

Chapter – 5

CONCLUSIONS

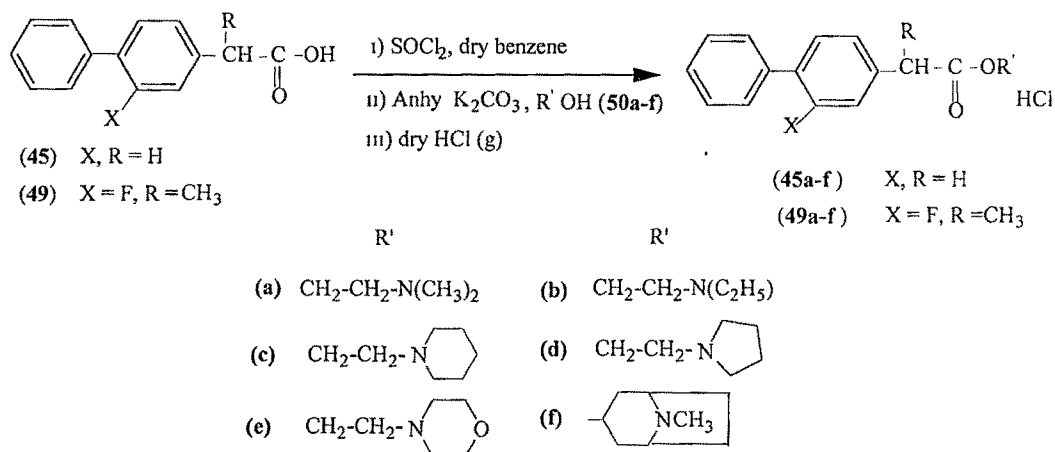
5. CONCLUSIONS

Chronic use of NSAIDs elicits the most common unwanted side effect of inducing gastric or intestinal ulceration. The direct contact effect (topical effect) due to the presence of free acidic carboxyl group in their structure, is one of the factors responsible for this gastric toxicity. Masking of the acidic carboxyl group by esterification or amidation has been exploited widely in the past to overcome this direct contact effect. Various attempts made for decreasing the gastrointestinal (GI) toxicity of NSAIDs by chemical modifications has been reviewed.

In the present study some clinically used acidic NSAIDs [4-biphenylacetic acid (**45**), (active metabolite of fenbufen which is three times more active than fenbufen (**44**) as an anti-inflammatory agent but at the same time much more toxic to GIT), flurbiprofen (**49**), diclofenac (**51**), indomethacin (**54**), aspirin (**56**) and ketorolac (**58**)] were esterified with six different aminoalcohols. The approach involved not only to mask the acidic carboxyl functionality but, also to incorporate the structural features of aminoalcohol ester class of anticholinergics into these derivatised NSAIDs. Hence, in contrast to the simple esters reported earlier (which simply prevent the local GI irritation by blocking the carboxyl group) the aminoesters designed by us are proposed to prevent GI irritation by two different mechanisms. The first one would be preventing the exposure of stomach mucosa to the acidic functionality of NSAIDs due to the blockade of free carboxyl group into ester function and secondly, these derivatives having inherent anticholinergic activity would inhibit gastric acid secretion.

The combination of these two properties of being anticholinergic when the derivatives are intact and anti-inflammatory after their hydrolysis may lead to a much wider scope for these esters with possible indications in ophthalmic inflammatory conditions (the anticholinergic activity should cause mydriasis, aiding in ophthalmic disc examination by the surgeon and after cleavage the liberated parent NSAID will elicit the normal anti-inflammatory activity) and in conditions of obstructive respiratory disorders (like ipratropium, an inhalational anticholinergic drug used in chronic obstructive pulmonary disease like asthma, which demand anticholinergic and anti-inflammatory activities simultaneously).

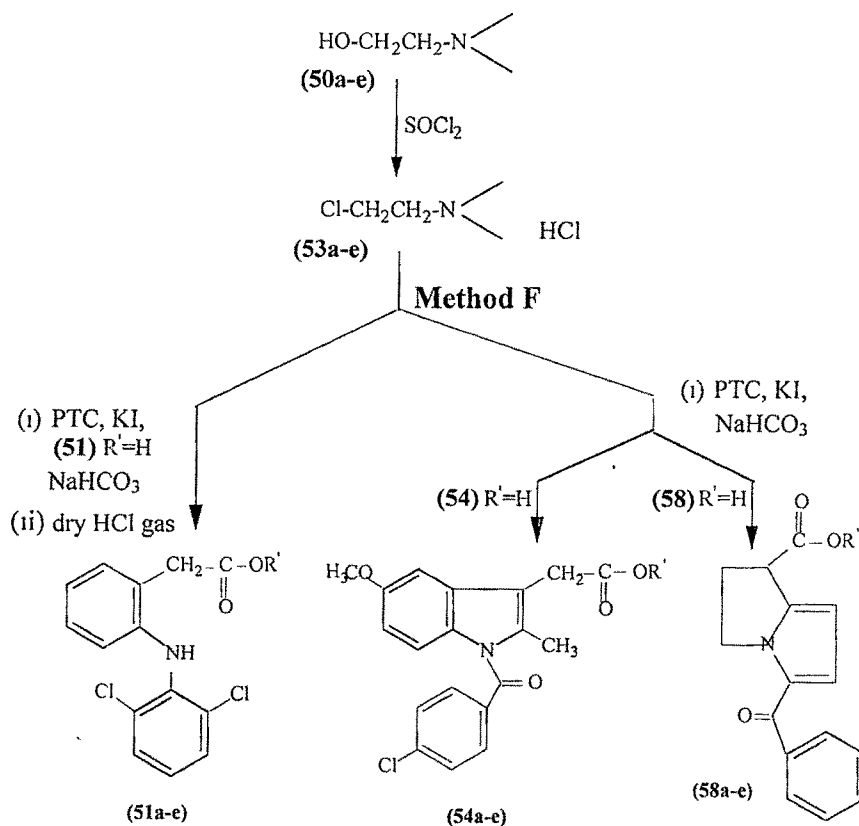
The derivatives were synthesized using different synthetic strategies 4-Biphenylacetic



SCHEME-A

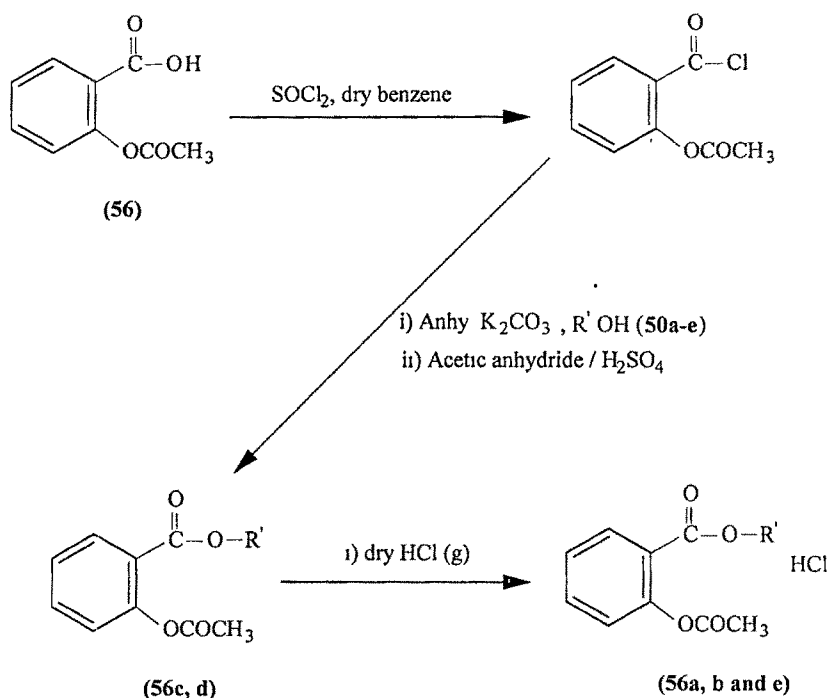
acid (45) and flurbiprofen (49) were reacted with the six different aminoalcohols as per Scheme-A

The aminoalcohol esters (51a-e, 54a-e and 58a-e) for drugs diclofenac (51), indomethacin (54) and ketorolac (58) were synthesized by adopting Scheme-B.



SCHEME-B

In case of aspirin (**56**) Scheme-A did not exactly work out as it offered ester derivatives of salicylic acid so, Scheme-C was adopted for the synthesis of these derivatives (**56a-e**).



SCHEME-C

All the synthesized amino ester derivatives were characterized by their spectral and elemental analyses.

All the synthesized compounds (**45a-f**, **49a-f**, **51a-e**, **54a-e**, **56a-e** and **58a-e**) were subjected to hydrolysis in hydrochloric acid buffer (pH 2.0) and phosphate buffer (pH 7.4) at $37 \pm 1^\circ\text{C}$ to assess their stability simulating the conditions prevalent in GIT. They were also subjected to enzymatic hydrolyses in human serum (80%) at $37 \pm 1^\circ\text{C}$. UV spectrophotometric methods, specific for estimating exclusively the liberated parent drugs in each case were developed. All the aminoalcohol ester derivatives were found to be stable in buffer (pH 2.0) and stable enough for sufficient period of time in buffer (pH 7.4) assuring them to be absorbed intact in the GIT. This would prevent damage to GIT by direct contact mechanism and would ensure anticholinergic activity in the gut. It was observed that dimethyl- and diethylaminoethyl ester derivatives of all the NSAIDs (**45**, **49**, **51**, **54** and **56**) exhibited a faster rate of chemical hydrolysis than the rest at pH 7.4. Increasing the chain length/bulk of the amine or introduction of polar grouping in the amine part (in morpholine) causes a decrease in the rate of hydrolysis of these esters. Derivatives of ketorolac (**58**) were found not to follow this generalisation, however. The

tropinol esters (**45f** and **49f**) were observed to be highly stable in both the buffers (pH 2.0 and 7.4). The high resistance exhibited by these tropinol esters towards the chemical hydrolysis in both the buffers might be due to the bulky amino alcohol part of the derivatives. In studies carried out in human serum it was found that except for the tropinol esters (**45f** and **49f**) all the other derivatives of drugs, 4-biphenylacetic acid (**45**), flurbiprofen (**49**), diclofenac (**51**), indomethacin (**54**) and ketorolac (**58**) exhibited a fast enzymatic hydrolyses to liberate the respective parent NSAIDs. The resistance exhibited by these tropinol ester derivatives (**45f** and **49f**) might be again due to the bulkiness of the alcohol part in comparison to the other esters of amino alcohols (**50a-e**). However, in case of aspirin (**56**), the derivatives (**56a-e**) were found to undergo enzymatic hydrolysis releasing salicylic acid instead of aspirin. Interestingly, like chemical hydrolysis, the derivatives of dimethyl and diethylamino alcohols (**50a** and **50b**) were found to undergo faster enzymatic cleavage than the ester derivatives of other aminoalcohols (**50c-f**) in human serum (80%), for all the derivatized NSAIDs.

All the synthesized aminoalcohol ester derivatives were screened for their anticholinergic activity on isolated rat ileum tissue preparation using acetylcholine as agonist and atropine sulphate as the standard antagonist. pA_2 Values were determined for each derivative. As hypothesized all the synthesized esters exhibited the expected anticholinergic activity, though much weaker than the standard drug. Among all the esters, the tropinol esters (**45f** and **49f**) were found to be the most potent (with highest pA_2 values). The pA_2 values observed for the derivatives (**45a-f**, **49a-f**) indicate that increase in chain length/cyclization and polarity of alcohol part has an influence on the anticholinergic potency. The activity was observed to increase with the increase in the number of carbons as the pA_2 values were obtained in the increasing order for the ester derivatives of the parent drugs (**45** and **58**). However, the observation was reverse in case of ester derivatives (**51a-e**) of diclofenac (**51**) with maximum anticholinergic potency residing in the dimethylaminoethyl ester derivative (**51a**) having the smallest chain length. The increase in chain length and polarity of the aminoalcohol part in these derivatives (**51a-e**) was observed to decrease the anticholinergic potency. It might be possible that the acid part of these esters (**51a-e**), which is bigger than that for compounds (**45a-f**, **49a-f**), is affecting the anticholinergic potency. No definite conclusions could be arrived at for the derivatives (**54a-e**) and (**56a-e**), but for the only common observation that the morpholinoethyl ester derivative (**54e** and **56e**) were the

most potent among all the other derivatives of **54** and **56**. The derivatives (**58a-e**) of the parent drug ketorolac (**58**) did not exhibit much difference in the pA_2 values except for the piperidinoethyl ester derivative (**58c**) which was the most potent among all of the other esters (**58a-e**).

The synthesized derivatives were evaluated for anti-inflammatory and analgesic activities, and for their ulcerogenic potential, at equimolar doses to their respective parent drugs within a span of 3-4 hours.

The anti-inflammatory activity for the synthesized ester derivatives was determined using the carrageenan-induced rat paw edema model. Most of the derivatives were found to possess either an enhanced or comparable anti-inflammatory activity to their respective parent drugs. Here too, the tropinol ester derivatives (**45f** and **49f**) of 4-biphenylacetic acid (**45**) and flurbiprofen (**49**) showed abnormal results. They were found to be devoid of any anti-inflammatory activity in this acute model of inflammation. This could be correlated to the resistance these derivatives exhibited towards chemical and enzymatic hydrolyses. The absence of the anti-inflammatory activity might be due to the failure of these derivatives to release the respective parent drugs.

The acetic acid-induced writhing model was used for evaluating the peripheral analgesic activity of these derivatives. A comparable activity, not significantly different from the respective parent drugs, was found to be possessed by most of the derivatives. The possession of analgesic activity by **49f** is inexplicable (since all other derivatives which exhibited analgesic activity have shown enzymatic hydrolysis which led us to the conclusion that the released parent moieties are responsible for their activities but, the absence of enzymatic susceptibility in case of **49f** and existence of analgesic activity could not be logically explained).

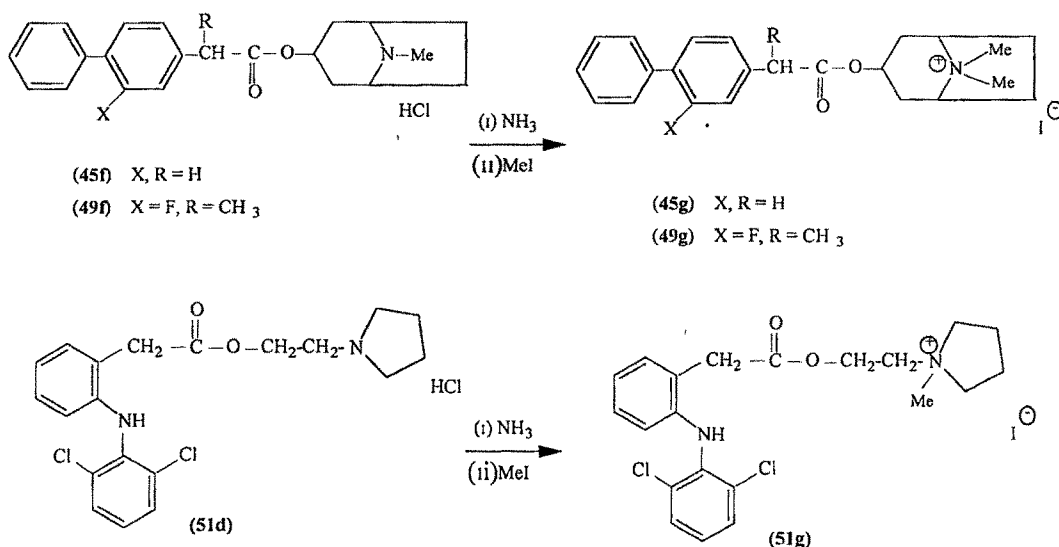
Finally, the ulcerogenic potential of the derivatives was determined *in vivo* in rats at an equimolar dose to their respective parent drugs. Significant reduction in the ulcerogenicity was observed for all the derivatives, which were evaluated for this activity when compared to their respective parent drugs. This observation supports the results of the kinetic studies performed for these derivatives in buffers indicating the successful

blockade of acidic carboxyl group to prevent local GIT irritation. Further, the existence of inherent anticholinergic activity in the derivatives might have also contributed towards decreasing the ulcerogenicity by decreasing the gastric acid secretions. Hence, it could be assumed that the decreased ulcerogenicity may be due to the blockade of the acidic functionality of the parent drugs as well as the possession of the anticholinergic activity by these compounds. The observed residual ulcerogenicity might be due to inhibition of COX-I enzyme by the parent NSAIDs after their liberation in systemic circulation.

In conclusion, these amino ester derivatives have resulted in moieties with much reduced GI side effects compared to the parent drugs, with a high water solubility and adequate stability in aqueous solutions, retaining the desired pharmacological activities except for **45f** and **49f**. A majority of them have also shown good susceptibility towards enzymatic hydrolysis. These derivatives due to their dual activity of being anticholinergic and anti-inflammatory may also have indications in such respiratory disorders which demand anti-inflammatory and bronchodilation effects simultaneously. Above all, the expected good aqueous solubility of the hydrochloride salts of these synthesized esters could also be exploited in formulating their parenteral and ophthalmic dosage forms.

The use of targeted or site-specific delivery to the affected tissues is one of the many approaches used to reduce a drug's undesirable effects. GIT toxicity is the limiting factor restricting the use of NSAIDs for chronic ailments. During the literature review we came across reports where quaternary ammonium compounds like hexamethonium and decamethonium have been found to show higher affinity for the cartilages. It has also been emphasized in literature that the localization of these quaternary compounds in the articular and epiphyseal cartilages and joint spaces might be of significance in relation to the distribution of drugs to inflamed joints and lesions of articular cartilage and raised the possibility of enhancing the duration and intensity of anti-inflammatory effect by chemically designing a drug combining both the antiarthritic activity and joint localization property. The aminoalcohol esters synthesized in the present work with the aim of improving the therapeutic efficacy of the clinically used NSAIDs possessed in common tertiary nitrogen in their structure. To explore the possibility of targeted delivery of the parent NSAIDs to joints of chronic arthritis patients it was thought of studying the localization of these synthesized derivatives after quaternization. To perform such a study some of the esters (**45f**, **49f** and **51d**) were quaternized using

methyl iodide to yield the quaternized ammonium esters (**45g**, **49g** and **51g**) as shown in Scheme-D. Four of these compounds were radiocomplexed with ^{99m}Tc and studied at Institute of Nuclear Medicine and Allied Sciences, New Delhi. The stability of each complex was assessed *in vitro* in human serum and saline. The complexes for the compounds (**51d** and **51g**) were not found to be stable in human serum hence, only the



Scheme-D

stable complexes for the compounds (**45f**, **45g**, **49f** and **49g**) were studied for their *in vivo* biodistribution in mice and the gamma imaging studies were performed in the rat carrageenan-induced hind paw edema model. In the biodistribution studies percent radioactivity in whole blood and various organs at different time intervals was determined. These studies revealed that the complexes of derivatives (**45f**, **45g**, **49f** and **49g**) were stable *in vivo*. The major fraction of the complexes was observed to be metabolized by liver, and kidney was the major route of elimination for these complexes in mice. The gamma images obtained in the inflammation model indicated that in comparison to the tertiary nitrogen containing derivatives (**45f** and **49f**), the quaternised derivatives (**45g** and **49g**) possessed a higher affinity for the inflamed sites in the animal model. A detailed study in this direction for expanding the scope or application of the present work may be planned in future.