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BIOLOGICAL ACTIVITY OF METHYL 2-[5-OXO-3,4-DI-(2-PIRYDYL)-1,4,5,6-TETRAHYDRO-1,2,4-TRIAZINE-6-YLIDENE] ACETATE

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The NCNN group is an essential part of various heterocycles bearing high biological activities. 1,2,4-Triazines and their condensed derivatives found applications as pharmaceuticals and in agriculture. For example vardenafil – phosphodiesterase inhibitor, is useful in the treatment of male erectile dysfunctions (1), apazone exhibit anti-inflammatory and analgesic actions (2), lamotrigine is used as anticonvulsant agent and in the treatment of bipolar depression (3). Moreover, ceftriaxon – well-known antibiotic (4) and also cytostatic nucleoside analog – azaribine (5), possess in the structure the 1,2,4-triazine ring. Pymetrozine represents a new chemical class of insecticides (6, 7). There is an interest in the synthesis and design of compound structurally based on this heterocyclic system because of their higher antimicrobial activity than that of standard drug and antifungal activity comparable with the standard drug (8, 9). They have been also reported to associate with antimycobacterial (10), antitumor (11, 12), antiviral (13-15), anxiolytic and antidepressant (16, 17) effects.

In our previous study on the reaction of the N^3-substituted amidrazones with dimethyl acetylenedicarboxylate in absolute ethanol at the temperature of -10°C, derivatives of dimethyl 2-[(1-aryl-amino-1-arylmethylidene)hydrazono]succinate were obtained. Only synthesis with amidrazone containing two 2-pirydyl substituents gave methyl 2-[5-oxo-3,4-di-(-2-pyridyl)-1,4,5,6-tetrahydro-1,2,4-triazine-6-ylidene)acetate under above-mentioned conditions. Cyclization of other linear products was carried out in methanol solution in the presence of triethylamine. The following substances were obtained: methyl 2-(5-oxo-3,4-diphenyl-1,4,5,6-tetrahydro-1,2,4-triazine-6-ylidene)acetate **(1)**, methyl 2-(5-oxo-3-phenyl-4-*p*-tolyl-1,4,5,6-tetrahydro-1,2,4-triazine-6-ylidene)acetate **(2)**, methyl 2-[5-oxo-4-phenyl-3-(2-pyridyl)-1,4,5,6-tetrahydro-1,2,4-triazine-6-ylidene]acetate **(3),** methyl 2-[5-oxo-3-(2-pyridyl)-4-*p*-tolyl-1,4,5,6-tetrahydro-1,2,4-triazine-6-ylidene]acetate **(4)**, methyl 2-[4-(4-nitrophenyl)-5-oxo-3-(2-pyridyl)-1,4,5,6-tetrahydro-1,2,4-triazine-6-ylidene]acetate **(5)**, methyl 2-[5-oxo-3,4-di-(-2-pyridyl)-1,4,5,6-tetrahydro-1,2,4-triazine-6-ylidene)acetate **(6)** (Figure 1, Table 1) (18). The acute toxicity of compounds **1-6** in mice was low (> 2000 mg/kg i.p.). Depending on the type of substituent methyl 2-(5-oxo-1,2,4-triazine-6-ylidene)acetate derivatives show different activity. The role of compounds **3** and **4** as biocidal plant protection agents such as herbicides, bactericidal, fungicidal, antimicrobial, protozacides, anticoccidial, parasiticides, insectides, acaricides and pestides, was presented in earlier report (19). Compounds **1** and **3** at a 500 μg mL^{-1} concentration did not affect the microflora of the human digestive tract (20) nor at a 10 – 400 μg mL^{-1} concentration, the morphotic elements of the green monkey kidney cells (21). Depending on the compound **3** dose and the viral species examined (VSV, EMCV and AV-5) it decreased proliferation comparable with the medical compound called Vratizolin (22).

We present here more extensive antimicrobial testing results for compound **6** because of promising activity of this type molecules.

EXPERIMENTAL

Material and methods

Bacteria were used as follows: *Acinetobacter baumannii* multidrug resistant (3 strains), *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* multidrug resistant (2 strains), *Esche-*

	R^1	R^2
1	C_6H_5	C_6H_5
2	C_6H_5	4-$CH_3C_6H_4$
3	2-C_5H_4N	C_6H_5
4	2-C_5H_4N	4-$CH_3C_6H_4$
5	2-C_5H_4N	4-$NO_2C_6H_4$
6	2-C_5H_4N	2-C_5H_4N

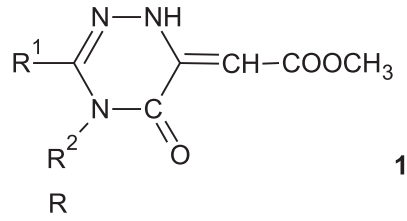

Figure 1. Structure of methyl 2-[5-oxo-3,4-di-substituted-1,4,5,6-tetrahydro-1,2,4-triazine-6-ylidene)acetate derivatives

Table 1. Distribution of MIC in μg/mL for bacteria and fungi.

Pathogen	Number of strains	MIC (μg/mL)
Gram-positive bacteria	10	512
Gram-negative bacteria	12	512
Yeasts: Candida albicans*	 20 2 1 1	 512 256 16 8
Candida non-albicans**	7 1	512 32

* 6 strains resistant to fluconazole and itraconazole
** 2 strains resistant to fluconazole and itraconazole

richia coli ATCC 35218, *Escherichia coli* ATCC 25922, *Escherichia coli* multidrug resistant (1 strain), *Klebsiella pneumoniae* ATCC 700603, *Klebsiella. pneumoniae* multidrug sensitive (2 strains), *Enterococcus faecalis* ATCC 21299, *Enterococcus faecalis* ATCC 29219, *Enterococcus faecalis* (1 strain), *Staphylococcus aureus* 209.P, *Staphylococcus aureus* MR-3, *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 25923, *Staphylococcus. aureus* ATCC 25213, *Staphylococcus aureus* ATCC 29213, *Staphylococcus lugduensis* ATCC 700328. The study was run also on 24 strains of *Candida albicans* (6 strains resistant on fluconazole and itraconazole), and 8 strains of *Candida* non-*albicans* (2 strains resistant on fluconazole and itraconazole), isolated from various clinical materials in University Hospital in Bydgoszcz. Antifungal activity was tested by the disc-diffusion method under standard conditions using Mueller-Hinton Agar (bio-Mérieux) as described by CLSI (Clinical and Laboratory Standards Institute) (23). All chemical and solvents (DMSO) were purchased from Sigma. The results were read following 24 h incubation at 35°C. Antifungal and antibacterial activity was expressed as Minimal Inhibitory Concentration (MIC) values in μg/mL.

RESULTS AND DISCUSSION

In vitro sensitivities to the 5-oxo-1,4,5,6-tetrahydro-1,2,4-triazine derivative **6** were similar for all tested bacterial strains, 20 strains of *Candida albicans* and 7 strains of *Candida* non-*albicans* with a MIC_{50} of 512 μg/mL. It was observed that the growth of only two strains of *Candida albicans* was inhibited by compound **6** at a concentration of 256 μg/mL. Surprisingly, this substance exhibited higher antifungal potency against three strains of *Candida*. The much lower MIC values of 8, 16 and 32 μg/mL have been found against two strains of *Candida albicans* and for one of *Candida* non-*albicans*, respectively (Table 2).

According to the literature, *Candida albicans* is an increasingly important opportunistic fungal pathogen and the incidence of fungal infections has risen significantly. Unfortunately, the arsenal of available antifungal drugs has not expanded to meet

the problem, and this serious disease often leads to death, even with treatment (24). Recently, Cernicka and co-workers reported that one of condensed derivatives of triazine, CTBT, displayed weak antifungal activity and strongly inhibited growth of yeast cells in combination with subinhibitory concentrations of other antifungals with a different mode of action. CTBT increased the sensitivity to fluconazole in multidrug-resistant cells overexpressing the efflux pumps. This novel chemosensitization by CTBT that can overcome multidrug resistance in yeast may prove useful in combined treatment of infections caused by drug-resistant fungal pathogens (25). Considering these findings, we would like to evaluate this type antifungal action of our compound in further research.

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