SEARCH FOR NEW DRUGS

SYNTHESIS AND ANTIOXIDANT ACTIVITY OF NEW 1,2,4-TRIAZOLE DERIVATIVES

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A series of new derivatives of 1,2,4-triazoles have been synthesized and their antioxidant properties have been studied. It was established that the obtained compounds exhibit a stabilizing effect on cell membranes and demonstrate antioxidant activity with respect to erythrocytes under oxidative stress conditions.

Key words: 1,2,4-triazole derivatives, erythrocytes, membrane stabilizing effect, antioxidant activity.

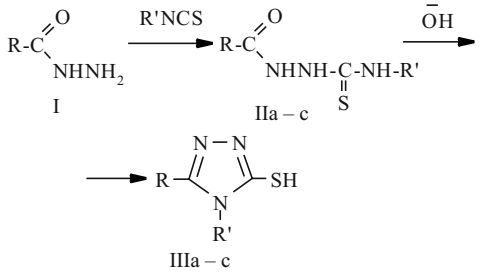
According to current thinking, excessive activation of free-radical peroxide oxidation processes is responsible for the pathogenesis of many diseases [1 – 3]. An enormous amount of evidence has accumulated in the last few decades and suggests that compounds of natural and synthetic origin that exhibit antioxidant properties are capable of preventing the onset and progression of diseases caused or mediated by the activation of free-radical oxidative stress [4 – 6].

1,2,4-Triazole derivatives have consistently attracted scientific and practical interest because of their widely varying chemical properties, synthetic versatility, and pharmacological activity. Herein we report the synthesis of new 1,2,4-triazole derivatives and the study of their antioxidant properties.

EXPERIMENTAL PART

The new 1,2,4-triazole derivatives were synthesized according to the following scheme. The reaction of carboxylic acid hydrazides (I) with isothiocyanates produced 1,4-disubstituted thiosemicarbazides (IIa – c) [7] that then cyclized intramolecularly in alkaline medium to transform into the corresponding 3,4-disubstituted-5-mercapto-1,2,4-triazoles (IIIa – c) [8].

It was found that the cyclization should be carried out in aqueous NaOH or KOH (10%). The reaction was completed quickly and provided high yields of the desired products.

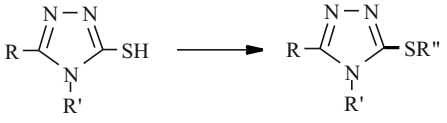


I IIa – c IIIa – c

R = *iso*-C_4H_9, R′ = furylmethyl;

R = *iso*-C_4H_9, R′ = methallyl

Sulfur-substituted 1,2,4-triazoles derivatives may also be interesting from a biological viewpoint. This would enable the biological activities of free and substituted thiols to be compared. Therefore, IIIa-c were alkylated by various halides.



IVa – c

R = *iso*-C_4H_9; R′ = methallyl; R″ = 4-chlorobenzoylmethyl;

R = *iso*-C_4H_9; R′ = furylmethyl; R″ = 4-nitrobenzoylmethyl;

R = *iso*-C_4H_9; R′ = furylmethyl; R″ = 4-chlorobenzoylmethyl.

It was found that the best results for the alkylation were obtained by reacting equimolar amounts of the reagents in anhydrous acetone.

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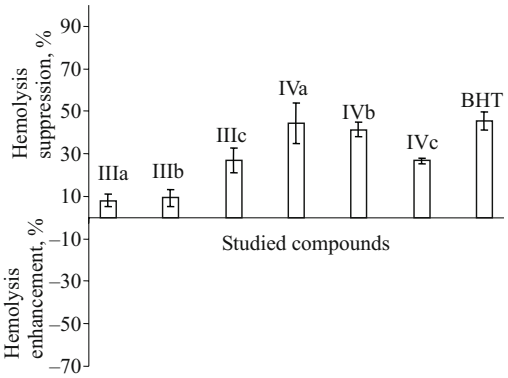

Fig. 1. Effect of 1,2,4-triazole derivatives III – IVa – c (10^{-3} M) on hemolytic stability of erythrocyte membranes (hematocrit 1%).

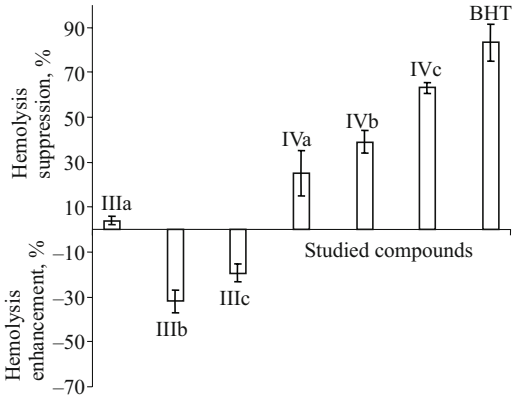

Fig. 2. Effect of 1,2,4-triazole derivatives III – IVa – c (10^{-3} M) on oxidative hemolysis of H_2O_2-treated erythrocytes (hematocrit 1%).

IR spectra of IIa – c, IIIa – c, and IVa – c in mineral oil were taken on a Nicolet Nexus FTIR. PMR spectra of $CDCl_3$ solutions were obtained on a Varian Model Mercury-300 spectrometer (300 MHz). TLC used Silufol UV-254 plates with detection by iodine vapor. Elemental analyses agreed with those calculated.

1,4-Disubstituted thiosemicarbazides (IIa – c). General method. The appropriate hydrazide (0.06 mol) in EtOH (30 mL) was placed into a flask, treated with the appropriate isothiocyanate (0.06 mol) in EtOH (30 mL), stirred vigorously, left for 1 h at room temperature, refluxed for 2 h, and cooled. The resulting crystals were filtered off, dried, and recrystallized.

1-Pentanoyl-4-β-phenylethylthiosemicarbazide (IIa). Yield 72%, mp 137 – 139°C (EtOH:H_2O, 1:1), R_f 0.58 (EtOH:C_6H_6, 1:5), $C_{14}H_{21}N_3O_2S$.

PMR spectrum (δ, ppm): 0.95 (t, 3H, CH_3), 1.20 (q, 2H, $C\underline{H}_2CH_3$), 1.65 (q, 2H, $CH_2C\underline{H}_2CH_2$), 2.40 (t, 2H, $CH_2CH_2C\underline{H}_2$), 2.80 (d, 2H, $C\underline{H}_2C_6H_5$), 3.75 (d, 2H, $NHC\underline{H}_2$), 7.10 m and 7.25 m (5H, H_{arom}), 7.45 (s, 1H, $N\underline{H}CH_2$), 9.10 (s, 1H, NH), 9.45 (s, 1H, NH).

1-Isopentanoyl-4-α-furylmethylthiosemicarbazide (IIb). Yield 83%, mp 153 – 154°C (EtOH:H_2O, 1:1), R_f 0.50 (EtOH:C_6H_6, 1:5), $C_{11}H_{17}N_3O_2S$.

PMR spectrum (δ, ppm): 0.93 (t, 6H, $2CH_3$), 2.00 (d, 2H, $C\underline{H}_2CO$), 2.15 [m, 1H, $CH(C\underline{H}_3)$], 4.20 (d, 2H, $NHC\underline{H}_2$), 6.10 (d, 1H, =CH), 6.25 (d, 1H, =CH), 6.35 (d, 1H, =CH), 7.45 (s, 2H, $N\underline{H}CH_2$), 9.10 (s, 1H, NH), 9.55 (s, 1H, NH).

1-Isopentanoyl-4-methallylthiosemicarbazide (IIc). Yield 85%, mp 143 – 144°C (EtOH:H_2O, 2:3), R_f 0.48 (EtOH:C_6H_6, 1:5), $C_{10}H_{19}N_3OS$.

PMR spectrum (δ, ppm): 0.93 (d, 6H, $2CH_3$), 1.80 (s, 3H, =C—CH_3), 1.95 (d, 2H, CH_2CO), 2.10 [m, 1H, $C\underline{H}(CH_3)_2$], 4.50 (s, 2H, CH_2N), 4.60 (s, 1H, CH_2=), 4.90 (s, 1H, CH_2=), 7.35 (s, 1H, $N\underline{H}CH_2$), 9.15 (s, 1H, NH), 9.40 (s, 1H, NH).

IR spectra of IIa – c showed the following characteristic absorption bands (ν, cm^{-1}): 1600 (C=C arom), 1640 (C=C), 1680 (C=O amide), 3050 (=CH), 3200 – 3300 (NH).

3,4-Disubstituted-5-mercapto-1,2,4-triazoles (IIIa – c). Aqueous KOH (3.4 g, 0.06 mol, 10%) was placed into a flask and treated with the appropriate 1,4-disubstituted thiosemicarbazide (0.04 mol). After the solids dissolved, the mixture was refluxed on a boiling water bath for 4 h, cooled, and acidified with dilute (1:1) HCl until the pH was 2 – 3. The resulting crystals were filtered off, washed with H_2O, dried, and recrystallized.

3-Butyl-4-β-phenylethyl-5-mercapto-1,2,4-triazole (IIIa). Yield 88%, mp 119 – 120°C (EtOH:H_2O, 1:1), R_f 0.65 (EtOH:C_6H_6, 1:6), $C_{14}H_{19}N_3S$.

PMR spectrum (δ, ppm): 0.95 (t, 3H, CH_3), 1.45 (q, 2H, $C\underline{H}_2CH_3$), 1.65 (q, 2H, $CH_2C\underline{H}_2CH_2$), 2.35 (t, 2H, $CH_2CH_2C\underline{H}_2$), 3.10 (t, 2H, $C\underline{H}_2C_6H_5$), 3.65 (d, 2H, NCH_2), 7.20 m and 7.35 m (5H, H_{arom}), 13.40 (s, 1H, SH).

3-Isobutyl-4-α-furylmethyl-5-mercapto-1,2,4-triazole (IIIb). Yield 73%, mp 135 – 136°C (EtOH:H_2O, 1:3), R_f 0.67 (EtOH:C_6H_6, 1:6), $C_{11}H_{15}N_3S$.

PMR spectrum (δ, ppm): 0.95 (d, 6H, $2CH_3$), 2.00 [m, 1H, $CH(CH_3)_2$], 3.80 (d, 2H, $CHCH_2$), 4.65 (s, 2H, CH_2N), 6.30 (d, 1H, CH=), 6.45 (d, 1H, CH=), 7.30 (t, 1H, CH=), 13.25 (s, 1H, SH).

3-Isobutyl-4-methallyl-5-mercapto-1,2,4-triazole (IIIc). Yield 85%, mp 148 – 150°C (EtOH:H_2O, 5:2), R_f 0.64 (EtOH:C_6H_6, 1:6), $C_{10}H_{17}N_3S$.

PMR spectrum (δ, ppm): 0.93 (d, 6H, CH_3), 1.70 (s, 3H, $=CCH_3$), 2.05 [m, 1H, $C\underline{H}(CH_3)_2$], 3.85 (d, 2H, $CHC\underline{H}_2$), 4.20 (s, 2H, NCH_2), 4.60 (s, 1H, CH_2=), 4.85 (s, 1H, CH_2=), 13.40 (s, 1H, SH).

IR spectra of IIIa-c contained the following characteristic absorption bands (ν, cm^{-1}): 1580 (C=N), 1600 (C=C arom), 1640 (C=C), 3050 (=CH).

3,4-Disubstituted 5-substituted thio-1,2,4-triazoles (IVa – c). The appropriate 3,4-disubstituted-5-mercapto-1,2,4-triazole (0.005 mol) and substituted bromoacetophenone (0.005 mol) in anhydrous acetone (10 ml) were placed into a dry flask and stirred for 1 h at room temperature and 2 h at 50 – 55°C. The acetone was distilled off. The solid was

cooled and made basic with aqueous NH_3 until the pH was 9 – 10. The resulting crystals were filtered off, washed with water until neutral, dried, and recrystallized.

3-Isobutyl-4-methallyl-5-(4′-chlorobenzoylmethyl)thio-1,2,4-triazole (IVa). Yield 93%, mp 52 – 53°C (hexane), R_f 0.59 (EtOH:C_6H_6, 1:4), $C_{18}H_{22}ClN_3OS$.

PMR spectrum (δ, ppm): 0.95 (d, 6H, $2CH_3$), 1.80 (s, 3H, $=CCH_3$), 2.05 [q, 1H, $C\underline{H}(CH_3)_2$], 2.25 (d, 2H, $CHC\underline{H}_2$), 3.95 (t, 2H, NCH_2), 4.25 (d, 1H, $=C\underline{H}_2$), 4.60 (d, 1H, $=C\underline{H}_2$), 5.00 (s, 2H, SCH_2), 7.15 m and 7.45 m (4H, H_{arom}).

3-Isobutyl-4-α-furylmethyl-5-(4′-nitrobenzoylmethyl)-thio-1,2,4-triazole (IVb). Yield 91%, mp 91 – 92°C (EtOH:H_2O, 2:1), R_f 0.54 (EtOH:C_6H_6, 1:4), $C_{19}H_{20}N_4O_4S$.

PMR spectrum (δ, ppm): 0.93 (d, 6H, $2CH_3$), 2.00 [q, 1H, $C\underline{H}(CH_3)_2$], 2.18 (d, 2H, $CHC\underline{H}_2$), 4.00 (t, 2H, NCH_2), 5.00 (s, 2H, SCH_2), 6.10 (d, 1H, $=C\underline{H}$), 6.25 (d, 1H, =CH), 6.30 (d, 1H, =CH), 7.15 m and 7.40 m (4H, H_{arom}).

3-Isobutyl-4-α-furylmethyl-5-(4′-chlorobenzoylmethyl)-thio -1,2,4-triazole (IVc). Yield 94%, mp 86 – 87°C (hexane), R_f 0.51 (EtOH:C_6H_6, 1:4), $C_{19}H_{20}ClN_3O_2S$.

PMR spectrum (δ, ppm): 0.95 (d, 6H, $2CH_3$), 2.10 [q, 1H, $C\underline{H}(CH_3)_2$], 2.20 (d, 2H, $CHC\underline{H}_2$), 3.95 (t, 2H, NCH_2), 5.05 (s, 2H, SCH_2), 6.15 (d, 1H, $=C\underline{H}$), 6.25 (d, 1H, =CH), 6.30 (d, 1H, =CH), 7.20 m and 7.40 m (4H, H_{arom}).

IR spectra of IVa – c exhibited the following characteristic absorption bands (ν, cm^{-1}): 1580 (C=N), 1600 (C=C arom), 1640 (C=C), 3050 (=CH).

EXPERIMENTAL BIOLOGICAL PART

Antioxidant properties of IIIa-IVc were studied using a model system of erythrocyte oxidative stress in which the ability of the compounds to prevent H_2O_2-stimulated hemolytic destruction of erythrocytes isolated from peripheral blood of healthy rats and resuspended in NaCl solution (150 mM, 1% hematocrit) was studied. Considering that the membrane-stabilizing properties of the compounds have a significant effect on the mechanisms of their biological activity, the effects of interaction of the new 1,2,4-triazole derivatives IIIa – IVc with cell membranes, in this instance with erythrocyte membranes, were studied in the same system but without H_2O_2.

The following control (C) and test (T) samples of erythrocyte suspensions were prepared in physiological solution:

a-C: suspension of erythrocytes without any additions;

a-T: suspension of erythrocytes with tested compounds added at a concentration of 10^{-3} M;

b-C: presence in the suspension of H_2O_2 at a final concentration of 0.015% (in order to stimulate oxidative damage of erythrocyte membranes);

b-T: parallel addition to erythrocyte suspension of H_2O_2 (0.015%) and tested compounds at a concentration of 10^{-3} M.

Samples were incubated for 1 h at 37°C with constant gentle shaking. Centrifugation at 3,000 rpm separated supernatant liquid containing hemoglobin released from the erythrocytes. The optical absorption of the supernatant at 541 nm (A_{541}) was determined by spectrophotometry. The change of A_{541} in a-T samples in percent relative to a-C enabled the membrane-stabilizing action of the tested compounds to be estimated. The decrease of optical absorption in b-T samples compared with b-C characterized the antioxidant activity of the tested compounds and their ability to quench active oxygen species and other free radicals induced in aqueous solution by addition of H_2O_2 to the erythrocyte suspension.

The optical absorption of the supernatant after incubation of control samples a-C for 1 h was 0.033 ± 0.004 and represented the background level of spontaneous hemolysis. Spontaneous hemolysis was taken as the null level; the effects of the tested compounds, the change of A_{541} in percent relative to the a-C value. Figure 1 shows that IIIa-b at a concentration of 10^{-3} M did not cause substantial shifts in the optical absorption of the samples. Therefore, they had no harmful action on the structure of the cell membranes. The background level of hemolysis decreased under the effect of the other tested compounds, namely IIIc and IVa-c, and butylated hydroxytoluene (BHT), which was used as a reference compound. Thus, these compounds exhibited a stabilizing action on the erythrocyte membranes.

H_2O_2-stimulated oxidative stress on erythrocytes that resulted from destruction of cell membranes and release of hemoglobin into the surrounding medium in control b-C caused a significant increase of A_{541} to 0.155 ± 0.008. Figure 2 shows this as the baseline null level. The experimental results showed that IIIa did not change the hemolysis level of H_2O_2-treated erythrocytes. Compounds IIIb and –c enhanced clearly hemolytic destruction of erythrocytes whereas IVa-c and BHT exhibited a distinct anti-hemolytic effect. Thus, they had a membrane-stabilizing action on the cells, probably due to a certain amount of antioxidant activity.

In general the experimental results led to the conclusion that the synthesized new 1,2,4-triazole derivatives IIIa-IVa-c did not have a harmful effect on the structural integrity of biological membranes. Moreover, IVa-c can be characterized as compounds capable of exhibiting membrane-stabilizing properties under conditions of oxidative stress on erythrocytes.

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