2 Identification of a PPAR-pan Agonist

2.1 Objective

The clinical benefits of Fibrates (PPARα agonists) and Glitazones (PPAR*γ* agonists) have established PPARs as potential targets for the treatment of metabolic syndrome. However, Fibrates require a high dose to have therapeutic effects, whereas the use of Glitazones causes weight gain, edema, carcinogenicity etc. in the rodents. The importance of controlling both the glucose and the lipid levels in metabolic syndrome, gave rise to the concept of identifying PPAR dual agonists, which can activate both PPARα and PPAR*γ*. In addition to their hypolipidemic effects, Fibrates control body weight gain in rodents, without affecting food intake, which led to a hypothesis that activation of PPARα may mitigate the weight gain induced by PPAR*γ* activation in humans. Consequently over the decades of the research in search of a dual PPAR*α*/*γ* agonist, several molecules were identified and studied in clinical trials. However, all of the dual

agonists except Saroglitazar failed in late stage clinical trials due to safety concerns, including potential carcinogenicity in rodents, signs of myopathy and rhabdomyolysis, increase in plasma creatinine and homocysteine, weight gain, fluid retention, peripheral edema and potential increased risk of cardiac failure.[156] Although the discontinued compounds share a few common side effects, the reason for discontinuation was always believed to be compound specific and interestingly the ratio of activation potential towards PPARα to PPAR*γ* is also believed to play a critical role in clinical effects of these dual agonists.

Failure of the PPAR dual agonists in clinical trials and emergence of PPAR*δ* as a potential target for the treatment of metabolic syndrome and inflammation, gave rise to an interesting approach of targeting all the subtypes of PPARs with a single molecule (PPAR-pan agonism). The aim of this approach is to activate each receptor subtype in order to provide maximal efficacy on appropriate target genes associated with specific pharmacological pathways while minimizing undesired adverse side effects. Hence it is believed that a PPAR-pan agonist could offer lucrative therapeutic potential to treat a broad spectrum of metabolic diseases which includes insulin sensitization, obesity, dyslipidemia, hypertension and inflammatory conditions. Currently development of PPAR-pan agonists has become an area of high thrust among several research groups as there is no potent PPAR-pan agonist in the market.

Based on the analysis of above results, it was hypothesized that a PPAR-pan agonist with moderate potency will serve a better, safer and effective alternative for the treatment of metabolic syndrome.

2.2 Introduction of Structurally Constrained oxime ether linker

2.2.1 Designing strategy

The gross protein structure of all PPAR subtypes is similar and possesses identical ligand binding pocket with 60-70% identity in amino acid sequence in the ligand binding domain.[30] As PPARδ has a smaller ligand binding pocket, It was thought that it would be feasible to enhance PPAR α and γ binding potential by incorporating chemical modifications in a PPARδ selective ligand rather than modifying PPARα, γ or dual agonist.

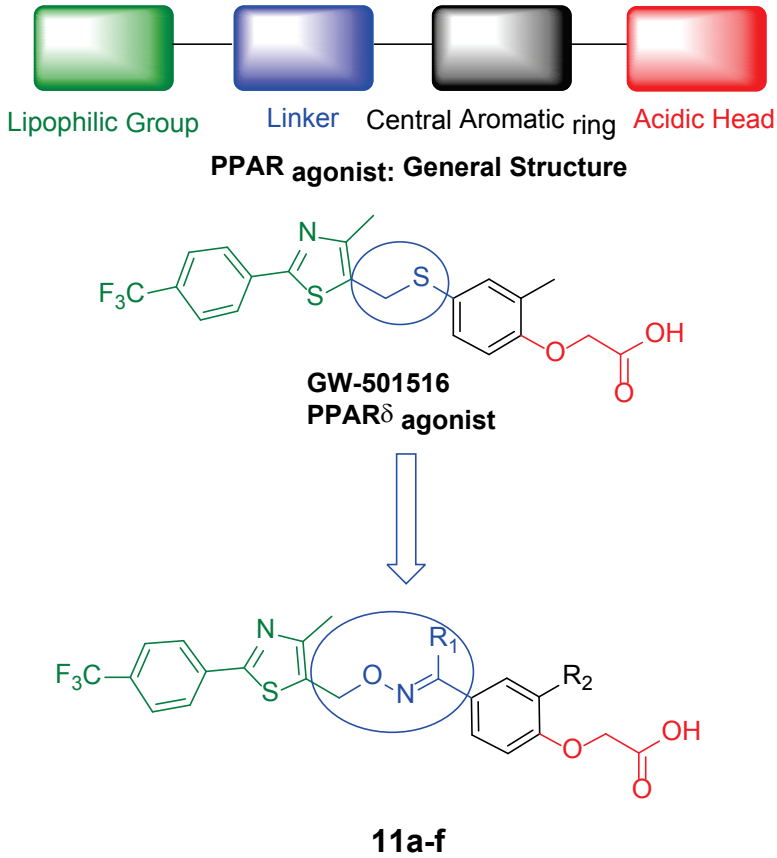


Figure 12: Designing PPAR-pan agonist: Introduction of structurally constrained linker

GW-501516[144] a potent PPARδ agonist (Figure 12) was selected as initial chemotype because along with the very high affinity towards PPARδ, GW-

501516 also activates PPARα and PPARγ moderately. Hence the minor modification in structural features of GW-501516 can enhance affinity towards PPARα and PPARγ without altering PPARδ affinity and eventually lead to an equipotent PPAR-pan agonist.

In course of the research directed towards the development of novel PPAR agonists, recent efforts have been incurred to modulate the subtype selectivity of ligands, results of which demonstrated that the subtype selectivity of PPAR agonists is sensitive to chemical modifications in the central spacer (tether) region of the molecules.[157-161] It has also been reported that highly potent PPARα/γ dual agonists can be obtained by replacing alkyl ether spacer with a rigid spacers such as alkenyl, alkynyl, indole ring, oxime-ether etc.[162-164] (Figure 13)

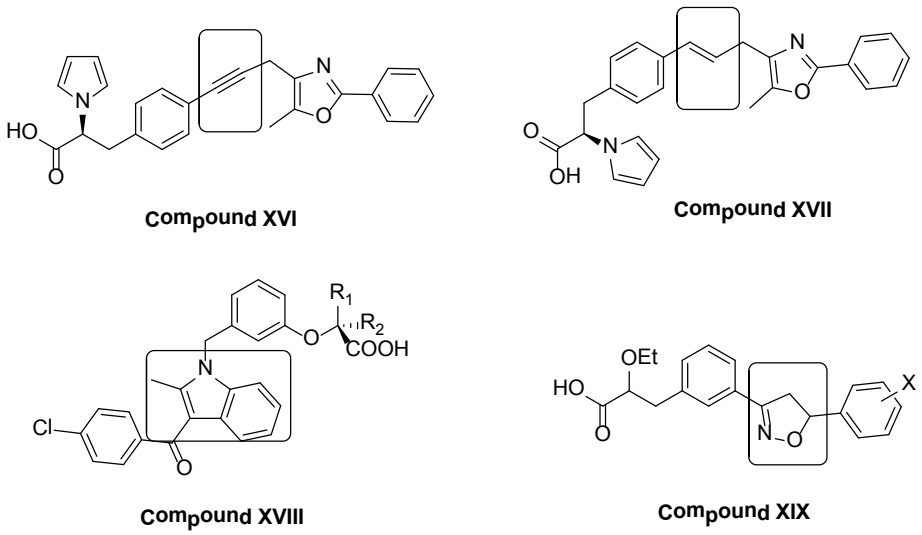


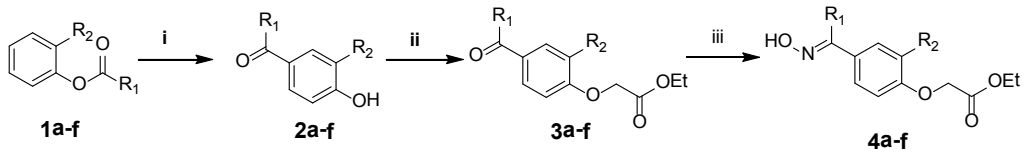
Figure 13: PPARα/γ dual agonists possessing structurally constrained linker

Based on the above findings and serendipity, it was hypothesized that incorporation of a structurally constrained oxime-ether linker in place of a flexible thio ether linker of GW-501516 (Figure 12) could provide us with new class of pan agonist and this led to designed a new series of compounds with oxime ether linker which is represented by compounds **(11a-f)**.

2.2.2 Results and Discussion

2.2.2.1 Chemistry

Compounds described in this section were synthesized according to Schemes 1, 2 and 3.



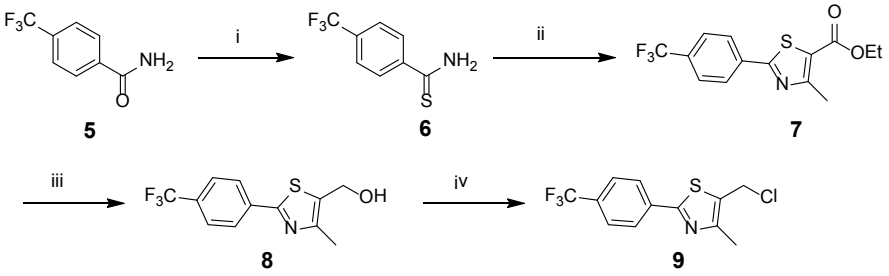
Compound	4a	4b	4c	4d	4e	4f
R_1	Me	Me	Et	Et	*n*-Pr	Cyclohexyl
R_2	Me	H	Me	H	Me	Me

Scheme 1: Reagents and conditions: (i) anhydrous $AlCl_3$, 160 ^oC, 2 h; (ii) Ethyl chloroacetate, K_2CO_3, DMF, 60 ^oC, 18 h; (iii) $NH_2OH.HCl$, CH_3COONa, Ethanol, reflux, 1 h

The synthesis route to oxime intermediates **4a-f** is outlined in Scheme 1. Starting materials **1a-f** were prepared in 80-92% yield by reacting phenol or *o*-cresol with respective acid chloride or anhydride, in the presence of triethylamine in dichloromethane. These *O*-acylatedphenols **1a-f** were subjected to the $AlCl_3$ mediated Fries Rearrangement, at 160 ^oC to obtain the 4-acylphenols or 4-acyl-o-cresols **2a-f,** in 27-58% yield. Since the ortho-position of phenyl ring in **2b** and **2d** is free of any substitution, the Fries migration gave a mixture of two products (*ortho* and *para* substituted products) with the major at *para* products (column chromatography). While **2a**, **2c**, **2e** and **2f** were obtained as exclusively para substituted products for an obvious reason that in the precursors of these compounds (**1a**, **1c**, **1e** and **1f**) one of the ortho-positions is occupied by methyl group. Nucleophilic substitution reaction of the above obtained intermediates **2a-f** with ethyl chloroacetate in the presence of K_2CO_3 in DMF at 60 ^oC gave the compounds **3a-f**. Treatment of the keto compounds **3a-f** with aqueous hydroxylamine hydrochloride, in the presence of sodium acetate for two hours in refluxing ethanol resulted in the corresponding oxime derivatives **4a-f**, which

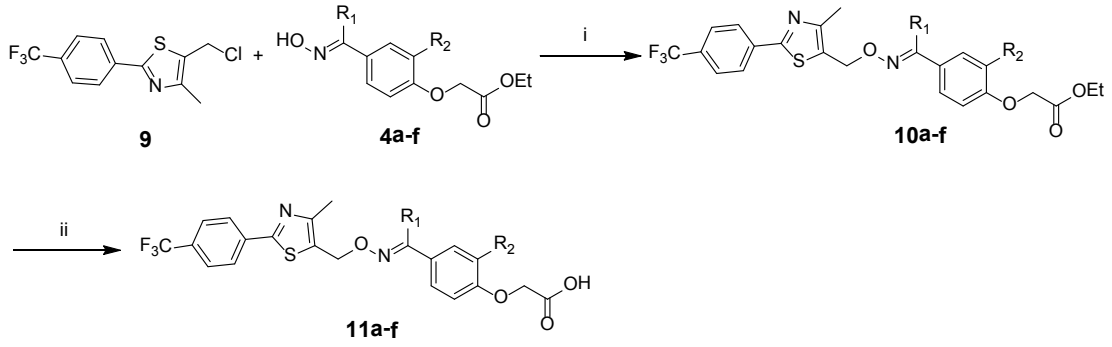
were purified by recrystallization from a mixture of ethyl acetate and *n*-hexane. The *E*-isomers were obtained as major products being thermodynamically more stable than the Z-isomers.[165]

The thiazole intermediate **9** was synthesized according to the procedure reported in the literature[144] (Scheme 2). 4-Trifluoromethylbenzamide **5** was treated with phosphorus pentasulfide in the presence of sodium bicarbonate in toluene at 90 ^{o}C for 1 hour to get 4-trifluoromethylthiobenzamide **6,** in 99% yield. Thiazole ring **7** was constructed by the reaction of thiamide **6** and ethyl-2-chloroacetoacetate in ethanol under refluxing condition over a period of 2 hours. Ester **7** was reduced to alcohol **8** by the treatment of lithium aluminum hydride in THF under nitrogen atmosphere for 30 minutes. Finally the synthesis of chloro intermediate **9** was accomplished in very good yield (99%) by reacting alcohol **8** with thionyl chloride in chloroform.



Scheme 2: Reagents and conditions: (i) P_2S_5, $NaHCO_3$, Toluene, 90 ^{o}C, 1 h; (ii) Ethyl 2-chloroacetoacetate, Ethanol, Reflux, 2 h; (iii) $LiAlH_4$, THF, 0-10 ^{o}C, 0.5 h; (iv) $SOCl_2$, $CHCl_3$, 25 ^{o}C, 4 h;

Synthesis of the final compounds **11a-f** is illustrated in Scheme 3. Coupling of thiazole intermediate **9** (Scheme 2) with oximes **4a-f** prepared as outlined in Scheme 1, in the presence of cesium carbonate in DMF at 60 ^{o}C provided the esters **10a-f.** The hydrolysis of these esters carried out under aqueous alkaline conditions yielded the carboxylic acids **11a-f**.



Compound	11a	11b	11c	11d	11e	11f
R_1	Me	Me	Et	Et	*n*-Pr	Cyclohexyl
R_2	Me	H	Me	H	Me	Me

Scheme 3: Reagents and conditions: (i) Cs_2CO_3, DMF, 60 ^oC, 18 h; (ii) $LiOH.H_2O$, THF, methanol, H_2O, 25 ^oC, 18 h

The detailed synthetic procedures and characterization data are included in the experimental section. Physical, analytical and spectral data of all the final as well as intermediate compounds are in confirmity with the structures assigned. In general, compounds **11a-f** (Scheme 3) were prepared in good yield under mild reaction conditions.

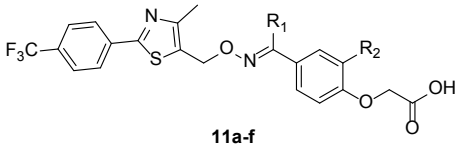
2.2.2.2 *In vitro* PPAR transactivation

Thiazole derivatives **11a-f** were screened for human PPAR (hPPAR) α, γ and δ agonistic activities on full length PPAR receptor transfected in HepG2 cells as described in the experimental section. WY-14643, Rosiglitazone and GW-501516 were used as positive controls for PPAR α, γ and δ respectively. The activities are reported as percent maximal activity of each compound compared to the reference compound and normalized to 100%, as summarized in Table 5.

Our aim in the present endeavor was to discover a PPAR-pan agonist with potent anti-hyperglycemic and anti-hyperlipidemic activities. The compounds were designed taking GW501516 as initial lead. As GW501516 is a potent PPARδ agonist and moderately activates PPARα and PPARγ, it was felt that chemical modifications in this chemotype in order to improve the activation

towards PPARα and PPARδ would be an appropriate strategy. The initial efforts towards this endeavor started with the introduction of structurally constrained oxime-ether as a linker in place of thio ether of GW501516, as represented in Figure 12. The thio ether linker was replaced with oxime-ether having different substitutions at R_1 (Figure 12) leading to a novel series of compounds **11a-f**.

Table 5: *In vitro* PPAR agonistic activity of the test compounds **11a-f**



11a-f

Compound	R_1	R_2	hPPAR Transactivation E_{max} (%)[a,b]		
			α	γ	δ
11a	Methyl	Methyl	99.8 ± 5.2	85.3 ± 2.1	90.1 ± 3.5
11b	Methyl	H	86.2 ± 4.1	58.4 ± 3.7	60.6 ± 3.0
11c	Ethyl	Methyl	96.4 ± 3.6	64.7 ± 3.2	61.9 ± 6.2
11d	Ethyl	H	97.5 ± 5.4	58.5 ± 3.2	41.4 ± 1.4
11e	*n*-Propyl	Methyl	58.7 ± 2.8	43.4 ± 2.4	48.3 ± 3.5
11f	Cyclohexyl	Methyl	57.9 ± 1.9	52.1 ± 2.2	98.7 ± 7.1
GW-501516			76.9 ± 7.1	61.1 ± 2.4	100.4 ± 4.3

[a]HepG2 cells transfected with pSG5 expression vector containing the cDNA of hPPARα or hPPARγ or hPPARδ and cotransfected with PPRE3-TK-luc. The luciferase activity determined using fire-fly luciferase assay and β-galactosidase activity determined in ELISA reader (n=3).

[b]E_{max} of test compounds compared to reference compounds (WY-14643 for α, Rosiglitazone for γ and GW-501516 for δ) normalized to 100%. Fold increase of WY-14643, Rosiglitazone and GW-501516 Vs DMSO is 8.40 ± 0.21 (PPARα), 13.49 ± 0.38 (PPARγ) and 28.54 ± 0.77 (PPARδ) respectively at 10 μM concentration.

As anticipated, compound **11a** possessing methyl group as R_1 showed significant improvement in affinity towards PPARα and PPARγ as compared to GW-501516. This compound exhibited high and balanced efficacy towards all the three PPAR subtypes with E_{max} of 99%, 85% and 90% towards PPAR α, γ and δ respectively. Replacement of methyl group as R_1 in **11a** with ethyl found to be detrimental to PPAR affinity as seen in the compound **11c**. Compounds **11b** and **11d** bearing no substitution at R_2 exhibited poor affinity towards PPARδ compared to their methyl substituted counterpart, suggesting the importance of

methyl group at R_2 for PPARδ activity. These results led to further optimize the size of alkyl groups as R_1. When the chain length at R_1 was further increased to propyl group, compound **11e** showed unfavorable PPAR affinities (Table 5). These results suggest that PPAR affinity decreases when chain length at position R_1 increases. To assess the effect of bulkier group, we synthesized compound **11f** with cyclohexyl group as R_1. Compared to compound **11a**, compound **11f** showed inferior affinity towards PPAR α and γ but interestingly found to be equipotent towards PPARδ (Table 5).

Based on the above results compounds **11a**, **11c** and **11f** were subjected for dose response curve (DRC) and EC_{50} (half maximal effective concentration) values are presented in Table 6.

Table 6: EC_{50} values of selected the test compounds

Compound	R_1	R_2	hPPAR Transactivation EC_{50} (μM)[a,b]		
			α	γ	δ
11a	Methyl	Methyl	0.008 ± 0.002	0.006 ± 0.002	0.01 ± 0.003
11c	Ethyl	Methyl	0.12 ± 0.01	0.8 ± 0.02	0.8 ± 0.05
11f	Cyclohexyl	Methyl	1.6 ± 0.21	1.0 ± 0.12	0.7 ± 0.09
Bezafibrate			42.5 ± 2.5	57.2 ± 5.4	18.9 ± 0.9
GW-501516			1.1 ± 0.32	0.8 ± 0.008	0.0012 ± 0.0006

[a]HepG2 cells transfected with pSG5 expression vector containing the cDNA of hPPARα or hPPARγ or hPPARδ and cotransfected with PPRE3-TK-luc. The luciferase activity determined using fire-fly luciferase assay and β-galactosidase activity determined in ELISA reader.
[b]EC_{50} is the concentration of the test compound that affords half-maximum transactivation activity.

Among the three compounds subjected for EC_{50} determination, compound **11a** emerged as highly potent and efficacious PPAR-pan agonist with balanced EC_{50} of 0.008 μM, 0.006 μM and 0.01 μM towards PPAR α, γ and δ respectively. The compound **11f** exhibited low *in vitro* potency, while compound **11c** showed moderate and balanced activity (EC_{50}: 0.12 μM, 0.8 μM and 0.8 μM for PPARα, γ and δ respectively) towards all three PPAR subtype. Keeping these results in mind, compound **11a** was selected for subsequent *in vivo* evaluation.

2.2.2.3 *In vivo* evaluation

Encouraged with the *in vitro* PPAR agonistic activity, compound **11a** being the most potent compound in the series, was selected for *in vivo* studies in *db/db* mice and HF-HC-Fructose (Food containing high cholesterol, high fat and fructose) fed *hamster* for their anti-hyperglycemic and anti-hyperlipidemic activities. Detailed experimental procedures of *in vivo* studies are described in experimental section.

In male *db/db* mice, compounds **11a** was dosed orally (po) at 10 mpk/day dose for 14 days. As included in Table 7, the test compound **11a** showed significant reduction of 23% and 36% in serum glucose and triglyceride (TG) respectively, however serum glucose and TG reduction with compound **11a** was found to be inferior compared to Rosiglitazone and Tesaglitazar.

Table 7: Anti-hyperglycemic activity of compound **11a** in *db/db* mice[a]

Compound	Dose (mpk/day/po)	% Change[b]	
		Serum Glucose	Serum TG
11a	10	-23.2 ± 3.1	-36.5 ± 2.5
Rosiglitazone	30	-41.1 ± 2.5	-53.8 ± 4.2
Tesaglitazar	3	-60.2 ± 6.1	-54.4 ± 5.0

[a]Male *db/db* mice dosed orally (po) with test compounds daily for 14 days and serum glucose, triglycerides (TG) were measured.
[b]Values expressed as % change of compound-treated mice vs vehicle control, indicated as M±SD (n=6), significant at $p < 0.05$.

Further, anti-hyperlipidemic activity of compound **11a** was evaluated in Female *hamsters* at 10 mpk/day/po dose, for 14 days. It showed statistically significant increase in serum HDL-C level along with substantial reduction in serum LDL-C level. The results are summarized in Figure 14.

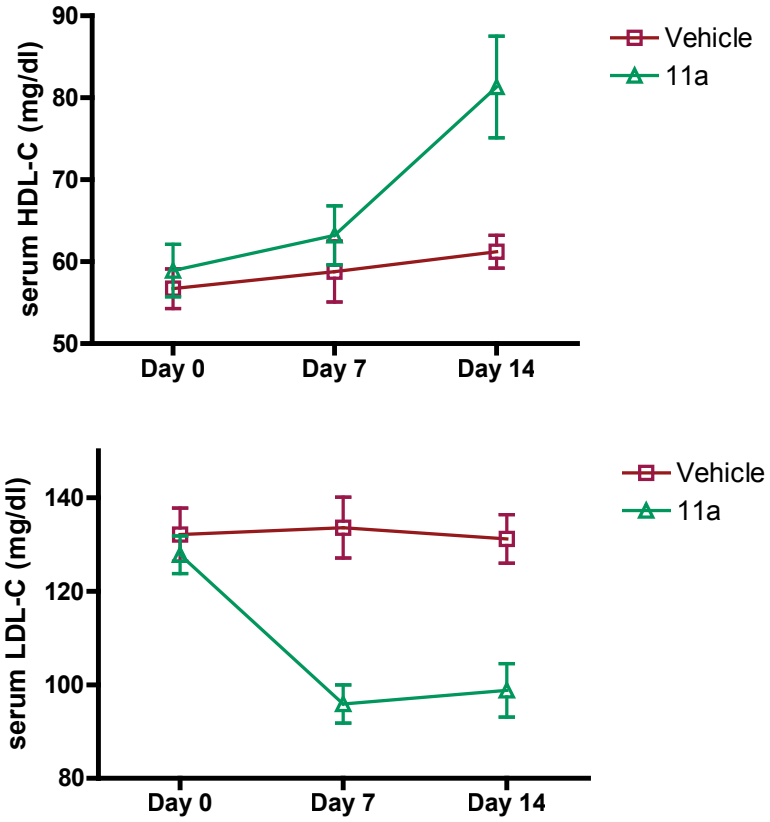


Figure 14: Anti-hyperlipidemic activity of the selected compound **11a** in *Hamster*

Female *Hamster* were fed with HF-HC-Sucrose diet (14 days), dosed orally with vehicle or test compounds (10 mpk) daily (14 days) and serum LDL cholesterol (LDL-C) / serum HDL-cholesterol (HDL-C) levels were estimated (0, 7 and 14 day), data represented as M±SD

All together above said *in vivo* results indicate that compound **11a** exhibits a significant anti-hyperlipidemic activity in female *hamster* and shows a reasonably good anti-hyperglycemic activity in male *db/db* mice.

Critical analysis of above described *in vitro* and *in vivo* experimental results led to the following conclusion.

- A highly potent and balanced PPAR-pan agonist **11a** with significant anti-hyperlipidemic and anti-hyperglycemic activity was identified
- Substitution on the oxime linker plays a crucial role in PPAR subtypes selectivity

2.3 Bioisosteric Replacement of Lipophilic Moiety

2.3.1 Designing strategy

The encouraging results of the thiazole containing oxiimino derivatives **11a-f** of previous section aspired us to investigate this scaffold more extensively. It was also believe that a compound with moderate but equipotent activity towards all the three PPAR subtype can show a similar efficacy with better safety window compared to the potent agonists. After studying the effect of different kind of substitutions over the oxime moiety, it was then intended to find an alternative to lipophilic thiazole group in order to prove the above mentioned hypothesis and to establish the findings of the preceding series.

Bioisosterism is a common phenomenon in the field of medicinal chemistry. Bioisosteres are the functional groups with similar chemical/physical properties that impart similar biological properties to a chemical compound. In order to overcome the safety related issues without altering efficacy, this concept is widely used in drug discovery research. Oxazole ring system is a well-accepted bioisostere of thiazole system. Apart from that 2-phenyloxazole and 2-(*p*-tolyl)oxazole are commonly used lipophilic parts in many clinical and preclinical PPAR agonists. Considering these points a novel series of 2-phenyloxazole and 2-(*p*-tolyl)oxazole derivatives represented by compounds **20a-f** and **21a-f** were designed by bioisosteric replacement of thiazole of compounds **11a-f** with oxazole.

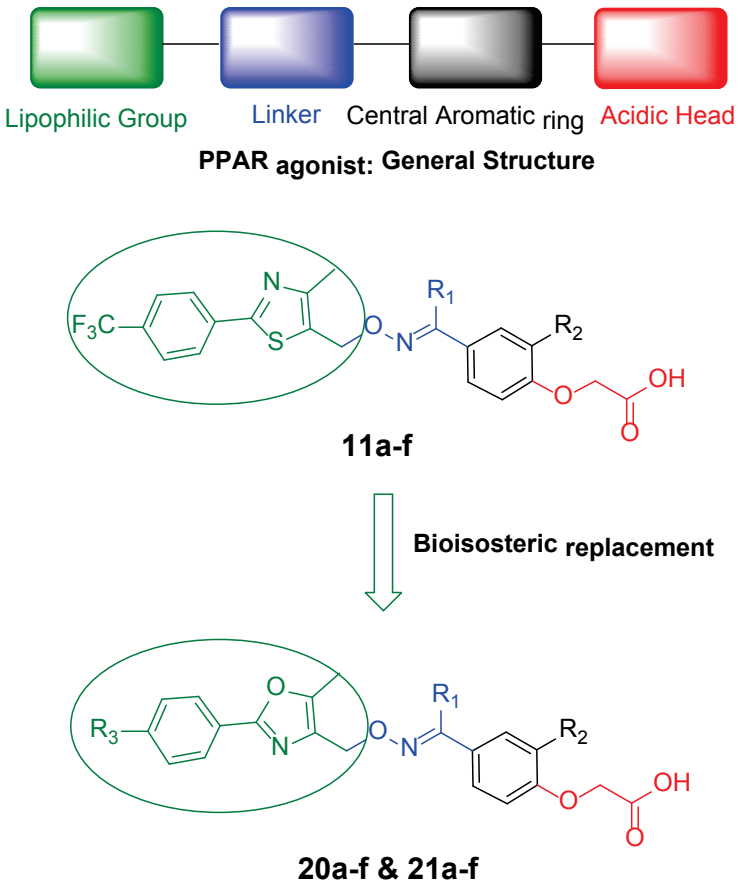


Figure 15: Designing PPAR-pan agonist: Bioisosteric Replacement of thiazole

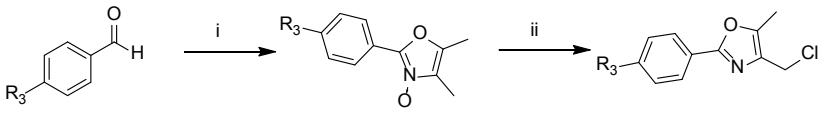
2.3.2 Results and Discussion

2.3.2.1 Chemistry

Synthesis of the newly designed compounds is outlined in Scheme 4 and Scheme 5.

The key intermediates **16** and **17** were synthesized in two steps as mentioned in scheme 4. Oxazole-N-oxides **14** and **15** were obtained in good yield by purging HCl gas into an ice cold solution of benzaldehyde (**12**)/p-tolualdehyde (**13**) and diacetylmonoxime in acetic acid. Regioselective chlorination of the 5-methyl group of the *N*-oxides **14** and **15** was carried out by the reacting *N*-oxides with phosphorous oxychloride at 60 ^{o}C over a period of 3

hours. 5-Chloromethyl oxazoles **16** and **17** were purified by recrystallization from hexane.



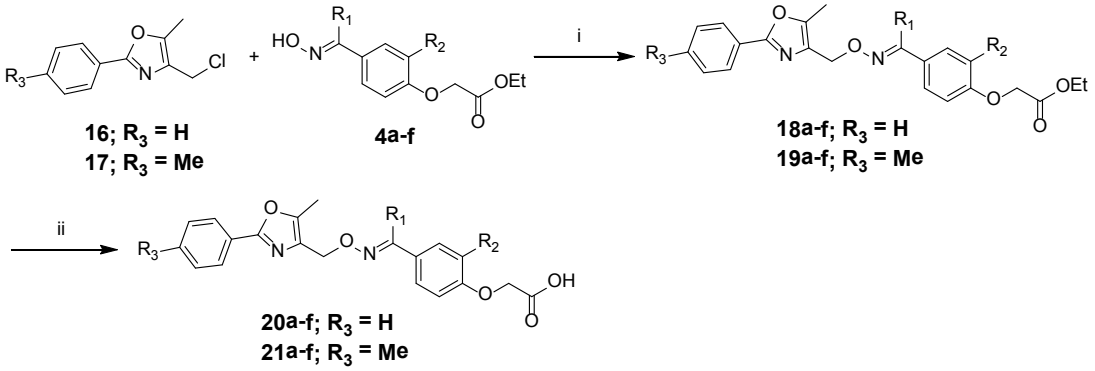
12; R_3 = H
13; R_3 = Me

14; R_3 = H
15; R_3 = Me

16; R_3 = H
17; R_3 = Me

Scheme 4: Reagents and conditions: (i) Diacetylmonoxime, AcOH, $HCl_{(g)}$, 0 ^oC, 3 h; (ii) $POCl_3$, 1,2-dichloroethane, 60 ^oC, 3 h

Synthesis of final compounds **20a-f** and **21a-f** is depicted in Scheme 5. Nucleophilic substitution of oximes **4a-f** (whose synthesis is described in the section 2.2) on 5-chloromethyl oxazoles (synthesized as per Scheme 4), in presence of cesium carbonate under nitrogen atmosphere at 60 ^oC for 18 hours resulted in the esters **18a-f** and **19a-f**. All the esters were purified by column chromatography and isolated in good yield (50-70%). The acids **20a-f** and **21a-f** were obtained in very good yield of 70-90% by hydrolysis of the esters **18a-f** and **19a-f** in the presence of lithium hydroxide.



16; R_3 = H
17; R_3 = Me

4a-f

18a-f; R_3 = H
19a-f; R_3 = Me

20a-f; R_3 = H
21a-f; R_3 = Me

Compound	a	b	c	d	e	f
R_1	Me	Me	Et	Et	*n*-Pr	Cyclohexyl
R_2	Me	H	Me	H	Me	Me

Scheme 5: Reagents and conditions: (i) Cs_2CO_3, DMF, 60 ^oC, 18 h; (ii) $LiOH.H_2O$, THF, methanol, H_2O, 25 ^oC, 18 h

The detailed synthesis procedures and characterization data are described in the experimental section. Physical, analytical and spectral data of all

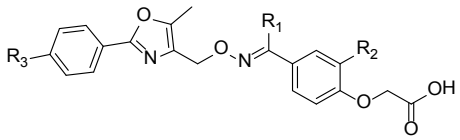
the final as well as intermediate compounds are in conformity with the structures assigned. The compounds **20a-f** and **21a-f** were prepared in good overall yields under mild reaction conditions.

2.3.2.2 *In vitro* PPAR transactivation

The novel oxazole derivatives **20a**-**f** and **21a**-**f** were tested for hPPAR *α*, γ and δ agonistic activities on full length PPAR receptor transfected in HepG2 cells as described in the experimental section. WY-14643, Rosiglitazone and GW-501516 were used as positive controls for PPAR *α*, γ and δ respectively. The *in vitro* PPAR subtype agonistic activities are reported as percent maximal activity of each compound compared to the reference compound and normalized to 100% as summarized in Table 8.

As mentioned above it was assumed that a moderate and balanced activator of all three PPAR subtypes would exhibit the anti-hyperglycemic and anti-hyperlipidemic activities like a potent selective/dual agonist while the adverse effects shown by many potent agonists would be minimized. Thus, the aim of this bioisosteric replacement of oxazole moiety was to identify equipotent PPAR-pan agonist with a moderate potency and significant anti-hyperglycemic and anti-hyperlipidemic activities. In the previous series a balanced and highly potent PPAR-pan agonist **11a** was identified**.** On which bases two novel series of compounds **20a-f** and **21a-f** containing oxazole scaffold and various substitutions at R_1 were designed and synthesized (Table 8).

Table 8: In vitro PPAR agonistic activity of test compounds **20a-f** and **21a-f**



Compound	R_1	R_2	R_3	hPPAR Transactivation E_{max} (%)[a,b]		
				α	γ	δ
20a	Methyl	Methyl	H	84.5 ± 5.5	89.5 ± 4.8	74.5 ± 4.4
20b	Methyl	H	H	58.8 ± 3.8	67.2 ± 4.6	52.1 ± 1.8
20c	Ethyl	Methyl	H	56.4 ± 4.5	72.8 ± 6.6	72.3 ± 2.5
20d	Ethyl	H	H	42.5 ± 1.2	44.7 ± 4.1	46.4 ± 3.8
20e	*n*-Propyl	Methyl	H	14.1 ± 0.9	31.4 ± 1.7	51.7 ± 5.5
20f	Cyclohexyl	Methyl	H	9.7 ± 1.5	18.1 ± 0.9	58.8 ± 1.9
21a	Methyl	Methyl	Methyl	45.3 ± 1.8	61.2 ± 4.3	67.6 ± 4.3
21b	Methyl	H	Methyl	51.1 ± 2.9	45.9 ± 5.5	48.1 ± 1.1
21c	Ethyl	Methyl	Methyl	72.6 ± 7.3	61.8 ± 6.4	56.3 ± 3.7
21d	Ethyl	H	Methyl	68.5 ± 6.1	53.3 ± 2.3	49.2 ± 4.5
21e	*n*-Propyl	Methyl	Methyl	33.3 ± 1.6	32.1 ± 3.1	46.8 ± 2.7
21f	Cyclohexyl	Methyl	Methyl	14.1 ± 0.8	29.4 ± 1.7	56.6 ± 5.1
GW-501516				76.9 ± 7.1	61.1 ± 2.4	100.4 ± 4.3

[a]HepG2 cells transfected with pSG5 expression vector containing the cDNA of hPPARα or hPPARγ or hPPARδ and cotransfected with PPRE3-TK-luc. The luciferase activity determined using fire-fly luciferase assay and β-galactosidase activity determined in ELISA reader (n=3).

[b]E_{max} of test compounds compared to reference compounds (WY-14643 for α, Rosiglitazone for γ and GW-501516 for δ) normalized to 100%. Fold increase of WY-14643, Rosiglitazone and GW-501516 Vs DMSO is 8.40 ± 0.21 (PPARα), 13.49 ± 0.38 (PPARγ) and 28.54 ± 0.77 (PPARδ) respectively at 10 µM concentration.

In this new series of the compounds **20a**-**f**, which contain oxazole as a lipophilic tail showed similar tendency as shown by thiazole compounds **11a**-**f** in the foregoing section. As anticipated, compound **20a** possessing methyl group as R_1 found to be equipotent towards all the three PPAR sub types with E_{max} of 85%, 89% and 75% towards PPARα, γ and δ respectively and emerged as the most efficient compound in the oxazole series. Whereas, compound **20c** having ethyl group as R_1 showed a good agonistic activity for all the three PPAR subtypes, but was found to be inferior as compared to **20a**. As observed earlier, compounds **20b** and **20d** where methyl group was replaced by H as R_2 showed a

decrease in PPAR*δ* activity as compared compounds **20a** and **20c**. Incorporation of *n*-propyl chain as R_1 (compound **20e**) resulted in complete loss of activity towards all the three PPAR subtypes which also supports the previous findings. In support of the former observation regarding bulkier substitution, the compound **20f** which possesses cyclohexyl group as R_1, showed an equipotent affinity to PPAR*δ* but failed to show a good affinity towards PPAR *α* and γ as compared to **20a**.

To further support the above findings, compounds **21a**-**f** were synthesized, wherein, phenyl on the oxazole ring at position-2 was substituted with 4-methyl group and substitutions on oxime were retained as in **20a-f**. As included in Table 8, a similar trend as shown by the compounds **20a**-**f** was observed. As expected, compound **21a**, which possess methyl group as R_1 is superior to the other compounds of the series. Compound **21c**, with ethyl group as R_1 exhibited inferior potency than compound **21a**. Removal of the methyl group as R_2 from **21a** and **21c** is found to be detrimental in terms of PPAR*δ* activity as showed by **21b** and **21d**. Linear elongation of the chain length in **21a** to propyl group resulted in decreased activity as evident from **21e**, while the compound **21f** bearing a bulkier cyclohexyl group as R_1 showed a good PPAR*δ* selectivity.

Compounds **20a** and **21a** among their respective series were selected (based on the above discussed *in vitro* results) for EC_{50} (half maximal effective concentration) determination and the results of which are briefed in Table 9. In conformity of the idea of replacing lipophilic group, both the compounds **20a** and **21a** demonstrated balanced and moderately potent EC_{50} values (**20a**: 0.5 μM, 0.3 μM and 0.8 μM for α, γ and δ respectively; **21a**: 0.15 μM, 0.4 μM and 0.3 μM for α, γ and δ respectively) compared to the earlier invented highly potent PPAR-pan agonist **11a**. Although EC_{50} values of **21a** was found to be comparable to that of **20a**, the compounds **21a** was found inferior in terms of E_{max} (Table 8) and therefore the compounds **20a** was selected for further in vivo evaluation.

Table 9: EC_{50} values of selected test compounds **20a** and **21a**

Compound	R_1	R_2	hPPAR Transactivation EC_{50} (μM)[a,b]		
			α	γ	δ
20a	Methyl	Methyl	0.5 ± 0.03	0.3 ± 0.05	0.8 ± 0.07
21a	Methyl	Methyl	0.15 ± 0.01	0.4 ± 0.01	0.3 ± 0.009
Bezafibrate			42.5 ± 2.5	57.2 ± 5.4	18.9 ± 0.9
GW-501516			1.1 ± 0.32	0.8 ± 0.008	0.0012 ± 0.0006

[a]HepG2 cells transfected with pSG5 expression vector containing the cDNA of hPPARα or hPPARγ or hPPARδ and cotransfected with PPRE3-TK-luc. The luciferase activity determined using fire-fly luciferase assay and β-galactosidase activity determined in ELISA reader.
[b]EC_{50} is the concentration of the test compound that affords half-maximum transactivation activity

Altogether, the above results reveal the crucial role of various substitutions at R_1 and R_2, wherein it is evident from the data that the methyl group at R_2 is essential for PPARδ activation. Further the linear elongation of the chain at R_1 was found to be unfavorable to PPAR activation, while the bulkier substitution at this position was found to be favorable for the PPARδ selectivity.

2.3.2.3 *In vivo* evaluation

The compound **20a** was evaluated for anti-hyperglycemic and anti-hyperlipidemic efficacy, in *db/db* mice and HF-HC-Fructose fed *hamster*. Detailed experimental procedures of *in vivo* studies are described in the experimental section. In male *db/db* mice, the compound **20a** was dosed orally (po) at 10 mpk/day for 14 days. The results are presented in Table 10.

Table 10: Anti-hyperglycemic activity of the selected compound 20a in *db/db* mice[a]

Compound	Dose (mpk/day/po)	% Change[b]	
		Serum Glucose	Serum TG
20a	10	-59.9 ± 5.4	-52.4 ± 4.7
Rosiglitazone	30	-41.1 ± 2.5	-53.8 ± 4.2
Tesaglitazar	3	-60.2 ± 6.1	-54.4 ± 5.0

[a]Male *db/db* mice dosed orally (po) with test compounds daily for 14 days and serum glucose, triglycerides (TG) were measured.
[b]Values expressed as % change of compound-treated mice vs vehicle control, indicated as M±SD (n=6), significant at $p < 0.05$.

As we anticipated even though the test compounds **20a** was the moderate activator of PPARs, it showed a significant anti-hyperglycemic activity compared to its thiazole analog **11a.** The compound **20a** showed a remarkable reduction of 60% and 52% in serum glucose and triglyceride (TG) respectively, which is comparable to highly potent and clinically proven PPAR agonists Tesaglitazar and Rosiglitazone (Table 10).

The inspiring results of the earlier study directed us to further evaluate anti-hyperlipidemic activities of the compound **20a** and therefore it was evaluated in Female *hamsters* at 10 mpk/day/po dose, the results of which are presented in Figure 16.

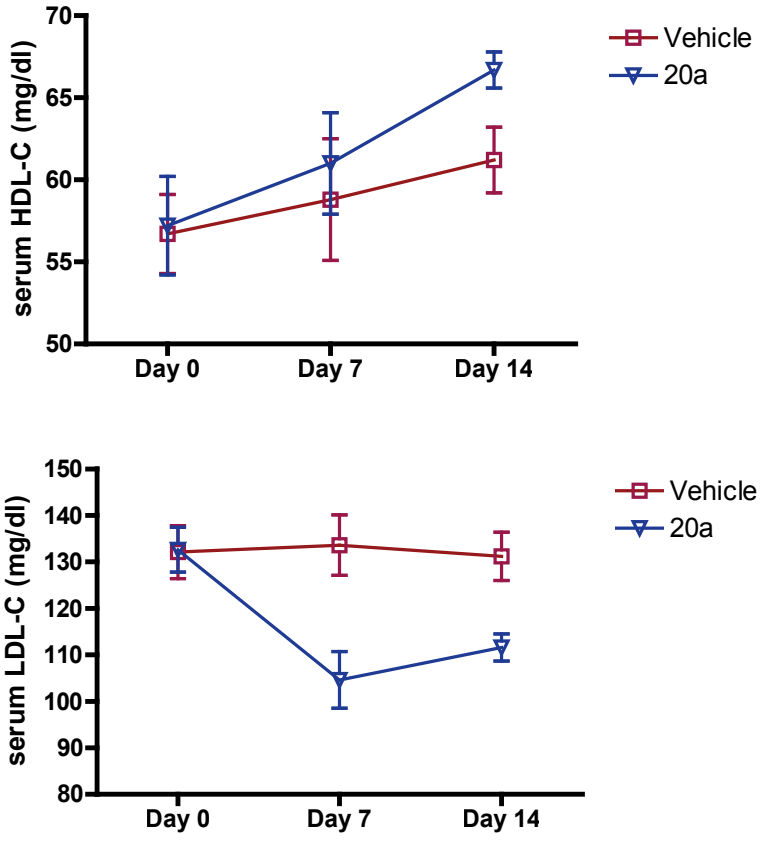


Figure 16: Anti-hyperlipidemic activity of the selected compound **11a** in *Hamster*

Female *Hamster* were fed with HF-HC-Sucrose diet (14 days), dosed orally with vehicle or test compounds (10 mpk) daily (14 days) and serum LDL cholesterol (LDL-C) / serum HDL-cholesterol (HDL-C) levels were estimated (0, 7 and 14 day), data represented as M±SD.

Female *Hamsters* were dosed orally daily for 14 days with compound **20a**. The detailed experimental procedure is given in experimental section. As shown in Figure 16, test compound **20a** was found to be effective in increasing serum HDL-C (9%) and reducing the serum LDL-C (21%), however it possesses inferior anti-hyperlipidemic activity, in comparison of compound **11a** (23% increase in HDL-C and 29% reduction in LDL-C).

In general, from *in vivo* studies compound **20a** is found to be a good alternative for the treatment of hyperglycemia as well as hyperlipidemia and is expected to be safer than the available options till the date.

2.3.2.4 Molecular Docking Study

To further understand the activity and selectivity profile of the compound **20a** at molecular level, docking studies were carried out using Glide version 5.6, the automated docking program implemented in the Schrodinger package. The geometry of the compound docked was subsequently optimized using the LigPrep version 2.6. The complexed X-ray crystal structure of the ligand binding domain (LBD) of PPARα with GW409544 (1K7L.pdb), PPARγ with Rosiglitazone (2PRG.pdb) and PPARδ with anthranilic acid derivative (3DY6.pdb) were obtained from RCSB Protein Data Bank.

When docked into PPARα binding pocket, the most stable docking models of compound **20a** adopted a conformation that allows the carboxylic group to form hydrogen bonds with Tyr 314, Tyr 464, and Ser280 (Figure 17A). The N of oxazole forms a hydrogen bond with -OH of Thr 279. Whereas, in PPARγ binding pocket, the carboxylic group formed hydrogen bonds with Tyr 473, Ser 289 and His 449. Other important reported residues His 223, Gln 286 are very close to the ligand (Figure 17B). In PPARδ binding pocket the carboxylic group formed hydrogen bonds with Tyr 473 and Thr 289. Other important residues like Thr 288 are also found to be close to the ligand (Figure 17C).

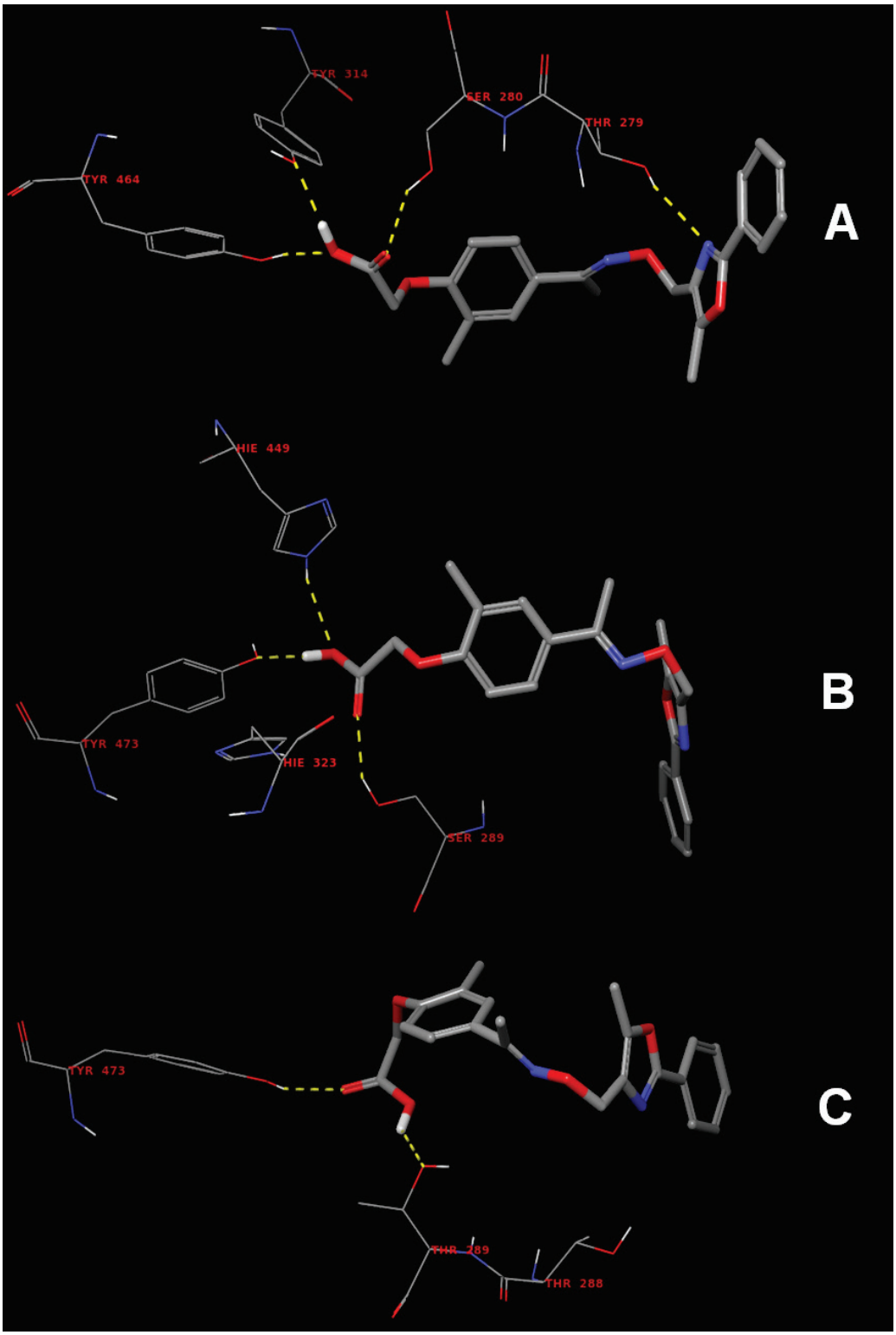


Figure 17: Molecular docking of Compound **20a** into PPARα (A), γ (B) and δ (C) binding pockets: H-bond interactions with amino acids are shown in dashed lines

In the light of these docking calculations, it can be inferred that the compound **20a** could equally selective towards PPARα, PPARγ and PPARδ.

Bearing in mind the above discussed *in vitro* and *in vivo* results of the present series of compounds, the following conclusions were made.

- The compound **20a** was identified as a potential compound for the treatment of hyperglycemia as well as hyperlipidemia
- Methyl group at the oxime moiety is more favorable for PPAR-pan agonism
- Methyl group at the *ortho* position of central aromatic ring is essential for PPARδ activity
- Increase in the chain length at oxime results in loss of activity
- Compound with the bulkier group on oxime seems to be more selective towards PPARδ

2.4 Modification in Lipophilic Moiety

2.4.1 Designing strategy

After identifying two compounds with very good *in vivo* and *in vitro* profile from the preceding two sections, the attempts were directed to know more about the role of lipophilic part of this scaffold, in the subtype selectivity of PPARs. It was also observed in the former section that changing the lipophilic group resulted in identification of a compound with better profile. Subsequently it was necessary to understand the effect of the replacement of lipophilic moiety with various heterocycles commonly used in many PPAR agonists.

With this reference a new series of compounds **36a-h**, containing several known heterocycles were designed and synthesized. Additionally to validate the findings from this series, another similar kind of series **44a-g** was synthesized**,** in

view of getting better PPAR-pan agonist. Designing aspects of these compounds are schematically represented in Figure 18.

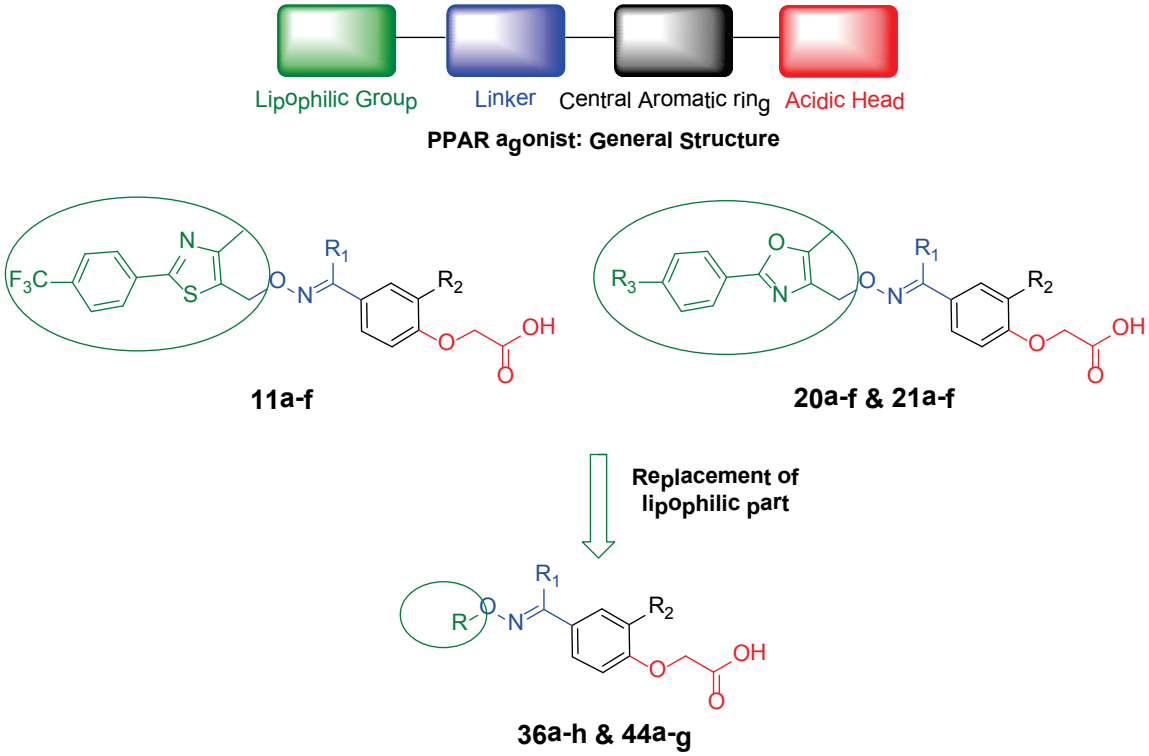


Figure 18: Designing PPAR-pan agonist: Replacement of Lipophilic Part

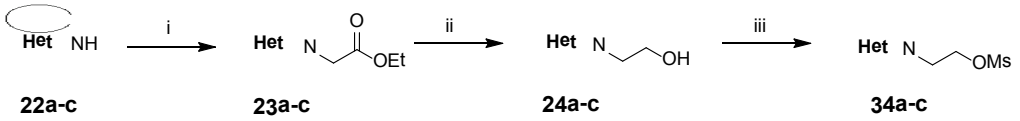
2.4.2 Results and Discussion

2.4.2.1 Chemistry

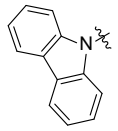
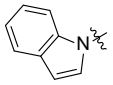
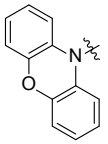
The synthesis of the newly designed compounds **36a-h** and **44a-g** described in this section was outlined in Schemes 6 to 13.

As demonstrated in Scheme 6, *N*-linked heterocyclic intermediates **34a-c** were synthesized from their corresponding heterocycles in three steps. The synthesis was carried out by reacting the corresponding heterocycle with ethyl-2-bromoacetate followed by reduction of ester, using lithium aluminum hydride and finally treating the hydroxy compounds with methanesulfonyl chloride. Initially these heterocycles were treated with 1,2-dibromoethane, in the presence of a

base. Since this method needed a strong base like sodamide and the yields were very low, the fore mentioned method was adopted.

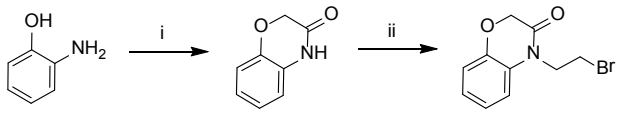


22a-c 23a-c 24a-c 34a-c

Compound	34a	34b	34c
Het			

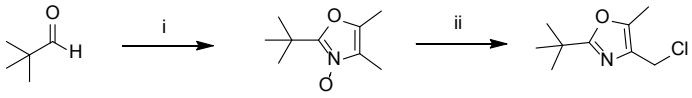
Scheme 6: Reagents and conditions: (i) NaH (50%), ethyl-2-bromoacetate, THF, 30 ^{o}C, 8h; (ii) $LiAlH_4$, THF, 25 ^{o}C, 2h (iii) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 10 ^{o}C, 3h

As depicted in Scheme 7, benzoxazinone intermediate **34d** was obtained by reacting 2-aminophenol (**25**) and chloroacetyl chloride, in the presence of sodium bicarbonate and a phase transfer catalyst benzyltrimethylammonium bromide, followed by the treatment with 1,2-dibromoethane, under strong alkaline condition like potassium hydroxide in dimethylsulfoxide..



25 26 34d

Scheme 7: Reagents and conditions: (i) Chloroacetyl chloride, $NaHCO_3$, benzyltrimethylammonium bromide, $CHCl_3$, 60 ^{o}C, 8 h; (ii) 1,2-dibromoethane, KOH, DMSO, 25 ^{o}C, 0.5 h



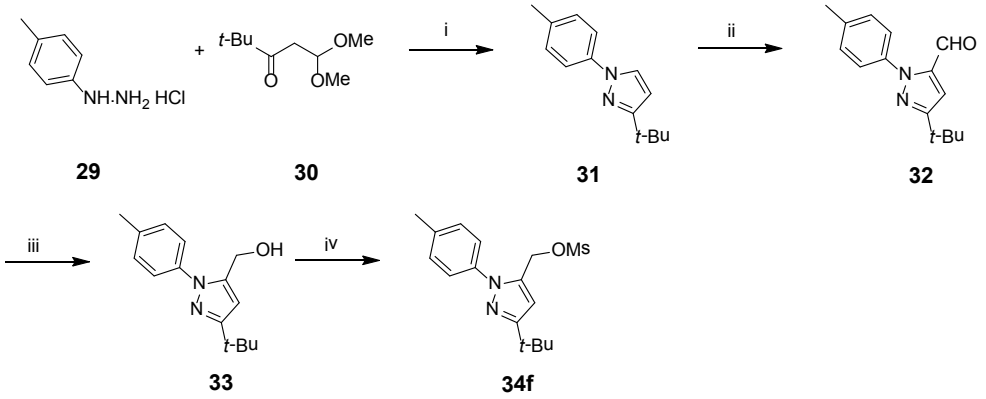
27 28 34e

Scheme 8: Reagents and conditions: (i) Diacetylmonoxime, AcOH, $HCl_{(g)}$, 0 ^{o}C, 3 h; (ii) $POCl_3$, dichloroethane, 60 ^{o}C, 3 h

When pivalaldehyde (**27**) was treated with diacetylmonoxime in the presence of $HCl_{(g)}$, in acetic acid, resulted in 2-*tert*-butyloxazole-N-oxide (**28**),

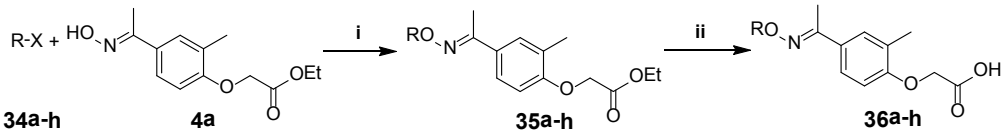
which upon reaction with phosphorous oxychloride, in 1,2-dichloroethane yielded the oxazole intermediate **34e** (Scheme 8).

The pyrazole intermediate **34f** was prepared as shown in Scheme 9. *p*-Tolylhydrazine hydrochloride (**29**) and acetal intermediate **30** when refluxed in ethanol for 2 hours, gave pyrazole **31,** which upon formylation using DMF in presence of a strong base *n*-butyllithium, resulted in the aldehyde **32**. The intermediate **34f** was finally obtained by reduction of aldehyde **32** with sodium borohydride and subsequently mesylation with methanesulfonyl chloride in the presence of triethylamine.

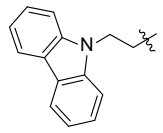
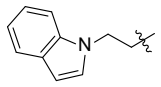
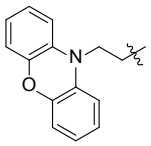
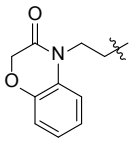


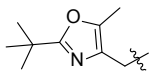
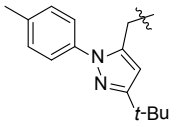
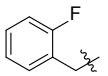
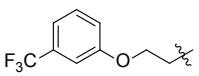
Scheme 9: Reagents and conditions: (i) EtOH, reflux, 2 h; (ii) DMF, *n*-BuLi, THF, -78 ^{o}C, 2 h; (iii) $NaBH_4$, MeOH, THF, 0-10 ^{o}C, 2 h; (iii) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 30 ^{o}C, 0.5 h.

Synthesis of the final compounds **36a-h** is elaborated in Scheme 10. Heterocyclic intermediates **34a-f** and the oxime intermediate **4a** (Section 2.2.2.1) were coupled in the presence of strong base cesium carbonate in N,N-dimethylformamide at 80 ^{o}C under nitrogen atmosphere to obtained corresponding esters **35a-f,** in good yields. These esters were purified using flash column chromatography using an appropriate mixture of ethyl acetate and hexane. Thereafter compounds **36a-f** were obtained by hydrolysis of the esters **35a-f** under alkaline condition as mentioned in Scheme 10.



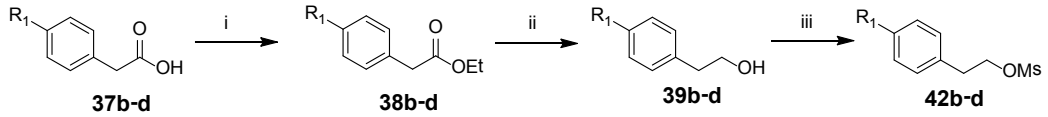
34a-h 4a 35a-h 36a-h

Compound	36a	36b	36c	36d
R				
X	OMs	OMs	OMs	OMs

Compound	36e	36f	36g	36h
R				
X	Cl	OMs	Br	Cl

Scheme 10: Reagents and conditions: (i) Cs_2CO_3, DMF, 60 ^oC, 18 h; (ii) $LiOH.H_2O$, THF, methanol, H_2O, 25 ^oC, 18 h

Furthermore another newly designed series of compounds **44a-g** was prepared following Schemes 11 to 13. Aryl intermediates **42b-d** were prepared in good yield over three steps, starting from their respective phenyl acetic acids **37b-d**. Esterification was carried by refluxing phenyl acetic acids **37b-d** in ethanol, in the presence of catalytic amount of sulfuric acid, for 5 hours. The esters **38b-d** were subjected to reduction with lithium aluminum hydride in THF, to yield 2-phenylethanols **39b-d**. Lastly mesylates **42b-d** were isolated by the treatment of compounds **39b-d** with methanesulfonyl chloride in the presence of triethylamine (Scheme 11).

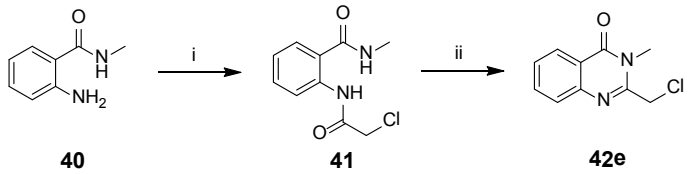


37b-d 38b-d 39b-d 42b-d

Compound	42b	42c	42d
R_1	CF_3	OMe	Me

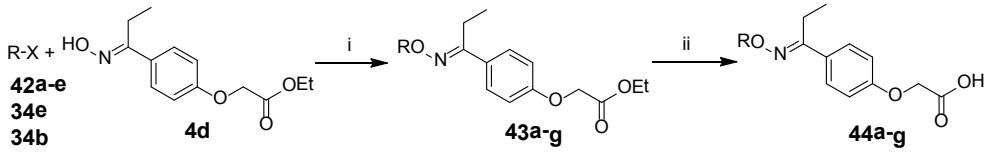
Scheme 11: Reagents and conditions: (i) H_2SO_4, EtOH, reflux, 5 h; (ii) $LiAlH_4$, THF, 25 ^oC, 2 h; (iii) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 25 ^oC, 2 h

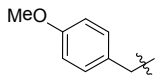
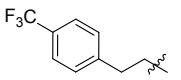
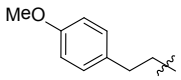
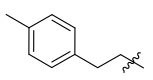
For the synthesis of quinazoline intermediate, anthranilamide **40** was converted into intermediate **41** by reacting with chloroacetyl chloride and triethyl amine in dichloromethane. Compound **42e** was obtained from the cyclization reaction of intermediate **41** by refluxing in a mixture of xylene and acetic acid for 15 hours (Scheme 12).

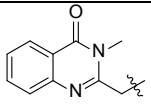
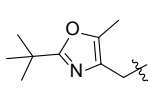
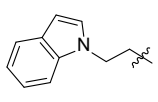


Scheme 12: Reagents and conditions: (i) chloroacetyl chloride, Et_3N, CH_2Cl_2, 30 ^oC, 3h; (ii) xylene, acetic acid, reflux, 15 h

For the synthesis of compounds **44a-g**, the Intermediate **42a** was commercially available, whereas the synthesis of key intermediates **42b-e**, **34b** and **34e** were prepared as described above. Unlike previous series the esters **43a-g** were obtained by the coupling reaction of intermediates **42a-e**, **34b** and **34e** with oxime **4d** (Section 2.2.2.1) in the presence of cesium carbonate at 60 ^oC under nitrogen atmosphere. Hydrolysis of these esters under alkaline condition resulted the final compounds **44a-g** (Scheme 13) which were purified by appropriate methods briefly described in experimental section.



Compound	44a	44b	44c	44d
R				

Compound	44e	44f	44g
R			

Scheme 13: Reagents and conditions: (i) Cs_2CO_3, DMF, 60 ^oC, 18 h; (ii) $LiOH.H_2O$, THF, methanol, H_2O, 25 ^oC, 18 h

The detailed synthetic procedures and characterization data are described in the experimental section. Physical, analytical and spectral data of all the final as well as intermediate compounds are in conformity with the structures assigned.

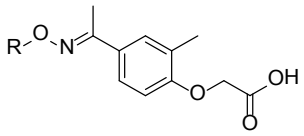
2.4.2.2 *In vitro* PPAR transactivation

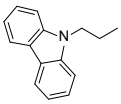
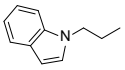
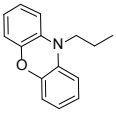
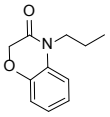
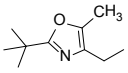
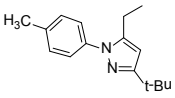
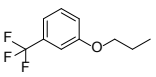
Compounds **36a-h** and **44a-g** were screened for hPPAR *α*, γ and δ agonistic activities on full length PPAR receptor transfected in HepG2 cells as described in the experimental section. WY-14643, Rosiglitazone and GW-501516 were used as positive controls for PPAR *α*, γ and δ respectively. The *in vitro* PPAR subtype agonistic activities are reported as percent maximal activity of each compound compared to the reference compound and normalized to 100%.

The aim in the existing study was to explore the modification in the lipophilic portion in the quest of a better molecule as a mono therapy for the effective treatment of various components of metabolic syndrome. In the former sections it was established that methyl group on oxime linker is essential for potency towards all the three PPARs. In earlier series, it was also observed that even a small change in lipophilic portion can lead to change the biological profile of the compound, in terms of potency as well as efficacy. For these reasons, the methyl group on oxime linker was retained and a novel series of compounds **36a-h** was designed by replacing thiazole/oxazole from the previous compounds with a variety of lipophilic portions known in the field of PPAR research in the present study, as represented in Figure 17. To accomplish the objective, various cyclic lipophilic parts of three different classes were chosen. First four nitrogen containing bicyclic or tricyclic ring systems known in the literature were selected and the compounds **36a-d** were synthesized. Secondly two more heterocycles 2-*tert*-butyloxazole and 1-*p*-tolylpyrazole that mimic 2-phenylthiazole and 2-phenyloxazoles parts of previously active compounds were chosen and the compounds **36e-f** were prepared. The compounds **36g-h** containing substituted

aryl group in place of heterocycle were also synthesized. The results of the *in vitro* transactivation of these compounds are given in Table 11.

Table 11: *In vitro* PPAR agonistic activity of test compounds **36a-h**



Compound	R	hPPAR Transactivation assay E_{max} (%)[a,b]		
		α	γ	δ
36a		32.2 ± 2.3	18.8 ± 1.9	88.3 ± 4.8
36b		38.6 ± 3.3	38.2 ± 1.1	80.9 ± 0.9
36c		27.1 ± 4.8	41.4 ± 2.2	25.3 ± 3.4
36d		24.0 ± 2.7	16.7 ± 1.8	7.5 ± 0.8
36e		48.3 ± 5.1	43.9 ± 4.9	89.0 ± 2.6
36f		172.7 ± 8.3	160.2 ± 5.5	138.3 ± 7.1
36g		34.0 ± 1.1	43.9 ± 1.9	30.5 ± 2.5
36h		118 ± 10.1	41.3 ± 1.9	61.6 ± 6.6
GW-501516		76.9 ± 7.1	61.1 ± 2.4	100.4 ± 4.3

[a]HepG2 cells transfected with pSG5 expression vector containing the cDNA of hPPARα or hPPARγ or hPPARδ and cotransfected with PPRE3-TK-luc. The luciferase activity determined using fire-fly luciferase assay and β-galactosidase activity determined in ELISA reader (n=3).

[b]E_{max} of test compounds compared to reference compounds (WY-14643 for α, Rosiglitazone for γ and GW-501516 for δ) normalized to 100%. Fold increase of WY-14643, Rosiglitazone and GW-501516 Vs DMSO is 8.40 ± 0.21 (PPARα), 13.49 ± 0.38 (PPARγ) and 28.54 ± 0.77 (PPARδ) respectively at 10 µM concentration.

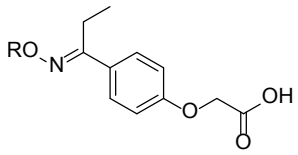
Surprisingly in spite of possessing known heterocyclic pharmacophores, the compounds **36a-d** having N-linked bicyclic/tricyclic heterocycles showed poor *in vitro* potency. Compounds **36a** and **36b** containing carbazole and indole heterocycles respectively activated PPAR*δ* moderately but were found to be detrimental towards PPARα and PPARγ. By incorporating phenoxazine and benzoxazinone as lipophilic tail, resulting compounds **36c** and **36d** failed to show affinity towards all the PPARs. These results indicate that incorporation of N-linked bicyclic or tricyclic heterocycles in an existing oxime scaffold is unfavorable for PPAR activation.

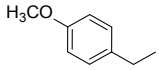
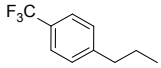
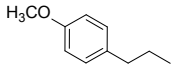
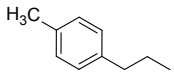
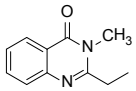
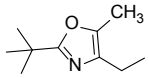
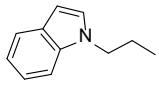
As expected, compounds **36e-f** that imitate active pharmacophore oxazole/thiazole were found to be active (Table 11). Particularly compound **36f** demonstrated E_{max} of 173%, 160% and 138% towards PPAR *α*, *γ* and *δ* respectively, which is even higher than the positive controls. While compound **36g** with 2-fluorobenzyl group did not show any effect, 4-trifluoromethylphenoxy group containing compound **36h** effectively activated PPARα but PPARγ and δ activation was moderate in this compound. That shows the significance of trifluoromethyl group on lipophilic portion in PPAR activation. From the *in vitro* activity results of the compounds **36a-h**, the compound **36f** was found to be a promising candidate for further studies.

To validate the observations made in the previous series, another series of compounds **44a-g,** containing oxime scaffold was designed, with a minor modification in substitution on oxime (ethyl group in place of methyl group) and with inclusion of selected heterocycles of previous series. As evident from the previous structure–activity relationship (SAR) discussion, the compound with trifluoromethyl substitution (**36h**) was found to be effective in PPAR activation. Keeping this in mind, the compounds **44a-d**, having different substitution on aromatic ring were designed. Additionally to validate the analysis of previous results, two heterocycles, 2-*tert*-butyloxazole and indole were chosen and compounds **44f-g** were prepared**.** In addition to that, the compound **44e** which

exhibits bicyclic, but C-linked heterocycle quinazolinonewas also prepared, to confirm whether the loss of activity in the previous N-linked heterocyclic compounds is due to N-link or not. The *in vitro* activity results of these compounds are given in Table 12.

Table 12: *In vitro* PPAR agonistic activity of test compounds **44a-g**



Compound	R	hPPAR Transactivation assay E_{max} (%)[a,b]		
		α	γ	δ
44a		78.0 ± 2.7	95 ± 7.9	42.3 ± 4.3
44b		77.3 ± 3.3	100.9 ± 9.9	122.6 ± 8.2
44c		68.6 ± 2.9	25.4 ± 2.5	44.0 ± 2.1
44d		45.1 ± 1.8	19.4 ± 1.2	28.3 ± 1.1
44e		20.6 ± 1.8	16.6 ± 1.6	23.9 ± 1.5
44f		52.9 ± 3.9	48.3 ± 3.8	30.5 ± 4.0
44g		12.2 ± 1.1	18.1 ± 1.5	26.4 ± 1.4
GW-501516		76.9 ± 7.1	61.1 ± 2.4	100.4 ± 4.3

[a]HepG2 cells transfected with pSG5 expression vector containing the cDNA of hPPARα or hPPARγ or hPPARδ and cotransfected with PPRE3-TK-luc. The luciferase activity determined using fire-fly luciferase assay and β-galactosidase activity determined in ELISA reader (n=3).

[b]E_{max} of test compounds compared to reference compounds (WY-14643 for α, Rosiglitazone for γ and GW-501516 for δ) normalized to 100%. Fold increase of WY-14643, Rosiglitazone and GW-501516 Vs DMSO is 8.40 ± 0.21 (PPARα), 13.49 ± 0.38 (PPARγ) and 28.54 ± 0.77 (PPARδ) respectively at 10 μM concentration.

Compounds consist of methoxy substitution as exemplified by compounds **44a** and **4c** were found to be inferior, in comparison with 4-trifluoromethyl substituted compound **44b**. Compound 4**4b** revealed good efficacy towards all the PPAR subtypes but exhibited poor potency, as found from EC_{50} results (EC_{50} of 0.18 μM, 2.41 μM 1.01 μM for PPAR α, γ and δ respectively). Whereas, substitution with methyl group resulted in complete loss of activity, as observed for compound **44d**. Compounds **44e-g** containing different heterocycles were also turned out to be non-efficacious.

Overall, trifluoromethyl group at *para* position of aryl ring is found to be essential for PPAR activation in this type of compounds. Apart from that, none of the compound was found to be prominent in terms of efficacy. As was assumed, the reason could be the change of methyl group to ethyl group on oxime linker.

Considering the above discussed *in vitro* results, the compound **36f** the most active compound among the compounds studied and was selected for further *in vivo* evaluation.

2.4.2.3 *In vivo* evaluation

With the aim of evaluating the anti-hyperglycemic activity of compound **36f** was subjected for *in vivo* studies in *db/db* mice. In male *db/db* mice, compounds **36f** was dosed orally (po) at 10 mpk/day for 14 days. Detailed experimental procedures of *in vivo* studies are described in experimental section. The results are included in Table 13.

Table 13: Anti-hyperglycemic activity of the selected compound **36f** in *db/db* mice[a]

Compound	Dose (mg/kg/day)	% Change[b]	
		Serum Glucose	Serum TG
36f	10	-18.4 ± 3.7	-32.2 ± 2.7
Rosiglitazone	30	-41.1 ± 2.5	-53.8 ± 4.2
Tesaglitazar	3	-60.2 ± 6.1	-54.4 ± 5.0

[a]Male *db/db* mice dosed orally (po) with test compounds daily for 14 days and serum glucose, triglycerides (TG) were measured.
[b]Values expressed as % change of compound-treated mice vs vehicle control, indicated as M±SD (n=6), significant at $p < 0.05$.

In contrast to its *in vitro* potency test compound **36f** did not exhibit prominent efficacy in animal model. This could be due to a poor pharmacokinetic profile of the molecule.

In conclusion;

- The compound **36f** was identified to be a highly potent PPAR-pan agonist with moderate anti-hyperglycemic activity.
- It was confirmed that the compounds with N-linked bicyclic/tricyclic ring systems are detrimental towards PPAR affinity.
- Phenyl substituted five membered heterocycles as lipophilic parts in current scaffold are favorable for PPAR activition.
- It has been observed that when aryl ring is used as lipophilic part the presence of trifluoromethyl substituent elevates the PPAR affinity.

2.5 Modification in acidic head

2.5.1 Designing strategy

So far in previous sections the newly designed scaffold was broadly explored and a number of compounds were designed. The impact of various substitutions on oxime linker was studied along with the significance of methyl group on the central ring. The variation in the lipophilic part was also extensively studied by using a diversified heterocycles in the former series. Now the attention was focused to study variations in the remaining acidic part. In the previous compounds acidic part is attached to the central aromatic ring through ether linker. As acetic acid or propionic acid without an oxygen linker are also widely employed acidic heads in many PPAR agonists, it was decided to study some molecules containing phenyl acetic acid as an acidic head (Figure 19).

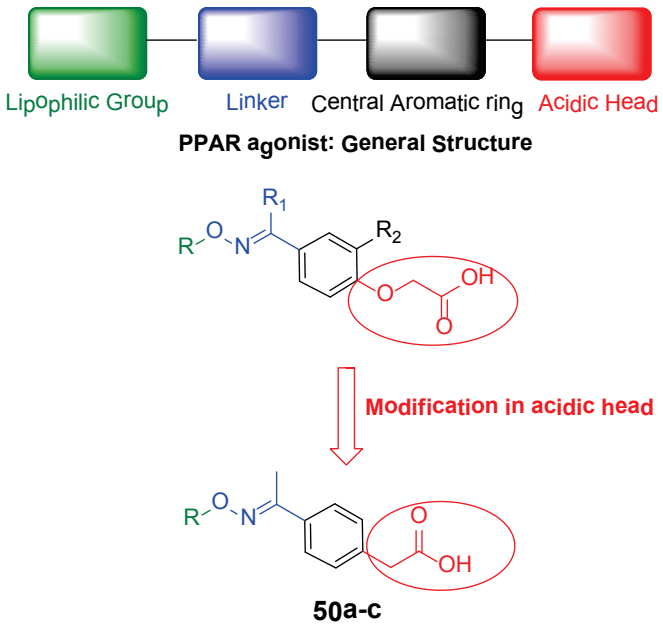


Figure 19: Designing PPAR-pan agonist: Modification in acetic head

The methyl group on oxime linker which was identified as an essential group for PPAR-pan agonism based on the previous studies was retained. In addition, three different types of heterocycles were selected as a lipophilic part and novel phenyl acetic acid derivatives **56a-c** were designed as outlined Figure 18.

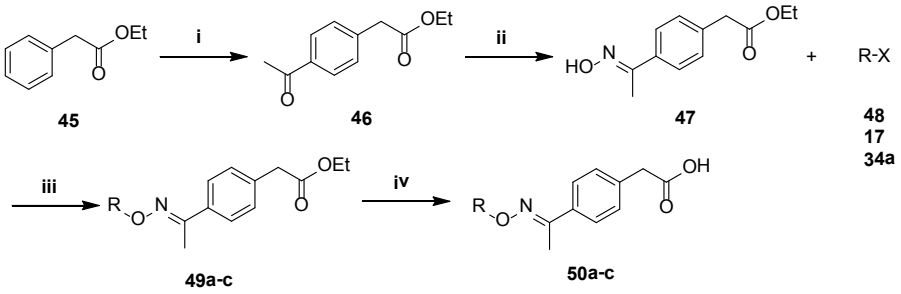
2.5.2 Results and Discussion

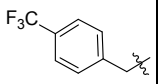
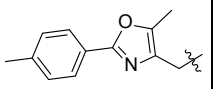
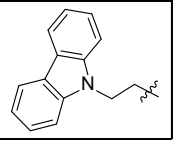
2.5.2.1 Chemistry

For the synthesis of compounds **50a-c**, the starting material phenylacetic acid ethyl ester **45** was prepared esterification of phenylacetic acid. The other starting materials **17**, **34a** and **14** were prepared as described previously (sections 2.2 and 2.3).

The Friedel Crafts acylation of **45** was carried out by reacting it with acetyl chloride in the presence of anhydrous aluminum chloride in carbon disulfide to yield compound **46.** On treatment with hydroxyl ammonium chloride and sodium

acetate in ethanol was converted to the oxime intermediate **47** (Scheme 14). The oxime **47** was isolated as more stable *E*-isomer.



Compound	50a	50b	50c
R			

Scheme 14: Reagents and conditions: (i) CH_3COCl, anhydrous $AlCl_3$, CS_2, 30 ^oC, 20 h; (ii) $NH_2OH.HCl$, CH_3COONa, Ethanol, reflux, 1 h; (iii) Cs_2CO_3, DMF, 60 ^oC, 18 h; (iv) $LiOH.H_2O$, THF, Ethanol, H_2O, 25 ^oC, 18 h

The synthesis of esters **49a-c** was accomplished by coupling of the oxime **47** and heterocyclic intermediates (4-trifluoromethylbenzyl methanesulfonate (**48**), **17** and **34a**) in presence of a strong base like cesium carbonate at an ambient temperature. Further final compounds **50a-c** were obtained by hydrolysis of their respective esters usinge lithium hydroxide.

The detailed synthetic procedures and characterization data are described in the experimental section. Physical, analytical and spectral data of all the final as well as intermediate compounds are in conformity with the structures assigned.

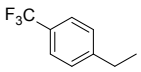
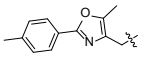
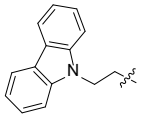
2.5.2.2 *In vitro* PPAR transactivation

The compounds **50a-c** were screened for hPPAR α, γ and δ agonistic activities on full length PPAR receptor transfected in HepG2 cells as described in the experimental section. WY-14643, Rosiglitazone and GW-501516 were used as positive controls for PPAR α, γ and δ respectively. The *in vitro* PPAR subtype

agonistic activities are reported as percent maximal activity of each compound compared to the reference compound and normalized to 100%.

As was observed, trifluoromethyl substituted aryl ring is desirable for PPAR activation, hence compound **50a** possessing 4-trifluoromethylbenzyl group as lipophilic portion was synthesized. *In vitro* data suggests that compound **50a** effectively activate PPAR*α* but affinity towards PPAR *γ* and *δ* is moderate. Another oxazole based compound **50b** which was designed based on earlier results of the oxazole series found to be equipotent with the E_{max} of 85%, 39% and 51% towards PPAR *α, γ* and *δ* respectively, similar to its trifluoromethyl phenoxyacetic acid analogue (Table 14).

Table 14: *In vitro* PPAR agonistic activity of test compounds **50a-c**

Compound	**R**	**hPPAR Transactivation E_{max} (%)[a,b]**		
		α	*γ*	*δ*
50a		85.4 ± 3.6	39.3 ± 1.7	51.4 ± 5.3
50b		88.8 ± 1.9	65.7 ± 3.1	72.1 ± 3.6
50c		22.6 ± 3.8	<1	<1
GW 501516		76.9 ± 7.1	61.1 ± 2.4	100.4 ± 4.3

[a]HepG2 cells transfected with pSG5 expression vector containing the cDNA of hPPAR*α* or hPPAR*γ* or hPPAR*δ* and cotransfected with PPRE3-TK-luc. The luciferase activity determined using fire-fly luciferase assay and *β*-galactosidase activity determined in ELISA reader (n=3).
[b]E_{max} of test compounds compared to reference compounds (WY-14643 for *α*, Rosiglitazone for *γ* and GW-501516 for *δ*) normalized to 100%. Fold increase of WY-14643, Rosiglitazone and GW-501516 Vs DMSO is 8.40 ± 0.21 (PPARα), 13.49 ± 0.38 (PPARγ) and 28.54 ± 0.77 (PPARδ) respectively at 10 µM concentration.

As was foreseen, introducing N-linked carbazole group as lipophilic part was unfavorable to PPAR activity as exemplified by compound **50c** that supports the earlier conclusion. The results show that there is not much difference in PPAR activity by replacing phenoxy acetic acid with phenyl acetic acid as acidic head.

2.6 Conclusion

With the objective of getting an equipotent PPAR-pan agonist with efficient anti-hyperglycemic and anti-hyperlipidemic activity, a new scaffold was designed and four different series of compounds were synthesized to explore various parts of the scaffold. After critical analysis of all the *in vitro* and *in vivo* results The following conclusions were made

- A concept of introducing structurally constrained oxime ether linker to obtain PPAR-pan agonism was practically implemented.
- The study led to a discovery of highly potent PPAR-pan agonist **11a** with significant anti-hyperlipidemic and very good anti-hyperglycemic activity. In addition to that it was also found that a molecule **20a** in spite of possessing moderate potency towards all PPARs shows a remarkable anti-hyperglycemic activity and very good anti-hyperlipidemic activity.
- While analyzing the role of substituents on oxime in PPAR activity it was observed that
  - Methyl group is most appropriate substituent for PPAR affinity.
  - Chain elongation from methyl to propyl resulted in a decreased PPAR activity.
  - Bulkier substituent like cyclohexyl increases PPARδ selectivity.
  - Methyl group at *ortho* position of central aromatic ring is found important for PPARδ activity.
- During extensive study of lipophilic portion, it was noticed that in this scaffold phenyl substituted 5-membered heterocycles enhance PPAR activity, whereas N-linked bicyclic or tricyclic heterocycles are detrimental to PPAR affinity.
- No difference in PPAR activity has been observed when acidic head is changed from phenoxyacetic acid to phenylacetic acid.