Chapter – 4

EXPERIMENTAL

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The melting points were taken in open capillaries and are uncorrected. The UV spectra were recorded on a Shimadzu UV-1601 spectrophotometer and the values in parentheses indicate the log molar absorptivity. The IR spectra were recorded on a Shimadzu-8300 FTIR instrument using KBr pellet/neat samples. The PMR spectra (DPX-200/300 MHz) were recorded for solutions in $CDCl_3$ (unless specified) and the chemical shifts are reported in parts per million (δ) relative to TMS as internal standard. TLC plates were prepared with silica gel G, activated at 110°C for about one hour and the spots were located by exposure to iodine vapors. Anhydrous sodium sulphate was used as drying agent.

4.1. SYNTHESES

4.1.1. AMINOALCOHOL ESTER DERIVATIVES OF 4-BIPHENYLACETIC ACID

4-Phenylacetophenone (47)

In a 100 ml two necked round bottom flask provided with a dropping funnel and a reflux condenser with guard tube, biphenyl (**46**) (5.0 g), anhydrous aluminium trichloride (9.7g) and anhydrous carbon disulphide (30 ml) were charged. The mixture was refluxed on a water bath for 20 minutes. Acetic anhydride (3 ml) in carbon disulphide (5 ml) was added dropwise over a period of 30 minutes with efficient stirring at 30-35°C. The reaction mixture was further refluxed for 3 hours and poured into crushed ice containing concentrated hydrochloric acid (15 ml) and the residual carbon disulphide removed by air bubbling. The precipitate so obtained was filtered, dried and recrystalized from methanol to give (**47**), (4.10g, 64.4%), m.p. 118-20°C (120-21°C)[103].

Anal. :

TLC : R_f 0.61 (Benzene : Pet. Ether; 9:1)

IR (KBr, cm^{-1}) : 1680, 1603, 1264 and 765

4-Biphenylacetic acid (45)

4-Phenylacetophenone (**47**) (10.0 g), morpholine (15 ml) and sulphur (4.0 g) were refluxed for 18 hours. The hot reaction mixture was poured into hot methanol (30 ml) and refrigerated over night. The precipitated matter i.e. the thiomorpholide (**48**) so obtained was filtered and washed with ice-cold methanol (5 ml). The solid so obtained was dissolved in methanol (40 ml) and sodium hydroxide solution (50%, 30 ml). The reaction mixture was refluxed for 16-18 hours. Excess of the solvent was removed and the residue was dissolved in water and filtered. The filtrate so obtained was chilled and acidified with dilute hydrochloric acid to yield a buff colored precipitate which was filtered, dried and crystallized from methanol to yield (**45**) (6.80 g, 63.1 %), m.p. 158-161°C (164-65°C)[103].

Anal :

TLC	: R_f 0.57 (Benzene, 1drop of acetic acid)
UV (MeOH, λ_{max})	: 252 nm (4.46)
IR (KBr, cm^{-1})	: 3400, 1690, 1489, 1257 and 764
PMR	: 3.70 (s, 2H) and 7.25-7.66 (m, 9H)

2-Dimethylaminoethyl 4-biphenylacetate hydrochloride (45a)

4-Biphenylacetic acid (**45**) (9.43 mmol, 2.0g) and thionyl chloride (27 mmol, 2.0 ml) were refluxed for 3 hours in dry benzene (5.0 ml) under anhydrous conditions. The excess of the thionyl chloride and solvent were removed off under vacuum and the acid chloride was obtained as a viscous liquid. The acid chloride so obtained was dissolved in dry, alcohol-free chloroform (30 ml) and anhydrous potassium carbonate (3.0 g) was added to it. 2-Dimethylaminoethanol (**50a**) (30.0 mmol, 3.0 ml) was dissolved in dry, alcohol-free chloroform (10 ml) and added gradually to the well-stirred acid chloride solution dropwise at room temperature. The reaction mixture was further stirred for 15 minutes followed by refluxing on a water bath for 3 hours. The reaction mixture was filtered and diluted with chloroform (200 ml). The filtrate so obtained was washed with ice-cold water till free from the excess of aminoalcohol (**50a**). The organic layer was dried and the solvent removed to give an oily product. The oily material was dissolved in dry isopropyl ether and dry hydrogen chloride gas was passed through the solution till

saturation. The solid hydrochloride so obtained was crystallized from acetone-isopropyl ether to give pure salt (**45a**), (1.44 g, 47.2%), m.p. 167-69°C.

Anal. :

TLC : R_f 0.57 (Benzene : $CHCl_3$, saturated with NH_3, 8 2)

UV (λ_{max}, MeOH) : 253 nm (4.41)

IR (KBr, cm^{-1}) : 3050, 1743, 1485, 1466, 1205, 756 and 694

PMR : 2.75 (s, 6H), 3.33 (t, 2H), 3.79 (s, 2H), 4.56-4.61 (m, 2H), 7.33-7.63 (m, 9H) and 12.75 (b, 1H)

Calculated for $C_{18}H_{22}ClNO_2$: C, 67.6; H, 6.9, N, 4.4. Found C, 67 1; H. 6 5, N, 4.6 %

2-Diethylaminoethyl 4-biphenylacetate hydrochloride (45b)

The acid chloride obtained on treatment of 4-biphenylacetic acid (**45**) (9 43 mmol. 2 0g) with thionyl chloride (27 mmol, 2.0 ml) was treated with 2-diethylaminoethanol (**50b**) (22.6 mmol, 3.0 ml) as described above for **45a** The work up of the reaction mixture yielded the solid hydrochloride (**45b**), (1 21 g, 85.4%), m p. 152-53°C.

Anal. :

TLC : R_f 0.62 (Benzene : $CHCl_3$, saturated with NH_3, 8.2)

UV (λ_{max}, MeOH) : 253 nm (4.31)

IR (KBr, cm^{-1}) : 3460, 1749, 1444, 1219, 1164,767 and 746

PMR : 1.30 (t, 6H), 3.02 (q, 4H), 3.26 (t, 2H), 3.75 (s. 2H), 4.60-4.65 (m, 2H), 7.27-7.59 (m, 9H) and 12.55 (b, 1H)

Calculated for $C_{20}H_{26}ClNO_2$: C, 69.1; H, 7.5; N, 4.0 Found C, 68.6; H, 7.2; N, 3.7%

1-[2-(1-Piperidino)]ethyl 4-biphenylacetate hydrochloride (45c)

4-Biphenylacetic acid (**45**) (9.43 mmol, 2.0g) was reacted with 2-piperidinoethanol (**50c**) (22.6 mmol, 3.0 ml) as described for compound (**45a**). Work up of the reaction mixture similar to that for compound (**45a**) afforded the solid hydrochloride which was

crystallized from acetone-isopropyl ether to afford compound (**45c**), (1.08 g, 31.9 %). m p. 161-63°C.

Anal. :

TLC . R_f 0.58 (Benzene : $CHCl_3$, saturated with NH_3, 8:2)
UV (λ_{max}, MeOH) . 253 nm (4.35)
IR (KBr, cm^{-1}) : 3460, 1743, 1444, 1219, 1164, 767 and 746
PMR : 1.25-1.77 (m, 6H), 2.45 (t, 2H), 3.19 (t, 2H), 3 38 (t, 2H), 3.74 (s, 2H), 4.63-4.66 (m, 2H), 7.27-7.57 (m, 9H) and 12.25 (b,1H; disappeared on deuterium exchange)
Calculated for $C_{21}H_{26}ClNO_2$: C, 70.1; H, 7 3; N, 3.9. Found C, 70 4; H, 7.5, N, 3.6%

1-[2-(1-Pyrrolidino)]ethyl 4-biphenylacetate hydrochloride (45d)

4-Biphenylacetic acid (**45**) (9 43 mmol, 2.0g) was treated with 2-pyrrolidinoethanol (**50d**) (25 65 mmol, 3 0 ml) following the procedure as described for compound (**45a**) The work up of the reaction mixture and crystallization afforded the solid hydrochloride (**45d**), (2 2 g, 67.5%), m p. 138-40°C.

Anal :

TLC : R_f 0.58 (Benzene : $CHCl_3$, saturated with NH_3, 8:2)
UV (λ_{max}, MeOH) : 253 nm (4.39)
IR (KBr, cm^{-1}) : 3480, 1743, 1486, 1456, 1218, 1168, 769 and 748
PMR : 2.02 (t, 4H), 2.25-2.60 (m, 6H), 3.78 (s, 2H), 4.58-4.63 (t, 2H), 7.27-7.58 (m, 9H) and 12.50 (b, 1H)
Calculated for $C_{20}H_{24}ClNO_2$: C, 69.5; H 6.9; N 4 1. Found C, 69.2; H, 7.3, N, 3.6 %

1-[2-(4-Morpholino)]ethyl 4-biphenylacetate hydrochloride (45e)

The acid chloride obtained on treatment of 4-biphenylacetic acid (**45**) (9.43 mmol, 2.0g) with thionyl chloride (27 mmol, 2.0 ml) was treated with 2-(4-morpholino)ethanol (24.79 mmol, 3.0 ml) following the procedure as described for compound (**45a**). The

work up of the reaction mixture and crystallization gave the solid hydrochloride (**45e**), (1.46 g, 43.0%), m.p. 150-52°C.

Anal. :

TLC : R_f 0.52 ($CHCl_3$: MeOH, 9.5.0.5)

UV (λ_{max}, MeOH) : 253 nm (4.28)

IR (KBr, cm^{-1}) : 3480, 1743, 1486, 1453, 1204, 1105, 1009, 907, 757 and 692

PMR : 2.60-2.67 (m, 2H), 3.25-3.29 (m, 4H), 3.76 (s, 2H), 3.80-4.22 (m, 4H), 4.67-4.70 (m, 2H), 7.33-7.58 (m, 9H) and 13.50 (b, 1H)

Calculated for $C_{20}H_{24}ClNO_3$: C, 66.4; H, 6.7; N, 3.9. Found C. 66 7, H. 7 2. N, 4.2%

Synthesis of tropinol (50f) from atropine sulphate

A solution of atropine sulphate (1.44 mmol, 1.1g) in distilled water (3 ml) was treated dropwise with potassium hydroxide solution (0.5 g in 4.0 ml distilled water) The precipitate obtained was filtered, dissolved in methanol (10 ml). potassium hydroxide (0.5 g) was added and the reaction mixture was refluxed for 1 hour. The excess solvent was removed and water (10 ml) was added to the solid obtained, extracted into chloroform (3x10 ml), dried and solvent removed to yield the solid compound. tropinol (**50f**) (0.42g, 95.5%) which was used as such for the next reaction.

8-Methyl-8-azabicyclo[3,2,1]octan-3-yl 4-biphenylacetate hydrochloride (45f)

4-Biphenylacetic acid (**45**) (9.43 mmol, 2.0g) was reacted with tropinol (**50f**) (24.8 mmol, 3.5g ml) following the procedure as described for compound (**45a**) followed by the work up of the reaction mixture and crystallization to yield the solid hydrochloride (**45f**), (1.70g, 54.14%), m.p. 256-58°C.

Anal. :

T.L.C. : R_f 0.43 ($CHCl_3$: MeOH, 9.5:0.5)

UV (λ_{max}, MeOH) : 253 nm (4.35)

IR (KBr, cm^{-1}) : 3450, 1727, 1486, 1453, 1223,.1167, 772 and 744

PMR : 1.62-1.65 (m, 2H),1.86-1.94 (m, 4H), 2.65 (s, 3H), 2.75-3.00 (m, 2H), 3.49-3.61 (m, 2H), 3.68 (s, 2H), 5.13-5.17 (m, 1H) and 7.31-7.60 (m, 9H)

Calculated for $C_{22}H_{26}ClNO_2$: C, 71.1; H, 7.1; N, 3.8. Found C, 70.7; H, 7 5, N, 4.1 %

4.1.2. AMINOALCOHOL ESTER DERIVATIVES OF FLURBIPROFEN

2-Dimethylaminoethyl 2-(2-fluoro-4-biphenyl)propionate hydrochloride (49a)

Flurbiprofen (**49**) (8.18 mmol, 2.0 g) and thionyl chloride (27 mmol, 2.0 ml) were refluxed for 3 hours in dry benzene (5.0 ml) under anhydrous conditions. The excess of the thionyl chloride and solvent were removed off under vacuum and the acid chloride so obtained was dissolved in dry, alcohol-free chloroform (30 ml) and anhydrous potassium carbonate (3.0 g) was added to it. 2-Dimethylaminoethanol (**50a**) (30.0 mmol, 3 0 ml) was dissolved in dry, alcohol-free chloroform (10 ml) and added to the well-stirred acid-chloride solution dropwise at room temperature. The reaction mixture was further stirred for 15 minutes followed by refluxing on a water bath for 3 hours. The reaction mixture was filtered and diluted with chloroform (200 ml). The filtrate so obtained was washed with ice-cold water till free from the excess of aminoalcohol (**50a**). The organic layer was dried and the solvent removed to give an oily product. The oily material was dissolved in dry isopropyl ether and dry hydrogen chloride gas was passed through the solution till saturation. The solid hydrochloride was crystallized from acetone-isopropyl ether to give pure salt (**49a**) (1.0 g, 32.7 %), m.p. 120-21°C.

Anal. :

TLC : R_f 0.63 (Benzene : $CHCl_3$, saturated with NH_3, 8:2)

UV (λ_{max}, MeOH) : 247 nm (4.46)

IR (KBr, cm^{-1}) : 3454 , 1732, 1168, 1134, 871, 748 and 692

PMR : 1.55 (d, 3H), 2.72 (s, 6H,), 3.27 (t. 2H,), 3.88 (q, 1H), 4.49-4.66 (m, 2H) and 7.09-7.53 (m, 8H)

Calculated for $C_{19}H_{23}ClFNO_2$: C, 64.9; H, 6.6; N, 3.9. Found C, 64.3; H, 7.0; N, 4.3 %

2-Diethylaminoethyl 2-(2-fluoro-4-biphenyl)propionate hydrochloride (49b)

Flurbiprofen (**49**) (8.18 mmol, 2 0 g) was treated with 2-diethylaminoethanol (22 6 mmol, 3.0ml) as described for compound (**49a**). The work up of the reaction mixture similar to that of compound (**49a**) yielded the solid hydrochloride (**49b**), (1.2 g, 27.9 %) m.p. 115-18°C.

Anal. :

TLC : R_f 0.32 (Benzene : $CHCl_3$ saturated with NH_3, 8·2)

UV (λ_{max}, MeOH) . 247 nm (4.30)

IR (KBr, cm^{-1}) . 3450, 1743, 1353, 1168, 769, 748 and 692

PMR : 1.28 (t, 6H), 1.55(d, 3H), 2.94-3.04 (m, 4H), 3 26 (t. 2H), 3.82 (q, 1H), 4.58-4.62 (m, 2H) and 7.08-7 53 (m. 8H)

Calculated for $C_{21}H_{27}ClFNO_2$: C, 66.4; H, 7 2; N 3.7. Found C, 66 2, H. 6.9. N, 3.4%

1-[2-(1-Piperidino)]ethyl 2-(2-fluoro-4-biphenyl)propionate hydrochloride (49c)

Compound (**49c**) was prepared by reacting flurbiprofen (**49**) (8.18 mmol. 2 0 g) with 2-piperidinoethanol (**50c**) (22.6 mmol, 3.0 ml) as described for compound (**49a**) The work up of the reaction mixture followed by crystallization yielded the solid hydrochloride (**49c**), (1.52 g, 44.7%), m.p. 153-54°C.

Anal. :

TLC : R_f 0.71 (Benzene : $CHCl_3$, saturated with NH_3, 8:2)

UV (λ_{max}, MeOH) : 247 nm, (4.34)

IR (KBr, cm^{-1}) : 3421, 1736, 1485,1417, 1186, 756, 698 and 667

PMR : 1.54 (d, 3H), 1.69-2.21 (m, 6H). 2.35-3 40 (m. 6H). 3.83 (q. 1H), 4.59-4.64 (m, 2H), 7.08-7.53 (m. 8H) and 12.25 (b,1H: disappeared on deuterium exchange)

Calculated for $C_{22}H_{27}ClFNO_2$: C, 67.4; H, 6.9; N, 3.6. Found C, 67.9; H, 7.4; N, 3.2%

1-[2-(1-Pyrrolidino)]ethyl 2-(2-fluoro-4-biphenyl)propionate hydrochloride (49d)

The acid chloride obtained on treatment of flurbiprofen (**49**) (8.18 mmol, 2.0 g) with thionyl chloride (27 mmol, 2.0 ml) was treated with 2-pyrrolidinoethanol (**50d**) (25.65 mmol, 3.0 ml) followed by the work up of the reaction mixture similar to that for (**49a**). Crystallization in similar fashion yielded the compound as solid hydrochloride (**49d**) (1.20 g, 29.3%), m.p. 95-96°C.

Anal. :

TLC : R_f 0.63 (Benzene : $CHCl_3$, saturated with NH_3, 8:2)

UV (λ_{max}, MeOH) : 247 nm (4.35)

IR (KBr, cm^{-1}) : 3356, 1733, 1620, 1581, 1484, 1132, 767, 724 and 699

PMR : 1.54 (d, 3H), 2.02-2.16 (m, 4H), 2.63-3.70 (m, 6H), 3 86 (q, 1H), 4.53-4.64 (m, 2H), 7.09-7.53 (m, 8H) and 12.45 (b, 1H)

Calculated for $C_{21}H_{25}ClFNO_2$: C, 66.8; H, 6.7; N, 3.7. Found C, 66.5, H, 7 1; N, 4.1%

1-[2-(4-Morpholino)]ethyl 2-(2-fluoro-4-biphenyl)propionate hydrochloride (49e)

The compound (**49e**) was prepared by reaction of flurbiprofen (**49**) (8.18 mmol, 2.0 g) with 2-(4-morpholino)ethanol (**50e**) (24.79 mmol, 3.0 ml) as described in Scheme-I. followed by the work up and crystallization to yield the solid hydrochloride (**49e**) (1.8 g, 52.6%). m.p. 118-20°C.

Anal. :

TLC : R_f 0.74 ($CHCl_3$: MeOH, saturated with NH_3, 9:1)

UV (λ_{max}, MeOH) : 247 nm (4.28)

IR (KBr, cm^{-1}) : 3420, 1740, 1484, 1418, 1266, 1132, 765 and 698

PMR : 1.54 (d, 3H), 2.58-3.29 (m, 6H), 3.75-4.20 (m, 5H), 4.66 (m, 2H), 7.09-7.53 (m, 8H) and 13.30 (b, 1H)

Calculated for $C_{21}H_{25}ClFNO_3$: C, 64.0; H, 6.4; N, 3.6. Found C, 64.2; H, 6.7; N, 3.2 %

8-Methyl-8-azabicyclo[3,2,1]octan-3-yl 2-(2-fluoro-4-biphenyl)propionate hydrochloride (49f)

The acid chloride obtained on treatment of flurbiprofen (**49**) (8.18 mmol, 2.0 g) with thionyl chloride (27 mmol, 2.0 ml) was treated with tropine (**50f**) (24.8 mmol, 3.5g), followed by the work up of the reaction mixture similar to that for **49a** to yield the solid hydrochloride (**49f**) (1.27 g, 39.9%), m.p. 196-198°C.

Anal. :

TLC : R_f 0.60 ($CHCl_3$: MeOH, saturated with NH_3, 9:1)

UV (MeOH, λ_{max}) : 247 (4.31)

IR (KBr, cm^{-1}) : 3300, 1727, 1580, 1484, 1452, 1281, 1165 and 776

PMR . 1.53 (d, 3H), 1.85-1.96 (m, 4H), 2.04-2 09 (m, 2H), 2.64 (s, 3H), 2.99-3.13 (m, 2H), 3.55-3.65 (m, 2H), 3.69- 3.76(m, 1H), 5.13-5.16 (m, 1H), 7.07-7.53 (m, 8H) and 12 51(b, 1H)

Calculated for $C_{23}H_{27}ClFNO_2$: C, 68.4; H, 6.7; N, 3.5. Found C. 68 7; H, 6 9; N, 3.4%

4.1.3. ATTEMPTED SYNTHESIS OF THE AMINOALCOHOL ESTER DERIVATIVES OF DICLOFENAC

Method A: Diclofenac (**51**) (3.39 mmol, 1.0 g) was dissolved in dichloromethane and treated dropwise with thionyl chloride (5.4 mol, 0.4 ml) at room temperature. The reaction mixture was stirred for 2 hours at room temperature followed by the addition of the 2-dimethylaminoethanol (**50a**) (15 mmol, 1.5 ml) and anhydrous potassium carbonate (1 g) with further stirring for 2 hours at room temperature. The reaction mixture was then diluted with chloroform (200 ml) and filtered. The organic layer was washed with ice-cold water to remove the excess of the aminoalcohol (**50a**). The organic layer was dried and the solvent removed to give a solid product that was crystallized using acetone-isopropyl ether. The above route was tried with the rest of aminoalcohols (**50b-d**). The products so-obtained were characterized to be the lactam (**52**) in all the cases with varying yields (40-53%), m.p. 112-15°C.

Anal. :

TLC : R_f 0.59, ($CHCl_3$: MeOH, 9:1)

IR (KBr, cm^{-1}) : 1732, 1613, 1490, 1483, 1437, 1302, 1171, 783 and 750

PMR : 3.78 (s, 2H), 6.39-6.41(d, 1H), 7.07-7.12 (m, 1H), 7.18-7.23 (m. 1H), 7.33-7.37 (m, 1H), 7.38-7.40 (d, 1H) and 7 49-7.52 (d. 2H).

Calculated for $C_{14}H_9Cl_2NO$: C, 60.5; H, 3.3; N 5.0. Found C, 60.8; H. 2 8; N, 4.7 %

Method B: Thionyl chloride (13.5 mmol, 1.0 ml) was added drop-wise with stirring to 2-dimethylaminoethanol (**50a**) (10.0 mmol, 1.0 ml) in dry isopropyl ether (10 ml) and the hydrochloride salt precipitated as solid was filtered and dried. The dried hydrochloride salt so obtained was added to a stirred slurry of diclofenac (**51**) (3.39 mmol, 1 0 g) and anhydrous potassium carbonate (1.0 g) in dried dimethylformamide (5 ml) over a period of half an hour. The reaction mixture was heated on a water bath for 2 hours. poured into ice-cold water (100 ml) and extracted with chloroform (4x 25 ml) The extract was washed with ice-cold water, dried and the solvent recovered to give a semisolid residue which was crystallized and identified to be the lactam (**52**). The reaction was repeated and the time of heating on water bath was varied from 2 to 4 hours with the isolation of the same oxindole (**52**) for all the attempts even with other amino alcohols (**50b-d**).

Method C : Diclofenac (**51**) (3.39 mmol, 1.0g) was dissolved in dry pyridine (4.0 ml) and acetic anhydride (1.0 ml) was added to it at room temperature and left overnight under anhydrous conditions. The excess of solvent and acetic anhydride was removed under vacuum and the residue was dissolved in chloroform and treated dropwise with thionyl chloride (8.4 mmol, 0.6 ml). The reaction mixture was stirred for 2 hours at room temperature followed by the addition of the 2-dimethylaminoethanol (**50a**) (15 mmol. 1 5 ml) and anhydrous potassium carbonate (1.0 g) with further stirring for 2 hours at room temperature. The reaction mixture was then diluted with chloroform (200 ml) and filtered. The organic layer was repeatedly washed with ice-cold water, dried and the solvent recovered to give a solid product, which was crystallized using acetone-isopropyl ether. The compound so obtained was identified to be lactam (**52**).

Method D : Diclofenac (**51**) (3.39 mmol, 1.0g) was dissolved in dry pyridine (4.0 ml) and acetic anhydride (1.0 ml) was added to it at room temperature. The reaction mixture

was left overnight, poured into ice-cold water, acidified and extracted with chloroform. The solvent was removed and the product crystallized from acetone-isopropyl ether to give the lactam (**52**).

4.1.4. AMINOALCOHOL ESTER DERIVATIVES OF DICLOFENAC

Method E : 2-(4-Morpholino)ethanol (**50e**) (8.3 mmol, 1.0 ml), 4-dimethylamino pyridine (50 mg) and dicyclohexylcarbodiimide (850 mg) in dry dichloromethane (40ml) were stirred[99] in an ice-bath. Diclofenac (**51**) (3.39 mmol, 1.0 g) was added in portions over a period of 2 hours and the reaction mixture was kept overnight at 0-4°C followed by filtration of the precipitated dicyclohexylurea (DCU). DCU was further precipitated by adding conc. hydrochloric acid (0.1 ml) into the reaction mixture kept in ice-bath for half an hour followed by filtration. The volume of the filtrate was made upto 150 ml with addition of chloroform. The organic layer was given several washings with ice-cold water, dried and complete removal of the solvent yielded an oily residue, which was purified by column chromatography using silica gel G as the stationary phase and toluene as the mobile phase. The oily residue was dissolved in dry isopropyl ether and filtered, dry hydrogen chloride passed through the filtrate till saturation to obtain solid hydrochloride salt (**51e**). The solid residue so obtained was filtered, dried and crystallized from dry acetone-isopropyl ether to afford pure compound (**51e**) in a yield of 5%. The reaction was also performed with 2-dimethylaminoethanol (**50a**) to afford the compound (**51a**) with a yield of 7 % only.

Method F

2-Dimethylaminoethyl 2-(2,6-dichlorophenylaminophenyl)acetate hydrochloride (51a)

2-Dimethylaminoethanol (**50a**) (10.0 mmol, 1.0 ml) was treated dropwise with thionyl chloride (0.02 mol, 1.5 ml) in dry chloroform (20 ml) under stirring at room temperature and refluxed for 15 minutes on a water bath. The reaction mixture was then cooled to room temperature and ice cold water (20 ml) was added to it. Phase transfer catalyst (PTC) (Tetraethylammonium bromide) (1.5 g), potassium iodide (300mg), diclofenac (**51**) (5.08 mmol, 1.5 g) and sodium bicarbonate (3.5 g) were added to the above solution and the reaction mixture was stirred for 15 -18 hours. The reaction mixture was

monitored by TLC, diluted with chloroform (200 ml) and washed several times with ice-cold water. The chloroform layer was dried and the solvent removed completely to afford the oily product. The oily residue was dissolved in dry isopropyl ether and filtered, dry hydrogen chloride passed through the filtrate till saturation to obtain solid hydrochloride salt. The solid residue so obtained was filtered, dried and crystallized from dry acetone-isopropyl ether to give (**51a**) (1.52 g, 55.6 %) m.p. 187-89°C.

Anal :

TLC	: R_f 0.59, ($CHCl_3$: MeOH, 9:1)
UV (λ_{max}, MeOH)	: 276 nm (4.09)
IR (KBr, cm^{-1})	: 3283, 1734, 1587, 1502, 1448, 1233, 1142, 1089 and 760
PMR	: 2.75 (s, 6H),3.32 (t, 2H), 3.93 (s,2H). 4 61-4 64 (m, 2H). 6.48-6.51(d, 1H), 6.62 (b, 1H). 6.90-6 95 (m, 1H). 6 99-7 04 (m, 1H). 7.09-7.14 (m, 1H). 7.22-7.25 (d. 1H) and 7 34-7 36 (d, 2H) and 12.74 (b, 1H)

Calculated for $C_{18}H_{21}Cl_3N_2O_2$: C, 53.5; H, 5.2; N, 6.9 Found C, 53.9; H. 5.6. N, 6.6%

2-Diethylaminoethyl 2-(2,6-dichlorophenylaminophenyl)acetate hydrochloride (51b)

The compound (**51b**) was prepared by the reaction between diclofenac (**51**) (5.08 mmol, 1.5 g) and 2-diethylaminoethanol (**50b**) (7.5 mmol, 1.0 ml) as described for compound (**51a**). The work up of the reaction mixture and crystallization yielded the crystalline salt (**51b**), (1.4 g, 47.8 %) m.p. 147-49°C.

Anal. :

TLC	: R_f 0.71, ($CHCl_3$: MeOH, 9:1)
UV (λ_{max}, MeOH)	: 276 nm (4.04)
IR (KBr, cm^{-1})	: 3315, 1730, 1588, 1506, 1448, 1228, 1135 and 782
PMR	: 1.31 (t, 6H), 3.06 (q, 4H), 3.28 (t, 2H), 3.86 (s, 2H), 4.66-4.69 (m, 2H), 6.50-6.53 (d, 1H), 6.59 (b, 1H), 6.92-6.97 (m, 1H), 6.99-7.04 (m, 1H), 7.10-7.16 (m, 1H), 7.20-7.23 (d, 1H), 7.34-7.37 (d, 2H) and 12.5 (b, 1H)

Calculated for $C_{20}H_{25}Cl_3N_2O_2$: C, 55.6; H, 5.8; N, 6.5. Found C, 55.9; H, 5.4; N, 6.8 %

1-(2-(1-Piperidino))ethyl 2-(2,6-dichlorophenylaminophenyl)acetate hydrochloride (51c)

Diclofenac (**51**) (5.08 mmol, 1.5 g) was reacted with 2-piperidinoethanol (**50c**) (7 5 mmol, 1.0 ml) as described for compound (**51a**) followed by work up to yield the hydrochloride salt salt (**51c**), (1.62 g, 53.5 %), m.p. 195-97°C

Anal. :

TLC : R_f 0.64, ($CHCl_3$: MeOH, 9:1)

UV (λ_{max}, MeOH) : 276 nm (4.06)

IR (KBr, cm^{-1}) : 3271, 1735, 1564, 1507, 1248, 1156 and 767

PMR : 1.21-1.79 (m, 6H), 2.17-3.44 (m, 6H), 3.87 (s, 2H), 4.70-4.73 (m, 2H), 6.50-6.52 (m, 1H), 6.53 (b, 1H), 6.92-6.97 (t, 1H), 7.00-7.06 (t, 1H), 7.10-7.16 (m, 1H), 7.21-7.23 (d, 1H), 7.36-7.38 (d, 2H) and 12.80 (b, 1H)

Calculated for $C_{21}H_{25}Cl_3N_2O_2$: C, 56.8; H, 5.7; N, 6.3. Found C, 56.4; H, 5.2; N, 6.5 %

1-[2-(1-Pyrrolidino)]ethyl 2-(2,6-dichlorophenylaminophenyl)acetate hydrochloride (51d)

The reaction of diclofenac (**51**) (5.08 mmol, 1.5 g) with 2-pyrrolidinoethanol (**50d**) (8.5 mmol, 1.0 ml) as depicted in Scheme-IV followed by crystallization yielded the desired compound (**51d**), (1.36 g, 46.7%) m.p. 192-94°C

Anal. :

T.L.C. : R_f 0.52, ($CHCl_3$: MeOH, 9:1)

UV (λ_{max}, MeOH) : 276 nm (4.04)

IR (KBr, cm^{-1}) : 3351, 1741, 1559, 1502, 1250, 1140, 780 and 753

PMR : 1.91-2.17 (m, 4H), 2.56-3.73 (m, 6H,), 3.90 (s, 2H), 4.66-4.69(m, 2H), 6.49-6.51(d, 1H), 6.58 (b, 1H), 6.91-6.96 (m, 1H), 7.00-7.05 (m, 1H), 7.09-7.15 (m, 1H), 7.22-7.25 (d, 1H), 7.35-7.37 (d, 2H) and 12.60 (b, 1H)

Calculated for $C_{20}H_{23}Cl_3N_2O_2$: C, 55.9; H, 5.4; N, 6.5. Found C, 55.4; H, 5.9; N, 6.1 %

1-[2-(4-Morpholino)]ethyl 2-(2,6-dichlorophenylaminophenyl)acetate hydrochloride (51e)

The compound (**51e**) was prepared by reacting diclofenac (**51**) (5.08 mmol, 1.5 g) and 2-(4-morpholino)ethanol (**50e**) (8.3 mmol, 1.0 ml) as described above for compound (**51a**). Upon work up of the reaction mixture compound (**51e**) was obtained as crystalline hydrochloride salt (1.50 g, 49.6 %), m.p. 173-75°C (177-79°C)[99].

Anal. :

TLC : R_f 0.80, ($CHCl_3$: MeOH, 9:1)

UV (λ_{max}, MeOH) : 276 nm (3.96)

IR (KBr, cm^{-1}) : 3348, 1738, 1559, 1506, 1238, 1124, 771 and 740

PMR : 2.65 (t, 2H), 3.23-3.41 (m, 4H,), 3.77-4.21 (m, 6H), 4.67 (s, 2H), 4.72-4.75 (m, 2H), 6.48-6.49 (d, 1H), 6.58 (b, 1H), 6.92-6.97 (m, 1H), 7.01-7.07 (m, 1H), 7.10-7.16 (m, 1H), 7.22-7.25 (d, 1H), 7.36-7.37 (d, 2H) and 13.30 (b, 1H)

Calculated for $C_{20}H_{23}Cl_3N_2O_3$: C, 53.9; H, 5.2; N, 6.3. Found C, 53.6; H, 4.9; N, 6.2 %

4.1.5. ATTEMPTED SYNTHESIS OF THE AMINOALCOHOL ESTER DERIVATIVES OF INDOMETHACIN

Method A

2-Dimethylaminoethyl 4-chlorobenzoate hydrochloride (55a)

Indomethacin (**54**) (2.8 mmol, 1.0 g) was dissolved in dichloromethane by refluxing and treated dropwise with thionyl chloride (8.4 mmol, 0.6 ml) after cooling to room

temperature. The reaction mixture was stirred for 2 hours at room temperature followed by the addition of the 2-dimethylaminoethanol (10 mmol, 1.0 ml) and anhydrous potassium carbonate (1.0 g) with further stirring for 2 hours at room temperature. The reaction mixture was then diluted with chloroform (200 ml) and filtered. The organic layer was washed with ice-cold water to remove the excess of the aminoalcohol, dried and solvent removed to give an oily product. The oily material was dissolved in dry iso-propyl ether and dry hydrogen chloride gas was passed through the solution till saturation. The solid hydrochloride was crystallized from acetone-isopropyl ether to give pure salt (**55a**) of 2-diethylaminoethyl 4-chlorobenzoate (0.5 g, 72.4%), m.p. 178-81°C.

Anal. :

TLC : R_f 0.41, ($CHCl_3$ saturated with NH_3)

IR (KBr, cm^{-1}) : 3400, 2654, 2489, 1731, 1593, 1265, 1104 and 753

PMR . 2.94 (s, 6H),3.47-3.50 (m, 2H), 4.82-4.85 (m, 2H), 7.43-7.46 (d, 2H), 8.02-8.05 (d, 2H)

Calculated for $C_{11}H_{15}Cl_2NO$: C, 53.2; H, 6.1; N,5.6. Found C, 53.1; H, 6.3; N, 5.4 %

2-Diethylaminoethyl 4-chlorobenzoate hydrochloride (55b)

Indomethacin (**54**) (2.8 mmol, 1.0 g) was reacted with 2-diethylaminoethanol (**50b**) (7.5 mmol, 1.0 ml) as described above for compound (**55a**) to yield an ester of 4-chloro-benzoic acid, as salt (**55b**), (0.35 g, 46.2 %), m.p. 167-69°C.

Anal. :

TLC : R_f 0.47, ($CHCl_3$ saturated with NH_3)

IR (KBr, cm^{-1}) : 3430, 1727, 1592, 1263, 1107 and 754

PMR : 1.44 (t, 6H), 3.19 3 24 (q, 4H), 3.41-3.45 (m, 2H,), 4.83-4.85 (m, 2H), 7.43-7.46 (d, 2H) and 7.97-8.04 (d, 2H)

Calculated for $C_{13}H_{19}Cl_2NO$: C, 56.5; H, 6.9; N,5.1. Found C, 56.1; H, 6.7; N, 5.3 %

1-[2-(1-Piperidino)]ethyl 4-chlorobenzoate hydrochloride (55c)

Indomethacin (**54**) (2.8 mmol, 1.0 g) was reacted with 2-piperidinoethanol (**50c**) (7.5 mmol, 1.0 ml) as described above for compound (**55a**) followed by crystallization to yield the compound (**55c**), (0.46 g , 59.7 %) m.p. 158-61°C.

Anal. :

TLC : R_f 0.53, ($CHCl_3$ saturated with NH_3)

IR (KBr, cm^{-1}) : 3410, 1727, 1590, 1261, 1117 and 754

1-[2-(1-Pyrrolidino)]ethyl 4-chlorobenzoate hydrochloride (55d)

The compound (**55d**) was obtained when indomethacin (**54**) (2.8 mmol, 1.0 g) was reacted with 2-pyrrolidinoethanol (**50d**) (7.5 mmol, 1.0 ml) as described above for compound (**55a**) to yield the hydrochloride (**55d**), (0.51 g , 67.1%), m.p 189-92°C

Anal. :

TLC : R_f 0.51, ($CHCl_3$ saturated with NH_3)

IR (KBr, cm^{-1}) : 3420, 1739, 1594, 1270, 1114 and 752

Method B : Thionyl chloride (13.5mmol, 1.0ml) was added drop-wise with stirring to 2-dimethylaminoethanol (**50a**) (10 mmol, 1.0 ml) in dry iso-propyl ether (10 ml) and the precipitated solid was filtered and dried. The dried hydrochloride salt was added to a stirred slurry of indomethacin (**54**) (2.8 mmol, 1.0 g) and anhydrous potassium carbonate (1.0 g) in dried dimethylformamide (5 ml) over a period of half an hour. The reaction mixture was heated on a water bath for 2 hours, poured into ice-cold water (100 ml) and extracted with chloroform (4x 25ml). The extract was washed with ice-cold water, dried and the solvent recovered to give an oily residue, which was dissolved in dry isopropyl ether and dry hydrogen chloride gas was passed through the solution till saturation. The solid hydrochloride was crystallized from acetone-isopropyl ether to give the salt (**55a**) m.p. 178-81°C. The above reaction was also repeated with the amino alcohols (**50b-d**) to yield solid hydrochloride salts (**55b-d**).

Method E : 2-Dimethylaminoethanol (**50a**) (10.0 mmol, 1.0 ml), 4-dimethylamino-pyridine (DMAP), (15 mg) and dicyclohexylcarbodiimide (DCC), (700 mg) were added to a solution of indomethacin (**54**), (2.7 mmol, 1.0 g) in chloroform (25 ml) with stirring in an ice-bath. The stirring was continued for 2 hours and the reaction mixture was kept overnight at a temperature of 0-4°C. Dicyclohexylurea (DCU) was precipitated by adding methanol (3.0 ml) and glacial acetic acid (1.0 ml) to the reaction mixture with stirring in ice-bath. The reaction mixture was filtered and chloroform (200 ml) was added to the filtrate followed by washing with 5% sodium bicarbonate (100 ml) and then with ice-cold water. The organic extract was dried and solvent removed completely, to yield yellow oil in a poor yield after purification by column chromatography (silica gel G and benzene), which was dissolved in methanol (2.0 ml). Methanolic hydrochloric acid was added to the above solution in cold. The solution turned dark in colour immediately giving a sweet smelling semisolid, which could not be salvaged

4.1.6. AMINOALCOHOL ESTER DERIVATIVES OF INDOMETHACIN

2-Dimethylaminoethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetate (54a)

2-Dimethylamino ethanol (**50a**) (10.0 mmol,1.0 ml) was treated dropwise with thionyl chloride (0.02 mol,1.5ml) in dry chloroform (20 ml) under stirring at room temperature and refluxed for 15 minutes on a water bath. The reaction mixture was then cooled to room temperature and ice cold water (20 ml) was added to it. Phase transfer catalyst (PTC) (Tetraethylammonium bromide) (1.5 g), potassium iodide (300mg), indomethacin (**54**) (2.8 mmol, 1.0 g) and sodium bicarbonate (3.5 g) were added to the above solution and the reaction mixture was stirred for 15 -18 hours. The reaction mixture was monitored by TLC. Diluted with chloroform (200 ml) and washed several times with ice-cold water. The chloroform layer was dried and removed completely to afford the yellow oily product (**54a**), (1.02 g, 85.7 %).

Anal. :

TLC : R_f 0.61, ($CHCl_3$: MeOH, 9:1)

UV (λ_{max}, MeOH) : 227 nm (4.40)

IR (neat, cm^{-1}) : 1733, 1683, 1593, 1457, 1356, 1088, 1036 and 754

PMR : 2.31 (s, 6H),2.40 (s, 3H), 2 63-2 69 (m, 2H), 3.65 (s, 2H), 3.80 (s, 3H), 4.24-4.26 (m, 2H), 6.64-6.68 (m, 1H), 6 81-6.87 (d, 1H), 6.94-6.95 (d, 1H), 7 47- 7.48 (d, 2H) and 7.63-7 67 (d, 2H)

2-Diethylaminoethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetate (54b)

The compound (**54b**) was prepared by reactıng indomethacin (**54**) (2.8 mmol. 1 0 g) and 2-diethylaminoethanol (**50b**) (7.5 mmol, 1.0 ml) Work up of the reactıon mixture as descrıbed for the compound (**54a**) yielded the product (**54b**), (1 03 g. 81.0 %)

Anal ·

TLC · R_f 0 73. ($CHCl_3$. MeOH 9:1)

UV (λ_{max}. MeOH) . 227 nm (4.48)

IR (neat, cm^{-1}) : 1733, 1683,1590, 1457, 1354, 1088, 1039 and 754

PMR . 1.11 (t, 6H). 2.35 (s, 3H).2.73-2 85 (q. 4H). 2 98 (t. 2H). 3.64 (s. 2H). 3 78 (s. 3H). 4 21- 4 26 (m. 2H). 6 60-6 65 (m. 1H). 6 84-6.88 (d, 1H), 6.93-6.95 (d. 1H). 7 43-7 48(d. 2H) and 7.60-7.66 (d, 2H)

1-[2-(1-Piperidino)]ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetate (54c)

Indomethacin (**54**) (2.8 mmol. 1.0 g) and 2-piperidinoethanol (**50c**) (7 5 mmol, 1.0 ml) were reacted as described in Scheme-IV to yield compound (**54c**), (0.90 g. 68 7 %)

Anal. :

TLC : R_f 0.76, ($CHCl_3$: MeOH, 9.1)

UV (λ_{max}, MeOH) : 227 nm (4.29)

IR (neat, cm^{-1}) : 1733, 1683, 1590, 1456, 1356, 1088, 1039 and 754

PMR : 1.25-1.68 (m. 6H), 2.34 (s, 3H), 2.55-2.77 (m, 4H), 2.93 (t, 2H), 3.60 (s, 2H), 3.79 (s, 3H). 4.29-4.55 (m, 2H).

6.65-6.74 (m, 1H), 6.85-6.93 (d, 1H), 7.01-7.02(d, 1H), 7.36-7.45 (d, 2H) and 7.92-7.97 (d, 2H)

1-[2-(1-Pyrrolidino)]ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetate (54d)

Compound (**54d**) was prepared by reacting indomethacin (**54**) (2.8 mmol, 1.0 g) with 2-pyrrolidinoethanol (**50d**) (8.5 mmol, 1.0 ml) as described above for the compound (**54a**) to yield the desired product as yellow oil, (1.1 g, 86.6 %).

Anal. :

TLC : R_f 0.61, ($CHCl_3$: MeOH, 9:1)
UV (λ_{max}, MeOH) : 227 nm (4.39)
IR (neat, cm^{-1}) : 1733, 1683, 1593, 1457, 1356, 1088,1036, and 754
PMR . 1.90-1.95 (m, 4H), 2.33 (s, 3H), 2 71-2.99 (m, 4H), 3.16 (t, 2H), 3.60 (s, 2H), 3.81 (s, 3H), 4 52-4 58 (t, 2H), 6.68-6.69 (m, 1H), 6.72-6.86 (d, 1H), 6.90-7.02 (d, 1H), 7.28-7.37 (d, 2H) and 7.92-7.97 (d, 2H)

1-[2-(4-Morpholino)]ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetate (54e)

The compound (**54e**) was obtained by reacting indomethacin (**54**) (2.8 mmol, 1 0 g) and 2-(4-morpholino)ethanol (**50e**) (8.3 mmol, 1.0 ml) as depicted in Scheme-IV. The desired product (**54e**) was obtained as yellow oil, (1.2 g, 60.9 %).

Anal. :

TLC : R_f 0.81, ($CHCl_3$: MeOH, 9:1)
UV (λ_{max}, MeOH) : 227 nm (4.40)
IR (neat, cm^{-1}) : 1733, 1684, 1593,1456, 1356, 1117, 1036 and 754
PMR : 2.40 (s, 3H), 2.43-2.75 (m, 6H), 3.57-3.77 (m, 4H), 3.68 (s, 2H), 3.83 (s, 3H), 4.24-4.27 (t, 2H), 6.64- 6.68 (m, 1H), 6.83-6.86 (d, 1H), 6.95-6.96 (d, 1H), 7.46-7.49 (d, 2H) and 7.65-7.67 (d, 2H)

4.1.7. AMINOALCOHOL ESTER DERIVATIVES OF ASPIRIN (2-ACETOXY-BENZOIC ACID)

2-Dimethylaminoethyl 2-hydroxybenzoate hydrochloride (57a)

Aspirin, (2-acetoxybenzoic acid) (**56**) (5.55 mmol, 1.0 g) and thionyl chloride (13.5 mmol, 1.0 ml) were refluxed for 3 hours in dry benzene (5.0 ml) under anhydrous conditions. The excess of the thionyl chloride and solvent were removed off under vacuum and the acid chloride was obtained as a viscous liquid. The acid chloride was dissolved in dry, alcohol-free chloroform (20 ml) and anhydrous potassium carbonate (2.0 g) was added to it. 2-Dimethylaminoethanol (**50a**) (20.0 mmol, 2.0 ml) was dissolved in dry, alcohol-free chloroform (10 ml) and added to the well-stirred acid chloride solution dropwise at room temperature. The reaction mixture was further stirred for 15 minutes followed by refluxing on a water bath for 3 hours. The reaction mixture was filtered and diluted with chloroform (200 ml). The filtrate so obtained was washed with ice-cold water till free from the excess aminoalcohol. The organic layer was dried and the solvent removed to give an oily residue. The oily material was dissolved in dry isopropyl ether and dry hydrogen chloride gas was passed through the solution till saturation. The solid hydrochloride so obtained was crystallized from acetone-isopropyl ether to give pure salt, (**57a**), (0.7g, 42.7%) m.p °C 128-30.

Anal. :

TLC	: R_f 0.81, ($CHCl_3$: MeOH, 9:1)
UV (λ_{max}, MeOH)	: 306 nm (3.66)
IR (KBr, cm^{-1})	: 2638, 1678, 1608, 1489, 1296, 1218, 984, 768. 737 and 706
PMR	: 2.97 (s, 6H), 3.58 (t, 2H), 4.88 (t, 2H), 6.87-7.89 (m, 4H), 10.40 (s, 1H), 12.81 (b, 1H)

2-Dimethylaminoethyl 2-acetoxybenzoate hydrochloride (56a)

Aspirin, (**56**) (5.55 mmol, 1.0 g) and thionyl chloride (13.5 mmol, 1.0 ml) were refluxed for 3 hours in dry benzene (5.0 ml) under anhydrous conditions. The excess of the thionyl chloride and solvent were removed off under vacuum and the acid chloride was

obtained as a viscous liquid. The acid chloride was dissolved in dry, alcohol-free chloroform (20 ml) and anhydrous potassium carbonate (2.0 g) was added to it. 2-Dimethylaminoethanol (**50a**) (20.0 mmol, 2.0 ml) was dissolved in dry, alcohol-free chloroform (10 ml) and added to the well-stirred acid chloride solution dropwise at room temperature. The reaction mixture was further stirred for 15 minutes followed by refluxing on a water bath for 3 hours. The reaction mixture was filtered and diluted with chloroform (200 ml). The filtrate so obtained was washed with ice-cold water till free from the excess aminoalcohol. The organic layer was dried and the solvent removed to give an oily residue.

The oily residue so obtained was vacuum dried, dissolved in acetic anhydride (3 0 ml) and a few drops of concentrated sulphuric acid were added to it followed by heating on a water bath for one hour with occasional shaking. The reaction mixture was added into crushed ice (100 g), basified with sodium bicarbonate, extracted with chloroform (5x20 ml), dried and the solvent removed completely to yield a dark brown oil The oily material was dissolved in dry isopropyl ether and dry hydrogen chloride passed through the solution till saturation (Scheme-VI). The solid hydrochloride so obtained was crystallized from acetone-isopropyl ether to give pure salt (**56a**). (0 70 g. 44%). m p 128-30°C.

Anal. :

TLC : R_f 0.67, ($CHCl_3$: MeOH, 9:1)

UV (λ_{max}, MeOH) : 279 nm (2.95)

IR (KBr, cm^{-1}) : 1763, 1736, 1489, 1369, 1606, 1258, 1192, 1092, 756 and 702

PMR : 2.35 (s, 3H), 2.60 (s, 6H), 3.48 (t, 2H), 4.75 (t, 2H), 7.08-7.15 (d, 1H), 7.29-7.38 (t, 1H), 7.55-7.65 (t, 1H), 8.02-8 08 (d, 1H) and 12.60 (b, 1H)

Calculated for $C_{13}H_{18}ClNO_4$: C, 54.3; H, 6.3; N, 4.9. Found C, 54.7; H, 5 9, N, 4.6%

2-Diethylaminoethyl 2-acetoxybenzoate hydrochloride (56b)

Aspirin (**56**) (5.55 mmol, 1.0 g) was reacted with 2-diethylaminoethanol (**50b**) (15 mmol, 2.0 ml) as described for compound (**56a**), Scheme-VI to yield compound (**56b**) (0.65 g, 34 95 %), m.p. 101-103°C.

Anal. :

TLC : R_f 0.69, ($CHCl_3$: MeOH 9·1)

UV (λ_{max}, MeOH) : 279 nm (3.21)

IR (KBr, cm^{-1}) : 1765, 1724, 1608, 1254, 1080, 754 and 704

PMR : 1.43 (t, 6H), 2.36 (s, 3H,), 3 15-3.29 (m, 4H), 3 38 (t, 2H), 4.85 (t, 2H), 7.11-7.15 (d, 1H), 7.30- 7 38 (m, 1H), 7 57-7 66 (m, 1H) and 8 00-8 01 (d, 1H)

Calculated for $C_{15}H_{22}ClNO_4$ C, 57 0, H, 7 0, N, 4.4 Found C, 57 3, H, 7 2, N, 4 2 %

1-[2-(1-Piperidino)]ethyl 2-acetoxybenzoate (56c)

The compound (**56c**) was prepared by reacting aspirin (**56**) (5.55 mmol, 1.0 g), and 2-piperidinoethanol (**50c**) (15 mmol, 2.0 ml) as described in Scheme-VI The work up of the reaction mixture followed by crystallization yielded the compound (**56c**), (0.65 g, 42.18%) as oil.

Anal. :

TLC : R_f 0.69, ($CHCl_3$: MeOH, 9:1)

UV (λ_{max}, MeOH) : 279 nm (3.21)

IR (neat, cm^{-1}) : 1769, 1725, 1608, 1254, 1080, 754 and 704

PMR : 1.72-1.76 (m, 6H , 2.35 (s, 3H), 2.98-3.04 (m, 4H), 3.07 (t, 2H), 4.60 (t, 2H), 7.14-7.19 (d, 1H), 7.28-7.33 (m, 1H), 7.55-7.59 (m, 1H) and 7.98-8.00 (d, 1H)

1-[2-(1-Pyrrolidino)]ethyl 2-acetoxybenzoate (56d)

Aspirin (**56**) (5.55 mmol, 1.0 g) was reacted with 2-pyrrolidinoethanol (**50d**) (15 mmol, 2.0 ml) as described for compound (**56c**), followed by work up to afford the compound (**56d**), (0.67 g, 41.36%) as oil.

Anal. :

TLC	: R_f 0.52, ($CHCl_3$: MeOH, 9:1)
UV (λ_{max}, MeOH)	: 279 nm (3.05)
IR (neat, cm^{-1})	: 1769, 1725, 1608, 1253, 1082, 754 and 704
PMR	: 1.93-1.96 (m, 4H), 2.35 (s, 3H), 3.04-3 07 (m, 4H), 3.21-3.27 (t, 2H), 4.61 (t, 2H), 7.09-7 11 (d, 1H), 7.28-7 33 (m. 1H), 7.55-7.59 (m, 1H) and 8.00-8.02 (d. 1H.)

1-[2-(4-Morpholino)]ethyl 2-acetoxybenzoate hydrochloride (56e)

The reaction of aspirin (**56**) (5.55 mmol, 1.0 g) with 2-(4-morpholino)ethanol (**50e**) (16 mmol, 2.0 ml) followed by the work up of the reaction mixture as described for compound (**56a**) yielded the hydrochloride salt (**56e**), (0.55 g, 30.1 %), m.p. 158-61°C

Anal. :

TLC	: R_f 0.60, ($CHCl_3$: MeOH 9:1)
UV (λ_{max}, MeOH)	: 279 nm (3.22)
IR (KBr, cm^{-1})	: 1769, 1722, 1608, 1253, 1082, 754 and 704
PMR	: 2.36 (s, 3H), 3.51 (t, 2H), 3.95-4.02 (m, 4H), 4.29 (t, 4H). 4.90 (t, 2H), 7.12-7.14 (d, 1H), 7.33-7.36 (m, 1H), 7.49-7.53 (m, 1H), 7.94-7.96 (d, 1H) and 13.74 (b, 1H)

Calculated for $C_{15}H_{20}ClNO_5$: C, 54.6; H, 6.1; N, 4.3. Found C, 54.2; H, 6.4; N, 3.9 %

4.1.8. AMINOALCOHOL ESTER DERIVATIVES OF KETOROLAC

2-Dimethylaminoethyl 5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylate (58a)

Ketorolac (**58**) (6.20 mmol, 1.5g) was reacted with 2-dimethylaminoethanol (**50a**) (15 mmol, 1.5 ml) by Method F [Compounds (**54a-e**)] in Scheme -IV, to yield the compound (**58a**), (1.3 g, 68.4%).

Anal. :

TLC	: R_f 0.38, ($CHCl_3$: MeOH, 9.1)
UV (λ_{max}, MeOH)	: 315 nm (4.23)
IR (neat, cm^{-1})	: 1733, 1625, 1575, 1432, 1269, 1176, 1046, 722 and 698
PMR	: 1.2-1.3 (m, 2H), 2.30-2.34 (s, 6H), 2 62-2 67 (t. 2H). 2 75-2.95 (m, 3H), 4.42-4 62 (m, 2H), 6 10-6.12 (d. 1H). 6 79-6.81 (d, 1H), 7.41-7 56 (m, 3H) and 7.79-7 81 (d. 2H)

2-Diethylaminoethyl 5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylate (58b)

The compound (**58b**) was prepared by reacting ketorolac (**58**) (6 20 mmol, 1 5g) with 2-diethylaminoethanol (**50b**) (12 mmol, 1.5 ml) as depicted by Method F. Work up of the reaction mixture yielded the desired product (**58b**)as a yellow oil (1 2 g, 56 9%).

Anal. :

TLC	: R_f 0.42, ($CHCl_3$: MeOH, 9:1)
UV (λ_{max}, MeOH)	: 314 nm (4.18)
IR (neat, cm^{-1})	: 1733, 1621, 1430, 1268, 1180, 1045, 724, 698 and 670
PMR	: 1.02-1.06 (t, 6H), 1.22-1.26 (m, 2H), 2.58-2.63 (m, 4H), 2.76 (t, 2H), 2.92-2.94 (m, 2H), 3.00-3.02 (m, 1H), 4.35-4.62 (m, 2H), 6.10-6.12 (d, 1H), 6 81-6.82 (d, 1H), 7.42-7.47 (m, 3H) and 7.77-7.82 (d, 2H)

1-[2-(1-Piperidino)]ethyl 5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylate (58c)

The reaction between ketorolac (**58**) (6.20 mmol, 1.5g) and 2-piperidinoethanol (**50c**) (12 mmol, 1.5 ml) as described for the compound (**58a**) followed by the work up yielded the compound (**58c**) (1.3 g, 59.6%).

Anal :

TLC	: R_f 0.44, ($CHCl_3$: MeOH, 9:1)
UV (λ_{max}, MeOH)	: 314 nm (4.21)
IR (neat, cm^{-1})	: 1733, 1621, 1574, 1431, 1269, 1180, 1045, 891 and 722
PMR	. 1.25-1.30 (m, 2H), 1.58-1.81 (t, 2H), 1.83-1 89 (m, 4H), 2.75-2 93 (m, 3H), 2.97-3.06 (m, 6H), 4.3-4.6 (m, 2H), 6.10-6.12 (d, 1H), 6.79-6.81(d, 1H), 7 38-7 51 (m, 3H), 7 77-7 81 (d, 2H)

1-[2-(1-Pyrrolidino)]ethyl 5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylate (58d)

The desired compound (**58d**) was synthesized by reacting ketorolac (**58**) (6 20 mmol, 1.5g) and 2-pyrrolidinoethanol (**50d**) (13 mmol, 1.5 ml) as described for compound (**58a**). The desired compound (**58d**) was obtained as yellow oil (1.3 g, 61.9%).

Anal :

TLC	: R_f 0.43, ($CHCl_3$: MeOH, 9:1)
UV (λmax, MeOH)	: 314 nm (4.19)
IR (neat, cm^{-1})	: 1739, 1623, 1578, 1432, 1269, 1177, 1046, 892, 722 and 698
PMR	: 1.2-1.3 (m, 2H), 1.80-1.84 (t, 2H), 1.99-2 03 (t, 2H), 2.65-2.90 (m, 6H), 3.08-3.12 (m, 3H), 4.32-4 59 (m, 2H), 6.10-6.11 (d, 1H), 6.79-6.81(d, 1H), 7.40-7.52 (m, 3H), 7.78-7.81 (d, 2H)

1-[2-(4-Morpholino)]ethyl 5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylate (58e)

Ketorolac (**58**) (6.20 mmol, 1.5g) was reacted with 2-(4-morpholino)ethanol (**50e**) (12 mmol, 1.5 ml) as described compound (**58a**) followed by work up of the reaction mixture, to yield the compound (**58e**), (1.3 g, 68.4%).

Anal. :

TLC	: R_f 0.45, ($CHCl_3$: MeOH, 9:1)
UV (λ_{max}, MeOH)	: 314 nm (4.11)
IR (neat, cm^{-1})	: 1739, 1623, 1578, 1454, 1270, 1177, 1046, 892, 722 and 698
PMR ($CDCl_3$+DMSO-d_6)	: 1.25 (t, 2H), 2 62-2 66 (m, 6H), 2.80-2 91 (m, 3H), 3 68-3 80 (m, 4H), 4 50-4 57 (m, 2H), 6 10-6 12 (d, 1H), 6.80-6 82 (d, 1H), 7 44-7 49 (m, 3H) and 7.77-7.81 (d, 2H)

4.2. HYDROLYSES STUDIES

All the synthesized ester derivatives were evaluated for their stability in buffers (pH 2.0 and 7.4), which simulate the pH conditions existing in the stomach and intestine. The enzymatic susceptibility towards serum esterases of all the derivatives was evaluated *in vitro* in pooled human serum (80%). UV spectrophotometric methods were developed for the determination of the parent drugs exclusively released from the respective derivatives over a definite period of time. The absorbance measurements were made on a UV-visible spectrophotometer (UV-1601, Shimadzu Corporation, Japan). The solutions and the buffers used in the study were prepared in distilled water. The buffers used were prepared according to the procedure as given in I. P. 1996. All the chemicals used were of analytical reagent grade. Anhydrous sodium sulphate was used as drying agent wherever required for the drying of organic solutions.

Solutions and Buffers

1. Potassium Chloride (0.2 M): Potassium chloride (1.491 g) was dissolved in distilled water and diluted to 100 ml.
2. Hydrochloric acid (0.2 N): Concentrated hydrochloric acid (1.7 ml) was diluted to 100 ml in a standard volumetric flask with distilled water.
3. Potassium dihydrogen phosphate (0.2 M): Potassium dihydrogen phosphate (2.722 g) was dissolved in distilled water and diluted to 100 ml in a standard volumetric flask.
4. Sodium hydroxide (0.x N): Sodium hydroxide (0.4x g) was dissolved in distilled water and volume made to 100 ml with distilled water.
5. Hydrochloric acid Buffer pH 2.0: 50 Ml of potassium chloride solution (0.2 M) was placed in a 200 ml volumetric flask and 13 ml of hydrochloric acid (0.2 N) was added and the volume made with distilled water.
6. Phosphate buffer pH 7.4: 50 Ml of potassium dihydrogen phosphate solution (0.2 M) was placed in a 200 ml volumetric flask and 39.1 ml of sodium hydroxide solution (0.2 N) was added and the volume made up with distilled water.

The hydrolyses studies performed are discussed under the following headings:

4.2.1. Hydrolyses studies of derivatives of 4-biphenylacetic acid (45a-f)

4.2.2. Hydrolyses studies of derivatives of flurbiprofen (49a-f)

4.2.3. Hydrolyses studies of derivatives of diclofenac (51a-e)

4.2.4. Hydrolyses studies of derivatives of indomethacin (54a-e)

4.2.5. Hydrolyses studies of derivatives of aspirin (56a-e)

4.2.6. Hydrolyses studies of derivatives of ketorolac (58a-e)

4.2.1. HYDROLYSES STUDIES OF DERIVATIVES OF 4-BIPHENYLACETIC ACID (45a-f)

4.2.1.1. Procedure for the preparation of calibration curve for estimating biphenylacetic acid (45) in buffers

A stock solution (1000 ug/ml) of biphenylacetic acid (**45**) was prepared in sodium hydroxide solution (0.1N). From this stock solution appropriate dilutions were prepared in sodium hydroxide solution (0.1N) to give solutions of concentration 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 ug/ml. Aliquots (1.0 ml) were withdrawn individually from these solutions, transferred separately into separating funnels containing buffer (9.0 ml, pH 2.0) and successively extracted with chloroform (2x5 ml) In each case the combined chloroform extract was extracted with sodium hydroxide solution (0.1N, 2x5 ml) and the pooled aqueous extract was heated in a water bath for 30 minutes, cooled to room temperature and the absorbance measured at 256 nm (λ_{max}). Sodium hydroxide solution (0.1N) after giving similar treatment without the drug solution was used as blank. Three such determinations were carried out and the regression analysis was performed.

4.2.1.2. Hydrolyses studies of (45a-f) in buffers (pH 2.0 and 7.4)

General procedure:

The general procedure carried out for these studies has been described by taking the example of one derivative (**45a**). A solution of concentration (1.0 mg/ml) of the appropriate derivative (**45a**) in buffer (pH 2.0 or pH 7.4 individually) was maintained at 37 $\pm$ 1°C in a water bath. Aliquots (1.0 ml) were withdrawn from this solution at regular time intervals of 0, 0.5 1.0, 2.0, 4.0, 6.0 and 8.0 hours and transferred separately into separating funnels containing buffer (9.0 ml, pH 2.0). In each case the solution was extracted with chloroform (2x5 ml). The combined organic extract was further extracted

with sodium hydroxide solution (0.1N, 2x5 ml). The combined aqueous extract was heated in a water bath for 30 minutes, cooled to room temperature and the absorbance measured at 256 nm (λ_{max}) against a blank after giving similar treatment but without the derivative. Samples were analysed in triplicate. The equation ($t_{1/2} = 0.693/k$) was used to calculate the half-lives. The above procedure was used to perform the studies for rest of the derivatives (**45b-f**) in both the buffer solutions.

4.2.1.3 Procedure for the preparation of calibration curve for estimating 4-biphenylacetic acid (45) in pooled human serum (80%)

A stock solution (1000 ug/ml) of biphenylacetic acid (**45**) was prepared in sodium hydroxide solution (0.1N). From this stock solution appropriate dilutions were prepared in sodium hydroxide solution (0.1N) to give solutions of concentration 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 ug/ml. Aliquots (1.0 ml) were withdrawn individually from these solutions and transferred into separating funnels containing trichloroacetic acid (1.0 ml, 10%) to precipitate the proteins. The volume was made upto 10.0 ml with buffer (pH 2.0) and successively extracted with isopropyl ether (2x5 ml) The combined organic extract was extracted with sodium hydroxide solution (0.1N, 2x5 ml) and the pooled aqueous extract was heated on a water bath for 30 minutes, cooled to room temperature and the absorbance measured at 256 nm (λ_{max}). Sodium hydroxide solution (0.1 N) after giving similar treatment without the drug solution was used as blank. Three determinations were carried out and the regression analysis was performed.

4.2.1.4 Hydrolyses studies of derivatives (45a-f) in pooled human serum (80%)

General procedure:

The procedure is exemplified for derivative (**45a**). Pooled human serum (4.0 ml) was taken in a stoppered conical flask and maintained at 37 $\pm$1°C in a water bath. A solution (1.0 ml, 5 mg/ml in pH 7.4) of derivative (**45a**) was added to the above pooled human serum. At appropriate time intervals (0, 15, 30, 60 and 120 minutes) aliquots (0.5 ml) were withdrawn and transferred separately to separating funnels containing trichloroacetic acid (1.0 ml, 10%). Further 8.5 ml of buffer (pH 2.0) was added and this solution was extracted with isopropyl ether (2x 5 ml) for all the aliquots. The combined ethereal extract was extracted with sodium hydroxide solution (0.1 N, 2x 5 ml) and the

pooled aqueous extract was heated in a water bath for 30 minutes, cooled to room temperature and the absorbance measured at 256 nm (λ_{max}). A blank was prepared by giving similar treatment to sodium hydroxide solution (0.1N).

4.2.2. HYDROLYSES STUDIES OF DERIVATIVES OF FLURBIPROFEN (49a-f)

4.2.2.1 Procedure for the preparation of calibration curve for the estimation of flurbiprofen (49) in buffers and pooled human serum (80%)

A stock solution (1000 ug/ml) of flurbiprofen (**49**) was prepared in sodium hydroxide solution (0.1N). From this stock solution appropriate dilutions were prepared in sodium hydroxide solution (0.1N) to give solutions of concentration 20, 40, 60, 80, 100, 120, 140, 180, 200, 220 and 240 ug/ml. Aliquots (1.0 ml) were withdrawn individually from these solutions and transferred into separating funnels containing buffer (9.0 ml, pH 2.0) and successively extracted with n-hexane (2x5 ml). The combined n-hexane extract was dried and absorbance measured at 237 nm (λ_{max}) for all the dilutions so treated. Sodium hydroxide (0.1 N) treated similarly but without the drug solution to afford n-hexane was used as blank. The study was repeated three times and regression analysis was performed for the closest sets of observations.

4.2.2.2 Hydrolyses studies of derivatives (49a-f) in buffers (pH 2.0 and 7.4)

General procedure:

The procedure carried out for the study of derivative (**49a**) in buffers has been described and was followed for rest of the compounds (**49b-f**). A solution of concentration (1.0 mg/ml) of the derivative (**49a**) in buffer (pH 2.0 or pH 7.4) was maintained at 37 ± 1°C in a water bath. Aliquots (1.0 ml) were withdrawn from this solution at regular time intervals of 0, 0.5 1.0, 2.0, 4.0, 6.0 and 8.0 hours and transferred separately into separating funnels containing buffer (9.0 ml, pH 2.0) and successively extracted with n-hexane (2x5 ml). The combined organic layer was dried, and absorbance was measured at 237 nm (λ_{max}) on a UV spectrophotometer against a blank after giving similar treatment but without the drug solution. Samples were analysed in triplicate. The equation ($t_{1/2} = 0.693/k$) was used to calculate the half-lives.

4.2.2.3 Hydrolysis studies of derivatives (49a-f) in pooled human serum (80%)

General procedure:

The procedure followed for the derivative (**49a**) as described below represents the general procedure adopted for all the compounds (**49b-f**). Pooled human serum (4.0 ml) was taken in a stoppered conical flask and maintained at 37 ±1°C in a water bath. A solution (1.0 ml, 5 mg/ml in pH 7.4) of the derivative (**49a**) was added to the above pooled human serum. At appropriate time intervals (0, 15, 30, 60 and 120 minutes) aliquots (0.5 ml) were withdrawn and transferred to separating funnels containing trichloroacetic acid solution (1.0 ml, 10%). Further 8.5 ml of buffer (pH 2.0) was added in each case and this solution was extracted with n-hexane (2x5 ml). The combined organic layer was dried, and its absorbance measured at 237 nm (λ_{max}) on a UV spectrophotometer against a blank.

4.2.3. HYDROLYSES STUDIES OF DERIVATIVES OF DICLOFENAC (51a-e)

4.2.3.1 Procedure for the preparation of calibration curve for the estimation of diclofenac (51) in buffers

A stock solution (1000 ug/ml) of diclofenac (**51**) was prepared in sodium hydroxide (0 1 ml). From this stock solution, appropriate dilutions were made to give solutions of concentration 50, 100, 150, 200, 250, 300, 350 400 and 450 ug/ml. Aliquots (1.0 ml) were withdrawn individually from these solutions, transferred separately into separating funnels containing buffer (9.0 ml, pH 2.0) and successively extracted with chloroform (2x5 ml). In each case the combined chloroform extract was extracted with sodium hydroxide solution (0.1N, 2x5 ml) and the pooled aqueous extract was heated on a water bath for 30 minutes, cooled to room temperature and the absorbance measured for the combined aqueous layer at 274 nm. The blank used was obtained by treating sodium-hydroxide solution (0.1 N) similarly but without the drug solution. Three determinations were carried out and the regression analysis was performed.

4.2.3.2 Hydrolyses studies of derivaitves (51a-e) in buffers (pH 2.0 and 7.4)

General procedure:

The general procedure carried out for these studies has been described by taking the example of the derivative (**51a**). A solution of concentration (1.0 mg/ml) of the derivative (**51a**) in buffer (pH 2.0 or pH 7.4 individually) was maintained at 37 ± 1°C in a water bath. Aliquots (1.0 ml) were withdrawn from this solution at regular time intervals of 0, 0.5 1.0, 2.0, 4.0, 6.0 and 8.0 hours and transferred separately into separating funnels containing buffer (9.0 ml, pH 2.0). In each case the solution was extracted with chloroform (2x5 ml). The combined organic extract was further extracted with sodium hydroxide solution (0.1N, 2x5 ml). The combined aqueous extract was heated in a water bath for 30 minutes cooled to room temperature and the absorbance measured at 274 nm using blank treated similarly but without the drug solution. Samples were analyzed in duplicate or triplicate and $t_{1/2}$ was calculated using equation ($t_{1/2}$ = 0.693/k). The above procedure was followed for carrying out the studies for the other derivatives (**51b-e**) in both the buffers.

4.2.3.3 Procedure for the preparation of calibration curve for estimating diclofenac (51) in pooled human serum (80%)

A stock solution (1000 ug/ml) of diclofenac (**51**) was prepared in sodium hydroxide (0.1 ml). From this stock solution appropriate dilutions were made in sodium hydroxide solution (0.1 N) to give solutions of concentration 50, 100, 150, 200, 250, 300, 350 400 and 450 ug/ml. Aliquots (1.0 ml) were withdrawn individually from these solutions and transferred into separating funnels containing trichloroacetic acid (1.0 ml, 10%) to precipitate out the proteins. The volume was made to 10.0 ml with buffer (pH 2.0) and successively extracted with isopropyl ether (2x5 ml). The combined organic extract was extracted with sodium hydroxide solution (0.1N, 2x5 ml) and the pooled aqueous extract in each case was heated on a water bath for 30 minutes, cooled to room temperature and the absorbance measured at 274 nm (λ_{max}). Sodium hydroxide solution (0.1 N) after giving similar treatment without the drug solution was used as blank. Three determinations were carried out and the closest sets were regressed to give a linear equation.

4.2.3.3 Hydrolyses studies of derivatives (51a-e) in pooled human serum (80%)

General procedure:

The procedure is exemplified for derivative (**51a**). Pooled human serum (4.0 ml) was taken in a stoppered conical flask and maintained at 37 $\pm$1°C in a water bath. A solution (1.0 ml, 5 mg/ml in pH 7.4) of derivative (**51a**) was added to the above pooled human serum. At appropriate time intervals (0, 15, 30, 60 and 120 minutes) aliquots (0.5 ml) were withdrawn and transferred separately to separating funnels containing trichloroacetic acid solution (1.0 ml, 10%). Further 8.5 ml of buffer (pH 2.0) was added and this solution was extracted with isopropyl ether (2x 5 ml) for all the aliquots. The combined ethereal extract was extracted with sodium hydroxide solution (0.1 N, 2x 5 ml) and the pooled aqueous extract was heated in a water bath for 30 minutes, cooled to room temperature and the absorbance measured at 274 nm (λ_{max}). A blank was prepared by giving similar treatment to sodium hydroxide solution (0.1N) but without the derivative solution.

4.2.4 HYDROLYSES STUDIES OF DERIVATIVES OF INDOMETHACIN (54a-e)

4.2.4.1 Procedure for the preparation of calibration curve for the estimation of indomethacin (54) in buffers and pooled human serum (80%)

A stock solution of indomethacin (**54**) (1000 ug/ml) was prepared in methanol. From this stock solution dilutions were made in pH 7.4 buffer to give solutions of concentration 20. 40, 60, 80, 100, 120, 140 and 160 ug/ml. Aliquots (1.0 ml) were withdrawn individually from these solutions, transferred into separating funnels containing buffer (9.0 ml, pH 2.0) and successively extracted with solvent ether (3x5ml). Solvent from the pooled organic extract was removed completely and methanol (10 ml) was added to the residue left and absorbance measured at 229 nm (λ_{max}) against a blank after giving similar treatment but without the drug solution. Three determinations were carried out and the regression analysis was performed for the closest sets of absorbance data recorded.

4.2.4.2 Hydrolyses studies of derivatives (54a-e) in buffers (pH 2.0 and 7.4)

General procedure:

The procedure carried out for the study of derivative (**54a**) in buffers has been described. Reaction was initiated by maintaining a solution of concentration (1.0 mg/ml) of the derivative (**54a**) in buffer (pH 2.0 or pH 7.4) at 37 ± 1°C in a water bath. Aliquots (1 0 ml) were withdrawn individually from these solutions at regular time intervals of 0. 0 5 1.0, 2.0, 4.0 and 6.0 hours and transferred separately to separating funnels containing buffer (9.0 ml, pH 2.0). This acidified solution was extracted with solvent ether (3x5ml). Solvent from the pooled organic extract was removed completely and methanol (10 ml) was added to the residue left and absorbance measured at 229 nm (λ_{max}) against a blank after giving similar treatment but without the compound solution. Samples were analyzed in duplicate or triplicate and $t_{1/2}$ was calculated using equation ($t_{1/2}$ = 0 693/k) The study for the other derivatives (**54b-e**) in both the buffers was carried out using the above described procedure.

4.2.4.3 Hydrolyses studies of derivatives (54a-e) in human serum (80%)

General procedure:

The procedure followed for the derivative (**54a**) as described below represents the general procedure adopted for all the other compounds (**54b-e**). Pooled human serum (4.0 ml) was taken in a stoppered conical flask and maintained at 37 ±1°C in a water bath. A solution (1.0 ml, 5 mg/ml in pH 7.4) of the derivative (**54a**) was added to the above pooled human serum. At appropriate time intervals (0, 15, 30, 60 and 120 minutes) aliquots (0.5 ml) were withdrawn and transferred separately into separating funnels containing trichloroacetic acid solution (1.0 ml, 10%). Further 8.5 ml of buffer (pH 2.0) was added in each case and this solution was extracted with solvent ether (3x5ml). Solvent from the pooled organic extract was removed completely, methanol (10 ml) was added to the residue left and absorbance measured at 229 nm (λ_{max}) Blank was prepared by similar treatment without the derivative solution. The experiment was repeated in duplicate.

4.2.5 HYDROLYSES STUDIES OF DERIVATIVES OF ASPIRIN (56a-e)

4.2.5.1 Procedure for the preparation of calibration curve for the estimation of aspirin (56) in buffers and pooled human serum (80%)

A stock solution of aspirin (**56**), (10,000 ug/ml) was prepared in methanol Appropriate dilutions from this stock solution were made with methanol to give solutions of concentration 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000 ug/ml Aliquots (1.0 ml) were withdrawn individually from these solutions and transferred separately into separating funnels containing buffer (9.0 ml, pH 2.0). The acidified solution was extracted into isopropyl ether (2x5 ml). The pooled organic extract, collected in stoppered test tubes was dried and the absorbance measured at 276 nm (λ_{max}). Blank was obtained by treating the isopropyl ether similarly but without the drug solution. Three determinations were carried out and for the closest sets regression analysis was performed.

4.2.5.2 Procedure for the preparation of calibration curve for the estimation of salicylic acid (57) in buffers and pooled human serum (80%)

A stock solution of salicylic acid (**57**), (10,000 ug/ml) was prepared in sodium hydroxide solution (0.1 N) From this stock solution, dilutions were made in sodium hydroxide solution (0.1 N) to give solutions of concentration 50, 100, 150, 200. 250, 300. 350 and 400 ug/ml. Aliquots (1.0 ml) were withdrawn individually from these solutions and transferred separately into separating funnels containing buffer (9.0 ml, pH 2.0). The acidified solution was extracted into isopropyl ether (2x5 ml). The pooled organic extract, collected in stoppered test tubes was dried and the absorbance measured at 306 nm (λ_{max}). The blank used was prepared by treating the isopropyl ether similarly but without the drug solution. Three determinations were carried out and the regression analysis was performed for the closest sets.

4.2.5.4 Hydrolyses studies of derivatives (56a-e) in buffers (pH 2.0 and 7.4)

General procedure:

The procedure carried out for the study of derivative (**56a**) in buffers has been described Reaction was initiated by maintaining a solution of concentration (1.0 mg/ml) of the derivative (**56a**) in buffer (pH 2.0 or pH 7.4) at 37 ± 1°C in a water bath. Aliquots (1 0 ml) were withdrawn from this solution at regular time intervals of 0, 0 5 1.0, 2.0, 4 0, 6.0 and 8.0 hours and transferred separately to separating funnels containing buffer (9.0 ml. pH 2.0). This acidified solution was extracted into isopropyl ether (2x 5 ml) for all the aliquots. The pooled organic extract, collected in stoppered test tubes was dried and absorbance measured at 276 and 306 nm. Blank was obtained by a similar treatment of buffer pH 2.0 but without the derivative solution. Study was performed in duplicate The above described procedure was followed for studying the derivatives (**56b-e**) in both the buffers.

4.2.5.5 Hydrolyses studies of derivatives (56a-e) in human serum (80%)

General procedure:

The procedure is exemplified for derivative (**56a**). Pooled human serum (4 0 ml) was taken in a stoppered conical flask and maintained at 37 ±1°C in a water bath. A solution (1.0 ml, 5 mg/ml in pH 7.4) of derivative (**56a**) was added to the above pooled human serum. At appropriate time intervals (0, 15, 30, 60 and 120 minutes) aliquots (0.5 ml) were withdrawn and transferred to separating funnels containing trichloroacetic acid (1.0 ml, 10%). Further 8.5 ml of buffer (pH 2.0) was added and this solution was extracted with isopropyl ether (2x 5 ml) for all the aliquots. The pooled organic extract collected in stoppered test tubes was dried and the absorbance measured at 276 and 306 nm. Blank was obtained by similar treatment of buffer pH 2.0 but without the derivative solution Study was performed in duplicate. The above described procedure was followed for studying the compounds (**56a-e**).

4.2.6 HYDROLYSIS OF DERIVATIVES OF KETOROLAC (58a-e)

4.2.6.1 Preparation of calibration curve for estimating ketorolac (58) in buffers and pooled human serum (80%)

A stock solution (1000 ug/ml) of ketorolac (**58**) was prepared in sodium hydroxide solution (0.1 N) and further dilutions were made in sodium hydroxide solution (0 1 N) to give concentrations of 20, 40, 60, 80, 100, 120, 140 and 160 ug/ml. Aliquots (1.0 ml) were withdrawn individually from these solutions, transferred separately into separating funnels containing buffer (9.0 ml, pH 2.0) and successively extracted with isopropyl ether (2x5 ml). The combined organic extract was collected in stoppered test tubes. dried and the absorbance measured at 307 nm. Sodium hydroxide solution (0.1 N) treated similarly but without the drug solution afforded the blank. Three determinations were carried out and the regression analysis was performed for the closest sets of observations

4.2.6.2 Hydrolyses studies of derivatives (58a-e) in buffers (pH 2.0 and 7.4)

General procedure:

The procedure carried out for the study of derivative (**58a**) in buffers has been described. Reaction was initiated by maintaining a solution of concentration (1 0 mg/ml) of the derivative (**58a**) in buffer (pH 2.0 or pH 7.4) at 37 $\pm$ 1°C in a water bath Aliquots (1.0 ml) were withdrawn from this solution at regular time intervals of 0, 0.5 1.0, 2.0, 4.0, 6 0 and 8.0 hours, and transferred separately to separating funnels containing buffer (9.0 ml. pH 2 0). This acidified solution was extracted into isopropyl ether (2x 5 ml) for all the aliquots. The pooled organic extract, collected in stoppered test tubes was dried and absorbance measured at 307 nm. Blank was obtained by similar treatment of buffer pH 2.0 but without the derivative solution. Study was performed in duplicate and half-life was calculated using the equation ($t_{1/2}$ = 0.693/k). The above described procedure was followed for performing the studies for the derivatives (**58b-e**) in both the buffers.

4.2.6.3 Hydrolyses studies of derivatives (58a-e) in human serum (80%)

General procedure:

The procedure is exemplified for derivative (**58a**). Pooled human serum (4.0 ml) was taken in a stoppered conical flask and maintained at 37 $\pm$1°C in a water bath. A solution (1.0 ml, 5 mg/ml in pH 7.4) of derivative (**58a**) was added to the above pooled human serum. At appropriate time intervals (0, 15, 30, 60 and 120 minutes) aliquots (0.5 ml) were withdrawn and transferred to separating funnels containing trichloroacetic acid solution (1.0 ml, 10%). Further 8.5 ml of buffer (pH 2.0) was added and this solution was extracted with isopropyl ether (2x 5 ml) for all the aliquots. The pooled organic extract, collected in stoppered test tubes was dried and the absorbance measured at 307 nm Blank was obtained by similar treatment of buffer pH 2.0 but without the derivative solution. Study was performed in duplicate. The procedure described above was followed for the compounds (**58b-e**).

4.3. BIOLOGICAL EVALUATION

The synthesized ester derivatives were evaluated for different types of biological activities. All the drugs were procured from Sigma chemicals. Chemicals were procured from local suppliers like S.D. Fine chemicals, Loba chemicals Ltd. and Suvidhinath chemicals, and are of AR grade. Distilled water was used for making the solutions wherever required. The methods followed for evaluation of each of these activities have been described in this chapter under the following headings:

4.3.1. Anticholinergic Activity

4.3.2. Antiinflammatory Activity

4.3.3. Analgesic Activity

4.3.4. Ulcerogenic Potencial

4.3.1. ANTICHOLINERGIC ACTIVITY

Materials:

Acetylcholine (ACh) solution: Acetylcholine hydrochloride (124.3 mg) equivalent to 100.0 mg of Ach was dissolved in distilled water in a 100 ml volumetric flask Dilutions were made to give solutions of concentration 100, 10, 1.0 and 0.1 ug/ml. All the solutions were stored in cold to prevent hydrolysis.

Atropine sulphate solution: Solution of atropine sulphate (1000 ug/ml) was prepared in distilled water. Appropriate dilutions were made to give solutions of concentration 100, 10, 1.0 and 0.1 ug/ml.

Physiological salt solution (Tyrode solution): Tyrode solution was prepared by dissolving various constituents in water as per the given procedure. Each constituent was dissolved individually in distilled water and mixed except for calcium chloride solution. which was added gradually with thorough mixing at the end of preparation.

Stock solutions of the Aminoalcohol ester derivatives: A stock solution (1000 ug/ml) of the required derivative was prepared in buffer solution (pH 2.0) and stored in cold conditions. Desired dilutions were made afresh in Tyrode solution whenever required.

Test solutions of the aminoalcohol ester derivatives: 5.0 Ml of the stock solution of

the aminoalcohol ester derivatives was diluted to make upto 1 litre with Tyrode solution to represent a final concentration of 5 ug/ml of the derivatives, just before use. Similarly, appropriate dilutions were made from the stock solution to give the Tyrode solution of desired higher or lower concentrations.

Experimental Conditions: The following experimental conditions[113] were used for finding out the anticholinergic activity of the synthesized aminoalcohol ester derivatives.

Bath capacity	: 30 ml
Temperature	: 37±1°C
Physiological salt solution	: Tyrode solution (with constant aeration)
Tissue preparation	: Isolated Rat ileum
Tension applied on the tissue	: 1.0 g

Procedure: The anticholinergic activity was determined[112] on isolated rat ileum Rats weighing 150-200g were housed single, fasted overnight with free access to water. Abdomen was dissected and the lower part of the intestine was taken out and immediately placed in Tyrode solution at 37±1°C with aeration. A piece of the tissue (1.0-1.5 cm) was mounted under tension (1.0 g) and was stabilized with washings of fresh Tyrode solution at intervals of every 10 minutes. The base line was recorded and if found straight, lowest dose (0.1 ug/ml) of Ach was injected into the bath and the response was recorded for 30 seconds. The tissue was relaxed to the base line by giving washings with fresh Tyrode solution and the dose response curve (DRC) was recorded for ACh till maximum response was obtained. The tissue was then allowed to be in contact with the Tyrode solution containing the derivative or atropine sulphate for half an hour and the DRC was repeated again for Ach till maximum response was obtained. The height was measured for each response obtained in absence and in presence of the antagonist (derivative or atropine sulphate). The percent response was calculated for both the DRCs and plotted against log molar concentration of acetylcholine concentration on the same graph paper to find the EC_{50} in absence and presence of the antagonist. pA_2 value was calculated by the following formula:

$$pA_2 = \log[M] + \log(x-1)$$

M = molar conc. of the antagonist and

x = EC_{50} in presence of antagonist / EC_{50} in absence of antagonist

4.3.2. ANTIINFLAMMATORY ACTIVITY

Materials

Animals : Sprague-Dawley rats of either sex (150-200 g).

Phlogistic agent (irritant) : Carrageenan (Lambda, Type IV) was procured from Sigma Aldrich and (1% w/v suspension in normal saline) was prepared 24 hr prior to its use.

Test solutions : Parent drugs as suspension (in 1% carboxymethylcellulose), Derivatives (solution in distilled water for the hydrochloride salts and as solution in 5% PEG 400 for the oily derivatives).

Instrument : Plethysmometre (Ugo –Basil) was used for the paw volume displacement measurements.

Procedure: Anti-inflammatory activity of the synthesized aminoalcohol ester derivatives was determined by the acute model of rat hind paw edema test as described by Winter *et al* [113]. Sprague-Dawley rats of either sex (n = 6) were used. The animals were fasted (24h) with water *ad libitum* prior to drug administration. Each animal was injected s.c. with the carrageenan suspension (0.1 ml) into the subplantar region of right hind paw, after one hour of compound/parent drug administration. The paw volume was measured immediately after injection and after 3 hours using a plethysmometer. The control group received the vehicle only. The derivatives were administered at a dose (mg/kg) equimolar to their respective parent drugs. The percentage inhibition of swelling was calculated in comparison to the control group by the formula,

$$\% \text{ Inhibition of paw edema} = (1 - Ed_{drug} / Ed_{control}) \times 100$$

Ed_{drug} = the edema volume in drug treated group

$Ed_{control}$ = the edema volume in control group rats.

4.3.3. ANALGESIC ACTIVITY

Materials:

Animals : Swiss albino mice (18-25 g).

Irritant :Acetic acid (0.6 % v/v, in normal saline) was used for inducing pain.

Test solutions : Parent drugs were prepared as suspension (1% w/v CMC)
Solid derivatives were prepared as solutions in distilled water and the oily derivatives as solutions in 5% PEG 400.

Procedure: Analgesic activity of derivatives/parent drugs was assessed by the acetic acid-induced writhing assay in the mouse as described by Koster *et al* [114]. The animals were divided by randomization in groups (each of six mice) and the derivatives were administered at dose (mg/kg) equimolar to their respective parent drugs. After one hour of oral administration the writhing syndrome was elicited by the intraperitoneal injection of acetic acid (10 ml/kg body weight) and the number of writhes for each mouse was counted after 5 minutes of injection for a period of 20 minutes The average number of writhes in each group of parent drug/derivative treated mice was compared with that of the control and degree of analgesia was expressed as percent inhibition calculated according to the formula:

% Inhibition of writhing = (1-T/S) x 100

S = number of writhes in control group of mice

T = number of writhes in drug treated group of mice.

4.3.4. ULCEROGENIC POTENCIAL

Materials

Animals : Sprague-Dawley rats of either sex (150-200 g).

Fixing solution : Formalin (5%) was prepared in distilled water.

Test solutions : Parent drugs were prepared as suspension (in 1% carboxymethyl cellulose), Solid derivatives (hydrochloride salts) were prepared as solutions in distilled water and the oily derivatives as solutions in 5% PEG 400.

Procedure: Sprague-Dawley rats (n = 5 or 6) of either sex were used[115] for the experiment. The rats were fasted for 36 hours with water *ad libitum* prior to administration of the derivative or the parent drug solutions. The animals were further kept on fasting for 4 hours after dosing. The derivatives were administered orally at a dose (mg/kg) equimolar to their respective parent drugs. The control group received the

plain vehicle The animals were sacrificed by cervical dislocation and their stomach was dissected out, cut along the greater curvature, washed with normal saline and kept in formalin solution (5%) for 15 minutes and gastric mucosa was observed for the lesions using a 2x2 binocular magnifier and the ulcer index was determined using the following formula as given below :

Ulcer index = 10 (Au/Am)

$Au = A_l + Ac + Ap$

A_l = area of linear lesions (l*b)

Ac = area of circular lesions (Πr^2)

Ap = total no. of piteaches/5

Am = total mucosal area ($\Pi D^2/8$)

(D = diameter of stomach)

4.4. BIODISTRIBUTION AND GAMMA IMAGING STUDIES

4.4.1 QUATERNIZATION OF DERIVATIVES (45f, 49f and 51d) TO AFFORD 45g, 49g and 51g.

Compound (**45f**) (0.001 mol, 0.2g) was dissolved in ammonia (5%, 10 ml) and extracted with chloroform (4x5 ml). The pooled organic extract was dried, removed completely to give an oily residue. Quaternization of this oily residue was carried out using methyl iodide as the methylating agent. Methyl iodide (0.5 ml) was added to a solution of the oily residue in methanol (3 ml) and refluxed for half an hour. The solvent on complete removal followed by drying under vacuum yielded the solid crystalline compound (**45g**) The procedure followed above was used for the quaternization of the derivatives (**49f** and **51d**) to yield compounds (**49g** and **51g**). The solid compounds (**45g**, **49g** and **51g**) so obtained were used as such for further studies.

4.4.2 RADIOLABELLING OF THE DERIVATIVES (45f, 45g, 49 f, 49g, 51d, and 51g)

Materials

Silica gel coated fiber sheets (Gelman Sciences. Inc., Ann Arbor, MI) were used for performing the instant TLC (ITLC). A well type gamma ray spectrophotometer (Type GRS23C, Electronics corporation of India ltd., Mumbai) was used for the gamma counting. The solutions were prepared in distilled water and all the chemicals and solvents used were of analytical grade.

4.4.2.1. Radiolabeling

The radiolabeling of the compounds (**45f, 45g, 49f, 49g, 51d** and **51g**) with reduced ^{99m}Tc were carried out as per the direct labeling method[124] ^{99m}Tc-$NaTcO_4$ (1.0 ml, 2 mCi / ml) was mixed with stannous chloride solution (0.1 ml, 1mg / ml in acetic acid (10%)) and mixed well. The pH was adjusted to 7.0 using sodium bicarbonate solution

(0.5 M). To this mixture, solution of the compound (**45f**) (1.0 ml, 2mg/ml) was added and the reaction mixture incubated for 15 minutes (37±1°C). The above experiment was repeated by varying the conditions one at a time i.e. using different moles of stannous chloride (0.05 ml to 0.2 ml), changing the pH of the medium between 6 and 8 at various values and varying the incubation time period (15, 30 and 60 minutes) of the reaction mixture. The procedure was repeated exactly in same manner for rest of the derivatives (**45g, 49f, 49g, 51d** and **51g**)

4.4.2.2. Evaluation of the labeling efficiency

Ascending instant thin layer chromatography (ITLC) was performed using acetone (100%) or saline (0.9%) as the mobile phase. 2-3 µl of the radiolabeled complex was applied at a point 1.0 cm away from one end of an ITLC-SG strip The strip was developed in acetone or saline and the solvent front was allowed to rise upto 8 cm from the origin. The strip was cut one cm below the solvent front and the radioactivity in each segment was determined in the gamma ray counter. The free pertechnetate which moved with the solvent ($R_f = 0.9$) and the reduced / hydrolysed (R/H) technetium along with the labeled complex remaining at the point of application were determined. ITLC was also run in pyridine-acetic acid- water (3: 5: 1.5 v/v) systems to determine the amount of reduced / hydrolysed (R/H) ^{99m}Tc (radio- colloids). The R/H ^{99m}Tc remained at the point of application while both the free pertechnetate and the labeled complex moved away with the solvent front in this solvent system (PAW). The difference between the activity for the spots which moved along with the solvent front using either acetone or saline from that obtained in the PAW system (at the point of application) gave the net amount of ^{99m}Tc-labeled complex. This procedure was repeated for the radiolabeling of all the derivatives (**45g, 49f, 49g** and **51d, 51g**) and for all the experiments conducted for optimization of labeling parameters like pH, incubation time and quantity of the reducing agent used.

4.4.3. *In vitro* stability study of ^{99m}Tc- labeled complex in human serum and saline

The *in vitro* stability study[124] of radiolabeled complex was determined in sodium chloride (0.9%) and in human serum separately by ascending thin layer chromatography. The ^{99m}Tc-labeled compound solution (**45f**) (0.1 ml) prepared in saline (0.9%) as described above was mixed separately with human serum (1.9 ml) and normal saline (1.9

ml) and incubated (37± 1°C). ITLC was performed at different time intervals (0, 0.25, 0.5, 1.0, 2.0, 4.0 and 24 hours) as described above, in acetone to assess the stability of the complex. Any decrease in percentage of ^{99m}Tc-labeled complex was considered as its degree of degradation.

4.4.4. BIODISTRIBUTION OF LABELED COMPLEX (45f)

Swiss albino mice (a group of 3 animals for each time interval) were used for the biodistribution studies of the ^{99m}Tc-labeled compounds. 24" Gauze needle was used for the i.v. injection. The ^{99m}Tc-labeled complex (0.1 ml), prepared as described above, was administered through the tail vein of each mouse. At different time intervals (0, 0.5, 1.0, 2.0, 4.0 and 24 hours) the animals (group of three mice for each time interval) were anaesthesized and blood was obtained by cardiac puncture. Blood was weighed and radioactivity measured in the gamma counter. The animals were sacrificed and tissues (heart, lung, liver, spleen, kidney, stomach and intestine) were dissected, washed with normal saline, made free from adhering tissues, weighed and their radioactivity measured. To correct for physical decay and to calculate radiopharmaceutical uptake in each organ as a fraction of the injected dose (% activity), aliquots (2.0 ul) of the complex solution, containing 2 % of the injected dose, were counted simultaneously at each time point. The percent activity for the whole organs and whole blood was determined for each time interval. The above described procedure was followed for studying the biodistribution of the complexes of compounds (**45g, 49f** and **49g**).

4.4.5 GAMMA IMAGING STUDIES IN ACUTE INFLAMMATION MODEL

Sprague-Dawley rats (2 in a group) were used for the studies. Carrageenan (1% w/v in normal saline) was used for inducing inflammation. Imaging was performed using a Single Photon Emission Computerized Tomography (SPECT, LC 75-005, Diacam, Siemens, USA) gamma camera.

Inflammation was induced in each rat by subcutaneous injection of carrageenan (0.1 ml) into the subplantar region of the right paw. Three hours later when maximum

inflammation was achieved, ^{99m}Tc-labeled complex (0.2 ml) was administered through the tail vein. The animals were anaesthetized (ketamine/diazepam i.m.), fixed on a board using the adhesive tapes and gamma imaging photographs were taken 1 hour after injecting the complex (i.v.).