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Analysis of some amino acid analogues of diclofenac as prodrugs

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Abstract
The main aim in this paper is to Diclofenac analogues as prodrug were synthesized and evaluated for antiinflammatory and analgesic activities. Hydrolysis studies of the prodrugs were performed in SGF and SIF. The synthesized compounds showed antiinflammatory and analgesic activity comparable to the parent drug.

Keywords: Amino acids, diclofenac as prodrugs

Introduction
Diclofenac is a potent and popular antiinflammatory drug but it suffers with various side effects such as peptic ulcer along with gastrointestinal bleeding, nausea, vomiting, abdominal pain, dyspepsia, diahorrehea etc. [1]. The ulcerogenic effect of Diclofenac is mainly due to its carboxylic acid functional group. In the present study various amino acids are used to make conjugates of diclofenac in order to mask the carboxylic acid functional group. As well as due to fact that amino acid possess marked antiinflammatory activity of their own and healing effect on gastric lesions produced by the NSAID's.

Materials and Methods
All chemicals used were of reagent and fine chemical grade. TLC was performed on silica gel G and KBr phase was used for IR on Shimadzu IR-47 spectrophotometer. Melting points of synthesized compounds were taken in open capillaries and are uncorrected.

1. Synthesis of diclofenac acid chloride
Diclofenac base (4.70 g) was dissolved in 60 mL of chloroform followed by the addition of 6 mL of thionyl chloride the reaction mixture was refluxed for 3-4 hours at 60-70°C. The solvent and excess thionyl chloride were distilled off at reduced pressure to obtain dark brown solid diclofenac acid chloride, Yield, 93%.

2. Synthesis of ethyl ester hydrochloride of amino acid [2]
General procedure: A solution of thionyl chloride (100 m mol) in dehydrated ethanol (250 mL) were placed in ice bath and the amino acid (100 mmol) was added slowly with stirring. The reaction mixture thus obtained was refluxed for 4 hours. The solvent was evaporated under reduced pressure giving the crude ethyl ester hydrochloride which was triturated with several portions of (20 mL) cold ether at 0°C until excess of dimethyl sulphide was removed. The resulting solid product was recrystallized from 25 mL of hot ethanol by slow addition of cold ether (150-200 ml) followed by cooling to 0°C. The crystals were collected and washed twice with an (5:1) ether: ethanol solution and washed with ether and dried under vacuum.

3. Synthesis of prodrugs (modified Schotten and Baumann reaction) [3]
General Procedure- Amino acid ethyl ester hydrochloride (10 mmol) was added in previously cooled solution of K_2CO_3 (10% 5 ml) at 10°C in ice bath. Followed by stirring until it became clear. Diclofenac acid chloride (10 mmol) was added to alkaline amino acid ester solution in portions and the content were vigorously stirred for one hour at 10°C. The solid separated was collected was dried in air. Followed by washing with NaOH solution (0.5%) and distilled water.

3.a. Synthesis of 2-(2',6'-dichloroanilino. Phenylacetamido ethyl acetate (Compound 1)

The compound was synthesized according to general procedure. Buff white solid amorphous, Yield: 79%, m.p.: 67-70,% N: 6.99 (7.14), IR (KBr) cm: 3350 (N-H stretch secondary amide), 3300 (N-H stretch secondary amine), 3030 (aromatic C-H stretch), 2900 (aliphatic C-H stretch), 1730 (C=O stretch of ester), 1550 (N- H bend), 1490 (C=C stretch of 1,2,3 tri substituted1,2 disubstituted aromatic ring), 1140 (C-O stretch of ester) 740,760, 710 (C-H bending out of plate of 4 adjacent H of aromatic ring). 610 (N-H wagging out of plane amide and secondary amine).

3.b. Synthesis of 2-(2',6'-dichloroanilino). phenylacetamido (2'-methyl) ethylacetate (Compound II)

Synthesized according to general procedure. Brownish orange solid amorphous, Yield: 75.37%, m.p.: 110-112,% N: 6.25 (6.8), IR (KBr) cm-4:3450 (N-H stretch secondary amide), 3350 (N-H stretch secondary amine), 3100 (aromatic C-H stretch), 2850 (aliphatic C- H stretch), 1730- (C=0 stretch of ester), 1563 (N-H band amide II), 1490 (C=C stretch of 1,2,3 tri substituted-1,2 disubstituted aromatic ring), 1380 (C=C stretch within aromatic ring), 1140 (C-0 stretch of ester), 1090 (C-CI aryl halide), 710 (C-H bending out of plane of 4 adjacent H of aromatic ring), 660.(N-H wagging out of plane amide and secondary amine).

3.c. Synthesis of 2-(2',6'-dichloroanilino). phenylacetamido '(2'-benzyl) ethyl acetate (Compound III)

Synthesized according to general procedure. Brownish white solid amorphous, Yield: 76.50%; m.p.:120-122,% N: 5.65 (5.8), IR (KBr) cm1: 3250 (N-H stretch secondary amide), 3280 (N-H stretch secondary amine), 3080 (aromatic C-H stretch), 2900 (aliphatic C-H stretch), 1732 (C=0 stretch of ester), 1562 (NHbend), 1485 (C=C stretch of 1,2,3 tri substituted1,2 disubstituted aromatic ring), 1090 (C-Cl aryl halide), 1140 (C-O stretch of ester), 710 (C-H bending out of plane of 4 adjacent H of aromatic ring), 630, 660 (N-H wagging out of plane amide and secondary amine).

3.d. Synthesis of 2-(2',6'-dichloroanilino) phenylacetamido (2"-hydroxy-methyl) ethyl acetate (Compound IV)

Synthesized according to general procedure. Light brown solid amorphous, Yield: 69.2%, m.p.: 186-190,% N: 5.94 (6.4), IR (KBr) cm: 3350 (N-H stretch secondary amide), 3300 (N-H stretch secondary amine), 3100 (aromatic C-H stretch), 2900 (aliphatic C-H stretch), 1740 (CEO stretch of ester), 1569 (N-H bend), 1440 (O-H out of plane bend), 1380 (C=C stretch of 1,2,3 tri substituted-1, 2 disubstituted aromatic ring), 1140 (C-O stretch of ester), 1090 (C-Claryl halide), 710 (C-H bending out of plane of 4 adjacent H of aromatic ring), 640, 660 (N-H wagging out of plane amide and secondary amine).

3.e. Synthesis of 2-(2,6-dichloroanilino) phenylacetamido (ethane-1-ol) ethyl acetate (Compound V)

Synthesized according to general procedure. brown solid amorphous, Yield: 60.62%, m.p.: 65-66,% N: 6:23 (6.4) IR (KBr) cm^{-1} 3350 (N-H stretch secondary amide), 3300 (N-H stretch secondary amine), 3100 (aromatic C-H stretch), 2900 (aliphatic C-H stretch), 1730 (CEO stretch of ester), 1570 (N-H bend), 1440 (O-H out of plane bend), 1490 (C=C stretch of 1,2,3 tri substituted-1, 2 disubstituted aromatic ring), 1240 (C-O stretch of alcohol), 1140 (C-O stretch of ester), 1092 (C-Claryl halide), 710 (C-H bending out of plane of 4 adjacent H of aromatic ring), 660 (N-H wagging out of plane amide and secondary amine).

3.f. Synthesis of 2-(2',6'-dichloroanilino) phenylacetamido-N-3"-carbo-ethoxy ethyl acetate (Compound VI)

Synthesized according to general procedure. buff white solid amorphous, Yield: 66.54%, m.p.: 80-82,% N: 6.52 (6.66), IR (KBr) cm": 3342 (N-H stretch secondary amide), 3286 (N-H stretch secondary amine), 3020 (aromatic C-H stretch), 2900 (aliphatic C-H stretch), 1740 (C=O stretch of ester), 1562 (N-H bend), 1140 (C=O stretch of ester), 1092 (C-Cl aryl halide), 710 (C-H bending out of plane of 4 adjacent H of aromatic ring), 640, 660 (N-H wagging out of plane amide and secondary amine).

4. Hydrolysis kinetics

Hydrolysis kinetic studies were carried in two media, i.e. hydrochloric acid buffer (pH 1.2) and phosphate buffer (pH 7.4). Prodrug equivalent to 10 mg of diclofenac free acid were transferred in the glass tubes fastoned with cellophane membrane. The tube was dipped 1 inch in a beaker containing SGF or SIF. Fluid inside the beaker was stirred on magnetic stirrer at 37±0.5°C. After every 30 minutes drug solution was taken out of beaker and followed by the addition of 1 ml of buffer solution to maintain the sink condition. The absorbance of withdrawn sample was measured agaisnt blank reagent at 287 nm by Shimadzu-UV 1601. Values are shown in Table 1.

Table 1: *In-vivo* Hydrolysis of Prodrugs

S. No.	Prodrug	Hydrolysis media	Cumulative% Prodrug hydrolysis after minutes							
			30	60	90	120	150	180	210	240
1.	I	SGF	11.9	18.2	2.40	25.0	26.5	28.2	29.3	30.2
		SIF	16.1	31.4	42.4	50.9	56.0	60.2	62.3	60.3
2.	II	SGF	11.7	18.4	20.8	22.8	24.7	25.6	26.2	27.2
		SIF	18.0	32.0	40.8	48.8	54.0	57.1	60.6	61.5
3.	III	SGF	12.2	18.4	21.7	24.6	26.4	27.1	28.2	28.9
		SIF	20.0	36.0	46.2	54.2	57.8	61.3	62.7	63.6
4.	IV	SGF	12.3	17.3	21.2	23.4	24.9	25.8	26.9	28.0
		SIF	16.2	30.2	40.5	50.0	56.1	61.2	63.7	65.1
5.	V	SGF	12.8	18.7	23.3	24.7	26.0	26.9	28.3	29.7
		SIF	16.3	29.5	38.9	44.3	48.7	52.6	55.6	58.0
6.	VI	SGF	13.0	18.8	22.4	25.5	27.1	28.6	29.5	30.9
		SIF	14.3	28.9	40.7	49.6	55.5	61.7	62.5	68.0

Table 2: Comparative study of the analgesic activity of synthesized prodrugs with diclofenac free base

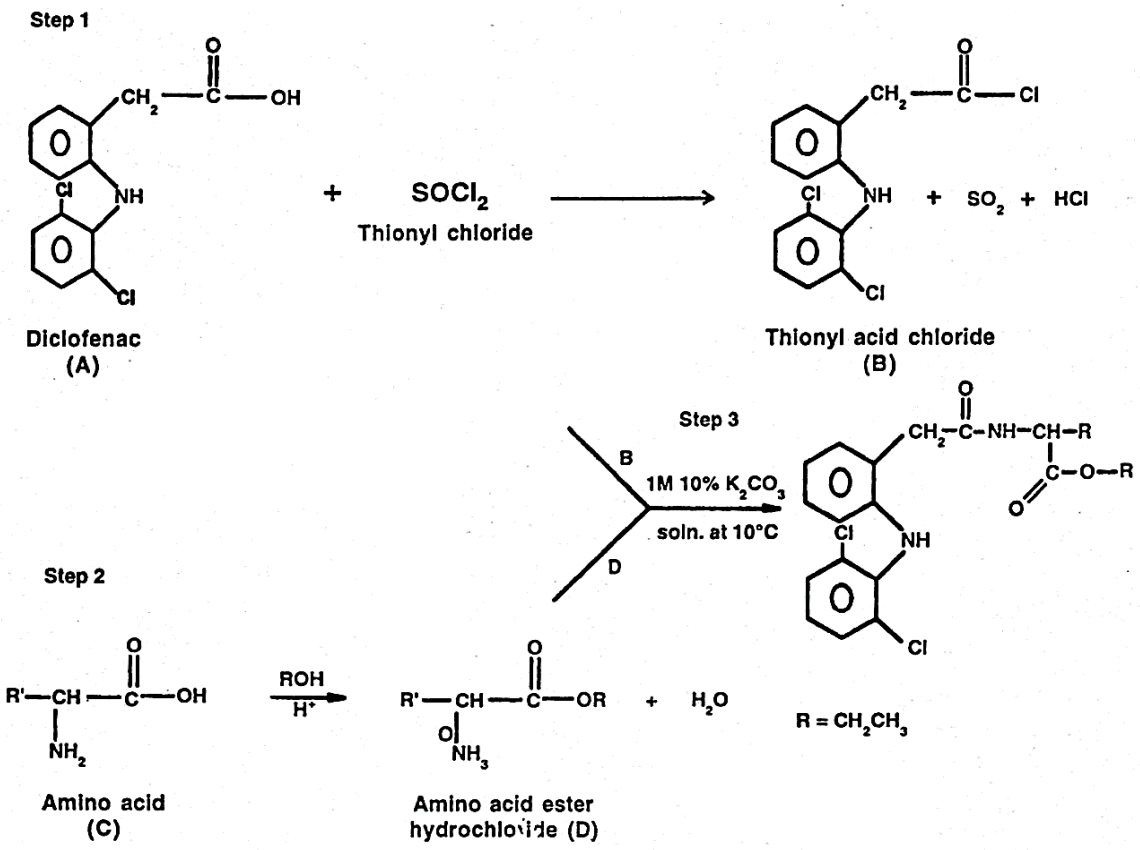
S. No.	Prodrugs	No. of rats taken	Normal reaction time (sec)	Reaction time after drug admn. (sec)	Mean change in reaction time (sec)	p value
1.	I	6	6.35	9.56	3.21	<0.05
2.	II	6	6.48	9.6	3.17	<0.05
3.	III	6	6.40	9.44	3.03	>0.05
4.	IV	6	6.40	9.53	3.121	<0.05
5.	V	6	6.23	9.2	3.056	<0.05
6.	VI	6	6.37	9.3	3.046	<0.05
7.	Control	6	6.23	–	–	–
8.	Standard	6	6.37	8.43	2.058	<0.05

Test dose equivalent to 25 mg/kg body weight of diclofenac
Standard-Diclofenac free base (25 mg/kg body weight)

Table 3: Comparative study of the analgesic activity of synthesized prodrugs with diclofenac free base

S. No.	Prodrugs	No of rats taken	Mean change in paw volume ± S.E.	Percentage Inhibition
1.	I	6	0.63	29.17
2.	II	6	0.625	30.54
3.	III	6	0.626	30.42
4.	IV	6	0.614	31.72
5.	V	6	0.623	30.69
6.	VI	6	0.649	27.80
7.	Control	6	0.9	–
8.	Standard	6	0.698	22.48

Test dose = equivalent to 25 mg/kg body weight of diclofenac
Standard = Diclofenac free base (25 mg/kg body weight)



(I) R'=H
(II) R'=CH_3
(III) R'=$-CH_2-C_6H_5$
(IV) R'=CH_2-OH
(V) R'=$CH(OH)-CH_3$
(VI) R'=$CH_2-C(=O)-OC_2H_5$

5. Biological evaluation

a) Anti-inflammatory activity

Anti-inflammatory activity' of the drug and synthesized prodrugs was performed using carageenan induced hind paw oedema method. Albino rats (100-200g) of either sex were divided into 8 groups having six in each group. The drug and prodrugs were administered as 1% gum acacia suspension orally 30 minutes before the injection of 0.1 mL of 1% suspension of carageenan into subplantar region of right hind paw. Paw volume was measured before the three hours after the carageenan administration by plethysometer. The change of paw volume was compared with that of control as well as with standard test group and expressed as percent inhibition (Table 2)

b) Analgesic activitys [5]

D'Amour smith tail flick test was adopted for the screening of analgesic activity. Albino rats (100-200 g) of either sex were divided into 8 groups having 6 animals each. The rats were placed in a rat holder through which tail of rat protruded out. Current was adjusted so that more than 90% rats gave tail flick response within five seconds and in no case it exceeds six seconds. Drug (25 mg/Kg body weight) and prodrug (equivalent to drug/Kg body weight) were administered orally. Percentage threshold was measured after one hour of administration of test compounds (Table 3).

c) Ulcerogenic activity [6]

It was determined by the method of Hitcha etal. The test compound and standard were administered orally as a suspension in 2% gum acacia. Male albino rats weighing between 100-200 g were starved for 18 hours but water was given as libitum. Animals were divided into groups of four and the compound fed orally. The control was fed only 2% gum acacia suspension. Approximately 5 hours after drug administration, the rats were sacrificed and the stomach was clamped with homostat at the oesophagal and pyloric ends and mounted on a flat surface and lesion were examined by means of binocular. All ulcer > 0.5 mm were counted and average number of ulcer were calculated (Table 4).

Table 4: Ulcerogenic index of the synthesized prodrugs with diclofenac free base prodrugs with diclofenac free base

S. No.	Prodrugs	No. of rats taken	Mean ulcer score (ulcerogenic index)
1.	I	2	0.5
2.	II	2	0.5
3.	III	2	0.5
4.	IV	2	0.5
5.	V	2	0.5
6.	VI	2	0.5
7.	Control	2	2

Test dose = equivalent to 25 mg/Kg body weight of diclofenac
Standard = Diclofenac free base (25 mg/Kg body weight)

Results and Discussion

Amino acid analogues of diclofenac as prodrugs were synthesized and characterized by IR, solubility studies. Purity was checked by TLC using silica gel G. Prodrugs show slow hydrolysis in SGF and were moderately hydrolysed in SIF (Table 1). All prodrugs showed significant analgesic activity as compared to parent drug (Table 3). The antiinflammatory activity followed excellent increase in percentage oedema inhibition with respect to the parent drug (Table 3). Study showed that ulcerogenic index is very less comparison to parent drug (diclofenac).

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