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Research Article

Search for compounds with antioxidant and antiradical activity among N9-substituted 2-(biphenyl-4-yl)imidazo[1,2-*a*]benzimidazoles

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Abstract

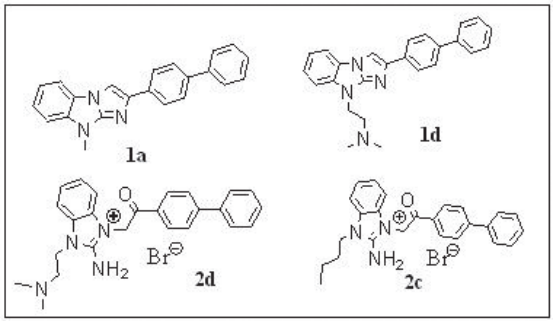
Introduction: Biphenyl and imidazobenzimidazole derivatives attract ongoing attention as a combination of these two privileged substructures with promising pharmacological activities. The aim of this study was to synthesize and investigate *in vitro* antioxidant activity of promising novel compounds: 2-(biphenyl-4-yl)imidazo[1,2-*a*]benzimidazoles.

Materials and methods: The newly synthesized compounds were characterized by IR, ^{1}H NMR and CHBr(Cl)NO analyses. All newly synthesized compounds were screened for their *in vitro* antioxidant activity: inhibition of lipid peroxidation (LPO), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation decolorization and inhibition of hemoglobin (Hb)-H_2O_2-induced luminol chemiluminescence.

Results and discussion: 2-Amino-3-[(2-biphenyl-4-yl)-2-oxo-ethyl)]-1-R-1*H*-benzimidazolium bromides were synthesized, and their cyclization into functionalized imidazo[1,2-*a*]benzimidazole derivatives was studied. The resulting compounds showed LPO inhibitory activity comparable to that of dibunol. Compounds **1a** and **1d** (see graphical abstract), containing a methyl or dimethylaminoethyl substituent in the N^9 position also proved to be equally highly active in the Hb-H_2O_2-induced luminol chemiluminescence model, while compound **1a** was somewhat more active than **1d** in the $ABTS^{\bullet}$ radical scavenging assay.

Conclusion: The study showed that compounds **1a** and **1d** have the highest antioxidant activity. Thus, this new class of 2-(biphenyl-4-yl)imidazo[1,2-*a*]benzimidazole derivatives represents a valuable leading series with great potential for use as antioxidants and as promising candidates for further efficacy evaluation.

Graphical abstract:



Keywords

antioxidant activity, cyclization, imidazo[1,2-*a*]benzimidazoles, quaternary benzimidazolium salts.

Introduction

One of the main mechanisms of the normal development of the body is to maintain a balance between the processes of free radical and peroxidation of various substrates and the state of antioxidant protection. Free radical oxidation is a necessary process for natural physiological reactions to occur in body cells, but it is also one of the universal mechanisms of their damage (Lankin et al. 2001; Jones 2008). Intensive formation of free radicals with insufficient activity of the endogenous antioxidant compensating system of the body leads to the occurrence of oxidative stress, which is involved in the development of numerous pathologies, for example, tumors (Kinnula and Crapo 2004; Valko et al. 2006), atherosclerosis (Förstermann et al. 2017; Kattoor et al. 2017; Marchio et al. 2019), cardiovascular diseases (Golikov et al. 2003; Petrie et al. 2018; Zhao et al. 2021), neurodegenerative diseases (Guidi et al. 2006; Chen and Zhong 2014; Tönnies and Trushina 2017), diabetes mellitus (Petrie et al. 2018; Luc et al. 2019; Zhang et al. 2020), non-alcoholic fatty liver disease (Cichoż-Lach and Michalak 2014; Masarone et al. 2018; Chen et al. 2020), etc. In cases where the mechanism that prevents and eliminates the consequences of damage caused by free radical oxidation, namely the endogenous antioxidant system, including antioxidants present in the cell in low concentrations, cannot cope with the pathological process, protection against the action of free radical oxidation can be enhanced by the intake of antioxidants.

By chemical nature, antioxidants represent a wide class of compounds: phenols and polyphenols (tocopherols, eugenol, pyrocatechol, gallic acid derivatives), flavonoids (rutin, quercetin), steroid hormones (lecithin, cephalin) and many other compounds (Belviranli and Okudan 2015; Neha et al. 2019). In addition, the N^9-substituted imidazo[1,2-*a*]benzimidazole derivatives, which we are actively studying, also demonstrate antioxidant properties, which allows us to consider this group of compounds as promising for further modification and development of new antioxidants (Anisimova et al. 2007, 2016; Kosolapov et al. 2013; Spasov et al. 2017).

When developing new pharmacologically active compounds, considerable attention is paid to the so-called "privileged" substructures (DeSimone et al. 2004). In continuation of research on the search for new pharmacologically active compounds, the present work describes the synthesis of previously unknown imidazo[1,2-*a*]benzimidazoles containing a biphenyl group in position 2, as well as various substituents at the nitrogen atom N^9. Biphenyls are of interest as pharmacologically important substructures whose derivatives are characterized by a number of pharmacological effects, including antioxidant ones (Severinsen et al. 2008; Jain et al. 2017). Their antioxidant potential lies in the ability to serve as scavengers of reactive oxygen species and inhibit lipid peroxidation (LPO) (Maddila et al. 2012; Shashikumar et al. 2014; Rikhi et al. 2015). In this regard, it is promising to study the activity of a combination of these two privileged substructures (Kim et al. 2014; Schneider and Schneider 2017), biphenyl and imidazobenzimidazole derivatives, for which high antioxidant activity should also be expected.

Materials and methods

Synthesis

IR spectra (n/cm^{-1}) of compounds obtained were recorded on a Varian Excalibur 3100 FT-IR spectrophotometer (Varian, USA), using the method of attenuated total reflection in powder; ^1H NMR spectra were recorded on Varian Unity-300 (Varian, USA) and Bruker Avance 600 N (Bruker, USA) spectrometers. Chemical shifts for ^1H are given relative to the signals of residual protons of a deuterated solvent (DMSO-d_6 and $CDCl_3$, δ 2.49 and 7.24, respectively). Melting points were measured on a Fisher-Johns Melting Point Apparatus (Fisher Scientific, USA). Elemental analysis was carried out using a classical method (Gel´man et al. 1987). Reaction progress and purity of synthesized compounds were monitored by TLC (plates with Al_2O_3 III degree of activity, eluent $CHCl_3$, visualization with iodine vapors in a moist chamber).

General procedure for synthesizing 2-amino-3-[(2-biphenyl-4-yl)-2-oxoethyl]-1-R-1H-benzimidazolium bromides 2

To a hot solution of 3 mmol of the corresponding amine **1** in acetone or acetonitrile at room temperature, 3 mmol of 4-(bromoacetyl)biphenyl was added. The reaction mixture was kept for 6–8 h at 25 °C. The hydrobromide precipitate was filtered off and washed thoroughly with acetone. The resulting chromatographically pure salts were dried in air and used in the next step without further purification. The structure of salts **2c-f** was confirmed by their transformation into imidazo[1,2-*a*]benzimidazoles, as well as by spectroscopic data.

2-Amino-3-[2-(biphenyl-4-yl)-2-oxoethyl]-1-butyl-1H-benzimidazolium bromide (2c)

Yield 97%, mp 246–248 °C. Found (%): C 64.48; H 5.45; Br 17.03; N 8.93. $C_{25}H_{26}BrN_3O$. Calculated (%): C 64.66; H 5.64; Br 17.21; N 9.05. IR spectrum, n/cm^{-1}:

3207, 3240 (NH_2), 1687 (C=O). ^{1}H NMR spectrum (300 MHz, DMSO-d_6), δ, ppm, J (Hz): 0.90–0.95 (t, 3H, $CH_2CH_2CH_2CH_3$, J=7.5); 1.34–1.41 (c, 2H, $CH_2CH_2CH_2CH_3$, J=6.9); 1.69–1.74 (t, 2H, $CH_2CH_2CH_2CH_3$, J=7.5); 4.22–4.26 (t, 2H, N_{Bzm}-CH_2, J=6.0); 6.01 (s, 2H, CH_2CO); 7.27–7.39 (m, 2H, H_{Ar}); 7.46–7.57 (m, 3H, H_{Ar}); 7.63–7.68 (t, 2H, H_{Ar}, J=7.5); 7.80–7.82 (d, 2H, H_{Ar}, J=6.0); 7.95–7.98 (d, 2H, H_{Ar}, J=9.0); 8.2 (s, 2H, H_{Ar}) 8.88 (br. s, 2H, N^+H_2).

2-Amino-3-[(2-biphenyl-4-yl)-2-oxoethyl)]-1-[2-(dimethylamino)ethyl-1H-benzimidazolium bromide (2d)

Yield 85%, mp 190–193 °C. IR spectrum, n/cm^{-1}: 3208, 3245 (NH_2), 1687 (C=O). Found (%): C 62.45; H 5.50; Br 16.48; N 11.50. $C_{25}H_{27}BrN_4O$. Calculated (%): C 62.63; H 5.68; Br 16.67; N 11.69. ^{1}H NMR spectrum (300 MHz, DMSO-d_6), δ, ppm, J (Hz): 2.26 (s, 6H, $N(CH_3)_2$), 2.67 (s, 2H, N-CH_2 exocycle); 4.33–4.37 (t, 2H, N_{Het}-CH_2, J=5.9); 6.01 (s, 2H, CH_2CO); 7.27–7.38 (m, 2H, H_{Ar}); 7.46–7.65 (m, 5H, H_{Ar}); 7.80–7.82 (d, 2H, H_{Ar}, J=7.2); 7.95–7.98 (d, 2H, H_{Ar}, J=8.4); 8.16–8.19 (d, 2H, H_{Ar}, J=8.4); 9.0 (s, 2H, N^+H_2).

2-Amino-3-[(2-biphenyl-4-yl)-2-oxoethyl)]-1-[2-(diethylamino)ethyl-1H-benzimidazolium bromide (2e)

Yield 98%, mp 208–210 °C. IR spectrum, n/cm^{-1}: 3208, 3245 (NH_2), 1687 (C=O). Found (%): C 63.80; H 6.23; Br 15.63; N 10.95 $C_{27}H_{31}BrN_4O$. Calculated (%): 63.92; H 6.15; Br 15.74; N 11.04. ^{1}H NMR spectrum (300 MHz, DMSO-d_6), δ, ppm, J (Hz): 0.81–0.85 (t, 6H, $N(CH_2CH_3)_2$, J=7.05), 2.54–2.50 (t, 4H, $N(CH_2CH_3)_2$, J=6.45), 2.74–2.77 (t, 2H, N-CH_2 exocycle, J=5.25); 4.33–4.30 (t, 2H, N_{Het}-CH_2, J=5.25); 6.01 (s, 2H, CH_2CO); 7.26–7.38 (m, 2H, H_{Ar}); 7.46–7.65 (m, 5H, H_{Ar}); 7.80–7.82 (d, 2H, H_{Ar} J=7.2); 7.95–7.98 (d, 2H, H_{Ar}, J=8.4); 8.16–8.19 (d, 2H, H_{Ar}, J=8.4), 9.0 (s, 2H, N^+H_2).

2-Amino-3-[(2-biphenyl-4-yl)-2-oxoethyl)]-1-[2-(morpholino)ethyl-1H-benzimidazolium bromide (2f)

Yield 98.2%, mp 219–221 °C. IR spectrum, n/cm^{-1}: 3208, 3245 (NH_2), 1688 (C=O). Found (%): C 62.09; H 5.65; Br 15.26; N 10.65. Calculated (%): C 62.19; H 5.57; Br 15.35; N 10.75. ^{1}H NMR spectrum (300 MHz, DMSO-d_6), δ, ppm, J (Hz): 2.4 (br. s, 4H, CH_2NCH_2), 2.7 (s, 2H, CH_2), 3.3 (br. s, 4H, CH_2OCH_2), 4.36 (s, 2H, CH_2), 6.01 (s, 2H, CH_2CO), 7.27–7.39 (m, 2H, H_{Ar}), 7.47–7.57 (m, 3H, H_{Ar}), 7.62–7.65 (m, 2H, H_{Ar}), 7.8–7.83 (t, 2H, H_{Ar}, J=7.2), 7.96–7.98 (d, 2H, H_{Ar}, J=8.4), 8.16–8.19 (d, 2H, H_{Ar}, J=8.1), 8.97 (s, 2H, N^+H_2).

Synthesis of 2-(biphenyl-4-yl)-9-[2-(dimethylamino)ethyl]-9H-imidazo[1,2-a]benzimidazole hydrochloride (1d)

A mixture of 1 mmol of bromide **2d** and 2 mmol of fused sodium acetate was refluxed in 7 mL of glacial acetic acid until the reaction was completed (3–4 h). The precipitate formed during cooling was filtered off, washed with water, and dried in air. The resulting base was purified by recrystallization from DMF. It was then converted to the hydrochloride by the action of concentrated HCl. Yield 80%, mp 225–227 °C. Found (%): C 72.12; H 6.15; Cl 8.36; N 13.54. $C_{25}H_{24}N_4$ HCl. Calculated (%): From 72.02; H 6.04; Cl 8.50; N 13.44. ^{1}H NMR spectrum (300 MHz, DMSO-d_6), δ, ppm, J (Hz): 2.95 (s, 6H, $2CH_3$), 3.69–3.73 (t, 2H, $CH_2N(CH_3)_2$, J=6.2) , 4.98 (s, N_{Het}-CH_2), 7.34–7.55 (m, 5H, H_{Ar}); 7.74–7.76 (d, 2H, H_{Ar}, J=7.2); 7.81–7.84 (d, 2H, H_{Ar}, J=8.4) 7.93–8.09 (m, 4H, H_{Ar}); 8.64 (s, 1H, H_{Ar}), 10.68 (br. s, 1H, N^+H).

Pharmacological activity

Inhibition of LPO

Antioxidant activity *in vitro* was studied in the ascorbate-induced LPO model (Lankin et al. 1975). A 4% rat liver homogenate was used as a substrate. The LPO reaction was induced by adding 50 mM of ascorbic acid (Chemapol, Czech Republic). The rate of oxidation was judged by the accumulation of products that give a positive reaction with 2-thiobarbituric acid (Fluka, Switzerland) (TBA-positive products). The optical density of the colored sample was measured at a wavelength of 532 nm on a spectrophotometer PD-303UV (APEL, Japan) in a cuvette with an optical path length of 10 mm. The activity of the studied compounds was expressed as a percentage relative to the control sample (without adding compounds). Butylated hydroxytoluene (dibunol) (Merck, Germany) and trolox (Sigma, USA) were used as reference substances. All compounds were tested in the concentration range from 0.1 to 10 μM to evaluate the concentration-effect relationship and calculate the median inhibitory concentration (IC_{50}).

$ABTS^{\bullet+}$ radical cation decolorization

Antiradical activity *in vitro* was studied on the model of the oxidation reaction of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet}$) (Rice-Evans and Miller 1994). The reaction medium with a total volume of 3 mL contained 0.1 mg of hemoglobin (hemoglobin, Hb) and 0.1 mg of $ABTS^{\bullet}$ (Sigma, USA) in phosphate-buffered saline (pH 6.8). The oxidation of $ABTS^{\bullet}$ was induced by adding a solution of H_2O_2 (0.612 mM) in phosphate-buffered saline. The optical density of the sample was measured at a wavelength of 734 nm for 30 min with an interval of 5 min on a spectrophotometer PD-303UV (APEL, Japan) in a cuvette with an optical path length of 10 mm. The activity of the studied compounds was expressed as a percentage relative to the control sample (without adding compounds) at the tenth minute of the reaction. Trolox (Sigma, USA) was used as a reference substance. All compounds were tested in the concentration range from 10 to 100 μM to evaluate the concentration-effect relationship and calculate the IC_{50}.

Inhibition of Hb-H_2O_2-induced luminol chemiluminescence

In addition, antiradical activity *in vitro* was studied in the model of free radical formation in the Hb-H_2O_2-luminol

system by measuring the chemiluminescence kinetics (Teselkin et al. 1997), which was recorded at 37 °C for 10 min on a Lum-100 chemiluminometer (OOO DISoft, Russia). The reaction medium with a total volume of 1 mL contained 0.01 mg Hb and 1 μM luminol (Serva, Germany) in phosphate-buffered saline (50 mM KH_2PO_4, 100 μM EDTA, pH 7.4). Free-radical oxidation of luminol was induced by adding 0.025% H_2O_2 solution in phosphate-buffered saline. EDTA was added to the buffer to prevent the decomposition of H_2O_2 by heavy metals present in trace amounts in water and chemical reagents. For all obtained chemiluminescence kinetic curves, the integral under the kinetic curve was calculated for a time equal to 10 min. The activity of the studied compounds was expressed as a percentage relative to the "control kinetics" of chemiluminescence of the model system without the addition of compounds. Trolox (Sigma, USA) was used as a reference substance. All compounds were tested in the concentration range from 0.1 to 10 μM to evaluate the concentration-effect relationship and calculate the IC_{50}.

Statistical data processing

Statistical processing of the results was carried out using the non-parametric Kruskal-Wallis test with Dunns multiple comparisons post-test and the regression analysis method for analyzing the concentration-effect relationship and calculating IC_{50} in the GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA).

Results and discussion

The synthesis of 9*H*-imidazo[1,2-*a*]benzimidazoles **1a-f** containing a biphenyl group directly linked to the benzimidazole tricycle is shown in Fig. 1.

The synthesis of biphenyl derivatives **1a-f** was carried out in two stages by quaternization of 1-R-2-aminobenimidazoles **3a-f** with 4-(bromoacetyl)biphenyl, followed by acid-catalyzed cyclization of the resulting 1-R-(4-biphenoyl)-methyl-2-iminobenzimidazole hydrobromides **2a-f.** Quaternary salts **2a-f** are formed in almost quantitative yield (92–95%) and can be used in the next step without further purification. The cyclization of bromides **2a-f** was carried out by boiling in acetic acid in the presence of fused sodium acetate for 4 h. The tricycles **1a-f** that precipitated from the reaction mass on cooling were washed with water, dried, and purified by crystallization. Mixing tests of these compounds did not show depression with the compounds previously prepared by basic catalyzed cyclization. Physicochemical characteristics of compounds **1a-c,e,f** and **2a,b** were published in (Spasov et al. 2017).

The structure of the obtained biphenyl derivatives **1d, 2a-f** was confirmed by IR and ^{1}H NMR spectroscopy and elemental analysis. IR spectra of quaternary benzimidazolium salts **2a-f** are characterized by the presence of absorption bands of the immonium group >N+=C (1687–1688 cm^{-1}) and stretching vibrations of the primary amino group (two bands in the region of 3150–3240 cm^{-1}). In the ^{1}H NMR spectra of bromides **2a-f**, in addition to other signals, there are two-proton singlets of the protons of the methylene groups of the biphenoylmethyl fragments (δ 5.9–6.0 ppm) and the protonated imino group (δ 8.83–8.88 ppm). In the spectra of cyclization products, imidazobenzimidazole bromohydrates **1a-f**, such signals are absent, but downfield signals of the N^+H fragment and the H(3) proton of the imidazole ring formed during the reaction are observed.

First of all, the antioxidant activity of the newly synthesized 2-(biphenyl-4-yl)imidazo[1,2-*a*]benzimidazoles **1a-1f** was studied *in vitro* using an ascorbate-induced LPO model. This model is a widely used method for primary testing chemical compounds for the presence of antioxidant activity and belongs to the so-called enzyme-independent methods (Alam et al. 2013; Romulo 2020). According to the results of the experiment, it was found that all the studied compounds **1a-1f** at a maximum concentration of 10 μM significantly suppressed the process of ascorbate-induced LPO (Table 1). Their activity was comparable to the activity of the reference substance dibunol and statistically significantly (p<0.05) exceeded the activity of the other reference substance trolox by almost 2 times. At a lower concentration of 1 μM, only two compounds, **1a** and **1d**, containing a methyl or dimethylaminoethyl substituent at the N^9 position of imidazo[1,2-*a*]benzimidazole, respectively, retained

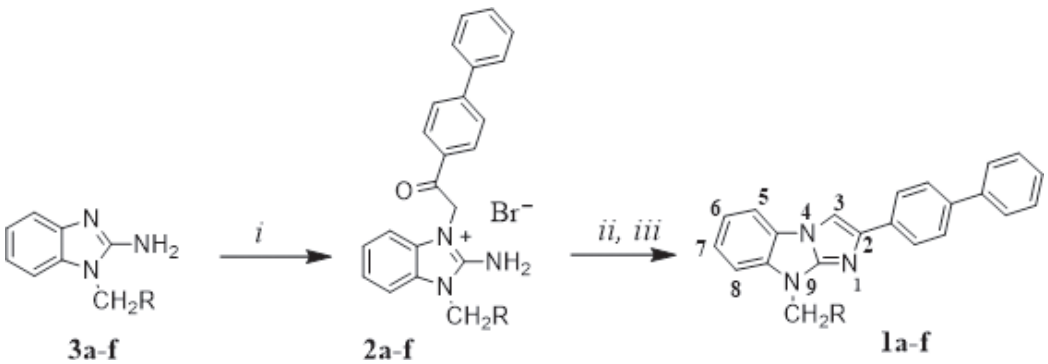


Figure 1. Scheme of 9*H*-imidazo[1,2-*a*]benzimidazole derivatives synthesis. **1-3**: R=H (**a**); CH_3 (**b**); $(CH_2)_2CH_3$ (**c**); $CH_2N(CH_3)_2$ (**d**); $CH_2N(C_2H_5)_2$ (**e**); $CH_2N(CH_2CH_2)_2O$ (**f**). **Reagents and conditions:** *i.* 4-(bromoacetyl)biphenyl, acetone; *ii.* CH_3COONa, glacial acetic acid CH_3COOH, boiling; *iii.* HCl.

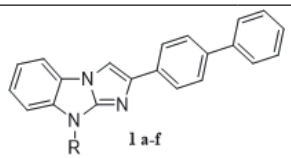
high antioxidant activity. At the same time, the IC_{50} of compounds **1a** and **1d** turned out to be similar to that for dibunol and was lower than the IC_{50} of trolox by 95 and 50 times, respectively.

Next, the antiradical properties of compounds **1a** and **1d** with the highest antioxidant activity according to the results of the first experiment were studied *in vitro* using the ABTS• oxidation reaction model (Rice-Evans and Miller 1994). When ABTS• is incubated in the presence of Hb and H_2O_2, a relatively stable $ABTS^{•+}$ radical cation is formed, and compounds with antiradical properties reduce $ABTS^{•+}$radical cation to ABTS• and decolorize its solution (Alam et al. 2013; Romulo 2020). According to the experimental results, both the reference substance trolox and compounds **1a** and **1d** at high concentrations of 50 and 100 μM significantly suppressed the ABTS• oxidation reaction. At a lower concentration of 10 μM, compounds **1a** and **1d**, but not trolox, also showed little antiradical activity (Table 2). At the same time, the IC_{50} of compound **1a** was slightly more than 2 times lower than the IC_{50} of trolox, while the IC_{50} of compound **1d** was similar to that of trolox.

In addition, the antiradical properties of compounds **1a** and **1d** were studied *in vitro* in a free radical formation model in the Hb-H_2O_2-luminol system by measuring the chemiluminescence kinetics (Teselkin et al. 1997). When interacting with some reactive molecules (free radicals, reactive oxygen species), luminol undergoes oxidation, during which chemiluminescence quanta are emitted. In this regard, luminol is used as a luminescent probe for reactive oxygen species. The introduction of inhibitors of free radical oxidation into the model chemiluminescence system leads to a change in the parameters of the chemiluminescence kinetics of luminol. This change is manifested in an increase in the latent period, a decrease in the light sum and the intensity of the glow. The nature of the change in these parameters depends on the mechanism of action of the test compound (Kobayashi et al. 2001). Compounds **1a** and **1d** and reference substance trolox were able to scavenge reactive oxygen species and luminol radicals formed in the reaction system in the model of Hb-H_2O_2-induced luminol chemiluminescence. At the same time, in compounds **1a** and **1d**, the antiradical properties turned out to be statistically significantly ($p<0.05$) more pronounced than in trolox, which is also confirmed by their IC_{50} values, which were approximately 5.5–5.9 times lower than that of trolox (Table 3).

The high antioxidant properties of 9H-imidazo[1,2-*a*] benzimidazoles are explained by the fact that fused benzimidazole derivatives are polynuclear aromatic compounds (Pozharskiy 1985) with a complex

Table 1. Antioxidant activity of N^9-substituted-2-biphenylimidazo[1,2-*a*]benzimidazoles and reference substances in the model of ascorbate-induced LPO *in vitro*

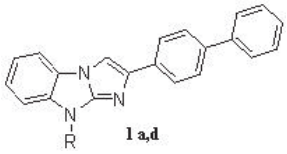


1 a-f

Compound	R	Inhibition of ascorbate-induced LPO, mean±SE, n=6 (%)		IC_{50} (μM)
		10 μM	1 μM	
1a	CH_3	94.27±2.70*	73.06±0.26	0.19
1b	C_2H_5	91.75±1.06*	25.06±1.20#	2.37
1c	C_4H_9	91.50±1.31*	46.12±1.29	1.22
1d	$CH_2CH_2N(CH_3)_2$	92.17±1.13*	67.99±3.47	0.36
1e	$CH_2CH_2N(C_2H_5)_2$	93.50±6.17*	33.64±1.39#	1.88
1f	$CH_2CH_2N(CH_2CH_2)_2O$	92.66±0.83*	49.93±0.60	1.01
Dibunol	–	92.95±0.78	77.58±2.49	0.27
Trolox	–	48.22±0.19	–[a]	18.1

Note: Statistical significance: *$p<0.05$ vs. Trolox, #$p<0.05$ vs. Dibunol (Kruskal-Wallis test with Dunn's multiple comparisons post-test); [a] – not tested.

Table 2. Effect of N^9-substituted-2-biphenylimidazo[1,2-*a*]benzimidazoles and reference substance on ABTS• oxidation reaction *in vitro*

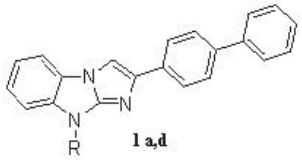


1 a,d

Compound	R	Inhibition of the ABTS• oxidation reaction, mean±SE, n=6 (%)		IC_{50} (μM)
		50 μM	10 μM	
1a	CH_3	90.87±1.19*	31.48±1.24*	22.4
1d	$CH_2CH_2N(CH_3)_2$	67.17±1.41	12.72±1.63	50.1
Trolox	–	67.04±2.56	0.77±0.32	49.5

Note: Statistical significance: *$p<0.05$ vs. Trolox (Kruskal-Wallis test with Dunn's multiple comparisons post-test).

Table 3. Antiradical activity of N^9-substituted-2-biphenylimidazo[1,2-*a*]benzimidazoles and reference substances in the model of Hb-H_2O_2-induced luminol chemiluminescence *in vitro*



1 a,d

Compound	R	Chemiluminescence inhibition, m±SE, n=6 (%)	IC_{50} (μM)
		1 μM	
1a	CH_3	97.37±0.31*	0.27
1d	$CH_2CH_2N(CH_3)_2$	97.23±0.46*	0.29
Trolox	–	40.04±5.11	1.6

Note: Statistical significance: *$p<0.05$ vs. Trolox (Kruskal-Wallis test with Dunn's multiple comparisons post-test).

π-electron system with unpaired electrons, which gives this condensed system the properties of "electron redundancy" and makes it vulnerable to attack by electrophilic particles (Grandberg and Nam 2016). The structure of imidazo[1,2-*a*]benzimidazole contains a 14π-electron system and two pairs of unpaired electrons in orbitals perpendicular to the π-system. Thus, imidazobenzimidazole derivatives have a high π-redundancy and can be donors of electron pairs that are not part of the aromatic π-system (Avdyunina 1979), and, therefore, are characterized by high reactivity and the ability to inhibit free-radical oxidation processes.

Thus, 2-(biphenyl-4-yl)imidazo[1,2-*a*]benzimidazoles showed pronounced antioxidant properties in the model of ascorbate-induced LPO, comparable with the activity of the reference substance dibunol. When evaluating the antiradical properties of the two most active compounds in the model of ascorbate-induced LPO, containing a methyl or dimethylaminoethyl substituent – **1a** and **1d**, respectively, in the N^9 position of imidazo[1,2-*a*]benzimidazole, they also turned out to be equally highly active in the model of Hb-H_2O_2-induced luminol chemiluminescence, whereas in the ABTS$^{\cdot}$ oxidation reaction model, compound **1a** was slightly more active than **1d**.

Conclusion

In conclusion, we have described a simple and efficient protocol for the synthesis of novel 2-(biphenyl-4-yl) imidazo[1,2-*a*]benzimidazole derivatives (**1a-1f**) in good yields. All synthesized compounds were tested for their antioxidant activity. The study showed that compounds **1a** and **1d** have the highest antioxidant activity. Thus, this new class of 2-(biphenyl-4-yl) imidazo[1,2-*a*]benzimidazole derivatives represents a valuable leading series with great potential for use as antioxidants and as promising candidates for further efficacy evaluation.

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Conflict of interests

The authors have declared that no competing interests exist.

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