Synthesis and evaluation of antiradical activity of 1,2,4-triazole derivatives substituted by cyclic ketals

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Abstract
1,2,4-Triazoles substituted by cyclic ketals were synthesized. As it was found, 2,2-disubstituted 4-(1,2,4-triazol-1-ylmethyl)-1,3-dioxolanes possess moderate antiradical activity. The antiradical activity of the compounds was evaluated by determination of DPPH free radical scavenging activity with trolox as a standard. Compounds having a hexylthiosulfonyl substituent, arylureido or arylthioureido fragments in the para-position of the aryl ring showed the greatest radical-scavenging activity.

Keywords- Alkylation, antiradical activity, antioxidant, arylsulfone, arylthiourea, aryurea, 1,3-dioxolane, determination of DPPH free radical scavenging activity, DPPH, ketol, 1,2,4-triazole.

INTRODUCTION
Numerous recent studies have proven that the imbalance in the system of free radical oxidation and antioxidant protection plays a key role in the molecular mechanisms of pathogenesis in many diseases [1]. Excess free radicals adversely affect on the cell structure, most intensively damaging its lipids. Activation of peroxide oxidation and accumulation of free radicals disrupt the structural and functional integrity of cell membranes, and, as a consequence, affect on the course of bioenergetic processes. At the same time, available data show relationship of increasing free radical activity and course of hypoxia [2], which leads to a vicious circle of cellular pathology, resulting in a loss of membrane barrier properties [3]. Nevertheless, the negative processes of free radical oxidation do not end only at the level of damage to the cell membrane. Active forms of oxygen may form inside the cells, disrupting metabolic processes, DNA synthesis, proliferation, and reducing enzyme activity. It leads to the development of a number of diseases: atherosclerosis, coronary heart disease, diabetes, Alzheimer's disease, Parkinson's disease, malignancies, and also causes cell death by apoptosis or necrosis [2].

In this regard, the search for new effective low-molecular and non-toxic antioxidants, capable of binding active radicals within cells, is an urgent task.

We have previously shown that substituted derivatives 1,2,4-triazoles having fragments of substituted cyclic ketals, namely 4-(1,2,4-triazol-1-ylmethyl)-1,3-dioxolanes, possess a pronounced fungicidal [4–7], growth-regulating [8], antibacterial [9], antymycobacterial [10] activity, have a cytotoxic effect against tumor cells [11]. These activities demonstrate that 4-(1,2,4-triazol-1-ylmethyl)-1,3-dioxolanes are able to penetrate through the cell membrane. This is apparently due to the presence in the molecule of hydrophilic fragments of 1,2,4-triazole and 1,3-dioxolane, and affinity for the lipid layer of the membrane is provided by lipophilic alkyl or aryl substituents in the 1,3-dioxolane fragment.

Derivatives of imidazole [12–22] and 1,2,4-triazole [23–33] have been extensively studied as antioxidants, while the antiradical activity of 1,3-dioxolane derivatives is much less described. It was shown for glycerol acetal [34], for benzodioxole [35] and acetaldehyde dimethyl acetal [36]. The antiradical activity of 1,2,4-triazoles substituted with the 1,3-dioxolane fragment in derivatives has not been studied yet.

MATERIALS AND METHODS
1H NMR spectra were recorded on Bruker AM-300 instrument (300.13 MHz). IR spectra were recorded on a Specord M-80 instrument (Nujol). The course of reaction was monitored and the purity of the compounds was checked by TLC (Silufol UV-254). The optical density was measured with the use of KFK-3-01 photoelectric colorimeter. The commercially available 2,2-diphenyl-2-picryl-1-hydrazyl (DPPH) and triis(hydroxymethyl) aminomethane (THAM) from Sigma Aldrich as well as high-purity dimethylsulfoxide (DMSO) and methanol were used.

We synthesized a wide series of 2,2-disubstituted 4-(1,2,4-triazol-1-ylmethyl)-1,3-dioxolanes and studied the antiradical activity of some representatives of each series. Compounds 1–9 were synthesized in several stages according to the procedures described by us earlier [4–11]. Compounds 11, 12 containing arylvinyl fragments were derived according to the procedure [7]. Compound 13 – the derivative of 4-tert-butyloxycyclohexanone – was derived according to the method [6]. Aromatic thiourea 14 and urea 15 were derived according to the procedure [11] based on described amines [37].

For further analysis of the structure-activity relationship, the lipophilicity of the synthesized compounds [38] was calculated, the values of which were close to the experimentally measured values [39].

New 1-(2-[4-chlorobenzyl]-2-[4-(hexylsulfonyl)phenyl]-1,3-dioxolan-4-yl)methyl)-1H-1,2,4-triazole (10), which showed the greatest antiradical activity, was synthesized in 5 stages. Hexitliobenzene was synthesized by alkylation of thiophenol with hexyl bromide; 2-(4-chlorophenyl)-1-[4-(hexylthio)phenyl]ethanone was derived by Friedel-Crafts alkylation of hexylthiobenzene with p-chlorophenylacetyl chloride.

2-(4-Chlorobenzyl)-4-(chloromethyl)-2-[4-(hexylthio)phenyl]-1,3-dioxolane was obtained by condensation of 2-(4-chlorophenyl)-1-[4-(hexylthio)phenyl]ethanone with 3-chloropropanediol catalyzed by TSA with azotropic water distillation. 1-(2-[4-Chlorobenzyl]-2-[4-(hexylthio)phenyl]-1,3-dioxolan-4-yl)methyl)-1H-1,2,4-triazole was synthesized by condensation of 2-(4-chlorobenzyl)-4-chloromethyl-2-(4-hexylthio)phenyl)-1,3-dioxolane with sodium salt of 1,2,4-triazole while boiling in DMF. Then it was oxidized by hydrogen peroxide in acetic acid to derive 1-(2-[4-Chlorobenzyl]-2-[4-(hexylsulfonyl)phenyl]-1,3-dioxolan-4-yl)methyl)-1H-1,2,4-triazole. At all stages, the substances were obtained in high yields.
Hexitolobenzene. A mixture 72 ml (77.3 g, 0.7 mol) of thiophenol and then 127 g (0.77 mol) of hexyl bromide were added to a solution of 46.14 g (0.82 mol) of potassium hydroxide in 150 ml of ethanol with stirring for 15 minutes. The mixture was stirred for 2 hours at 50°C and filtered. The filtrate was evaporated, the oily residue was distilled under vacuum, fractions with b.p. 146–149/20 mm Hg were picked up. 124.1 g (91%) of hexitotolobenzene were obtained as a colorless liquid.

1-[2-(2-Methyl-1,3-dioxolan-4-ylmethyl)-1H,1,2,4-triazole (1). Yield 23%, m.p. 86–87°C. NMR (CDCl3, δ, ppm, J/Hz): 1.63 (s, 3H, CH3); 3.70-3.97 (m, 1.66H, CH2O); 4.05 (d, J=3.3, CH2O); J=6.3; J=8.8); 4.25-4.45 (m, 2.66H, CH2N + CHO); 4.65 (q, 0.34H, CHO, J=5.8); 7.38 (d, 2H, Ar, J=8.6); 7.23-7.52 (m, 5H, Ar); 7.96 (s, 1H, CH3triaz.) 8.25(s, 1H, CH3triaz.) IR (Nujol, v/sm-1): 1274 (β CH3triaz.); 1245, 1222, 1190, 1168, 1088 (COCCOC)792 (C=C); 688 (C=S).

The following compounds were prepared according to the procedure described above.

1-[2-(2-Methyl-1,3-dioxolan-4-ylmethyl)-1H,1,2,4-triazole (2). Yield 82%, m.p. 90–92°C. NMR (CDCl3, δ, ppm, J/Hz): 1.56 (s, 3H, CH3); 3.66 (d, J=0.38H, CH2O, J=7.81, J=8.8); 3.75 (d, 0.62H, CH2O, J=6.4; J=8.8); 3.97 (d, 0.62H, CH2O, J=5.1; J=8.7); 4.17 (d, 0.38H, CH2O, J=6.4; J=8.8); 4.24-4.29 (m, 0.74H, CH2N + CHO); 4.36 (d, 0.62H, CH2N, J=6.8; J=8.5); 4.42 (d, 1H, CH3N, J=6.4; J=8.5); 4.61 (q, 0.38H, CHO, J=5.6); 7.64 (d, 0.62H, Ar, J=8.6); 7.64 (d, 0.38H, Ar, J=8.6); 7.79 (s, 0.38H, CH3triaz.); 9.18 (s, 0.62H, CH3triaz.); 8.18 (d, 1H, Ar, J=8.6); 8.37 (s, 0.38H, CH3triaz.); 8.53(s, 0.62H, CH3triaz.). IR (Nujol, v/sm-1): 1270 (β CH3triaz.); 1240, 1220, 1175, 1127, 1087 (COCCOC).

1-[2-(2-Methyl-1,3-dioxolan-4-ylmethyl)-1H,1,2,4-triazole (3). Yield 63%, m.p. 85–86°C. NMR (CDCl3, δ, ppm, J/Hz): 1.56 (s, 3H, CH3); 3.66 (d, J=0.38H, CH2O, J=7.81, J=8.8); 3.75 (d, 0.62H, CH2O, J=6.4; J=8.8); 3.97 (d, 0.62H, CH2O, J=5.1; J=8.7); 4.17 (d, 0.38H, CH2O, J=6.4; J=8.8); 4.24-4.29 (m, 0.74H, CH2N + CHO); 4.36 (d, 0.62H, CH2N, J=6.8; J=8.5); 4.42 (d, 1H, CH3N, J=6.4; J=8.5); 4.61 (q, 0.38H, CHO, J=5.6); 7.64 (d, 0.62H, Ar, J=8.6); 7.64 (d, 0.38H, Ar, J=8.6); 7.89 (s, 0.38H, CH3triaz.); 9.18 (s, 0.62H, CH3triaz.); 8.18 (d, 1H, Ar, J=8.6); 8.37 (s, 0.38H, CH3triaz.); 8.53(s, 0.62H, CH3triaz.). IR (Nujol, v/sm-1): 1270 (β CH3triaz.); 1240, 1220, 1175, 1127, 1087 (COCCOC).

1-[2-(2-Cyclohexyl-2-methyl-1,3-dioxolan-4-ylmethyl)-1H,1,2,4-triazole (4). Yield 80%, m.p. 126–127°C. NMR (DMSO-d6, δ, ppm, J/Hz): 1.21-1.52 (m, 6H, (CH2)2cycl.); 1.62 (s, 3H, CH3); 1.76-1.96 (m, 4H, (CH2)3); 2.50 (t, 1H, CH2CH2CH2J=11.8); 3.78-3.95 (m, 2H, CH2O); 4.28-4.44 (m, 3H, CH3N + CHO); 7.16 (d, 2H, CH3H, J=8.3; 7.33 (d, 2H, CH3H, J=8.3); 7.97 (s, 1H, CH3triaz.); 8.26 (s, 1H, CH3triaz.). IR (Nujol, v/sm-1): 1268 (β CH3triaz.); 1195, 1125, 1085 (COCCOC).

1-[2-(2-Chlorobenzyl)-2-(4-methylthiophenyl)-1,3-dioxolan-4-ylmethyl]-1H,1,2,4-triazole (5). Yield 76%, m.p. 127–128°C. NMR (DMSO-d6, δ, ppm, J/Hz): 1.04 (s, 9H, (CH3)3); 1.92 (d, 2H, CH2C(CH3)J=12.6); 3.67 (d, d, CH2O, J=11.8); 7.63 (s, 1H, CHO, J=7.2; J=8.8); 4.13-4.46 (m, 3H, CH3N + CHO); 7.55 (d, 2H, Ar, J=8.2); 7.78(s, 1H, CH3triaz.); 8.72(s,1H,CH3triaz.). IR (Nujol, v/sm-1): 1265 (β CH3triaz.); 1190, 1150, 1085 (COCCOC).

1-[2-(4-Methoxyphenyl)-2-(phenylthiobenzenewere obtained as a colorless liquid. IR spectrum (vaseline oil, v/sm-1): 1610 (CO); 778 (C=C); 700 (C-S).

1-[2-(2-Chlorobenzyl)-2-(4-methylthiophenyl)-1,3-dioxolan-4-ylmethyl]-1H,1,2,4-triazole (6). Yield 69%, m.p. 151–152°C. NMR (DMSO-d6, δ, ppm, J/Hz): 2.72 (d, 3H, CH3); 3.10 (s, 2H, p-CIPhCH2); 3.51-4.43 (m, 5H, CH2O, CH2N + CHO); 7.04 (d, 2H, C6H5 + PhCH2J=8.1; J=9.9); 7.10 (d, d, 2H, C6H5 + p-CIPhCH2J=7.4; J=9.9); 7.15-7.28 (m, 4H, C6H5 + C6H5 + p-CIPhCH2); 7.93 (s, 1H, CH3triaz.); 7.99 (s, 1H, CH3triaz.); 8.32 (s, 1H, CH3triaz.); 8.48 (s, 1H, CH3triaz.). IR (Nujol, v/sm-1): 1272 (β CH3triaz.); 1245, 1220, 1195, 1160, 1075 (COCCOC); 784(C-C).

1-[2-(4-Methoxyphenyl)-2-(phenylthiobenzenewere obtained as a colorless liquid. IR spectrum (vaseline oil, v/sm-1): 1610 (CO); 778 (C=C); 700 (C-S).
The following compound was prepared according to the technique described below.

1-[[2-[(4-Chlorobenzyl)-2-(4-hexylsulfonyl)phenyl]-1,3-dioxolan-4-yl]-methyl]-1H-1,2,4-triazole (10). A solution 7 ml (0.068 mol) of 30% hydrogen peroxide were added to a solution of 1 g (0.0021 mol) of 1-[[2-(4-chlorobenzyl)-2-[4-(hexylthio)phenyl]-1,3-dioxolan-4-yl]-methyl]-1H-1,2,4-triazole in 7 ml of glacial acetic acid and stirred for 7 days at room temperature, then poured 20 g of crushed ice and 22 ml of 50% sodium hydroxide solution into a mixture, extracted with ethyl acetate (2x50 ml), washed with water until a neutral reaction was achieved, dried over magnesium sulfate, after test ethyl acetate was evaporated under vacuum. 0.753 g (76%) of 1-[[2-[4-(chlorobenzyl)-2]-[4-(hexylsulfonyl)phenyl]-1,3-dioxolan-4-yl]-methyl]-1H-1,2,4-triazole were derived as yellowish crystals with m. 157–158. NMR (CDCl 3, δ, ppm, J/Hz) 8.22 (s, 1H, CHO, J = 7.3, 4H, CHO=J = 5.8, 5H, J = 8.0); 4.02 (d, 2H, CH2O, J = 5.6, 6.2); 2.45-4.43 (m, 4H, CH2N); 7.94 (s, t, t, t, CH3); 8.17 (s, 1H, CH3); 8.01 (s, 1H, CH3); 8.09 (s, 1H, NH). IR (Nujol, v/sm): 3370, 3311 (NH); 1622 (CO); 1245, 1175, 1089(COCOC), 745 (C–Cl).

The compound 9 was oxidized to a sulfox 10 by the technique described below.

1-[[2-[(4-Chlorobenzyl)-2-(4-hexylsulfonyl)phenyl]-1,3-dioxolan-4-yl]-methyl]-1H-1,2,4-triazole (6). Yield 72%, m. 125–129°C NMR (CDCl 3, δ, ppm, J/Hz): 4.04 (d, 1H, CH2O, J = 5.2, J = 8.8); 4.09 (d, 1H, CH2O, J = 6.6, J = 8.8); 4.33 (d, 2H, CH2N, J = 5.2); 4.58 (q, 1H, CHO, J = 5.2); 7.23–7.42 (m, 6H, Ar); 7.47 (d, 2H, Ar, J = 8.8); 7.94 (s, 1H, C=CH triaz); 8.01 (s, 1H, C=H triaz). IR (Nujol, v/sm): 1270 (β CH triaz); 1245, 1215, 1177, 1115, 1080 (COCOC); 715 (CCl).

The antiradical activity of the compounds was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the previously described procedures [40, 41].

The 3 ml of 0.3 mmol solution of DPPH in methanol, 1 ml of THAM-HCl buffer solution with pH = 7.4 were added to a solution of 2x10⁻⁵ mol of the test substance 1–15 or reference standard - trolox in DMSO. The solution was held at room temperature (25°C) for 30 minutes, and then its optical density was measured at a wavelength of 517 nm in cells with an absorbing layer thickness of 0.5 cm. The same solutions were used as the comparison solution, but without the test substance.

Known antioxidant Trolox were used as standard of antioxidant activity. The obtained measurements were processed in a standard way using the Student’s-t test (the confidence probability was 0.95).

RESULTS AND DISCUSSION

The antiradical activity of the compounds was evaluated by determination of DPPH free radical scavenging activity. Antiradical activity of the testing compounds is below the standard – trolox, but the compounds showed a pronounced DPPH free radical scavenging activity (Table). Compounds 10, 14, 15 showed the highest activity, leading to a nearly 50% reduction in the number of active radicals. Apparently, introduction of a fragment of sulfone, thiourea and urea,
conjugated with an aromatic ring, enhances antiradical activity in series of the studied compounds. Introduction of alkenyl substituents conjugated with an aromatic ring also increased antiradical activity, and introduction of the nitro group reduced activity.

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* calculated values of logP_{ow} [38]; ** Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; *** Measured at pH=7.4 [42].

**CONCLUSIONS**

Substituted 4-(1,2,4-triazol-1-ylmethyl)-1,3-dioxolanes exhibit DPPH free radical scavenging activity. The most radical binding activity was demonstrated by compounds having a hexylthiosulfone substituent or fragments of arylthiourea or arylurea in the para position of the aryl ring. The studied series of compounds will allow to optimize the search for new radical-scavengers in the series of 1,2,4-triazoles, substituted by cyclic ketals.

**REFERENCES**


