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# Synthesis and Biological Activity of some Amino Acid Conjugates of Oxaprozin

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**Amino acid conjugates between various amino acid methyl esters and the carboxylic acid group of oxaprozin were synthesized using N,N'-dicyclohexylcarbodiimide as a coupling agent. These compounds were characterized by analytical and spectral data. Kinetics of hydrolysis of these conjugates was studied. The esters were evaluated for analgesic and antiinflammatory activity and ulcerogenicity. The esters showed comparable analgesic and anti-inflammatory activity with negligible ulcerogenicity.**

Oxaprozin, chemically 4,5-biphenyl-2-oxazole propanoic acid, is a newer non-steroidal antiinflammatory drug belonging to aryl alkanoic acid subclass of compounds[1]. As in the case with some other newer non-steroidal antiinflammatory drugs, oxaprozin offers the convenience of once daily administration. As with other non-steroidal antiinflammatory drugs, this drug also suffers from mild dyspepsia, gastric discomfort to gastric bleeding. Reported literature confirms that gastric side effects of oxaprozin are due to the presence of COOH group in the parent drug moiety[2,3]. Therefore, in order to reduce this side effect, a structural modification has to be carried out which will mask the COOH group. Amino, amide or ester derivatives are the possible modifications of COOH group. A strategic group attached to mask COOH group will not only protect the otherwise vulnerable group and stabilize the molecule, but it will also direct the drug to its target site. The salient features of the usefulness of conjugation of amino acids with NSAIDs are many[4,5]. A drug with free carboxyl group can be derivatized into corresponding esters or amide of amino acids, so as to alter the physical properties of parent drug with one or more of the hydrolase enzymes serving as the *in vivo* reconversion sites. The body's handling of nutritional substances suggests that the use of a nutrient moiety as a derivatizing group to modify physico-chemical properties which limit oral absorption, might also permit nonspecific targeting of enzymes involved in the terminal phase of digestion. These drugs have the additional advantage of producing non-toxic nutrient supplements upon cleavage, since amino acids are normal dietary constituents and they are less toxic to body in moderate doses. Classifying the amino acid as non-polar, polar, acidic and basic, a given drug molecule can be made more or less soluble in a given solvent. Acid/base properties can also be changed or altered completely. Hydrolysis of amino acids is likely to be catalyzed by, nucleophiles or bases, even in the absence of enzyme-facilitated hydrolysis. Amino acids have healing effect on gastric lesions produced by NSAIDS. An important finding is that many amino acids possessed marked antiinflammatory activity against gelatin-induced hind paw edema in rats[6]. The present report describes the synthesis of conjugates of oxaprozine with amino acids such as glycine or L-histidine or L-tryptophan and evaluation of the physicochemical properties, hydrolysis kinetics and biological activities.

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## MATERIALS AND METHODS

All the chemicals used were of synthetic grade. Oxaprozin was obtained as a gift sample from Ciba Giegy Ltd., Switzerland. The purity of the compounds was ascertained by TLC on precoated silica gel $F_{254}$ plates (Merck, Mumbai) using iodine vapours and UV light as detecting agents. The melting points of the synthesized compound and intermediates were determined by open capillary method and are uncorrected. The IR spectra of the synthesized com-

pounds were recorded on a Jasco V-530 FTIR in potassium bromide pellets. The NMR spectra of the synthesized compounds were recorded in $CDCl_3$ using a Varian VRX-300 (300 MHz NMR) at RSC, IIT, Powai, Mumbai. Tetramethyl silane was used as the internal standard. The elemental analysis (C, H, N) was carried out on a CarloErba 1108 Heraeus instrument at the RSIC, CDRI, Lucknow. The absorbance maxima ($\lambda_{max}$) of the synthesized compounds were determined on a Jasco V-530 UV/Vis double beam spectrophotometer in methanol or hydrochloric acid buffer (pH 1.2) or phosphate buffer (pH 7.4). The aqueous solubilities of all compounds prepared were determined at room temperature (25±1°). The partition coefficients of all the synthesized compounds were determined in octanol/phosphate buffer (pH 7.4) at room temperature[6].

**Synthesis of methyl ester hydrochloride of glycine (GME.HCl) (A1):**

Freshly distilled thionyl chloride [0.05 mol+30% excess; 5 ml] was slowly added to methanol (100 ml) with cooling and glycine (0.1 mol, 7.5 g) was added to it. The mixture was refluxed for 6 h at 60-70° with continuous stirring on a magnetic stirrer. Excess of thionyl chloride and solvent was removed under reduced pressure giving crude GME.HCl. The crude product was triturated with 20 ml portions of cold ether at 0°, until excess dimethyl sulphite was removed. The resulting solid product was collected and dried under high vacuum to give dried GME.HCl. It was recrystallized from hot methanol by slow addition of 15-20 ml of ether, followed by cooling at 0°. 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 GME.HCl. Melting point: 174°, $R_f$ = 0.76 in chloroform:methanol (2:1), % yield: 89.03, log $P_{oct}$ (GME): 0.56

**Synthesis of histidine methyl ester hydrochloride (HME.HCl) (A2):**

Freshly distilled thionyl chloride (0.05 mol+30% excess, 5 ml) was added to methanol (100 ml) with cooling. To this, L-histidine (0.1 M, 15.52 g) was added. The reaction mixture was refluxed for 7 h at 60-70° with continuous stirring

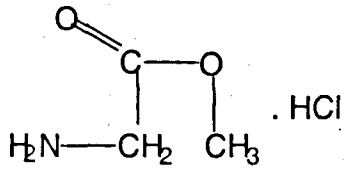


**Fig. 1: (A1) 2-methyl amino acetate hydrochloride.**

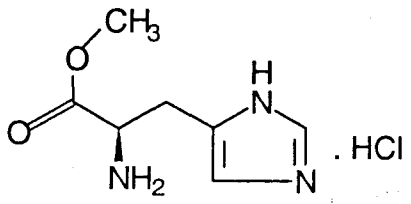


**Fig. 2: (A2) (-) 2-amino-3-(imidazol-4-yl) methyl propionate hydrochloride.**

on a magnetic stirrer. Same procedure was followed for workup as mentioned for GME.HCl. Melting point: 202-203°, $R_f$: 0.49 in chloroform:methanol (2:1), % yield: 72.1, log $P_{oct}$ HME: 0.77.

**Synthesis of tryptophan methyl ester hydrochloride (TME.HCl) (A3):**

Freshly distilled thionyl chloride (0.05 mol+30% excess, 5 ml) was slowly added to methanol (100 ml) with cooling and L-tryptophan (0.1 mol, 20.42 g) was added to it. The mixture was refluxed for 6 h at 60-70° with continuous stirring on a magnetic stirrer. Same procedure was followed as mentioned in synthesis of GME.HCl. Melting point: 220-223°, $R_f$: 0.86 in chloroform:methanol (2:1), % yield: 63.10, log $P_{oct}$ (TME): 2.09.

**Synthesis of oxaprozin glycine methyl ester (SJK 1):**

To a solution of glycine methyl ester hydrochloride (0.1 mol, 12.6 g, A1) dissolved in methanol, triethylamine (0.1 mol 10.1 g) was added slowly and stirred for 2 h at 0°. The reaction mixture was then filtered and the methanol distilled off to get sticky glycine methyl ester. The ester was then dried under reduced pressure. N,N'-dicyclohexylcarbodiimide (0.11 mol, 22.7 g) was then added to a solution of oxaprozin (0.1 M) and N-hydroxy succiniimide (0.2 mol) in dry dichloromethane (250 ml) at 0°. Subsequently, glycine methyl ester (0.1 mol) in dichloromethane (100 ml) was added to the reaction mixture. It was stirred at 0° for 2 h and then at room temperature for 36 h. The reaction mixture was filtered to remove the precipitated

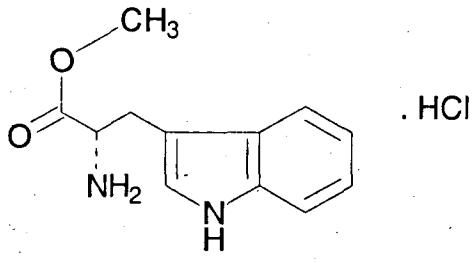


**Fig. 3: (A3) (-) 2-amino-3-(indol-3-yl) methyl propionate hydrochloride.**

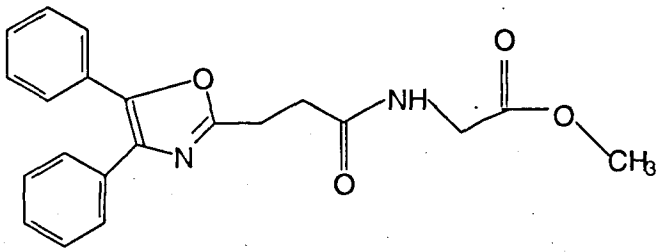

**Fig. 4: (SJK1) N-{(methoxycarbonyl)-methyl}–2–{(4,5-diphenyl) Oxazol–2 –yl} propionamide.**

dicyclohexyl urea and the filtrate was washed with 1 M hydrochloric acid, 5 % sodium bicarbonate and saturated solution of sodium chloride respectively. The organic layer was dried with anhydrous sodium sulphate and concentrated under reduced pressure to give oxaprozin glycine methyl ester. Melting point: 98°, $R_f$: 0.26 in benzene:ethyl acetate (2:1), % yield: 45, log $P_{oct}$: 2.67, aqueous solubility: 1.57 mg/ml. $\lambda_{max}$-methanol: (286 nm), hydrochloric acid buffer pH 1.2: (265 nm) phosphate buffer pH 7.4: (284 nm). The IR spectra of SJK1 showed absorption band at 3405 $cm^{-1}$ (-NH stretch), 1730 $cm^{-1}$ (ester carbonyl group), 1685 $cm^{-1}$ (amide carbonyl bend) while $^1H$ NMR of SJK1 showed presence of signals (in ppm) at d 2.90 (t, 2H, J=6Hz), 3.20 (t, 2H, J=6Hz), 3.70 (s, 3H), 4.10 (d, 2H), 6.50 –6.60 (bs, 1H), 7.2 –7.40 (m, 5 H), 7.5 –7.70 (m, 5H).

**Synthesis of oxaprozin histidine methyl ester (SJK 2):**

For this synthesis, histidine methyl ester (0.1 mol, A2) was used and the same procedure was followed as mentioned for SJK1. Melting point: 178°, $R_f$: 0.35 in benzene:ethyl acetate (2:1), % yield: 61.5, log $P_{oct}$: 2.81, aqueous solubility: 1.73 mg/ml. Elemental analysis, calculated for $C_{25}H_{24}N_4O_4$ C: 67.55; H: 5.44; N: 12.60 found: C: 68.51; H: 5.71; N: 12.89. $UV_{max}$: methanol (284 nm), hydrochloric acid buffer pH: 1.2 (253.2 nm), phosphate buffer pH: 7.4 (282.7 nm). IR spectra showed absorption bands at 3320 (-NH stretch), 1734 $cm^{-1}$ (ester carbonyl group), 1620 (amide carboxyl bend). $^1H$ NMR ($CDCl_3$, 300 MHz) of SJK2 showed the presence of signals (in ppm) at δ 2.3 (d, 2H), 2.90 (t, 2H, J=Hz), 3.20 (t, 2H, J=6Hz), 3.70 (s, 3H), 3.80 (m, 1H), 7.20–7.40 (m, 6H), 7.60–7.80 (m, 5H).

**Synthesis of oxaprozin tryptophan methyl ester (SJK 3):**

For this tryptophan methyl ester (0.1 mol, A3) was used and the same procedure was followed as mentioned for SJK1. Melting point: 222°, $R_f$: 0.32 in benzene: ethyl acetate (2:1), % yield: 48.3, log $P_{oct}$: 5.45, aqueous solubility: 0.07 mg/ml. UVmax: methanol (284 nm), hydrochloric acid buffer pH 1.2 (271 nm), phosphate buffer pH 7.4 (282.7 nm). IR spectra showed absorption bands at 3305 $cm^{-1}$ (-NH stretch), 1720 $cm^{-1}$ (ester carbonyl group), 1635 $cm^{-1}$ (amide carboxyl bend) $^1H$ NMR ($CDCl_3$, 300 MHz) showed signal (in ppm) at δ 2.3 (d, 2 H), 2.90 (t, 2H, J= $5H_2$), 3.20 (t, 2H, J= $5H_2$), 3.70 (S, 3H), 3.80 (m, 1H), 7.20–7.40 (m, 6H), 7.50–7.80 (m, 9H).

**Kinetics of hydrolysis:**

Hydrolysis studies of the amino acid conjugates were carried out in aqueous buffer solutions at 37±1°, hydrochloric acid (pH 1.2) and phosphate buffer (pH 7.4). The total buffer concentration was generally 0.05 M and a constant ionic strength ($\mu$) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride[10]. The hydrolysis of amino acids conjugates was also studied in 0.05 M phosphate buffer (pH 7.4) containing 80 % human plasma at 37±1°. The aliquots withdrawn were estimated directly, after appropriate dilutions by injecting 20 ml of the solution onto a HPLC column and detected using an UV detector. The kinetics of hydrolysis was monitored by the

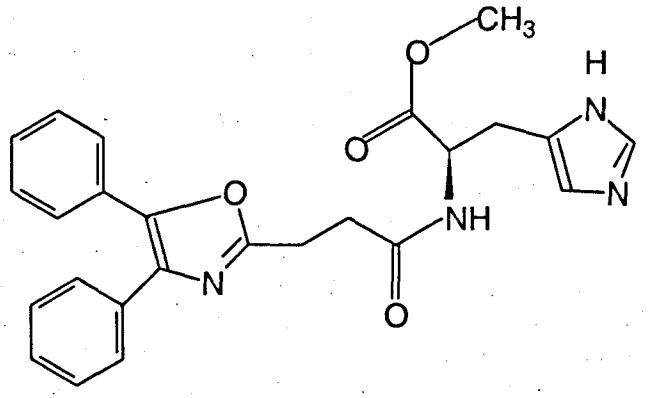

**Fig. 5: (SJK2) (-)N-{(methoxycarbonyl)-2-imidazol-4-yl-ethyl}–2–{(4,5-diphenyl)–oxazol–2-yl} propionamide.**

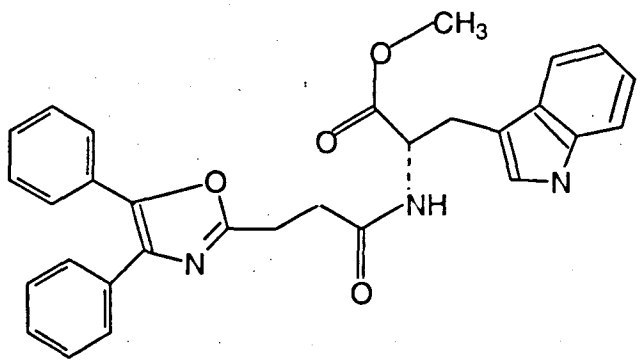

**Fig. 6: (SJK3) (-)N-{(methoxycarbonyl)-2-(indol-3yl-ethyl}–2–{(4,5-diphenyl)–oxazol–2-yl} propionamide.**

increase of free drug concentration with time and the order of reaction and half-life ($t_{1/2}$) was 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 minutes, a is the initial concentration of prodrug, x is the amount of prodrug hydrolyzed, and a-x is the amount of prodrug remaining. The results of the hydrolysis kinetic studies are summarized in Table 1.

**Pharmacological studies:**

Suspensions of the test compounds were prepared in distilled water using 1% carboxy methylcellulose (CMC). In all cases controls received the same quantity of CMC solution. Antiinflammatory activity was evaluated by carrageenan-induced rat hind paw edema method of Winter *et al*[12,13]. Sprague Dawley rats of either sex weighing between 120-150 g were distributed into control, standard and test (6 animals each) groups. The test compounds were administered orally at doses equimolar to oxaprozin. One hour after this treatment, an inflammatory edema was induced in hind paw by injection of 0.1 ml of 1% carrageenan (C-3889 Type IV, Sigma Chemical, St. Louis, MO) in distilled water, in the subplanter tissues of hind paw. Paw volumes were measured before and after administration of carrageenan using a plethysmometer. The percent inhibition was calculated using formula, $(1-V_t/V_c)x100$, where $V_t$ and $V_c$ are the mean relative changes in the paw volume in test and control, respectively.

Analgesic activity was evaluated by Randal Selitto method[14]. The apparatus used for determination of analgesic activity, by carrageenan-induced paw hyperalgesia, was Ugo Basile Analgesy meter. The suspensions of test compounds were prepared in distilled water using 1% CMC. In all cases control received the same quantity of CMC. Sprague Dawley rats of either sex were randomly distributed in control and experimental group of six animals. At 0 h hyperalgesia was induced by injecting 0.1 ml of 1 % carragenan in distilled water, into the subplanter tissues of hind paw. The rats received drug or test compounds at equimolar doses, 2 h after carrageenan injection and were evaluated for paw hyperalgesia 1 h later. The force at which a rat withdrew its hind paw, vocalized or struggled was multiplied by 10, (as recommended by the manufacturer), and recorded as the withdrawl force (g) or pain threshold (g)[15].

TABLE 1: HYDROLYSIS OF OXAPROZIN AMINO ACID CONJUGATES (SJK1-3).

| Compound | t1/2 (min) | | |
|---|---|---|---|
| | hydrochloric acid | phosphate buffer | 80% human plasma |
| SJK1 | -- | 134.8 | 21.1 |
| SJK2 | -- | 112.3 | 25.1 |
| SJK3 | -- | 128.5 | 32.2 |

Hydrolytic rate of the amino acid conjugates of oxaprozin were determined in hydrochloric acid, phosphate buffer (pH 7.4) and 80% human plasma and the time necessary for 50% hydrolysis (t1/2) are calculated.

Ulcerogenicity was determined by the method of Rainsford and Whitehouse[16], taking oxaprozin as standard. Sprague Dawley rats of either sex were randomly distributed in control and experimental group of six animals. Following oral administration of 5 ml of the aqueous drug suspensions, the animals were stressed by exposure to cold (-15° for 1 h). The animals were placed in separate polypropylene cages. After 2 h of drug administration, the animals were sacrificed. The stomach was opened along the greater curvature and the number of lesions was examined by means of a magnifying lens. All ulcers larger than 0.5 mm were counted. The ulcers were scored according to the method reported by Cioli *et al.*[17] and the ulcer index was determined. The results of pharmacological activities are summarized in Table 2.

## RESULTS AND DISCUSSION

The synthesized compounds were subjected to physicochemical characterization. Partition coefficient studies indicated change in lipophilicity of the amino acid conjugates and corresponding change in aqueous solubilities. The IR spectrum of the synthesized compounds showed –NH stretching and amide carboxyl bending vibrations characteristic for amides. Hydrolysis studies of the synthesized compounds show that they did not undergo hydrolysis in 0.05 M hydrochloric acid buffer (pH 1.2) that is to say that these amino acid conjugates did not release the active drug in acidic pH of the stomach. The hydrolysis of amino acid conjugates of oxaprozin in 0.05 M phosphate buffer (pH 7.4) and in 80 % human plasma (pH 7.4) indicated that all the conjugates were hydrolysing by first order kinetics. The half-life of amino acid conjugates of oxaprozin in 0.05 M phosphate buffer (pH 7.4) ranged from 110 min to 140 min suggesting that the prodrugs are adequately stable to be absorbed intact from the intestine. The short half-life of the conjugates in plasma indicates the influence of enzymes (amidases) present in the plasma.

TABLE 2: PHARMACOLOGICAL ACTIVITIES OF OXAPROZIN AND ITS AMINO ACID CONJUGATES (SJK1-3).

| Compound | Antiinflammatory activity % inhibition | Analgesic activity pain threshold | Ulcer index |
|---|---|---|---|
| SJK1 | 51.8 | 75 | NIL |
| SJK2 | 63.4 | 65 | NIL |
| SJK3 | 43.1 | 88 | 1.5± 0.38 |
| OXAPROZIN | 44.1 | 80 | 40.2±7.36 |

The antiinflammatory, analgesic and ulcerogenic activities of oxaprozin in comparison with its amino acid conjugates, SJK 1 (glycine methyl ester), SJK 2 (histidine methyl ester) and SJK 3 (tryptophan methyl ester).

Results of antiinflammatory activity reveal that SJK1 and SJK2 showed better inhibitory activity i.e. 51.8% and 63.4%, respectively, as compared to oxaprozin. Results of analgesic activity revealed that SJK3 (oxaprozin tryptophan methyl ester) showed better activity (88%) as compared to oxaprozin (80%) whereas all other compounds showed comparable activity to that of oxaprozin. The most common side effect that all nonsteroidal antiinflammatory drugs possess is their ulcerogenicity tendency on prolonged use. Evaluation of ulcerogenicity revealed that all the amino acid conjugates of oxaprozin show no or very less ulcerogenic tendency. Thus the basic purpose of circumventing the ulcerogenic potential of oxaprozin without affecting its other biological activity has been accomplished.

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