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Antibacterial activity of certain fused 1,2,4-triazole derivatives

1,2,4-Triazole scaffold 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) (4).

Besides, it follows from the literature data that depending on the nature of substituent derivatives of 1,2,4-triazole show antibacterial (1–2, 8–9), antifungal (9–10) and anti-inflammatory (7) properties.

Previous studies concerning bridgehead nitrogen-heterobicyclic compounds obtained by fusion of 4,5-dihydroimidazole and 1,2,4-triazole nuclei, have identified one compound having a sulfanyl group at position 3 and a 4-chlorophenyl substituent at position 7 (e.g., 7-(4-chlorophenyl)-3-thiolo-5*H*-6,7-dihydroimidazo[2,1-*c*][1,2,4]triazole) with significant antifungal activity (11). It is of interest that some derivatives having the same heterocyclic skeleton, e.g., 7-aryl-5-methyl-3-thiolo-imidazo[2,1-*c*][1,2,4]triazol-6-ones described in the literature have been evaluated for their general pharmacological activities and have been found to possess high antifungal activity. These compounds exhibited fungicidal action almost equivalent to that of mancozeb (Dithane M-45) at 1000 ppm concentration and inhibited the growth of *Aspergillus niger* and *Fusarium oxysporum* by more than 48% and 47 % even at 10 ppm concentration (10). Furthermore, previously examined the 7-(4-methylphenyl)-3-methylthio-5*H*-6,7-dihydroimidazo[2,1-*c*][1,2,4]triazole was strongly active against *Staphylococcus aureus* ATCC 25923, with MIC results at 31.7 μM. Its antibacterial potency was hardly 1.1-times higher than that of ampicillin and 2.6-fold lower in comparison to chloramphenicol. This compound was also found to exhibit activity against *Staphylococcus epidermidis* in the disc-diffusion assay (12).

Prompted by these reports and in attempt to prepare heterocyclic compounds of biological interest (12–15), it seemed worthwhile to design and evaluate the two of hitherto untested 7-aryl-5*H*-6,7-dihydroimidazo[2,1-*c*][1,2,4]triazoles containing the methylthio (I) and hydrazino (II) groups as expected pharmacophores of antimicrobial activity. In the present paper we would like to report the results on the antibacterial potency investigation of following compounds:

I 7-(2,3-dimethylphenyl)-3-methylthio-5*H*-6,7-dihydroimidazo[2,1-*c*][1,2,4]triazole;

II 7-(2,3-dimethylphenyl)-3-hydrazino-5*H*-6,7-dihydroimidazo[2,1-*c*][1,2,4]triazole.

These derivatives were obtained according to our procedures as previously described (12, 16). The tested compounds were obtained from 7-(2,3-dimethylphenyl)-2,5,6,7-tetrahydroimidazo[2,1-*c*][1,2,4]triazol-(3*H*)-thiones (17) by alkylation with methyl iodide (I) and further treatment with hydrazine hydrate (II). Their chemical structures were confirmed on the basis of elemental analysis and spectral data (nuclear magnetic resonance, infrared and mass spectra). These compounds were characterized by solubility in propan-2-ol, dimethylformamide and dimethyl sulfoxide.

MATERIALS AND METHODS

Assay of antibacterial activity *in vitro* Imidazotriazoles of types I, II were tested for their antibacterial activity. The tested microorganisms were isolated from clinical specimens of the Laboratory of Medical Microbiology Department, Medical University of Lublin. The assayed collection included 48 strains of Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*) and 16 strains of Gram-negative bacteria (*Escherichia coli*) (Table 1).

Table 1. Microorganism cultures used to antibacterial screening

Group	Strain	Number of strains
Gram-positive bacteria	*Staphylococcus aureus*	21
	Staphylococcus epidermidis	15
	Streptococcus pyogenes	12
Gram-negative bacteria	*Escherichia coli*	16

The minimal inhibitory concentration (MIC) values of the compounds tested were determined by using microdilution broth method according to NCCLS standard (3, 5). The inocula of microorganisms were prepared from 24 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The test compounds dissolved in dimethyl sulfoxide (DMSO) were first diluted to the highest concentration (500 μg mL^{-1}) to be tested. Then serial twofold dilutions were made at concentration ranging from 1.95 to 500 μg mL^{-1} in 10 mL sterile tubes. Prepared suspensions of the standard microorganisms were added to each dilution at a 1:1 ratio. Growth (or its lack) of microorganisms was determined visually after incubation at 37°C for 24 h. The lowest concentration at which there was no visible growth (turbidity) was taken as the MIC. Ampicillin was used as a standard drug for comparison in the antibacterial studies. Furthermore, control experiments using dimethyl sulfoxide were done for antibacterial activity studies. The presented results (Table 2) were obtained from three independent measurements. The investigations were carried out at the Department of Medical Microbiology, Medical University, Lublin.

Table 2. Antibacterial activity expressed as MIC (μM) of the tested imidazotriazoles using the microdilution broth method

Comp.	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Streptococcus pyogenes*	*Escherichia coli*
I	76.8	38.4	19.2	96.0
II	51.2	81.9	40.9	81.9
Standard	35.8	35.8	nt	nt

Standard: ampicillin, nt – not tested

RESULTS AND DISCUSSION

Based on conducted microbiological tests, it was shown that both studied compounds of the type I-II were proved to inhibit growth of all the investigated bacteria. The examined 7-(2,3-dimethylphenyl)-3-methylthio-5*H*-6,7-dihydroimidazo[2,1-*c*][1,2,4]triazole (I) was strongly active against *Streptococcus pyogenes* and *Staphylococcus epidermidis* with MICs results at 19.2 and 38.4 μM, respectively. This compound revealed similar potency against *Staphylococcus epidermidis* to that of ampicillin. However, its activity against *Staphylococcus aureus* was 2.1-fold lower than that of ampicillin. The 7-(2,3-dimethylphenyl)-3-hydrazino-5*H*-6,7-dihydroimidazo[2,1-*c*][1,2,4]triazole (II) was effective on *Streptococcus pyogenes* and *Staphylococcus aureus* with MIC results at 40.9 and 51.2 μM. Its potency against *Staphylococcus aureus* and *Staphylococcus epidermidis* was about 1.4 and 2.3-fold lower, respectively, than that of ampicillin. Moreover, compound I was found to be 2.1-fold more active than II against *Streptococcus pyogenes* and *Staphylococcus epidermidis*, whereas imidazotriazole of type II was 1.5 and 1.2–fold more potent than I against *Staphylococcus aureus* and *Escherichia coli*, respectively.

According to the results obtained, compound I was found to exhibit almost equipotent to ampicillin antibacterial *in vitro* activity against *Staphylococcus epidermidis* with a MIC value of 38.4 μM and, therefore, may be considered promising for the development of new antibacterial agents.

Taking into account significant antibacterial activities of the presented compounds, the research in this field will be continued. It is likely to happen that their structural analogues should also be active.

CONCLUSIONS

1. Both tested compounds (I and II) were active against all Gram-positive and Gram-negative bacterial strains tested.

2. The examined compounds revealed MIC values in the range 19.2–96.0 μM.

3. Compound I was found to exhibit almost equipotent to ampicillin *in vitro* activity against *Staphylococcus epidermidis* with a MIC value of 38.4 μM and, therefore, may be considered promising for the development of new antibacterial agents.

4. The microbiological screening tests afforded to confirm the antibacterial activity of the investigated compounds.

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SUMMARY

1,2,4-Triazole is the structural element of many drugs that have a wide biological spectrum of activity. Besides, it follows from the literature data that 1,2,4-triazoles and their fused systems show antibacterial, antifungal and antinflammatory properties. The obtained compounds were tested for their antibacterial potency. Microbiological tests conducted on 64 strains of bacteria showed that the examined compounds (I and II) revealed MIC values in the range 19.2–96.0 μM. Compound I was found to exhibit almost equipotent to ampicillin *in vitro* activity against *Staphylococcus epidermidis* with a MIC value of 38.4 μM.

Aktywność przeciwbakteryjna niektórych skondensowanych pochodnych 1,2,4-triazolu

Układ 1,2,4-triazolu występuje w strukturze leków wykazujących szerokie spektrum aktywności biologicznej. 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 otrzymanych związków. Przeprowadzone na 64 szczepach bakteryjnych testy aktywności przeciwdrobnoustrojowej wykazały, że badane związki posiadają wartości MIC w granicach 19.2–96.0 μM. Związek I wykazał podobną do ampicyliny aktywność w stosunku do *Staphylococcus epidermidis* z wartością MIC równą 38.4 μM.