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ANTIBACTERIAL AND CENTRAL NERVOUS SYSTEM ACTIVITY OF (4,5-DIARYL-4*H*-1,2,4-TRIAZOL-3-YL)METHACRYLIC ACID DERIVATIVES

RENATA PAPROCKA[1*], BOŻENA MODZELEWSKA-BANACHIEWICZ[1], JOLANTA KUTKOWSKA[2], KAMIL PAWŁOWSKI[3], IWONA PIĄTKOWSKA-CHMIEL[3] and EWA JAGIEŁŁO-WÓJTOWICZ[3]

[1] Department of Organic Chemistry, Faculty of Pharmacy, Nicolaus Copernicus University in Toruń, A. Jurasza 2, 85-089 Bydgoszcz, Poland
[2] Department of Genetics and Microbiology, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland
[3] Department of Toxicology, Medical University, Chodźki 8, 20-093 Lublin, Poland

Abstract: The series of 1,2,4-triazole derivatives containing methacrylic acid moiety were synthesized in reaction of N^3-substituted amidrazones with itaconic anhydride. Preliminary calculated bioavailability parameters of obtained compounds suggested good penetration *via* cell membranes and their good absorption after oral intake. Antimicrobial evaluation *in vitro* showed diverse activity of obtained triazoles mainly on Gram-positive bacterial strains. One derivative was also examined to determine the effect on the central nervous system of mice.

Keywords: triazoles, methacrylic acid, propenoic acid, antibacterial activity, CNS activity

Triazole ring is an important pharmacophore present in numerous synthetic compounds with diverse biological activity (1). There are two main kinds of pharmacological effects displayed by triazole derivatives: antibacterial (2), antiviral (3), antifungal (2, 4) and central nervous system activity: anticonvulsant (5), antidepressant (5), analgesic (6). Triazoles were also described as anti-inflammatory and anticancer agents (7). Triazoles containing propenoic acid moiety showed high antibacterial activity against *Staphylococcus aureus, Escherichia coli* and *Mycobacterium smegmatis* (8), moreover they were described as potent anticonvulsant and antinociceptive agents (9). Due to this premise we decided for closer examination of antimicrobial properties of 1,2,4-triazole derivatives possessing similar methacrylic acid moiety. Those compounds showed anti-inflammatory activity similar to ibuprofen (10), which also give reasons for investigation of their influence on central nervous system to identify their possible side effects. The aim of this study was to evaluate the antimicrobial and central nervous system activity of 1,2,4-triazole derivatives containing methacrylic acid moiety. Obtained results enable to compare the influence of propenoic and methacrylic acid moieties present in 1,2,4-triazole derivatives on the activity of these compounds.

EXPERIMENTAL

Antimicrobial activity of compounds 1-9 *in vitro*

To evaluate the antimicrobial activity of triazoles **1-9** the broth microdilution method, in 96-well microtiter plates (Kartell), was used. The following bacterial strains were tested: Gram-negative: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Yersinia enterocolitica* O3; Gram-positive: *Staphylococcus aureus* ATCC 25923*, Enterococcus faecalis* ATCC 29212, *Sarcina lutea*; *Mycobacterium smegmatis, Nocardia corralina,* and the pathogenic fungus *Candida albicans*. The tested strains at final concentration of 10^5 CFU/mL were inoculated into a liquid Luria-Bertani (LB) medium in the presence of different concentrations (25, 50, 75, 100, and 250 mg/mL) of compounds dissolved in dimethyl sulfoxide (DMSO). Tests were performed in triplicate for each concentration, in all the tests, DMSO was used as the control. The microbial growth was measured at a wavelength of 550 nm after 18 h (bacteria) or 48 h

* Corresponding author: e-mail: renatabursa@o2.pl; phone: (+48) 525853905

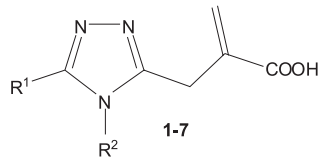
(*Candida*) incubation. The MIC (minimal inhibitory concentration) values were calculated based on the density of the growth and were the lowest triazoles concentration that reduced microbial growth as compared with the drug-free growth control.

Pharmacological screening of compound 2

The experiments were carried out on male Albino-Swiss mice (20-21 g) kept at room temperature of 21 ± 1°C under natural day-night cycle with free access to food and water *ad libitum*. Compound **2** was administered intraperitoneally (*i.p.*) as suspension in a 1% Tween 80 solution in the constant volume of 0.1 mL/10 g b.w. of mice. Control animals received the same volumes of the solvent. In all experiments the compound was used in doses starting from 0.1 to 0.00625 of LD_{50}. Each experimental group consisted of eight animals. The experiments were performed between 9 a.m. and 2 p.m. Permission for the animal tests and experiments was given by the Ethical Board of the Medical University of Lublin.

Screening of the CNS activity in mice was performed in 10 tests described below. Motor coordination was evaluated with the chimney test of Boissier et al. (9). The rectal body temperature in mice was measured by an Ellab thermometer. Exploratory and anxiety-related behavior was assessed according to Boissier and Simon (12). Anxiolytic activity was measured by the four plate test in mice according to Aron et al. (13). The passive avoidance task, which is considered to be a measure of long-term memory in animals, followed

Table 1. Structures and molecular properties of compounds **1-9** (TPSA - topological planar surface area, log P - octanol-water partition coefficient, nOHNH - number of hydrogen bond donors, nON - number of hydrogen bond acceptors, M.w. - molecular weight).



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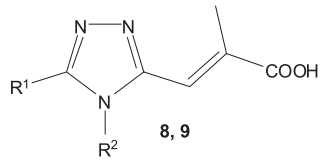

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No.	R^1	R^2	TPSA	log P	nOHNH	nON	M.w.
1	4-pyridyl	4-methylphenyl	80.91	2.21	6	1	320.35
2	2-pyridyl	4-methylphenyl	80,91	2.27	6	1	320.35
3	2-pyridyl	2-pyridyl	93.80	1.34	7	1	307.31
4	2-pyridyl	phenyl	80.91	1.82	6	1	306.32
5	phenyl	phenyl	68.02	2.96	5	1	305.34
6	phenyl	4-nitrophenyl	113.84	2.92	8	1	350.33
7	2-pyridyl	4-nitrophenyl	126.73	1.77	9	1	351.32
8	2-pyridyl	2-pyridyl	93.80	1.67	7	1	307.31
9	2-pyridyl	phenyl	80.91	2.15	6	1	306.32

Table 2. MIC values for compounds **1-9**.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Escherichia coli ATCC 25922	250	>250	250	>250	100	250	250	250	>250
Pseudomonas aeruginosa ATCC 27853	250	>250	250	>250	100	>250	250	>250	>250
Yersinia enterocolitica O:3	250	250	>250	250	250	250	250	>250	250
Staphylococcus aureus ATCC 25923	100	>250	250	>250	100	100	100	100	>250
Enterococcus faecalis ATCC 29212	>250	100	>250	100	>250	50	250	250	>250
Sarcina lutea	250	100	250	100	250	250	>250	250	>250
Mycobacterium smegmatis	250	>250	250	>250	250	250	100	250	250
Nocardia corralina	100	>250	250	250	>250	>250	250	250	250
Candida albicans	>250	250	250	250	>250	250	>250	>250	250

Table 3. The influence of compound **2** on thiopental-induced sleep in mice (n = 8).

Compound	Treatment	Sleeping time (min)	
	(mg/kg *i.p.*)	$\bar{X}$ ± S.E.M.	(%)
control	-	33.8 ± 8.2	100 ± 24,3
2	6.25	28.6 ± 9.3	84.6 ± 27,5
2	12.5	53.2 ± 6.4 *	157.4 ± 18,9 *
2	25	88.1 ± 18.4 *	260.7 ± 54,4 *
2	50	120 ± 18 *	355 ± 51,3 *
2	100	193 ± 12 *	571 ± 35,5 *

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

Table 4. The influence of compound **2** on pentetrazole-induced seizures in mice (n = 8).

Compound	Treatment	Seizures number (%)		Mortality
	(mg/kg i.p.)	clonic	tonic	
control	-	100	87.5	87.5
2	50	100	62.5	62.5
2	100	100	37.5 *	37.5 *

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

the procedure of Venault et al. (14). Antidepresive activity was assessed by the "forced swimming" test in mice according to Porsolt et al. (15). Thiopental-induced sleep was measured too. Pain reactivity was measured by the "writhing syndrome" test (9). Antiepileptic effects was tested by reduction of pentetrazole (105 mg/kg b.w.)-induced seizures (9). Antiserotoninergic effect was evaluated by the Corne test in mice according to Corne et al. (16). Student's *t*-test was used to determine the significance of differences between mean values of the control and investigated groups.

RESULTS AND DISCUSSION

The series of 1,2,4-triazole derivatives **1-9** (Table 1) were synthesized according to previously described procedures (10, 11). The structures of obtained compounds were confirmed by elemental analysis and spectral methods: IR, ^{1}H NMR, ^{13}C NMR, and MS as well as X-ray crystallography. The purity of compounds **1-9** were evaluated by reversed-phase thin layer chromatography (17).

Molecular properties of compounds **1-9** important for drug pharmacokinetics in the human body were calculated online by Molinspiration Chemoinformatics (18) and are compared in Table 1. According to Lipinski rule of five an oral active drug cannot violate more than one of the following criteria: = 5 hydrogen donors (nOHNH), = 10 hydrogen acceptors (nON), MW = 500 Da, log $P_{calc.}$ = 5 (19). All compounds **1-9** fulfilled the rule of five which indicates their good absorption after oral intake. Topological polar surface area (TPSA) is another useful indicator of drug absorbance and blood-brain barrier crossing (20). TPSA values lower than 140 Å suggest good cell membranes permeability of derivatives **1-9**, while values lover than 90 Å indicate chances to penetrate blood-brain barrier for compounds **1**, **2**, **4**, **5** and **9**. Basing on this calculations derivative **2** characterized by the highest lipophilicity among compounds possessing 2-pyridine ring was chosen for CNS activity pharmacological tests.

Antimicrobial activity of compounds 1-9

Obtained MIC values of the triazoles **1-9** against tested microorganisms are presented in Table 2. Only compound **5** possessing two phenyl substituents was effective against *E. coli* and *P. aeruginosa* Gram-negative bacterial strains at concentration 100 μg/mL. The most susceptible to tested compounds Gram-positive strain was *S. aureus*, its growth was inhibited by five derivatives: **1** and **5-8** at the concentration of 100 μg/mL. The strongest bacteriostatic effect against *E. faecalis* showed at concentration 50 μg/mL compound **6** possessing phenyl and 4-nitrophenyl substituents. Compounds **2** and **4** possessing 2-pyridine ring were active

against *E. faecalis* and *S. lutea* (MIC = 100 μg/mL), however other compounds with this substituent were less effective. Only derivative **1** carrying a 4-pyridyl moiety inhibited growth of *Nocardia corralina* at the concentration of 100 μg/mL; whereas triazole **7** with 2-pyridyl and 4-nitrophenyl substituents was active against *Mycobacterium smegmatis* at 100 μg/mL. The weakest antibacterial effect was observed for compounds **3**. Compounds **1-9** showed no significant antifungal activity against *Candida albicans*.

Pharmacological screening of compound 2

The effects of investigated compound **2** in behavioral studies was carried out on male Albino Swiss mice. Acute toxicity was low (LD_{50} value 1000 mg/kg *i.p.*). Preliminary pharmacological studies showed that compound **2** do not display the neurotoxic activity and weakly affect the CNS of mice. Compound **2** in a wide range prolonged the time of sleep induced by thiopental (Table 3). A significant relationship between the dose and effect was observed. Compound **2** given only in a dose of 100 mg/kg significantly decreased the number of clonic and tonic seizures induced by pentetrazole in comparison with control group (Table 4). In the remaining tests compound **2** was inactive.

CONCLUSION

Biological properties of nine 1,2,4-triazole derivatives possessing methacrylic acid moiety were experimentally evaluated. Triazoles **1-9** showed moderate antimicrobial activity. Most of them exhibited antibacterial effect against Gram-positive bacteria strains with MIC values ranging from 50 to 100 μg/mL; but no antifungal and antimycobacterial activity was observed. Similar 1,2,4-triazoles containing propenoic acid substituent (8) have been shown to possess a higher antibacterial activity, characterized by lower MIC values. The pharmacological effects exerted of compound **2** on the central nervous system of mice was relatively low; only at the highest dose the anticonvulsant activity was observed. The presence of methacrylic acid group in triazoles may be responsible for the decrease of antibacterial and loss of antinociceptive activities in comparison to analogous propenoic acid derivatives.

Authors declare no conflict of interests.

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