



# **SYNTHESIS, CHARACTERIZATION AND ULCEROGENIC-ANALGESIC PROFILE OF SOME AMINO ACID-DICLOFENAC CONJUGATES**

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## **ABSTRACT**

Various amino acid conjugates of diclofenac were synthesized by reacting amino acid methyl esters with the carboxylic acid moiety of diclofenac using N, N'-dicyclohexylcarbodiimide as a coupling agent. The synthesized compounds were characterized by physico-chemical and spectral studies like IR and <sup>1</sup>H NMR. The compounds synthesized were screened for analgesic and ulcerogenic activity by Acetic acid induced method and pyloric ligation induced ulceration method, respectively. All the compounds exhibited significant analgesic activity as compared to diclofenac sodium with less ulcerogenic activity.

**Key words:** Diclofenac, Amino acid methyl esters, Analgesic activity, Ulcerogenic activity

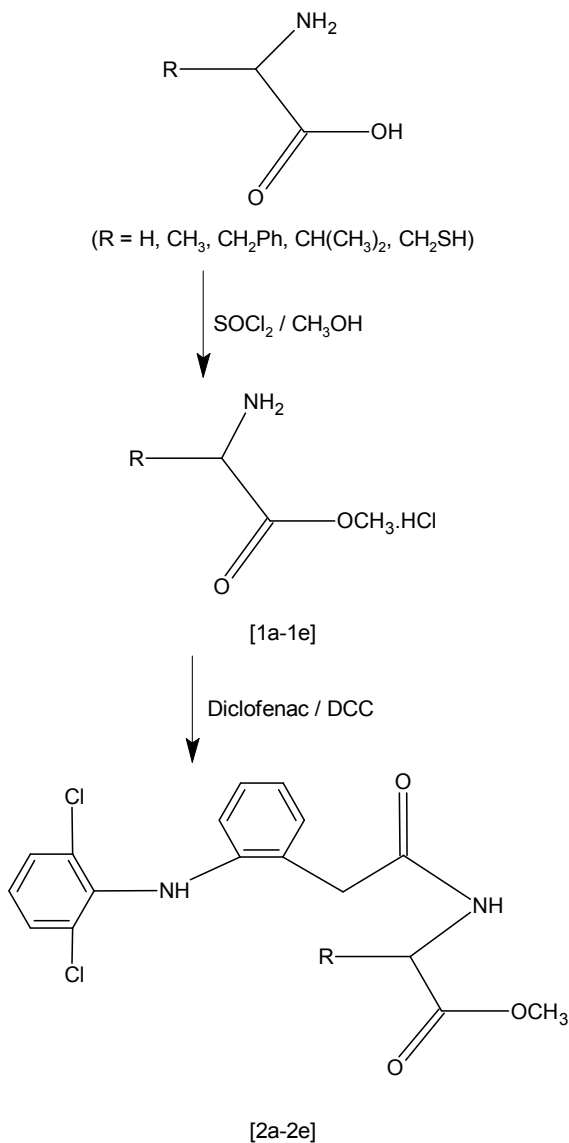
## **INTRODUCTION**

Diclofenac is a traditional NSAIDs drug belonging to propionic acid subclass of compounds<sup>1</sup>. As in the case with other NSAIDs, diclofenac also suffers from gastric discomfort to gastric bleeding<sup>2</sup>. The production of gastrointestinal lesions is probably a combination of local irritation produced by the free carboxylic acid group of NSAIDs and local inhibition of cytoprotective action of prostaglandins on gastric mucosa. The considerable GI distress associated with chronic use of these compounds and their low half life constitutes the main disadvantage in clinical use of NSAIDs<sup>3</sup>. So in order to suppress the GI irritation produced by diclofenac, which is believed to cause mainly the direct contact of acidic group with GI mucosa, a structural modification has to be carried out

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which will mask the carboxylic group<sup>4</sup>. The incorporation of amino acid will not only protect the otherwise vulnerable group and stabilize the molecule, but it will also direct the drugs to in specific target site. Moreover amino acids have healing effect on gastric lesions produced by NSAIDs. In the present work, we report the synthesis of conjugates of diclofenac with various amino acids glycine, alanine, phenylalanine, valine and cysteine and their evaluation for analgesic and ulcerogenic profile.

**Scheme**

## EXPERIMENTAL

### Materials and methods

Progress of all the reactions was monitored by thin-layer chromatography using Merck precoated silica gel GF 254. Compounds were purified by column chromatography using silica gel 60-120 mesh from Merck. Melting points were determined by open capillary method and are uncorrected. The IR spectra of the compounds were recorded on FTIR Shimadzu 8400 FT- IR spectrophotometer by KBr disc method. The  $^1\text{H}$  NMR spectra were recorded on Bruker Advance II 400 NMR spectrophotometer at 300 MHz using DMSO as solvent. Elemental analysis results were within  $\pm 0.4\%$  of theoretical values. The physical, analytical and spectral data are given in Tables 1 and 2.

All the protocols of animal experiments have been approved by the Institutional animal ethics committee (IAEC).

### Synthesis of methyl ester hydrochloride of glycine (1a)

Fresh distilled thionyl chloride (0.05 mole + 30 % excess; 5 mL) was slowly added to precooled 100 mL of methanol followed by addition of glycine (0.1 mol). The reaction mixture was refluxed for 5 hrs at 60-70°C with continuous stirring under anhydrous condition. Excess of thionyl chloride was removed by distillation. The crude product so obtained was triturated with 20 mL of cold ether at 0°C to remove excess dimethyl sulphite to afford methyl ester hydrochloride of glycine. The crude product was recrystallized from hot methanol with slow addition of 15-20 mL of ether and kept over night in refrigerator. The crystals were collected and washed twice with ether : methanol mixture (5 : 1) followed by pure ether. Similarly, other compounds (**1b-1e**) were prepared.

### Synthesis of diclofenac conjugate of glycine methyl ester (2a)

Triethylamine (0.01 mol, 1.01 g, 1.39 mL) was slowly added to a methanolic solution of glycine methyl ester hydrochloride (0.01mol) and stirred for 2 hrs at 0°C and filtered. The excess of methanol was removed by rotatory evaporator to get sticky glycine methyl ester and dried under reduce pressure. N, N'-Dicyclohexylcarbodiimide (0.01 mol, 2.063 g) was added at 0°C to a solution of diclofenac (0.01 mol, 2.96 g) and dimethyl aminopyridine (0.001 mol, 0.122 g) in dry dichloromethane. To this reaction mixture, glycine methyl ester in dry dichloromethane was added and stirred at 0°C for 2 hrs followed by 30 hrs at room temperature<sup>5</sup>.

Table 1: Physical and analytical data of synthesized compounds

Compound	R	Mol. formula/ Mol. wt.	m. p. (°C)	Yield (%)	R <sub>f</sub> (cm)*	λ <sub>max</sub> in methanol (nm)	% of N (Calculated/ found)
<b>2 a</b>	-H	C <sub>17</sub> H <sub>16</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>3</sub> / 366.05	155-157	56	0.46	331	7.63/7.61
<b>2 b</b>	-CH <sub>3</sub>	C <sub>18</sub> H <sub>18</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>3</sub> / 380.07	167-169	38	0.44	324	7.35/7.31
<b>2 c</b>	-CH <sub>2</sub> Ph	C <sub>24</sub> H <sub>22</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>3</sub> / 456.10	163-164	48	0.32	322	6.13/6.16
<b>2 d</b>	-CH(CH <sub>3</sub> ) <sub>2</sub>	C <sub>20</sub> H <sub>22</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>3</sub> / 408.10	179-180	37	0.47	326	6.84/6.80
<b>2 e</b>	-CH <sub>2</sub> SH	C <sub>18</sub> H <sub>18</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>3</sub> S / 413.00	165-166	53	0.29	329	6.78/6.79

\*Solvent system : Benzene: Ethyl Acetate (2:1)

**Table 2: Spectral data of synthesized compounds**

Compound	IR (KBr) $\text{cm}^{-1}$ and $^1\text{H}$ NMR (DMSO- $d_6$ ) ( $\delta$ )
<b>2 a</b>	IR : 3405.04 (N-H), 1730.64 (C=O), 1684.6 (CONH) and 3057 (Ar-H); $^1\text{H}$ NMR : 7.19-7.23 (m, 3H, Ar-H), 2.12 (s, 1H, Ar-NH), 6.86-6.89 (m, 4H, Ar-H), 3.66 (s, 2H, $\text{CH}_2$ ), 7.44-7.45 (d, 1H, $\text{CH}_2$ ), 1.83 (s, 3H, $\text{CH}_3$ ) and 4.1-4.4 (t, 2H, $\text{CH}_2$ )
<b>2 b</b>	IR : 3412.06 (N-H), 1734.07 (C=O), 1685.1 (CONH) and 3054 (Ar-H); $^1\text{H}$ NMR : 7.18-7.22 (m, 3H, Ar-H), 2.10 (s, 1H, Ar-NH), 6.86-6.88 (m, 4H, Ar-H), 3.7 (s, 2H, $\text{CH}_2$ ), 7.42-7.44 (d, 1H, CONH), 4.1-4.2 (d, 3H, $\text{CH}_3$ ), 2.8-2.9 (m, 1H, CH) and 1.82 (s, 3H, $\text{CH}_3$ )
<b>2 c</b>	IR : 3392 (N-H), 1737.3 (C=O), 1652 (CONH) and 3048 (Ar-H); $^1\text{H}$ NMR : 7.17-7.22 (m, 3H, Ar-H), 2.10 (s, 1H, Ar-NH), 6.86-6.88 (m, 4H, Ar-H), 3.7 (s, 2H, $\text{CH}_2$ ), 7.42-7.44 (d, 1H, CONH), 2.85-2.91 (m, 1H, CH), 4.35-4.37 (d, 2H, $\text{CH}_2$ ) and 1.82 (s, 3H, $\text{CH}_3$ )
<b>2 d</b>	IR : 3370.24 (N-H), 1754.6 (C=O), 1654.2 (CONH), 3024.7 (Ar-H) and 1382 ( $\text{CH}(\text{CH}_3)_2$ ); $^1\text{H}$ NMR : 7.17-7.22 (m, 3H, Ar-H), 7.40-7.41 (d, 1H, CONH), 6.86-6.88 (m, 4H, Ar-H), 2.10 (s, 1H, Ar-NH), 3.7 (s, 2H, $\text{CH}_2$ ), 3.08-3.14 (d, 6H, $\text{CH}_3$ ), 2.85-2.91 (m, 1H, CH) and 1.81 (s, 3H, $\text{CH}_3$ )
<b>2 e</b>	IR : 3317.8 (N-H), 1732 (C=O), 1654 (CONH), 3034 (Ar-H) and 2568.2 (SH); $^1\text{H}$ NMR : 7.17-7.22 (m, 3H, Ar-H), 7.41-7.42 (d, 1H, CONH), 6.86-6.88 (m, 4H, Ar-H), 1.66 (s, 1H, SH), 3.7 (s, 2H, $\text{CH}_2$ ), 2.85-2.91 (m, 1H, CH), 2.10 (s, 1H, Ar-NH) and 1.81 (s, 3H, $\text{CH}_3$ )

The reaction mixture was filtered and filtrate was washed with 1M hydrochloric acid, 5 % sodium bicarbonate and saturated sodium chloride solution respectively. The organic layer was dried over sodium sulphate and concentrated under reduced pressure to yield the product. Other compounds (**2b-2e**) were prepared by similar method with slight variation in reaction time.

### Conversion of diclofenac sodium to diclofenac base:

Saturated solution of diclofenac sodium (20 g) in methanol was prepared followed by addition of 1 M hydrochloric acid until the precipitate occurs. The crude product was filtered, washed and recrystallized from methanol. Yield: 18.94 g (94.7 %), m. p.: 64-66°C.

### Pharmacological Studies

Ulcerogenic activity was determined by Pylorus ligation induced ulcer model, using diclofenac sodium as standard. Wister Albino rats, weighing  $180 \pm 20$ g, were randomly distributed in control and test groups of six animals each and kept under fasting state for 12 hrs. Parent drug and test compounds were administered orally by gastric probe, using 2% w/v tween 80 as a vehicle, 30 minutes prior to pyloric ligation of the respective groups. To the control, only 2% w/v tween 80, the vehicle was given. Under phenobarbitone anesthesia, the abdomen was opened and the pylorus was ligated. The abdomen was then sutured. At the end of 4 hrs after ligation, the animals were sacrificed by chloroform inhalation, and the stomach was dissected out. The gastric content was collected and the volume was measured. The stomach was then opened along the greater curvature and the ulcer index was determined by examining the inner lining of each stomach<sup>6-8</sup>.

Analgesic activity was evaluated by acetic acid writhing induced model. Albino mice weighing  $50 \pm 20$  g were randomly distributed in control and test groups of six animals each. Each of the synthesized compounds and standard were orally administered in 2% w/v tween 80 suspension. After 1 hr, 0.6 % of acetic acid in 0.9 % saline solution was given intraperitoneally and the number of writhes for each mice was counted for 20 minutes period between 5 and 25 minutes after the acetic acid injection<sup>9,10</sup>. The average number of writhes in each group of drug treated animals was compared with that in the control group and the degree of analgesia was expressed as percent inhibition according to the following formula –

$$\% \text{ Inhibition in writhes} = \left( 1 - \frac{\text{Average number of writhes with standard or test}}{\text{Average number of writhes with control}} \right) \times 100$$

The results of pharmacological activities are summarized in Table 3.

**Table 3: Pharmacological activities of diclofenac - amino acid conjugates**

Design of treatment	Analgesic activity (% inhibition)	Ulcerogenic activity		
		Vol. of gastric secretion after 24 hrs (ml)	Total acid output (meq/ml)	Ulcer index
Control [2%(w/v) Tween 80]	-	9.5 ± 0.23 <sup>a</sup>	24.3 ± 0.19 <sup>a</sup>	2.965
Diclofenac Sodium	16.00 ± 1.80	6.1 ± 0.01 <sup>b</sup>	60 ± 0.30 <sup>b</sup>	4.125
<b>2a</b>	25.70 ± 1.28	5.2 ± 0.02 <sup>b</sup>	22.4 ± 0.11 <sup>b</sup>	2.475
<b>2b</b>	38.00 ± 1.90	5.3 ± 0.03 <sup>b</sup>	23.8 ± 0.15 <sup>b</sup>	2.624
<b>2c</b>	29.71 ± 2.49	5.1 ± 0.02 <sup>b</sup>	21.9 ± 0.13 <sup>b</sup>	2.128
<b>2d</b>	37.49 ± 1.87	4.9 ± 0.03 <sup>b</sup>	22.3 ± 0.17 <sup>b</sup>	2.364
<b>2e</b>	29.55 ± 3.33	5.6 ± 0.01 <sup>b</sup>	24.1 ± 0.12 <sup>b</sup>	2.749

Six rats were used in each treatment. Values (Mean ± SEM)

The statistical significance of difference between means was calculated by student's unpaired 't' test.

<sup>a</sup>Insignificant as compared to normal control;

<sup>b</sup>p < 0.05. Significant as compared to normal control.

## RESULTS AND DISCUSSION

Amino acids on reaction with thionyl chloride and methanol yielded methyl ester of amino acids which on reaction with diclofenac base using DDC gave the title compounds. The synthesized compound were evaluated for their analgesic activity by acetic acid writhing induced model. All the compounds (**2a-2e**) have shown significant activity as compared to standard diclofenac sodium.

Ulcerogenic activity was determined by pyloric ligation model using diclofenac sodium as standard. All the compounds have shown to possess very less ulcerogenic tendency.

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