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CHEMISTRY

Study of Antioxidant Activity of New Compounds with 1,3-Thiazin-2,6-Dione and Pyrrolidine Fragments[1]

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Abstract—Antioxidant activity of new compounds with 1,3-diazin-2,6-dione and pyrrolidine fragments in comparison with 2,6-di-*tert*-butyl-4-mercaptophenol has been studied in the model system of adrenaline autooxidation in alkaline medium in DPPH, NBT, and CUPRAC tests. In the series of studied compounds, a leader has been revealed, which shows moderate antiradical activity toward DPPH radical, prevents accumulation of toxic products of adrenaline quinoid oxidation, and exhibits higher efficiency as one-electron Cu^{2+} reducing reagent as compared with known antioxidants, trolox and 2,6-di-*tert*-butyl-4-mercaptophenol.

Keywords: 1,3-thiazin-2,6-dione fragment, pyrrolidine, DPPH test, CUPRAC test, NBT test, antioxidant activity, adrenaline, xanthine oxidaze, superoxide anion radical

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Multifactor nature of different pathologies related to oxidative stress development causes urgency of directed search for new efficient polyfunctional therapeutics, including inhibitors of oxidative processes [1–3]. Promising class of therapeutics with antioxidant activity is nitrogen- and sulfur-containing heterocycles, including 1,3-thiazine [4–6] and pyrrolidine [7–9] derivatives, which are considered in recent time as a universal scaffold for designing new biologically active compounds. Compounds showing wide spectrum of biological activity, antioxidant including, were revealed among derivatives of these heterocycles.

The combination of several pharmacophoric fragments showing different mechanisms of antioxidant action in molecule increases the probability of emergence of intramolecular synergic effect of their antioxidant activity. Hydrazine carboxamide and carbamate derivatives may be considered as such pharmacophores. It was found previously that the presence of hydrazine carboxamide group in compound structure in combination with other pharmacophores provides emergence of other kinds of activity (antiglycemic, antibacterial, anticonvulsive, antituberculous, and antitumor) along with antioxidant activity [10–12]. The detection of antioxidant properties of carbamates showing inhibiting activity toward such enzymes as cholinesterase and monoamine oxidaze is important for designing multitarget preparations affecting simultaneously on several molecular targets involved in pathogenesis of many neurodegeneratrive diseases [13, 14].

Therefore, we studied antioxidant activity of new heterocyclic compounds **1–3** (Fig. 1) in comparison with activity of known synthetic sterically hindered phenolic antioxidant, 2,6-di-*tert*-butyl-4-mercaptophenol (**4**) (Sigma-Aldrich).

Compound **1** was obtained by condensation of malonic acid, potassium thiocyanate, and acetic anhydride in glacial acetic acid by procedure reported in the work [15]. Compound **3** was synthesized by three-component condensation of the corresponding chalcone with sarcosine and paraform on refluxing in toluene for four hours [16].

Compound **2** was prepared by heating of a mixture of 0.374 g (2 mmol) of 5-acetyl-4-hydroxy-2*H*-1,3-thiazine-2,6(3*H*)-dione (**1**) and 0.23 g (2 mmol) of semicarbazide hydrochloride in 10 mL of ethanol for 5 h. After cooling, the resultant precipitate was filtered off, dried in air, and recrystallized from dioxane. The

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Table 1. Antioxidant activity of compounds **1**–**4**

Compound	Action efficiency (AE), (%)			$TEAC_{CUPRAC}$ [b]
	DPPH test[a]	NBT test[a]	adrenaline autooxidation system [b]	
1	7.17 ± 0.02	−63.60 ± 0.11	−12.5 ± 0.01	0.06 ± 0.01
2	40.92 ± 0.05	not active	61.1 ± 0.02	1.57 ± 0.09
3	−0.90 ± 0.04	−80.32 ± 0.15	31.3 ± 0.03	Not active
4	90.40 ± 0.25	45.70 ± 0.07	43.1 ± 0.06	0.74 ± 0.04

Concentration in test system: [a] 100 μM; [b] 25 μM.

yield of the product as colorless crystals was 0.52 g (97%), m. p. 249–252°C. IR (ν, cm^{-1}): 3100–3500 (NH, OH), 1712, 1700 (C=O), 1648 (C=N). ^1H NMR (δ, ppm): 1.96 s (3H, CH_3), 6.74 s (2H, NH_2), 10.48 s [1H, NH(CO)], 11.52 s (1H, thiazine NH), 12.59 s (1H, OH). ^{13}C NMR (δ, ppm): 19.2 (CH_3), 98.5 (C^5), 161.4 ($CONH_2$), 163.6 (C^6), 167.4 (C^4), 173.7 (C^2), 179.8 (C=N). Found (%): C, 32.20; H, 3.16; N, 22.63. For $C_7H_8N_4O_4S$ anal. calcd. (%): C, 32.42; H, 3.30; N, 22.94.

The antiradical activity of compounds **1**–**4** was determined in vitro by spectral method in different model systems: toward 2,2-dipheny-1-picrylhydrazyl radical (DPPH test) ($O_2^{\bullet-}$) [17], toward superoxide anion radical generated in a xanthine/xanthine oxidaze enzymatic system (NBT test) [18], and in nonenzymatic adrenaline oxidation into adrenochrome in alkaline medium [19]. We calculated action efficiency (AE) of the studied compounds in these test systems by the formula:

$$AE\ (\%) = [(1 - \Delta D_i/\Delta D_0) \times 100\%],$$

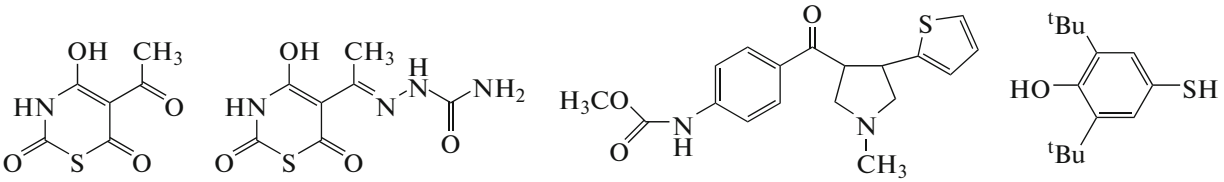
where ΔD_i is change in optical density in the presence of studied compound, ΔD_0 is change in optical density in control sample (without added compounds). The positive value of AE indicates antioxidant activity of tested compound, while negative value indicates prooxidant activity.

The reducing activity of compounds **1**–**4** was determined also in the reaction of electron transfer on Cu^{2+} (CUPRAC test) [20] and calculates as trolox equivalents (TEAC, *trolox equivalent antioxidant capacity*). The activity of trolox, water-soluble tocopherol analog, was taken as one, while the value of parameter TEAC > 1 indicates higher reducing activity of tested compound as compared with trolox.

DPPH test is widely used for detection of antiradical activity of potential antioxidants. The study of ability of compound to react with stable N-centered chromogen radical 2,2-diphenyl-1-picrylhydrazyl indicates that all compounds, except for compound **3**, show antiradical activity by reaction with DPPH radical (Table 1). The reaction of 1,3-thiazine derivatives with DPPH radical proceeds by the mechanism of homolytic abstraction of hydrogen atom from hydroxyl group of phenol.

Among studied heterocyclic compounds, compound **2** displays the highest antiradical activity, which is explained probably by the possibility to form more stable intermediate due to conjugation in thiazine ring, stabilization of sulfur atom charge, and the presence in this compound of hydrazine carboxamide group showing antioxidant activity. The activity of compound **2** in this model system is half as much the activity of 2,6-di-*tert*-butyl-4-mercaptophenol **4**.

The study of reducing activity of compounds **1**–**4** showed that 1,3-thiazine derivative of compound **2** is more active reducing agent as compared with trolox by factor of 1.57, while compounds **1** and **3** do not reduce Cu^{2+} in this model system. It should be noted that the reduction of Cu^{2+} by antioxidants in a real biosystem could cause their prooxidant activity [21].



1 **2** **3** **4**

Fig. 1. Chemical structures of compounds: **1**, 5-acetyl-4-hydroxy-2*H*-1,3-thiazine-2,6(3*H*)-dione; **2**, 2-[(*E*)-1-(4-hydroxy-2,6-dioxo-3,6-dihydro-2*H*-1,3-thiazin-5-yl)ethylidene]-1-hydrazine carboxamide; **3**, methyl *N*-(4-{4-[4-(2-thienyl)-1-methyltetrahydro-1*H*-pyrrol-3-yl]carbonyl}phenyl)carbamate; **4**, 2,6-di-*tert*-butyl-4-mercaptophenol.

The study of antiradical activity of compounds **1–4** toward $O_2^{\bullet-}$ formed under in xanthine/xanthine oxidaze enzymatic system the enzymatic system (NBT test) showed that, in contrast to mercaptophenol **4**, compound **2** does not show activity toward this reactive oxygen species (ROS), while compounds **1** and **3** were found to promote reduction of nitro blue tetrazolium into formazane by superoxide, as compared with control experiment. Superoxide trapping activity of compound **4** is caused by the possibility of HO and HS groups oxidation by $O_2^{\bullet-}$ anion radical to form stable aroxyl and thiyl radicals [22, 23]. It should be noted that the promoting effect of compounds **1** and **3** on reduction of nitro blue tetrazolium may be due to their ability to increase xanthine oxidaze activity [24].

In the model system of adrenaline autooxidation in alkaline medium, we detected antioxidant activity of all compounds, except for **1**, adrenochrome is accumulated in the presence of the latter. The inhibiting activity of compound **2** is considerably higher than the activity of antioxidant **4**. Taking into account that adrenaline oxidation in alkaline medium leads to different ROS along with $O_2^{\bullet-}$ and hydrogen peroxide: carbon dioxide anion radicals and bicarbonate anion radicals [25, 26], the revealed inhibiting activity of compounds in this system indicates their general inhibiting activity toward the noted radicals.

Thus, our study of antioxidant activity of new heterocyclic compounds **1–3** revealed leader compound **2**, whose activity is higher than that of reference antioxidant in model systems of adrenaline autooxidation and CUPRAC test. This derivative of 1,3-thiazine shows moderate antiradical activity toward DPPH radical, prevents accumulation of toxic products of adrenaline quinoid oxidation, and behaves as more efficient one-electron reducing agent as compared with the known antioxidants: trolox and 2,6-di-*tert*-butyl-4-mercaptophenol. Further studies of activity of this compound are necessary to design more efficient and safe multitarget preparations on the basis of compound **2** for the treatment of diseases of multifactor nature.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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