Synthesis and study of new phenolic antioxidants with nitroaromatic and heterocyclic substituents

O. V. Mikhalev, D. B. Shpakovsky, Yu. A. Gracheva, T. A. Antonenko, D. V. Albov, L. A. Aslanov, and E. R. Milaeva*

M. V. Lomonosov Moscow State University,
1, Build. 3, Leninskie Gory, 119991 Moscow, Russian Federation.
Fax: +7 (495) 932 8846. E-mail: mov@med.chem.msu.ru

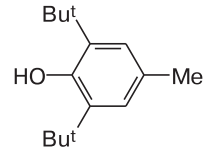
New polyfunctional aromatic, nitroaromatic, and heterocyclic compounds linked to the 2,6-di-*tert*-butylphenol moiety *via* —NH—, —C(O)NH—, —S—, or—C=N— spacers were synthesized. These structures provide intramolecular charge transfer (ICT) and exhibit antioxidant activity. The structures of the new compounds were established by X-ray diffraction. The novel compounds were evaluated for antioxidant activity using the DPPH assay. The presence of the 2,4,6-trinitrophenyl moiety in combination with the —NH— spacer leads to a considerable increase in the antioxidant activity of 2,6-di-*tert*-butylphenols. These compounds are also weak lipoxygenase inhibitors. The results of this study provide an opportunity to search for new types of antioxidants with ICT.

Key words: 2,6-di-*tert*-butylphenol, antioxidant activity, DPPH assay, intramolecular charge transfer, X-ray diffraction analysis.

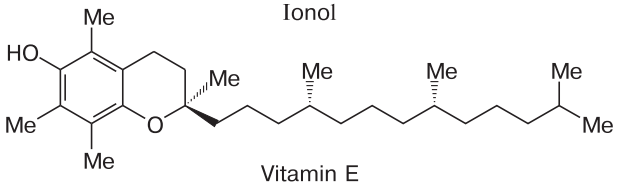
Oxidative destruction plays a significant role in biochemical processes, oxidative stress being a cause of many body pathologies. Natural phenolic products are involved in the antioxidant defense system in the living organism.[1] The corresponding phenoxyl radicals are key intermediates responsible for antioxidant activity of such compounds that are able to interact with reactive free radicals.

2,6-Dialkylphenols mimic natural systems, in particular, tocopherols (*e.g.*, vitamin E), tyrosine, or thyroxine. These compounds have low toxicity and, consequently, are of interest as biologically active compounds for medicine and also as industrially used inhibitors of radical chain oxidation of organic molecules.[2] Sterically hindered phenols also meet requirements for effective antioxidants and serve as inhibitors of oxidation of various organic substrates.[3] 2,6-Di-*tert*-butyl-4-methylphenol (ionol, commercial index E321) is the most widely utilized synthetic antioxidant. The efficacy of 2,6-di-*tert*-butylphenols as inhibitors of oxidative destruction of hydrocarbons is determined by the nature of *ortho*-alkyl groups and the group in the *para*-position of the aromatic ring, which affects the stability of the phenoxyl radicals generated during oxidation. Groups of different nature and having different structures, including metal-containing moieties, can serve as substitutes at the *para*-position.[4—6] A search for new metal-containing phenolic antioxidants for prevention of oxidative stress-related carcinogenesis in the cell is currently underway.[5,7]

Various N,S,O-containing amine and amide derivatives bearing the 2,6-di-*tert*-butylphenol moiety belong to



Ionol



Vitamin E

cyclooxygenase inhibitors and exhibit pronounced anti-inflammatory activity.[8]

The living cells include nucleotides, porphyrins, flavins, quinones, and peptides as essential low-molecular-weight components, all of which being characterized by a relatively low electronic excitation energy, low ionization potentials, and high electron affinity. Therefore, these reactive compounds can be involved in charge transfer processes.

The energy transfer in the electron transport chain is associated with the formation of charge transfer complexes between purines that act as electron donors and riboflavin (vitamin B_2) as an electron acceptor.[9]

Organic compounds with intramolecular charge transfer (CICT), comprising donor and acceptor moieties separated by a group of atoms or a spacer, are of considerable interest due to specific physicochemical, optical, and biological properties. The spatial arrangement of the interacting

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 4, pp. 0712—0720, April, 2018.

donor and acceptor groups and the character of charge transfer are determined by the spacer (bridge) type and length. The following three cases can be distinguished: (1) the acceptor and the donor are not involved in conjugation, (2) the spacer is involved in conjugation with the acceptor or the donor, and (3) the donor is conjugated with the acceptor.[10]

Organic compounds with intramolecular charge transfer can be classified according to the number of atoms comprising the spacer (one-atom, two-atom, *etc.*). Derivatives of the picryl series containing donor groups of different nature and donor strength were synthesized.[11—13] An example is a compound containing the aminomethoxyquinoline moiety, which is present in the known antimalarial drugs, such as pentaquine and primaquine.[14] In picryl derivatives, the overlap of π-orbitals of benzene rings along the conjugation chain is insignificant and depends on the angle of rotation of the donor ring plane with respect to the acceptor moiety. In any electronic donor-acceptor system, the electron transfer can be considered as the electron density redistribution from the highest occupied molecular orbital (HOMO) of the donor moiety to the lowest unoccupied molecular orbital (LUMO) of the acceptor. Intramolecular charge-transfer complexes include molecules, in which HOMO and LUMO are strictly localizes on the corresponding donor and acceptor moieties of the molecule, with no direct conjugation between their π-electron systems, for example, if the donor and the acceptor are separated by at least one sp^3-hybridized carbon atom.[12,15] The steric factor plays an important role in the formation of CICTs. Some of such compounds are characterized by the alternating arrangement of the donor and acceptor rings in parallel planes.[16] The introduction of substituents, which hinder coplanarity of the donor and acceptor rings (alkyl substituents in the *ortho* position), decreases the constant for the formation of 1 : 1 charge transfer complexes. Molecular CICTs can be used as ligands for the preparation of coordination compounds that hold promise in studying metal-containing donor-acceptor systems.

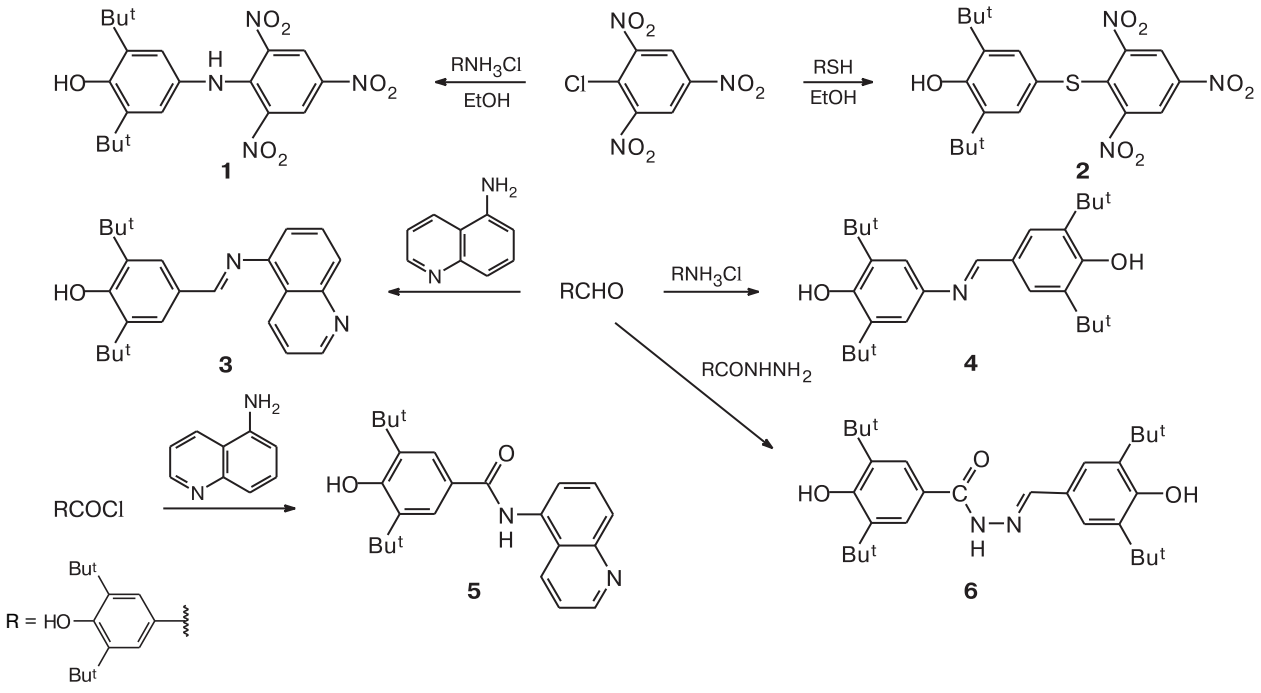
The construction of polyfunctional antioxidants is a new promising approach to search for physiologically active compounds, because a combination of the antioxidant 2,6-dialkylphenol group and different functional moieties allows the targeted design of antioxidants with controlled activity.

The goal of this study is to find new antioxidants. For this purpose, we synthesized compounds **1**—**6** based on 2,6-di-*tert*-butylphenol (Scheme 1). Antioxidant activity of compounds **1**—**6** was evaluated by spectrophotometry using the phenolic hydrogen transfer to the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the enzymatic oxidation of linoleic acid by the enzyme lipoxygenase.

Results and Discussion

The known nucleophilic substitution of halogen in activated 2,4,6-trinitrochlorobenzene in the reactions with *N*- or *S*-nucleophiles affords arylation products, *viz.*, derivatives **1** and **2** containing the picryl moiety. We synthesized compound **1**[17] by a modified procedure, which made it possible to substantially increase the yield of the target product and decrease the reaction time.

Scheme 1



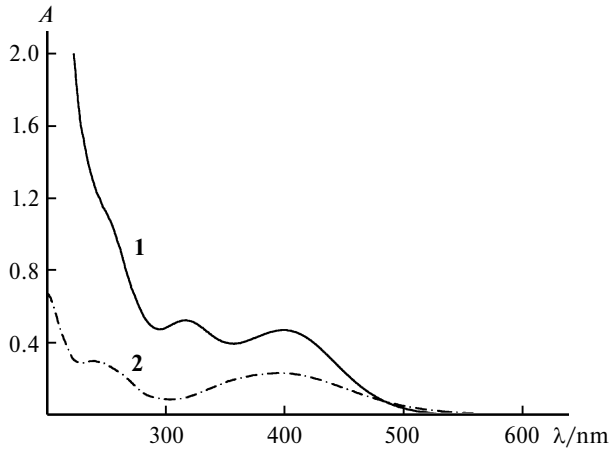


Fig. 1. Absorption spectra of compounds **1** (20 μmol L^{-1}) and **2** (100 μmol L^{-1}) in MeCN.

With the aim of varying the spacer type, we synthesized Schiff bases **3** and **4** with the RCH=N group (R = 3,5-di-*tert*-butyl-4-hydroxyphenyl). The reaction of 5-aminoquinoline with 3,5-di-*tert*-butyl-4-hydroxybenzoyl chloride in CH_2Cl_2 in the presence of NEt_3 gave the acylation product, amide **5** containing the phenol group and the —CONH— spacer. 3,5-Di-*tert*-butyl-4-hydroxybenzohydrazide was used to synthesize derivative **6**. Air- and solution-stable compounds **1**—**6** were characterized by IR, UV, and NMR spectroscopy and elemental analysis.

Compounds **1**—**4** are brightly colored substances, as opposed to colorless derivatives **5** and **6** with the —CONH— spacer. Apart from the absorption band of nitrobenzene that is often masked by absorption of the solvent, the electronic absorption spectra of compounds **1** and **2** show bands assigned to charge transfer from the donor aryl moiety to the acceptor picryl group *via* a spacer (—NH—) and also a band arising from through-space intramolecular charge transfer (ICT) in a stacking interaction mode between one of the *ortho*-nitro groups of the acceptor picryl moiety and the π-electron system of the donor.[12,14,18]

The electronic absorption spectra of all the synthesized compounds are characterized by a long-wavelength maximum undergoing a bathochromic shift with increasing donor strength of the aryl moiety. The long-wavelength maximum at 360—390 nm should be assigned to the charge transfer from the donor moiety of the molecule to the planar *ortho*-nitro group. Then, with increasing excitation energy, the *para*-transition should be observed, appearing as an inflection point or a maximum at 330—340 nm. Since compounds **1** and **2** are quite intensely colored, which is an evidence of a long-wavelength charge transfer band, we compared the electron transfer energies (E_{ICT}) for related compounds of a picryl series. These energies can be evaluated by the equation $E_{ICT} = 1239.8/\lambda_{max}$ (see Ref. 19). For compound **1** (λ_{max} = 397 nm) containing the—NH— spacer, E_{ICT} is 3.12 eV; for compound **2** (λ_{max} = 402 nm), 3.08 eV (Fig. 1).

The molecular structures of compounds **1**, **2**, **3**, and **5** were determined by X-ray diffraction. The crystallographic characteristics and selected bond lengths and bond angles for these compounds are summarized in Tables 1 and 2. The molecular structures of compounds **1** and **2** are shown in Fig. 2. In structure **1**, the N(1) atom forms the intra-

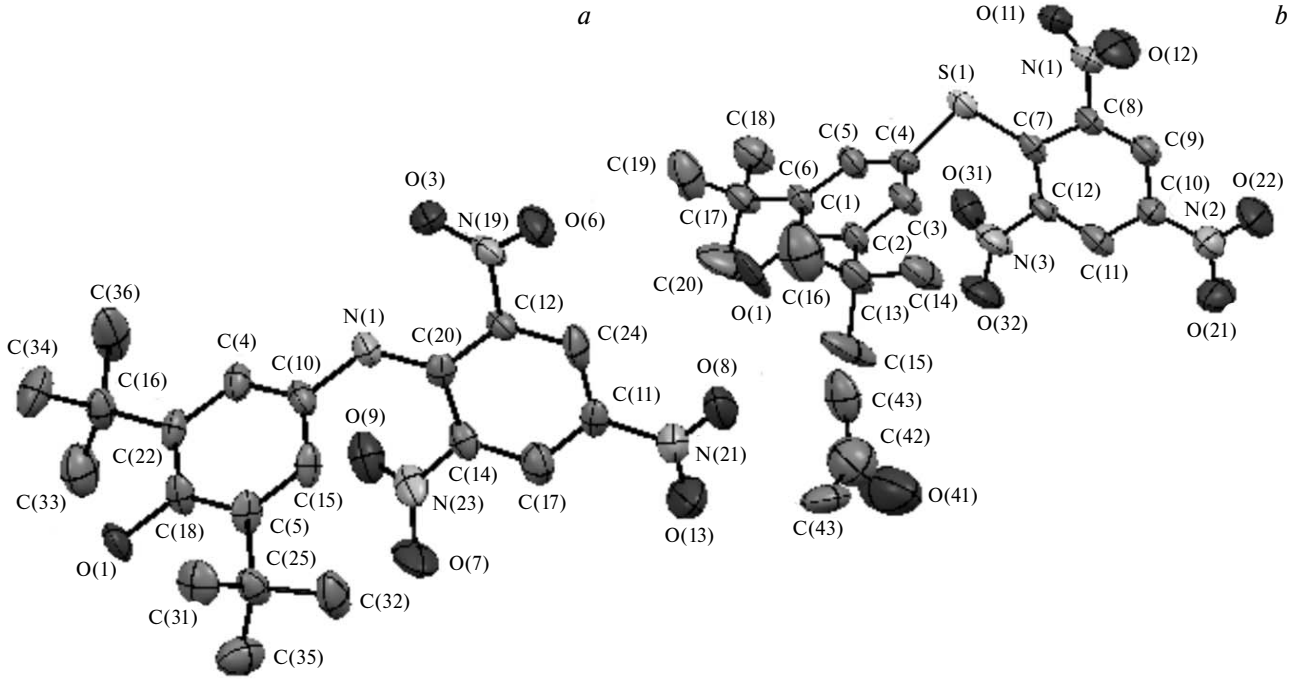


Fig. 2. Molecular structures of compounds **1** (*a*) and **2** (*b*) with thermal ellipsoids drawn at 50% and 35% probability level for **1** and **2**, respectively. Hydrogen atoms are not shown.

Table 1. Crystallographic characteristics of compounds **1—3** and solvates **5** · MeCN and **5** · Me_2CO

Compound	**1**	**2**	**3**	**5**·MeCN	**5**·Me_2CO
Molecular formula	$C_{20}H_{24}N_4O_7$	$C_{23}H_{23}N_3O_7S$	$C_{24}H_{28}N_2O$	$C_{24}H_{28}N_2O_2$ · · MeCN	$C_{24}H_{28}N_2O_2$ · · Me_2CO
Molecular weight	432.43	485.53	360.48	417.54	434.56
Crystal system	Monoclinic	Monoclinic	Triclinic	Tricli nic	Triclinic
Space group	$P2_1/n$	$C2/c$	$P\bar{1}$	$P\bar{1}$	$P\bar{1}$
a/Å	5.7884(5)	34.836(4)	6.5791(10)	9.046(3)	9.4903(4)
b/Å	11.6766(6)	7.5321(6)	11.4699(18)	10.164(3)	9.8061(5)
c/Å	32.854(2)	18.923(2)	14.432(3)	13.588(4)	13.6912(6)
α/deg	90.00	90.00	85.177(14)	93.01(2)	102.941(4)
β/deg	91.292(6)	102.747(10)	80.374(13)	99.31(2)	92.855(4)
γ/deg	90.00	90.00	83.385(13)	102.99(2)	101.787(4)
T/K	293(2)	293(2)	293(2)	293(2)	293(2)
V/Å^3	2220.0(3)	4842.8(9)	1064.2(3)	1196.2(6)	1209.48(10)
Z	4	1	2	2	2
d_{calc}/g cm^{-3}	1.258	1.352	1.125	1.159	1.193
Radiation	CuK_α	MoK_α	MoK_α	MoK_α	CuK_α
$\mu(K_\alpha)$/mm^{-1}	0.575	0.183	0.069	0.074	0.613
θ-Angle range/deg	4—68.8	2—25.5	3—30	3.5—28	5—70
h, k, l ranges	$-6 \leqslant h \leqslant 3$, $-13 \leqslant k \leqslant 13$, $-36 \leqslant l \leqslant 39$	$-42 \leqslant h \leqslant 42$, $-6 \leqslant k \leqslant 9$, $-22 \leqslant l \leqslant 20$	$-9 \leqslant h \leqslant 3$, $-16 \leqslant k \leqslant 14$, $-20 \leqslant l \leqslant 20$	$-11 \leqslant h \leqslant 11$, $-13 \leqslant k \leqslant 13$, $-8 \leqslant l \leqslant 17$	$-11 \leqslant h \leqslant 11$, $-11 \leqslant k \leqslant 11$, $0 \leqslant l \leqslant 16$
Crystal size/mm	0.1×0.1×0.1	0.2×0.2×0.2	0.3×0.2×0.2	0.2×0.2×0.2	0.3×0.2×0.2
Total number of reflections	3088	4421	6090	5468	4158
Number of unique reflections	1803	1051	683	1306	2463
GOOF	2.304	0.846	0.414	0.552	0.850
R_1/wR_2 ($I \geqslant 2\sigma(I)$)	0.1712/0.3844	0.1491/0.3270	0.0363/0.0810	0.0402/0.0676	0.0834/0.0834
Residual electron density ($\Delta\rho_{max}/\Delta\rho_{min}$)/e Å^{-3}	0.591/−0.654	0.694/−0.548	0.095/−0.126	0.112/−0.134	0.178/−0.171

molecular N(1)—H(1)...O(1) and N(1)—H(1)...O(9) hydrogen bonds with oxygen atoms of two nitro groups (H(1)...O(1) and H(1)...O(9) are 2.657 and 3.543 Å, respectively). In structure **2**, the S(1) atom is linked to adjacent nitro oxygen atoms through the intermolecular S(1)...O(11) contact (2.744 Å) and two short intermolecular S(1)...O(11) and S(1)...O(31) contacts (3.117 and 3.110 Å, respectively). The O(11) and O(31) atoms form short intermolecular contacts with one another (3.039 Å). The phenol oxygen atom is involved in the intermolecular O(1)—H(1)...O(41) hydrogen bond (2.331 Å) with the oxygen atom of the acetone molecule.

Compound **3** (Fig. 3) was found to be a covalent monomer stabilized by the intermolecular O(1)—H(1)...N(1) hydrogen bond (H(1)...N(1), 2.284 Å).

Compound **5** showed a tendency to form solvates. The structures of the solvates with the solvents acetonitrile (**5** · MeCN) and acetone (**5** · Me_2CO) were determined by X-ray diffraction. In the structure of **5** · MeCN (see Fig. 3), there are the intermolecular N(11)—H(11)...N(31) and O(1)—H(1)...N(1) hydrogen bonds with the H(11)...N(31) and H(1)...N(1) distances of 2.107 and 2.275 Å, respectively. The structure of **5** · Me_2CO is stabilized by the intermolecular N(11)—H(11)...O(3) and O(1)—H(1)...N(1) hydrogen bonds with the H(11)...O(3) and H(1)...N(1), distances of 2.139 and 2.193 Å, respectively.

Evaluation of antioxidant activity. Due to the presence of the 2,6-di-*tert*-butylphenol moiety, the synthesized compounds would be expected to exhibit antioxidant activ-

Scheme 2

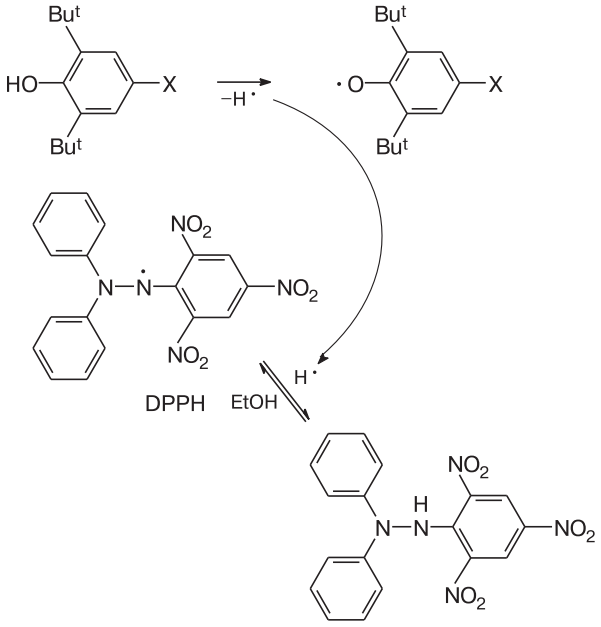


Table 2. Selected bond lengths and bond angles in compounds **1**—**3** and solvates **5** · MeCN and **5** · Me_2CO

Bond	*d*/Å	Angle	ω/deg
		Compound **1**	
N(1)—C(20)	1.352(11)	C(20)—N(1)—C(10)	128.1(9)
N(1)—C(10)	1.417(11)	C(4)—C(10)—N(1)	121.9(10)
O(1)—C(18)	1.389(10)	N(1)—C(10)—C(15)	118.0(8)
O(3)—N(19)	1.241(10)	N(1)—C(20)—C(12)	123.4(10)
O(6)—N(19)	1.198(10)	N(1)—C(20)—C(14)	122.0(9)
O(7)—N(23)	1.287(14)	O(1)—C(18)—C(22)	118.0(8)
O(9)—N(23)	1.210(12)	O(6)—N(19)—O(3)	124.3(9)
O(8)—N(21)	1.194(11)	O(8)—N(21)—O(13)	124.4(10)
O(13)—N(21)	1.230(13)	O(9)—N(23)—O(7)	121.5(10)
C(11)—N(21)	1.448(12)	C(5)—C(18)—O(1)	120.2(9)
C(12)—N(19)	1.468(13)	O(13)—N(21)—C(11)	115.7(9)
C(14)—N(23)	1.408(12)	O(9)—N(23)—C(14)	120.5(11)
		Compound **2**	
S(1)—C(7)	1.738(7)	C(7)—S(1)—C(4)	104.1(3)
S(1)—C(4)	1.750(5)	C(3)—C(4)—S(1)	121.9(5)
O(1)—C(1)	1.384(7)	C(5)—C(4)—S(1)	117.4(5)
C(8)—N(1)	1.436(8)	C(12)—C(7)—S(1)	128.2(4)
C(10)—N(2)	1.405(9)	C(8)—C(7)—S(1)	120.1(5)
C(12)—N(3)	1.452(8)	C(6)—C(1)—O(1)	117.9(6)
N(1)—O(11)	1.210(8)	O(1)—C(1)—C(2)	118.0(6)
N(1)—O(12)	1.202(8)	O(11)—N(1)—O(12)	22.5(6)
N(2)—O(21)	1.232(7)	O(21)—N(2)—O(22)	19.8(7)
N(2)—O(22)	1.268(7)	O(32)—N(3)—O(31)	24.8(6)
N(3)—O(32)	1.172(7)	N(1)—C(8)—C(7)	119.1(6)
N(3)—O(31)	1.234(9)	N(2)—C(10)—C(11)	20.0(6)
		Compound **3**	
N(1)—C(2)	1.302(6)	C(2)—N(1)—C(9)	116.7(5)
N(1)—C(9)	1.388(6)	N(1)—C(2)—C(3)	125.3(6)
C(5)—N(11)	1.424(6)	N(11)—C(5)—C(10)	117.4(5)
N(11)—C(12)	1.271(5)	N(1)—C(9)—C(8)	117.8(5)
C(16)—O(1)	1.369(6)	N(1)—C(9)—C(10)	121.7(5)
O(1)—H(1)	0.75(5)	N(11)—C(12)—C(13)	124.2(5)
C(12)—C(13)	1.458(6)	C(16)—O(1)—H(1)	117.0(4)
C(5)—C(10)	1.431(6)		
C(5)—C(6)	1.352(6)		
		Compound **5** · MeCN	
N(1)—C(2)	1.319(4)	C(2)—N(1)—C(9)	116.8(3)
N(1)—C(9)	1.374(3)	N(1)—C(2)—C(3)	124.7(3)
C(5)—N(11)	1.435(4)	C(6)—C(5)—N(11)	119.2(3)
N(11)—C(12)	1.367(3)	C(10)—C(5)—N(11)	119.8(3)
N(11)—H(11)	0.920(2)	N(1)—C(9)—C(8)	118.0(3)
C(12)—O(2)	1.229(3)	N(1)—C(9)—C(10)	122.8(3)
C(16)—O(1)	1.370(3)	O(2)—C(12)—N(11)	120.2(3)
O(1)—H(1)	0.780(3)	C(16)—O(1)—H(1)	119.0(2)
N(31)—C(32)	1.103(4)		
		Compound **5** · Me_2CO	
N(1)—C(2)	1.311(3)	C(2)—N(1)—C(9)	118.53(19)
N(1)—C(9)	1.374(3)	N(1)—C(2)—C(3)	124.0(2)
C(5)—N(11)	1.423(2)	C(6)—C(5)—N(11)	120.0(2)
N(11)—C(12)	1.353(3)	C(10)—C(5)—N(11)	119.12(18)
N(11)—H(11)	0.821(17)	N(1)—C(9)—C(8)	119.87(18)
C(12)—O(2)	1.209(2)	N(1)—C(9)—C(10)	121.05(19)
C(16)—O(1)	1.353(2)	O(2)—C(12)—N(11)	122.51(18)
O(1)—H(1)	0.740(4)	C(16)—O(1)—H(1)	125.0(3)
C(32)—O(3)	1.199(3)		

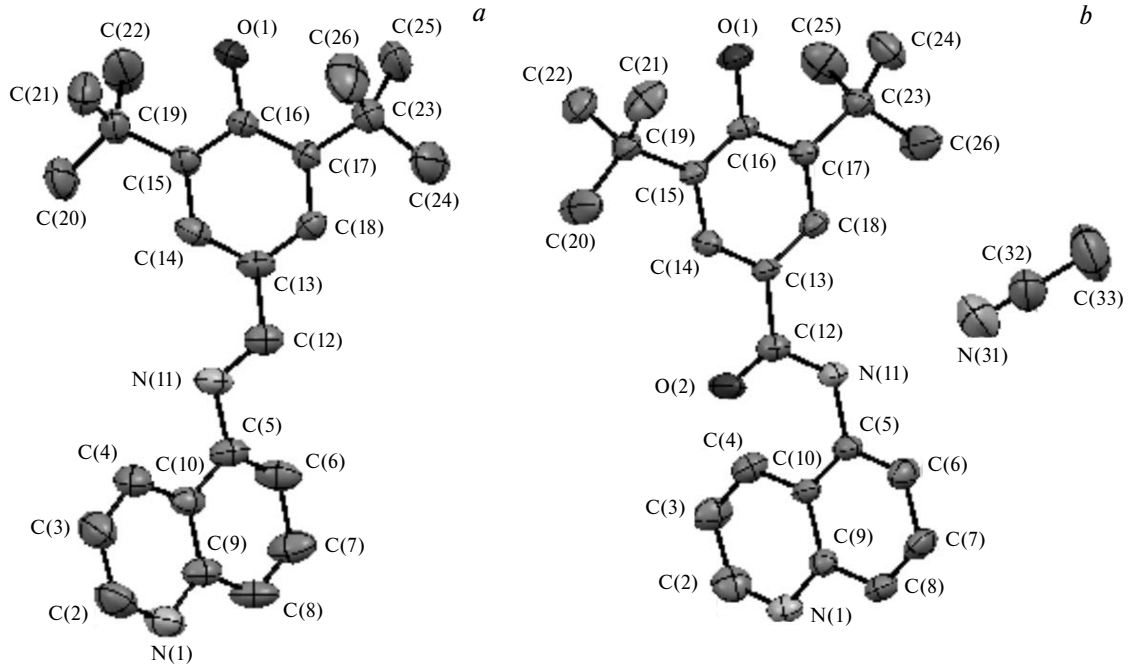


Fig. 3. X-ray molecular structures of compounds **3** (*a*) and **5**·Me_2CO (*b*) with thermal ellipsoids drawn at 50% probability level. Hydrogen atoms are not shown.

ity. We evaluated the antioxidant activity of the synthesized compounds using the model reaction (DPPH assay) and the inhibition of linoleic acid oxidation by lipoxygenase.

Evaluation of antioxidant activity of compounds using the DPPH assay. The activity of 2,6-di-*tert*-butylphenols as free-radical scavengers was evaluated using a known method[20] based on the ability of the compounds to reduce the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Scheme 2).

2,2-Diphenyl-1-picrylhydrazyl is one of a few stable *N*-centered organic radicals. Its solution is deep violet in color. The absorbance (*A*) of 0.1 m*M* DPPH solutions in ethanol containing the test compounds at different concentrations was measured for 30 min at a wavelength corresponding to the absorption maximum of DPPH. By comparing the effective concentrations of compounds **1**—**6** that lead to 50% reduction of the initial DPPH concentration (EC_{50}, see Fig. 4), the following conclusion was made: the nature of the spacer and the number of phenol groups considerably affect the antioxidant activity of compounds. The presence of the amide moiety CONH leads to a decrease in the antioxidant activity due apparently to the absence of conjugation between the donor phenol group and the acceptor moiety.

Compounds **4** and **6** containing two phenol groups are much more active than the standard antioxidant ionol. Compound **1** containing the one-atom NH spacer is the most effective antioxidant. The presence of the acceptor picryl moiety conjugated with the phenol group leads to an increase in stability of the resulting phenoxyl radical.

Inhibition of the enzyme lipoxygenase. Lipoxygenase (LOX), which is of interest as a pharmaceutical target, catalyzes the oxidation of polyunsaturated fatty acids. This oxidation process affords reactive oxygen metabolites as by-products that cause oxidative stress.[21] The oxidation of linoleic acid catalyzed by soybean lipoxygenase (LOX-1-B) was performed at room temperature. The course of the reaction was monitored by measuring the increase in absorbance of the reaction mixture at a wavelength of 234 nm corresponding to the absorption maximum of the reaction products, isomeric 9-hydroperoxy-*trans*-10,*cis*-12- and 13-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acids (LOOH). An increase in the concentration of isomeric linoleic acid hydroperoxides in the presence of different compounds is shown in Fig. 5.

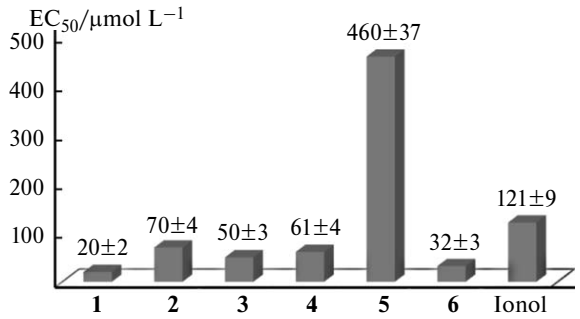


Fig. 4. Effective concentrations EC_{50} (μmol L^{-1}) for compounds **1**—**6** and ionol evaluated using the DPPH assay.

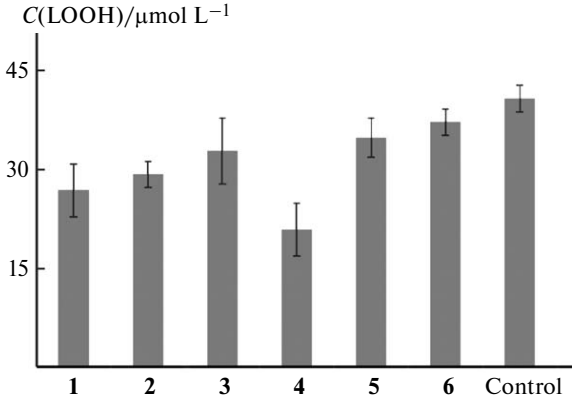


Fig. 5. Accumulation of linoleic acid hydroperoxides after 5 min oxidation of linoleic acid by lipoxygenase LOX 1-B in the presence of compounds **1—6** at a concentration of 100 μmol L^{-1}; control, in the absence of the compounds.

A comparative analysis of the inhibition of linoleic acid oxidation by compounds **1—6** demonstrates that they are weak inhibitors of linoleic acid oxidation.

The 2,6-di-*tert*-butylphenol moiety imparts inhibitory activity to the compounds. Schiff base **4** exhibits the highest activity. The nature of the spacer also affects the degree of inhibition of lipoxygenase by the compounds under examination.

The development of potential pharmacological agents is aimed at reducing the side effect on healthy cells. For this purpose, we propose to introduce groups that have proven antioxidant activity. Sterically hindered phenols are mimetics of vitamin E and inhibitors of free-radical oxidation processes. We synthesized aromatic and heterocyclic compounds containing 2,6-di-*tert*-butylphenol moieties. The molecular structures in the crystals were studied by X-ray diffraction.

The synthesized compounds were evaluated for antioxidant activity using the hydrogen transfer reaction (DPPH assay) and enzymatic oxidation of linoleic acid. It was demonstrated that the introduction of the 2,4,6-trinitrophenyl moiety into the molecule in combination with the —NH— spacer leads to a significant increase in antioxidant activity. The synthesized compounds proved to be weak lipoxygenase inhibitors. The results of this study provide an opportunity to search for new types of antioxidants.

Experimental

The following commercially available reactants were used: 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde hemihydrate (99%, Sigma-Aldrich), 5-aminoquinoline (97%, Sigma-Aldrich), and Et_3N (99%, Sigma-Aldrich). 2,4,6-Trinitrochlorobenzene (picryl chloride),[22] 3,5-di-*tert*-butyl-4-hydroxybenzoyl chloride,[23] 2,6-di-*tert*-butyl-4-aminophenol hydrochloride,[24] 2,6-di-*tert*-butyl-4-mercaptophenol,[25] and 3,5-di-*tert*-butyl-4-hydroxybenzohydrazide[26] were synthesized by known procedures. The solvents (EtOH (95%), $CHCl_3$, CH_2Cl_2, MeOH, MeCN, toluene, acetone, hexane (all of reagent grade) and petroleum ether (b.p. 40—70 °C)) were used as-received.

The IR spectra were recorded on an IR200 Fourier-transform IR spectrophotometer (Thermo Nicolet) as KBr pellets. The NMR spectra were measured on a Bruker AMX-400 spectrometer in $CDCl_3$ (^{1}H, 400 MHz; ^{13}C, 100 MHz). The electronic absorption spectra were recorded on Evolution 300 (Termo Scientific) and Zenyth200rt (Anthos) spectrophotometers.

***N*-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2,4,6-trinitrophenylamine (1).** A solution of picryl chloride (247 mg, 1 mmol) in ethanol (5 mL) was added dropwise to a solution of 3,5-di-*tert*-butyl-4-hydroxyphenylamine hydrochloride (258 mg, 1 mmol) in ethanol (5 mL). The mixture was refluxed for 30 min, cooled to room temperature, concentrated *in vacuo* to 3 mL, and kept until crystals formed. The dark-red crystals were filtered off, washed with ethanol, and dried *in vacuo* at 80 °C. The crystals were studied by X-ray diffraction. The yield was 374 mg (86%). M.p. 190—191 °C (decomp.) (*cf.* Ref. 17: m.p. 187—189 °C (propan-1-ol)). IR, ν/cm^{-1}: 3619 (OH); 3335 (NH); 3102; 3088 (CH arom); 2963; 2877 (CH); 1624; 1591; 1534 (NO_2); 1437; 1356; 1338; 1287. ^{1}H NMR, δ: 1.43 (s, 18 H, Bu^t); 5.36 (s, 1 H, OH); 6.88 (s, 2 H, CH arom); 9.06 (s, 2 H, CH arom (picryl)); 10.40 (s, 1 H, NH). ^{13}C NMR, δ: 29.9 (C(<u>C</u>H_3)$_3$); 34.5 (<u>C</u>(CH_3)$_3$); 118.3; 120.6; 127.3; 129.0; 134.7; 137.5; 139.5; 153.2 (C_{arom}). Found (%): C, 55.40; H, 5.50; N, 12.76. $C_{20}H_{24}N_4O_7$. Calculated (%): C, 55.55; H, 5.59; N, 12.95. UV (MeCN), λ_{max}/nm (ε): 239 (15100); 397 (11600).

3,5-Di-*tert*-butyl-4-hydroxyphenyl 2,4,6-trinitrophenyl sulfide (2). A solution of picryl chloride (248 mg, 1 mmol) in ethanol (5 mL) was added to a solution of 2,6-di-*tert*-butyl-4-mercaptophenol (238 mg, 1 mmol) in ethanol (5 mL). The mixture was refluxed for 10 min, cooled to room temperature, and kept for 16 h. The orange crystals that formed were filtered off, washed with cold ethanol, dried *in vacuo*, and recrystallized from acetone. The crystals were studied by X-ray diffraction. The yield was 399 mg (89%). M.p. 179 °C (decomp.). IR, ν/cm^{-1}: 3629 (OH); 3105; 3078 (CH arom); 2960; 2876 (CH); 1599; 1537 (NO_2); 1427; 1337. ^{1}H NMR, δ: 1.41 (s, 18 H, Bu^t); 5.57 (s, 1 H, OH); 7.14 (s, 2 H, CH (arom)); 8.71 (s, 2 H, CH arom (picryl)). ^{13}C NMR, δ: 29.9 (C(<u>C</u>H_3)$_3$); 34.5 (<u>C</u>(CH_3)$_3$); 118.3; 122.6 (2 C); 131.5 (2 C); 137.8; 140.8; 144.0; 151.0; 156.1 (C_{arom}). Found (%): C, 53.46; H, 5.20; N, 9.12; S, 7.05. $C_{20}H_{23}N_3O_7S$. Calculated (%): C, 53.44; H, 5.16; N, 9.35; S, 7.13. UV (MeCN), λ_{max}/nm (ε): 317 (5300); 402 (4800).

2,6-Di-*tert*-butyl-4-[(quinolin-5-ylimino)methyl]phenol (3). 3,5-Di-*tert*-butyl-4-hydroxybenzaldehyde hemihydrate (1.12 g, 5 mmol) and several crystals of *p*-toluenesulfonic acid were added to a solution of 5-aminoquinoline (0.72 g, 5 mmol) in ethanol (30 mL). The mixture was refluxed for 1 h, the solvent was removed *in vacuo* to one-third of the initial volume, toluene (15 mL) was added to the residue, and the mixture was refluxed for 6 h. Then petroleum ether (3×5 mL, 15 mL in total) was added, the mixture was refluxed for 5 min, and the insoluble residue was filtered off. The solution was cooled to 5 °C, and the orange crystals that formed were washed with petroleum ether and dried *in vacuo* (8 Torr). The yield was 1.41 g (79%). M.p. 197 °C. IR, ν/cm^{-1}: 3621 (OH free); 3029; 2870 (CH); 1620 (C=N); 1569; 1467; 1427; 1211. ^{1}H NMR, δ: 1.55 (s, 18 H, Bu^t); 5.74 (s, 1 H, OH); 7.12 (d, 1 H, quinol, $^3J_{HH}$ = 8.1 Hz); 7.41—7.45

(m, 1 H, quinol); 7.71 (t, 1 H, quinol, $^3J_{HH}$ = 8.0 Hz); 7.89 (s, 2 H, CH arom.); 7.97 (d, 1 H, quinol, $^3J_{HH}$ = 8.0 Hz); 8.50 (s, 1 H, CH=N); 8.74 (d, 1 H, quinol, $^3J_{HH}$ = 8.6 Hz); 8.96 (dd, 1 H, quinol, J_{HH} = 4.0 Hz, J_{HH} = 2.5 Hz). ^{13}C NMR, δ: 30.2 (C($\underline{C}H_3)_3$); 34.5 ($\underline{C}(CH_3)_3$); 113.1 (CH quinol); 120.6 (CH quinol); 124.3; 126.4 (CH quinol); 126.5 (2 C); 127.8; 129.6 (CH quinol); 132.8 (CH quinol); 136.6 (2 C); 148.6; 149.9; 150.7 (CH quinol); 157.5; 161.7 (C=N). Found (%): C, 79.76; H, 7.67; N, 7.64. $C_{24}H_{28}N_2O$. Calculated (%): C, 79.96; H, 7.83; N, 7.77. UV ($CHCl_3$), λ_{max}/nm (ε): 246 (20029); 296,5 (9301); 342 (11394).

4-{(3,5-Di-*tert*-butyl-4-hydroxyphenyl)imino]methyl}-2,6-di-*tert*-butylphenol (4). 2,6-Di-*tert*-butyl-4-aminophenol hydrochloride (309 mg, 1.2 mmol) and Et_3N (0.167 mL, 1.2 mmol) were added to a solution of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde hemihydrate (244 mg, 1 mmol) in ethanol (15 mL) under argon atmosphere on heating to 40 °C. The mixture was stirred for 30 min and then cooled to −18 °C. The yellow-orange precipitate that formed was filtered off, washed with hexane, dried *in vacuo* (8 Torr), and recrystallized from ethanol. The yield was 324 mg (71%). M.p. 227 °C (with decomp.). IR, ν/cm^{-1}: 3604 br. (OH free); 3000; 2858 (CH); 1620 (C=N); 1462; 1377; 1236. ^{1}H NMR, δ: 1.50 (s, 18 H, Bu^t); 5.14 (s, 1 H, OH); 5.57 (br.s, 1 H, OH); 7.10 (s, 2 H); 7.74 (s, 2 H); 8.40 (br.s, 1 H, CH=N). ^{13}C NMR, δ: 30.2 (C($\underline{C}H_3)_3$); 30.3 (C($\underline{C}H_3)_3$); 34.4 ($\underline{C}(CH_3)_3$); 34.5 ($\underline{C}(CH_3)_3$); 117.7; 125.9; 127.7; 128.3; 136.3; 136.6; 152.0; 158.8 (C=N). Found (%): C, 76.68; H, 10.05; N, 3.01. $C_{29}H_{43}NO_2 \cdot H_2O$. Calculated (%): C, 76.44; H, 9.95; N, 3.07. UV ($CHCl_3$), λ_{max}/nm (ε): 287 (12730); 339.5 (12172).

***N*-(Quinolin-5-yl)-3,5-di-*tert*-butyl-4-hydroxybenzamide (5).** 3,5-Di-*tert*-butyl-4-hydroxybenzoyl chloride (1.35 g, 5 mmol) was added with stirring to a solution of 5-aminoquinoline (0.72 g, 5 mmol) in CH_2Cl_2 (5 mL) cooled to 5—10 °C. The mixture was stirred for 10 min at room temperature and then refluxed for 30 min. The red-brown precipitate that formed on cooling was filtered off. The crystals were washed with acetone (3×3 mL) and dried in air. The precipitate was dissolved in water and treated with $NaHCO_3$ (0.42 g, 5 mmol). The colorless precipitate that formed was filtered off, washed with water, and recrystallized from an H_2O—EtOH mixture. The yield was 1.75 g (83%). M.p. 235 °C. IR, ν/cm^{-1}: 3551 (OH free); 2958; 2874 (CH); 1703 (C=O); 1697; 1600; 1304; 1238; 1103. ^{1}H NMR, δ: 1.26 (t, 3 H, C$\underline{H}_3$CH$_2$, $^3J_{HH}$ = 4.0 Hz); 1.50 (s, 18 H, Bu^t); 3.74 (q, 2 H, CH$_3$C$\underline{H}_2$, $^3J_{HH}$ = 4.0 Hz); 5.74 (s, 1 H, OH); 7.43—7.45 (m, 1 H, quinol); 7.74 (t, 1 H, quinol, $^3J_{HH}$ = 8.0 Hz); 7.83 (s, 2 H, CH arom.); 7.86 (d, 1 H, quinol, $^3J_{HH}$ = 8.0 Hz); 8.04 (d, 1 H, quinol, $^3J_{HH}$ = 8.0 Hz); 8.13 (br.s, 1 H, NH); 8.26 (d, 1 H, quinol, $^3J_{HH}$ = 8.0 Hz); 8.94—8.97 (m, 1 H, quinol). ^{13}C NMR, δ: 30.1 (C($\underline{C}H_3)_3$); 34.6 ($\underline{C}(CH_3)_3$); 121.0; 123.4; 123.6; 124.6 (2 C); 125.3 127.7; 129.2; 130.5; 133.1; 136.3; 148.8; 150.4; 157.5; 167.3 (C=O). Found (%): C, 73.56; H, 8.03; N, 6.32. $C_{24}H_{28}N_2O_2 \cdot C_2H_5OH$. Calculated (%): C, 73.90; H, 8.11; N, 6.63. Single crystals suitable for X-ray diffraction were obtained by slow evaporation of the solvent from a solution in MeCN (**5**·MeCN) or acetone (**5**·Me_2CO). Found (%): C, 74.29; H, 7.39; N, 9.66. $C_{24}H_{28}N_2O_2 \cdot CH_3CN$. Calculated (%): C, 74.79; H, 7.48; N, 10.06. Found (%): C, 74.85; H, 7.73; N, 6.58. $C_{24}H_{28}N_2O_2 \cdot (CH_3)_2CO$. Calculated (%): C, 74.62; H, 7.89; N, 6.45.

***N′*-[(3,5-Di-*tert*-butyl-4-hydroxyphenyl)methylidene]-4-hydroxy-3,5-di-*tert*-butylbenzohydrazide (6).** A mixture of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde hemihydrate (368 mg, 1.5 mmol) and 3,5-di-*tert*-butyl-4-hydroxybenzohydrazide (400 mg, 1.5 mmol) in toluene (30 mL) was refluxed for 3 h with a Dean—Stark trap. Then the mixture was cooled to room temperature, and the solution was concentrated *in vacuo* to a small volume. The pale-beige precipitate that formed was filtered off, washed with hexane, dried in air, and recrystallized from a $CHCl_3$—hexane mixture. The yield was 493 mg (68%), colorless powder. M.p. 290 °C. IR, ν/cm^{-1}: 3635 (OH free); 3205 (NH); 3041; 2856 (CH); 1648 (C=O); 1537; 1462; 1375; 1306; 1238. ^{1}H NMR, δ: 1.45 (s, 18 H, Bu^t); 1.48 (s, 18 H, Bu^t); 5.51 (s, 1 H, OH); 5.62 (s, 1 H, OH); 7.57 (s, 2 H); 7.72 (s, 2 H); 8.47 (br.s, 1 H, CH=N); 9.46 (br.s, 1 H, NH). ^{13}C NMR, δ: 30.2 (C($\underline{C}H_3)_3$); 30.2 (C($\underline{C}H_3)_3$); 34.4 ($\underline{C}(CH_3)_3$); 34.4 ($\underline{C}(CH_3)_3$); 124.5; 125.1 (2 C); 127.7; 136.3 (2 C); 146.0; 150.0; 156.2; 157.1; 162.5; 164.9 (C=O). Found (%): C, 75.07; H, 9.25; N, 5.81. $C_{30}H_{44}N_2O_3$. Calculated (%): C, 75.00; H, 9.16; N, 5.83. UV (MeCN), λ_{max}/nm (ε): 316 (26919).

X-ray diffraction study. X-ray diffraction intensities for the compounds were measured on a STOE StadiVari Pilatus100K diffractometer, λ(CuKα) = 1.5418 Å, λ(MoKα) = 0.71073 Å, ω-scanning technique.[27] The X-ray diffraction data sets were processed with the WinGX suite.[28] All subsequent calculations were performed using the SHELX-97 program package.[29] The crystal structures were solved by direct methods and then refined with anisotropic displacement parameters for all nonhydrogen atoms. The hydrogen atoms were positioned geometrically and refined isotropically using a riding model. The structure drawings were prepared with the MERCURY CSD 3.1 program.[30] The atomic coordinates and other crystal structure parameters of compounds **1—3** and solvates **5**·MeCN and **5**·Me_2CO were deposited with the Cambridge Crystallographic Data Centre (CCDC 1575906 (**1**), 1575907 (**2**), 1582462 (**3**), 1582463 (**5**·MeCN), and 1582464 (**5**·Me_2CO)) and are available at www.ccdc.cam.ac.uk/data_request/cif. The X-ray diffraction data and the principal crystallographic characteristics for compounds **1—3** and solvates **5**·MeCN and **5**·Me_2CO are given in Tables 1 and 2.

Evaluation of antioxidant activity of compounds using the DPPH assay. The antioxidant activity was evaluated using the stable radical 2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich)[20] by spectrophotometry at λ_{max} = 517 nm. The known procedure[31] was modified for a microplate spectrophotometer. The reaction was performed in plate wells (96 wells). The reaction mixture contained DPPH (0.1 mL, 0.2 mmol L^{-1}) and a solution of the test compound at different concentrations (0.01, 0.02, 0.05, and 0.1 mmol L^{-1}) in EtOH (0.1 mL). The experiments were performed in three parallel runs. The reaction was accomplished at 25 °C for 30 min. The data were processed and the EC_{50} values were calculated using the Microsoft Excel 2010 and GraphPad Prism 5 programs. The antioxidant activity I (%) was calculated according to the formula

$$I = (A_0 - A_1)/A_0 \cdot 100,$$

where A_0 is the absorbance of the control DPPH solution, A_1 is the absorbance of the reaction mixture in the presence of the test compound after 30 min.

Effect of compounds on enzymatic oxidation of linoleic acid by lipoxygenase. The lipoxygenase activity was evaluated by spectrophotometry[32] with a Zenyth200rt 96-well plate spectrophotometer (Anthos) using lipoxygenase (LOX I-B, Sigma-Aldrich, 15 MU) and linoleic acid (99%, Sigma-Aldrich). The concentrations of linoleic acid oxidation products, isomeric hydroperoxides,

were measured at λ_{max} = 234 nm (ε = 25000 L mol^{-1} cm^{-1}).[33] The analyte mixture contained a linoleic acid solution (2 mL, 0.3 mmol L^{-1}), borate buffer (0.89 mL), pH 9.0, and a solution of the test compound in DMSO (0.01 mL). The reaction was initiated by the addition of 0.1 mL of the enzyme solution (500 U). The measurements were performed for 5 min at 20 °C with different concentrations of the test compounds. All experiments were performed in three parallel runs.

This study was financially supported by the Russian Foundation for Basic Research (Project No. 17-03-01070) using facilities of the Center for Collective Use of the M. V. Lomonosov Moscow State University.

References

1. E. Denisov, *Handbook of Antioxidants*, CRC Press, Boca Raton—New York, 1995, 175 pp.
2. V. V. Ershov, G. A. Nikiforov, A. A. Volod´kin, *Prostranstvenno-zatrudnennye fenoly* [*Sterically Hindered Phenols*], Khimiya, Moscow, 1972, 352 pp. (in Russian).
3. V. A. Roginskii, *Fenol´nye antioksidanty: reaktsionnaya sposobnost´ i effektivnost´* [*Phenolic Antioxidants: Reactivity and Efficiency*], Nauka, Moscow, 1988, 274 pp. (in Russian).
4. E. R. Milaeva, *Russ. Chem. Bull.*, 2001, **50**, 573—586.
5. E. R. Milaeva, *Curr. Top. Med. Chem.,* 2011, **11**, 2703—2713.
6. E. R. Milaeva, D. B. Shpakovsky, Yu. A. Gracheva, S. I. Orlova, V. V. Maduar, B. N. Tarasevich, N. N. Meleshonkova, L. G. Dubova, E. F. Shevtsova, *Dalton Trans.*, 2013, **42**, 6817—6828.
7. E. A. Kuchuk, K. V. Zaitsev, F. A. Mamedova, A. V. Churakov, G. S. Zaitseva, D. A. Lemenovsky, S. S. Karlov, *Russ. Chem. Bull.*, 2016, **65**, 1743—1749.
8. G. N. Ziakas, E. A. Rekka, A. M. Gavalas, *Bioorg. Med. Chem.*, 2006, **14**, 5616—5624.
9. I. Bertini, H. B. Gray, E. I. Stiefel, J. S. Valentine, *Biological Inorganic Chemistry*, Univ. Sci. Books, 2007, 766 p.
10. Ya. F. Freimanis, *Organicheskie soedineniya s vnutrimolekulyarnym perenosom zaryada* [*Organic Compounds with Intramolecular Charge Transfer*], Zinatne, Riga, 1985, 191 pp. (in Russian).
11. O. V. Mikhalev, I. V. Fedyanin, B. N. Tarasevich, I. G. Il´ina, K. P. Butin, *Moscow Univ. Chem. Bull.*, 2007, **62**, 99—104.
12. I. G. Il´ina, O. V. Mikhalev, K. P. Butin, L. D. Ashkinadze, Z. G. Nosan´, *Russ. J. Org. Chem.*, 2003, **39**, 99—102.
13. G. V. Gridunova, V. N. Petrov, I. G. Il´ina, Yu. T. Struchkov, O. V. Mikhalev, *Kristallografiya* [*Sov. Phys. Crystallogr.*], 1990, **35**, 54—58. (in Russian).
14. K. I. Grandberg, L. G. Kuz´mina, G. G. Aleksandrov, I. G. Il´ina, O. V. Mikhalev, R. D. Rakhimov, K. P. Butin, *Chem. Heterocycl. Compd.*, 1999, **35**, 900—906.
15. I. G. Il´ina, O. V. Mikhalev, *Russ. J. Org. Chem.*, 1997, **33**, 1424—1426.
16. G. V. Gridunova, V. N. Petrov, Yu. T. Struchkov, I. G. Il´ina, O. V. Mikhalev, *Kristallografiya* [*Sov. Phys. Crystallogr.*], 1990, **35**, 59—62 (in Russian).
17. E. Manda, T. Shimura, *Bull. Chem. Soc. Jpn.*, 1976, **49**, 1431—1432.
18. V. V. Redchenko, S. G. Semenov, O. V. Mikhalev, I. G. Il´ina, *Zh. Obshch. Khim.* [*J. Gen. Chem. USSR*], 1987, **57**, 2599—2606 (in Russian).
19. L. A. Kazitsina, N. B. Kupletskaya, *Primenenie UF-, IK-, YaMR- i mass-spektroskopii v organicheskoi khimii* [*Application of UV, IR, NMR Spectroscopy and Mass Spectrometry in Organic Chemistry*], Izd-vo MGU, Moscow, 1979, 240 pp. (in Russian).
20. V. Bondet, W. Brand-Williams, C. Berset, *Food Sci. Technol.*, 1997, **30**, 609—615.
21. A. Andreou, I. Feussner, *Phytochem.*, 2009, **70**, 1504—1510.
22. I. G. Il´ina, E. B. Ivanova, O. B. Mikhalev, B. M. Potapov, *Elektronika organicheskikh materialov* [*Electronics of Organic Materials*], Nauka Moscow, 1985, 228 pp. (in Russian).
23. E. P. Ivakhnenko, A. I. Shif, A. I. Prokof´ev, L. P. Olekhnovich, V. I. Minkin, *Zh. Org. Khim.* [*J. Org. Chem. USSR*], 1989, **25**, 357—367 (in Russian).
24. A. Rieker, K. Scheffler, R. Mayer, B. Narr, E. Muller, *Justus Liebigs Ann. Chem.*, 1966, **693**, 10—19.
25. E. Muller, H. B. Stegman, K. Scheffler*, Justus Liebigs Ann. Chem.*, 1961, **645**, 79—91.
26. I. A. Zav´ialov, O. V. Polyakova, E. R. Milaeva, A. I. Prokof´ev, *Russ. Chem. Bull.*, 1995, **44**, 1725—1728.
27. *Stoe & Cie* (2012), X-AREA, X-RED32; Stoe & Cie, Darmstadt, Germany.
28. L. J. Farrugia, *J. Appl. Crystallogr.*, 2012, **45**, 849—854.
29. G. M. Sheldrick, *Acta Crystallogr.*, *Sect. A: Found Crystallogr.*, 2008, **64**, 112—122.
30. C. F. Macrae, P. R. Edington, P. McCabe, E. Pidcock, G. P. Shields, R. Taylor, M. Towler, J. Van De Streek, *J. Appl. Crystallogr.*, 2006, **39**, 453—457.
31. W. Brand-Williams, M. Cuvelier, C. Berset, *Food Sci. Technol.*, 1995, **28**, 25—30.
32. M. N. Xanthopoulou, S. K. Hadjikakou, N. Hadjiliadis, E. R. Milaeva, J. A. Gracheva, V. Yu. Tyurin, N. Kourkoumelis, K. C. Christoforidis, A. K. Metsios, S. Karkabounas, K. Charalabopoulos, *Eur. J. Med. Chem.*, 2008, **43**, 327—335.
33. J. M. Lopez-Nicolas, R. Bru, A. Sanchez-Ferrer, F. Carcia-Carmona, *Anal. Biochem.*, 1994, **221**, 410—415.

Received December 25, 2017;
in revised form February 9, 2018;
accepted February 17, 2018