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Antimicrobial screening of certain fused 1,2,4-triazine derivatives

Imidazotriazines display a wide spectrum of biological activity. Till now all known imidazo[2,1-c]triazines have been prepared and tested as cardiovascular agents (7), bactericides (6), central nervous system stimulants (2). Other derivatives of this ring system have been received as the Maillard reaction inhibitors. Nowadays it is considered that the Maillard reaction takes part in various diseases relating to diabetes and ageing. Some of imidazo[2,1-c]triazines described in the literature have been synthesized for the treatment or prevention of various diabetes complications such as a coronary disease, a periphery circulatory disorder, a renal disease, a cerebrovascular disorder, a diabetic neurosis, a retinitis, an articular sclerosis or the diseases caused by ageing such as a senile cataract, an atherosclerosis etc. by inhibiting the Maillard reaction (4).

On the other hand, Azaribine is a new antiviral agent structurally based on the 1,2,4-triazine heterocyclic system.

Imidazo[2,1-c]triazines reported herein contain in their chemical structure similar features (potential pharmacophore formations: the phenyl ring, the additional carbonyl group as the potential acceptor center of hydrogen bond) to many morphine-like analgesics, such as benzitramid, fentanyl, petidine and selective ligand of δ-opioid receptors – SCN-80. Besides, these compounds exhibited antinociceptive activity as the result of the "writhing syndrome" test indicated.

Taking into account the present activity of the above mentioned compounds on the central nervous system and the results of previous studies concerning the synthesis and biological action of structurally similar derivatives of the same heterocyclic ring system whose antinociceptive action was confirmed (9, 10), it seemed worthwhile to carry out antimicrobial screening to exclude their potential antimicrobial activity resulting from the literature data (6).

The *in vitro* antimicrobial activities against the bacterial, yeast-like fungi and moulds strains were determined by disc-diffusion method by Kirby-Bauer.

The following compounds were tested in relation to bacterial, fungal and moulds strains:

I. 3-methyl-8-phenyl-7,8-dihydro-6H-imidazo[2,1-c][1,2,4]triazin-4-one;
II. 3-methyl-8-(4-methylphenyl)-7,8-dihydro-6H-imidazo[2,1-c][1,2,4]triazin-4-one;
III. 3-methyl-8-(4-methoxyphenyl)-7,8-dihydro-6H-imidazo[2,1-c][1,2,4]triazin-4-one;
IV. 3-methyl-8-(3-chlorophenyl)-7,8-dihydro-6H-imidazo[2,1-c][1,2,4]triazin-4-one;
V. 3-methyl-8-(4-chlorophenyl)-7,8-dihydro-6H-imidazo[2,1-c][1,2,4] triazin-4-one;
VI. 3-methyl-8-(3,4-dichlorophenyl)-7,8-dihydro-6H-imidazo[2,1-c][1,2,4]triazin-4-one.

These compounds were obtained by cyclocondensation of adequate 1-arylimidazolidine-2-one hydrazones (1-aryl-2-hydrazinoimidazolines) with pyruvic acid. The structure of the above compounds was confirmed by elemental analysis and spectral data: infrared spectra (IR), nuclear magnetic resonance

(^{1}H NMR) and mass spectra (MS). Their purity was tested by means of thin layer chromatography (TLC). These compounds were characterized by solubility in dimethylformamide and dimethylsulfoxide (8).

Based on preliminary behavioral animal tests conducted on male Albino-Swiss mice (22–25 g) in the Department of Toxicology, Medical University of Lublin it has been shown that these compounds have low toxicity (LD_{50} value ranging from 850 mg kg^{-1} to above 1000 mg kg^{-1} b.w. via i.p.) and analgesic activity. Their further pharmacological activity is still being investigated in the Department of Toxicology, Medical University of Lublin.

MATERIAL AND METHODS

Assay of antimicrobial activity *in vitro*. The synthesized compounds were tested for their antimicrobial (antibacterial and antifungal) activities by the disc-diffusion method by Kirby-Bauer, using Mueller-Hinton medium for bacteria and the same medium with 4% glucose for fungi. The tested microorganisms were isolated from clinical specimens of the Laboratory of the Medical Microbiology Department, Medical University of Lublin. The assayed collection included 54 strains of Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus agalactiae*), 52 strains of Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Proteus spp., Klebsiella pneumoniae, Enterobacter aerogenes*), 6 strains of yeast-like fungi (*Candida albicans*), 3 strains of moulds (*Aspergillus spp.*) – Table 1.

Table 1. Microorganism cultures used to microbiological screening

Group	Strain	Number of strains
Gram-positive bacteria	*Staphylococcus aureus*	21
	Staphylococcus epidermidis	15
	Streptococcus pyogenes	12
	Streptococcus agalactiae	6
Gram-negative bacteria	*Escherichia coli*	16
	Pseudomonas aeruginosa	12
	Proteus spp.	10
	Klebsiella pneumoniae	8
	Enterobacter aerogenes	6
Yeast-like fungi	*Candida albicans*	6
Moulds	*Aspergillus spp.*	3

In the disc-diffusion method, sterile paper disc (ϕ5mm) impregnated with dissolved in dimethylsulfoxide (DMSO) compound at concentrations of 100 μg ml^{-1} and 200 μg ml^{-1} were used. Discs containing DMSO were used as control. The microorganisms cultures were spread over the following appropriate media: Mueller-Hinton agar for *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus agalactiae, Escherichia coli, Pseudomonas aeruginosa,* Proteus *spp., Klebsiella pneumoniae, Enterobacter aerogenes*, and Saburoud agar for the yeast-like fungi (*Candida albicans*) and for the moulds (*Aspergillus spp.*) in Petri dishes. Then, the

paper discs impregnated with the solutions of the compound tested were placed on the surface of the media inoculated with the microorganism. The plates were incubated at 35°/24 h for the microorganisms cultures. After incubation, the zones of growth inhibition around the discs were observed indicating that the examined compound inhibits the growth of microorganism (1, 3, 5).

RESULTS AND DISCUSSION

In connection with the influence of the above-mentioned compounds on the central nervous system it seemed worthwhile to carry out antimicrobial screening (antibacterial and antifungal) to exclude their potential antimicrobial activity resulting from the literature data.

Antibacterial and antifungal activities of the obtained compounds were tested in relation to 54 strains of Gram-positive and 52 strains of Gram-negative bacteria, 6 strains of yeast-like fungi and 3 strains of moulds. It can be concluded from microbiological screening tests that compounds I–VI in the examined concentrations (100 $\mu g\ ml^{-1}$ and 200 $\mu g\ ml^{-1}$) had no influence on the growth of microorganisms tested.

Lack of antimicrobial activity of the tested compounds seemed to be profitable in the case of compounds possessing effect on the central nervous system i.e. showing antinociceptive activity.

CONCLUSIONS

1. It can be concluded from microbiological tests that compounds 1–6 in examined concentrations (100 $\mu g\ ml^{-1}$ and 200 $\mu g\ ml^{-1}$) had no influence on the growth of microorganisms tested.

2. These results afforded to limit the possible biological spectrum of activity of fused 1,2,4-triazines.

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SUMMARY

The synthesized fused 1,2,4-triazines were tested for their potential antimicrobial activity resulting from the literature data. Microbiological screening tests conducted in relation to 54 strains of Gram-positive and 52 strains of Gram-negative bacteria, 6 strains of yeast-like fungi and 3 strains of moulds showed that these compounds in examined concentrations 100 mg ml^{-1} and 200 mg ml^{-1} had no influence on the growth of microorganisms tested. The microbiological screening tests afforded to limit the possible biological spectrum of activity of tested compounds.

Badanie aktywności przeciwdrobnoustrojowej
niektórych skondensowanych pochodnych 1,2,4-triazyny

Otrzymane skondensowane pochodne 1,2,4-triazyny przebadano pod względem ich potencjalnej aktywności przeciwdrobnoustrojowej. W oparciu o przeprowadzone – na 54 szczepach bakterii Gram-dodatnich, 52 szczepach bakterii Gram-ujemnych, 6 szczepach drożdżaków i 3 szczepach pleśni – testy aktywności przeciwdrobnoustrojowej otrzymanych związków wykazano ich brak wpływu w badanych stężeniach (100 μg ml^{-1} i 200 μg ml^{-1}) na wzrost testowanych drobnoustrojów. Wyniki badań pozwoliły zawęzić szerokie spektrum aktywności biologicznej skondensowanych pochodnych 1,2,4-triazyny.