

SYNTHESIS, ANTIBACTERIAL, ANTIFUNGAL AND GENOTOXIC ACTIVITY OF BIS-1,3,4-OXADIAZOLE DERIVATIVES

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In the present investigation, four 1,3,4-bis-oxadiazole derivatives were synthesized as potential antimicrobial agents. The compounds are: 5,5'-dimercapto-bis-[1,3,4-oxadiazol-2-yl]propane (2a), 5,5'-dimercapto-bis-[1,3,4-oxadiazol-2-yl]butane (2b), 5,5'-dimercapto-bis-[1,3,4-oxadiazol-2-yl]octane (2c) and 5,5'-dibenzylthio-bis-[1,3,4-oxadiazol-2-yl]butane (3). The above newly synthesized compounds were investigated for their antibacterial, antifungal and mutagenic activities. The results of the biological activities revealed that the compounds 2a-c exhibited both antibacterial and antifungal activities against *S. aureus* and *B. subtilis*. Compound 2a also showed activity against *P. aeureginosa*. All the above compounds and compound 3 exhibited activity against *C. albicans*. Genotoxic studies showed that compound 2a had a weak base pair substitution mutagenicity but none of them exhibited a frameshift mutagenic action using Ames test.

Key words: *bis-1,3,4-oxadiazole, chemical mutagenicity, antibacterial, antifungal*

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INTRODUCTION

1,3,4-Oxadiazole derivatives are well known to have a wide range of biological activities. Examples of such activities are anti-inflammatory [21], antifungal [7, 12, 29], antiparasitic [20] and antimicrobial [8, 16, 22, 25, 29] effects. Furthermore, bis-mercapto-1,3,4-oxadiazoles were used as active substances to control *Meloidogyne incognita* in tomato [1]. In the present investigation, a series of substituted bis-1,3,4-oxadiazoles derivatives were synthesized as potential antibacterial and antifungal agents.

On the other hand, Ames test is a well established method to examine the mutagenicity of chemical compounds. This is due in part to its ability of prescreening a large number of chemicals for any mutagenic and thus for any suspected carcinogenic effects. In addition, the test makes possible to distinguish between frameshift and base-pair substitution mutagens [17]. It is worth to mention that various experimental results support the hypothesis that environmental factors are major cause of cancer [10]. That prompted us to investigate both the antibacterial and the antifungal as well as the genotoxic activities of the above-mentioned compounds.

MATERIALS and METHODS

Preparation of 5,5'-dimercapto[bis-1,3,4-oxadiazol-2-yl]alkanes (1a-c)

To a solution of potassium hydroxide (10.0 mmol) in absolute ethanol (50 ml), alkanedioic acid dihydrazide, **1**, (5.0 mmol) was added. Carbon disulfide (11.0 mmol) was added to the reaction mixture, which led to a pale yellow precipitate formation. The reaction mixture was heated under reflux for 4 h, during which time the mixture became clear. The solution was concentrated by distillation and acidified with dilute hydrochloric acid to obtain the products. The following compounds were prepared according to the above procedure:

5,5'-dimercapto-bis[1,3,4-oxadiazol-2-yl]propane **2a**
yield: 80%; m.p.: 218 (d). ¹H-NMR (DMSO-d₆): 14.1 (bs, 2H, SH), 2.84 (t, 4H, J = 7.5 Hz, CH₂), 1.93 (p, 2H, CH₂).

5,5'-dimercapto-bis-[1,3,4-oxadiazol-2-yl]butane, **2b**
yield: 75%; m.p. 208–210. ¹H-NMR (DMSO-d₆): 14.30 (bs, 2H, SH), 2.77 (t, 4H, J = 7.2 Hz, CH₂), 1.80 (m, 4H, CH₂).

5,5'-dimercapto-bis-[1,3,4-oxadiazol-2-yl]octane, **2c**
yield: 83%; m.p. 82–85°C ¹H-NMR (DMSO-d₆): 14.25 (bs, 2H, SH), 2.49 (t, 4H, J = 7.0 Hz, CH₂); 1.62 (m, 4H, CH₂); 1.29 (m, 8H, CH₂).

Preparation of 5,5'-dibenzylthio-bis-[1,3,4-oxadiazol-2-yl]butane (3)

To a solution of potassium hydroxide (10 mmol) in absolute ethanol (50 ml), compound **2b** (5 mmol) was added with stirring. A solution of benzyl bromide (10 mmol) in ethanol (20 ml) was added. The reaction mixture was heated under reflux for 2 h. After cooling, water (100 ml) was added, which led to a precipitate formation. The resulting solid residue was crystallized from chloroform/hexane (1 : 2).

Yield: 68%, m.p. 105–106°C. ¹H-NMR (CDCl₃) δ: 7.30 (m, 10 H, aromatic), 4.44 (s, 4H, CH₂), 2.83 (t, 4H, CH₂), 1.8 (m, 4H, CH₂).

Bacterial and fungal strains

For antibacterial activity, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* PA 0303 were used. In antifungal studies *Candida albicans* was used. For mutagenicity tests, *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA100 were used. The strains were kindly supplied by Prof. B.N. Ames (Department of Biochemistry, University of California, Berkeley, USA).

Antimicrobial activity

Stock solutions of the chemical compounds were dissolved in dimethylsulfoxide, then diluted in dextrose broth to give an initial concentration of 4 mg/ml. Serial dilutions in dextrose broth were made until final concentrations of 450, 300, 200, 100, 50 and 25 µg/ml were reached for the agar dilution method. In broth dilution method, the final concentrations were 450, 200, 100, 50, 25, 12 and 6 µg/ml. The antifungal drug miconazole was used as a reference substance [14]. The microorganisms were grown overnight in dextrose broth at 35°C and diluted to 10⁻³ just before being used. Four control tubes were prepared as follows: one with dextrose broth only, one with dextrose broth and the test organism, one with the highest drug concentration and the last one with the lowest drug concentration. Test tubes containing 2 ml of dextrose broth were inoculated with 0.05 ml of the di-

luted overnight cultures. All tubes were then incubated at 35°C for 18 h. The lowest concentration at which there was no growth was considered as the minimum inhibitory concentration (MIC).

Mutagenic studies

Stock solutions of the test chemicals were prepared by dissolving 10 mg of the compound in dimethylsulfoxide. Serial dilutions ranging from 4 mg/ml to 0.01 mg/ml were made. Vogel-Bonner medium E (50X), histidine-biotin solution (0.5 M), top agar, minimal glucose plates, histidine-biotin plates and ampicillin plates were prepared as described by Maron and Ames [15]. The plate incorporation test was followed as described by Maron and Ames [15]. The top agar was distributed into capped culture tubes, which were held at 45°C in a water bath. To each tube, 0.1 ml of a fresh overnight culture of the tested strain was added, followed by the addition of 0.1 ml of the tested compound. Sodium azide, 4-nitro-o-phenylenediamine and methylmethane sulfonate were used as positive controls. The test components were mixed by vortexing the tube for about 3 s at low speed and directly poured onto a minimal glucose agar plates. After 45 min, the plates were inverted and placed in a dark incubator at 37°C. The revertant colonies on the treated as well as on the negative control plates were counted.

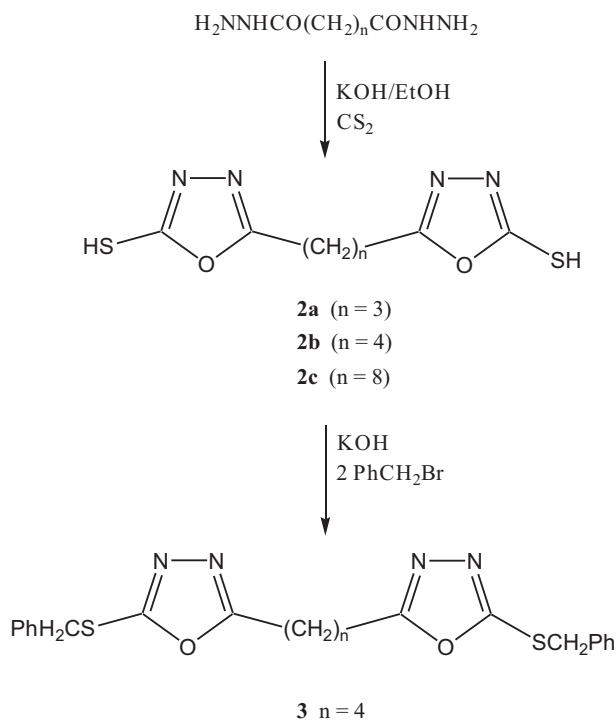
RESULTS

Synthesis of the compounds

Bis-1,3,4-oxadiazoles **2a-c** used in this study, were prepared by the reactions of alkanedioic acid dihydrazides **1a-c** with carbon disulfide in alcoholic potassium hydroxide solutions, Scheme 1. Compound **2b** was converted to the dipotassium salt via treatment with KOH in alcohol. Addition of benzyl bromide to the latter salt afforded the dibenzylmercapto derivative **3** with good yield. The chemical and physical data of these compounds are given in the experimental section.

Antimicrobial activity

Evaluation of the above-mentioned compounds for their antimicrobial activities showed that mercapto substituted bis-1,3,4-oxadiazole derivatives **2a-c** exhibited both antibacterial and antifungal ac-



Scheme 1.

tives. The results are presented in Table 1. The tested compounds showed activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans*. In addition, **2a** showed inhibitory activity against *Pseudomonas aeruginosa*. However, compound **3** showed a weak activity against one bacterial strain, but a clear activity against *Candida albicans*. It should be mentioned that the antimicrobial results were obtained at concentrations of 450 µg/ml for all tested compounds.

Table 1. Antibacterial and antifungal activities of the tested oxadiazole derivatives*

| Compound (450 µg/ml) | <i>E. coli</i> | <i>Ps. aeruginosa</i> | <i>Staph. aureus</i> | <i>B. subtilis</i> | <i>C. albicans</i> |
|-------------------------|----------------|---------------------------|--------------------------|------------------------|------------------------|
| 2a | – | + | + | + | + |
| 2b | – | – | + | + | + |
| 2c | – | – | + | + | + |
| 3 | – | – | – | + | + |

*+ – active compound against the specified microorganism;
 -- inactive compound against the specified microorganism

Mutagenic activities

Tested compounds were investigated for their mutagenicity. The results are presented in Table 2.

This table shows that compound **2b** is a weak base-pair substitution mutagen in strain TA100 of *Salmonella typhimurium*. Strains TA1535 and TA100 are base-pair substitution detector strains, while TA1537 and TA98 are frameshift detector strains. However, no frameshift mutation was detected in either TA98 or TA1537 strains of *Salmonella* after exposure to any of the tested oxadiazole derivatives. Furthermore, no mutagenic activity was detected in TA1535.

Table 2. Mutagenicity of the tested oxadiazole derivatives*

| Compound | TA1535 | TA1537 | TA98 | TA100 |
|------------------|--------|--------|------|-------|
| 2a | – | – | – | – |
| 2b (1 µg) | – | – | – | 200** |
| 2c | – | – | – | – |
| 3 | – | – | – | – |

* – indicates nonmutagenic compound; ** – an average of three plates after subtracting the background revertants

DISCUSSION

The bis-1,3,4-oxadiazole derivatives were prepared in this study as potential antibacterial and/or antifungal drugs. The idea came from our previous interest in these compounds, in addition to the data that were published in the literature about the promising effects of some related compounds. The importance of developing new antibacterial active compounds needs not to be emphasized, specially if one considers the problem of resistance and multiresistance properties arising continuously among pathogenic bacteria.

Oxadiazole derivatives were synthesized and evaluated for their biological activities [19]. Some bis-1,3,4-oxadiazole derivatives have also been tested for antibacterial activity [2, 3, 13, 23, 30]. The compounds investigated in the present study showed antibacterial and antifungal activities against the strains used in this investigation. These results are very encouraging and extend our research in two directions. The first one is to synthesize other derivatives in order to increase their antibacterial and/or antifungal activity. Antifungal and antiviral activities were detected in other studies using different derivatives [4, 6, 9, 11, 27, 28]. The second one is to carry out more investigations of the synthesized compounds in terms of any possible cytotoxic, antiviral, anti-inflammatory, enzyme inhibit-

ing and even anticancer activities. It is worth to mention here, that other related compounds were found to possess toxic, anticancer and anti-inflammatory effects [5, 18, 24, 26]. Compound **3**, which showed a very strong activity against *Candida albicans* is a very promising antifungal drug to be modified. The same applies to compound **2a** concerning its activity against *Pseudomonas aeruginosa*. Our results indicate that the addition of benzyl groups as substituents enhances the antifungal activity of the prepared compounds. It indicates further that the length of the alkane is an important factor for the activity. The butane and octane derivatives seem to be more active than the propane derivative. It is notable, also, that benzyl substitution in compound **2b** leads to not only to an increase in the antimicrobial activity, but also to a complete loss of its mutagenic activity. This is an important finding since the mutagenic compounds are strongly suspected to be carcinogenic ones.

The results of the present investigation may encourage us to develop and/or improve similar other related compounds and test them for a wide range of biological activities.

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