



Catechol thioethers with physiologically active fragments: Electrochemistry, antioxidant and cryoprotective activities

Ivan V. Smolyaninov^{a,b,*}, Olga V. Pitikova^a, Eugenia O. Korchagina^a, Andrey I. Poddel'sky^c, Georgy K. Fukin^c, Svetlana A. Luzhnova^d, Andrey M. Tichkomirov^a, Elena N. Ponomareva^{a,b}, Nadezhda T. Berberova^a

^a Department of Chemistry, Astrakhan State Technical University, 16 Tatisheva str., Astrakhan 414056, Russia

^b Toxicology Research Group of Southern Scientific Centre of Russian Academy of Science, 41 Chekhova str., Rostov-on-Don 344006, Russia

^c G.A. Razuvaev Institute of Organometallic Chemistry, Russian Academy of Sciences, 49 Tropinina str., 603137 Nizhny Novgorod, Russia

^d Department of Microbiology and Immunology, Pyatigorsk Medicinal and Pharmaceutical Institute, 11 Kalinina str., Pyatigorsk 357500, Russia

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ABSTRACT

A number of asymmetrical thioethers based on 3,5-di-*tert*-butylcatechol containing sulfur atom bonding with physiologically active groups in the sixth position of aromatic ring have been synthesized and the electrochemical properties, antioxidant, cryoprotective activities of new thioethers have been evaluated. Cyclic voltammetry was used to estimate the oxidation potentials of thioethers in acetonitrile. The electrooxidation of compounds at the first stage leads to the formation of *o*-benzoquinones. The antioxidant activities of the compounds were determined using 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) assay, experiments on the oxidative damage of the DNA, the reaction of 2,2'-azobis(2-amidinopropane hydrochloride) (AAPH) induced glutathione depletion (GSH), the process of lipid peroxidation of rat liver (Wistar) homogenates *in vitro*, and iron(II) chelation test. Compounds 1–9 have greater antioxidant effectiveness than 3,5-di-*tert*-butylcatechol (CatH₂) in all assays. The variation of physiologically active groups at sulfur atom allows to regulate lipophilic properties and antioxidant activity of compounds. Thioethers 3, 4 and 7 demonstrate the combination of radical scavenging, antioxidant activity and iron(II) binding properties. The researched compounds 1–9 were studied as possible cryoprotectants of the media for cryopreservation of the Russian sturgeon sperm. Novel cryoprotective additives in cryomedium reduce significantly the content of membrane-permeating agent (DMSO). A cryoprotective effect of an addition of the catechol thioethers depends on the structure of groups at sulfur atom. The cryoprotective properties of compounds 3, 4 and 7 are caused by combination of catechol fragment, bonded by a thioether linker with a long hydrocarbon chain and a terminal ionizable group or with a biologically relevant acetylcysteine residue.

1. Introduction

Catechol (hydroquinone) fragment is a versatile platform that has been found in many natural and synthetic compounds [1–4]. Several classes of biological active substances including flavonoids, amines, alkaloids and phenolic acids contain this moiety. The different structural modifications of catechol (hydroquinone) have been performed to change the properties of these compounds via modulation of lipophilicity, introduction of the additional physiologically active groups, variation of the different redox active fragments. The successful synthetic examples of such targeted modification of the structure are derivatives of hydroxycinnamic acid, dopamine, hydroxytyrosol, urishinol

[5–11]. Catechols and their modified analogues demonstrate antibacterial, antitumor, anti-inflammatory, cytotoxic activities, as well as neuroprotective effect [12–18]. This class of compounds exhibits antioxidant activity inhibiting free radical processes. However, catechols (hydroquinones) may undergo redox-transformations with a generation of semiquinone radicals which are producers of reactive oxygen species (ROS): superoxide radical anion, hydrogen peroxide [19,20].

We are interested in the antioxidant properties of polyatomic phenols, in particular containing catechol moiety, since some of them have been considered as potential therapeutic agents against inflammation processes and neurodegenerative diseases [21–24]. Antioxidants have a presumably therapeutic effect in such diseases. Oxidative stress and

* Corresponding author at: Department of Chemistry, Astrakhan State Technical University, 16 Tatisheva str., Astrakhan 414056, Russia.

E-mail addresses: ivsmolyaninov@gmail.com, berberova@astu.org (I.V. Smolyaninov).

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inflammation are intrinsically related. The imbalance between oxidants and antioxidants in favor of the oxidants is closely associated with the initiation of neurodegenerative disorders and leads to the molecular damage in cells component such as proteins, lipids, DNA. Catechol containing compounds possess anti-inflammatory effects as they can induce the expression of heme oxygenase-1 (HO-1), as a consequence of Nrf-2 activation [17,25]. It was found that modified analogs of dopamine also exhibit anti-inflammatory properties [26], inhibits tumor necrosis factor alpha (TNF α) [27], exert cytoprotective properties including prevention of cold inflicted injury [8]. These peculiarities make such type of compounds interesting molecules to decrease impacts caused by inflammation or oxidative stress.

Thioethers prevail in the structure of biologically active compounds and pharmaceuticals [28]. Recently, antioxidants bearing sulfur atoms attracted the particular attention [29]. The introduction of sulfur atom into the structure of redox-active compounds – phenols, synthetic derivatives of tocopherols, ascorbic acid – develops new perspectives for design of polyfunctional antioxidants [30–32]. The unsubstituted hydroquinone/catechol thioethers with glutathione or acetylcysteine residues are well-known compounds [33–35]. In coordination chemistry, sulfur-modified sterically hindered catechols are widely used as chelating ligands [36–40]. Catechol-based compounds have attracted interest owing to their antioxidant and radical scavenging properties as well as their application in the synthesis of coordination polymers as multitopic ligands, in supramolecular and material chemistry [41–43].

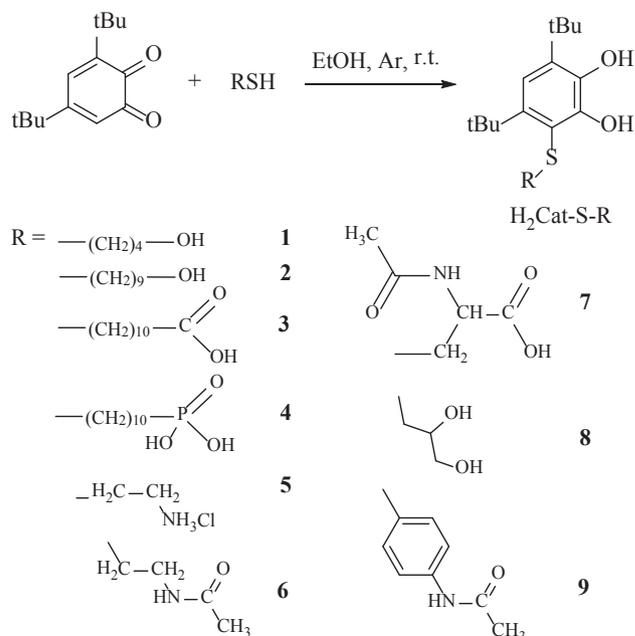
At the same time few results oriented to antioxidant researches were reported [44–46]. Catechol thioethers are attractive objects for a design of effective exogenous antioxidants as the combination of two different fragments covalently linked by sulfide bond can convey synergy from the effects of both acting moieties. In this context, herein we research hybrid antioxidants structurally based on sterically hindered catecholic scaffold.

Natural catechols (hydroquinones) are the redox active molecules. The important feature is that catechols (quinones) readily donate, store and accept electrons under biologically relevant conditions. Redox activity of quinone-type compounds deals with the possibility of the existence in three different redox states: catecholate, *o*-semiquinone radical anion, *o*-benzoquinone. Thioether group also possesses redox activity as it relates oxidative sequence with sulfoxides and sulfones. From this viewpoint, electrochemical methods can be promising tools in the study of catechol containing thioethers. Electrochemical approaches have often been used to evaluate the reducing power of antioxidants, to establish the interrelation between redox potentials and antioxidant activities of compounds [47,48].

The present research is focused on the development of the effective antioxidants based on the Michael addition reaction of sulfur containing compounds with 3,5-di-*tert*-butyl-*o*-benzoquinone (Scheme 1).

The synthesized thioethers contain three constituent parts: redox-active sterically hindered catechol moiety donates hydrogen atom and plays a role of primary antioxidant; thioether linker is able to appear secondary antioxidants properties; the terminal polar group is a physiologically active residue. In this paper we discuss the influence of the different polar groups at sulfur atom on the electrochemical properties; antioxidant and cryoprotective activity of thioethers 1–9. The radical scavenging and antioxidant properties of the compounds were evaluated using 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) assay, the reaction of 2,2'-azobis(2-amidinopropane hydrochloride) (AAPH) induced glutathione depletion (GSH), the oxidative damage of the DNA, the process of lipid peroxidation of rat liver (Wistar) homogenates *in vitro* and iron(II) chelating test.

Cryopreservation is a promising way for conservation of rare and endangered species, such as sturgeon. The fish sperm is favorable object for these purposes, as it can be stored in liquid nitrogen during several decades without change of its productive qualities [49]. However, cryopreservation of spermatozoa produces the injury of the majority of the cells because of intra- and extracellular water crystallization which



Scheme 1. Reagents and conditions of synthesis compounds 1–9.

leads to the damage cellular membranes, mitochondria, DNA and hyper-production of ROS [50,51]. To minimize these impacts the various methods are used. The addition of antioxidants in cryoprotective medium is one of them [52,53]. The studied thioethers were considered as cryoprotectants of the media for cryopreservation of the Russian sturgeon sperm.

2. Results and discussion

2.1. Chemistry

The thioethers 1–9 were synthesized as shown on Scheme 1 with the yields from 35 to 71%. Optimal conditions for Michael addition reaction of the sulfur containing organic compounds to 3,5-di-*tert*-butyl-*o*-benzoquinone are the use of ethanol and inert atmosphere at the room temperature. The reagent ratio “*o*-benzoquinone: thiol” varies from 1 to 2. It was found that the maximal target product formation takes place at the ratio “*o*-benzoquinone: thiol” as 1.0: 1.5. The structure of the synthesized compounds was confirmed by ^1H and ^{13}C NMR, IR spectroscopy and elemental analysis. Compounds 7 and 8 containing the chiral center were isolated as a racemic mixture of isomers.

X-ray quality crystals of 8 were grown from acetonitrile solution as solvate 8·CH $_3$ CN. The thioglycerine fragments in molecules of 8 in crystal are disordered over two positions (Fig. 1). The six-membered carbon ring C(1–6) is aromatic with C–C bond distances of 1.385(3)–1.404(3) Å. The equal carbon-oxygen bonds C(1)–O(1) and C(2)–O(2) of 1.365(3) Å are usual for catechols [54]. The thioglycerine group leaves the plane of the aromatic ring of catechol moiety: the torsion angle C(1)–C(6)–S(1)–C(15) is 69.8(7)°, the torsion angle C(1)–C(6)–S(1)–C(15') is 88.5(8)°.

We have found a lot of intra- and intermolecular hydrogen bondings in crystals 8·CH $_3$ CN (Fig. 2). The intramolecular hydrogen bondings O(2)–H(2)...O(1) and O(1)–H(1)...O(3) were observed, the corresponding distances O(2)...O(1) and O(1)...O(3) are 2.62 Å and 2.78 Å, respectively; angles O(2)–H(2)–O(1) and O(1)–H(1)–O(3) are 115.68 and 163.04°, respectively. Molecules of 8 form dimers through the intermolecular hydrogen bonds O(2)–H(2)...O(4) (the distance O(2)...O(4) is 2.97 Å), O(3)–H(3)...O(1) (the distance O(3)...O(1) is 3.03 Å). The corresponding bond angles are 136.46 and 131.56 Å.

A wide choice of commercially available thio-derivatives, 3,5-di-

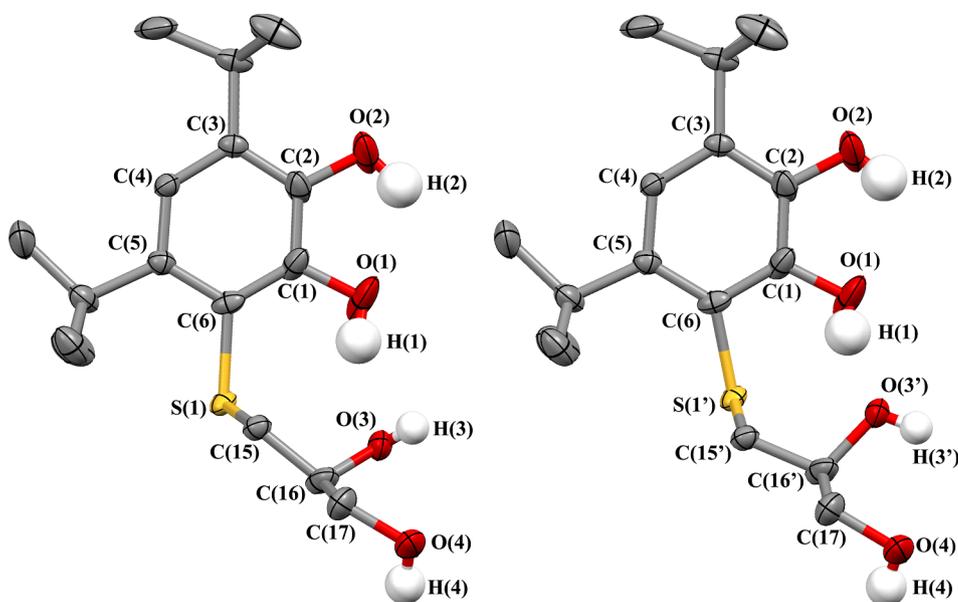


Fig. 1. The X-ray structure of catechol **8** with the indication of disordered thioglycerine fragment. The ellipsoids are given with 50% probability. The hydrogen atoms excepting hydroxyl groups hydrogens are omitted for clarity. The selected bond lengths: O(1)–C(1) 1.365(3), O(2)–C(2) 1.365(3), O(3)–C(16) 1.433(7), O(3')–C(16') 1.431(7), O(4)–C(17) 1.430(3), S(1)–C(6) 1.811(3), S(1)–C(15) 1.827(4), S(1')–C(6) 1.768(3), S(1')–C(15') 1.827(5), C(1)–C(2) 1.385(3), C(1)–C(6) 1.394(3), C(2)–C(3) 1.386(3), C(3)–C(4) 1.393(3), C(4)–C(5) 1.388(3), C(5)–C(6) 1.404(3), C(15)–C(16) 1.506(6), C(15')–C(16') 1.506(6), C(16)–C(17) 1.499(4), C(16')–C(17) 1.498(5), O(1)–H(1) 0.82(1), O(2)–H(2) 0.84(1), O(3)–H(3) 0.86(1), O(3')–H(3') 0.80(1), O(4)–H(4) 0.82(1).

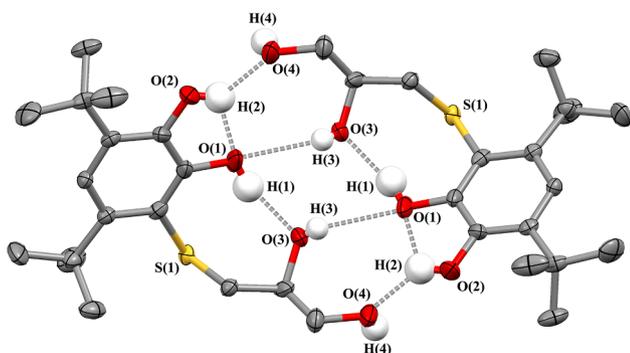


Fig. 2. The intra- and intermolecular hydrogen bondings between two neighboring molecules **8** in crystals $8 \cdot \text{CH}_3\text{CN}$. The hydrogen atoms excepting atoms H(1), H(2), H(3) and H(4) are omitted for clarity.

tert-butyl-*o*-benzoquinone, and the simplicity of the reaction allows to modify the catechol ring by the various functional groups bonded by sulfide linker. The presence of blocking *tert*-butyl groups inhibits the reaction polymerization which is known for dopamine. The target compounds contain a catechol unit and polar fragments such as aliphatic hydroxyl group, carboxylic or phosphonic acid moieties, amino-, acetamido-, thioether functionalities. The presence of *tert*-butyl substituents in catecholic fragment leads to increasing hydrophobicity of this part of molecules. The combination of terminal polar physiologically active groups and catecholic fragment allow to consider these thioethers as amphiphilic substances. The researched compounds can reveal different type of activity: redox-active catechol fragment has the three different redox levels and possesses radical scavenging activity, antioxidant or prooxidant properties; polar groups raise hydrophilicity and metal binding ability.

2.2. Evaluation of electrochemical properties by cyclic voltammetry

The electrochemical properties of compounds **1–9** have been investigated by cyclic voltammetry (CV) in acetonitrile (MeCN) solutions containing 0.1 M NBu_4ClO_4 (TBAP) as supporting electrolyte at a glassy carbon working electrode. The redox potentials are referenced to Ag/AgCl/KCl electrode (Table 1).

In acetonitrile the CVs of compounds **1–8** display two successive oxidation peaks in the potential range up to 1.80 V (Fig. 3, Figs. S1–S3). The first two electron electrochemical stage is ascribed to the

Table 1

Electrochemical oxidation potentials of compounds **1–9** (MeCN, GC-anode, C = 2.0 mM, 0.1 M TBAP, scan rate 200 mV s^{-1} , Ag/AgCl/KCl).

Compound	E^{ox1}/V	E^{ox2}/V
1	1.18	1.56
2	1.21	1.57
3	1.17	1.57
4	1.06	1.55
5	1.21	1.63
6	1.06	1.63
7	1.14	1.70
8	1.15	1.47
9	1.15	1.44
$^{**}\text{H}_2\text{Cat-S-C}_4\text{H}_9$	1.20	1.60

E^{ox1} E^{ox2} – potentials of anodic peaks; $^{*}E^{\text{ox3}}$ – potential of third anodic peak; ** data for 4,6-di-*tert*-butyl-3-(butylthio)benzene-1,2-diol ($\text{H}_2\text{Cat-S-C}_4\text{H}_9$) from Ref. [55].

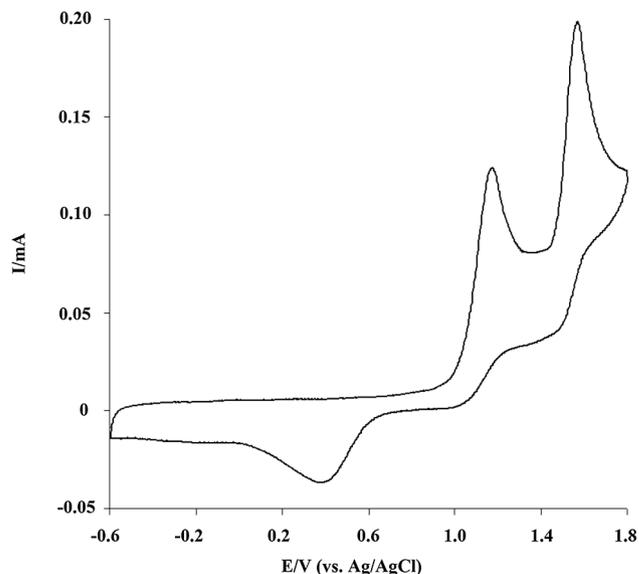
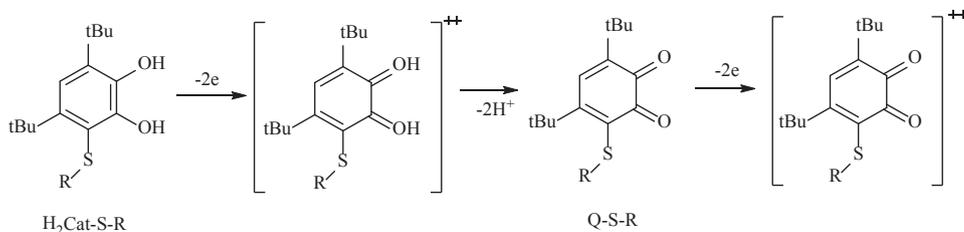


Fig. 3. Cyclic voltammogram of thioether **1** in the potential switch from -0.6 to 1.8 V (MeCN, C = 3 mM, 0.1 M TBAP, scan rate 200 mV s^{-1}).



Scheme 2. Electrochemical transformations of compounds 1–9.

irreversible oxidation of catechol fragment.

The one quasi-reversible two-electron oxidation peak appears on the CVs of catechols or hydroquinones at different values of pH (most frequently neutral or acidic media) in aqueous solutions containing phosphate buffer or water/acetonitrile solutions [56–58]. However electrochemical behavior of catechols and hydroquinones in aprotic solvents is significantly different in water media. The typical irreversible two-electron oxidation wave is observed [59,60]. In the case of hydroquinones, a reversible two-electron wave is observed only in the presence of strong acids [59].

In aprotic media ECEC mechanism takes place for thioethers 1–8. This mechanism involves the electron transfer (E) followed by the fast deprotonation (C) and the subsequent loss of a second electron. At the same time it is known for catechols two one-electron steps are often joined in one dielectron stage [57]. The cathodic peak observed on the reverse scan of the voltammograms (in the range from 0.50 to 0.30 V) corresponds presumably to the reduction of the $\text{HQ}^+-\text{S-R}$ intermediate [59]. The further deprotonation step leads to *o*-quinone formation as a final product of electrooxidation (Scheme 2) at the first stage. This electrochemical picture is close to that one for recently researched catechol-thioethers [55]. The introduction of thioether group in catechol moiety leads to the extension of the redox potential range by the occurrence of additional irreversible two-electron electrochemical stage at 1.47–1.70 V (Table 1).

The instability of the electrogenerated dications of compounds 1–8 indicates the chemical reaction (C) following the electron transfer: the C–S bond cleavage or the formation of sulfoxides, sulfones, sulfonium salts [61]. In case of compound 9, three oxidation peaks are observed on CV curve (Fig. 4).

The oxidation potentials of the first and the third oxidation peaks of compound 9 have the close values with other thioethers (Table 1). The first two-electron peak is responsible for the oxidation of catecholic

fragment. The presence of additional electroactive amide group bonded by *p*-phenylene spacer gives the quasi-reversible oxidation process at 1.44 V which may be found between the electrooxidation of catecholic group and the oxidation of thioether linker. Compounds 6 and 7 also contain amide group, but no one additional peak was not observed on CVs at potential switch up to 1.8 V. In the case of thioether 6 this oxidation peak was observed at 1.93 V. We think the combination of electron donor *p*-phenylene spacer and amide group could be responsible for the second oxidation process.

To confirm the formation of *o*-quinones at the first stage of electrochemical process a controlled-potential microelectrolysis of compounds 1, 3, 4, 6, 7 was performed at 1.30 or 1.20 V during 2 h. There is quasi-reversible one-electron reduction peak in the potential range -0.20 – (-0.40) V was observed on the cyclic voltammograms in the cathode area of potentials after electrolysis. The redox process corresponds to the reduction of electrogenerated *o*-benzoquinone to *o*-benzosemiquinone. The recorded UV–vis. absorption spectra of the electrolysis products display the absorption bands with a maximum in the range 490–505 nm. A wide absorption band in the visible spectral range is a characteristic feature of compounds containing a quinoid fragment and a thioether group [62]. These results are consistent with previously obtained data for catechol thioethers [55].

The values of oxidation potentials have often been used to the prediction of the antioxidant activity since this parameter is interrelated to the electron-donating properties of molecules as one of the factor of radical scavenging ability. Low oxidation potential values point out the higher antioxidant, and antiradical activity. Compounds 1–3 and 5 are characterized by the similar oxidation potentials with previously studied $\text{H}_2\text{Cat-S-C}_4\text{H}_9$. It is worth noting that the thioesters 4 and 6 with minimal values of E_p^{ox} are compounds with potentially higher antioxidant activity. The oxidation potentials of the catechol thioethers are close to those for 11-(2,3-dihydroxyphenyl) undecanoic acid and alkyl gallates [18,63]. In the case of compounds 7–9 we also found the shift of oxidation potentials to the cathodic area as compared to $\text{H}_2\text{Cat-S-C}_4\text{H}_9$ that indicates an increase in the reduction activity. The feature of compounds with catechol moiety is the most pronounced antiradical power and lowest oxidation peak potential [64].

However, not only the value of redox potential affects antioxidant properties, but also the ability to ionize polar groups, the number of possible redox states, and the stability of intermediates formed during the oxidation. Groups with acid-base properties dominate in the structure of antioxidants, the influence of pH on the electrochemical characteristics can be observed. In most cases with increasing pH the oxidation potential has a tendency to become less positive, and electrooxidation occurs with greater facility [64]. The thioether 5 has a protected amino-group, while the compounds 3, 4 and 7 contain the acidic carboxylic or phosphonic groups. Under physiological conditions these groups can be ionized, consequently this process would affect the antioxidant properties of compounds. We researched the electrochemical behavior of thioethers 3–5 and 7 in the presence of bases (Bu_4NOH or Et_3N). The additive of one equivalent of Bu_4NOH into a solution of thioethers 3, 4 and 7 leads to the splitting the first two-electron oxidation peak of catechol fragment to two peaks (Fig. 5, Fig. S4). The change of oxidation mechanism from two-electron step to two stages occurs. New irreversible one-electron oxidation peaks are

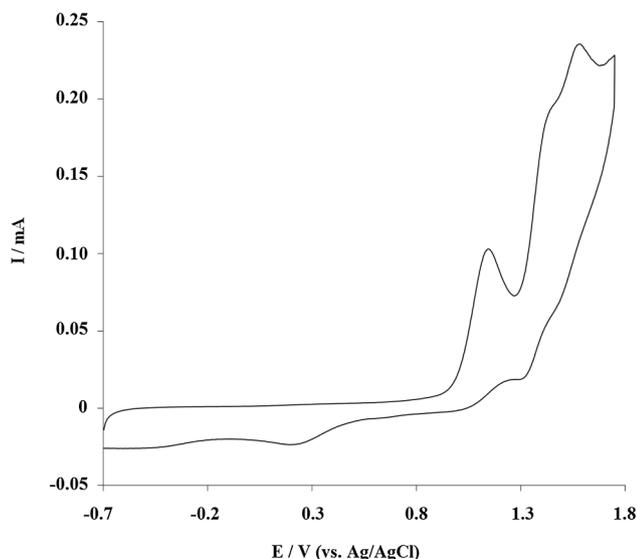


Fig. 4. Cyclic voltammogram of thioether 9 in the potential switch from -0.7 to 1.8 V (MeCN, $C = 3$ mM, 0.1 M TBAP, scan rate 200 mV s^{-1}).

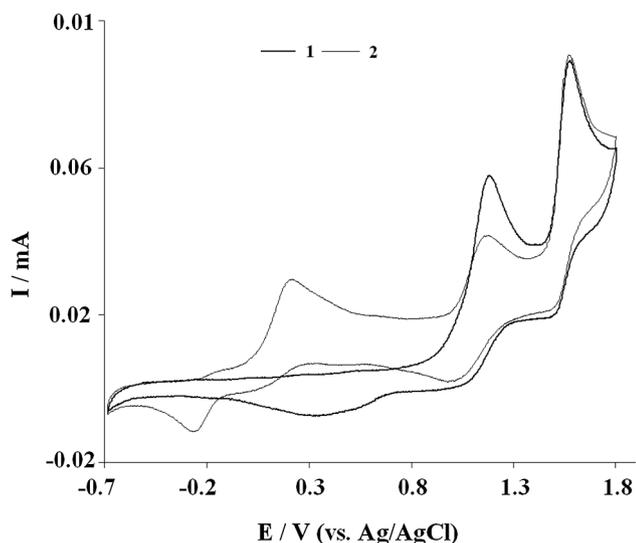
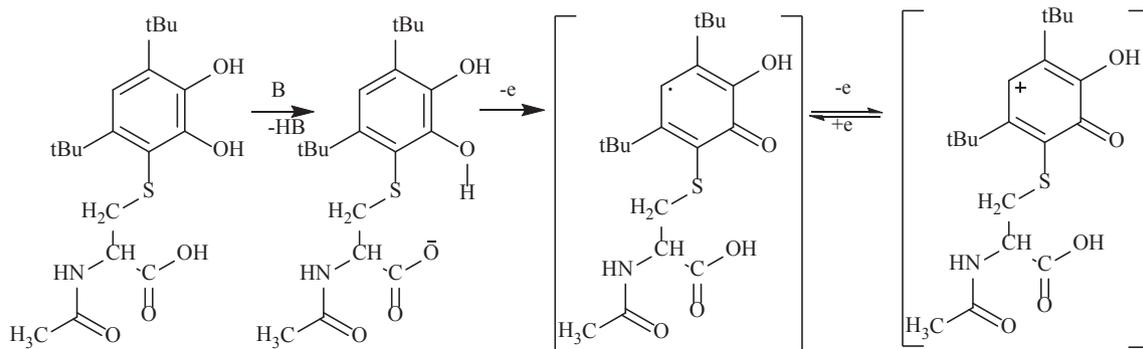


Fig. 5. Cyclic voltammograms of thioether 3 (curve 1); in the presence 1 eq. of Bu_4NOH (curve 2) (the potential switch from -0.7 to 1.8 V, MeCN, $C = 2$ mM, 0.1 M TBAP, scan rate 200 mV s^{-1}).

observed at 0.21 , 0.27 , 0.48 V for compounds 3, 4 and 7, respectively.

The second oxidation peak has the reversible one electron character. The current ratio (I_c/I_a) is 0.9 for compounds 3 and 7 (for compound 4 the value of $I_c/I_a = 0.4$). The reversibility of this redox process points out the formation of the relatively stable intermediate in the time of CV experiment. It should be mentioned that the anodic stage corresponding to the oxidation at sulfide linker remains unchanged. The deprotonation of carboxylic (phosphonic) group favors to formation of anion. The presence of two hydroxyl groups in catechol unit may provide the intramolecular or intermolecular hydrogen bonding with a carboxylate anion. The intramolecular electron transfer for redox-asymmetric systems accompanies proton-coupled electron transfer (PCET), as the combination of proton transfer reactions facilitates electron transfer [65–69]. Numerous papers devote the investigation of sterically hindered phenol compounds containing nitrogen base group [70–73]. In these systems, the phenol oxidation process occurs at a much lower potential than that of a non-H-bonded phenols. In the presence of a base the oxidation of catechol moiety is also observed at more negative potential. However, in our case the first oxidation is irreversible one-electron process with following chemical stage in the solution. This reaction may be the proton transfer from oxidized catechol fragment to anion with the formation of the stable 2-hydroxyphenoxy radical. The reversible oxidation of this radical to cation occurs at the same potentials ($E_{1/2} = 1.06$ – 1.08 V) for three compounds that confirms the generation of the same type intermediates (Scheme 3).

The value of oxidation potentials of the second stage is close to a well-known reversible process of one-electron oxidation of phenoxyl



Scheme 3. Electrochemical transformations of 7 in the presence of one equivalent of base.

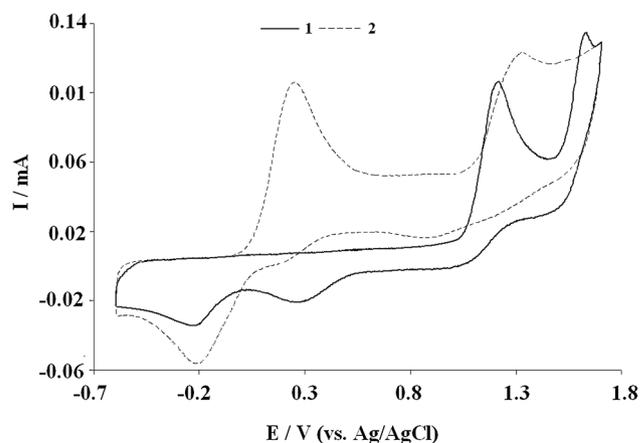
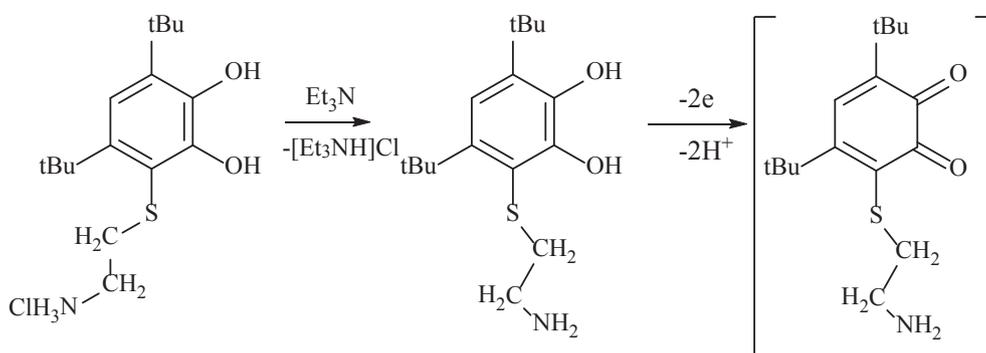


Fig. 6. Cyclic voltammograms of thioether 5 (curve 1); in the presence 1 eq. of Et_3N (curve 2) (the potential switch from -0.6 to 1.7 V, MeCN, $C = 3$ mM, 0.1 M TBAP, scan rate 200 mV s^{-1}).

radical [74]. The indicator of current ratio (0.9 and 0.4) suggests the possibility of realization of the next chemical stage – the deprotonation of cation to *o*-benzoquinone. In the course of time the generated monoanions is unstable under oxygen condition that leads to coloration of the solutions. In the UV–vis. spectra we observed the absorption with maximum in the range of 490 – 505 nm proper to *o*-benzoquinones.

The interaction of thioether 5 with triethylamine (or Bu_4NOH) leads to a change of the electrochemical picture (Fig. 6(2)). The first oxidation process (1.21 V) shifts to 0.27 V, the second stage is disappeared, while a new oxidation peak at 1.35 V is observed on the CV curve. The intensity of current of the first peak is equal to one without the additive of base, this fact points out the two-electron transfer during the oxidation process. The deblocking of amine-group favors to decreasing the oxidation potential due to the hydrogen bonding as in case of phenol compounds with amino-, imidazole or pyridine groups. However, the number of transferred electrons remains unchanged as well as the irreversibility of electrochemical process. The electrooxidation coupled with proton transfer promotes the formation of unstable *o*-benzoquinone at the first stage (Scheme 4). The second oxidation step may be related to the oxidation of the product of intramolecular cyclization forming from generated *o*-benzoquinone [36].

It is interesting to estimate the modulation of antioxidant activity of the initial and ionized forms of target compounds using cyclic voltammetry. The cathodic shift of the oxidation peaks of anionic forms of thioethers correlates with the increase of antioxidant properties. The CV method can be applied for the assessment of the ease of electron transfer through the shift of the redox potential (E) ($\Delta G = -nF\Delta E$) [75]. The differences in the redox potentials of the initial and ionized forms for compounds 3–5 and 7 give the values of $\Delta G = -22.6$; -18.2 ; -21.7 ; -16.8 kcal mol^{-1} , respectively. It points out the easier



Scheme 4. Electrochemical transformations of compound 5 in the presence of one equivalent of triethylamine.

oxidation of ionized forms of these compounds. Thus, according to electrochemical data the deprotonation of NH₃-cation in 5 or carboxylic (phosphonic) groups in compounds 3, 4, 7 lead to the growth of antioxidant activities. Another method to estimate the antioxidant properties often combined with electrochemical data is a DPPH radical scavenging activity assay.

2.3. Antioxidant activity assay

2.3.1. Scavenging ability of DPPH radical

A comparative evaluation of antioxidant activity of compounds 1–9, 3,5-di-*tert*-butylcatechol (CatH₂) with known antioxidant such as α -tocopherol was performed in a reaction with 2,2-diphenyl-1-picrylhydrazyl radical. The main parameters of DPPH scavenging activity (EC₅₀, TEC₅₀, AE) of the compounds were determined in acetonitrile at 298 K. The introduction of compounds 1–9 in solution of DPPH results in a decrease in the intensity of the absorption maximum at 517 nm. The comparative data on the antiradical activity of compounds 1–9 and α -tocopherol are presented in Table 2.

The thioethers 1–9 are characterized by close EC₅₀ parameters in a range of 19.0–14.5 μ mol. Phosphonic acid 4 has lower value of EC₅₀ among the set of compounds. In the case of 2 with the terminal hydroxyl group the parameter EC₅₀ is comparable with natural analog hydroxytyrosol (13.4 \pm 0.6 μ mol). Our results are consistent with the data obtained earlier for catechol containing 2-isothiocyanatoethyl group in the 4th position of aromatic ring (16.6 \pm 1.0 μ mol) and dopamine hydrochloride (11.4 \pm 1.2 μ mol) [77]. At the same time the index EC₅₀ for compounds 1–9 is lower than EC₅₀ in case of synthetic mitochondrial antioxidants with catechol ring [4,78]. The considering thioethers have higher indicators of EC₅₀ in comparison with catechol thioethers with hydrocarbon substituent at sulfur atom (H₂Cat-S-C₄H₉) or α -tocopherol [55]. However, these EC₅₀ values point out the

Table 2

Radical scavenging activity of target compounds in the reaction with DPPH radical (MeCN, 298 K).

Compound	EC ₅₀ / μ mol	TEC ₅₀ /min	AE $\cdot 10^3$
1	18.5 \pm 0.8	10.0 \pm 0.5	5.4 \pm 0.4
2	15.2 \pm 0.4	30.1 \pm 0.6	2.2 \pm 0.1
3	17.5 \pm 0.6	10.1 \pm 0.3	5.7 \pm 0.4
4	14.5 \pm 0.9	10.0 \pm 0.8	6.9 \pm 0.6
5	19.0 \pm 0.5	10.3 \pm 0.9	5.1 \pm 0.5
6	17.1 \pm 0.3	9.1 \pm 1.0	6.5 \pm 1.2
7	15.5 \pm 0.7	1.3 \pm 0.7	64.3 \pm 14.3
8	15.5 \pm 1.0	5.2 \pm 0.8	12.7 \pm 2.8
9	15.0 \pm 0.8	10.0 \pm 1.0	6.7 \pm 1.1
^a H ₂ Cat-S-C ₄ H ₉	12.0 \pm 0.2	50.0 \pm 0.5	1.7 \pm 0.5
^b H ₂ Cat-S-CH ₂ -COOH	13.2 \pm 0.9	10.0 \pm 0.9	7.6 \pm 0.8
^b α -tocopherol	12.0 \pm 0.5	15.1 \pm 0.4	5.55 \pm 0.1

^a Data for H₂Cat-S-C₄H₉ are from Ref. [49].

^b Data for H₂Cat-S-CH₂-COOH and α -tocopherol are from Ref. [76].

pronounced radical scavenging activity.

The times of achievement of an equilibrium state (TEC₅₀) for compounds 1–9 differ considerably. This parameter deals with the reactivity of the compounds toward short living radicals formed in biological systems. The most of compounds (1, 3–6, 9) have identical values of TEC₅₀ exceeded the data for α -tocopherol (Table 2). These results are consistent with the indicator TEC₅₀ for 2-((4,6-di-*tert*-butyl-2,3-dihydroxyphenyl)thio)acetic acid (H₂Cat-S-CH₂-COOH) [76]. The presence of the polar groups favors to reducing TEC₅₀ of the compounds in comparison with H₂Cat-S-C₄H₉ and this indicates an increase of antiradical activity. The deprotonation of cationic group in 5 by triethylamine does not significantly influence the value of EC₅₀ (20.0 \pm 0.8 μ mol) but the indicator of TEC₅₀ reduces to 1.1 \pm 0.4 min in comparison with that one for the initial catechol thioether (10.3 \pm 0.9 min). The ability of an intramolecular H-bonding between amino-group and catechol hydroxyl has a profound influence on the rate of H-atom abstraction. These data are consistent with results for the reaction of DPPH with hydroquinone containing pendant piperidine fragment, which is caused by the presence of H-bond [79].

Compounds 7 and 8 with chiral carbon atom and the possibility the formation of intramolecular H-bonds are characterized the minimal time of achievement of an equilibrium state in the reaction with DPPH. The antiradical efficiency (AE) combines two parameters, *viz.*, EC₅₀ and TEC₅₀, and makes it possible to estimate both the capacity of substance of electron or hydrogen atom donating and the rate of the reaction with DPPH radical. According to the earlier proposed classification [80] only compound 2 reveals a medium antiradical efficiency, whereas compounds 3, 4, 6–9 have the high values AE which exceed data for α -tocopherol and alkyl gallates [81]. In DPPH test the most active compounds are catechol thioethers 7 and 8 containing acetylcysteine and ethylene glycolic fragments, respectively.

2.3.2. AAPH-induced oxidation of DNA

We have performed the experiments on the oxidative damage of DNA *in vitro* in the presence of compounds 1–9 and trolox. 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) decomposes at physiological temperature (37 °C) with the generation of the peroxide radicals which induce the supercoiled DNA strand. In the result of this process, the formed carbonyl compounds react with thiobarbituric acid (TBA) to obtain TBA reactive substances (TBARS) [82,83]. The AAPH induced oxidation of DNA was carried out at 50 μ mol thioethers concentration and the absorbance of TBARS in UV-vis. spectra was measured after the oxidation during 150 min in comparison with that in the blank experiment (Fig. 7).

In the presence of compounds 1–9 and trolox the absorbances of TBARS are lower than those in the blank experiment (Fig. 5, control) indicating that the substances additives inhibit the degradation of DNA. Compounds 1–9 act as antioxidants in this test. The catechol thioethers 2, 3 containing the long hydrocarbon chain and terminal polar hydroxyl or carboxylic groups are less active: the level of TBARS decreases by

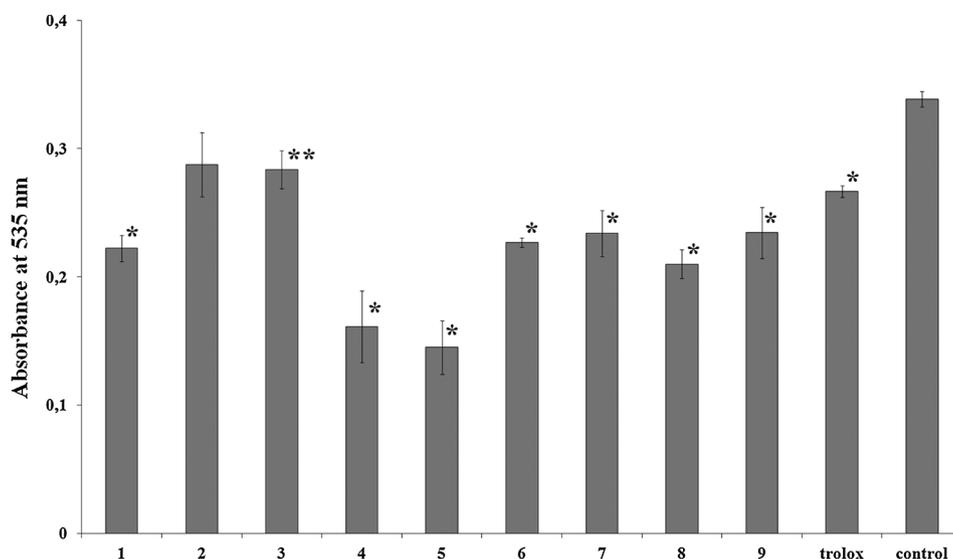


Fig. 7. The variety of the absorbance (at the thickness of the absorbing layer – 1 cm) of TBARS in the DNA oxidation (2.0 mg ml^{-1}) induced by 40 mM AAPH in the presence of 1–9 and trolox (the results are expressed as mean \pm standard deviation ($^*p < 0.005$; $^{**}p < 0.05$)).

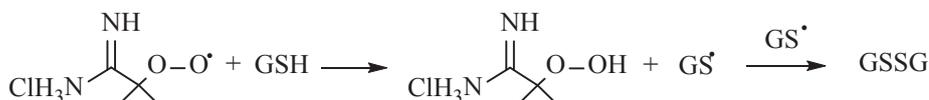
18.0%. The compounds 1, 6–9 with acetamide or hydroxyl groups have more pronounced inhibition effect: they reduce the TBARS amounts by 30.7–38.0%. The antioxidant activity of these thioethers is higher than the activity of trolox. The combination of polar group and the thioether linker with catechol ring favors to the appearance of the inhibition activity of compounds 1–9. It was earlier shown that 3,5-di-*tert*-butylcatechol (CatH₂) displays a weak promotion effect on the oxidation of DNA ($103.1 \pm 3.8\%$) [76]. In this assay the most active compounds are catechols 4 and 5 with phosphonic acid and cystamine hydrochloride fragments, respectively. In comparison with the control experiment, the absorbance of TBARS reduces by 52.5 and 57.2%. These results exceed data for catechol thioether with additional phenolic group (37.4%) [76].

In this test we have shown that catechol thioethers 1, 4–9 can demonstrate more pronounced inhibiting activity than the known antioxidant trolox. These results suggest that the thioethers 1–9 may act as antioxidant in the presence of radical promoter. On the one hand, the activity of 1–9 in the reaction with DPPH, in the test with the AAPH induced oxidation of DNA suggest a demonstration of radical scavenging properties of thioethers. On the other hand, electrochemical data point out the formation of *o*-quinones during the oxidation process. Consequently, such type of compounds may exhibit a dual anti- or pro-oxidant activity. An interaction of quinones with glutathione, ascorbic acid or NADH will cause the disruption of intracellular redox balance, induce the lipid peroxidation (LP) process. Glutathione (GSH) is involved in the functioning of the organism antioxidant defense system, and the ratio of a reduced to an oxidized form of glutathione (GSSG) relates to the disturbance of redox balance in the cell. In order to evaluate the potential pro-oxidant effect of thioethers 1–9, their influence on the AAPH induced glutathione oxidation was investigated.

2.3.3. The influence of compounds on AAPH induced glutathione depletion and process of lipid peroxidation in vitro

The water-soluble radical initiator AAPH is also used in the reaction with glutathione. Interaction of ROO-radicals with glutathione accompanies a GSH depletion (Scheme 5).

The addition of the promoter contributes a regular decrease of



Scheme 5. The interaction of glutathione (GSH) with ROO-radicals generated at physiological temperature (37 °C) leads to oxidized form of glutathione (GSSG).

Table 3

The change of GSH concentration in time in the presence of AAPH and effect of compounds 1–9, CatH₂, Trolox, (C = 0.1 mM) on remaining level of GSH. The reaction system (GSH + AAPH) consisted 2.0 mM AAPH and 0.1 mM GSH in PBS, incubated at 37 °C during 180 min.

Compound	1 h	2 h	3 h
1	76.1 \pm 0.9	54.3 \pm 0.7	35.2 \pm 0.5
2	86.1 \pm 1.9	74.9 \pm 1.1	47.6 \pm 0.9
3	84.7 \pm 0.8	72.9 \pm 2.3	47.1 \pm 1.2
4	79.5 \pm 0.6	66.8 \pm 1.3	43.1 \pm 0.7
5	74.3 \pm 2.0	63.4 \pm 0.9	40.8 \pm 1.0
6	77.9 \pm 1.8	68.1 \pm 2.4	39.6 \pm 1.8
7	58.2 \pm 0.4	32.4 \pm 1.7	14.3 \pm 1.5
8	57.4 \pm 1.0	37.7 \pm 0.8	17.4 \pm 0.7
9	83.1 \pm 1.6	69.1 \pm 2.3	39.8 \pm 0.8
CatH ₂	59.6 \pm 0.8	42.6 \pm 0.6	30.3 \pm 0.7
Trolox	80.2 \pm 0.4	68.3 \pm 0.4	46.6 \pm 0.3
Control	75.3 \pm 0.8	62.4 \pm 3.0	40.2 \pm 2.8

glutathione concentration in the reaction medium on the 60 μmol of the initial value (Table 3).

The thioethers 1, 4–6 and 9 can be marked out in the series of compounds because they don't change significantly the level of depletion of GSH during the time of test in comparison with control experiment data (Table 3). Consequently, these catechols do not show pronounced anti- or pro-oxidant effect on AAPH induced oxidation of GSH. Unlike above mentioned compounds the thioethers 7 and 8, as well as 3,5-di-*tert*-butylcatechol have a remarkable promoting effect. The concentration of GSH decreases by 10–30 μmol in comparison with the results obtained for AAPH. It should be mentioned that these thioethers are the most active in the DPPH assay. The promoting effect of these substances relates possibly to the fast formation of the corresponding *o*-quinones. In the presence of a radical initiator and a reducing agent (GSH), a redox cycling catechol/*o*-quinone is possible resulting in a significant reduction of glutathione concentration in the reaction medium. This behaviour has been previously observed for 2-((4,6-di-*tert*-butyl-2,3-dihydroxyphenyl)thio)benzoic acid and the derivatives of gallic acid [75,80].

In the set of compound only **2** and **3** catechols have the antioxidant effect on the AAPH induced depletion of GSH. These compounds are characterized by the high hydrophobicity. Such behavior has been also observed for natural compounds containing a catechol moiety and hydrophobic hydrocarbon groups [84]. Earlier we have shown that the catechol thioethers with pentyl- and cyclopentyl substituents at sulfur atom possessed an analogical activity [76]. It is possible that compounds **2** and **3** could participate in the micellization process facilitating their existence as micelles or other aggregates in the water phase.

In the assay with AAPH induced glutathione depletion the several compounds reveal the absence of pronounced effect or prooxidant properties. It is interesting to estimate the behaviour of these substances in the model reaction of lipid peroxidation (LP) *in vitro*. Here we evaluate the influence of compounds **1–9** and CatH₂ on Fe²⁺-induced lipid peroxidation of the rat (Wistar) liver homogenate as a non-enzymatic process by addition of (NH₄)Fe(SO₄)₂. The intensity of lipid peroxidation of the rat liver homogenates was assessed by the accumulation of TBARS products [85]. The samples of the rat liver homogenates were divided as following: one control (blank experiment) and homogenates with additives of thioethers. TBARS concentrations were determined by measuring the intensity of absorbance of solutions at 535 nm using UV-vis spectroscopy (Fig. 8).

All examined catechol derivatives showed generally antioxidant properties than the catechol CatH₂ which had a weak promotion action. In the presence of compounds **1–4**, **7**, **8** decrease of the TBARS content (16.0–39.4%) was observed. This fact points out the antioxidant influence of these substances on the lipid peroxidation process. The most active thioethers were **1–4** and **7**. At the same time, the TBARS concentration remains practically equal to the control in the rat liver homogenates with the additives of compounds **5**, **6** and **9**. The variation of the groups at sulfur atom allows to modulate anti-, pro-oxidant properties of this type compounds. For example, catechol thioether with phenolic fragment reveals only weak antioxidant activity [76]. The presence of polar hydroxyl, acidic groups bonding with sulfur atom and hydrophobic carbon chain or acetylcysteine moiety in the structure of thioethers gives the better effect on antioxidant properties. While acetamide derivatives **7**, **9** and catechol with the residue of cystamine hydrochloride **5** haven't pronounced influence on the lipid peroxidation the rat liver homogenate. While the compounds **5**, **6** or **9** can possibly undergo the hydrolysis or the deprotonation in the biological conditions giving amino derivatives. Nevertheless the pronounced antioxidant

effect is absence for these compounds.

The increase of activity of compounds **1–4** may be attributed to the higher lipophilicity achieved through the elongation of carbon chain. Calculated values of ClogP are equal 5.04 (**1**), 7.56 (**2**), 8.37 (**3**), 7.95 (**4**) 8.37 [86]. Compounds **2–4** possess high lipophilicity. This important parameter for antioxidant molecules is due to their partition into lipid bilayers. The absence of antioxidant effect was observed for catechol compounds containing hydrophobic group at sulfur atom, conversely this type of compounds had pro-oxidant influence [76]. Also the presence only carboxylic group in the catechol derivatives led to the analogical effect. The catechol fragment bonded via sulfur linker with hydrocarbon chain containing the terminal polar group favors to appear the antioxidant activity. This behavior may be due to the interaction with metal ion (Fe²⁺) and occurring chelation activity. Consequently, we researched the iron binding properties of thioethers.

2.3.4. Evaluation of iron(II) chelation properties

Iron participates in many vital essential biochemical processes. A disbalance of iron homeostasis (the generation of free iron or its overload) may provoke numerous disorders and diseases. It has been shown that Fe overload plays an important role in free radical production (Fenton reaction), initiation of lipid peroxidation leading to the progress of oxidative stress [87,88]. The iron imbalance is one of assumed reason in the pathogenesis of neurodegenerative diseases (Parkinson's and Alzheimer's diseases) [89]. Besides, ferroptosis is one of form of programmed cell death which is caused by iron metabolism and the accumulation of lipid peroxidation products [90]. It has been proved that iron-dependent ROS generation and lipid peroxidation is involved in this way of regulated cell death [91]. Ferroptosis can be inhibited by the application of iron chelators, lipophilic antioxidants, inhibitors of lipid peroxidation [92]. Catechol-based compounds are known iron chelators [1]. Catechol containing siderophores possess a higher affinity to iron ions [93,94]. The different types of metal chelators with catechol, galloyl moieties, acid residues or amide linkage were studied [95,96].

In this context it is interesting to estimate the iron-binding activity of the target catechol thioethers and to define an existence of the correlation with the structure of antioxidants. The iron(II) chelating ability of catechols **1–9** was evaluated by ferrozine test. In the presence of **1–9** the lowering absorbance is observed. EDTA was used as a reference compound. The percentage of iron(II) binding for the thioethers is displayed in Fig. 9. It has been found that EDTA chelates completely all

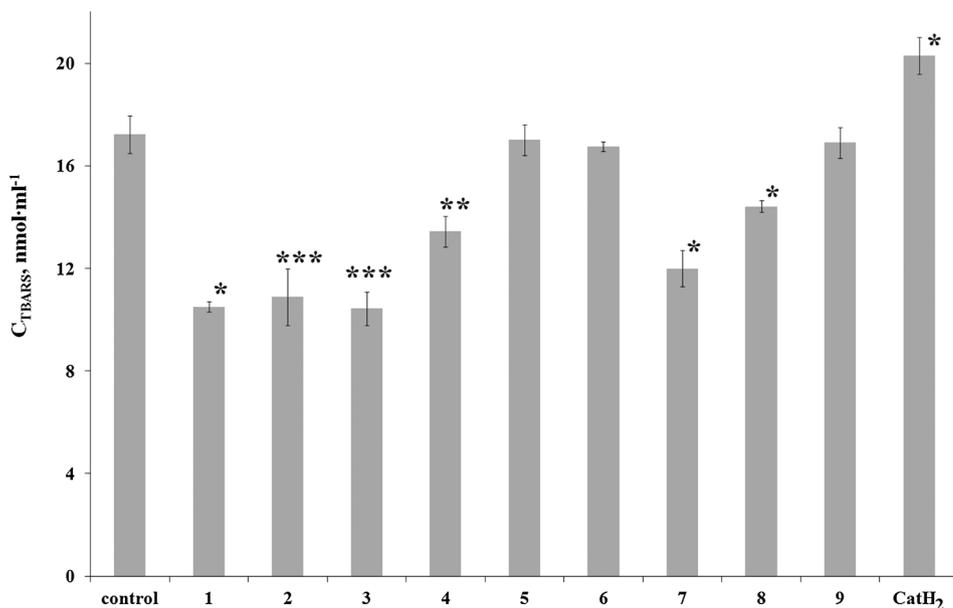


Fig. 8. The level of TBARS in rat liver homogenates (Wistar) *in vitro*, in the presence of compounds **1–9** and CatH₂ during the incubation 3 h (concentration of compounds in the reaction medium is 0.1 mM; without additives – control; the results are expressed as mean ± standard deviation (*p < 0.005; **p < 0.01; ***p < 0.05)).

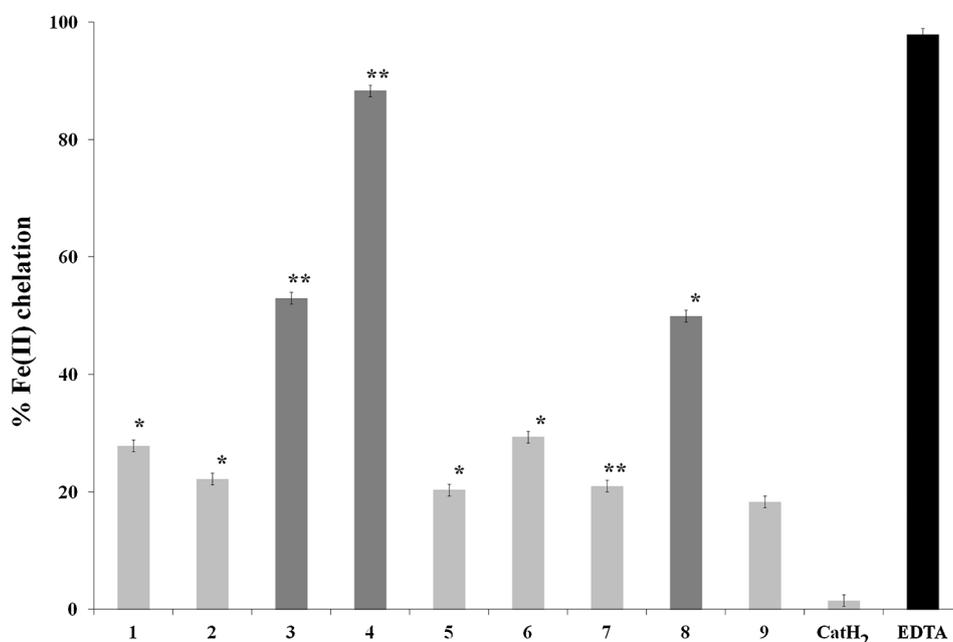


Fig. 9. Iron(II) chelation activity of target compounds 1–9, CatH₂ and EDTA. Results are expressed as mean \pm standard deviation (* $p < 0.001$; ** $p < 0.05$).

Fe²⁺ ions and inhibits the formation of the colored ferrozine-iron(II) complex. In case of compounds 1, 2, 5–7 and 9 the introduction of additional polar group increases insignificantly the iron-binding activity varying from 18 to 29%.

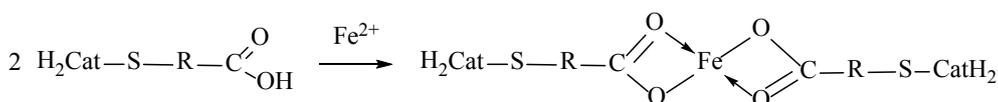
The thioether 4 with terminal phosphonic group has maximal iron (II) chelation capacity (88%). A large number of coordination modes are accessible for phosphonic acids with a maximal coordination number of nine [97]. These compounds as carboxylate ligands are able to form polynuclear metal containing compounds [98]. The replacement of phosphonic by carboxylic group in compound 3 leads to the decrease of chelation properties (53%). In the case of compounds 3, the chelating activity may be caused by the formation of carboxylate complexes with iron(II) ion (Scheme 6).

In the transition from compounds 1 and 2 with one hydroxyl group to the thioether 8 containing ethylene glycolic fragment the activity doubles. Compound 7 with acetylcysteine group has not pronounced iron-binding activity in comparison with 3 and 4. This behavior may be caused by the intramolecular hydrogen bonding due to the closeness of catechol fragment with acetylcysteine residue. In contrast to the researched compounds, a sterically hindered catechol (CatH₂) doesn't exhibit chelation ability due to the presence of hydrophobic *tert*-butyl substituents in catechol ring. However, it is known fact that the caffeic acid derivatives possess the iron-binding activity [96]. Modified catechol containing compounds with acetamide group and lipophilic triphenylphosphonium cation reveal the high chelation properties (90%) [78]. The elongation of hydrocarbon linker is accompanied by the growth of hydrophobicity and results in the reduction of iron-binding ability (60–65%). The difference between researched compounds and aforementioned catechols is the presence hydrophobic substituents in catechol ring. In our case the *tert*-butyl groups in aromatic moiety reduce significantly iron-chelation properties in comparison with unsubstituted catechols. The chelation activity of target catechol thioethers is mostly determined by the presence of polar terminal groups.

2.4. Cryoprotective activity

Cryopreservation of generative cells is one of the effective methods to safe endangered species. The reactive oxygen species (ROS) may be generated in the process of cryopreservation and storage of sperm leading to deterioration of sperm quality. A sufficiently high concentration of polyunsaturated acids in sperm makes it very sensitive to the harmful effects of free radicals and heavy metals. Sperm contains small amounts of antioxidant enzymes that prevent or eliminate damage caused by an oxidation [99]. The addition of different types of antioxidants in cryomedia is one of ways to reduce the impact of oxidative stress [100]. In papers of our colleagues it was shown that the introduction of antioxidants bearing phenolic group in cryoprotective medium caused the inhibition effect on lipid peroxidation process and improved the quality of thawed fish sperm [101,102]. Keeping in mind this fact, the influence of dopamine derivatives during hypothermic organ preservation and antioxidation properties of researched compounds, we decided to verify the protective ability of catechol thioethers in the process cryopreservation of sperm Russian Sturgeon.

Solvents such as methanol, dimethylsulfoxide is present in most types of cryoprotective media in the concentration of 10–12% [102,103]. These additives prevent the formation of intracellular ice crystals and play a role of cell membrane-permeating cryoprotectants. However, the most frequently used DMSO has few negative effects that create some difficulties to apply it in routine procedures [104]. In this part of the work we attempted to carry out the cryopreservation of fish sperm with minimal quantity of DMSO necessary for the dissolution of substances (0.2 ml). Earlier it was shown that optimal concentration in case of phenolic antioxidant for cryopreservation of fish sperm was 100 μ mol [101,102]. The target compounds 1–9 were administrated in cryoprotective medium in the same concentration. The effect of catechol thioethers on the time of motility of Russian sturgeon sperm was studied in the modified Stein's medium after cryopreservation during 3 days of freezing in liquid nitrogen (Fig. 10). In the control experiment all spermatozoa died without enough of DMSO.



Scheme 6. The proposed structure of the resulting chelate iron-containing complex at the chelation of compound 3.

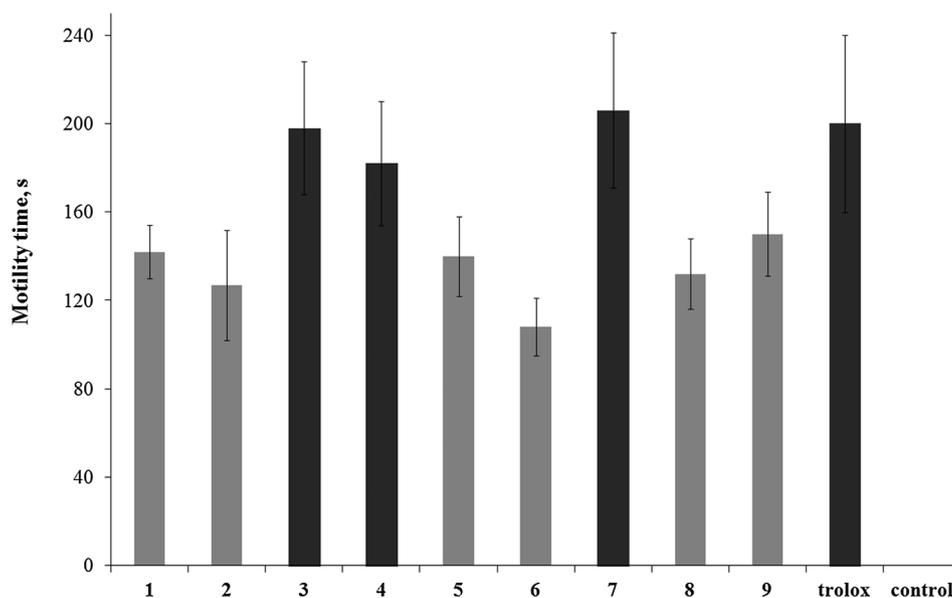


Fig. 10. The variation of the post thaw spermatozoa motility in dependence of additive of target compounds 1–9. Results are expressed as as mean \pm standard deviation.

The addition of catechols resulted in keeping sperm motility during 108–206 s. Under artificial conditions of reproduction of Russian Sturgeon the minimal time for fertilization of sturgeon roe is 180 s. According this parameter, the compounds can be divided into two groups. The first one contains catechol thioethers 1, 2, 5, 6, 8, 9 with lower threshold value of motility time (109–130 s). However, in comparison with control group the fact of saving viability spermatozoa points out the protective effect of these compounds in the cryopreservation process. The second group consists of thioethers 3, 4 and 7 having motility time more than 180 s. The cryoprotective activity of these thioethers is comparable with results for water-soluble antioxidant – trolox. The feature of compounds 3 and 4 is the presence of the head catechol moiety, long hydrocarbon chain and terminal ionized group (carboxylic or phosphonic). These acids are hydrophobic compounds with ClogP 8.37 and 7.95, respectively [86]. Nevertheless in the presence of catechol thioether 2 with similar structure and the parameter ClogP the motility time does not exceed 180 s. At the same time compound 7 is not so lipophilic (ClogP = 2.05) but it contains acetylcysteine residue that can be transformed into catechol with cysteine fragment under hydrolysis conditions. It is known fact that cysteine improves the motility and fertility of sperm cells during cryopreservation [105,106].

Earlier it was shown that there is a correlation between the inhibition action of antioxidant in the process of fish sperm lipid peroxidation and sperm motility [101,102]. In our case catechols 1–4 and 7 possess the antioxidant effect on lipid peroxidation of the rat liver homogenates while compounds 3, 4 and 7 in this series have the cryoprotective activity. The occurrence of cryoprotective activity for trolox suggests that the contribution of antioxidant function is also one of the factors determining the cryoprotective properties of catechol thioethers. It can be suggested that compounds 3 and 4 have the ability to integrate in plasma membrane of sperm cells and to stabilize it in the process of cryopreservation. The presence of acetylcysteine fragment increases cryoprotective activity of 7.

3. Conclusions

At the first stage, the electrooxidation of catechol thioether 1–9 results in the generation of *o*-benzoquinones. The second oxidation step for compounds involves a sulfide linker. In the case of compounds 6 and 9 the presence of amide group at the sulfur atom leads to appearance of

the third oxidation process at higher anodic potential. Catechol thioethers 4 and 6 with minimum values of E_{p}^{ox1} are compounds with potentially higher antioxidant activity. Compounds 3–5 and 7 contain ionize polar moieties. The deprotonation of these group shifts oxidation potentials in cathodic area and changes a mechanism of electrochemical oxidation. In case of compounds 3, 4, 7 electrooxidation occurs in two consecutive stages: the first oxidation is one-electron irreversible process with following deprotonation that leads to the formation of the stable 2-hydroxyphenoxy radical. These intermediates are reversibly oxidized at the potentials in the range from 1.06 to 1.08 V. The differences of the redox potentials of the initial and ionized forms for compounds 3–5 and 7 give the values of $\Delta G = -22.6$; -18.2 ; -21.7 ; $-16.8 \text{ kcal mol}^{-1}$, respectively. The deprotonation of polar group in these compounds leads to the growth of antioxidant activities.

Similar EC_{50} values for test compounds suggest the participation of the catechol fragment in the reaction with DPPH. The additional groups at the sulfur atom affect the values TEC_{50} that for compounds 1, 3–6, 9 exceeded the data for α -tocopherol. For example, it has been shown for compound 5 that the deprotonation promotes a 10-fold reduction in the index of TEC_{50} in comparison with the initial catechol thioether. In the course of the AAPH induced oxidative DNA damage, all tested compounds exhibit inhibitory activity. In this test we have shown that catechols thioethers 1, 4–9 demonstrate more pronounced inhibiting activity than the known antioxidant – trolox. The most active compounds are catechols 4 and 5 with phosphonic acid and cystamine hydrochloride residues.

The catechol thioethers 1, 4–6 and 9 can be marked out in the series of compounds because they do not show pronounced anti- or pro-oxidant effect on AAPH induced oxidation of GSH. This fact points out the absence of potential toxic effect of these compounds on GSH redox cycle. Unlike above mentioned compounds the thioethers 7 and 8, as well as 3,5-di-*tert*-butylcatechol have a remarkable promoting effect. Compound 1–4 and 7 possess pronounced antioxidant activity in the model reaction of lipid peroxidation of the rat (Wistar) liver homogenate. The combination of catechol fragment, the terminal polar group and hydrocarbon chain favors to appear the antioxidant activity. The presence of the *tert*-butyl groups in aromatic moiety reduces significantly iron chelation properties for most compounds in comparison with unsubstituted catechols. The chelation activity of catechol thioethers 3, 4, and 8 is mostly determined by the presence of polar terminal

groups.

The application of catechol thioethers as cryoprotective additives shows their perspective in the process of cryopreservation of the Russian sturgeon sperm. The modified cryomedium contributes to the safe of the viability of spermatozoa after cryopreservation and a decrease of concentration of cell membrane-permeating agents. Compounds **3**, **4** and **7** are the most suitable substances according to the time required for artificial reproduction.

These compounds in the majority of tests exhibit pronounced antioxidant properties, inhibit the reaction of oxidative DNA damage, lipid peroxidation process and possess iron(II) binding activity. The found properties of these substances are due to a combination of catechol fragment, bonded by a thioether linker with a long hydrocarbon chain and a terminal ionizable group or with a biologically relevant acetylcysteine residue.

4. Experimental

4.1. Synthesis and characterization

4.1.1. Materials and methods

All starting reagents were commercially available (3,5-di-*tert*-butyl-*o*-benzoquinone (Alfa Aesar, 98+%), L-glutathione reduced (GSH) (Sigma Aldrich, ≥97%), 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) (Aldrich, 97%), 11-mercaptoundecylphosphonic acid (Aldrich, 97%), 9-mercapto-1-nonanol (Aldrich, 96%), 11-mercaptoundecanoic acid (Aldrich, 95%), 4-acetamidothiophenol (Acros Organics, 95%), 4-mercapto-1-butanol (Aldrich, 95%), *N*-acetylcysteamine (Aldrich, 95%), 2-aminoethanethiol (Acros Organics, 98%), 1-thioglycerol (Sigma Aldrich, ≥99%), *N*-acetyl-L-cysteine (Aldrich, 99%), 2,2-diphenyl-1-picrylhydrazyl (Aldrich), thiobarbituric acid (≥98%, Sigma-Aldrich), deoxyribonucleic acid sodium salt (DNA) from salmon testes (Sigma), phosphate buffered saline (PBS) pH 7.4 (Sigma), 3,5-di-*tert*-butylcatechol (98%, Aldrich), (±)- α -tocopherol, (96+%, Sigma), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (97%, Aldrich), 5,5'-dithio-bis(2-nitrobenzoic acid) (DNTB) (98%) (Sigma), trichloroacetic acid (≥99%, Sigma-Aldrich) were used without further purification. Solvents were purified following standard procedures [107].

The IR spectra were recorded on an FSM-1201 FT-IR spectrometer in KBr pellets. The NMR spectra were measured in CDCl₃ or DMSO-*d*₆ on a Bruker Avance DPX-200, Bruker Avance HD 400 spectrometers with a frequency of 200 MHz (¹H) or 400 MHz (¹H) and 50 MHz (¹³C) or 100 MHz (¹³C) using Me₄Si as an internal standard. The chemical shift values are given in ppm with the reference to solvent and the coupling constants (J) are given in Hz. The elemental analysis was carried out on a Euro EA 3000 (C,H,N) and Analytik Jena multi EA 5000 (C,S,N,Cl) elemental analyzers. The UV-VIS spectra were recorded with a SF-103 spectrophotometer (in a range of 350–900 nm) at room temperature in the quartz cells with the thickness of 1.0 cm. Electrochemical studies were carried out using IPC-Pro potentiostat in three-electrode mode. The stationary glassy carbon (d = 2 mm) disk was used as working electrode; the auxiliary electrode was platinum-flag electrode. The reference electrode was Ag/AgCl/KCl (sat.) with watertight diaphragm. All measurements were carried out under argon. The samples were dissolved in the pre-deaerated solvent. The scan rate (ν) was 200 mV s⁻¹. The supporting electrolyte 0.1 M Bu₄NClO₄ (99%, electrochemical grade, Fluka) was dried in vacuum (48 h) at 50 °C. The concentration of compounds was 1–3 mmol. The compounds **1–9** show a linear dependency of the first anodic current peak with the square root of the scan rate ($\nu^{1/2}$), indicating a diffusion controlled system.

Microelectrolysis of compounds **1**, **3**, **4**, **6**, **7** (2 h) was performed at 25 °C under anaerobic condition (argon) in an undivided three-electrode cell (2 ml), in MeCN with 0.1 M Bu₄NClO₄. Platinum flag electrodes with the surface area of 0.7 cm² were applied. The concentration of compounds was 2 mmol. The electrolysis at controlled-potential was

performed at 1.20 V (**4**, **6**) or 1.30 V (**1**, **3**, **7**). A platinum wire and the standard Ag/AgCl reference electrode with watertight diaphragm were used.

4.1.2. X-ray diffraction

The X-ray diffraction data for **8-CH₃CN** were collected on a Bruker D8 Quest diffractometer (Mo-K α radiation, ω -scan technique, $\lambda = 0.71073 \text{ \AA}$). The intensity data were integrated by SAINT [108] program. SADABS [109,110] was used to perform area-detector scaling and absorption corrections. The structure was solved by dual-space [111] method and was refined on F² using all reflections with the SHELXTL package [112]. All non-hydrogen atoms were refined anisotropically. The H atoms were placed in calculated positions and refined in the "riding-rotating model". The H atoms of -OH groups were treated with different distances using AFIX 148 instruction. The thioglycerine fragment of molecule **8** is disordered over two positions (~0.54:0.46 ratio). In the crystal of **8-CH₃CN** a solvate molecule of acetonitrile was found to be disordered at the special position. The details of crystallographic, collection and refinement data for **8** are presented in Table S1. CCDC-1900814 contain the supplementary crystallographic data of **8-CH₃CN** for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

4.1.3. Synthesis of catechol thioethers **1–9**

The target compounds were prepared according to reported procedure [55] with some modifications. The appropriate thiol (3 mmol in 10 ml ethanol) was added dropwise to a solution of 3,5-di-*tert*-butyl-*o*-benzoquinone (2.0 mmol, 0.44 g) in 15 ml ethanol over a period of 4–5 h and the mixture was stirred at room temperature under argon till decolouration of the reaction media. The volume was concentrated under a reduced pressure to yield a crude solid which was recrystallized from acetonitrile.

4,6-Di-*tert*-butyl-3-((4-hydroxybutyl)thio)benzene-1,2-diol (**1**)

Yield: 0.38 g (58%). White powder. M.p. 94 °C. FT-IR (KBr) ν , cm⁻¹: 3372s, 2995m, 2986s, 2910w, 2870m, 1474s, 1391s, 1360m, 1291s, 1222s. ¹H NMR (400 MHz, CDCl₃) δ , ppm: 1.40 (s, 9H, tBu), 1.49 (s, 9H, tBu), 1.52 (s, 1H, OH), 1.65–1.83 (m, 4H, CH₂), 2.70 (t, *J* = 7.2 Hz, 2H, CH₂), 3.69 (t, *J* = 6.2 Hz, 2H, CH₂), 5.63 (s, 1H, OH), 6.91 (s, 1H, C₆H₁), 7.29 (s, 1H, OH). ¹³C NMR (100 MHz, CDCl₃) δ , ppm: 25.83, 29.27, 31.27, 31.84, 35.08, 36.81, 37.47, 62.33, 115.63, 115.93, 135.90, 140.58, 143.11, 145.21. Anal. calc. for C₁₈H₃₀O₃S: C, 66.22; H, 9.26; S, 9.82. Found: C, 66.18; H, 9.30; S, 9.87.

4,6-Di-*tert*-butyl-3-((9-hydroxynonyl)thio)benzene-1,2-diol (**2**)

Yield: 0.33 g (42%). White powder. M.p. 63–64 °C. FT-IR (KBr) ν , cm⁻¹: 3540s, 3492w, 3350m, 2995m, 2954s, 2924w, 2852m, 1483s, 1395s, 1361m, 1293m, 1242s. ¹H NMR (200 MHz, CDCl₃) δ , ppm: 1.25–1.37 (m, 10H, CH₂), 1.41 (s, 9H, tBu), 1.49 (s, 9H, tBu), 1.50–1.75 (m, 4H, CH₂), 2.65 (t, *J* = 7.4 Hz, 2H, CH₂), 3.65 (t, *J* = 6.5 Hz, 2H, CH₂), 3.75 (br.s, 3H, 3OH), 6.91 (s, 1H, C₆H₁ arom.). ¹³C NMR (50 MHz, CDCl₃) δ , ppm: 25.65, 28.93, 29.07, 29.27, 29.29, 29.34, 31.44, 32.67, 35.07, 36.80, 37.79, 63.00, 115.86, 115.91, 135.83, 140.55, 143.09, 146.20. Anal. calc. for C₂₃H₄₀O₃S: C, 69.65; H, 10.17; S, 8.08. Found: C, 69.70; H, 10.21; S, 8.12.

11-((4,6-Di-*tert*-butyl-2,3-dihydroxyphenyl)thio)undecanoic acid (**3**)

Yield: 0.63 g (71%). White powder. M.p. 68 °C. FT-IR (KBr) ν , cm⁻¹: 3525m, 3489m, 3325m, 2991m, 2953s, 2923m, 2856m, 1708s, 1492m, 1468m, 1397s, 1362m, 1293s, 1239s, 1198m. ¹H NMR (200 MHz, CDCl₃) δ , ppm: 1.25–1.37 (m, 12H, CH₂), 1.41 (s, 9H, tBu), 1.49 (s, 9H, tBu), 1.55–1.75 (m, 4H, CH₂), 2.36 (t, *J* = 7.4 Hz, 2H, CH₂), 2.65 (t, *J* = 7.4 Hz, 2H, CH₂), 5.61 (br.s, 1H, OH), 6.91 (s, 1H, C₆H₁ arom.), 7.29 (s, 1H, OH), 10.75 (br.s, 1H, COOH). ¹³C NMR (50 MHz, CDCl₃) δ , ppm: 24.64, 28.97, 28.99, 29.14, 29.28, 29.30, 29.38, 31.45, 34.02, 35.08, 36.81, 37.82, 115.87, 115.91, 135.82, 140.54, 143.11, 145.20, 180.04. Anal. calc. for C₂₅H₄₂O₄S: C, 68.45; H, 9.65; S, 7.1. Found: C, 68.40; H, 9.74; S, 7.37.

(11-((4,6-Di-tert-butyl-2,3-dihydroxyphenyl)thio)undecyl)phosphonic acid (4)

Yield: 0.36 g (37%). White powder. M.p. 97 °C. FT-IR (KBr) ν , cm^{-1} : 3543m, 3326m, 2995m, 2954m, 2924m, 2852s, 1486m, 1465m, 1397s, 1362m, 1235s, 1160m. ^1H NMR (400 MHz, DMSO- d_6) δ , ppm: 1.22 (br.s, 10H, 5 CH_2), 1.25–1.35 (m, 4H, 2 CH_2), 1.32 (s, 9H, tBu), 1.38–1.50 (m, 4H, 2 CH_2), 1.43 (s, 9H, tBu), 1.56 (qv, $J = 7.5$ Hz, 2H, CH_2), 2.65 (d, $J = 7.5$ Hz, 2H, SCH_2), 5.85 (br.s, 1H, OH), 6.75 (s, 1H, arom. C_6H_1), 7.99 (br.s, 1H, OH). ^{13}C NMR (100 MHz, DMSO- d_6) δ , ppm: 22.78, 26.98, 28.33, 28.53, 28.61, 28.69, 28.76, 29.00, 29.31, 30.08, 30.23, 31.26, 34.66, 35.64, 36.43, 114.81, 117.88, 134.78, 141.31, 142.32, 147.29. Anal. calc. for $\text{C}_{25}\text{H}_{45}\text{O}_5\text{PS}$: C, 61.45; H, 9.28; S, 6.56. Found: C, 61.42; H, 9.33; S, 6.60.

3-[(2-Aminoethyl)-sulfonyl]-4,6-di-tert-butylbenzene-1,2-diol hydrochloride (5) was synthesized according to previous report [36]. Yield: 0.21 g (40%). FT-IR (KBr) ν , cm^{-1} : 3350m, 2998m, 2958s, 291w, 2873m, 2600m, 1483m, 1332m, 1232m. Anal. calc. for $\text{C}_{16}\text{H}_{28}\text{NO}_2\text{S}\cdot\text{Cl}$: C, 57.55; H, 8.45; Cl, 10.62; N, 4.19; S, 9.60. Found: C, 56.75; H, 8.60; Cl, 10.1; N, 4.06; S, 9.71.

***N*-(2-((4,6-Di-tert-butyl-2,3-dihydroxyphenyl)thio)ethyl)acetamide (6)**

Yield: 0.39 g (57%). White powder. M.p. 167 °C. FT-IR (KBr) ν , cm^{-1} : 3384s, 3262m, 2997m, 2958s, 2904w, 2870m, 1645s, 1594m, 1486m, 1396s, 1231m. ^1H NMR (200 MHz, CDCl_3) δ , ppm: 1.39 (s, 9H, tBu), 1.47 (s, 9H, tBu), 2.00 (s, 3H, CH_3), 2.83 (t, $J = 6.3$ Hz, 2H, CH_2S), 3.54 (q, $J = 6.3$ Hz, 2H, CH_2), 5.70 (br.s, 1H, OH), 6.02 (br.t, $J = 6.2$ Hz, 1H, NH), 6.91 (s, 1H, arom. C_6H_1), 7.57 (br.s, 1H, OH). ^{13}C NMR (50 MHz, CDCl_3) δ , ppm: 23.13, 29.26, 31.52, 35.09, 36.84, 38.09, 39.55, 114.92, 116.23, 136.17, 140.91, 143.23, 145.61, 170.77. Anal. calc. for $\text{C}_{18}\text{H}_{29}\text{NO}_3\text{S}$: C, 63.68; H, 8.61; N, 4.13; S, 9.44. Found: C, 63.85; H, 8.90; N, 4.08; S, 9.52.

***N*-Acetyl-S-(4,6-di-tert-butyl-2,3-dihydroxyphenyl)cysteine (7)** was isolated as a racemic mixture of isomers. Yield: 0.47 g (61%). White powder. M.p. 193 °C. FT-IR (KBr) ν , cm^{-1} : 3520m, 3360m, 2995m, 2961s, 2913w, 2866m, 1736s, 1624s, 1540m, 1486m, 1395s, 1239s. ^1H NMR (200 MHz, CDCl_3) δ , ppm: 1.32 (s, 9H, tBu), 1.40 (s, 9H, tBu), 1.86 (s, 3H, CH_3), 2.86–3.05 (m, 2H, CH_2), 4.39 (m, 1H, CH), 6.77 (s, 1H, C_6H_1), 8.11 (s, 1H, OH), 8.37 (d, $J = 7.4$ Hz, 1H, NH), 8.39 (br. s, 1H, OH), 12.84 (br.s, 1H, COOH). ^{13}C NMR (50 MHz, CDCl_3) δ , ppm: 22.19, 29.12, 31.08, 34.49, 36.17, 37.35, 51.67, 114.80, 116.61, 135.20, 141.56, 142.15, 147.17, 169.28, 171.80. Anal. calc. for $\text{C}_{19}\text{H}_{29}\text{NO}_5\text{S}$: C, 59.51; H, 7.62; N, 3.65; S, 8.36. Found: C, 59.53; H, 7.80; N, 3.59; S, 8.41.

4,6-Di-tert-butyl-3-((2,3-dihydroxypropyl)thio)benzene-1,2-diol (8) was isolated as a racemic mixture of isomers. Yield: 0.35 g (54%). White powder. M.p. 110 °C. FT-IR (KBr) ν , cm^{-1} : 3394s, 2997m, 2960s, 2910w, 2870m, 1484m, 1396m, 1366m, 1239m, 1170w. ^1H NMR (400 MHz, DMSO- d_6) δ , ppm: 1.32 (s, 9H, tBu), 1.45 (s, 9H, tBu), 2.60 (dd, $J = 8.1$ Hz, $J = 12.8$ Hz, 1H, CH_2), 2.85 (dd, $J = 3.2$ Hz, $J = 12.5$ Hz, 1H, CH_2), 3.28 (m, 2H, SCH_2), 3.63–3.70 (m, 1H, CH), 4.79 (br.s, 1H, OH), 5.85 (br.s, 1H, OH), 6.75 (s, 1H, arom. C_6H_1), 8.12 (br.s, 1H, OH), 8.84 (br.s, 1H, OH). ^{13}C NMR (100 MHz, DMSO- d_6) δ , ppm: 29.25, 31.45, 34.67, 36.43, 42.27, 64.58, 69.96, 114.50, 117.34, 134.78, 141.67, 142.15, 147.35. Anal. calc. for $\text{C}_{17}\text{H}_{28}\text{O}_4\text{S}$: C, 62.16; H, 8.59; S, 9.76. Found: C, 62.21; H, 8.72; S, 9.80.

***N*-(4-((4,6-Di-tert-butyl-2,3-dihydroxyphenyl)thio)phenyl)acetamide (9)**

Yield: 0.40 g (52%). White powder. M.p. 215 °C. FT-IR (KBr) ν , cm^{-1} : 3533 s, 3407 s, 3283 m, 3184 m, 2961 s, 2910 m, 2873 m, 1671 s, 1531 m, 1492 m, 1398 s, 1235 m. ^1H NMR (200 MHz, CDCl_3) δ , ppm: 1.42 (s, 9H, tBu), 1.44 (s, 9H, tBu), 2.11 (s, 3H, CH_3), 5.37 (br.s, 1H, OH), 6.87 (d, $J = 8.6$ Hz, 2H, C_6H_4 arom.), 6.89 (s, 1H, arom. C_6H_1), 7.36 (d, $J = 8.6$ Hz, 2H, C_6H_4 arom.), 7.52 (br.s, 1H, OH). ^{13}C NMR (50 MHz, CDCl_3) δ , ppm: 23.13, 29.26, 31.52, 35.09, 36.84, 38.09, 39.55, 114.92, 116.23, 136.17, 140.91, 143.23, 145.61, 170.77. Anal. calc. for $\text{C}_{22}\text{H}_{29}\text{NO}_3\text{S}$: C, 68.18; H, 7.54; N, 3.61; S, 8.27. Found: C, 68.24; H, 7.70; N, 3.44; S, 8.32.

4.2. Antioxidant activity assay

4.2.1. DPPH radical scavenging activity assay

DPPH radical scavenging activity was performed according to the method of Bondet et al. [113] with some modification. A MeCN solution ($C_0 = 50 \mu\text{mol}$) of the radical DPPH was prepared daily and protected from light. An absorbance was recorded to check the stability of the radical throughout the time of analysis. For each antioxidant, different concentrations were tested (expressed as the number of moles of antioxidant/mole DPPH). The antioxidant solution in acetonitrile (0.05 ml) was added to 2 ml of a 50 μmol solution of DPPH in MeCN. The decrease in absorbance was determined at 517 nm at 0, 1, 2, 3, 4, 5 min and every next 5 min until the reaction reached a plateau at room temperature.

The parameter EC_{50} is the concentration of an antioxidant necessary for decreasing the amount of DPPH radical by 50% of the initial value. The value of EC_{50} was determined at equilibration in 0–30 min depending on the compound used. To determine EC_{50} , the plot of the residual concentration of the stable radical vs molar ratio, expressed as the number of moles of the antioxidant per 1 mol of the stable radical, was constructed. The parameter (n_{DPPH}) is the number of molecules of converted DPPH radical per one molecule of the compound ($n_{\text{DPPH}} = C_0 / (2 \times \text{EC}_{50})$, where C_0 is the initial concentration of radical). TEC_{50} is the time of achievement of an equilibrium state at the antioxidant concentration equal to EC_{50} . The antiradical efficiency (AE) was determined with the equation $\text{AE} = 1 / (\text{EC}_{50} \times \text{TEC}_{50})$ [114]. To determine EC_{50} , the plot of the residual concentration of the stable radical vs molar ratio, expressed as the number of moles of the antioxidant per 1 mol of the stable radical, was constructed. All experiments were performed in triplicate at room temperature.

4.2.2. AAPH-induced oxidation of DNA assay

The deoxyribonucleic acid sodium salt (DNA) from salmon testes, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) and thio-barbituric acid (TBA) were purchased from Sigma and used without purification. AAPH-induced oxidation of DNA was carried out following the known method with a little modifications [82,83]. Briefly, 0.02 ml of stock solutions of the research compounds in DMSO were added to PBS (pH 7.4) solutions of AAPH and DNA, in which the final concentration of DNA and AAPH was kept 2.5 mg ml^{-1} and 40 mmol, respectively. Then, the above solution was dispatched into test tubes with 2.0 ml solution contained in every one. All the tubes were incubated in a water bath during 2.5 h at 37 °C to initiate the oxidation. Test tubes were taken out and cooled immediately, to which 1.0 ml of TBA (1.00 g TBA and 0.40 g NaOH dissolved in 100 ml PBS (pH 7.4)) and 1.0 ml of 3.0% trichloroacetic acid aqueous solution was added. The tubes were heated in a boiling water bath for 15 min. After cooling, 2.0 ml of *n*-butanol was added and shaken vigorously to extract TBARS. The absorbance of *n*-butanol layer was measured at 535 nm. Finally, the average value of three absorbance data (within 10% experimental error) was determined. The absorbance in the blank experiment and in the presence of the compounds (catechol thioethers 1–6, 2-((4,6-di-tert-butyl-2,3-dihydroxyphenyl)thio)acetic acid (7), 3,5-di-tert-butylcatechol (CatH₂) and trolox were assigned as A_0 and A_i . The antioxidant effect of the tested compounds (in percentage of forming TBARS) on the AAPH induced oxidation of DNA was expressed by $A_i/A_0 \times 100$.

4.2.3. AAPH induced glutathione depletion

The AAPH-induced depletion of GSH was carried out following the known method [67]. A solution of GSH (0.1 mmol) in PBS (pH 7.4) was incubated with 10 mmol AAPH in PBS at 37 °C for 3.0 h. Compound 1–7, CatH₂ and trolox (0.02 ml in ethanol solution) were added before the addition of AAPH. A final concentration of the compounds in the reaction mixture was 0.1 mmol. Three samples with volume of 0.5 ml were collected after each hour of the incubation, diluted with PBS to 1.5 ml and 0.1 ml of DNTB (5,5'-dithiobis (2-nitrobenzoic acid)

0.0396 g in 10 ml ethanol) was added. The absorbance at 412 nm was recorded against a blank consisting of 2 ml of PBS and 0.1 ml of DTNB. A residual glutathione concentration was evaluated by the analytical curve which was produced using standard GSH solutions in the range from 5 to 100 μ M concentrations.

4.2.4. Lipid peroxidation of rat liver homogenate

On the day of the experiment, adult Wistar male rats fasted overnight were euthanized in a CO₂- chamber followed by decapitation. The procedure was in compliance with the Good Laboratory Practice and Guidelines for Animal Experiments at Leprosy Research Institute, Ministry of Health of the Russian Federation. Homogenates of liver brain (1:10 w/v) were prepared immediately before use in phosphate buffer, pH 7.4 media using a homogeniser. The extent of lipid peroxidation was estimated by using the thiobarbituric acid reactive substances (TBARS) assay [85].

The influence of compounds 1–9 and CatH₂ on lipid peroxidation of the rat liver homogenates was carried out at 37 °C for 3 h in phosphate buffer (pH 7.4) in the presence or absence of compounds or vehicle (DMSO). The concentration of compounds in the medium was 0.1 mM. The level of lipid peroxidation was measured in the rat (Wistar) liver homogenates as a non-enzymatic process by the addition of ascorbic acid and (NH₄)₂Fe(SO₄)₂. The homogenate was divided into following experimental groups: one control homogenate and ten samples of homogenate with the addition of compounds 1–9, CatH₂. Solutions of ascorbic acid (0.1 ml, 2.6 mmol), Fe(NH₄)₂(SO₄)₂ (0.1 ml, 0.4 mM) and trichloroacetic acid (1 ml, 40%) were injected into the probe. The test tubes were incubated at 37 °C and then probes were centrifuged (10 min at 3000g). The supernatants (2 ml) were transferred to new test tubes and mixed with 1 ml TBA solution (0.8%). The probes were heated at 95 °C for 10 min, cooled at 4 °C, then probes centrifuged (10 min at 10 000g) and the supernatants absorbance was measured at 535 nm at the SF-103 spectrophotometer. All the experiments were performed using four independent experiments. Data are normalized to control probe with oxidant. Preliminary experiments were done in the absence of compounds interaction with thiobarbituric acid. The values are expressed as mean \pm SD.

4.2.5. Iron (II) chelation activity

The iron(II) chelation ability of catechol thioethers was evaluated by the spectrophotometric ferrozine method measuring the absorbance of the [Fe(ferrozine)₃]²⁺ complex at 562 nm [115]. Ammonium iron (II) sulphate in ammonium acetate buffer (pH 6.7) was used. All solutions were prepared in a plastic material in order to avoid iron contamination. A solution of the test compound (0.5 mmol) 200 μ l in ethanol and ammonium iron (II) sulphate (0.1 ml) in ammonium acetate (200 μ mol) were added, incubated for 10 min and the absorbance was read at 562 nm. An aqueous 5 mmol solution of ferrozine was freshly prepared and then added (0.2 ml) to each test tube (100 μ M final concentration). Addition of ferrozine solution was followed by a new incubation at 37 °C for a 10 min period, and measurement of [Fe(ferrozine)₃]²⁺ complex absorbance at 562 nm. In the control researched compounds solution was replaced by blank test tubes with ethanol. The absorbance of the first reading was subtracted to the final values to discard any absorbance due to the test compounds. EDTA used as reference compound. It was found that EDTA chelates practically all available iron and inhibits the formation of the colored ferrozine-Fe(II) complex. The percentage of iron(II) chelation (% Fe(II) chelation) values was expressed as mean \pm standard deviation of five independent experiments.

4.3. Cryoprotective activity of thioethers in cryopreservation of Russian sturgeon (*Acipenser gueldenstaedti*) sperm

4.3.1. Sperm collection

Russian sturgeon, received from sturgeon hatchery

(Aleksandrovsky) was used in the study. Tests were carried out during the period from 2017 to 2018. Every year naturally mature fish (8–10 male fish, weighing 65 kg individually, 10–12 years old) were obtained during peak stage of the spawning season (from the end of April to the middle of May). The sperm was collected by the hypophysial injections method. Maturation and spawning were induced with an intramuscular injection of 4 mg/kg of the LH-RHa (Luteinizing Hormone – Releasing Hormone Ethylamide) at water temperature 9 °C. The response time to the hormone injection was approximately 12 h. The sperm was collected by catheter. Sperm was stripped from three individuals of each species by gentle abdominal massage, taking care to avoid contamination with blood, urine or faeces.

The percentage of motile sperm cells was estimated subjectively using binocular microscope Micmed-5 with video-eyepiece HB-200 (LOMO, Russia) with \times 800 total magnifications after addition of river water as an activating solution to the post-thaw sperm at a ratio of 1:250, and the fresh sperm was activated at a ratio of 1:1000. For cryopreservation we used the sperm samples showing less than 90% motility were discarded. Motility time (total period of sperm movement, sec) was defined as the time from the activation to the termination of movement using stopwatch. Duration time of sperm activation was measured by the same operator for three times.

4.3.2. Sperm cryopreservation

Thioethers were dissolved in 0.2 ml DMSO a then added into in the modified Steins cryomedium (130 mmol NaCl, 5 mmol KCl, 20 mM NaHCO₃, 5.5 mmol sucrose, 12.5% egg yolk) to make a stock solution with the sperm at a compound concentration of 0.1 mM. Sperm cryopreservation was carried out according to the methods of Tsvetkova et al. [116]. The sperm diluted with the cryoprotective medium was distributed in labeled 1.5 ml Eppendorf tubes and placed in the refrigerator for 40 min for equilibration [117]. The ratio of sperm and cryomedium was 1:1. Freezing temperature was measured with an electronic thermometer. After equilibration deep freezing was performed in three stages: from 5 °C to –15 °C with rate 2–5 °C/min (freezing time 2–5 min); from –15 °C to –70 °C with rate 20–25 °C/min (freezing time about 3 min); deep freezing in liquid nitrogen. Thawing of sperm was performed in a water bath during 30–40 s at a temperature of 38–40 °C. Sperm survival was evaluated using the criteria of the post thaw spermatozoa motility.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioorg.2019.103003>.

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