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Pharmacological activities of some synthesized chiral macrocyclic pentapeptide Schiff base candidates.

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Abstract

Macrocyclic peptides are very important in bioorganic and medicinal chemistry investigations. Synthesis and chemical modifications of existing antibacterial agents in order to generate novel macromolecules with better therapeutic properties are necessary. A series of macrocyclic pentapeptide Schiff bases 3-10 were synthesized from the reaction of Nα-dinicotinoyl-bis (L-leucyl-L-phenylalaninyl acid hydrazide) (1) and the macrocyclic pentapeptide ester (2) with selected dibasic amino acid and active reagents. All prepared compounds were tested as anti-inflammatory, analgesic and anticonvulsant agents. Some of the screened compounds exhibited better anti-inflammatory, analgesic and anticonvulsant activities comparable to prednisolone®, valdecoxib and carbamazepine as reference drugs.

Keywords: Macrocyclic pentapeptides, Amino acids, Schiff bases, Pharmacological activities.

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Introduction

Macrocyclic peptides are very important and represent a fascinating area of bioorganic and medicinal chemistry investigations [1,2]. Synthesis and chemical modifications of existing antibacterial agents in order to generate novel macromolecules with better therapeutic properties are necessary [3]. Peptides are function well as drugs due to their low bioavailability and rapid degradation within cells [4]. In continuation to our previous work, the synthesis of some new macrocyclic peptide candidates from pyridine dicarboxylic acids with amino acids and screening of their biological activities were reported [5-11]. In view of these observations and continuation of our previous works in macrocyclic and heterocyclic chemistry, we have screened some synthesized of macrocyclic pentapeptides Schiff bases containing pyridine moiety and screening for their analgesic and anticonvulsant activities.

Results and Discussion

Chemistry

A series of macrocyclic pentapeptide derivatives 3-10 were synthesized from compounds 1 and 2, which was obtained from 3, 5-pyridinedicarbonyl dichloride, according to the previous published procedures (Figure 1) [12,13]. A series of derivatives 3-10 were synthesized from compounds 1 and 2 in advance and screened as antimicrobial agents [14]. Herein, we used these compounds 1-10 for evaluation as anti-inflammatory, analgesic and anticonvulsant agents.

Pharmacological screening

Initially the acute toxicity of the tested compounds was assayed *via* the determination of their LD_{50} (Table 1). All the tested compounds were interestingly less toxic than the reference drug (Table 1). The synthesized compounds were

pharmacologically screened on male albino rats for their anti-inflammatory potency (Tables 2 and 3) and on Webster mice for their analgesic and anticonvulsant activities (Tables 4 and 5).

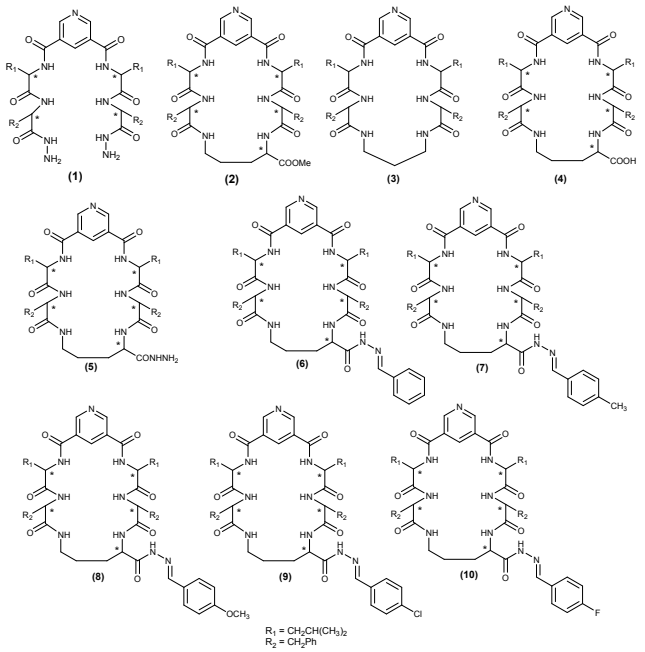

Figure 1. The chemical structures of the tested compounds 1-10.

Table 1. Acute toxicity (LD_{50}) of the tested compounds 1-10.

Comp. no	LD_{50} (mg/kg)
1	1.980 ± 0.016
2	1.745 ± 0.013
3	2.716 ± 0.011
4	1.918 ± 0.014
5	1.812 ± 0.011
6	2.313 ± 0.013
7	2.816 ± 0.012
8	2.547 ± 0.016
9	2.012 ± 0.013
10	2.810 ± 0.016
Prednisolone®	1.618 ± 0.016

Anti-inflammatory screening: For the determination of the antiphlogistic potency of the tested compounds, two standard tests were realized at 25 and 50 mg/kg rat body weight namely, the protection against carrageenan® induced edema according Winter et al. [15] and the inhibition of plasma PGE2. The latter is known as a good confirming indicator for the carrageenan® induced rat paw edema [16]. From the obtained results in Table 2, the protection against carrageenan® induced edema, these compounds 1, 2, 4, 5, 7-10 were found more activity than prednisolone®. Where, their protection percentage against carrageenan induced edema at two dose levels 25 and 50 mg/kg are 92.18/99.22, 88.35/98.56, 92.35/99.55, 93.18/95.45, 88.65/98.14, 92.17/99.20, 93.60/99.18, and 94.66/99.72, respectively (Prednisolone® 81/93).

Table 2. Anti-inflammatory potencies of the synthesized compounds 1-10 (protection against carrageenan-induced edema).

Compound no	Dose (mg/kg)	Protection against carrageenan-induced edema (%)*
1	25	92.18 ± 0.078
	50	99.22 ± 0.072
2	25	88.35 ± 0.0757
	50	98.56 ± 0.078
3	25	67.17 ± 0.058
	50	78.13 ± 0.062
4	25	92.35 ± 0.075
	50	99.55 ± 0.078
5	25	93.18 ± 0.080
	50	95.45 ± 0.075
6	25	64.68 ± 0.065
	50	84.22 ± 0.064
7	25	88.65 ± 0.075
	50	98.14 ± 0.078
8	25	92.17 ± 0.078
	50	99.20 ± 0.082
9	25	93.60 ± 0.089
	50	99.18 ± 0.086
10	25	94.66 ± 0.069
	50	99.72 ± 0.070
Prednisolone®	25	81.00 ± 0.100
	50	93.00 ± 0.082

*The doses tested were 25, 50 mg and carryout three determinations for each dose.

Additionally, from the obtained results in Table 3, we found the inhibition of plasma PGE2 for the compounds 1, 2, 4, 5, 7-10 more potent than prednisolone® at two tested doses levels 25 and 50 mg/kg. The inhibition percentage for potent compounds 1, 2, 4, 5, 7-10 was found as: 84.64/95.10, 86.26/91.62, 89.34/93.25, 85.26/91.62, 93.35/96.54, 92.28/96.48, 88.16/92.35 and 85.78/95.42, respectively (Prednisolone® 77.00/91.00).

Table 3. Anti-inflammatory potencies of the synthesized compounds 1-10 (Inhibition of plasma PGE2).

Compound no	Dose (mg/kg)	Inhibition of plasma PGE2 (%)*

1	25	84.64 ± 0.113
	50	95.10 ± 0.122
2	25	86.26 ± 0.089
	50	91.62 ± 0.108
3	25	43.18 ± 0.088
	50	63.13 ± 0.078
4	25	89.34 ± 0.080
	50	93.25 ± 0.094
5	25	85.26 ± 0.089
	50	91.62 ± 0.100
6	25	41.16 ± 0.077
	50	54.17 ± 0.098
7	25	93.35 ± 0.085
	50	96.54 ± 0.114
8	25	92.28 ± 0.088
	50	96.48 ± 0.112
9	25	88.16 ± 0.078
	50	92.35 ± 0.098
10	25	85.78 ± 0.114
	50	95.42 ± 0.098
Prednisolone®	25	77.00 ± 0.084
	50	91.00 ± 0.087

*The doses tested were 25, 50 mg and carryout three determinations for each dose.

Analgesic activity: All compounds tested exhibited analgesic activities in a hot-plate assay (Table 4). The compounds 5 and 9 are most potent activities than valdecoxib, by nearly 110-140% (5 showed the most pronounced effect). Also, from the results in Table 4, we showed the analgesic activities of 1-4, 6-8, and 10 are 60-90% activities as compared to valdecoxib as standard drug (100% activity) (Table 4).

Table 4. *Analgesic activities of some synthesized compounds 1-10.*

Compound	Analgesic potency relative to valdecoxib ± SE						
	10 min	20 min	30 min	45 min	60 min	90 min	120 min
1	0.85 ± 0.012	0.90 ± 0.017	0.92 ± 0.017	0.95 ± 0.020	0.96 ± 0.032	0.94 ± 0.018	0.93 ± 0.026
2	0.90 ± 0.010	0.92 ± 0.012	0.93 ± 0.016	0.88 ± 0.017	0.83 ± 0.021	0.79 ± 0.016	0.65 ± 0.012
3	0.88 ± 0.010	0.89 ± 0.012	0.89 ± 0.011	0.91 ± 0.019	0.92 ± 0.016	0.93 ± 0.014	0.91 ± 0.014
4	0.65 ± 0.011	0.64 ± 0.017	0.73 ± 0.013	0.73 ± 0.016	0.74 ± 0.019	0.75 ± 0.015	0.78 ± 0.012
5	1.15 ± 0.180	1.35 ± 0.160	1.28 ± 0.130	1.42 ± 0.190	1.40 ± 0.318	1.45 ± 0.288	1.40 ± 0.270
6	0.62 ± 0.011	0.73 ± 0.014	0.79 ± 0.001	0.81 ± 0.014	0.84 ± 0.014	0.84 ± 0.015	0.84 ± 0.035
7	0.66 ± 0.015	0.63 ± 0.012	0.88 ± 0.012	0.88 ± 0.016	0.88 ± 0.021	0.89 ± 0.015	0.89 ± 0.017
8	0.64 ± 0.014	0.65 ± 0.010	0.74 ± 0.012	0.75 ± 0.015	0.77 ± 0.011	0.77 ± 0.012	0.77 ± 0.013
9	0.97 ± 0.012	0.98 ± 0.015	1.40 ± 0.13	1.43 ± 0.210	1.45 ± 0.350	1.41 ± 0.340	1.40 ± 0.450
10	0.77 ± 0.013	0.85 ± 0.012	0.84 ± 0.012	0.87 ± 0.016	0.88 ± 0.017	0.84 ± 0.012	0.83 ± 0.017
Valdecoxib	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Anticonvulsant activity: Antagonism against yohimbine-induced clonic seizures in mice is considered a predictive model of anticonvulsant and GABA-mimetic potential [17]. From the obtained results in Table 5, the compounds 2, 4, 5, 8, and 10 were more potent than carbamazepine with relative potencies of 2.18, 2.05, 1.85, 1.84, and 2.12, respectively. Compounds 3 and 6 were devoid of anticonvulsant activity in the yohimbine-induced clonic seizures assay, while compounds 1, 7, and 9 showed interesting anticonvulsant activities. Their relative potencies are 0.75, 0.76, and 0.82, respectively with compared to carbamazepine (1.0).

Table 5. *Anticonvulsant activities of selected compounds (as ED_{50} values) antagonizing yohimbine-induced clonic seizure, relative to the anticonvulsant activity of carbamazepine.*

Compound (mg/kg BW)	ED50 ± SE	Relative potency compared to carbamazepine ± SE
Control	0	0
Carbamazepine	29 ± 0.31	1.0 ± 0.01
1	58 ± 0.45	0.75 ± 0.011
2	14 ± 0.112	2.18 ± 0.022
3	No protection	No protection
4	15 ± 0.118	1.85 ± 0.0175
5	16 ± 0.115	2.05 ± 0.024
6	No protection	No protection
7	42 ± 0.35	0.76 ± 0.012
8	15 ± 0.114	1.84 ± 0.0182
9	36 ± 0.30	0.82 ± 0.010
10	14 ± 0.116	2.12 ± 0.020

Experimental Section

Chemistry

Synthesis, physicochemical and spectral data for the compounds have been reported in advance [12-14].

Pharmacological screening

Animals: Biological experiments were conducted according to the ethical rules and animals were obtained from the Animal House Colony, Research Institute of Ophthalmology, Giza, Egypt. Approval of the institutional animal ethical committee for the animals studies was obtained from the Office of Environmental Health and Radiation Safety, ACUC Protocol 1096-5. All animals were allowed free access to water and were kept on a constant standard diet.

Determination of acute toxicity (LD_{50}): The LD_{50} for compounds were determined by injected different gradual increased doses of the tested compounds to adult mail albino rats, then calculate the dose cause 50% animal death, according to Austen et al. [18].

Anti-inflammatory activity: *Carrageenan® induced rat's paw: Procedure:* Groups of adult male albino rats (150-180 g), each of 8 animals were orally dosed with tested compounds at a dose level of 25-50 mg/kg one hour before carrageenan® challenge. Foot paw edema was induced by subplenter injection of 0.05 ml of 1% suspension of carrageenan® in saline into the planter tissue of one hind paw. An equal volume of saline was injected to the other hind paw and served as control. Four hours after drug administration the animals were decapitated, blood was collected and the paws were rapidly excised. The average weight of edema was examined for the treated as well as the control group and the percentage inhibition of weight of edema was also evaluated. Prednisolone® (5 mg/kg) was employed as standard reference against which the tested compounds were compared.

Calculation and evaluation: Thirty minutes after the rats are challenged by subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the planter side of the lift hind paw. The paw is marked with ink at the level of the lateral malleolus, the paw volume was measured by a sensitive method developed by Webb et al. [19] that calculated by interfacing a yridi Delta range top-loading balance with a micro-computer.

% Protection=(A-B) × 100/A

A=the paw volume of non-treated group

B=the paw volume of treated group

Estimation of plasma prostaglandin E2 (PGE2): The experimental method which was used in analgesic activity has been adopted according to the reported procedure [19].

Calculation and evaluation: The PGE2 was calculated for the treated and control groups, then the PGE2 percentage inhibition is determined by the following equation:

% inhibition=(A-B) × 100/A

A=PGE2 in the control group

B=PGE2 in the treated group

Analgesic activity: The experimental method which was used in analgesic activity has been adopted according to the reported procedure [18]. Potencies relative to that of valdecoxib were determined (Table 4).

Anticonvulsant activity: The experimental method which was used in anticonvulsant activity has been adopted from Tyahr [20].

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