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STUDIES ON O-[2, 6-DICHLOROPHENYL-1-AMINO] PHENYL ACETIC ACID, (DICHLOFENAC OR VOLTAREN).

I- Synthsis Anti - inflammatory, Analgesic and Ulcerogenic Activities of some New Amino Acid Conjugates

By

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ABSTRACT

o - [2, 6 - Dichlorophenyl - 1 - amino] phenyl acetic acid (dichlofenac, or voltaren) (I) reacted with some amino acid esters, namely glycine, L - valine, L - diiodotyrosine and L - tryptophan through the active ester (B) to give the corresponding o - [2, 6 - dichlorophenyl - 1 - amino] benzyl carboxy - N - amino acid ester of the type (IIa - d), respectively, which were hydrolysed in alkaline medium to yeild the free amino acids (IIIa - d). Condensation of (IIa - d) with hydrazine hydrate gave the corresponding acid hydrazides (IVa - d), which in turn reacted with trimethoxybenzal - dehyde to give the corresponding Schiff's bases (Va - d), respectively. The

anti - inflammatory, ulcerogenic and analgesic activities of the obtained compounds were investigated.

INTRODUCTION

Dichlofenac (I) is one of the most potent anti - inflammatory and analgesic drugs used nowadays[1 - 6]. Also it is reported that amino acid conjugates improve the pharmacokinetic of drugs[7 - 13].

We aimed in this work to combine some amino acids with dichlofenac in an attempt to prepare new derivatives having the potency of the parent drug and lacking its undesirable effects.

DISCUSSION

1 - Hydroxybenzotriazole (A) is used in this work as the most promising catalyst[14]. This procedure gave a much higher yield than those obtained from the classical method[15]. Thus the formation of o - [2, 6 - dichloroanilino] phenyl acetic acid - 1 - hydroxybenzotriazole ester (B) is followed by aminolysis with some amino acid methyl ester namely, glycine L - valine, L - diiodotyrosine and L - tryptophan led to the formation of N - subsituted amino acid ester (IIa - d), respectively [cf. Shceme 1]. The structure of the obtained compounds was confirmed by their correct microanalysis, IR, ^{1}H - NMR and mass spectra. The physical and analytical data of these compounds are illustrated in Table (1).

The IR spectrum of compound (IIb), as an example, showed a sharp

band in the 1740 cm^{-1} region, which is characteristic for the ester carbonyl group, 1640 cm^{-1} characteristic for the amide carbonyl group and 3300 cm^{-1} for NH group. The ^1H - NMR spectrum of (IIa) (DMSO - d_6) showed signals (δ = ppm) at 8.85 (1H, NH), 8.05 (1H, s, ph - NH - ph), 6.3 - 7.7 (7H, m, aromatic protons), 3.9 (2H, d, CH_2 - N), 3.65 (2H, s, CH_2 - ph) and 3.6 (3H, s, CH_3). The mass spectrum of (IIa) is in agreement with its structure. It showed the molecular ion peak M^+ and M^+ +2 at 367 and 369, respectively. The two molecular ion peaks are due to the two isotopes of chloride element. The percentage of these two molecular ions are 18.25 % and 12.28 %, respectively of the base peak which appeared at m / e = 277. The fragmentation pattern is illustrated in Figure (1).

Similar results were obtained by the reaction of L - valine, L - diiodotyrosine and L - tryptophan with dichlofenac to give the corresponding amino acid ester conjugates (IIa, c, d), respectively (cf. Scheme 1).

Their structures were confirmed by their spectral and analytical data (cf. Table 1).

The N - substituted amino acid esters (IIa - d) were hydrolysed using 1N potassium hydroxide to give the corresponding free amino acids (IIa - d) (cf. Scheme 2). The physical and analytical data of these compounds are illustrated in Table (1).

Condensation of N - substituted amino acid methyl esters (IIa - d) with hydrazine hydrate 80 % in absolute ethanol led to the formation of the corresponding o - [2, 6 - dichlorophenyl - 1 - amino] carboxy - N - amino

acid hydrazides (IVa - d), respectively. The structures of these compounds were confirmed by their correct microanalyses (cf. Table 1), IR and ^1H - NMR spectra. The IR spectrum of (IVb), as an example, showed bands at 1750 cm^{-1} (C = O), 1655 cm^{-1} (CO - NH - NH_2), 3455 cm^{-1} (NH) and 3320 and 3340 cm^{-1} (NH_2). The ^1H - NMR spectrum of (IVb) ($CDCl_3$), showed signals at 7.3 - 7.6 (7H, m,aromatic protons), 4.3 (2H, S,ph- CH_2-), 4.0 (1H,d, C-CH - CO), 2.9 (1H, m, $CH(CH_3)_2$) and 1.9 (6H, d, CH_3). The physical and analytical data are illustrated in Table (1).

On the other hand, the formation of the Schiff's bases was achieved by the condensation of the acid hydrazides (IVa-d) with trimethoxybenzaldehyde in boiling ethanol. The structure of (Va - d) was confirmed by their correct microanalyses, IR and ^1H - NMR spectra. The IR spectrum of (Vc), as an example, showed bands at 3450 cm^{-1} (OH), 3200 cm^{-1} (NH), 1710 and 1670 cm^{-1} (CH_2 - CO - and CH - CO -) and 1560 cm^{-1} (C = N). The ^1H - NMR spectrum of (Vc) ($CDCl_3$) revealed signals at 7.1 - 7.7 (11H, m, aromatic protons), 8.6 (1H, s, CH = N), 4.1 (2H, s, ph - CH_2), 4.2 (2H, d, CH_2 - diiodophenol), 3.9 (1H, t, CH - CO -) and 3.7, 3.8 (9H, 2s, 3(OCH_3)).

The chemical reactions of (IIa-d) with hydrazine hydrate and (IVa-d) with trimethoxybenzaldehyde to form (Va-d) are illustrated in Scheme (2).

Biological data :

From Table (2), most of the tested compounds show anti - inflammatory activity especially compounds (IIIb), (IVa), (IIIa), (IVb), (IIa),

(Va), (IIc), (Vc), (IVc), (IIIc), (IIId), and (IIb) in descending order, where the first three compounds showed activity superior to dichlofenac itself in respect to the % oedema, volume of the plural fluid and specially the number of ulcers found which reached almost zero in the compounds (IIIb), (IVa) and (IIa).

The tested compounds (IIIc), (IIb), (IVd), (IIc), (IVb), (IVa), (IIIa), (IVc), (IIId), (IIIa) and (IIa) were found to have significant analgesic activity in descending order. This latter finding represent an important advantage to their anti - inflammatory activity. However, they all showed analgesic activity significantly different from the control values, none of them reached the analgesic activity of our standard drug.

Menawhile, the animals tolerated well the tested compounds and no mortalities were obserbed during the preliminary results are encouraging for further studies where some diclofenac amino acid conjugates did not alter its anti - inflammatory activity and in addition it improved its ulcerogenic effect on the expense of its analgesic activity.

Experimental Part :

Melting points are not corrected. The infrared spectra were carried out on a Karl Zeiss IMR 16 spectrophotometer. The ^{1}H - NMR spectra were measured on Jeol spectrometer Ex - 270. Mass spectra were recorded on Ms 30 (AEL) 70 eV. All thin layer chromatography (TLC) were done on aluminium sheets silica gel (60 F 245-Merck) using chloroform - methanol - acetic acid (85 + 10 + 5) (petrolium ether) (1 : 1) as a solvent system.

Ninhydrin and starch reagents were used for the detection of amino acid derivatives.

Preparation of o - (2, 6 - dichlorophenyl - 1 - amino) benzyl carboxy - N - amino acid esters (IIa - d) :

To a cold solution of N - hydroxybenzotriazole (A) (1 mmol) in dry tetrahydrofuran (10 ml), was added a solution of voltaren (1 mmol) in 10 ml THF was then added protionwise (within about 15 min.) at 0°C. The reaction mixture was stirred until its temperature reached the room temperature and the reaction progress was followed by TLC. the formed dicyclohexylurea, after cooling, was filtered off and the filtrate was evaporated under vacuum to give the active ester (B). To a cold solution of active ester (B) (1 mmol) and triethylamine (1 mmol) in 10 ml THF at 0°C and while stirring, was added a solution of amino acid ester hydrochloride (1 mmol) in 10 ml THF portionwise within 15 min. The pH of the reaction mixture was adjusted to 8 - 9 by adding triethylamine. The reaction mixture was stirred at room temperature for another 3 hours and followed by TLC. The solvent was then driven off under vacuum and the residue was desolved in ethyl acetate and then filtered. The filtrate was washed with 5% sodium hydrogen carbonate, 1N hydrochloric acid and finally with water and then dried over anhydrous sodium sulfate. The solvent was evaporated and the obtained product was recrystallized from aqueous methanol. The physical and analytical data of the obtained products are illustrated in Table (1).

Preparation of o - (2, 6 - dichlorophenyl - 1 - amino) benzyl caboxy-N- amino acids (IIIa - d) :

To a solution of (IIa, IIb, IIc or IId) (1 mmol), in 10 ml ethanol, was added a solution of 1N (10 ml) potassium hydroxide. The reaction mixture was refluxed for 2 min, then kept at 50°C for 3 h and then at room temperature for 24 h. After filtration, the solvent was evaporated under vacuum till dryness and 10 ml water was added. The reaction mixture was cooled and then neutrallized with 1N hydrochloric acid. The formed product was filtered off, washed with water, then dried and finally, recrystallized from ethanol/water to give the title compounds. The physical and analytical data of the prepared compounds are illustrated in Table (1).

Preparation of o - (2, 6 - dichlorophenyl - 1 - amino) benzyl carboxy - N -amino acid hydrazides (IVa - d) :

To a solution of (IIa-d) (1 mmol), in ethanol (10 ml), was added hydrazine hydrate (80%) (20 mmol). The reaction mixture was refluxed for 3 h and then left overnight at room temperature. The formed product was filtered off, washed with water, diethyl ether and then recrystallized from aqueous methanol to give the title compounds. The physical and analytical data of the obtained compounds are illustrated in Table (1).

Preparation of o - (2, 6 - dichlorophenyl - 1 - amino) benzyl carboxy - N - amino acid hydrazones (Va - d) :

A mixture of the amino acid hydrazide (IVa-d) (1 mmol) in 10 ml ethanol and trimethoxybenzaldehyde (1 mmol) was refluxed for 6 h and then left at room temperature overnight. The formed product was filtered off and

recrystallized from aqueous ethanol to give the title compounds (Table 1).

PHARMACOLOGY

Materials and methods :

Materials :

Animals :

Male albino rats (100-110 g body weight) were obtained from the animal house colony of the National Research Centre, Dokki, Cairo (Egypt). They were randomly assigned to groups each 6 animals. Each group was housed individually and fed on a standard laboratory diet and water ad. libitum.

Chemicals and Drugs:

Diclofenac sod. (Ciba-Geigy);

Carrageenan (BDH).

Acute Anti - inflammatory activity:

The inflammatory oedema was induced in one hind paw of the rats by s.c injection of 0.05 ml of 1% carrageenan into the subplantar tissue according to the method of Winter et al.[16]. The first group (negative control group) received propylene glycol and water 10% v/v. the second group (positive control group) received diclofenac Na drug in a single oral dose of 20 mg/kg orally. The other group received the tested compounds dissolved in a mixture of propylene glycol and water 10% v/v. After the treatment by

one hour, carrageenan 1% was injected into the subplantar tissue of the right hind paw (0.05 ml s.c), and 0.05 ml saline in the left hind paw. Three hours later, the volume of the injected paw was measured and the percent oedema was calculated. Results are shown in Table (2).

Carrageenan induced pleurisy:

Pleurisy was induced by injection of 0.2 ml of 2% carrageenan in 0.9% NaCl solution between the 8th and 9th left rib, into the pleural cavity of rats (Velo et al.[17]). Exudates were collected after 3 h and its volume determined (Corell and Hasselman[18]).

The first gruop of animals was the negative control group, the second group was the positive control group and received diclofenac sodium (20mg/kg). The other groups received the tested compounds one hour before induction of plenisy. The inhibition of exudate formation in treated animals was calculated and compared with that of control rats.

$$\% \text{ Oedema} = \frac{\text{(weight of right paw) - (weight of left paw)}}{\text{weight of left paw}} \times 100$$

The results are shown in Table (2).

Analgesic activity :

Electric current as a noxious stimulus used by Charlier et al.[19] and the minimum voltage for the current was determined, that causes the animal to emit a cry. Electrical stimulation to the tail of the rat was applied by means

of 515 Master Schoker (Lafayette Inst. Co.) stimutation was carried out by an alternative current of 50 cycles / sec for 0.2 sec. The internal resistance was set to 400 K, groups of male albino rats (each 100 - 110 g in weight). The first group was the negative control group. The second group was the

positive control group and received diclofenac sodium (20 mg / kg). The other groups received the tested compounds. Four hours latter the electric current was applied (cf. Table 2).

Ulcerogenic activity :

The acute ulcerogenic or gastric nucosal eroding action of compounds were examined by a modification of the method reported by Corell et al.[20]. Group of animals each of 6 animals were starved for 18 h. The first group was the negative control group. The second group was the positive control group and received diclofenac - sodium. The other groups received the tested compounds (single oral dose 20 mg / kg b. wt) 4 h later, the rats were sacrified. The stomach of each rat was removed, cut along the greater curvature, mounted on a flat surface examined for the presence of any eroded or ulcerated area (cf. Table 2).

Statistics :

Statistical analysis of the data was made using student's "t test" and the 0.05 level of propability was regarded as significant (Sendecor and Cochran)[21].

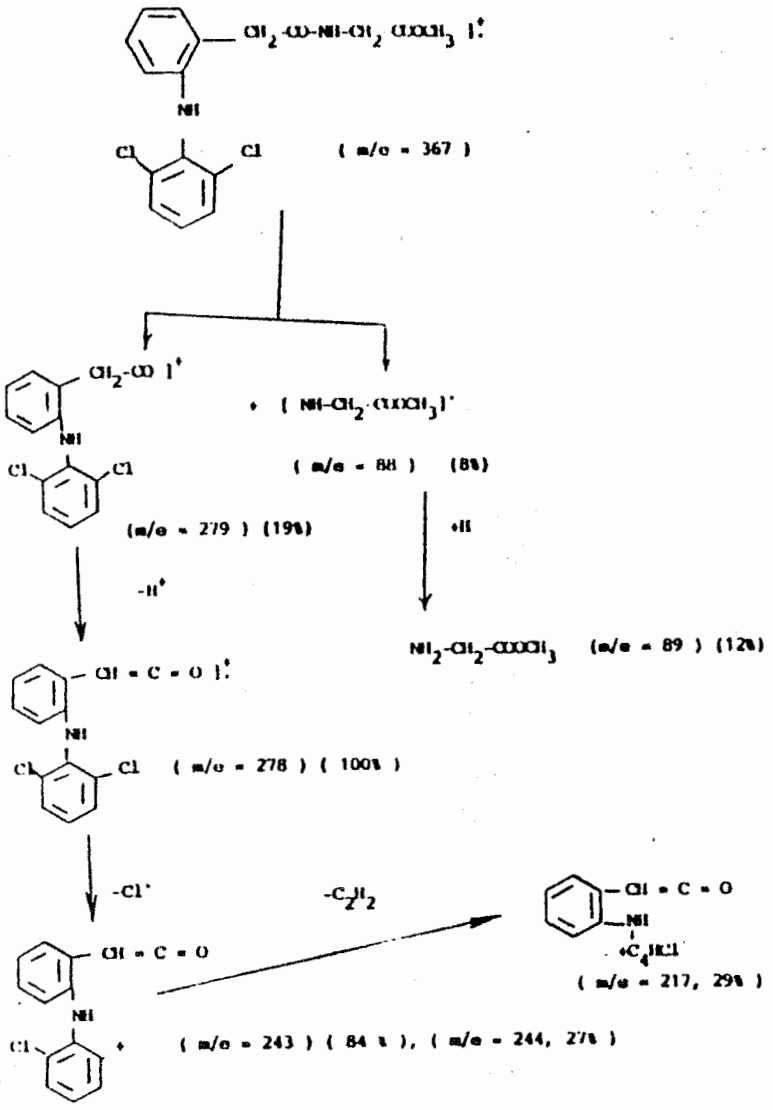
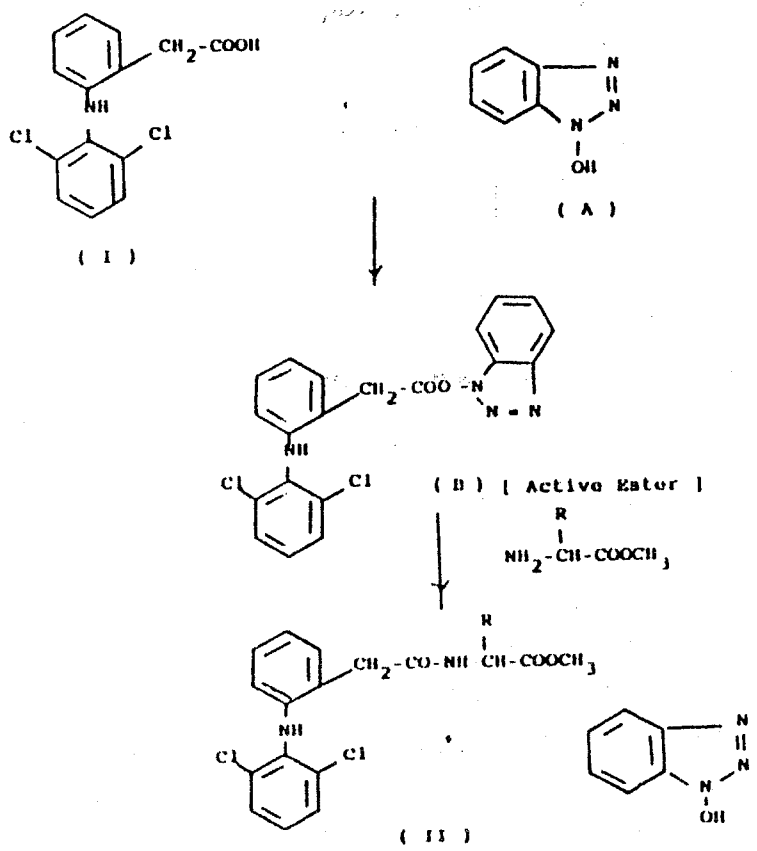
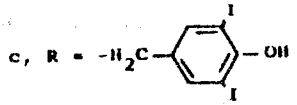


Figure (1)
The fragmentation patrren of compound (IIa)

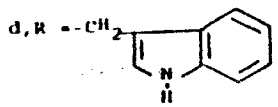


(II), a, R = H (Gly)

b, R = -CH-$(CH_3)_2$ (L-Val)

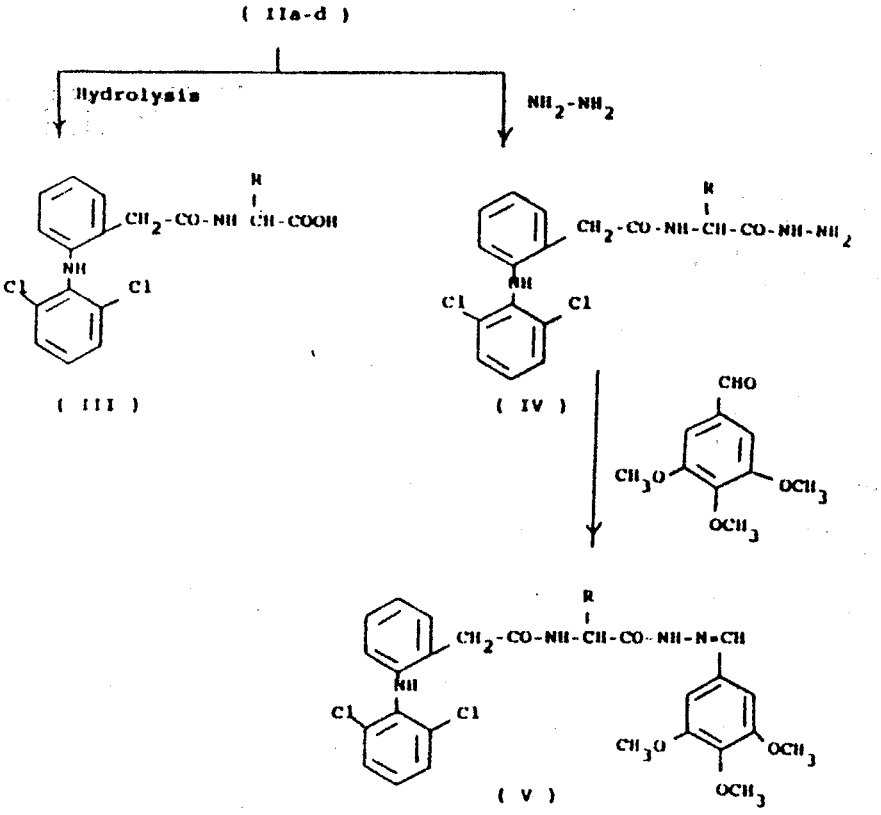


(L-Diiodotyrosine)



(L-Tryptophane)

(Scheme (I)



III, IV and V, a, R = H (Gly)

b, R = -CH-(CH3)2 (L-Val)

c, R = -CH2- OH (L-Diiodotyrosine)

d, R = -CH2- (L-Tryptophane)

Scheme (2)

Table (1): Physical and analytical data of the prepared compounds.

Compd. No.	M. formula M. wt.	M.p. °C	Yield (%)	Analysis Calcld./Found		
				C%	H%	N%
IIa	$C_{17}H_{16}Cl_2N_2O_3$ (367)	156-8	82	55.58 55.0	4.36 4.8	7.63 8.0
IIb	$C_{20}H_{22}Cl_2N_2O_3$ (409)	85-7	76	58.68 58.4	5.38 5.4	6.84 7.2
IIc	$C_{24}H_{20}Cl_2N_2O_4$ (725)	160-2	66	39.72 40.1	2.76 2.5	3.86 3.5
IId	$C_{26}H_{23}Cl_2N_3O_3$ (496)	oily	-	62.90 63.2	4.63 4.7	8.46 8.6
IIIa	$C_{16}H_{14}Cl_2N_2O_3$ (353)	176-8	55	54.39 54.4	3.96 4.3	7.93 8.1
IIIb	$C_{19}H_{20}Cl_2N_2O_3$ (395)	150-2	63	57.72 57.4	5.06 4.8	7.09 6.7
IIIc	$C_{23}H_{18}Cl_2I_2N_2O_4$ (711)	175-7	53	38.82 39.2	2.53 2.7	3.94 4.1
IIId	$C_{25}H_{21}Cl_2N_3O_3$ (482)	oily	-	62.24 62.3	4.35 4.8	8.71 9.1
IVa	$C_{16}H_{16}Cl_2N_4O_2$ (367)	199-200	66	52.31 52.3	4.36 4.6	15.26 15.5
IVb	$C_{19}H_{22}Cl_2N_4O_2$ (409)	110-12	54	55.74 56.1	5.38 5.6	13.69 13.5
IVc	$C_{23}H_{20}Cl_2I_2N_4O_3$ (725)	222-24	45	38.07 38.3	2.76 2.4	7.72 7.3
IVd	$C_{25}H_{23}Cl_2N_5O_2$ (496)	130-2	64	60.48 60.6	4.63 4.3	14.11 14.3
Va	$C_{26}H_{26}Cl_2N_4O_5$ (545)	174-6	53	57.24 57.7	4.77 4.3	10.27 10.3
Vb	$C_{29}H_{32}Cl_2N_4O_5$ (587)	190-2	76	59.28 59.4	5.45 5.5	9.54 9.7
Vc	$C_{33}H_{30}Cl_2I_2N_4O_6$ (903)	205-7	69	43.85 43.5	3.32 3.5	6.20 6.5
Vd	$C_{35}H_{33}Cl_2N_5O_5$ (674)	152-4	58	62.31 62.5	4.89 5.2	10.38 10.2

Table (2): Biological data.

Animal group	Tested material	% Oedema (mean±S.E.)	Pleural fluid (ml) (mean±S.E.)	Analgesic activity (volts) (mean±S.E.)	Number of ulcers (mean±S.E.)
Control	propylene glycol and water	57.89 ± 1.24	0.65 ± 0.009	80 ± 1.23	0 ± 0
Positive control	diclofenac ood	14.52* ± 1.03	0.22* ± 0.007	165* ± 4.99	13.33 ± 1.66
1	IIa	19.93* ± 1.83	0.070* ± 0.012	98.75* ± 4.27	0.83 ± 0.31
2	IIIa	13.60* ± 0.99	0.218* ± 0.002	100.83* ± 0.477	2.25 ± 0.82
3	IVa	11.96* ± 1.72	0.134* ± 0.004	102.00* ± 1.23	0 ± 0
4	Va	26.85* ± 3.82	0.02* ± 0.001	98.50* ± 7.500	3.4 ± 1.12
5	IIb	43.39* ± 4.50	0.30* ± 0.01	128.3* ± 7.38	0 ± 0
6	IIIb	11.66* ± 1.60	0.047* ± 0.003	112.50* ± 6.921	0.5 ± 0.499
7	IVb	15.08* ± 2.71	0.128* ± 0.006	117.83* ± 0.79	2.33 ± 1.18
8	IIc	28.53* ± 1.87	0.668 ± 0.101	118.33* ± 3.33	0.333 ± 0.21
9	IIIc	37.40* ± 2.94	0.620 ± 0.180	132.5* ± 4.23	0.833 ± 0.54
10	IVc	33.43* ± 2.86	0.21* ± 0.021	100.29* ± 9.33	nulti[0]
11	Vc	32.83* ± 3.83	0.40* ± 0.026	84.16* ± 3.96	0.667 ± 0.49
12	IIId	38.72* ± 0.49	0.4* ± 0.020	103.00* ± 5.03	0 ± 0
13	IVd	55.39* ± 1.94	0.616 ± 0.016	119.00* ± 5.42	0 ± 0

* Significantly different from control values at ≤ 0.05

[0] Severe gastric ulceration

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دراسة على ارثو (٢، ٦ - ثنائى الفنيل - ١ - أمينو) فينيل حامض الخليك (الفولتارين)

I - تخليق ودراسة الفعالية ضد الإلتهاب وكمسكنات ولقرحة المعدة لبعض الأحماض الأمينية

أحمد محمد شلبى ووفاء العراقى

المركز القومى للبحوث - القاهرة

فى هذا البحث تم تفاعل الفولتارين (I) مع بعض أسترات الأحماض الأمينية مثل الجليسين، الفالين، ثنائى أيودو تيروزين والتربتوفان، وذلك من خلال تحضير الإستر البسيط من مركب الفولتارين (B) ليعطى بدوره المركبات أرثو (٢، ٦ ثنائى كلور فينيل-١-أمينو) بنزيل - كاربوكسى إستر لحامض أمينى (IIa - d).

وبالتحليل القلوى للمركبات (IIa - d) تم الحصول على الحامض الحر (IIIa - d).

ومن خلال تكاثف (IIa - d) مع الهيدرازين المائى تم الحصول على الحامض الهيدرازينى المقابل (IVa - d) الذى تم تفاعله بدوره مع ثلاثى الميثوكسى بنزالدهيد ليعطى قواعد شيف (Va - d) المقابلة.

ولقد تم إثبات التركيب البنائى للمركبات السابقة بالتحاليل الدقيقة والأشعة تحت الحمراء وكذلك الرنين النووى المغناطيسى.

ومن ناحية أخرى فلقد تم إختبار المواد الناتجة كمضادات للإلتهابات وكمسكنات وكذلك لقرحة المعدة على فئران التجارب.