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Biological activity of 1,2,4-triazineand 1,2,4-triazole derivatives

Aktywność biologiczna pochodnych 1,2,4-triazin i 1,2,4-triazoli

SUMMARY

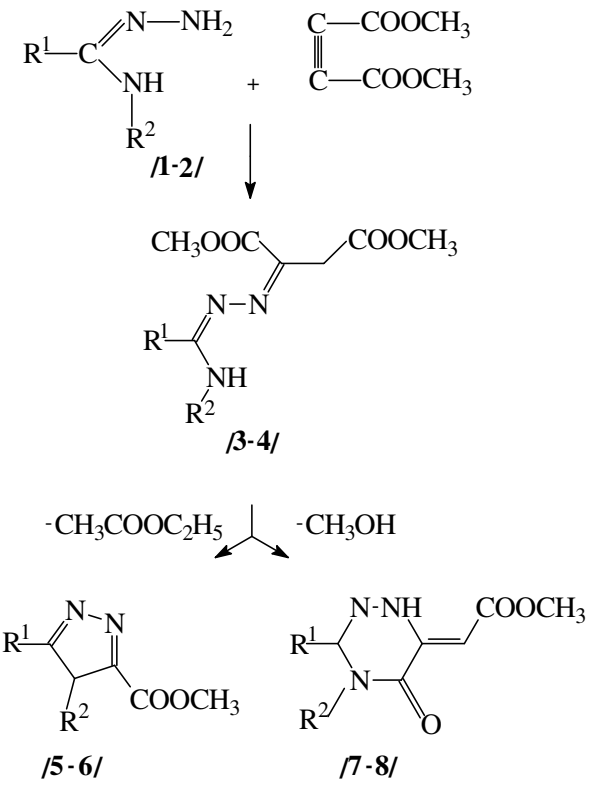
Triazine and triazole are used in medicine, agriculture and industry and they are also used as effective herbicides and fungicides. In continuation of our studies on condensed heterocycles 1,2,4-triazine and 1,2,4-triazole derivatives were prepared. This newly synthesized compounds were screened for their antibacterial and antifungal activity. The results of such studies are discussed in this paper.

Key words: 1,2,4-triazine, 1,2,4-triazole, antimicrobial activity, *in vitro* study

INTRODUCTION

The reaction of the N^3-substituted amidrazones with dimethyl acetylenedicarboxylate led to the formation of derivatives of dimethyl 2-[(1-arylamino-1-arylmethylidene)hydrazono] succinate /3-4/ [Modzelewska-Banachiewicz and Kałabun 1999] Cyclization of compounds **3, 4** carried out in methanol solution in the presence of triethylamine led to the formation of methyl 2-(5-oxo-3,4-diaryl-1,4,5,6-tetrahydro-1,2,4-triazine-6-ylidene)-acetates **/5–6/** [Modzelewska-Banachiewicz and Kałabun 1999]. The cyclization reaction of dimethyl 2-[(1-arylamino-1-arylmethylidene)hydrazono] succinate when performed in boiling n-butanol can lead to the formation of derivatives of 5-oxo-1,2,4-triazine-6-carboxylic acid **/5–6/** (with the liberation of a methanol molecule) and 1,2,4-triazole-5-carbixylic acid **/7–8/** (with concomitant liberation of a molecule of methyl acetate) with ratio of ca. 1:1. Separation of both reaction products was possible based on their different solubility in ethyl ether [Modzelewska-Banachiewicz and Kamińska 2001].

	R^1	R^2
1, 3, 5, 7	2-C_5H_4N	C_6H_5
2, 4, 6, 8	2-C_5H_4N	p-$CH_3C_6H_4$



The chemical structures of compounds **5–8** were confirmed by IR, ^{1}H, ^{13}C and ^{15}N NMR, ^{13}C CPMAS NMR and X-ray. Molecular modeling shows that there is not enough space for free rotation of neighboring substituent at C^3 and N^4 and two rings, especially in **5, 6**, should be twisted with respect to the central heterocyclic system. The X-ray diffraction analysis of **7** and **5** evidenced that in the crystal of **7** the twist angles of 2-pyrydyl and phenyl rings are 20.4 and 74.0^0, whereas in **5** they are 51.6 and 80.5^0, respectively [Bednarek *et al.* 2001]. The purity of the compounds was confirmed by the TLC method **/5–8/** [Modzelewska and Pyra 1995–1996, Modzelewska-Banachiewicz and Kamińska 2001].

Acute toxicity LD_{50} of compounds **5, 6** in mice was over 2000 mg kg^{-1} i.p. when that of compounds **7, 8** was over 1750 mg kg^{-1}. In behavioral tests, compound 7 exhibited only weak analgesic activity in the „writhing test", while compound **5** showed no effect on the central nervous system of mice in all behavioral tests applied [Modzelewska and Pyra 1995–1996, Modzelewska-Banachiewicz and Kamińska 2001].

Compound **5** from this group also exhibited moderate antibacterial and antifungal activity with MIC values of 200–300 μg ml^{-1} against *Escherichia coli, Brucella abortus, Mycobacterium smegmatis, Candida albicans* and *Epidermophyton floccosum* [Modzelewska and Pyra 1995–1996]. Compounds **5** and **7** at a 500 μg ml^{-1} concentration did not affect the microflora of the human digestive tract [Szcześniak and Modzelewska 2001] nor, at a 10–400 μg ml^{-1} concentration, the morphotic elements of the green monkey kidney cells [Truchliński *et al.* 2000]. The effects of compounds **5** and **7** upon replication (proliferation in the virus-susceptible cells) of VSV, EMCV and AV-5 were

different. Depending on the compound dose and the viral species examined they decreased proliferation by 3.3–10 times, which is comparable with the medical compound called Vratizolin used therapeutic ably. In a similar experimental model Vratizolin decreased AV – 5 titre ca. 10 times. The compounds affected early stages of virus replication (absorption and penetration); however, they did not affect later stages of proliferation [Modzelewska-Banachiewicz and Kamińska 2001].

This paper presents biological activity of methyl-2-[5-oxo-3-(2-pyridyl)-4-phenyl-1,4,5,6-tetrahydro-1,2,4-triazine-6-ylidene]acetate **/5/**, methyl-2-[5-oxo-3-(2-pyridyl)-4-(4-tolyl)-1,4,5,6-tetrahydro-1,2,4-triazine-6-ylidene]acetate **/6/**, methyl-[3-(2-pyridyl)-4-phenyl-1,2,4-triazole]-5-carboxylate **/7/** and methyl-[3-(2-pyridyl)-4-(4-tolyl)-1,2,4-triazole]-5-carboxylate **/8/**.

MATERIAL AND METHODS

Antimicrobial screening

Antibacterial and antifungal activities of newly synthesized compounds were tested *in vitro* in relation to 5 bacterial and 20 fungal strains. All strains under study were clinical isolates identified by means of conventional microbiological methods. Dilution method on Mueller-Hinton Agar (Merck) for estimation of MIC values (MIC caused full inhibition of growth) was applied to evaluation the antimicrobial activity.

The newly synthesized compounds were screened for their *in vitro* antibacterial activity against *Staphylococcus aureus* (n = 2), *Streptococcus pneumans*, *Enterococcus faecalis, Escherichia coli* and antifungal activity against yeasts: *C. albicans* (n = 5*), Malassezia pachydermatis* (n = 5) and dermatophytes *Trichophyton mentagrophytes* (n = 5), *Microsporum canis* (n = 5). These were either reference strains or the strains isolated directly from clinical materials.

Microorganisms were multiplied on the Muller-Hinton agar (bacteria) and Muller-Hinton agar enriched with 4% glucose and adjusted to pH 6.0. (fungi). The tested compounds were dissolved in DMSO, whose influence on microorganisms was parallelly tested. A medium having a maximum compounds concentration (200 μg ml^{-1}) contained 3% DMSO. Amounts of 0.02 ml microorganism cultures (about 10^5 cfu of bacterial cells, and 10^4 cfu of fungi) were put onto Petri dishes containing 20 ml medium with the addition of decreasing concentrations of compounds (200–0.01 μg ml^{-1}). The plates were incubated for 24 hrs at 37°C (bacteria) or for 48 h at 37°C (Yeast-like fungi) and for 3–7 days at 37°C (genus *Trichophyton*) or 25°C (genus *Microsporum*). At the same time, the sensitivity of the strains to DMSO was determined.

The presented results were obtained from three independent measurements. The investigations were carried out in the Department of Pharmacology and the Department of Microbiology, Agricultural University, Lublin.

RESULTS AND DISCUSSION

The antifungal and antibacterial *in vitro* activities of the synthesized compounds were studied through applying the broth dilution method, which is one of the most precise and reliable methods for determining the degree of sensitivity of microbes to antibiotics. Other 1,2,4-triazole and 1,2,4-triazine heterocyclic entities that are very interesting components in terms of their biological properties, such as antifungal, antibacterial and herbicidal were studied.

Table 1. Antifungal activities of 1,2,4-triazine and 1,2,4-triazole tested *in vitro*
Tabela 1. Aktywność antygrzybicza pochodnych 1,2,4-triazin i 1,2,4-triazoli w badaniach *in vitro*

Group Grupa	Species Gatunek	Number of strains Liczba szczepów	MIC μg ml^{-1}
Yeast-likes fugi Grzyby drożdżopodobne	*Candida albicans* *Malassezia pachydermatis*	5 5	> 200 > 200
Dermatophytes Dermatofity	*Trichophyton mentagrophytes* *Microsporum canis*	5 5	> 200 > 200

Table 2. Antibacterial activities of 1,2,4-triazine and 1,2,4-triazole tested *in vitro*
Tabela 2. Aktywność antybakteryjna pochodnych 1,2,4-triazin (3,4) i 1,2,4-triazoli (1,2) w badaniach *in vitro*

Species Gatunek	Number of strains Liczba szczepów	MIC μg ml^{-1}			
		1	2	3	4
Staphylococcus aureus	5	0.012	0.05	0.025	0.025
Staphylococcus aureus ®	2	0.39	0.78	1.65	3.15
Streptococcus pheumoniae	3	0.20	0.10	0.25	0.20
Enterococcus faecalis	5	0.05	0.05	0.05	0.05
Escherichia coli	5	0.10	0.10	0.10	0.15

The role of uncondensed 1,2,4-triazine derivatives and the related compounds as biocidal plant protection agents such as herbicides, bactericidal, fungicidal, antimicrobial, protozacides, anticoccidial, parasiticides, insecticides, acaricdes and pesticides, is presented. The results of the antibacterial effect of the newly synthesized compounds were reported as MIC against Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*. The other compounds had no inhibitory activity.

It can be concluded from microbiological tests that the newly synthesized compounds /1–4/ have low antifungal activity. They did not inhibit the growth of any strains studied even at 200 μg ml^{-1} concentration (Tab. 1).

The results of studies on antibacterial activity these compounds are given in Tab. 2. 1,2,4- triazine derivatives exhibit higher activity against Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus pneumoniae* and *Enterococcus faecalis*) and low activity against Gram-negative bacteria (*Escherichia coli*) and strains *Staphylococcus aureus*^{R}.

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STRESZCZENIE

Triaziny, triazole oraz ich pochodne znajdują szerokie zastosowanie w medycynie, rolnictwie oraz przemyśle spożywczym, głównie jako wysokiej skuteczności preparaty o właściwościach chwasto- i grzybobójczych. Obecne badania dotyczą dwóch nowych pochodnych: 1,2,4-tiazyny i 1,2,4-triazole oraz ich antybakteryjnej i antygrzybiczej aktywności. Wykazano stosunkowo wysoką aktywność badanych preparatów w stosunku do bakterii gram-dodatnich (*S. aureus, S. pneumoniae i E. faecalis*).

Słowa kluczowe: 1,2,4-tiazyny, 1,2,4-tiazole, aktywność antybakteryjna, aktywność antygrzybicza, badania *in vitro*