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The susceptibility of certain bacterial strains to imidazotriazine 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). The 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 retinities, an articular sclerosis or the diseases caused by ageing such as a senile cataract, an atherosclerosis etc. by inhibiting the Maillard reaction (4).

Taking into account the presence of certain substituents and formations in the structure of synthesized imidazotriazine derivatives which are also present in certain antimicrobial agents, the *in vitro* antimicrobial activities against the bacterial, yeast-like fungi and moulds strains were determined by the disc-diffusion method by Kirby-Bauer.

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

1. 3-[4'-(2'-aminothiazolyl)]-8-phenyl-7,8-dihydro-6H-imidazo[2,1-c][1,2,4]triazin-4-one;
2. 3-[4'-(2'-aminothiazolyl)]-8-(2-methylphenyl)-7,8-dihydro-6H-imidazo[2,1-c][1,2,4] triazin-4-one;
3. 3-[4'-(2'-aminothiazolyl)]-8-(4-methylphenyl)-7,8-dihydro-6H-imidazo[2,1-c][1,2,4] triazin-4-one;
4. 3-[4'-(2'-aminothiazolyl)]-8-(4-ethoxyphenyl)-7,8-dihydro-6H-imidazo[2,1-c][1,2,4] triazin-4-one;
5. 3-[4'-(2'-aminothiazolyl)]-8-(2,3-dimethylphenyl)-7,8-dihydro-6H-imidazo[2,1-c][1,2,4] triazin-4-one;
6. 8-phenyl-6,7-dihydro-4-oxoimidazo[2,1-c][1,2,4]triazine-3-acetic acid hydrazide;
7. 8-(4-methylphenyl)-6,7-dihydro-4-oxoimidazo[2,1-c][1,2,4]triazine-3-acetic acid hydrazide;
8. 8-(4-methoxyphenyl)-6,7-dihydro-4-oxoimidazo[2,1-c][1,2,4]triazine-3-acetic acid hydrazide;
9. 8-(4-ethoxyphenyl)-6,7-dihydro-4-oxoimidazo[2,1-c][1,2,4]triazine-3-acetic acid hydrazide;
10. 8-(4-chlorophenyl)-6,7-dihydro-4-oxoimidazo[2,1-c][1,2,4]triazine-3-acetic acid hydrazide;
11. 3,8-diphenyl –7,8-dihydro-6H-imidazo [2,1-c][1,2,4]triazin-4-one;
12. 3-phenyl-8-(2-methylphenyl) –7,8-dihydro-6H-imidazo [2,1-c][1,2,4]triazin-4-one;
13. 3-phenyl-8-(4-methylphenyl) –7,8-dihydro-6H-imidazo [2,1-c][1,2,4]triazin-4-one;
14. 3-phenyl-8-(2-methoxyphenyl) –7,8-dihydro-6H-imidazo [2,1-c][1,2,4]triazin-4-one;
15. 3-phenyl-8-(4-methoxyphenyl) –7,8-dihydro-6H-imidazo [2,1-c][1,2,4]triazin-4-one;
16. 3-phenyl-8-(4-ethoxyphenyl) –7,8-dihydro-6H-imidazo [2,1-c][1,2,4]triazin-4-one;
17. 3-phenyl-8-(2,3-dimethylphenyl) –7,8-dihydro-6H-imidazo [2,1-c][1,2,4]triazin-4-one;
18. 3-phenyl-8-(4-chlorophenyl) –7,8-dihydro-6H-imidazo [2,1-c][1,2,4]triazin-4-one;
19. 8-(4-chlorophenyl)-4-oxo-2H-3,4,6,7-tetrahydroimidazo[2,1-c][1,2,4]triazine-3-acetic acid.

These compounds were obtained in the reaction of 1-arylimidazolidine-2-one hydrazones with ethyl 2-(2-amino-4-thiazolyl)glyoxylate (compounds 1–5), ethyl phenylglyoxylate (compounds 11–18), fumaric acid (compound 19) and in the two step reaction of 1-aryl-2-hydrazinoimidazolines with dimethyl acetylenedicarboxylate and hydrazine hydrate (compounds 6–10). The structure of above compounds was confirmed by elemental analysis and spectral data: infrared spectra (IR), nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) and mass spectra. Their purity was tested by means of chromatography. These compounds were characterized by solubility in dimethylformamide (compounds 1–10, 13, 19), mixture of methanol/dichloromethane (compounds 12, 14–18), and dimethylsulfoxide (8, 9, 10, 11).

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 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 for 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 (ϕ 5mm) 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

Antibacterial and antifungal activities of the obtained compounds were tested in relation to 54 strains of Gram-possitive 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 1–18 in examined concentrations (100μ ml^{-1} and 200μ ml^{-1}) had no influence on the growth of microorganisms tested. Compound 19 significantly inhibited the growth of certain Gram-negative bacterial strains studied. It was active against 16 strains of *Escherichia coli*, 12 strains of *Pseudomonas aeruginosa*, 10 strains of *Proteus spp.*, 8 strains of *Klebsiella pneumoniae*, 6 strains of *Enterobacter aerogenes* in the examined concentrations of 100 μg ml^{-1} and 200 μg ml^{-1}. It had no influence on the growth of certain Gram-possitive bacterial strains: 21 strains of *Staphylococcus aureus*, 15 strains of *Staphylococcus epidermidis*, 12 strains of *Streptococcus pyogenes*, 6 strains of *Streptococcus agalactiae* and 6 strains of yeast-like fungi (*Candida albicans*) and 3 strains of moulds (*Aspergillus spp.*) in the examined concentrations.

Taking into account the significant activity of compound 19, particularly against Gram-negative strains of bacteria, the research on this field will be continued. It is likely to happen that its structural analogues should also be active.

CONCLUSIONS

1. It can be concluded from microbiological tests that compounds 1–18 in the examined concentrations (100 μg ml^{-1} and 200 μg ml^{-1}) had no influence on the growth of the tested microorganisms.

2. Compound 19 was active against all the tested strains of Gram-negative bacteria and inactive against all the tested strains of Gram-positive bacteria, 6 strains of yeast-like fungi and 3 strains of moulds.

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SUMMARY

The synthesized imidazo[2,1-c]triazines were tested for their antimicrobial activity. 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 significant activity of compound 19 against 52 Gram-negative bacterial strains. This compound was active against 16 strains of *Escherichia coli*, 12 strains of *Pseudomonas*, 10 strains of *Proteus spp.*, 8 strains of *Klebsiella pneumoniae* and 6 strains of *Enterobacter aerogenes* in the examined concentrations (100 μg ml^{-1} and 200 μg ml^{-1}).

Wrażliwość pewnych szczepów bakteryjnych na pochodne imidazotriazyny

Określono aktywność przeciwdrobnoustrojową zsyntetyzowanych związków. Na podstawie przeprowadzonych na 54 szczepach bakterii Gram-dodatnich, 52 szczepach bakterii Gram-ujemnych, 6 szczepach drożdżaków i 3 szczepach pleśni testów aktywności przeciwdrobnoustrojowej otrzymanych związków wykazano aktywność przeciwbakteryjną związku 19. Związek ten w badanych stężeniach (100 μg ml^{-1} i 200 μg ml^{-1}) silnie hamował wzrost 52 Gram-ujemnych szczepów bakteryjnych (16 szczepów *Escherichia coli*, 12 szczepów *Pseudomonas*, 10 szczepów *Proteus spp.*, 8 szczepów *Klebsiella pneumoniae* oraz 6 szczepów *Enterobacter aerogenes*).