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The susceptibility of certain microbial strains to some imidazoline derivatives

Imidazoline (4,5-dihydroimidazole) and its derivatives form an important group of organic compounds and many of them show pharmacological activity as ligands of the imidazoline receptor. Imidazoline system is the structural element of many drugs those have different pharmacological activity. The following adrenergic imidazoline derivatives are applicable in medicine: naphazoline, xylometazoline, oxymetazoline, fenoxazoline, tetryzoline. The others: tolazoline and phentolamine are used as α-adrenolytics, cifenline as antiarrhythmic, clonidine as hypotensive and antazoline as antihistaminic (5, 8).

Besides, from the literature data it follows that depending on the type of substituent derivatives of imidazoline may also show antimicrobial properties (2, 4, 6).

The following compounds obtained as potential ligands of the imidazoline receptor due to the reaction of 1-arylimidazolidin-2-one hydrazones (1-aryl-2-hydrazinoimidazolines) with dimethyl acetylenedicarboxylate (compounds I-III) or dimethyl-3-oxoglutarate (IV-VI) were tested *in vitro* in relation to bacterial, fungal and moulds strains to exclude or confirm their potential antimicrobial activity:

I. 2-(1-phenyl-Δ^2-imidazolin-2-ylhydrazono) succinate.
II. 2-[1-(4-methoxyphenyl)-Δ^2-imidazolin-2-ylhydrazono] succinate.
III. 2-[1-(4-chlorophenyl)-Δ^2-imidazolin-2-ylhydrazono] succinate.
IV. 3-[1-(4-methylphenyl)-Δ^2-imidazolin-2-ylhydrazono] glutarate.
V. 3-[1-(4-chlorophenyl)-Δ^2-imidazolin-2-ylhydrazono] glutarate.
VI. 3-[1-(3,4-dichlorophenyl)-Δ^2-imidazolin-2-ylhydrazono] glutarate.

Their chemical structures were confirmed on the basis of elemental analysis and spectral data: infrared (IR), nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) and mass spectra (MS). Their purity was tested by means of chromatography. All the compounds were characterized by solubility in methanol, dimethylformamide and dimethylsulfoxide (9, 10).

MATERIAL AND METHODS

Assay of antimicrobial activity *in vitro*. The synthesized compounds were tested for their antimicrobial (antibacterial and antifungal) activities by 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. 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 (ϕ 5 mm) 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, 7).

RESULTS AND DISCUSSION

Antibacterial and antifungal activities of obtained compounds were tested by the disc-diffusion method to Kirby-Bauer in relation to 54 Gram-positive and 52 Gram-negative bacterial strains, 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 of 100 μg ml^{-1} and 200 μg ml^{-1} had no influence on the growth of microorganisms tested. The conducted tests afforded to limit the possible biological spectrum of activity of synthesized imidazoline derivatives and exclude their potential antimicrobial activity.

CONCLUSIONS

1. All the tested compounds were inactive against of 54 Gram-positive and 52 Gram-negative bacterial strains, 6 strains of yeast-like fungi and 3 strains of moulds.

2. The microbiological screening tests afforded to limit the possible biological spectrum of activity of the tested compounds.

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SUMMARY

Imidazoline ring system is the structural element of many drugs which have different pharmacological spectrum of activity as ligands of the imidazoline receptor. Besides, from the literature data it follows that depending on the type of substituent certain derivatives of imidazoline may also show antimicrobial properties. The obtained compounds were tested for their potential antimicrobial activity. Microbiological tests conducted on 106 strains of bacteria, 6 strains of yeast-like fungi and 3 strains of moulds have shown that all the tested compounds in the examined concentrations (100 mg ml^{-1} and 200 mg ml^{-1}) had no influence on the growth of tested bacteria, yeast-like fungi and moulds.

Wrażliwość pewnych szczepów bakteryjnych na wybrane pochodne imidazoliny

Układ imidazoliny jest obecny w strukturze leków wykazujących różnorodne działanie farmakologiczne. Ponadto z danych literaturowych wynika, że w zależności od podstawnika niektóre pochodne imidazoliny mogą wykazywać aktywność przeciwdrobnoustrojową. Określono aktywność przeciwbakteryjną i przeciwgrzybiczą otrzymanych związków. Przeprowadzone na 106 szczepach bakteryjnych, 6 szczepach drożdżaków i 3 szczepach pleśni testy aktywności przeciwdrobnoustrojowej wykazały, że otrzymane związki w badanych stężeniach (100 μg ml^{-1} i 200 μg ml^{-1}) nie hamowały wzrostu bakterii, drożdżaków i pleśni.