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SHORT COMMUNICATION

# Antimicrobial Activity of 2,4-dihydro-[1,2,4]triazol-3-one Derivatives

JOANNA STEFAŃSKA[1], MARTA STRUGA[2*], STEFAN TYSKI[1, 3], JERZY KOSSAKOWSKI[2] and MARIA DOBOSZ[4]

[1] Department of Pharmaceutical Microbiology and [2] Department of Medical Chemistry, Medical University, Department of Pharmaceutical Microbiology, Warsaw, Poland
[3] Department of Antibiotics and Microbiology, National Medicines Institute, Warsaw, Poland
[4] Department of Organic Chemistry, Medical University, Lublin, Poland

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Abstract

Antibacterial and antifungal activity of 2,4-dihydro-[1,2,4]triazol-3-one derivatives were examined by the disc-diffusion method (growth inhibition zone diameter in agar medium). The MIC's for the most active agents were determined. Of all the tested compounds, aminomethyl derivatives of 2,4-dihydro-[1,2,4]triazol-3-one exhibit activity against the majority of microorganisms studied.

Key words: 2,4-dihydro-[1,2,4]triazol-3-one derivatives; antimicrobial activity

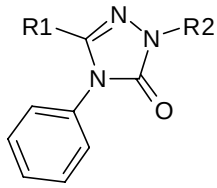
1,2,4-Triazole and 1,2,4-triazol-3-one derivatives show a broad spectrum of biological activities such as herbicides (Schmitzer *et al.*, 2000; Frecht and Dahmen, 2005), fungicides (Mueller and Kluth, 1991; Mueller and Kirsten, 1996), antivirals (Witkowski *et al.*, 1973; Burzozowski *et al.*, 1998) as well as analgesic (Temple *et al.*, 1983), antihypertensive (Ashton and Chakravarty, 1995) and antitumor (Ikizler *et al.,* 1998) action. A great number of 1,2,4-triazole and 1,2,4-triazol-3-one derivatives display interesting antifungal and antimicrobial activities (Bhat *et al.*, 2001; Hui *et al.*, 2000; Colancesca-Ragenovic *et al.*, 2001; Yüksek *et al.*, 1997; Ikizler *et al.*, 1997; Latthe *et al.*, 2006). Extending the research in this area, we decided to investigate derivatives, generally differently substituted in the 4-dihydro-[1,2,4]triazol-3-one ring. Their activity against bacteria and fungi was examined.

The antimicrobial activity of compounds was tested against a series of Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* NCTC 4163, *Bacillus subtilis* ATCC 6633, *Enterococcus hirae* ATCC 10541); Gram-negative bacteria (*Escherichia coli* ATCC 10538, *Eschericia coli* NCTC 8196, *Proteus vulgaris* NCTC 4635, *Pseudomonas aeruginosa* ATCC 15442, *Pseudomonas aeruginosa* NCTC 6749, *Bordetella bronchiseptica* ATCC 4617) and yeasts (*Candida albicans* ATCC 10231, *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019). Microorganisms used in this study were obtained from the collection of the Department of Pharmaceutical Microbiology, Medical University of Warsaw, Poland.

All chemicals were of analytical grade (Aldrich) and were used without further purification. The list of 2,4-dihydro-[1,2,4]triazol-3-one derivatives that were studied is shown in Fig. 1. Compounds used in the study were previously synthesized (Dobosz and Struga, 1997; 2000; Dobosz *et al.*, 2000). 4-Phenyl-2,4-dihydro-[1,2,4]triazol-3-one and 4,5-diphenyl-2,4-dihydro-[1,2,4]triazol-3-one were prepared by cyclization of semicarbazide derivatives of formic and benzoic acids in basic medium and were used as starting products. These compounds were subjected to reaction with *e.g.* ethyl chloroformate, allyl bromide, acetic anhydride and benzoyl chloride. Aminometylation and cyanoethylation reactions were also carried out.

The substitution reaction of 4-phenyl-2,4-dihydro-[1,2,4]triazol-3-one and 4,5-diphenyl-2,4-dihydro-[1,2,4]triazol-3-one with ethyl bromoacetate gave the corresponding *N*-derivatives such as (4-phenyl- or 3,4-diphenyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl)-acetic acid ethyl ester and were later subjected to reaction with ammonia solution to be transformed into

* Corresponding author. M. Struga, Department of Medical Chemistry, Medical University, Oczki 3, 02-007 Warsaw, Poland; phone: (48) 22 6280679; **e-mail: marta.struga@am.edu.pl**




| | R1 | R2 | | R1 | R2 |
|---|---|---|---|---|---|
| **1** | H | H | **21** | H | $CH_2CH_2COOH$ |
| **2** | $C_6H_5$ | H | **22** | H | $CH_2CH(OH)CH_2$-piperidine |
| **3** | $CH_3$ | H | **23** | $C_6H_5$ | $CH_2CH(OH)CH_2$-piperidine |
| **4** | $C_6H_5$ | $CH_2COOC_2H_5$ | **24** | H | $CH_2CH(OH)CH_2$-morpholine |
| **5** | $CH_3$ | $CH_2COOC_2H_5$ | **25** | H | $CH_2CH(OH)CH_2$-pirrolidine |
| **6** | H | $COOC_2H_5$ | **26** | $C_6H_5$ | $CH_2CH(OH)CH_2$-pirrolidine |
| **7** | H | $CH_2CH=CH_2$ | **27** | H | $CH_2CH(OH)CH_2$-diethylamine |
| **8** | $CH_3$ | $CH_2CH=CH_2$ | **28** | $C_6H_5$ | $CH_2CH(OH)CH_2$-diethylamine |
| **9** | $C_6H_5$ | $COCH_3$ | **29** | H | $CH_2CH(OH)CH_2$-N-phenyl-N-benzyl |
| **10** | $CH_3$ | $COCH_3$ | **30** | $C_6H_5$ | $CH_2CH(OH)CH_2$-N-phenyl-N-benzyl |
| **11** | H | $CH_2$-piperidine | **31** | H | $CH_2CONH_2$ |
| **12** | H | $CH_2$-morpholine | **32** | H | $CH_2CONHCH_2$-piperidine |
| **13** | $C_6H_5$ | $CH_2$-morpholine | **33** | $C_6H_5$ | $CH_2CONHCH_2$-piperidine |
| **14** | H | $CH_2$-pirrolidine | **34** | H | $CH_2CONHCH_2$-morpholine |
| **15** | $C_6H_5$ | $CH_2$-pirrolidine | **35** | H | $CH_2CONHCH_2$-pirrolidine |
| **16** | H | $CH_2$-$N(C_2H_5)_2$ | **36** | $C_6H_5$ | $CH_2CONHCH_2$-pirrolidine |
| **17** | H | $CH_2$-N-phenyl-N-benzyl | **37** | H | $CH_2CONHCH_2$-diethylamine |
| **18** | $C_6H_5$ | $CH_2$-N-phenyl-N-benzyl | **38** | $C_6H_5$ | $CH_2CONHCH_2$-diethylamine |
| **19** | H | $CH_2CH_2CN$ | **39** | H | $CH_2CONHCH_2$-triazole |
| **20** | H | $CH_2CH_2CN$ | **40** | $C_6H_5$ | $CH_2CONHCH_2$-triazole |

Fig. 1. Chemical structures of 2,4-dihydro-[1,2,4]triazol-3-one derivatives used in this study

the corresponding amides. Aminomethylation reaction of obtained compounds was carried out by using pyrrolidine, piperidine, morpholine and diethylamine or 1,2,4-triazole as amines. 4-Phenyl-2,4-dihydro-[1,2,4]triazol-3-one and 4,5-diphenyl-2,4-dihydro-[1,2,4]triazol-3-one were used in reaction with 2-chloromethyloxirane. Then, they were converted with piperidine, pyrrolidine, morpholine, diethylamine, *N*-benzylamine and 1,2,4-triazole into corresponding aminoalkanol derivatives.

Antimicrobial activity was determined using Mueller Hinton II agar (Becton Dickinson) for bacteria and RPMI agar (Sigma) for fungi. Solutions of the tested agents were prepared in ethanol or dimethylsulfoxide. Preliminary investigations were carried out using the disc-diffusion method according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2003). The tested compound solutions were dripped onto sterile filter paper discs (Whatman No 3 chromatography paper, 9 mm dimeter) to obtain 400 µg of substance per disc. Dry discs were placed on the surface of appropriate agar medium. Minimal Inhibitory Concentrations (MIC's) were determined by the agar dilution method (CLSI, 2003). Concentrations of the tested agents in solid medium ranged from 6.25 to 400 µg/ml. The final inoculum of test microorganisms was $10^4$ CFU/ml. Results of antimicrobial activity were read after 18 h incubaton at 35°C for bacteria and at 30°C for fungi.

The antimicrobial activity of 2,4-dihydro-[1,2,4]triazol-3-one derivatives was tested against a wide range of microorganisms, including Gram-positive and Gram-negative bacteria and fungi. At first we performed analysis using disc-diffusion method. Compounds showing activity in these tests were later examined in order to determine their minimal inhibitory concentration (MIC). The results are summarized in Table I.

In the present work a series of 40 derivatives of 2,4-dihydro-[1,2,4]-triazol-3-one was investigated and 12 of them demonstrated some antimicrobial activity. This fact was evaluated and confirmed by MIC determination and the obtained data may serve as a good reason for discussion of the influence of the compound's molecular structure on its biological activity.

Table I
Antimicrobial activities of of 2,4-dihydro-[1,2,4]triazol-3-one derivatives – diameter of the growth inhibition zone [mm] and Minimal Inhibitory Concentration (MIC in parentheses) [μg/ml]

| Strain \ Compound No | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **32** | **33** | **35** | **36** | **37** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Staphylococcus aureus* ATCC 6538P | 16 (>400) | 16 (400) | 14 (400) | 17 (>400) | 14 (>400) | 19 (400) | 15 (400) | 13 (>400) | 15 (>400) | 13 (>400) | 15 (>400) | 20 (200) |
| *Staphylococcus aureus* NCTC 4163 | 15 (>400) | 16 (400) | 15 (400) | 17 (>400) | 14 (>400) | 20 (400) | 15 (400) | 13 (>400) | 16 (>400) | 11(>400) | 14 (>400) | 22 (200) |
| *Staphylococcus aureus* ATCC 29213 | 16 (>400) | 14 (400) | 14 (>400) | 14 (>400) | 13 (>400) | 20 (400) | 16 (400) | 13 (>400) | 15 (>400) | 11(>400) | 14 (>400) | na |
| *Staphylococcus aureus* ATCC 25923 | 16 (>400) | 16 (400) | 14 (>400) | 17 (>400) | 15 (>400) | 20 (400) | 17 (400) | 14 (>400) | 15 (>400) | 11 (>400) | 13 (>400) | na |
| *Bacillus subtilis* ATCC 6633 | 15 (400) | 15 (400) | 11 (400 | 13 (>400) | na | 17 (400) | 13 (400) | na | na | na | na | 16 (200) |
| *Enterococcus hirae* ATCC 10541 | na | na | na | na | na | na | na | na | na | na | na | na |
| *Escherichia coli* ATCC 10538 | na | na | na | 13 (>400) | na | 11 (>400) | na | na | na | na | na | na |
| *Escherichia coli* NCTC 8196 | 11 (>400) | 11 (>400) | 11 (>400) | 13 (>400) | na | 13 (>400) | 11 (>400) | na | na | na | na | na |
| *Proteus vulgaris* ATCC 4635 | na | na | na | 13 (>400) | na | 13 (>400) | na | na | na | na | na | na |
| *Pseudomonas aeruginosa* NCTC15442 | na | na | na | 13 (>400) | na | 11 (>400) | na | na | na | na | na | na |
| *Pseudomonas aeruginosa* NCTC 6749 | na | na | na | 13 (>400) | na | 11 (>400) | na | na | na | na | na | na |
| *Bordetella bronchiseptica* ATCC 4617 | 21 (200) | 20 (200) | 16 (400) | 20 (200) | 18 (400) | 24 (200) | 19 (100) | 16 (400) | 16 (400) | 16 (>400) | 16 (>400) | na |
| *Candida albicans* ATCC 10231 | na | na | na | na | na | na | na | na | na | na | na | na |
| *Candida albicans* ATCC 90028 | na | na | na | na | na | na | na | na | na | na | na | na |
| *Candida parapsilosis* ATCC 22019 | na | na | na | na | na | 25 (200) | na | na | na | na | na | na |

na – no activity Derivatives not listed above were completely inactive in concentration up to 400 μg per disc

It is possible to find out analyzing the structure of 2,4-dihydroxo-[1,2,4]-triazol-3-one that general antibacterial activity is connected with the groups of a certain substituent in the position 2 of a 1,2,4-triazol-3-one ring. Also substituents in positions 4 and 5 of the above ring may determine specific substance activity.

Twelve compounds with the presence of characteristic substituents in the position 2 were found to be active. They can be divided as follows depending on their structure:

Group I: compounds no **11, 12, 13, 14, 15, 16, 18**

Group II: compounds no **32, 33, 35, 36, 37**

In the first distinguished group, the typical constituent in position 2 is a methylene group (-$CH_2$-) connected with both – 2,4-dihydro-[1,2,4]-triazol-3-one ring and nitrogen atom (*N*). Nitrogen can be present either as the constituent of the ring structure, ex. pyrolidyne (comp. no **14**, **15**), morfoline (comp. no **12**, **13**), piperidine (comp. no **11**) or as a disubstituted amine (comp. no **16**, **18**). Depending on the amine's type an increase or a decrease of antibacterial activity can be observed. Best results were obtained for compounds no **14** (*N* in a pyrolydine ring) and **16** (*N* as a component of *N, N*-diethylamine) as they were characterized by stronger and broader spectra of activity. Both compound no **14** and **16** were active against all Gram-negative bacterial strains and most of the tested Gram-positive bacteria except *E. hirae* ATCC 10541. Moreover, compound no. **16** showed antifungal activity against *C. parapsilosis* ATCC 22019.

It is not possible to point out undoubtedly what exactly the range of antibacterial activity depends on. It is surely connected with the substituents present in positions 4 and 5 of the 2,4-dihydro-[1,2,4]-triazol-3-one ring. For example, a phenyl group in position 5 of compound no **15** limits the spectrum of its activity (only to Gram-positive bacteria) in comparison with compound no **14**. This is also reflected in the case of compounds **17** and **18**. Moreover compound no **17** (not substituted in 5 position) doesn't exhibit any activity against tested yeasts and bacteria of different species. In case of compounds **12** and **13** any changes in activity depending on the type of substituents in 4 and 5 positions were not observed.

In Group II, the structure of substituents found in position 2 of the ring is far more complicated. It is composed of an amide group connected with 2 methylene groups by nitrogen and carbon atoms. One methylene group is combined with 2,4-dihydro-[1,2,4]-triazol-3-one and the other with an amine. The activity of substances classified as Group II doesn't differ significantly. Any differences can derive from the presence of substituents in 4 and 5 positions of the ring. The phenyl group found in compound **33** may be the reason of its stronger antistaphylococcal activity in comparison with compound **32**.

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