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CHEMISTRY

Activity of Hydroxy Derivatives of Chalcones toward Superoxide Anion Radical

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Abstract—Trapping activity of new hydroxy derivatives of chalcones toward electrochemically generated superoxide anion radical ($O_2^{\bullet-}$) in a xanthine/xanthine oxidase enzymatic system upon reduction of nitro blue tetrazolium and during non-enzymatic quinoid oxidation of adrenaline in alkaline medium has been studied. Antiradical activity of new hydroxy derivatives of chalcones toward $O_2^{\bullet-}$ has been established for the first time. Reaction with electrochemically generated $O_2^{\bullet-}$ and higher antiradical activity under adrenaline autooxidation conditions in alkaline medium has been revealed for chalcone containing 2,6-di-*tert*-butylphenol fragment. Chalcone with two non-shielded OH groups showed the highest trapping activity in xanthine/xanthine oxidase enzymatic system and SOD-protective activity. The inhibiting activity of hydroxy derivatives of chalcones toward $O_2^{\bullet-}$ agrees well with in silico prediction and indicates their ability to decrease probability of oxidative stress development. Correlation has been found between the ability of the studied chalcones to consume superoxide anion radical and inhibiting activity in model systems of peroxide oxidation of oleic acid and tilapia liver lipids.

Keywords: hydroxy derivatives of chromenes, sterically hindered phenolic fragment, antioxidant activity, superoxide anion radical

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Unbalanced biochemical disorders appeared in biological systems lead to formation of reactive oxygen species (ROS), which induce development of many pathological states [1]. Superoxide anion radical (superoxide anion $O_2^{\bullet-}$) is one of active oxygen forms in living organisms which forms during one-electron reduction of O_2 [2]. The role of $O_2^{\bullet-}$ in organism is dual: under normal conditions, it is involved in cellular activity in controllable mode, while its excess can damage biological molecules including nucleic acids, lipids, proteins to cause acute and chronical disorders of metabolic routes [3].

There is a natural endogeneous protective antioxidant system, for example, superoxide dismutase (SOD), superoxide reductase, which efficiently removes $O_2^{\bullet-}$ and other ROS. On metabolism violation or under the action of external factors, endogenous cell protection copes with oxidative stress consequences [4]. Natural and synthetic compounds capable of fast consumption of $O_2^{\bullet-}$ are promising antioxidants because they can decrease toxic effect of ROS due to termination of free-radical processes, which is important for the prevention and treatment of pathological states.

To decrease the risk of adverse side effects, it is preferable to employ phytogenic compounds, for example flavonoids, showing wide scope of biological activity, antioxidant properties including [5, 6]. At the same time, many natural antioxidants, flavonoids including, exhibit a number of drawbacks such as low solubility, insufficient oral bioavailability, low system absorption, high excretion rate, and formation of toxic metabolites [7, 8]. Moreover, it was found earlier that flavonoids might exhibit prooxidant properties at high concentrations [9]. All above factors confine application of plant antioxidants for therapeutic purposes and favor to the development of new efficient and safe pharmaceutical compounds containing different bio-

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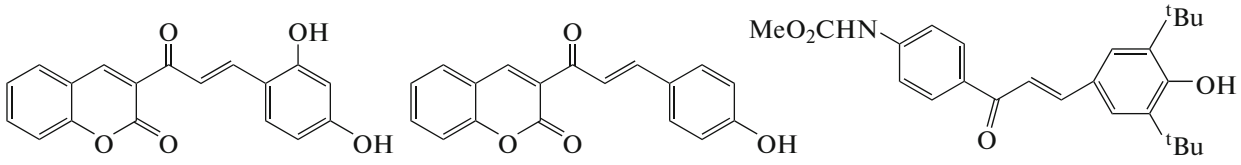


1 **2** **3**

Fig. 1. Chemical structures of compounds: **1**, 3-[(*E*)-3-(2,4-dihydroxyphenyl)-2-propenoyl]-2*H*-chromen-2-one; **2**, 3-[(*E*)-3-(4-hydroxyphenyl)-2-propenoyl]-2*H*-chromen-2-one; and **3**, methyl N-(4-{(*E*)-3-[3,5-di(*tert*-butyl)-4-hydroxyphenyl]-2-propenoyl}phenyl)carbamate.

mimetic redox-active and pharmacophoric groups which differ in mechanism of antioxidant action.

New hydroxy derivatives of chalcones **1**–**3** (Fig. 1) were prepared previously, it was predicted in silico that the derivatives can behave as antioxidants, traps for free radicals, oxygen, and nitrogen oxide [10].

The work deals with the trapping activity of chalcones **1**–**3** toward electrochemically generated $O_2^{\bullet-}$ in a xanthine/xanthine oxidase enzymatic system and during non-enzymatic adrenaline quinoid oxidation in alkaline medium.

The antioxidant effect of potential inhibitors of oxidation processes is caused mainly by activity toward different ROS kinds. Superoxide anion radical $O_2^{\bullet-}$ forms during oxygen one-electron reduction, therefore electrochemical methods of investigation are convenient to study reactions of potential antioxidants with this ROS. Advantages of electrochemical method are high sensitivity, rate and reproducibility of data and lack of byproducts because aprotic solvent favors to radical stabilization and prevents $O_2^{\bullet-}$ dismutation, which provides much more accurate measurement of radical trapping ability [11].

Electrochemical reduction of oxygen on Pt electrode in acetonitrile leads to formation of $O_2^{\bullet-}$ detected on cyclic voltammogram (CV) (CH_3CN, Pt, 0.1 M $^nBu_4NClO_4$, $C = 5$ mM, Ag/AgCl, $v = 0.2$ V s^{-1}) [12]. In the presence of hydroxy derivatives of chalcones **1** and **2**, no changes were observed on CV indicating the absence of their activity toward electrochemically generated $O_2^{\bullet-}$ in spite of the presence in the structure of OH groups as hydrogen and electron transfer systems.

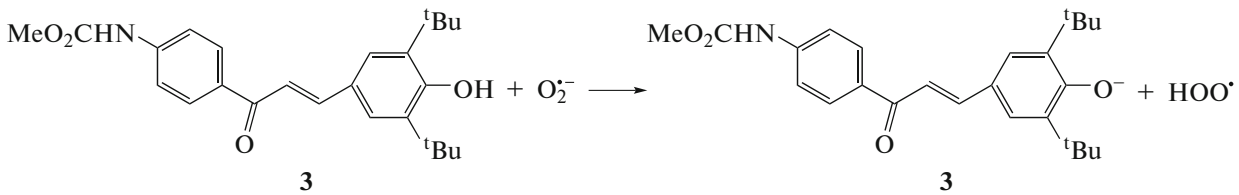
The reaction of compound **3** with $O_2^{\bullet-}$ leads to disappearance of reversibility of O_2 reduction stage (Fig. 2).

Decrease of anode peak height for superoxide anion radical oxidation and increase in cathode peak height indicates the irreversible reaction of compound **3** with $O_2^{\bullet-}$. The reverse branch of CV curve shows emergence of a new anode peak at +0.05 V, which is probably explained by the formation of the corresponding phenolate anion when $O_2^{\bullet-}$ abstracts hydrogen atom from OH group of chalcone **3** as shown in Scheme 1.

The obtained results indicate the activity of compound **3** toward electrochemically generated $O_2^{\bullet-}$, which is explained by the presence of sterically hindered phenol fragment which favors to formation of stable intermediate.

We studied the inhibiting activity of compounds **1**–**3** toward $O_2^{\bullet-}$ generated in xanthine/xanthine oxidase enzymatic system on reduction of nitro blue tetrazolium (NBT test) into blue formazane with absorption maximum at 560 nm. The amount of reduced nitro blue tetrazolium is directly proportional to amount of $O_2^{\bullet-}$ formed in the model system, therefore the decrease of reduced formazane concentration indicates the antiradical activity of compound.

We revealed insignificant inhibiting activity of chalcones **1**–**3** toward $O_2^{\bullet-}$ formed in the enzymatic system (Fig. 3). A high ability to consume $O_2^{\bullet-}$ in NBT test was revealed for compound **1** (41.3 ± 0.09% inhibition), which contains two hydroxyl groups in con-



3 **3**

Scheme 1.

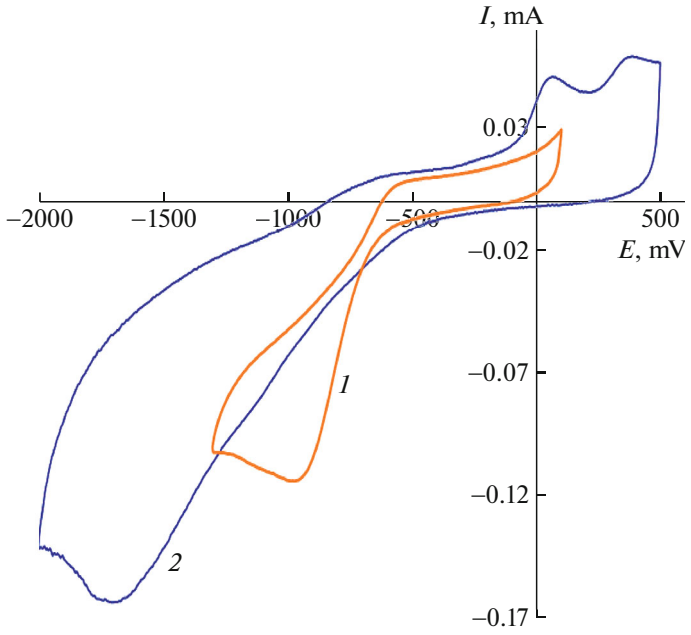


Fig. 2. CV of $O_2^{\bullet-}$ reduction (curve *1*) in the absence and (curve *2*) in the presence of compound **3** (CH_3CN, Pt, 0.1 M $^nBu_4NClO_4$, $C = 5$ mM, Ag/AgCl, $v = 0.2\ V\ s^{-1}$).

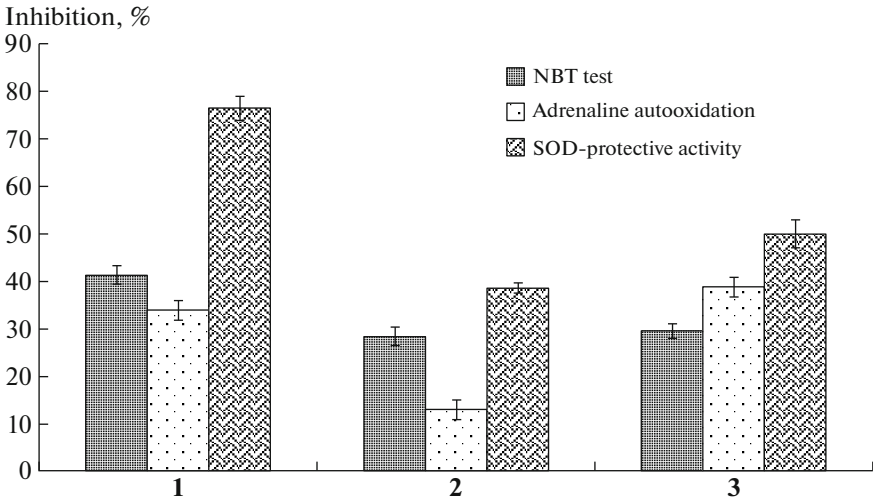


Fig. 3. Trapping activity of compounds **1**–**3** toward $O_2^{\bullet-}$, generated in a xanthine/xanthine oxidase enzymatic system, during adrenaline quinoid oxidation in alkaline medium, in the presence of supernatant of Russian sturgeon liver homogenate (adrenaline autooxidation). The figures show average values obtained in independent experiments in five parallel measurements in each experiment relative to control, difference from control experimental group $p < 0.05$.

trast to compounds **2** and **3**, whose activity was 28.4 ± 0.04 and 29.5 ± 0.06, respectively (Fig. 3).

Taking into account that this model system depending on medium pH allows autooxidation of flavonoids and the fact that resulting formazane is poorly soluble in water [13], the obtained figures provide no correct evidence on the ability of the studied compounds to consume $O_2^{\bullet-}$. Therefore, we studied activity hydroxy derivatives of chalcones toward $O_2^{\bullet-}$ obtained in the model system of adrenaline autooxidation in alkaline medium (pH 10.65) into adrenochrome detected at λ = 347 nm [14]. The feature of this method is that the quinoid oxidation of adrenaline produces not only $O_2^{\bullet-}$ but also radicals of carbonate–bicarbonate anions and CO_2, consequently, as a whole, one can judge the antiradical activity of chalcones. Furthermore, the model system of quinoid oxidation of adrenaline allows one to assess the ability of

biopreparations (supernatant of Russian sturgeon liver homogenate) to inhibit $O_2^{\bullet-}$ generation, i.e., to determine protecting ability of compound toward superoxide dismutase.

Under adrenaline autooxidation conditions, all chalcones inhibit formation of $O_2^{\bullet-}$: compounds **1** and **3** by 33.9 and 38.8%, respectively (Fig. 3). Compound **2** shows the least activity in the non-enzymatic system (13.0% inhibition), which is probably due to the presence of one unshielded OH group in chalcone structure. Addition of supernatant of Russian sturgeon liver homogenate into alkaline bicarbonate buffer leads to decrease of rate of adrenaline autooxidation, which indicates consumption of $O_2^{\bullet-}$ by cytosol SOD. The highest SOD-protective activity of biopreparation was found for chalcone derivative **1** (76.4 ± 0.01% inhibition), while compounds **2** and **3** inhibit adrenochrome formation by 38.6 ± 0.01 and 49.9 ± 0.03%, respectively.

Obtained results on the ability of hydroxy derivatives of chalcones **1**–**3** to consume superoxide anion radical correlate with data on the highest inhibiting effect of chalcone **1** in the model system of non-enzymatic peroxide oxidation of oleic acid and compound **3** in the model system of long-term oxidation of tilapia liver lipids [10].

Thus, in vitro experiments showed that chalcone derivatives **1**–**3** exhibit properties of primary antioxidants, whose antiradical properties are explained by possibility to behave as donors of electrons and/or hydrogen atoms. Trapping activity of the compounds toward $O_2^{\bullet-}$ generated in different model systems agrees well with in silico prediction data [10]. The highest inhibiting activity in xanthine/xanthine oxidase enzymatic system and SOD-protective activity was found for chalcone **1** containing two unshielded OH groups. The reaction of electrochemically generated $O_2^{\bullet-}$ was revealed only for chalcone **3**, which shows high antiradical activity also under conditions of adrenaline autooxidation in alkaline medium, which is explained by the presence of 2,6-di-*tert*-butylphenol fragment capable of formation of persistent aroxyl radical. Taking into account that compounds **1**–**3** are well soluble in ethanol and DMSO and the fact that in silico modeling predicts low potential toxicity (4th class), further experimental study of biological activity and acute toxicity of these chalcones in vivo will allow us to reveal efficient and safe pharmacologically active compounds.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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