ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LIX, N 2, 113 SECTIO D 2004

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# *The susceptibility of certain microbial strains to fused 1,2,4-triazole derivatives*

1,2,4-Triazole system is the structural element of many drugs that have different pharmacological activity. The following 1,2,4-triazole derivatives are applicable in medicine: alprazolam (tranquilizer), estazolam (hypnotic, sedative, tranquilizer), rilmazafon (hypnotic, anxiolytic, used in the case of neurotic insomnia), benatradin (diuretic), trapidil (hypotensive), trazodon (antidepressant, anxiolytic), etoperidone (antidepressant), nefazodone (antidepressant, 5-$HT_2$ A-antagonist), anastazole (antineoplastic, non-steroidal aromatase inhibitor), letrozole (antineoplastic, aromatase inhibitor), ribavirin (antiviral), fluconazole, itraconazole, terconazole (antifungal) (5).

Besides, from the literature data it follows that depending on the type of substituent derivatives of 1,2,4-triazole show antibacterial (1, 3, 9, 10), antifungal (10, 11) and anti-inflammatory (8) properties.

The following compounds obtained due to the reaction of 1-arylimidazolidin-2-one hydrazones with triethyl orthoformate and succinic anhydride were tested *in vitro* in relation to bacterial, fungal and moulds strains:

I. 7-phenyl-5H-6,7-dihydroimidazo[2,1-c][1,2,4]triazole;
II. 7-(4-methylphenyl)-5H-6,7-dihydroimidazo[2,1-c][1,2,4]triazole;
III. 7-(4-methoxyphenyl)-5H-6,7-dihydroimidazo[2,1-c][1,2,4]triazole;
IV. 7-(4-chlorophenyl)-5H-6,7-dihydroimidazo[2,1-c][1,2,4]triazole;
V. 7-(4-chlorophenyl)-3-thiolo-5H-6,7-dihydroimidazo[2,1-c][1,2,4]triazole;
VI. 3-[7-(2-methylphenyl)-5H-6,7-dihydroimidazo[2,1-c][1,2,4]triazol-3-yl]propionic acid monohydroiodide;
VII. 3-[7-(4-methylphenyl)-5H-6,7-dihydroimidazo[2,1-c][1,2,4]triazol-3-yl]propionic acid monohydroiodide;
VIII. 3-[7-(2,3-dimethylphenyl)-5H-6,7-dihydroimidazo[2,1-c][1,2,4]triazol-3-yl]propionic acid monohydroiodide.

Their chemical structures were confirmed on the basis of elemental analysis and spectral data (nuclear magnetic resonance and mass spectrum). All the compounds were characterized by solubility in ethanol/diethyl ether mixture, dimethylformamide and dimethylsulfoxide (12).

## 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 (2, 4, 6).

## RESULTS AND DISCUSSION

Based on microbiological tests conducted on eight compounds it has been shown that all the tested compounds in the examined concentrations of 100 μg $ml^{-1}$ and 200 μg $ml^{-1}$ had no influence on the growth of 54 Gram-positive and 52 Gram-negative bacterial strains and 3 strains of moulds. Among the variously substituted imidazotriazole derivatives compound V in concentrations of 100 μg $ml^{-1}$ and 200 μg $ml^{-1}$ significantly inhibited growth of 6 strains of yeast-like fungi (*Candida albicans*). It is of interest that some derivatives having the same heterocyclic skeleton –7-aryl-5--methyl-3-thiolo-imidazo[2,1-c][1,2,4]triazol-6-ones-described in the literature have shown also antifungal activity. Their structure is similar to compound V, which had also the thiol group in the 3rd position and the substituted phenyl ring in the 7th position. This compound had no methyl substituent in the 5th position and no oxo group in the 6th position in comparison with compounds of this heterocyclic skeleton described in the literature (11).

All the tested compounds had the same heterocyclic skeleton – imidazo[2,1-c] [1,2,4]triazole ring system. All were substituted in the 7th position by phenyl or substituted phenyl ring. Compounds I, II, III, IV were unsubstituted in the 3rd position and were inactive in microbial tests. Compound V had

thiol group in this position and was active against 6 strains of yeast-like fungi (*Candida albicans*). Compounds VI, VII, VIII possessed the hydroxycarbonylethyl formation in the 3rd position and were inactive in microbiological tests.

Based on performed antimicrobial screening the structure-activity relationship in the group of imidazo[2,1-c][1,2,4]triazole derivatives was observed. In the same ring system introduction of the thiol group in the 3rd position was necessary to show antifungal activity. The conducted tests afforded to limit the possible spectrum of microbiological activity of the examined compounds.

Taking into account the significant activity of compound V, particularly against yeast-like fungi, the research in this field will be continued. This refers to modifications of the presented structures and the synthesis of analogous derivatives of compound V.

## CONCLUSIONS

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

2. Compound V showed significant antifungal activity in the tested concentrations and the lack of activity against all the examined bacterial and moulds strains.

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SUMMARY

1,2,4-Triazole is the structural element of many drugs that have different pharmacological spectrum of activity. Besides, from the literature data it follows that 1,2,4-triazoles and its fused systems show antibacterial, antifungal and anti-inflammatory activities. 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 $\mu g\ ml^{-1}$ and 200 $\mu g\ ml^{-1}$) had no influence on the growth of the tested bacteria and moulds. It has been found that among variously substituted derivatives of imidazotriazole system compound V significantly inhibited the growth of 6 strains of *Candida albicans* in the examined concentrations. Its chemical structure was similar to novel antifungal agents described in the literature.

Wrażliwość pewnych szczepów bakteryjnych na skondensowane pochodne 1,2,4-triazolu

Układ 1,2,4-triazolu występuje w strukturze leków wykazujących szerokie spektrum aktywności farmakologicznej. Ponadto z danych literatury wynika, że 1,2,4-triazole, a także układy skondensowane zawierające w swojej strukturze ten układ wykazują aktywność przeciwbakteryjną, przeciwgrzybiczą i przeciwzapalną. 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 nie hamowały wzrostu bakterii i pleśni, natomiast związek V znacząco hamował wzrost drożdżaków (*Candida albicans*). Związek ten jest strukturalnie podobny do opisanych w literaturze nowych leków przeciwgrzybiczych.