Novel diphenylsulfimide antioxidants containing 2,6-di-*tert*-butylphenol moieties*

E. R. Milaeva,[a,b]★ D. B. Shpakovsky,[a] I. A. Maklakova,[a] K. A. Rufanov,[a] M. E. Neganova,[b] E. F. Shevtsova,[b] A. V. Churakov,[c] V. A. Babkova,[d] D. A. Babkov,[d] V. A. Kosolapov,[d] and A. A. Spasov[d]

[a]*M. V. Lomonosov Moscow State University, 1, Build. 3, Leninskie Gory, 119991 Moscow, Russian Federation. Fax: +7 (495) 932 8846. E-mail: milaeva@med.chem.msu.ru*
[b]*Institute of Physiologically Active Compounds, Russian Academy of Sciences, 1 Severnyi proezd, 142432 Chernogolovka, Moscow Region, Russian Federation.*
[c]*N. S. Kurnakov Institute of General and Inorganic Chemistry, Russian Academy of Sciences, 31 Leninsky prosp., 119991 Moscow, Russian Federation.*
[d]*Volgograd State Medical University, 1 pl. Pavshikh Bortsov, 400131 Volgograd, Russian Federation*

New diphenylsulfimide derivatives containing substituents with the 2,6-di-*tert*-butylphenol moiety at the nitrogen atom were synthesized. Their molecular structures were established by X-ray diffraction. Antioxidant activity was experimentally evaluated by spectrophotometry based on hydrogen transfer to the stable radicals, namely, 2,2-diphenyl-1-picrylhydrazyl and the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$), and using *in vitro* lipid peroxidation in rat brain and liver homogenates. The presence of phenol and diphenylsulfimide moieties in one molecule leads to a significant enhancement of antioxidant activity. The new compounds exhibit moderate inhibitory activity against enzymes involved in carbohydrate and lipid metabolism. The evaluation of antiglycation activity showed that the new sulfimides taken at a concentration of 100 μmol L^{-1} have activity comparable with that of aminoguanidine.

Key words: sulfimides, 2,6-di-*tert*-butylphenol, antioxidant activity, lipid peroxidation, antiglycation activity, X-ray diffraction.

Sulfimides are compounds with the general formula $R^1R^2S{=}NR^3$.[1—3] They have attracted interest because of their considerable synthetic potential associated with the presence of the labile $S^{IV}{=}N$ bond, at which both nucleophilic and electrophilic reagents can react. The reaction of free-base *S*,*S*-diphenylsulfimide with aldehydes and ketones in the presence of the pyridine borane complex in acetic acid under mild conditions provides a convenient synthetic route to *N*-alkyl-*S*,*S*-diphenylsulfimides.[4] *N*-Acylsulfilimines react with ketenes to form oxazolinones and indolinones.[5] Thermal decomposition and photolysis of sulfimides $Ph_2S{=}NC(O)R$ afford isocyanates RNCO and Ph_2S.[6] The mechanism of formation of isocyanates is attributed to the involvement of nitrene in the S—N bond cleavage during the Curtius rearrangement.[7] The free-base Ph_2SNH forms complexes with Ag, Co, Cu, Fe, and Pt ions, the structures of which were established by X-ray diffraction.[8—11] Diarylsulfimides, diarylsulfoximines, and other related compounds have long been considered to be of little use for the design of pharmacologically active compounds. However, recent results showed that there is a broad range of sulfimide-based structures belonging to targeted physiologically active substances. Some sulfoximines were found to have bactericidal, herbicidal, and antioxidant properties. However, relatively high toxicity limits their use as drugs.[12—14]

Oxidative damage reactions play a significant role in biochemical processes in the body. The cellular redox imbalance leads to oxidative stress that induces different diseases. Phenol derivatives, in particular, tocopherols, tyrosine, or tyroxine, that form the antioxidant defense system are involved in radical biochemical processes and are essential for cellular homeostasis. Synthetic 2,6-dialkylphenols are less toxic than their unsubstituted precursors and are widely used as antioxidants. The mechanism of antioxidant action of sterically hindered phenols is associated with the formation of relatively stable phenoxide radicals.[15] The protective action of multifunctional antioxidants is diverse and can be additive, antagonistic, or

* Dedicated to Academician of the Russian Academy of Sciences B. A. Trofimov on the occasion of his 80th birthday.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 11, pp. 2025—2034, November, 2018.
1066-5285/18/6711-2025

synergistic. For instance, a mixture of sterically hindered phenols and aromatic amines exerts a synergistic effect in the carbohydrate oxidation, which can be attributed to interactions of inhibitors and their radicals.[16] Phenolic antioxidants containing different organic and heterorganic substituents in the *para* position are currently in trials as potential regulators of cellular oxidative stress[17—20] and as cytotoxic agents.[21,22]

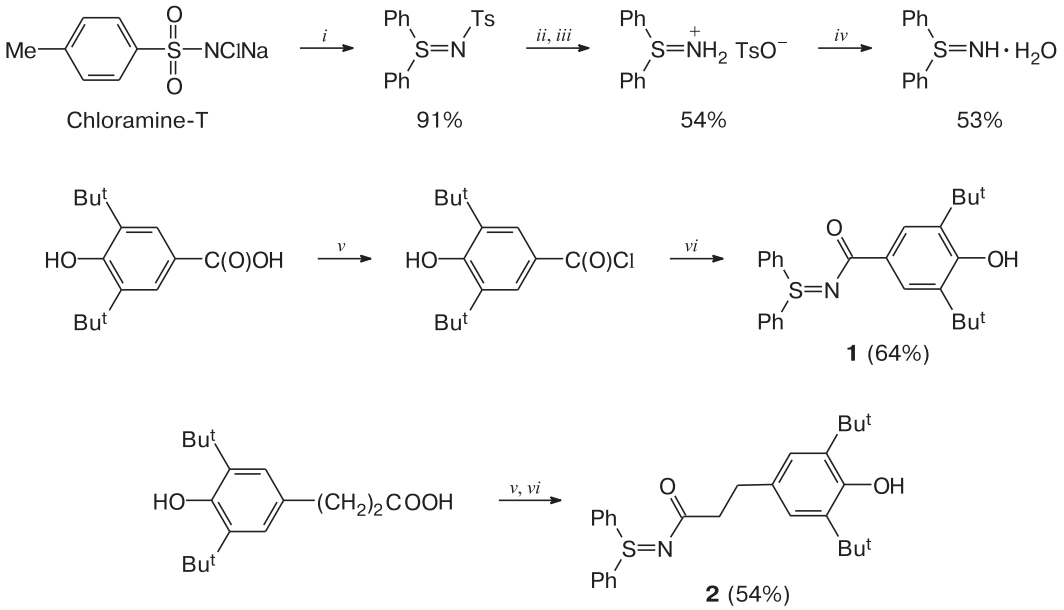
The goal of this study was to prepare new multifunctional compounds containing, apart from the S^{IV}=N—R bond, the 2,6-di-*tert*-butylphenol moiety that reduces the general toxicity and simulates the action of α-tocopherol (vitamin E). We synthesized new multifunctional compounds with desired biological activity based on diphenylsulfimide derivatives **1** and **2** containing the 2,6-di-*tert*-butylphenol moiety (Scheme 1). We evaluated antioxidant activity using model reactions with the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$), lipid peroxidation in rat brain and liver homogenates, and inhibition of enzymes, such as lipoxygenase (LOX 1-B), dipeptidyl peptidase 4 (DPP-4), glycogen phosphorylase (GP), and α-glycosidase. Besides, we assessed antiglycation activity of the new compounds.

Results and Discussion

Synthesis of compounds and structural study. The starting compound Ph_2SNH used for the synthesis of sulfimide derivatives was prepared by oxidative amination of diphenyl sulfide with chloramine T (see Scheme 1) described in the literature.[23] The acid hydrolysis of the *N*-tosyl derivative of *S*,*S*-diphenylsulfimide affords, depending on the reaction conditions, different products, in particular Ph_2SO. The temperature plays a key role in determining the reaction pathway.[24] For instance, heating of Ph_2S=N—Ts under reflux in the $CHCl_3$—H_2SO_4 system for 15 min affords, after hydrolysis, diphenyl sulfoxide Ph_2SO in 52% yield, which may be attributed to the possible oxidation of the product in the presence of a 22-fold excess of H_2SO_4. We modified this procedure for the synthesis of sulfimide Ph_2S=NH by decreasing the amount of the acid (to a molar ratio of 1 : 8), which is required for dissolution of Ph_2S=N—Ts, and performing the reaction at room temperature for 1 h. The reaction first gives hydrotosylate of the target product,[24] which reacts with alkali to form free-base Ph_2S=NH as hydrate. The water solvate molecule can easily be removed by azeotropic distillation with benzene. The reaction of anhydrous sulfimide Ph_2S=NH with 3,5-di-*tert*-butyl-4-hydroxybenzoyl and 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propanoyl chlorides in CH_2Cl_2 in the presence of the base NEt_3 at 20 °C yields acylation products **1** and **2** containing the 2,6-di-*tert*-butylphenol moiety, which is conjugated with the electronic system of Ph_2S=N—C(=O)— in one of them and is separated by the saturated —CH_2CH_2— spacer in the other one.

Compounds **1** and **2** are crystalline compounds that are stable in air and solutions. Their structures were confirmed by IR spectroscopy, ^1H and ^{13}C NMR spectroscopy, and elemental analysis. The IR spectra of these compounds

Scheme 1



Reagents and conditions: *i*. Ph_2S, MeOH, HOAc; *ii*. H_2SO_4. *iii*. H_2O, $CHCl_3$; *iv*. 20% NaOH, H_2O; *v*. $SOCl_2$, hexane, Δ; *vi*. Ph_2S=NH, NEt_3, CH_2Cl_2.

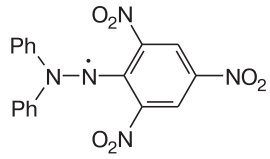
show absorption bands in the 3558—3640 cm^{-1} region corresponding to O—H stretching vibrations of the sterically hindered non-associated phenol group. The C=O stretching vibrations of the carbonyl group in compounds **1** (1593 cm^{-1}) and **2** (1589 cm^{-1}) have moderate intensity and appear at lower frequencies compared to ν(CO) for the starting acids (1704 and 1707 cm^{-1}, respectively). Evidently, the frequencies ν(NH) for **1** and **2**, as opposed to the starting sulfimide, do not appear. The recrystallization of compound **1** from benzene gave single crystals suitable for X-ray diffraction (Figs 1 and 2).

In the crystal structure of compound **1**, there are two crystallographically independent molecules with similar geometric parameters. The central >S=N—C(=O)—C moiety in compounds **1** and **2** is planar within 0.07 Å. An analysis of the Cambridge Structural Database[25] shows that the geometric parameters of molecules **1** and **2** (Table 1) are similar to those in related *N*-carboxysulfimines.

The S=N—C double bond in compounds **1** and **2** adopts a transoid conformation, one phenyl substituent lying virtually in the >S=N—C(=O)—C basal plane with the corresponding torsion angles larger than 157°, whereas the second phenyl substituent being perpendicular to this plane with the torsion angles close to 90°. In the crystals of **1** and **2**, adjacent molecules are linked by O—H...O=C hydrogen bonds to form chains (see Figs 1, *b* and 2, *b*).

Nitrogen and sulfur atoms are not involved in hydrogen bonding. The carbonyl group of each molecule **1** forms a hydrogen bond with the phenol group of the adjacent molecule; the H(1) O(11) and H(2) O(21) distances are 2.57(3) and 2.66(3) Å, respectively. Compound **2** forms a shorter intermolecular O(1)—H(1) O(2) hydrogen bond with the H(1) O(2) distance of 2.09(2) Å (see Table 1).

Evaluation of radical scavenging and antioxidant activity. The introduction of the 2,6-di-*tert*-butylphenol moiety into compounds **1** and **2** provides the appearance of radical scavenging and antioxidant activity. The radical scavenging activity of the compounds was evaluated by spectrophotometry using the reaction with the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH).[26]



DPPH

The absorbance of 0.1 *mM* DPPH solutions in the presence of compounds **1**, **2**, and Ph_2S=NH was measured within 30 min at a wavelength of 517 nm corresponding to the absorption maximum of DPPH. At a concentration of 0.1 mmol L^{-1}, Ph_2S=NH did not exhibit antioxidant activity in the DPPH assay.

The effective concentration EC_{50} (the concentration of the compound required for decreasing the concentration of the DPPH radical by 50%)[27] was determined from the

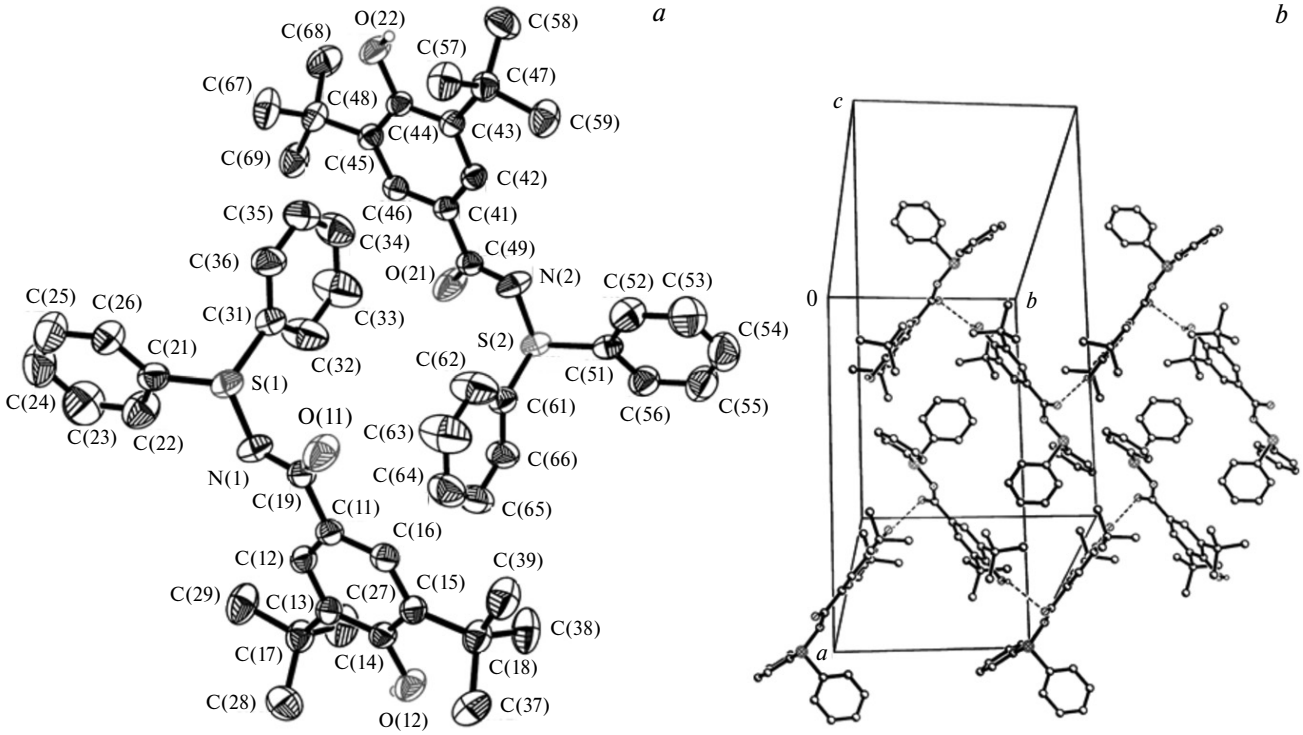


Fig. 1. Molecular structure of compound **1** (*a*) and its crystal packing (*b*). Thermal ellipsoids are drawn at the 50% probability level. Hydrogen atoms and benzene solvate molecules are omitted for clarity.

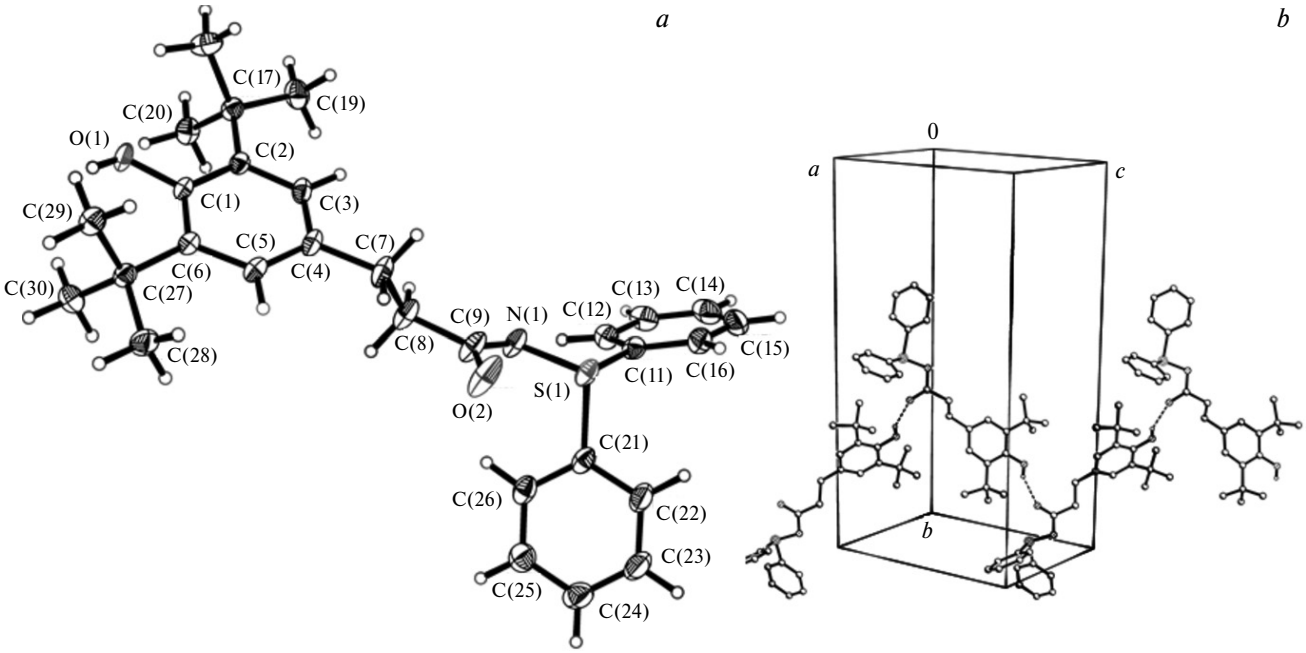


Fig. 2. Molecular structure of compound **2** (*a*) and its packing showing hydrogen-bonded chains (*b*). Thermal ellipsoids are drawn at the 50% probability level.

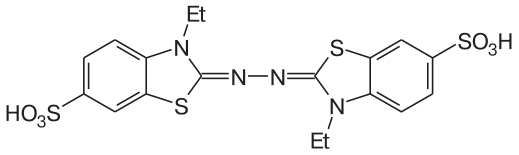
dependence of the concentration of DPPH, which remained unconsumed (in %) 30 min after the beginning of the reaction, on the initial concentration of the compound (0.01—0.1 mmol L^{-1}). A comparison of the EC_{50} values (Table 2) for phenol derivatives **1** and **2** containing the diphenylsulfimide moiety shows that their activity is

Table 1. Selected bond lengths and bond angles in compounds **1** and **2**

Bond	*d*/Å	Angle	ω/deg
	Compound **1** (molecule A)		
S(1)—N(1)	1.6366(18)	N(1)—S(1)—C(21)	101.90(11)
S(1)—C(21)	1.778(3)	N(1)—S(1)—C(31)	104.89(11)
S(1)—C(31)	1.794(2)	C(21)—S(1)—C(31)	101.40(11)
N(1)—C(19)	1.352(3)	C(19)—N(1)—S(1)	110.08(16)
C(19)—O(11)	1.226(3)	O(11)—C(19)—N(1)	125.2(2)
C(14)—O(12)	1.372(3)	O(11)—C(19)—C(11)	120.9(2)
O(12)—H(1)	0.70(2)		
	Compound **1** (molecule B)		
S(2)—N(2)	1.6307(19)	N(2)—S(2)—C(51)	101.26(11)
S(2)—C(51)	1.776(3)	N(2)—S(2)—C(61)	104.43(11)
S(2)—C(61)	1.790(2)	C(51)—S(2)—C(61)	101.31(11)
N(2)—C(49)	1.339(3)	C(49)—N(2)—S(2)	112.16(16)
C(49)—O(21)	1.223(3)	O(21)—C(49)—N(2)	125.3(2)
C(44)—O(22)	1.367(3)	O(21)—C(49)—C(41)	121.0(2)
O(22)—H(2)	0.69(2)		
	Compound **2**		
S(1)—N(1)	1.6543(10)	N(1)—S(1)—C(11)	101.19(6)
S(1)—C(11)	1.7819(12)	N(1)—S(1)—C(21)	107.43(6)
S(1)—C(21)	1.8016(14)	C(11)—S(1)—C(21)	102.46(5)
N(1)—C(9)	1.3526(16)	C(9)—N(1)—S(1)	108.64(8)
C(1)—O(1)	1.3720(13)	O(2)—C(9)—N(1)	124.14(11)
C(9)—O(2)	1.2406(15)	O(2)—C(9)—C(8)	120.50(11)
O(1)—H(1)	0.78(2)		

significantly lower than that of the known antioxidant ionol but is comparable with that of 3,5-Bu^t_2-4-HOC_6H_2-$(CH_2)_2COOH$ (EC_{50} >380 μmol L^{-1}). The antioxidant activity of the compounds depends on the nature of the spacer. The activity of compound **1**, which contains the sulfimide moiety in resonance with the phenol group, is less pronounced that that of derivative **2**, in which the phenol and sulfimide moieties are separated by the $—CH_2CH_2—$ spacer that does not provide electron density transfer.

The stable $ABTS^{\bullet+}$ radical cation is widely used to evaluate the ability of antioxidants to react with radicals.[28,29] The $ABTS^{\bullet+}$ species is generated by the reaction of ABTS with hydrogen peroxide immediately before the measurement. The advantage of this radical is that it is soluble in water and organic solvents.



ABTS

The ABTS assay also showed that compound **2** has significant activity and its EC_{50} (Table 2) is higher than that of 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox).

Inhibition of lipoxygenase. Lipoxygenase (LOX 1-B) is the enzyme that catalyzes oxidation of polyunsaturated fatty acids.[30] The oxidation of linoleic acid catalyzed by this enzyme in the presence of different compounds was performed at room temperature. The course of the reaction was monitored by measuring a decrease in the absorbance of the reaction mixture at a wavelength of 234 nm corresponding to the absorption maximum of the reaction products, isomeric (10*E*,12*Z*)-9-hydroperoxyoctadeca-10,12-dienoic and (9*Z*,11*E*)-13-hydroperoxyoctadeca-9,11-dienoic acids. The activity of the compound as a lipoxygenase inhibitor is characterized by the degree of inhibition (I (%)) of hydroperoxide accumulation after 5 min incubation with the tested compound (1 mmol L^{-1}). It was found that diphenylsulfimide does not inhibit the linoleic acid oxidation, whereas the introduction of the 2,6-di-*tert*-butylphenol moiety into compounds **1** and **2** imparts moderate inhibitory activity to the compounds. Compound **1** containing the sulfimide moiety in resonance with the phenol group displays less pronounced inhibitory activity (18±1%) than analog **2** (24±2%) with the $—CH_2CH_2—$ spacer.

Table 2. The IC_{50} values for the tested compounds in the DPPH and ABTS assay

Compound	DPPH assay EC_{50}/μmol L^{-1}	ABTS assay EC_{50}/mmol L^{-1}
1	>400	0.130
2	>320	0.017
Trolox	36±1	0.091
Ionol	102±2	—

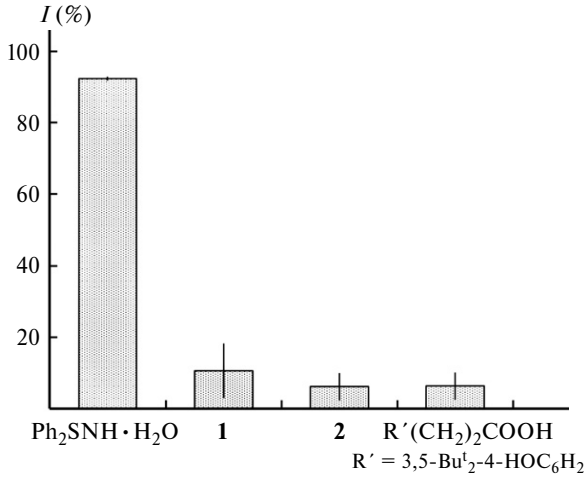


Fig. 3. Inhibition of Fe^{3+}-induced LPO (*I* (% of the control)) in a rat brain homogenate in the presence of compounds **1**, **2**, $Ph_2SNH \cdot H_2O$, and 3,5-Bu^t_2-4-$HOC_6H_2(CH_2)_2COOH$. The rate of LPO was estimated by measuring accumulation of malonic dialdehyde (MDA).

Influence of compounds on lipid peroxidation in rat brain and liver homogenates. Oxidative stress that induces lipid peroxidation (LPO) underlies many pathologies, including neurodegenerative diseases. We evaluated the influence of the synthesized compounds on the LPO in rat brain homogenates. We used the Fe^{3+} salt ($FeNH_4(SO_4)_2 \cdot 12\ H_2O$)B to initiate LPO. Our results show that $Ph_2SNH \cdot H_2O$ does not exhibit antioxidant activity, whereas phenol-containing compounds **1**, **2**, and 3,5-Bu^t_2-4-$HOC_6H(CH_2)_2COOH$ efficiently inhibit Fe^{3+}-induced LPO in a concentration-dependent manner, virtually completely preventing the latter process at concentrations higher than 10 μmol L^{-1} (Fig. 3). The IC_{50} values (the concentration of a compound required for achieving *in vitro* 50% inhibition of the LPO reaction) are given in Table 3.

The ascorbate-dependent LPO assay in rat liver homogenates revealed high antioxidant activity of compounds **1** and **2** (at a concentration of 10 μmol L^{-1}). The half-maximal inhibitory concentration IC_{50} of compound **1**

Table 3. The IC_{50} values for the tested compounds as inhibitors of Fe^{3+}-induced LPO

Compound	IC_{50}/μmol L^{-1}
1	4.3±2.9
2	3.1±2.4
3,5-Bu^t_2-4-$HOC_6H_2(CH_2)_2COOH$	13.7±7.9

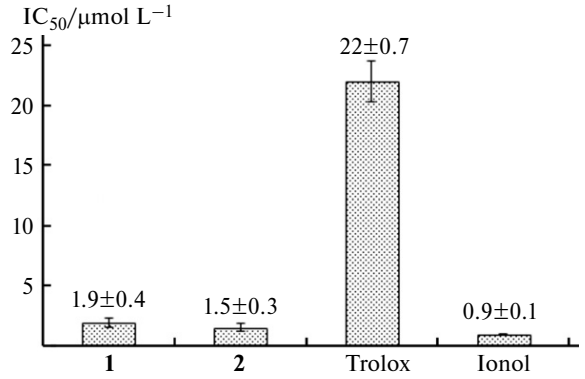


Fig. 4. Inhibitory concentrations against ascorbate-dependent LPO in rat liver homogenates at different concentrations of compounds **1**, **2**, Trolox, and ionol.

(Fig. 4) indicates that it outperforms Trolox in activity and is only slight inferior to ionol. Compound **2** exhibited the highest activity comparable with that of the ionol used as the reference compound. The antioxidant activity of compounds **1** and **2** suggests that they can be considered as potential pharmacological agents.

Evaluation of antiglycation activity. Type 2 diabetes mellitus is a socially significant disease in view of high prevalence, long-term chronic nature, and a high rate of mortality associated with the progression of cardiovascular complications. The current development of antidiabetic drugs is aimed at reducing side effects and overcoming drug resistance. Therefore, the design of new highly efficient low-toxic agents is a challenge. There are two approaches to the discovery of new hypoglycemic compounds: the structural optimization of available drugs in order to reduce their toxicity and the target-oriented design. Previously, we have shown that ferrocene—2,6-di-*tert*-butylphenol conjugates are efficient inhibitors of protein glycosylation, and their activity is comparable with or higher than that of the standard agent aminoguanidine.[31] Testing of these compounds in *in vitro* models, which are employed to develop antidiabetic agents, is of interest for the design of efficient drugs for therapy of diseases the pathogenesis of which involves non-enzymatic glycosylation.

The reaction of monosaccharides, in particular glucose, with free amino groups of proteins, the so-called Maillard glycation reaction, affords final glycosylation products and underlies one the theories of aging. The accumulation of these products facilitates the progression of such diseases as diabetes mellitus, atherosclerosis, heart failure, and neurodegenerative disorders. Glycosylation products promote intracellular oxidative stress accompanied by accumulation of reactive oxygen species, which are also involved in the damage of cellular structures. Scientific efforts aimed at discovery of of inhibitors of glycosylation of biomolecules in the body, in particular among antioxidants, is currently underway.[31]

Table 4. Antiglycation activity in the presence of compounds **1** and **2** and aminoguanidine at different concentrations

Compound	Antiglycation activity (%)	
	1 mmol L^{-1}	0.1 mmol L^{-1}
1	31.2±2.3	1.7±0.7
2	13.1±2.4	6.0±2.4
Aminoguanidine	57.8±0.6	6.0±2.1

Aminoguanidine is known to inhibit *in vitro* glycosylation. The evaluation of antiglycation activity (Table 4) demonstrated that compound **1** inhibited fluorescence of glycated bovine serum albumin by 31% at a concentration of 1 mmol L^{-1}, but it was almost inactive at a tenfold lower concentration. Compound **2** inhibited glycation of proteins by 13.1 and 6.0% at concentrations of 1 and 0.1 mmol L^{-1}, respectively. Both compounds were inferior to aminoguanidine used as the reference compound at a concentration of 1 mmol L^{-1}, while the activity of compound **2** at a concentration of 0.1 mmol L^{-1} was comparable with that of aminoguanidine.

Inhibition of enzymes. Dipeptidyl peptidase 4 (DPP-4) is a membrane enzyme that catalyzes the hydrolysis of the peptide bond of N-terminal proline and is involved in the immune regulation and apoptosis. Inhibitors of DPP-4 belong to hypoglycemic agents. Vildagliptin (Galvus®) was approved in 2008 in the Russian Federation as a DPP-4 inhibitor that improves glycemic control and reduces the level of glycated hemoglobin HbA1c and plasma glucose after eating in patients with type 2 diabetes mellitus. At a concentration of 0.1 mmol L^{-1}, compound **1** (27.3±6.4%) significantly inhibited DPP-4 activity, being inferior to the reference drug vildagliptin (97.8±1.8 %) approximately by a factor of three, while compound **2** (0.8±1.3%) was virtually inactive in this assay.

Glycogen phosphorylase (GP) is a regulatory enzyme involved in glycogen breakdown to glucose 1-phosphate. The enzyme GP is considered as one of targets for the development of a new group of agents for the therapy of type 2 diabetes mellitus. Compound **1** did not exhibit activity in this model at a concentration of 0.1 mmol L^{-1}, whereas compound **2** significantly inhibited GP activity (by 9.4±1.6%), being approximately tenfold less efficient than the reference compound CP-316819 (94.1±4.8%).

α-Glucosidase inhibitors are used in the complex therapy of type 2 diabetes mellitus along with insulin. Drugs of this group retard digestion of complex carbohydrates (including starch and sugar) and glucose absorption in the small intestine. We found that at a concentration of 1 mmol L^{-1}, compound **2** acts as a weak inhibitor (4.9±1.6 %), being ~11 times weaker than acarbose used as the reference agent (56.9±0.8%). Compound **1** precipitated from the reaction mixture as an opalescent substance and did not exhibit activity.

To summarize, we synthesized and structurally characterized new sulfimide derivatives containing 2,6-di-*tert*-butylphenol moieties. Antioxidant activity of these compounds appeared as an efficient inhibition of LPO in liver cell and brain cell membranes. We investigated possible mechanisms of antioxidant activity, such as lipoxygenase inhibition or radical scavenging activity based on reactions with stable radicals (DPPH and $ABTS^{\bullet+}$). The combination of phenol and diphenylsulfimide moieties in one molecule was found to significantly enhance antiradical activity. Antiglycation activity of the new compounds and their ability to inhibit a number of enzymes provide evidence that they can be considered as biologically active compounds promising for further studies.

Experimental

The NMR spectra were recorded on a Bruker AMX-400 spectrometer in $CDCl_3$ (^1H, 400 MHz; ^{13}C, 100 MHz). The IR spectra were measured on an IR200 Fourier-transform IR spectrophotometer (ThermoNicolet) as Nujol mulls and KBr pellets. The electronic absorption spectra were recorded on Evolution 300 (Termo Scientific, USA) and Zenyth200rt (Anthos, Austria) spectrophotometers. Elemental analysis was performed on a Vario micro cube elemental analyzer (Elementar).

The following commercially available reactants (Sigma-Aldrich) were used: diphenyl sulfide (98%), 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (99%), sodium *N*-chloro-*p*-toluenesulfonamide trihydrate (chloramine T) (99%), NEt_3 (99%), 2,2-diphenyl-1-picrylhydrazyl, ABTS, lipoxygenase (LOX I-B, 15 MU), linoleic acid (99%), 2-thiobarbituric acid, ascorbic acid, bovine serum albumin, sodium azide, glucose, Gly-Pro *p*-nitroanilide, vildagliptin, glucose 1-phosphate, rabbit muscle glycogen phosphorylase, and CP-316819. 3,5-Di-*tert*-butyl-4-hydroxybenzoyl chloride[32] and 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propanoic acid[33] were synthesized according to known procedures.

The solvents $CHCl_3$, CH_2Cl_2, MeOH, EtOH, AcOH, DMSO, benzene, acetone, and hexane (all of reagent grade) were used as received.

Synthesis of *N*-(diphenyl-λ⁴-sulfanylidene)-4-hydroxy-3,5-di-*tert*-butylbenzamide (1).

Step 1. Synthesis of *N*-(diphenyl-λ⁴-sulfanylidene)-4-methylbenzenesulfonamide. Diphenyl sulfide (9 mL, 50 mmol) was added to a solution of chloramine T (15.49 g, 55 mmol) in MeOH (70 mL). Then a solution of AcOH (2.5 mL) in MeOH (12.5 mL) was added dropwise. The mixture was stirred for 1 h at room temperature and then poured into a cold solution of NaOH (2.5 g) in water (225 mL). The white precipitate that formed was filtered off, washed with water, and dried in air. The product was recrystallized from MeOH. Yield 16.14 g (91%), m.p. 112—113 °C (*cf.* lit. data[23]: m.p. 113 °C, *cf.* lit. data[34]: 123—124 °C).

Step **2. Synthesis of *S*,*S*-diphenylsulfimide hydrotosylate.** *N*-(Diphenyl-λ⁴-sulfanylidene)-4-methylbenzenesulfonamide (product of Step 1; 7.60 g, 21.4 mmol) was dissolved with stirring in 95% H_2SO_4 (9 mL) for 1 h. The solution was poured onto ice (20 g), extracted with $CHCl_3$ (3×10 mL), and concentrated *in vacuo* to a small volume. Then acetone (4 mL) was added. The crystals that formed were filtered off and washed with a small volume of acetone. Yield 4.32 g (54%), m.p. 127—128 °C (*cf.* lit. data[24]: m.p. 128.5 °C).

Step 3. Synthesis of *S*,*S*-diphenylsulfimide monohydrate ($Ph_2SNH \cdot H_2O$). *S*,*S*-Diphenylsulfimide hydrotosylate (product of Step 2; 4.32 g, 11.6 mmol) was dissolved in $CHCl_3$ (10 mL) and treated with a 20% NaOH solution (10 mL). The organic layer was washed with water (3×10 mL), separated, and dried over Na_2SO_4. The chloroform was distilled off *in vacuo*. Methanol (1 mL) and several drops of water were added to the oily residue, and the solution was allowed to crystallize. The colorless needle-like crystals were filtered off, washed with water, and dried in air. Yield 1.35 g (53%), m.p. 59—62 (*cf.* lit. data[34]: m.p. 56—59 °C (toluene), *cf.* lit. data[24]: 70—71 °C (benzene—hexane)).

Step 4. Synthesis of compound $1 \cdot C_6H_6$. Anhydrous benzene (15 mL) was added to a solution of $Ph_2SNH \cdot H_2O$ (219 mg, 1 mmol) in CH_2Cl_2 (2 mL). Then the solvents were distilled off *in vacuo* (10 Torr) on heating to 70 °C to remove solvating water. The residue was dissolved in CH_2Cl_2 (7 mL), NEt_3 (139 μL, 1 mmol) was added, and a solution of 3,5-di-*tert*-butyl-4-hydroxybenzoyl chloride (268 mg, 1 mmol) in CH_2Cl_2 (3 mL) was added dropwise. The mixture was stirred for 1 h, the solvent was distilled off *in vacuo*, the residue was dissolved in benzene (10 mL), the precipitate of triethylamine hydrochloride was filtered off, the benzene solution was concentrated *in vacuo* to a small volume, and the resulting solution was allowed to crystallize. The colorless crystalline precipitate that formed was filtered off, washed with hexane, and dried *in vacuo*. Yield 329 mg (64%). M.p. 155—157 °C (with decomp.). The crystals were used for X-ray diffraction measurements. IR (Nujol mulls), ν/cm^{-1}: 3558 (OH); 2951—2858 (CH); 1593 (CO); 1556; 1464; 1377, 928 (S=N). ^1H NMR, δ: 1.51 (s, 18 H, Bu^t); 5.51 (s, 1 H, OH); 7.39 (s, 6 H, C_6H_6); 7.45—7.55 (m, 6 H, CH_{arom}); 7.80—7.90 (m, 4 H, CH_{arom}); 8.19 (s, 2 H, CH_{arom}). ^{13}C NMR, δ: 30.30 ($C(\underline{C}H_3)_3$); 34.36 ($\underline{C}(CH_3)_3$); 126.28 (2 C); 127.80 (4 C); 128.34 (C_6H_4); 129.73 (4 C); 131.67 (2 C); 134.97; 136.96; 156.61 (HOC_{arom}); 177.23 (C=O). Found (%): C, 77.22; H, 7.20; N, 2.92; S, 5.99. $C_{27}H_{31}NO_2S \cdot C_6H_6$. Calculated (%): C, 77.46; H, 7.29; N, 2.74; S 6.27. UV ($CHCl_3$), λ_{max}/nm (ε): 271 (14730).

Biochemical assays were performed using compound **1** containing no benzene solvate molecules. For this purpose, the solvate was recrystallized from a chloroform—petroleum ether mixture followed by vacuum drying (1 Torr) at 80 °C.

***N*-(Diphenyl-λ⁴-sulfanylidene)-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propanamide (2).** Anhydrous benzene (15 mL) was added to a solution of $Ph_2SNH \cdot H_2O$ (219 mg, 1 mmol, see Step 3 of the synthesis of compound **1**) in CH_2Cl_2 (2 mL). Then the solvents were distilled off *in vacuo* (10 Torr) on heating to 70 °C. The residue was dissolved in CH_2Cl_2 (10 mL), NEt_3 (139 μL, 1 mmol) was added, and a solution of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propanoyl chloride (297 mg, 1 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The mixture was stirred for 1 h and then kept for 16 h. The solvent was distilled off *in vacuo*, the residue was dissolved in benzene (15 mL), the precipitate of triethylamine hydrochloride was filtered off, the benzene solution was concentrated *in vacuo* to a small volume, acetone (3 mL) was added, and the solution was allowed to crystallize. The resulting colorless crystalline precipitate was filtered off, washed with hexane, and dried *in vacuo*. Yield 247 mg (54%). M.p. 148 °C. The crystals were used for X-ray diffraction measurements. IR (KBr), ν/cm^{-1}: 3639 (OH free); 3470 (OH bond); 3055—2853 (CH); 1762 (C=O); 1590; 1569; 1463; 1376; 1109; 908 (S=N). ^1H NMR, δ: 1.44 (s, 18 H, Bu^t); 2.81 (t, 2 H, CH_2, $^3J_{H,H}$ = 8.0 Hz); 3.01 (t, 2 H, CH_2, $^3J_{H,H}$ = 8.0 Hz); 5.06 (s, 1 H, OH); 7.11

(s, 2 H, C_6H_2); 7.45—7.55 (m, 6 H, Ph); 7.60—7.75 (m, 4 H, CH_{Ph}). ^{13}C NMR, δ: 30.39 ($C(\underline{C}H_3)_3$); 32.78 ($\underline{C}H_2$); 34.32 ($\underline{C}(CH_3)_3$); 39.73 ($\underline{C}H_2$); 125.00 (2 C); 127.94 (4 C); 129.80 (4 C); 131.82 (2 C); 132.55; 135.64; 136.00; 151.81 (C_{arom}); 184.09 (C=O). Found (%): C, 75.45; H, 7.64; N, 3.03; S, 6.95. $C_{29}H_{35}NO_2S$. Calculated (%): C, 76.19; H, 8.22; N, 3.33; S 6.37.

X-ray diffraction study. X-ray diffraction data sets were collected on a Bruker SMART II automated diffractometer at 295 K (for compound **1**) and 150 K (for compound **2**) using Mo*K*α radiation (λ = 0.71073 Å, graphite monochromator) and ω-scanning technique. Absorption corrections were applied based on intensities of equivalent reflections.[35] The structures were solved by direct methods and refined by the full-matrix least-squares method based on F^2 with anisotropic displacement parameters for all nonhydrogen atoms (SHELXTL-Plus).[36] The hydroxyl hydrogen atoms H(1) and H(2) in the structure of **1** were found in difference Fourier maps and refined isotropically. The other hydrogen atoms were positioned geometrically and refined using a riding model. In the crystal of **1**, both benzene solvate molecules are rotationally disordered over two positions with an occupancy ratio of 0.71/0.29 and 0.65/0.35. In the structure of **2**, all hydrogen atoms were found in electron density maps, and their positional and thermal parameters were refined. Crystallographic data for **1** and **2** were deposited with the Cambridge Crystallographic Data Centre (CCDC 1541082 (**1**) and CCDC 1541083 (**2**) and are available at www.ccdc.cam.ac.uk/data_request/cif. The X-ray diffraction data statistics and principal crystallographic characteristics for compounds **1** and **2** are given in Tables 1 and 5.

Evaluation of antioxidant activity. DPPH assay. The antioxidant activity was evaluated using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH)[27] by spectrophotometry at λ_{max} = = 517 nm. The known procedure[26] was modified for a Zenyth200rt 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 tested 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 triplicate. The reaction was accomplished at 25 °C for 30 min. 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, and A_1 is the absorbance of the reaction mixture in the presence of the tested compound after 30 min.

The data were processed and the EC_{50} values were calculated using the Microsoft Excel 2010 and GraphPad Prism 5 software.

ABTS assay. For the ABTS assay,[37] the reaction mixture was prepared from an ABTS solution (600 μL, 0.5 mg mL^{-1}) and a hemoglobin solution (300 μL, 1 mg mL^{-1}). The tested compound was added to the sample in a specified concentration range in an amount of 300 μL. Then phosphate buffer (1.2 mL), pH 6.8, and hydrogen peroxide (600 μL, 0.612 nm) were added. The absorbance was measured at a wavelength of 734 nm for 30 min with a time interval of 5 min. The activity of the compounds was

Table 5. Crystallographic characteristics and X-ray diffraction data statistics for compounds **1** and **2**

Compound	**1**	**2**
Molecular formula	$C_{33}H_{37}NO_2S$	$C_{29}H_{35}NO_2S$
F_w	511.70	461.64
Crystal size/mm	0.35×0.25×0.10	0.40×0.20×0.20
Crystal system	Monoclinic	Monoclinic
Monoclinic	$P2_1/c$	$P2_1/n$
a/Å	22.6547(17)	8.6960(9)
b/Å	12.2146(9)	24.364(3)
c/Å	21.9753(16)	12.2616(13)
β/deg	102.073(1)	99.637(2)
V/Å^3	5946.5(8)	2561.2(5)
Z	8	4
d_{calc}/g cm^{-3}	1.143	1.197
μ(Mo*K*α)/mm^{-1}	0.137	0.152
F(000)	2192	992
θ-Angle range/deg	0.92—25.50	1.67—29.00
h, k, l ranges	$-27 \le h \le 27$	$-11 \le h \le 11$
	$-14 \le k \le 14$	$-32 \le k \le 33$
	$-26 \le l \le 26$	$-16 \le l \le 16$
Number of reflections		
total	50681	28183
unique	11074	6813
(R_{int})	(0.0437)	(0.0203)
Number of variables	687	438
R_1 based on $I > 2\sigma(I)$	0.0540	0.0418
wR_2 (all data)	0.1782	0.1126
Goodness-of-fit based on F^2	1.013	1.041
Residual electron density, $\Delta\rho_{min}/\Delta\rho_{max}$/e Å^{-3}	−0.258/0.354	−0.483/0.733

calculated from the results of the ABTS assay 10 min after the beginning of the reaction.

Influence of compounds on enzymatic oxidation of linoleic acid by lipoxygenase. The lipoxygenase activity was evaluated by spectrophotometry.[38] The concentrations of linoleic acid oxidation products, isomeric hydroperoxides, were measured at λ_{max} = 234 nm (ε = 25000 L mol^{-1} cm^{-1}) with a 96-well microplate spectrophotometer.[39] The analyte solution 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 tested 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 at different concentrations of the tested compounds (0.1, 0.5, 1, and 2 mmol L^{-1}). The degree of inhibition in the presence of the tested compounds was calculated according to the formula:

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

where A_0 is the absorbance of the control solution, and A_1 is the absorbance of the reaction mixture in the presence of the tested compound 5 min after the beginning of the reaction.

All experiments were performed in triplicate.

Influence of compounds on lipid peroxidation in rat brain and liver homogenates. The intensity of lipid peroxidation (LPO) in a rat brain homogenate was assessed using the modified TBA assay.[40] The TBA assay is based on the reaction of thiobarbituric acid (TBA) with LPO intermediates giving a colored trimethine complex with λ_{max} = 540 nm. It is assumed that malonic dialdehyde (MDA) plays a key role in the formation of this colored product.

Antioxidant activity was evaluated by measuring inhibition of *in vitro* ascorbate-dependent LPO in liver homogenates.[41] A 4% rat liver homogenate was used as the substrate. The compounds were studied at concentrations of 1000, 100, 10, and 1 μmol L^{-1}. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) served as the reference compound. The reaction was initiated with 50 *mM* ascorbic acid. The rate of LPO was determined from accumulation of MDA in the reaction with thiobarbituric acid. The absorbance of the colored product was measured at a wavelength of 532 nm on an Apel PD-303UV spectrophotometer (Japan) in a cell with an optical path length of 10 mm. The activity of compounds was expressed in percentage and was calculated according to the formula:

$$I\ (\%) = 100 - (A_s/A_{contr} \cdot 100),$$

where I is inhibition, A_s is the absorbance of the sample containing the tested compound, and A_{contr} is the absorbance of the blank sample.

Compounds with a high activity level were studied in a broad concentration range, and the IC_{50} values (the concentration required for achieving 50% inhibition of the reaction) were calculated. The statistical analysis of the results was performed using parametric Student's t-test with the Statistica 6.0 software (StatSoft, USA).

Evaluation of antiglycation activity. The protein glycation was simulated in a reaction mixture containing glucose (500 mmol L^{-1}) and BSA (1 mg mL^{-1}) dissolved in phosphate buffer, pH 7.4, supplemented with sodium azide at a concentration of 0.02% to prevent bacterial growth.[42] All compounds were dissolved in DMSO. The tested compounds were added to experimental samples to the final concentrations of 1 and 0.1 mmol L^{-1}; equal volumes of the solvent were added to the control samples. All experimental samples were incubated for 24 h at 60 °C in a TS-80M-2 thermostat (Russia).

After completion of incubation, the specific fluorescence of glycated bovine serum albumin was measured on a F-7000 spectrofluorimeter (Hitachi, Japan) at λ_{ex} = 370 nm and λ_{em} = 440 nm. The antiglycation activity was calculated relative to the fluorescence of the control samples. Aminoguanidine, which is the inhibitor of non-enzymatic glycosylation, was used as the reference compound.[43]

Evaluation of *in vitro* dipeptidyl peptidase 4 activity. The inhibitory activity of compounds against dipeptidyl peptidase 4 was evaluated as follows. Donor blood plasma (40 μL) was added to 0.1 *M* Tris-HCl buffer (50 μL), pH 8.0, followed by the addition of a solution of the tested compound (10 μL) at a concentration of 0.1 mmol L^{-1} in Tris buffer. The solution was incubated at 37 °C for 5 min, and then a 1 *mM* solution of Gly-Pro *p*-nitroanilide (100 μL) as the dipeptidyl peptidase 4 substrate was added to the reaction mixture. The incubation was performed at 37 °C for 15 min, and the formation of *p*-nitroanilide was determined from the absorbance at a wavelength of 405 nm[44] using an Infinite M200 PRO microplate reader (Tecan, Austria). Vildagliptin was used as the negative control.[45]

Evaluation of *in vitro* glycogen phosphorylase activity. The inhibitory activity of compounds against glycogen phosphorylase was evaluated as follows. A 50 *mM* HEPES buffer solution (100 μL), pH 7.2, supplemented with 100 *mM* KCl, 2.5 *mM* $MgCl_2$, 0.5 *mM* glucose 1-phosphate, and glycogen (1 mg mL^{-1}) was preincubated with rabbit muscle glycogen phosphorylase and a solution (5 μL) of the tested compound at a concentration of 0.1 mmol L^{-1} at 30 °C for 30 min. Then a solution (150 μL) containing 1.05% $(NH_4)_2MoO_4$ and 0.034% Malachite green was added to the reaction mixture. The incubation was performed at 30 °C for 20 min. The amount of released phosphate was determined from the absorbance at a wavelength of 620 nm[46] using an Infinite M200 PRO microplate reader (Tecan, Austria). The compound CP-316819 was used as the negative control.[47]

Statistical data processing. The statistical data processing was performed using the Mann—Whitney U test with the Statistica 6.0 software (StatSoft, USA).

This study was financially supported by the Russian Science Foundation (Project No. 14-13-00483, synthesis of compounds; Project No. 14-25-00139, experiments with enzymes), the Russian Foundation for Basic Research (Project No. 17-03-01070, peroxidation experiments; Project No. 18-03-00203, antioxidant activity evaluation), and the Council on Grants at the President of the Russian Federation (SP-595.2018.4, antiglycation activity evaluation). The X-ray diffraction study was performed at the Shared Facility Center of the N. S. Kurnakov Institute of General and Inorganic Chemistry, Russian Academy of Sciences.

References

1. N. Furukawa, S. Oae, *Ind. Eng. Chem. Prod. Res. Dev.*, 1981, **20**, 260—270.

2. T. L. Gilchrist, J. C. Moody, *Chem. Rev.*, 1977, **77**, 409—435.
3. I. V. Koval´, *Russ. Chem. Rev.*, 1990, **59**, 819—831.
4. T. Fujie, T. Iseki, H. Iso, Y. Imai, E. Tsukurimichi, T. Yoshimura, *Synthesis*, 2008, No. 10, 1565—1569.
5. M. Abou-Gharbia, D. M. Ketcha; D. E.Zacharias, D. Swern, *J. Org. Chem.*, 1985, **50**, 2224—2228.
6. N. Furukawa, M. Fukumura, T. Nishio, S. Oae, *J. Chem. Soc., Perkin Trans. 1*, 1977, 96—98.
7. N. Furukawa, T. Nishio, M. Fukumura, S. Oae, *Chem. Lett.*, 1978, 209—210.
8. S. Gumus, S. Hamamci, V. T. Yilmaz, C. Kazak, *J. Molec. Struct.*, 2007, **828**, 181—187.
9. S. H. Dale, M. R. J. Elsegood, K. E. Holmes, P. F. Kelly, *Acta Crystallogr., Sec. C: Cryst. Struct. Comm.*, 2005, **C61**, m34-m39.
10. P. F. Kelly, S.-M. Man, A. M. Z. Slawin, K. W. Waring, *Polyhedron*, 1999, **18**, 3173—3179.
11. P. F. Kelly, A. C. Macklin, A. M. Z. Slawin, K. W. Waring, *Polyhedron*, 2000, **19**, 2077—2081.
12. S. Zhou, Z. Li, *J. Phys. Chem. Biophys.*, 2015, **5**, 174.
13. C. M. M. Hendriks, P. Nürnberg, C. Bolm, *Synthesis*, 2015, **47**, 1190—1194.
14. U. Lücking, *Angew. Chem., Int. Ed.*, 2013, **52**, 2—12.
15. E. T. Denisov, *Handbook of Antioxidants: Bond Dissociation Energies, Rate Constants, Activation Energies, and Enthalpies of Reactions*, CRC Press, Boca Raton, New York, 1995, 174 pp.
16. E. T. Denisov, I. B. Afanas´ev, *Oxidation and Antioxidants in Organic Chemistry and Biology*. Boca Raton: Taylor & Francis, 2005. 981 p.
17. E. R. Milaeva, *Curr. Top. Med. Chem.*, 2011, **11**, 2703—2713.
18. 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.
19. O. V. Mikhalev, D. B. Shpakovsky, Yu. A. Gracheva, T. A. Antonenko, D. V. Albov, L. A. Aslanov, E. R. Milaeva, *Russ. Chem. Bull.*, 2018, **67**, 712—720.
20. I. V. Zhigacheva, M. M. Rasylov, *Russ. Chem. Bull.*, 2018, **67**, 721—725].
21. E. R. Milaeva, D. B. Shpakovsky, Yu. A. Gracheva, T. A. Antonenko, D. I. Osolodkin, V. A. Palyulin, P. N. Shevtsov, M. E. Neganova, D. V. Vinogradova, E. F. Shevtsova, *J. Organomet. Chem.*, 2015, **782**, 96—102.
22. T. A. Antonenko, D. B. Shpakovsky, M. A. Vorobyov, Yu. A. Gracheva, E. V. Kharitonashvili, L. G. Dubova, E. F. Shevtsova, V. A. Tafeenko, L. A. Aslanov, A. G. Iksanova, Yu. G. Shtyrlin, E. R. Milaeva, *Appl. Organomet. Chem.*, 2018, **32**, e4381.
23. A. Kucsman, I. Kapovits, M. Balla, *Tetrahedron*, 1962, **18**, 75—78.
24. T. Yoshimura, T. Omata, N. Furukawa, S. Oae, *J. Org. Chem.*, 1976, **41**, 1728—1733.
25. F. H. Allen, *Acta. Crystallogr. B.*, 2002, **B58**, 380—388.
26. W. Brand-Williams, M. Cuvelier, C. Berset, *Lebensm.-Wiss.-Technol.,* 1995, **28**, 25—30.
27. V. Bondet, W. Brand-Williams, C. Berset, *LWT-Food Sci. Tech.*, 1997, **30**, 609—615.
28. A. Cano, O. Alcaraz, M. Acosta, M. Arnao, *Redox Rep.*, 2002, **7**, 103—109.
29. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, *Free Radic. Biol. Med.*, 1999, **26**, 1231—1237.
30. A. Andreou, I. Feussner, *Phytochem.*, 2009, **70**, 1504—1510.
31. E. R. Milaeva, D. B. Shpakovsky, N. N. Meleshonkova, S. I. Orlova, E. F. Shevtsova, L. G. Dubova, E. G. Kireeva, V. A. Kosolapov, V. A. Kusnetsova, D. V. Sorotsky, O. A. Solov´eva, A. A. Spasov, *Russ. Chem. Bull.*, 2015, **64**, 2195—2202.
32. E. P. Ivakhnenko, A. I. Shif, A. I. Prokif'ev, L. P. Olekhnovich, V. I. Minkin, *J. Org. Chem. USSR*, 1989, **25**, 357—367.
33. T. H. Coffield, A. H. Filbey, G. G. Ecke, A. J. Kolka, *J. Am. Chem. Soc.*, 1957, **79**, 5019—5023.
34. P. Wyatt, A. Hudson, J. Charmant, A. G. Orpen, H. Phetmung, *Org. Biomol. Chem.*, 2006, **4**, 2218—2232.
35. G. M. Sheldrick, *SADABS. Program for Scaling and Correction of Area Detector Data*, University of Göttingen, Germany, 1997.
36. G. M. Sheldrick, *Acta. Crystallogr. A*, 2008, **A64**, 112—122.
37. C. A. Rice-Evans, N. J. Miller, *Methods Enzymol.*, 1994, **234**, 279—293.
38. 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.
39. J. M. Lopez-Nicolas, R. Bru, A. Sanchez-Ferrer, F. Carcia-Carmona, *Anal. Biochem.*, 1994, **221**, 410—415.
40. M. E. Neganova, V. A. Blik, S. G. Klochkov, N. E. Chepurnova, E. F. Shevtsova, *Neurochemical J.*, 2011, **5**, 208—214..
41. V. Z. Lankin, S. M. Gurevich, E. B. Burlakova, *Trudy moskovskogo obshchestva ispytatelei prirody* [*Proceeding of the Moscow Society of Naturalists*], 1975, **52**, 73—78 (in Russian).
42. A. Jedsadayanmata, *Naresuan University J.*, 2005, **13**, 35—41.
43. P. J. Thornalley, *Arch. Biochem. Biophys.*, 2003, **419**, 31—40.
44. V. Matheeussen, A.-M. Lambeir, W. Jungraithmayr, N. Gomez, K. Mc Entee, P. Van der Veken, S. Scharpé, I. De Meester, *Clin. Chim. Acta*, 2012, **413**, 456—462.
45. F. X. Pi-Sunyer, A. Schweizer, D. Mills, S. Dejager, *Diabetes Res. Clin. Pract.*, 2007, **76**, 132—138.
46. H. H. Hess, J. E. Derr, *Anal. Biochem.*, 1975, **63**, 607—613.
47. S. W. Suh, J. P. Bergher, C. M. Anderson, J. L. Treadway, K. Fosgerau, R. A. Swanson, *J. Pharmacol. Exp. Ther.*, 2007, **321**, 45—50.

Received July 23, 2018;
in revised form October 12, 2018;
accepted October 17, 2018