Anticonvulsant and Antiparkinsonian Evaluation of Some Synthesized Chiral Peptide Derivatives Using 3-benzoyl-4, 5-dioxo-2-phenyl-pyrrolidin-1-yl) Acetic Acid

Abd El-Galil E. Amr

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, King Saud University,
P.O.Box 2457, Riyadh 11451, Saudi Arabia

Abstract: A series of heterocyclic derivatives were synthesized using 3-benzoyl-4,5-dioxo-2-phenyl-pyrrolidin-1-yl)acetic acid (1) as starting material. Treatment of 1 with valinyl methyl ester [Mixed anhydride method] to give the corresponding peptide methyl ester 2, which was reacted with several reagents to produce some newly, synthesized peptidohetero-organic derivatives 3-11. The pharmacological screening showed that many of these obtained compounds have good anticonvulsant and antiparkinsonian activities comparable to Carbamazapine® and Benzotropene® as reference drugs.

Key words: 3-Benzoyl-4 · 5-dioxo-2-phenyl-pyrrolidin-1-yl) acetic acid · Amino acids · Anticonvulsant · Antiparkinsonian activities

INTRODUCTION

Recently, in previous work we reported that certain substituted peptide-heterocyclic systems as anti-inflammatory and analgesic activities [1]. However, because the peptide derivatives have antimicrobial and antiinflammatory activities [2-7] and antitumor properties [8-11]. We also demonstrated that some peptidoheterocyclic derivatives exhibit a general ionophoric potency for divalent cations [12] and are useful for assembling novel thiocyanate-selective membrane sensors [13]. In addition, some new heterocyclic derivatives were synthesized which exhibit analgetic, anti-parkinsonian androgenic anabolic and antiinflammatory activities [14-19]. On the other hand, semicarbazide, thiosemicarbazide and imide derivatives show promising biological and pharmacological activities [20-22]. In view of these observations and as continuation of our previous work on peptido-heterocyclic chemistry, we have synthesized some new compounds containing amino acid and hetero-organic moieties and tested their anticonvulsant and antiparkinsonian activities.

Experimental: Melting points were determined on open glass capillaries using an Electrothermal IA 9000 SERIES digital melting point apparatus and are uncorrected. Elemental analyses were performed in the Microanalytical Unit, National Research Centre, Cairo Egypt and were found within ±0.4% of the theoretical values. The IR spectra (KBr,v, cm^{-1}) were recorded on a FT IR-8201 PC Spectrophotometer. The ^{1}H NMR spectra were measured with Jeol 270 MHz (Japan) in DMSO-d$_6$. The chemical shifts were recorded (δ, ppm) relative to TMS. The Mass spectra were run at 70 eV with a Finnigan SSQ 7000 spectrometer using EI and the values of m/z are indicated in Dalton. The starting material (1) was prepared in the previous work [1].

Methyl 2-(2-(3-benzoyl-4, 5-dioxo-2-phenylpyrrolidin-1-yl) acetamido)-3-methylbutanoate 2: To a cold and stirred dry dichloromethane solution (25 ml, -20°C) of the acid 1 (0.37 g, 1 mmol), ethyl chloroformate (0.1 g, 1 mmol) and triethylamine (0.1 ml, 1 mmol) were added. Ten minutes later, a cold methylene chloride solution (10 ml, -20°C) of L-valine methyl ester (0.13 g, 1 mmol) was added. Stirring of the cold reaction mixture (-20°C) was continued for 3 h and at room temperature for overnight. The solution was then washed with water, 1N hydrochloric acid, 1N sodium bicarbonate and finally with water. The solvent was dried over calcium chloride anhydrous, evaporated and the obtained residue was solidified by dry ether trituration, filtered off, dried under vacuum and crystallized to afford the ester 2. Yield 65%, mp: 132-4°C (ether); IR: 3430-3350 (NH), 1745 (C=O, ester), 1690-1684 (C=O); ^{1}H-NMR: 0.85 (d, 6H, 2CH$_3$), 1.12 (m, 1H, CH), 3.48 (s, 3H, OCH$_3$), 3.62 (d,

Corresponding Author: Dr. Abd El-Galil E. Amr, Department of Pharmaceutical Chemistry, Faculty of Pharmacy,
King Saud University P.O Box 2457, Riyadh 11451, Saudi Arabia

1H, CH), 3.80 (s, 2H, CH$_2$), 4.35, 5.28 (2d, 2H, 2CH), 7.15-7.88 (m, 10H, Ar-H), 8.14 (s, 1H, NH, exchangeable with D$_2$O); MS m/z (%): 450 (M$^+$, 12), corresponding to the molecular formula C$_{25}$H$_{26}$N$_2$O$_6$ and at 243 (100, base peak).

2-(2-(3-Benzoyl-4, 5-dioxo-2-phenylpyrrolidin-1-yl) acetamido)-3-methylbutanoic acid 3: Sodium hydroxide (1N, 25 ml) was drop-wisely added to a cold and stirred ethanolic solution (0.45g, 1 mmol, -5°C) of the ester 2. Stirring was continued at that temperature for 2 h and then for 12 h at room temperature followed by evaporation of the solvent. The cold reaction mixture was acidified with 1N hydrochloric acid to pH ~3 and the obtained solid was filtered off, washed with cold water, dried and crystallized to afford the acid 3. Yield 78%, mp: 224-6°C (EtOH/ether); IR: 3545-3336 (OH, NH), 1718 (C=O, acid), 1695-1685 (C=O); ^{1}H-NMR: 0.90 (d, 6H, 2CH$_3$), 1.85 (m, 1H, CH), 3.82 (s, 2H, CH$_2$), 4.18 (m, 1H, CH), 4.60, 5.56 (2d, 2H, 2CH), 7.05-7.92 (m, 10H, Ar-H), 8.18 (s, 1H, NH, exchangeable with D$_2$O), 11.50 (s, 1H, OH, exchangeable with D$_2$O); MS m/z (%): 436 (M$^+$, 42), corresponding to the molecular formula C$_{24}$H$_{24}$N$_2$O$_6$ and at 419 (100, base peak).

N-(1-Hydrazinyl-3-methyl-1-oxobutan-2-yl)-2-(3-(hydrazono (phenyl) methyl)-4, 5-dioxo-2-phenylpyrrolidin-1-yl) acetamide 4: Hydrazine hydrate (0.8 ml, 16 mmol) was added to a ethanolic solution (50 ml) of the ester 2 (0.45g, 1 mmol). The reaction mixture was refluxed for 3 h after which the solvent was evaporated. The obtained residue was triturated with ether, the formed solid was filtered off and crystallized to afford the hydrazinyl-hydrazide derivative 4. Yield 87%, mp: 243-5°C (EtOH/n-hexane); IR: 3480-3335 (NH, NH$_2$), 1695-1686 (C=O); ^{1}H-NMR: 0.86 (d, 6H, 2CH$_3$), 2.35 (m, 1H, CH), 3.76 (s, 2H, CH$_2$), 3.98 (m, 1H, CH), 4.55, 5.35 (2d, 2H, 2CH), 5.85, 6.42 (2s, 4H, 2NH$_2$ exchangeable with D$_2$O), 7.22-7.98 (m, 10H, Ar-H), 8.22, 9.00 (2s, 2H, 2 NH, exchangeable with D$_2$O); MS m/z (%): 464 (M$^+$, 100), corresponding to the molecular formula C$_{24}$H$_{28}$N$_6$O$_4$ and as base peak.

Methyl2-(2-(2-(3-benzoyl-4,5-dioxo-2-phenylpyrrolidin-1-yl) acetamido)-3-methylbutan-amido)-4-methylpentanoate 5: To a cold and stirred dry dichloromethane solution (25 ml, -20°C) of the acid 3 (0.46g, 1 mmol), ethyl chloroformate (0.1 g, 1 mmol) and triethylamine (0.1 g, 1 mmol) were successively added. Ten minutes later, a cold methylene chloride solution (10 ml, -20°C) of L-leucine methyl ester (0.15 g, 1 mmol) was added. Stirring of the cold reaction mixture was continued for 3 h and at room temperature for overnight. The

solution was then washed with water, 1N hydrochloric acid, 1N sodium bicarbonate and finally with water. The dried solution (anhydrous CaCl$_2$) was evaporated and the obtained oily residue was solidified by dry ether trituration, filtered off, dried and crystallized to afford the ester 5. Yield 68%, mp: 154-6°C (MeOH); IR: 3418-3350 (NH),1747(C=O,ester),1698-1678(C=O); ^{1}H-NMR:0.88-0.85 (m, 12, 4CH$_3$), 1.48-1.45 (m, 1H, CH), 1.80 (m, 2H, CH$_2$), 2.40-2.35 (m, 1H, CH), 3.56 (s, 3H, OCH$_3$), 3.74 (s, 2H, CH$_2$), 4.50-4.48 (m, 2H, 2CH), 4.62, 5.45 (2d, 2H, 2CH), 7.15-7.95 (m, 10H, Ar-H), 8.16, 8.18 (2s, 2H, 2 NH, exchangeable with D$_2$O); MS m/z (%): 563 (M$^+$, 8), corresponding to the molecular formula C$_{31}$H$_{37}$N$_3$O$_7$ and at 243 (100, base peak).

N-(1-((E)-2-(4-fluorobenzylidene)hydrazinyl)-3-methyl-1-oxobutan-2-yl)-2-(3-((Z)-((Z)-(4-fluorobenzylidene) hydrazono)(phenyl)methyl)-4,5-dioxo-2-phenylpyrrolidin-1-yl)acetamide 6: A stirred solution of hydrazide 4 (0.46g, 1 mmol) and *p*-flourobenzaldehyde (0.25g, 2 mmol) in absolute methanol (50 ml) was refluxed for 3 h. The reaction mixture was allowed to left at room temperature overnight, then evaporated under reduced pressure. The obtained oily product was solidified by trituration with ethanol/pet ether (40-60°C), the remaining solid was filtered off, dried and crystallized to give the corresponding hydrazone 6. Yield 82%, mp: 256-8°C (EtOH); IR: 3445-3325 (NH), 1698-1688 (C=O), 1665 (C=N); ^{1}H-NMR: 0.92 (d, 6H, 2CH$_3$), 2.38 (m, 1H, CH), 3.75 (s, 2H, CH$_2$), 4.14 (m, 1H, CH), 4.48, 5.36 (2d, 2H, 2CH), 7.08-7.96 (m, 20H, Ar-H + 2CH=N), 8.30, 10.16 (2s, 2H, 2 NH, exchangeable with D$_2$O); MS m/z (%): 676 (M$^+$, 10), corresponding to the molecular formula C$_{38}$H$_{34}$F$_2$N$_6$O$_4$ and at 122 (100, base peak).

N-(1-(2-(Ethylcarbamothioyl)hydrazinyl)-3-methyl-1-oxobutan-2-yl)-2-(3-((2-(ethylcarbamo-thioyl) hydrazono)(phenyl)methyl)-4,5-dioxo-2-phenylpyrrolidin-1-yl)acetamide 7: A mixture of hydrazide 4 (0.46 g, 1 mmol) and ethyl isothiocyanate (~0.2 g, 2 mmol) in dry dioxane (50 ml) containing of triethylamine (2 ml) was heated under reflux for 10 h. After cooling, the obtained solid was filtered off, washed with n-hexane, dried and crystallized to give thiosemicarbazide derivative 7. Yield 76%, mp: 135-7°C (dioxane/ether); IR: 3460-3325 (br, NH), 1698-1680 (C=O), 1228 (C=S); ^{1}H-NMR: 0.88 (d, 6H, 2CH$_3$), 1.24-1.20 (m, 6H, 2CH$_3$), 2.38 (m, 1H, CH), 3.68 (s, 2H, CH$_2$), 4.05 (m, 1H, CH), 4.30-4.22 (m, 4H, 2CH$_2$), 4.45, 5.40 (2d, 2H, 2CH), 7.15-7.92 (m, 10H, Ar-H), 8.30, 8.45, 8.56, 9.10, 9.86 (5s, 6H, 6NH, exchangeable with D$_2$O); MS m/z (%): 638 (M$^+$, 56), corresponding to the molecular formula C$_{30}$H$_{38}$N$_8$O$_4$S$_2$ and at 243 (100, base peak).

Synthesis of phenylpyrrolidine butanamide derivatives **8 and 9:** A stirred glacial acetic acid suspension (50 ml) of hydrazide 4 (0.46g, 1 mmol) and phthalic or 1,2,4,5-tetrachlorophthalic anhydrides (2 mmol) was heated (80°C) for 4 h. The reaction mixture was concentrated under reduced pressure, cooled and the separated solid was collected by filtration, dried and crystallized to yield the corresponding imide derivative 8 and 9, respectively.

N-(1,3-Dioxoisoindolin-2-yl)-2-(2-(3-((1,3-dioxoisoindolin-2-ylimino)(phenyl)methyl)-4,5-dioxo-2-phenylpyrrolidin-1-yl)acetamido)-3-methylbutanamide 8:

Yield 92%, mp: > 280°C (AcOH/H_2O); IR: 3466-3296 (NH), 1728, 1725 (CO), 1698-1690 (C=O); ^{1}H-NMR: 0.90 (d, 6H, 2CH$_3$), 2.32 (m, 1H, CH), 3.78 (s, 2H, CH$_2$), 4.10 (m, 1H, CH), 4.46, 5.42 (2d, 2H, 2CH), 7.15-7.98 (m, 18H, Ar-H), 8.85, 9.44 (3s, 3H, 3 NH, exchangeable with D_2O); MS m/z (%): 724 (M$^+$, 8), corresponding to the molecular formula $C_{40}H_{32}N_6O_8$ and at 320 (100, base peak).

2-(2-(2,3-Dioxo-5-phenyl-4-(phenyl(4,5,6,7-tetrachloro-1,3-dioxoisoindolin-2-ylimino)methyl)-pyrrolidin-1-yl)acetamido)-3-methyl-N-(4,5,6,7-tetrachloro-1,3-dioxoisoindolin-2-yl)butan-amide 9:

Yield 86%, mp: > 300°C (DMF/H_2O); IR: 3458-3298 (NH), 1732, 1727 (CO), 1695-1685 (C=O); ^{1}H-NMR: 0.88 (d, 6H, 2CH$_3$), 2.36 (m, 1H, CH), 3.75 (s, 2H, CH$_2$), 4.15 (m, 1H, CH), 4.38, 5.46 (2d, 2H, 2CH), 7.18-7.94 (m, 10H, Ar-H), 8.78, 9.65 (3s, 3H, 3 NH, exchangeable with D_2O); MS m/z (%): 995 (M$^+$, 32), corresponding to the molecular formula $C_{40}H_{24}Cl_8N_6O_8$ and at 167 (100, base peak).

N-(1-Hydrazinyl-4-methyl-1-oxopentan-2-yl)-2-(2-(3-(hydrazono (phenyl) methyl)-4, 5-dioxo-2-phenylpyrrolidin-1-yl) acetamido)-3-methylbutanamide 10:

Hydrazine hydrate (0.8 ml, 16 mmol) was added to a methanolic solution (10 ml) of 5 (0.56g, 1 mmol). The reaction mixture was refluxed for 3 h. after which the solvent was evaporated under reduced pressure. The obtained residue was triturated with ether, filtered off and crystallized to afford the corresponding hydrazide 10. Yield 78%, mp: 232-4°C (Dioxane); IR: 3418-3250 (NH, NH$_2$), 1705 (C=O), 1695-1685 (C=O); ^{1}H-NMR: 0.92-0.84 (m, 12, 4CH$_3$), 1.52-1.46 (m, 1H, CH), 1.80 (m, 2H, CH$_2$), 2.42-2.35 (m, 1H, CH), 3.76 (s, 2H, CH$_2$), 4.15 (s, 2H, NH$_2$, exchangeable with D_2O), 4.48-4.45 (m, 2H, 2CH), 4.64, 5.48 (2d, 2H, 2CH), 5.65 (s, 2H, NH$_2$, exchangeable with D_2O), 7.10-7.95 (m, 10H, Ar-H), 8.16, 8.28, 8.36 (3s, 3H, 3 NH, exchangeable with D_2O); MS m/z (%): 577 (M$^+$, 28), corresponding to the molecular formula $C_{30}H_{39}N_7O_5$ and at 243 (100, base peak).

N-(1-((E)-2-(4-fluorobenzylidene)hydrazinyl)-4-methyl-1-oxopentan-2-yl)-2-(2-(3-((Z)-((E)-(4-fluorobenzylidene) hydrazono)(phenyl)methyl)-4,5-dioxo-2-phenylpyrrolidin-1-yl)acetamido)-3-methylbutanamide 11:

A stirred solution of hydrazide 10 (0.6g, 1 mmol) and *p*-flourobenzaldehyde (0.25g, 2 mmol) in absolute methanol (50 ml) was refluxed for 4 h. The reaction mixture was allowed to left at room temperature overnight and evaporated under reduced pressure. The obtained product was solidified by trituration with ethanol/ether; the remaining solid was filtered off, dried and crystallized to give the corresponding hydrazone 11. Yield 65%, mp: > 250°C (Ac-OH); IR: 3398-3245 (NH), 1700-1695 (C=O), 1660 (C=N); ^{1}H-NMR: 0.86-0.82 (m, 12, 4CH$_3$), 1.50-1.45 (m, 1H, CH), 1.75 (m, 2H, CH$_2$), 2.46-2.38 (m, 1H, CH), 3.75 (s, 2H, CH$_2$), 4.52-4.46 (m, 2H, 2CH), 4.66, 5.50 (2d, 2H, 2CH), 7.18-7.98 (m, 20H, Ar-H+ 2CH=N), 8.24, 8.38, 9.12 (3s, 3H, 3 NH, exchangeable with D_2O); MS m/z (%): 789 (M$^+$, 8), corresponding to the molecular formula $C_{44}H_{45}F_2N_7O_5$ and at 419 (100, base peak).

Pharmacological Screening

Anticonvulsant Activity: Male Webster mice (20-30 g) were individually placed in clear plastic cylinder and the tested compounds were administrated intrapretonial (5 mg/kg), 30 min prior to a dose of 45 mg/kg of yohimbine.HCl. The animals were observed for onset and number of clonic seizures. Evaluation ED$_{50}$ values of compounds with 95% confidence limit were calculated for the antagonism of yohimbine-induced clonic seizures by means of the Lichtfield-Wilcoxon procedure [23,24] (Table 1).

Antiparkinsonian Activity: The muscarinic agonists Tremorine® and Oxotremorine® induce parkinisonisin-like signs such as tremor, ataxia, spasticity, salivation, lacrimation and hypothermia. These signs are antagonized by antiparkinsonian agents. Groups of eight mail mice (18–20 g) were used. They were dosed orally with the tested compounds (5 mg/kg) or the standard (Benzotropene® mesilate, 5 mg/kg) [25] 1 h prior the administration of 0.5mg/kg of Oxotremerine® S.C. Rectal temperature was measured before administration of the compounds and 1 h after Oxotremerine® dosage. The scores for the recorded signs are zero (absent), one (slight), two (medium) and three (high) (Table 2).
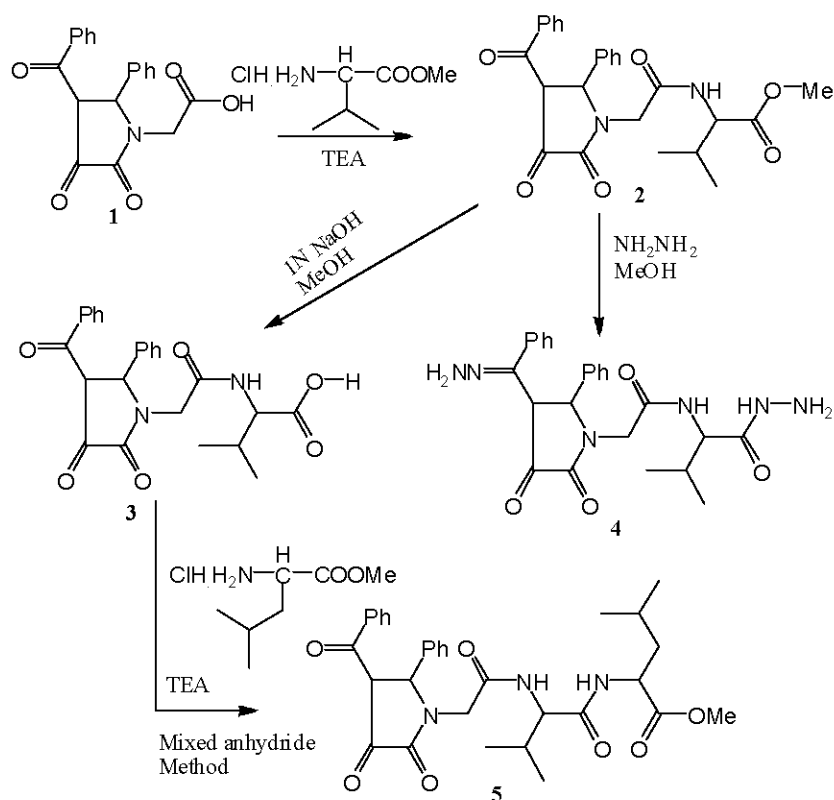
RESULTS AND DISCUSSION

Chemistry: The reaction of L-valine methyl ester hydrochloride with the acid derivative 1 [1] and ethyl

Table 1: Anticonvulsant activity of new synthesized compounds as compared to Carbamazepine® in mice
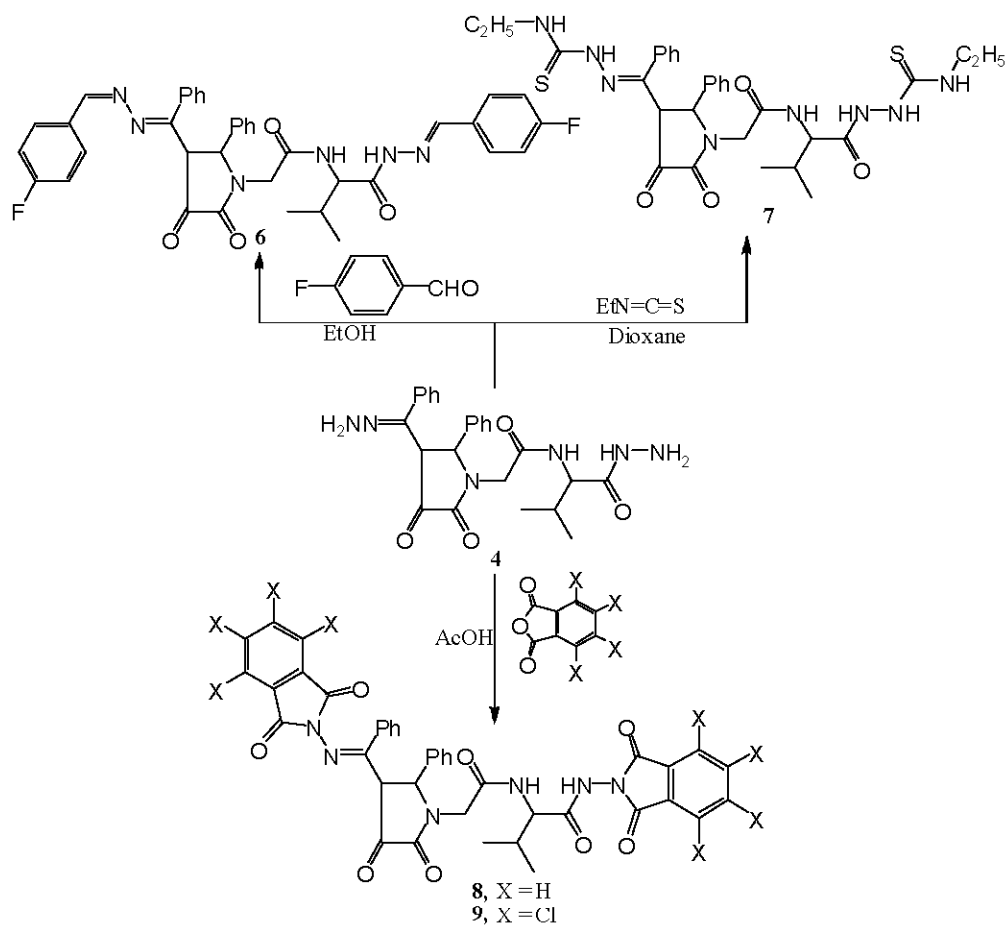
Comp. No	ED$_{50}$ Value (mg/kg)	Relative potency of Carbamazepine®
Control	0	0
Carbamazepine®	29	1
1	no protection	-
2	50	0.62
3	no protection	-
5	13	2.18
8	31	0.95
10	35	1.70
11	15	1.92

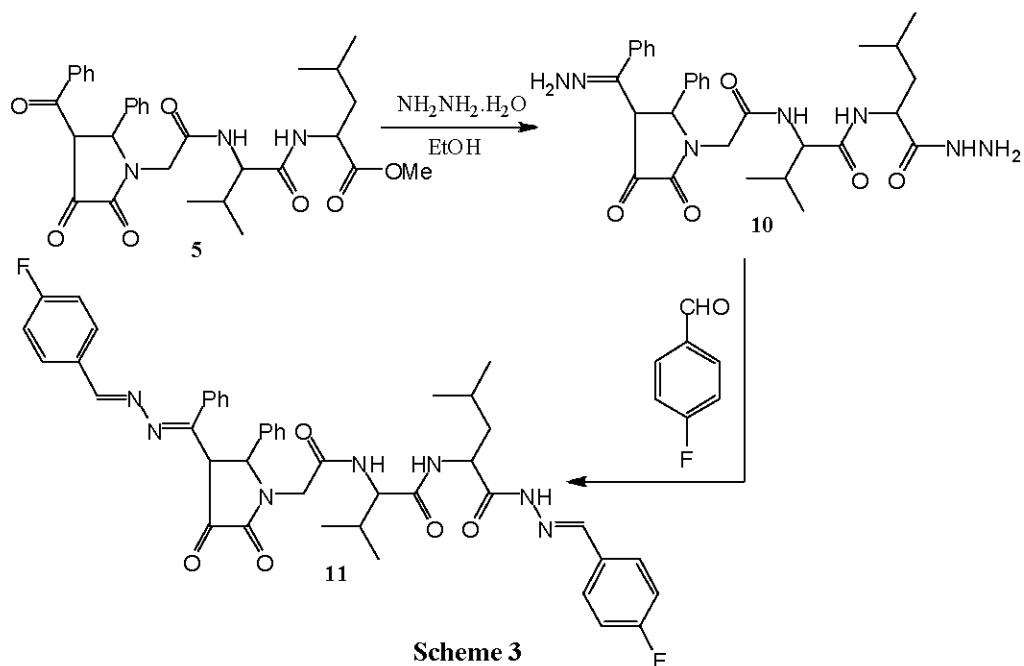Table 2: Antiparkinsonian activity of new synthesized compounds as compared with Benzotropene®

Comp. no.	Salivation and lacrimation score	Tremors score	% decrease from Oxotremerine® rectal temp.	Relative potency to Benzotropene®
Control	0	0	0	0
Benzotropene®	1	1	26	1
1	2	2	12	0.44
2	2	2	16	0.60
3	2	2	11	0.40
5	1	1	19	0.80
8	3	3	5	0.16
10	1	1	21	0.81
11	3	3	4	0.15

Scheme 1

Scheme 2

Scheme 3

chloroformate in the presence of triethylamine [Mixed anhydride method] afforded the corresponding peptide ester derivative 2 [Mixed anhydride method]. When treatment of the ethyl ester 2 with 1N NaOH or hydrazine hydrate, the corresponding acid 3 and acid hydrazide 4 were isolated, respectively. On the other hand, the reaction of L-leucine methyl ester hydrochloride with the acid derivative 3 and ethyl chloroformate in the presence of triethylamine afforded the corresponding peptide ester derivative 5 [Mixed anhydride method] [8] (Scheme 1).

The hydrazino hydrazide 4 was condensed with *p*-flourobenzaldehyde in refluxing ethanol to afford the corresponding hydrazone 6, while, it was treated with ethyl isothiocyanate in refluxing dioxane to afford the thiosemicarbazide derivative 7. Condensation of 4 with phthalic and 1,2,4,5-tetrachlorophethalic anhydrides in refluxing glacial acetic acid afforded the corresponding imide derivatives 8 and 9, respectively (Scheme 2).Treatment of peptide ester 5 with hydrazine hydrate in boiling ethanol to give the corresponding hydrazide 10, which was reacted with 1,2,4,5-tetrachlorophthalic anhydride in refluxing acetic acid to afford the corresponding imide derivative 11 (Scheme 3).

Pharmacological Screening: All animals were obtained from the Animal House Colony, Research Institute of Ophthalmology, Giza, Egypt. Then the newly synthesized compounds were pharmacologically screened for their anticonvulsant and antiparkinsonian activities using male albino rats (Tables 1 and 2).

Anticonvulsant Activity: While compounds 1 and 3 were devoid of any anticonvulsant activity and provided no protection against yohinobine-induced clonic seizures, compounds 2, 8 and 10 showed interesting anticonvulsant activities. Their relative potencies to Carbamazebene® were 0.62, 0.95 and 1.70, respectively. Compounds 5 and 11 were even more potent than Carbamazebene® (2.18 and 1.92, relative potency) (Table 1).

Antiparkinsonian Activity: Compounds 1, 2 and 3 showed moderate activity (relative potencies to Benzotropene® 0.44, 0.60 and 0.40). Compounds 5 and 10 are the most potent antiparkinsonic agents (0.80 relative potency) (Table 2).

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