Pharmacological Study on Some New 3-[(1-Methylpyrrol-2-yl)-methyl]-4-Substituted 4,5-Dihydro-1*H*-1,2,4-triazol-5-ones

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3-[(1-Methylpyrrol-2-yl)methyl]-4-substituted 4,5-dihydro-1*H*-1,2,4-triazol-5-ones were obtained by the cyclization reaction of 1-[(1-methylpyrrol-2-yl)acetyl]-4-substituted semicarbazides in alkaline medium. The effects of the synthesized compounds of the central nervous system of mice were studied.

Key words: 1,2,4-Triazol-5-one, Pharmacological Screening

Introduction

Triazoles and their heterocyclic derivatives represent an interesting class of compounds possessing a wide spectrum of pharmacological activities. Depending on the type of substituents these can show various biological activities such as anticonvulsant (Kane *et al*., 1990), analgesic (Turan-Zitouni *et al.*, 1999), anti-inflammatory (Wade *et al.*, 1982), antitumour (Demirbaş *et al.*, 2002), antimicrobial (Demirbaş *et al.*, 2005), and antiviral (Kritsanida *et al*., 2002). Several compounds containing an 1,2,4-triazole ring, for example fluconazole and itraconazole, are well known drugs. 1,2,4-Triazol-3-one drugs such as trazodone-HCl [2-{3-[4-(3-chlorophenyl)-1-piperazinyl]propyl}-1,2,4-triazol-[4,3-α]-pyridin-3-(2*H*)-one hydrochloride] (Brodgen *et al.,* 1981), nefazodone-HCl [2-{3-[4-(3-chlorophenyl)-1-piperazinyl]propyl}-5-ethyl-2,4-dihydro-4-(2-phenoxyethyl)-3*H*-1,2,4-triazol-3-one hydrochloride] (Davis *et al.*, 1997), and etoperidone-HCl [2-{3-[4-(3-chlorophenyl)-1-piperazinyl]propyl}-4,5-diethyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one hydrochloride] (Ramacci *et al.*, 1979) are known for their therapeutic use in treating depression. These drugs selectively block postsynaptic serotonin 5-hydroxytryptamine (5-HT) receptors (Rafa *et al.*, 1992). Additionally, pyrrole and its derivatives have shown to possess biological activities such as antibacterial (Demirayak *et al.*, 1999), antitumour (Halazy and Magnus, 1984), and antitubercular (Sbardella *et al.,* 2004).

In view of the pharmacological profiles of the two chemical moieties described above, we synthesized hybrid compounds that comprise both the pyrrole and the 1,2,4-triazol-5-one ring system. The synthesis, determination of lipophilicity and antibacterial activities were described earlier (Pitucha *el al.*, 2009). This paper reports results of preliminary pharmacological investigations of the synthesized compounds. In particular, some behavioural tests were performed in order to check the influence of derivatives of 1,2,4-triazol-5-one on the central nervous system (CNS) of mice.

Results and Discussion

3-[(1-Methylpyrrol-2-yl)methyl]-4-substituted 4,5-dihydro-1*H*-1,2,4-triazol-5-ones were obtained by the intramolecular dehydrative cyclization reaction of 1-[(1-methylpyrrol-2-yl)acetyl]-4-substituted semicarbazides in basic medium (Pitucha *et al.*, 2009). The chemical strucures of the investigated compounds are shown in Fig. 1.

Preliminary pharmacological studies showed that these derivatives of 1,2,4-triazol-5-one have a slight depressive effect on the CNS of mice. All tested compounds prolonged the thiopental-induced sleep but only when administered in a dose of 100 mg/kg i.p. (Table I). None of the compounds had a neurotoxic effect because they did not disturb motor coordination in the "chimney test" in mice.

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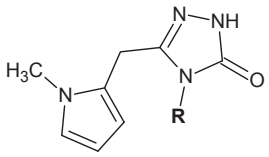

1: R = 4-BrC_6H_4
2: R = C_6H_{11}
3: R = C_2H_5

Fig. 1. Chemical structures of compounds **1**–**3**.

Compounds **1**–**3**, differing in the substituents at position 4 of the triazole ring, showed analgesic effects. Compound **2** [4-cyclohexyl-3-[(1-methylpyrrol-2-yl)methyl]-4,5-dihydro-1*H*-1,2,4-triazol-5-one] was most active. In a wide dose range, it lowered the pain reactivity of mice in the "writhing syndrome" test (Table II). Compounds **2** and **3**, at a dose of 100 mg/kg i.p., showed an anticonvulsive effect as they reduced the number of mice reacting with tonic seizures (Table III). Compound **3** [4-ethyl-3-[(1-methylpyrrol-2-yl)methyl]-4,5-dihydro-1*H*-1,2,4-triazol-5-one] significantly decreased their mortality. None of the tested compounds at 100 mg/kg i.p. showed anxiolytic, antidepressant or antiserotoninergic activities.

In conclusion, we found that the analgesic effect of the tested compounds is interesting and should be examined in more detail.

Table I. The influence of compounds **1**–**3** on thiopental-induced sleep ($N = 8$).

Compound	Treatment [mg/kg i.p.]	Sleeping time	
		[min ± SEM]	(%)
Control	–	30.5 ± 3.3	100.0 ± 10.8
1	50	36.6 ± 8.1	120 ± 26/5
	100	96.2 ± 18.6 *	315.6 ± 61 *
2	25	32 ± 2.6	104.9 ± 8.5
	50	124.4 ± 5.6 *	407.8 ± 18.3 *
	100	154.4 ± 3.8 *	506.3 ± 12.4 *
Control	–	30.0 ± 6.6	100.0 ± 22.0
3	25	46.4 ± 7.1	154.8 ± 23.7
	50	108.3 ± 0.69 *	361 ± 2.3 *
	100	107.1± 24.1 *	357. 1 ± 80.3 *

* $p < 0.001$ vs. the control group.

Table II. The antinociceptive activity of compounds **1**–**3** in the "writhing syndrome" test in mice ($N = 8$).

Compound	Treatment [mg/kg i.p.]	Mean writhing number	Inhibition[a] (%)
Control	–	26.8 ± 5.3	0
1	25.0	17.8 ± 5.8	33.6
	50.0	15.8 ± 1.3*	58.9*
	100.0	7.4 ± 2.5*	72.4*
2	6.25	22.5 ± 5.6	16
	12.5	16.0 ± 1.8*	40*
	25.0	8.6 ± 2.1*	67.9*
	50.0	2.1 ± 0.9*	92.2*
	100.0	1.7 ± 0.2*	93.7*
Control	–	28.6 ± 5.3	0
3	12.5	23.1 ± 8.4	19.2
	25.0	11.7 ± 4.2*	59.1*
	50.0	2.6 ± 0.9*	90.9*
	100.0	3.1 ± 1.2*	89.2*

[a] % of inhibition obtained by comparison with the control group.
* $p < 0.001$ vs. the control group.

Table III. The influence of the investigated compounds **1**–**3** on pentetrazole-induced convulsions in mice (N = 8).

Compound	Treatment [mg/kg i.p.]	Tonic seizure (%)	Lethality (%)
Control	–	75	50
1	100.0	62.5	37.5
2	50.0	87.5	75
	100.0	37.5*	25*
3	50.0	87.5	75
	100	25*	75

* $p < 0.05$ vs. the control group.

Experimental

Animals

The study was performed on male Albino Swiss mice (22–25 g) purchased from a licensed dealer (Górzkowska, Warsaw, Poland). They were kept in colony cages with free access to tap water and food (standard laboratory pellets; Bacutil, Motycz, Poland) and maintained under a natural day-night cycle. The experiments were performed between 8 a.m. and 3 p.m. All the experiments were approved by the Ethical Board of the Medical University of Lublin, Poland. The investigated compounds **1**–**3** were administered intraperitoneally (i.p.) in doses of 6.25, 12.5, 25, 50, and 100 mg/kg as suspensions in 1% aqueous Tween 80 solution at a constant volume of 0.1 ml per 10 g body weight of mice. Control animals received the same volume of solvent. Each experimental group consisted of eight animals.

Motor coordination in the "chimney test" (Boisser et al., 1960)

Briefly, mice had to climb up backwards in a plastic tube (3 cm inner diameter, 25 cm long). Mice unable to perform the task within 60 s were considered to display motor impairment. Motor impairment was quantified as the percentage of animals that failed to complete the test.

Body temperature

The rectal body temperatures of mice (measured with an Ellab thermometer) were recorded 15, 30, 45, 60, 90, and 120 min after injection of the investigated compounds in a dose of 100 mg/kg i.p.

Anxiolytic activity in the "four plate" test (Aron et al., 1971)

The anxiolytic activity was assessed 30 min after administration of **1**–**3** in a dose of 100 mg/kg i.p. The number of punished crossings was counted for 1 min.

Immobilization time in the despair test (Porsolt et al., 1977)

The compounds **1**–**3** were given in dose of 100 mg/kg i.p. before the testing. Mice were individually placed and forced to swim in a glass beaker (27 cm height, 16 cm diameter) containing 15 cm of water (25 ºC). A mouse was considered immobile when it floated in the water, in upright position, and made only small movements to keep its head above the water. The total immobility time of mice was measured during the last 4 min of the 6-min test.

Thiopental-induced sleep

60 mg/kg i.p. of thiopental (Sandoz, Vienna, Austria) were given 30 min after administration of **1**–**3** in doses of 50 and 100 mg/kg i.p.. The period during which the animals lost the righting reflex was regarded as the sleeping time.

Analgesic activity in the "writhing syndrome" test (Witkin et al., 1961)

Pain reactivity was measured in mice by the "writhing syndrome" test. 30 min after administration of **1**–**3** in doses of 100, 50, 25, 12.5 or 6.25 mg/kg i.p., the animals were injected i.p. with 0.6% acetic acid and the number of writhing episodes was counted for 30 min.

Pentetrazole-induced seizures

30 min after administration of compounds **1**–**3** in a dose of 100 mg/kg i.p., the mice were injected subcutaneously (sc) with pentetrazole (115 mg/kg). Right afterwards the animals were placed singly in organic cages (25 × 15 × 10 cm). The clonic and tonic seizures as well as mortality were recorded for 30 min.

Head-twitch responses induced by 5-hydroxytryptophan (5-HTP) (Corne et al., 1963)

The tested compounds **1**–**3** in a dose of 100 mg/kg i.p. were administered 30 min before of 5-HTP in a dose administration of 180 mg/kg. Immediately after the treatment the mice were separated in organic glasses and the number of head-twitch episodes was counted for 60 min.

Statistical analysis

The Student *t*-test (for analyzing the data from Tables I and II) or the Fisher two-tailed exact probability test (for analyzing the data from Table III) were used to determine the significance of differences between mean values of the control and investigated groups. Differences were considered significant when $p < 0.05$.

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