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Synthesis and anti-phlogistic potency of some new non-proteinogenic amino acid conjugates of "Diclofenac"*

Review Article

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Summary. In search for more potent, particularly less ulcerogenic gastritis that hopefully replace the universal NSAID "Diclofenac", (2-[(2,6-dichlorophenyl)amino]-phenylacetic acid, C.A.S. 15307-86-5), twelve new non-proteinogenic amino acid conjugates of the drug, namely that of sarcosine, β-alanine, D-leucine and D-phenylalanine, were synthesized and biologically screened for their anti-inflammatory, analgesic and ulcerogenic activity in rats.

"Diclofenac" amino acid esters (IIa–d), were synthesized *via* the corresponding HOSu or HOBt active esters. Alkaline hydrolysis (NaOH) followed by acidification ($KHSO_4$) or thioamide formation (Lawsson's Reagent, C.A.S. 19172-47-5), afforded the corresponding free acids IIIa–d or the thioamides IVa–d respectively.

Interestingly, in contrary to the parent "Diclofenac", the synthesized candidates (except IIId), were entirly nonulcerogenic in rats. Further, they considerably retained a generelized anti-phlogistic activity. The major "Diclofenac" irritating gastric side effect was thus eliminated.

Particularly, the sarcosine conjugate IIa and its thiomimic IVa exhibit promising therapeutic perspectives.

Keywords: Amino acids – Diclofenac – Anti-phlogistics – Non-proteinogenic amino acids – Voltaren®. Thionation – Cyclo-oxygenase inhibitors – Ulcerogenic gastritis

1 Introduction

The anti-phlogistic NSAID "Diclofenac" (I) is a potent cyclo-oxygenase inhibitor. It therapeutically interferes, thereby, with the arachidonic acid cas-

* Preliminary results were presented and discussed at the 5th International Congress on Amino Acids, August 25–29, 1997, Chalkidiki, Greece and abstracted in Amino Acids (1997) 15/1: 75.

cade, prior to the biosynthesis of the inflammatory prostaglandins. The drug has, consequently, a universal anti-phlogistic potency represented by generalized anti-inflammatory, anti-pyretic, anti-rheumatic and analgesic characteristics (Robinson, 1977; Kass, 1982, Evens, 1979; Scherrer and Whitehouse, 1974).

However, several undesired, side effects of the drug, namely its ulcerogenicity, frequently restrict its remedial recommendation. The drug is, thus, contra-indicated to highly gastro-intestinal ulcer-risk subjects. The search for potential substitutes of the drug, while essentially having a facile synthetic accessibility is, therefore, an updated area of anti-pholgistic drug research.

In this context, the conception, optimized synthesis, purification and investigation of the anti-phlogistic potency of twelve new non-proteinogenic amino acid conjugates of the drug, were undertaken.

2 Materials and methods

2.1 Chemistry

General methodology

"Diclofenac" sodium, (Voltaren)®, was kindly supplied by Ciba-Geigy, Egypt. The salt was quantitatively liberated, affording the free acid by a method analogous to that followed by Spangenberg et al., 1971, for t-butyloxycarbonyl amino acid dicyclohexylamine salts.

The used chemicals and solvents were obtained from Aldrich Chemical Co. (Steinheim, Germany) and E. Merck (Darmstadt, Germany).

Solvents were, of an analytical grade and were dried over the suitable molecular sieve (4A° or 3A°). Their removal from the reaction mixtures was attained under reduced pressure and at room temperature in a rotatory evaporator.

Chromatographic (TLC, silica gel aluminium sheets $60F_{254}$ (E. Merck) monitoring of reactions and purity check were identical to the methodology and under the experimental conditions followed for other amino acid derivatives (Abo-Ghalia and Soliman, 1996). While "S" stands for a chromatographic solvent system of chloroform/acetic acid methanol, 85/5/10 by volume, S_1 and S_2 represent the same solvent systeme to which petroleum ether was added, in an equal or half ratio volume respectively.

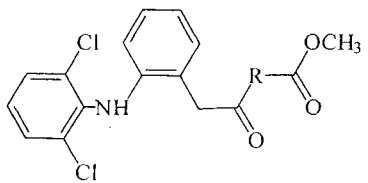
Melting points were determined in glass capillary tubes on an "Electrothermal SMP-1-Sturat" (England) apparatus and were uncorrected. Optical rotations were measured by a "Polax-D polarimeter (ATAGO, Japan), provided by a SI-Na-1 Sodium lamp (Toshiba, Japan). IR spectra (KBr), were carried out on "Jasco FT/IR-300E Fourier transform" infrared spectrometer. ^{1}H-NMR spectra were run on Jeol Ex-270 MHz spectrometer. Mass spectra run on "Finnigan SSQ-7000" spectrometer. Elemental microanalysis for carbon, hydrogen and nitrogen was performed in the "Micro-Analytical" unit of Cairo University.

2.2 Synthetic methods

Synthesis of "Diclofenac" amino acid esters (**IIa–d**, Table 1). Active ester method

To a stirred cold solution (≈−5°C) of "Diclofenac" (1 g, 3.38 mmol) and N-hydroxysuccinimide (0.46 g, 3.38 mmol) in THF (30 ml), DCCI solution (≈0.7 g, 3.38 mmol) in THF (5 ml), was added over 30 min. A cold (0°C) stirred suspension of the amino acid ester hydrochloride (3.38 mmol), containing Et_3N (≈0.5 ml, 3.38 mmol) in

Table 1. Physical data of "Diclofenac" amino acid esters (**IIa–d**)



Comp. No.	Configuration	Amino acid residue	M.P. (°C)	yield %	$[\alpha]^{30}$ D (C = 1, THF)	Molecular formula (M.Wt.)	Analysis found/required			$R_f \times 100$	
							C	H	N	S_1	S_2
IIa	–	Sar	117–118	30	–	$C_{18}H_{18}Cl_2N_2O_3$ (381.24)	56.71 56.90	4.76 4.70	7.35 7.30	69	45
b	–	β-Ala	110–12	60	–	$C_{18}H_{18}Cl_2N_2O_3$ (381.24)	56.71 56.64	4.76 4.80	7.35 7.40	46	40
c	D-	Leu	98–100	35	+60	$C_{21}H_{24}Cl_2N_2O_3$ (423.32)	59.58 59.50	5.71 5.70	6.62 6.58	70	50
d	D-	Phe	143–4	60	−55	$C_{24}H_{22}Cl_2N_2O_3$ (457.34)	63.03 62.80	4.85 5.02	6.13 6.18	77	55

IIa, R = -$N(CH_3)CH_2$- (Sar); **IIb, R** = -$NH(CH_2)_2$- (β-Ala); **IIc, R** = -$NHCH[CH_2CH(CH_3)_2]$- (D-Leu); **IId, R** = -$NHCH(CH_2$-$C_6H_5)$- (D-Phe).

THF (10 ml), was then added. Stirring was continued at the same temp. for 3 h, at pH 8 (Et_3N) and at 0°C over night, then at room temp. for 48 h. Drops of glacial acetic acid were added to the cold (0°C) suspension and the reaction mixture was filtered off. The filtrate was evaporated, and the residue was taken up in EtOAc, washed with $NaHCO_3$ (5%, 3 × 10 ml), distilled water (3 × 10 ml), $KHSO_4$ (5%, 3 × 10 ml) and distilled water (3 × 10 ml), and concentrated to dryness. The residue was chromatographically purified (TLC), giving rise to the desired products.

Synthesis of "Diclofenac" amino acids (**III a–d**, Table 2)

To a methanolic solution (10 ml), of compounds IIa–d (≈1 mmol), a NaOH solution (0.5 N, 26 ml) was added and the mixture was stirred for ≈1 h. The mixture was then neutralized ($KHSO_4$, 1 N, ≈3 ml), and evaporated. Water (50 ml), was then added and the mixture was washed with EtOAc (3 × 10 ml). The cold reaction mixture (0°C) was acidified (pH 2–3, $KHSO_4$, 1 N), and extracted with EtOAc (3 × 30 ml). The combined extracts were washed with water (3 × 10 ml), and dried (Na_2SO_4), evaporated, and finally solidified by addition of pet. ether (b.p. 40–60°C).

Synthesis of thionated "Diclofenac" amino acid esters (**IVa–d**, Table 3)

Compound **IIa–d** (≈0.5 mmol), and Lawesson's Reagent (0.1 g, ≈0.25 mmol), were refluxed at 80°C in anhydrous benzene (10 ml) for ≈90 min. The reaction mixture was decolorized (charcoal), kept cold (0°C) overnight, filtered off and the solvent was then evaporated. The oily residue was chromatographed on preparative silica gel G_{60} plates affording the compounds **IVa–d**.

2.3 Anti-phlogistic potency

Rats (adult male Albino, 100–110 gm.), were obtained from the animal house colony of the National Research Centre, Dokki, Cairo (Egypt). They were randomly assigned to groups of tens. Each group was individually housed and fed on a standard laboratory diet. Rats were orally dosed with 10% aqueous propylene glycol (negative control group) or with Voltaren® (Ciba-Geigy, 20 mg, ≈0.068 mmol/kg rat body weight, positive control group) or with a tested candidate (0.068 mmol/kg rat body weight) both in the same glycol medium. For each group, the mean value of the obtained results were then considered.

Statistical analysis of the data was computed via the Student's "t-test". A 0.05 level of propability was regarded as significant according to Sendecor and Cochran (1971).

The following investigative tests were carried out and the results are listed in Table 5:

A Ulcerogenic effect
The acute ulcerogenicity (gastric mucosol eroding action), of the compounds was examined according to the methodology reported by Corell et al. (1979).

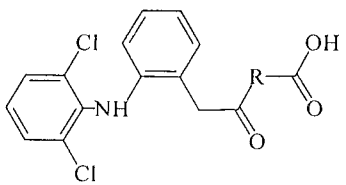
B Carrageenan foot pad oedema (Acute anti-inflammatory effect)
The procedure of Winter et al. (1962), was adopted for that activity.

C Carrageenan induced pleurisy (Acute anti-inflammatory effect)
The method of Velo et al. (1973), was followed. The volume of the pleural exudate was measured according to the methodology of Corell and Hasselmann (1983).

D Cotton pellet mean gain (chronic anti-inflammatory effect)
The activity was evaluated according to Meier et al. (1950).

E Analgesic activity (Electric stimulation test)
The activity was investigated as described by Charlier et al. (1961).

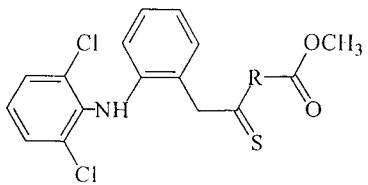
Table 2. Physical data of "Diclofenac" amino acids (**IIIa–d**)



Comp. No.	configuration	Amino acid residue	M.P. (°C)	yield %	$[\alpha]^{30}$ D (C = 1, THF)	Molecular formula (M.Wt.)	Analysis found/required			$R_f \times 100$	
							C	H	N	S_1	S_2
IIIa	–	Sar	160–161	80	–	$C_{17}H_{16}Cl_2N_2O_3$ (367.21)	55.61 55.51	4.39 4.41	7.63 7.61	38	11
b	–	β-Ala	180–183	85	–	$C_{17}H_{16}Cl_2N_2O_3$ (367.21)	55.61 55.62	4.39 4.50	7.63 7.70	28	10
c	D-	Leu	95–96	75	−20	$C_{20}H_{22}Cl_2N_2O_3$ (409.29)	59.69 58.80	5.42 5.45	6.84 6.70	39	12
d	D-	Phe	138–140	90	+45	$C_{23}H_{20}Cl_2N_2O_3$ (443.31)	62.32 62.40	4.55 4.50	6.32 6.40	36	15

IIIa, R = -$N(CH_3)CH_2$- (Sar); **IIIb, R** = -$NH(CH_2)_2$- (β-Ala); **IIIc, R** = -$NHCH[CH_2CH(CH_3)_2]$- (D-Leu); **IIId, R** = -$NHCH(CH_2\text{-}C_6H_5)$- (D-Phe).

Table 3. Physical data of thionated "Diclofenac" amino acid esters (**IVa–d**)



Comp. No.	Configuration	Amino acid residue	M.P. (°C)	yield %	$[\alpha]^{30}$ D (C = 1, THF)	Molecular formula (M.Wt.)	Analysis found/required				$R_f \times 100$	
							C	H	N	S	S_1	S_2
IVa	–	Sar	84–85	85	–	$C_{18}H_{18}Cl_2N_2O_2S$ (397.31)	54.42 54.30	4.57 4.50	7.05 7.10	8.07 8.10	76	64
b	–	β-Ala	126–8	80	–	$C_{18}H_{18}Cl_2N_2O_2S$ (397.31)	54.42 54.30	4.57 4.50	7.05 7.10	8.07 8.10	60	59
c	D-	Leu	118–20	85	−30	$C_{21}H_{24}Cl_2N_2O_2S$ (439.39)	57.41 57.30	5.51 5.50	6.38 6.41	7.30 7.31	89	80
d	D-	Phe	80–82	90	−80	$C_{24}H_{22}Cl_2N_2O_2S$ (473.40)	60.89 60.88	4.68 4.65	5.92 5.79	6.77 6.80	90	76

IVa, R = -$N(CH_3)CH_2$- (Sar); **IVb, R** = -$NH(CH_2)_2$- (β-Ala); **IVc, R** = -NHCH[$CH_2CH(CH_3)_2$]- (D-Leu); **IVd, R** = -NHCH(CH_2-C_6H_5)- (D-Phe).

3 Results

3.1 Chemistry

Scheme 1 resumes the followed synthetic routes and Table 1–4, group the obtained convenient analytical, physical and spectroscopic data.

3.2 Anti-phlogistic potencies

Table 5 presents the obtained anti-phlogistic potencies of the candidates.

4 Discussion

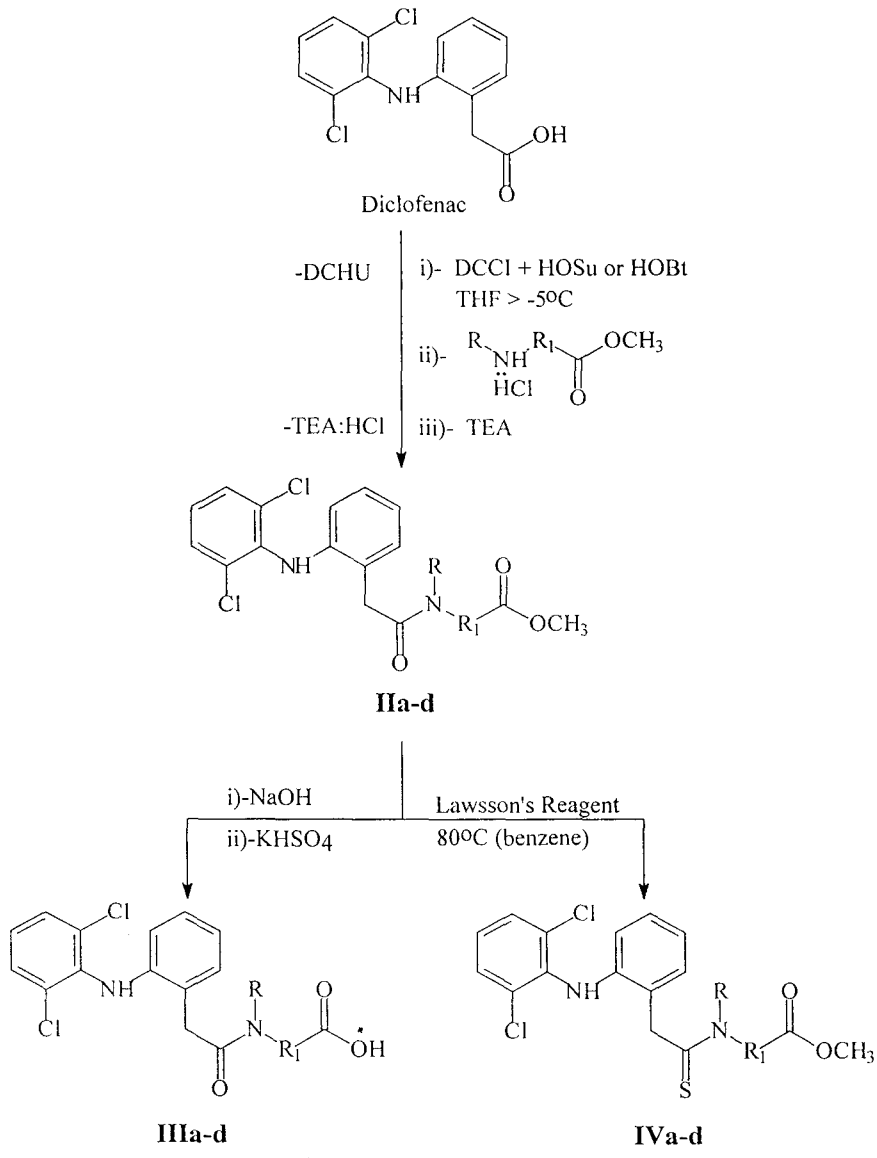
4.1 Preconception of the conjugates

Due to their several unwanted side effects, the search for more potent anti-phlogistics is still an updated area of drug research.

Alternatively, substantial literature evidence, examplified by our works (Abo-Ghalia et al., 1979, 1988, 1996) indicated that, amino acids and peptides, being taxonomically natural, physiologically non-toxic and chemically multi-functional, their rationalized conjugates with biologically active synthetic organic agents, are hypothesized to be more potent, particularly less toxic than their parent biologicals. Such presumed potentiation could be attributed to a possible enhanced receptor binding selectivity, better solubility, more favorable transport properties, pharmacologically adequate pattern of enzymatic degradation and a general physiological compatibility. Further, the probability of these conjugates for acting as effective pro-drugs or pharmacophores, that more effectively liberate their active principles, is not excluded.

Consequently, one of the ever considering principles of recent drug design research, is the conception of their amino acid and peptide structurally variable congeners. These probing hybrids, by *turns*, could shed light on the acting biological mechanisms. Establishing structure-activity relationships, as well as, facilitating the conception of new drug generations are, likewise, more feasibly achieved.

In fact, as a potentiation approach to suppress its ulcerogenicity, "Diclofenac" was occasionally reported, to be concurrently adminstrated with a variety of amino acids, particularly basic amino acids (Yaginuma et al., 1981, 1982a,b; Morishita, 1982; Kogyo, 1982). Thus, suppository formulations were prepared and tested. In spite of the fact that such formulations were mere drug/amino acid mechanical salt mixtures, yet it was reported that less irritating effect on rat rectal mucosa in rats, was achieved. However, interestingly, except for some promising exploratory trials from this department (Shalaby et al., 1997), null approach for the synthesis and investigation of amino acid covalent condensates of the drug, was yet reported.



R	R_1
CH_3	$-CH_2-$ (Sar)
H	$-CH_2-CH_2-$ (β-Ala)
H	$-CH(CH_2CH(CH_3)_2-$ (D-Leu)
H	$-CH(CH_2-C_6H_5)-$ (D-Phe)

Scheme 1. Synthesis of "Diclofenace" amino acid conjugates

Table 4. ^{1}H-NMR Spectral data of "Diclofenac" amino acid conjugates (**II–IVa–d**)

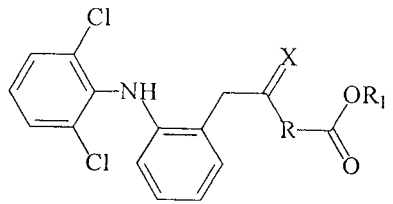
Assignment	Protons chemical shift (δ ppm)						
Comp. No.	Aromatic & NH	CH_2- benzyl	α-CH-	β-CH-	γ-CH-	Ester protons	Other protons
IIa	6.25–7.50 (m,7H)	3.85(s)	3.15(s,2H)	–	–	3.60 (s,3H)	4.10(s,3H,N-CH_3), 7.75(s,1H, NH)
b	6.49–7.41 (m,8H)	3.66(s)	2.51–2.57 (t,2H)	3.48–3.50 (m,2H)	–	3.66 (s,3H)	6.36(bt,1H,NH)
c	6.14–7.35 (m,9H)	3.70(s)	4.67–4.68 (m,1H)	1.57–1.60 (m,2H)	1.57–1.60 (m,1H)	3.70 (s,3H)	0.9(m,6H,2CH3 ipr)
d	6.25–7.45 (m,12H)	3.55(s)	4.55(q,1H)	2.80–3.15 (m,2H)	–	3.55 (s,3H)	7.8(s,1H,NH), 8.75(d,1H,NH)
IIIa	6.51–7.35 (m,7H)	3.92(s)	3.27(s,2H)	–	–	–	4.19(s,3H,NH, N-CH3), 3.03(s,1H,NH)
b	6.48–7.36 (m,9H)	3.67(s)	2.50–2.60 (bm,2H)	3.50–3.54 (bm,2H)	–	–	–
c	6.20–7.40 (m,9H)	3.75(s)	4.50–4.70 (m,1H)	1.50–1.80 (m,2H)	1.20–1.45 (m,1H)	–	0.8–1.0(m,6H, 2CH3, ipr.)
d	6.40–7.30 (m,14H)	3.13(s)	4.85 (bm,1H)	3.63–3.66 (q,2H)	–	–	–

Table 4. *Continued*

Assignment	Protons chemical shift (δ ppm)						
Comp. No.	Aromatic & NH	CH_2- benzyl	α-CH-	β-CH-	γ-CH-	Ester protons	Other protons
IVa	6.48–7.35 (m,8H)	4.33(s)	3.49(s,2H)	–	–	3.78 (s,3H)	4.80(s,3H,N-CH3)
b	6.46–7.37 (m,8H)	4.18(s)	2.60–3.70 (t,2H)	3.89–3.90 (q,2H)	–	3.60 (s,3H)	8.06(bs,1H,NH)
c	6.49–7.36 (m,8H)	4.20(s)	5.15–5.18 (q1H)	1.66–1.76 (m,2H)	1.56–1.66 (m,1H)	3.72 (s,3H)	0.85–0.90(m,6H 2CH3. ipr), 7.65–7.75(bs,1H,NH)
d	6.50–7.37 (m,12H)	3.73(s)	4.08–4.30 (q,1H)	3.19–3.30 (m,2H)	–	3.73 (s,3H,CH3)	7.75–7.58(bd,1H,NH), 5.37–5.40(m,1H,NH)

-Frequentely the NH protons were identified by their disappearance after D_2O exchange.
-Otherwise stated the used solvent is CDCl3
-Tetramethylsilane (TMS) is the standard reference
-α,β,γ-refere to the amino acid protons
DMSO-d_6 is the used solvent
Multiplicity: *s* singlet, *d* douplet, *t* triplet, *q* quartet, *m* massive, *b* broad.

Table 5. Anti-phlogistic activity of Diclofenac amino acid conjugates

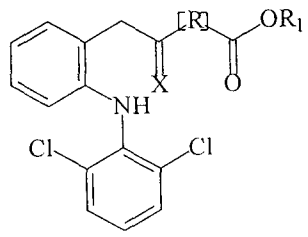


(II,III,IV) a, R = -N(CH_3)-CH_2-, (II,III,IV) b, R = -NH-(CH_2)$_2$-,
(II,III,IV) c, R = -NHCHCH_2(CH_3)$_2$-, (II,III,IV) d, R = -NHCH(CH_2Ph)-

R_1	X	Amino acid	Comp. No.	Anti-inflamatory activity			Analgesic activity (mean volt ±S.E.)	Ulcer number (mean ±S.E.)
				Acute		Cronic		
				% Oedema mean (±S.E.)	Pleural fluid (μl volume ±S.E.)	Mean gain in pellet wt. (mg ±S.E.)		
CH_3	O		**IIa**	18.950 ± 0.090	0.154 ± 0.013	70.110 ± 3.910	150.00 ± 3.190	1.00 ± 0.630
H	O	Sarcosine	**IIIa**	35.380 ± 0.890	0.057 ± 0.012	63.090 ± 3.900	109.00 ± 6.211	1.00 ± 0.010
CH_3	S		**IVa**	17.150 ± 2.360	0.027 ± 0.070	65.110 ± 3.330	180.00 ± 3.930	0.17 ± 0.160
CH_3	O	β-Alanine	**IIb**	59.190 ± 0.710	0.590 ± 0.021	35.440 ± 3.170	156.00 ± 1.990	4.25 ± 2.010
H	O		**IIIb**	45.660 ± 0.940	0.213 ± 0.011	70.010 ± 3.590	137.00 ± 4.360	0 ± 0
CH_3	S		**IVb**	57.190 ± 0.330	0.110 ± 0.043	53.910 ± 6.170	143.57 ± 2.520	1.00 ± 0.210
CH_3	O	D-	**IIc**	43.000 ± 1.900	0.058 ± 0.016	69.010 ± 5.610	116.00 ± 2.920	0.31 ± 0.290
H	O	Leucine	**IIIc**	33.490 ± 3.570	0.314 ± 0.120	67.570 ± 0.600	115.00 ± 3.880	0.21 ± 0.990
CH_3	S		**IVc**	42.510 ± 3.320	0.043 ± 0.014	67.730 ± 0.070	120.00 ± 2.170	3.21 ± 1.170
CH_3	O	D-	**IId**	46.790 ± 0.730	0.411 ± 0.033	65.330 ± 4.110	130.00 ± 2.740	0.40 ± 0.990
H	O	Phenyl	**IIId**	58.110 ± 0.390	0.579 ± 0.014	50.340 ± 3.990	117.51 ± 3.520	+++
CH_3	S	alanine	**IVd**	60.110 ± 0.190	0.410 ± 0.007	43.190 ± 4.110	110.17 ± 1.630	0 + 0
−Ve control group (propylene glycol/H_2O)				57.220 ± 0.310	0.576 ± 0.022	150.990 ± 18.21	81.440 ± 7.770	0 + 0
+Ve control group (Voltarine 20 mg/kg)				12.870 ± 0.010	0.310 ± 0.009	60.370 ± 6.115	165.91 ± 3.330	13.43 ± 0.95

+++ Uncountable number of ulcers; *S.E.* Standard error.

Thereby, it was, herein, appeared plausible to investigate such condensates typfied by the structure:



R = amino acid residue, X = O or S, R_1 = Me or H

Four nonproteinogenic amino acids namely sarcosine, β-alanine, D-leucine and D-phenylalanine, were selected to be chemically condensed with the drug.

Our rational for the selection of the non-proteinogenic amino acids rested upon the fact that, being non-proteinogenic, the *in vivo* resistance of these conjugates to natural hydrolases, is prejudged.

Further, a particular elucidative and supportive rational could be proposed for each of these amino acids as developed in the following:

Sarcosine: Since in the above refered, preliminary study (Shalaby et al., 1997) glycine conjugates of "Diclofenac" seemed to be promising investigatable substitutes of the drug, it was, herein, found advantageous to incorporate sarcosine as the N-methyl positional isomer of glycine. The amino acid, by its turn, is a known glycine natural precursor in the choline/glycine metabolic chain.

β-Alanine: The amino acid is structurally the next higher linear homologue of glycine. Although non-proteinogenic, it is known in other metabolic pathways. Its traces are generally detected in body tissues and its dipeptides with L-histidine are the frequent human skeletal muscle peptides, namely carnosin and anserine. When acylated with pantoic acid, the product, pantothenic acid is a member of indispensable vitamin B-complex group.

D-Leucine and D-phenylalanine: Similar to the other D-amino acids, traces of these isomers are detected in metabolically inert proteins (eg. lenses teeth . . .). When ingested, these amino acids are degraded by D-amino acid oxidases to be used as energy source.

In addition to the conjugation of these four amino acids, carboxyl terminal modulation, as ester or free acid was also intended. Thus, while the free acid conjugates (**IIIa–d**) insured the investigation of less lipophilic, complex forming and/or salt forming ligands, their corresponding esters (**IIa–d**) permited the study of their less ionisable and more lipophilic analogues.

Complementry, since full or partial thionation of biologically active agents is a fascinating proposal when their potentiation is searched for (Clausen, 1981; Yde et al., 1983; Andersen et al., 1983; Thorsen et al., 1983),

it seemed of interest to synthesize and investigate the corresponding thioamide conjugates.

4.2 Synthetic chemistry of "Diclofenac" amino acid conjugates

Since the optical purity equates the chemical purity, as a major concern upon which the chemical structure-biological activity is related, mild conditions (low temperature & moderate pH) and practically non-racemizing amide bond forming methods were principally envisoned for the synthesis of the conjugates. Both the classical carbodiimide coupling method (DCCI) of Sheehan and Hess (1955) and that modified by the manipulation of some recommended additives were experimented. Two additives were applied namely N-hydroxy succinimide (HOSu) and 1-hydroxybenzotriazole (HOBt) were used (Bodanszky and Bodanszky, 1984). The classical DCCI method, although tested with the recently recommended solvents media (THF, CH_2Cl_2), rather than the commonly used DMF and acetonitrile, proved quite inferior to the "additive methodology". The formation of N-"Diclofenac" urea as undesirable significant contaminant, was the essential drawback leading to low yields and served equally well for the obtainment of the desired products with satisfactory yields and purity, as reflected by their recorded chemical, spectroscopic and analytical data.

The application of Lawesson's reagent [2,4-bis(p-methoxyphenyl)-1,3-dithiadiphosphetane, 2,4-disulfide, LR], as a mild and popular thionating agent, rendered attractive the synthesis of the thio mimics of the carbonyls namely amide biologicals to evaluate their activities (Clausen, 1981).

The herein intensive application of manually prepared preparative silica gel thin layer chromatography with U.V. tracing of the desired and side products, rendered facile, their separation in a pure form.

4.3 Apparent toxicity and anti-phlogistic potencies of "Diclofenac"

The investigation of the obtained anti-phlogistic results (Table 5), revealed the following significant criteria:

I Except for the D-phenylalanine conjugate (**IIId**), whose results seemed nonconformist, the other candidates were entirly devoid of ulcerogenicity. The major drawback of the drug, namely its severe pathogenic irritability, with its occasional lethal consequences, was thus, eliminated.

II Again, apart from **IIId**, the consideration of the data of the two evaluation extremes of the candidates, namely the results of both the negative control and the positive control groups of rats, the totality of the candidates retained significant variable anti-phlogistic potencies that displayed by "Diclofenac". Such deduction could be developed via the following remarks:

a Relative to the negative control group (~80 volt) and the drug receiving group (positive group) of rats (~160 volt), all the candidates exhibited

significant, analgesia. (110–156 volt), except for IVa, which proved to be more potent (180 volt).

b Similarly, reviewing the chronic anti-inflammatory data, as reflected by the mean gain in pellet weight test, for both the negative (~150mg) and the positive group of rats (~60mg), all the candidates were either more potent (~30–40mg) or of comparable potency to "Diclofenac" (~70mg).

c The acute anti-inflammatory potency as tested by the induced pleuristy test after a carageenan challenge, revealed that, **6** of the **12** candidates (**IIa**, **IIc**, **IIIa**, **IIIb**, **Iva**, **IVc**), were more potent than the drug ($<0.31\mu l$) for Diclofenac.

d The determined induced oedema (carageenan challenge), reflecting the acute anti-inflammatory potency, indicated that two candidates (**IIa**, **IVa**) were fairly comparable (~17%) to the drug (13%), while the others were either moderately potent or practically inactive.

Besides: if a general view of the obtained results is attempted, some major findings could be outlined in the following:

- Although still retaining a general anti-phlogistic potency, the obtained moderate analgesic activity of both D-leucine and D-phenylalanine conjugates (**II–IV**)**c** and (**II–IV**)**d**, for which the free amino acids were reported to have analgesia by themselves, (Seymor et al., 1984) could indicate that no apparent synergy between the two combined analgesic segments, namely "Diclofenac" and the D-amino acid.
- It was the idenity of the amino acid residue rather than a carboxyl terminal modulation (acid and ester) or a skeletal amide- thioamide transformation that had a major impact on the reflected anti-phlogistic potency of a candidate. In this context, when the data of the five experimented anti-phlogistic parameters were compromisingly compiled, *sarcosine conjugates* (**II–IV**)**a** appeared as the most promissing candidates. Thus, the ester **IIa**, while was confirmed more potent than "Diclofenac" in acute anti-inflammation (pleural fluid test), it appeared, for the same property slightly less active in the induced oedema test. Analogously, fairly comparable chronic anti-inflammatory (pellet weight test) and analgesic activties (electric stimulation test), were found. Thionation of the ester affording the thionated mimic **IVa**, was accompanied by an overall augmentation of the entire anti-phlogistic potency. Its analgesia, however, turned superior then the parent "Diclofenac".

5 Conclusion and perspectives

The ample of the afore-cited data and deductions particularly the confirmed non-ulcerogenicity of the synthesized candidates, strongly plead in favour or our intially proposed hypothesis of the potentiation capability of drug-amino acid conjugation. However, for a more integral view, rathre than the herein presented individual case of "Diclofenac", a more diversification of the conjugatable amino acids seems to be indispensable.

Yet, sarcosine conjugates appear herein as promising substitutes of "Diclofenac" with the *proviso* that the other, not yet studied, pharmacological parameters, as the drug LD_{50}/LE_{50}, the gastro-intestinal/tolerability indices and the hepato- toxicity profiles prove to be satisfactory. If it would be the case and considering the non-ulcerogenicity of the candidates, higher doses, rather than the herein adotepted strict molar equivalent of "Diclofenac", could be then viewed to compromise the inferiorty of one or more encountered anti-phlogistic property of a promissing candidate.

In addition, enzymatic inhibitory studies on cyclo-oxygenase would be of interest to shed lights on the actual functioning anti-phlogistic mechanism of the candidates.

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