“Design, Synthesis and Biological Evaluation of Novel Selective PTP-1B Inhibitors as a New Class of Antidiabetic Agents’’

A Thesis Submitted to

The Maharaja Sayajirao University of Baroda

For the Degree of

Doctor of Philosophy

IN

CHEMISTRY

BY

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CERTIFICATE

This is to certify that the thesis entitled **"Design, Synthesis and Biological Evaluation of Novel Selective PTP-1B Inhibitors as a New Class of Antidiabetic Agents''** which is being submitted to The Maharaja Sayajirao University of Baroda, Vadodara for the award of the degree of **DOCTOR OF PHILOSOPHY IN CHEMISTRY** is the result of the original research work conducted by **Mr. Dipam Patel** under our supervision and guidance at Zydus Research Centre, Ahmedabad and the work embodied in this thesis has not formed earlier the basis for the award of any degree or similar title of this or university or examining body.

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DECLARATION

I hereby declare that the topic entitled "**Design, Synthesis and Biological Evaluation of Novel Selective PTP-1B Inhibitors as a New Class of Antidiabetic Agents**" submitted herewith to The Maharaja Sayajirao University of Baroda, Vadodara of the fulfillment for the award of the degree of **DOCTOR OF PHILOSOPHY IN CHEMISTRY** is the result of the work carried out by me in Chemistry Department, Zydus research Centre, Ahmedabad.

The result of this work has not been previously submitted for any degree/fellowship to any university or institution.

Date:

Place:

Dipam Patel

PREFACE

This thesis is the outcome of my Ph.D. work at Zydus Research Centre and the department of Chemistry, The Maharaja Sayajirao University of Baroda, Vadodara, India.

The thesis consists of four major sections, Introduction, Designing, Results & discussion, experimental and overall summary part which cover various aspects of metabolic diseases and development of PTP-1B inhibitors for the treatment of the Type 2 diabetes. Three papers have been published in international journals.

The '**Introduction**" section deals with the general information about metabolic diseases, wherein detailed pathophysiology and the current therapeutic option are discussed with its limitation, followed by an introduction to PTP-1B inhibitors as novel target for the treatment of diabetes.

The "**Designing PTP-1B inhibitors**" section deals with the strategies and rationale for designing novel and sub-type selective PTP-1B inhibitors.

The "**Results & Discussion**" section summarized discussion on synthesis, biological activities and molecular modeling studies of the novel compounds.

In the "**Experimental**" section, detailed procedures for the synthesis of the compounds as well as the characterization data are presented. The details of various biological experiments are also described in this section.

In the '**Appendix-V**" copy of spectra (^{1}H-NMR, ESI-MS, ^{13}C-NMR and HPLC) of representative compounds from each series (intermediates and final compounds) are included. Data of all the compounds can be found in the experimental section, followed by copies of our publications.

Working for this thesis has been a great learning experience for me. Understanding the physiological pathway involved in metabolic syndrome and the biological roles of PTP-1B in this complex disease was very interesting and stimulative. Molecular modeling experiments provided good learning and were instrumental in understanding the ligand receptor interaction and structural requirements of the compounds to be synthesized. Presenting the work in the form of publications was equally a good learning experience.

Since this work has been carried out at Zydus Research Centre which is an industrial R&D centre as a medicinal chemist, it gives me a feeling of satisfaction that my drug design strategies and the studies described in this thesis may form the basis for the development of novel PTP-1B inhibitors. The feeling of satisfication is not only for the scientific outcome of the research work but also as it contributes for the social cause, since the ultimate need for the treatment of metabolic disorders such as diabetes. My philosophy is aline with the mission statement of my organization mission which says that,

"*ZRC aims to be the most admired pharmaceutical research centre for innovation in life science dedicated to alleviating human suffering*"

Human suffering is increasing day by day owing to various life threatening diseases and due to absence of treatment or resistance to treatment. Current understanding of metabolic diseases and treatment options are good but not adequate enough. Hence every endeavor in the direction of developing novel therapies in this area would be a significant contribution towards alleviating human suffering.

Dipam Patel

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My special thanks to lab-friends ***Pradip Jadav, Brijesh Darji, Yernaidu Siriki, Jignesh Pethani, Ganesh Rahane, Darshan Joshi, Rajendra Chopade, Vijay Prajapati, Pinkal Prajapati, Bhushan Dave, Hardik Shah*** *and* ***Krunal Kothari*** *for their cheerful co-operation and the help in every aspect through out this work.*

The work would have not completed without the help provided by the analytical group. My sincere thanks to ***Dr. R Murugan, Jigar Gajjar, Timar Patel*** *and* ***Jignesh Chauhan*** *for recording the NMR, Mass and HPLC spectra.*

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Finally, no words can express the feeling towards my parents and family members, who have contributed and sacrified a lot to reach me at this stage and will always remain a sole source of inspiration in my life.

Dipam Patel

Appendix- I: Abbreviations used

MS	Metabolic syndrome
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
GIT	Gastrointestinal tract
T1DM	Type I diabetes mellitus
IDDM	Insulin-dependent diabetes mellitus
T2DM	Type II diabetes mellitus
GDM	Gestational diabetes mellitus
GLP-1	Glucagon Like Peptide-1
DPP-IV	Dipeptidyl peptidase type IV
NEP	Neutral endopeptidases
GI	Gastrointestinal
PPARγ	Peroxisome proliferators-activated receptor gamma
11β-HSD-1	11β-hydroxysteroid dehydrogenase type-1
FBPase	Fructose 1 6-bisphosphatase
GSK-3	Glycogen synthase kinase-3
SGLT2	Sodium-dependent glucose cotransporters 2
PTP-1B	Protein tyrosine phosphatases 1B
GIP	Gastric inhibitory peptide
PPs	Protein phosphatases
PTPs	Protein tyrosine phosphatases
*p*Tyr	phosphotyrosine
*p*Thr	phosphothreonine
*p*Ser	phosphoserine

RPTPs	receptor-like PTPs
TC-PTP	T-cell protein tyrosine phosphatase
LAR	leukocyte common antigen-related
DIO	Diet-induced obesity
IR	Insulin receptor
IRTK	Insulin receptor tyrosine kinase
IRS	Insulin receptor substrate
PI3K	Phosphatidyl inositol 3 kinase
GLUT-4	Glucose transporter-4
MOA	Mechanism of action
LMW-PTP	Low-molecular-weight PTP
OBA	Oxalylamino benzoic acid
F2pmp	Phosphonodifluoromethyl phenyl
IZD	Isothiazolidinone
TZD	Thiadiazolidinone
PK	Pharmacokinetic
SAR	Structure activity relationship
DAST	Diethylsulfourtrifluoride
NFSi	*N*-fluorobenzenesulfonimide
KHMDS	Potassium bis(trimethylsilyl)amide
NaHMDS	Sodium hexamethyldisilazane
TFA	Trifluoroacetic acid
$Pd(OAc)_2$	Palladium acetate
$LiAlH_4$	Lithium aluminiumhydride
$SOCl_2$	Thionyl chloride

NBS	*N*-bromosuccinamide
CCl_4	Carbon tetrachloride
$NaBH_4$	Sodium borohydride
PPh_3	Triphenyl phosphine
DMF	*N*,*N*-dimethyl formamide
H_2	Hydrogen
*p*NNP	*p*-Nitro phenyl phosphate
IPGTT	Intraperitoneal glucose tolerance test
i.v.	Intravenous
i.p.	Intraperitoneal
p.o.	Oral
AUC	Area under curve
IFD	induced-fit docking
DIEA	*N*, *N*-diisopropylethylamine
TMSBr	Trimethylsilyl bromide
DIC	1,3-diisopropylcarbonamide
SPPS	Solid Phase Peptide Synthesis
EDT	1,2-ethanedithiol
DMAP	*N*,*N*-dimethyl aminopyridine

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Chapter I: Introduction

1. Introduction

1.1. Diabetes

The term “Metabolic syndrome” (MS) refers to a cluster of medical disorders such as, hyperinsulinaemia, hyperglycemia, dyslipidemia, high blood pressure, insulin resistance and obesity. When occurring together then they increase the risk of developing cardiovascular diseases and diabetes. The incidences of metabolic syndrome have reached global epidemic proportions **[1-2]**. Metabolic syndrome is also known as metabolic syndrome X, cardiometabolic syndrome, syndrome X, insulin resistance syndrome, Reaven’s syndrome (named after Gerald Reaven) and CHAOS (in Australia).

Obesity, is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health and is a major risk factor for developing the other metabolic diseases and diabetes and cardiovascular disease are the major manifestations of metabolic syndrome. Obesity as a metabolic disorder was reported in 1947 **[3]** and subsequently described as a syndrome, which comprised of hypertension, hyperglycemia **[4]**. After a gap of four decades, in 1988 a cluster of risk factors for diabetes and cardiovascular diseases were defined and was named as Syndrome X **[5]**. The introduction of concept of insulin resistance and its inclusion in the group of metabolic syndrome was the most significant move in this area and renamed as ‘The Deadly Quartet’ **[6]** and “The Insulin Resistance Syndrome” **[7]**.

Diabetes mellitus is a group of metabolic diseases in which hyperglycemia arises as a result of a relative or absolute deficiency of insulin secretion, resistance to insulin action, or both **[8]**. Diabetes is an ailment in

which the body does not produce or properly use insulin. Insulin is a regulatory hormone required for energy management. The cause of diabetes continues to be anonymity, although both genetics and environmental factors such as obesity and lack of exercise appear to play roles. Diabetes mellitus is a major and growing public health problem throughout the world, with an estimated worldwide prevalence of 220 million people in 2010 and it is expected to increase to 366 million people by 2030 [**9**]. Many people also have other abnormalities of glucose metabolism (sometimes called "prediabetes") manifest either as impaired fasting glucose (IFG) levels or as impaired glucose tolerance (IGT). The criteria for diagnosis of diabetes and prediabetes conditions are summarized in **Table 1**.

Table 1. Diagnostic Criteria for Diabetes Mellitus and Prediabetes conditions

Types of Diabetes	Pre-prandial plasma glucose mg/dL (mmol/L)	Post-prendial plasma glucose mg/dL (mmol/L)
Normal	**< 110 (< 6.1)**	**< 140 (< 7.8)**
Impaired fasting glucose (IFG)	**≥ 100 (≥ 6.1) & < 120 (< 7.0)**	**< 140 (< 7.8)**
Impaired glucose tolerance (IGT)	**< 126 (< 7.0)**	**≥ 140 (≥ 7.8)**
Diabetes mellitus	**≥ 126 (≥ 7.0)**	**≥ 200 (≥ 11.1)**

Majority of diabetic patients can be treated with the agents that reduce hepatic glucose production (glucagon antagonist), reduce glucose absorption from gastrointestinal track (GIT), stimulate β-cell function (insulin

secretagogues) or with the agents that enhance the tissue sensitivity of the patients towards insulin (insulin sensitizers). The drugs presently used to treat diabetes include α-glucosidase inhibitors, insulin sensitizers, insulin secretagogues and K_{ATP} channel blockers **[10]**. However, almost one-half of diabetic subjects lose their response to these agents, over a period of time and thereby to insulin therapy. Insulin treatment has several drawbacks, it is injectable, causes hypoglycemia and weight gain **[11]**.

1.1.1. Types of diabetes

Although several pathogenic processes may be involved in the development of diabetes, the vast majority of cases fall into two main categories: Type I diabetes and Type II diabetes. Gestational diabetes, yet another type of diabetes diagnosed in pregnant women.

1.1.1.1. Type 1 diabetes mellitus (T1DM)

Type I diabetes occurs usually due to an immune-mediated destruction of pancreatic islet β-cells with consequent insulin deficiency. Although usually having an abrupt clinical onset, the disease process unfolds slowly, with progressive loss of β-cells. In T1DM, >90% β-cells are destroyed by autoimmune-mediated islet cell destruction, and hence T1DM patient rely on insulin injections for survival. T1DM is usually diagnosed in children and young adults, it is also called as juvenile diabetes or insulin-dependent diabetes mellitus (IDDM). Conditions associated with T1DM include hyperglycemia and ketoacidosis (**Figure 1**). T1DM increases risk for many serious complications. Some complications of T1DM includes: heart disease (cardiovascular disease), blindness (retinopathy), nerve damage

(neuropathy), kidney damage (nephropathy), foot and skin complications and depression.

1.1.1.2. Type 2 diabetes mellitus (T2DM)

Type II diabetes mellitus (T2DM), the most common type of diabetes usually occurs due to insulin resistance, defect in the insulin production or increase in the hepatic glucose production and is usually associated with dyslipidemia, hypertension and obesity **[12]**.

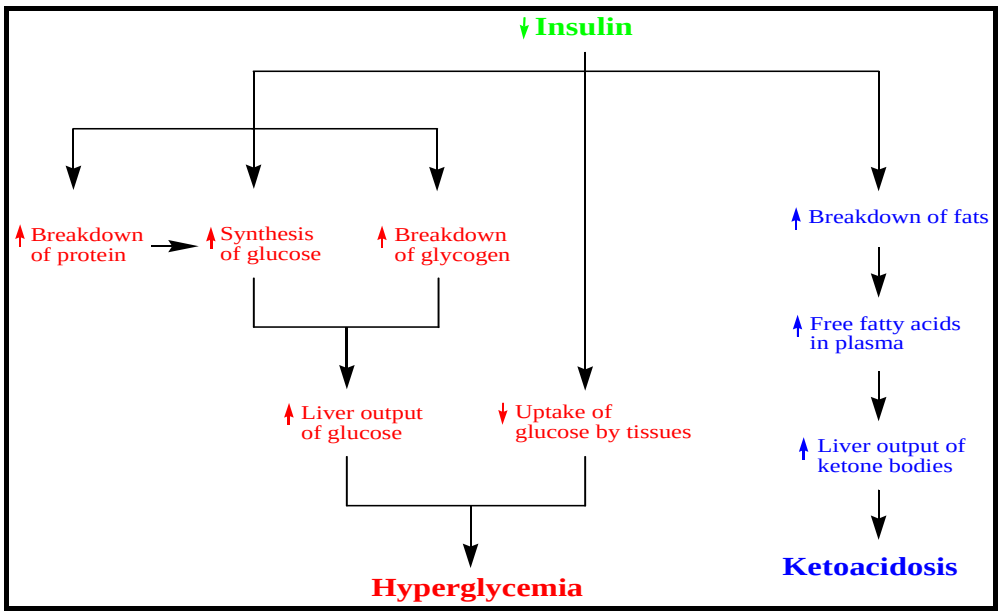


Figure 1. Metabolic Changes in T1DM

In T2DM, >50% of β-cells are already lost at the time of diagnosis continue to decline throughout the course of T2DM, mainly due to apoptosis **[13]**. As depicted in **Figure 2**, insulin resistance arises as a consequence of multiple factors such as sedentary lifestyle, aging and obesity which results in hyperglycemia, blood pressure elevation, and dyslipidemia. The important contributing factors for T2DM include resistance to insulin, increased hepatic glucose production, decreased insulin-mediated glucose transport into

adipose tissues and impaired β-cell function leading to loss of early phase of insulin release.

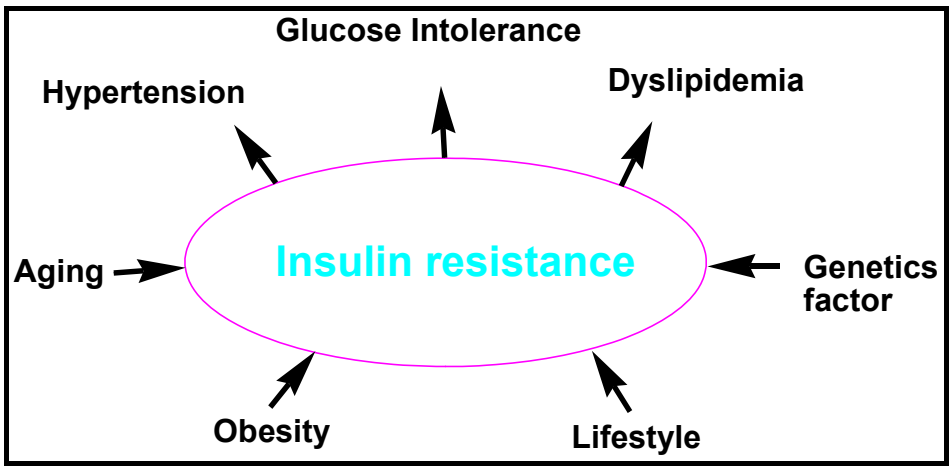


Figure 2. Causes and consequences of Insulin resistance

In T2DM, patients begin with insulin resistance and often treated with various oral antihyperglycemic agents; however, over a period of time, almost one-half of T2DM subjects lose their response to these agents and thereby require insulin therapy **[14]**. The decline in β-cells in T2DM drives the progressive deterioration in glycemic control and develops secondary complications.

1.1.1.3. Gestational diabetes mellitus (GDM)

Gestational diabetes mellitus (GDM) is a condition in which women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy (especially during third trimester of pregnancy). Gestational diabetes occurs when the body of a pregnant woman does not secrete excess insulin required during pregnancy leading to increased blood sugar levels.

Gestational diabetes generally has few symptoms and it is most commonly diagnosed by screening during pregnancy. Diagnostic tests detect

inappropriately high levels of glucose in blood samples. Gestational diabetes affects 3-10% of pregnancies, depending on the population studied **[15]**. In general, babies born to mothers with gestational diabetes are typically at increased risk of problems such as being large for gestational age (which may lead to delivery complications), low blood sugar, and jaundice. Gestational diabetes is a treatable condition and women who have adequate control of glucose levels can effectively decrease these risks **[16]**.

1.2. Pathogenesis of T2DM

The pathological sequence of T2DM is complex and involves many different elements that act together and make T2DM condition more complex (**Figure 3**). As described earlier, T2DM is characterized by varying degree of insulin resistance and insulin deficiency. It is thought that the earliest defect in the pathogenesis of T2DM is impaired insulin action or insulin resistance.

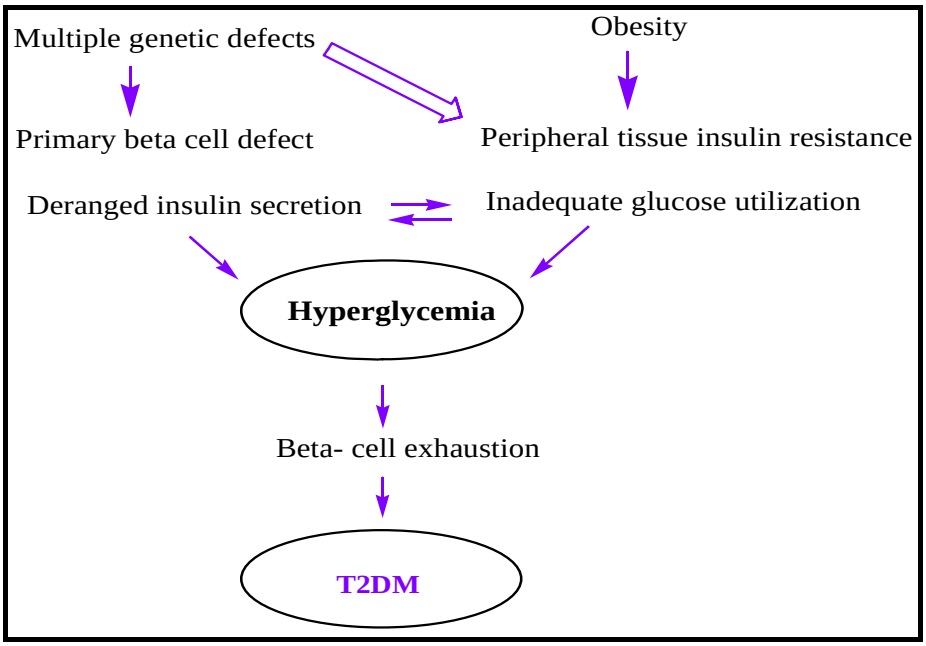


Figure 3. Proposed Pathogenesis of Type 2 Diabetes

Resistance to the action of insulin results in impaired insulin mediated glucose uptake by muscles, incomplete suppression of hepatic glucose output and impaired triglyceride uptake by fat. To overcome the insulin resistance, beta islet cells will increase the amount of insulin secreted.

Along with different factors, both endogenous hormone Glucagon like peptide-1 (GLP-1) and glucagon play an important role in pathogenesis of T2DM **[17]**. GLP-1 (7-36) amide is a product of the preproglucagon gene, which is secreted from intestinal L-cells, in response to the ingestion of food. Endogenous GLP-1 binds to a membrane GLP-1 receptor. As a consequence of this, insulin release from the pancreatic β-cells is increased **[17]**. The major problem of GLP-1 is its shorter half life. Glucagon (29 amino acid peptide) hormone is produced from proglucagon in pancreatic α-cell by PC2 **[17]**. The main physiological role of glucagons is to stimulate hepatic glucose output, thereby leading to hypoglycemia. Therefore two defects, insulin resistance and insulin deficiency are responsible factors for the development of T2DM.

1.3. Current & Newer therapies for the treatment of T2DM

1.3.1. Current therapies for the treatment of T2DM

The cornerstone of treatment and prevention of T2DM is lifestyle modification through increased physical activity and attention to food intake, particularly among the subjects, where in weight loss is the principal goal. When lifestyle modifications do not result in normalization or near normalization of metabolic abnormalities, pharmacological therapy is required. Based on route of administration, current therapies are divided in two groups, (1) Injectable and (2) Oral therapies.

1.3.1.1. Injectable therapies for the treatment of T2DM

Insulin facilitates glucose entry into adipose tissues, muscles, and liver by stimulating several enzymatic reactions that start at the insulin receptors. The stimulation of an intrinsic tyrosine kinase of the insulin receptor results in an increase in membrane phosphorylation that consequently increases the membrane permeability to glucose through a complicated cascade of intracellular events. Currently available injectable analogus are divided into two groups, (1) insulin analogues and (2) incretin mimetics as shown in **Table 2**. Further, insulin analogues are sub-divided into three groups depending upon their duration of action.

Table 2. Insulin and insulin analogues

Drug	Source	Trade name
Insulin product **Fast acting:** **Regular**	Recombinant DNA Pork	Humulin R, Novolin R Iletin II
Lispro	Recombinant DNA	Humalog, Humalog, Lispro-PFC
Aspart	Recombinant DNA	Novolog, Flexpen
Glulisine	Recombinant DNA	Apidra
Intermediate acting: **Isophane Insulin**	Pork	Iletin II NPH Purified Pork
Isophane Insulin	Recombinant DNA	Humulin N, Humulin N Pen Novolin N
Insulin zinc	Pork	Iletin II Lente
Insulin humane zinc	Recombinant DNA	Humulin L, Novolin Ge Lente
Long acting: **Extended insulin human zinc suspension**	Recombinant DNA	Humulin U, Novolin ge Ultralente
Insulin glargine	Recombinant DNA	Lantus

Inceretin mimetics **Exenatide**	Saliva of the Gila monster	Byetta
Liraglutide	----	Victoza

As described earlier, in T1DM >90% β-cells are destroyed and hence T1DM patients rely on insulin injection for survival. While T2DM begins with insulin resistance and over a period of time almost one-half of T2DM pateients lose their response to antihyperglycemic agents and thereby require insulin therapy **[13]**.

The effect of insulin on glucose uptake and metabolism is shown in **Figure 4**. Secreted insulin binds to its receptor, which in turn starts many protein activation cascades. These cascades include translocation of Glut-4 transporter to the plasma membrane and influx of glucose, glycogen synthesis, glycolysis and fatty acid synthesis. The main action of insulin on cells includes increased glucogen synthesis, increased fatty acid synthesis, increased esterification of fatty acid, decreased proteolysis, decreased lipolysis and decreased gluconeogenesis.

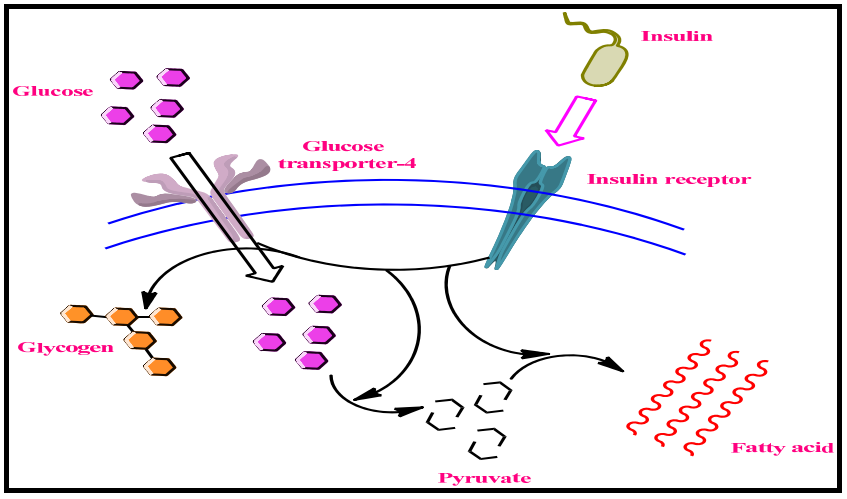


Figure 4. Mode of action of insulin on glucose uptake

Exenatide and liraglutide belong to group of inceretin mimetics. Exenatide is a 39-amino-acid peptide, an insulin secretagogue, with glucoregulatory effects. It bears a 50% amino acid homology to GLP-1 and it has a longer half-life. Liraglutide is an acylated human GLP-1 receptor agonist, with a 97% amino acid sequence identity to endogenous human GLP-1(7-37).

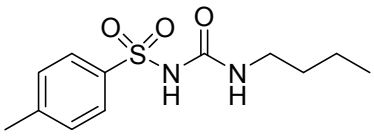
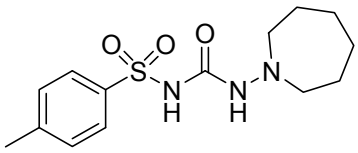
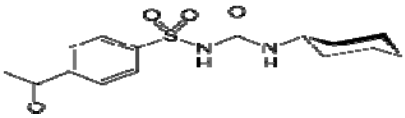
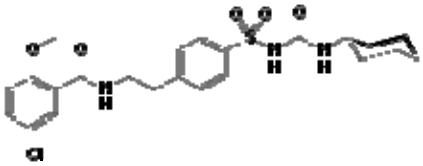
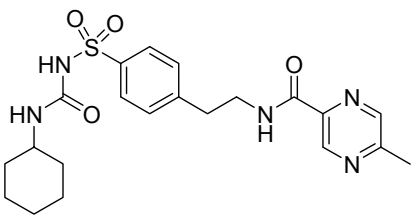
Liraglutide is stable against metabolic degradation by both peptidases, dipeptidyl peptidase IV (DPP-IV) and neutral endopeptidases (NEP). Exenatide augments pancreas response **[18]** (i.e. increases insulin secretion) in response to eating meals; the result is the release of a higher, more appropriate amount of insulin that helps lower the rise in blood sugar from eating. It also suppresses pancreatic release of glucagon in response to eating. Exenatide helps to slow down gastric emptying and reduces liver fat content. While liraglutide acts in a glucose-dependent manner, meaning it will stimulate insulin secretion only when blood glucose levels are higher than normal. It has the potential for inhibiting apoptosis and stimulating regeneration of beta cells. It decreases appetite and maintains body weight and lowers blood triglyceride levels.

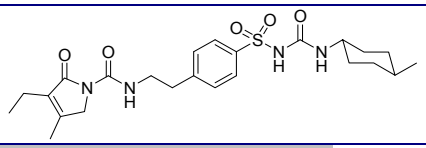
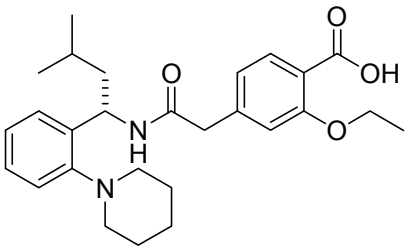
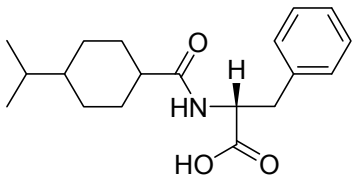
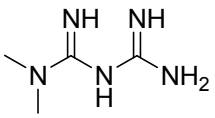
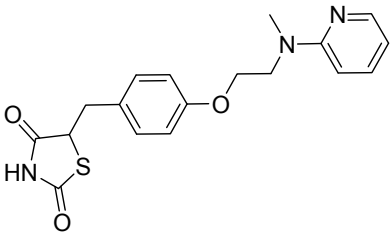
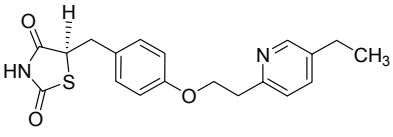
These injectable therapies & inceretin mimetics have several drawbacks, it is injectable, produces hyperglycemia and causes weight gain, which is believed to be a potential cause for the development of diabetes complications **[19]**. Thus, there is an urgent need to develop some oral antihyperglycemic agents that can complement with the existing injectable therapies.

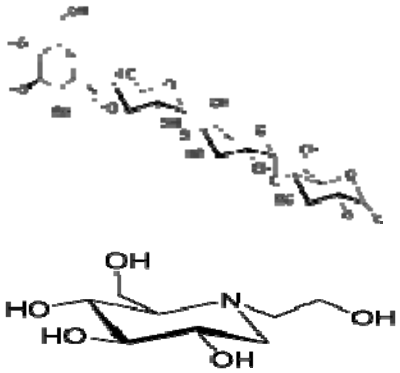
1.3.1.2. Oral antidiabetic agents for the treatment of T2DM

Before 1995, sulfonylureas were the only oral antidiabetic agents available for the treatment of T2DM. Since 1995, there has been an explosion of introduction of new classes of pharmacologic agents. Currently available oral antidiabetic therapies include agents which cause insulin production (sulfonylureas, secretagogues), agents which decrease hepatic glucose production (biguanides), agents which act as insulin sensitizers (glitazones), and R-glucosidase inhibitors which are listed in **Table 3**.

Table 3. Orally administered antidiabetic agents for T2DM treatment

Drug class	Agent (Brand name)	Structure
Sulfonylureas [20] **First generation**	Tolbutamide (Oramide, Orinase)	
	Tolazamide (Tolamide, Tolinase)	
Second generation	Acetohexamide (Demylor)	
	Glyburide (Micronase, Diabeta)	
	Glipizide (Glucotrol)	

Third generation	Glimepride (Amaryl)	
M.O.A- Stimulating insulin production by inhibiting the K_{ATP} channel in pancreatic β- cells		
Non-Sulfonylurea [21] Secretagogues	Repaglinide (Prandin)	
	Nateglinide (Starlix)	
M.O.A-enhance insulin secretion in pancreatic β- cells		
Biguanides [22]	Metformin (Glucophage)	
M.O.A- Decreases insulin resistance in liver		
Insulin receptors sensitizers [23]	Rosiglitazone (Avandia)	
	Pioglitazone (Actors)	
M.O.A- stimulates PPAR-γ and PPAR-α, reduces insulin resistance in the liver and peripheral tissues		

Alpha-Glucosidase Inhibitors [24]	Acarbose (Prelose) Miglitol (Glyset)	
M.O.A- Reduces intestinal glucose absorption in G.I tract		

The usual treatment strategy in T2DM is to start with either metformin **[22]** or a secretagogue **[21]**. If adequate control is still not achieved, the second step is to add a complementary drug, i.e. one working by a different pathway. The most common such combination is metformin plus a secretagogue. If adequate glycemic control is still not attained, the choice is to add a third class of oral drugs (e.g. glitazone or glucosidase inhibitors).

However, most of the oral antihyperglycemic agents are also associated with side effects and adverse events. In case of sulfonylureas, they work by stimulating endogeneous release of insulin and exhibit hypoglycemia as the major side effects. The other adverse effect consists of digestive manifestation (nausea, epigastic pain, liver pain) and of hematological manifestations (pancytopenia, autoimmune hemolytic anemia, thrombocytopenia) **[20]**.

Biguanides reduce hepatic glucose output and increase uptake of glucose by periphery. They are associated with side effects such as digestive manifestations especially epigastric pain and diarrhea. Lactic acidosis is another adverse effect associated with biguanides. The major side effects associated with glitazones are mild edema of the lower limbs, through the loss

of elimination of salt and water. The other adverse effect is decrease in hemoglobin, with the appearance of anemia. Glitazones can also cause hypercholesterolemia and triglycerides disorders. Alpha glucosidase inhibitors exhibit side effects such as weight gain, abdominal bloating, flatulence, abdominal discomfort and diarrhea.

1.3.2. Newer therapies for the treatment of T2DM

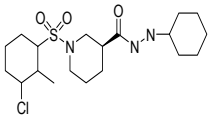
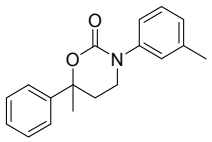
As described earlier, most of the injectable and oral therapies exhibit serious side effects and adverse events such as hypoglycemia, GI side effects, lactate production, fluid retention, hepatoxicity, allergic reaction and cardiovascular effects. Also long term use exhibits side effects such as body weight gain and progressive loss of β-cell function. To overcome the side effects and safety concern of these current drugs, several newer pharmacologic approaches have been developed for the safe and effective treatment of T2DM. Currently several new therapies are in various stages of clinical development for the treatment of T2DM as shown in **Table 4**.

Table 4. New therapies under clinical development for the treatment of T2DM

Target	Structure/ Name	Company	Clinical status	Ref.
GLP-1 agonist*	ZYD1	Zydus Cadila	Phase I	**[25]**
	PB-1023	PhaseBio	Phase II	**[26]**
	NNC-0113-0987	Novo Nordisk	Phase I	**[27]**

Target	Structure/ Name	Company	Clinical status	Ref.
GLP-1 agonist*	NOX-G15	Noxxon	Preclinical	**[28]**
DPP-IV Inhibitors**	Vidagliptin (Galves; LAF-237)	Novartis	Launched	**[29-32]**
	Saxagliptin (BMS-477118)	Bristol-Mayers Squibb	Launched	**[33-35]**
	(KRP-104)	Active Biosciences (Kyorin)	Phase II	**[36]**
	Anagliptin (SK-0403)	Kowa JW Pharmaceutical	Phase III	**[37-38]**
	Gemigliptin (LC15-444)	LG Life Sciences	Phase III	**[39]**
	KR-66223	LG Life Sciences	Preclinical	**[40]**
	Melogliptin (GRC-8200)	Merck	Phase II	**[41-42]**
	Teneligliptin (MP-513)	Mitsubishi Tanabe Pharma	Registered	**[43]**

Target	Structure/ Name	Company	Clinical status	Ref.
DPP-IV Inhibitors**	Linagliptin (BI-1356)	Boehringer Ingelheim	Launched	**[44]**
	Gosogliptin (PF-734200)	SatRx	Preclinical	**[45-49]**
	Trelagliptin (SYR-472)	Takeda	Phase III	**[50]**
	ARI-2243	Arisaph Pharmaceutical	Phase I	**[51-52]**
	MK-3102	Merck & Co	Phase II	**[53]**
	DA-1229	Dong-A	Phase II	**[54-55]**
	PBL-1427	Panacea Biotech	Preclinical	**[56]**
PPARγ dual agonist**	Efatutazone (CS-7017)	Daiichi Sankyo	Phase II	**[57]**
	Chiglitzar (CS-0038)	Chipscreen Biosciences	Phase III	**[58]**

Target	Structure/Name	Company	Clinical status	Ref.
PPARγ dual agonist**	Libeglitazone (CKD-501)	Chong Kun Dang	Phase III	**[59-60]**
	PPARγ agonist	Takeda	Preclinical	**[61-62]**
11β-HSD-1 inhibitors**		Bristol-Mayers Squibb	Preclinical	**[63]**
	RG-4929	Roche	Phase II	**[64]**
	RG-7234	Roche	Phase I	**[64]**
	BVT-3498	Biovitrum	Discontinued Phase II	**[64]**
	PF-915275	Pfizer	Discontinued Phase I	**[65]**
		Vitae Pharmaceutical	Preclinical	**[66]**
	INCB-13739	Incyte Corporation	Phase IIb	**[67]**
	INCB-20817	Incyte Corporation	Discontinued Phase I	**[68]**

Target	Structure/ Name	Company	Clinical status	Ref.
11β-HSD-1 inhibitors**	AZD4017	AstraZeneca	Phase I	**[69]**
	AMG-221	Biovitrum	Phase I	**[70]**
FBPase inhibitors**	CS-917	Metabasis Therapeutics	Discontinued (Phase II)	**[71]**
GSK-3 inhibitors**	DM-199	DiaMedica	Preclinical	**[72]**
	DM-204	DiaMedica	Preclinical	**[73]**
SGLT2 Inhibitors**	Canagliflozin (TA-7284)	Mitsubishi Tanabe Pharma	Under development	**[74]**
	Dapagliflozin (TA-7284)	Bristol-Myers Squibb	Pre-registration	**[75]**
	Empagliflozin (BI-10773)	Boehringer Ingelheim	Phase III	**[76-77]**
	Tofogliflozin (RG-7201)	Roche	Phase III	**[78]**

Target	Structure/ Name	Company	Clinical status	Ref.
SGLT2 Inhibitors**	Remogliflozin etabonate (KGT-1681)	Kissei	Phase II	**[79-82]**
	Ipragliflozin (ASP-1941)	Kotobuki Pharmaceutical	Phase III	**[83]**
	Luseogliflozin (TS-071)	Taisho	Phase III	**[84-85]**
	SBM-TFC-039	Sirona Biochem	Preclinical	**[86]**
	THR-1474	Theracos	Phase II	**[87]**
	Ertugliflozin (PF-04971729)	Pfizer	Phase II	**[88-90]**
	EGT-0001442	Theracos	Phase II	**[91]**
PTP-1B Inhibitors**	Ertiprotafib	Wyeth Pharmaceutical	Discontinued	**[92]**
	TTP-814	Trans Tech Pharma	Phase II	**[93]**
	ISIS-PTP1BRx	Isis Pharmaceutical	Phase I	**[94]**
	PTP1B Inhibitors	Advinus	Preclinical	**[95]**

GLP-1: Glucagon-like peptide 1; **DPP-IV**: Dipeptidyl peptidase IV; **PPARγ:** Peroxisome proliferators-activated receptor gamma; **11β-HSD-1**: 11β-hydroxysteroid dehydrogenase type-1; **FBPase**: Fructose 1 6-bisphosphatase ; **GSK-3**: Glycogen synthase kinase-3; **SGLT2**: Sodium-dependent glucose cotransporters; **PTP-1B**: Protein tyrosine phosphatases 1B. ***** Injectable; ****** Oral

As described in **Table 4**, currently several new therapies are in various stages of clinical development for the treatment of T2DM. Among these new therapies, DPP-IV inhibitors and PTP-1B inhibitors are most promising. Endogenous dipeptidyl peptidase type IV (DPP-IV) enzyme has been shown to be a key physiological regulator of incretin activity. DPP-IV is a serine protease and *in vivo*, it inactivates both the incretin hormones GLP-1 & Gastric inhibitory peptide (GIP) **[96-98]**, which in-turn stimulates glucose dependent insulin secretion. Thus, inhibition of DPP-IV activity results in increased level of intact bioactive GIP and GLP-1 peptides, which cause an increase in the amount of insulin release from β-cell of the islets of Langerhans after eating, even before blood glucose levels become elevated, thereby, it acts as an antidiabetic agent.

Protein tyrosine phosphatase-1B (PTP-1B) enzyme leads to dephosphorylation of insulin receptor and acts as a negative regulator in insulin signaling pathway **[99-100]**. The two different reports suggest that PTP-1B knock-out mice showed improved insulin sensitivity **[101-102]**. Thus, inhibition of PTP-1B could be the most effective and safer for the treatment of T2DM.

Among the newer therapies, GLP-1 agonists have not been very successful due to little risk of hypoglycemia **[103]**, gastrointestinal side effects **[104]** and their injectable route of administration. Main side effect of PPARγ

dual agonist is water retention, leading to edema, an increased risk of coronary heart disease **[105-106]**. Compounds of FBPase inhibitors show hypoglycemia as a major side effect **[71]**. Inhibitors of GSK-3 are associated with side effects such as neuronal disorders **[107]**, while inhibitors SGLT2 and 11β-HSD-1 have low potential for hypoglycemia.

As discuss earlier, inhibitors of DPP-IV and PTP-1B are the most up coming therapies for T2DM treatment. DPP-IV inhibitors can apply to lower post-prandial blood glucose **[108]**, but they could not address the issue of lowering fasting blood glucose and other metabolic disease such as obesity. The major disadvantage of these DPP-IV inhibitors is that they allow the risk of cancer **[109-111]**. Recently, a study of DPP-IV inhibition on human non-small cell lung cancer (NSCLC) concludes that "DPP-IV functions as a tumor suppresser, and its down regulation may contribute to the loss of growth control in NSCLC cells **[112]**. While, PTP-1B inhibitors exhibit potential for enhancing insulin action by prolonging the phosphorylated state of the insulin receptor **[113]**. Moreover, PTP-1B acts as a negative reglulator of leptin signaling which is an adipocyte-derived hormone, which acts on the hypothalamus to decrease food intake and increase energy expenditure **[114]**. Recently, it has been proved that over expression of PTP-1B is sufficient to drive tumorigenesis in mice, providing additional support for the use of PTP-1B inhibitors for cancer therapy **[115-117]**. On the basis of these data, PTP-1B is currently considred one of the best biological target for metabolic diseases such as T2DM and obesity.

Overall among different therapies such as agents which cause insulin secretion, agents which decrease hepatic glucose production and agents

which decrease insulin resistance, it can be concluded that the insulin resistance is the most dominant cause for T2DM, hence increasing insulin sensitivity can form a promising therapy for the treatment of T2DM. Various drugs are available that directly or indirectly increase insulin sensitivity but their high cost, selectivity and route of administration are limiting factors. This invites research to develop small molecule based PTP-1B inhibitors may provide safe and cost effective option for the treatment of diabetes. Although, many small molecule based PTP-1B inhibitors are under development at various research laboratories throughout the world. The present scenario of non availability of safe, cost effective and efficacious small molecule based PTP-1B inhibitors, prompted us to hunt for novel PTP-1B inhibitors. The research presented in this thesis focus on the synthesis, biological evaluation of potent, selective and orally bioavailable PTP-1B inhibitors. In the next section, an overview on PTP-1B inhibitors is presented.

1.4. Introduction to PTP-1B inhibitors

1.4.1. Protein Phosphatase and their importance

Protein phosphatases (PPs) are an important sub family of enzymes that remove phosphate groups from proteins **[118]** and work exactly opposite to the function of protein kinases which phosphorylate proteins and together these opposing enzymes maintain the equilibrium at right levels. Protein tyrosine phosphatases (PTPs) are characterized by a conserved active site sequence (H/V)C(X)$_5$R(S/T) **[119]**, called the PTP signature motif, in which the cysteine residue functions as a nucleophile and is essential for catalysis. The PTP family can be divided into two major groups based on their substrate specificity: classical phosphotyrosine (*p*Tyr)-specific PTPs (38 phosphatases

belong to this subfamily) and dual specificity PTPs which recognize phosphotyrosine (*p*Tyr), phosphotheonine (*p*Thr) and phosphoserine (*p*Ser) residues **[120-122]**.

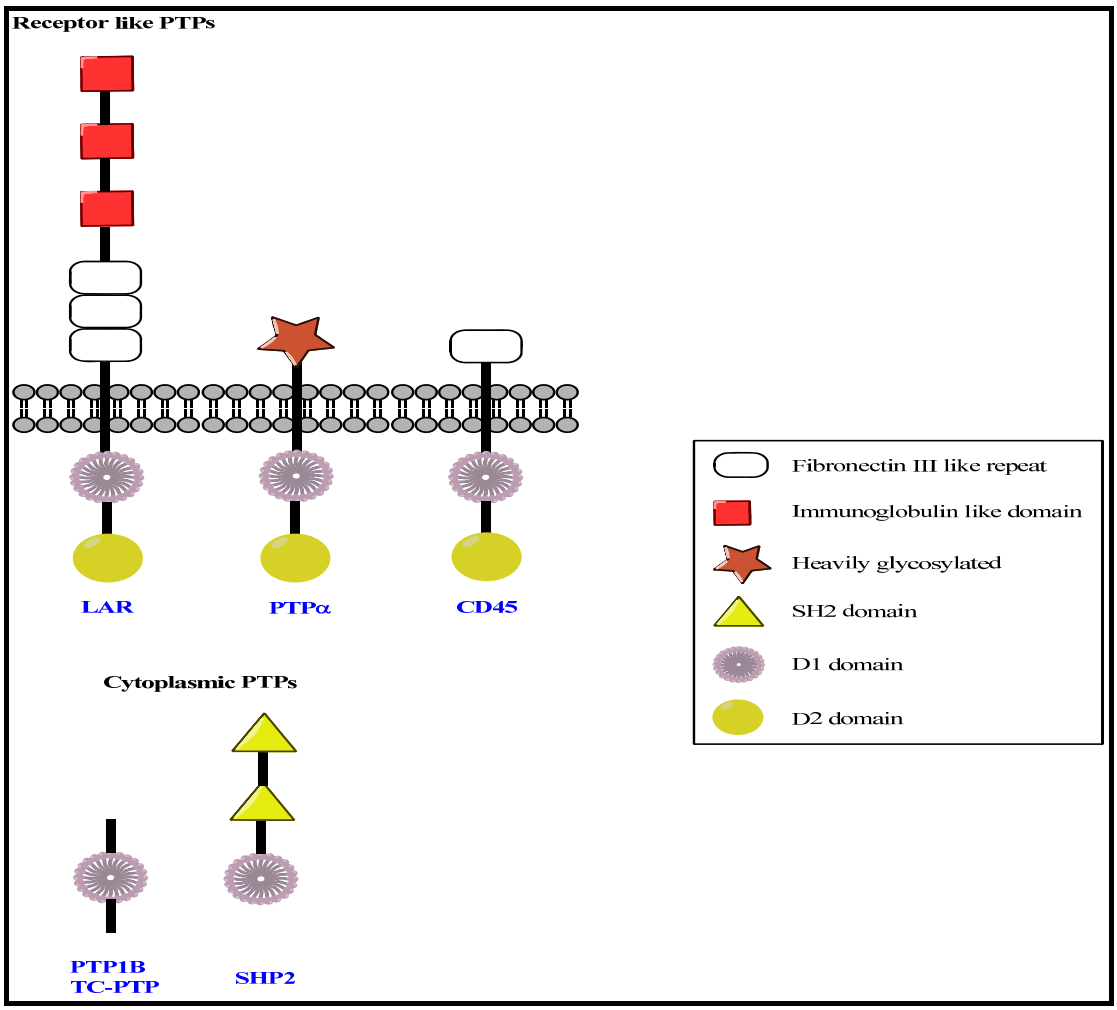


Figure 5. Classical representation of various PTPs

The classical *p*Tyr-specific PTPs can be further sub-divided as intracellular or receptor-like PTPs (RPTPs) as shown in **Figure 5**. The RPTPs have the potential to regulate signaling by extra-cellular ligand-controlled protein Tyr dephosphorylation and posses a tandem arrangement of two PTP domains in the intracytoplasmic region. The membrane-proximal (D1) domain

contains all the active residues, where as the membrane-distal domain (D2) is inactive and plays a regulatory role. All the cytoplasmic PTPs are characterized by regulatory sequences that control the intracellular localization and their catalytic activity **[120]**.

1.4.2. Protein tyrosine phosphatase (PTPs)

Protein tyrosine phosphatases (PTPs) are an important super family of PPs which remove phosphate groups from phosphorylated tyrosine residues on proteins and regulate many signal transduction pathways, including growth initiation, propagation and termination, by regulating the extent of phosphorylation by working in association with the kinases in reciprocal directions **[123]**. Defect in PTPase activity can lead to aberrations in the phosphorylation of tyrosine, eventually leading to protein malfunction, which contributes to the many human diseases like cancer, diabetes, obesity and rheumatoid arthritis. In recent days, PTPases have gained importance as the drug discovery target because of its critical role in bioprocesses. Some important phosphatases include CD45, PTP-1B, PTP1N, T-cell protein tyrosine phosphatase (TC-PTP), SHP-2, leukocyte common antigen-related (LAR), PP2C, Ppz1p, Ppz2p. CD45 is present in human hematopoietic cells and its mutation may lead to dysfunction of B lymphocytes and low T cell production which can lead to immunodeficiency.

Among all phosphatases, TC-PTP and PTP-1B play an important role in bioprocesses. TC-PTP-deficient mice died within 3-5 week of age because of impaired B cell and T cell functions, signifies that TC-PTP is important in insulin signaling **[124]**. While Inhibition of PTP-1B results in sensitization to insulin signaling and protection against diet-induced obesity. This dual effect

of PTP-1B makes it a promising target in the treatment of T2DM by addressing key issues not filled by current therapies.

1.4.3. Role of PTP-1B in metabolic diseases

Modification of proteins by tyrosine phosphorylation is a major mechanism to control their functions, and plays a key role in transmembrane and intracellular signaling. PTP-1B is the prototype for the superfamily of PTPs and has been the most extensively studied within the group. Growing biochemical evidence was accumulating for the involvement of the prototypical phosphatase, PTP-1B, in the intracellular dephosphorylation of the insulin receptor and insulin receptor proteins and thus its involvement in the down-regulation of insulin signaling. Inhibition of PTP-1B activity has the potential for enhancing insulin action by prolonging the phosphorylated stage of the insulin receptor **[113]**. Gene knockout studies in animals have also demonstrated that PTP-1B-deficient mice maintain lower nonfasting blood glucose and insulin levels compared to their wild-type **[101-102]**. PTP-1B deficiency does not induce hypoglycemia in the fasted state. PTP-1B knockout mice has an unexpected protective effect against high-fat diet-induced obesity (DIO) **[125]**. Both basal metabolic rate and total energy expenditure are enhanced in PTP-deficient mice **[126]**. This mice are reported to have increased sensitivity to leptin **[127]**. On the basis of these data, PTP-1B is currently considered one of the best validated biological targets for non-insulin dependent diabetes and obesity.

1.4.3.1. PTP-1B as a negative regulator of insulin signaling

The role of PPT1B in the regulation of insulin signaling is shown in **Figure 6**. Insulin binds to its receptor, leads to insulin induced activation of

insulin receptor tyrosine kinase (IRTK) through autophosphorylation **[128-132]**. This leads to recruitment of insulin receptor substrate (IRS) proteins, followed by activation of phosphatidyl inositol 3 kinase (PI3K) and subsequent translocation of glucose transporter-4 (GLUT-4), from the cytoplasm into plasma membrane thus allowing glucose to enter the cell. The activated IR complex is moved to the endoplasma by the process which is dependent on tyrosine autophosphorylation.

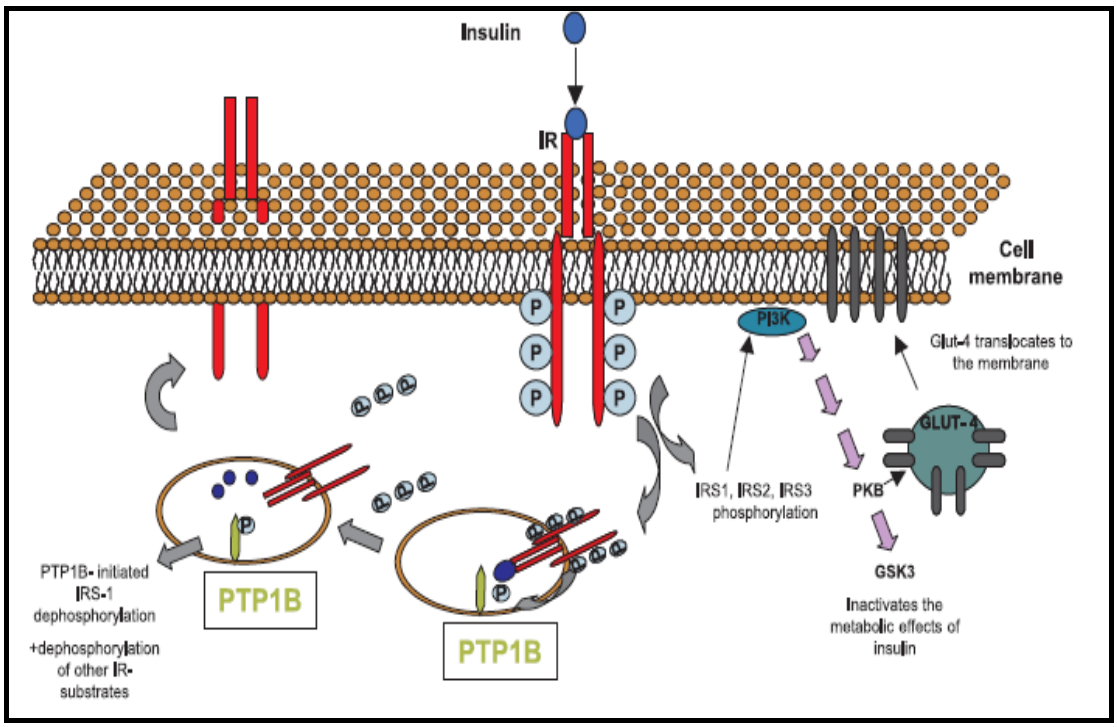


Figure 6. Role of PTP-1B in insulin signaling

In the endosomal compartment, insulin dissociates from its receptor which dephosphorylates leading to translocation and recycling of the receptor to the plasma membrane. PTP-1B plays an important role in the negative regulation of insulin signaling. The phosphorylated and also activated IR phosphorylates also tyrosine residues of PTP-1B which increases its catalytic

activity. It interacts with the IR and removes tyrosine phosphates induced by autophosphorylation in response to insulin binding. PTP-1B also dephosphorylate IRSs thus attenuating the insulin action.

1.5. Crystal structure of PTP-1B

PTP-1B was the first PTP enzyme to be purified from human placental tissue. The native protein consists of 435 amino-acid residues, 50KDa protein belonging to the non-transmemnrenane PTP family **[133-137]**. The conserved core PTP catalytic domain is coded by residues 30-278 amino acids and contains the consensus tyrosine phosphatase signature motif: (I/V)HC_xAG_xGR(S/T)G which shares an average of 40% of sequence identity with other members of PTP family **[119]**. The following 80 to 100 residues constitute the regulatory section and, finally the 35-carboxy-terminal amino acid residues (residues 400 to 435) are rich in Pro, and constitute the membrane localization region, responsible for binding the enzyme to the cytoplasmic face of the IR. For biological purpose, shorter version of the protein (298 or 321 residue) are usually employed.

The 321-residue version is composed of a single domain, organized in eight α-helices and twelve β strands. **Figure 7** represents a cartoon representation of the secondary structure of PTP-1B. The main structural features are (1) catalytic loop, (2) WPD loop, (3) Secondary aryl-phosphate-binding site and (4) YRD motif.

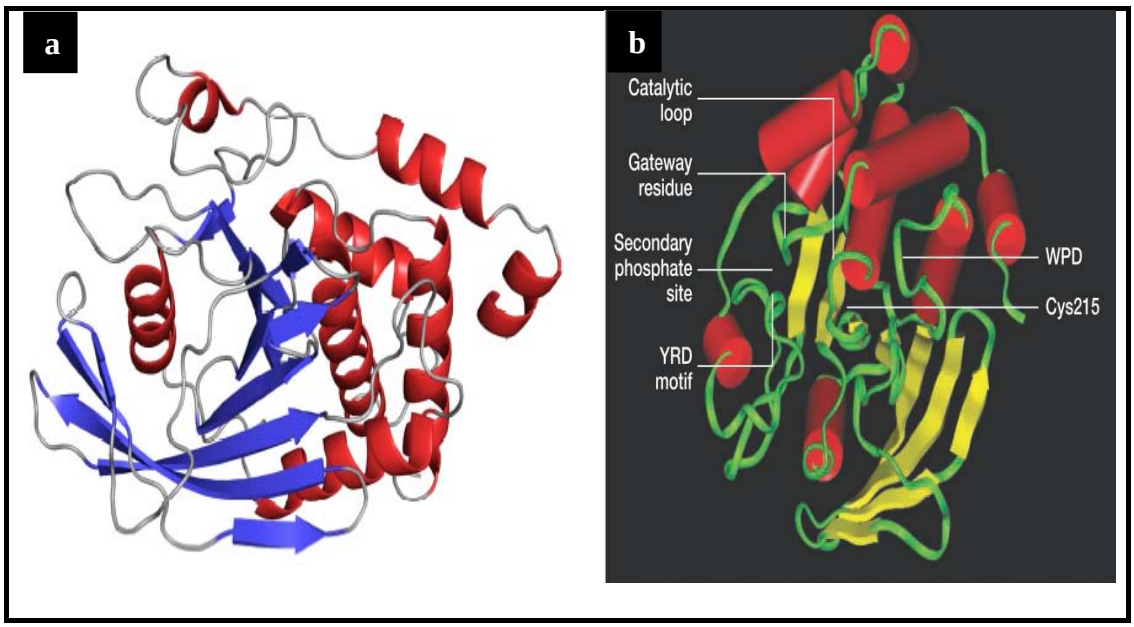


Figure 7. (a) Cartoon representation of the 321-residue version of PTP-1B; **(b)** A ribbion view of crystal structure of PTP-1B, highlighting the main region of the protein

Catalytic Loop (Active site 1/A): The active site of PTP-1B contains a common structure motif of PTPs. The base of the catalytic site is defined by the 214-221 PTP signature motif (His-Cys-Ser-Ala-Gly-Ile-Gly-Arg) in PTP, a loop of eight amino-acid residues that forms a rigid, cradle-like structure that coordinates to the aryl-phosphate moiety of the substrate. This loop also contains the active-site nucleophile Cys215. Four other loops bearing invariant residues form the sides of the catalytic cleft and contribute to catalysis and substrate recognition [aspartic acid (Asp181), Phenylalanine (Phe182), Tyrosine (Tyr46), valine (Val49), lysine (Lys120) and glutamine (Gln262) as shown in **Figure 8**. The depth of this cleft is 8-9 Å which provides the substrate selectivity, as serine and threonine are not enough to reach Cys215 of PTP-1B.

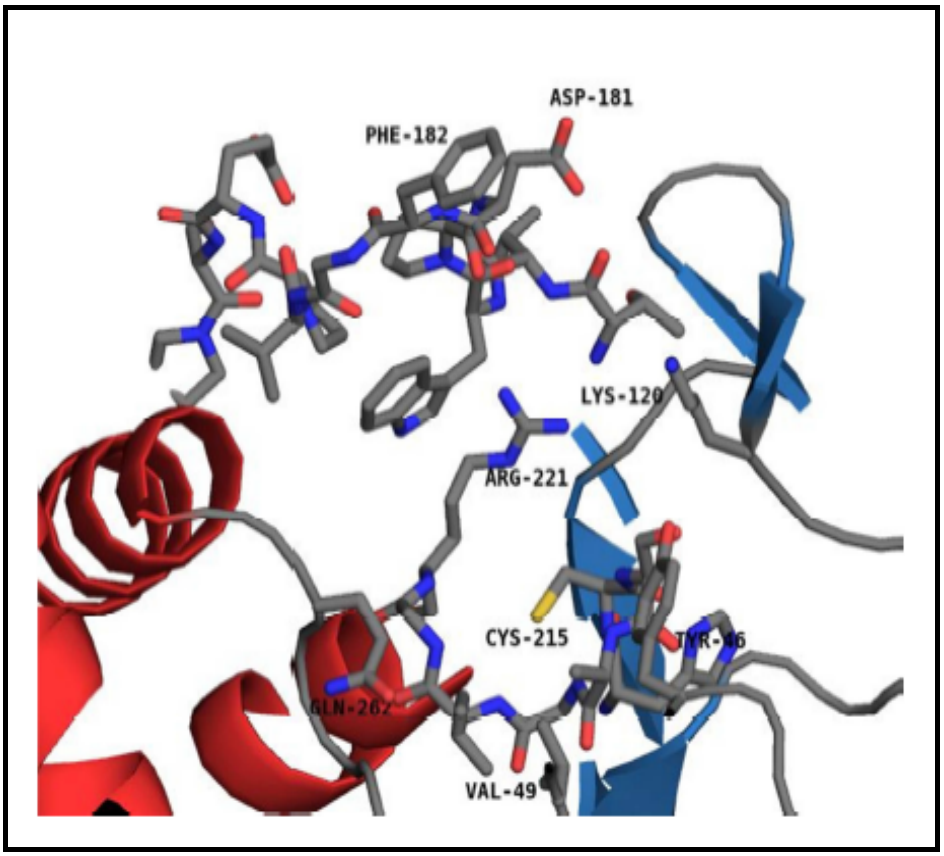


Figure 8. PTP-1B's active site. The important residues for catalysis are shown

WPD Loop: The WPD loop (79-187 amino acids) moves up to 12 Å to close down on the phenyl ring of the substrate, which maximizes hydrophobic interaction. Asp181 acts as a general acid to protonate the tyrosyl leaving group. As there are two conformations of PTP-1B, open and closed-inhibitors could be designed to target either conformations as shown in **Figure 9**. The crystallographic data indicates that the inhibition of PTP-1B by binding to open conformation leads to weaker inhibitors than the molecule that induce the closed conformation **[138]**.

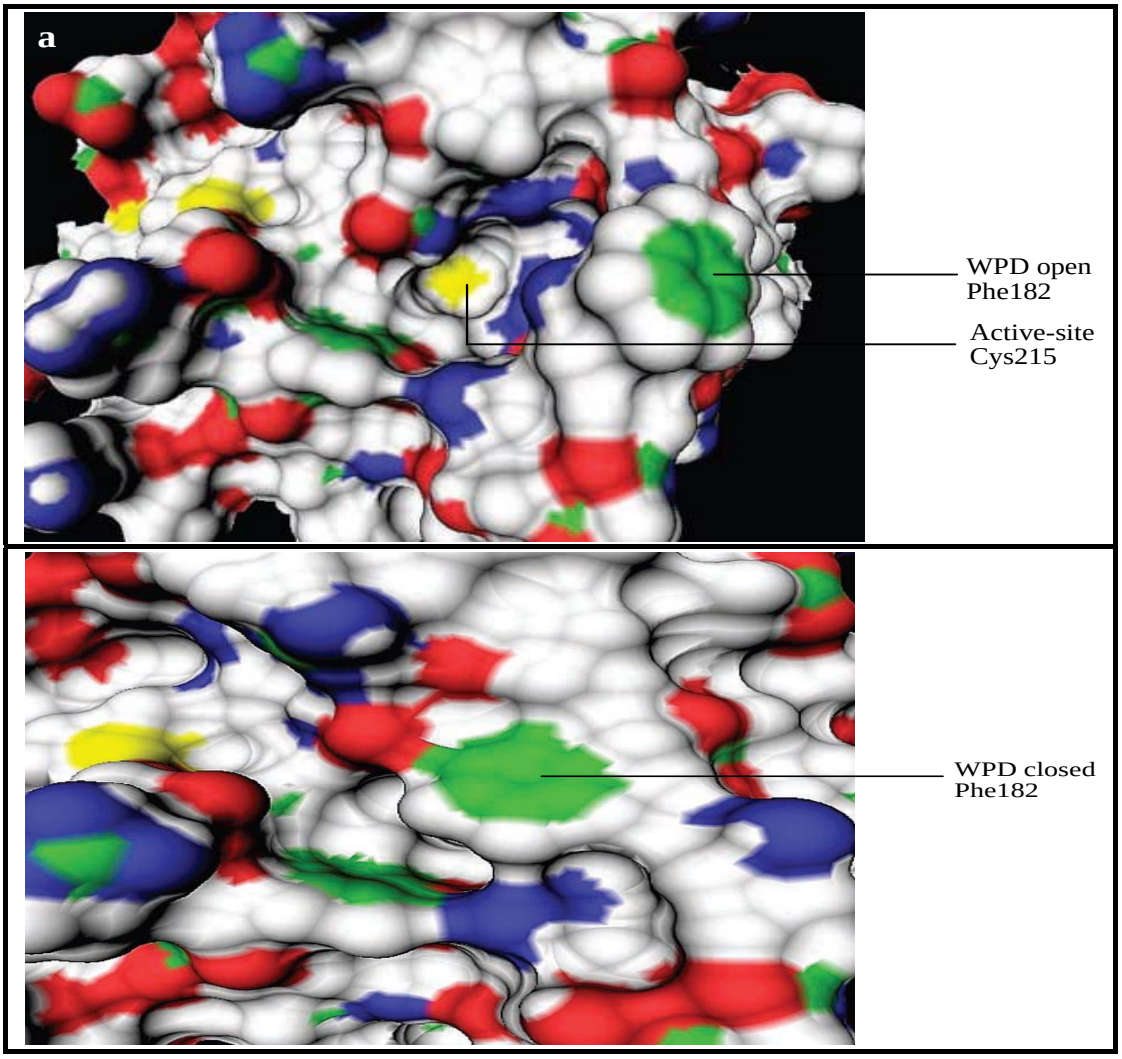


Figure 9. (a) Open conformation of active site; **(b)** The active site after closure of the WPD (tyrosine, proline, aspartic acid) loop

Secondary aryl-phosphate-binding site: Crystallographic studies of the complex between *bis*-(*p*-phosphophenyl) methane (BPPM) and the Cys215Ala mutant led to the discovery of a second aryl-phosphate-binding site adjacent to the catalytic site **[139]**. The second site is catalytically inactive, and provides weaker binding interactions compared with the primary site, owing to its more open exposure to solvent. With the help of these binding sites, it opens the possibility of using the strategy of independently

finding molecules that bind to each site, then linking them together to get a much more potent inhibitor.

YRD Loop: YRD motif (Arg47 and Asp48), which are close to the active site, has allowed selectivity to be achieved over most other PTPs as shown in **Figure 10**. Arg47 and Asp48 form a charged region at the top of the binding pocket of PTP-1B. Asp48 contributes in salt-bridge formation with an inhibitors containing basic nitrogen in that region, allowed for achieving selectivity over other PTPs **[140-141]**.

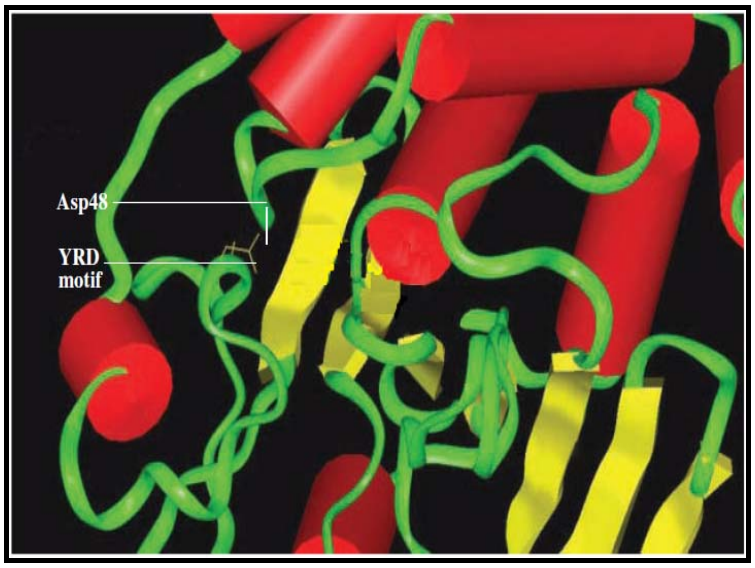


Figure 10. YRD motif provides an opportunity for selective PTP inhibition

Other residues in PTP-1B provide unique structural insight and opportunities for achieving inhibitor selectivity. Recently, a phospho-tyrosine-binding site adjacent to the PTP-1B active site was identifies which differ from TC-PTP that provided an alternative pocket to be use in inhibitor design. A gateway to this pocket is provided in PTP-1B through Gly259. Many other PTPs have bulkier residues at this position that restrict access to the

secondary aryl-phosphate site. Gly259 has been shown to control both substrate and inhibitors access to the secondary aryl-phosphate site in PTPs that are closely related to PTP-1B as shown in **Figure 11**. Thus, selectivity over most other PTPs can be achieved by capitalizing the differences in the YRD region through Asp48 and the gateway opened by Gly259 **[142-143]**.

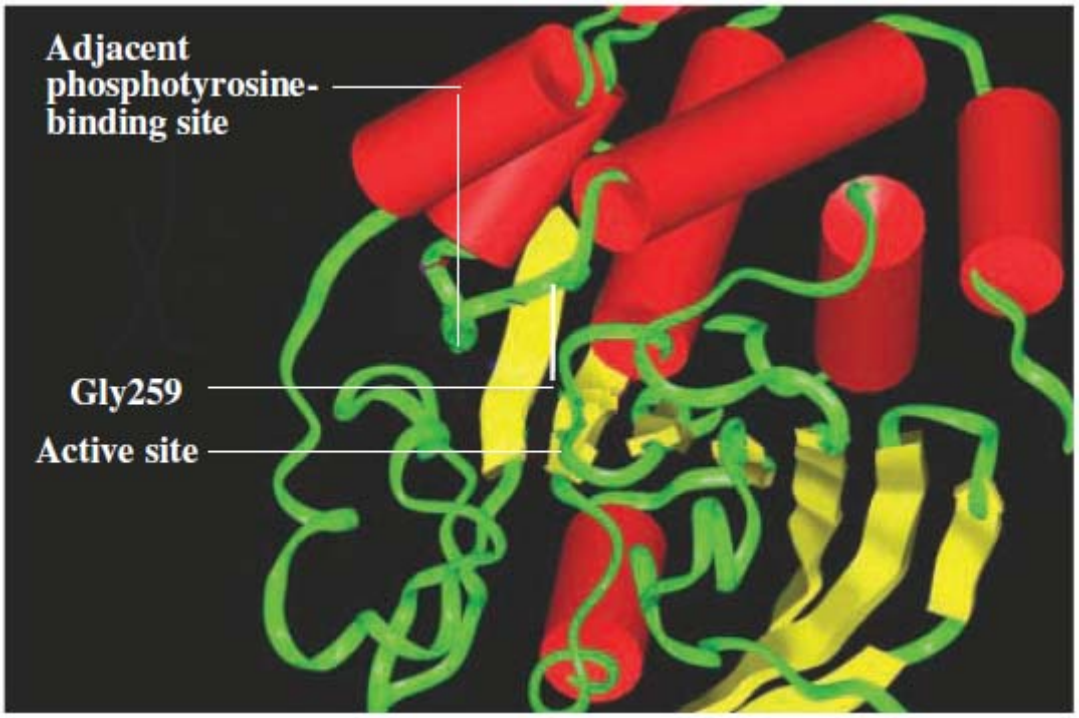


Figure 11. Gly259 allows access to the adjacent phosphotyrosine-binding site in PTP-1B

1.6. PTP-1B mechanism of action (MOA)

As describe earlier, PTP-1B acts as a negative regulator of the insulin signaling pathway acting by dephosphorylating phosphotyrosine residues in the insulin receptors (IRs), insulin receptor substrates (IRSs) and its MOA is shown in **Figure 12**. Recognition of the substrate peptide sequence by PTP-1B and binding of the phosphotyrosine deep in the catalytic site of the

phosphate-binding loop (p-loop) are mediated by residues 214-221 (His-Cys-Ser-Ala-Gly-Ile-Gly-Arg) **[144]**. It contains an active site nucleophile, Cys215. The dephosphorylation of tyrosine takes place via two steps **[145]**. In the first step there is a nucleophilic attack on the substrate phosphate by catalytic intermediate and release of phosphate sulphur atom of Cys, coupled with protonation of tyrosyl leaving group by Asp181 acting as a general acid. This leads to the formation of cysteinyl-phosphate intermediate. The second step is mediated by Glu262 and Asp181, leads to the hydrolysis of catalytic intermediate and release of phosphate.

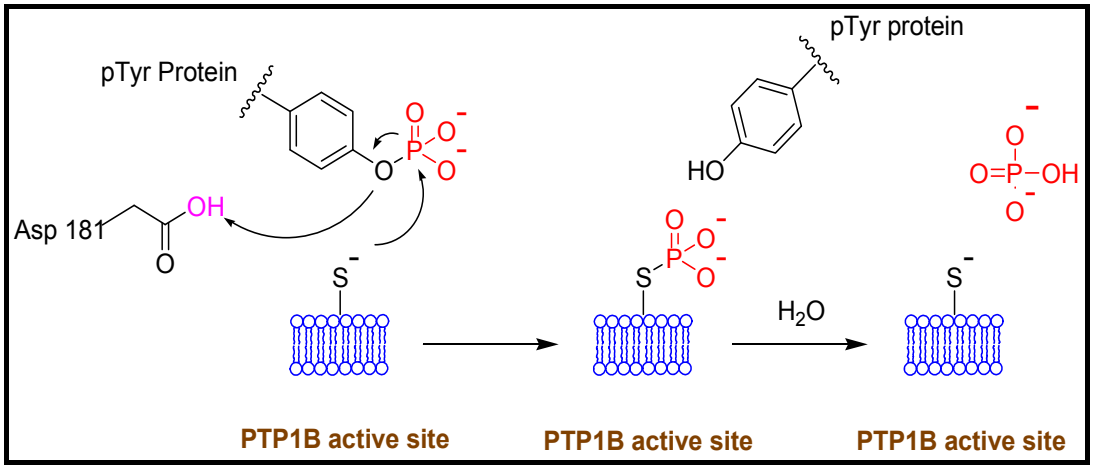


Figure 12. PTP-1B mechanism of action

1.7. Challenges in developing potent and selective PTP-1B inhibitors

Knowing the importance of PTP-1B inhibitors in insulin signaling, the major medicinal chemistry challenges of PTP-1B inhibitor development are two fold: cell permeability and selectivity. Selectivity is one of the major issues in the development of PTP-1B inhibitors as drugs. Because all PTPs share a high degree of structural conservation in the active site signature motif [the (H/V)CX_5R(S/T) **[119]** amino acid sequence, which adopts a unique loop

structure and employ a common catalytic mechanism. They have similar core structure made of a central parallel beta sheet with flanking alpha helices containing a β-loop and α-loop surrounding signature motif. The function and specificity of these proteins are attributed to the regulatory domains and subunits. Because of the close similarities among the PTPs, it is known to be a very difficult to selectively target specific PTPase.

Among the various PTPase, the most closely associated PTPs are leukocyte common antigen-related IR (LAR), protein tyrosine phosphatase α (PTPα), SH2-domain containing phosphatase-2 (SHP-2), TC-PTP, low-molecular-weight PTP (LMW-PTP) and PTP-MEG2. LAR is associated with IR and acts as a down-regulator of insulin signaling **[146-149]**. LAR KO mice exhibits a phenotype consistent with insulin resistance **[150]**. PTPα acts as a negative regulator of IR in BHK-IR cells **[151-152]**. PTPα antisense studies showed that it is not required for insulin signaling **[153]** and KO mice study showed no defect in glucose homeostasis **[154]**.

SHP-2 binds to IR and IRS-1 and acts as a negative regulator of insulin signaling **[155-157]**. Absence of SHP-2 in mice was embryonic lethal and had no defect in glucose homeostasis **[158]**. TC-PTP is found to interact with IR upon insulin signaling and acts as down-regulator of AKT activation upon insulin stimulation **[159-160]**. A recent study showed that TC-PTP KO mice die at 3-5 weeks of age because of impaired β-cell and T cell functions **[124]**.

LMW-PTP acts as a negative regulator of insulin action **[161]**. While, PTP-MEG2 is a negative regulator of hepatic insulin signaling **[162]**. KO mice

study of both, LMW-PTP and PTP-MEG2 showed no defects in glucose homeostasis.

Among the PTPase, PTP-1B and TC-PTP play an important role in insulin signaling pathway. TC-PTP is the most homologus phosphatase to PTP-1B with 80% sequence identity in the catalytic domain and identical active sites as shown in **Figure 13 [163]**. This makes it very challenging to control selectivity. Lack of oral bioavailability is another important issue in the development of PTP-1B-based therapeutics as they exhibit limited cell permeability due to the presence of negatively charged polar groups **[164-179]**.

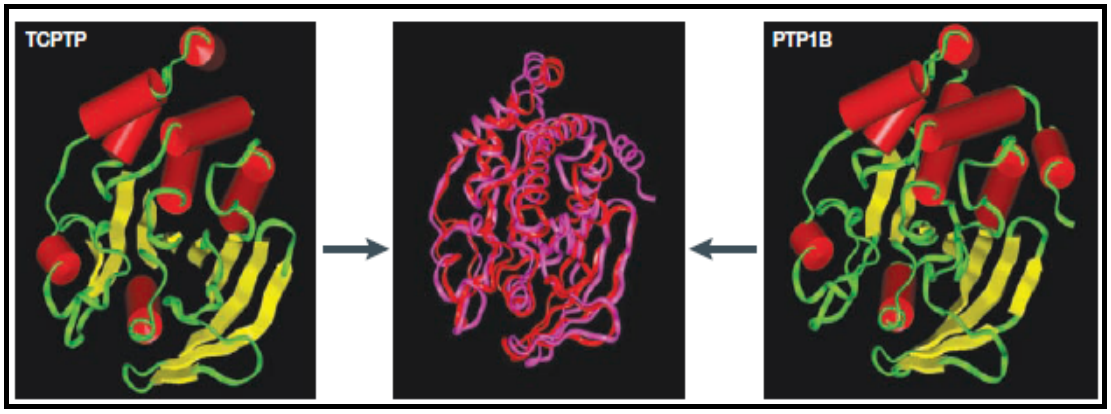


Figure 13. The X-ray crystal structure of PTP-1B and TC-PTP. The overlay of PTP-1B (purple) on TC-PTP (red) shows the high level of similarity, which poses a problem for developing selective inhibitors

The PTP-1B inhibitors which exhibit selectivity over closely associated TC-PTP have been recently developed by (1) targeting second aryl phosphate binding site adjacent to the active site and (2) identifying dual-binding site phosphotyrosine mimetics (*p*Tyr mimetics) to address the cell permeability issue.

1.7.1. Identification of noncatalytic aryl phosphate-binding site

As describe earlier, one of the major challenge in designing PTP-1B inhibitor is achieving selectivity over highly homologous TC-PTP. To address this problem, Zhang and colleagues identified an additional noncatalytic aryl phosphate binding site-B adjacent to the catalytic site. Site-B of PTP-1B differs from that of TC-PTP by a few amino acids (F52Y and A27S) as shown in **Figure 14** and thus offers an opportunity to improve selectivity **[139]**. The most important residues in site B appear to be Arg-24 and Arg-254, which co-ordinate the phosphate. Additional favourable interactions include water-mediated hydrogen bonding, weakly polar interaction with Met-258 and Gln-262, and van der Waals contacts with Ile-219, Asp-48 and Val-49. Thus, the discovery of additional second aryl phosphate binding site-B provides a new concept for the designing of tight-binding, highly specific PTP-1B inhibitors.

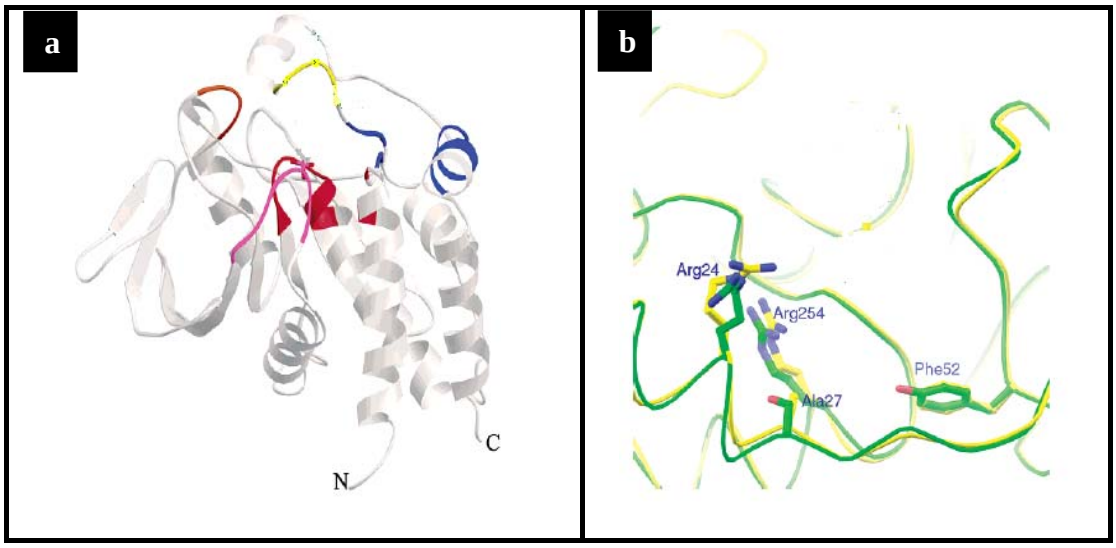


Figure 14. (**a**) PTP1B primary and secondary aryl-binding sites. Red: primary binding (or catalytic) site; blue: secondary aryl phosphate binding site; yellow: YRD loop; magenta: WPD loop; (**b**) The secondary aryl binding site in PTP1B and TC-PTP. Ribbon diagram of the secondary aryl binding site in PTP1B (yellow) and TC-

PTP (green), identified by Arg24 and Arg254. The only differences between PTP1B and TC-PTP among the first shell residues are the F52Y and A27S

1.7.2. Identification of dual-binding site phosphotyrosine mimetics (*p*Tyr mimetics)

As described earlier, other than selectivity issue, the major challenges in developing PTP-1B inhibitors are cell permeability and oral bioavailability. These findings led to the concept of development of *p*Tyr mimetics such as carboxylic acid **[180-181]**, oxalylamino benzoic acid (OBA) **[182]**, and nonhydrolyzable phosphonodifluoromethyl phenyl group (DFMP group) **[183]**, as shown in **Figure 15**. The limited cell permeability with DFMP, OBA and carboxylic acid containing inhibitors inspired efforts towards the rational design of phosphonate mimetics with improved cellular activity.

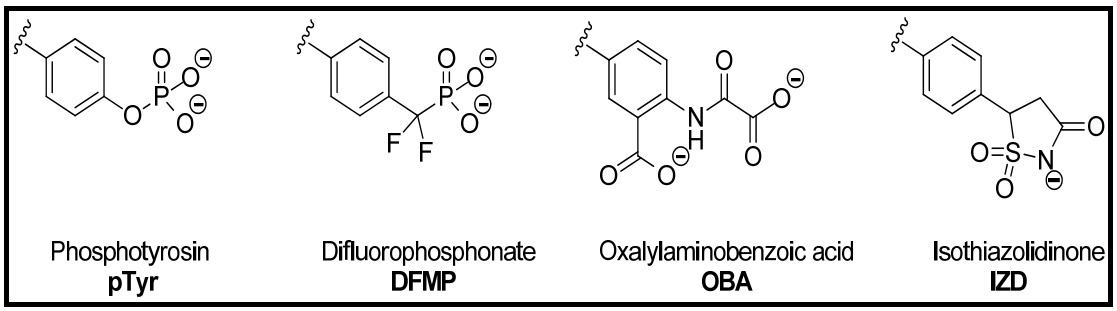


Figure 15. Potent *p*Tyr mimetics

In the area of cell permeable *p*Tyr mimetic, novel five-membered ring phosphonate mimetics, isothiazolidinone (IZD) **[184]** and thiadiazolidinone (TZD) **[185]** were discovered independently by several pharma companies such as Novartis, AstraZeneca, Vertex and Incyte (**Figure 15)** having significant cellular activity, therby providing a major advance in designing cell

permeable PTP-1B inhibitors. Thus, the discovery of cell permeable *p*Tyr mimetics provides a new concept of designing orally active and cell permeable PTP-1B inhibitors.

1.8. Overview on PTP-1B inhibitors under development

Most of the PTP-1B inhibitors reported initially were nonspecific and they also exhibited poor cell permeability (**Figure 16**). Only Ertiprotafib (**Compound A**) developed by Wyeth for T2DM, entered clinical trials but stopped at Phase II, owing to its unsatisfactory efficacy and side effects **[92]**.

Albert Einstein college developed a potent peptide (**Compound B**) containing bis DFMP group located at both N & C terminal of peptide. This compound dramatically improved enzyme potency (PTP-1B, K_i = 2.4 nm) and specificity over the highly homologous TC-PTP (10 fold). Unfortunately, the higher polar nature of peptide leads to poor cell permeability and low oral bioavailability **[186]**.

An effort for developing non-peptide scaffolds by incorporating the DFMP group has been carried out by several pharma companies. In this field Merck Frosst initiated its efforts which led to promising PTP-1B inhibitors such as, benzotriazole (**Compound C**) **[187]**, arylketone (**Compound D**) **[188]** and naphthyl (**Compound E**) **[189]** derivatives. These compounds are very potent *in vitro*, but poor selective against TC-PTP limited their further clinical development.

Recently, Affymax has identified a sulfonamide scaffold bearing the DFMP group as *p*Tyr mimetics (**Compound F**) **[190]** and novel ketophosphonate *p*Tyr mimetic (**Compound G**) **[190]**. These compounds were found to be nonselective against TC-PTP.

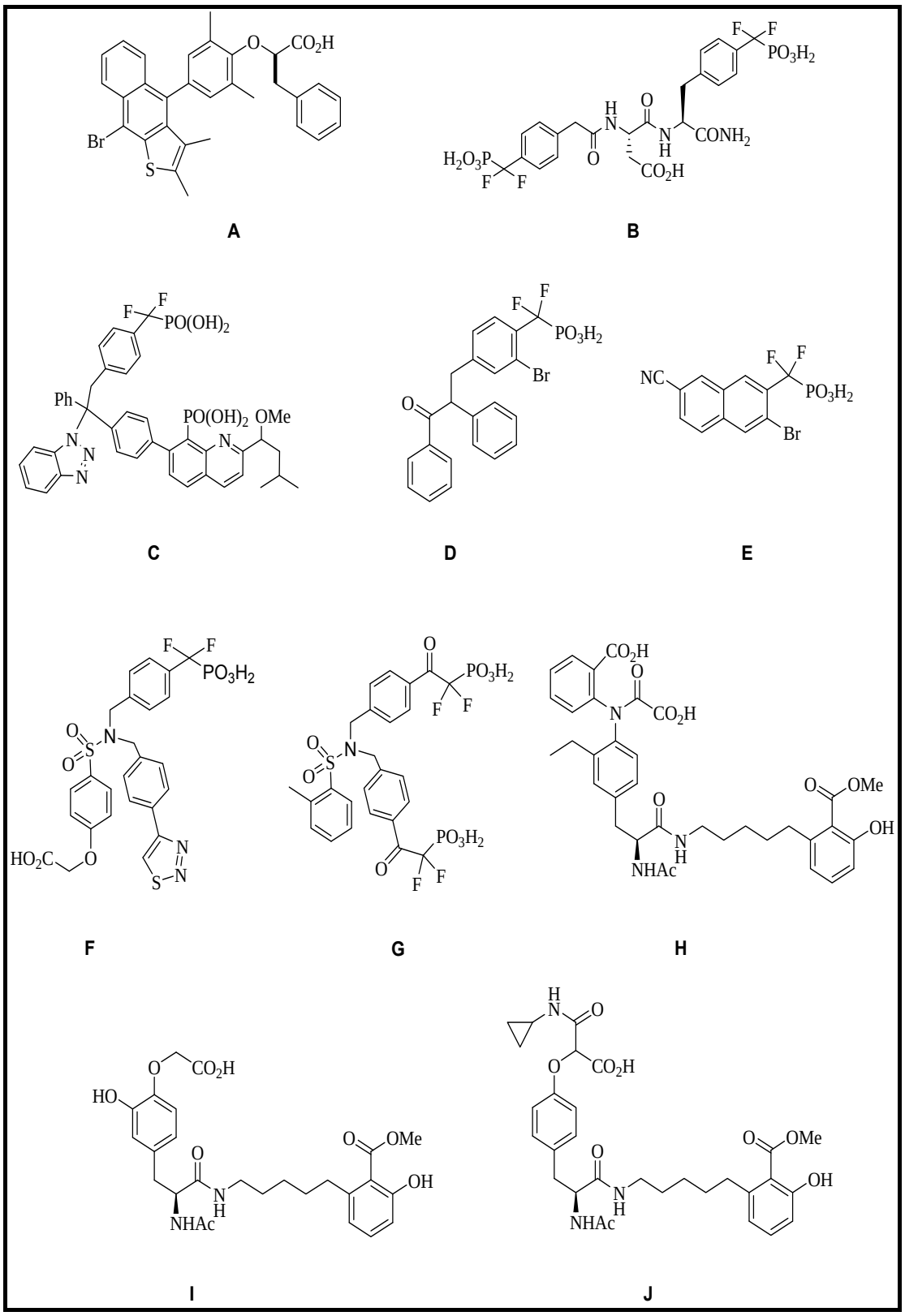


Figure 16. Potent PTP-1B inhibitors under development

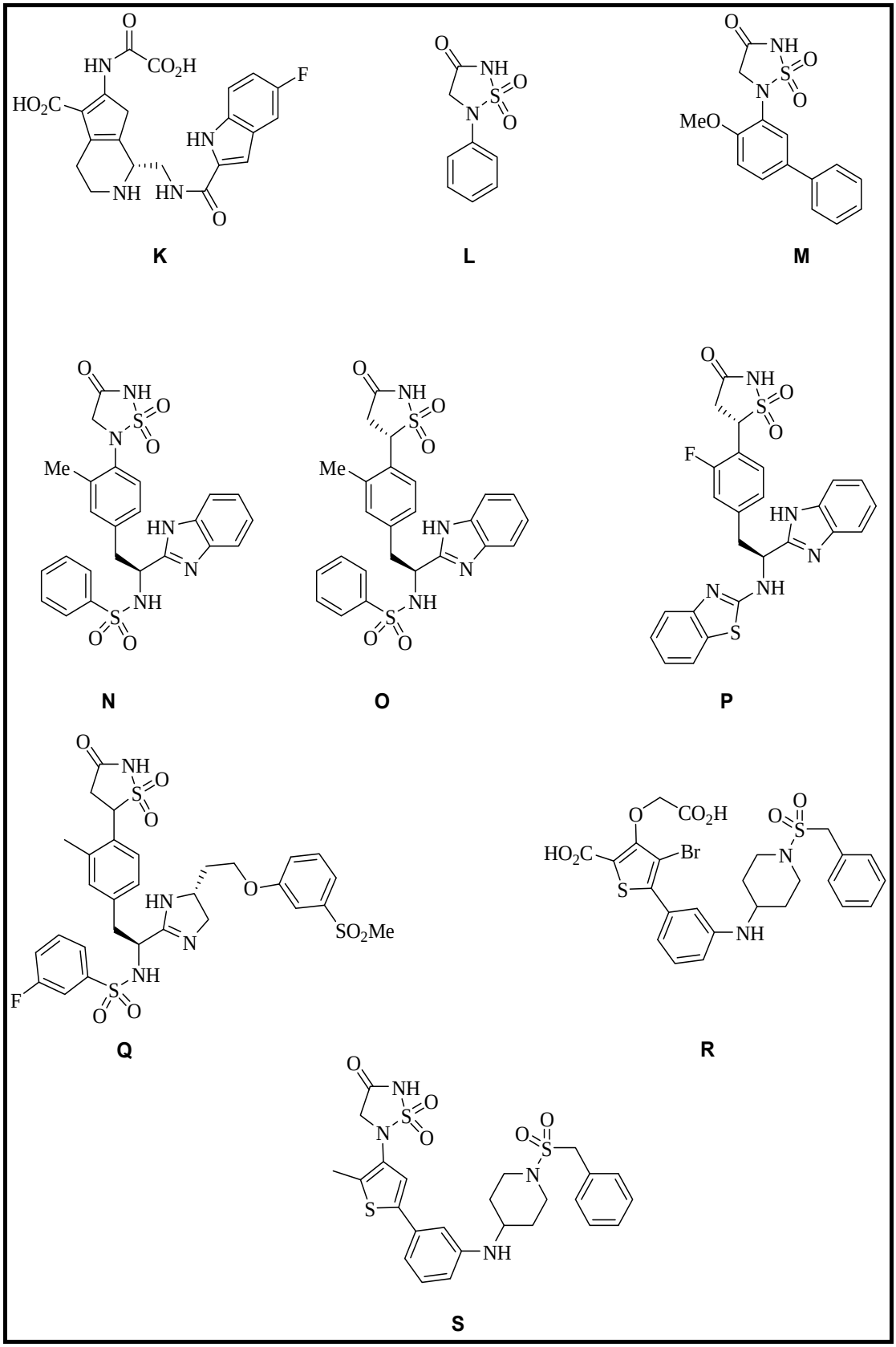


Figure 16. Potent PTP-1B inhibitors under development (continued)

Abbott group initiated their efforts in area of developing potent PTP-1B inhibitors. They identified a salicylic acid fragment bound in the site-B of PTP-1B and that moiety could be linked to several scaffolds bearing carboxylic acid *p*Tyr mimetics providing inhibitors with improved enzyme potency and selectivity.

Abbott reported 2-oxalyarylaminobenzoic acid derivative (**Compound H**) **[191]** with enhanced potency and poor TC-PTP selectivity (3.6-fold). **Compound I** and **J [192]** were developed by the modification of **compound H**, which showed inhibition of PTP-1B at micromolar concentration with greater than 20-fold selectivity over TC-PTP.

Novo Nordisk reported 2-oxalylaminobenzoic acid (OBA) containing *p*Tyr mimetics on thiophene-based scaffold (**Compound K**) **[182]** as a potent PTP-1B inhibitor. The major drawback of this acid was lack of cellular activity, mainly due to its poor permeability.

Lack of adequate membrane permeability with bis-anionic DFMP and poor inhibition provided impetus to search for novel *p*Tyr mimetics. To address the above problem, many pharma companies such as AstraZeneca (**Compound L-M**) **[185]** and Incyte (**Compound N-Q**) **[193-195]** discovered novel five-membered heterocyclic *p*Tyr mimetics (IZD & TZD) containing PTP-1B inhibitors which showed an improved cell permeability.

Wyeth has published a comparision of thiophene-based inhibitors bearing dicarboxylic acid (**Compound R**) **[196]** and a TZD (**Compound S**) **[197]**. These compounds showed potent in vitro PTP-1B inhibitory activity.

Currently various pharma companies have initiated PTP-1B inhibitors program, such as Merck Frosst Canada Ltd., Janssen Pharmaceutica,

Sunesis Pharmaceuticals, Inc., Abbott Laboratories and Ri-bozyme Pharmaceuticals Inc., have filed several patent applications (WO2008089581, WO2005044277, WO2004065411, WO2003073987, WO2003072537, WO2003070881, WO2003006643, WO2003002569) with very broad claims that indicate their active involvement in this field. However, search for potent, selective and orally bioavailable PTP-1B inhibitor is a major quest for a safe & effective treatment of T2DM and obesity. In this regard, knowing the potential of PTP-1B inhibitor target, we have attempted to design novel series of PTP-1B inhibitors and this design strategy is described in the next chapter.

1.9. Conclusion

Diabetes mellitus is the most prevalent and serious metabolic disorder. Among T1DM & T2DM, T2DM is one of the major public health challenges of 21st century. Currently available therapies have several drawbacks. Therefore several new therapies are being developed among which inhibition of PTP-1B is the most promising approach for the safe and effective treatment of Type II diabetes. However achieving selectivity and oral bioavailibity are major challenges with the design & development of PTP-1B inhibitors. To address these concern, in the next section, we have outlined designing of novel series of PTP-1B inhibitors in an attempt to develop next generation therapies for treatment of metabolic disorder such T2DM and obesity.

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Chapter II: Designing Strategy Of PTP-1B inhibitors

2. Design strategy of PTP-1B inhibitors

2.1. Orally active, potent and selective PTP-1B inhibitors

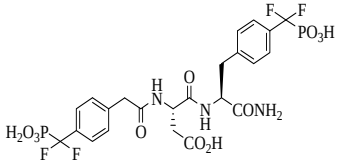
As discussed in the previous chapter, deregulation of PTP activity contributes to the pathogenesis of several human diseases, including, diabetes, obesity, cancer and immune disorders **[1-6]**. Knowing that inhibition of PTP-1B activity has the potential for enhancing insulin action by prolonging the phosphorylated state of the insulin receptor. The importance of the PTPs in diverse pathophysiology has made them the focus of intense interest as a new class of drug targets. Thus, inhibitions of the PTP are expected to have therapeutic value with novel mode of action. Among various members of the PTP superfamily, PTP-1B has emerged as the best-validated drug target **[7]**. However, it has become apparent that the conserved structural and mechanistic features of the PTP active site present substantial challenges to drug development. Many compounds that are highly potent PTPs inhibitors *in vitro* have been identified; however, achieving selectivity and cell permeability is an ongoing challenge.

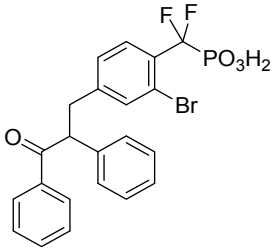
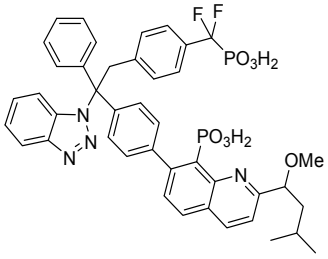
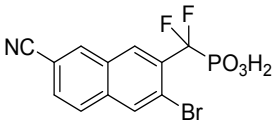
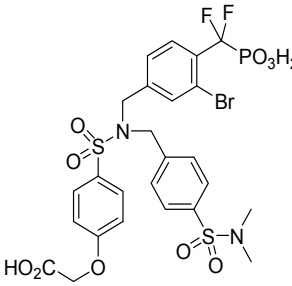
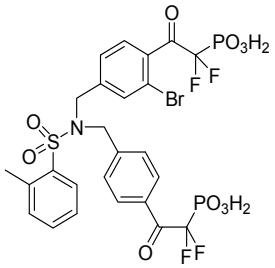
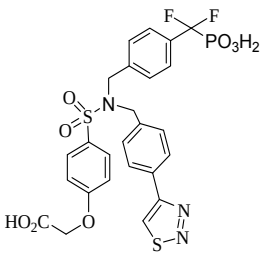
For PTP-1B, one strategy to achieve selectivity has been to explore the secondary site pocket. It now appears that the approach of targeting the secondary site pocket, may give PTP-1B inhibitors selectivity over many phosphatase, but is often not sufficient to give the desired selectivity over TC-PTP **[8]** and, therefore, other inhibitor design strategies are required in addition. One such approach may be the design of inhibitors that explore three sites: the active site, the secondary site and the β*5*- β*6* loop to achieve selectivity and has already been used successfully in the designing of PTP-1B inhibitors. Combined targeting of these sites may lead to a several-fold

increase in selectivity over TC-PTP. Nevertheless, great progress has been made to address the inherent potency, selectivity and bioavailability problems associated with targeting PTPs for therapeutic development. However none of these inhibitors have been marketed. Thus the development of PTP-1B inhibitors with potent biological activity and selectivity has been an attractive target among the several research groups working in the area of metabolic disorders all over the globe. Knowing therapeutic potential of these inhibitors, we attempt to design novel series of molecules. In this section, we have summarized designing of three novel series. First series: Benzotriazole-based derivatives, Second series: Tri-aryl sulfonamide based derivatives and Third series: Peptidomimetic based derivatives as novel PTP-1B inhibitors.

Several attempts were made in past to develop potent selective PTP-1B inhibitors as shown in **Table 5**. Among the various DFMP based inhibitors, peptide containing DFMP, showed excellent *in vitro* potency against PTP-1B (IC_{50} = 2.4 nM), but its further development was stopped due to poor bioavailability **[9]**.

Table 5. Classifications DFMP based PTP-1B inhibitors

Class	Structure/Name	Company/ Institution	Clinical Status	Ref.
Peptidomimetic DFMP Inhibitors		Albert Einstein College	Hit*	**[9]**

Arylketone DFMP inhibitors		Merck Frosst	Hit*	**[10]**
Benzotriazole DFMP inhibitors		Merck Frosst	Hit*	**[11]**
Naphthyl DFMP inhibitors		Merck Frosst	Hit*	**[12]**
Sulfonamide DFMP inhibitors		Affymax	Hit*	**[13]**
		Affymax	Hit*	**[13]**
		Affymax	Hit*	**[13]**

* Hit indicates early discovery stage molecule with or without preclinical activity

Scientist at Merck Frosst designed and developed non-peptide scaffolds containing DFMP group as potent PTP-1B inhibitors **[10-11]**. Oral administration of these compounds demonstrated good antidiabetic activity and good oral bioavailability in different animal species. But its further development was stopped due to poor selectivity over TC-PTP.

Recently, Affymax has identified a sulfonamide scaffold bearing DFMP group as a potent PTP-1B inhibitor. But like other PTP-1B inhibitors, sulfonamide inhibitors are nonselective over TC-PTP **[12]**.

2.1.1. Rationale for designing Benzotriazole-based PTP-1B inhibitors (First series)

Recently, Lau et al. reported a series of potent non-peptidic benzotriazole-based PTP-1B inhibitors **[14]**, but it showed poor selectivity over TC-PTP. With identification of an additional noncatalytic aryl phosphate binding site (site 2/B) proximal to the catalytic phosphate binding site **[15]** and taking the advantage of difference in Site B amino acids of PTP-1B and TC-PTP provided an opportunity to improve selectivity over TC-PTP **[15]**. Consequently, dual-site inhibitors were designed to bind across both site A and B, to achieve additive effects and thereby improve potency and selectivity towards PTP-1B over closely associated PTPs **[16]**.

The X-ray crystal structure of PTP 1B in complex with compound **I** reveals that sites A and B each have a DFMP moiety (difluoromethylphosphonic acid) anchored into it **[14]**. The benzotriazole ring system also functions as an anchor and is located into the active site and providing good selectivity for PTP-1B over other PTPs. The fourth substituent (benzene ring) occupies a hydrophobic pocket. Altogether, this signifies that

the presence of all four substituents oriented rigidly by the molecule's stereo centre is essential for high potency and selectivity **[11]**. Recently, Incyte Ltd. reported IZD-based (isothiazolidinone) potent *p*Tyr mimetic **[17]**. The IZD heterocycle were designed based on the hypothesis that two sulfonyl oxygen atoms mimic the oxygen atoms of the DFMP group, whereas the carbonyl and the ionized NH groups mimic the DFMP anion **[18-20]**.

As a part of our on going research in the development of potent and selective PTP-1B inhibitors as a potentially viable approach for the safe and effective treatment of T2DM, we wished to design novel compounds with possessing the features of dual binding site so that these compounds can be developed as potent and selective PTP-1B inhibitors. We reported the design of novel benzotriazole-based dual-site PTP-1B inhibitors. These newly designed compounds consist of four key structural components: a) benzotriazole ring, b) acetophenone, c) benzyl, naphthyl, or quinoline ring system suitably substituted with DFMS, DFMP, or IZD groups. The benzotriazole ring system was introduced as a basic pharmacophore to obtain superior PTP-1B selectivity. The two DFMP groups were specifically incorporated as *p*Tyr mimetics to access both binding sites A and B, thereby improving potency and selectivity for PTP-1B over TC-PTP. Furthermore, bioisosteric replacement of DFMP was carried out with DFMS/IZD, to improve membrane permeability and binding affinity for site B. The acetophenone, benzyl, naphthyl, and quinolinyl ring systems were integrated to improve overall lipophilicity and thus oral efficacy.

The SAR studies of benzotriazole based dual binding site PTP-1B inhibitors shown in **Figure 17** demonstrated that selectivity for PTP-1B over

TC-PTP was greatly enhanced due to incorporation of newly *p*Tyr mimics on benzotriazole scaffold which specifically get accommodated into the site A & B of PTP-1B, mainly due to the acquisition of its favorable conformations. Thus, the difference in the site B residues of PTP-1B over TC-PTP could be exploited to design potent and selective PTP-1B inhibitors.

Keeping this motive in mind, compounds **II**, **III** and **IV** (**Figure 17**) were designed. Altogether, two sets of compounds on benzotriazole nucleus containing newly designed *p*Tyr mimetics mimics (**Figure 18**) have been prepared.

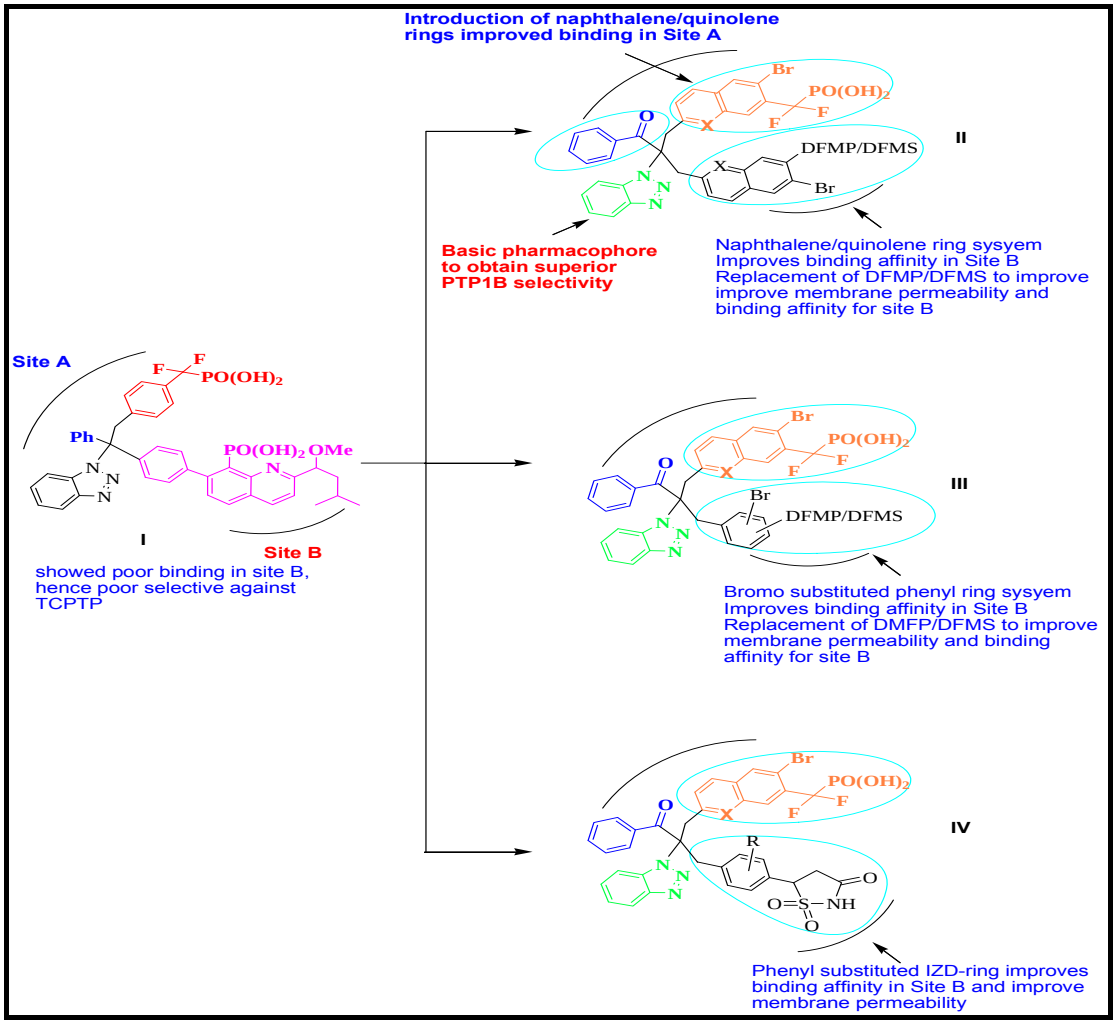


Figure 17. Design Strategy of Benzotriazole-Based PTP-1B Inhibitors

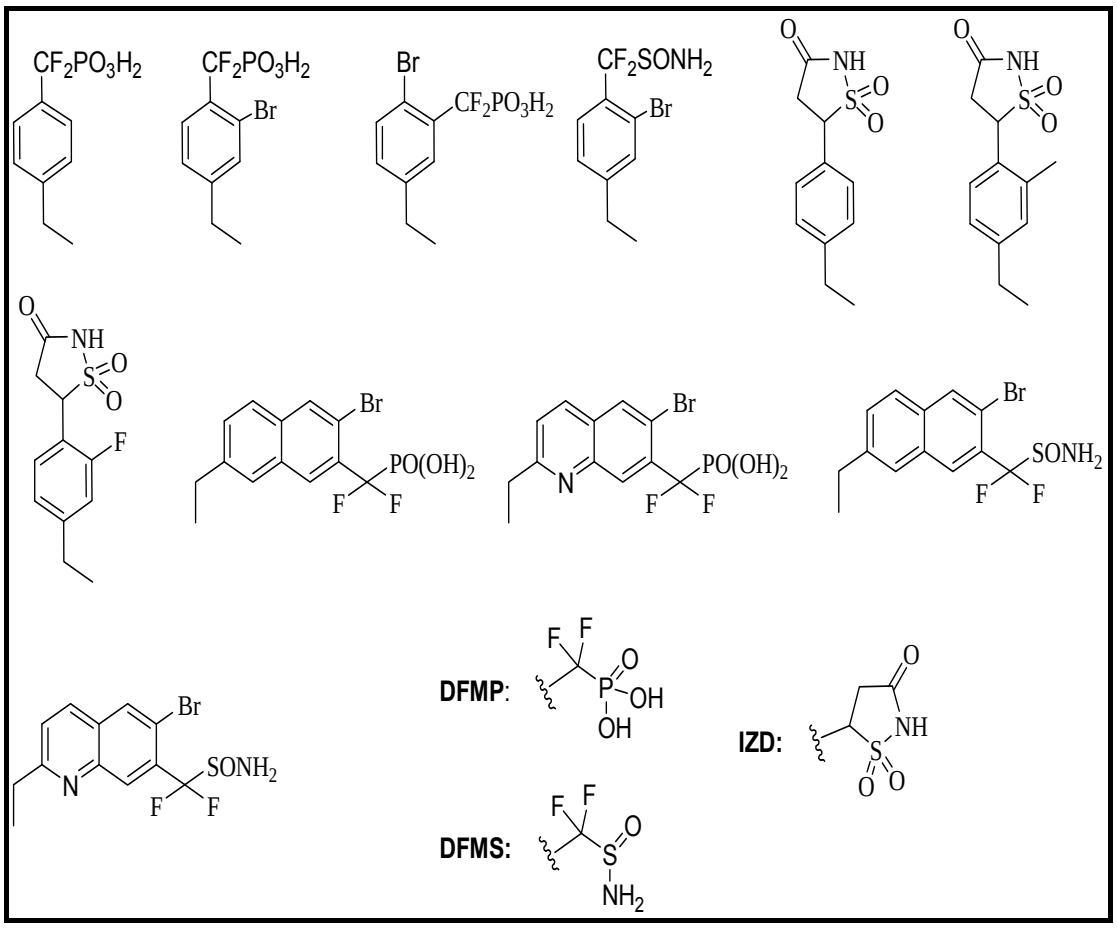


Figure 18. Structures of Potent *p*Tyr mimetics

Thus, in this series, all the the favorable structure components of patent compound **I** essential for the key interaction with PTP-1B were retained, except suitable changes were made to improve potency, selectivity. In this, series, total fifteen compounds **7a-f**, **10a-c** and **13a-f** have been prepared and their synthetic schemes are explained in detail in **Chemistry section 3.1.1**.

2.1.2. Rationale for designing Triaryl-Sulfonamide based PTP-1B inhibitors (Second series)

Knowing the fact that the development of selective and orally bioavailable PTP-1B inhibitors (selectivity against TC-PTP) could be viable

approach for the safe and effective treatment of T2DM, recently, Homles et. al. reported a novel series of triarly-sulfonamide based potent PTP-1B inhibitor (compound **V**) and some of the test compounds showed nM range PTP-1B inhibitory activity (**Figure 19**). In addition, the sulfonamide framework may display a different three-dimensional orientation which provides significant interaction with the enzyme active site **[21]**. Also sulfonamides have been used to mimic a water molecule of hydration in the active site **[22]** or as potential *p*Tyr mimetics **[20]**.

With successive achievement of additive effects of bidented *p*Tyr mimetics [difluoromethylphosphonates (DFMP)/ difluoromethylsulfonamide (DFMS)/ isothiazolidinone (IZD)] on benzotirazole-scaffold, encourage the present further research on dual binding site concept. It has been attempted to design triaryl sulfonamide based PTP-1B inhibitors to understand the effect of incorporation such dual binding *p*Tyr mimetics. These newly designed compounds consists of three key structural components: a) phenyl ring containing oxo-acetic acid, b) thiadiazolybenzyl ring, c) benzyl, naphthyl, or quinoline ring system suitably substituted with DFMP or its bioisostere DFMS, or IZD groups. All three key components were attached together with sulfonamide frame, introduced as a basic pharmacophore to obtain superior PTP-1B selectivity (**Figure 19**).

Typical tri-aryl sulfonamide scaffolds were designed compounds **VI**, V**II** and **VIII** as dual binding site PTP-1B inhibitors (**Figure 19**), comprises of a newly designed *p*Tyr mimetics (**Figure 20**).

In this series, we prepared total nineteen compounds **50a-g**, **52a-d**, and **54a-h**. Their synthetic methodology and characterization are explained in detail in **Chemistry section 3.2.1**.

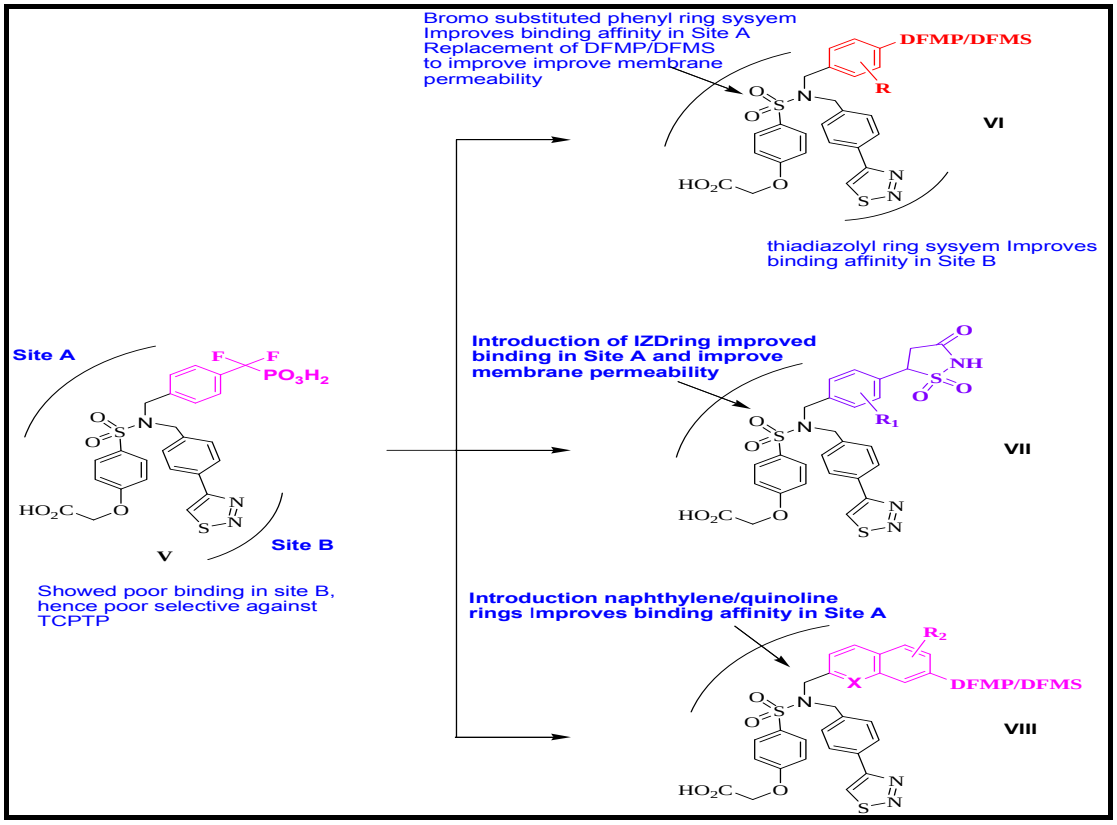


Figure 19. Design Strategy of triaryl sulfonamide-based PTP-1B Inhibitors

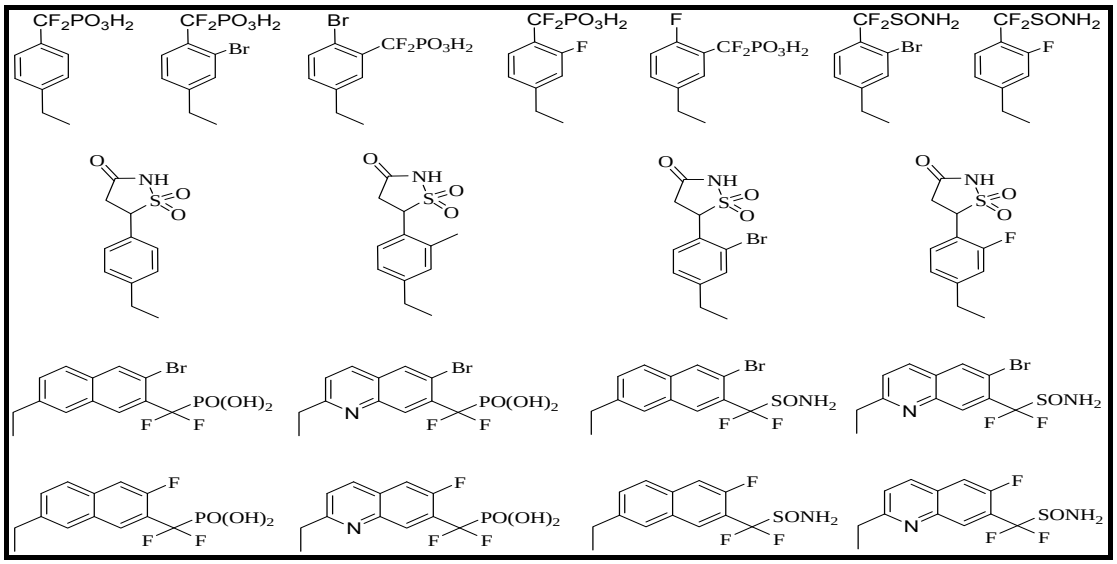


Figure 20. Structures of Potent *p*Tyr mimetic

2.1.3. Rationale for designing Peptidomimetic PTP-1B inhibitors (Third series)

In continuation with dual binding site concept, Zhang et al., reported tri-peptide containing two DFMP group as *p*Tyr mimic, as a potent PTP-1B inhibitors (**Table 5**) **[9]**. These *p*Tyr mimics were located at C and N terminals of peptide, which was linked with a suitable linker which allows the molecule to interact with both binding sites. The X-ray crystal structure of PTP-1B in complex with compound **IX** (**Figure 23**) illustrated that one of DFMP group binds at site-A. From co-crystallography data, it was observed that second DFMP group does not to bind to site-B but interacts with Arg47, so-called site-C of PTP-1B, and hence a moderate selectivity (10-fold) over TC-PTP (IC_{50}: 26 nM) was observed **[9]**. Due to potent *in vitro* PTP-1B inhibitory activity and as a part of our ongoing research on dual binding-site PTP-1B inhibitors, a novel library of tripeptide as a potent, selective and dual binding PTP-1B inhibitors containing two *p*Tyr mimetics (DFMP/DFMS/IZD), one targeted to the site-A, and the other targeted to a unique adjacent noncatalytic site-B have been designed.

These newly designed compounds consists of three key structural components: a) basic *p*Tyr mimic unnatural amino acid substituted with DFMP, b) linker, c) N-terminal diversity elements suitably substituted with DFMP or its bioisostere DFMS, or IZD groups. Recently, we reported DFMP-substituted naphthyl/ quinolinyl templates as a potent *p*Tyr mimic which allow the molecule to interact with both binding site **[23-24]**, and hence we selected as difluorophosphonate-substituted naphthyl/ quinolinyl templates were selected as basic *p*Tyr mimic unnatural amino acids (**Figure 21**).

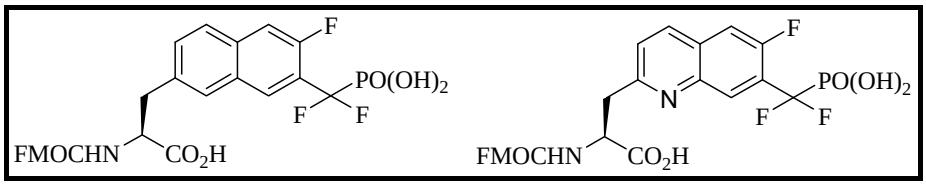


Figure 21. Structures of Potent *p*Tyr mimetic unnatural amino acid

A small array of N-terminal diversity elements were chosen as a second *p*Tyr mimics and linked to *p*Tyr mimic unnatural amino acid using aspartic acid to access binding interactions at both the site (**Figure 22**).

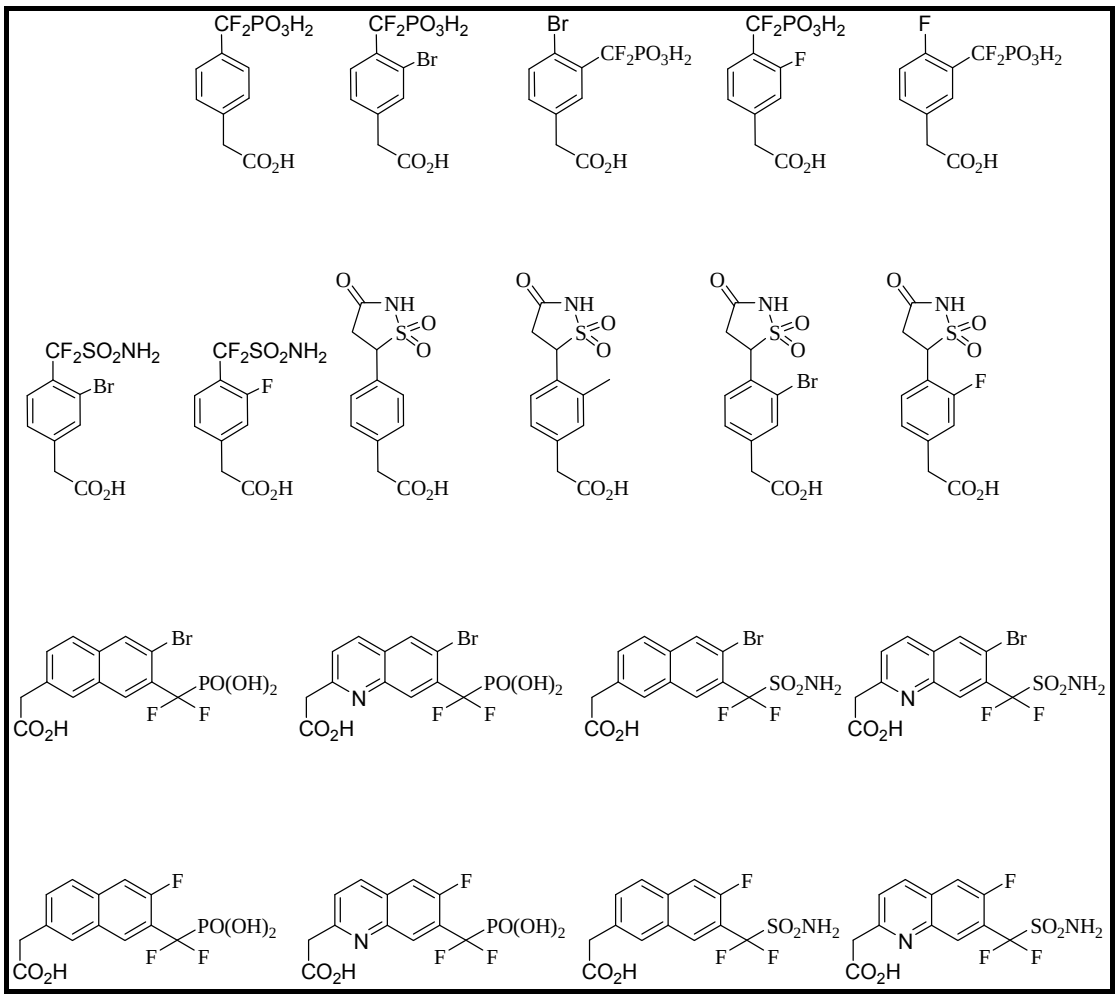


Figure 22. Structures of N-terminal diversity elements

A typical peptidomimetics were designed as dual binding site PTP 1B inhibitors, comprising of new *p*Tyr mimetics. Keeping this motive in mind, compounds **X**, **XI** and **XII** (**Figure 23**) have been designed.

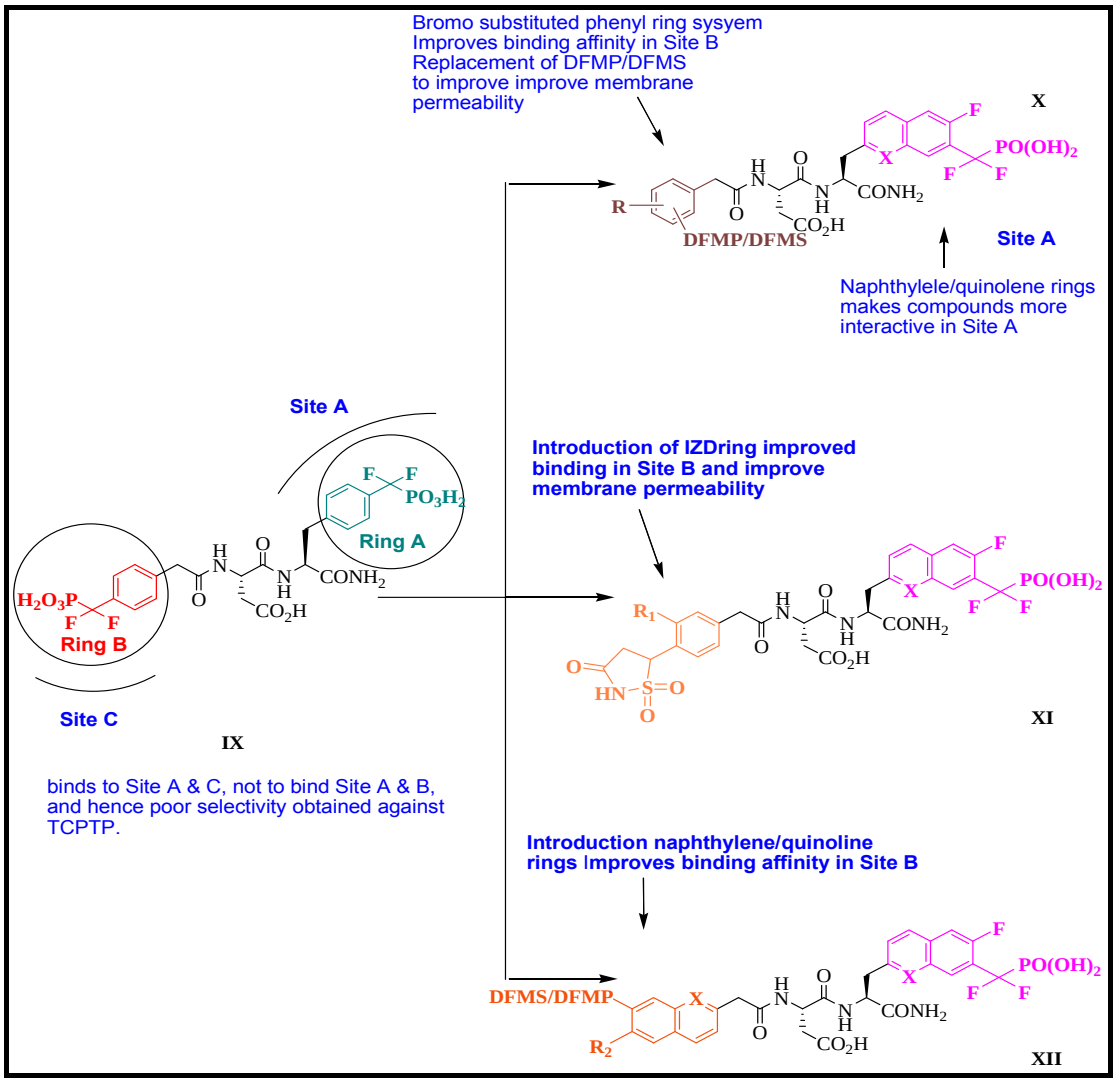


Figure 23. Design Strategy peptidomimetic PTP-1B Inhibitors

In this series, total twenty three compounds **61a-i**, **64a-d** and **67a-j**, are prepared. Their synthetic methodology and chemical characterization are explained in detail in **Chemistry section 3.3.1**.

2.1.4. Conclusion

In the present work, total three series (First series: benzotriazole-based PTP-1B inhibitors, second series: tri-aryl sulfonamide-based PTP-1B inhibitors and Third series: peptidomimetic based PTP-1B inhibitors) were designed as potent and site-specific PTP-1B inhibitors for a safe and effective treatment of metabolic diseases such as T2DM and obesity. In the first series, benzotriazole-based derivatives were designed and total fifteen compounds have been prepared. In the second series, tri-aryl sulfonamide based derivatives were designed and total nineteen compounds are reported. In the third series, peptidomimetic- based derivatives were designed and total twenty three compounds have been synthesized. Altogather fifty-seven new compounds have been synthesized as PTP-1B inhibitors. All the test compounds were purified and are well characterized, and have been subjected for *in vitro*, *in vivo* and pharmacokinetic (PK) studies and results are summarized in the results and discussion section.

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Chapter III: Results & Discussion

3. Results and discussion

As described in the previous chapter, three novel series of PTP-1B inhibitors have been designed and synthesized. The first series is benzotriazole based derivatives, the second series triaryl-sulfonamide bases derivatives and the third series is peptidomimetic based derivatives. All synthesized compounds were purified, characterized and subjected to *in-vitro* evaluation to establish structure activity relationship (SAR) of individual series. Selected short listed compounds (most potent compounds) from each series were subjected to *in vitro* sub-type selectivity studies (against TC-PTP). The most potent and selective compounds were further subjected to *in vivo* antidiabetic activity. Selected short listed compounds (most potent compounds : both *in vitro* & *in vivo*) were also subjected to PK studies.

In this section, we summarize results and discussion of :

a) Benzotriazole based PTP-1B inhibitors (First series)

b) Triaryl-sulfonamide based PTP-1B inhibitors (Second series)

c) Peptidomimetic based PTP-1B inhibitors (Third series),

in following order:

- Synthesis of three different series (Chemistry)
- *In vitro* PTP-1B inhibitory activity, selectivity and SAR
- *In vivo (*antidiabetic activity) evaluation of PTP-1B inhibitors
- PK studies of selected compounds
- Docking studies

3.1. Benzotriazole based PTP-1B inhibitors (First series)

3.1.1. Chemistry

In the previous section rationale for designing dual binding site benzotriazole based PTP-1B inhibitors containing potent *p*Tyr mimetic has been described, wherein it was intended to synthesize the compounds represented by general structures **7a-f**, **10a-c** and **13a-f** (**Figure 24**). Synthetic methodology was designed based on the retrosynthetic analysis and according to the synthesis schemes shown on the following pages. Synthetic methods reported in literature were adapted for the synthesis of **2**, **3a** and **3b** which were the common intermediates for the synthesis of the title compounds **7a-f**, **10a-c** and **13a-f** respectively. Substituted phenyl, naphthalene and quinoline derivatives were also synthesized as potent *p*Tyr mimic following the procedure reported earlier by choosing the appropriate starting materials and optimizing reaction conditions.

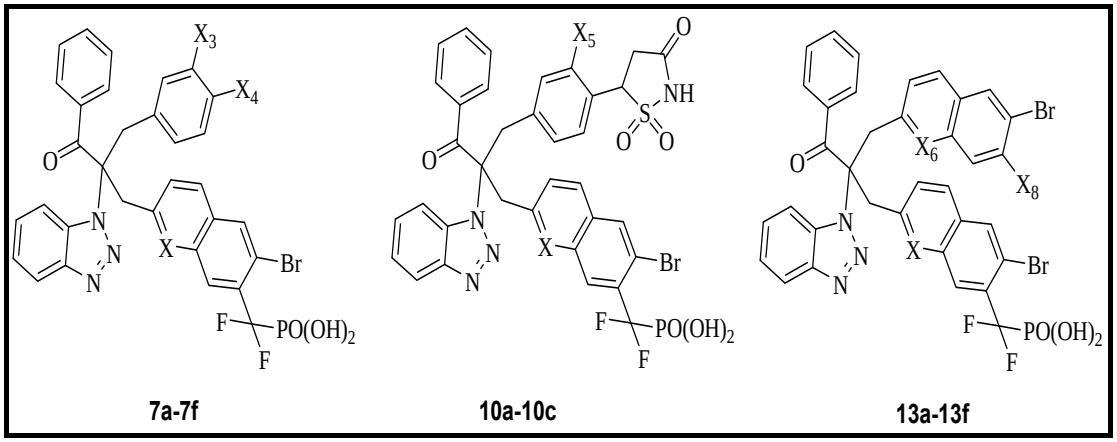
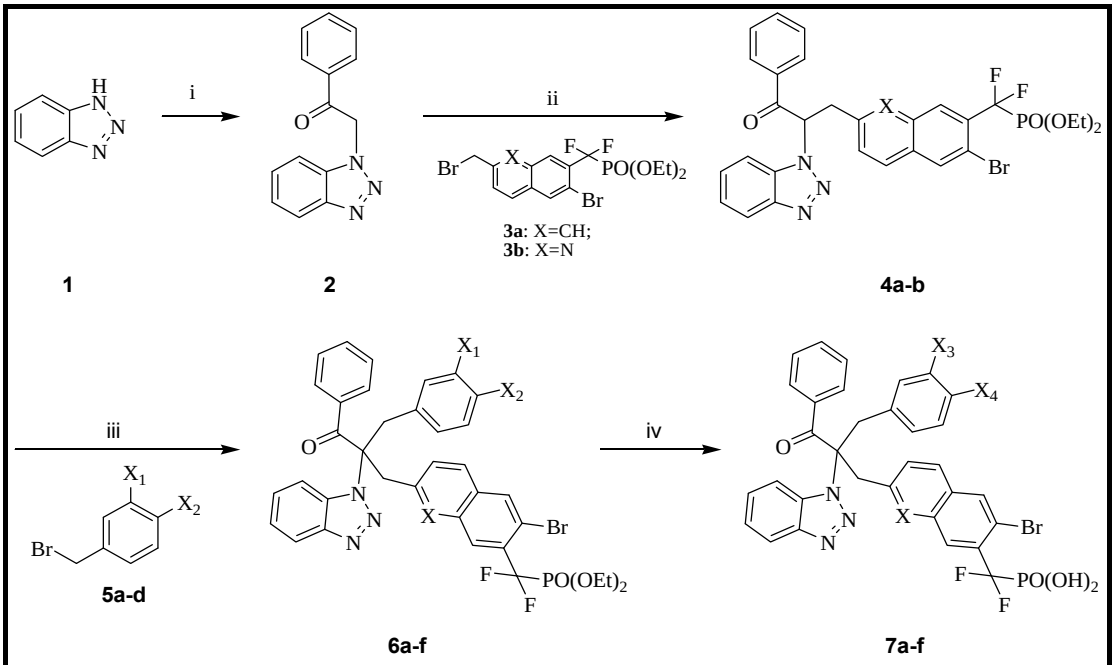


Figure 24. Benzotriazole based PTP-1B inhibitors



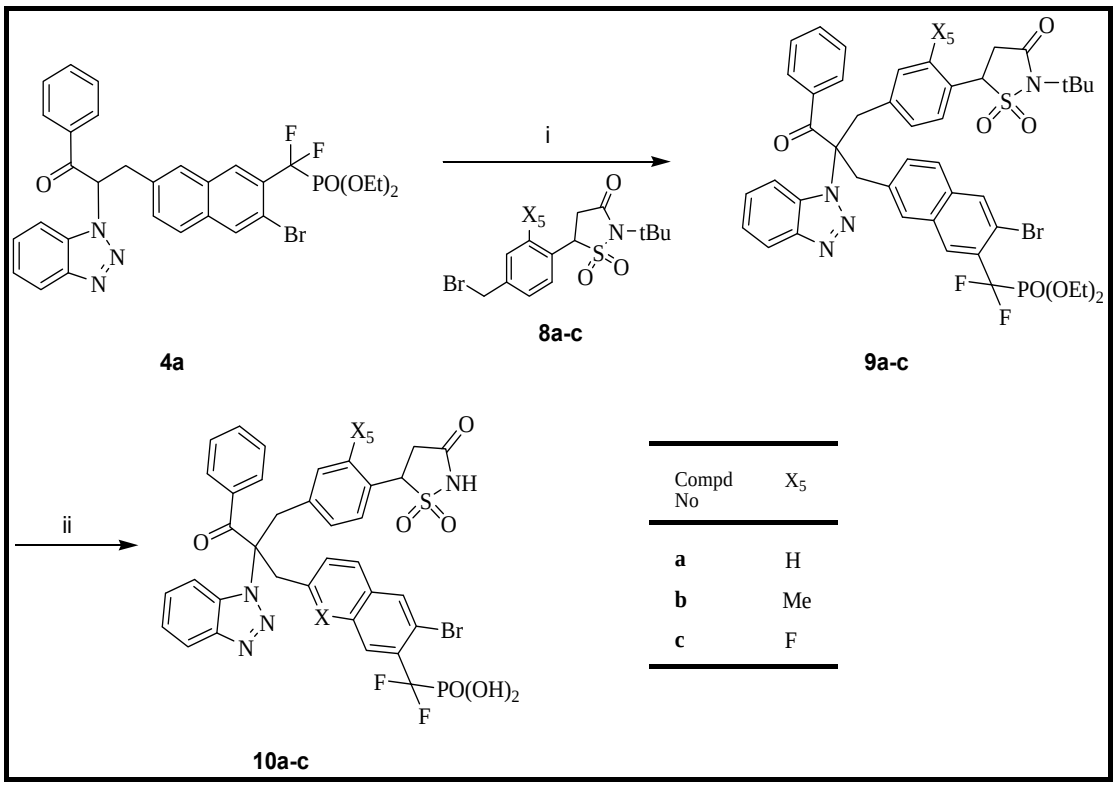
Compd No	X_1	X_2
5a	H	$CF_2PO(OEt)_2$
5b	Br	$CF_2PO(OEt)_2$
5c	$CF_2PO(OEt)_2$	Br
5d	Br	$CF_2SON(tBu)_2$

Compd No	X	X_1	X_2
6a	CH	H	$CF_2PO(OEt)_2$
6b	CH	Br	$CF_2PO(OEt)_2$
6c	CH	$CF_2PO(OEt)_2$	Br
6d	CH	Br	$CF_2SON(tBu)_2$
6e	N	Br	$CF_2PO(OEt)_2$
6f	N	Br	$CF_2SON(tBu)_2$

Compd No	X	X_3	X_4
7a	CH	H	$CF_2PO(OH)_2$
7b	CH	Br	$CF_2PO(OH)_2$
7c	CH	$CF_2PO(OH)_2$	Br
7d	CH	Br	CF_2SONH_2
7e	N	Br	$CF_2PO(OH)_2$
7f	N	Br	CF_2SONH_2

Reagents and conditions: (i) NaH (60%), $BrCH_2COPh$, DMF, 25 ^oC, 5 h; (ii) n-BuLi, THF, -78 ^oC ⟶ 25 ^oC, 4 h; (iii) n-BuLi, THF, -78 ^oC ⟶ 25 ^oC, 6 h; (iv) TMSBr, CH_2Cl_2, -15 ^oC, 3 h and/or TFA CH_2Cl_2, 25 ^oC, 2 h.

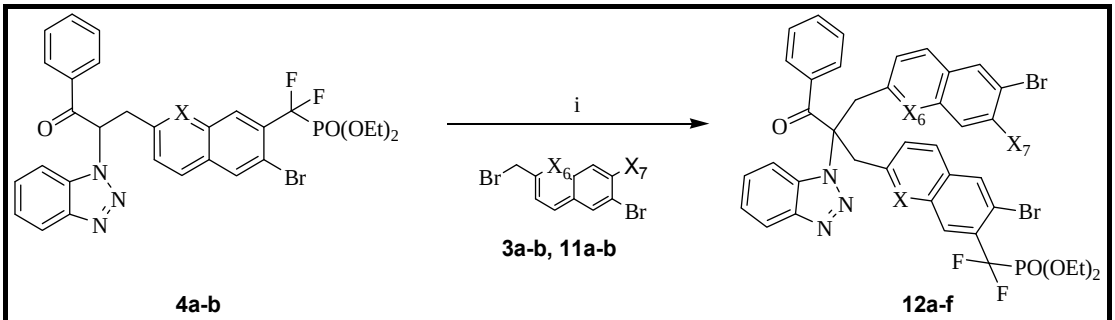
Scheme 1. Synthesis methodology for the preparation of the compounds (**7a-f**)



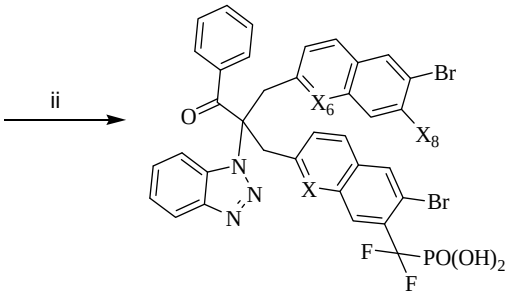
Reagents and conditions: (i) n-BuLi, THF, -78 ^{o}C ⟶ 25 ^{o}C, 6 h; (ii) TMSBr, CH_2Cl_2, -15 ^{o}C, 3 h and TFA CH_2Cl_2, 25 ^{o}C, 2 h.

Scheme 2. Synthesis methodology for the preparation of the compounds (**10a-c**)

Synthesis methodology of the designed compounds **7a-f**, **10a-c** and **13a-f** is outlined in **Schemes 1-3 [1].** Treatment of **1** with phenacyl bromide in the presence of sodium hydride gave **2**. Deprotonation of **2** with n-BuLi in THF gave **4a-b**. Subsequent alkylation of **4a-b** with various substituted benzyl bromides **5a-d** or **8a-c** or naphthyl/quinolinyl analogues **3a-b**/**11a-b** led to the formation of **6a-f**, **9a-c** and **12a-f**, which upon deprotection with trimethylsilyl bromide/trifluoroacetic acid results in the formation of title compounds **7a-f**, **10a-c** and **13a-f**.



Compd No	X_6	X_7
3a	CH	$CF_2PO(OEt)_2$
3b	N	$CF_2PO(OEt)_2$
11a	CH	$CF_2SON(tBu)_2$
11b	N	$CF_2SON(tBu)_2$



Compd No	X	X_6	X_7
12a	CH	CH	$CF_2PO(OEt)_2$
12b	CH	N	$CF_2PO(OEt)_2$
12c	CH	CH	$CF_2SON(tBu)_2$
12d	CH	N	$CF_2SON(tBu))_2$
12e	N	N	$CF_2PO(OEt)_2$
12f	N	N	$CF_2SON(tBu)_2$

Compd No	X	X_6	X_8
13a	CH	CH	$CF_2PO(OH)_2$
13b	CH	N	$CF_2PO(OH)_2$
13c	CH	CH	CF_2SONH_2
13d	CH	N	CF_2SONH_2
13e	N	N	$CF_2PO(OH)_2$
13f	N	N	CF_2SONH_2

Reagents and conditions: (i) n-BuLi, THF, -78 $^oC \longrightarrow$ 25 ^oC, 6 h; (ii) TMSBr, CH_2Cl_2, -15 ^oC, 3 h and/or TFA CH_2Cl_2, 25 ^oC, 2 h.

Scheme 3. Synthesis methodology for the preparation of the compounds (**13a-f**)

For the preparation of the compounds **7a**-**f**, **10a**-**c** and **13a**-**f** (**Scheme 1**-**3**), it was required to synthesize DFMP derivatives (**3a**-**b, 5a**-**c**), DFMS derivatives (**5d**, **11a**-**b**) and IZD derivatives (**8a**-**c**) as potent *p*Tyr mimics.

Synthesis of DFMP derivatives (3a-b, 5a-c)

In general, synthesis of DFMP group is a major challenge for organic chemists. Several methods are reported in literature for the preparation of DFMP group (**XIII**, **Figure 25**). Dufresne et al., prepared DFMP derivatives (**Method A**), starting from aldehyde (structure **XIV**) **[2]** and reaction with lithium salt of diethyl phosphite followed by a Swern oxidation gave ketophosphonate. The last step required reaction with diethylsulfourtrifluoride (DAST) to give DFMP group (structure **XIII**). The disadvantage of this method was poor yield and handling of expensive DAST as well as the unpredictable exothermic reaction.

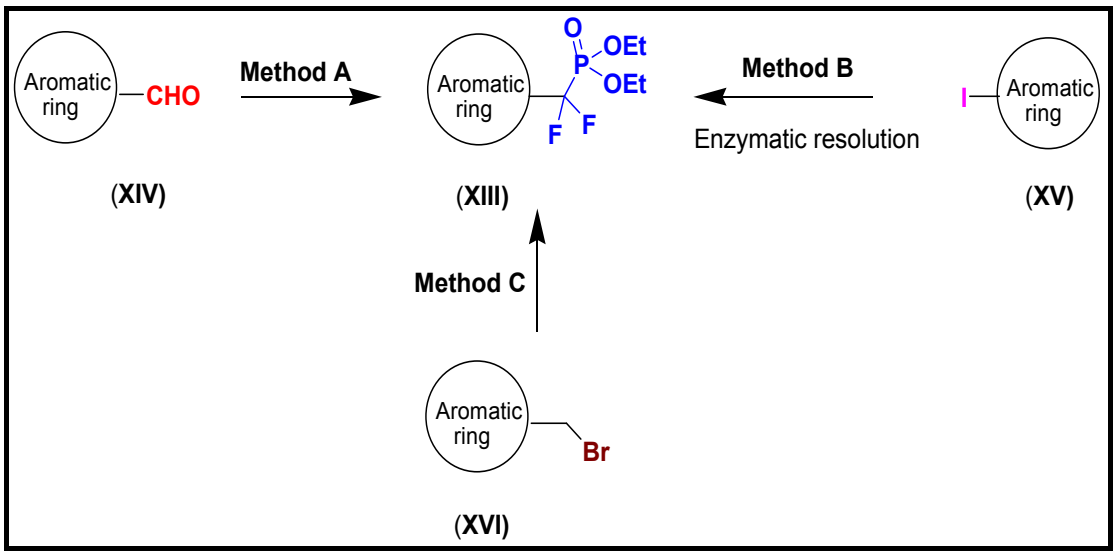


Figure 25. Different approaches for the synthesis of DFMP group

In an alternative method for the preparation of DFMP group (**Method B**), Yokomatsu et al., used aryl halide (structure **XV**) and metalated dialkyl difluoromethylphosphonates catalyzed by transition metal complexes **[3]**, which was a major limitation of this method. In another **Method C**, Hussain et al., used substituted benzyl bromide (structure **XVI**) as a starting material.

They reported more efficient and DAST-free synthesis by introducing flourines by electrophilic fluorination of the benzylic phosphonate using *N*-fluorobenzenesulfonimide (NFSi) as a fluoranating agent **[4]**. This method offers some distinct advantages over the conventional one using DAST reagent, such as low cost, simplicity and safety.

Among the various reported methods, we used the method C which was slightly modified this method further by using potassium bis(trimethylsilyl)amide (KHMDS), instead of sodium hexamethyldisilazane (NaHMDS) to yield benzylic phosphites from benzylic bromides. This method was cost effective, with improvement in yield and was found to be safe for synthesis of DFMP derivatives.

Synthesis of DFMS derivatives (**5d**, **11a**-**b**)

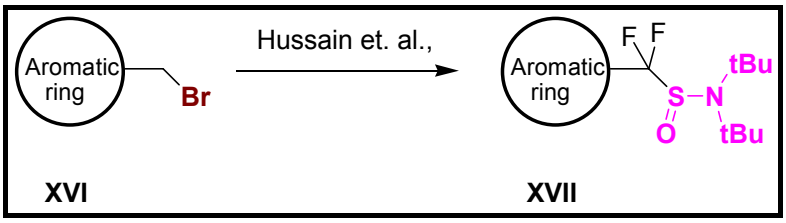


Figure 26. Practical approach for the synthesis of DFMS group

In general for the synthesis of DFMS group, Hussain et. al., used substituted benzyl bromide (structure **XVI**, **Figure 26**) as a starting material **[4]**. They reported more efficient and DAST-free synthesis for DFMS by introducing the fluorines by electrophilic fluorination of a benzylic sulfonamide using NaHMDS/NFSi. They used allyl protection of sulfonamide which was removed by using Pd-catalyst and barbituric acid, which was a major limitation of this method.

We used Hussain et. al., method for synthesis of DFMS derivatives and modified this method further by using KHMDS instead of NaHMDS for the preparation of benzylic sulfonamide from benzylic bromide and also used t-butyl protecting group was employed instead of allyl protection and it can easily removed by using trifluoroacetic acid (TFA). This method was cost effective, good in yield for the synthesis of DFMS (**XVII**, **Figure 26**) derivatives.

Synthesis of IZD derivatives (**8a**-**8c**)

Several methods are reported in literature for the preparation of IZD derivatives (structure **XIX**, **Figure 27**). Douty et. al., used boronic acid (structure **XV**, **Figure 27**) **[5]** in a Suzuki-coupling reaction using Pd-catalyst such as $PdCl_2(dppf).CH_2Cl_2$, which was major limitation of this method. In another **Method B**, Combs et. al., used aromatic nitro derivatives **[6]** as starting material (structure **XVIII**, **Figure 27**). The disadvantage of this method was yield remain lower.

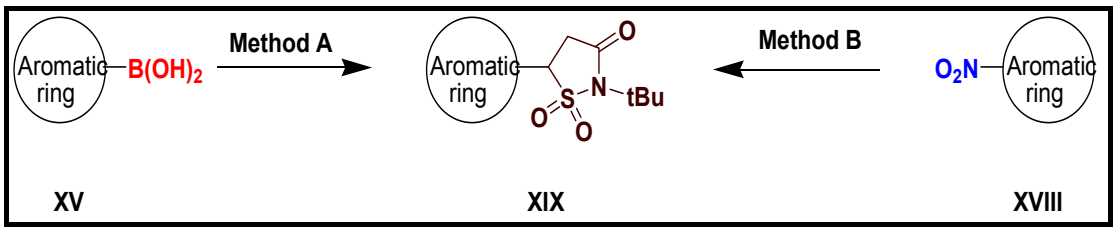
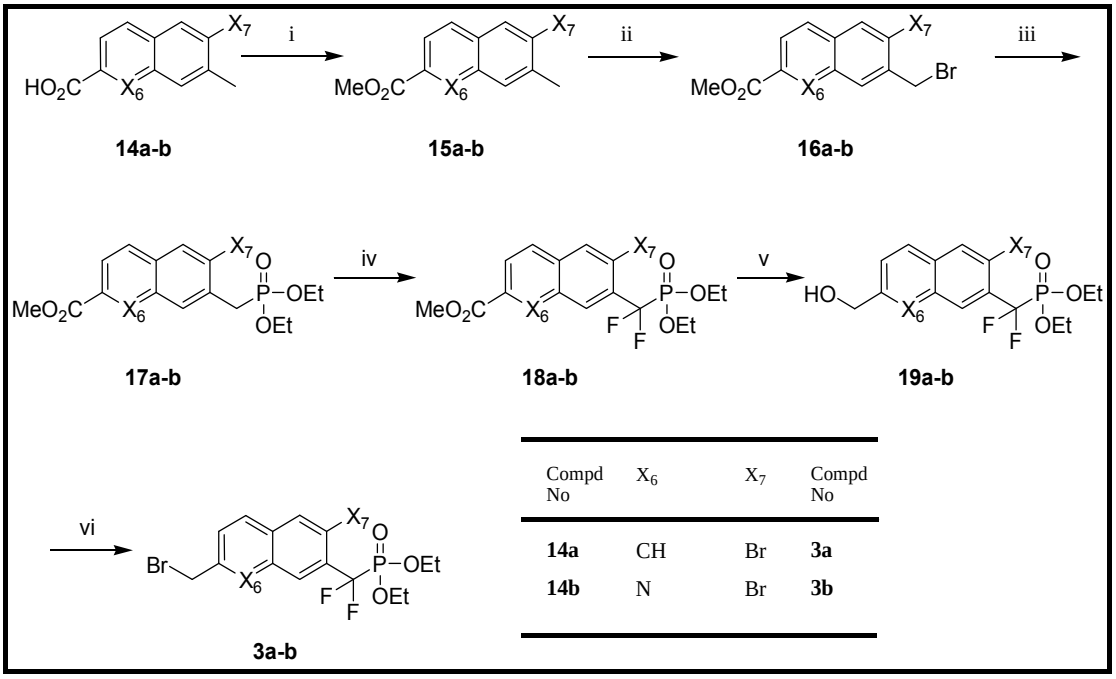


Figure 27. Practical approaches for the synthesis of IZD derivatives

Among various reported methods, we used method B which was modified by using $Pd(OAc)_2$ instead of $PdCl_2(dppf).CH_2Cl_2$ for Suzuki-coupling reaction. For olefinic double bond reduction, we used 10% Pd/C instead of

lithium aluminiumhydride ($LiAlH_4$). This method was cost effective and was found easy for synthesis.

By using modified Hussain method, synthesis of common intermediates **3a**-**b** is outlined in **Scheme 4**. Acid derivatives of substituted naphthalene/quinoline moiety **14a-b** were converted to their methyl ester **15a-b** by refluxing in CH_3OH in presence of thionyl chloride ($SOCl_2$). Bromination of methyl group **15a-b** was carried out using *N*-bromosuccinamide (NBS) in the presence of a catalytic amount of benzoyl peroxide in carbon tetrachloride (CCl_4) resulting $–CH_2Br$ derivatives **16a-b**, which were converted to their phosphonate derivatives **17a-b** via a Michaekis-Arbuzov reaction using triethylphosphite **[7-11]**. Electrophilic fluorination of phosphonate **17a-b** using KHMDS/NFSi gave fluorophosphonate **18a-b [12-14]**. Reduction of methyl ester **18a-b** using sodium borohydride ($NaBH_4$) gave alcohol **19a-b** which were converted into bromide **3a-b** using CBr_4/ triphenylphosphine (PPh_3).

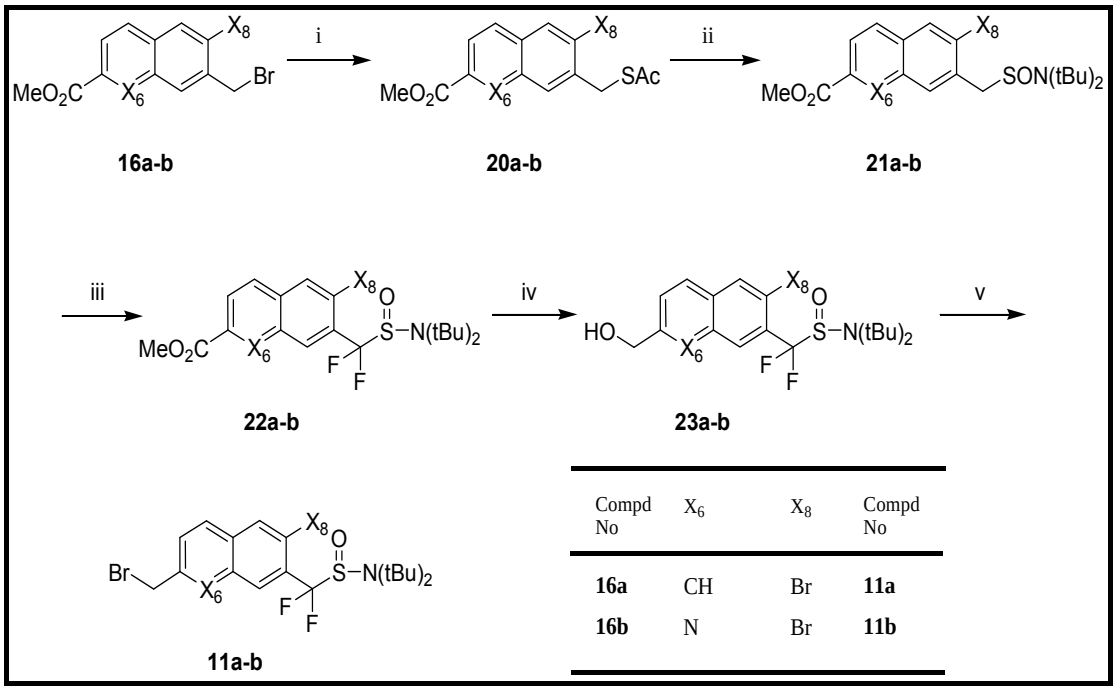


Compd No	X_6	X_7	Compd No
14a	CH	Br	**3a**
14b	N	Br	**3b**

Reagents and conditions: (i) $SOCl_2$, CH_3OH, 70 ^oC, 3 h: (ii) NBS, benzoyl peroxide, CCl_4, 80 ^oC, 4 h; (iii) Triethylphoshite, reflux, 5 h; (iv) KHMDS, NFSi, THF, -78 $^oC \longrightarrow$ 25 ^oC, 18 h; (v) $NaBH_4$, CH_3OH, 60 ^oC, 3 h; (vi) CBr_4, PPh_3, CH_2Cl_2, 0 ^oC, 3 h.

Scheme 4. Synthesis methodology for the preparation of the compounds (**3a-b**)

By using modified Hussain method, synthesis of intermediates **11a-b** is illustrated as **Scheme 5 [4]**. Reaction of bromide **16a-b** with potassium thioacetate gave thioacetate **20a-b**. Control oxidative chlorination of **20a-b** gives crude sulfinyl chloride, which were reacted with di-tertbutyl amine to give protected sulfonamide **21a-b**. Fluorines could be readily introduced by electrophilic fluorination of benzylic sulfonamide **21a-b** using KHMDS/NFSi gave desired difluoromethylenesulfinamide **22a-b [12-14]**. Reduction of methyl ester **22a-b** using $NaBH_4$ gave alcohols (**23a-b**) which were converted into bromide **11a-b** using CBr_4/PPh_3.

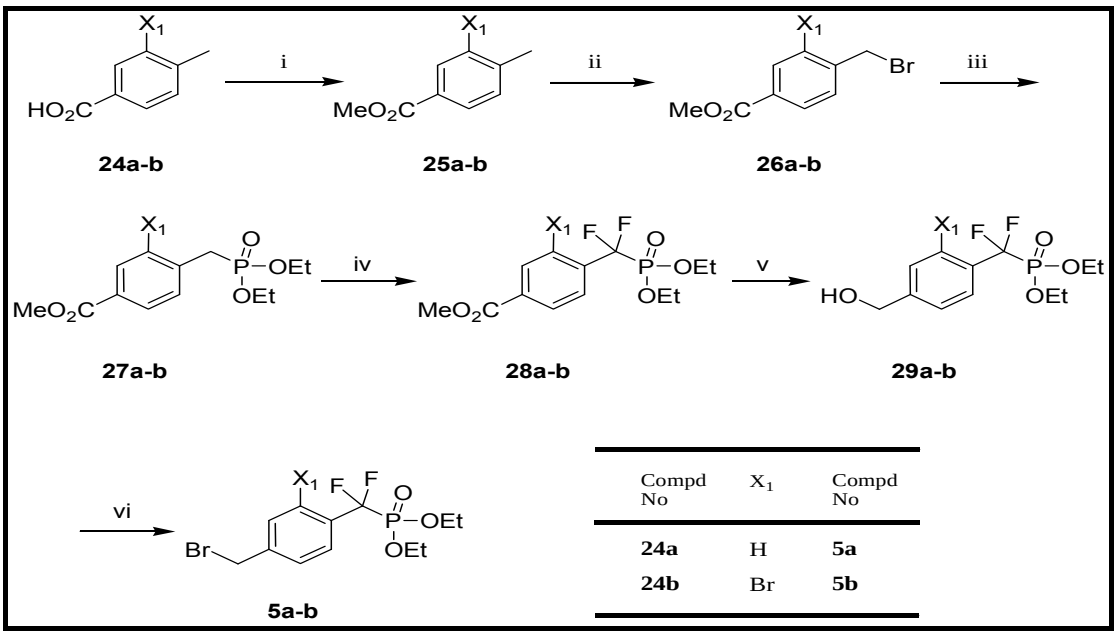


Compd No	X_6	X_8	Compd No
16a	CH	Br	**11a**
16b	N	Br	**11b**

Reagents and conditions: (i) KSAc, DMF, 30 min, 25 ^{o}C; (ii) Cl_2, HOAc/H_2O, 0 ^{o}C, 1 h then CH_2Cl_2, ditert-butyl amine, 25 ^{o}C, 2 h; (iii) KHMDS, THF, NFSi, -78 ^{o}C ⟶ 25 ^{o}C, 18 h; (v) $NaBH_4$, CH_3OH, 65 ^{o}C, 2 h; (vi) CBr_4, PPh_3, CH_2Cl_2, 0 ^{o}C, 3 h.

Scheme 5. Synthesis methodology for the preparation of the compounds (**11a-b**)

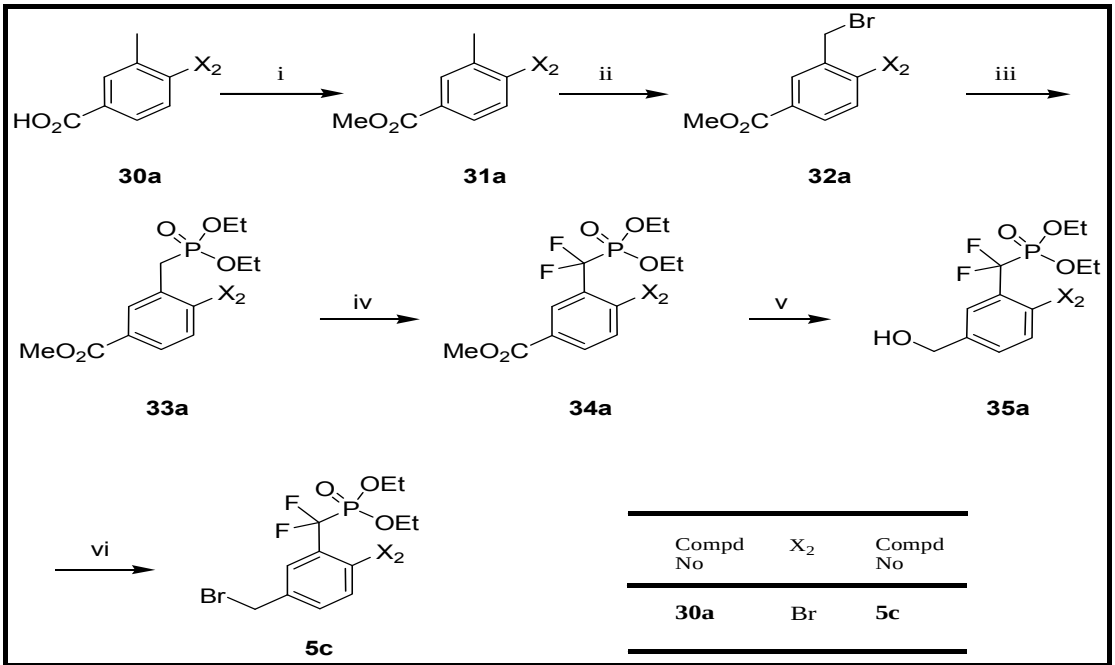
Synthesis of intermediates **5a-b**, and **5c** are illustrated as **Scheme 6** & **Scheme 7**. A methyl derivatives of substituted benzoic acid **24a**-**b** or **30a** were converted to their methyl ester **25a**-**b**, **31a** by refluxing in CH_3OH in presence of $SOCl_2$. Free radical bromination of methyl group was carried out using NBS in the presence of a catalytic amount of benzoyl peroxide in CCl_4 resulting benzyl bromide derivatives **26a**-**b**, **32a** which were converted to their phosphonate derivatives **27a**-**b**, **33a** using triethylphosphite. Electrophilic fluorination of phosphonate using KHMDS and NFSi gave difluoromethylenephosphonate **28a**-**b**, **34a**. Reduction of methyl ester using $NaBH_4$ gave benzyl alcohol **29a**-**b**, **35a** which were converted into benzyl bromide **5a-b**, & **5c** using CBr_4/PPh_3.



Compd No	X_1	Compd No
24a	H	**5a**
24b	Br	**5b**

Reagents and conditions: (i) $SOCl_2$, CH_3OH, 70 ^oC, 3 h: (ii) NBS, benzoyl peroxide, CCl_4, 80 ^oC, 4 h; (iii) Triethylphoshite, reflux, 5 h; (iv) KHMDS, NFSi, THF, -78 $^oC \longrightarrow$ 25 ^oC, 18 h; (v) $NaBH_4$, CH_3OH, 60 ^oC, 3 h; (vi) CBr_4, PPh_3, CH_2Cl_2, 0 ^oC, 3 h.

Scheme 6. Synthesis methodology for the preparation of the compounds (**5a-b**)

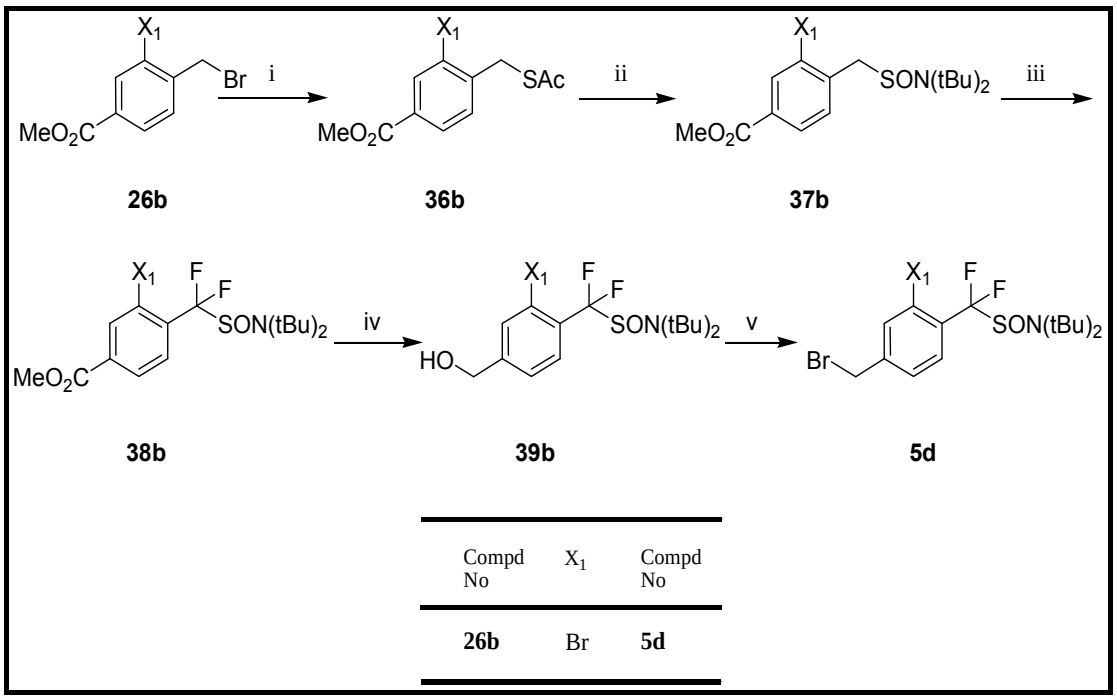


Reagents and conditions: (i) $SOCl_2$, CH_3OH, 70 ^oC, 3 h: (ii) NBS, benzoyl peroxide, CCl_4, 80 ^oC, 4 h; (iii) Triethylphoshite, reflux, 5 h; (iv) KHMDS, NFSi, THF, -78 $^oC \longrightarrow$ 25 ^oC, 18 h; (v) $NaBH_4$, CH_3OH, 60 ^oC, 3 h; (vi) CBr_4, PPh_3, CH_2Cl_2, 0 ^oC, 3 h.

Scheme 7. Synthesis methodology for the preparation of the compounds (**5c**)

Synthesis of intermediate **5d** is illustrated as **Scheme 8**. Reaction of bromide **26b** with potassium thioacetate gave thioester **36b**. Controlled oxidative chlorination gave sulfonyl chloride, which was reacted with di-tertbutyl amine to give protected sulfonamide **37b**. Fluorines could be readily introduced by electrophilic fluorination of benzylic sulfonamide using KHMDS/NFSi gave desired difluoromethylenesulfinamide **38b**. Reduction of

methyl ester to benzyl alcohol **39b** was carried out using $NaBH_4$ which then converted into bromide **5d** using CBr_4/PPh_3.



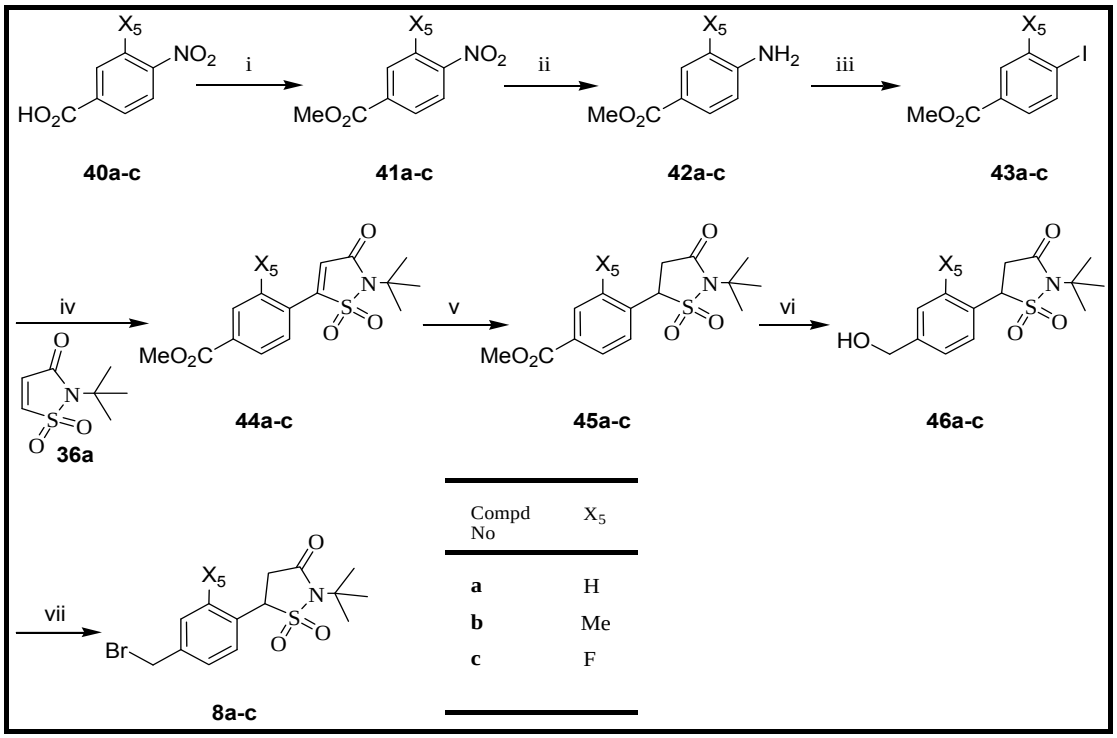
Compd No	X_1	Compd No
26b	Br	**5d**

Reagents and conditions: (i) KSAc, DMF, 30 min, 25 ^oC; (ii) Cl_2, $HOAc/H_2O$, 0 ^oC, 1 h then CH_2Cl_2, ditert-butyl amine, 25 ^oC, 2 h; (iii) KHMDS, THF, NFSi, -78 $^oC \longrightarrow$ 25 ^oC, 18 h; (v) $NaBH_4$, CH_3OH, 65 ^oC, 2 h; (vi) CBr_4, PPh_3, CH_2Cl_2, 0 ^oC, 3 h.

Scheme 8. Synthesis methodology for the preparation of the compounds (**5d**)

By using modified Combs method, synthesis of intermediates **8a-c** is illustrated as **Scheme 9**. Nitro derivatives of substituted benzoic acid **40a-c** were converted to their methyl ester **41a-c** using CH_3OH and $SOCl_2$. The nitro group in **41a-c** was reduced to amino group using zinc and ammonium chloride in methanol to get **42a-c**, followed by diazotization using sodium nitrite under acidic conditions and the resulting intermediates were trapped with potassium iodide to afford **43a-c**. The iodo core was coupled with **36a** with classical ligandless conditions utilizing $Pd(OAc)_2$, tetra-butyl ammonium chloride, and triethyl amine in *N*,*N*-dimethyl formamide (DMF) results **44a-c**

[6]. Reduction of unsaturated IZD (**44a-c**) to saturated IZD (**45a-c**) was carried out using 10% Pd/C in methanol under hydrogen (H_2) atmosphere. Methyl esters were reduced to corresponding alcohols **46a-c** using $NaBH_4$ which were then converted into bromides (**8a-c**) using CBr_4/PPh_3.



Reagents and conditions: (i) $SOCl_2$, CH_3OH, 70 ^oC, 3 h; (ii) Zn^0, NH_4Cl, CH_3OH, H_2O, 65 ^0C, 1 h; (iii) $NaNO_2$, 1 *N* aq. HCl, KI, 0 ^oC for 30 min then 45 ^oC for 45 min; (iv) $Pd(OAc)_2$, Bu_4NCl, **36a**, Et_3N, DMF, 100 ^oC, 3 h; (v) 10% Pd/C, EtOH, 25 ^oC, 16 h; (vi) $NaBH_4$, CH_3OH, 65 ^oC, 3 h; (vii) CBr_4, PPh_3, CH_2Cl_2, 0 ^oC, 2 h.

Scheme 9. Synthetic methods for the preparation of title compounds (**8a-c**)

Using above synthetic routes (**Scheme 1**, **2** and **3**), all together 15 new compounds (**7a**-**f**, **10a**-**c** and **13a**-**f**) were prepared. All the final compounds and intermediates were purified, characterized and spectral data of compounds were found to be in conformity with the structures assigned. Detailed experimental procedures and spectral characterization data are

presented in **experimental section 5.1.** and representative spectra's of selected compounds are given **spectral data Section 6**.

3.1.2. *In vitro* PTP-1B inhibitory activity, selectivity and structure activity relationship (SAR)

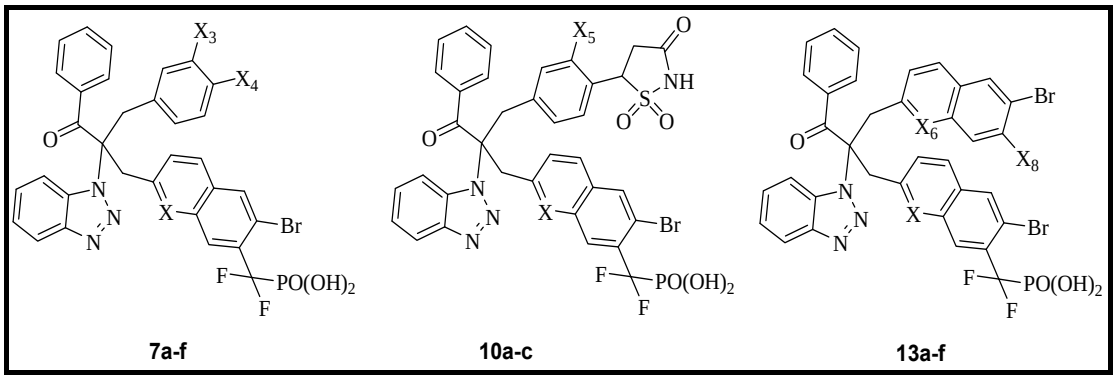
All the final compounds **7a-f**, **10a-c** and **13a-f** were screened for *in vitro* PTP-1B inhibitory activity using *p*-Nitro phenyl phosphate *(p*NPP) enzymatic assay (detailed experimental protocol is given in **experimental Section 5.3**) **[15]**, mainly to establish the SAR of new series of benzotriazole based PTP-1B inhibitors. As depicted in **Table 6**, depending upon the nature of substitution, all the compounds showed different degree of PTP-1B inhibition (IC_{50}).

As described earlier in designing strategy section of PTP-1B inhibitors comprised of a basic pharmacophore attached to potent *p*Tyr mimetic. Here, benzotriazole ring system was retained as a basic pharmacophore to obtain superior PTP-1B selectivity over other PTPases. Based on design strategy, compounds were mainly divided in two series, either having naphthyl **7a-d**, **10a-c** and **13a-d** or quinolinyl **7e-f** and **13e-f** templates. Within the first series **7a-d**, **10a-c** and **13a-d,** three distinct sets of compounds were prepared by introducing DFMP/DFMS/IZD benzyl group **7a-d, 10a-c** or with DFMP/DFMS-substituted naphthyl/quinolinyl templates **13a-d**. In the second series, **7e-f**, **13e-f,** DFMP/DFMS-substituted quinolinyl analogues of the selected compounds for the first series were prepared.

The first set of compounds containing DFMP/DFMS-substituted benzyl groups showed different degrees of PTP-1B inhibitory activity depending on the DFMP group position (*meta* Vs *para*) on the benzyl ring and *ortho*

substitutents (hydrogen or halogen). Compound **7c** with *m*-DFMP substitutent showed weak PTP-1B inhibitory activity relative to that of p-DFMP **7a**, whereas compound **7b** with *p*-DFMP group and *ortho*-bromo substitution to DFMP, showed good PTP-1B inhibitory activity.

Table 6. The *In vitro* PTP-1B inhibitory activity of test compounds **7a-f**, **10a-c** & **13a-f**.



Compd No	X	X_3	X_4	X_5	X_6	X_8	PTP-1B[a] (IC_{50} nM)
7a	CH	H	$CF_2PO(OH)_2$	-----	-----	-----	46
7b	CH	Br	$CF_2PO(OH)_2$	-----	-----	-----	20
7c	CH	$CF_2PO(OH)_2$	Br	-----	-----	-----	404
7d	CH	Br	CF_2SONH_2	-----	-----	-----	19
7e	N	Br	$CF_2PO(OH)_2$	-----	-----	-----	21
7f	N	Br	CF_2SONH_2	-----	-----	-----	22

10a	CH	-----	-----	H	-----	-----	930
10b	CH	-----	-----	Me	-----	-----	924
10c	CH	-----	-----	F	-----	-----	919
13a	CH	-----	-----	-----	CH	$CF_2PO(OH)_2$	5
13b	CH	-----	-----	-----	N	$CF_2PO(OH)_2$	3
13c	CH	-----	-----	-----	CH	CF_2SONH_2	4
13d	CH	-----	-----	-----	N	CF_2SONH_2	6
13e	N	-----	-----	-----	N	$CF_2PO(OH)_2$	5
13f	N	-----	-----	-----	N	CF_2SONH_2	4

[a] Enzymatic assay was carried out in 96-well plates. To an assay buffers, *p*NPP and test compounds were added and the reaction was initiated by addition of PTP-1B/TC-PTP (10 -100 nM). The initial rate of PTPase-catalyzed hydrolysis of *p*NPP was measured at 405 nm. IC_{50} value was determined under fixed *p*NPP concentration of 1 mM (n=3).

Bioisosteric replacement of the *p*-DFMP substitution **7b** with *p*-DFMS **7d** showed nearly similar *in vitro* PTP-1B inhibitory activity, suggesting that the highly negatively charged DFMP group can be replaced with a DFMS group to overcome the issue of low permeability. In second set of compounds containing IZD-substituted benzyl groups showed week inhibitory activity, irrespective of their *ortho* substitutents. The third set of the compounds with

DFMP/DFMS-substituted naphthyl/quinolinyl templates **13a-d** showed potent PTP-1B inhibitory activity.

In second series of compounds DFMP/DFMS-substituted quinolinyl derivatives **7e-f**, **13e-f** showed similar PTP-1B inhibitory activity as compare to the naphthyl derivatives **7b**, **7d**, **13b** and **13d**.

From above SAR study, out of fifteen compounds, short listed compounds **7b**, **7d**, **13a-f** showed excellent PTP-1B inhibition **[15]**. These compounds were evaluated for *in vitro* selectivity over other PTPs (PTPα, LAR, CD45, VHR, SHP-1, SHP-2 and TC-PTP), by using the *p*NPP assay, and IC_{50} values are described in **Table 7** (detailed experimental protocol is given in **experimental Section 5.3**).

As shown in **Table 7**, compounds **7b** and **7d** containing DFMP/DFMS-substituted benzyl group showed ~10-fold selectivity, while **13b** and **13d,** i.e. DFMP/DFMS-substituted quinolinyl templates showed ~30-fold selectivity over TC-PTP. The most potent compounds **13a** and **13c,** containing DFMP/DFMS-substituted naphthyl templates showed >115-fold selectivity, while **13e** & **13f** showed ~20-fold selectivity over TC-PTP, indicating that among the three different ring systems (benzyl, naphthyl, and quinolinyl), only naphthyl derivatives showed best selectivity, due to favorable orientation of the naphthyl ring system across the both binding sites A and B of PTP-1B. Both the selected test compounds (**13a** & **13c**) showed >5000-fold selectivity over other PTPs (PTPα, LAR, CD45, VHR, SHP-1 and SHP-2). Based on PTP-1B inhibitory activity and sub-type selectivity results, short listed compounds (**13a** and **13c**) were further subjected for their *in vivo* (antidiabetic acitivity) and PK evaluations.

Table 7. Subtype-selectivity data selected compounds (**7b**, **7d** and **13a-f)**

Compound	IC_{50} nM		Fold Selectivity[b]
	PTP-1B[a]	TC-PTP[a]	
7b	20	201	~ 10 fold
7d	19	192	~ 10 fold
13a	5	580	~ 116 fold
13b	3	90	~ 30 fold
13c	4	480	~ 120 fold
13d	6	185	~ 31 fold
13e	5	101	~ 20 fold
13f	4	82	~ 20 fold

[a] Enzymatic assay was carried out in 96-well plates. The initial rate of PTPase-catalyzed hydrolysis of *p*NPP was measured at 405 nm. IC_{50} value was determined under fixed *p*NPP concentration of 1 mM (n=3); Data represent the mean ± SD; **7b**, **7d** and **13a-f** showed > 5000-fold selectivity over PTPα, CD45, LAR, SHP-1 and SHP-2 enzymes.
[b] Fold selectivity calculated as ratio of IC_{50} values of TC-PTP/PTP-1B inhibitions

3.1.3. *In vivo* antidiabetic activity selected compounds (**13a** and **13c**)

Subsequently the most potent and selective compounds **13a** and **13c** were tested for *in vivo* antidiabetic activity in male C57BL/6j mice using the intraperitoneal glucose tolerance test (IPGTT) protocol (detail experimental protocol is given in **experimental Section 5.3**) **[16-17]**. As shown in **Figure 28**, both the potent compounds showed significant decrease in blood glucose when administrated by the i.p. route. Compound **13a** showed excellent antidiabetic activity orally, whereas compound **13c** showed moderate activity upon oral (p.o.) administration. Based on the designing concept, replacement

of DFMP group of **13a** with DFMS group **13c** to overcome the cell permeability issue. However, these comparative oral antidiabetic activity results indicate that the DFMP group is more favorable for oral antidiabetic activity than the DFMS group.

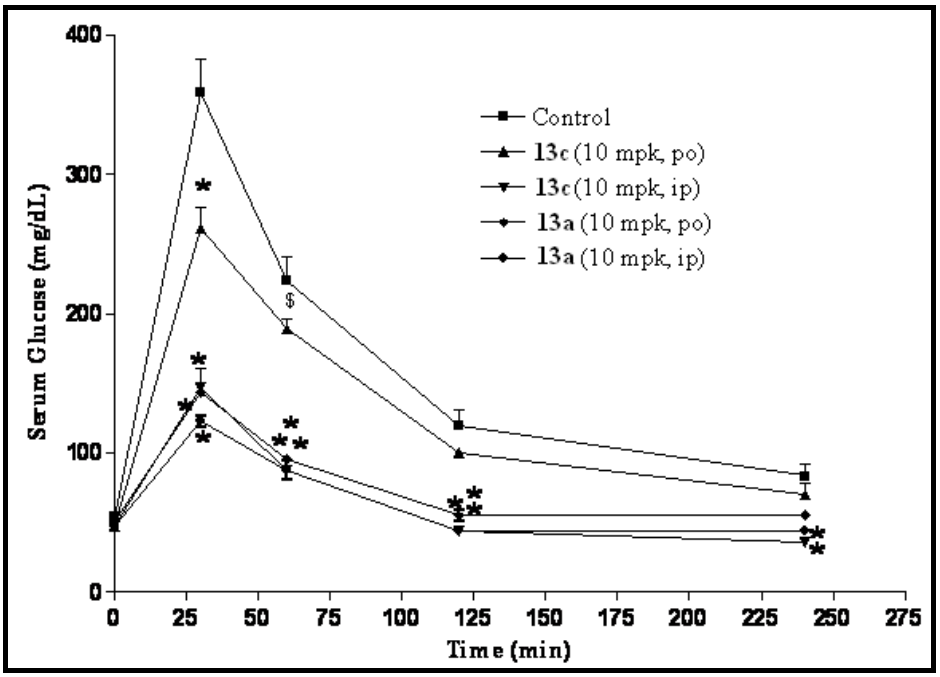


Figure 28. Antidiabetic activity of compounds **13a** and **13c** in C57 mice. (IPGTT): Overnight-fasted male C57BL/6J mice (n=6) were dosed p.o./i.p. with vehicle or test compounds (10 $mgkg^{-1}$) 0.5 h prior to IPGTT (1.5 gkg^{-1}, 10 mL); serum glucose levels were determined at 0, 30, 60, 120, and 240 min. Values represent the mean ± SEM; *p<0.01 and $p<0.05 by twoway ANOVA followed by Bonferronipost-test.

3.1.4. Pharmacokinetic (PK) studies of selected compounds (**13a** and **13c**)

Based on the *in vitro* PTP-1B inhibitory activity, selectivity and *in vivo* antidiabetic activity, compound **13a** and **13c** were selected as the lead compounds to study their pharmacokinetic profile in male Wister rats (detail experimental protocol is given in **experimental Section 5.3**). In order to understand the PK profile of test compounds, a comparative single dose (10

mpk; iv/po) PK study of our most potent compounds (**13a** & **13c**) was carried out and the various PK parameters are summarized in **Table 8** . Compound **13a** exhibits extended t_{max}, half-life ($t_{1/2}$) and moderate area under the curve (AUC), clearance at a dose of 10 $mgkg^{-1}$. While compound **13c** showed rapid t_{max} and clearance, good area under the curve (AUC), and moderate half-life ($t_{1/2}$). Based on PK result, compound **13a** showed roughly sevenfold higher bioavailability (F~68%) compare to **13c**. These improved PK profile of compound **13a** supports its excellent pharmacodynamic effects (antidiabetic activity) in C57 mice, administered orally.

Table 8. Pharmacokinetic parameters of compounds **13a** and **13c** in male *wistar* rat

	PK parameters[#]	**13a**	**13c**
i.v	$t_{1/2}$ (h) K_{elim} (h^{-1}) AUC (h μg/ml)	1.01 ± 0.2 0.99 ± 0.1 15.499 ± 0.191	2.41 ± 0.3 0.29 ± 0.1 29.341 ± 0.795
p.o	t_{max} (h) $t_{1/2}$ (h) K_{elim} (h^{-1}) AUC (h μg/ml) F[%]	0.33 ± 0.2 3.81 ± 0.2 0.44 ± 0.01 10.562 ± 0.105 68.14%	1.21 ± 0.01 8.67 ± 0.26 0.08 ± 0.05 3.031 ± 0.312 10.33 %

[#]Single dose (10 $mgkg^{-1}$; i.v./p.o.) PK study for compound **13a** and **13c** was carried out in fasted male wistar rats (n=6) and plasma concentration of compounds were determined by LC-MS-MS, data represented as mean ± SD.

3.1.5. Molecular docking Study

For a better understanding of selectivity profile of most potent compound **13a** at molecular level, a molecular docking analysis was carried using Glide docking (version 5.6) software to understand selectivity profile and its critical interaction with both binding sites A & B of PTP-1B **[18]**. The initial

Glide docking studies for **13a** gave poor binding conformation. Based on this observation, induced-fit docking (IFD) was carried out for **13a** and docked in the active site of PTP-1B (Protein Data Bank code: 1Q6T).

Briefly, IFD involves the adjustment in the residues surrounding the binding sites to obtain an alternative structure that can accommodate ligands that would otherwise not fit into the original binding sites. The IFD procedure is based on the Glide docking program which involves the refinement molecule in Prime (Schrödinger LLC), which was reported to accurately predict the ligand binding modes and concomitant structural changes in the receptor. Prime was used for side chain rotamer prediction and energy minimization of the conformation of residues located within 5 Å of the ligand in any of the poses.

Upon IFD, residues both binding sites A and B adopt new conformations, allowing better ligand fit **13a** at both the sites. A comparative binding sites pocket of PTP-1B with respect to **13a** and in comparison with known PTP 1B inhibitor **I** is shown (**Figure 29**). Upon IFD, Arg524 and Phe682 rotomers are changed; particular flipping of the Phe682 aromatic ring and the side chain of Arg524 was predominant. Because of this, site B cavity is enlarged, thereby accommodating ligand **13a** quite well, and site B best fit with the overall shape of 13a (**Figure 30c**). The favorable hydrogen bond interactions between **13a** and both sites A and B of PTP-1B support its excellent *in vitro* PTP-1B selectivity over TC-PTP.

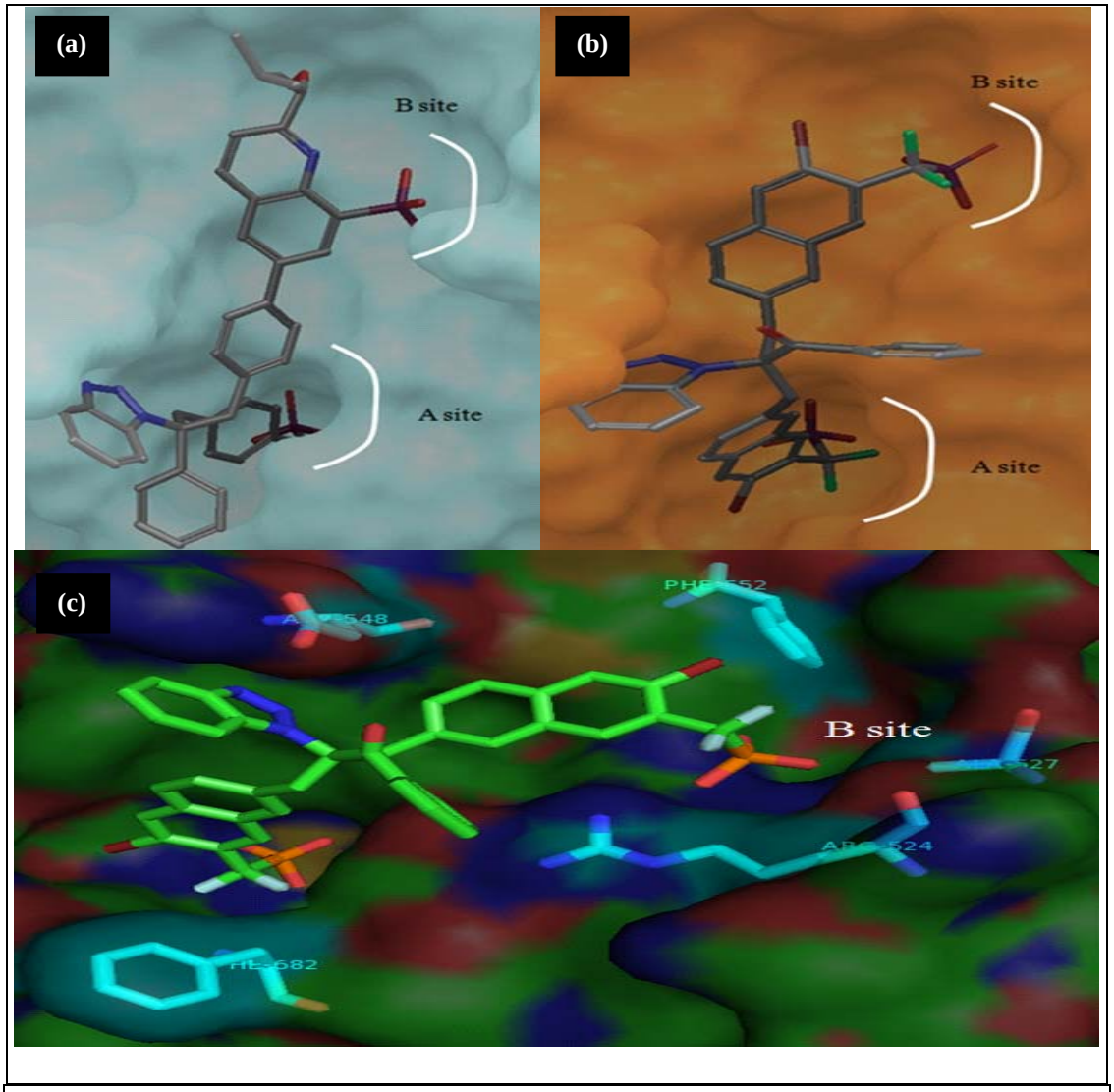


Figure 29. Binding pose of **I** and **13a** in the PTP-1B active site; site A and B are indicated; **(a)** X-ray crustal structure of PTP-1B in complex with known inhibitor I (PDB code: 1Q6T); **(b)** Binding pose of **13a** in the PTP-1B active site; **(c)** Interaction of 13a with key residues of PTP-1B site A & B. Residues numbering is as per current numbering (PDB code: 1QT6): Arg24 (524), Phe52 (552), Arg254 (754), and Met258 (758).

3.1.6. Conclusion

As a part of the ongoing research on dual binding-sites PTP-1B inhibitors, it was envisioned to design novel dual binding-site PTP-1B inhibitors by incorporating potent *p*Tyr mimetic in a single scaffold. Based on this concept, dual binding-sites novel tetrasubstituted benzotriazole-based PTP-1B inhibitors were designed. These compounds were tested for their *in vitro* PTP-1B inhibitory activity, selectivity over TC-PTP and *in vivo*

antidiabetic activity in animal models. Novel tetrasubstituted-based compounds exhibit favorable orientation across both the binding sites of the PTP-1B, thus establishing the evidence for our hypothesis of incorporating the dual binding features in a single chemotype in order to develop novel PTP-1B inhibitors. The lead compound **13a** is one such dual binding-site PTP-1B inhibitor, indicating that among three different ring systems (benzyl, naphthyl, and quinolinyl), the naphthyl derivatives show selectivity owing to favorable orientation of the naphthyl ring system across both the binding sites of PTP-1B enzyme. Compound **13a** exhibits excellent anti-hyperglycemic effects in animal models, along with improved oral bioavailability. The results of our in silico docking study are found to be in agreement with the observed *in vitro* PTP-1B selectivity. Thus discovery of novel benzotriazole based series suggests that this new class of compounds could be useful approach towards a safe and effective prevention of T2DM and need to be subjected to further pre-clinical evaluation.

3.2. Triaryl-sulfonamide based PTP-1B inhibitors (Second series)

3.2.1. Chemistry

Previously described the rationale for designing dual binding tri-aryl sulfonamide-based PTP 1B inhibitors containing newly, potent *p*Tyr mimetics, the title compounds represented by general structures **50a-g**, **52a-d** and **54a-h** (**Figure 30**). Synthetic methodology was designed following a retro-synthetic analysis and the synthetic schemes shown in the following discussion. Synthetic methods reported in literature were adopted for the synthesis of **48 [19]** which are the common intermediates for the synthesis of the title compounds **50a-g**, **52a-d** and **54a-h** respectively.

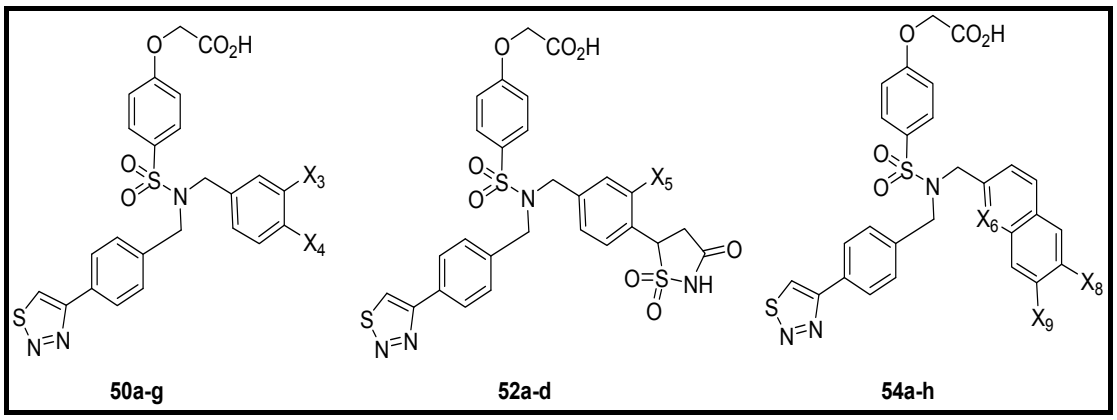
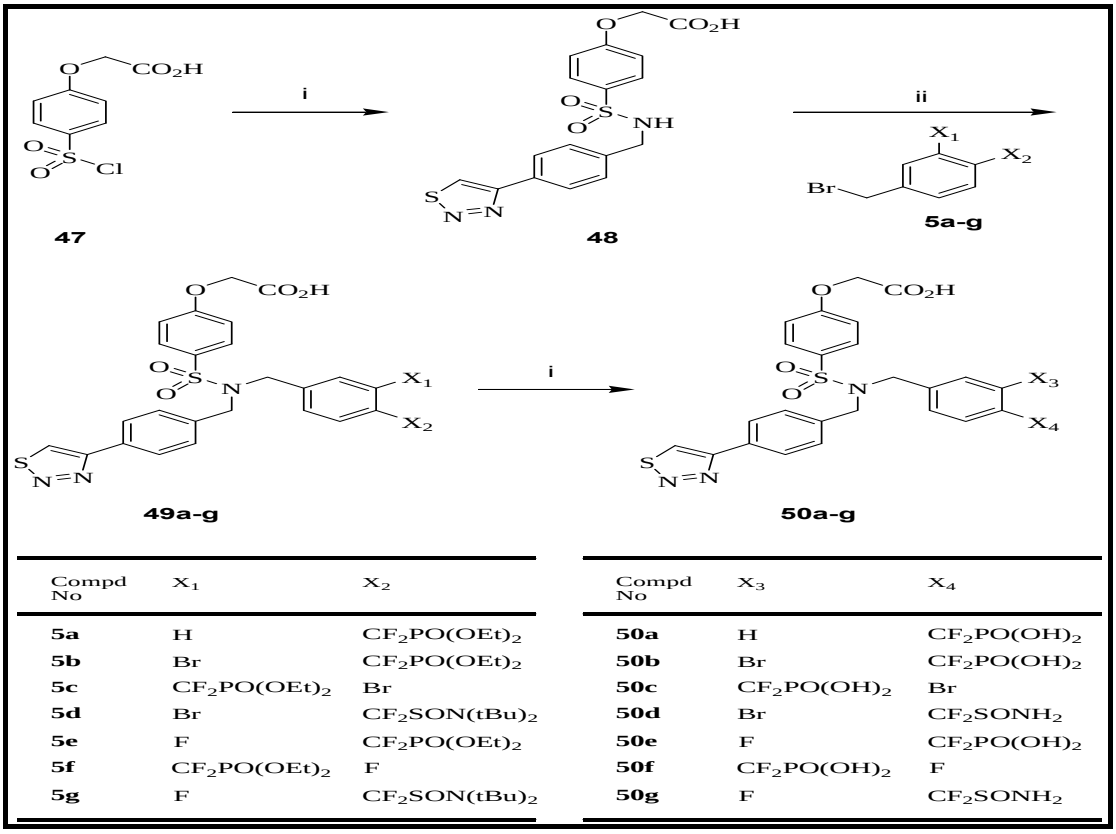


Figure 30. Tri-aryl sulfonamide-Based PTP-1B Inhibitors

Substituted phenyl, naphthalene and quinoline derivatives as a potent *p*Tyr mimic were also synthesized following the procedure reported earlier by choosing the appropriate starting materials and optimizing reaction conditions.

Synthesis of the designed compounds **50a-g**, **52a-d** and **54a-h** is illustrated in **Schemes 10-12 [19].** Treatment of the substituted benzene sulfonyl chloride **47** with thiadiazolyl-benzyl amine in presence of *N*, *N*-diisopropylethylamine (DIEA) gave mono–substituted sulfonamide **48**. Alkylation of **48** with **5a-g** or **8a-d** or **3a-b** or **11a-f** gave di-substituted protected sulfonamide **49a-g** or **51a-d** or **53a-h**, which were subsequently converted to **50a-g** or **52a-d** or **54a-h** by the treatment with trimethylsilyl bromide (TMSBr) or TFA to hydrolyze the phosphonate diethylesters and the t-butyl esters.

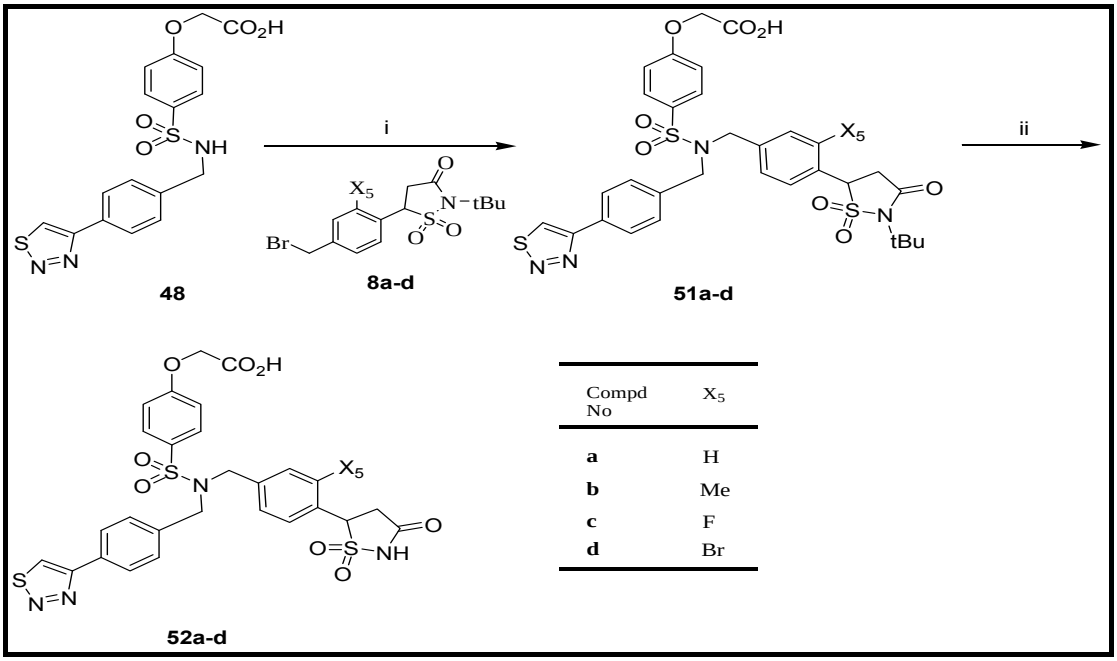


Compd No	X_1	X_2
5a	H	$CF_2PO(OEt)_2$
5b	Br	$CF_2PO(OEt)_2$
5c	$CF_2PO(OEt)_2$	Br
5d	Br	$CF_2SON(tBu)_2$
5e	F	$CF_2PO(OEt)_2$
5f	$CF_2PO(OEt)_2$	F
5g	F	$CF_2SON(tBu)_2$

Compd No	X_3	X_4
50a	H	$CF_2PO(OH)_2$
50b	Br	$CF_2PO(OH)_2$
50c	$CF_2PO(OH)_2$	Br
50d	Br	CF_2SONH_2
50e	F	$CF_2PO(OH)_2$
50f	$CF_2PO(OH)_2$	F
50g	F	CF_2SONH_2

Reagents and conditions: (i) thiadiazolyl-C_6H_4-CH_2NH_2, DIEA, CH_2Cl_2, 25 ^oC, 5 h; (ii) K_2CO_3, CH_3CN, 70 ^oC, 4 h; (iii) TMSBr, CH_2Cl_2, -15 ^oC, 3 h.

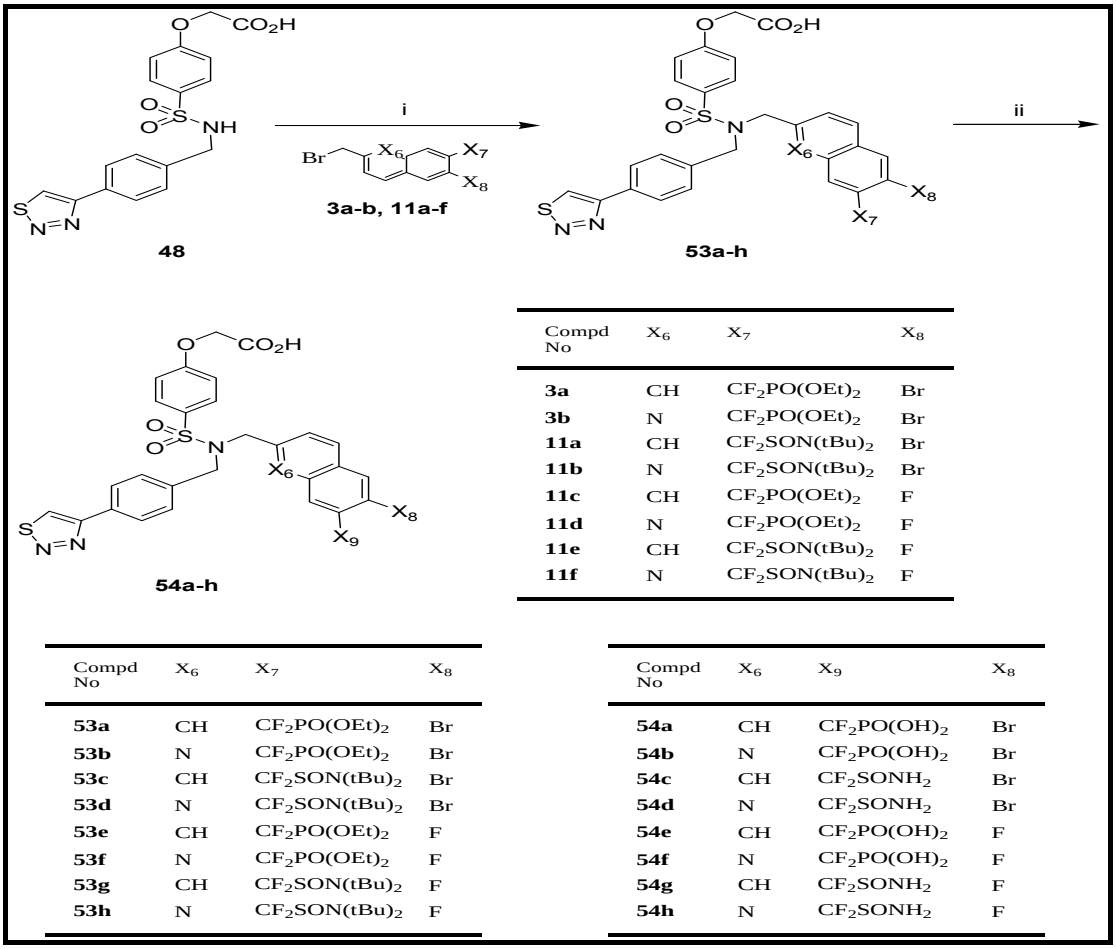
Scheme 10. Synthesis methodology for the preparation of the compounds (**50a-g**)



Compd No	X_5
a	H
b	Me
c	F
d	Br

Reagents and conditions: (i) K_2CO_3, CH_3CN, 70 ^oC, 4 h; (ii) TFA, CH_2Cl_2, 25 ^oC, 2 h.

Scheme 11. Synthesis methodology for the preparation of the compounds (**52a-d**)



Compd No	X_6	X_7	X_8
3a	CH	$CF_2PO(OEt)_2$	Br
3b	N	$CF_2PO(OEt)_2$	Br
11a	CH	$CF_2SON(tBu)_2$	Br
11b	N	$CF_2SON(tBu)_2$	Br
11c	CH	$CF_2PO(OEt)_2$	F
11d	N	$CF_2PO(OEt)_2$	F
11e	CH	$CF_2SON(tBu)_2$	F
11f	N	$CF_2SON(tBu)_2$	F

Compd No	X_6	X_7	X_8
53a	CH	$CF_2PO(OEt)_2$	Br
53b	N	$CF_2PO(OEt)_2$	Br
53c	CH	$CF_2SON(tBu)_2$	Br
53d	N	$CF_2SON(tBu)_2$	Br
53e	CH	$CF_2PO(OEt)_2$	F
53f	N	$CF_2PO(OEt)_2$	F
53g	CH	$CF_2SON(tBu)_2$	F
53h	N	$CF_2SON(tBu)_2$	F

Compd No	X_6	X_9	X_8
54a	CH	$CF_2PO(OH)_2$	Br
54b	N	$CF_2PO(OH)_2$	Br
54c	CH	CF_2SONH_2	Br
54d	N	CF_2SONH_2	Br
54e	CH	$CF_2PO(OH)_2$	F
54f	N	$CF_2PO(OH)_2$	F
54g	CH	CF_2SONH_2	F
54h	N	CF_2SONH_2	F

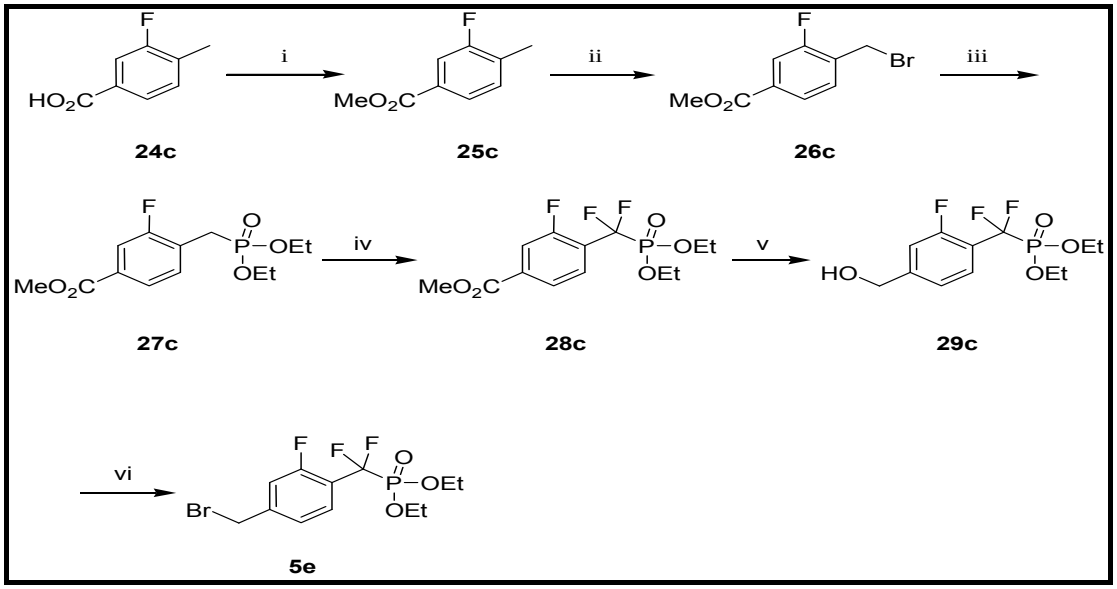
Reagents and conditions: (i) K_2CO_3, CH_3CN, 70 ^oC, 4 h; (ii) TMSBr, CH_2Cl_2, -15 ^oC, 3 h.

Scheme 12. Synthesis methodology for the preparation of the compounds (**54a-h**)

Synthesis of common electrophiles **5a-d**, **8a-c**, **3a-b** and **11a-b** are outlined in the previous section.

Synthesis of the newly designed electrophiles **5e-g**, **8d** and **11c-f** are outlined in **Scheme 13-18**. For **5e** & **5f** (**Scheme 13**-**14**), acid moieties **24c** or **30b** were converted to their methyl esters **25c** or **31b** by refluxing in CH_3OH in presence of $SOCl_2$. Bromination of methyl group **25c** or **31b** was carried out using NBS in the presence of a catalytic amount of benzoyl peroxide in CCl_4

resulting $-CH_2Br$ derivatives **26c** or **32b**, which were converted to their phosphonate derivatives **27c** or **33b** via the Michaekis-Arbuzov reaction using triethylphosphite **[7-11]**. Electrophilic fluorination of phosphonate **27c** or **33b** using KHMDS and NFSi gave fluorophosphonate **28c** or **34b [12-14]**. Reduction of methyl ester **28c** or **34b** using $NaBH_4$ gave alcohol **29c** or **35b** which was converted into bromide **5e** or **5f** using CBr_4/PPh_3.

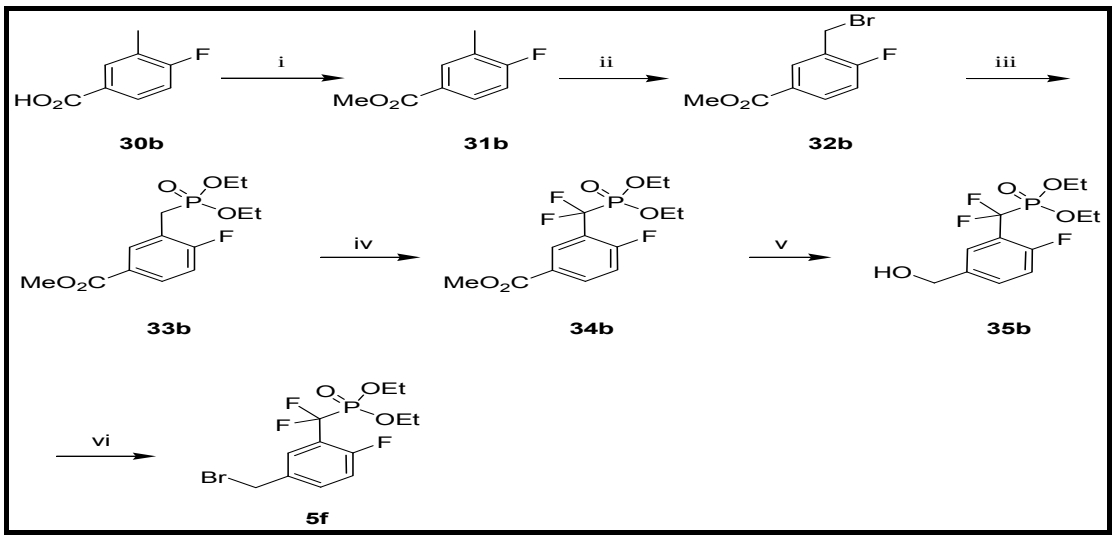


Reagents and conditions: (i) $SOCl_2$, CH_3OH, 70 ^oC, 3 h: (ii) NBS, benzoyl peroxide, CCl_4, 80 ^oC, 4 h; (iii) Triethylphoshite, reflux, 5 h; (iv) KHMDS, NFSi, THF, -78 $^oC \longrightarrow$ 25 ^oC, 18 h; (v) $NaBH_4$, CH_3OH, 60 ^oC, 3 h; (vi) CBr_4, PPh_3, CH_2Cl_2, 0 ^oC, 3 h.

Scheme 13. Synthesis methodology for the preparation of the compounds (**5e**)

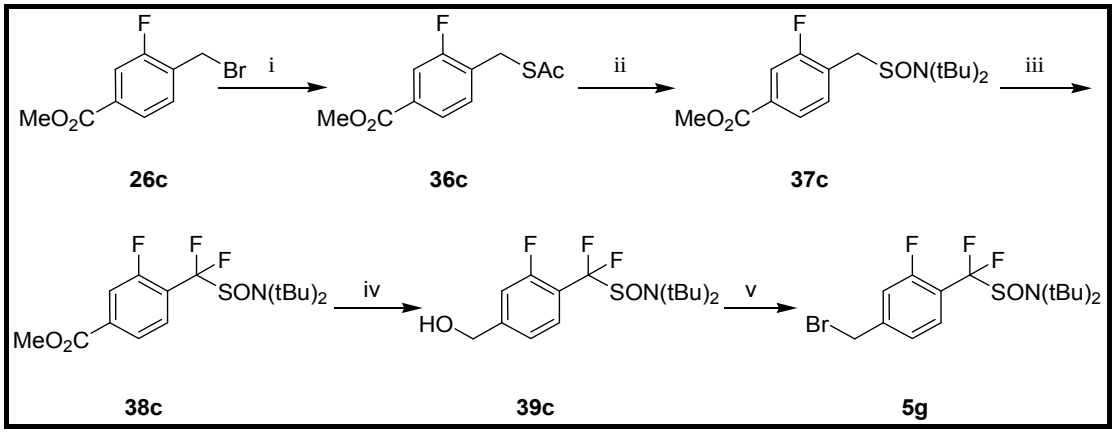
Synthesis of intermediate **5g** is illustrated as **Scheme 15**. Reaction of bromide **26c** with potassium thioacetate gave the thioester **36c**. Controled oxidative chlorination gave sulfonyl chloride, which was reacted with di-tertbutyl amine to give protected sulfonamide **37c**. Fluorines could be readily introduced by electrophilic fluorination of benzylic sulfonamide using KHMDS/NFSi gave desired difluoromethylenesulfinamide **38c [12-14]**.

Reduction of the methyl ester to benzyl alcohol **39c** was carried out using $NaBH_4$ and then was converted into the bromide **5g** using CBr_4/PPh_3.



Reagents and conditions: (i) $SOCl_2$, CH_3OH, 70 ^oC, 3 h: (ii) NBS, benzoyl peroxide, CCl_4, 80 ^oC, 4 h; (iii) Triethylphoshite, reflux, 5 h; (iv) KHMDS, NFSi, THF, -78 $^oC \longrightarrow$ 25 ^oC, 18 h; (v) $NaBH_4$, CH_3OH, 60 ^oC, 3 h; (vi) CBr_4, PPh_3, CH_2Cl_2, 0 ^oC, 3 h.

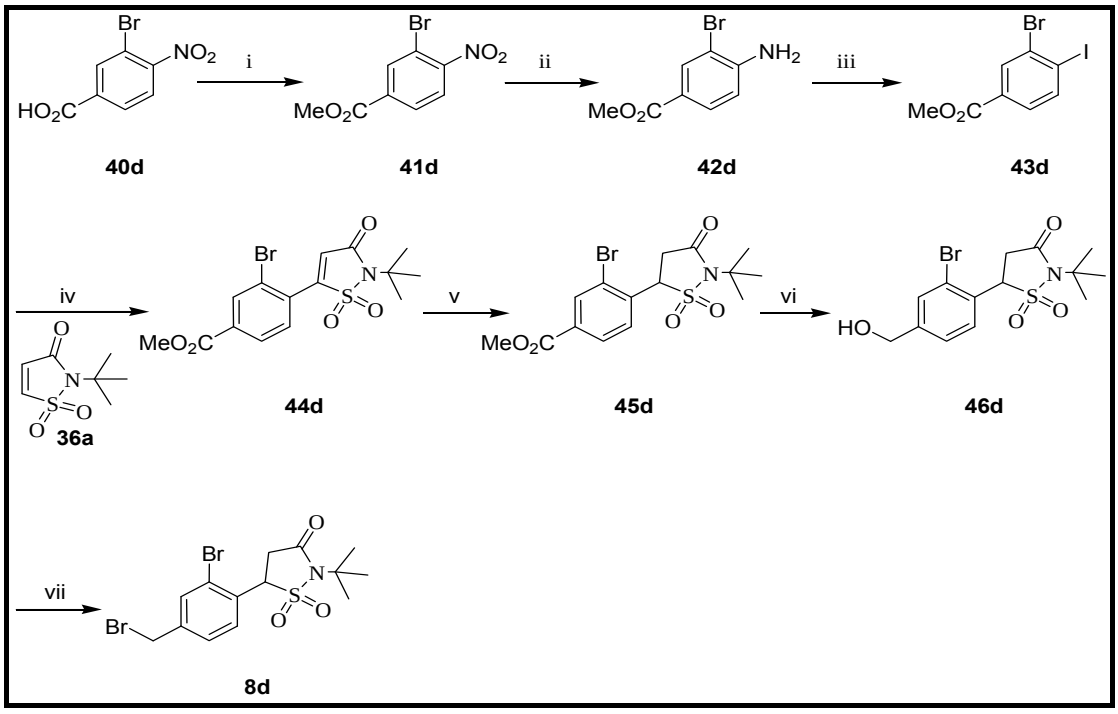
Scheme 14. Synthesis methodology for the preparation of the compounds (**5f**)



Reagents and conditions: (i) KSAc, DMF, 30 min, 25 ^oC; (ii) Cl_2, $HOAc/H_2O$, 0 ^oC, 1 h then CH_2Cl_2, ditert-butyl amine, 25 ^oC, 2 h; (iii) KHMDS, THF, NFSi, -78 $^oC \longrightarrow$ 25 ^oC, 18 h; (v) $NaBH_4$, CH_3OH, 65 ^oC, 2 h; (vi) CBr_4, PPh_3, CH_2Cl_2, 0 ^oC, 3 h.

Scheme 15. Synthesis methodology for the preparation of the compounds (**5g**)

Synthesis of the intermediate **8d** is illustrated as **Scheme 16**. Nitro derivative of substituted benzoic acid **40d** is converted to its methyl ester **41d** using CH_3OH and $SOCl_2$. The nitro group of **41d** was reduced to amino group using zinc and ammonium chloride in methanol **42d**, followed by diazotized using sodium nitrite under acidic conditions and the resulting intermediate was trapped with potassium iodide to afford **43d**. The iodo core was coupled with **36a** with classical ligandless conditions utilizing palladium acetate, tetra-butyl ammonium chloride, and triethyl amine in DMF resulted in **44d [6]**. Reduction of the IZD **44d** to the saturated IZD **45d** was carried out using 10% Pd/C in methanol under H_2 atmosphere. Methyl ester was reduced to the corresponding alcohol **46d** using $NaBH_4$ which then was converted into bromide **8d** using CBr_4/PPh_3.

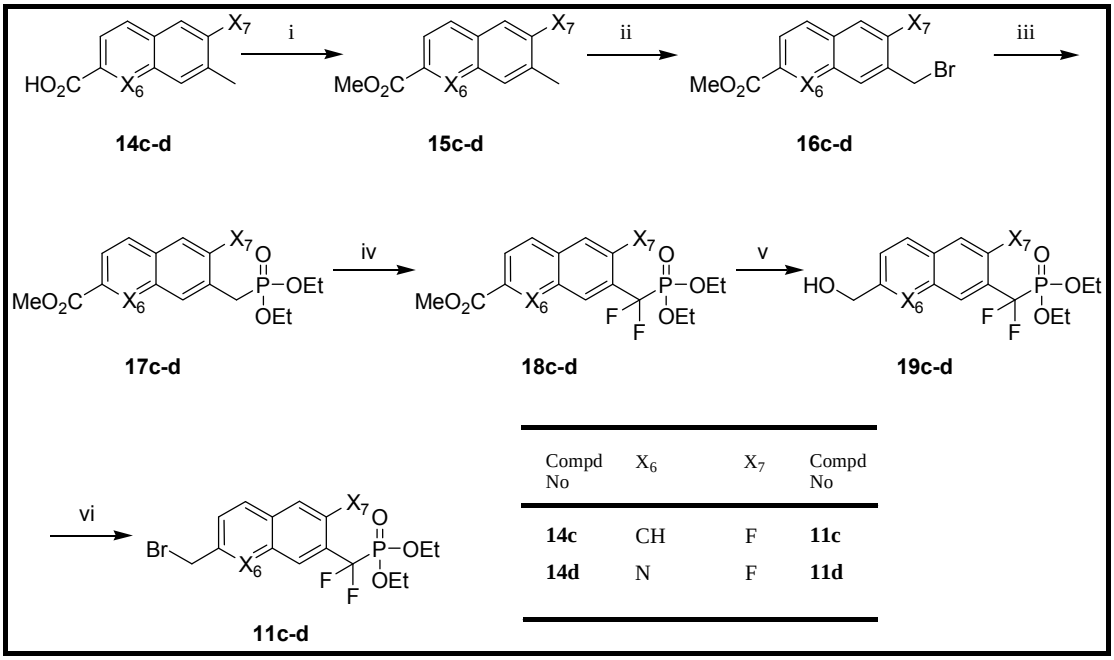


Reagents and conditions: (i) $SOCl_2$, CH_3OH, 70 ^oC, 3 h: (ii) Zn^0, NH_4Cl, CH_3OH, H_2O, 65 ^0C, 1 h; (iii) $NaNO_2$, 1 *N* aq. HCl, KI, 0 ^oC for 30 min then 45 ^oC for 45 min; (iv) $Pd(OAc)_2$,

Bu_4NCl, **36a**, Et_3N, DMF, 100 ^oC, 3 h; (v) 10% Pd/C, EtOH, 25 ^oC, 16 h; (vi) $NaBH_4$, CH_3OH, 65 ^oC, 3 h; (vii) CBr_4, PPh_3, CH_2Cl_2, 0 ^oC, 2 h.

Scheme 16. Synthesis methodology for the preparation of the compounds (**8d**)

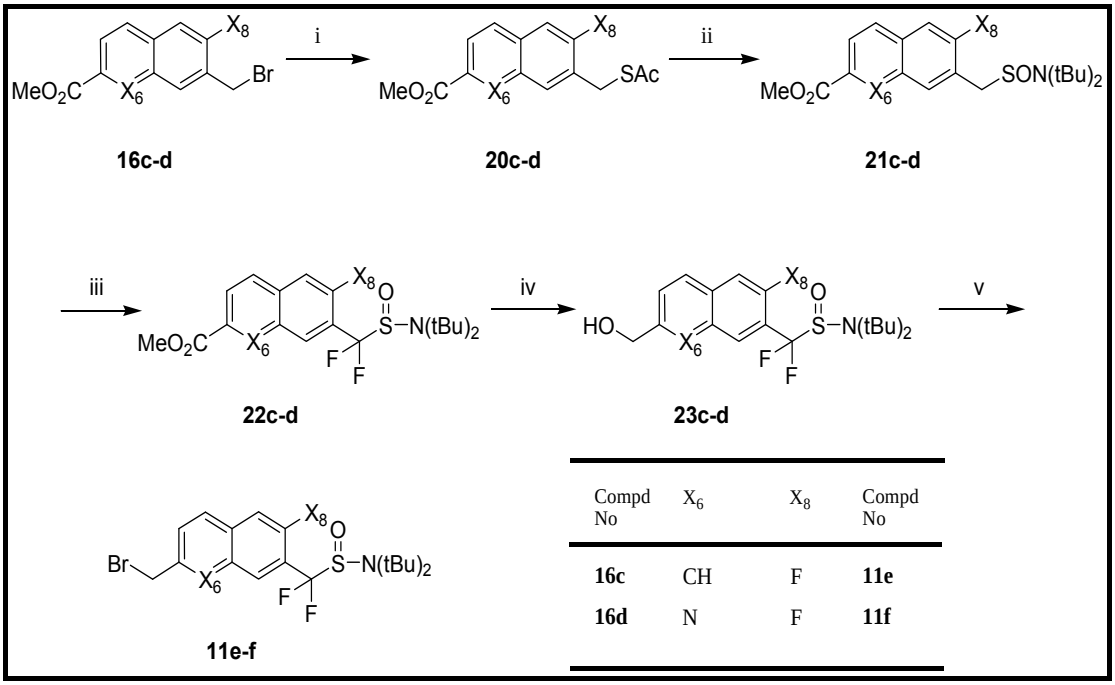
Synthetic methodology of the intermediates **11c-d** is outline in **Scheme 17.** Acid derivatives of substituted naphthalene/quinoline moiety **14c-d** were converted to their methyl ester **15c-d** by refluxing in CH_3OH in the presence of $SOCl_2$. Bromination of methyl group in **15c-d** was carried out using *N*-bromosuccinamide (NBS) in the presence of a catalytic amount of benzoyl peroxide in CCl_4 resulting $–CH_2Br$ derivatives **16c-d**, which were converted to their phosphonate derivatives **17c-d** via the Michaekis-Arbuzov reaction using triethylphosphite. Electrophilic fluorination of phosphonate **17c-d** using NaHMDS and NFSi gave fluorophosphonate **18c-d**. Reduction of methyl ester **18c-d** using $NaBH_4$ gave alcohol **19c-d** which were converted into the bromide **11c-d** using CBr_4/PPh_3.



Compd No	X_6	X_7	Compd No
14c	CH	F	**11c**
14d	N	F	**11d**

Reagents and conditions: (i) $SOCl_2$, CH_3OH, 70 ^oC, 3 h: (ii) NBS, benzoyl peroxide, CCl_4, 80 ^oC, 4 h; (iii) Triethylphoshite, reflux, 5 h; (iv) KHMDS, NFSi, THF, -78 ^oC ⟶ 25 ^oC, 18 h; (v) $NaBH_4$, CH_3OH, 60 ^oC, 3 h; (vi) CBr_4, PPh_3, CH_2Cl_2, 0 ^oC, 3 h.

Scheme 17. Synthesis methodology for the preparation of the compounds (**11c**-**d**)



Compd No	X_6	X_8	Compd No
16c	CH	F	**11e**
16d	N	F	**11f**

Reagents and conditions: (i) KSAc, DMF, 30 min, 25 ^oC; (ii) Cl_2, $HOAc/H_2O$, 0 ^oC, 1 h then CH_2Cl_2, ditert-butyl amine, 25 ^oC, 2 h; (iii) KHMDS, THF, NFSi, -78 ^oC ⟶ 25 ^oC, 18 h; (v) $NaBH_4$, CH_3OH, 65 ^oC, 2 h; (vi) CBr_4, PPh_3, CH_2Cl_2, 0 ^oC, 3 h.

Scheme 18. Synthesis methodology for the preparation of the compounds (**11e**-**f**)

Synthesis of intermediate **11e-f** is illustrated as **Scheme 18**. Reaction of bromide **16c-d** with potassium thioacetate gave thioester which on controled oxidative chlorination gave sulfonyl chlorides, which were reacted with di-tertbutyl amine to give protected sulfonamide. Fluorines were readily introduced by electrophilic fluorination of benzylic sulfonamide using KHMDS/NFSi gave desired difluoromethylenesulfinamide. The methyl esters

then were reduced to alcohol using $NaBH_4$ which then, converted into bromides **11e-f** using CBr_4/PPh_3.

Using above synthetic routes (**Scheme 10**-**12**), all together nineteen new compounds (**50a-g**, **52a-d** and **54a-h**) were prepared. All the final compounds, intermediates were purified, characterized and the spectral data of compounds were found to be in confirmity with the structures assigned. Detailed experimental procedures are described in **experimental Section 5.1** and the representative spectra's of selected compounds are given in **spectral data section 6**.

3.2.2. *In vitro* PTP-1B inhibitory activity, selectivity and structure activity relationship (SAR)

All the compounds **50a-g**, **52a-d** and **54a-h** were screened using *p*NPP enzymatic assay for PTP 1B inhibitory activity (detailed experimental protocol is given in **experimental Section 5.3**) **[15]**, mainly to establish the SAR of the new series of triaryl-sulfonamide based PTP-1B inhibitors. As deplicated in **Table 9**, depending upon the nature of substitution, the compounds showed varying degree of PTP-1B inhibition (IC_{50}).

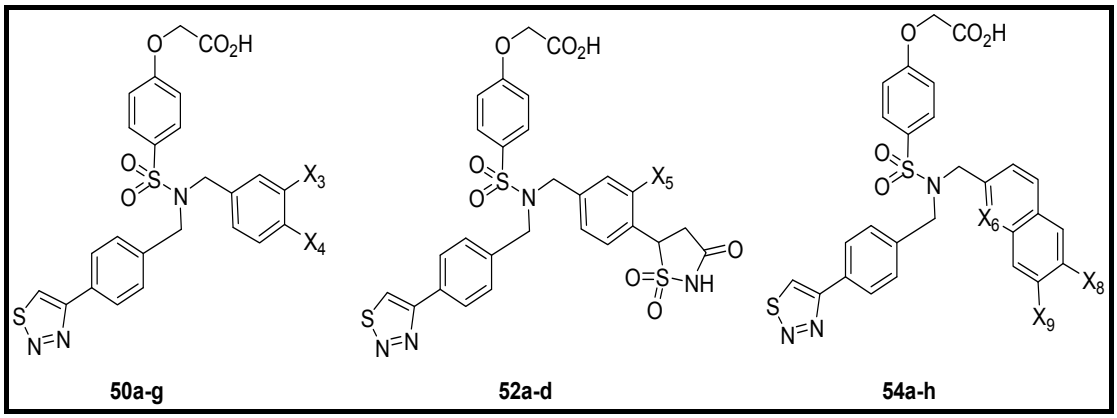
The adopted designing strategy for potent & selective PTP-1B inhibitors comprises of a basic pharmacophore which is attached to a potent *p*Tyr mimetic. In this series, sulfonamide scaffold was specifically selected to design dual binding PTP-1B inhibitors to obtain superior selectivity over other PTPases. The title compounds were mainly divided in two series, either by substituting DFMP/DFMS/IZD-benzyl group **50a-g**, **52a-d** or with DFMP/DFMS-substituted naphthyl/quinolinyl templates **54a-h**. Within the first series **50a-g** & **52a-d**, two sets of compounds were prepared by substituting

DFMP/DFMS benzyl group **50a-g** or IZD-substituted benzyl groups **52a-d**, while in the second series **54a-h**, two distinct sets of compounds were prepared containing DFMP/DFMS-substituted naphthyl/quinolinyl templets **54a-d** or their fluoro analogues **54e-h**.

Within the first series, the first set of compounds **50a-g** containing DFMP-substituted benzyl group showed different degree of PTP-1B inhibitory activity depending on the DFMP group position on the benzyl ring and the ortho substituents. Compound **50c** with *m*-DFMP group showed a weak inhibitory activity compare to **50a** with *p*-DFMP. Whereas compound **50b**, containing *p*-DFMP and *o*-bromo substitution to DFMP, showed a good inhibitory activity. Replacement of *o*-bromo analogues **50b-c** with more electronegative *o*-fluoro derivatives **50e-f**, showed improved PTP 1B inhibitory activity.

Bioisosteric replacement of the DFMP with DFMS **50d** and **50g**, showed comparable *in vitro* inhibitory activity, suggesting that the highly negatively charged DFMP group can be replaced with DFMS group to overcome the issue of low permeability. Among the **50d** and **50g**, compound **50g** showed better inhibitory activity, which could be due to increase in the electronegativity and decrease in the steric bulk (**50d**: ortho-bromo vs **50g**:ortho-fluoro) adjacent to the DFMS group. The second set of compounds containing IZD-benzyl ring **52a-d**, showed a weak inhibitory activity, irrespective of their ortho substituents. Among the series, ortho-fluoro compound **52d** was found to be the most potent, whereas the ortho-methyl **52b** and ortho-bromo **52c** derivatives were found to be equipotent. Un-substituted derivative **52a** was found to be the least active.

Table 9. The *In vitro* PTP-1B inhibitory activity of test compounds **50a-g**, **52a-d** & **54a-h**.



Compd No	X_3	X_4	X_5	X_6	X_8	X_9	PTP-1B[a] (IC_{50} nM)
50a	H	$CF_2PO(OH)_2$	-----	-----	-----		78 ± 02
50b	Br	$CF_2PO(OH)_2$	-----	-----	-----		50 ± 03
50c	$CF_2PO(OH)_2$	Br	-----	-----	-----		288 ± 08
50d	Br	CF_2SONH_2	-----	-----	-----		47 ± 04
50e	F	$CF_2PO(OH)_2$	-----	-----	-----		35 ± 04
50f	$CF_2PO(OH)_2$	F	-----	-----	-----		141 ± 10
50g	F	CF_2SONH_2	-----	-----	-----		31 ± 02
52a	-----	-----	H	-----	-----		500 ± 10
52b	-----	-----	Me	-----	-----		470 ± 11

52c	-----	-----	F	-----	-----		460 ± 12
52d	-----	-----	Br	-----	-----		480 ± 11
54a	-----	-----	-----	CH	Br	$CF_2PO(OH)_2$	17 ± 01
54b	-----	-----	-----	N	Br	$CF_2PO(OH)_2$	18 ± 03
54c	-----	-----	-----	CH	Br	CF_2SONH_2	39 ± 03
54d	-----	-----	-----	N	Br	CF_2SONH_2	41 ± 05
54e	-----	-----	-----	CH	F	$CF_2PO(OH)_2$	9 ± 01
54f	-----	-----	-----	N	F	$CF_2PO(OH)_2$	11 ± 04
54g	-----	-----	-----	CH	F	CF_2SONH_2	35 ± 07
54h	-----	-----	-----	N	F	CF_2SONH_2	38 ± 08

[a] Enzymatic assay was carried out in 96-well plates. To an assay buffers, *p*NPP and test compounds were added and the reaction was initiated by addition of PTP-1B/TC-PTP (10 -100 nM). The initial rate of PTPase-catalyzed hydrolysis of *p*NPP was measured at 405 nm. IC_{50} value was determined under fixed *p*NPP concentration of 1 mM (n=3).

The second series of compounds with DFMP/DFMS-substituted naphthyl/quinolinyl compounds **54a-h** showed potent inhibitory activity. Within the the second series, the first set of compounds containing DFMP-substituted naphthyl/quinolinyl moiety **54a-b** showed better PTP-1B inhibitory activity compared to its bioisostere DFMS-substituted templates **54c-d**.The reason could be that at pH 6.5 (*in vitro* experimental system) monoanionic

DFMP exhibit an equilibrium with its dianionic form and the PTP-1B enzyme show strong binding interaction with the dianionic form **[20-21]**.

In second set, replacement of bromo analogues **54a-d** with more electronegative fluoro analogues **54e-h**, found to be more potent compare to its bromo analogues.

In conclusion, *para*-substituted *p*Tyr mimetics exhibit favorable PTP-1B inhibitory activity. The compound containing ortho-halogen substitution next to *p*Tyr mimetics showed higher inhibitory activity. Among the three different *p*Tyr mimetics, DFMP exhibits the highest potency. Fluoro analogues of naphthyl/quinolinyl rings containing DFMP as a *p*Tyr mimic found to be the most potent compounds among the both series.

From the above SAR study, out of nineteen compounds, short listed compounds (**50a-b**, **50e**, **50g and 54e-f)** showed satisfactory *in vitro* PTP-1B inhibitory activity within this series. Compared to parent compound **V**, these compounds showed potent PTP-1B inhibitory activity and were subjected to evaluate their *in vitro* selectivity over other PTPs (LAR, CD45, SHP-1, SHP-2 and TC-PTP) by using the *p*NPP assay, and IC_{50} values are described in **Table 10** (detailed experimental protocol is given in **experimental Section 5.3**).

As deplicated in **Table 10**, compound **50a** containing DFMP-substituted benzyl group showed ~7-fold selectivity, whereas **50b** and **50e,** i.e. ortho-bromo or ortho-fluoro analogues of DFMP-substituted benzyl templates showed ~15 to 25-fold selectivity over TC-PTP, while compound **50g**, the ortho-fluoro analogue of DFMS-substituted benzyl templates showed ~20-fold selectivity. The most potent compounds **54e** and **54f,** containing ortho-fluoro

analogues of DFMP-substituted naphthyl/quinolinyl templates showed ~90 to 96-fold selectivity over TC-PTP. While all the selected compounds showed >5000-fold selectivity over other PTPs.

Table 10. Subtypes-selectivity data selected compounds (**50a-b**, **50e**, **50g and 54e-f**)

Compound	IC_{50} nM		Fold Selectivity[b]
	PTP-1B[a]	TC-PTP[a]	
50a	78 ± 02	546 ± 12	~ 7 fold
50b	50 ± 03	750 ± 13	~ 15 fold
50e	35 ± 04	870 ± 07	~ 24 fold
50g	31 ± 02	620 ± 08	~ 20 fold
54e	9 ± 01	870 ± 05	~ 96 fold
54f	11 ± 04	990 ± 08	~ 90 fold

[a] Enzymatic assay was carried out in 96-well plates. The initial rate of PTPase-catalyzed hydrolysis of *p*NPP was measured at 405 nm. IC_{50} value was determined under fixed *p*NPP concentration of 1 mM (n=3); Data represent the mean ± SD; **50a-b**, **50e**, **50g** and **54e-f** showed > 5000-fold selectivity over PTPα, CD45, LAR, SHP-1 and SHP-2 enzymes.

[b] Fold selectivity calculated as ratio of IC_{50} values of TC-PTP/PTP-1B inhibitions

In conclusion, based upon *in vitro* PTP-1B inhibitory activity and *in vitro* sub-type selectivity results, compounds with DFMP/DFMS-substituted benzyl groups showed poor selectivity. While compounds with DFMP-substituted naphthyl/quinolinyl templates showed excellent inhibitory activity and selectivity over TC-PTP, indicating that among the two different ring systems (benzyl/fused-ring system), only fused-ring system showed excellent

selectivity. Based on PTP-1B inhibitory activity and sub-type selectivity results, short listed compounds (**54e** and **54f**) were further subjected to *in vivo* (antidiabetic activity) and PK evaluations.

3.2.3. *In vivo* antidiabetic activity of the selected compounds (**54e** and **54f**)

Based on *in vitro* potency and selectivity, short listed compounds (**54e** and **54f**; @ 20 mpk, po) were tested for *in vivo* antidiabetic activity in male C57BL/6j mice using the intraperitoneal glucose tolerance test (IPGTT) protocol (detailed experimental protocol is given in **experimental Section 5.3**) **[16-17]** and serum glucose levels (AUC glucose @ 240 min; mg dL^{-1}). As shown in **Figure 31**, compound **54e** showed excellent antidiabetic activity upon oral administration, whereas **54f** showed moderate activity (-52.8 ± 5.36 and -19.18 ± 5.8, respectively).

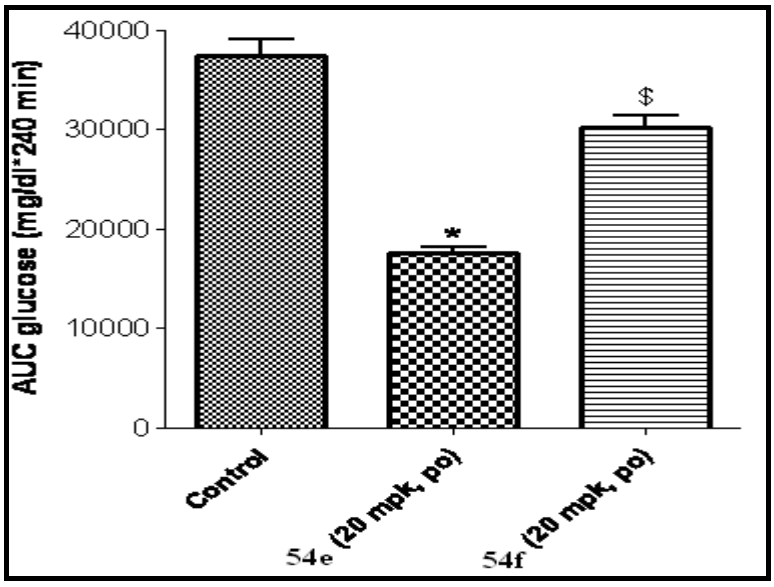


Figure 31. *In vivo* antidiabetic activity of compounds 54e and 54f in C57 mice (IPGTT). *P<0.05, $P<0.01, Two-Way ANOVA followed by Bonferroni post test, M ± SEM.

Based on the designing concept, DFMP-substituted naphthalene ring **54e** was replaced with DFMP-substituted quinoline ring **54f** to overcome the cell permeability issue. However, these comparative oral antidiabetic activity results also support our hypothesis that the DFMP-naphthalene ring is more favorable for oral antidiabetic activity than the quinoline ring.

3.2.4. Pharmacokinetic (PK) studies of the selected compounds (**54e** and **54f**)

Based on the *in vitro* PTP-1B inhibitory activity, selectivity and *in vivo* antidiabetic activity, compound **54e** and **54f** were selected as the lead compounds to study their pharmacokinetic profile in male C57BL/6J mice (n = 6) (detailed experimental protocol is given in **experimental Section 5.3**). In order to understand the PK profile of test compounds, a comparative single dose (20 mpk; iv or po) PK study of our most potent compounds (**54e** & **54f**) was carried out and the various PK parameters are summarized in **Table 11**.

Table 11. Pharmacokinetic (PK) study parameters of compounds **54e** and **54f** in male C57BL/6J mice

PK parameters[a]	54e	54f
t_{max} (h)	0.31 ± 0.01	0.69 ± 0.10
C_{max} (µg/ml)	6.3 ± 0.51	0.96 ± 0.32
$T_{1/2}$ (h)	6.96 ± 0.91	0.99 ± 0.76
AUC (h □g/ml)	8.91 ± 0.14	1.32 ± 0.32
F[%][b]	6.51%	0.9 %

[a]Single dose (20 $mgkg^{-1}$; i.v./p.o.) PK study for compound **54e** and **54f** was carried out in fasted male C57BL/6j mice (n=6) and plasma concentration of compounds were determined by LC-MS, data represented as mean ± SD.
[b]Oral bioavailability (%F) was calculated wrt to iv AUC (**54e**: 137.07 ± 4.28 and **54f**: 146.66 ± 9.81 h µg/ml administered at 20 mpkg dose iv.

Compound **54e** exhibits rapid t_{max}, extended half-life ($t_{1/2}$) and good area under the curve (AUC), clearance at a dose of 20 $mgkg^{-1}$. While compound **54f** showed extended t_{max}, short half-life ($t_{1/2}$) and moderate area under the curve (AUC). Based on PK result, compound **54e** showed roughly seven-fold higher bioavailability (F~6.5%) compared to **54f**. These improved PK profile of compound **54e** supports its excellent pharmacodynamic effects (antidiabetic activity) in C57 mice, when administered orally.

3.2.5. Molecular docking Study

To understand the selectivity profile of **54e** and **50a** (compound **V**) at molecular level, a molecular docking analysis was carried using Glide docking software (version 5.6) **[18]**. As shown in **Figure 32**, upon IFD, compound **50a** completely docks in the binding site A and the DFMP substituted phenyl ring strongly coordinates with Phe682 (site A residue), while the oxy acetic acid and thiadiazolyl ring partially oriented in site B and dock partly with key residues of site B and hence poor (~7-fold) *in vitro*, selectivity over TC-PTP was observed (**Figure 32a**). While replacement of DFMP-substituted benzyl ring with DFMP-substituted naphthyl ring as in **54e** allows the molecule to adopt a new conformation, as a result it docks very well at the both binding sites (**Figure 32b**). It was observed upon IFD, flipping of thiadiazolyl ring was observed in site B and as a result of this, **54e** docks very well in site A and B. Thus, PTP 1B selectivity over TC-PTP was achieved by taking advantage of amino acid differences in the site B. In particular, flipping of thiadiazolyl ring in site B and its strong interaction with key residues of site B (Asp548, Arg524 and Arg754). Thus the favourable hydrogen bond interaction of **54e** with site

A and B of PTP-1B support *in vitro* PTP-1B activity and its excellent selectivity over TC-PTP.

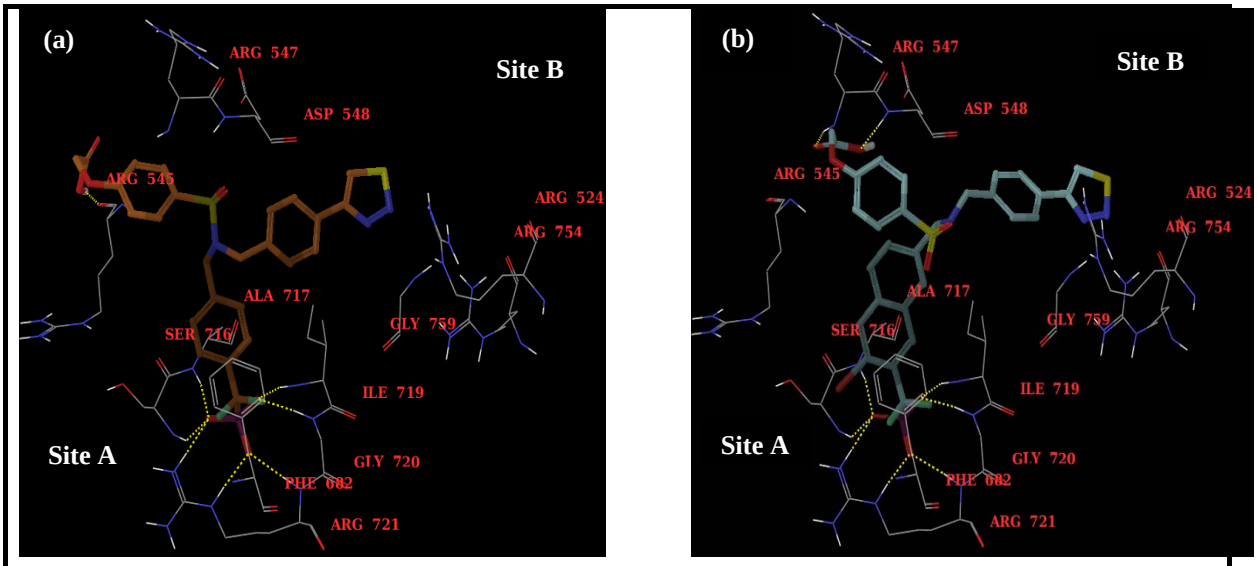


Figure 32. (a) Binding pose of **V** in the PTP-1B active site; site A and B are indicated, wherein it interact closely with key residues of site A and marginally with site B **(b)** compound **54e** docks very well into both site A & B. Residues numbering is as per current numbering (PDB code: 1QT6): Arg24 (524), Phe52 (552), Arg254 (754), and Met258 (758).

3.2.6. Conclusion

Continuating the concept of dual binding-sites PTP-1B inhibitors, it was envisioned to design novel dual binding-site PTP-1B inhibitors by incorporating potent *p*Tyr mimetic in a single scaffold. Based on this concept, novel tri-aryl sulfonamide-based PTP-1B inhibitors were designed and tested for their *in vitro* PTP-1B inhibitory activity, selectivity over TC-PTP and *in vivo* antidiabetic activity in relevant animal models. Novel sulfonamide-based compounds exhibit favourable orientation across both the binding sites of the PTP-1B, thus establishing the evidence for our hypothesis of incorporating the dual binding features in a single chemotype in order to develop novel PTP-1B inhibitors. The lead compound **54e** is one such dual binding-site PTP-1B

inhibitors, indicating that only fluoro analogue of naphthyl derivatives showed selectivity owing to favourable orientation of the molecule across both the binding sites of PTP 1B enzyme. Compound **54e** exhibited excellent anti-hyperglycemic effects in animal models, along with improved oral bioavailability. The results of our in silico docking study are also in agreement with the observed in vitro PTP-1B selectivity. Thus preliminary PK/PD studies results reveal that further modification on **54e** may lead to the identification of a potent compound from this series, with improved PK/PD profile for an efficient treatment of T2DM.

3.3. Peptidomimetics based PTP-1B inhibitors (Third series)

3.3.1. Chemistry

As previously described the rationale for designing dual binding tripeptide-based PTP-1B inhibitors containing newly, potent *p*Tyr mimetics, the title compounds represented by general structures **61a-i**, **64a-d** and **67a-j** (**Figure 33**). Synthetic methodology was designed based on the retro-synthetic analysis and the synthesis schemes shown in the following discussion. Synthetic methods reported in literature were adopted for synthesis of **56a-b** which were the common intermediates for the synthesis of the compounds **61a-i**, **64a-d** and **67a-j.** Substituted acetic acid derivatives as a potent *p*Tyr mimic were synthesized following the procedure reported earlier by choosing the appropriate starting materials and by optimizing reaction conditions.

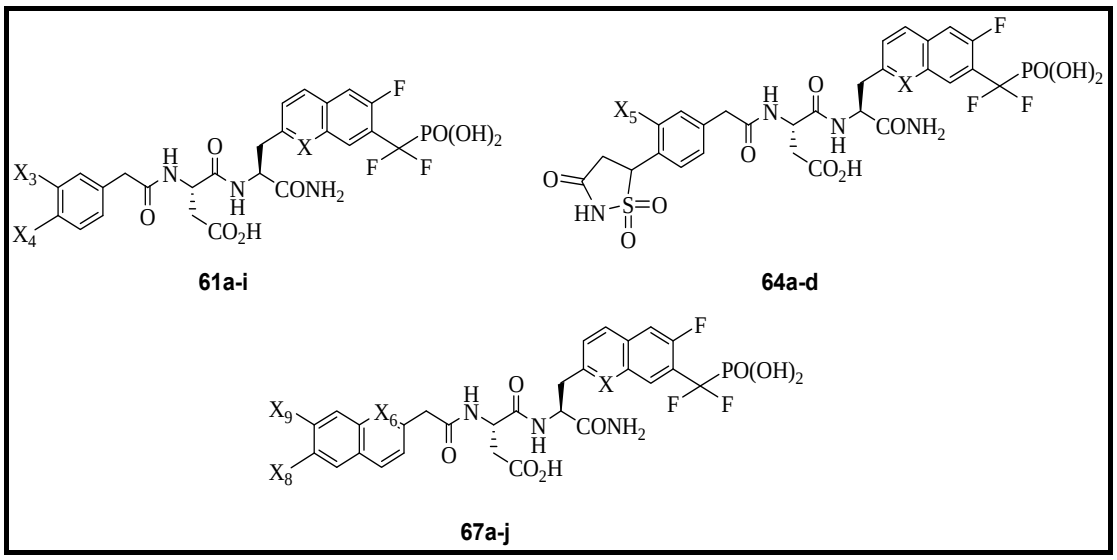
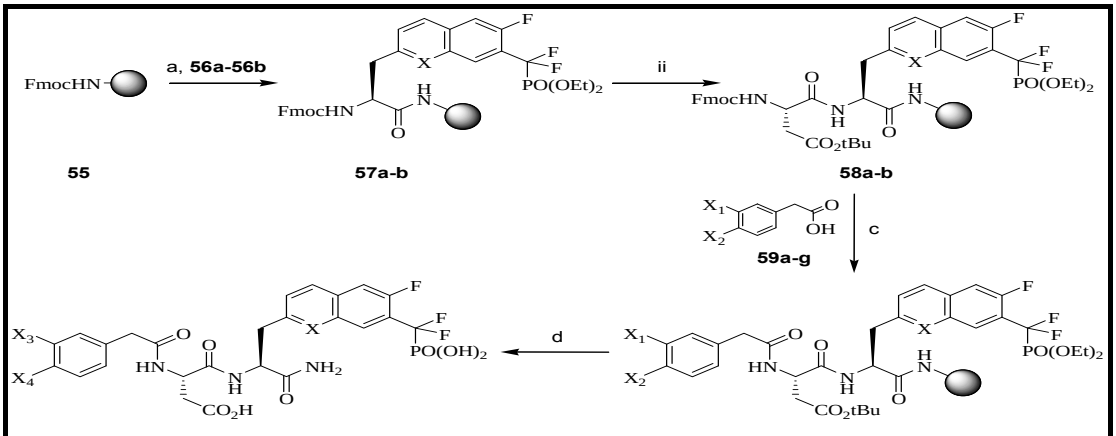


Figure 33. Peptidomimetic-Based PTP-1B Inhibitors

Synthesis of the designed compounds **61a-i**, **64a-d** and **67a-j** is illustrated in **Schemes 19-21.** Novel peptidomimetics were synthesized using Fmoc-based Solid Phase Peptide Synthesis (SPPS) approach **[22]**, starting from commercially available Fmoc Rink Amide MBHA resin **55**. Deprotection of **55** with piperidine (20% in DMF) and 1,3-diisopropylcarbonamide (DIC) coupling with Fmoc-protected difluorophosphonate-substituted naphthyl/ quinolinyl based unnatural amino acid **56a-b** gives the resin-bouned Fmoc-protected amino acid **57a-b**. Deprotection of **57a-b** with 20% piperidine in DMF and DIC coupling with Fmoc-Asp(O^{t}Bu)-OH gives Fmoc-protected resin bound dipeptides **58a-b**. Deprotection of **58a-b** with 20% piperidine in DMF and DIC coupling with substituted phenyl /naphthyl/quinolinyl acetic acids (**59a-g** or **62a-d** or **65a-h**) gives fully-protected resin bounded tripeptides **60a-i** or **63a-d** or **66a-j**. Final cleavage from resin and side chain deprotection of was achieved by treatment with 1M TMSBr in trifluoroacetic acid (TFA) with

5% 1,2-ethanedithiol (EDT) and 1% *m*-cresol at 0 ^{o}C for 5h and then at room temperature for 16h afforded the compounds **61a-i**, **64a-d** and **67a-j**.



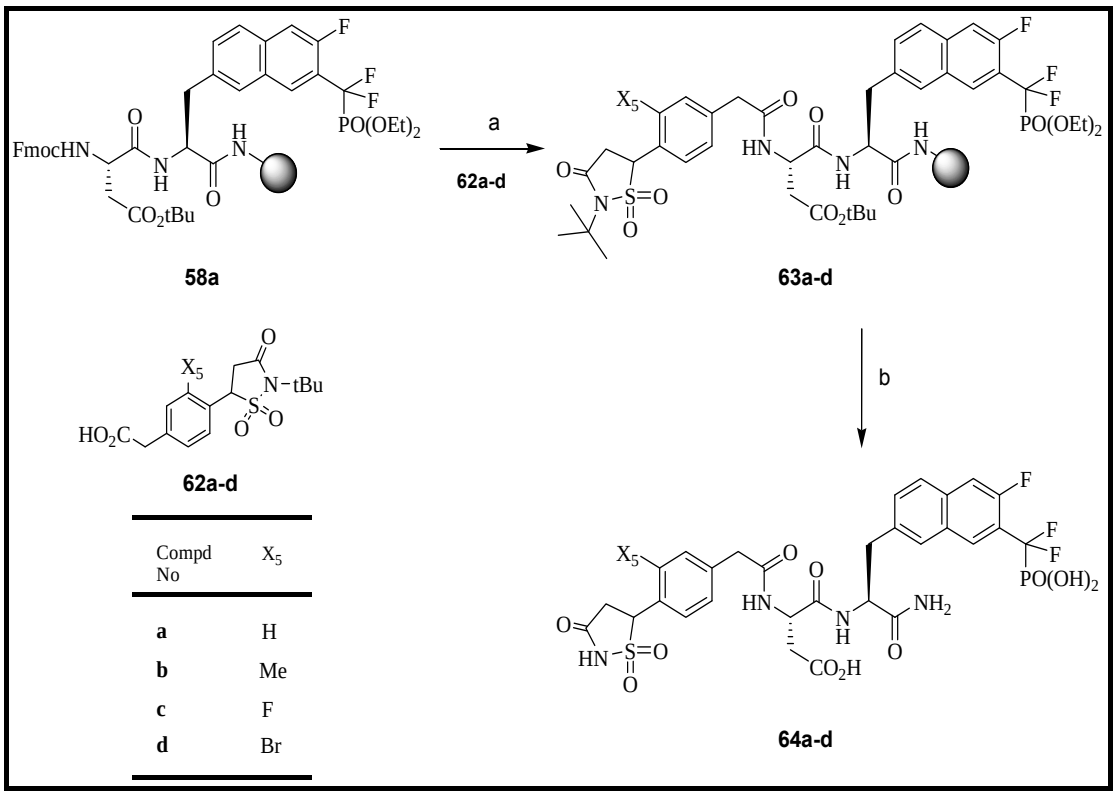
Compd No	X_1	X_2
59a	H	$CF_2PO(OEt)_2$
59b	Br	$CF_2PO(OEt)_2$
59c	F	$CF_2PO(OEt)_2$
59d	$CF_2PO(OEt)_2$	Br
59e	$CF_2PO(OEt)_2$	F
59f	Br	$CF_2SO_2N(tBu)_2$
59g	F	$CF_2SO_2N(tBu)_2$

Compd No	X	X_1	X_2
60a	CH	H	$CF_2PO(OEt)_2$
60b	CH	Br	$CF_2PO(OEt)_2$
60c	CH	F	$CF_2PO(OEt)_2$
60d	CH	$CF_2PO(OEt)_2$	Br
60e	CH	$CF_2PO(OEt)_2$	F
60f	CH	Br	$CF_2SO_2N(tBu)_2$
60g	CH	F	$CF_2SO_2N(tBu)_2$
60h	N	Br	$CF_2PO(OEt)_2$
60i	N	F	$CF_2PO(OEt)_2$

Compd No	X	X_3	X_4
61a	CH	H	$CF_2PO(OH)_2$
61b	CH	Br	$CF_2PO(OH)_2$
61c	CH	F	$CF_2PO(OH)_2$
61d	CH	$CF_2PO(OH)_2$	Br
61e	CH	$CF_2PO(OH)_2$	F
61f	CH	Br	$CF_2SO_2NH_2$
61g	CH	F	$CF_2SO_2NH_2$
61h	N	Br	$CF_2PO(OH)_2$
61i	N	F	$CF_2PO(OH)_2$

Reagents and conditions: (a) **i.** 20% Piperidine in DMF; **ii. 56a-56b**, HOBt, DIC, DMF, N_2; (b) **i.** 20% Piperidine in DMF; **ii.** Fmoc-Asp(O^{t}Bu)-OH, HOBt, DIC, DMF, N_2; (c) **i.** 20% Piperidine in DMF; **ii. 59a-59g**, HOBt, DIC, DMF, N_2; (d) 1M TSMBr in TFA: 1,2-ethanedithiol: m-cresol (94:5:1), 0 ^{o}C for 5h and then at RT for 16 h.

Scheme 19. Synthesis methodology for the preparation of the compounds (**61a**-**i**)

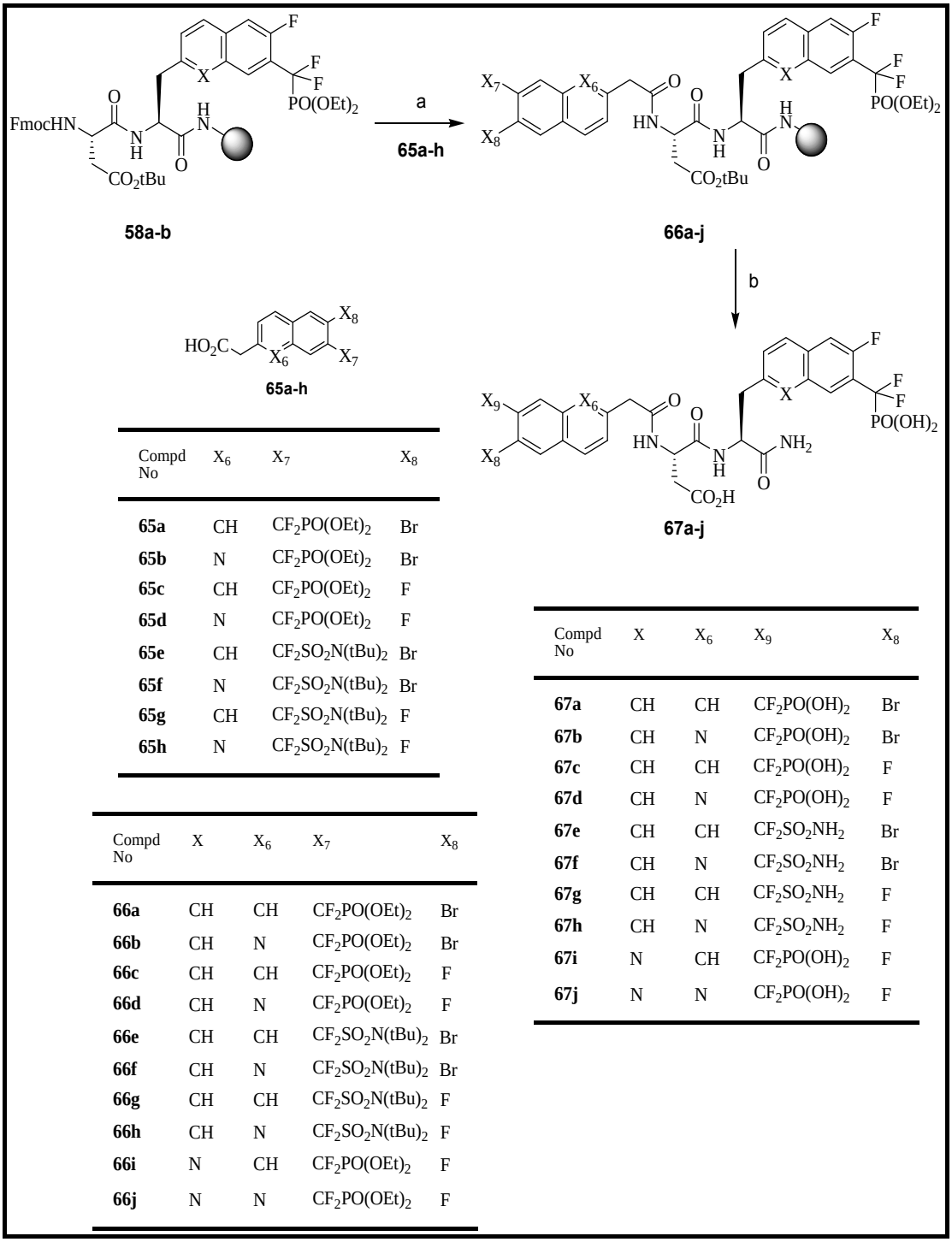


Reagents and conditions: (a) **i.** 20% Piperidine in DMF; **ii. 62a-62d**, HOBt, DIC, DMF, N_2; (d) 1M TSMBr in TFA: 1,2-ethanedithiol: m-cresol (94:5:1), 0 ^{o}C for 5h and then at RT for 16 h.

Scheme 20. Synthesis methodology for the preparation of the compounds (**64a**-**d**)

Crude peptides were purified using semi-preparative HPLC on a Shimadzu model LC-8A liquid chromatography. Desired fractions were pooled together, frozen and lypholized to give the final compounds.

For the preparation of peptidomimetic **61a-i**, **64a-d** and **67a-j** (**Scheme 19**-**21**), we need to first synthesis of chirally pure potent *p*Tyr mimic-unnatural amino acid: Fmoc-protected difluorophosphonate-substituted naphthyl/quinolinyl based amino acids **56a**-**b**.



Compd No	X_6	X_7	X_8
65a	CH	$CF_2PO(OEt)_2$	Br
65b	N	$CF_2PO(OEt)_2$	Br
65c	CH	$CF_2PO(OEt)_2$	F
65d	N	$CF_2PO(OEt)_2$	F
65e	CH	$CF_2SO_2N(tBu)_2$	Br
65f	N	$CF_2SO_2N(tBu)_2$	Br
65g	CH	$CF_2SO_2N(tBu)_2$	F
65h	N	$CF_2SO_2N(tBu)_2$	F

Compd No	X	X_6	X_7	X_8
66a	CH	CH	$CF_2PO(OEt)_2$	Br
66b	CH	N	$CF_2PO(OEt)_2$	Br
66c	CH	CH	$CF_2PO(OEt)_2$	F
66d	CH	N	$CF_2PO(OEt)_2$	F
66e	CH	CH	$CF_2SO_2N(tBu)_2$	Br
66f	CH	N	$CF_2SO_2N(tBu)_2$	Br
66g	CH	CH	$CF_2SO_2N(tBu)_2$	F
66h	CH	N	$CF_2SO_2N(tBu)_2$	F
66i	N	CH	$CF_2PO(OEt)_2$	F
66j	N	N	$CF_2PO(OEt)_2$	F

Compd No	X	X_6	X_9	X_8
67a	CH	CH	$CF_2PO(OH)_2$	Br
67b	CH	N	$CF_2PO(OH)_2$	Br
67c	CH	CH	$CF_2PO(OH)_2$	F
67d	CH	N	$CF_2PO(OH)_2$	F
67e	CH	CH	$CF_2SO_2NH_2$	Br
67f	CH	N	$CF_2SO_2NH_2$	Br
67g	CH	CH	$CF_2SO_2NH_2$	F
67h	CH	N	$CF_2SO_2NH_2$	F
67i	N	CH	$CF_2PO(OH)_2$	F
67j	N	N	$CF_2PO(OH)_2$	F

Reagents and conditions: (a) **i.** 20% Piperidine in DMF; **ii. 65a-65h**, HOBt, DIC, DMF, N_2; (d) 1M TSMBr in TFA: 1,2-ethanedithiol: m-cresol (94:5:1), 0 ^{o}C for 5h and then at RT for 16 h.

Scheme 21. Synthesis methodology for the preparation of the compounds (**67a-j**)

Synthesis of unnatural amino acid

In general synthesis of chirally pure unnatural amino acid is a major challenge for organic scientists. Several methods are reported in literature for the preparation of amino acid (structure **XX**, **Figure 34**). Roumcstant et al., prepared unnatural amino acid (**Method A**), starting from bicyclic Schiff base (structure **XXI**) and reacting with lithium bis(trimethylsilyl)amide followed by the alkylation of the enolate with alkyl halide providing chirally pure amino acid **[23]**. The disadvantage of this method was poor yield.

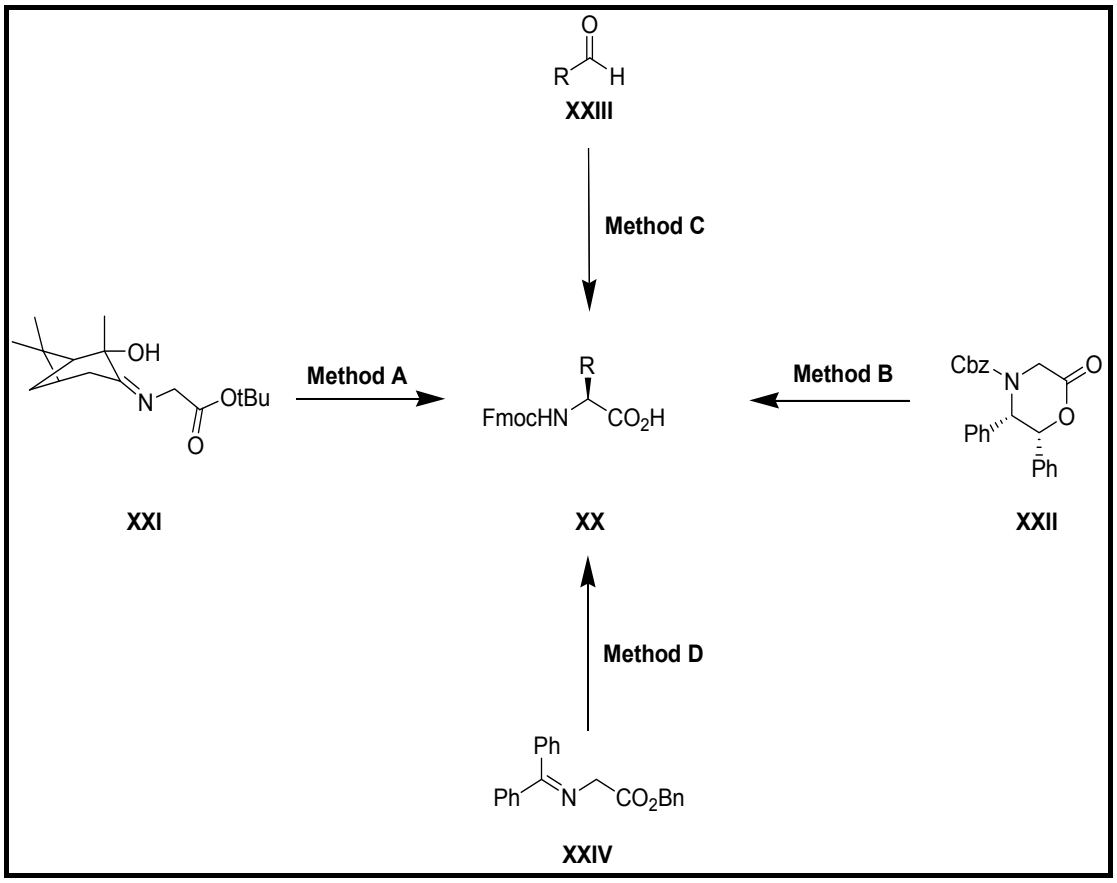


Figure 34. Different approaches for the synthesis chirally pure unnatural amino acid

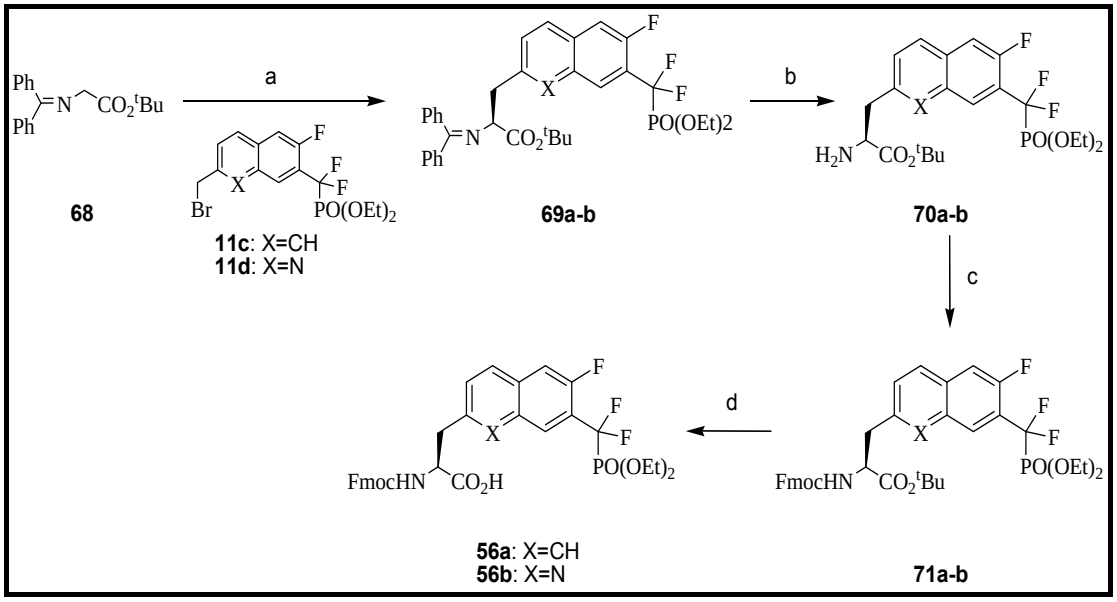
In alternative method for the preparation unnatural amino acid (**Method B**), Williams et al., used imino lactone (structure **XXII**) as a chiral

auxiliary **[24-26]**. This method provides the alkylation product in high diastereomeric excess in poor yield, which is a major limitation of this method. In another **Method C**, Strecker et al., used aldehyde (structure **XXIII**) as a starting material for the synthesis of unnatural amino acid, to give racemic compound which is a limitation of this method **[27-30]**. In **Method D**, O'Donnell et. al., used Schiff base derived from the corresponding phenyl glycinate **[31]**. They achieved alkylation via enolate formation with lithium diisopropylamine (LDA)/ 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU) to give racemic product which was a major limitation of this method.

Among the various reported methods, we used O'Donnell et. al., method (**Method D**) and we modified this method by using 2-t-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine, instead of LDA for the preparation of enolate from tert-butyl 2-(diphenylmetheleneamino)acetate used as Schiff base. Further this method was modified by using O-allyl-N-(9-anthracenylmethyl)cinchonidium bromide as a phase-transfer catalysed alkylation to give chirally pure amino acid in better yield. This method was cost effective, with improvement in yield and was found to be safe for the synthesis unnatural amino acid.

By using modified O'Donnell method, synthesis of common unnatural amino acids, such as Fmoc-protected difluorophosphonate-substituted naphthyl/quinolinyl based unnatural amino acids **56a**-**b** is outlined in **Scheme 22**. Bromo derivatives of substituted naphthalene/quinoline moiety **11c-d** were reacted with tert-butyl diphenyl glycenate **68** in the presence of chiral auxiliary and base giving both ends protected amino acid **69a-b**. Deprotection of N-terminal **69a-b** was achieved using 15% citric acid to give N-terminal free

enantiomerically pure amino acid **69a-b**. Deprotection of N-terminal **69a-b** was achieved using 15% citric acid gives N-terminal free chirally pure amino acid **70a-b**. N-terminal protection of **70a-b** was carried out using Fmoc-OSu in presence of base gives Fmoc-protected amino acid **71a-b**. Deprotection of tert-butyl group of **71a-b** was affected with trifluoro acetic acid to give the compounds **56a-b** as chirally pure amino acid.

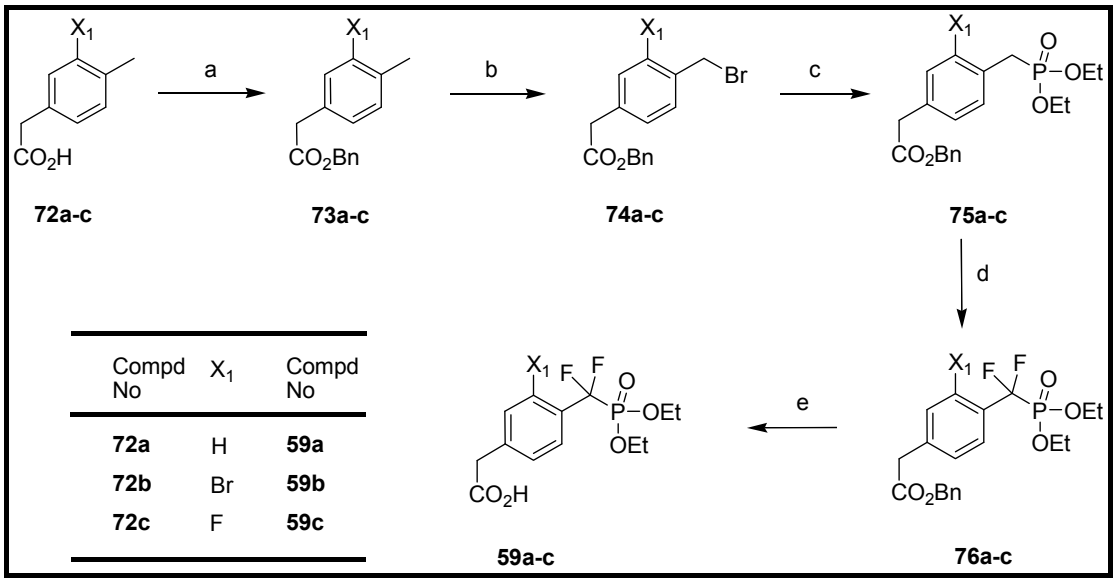


Reagents and conditions: (a) O-Allyl-N-(9-anthracenylmethyl)cinchonidium bromide, 2-t-Butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine, **11c-11d**, CH_2Cl_2, -20 ^oC, 24hrs; (b) 15% citric acid, THF, 25 ^oC, 3hrs; (c) Fmoc-Cl, Na_2CO_3, Acetone, 25 ^oC, 24hrs; (d) TFA or 1*M* HCl/ether, 25 ^oC, 4h.

Scheme 22. Synthesis methodology for the preparation of DFMP-protected unnatural amino acid (**56a**-**b**)

By using modified Hussain et. al. method, synthesis of common intermediates **59a**-**e** is outlined in **Scheme 23** and **Scheme 24**. Acid derivatives of substituted phenyl acetic acid **72a-e** were converted to the benzyl esters **73a-e** by reacting with benzyl alcohol in presence of *N*,*N*-

dimethyl aminopyridine (DMAP) and DIC. Bromination of methyl group in **73a-e** was carried out using NBS in the presence of a catalytic amount of benzoyl peroxide in CCl_4 resulting **74a-e**, which were converted to the phosphonate derivatives **75a-e** via the Michaekis-Arbuzov reaction using triethylphosphite. Electrophilic fluorination of phosphonate **75a-e** using KHMDS and NFSi gave fluorophosphonate **76a-e**. Compounds **76a-e** were deprotected in the presence of H_2 and Pd/C (10%) results the formation of the compounds **59a-e**.



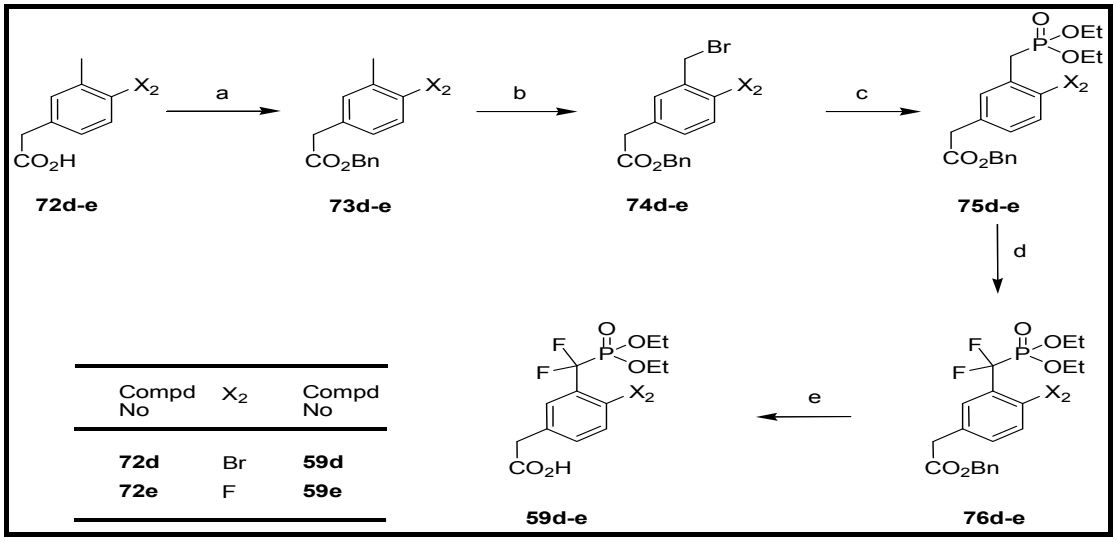
72a-c **73a-c** **74a-c** **75a-c**

Compd No	X_1	Compd No
72a	H	**59a**
72b	Br	**59b**
72c	F	**59c**

59a-c **76a-c**

Reagents and conditions: (a) BnOH, DIC, DMAP, CH_2Cl_2, 25 ^oC, 6h; (b) NBS, benzoyl peroxide, CCl_4, 85 ^oC, 4 h; (c) Triethylphoshite, reflux, 5 h; (d) KHMDS, NFSi, THF, -78 $^oC \longrightarrow$ 25 ^oC, 18 h; (e) H_2, Pd/C, CH_3OH, 60psi, 4h.

Scheme 23. Synthesis methodology for the preparation of the compounds (**59a**-**c**)

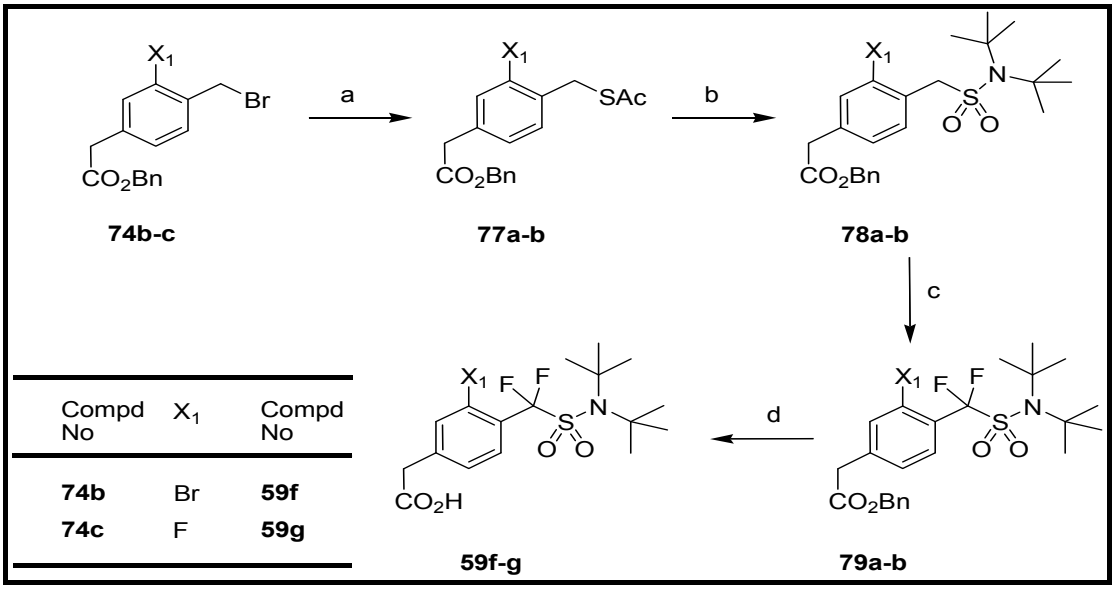


Compd No	X_2	Compd No
72d	Br	**59d**
72e	F	**59e**

Reagents and conditions: (a) BnOH, DIC, DMAP, CH_2Cl_2, 25 ^oC, 6h; (b) NBS, benzoyl peroxide, CCl_4, 85 ^oC, 4 h; (c) Triethylphoshite, reflux, 5 h; (d) KHMDS, NFSi, THF, -78 $^oC \longrightarrow$ 25 ^oC, 18 h; (e) H_2, Pd/C, CH_3OH, 60psi, 4h.

Scheme 24. Synthesis methodology for the preparation of the compounds (**59d**-**e**)

By using modified Hussain method, synthesis of intermediates **59f-g** is illustrated as **Scheme 25**. Reaction of **74b-c** with potassium thioacetate gave thioester **77a-b**. Oxidative chlorination results in sulfonyl chlorides, which were reacted with ditert-butyl amine to give protected sulfonamide **78a-b**. Fluorines could be readily introduced by electrophilic fluorination of benzylic sulfonamide using KHMDS/NFSi giving desired difluoromethylenesulfonamides **79a-b**. Debenzylation of **79a-b** resulted in free acids **59f-g**.



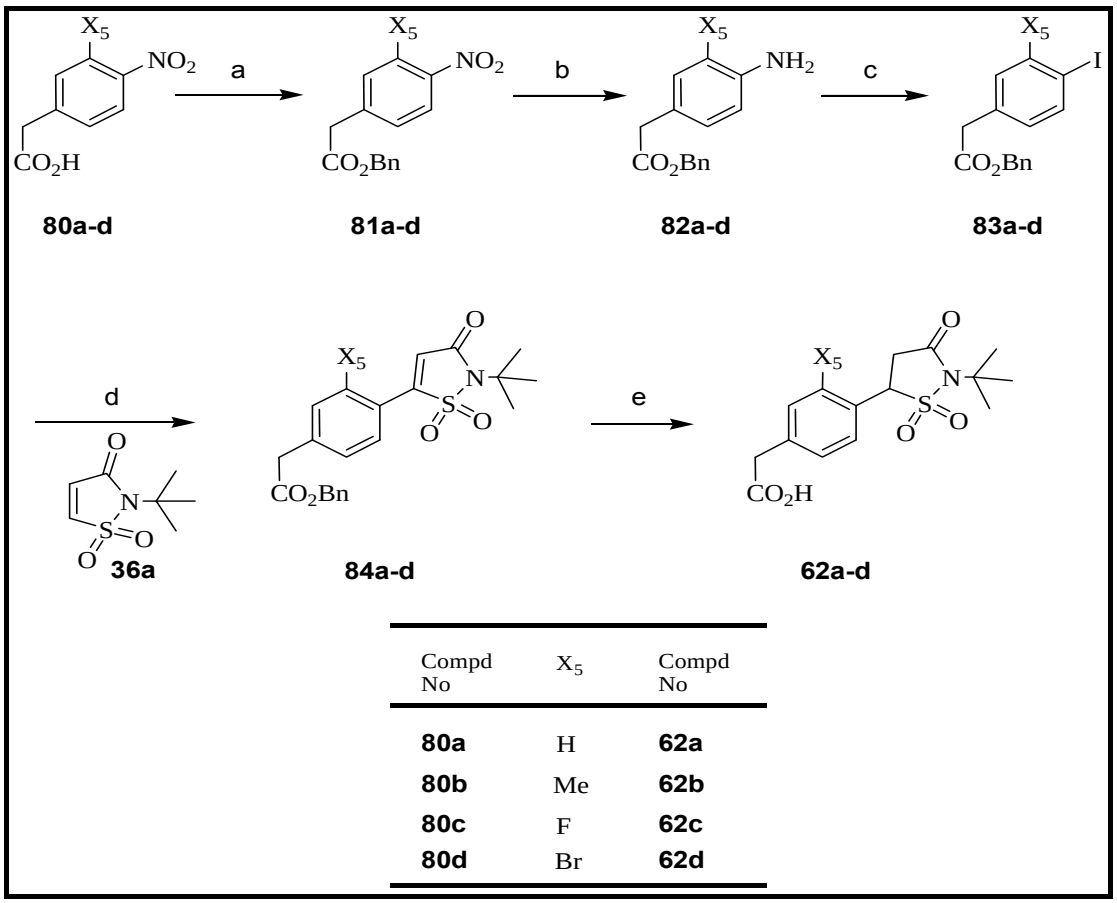
74b-c 77a-b 78a-b 79a-b 59f-g

Compd No	X_1	Compd No
74b	Br	**59f**
74c	F	**59g**

Reagents and conditions: (a) KSAc, DMF, 30 min, 25 ^{o}C; (b) Cl_2, HOAc/H_2O, 0 ^{o}C, 3 h then CH_2Cl_2, ditert-butyl amine, 25 ^{o}C, 2 h; (c) KHMDS, THF, NFSi, -78 ^{o}C ⟶ 25 ^{o}C, 18 h; (d) H_2, Pd/C, CH_3OH, 60psi, 4h.

Scheme 25. Synthesis methodology for the preparation of the compounds (**59f**-**g**)

By using modified Combs method, synthesis of intermediates **62a-d** is illustrated as **Scheme 26**. Nitro substituted phenyl acetic acid moieties **80a-d** were converted to the benzyl esters **81a-d** by reacting with benzyl alcohol in the presence of DMAP and DIC. The nitro group of **81a-d** was reduced to an amino group using zinc and ammonium chloride in methanol **82a-d**, followed by diazotization using sodium nitrite under acidic condition and the resulting intermediate was trapped with potassium iodide to afford **83a-d**. The iodo core was coupled with **36a** with classical ligandless conditions utilizing palladium acetate, tetra-butyl ammonium chloride, and triethyl amine in DMF results **84a-d**. Reduction of unsaturated IZD to saturated IZD & debenzylation of benzyl ester were carried out using 10% Pd/C in methanol under H_2 atmosphere to get **62a-d**.



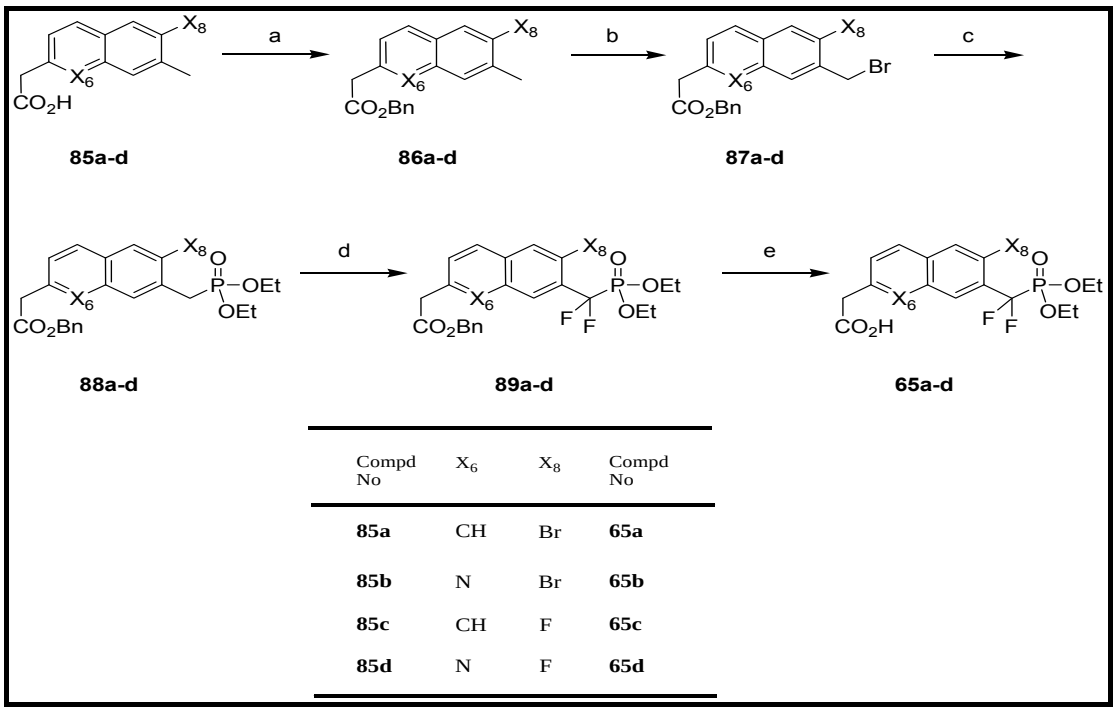
Compd No	X_5	Compd No
80a	H	**62a**
80b	Me	**62b**
80c	F	**62c**
80d	Br	**62d**

Reagents and conditions: (a) BnOH, DIC, DMAP, CH_2Cl_2, 25 ^oC, 6h; (b) Zn^0, NH_4Cl, CH_3OH, H_2O, 65 ^0C, 1 h; (c) $NaNO_2$, 1 *N* aq. HCl, KI, 0 ^oC for 30 min then 45 ^oC for 45 min; (d) $Pd(OAc)_2$, Bu_4NCl, **36a**, Et_3N, DMF, 100 ^oC, 3 h; (e) 10% Pd/C, EtOH, 25 ^oC, 16 h.

Scheme 26. Synthesis methodology for the preparation of the compounds (**62a**-**d**)

Synthesis of common intermediates **65a**-**d** is outlined in **Scheme 27**. Acid derivatives **85a-d** were converted to the benzyl esters **86a-d** by reacting with benzyl alcohol in the presence of DMAP and DIC. Bromination of methyl group **86a-d** was carried out using NBS in the presence of a catalytic amount of benzoyl peroxide in CCl_4 resulting in **87a-d**, which were converted to its phosphonate derivatives **88a-d** via the Michaekis-Arbuzov reaction using triethylphosphite. Electrophilic fluorination of phosphonates **88a-d** using KHMDS and NFSi gave fluorophosphonate **89a-d**. Compounds **89a-d** were

subjected to hydrogenation in the presence of 10% Pd/C resulted in the formation of the **65a-d**.

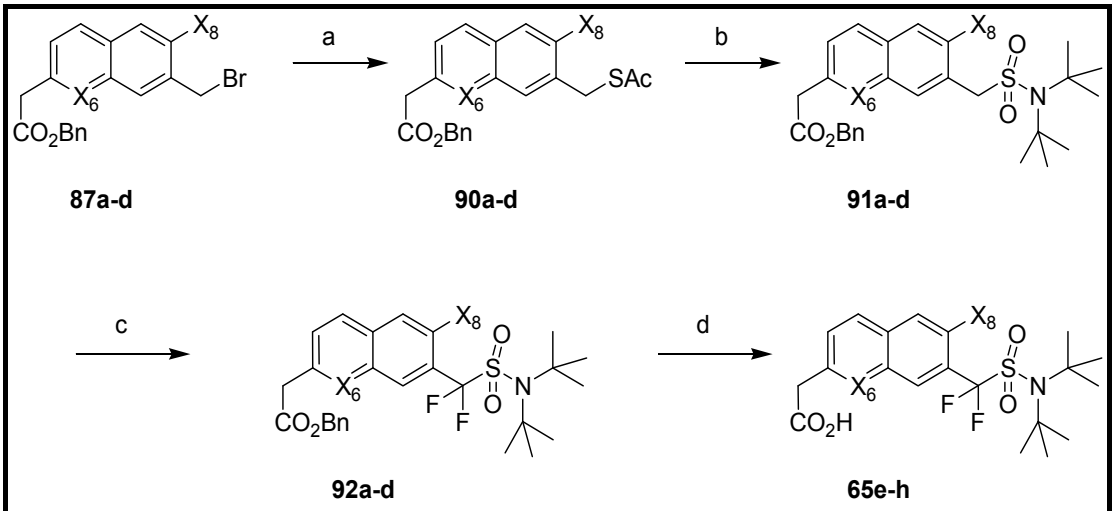


Compd No	X_6	X_8	Compd No
85a	CH	Br	**65a**
85b	N	Br	**65b**
85c	CH	F	**65c**
85d	N	F	**65d**

Reagents and conditions: (a) BnOH, DIC, DMAP, CH_2Cl_2, 25 ^oC, 6h; (b) NBS, benzoyl peroxide, CCl_4, 85 ^oC, 4 h; (c) Triethylphoshite, reflux, 5 h; (d) KHMDS, NFSi, THF, -78 $^oC \longrightarrow$ 25 ^oC, 18 h; (e) H_2, Pd/C, CH_3OH, 60psi, 5h.

Scheme 27. Synthesis methodology for the preparation of the compounds (**65a**-**d**)

Synthesis of intermediate **65e-h** is illustrated as **Scheme 28**. Reaction of **87a-d** with potassium thioacetate gave the thioesters **90a-d**. Oxidative chlorination gave sulfonyl chlorides, which were reacted with ditert-butyl amine to give protected sulfonamide **91a-d**. Fluorines could be readily introduced by electrophilic fluorination of benzylic sulfonamide using KHMDS/NFSi giving the desired difluoromethylenesulfonamides **92a-d**. Debenzylation of **92a-d** using 10% Pd/C gave the free acids **65e-h**.



87a-d **90a-d** **91a-d**

92a-d **65e-h**

Compd No	X_6	X_8	Compd No
87a	CH	Br	**65e**
87b	N	Br	**65f**
87c	CH	F	**65g**
87d	N	F	**65h**

Reagents and conditions: (a) KSAc, DMF, 30 min, 25 ^{o}C; (b) Cl_2, HOAc/H_2O, 0 ^{o}C, 1 h then CH_2Cl_2, ditert-butyl amine, 25 ^{o}C, 2 h; (c) KHMDS, THF, NFSi, -78 ^{o}C ⟶ 25 ^{o}C, 18 h; (d) H_2, Pd/C, CH_3OH, 60psi, 4h.

Scheme 28. Synthetic methods for the preparation of title compounds (**65a**-**d**)

Using above synthetic routes (**Scheme 19**-**21**), all together twenty three compounds (**61a-i**, **64a-d** and **67a-j**) were prepared. All the final compounds, intermediates were purified, characterized and the spectral data of compounds were found to be in confirmily with the structure assigned. Detailed experimental procedures are described in **experimental Section 5.1** and the representative spectra's of the selected compounds are given in **spectral data Section 6**.

3.3.2. In-vitro PTP 1B inhibitory activity, selectivity and structure activity relationship (SAR)

All the compounds **61a-i**, **64a-d** and **67a-j** were screened using *p*NPP enzymatic assay for PTP 1B inhibitory activity (detailed experimental protocol is given in **experimental Section 5.3**) **[15]**, mainly to establish the SAR of the new series of peptidomimetic based PTP-1B inhibitors. As deplicated in **Table 12**, depending upon the nature of substitution, all the compounds showed different degree of PTP-1B inhibition as reflected by their IC_{50} values.

In continuation with our ongoing research on dual binding-site PTP-1B inhibitors, a novel library of tripeptides as potent, selective and dual binding PTP-1B inhibitors containing two *p*Tyr mimetics (DFMP/DFMS/IZD) is reported, one targeted to the site-A, and the other targeted to a unique adjacent noncatalytic site-B. The title compounds were divided in two series, either substituted with DFMP/DFMS/IZD-benzyl group **61a-g**, **64a-d** or with DFMP/DFMS-naphthyl/quinolinyl templates **66a-h**.

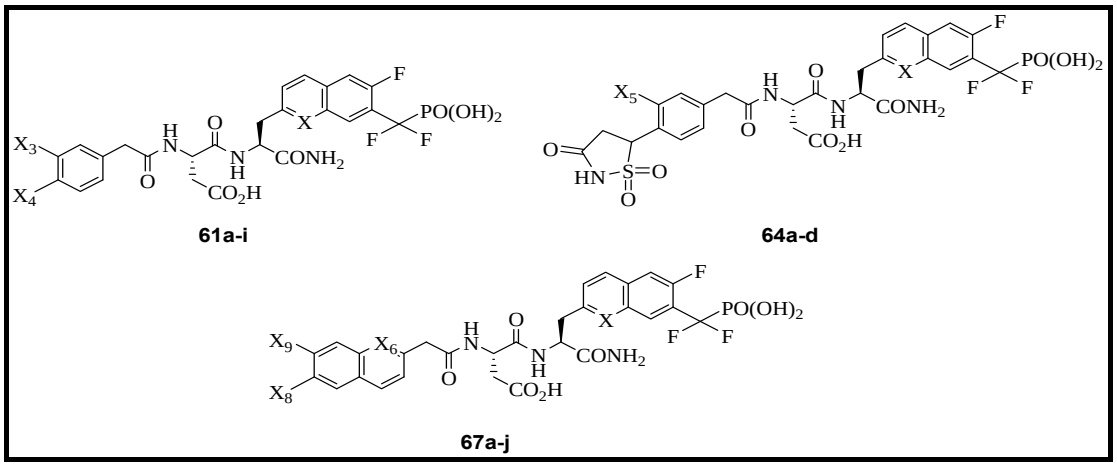
Within the first series **61a-g**, **64a-d**, the first set of compounds **61a-g** containing DFMP/DFMS-substituted benzyl groups **61a-e** showed a diverse PTP-1B inhibitory activity depending on the DFMP/DFMS group's relative position (*meta* or *para*) on the phenyl ring and the *ortho* substitutents (hydrogen or halogen). Replacement of DFMP-substituted phenyl group in **IX** (**at ring A**) with DFMP-substituted naphthyl templates as in **61a** showed two fold improvement in PTP-1B inhibitory activity. Whereas compounds with *m*-DFMP and *o*-bromo to DFMP **61d**, showed a weak PTP-1B inhibitory activity relative to that of the un-substituted **61a**. Compound **61b**, containing *p*-DFMP

and *o*-bromo substituted to DFMP, showed more potent PTP-1B inhibitory activity.

Replacement of *o*-bromo analogues **61b** & **61d** with more electronegative *o*-fluoro substitution to DFMP **61c** & **61e**, showed improved inhibitory activity, which could be due to increase in the electronegativity and decrease in the bulk (*o*-bromo vs *o*-fluoro) adjacent to the DFMP group. Bioisosteric replacement of the *p*-DFMP in **61b** & **61c** with *p*-DFMS, **61f** & **61g** showed a moderate *in vitro* PTP-1B inhibitory activity. The reason could be that at pH 6.5 (simulate with *in vitro* system), it has been reported that the monoanioninc DFMP exhibit an equilibrium with its dianionic form and the PTP-1B enzyme show strong binding interaction with the dianionic form. Thus the compounds containing DFMP-substituted templates showed better PTP-1B inhibitory activity compared to the DFMS-substituted templates, may be due to the preference of PTP-1B enzyme binding with the dianionic form of DFMP. Among **61f** & **61g**, compound **61g** showed better PTP-1B inhibitory activity.

The second set of the compounds containing IZD-substituted benzyl groups **64a-d** showed promising PTP-1B inhibitory activity, depending upon the nature of *ortho* substituents. Within the set, un-substituted derivative **64a** was found to be least active, the *ortho*-methyl **64b** and *ortho*-bromo **64d** were found to be equipotent, while *ortho*-fluoro **64c** was found to be the most potent, due to increase in the electro negative and decrease in the steric bulk (*o*-bromo vs *o*-fluoro) adjacent to the IZD group.

Table 12. The *In vitro* PTP-1B inhibitory activity of test compounds **61a-i**, **64a-d** and **67a-j**.



Comp No	X_3	X_4	X_5	X_6	X_8	X_9	IC_{50} nM PTP-1B[a]
61a X=CH	H	$CF_2PO(OH)_2$	----	-----	----	-----	1.2 ± 0.09
61b X=CH	Br	$CF_2PO(OH)_2$	----	-----	----	-----	0.8 ± 0.002
61c X=CH	F	$CF_2PO(OH)_2$	----	-----	----	-----	0.43 ± 0.005
61d X=CH	$CF_2PO(OH)_2$	Br	----	-----	----	-----	3.22 ± 0.04
61e X=CH	$CF_2PO(OH)_2$	F	----	-----	----	-----	3.01 ± 0.01
61f X=CH	Br	$CF_2SO_2NH_2$	----	-----	----	-----	1.14 ± 0.007
61g X=CH	F	$CF_2SO_2NH_2$	----	-----	----	-----	1.01 ± 0.006
61h X=N	Br	$CF_2PO(OH)_2$	----	-----	----	-----	1.77 ± 0.02
61i X=N	F	$CF_2PO(OH)_2$	----	-----	----	-----	1.26 ± 0.009
64a X=CH	-----	-----	H	-----	----	-----	0.61 ± 0.001
64b X=CH	-----	-----	Me	-----	----	-----	0.47 ± 0.003
64c X=CH	-----	-----	F	-----	----	-----	0.19 ± 0.004
64d X=CH	-----	-----	Br	-----	----	-----	0.43 ± 0.003
67a X=CH	-----	-----	----	CH	Br	$CF_2PO(OH)_2$	0.24 ± 0.005

67b X=CH	-----	-----	----	N	Br	$CF_2PO(OH)_2$	0.31 ± 0.007
67c X=CH	-----	-----	----	CH	F	$CF_2PO(OH)_2$	0.13 ± 0.001
67d X=CH	-----	-----	----	N	F	$CF_2PO(OH)_2$	0.15 ± 0.002
67e X=CH	-----	-----	----	CH	Br	$CF_2SO_2NH_2$	0.4 ± 0.004
67f X=CH	-----	-----	----	N	Br	$CF_2PO(OH)_2$	0.55 ± 0.006
67g X=CH	-----	-----	----	CH	F	$CF_2SO_2NH_2$	0.29 ± 0.007
67h X=CH	-----	-----	----	N	F	$CF_2SO_2NH_2$	0.27 ± 0.008
67i X=N	-----	-----	----	CH	F	$CF_2PO(OH)_2$	1.32 ± 0.01
67j X=N	-----	-----	----	N	F	$CF_2PO(OH)_2$	1.94 ± 0.06

[a] Enzymatic assay was carried out in 96-well plates. To an assay buffers, *p*NPP and test compounds were added and the reaction was initiated by addition of PTP-1B/TC-PTP (10 -100 nM). The initial rate of PTPase-catalyzed hydrolysis of *p*NPP was measured at 405 nm. IC_{50} value was determined under fixed *p*NPP concentration of 1 mM (n=3).

The third set of compounds with DFMP/DFMS-substituted naphthyl/quinolinyl templates **67a-h** showed potent PTP-1B inhibitory activity. Withinin this set, DFMP-substituted naphthyl/quinolinyl templates **67a-b** showed a better PTP-1B inhibitory activity compared to its bioisostere DFMS-substituted templates **67e-f**. Replacement of the bromo analogues **67a-b** & **67e-f** with more electronegative fluoro analogues **67c-d** & **67g-h** led to highly potent PTP-1B inhibitors among all series.

While the second series of the compounds (DFMP-substituted quinolinyl derivatives **61h-i** & **67i & 67j** showed 2-10 fold less PTP-1B inhibitory activity as compared to the first series (**61b-c** & **67c-d**).

In conclusion, among the two series, compounds with DFMP-substituted naphthyl templates **61a-g**, **64a-d** & **67a-h** (series **1)** were found to be more potent than quinolinyl system **61h-g** & **67i-j** (series **2)**, as a *p*Tyr

mimic on ring-A of compound **IX**. Within the series 1, *p*-substituted DFMP/DFMS/IZD exhibit favourable PTP-1B inhibitory activity compared to *m*-substituent on benzyl templates of ring-B in **IX**. The compounds with *o*-halogen substituents next to *p*Tyr mimetics showed potent PTP-1B inhibitory activity and the fluoro analogues were found to be more potent than their corresponding bromo analogues. Among the three-different *p*Tyr mimetics (DFMP/DFMS/IZD) on benzyl templates of ring-B in **IX**, DFMP exhibit the highest PTP-1B inhibitory activity, followed by IZD and DFMS. Among the three-different *p*Tyr mimetics substituted on benzyl/ naphthyl/ quinolinyl templates on ring-B of **IX**, naphthyl exhibited the highest PTP-1B inhibitory activity, followed by quinolinyl and benzyl. Overall, *in vitro* PTP-1B inhibitory activity results clearly suggest that the potency of peptidomimetics based PTP-1B inhibitors can be modulated by introducing a suitable *p*Tyr mimic on ring-A and ring-B of **IX**.

From the above SAR study, out of twenty three compounds, short listed compounds (**64c**, **67c** & **67d)** showed potent *in vitro* PTP-1B inhibitory activity within this series as compared to parent compound **IX** and were subjected to evaluate their *in vitro* selectivity over other PTPs (LAR, CD45, SHP-1, SHP-2 and TC-PTP) by using the *p*NPP assay, and IC_{50} values are described in **Table 13** (detailed experimental protocol is given in **experimental Section 5.3**).

As deplicated in **Table 13**, compound **64c** showed ~25-fold selectivity, while **67c** & **67d** showed ~95 to 110-fold selectivity over TC-PTP. All the three compounds **64c**, **67c** & **67d** showed > 5000-fold selectivity over PTPα, LAR, CD45, SHP-1, SHP-2 enzymes. The compound containing IZD-substituted

benzyl groups as a *p*Tyr mimic showed moderate selectivity, while compounds containing DFMP-substituted naphthyl/quinolinyl templates as a *p*Tyr mimic, showed excellent selectivity, indicated that among two different ring systems (benzyl / fused-ring system), the fused-ring system (naphthyl/quinolinyl derivatives) showed greater selectivity over TC-PTP.

Table 13. Subtypes-selectivity data selected compounds (**64c, 67c** and **67d**)

Compound	IC_{50} nM		Fold Selectivity[b]
	PTP-1B[a]	TC-PTP[a]	
64c	0.19 ± 0.004	4.75 ± 0.09	~ 25 fold
67c	0.13 ± 0.001	14.22 ± 0.8	~ 110 fold
67d	0.15 ± 0.002	14.61 ± 0.9	~ 97 fold

[a] Enzymatic assay was carried out in 96-well plates. The initial rate of PTPase-catalyzed hydrolysis of *p*NPP was measured at 405 nm. IC_{50} value was determined under fixed *p*NPP concentration of 1 mM (n=3); Data represent the mean ± SD; **64c**, **67c** and **67d** showed > 5000-fold selectivity over PTPα, CD45, LAR, SHP-1 and SHP-2 enzymes.

[b] Fold selectivity calculated as ratio of IC_{50} values of TC-PTP/PTP-1B inhibitions

3.3.3. Molecular docking Study

In order to understand the selectivity profile and dual binding interaction with both binding sites A & B of PTP-1B, the short listed compounds **64c**, **67c** & **67d** at molecular level, a molecular docking analysis was carried using Glide docking software (version 5.6). The initial Glide docking studies for **64c**, **67c** & **67d** gave poor results in terms of binding conformation. Based on this observation, the compounds were docked using the induced fit docking (IFD) protocol (**Figure 35**). Docking study of **IX** was

also carried out to validate our model and compare our docking results with the published X-ray co-crystallographic data of **IX** with PTP-1B (**Figure 35a**).

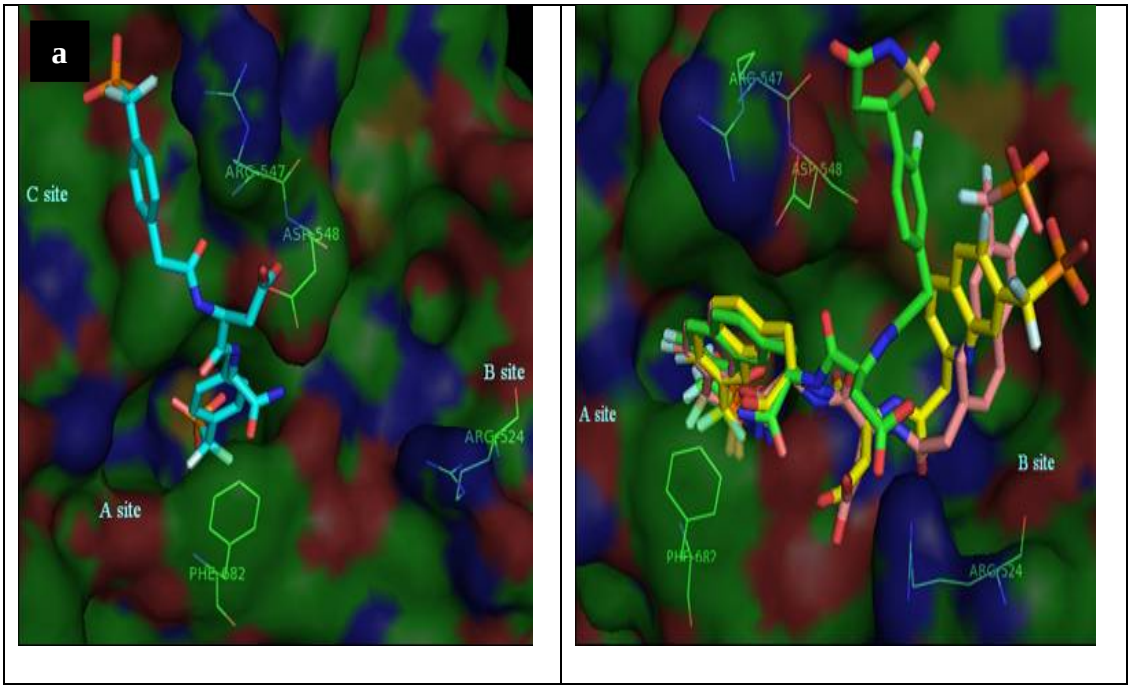


Figure 35. (a) Binding pose of **IX** in the PTP-1B active site; **IX** docks very well in sites A & C instead of A & B; **(b)** Overlay binding poses of **64c** (green), **67c** (pink) and **67d** (yellow in the PTP-1B active sites. **67c** & **67d** best fits into site A & B, while **64c**, docks in site-A and partially flips towards site-C. Residues numbering is as per current numbering (PDB code: 1QT6): Arg24 (524), Phe52 (552), Arg254 (754), and Met258 (758)

For acheiving selectivity over TC-PTP, site B interaction is essential. The IFD study results of **IX** illustrate (**Figure 35a**) that the compound **IX** docks very well in site-A [ring A, (DFMP-phenyl moiety) interact with Phe682 of site A], ring-B (DFMP-benzyl moiety) flip into site-C (interact with Arg547) and marginally interact with site-B residues (Asp548 and Arg524), thereby favors best fit of **IX** in sites A & C instead of sites A & B. Thereby, our docking study

results of **IX** correlates with the literature X-ray co-crystallographic data and thus validate our model.

Overlay binding poses of **64c**, **67c** & **67d** in the PTP-1B active sites are shown in **Figure 35b**. All the three compounds docks very well in site-A [ring-A (DFMP-naphthalene) interact with Phe682 of site-A]. Naphthalene/ quinolinyl moieties (ring-B) of **67c** & **67d**, flip into site-B, make strong interaction with the key residues of site-B (Asp548 & Arg524), thereby favours best fit of **67c** & **67d** into site A and B, which may account for good selectivity against TC-PTP (*in vitro*).

While IZD-benzyl moiety (ring B) of **64c** makes weak interactions with the key residue of site-B and partially flips towards site-C (Arg547), which may account for its moderate selectivity against TC-PTP.

3.3.4. Conclusion

The data generated on specificity of designed molecules confirms the hypothesis that potent and selective dual binding-site PTP-1B inhibitors can be developed by incorporating potent *p*Tyr mimetic in a single scaffold. These novel peptidomimetics designed based on dual binding site hypothesis, exhibits favorable orientation across the both binding sites A and B of the PTP-1B. The SAR around the lead compounds **67c & 67d**, indicating that only fluoro analogue of naphthyl derivatives showed selectivity owing to favorable orientation of the molecule across both the binding sites of PTP-1B enzyme. The results of our in silico docking study are also in agreement with the observed *in vitro* PTP-1B selectivity. Thus discovery of potent peptidomimetics suggests that it is a new approach towards a safe and effective prevention of T2DM.

Compared to previous two series, short listed compounds **67c** and **67d** were not subjected to further detailed pharmacokinetic evolution, due to their metabolic instability and oral PK issue. However these compounds (**67c** and **67d)** can be considered as primary lead from this series, which can be converted into clinical candidates by improving their metabolic stability and oral PK, thereby this series further expands the scope for suitable structural modifications to treat T2DM.

3.4. References

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Chapter IV: Overall summary and Future prospects

4. Overall Summary and Future prospects

4.1 Overall summary of the present investigation

T2DM is a complex multifactorial disease affecting quality of life of an affected individual. T2DM is a progressive disorder accompanied by deterioration in β cell function and insulin resistance. Despite this fact, there is now clear evidence that tight control of blood glucose significantly reduces the risk of complications of diabetes. PTP-1B inhibitors, which control the activity of PTP-1B, known as negative regulator of insulin signaling offers safe and effective mean for treating metabolic diseases.

In last few decades, several small molecules based-PTP-1B inhibitors are reported in the literature, however, no PTP-1B inhibitor has reached to the market, mainly due to their lack of selectivity over TC-PTP and poor oral bioavailability.

For my PhD dissertation work, altogether three series of PTP-1B inhibitors were designed. In the first series, as benzotriazole based PTP-1B inhibitors, total fifteen compounds were prepared. In the second series, based on triaryl-sulfonamide derivatives as PTP-1B inhibitors, total nineteen compounds were prepared. In the third series, total twenty three peptidomimetics derivatives were prepared as PTP-1B inhibitors. Altogether fifty-seven compounds were synthesized, purified, characterized and were subjected to *in vitro* PTP-1B inhibitory activity. The most potent selected PTP-1B inhibitors from each series were further subjected to the *in vitro* selectivity over other PTPs (especially over TC-PTP). From each series, the most potent and selective compounds were subjected to the *in vivo* antidiabetic

activity including PK studies. All the three series were found to be potent and selective PTP-1B inhibitors.

In the first series, test compounds **13a** and **13c** showed excellent PTP-1B inhibition (*in vitro*) along with selectivity over PTPs, therefore **13a** and **13c** were considered as optimized lead from this series.

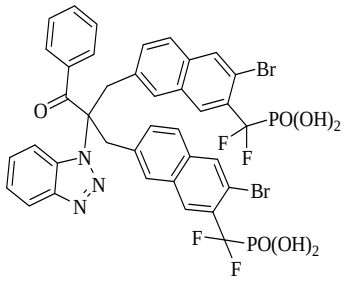
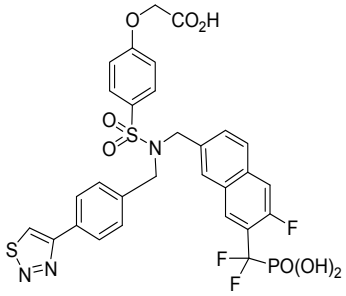
In vivo and *in vitro* PTP-1B inhibitory activity and molecular docking studies results of **13a** and **13c** clearly demonstrated that the potency and selectivity of benzotriazole based PTP-1B inhibitors can be modulated using suitable introduction of *p*Tyr mimetic. Furthermore, it was observed that suitable introduction of *p*Tyr mimetics on benzotriazole scaffold contributed significantly towards improvement in the *in vivo* PTP-1B inhibitory activity, which could be correlated with the improved oral bioavailability. The *in vitro* and *in vivo* studies, results of **13a** demonstrated potent *in vitro* PTP-1B inhibitory activity and more than 100-fold selectivity against TC-PTP. In PK studies, compound **13a** showed good oral bioavailability, indicating that the test compound **13a** is a promising candidate for safe and effective treatment of T2DM and need to subject to further pre-clinical evaluation.

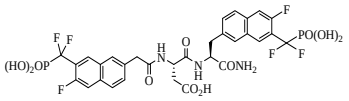
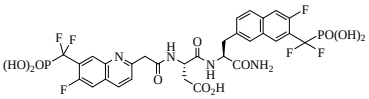
In the second series, our attempt to replace *p*Tyr mimietic of compound **V** with its more potent *p*Tyr mimetics, lead to the development of novel, potent, selective and structurally diverse scaffold as PTP-1B inhibitors. Most potent compound **54e** from this series showed nM potency (PTP-1B inhibitory activity), which was found to be better than **V**. It also showed improvement in the fold selectivity over various PTPs. Thus, preliminary PK studies results reveal that a further modification on **54e** may lead to the

identification of a potent compound from this series, with improved bioavailability for the efficient treatment of T2DM.

In the third series, modification of peptidomimetic derivatives of **IX** (preliminary hit) was carried out by suitable changes at C and N terminals of compound **IX**, and we identified progressive lead compounds **67c** and **67d**, which showed PTP-1B inhibitory activity in nM range with more than 95-fold selectivity over TC-PTP.

Table. 14 Short listed key compounds from 3 different series

Series	**Structure**	***In vitro*** **(PTP-1B)** IC_{50} **nM**	***In vitro*** **(TC-PTP)** IC_{50} **nM**	**Fold Selectivity**	**PK** **hμgmL^{-1}**
1	 **13a**	5	580	116	10.562
2	 **54e**	9	870	96	8.91

3	 **67c**	0.13	14.22	110	NA
	 **67d**	0.15	14.61	97	NA

Compared to the previous two series, compounds **67c** and **67d** were not subjected to further *in vivo* evaluation, due to their metabolic instability and oral PK issues. However compounds **67c** and **67d** can be considered as primary lead from this series, which can be converted into clinical candidates by improving metabolic stability and oral PK. This series expands further scope for studying suitable structural modifications to treat T2DM. The key compounds of each series are listed in **Table 14**.

4.2 Future Prospects

From the first series, compound **13a** showed excellent PTP-1B inhibitory activity (*in vitro*) & antidiabetic activity (*in vivo*). The PK profile of **13a** was found to be satisfactory compared to the standard compound and therefore **13a** represents a promising candidate to work on. Future work includes same additional safety study and pre-clinical studies before it can be subjected to clinical development. Compound **13a** should be considered for chronic efficacy studies and for long term toxicological evolution, along with its PK profile in higher animals such as dog or monkey.

In the second series, compound **54e** showed better *in vitro* PTP-1B inhibitory activity due to its strong interaction with site A and showed more than 90-fold selectivity over TC-PTP due to its strong interaction with site B, which was not observed in **V**. This discovery encourages us to move one step further ahead in designing potent and PTP-1B-specific inhibitors, which demands further structural modification. Compounds **XIII** & **XIV** are proposed modification in **54e**, expected to improve its PTP-1B inhibitory activity, selectivity over TC-PTP and may result in a better PK profile (**Figure 36**).

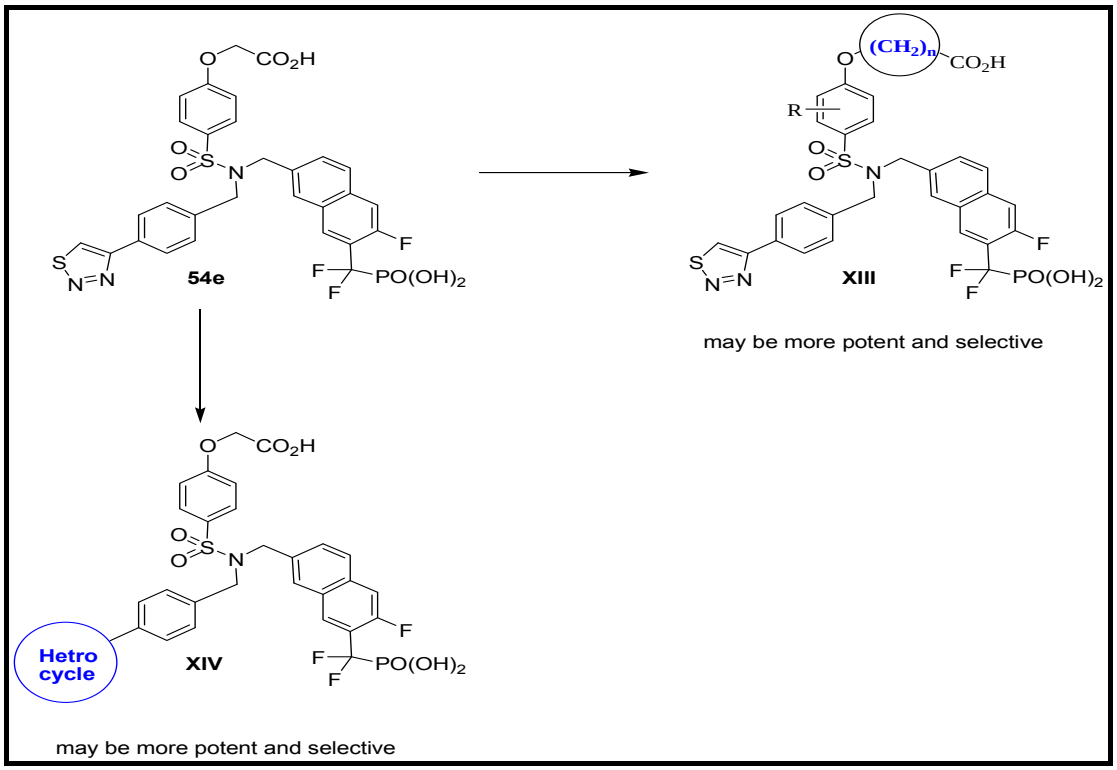


Figure 36. Future plans for series 2 modification

In case of the third series, compound **67c** was found to be most active among all the three series, but oral bioavailability was a major issue in the development of peptidomimetic based PTP-1B inhibitors. Thus, we may need to improve metabolic stability to achieve good oral bioavailability by oral route.

For this, the following future modifications in **67c** are proposed to improve its *ex vivo* and *in vivo* activities along with its PK profile (**Figure 37**). Bioisosteric replacement of central amino acid (Aspartic acid) with unnatural amino acid (**XV**) or with spacer $[(-CH)_2]_n$ (**XVI**), may improve its *ex-vivo* stability to over come oral bioavailabity.

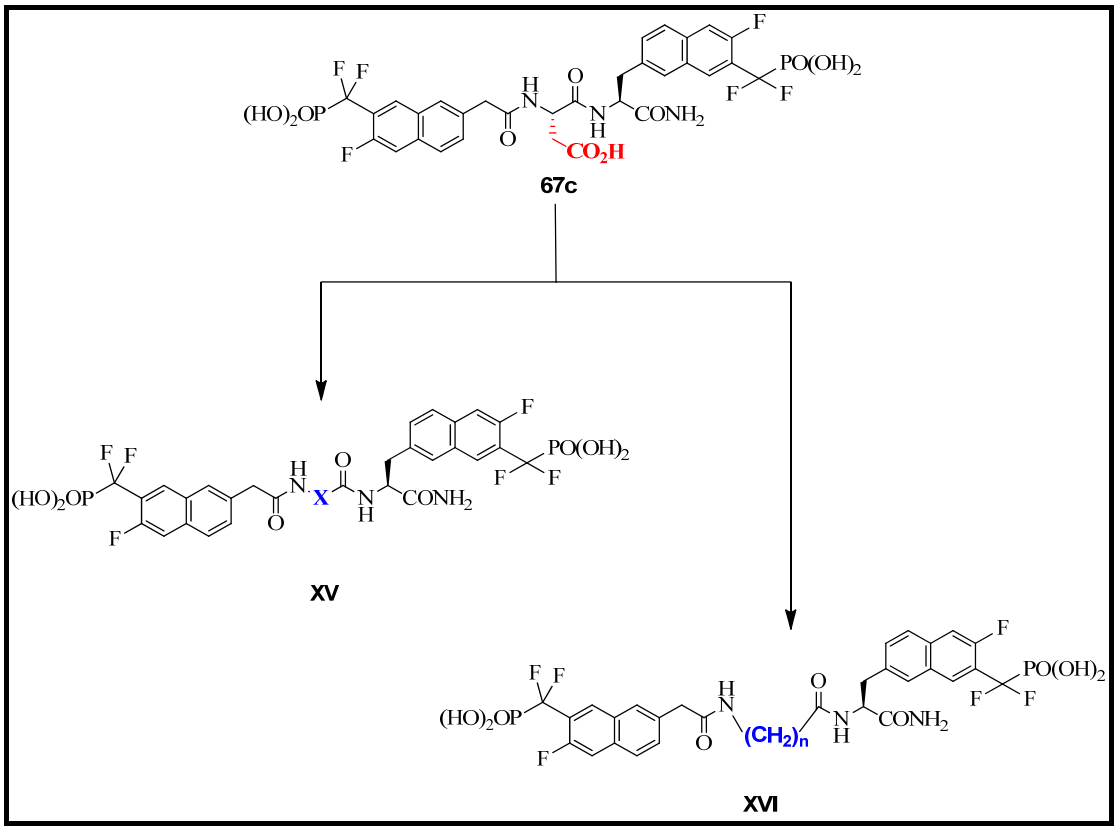


Figure 37. Future plans for series 3 modifications

Chapter V: Experimental

5. Experimental

5.1 Chemistry

5.1.1. Materials and Methods

All the reagents used for the synthesis were purchased from Aldrich Company Limited, Dorset and were used without further purification. Solvents were procured from commercial source and used after distilling or drying according to the known methods. All the air and/or moisture sensitive reactions were carried out in dry solvents under nitrogen atmosphere. Melting points were recorded on open glass capillaries, using scientific melting point apparatus and are uncorrected.

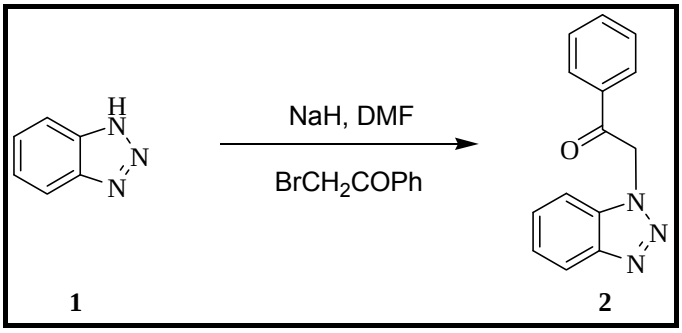
The ^{1}H NMR spectra were recorded on a Bruker Avance-400 (400 MHz) spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) relative to TMS either in $CDCl_3$ or Acetone-d_6. Signal multiplicities are represented by a s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), bs (broad singlet), and m (multiplet). ^{13}C NMR spectra were recorded on Bruker Avance-400 at 100 MHz either in $CDCl_3$ or Acetone-d_6. Mass spectra (ESI-MS) were obtained on Shimadzu LCMS 2010-A spectrometer.

HPLC analyses were carried out at λ_{max} 220 nm using column. Progress of the reactions was monitored by TLC using precoated TLC plates (E. Merck Kieselgel 60 F254) and the spots were visualized by UV and/or iodine vapors. The chromatographic purification was performed on silica gel (200-400 mesh). Few compounds directly used for next step without purification and analysis. Detailed synthetic procedures and characterization

data of all the final compounds and intermediates are described in next section.

5.1.2. Experimental Details

5.1.2.1. 1-phenacyl-1*H*-1,2,3-benzotriazole (2)

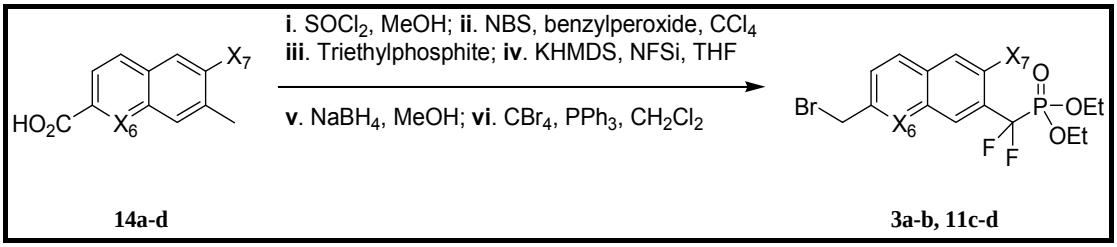


To a suspension of NaH (60%) (2.90 g, 0.201 mol) in dry DMF (60 mL), a solution of 1 (20.0 g, 0.168 mol) in dry DMF (100 mL) was added drop wise over a period of 30 min at 0-5 ^{o}C and stirred for 30 min. Phenacyl bromide ($BrCH_2COPh$) (36.79 g, 0.185 mol) was added to the reaction mixture at same temperature and stirred at 25 ^{o}C for 5 hours. The reaction was poured into ice cold water (480 mL) and extracted with ethyl acetate (3 X 240 mL). The combined organic layers was successively washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum to get the crude product which was purified by column chromatography using 10% ethyl acetate in hexane as eluent to furnish the title compound 2 (30.2 g, 75%) as a white solid. mp: 77-78 ^{o}C; Purity by HPLC: 95.96%.

^{1}HNMR (Acetone-*d6*) : δ5.93 (s, 2H), 7.26-7.35 (m, 5H), 7.38-7.42 (m, 1H), 7.46-7.50 (m, 1H), 7.62-7.64 (m, 1H), 7.96-7.99 (m, 1H)

ESI/MS (m/z) : 238.4 $(M+H)^{+}$.

5.1.3. General procedure for the synthesis of Compounds (3a-b, 11c-d)



Step I: To a solution of acids **14a-d** (1 mole equivalent) in MeOH (10 fold), $SOCl_2$ (3.0 mole equivalent) was added drop wise over a period of 30 min at 0-10 ^oC, and then heated at 70 ^oC for 3 hours. Methanol was distilled out under vacuum; residue was poured into ice cold water (10 fold) and extracted with ethyl acetate (2 X 10 fold). The combined organic layer was successively washed with saturated aqueous $NaHCO_3$, water, and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum to furnish the title compounds **15a-d** as white solids.

Step II: To a stirred solution of **15a-d** (1 mole equivalent) in CCl_4 (10 fold) were added *N*-bromosuccanamide (1.2 mole equivalent) and benzoylperoxide (0.01 mole equivalent). The mixture was gently refluxed for 4 hours under N_2 atmosphere. After 4 hours, water (10 fold) was added and the mixture was extracted with CH_2Cl_2 (2 X 10 fold). The combined organics were washed with saturated aqueous $NaHCO_3$, then dried over Na_2SO_4, and concentrated. The resulting residue was subjected to flash chromatography using 10% ethyl acetate in hexane as eluent to furnish title compound **16a-d** as a white needles.

Step III: To a mixture of **16a-d** (1 mole equivalent) and triethyl phosphite (2.4 mole equivalent) was refluxed for 5 hours. The excess triethyl phosphite was removed by distillation and the residue was subjected to flash

chromatography using 10% ethyl acetate in hexane as an eluent which gave **17a-d** as white solids.

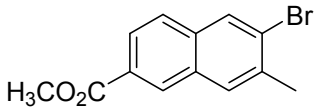
Step IV: To a solution of **17a-d** (1 mole equivalent) and N-fluorobenzenesulfonamide (2.5 mole equivalent) in dry THF (10 fold) at -78 ^{o}C was added KHMDS (1.0 M in THF, 2.5 mole equivalent) over 10 min. The mixture was stirred for 2 hours at -78 ^{o}C, then allowed to warm to 25 ^{o}C, and stirred for 18 hours. The reaction was quenched with a saturated aqueous NH_4Cl solution and extracted with ethyl acetate (2 X 20 fold). The combined organic layers were washed successively with water, followed by brine, and dried over Na_2SO_4, filtered and concentrated under vacuum to get the crude product which was subjected to flash by chromatography using 15% ethyl acetate in hexane as eluent to furnish title compounds **18a-d** as white solids.

Step V: A suspension of **18a-d** (1 mole equivalent) and $NaBH_4$ (6.0 mole equivalent) in MeOH (10 fold) under N_2 was stirred at 65 ^{o}C for 3 hours. The reaction mixture was quenched with a saturated aqueous NH_4Cl solution, water was added and the mixture was extracted with ethyl acetate (3 X 20 fold). The combined organics were dried over Na_2SO_4 and concentrated. The residue was subjected to column chromatography (10% ethyl acetate in hexane) which gave pure product **19a-d** white in colour.

Step VI: To a solution of **19a-d** (1 mole equivalent) in dry CH_2Cl_2 (20 fold) at 0 ^{o}C were added carbon tetrabromide (1.1 mole equivalent) and triphenylphosphine (1.1 mole equivalent) and stirred for 2 hours. The mixture was concentrated at room temperature on rotary

evaporation and the resulting residue subjected to flash chromatography using 10% ethyl acetate in hexane which gave corresponding bromo derivatives **3a-b, 11c-d** as white solids.

5.1.3.1. Methyl 6-bromo-7-methyl-2-naphthoate (15a)

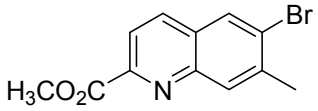


15a (25 g, 95%) was prepared from **14a** (25 g, 0.094 mole) by means of the general procedure described above in Step-I as a white solid. mp: 99-100 ^{o}C; Purity by HPLC: 94.48%.

^{1}HNMR (Acetone-*d6*) : δ 2.33 (s, 3H), 3.91 (s, 3H), 7.50 (s, 1H), 7.79 (d, *J* = 8.4 Hz, 1H), 7.89 (s, 1H), 7.99 (d, *J* = 8.1 Hz, 1H), 8.20 (s, 1H)

ESI/MS (m/z) : 280.2 $(M+H)^{+}$.

5.1.3.2. Methyl 6-bromo-7-methylquinoline-2-carboxylate (15b)

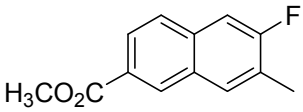


15b (20 g, 76%) was prepared from **14b** (25 g, 0.094 mole) by means of the general procedure described above in Step-I as a white solid. mp: 88-90 ^{o}C; Purity by HPLC: 95.33%.

^{1}HNMR (Acetone-*d6*) : δ 2.35 (s, 3H), 3.87 (s, 3H), 7.88 (s, 1H), 7.93 (s, 1H), 7.98 (d, *J* = 7.7 Hz, 1H), 7.98 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 281.5 $(M+H)^{+}$.

5.1.3.3. Methyl 6-fluoro-7-methyl-2-naphthoate (15c)

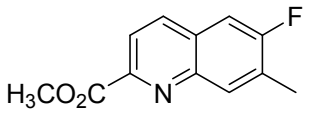


15c (9.61 g, 90%) was prepared from **14c** (10 g, 0.049 mole) by means of the general procedure described above in Step-I as a white solid. mp: 88-90 ^{o}C; Purity by HPLC: 97.62%.

^{1}HNMR (Acetone-*d6*) : δ 2.47 (s, 3H), 3.87 (s, 3H), 7.45 (d, *J* = 1.9 Hz, 1H), 7.66 (s, 1H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.99 (d, *J* = 7.9 Hz, 1H), 8.59 (s, 1H)

ESI/MS (m/z) : 219.4 $(M+H)^{+}$.

5.1.3.4. Methyl 6-fluoro-7-methylquinoline-2-carboxylate (15d)

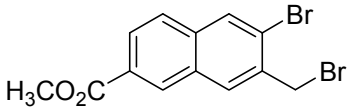


15d (9.12 g, 85%) was prepared from **14d** (10 g, 0.048 mole) by means of the general procedure described above in Step-I as a white solid. mp: 98-99 ^{o}C; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : δ 2.39 (s, 3H), 3.91 (s, 3H), 7.46 (d, *J* = 1.8 Hz, 1H), 7.89 (d, *J* = 7.9 Hz, 1H), 7.99 (s, 1H), 8.15 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 220.4 $(M+H)^{+}$.

5.1.3.5. Methyl 6-bromo-7-(bromomethyl)-2-naphthoate (16a)

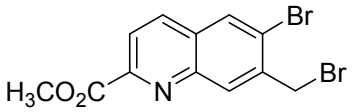


16a (25 g, 78%) was prepared from **15a** (25 g, 0.089 mole) by means of the general procedure described above in Step-II as a white solid. mp: 77-79 ^{o}C; Purity by HPLC: 96.94%.

^{1}HNMR (Acetone-*d6*) : δ 3.92 (s, 3H), 4.69 (s, 2H), 7.53 (s, 1H), 7.77 (d, *J* = 7.9 Hz, 1H), 7.94 (s, 1H), 8.05 (d, *J* = 7.9 Hz, 1H), 8.19 (s, 1H)

ESI/MS (m/z) : 359.0 $(M+H)^{+}$.

5.1.3.6. Methyl 6-bromo-7-(bromomethyl)quinoline-2-carboxylate (16b)

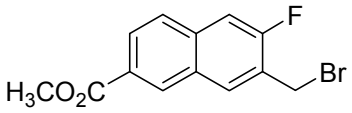


16b (20 g, 78%) was prepared from **15b** (20 g, 0.071 mole) by means of the general procedure described above in Step-II as a white solid. mp: 91-93 ^{o}C; Purity by HPLC: 96.66%.

^{1}HNMR (Acetone-*d6*) : δ 3.80 (s, 3H), 4.66 (s, 2H), 7.88 (s, 1H), 7.92 (s, 1H), 7.95 (d, *J* = 7.7 Hz, 1H), 8.11 (d, *J* = 8.0 Hz, 1H)

ESI/MS (m/z) : 360.3 $(M+H)^{+}$.

5.1.3.7. Methyl 7-(bromomethyl)-6-fluoro-2-naphthoate (16c)

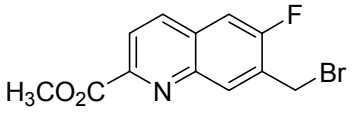


16c (9.9 g, 77%) was prepared from **15c** (9.6 g, 0.044 mole) by means of the general procedure described above in Step-II as a white solid. mp: 68-66 ^{o}C; Purity by HPLC: 96.47%.

^{1}HNMR (Acetone-*d6*) : δ 3.71 (s, 3H), 4.61 (s, 2H), 7.44 (d, *J* = 1.9 Hz, 1H), 7.66 (s, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 7.99 (d, *J* = 7.8 Hz, 1H), 8.51 (s, 1H)

ESI/MS (m/z) : 298.3 $(M+H)^{+}$.

5.1.3.8. Methyl 7-(bromomethyl)-6-fluoroquinoline-2-carboxylate (16d)

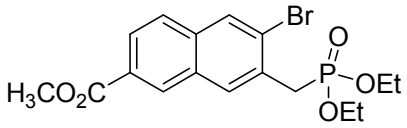


16d (7.34 g, 60%) was prepared from **15d** (9 g, 0.041 mole) by means of the general procedure described above in Step-II as a white solid. mp: 89-91 ^{o}C; Purity by HPLC: 94.44%.

^{1}HNMR (Acetone-*d6*) : δ 3.93 (s, 3H), 4.67 (s, 2H), 7.39 (d, *J* = 1.9 Hz, 1H), 7.83 (d, *J* = 7.9 Hz, 1H), 7.99 (s, 1H), 8.19 (d, *J* = 8.2 Hz, 1H)

ESI/MS (m/z) : 299.4 $(M+H)^+$.

5.1.3.9. Methyl 6-bromo-7-[(diethoxyphosphoryl)methyl]-2-naphthoate (17a)

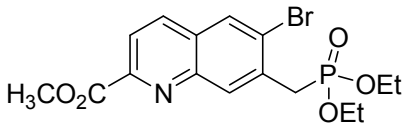


17a (20 g, 69%) was prepared from **16a** (25 g, 0.070 mole) by means of the general procedure described above in Step-III as a white solid. mp: 119-121 ^oC; Purity by HPLC: 97.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.09 (t, *J* = 6.9 Hz, 6H), 3.17 (s, 2H), 3.89 (s, 3H), 4.21 (m, 4H), 7.49 (s, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.95 (s, 1H), 8.00 (d, *J* = 7.8 Hz, 1H), 8.19 (s, 1H)

ESI/MS (m/z) : 416.6 $(M+H)^+$.

5.1.3.10. Methyl 6-bromo-7-[(diethoxyphosphoryl)methyl]-quinoline-2-carboxylate (17b)

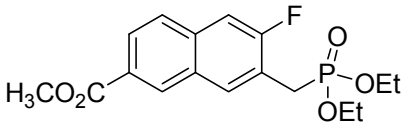


17b (18 g, 78%) was prepared from **16b** (20 g, 0.055 mole) by means of the general procedure described above in Step-III as a white solid. mp: 101-103 ^oC; Purity by HPLC: 96.66%.

^{1}HNMR (Acetone-*d6*) : δ 1.13 (t, *J* = 7.1 Hz, 6H), 3.03 (s, 2H), 3.85 (s, 3H), 4.05 (m, 4H), 7.90 (s, 1H), 7.93 (s, 1H), 7.97 (d, *J* = 7.7 Hz, 1H), 8.13 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 417.2 $(M+H)^+$.

5.1.3.11. Methyl 7-[(diethoxyphosphoryl)methyl]-6-fluoro-2-naphthoate (17c)

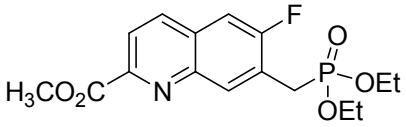


17c (7.08 g, 60%) was prepared from **16c** (9.9 g, 0.033 mole) by means of the general procedure described above in Step-III as a white solid. mp: 109-110 ^{o}C; Purity by HPLC: 97.63%.

^{1}HNMR (Acetone-*d6*) : δ 1.13 (t, *J* = 7.1 Hz, 6H), 3.11 (s, 2H), 3.77 (s, 3H), 4.11 (m, 4H), 7.44 (d, *J* = 1.9 Hz, 1H), 7.65 (s, 1H), 7.81 (d, *J* = 7.9 Hz, 1H), 8.03 (d, *J* = 7.9 Hz, 1H) 8.57 (s, 1H)

ESI/MS (m/z) : 355.5 $(M+H)^{+}$.

5.1.3.12. Methyl 7-[(diethoxyphosphoryl)methyl]-6-fluoroquinoline-2-carboxylate (17d)

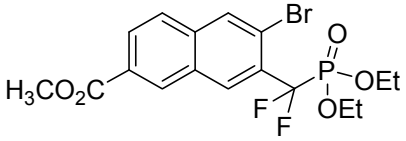


17d (6.04 g, 70%) was prepared from **16d** (7.25 g, 0.024 mole) by means of the general procedure described above in Step-III as a white solid. mp: 117-119 ^{o}C; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.10 (t, *J* = 7.0 Hz, 6H), 3.07 (s, 2H), 3.91 (s, 3H), 4.10 (m, 4H), 7.39 (d, *J* = 1.7 Hz, 1H), 7.87 (d, *J* = 7.7 Hz, 1H) 7.99 (s, 1H), 8.19 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 356.6 $(M+H)^{+}$.

5.1.3.13. Methyl 6-bromo-7-[(diethoxyphosphoryl)difluoromethyl]-2-naphthoate (18a)

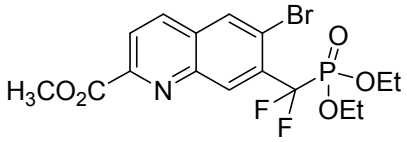


18a (20 g, 92%) was prepared from **17a** (20 g, 0.048 mole) by means of the general procedure described above in Step-IV as a white solid. mp: 77-79 ^{o}C; Purity by HPLC: 99.34%.

^{1}HNMR (Acetone-*d6*) : δ 1.11 (t, *J* = 7.1 Hz, 6H), 3.91 (s, 3H), 4.17 (m, 4H), 7.55 (s, 1H), 7.79 (d, *J* = 8.2 Hz, 1H), 7.97 (s, 1H), 8.07 (d, *J* = 7.9 Hz, 1H), 8.21 (s, 1H)

ESI/MS (m/z) : 452.2 $(M+H)^{+}$.

5.1.3.14. Methyl 6-bromo-7-[(diethoxyphosphoryl)difluoromethyl]-quinoline-2-carboxylate (18b)

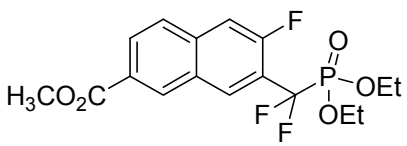


18b (15 g, 77%) was prepared from **17b** (18 g, 0.043 mole) by means of the general procedure described above in Step-IV as a white solid. mp: 83-85 ^{o}C; Purity by HPLC: 98.10%.

^{1}HNMR (Acetone-*d6*) : δ 1.02 (t, *J* = 7.0 Hz, 6H), 3.81 (s, 3H), 4.21 (m, 4H), 7.88 (s, 1H), 7.93 (s, 1H), 7.99 (d, *J* = 7.2 Hz, 1H), 8.11 (d, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 453.7 $(M+H)^{+}$.

5.1.3.15. Methyl 7-[(diethoxyphosphoryl)difluoromethyl]-6-fluoro-2-naphthoate (18c)

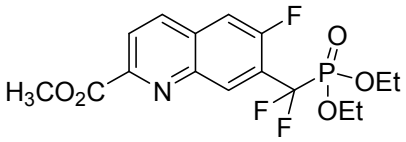


18c (5.09 g, 66%) was prepared from **17c** (7.0 g, 0.019 mole) by means of the general procedure described above in Step-IV as a white solid. mp: 88-90 ^{o}C; Purity by HPLC: 95.77%.

^{1}HNMR (Acetone-*d6*) : δ 1.07 (t, *J* = 7.2 Hz, 6H), 3.93 (s, 3H), 4.13 (m, 4H), 7.47 (d, *J* = 1.9 Hz, 1H), 7.62 (s, 1H), 7.81 (d, *J* = 7.9 Hz, 1H), 8.02 (d, *J* = 7.8 Hz, 1H) 8.47 (s, 1H)

ESI/MS (m/z) : 391.3 $(M+H)^+$.

5.1.3.16. Methyl 7-[(diethoxyphosphoryl)difluoromethyl]6-fluoro Quinoline-2-carboxylate (18d)

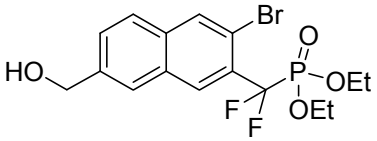


18d (4.95 g, 75%) was prepared from **17d** (6.0 g, 0.017 mole) by means of the general procedure described above in Step-IV as a white solid. mp: 100-101 ^oC; Purity by HPLC: 96.30%.

^{1}HNMR (Acetone-*d6*) : δ 1.13 (t, *J* = 6.9 Hz , 6H), 3.93 (s, 3H), 4.17 (m, 4H), 7.39 (d, *J* = 1.9 Hz, 1H), 7.88 (d, *J* = 7.6 Hz, 1H), 8.03 (s, 1H), 8.14 (d, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 392.3 $(M+H)^+$.

5.1.3.17. Diethyl [3-bromo-7(hydroxymethyl)naphthalene-2-yl]difluoro methylphosphonate (19a)



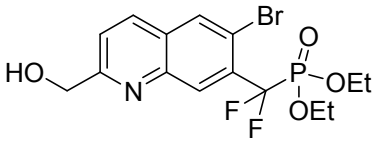
19a (15 g, 80%) was prepared from **18a** (20 g, 0.044 mole) by means of the general procedure described above in Step-V as a white solid. mp: 100-102 ^oC; Purity by HPLC: 99.33%.

^{1}HNMR (Acetone-*d6*) : δ 1.10 (t, *J* = 7.3 Hz, 6H), 2.05 (s, 1H), 4.05 (m, 4H), 4.91 (s, 2H), 7.18 (d, *J* = 7.7 Hz, 1H), 7.29 (s, 1H), 7.35 (s, 1H), 7.60 (d, *J* =

7.9 Hz, 1H), 7.89 (s, 1H)

ESI/MS (m/z) : 424.4 $(M+H)^+$.

5.1.3.18. Diethyl [6-bromo-2-(hydroxymethyl)quinolin-7-yl]difluoromethyl phosponate (19b)

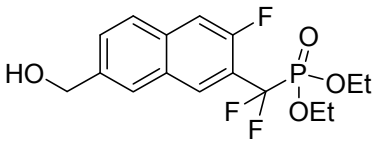


19b (10 g, 71%) was prepared from **18b** (15 g, 0.033 mole) by means of the general procedure described above in Step-V as a white solid. mp: 100-102 ^oC; Purity by HPLC: 97.77%.

^{1}HNMR (Acetone-*d6*) : δ 1.15 (t, *J* = 7.5 Hz, 6H), 2.10 (s, 1H), 4.17 (m, 4H), 5.21 (s, 2H), 7.17 (d, *J* = 7.9 Hz, 1H), 7.73 (s, 1H), 7.87 (s, 1H), 7.99 (d, *J* = 7.3 Hz, 1H)

ESI/MS (m/z) : 425.7 $(M+H)^+$.

5.1.3.19 Diethyl [3-fluoro-7(hydroxymethyl)naphthalene-2-yl]difluoro methylphosphonate (19c)

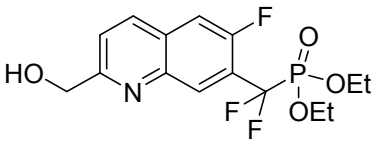


19c (3.15 g, 68%) was prepared from **18c** (5.0 g, 12.82 mmole) by means of the general procedure described above in Step-V as a white solid. mp: 105-106 ^oC; Purity by HPLC: 98.14%.

^{1}HNMR (Acetone-*d6*) : δ 1.11 (t, *J* = 7.2 Hz, 6H), 2.11 (s, 1H), 4.09 (m, 4H), 4.87 (s, 2H), 7.13 (d, *J* = 7.9 Hz, 1H), 7.33 (m, 1H), 7.41-7.44 (m, 2H), 7.69 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 363.3 $(M+H)^+$.

5.1.3.20. Diethyl [6-fluoro-2-(hydroxymethyl)quinolin-7-yl]difluoromethyl phosphonate (19d)

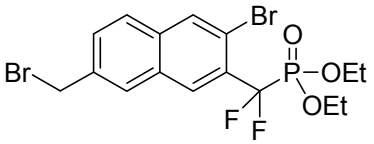


19d (2.91 g, 66%) was prepared from **18d** (4.75 g, 12.14 mmole) by means of the general procedure described above in Step-V as a white solid. mp: 110-112 ^{o}C; Purity by HPLC: 96.96%.

^{1}HNMR (Acetone-*d6*) : δ 1.09 (t, *J* = 7.2 Hz, 6H), 2.11 (s, 1H), 4.11 (m, 4H), 5.25 (s, 2H), 7.14 (d, *J* = 7.8 Hz, 1H), 7.37 (d, *J* = 1.9 Hz, 1H), 7.81(s, 1H), 7.97 (s, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 364.5 $(M+H)^{+}$.

5.1.3.21. Diethyl [3-bromo-7-(bromomethyl)naphthalen-2-yl]difluoro methylphosphonate (3a)

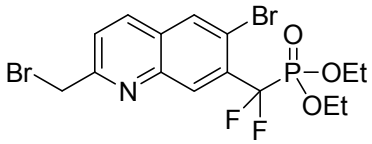


3a (10 g, 58%) was prepared from **19a** (15 g, 0.035 mole) means of the general procedure described above in Step-VI as a white solid. mp: 102-103 ^{o}C; Purity by HPLC: 99.42%.

^{1}HNMR (Acetone-*d6*) : δ 1.33 (t, *J* = 7.1 Hz, 6H), 4.21 (m, 4H), 4.67 (s, 2H), 7.19 (d, *J* = 8.1 Hz, 1H), 7.31 (s, 1H), 7.42 (s, 1H), 7.63 (d, *J* = 7.9 Hz, 1H), 7.89 (s, 1H)

ESI/MS (m/z) : 487.3 $(M+H)^{+}$.

5.1.3.22. Diethyl [6-bromo-2-(bromomethyl)quinolin-7-yl]difluoromethyl phosphonate (3b)

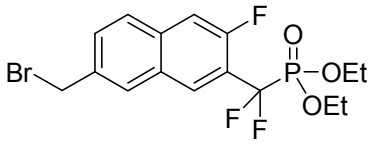


3b (10 g, 87%) was prepared from **19b** (10 g, 0.023 mole) by means of the general procedure described in Step-VI above as a white solid. mp: 111-113 ^{o}C; Purity by HPLC: 98.09%.

^{1}HNMR (Acetone-*d6*) : δ 1.23 (t, *J* = 7.1 Hz, 6H), 4.09 (m, 4H), 4.91 (s, 2H), 7.15 (d, *J* = 8.1 Hz, 1H), 7.70 (s, 1H), 7.87 (s, 1H), 7.99 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 488.6 $(M+H)^{+}$.

5.1.3.23. Diethyl [7-(bromomethyl)-3-fluoronaphthalen-2-yl]difluoro methylphosphonate (11c)

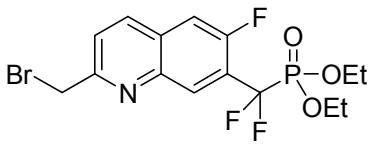


11c (1.93 g, 55%) was prepared from **19c** (3.0 g, 8.28 mmole) means of the general procedure described above in Step-VI as a white solid. mp: 111-113 ^{o}C; Purity by HPLC: 98.95%.

^{1}HNMR (Acetone-*d6*) : δ 1.35 (t, *J* = 7.3 Hz, 6H), 4.23 (m, 4H), 471 (s, 2H), 7.17 (d, *J* = 8.0 Hz, 1H), 7.37 (s, 1H), 7.42-7.45 (m, 2H), 7.67 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 426.6 $(M+H)^{+}$.

5.1.3.24. Diethyl [2-(bromomethyl)-6-fluoroquinolin-7-yl]difluoromethyl phosphonate (11d)



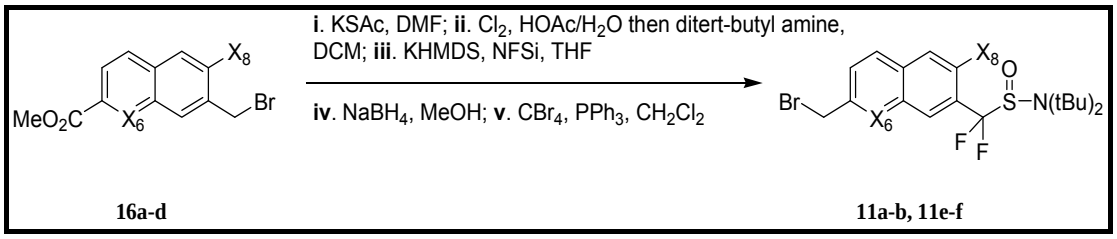
11d (1.7 g, 52%) was prepared from **19d** (2.8 g, 7.71 mmole) by means of the

general procedure described in Step-VI above as a white solid. mp: 110-111 ^{o}C; Purity by HPLC: 98.80%.

^{1}HNMR (Acetone-*d6*) : δ 1.27 (t, *J* = 7.2 Hz , 6H), 4.06 (m, 4H), 4.90 (s, 2H), 7.11 (d, *J* = 8.0 Hz, 1H), 7.32 (d, *J* = 1.9 Hz, 1H), 7.79 (s, 1H), 7.96 (d, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 427.4 $(M+H)^{+}$.

5.1.4. General procedure for the synthesis of Compounds (11a-b, 11e-f)



Step I: A solution of **16a-d** (1 mole equivalent) and potassium thioacetate (1.03 mole equivalent) in DMF (3 fold) was stirred at 25 ^{o}C for 30 min. The reaction was diluted with water (6 fold) and extracted with CH_2Cl_2 (2 X 3 fold). The combined organics were dried over Na_2SO_4, and then concentrated, and the resulting solid residue recrystalized in CH_2Cl_2/hexane which gave title compound **20a-d** as crystalline white solids.

Step II: Chlorine gas was bubbled through a suspension of thioacetate **20a-d** (1 mole equivalent) in glacial acetic acid (3 fold) and ice water (1 fold) at 0 ^{o}C for 1 hour. Nitrogen was then bubbled through the solution to remove excess Cl_2 and then mixture was diluted with water (2 fold) and extracted with CH_2Cl_2 (2 X 2 fold). The combined organics were dried over Na_2SO_4 and concentrated. The resulting sulfonyl chloride was unstable and used immediately for the next step. To a solution of crude product in CH_2Cl_2 (10 fold) was added ditert-butyl amine (2.5 mole equivalent) and the mixture was

stirred for 2 hours at 25 ^{o}C. Water (3 fold) was added and the mixture was extracted with CH_2Cl_2 (2 X 10 fold) and the combined organics were dried over Na_2SO_4 and concentrated. The resulting residue was subjected to flash chromatography using 5% ethyl acetate in hexane as eluent gave the desired product **21a-d** as white solids.

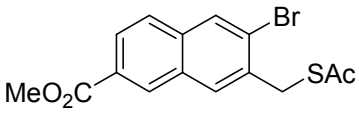
Step III: To a solution of **21a-d** (1 mole equivalent) and N-fluorobenzenesulfonamide (2.5 mole equivalent) in dry THF (8 fold) at -78 ^{o}C was added KHMDS (1.0 M in THF, 2.5 mole equivalent) over 30 min. The mixture was stirred for 2 hours at -78 ^{o}C, then allowed to warm to 25 ^{o}C, and stirred for 18 hours. The reaction was quenched with a saturate aqueous NH_4Cl solution, extracted with ethyl acetate (2 X 20 fold). The combined organic layers were washed successively with water, followed by brine, and dried over Na_2SO_4, filtered and concentrated under vacuum to get the crude product which was subjected to flash by chromatography using 15% ethyl acetate in hexane as eluent to furnish title compound **22a-d** as white solids.

Step IV: A suspension of **22a-d** (1 mole equivalent) and $NaBH_4$ (6.0 mole equivalent) in MeOH (15 fold) under N_2 atmosphere, was stirred at 65 ^{o}C for 3 hours. The reaction mixture was quenched with a saturated aqueous NH_4Cl solution, water was added and the mixture was extracted with ethyl acetate (3 X 20 fold). The combined organics were dried over Na_2SO_4 and concentrated. The residue was subjected to column chromatography (10% ethyl acetate in hexane) which gave pure products **23a-d** as white products.

Step V: To a solution of **23a-d** (1 mole equivalent) in dry CH_2Cl_2 (20 fold) at 0 ^{o}C were added carbon tetrabromide (1.2 mole equivalent) and triphenylphosphine (1.2 mole equivalent) and stirred for 2 hours. The mixture

was concentrated at room temperature by rotary evaporation and the resulting residue subjected to flash chromatography using 10% ethyl acetate in hexane which gave corresponding bromo derivatives **11a-b, 11e-f** as white solids.

5.1.4.1. Methyl 7-(acetylthiomethyl)-6-bromo-2-naphthoate (20a)

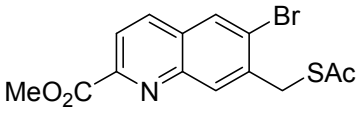


20a (20 g, 81%) was prepared from **16a** (25 g, 0.069 mole) by means of the general procedure described in Step-I above as a white solid. mp: 66-64 ^{o}C; Purity by HPLC: 95.55%.

^{1}HNMR (Acetone-*d6*) : *δ* 2.23 (s, 3H), 3.98 (s, 3H), 4.31 (s, 2H), 7.35 (s, 1H), 7.77 (d, *J* = 7.9 Hz, 1H), 7.98 (s, 1H), 8.10 (d, *J* = 7.8 Hz, 1H), 8.39 (s, 1H)

ESI/MS (m/z) : 354.4 $(M+H)^{+}$.

5.1.4.2. Methyl 7-(acetylthiomethyl)-6-bromoquinoline-2-carboxylate (20b)

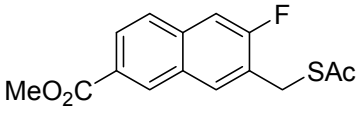


20b (11 g, 74%) was prepared from **16b** (15 g, 0.041 mole) by means of the general procedure described in Step-I above as a white solid. mp: 88-84 ^{o}C; Purity by HPLC: 97.55%.

^{1}HNMR (Acetone-*d6*) : *δ* 2.33 (s, 3H), 3.82 (s, 3H), 4.17 (s, 2H), 7.68 (s, 1H), 7.77 (s, 1H), 7.98 (d, *J* = 7.7 Hz, 1H), 8.19 (d, *J* = 7.3 Hz, 1H)

ESI/MS (m/z) : 355.5 $(M+H)^{+}$.

5.1.4.3. Methyl 7-(acetylthiomethyl)-6-fluoro-2-naphthoate (20c)

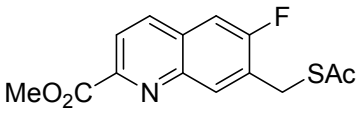


20c (8.85 g, 90%) was prepared from **16c** (10 g, 0.033 mole) by means of the general procedure described in Step-I above as a white solid. mp: 77-78 ^{o}C; Purity by HPLC: 93.10%.

^{1}HNMR (Acetone-*d6*) : δ 2.17 (s, 3H), 3.81 (s, 3H), 4.27 (s, 2H), 7.39 (d, *J* = 1.9 Hz, 1H), 7.62 (s, 1H), 7.78 (d, *J* = 7.9 Hz, 1H), 7.96 (d, *J* = 7.7 Hz, 1H), 8.43 (s, 1H)

ESI/MS (m/z) : 293.7 $(M+H)^{+}$.

5.1.4.4. Methyl 7-(acetylthiomethyl)-6-fluoroquinoline-2-carboxylate (20d)

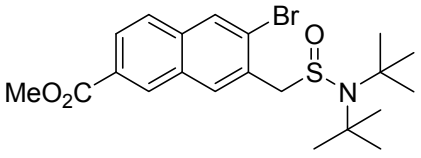


20d (3.93 g, 80%) was prepared from **16d** (5 g, 0.016 mole) by means of the general procedure described in Step-I above as a white solid. mp: 98-99 ^{o}C; Purity by HPLC: 96.60%.

^{1}HNMR (Acetone-*d6*) : δ 2.33 (s, 3H), 3.91 (s, 3H), 4.21 (s, 2H), 7.45 (d, *J* = 1.7 Hz, 1H), 7.88-7.96 (m, 2H), 8.09 (d, *J* = 7.3 Hz, 1H)

ESI/MS (m/z) : 294.4 $(M+H)^{+}$.

5.1.4.5. Methyl 6-bromo-7-(*N*,*N*-di-tert-butylsulfinylmethyl)-2-naphthoate (21a)

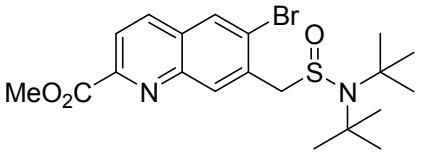


21a (20 g, 77%) was prepared from **20a** (20 g, 0.056 mole) by means of the general procedure described in Step-II above as a white solid. mp: 81-83 ^{o}C; Purity by HPLC: 94.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.18 (s, 18H), 3.78 (s, 3H), 3.99 (s, 2H), 7.53 (s, 1H), 7.80 (d, *J* = 7.7 Hz, 1H), 7.95 (s, 1H), 8.14 (d, *J* = 7.7 Hz, 1H), 8.22 (s, 1H)

ESI/MS (m/z) : 455.7 $(M+H)^+$.

5.1.4.6. Methyl 6-bromo-7-(*N*,*N*-di-tert-butylsulfinylmethyl)quinoline-2-carboxylate (21b)

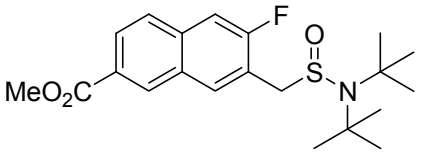


21b (13 g, 92%) was prepared from **20b** (15 g, 0.031 mole) by means of the general procedure described in Step-II above as a white solid. mp: 97-99 ^oC; Purity by HPLC: 96.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.21 (s, 18H), 3.80 (s, 2H), 3.92 (s, 3H), 7.88 (s, 1H), 7.93 (s, 1H), 7.99 (d, *J* = 7.9 Hz, 1H), 8.15 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 456.7 $(M+H)^+$.

5.1.4.7. Methyl 7-(*N*,*N*-di-tert-butylsulfinylmethyl)-6-fluoro-2-naphthoate (21c)

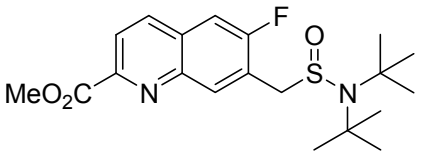


21c (8.6 g, 75%) was prepared from **20c** (8.5 g, 0.029 mole) by means of the general procedure described in Step-II above as a white solid. mp: 69-71 ^oC; Purity by HPLC: 95.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.21 (s, 18H), 3.77 (s, 3H), 3.89 (s, 2H), 7.38 (d, *J* = 1.9 Hz, 1H), 7.60 (s, 1H), 7.80 (d, *J* = 7.7 Hz, 1H), 7.99 (d, *J* = 7.9 Hz, 1H), 8.53 (s, 1H)

ESI/MS (m/z) : 394.5 $(M+H)^+$.

5.1.4.8. Methyl 7-(*N*,*N*-di-tert-butylsulfinylmethyl)-6-fluoroquinoline-2-carboxylate (21d)

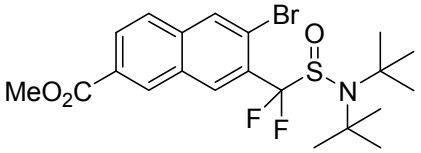


21d (3.67 g, 70%) was prepared from **20d** (3.9 g, 0.013 mole) by means of the general procedure described in Step-II above as a white solid. mp: 88-90 ^oC; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.17 (s, 18H), 3.79 (s, 2H), 3.94 (s, 3H), 7.39 (d, *J* = 1.9 Hz, 1H), 7.87 (d, *J* = 7.7 Hz, 1H), 8.01-8.13 (m, 2H)

ESI/MS (m/z) : 395.7 $(M+H)^+$.

5.1.4.9. Methyl 6-bromo-7-(*N*,*N*-di-tert-butyldifluoromethanesulfinyl)-2-naphthoate (22a)

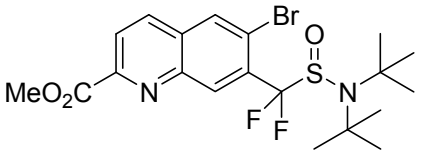


22a (15 g, 69%) was prepared from **21a** (20 g, 0.044 mole) by means of the general procedure described in Step-III above as a white solid. mp: 81-83 ^oC; Purity by HPLC: 94.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.11 (s, 18H), 3.88 (s, 3H), 7.57 (s, 1H), 7.77 (d, *J* = 7.9 Hz, 1H), 7.97 (s, 1H), 8.10 (d, *J* = 7.9 Hz, 1H), 8.23 (s, 1H)

ESI/MS (m/z) : 491.6 $(M+H)^+$.

5.1.4.10. Methyl 6-bromo-7-(*N*,*N*-di-tert-butuldifluoromethane sulfinyl)quioline-2-carboxylate (22b)

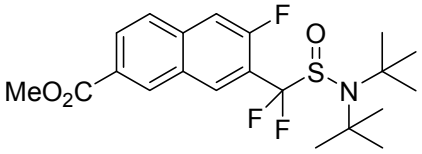


22b (10 g, 71%) was prepared from **21b** (20 g, 0.028 mole) by means of the general procedure described in Step-III above as a white solid. mp: 88-90 ^{o}C; Purity by HPLC: 99.07%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.23 (s, 18H), 3.98 (s, 3H), 7.88 (s, 1H), 7.95 (s, 1H), 7.99 (d, *J* = 7.9 Hz, 1H), 8.19 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 492.7 $(M+H)^{+}$.

5.1.4.11. Methyl 7-(*N*,*N*-di-tert-butyldifluoromethanesulfinyl)-6-fluoro-2-naphthoate (22c)

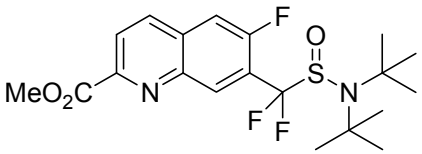


22c (6.03 g, 65%) was prepared from **21c** (8.5 g, 0.021 mole) by means of the general procedure described in Step-III above as a white solid. mp: 103-104 ^{o}C; Purity by HPLC: 93.66%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.19 (s, 18H), 3.91 (s, 3H), 7.43 (d, *J* = 1.9 Hz, 1H), 7.60 (s, 1H), 7.79 (d, *J* = 7.9 Hz, 1H), 7.97 (d, *J* = 7.8 Hz, 1H), 8.53 (s, 1H)

ESI/MS (m/z) : 430.4 $(M+H)^{+}$.

5.1.4.12. Methyl 7-(*N*,*N*-di-tert-butuldifluoromethanesulfinyl)-6-fluoro-quinoline-2-carboxylate (22d)

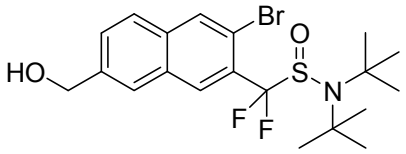


22d (3.05 g, 80%) was prepared from **21d** (3.5 g, 8.87 mmole) by means of the general procedure described in Step-III above as a white solid. mp: 111-112 ^{o}C; Purity by HPLC: 98.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.09 (s, 18H), 3.93 (s, 3H), 7.47 (d, *J* = 1.8 Hz, 1H), 7.88-8.10 (m, 3H)

ESI/MS (m/z) : 431.6 $(M+H)^{+}$.

5.1.4.13. [3-bromo-7-(hydroxymethyl)naphthalen-2-yl]-*N*,*N*-di-tert-butyldifluoromethanesulfinamide (23a)

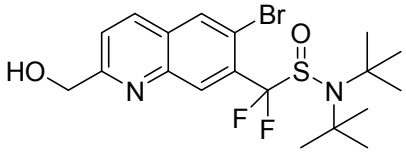


23a (13 g, 91%) was prepared from **22a** (15 g, 0.030 mole) by means of the general procedure described in Step-IV above as a white solid. mp: 99-101 ^{o}C; Purity by HPLC: 97.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.21 (s, 18H), 4.91 (s, 2H), 7.19 (d, *J* = 7.9 Hz, 1H), 7.31 (s, 1H), 7.41 (s, 1H), 7.63 (d, *J* = 7.8 Hz, 1H), 7.88 (s, 1H)

ESI/MS (m/z) : 463.7 $(M+H)^{+}$.

5.1.4.14. [6-bromo-2-(hydroxymethyl)quinolin-7-yl]-*N*,*N*-di-tert-butyl difluoromethanesulfinamide (23b)

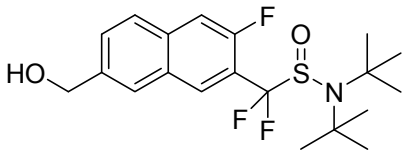


23b (8 g, 85%) was prepared from **22b** (10 g, 0.020 mole) by means of the general procedure described in Step-IV above as white solid. mp: 117-119 ^{o}C; Purity by HPLC: 97.10%.

^{1}HNMR (Acetone-*d6*) : δ 1.15 (s, 18H), 5.19 (s, 2H), 7.17 (d, *J* = 7.8 Hz, 1H), 7.77 (s, 1H), 7.87 (s, 1H), 7.99 (d, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 464.7 $(M+H)^+$.

5.1.4.15. [3-fluoro-7-(hydroxymethyl)naphthalen-2-yl]-*N*,*N*-di-tert-butyl difluoromethanesulfonimide (23c)

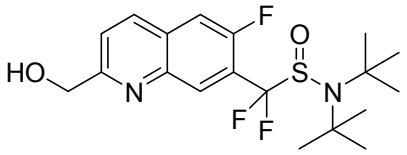


23c (3.36 g, 60%) was prepared from **22c** (6.0 g, 0.014 mole) by means of the general procedure described in Step-IV above as a white solid. mp: 117-119 ^oC; Purity by HPLC: 95.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.23 (s, 18H), 4.87 (s, 2H), 7.13 (d, *J* = 7.7 Hz, 1H), 7.35 (d, *J* = 1.8 Hz, 1H), 7.40 (s, 1H), 7.47 (s, 1H), 7.68 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 402.4 $(M+H)^+$.

5.1.4.16. [6-fluoro-2-(hydroxymethyl)quinolin-7-yl]-*N*,*N*-di-tert-butyl difluoromethanesulfonamide (23d)

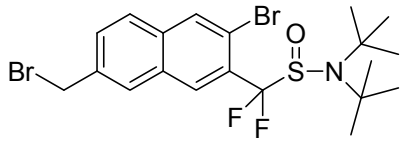


23d (1.68 g, 60%) was prepared from **22d** (3.0 g, 6.96 mmole) by means of the general procedure described in Step-IV above as a white solid. mp: 123-124 ^oC; Purity by HPLC: 96.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.21 (s, 18H), 5.13 (s, 2H), 7.08 (d, *J* = 7.9 Hz, 1H), 7.36 (d, *J* = 1.9 Hz, 1H), 7.80 (m, 1H), 7.96 (d, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 403.8 $(M+H)^+$.

5.1.4.17. [3-bromo-7-(bromomethyl)naphthalen-2-yl]-*N*,*N*-di-tert-butyl difluoromethanesulfinamide (11a)

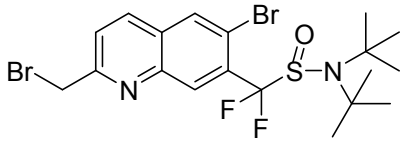


11a (10 g, 67%) was prepared from **23a** (13 g, 0.028 mole) by means of the general procedure described in Step-V above as a white solid. mp: 92-94 ^{o}C; Purity by HPLC: 97.77%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.23 (s, 18H), 4.77 (s, 2H), 7.21 (d, *J* = 8.0 Hz, 1H), 7.30 (s, 1H), 7.43 (s, 1H), 7.67 (d, *J* = 7.9 Hz, 1H), 7.90 (s, 1H)

ESI/MS (m/z) : 526.4 $(M+H)^{+}$.

5.1.4.18. (6-bromo-2-(bromomethyl)quinolin-7-yl)-*N*,*N*-di-tert-butyl difluoromethanesulfinamide (11b)

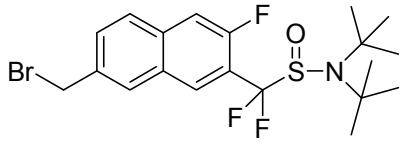


11b (5 g, 55%) was prepared from **23b** (8 g, 0.072 mole) by means of the general procedure described in Step-V above as a white solid. mp: 89-91 ^{o}C; Purity by HPLC: 97.66%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.17 (s, 18H), 4.91 (s, 2H), 7.15 (d, *J* = 8.2 Hz, 1H), 7.71 (s, 1H), 7.87 (s, 1H), 7.99 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 527.5 $(M+H)^{+}$.

5.1.4.19. [7-(bromomethyl)-3-fluoronaphthalen-2-yl]-*N*,*N*-di-tert-butyl difluoromethanesulfinamide (11e)

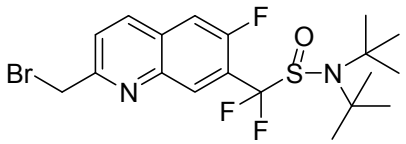


11e (2.29 g, 62%) was prepared from **23c** (3.2 g, 7.97 mmole) by means of the general procedure described in Step-V above as a white solid. mp: 99-100 ^{o}C; Purity by HPLC: 98.10%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.18 (s, 18H), 4.65 (s, 2H), 7.13 (d, *J* = 8.1 Hz, 1H), 7.29 (d, *J* = 1.7 Hz, 1H), 7.41 (s, 1H), 7.47 (s, 1H), 7.69 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 465.6 $(M+H)^{+}$.

5.1.4.20. [2-(bromomethyl)-6-fluoroquinolin-7-yl]-*N*,*N*-di-tert-butyldifluoro methanesulfinamide (11f)

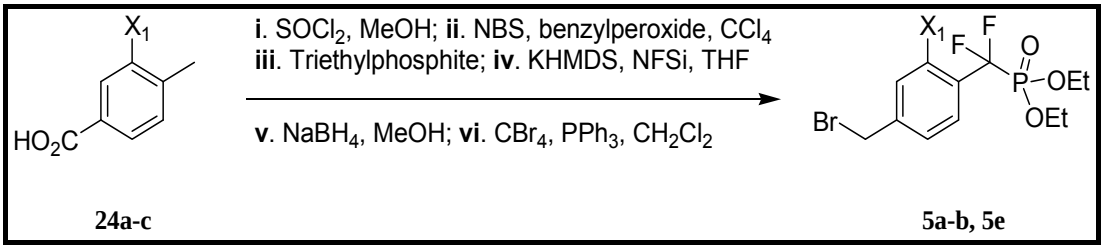


11f (1.73 g, 67%) was prepared from **23d** (1.5 g, 3.72 mmole) by means of the general procedure described in Step-V above as a white solid. mp: 87-88 ^{o}C; Purity by HPLC: 99.10%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.20 (s, 18H), 4.85 (s, 2H), 7.07 (d, *J* = 8.0 Hz, 1H), 7.29 (d, *J* = 1.9 Hz, 1H), 7.79 (s, 1H), 7.98 (d, *J* = 7.7 Hz, 1H)

ESI/MS (m/z) : 466.6 $(M+H)^{+}$.

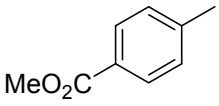
5.1.5. General procedure for the synthesis of Compounds (5a-c)



24a-c **5a-b, 5e**

Title compounds **5a-b, 5e** were prepared using the same procedure as that used for compound **3a-b** using appropriate starting material.

5.1.5.1. Methyl 4-methylbenzoate (25a)

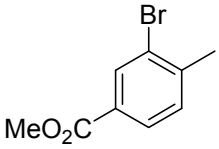


25a (10 g, 91%) was prepared from **24a** (10 g, 0.073 mole) by means of the general procedure described in **5.1.3.** (step-I) as a white solid. mp: 33-35 ^{o}C; Purity by HPLC: 99.00%.

^{1}HNMR (Acetone-*d6*) : δ 2.29 (s, 3H), 3.90 (s, 3H), 7.29 (d, *J* = 7.0 Hz, 2H), 7.89 (d, *J* = 8.0 Hz, 2H)

ESI/MS (m/z) : 151.4 $(M+H)^{+}$.

5.1.5.2. Methyl 3-bromo-4-methylbenzoate (25b)

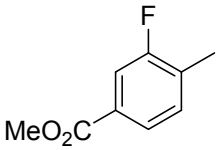


25b (10 g, 93%) was prepared from **24b** (10 g, 0.046 mole) by means of the general procedure described in **5.1.3.** (step-I) as a white solid. mp: 66-67 ^{o}C; Purity by HPLC: 98.78%.

^{1}HNMR (Acetone-*d6*) : δ 2.11 (s, 3H), 3.77 (s, 3H), 7.20 (d, *J* = 7.7 Hz, 1H), 7.88 (d, *J* = 7.9 Hz, 1H), 8.10 (s, 1H)

ESI/MS (m/z) : 230.6 $(M+H)^{+}$.

5.1.5.3. Methyl 3-fluoro-4-methylbenzoate (25c)

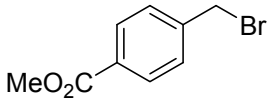


25c (24.5 g, 90%) was prepared from **24c** (25 g, 0.162 mole) by means of the general procedure described in **5.1.3.** (step-I) as a white solid. mp: 44-46 ^{o}C; Purity by HPLC: 93.40%.

^{1}HNMR (Acetone-*d6*) : δ 2.39 (s, 3H), 3.87 (s, 3H), 7.17 (d, *J* = 7.8 Hz, 1H), 7.59 (d, *J* = 1.9 Hz, 1H), 7.71 (d, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 169.2 $(M+H)^+$.

5.1.5.4. Methyl 4-(bromomethyl)benzoate (26a)

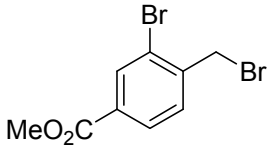


26a (10 g, 65%) was prepared from **25a** (10 g, 0.066 mole) by means of the general procedure described in **5.1.3.** (step-II) as a white solid. mp: 57-58 ^oC; Purity by HPLC: 96.96%.

^{1}HNMR (Acetone-*d6*) : δ 3.69 (s, 3H), 4.55 (s, 2H), 7.22 (d, *J* = 7.9 Hz, 2H), 7.93 (d, *J* = 7.8 Hz, 2H)

ESI/MS (m/z) : 230.7 $(M+H)^+$.

5.1.5.5. Methyl 3-bromo-4-(bromomethyl)benzoate (26b)

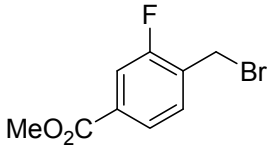


26b (10 g, 74%) was prepared from **25b** (10 g, 0.043 mole) by means of the general procedure described in **5.1.3.** (step-II) as a white solid. mp: 72-74 ^oC; Purity by HPLC: 95.50%.

^{1}HNMR (Acetone-*d6*) : δ 3.73 (s, 3H), 4.44 (s, 2H), 7.13 (d, *J* = 7.1 Hz, 1H), 7.72 (d, *J* = 7.5 Hz, 1H), 8.01 (s, 1H)

ESI/MS (m/z) : 309.4 $(M+H)^+$.

5.1.5.6. Methyl 4-(bromomrthyl)-3-fluorobenzoate (26c)

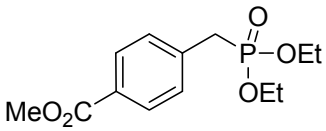


26c (21.1 g, 60%) was prepared from **25c** (24 g, 0.142 mole) by means of the general procedure described in **5.1.3.** (step-II) as a white solid. mp: 68-70 ^{o}C; Purity by HPLC: 96.60%.

^{1}HNMR (Acetone-*d6*) : δ 3.82 (s, 3H), 4.61 (s, 2H), 7.13 (d, *J* = 7.9 Hz, 1H), 7.52 (d, *J* = 1.8 Hz, 1H), 7.68 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 248.4 $(M+H)^{+}$.

5.1.5.7. Methyl 4-[(diethoxyphosphoryl)methyl]benzoate (27a)

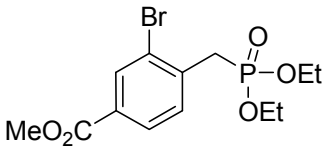


27a (9 g, 72%) was prepared from **26a** (10 g, 0.043 mole) by means of the general procedure described in **5.1.3.** (step-III) as a white solid. mp: 63-65 ^{o}C; Purity by HPLC: 95.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.19 (t, *J* = 7.1 Hz, 6H), 3.19 (s, 2H), 3.87 (s, 3H), 4.10-4.19 (m, 4H), 7.29 (d, *J* = 7.3 Hz, 2H), 7.89 (d, *J* = 7.3 Hz, 2H)

ESI/MS (m/z) : 287.7 $(M+H)^{+}$.

5.1.5.8. Methyl 3-bromo-4-[(diethoxyphosphoryl)methyl]benzoate (27b)

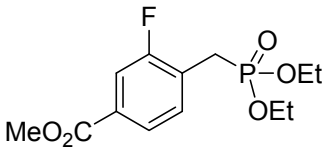


27b (9 g, 76%) was prepared from **26b** (10 g, 0.032 mole) by means of the general procedure described in **5.1.3.** (step-III) as a white solid. mp: 89-91 ^{o}C; Purity by HPLC: 94.44%.

^{1}HNMR (Acetone-*d6*) : δ 1.21 (t, *J* = 7.1 Hz, 6H), 3.39 (s, 2H), 3.88 (s, 3H), 4.01-4.11 (m, 4H), 7.43 (d, *J* = 8.0 Hz, 1H), 7.87 (d, *J* = 7.9 Hz, 1H), 8.15 (s, 1H)

ESI/MS (m/z) : 366.6 $(M+H)^+$.

5.1.5.9. Methyl 4-[(diethoxyphosphoryl)methyl]-3-fluorobenzoate (27c)

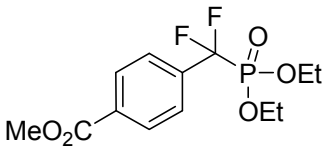


27c (18.1 g, 70%) was prepared from **26c** (21 g, 0.085 mole) by means of the general procedure described in **5.1.3.** (step-III) above as a white solid. mp: 71-73 ^oC; Purity by HPLC: 94.10%.

^{1}HNMR (Acetone-*d6*) : δ 1.15 (t, *J* = 6.9 Hz, 6H), 2.99 (s, 2H), 3.91 (s, 3H), 4.09 (m, 4H), 7.09 (d, *J* = 7.8 Hz, 1H), 7.49 (d, *J* = 1.9 Hz, 1H), 7.61 (d, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 305.4 $(M+H)^+$.

5.1.5.10. Methyl 4-[(diethoxyphosphoryl)difluoromethyl]benzoate (28a)

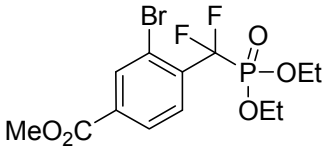


28a (8 g, 79%) was prepared from **27a** (10 g, 0.031 mole) by means of the general procedure described in **5.1.3.** (step-IV) as a white solid. mp: 99-101 ^oC; Purity by HPLC: 93.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.27 (t, *J* = 7.3 Hz, 6H), 3.91 (s, 3H), 4.07-4.21 (m, 4H), 7.37 (d, *J* = 7.7 Hz, 2H), 7.97 (d, *J* = 7.2 Hz, 2H)

ESI/MS (m/z) : 323.4 $(M+H)^+$.

5.1.5.11. Methyl 3-bromo-4-[(diethoxyphosphoryl)difluoromethyl] benzoate (28b)

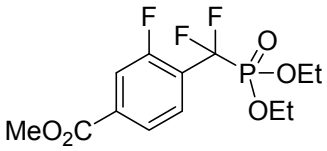


28b (8 g, 81%) was prepared from **27b** (9 g, 0.024 mole) by means of the general procedure described in **5.1.3.** (step-IV) as a white solid. mp: 35-37 ^{o}C; Purity by HPLC: 95.55%.

^{1}HNMR (Acetone-*d6*) : δ 1.10 (t, *J* = 7.2 Hz, 6H), 3.69 (s, 3H), 3.99-4.07 (m, 4H), 7.47 (d, *J* = 8.3 Hz, 1H), 7.77 (d, *J* = 7.9 Hz, 1H), 8.07 (s, 1H)

ESI/MS (m/z) : 402.2 $(M+H)^{+}$.

5.1.5.12. Methyl 4-[(diethoxyphosphoryl)difluoromethyl]-3-fluoro benzoate (28c)

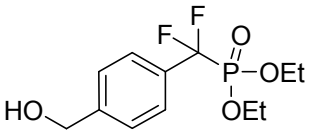


28c (13 g, 65%) was prepared from **27c** (18 g, 0.059 mole) by means of the general procedure described in **5.1.3.** (step-IV) as white solid. mp: 100-102 ^{o}C; Purity by HPLC: 95.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.17 (t, *J* = 6.8 Hz, 6H), 3.83 (s, 3H), 4.13 (m, 4H), 7.11 (d, *J* = 7.9 Hz, 1H), 7.53 (d, *J* = 1.7 Hz, 1H), 7.67 (d, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 340.2 $(M+H)^{+}$.

5.1.5.13. Diethyl [(4-hydroxymethylphenyl)difluoromethyl]phosphonate (29a)

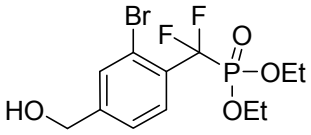


29a (6 g, 82%) was prepared from **28a** (8 g, 0.024 mole) by means of the general procedure described in **5.1.3.** (step-V) as a white solid. mp: 100-101 ^{o}C; Purity by HPLC: 97.77%.

^{1}HNMR (Acetone-*d6*) : δ 1.22 (t, *J* = 7.1 Hz, 6H), 3.69 (s, 1H), 4.02-4.10 (m, 4H), 4.55 (s, 2H), 7.15 (d, *J* = 8.2 Hz, 2H), 7.24 (d, *J* = 8.0 Hz, 2H)

ESI/MS (m/z) : 295.6 $(M+H)^+$.

5.1.5.14. Diethyl [(2-bromo-4-hydroxymethylphenyl)difluoromethyl] phosphonate (29b)

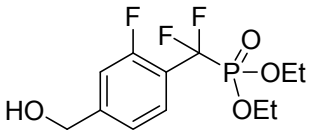


29b (5 g, 67%) was prepared from **28b** (8 g, 0.020 mole) by means of the general procedure described in **5.1.3.** (step-V) as a white solid. mp: 93-95 ^oC; Purity by HPLC: 96.66%.

^{1}HNMR (Acetone-*d6*) : δ 1.27 (t, *J* = 7.3 Hz, 6H), 3.59 (s, 1H), 4.07-4.17 (m, 4H), 4.67 (s, 2H), 7.22 (d, *J* = 7.2 Hz, 1H), 7.45 (d, *J* = 8.1 Hz, 1H), 7. 61 (s, 1H)

ESI/MS (m/z) : 374.5 $(M+H)^+$.

5.1.5.15. Diethyl difluoro[2-fluoro-4-(hydroxymethyl)phenyl)methyl phosphonate (29c)

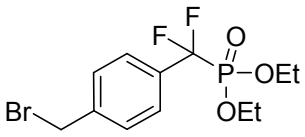


29c (9.17 g, 80%) was prepared from **28c** (12.5 g, 0.036 mole) by means of the general procedure described in **5.1.3.** (step-V) as a white solid. mp: 103-104 ^oC; Purity by HPLC: 97.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.09 (t, *J* = 6.9 Hz, 6H), 4.19 (m, 4H), 4.81 (s, 2H), 6.81 (d, *J* = 7.8 Hz, 1H), 6.81 (d, *J* = 1.9 Hz, 1H), 7.09 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 313.3 $(M+H)^+$.

5.1.5.16. Diethyl [(4-bromomethylphenyl)difluoromethyl]phosphonate (5a)

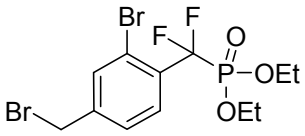


5a (5 g, 68%) was prepared from **29a** (6 g, 0.020 mole) by means of the general procedure described in **5.1.3.** (step-VI) as a white solid. mp: 55-57 ^{o}C; Purity by HPLC: 98.50%.

^{1}HNMR (Acetone-*d6*) : δ 1.17 (t, *J* = 7.3 Hz, 6H), 4.03-4.17 (m, 4H), 4.57 (s, 2H), 7.03 (d, *J* = 8.1 Hz, 2H), 7.17 (d, *J* = 8.1 Hz, 2H)

ESI/MS (m/z) : 358.1 $(M+H)^{+}$.

5.1.5.17. Diethyl [(2-bromo-4-bromomethylphenyl)difluoromethyl] phosphonate (5b)

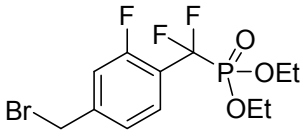


5b (4 g, 68%) was prepared from **29b** (5 g, 0.013 mole) by means of the general procedure described in **5.1.3.** (step-IV) as a white solid. mp: 88-90 ^{o}C; Purity by HPLC: 98.01%.

^{1}HNMR (Acetone-*d6*) : δ 1.22 (t, *J* = 7.0 Hz, 6H), 4.06-4.21 (m, 4H), 4.35 (s, 2H), 7.29 (d, *J* = 7.9 Hz, 1H), 7.49 (d, *J* = 8.2 Hz, 1H), 7. 66 (s, 1H)

ESI/MS (m/z) : 437.2 $(M+H)^{+}$.

5.1.5.18. Diethyl [4-(bromomethyl)-2-fluorophenyl]difluoromethyl phosphonate (5e)

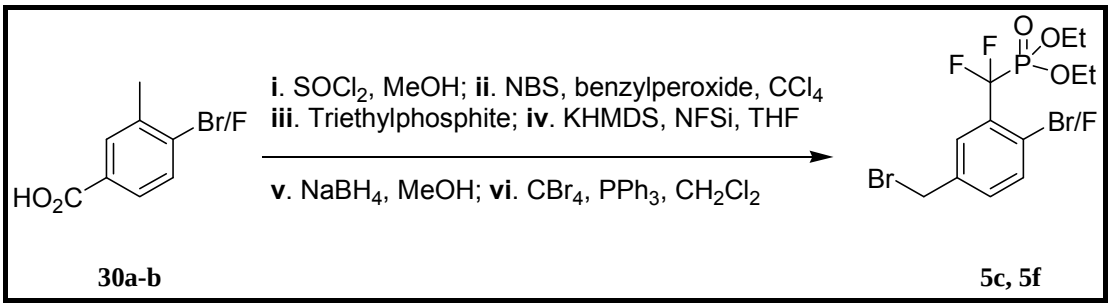


5e (7.78 g, 72%) was prepared from **29c** (9 g, 0.028 mole) by means of the general procedure described in **5.1.3.** (step-VI) as a white solid. mp: 123-124 ^{o}C; Purity by HPLC: 96.70%.

^{1}HNMR (Acetone-*d6*) : δ 1.14 (t, *J* = 7.0 Hz, 6H), 4.03 (m, 4H), 4.61 (s, 2H), 6.66 (d, *J* = 1.8 Hz, 1H), 6.79 (d, *J* = 7.9 Hz, 1H), 6.99 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 376.4 $(M+H)^{+}$.

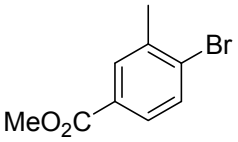
5.1.6. General procedure for the synthesis of Compounds (5c, 5f)



30a-b **5c, 5f**

Title compounds **5c, 5f** was prepared using the same procedure as that used for compound **3a-b** using appropriate starting material.

5.1.6.1. Methyl 4-bromo-3-methylbenzoate (31a)

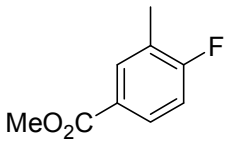


31a (8 g, 75%) was prepared from **30a** (10 g, 0.046 mole) by means of the general procedure described in **5.1.3.** (step-I) as a white solid. mp: 43-44 ^{o}C; Purity by HPLC: 98.12%.

^{1}HNMR (Acetone-*d6*) : δ 2.37 (s, 3H), 3.91 (s, 3H), 7.44 (d, *J* = 7.9 Hz, 1H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.80 (s, 1H)

ESI/MS (m/z) : 230.3 $(M+H)^{+}$.

5.1.6.2. Methyl 4-fluoro-3-methylbenzoate (31b)

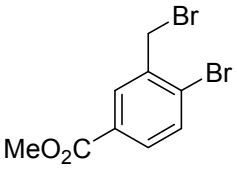


31b (7.63 g, 70%) was prepared from **30b** (10 g, 0.064 mole) by means of the general procedure described in **5.1.3.** (step-I) as a white solid. mp: 42-43 ^{o}C; Purity by HPLC: 94.70%.

^{1}HNMR (Acetone-*d6*) : δ 2.29 (s, 3H), 3.93 (s, 3H), 6.93 (m, 1H), 7.68-7.78 (m, 2H)

ESI/MS (m/z) : 169.3 $(M+H)^{+}$.

5.1.6.3. Methyl 4-bromo-3-(bromomethyl)benzoate (32a)

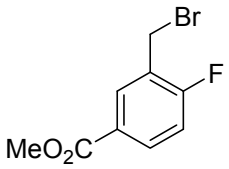


32a (10 g, 93%) was prepared from **31a** (8 g, 0.035 mole) by means of the general procedure described in **5.1.3.** (step-II) as a white solid. mp: 69-71 ^{o}C; Purity by HPLC: 97.77%.

^{1}HNMR (Acetone-*d6*) : δ 3.89 (s, 3H), 4.57 (s, 2H), 7.44 (d, J = 7.9 Hz, 1H), 7.62 (d, J = 7.9 Hz, 1H), 7.91 (s, 1H)

ESI/MS (m/z) : 309.2 $(M+H)^{+}$.

5.1.6.4. Methyl 3-(bromomethyl)-4-fluorobenzoate (32b)

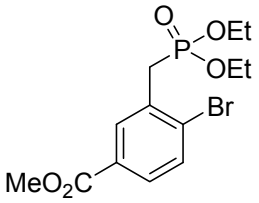


32b (6.6 g, 60%) was prepared from **31b** (7.5 g, 0.044 mole) by means of the general procedure described in **5.1.3.** (step-II) as a white solid. mp: 93-95 ^{o}C; Purity by HPLC: 95.70%.

^{1}HNMR (Acetone-*d6*) : δ 3.91 (s, 3H), 4.47 (s, 2H), 6.99 (m, 1H), 7.72-7.77 (m, 2H)

ESI/MS (m/z) : 248.1 $(M+H)^+$.

5.1.6.5. Methyl 4-bromo-3-[(diethoxyphosphoryl)methyl]benzoate (33a)

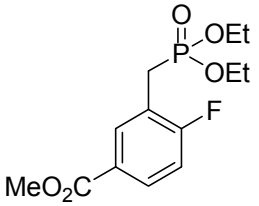


33a (7 g, 59%) was prepared from **32a** (8 g, 0.032 mole) by means of the general procedure described in **5.1.3.** (step-III) as white solid. mp: 88-90 ^oC; Purity by HPLC: 96.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.19 (t, *J* = 7.2 Hz, 6H), 3.07 (s, 2H), 3.78 (s, 3H), 4.03-4.13 (m, 4H), 7.47 (d, *J* = 7.9 Hz, 1H), 7.77 (s, 1H), 7.91 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 366.6 $(M+H)^+$.

5.1.6.6. Methyl 3-[(diethoxyphosphoryl)methyl]-4-fluorobenzoate (33b)

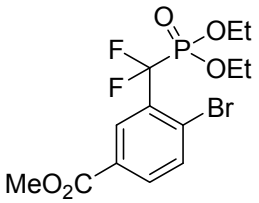


33b (4 g, 50%) was prepared from **32b** (6.5 g, 0.026 mole) by means of the general procedure described in **5.1.3.** (step-III) as a white solid. mp: 101-103 ^oC; Purity by HPLC: 93.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.04 (t, *J* = 6.8 Hz, 6H), 3.09 (s, 2H), 3.89 (s, 3H), 4.17 (m, 4H), 7.09 (m, 1H), 7.72-7.76 (m, 2H)

ESI/MS (m/z) : 305.7 $(M+H)^+$.

5.1.6.7. Methyl 4-bromo-3-[(diethoxyphosphoryl)difluoromethyl]benzoate (34a)

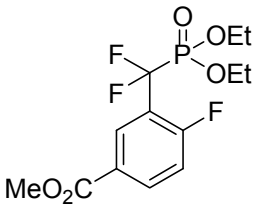


34a (5 g, 65%) was prepared from **33a** (7 g, 0.019 mole) by means of the general procedure described in **5.1.3.** (step-IV) as a white solid. mp: 77-79 ^{o}C; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.21 (t, *J* = 7.1 Hz, 6H), 3.91 (s, 3H), 4.01-4.14 (m, 4H), 7.49 (d, *J* = 8.0 Hz, 1H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.99 (s, 1H)

ESI/MS (m/z) : 402.4 $(M+H)^{+}$.

5.1.6.8. Methyl 3-[(diethoxyphosphoryl)difluoromethyl]-4-fluorobenzoate (34b)

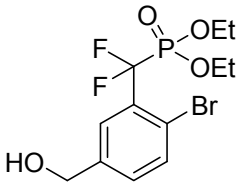


34b (2.18 g, 50%) was prepared from **33b** (3.9 g, 12.82 mmole) by means of the general procedure described in **5.1.3.** (step-IV) as a white solid. mp: 111-112 ^{o}C; Purity by HPLC: 94.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.23 (t, *J* = 7.1 Hz, 6H), 3.93 (s, 3H), 4.10 (m, 4H), 6.99 (m, 1H), 7.69-7.78 (m, 2H)

ESI/MS (m/z) : 341.3 $(M+H)^{+}$.

5.1.6.9. Diethyl [(2-bromo-5-hydroxymethylphenyl)difluoromethyl] phosphonate (35a)

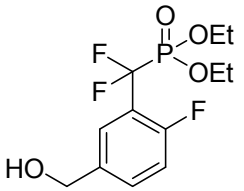


35a (4 g, 86%) was prepared from **34a** (5 g, 0.012 mole) by means of the general procedure described in **5.1.3.** (step-V) as a white solid. mp: 91-93 ^{o}C; Purity by HPLC: 94.60%.

^{1}HNMR (Acetone-*d6*) : δ 1.19 (t, *J* = 7.0 Hz, 6H), 3.57 (s, 1H), 4.09-4.19 (m, 4H), 4.59 (s, 2H), 7.01 (d, *J* = 7.7 Hz, 1H), 7.19 (s, 1H), 7.51 (d, *J* = 7.1 Hz, 1H)

ESI/MS (m/z) : 374.5 $(M+H)^{+}$.

5.1.6.10. Diethyl difluoro[2-fluoro-5-(hydroxymethyl)phenyl)methyl phosphonate (35b)

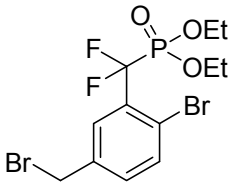


35b (1.28 g, 70%) was prepared from **34b** (2 g, 5.88 mmole) by means of the general procedure described in **5.1.3.** (step-V) as a white solid. mp: 97-99 ^{o}C; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.11 (t, *J* = 6.8 Hz, 6H), 4.09 (m, 4H), 4.78 (s, 2H), 6.78-6.99 (m, 3H)

ESI/MS (m/z) : 313.5 $(M+H)^{+}$.

5.1.6.11. Diethyl [(2-bromo-5-bromomethylphenyl)difluoromethyl] phosphonate (5c)

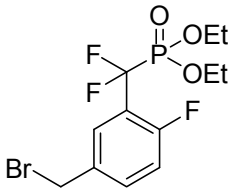


5c (3 g, 63%) was prepared from **35a** (4 g, 0.010 mole) by means of the general procedure described in **5.1.3.** (step-VI) above as a white solid. mp: 101-103 ^{o}C; Purity by HPLC: 97.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.31 (t, *J* = 7.3 Hz, 6H), 4.01-4.11 (m, 4H), 4.49 (s, 2H), 6.93 (d, *J* = 7.8 Hz, 1H), 7.01 (s, 1H), 7.44 (d, *J* = 8.0 Hz, 1H)

ESI/MS (m/z) : 437.5 $(M+H)^{+}$.

5.1.6.12. Diethyl [5-(bromomethyl)-2-fluorophenyl]difluoromethyl phosphonate (5f)

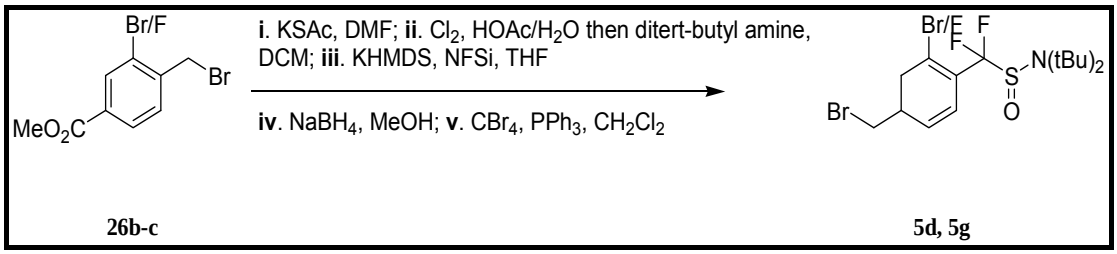


5f (0.79 g, 66%) was prepared from **35b** (1 g, 3.20 mmole) by means of the general procedure described in **5.1.3.** (step-VI) as a white solid. mp: 119-121 ^{o}C; Purity by HPLC: 97.50%.

^{1}HNMR (Acetone-*d6*) : δ 1.07 (t, *J* = 7.2 Hz, 6H), 4.11 (m, 4H), 4.92 (s, 2H), 6.73 (m, 1H), 6.84-6.94 (m, 2H)

ESI/MS (m/z) : 376.2 $(M+H)^{+}$.

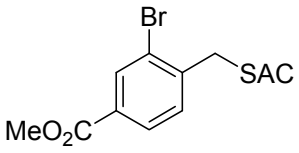
5.1.7. General procedure for the synthesis of Compounds (5d, 5g)



26b-c **5d, 5g**

Title compounds **5d** & **5g** were prepared using the same procedure as that used for compound **11a-b** using appropriate starting material.

5.1.7.1. Methyl 4[(acetylthio)methyl]-3-bromobenzoate (36b)

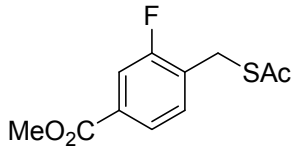


36b (7 g, 71%) was prepared from **26b** (10 g, 0.032 mole) by means of the general procedure described in **5.1.4.** (step-I) as a white solid. mp: 62-63 ^{o}C; Purity by HPLC: 96.90%.

^{1}HNMR (Acetone-*d6*) : δ 2.32 (s, 3H), 3.87 (s, 3H), 4.29 (s, 2H) 7.37 (d, *J* = 7.5 Hz, 1H), 7.87 (d, *J* = 7.4 Hz, 1H), 8.10 (s, 1H)

ESI/MS (m/z) : 304.5 $(M+H)^{+}$.

5.1.7.2. Methyl 4-[(acetylthio)methyl]-3-fluorobenzoate (36c)

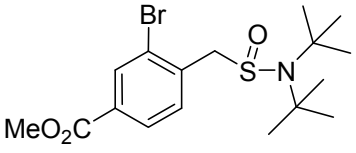


36c (8.81 g, 90%) was prepared from **26c** (10 g, 0.040 mole) by means of the general procedure described in **5.1.4.** (step-I) as a white solid. mp: 88-90 ^{o}C; Purity by HPLC: 93.90%.

^{1}HNMR (Acetone-*d6*) : δ 2.25 (s, 3H), 3.77 (s, 3H), 4.16 (s, 2H) 7.17 (m, 1H), 7.55-7.63 (m, 2H)

ESI/MS (m/z) : 243.4 $(M+H)^{+}$.

5.1.7.3. Methyl 3-bromo-4-[(*N*,*N*-di-tert-butylsulfinamoyl)methyl]benzoate (37b)

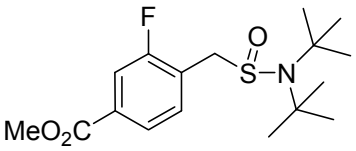


37b (5 g, 53%) was prepared from **36b** (7 g, 0.023 mole) by means of the general procedure described in **5.1.4.** (step-II) as a white solid. mp: 82-84 ^{o}C; Purity by HPLC: 96.10%.

^{1}HNMR (Acetone-*d6*) : δ 1.33 (s, 18H), 3. 86 (s, 2H), 3.91 (s, 3H), 7.23 (d, *J* = 7.9 Hz, 1H), 7.91 (d, *J* = 7.7 Hz, 1H), 8.17 (s, 1H)

ESI/MS (m/z) : 405.7 $(M+H)^{+}$.

5.1.7.4. Methyl 4-[(*N*,*N*-di-tert-butylsulfinamoyl)methyl]-3-fluorobenzoate (37c)

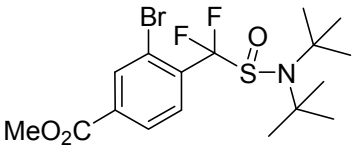


37c (6 g, 50%) was prepared from **36c** (8.5 g, 0.035 mole) by means of the general procedure described in **5.1.4.** (step-II) as a white solid. mp: 77-79 ^{o}C; Purity by HPLC: 95.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.27 (s, 18H), 3.79 (s, 2H), 3.88 (s, 3H), 7.14 (m, 1H), 7.60-7.68 (m, 2H)

ESI/MS (m/z) : 344.4 $(M+H)^{+}$.

5.1.7.5. Methyl 3-bromo-4-[(*N*,*N*-di-tert- butylsulfinamoyl)difluoromethyl] benzoate (38b)

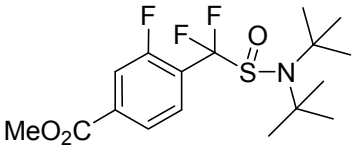


38b (3 g, 55%) was prepared from **37b** (5 g, 0.012 mole) by means of the general procedure described in **5.1.4.** (step-III) as a white solid. mp: 77-78 ^{o}C; Purity by HPLC: 94.96%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.29 (s, 18H), 3. 91 (s, 3H), 7.31 (d, *J* = 7.2 Hz, 1H), 7.89 (d, *J* = 7.9 Hz, 1H), 8.09 (s, 1H)

ESI/MS (m/z) : 441.1 $(M+H)^+$.

5.1.7.6. Methyl 4-[(*N*,*N*-di-tert-butylsulfinamoyl)difluoromethyl]-3-fluoro benzoate (38c)

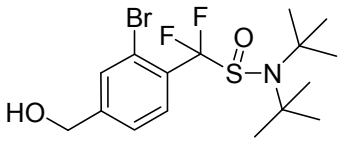


38c (3.91 g, 60%) was prepared from **37c** (5.9 g, 0.017 mole) by means of the general procedure described in **5.1.4.** (step-III) as a white solid. mp: 111-113 ^oC; Purity by HPLC: 96.96%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.18 (s, 18H), 3. 87 (s, 3H), 7.11 (m, 1H), 7.55-7.68 (m, 2H)

ESI/MS (m/z) : 380.1 $(M+H)^+$.

5.1.7.7. Methyl 3-bromo-4-[(*N*,*N*-di-tert- butylsulfinamoyl)difluoromethyl] benzoate (39b)



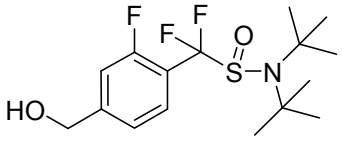
39b (2 g, 71%) was prepared from **38b** (3 g, 6.81 mmole) by means of the general procedure described in **5.1.4.** (step-IV) as a white solid. mp: 99-101 ^oC; Purity by HPLC: 97.96%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.37 (s, 18H), 3.69 (s, 1H), 4. 77 (s, 2H), 7.00 (d, *J* = 7.7 Hz, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 7.49 (s, 1H)

ESI/MS (m/z) : 413.3 $(M+H)^+$.

5.1.7.8. Methyl 4-[(*N*,*N*-di-tert-butylsulfinamoyl)difluoromethyl]-3-fluoro

benzoate (39c)

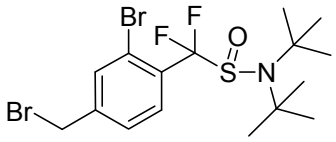


39c (2.81 g, 80%) was prepared from **38c** (3.8 g, 0.01 mmole) by means of the general procedure described in **5.1.4.** (step-IV) as a white solid. mp: 121-123 ^{o}C; Purity by HPLC: 98.50%.

^{1}HNMR (Acetone-*d6*) : δ 1.21 (s, 18H), 3.77 (s, 1H), 4. 83 (s, 2H), 6.77-6.81 (m, 2H), 7.03 (m, 1H)

ESI/MS (m/z) : 352.4 $(M+H)^{+}$.

5.1.7.9. 1-[2-bromo-4-(bromomethyl)phenyl]-*N*,*N*-di-tert-butyl-1,1-difluoro methane-sulfinamide (5d)

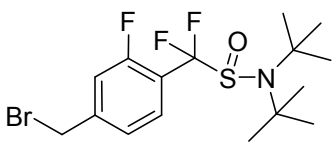


5d (1.5 g, 65%) was prepared from **39b** (2 g, 4.85 mmole) by means of the general procedure described in **5.1.4.** (step-V) as a white solid. mp: 111-113 ^{o}C; Purity by HPLC: 98.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.29 (s, 18H), 4. 55 (s, 2H), 6.99 (d, J = 8.0 Hz, 1H), 7.17 (d, J = 7.9 Hz, 1H), 7.36 (s, 1H)

ESI/MS (m/z) : 476.9 $(M+H)^{+}$.

5.1.7.10. 1-[4-(bromomethyl)-2fluorophenyl]-*N*,*N*-di-tert-butyl-1,1-difluoro methanesulfinamide (5g)

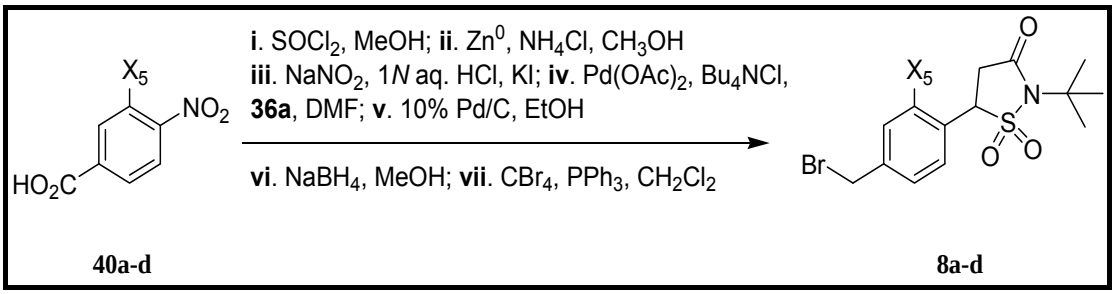


5g (1.62 g, 55%) was prepared from **39c** (2.5 g, 7.12 mmole) by means of the general procedure described in **5.1.4.** (step-V) as a white solid. mp: 99-101 ^{o}C; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.33 (s, 18H), 4. 61 (s, 2H), 6.63-6.72 (m, 2H), 6.99 (m, 1H)

ESI/MS (m/z) : 415.3 $(M+H)^{+}$.

5.1.8. General procedure for the synthesis of Compounds (8a-d)



40a-d **8a-d**

Step I: To an ice-cold solution of acid **40a-d** (1 mole equivalent) in MeOH (10 fold), $SOCl_2$ (3.0 mole equivalent) was added drop wise over a period of 30 min at 0-15 ^{o}C, and then heated at 70 ^{o}C for 3 hours. Methanol was distilled out under vacuum; residue was poured into ice cold water (10 fold) and extracted with ethyl acetate (2 X 10 fold). The combined organic layers were successively washed with saturated aqueous $NaHCO_3$, followed by water, and finally with brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum to furnish title compound **41a-d** as solids.

Step II: A stirred solution of **41a-d** (1 mole equivalent) in methanol (20 fold) and water (2 fold) was treated with ammonium chloride (1.5 mole equivalent) and then zinc (8 mole equivalent). The solution was stirred at reflux for 1 h. The solution was filtered through celite, the residue was concentrated under

vacuum to remove methanol, and the water solution was extracted with ethyl acetate (2 x 10 fold) and dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum to furnish the compounds **42a-d** as yellow foam.

Step III: To a suspended solution of **42a-d** (1 mole equivalent) in 1 *N* aqueous hydrochloric acid (25 fold) was treated dropwise with sodium nitrite (1 mole equivalent) in water (4 fold) at 0 ^{o}C. After 15 min, potassium iodide (1 mole equivalent) in water (4 fold) was added, and the solution was heated at 40 ^{o}C for 30 min. The solution was quenched with a saturated aqueous sodium thiosulfate solution and extracted with ethyl acetate (2 x 20 fold). The combined extracts were washed with a 0.1 *N* hydrochloric acid solution followed by saturated aqueous sodium bicarbonate solution and dried over sodium sulfate, filtered and concentrated under vacuum to furnish the products, purified by silica gel chromatography (10-40% ethyl acetate/hexane) afforded the product **43a-d** as solids.

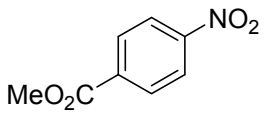
Step IV: Compound **43a-d** (1 mole equivalent), 2-tert-butylisothiazol-3(2H)-one 1,1-dioxide 36a (1.75 mole equivalent), palladium acetate (0.2 mole equivalent), tetra-butylammonium chloride (1 mole equivalent), and then triethylamine (3 mole equivalent) were dissolved in DMF (20 fold). The solution was degassed and then stirred with heating at 70 ^{o}C under nitrogen atmosphere for 2 h. The solution was diluted with ethyl acetate (10 fold) and washed with water and a 1 *N* aqueous hydrochloric acid solution. The organic phase was filtered through Celite with ethyl acetate washing. The organic solution was dried over sodium sulfate, concentrated in vacuo, and then purified by silica gel chromatography (10-15% ethyl acetate/hexane) to afford the desired product **44a-d** as solids.

Step V: Compound **44a-d** and 10% palladium on carbon (0.5 fold) in ethanol (10 fold) were shaken on a Parr hydrogenation apparatus for 3 h. The mixture was filtered through Celite and concentration gave **45a-d** as a solid.

Step VI: To a solution of **45a-d** (1 mole equivalent) in methanol (10 fold) was chilled to 0 ^{o}C. Sodium borohydride (3 mole equivalent) was added and solution was stirred at 0 ^{o}C for 30 min. Mixture was acidified with acetic acid, the solution was diluted with ethyl acetate (2 x 20 fold), washed with water, and dried over sodium sulfate. Silica gel chromatography (10-30% ethyl acetate/hexane) afforded the product **46a-d** as a solid.

Step VII: To a solution of **46a-d** (1 mole equivalent) in dry CH_2Cl_2 (20 fold) at 0 ^{o}C were added carbon tetrabromide (1.2 mole equivalent) and triphenylphosphine (1.1 mole equivalent) and stirred for 3 hours. The mixture was concentrated at room temperature on rotary evaporation and the resulting residue subjected to flash chromatography using 10% ethyl acetate in hexane which gave the corresponding bromo derivatives **8a-d** as white solids.

5.1.8.1. Methyl 4-nitrobenzoate (41a)

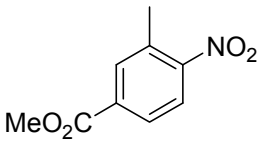


41a (19.5 g, 90%) was prepared from **40a** (20 g, 0.119 mole) by means of the general procedure described in Step-I above as a yellow solid. mp: 94-96 ^{o}C; Purity by HPLC: 96.66%.

^{1}HNMR (Acetone-*d6*) : δ 3.90 (s, 3H), 8.22 (d, *J* = 7.0 Hz, 2H), 8.39 (d, *J* = 8.0 Hz, 2H)

ESI/MS (m/z) : 182.5 $(M+H)^{+}$.

5.1.8.2. Methyl 3-methyl-4-nitrobenzoate (41b)

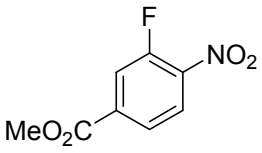


41b (18.3 g, 85%) was prepared from **40b** (20 g, 0.110 mole) by means of the general procedure described in Step-I above as a yellow solid. mp: 80-81 ^{o}C; Purity by HPLC: 97.03%.

^{1}HNMR (Acetone-*d6*) : *δ* 2.55 (s, 3H), 3.85 (s, 3H), 8.00 (s, 1H), 8.12 (d, *J* = 7.7 Hz, 1H), 8.31 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 196.6 $(M+H)^{+}$.

5.1.8.3. Methyl 3-fluoro-4-nitrobenzoate (41c)

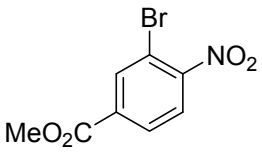


41c (18.7 g, 87%) was prepared from **40c** (20 g, 0.108 mole) by means of the general procedure described in Step-I above as a yellow solid. mp: 58-60 ^{o}C; Purity by HPLC: 97.77%.

^{1}HNMR (Acetone-*d6*) : *δ* 3.79 (s, 3H), 7.88 (d, *J* = 1.8 Hz, 1H), 8.09 (d, *J* = 7.9 Hz, 1H), 8.39 (d, *J* = 8.2 Hz, 1H)

ESI/MS (m/z) : 200.4 $(M+H)^{+}$.

5.1.8.4. Methyl 3-bromo-4-nitrobenzoate (41d)

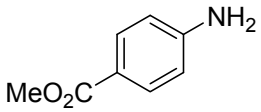


41d (3.17 g, 60%) was prepared from **40d** (5 g, 0.02 mole) by means of the general procedure described in Step-I above as a yellow solid. mp: 39-40 ^{o}C; Purity by HPLC: 94.50%.

^{1}HNMR (Acetone-*d6*) : δ 3.91 (s, 3H), 8.19 (d, *J* = 7.9 Hz, 1H), 8.31 (d, *J* = 8.0 Hz, 1H), 8.59 (s, 1H)

ESI/MS (m/z) : 261.4 $(M+H)^+$.

5.1.8.5. Methyl 4-aminobenzoate (42a)

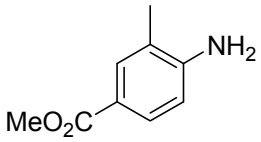


42a (11.1 g, 70%) was prepared from **41a** (19 g, 0.104 mole) by means of the general procedure described in Step-II above as a yellow foam. mp: 110-112 ^oC; Purity by HPLC: 94.09%.

^{1}HNMR (Acetone-*d6*) : δ 3.77 (s, 3H), 6.77 (d, *J* = 8.4 Hz, 2H), 7.69 (d, *J* = 8.3 Hz, 2H)

ESI/MS (m/z) : 152.1 $(M+H)^+$.

5.1.8.6. Methyl 4-amino-3-methylbenzoate (42b)

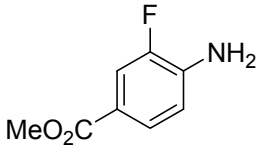


42b (10.9 g, 72%) was prepared from **41b** (18 g, 0.092 mole) by means of the general procedure described in Step-II above as a yellow foam. mp: 61-63 ^oC; Purity by HPLC: 93.40%.

^{1}HNMR (Acetone-*d6*) : δ 2.55 (s, 3H), 3.85 (s, 3H), 8.00 (s, 1H), 8.12 (d, *J* = 7.7 Hz, 1H), 8.31 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 166.7 $(M+H)^+$.

5.1.8.7. Methyl 4-amino-3-fluorobenzoate (42c)

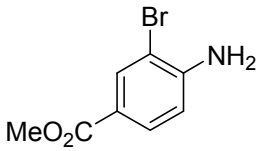


42c (10.4 g, 68%) was prepared from **41c** (18 g, 0.09 mole) by means of the general procedure described in Step-II above as a yellow foam. mp: 73-75 ^{o}C; Purity by HPLC: 95.60%.

^{1}HNMR (Acetone-*d6*) : δ 3.89 (s, 3H), 6.77 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 1.8 Hz, 1H), 7.55 (d, *J* = 8.3 Hz, 1H)

ESI/MS (m/z) : 170.2 $(M+H)^{+}$.

5.1.8.8. Methyl 4-amino-3-bromobenzoate (42d)

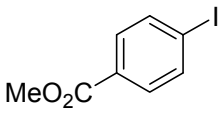


42d (1.78 g, 65%) was prepared from **41d** (3.1 g, 11.92 mmole) by means of the general procedure described in Step-II above as a yellow foam. mp: 103-105 ^{o}C; Purity by HPLC: 95.00%.

^{1}HNMR (Acetone-*d6*) : δ 3.99 (s, 3H), 6.81 (d, *J* = 8.1 Hz, 1H), 7.61 (d, *J* = 8.2 Hz, 1H), 8.03 (s, 1H)

ESI/MS (m/z) : 231.4 $(M+H)^{+}$.

5.1.8.9. Methyl 4-iodobenzoate (43a)

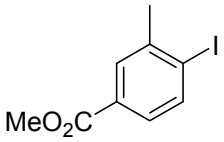


43a (10.5 g, 55%) was prepared from **42a** (11 g, 0.072 mole) by means of the general procedure described in Step-III above as a yellow solid. mp: 112-113 ^{o}C; Purity by HPLC: 96.97%.

^{1}HNMR (Acetone-*d6*) : δ 3.91 (s, 3H), 7.81 (d, *J* = 82 Hz, 2H), 7.93 (d, *J* = 7.9 Hz, 2H)

ESI/MS (m/z) : 263.5 $(M+H)^{+}$.

5.1.8.10. Methyl 4-iodo-3-methylbenzoate (43b)

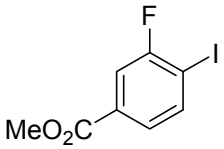


43b (10.0 g, 60%) was prepared from **42b** (10 g, 0.06 mole) by means of the general procedure described in Step-III above as a yellow solid. mp: 58-60 ^{o}C; Purity by HPLC: 92.55%.

^{1}HNMR (Acetone-*d6*) : δ 2.22 (s, 3H), 3.73 (s, 3H), 7.49 (s, 1H), 7.59 (d, *J* = 8.1 Hz, 2H), 7.80 (d, *J* = 8.0 Hz, 2H)

ESI/MS (m/z) : 277.4 $(M+H)^{+}$.

5.1.8.12. Methyl 3-fluoro-4-iodobenzoate (43c)

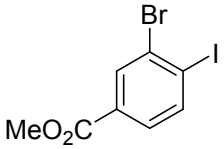


43c (11.2 g, 68%) was prepared from **42c** (10 g, 0.059 mole) by means of the general procedure described in Step-III above as a yellow solid. mp: 94-95 ^{o}C; Purity by HPLC: 92.20%.

^{1}HNMR (Acetone-*d6*) : δ 3.94 (s, 3H), 7.39 (d, *J* = 1.8 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 1H), 7.91 (d, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 281.2 $(M+H)^{+}$.

5.1.8.13. Methyl 3-bromo-4-iodobenzoate (43d)

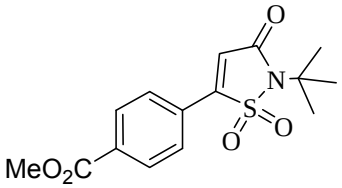


43d (1.61 g, 68%) was prepared from **42d** (1.6 g, 6.95 mmole) by means of the general procedure described in Step-III above as a yellow solid. mp: 98-100 ^{o}C; Purity by HPLC: 93.50%.

^{1}HNMR (Acetone-*d6*) : δ 3.81 (s, 3H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.81 (d, *J* = 8.1 Hz, 1H), 8.09 (s, 1H)

ESI/MS (m/z) : 342.3 $(M+H)^+$.

5.1.8.14. Methyl 4-[2-(tert-butyl)-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl]benzoate (44a)

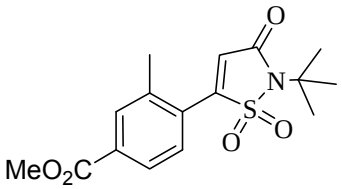


44a (7.4 g, 60%) was prepared from **43a** (10 g, 0.038 mole) by means of the general procedure described in Step-IV above as a yellow solid. mp: 102-103 ^oC; Purity by HPLC: 95.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.39 (s, 9H), 3.91 (s, 3H), 7.48 (d, *J* = 8.2 Hz, 2H), 7.66 (s, 1H), 7.73 (d, *J* = 7.8 Hz, 2H)

ESI/MS (m/z) : 324.2 $(M+H)^+$.

5.1.8.15. Methyl 4-[2-(tert-butyl)-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl]-3-methylbenzoate (44b)

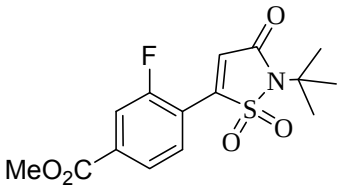


44b (8.3 g, 68%) was prepared from **43b** (10 g, 0.036 mole) by means of the general procedure described in Step-IV above as a yellow solid. mp: 111-113 ^oC; Purity by HPLC: 96.78%.

^{1}HNMR (Acetone-*d6*) : δ 1.44 (s, 9H), 2.45 (s, 3H), 3.87 (s, 3H),7.21 (s, 1H), 7.38 (d, *J* = 8.1 Hz, 1H), 7.51 (d, *J* = 7.9 Hz, 1H), 7.81 (s, 1H)

ESI/MS (m/z) : 338.1 $(M+H)^+$.

5.1.8.16. Methyl 4-[2-(tert-butyl)-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl]-3-fluorobenzoate (44c)

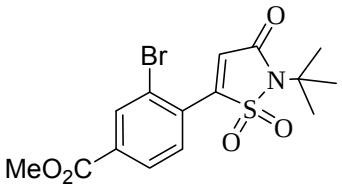


44c (8.3 g, 62%) was prepared from **43c** (11 g, 0.039 mole) by means of the general procedure described in Step-IV above as a yellow solid. mp: 109-111 ^{o}C; Purity by HPLC: 94.46%.

^{1}HNMR (Acetone-*d6*) : δ 1.49 (s, 9H), 3.77 (s, 3H),7.41 (s, 1H), 7.48 (d, *J* = 8.3 Hz, 1H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.69 (d, *J* = 1.9 Hz, 1H)

ESI/MS (m/z) : 342.3 $(M+H)^{+}$.

5.1.8.17. Methyl 3-bromo-4-[2-(tert-butyl)-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl]benzoate (44d)

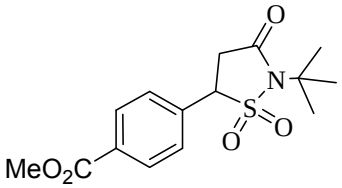


44d (1.23 g, 70%) was prepared from **43d** (1.5 g, 4.39 mmole) by means of the general procedure described in Step-IV above as a yellow solid. mp: 103-105 ^{o}C; Purity by HPLC: 95.55%.

^{1}HNMR (Acetone-*d6*) : δ 1.42 (s, 9H), 3.89 (s, 3H),7.39 (s, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.69 (d, *J* = 7.9 Hz, 1H), 8.21 (s, 1H)

ESI/MS (m/z) : 403.5 $(M+H)^{+}$.

5.1.8.18. Methyl 4-[2-(tert-butyl)-1,1-dioxido-3-oxoisothiazolidin-5-yl]-benzoate (45a)

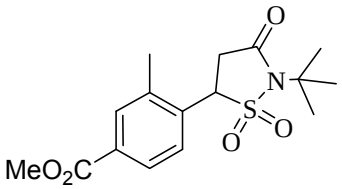


45a (4.0 g, 55%) was prepared from **44a** (7.2 g, 0.022 mole) by means of the general procedure described in Step-V above as a yellow solid. mp: 119-121 ^{o}C; Purity by HPLC: 95.03%.

^{1}HNMR (Acetone-*d6*) : δ 1.63 (s, 9H), 3.07 (dd, *J* = 17.2, 7.3 Hz, 1H), 3.32 (dd, *J* = 17.3, 8.2 Hz, 1H), 3.86 (s, 3H), 4.99 (dd, *J* = 8.2, 7.3 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.98 (d, *J* = 7.8 Hz, 2H)

ESI/MS (m/z) : 326.6 $(M+H)^{+}$.

5.1.8.19. Methyl 4-[2-(tert-butyl)-1,1-dioxido-3-oxoisothiazolidin-5-yl]-3-methylbenzoate (45b)

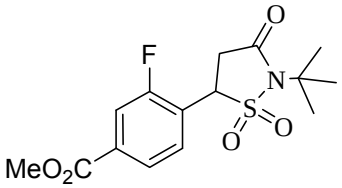


45b (5.2 g, 63%) was prepared from **44b** (8.2 g, 0.024 mole) by means of the general procedure described in Step-V above as a yellow solid. mp: 119-121 ^{o}C; Purity by HPLC: 97.50%.

^{1}HNMR (Acetone-*d6*) : δ 1.57 (s, 9H), 2.41 (s, 3H), 3.01 (dd, *J* = 17.4, 7.8 Hz, 1H), 3.21 (dd, *J* = 17.1, 7.9 Hz, 1H), 3.91 (s, 3H), 5.07 (dd, *J* = 8.1, 7.0 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.72 (s, 1H), 7.84 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 340.4 $(M+H)^{+}$.

5.1.8.20. Methyl 4-[2-(tert-butyl)-1,1-dioxido-3-oxoisothiazolidin-5-yl]-3-fluorobenzoate (45c)

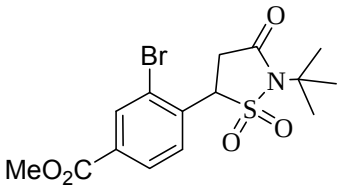


45c (5.77 g, 70%) was prepared from **44c** (8.2 g, 0.024 mole) by means of the general procedure described in Step-V above as a yellow solid. mp: 103-105 ^{o}C; Purity by HPLC: 98.40%.

^{1}HNMR (Acetone-*d6*) : δ 1.49 (s, 9H), 2.99 (dd, *J* = 17.7, 7.9 Hz, 1H), 3.27 (dd, *J* = 17.0, 8.1 Hz, 1H), 3.90 (s, 3H), 5.11 (dd, *J* = 8.3, 7.3 Hz, 1H), 7.41 (d, *J* = 8.1 Hz, 1H), 7.60 (d, *J* = 1.9 Hz, 1H), 7.80 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 344.4 $(M+H)^{+}$.

5.1.8.21. Methyl 3-bromo-4-[2-(tert-butyl)-1,1-dioxido-3-oxo-2,3-dihydroisothiazolidin-5-yl]benzoate (45d)

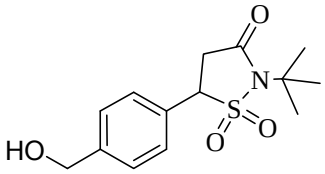


45d (0.76 g, 63%) was prepared from **44d** (1.2 g, 2.98 mmole) by means of the general procedure described in Step-V above as a yellow solid. mp: 103-105 ^{o}C; Purity by HPLC: 98.40%.

^{1}HNMR (Acetone-*d6*) : δ 1.57 (s, 9H), 3.11 (dd, *J* = 17.9, 8.2 Hz, 1H), 3.33 (dd, *J* = 17.3, 7.9 Hz, 1H), 3.83 (s, 3H), 5.13 (dd, *J* = 8.2, 7.7 Hz, 1H), 7.35 (d, *J* = 7.9 Hz, 1H), 7.91 (d, *J* = 7.9 Hz, 1H), 8.23 (s, 1H)

ESI/MS (m/z) : 405.4 $(M+H)^{+}$.

5.1.8.22. 2-(tert-butyl)-5-[4-(hydroxymethyl)phenyl]isothiazolidin-3-one 1,1-dioxide (46a)

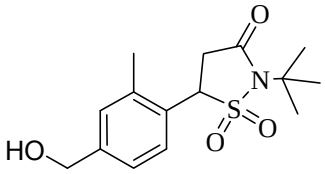


46a (2.7 g, 78%) was prepared from **45a** (3.8 g, 0.011 mole) by means of the general procedure described in Step-VI above as a white solid. mp: 131-133 ^{o}C; Purity by HPLC: 97.10%.

^{1}HNMR (Acetone-*d6*) : δ 1.49 (s, 9H), 3.01 (dd, *J* = 17.9, 8.3 Hz, 1H), 3.37 (dd, *J* = 17.0, 7.2 Hz, 1H), 4.66 (s, 2H), 5.05 (dd, *J* = 8.0, 7.7 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 2H), 7.32 (d, *J* = 7.9 Hz, 2H)

ESI/MS (m/z) : 298.8 $(M+H)^{+}$.

5.1.8.23. 2-(tert-butyl)-5-[4-(hydroxymethyl)-2-methylphenyl] Isothiazolidin-3-one 1,1-dioxide (46b)

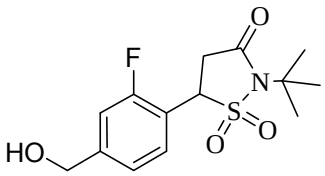


46b (3.67 g, 77%) was prepared from **45b** (5.0 g, 0.014 mole) by means of the general procedure described in Step-VI above as a white solid. mp: 131-132 ^{o}C; Purity by HPLC: 98.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.49 (s, 9H), 2.37 (s, 3H), 3.10 (dd, *J* = 17.1, 8.1 Hz, 1H), 3.23 (dd, *J* = 17.9, 7.7 Hz, 1H), 4.67 (s, 2H), 5.13 (dd, *J* = 7.9, 6.9 Hz, 1H), 6.99 (d, *J* = 7.8 Hz, 1H), 7.12 (d, *J* = 7.7 Hz, 1H), 7.23 (s, 1H)

ESI/MS (m/z) : 312.2 $(M+H)^{+}$.

5.1.8.24. 2-(tert-butyl)-5-[2-fluoro-4-(hydroxymethyl)phenyl]isothiazolidin-3-one 1,1-dioxide (46c)

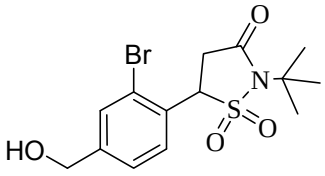


46c (4.14 g, 82%) was prepared from **45c** (5.5 g, 0.016 mole) by means of the general procedure described in Step-VI above as a white solid. mp: 123-125 ^{o}C; Purity by HPLC: 97.70%.

^{1}HNMR (Acetone-*d6*) : δ 1.49 (s, 9H), 3.06 (dd, *J* = 17.9, 7.7 Hz, 1H), 3.17 (dd, *J* = 17.3, 8.2 Hz, 1H), 4.71 (s, 2H), 5.09 (dd, *J* = 8.0, 7.1 Hz, 1H), 6.81 (d, *J* = 1.9 Hz, 1H), 6.98 (d, *J* = 7.1 Hz, 1H), 7.23 (d, *J* = 7.7 Hz, 1H)

ESI/MS (m/z) : 316.6 $(M+H)^{+}$.

5.1.8.25. 5-[2-bromo-4-(hydroxymethyl)phenyl]- 2-(tert-butyl) isothiazolidin-3-one 1,1-dioxide (46d)

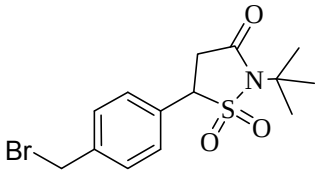


46d (0.4 g, 61%) was prepared from **45d** (0.7 g, 1.73 mmole) by means of the general procedure described in Step-VI above as a white solid. mp: 131-133 ^{o}C; Purity by HPLC: 98.80%.

^{1}HNMR (Acetone-*d6*) : δ 1.48 (s, 9H), 2.91 (dd, *J* = 17.8, 8.0 Hz, 1H), 3.15 (dd, *J* = 17.7, 7.8 Hz, 1H), 4.59 (s, 2H), 5.11 (dd, *J* = 8.0, 7.4 Hz, 1H), 7.11 (d, *J* = 7.7 Hz, 1H), 7.29 (d, *J* = 7.9 Hz, 1H), 7.51 (s, 1H)

ESI/MS (m/z) : 377.4 $(M+H)^{+}$.

5.1.8.26. 5-[4-(bromomethyl)phenyl]-2-(tert-butyl)isothiazolidin-3-one 1,1-dioxide (8a)

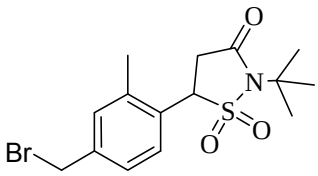


8a (1.8 g, 60%) was prepared from **46a** (2.5 g, 8.41 mmole) by means of the general procedure described in Step-VII above as a white solid. mp: 122-123 ^{o}C; Purity by HPLC: 97.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.53 (s, 9H), 3.17 (dd, *J* = 17.7, 8.0 Hz, 1H), 3.31 (dd, *J* = 17.2, 7.0 Hz, 1H), 4.57 (s, 2H), 5.13 (dd, *J* = 8.2, 7.9 Hz, 1H), 7.19 (d, *J* = 8.2 Hz, 2H), 7.29 (d, *J* = 7.8 Hz, 2H)

ESI/MS (m/z) : 361.1 $(M+H)^{+}$.

5.1.8.27. 5-[4-(bromomethyl)-2-methylphenyl]2-(tert-butyl)isothiazolidin-3-one 1,1-dioxide (8b)

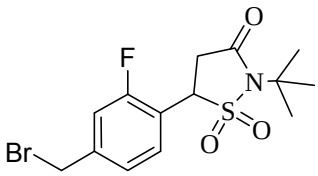


8b (2.7 g, 65%) was prepared from **46b** (3.5 g, 0.011 mole) by means of the general procedure described in Step-VII above as a white solid. mp: 126-128 ^{o}C; Purity by HPLC: 95.55%.

^{1}HNMR (Acetone-*d6*) : δ 1.63 (s, 9H), 2.43 (s, 3H), 3.06 (dd, *J* = 17.8, 7.9 Hz, 1H), 3.31 (dd, *J* = 17.8, 7.3 Hz, 1H), 4.41 (s, 2H), 5.02 (dd, *J* = 8.2, 7.1 Hz, 1H), 6.99-7.15 (m, 3H)

ESI/MS (m/z) : 375.5 $(M+H)^{+}$.

5.1.8.28. 5-[4-(bromomethyl)-2-fluorophenyl]-2-(tert-butyl)isothiazolidin-3-one 1,1-dioxide (8c)

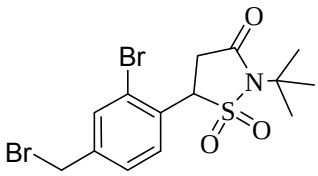


8c (3.26 g, 68%) was prepared from **46c** (4.0 g, 0.012 mole) by means of the general procedure described in Step-VII above as a white solid. mp: 113-115 ^{o}C; Purity by HPLC: 96.10%.

^{1}HNMR (Acetone-*d6*) : δ 1.41 (s, 9H), 2.91 (dd, *J* = 17.7, 7.3 Hz, 1H), 3.21 (dd, *J* = 17.9, 8.5 Hz, 1H), 4.69 (s, 2H), 5.17 (dd, *J* = 8.3, 7.0 Hz, 1H), 6.70-6.99 (m, 2H), 7.17 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 379.3 $(M+H)^{+}$.

5.1.8.29. 5-[2-bromo-4-(bromomethyl)phenyl]-2-(tert-butyl)isothiazolidin-3-one 1,1-dioxide (8d)

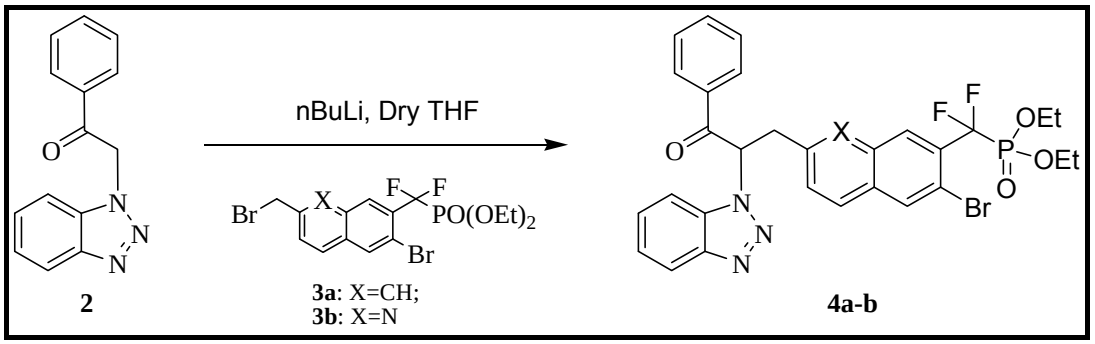


8d (0.3 g, 69%) was prepared from **46d** (0.38 g, 1.01 mmole) by means of the general procedure described in Step-VII above as a white solid. mp: 133-135 ^{o}C; Purity by HPLC: 97.00%

^{1}HNMR (Acetone-*d6*) : δ 1.47 (s, 9H), 2.99 (dd, *J* = 17.9, 8.1 Hz, 1H), 3.21 (dd, *J* = dd, *J* = 17.9, 8.1 Hz, 1H), 4.49 (s, 2H), 5.03 (dd, *J* = 8.2, 7.0 1H), 7.07 (d, *J* = 7.0 Hz, 1H), 7.21 (d, *J* = 7.8 Hz, 1H), 7.43 (s, 1H)

ESI/MS (m/z) : 440.3 $(M+H)^{+}$.

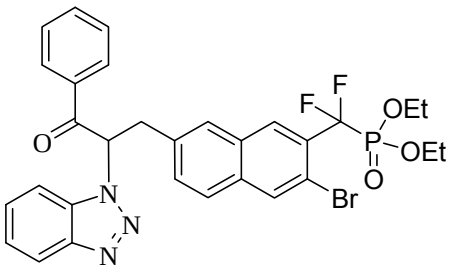
5.1.9. General procedure for the synthesis of Compounds (4a-b)



To a solution of **2** (1 mole equivalent) in dry THF (3 fold), a solution of n-BuLi (1.2 mole equivalent, 1.6 M in Hexane) was added drop wise over a period of 30 min at -78 ^{o}C and stirred at the same temperature for 30 min. **3a** or **3b** (1.2 mole equivalent) was added in THF

(1 fold) to the reaction mixture at same temperature and further stirred at 25 ^{o}C for 4 hours. The reaction was quenched with a saturate aqueous NH_4Cl solution, extracted with ethyl acetate (2 X 3 fold). The combined organic layers were washed successively with water, followed by brine, and dried over Na_2SO_4, filtered and concentrated under vacuum to get the crude product which was subjected to flash by chromatography using 10% ethyl acetate in hexane as eluent to furnish the compound **4a** and **4b** as white solids.

5.1.9.1. Diethyl [7-{2-(1H-benzo[d][1,2,3]triazol-1-yl)-3-oxo-3-phenylpropyl}-3-bromonaphthalen-2-yl]difluoromethylphosphonate (4a)

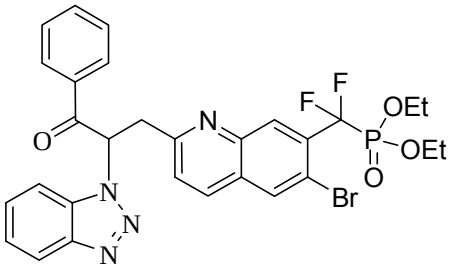


4a (15 g, 55%) was prepared from **2** (10 g, 0.042 mole) by means of the general procedure described as above as a white solid. mp: 122-124 ^{o}C; Purity by HPLC: 97.99%.

^{1}HNMR (Acetone-*d6*) : δ 1.09 (t, *J* = 7.0 Hz, 6H), 3.16-3.26 (dd, *J* = 7.9 Hz, 1H), 3.67-3.77 (dd, *J* = 7.0 Hz, 1H), 4.11 (m, 4H), 5.91-6.02 (m, 1H), 7.21 (d, *J* = 8.0 Hz, 1H), 7.31-7.39 (m, 7H), 7.62 (d, *J* = 7.8 Hz, 1H), 7.88 (s, 1H), 7.95-7.99 (m, 4H)

ESI/MS (m/z) : 643.7 $(M+H)^{+}$.

5.1.9.2. Diethyl [2-{2-(1H-benzo[d][1,2,3]triazol-1-yl)-3-oxo-3-phenyl propyl}-6-bromoquinolin-7-yl]difluoromethylphosphonate (4b)

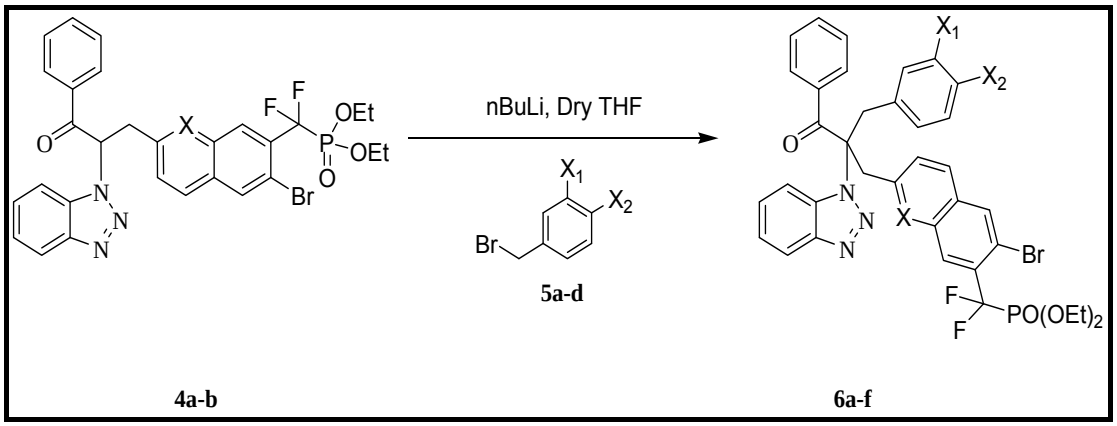


4b (12g, 56%) was prepared from **2** (8 g, 0.033 mole) by means of the general procedure described as above as a white solid. mp: 102-104 ^{o}C; Purity by HPLC: 97.98%.

^{1}HNMR (Acetone-*d6*) : δ 1.11 (t, *J* = 7.1 Hz, 6H), 3.28-3.36 (dd, *J* = 7.8 Hz, 1H), 3.63-3.70 (dd, *J* = 7.1 Hz, 1H), 4.09 (m, 4H), 5.58-5.69 (m, 1H), 7.13 (d, *J* = 8.1 Hz, 1H), 7.29-7.37 (m, 5H), 7.69 (s, 1H), 7.82 (s, 1H), 7.94-7.99 (m, 5H)

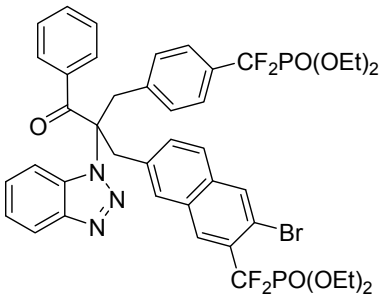
ESI/MS (m/z) : 644.9 $(M+H)^{+}$.

5.1.10. General procedure for the synthesis of Compounds (6a-f)



To a solution of **4a** or **4b** (1 mole equivalent) in dry THF (3 fold), a solution of n-BuLi (1.2 mole equivalent, 1.6 M in Hexane) was added drop wise over a period of 30 min at -78 ^{o}C and stirred at the same temperature for 30 min. **5a-d** (1.2 mole equivalent) was added in THF (1 fold) to the reaction mixture at same temperature and further stirred at 25 ^{o}C for 6 hours. The reaction was quenched with a saturate aqueous NH_4Cl solution, extracted with ethyl acetate (2 X 3 fold). The combined organic layers were washed successively with water, followed by brine, and dried over Na_2SO_4, filtered and concentrated under vacuum to get the crude product which was subjected to flash by chromatography using 5% ethyl acetate in hexane as eluent to furnish the compound **6a-6f** as white solids.

5.1.10.1. Compound - 6a

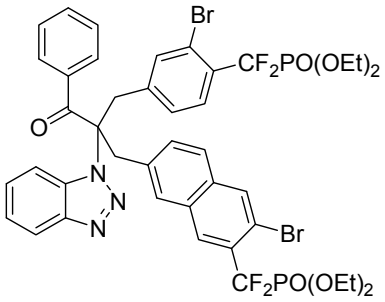


6a (1g, 70%) was prepared from **4a** (1 g, 1.55 mmole) by means of the general procedure described as above as a white solid. mp: 132-134 ^{o}C; Purity by HPLC: 94.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.07-1.15 (t, *J* = 7.0 Hz, 12H), 2.77 (s, 2H), 3.15 (s, 2H), 4.05-4.15 (m, 8H), 7.00-7.19 (m, 4H), 7.29-7.32 (m, 4H), 7.40-7.59 (m, 4H), 7.79-7.85 (m, 4H), 7.90-7.99 (m, 2H)

ESI/MS (m/z) : 919.9 $(M+H)^{+}$.

5.1.10.2. Compound – 6b

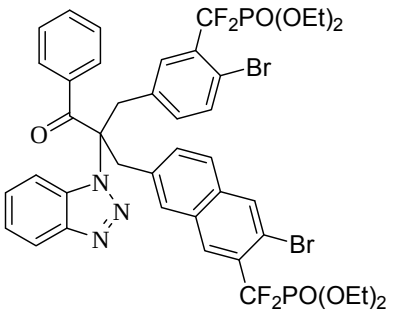


6b (1.2 g, 77%) was prepared from **4a** (1 g, 1.55 mmole) by means of the general procedure described as above as a white solid. mp: 129-131 ^{o}C; Purity by HPLC: 95.55%.

^{1}HNMR (Acetone-*d6*) : δ 1.09-1.14 (t, *J* = 7.1 Hz, 12H), 2.97 (s, 2H), 3.22 (s, 2H), 4.11-4.19 (m, 8H), 6.99-7.10 (m, 4H), 7.29-7.39 (m, 4H), 7.44-7.67 (m, 4H), 7.81-7.91 (m, 3H), 7.95-8.02 (m, 2H)

ESI/MS (m/z) : 998.9 $(M+H)^{+}$.

5.1.10.3. Compound – 6c

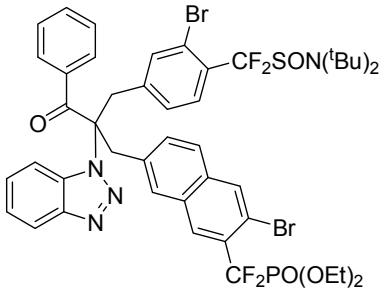


6c (1.1 g, 70%) was prepared from **4a** (1 g, 1.55 mmole) by means of the general procedure described as above as a white solid. mp: 119-121 ^{o}C; Purity by HPLC: 95.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.05-1.13 (t, *J* = 7.3 Hz, 12H), 2.87 (s, 2H), 3.19 (s, 2H), 4.09-4.17 (m, 8H), 7.00-7.23 (m, 4H), 7.33-7.39 (m, 4H), 7.50-7.67 (m, 4H), 7.79-7.91 (m, 3H), 7.95-7.99 (m, 2H)

ESI/MS (m/z) : 998.9 $(M+H)^{+}$.

5.1.10.4. Compound – 6d

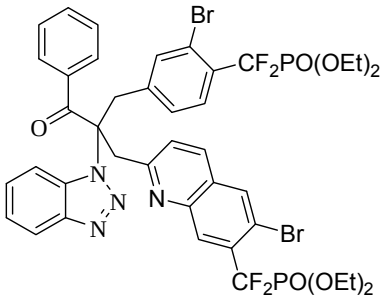


6d (1.1 g, 68%) was prepared from **4a** (1 g, 1.55 mmole) by means of the general procedure described as above as a white solid. mp: 141-143 ^{o}C; Purity by HPLC: 93.77%.

^{1}HNMR (Acetone-*d6*) : δ 1.07-1.11 (t, *J* = 7.2 Hz, 6H), 1.21 (s, 18H), 2.91 (s, 2H), 3.31 (s, 2H), 4.05 (m, 4H), 6.77-6.89 (m, 2H), 6.99-7.21 (m, 2H), 7.29-7.39 (m, 4H), 7.50-7.66 (m, 4H), 7.79-7.87 (m, 3H), 7.95-7.98 (m, 2H)

ESI/MS (m/z) : 1037.7 $(M+H)^{+}$.

5.1.10.5. Compound – 6e

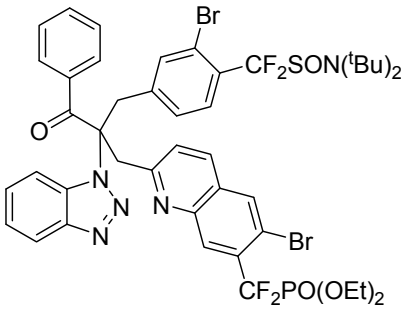


6e (1.3 g, 83%) was prepared from **4a** (1 g, 1.55 mmole) by means of the general procedure described as above as a white solid. mp: 129-131 ^{o}C; Purity by HPLC: 97.77%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.05-1.11 (t, *J* = 7.3 Hz, 12H), 2.93 (s, 2H), 3.31 (s, 2H), 4.10-4.17 (m, 8H), 7.00-7.19 (m, 3H), 7.28-7.39 (m, 3H), 7.49-7.65 (m, 4H), 7.77-7.82 (m, 3H), 7.90-7.99 (m, 3H)

ESI/MS (m/z) : 999.6 $(M+H)^{+}$.

5.1.10.6. Compound – 6f

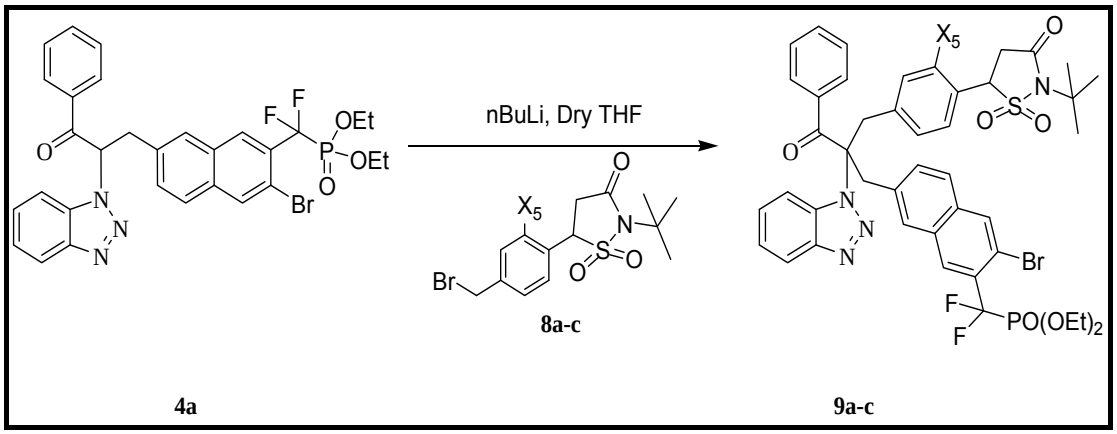


6f (1.3 g, 81%) was prepared from **4a** (1 g, 1.55 mmole) by means of the general procedure described as above as a white solid. mp: 138-140 ^{o}C; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.04-1.14 (t, *J* = 7.2 Hz, 6H), 1.23 (s, 18H), 2.77 (s, 2H), 3.24 (s, 2H), 4.19 (m, 4H), 6.87-7.10 (m, 3H), 7.33-7.51 (m, 6H), 7.61-7.77 (m, 2H), 7.99-8.27 (m, 5H)

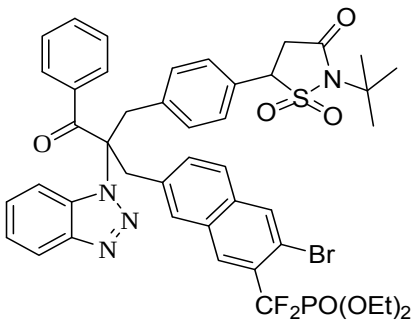
ESI/MS (m/z) : 1038.8 $(M+H)^{+}$.

5.1.11. General procedure for the synthesis of Compounds (9a-c)



To a solution of **4a** (1 mole equivalent) in dry THF (3 fold), a solution of n-BuLi (1.2 mole equivalent, 1.6 M in Hexane) was added drop wise over a period of 30 min at -78 ^{o}C and stirred at the same temperature for 30 min. **8a-c** (1.2 mole equivalent) was added in THF (1 fold) to the reaction mixture at the same temperature and further stirred at 25 ^{o}C for 6 hours. The reaction was quenched with a saturate aqueous NH_4Cl solution, extracted with ethyl acetate (2 X 3 fold). The combined organic layers were washed successively with water, followed by brine, and dried over Na_2SO_4, filtered and concentrated under vacuum to get the crude product which was subjected to flash by chromatography using 5% ethyl acetate in hexane as eluent to furnish title compound **9a-c** as white solids.

5.1.11.1. Compound - 9a

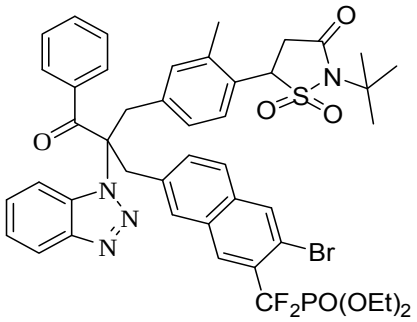


9a (0.9 g, 63%) was prepared from **4a** (1 g, 1.55 mmole) by means of the general procedure described as above as a white solid. mp: 153-155 ^{o}C; Purity by HPLC: 93.33%.

^{1}HNMR (Acetone-*d6*) : δ 1.03-1.12 (t, *J* = 7.2 Hz, 6H), 1.31 (s, 9H), 2.91 (s, 2H), 3.03 (dd, *J* = 13.8, 8.9 Hz, 1H), 3.11 (dd, *J* = 14.9, 6.2 Hz, 1H), 3.21 (s, 2H), 4.05 (m, 4H), 4.33 (t, *J* = 7.8 Hz, 1H), 7.03-7.23 (m, 5H), 7.30-7.39 (m, 4H), 7.44-7.67 (m, 4H), 7.83-7.87 (m, 3H), 7.93-7.99 (m, 2H)

ESI/MS (m/z) : 922.8 $(M+H)^{+}$.

5.1.11.2. Compound – 9b

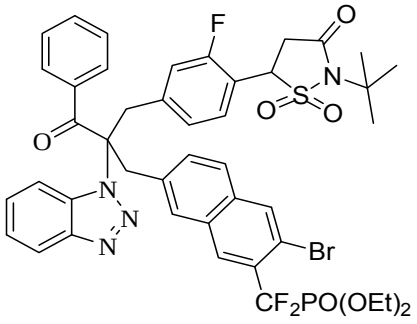


9b (1.01 g, 73%) was prepared from **4a** (1 g, 1.55 mmole) by means of the general procedure described as above as a white solid. mp: 139-141 ^{o}C; Purity by HPLC: 96.45%.

^{1}HNMR (Acetone-*d6*) : δ 1.01-1.11 (t, *J* = 7.0 Hz, 6H), 1.29 (s, 9H), 2.31 (s, 3H), 2.77 (s, 2H), 3.11 (dd, *J* = 13.9, 8.5 Hz, 1H), 3.19 (dd, *J* = 14.7, 6.0 Hz, 1H), 3.35 (s, 2H), 4.11 (m, 4H), 4.39 (t, *J* = 7.8 Hz, 1H), 6.73-6.96 (m, 3H), 7.07-7.23 (m, 1H), 7.31-7.41 (m, 4H), 7.50-7.68 (m, 4H), 7.83-7.88 (m, 3H), 7.94-8.03 (m, 2H)

ESI/MS (m/z) : 936.6 $(M+H)^{+}$.

5.1.11.3. Compound – 9c

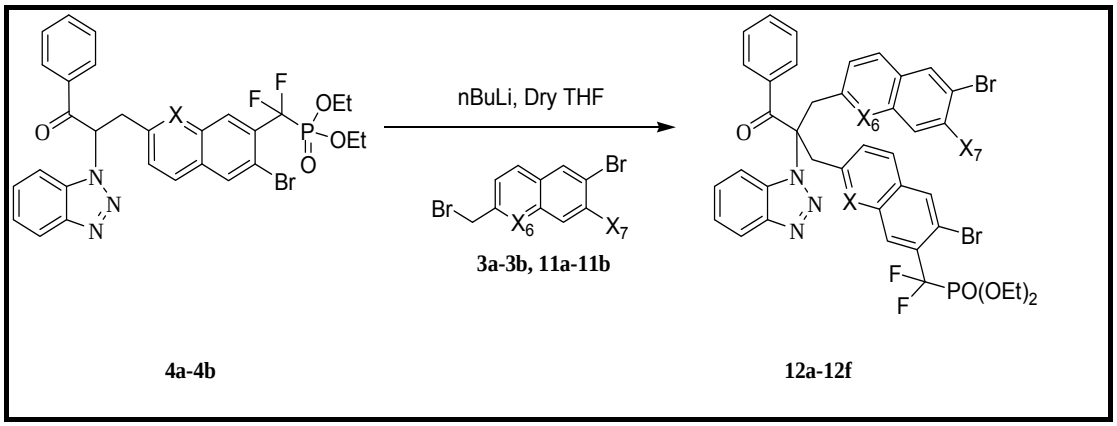


9c (1.1 g, 75%) was prepared from **4a** (1 g, 1.55 mmole) by means of the general procedure described as above as a white solid. mp: 121-123 ^{o}C; Purity by HPLC: 97.03%.

^{1}HNMR (Acetone-*d6*) : δ 1.05-1.11 (t, *J* = 7.2 Hz, 6H), 1.33 (s, 9H), 2.87 (s, 2H), 3.07 (dd, *J* = 14.2, 8.8 Hz, 1H), 3.21 (dd, *J* = 14.9, 5.9 Hz, 1H), 3.42 (s, 2H), 4.21 (m, 4H), 4.41 (t, *J* = 7.9 Hz, 1H), 6.81-6.91 (m, 2H), 7.03-7.22 (m, 2H), 7.31-7.41 (m, 4H), 7.55-7.69 (m, 4H), 7.81-7.89 (m, 3H), 7.93-7.97 (m, 2H)

ESI/MS (m/z) : 940.4 $(M+H)^{+}$.

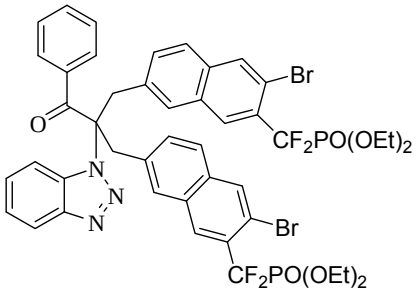
5.1.12. General procedure for the synthesis of Compounds (12a-f)



To a solution of **4a** or **4b** (1 mole equivalent) in dry THF (3 fold), a solution of n-BuLi (1.2 mole equivalent, 1.6 M in Hexane) was added drop wise over a period of 30 min at -78 ^{o}C and stirred at the same temperature for 30 min. **3a-b**, **11a-b** (1.2 mole equivalent) was added in THF (1 fold) to the reaction

mixture at same temperature and further stirred at 25 ^{o}C for 6 hours. The reaction was quenched with a saturate aqueous NH_4Cl solution, extracted with ethyl acetate (2 X 3 fold). The combined organic layers were washed successively with water, followed by brine, and dried over Na_2SO_4, filtered and concentrated under vacuum to get the crude product which was subjected to flash by chromatography using 5% ethyl acetate in hexane as eluent to furnish title compound **12a-f** as a white solid.

5.1.12.1. Compound - 12a

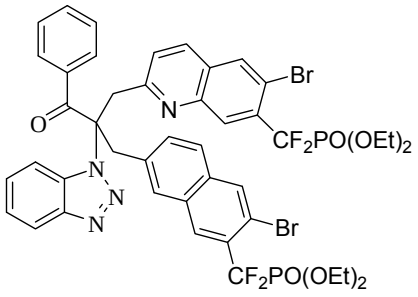


12a (0.95 g, 58%) was prepared from **4a** (1 g, 1.55 mmole) by means of the general procedure described as above as a white solid. mp: 129-131 ^{o}C; Purity by HPLC: 99.43%.

^{1}HNMR (Acetone-*d6*) : δ 1.11-1.19 (t, *J* = 7.2 Hz, 12H), 2.88 (s, 2H), 3.17 (s, 2H), 4.07-4.11 (m, 8H), 7.01-7.19 (m, 2H), 7.29-7.39 (m, 6H), 7.55-7.66 (m, 5H), 7.81-7.87 (m, 4H), 7.94-7.98 (m, 2H)

ESI/MS (m/z) : 1048.8 $(M+H)^{+}$.

5.1.12.2. Compound – 12b

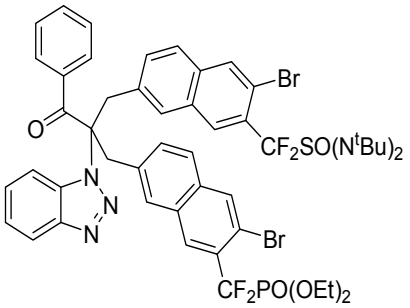


12b (1.2 g, 74%) was prepared from **4a** (1 g, 1.55 mmole) by means of the general procedure described as above as a white solid. mp: 137-139 ^{o}C; Purity by HPLC: 96.77%.

^{1}HNMR (Acetone-*d6*) : δ 1.09-1.17 (t, *J* = 7.0 Hz, 12H), 2.77 (s, 2H), 3.13 (s, 2H), 4.09-4.13 (m, 8H), 7.07-7.21 (m, 2H), 7.30-7.39 (m, 4H), 7.49-7.63 (m, 5H), 7.80-7.84 (m, 4H), 7.90-7.96 (m, 3H)

ESI/MS (m/z) : 1049.9 $(M+H)^{+}$.

5.1.12.3. Compound – 12c

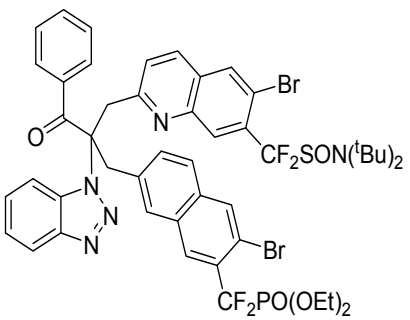


12c (1.4 g, 82%) was prepared from **4a** (1 g, 1.55 mmole) by means of the general procedure described as above as a white solid. mp: 162-164 ^{o}C; Purity by HPLC: 98.10%.

^{1}HNMR (Acetone-*d6*) : δ 1.10 (t, *J* = 7.2 Hz, 6H), 1.22 (s, 18H), 2.91 (s, 2H), 3.27 (s, 2H), 4.07-4.12 (m, 4H), 7.07-7.23 (m, 2H), 7.33-7.39 (m, 4H), 7.47-7.54 (m, 4H), 7.66-7.78 (m, 2H), 7.99-8.21 (m, 7H)

ESI/MS (m/z) : 1087.4 $(M+H)^{+}$.

5.1.12.4. Compound – 12d

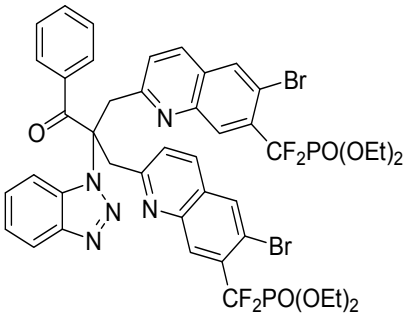


12d (1.4 g, 82%) was prepared from **4a** (1 g, 1.55 mmole) by means of the general procedure described as above as a white solid. mp: 159-161 ^{o}C; Purity by HPLC: 97.55%.

^{1}HNMR (Acetone-*d6*) : δ 1.02 (t, *J* = 7.0 Hz, 6H), 1.17 (s, 18H), 2.88 (s, 2H), 3.24 (s, 2H), 4.11-4.15 (m, 4H), 7.03-7.19 (m, 2H), 7.29-7.37 (m, 3H), 7.44-7.50 (m, 4H), 7.63-7.80 (m, 2H), 7.94-8.31 (m, 7H)

ESI/MS (m/z) : 1088.5 $(M+H)^{+}$.

5.1.12.5. Compound – 12e

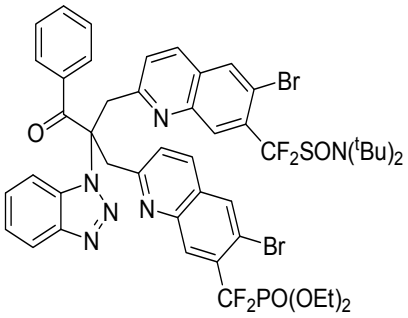


12e (1.1 g, 67%) was prepared from **4b** (1 g, 1.55 mmole) by means of the general procedure described as above as a white solid. mp: 159-161 ^{o}C; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.07-1.19 (t, *J* = 7.2 Hz, 12H), 2.85 (s, 2H), 3.27 (s, 2H), 4.07-4.12 (m, 8H), 7.01-7.22 (m, 2H), 7.32-7.42 (m, 2H), 7.49-7.69 (m, 5H), 7.83-7.89 (m, 4H), 7.94-7.98 (m, 4H)

ESI/MS (m/z) : 1050.3 $(M+H)^{+}$.

5.1.12.6. Compound – 12f

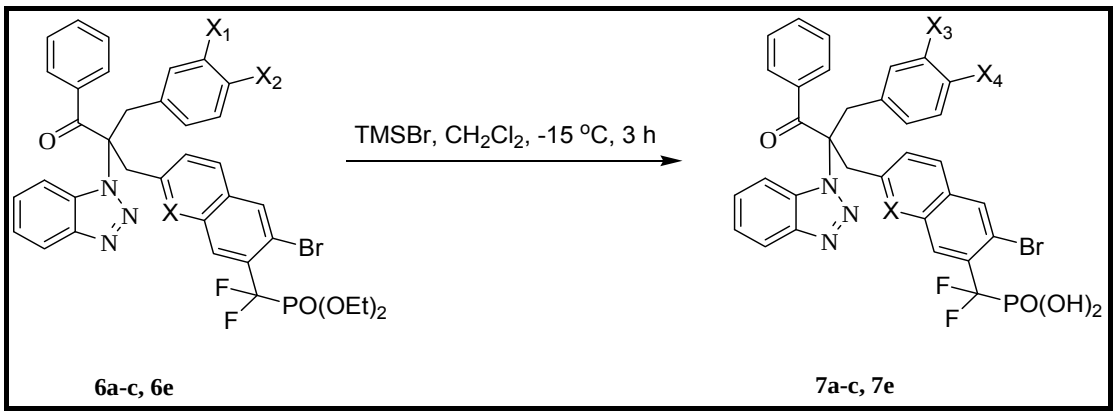


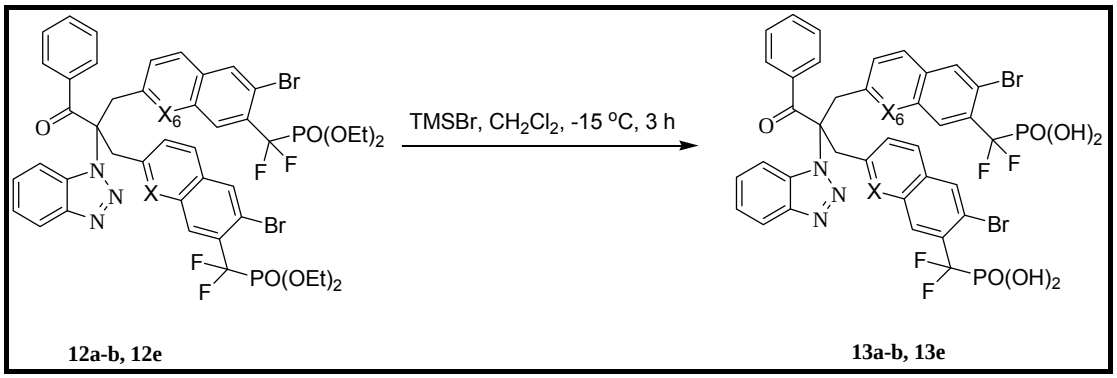
12f (1.3 g, 76%) was prepared from **4b** (1 g, 1.55 mmole) by means of the general procedure described as above as a white solid. mp: 133-135 ^{o}C; Purity by HPLC: 95.77%.

^{1}HNMR (Acetone-*d6*) : δ 1.09 (t, *J* = 7.2 Hz, 6H), 1.19 (s, 18H), 2.83 (s, 2H), 3.29 (s, 2H), 4.10-4.13 (m, 4H), 7.13-7.21 (m, 2H), 7.39-7.43 (m, 2H), 7.50-7.61 (m, 4H), 7.68-7.77 (m, 2H), 7.91-8.29 (m, 7H)

ESI/MS (m/z) : 1089.4 $(M+H)^{+}$.

5.1.13. General procedure for the synthesis of Compounds (7a-c, 7e & 13a-b, 13e)

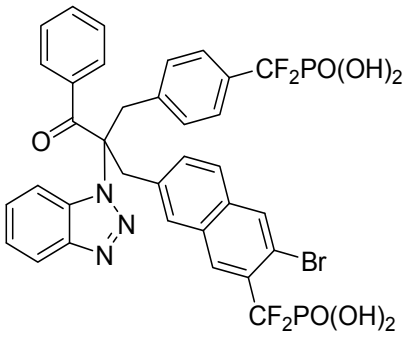




To a solution of **6a-c** or **16e** or **12a-b** or **12e** (1 mole equivalent) in CH_2Cl_2 (2 fold) chilled at -15 ^{o}C, bromotrimethylsilane (23 mole equivalent) was added drop wise over a period of 30 min and stirred at the same temperature for 3 h. The solution was concentrated by rotary evaporation to an oily residue which

was suspended in water, and extracted with ethyl acetate (2 X 3 fold). The combined organic layers were washed successively with saturated aqueous $NaHCO_3$ and water, followed by brine, and dried over Na_2SO_4, filtered and concentrated under vacuum to get the crude product which was subjected to flash chromatography by using 15% ethyl acetate in hexane as eluent to furnish the compounds **7a-c, 7e, 13a-b, 13e** as solids.

5.1.13.1. Compound – 7a



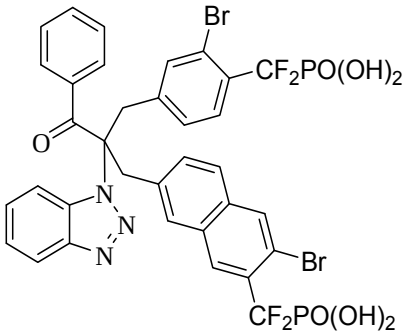
7a (0.59 g, 75%) was prepared from **6a** (0.9 g, 0.98 mmole) by means of the general procedure described as above as a white solid. mp: 163-164 ^{o}C; Purity by HPLC: 98.53%.

^{1}HNMR (Acetone-*d6*) : *δ* 2.89 (s, 2H), 3.25 (s, 2H), 7.01-7.21 (m, 4H), 7.30-7.32 (m, 4H), 7.45-7.65 (m, 4H), 7.85-7.91 (m, 4H), 7.90-7.98 (m, 2H)

^{13}CNMR (Acetone-*d6*) : *δ* 33.4, 34.9, 77.4, 118.8, 123.5, 125.7, 127.2, 127.6, 127.9, 128.1, 128.4, 128.7, 133.1, 134.5, 134.9, 135.9, 137.0, 137.4, 138.2, 203.3

ESI/MS (m/z) : 807.1 $(M+H)^{+}$.

5.1.13.2. Compound – 7b



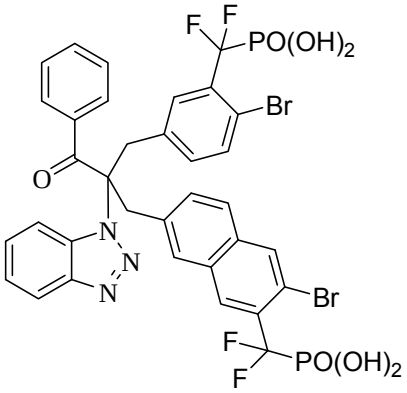
7b (0.56 g, 63%) was prepared from **6b** (1.0 g, 0.98 mmole) by means of the general procedure described as above as a white solid. mp: 173-174 ^{o}C; Purity by HPLC: 95.36%.

^{1}HNMR (Acetone-*d6*) : *δ* 2.93 (s, 2H), 3.27 (s, 2H), 7.00-7.21 (m, 4H), 7.30-7.36 (m, 4H), 7.47-7.65 (m, 4H), 7.85-7.91 (m, 3H), 7.93-7.98 (m, 2H)

^{13}CNMR (Acetone-*d6*) : *δ* 33.1, 34.7, 77.8, 110.3, 118.8, 123.5, 125.8, 127.1, 127.5, 127.9, 128.1, 128.4, 128.7, 133.5, 134.3, 134.9, 135.9, 137.0, 137.4, 138.2, 201.3

ESI/MS (m/z) : 886.3 $(M+H)^{+}$.

5.1.13.3. Compound – 7c



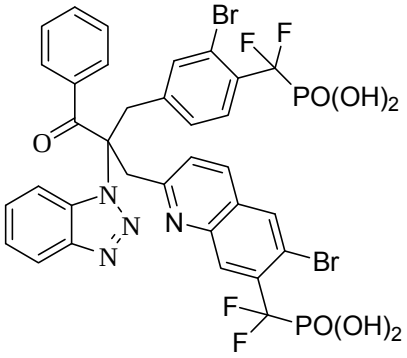
7c (0.59 g, 67%) was prepared from **6c** (1.0 g, 0.98 mmole) by means of the general procedure described as above as a white solid. mp: 193-195 ^{o}C; Purity by HPLC: 96.44%.

^{1}HNMR (Acetone-*d6*) : δ 2.90 (s, 2H), 3.25 (s, 2H), 7.00-7.21 (m, 4H), 7.32-7.36 (m, 4H), 7.49-7.65 (m, 4H), 7.85-7.88 (m, 3H), 7.93-7.97 (m, 2H)

^{13}CNMR (Acetone-*d6*) : δ 33.1, 34.6, 77.8, 110.6, 118.8, 119.9, 123.5, 125.9, 127.2, 127.5, 127.9, 128.1, 128.4, 128.7, 133.5, 134.3, 134.9, 135.9, 137.0, 137.4, 138.2, 140.2, 158.2, 201.3

ESI/MS (m/z) : 886.4 $(M+H)^{+}$.

5.1.13.4. Compound – 7e



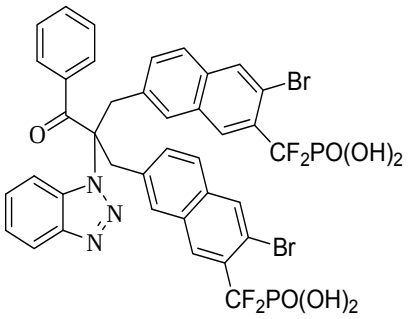
7e (0.66 g, 75%) was prepared from **6e** (1.0 g, 1.00 mmole) by means of the general procedure described as above as a pale yellow solid. mp: 159-161 ^{o}C; Purity by HPLC: 96.49%.

^{1}HNMR (Acetone-*d6*) : δ 2.99 (s, 2H), 3.29 (s, 2H), 7.00-7.21 (m, 3H), 7.30-7.36 (m, 3H), 7.47-7.65 (m, 4H), 7.85-7.91 (m, 3H), 7.93-7.98 (m, 3H)

^{13}CNMR (Acetone-*d6*) : δ 33.2, 34.6, 110.1, 118.6, 122.7, 123.1, 125.9, 127.0, 127.5, 127.8, 128.2, 128.5, 128.7, 133.6, 134.1, 134.6, 135.6, 137.0, 137.2, 138.2, 141.0, 147.2, 162.8, 200.0

ESI/MS (m/z) : 887.1 $(M+H)^{+}$.

5.1.13.5. Compound – 13a



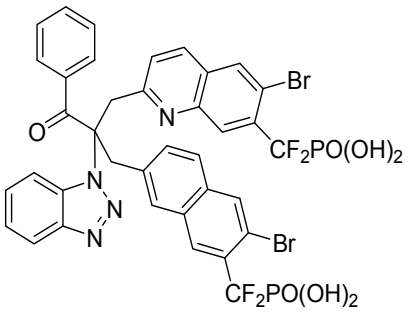
13a (0.58 g, 73%) was prepared from **12a** (0.9 g, 0.86 mmole) by means of the general procedure described as above as an off white solid. mp: 131-133 ^{o}C; Purity by HPLC: 99.16%.

^{1}HNMR (Acetone-*d6*) : δ 2.95 (s, 2H), 3.27 (s, 2H), 7.05-7.21 (m, 2H), 7.32-7.35 (m, 6H), 7.43-7.63 (m, 5H), 7.85-7.89 (m, 4H), 7.90-7.98 (m, 2H)

^{13}CNMR (Acetone-*d6*) : δ 33.2, 34.8, 77.3, 110.3, 118.3, 119.1, 123.4, 125.3, 126.7, 127.2, 127.9, 128.2, 128.7, 128.9, 133.0, 134.2, 134.7, 135.9, 137.1, 137.9, 138.3, 200.3

ESI/MS (m/z) : 936.2 $(M+H)^{+}$.

5.1.13.6. Compound – 13b



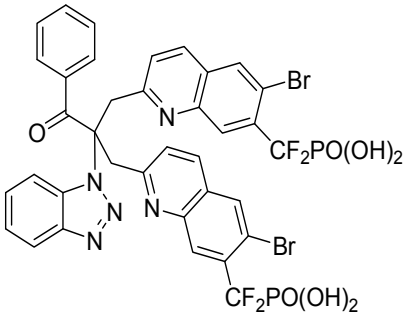
13b (0.65 g, 73%) was prepared from **12b** (1.0 g, 0.95 mmole) by means of the general procedure described as above as an off white solid. mp: 101-103 ^{o}C; Purity by HPLC: 97.20%.

^{1}HNMR (Acetone-*d6*) : δ 2.97 (s, 2H), 3.29 (s, 2H), 7.05-7.21 (m, 2H), 7.32-7.35 (m, 4H), 7.43-7.63 (m, 5H), 7.84-7.89 (m, 4H), 7.96-7.98 (m, 3H)

^{13}CNMR (Acetone-*d6*) : δ 33.8, 34.9, 77.0, 118.1, 118.5, 119.6, 123.5, 125.8, 126.9, 127.3, 127.9, 128.3, 128.7, 128.9, 133.4, 134.2, 134.7, 135.9, 137.1, 137.9, 138.3, 141.4, 147.4, 162.3, 200.3

ESI/MS (m/z) : 937.0 $(M+H)^+$.

5.1.13.7. Compound – 13e



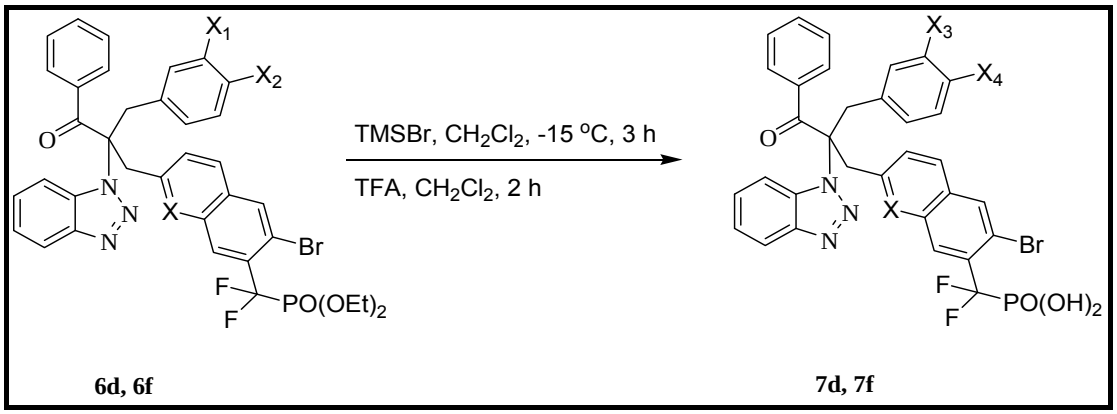
13e (0.59 g, 66%) was prepared from **12e** (1.0 g, 0.95 mmole) by means of the general procedure described as above as a yellow solid. mp: 151-153 ^oC; Purity by HPLC: 97.24%.

^{1}HNMR (Acetone-*d6*) : δ = 2.97 (s, 2H), 3.29 (s, 2H), 7.05-7.21 (m, 2H), 7.32-7.35 (m, 2H), 7.43-7.72 (m, 5H), 7.84-7.89 (m, 4H), 7.96-7.98 (m, 4H)

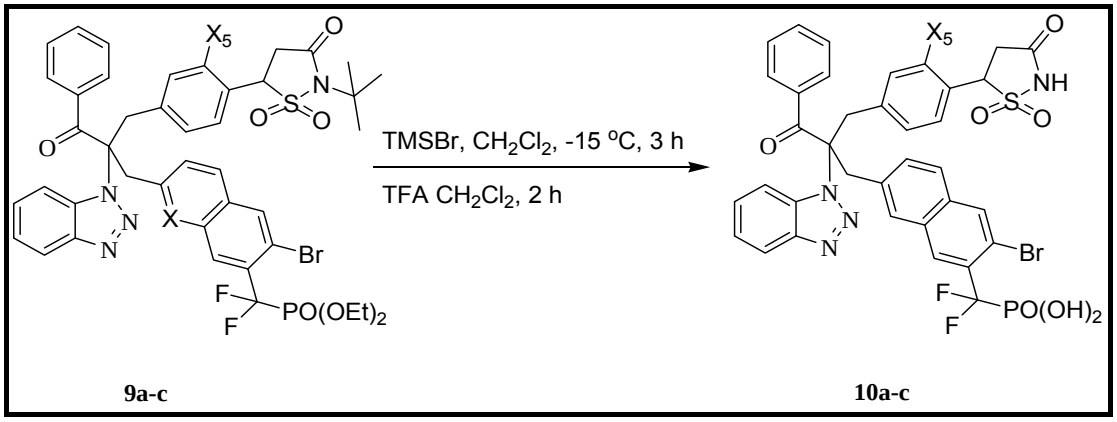
^{13}CNMR (Acetone-*d6*) : δ 33.8, 34.9, 77.4, 118.1, 118.5, 119.5, 121.0, 123.7, 125.8, 126.9, 127.3, 128.3, 128.7, 128.9, 133.4, 134.2, 134.7, 135.9, 137.9, 138.3, 141.4, 147.4, 162.3, 201.3

ESI/MS (m/z) : 938.1 $(M+H)^+$.

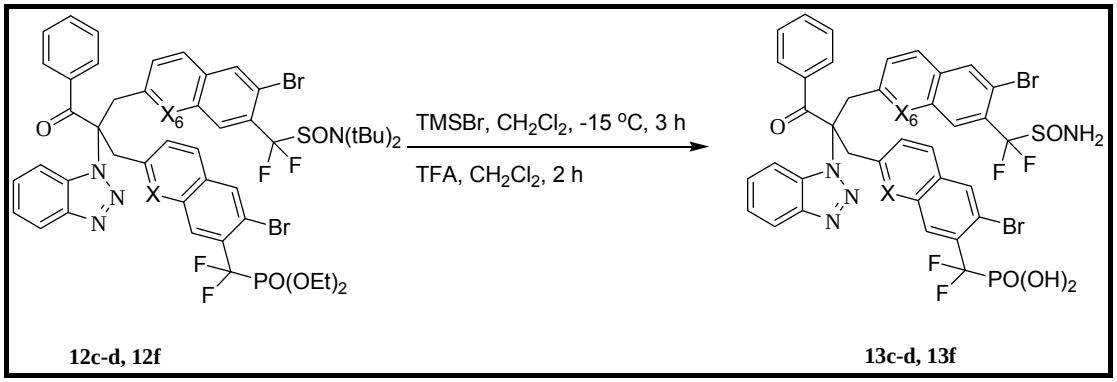
5.1.14. General procedure for the synthesis of Compounds (7d, 7f, 10a-c, 13c-d & 13f)



6d, 6f **7d, 7f**



9a-c **10a-c**

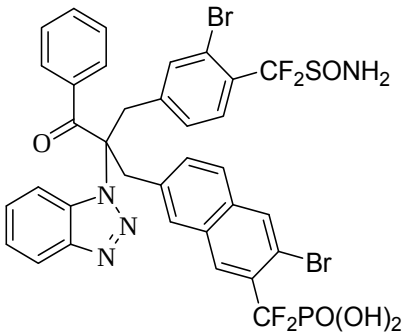


12c-d, 12f **13c-d, 13f**

To a solution of **6d** or **6f** or **9a-c** or **12c-d** or **12f** (1 mole equivalent) in CH_2Cl_2 (2 fold) chilled at -15 ^oC, bromotrimethylsilane (23 mole equivalent) was added drop wise over a period of 30 min at -15 ^oC and stirred at the same temperature for 3 h. The solution was concentrated by rotary evaporation to

an oily residue, dissolved in a mixture of CH_2Cl_2 (2 fold) and trifluoroacetic acid (2 fold) and stirred for 2 h. The solution was then evaporated to dryness, suspended in water, and extracted with ethyl acetate (2 X 3 fold). The combined organic layers were washed successively with saturated aqueous $NaHCO_3$, water, followed by brine, and dried over Na_2SO_4, filtered and concentrated under vacuum to get the crude product which was subjected to flash chromatography by using 15% ethyl acetate in hexane as eluent to furnish title compounds **7d, 7f, 10a-c, 13c-d** & **13f** as solids.

5.1.14.1. Compound – 7d



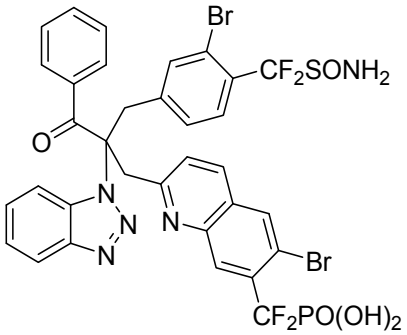
7d (0.56 g, 67%) was prepared from **6d** (1.0 g, 0.96 mmole) by means of the general procedure described as above as a white solid. mp: 181-183 ^oC; Purity by HPLC: 96.26%.

^{1}HNMR (Acetone-*d6*) : δ 2.94 (s, 2H), 3.27 (s, 2H), 6.80-6.89 (m, 2H), 7.00-7.21 (m, 2H), 7.32-7.38 (m, 4H), 7.49-7.65 (m, 4H), 7.84-7.89 (m, 3H)

^{13}CNMR (Acetone-*d6*) : δ 33.4, 34.6, 77.4, 110.8, 118.9, 119.9, 123.5, 125.7, 127.1, 127.5, 127.9, 128.1, 128.4, 128.9, 133.5, 134.3, 134.9, 135.9, 137.0, 137.4, 138.4, 140.6, 158.8, 200.3

ESI/MS (m/z) : 869.3 $(M+H)^+$.

3.1.14.2. Compound – 7f



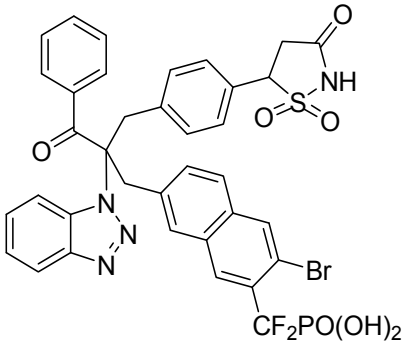
7f (0.68 g, 67%) was prepared from **6f** (1.2 g, 1.15 mmole) by means of the general procedure described as above as a white solid. mp: 161-163 ^{o}C; Purity by HPLC: 96.46%.

^{1}HNMR (Acetone-*d6*) : *δ* 2.97 (s, 2H), 3.29 (s, 2H), 7.21-6.90 (m, 3H), 7.38-7.55 (m, 6H), 7.64-7.77 (m, 2H), 7.90-8.35 (m, 5H)

^{13}CNMR (Acetone-*d6*) : *δ* 33.5, 34.7, 87.4, 110.2, 118.6, 119.8, 123.5, 125.7, 127.0, 127.5, 127.9, 128.1, 128.4, 128.9, 133.4, 134.3, 134.6, 135.9, 137.0, 137.8, 138.4, 140.6, 158.8, 162.0, 200.3

ESI/MS (m/z) : 870.3 $(M+H)^{+}$.

5.1.14.3. Compound – 10a



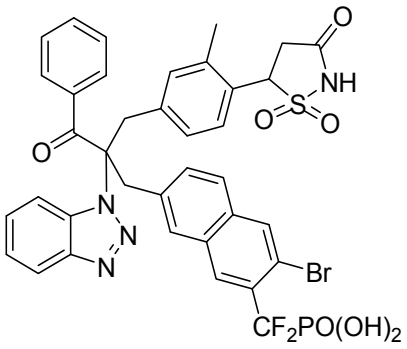
10a (0.55 g, 78%) was prepared from **9a** (0.8 g, 0.86 mmole) by means of the general procedure described as above as a white solid. mp: 119-121 ^{o}C; Purity by HPLC: 98.77%.

^{1}HNMR (Acetone-*d6*) : δ 2.95 (s, 2H), 3.01 (dd, *J* = 13.9, 8.5 Hz, 1H), 3.15 (dd, *J* = 14.8, 6.0 Hz, 1H), 3.27 (s, 2H), 4.50 (t, *J* = 7.9 Hz, 1H), 7.05-7.21 (m, 5H), 7.32-7.35 (m, 4H), 7.43-7.63 (m, 4H), 7.84-7.87 (m, 3H), 7.90-7.99 (m, 2H)

^{13}CNMR (Acetone-*d6*) : δ 33.4, 34.7, 37.1, 54.1, 77.1, 110.1, 118.9, 119.3, 123.6, 125.3, 126.8, 127.3, 127.9, 128.3, 128.7, 128.9, 133.0, 134.1, 134.7, 135.7, 137.0, 137.7, 138.1, 140.1, 177.0, 201.3

ESI/MS (m/z) : 810.3 $(M+H)^{+}$.

5.1.14.4. Compound – 10b



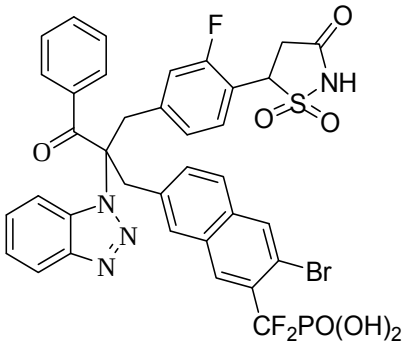
10b (0.63 g, 80%) was prepared from **9b** (0.9 g, 0.96 mmole) by means of the general procedure described as above as an off white solid. mp: 159-160 ^{o}C; Purity by HPLC: 97.78%.

^{1}HNMR (Acetone-*d6*) : δ 2.35 (s, 3H), 2.99 (s, 2H), 3.07 (dd, *J* = 13.7, 9.9 Hz, 1H), 3.17 (dd, *J* = 14.7, 4.4 Hz, 1H), 3.25 (s, 2H), 4.91 (t, *J* = 7.9 Hz, 1H), 6.78-6.89 (m, 3H), 7.05-7.21 (m, 1H), 7.32-7.38 (m, 4H), 7.49-7.65 (m, 4H), 7.86-7.89 (m, 3H), 7.93-7.98 (m, 2H)

^{13}CNMR (Acetone-*d6*) : δ 18.4, 33.2, 34.5, 37.4, 47.1, 77.3, 110.8, 118.6, 118.9, 123.1 125.6, 126.9, 127.1, 127.9, 128.1, 128.4, 128.9, 133.9, 134.3, 134.9, 135.7, 137.0, 137.2, 138.9, 140.7, 158.9, 177.9, 202.3

ESI/MS (m/z) : 824.3 $(M+H)^{+}$.

5.1.14.5. Compound – 10c



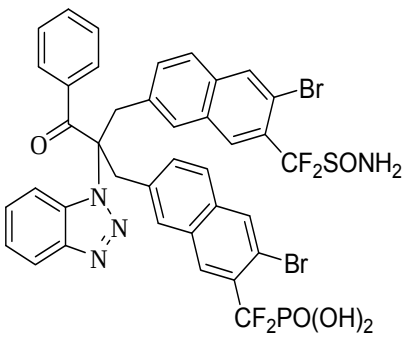
10c (0.65 g, 74%) was prepared from **9c** (1.0 g, 1.06 mmole) by means of the general procedure described as above as an off white solid. mp: 165-167 ^{o}C; Purity by HPLC: 95.10%.

^{1}HNMR (Acetone-*d6*) : δ 2.91 (s, 2H), 3.05 (dd, *J* = 13.8, 8.5 Hz, 1H), 3.19 (dd, *J* = 14.9, 6.0 Hz, 1H), 3.27 (s, 2H), 4.55 (m, 1H), 6.78-6.89 (m, 2H), 7.05-7.21 (m, 2H), 7.32-7.38 (m, 4H), 7.49-7.65 (m, 4H), 7.85-7.89 (m, 3H), 7.90-7.98 (m, 2H)

^{13}CNMR (Acetone-*d6*) : δ 33.4, 34.6, 37.1, 42.8, 77.2, 110.3, 118.3, 118.9, 123.6, 125.6, 127.0, 127.4, 127.9, 128.0, 128.4, 128.9, 133.8, 134.2, 134.9, 135.8, 137.1, 137.2, 138.9, 140.7, 158.9, 177.0, 201.3

ESI/MS (m/z) : 828.3 $(M+H)^{+}$.

5.1.14.6. Compound – 13c



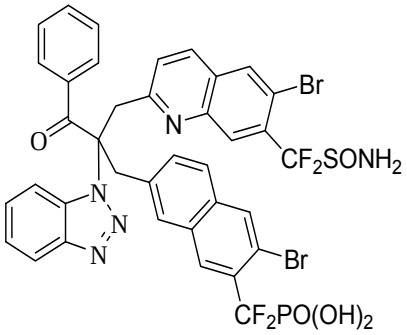
13c (0.78 g, 71%) was prepared from **12c** (1.3 g, 1.19 mmole) by means of the general procedure described as above as an off white solid. mp: 151-153 ^{o}C; Purity by HPLC: 98.20%.

^{1}HNMR (Acetone-*d6*) : *δ* 2.95 (s, 2H), 3.27 (s, 2H), 7.05-7.21 (m, 2H), 7.31-7.35 (m, 4H), 7.40-7.53 (m, 4H), 7.63-7.75 (m, 2H), 7.90-8.16 (m, 7H)

^{13}CNMR (Acetone-*d6*) : *δ* 33.2, 34.8, 87.3, 110.3, 118.3, 119.1, 123.4, 125.3, 126.7, 127.2, 127.9, 128.2, 128.7, 128.9, 133.0, 134.2, 134.7, 135.9, 137.1, 137.9, 138.3, 142.2, 162.3, 200.3

ESI/MS (m/z) : 919.2 $(M+H)^{+}$.

5.1.14.7. Compound – 13d



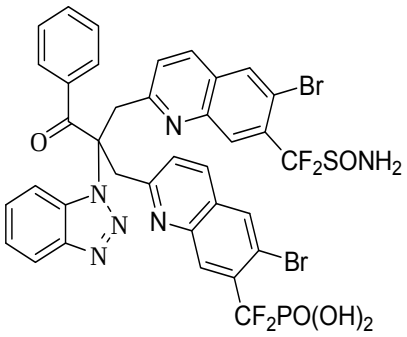
13d (0.70 g, 76%) was prepared from **12d** (1.1 g, 1.01 mmole) by means of the general procedure described as above as an off white solid. mp: 121-123 ^{o}C; Purity by HPLC: 97.20%.

^{1}HNMR (Acetone-*d6*) : *δ* 2.93 (s, 2H), 3.29 (s, 2H), 7.05-7.21 (m, 2H), 7.31-7.35 (m, 3H), 7.40-7.55 (m, 4H), 7.60-7.79 (m, 2H), 7.90-8.35 (m, 7H)

^{13}CNMR (Acetone-*d6*) : *δ* 33.2, 34.7, 87.3, 110.3, 118.3, 119.1, 123.5, 125.3, 126.8, 127.2, 127.9, 128.2, 128.7, 128.9, 133.5, 134.2, 134.7, 135.9, 137.1, 137.9, 138.3, 142.3, 162.3, 200.7

ESI/MS (m/z) : 920.2 $(M+H)^{+}$.

5.1.14.8. Compound – 13f



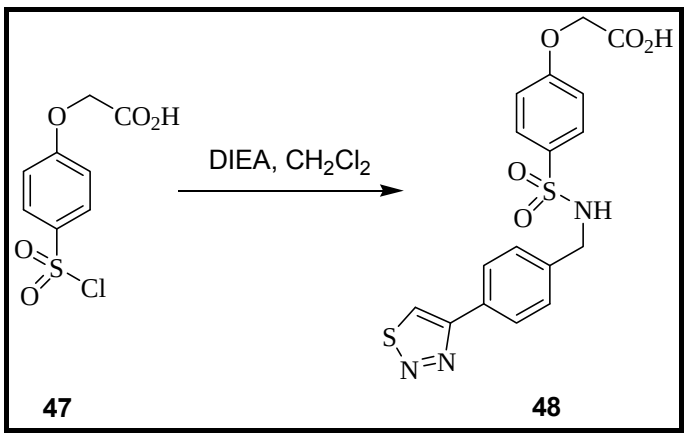
13f (0.77 g, 76%) was prepared from **12f** (1.2 g, 1.10 mmole) by means of the general procedure described as above as an off white solid. mp: 121-123 ^{o}C; Purity by HPLC: 98.20%.

^{1}HNMR (Acetone-*d6*) : δ 2.93 (s, 2H), 3.29 (s, 2H), 7.15-7.21 (m, 2H), 7.35-7.41 (m, 2H), 7.50-7.55 (m, 4H), 7.60-7.79 (m, 2H), 7.90-8.36 (m, 7H)

^{13}CNMR (Acetone-*d6*) : δ 33.4, 34.7, 87.3, 110.1, 118.2, 119.1, 123.2, 125.3, 126.9, 127.2, 127.9, 128.3, 128.7, 128.9, 133.5, 134.2, 134.7, 135.9, 137.1, 137.9, 138.3, 141.8, 142.3, 162.3, 201.7

ESI/MS (m/z) : 921.2 $(M+H)^{+}$.

5.1.15. 2-[4-{*N*-4(1,2,3-thiadiazol-4-yl)benzyl}-benzenesulfonamide)phenoxy]-acetic acid (48)



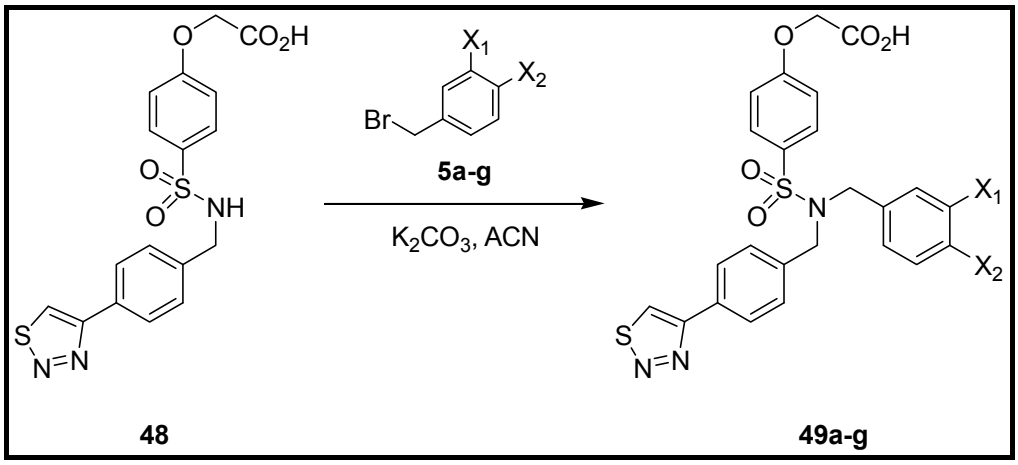
To a stirred solution of **47** (20 g, 0.08 mol) and thiadiazolylbenzyl amine (15.3 g, 0.08 mol) in dry CH_2Cl_2 (80 mL) was added diisopropylethylamine (0.088

mol) at 25 ^{o}C for 5 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with water followed by brine solution, dried over sodium sulfate and concentrated to afford a solid product which was purified using column chromatography using 0-30% ethyl acetate in hexane as eluent to furnish the title compound **48** (25.9 g, 80%) as a off white solid. mp: 93-94 ^{o}C; Purity by HPLC: 98.19%.

^{1}HNMR (Acetone-*d6*) : δ 4.37 (s, 2H), 4.94 (s, 2H), 7.01-7.15 (m, 4H), 7.32-7.36 (m, 2H), 7.79-7.85 (m, 2H), 8.21 (s, 1H), 11.1 (s, 1H)

ESI/MS (m/z) : 407.4 $(M+H)^{+}$.

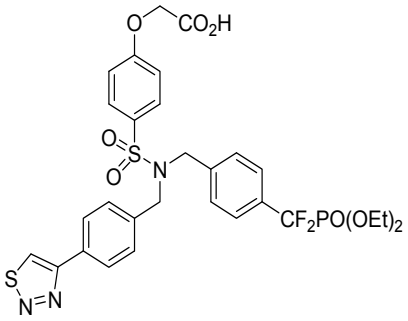
5.1.16. General procedure for the synthesis of Compounds (49a-g)



48 **49a-g**

A mixture of **48** (1 mole equivalent), **5a-g** (1 mole equivalent) and anhydrous K_2CO_3 (1.2 mole equivalent) in ACN (10 fold) under nitrogen was heated at 70 ^{o}C for 4 h. The reaction mixture was cooled and concentrated under reduced pressure. Residue was diluted with ethyl acetate (10 fold), washed with water followed by brine, dried over sodium sulfate and concentrated to afford a solid product which was purified using column chromatography using 0-50% ethyl acetate in hexane as eluent to furnish title compound **49a-g** as a solid.

5.1.16.1. Compound – 49a

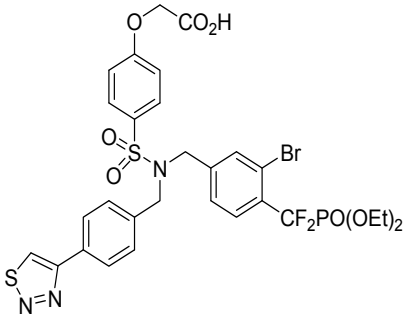


49a (1.17 g, 70%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described above as a white solid. mp: 123-124 ^{o}C; Purity by HPLC: 97.70%.

^{1}HNMR (Acetone-*d6*) : δ 1.11 (t, J = 7.1 Hz, 6H), 4.00-4.13 (m, 4H), 4.24 (s, 2H), 4.47 (s, 2H), 4.91 (s, 2H), 6.94-7.00 (m, 4H), 7.07-7.19 (m, 4H), 7.33-7.39 (m, 2H), 7.83-7.92 (m, 2H), 8.21 (s, 1H), 11.0 (s, 1H)

ESI/MS (m/z) : 683.7 $(M+H)^{+}$.

5.1.16.2. Compound – 49b

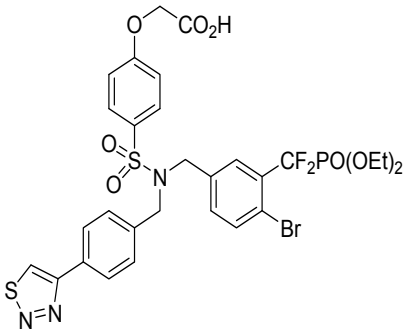


49b (1.55 g, 83%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described above as a white solid. mp: 133-134 ^{o}C; Purity by HPLC: 96.60%.

^{1}HNMR (Acetone-*d6*) : δ 1.07 (t, J = 6.9 Hz, 6H), 4.05-4.15 (m, 4H), 4.28 (s, 2H), 4.44 (s, 2H), 4.87 (s, 2H), 6.82-6.90 (m, 2H), 7.07-7.21 (m, 5H), 7.30-7.38 (m, 2H), 7.77-7.80 (m, 2H), 8.17 (s, 1H), 11.4 (s, 1H)

ESI/MS (m/z) : 761.6 $(M+H)^{+}$.

5.1.16.3. Compound – 49c

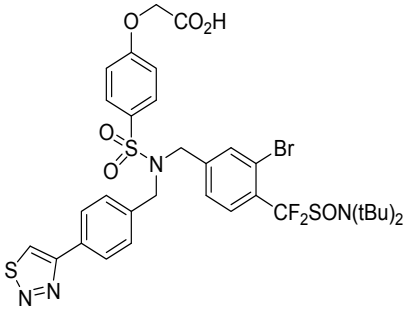


49c (1.49 g, 80%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described above as a white solid. mp: 100-101 ^{o}C; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.13 (t, J = 7.0 Hz, 6H), 4.08-4.16 (m, 4H), 4.32 (s, 2H), 4.45 (s, 2H), 4.93 (s, 2H), 6.77 (s, 1H), 6.88 (m, 1H), 7.05-7.19 (m, 5H), 7.32-7.36 (m, 2H), 7.80-7.84 (m, 2H), 8.20 (s, 1H), 11.1 (s, 1H)

ESI/MS (m/z) : 761.4 $(M+H)^{+}$.

5.1.16.4. Compound – 49d

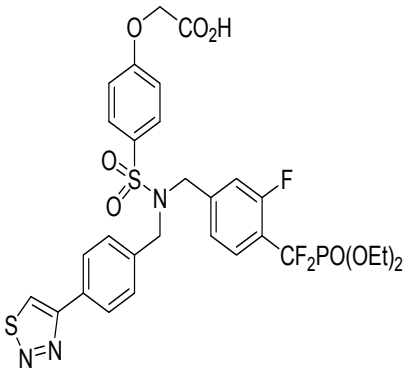


49d (1.18 g, 60%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described above as a white solid. mp: 139-141 ^{o}C; Purity by HPLC: 94.40%.

^{1}HNMR (Acetone-*d6*) : δ 1.17 (s, 18H), 4.32 (s, 2H), 4.44 (s, 2H), 4.77 (s, 2H), 6.80-6.89 (m, 2H), 7.03-7.13 (m, 5H), 7.36-7.42 (m, 2H), 7.80-7.90 (m, 2H), 8.16 (s, 1H), 11.1 (s, 1H)

ESI/MS (m/z) : 800.4 $(M+H)^{+}$.

5.1.16.5 Compound – 49e

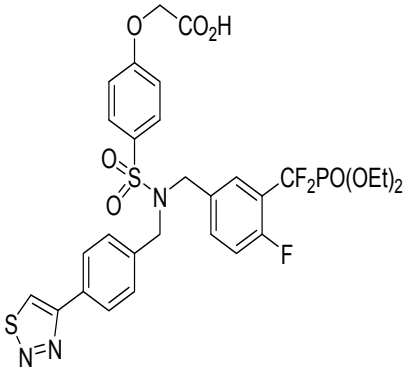


49e (1.11 g, 65%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described above as a white solid. mp: 119-120 ^{o}C; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.13 (t, *J* = 6.8 Hz, 6H), 4.05-4.19 (m, 4H), 4.30 (s, 2H), 4.42 (s, 2H), 4.83 (s, 2H), 6.69 (d, *J* = 1.9 Hz, 1H), 6.79-6.99 (m, 2H), 7.09-7.19 (m, 4H), 7.32-7.37 (m, 2H), 7.79-7.81 (m, 2H), 8.13 (s, 1H), 11.0 (s, 1H)

ESI/MS (m/z) : 700.5 $(M+H)^{+}$.

5.1.16.6 Compound – 49f

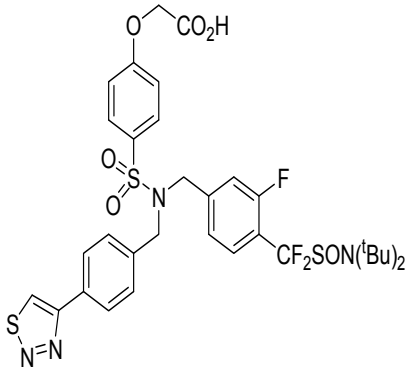


49e (0.85 g, 50%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described above as a white solid. mp: 99-100 ^{o}C; Purity by HPLC: 95.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.21 (t, J = 7.1 Hz, 6H), 4.07-4.12 (m, 4H), 4.32 (s, 2H), 4.44 (s, 2H), 4.91 (s, 2H), 6.74-6.87 (m, 3H), 7.11-7.20 (m, 4H), 7.36-7.40 (m, 2H), 7.73-7.77 (m, 2H), 8.09 (s, 1H), 11.2 (s, 1H)

ESI/MS (m/z) : 700.5 $(M+H)^+$.

5.1.16.7 Compound – 49g

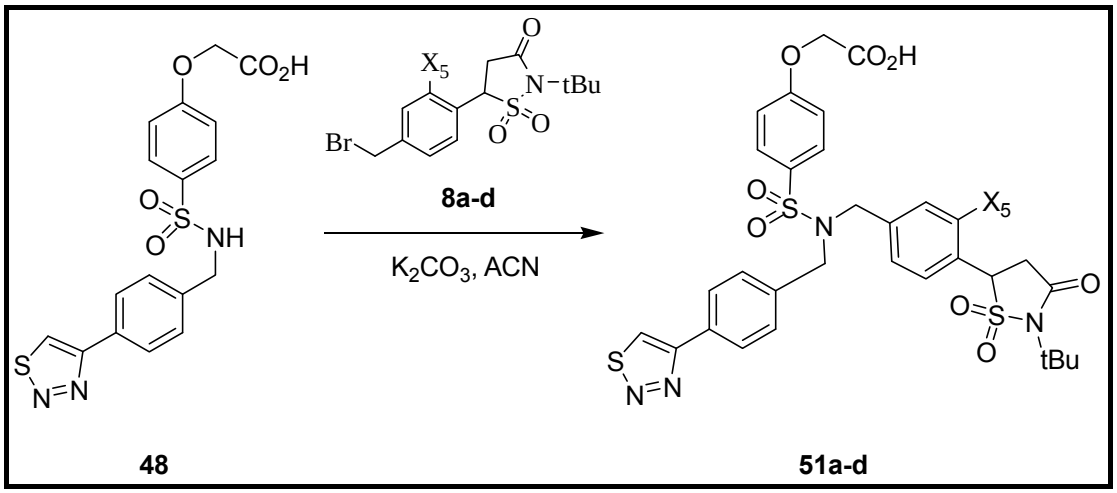


49g (1.00 g, 55%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described above as a white solid. mp: 121-122 ^oC; Purity by HPLC: 95.55%.

^{1}HNMR (Acetone-*d6*) : δ 1.21 (s, 18H), 4.33 (s, 2H), 4.42 (s, 2H), 4.93 (s, 2H), 6.71-6.94 (m, 3H), 7.07-7.17 (m, 4H), 7.33-7.40 (m, 2H), 7.79-7.87 (m, 2H), 8.21 (s, 1H), 11.5 (s, 1H)

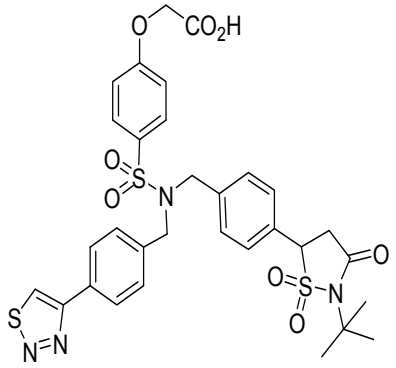
ESI/MS (m/z) : 740.2 $(M+H)^+$.

5.1.17. General procedure for the synthesis of Compounds (51a-d)



Title compound **51a-d** was prepared using the same procedure as that used for compound **49a-g** using appropriate starting material.

5.1.17.1 Compound – 51a

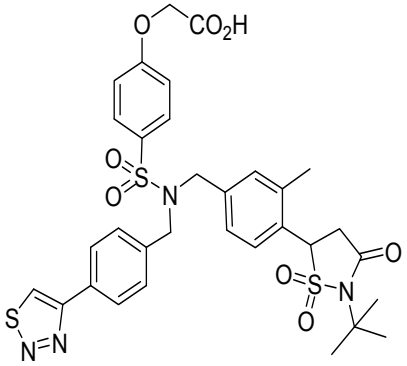


51a (1.18 g, 70%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described in **5.1.16.** as a white solid. mp: 163-164 ^{o}C; Purity by HPLC: 96.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.33 (s, 9H), 3.09 (dd, *J* = 17.5, 8.3 Hz, 1H), 3.21 (dd, *J* = 17.0, 7.3 Hz, 1H), 4.17 (s, 2H), 4.33 (s, 2H), 4.62 (t, *J* = 7.8, 1H), 4.91 (s, 2H), 6.99-7.00 (m, 4H), 7.10-7.19 (m, 4H), 7.37-7.41 (m, 2H), 7.80-7.88 (m, 2H), 8.27 (s, 1H), 11.2 (s, 1H)

ESI/MS (m/z) : 686.6 $(M+H)^{+}$.

5.1.17.2. Compound – 51b

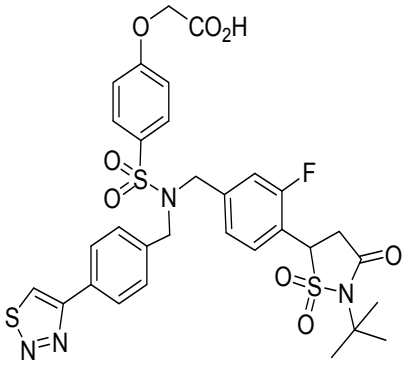


51b (1.20 g, 70%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described in **5.1.16.** as a white solid. mp: 155-157 ^{o}C; Purity by HPLC: 93.70%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.30 (s, 9H), 2.37 (s, 3H), 3.01 (dd, *J* = 16.9, 7.9 Hz, 1H), 3.17 (dd, *J* = 17.3, 7.5 Hz, 1H), 4.23 (s, 2H), 4.37 (s, 2H), 4.69 (t, *J* = 7.9, 1H), 4.88 (s, 2H), 6.80-6.91 (m, 3H), 7.09-7.17 (m, 4H), 7.30-7.40 (m, 2H), 7.77-7.87 (m, 2H), 8.12 (s, 1H), 11.3 (s, 1H)

ESI/MS (m/z) : 700.1 (M+H) $^{+}$.

5.1.17.3. Compound – 51c

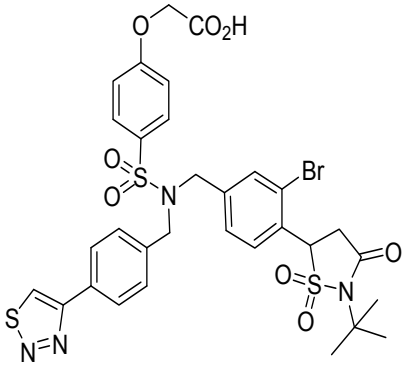


51c (1.38 g, 80%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described in **5.1.16.** as a white solid. mp: 139-141 ^{o}C; Purity by HPLC: 94.40%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.28 (s, 9H), 2.93 (dd, *J* = 16.8, 7.7 Hz, 1H), 3.19 (dd, *J* = 17.0, 7.2 Hz, 1H), 4.27 (s, 2H), 4.38 (s, 2H), 4.67 (t, *J* = 7.2, 1H), 4.93 (s, 2H), 6.70-6.91 (m, 3H), 7.10-7.15 (m, 4H), 7.37-7.42 (m, 2H), 7.84-7.91 (m, 2H), 8.13 (s, 1H), 11.4 (s, 1H)

ESI/MS (m/z) : 703.6 (M+H) $^{+}$.

5.1.17.4. Compound – 51d

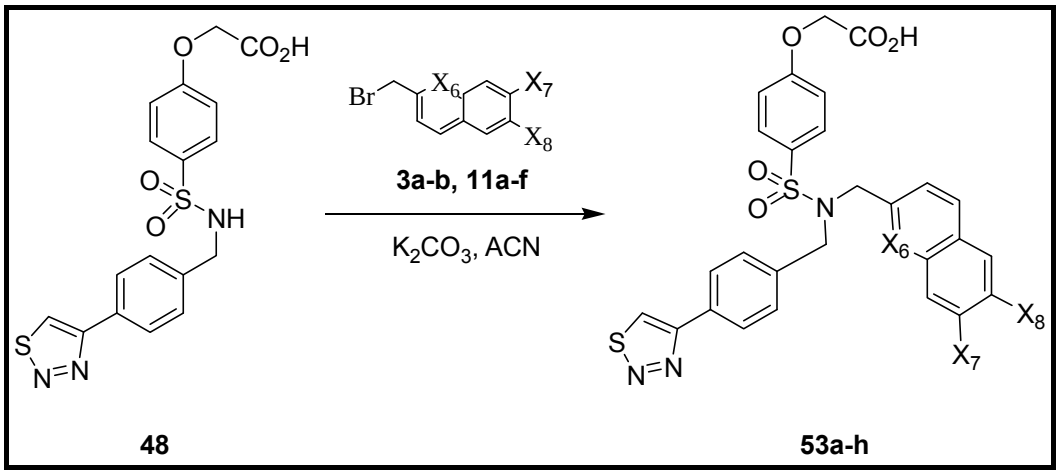


51c (1.22 g, 65%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described in **5.1.16.** as a white solid. mp: 122-123 ^{o}C; Purity by HPLC: 95.80%.

^{1}HNMR (Acetone-*d6*) : δ 1.31 (s, 9H), 2.99 (dd, *J* = 17.1, 7.9 Hz, 1H), 3.17 (dd, *J* = 17.1, 7.1 Hz, 1H), 4.21 (s, 2H), 4.40 (s, 2H), 4.63 (t, *J* = 7.4, 1H), 4.93 (s, 2H), 6.88-6.93 (m, 2H), 7.09-7.13 (m, 4H), 7.21 (s, 1H), 7.35-7.39 (m, 2H), 7.83-7.87 (m, 2H), 8.10 (s, 1H), 11.5 (s, 1H)

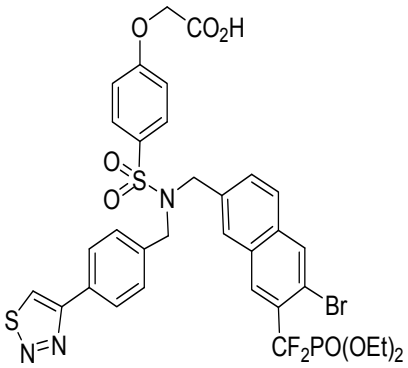
ESI/MS (m/z) : 764.8 $(M+H)^{+}$.

5.1.18. General procedure for the synthesis of Compounds (53a-h)



Title compound **53a-h** was prepared using the same procedure as that used for compound **49a-g** using appropriate starting material.

5.1.18.1. Compound – 53a

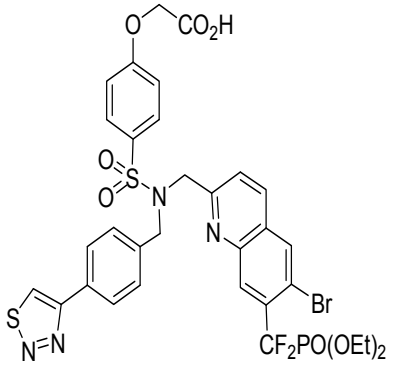


53a (1.25 g, 63%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described in **5.1.16.** as a white solid. mp: 117-118 ^{o}C; Purity by HPLC: 96.10%.

^{1}HNMR (Acetone-*d6*) : δ 1.12 (t, J = 7.2 Hz, 6H), 4.03-4.10 (m, 4H), 4.22 (s, 2H), 4.57 (s, 2H), 4.89 (s, 2H), 7.09-7.18 (m, 4H), 7.23-7.37 (m, 5H), 7.63 (m, 1H), 7.81-7.89 (m, 3H), 8.15 (s, 1H), 11.0 (s, 1H)

ESI/MS (m/z) : 811.7 $(M+H)^{+}$.

5.1.18.2. Compound – 53b

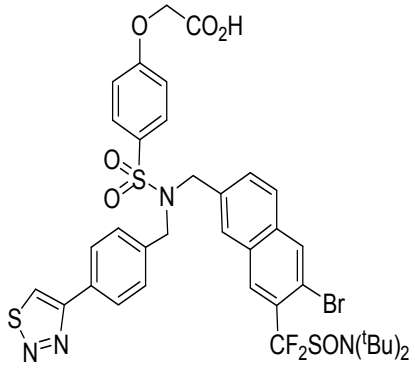


53b (0.99 g, 50%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described in **5.1.16.** as a white solid. mp: 123-124 ^{o}C; Purity by HPLC: 95.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.17 (t, *J* = 7.0 Hz, 6H), 4.05-4.09 (m, 4H), 4.22 (s, 2H), 4.78 (s, 2H), 4.91 (s, 2H), 7.02-7.19 (m, 5H), 7.29-7.36 (m, 2H), 7.77-7.96 (m, 5H), 8.20 (s, 1H), 11.4 (s, 1H)

ESI/MS (m/z) : 812.6 $(M+H)^+$.

5.1.18.3. Compound – 53c

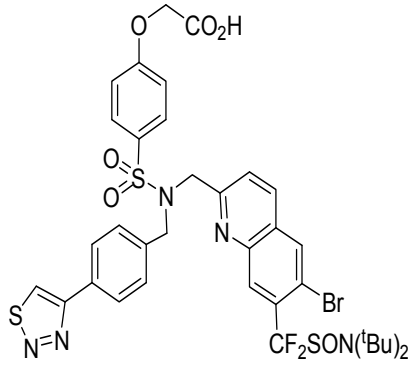


53c (1.25 g, 60%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described in **5.1.16.** as a white solid. mp: 133-135 ^oC; Purity by HPLC: 97.70%.

^{1}HNMR (Acetone-*d6*) : δ 1.19 (s, 18H), 4.38 (s, 2H), 4.55 (s, 2H), 4.96 (s, 2H), 7.03-7.20 (m, 4H), 7.25-7.39 (m, 5H), 7.63-7.83 (m, 4H), 8.23 (s, 1H), 11.2 (s, 1H)

ESI/MS (m/z) : 851.3 $(M+H)^+$.

5.1.18.4. Compound – 53d

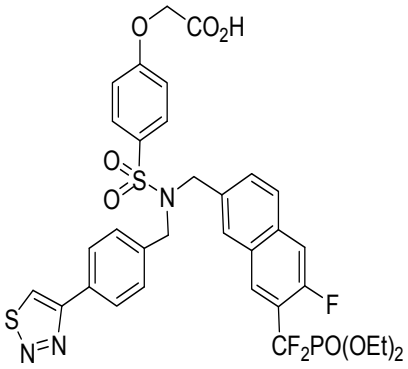


53d (1.46 g, 70%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described in **5.1.16.** as a white solid. mp: 143-144 ^{o}C; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.21 (s, 18H), 4.44 (s, 2H), 4.78 (s, 2H), 4.93 (s, 2H), 7.05-7.17 (m, 5H), 7.36-7.40 (m, 2H), 7.70-7.93 (m, 5H), 8.18 (s, 1H), 10.9 (s, 1H)

ESI/MS (m/z) : 852.8 $(M+H)^{+}$.

5.1.18.5. Compound – 53e

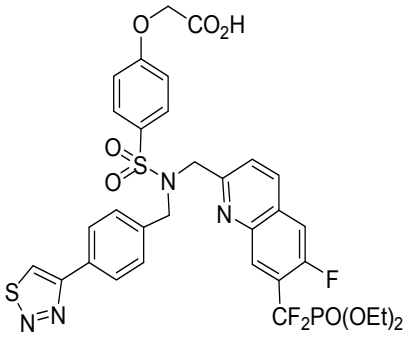


53e (1.21 g, 66%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described in **5.1.16.** as a white solid. mp: 121-123 ^{o}C; Purity by HPLC: 98.20%.

^{1}HNMR (Acetone-*d6*) : δ 1.19 (t, J = 7.0 Hz, 6H), 4.07-4.11 (m, 4H), 4.33 (s, 2H), 4.61 (s, 2H), 4.92 (s, 2H), 7.04-7.17 (m, 5H), 7.32-7.45 (m, 5H), 7.69 (m, 1H), 7.85-7.91 (m, 2H), 8.21 (s, 1H), 11.2 (s, 1H)

ESI/MS (m/z) : 751.3 $(M+H)^{+}$.

5.1.18.6. Compound – 53f

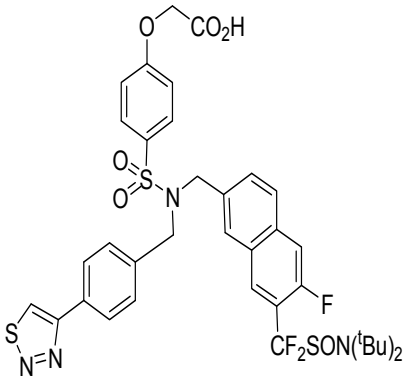


53f (1.01 g, 55%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described in **5.1.16.** as a white solid. mp: 119-121 ^{o}C; Purity by HPLC: 92.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.17 (t, J = 7.3 Hz, 6H), 4.09-4.12 (m, 4H), 4.39 (s, 2H), 4.73 (s, 2H), 4.94 (s, 2H), 7.03-7.13 (m, 5H), 7.30-7.35 (m, 3H), 7.80-7.96 (m, 4H), 8.23 (s, 1H), 11.2 (s, 1H)

ESI/MS (m/z) : 751.9 $(M+H)^{+}$.

5.1.18.7. Compound – 53g

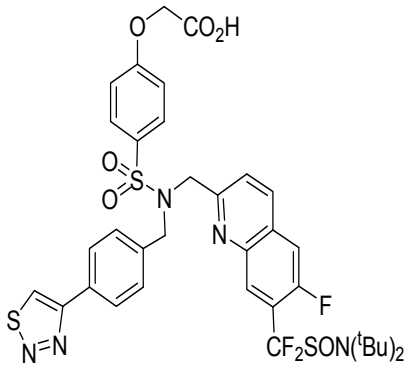


53g (1.32 g, 68%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described in **5.1.16.** as a white solid. mp: 169-171 ^{o}C; Purity by HPLC: 98.20%.

^{1}HNMR (Acetone-*d6*) : δ 1.17 (s, 18H), 4.41 (s, 2H), 4.57 (s, 2H), 4.89 (s, 2H), 7.01-7.17 (m, 5H), 7.33-7.43 (m, 5H), 7.62 (m, 1H), 7.81-7.85 (m, 2H), 8.20 (s, 1H), 11.1 (s, 1H)

ESI/MS (m/z) : 790.6 $(M+H)^+$.

5.1.18.8 Compound – 53h

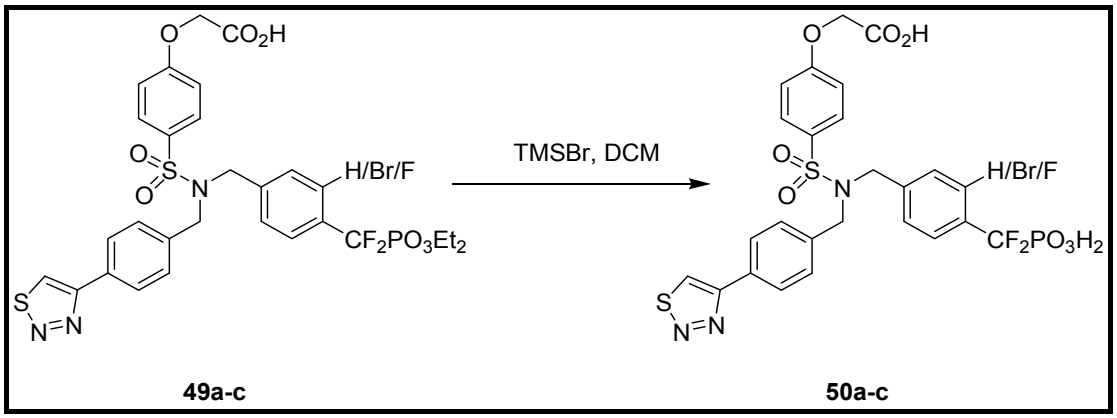


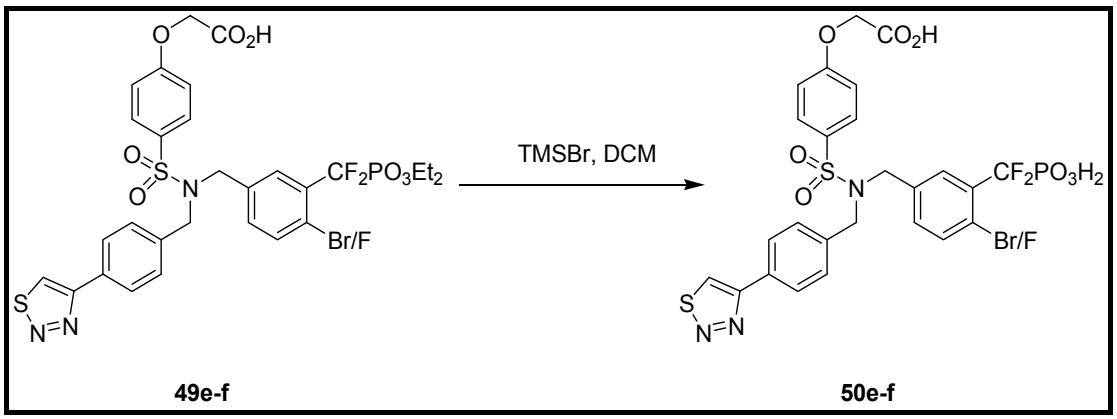
53h (1.30 g, 67%) was prepared from **48** (1.0 g, 2.45 mmole) by means of the general procedure described in **5.1.16.** as a white solid. mp: 155-156 ^oC; Purity by HPLC: 94.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.16 (s, 18H), 4.39 (s, 2H), 4.78 (s, 2H), 4.93 (s, 2H), 7.04-7.13 (m, 5H), 7.32-7.39 (m, 3H), 7.80-7.96 (m, 4H), 8.23 (s, 1H), 11.0 (s, 1H)

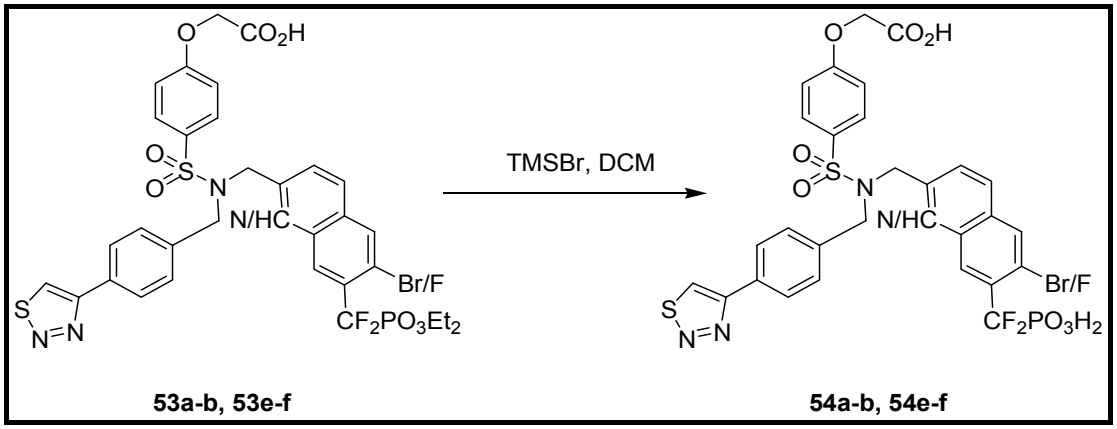
ESI/MS (m/z) : 791.9 $(M+H)^+$.

5.1.19. General procedure for the synthesis of Compounds (50a-c, 50e-f, 54a-b & 54e-f)





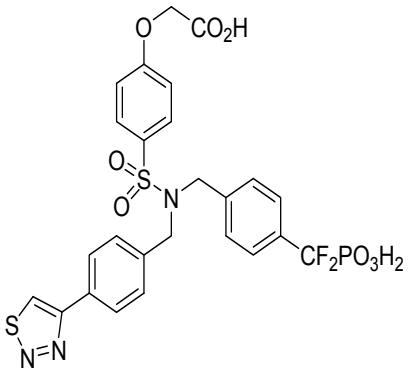
49e-f **50e-f**



53a-b, 53e-f **54a-b, 54e-f**

To a stirred solution of **49a-c, 49e-f, 53a-b and 53e-f** (1 mole equivalent) in dry CH_2Cl_2 (10 fold) was added trimethylsilyl bromide (2.2 mole equivalent) at -15 ^oC and stirred for 3 h. The reaction mixture was concentrated under reduced pressure and resulting residue was purified by reverse-phase prep-HPLC using water (0.05% TFA)/acetonitrile (0.05% TFA) gradient. All desired fractions were pooled together, frozen and lypholized to afford the compounds **50a-c**, **50e-f**, **54a-b** and **54e-f**.

5.1.19.1. Compound – 50a



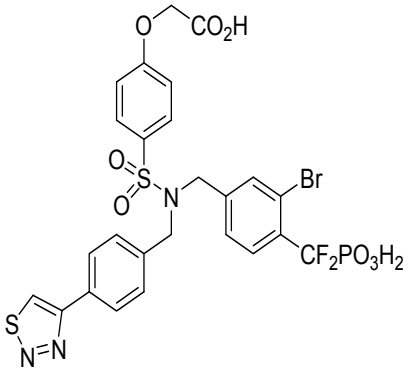
50a (0.75 g, 71%) was prepared from **49a** (1.15 g, 2.45 mmole) by means of the general procedure described above as an off white solid. mp: 143-144 ^{o}C; Purity by HPLC: 98.10%.

^{1}HNMR (Acetone-*d6*) : δ 4.21 (s, 2H), 4.38 (s, 2H), 4.85 (s, 2H), 6.94-7.05 (m, 6H), 7.12-7.18 (m, 2H), 7.30-7.36 (m, 2H), 7.80-7.85 (m, 2H), 8.17 (s, 1H), 11.2 (s, 1H)

^{13}CNMR (Acetone-*d6*) : δ 49.4, 50.5, 67.4, 113.8, 114.8, 127.2, 127.6, 127.9, 128.1, 128.4, 128.7, 130.1, 131.4, 132.1, 133.1, 135.5, 136.7, 137.2, 161.4, 163.2, 175.3

ESI/MS (m/z) : 626.1 $(M+H)^{+}$.

5.1.19.2. Compound – 50b



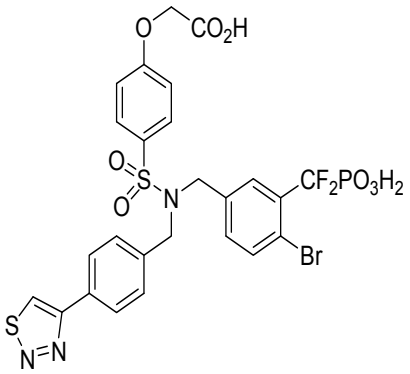
50b (0.96 g, 69%) was prepared from **49b** (1.5 g, 1.97 mmole) by means of the general procedure described above as a white solid. mp: 133-135 ^{o}C; Purity by HPLC: 97.00%.

^{1}HNMR (Acetone-*d6*) : *δ* 4.19 (s, 2H), 4.42 (s, 2H), 4.82 (s, 2H), 6.80-7.09 (m, 4H), 7.12-7.17 (m, 3H), 7.29-7.39 (m, 2H), 7.79-7.83 (m, 2H), 8.19 (s, 1H), 11.0 (s, 1H)

^{13}CNMR (Acetone-*d6*) : *δ* 49.8, 50.5, 67.8, 113.2, 114.7, 122.1, 125.3, 127.1, 127.4, 127.8, 128.0, 128.3, 128.9, 129.8, 130.3, 131.2, 132.0, 133.3, 136.5, 138.2, 139.5, 161.3, 163.9, 173.3

ESI/MS (m/z) : 704.4 $(M+H)^{+}$.

5.1.19.3 Compound – 50c



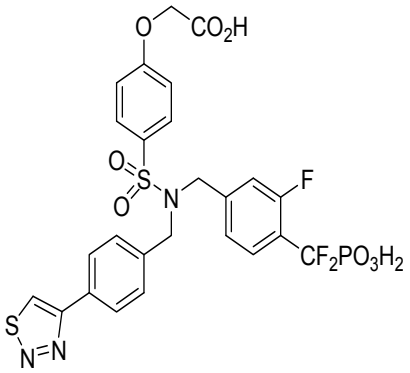
50c (0.99 g, 77%) was prepared from **49c** (1.4 g, 1.84 mmole) by means of the general procedure described above as a white solid. mp: 129-131 ^{o}C; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : *δ* 4.29 (s, 2H), 4.45 (s, 2H), 4.91 (s, 2H), 6.73-7.09 (m, 4H), 7.11-7.20 (m, 3H), 7.30-7.39 (m, 2H), 7.77-7.83 (m, 2H), 8.21 (s, 1H), 11.4 (s, 1H)

^{13}CNMR (Acetone-*d6*) : *δ* 50.1, 50.8, 68.2, 113.8, 114.5, 120.7, 125.6, 127.3, 128.0, 128.4, 128.8, 130.1, 130.6, 131.1, 131.9, 132.5, 135.8, 136.7, 161.7, 164.2, 173.0

ESI/MS (m/z) : 704.1 $(M+H)^+$.

5.1.19.4. Compound – 50e



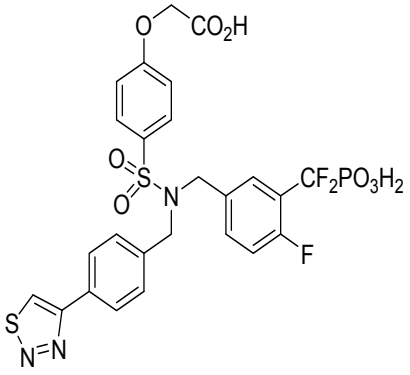
50e (0.8 g, 70%) was prepared from **49e** (1.19 g, 1.70 mmole) by means of the general procedure described above as a white solid. mp: 143-145 ^oC; Purity by HPLC: 96.29%.

^{1}HNMR (Acetone-*d6*) : *δ* 4.21 (s, 2H), 4.45 (s, 2H), 4.88 (s, 2H), 6.83-7.07 (m, 4H), 7.11-7.19 (m, 3H), 7.30-7.39 (m, 2H), 7.77-7.83 (m, 2H), 8.21 (s, 1H), 11.1 (s, 1H)

^{13}CNMR (Acetone-*d6*) : *δ* 48.8, 51.5, 68.8, 112.9, 114.5, 115.5, 122.3, 124.0, 125.6, 127.0, 127.4, 127.7, 128.0, 128.5, 128.9, 129.7, 130.1, 131.5, 132.4, 133.8, 136.6, 138.5, 139.9, 160.2, 161.3, 163.9, 173.0

ESI/MS (m/z) : 644.4 $(M+H)^+$.

5.1.19.5. Compound – 50f



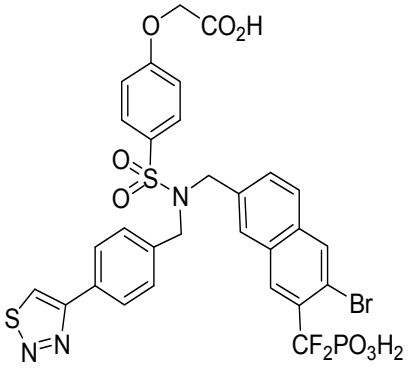
50f (0.55 g, 76%) was prepared from **49f** (0.8 g, 1.14 mmole) by means of the general procedure described above as a white solid. mp: 118-119 ^{o}C; Purity by HPLC: 97.10%.

^{1}HNMR (Acetone-*d6*) : *δ* 4.32 (s, 2H), 4.47 (s, 2H), 4.93 (s, 2H), 6.77-7.09 (m, 4H), 7.09-7.20 (m, 3H), 7.29-7.39 (m, 2H), 7.73-7.83 (m, 2H), 8.20 (s, 1H), 11.4 (s, 1H)

^{13}CNMR (Acetone-*d6*) : *δ* 49.1, 50.5, 67.2, 114.1, 114.5, 122.7, 125.7, 127.2, 127.9, 128.4, 128.8, 130.1, 130.6, 131.3, 131.9, 132.4, 135.8, 136.7, 158.5, 161.8, 163.2, 173.1

ESI/MS (m/z) : 644.7 $(M+H)^{+}$.

5.1.19.6. Compound – 54a



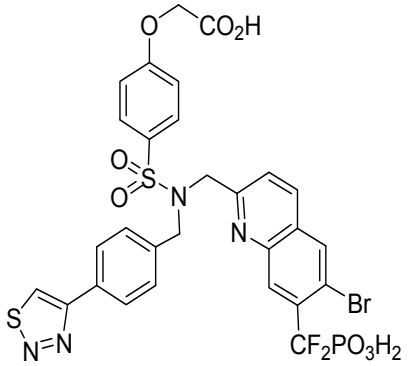
54a (0.78 g, 70%) was prepared from **53a** (1.2 g, 1.48 mmole) by means of the general procedure described above as an off white solid. mp: 131-133 ^{o}C; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : *δ* 4.39 (s, 2H), 4.55 (s, 2H), 4.91 (s, 2H), 7.03-7.14 (m, 4H), 7.21-7.39 (m, 5H), 7.41-7.85 (m, 4H), 8.22 (s, 1H), 11.3 (s, 1H)

^{13}CNMR (Acetone-*d6*) : *δ* 48.8, 50.5, 66.9, 113.9, 114.5, 118.9, 125.3, 125.7, 127.3, 127.7, 128.3, 128.7, 128.9, 130.2, 132.4, 134.6, 135.9, 138.1, 139.9, 161.3, 163.9, 173.7

ESI/MS (m/z) : 755.4 $(M+H)^{+}$.

5.1.19.7. Compound – 54b



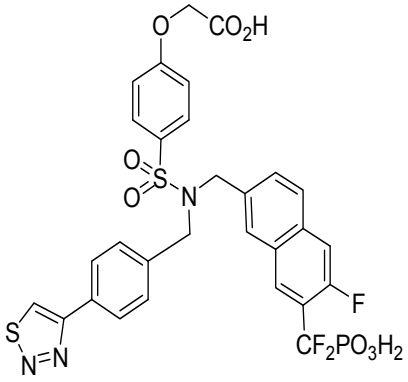
54b (0.61 g, 69%) was prepared from **53b** (0.95 g, 1.17 mmole) by means of the general procedure described above as a pale yellow solid. mp: 141-143 ^{o}C; Purity by HPLC: 97.77%.

^{1}HNMR (Acetone-*d6*) : *δ* 4.39 (s, 2H), 4.77 (s, 2H), 4.94 (s, 2H), 7.07-7.17 (m, 5H), 7.30-7.39 (m, 2H), 7.70-7.97 (m, 5H), 8.18 (s, 1H), 11.1 (s, 1H)

^{13}CNMR (Acetone-*d6*) : *δ* 50.8, 53.5, 66.7, 113.9, 114.6, 121.1, 121.8, 122.4, 125.3, 127.3, 127.7, 128.1, 128.5, 128.9, 129.7, 130.1, 131.4, 132.0, 134.1, 136.5, 141.3, 147.6, 159.9, 161.2, 163.8, 173.2

ESI/MS (m/z) : 756.4 $(M+H)^{+}$.

5.1.19.8. Compound – 54e



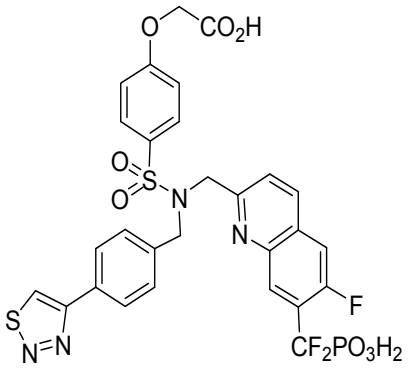
54e (0.82 g, 74%) was prepared from **53e** (1.2 g, 1.6 mmole) by means of the general procedure described above as an off white solid. mp: 121-123 ^{o}C; Purity by HPLC: 97.83%.

^{1}HNMR (Acetone-*d6*) : δ 4.43 (s, 2H), 4.55 (s, 2H), 4.90 (s, 2H), 7.02-7.15 (m, 4H), 7.23-7.39 (m, 5H), 7.45-7.85 (m, 4H), 8.25 (s, 1H), 11.2 (s, 1H)

^{13}CNMR (Acetone-*d6*) : δ 49.4, 50.4, 66.7, 109.7, 113.7, 114.8, 118.9, 125.4, 125.7, 127.4, 127.7, 128.2, 128.7, 128.9, 130.8, 132.6, 134.6, 135.9, 138.7, 139.9, 161.0, 163.8, 173.6

ESI/MS (m/z) : 694.7 $(M+H)^{+}$.

5.1.19.9. Compound – 54f



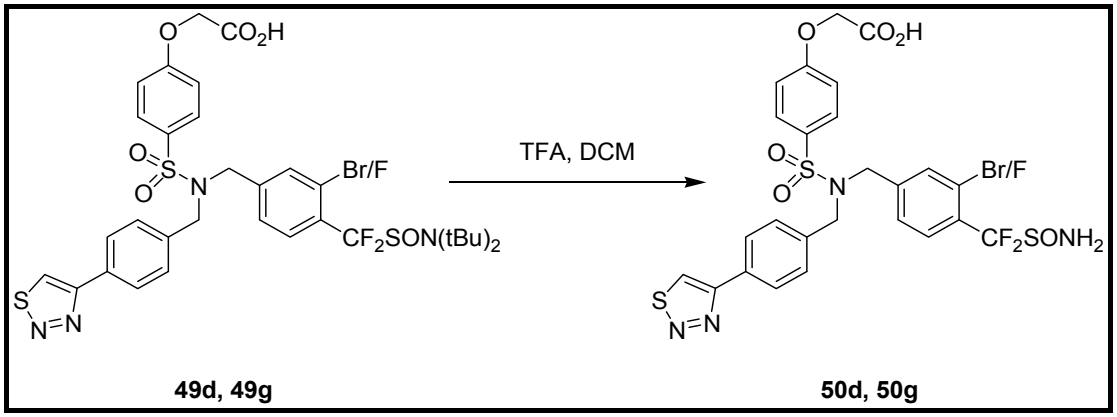
54f (0.62 g, 67%) was prepared from **53f** (1.0 g, 1.33 mmole) by means of the general procedure described above as a pale yellow solid. mp: 131-133 ^{o}C; Purity by HPLC: 96.90%.

^{1}HNMR (Acetone-*d6*) : δ 4.42 (s, 2H), 4.75 (s, 2H), 4.89 (s, 2H), 7.05-7.17 (m, 5H), 7.29-7.39 (m, 2H), 7.72-7.97 (m, 5H), 8.21 (s, 1H), 11.2 (s, 1H)

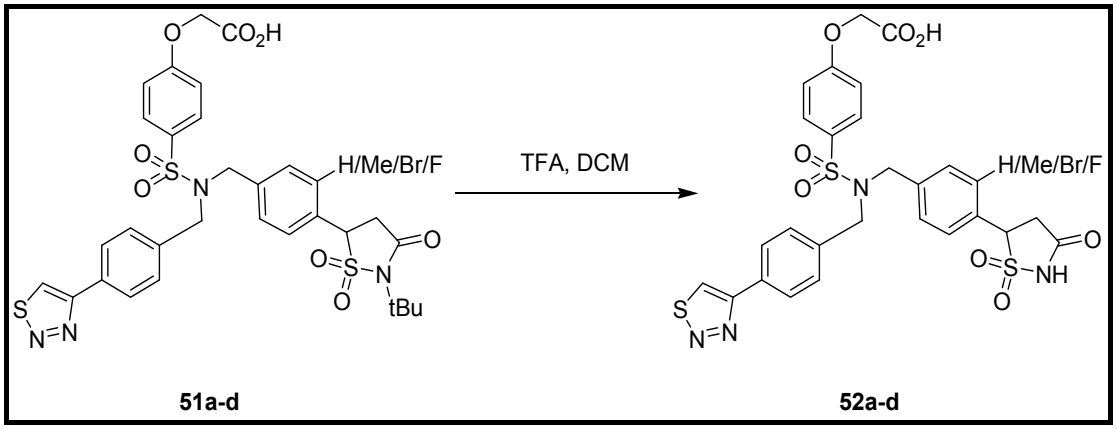
^{13}CNMR (Acetone-*d6*) : δ 50.9, 53.7, 66.9, 108.8, 113.7, 114.5, 121.3, 121.9, 122.7, 125.9, 127.2, 127.9, 128.2, 128.6, 128.9, 129.7, 130.7, 131.6, 132.0, 134.5, 136.5, 141.3, 143.7, 147.6, 160.0, 161.2, 162.3, 163.9, 173.0

ESI/MS (m/z) : 695.6 $(M+H)^{+}$.

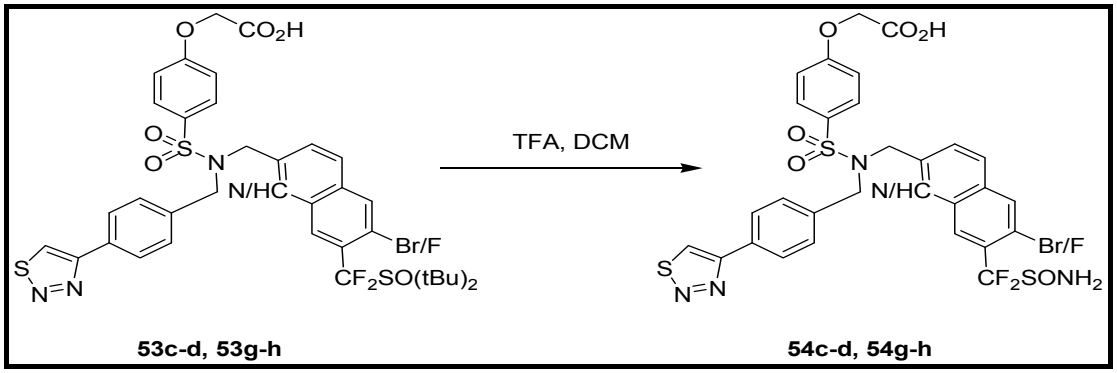
5.1.20. General procedure for the synthesis of Compounds (50d, 50g, 52a-d, 54c-d & 54g-h)



49d, 49g **50d, 50g**



51a-d **52a-d**

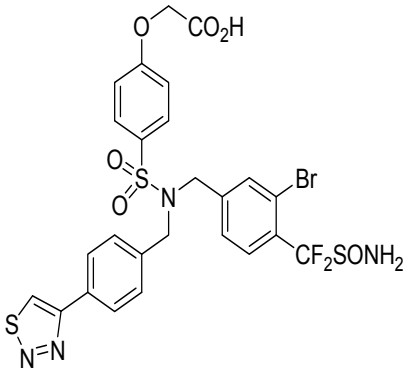


53c-d, 53g-h **54c-d, 54g-h**

To a stirred solution of **49d, 49g, 51a-d, 53c-d and 53g-h** (1 mole equivalent) in dry CH_2Cl_2 (10 fold) was added trifluoroacetic acid (10 fold) at 25 ^oC and stirred for 2 h. The reaction mixture was concentrated under reduced pressure and resulting residue was purified by reverse-phase prep-HPLC using water (0.05% TFA)/acetonitrile (0.05% TFA) gradient. All desired fractions were

pooled together, frozen and lypholized to afford the compounds **50d, 50g**, **52a-d**, **54c-d** and **54g-h**.

5.1.20.1. Compound – 50d



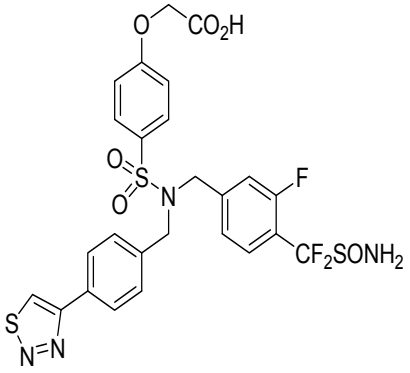
50d (0.70 g, 74%) was prepared from **49d** (1.1 g, 1.37 mmole) by means of the general procedure described above as an off white solid. mp: 125-127 ^{o}C; Purity by HPLC: 95.50%.

^{1}HNMR (Acetone-*d6*) : δ 4.23 (s, 2H), 4.44 (s, 2H), 4.91 (s, 2H), 6.79-7.09 (m, 4H), 7.11-7.17 (m, 3H), 7.30-7.39 (m, 2H), 7.79-7.87 (m, 2H), 8.17 (s, 1H), 11.2 (s, 1H)

^{13}CNMR (Acetone-*d6*) : δ 49.9, 50.7, 67.6, 113.8, 114.3, 122.4, 127.1, 127.4, 127.8, 128.0, 128.2, 128.6, 129.8, 130.1, 131.5, 132.0, 133.0, 136.5, 138.3, 139.4, 157.9, 161.7, 162.9, 173.5

ESI/MS (m/z) : 688.3 $(M+H)^{+}$.

5.1.20.2. Compound – 50g



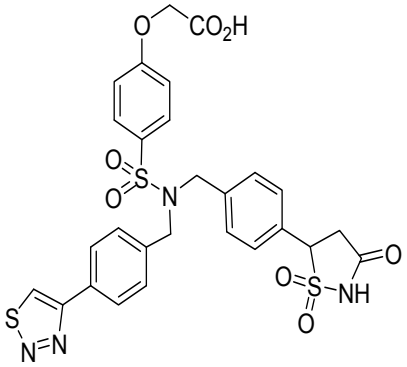
50g (0.53 g, 70%) was prepared from **49g** (0.9 g, 1.21 mmole) by means of the general procedure described above as an off white solid. mp: 121-123 ^{o}C; Purity by HPLC: 98.20%.

^{1}HNMR (Acetone-*d6*) : *δ* 4.20 (s, 2H), 4.47 (s, 2H), 4.94 (s, 2H), 6.77-7.10 (m, 4H), 7.13-7.17 (m, 3H), 7.29-7.39 (m, 2H), 7.80-7.87 (m, 2H), 8.19 (s, 1H), 11.1 (s, 1H)

^{13}CNMR (Acetone-*d6*) : *δ* 49.7, 50.5, 67.8, 113.7, 114.6, 115.6, 122.3, 127.0, 127.3, 127.8, 128.0, 128.2, 128.6, 129.1, 130.6, 131.7, 132.1, 133.3, 136.7, 138.5, 139.4, 154.9, 157.0, 161.7, 163.9, 173.3

ESI/MS (m/z) : 627.8 $(M+H)^{+}$.

5.1.20.3. Compound – 52a



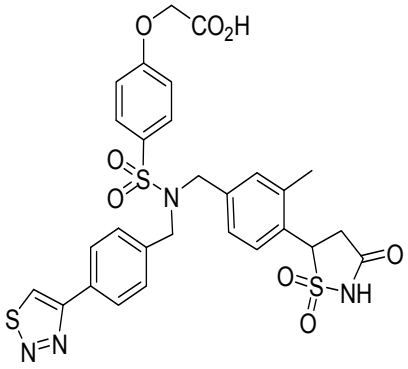
52a (0.69 g, 69%) was prepared from **51a** (1.1 g, 1.60 mmole) by means of the general procedure described above as an off white solid. mp: 109-111 ^{o}C; Purity by HPLC: 99.82%.

^{1}HNMR (Acetone-*d6*) : *δ* 3.01 (dd, *J* = 13.8, 8.6 Hz, 1H), 3.17 (dd, *J* = 14.7, 6.1 Hz, 1H), 4.17 (s, 2H), 4.44 (s, 2H), 4.57 (t, *J* = 7.8 Hz, 1H), 4.93 (s, 2H), 6.99-7.05 (m, 6H), 7.09-7.15 (m, 2H), 7.29-7.39 (m, 2H), 7.79-7.85 (m, 2H), 8.13 (s, 1H), 11.2 (s, 1H)

^{13}CNMR (Acetone-*d6*) : δ 37.8, 49.9, 50.9, 53.6, 66.6, 113.4, 114.4, 127.2, 127.6, 127.8, 128.0, 128.2, 128.9, 129.8, 130.0, 131.4, 132.2, 133.7, 136.6, 161.8, 163.9, 173.2, 177.3

ESI/MS (m/z) : 629.7 $(M+H)^+$.

5.1.20.4. Compound – 52b



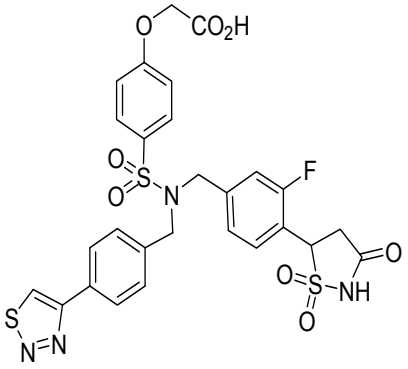
52b (0.83 g, 77%) was prepared from **51b** (1.18 g, 1.68 mmole) by means of the general procedure described above as an off white solid. mp: 101-103 ^oC; Purity by HPLC: 95.50%.

^{1}HNMR (Acetone-*d6*) : δ 2.37 (s, 3H), 3.03 (dd, *J* = 13.9, 8.5 Hz, 1H), 3.14 (dd, *J* = 14.8, 6.0 Hz, 1H), 4.23 (s, 2H), 4.45 (s, 2H), 4.55 (t, *J* = 7.9 Hz, 1H), 4.88 (s, 2H), 6.85-7.09 (m, 5H), 7.10-7.15 (m, 2H), 7.31-7.38 (m, 2H), 7.81-7.85 (m, 2H), 8.19 (s, 1H), 11.0 (s, 1H)

^{13}CNMR (Acetone-*d6*) : δ 19.2, 37.4, 48.1, 50.2, 51.7, 67.9, 114.0, 114.7, 124.8, 127.0, 127.3, 127.7, 128.1, 128.5, 128.9, 129.7, 130.3, 131.6, 132.1, 133.4, 136.3, 139.0, 161.5, 173.5, 177.1

ESI/MS (m/z) : 643.7 $(M+H)^+$.

5.1.20.5. Compound – 52c



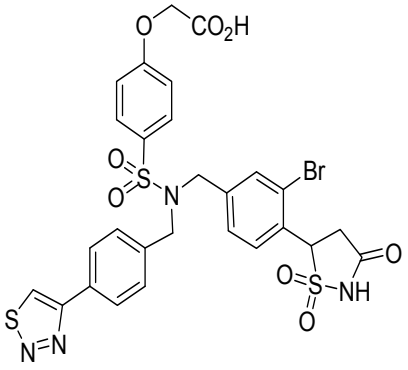
52c (0.92 g, 77%) was prepared from **51c** (1.3 g, 1.85 mmole) by means of the general procedure described above as a white solid. mp: 143-145 ^{o}C; Purity by HPLC: 95.53%.

^{1}HNMR (Acetone-*d6*) : δ 2.99 (dd, *J* = 13.9, 8.5 Hz, 1H), 3.15 (dd, *J* = 14.8, 6.0 Hz, 1H), 4.33 (s, 2H), 4.45 (s, 2H), 4.50 (t, *J* = 7.9 Hz, 1H), 4.92 (s, 2H), 6.70-6.92 (m, 3H), 7.02-7.06 (m, 2H), 7.09-7.15 (m, 2H), 7.32-7.37 (m, 2H), 7.78-7.83 (m, 2H), 8.16 (s, 1H), 11.2 (s, 1H)

^{13}CNMR (Acetone-*d6*) : δ 37.1, 42.9, 48.9, 50.1, 67.8, 114.2, 114.9, 123.8, 125.8, 126.9, 127.2, 127.9, 128.1, 128.5, 129.2, 130.0, 131.5, 132.0, 135.4, 136.5, 160.1, 161.7, 163.9, 173.1, 177.3

ESI/MS (m/z) : 647.7 $(M+H)^{+}$.

5.1.20.6. Compound – 52d



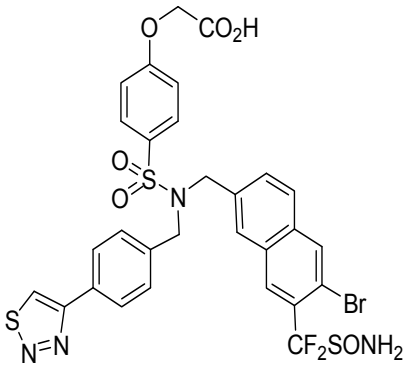
52d (0.84 g, 76%) was prepared from **51d** (1.2 g, 1.57 mmole) by means of the general procedure described above as a white solid. mp: 133-135 ^{o}C; Purity by HPLC: 96.90%.

^{1}HNMR (Acetone-*d6*) : δ 2.97 (dd, *J* = 13.8, 8.7 Hz, 1H), 3.13 (dd, *J* = 14.9, 6.2 Hz, 1H), 4.32 (s, 2H), 4.47 (s, 2H), 4.52 (t, *J* = 7.8 Hz, 1H), 4.94 (s, 2H), 6.72-6.92 (m, 3H), 7.01-7.06 (m, 2H), 7.10-7.15 (m, 2H), 7.33-7.37 (m, 2H), 7.75-7.83 (m, 2H), 8.14 (s, 1H), 11.0 (s, 1H)

^{13}CNMR (Acetone-*d6*) : δ 36.1, 42.7, 48.8, 50.3, 67.7, 114.6, 114.9, 122.5, 123.6, 125.7, 126.8, 127.3, 127.8, 128.0, 128.5, 129.6, 130.0, 131.6, 132.0, 135.4, 136.7, 160.4, 161.9, 163.9, 173.0, 177.1

ESI/MS (m/z) : 708.7 $(M+H)^{+}$.

5.1.20.7. Compound – 54c



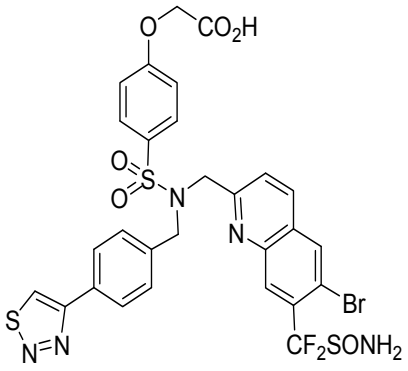
54c (0.74 g, 71%) was prepared from **53c** (1.2 g, 1.41 mmole) by means of the general procedure described above as an off white solid. mp: 161-163 ^{o}C; Purity by HPLC: 99.00%.

^{1}HNMR (Acetone-*d6*) : δ 4.42 (s, 2H), 4.53 (s, 2H), 4.95 (s, 2H), 7.01-7.25 (m, 5H), 7.31-7.39 (m, 4H), 7.59-7.89 (m, 4H), 8.19 (s, 1H), 11.5 (s, 1H)

^{13}CNMR (Acetone-*d6*) : δ 49.8, 50.9, 67.9, 114.1, 114.7, 118.3, 125.7, 127.3, 127.8, 128.3, 128.5, 128.9, 130.2, 132.0, 134.3, 135.2, 138.1, 158.6, 161.2, 163.7, 173.0

ESI/MS (m/z) : 738.7 $(M+H)^+$.

5.1.20.8. Compound – 54d



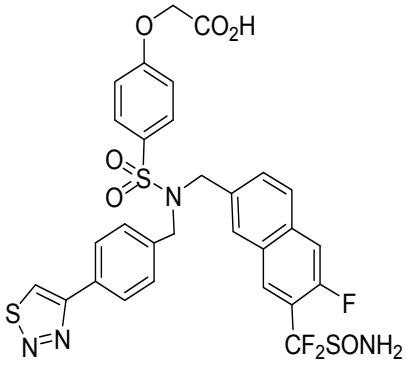
54d (0.89 g, 74%) was prepared from **53d** (1.4 g, 1.64 mmole) by means of the general procedure described above as a pale yellow solid. mp: 167-169 ^oC; Purity by HPLC: 95.10%.

^{1}HNMR (Acetone-*d6*) : *δ* 4.43 (s, 2H), 4.79 (s, 2H), 4.91 (s, 2H), 7.03-7.19 (m, 5H), 7.30-7.39 (m, 2H), 7.60-7.97 (m, 5H), 8.23 (s, 1H), 11.0 (s, 1H)

^{13}CNMR (Acetone-*d6*) : *δ* 50.3, 53.8, 65.7, 113.8, 114.6, 121.0, 121.6, 122.3, 127.3, 127.7, 128.1, 128.4, 128.9, 129.7, 130.0, 131.4, 132.4, 134.1, 136.5, 141.4, 147.4, 159.8, 161.7, 163.9, 173.0

ESI/MS (m/z) : 739.7 $(M+H)^+$.

5.1.20.9. Compound – 54g



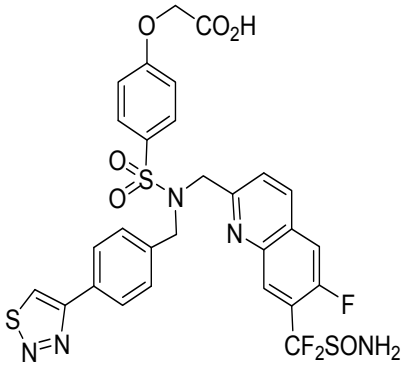
54g (0.78 g, 70%) was prepared from **53g** (1.3 g, 1.64 mmole) by means of the general procedure described above as an off white solid. mp: 151-153 ^{o}C; Purity by HPLC: 97.70%.

^{1}HNMR (Acetone-*d6*) : *δ* 4.44 (s, 2H), 4.55 (s, 2H), 4.93 (s, 2H), 7.00-7.25 (m, 5H), 7.29-7.39 (m, 4H), 7.61-7.89 (m, 4H), 8.22 (s, 1H), 10.9 (s, 1H)

^{13}CNMR (Acetone-*d6*) : *δ* 49.9, 51.2, 68.9, 114.0, 114.9, 118.0, 125.6, 126.4, 127.0, 127.9, 128.2, 128.6, 128.9, 130.6, 132.1, 134.6, 135.0, 138.6, 158.9, 161.1, 163.6, 172.0

ESI/MS (m/z) : 677.7 $(M+H)^{+}$.

5.1.20.10. Compound – 54h



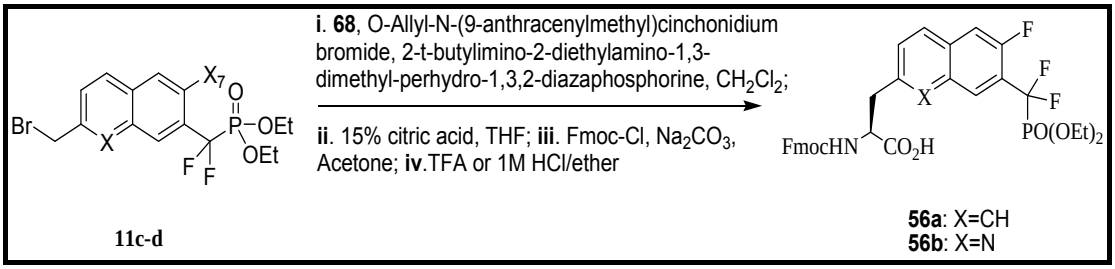
54h (0.68 g, 64%) was prepared from **53h** (1.25 g, 1.58 mmole) by means of the general procedure described above as a pale yellow solid. mp: 157-159 ^{o}C; Purity by HPLC: 96.30%.

^{1}HNMR (Acetone-*d6*) : *δ* 4.39 (s, 2H), 4.75 (s, 2H), 4.89 (s, 2H), 7.00-7.19 (m, 5H), 7.28-7.39 (m, 2H), 7.59-7.97 (m, 5H), 8.27 (s, 1H), 11.2 (s, 1H)

^{13}CNMR (Acetone-*d6*) : *δ* 50.5, 53.9, 66.7, 108.9, 113.8, 114.7, 121.1, 121.7, 122.0, 127.0, 127.7, 128.0, 128.4, 128.9, 129.7, 130.1, 131.4, 132.4, 134.5, 136.5, 141.4, 147.4, 159.8, 161.7, 162.4, 163.8, 173.4

ESI/MS (m/z) : 678.8 $(M+H)^{+}$.

5.1.21. General procedure for the synthesis of Compounds (56a-b)



Step I: To a stirred mixture of **11c-d** (1 mole equivalent), tert-butyl 2-(diphenylmetheleneamino)acetate **68** (1 mole equivalent) and O-Allyl-N-(9-anthracenylmethyl)cinchonidium bromide (0.1 mole equivalent) in dry CH_2Cl_2 (10 fold), at -78 ^oC under argon was added 2-t-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine (1.5 mole equivalent) over 5 min. The reaction mixture was stirred at – 20 ^oC for 24h and then allowed to warm to room temperature. The reaction mixture was concentrated, directly applied to column chromatography (100-200 mesh size silica gel column, 0-30% ethyl acetate: hexane) afforded the title compounds **69a-b** as solid.

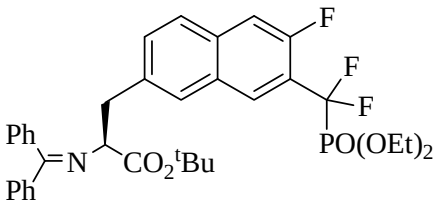
Step II: To a stirred solution of **69a-b** (1 mole equivalent) in THF (10 fold) at 25 ^oC under argon atmosphere was added 15 % citric acid (6.5 mole equivalent) in water. After 20h, mixture was concentrated, residue was dissolved in water (2 fold), washed with diethyl ether. Aqueous layer was basified with saturated sodium bicarbonate upto 8 pH, extracted with ethyl acetate (3 x 2 fold), washed with water, finally with brine. Organic phase was dried over sodium sulphate, concentrated under reduced pressure afforded the title compound **70a-b** as solids.

Step III: A stirred of solution of **70a-b** (1 mole equivalent) in acetone (10 fold) was treated with Na_2CO_3 (1.5 mole equivalent) in water (10 fold) at 25 ^oC

and stirred for 30 min, mixture was cooled to 5 ^{o}C, and Fmoc-Cl (1.1 mole equivalent) in acetone (5 fold) was added dropwise. After stirring at same temperature, mixture was brought at 25 ^{o}C and stirred for 24h. After 24h, mixture was concentrated, residue was dissolved in water (10 fold), extracted with ethyl acetate (2 x 3 fold), dried over Na_2SO_4, concentrated under reduced pressure, crude product was purified using column chromatography (100-200 mesh size silica gel column, 0-30% ethyl acetate: hexane) afforded the title compounds **71a-b** as a solid.

Step IV: A stirred solution of **71a-b** (1 mole equivaent) in TFA (10 fold) or 1*M* HCl/ether, protected with the atmosphere by a calcium chloride filling drying tube was stirred at 25 ^{o}C for 4h. Then, reaction mixture was concentrated under vacuum at less then 40 ^{o}C, resulting oil was extracted with ethyl acetate (2 x 2 fold), washed with water, followed by brine, dried over Na_2SO_4 and concentrated under vacuum afforded the compounds **56a-b** as a solid.

5.1.21.1. (2S)-tert-butyl 2-(diphenylmethyleneamino)-3-(6-fluoro-7-(diethyldifluoro)methyl phosphonatenaphthalen-2-yl)propanoate (69a)



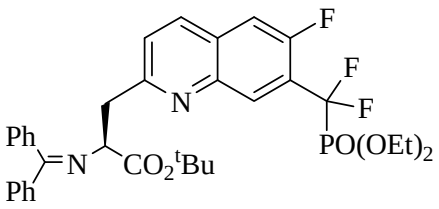
69a (10.67 g, 71%) was prepared from **11c** (10 g, 0.023 mole) by means of the general procedure described above in Step-I as a white solid. mp: 107-108 ^{o}C; Purity by HPLC: 95.07%.

^{1}HNMR (Acetone-*d6*) : δ 1.27 (t, *J* = 7.2 Hz, 3H), 1.35 (t, *J* = 7.2 Hz, 3H), 1.46 (s, 9H), 3.15 (dd, *J* = 8.5 Hz, 13.9 Hz, 1H), 3.22 (dd, *J* = 4.3 Hz, 14.1 Hz,

1H), 4.21 (m, 4H), 4.39 (m, 1H), 7.13 (d, *J* = 8.1 Hz, 1H), 7.31 (s, 1H), 7.41 (s, 1H), 7.43 (s, 1H), 7.57-7.66 (m, 10H), 7.96 (d, *J* = 8.0 Hz, 1H)

ESI/MS (m/z) : 640.4 $(M+H)^+$.

5.1.21.2. (2S)-tert-butyl 2-(diphenylmethyleneamino)-3-(6-fluoro-7-diethyldifluoro)methyl phosphonatequinolin-2-yl)propanoate (69b)

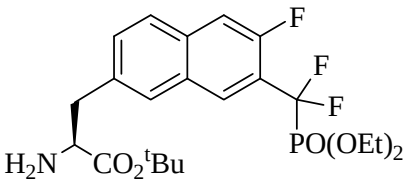


69b (9.0 g, 60%) was prepared from **11d** (10 g, 0.023 mole) by means of the general procedure described above in Step-I as an off white solid. mp: 115-117 ^oC; Purity by HPLC: 96.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.20 (t, *J* = 7.5 Hz, 3H), 1.29 (t, *J* = 7.4 Hz, 3H), 1.38 (s, 9H), 3.46 (dd, *J* = 8.2 Hz, 13.8 Hz, 1H), 3.71 (dd, *J* = 4.2 Hz, 14.3 Hz, 1H), 4.19 (m, 4H), 4.35 (m, 1H), 7.07 (d, *J* = 8.0 Hz, 1H), 7.34 (s, 1H), 7.61 (s, 1H), 7.57-7.97 (m, 10H), 8.29 (d, *J* = 8.2 Hz, 1H)

ESI/MS (m/z) : 641.6 $(M+H)^+$.

5.1.21.3. (2S)-tert-butyl2-amino-3-(6-fluoro-7-(diethylfluoro)methyl phosphonatenaphthalen-2-yl)propanoate (70a)

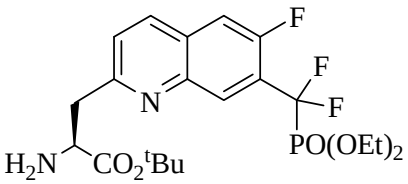


70a (5.2 g, 70%) was prepared from **69a** (10 g, 0.015 mole) by means of the general procedure described above in Step-II as a white solid. mp: 123-124 ^oC; Purity by HPLC: 94.51%.

^{1}HNMR (Acetone-*d6*) : δ 1.21 (t, *J* = 7.0 Hz, 3H), 1.23 (t, *J* = 7.2 Hz, 3H), 1.31 (s, 9H), 3.40 (dd, *J* = 8.2 Hz, 13.8 Hz, 1H), 3.65 (dd, *J* = 4.2 Hz, 13.9 Hz, 1H), 4.09 (m, 4H), 4.19 (m, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 7.29 (s, 1H), 7.39 (s, 1H), 7.45 (s, 1H), 7.90 (d, *J* = 7.7 Hz, 1H)

ESI/MS (m/z) : 476.4 $(M+H)^+$.

5.1.21.4. (2S)-tert-butyl 2-amino-3-(6-fluoro-7-(diethylfluoro)methyl phosphonatequinolin-2-yl)propanoate (70b)

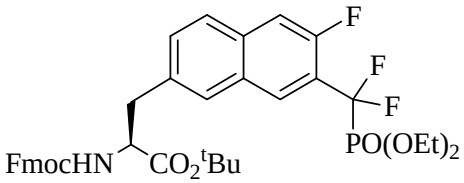


70b (4.08 g, 61%) was prepared from **69b** (9 g, 0.014 mole) by means of the general procedure described above in Step-II as an off white solid. mp: 111-112 ^oC; Purity by HPLC: 95.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.21 (t, *J* = 7.0 Hz, 3H), 1.23 (t, *J* = 7.2 Hz, 3H), 1.31 (s, 9H), 3.40 (dd, *J* = 8.2 Hz, 13.8 Hz, 1H), 3.65 (dd, *J* = 4.2 Hz, 13.9 Hz, 1H), 4.09 (m, 4H), 4.19 (m, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 7.29 (s, 1H), 7.39 (s, 1H), 7.45 (s, 1H), 7.90 (d, *J* = 7.7 Hz, 1H)

ESI/MS (m/z) : 477.4 $(M+H)^+$.

5.1.21.5. (2S)-tert-butyl 2-[{(9H-fluoren-9-yl)methoxy}carbonylamino]-3-(6-fluoro-7-(diethyldifluoro)methylphosphonate-naphthalen-2-yl)propanoate (71a)

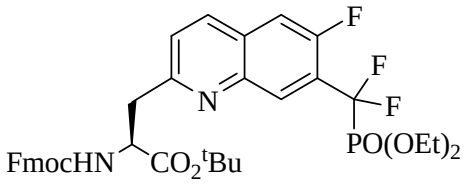


71a (4.6 g, 63%) was prepared from **70a** (5.0 g, 0.010 mole) by means of the general procedure described above in Step-III as a white solid. mp: 153-154 ^{o}C; Purity by HPLC: 94.35%.

^{1}HNMR (Acetone-*d6*) : δ 1.18 (t, *J* = 7.2 Hz, 3H), 1.21 (t, *J* = 7.2 Hz, 3H), 1.38 (s, 9H), 3.10 (dd, *J* = 8.0 Hz, 13.9 Hz, 1H), 3.35 (dd, *J* = 4.1 Hz, 14.9 Hz, 1H), 4.09 (m, 4H), 4.21 (t, *J* = 6.9 Hz, 1H), 4.58-4.68 (m, 3H), 7.14 (d, *J* = 8.2 Hz, 1H), 7.33 (s, 1H), 7.41 (s, 1H), 7.47 (s, 1H), 7.55-7.88 (m, 8H), 7.98 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 698.8 $(M+H)^{+}$.

5.1.21.6. (2S)-tert-butyl 2-[{(9H-fluoren-9-yl)methoxy}carbonylamino]-3-(6-fluoro-7-(diethyldifluoro)methylphosphonate-quinolin-2-yl)propanoate (71b)

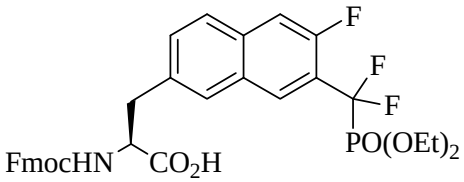


71b (3.9 g, 67%) was prepared from **70b** (4.0 g, 0.008 mole) by means of the general procedure described above in Step-III as a white solid. mp: 133-135 ^{o}C; Purity by HPLC: 94.60%.

^{1}HNMR (Acetone-*d6*) : δ 1.13 (t, *J* = 7.7 Hz, 3H), 1.29 (t, *J* = 7.1 Hz, 3H), 1.41 (s, 9H), 3.37 (dd, *J* = 8.3 Hz, 14.6 Hz, 1H), 3.63 (dd, *J* = 4.4 Hz, 13.9 Hz, 1H), 4.19 (m, 4H), 4.46 (t, *J* = 7.2 Hz, 1H), 4.57-4.70 (m, 3H), 7.10 (d, *J* = 8.0 Hz, 1H), 7.35 (s, 1H), 7.55-7.89 (m, 9H), 8.30 (d, *J* = 8.3 Hz, 1H)

ESI/MS (m/z) : 699.8 $(M+H)^{+}$.

5.1.21.7. (2S) 2-[{(9H-fluoren-9-yl)methoxy}carbonylamino]-3-(6-fluoro-7-(diethylfluoro)methylphosphonate-naphthalen-2-yl)propanoic acid (56a)

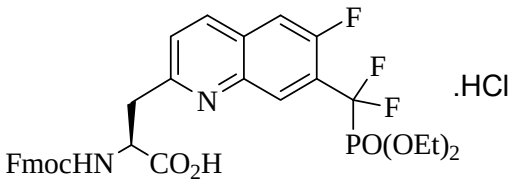


56a (2.77 g, 67%) was prepared from **71a** (4.5 g, 0.006 mole) by means of the general procedure described above in Step-IV as a white solid. mp: 129-130 ^{o}C; Purity by HPLC: 93.76%.

^{1}HNMR (Acetone-*d6*) : δ 1.19 (t, *J* = 7.4 Hz, 3H), 1.23 (t, *J* = 7.4 Hz, 3H), 2.99 (dd, J = 8.1 Hz, 14.3 Hz, 1H), 3.23 (dd, *J* = 4.0 Hz, 14.7 Hz, 1H), 4.11 (m, 4H), 4.19 (t, *J* = 7.2 Hz, 1H), 4.69-4.75 (m, 3H), 7.11 (d, *J* = 8.0 Hz, 1H), 7.34 (s, 1H), 7.39 (s, 1H), 7.43 (s, 1H), 7.51-7.77 (m, 8H), 7.91 (d, *J* = 7.7 Hz, 1H)

ESI/MS (m/z) : 642.8 $(M+H)^{+}$.

5.1.21.8. (2S) 2-[{(9H-fluoren-9-yl)methoxy}carbonylamino]-3-(6-fluoro-7-(diethylfluoro)methylphosphonatequinolin-2-yl)propanoic acid hydrochloride salt (56b)

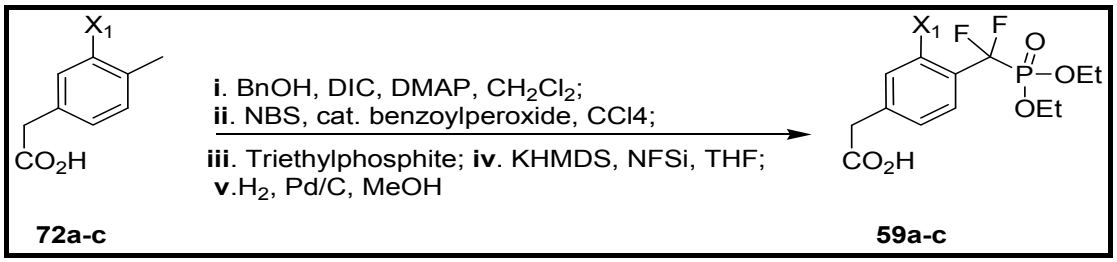


56b (2.18 g, 61%) was prepared from **71b** (3.9 g, 0.005 mole) by means of the general procedure described above in Step-IV as an off white solid. mp: 144-145 ^{o}C; Purity by HPLC: 95.40%.

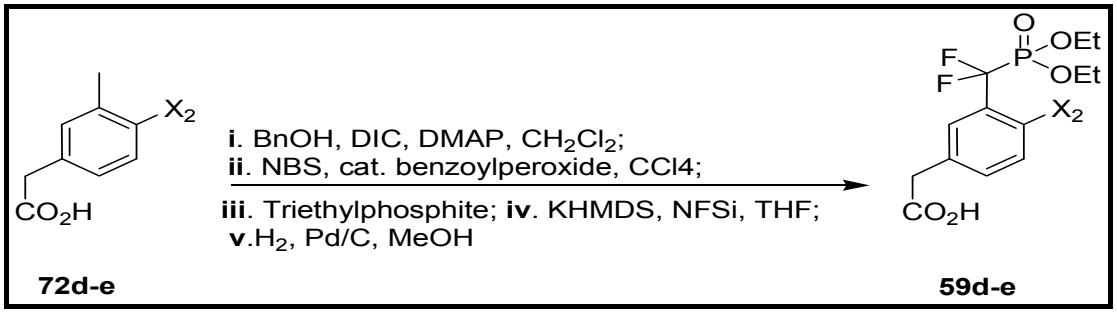
^{1}HNMR (Acetone-*d6*) : δ 1.21 (t, *J* = 7.6 Hz, 3H), 1.29 (t, *J* = 7.5 Hz, 3H), 3.27 (dd, *J* = 8.0 Hz, 14.3 Hz, 1H), 3.50 (dd, *J* = 4.0 Hz, 14.7 Hz, 1H), 4.19 (m, 4H), 4.46 (t, *J* = 7.3 Hz, 1H), 4.70-4.75 (m, 3H), 7.11 (d, *J* = 8.2 Hz, 1H), 7.37 (s, 1H), 7.49-7.87 (m, 9H), 8.21 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 643.8 $(M+H)^+$.

5.1.22. General procedure for the synthesis of Compounds (59a-c, 59d-e)



72a-c **59a-c**



72d-e **59d-e**

Step I: To a solution of substituted phenylacetic acid **72a-e** (1.0 mole equivalent) in CH_2Cl_2 (10 fold) was added benzyl alcohol (10 mole equivalent) and DMAP (0.5 mole equivalent). The solution was chilled to 0 ^oC, and 1,3-diisopropylcarbodiimide (1 mole equivalent) was added in a dropwise manner. The mixture was stirred at room temperature for 6h. The reaction mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ (2 x 2 fold), water and finally with saturated aqueous brine. Organic layer was dried over sodium sulfate and concentrated to afford a crude solid product. Chromatography (100-200 mesh size silica gel column, 0-30% ethyl acetate: hexane) afforded the title compound **73a-e** as a solid.

Step II: To a stirred solution of **73a-e** (1 mole equivalent) in CCl_4 (10 fold) were added *N*-bromosuccanamide (1.1 mole equivalent) and benzoylperoxide (0.01 mole equivalent). The mixture was gently refluxed for 4 hours under N_2 atmosphere. After 4 hours, water (10 fold) was added and the mixture was

extracted with CH_2Cl_2 (2 X 10 fold). The combined organics were washed with saturated aqueous $NaHCO_3$, then dried over Na_2SO_4, and concentrated. The resulting residue was subjected to flash chromatography using 10% ethyl acetate in hexane as eluent to furnish the compound **74a-e** as a white needles.

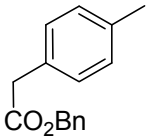
Step III: To a mixture of **74a-e** (1 mole equivalent) and triethyl phosphite (2.5 mole equivalent) was refluxed for 3 hours. The excess triethyl phosphite was removed by distillation and the residue was subjected to flash chromatography using 10% ethyl acetate in hexane as an eluent which gave **75a-75e** as white solids.

Step IV: To a solution of **75a-e** (1 mole equivalent) and N-fluorobenzenesulfonamide (2.5 mole equivalent) in dry THF (15 fold) at -78 ^oC was added KHMDS (1.0 M in THF, 2.5 mole equivalent) over 10 min. The mixture was stirred for 2 hours at -78 ^oC, then allowed to warm to 25 ^oC, and stirred for 18 hours. The reaction was quenched with a saturated aqueous NH_4Cl solution, extracted with ethyl acetate (2 X 10 fold). The combined organic layers were washed successively with water, followed by brine, and dried over Na_2SO_4, filtered and concentrated under vacuum to get the crude product which was subjected to flash by chromatography using 15% ethyl acetate in hexane as eluent to furnish title compound **76a-e** as a colourless liquid.

Step V: A solution of benzyl ester **76a-e** (1 mole equivalent) in CH_3OH (10 mL), add 10% Pd/C (10% w/w) and hydrogenated using parr hydrogenation apparatus at 60 psi for 4h. After 4h, Pd/C was filtered through Celite, CH_3OH

was evaporated, and resulting residue was triturated using hexane offered the title compounds **59a-e** as solids.

5.1.22.1. Benzyl 2-(*p*-tolyl)acetate (73a)

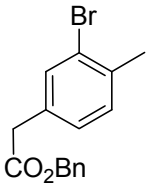


73a (12.8 g, 80%) was prepared from **72a** (10 g, 0.066 mole) by means of the general procedure described above in Step-I as a white solid. mp: 41-42 ^{o}C; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : δ 2.29 (s, 3H), 3.69 (s, 2H), 5.21 (s, 2H), 7.07 (d, *J* = 8.2 Hz, 2H), 7.19 (d, *J* = 7.8 Hz, 2H), 7.39-7.51 (m, 5H)

ESI/MS (m/z) : 241.4 $(M+H)^{+}$.

5.1.22.2. Benzyl 2-(3-bromo-4-methylphenyl)acetate (73b)

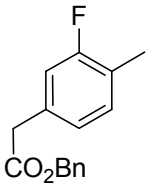


73b (11.42 g, 82%) was prepared from **72b** (10 g, 0.043 mole) by means of the general procedure described above in Step-I as a white solid. mp: 144-145 ^{o}C; Purity by HPLC: 97.10%.

^{1}HNMR (Acetone-*d6*) : δ 2.33 (s, 3H), 3.77 (s, 2H), 5.19 (s, 2H), 7.01 (d, *J* = 7.9 Hz, 1H), 7.14 (d, *J* = 7.8 Hz, 1H), 7.29 (s, 1H), 7.41-7.50 (m, 5H)

ESI/MS (m/z) : 320.3 $(M+H)^{+}$.

5.1.22.3. Benzyl 2-(3-fluoro-4-methylphenyl)acetate (73c)

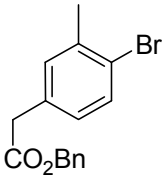


73c (11.82 g, 77%) was prepared from **72c** (10 g, 0.059 mole) by means of the general procedure described above in Step-I as a white solid. mp: 144-145 ^{o}C; Purity by HPLC: 94.60%.

^{1}HNMR (Acetone-*d6*) : *δ* 2.18 (s, 3H), 3.65 (s, 2H), 5.20 (s, 2H), 6.77 (d, *J* = 8.3 Hz, 1H), 6.91 (d, *J* = 7.7 Hz, 1H), 7.13 (d, *J* = 7.8 Hz, 1H), 7.38-7.49 (m, 5H)

ESI/MS (m/z) : 259.5 $(M+H)^{+}$.

5.1.22.4. Benzyl 2-(4-bromo-3-methylphenyl)acetate (73d)

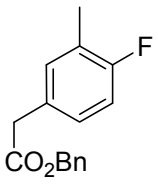


73d (9.75 g, 70%) was prepared from **72d** (10 g, 0.043 mole) by means of the general procedure described above in Step-I as a white solid. mp: 139-141 ^{o}C; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : *δ* 2.38 (s, 3H), 3.55 (s, 2H), 5.37 (s, 2H), 6.73-6.80 (m, 2H),7.21 (d, *J* = 7.9 Hz, 1H), 7.39-7.49 (m, 5H)

ESI/MS (m/z) : 320.4 $(M+H)^{+}$.

5.1.22.5. Benzyl 2-(4-fluoro-3-methylphenyl)acetate (73e)

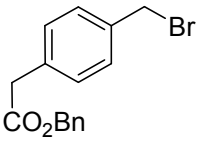


73e (10.75 g, 70%) was prepared from **72e** (10 g, 0.059 mole) by means of the general procedure described above in Step-I as a white solid. mp: 121-123 ^{o}C; Purity by HPLC: 98.20%.

^{1}HNMR (Acetone-*d6*) : *δ* 2.34 (s, 3H), 3.49 (s, 2H), 5.31 (s, 2H), 6.69 (d, *J* = 8.2 Hz, 1H), 6.83 (d, *J* = 7.9 Hz, 1H), 7.04 (s, 1H), 7.32-7.42 (m, 5H)

ESI/MS (m/z) : 259.3 $(M+H)^{+}$.

5.1.22.6. Benzyl 2-[4-(bromomethyl)phenyl]acetate (74a)

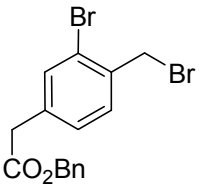


74a (10.8 g, 65%) was prepared from **73a** (12.5 g, 0.052 mole) by means of the general procedure described above in Step-II as a white solid. mp: 144-145 ^{o}C; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : *δ* 3.77 (s, 2H), 4.57 (s, 2H), 5.31 (s, 2H), , 7.10 (d, *J* = 7.9 Hz, 2H), 7.17 (d, *J* = 7.9 Hz, 2H), 7.41-7.50 (m, 5H)

ESI/MS (m/z) : 320.1 $(M+H)^{+}$.

5.1.22.7. Benzyl 2-(3-bromo-4-(bromomethyl)phenyl)acetate (74b)

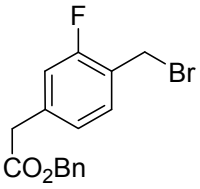


74b (7.54 g, 55%) was prepared from **73b** (11.0 g, 0.034 mole) by means of the general procedure described above in Step-II as a white solid. mp: 144-145 ^{o}C; Purity by HPLC: 95.10%.

^{1}HNMR (Acetone-*d6*) : *δ* 3.69 (s, 2H), 4.61 (s, 2H), 5.23 (s, 2H), , 7.03 (d, *J* = 7.7 Hz, 1H), 7.17 (d, *J* = 7.9 Hz, 1H), 7.31 (s, 1H), 7.38-7.52 (m, 5H)

ESI/MS (m/z) : 399.6 $(M+H)^{+}$.

5.1.22.8. Benzyl 2-(4-(bromomethyl)-3-fluorophenyl)acetate (74c)

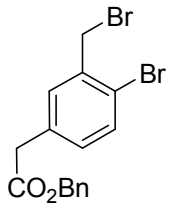


74c (10.5 g, 70%) was prepared from **73c** (11.5 g, 0.044 mole) by means of the general procedure described above in Step-II as a white solid. mp: 144-145 ^{o}C; Purity by HPLC: 97.00%.

^{1}HNMR (Acetone-*d6*) : *δ* 3.79 (s, 2H),4.69 (s, 2H), 5.31 (s, 2H), 6.69 (d, *J* = 8.1 Hz, 1H), 6.88 (d, *J* = 7.9 Hz, 1H), 7.17 (d, *J* = 7.8 Hz, 1H), 7.43-7.55 (m, 5H)

ESI/MS (m/z) : 338.4 $(M+H)^{+}$.

5.1.22.9. Benzyl 2-[4-bromo-3-(bromomethyl)phenyl]acetate (74d)

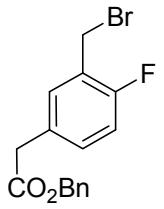


74d (6.51 g, 55%) was prepared from **73d** (9.5 g, 0.029 mole) by means of the general procedure described above in Step-II as a white solid. mp: 95-96 ^{o}C; Purity by HPLC: 94.44%.

^{1}HNMR (Acetone-*d6*) : *δ* 3.47 (s, 2H), 4.57 (s, 2H), 5.39 (s, 2H), 6.77-6.82 (m, 2H),7.23 (d, *J* = 7.8 Hz, 1H), 7.44-7.51 (m, 5H)

ESI/MS (m/z) : 399.2 $(M+H)^{+}$.

5.1.22.10. Benzyl 2-[3-(bromomethyl)-4-fluorophenyl]acetate (74e)

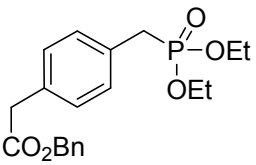


74e (8.22 g, 60%) was prepared from **73e** (10.5 g, 0.04 mole) by means of the general procedure described above in Step-II as a white solid. mp: 111-112 ^{o}C; Purity by HPLC: 93.50%.

^{1}HNMR (Acetone-*d6*) : *δ* 3.49 (s, 2H), 4.61 (s, 2H), 5.41 (s, 2H), 6.71 (d, *J* = 7.9 Hz, 1H), 6.83 (d, *J* = 7.9 Hz, 1H), 6.(3 (d, *J* = 7.8 Hz, 1H), 7.43-7.55 (m, 5H)

ESI/MS (m/z) : 338.1 $(M+H)^{+}$.

5.1.22.11. Benzyl 2-[4-{(diethoxyphosphoryl)methyl}phenyl]acetate (75a)

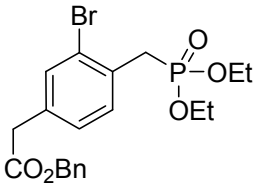


75a (8.66 g, 70%) was prepared from **74a** (10.5 g, 0.033 mole) by means of the general procedure described above in Step-III as a white solid. mp: 99-100 ^{o}C; Purity by HPLC: 94.40%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.13 (t, *J* = 7.2 Hz, 3H), 1.23 (t, *J* = 7.1 Hz, 3H), 3.01 (s, 2H), 3.77 (s, 2H), 4.2 (m, 4H), 5.31 (s, 2H), 7.10 (d, *J* = 7.8 Hz, 2H), 7.19 (d, *J* = 7.9 Hz, 2H), 7.33-7.49 (m, 5H)

ESI/MS (m/z) : 377.3 $(M+H)^{+}$.

5.1.22.12. Benzyl 2-[3-bromo-4-{(diethoxyphosphoryl)methyl} phenyl]acetate (75b)

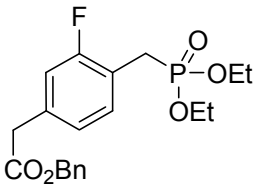


75b (6.0 g, 75%) was prepared from **74b** (7.0 g, 0.017 mole) by means of the general procedure described above in Step-III as a white solid. mp: 101-102 ^{o}C; Purity by HPLC: 96.60%.

^{1}HNMR (Acetone-*d6*) : δ 1.19 (t, *J* = 7.1 Hz, 3H), 1.22 (t, *J* = 7.1 Hz, 3H), 3.11 (s, 2H), 3.81 (s, 2H), 4.29 (m, 4H), 5.19 (s, 2H), 7.01 (d, *J* = 7.9 Hz, 1H), 7.17 (d, *J* = 7.9 Hz, 1H), 7.36 (s, 1H), 7.41-7.53 (m, 5H)

ESI/MS (m/z) : 456.6 $(M+H)^{+}$.

5.1.22.13. Benzyl 2-(4-((diethoxyphosphoryl)methyl)-3-fluorophenyl) acetate (75c)

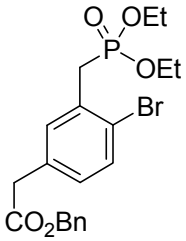


75c (8.06 g, 69%) was prepared from **74c** (10.0 g, 0.029 mole) by means of the general procedure described above in Step-III as a white solid. mp: 111-112 ^{o}C; Purity by HPLC: 93.90%.

^{1}HNMR (Acetone-*d6*) : δ 1.11 (t, *J* = 7.2 Hz, 3H), 1.19 (t, *J* = 7.1 Hz, 3H), 3.13 (s, 2H), 3.57 (s, 2H), 4.29 (m, 4H), 5.19 (s, 2H), 6.79 (d, *J* = 7.9 Hz, 1H), 7.01 (d, *J* = 7.7 Hz, 1H), 7.14 (d, *J* = 7.9 Hz, 1H), 7.41-7.53 (m, 5H)

ESI/MS (m/z) : 395.5 $(M+H)^{+}$.

5.1.22.14. Benzyl 2-[4-bromo-3-{(diethoxyphosphoryl)methyl}phenyl] acetate (75d)

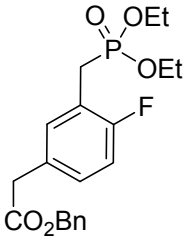


75d (4.53 g, 62%) was prepared from **74d** (6.4 g, 0.016 mole) by means of the general procedure described above in Step-III as a white solid. mp: 77-78 ^{o}C; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.09 (t, *J* = 7.0 Hz, 3H), 1.23 (t, *J* = 7.2 Hz, 3H), 3.09 (s, 2H), 3.59 (s, 2H), 4.11 (m, 4H), 5.37 (s, 2H), 6.72-6.83 (m, 2H), 7.17-7.23 (m, 6H)

ESI/MS (m/z) : 456.5 $(M+H)^{+}$.

5.1.22.15. Benzyl 2-[3-{(diethoxyphosphoryl)methyl}-4-fluorophenyl] acetate (75e)

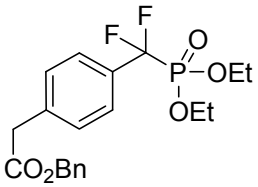


75e (5.14 g, 55%) was prepared from **74e** (8.0 g, 0.023 mole) by means of the general procedure described above in Step-III as a white solid. mp: 119-121 ^{o}C; Purity by HPLC: 95.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.13 (t, *J* = 7.3 Hz, 3H), 1.21 (t, *J* = 7.0 Hz, 3H), 3.09 (s, 2H), 3.49 (s, 2H), 4.31 (m, 4H), 5.37 (s, 2H), 6.73-6.84 (m, 3H), 7.41-7.53 (m, 5H)

ESI/MS (m/z) : 395.5 $(M+H)^{+}$.

5.1.22.16. Benzyl 4-[(diethylphosphono)difluoromethyl]phenylacetate (76a)

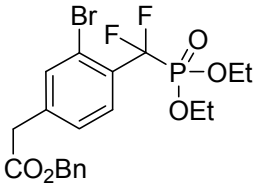


76a (5.12 g, 55%) was prepared from **75a** (8.5 g, 0.022 mole) by means of the general procedure described above in Step-IV as a colourless liquid. Purity by HPLC: 94.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.29 (t, *J* = 7.1 Hz, 6H), 3.65 (s, 2H), 4.18 (m, 4H), 5.11 (s, 2H), 7.22-7.41 (m, 7H), 7.49 (d, *J* = 7.8 Hz, 2H)

ESI/MS (m/z) : 413.3 $(M+H)^+$.

5.1.22.17. Benzyl 2-[3-bromo-4-(diethylphosphono)difluoromethyl]phenyl acetate (76b)

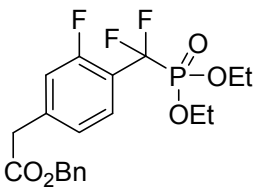


76b (3.82 g, 60%) was prepared from **75b** (5.9 g, 0.013 mole) by means of the general procedure described above in Step-IV as a colourless liquid. Purity by HPLC: 95.10%.

^{1}HNMR (Acetone-*d6*) : δ 1.27 (t, *J* = 7.2 Hz, 6H), 3.59 (s, 2H), 4.09 (m, 4H), 5.37 (s, 2H), 6.83-6.99 (m, 2H), 7.11-7.22 (m, 6H)

ESI/MS (m/z) : 492.2 $(M+H)^+$.

5.1.22.18. Benzyl 2-[4-(diethylphosphono)difluoromethyl-3-fluoro] phenylacetate (76c)

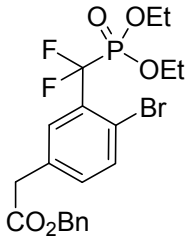


76c (4.97 g, 57%) was prepared from **75c** (8.0 g, 0.020 mole) by means of the general procedure described above in Step-IV as a colourless liquid. Purity by HPLC: 98.00%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.25 (t, *J* = 7.1 Hz, 6H), 3.51 (s, 2H), 4.05 (m, 4H), 5.41 (s, 2H), 6.71-6.99 (m, 3H), 7.15-7.22 (m, 5H)

ESI/MS (m/z) : 431.4 $(M+H)^+$.

5.1.22.19. Benzyl 2-[4-bromo-3-(diethylphosphono)difluoromethyl]phenyl acetate (76d)

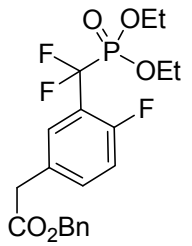


76d (3.2 g, 66%) was prepared from **75d** (4.5 g, 0.009 mole) by means of the general procedure described above in Step-IV as a white solid. mp: 100-101 ^oC; Purity by HPLC: 97.10%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.22 (t, *J* = 7.1 Hz, 6H), 3.61 (s, 2H), 4.11 (m, 4H), 5.39 (s, 2H), 6.73-6.79 (m, 2H), 7.10-7.23 (m, 6H)

ESI/MS (m/z) : 492.2 $(M+H)^+$.

5.1.22.20. Benzyl 2-[3-(diethylphosphono)difluoromethyl-4-fluoro] phenylacetate (76e)

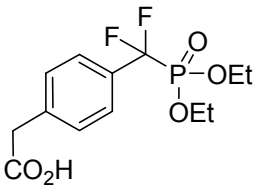


76e (3.54 g, 65%) was prepared from **75e** (5.0 g, 0.012 mole) by means of the general procedure described above in Step-IV as a white solid. mp: 133-134 ^{o}C. Purity by HPLC: 99.10%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.27 (t, *J* = 7.2 Hz, 6H), 3.49 (s, 2H), 4.09 (m, 4H), 5.49 (s, 2H), 6.73-7.09 (m, 3H), 7.11-7.20 (m, 5H)

ESI/MS (m/z) : 431.3 $(M+H)^{+}$.

5.1.22.21. 4-[(Diethylphosphono)difluoromethyl]phenylacetic acid (59a)



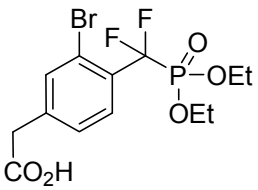
59a (3.5 g, 90%) was prepared from **76a** (5.0 g, 0.012 mole) by means of the general procedure described above in Step-V as a white solid. mp: 98-99 ^{o}C; Purity by HPLC: 97.80%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.24 (t, *J* = 7.2 Hz, 6H), 3.78 (s, 2H), 4.18 (m, 4H), 7.41 (d, *J* = 7.8

Hz, 2H), 7.63 (d, *J* = 7.9 Hz, 2H), 11.0 (s, 1H)

ESI/MS (m/z) : 323.4 $(M+H)^{+}$.

5.1.22.22. 2-[3-bromo-4-{(diethylphosphono)difluoromethyl}]phenylacetic acid (59b)

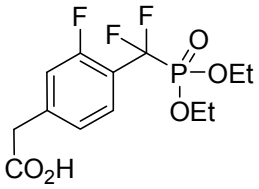


59b (2.78 g, 92%) was prepared from **76b** (3.7 g, 0.007 mole) by means of the general procedure described above in Step-V as a white solid. mp: 109-110 ^{o}C; Purity by HPLC: 95.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.21 (t, *J* = 7.1 Hz, 6H), 3.49 (s, 2H), 4.21 (m, 4H), 6.99 (d, *J* = 7.9 Hz, 2H), 7.13 (m, 1H), 11.3 (s, 1H)

ESI/MS (m/z) : 402.3 $(M+H)^+$.

5.1.22.23. 2-[4-{(diethylphosphono)difluoromethyl}-3-fluoro]phenylacetic acid (59c)

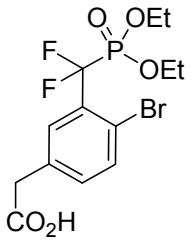


59c (3.34 g, 88%) was prepared from **76c** (4.8 g, 0.011 mole) by means of the general procedure described above in Step-V as a white solid. mp: 77-78 ^oC; Purity by HPLC: 98.30%.

^{1}HNMR (Acetone-*d6*) : δ 1.19 (t, *J* = 7.0 Hz, 6H), 3.51 (s, 2H), 4.27 (m, 4H), 6.63-6.67 (m, 2H), 6.98 (m, 1H), 11.5 (s, 1H)

ESI/MS (m/z) : 341.2 $(M+H)^+$.

5.1.22.24. 2-[4-bromo-3-(diethylphosphono)difluoromethyl]phenylacetic acid (59d)

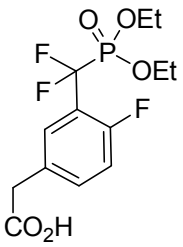


59d (176 g, 72%) was prepared from **76d** (4.5 g, 0.006 mole) by means of the general procedure described above in Step-V as a white solid. mp: 115-116 ^oC; Purity by HPLC: 98.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.30 (t, *J* = 7.3 Hz, 6H), 3.49 (s, 2H), 4.21 (m, 4H), 6.74-6.80 (m, 2H), 7.19 (m, 1H), 10.9 (s, 1H)

ESI/MS (m/z) : 402.4 $(M+H)^+$.

5.1.22.25. 2-[3-(diethylphosphono)difluoromethyl-4-fluoro]phenylacetic acid (59e)

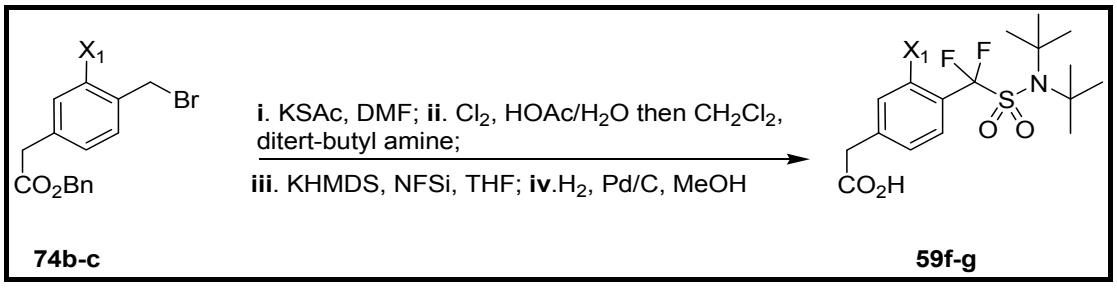


59e (1.88 g, 68%) was prepared from **76e** (3.5 g, 0.008 mole) by means of the general procedure described above in Step-V as a white solid. mp: 99-100 ^oC. Purity by HPLC: 97.70%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.24 (t, *J* = 7.7 Hz, 6H), 3.59 (s, 2H), 4.15 (m, 4H),), 6.73-6.87 (m, 3H), 11.2 (s, 1H)

ESI/MS (m/z) : 341.1 $(M+H)^+$.

5.1.23. General procedure for the synthesis of Compounds (59f-59g)



74b-c **59f-g**

Step I: A solution of **74b-c** (1 mole equivalent) and potassium thioacetate (1.05 mole equivalent) in DMF (5 fold) was stirred at 25 ^oC for 30 min. The reaction was diluted with water (3 fold) and extracted with CH_2Cl_2 (2 X 2 fold). The combined organics were dried over Na_2SO_4, and then concentrated, and the resulting solid residue was recrystalized in CH_2Cl_2/hexane which gave the compound **77a-b** as crystalline white solids.

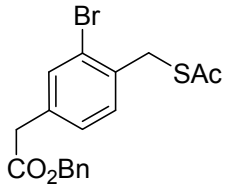
Step II: Chlorine gas was bubbled through a suspension of thioacetate **77a-b** (1 mole equivalent) in glacial acetic acid (3 fold) and ice water (1 fold) at 0 ^{o}C for 3 hour. Nitrogen was then bubbled through the solution to remove excess Cl_2 and then mixture was diluted with water (4 fold) and extracted with CH_2Cl_2 (2 X 4 fold). The combined organics were dried over Na_2SO_4 and concentrated. The resulting sulfonyl chloride was unstable and used immediately for the next step. To a solution of crude product in CH_2Cl_2 (10 fold) was added ditert-butyl amine (2.5 mole equivalent) and the mixture was stirred for 2 hours at 25 ^{o}C. Water (5 fold) was added and the mixture was extracted with CH_2Cl_2 (2 X 10 fold) and the combined organics were dried over Na_2SO_4 and concentrated. The resulting residue was subjected to flash chromatography using 7% ethyl acetate in hexane as eluent gave the desired product **78a-b** as a white solids.

Step III: To a solution of **78a-b** (1 mole equivalent) and N-fluorobenzenesulfonamide (2.5 mole equivalent) in dry THF (10 fold) at -78 ^{o}C was added KHMDS (1.0 M in THF, 2.5 mole equivalent) over 30 min. The mixture was stirred for 2 hours at -78 ^{o}C, then allowed to warm to 25 ^{o}C, and stirred for 18 hours. The reaction was quenched with a saturate aqueous NH_4Cl solution, extracted with ethyl acetate (2 X 20 fold). The combined organic layers was washed successively with water, followed by brine, and dried over Na_2SO_4, filtered and concentrated under vacuum to get the crude product which was subjected to flash chromatography by using 20% ethyl acetate in hexane as eluent to furnish the compounds **79a-b** as white solids.

Step IV: A solution of benzyl ester **79a-b** (1 mole equivalent) in CH_3OH (10 fold), add 10% Pd/C (10% w/w) and hydrogenated using parr hydrogenation

apparatus at 60 psi for 4h. After 4h, Pd/C was filtered through Celite, CH_3OH was evaporated, and resulting residue was triturated using hexane which offered the title compound **59f-g** as a solids.

5.1.23.1. Benzyl 2-[4-(acetylthiomethyl)-3-bromophenyl]acetate (77a)

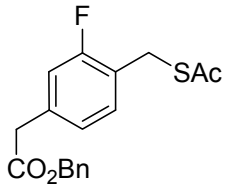


77a (5.92 g, 60%) was prepared from **74b** (10.0 g, 0.025 mole) by means of the general procedure described above in Step-I as a white solid. mp: 131-132 ^{o}C; Purity by HPLC: 92.70%.

^{1}HNMR (Acetone-*d6*) : δ = 2.30 (s, 3H), 3.51 (s, 2H), 4.13 (s, 2H), 5.37 (s, 2H), 6.90-6.99 (m, 2H), 7.13-7.20 (m, 6H)

ESI/MS (m/z) : 394.4 $(M+H)^{+}$.

5.1.23.2. Benzyl 2-[4-(acetylthiomethyl)-3-fluorophenyl]acetate (77b)

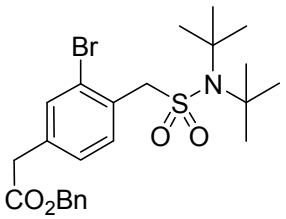


77b (5.41 g, 55%) was prepared from **74b** (10.0 g, 0.029 mole) by means of the general procedure described above in Step-I as a white solid. mp: 121-122 ^{o}C; Purity by HPLC: 94.00%.

^{1}HNMR (Acetone-*d6*) : δ = 2.34 (s, 3H), 3.47 (s, 2H), 4.17 (s, 2H), 5.39 (s, 2H), 6.90-6.99 (m, 2H), 7.13-7.20 (m, 6H)

ESI/MS (m/z) : 333.5 $(M+H)^{+}$.

5.1.23.3. Benzyl N,N-ditertbutyl-2-[3-bromo-4-(methanesulfonamide) phenyl]acetate (78a)

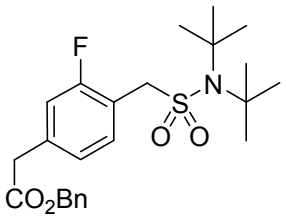


78a (3.76 g, 50%) was prepared from **77a** (5.8 g, 0.014 mole) by means of the general procedure described above in Step-II as a white solid. mp: 89-91 ^{o}C; Purity by HPLC: 96.60%.

^{1}HNMR (Acetone-*d6*) : δ = 1.27 (s, 18H), 3.61 (s, 2H) 4.66 (s, 2H), 5.37 (s, 2H), 6.88-6.97 (m, 2H), 7.12-7.19 (m, 6H)

ESI/MS (m/z) : 511.5 $(M+H)^{+}$.

5.1.23.4. Benzyl N,N-ditertbutyl-2-[3-fluoro-4-(methanesulfonamide) phenyl]acetate (78b)

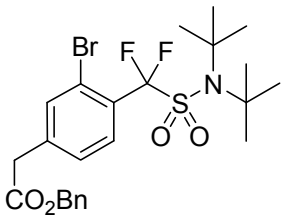


78b (4.3 g, 60%) was prepared from **77b** (5.3 g, 0.016 mole) by means of the general procedure described above in Step-II as a white solid. mp: 104-105 ^{o}C; Purity by HPLC: 95.50%.

^{1}HNMR (Acetone-*d6*) : δ = 1.17 (s, 18H), 3.46 (s, 2H) 4.71 (s, 2H), 5.41 (s, 2H),6.81-6.97 (m, 2H), 7.11-7.20 (m, 6H)

ESI/MS (m/z) : 450.7 $(M+H)^{+}$.

5.1.23.5. Benzyl N,N-ditertbutyl-2-[3-bromo-4-(difluoromethane sulfonamide)phenyl]acetate (79a)

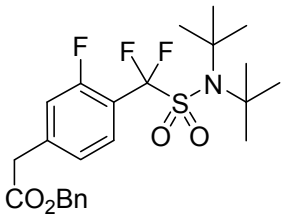


79a (2.28 g, 61%) was prepared from **78a** (3.5 g, 0.006 mole) by means of the general procedure described above in Step-III as a white solid. mp: 99-100 ^{o}C; Purity by HPLC: 95.10%.

^{1}HNMR (Acetone-*d6*) : δ = 1.28 (s, 18H), 3.67 (s, 2H), 5.29 (s, 2H), 6.78-6.96 (m, 2H), 7.08-7.21 (m, 6H)

ESI/MS (m/z) : 547.8 $(M+H)^{+}$.

5.1.23.6. Benzyl N,N-ditertbutyl-2-[4-(difluoromethanesulfonamide)-3-fluorophenyl]acetate (79b)

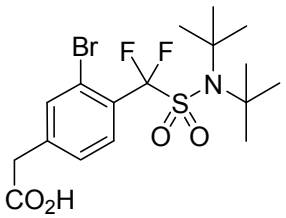


79b (3.17 g, 70%) was prepared from **78b** (4.2 g, 0.009 mole) by means of the general procedure described above in Step-III as a white solid. mp: 113-114 ^{o}C; Purity by HPLC: 95.00%.

^{1}HNMR (Acetone-*d6*) : δ = 1.17 (s, 18H), 3.49 (s, 2H), 5.21 (s, 2H), 6.77-6.99 (m, 2H), 7.07-7.19 (m, 6H)

ESI/MS (m/z) : 486.8 $(M+H)^{+}$.

5.1.23.7. N,N-ditertbutyl-2-[3-bromo-4-(difluoromethanesulfonamide) phenyl]acetic acid (59f)

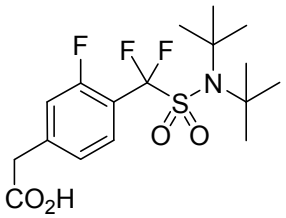


59f (1.28 g, 70%) was prepared from **79a** (2.2 g, 0.004 mole) by means of the general procedure described above in Step-IV as a white solid. mp: 121-122 ^{o}C; Purity by HPLC: 97.30%.

^{1}HNMR (Acetone-*d6*) : δ = 1.28 (s, 18H), 3.57 (s, 2H), 6.73 (d, *J* = 8.3 Hz, 1H), 6.91 (d, *J* = 8.2 Hz, 1H), 7.11 (s, 1H)

ESI/MS (m/z) : 457.7 $(M+H)^{+}$.

5.1.23.8. N,N-ditertbutyl-2-[4-(difluoromethanesulfonamide)-3-fluoro phenyl]acetic acid (59g)

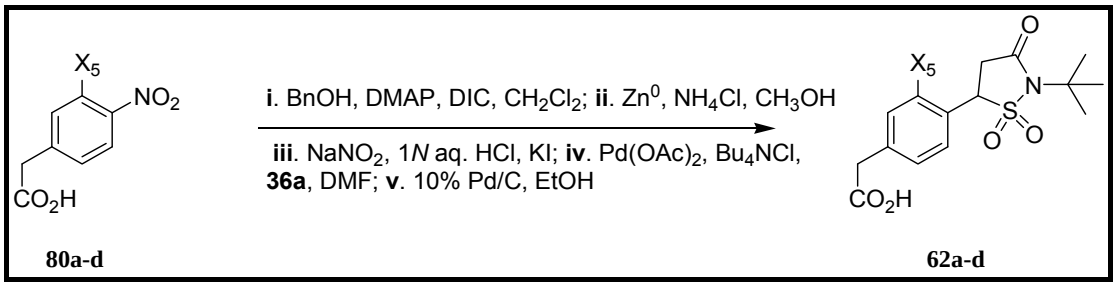


59g (1.1 g, 70%) was prepared from **79b** (2.0 g, 0.003 mole) by means of the general procedure described above in Step-IV as a white solid. mp: 133-134 ^{o}C; Purity by HPLC: 97.00%.

^{1}HNMR (Acetone-*d6*) : δ = 1.31 (s, 18H), 3.49 (s, 2H), 6.71 (d, *J* = 8.5 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 7.13 (s, 1H)

ESI/MS (m/z) : 396.7 $(M+H)^{+}$.

5.1.24. General procedure for the synthesis of Compounds (62a-d)



Step I: To a solution of *p*-nitro substituted phenylacetic acid **80a-d** (1.0 mole equivalent) in CH_2Cl_2 (10 fold) was added benzyl alcohol (10 mole equivalent) and DMAP (0.5 mole equivalent). The solution was then cooled to 0 ^oC, and DIC (1 mole equivalent) was added in a dropwise manner. The mixture was stirred at room temperature for 6h. The reaction mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ (2 x 2 fold), water and finally with saturated aqueous brine. Organic layer was dried over sodium sulfate and concentrated to afford a crude solid product. Chromatography (100-200 mesh size silica gel column, 0-15% ethyl acetate: hexane) afforded the title compound **81a-d** as a solid.

Step II: A stirred solution of **81a-d** (1 mole equivalent) in methanol (20 fold) and water (2 fold) was treated with ammonium chloride (1.5 mole equivalent) and then zinc (8 mole equivalent). The solution was stirred at reflux for 1 h. The solution was filtered through celite, the residue was concentrated under vacuum to remove methanol, and the water solution was extracted with ethyl acetate (2 x 10 fold) and dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum to furnish title compound **82a-d** as yellow solid.

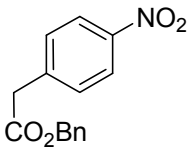
Step III: To a suspension solution of **82a-d** (1 mole equivalent) in 1 *N* aqueous hydrochloric acid (25 fold) was treated dropwise with sodium nitrite

(1 mole equivalent) in water (4 fold) at 0 ^{o}C. After 15 min, potassium iodide (1 mole equivalent) in water (4 fold) was added, and the solution was heated at 40 ^{o}C for 30 min. The solution was quenched with a saturated aqueous sodium thiosulfate solution and extracted with ethyl acetate (2 x 20 fold). The combined extracts was washed with a 0.1 *N* hydrochloric acid solution followed by saturated aqueous sodium bicarbonate solution and dried over sodium sulfate, filtered and concentrated under vacuum to furnish the residue, purified by silica gel chromatography (10-40% ethyl acetate/hexane) afforded the product **83a-d** as solid.

Step IV: Compound **83a-d** (1 mole equivalent), 2-tert-butylisothiazol-3(2H)-one 1,1-dioxide **36a** (1.75 mole equivalent), palladium acetate (0.2 mole equivalent), tetra-butylammonium chloride (1 mole equivalent), and triethylamine (3 mole equivalent) were dissolved in DMF (20 fold). The solution was degassed and then stirred with heating at 70 ^{o}C under nitrogen atmosphere for 2 h. The solution was diluted with ethyl acetate (10 fold) and washed with water and a 1 *N* aqueous hydrochloric acid solution. The organic phase was filtered through Celite and washed ethyl acetate. The organic solution was dried over sodium sulfate, concentrated in vacuo, and purified by silica gel chromatography (10-15% ethyl acetate/hexane) to afford the desired product **84a-d** as a solid.

Step V: Compound **84a-d** and 10% palladium on carbon (0.5 fold) in ethanol (10 fold) were shaken on a parr hydrogenation apparatus for 3 h. The mixture was filtered through Celite and concentrated to give **62a-d** as a solids.

5.1.24.1. Benzyl 2-(4-nitrophenyl)acetate (81a)

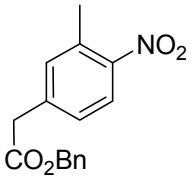


81a (12.72 g, 85%) was prepared from **80a** (10.0 g, 0.055 mole) by means of a general procedure described above in Step-I as white solid. mp: 77-78 ^{o}C; Purity by HPLC: 94.60%.

^{1}HNMR (Acetone-*d6*) : δ = 3.51 (s, 2H), 5.39 (s, 2H), 6.32 (d, *J* = 7.7 Hz, 2H), 6.85 (d, *J* = 7.9 Hz, 2H) 7.32-7.44 (m, 5H)

ESI/MS (m/z) : 272.2 $(M+H)^{+}$.

5.1.24.2. Benzyl 2-(3-methyl-4-nitrophenyl)acetate (81b)

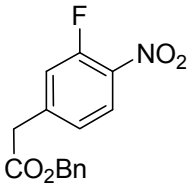


81b (10.23 g, 70%) was prepared from **80b** (10.0 g, 0.051 mole) by means of the general procedure described above in Step-I as a white solid. mp: 88-89 ^{o}C; Purity by HPLC: 95.55%.

^{1}HNMR (Acetone-*d6*) : δ = 2.37 (s, 3H), 3.47 (s, 2H), 5.37 (s, 2H), 7.12 (s, 1H), 7.15-7.21 (m, 6H) 7.97 (m, 1H)

ESI/MS (m/z) : 286.4 $(M+H)^{+}$.

5.1.24.3. Benzyl 2-(3-bromo-4-nitrophenyl)acetate (81c)

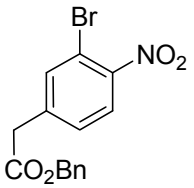


81c (10.6 g, 73%) was prepared from **80c** (10.0 g, 0.05 mole) by means of the general procedure described above in Step-I as a white solid. mp: 103-104 ^{o}C; Purity by HPLC: 96.90%.

^{1}HNMR (Acetone-*d6*) : δ = 3.54 (s, 2H), 5.37 (s, 2H), 7.02-7.05 (m, 2H), 7.19-7.26 (m, 5H), 8.07 (d, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 290.3 $(M+H)^{+}$.

5.1.24.4. Benzyl 2-(3-bromo-4-nitrophenyl)acetate (81d)

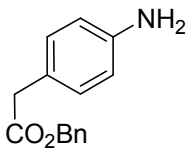


81d (10.9 g, 81%) was prepared from **80d** (10.0 g, 0.038 mole) by means of the general procedure described above in Step-I as a white solid. mp: 122-124 ^{o}C; Purity by HPLC: 93.70%.

^{1}HNMR (Acetone-*d6*) : δ = 3.55 (s, 2H), 5.41 (s, 2H), 7.12-7.23 (m, 5H), 7.26 (dd, *J* = 8.0, 1.9 Hz, 1H), 7.46 (d, *J* = 1.8 Hz, 1H), 7.95 (d, *J* = 8.0 Hz, 1H)

ESI/MS (m/z) : 351.3 $(M+H)^{+}$.

5.1.24.5. Benzyl 2-(4-aminophenyl)acetate (82a)

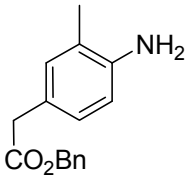


82a (6.67 g, 60%) was prepared from **81a** (12.5 g, 0.046 mole) by means of the general procedure described above in Step-II as yellow solid. mp: 108-109 ^{o}C; Purity by HPLC: 94.10%.

^{1}HNMR (Acetone-*d6*) : δ = 3.47 (s, 2H), 5.29 (s, 2H), 7.32 (d, *J* = 7.8 Hz, 2H), 7.32-7.44 (m, 5H), 8.09 (d, *J* = 7.7 Hz, 2H)

ESI/MS (m/z) : 242.5 $(M+H)^{+}$.

5.1.24.6. Benzyl 2-(4-amino-3-methylphenyl)acetate (82b)

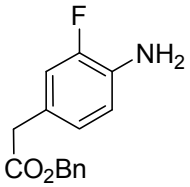


82b (6.0 g, 67%) was prepared from **81b** (10.0 g, 0.035 mole) by means of the general procedure described above in Step-II as a yellow foam. mp: 122-123 ^{o}C; Purity by HPLC: 93.30%.

^{1}HNMR (Acetone-*d6*) : δ = 2.32(s, 3H), 3.49 (s, 2H), 5.39 (s, 2H), 7.10 (s, 1H), 7.13-7.25 (m, 6H) 7.57 (m, 1H)

ESI/MS (m/z) : 256.4 $(M+H)^{+}$.

5.1.24.7. Benzyl 2-(4-amino-3-fluorophenyl)acetate (82c)

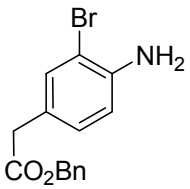


82c (6.54 g, 73%) was prepared from **81c** (10.0 g, 0.034 mole) by means of the general procedure described above in Step-II as a yellow foam. mp: 129-130 ^{o}C; Purity by HPLC: 92.00%.

^{1}HNMR (Acetone-*d6*) : δ = 3.59 (s, 2H), 5.41 (s, 2H), 6.32 (d, J = 8.0 Hz, 1H), 6.55 (s, 1H), 6.59 (d, J = 8.1 Hz, 1H), 7.12-7.25 (m, 5H)

ESI/MS (m/z) : 260.4 $(M+H)^{+}$.

5.1.24.8. Benzyl 2-(4-amino-3-fluorophenyl)acetate (82d)

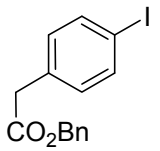


82d (7.39 g, 77%) was prepared from **81d** (10.5 g, 0.03 mole) by means of the general procedure described above in Step-II as a yellow foam. mp: 111-112 ^{o}C; Purity by HPLC: 94.00%.

^{1}HNMR (Acetone-*d6*) : δ = 3.49 (s, 2H), 5.37 (s, 2H), 6.23 (d, *J* = 7.9 Hz, 1H), 6.77 (d, *J* = 8.2 Hz, 1H), 6.98 (s, 1H), 7.17-7.24 (m, 5H)

ESI/MS (m/z) : 321.3 $(M+H)^{+}$.

5.1.24.9. Benzyl 2-(4-iodophenyl)acetate (83a)

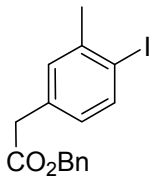


83a (4.64 g, 49%) was prepared from **82a** (6.5 g, 0.027 mole) by means of the general procedure described above in Step-III as a pale yellow solid. mp: 122-123 ^{o}C; Purity by HPLC: 93.10%.

^{1}HNMR (Acetone-*d6*) : δ = 3.59 (s, 2H), 5.37 (s, 2H), 6.88 (d, *J* = 7.8 Hz, 2H), 7.09-7.24 (m, 5H), 7.55 (d, *J* = 7.9 Hz, 2H)

ESI/MS (m/z) : 353.5 $(M+H)^{+}$.

5.1.24.10. Benzyl 2-(4-iodo-3-methylphenyl)acetate (83b)

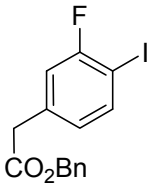


83b (3.58 g, 43%) was prepared from **82b** (5.8 g, 0.022 mole) by means of the general procedure described above in Step-III as an off white solid. mp: 131-132 ^{o}C; Purity by HPLC: 94.20%.

^{1}HNMR (Acetone-*d6*) : δ = 2.35 (s, 3H), 3.53 (s, 2H), 5.33 (s, 2H), 6.63 (s, 1H), 6.65 (d, *J* = 8.1 Hz, 1H), 7.15-7.21 (m, 5H) 7.39 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 367.3 $(M+H)^{+}$.

5.1.24.11. Benzyl 2-(3-fluoro-4-iodophenyl)acetate (83c)

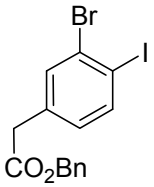


83c (4.36 g, 47%) was prepared from **82c** (6.5 g, 0.025 mole) by means of the general procedure described above in Step-III as a white solid. mp: 144-145 ^{o}C; Purity by HPLC: 95.10%.

^{1}HNMR (Acetone-*d6*) : δ = 3.47 (s, 2H), 5.27 (s, 2H), 6.54 (s, 1H), 6.61 (d, *J* = 8.0 Hz, 1H), 7.14-7.29 (m, 5H), 7.52 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 371.3 $(M+H)^{+}$.

5.1.24.12. Benzyl 2-(3-bromo-4-iodophenyl)acetate (83d)

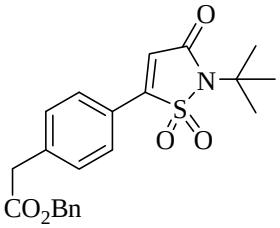


83d (4.84 g, 50%) was prepared from **82d** (7.2 g, 0.022 mole) by means of the general procedure described above in Step-III as a white solid. mp: 130-131 ^{o}C; Purity by HPLC: 93.90%.

^{1}HNMR (Acetone-*d6*) : δ = 3.53 (s, 2H), 5.32 (s, 2H), 6.77 (d, *J* = 8.1 Hz, 1H), 7.01 (s, 1H), 7.17-7.24 (m, 5H), 7.39 (d, *J* = 8.2 Hz, 1H)

ESI/MS (m/z) : 432.3 $(M+H)^{+}$.

5.1.24.13. Benzyl 2-[4-(2-tert-butyl-1,1-dioxide-3-oxo-2,3-dihydro isothiazole-5-yl)]phenylacetate (84a)

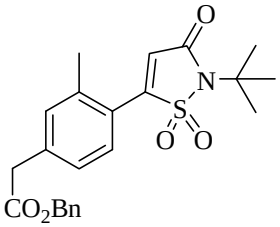


84a (3.27 g, 62%) was prepared from **83a** (4.5 g, 0.012 mole) by means of the general procedure described above in Step-IV as a white solid. mp: 149-150 ^{o}C; Purity by HPLC: 96.30%.

^{1}HNMR (Acetone-*d6*) : δ = 1.37 (s, 9H), 3.49 (s, 2H), 5.31 (s, 2H), 7.04 (d, *J* = 7.7 Hz, 2H), 7.09-7.24 (m, 7H), 7.59 (s, 1H)

ESI/MS (m/z) : 414.7 $(M+H)^{+}$.

5.1.24.14. Benzyl 2-[3-methyl-4-(2-tert-butyl-1,1-dioxide-3-oxo-2,3-dihydro isothiazole-5-yl)]phenylacetate (84b)

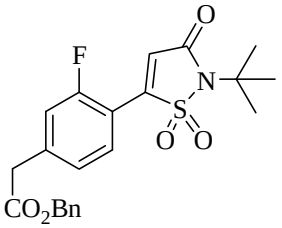


84b (2.73 g, 67%) was prepared from **83b** (3.5 g, 0.009 mole) by means of the general procedure described above in Step-IV as a white solid. mp: 127-128 ^{o}C; Purity by HPLC: 94.90%.

^{1}HNMR (Acetone-*d6*) : δ = 1.30 (s, 9H), 2.37 9s, 3H), 3.51 (s, 2H), 5.37 (s, 2H), 6.81 (s, 1H), 6.94 (d, *J* = 8.3 Hz, 1H), 7.03 (d, *J* = 7.9 Hz, 1H), 7.15-7.30 (m, 5H), 7.41 (s, 1H)

ESI/MS (m/z) : 428.7 $(M+H)^{+}$.

5.1.24.15. Benzyl 2-[3-fluoro-4-(2-tert-butyl-1,1-dioxide-3-oxo-2,3-dihydro isothiazole-5-yl)]phenylacetate (84c)

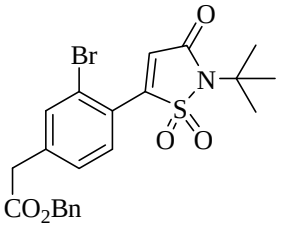


84c (3.37 g, 69%) was prepared from **83c** (4.2 g, 0.011 mole) by means of the general procedure described above in Step-IV as a white solid. mp: 111-112 ^{o}C; Purity by HPLC: 97.20%.

^{1}HNMR (Acetone-*d6*) : δ = 1.27 (s, 9H), 3.47 (s, 2H), 5.31 (s, 2H), 6.77 (d, *J* = 8.0 Hz, 1H), 6.72 (s, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 7.19-7.29 (m, 5H), 7.39 (s, 1H)

ESI/MS (m/z) : 432.7 $(M+H)^{+}$.

5.1.24.16. Benzyl 2-[3-bromo-4-(2-tert-butyl-1,1-dioxide-3-oxo-2,3-dihydro isothiazole-5-yl)]phenylacetate (84d)

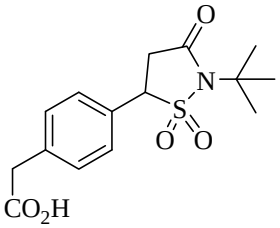


84d (3.39 g, 66%) was prepared from **83d** (4.5 g, 0.010 mole) by means of the general procedure described above in Step-IV as a white solid. mp: 94-95 ^{o}C; Purity by HPLC: 96.60%.

^{1}HNMR (Acetone-*d6*) : δ = 1.30 (s, 9H), 3.53 (s, 2H), 5.37 (s, 2H), 6.97 (d, *J* = 8.2 Hz, 1H), 7.08 (d, *J* = 8.0 Hz, 1H), 7.18 (s, 1H), 7.20-7.27 (m, 5H), 7.49 (s, 1H)

ESI/MS (m/z) : 493.7 $(M+H)^{+}$.

5.1.24.17. 2-[4-(2-tert-butyl-1,1-dioxide-3-oxoisothiazole-5-yl)]phenyl acetic acid (62a)

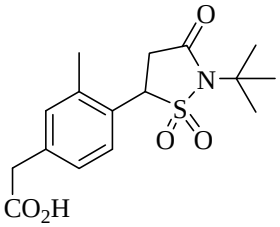


62a (1.81 g, 77%) was prepared from **84a** (3.0 g, 0.007 mole) by means of the general procedure described above in Step-V as a white solid. mp: 105-106 ^{o}C; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : δ = 1.27 (s, 9H), 3.50 (s, 2H), 2.98 (dd, *J* = 13.6, 4.3 Hz, 1H), 3.15 (dd, *J* = 17.2, 8.3 Hz, 1H), 5.23 (t, *J* = 9.6 Hz, 1H), 6.98 (d, *J* = 7.8 Hz, 2H), 7.05 (d, *J* = 7.8 Hz, 2H)

ESI/MS (m/z) : 326.4 $(M+H)^{+}$.

5.1.24.18. 2-[3-methyl-4-(2-tert-butyl-1,1-dioxide-3-oxoisothiazole-5-yl)] phenylacetic acid (62b)

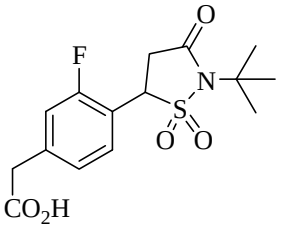


62b (1.72 g, 77%) was prepared from **84b** (2.5 g, 0.005 mole) by means of the general procedure described above in Step-V as a white solid. mp: 91-92 ^{o}C; Purity by HPLC: 96.80%.

^{1}HNMR (Acetone-*d6*) : δ = 1.21 (s, 9H), 2.41 (s, 3H), 2.91 (dd, *J* = 13.8, 4.5 Hz, 1H), 3.17 (dd, *J* = 17.1, 8.1 Hz, 1H), 3.51 (s, 2H), 4.53 (t, *J* = 9.8 Hz, 1H), 6.81 (s, 1H), 6.85 (d, *J* = 7.9 Hz, 1H), 6.99 (d, *J* = 7.7 Hz, 1H)

ESI/MS (m/z) : 340.6 $(M+H)^{+}$.

5.1.24.19. 2-[3-fluoro-4-(2-tert-butyl-1,1-dioxide-3-oxoisothiazole-5-yl)] phenylacetic acid (62c)

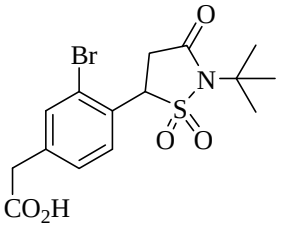


62c (1.93 g, 76%) was prepared from **84c** (3.2 g, 0.007 mole) by means of the general procedure described above in Step-V as a white solid. mp: 103-104 ^{o}C; Purity by HPLC: 96.50%.

^{1}HNMR (Acetone-*d6*) : δ = 1.24 (s, 9H), 2.88 (dd, *J* = 14.0, 4.5 Hz, 1H), 3.21 (dd, *J* = 17.6, 8.4 Hz, 1H), 3.33 (s, 2H), 4.51 (t, *J* = 9.8 Hz, 1H), 6.72 (s, 1H), 6.77 (d, *J* = 8.0 Hz, 1H), 6.99 (d, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 344.6 $(M+H)^{+}$.

5.1.24.20. 2-[3-bromo-4-(2-tert-butyl-1,1-dioxide-3-oxoisothiazole-5-yl)] phenylacetic acid (62d)

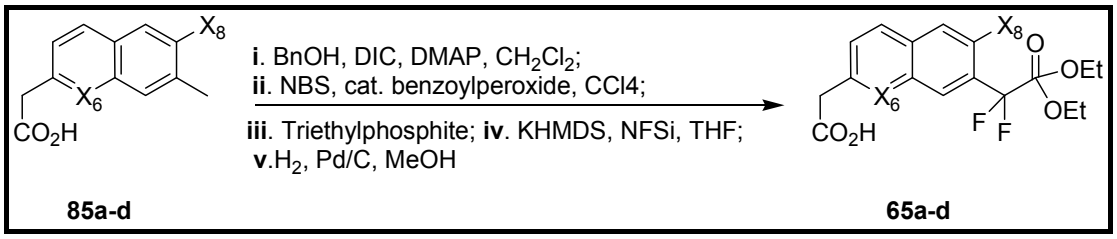


62d (1.81 g, 69%) was prepared from **84d** (3.2 g, 0.006 mole) by means of the general procedure described above in Step-V as a white solid. mp: 111-112 ^{o}C; Purity by HPLC: 95.50%.

^{1}HNMR (Acetone-*d6*) : δ = 1.19 (s, 9H), 2.93 (dd, *J* = 13.9, 4.2 Hz, 1H), 3.15 (dd, *J* = 17.2, 8.0 Hz, 1H), 3.49 (s, 2H), 4.61 (t, *J* = 9.7 Hz, 1H), 6.89 (d, *J* = 8.3 Hz, 1H), 6.97 (d, *J* = 7.9 Hz, 1H), 7.21 (s, 1H)

ESI/MS (m/z) : 405.3 $(M+H)^{+}$.

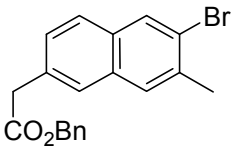
5.1.25. General procedure for the synthesis of Compounds (65a-d)



85a-d **65a-d**

Title compound **65a-d** was prepared using the same procedure as that used for compound **59a-c** (**5.1.22.**) using appropriate starting material.

5.1.25.1. Benzyl 2-(6-bromo-7-methylnaphthalen-2-yl)acetate (86a)

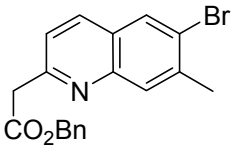


86a (10.58 g, 70%) was prepared from **85a** (10 g, 0.035 mole) by means of a general procedure described in **5.1.22.** (step-I) as a white solid. mp: 149-151 ^{o}C; Purity by HPLC: 94.20%.

^{1}HNMR (Acetone-*d6*) : δ 2.42 (s, 3H), 3.87 (s, 2H), 5.23 (s, 2H), 7.10-7.19 (m, 5H), 7.27 (d, *J* = 7.7 Hz, 1H), 7.37 (s, 1H), 7.44 (s, 1H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.89 (s, 1H)

ESI/MS (m/z) : 370.4 $(M+H)^{+}$.

5.1.25.2. Benzyl 2-(6-bromo-7-methylquinolin-2-yl)acetate (86b)

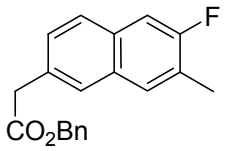


86b (11.9 g, 90%) was prepared from **85b** (10 g, 0.035 mole) by means of the general procedure described in **5.1.22.** (step-I) as a white solid. mp: 137-138 ^{o}C; Purity by HPLC: 95.10%.

^{1}HNMR (Acetone-*d6*) : δ 2.37 (s, 3H), 3.91 (s, 2H), 5.31 (s, 2H), 7.11 (d, *J* = 7.9 Hz, 1H), 7.19-7.22 (m, 5H), 7.72 (s, 1H), 7.89 (s, 1H), 7.99 (d, *J* = 7.7 Hz, 1H)

ESI/MS (m/z) : 371.2 $(M+H)^+$.

5.1.25.3. Benzyl 2-(6-fluoro-7-methylnaphthalen-2-yl)acetate (86c)

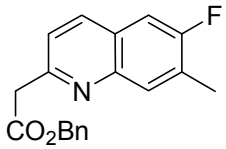


86c (10.88 g, 77%) was prepared from **85c** (10 g, 0.045 mole) by means of the general procedure described in **5.1.22.** (step-I) as a white solid. mp: 121-122 ^oC; Purity by HPLC: 95.06%.

^{1}HNMR (Acetone-*d6*) : δ 2.51 (s, 3H), 3.67 (s, 2H), 5.42 (s, 2H), 7.19-7.22 (m, 6H), 7.34 (m, 1H), 7.49 (s, 1H), 7.67 (d, *J* = 7.7 Hz, 1H)

ESI/MS (m/z) : 309.4 $(M+H)^+$.

5.1.25.4. Benzyl 2-(6-fluoro-7-methylquinolin-2-yl)acetate (86d)

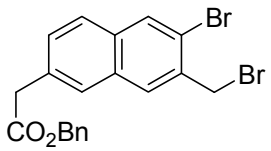


86d (11.43 g, 81%) was prepared from **85d** (10 g, 0.045 mole) by means of the general procedure described in **5.1.22.** (step-I) as a white solid. mp: 113-114 ^oC; Purity by HPLC: 94.80%.

^{1}HNMR (Acetone-*d6*) : δ 2.31 (s, 3H), 3.89 (s, 2H), 5.39 (s, 2H), 7.09 (d, *J* = 7.9 Hz, 1H), 7.20-7.26 (m, 5H), 7.35 (m, 1H), 7.81 (s, 1H), 7.98 (d, *J* = 7.7 Hz, 1H)

ESI/MS (m/z) : 310.1 $(M+H)^+$.

5.1.25.5. Benzyl 2-[6-bromo-7-(bromomethyl)naphthalen-2-yl]acetate (87a)

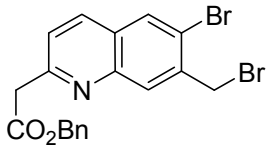


87a (8.41 g, 66%) was prepared from **86a** (10.5 g, 0.028 mole) by means of the general procedure described in **5.1.22.** (step-II) as a white solid. mp: 101-102 ^{o}C; Purity by HPLC: 93.30%.

^{1}HNMR (Acetone-*d6*) : δ 3.59 (s, 2H), 4.71 (s, 2H), 5.41 (s, 2H), 7.16-7.22 (m, 6H) 7.36 (s, 1H), 7.43 (s, 1H), 7.69 (d, *J* = 7.8 Hz, 1H), 7.87 (s, 1H)

ESI/MS (m/z) : 449.3 $(M+H)^{+}$.

5.1.25.6. Benzyl 2-[6-bromo-7-(bromomethyl)quinolin-2-yl]acetate (87b)

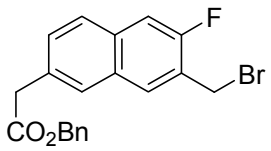


87b (7.8 g, 55%) was prepared from **86b** (11.7 g, 0.031 mole) by means of the general procedure described in **5.1.22.** (step-II) as a white solid. mp: 88-89 ^{o}C; Purity by HPLC: 96.40%.

^{1}HNMR (Acetone-*d6*) : δ 3.87 (s, 2H), 4.57 (s, 2H), 5.39 (s, 2H), 7.12-7.19 (m, 6H), 7.69 (s, 1H), 7.88 (s, 1H), 7.99 (d, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 450.4 $(M+H)^{+}$.

5.1.25.7. Benzyl 2-[7-(bromomethyl)-6-fluoronaphthalen-2-yl]acetate (87c)

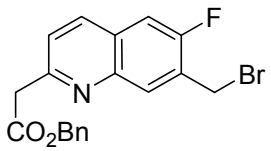


87c (6.98 g, 53%) was prepared from **86c** (10.5 g, 0.034 mole) by means of the general procedure described in **5.1.22.** (step-II) as a white solid. mp: 101-103 ^{o}C; Purity by HPLC: 93.33%.

^{1}HNMR (Acetone-*d6*) : *δ* 3.66 (s, 2H), 4.72 (s, 2H), 5.47 (s, 2H), 7.11-7.19 (m, 6H), 7.35 (m, 1H), 7.42-7.45 (s, 2H), 7.67 (d, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 388.2 $(M+H)^{+}$.

5.1.25.8. Benzyl 2-[7-(bromomethyl)-6-fluoroquinolin-2-yl]acetate (87d)

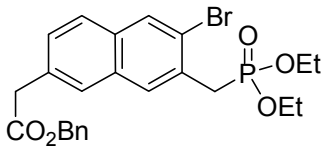


87d (7.45 g, 53%) was prepared from **86d** (11.2 g, 0.036 mole) by means of the general procedure described in **5.1.22.** (step-II) as a white solid. mp: 111-112 ^{o}C; Purity by HPLC: 94.68%.

^{1}HNMR (Acetone-*d6*) : *δ* 3.88 (s, 2H), 4.55 (s, 2H), 5.39 (s, 2H), 7.10 (d, *J* = 7.8 Hz, 1H), 7.13-7.19 (m, 5H), 7.39 (m, 1H), 7.80 (s, 1H), 7.97 (d, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 388.2 $(M+H)^{+}$.

5.1.25.9. Benzyl 2-[6-bromo-7-{(diethoxyphosphoryl)methyl}naphthalen-2-yl]acetate (88a)

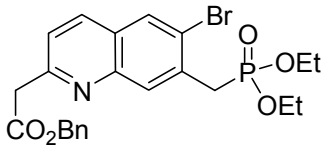


88a (6.47 g, 70%) was prepared from **87a** (8.2 g, 0.018 mole) by means of the general procedure described in **5.1.22.** (step-III) as a white solid. mp: 133-134 ^{o}C; Purity by HPLC: 97.60%.

^{1}HNMR (Acetone-*d6*) : δ 1.09 (t, *J* = 7.0 Hz, 3H), 1.22 (t, *J* = 7.3 Hz, 3H), 3.09 (s, 2H), 3.66 (s, 2H), 4.11 (m, 4H), 5.41 (s, 2H), 7.09-7.19 (m, 5H), 7.25 (d, *J* = 7.9 Hz, 1H), 7.32 (s, 1H), 7.42 (s, 1H), 7.66 (d, *J* = 7.7 Hz, 1H), 7.91 (s, 1H)

ESI/MS (m/z) : 506.5 $(M+H)^+$.

5.1.25.10. Benzyl 2-[6-bromo-7-{(diethoxyphosphoryl)methyl}quinolin-2-yl]acetate (88b)

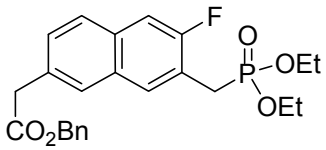


88b (5.24 g, 62%) was prepared from **87b** (7.5 g, 0.016 mole) by means of the general procedure described in **5.1.22.** (step-III) as a white solid. mp: 144-145 ^oC; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.08 (t, *J* = 7.2 Hz, 3H), 1.19 (t, *J* = 7.2 Hz, 3H), 3.17 (s, 2H), 3.85 (s, 2H), 4.17 (m, 4H), 5.35 (s, 2H), 7.11-7.23 (m, 6H), 7.74 (s, 1H), 7.88 (s, 1H), 7.97 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 507.7 $(M+H)^+$.

5.1.25.11. Benzyl 2-[7-{(diethoxyphosphoryl)methyl}-6-fluoronaphthalen-2-yl]acetate (88c)

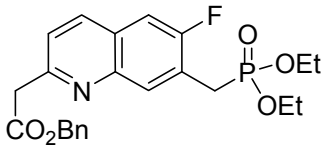


88c (5.38 g, 69%) was prepared from **87c** (6.8 g, 0.017 mole) by means of the general procedure described in **5.1.22.** (step-III) as a white solid. mp: 116-117 ^oC; Purity by HPLC: 97.15%.

^{1}HNMR (Acetone-*d6*) : δ 1.09 (t, *J* = 7.0 Hz, 3H), 1.22 (t, *J* = 7.3 Hz, 3H), 3.15 (s, 2H), 3.72 (s, 2H), 4.08 (m, 4H), 5.39 (s, 2H), 7.13-7.23 (m, 6H), 7.34 (m, 1H), 7.42 (s, 1H), 7.51 (s, 1H), 7.69 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 445.6 $(M+H)^+$.

5.1.25.12. Benzyl 2-[7-{(diethoxyphosphoryl)methyl}-6-fluoroquinolin-2-yl]acetate (88d)

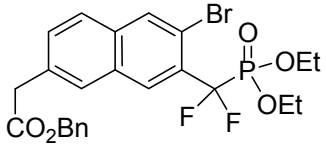


88d (5.19 g, 62%) was prepared from **87d** (6.8 g, 0.017 mole) by means of the general procedure described in **5.1.22.** (step-III) as white solid. mp: 122-123 ^oC; Purity by HPLC: 96.55%.

^{1}HNMR (Acetone-*d6*) : δ 1.13 (t, *J* = 7.2 Hz, 3H), 1.24 (t, *J* = 7.2 Hz, 3H), 3.03 (s, 2H), 3.85 (s, 2H), 4.11 (m, 4H), 5.42 (s, 2H), 7.04 (d, *J* = 7.7 Hz, 1H), 7.15-7.20 (m, 5H), 7.37 (m, 1H), 7.77 (s, 1H), 7.99 (d, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 446.8 $(M+H)^+$.

5.1.25.13. Benzyl 2-[6-bromo-7-(diethyldifluoro)methylphosphonate naphthalen-2-yl]acetate (89a)

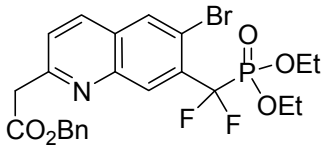


89a (4.65 g, 69%) was prepared from **88a** (6.3 g, 0.012 mole) by means of the general procedure described in **5.1.22.** (step-IV) as a white solid. mp: 129-131 ^oC; Purity by HPLC: 96.70%.

^{1}HNMR (Acetone-*d6*) : δ 1.20 (t, *J* = 7.1 Hz, 3H), 1.37 (t, *J* = 7.2 Hz, 3H), 3.86 (s, 2H), 4.28 (m, 4H), 5.19 (s, 2H), 7.19 (d, *J* = 7.8 Hz, 1H), 7.33 (s, 1H), 7.37 (s, 1H), 7.41-7.55 (m, 5H), 7.98 (d, *J* = 7.7 Hz, 1H), 8.17 (s, 1H)

ESI/MS (m/z) : 542.3 $(M+H)^+$.

5.1.25.14. Benzyl 2-[6-bromo-7-(diethyldifluoro)methylphospho natequinilin-2-yl]acetate (89b)

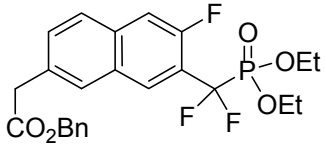


89b (3.0 g, 57%) was prepared from **88b** (5.0 g, 0.009 mole) by means of a general procedure described in **5.1.22.** (step-IV) as a white solid. mp: 113-114 ^{o}C; Purity by HPLC: 97.10%.

^{1}HNMR (Acetone-*d6*) : δ 1.17 (t, *J* = 7.2 Hz, 3H), 1.31 (t, *J* = 7.0 Hz, 3H), 4.09 (s, 2H), 4.17 (m, 4H), 5.21 (s, 2H), 7.14 (d, *J* = 7.8 Hz, 1H), 7.32-7.49 (m, 5H), 7.53 (s, 1H), 8.31 (s, 1H), 8.37 (d, *J* = 7.7 Hz, 1H)

ESI/MS (m/z) : 543.1 $(M+H)^+$.

5.1.25.15. Benzyl 2-[6-fluoro-7-(diethyldifluoro)methylphosphonate naphthalen-2-yl]acetate (89c)

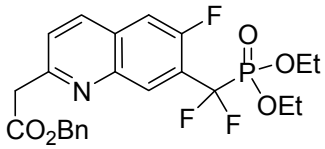


89c (3.76 g, 67%) was prepared from **88c** (5.2 g, 0.011 mole) by means of the general procedure described in **5.1.22.** (step-IV) as a white solid. mp: 111-112 ^{o}C; Purity by HPLC: 96.01%.

^{1}HNMR (Acetone-*d6*) : δ 1.27 (t, *J* = 7.0 Hz, 3H), 1.29 (t, *J* = 7.5 Hz, 3H), 3.79 (s, 2H), 4.18 (m, 4H), 5.23 (s, 2H), 7.14 (d, *J* = 7.9 Hz, 1H), 7.31 (s, 1H), 7.39 (s, 1H), 7.43 (s, 1H), 7.45-7.55 (m, 5H), 7.99 (d, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 481.6 $(M+H)^+$.

5.1.25.16. Benzyl 2-[6-fluoro-7-(diethyldifluoro)methylphosphonate quinolin-2-yl]acetate (89d)

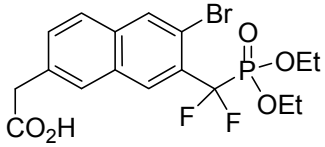


89d (3.51 g, 65%) was prepared from **88d** (5.0 g, 0.011 mole) by means of the general procedure described in **5.1.22.** (step-IV) as white solid. mp: 93-94 ^oC; Purity by HPLC: 94.40%.

^{1}HNMR (Acetone-*d6*) : δ 1.14 (t, *J* = 7.4 Hz, 3H), 1.29 (t, *J* = 7.2 Hz, 3H), 4.11 (s, 2H), 4.19 (m, 4H), 5.27 (s, 2H), 7.08 (d, *J* = 7.8 Hz, 1H), 7.34 (s, 1H), 7.38-7.49 (m, 5H), 7.59 (s, 1H), 8.33 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 482.4 $(M+H)^+$.

5.1.25.17. 2-[6-bromo-7-{(diethoxyphosphoryl)difluoromethyl} naphthalene-2-yl]acetic acid (65a)

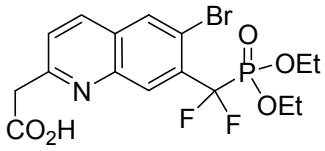


65a (3.11 g, 83%) was prepared from **89a** (4.5 g, 0.008 mole) by means of the general procedure described in **5.1.22.** (step-V) as a white solid. mp: 147-149 ^oC; Purity by HPLC: 98.20%.

^{1}HNMR (Acetone-*d6*) : δ 1.18 (t, *J* = 7.2 Hz, 3H), 1.22 (t, *J* = 7.2 Hz, 3H), 3.74 (s, 2H), 4.19 (m, 4H), 7.19 (d, *J* = 7.7 Hz, 1H), 7.31 (s, 1H), 7.37 (s, 1H), 7.93 (d, *J* = 7.7 Hz, 1H), 8.09 (s, 1H)

ESI/MS (m/z) : 452.4 $(M+H)^+$.

5.1.25.18. 2-[6-bromo-7-{(diethoxyphosphoryl)difluoromethyl}-quinolin-2-yl]acetic acid (65b)

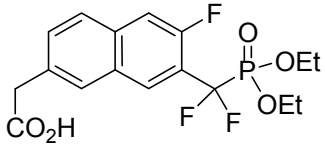


65b (1.82 g, 78%) was prepared from **89b** (2.8 g, 0.005 mole) by means of the general procedure described in **5.1.22.** (step-V) as a white solid. mp: 130-131 ^oC; Purity by HPLC: 96.10%.

^{1}HNMR (Acetone-*d6*) : δ 1.09 (t, *J* = 7.2 Hz, 3H), 1.24 (t, *J* = 7.2 Hz, 3H), 4.01 (s, 2H), 4.23 (m, 4H), 7.18 (d, *J* = 7.7 Hz, 1H), 7.52 (s, 1H), 8.29 (s, 1H), 8.33 (d, *J* = 7.7 Hz, 1H)

ESI/MS (m/z) : 453.3 $(M+H)^+$.

5.1.25.19. 2-[7-{(diethoxyphosphoryl)difluoromethyl}-6-fluoronaphthalen-2-yl]acetic acid (65c)

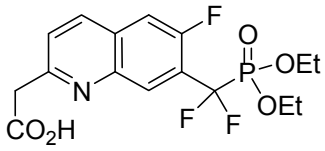


65c (2.3 g, 81%) was prepared from **89c** (3.5 g, 0.007 mole) by means of the general procedure described in **5.1.22.** (step-V) as a white solid. mp: 151-152 ^oC; Purity by HPLC: 95.57%.

^{1}HNMR (Acetone-*d6*) : δ 1.05 (t, *J* = 7.3 Hz , 3H), 1.21 (t, *J* = 7.2 Hz , 3H), 3.83 (s, 2H), 4.29 (m, 4H), 7.14 (d, *J* = 7.8 Hz, 1H), 7.33 (s, 1H), 7.41 (s, 1H), 7.45 (s, 1H) 7.97 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 391.4 $(M+H)^+$.

5.1.25.20. 2-[7-{(diethoxyphosphoryl)difluoromethyl}-6-fluoroquinolin-2-yl]acetic acid (65d)

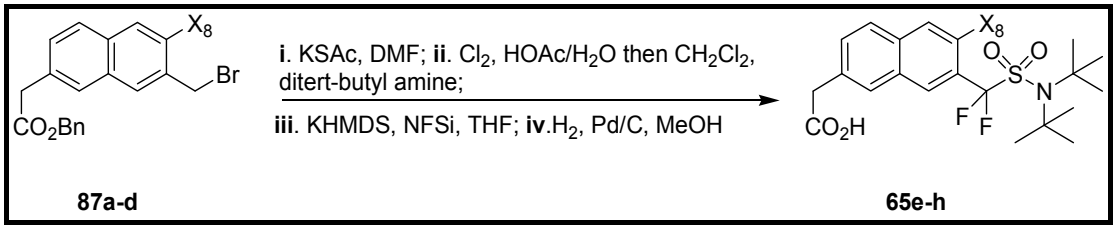


65d (2.21 g, 78%) was prepared from **89d** (3.5 g, 0.007 mole) by means of the general procedure described in **5.1.22.** (step-V) as a white solid. mp: 130-131 ^oC; Purity by HPLC: 96.00%.

^{1}HNMR (Acetone-*d6*) : δ 1.06 (t, *J* = 7.3 Hz, 3H), 1.19 (t, *J* = 7.2 Hz, 3H), 4.11 (s, 2H), 4.42 (m, 4H), 7.11 (d, *J* = 8.3 Hz, 1H), 7.34 (s, 1H), 7.61 (s, 1H), 8.39 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 392.5 $(M+H)^+$.

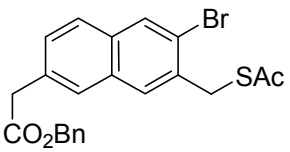
5.1.26. General procedure for the synthesis of Compounds (65e-h)



87a-d **65e-h**

Title compound **65e-h** was prepared using the same procedure as that used for compound **59f-g** (**5.1.23.**) using appropriate starting material.

5.1.26.1. Benzyl 2-[7-(acetylthiomethyl)-6-bromonaphthalen-2-yl]acetate (90a)

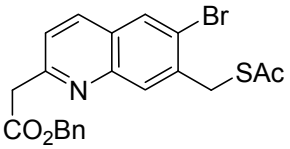


90a (8.9 g, 90%) was prepared from **87a** (10.0 g, 0.022 mole) by means of the general procedure described in **5.1.23.** (step-I) as a white solid. mp: 88-90 ^{o}C; Purity by HPLC: 94.20%.

^{1}HNMR (Acetone-*d6*) : *δ* 2.31 (s, 3H), 3.60 (s, 2H), 4.24 (s, 2H), 5.30 (s, 2H), 7.11-7.18 (m, 5H), 7.23 (d, *J* = 7.9 Hz, 1H), 7.30 (s, 1H), 7.34 (s, 1H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.89 (s, 1H)

ESI/MS (m/z) : 444.4 $(M+H)^{+}$.

5.1.26.2. Benzyl 2-[7-(acetylthiomethyl)-6-bromoquinolin-2-yl]acetate (90b)

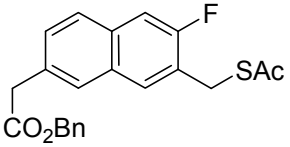


90b (8.4 g, 85%) was prepared from **87b** (10.0 g, 0.022 mole) by means of the general procedure described in **5.1.23.** (step-II) as a white solid. mp: 66-67 ^{o}C; Purity by HPLC: 93.55%.

^{1}HNMR (Acetone-*d6*) : *δ* 2.27 (s, 3H), 3.88 (s, 2H), 4.13 (s, 2H), 5.37 (s, 2H), 7.15 (d, *J* = 8.5 Hz, 1H), 7.16-7.21 (m, 5H), 7.73 (s, 1H), 7.88 (s, 1H), 8.01 (d, *J* = 7.7 Hz, 1H)

ESI/MS (m/z) : 445.5 $(M+H)^{+}$.

5.1.26.3. Benzyl 2-[7-(acetylthiomethyl)-6-fluoronaphthalen-2-yl]acetate (90c)

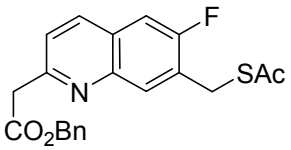


90c (8.69 g, 88%) was prepared from **87c** (10.0 g, 0.025 mole) by means of the general procedure described in **5.1.23.** (step-I) as a white solid. mp: 101-103 ^{o}C; Purity by HPLC: 95.55%.

^{1}HNMR (Acetone-*d6*) : *δ* 2.27 (s, 3H), 3.66 (s, 2H), 4.31 (s, 2H), 5.39 (s, 2H), 7.13 (d, *J* = 8.0 Hz, 1H), 7.17-7.19 (m, 5H), 7.31 (s, 1H), 7.41 (s, 1H), 7.45 (s, 1H), 7.69 (d, *J* = 7.7 Hz, 1H)

ESI/MS (m/z) : 383.6 $(M+H)^{+}$.

5.1.26.4. Benzyl 2-[7-(acetylthiomethyl)-6-fluoroquinolin-2-yl]acetate (90d)

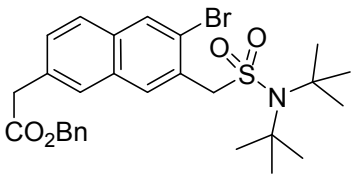


90d (8.49 g, 86%) was prepared from **87d** (10.0 g, 0.025 mole) by means of the general procedure described in **5.1.23.** (step-I) as a white solid. mp: 93-94 ^{o}C; Purity by HPLC: 97.10%.

^{1}HNMR (Acetone-*d6*) : *δ* 2.33 (s, 3H), 3.87 (s, 2H), 4.17 (s, 2H), 5.41 (s, 2H), 7.07 (d, *J* = 8.3 Hz, 1H), 7.17-7.20 (m, 5H), 7.36 (s, 1H), 7.80 (s, 1H), 7.97 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 384.8 $(M+H)^{+}$.

5.1.26.5. Benzyl *N*,*N*-ditertbutyl-2-(6-bromo-7-methanesulfonamide naphthalen-2-yl)acetate (91a)

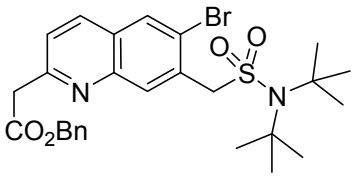


91a (6.67 g, 60%) was prepared from **90a** (8.8 g, 0.019 mole) by means of the general procedure described in **5.1.23.** (step-II) as a white solid. mp: 100-101 ^{o}C; Purity by HPLC: 96.66%.

^{1}HNMR (Acetone-*d6*) : δ 1.21 (s, 18H), 3.66 (s, 2H), 4.80 (s, 2H), 5.41 (s, 2H), 7.15-7.19 (m, 5H), 7.23 (d, *J* = 8.1 Hz, 1H), 7.33-7.37 (m, 2H), 7.63 (d, *J* = 7.2 Hz, 1H), 7.87 (s, 1H)

ESI/MS (m/z) : 561.6 $(M+H)^{+}$.

5.1.26.6. Benzyl *N*,*N*-ditertbutyl-2-(6-bromo-7-methanesulfonamide quinolin-2-yl)acetate (91b)

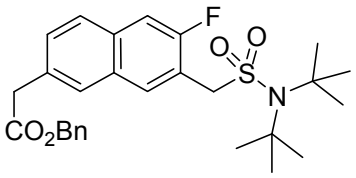


91b (7.02 g, 67%) was prepared from **90b** (8.3 g, 0.018 mole) by means of the general procedure described in **5.1.23.** (step-II) as a white solid. mp: 118-120 ^{o}C; Purity by HPLC: 95.15%.

^{1}HNMR (Acetone-*d6*) : δ 1.19 (s, 18H), 3.88 (s, 2H), 4.68 (s, 2H), 5.33 (s, 2H), 7.15-7.19 (m, 6H), 7.73 (s, 1H), 7.87 (s, 1H), 7.99 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 562.7 $(M+H)^{+}$.

5.1.26.7. Benzyl N,N-ditertbutyl-2-(6-fluoro-7-methanesulfonamide naphthalen-2-yl)acetate (91c)

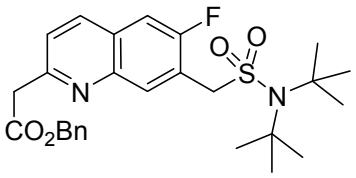


91c (7.99 g, 72%) was prepared from **90c** (8.5 g, 0.022 mole) by means of the general procedure described in **5.1.23.** (step-II) as a white solid. mp: 121-122 ^{o}C; Purity by HPLC: 94.10%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.13 (s, 18H), 3.62 (s, 2H), 4.77 (s, 2H), 5.39 (s, 2H), 7.14 (d, *J* = 8.0 Hz, 1H), 7.18-7.20 (m, 5H), 7.36 (s, 1H), 7.41-7.45 (m, 2H), 7.66 (d, *J* = 7.3 Hz, 1H)

ESI/MS (m/z) : 500.7 $(M+H)^{+}$.

5.1.26.8. Benzyl N,N-ditertbutyl-2-(6-fluoro-7-methanesulfonamide quinolin-2-yl)acetate (91d)

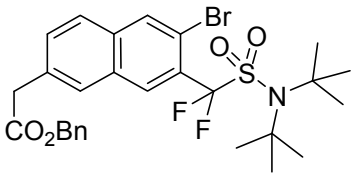


91d (7.15 g, 66%) was prepared from **90d** (8.3 g, 0.021 mole) by means of the general procedure described in **5.1.23.** (step-II) as a white solid. mp: 111-112 ^{o}C; Purity by HPLC: 94.90%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.17 (s, 18H), 3.82 (s, 2H), 4.67 (s, 2H), 5.37 (s, 2H), 7.09 (d, *J* = 7.8 Hz, 1H), 7.16-7.19 (m, 5H), 7.39 (s, 1H), 7.79 (s, 1H), 8.09 (d, *J* = 8.4 Hz, 1H)

ESI/MS (m/z) : 501.8 $(M+H)^{+}$.

5.1.26.9. Benzyl *N*,*N*-ditertbutyl-2-[6-bromo-7-(difluoromethane sulfonamide)naphthalen-2-yl]acetate (92a)

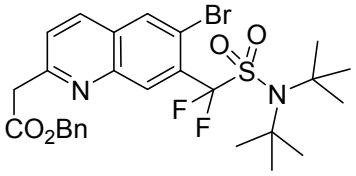


92a (4.0 g, 58%) was prepared from **91a** (6.5 g, 0.011 mole) by means of the general procedure described in **5.1.23.** (step-III) as a white solid. mp: 144-145 ^{o}C; Purity by HPLC: 96.90%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.23 (s, 18H), 3.59 (s, 2H), 5.39 (s, 2H), 5.79-5.81 (m, 2H), 7.15-7.19 (m, 5H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.33-7.39 (m, 2H), 7.66 (d, *J* = 7.9 Hz, 1H), 7.89 (s, 1H)

ESI/MS (m/z) : 597.7 $(M+H)^{+}$.

5.1.26.10. Benzyl N,N-ditertbutyl-2-[6-bromo-7-(difluoromethane sulfonamide)quinolin-2-yl]acetate (92b)

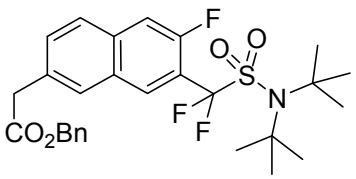


92b (4.55 g, 62%) was prepared from **91b** (6.9 g, 0.012 mole) by means of the general procedure described in **5.1.23.** (step-III) as a white solid. mp: 122-123 ^{o}C; Purity by HPLC: 93.10%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.09 (s, 18H), 3.81 (s, 2H), 5.39 (s, 2H), 5.80-5.83 (m, 2H), 7.15-7.18 (m, 6H), 7.75 (s, 1H), 7.89 (s, 1H), 8.01 (d, *J* = 8.2 Hz, 1H)

ESI/MS (m/z) : 598.8 $(M+H)^{+}$.

5.1.26.11. Benzyl N,N-ditertbutyl-2-[7-(difluoromethanesulfonamide)-6-fluoronaphthalen-2-yl]acetate (92c)

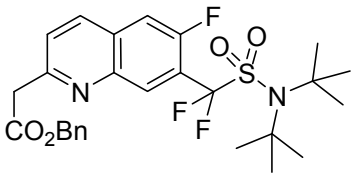


92c (5.26 g, 63%) was prepared from **91c** (7.8 g, 0.015 mole) by means of the general procedure described in **5.1.23.** (step-III) as a white solid. mp: 147-148 ^{o}C; Purity by HPLC: 94.55%.

^{1}HNMR (Acetone-*d6*) : δ 1.17 (s, 18H), 3.63 (s, 2H), 5.35 (s, 2H), 7.14-7.18 (m, 6H), 7.31 (s, 1H), 7.40 (s, 1H), 7.43 (s, 1H), 7.67 (d, *J* = 8.0 Hz, 1H), 7.89 (s, 1H)

ESI/MS (m/z) : 536.6 $(M+H)^{+}$.

5.1.26.12. Benzyl *N*,*N*-ditertbutyl-2-[7-(difluoromethanesulfonamide)-6-fluoroquinolin-2-yl]acetate (92d)

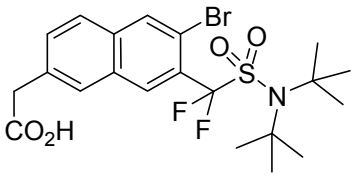


92d (4.95 g, 66%) was prepared from **91d** (7.0 g, 0.014 mole) by means of the general procedure described in **5.1.23.** (step-III) as a white solid. mp: 133-134 ^{o}C; Purity by HPLC: 92.70%.

^{1}HNMR (Acetone-*d6*) : δ 1.11 (s, 18H), 3.87 (s, 2H), 5.31 (s, 2H), 7.03 (d, *J* = 8.0 Hz, 1H), 7.16-7.19 (m, 5H), 7.35 (s, 1H), 7.77 (s, 1H), 7.97 (d, *J* = 8.1 Hz, 1H)

ESI/MS (m/z) : 537.7 $(M+H)^{+}$.

5.1.26.13. N,N-ditertbutyl-2-[6-bromo-7-(difluoromethanesulfonamide) Naphthalen-2-yl]acetic acid (65e)

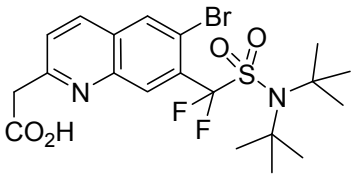


65e (2.25 g, 70%) was prepared from **92a** (3.8 g, 0.006 mole) by means of the general procedure described in **5.1.23.** (step-IV) as a white solid. mp: 122-123 ^{o}C; Purity by HPLC: 96.10%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.21 (s, 18H), 3.61 (s, 2H), 7.20 (d, *J* = 7.7 Hz, 1H), 7.33-7.35 (m, 2H), 7.63 (d, *J* = 7.9 Hz, 1H), 7.91 (s, 1H)

ESI/MS (m/z) : 507.7 $(M+H)^{+}$.

5.1.26.14. *N*,*N*-ditertbutyl-2-[6-bromo-7-(difluoromethanesulfonamide) naphthalen-2-yl]acetic acid (65f)

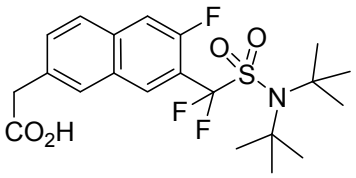


65f (2.94 g, 77%) was prepared from **92b** (4.5 g, 0.007 mole) by means of the general procedure described in **5.1.23.** (step-IV) as a white solid. mp: 141-142 ^{o}C; Purity by HPLC: 94.90%.

^{1}HNMR (Acetone-*d6*) : *δ* 1.19 (s, 18H), 3.88 (s, 2H), 7.16 (d, *J* = 7.9 Hz, 1H), 7.73 (s, 1H), 7.87 (s, 1H), 7.97 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 508.3 $(M+H)^{+}$.

5.1.26.15. N,N-ditertbutyl-2-[7-(difluoromethanesulfonamide)-6-fluoro naphthalen-2-yl]acetic acid (65g)

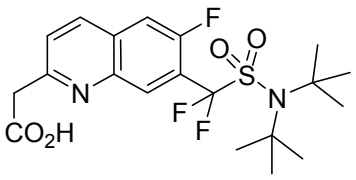


65g (3.24 g, 77%) was prepared from **92c** (5.0 g, 0.009 mole) by means of the general procedure described in **5.1.23.** (step-IV) as a white solid. mp: 137-139 ^{o}C; Purity by HPLC: 95.55%.

^{1}HNMR (Acetone-*d6*) : δ 1.29 (s, 18H), 3.59 (s, 2H), 7.14 (d, *J* = 7.9 Hz, 1H), 7.33 (s, 1H), 7.41-7.44 (m, 2H), 7.66 (d, *J* = 7.9 Hz, 1H)

ESI/MS (m/z) : 446.7 $(M+H)^+$.

5.1.26.16. N,N-ditertbutyl-2-[7-(difluoromethanesulfonamide)-6-fluoro quinolin-2-yl]acetic acid (65h)

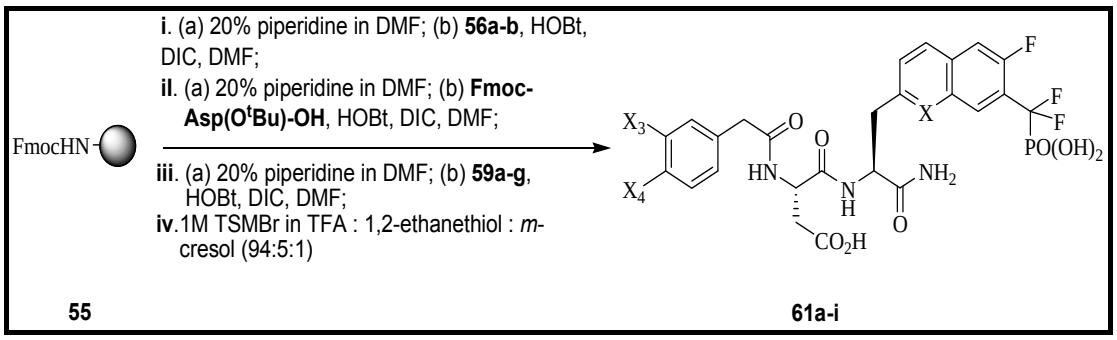


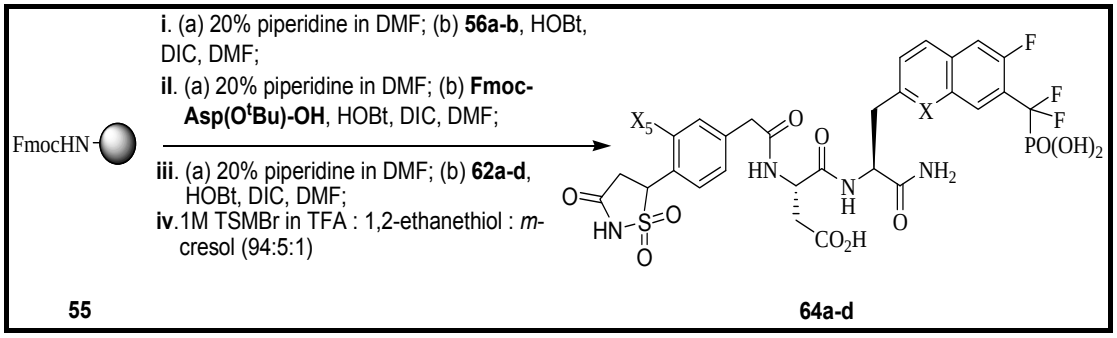
65h (2.71 g, 68%) was prepared from **92d** (5.0 g, 0.009 mole) by means of the general procedure described in **5.1.23.** (step-IV) as a white solid. mp: 121-122 ^oC; Purity by HPLC: 93.50%.

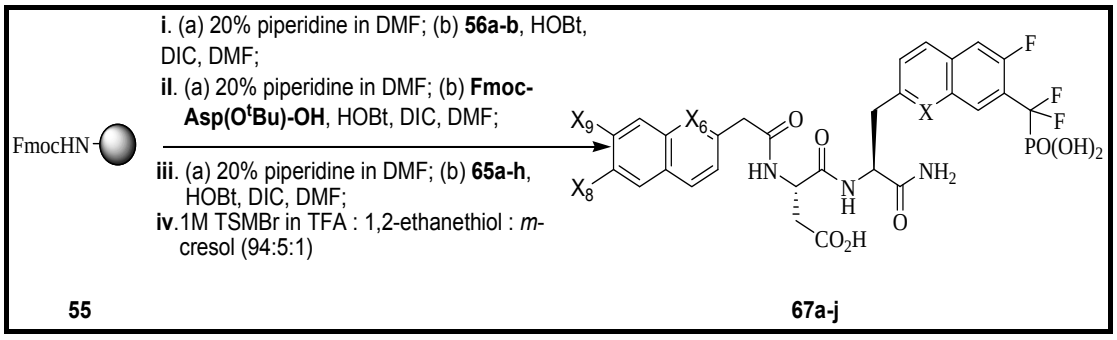
^{1}HNMR (Acetone-*d6*) : δ 1.20 (s, 18H), 3.82 (s, 2H), 7.09 (d, *J* = 7.8 Hz, 1H), 7.35 (s, 1H), 7.81 (s, 1H), 7.95 (d, *J* = 7.8 Hz, 1H)

ESI/MS (m/z) : 447.6 $(M+H)^+$.

5.1.27. General procedure for the synthesis of Compounds (61a-i, 64a-d, 67a-j)







Title compounds **61a-i, 64a-d & 67a-j** were prepared using following procedure by solid phase peptide synthesis (SPPS) protocol.

Fmoc protected Rink amide MBHA resin **55** (2.0 g, 0.58 mmol/g) was swollen in DMF for 30 min and washed with DMF (3 x 25 mL). Fmoc group of the resin was removed by agitating Fmoc-protected resin in 20% piperidine solution (25 mL, 1 x 5 min and 1 x 30 min) for the next coupling reaction. Fmoc-difluorophosphonate-substituted naphthyl/quinolinyl based unnatural amino acid **56a/b** was coupled to the deprotected resin by agitating their respective pre-activated solution under N_2 atmosphere. [i.e. Fmoc-unnatural amino acid (4 eq), HOBt (4 eq), and DIC (4 eq)] in DMF (5mL) for 30 min). After completion of the coupling reaction confirmed by Kaiser Ninhydrin and TNBS tests, peptidyl resin was washed with DMF, DCM and ether (3 x 25 mL each) and treated with 20% piperidine solution (25 mL, 1 x 5 min and 1 x 30 min) to remove Fmoc group. Deprotected peptidyl resin was then washed with DMF,

DCM and ether (3 x 25 mL) and swollen in DMF for 10 min for the coupling of Fmoc-Asp(O^tBu)-OH. After completion of the coupling reaction confirmed by Kaiser Ninhydrin and TNBS tests, peptidyl resin was washed with respective solvent and treated with 20% piperidine solution (25 mL, 1 x 5 min and 1 x 30 min) to remove Fmoc group. Di-peptidyl resin was washed with DMF, DCM and ether and swollen in DMF for 30 min for the coupling of substituted phenyl/naphthyl/quinolinyl acetic acid **59a-g**, **62a-d** & **65a-h**. Substituted acetic acids were incorporated to the respective di-peptidyl resin by agitating peptidyl resin in the preactivated coupling solution of respective acetic acid under N_2 atm. over a period of 2-4 hrs. Complection of coupling was confirmed by Kaiser Ninhydrin and TNBS tests, whenever coupling was found incomplete one more coupling cycle was performed. Then, the fully protected resin was washed with DMF, DCM and ether (3 x 25 mL) and dried under vacuum for the globle cleavage to get the desired peptidomimetics.

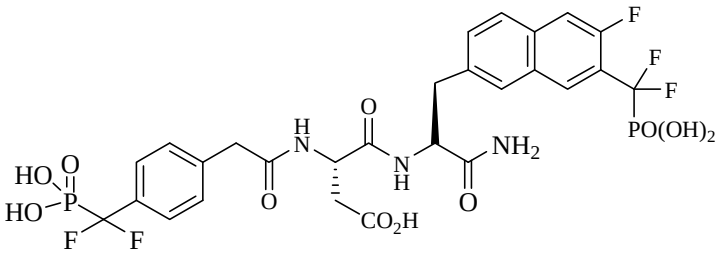
Cleavage:

The desired peptidomimetics were cleaved and deprotected from their respective peptidyl-resin by treatment wit TFA cleavage mixture as follows. A solution of TFA / bromotrimethylsilane / 1,2-ethanethiol / m-cresol (93: 1: 5: 1) (20 mL / 100 mg of peptidyl resin) was added to peptidyl-resin and the mixture was kept at 0 ^oC for 5 hrs and then at RT for 16 hrs with occasional shaking. The resin was filtered, washed with a cleavage mixture and the combined filterate was evapourated to dryness. Residue obtained was triturated with ether (20 mL) to yield crude compounds, typically in quantitative yield (Ca 200-250 mg).

Purification:

The reverse-phase prep-HPLC was carried out on a Shimadzu LC-8A liquid chromatography. A solution of crude compounds dissolved in water: ACN (1:1, 5 mL) or methanol (5mL) was injected into a semi-prep column (Luna 10 µ; C_{18}; 210-220 nm), dimension 250 x 21.2 mm and eluted with a linear gradient of ACN: water, both buffered wit 0.1% TFA, using a flow rate of 15 mL/min, with effluent monitoring by PDA detector at 220 nm. A typical gradient of 20% to 65% of water-ACN mixture, buffered with 0.1% TFA was used, over a period of 100 minutes, with 1% gradient change per minute. The desired products were collected in a single 50-80 mL fraction. The desired fractions were pooled, frozen and lypholized afforded the desired compounds as white solids.

5.1.27.1. (S)-4-[{R}-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoro naphalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(4-(difluoro(phosphono) methyl)phenyl)acetamido)-4-oxobutanoic acid (61a)



61a (70 mg, 67%) was prepared from **55** (250 mg, Loading capacity: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 157-158 ^{o}C; Purity by HPLC: 98.89%.

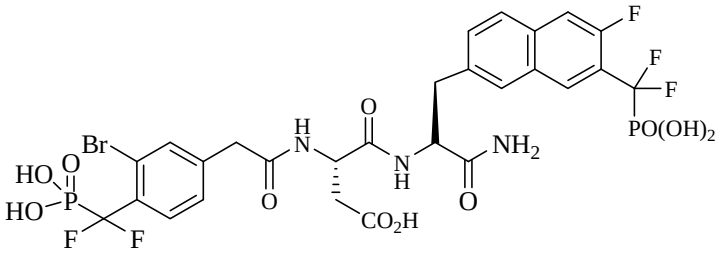
^{1}HNMR (D_2O) : δ 2.61 (dd, J = 7.9 Hz, 17.1 Hz, 1H), 2.89 (dd, J = 6.1 Hz, 16.9 Hz, 1H), 3.27 (dd, J = 8.9 Hz, 14.3 Hz, 1H), 3.39 (dd, J = 5.8 Hz, 14.1

Hz, 1H), 3.68 (s, 2H), 4.85 (m, 1H), 4.93 (m, 1H), 7.09-7.11 (m, 4H), 7.14 (d, *J* = 8.9 Hz, 1H), 7.31 (s, 1H), 7.39 (s, 1H), 7.47 (s, 1H), 7.99 (d, *J* = 8.0 Hz, 1H)

^{13}CNMR (Acetone-*d6*) : *δ* 35.4, 37.8, 43.1, 53.4, 59.8, 109.8, 125.6, 126.2, 127.8, 128.2, 128.8, 130.0, 130.4, 134.2, 135.2, 136.4, 139.1, 145.9, 161.2, 170.3, 171.4, 173.2, 174.8

ESI/MS (m/z) : 726.4 $(M+H)^+$.

5.1.27.2. (S)-4-[{R}-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoronaphalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(3-bromo-4-(difluoro (phosphono)methylphenyl)acetamido)-4-oxobutanoic acid (61b)



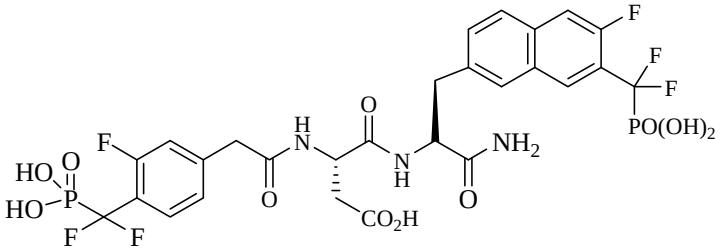
61b (70 mg, 60%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 144-145 ^oC; Purity by HPLC: 97.70%.

^{1}HNMR (D_2O) : *δ* 2.67 (dd, *J* = 7.8 Hz, 17.0 Hz, 1H), 2.87 (dd, *J* = 6.5 Hz, 17.2 Hz, 1H), 3.29 (dd, *J* = 8.7 Hz, 14.5 Hz, 1H), 3.53 (dd, *J* = 6.0 Hz, 14.3 Hz, 1H), 3.57 (s, 2H), 4.88 (m, 1H), 4.92 (m, 1H), 7.02 (d, *J* = 8.2 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 7.26 (s, 1H), 7.30 (s, 1H), 7.40 (s, 1H), 7.47 (s, 1H), 7.94 (d, *J* = 8.2 Hz, 1H)

^{13}CNMR (Acetone-*d6*) : *δ* 35.2, 36.2, 37.5, 53.7, 59.5, 109.6, 125.4, 126.4, 127.5, 128.8, 130.1, 130.5, 132.1, 134.9, 136.2, 137.4, 138.1, 144.2, 161.7, 170.1, 171.6, 173.0, 174.9

ESI/MS (m/z) : 805.4 $(M+H)^+$.

5.1.27.3. (S)-4-[{R}-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoro naphalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(4-(difluoro(phosphono) methyl)-3-fluorophenyl)acetamido)-4-oxobutanoic acid (61c)



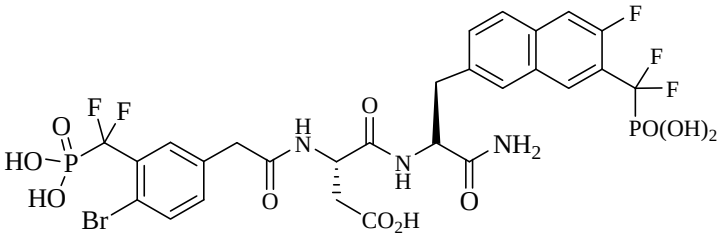
61c (75 mg, 70%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 121-122 ^{o}C; Purity by HPLC: 97.10%.

^{1}HNMR (D_2O) : δ 2.59 (dd, *J* = 7.1 Hz, 17.8 Hz, 1H), 2.77 (dd, *J* = 6.3 Hz, 17.4 Hz, 1H), 3.21 (dd, *J* = 8.5 Hz, 14.6 Hz, 1H), 3.57 (dd, *J* = 6.1 Hz, 15.3 Hz, 1H), 3.69 (s, 2H), 4.87 (m, 1H), 4.96 (m, 1H), 6.67 (s, 1H), 6.89 (d, *J* = 7.9 Hz, 1H), 7.10 (d, *J* = 8.1 Hz, 1H), 7.11 (d, *J* = 8.0Hz, 1H), 7.29 (s, 1H), 7.33 (s, 1H), 7.41 (s, 1H), 7.45 (s, 1H), 7.99 (d, *J* = 8.1 Hz, 1H)

^{13}CNMR (Acetone-*d6*) : δ 34.2, 38.2, 42.5, 53.3, 59.0, 109.7, 117.1, 122.8, 125.6, 126.2, 127.4, 128.7, 129.7, 130.5, 134.1, 136.3, 137.7, 138.8, 139.5, 160.7, 170.3, 171.5, 173.2, 175.7

ESI/MS (m/z) : 744.4 $(M+H)^{+}$.

5.1.27.4. (S)-4-[{R}-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoronaphalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(4-bromo-3-(difluoro(phosphono)methyl)phenyl)acetamido)-4-oxobutanoic acid (61d)



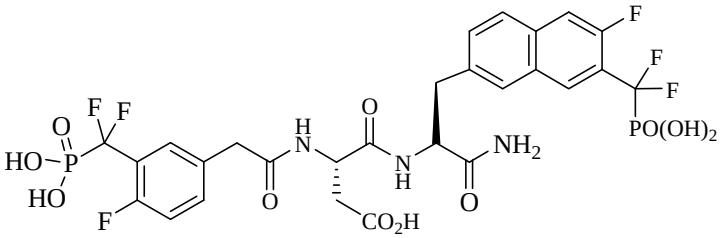
61d (77 mg, 67%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 143-144 ^{o}C; Purity by HPLC: 99.20%.

^{1}HNMR (D_2O) : δ 2.59 (dd, *J* = 7.9 Hz, 17.2 Hz, 1H), 2.92 (dd, *J* = 6.0 Hz, 17.0 Hz, 1H), 3.21 (dd, *J* = 8.9 Hz, 14.7 Hz, 1H), 3.51 (dd, *J* = 6.1 Hz, 14.6 Hz, 1H), 3.55 (s, 2H), 4.86 (m, 1H), 4.90 (m, 1H), 6.94 (d, *J* = 8.0 Hz, 1H), 6.99 (s, 1H), 7.13 (d, *J* = 8.1 Hz, 1H), 7.32 (s, 1H), 7.37 (s, 1H), 7.42 (s, 1H), 7.46 (s, 1H), 7.92 (d, *J* = 8.2 Hz, 1H)

^{13}CNMR (Acetone-*d6*) : δ 35.0, 37.2, 43.4, 53.3, 59.0, 109.9, 121.5, 125.2, 126.2, 127.4, 128.6, 131.0, 132.5, 134.1, 136.0, 139.2, 140.8, 142.8, 161.2, 170.3, 171.4, 173.2, 175.0

ESI/MS (m/z) : 805.6 $(M+H)^{+}$.

5.1.27.5. (S)-4-[{R}-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoronaphalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(3-(difluoro (phosphono)methyl)-4-fluorophenyl)acetamido)-4-oxobutanoic acid (61e)



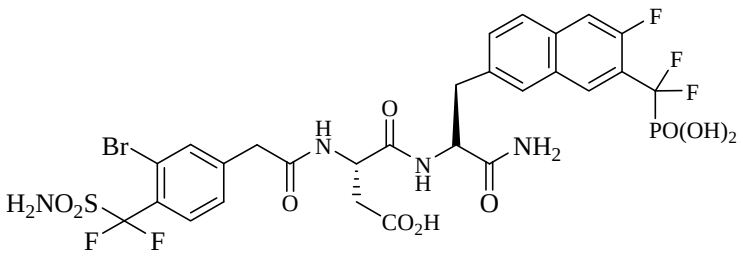
61e (66 mg, 62%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 121-122 ^{o}C; Purity by HPLC: 98.80%.

^{1}HNMR (D_2O) : δ 2.67 (dd, *J* = 7.0 Hz, 17.8 Hz, 1H), 2.87 (dd, *J* = 6.0 Hz, 17.0 Hz, 1H), 3.31 (dd, *J* = 8.2 Hz, 14.5 Hz, 1H), 3.54 (dd, *J* = 6.0 Hz, 15.9 Hz, 1H), 3.73 (s, 2H), 4.86 (m, 1H), 4.92 (m, 1H), 7.01 (d, *J* = 8.1 Hz, 1H), 7.10 (s, 1H), 7.13 (d, *J* = 7.9 Hz, 1H), 7.18 (d, *J* = 8.4 Hz, 1H), 7.33 (s, 1H), 7.39 (s, 1H), 7.47 (s, 1H), 8.03 (d, *J* = 7.7 Hz, 1H)

^{13}CNMR (Acetone-*d6*) : δ 34.9, 37.7, 43.5, 53.7, 59.7, 109.2, 116.4, 124.4, 125.4, 126.0, 127.5, 128.8, 130.2, 131.4, 131.7, 134.0, 136.2, 139.1, 159.2, 161.7, 170.0, 171.7, 173.4, 174.9

ESI/MS (m/z) : 744.4 $(M+H)^+$.

5.1.27.6. (S)-4-[{R}-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoronaphalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(3-bromo-4-(difluoro(sulfonamide)methyl)phenyl)acetamido)-4-oxobutanoic acid (61f)



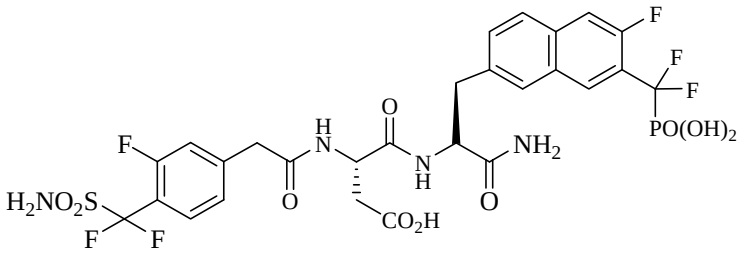
61f (81 mg, 70%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 162-163 ^oC; Purity by HPLC: 98.90%.

^{1}HNMR (D_2O) : δ 2.57 (dd, *J* = 8.4 Hz, 16.9 Hz, 1H), 2.98 (dd, *J* = 7.0 Hz, 17.0 Hz, 1H), 3.33 (dd, *J* = 8.9 Hz, 15.5 Hz, 1H), 3.61 (dd, *J* = 6.1 Hz, 15.3 Hz, 1H), 3.55 (s, 2H), 4.81 (m, 1H), 4.89 (m, 1H), 7.00 (d, *J* = 8.7 Hz, 1H), 7.05 (d, *J* = 7.0 Hz, 1H), 7.14 (d, *J* = 8.2 Hz, 1H), 7.29 (s, 1H), 7.35 (s, 1H), 7.42 (s, 1H), 7.50 (s, 1H), 7.99 (d, *J* = 8.0 Hz, 1H)

^{13}CNMR (Acetone-*d6*) : δ 34.1, 37.1, 42.2, 53.3, 59.9, 109.0, 123.2, 125.0, 126.1, 127.4, 128.4, 129.0, 130.3, 130.7, 134.1, 134.9, 136.0, 138.3, 139.5, 161.9, 169.9, 171.9, 172.0, 175.9

ESI/MS (m/z) : 804.4 $(M+H)^+$.

5.1.27.7. (S)-4-[{R}-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoronaphalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(4-(difluoro (sulfonamide)methyl)-3-fluorophenyl)acetamido)-4-oxobutanoic acid (61g)



61g (82 mg, 77%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 152-153 ^{o}C; Purity by HPLC: 99.50%.

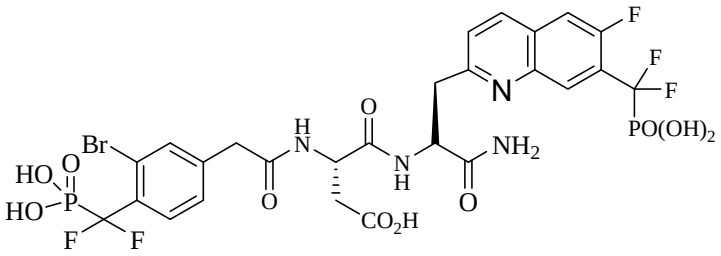
^{1}HNMR (D_2O) : δ 2.70 (dd, *J* = 7.9 Hz, 16.9 Hz, 1H), 2.97 (dd, *J* = 7.8 Hz, 17.9 Hz, 1H), 3.33 (dd, *J* = 8.3 Hz, 15.0 Hz, 1H), 3.43 (dd, *J* = 5.8 Hz, 15.0 Hz, 1H), 3.49 (s, 2H), 4.80 (m, 1H), 4.96 (m, 1H), 7.13-7.21 (m, 2H), 7.33-7.36 (m, 3H), 7.40-7.44 (m, 2H), 7.89-7.96 (m, 2H), 8.18 (s, 1H)

^{13}CNMR (Acetone-*d6*) : δ 34.9, 37.5, 42.9, 53.6, 59.6, 109.7, 117.1, 122.8, 125.1, 125.6, 126.2, 127.5, 128.0, 129.7, 130.5, 134.0, 136.2, 137.7, 139.1, 160.8, 161.7, 165.3, 170.9, 171.8, 173.0, 175.5

ESI/MS (m/z) : 743.7 $(M+H)^+$.

5.1.27.8. (S)-4-[(R)-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoroquinolin-2-yl)-1-oxopropan-2-ylamino)-3-(2-(3-bromo-4-

(difluoro(phosphono)methyl)phenyl)acetamido)-4-oxobutanoic acid (61h)



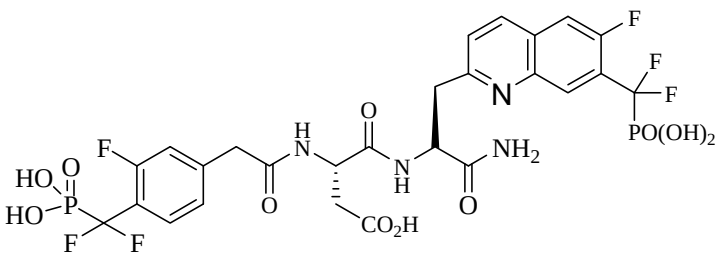
61h (92 mg, 80%) was prepared from **55** (250 mg, Loading capacity: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 132-133 ^{o}C; Purity by HPLC: 97.89%.

^{1}HNMR (D_2O) : δ 2.60 (dd, *J* = 7.0 Hz, 17.2 Hz, 1H), 2.90 (dd, *J* = 6.6 Hz, 17.6 Hz, 1H), 3.25 (dd, *J* = 8.8 Hz, 15.5 Hz, 1H), 3.37 (s, 2H), 3.59 (dd, *J* = 6.3 Hz, 14.9 Hz, 1H), 4.81 (m, 1H), 4.87 (m, 1H), 7.00-7.10 (m, 3H), 7.27 (s, 1H), 7.33 (s, 1H), 7.37 (s, 1H), 7.60 (s, 1H)

^{13}CNMR (Acetone-*d6*) : δ 34.4, 35.2, 42.5, 53.6, 59.9, 108.6, 122.1, 123.3, 128.7, 129.0, 130.3, 130.6, 134.5, 138.3, 138.8, 139.2, 141.8, 143.2, 145.3, 161.7, 162.4, 170.3, 171.6, 173.2, 175.2

ESI/MS (m/z) : 806.6 $(M+H)^{+}$.

5.1.27.9. (S)-4-[(R)-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoroquinolin-2-yl)-1-oxopropan-2-ylamino)-3-(2-(4-(difluoro (phosphono)methyl)-3-fluorophenyl)acetamido)-4-oxobutanoic acid (61i)



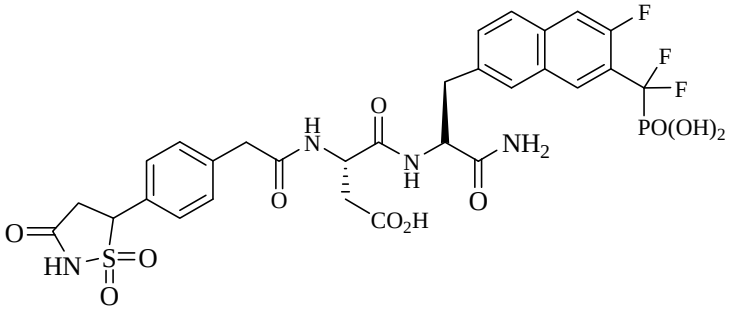
61i (85 mg, 79%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 159-160 ^{o}C; Purity by HPLC: 98.00%.

^{1}HNMR (D_2O) : δ 2.67 (dd, *J* = 6.9 Hz, 17.0 Hz, 1H), 2.93 (dd, *J* = 6.7 Hz, 17.7 Hz, 1H), 3.23 (dd, *J* = 8.0 Hz, 16.1 Hz, 1H), 3.41 (s, 2H), 3.50 (dd, *J* = 6.7 Hz, 14.0 Hz, 1H), 4.84 (m, 1H), 4.90 (m, 1H), 6.67 (s, 1H), 6.88-7.11 (m, 3H), 7.34 (s, 1H), 7.58 (s, 1H), 8.39 (d, *J* = 8.2 Hz, 1H)

^{13}CNMR (Acetone-*d6*) : δ 34.9, 35.4, 42.9, 53.3, 59.5, 108.8, 117.7, 122.0, 125.4, 128.1, 129.7, 130.8, 134.1, 137.7, 138.0, 138.8, 143.4, 145.2, 161.6, 162.7, 170.7, 172.0, 173.0, 174.8

ESI/MS (m/z) : 745.6 $(M+H)^{+}$.

5.1.27.10. (S)-4-[(R)-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoro naphthalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(4-(1,1-dioxide-3-oxoiso thiazolidin-5-yl)]phenyl)acetamido)-4-oxobutanoic acid (64a)



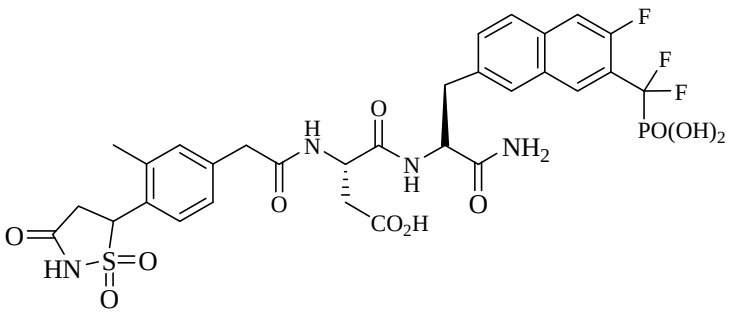
64a (63 mg, 60%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 173-174 ^{o}C; Purity by HPLC: 96.90%.

^{1}HNMR (D_2O) : δ 2.49 (dd, *J* = 6.8 Hz, 15.8 Hz, 1H), 2.88 (dd, *J* = 6.2 Hz, 16.7 Hz, 1H), 2.98 (dd, *J* = 4.3 Hz, 13.8 Hz, 1H), 3.17 (dd, *J* = 8.1 Hz, 17.2 Hz, 1H), 3.31 (dd, *J* = 7.1 Hz, 16.8 Hz, 1H), 3.37 (s, 2H) 3.54 (dd, *J* = 6.1 Hz, 15.4 Hz, 1H), 4.80 (m, 1H), 4.91 (m, 1H), 5.11 (t, *J* = 9.6 Hz, 1H), 7.11 (d, *J* = 7.8 Hz, 1H), 7.16-7.19 (m, 4H), 7.33 (s, 1H), 7.43 (s, 1H), 7.48 (s, 1H), 7.99 (d, *J* = 7.0 Hz, 1H)

^{13}CNMR (Acetone-*d6*) : δ 34.2, 37.3, 42.6, 58.1, 62.3, 109.7, 125.4, 126.0, 128.2, 128.8, 129.6, 130.5, 132.1, 132.8, 134.0, 136.3, 139.6, 160.5, 170.0, 170.2, 172.9, 174.5, 178.9

ESI/MS (m/z) : 729.7 $(M+H)^+$.

5.1.27.11. (S)-4-[(R)-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoro nappthalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(3-methyl-4-(1,1-dioxide-3-oxoisothiazolidin-5-yl)]phenyl)acetamido)-4-oxobutanoic acid (64b)



64b (74 mg, 60%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 155-156 ^oC; Purity by HPLC: 96.00%.

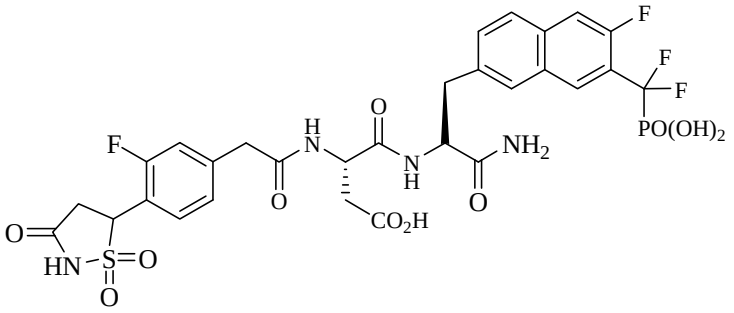
^{1}HNMR (D_2O) : δ 2.31 (s, 3H), 2.41 (dd, *J* = 6.9 Hz, 15.0 Hz, 1H), 2.87 (dd, *J* = 6.1 Hz, 16.9 Hz, 1H), 2.95 (dd, *J* = 5.3 Hz, 14.8 Hz, 1H), 3.19 (dd, *J* = 8.0 Hz, 17.4 Hz, 1H), 3.35 (dd, *J* = 7.0 Hz, 16.0 Hz, 1H), 3.39 (s, 2H) 3.57 (dd, *J* = 5.1 Hz, 14.4 Hz, 1H), 4.78 (m, 1H), 4.90 (m, 1H), 5.15 (t, *J* = 9.8 Hz, 1H), 6.99

(d, *J* = 7.9 Hz, 1H), 7.05-7.09 (m, 2H), 7.17 (d, *J* = 8.0 Hz, 1H), 7.29 (s, 1H), 7.40 (s, 1H), 7.44 (s, 1H), 7.98 (d, *J* = 7.0 Hz, 1H)

^{13}CNMR (Acetone-*d6*) : *δ* 19.7, 35.0, 37.4, 43.6, 59.6, 109.2, 125.0, 126.5, 128.1, 128.8, 130.6, 131.4, 132.7, 136.0, 139.2, 139.8, 161.5, 170.1, 172.3, 173.3, 174.4, 177.8

ESI/MS (m/z) : 743.4 $(M+H)^+$.

5.1.27.12. (S)-4-[(R)-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoro nappthalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(3-fluoro-4-(1,1-dioxide-3-oxoisothiazolidin-5-yl)]phenyl)acetamido)-4-oxobutanoic acid (64c)



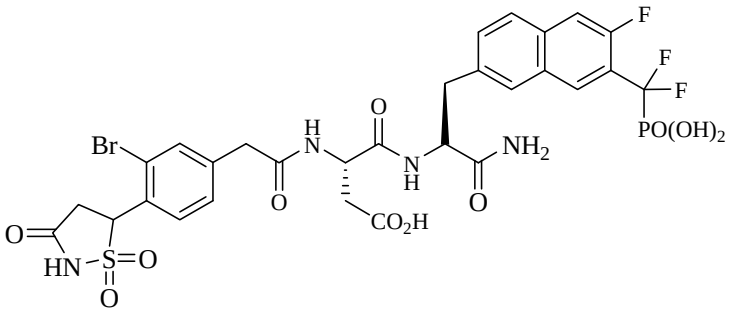
64c (65 mg, 60%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 133-134 ^oC; Purity by HPLC: 97.58%.

^{1}HNMR (D_2O) : *δ* 2.67 (dd, *J* = 6.9 Hz, 16.5 Hz, 1H), 2.91 (dd, *J* = 6.5 Hz, 16.5 Hz, 1H), 2.98 (dd, *J* = 6.0 Hz, 15.3 Hz, 1H), 3.17 (dd, *J* = 7.0 Hz, 16.9 Hz, 1H), 3.35 (dd, *J* = 7.0 Hz, 16.9 Hz, 1H), 3.43 (s, 2H) 3.55 (dd, *J* = 5.9 Hz, 15.0 Hz, 1H), 4.86 (m, 1H), 4.99 (m, 1H), 5.23 (t, *J* = 8.4 Hz, 1H), 6.77 (s, 1H), 6.99 (d, *J* = 7.9 Hz, 1H), 7.10-7.15 (m, 2H), 7.33-7.44 (m, 3H), 7.96 (d, *J* = 8.0 Hz, 1H)

^{13}CNMR (Acetone-*d6*) : δ 34.2, 37.1, 37.5, 42.6, 53.2, 55.5, 59.5, 108.0, 125.1, 126.1, 127.9, 129.8, 130.6, 134.1, 134.7, 136.2, 139.2, 160.4, 161.9, 170.3, 171.1, 173.2, 175.9, 178.0

ESI/MS (m/z) : 747.9 $(M+H)^+$.

5.1.27.13. (S)-4-[(R)-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoro napthalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(3-bromo-4-(1,1-dioxide-3-oxoisothiazolidin-5-yl)]phenyl)acetamido)-4-oxobutanoic acid (64d)



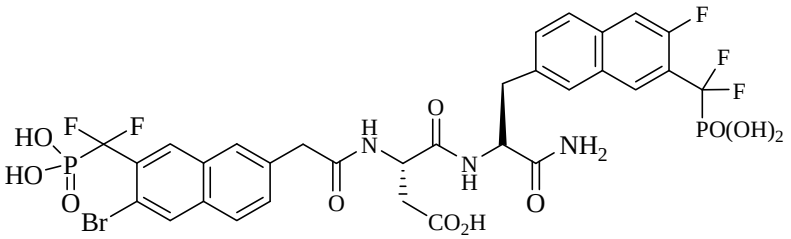
64d (80 mg, 69%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 149-150 ^oC; Purity by HPLC: 98.70%.

^{1}HNMR (D_2O) : δ 2.63 (dd, *J* = 6.8 Hz, 15.5 Hz, 1H), 2.90 (dd, *J* = 6.6 Hz, 16.6 Hz, 1H), 2.93 (dd, *J* = 5.9 Hz, 14.9 Hz, 1H), 3.29 (dd, *J* = 8.0 Hz, 17.5 Hz, 1H), 3.33 (dd, *J* = 7.1 Hz, 16.2 Hz, 1H), 3.41 (s, 2H) 3.54 (dd, *J* = 5.5 Hz, 14.9 Hz, 1H), 4.82 (m, 1H), 4.92 (m, 1H), 5.19 (t, *J* = 9.4 Hz, 1H), 7.06 (d, *J* = 7.8 Hz, 1H), 7.10 (d, *J* = 7.9 Hz, 1H), 7.15 (d, *J* = 8.2 Hz, 1H), 7.29-7.34 (m, 2H), 7.42 (s, 1H), 7.47 (s, 1H), 8.01 (d, *J* = 7.9 Hz, 1H)

^{13}CNMR (Acetone-*d6*) : δ 35.2, 36.4, 37.5, 41.6, 58.8, 59.5, 109.0, 123.3, 125.2, 126.3, 128.0, 128.5, 130.4, 134.3, 135.7, 136.0, 139.0, 142.4, 161.7, 170.6, 172.0, 173.1, 174.9, 178.8

ESI/MS (m/z) : 808.8 $(M+H)^+$.

5.1.27.14. (S)-4-[(R)-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoro nappthalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(6-bromo-7-(difluoro (phoshono)methyl)naphthalen-2-yl)acetamido)-4-oxobutanoic acid (67a)



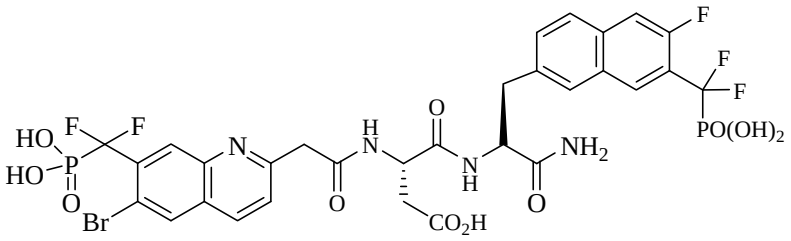
67a (75 mg, 61%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 161-162 ^{o}C; Purity by HPLC: 98.10%.

^{1}HNMR (D_2O) : δ = 2.60 (dd, *J* = 8.3 Hz, 17.2 Hz, 1H), 2.90 (dd, *J* = 7.1 Hz, 17.2 Hz, 1H), 3.27 (dd, *J* = 8.8 Hz, 15.1 Hz, 1H), 3.55 (dd, *J* = 6.0 Hz, 15.7 Hz, 1H), 3.59 (s, 2H), 4.79 (m, 1H), 4.92 (m, 1H), 7.11 (d, *J* = 8.0 Hz, 1H), 7.23 (d, *J* = 8.3 Hz, 1H), 7.30-7.38 (m, 3H), 7.40 (s, 1H), 7.47 (s, 1H), 7.93-7.97 (m, 2H), 8.19 (d, *J* = 8.7 Hz, 1H)

^{13}CNMR (Acetone-*d6*) : δ 33.9, 37.0, 43.3, 53.9, 59.9, 109.1, 118.8, 125.0, 125.7, 125.9, 126.0, 127.2, 127.9, 128.2, 129.6, 130.2, 134.3, 135.4, 136.1, 137.8, 138.3, 139.3, 142.5, 161.8, 170.3, 171.5, 173.2, 174.9

ESI/MS (m/z) : 855.7 $(M+H)^{+}$.

5.1.27.15. (S)-4-[(R)-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoro napthalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(6-bromo-7-(difluoro (phoshono)methyl)quinolin-2-yl)acetamido)-4-oxobutanoic acid (67b)



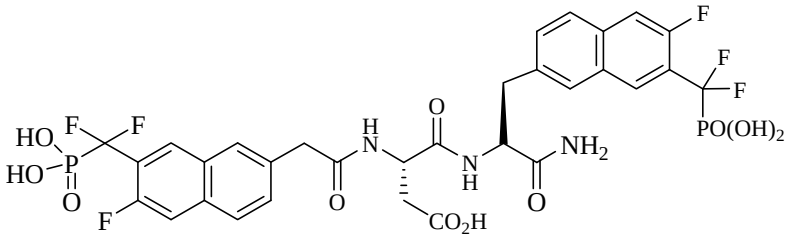
67b (75 mg, 61%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 137-138 ^{o}C; Purity by HPLC: 98.50%.

^{1}HNMR (D_2O) : δ = 2.47 (dd, *J* = 7.3 Hz, 17.9 Hz, 1H), 2.91 (dd, *J* = 7.0 Hz, 17.0 Hz, 1H), 3.23 (dd, *J* = 8.2 Hz, 15.6 Hz, 1H), 3.59 (dd, *J* = 6.1 Hz, 14.7 Hz, 1H), 3.77 (s, 2H), 4.87 (m, 1H), 4.90 (m, 1H), 7.13-7.17 (m, 2H), 7.31 (s, 1H), 7.41 (s, 1H), 7.44 (s, 1H), 7.57 (s, 1H), 7.99 (d, *J* = 8.8 Hz, 1H), 8.30 (s, 1H), 8.39 (d, *J* = 8.1 Hz, 1H)

^{13}CNMR (Acetone-*d6*) : δ 34.9, 36.0, 44.3, 55.5, 60.9, 109.7, 121.0, 121.7, 125.4, 126.2, 127.9, 128.8, 129.7, 130.5, 134.0, 136.2, 139.3, 141.4, 141.8, 148.9, 159.3, 161.0, 170.0, 171.8, 173.0, 175.8

ESI/MS (m/z) : 856.6 $(M+H)^{+}$.

5.1.27.16. (S)-4-[(R)-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoro napthalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(7-(difluoro(phoshono) methyl)-6-fluoronaphthalen-2-yl)acetamido)-4-oxobutanoic acid (67c)



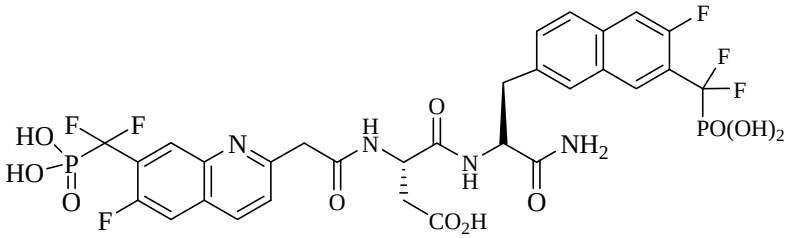
67c (80 mg, 61%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 169-170 ^{o}C; Purity by HPLC: 94.34%.

^{1}HNMR (D_2O) : δ = = 2.59 (dd, *J* = 7.3 Hz, 17.0 Hz, 1H), 2.89 (dd, *J* = 6.1 Hz, 16.2 Hz, 1H), 3.31 (dd, *J* = 8.1 Hz, 15.0 Hz, 1H), 3.57 (dd, *J* = 6.1 Hz, 15.3 Hz, 1H), 3.61 (s, 2H), 4.88 (m, 1H), 4.96 (m, 1H), 7.14-7.17 (m, 2H), 7.29-7.33 (m, 2H), 7.40-7.48 (m, 4H), 7.89-7.97 (m, 2H)

^{13}CNMR (Acetone-*d6*) : δ 36.9, 39.0, 43.9, 54.8, 60.2, 109.1, 109.5, 125.2, 125.9, 126.9, 127.5, 128.0, 128.8, 130.1, 134.2, 136.4, 139.2, 161.7, 170.6, 171.6, 173.7, 174.0

ESI/MS (m/z) : 794.9 $(M+H)^+$.

5.1.27.17. (S)-4-[(R)-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoro napthalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(7-(difluoro(phoshono) methyl)-6-fluoroquinolin-2-yl)acetamido)-4-oxobutanoic acid (67d)



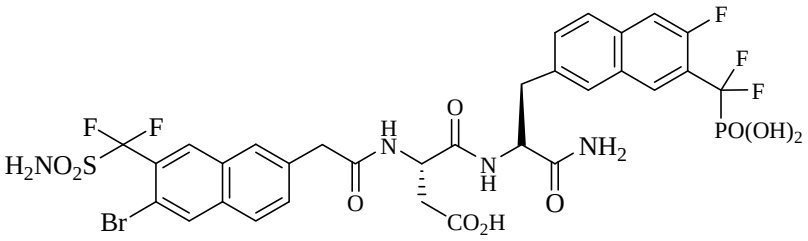
67d (70 mg, 61%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 155-156 ^oC; Purity by HPLC: 95.47%.

^{1}HNMR (D_2O) : δ = 2.38 (dd, *J* = 7.1 Hz, 17.1 Hz, 1H), 2.88 (dd, *J* = 6.0 Hz, 15.1 Hz, 1H), 3.29 (dd, *J* = 7.2 Hz, 16.1 Hz, 1H), 3.54 (dd, *J* = 6.3 Hz, 14.4 Hz, 1H), 3.75 (s, 2H), 4.88 (m, 1H), 4.91 (m, 1H), 7.09 (d, *J* = 8.1 Hz, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 7.32-7.34 (m, 2H), 7.39 (s, 1H), 7.47 (s, 1H), 7.61 (s, 1H), 7.95 (d, *J* = 8.4 Hz, 1H), 8.30 (d, *J* = 8.0 Hz, 1H)

^{13}CNMR (Acetone-*d6*) : δ 35.2, 37.0, 42.3, 53.5, 59.9, 108.6, 109.5, 122.0, 125.2, 126.4, 128.7, 129.9, 130.6, 134.1, 134.5, 136.2, 138.8, 139.2, 143.4, 145.5, 158.4, 162.1, 170.3, 171.6, 173.2, 175.9

ESI/MS (m/z) : 795.6 $(M+H)^+$.

5.1.27.18. (S)-4-[(R)-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoro napthalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(6-bromo-7-(difluoro (slufonamide)methyl)naphthalen-2-yl)acetamido)-4-oxobutanoic acid (67e)



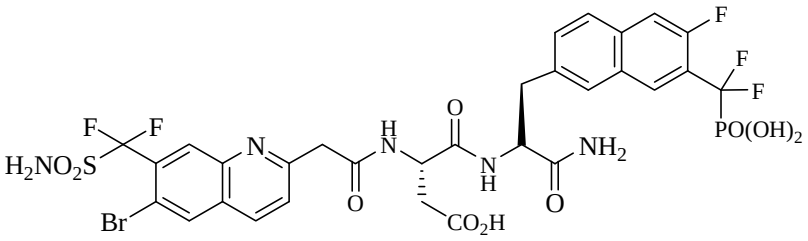
67e (95 mg, 77%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 141-142 ^oC; Purity by HPLC: 98.00%.

^{1}HNMR (D_2O) : δ = 2.67 (dd, *J* = 7.6 Hz, 17.5 Hz, 1H), 2.92 (dd, *J* = 5.1 Hz, 16.8 Hz, 1H), 3.29 (dd, *J* = 7.1 Hz, 15.5 Hz, 1H), 3.54 (dd, *J* = 6.0 Hz, 15.5 Hz, 1H), 3.60 (s, 2H), 4.89 (m, 1H), 4.92 (m, 1H), 7.14 (d, *J* = 7.7 Hz, 1H), 7.23 (d, *J* = 7.9 Hz, 1H), 7.33-7.39 (m, 3H), 7.42 (s, 1H), 7.47 (s, 1H), 7.91-7.96 (m, 2H), 8.17 (s, 1H)

^{13}CNMR (Acetone-*d6*) : δ 36.0, 37.8, 43.1, 54.3, 60.5, 109.0, 118.5, 125.1, 126.7, 127.4, 128.6, 129.6, 130.5, 134.4, 135.2, 136.1, 137.8, 138.1, 139.1, 161.4, 168.8, 170.5, 171.2, 173.2, 174.9

ESI/MS (m/z) : 854.5 $(M+H)^+$.

5.1.27.19. (S)-4-[(R)-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoro napthalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(6-bromo-7-(difluoro (sulfonamide)methyl)quinolin-2-yl)acetamido)-4-oxobutanoic acid (67f)



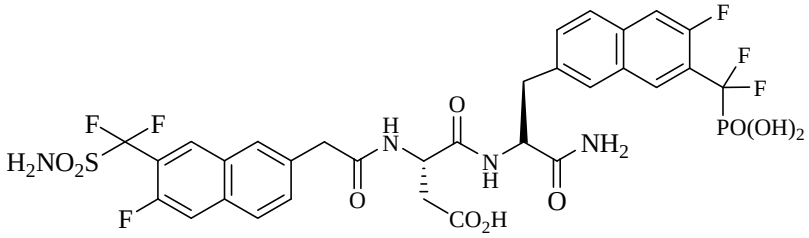
67f (78 mg, 63%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 135-136 ^{o}C; Purity by HPLC: 97.55%.

^{1}HNMR (D_2O) : δ = 2.59 (dd, *J* = 7.1 Hz, 17.1 Hz, 1H), 2.83 (dd, *J* = 5.9 Hz, 16.0 Hz, 1H), 3.27 (dd, *J* = 7.0 Hz, 15.9 Hz, 1H), 3.59 (dd, *J* = 6.1 Hz, 15.9 Hz, 1H), 3.73 (s, 2H), 4.80 (m, 1H), 4.95 (m, 1H), 7.13-7.17 (m, 2H), 7.31 (s, 1H), 7.41 (s, 1H), 7.45 (s, 1H), 7.53 (s, 1H), 7.96 (d, *J* = 7.9 Hz, 1H), 8.27 (s, 1H), 8.39 (d, *J* = 7.8 Hz, 1H)

^{13}CNMR (Acetone-*d6*) : δ 35.2, 37.5, 43.5, 55.3, 59.5, 109.2, 121.1, 121.7, 125.4, 126.1, 127.9, 128.8, 129.7, 130.0, 134.1, 136.3, 139.2, 141.4, 148.7, 158.9, 161.7, 168.2, 169.5, 171.0, 173.8, 176.9

ESI/MS (m/z) : 855.7 $(M+H)^{+}$.

5.1.27.20. (S)-4-[(R)-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoro napthalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(7-(difluoro(slufonamide) methyl)-6-fluoronaphthalen-2-yl)acetamido)-4-oxobutanoic acid (67g)



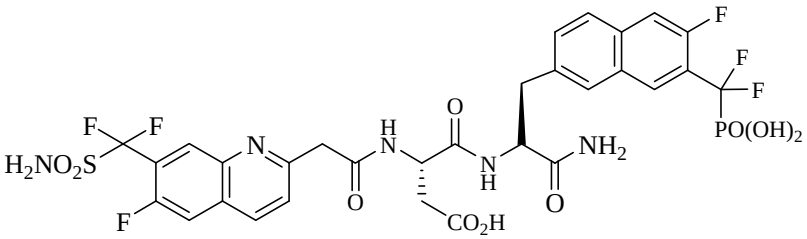
67g (90 mg, 79%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 166-168 ^{o}C; Purity by HPLC: 97.10%.

^{1}HNMR (D_2O) : δ = 2.61 (dd, *J* = 7.8 Hz, 18.5 Hz, 1H), 2.90 (dd, *J* = 5.0 Hz, 16.9 Hz, 1H), 3.33 (dd, *J* = 7.0 Hz, 15.0 Hz, 1H), 3.59 (dd, *J* = 6.0 Hz, 15.9 Hz, 1H), 3.63 (s, 2H), 4.82 (m, 1H), 4.96 (m, 1H), 7.14-7.19 (m, 2H), 7.33-7.45 (m, 6H), 7.89-7.97 (m, 2H)

^{13}CNMR (Acetone-*d6*) : δ 34.4, 38.8, 43.0, 54.9, 62.5, 108.7, 125.4, 126.0, 127.5, 128.8, 130.5, 134.1, 134.4, 136.6, 139.3, 161.7, 165.4, 169.5, 171.6, 173.9, 175.0

ESI/MS (m/z) : 793.7 $(M+H)^{+}$.

5.1.27.21. (S)-4-[(R)-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoro napthalen-2-yl)-1-oxopropan-2-ylamino)-3-(2-(7-(difluoro(sulfonamide) methyl)-6-fluoroquinolin-2-yl)acetamido)-4-oxobutanoic acid (67h)



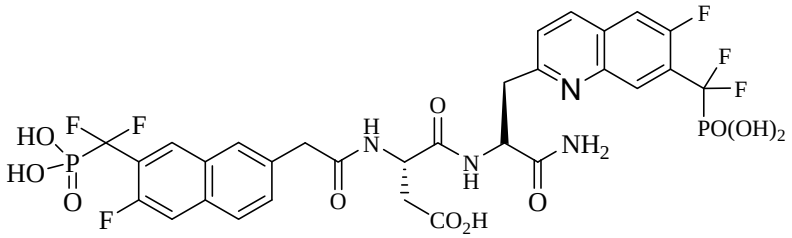
67h (77 mg, 67%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 122-123 ^{o}C; Purity by HPLC: 96.60%.

^{1}HNMR (D_2O) : δ = 2.66 (dd, *J* = 7.0 Hz, 17.8 Hz, 1H), 2.92 (dd, *J* = 6.9 Hz, 16.9 Hz, 1H), 3.34 (dd, *J* = 7.2 Hz, 16.2 Hz, 1H), 3.61 (dd, *J* = 6.0 Hz, 15.0 Hz, 1H), 3.83 (s, 2H), 4.84 (m, 1H), 4.92 (m, 1H), 7.08 (d, *J* = 7.9 Hz, 1H), 7.17 (d, *J* = 7.1 Hz, 1H) 7.31-7.35 (m, 2H), 7.42 (s, 1H), 7.49 (s, 1H), 7.63 (s, 1H), 7.98 (d, *J* = 7.7 Hz, 1H), 8.29 (d, *J* = 7.9 Hz, 1H)

^{13}CNMR (Acetone-*d6*) : δ 33.2, 39.5, 41.5, 57.3, 61.3, 108.2, 122.1, 124.4, 126.9, 127.5, 128.8, 130.6, 134.4, 135.3, 139.1, 143.4, 145.7, 158.4, 161.5, 162.4, 165.7, 170.5, 171.2, 172.8, 175.5

ESI/MS (m/z) : 794.7 $(M+H)^{+}$.

5.1.27.22. (S)-4-[(R)-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoroquin-olin-2-yl)-1-oxopropan-2-ylamino)-3-(2-(7-(difluoro(phoshono) methyl)-6-fluoronaphthalen-2-yl)acetamido)-4-oxobutanoic acid (67i)



67i (65 mg, 61%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 171-172 ^{o}C; Purity by HPLC: 97.00%.

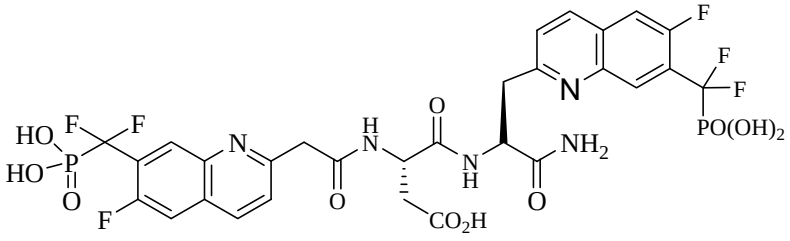
^{1}HNMR (D_2O) : δ = 2.60 (dd, *J* = 7.0 Hz, 17.1 Hz, 1H), 2.88 (dd, *J* = 6.0 Hz, 16.6 Hz, 1H), 3.27 (dd, *J* = 7.1 Hz, 15.9 Hz, 1H), 3.49 (dd, *J* = 6.0 Hz, 15.8 Hz, 1H), 3.59 (s, 2H), 4.87 (m, 1H), 4.91 (m, 1H), 7.07-7.16 (m, 2H), 7.33-7.37

(m, 2H), 7.41-7.47 (m, 2H), 7.61 (s, 1H), 7.98 (d, *J* = 8.0 Hz, 1H), 8.31 (d, *J* = 8.9 Hz, 1H)

^{13}CNMR (Acetone-*d6*) : *δ* 35.0, 35.5, 43.3, 53.8, 59.2, 108.6, 109.7, 122.0, 125.4, 128.1, 128.8, 130.5, 134.1, 134.5, 138.2, 139.2, 161.6, 162.0, 170.3, 171.5, 173.2, 174.9

ESI/MS (m/z) : 795.5 $(M+H)^+$.

5.1.27.23. (S)-4-[(R)-1-Amino-3-(7-(difluoro(phosphono)methyl)-6-fluoroquinolin-2-yl)-1-oxopropan-2-ylamino)-3-(2-(7-(difluoro(phoshono) methyl)-6-fluoroquinolin-2-yl)acetamido)-4-oxobutanoic acid (67j)



67j (70 mg, 61%) was prepared from **55** (250 mg, LC: 0.58 mmol/g) by means of the general procedure described above as a white solid. mp: 129-130 ^oC; Purity by HPLC: 96.00%.

^{1}HNMR (D_2O) : *δ* = 2.49 (dd, *J* = 7.1 Hz, 17.9 Hz, 1H), 2.90 (dd, *J* = 6.3 Hz, 16.9 Hz, 1H), 3.24 (dd, *J* = 7.1 Hz, 16.1 Hz, 1H), 3.51 (dd, *J* = 6.1 Hz, 15.9 Hz, 1H), 3.77 (s, 2H), 4.86 (m, 1H), 4.94 (m, 1H), 7.02-7.11 (m, 2H), 7.29-7.34 (m, 2H), 7.55-7.62 (m, 2H), 8.21-8.30 (m, 2H)

^{13}CNMR (Acetone-*d6*) : *δ* 34.9, 35.4, 42.3, 53.3, 59.9, 108.3, 108.7, 122.1, 128.7, 130.6, 134.5, 138.2, 138.8, 143.2, 145.7, 158.5, 161.8, 162.7, 169.3, 171.0, 173.3, 175.0

ESI/MS (m/z) : 796.6 $(M+H)^+$.

5.2. Docking study

The molecular docking analysis of test compounds was carried out using extra precision (XP) glide version 5.6 docking software of Schrodinger **[1]**, to understand their critical interaction with PTP-1B crystal structure. The three-dimential protein structure of PTP-1B (PDB id: 1Q6T) was obtained from the protein data bank and the protein structure was prepared using protein preparation wizard module of Schrodinger. After protein structure was prepared, the bound ligand of receptor was defined as grid binding box. For docking study, the ligand structures were prepared using the Ligprep module of Schrodinger to generate energy-minimized correct 3D ligand structures. The initial Glide docking studies gave poor results in terms of binding conformations. Based on this observation, the test compounds were docked using the induced fit docking (IFD) protocol. The IFD is based on the docking program Glide with the refinement module in Prime (Schrodinger, Inc.), which was reported to accurately predict the ligand binding modes and concomitant structural changes in the receptor.

5.3. Biology

5.3.1. PTP-1B inhibitory and selectivity (over TC-PTP) enzymatic assay (*in vitro*)

Test compounds were assessed for their ability to inhibit the purified enzyme in a colorimetric-based *p*-NPP assay as per modified literature procedure **[2]**. The enzymatic assay was carried out at room temperature in 96-well plates. The assay buffer contained 50 mM 3, 3-dimethyl glutarate, 1 mM EDTA, 1 mM TCEP, and 0.01% Triton (pH 7.0 with ionic strength of 0.15 M adjusted by sodium chloride). The reaction was initiated by addition of the

enzyme at a final concentration of 10 or 100 nM for PTP-1B and 20 nM for TC-PTP, respectively. The initial rate of the PTPase-catalyzed hydrolysis of p-nitro phenylphosphate (*p*NPP) was measured by following the absorbance change at 405 nm. IC_{50} values were determined under a fixed *p*NPP concentration of 1 nM. All the assays were carried out in duplicate or triplicate, and the average results are presented. PTPases used in the assay were recombinant human PTP-1B (hPTP-1B catalytic domain, residues 1-299, expressed and purified according to literature procedures), recombinant human TC-PTP (residues 1-299).

5.3.2. Antidiabetic activity (*in vivo*)

Study (*in vivo* antidiabetic activity) was conducted using the intraperitoneal glucose tolerance test (IPGTT) protocol **[3-4]**. This study was conducted on C57BL/6j mice of either sex (age 8-12 weeks), weighing between 20 to 25g. The animals were divided into two groups (control and standard) and each experimental group consisted of six animals. All the animals were left for 2 days under laboratory conditions for acclimatization and maintained on a standard pellet diet and water *ad libitum* before the day of the experiment. On the last day food was withdrawn and they were given water only. A 12 hours dark/light cycle was also maintained. All the animal experiments were conducted according to the internationally valid guidelines following approval by the 'Zydus Research Center Animal Ethical Committee'.

Overnight fasted mice were dosed orally (po) with the test compounds (10 or 20 mpkg), on a body weight basis, 30-min prior to the intraperitoneal (ip) glucose load (1.5 gpkg). Blood samples were collected at various time points (0, 30, 60, 120 and 240 min). Blood samples were centrifuged (3000

rpm, 15 min at 40 °C) and the separated serum was immediately subjected for the glucose estimation. The glucose estimation was carried out with DPEC-GOD/POD method (Ranbaxy Fine Chemicals Limited, Diagnostic division, India), using Spectramax-190, in 96-microwell plate reader (Molecular devices Corporation, Sunnyvale, Californai). Mean values of duplicate samples were calculated using Microsoft excel and the Graph Pad Prism software (version 4.0) was used to plot a area under the curve (0-240 min AUC). The AUC obtained from graphes were analyzed for two-way ANOVA, followed by Bonferroni post test, using Graph Pad Prism software.

5.3.3. Pharmacokinetic study

The pharmacokinetic parameters of test compounds were determined in male C57BL/6j mice or wister rat. Briefly, test compounds were administered orally / iv on a body weight basis to overnight fasted rats or mice. Serial blood samples were collected in microcentrifuge tubes containing EDTA at pre-dose as well as 0.15, 0.3, 0.5, 0.75, 1, 2, 4, 6, 8, 24 and 30 hrs post-dose after compounds administration. Approximately 0.3 mL of blood was collected at each time point and centrifuged at 4 ^{o}C. The obtained plasma was frozen, stored at -70 ^{o}C and the concentrations of compounds in plasma were determined by the LC-MS/MS (Shimadzu LC10AD, USA), using YMC hydrosphere C18 (2.0 x 50 mm, 3 µm) column (YMC Inc., USA). The pharmacokinetic parameters, such T_{max}, $t_{1/2}$, C_{max}, AUC and *%F* were calculated using a non-compartmental model of WinNonlin software (version 5.2).

5.4. References

[1] Schrodinger Suite 2010, Glide version 5.6, Prime version 2.2, Schrodinger LLC, New York, NY (USA) 2010.

[2] Moretto, A. F.; Kirincich, S. J.; Xu, W. X.; Smith, M. J.; Wan, Z.-K.; Wilson, D. P.; Follows, B. C.; Binnun, E.; Joseph-McMarthy, D.; Foreman, K.; Erbe, D. V.; Zhang, Y.-L.; Tam, S. K.; Tam, S. Y.; Lee, J. *Bioorg. Med. Chem. Lett.* 2006, *14*, 2162.

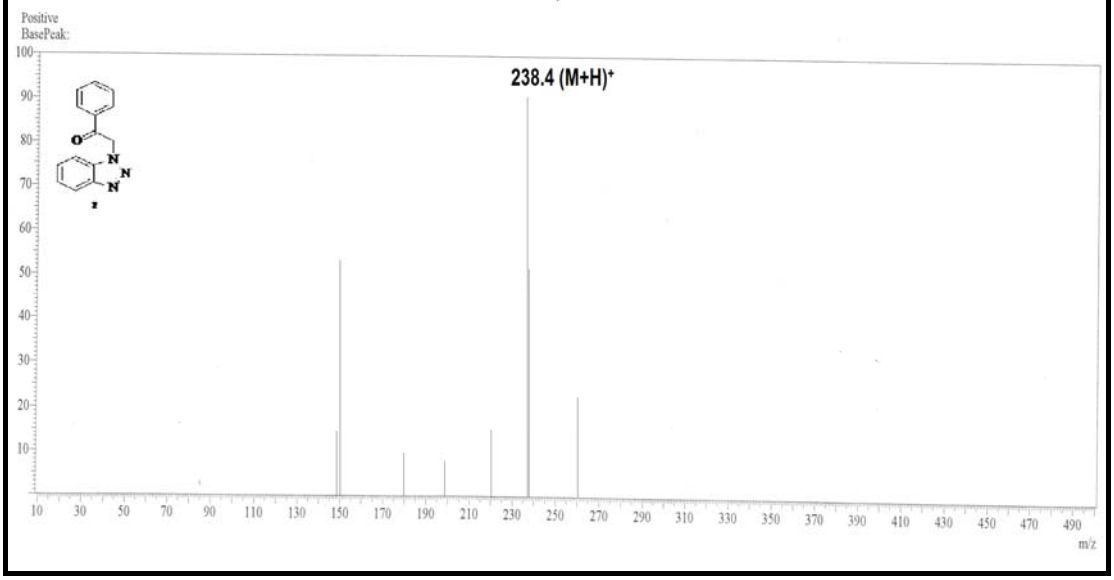
[3] Chen, D.; Wang, M. *Diabetes Obes. Metab.* 2005, *7(4)*, 307.

[4] Kim, J. G.; Baggio, L. L.; Bridon, D. P.; Castaigne, J. P.; Robitaille, M. F.; Jette, L.; Benquet, C.; Drucker, D. J. *Diabetes* 2003, *52(3)*, 751.

Appendix- VI: Spectra

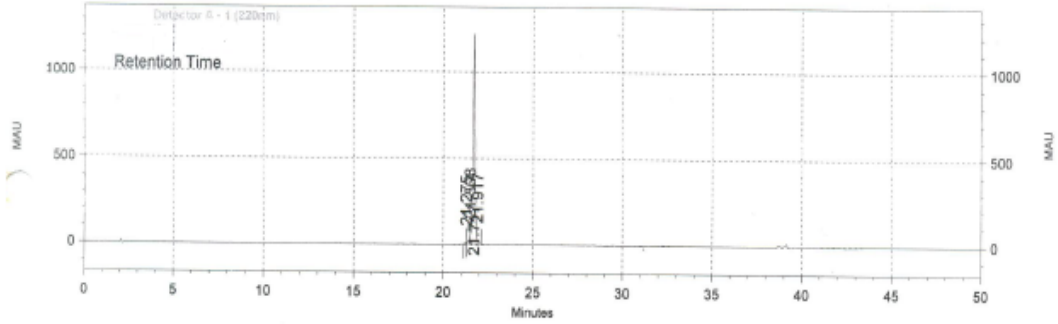
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum



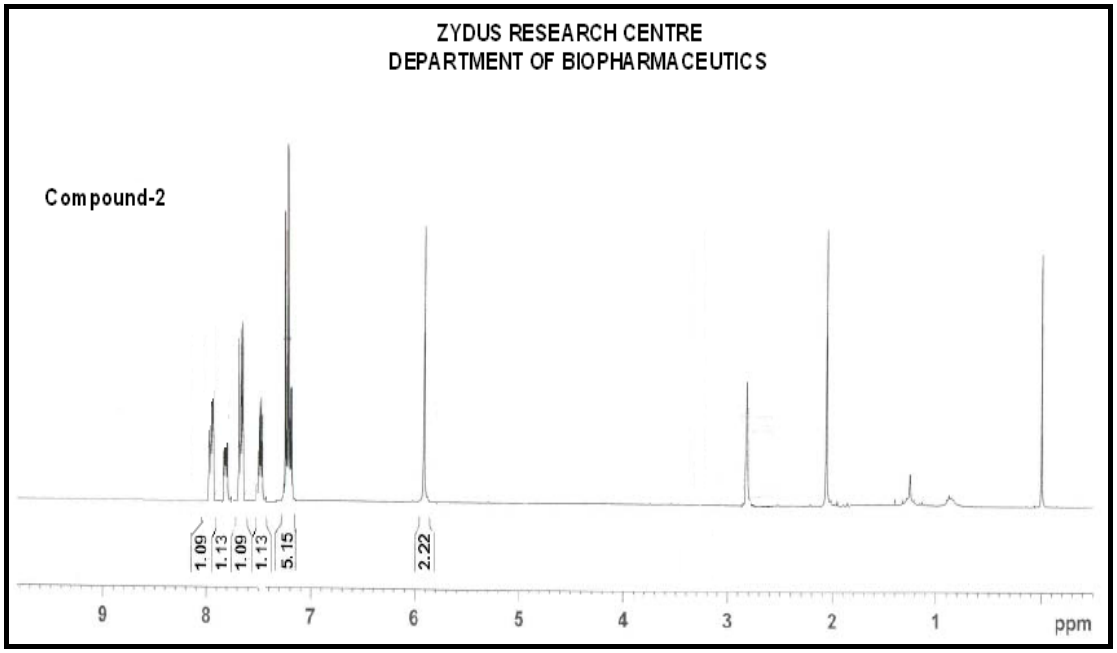
Compound-2

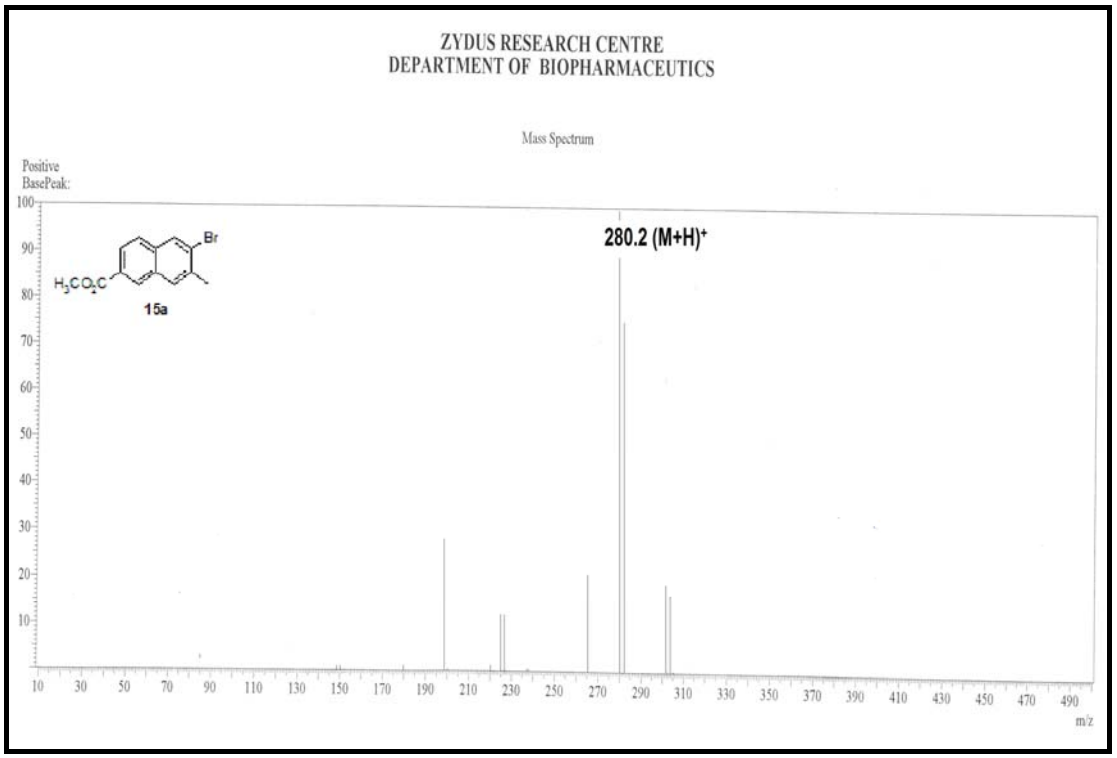
Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm



Detector A - 1 (220nm)

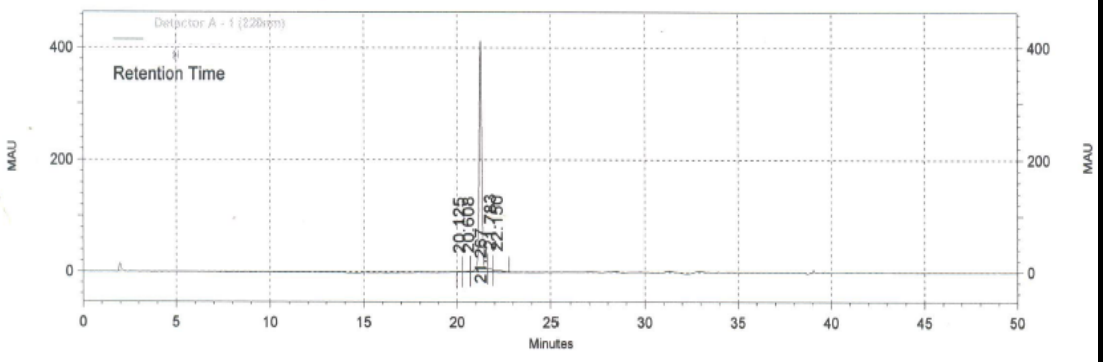
Pk #	Retention Time	Area	Area %
1	21.27	34459	0.31
2	21.51	271552	2.46
3	21.72	10587625	95.96
4	21.92	139513	1.26
Totals		11033149	100.00


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-2
1.09
1.13
1.09
1.13
5.15
2.22
9
8
7
6
5
4
3
2
1
ppm


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
Br
H3CO2C
15a
280.2 (M+H)+
m/z

Compound-15a

Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm

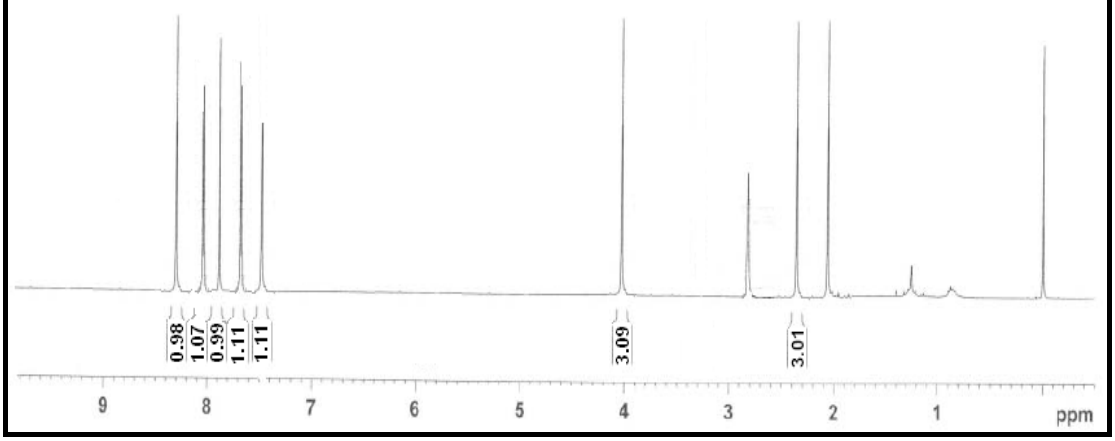


Detector A - 1 (220nm)

Pk #	Retention Time	Area	Area %
1	20.13	6087	0.14
2	20.61	16327	0.39
3	21.27	3992522	94.48
4	21.78	106691	2.52
5	22.15	104263	2.47
Totals		4225890	100.00

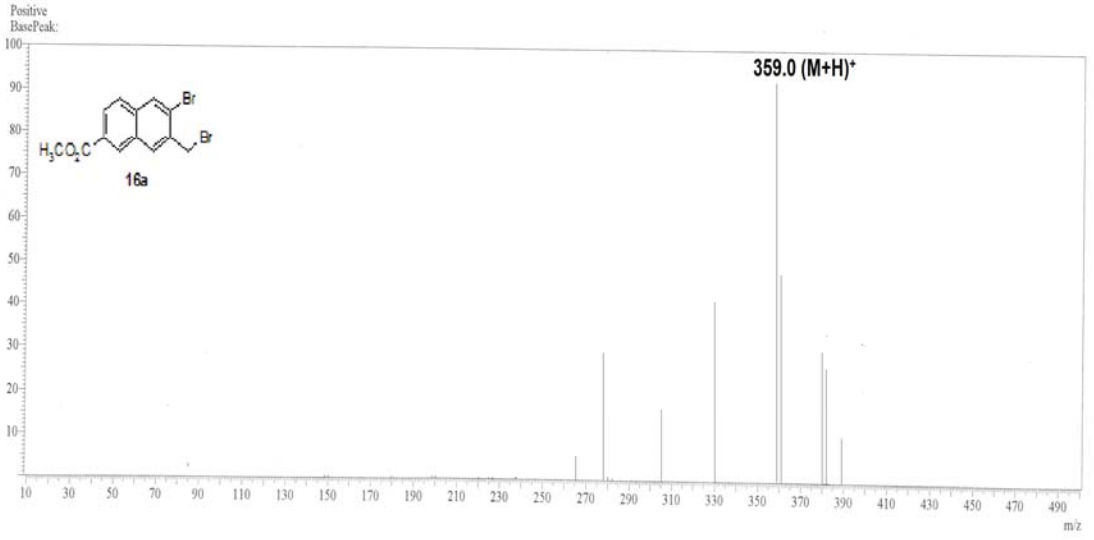
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Compound-15a



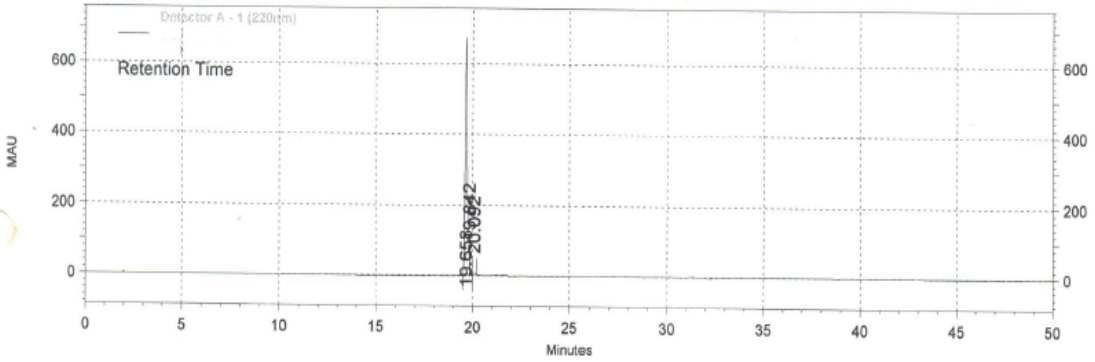
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum



Compound-16a

Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm

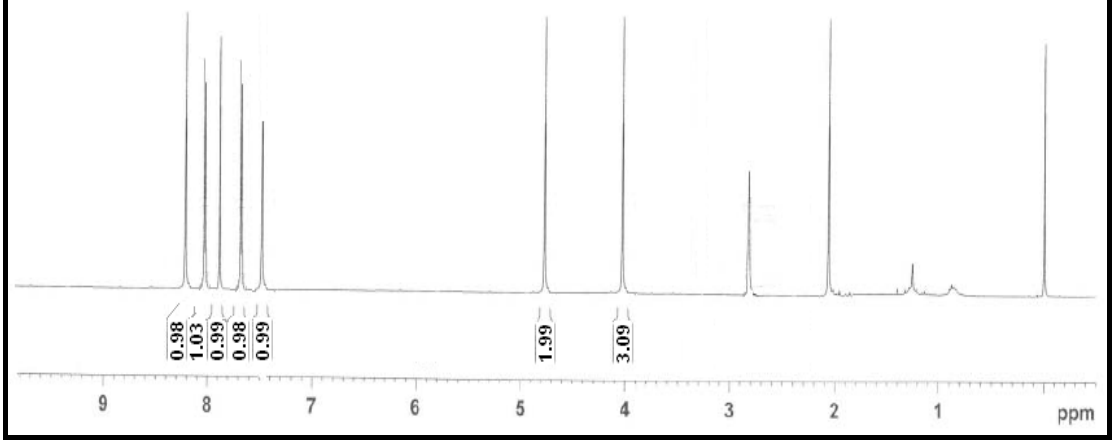


Detector A - 1 (220nm)

Pk #	Retention Time	Area	Area %
1	19.66	5436267	96.94
2	19.84	126127	2.25
3	20.09	45309	0.81
Totals		5607703	100.00

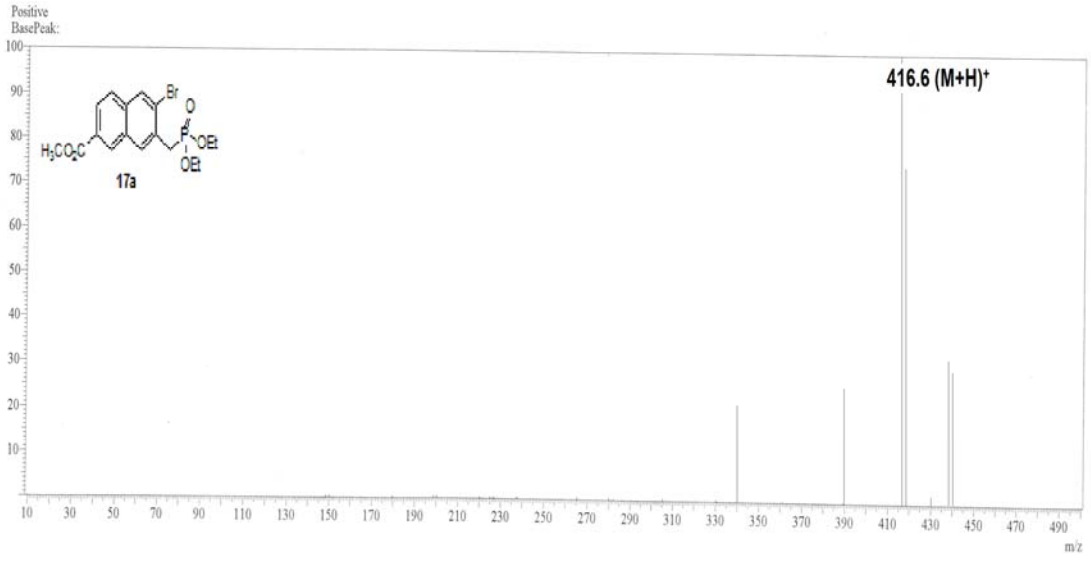
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Compound-16a



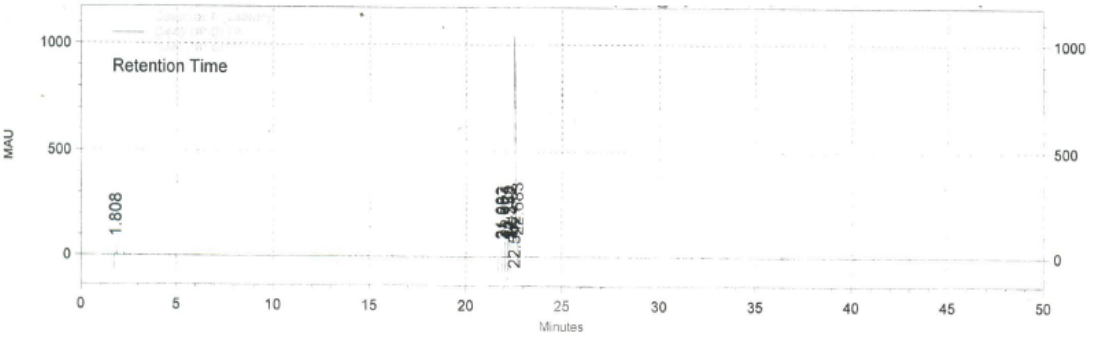
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum



Compound-17a

Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm

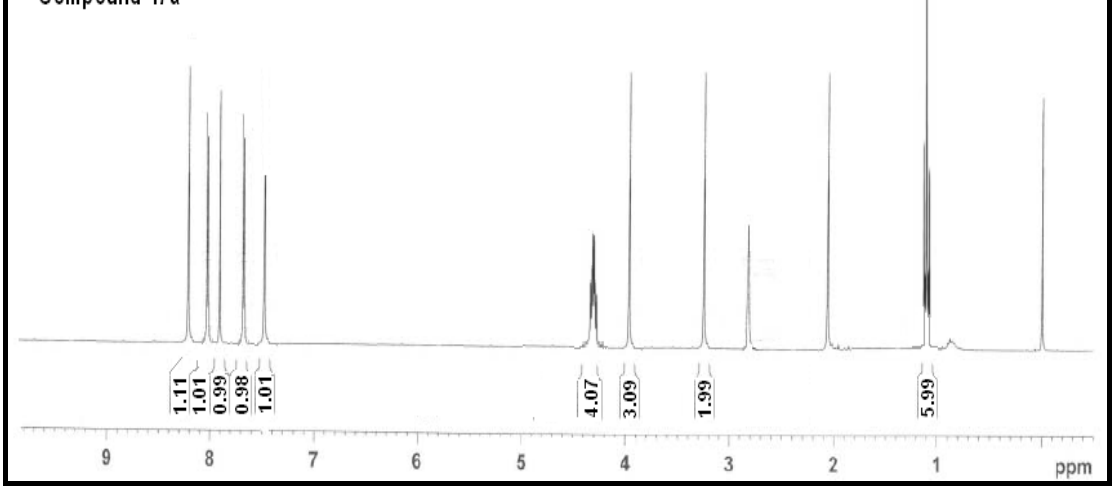


Detector A (220nm)

Pk #	Retention Time	Area	Area %
1	1.81	16711	0.30
2	21.88	7435	0.13
3	21.97	13674	0.24
4	22.13	47801	0.85
5	22.26	17887	0.32
6	22.34	22820	0.41
7	22.54	5441847	97.00
8	22.68	41865	0.75
Totals		5610040	100.00

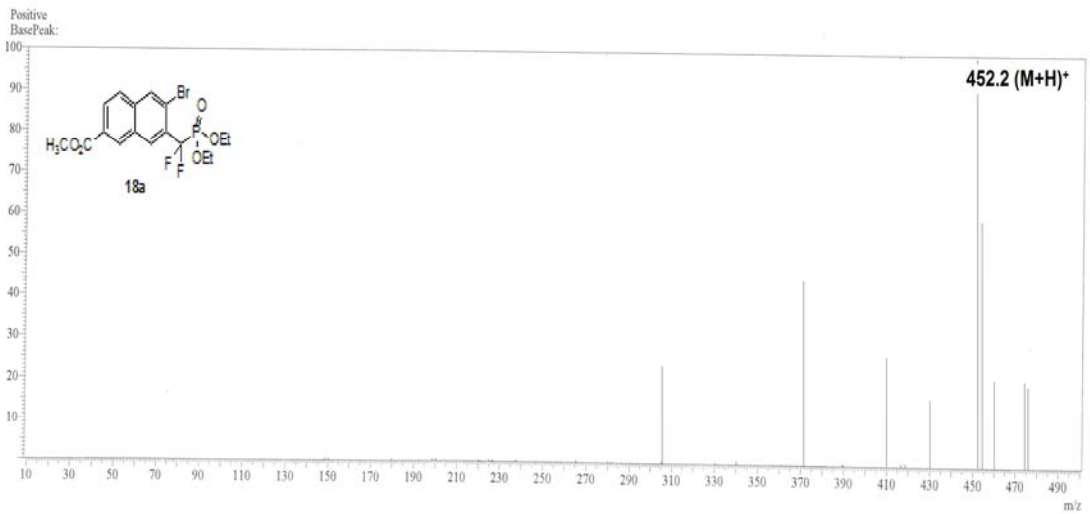
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Compound-17a



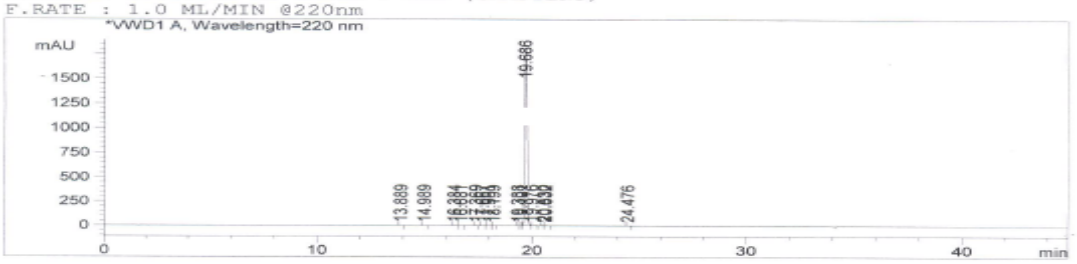
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum



Compound-18a

COLUMN : YMC J'SPHERE C18 (150 X4.6)4u
M.PHASE: 0.05%TFA IN WATER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN @220nm



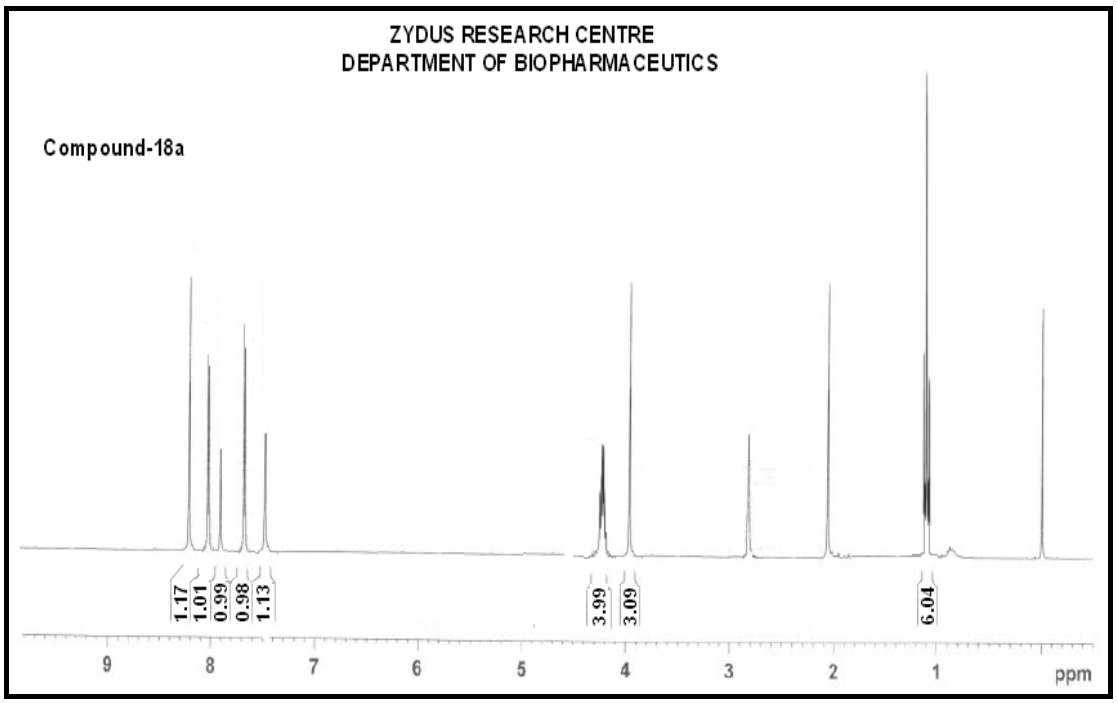
Area Percent Report

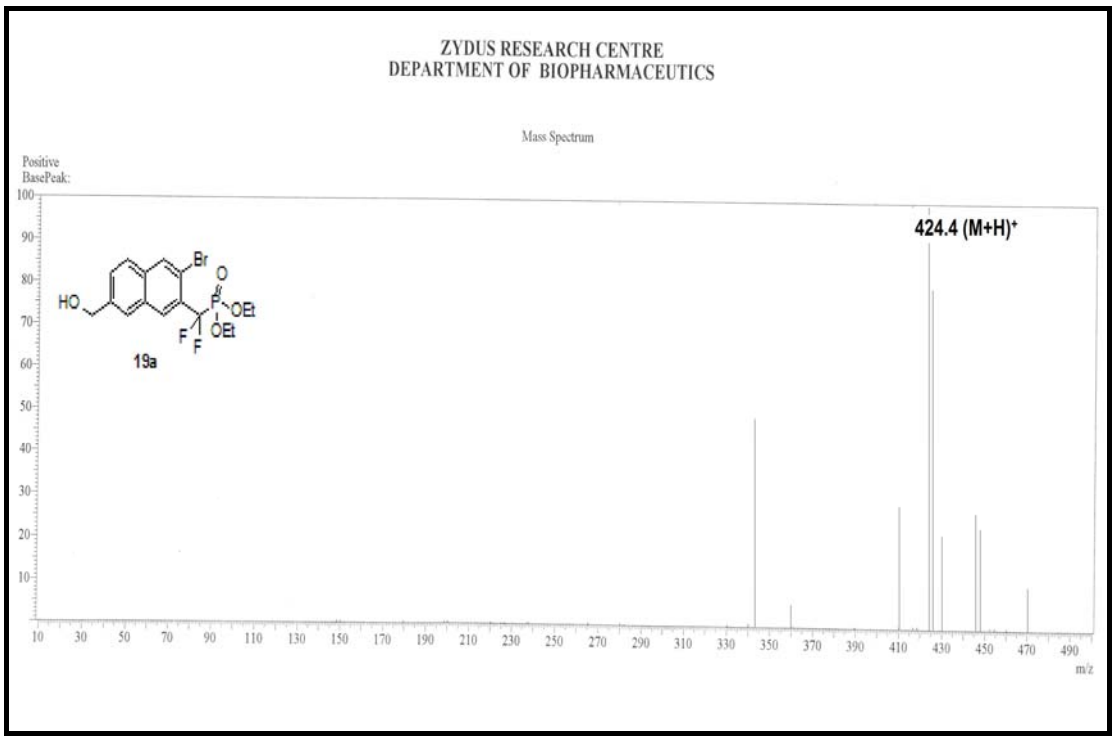
Sorted By : Signal
Multiplior : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	13.889	BBA	0.0859	3.91594	6.82002e-1	0.0300
2	14.989	BBA	0.0784	4.08873	8.01853e-1	0.0314
3	16.384	BP	0.0864	13.21684	2.33607	0.1014
4	16.681	BBA	0.1024	2.38064	3.71543e-1	0.0183
5	17.369	PBA	0.0864	11.86920	2.09666	0.0911
6	17.667	PB	0.0885	4.10001	7.02081e-1	0.0315
7	17.991	PV	0.0873	4.61685	8.05447e-1	0.0354
8	18.199	VBA	0.0850	1.96662	3.55334e-1	0.0151
9	19.338	PV	0.0823	4.46023	8.21172e-1	0.0342
10	19.491	VV	0.0823	3.31299	6.24680e-1	0.0254
11	19.686	MF R	0.1194	1.29478e4	1807.71924	99.3484
12	19.976	FM R	0.0825	11.02906	2.22781	0.0846
13	20.530	MF R	0.0662	1.77854	4.47447e-1	0.0136
14	20.632	FM R	0.1429	15.68794	1.83033	0.1204
15	24.476	BP	0.0865	2.49156	4.39625e-1	0.0191
Totals :				1.30327e4	1822.26130	

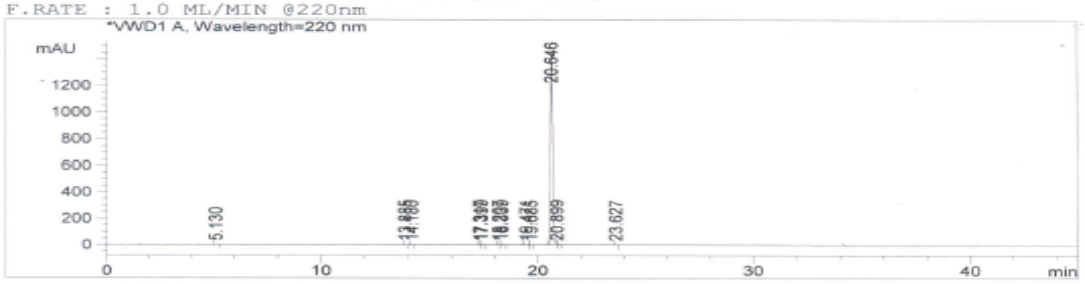
Results obtained with enhanced integrator!


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-18a
1.17
1.01
0.99
0.98
1.13
3.99
3.09
6.04
9
8
7
6
5
4
3
2
1
ppm


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
424.4 (M+H)+
HO
Br
O
P
OEt
OEt
F
F
19a
m/z

Compound-19a

COLUMN : YMC J'SPHERE C18 (150 X4.6)4u
M.PHASE: 0.05%TFA IN WATER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN @220nm



Area Percent Report

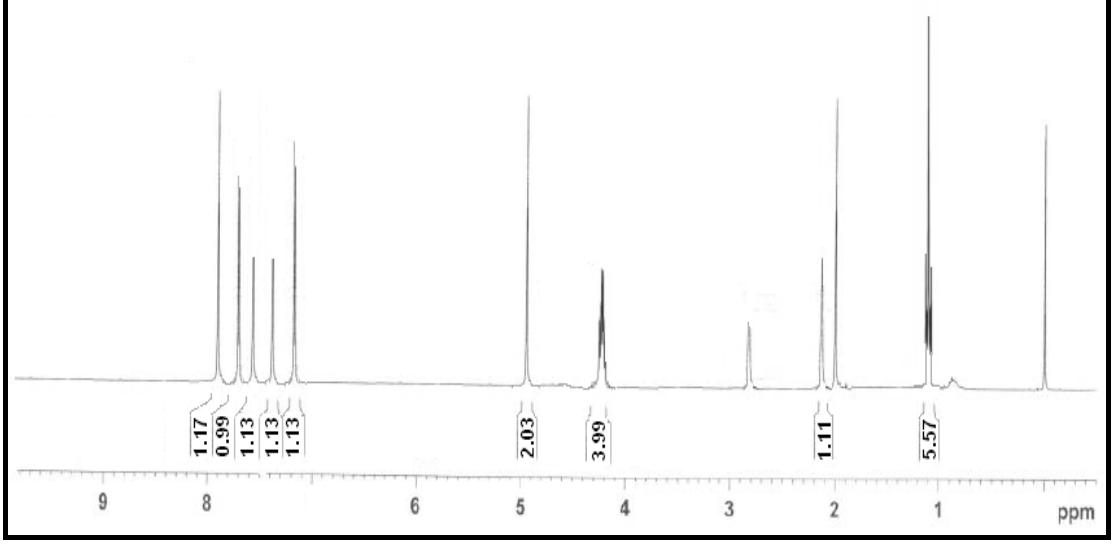
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	5.130	BP	0.1280	4.87836	5.75624e-1	0.0486
2	13.885	BB	0.0856	3.79475	6.78440e-1	0.0378
3	14.180	PBA	0.0753	1.11681	2.14490e-1	0.0111
4	17.317	MF R	0.0646	1.06985	2.76112e-1	0.0107
5	17.399	FM R	0.0995	14.34052	2.40300	0.1429
6	18.207	PV	0.0878	3.60624	6.38239e-1	0.0359
7	18.339	VBA	0.0988	17.08670	2.49433	0.1703
8	19.471	BV	0.1241	6.49813	8.23126e-1	0.0648
9	19.685	VBA	0.1046	2.97078	4.26130e-1	0.0296
10	20.646	MF R	0.1136	9967.28027	1462.34070	99.3355
11	20.899	FM R	0.0712	9.92987	2.32580	0.0990
12	23.627	BBA	0.0916	1.38779	2.22775e-1	0.0138
Totals :				1.00340e4	1473.41877	

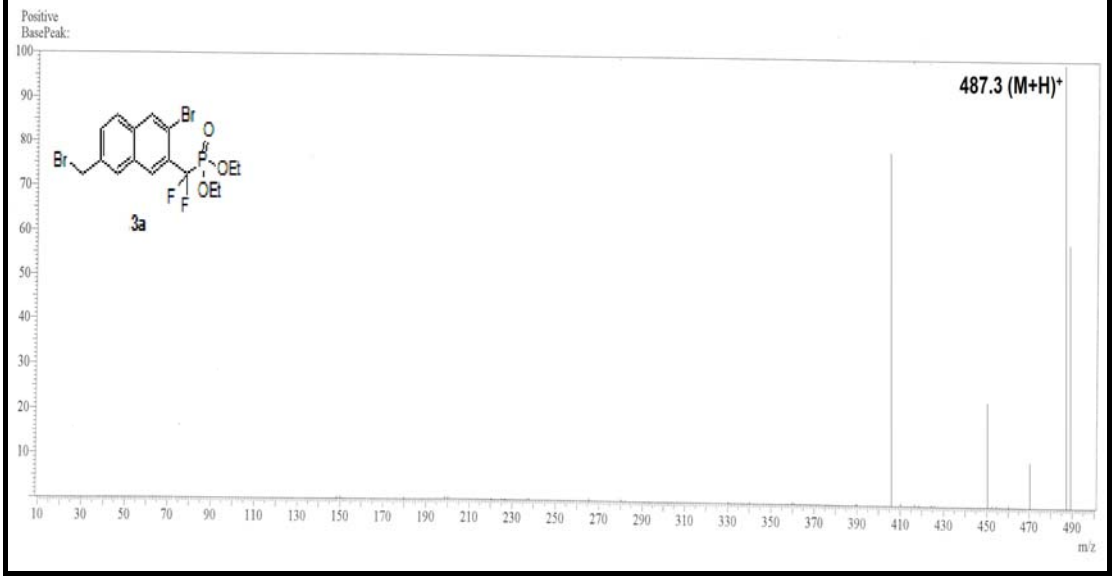
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Compound-19a



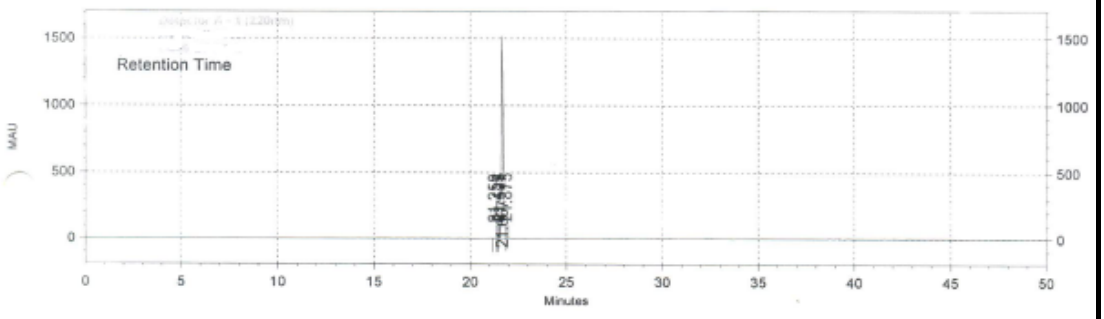
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum



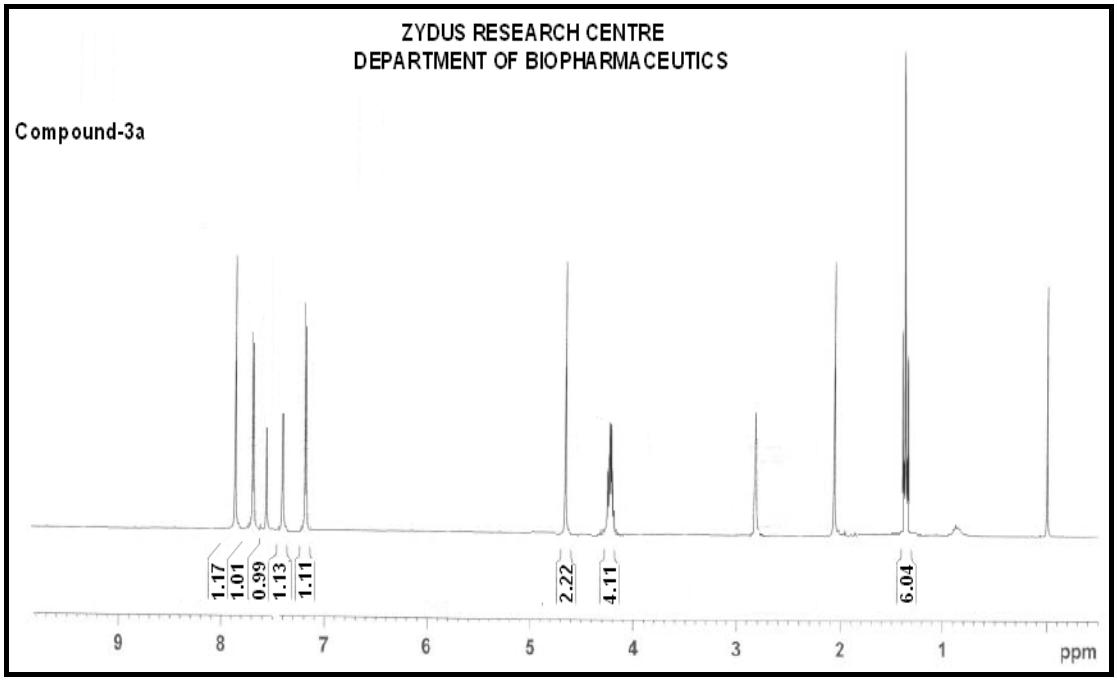
Compound-3a

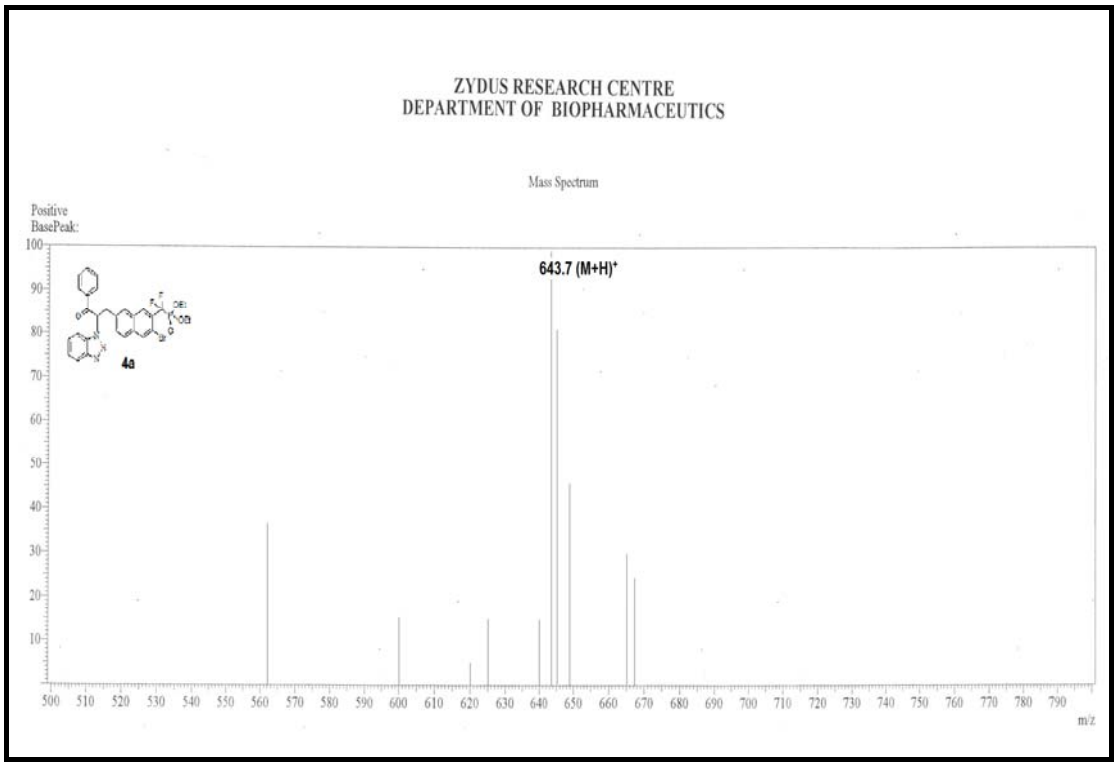
Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm



Detector A - 1 (220nm)

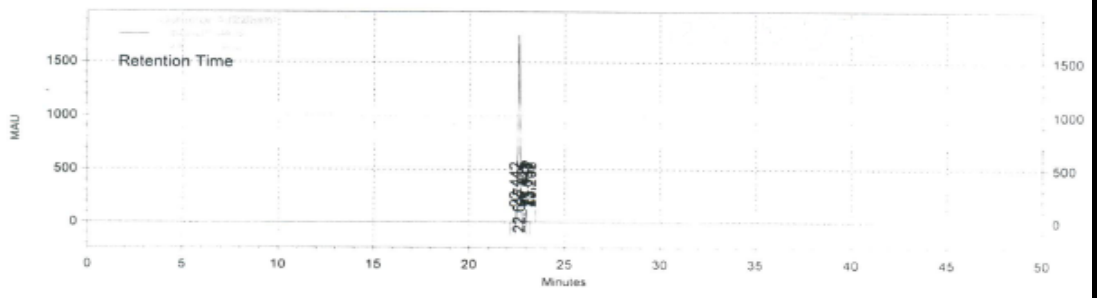
Pk #	Retention Time	Area	Area %
1	21.25	5398	0.05
2	21.43	7875	0.08
3	21.52	30991	0.30
4	21.67	10284564	99.42
5	21.88	15452	0.15
Totals		10344280	100.00


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-3a
1.17
1.01
0.99
1.13
1.11
2.22
4.11
6.04
9
8
7
6
5
4
3
2
1
ppm


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
643.7 (M+H)+
4a
m/z

Compound-4a

Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm

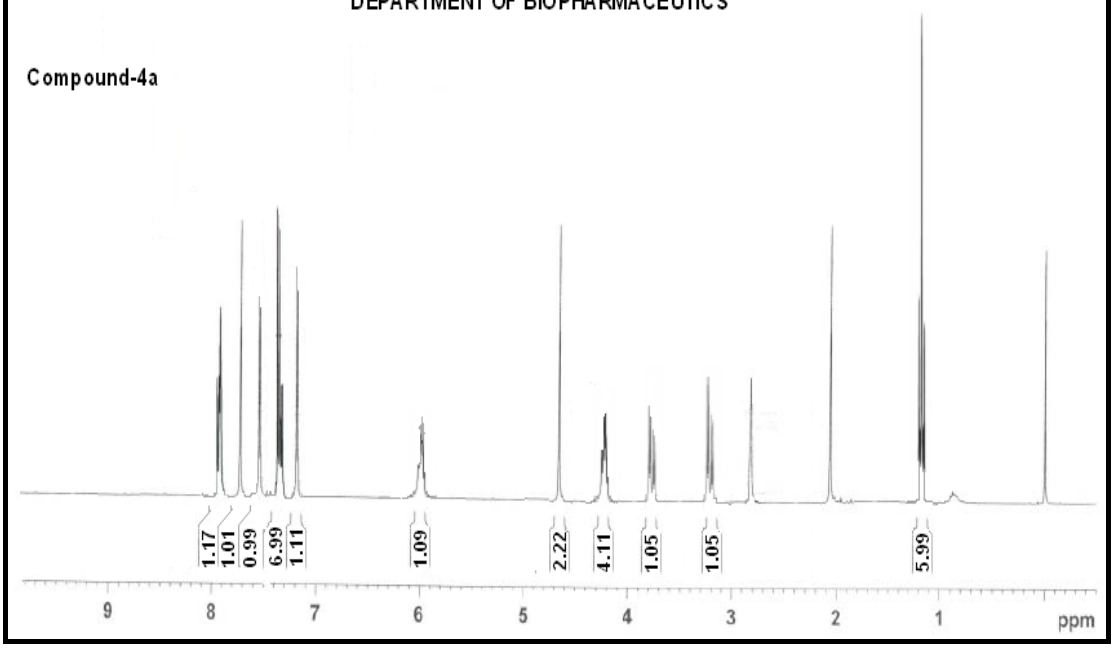


Detector A (220nm)

Pk #	Retention Time	Area	Area %
1	22.44	26758	0.22
2	22.62	12049403	97.99
3	22.82	94297	0.77
4	23.03	15656	0.13
5	23.12	93176	0.76
6	23.21	17336	0.14
Totals		12296626	100.00

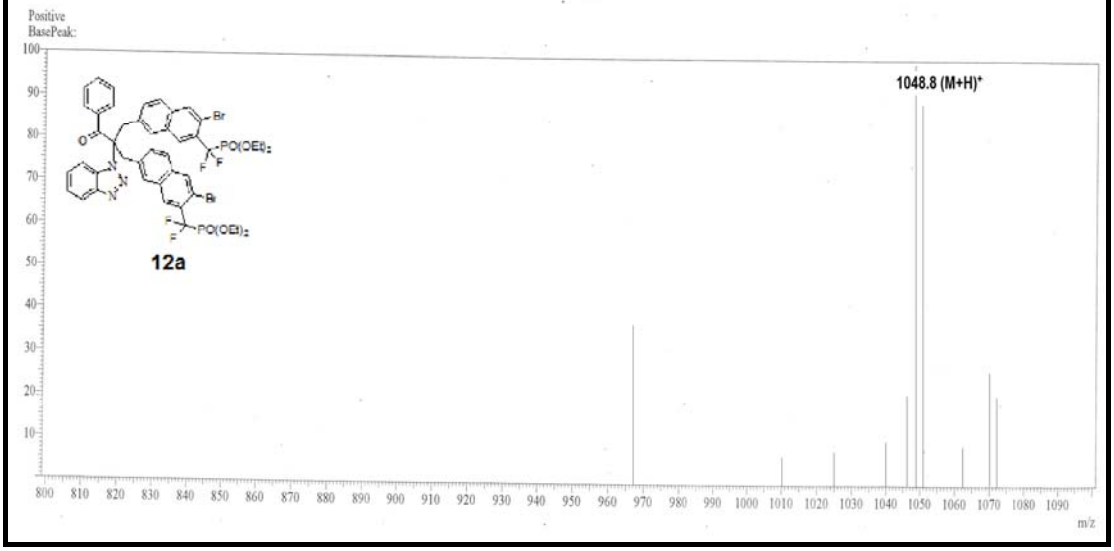
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Compound-4a



ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

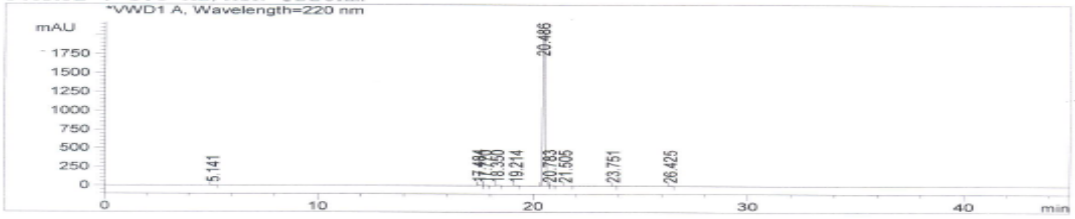
Mass Spectrum



12a

Compound-12a

COLUMN : YMC J'SPHERE C18 (150 X4.6)4u
M.PHASE: 0.05%TFA IN WATER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN @220nm



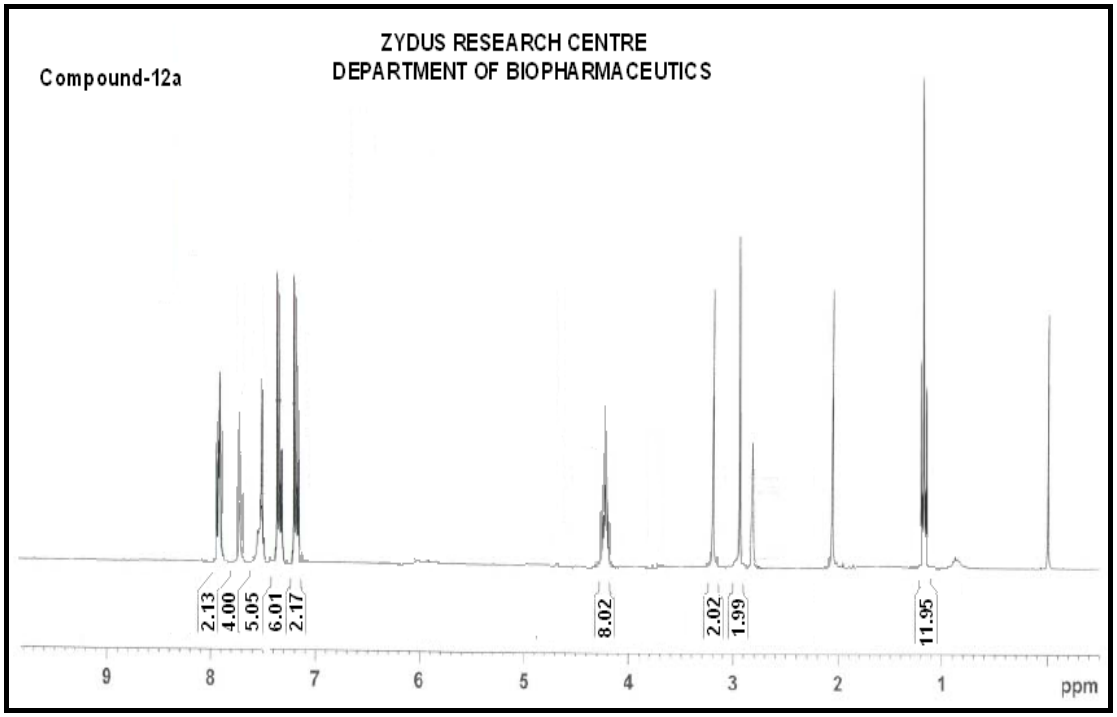
Area Percent Report

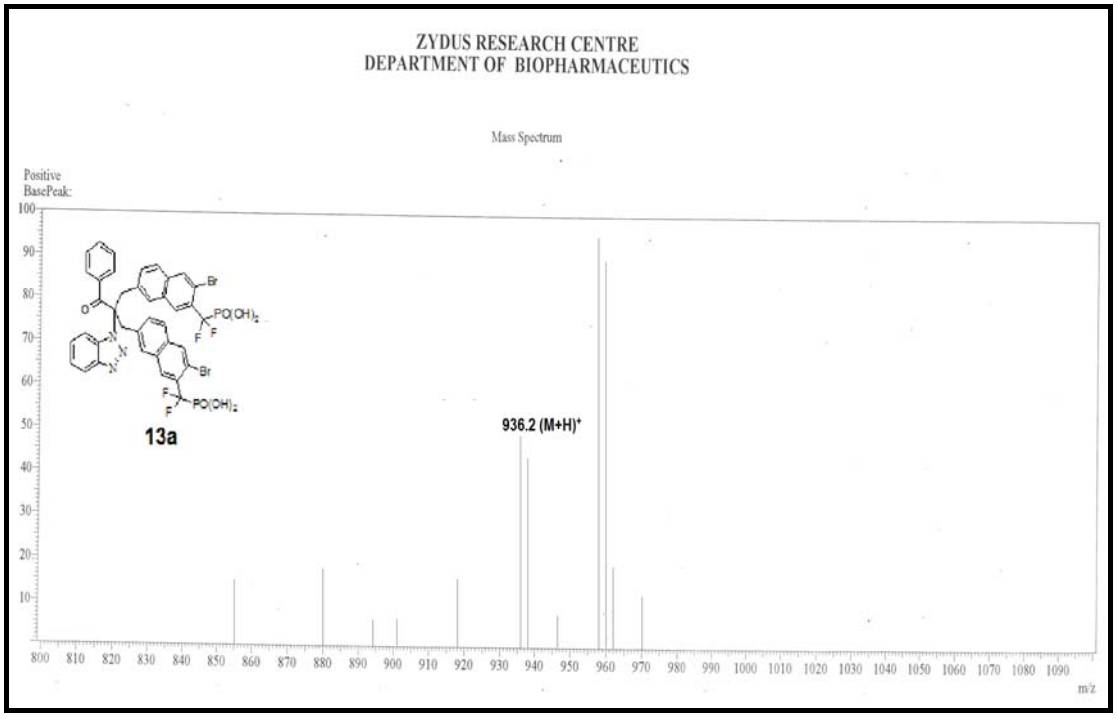
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	5.141	BBA	0.1186	3.09604	3.90868e-1	0.0178
2	17.484	BB	0.0882	36.43892	6.27047	0.2090
3	17.770	BP	0.0930	6.30189	9.92022e-1	0.0361
4	18.350	PP	0.0895	15.90040	2.68446	0.0912
5	19.214	BBA	0.0942	2.13323	3.50807e-1	0.0122
6	20.486	MF R	0.1396	1.73381e4	2069.49756	99.4347
7	20.783	FM R	0.0769	18.91316	4.09811	0.1085
8	21.505	BP R	0.1423	3.78884	3.95490e-1	0.0217
9	23.751	PBA	0.0905	10.05057	1.67282	0.0576
10	26.425	BBA	0.0814	1.95218	3.64740e-1	0.0112
Totals :				1.74367e4	2086.71735	

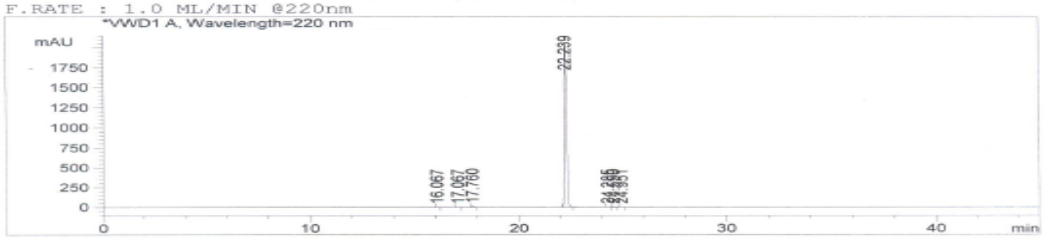
Results obtained with enhanced integrator!


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-12a
2.13
4.00
5.05
6.01
2.17
8.02
2.02
1.99
11.95
9
8
7
6
5
4
3
2
1
ppm


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
13a
936.2 (M+H)+
m/z

Compound-13a

COLUMN : YMC J'SPHERE C18 (150 X4.6)4u
M.PHASE: 0.05%TFA IN WATER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN @220nm



Area Percent Report

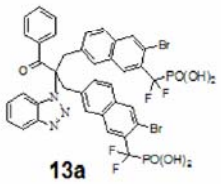
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

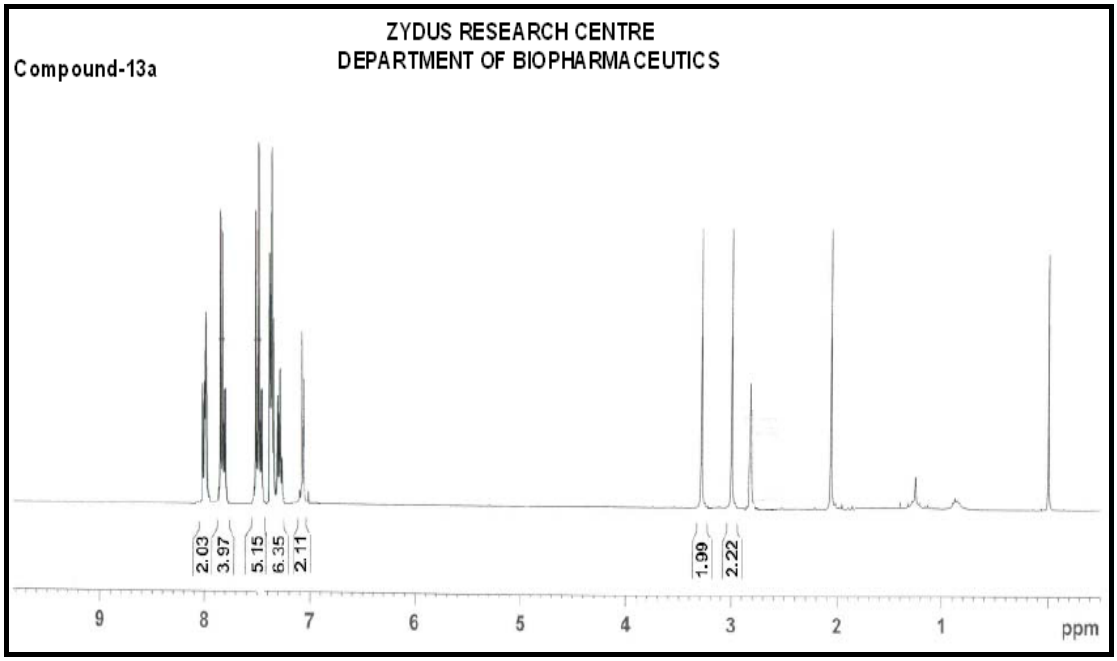
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	16.067	BB	0.0810	8.32541	1.52920	0.0515
2	17.067	BBA	0.1026	5.46827	7.90139e-1	0.0339
3	17.760	BBA	0.0873	74.40192	12.69425	0.4607
4	22.239	PBA	0.1231	1.60154e4	2052.34204	99.1650
5	24.285	BV	0.1112	6.57687	8.44534e-1	0.0407
6	24.550	VBA	0.0935	32.76357	5.22569	0.2029
7	24.951	BP	0.0887	7.31327	1.24907	0.0453
Totals :				1.61503e4	2074.67492	

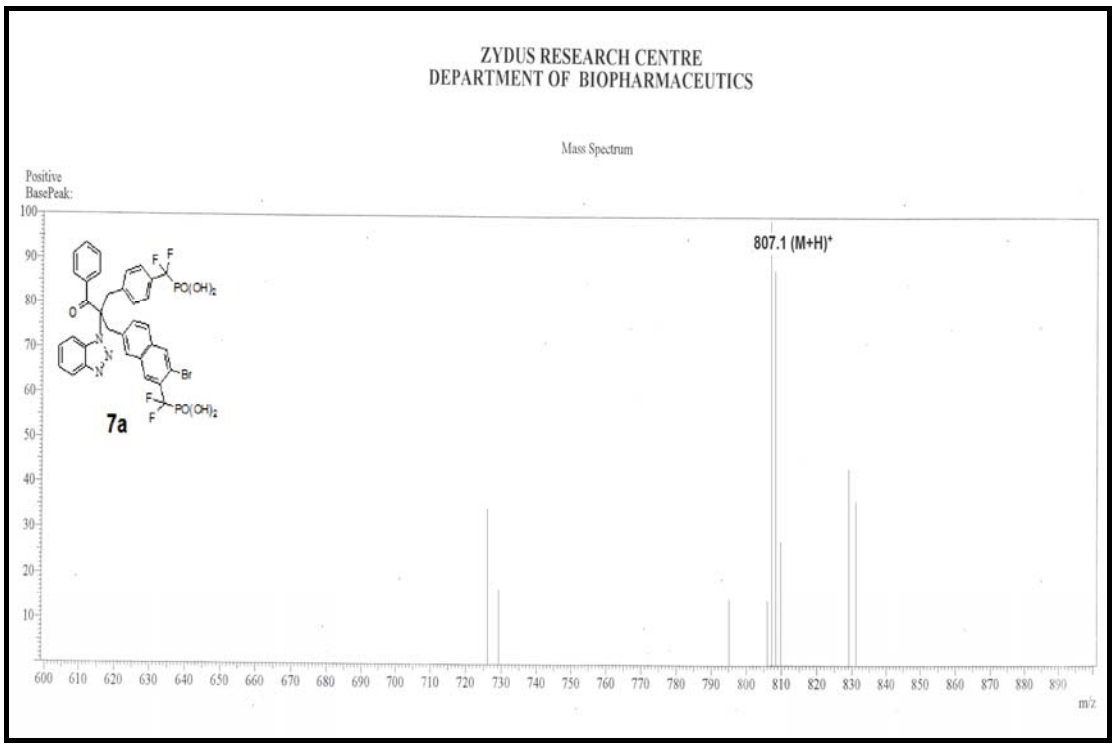
Results obtained with enhanced integrator!

ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS



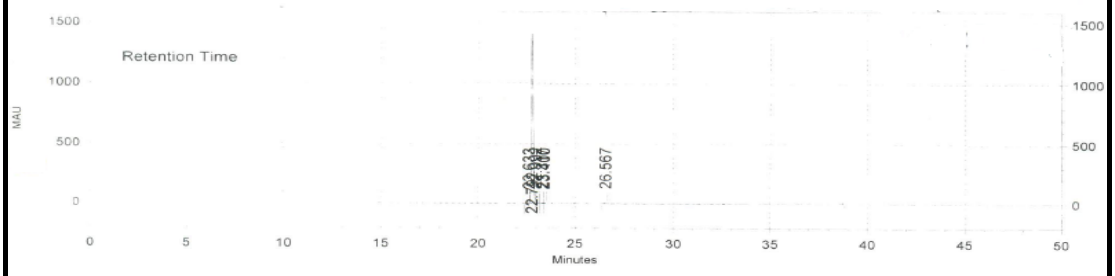
13a


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-13a
2.03
3.97
5.15
6.35
2.11
1.99
2.22
9
8
7
6
5
4
3
2
1
ppm


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
807.1 (M+H)+
PO(OH)2
Br
7a
m/z

Compound-7a

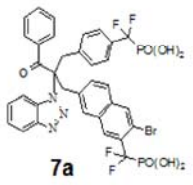
Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm



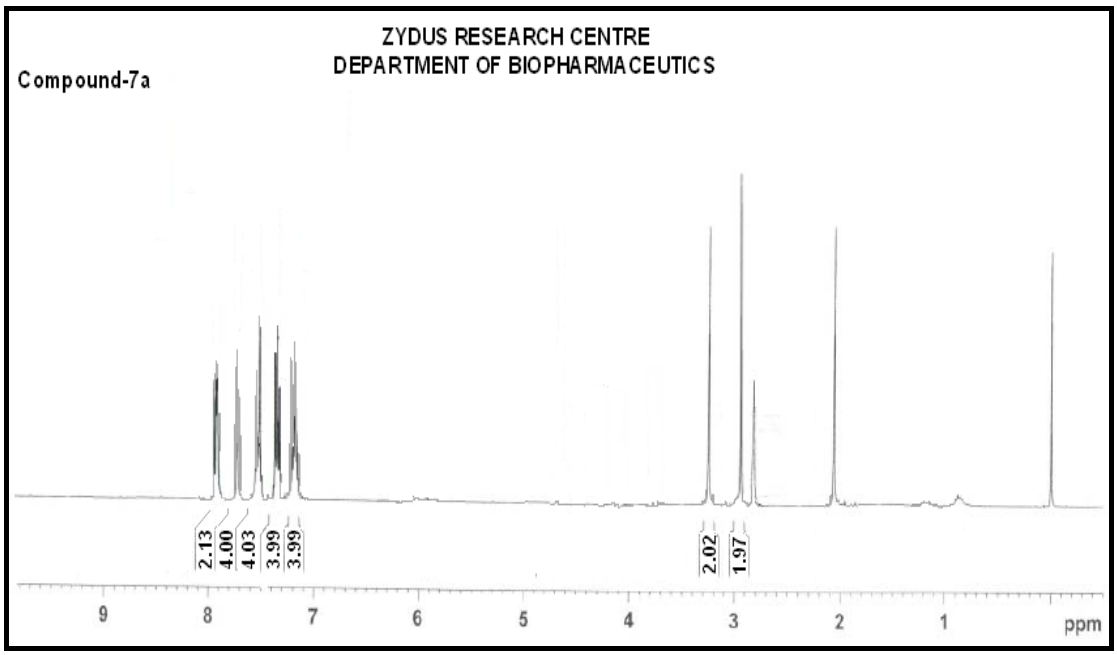
Detector A (220nm)

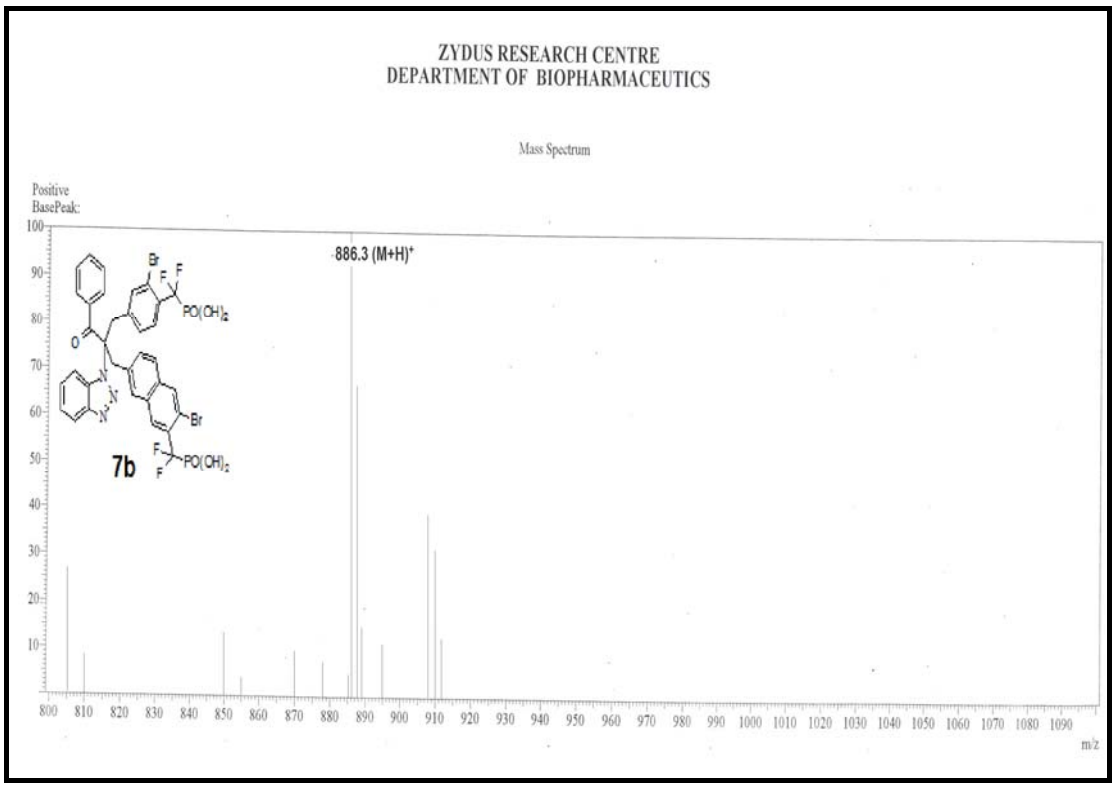
Pk #	Retention Time	Area	Area %
1	22.63	19025	0.22
2	22.79	8482320	98.53
3	22.99	30585	0.36
4	23.32	13956	0.16
5	23.40	17200	0.20
6	26.57	45598	0.53
Totals		8608684	100.00

ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

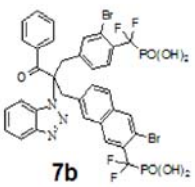


7a


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-7a
2.13
4.00
4.03
3.99
3.99
2.02
1.97
9
8
7
6
5
4
3
2
1
ppm


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
886.3 (M+H)+
7b
m/z

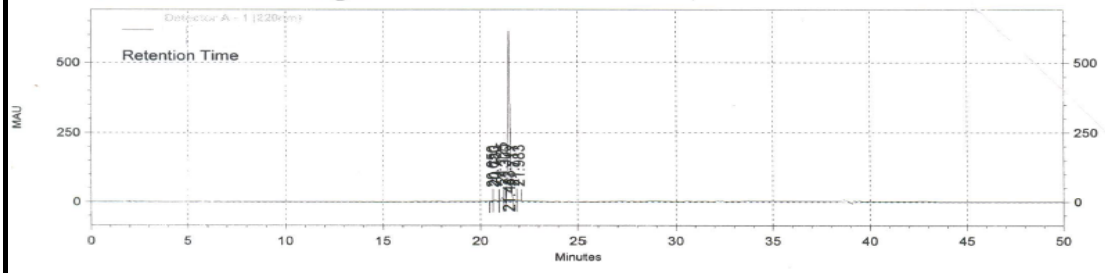
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS



7b

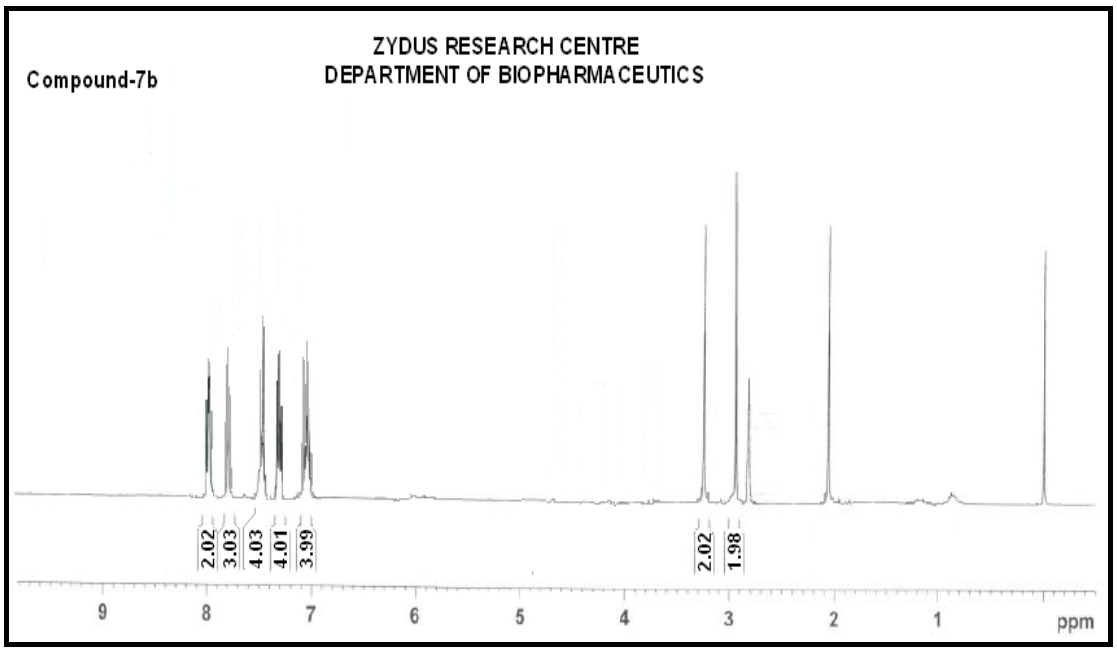
Compound-7b

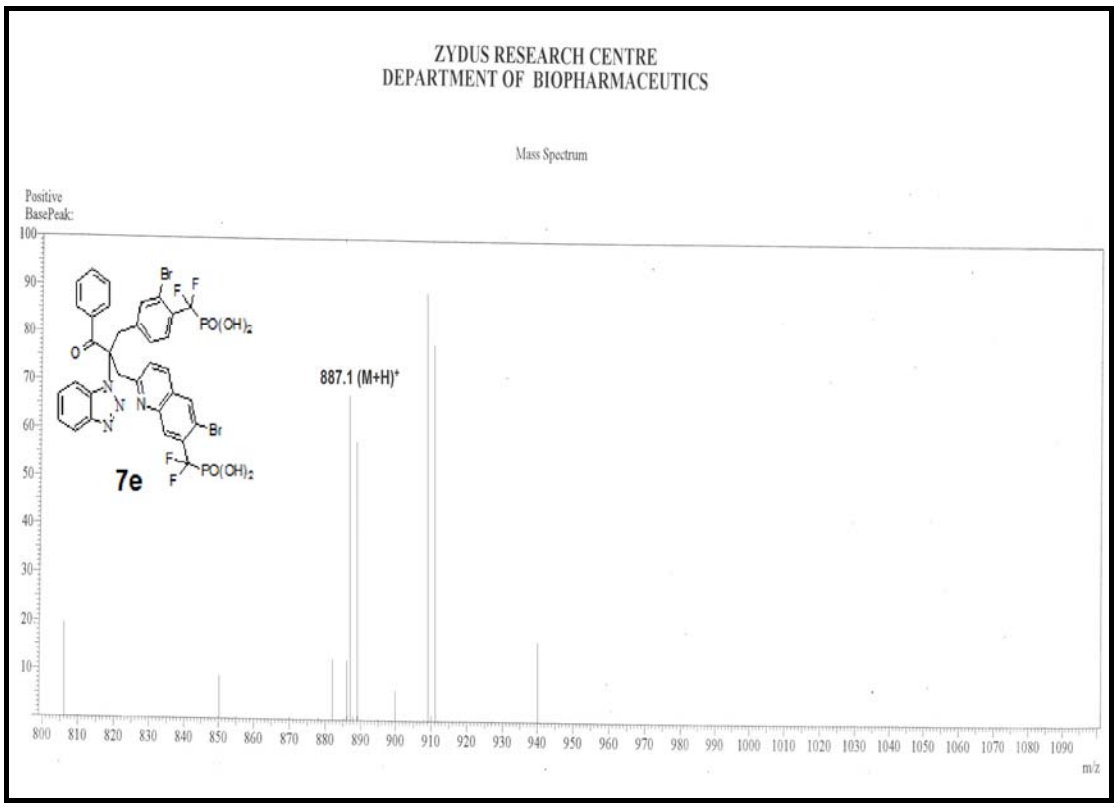
Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm



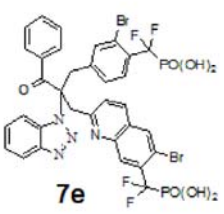
Detector A - 1 (220nm)

Pk #	Retention Time	Area	Area %
1	20.65	14522	0.27
2	20.78	51475	0.96
3	21.13	106898	1.98
4	21.30	19422	0.36
5	21.48	5136361	95.36
6	21.72	27301	0.51
7	21.98	30145	0.56
Totals		5386124	100.00


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-7b
2.02
3.03
4.03
4.01
3.99
2.02
1.98
9
8
7
6
5
4
3
2
1
ppm


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
887.1 (M+H)+
7e
m/z

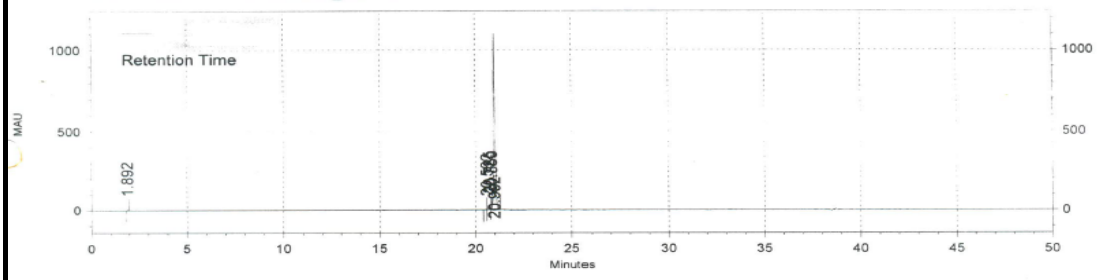
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS



7e

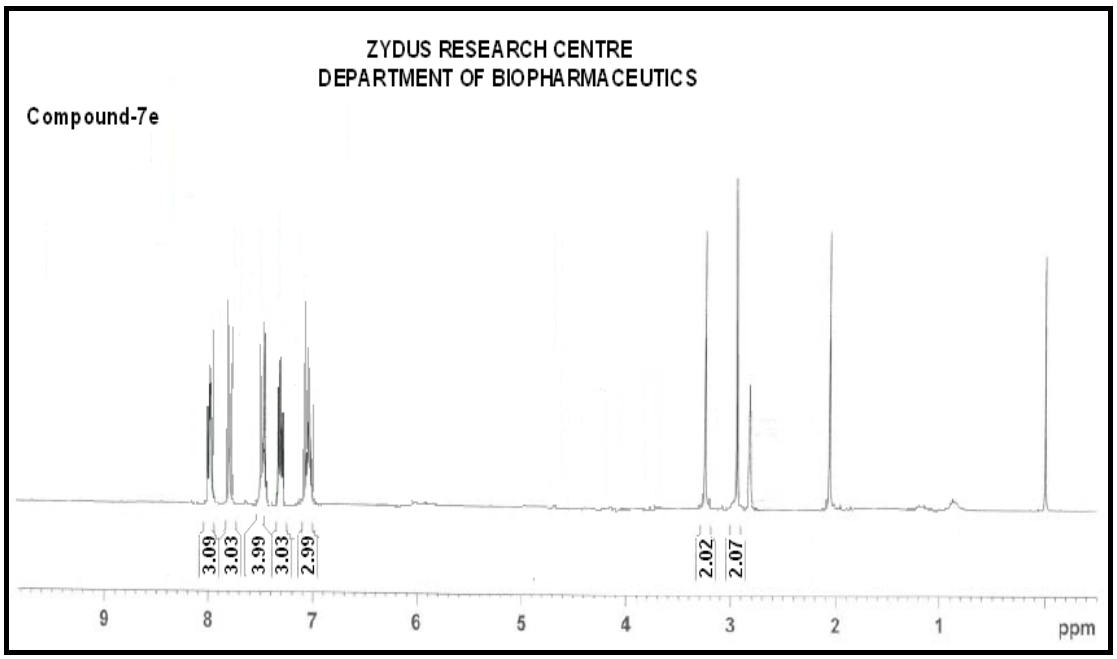
Compound-7e

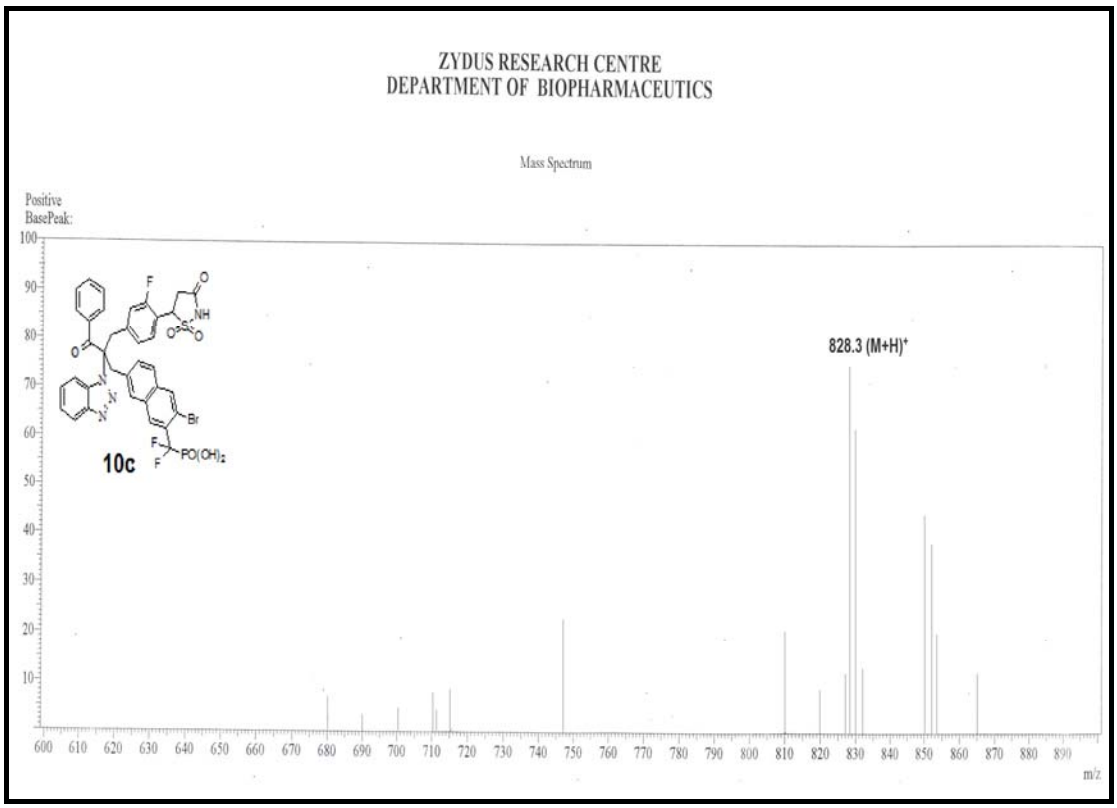
Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm



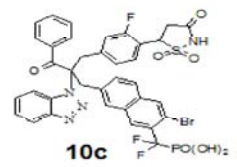
Detector A (220nm)

Pk #	Retention Time	Area	Area %
1	1.89	17710	0.28
2	20.59	12559	0.20
3	20.73	129427	2.01
4	20.85	66339	1.03
5	20.99	6213516	96.49
Totals		6439551	100.00


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-7e
3.09
3.03
3.99
3.03
2.99
2.02
2.07
9
8
7
6
5
4
3
2
1
ppm


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
828.3 (M+H)+
10c

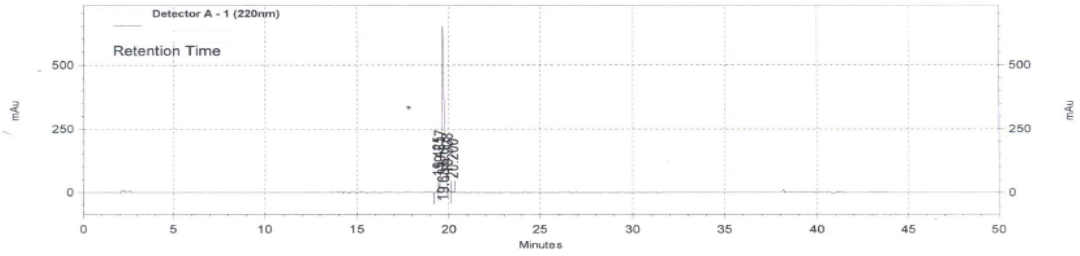
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS



10c

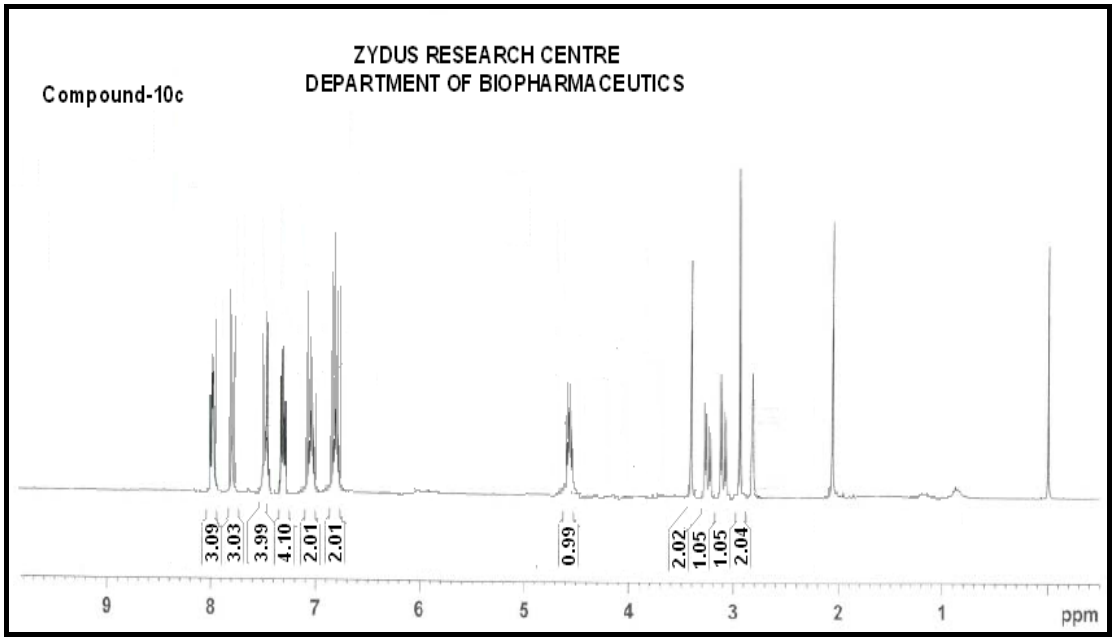
Compound-10c

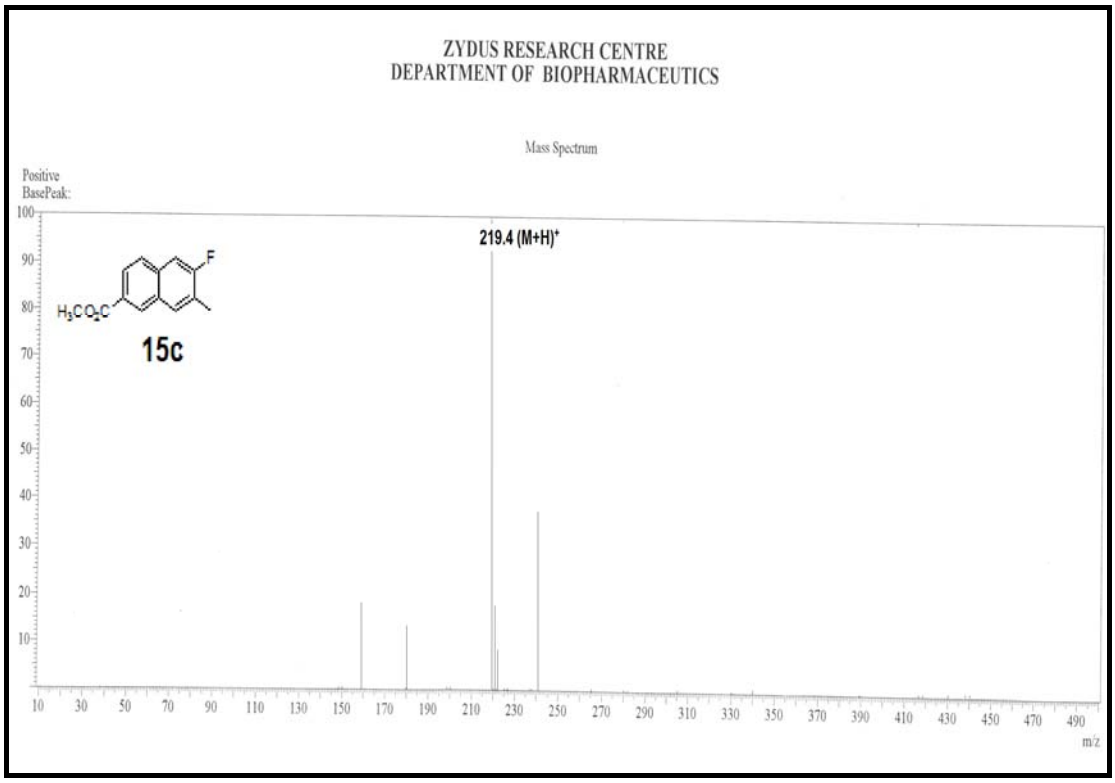
Column : Thermo-Syncronis-AQ[150*4.6mm] 5um
M.P. : 0.1%TFA IN WATER: ACN[GRADIENT]
Flow : 1.0 ml/min@220nm



Detector A - 1 (220nm)

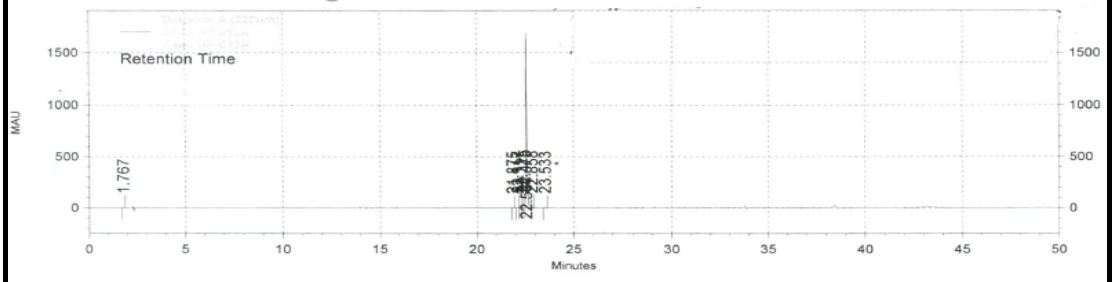
Pk #	Retention Time	Area	Area Percent
1	19.425	53381	0.83
2	19.517	131777	2.05
3	19.683	6103693	95.10
4	19.908	111891	1.74
5	20.200	17316	0.27
Totals		6418058	100.00


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-10c
3.09
3.03
3.99
4.10
2.01
2.01
0.99
2.02
1.05
1.05
2.04
9
8
7
6
5
4
3
2
1
ppm


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
219.4 (M+H)+
F
H3CO2C
15c
m/z

Compound-15c

Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm

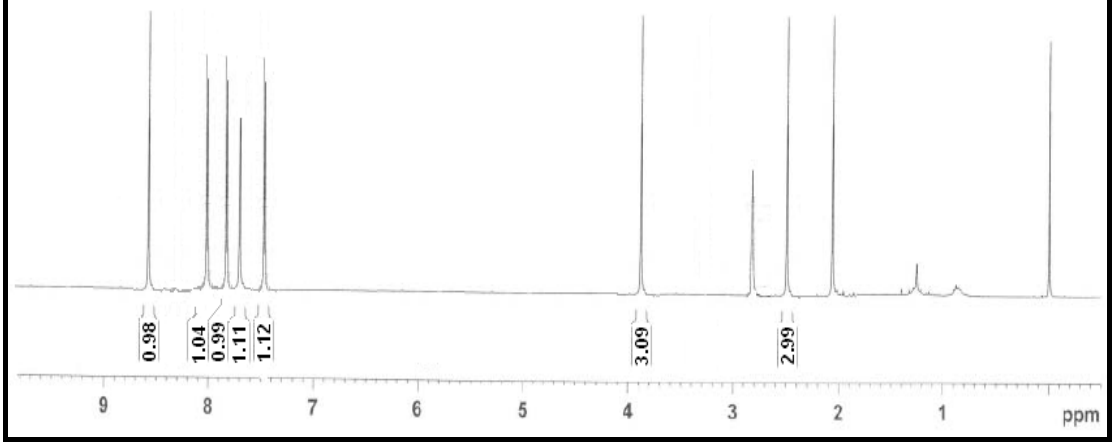


Detector A (220nm)

Pk #	Retention Time	Area	Area %
1	1.77	7288	0.09
2	21.88	5356	0.06
3	22.12	7569	0.09
4	22.32	41792	0.51
5	22.48	93622	1.13
6	22.57	8066677	97.62
7	22.86	25452	0.31
8	23.53	15236	0.18
Totals		8262992	100.00

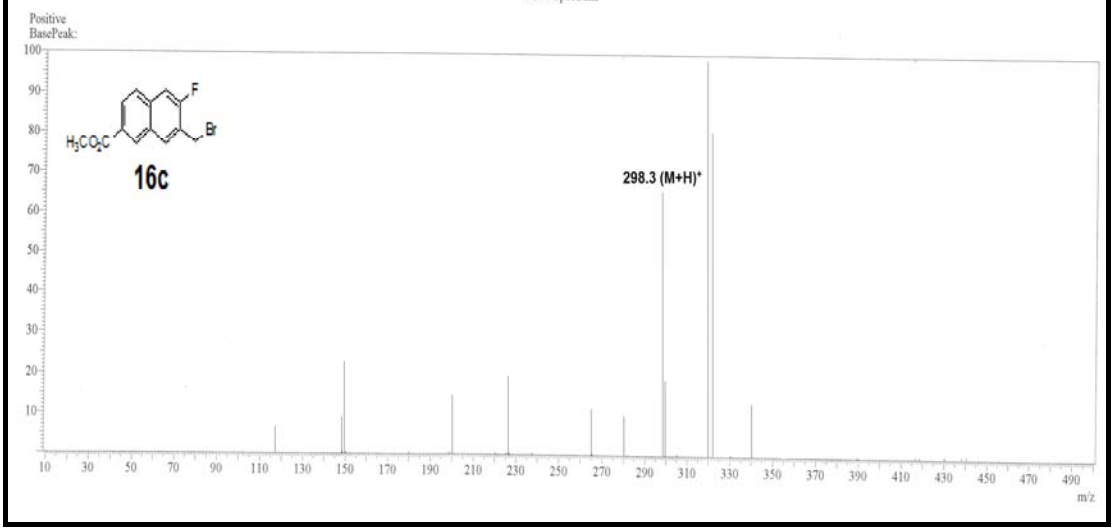
Compound-15c

ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS



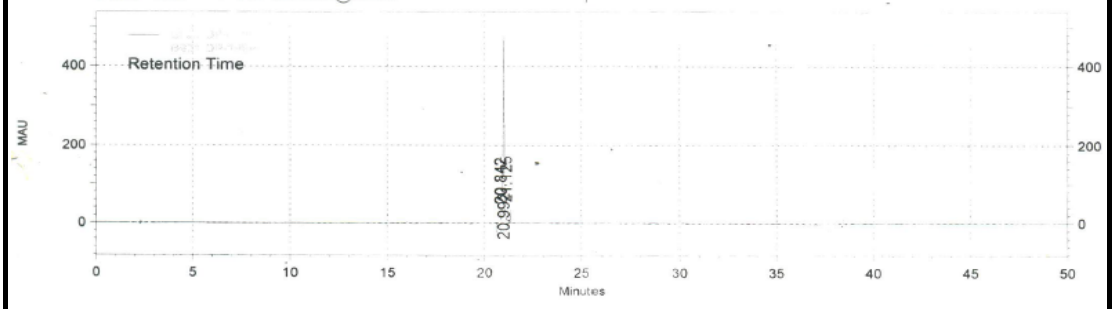
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum



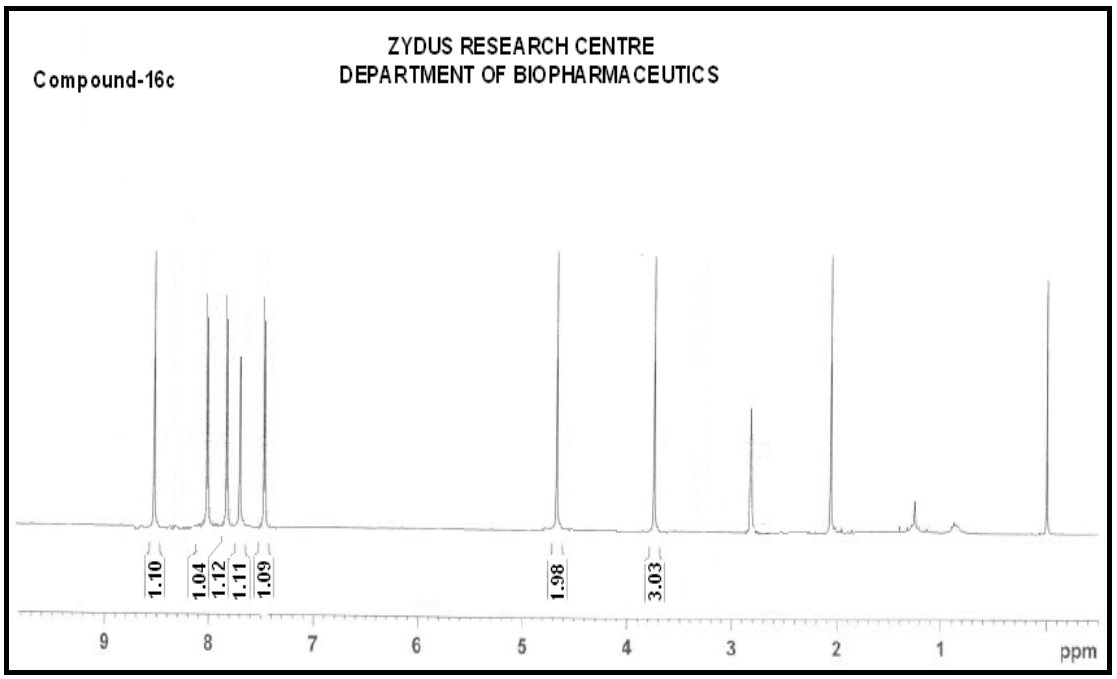
Compound-16c

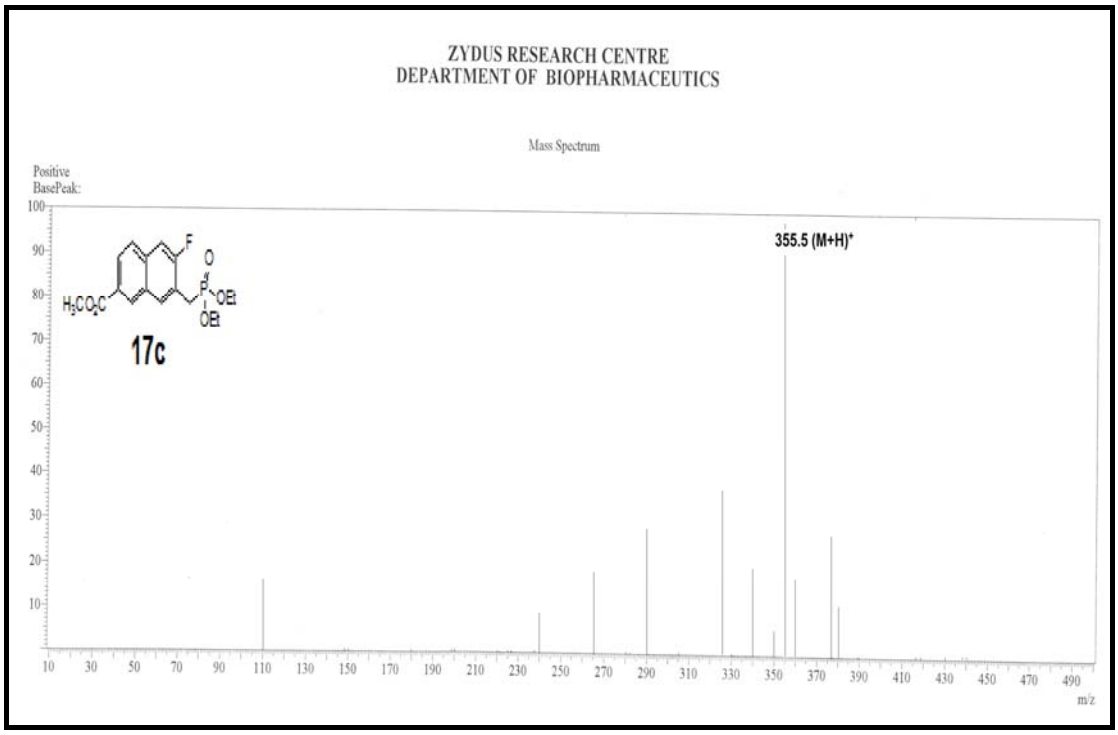
Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220n



Detector A (220nm)

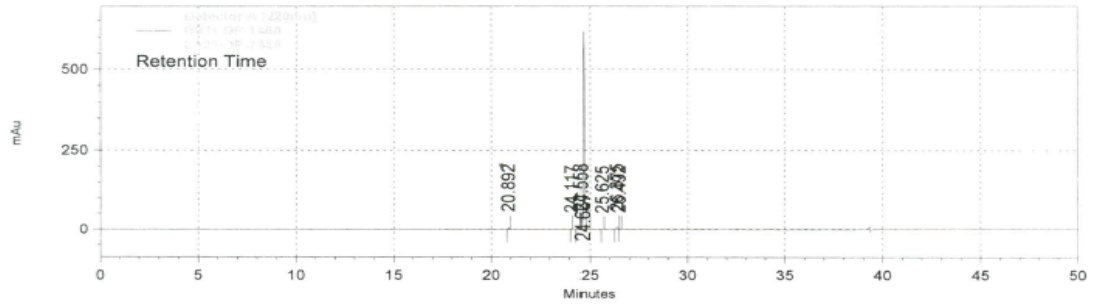
Pk #	Retention Time	Area	Area %
1	20.84	36742	1.51
2	20.99	2350072	96.47
3	21.13	49305	2.02
Totals		2436119	100.00


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-16c
1.10
1.04
1.12
1.11
1.09
1.98
3.03
9
8
7
6
5
4
3
2
1
ppm


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
355.5 (M+H)+
17c
m/z

Compound-17c

Column :YMC-ODS-AQ,[150x4.6mm],5um Injection volume:10 uL
Mobile phase :[0.1% TFA in water:ACN] (Gradient) Vial no.: 10008
Flow rate :1.0 mL/min@220nm

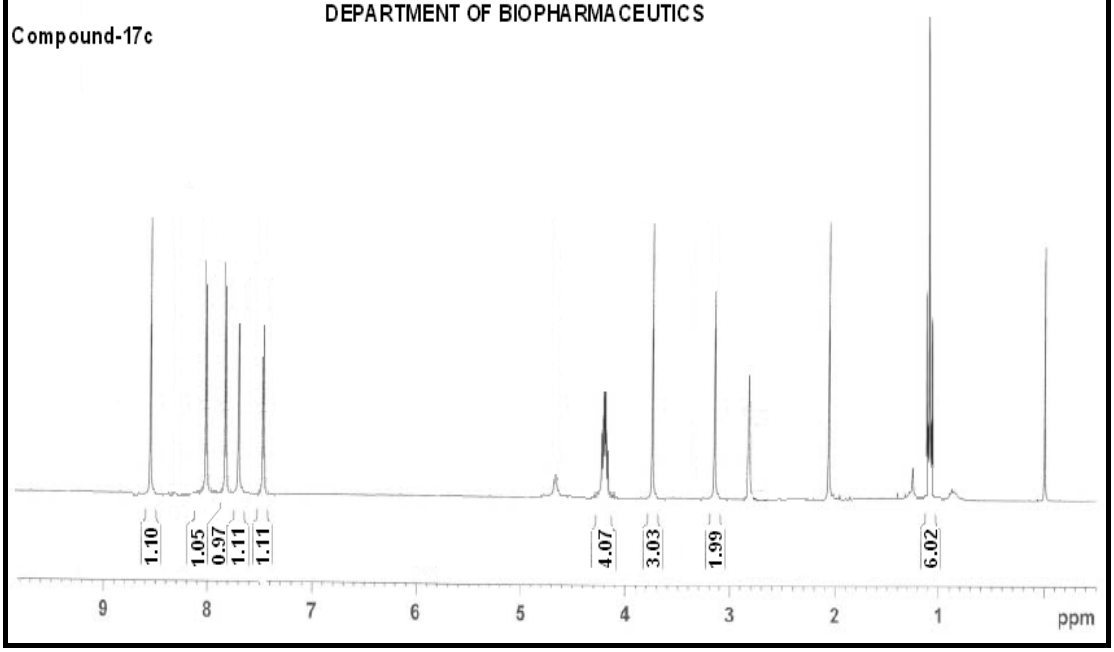


Detector A
(220nm)

Pk #	Retention Time	Area	Area Percent
1	20.892	25003	0.65
2	24.117	2747	0.07
3	24.558	19764	0.51
4	24.667	3773152	97.63
5	25.625	2321	0.06
6	26.375	32875	0.85
7	26.492	8916	0.23
Totals		3864778	100.00

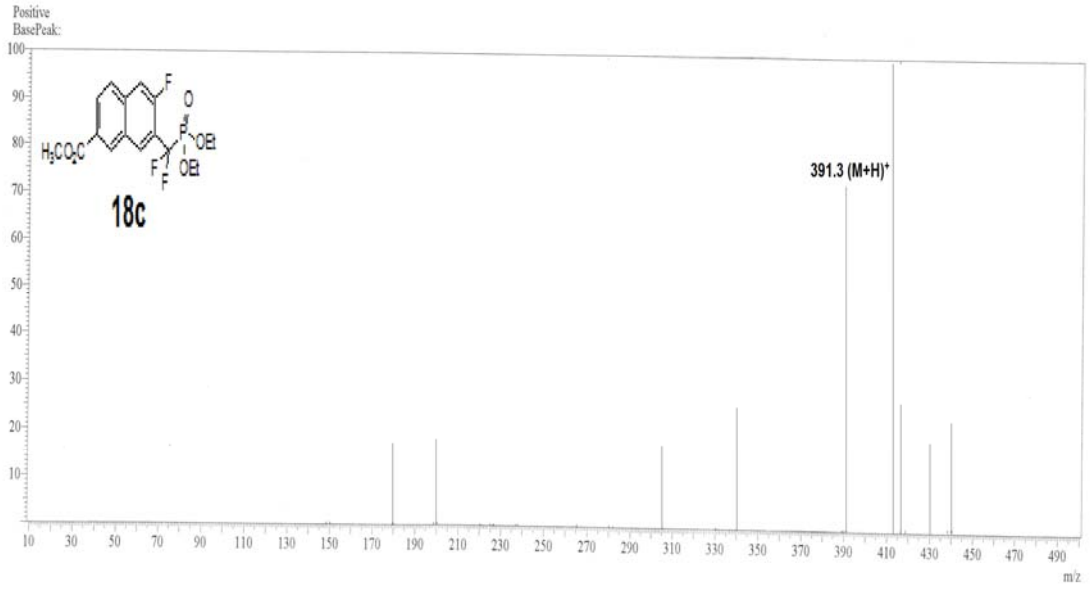
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Compound-17c



ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

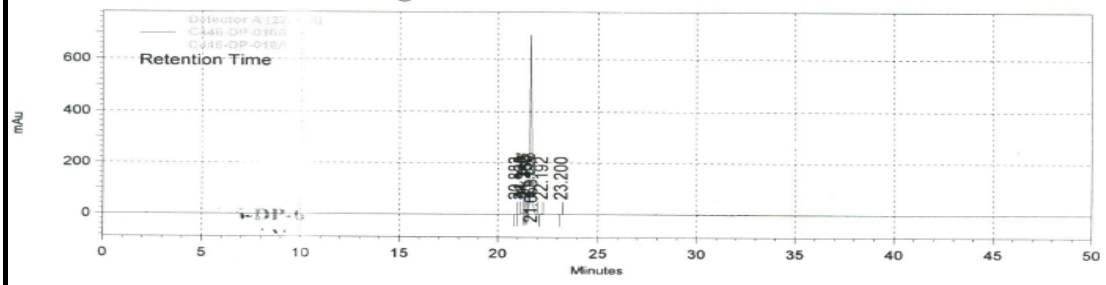
Mass Spectrum



18c

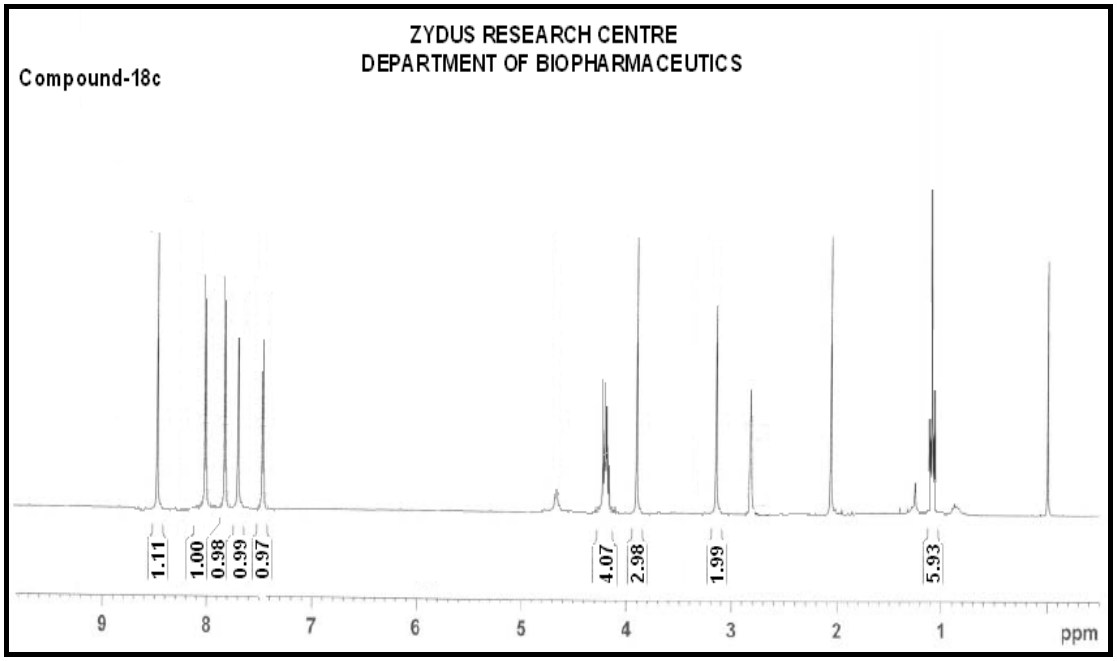
Compound-18c

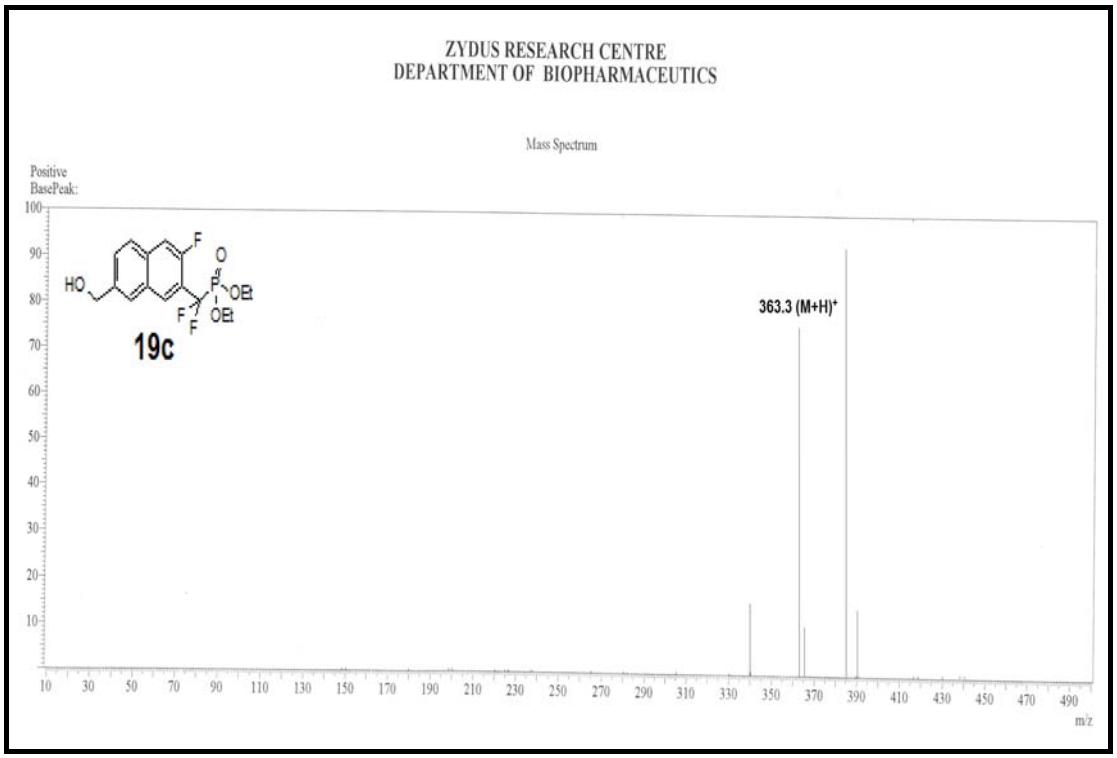
Column :YMC-ODS-AQ,[150x4.6mm],5um
Mobile phase :[0.1% TFA in water:ACN] (Gradient)
Flow rate :1.0 mL/min@220nm
Injection volume:10 uL
Vial no.: 10002



Detector A
(220nm)

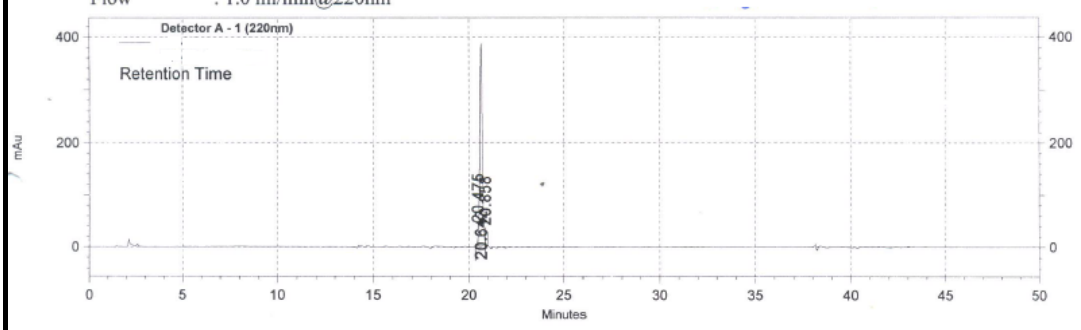
Pk #	Retention Time	Area	Area Percent
1	20.883	6589	0.13
2	21.125	11021	0.23
3	21.242	50923	1.04
4	21.333	26829	0.55
5	21.450	36402	0.74
6	21.533	62572	1.28
7	21.658	4683474	95.77
8	22.192	9692	0.20
9	23.200	2665	0.05
Totals		4890167	100.00


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-18c
1.11
1.00
0.98
0.99
0.97
4.07
2.98
1.99
5.93
9
8
7
6
5
4
3
2
1
ppm


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
HO
F
O
P
OEt
F
F
OEt
19c
363.3 (M+H)+
m/z

Compound-19c

Column : Thermo-Syncronis-AQ[150*4.6mm] 5um
M.P. : 0.1%TFA IN WATER: ACN[GRADIENT]
Flow : 1.0 ml/min@220nm

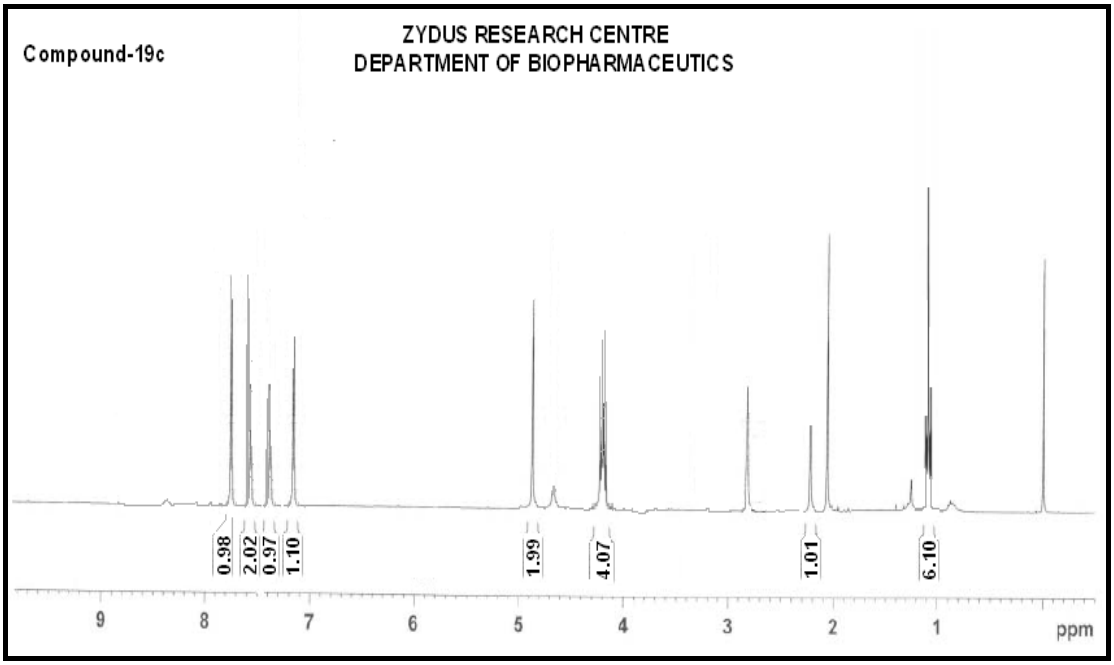


Detector A - 1 (220nm)

Pk #	Retention Time	Area	Area Percent
1	20.475	40693	1.12
2	20.642	3575413	98.14
3	20.858	27150	0.75
Totals		3643256	100.00

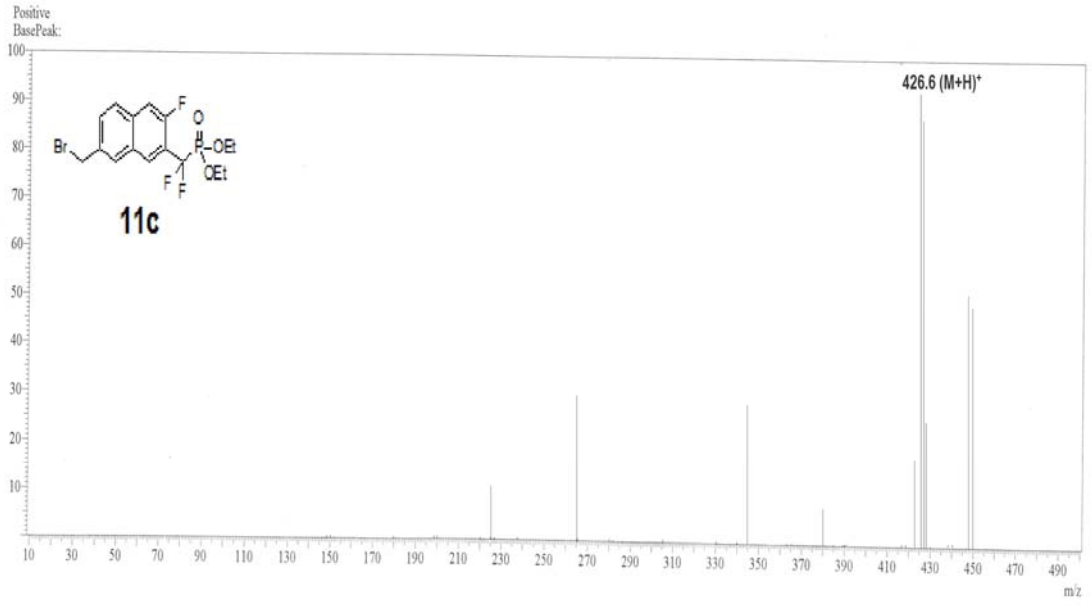
Compound-19c

ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS



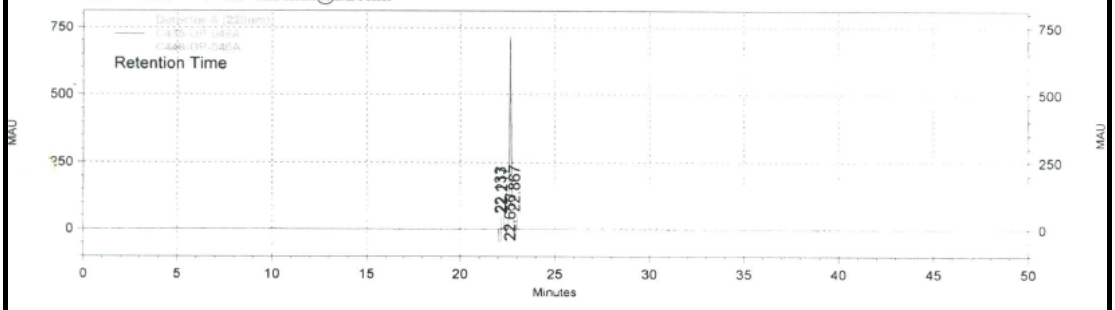
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum



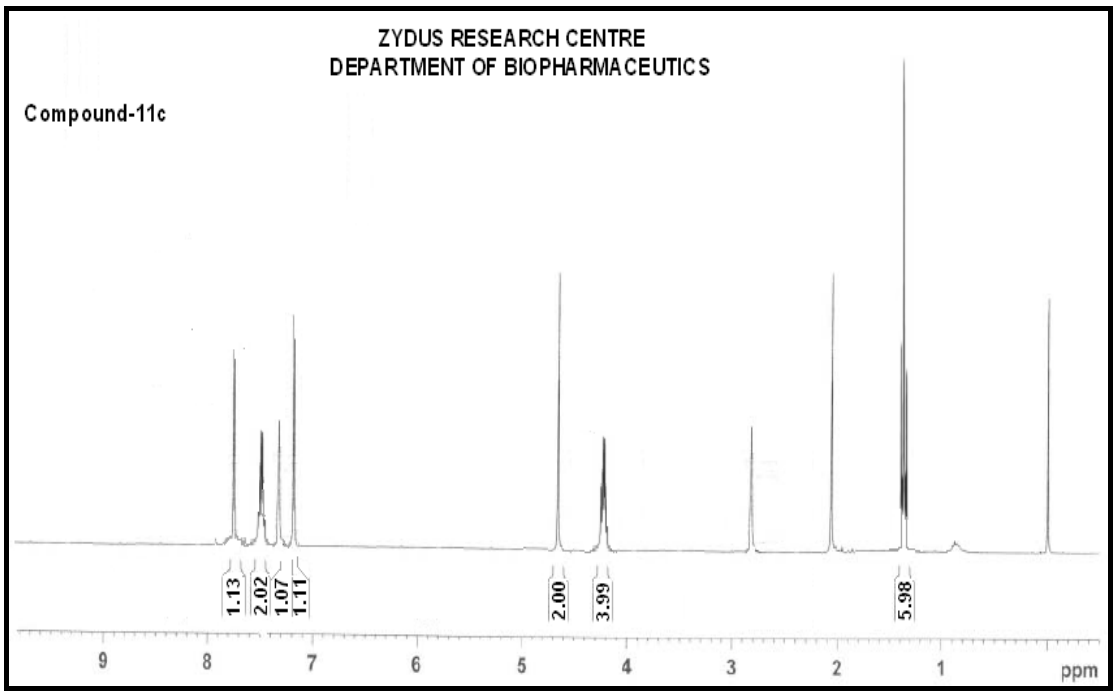
Compound-11c

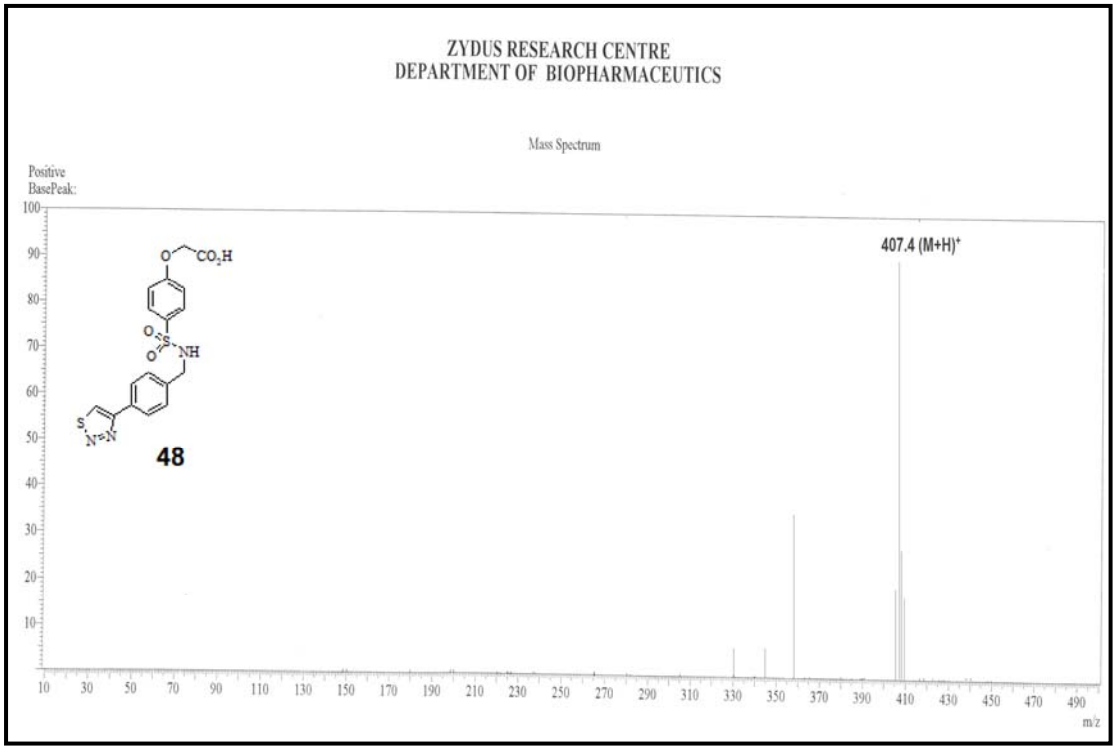
Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm



Detector A (220nm)

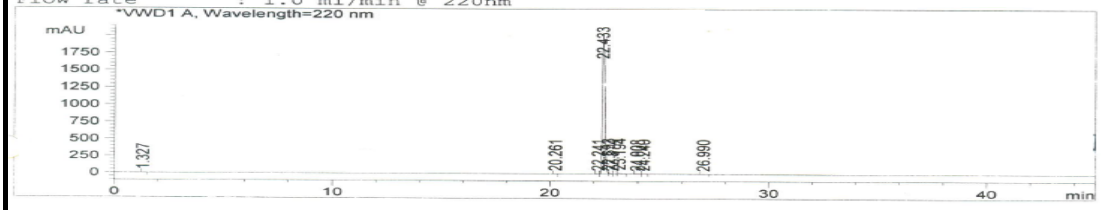
Pk #	Retention Time	Area	Area %
1	22.13	1500	0.03
2	22.22	11279	0.26
3	22.65	4299244	98.95
4	22.87	32892	0.76
Totals		4344915	100.00


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-11c
1.13
2.02
1.07
1.11
2.00
3.99
5.98
9
8
7
6
5
4
3
2
1
ppm


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
407.4 (M+H)+
48

Compound-48

Column : YMC J'Sphere C18(150*4.6mm)4u
Mobile phase : 0.05% TFA IN WATER:ACN (Gradient)
Flow rate : 1.0 ml/min @ 220nm



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

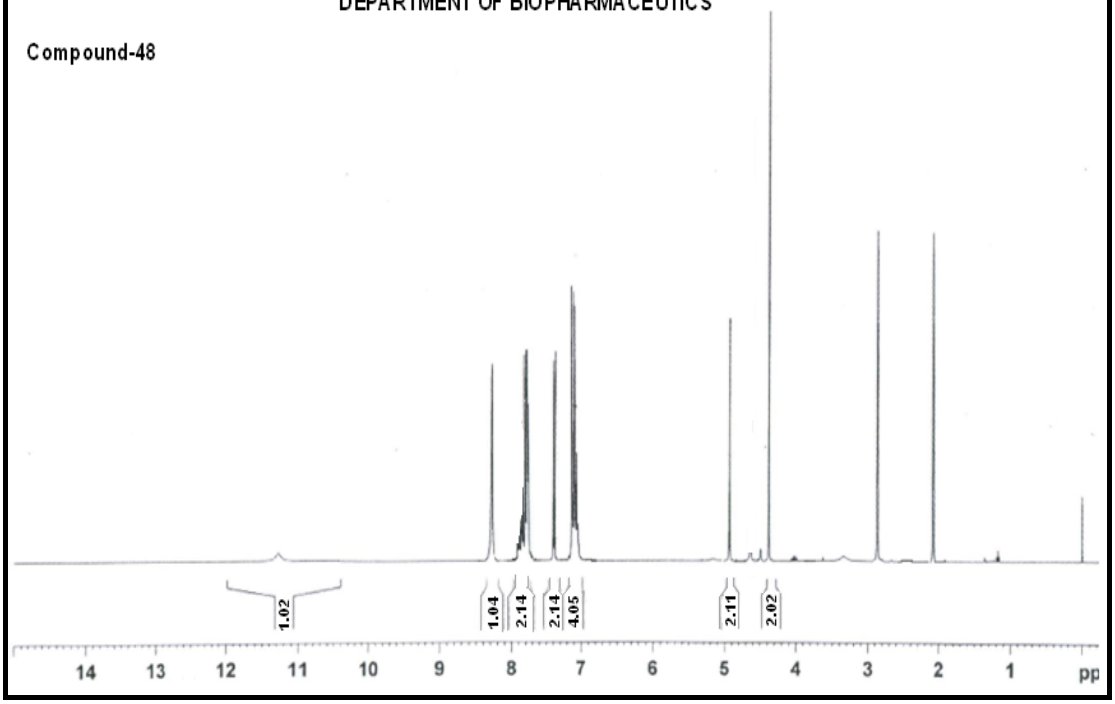
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	1.327	PBA	0.0803	5.75686	1.04404	0.0335
2	20.261	BBA	0.0826	2.15768	4.04431e-1	0.0126
3	22.241	BV	0.0803	12.64238	2.40412	0.0736
4	22.433	MF R	0.1371	1.68774e4	2051.78613	98.1928
5	22.693	MF R	0.0764	98.85528	15.85470	0.5751
6	22.874	FM R	0.1182	20.38837	2.87487	0.1186
7	23.194	VBA	0.1103	125.78972	17.15904	0.7318
8	24.008	BV	0.1037	3.78015	5.79817e-1	0.0220
9	24.240	VBA	0.1092	31.34989	4.48431	0.1824
10	26.990	BBA	0.1629	9.90734	9.46253e-1	0.0576

Totals : 1.71881e4 2097.53771

Results obtained with enhanced integrator!

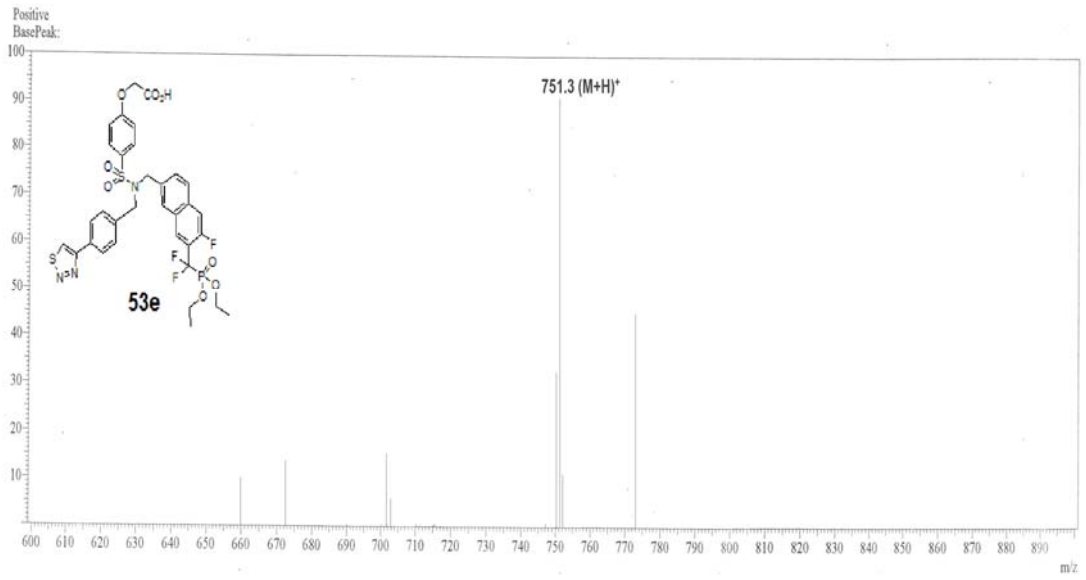
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Compound-48



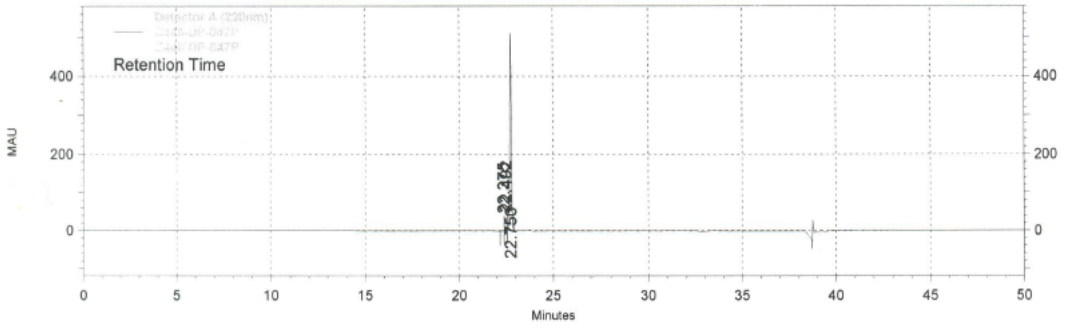
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum



Compound-53e

Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm

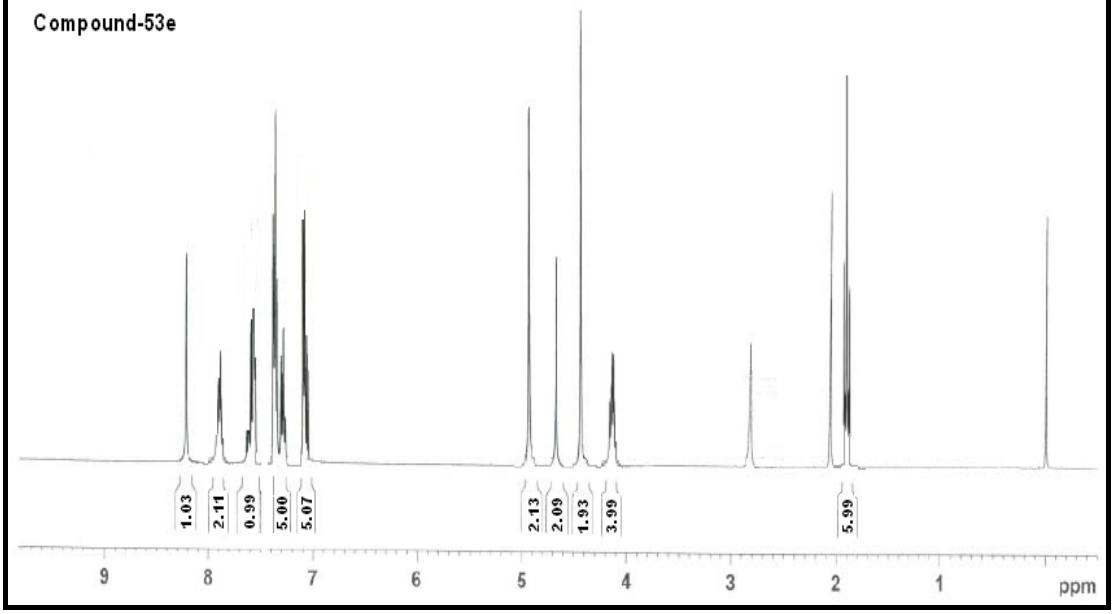


Detector A (220nm)

Pk #	Retention Time	Area	Area %
1	22.38	11990	0.37
2	22.49	46248	1.43
3	22.75	3184403	98.20
Totals		3242641	100.00

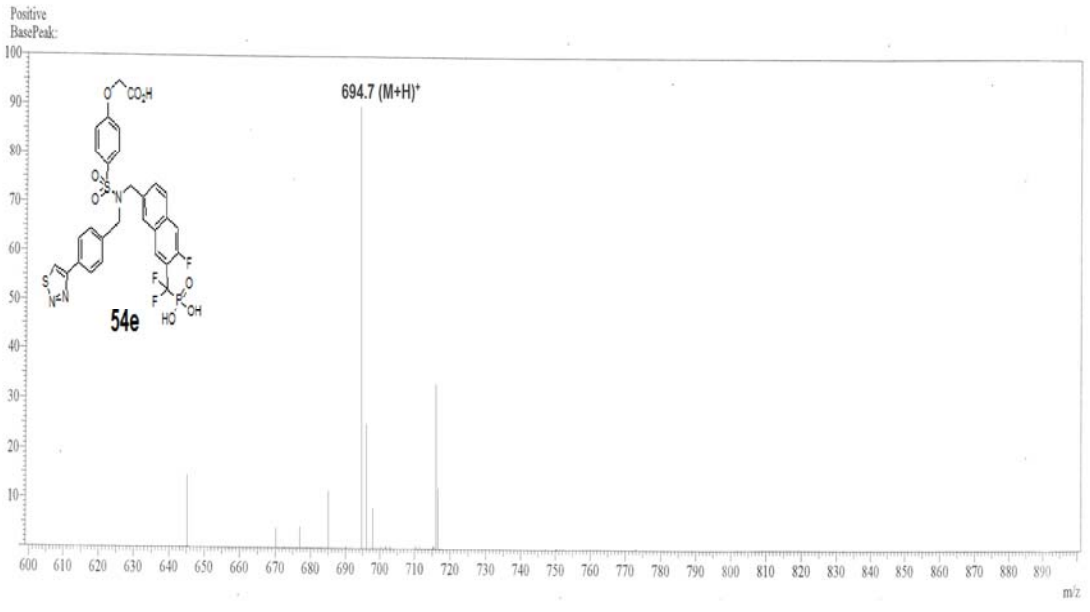
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Compound-53e

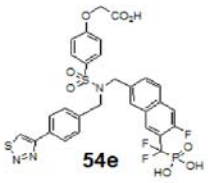


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum



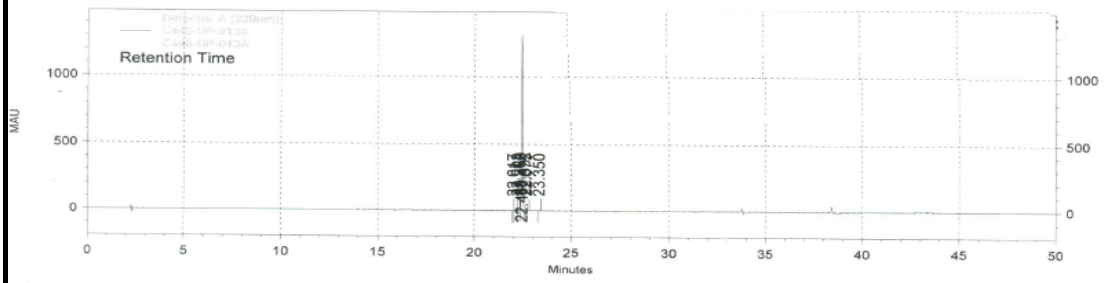
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS



54e

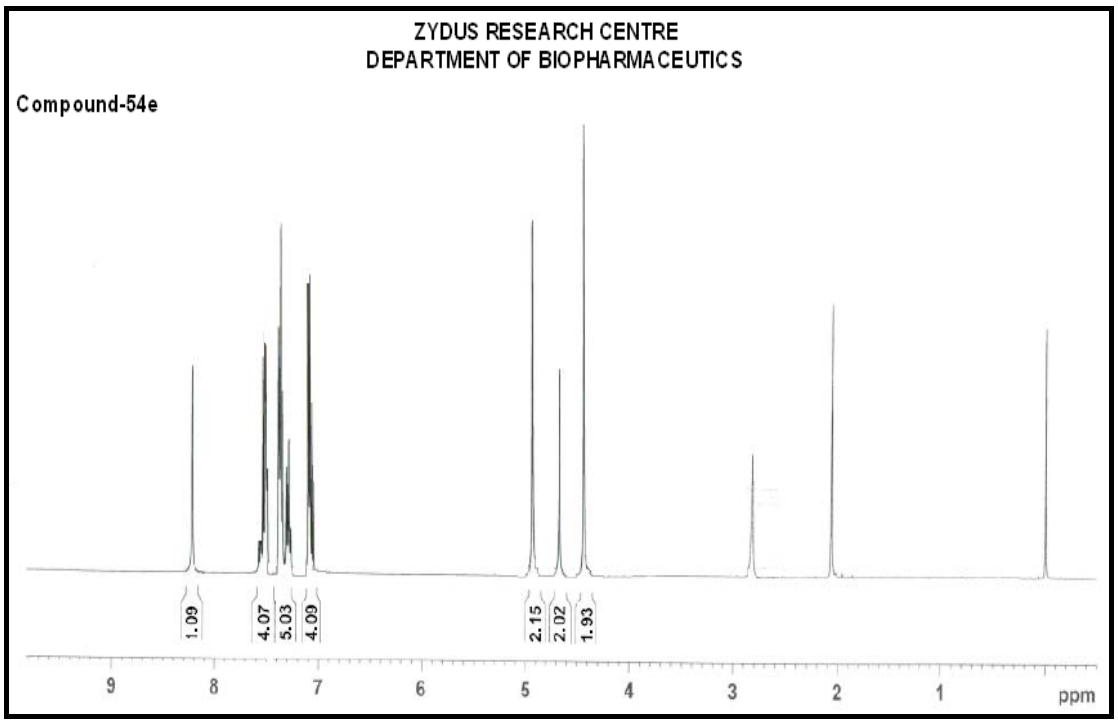
Compound-54e

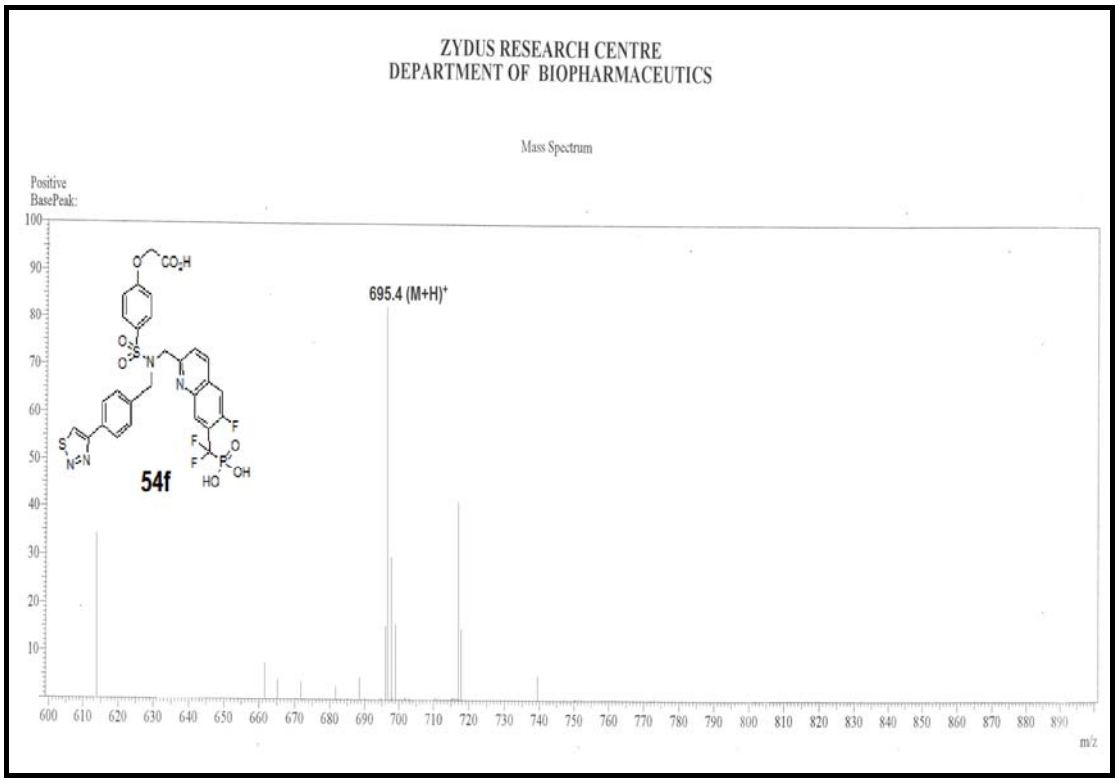
Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm



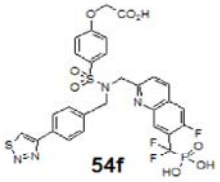
Detector A (220nm)

Pk #	Retention Time	Area	Area %
1	22.02	2837	0.05
2	22.21	27675	0.44
3	22.35	32481	0.52
4	22.45	6130578	97.83
5	22.59	48721	0.78
6	22.68	13935	0.22
7	23.35	10505	0.17
Totals		6266732	100.00


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-54e
1.09
4.07
5.03
4.09
2.15
2.02
1.93
9
8
7
6
5
4
3
2
1
ppm


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
695.4 (M+H)+
54f
m/z

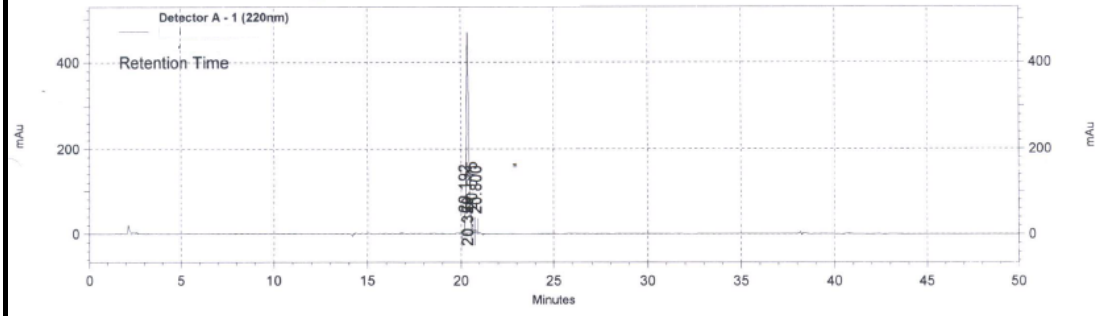
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS



54f

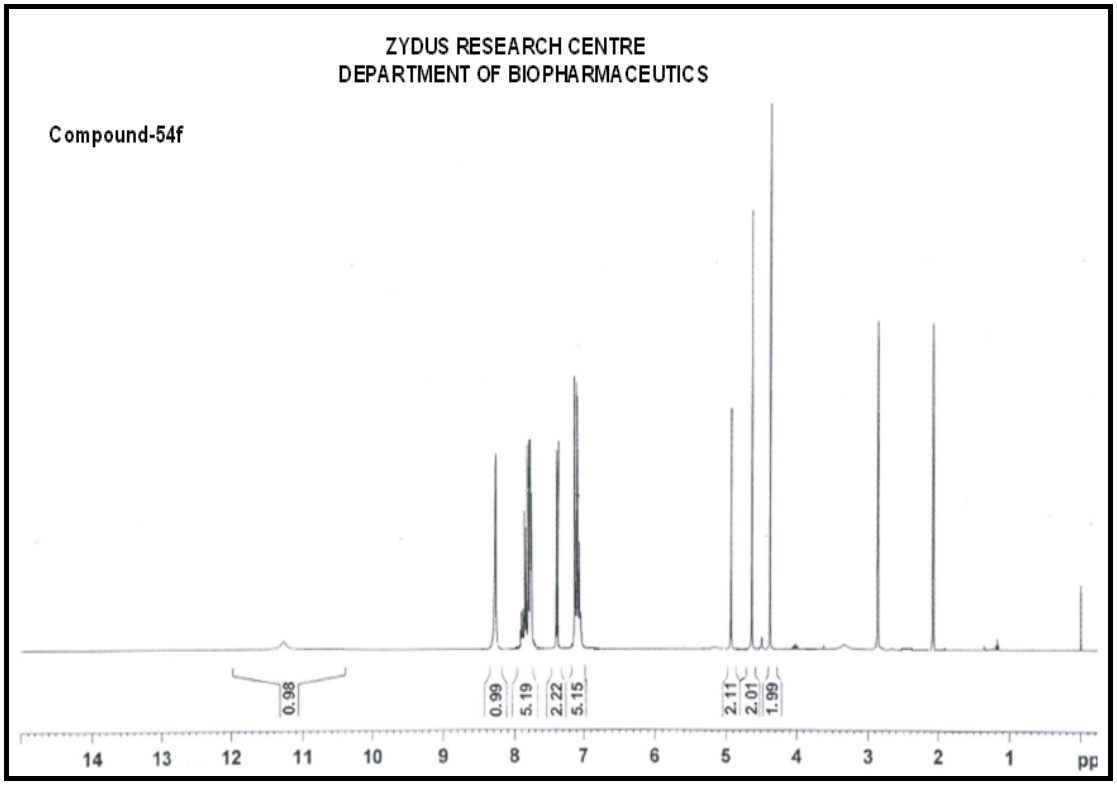
Compound-54f

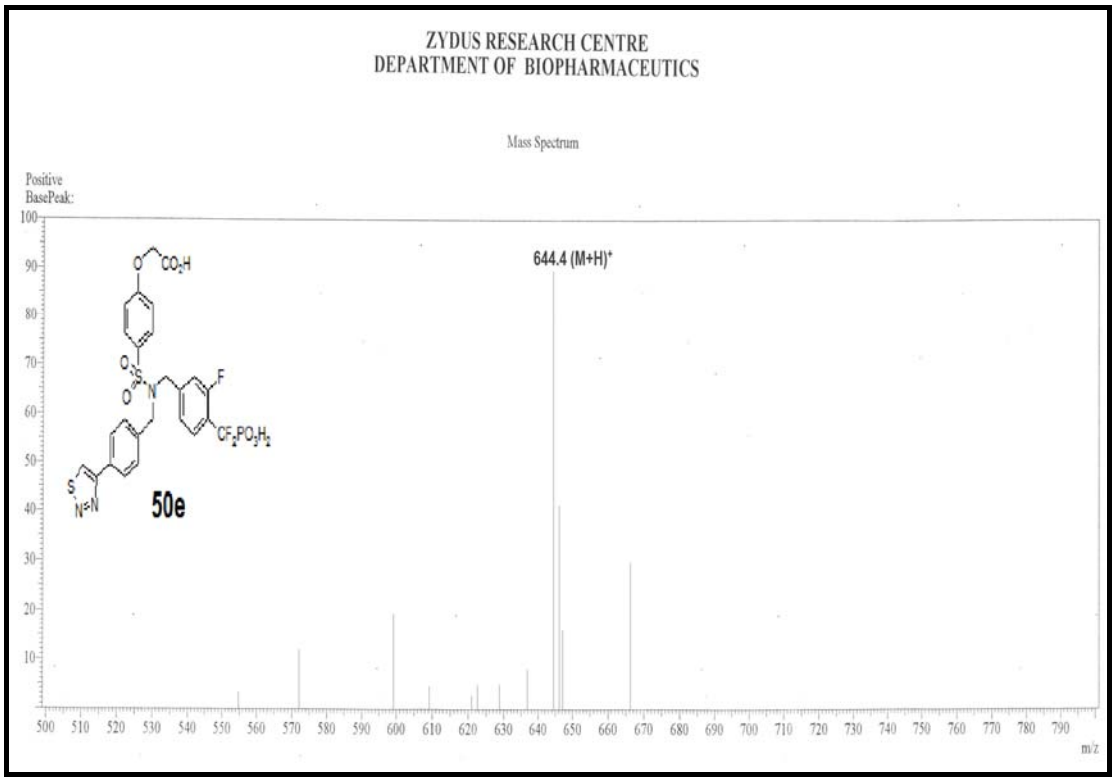
Column : Thermo-Syncronis-AQ[150*4.6mm] 5um
M.P. : 0.1%TFA IN WATER: ACN[GRADIENT]
Flow : 1.0 ml/min@220nm



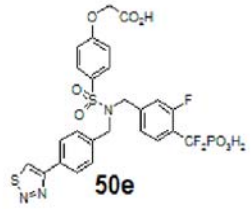
Detector A - 1 (220nm)

Pk #	Retention Time	Area	Area Percent
1	20.192	22658	0.54
2	20.358	4054305	96.90
3	20.575	72876	1.74
4	20.800	34096	0.81
Totals		4183935	100.00


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-54f
0.98
0.99
5.19
2.22
5.15
2.11
2.01
1.99
14
13
12
11
10
9
8
7
6
5
4
3
2
1
pp


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
644.4 (M+H)+
50e

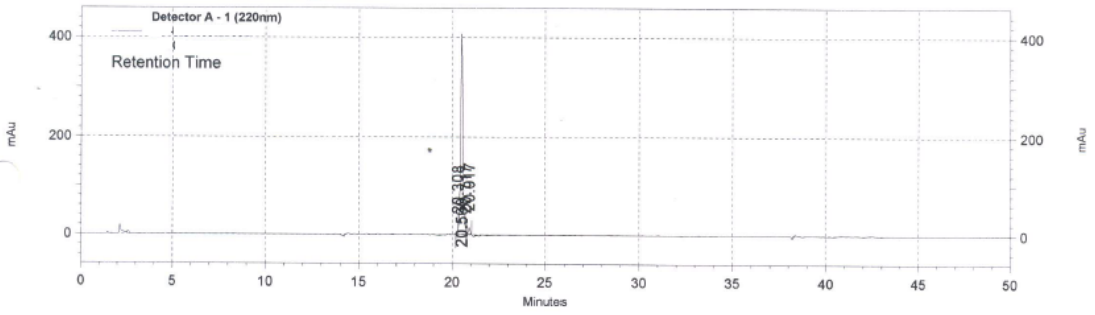
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS



50e

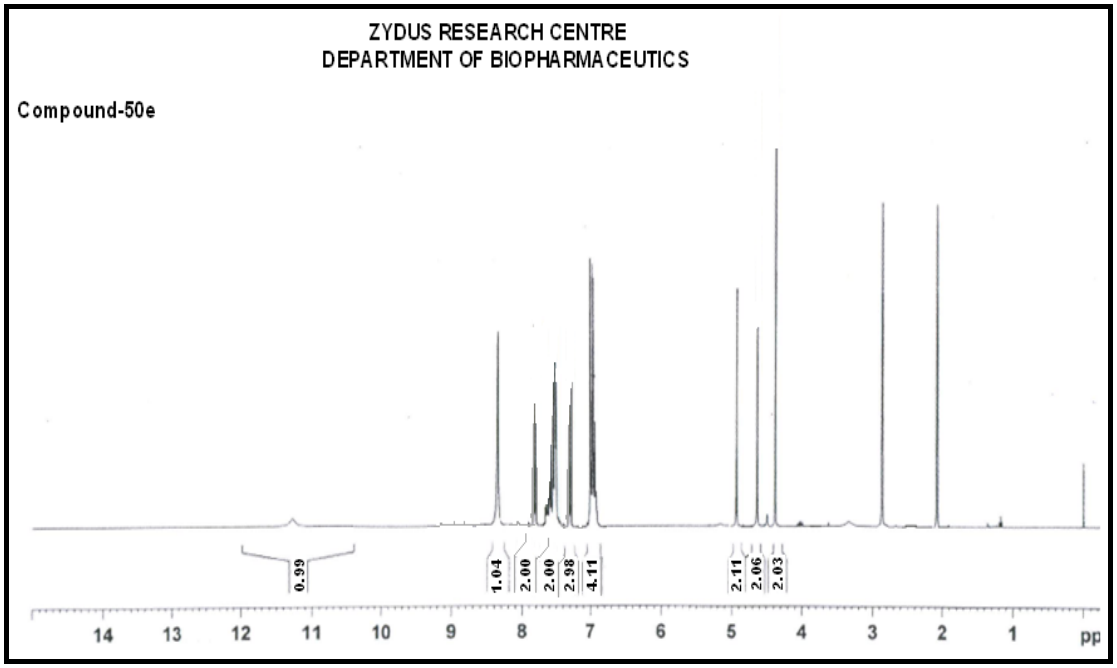
Compound-50e

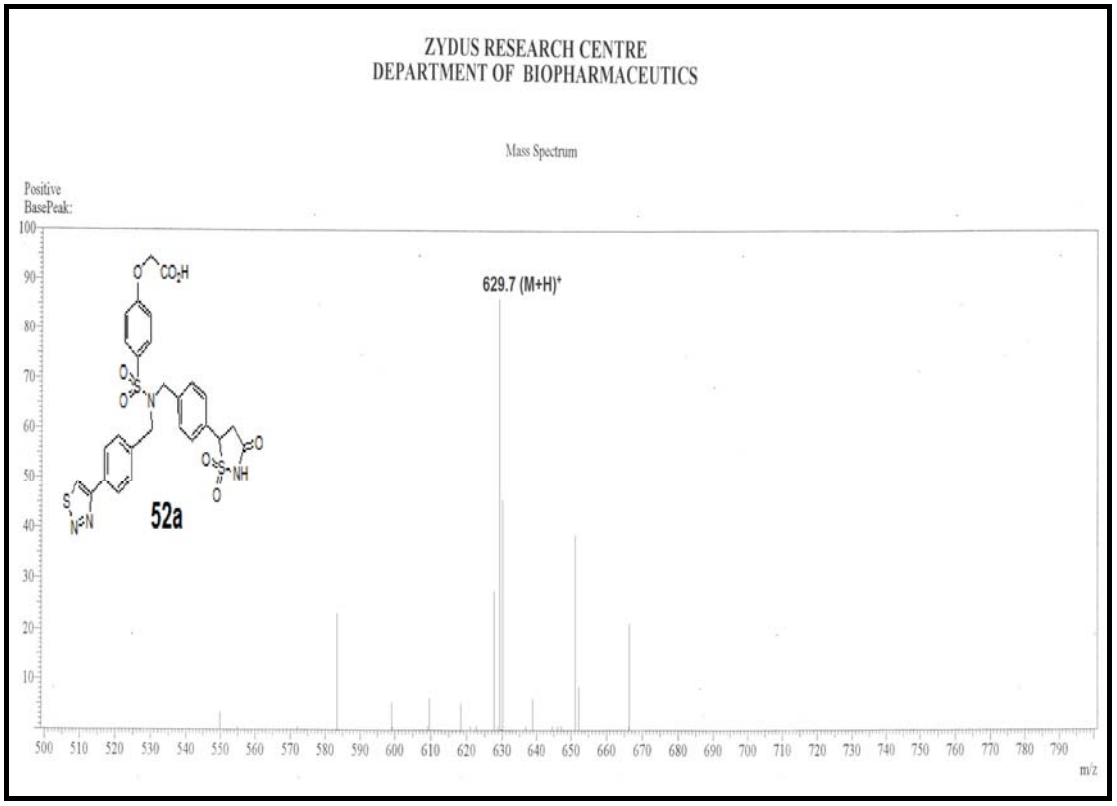
Column : Thermo-Syncronis-AQ[150*4.6mm] 5um
M.P. : 0.1%TFA IN WATER: ACN[GRADIENT]
Flow : 1.0 ml/min@220nm



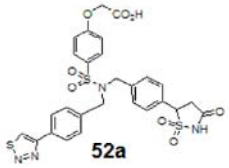
Detector A - 1 (220nm)

Pk #	Retention Time	Area	Area Percent
1	20.308	22560	0.59
2	20.500	3704758	96.29
3	20.717	29733	0.77
4	20.917	90616	2.36
Totals		3847667	100.00


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-50e
0.99
1.04
2.00
2.00
2.98
4.11
2.11
2.06
2.03
14 13 12 11 10 9 8 7 6 5 4 3 2 1 pp


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
629.7 (M+H)+
52a
m/z

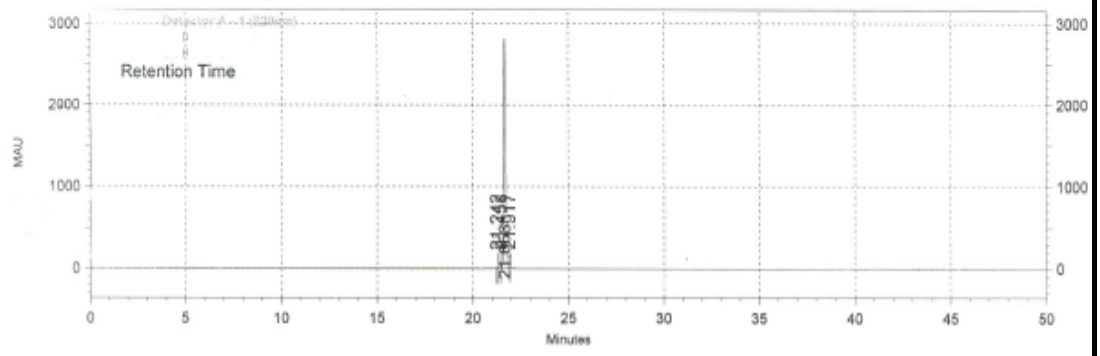
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS



52a

Compound-52a

Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm

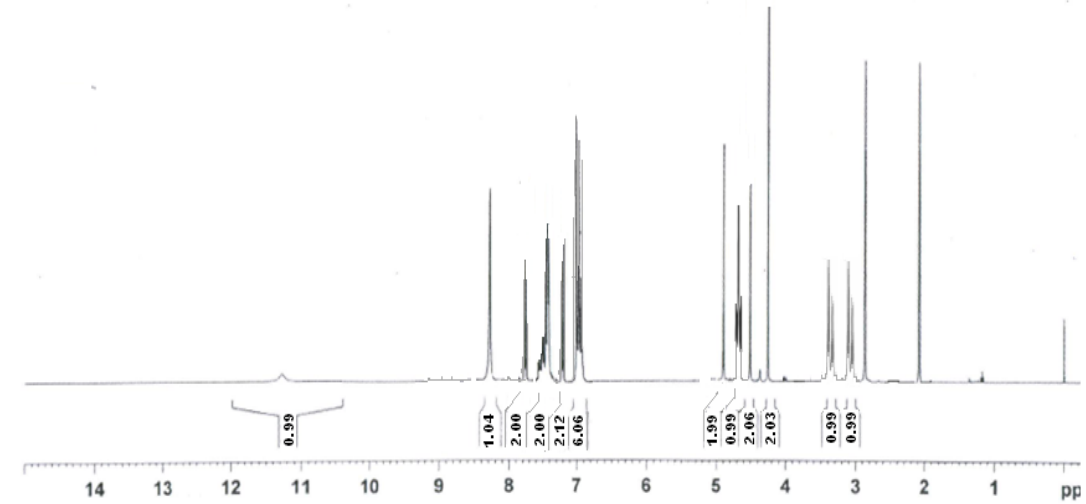


Detector A - 1 (220nm)

Pk #	Retention Time	Area	Area %
1	21.24	14498	0.07
2	21.46	15197	0.07
3	21.66	20413079	99.82
4	21.92	7546	0.04
Totals		20450320	100.00

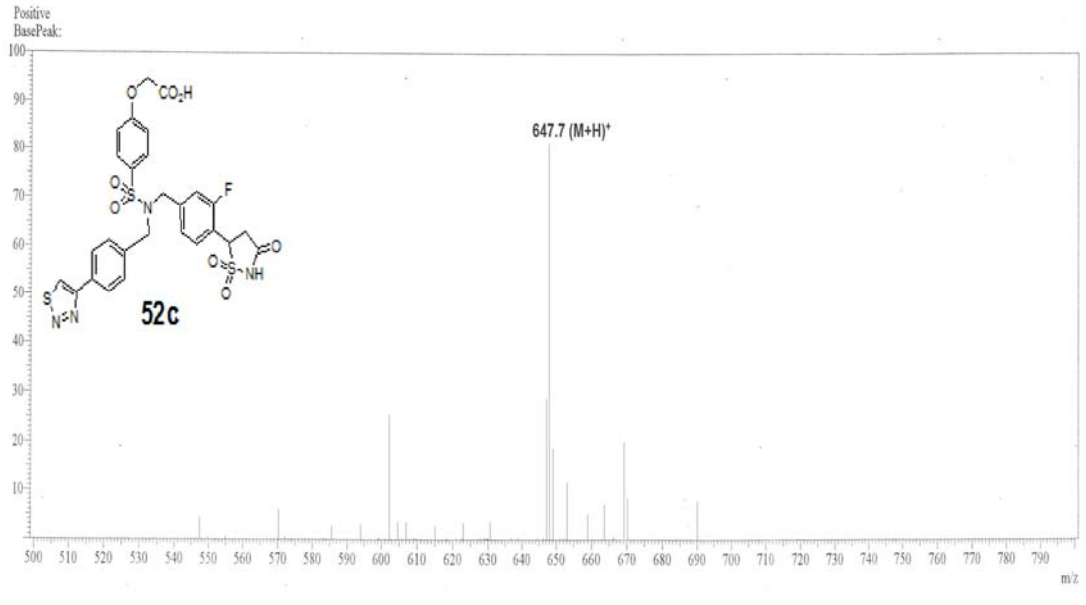
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Compound-52a

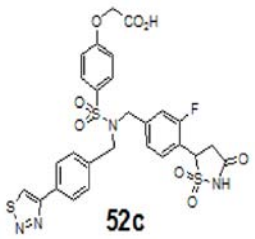


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum



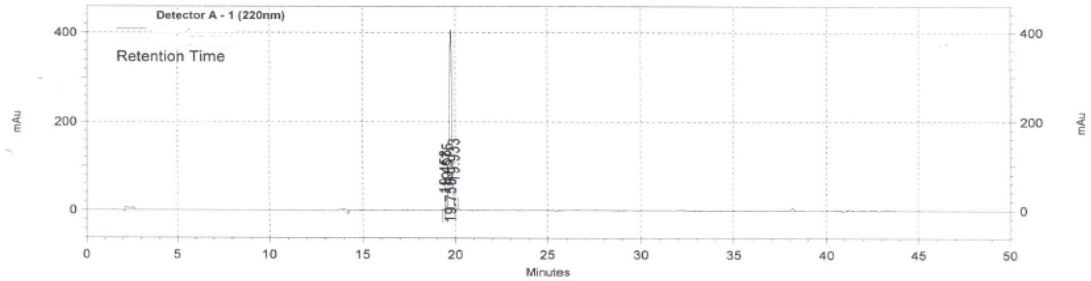
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS



52c

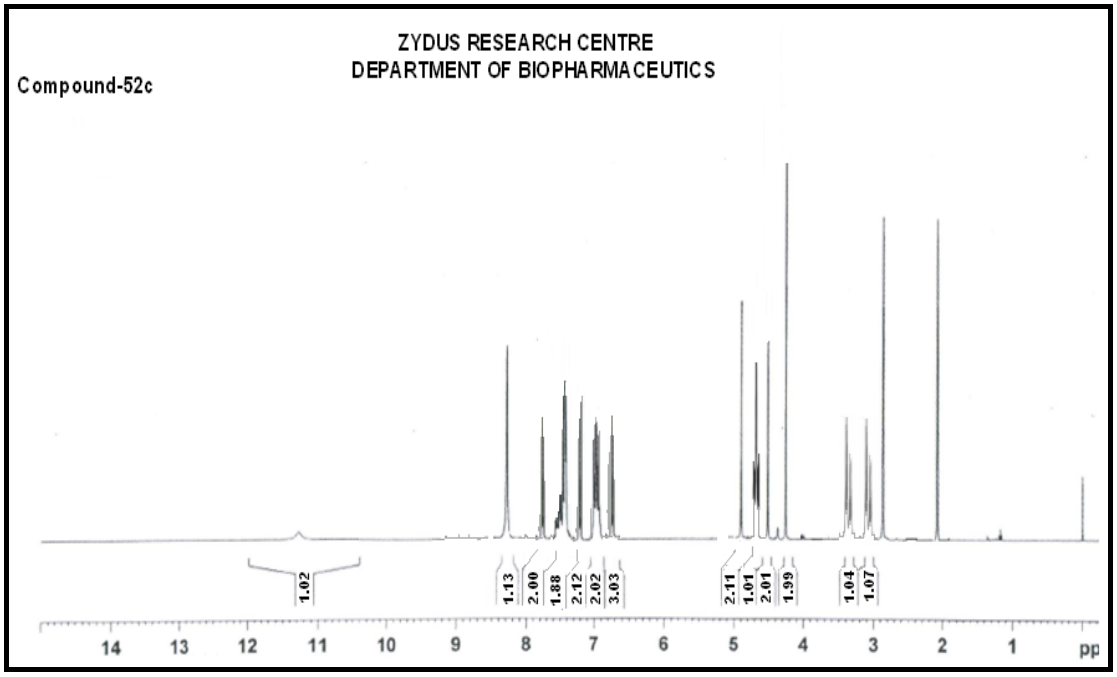
Compound-52c

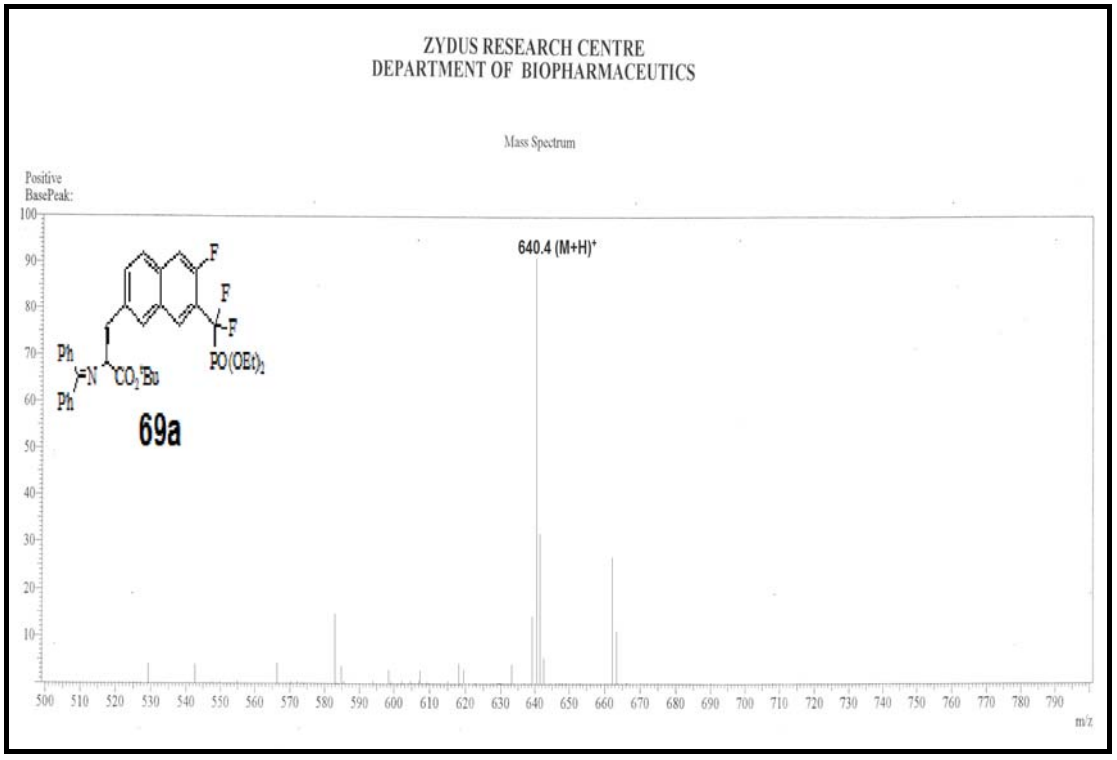
Column : Thermo-Syncronis-AQ[150*4.6mm] 5um
M.P. : 0.1%TFA IN WATER: ACN[GRADIENT]
Flow : 1.0 ml/min@220nm



Detector A - 1 (220nm)

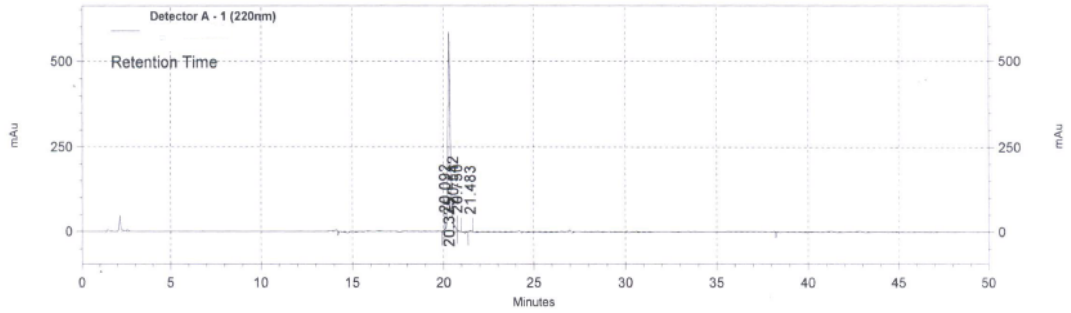
Pk #	Retention Time	Area	Area Percent
1	19.458	11336	0.29
2	19.575	57120	1.44
3	19.750	3782002	95.53
4	19.933	108572	2.74
Totals		3959030	100.00


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-52c
1.02
1.13
2.00
1.88
2.12
2.02
3.03
2.11
1.01
2.01
1.99
1.04
1.07
14 13 12 11 10 9 8 7 6 5 4 3 2 1 pp


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
640.4 (M+H)+
69a
100 90 80 70 60 50 40 30 20 10
500 510 520 530 540 550 560 570 580 590 600 610 620 630 640 650 660 670 680 690 700 710 720 730 740 750 760 770 780 790
m/z

Compound-69a

Column : Thermo-Syncronis-AQ[150*4.6mm] 5um
M.P. : 0.1%TFA IN WATER: ACN[GRADIENT]
Flow : 1.0 ml/min@220nm

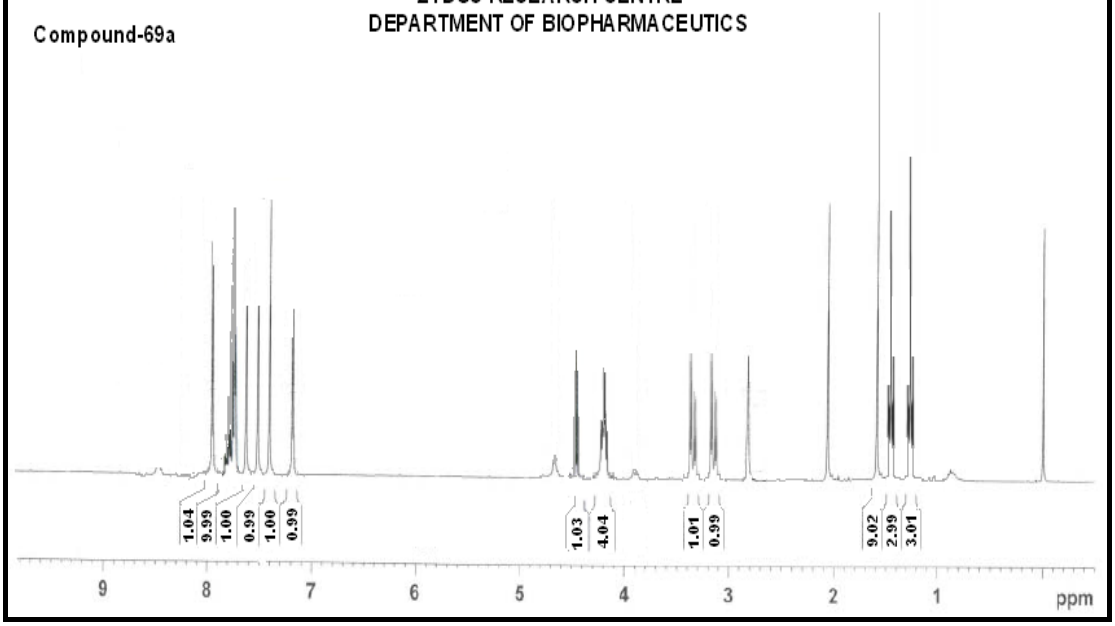


Detector A - 1 (220nm)

Pk #	Retention Time	Area	Area Percent
1	20.092	30736	0.54
2	20.325	5449365	95.07
3	20.542	196750	3.43
4	20.750	34088	0.59
5	21.483	21043	0.37
Totals		5731982	100.00

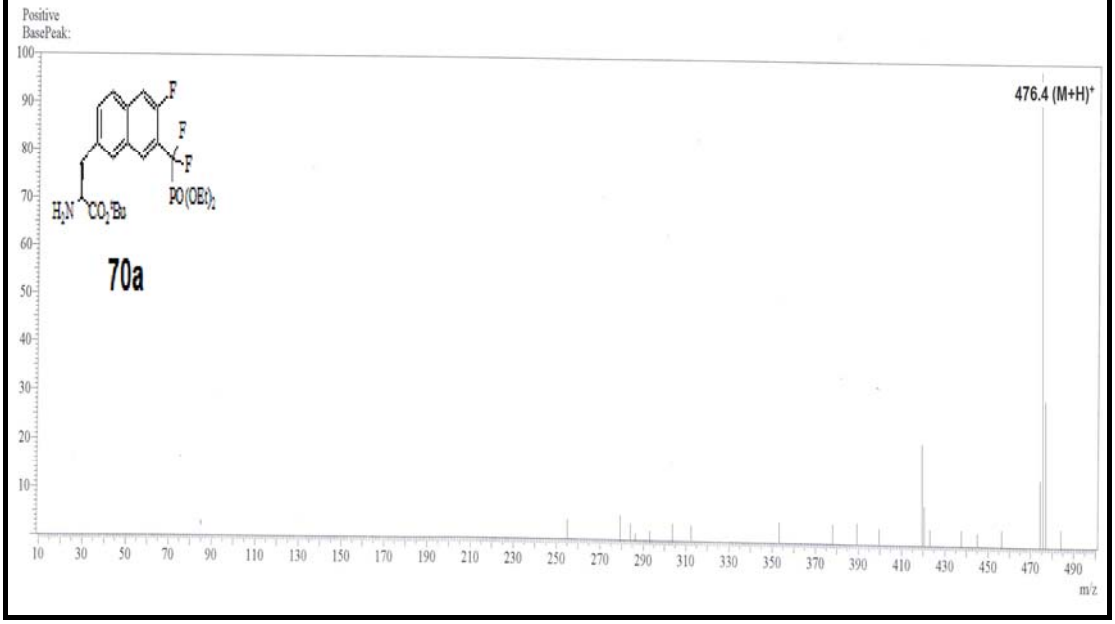
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Compound-69a



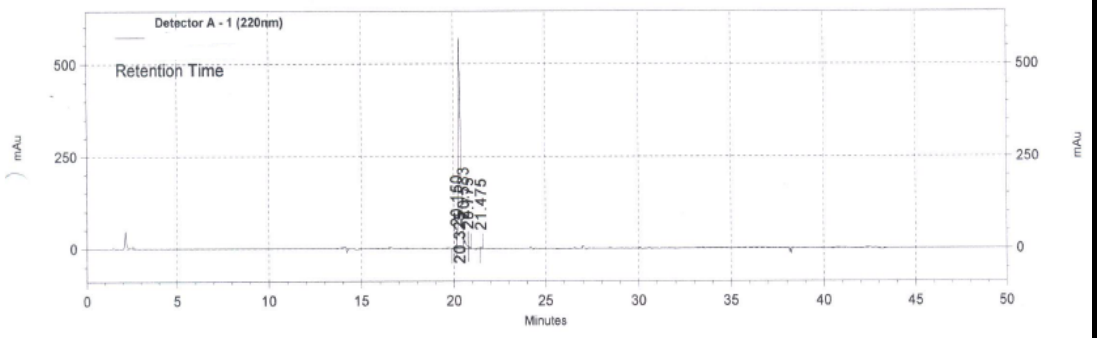
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum



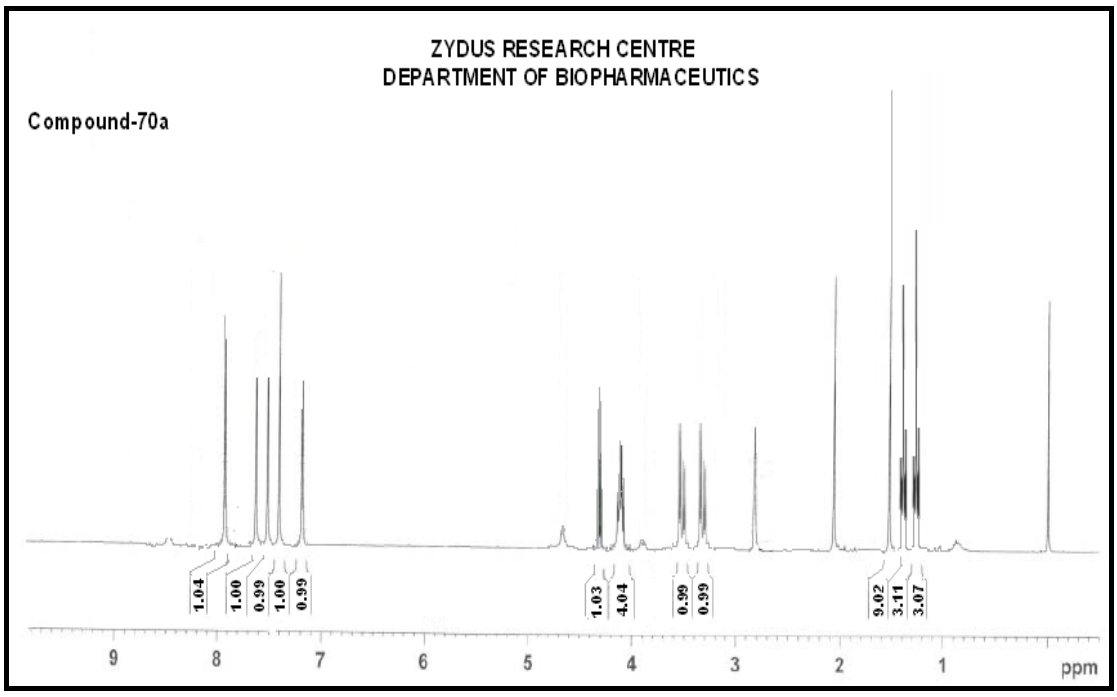
Compound-70a

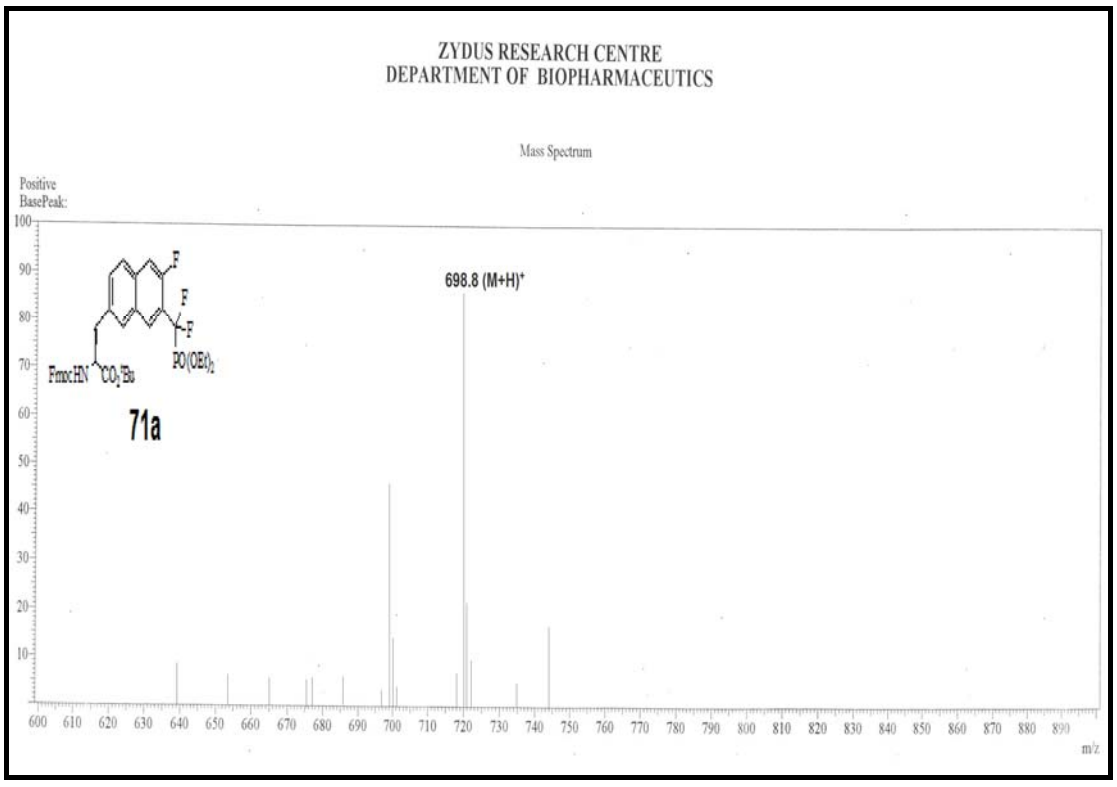
Column : Thermo-Syncronis-AQ[150*4.6mm] 5um
M.P. : 0.1%TFA IN WATER: ACN[GRADIENT]
Flow : 1.0 ml/min@220nm



Detector A - 1 (220nm)

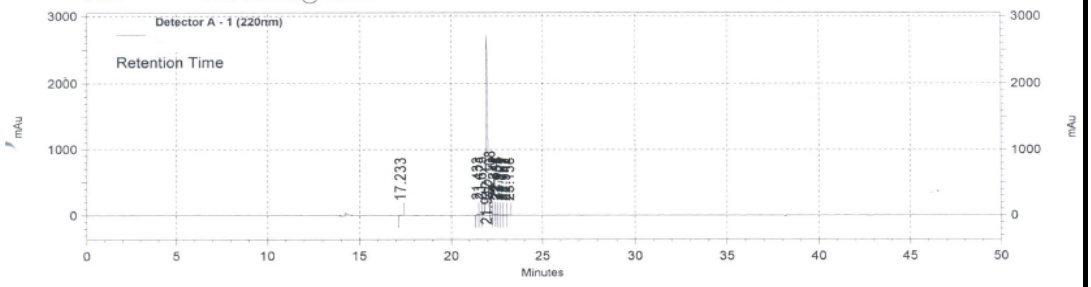
Pk #	Retention Time	Area	Area Percent
1	20.150	63628	1.13
2	20.325	5312158	94.51
3	20.533	206153	3.67
4	20.775	27856	0.50
5	21.475	10788	0.19
Totals		5620583	100.00


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-70a
1.04
1.00
0.99
1.00
0.99
1.03
4.04
0.99
0.99
9.02
3.11
3.07
9
8
7
6
5
4
3
2
1
ppm


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
698.8 (M+H)+
71a
m/z

Compound-71a

Column : Thermo-Syncronis-AQ[150*4.6mm] 5um
M.P. : 0.1%TFA IN WATER: ACN[GRADIENT]
Flow : 1.0 ml/min@220nm

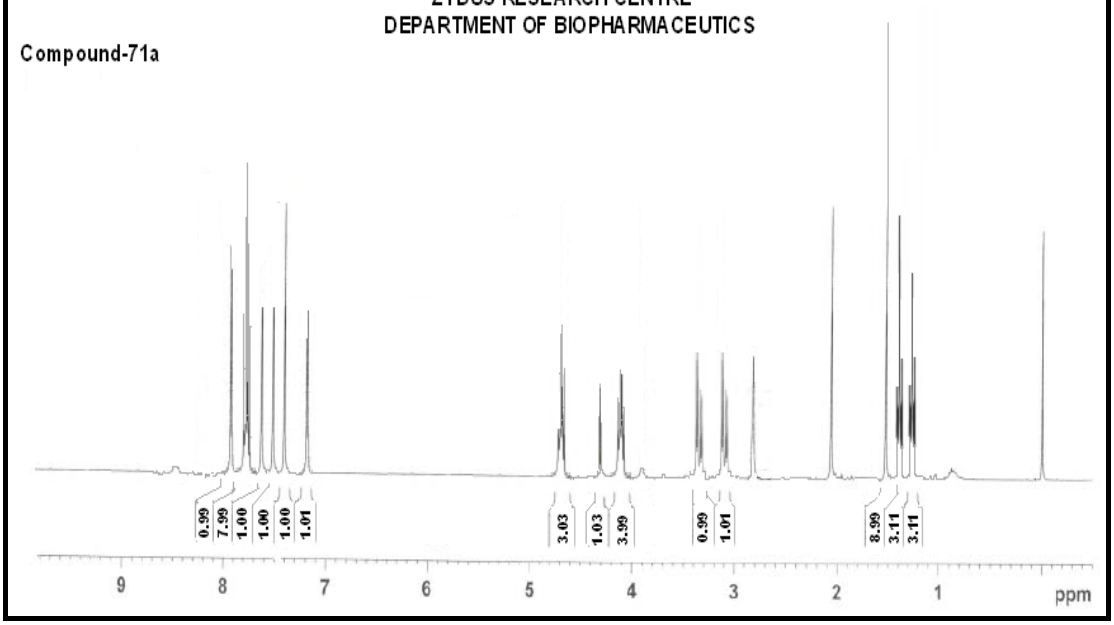


Detector A - 1 (220nm)

Pk #	Retention Time	Area	Area Percent
1	17.233	27449	0.13
2	21.433	94398	0.46
3	21.625	140510	0.69
4	21.942	19339400	94.35
5	22.108	485474	2.37
6	22.342	185300	0.90
7	22.458	114921	0.56
8	22.558	27277	0.13
9	22.767	33384	0.16
10	22.942	33395	0.16
11	23.158	16802	0.08
Totals		20498310	100.00

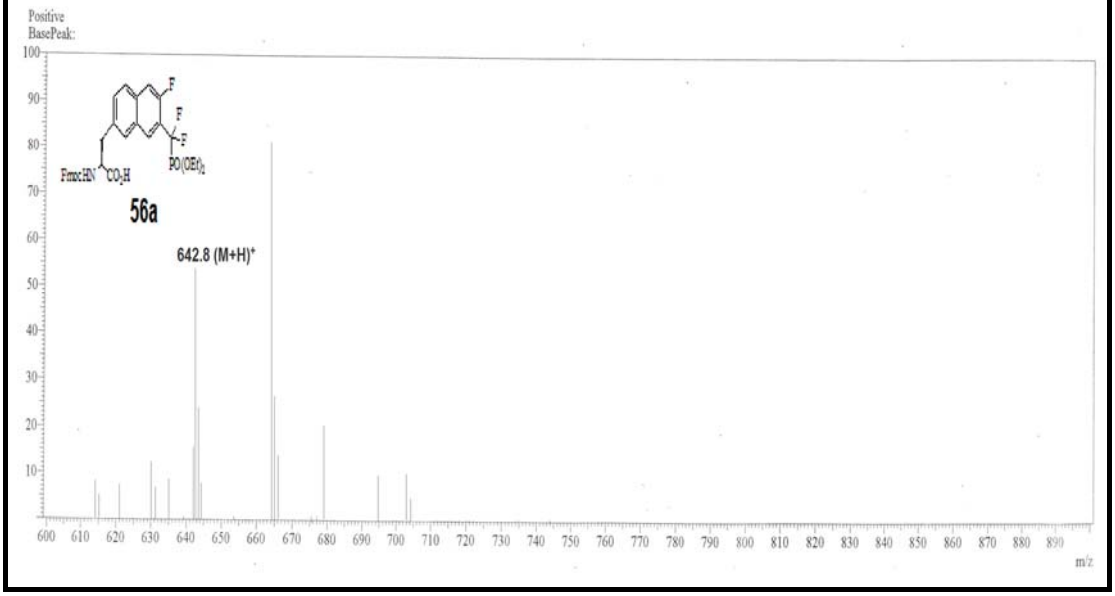
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Compound-71a



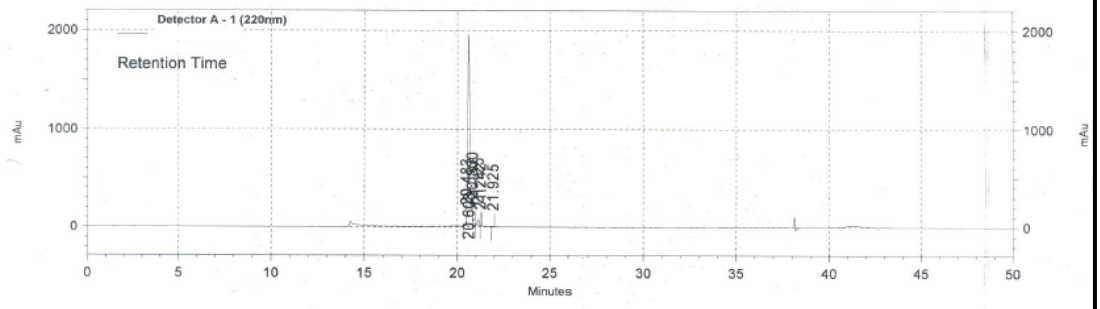
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum



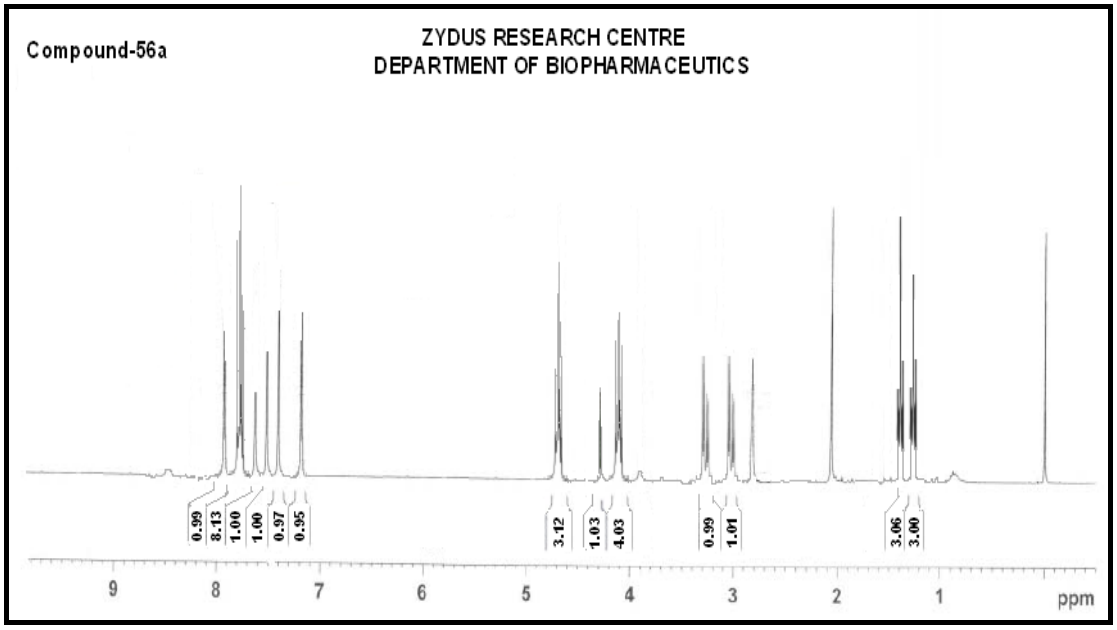
Compound-56a

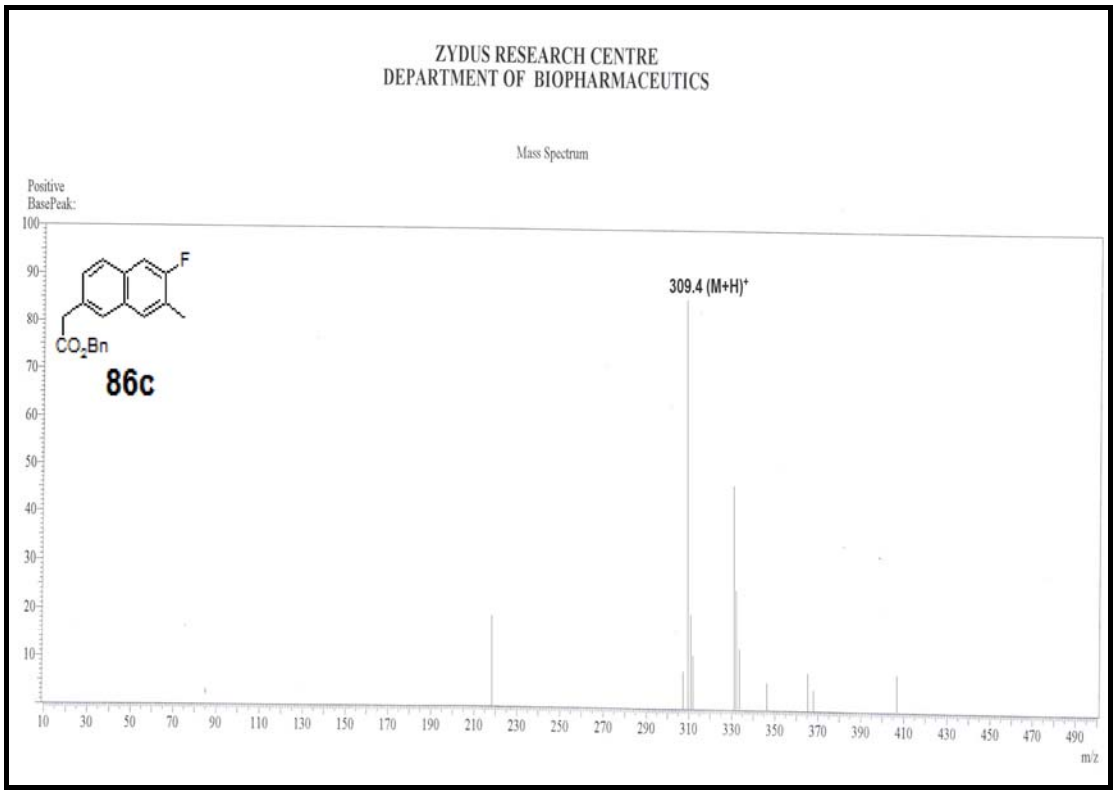
Column : Thermo-Syncronis-AQ[150*4.6mm] 5um
M.P. : 0.1%TFA IN WATER: ACN[GRADIENT]
Flow : 1.0 ml/min@220nm



Detector A - 1 (220nm)

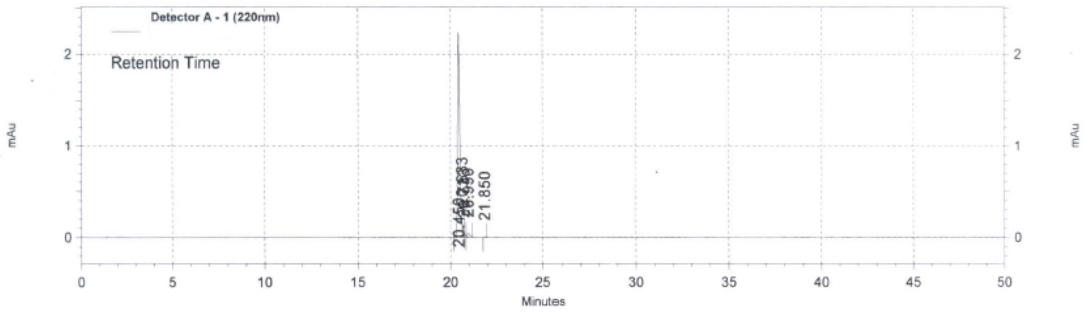
Pk #	Retention Time	Area	Area Percent
1	20.483	112745	0.66
2	20.608	16096620	93.76
3	20.800	462324	2.69
4	21.125	467044	2.72
5	21.242	14243	0.08
6	21.925	15420	0.09
Totals		17168396	100.00


Compound-56a
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
0.99
8.13
1.00
1.00
0.97
0.95
3.12
1.03
4.03
0.99
1.01
3.06
3.00
9
8
7
6
5
4
3
2
1
ppm


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
F
CO2Bn
86c
309.4 (M+H)+
m/z

Compound-86c

Column : Thermo-Syncronic-AQ[150*4.6mm] 5um
M.P. :0.1%TFA IN WATER: ACN[GRADIENT]
Flow : 1.0 ml/min@220nm

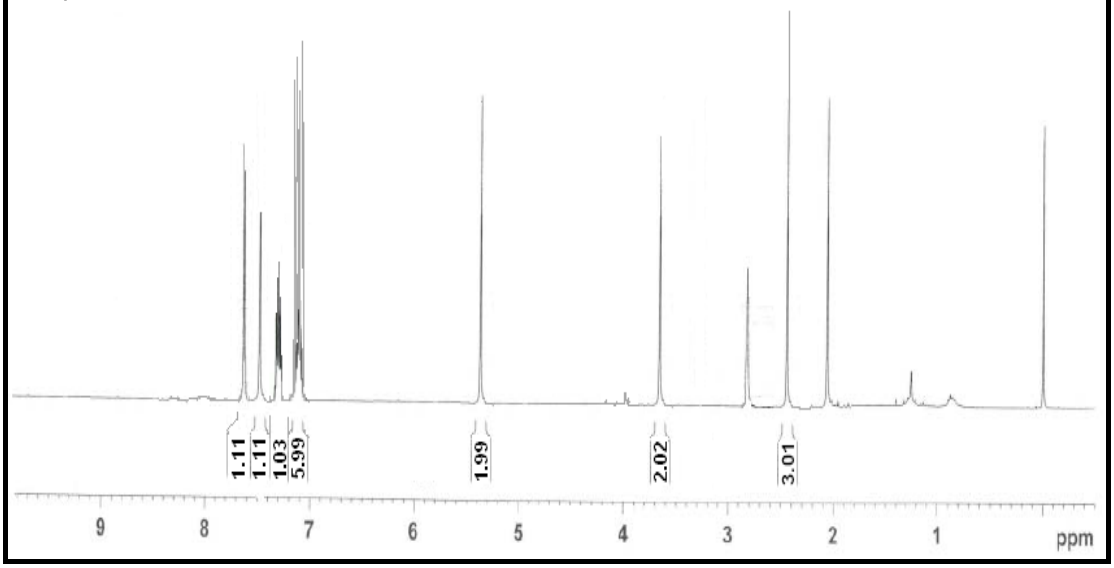


Detector A - 1 (220nm)

Pk #	Retention Time	Area	Area Percent
1	20.450	18685562	95.06
2	20.633	456135	2.32
3	20.733	117581	0.60
4	20.950	376167	1.91
5	21.850	21573	0.11
Totals		19657018	100.00

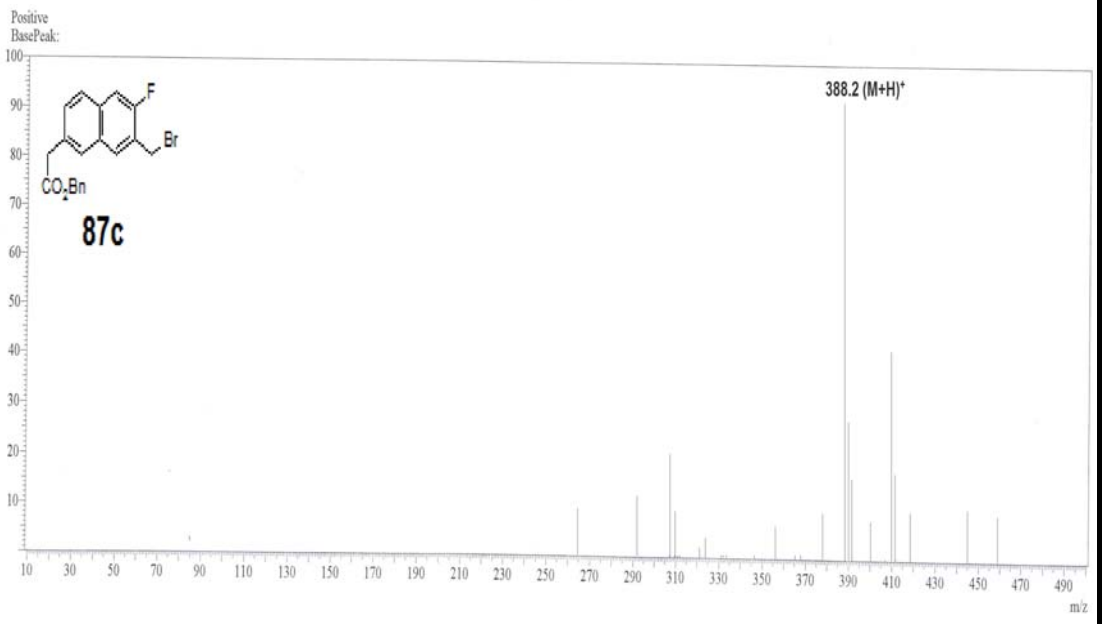
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Compound-86c



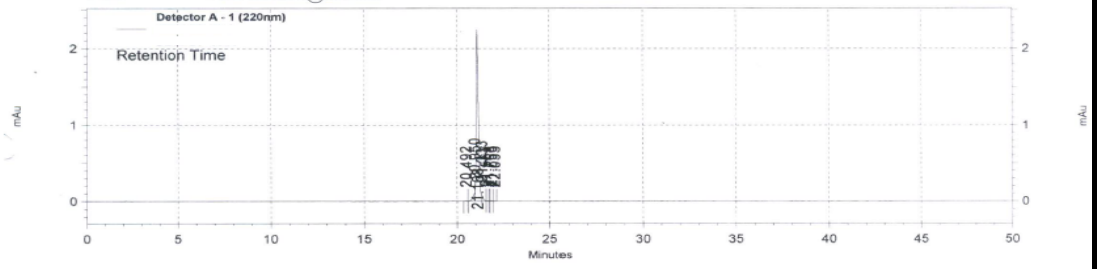
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum



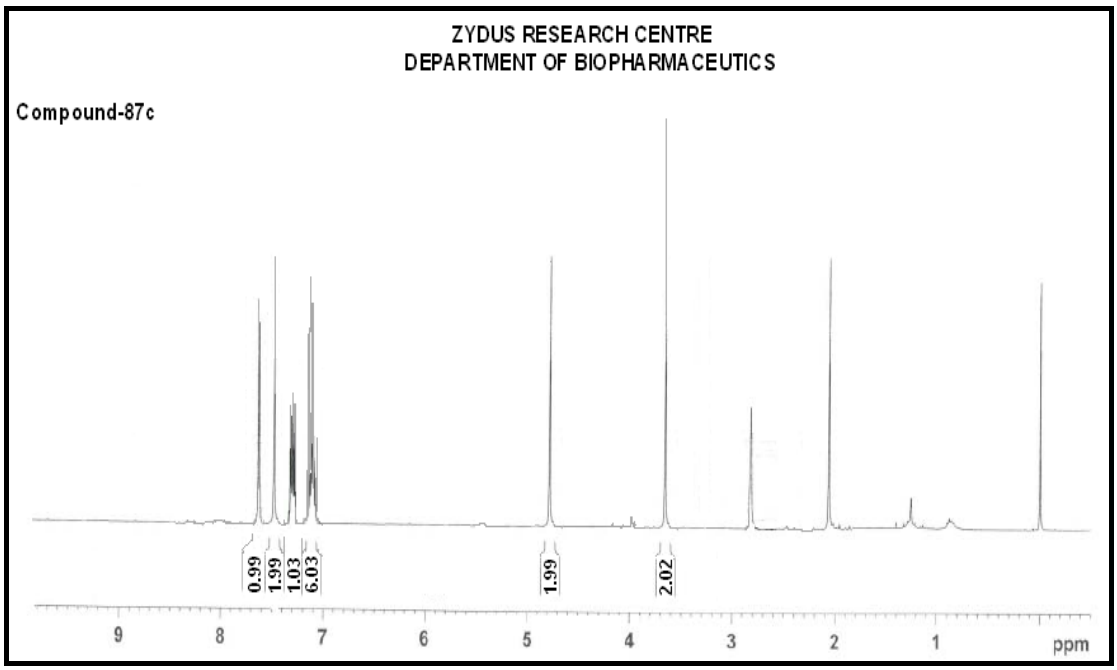
Compound-87c

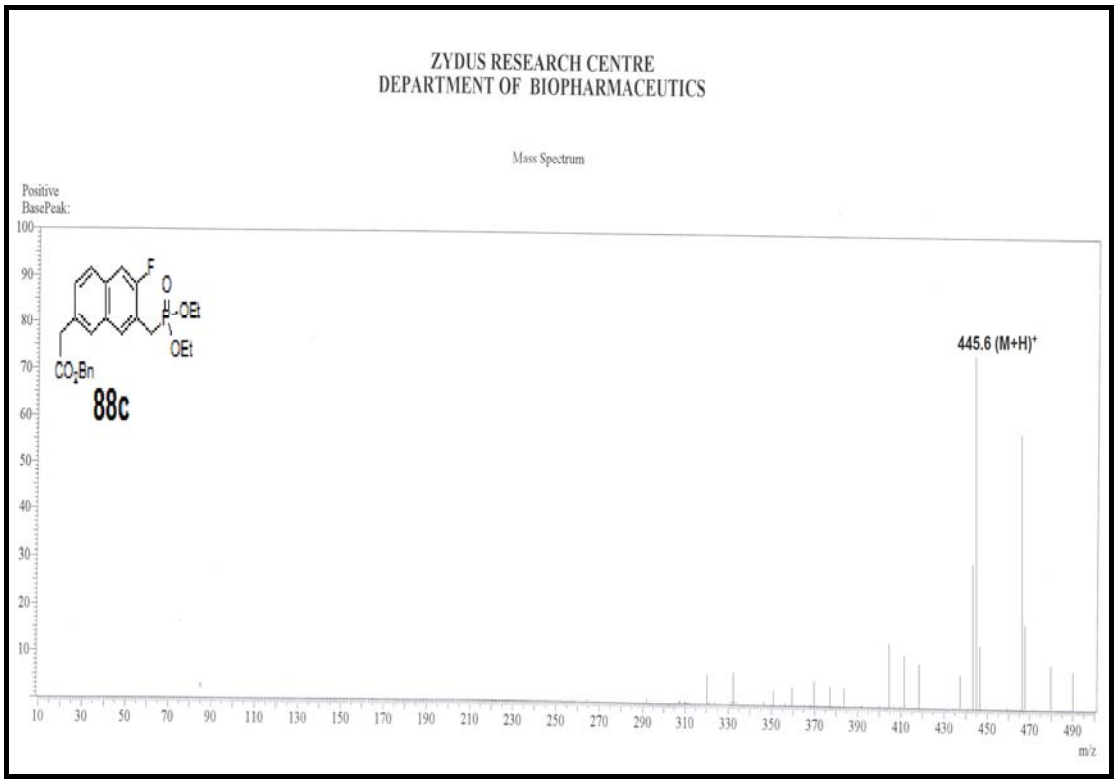
Column : Thermo-Syncronic-AQ[150*4.6mm] 5um
M.P. :0.1%TFA IN WATER: ACN[GRADIENT]
Flow : 1.0 ml/min@220nm



Detector A - 1 (220nm)

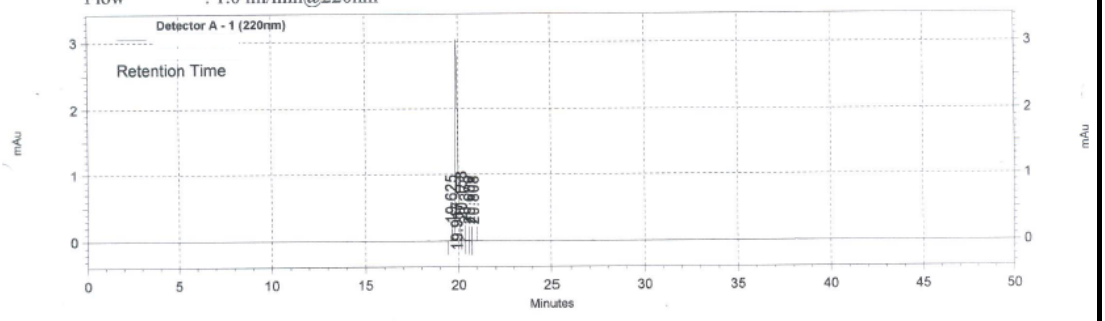
Pk #	Retention Time	Area	Area Percent
1	20.492	25521	0.13
2	20.950	802768	3.97
3	21.108	18881370	93.33
4	21.333	397037	1.96
5	21.567	44960	0.22
6	21.708	14509	0.07
7	21.858	49509	0.24
8	22.033	14194	0.07
Totals		20229868	100.00


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-87c
0.99
1.99
1.03
6.03
1.99
2.02
9
8
7
6
5
4
3
2
1
ppm


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
F
O
P
OEt
OEt
CO2Bn
88c
445.6 (M+H)+
m/z

Compound-88c

Column : Thermo-Syncronic-AQ[150*4.6mm] 5um
M.P. :0.1%TFA IN WATER: ACN[GRADIENT]
Flow : 1.0 ml/min@220nm

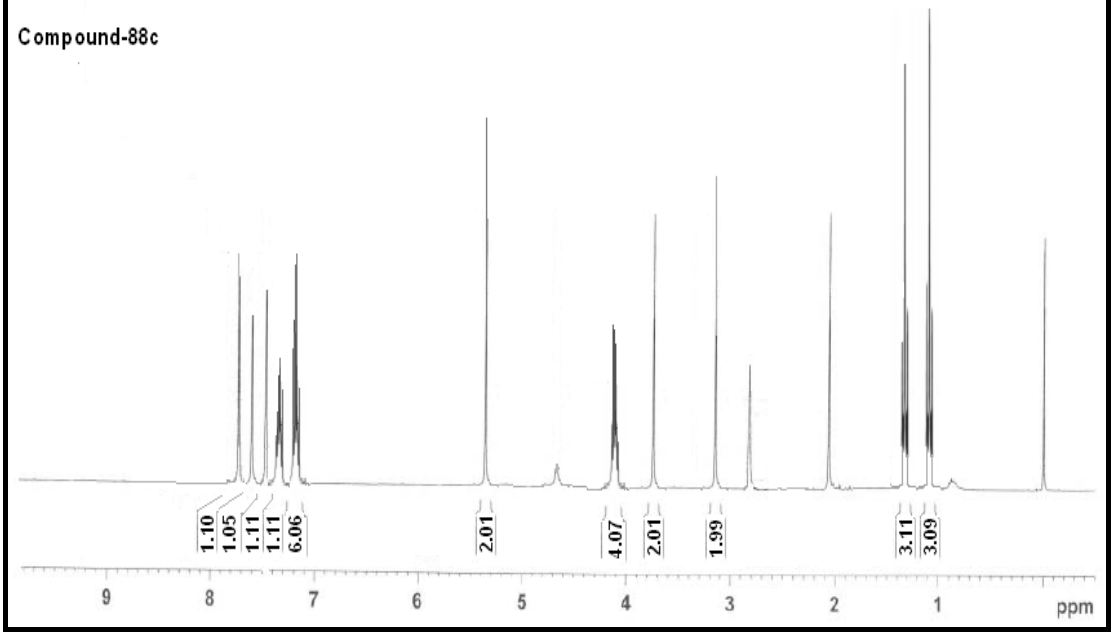


Detector A - 1 (220nm)

Pk #	Retention Time	Area	Area Percent
1	19.625	117813	0.47
2	19.917	24497074	97.15
3	20.158	442796	1.76
4	20.375	98060	0.39
5	20.600	44930	0.18
6	20.808	16038	0.06
Totals		25216711	100.00

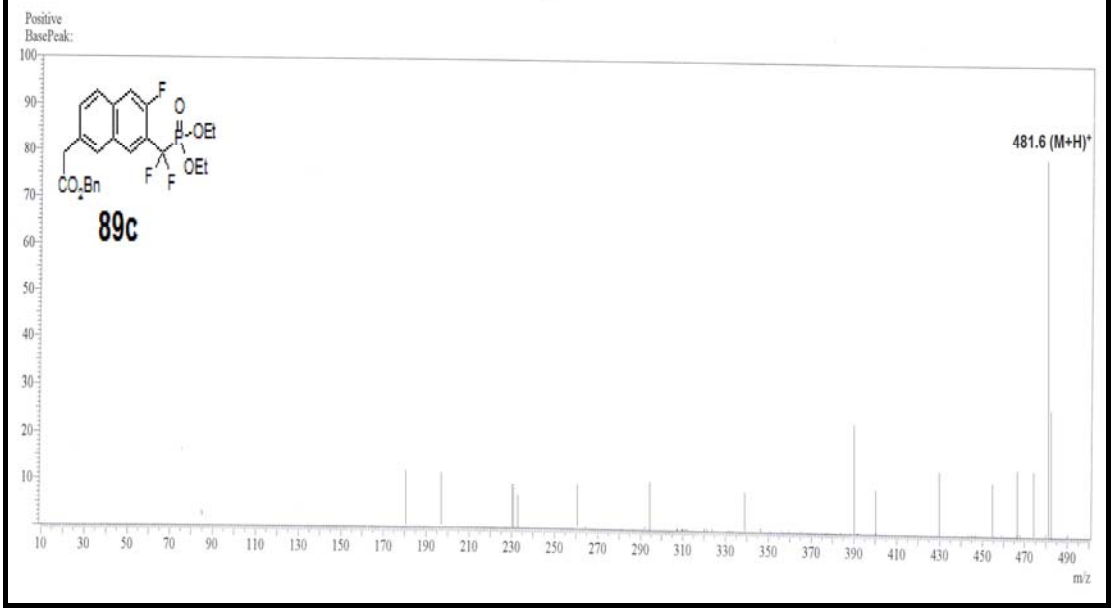
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Compound-88c



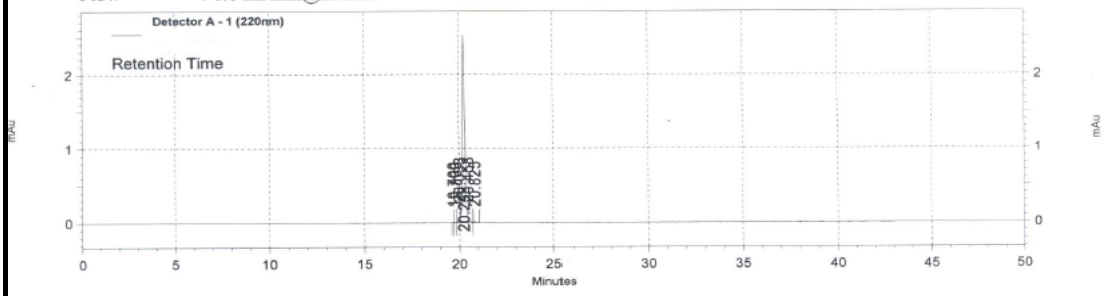
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum



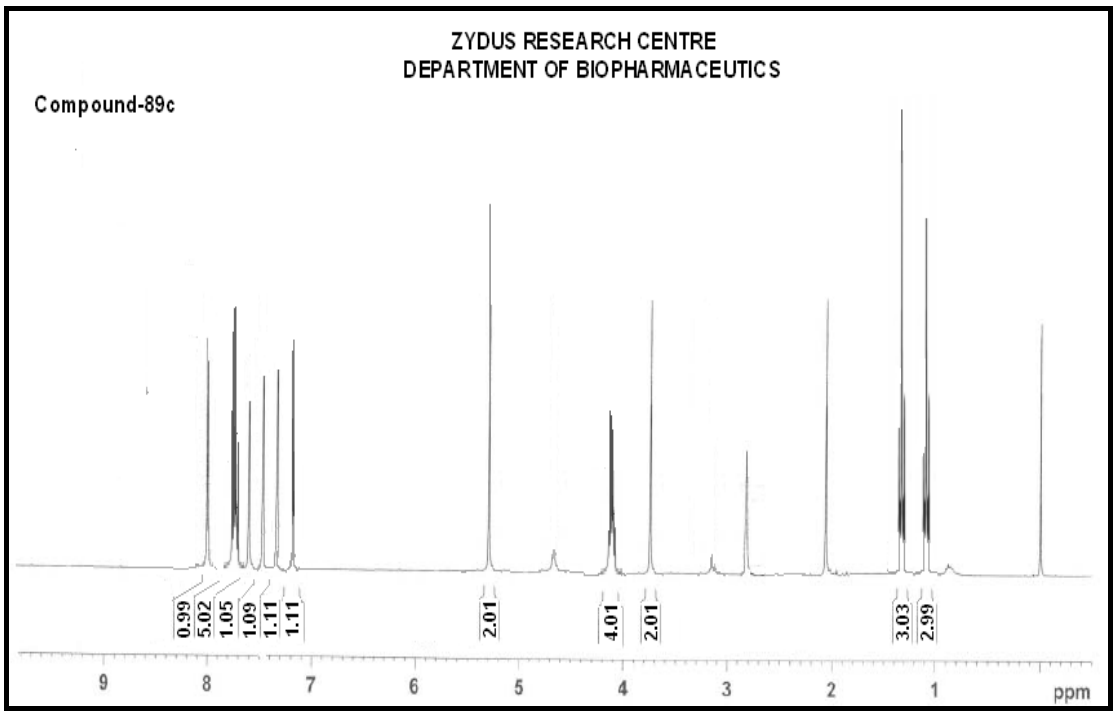
Compound-89c

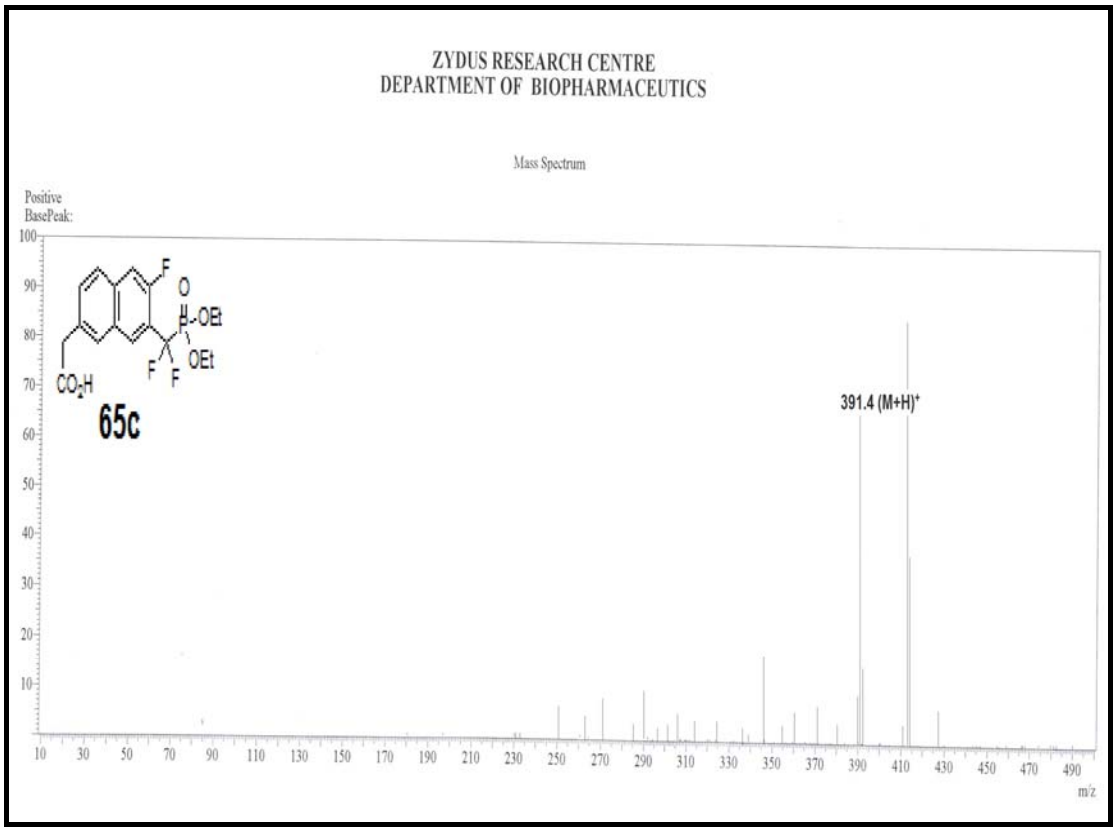
Column : Thermo-Syncronic-AQ[150*4.6mm] 5um
M.P. :0.1%TFA IN WATER: ACN[GRADIENT]
Flow : 1.0 ml/min@220nm



Detector A - 1 (220nm)

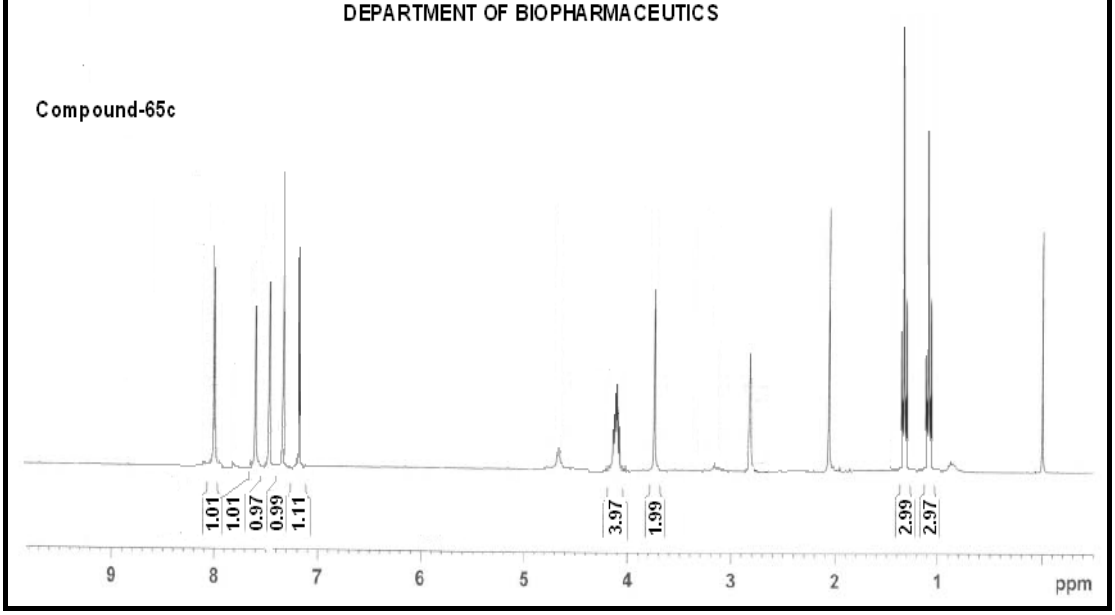
Pk #	Retention Time	Area	Area Percent
1	19.700	10083	0.05
2	19.833	40887	0.18
3	20.033	339673	1.53
4	20.258	21321789	96.01
5	20.458	386618	1.74
6	20.825	108332	0.49
Totals		22207382	100.00


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Compound-89c
0.99
5.02
1.05
1.09
1.11
1.11
2.01
4.01
2.01
3.03
2.99
9
8
7
6
5
4
3
2
1
ppm


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
Mass Spectrum
Positive
BasePeak:
F
O
P
OEt
OEt
F
F
CO2H
65c
391.4 (M+H)+
m/z

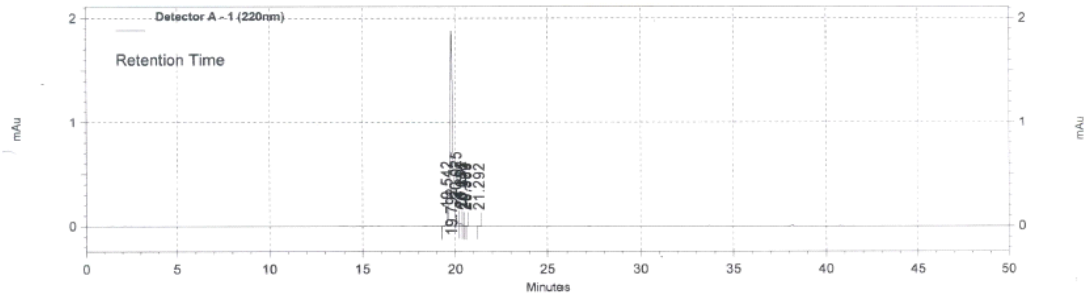
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Compound-65c



Compound-65c

Column : Thermo-Syncronic-AQ[150*4.6mm] 5um
M.P. :0.1%TFA IN WATER: ACN[GRADIENT]
Flow : 1.0 ml/min@220nm

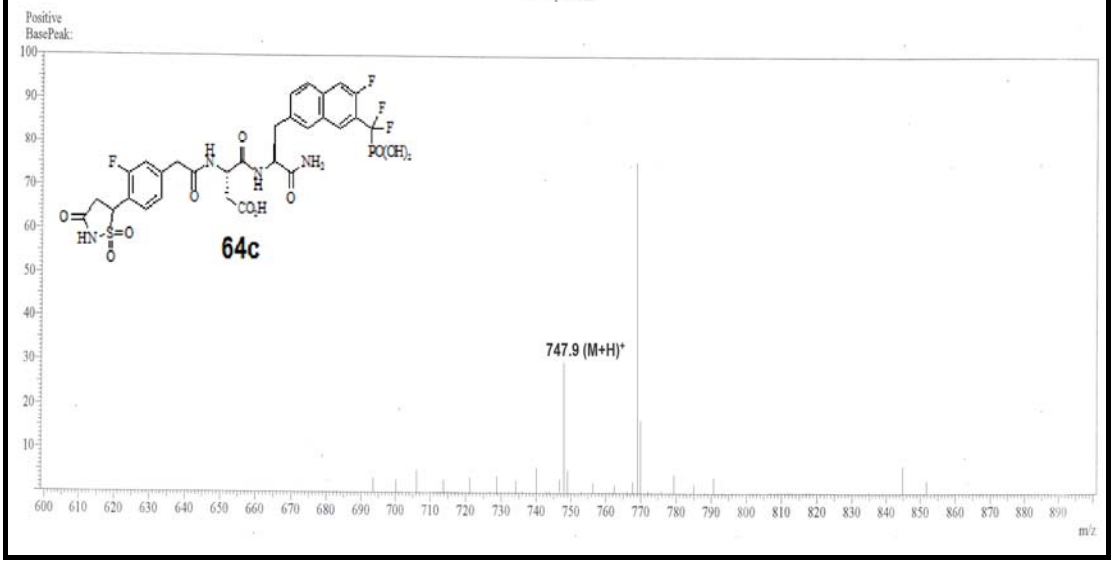


Detector A - 1 (220nm)

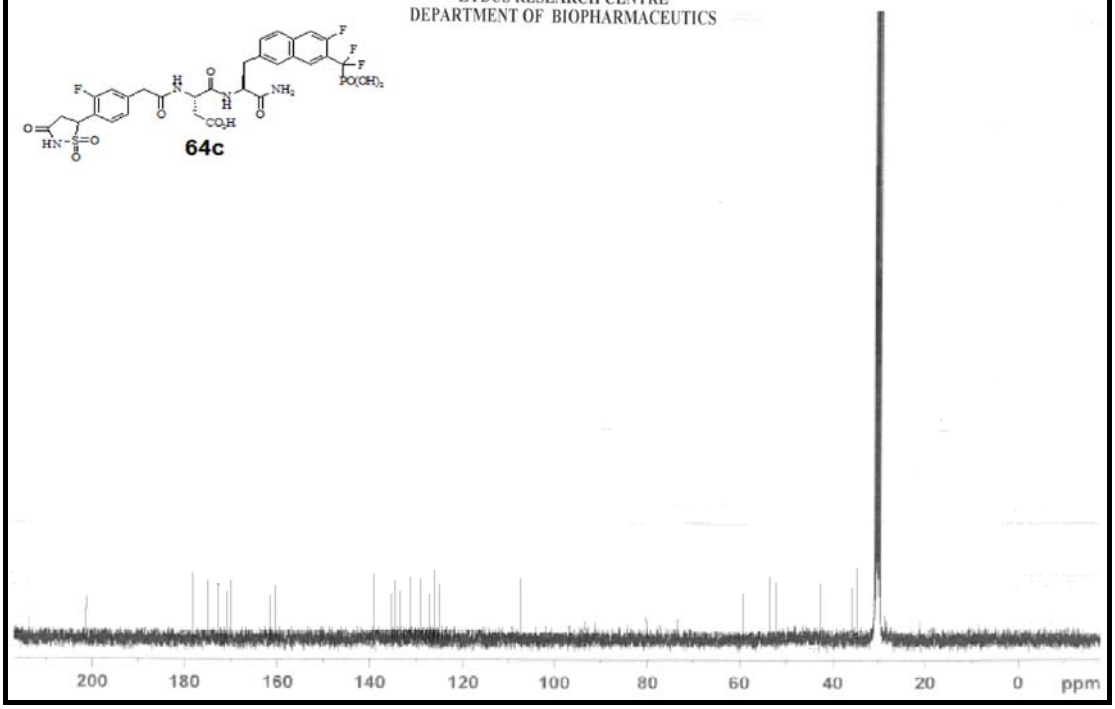
Pk #	Retention Time	Area	Area Percent
1	19.542	101924	0.52
2	19.792	18638641	95.57
3	20.025	515451	2.64
4	20.292	152094	0.78
5	20.375	47094	0.24
6	20.500	25923	0.13
7	20.583	9684	0.05
8	21.292	11161	0.06
Totals		19501972	100.00

ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum



ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS



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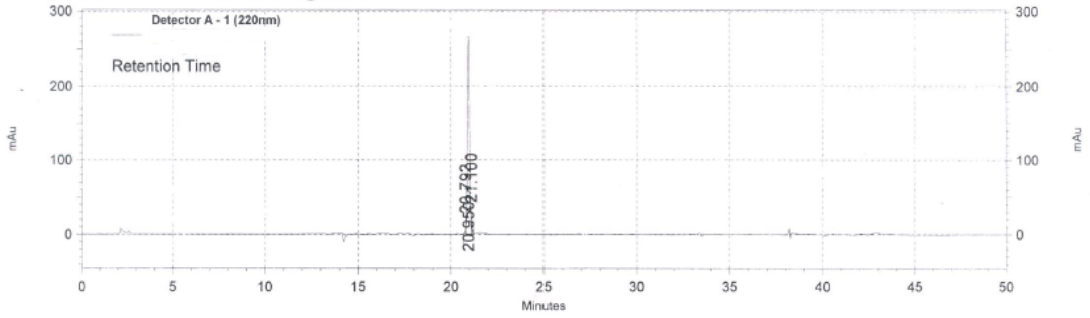

Detector A - 1 (220nm)

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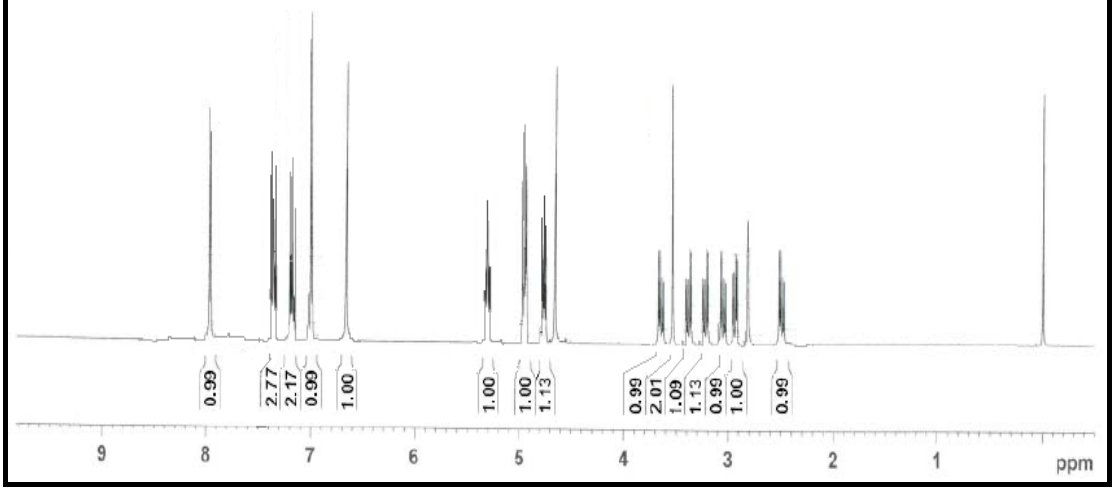
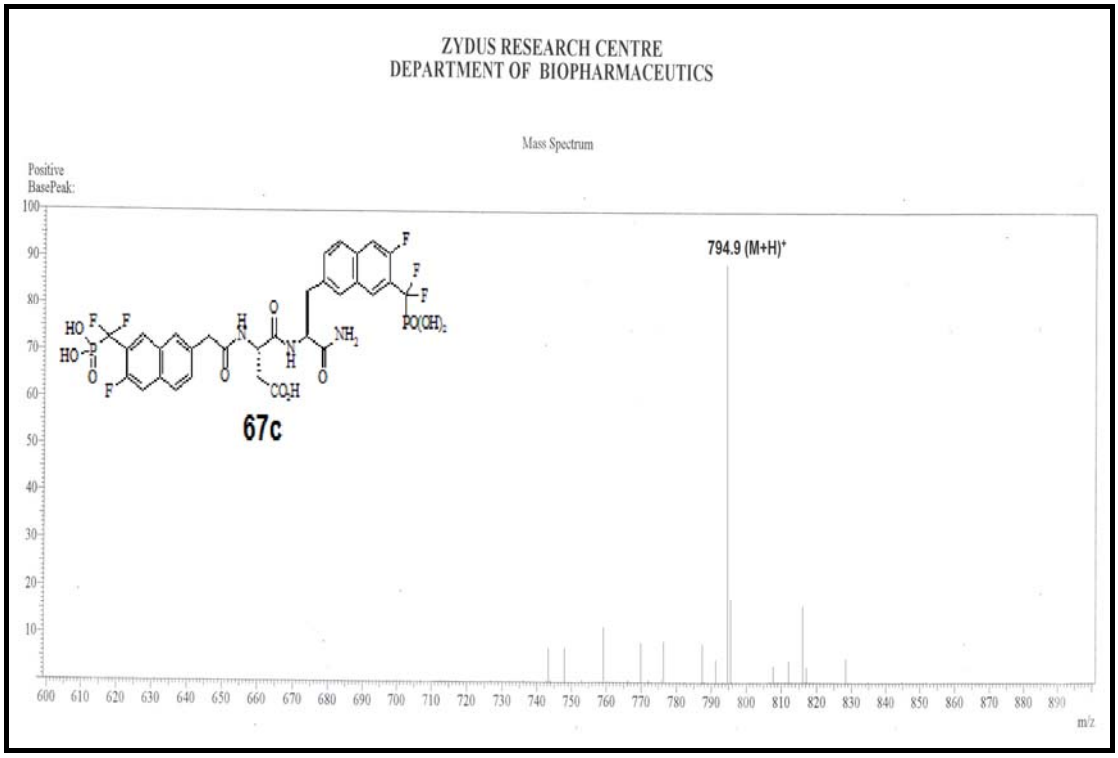
Compound-64c




ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
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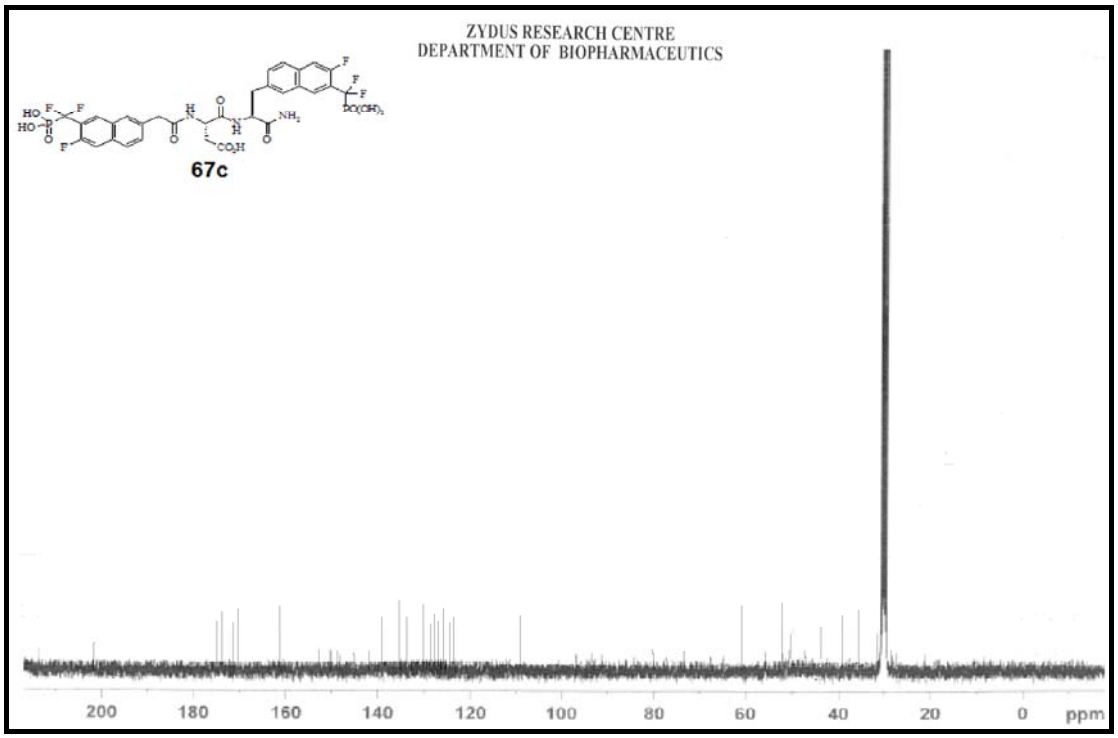
67c
m/z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DEPARTMENT OF BIOPHARMACEUTICS
67c
ppm

Compound-67c

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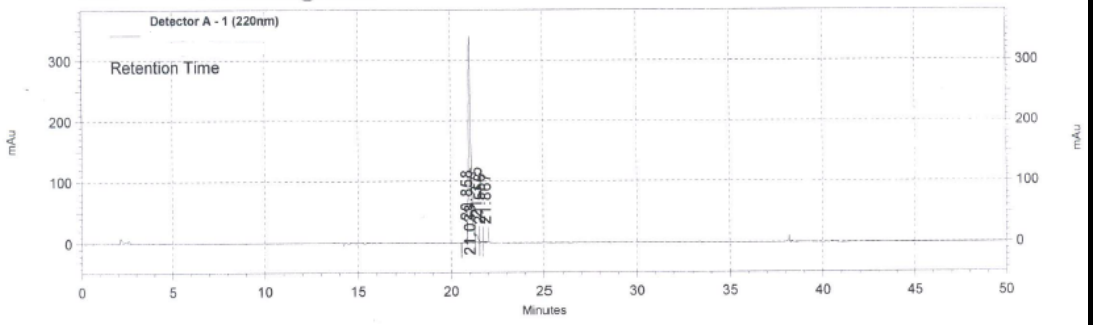
M.P. : 0.1%TFA IN WATER: ACN[GRADIENT]
Flow : 1.0 ml/min@220nm



Detector A - 1 (220nm)

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4	21.558	11229	0.33
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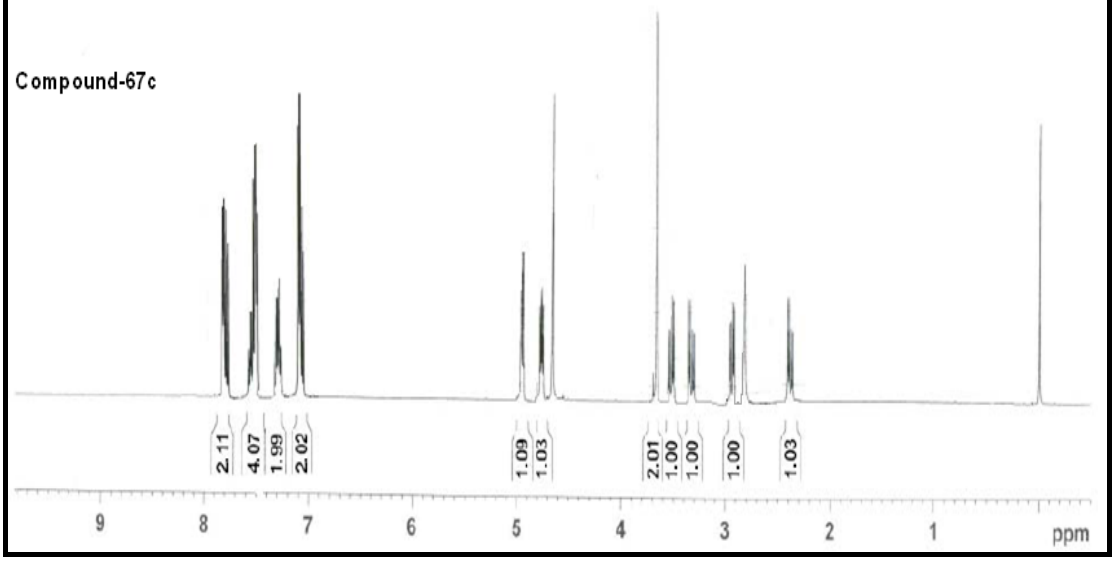
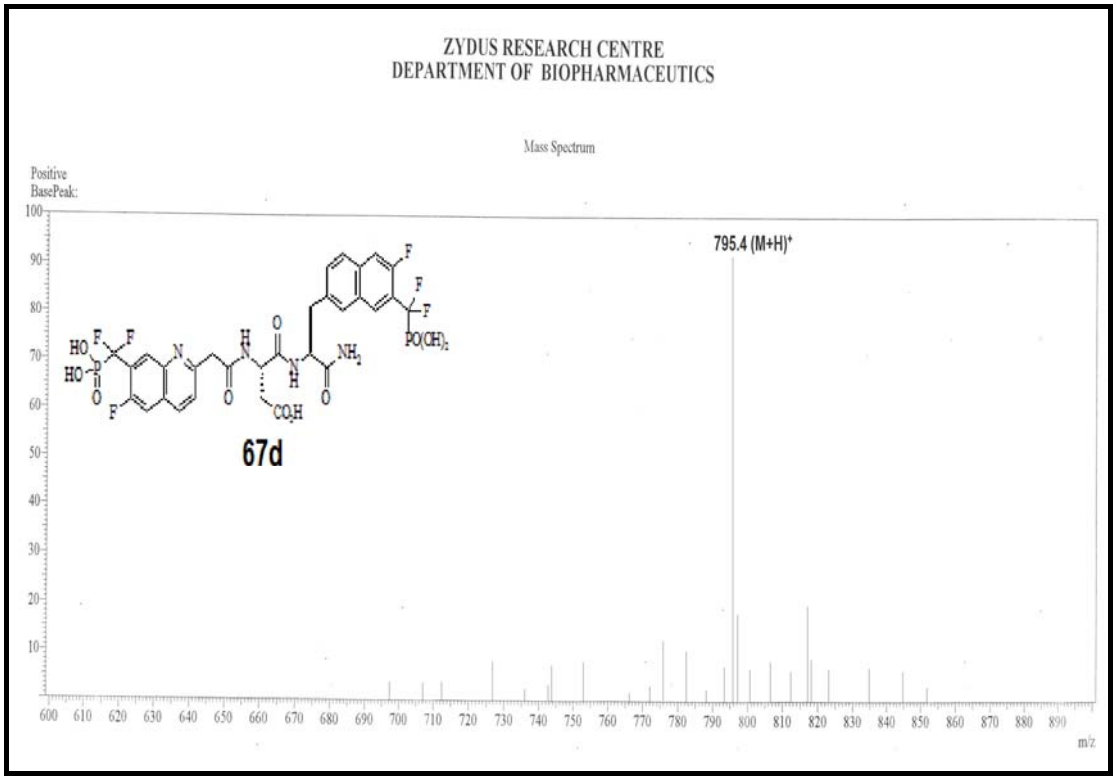
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Compound-67c

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DEPARTMENT OF BIOPHARMACEUTICS
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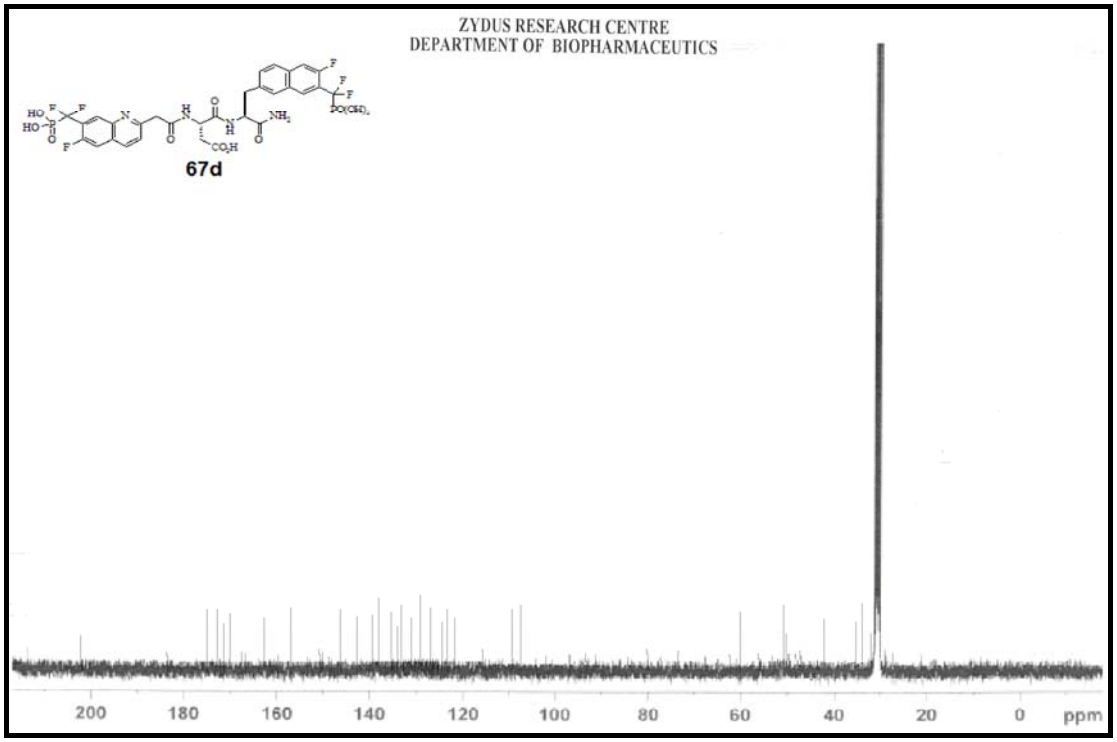
67d
m/z


ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS
67d
ppm

Compound-67d

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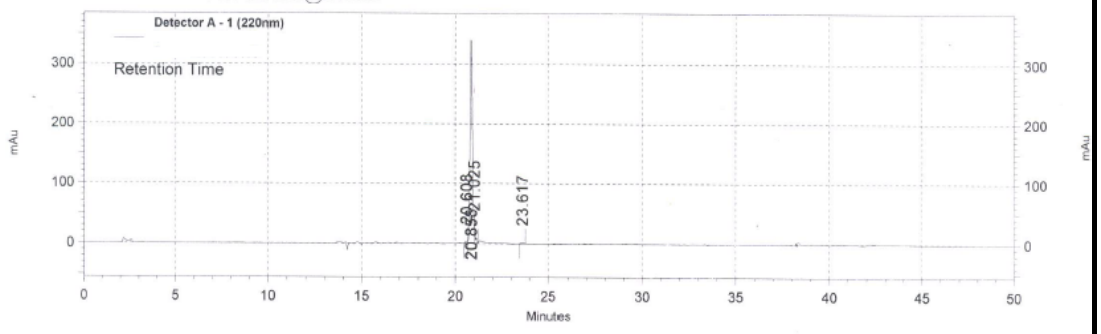
M.P. : 0.1%TFA IN WATER: ACN[GRADIENT]
Flow : 1.0 ml/min@220nm



Detector A - 1 (220nm)

Pk #	Retention Time	Area	Area Percent
1	20.608	24674	0.81
2	20.858	2909173	95.47
3	21.025	96809	3.18
4	23.617	16516	0.54
Totals		3047172	100.00

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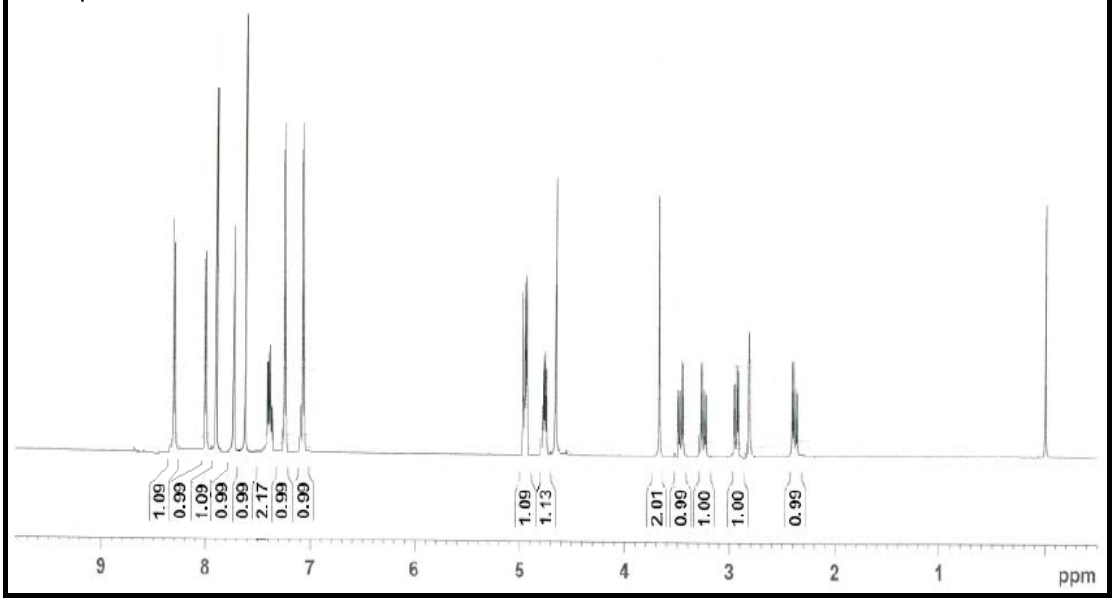
DEPARTMENT OF BIOPHARMACEUTICS

Compound-67d



Chapter VII: Publications

7. Publication

List of Publications from the PhD work

[1] **Dipam Patel**, Mukul Jain, Shailesh R. Shah, Rajesh Bahekar, Pradip Jadav, Brijesh Darji, Yernaidu Siriki, Debdutta Bandyopadhyay, Amit Joharapurkar, Samadhan Kshirsagar, Harilal Patel, Mubeen, Shaikh, Kalapatapu, V. V. M. Sairam and Pahkaj Patel. Discovery of Orally Active, Potent and Selective Benzotriazole- Based PTP1B Inhibitors. *ChemMedChem* **2011**, *6*, 1011-1016.

[2] **Dipam Patel**, Mukul Jain, Shailesh R. Shah, Rajesh Bahekar, Pradip Jadav, Amit Joharapurkar, Nirav Dhanesha, Mubeen Shaikh, Kalapatapu V. V. M. Sairam and Prashant Kapadnis. Discover of potent, selective and orally bioavailable triaryl-sulfonamide based PTP1B inhibitors. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1111-1117.

[3] **Patel D**, Jain M, Shah S R, Bahekar R, Jadav P, Shah K, Joharapurakar A, Shaikh M and Sairam K V. Peptidomimetic as Potent and Selective PTP1B Inhibitors. *Medicinal Chemistry* **2012** (In Press).

DOI: 10.1002/cmdc.201100077

Discovery of Orally Active, Potent, and Selective Benzotriazole-Based PTP1B Inhibitors**

Dipam Patel,[a, b] Mukul Jain,*[a] Shailesh R. Shah,*[b] Rajesh Bahekar,*[a] Pradip Jadav,[a] Brijesh Darji,[a] Yernaidu Siriki,[a] Debdutta Bandyopadhyay,[a] Amit Joharapurkar,[a] Samadhan Kshirsagar,[a] Harilal Patel,[a] Mubeen Shaikh,[a] Kalapatapu V. V. M. Sairam,[a] and Pankaj Patel[a]

The worldwide incidence of metabolic syndromes such as obesity and diabetes are increasing at an alarming rate.[1,2] Patients that suffer from obesity-induced type 2 diabetes (informally known as *diabesity*) are at increased risk of cardiovascular disease; their numbers pose a significant economic burden on health services.[3] Type 2 diabetes mellitus (T2DM) is clinically characterized by increased blood glucose levels, either due to defects in insulin secretion, insulin resistance, or both.[4] Current treatments for diabetic patients include various oral anti-hyperglycemic agents; however, over a period of time nearly half of T2DM sufferers lose their response to these agents and thereby require insulin therapy. Except incretin therapies, most of the available anti-hyperglycemic agents including insulin promote weight gain, which further aggravates obesity-associated cardiovascular risk and insulin resistance.[5–9] Thus, there is an urgent need to develop novel agents for glycemic control that can complement existing therapies and prevent the progression of secondary complications associated with diabesity.

In recent years, development of protein tyrosine phosphatase 1B (PTP1B) inhibitors has been considered as one of the best validated biological targets for the treatment of T2DM.[10] PTP1B acts as a negative regulator in insulin signaling pathways; it dephosphorylates key tyrosine residues within the regulatory domain of the β-subunit of the insulin receptor.[11] Thus, the inhibition of PTP1B activity has the potential for enhancing insulin action by prolonging the phosphorylated state of the insulin receptor.[12] Gene knockout studies in animals have also demonstrated that PTP1B$^{-/-}$ mice show increased insulin sensitivity and are resistant to diet-induced obesity.[13,14]

Over the past two decades, several structurally diverse small-molecule-based PTP1B inhibitors have been developed, including Ertiprotafib, which was discontinued in phase II clinical trials owing to lack of efficacy and dose-dependent side effects.[15] Most of the initial PTP1B inhibitors, such as phosphonates, carboxylic acids, and difluoromethylphosphonates (DFMPs), were designed to bind to the active site (site 1/A) by mimicking the phosphotyrosine (pTyr) substrate.[16] However, achieving PTP1B selectivity over closely associated PTPs (PTPα, LAR, CD45, VHR, SHP-1, SHP-2, and T-cell protein tyrosine phosphatase (TCPTP)) is one of the major challenges, as most of the closely associated PTPs, particularly TCPTP, share a high degree of primary sequence identity (92%) in the active site (pTyr binding pocket).[17] Lack of oral bioavailability is another important issue in the development of potent and selective PTP1B inhibitors, as the majority of the active-site-directed PTP1B inhibitors exhibit limited cell permeability due to the presence of negatively charged polar groups.[18,19]

To address this problem, Zhang and colleagues identified an additional noncatalytic aryl phosphate binding site (site 2/B) proximal to the catalytic phosphate binding site.[17] Site B of PTP1B differs from that of TCPTP by a few amino acids (F52Y and A27S) and thus offers an opportunity to improve selectivity over TCPTP.[20] Consequently, dual-site inhibitors were designed to bind across both sites A and B, to achieve additive effects and thereby improve potency and selectivity toward PTP1B over closely associated PTPs.[21] Based on this dual binding site concept, various DFMP-based PTP1B inhibitors such as arylketone **1**, benzotriazoles **2a** and **2b**, and naphthyl derivative **3** were developed (Figure 1).[22] The X-ray crystal structure of PTP1B in complex with compound **2b** reveals that sites A and B each have a DFMP moiety anchored into it.[23] The benzotriazole ring system also functions as an anchor and is located under the YRD loop, thereby rigidly locking the molecule into the active site and providing good selectivity for PTP1B over other PTPs. The fourth substituent (benzene ring) occupies a hydrophobic pocket. Altogether, this signifies that the presence of all four substituents oriented rigidly by the molecule's stereocenter is essential for high potency and selectivity.[22]

Although results of oral bioavailability and in vivo antidiabetic activity assays for compound **2a** have yet to be published, in vitro results show improved PTP1B inhibitory activity (IC_{50} = 5 nM) and moderate selectivity (sevenfold) over TCPTP (IC_{50} = 36 nM). The X-ray crystal structure of PTP1B in complex with compound **2a** illustrates that a methoxy group aligns very closely (3.7 Å) to the side chain of F52 (site B).[23] Oral administration of compounds **1** and **3** demonstrated good antidiabetic activity (compound **3**: ED_{50} = 0.8 mg kg^{-1}, p.o.) and oral bioavailability (compounds **1** and **3**: F = 13 and 24%, respectively) in different animal species, despite their moderate in vitro PTP1B inhibitory activity (IC_{50} = 120 nM) and poor selectivity

[a] D. Patel, Dr. M. Jain, Dr. R. Bahekar, P. Jadav, B. Darji, Y. Siriki, Dr. D. Bandyopadhyay, Dr. A. Joharapurkar, S. Kshirsagar, H. Patel, M. Shaikh, Dr. K. V. V. M. Sairam, P. Patel
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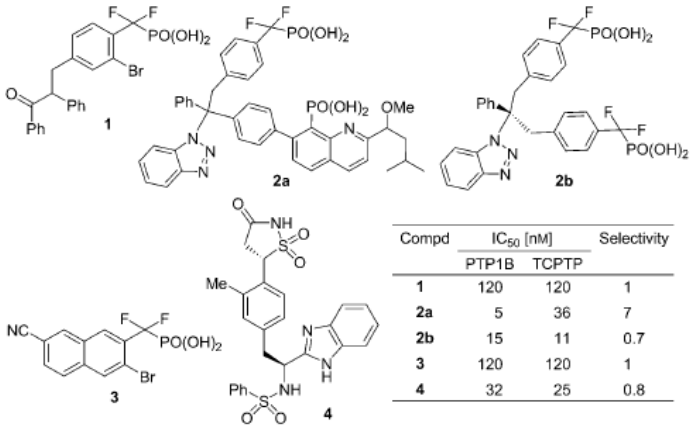
[**] ZRC communication No. 378 (part of PhD thesis work of D.P.)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201100077.



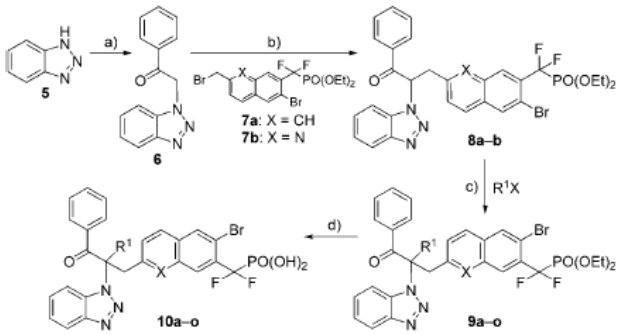
Compd	IC_{50} [nM]		Selectivity
	PTP1B	TCPTP	
1	120	120	1
2a	5	36	7
2b	15	11	0.7
3	120	120	1
4	32	25	0.8

Figure 1. Structurally diverse small-molecule-based PTP1B inhibitors **1**–**4**.

(<1-fold over TCPTP).[22] A few years ago researchers at Incyte Ltd. (Wilmington, DE, USA) reported isothiazolidinone (IZD)-based pTyr mimetics as potent PTP1B inhibitors (Figure 1, **4**: IC_{50} = 32 nM).[24] The IZD heterocycles were designed based on the hypothesis that the two sulfonyl oxygen atoms mimic the oxygen atoms of the DFMP group, whereas the carbonyl and the ionized NH group mimic the DFMP anion.[25] Most importantly, compounds bearing this diffusely mono-anionic heterocyclic pTyr mimetic (IZD) showed improved membrane permeability and high binding affinity toward site B of PTP1B.[26]

With the development of potent and selective PTP1B inhibitors as a potentially viable approach for the safe and effective treatment of T2DM, herein we report the design of novel benzotriazole-based dual-site PTP1B inhibitors (compound **10a–o**). There are four key structural components: a) benzotriazole ring, b) acetophenone, c) DFMP-substituted naphthyl or quinolinyl derivatives, and d) benzyl, naphthyl, or quinolinyl ring systems suitably substituted with difluoromethylsulfonamide (DFMS), DFMP, or IZD groups. The benzotriazole ring system was introduced as a basic pharmacophore to obtain superior PTP1B selectivity over other PTPs.[22] The two DFMP groups were specifically incorporated as pTyr mimetics to access both binding sites A and B, thereby improving potency and selectivity for PTP1B over TCPTP. Furthermore, bioisosteric replacement of DFMP was carried out with DFMS/IZD, to improve membrane permeability and binding affinity for site B.[26] The acetophenone, benzyl, naphthyl, and quinolinyl ring systems were integrated to improve overall lipophilicity and thus oral efficacy. The in vitro PTP1B inhibitory activity and subtype-selectivity of all test compounds **10a–o** were assessed with an in vitro *para*-nitrophenylphosphate (*p*NPP) enzyme assay.[27] Furthermore, based on the in vitro results, highly potent and selective test compounds **10h** and **10j** were subjected to in vivo studies to determine their antidiabetic effects and pharmacokinetic (PK) profiles.[28]

Synthesis of the title compounds **10a–o** was carried out as shown in Scheme 1 by following a modified published procedure.[22b] Treatment of benzotriazole **5** with phenacyl bromide in the presence of sodium hydride gave the 1-phenacyl-1*H*-1,2,3-benzotriazole **6**. Deprotonation of compound **6** with *n*-butyllithium followed by alkylation with the appropriate electrophiles (**7a** or **7b**) gave the intermediate **8a,b** in good yield. Subsequent alkylation of compounds **8a,b** with variously substituted benzyl



Scheme 1. *Reagents and conditions:* a) $BrCH_2COPh$, NaH, DMF, 25 °C, 5 h; b) *n*BuLi, THF, −78–25 °C, 4 h; c) *n*BuLi, THF, R^1X −78–25 °C, 6 h; d) TMSBr, CH_2Cl_2, −15 °C, 3 h and/or TFA, CH_2Cl_2, 2 h.

bromides or the naphthyl or quinolinyl analogues in the presence of *n*-butyllithium led to the formation of compounds **9a–o**, which, upon deprotection with trimethylsilyl bromide/trifluoroacetic acid, yielded the final compounds **10a–o**. The structures of all final compounds and their intermediates were confirmed by physical, analytical, and spectral data (^{13}C NMR, ^1H NMR, and ESIMS). In general, compounds **10a–o** were prepared in good yields under mild reaction conditions. The overall yields at the final step were found to be in the range of 60–80%. ESIMS data showed molecular ion peaks $[M]^+$ with different intensities, corresponding to the molecular weights of the compounds. Elemental analyses were determined within ±0.04% of theoretical values. The IR and NMR spectroscopic data for all synthesized compounds were also found to be in agreement with the structures assigned (see the Supporting Information).

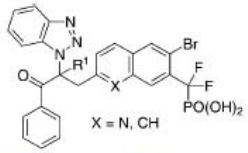
The in vitro PTP1B inhibitory activity (*p*NPP assay) was determined in order to establish the structure–activity relationship

(SAR). The compounds were prepared in two main series, either by introducing naphthyl (**10a–k**) or quinolinyl (**10l–o**) templates. Within the first series **10a–k**, distinct sets of compounds were prepared by substituting DFMP/DFMS/IZD benzyl groups at position R^1 (**10a–g**) or with DFMP/DFMS-substituted naphthyl/quinolinyl templates (**10h–k**). In the second series **10l–o**, DFMP/DFMS-substituted quinolinyl analogues of the selected test compounds of the first series (**10b**, **10d**, **10i**, and **10k**) were prepared. As shown in Table 1, all the tested compounds showed varying degrees of PTP1B inhibition (IC_{50}) depending on the nature of the substituents.

The first set of compounds containing DFMP-substituted benzyl groups at R^1 (compounds **10a–c**) showed diverse PTP1B inhibitory activity depending on the DFMP group position (*meta* versus *para*) on the benzyl ring and the *ortho* substituents (hydrogen or halogen). Compound **10c**, with a *m*-DFMP group, showed weak PTP1B inhibitory activity relative to that of *p*-DFMP (in **10a**), whereas compound **10b**, containing *p*-DFMP and *o*-bromo substitution to DFMP, showed good PTP1B inhibitory activity. Bioisosteric replacement of the *p*-DFMP group in compound **10b** with *p*-DFMS (**10d**) showed similar in vitro PTP1B inhibitory activity, indicating that the highly negatively charged DFMP group can be replaced with a DFMS group to overcome the issue of low permeability.

The second set of compounds containing IZD-substituted benzyl groups at R^1 (**10e–g**) showed weak PTP1B inhibitory activity, irrespective of their *ortho* substituents. The third set of the compounds with position R^1 as DFMP/DFMS-substituted naphthyl/quinolinyl templates (**10h–k**) showed potent PTP1B inhibitory activity. The second series of compounds (DFMP/DFMS-substituted quinolinyl derivatives **10l–o**) showed promising and similar PTP1B inhibitory activities as observed for the

Table 1. In vitro PTP1B inhibitory activity and selectivity of test compounds **10a–o**.



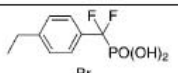
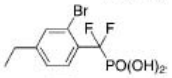
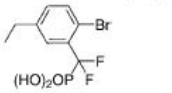
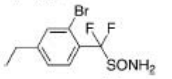
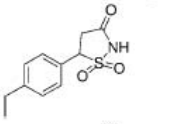
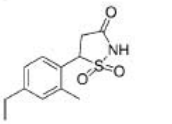
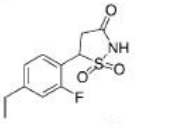
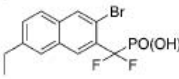
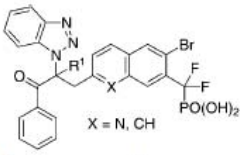
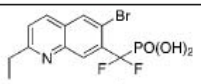
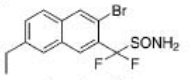
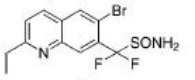
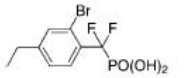
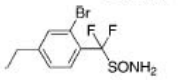
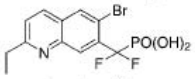
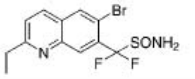
Compd	R^1	X	PTP1B[a]	TCPTP[b]
			IC_{50} [nM]	
10a		CH	46	
10b		CH	20	201 (10)[c]
10c		CH	404	
10d		CH	19	192 (10)[c]
10e		CH	930	
10f		CH	924	
10g		CH	919	
10h		CH	5	580 (116)[c]

Table 1. (Continued)



Compd	R^1	X	PTP1B[a]	TCPTP[b]
			IC_{50} [nM]	
10i		CH	3	90 (30)[c]
10j		CH	4	480 (120)[c]
10k		CH	6	185 (30.8)[c]
10l		N	21	
10m		N	22	
10n		N	5	101 (20.2)[c]
10o		N	4	82 (20.5)[c]

[a] Enzyme assays were carried out in 96-well plates; *para*-nitrophenylphosphate (*p*NPP) and test compounds were added to assay buffer, and reactions were initiated by the addition of PTP1B or TCPTP (10–100 nM). The initial rate of PTPase-catalyzed hydrolysis of *p*NPP was measured at λ 405 nm. IC_{50} values were determined under a fixed *p*NPP concentration of 1 mM ($n=3$). [b] Selected test compounds that were screened for TCPTP inhibitory activity also showed >5000-fold selectivity over CD45, LAR, SHP-1, and SHP-2 enzymes (data not shown). [c] Fold selectivity calculated as the ratio of IC_{50} values of TCPTP/PTP1B inhibition.

DFMP/DFMS-substituted naphthyl derivatives **10b**, **10d**, **10i**, and **10k**.

As mentioned above, our goal was to develop potent and selective tetrasubstituted benzotriazole-based PTP1B inhibitors, and in this attempt in the first series, three sets of compounds were prepared by introducing DFMP, DFMS, or IZD as pTyr mimetics either on benzyl or naphthyl/quinolinyl templates. The SAR study revealed that the *para*-substituted pTyr mimetics (DFMP, DFMS, and IZD) exhibit favorable PTP1B inhibitory activity. The compounds with *ortho*-bromo substitution next to pTyr mimetics show potent PTP1B inhibitory activity, and among three different pTyr mimetics, DFMP and DFMS exhibited the highest PTP1B inhibitory activity. No significant difference in activity was observed among the compounds with naphthyl or quinolinyl templates. However, these compounds were found to be more potent than the benzyl derivatives. Overall, the in vitro PTP1B inhibition results clearly show that the potency of tetrasubstituted dual-site benzotriazole-based PTP1B inhibitors can be modulated with suitable substituents at the R^1 position.

The in vitro selectivity over other PTPs (PTPα, LAR, CD45, VHR, SHP-1, SHP-2, and TCPTP) was evaluated for the most potent compounds (**10b**, **10d**, **10h–k**, **10n–o**) by using the *p*NPP assay, and IC_{50} values are listed in Table 1.[27] Compounds **10b** and **10d** showed ~10-fold selectivity, **10h** and **10j** showed >115-fold selectivity, **10i** and **10k** showed ~30-fold selectivity, and compounds **10n–o** showed ~20-fold selectivity over TCPTP. All the selected test compounds showed >5000-fold selectivity over PTPα, LAR, CD45, VHR, SHP-1, and SHP-2 enzymes. Compounds **10b** and **10d**, containing DFMP/DFMS-substituted benzyl groups at R^1, showed poor selectivity. Compounds **10i** and **10k**, containing DFMP/DFMS-substituted quinolinyl templates at R^1, and the second series of compounds **10n–o**, containing additional DFMP/DFMS-substituted quinolinyl templates, showed moderate TCPTP selectivity, while **10h** and **10j**, containing DFMP/DFMS-substituted naphthyl templates at R^1, showed excellent selectivity over TCPTP, indicating that among the three different ring systems (benzyl, naphthyl, and quinolinyl) selected as R^1, only naphthyl derivatives showed the best selectivity, perhaps due to the favorable orientation of the naphthyl ring system across both the binding sites A and B of PTP1B.

The in vivo antidiabetic activity of the most potent and selective compounds **10h** and **10j** was evaluated in male C57BL/6J mice using the intraperitoneal glucose tolerance test (IPGTT) protocol.[28] Briefly, mice fasted overnight ($n=6$) were dosed orally (p.o.)/intraperitoneally (i.p.) with the test compounds **10h** or **10j** (10 mg kg^{-1}) 0.5 h prior to the i.p. glucose load (1.5 g kg^{-1}, 10 mL). Blood samples were collected at various time points (0, 30, 60, 120, and 240 min), and the serum was separated, subjected to glucose estimation, and serum glucose levels (mg dL^{-1}) were recorded (Figure 2). Both the test compounds effected significant decreases in blood glucose when administered by the i.p. route. Compound **10h** showed excellent antidiabetic activity orally, whereas compound **10j** showed moderate activity upon p.o. administration. In the design of compound **10j** (R^1: DFMS-substituted naphthyl derivative), it was assumed that the highly negatively charged DFMP group of **10h** could be replaced with a DFMS group to alleviate the permeability issue; however, these comparative oral antidiabetic activity results indicate that the DFMP group is more favorable for oral antidiabetic activity than the DFMS group.

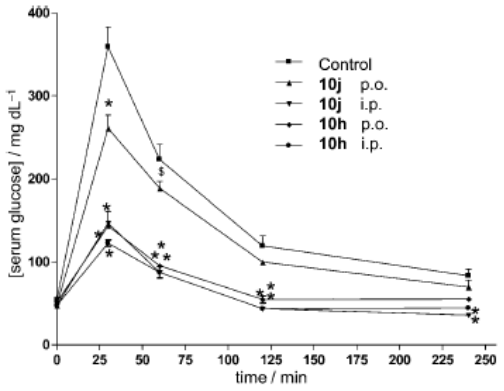


Figure 2. Antidiabetic activity of compounds **10h** and **10j** in C57 mice (IPGTT): Overnight-fasted male C57BL/6J mice ($n=6$) were dosed p.o./i.p. with vehicle or test compounds (10 mg kg^{-1}) 0.5 h prior to IPGTT (1.5 g kg^{-1}, 10 mL); serum glucose levels were determined at 0, 30, 60, 120, and 240 min. Values represent the mean ±SEM; $^{*}p<0.01$ and $^{\$}p<0.05$ by two-way ANOVA followed by Bonferroni post-test.

Further to understand the PK profile, a comparative single dose (10 mg kg^{-1} i.v. or p.o.) study of **10h** and **10j** was carried out in male Wistar rats ($n=6$), and the various PK parameters were recorded (Table 2). In a single-dose PK study, compound **10j** showed rapid t_{max} and clearance, good area under the curve (AUC), and moderate half-life ($t_{1/2}$), whereas **10h** showed extended t_{max} and $t_{1/2}$ values, and moderate AUC and clearance. Relative to **10j**, compound **10h** showed roughly sevenfold higher bioavailability (F~68%). Thus, the improved PK profile of compound **10h** supports its excellent pharmacodynamic effects (antidiabetic activity) in C57 mice when administered orally.

Table 2. Comparison of pharmacokinetic parameters of compounds **10h** and **10j**.

Route	Parameter[a]	10h	10j
i.v.	$t_{1/2}$ [h]	1.01 ±0.2	2.41 ±0.3
	K_{elim} [h^{-1}]	0.99 ±0.1	0.29 ±0.1
	AUC [h μg mL^{-1}]	15.499 ± 0.191	29.341 ±0.795
p.o.	t_{max} [h]	0.33 ±0.2	1.21 ±0.01
	$t_{1/2}$ [h]	3.81 ±0.2	8.67 ±0.26
	K_{elim} [h^{-1}]	0.44 ±0.01	0.08 ±0.05
	AUC [h μg mL^{-1}]	10.562 ± 0.105	3.031 ±0.312
	F [%]	68.14	10.33

[a] Single-dose (10 mg kg^{-1} i.v./p.o.) PK studies for compounds **10h** and **10j** were carried out in fasted male Wistar rats ($n=6$), and plasma compound concentrations were determined by LC–MS–MS; data represent the mean ±SD.

A molecular docking analysis of **10h** was carried with Glide docking software (ver. 5.6) to understand its selectivity profile and critical interactions with both binding sites A and B of PTP1B.[29] The initial Glide docking studies for **10h** gave poor results in terms of binding conformation. Based on this observation, the compound **10h** was docked in the active site of PTP1B (PDB code: 1Q6T) using the induced-fit docking (IFD) protocol, which involves adjustments in the residues surrounding the binding sites to obtain an alternative structure that can accommodate ligands that would otherwise not fit into the original binding sites. The IFD procedure is based on the Glide docking program with the refinement module in Prime (Schrödinger LLC), which was reported to accurately predict the ligand binding modes and concomitant structural changes in the receptor.[29] Prime was used for side chain rotamer prediction and energy minimization of the conformation of residues located within 5 Å of the ligand in any of the poses.

The IFD results illustrate that the residues of both binding sites A and B adopt new conformations, allowing better ligand fit (**10h**) at both sites. The comparative binding site pockets of PTP1B with respect to compound **10h** and in comparison with the X-ray crystal structure of the known PTP1B inhibitor **2a** are shown in Figure 3A and 3B. It was observed that upon IFD, Arg524 and Phe682 rotamers are changed, and because of this, compound **10h** docks very well into both sites. Thus PTP1B selectivity over TCPTP was achieved by taking advantage of amino acid differences in site B (F552Y and A527S). In particular, the flipping of the Phe682 aromatic ring and the side chain of Arg524 was predominant. As a result, the site B cavity is enlarged, thereby accommodating ligand **10h** quite well, and hence site B best fits with the overall shape of **10h** (Figure 3C). The favorable hydrogen bond interactions between compound **10h** and both sites A and B of PTP1B support its excellent in vitro PTP1B selectivity over TCPTP.

In summary, novel tetrasubstituted benzotriazole-based PTP1B inhibitors containing a DFMP-substituted naphthyl template at R^1 show excellent in vitro potency and selectivity over TCPTP, indicating that among three different ring systems (benzyl, naphthyl, and quinolinyl) selected as R^1, only naphthyl derivatives show high selectivity owing to favorable orientation of the naphthyl ring system across both the binding sites of the PTP1B enzyme. The lead compound **10h** shows excellent anti-hyperglycemic effects in animal models, along with improved oral bioavailability. The results of our in silico docking study are in agreement with the observed in vitro PTP1B selectivity. The results of this preliminary study confirm that highly potent and selective PTP1B inhibitors could represent a viable approach toward the safe and effective regulation of glucose homeostasis in T2DM patients. Further evaluations of these lead compounds (chronic pharmacodynamic studies and toxicological evaluations) are currently underway, and results will be communicated in due course.

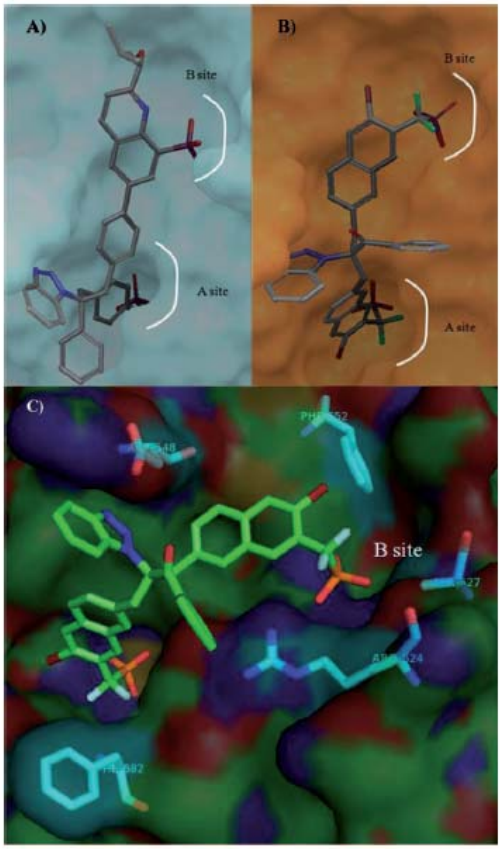


Figure 3. A) X-ray crystal structure of PTP1B in complex with the known inhibitor **2a** (PDB code: 1Q6T). B) Binding pose of **10h** in the PTP1B active site; sites A and B are indicated. Compound **10h** occupies both sites which is essential for PTP1B selectivity over TCPTP. C) Key residues in binding site B surrounding **10h**: upon IFD, Arg524 and Phe682 rotamers change and as a result **10h** docks very well into both sites. Residue numbering is as per current numbering (PDB code: 1Q6T): Arg24 (524), Ala27 (527), Phe52 (552), Arg254 (754), and Met258 (758).

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Keywords: benzotriazoles · inhibitors · metabolic disorders · PTP1B · TCPTP

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[29] Schrödinger Suite 2010, Glide version 5.6, Prime version 2.2, Schrödinger LLC, New York, NY (USA) 2010.

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Discovery of potent, selective and orally bioavailable triaryl-sulfonamide based PTP1B inhibitors ☆

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ABSTRACT

A novel series of *p*Tyr mimetics containing triaryl-sulfonamide derivatives (**5a–r**) are reported as potent and selective PTP1B inhibitors. Some of the test compounds (**5o** and **5p**) showed excellent selectivity towards PTP1B over various PTPs, including TCPTP (in vitro). The lead compound **5o** showed potent anti-diabetic activity (in vivo), along with improved pharmacokinetic profile. These preliminary results confirm discovery of highly potent and selective PTP1B inhibitors for the treatment of T2DM.

Over the past decade, there has been an alarming increase in the metabolic syndrome such as obesity and diabetes.[1,2] Patients suffer from obesity-induced type 2 diabetes (diabesity) are at increased risk of cardiovascular diseases and pose huge economic burden on healthcare services.[3] Clinically type 2 diabetes mellitus (T2DM) is characterized by persistent hyperglycemia, either due to defects in insulin secretion, insulin resistance or both.[4] Currently diabetic patients are treated with various oral antihyperglycemic agents; however, over a period of time, T2DM subjects lose their response to these agents and thereby require insulin therapy. Except incretin therapies, most of the available antihyperglycemic agents, including insulin promote weight gain, which further aggravates obesity associated cardiovascular risk and insulin resistance.[5–9] Thus, there is an urgent need to develop novel agents for glycemic control.

Protein tyrosine phosphatase 1B (PTP1B) enzyme acts as a negative regulator in insulin signaling pathways. Inhibition of PTP1B enzyme activity exhibits potential for enhancing insulin action by prolonging the phosphorylated state of the insulin receptor.[10] Several structurally diverse small-molecule based PTP1B inhibitors have been developed.[11] Initially, PTP1B inhibitors were designed to bind to the active site (Site-1/A).[12] However, achieving PTP1B selectivity over closely associated PTPs (PTPα, LAR, CD45, VHR, SHP-1, SHP-2 and T-cell protein tyrosine phosphatase (TCPTP)) is one of the major challenge, as the closely associated PTPs share a high degree of sequence homology (92%).[13] Lack of oral bioavailability is another aspect in the development of potent and selective PTP1B inhibitors, as the majority of the active site directed PTP1B inhibitors exhibit limited cell permeability.[14,15]

An additional non-catalytic aryl-phosphate binding site (site-2/B) was also identified, proximal to the catalytic-phosphate binding site.[13] The site B of PTP1B differs from that of TCPTP by a few amino acids (F52Y and A27S) and thus offers an opportunity to improve selectivity over TCPTP.[16] Based upon this dual binding site concept, recently, we reported, effect of bidentate *p*Tyr mimetics (Difluoromethylphosphonates (DFMP)/Difluoromethylsulfonamide (DFMS)/Isothiazolidinone (IZD)) on benzotriazole-scaffold which lead to a discovery of an orally active dual binding-site PTP1B inhibitors (Compound **1**; Fig. 1).[17] As a part of our ongoing research on PTP1B inhibitors, herein, we report incorporation of monodentate *p*Tyr mimetics (DFMP/DFMS/IZD) on triaryl sulfonamide based-scaffold (Compound **2**; Fig. 1).[18] Although selectivity, pharmacodynamic (PD) and pharmacokinetic (PK) profile of compound **2** is not reported, however, in vitro it showed good PTP1B inhibitory activity (IC_{50}: 74 nM).[18] Therefore, the triaryl sulfonamide based scaffolds were specifically selected to design dual binding PTP1B inhibitors (**5a–r**). The in vitro PTP1B inhibitory activity and subtypes-selectiv

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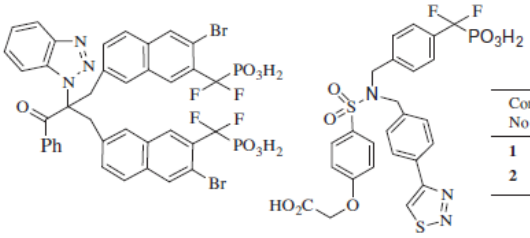


Compd No	PTP1B (IC_{50}; μM)	TCPTP (IC_{50}; μM)	Selectivity
1	0.005	0.580	116
2	0.074	NA	NA

1 **2**

Figure 1. Structurally diverse small molecule based-PTP1B inhibitors.

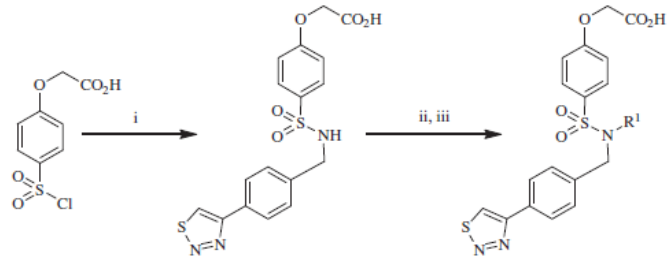
ity of all the test compounds **5a–r** were assessed using enzymatic assay (in vitro).[19] Furthermore, based on the in vitro results, highly potent and selective test compounds **5o** and **5p** were subjected in vivo, to determine their antidiabetic effect and PK profile.[20,21]

Synthesis of the title compounds **5a–r** was carried out as depicted in Scheme 1, following the modified literature procedure.[18c] Treatment of substituted benzenesulfonyl chloride **3** with primary amine in the presence of *N,N*-diisopropylethylamine gave mono-substituted sulfonamide **4**. Alkylation of the compound **4** with appropriate electrophile gave the di-substituted sulfonamides, which were subsequently converted to **5a–r** by treatment with trimethylsilyl bromide/trifluoracetic acid to hydrolyze the *t*-butyl esters and the phosphonate diethylesters. All the titled compounds and intermediates were characterized with their physical, analytical and spectral data (^{13}C NMR, ^{1}H NMR and ESI MS). Elemental analyses were determined within 0.04% of theoretical values. Overall, **5a–r** was prepared in good yield, under the mild reaction conditions. The percentage yield in the final step was found to be in the range of 60–80%. The ESI MS and NMR spectral data of all the synthesized compounds were also found to be in conformity with the structures assigned and ensure the formation of the compounds **5a–r** (see Supplementary data for analytical and spectral data).

The in vitro PTP1B inhibitory activity (*p*-NPP assay) was determined in order to establish the structure–activity relationship (SAR). As shown in Tables 1 and 2, two series of the compounds were prepared by substituting DFMP/DFMS/IZD-benzyl groups (**5a–j**) or with DFMP/DFMS-substituted naphthyl/quinolinyl templates (**5k–r**) at position R^1. In the first series (**5a–j**), two sets of compounds were prepared by substituting DFMP/DFMS benzyl groups (**5a–f**) or IZD-substituted benzyl templates (**5g–j**), while in the second series (**5k–r**), two sets of compounds were prepared by substituting DFMP/DFMS-substituted naphthyl/quinolinyl templates (**5k–n**) or its fluoro analogues (**5o–r**). Depending on the nature of the substitutents, the tested compounds showed varying degrees of PTP1B inhibitory activity (Tables 1 and 2).

Within the first series (**5a–j**), the first set of compounds (**5a–f**) containing DFMP-substituted benzyl groups at R^1 (**5a–d**) showed diverse PTP1B inhibitory activity depending on the DFMP group position (*meta* vs *para*) on the benzyl ring and the *ortho* substitutents (hydrogen or halogen). Compound **5b**, with a *m*-DFMP group, showed weak PTP1B inhibitory activity relative to that of *p*-DFMP (**2**), whereas compound **5a**, containing *p*-DFMP and *o*-bromo substitution to DFMP, showed good PTP1B inhibitory activity. Replacement of *o*-bromo analogues (**5a–b**) with more electronegative *o*-fluoro derivatives (**5c–d**), showed improved PTP1B inhibitory activity. Bioisosteric replacement of the *p*-DFMP group in compound **5a** with *p*-DFMS (**5e** and **5f**) showed comparable in vitro PTP1B inhibitory activity, suggesting that the highly negatively charged DFMP group can be replaced with a DFMS group to overcome the issue of low permeability. Among the **5e** and **5f** tested, **5f** showed improved PTP1B inhibitory activity, which could be due to increase in the electronegativity and decrease in the steric bulk (**5e**: ortho-bromo vs **5f**: ortho-fluoro) adjacent to the DFMS group. The second set of compounds containing IZD-substituted benzyl groups at R^1 (**5g–j**) showed weak PTP1B inhibitory activity, irrespective of their ortho substitutents. Among the second set (**5g–j**), ortho-fluoro derivative (**5j**) was found to be most potent, whereas the ortho-methyl (**5h**) and ortho-bromo (**5i**) derivatives were found to be equipotent. Unsubstituted derivative (**5g**) was found to be least active, with the set.

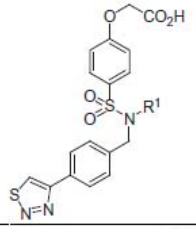
The second series of compounds with position R^1 as DFMP/DFMS-substituted naphthyl/quinolinyl templates (**5k–r**) showed potent PTP1B inhibitory activity. Within the second series, the first set of compounds containing DFMP-substituted naphthyl/quinoli-



3 **4** **5(a-r)**

Scheme 1. Synthesis of compounds **5a–r**. Reagents and conditions: (i) thiadiazolyl-C_6H_4-CH_2-NH_2, CH_2Cl_2, DIEA, 25 °C, 5 h; (ii) R^1-X, K_2CO_3, CH_3CN, 70 °C, 4 h; (iii) TMSBr, CH_2Cl_2, −15 °C, 3 h or TFA, CH_2Cl_2, 2 h.

Table 1
The In vitro PTP1B inhibitory activity and selectivity of test **5a–j**



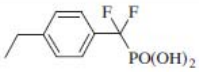
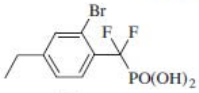
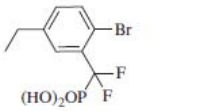
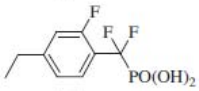
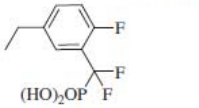
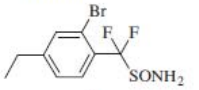
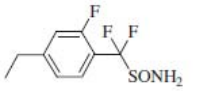
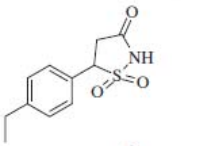
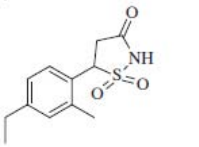
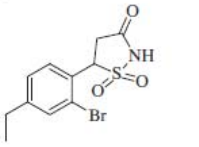
Compd No.	R^1	PTP1B[a] IC_{50} (nM)	TCPTP[b] IC_{50} (nM)
2		78 ± 02	546 ± 12 (~7)[c]
5a		50 ± 03	750 ± 13 (~15)[c]
5b		288 ± 08	
5c		35 ± 04	870 ± 07 (~24)[c]
5d		141 ± 10	
5e		47 ± 04	
5f		31 ± 02	620 ± 08 (~20)[c]
5g		500 ± 10	
5h		470 ± 11	
5i		480 ± 11	

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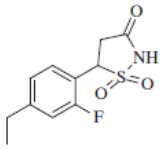
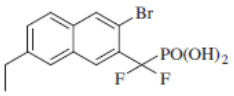
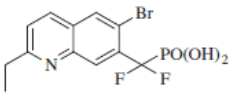
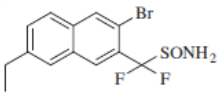
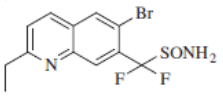
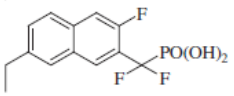
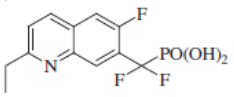
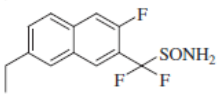
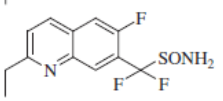
Table 1 (*continued*)

Compd No.	R^1	PTP1B[a] IC_{50} (nM)	TCPTP[b] IC_{50} (nM)
5j		460 ± 12	

[a] Enzymatic assay was carried out in 96-well plates. The initial rate of PTPase-catalyzed hydrolysis of pNPP was measured at 405 nm. IC_{50} value was determined under fixed pNPP concentration of 1 mM (n = 3; represents Mean ± SD).
[b] Selected test compounds which were screened for TCPTP inhibitory activity also showed >5000-fold selectivity over CD45, LAR, SHP-1 and SHP-2 enzymes (data not shown).
[c] Fold selectivity calculated as ratio of average IC_{50} values of TCPTP/PTP1B inhibitions.

Table 2
The In vitro PTP1B inhibitory activity and selectivity of test **5k–r**

Compd No.	R^1	PTP1B[a] IC_{50} nM	TCPTP[b] IC_{50} nM
5k		17 ± 01	
5l		18 ± 03	
5m		39 ± 03	
5n		41 ± 05	
5o		9 ± 01	870 ± 05 (~96)[c]
5p		11 ± 04	990 ± 08 (~90)[c]
5q		35 ± 07	
5r		38 ± 08	

[a] Enzymatic assay was carried out in 96-well plates. The initial rate of PTPase-catalyzed hydrolysis of pNPP was measured at 405 nm. IC_{50} value was determined under fixed pNPP concentration of 1 mM (*n* = 3; represents Mean ± SD).
[b] Selected test compounds which were screened for TCPTP inhibitory activity also showed >5000-fold selectivity over CD45, LAR, SHP-1 and SHP-2 enzymes (data not shown).
[c] Fold selectivity calculated as ratio of average IC_{50} values of TCPTP/PTP1B inhibitions.

nyl templates (**5k–l**) showed better PTP1B inhibitory activity compare to its bioisostere DFMS-substituted templates (**5m–n**). At pH 6.5 (simulate with in vitro system), it has been reported that the monoanionic DFMP exhibit an equilibrium with its dianionic form and the PTP1B enzyme show strong binding interaction with the dianionic form.[22] Thus compounds containing DFMP-substituted templates (**5k–l**) showed better PTP1B inhibitory activity compare to DFMS-substituted templates (**5m–n**), may be due to the preference of PTP1B enzyme binding with the dianionic form of DFMP. In second set, replacement of bromo analogues (**5k–n**) with its more electronegative fluoro analogues (**5o–r**), leads to a highly potent PTP1B inhibitors among all series. As observed in the previous series, electronegative fluoro analogues (**5o–r**) were found to be more potent compare to its bromo analogues (**5k–n**), which could

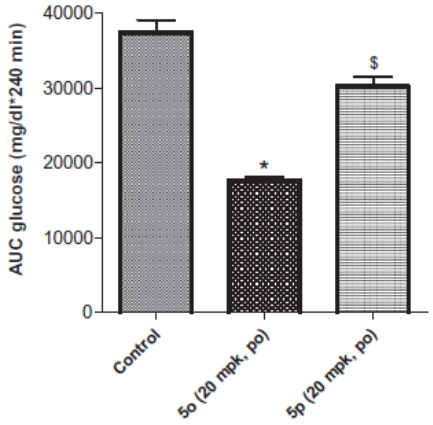
be due to increase in the electronegativity and decrease in the steric bulk (**5k–n**: ortho-bromo vs **5o–r**: ortho-fluoro) adjacent to the DFMS/DFMP groups.

The SAR study reveals that the *para*-substituted *p*Tyr mimetics exhibit favorable PTP1B inhibitory activity. The compounds with *ortho*-halogen substitution next to *p*Tyr mimetics showed potent PTP1B inhibitory activity and among three-different *p*Tyr mimetics, DFMP exhibited the highest PTP1B inhibitory activity. Similarly, the fluoro analogues of naphthyl/quinolinyl templates containing DFMP as a *p*Tyr mimic were found to be more potent than their corresponding bromo analogues. Among two series tested, compounds with DFMP/DFMS-substituted naphthyl/quinolinyl templates (**5k–r**; second series) were found to be more potent than the benzyl derivatives (**5a–j**; first series). One possible explanation for this is that naphthyl/quinolinyl ring possibly involves more pi-stacking interactions with aromatic residues located close to the active site of PTP1B, compare to benzyl ring. Overall, in vitro PTP1B inhibitory activity results clearly suggest that the potency of triaryl sulfonamide-based PTP1B inhibitors can be modulated using suitable substitutents at R^1 position.

The in vitro selectivity over PTPs (PTPα, LAR, CD45, VHR, SHP-1, SHP-2 and TCPTP) was evaluated for most potent compounds (**5a**, **5c**, **5f**, **5o** and **5p**), using *p*-NPP assay and IC_{50} values are listed in (Tables 1 and 2).[19] Compounds **5a** and **5c** showed ~15 to 25-fold selectivity, **5f** showed ~20-fold selectivity, **5o** and **5p** showed ~90 to 96-fold selectivity over TCPTP enzyme, while all the selected test compounds showed >5,000-fold selectivity over PTPα, LAR, CD45, VHR, SHP-1 and SHP-2 enzymes. The compounds **5a**, **5c** and **5f** containing R^1 as DFMP/DFMS-substituted benzyl groups showed poor selectivity. Compounds **5o** and **5p** containing DFMP/DFMS-substituted naphthyl/quinolinyl templates at R^1, showed excellent selectivity over TCPTP, indicated that among the two different ring systems (benzyl/fused-ring system) selected as R^1, only fused-ring templates (naphthyl/quinolinyl derivatives) showed best selectivity. The in vitro PTP1B inhibitory activity and PTPs selectivity was also determined for standard compound **2** (Table 1; IC_{50}: 78 ± 02 nM, with ~7-fold selectivity over TCPTP) and the IC_{50} values (PTP 1B inhibitory activity) were found to be in agreement with the literature (IC_{50}: 74 nM).[18]

The in vivo antidiabetic activity of the most potent and selective compounds (**5o** and **5p**; @ 20 mpk, po) was evaluated in male C57BL/6J mice, using IPGTT (Intraperitoneal glucose tolerance test) protocol and changes in serum glucose levels (AUC glucose @ 240 min; mg dL^{-1}), with compounds **5o** and **5p** are shown in Figure 2.[20] Compound **5o** showed excellent antidiabetic activity orally, whereas compound **5p** showed moderate activity upon oral administration (−52.8 ± 5.36 and −19.18 ± 5.8, respectively). In vivo antidiabetic activity was also evaluated for standard compound **2** (as positive control). Compared to vehicle control, there was no change in the serum glucose levels (AUC glucose) was observed with compound **2**, when it was administered @ 20 mpk, po. Compound **2** was found to be inactive, which could be due to its poor oral bioavailability.

Further to understand the pharmacokinetic (PK) profile, a comparative single dose (20 $mgkg^{-1}$ iv or po) study of **5o** and **5p** was carried out in male C57BL/6J mice ($n = 6$) and the various PK parameters such as T_{max}, $t_{1/2}$, C_{max}, AUC and %*F* were calculated and recorded (Table 3).[21] In a single-dose PK study, compound **5o** showed rapid t_{max}, good area under the curve (AUC), and extended half-life ($t_{1/2}$), whereas **5p** showed extended t_{max}, short $t_{1/2}$ and moderate AUC. Relative to **5p**, compound **5o** showed ~7-fold



*P<0.05, $P<0.01, Two-Way ANOVA followed by Bonferroni post test, M ± SEM;
Standard compound **2** was also tested @ 20 mpk, po, but no change in the AUC glucose was observed with respect to vehicle control.

Figure 2. In vivo antidiabetic activity of compounds **5o** and **5p** in C57 mice (IPGTT).

Table 3
Pharmacokinetic (PK) study parameters[a] of compounds **5o** and **5p**

Compd No.	T_{max} (h)	C_{max} (μg/ml)	$T_{1/2}$ (h)	AUC (0–∞) h μg/ml	%*F*[b]
5o	0.31 ± 0.01	6.3 ± 0.51	6.96 ± 0.91	8.91 ± 0.14	6.51
5p	0.69 ± 0.10	0.96 ± 0.32	0.99 ± 0.76	1.32 ± 0.31	0.9

[a] In male C57BL/6J mice ($n = 6$), compounds **5o** and **5p** were administered orally (po) at 20 $mgkg^{-1}$ dose and plasma concentration was analyzed by LC–MS, values indicate Mean ± SD.
[b] Oral bioavailability (%F) was calculated wrt to iv AUC (**5o**: 137.07 ± 4.28 and **5p**: 146.66 ± 9.81 h μg/ml) administered at 20 $mgkg^{-1}$ dose, iv.

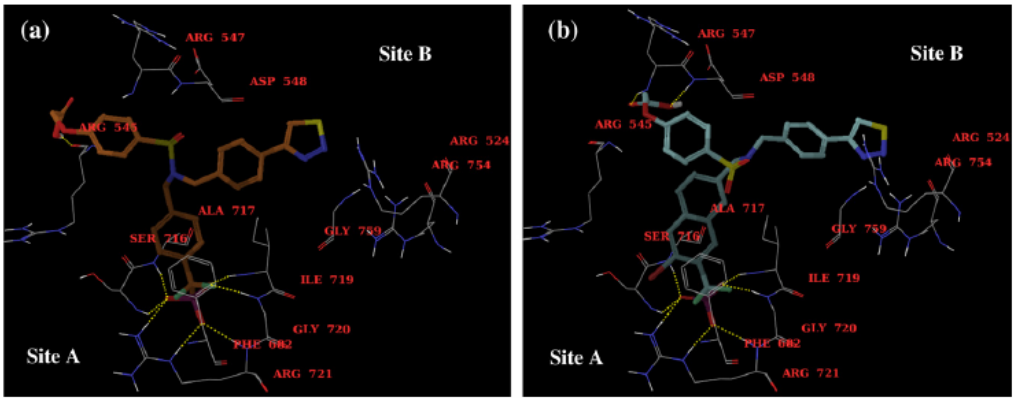


Figure 3. Key interaction of compounds **2** and **5o** with active site-A and secondary aryl-binding site-B. (a) Binding pose of compound **2** in the PTP1B active site is indicated, wherein it interact closely with key residues of site A and marginally with site B. (b) Compound **5o** docks very well into both the sites (A & B), particularly, flipping of thiadiazolyl moiety in site B and its strong interactions with key resides of site B (Asp548, Arg524 and Arg754) favors best fits of **5o**. Residue numbering is as per current numbering (PDB code: 1Q6T): Arg24 (524), Asp48 (548) and Arg254 (754).

higher bioavailability (%*F*: ~6.5%). Thus improved pharmacokinetic profile of compound **5o** justifies its excellent pharmacodynamic effects (antidiabetic activity) in C57 mice, when administered orally.

The molecular docking analysis of **5o** was carried in Glide, to understand its critical interactions with both the binding sites (A and B) of PTP1B enzyme.[23] The initial Glide docking studies for **5o** gave poor results in terms of binding conformation. Based on this observation, the compound **5o** was docked using the induced fit docking (IFD) protocol. The IFD is based on the docking program Glide with the refinement module in Prime (Schrodinger, Inc.), which was reported to accurately predict the ligand binding modes and concomitant structural changes in the receptor.[23]

Since the interaction of compound **2** with the PTP1B enzyme is unpublished, molecular docking analysis was also carried out for compound **2** (Fig. 3a). As described earlier, for achieving selectivity over TCPTP, interactions in site B is essential. The IFD results illustrate that compound **2** completely docks in the binding site A and the DFMP substituted phenyl ring of compound **2** strongly coordinate with Phe682 (site A residue), while the oxy acetic acid and thiadiazolyl ring of compound **2** partially project in site B and interact partly with key residues of site B. In vitro, ~7-fold selectivity over TCPTP enzyme was observed with compound **2** and it could be attributed to its partial orientation in site B, which is essential for achieving selectivity over TCPTP enzyme.

The IFD results of compound **5o** illustrated that introduction of DFMP-naphthyl template allows to adopt new conformation; as a result, it docks very well at the both binding sites (Fig. 3b). It was observed that upon IFD, flipping of thiadiazolyl ring was observed in site B and because of this change, compound **5o** docks very well into both the sites (A and B). Thus the PTP1B selectivity over TCPTP was achieved by taking advantage of amino acid differences in the site B. In particular, the flipping of thiadiazolyl moiety in site B and its strong interaction with key resides of site B (Asp548, Arg524 and Arg754). The favorable hydrogen bond interactions of compound **5o** with both the sites (A and B) of PTP1B enzyme support its potent in vitro PTP1B activity and excellent selectivity over TCPTP.

In summary, novel triaryl sulfonamide-based PTP1B inhibitors containing DFMP-substituted naphthyl template at R^1 show excellent in vitro potency and selectivity over TCPTP, indicating that among three different ring systems selected as R^1, only naphthyl derivatives shows best selectivity, due to favorable orientation of ligand across both the binding sites of the PTP1B enzyme. The lead compound **5o** shows significant antihyperglycemic effects (in vivo), along with oral bioavailability. Thus, preliminary study results confirm that highly potent and selective PTP1B inhibitor could be viable approach for the effective treatment of T2DM.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.122.

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21. Briefly, for single dose PK study, test compounds were administered orally/iv on a body weight basis (20 $mgkg^{-1}$) to overnight fasted male C57BL/6J mice. Serial blood samples were collected in microcentrifuge tubes containing EDTA at pre-dose, 0.15, 0.3, 0.5, 0.75, 1, 2, 4, 6, 8, 24 and 30 h post-dose after compounds administration. Approximately 0.3 ml of blood was collected at each time point and centrifuged at 4 °C. The obtained plasma was frozen, stored at −70 °C and the concentrations of compounds in plasma were determined by the LC–MS/MS (Shimadzu LC10AD, USA), using YMC hydrosphere C18 (2.0 × 50 mm, 3 μm) column (YMC Inc., USA). The pharmacokinetic parameters, such as T_{max}, $t_{1/2}$, C_{max}, AUC and $\%F$ were calculated using a non-compartmental model of WinNonlin software version 5.2.
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Peptidomimetics as Potent and Selective PTP1B Inhibitors.

Patel D, Jain M, Shah SR, Bahekar R, Jadav P, Shah K, Joharapurakar A, Shaikh M, Sairam KV.

Source

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Abstract

A series of peptidomimetics containing bidentate pTyr mimetics (9a-w) are reported as potent and selective PTP1B inhibitors. Compounds (9p and 9q) showed excellent selectivity towards PTP1B over various PTPs, including TCPTP (in vitro), confirms discovery of highly potent and selective PTP1B inhibitors.

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Vitae

8. Vitae

The author was born on 28th June, 1978 at Samsabad, district Baroda, Gujarat. After obtaining S.S.C & H.S.C. from Sardar Vinay Mandir, Baroda, he joined M. S. University, Baroda and obtained B.Sc degree in 1999 and M.Sc degree in 2001. He then joined Medicinal Chemistry Department at Zydus Research Centre in May 2001 where he is currently working as a scientist. The author is co-inventor of the molecule ZYH1 as a lipid lowering agent which phase-III clinical trials have been completed successfully. The author is also co-inventor of the molecule ZYD1 and ZYOG1 as a GLP-1 agonist for the treatment of diabetes which phase-I clinical trials have been completed successfully.

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List of Publications

[1] Rajesh H. Bahekar, Mukul R. Jain, Arun A. Gupta, Ashish Goel, Pradip A. Jadav, **Dipam N. Patel**, Vijay M. Prajapati, and Pankaj R. Patel. Synthesis and Antidiabetic Activity of 3,6,7-Trisubstituted-2-(1H-imidazol-2-ylsulfanyl)quinoxalines and Quinoxalin-2-ylisothioureas. *Arch. Pharm. Chem. Life Sci.* **2007**, *340*, 359-366.

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List of Posters

[1] Bahekar R. H., Gupta A., **Patel D**. and Loharay V. B. Synthesis of Peptides by Solid Phase Peptide Synthesis Approach (SPPS). Poster presented in 2nd RBF international Symposium, *Current Trends in*

Pharmaceutical Sciences: Role of Genomics and Proteomics held at Zydus Research Centre, Ahmedabad in January 23-25, **2005**.

[2] **Dipam N. Patel**, Pradip A. Jadav, Brijesh A. Darji, Yernaidu Siriki, Mukul R. Jain and Rajesh H. Bahekar. Synthesis and Glucose Dependent Insulinotropic Activity of Substituted-Hetero-Aryl Oxazolyl Derivatives. Poster presented in 4th RBF international Symposium, *Advances in Cardiometabolic Research-Basic Science and Clinical Aspects* held at Zydus Research Centre, Ahmedabad in February 2-5, **2009**.

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