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Synthesis, Hydrolysis Kinetics and Pharmacodynamic Profile of Novel Prodrugs of Flurbiprofen

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Amide conjugates of flurbiprofen with various amino acid methyl esters were synthesized by Schotten-Baumann technique using prodrug concept. Their physico-chemical characterization was carried out by analytical and spectral methods. They were subjected to *in vitro* hydrolysis in hydrochloric acid buffer (pH 1.2), phosphate buffer (pH 7.4) and 80% human plasma (pH 7.4). The amides were screened for analgesic, antiinflammatory and ulcerogenic activities. They showed comparable analgesic and antiinflammatory activities with appreciable decrease in the ulcer index. Amide conjugate of flurbiprofen with phenylalanine was found to be the most active, even more than the parent drug flurbiprofen.

Nonsteroidal antiinflammatory drugs (NSAIDs) form a class of therapeutic agents that are most widely used world over because of their analgesic, antipyretic and antiinflammatory effects. It is estimated that, currently, over 40 million people worldwide take NSAIDs daily. Although they are very effective agents, their gastrointestinal (GI) side effects are significant and serious with endoscopically visible gastroduodenal ulceration occurring in 30% patients after 12 weeks therapy. In fact, between 55% to 60% of the hospital admissions for GIT bleedings are related to NSAIDs and the treatment to GI adverse effects accounts for 30% of the total cost of the treatment. The study of Cioli *et al.*[1] suggests that the direct tissue contact of NSAIDs plays an important role in the production of GIT lesions and the reported literature confirms that gastric side effects of flurbiprofen, 2-(2-fluorobiphenyl-4-yl)-propionic acid are due to presence of –COOH group in the parent drug moiety[2]. In order to reduce this side effect, a structural modification like an amide or ester, has to be carried out to mask COOH group temporarily. A strategic group attached to mask COOH group will not only protect the vulnerable group and stabilize the molecule but it will also direct the drug to its target site.

The rational behind selection of amino acid as a promoiety is as follows[3-5]. Important reporting has been made by Meyers *et al.* in 1979 that many amino acids possess marked antiinflammatory activity against gelatin induced hind paw edema in rats and have healing effect on gastric lesions produced by NSAIDs. So these prodrugs may have additional advantage of producing nontoxic amino acids as byproducts, which upon cleavage may give a synergistic antiinflammatory effect with NSAIDs along with gastro protective effect. By proper selection of amino acids, polarity, solubility profile and acid -base properties of a given drug molecule can be altered completely. The body's handling of nutritional substances suggests that use of a nutrient moiety as a derivatizing group might also permit more specific targeting for enzymes involved in the terminal phase of digestion along with modifying physico-chemical properties, which limit GI drug absorption. N-acylation of amines to give amide

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prodrugs has been used to a limited extent due to their relative stability *in vivo*. But certain amides formed with amino acids may be susceptible to enzymatic cleavage *in vivo*[6].

The present report describes the synthesis of amide prodrugs of flurbiprofen with methyl esters of L-tryptophan, L-histidine, L-phenylalanine, DL-alanine, (fig. 1) their physico-chemical characterization, hydrolysis kinetics and pharmacological studies, which may prove to be less irritant to GIT with an added advantage of synergistic antiinflammatory effect.

All chemicals used were of synthetic grade. Flubiprofen was obtained as a gift sample from FDC, Roha Dhatav MIDC. The purity of compounds was ascertained by TLC on precoated silica F_{254} plates (Merck, Mumbai) using iodine vapours and UV light as detecting agents. The melting points of the synthesized compounds and intermediates were determined by open capillary method and are uncorrected. The IR spectra of synthesized compounds were recorded on a Perken Elmer IR spectrophotometer model 841 in KBr pellets. The NMR spectra of the synthesized compounds were recorded on a Varian EM-360 L (60 MHz) at Department of Pharmaceutical Sciences, Punjab University, Chandigarh. The molecular weights of compounds were determined from their Mass spectra which were recorded on Jeol D-300 at RSIC, CDRI, Lucknow. The elemental analysis of synthesized compounds was carried out on Carlo Erba 1108 Heraeus at RSIC, CDRI, Lucknow.

The absorbance maxima (λ_{max}) of the compounds were determined on a Shimadzu 160A UV double beam spectrophotometer in hydrochloric acid buffer (pH 1.2) and phosphate buffer (pH 7.4). The aqueous solubilities and the partition coefficients were determined in octanol/phosphate buffer (pH 7.4) at room temperature[7].

Synthesis of methyl ester hydrochlorides[8] of various amino acids i.e. L-tryptophan (A1), L-histidine (A2), L-phenylalanine (A3) and DL-alanine (A4) was carried out by the procedure described below. Freshly distilled thionyl chloride (0.05 mol+30% excess) was slowly added to methanol (100 ml) with cooling and amino acid (0.1 mol) was added to it. The mixture was refluxed for 6-8 h at 60-70° with continuous stirring on a magnetic stirrer. Excess thionyl chloride and solvent was removed under reduced pressure giving crude amino acid methyl ester hydrochloride. It was triturated with 20 ml portion of cold ether at 0° until excess of dimethyl sulfite was removed. The resulting solid product was collected and dried under vacuum. It was recrystallized from hot methanol by slow addition of 15-20 ml ether, followed by cooling at 0°. The crystals were collected on next day and washed twice with ether: methanol mixture (5:1) followed by pure ether and dried under vacuum to give pure amino acid methyl ester hydrochloride.

For synthesis of flubiprofen acid chloride (FAC), flubriprofen (0.05 mol) was dissolved in minimum amount of chloroform and freshly distilled thionyl chloride (0.05 mol+30% excess) was added slowly to it. The mixture was refluxed for 15 h at 60-70° with continuous stirring on magnetic stirrer. The viscous liquid was immediately poured on petridish and was vacuum dried to give yellow white crude acid chloride. The physico-chemical characteristics of all the intermediates are summarized in Table 1.

TABLE 1: PHYSICO-CHEMICAL CHARACTERSTICS OF INTERMEDIATES

Compound	Melting point*	R_f#	% yield
A1	220-223°	0.86	63.1
A2	202-203°	0.49	72.1
A3	150-152°	0.66	50.02
A4	115-118°	0.47	75.9
FAC	100-103°	0.82	82.06

*Uncorrected, # chloroform/methanol; 2:1

The synthesis of prodrugs of flubiprofen[9] with methyl esters of tryptophan (FT), histidine (FH), phenylalanine (FP), alanine (FA) was carried out as described below. Ice-cold, aqueous sodium hydroxide solution 5% (125 ml) was taken in 250 ml beaker and amino acid methyl ester hydrochloride (0.05 mol) was added to it. The reaction mixture was mechanically stirred for 30 min at room temperature, after which the beaker was transferred to an ice-bath kept on mechanical stirrer, maintaining the temperature at 10°. FAC (0.01 mol+10% excess) was added in small portions, with continuous stirring for 7-8 h. The solid that separated out was filtered at vacuum pump and dried. The crude product was recrystallized from methanol. FT, (N-[(β-indole-3-yl)-α-(methylpropionate)]-2-[(2-fluorobiphenyl)-4-yl]-propionamide): mp: 262-265°, R_f: 0.68 in benzene:ethyl acetate (2:1), % yield: 50.2, log P_{oct} (FT): 1.04, aqueous solubility: 8.98 mg/ml. λ_{max} in HCl buffer pH 1.2: 251.8 nm and in phosphate buffer pH 7.4: 247 nm. Elemental analysis: calculated for $C_{27}H_{25}FN_2O_3$: C: 72.89; H: 5.62; N: 6.29, found: C: 71.19; H: 5.94; N: 6.02. IR KBr,(cm^{-1}): 3395 (-NH stretch

amide), 1735 (-C=O stretch, saturated ester), 1680 (-C=O stretch amide), 1520 (-NH bending amide), 1260 (-C-F stretch). ^{1}H NMR (CD_3OD, ppm): δ 1.36[(s), 3H, -CH_3 saturated], δ 1.45 [(d), 2H, -CH_2 saturated], δ 2.54 [(d), 3H, CH-CH_2 saturated], δ 3.24-3.36 [(q), 4H, -CH-CH_3], δ 3.66[(d), 3H, -OCH_3], δ 5.30[(d), 1H, CH=C- cyclic conjugation], δ 7.12 [(d), 1H, NH-C- acyclic conjugation], δ 7.27-7.42 [(m), Ar – H], δ 7.57 [(s), NH- hetroaromatic], MS: m/z 446 (M^+).

FH, (N-[(β-imidazole-3-yl)-α-methyl propionate]-2-[(2-fluorobiphenyl)-4-yl]-propionamide): mp: 220-222°, R_f: 0.79 in acetone:water (5:1), % yield: 81.4, log P_{oct}: 0.95, aqueous solubility: 5.27 mg/ml, λ_{max} in HCl buffer pH 1.2: 249 nm and in phosphate buffer pH 7.4: 247 nm. Elemental analysis calculated for $C_{22}H_{23}FN_3O_3$: C: 66.79; H: 5.81; N: 10.62, found: C: 67.82; H: 5.54; N: 9.57. IR KBr, (cm^{-1}): 3360 (-NH stretch amide), 1730 (-C=O stretch saturated ester), 1660 (-C=O stretch amide), 1530 (-NH bending amide), 1260 (C-F stretch). ^{1}H NMR ($CDCl_3$+DMSO-d_6, ppm): δ 0.97 [(s), 3H] δ1.05 [(s), 2H], CH_3 saturated, δ1.35-1.45[(d), 2H]-CH_2 saturated, δ 2.55[(s), 1H]-HC-C=O saturated, δ3.4-3.72 [(q), 4H]-CH-CH_3, δ3.78 [(s), 3H] –OCH_3, δ5.30 [(d),1H]-CH=C, δ7.12 [(d), 1H] CO-NH, δ7.15-7.4 [(m), 5H], δ7.7 [(m), 3H] Ar-H, δ7.7 [(s), 1H] NH- hetreocyclic, MS: m/z 395 (M^+).

FP,(N-[β-benzyl-α-methylpropionate]-2-[(2-fluorobiphenyl)- 4-yl] propionamide): mp: 251-254°, R_f: 0.73 in chloroform:benzene (2:5), % yield: 61.1%, log P_{oct}: 0.71, aqueous solubility: 2.28 mg/ml, λmax in HCl buffer pH 1.2: 254 nm, and in phosphate buffer pH 7.4: 249 nm. Elemental analysis, calculated for $C_{22}H_{24}FNO_3$: C, 73.99; H, 5.91; N, 3.45, found: C, 72.69;H, 4.98;N, 3.21. IR KBr, (cm^{-1}): 3360 (-NH stretch amide), 1735 (-C=O stretch sat. ester), 1660 (C=O stetch amide), 1535 (NH bending amide), 1480 (C=N stretch), 1260 (C-F stretch). H^1 NMR (CD_3OD, ppm): δ1.42 [(s), 3H], CH_3 saturated, δ1.56 [(d), 2H]-CH_2 saturated, δ2.30 [(d), 1H]-CH saturated, δ2.60 [(d), 1H]-HC-C=O, δ3.30-3.39 [(q),4H]-CH-CH_3, δ3.69 [(d),3H]-OCH_3, δ7.12 [(d),1H] CO-NH, δ7.33-7.48 [(m), 5H], δ7.84 [(m), 3H] Ar-H. MS: m/z 405(M^+).

FA, (N-[-α-methyl propionate]-2-[(2-fluorobiphenyl)- 4-yl]-propionamide): mp: 228-230°, R_f: 0.53 in methanol:water (3:1), % yield: 64.5, log P_{oct}: 0.97, aqueous solubility: 3.57 mg/ml. λmax in HCl buffer pH 1.2: 248 nm and in phosphate buffer pH 7.4: 247 nm. Elemental analysis, calculated for $C_{19}H_{20}FNO_3$: C, 69.22; H, 6.07; N, 4.25, found, 69.37; H, 6.22; N, 3.89. IR KBr, (cm^{-1}): 3360 (-NH stretch amide), 1730 (-C=O stretch saturated ester), 1670 (-C=O stretch amide), 1530 (-NH bending amide), 1260 (-C-F stretch). H^1 NMR (CD_3OD, ppm): δ1.39 [(s), 3H]-CH_3 saturated, δ1.48 [(d), 2H]-CH_2 saturated, δ2.13 [(d), 1H]-CH saturated, δ2.54 [(s), 1H]-HC-C=O, δ3.27-3.36 [(q), 4H]-CH-CH3, δ3.66 [(d), 3H]-OCH_3, δ5.30 [(d), 1H], δ7.12 [(d), 1H]-CO-NH, δ7.27-7.42 [(m), 5H], δ7.81 [(m), 3H] Ar-H. MS: m/z 317 (M^+).

In vitro hydrolysis studies[10] of amide prodrugs were carried out in hydrochloric acid buffer (pH 1.2), phosphate buffer (pH 7.4) and 80% human plasma (pH 7.4) at 37±1°. Total buffer concentration was generally 0.05 mol and a constant ionic strength (μ) of 0.5 mol was maintained for each buffer by adding calculated amount of potassium chloride. Free flubiprofen which was supposed to be released after hydrolysis of conjugate was extracted with two 5 ml portions of chloroform. The chloroform layer was estimated on UV spectrophotometer for the amount of free flubiprofen released after hydrolysis of conjugate in HCl buffer and phosphate buffer at 248.4 nm and at 227 nm for hydrolysis in 80% human plasma. The kinetics of hydrolysis was monitored by increase of free drug concentration with time and the order of reaction and half lives ($t^1/_2$) were calculated. Rate of hydrolysis was calculated using equation, K=(2.303/t)log(a/a-x), where K represents hydrolysis constant, t is the time in min, a is the initial concentration of prodrug, x is the amount of prodrug hydrolyzed and (a-x) is the amount of the prodrug remaining. The results of hydrolysis kinetics studies are summarized in Table 2.

For the pharmacological studies, the suspensions of test compounds (at equimolar doses) were prepared in the distilled water using 2% gum acacia. In all cases, control received the same quantity of gum acacia. Haffkine strain albino rats of either sex weighing between 200-300 g ran-

TABLE 2:HYDROLYSIS OF FLURBIPROFEN AMINO ACID CONJUGATES

Conjugates	Half life ($t_{1/2}$) min		
	Hydrochloric acid buffer	Phosphate buffer	80% human plasma
FT	-	76.09	25.55
FH	-	55.13	29.31
FP	-	41.36	16.24
FA	-	30.19	20.35

Rate of hydrolysis of amino acid conjugates of flurbiprofen was determined in hydrochloric acid buffer (pH 1.2), phosphate buffer (pH 7.4) and 80% human plasma and time necessary for 50% hydrolysis ($t^1/_2$) was calculated.

domly distributed in control; standard and test groups (6 animals each) were used for antiinflammatory and ulcerogenic activities. For analgesic activity the weight range of rats was 150-200 g. Antiinflammatory activity was evaluated by carrageenan- induced rat paw edema method of Winter *et al*[11] using UGO Basile plethysmometer. The experimental protocols for the same have been approved by the Institutional Animal Ethics Committee. Edema was induced in hind paw by injection of 0.1 ml of 1% carrageenan (C-3889 Type IV Sigma Chemicals, St.Louis, MO) in distilled water into planter tissue of paw using 27-gauge needle. The percent inhibition of edema was calculated by using formula $(1-V_t/V_c)x100$, where V_t and V_c are the mean relative changes in paw volume in test and control, respectively. Analgesic activity of the synthesized compounds was determined by tail flick method[12], using analgesiometer (Inco, Ambala). The tail flick latency of each group was noted after every 15 min upto $2^1/_2$ h. The ulcerogenic activity was determined by method of Hitchens *et al.*[13] taking flurbiprofen as standard. The test compounds and standards were administered orally (at ten times higher doses), as a suspension in 2% gum acacia at a constant volume. All the ulcers greater than 0.5 mm were counted, using a method reported by Cioli *et al.*[1]. Ulcer index was determined for each compound. The results of antiinflammatory, analgesic and ulcerogenic activities are summarized in Table 3.

The synthesized compounds were subjected to physicochemical characterization. Partition coefficient studies indicated improved aqueous solubility of amide prodrugs as confirmed by their aqueous solubility values. The IR spectrum of synthesized compounds showed –NH stretching and carboxyl bending vibrations characteristic for amides. Hydrolysis studies of the synthesized compounds show that they did not undergo hydrolysis in 0.05 mol hydrochloric acid buffer (pH 1.2) implying that these amino acid conjugates would be stable in the acidic pH of stomach. The hydrolysis of amino acid conjugates of flubiprofen in 0.05 mol phosphate buffer (pH 7.4) indicated that the hydrolysis of all the conjugates followed first order kinetics where as hydrolysis in 80% human plasma revealed that FT and FP hydrolyzed following initial zero and then first order kinetics throughout. The half lives of amino acid conjugates of flubiprofen in 0.05 mol phosphate buffer (pH 7.4) ranged from 30.19 min to 70.09 min suggesting that the prodrugs are adequately stable to be absorbed intact from the intestine. The short half-lives of the conjugates in plasma ranging from 16.2 to 29.3 min indicate the influence of enzymes (amidases) present in plasma.

Result of antiinflammatory activity reveal that FP and FA showed better antiinflammatory activity (86% and 75.3%, respectively), where as FT and FH had comparable antiinflammatory activity (68.8% and 71%, respectively) as compared to flubiprofen (73%). Results of analgesic activity showed that peak activity was observed at 1.5 h for FH, FP and FA and at 1 h in case of FT as compared to 2.5 h with flubiprofen. This decrease in time can be attributed to increase in the aqueous solubility of these compounds. Amino acid conjugates showed appreciable decrease in ulcerogenic tendency as compared to flurbiprofen. Thus it is quite natural to presume that ulerogenicity, which is one of the serious side effects of flubiprofen has been successfully overcome without affecting it's other biological activities.

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TABLE 3: PHARMACOLOGICAL ACTIVITIES OF FLURBIPROFEN AND ITS AMINO ACID CONJUGATES

Compound	Doses (mg/kg)	Antiinflammatory activity % Inhibition*	Analgesic activity Test latency[a] s±S.E	Ulcer Index[a] ± S.E
FT	2.59	68.81	14.0±0.55	1.50±0.84
FH	2.30	70.96	14.6±0.22	1.66±0.52
FP	2.37	86.02	12.6±0.28	2.00±1.41
FA	1.92	75.26	11.2±0.8	2.33±2.61
Flurbiprofen	1.43	73.11	11.2±0.8	7.62±2.89

The antiinflammatory, analgesic and ulcerogenic activities of flurbiprofen in comparison with its amino acid conjugates,* % Inhibition was calculated by taking into consideration mean relative changes in paw volume in test and control groups respectively ± S.E. therefore is not accompanied by standard deviation value. **a** -$p < 0.01$.

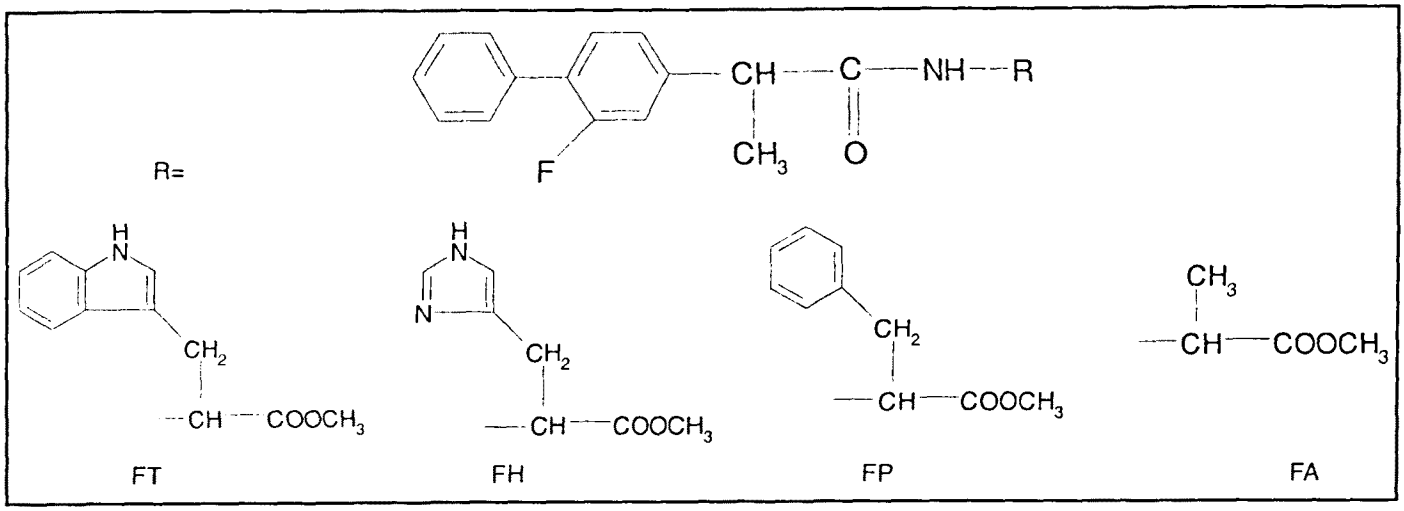

Fig. 1: Structures of prodrugs of flurbiprofen with amino acids.

tance to carry out this work.

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Pharmacological Evaluation of Synthetic Imidazolinones and their Schiff Bases

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Some imidazolines were synthesized from oxazolinones and upon treatment with benzaldehyde the Schiff's bases were synthesized from the respective imidazolinones. All these compounds

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