# Synthesis and antioxidant properties of benzimidazole derivatives with isobornylphenol fragments

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A series of new 2-substituted-1*H*-benzimidazoles was synthesized on the basis of 4-hydroxybenzaldehydes. Their antioxidant properties were evaluated in the *in vitro* models and compared with those of known analogs. The structural specific features of the molecules responsible for the high antioxidant activity were revealed. Among tested derivatives, 2-substituted 1*H*-benzimidazole containing a phenol fragment with isobornyl and *tert*-butyl groups was found to have the greatest ability to protect biomolecules and cells under conditions of acute oxidative stress.

**Key words:** 1*H*-benzimidazoles, sterically hindered phenols, isobornylphenols, antioxidant activity, membrane-protective activity, red blood cells, oxidative hemolysis.

A promising approach to the development of drugs with a wide range of action is the synthesis of biologically active compounds containing several different pharmacophore groups in their structure. One of these groups of interest for medicinal chemistry is a benzimidazole core. Compounds containing a benzimidazole fragment exhibit a variety of pharmacological properties.[1—7] 2-Phenyl-substituted 1*H*-benzimidazoles are known to manifest antimicrobial,[8] antiviral,[9] antiparasitic,[10] antibacterial,[11] anticancer,[12] antiinflammatory,[13] hypotensive,[14] antidiabetic,[15] immunomodulatory,[13] antioxidant (AO),[8] and other activities. At the same time, sterically hindered phenols are biologically active compounds with a wide range of activities. A special place among them is taken by isobornylphenols, which exhibit hemorheological, antiplatelet, and cardioprotective properties, have low toxicity and are efficient inhibitors of free-radical processes.[16—20] A combination of two biologically active structures in one molecule can increase the efficiency of pharmacological action of the constituents or give new properties to the resulting hybrid drug.

The present work reports the synthesis of a number of new hybrid structures containing fragments of benzimidazole and sterically hindered phenol with terpene substituents. The effect of the benzimidazole fragment on the toxicity of the synthesized compounds was studied, and the AO activity (AOA) was evaluated using *in vitro* models. Compounds promising for further modification and pharmacological studies were identified.

## Results and Discussion

2-Phenyl-1*H*-benzimidazoles were synthesized by the reaction of *ortho*-phenylenediamine with the corresponding aldehyde according to the procedure described in the literature[21] (Scheme 1).* To evaluate the effect of substituents in the phenol fragment on the AO properties of substituted benzimidazoles, we synthesized compounds **7** and **8** with two isobornyl substituents (*meso*-stereoisomer and racemate, respectively) and compounds **9** and **10** with isobornyl and *tert*-butyl or methyl substituents. The known[22,23] benzimidazoles **11** (with two *tert*-butyl groups) and **12** (without substituents in the phenol ring) were synthesized for a comparative evaluation of biological activity.

The structure of benzimidazoles synthesized for the first was established based on the spectral data. The $^1$H and $^{13}$C NMR spectra of compounds **7**—**10** contain signals for isobornylphenol and benzimidazole fragments. In the $^1$H NMR spectra, the signal for the NH proton appeared as a singlet in the region of $\delta_H$ 12.62—12.71; in the $^{13}$C NMR spectra, the signal for the C(2) atom is observed in the region of $\delta_C$ 155.92—157.13. The ratio of the integral intensities of the protons of aromatic rings and terpene

* The numbering system of C atoms is introduced for the convenience of reading the NMR spectra. Compounds **1** and **7** are *meso*-stereoisomers, **2**—**4**, **8**—**10** are racemates.

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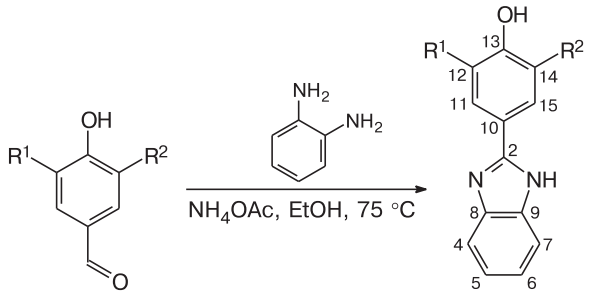
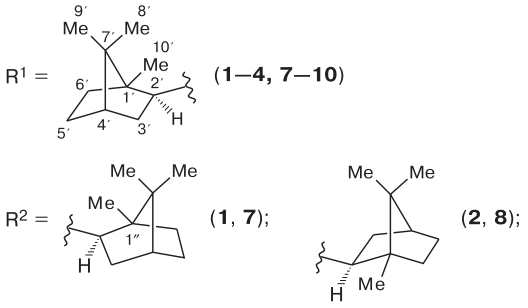
**Scheme 1**




**1—6** **7—12**




$R^2$ = Bu$^t$ (**3**, **9**); Me (**4**, **10**);

$R^1 = R^2$ = Bu$^t$ (**5**, **11**); $R^1 = R^2$ = H (**6**, **12**)

fragments in the $^1$H NMR spectra indicates the formation of 2-substituted benzimidazoles.

For derivatives **7—12**, the radical scavenging activity (RSA) was evaluated in the diphenylpycrylhydrazyl (DPPH) test, AOA was studied on a substrate obtained from the brain of laboratory mice; the hemolytic activity, AOA, and membrane-protective (MP) properties were studied using red blood cells of laboratory mice. 2,6-Diisobornyl-4-methylphenol (**13**) possessing a high AOA[24] was used as a reference compound. The listed test systems were previously used by us for the comparative evaluation of AOA of different phenolic compounds.[19,25—27]

Using a substrate obtained from the brain tissue of animals, it was shown that the presence of substituents and their structure are essential for the AOA of benzimidazoles (Table 1). The highest AOA at concentrations of 100 and 10 μmol L$^{-1}$ (at the level of compound **13** and above) was observed in compounds **9** and **10**, each containing one isobornyl and one alkyl substituent at *ortho*-position to the phenol hydroxy group. Derivatives **7** and **8** with two isobornyl fragments showed somewhat lower activity. 2-Phenyl-1*H*-benzimidazoles **11** and **12** without terpene substituents possess the lowest AOA. The enhancement of AOA after the introduction of an isobornyl substituent was observed by us earlier for 2,2-dimethylchroman-6-ol.[28] The radical scavenging activity of benzimidazoles **7—12** varied within a narrower range, which

**Table 1.** Comparative evaluation of antioxidant* and radical scavenging** activities of compounds **7—13**

| Compound | TBA-RS/nmol mL$^{-1}$ | | RSA (% of inhibition) |
|---|---|---|---|
| | 100 μmol L$^{-1}$ | 10 μmol L$^{-1}$ | |
| **7** | 23.4±0.2 | 37.6±0.1 | 16.4±1.5 |
| **8** | 4.5±0.3 | 20.6±0.6 | 25.4±1.1 |
| **9** | 4.6±0.3 | 5.2±0.2 | 15.6±0.2 |
| **10** | 4.5±1.5 | 5.8±0.1 | 17.1±1.1 |
| **11** | 49.9±0.2 | 59.4±0.8 | 22.6±0.4 |
| **12** | 22.8±0.4 | 70.3±0.2 | 0.3±0.1 |
| **13** | 4.7±0.2 | 7.1±0.2 | 75.3±1.2 |

* The antioxidant activity (test on the substrate from the brain of laboratory mice) of compounds under study at concentrations of 100 and 10 μmol L$^{-1}$ was evaluated by the ability to inhibit the accumulation of secondary lipid peroxidation (LPO) products reacting with 2-thiobarbituric acid (TBA-RS) in the substrate obtained from the brain of laboratory mice (1 h after the $Fe^{2+}$/ascorbate initiation of LPO). The concentration of TBA-RS in the control (without compounds) and in the intact (without initiation of oxidation) samples was 88.0±1.3 and 42.9±0.8 nmol mL$^{-1}$, respectively.

** The radical scavenging activity of compounds at a concentration of 100 μmol L$^{-1}$ was determined in a test with DPPH.

suggests an insignificant influence of the structure of *ortho*-substituents of the phenol ring on the ability of compounds to reduce the stable DPPH radical (see Table 1). An exception is compound **12**, which does not contain any substituents at *ortho*-position with respect to the phenol hydroxy group and shows almost no RSA even at a concentration of 100 μmol L$^{-1}$. Using non-cellular model systems, it was shown that of diastereomers **7** and **8**, racemate **8** is more active in all respects (see Table 1).

All the synthesized benzimidazoles showed extremely low hemolytic activity in red blood cells as a model object. The death of erythrocytes during 5 h of incubation with compounds **7—12** at a concentration of 10 μmol L$^{-1}$ did not exceed 5%.

The highest MP activity (MPA) among the synthesized compounds was observed for benzimidazole **9**, compound **10** was found to be a little less active in the indicated tests (Table 2). The same derivatives most actively inhibited the oxidation of native hemoglobin and the accumulation of secondary lipid peroxidation (LPO) products in erythrocytes. According to the regression analysis, the MPA of the test compounds in the cellular model system closely correlates with the results of the study of AOA on brain lipids ($R$s = 0.90, $p$ = 0.002, $n$ = 8).

To clarify the differences in the MPA between compounds **9**, **13** and the well-known antioxidant 2,6-di-*tert*-butyl-4-methylphenol (BHT), a similar experiment was conducted, in which the concentration of compounds was reduced to 0.1 μmol L$^{-1}$ (Table. 3). It was found that at

**Table 2.** Comparative evaluation of membrane-protective and antioxidant activities of compounds **7—13** at a concentration of 0.1 μmol $L^{-1}$, using red blood cells of laboratory mice

| Compound | MPA (% of hemolysis) | | | TBA-RS/ nmol $mL^{-1}$ | metHb* / oxyHb | ferrylHb** / oxyHb |
|---|---|---|---|---|---|---|
| | 1 h | 3 h | 5 h | | | |
| Control | 31.0±1.5 | 43.7±0.9 | 49.8±0.7 | 1.87±0.01 | 2.003±0.055 | 0.945±0.012 |
| **7** | 22.0±1.3 | 37.2±3.0 | 41.2±2.1 | 1.83±0.08 | 1.860±0.231 | 0.844±0.050 |
| **8** | 17.3±2.3 | 35.4±0.8 | 38.7±1.1 | 1.39±0.01 | 1.873±0.095 | 0.753±0.021 |
| **9** | 2.1±0.2 | 4.5±0.1 | 5.8±0.3 | 1.17±0.06 | 0.878±0.102 | 0.537±0.023 |
| **10** | 3.1±0.1 | 12.8±0.9 | 16.1±0.7 | 1.23±0.07 | 1.001±0.041 | 0.598±0.026 |
| **11** | 14.1±1.0 | 33.4±1.1 | 39.1±1.5 | 2.11±0.09 | 1.785±0.123 | 0.808±0.059 |
| **12** | 23.8±1.0 | 38.1±2.3 | 42.4±2.1 | 1.50±0.06 | 1.395±0.111 | 0.649±0.008 |
| **13** | 1.6±0.0 | 4.0±0.3 | 5.3±0.1 | 1.41±0.01 | 1.145±0.063 | 0.563±0.014 |

* metHb is methemoglobin, oxyHb is oxyhemoglobin.
** ferrylHb is ferrylhemoglobin.

this concentration, benzimidazole **9** containing isobornyl and *tert*-butyl groups is slightly more active than BHT in certain indices, but inferior to compound **13**.

In conclusion, new hybrid compounds **7—10** containing a benzimidazole ring and an isobornylphenol fragment were synthesized, and it was found that the introduction of a benzimidazole fragment does not lead to the appearance of toxicity. It was shown that the presence and structure of substituents in the phenol fragment of the compounds obtained significantly affect their AO properties in various model systems. It was noted that the fact of the presence of an alkyl and/or a terpene substituent is important for RSA in the test with DPPH, while the AOA on a substrate containing natural lipids significantly depends on their structure. A close positive relationship was found between the MPA of the studied compounds in the cell model system and their AOA on a substrate containing brain lipids. All the derivatives with isobornyl substituents are promising for further research. 2-(3-*tert*-Butyl-5-isobornyl-4-hydroxyphenyl) benzimidazole (**9**) was found to have the highest ability to protect biomolecules and cells under conditions of acute oxidative stress.

## Experimental

$^1$H and $^{13}$C NMR spectra were recorded on a Bruker Avance II 300 spectrometer (300.17 and 75.48 MHz, respectively) for solutions of compounds in $CDCl_3$ and DMSO-$d_6$. The signals were assigned using $^{13}$C NMR spectra recorded in the *J*-modulation mode; the HSQC procedure was used in some cases. IR diffuse reflectance spectra were recorded on a Shimadzu IR Prestige 21 Fourier-transform IR spectrometer in KBr pellets. Melting points were determined on a Sanyo Gallenkamp MPD 350 apparatus and were not corrected. Elemental analysis was performed on a vario Micro cube automatic analyzer in the CHNS mode.

The reaction progress and the purity of the synthesized compounds were monitored by TLC on the Sorbfil plates (IMID Ltd.). Column chromatography was carried out on 0.06—0.2 mm silica gel (Alfa Aesar). All solvents were dried and purified by standard procedures.

The starting aldehydes **1**,[29] **2**,[30] **4**,[31] and **5**[32] were prepared according to procedures described previously; 4-hydroxybenzaldehyde (**6**) (Alfa Aesar) was used without additional purification.

The analysis of the synthesized compounds is partially performed using the equipment of the "Chemistry" Center for Collective Use at the Institute of Chemistry, Komi Scientific Centre (SC), Ural Branch of the Russian Academy of Sciences (UB RAS). The activity of compounds was studied using the equipment of the Molecular Biology Center for Collective Use of the Institute of Biology, Komi SC UB RAS. The animals used in the work were from a scientific collection of experimental animals of the Institute of Biology, Komi SC UB RAS (http://www.ckp-rf.ru/usu/471933/).

**3-(*tert*-Butyl)-4-hydroxy-5-{1,7,7-trimethylbicyclo[2.2.1]-hept-*exo*-2-yl}benzaldehyde (3)** was obtained according to the procedure described earlier[32] with some modifications. 2-*tert*-Butyl-6-isobornyl-4-methylphenol[33] (1.43 g, 4.76 mmol) was

**Table 3.** Comparative evaluation of membrane-protective and antioxidant activity of derivative **9** at a concentration of 0.1 μmol $L^{-1}$, using red blood cells of laboratory mice

| Compound | MPA (% of hemolysis) | | | TBA-RS/ nmol $mL^{-1}$ | metHb/ oxyHb | $I_{fl}$ /arb. units* |
|---|---|---|---|---|---|---|
| | 1 h | 3 h | 5 h | | | |
| Control | 22.3±0.4 | 33.7±0.6 | 43.2±0.7 | 1.36±0.04 | 1.591±0.079 | 7.38±0.08 |
| BHT | 10.7±0.2 | 29.2±0.7 | 36.0±0.6 | 1.41±0.05 | 1.129±0.137 | 7.30±0.09 |
| **9** | 8.9±0.8 | 25.5±1.3 | 35.3±1.4 | 1.34±0.05 | 1.311±0.049 | 6.56±0.14 |
| **13** | 5.3±0.7 | 10.5±0.7 | 17.3±0.6 | 1.30±0.10 | 0.964±0.088 | 6.27±0.08 |

* The fluorescence intensity ($I_{fl}$) in the intact sample was 2.49±0.01 arb. units.

dissolved in Bu$^t$OH (50 mL) with heating. The solution was cooled to ~40 °C, followed by the addition of $Br_2$ (0.56 mL, 9.52 mmol) in small portions. The reaction mixture was stirred for 3 h at 20 °C and left for 16 h. The volatile components were removed under reduced pressure, the residue was dissolved in $CHCl_3$ (25 mL), washed with saturated solution of $Na_2S_2O_3$ (3×10 mL) and water (2×10 mL), dried with $Na_2SO_4$. The solvent was removed under reduced pressure, the reaction product was precipitated from PhH. A colorless powder, m.p. 210—212 °C (decomp.). The yield was 0.71 g (47%). Found (%): C, 80.11; H, 9.46. $C_{21}H_{30}O_2$. Calculated (%): C, 80.21; H, 9.62. IR, ν/cm$^{-1}$: 3414 (OH); 2955, 2878, 1435, 1385 ($CH_3$, $CH_2$); 1668 (C=O). $^1$H NMR ($CDCl_3$), δ: 0.79 (s, 3 H, C(10′)$H_3$); 0.86, 0.87 (both s, 3 H each, C(8′)$H_3$, C(9′)$H_3$); 1.45 (s, 9 H, C($CH_3$)$_3$); 1.31—1.49, 1.61—1.81, 1.85—2.05 (all m, 2 H each, H(3′), H(4′), C(5′)$H_2$, C(6′)$H_2$); 2.31—2.43 (m, 1 H, H(3′)); 2.88 (t, 1 H, H(2′), $J$ = 8.4 Hz); 5.60 (s, 1 H, OH); 7.69, 7.74 (both s, 1 H each, H(11), H(15)); 9.85 (1 H, CHO). $^{13}$C NMR ($CDCl_3$), δ: 12.27 (C(10′)); 20.18 (C(9′)); 21.29 (C(8′)); 27.53 (C(5′)); 29.57 (C($\underline{C}H_3$)$_3$); 34.30 (C(3′)); 34.58 ($\underline{C}$($CH_3$)$_3$); 40.37 (C(6′)); 45.27 (C(4′)); 46.19 (C(2′)); 48.55 (C(7′)); 49.63 (C(1′)); 127.30, 128.12 (C(11), C(15)); 128.64, 129.48, 136.31 (C(10), C(12), C(14)); 159.28 (C(13)); 191.71 (CHO).

**Synthesis of 2-phenylbenzimidazoles 7—12 (general procedure).** A procedure similar to that described in the work[21] was used. A mixture of *o*-phenylenediamine (0.053 g, 0.49 mmol), aldehyde **1**—**6** (0.51 mmol), and $NH_4OAc$ (0.038 g, 0.49 mmol) in anhydrous EtOH (15 mL) was stirred for 10 h at 75 °C. The reaction mixture was cooled to room temperature, the precipitate was collected by filtration, washed with EtOAc, and dried. An additional amount of the product was obtained after evaporation and precipitation from the mother liquor. Compounds **9**, **10**, and **12** were isolated by column chromatography (eluent PhH—MeOH (**9**), petroleum ether—EtOAc (**10**, **12**)).

**4-(1*H*-Benzo[*d*]imidazol-2-yl)-2,6-di-{1,7,7-trimethylbicyclo[2.2.1]hept-*exo*-2-yl}phenol (7) (*meso*-stereoisomer).** A white powder, m.p. >300 °C. The yield was 63%. Found (%): C, 81.79; H, 8.78; N, 5.78. $C_{33}H_{42}N_2O$. Calculated (%): C, 82.11; H, 8.77; N, 5.80. IR, ν/cm$^{-1}$: 3597, 2949, 2875, 1620, 1537, 1458, 1431, 1361, 1321, 1273, 1186, 742. $^1$H NMR (DMSO-$d_6$), δ: 0.78 (s, 6 H, C(10′)$H_3$, C(10″)$H_3$); 0.81 (s, 6 H, C(8′)$H_3$, C(8″)$H_3$); 0.90 (s, 6 H, C(9′)$H_3$, C(9″)$H_3$); 1.26—1.41 (m, 2 H, C(5′)H, C(5″)H); 1.51—1.68 (m, 6 H, C(3′)H, C(3″)H, C(6′)$H_2$, C(6″)$H_2$); 1.81—1.88 (m, 4 H, C(5′)H, C(5″)H, H(4′), H(4″)); 2.34—2.47 (m, 2 H, C(3′)H, C(3″)H); 3.25—3.40 (m, 2 H, H(2′), H(2″), overlapped by the signal of HOD); 7.07—7.20 (m, 2 H, H(5), H(6)); 7.47, 7.61 (both d, 1 H each, H(4), H(7), $J$ = 7.4 Hz, $J$ = 7.1 Hz); 8.00 (s, 2 H, H(11), H(15)); 8.03 (s, 1 H, OH); 12.70 (s, 1 H, NH). $^{13}$C NMR (DMSO-$d_6$), δ: 12.28 (C(10′), C(10″)); 20.00, 21.21 (C(8′), C(8″), C(9′), C(9″)); 27.09 (C(5′), C(5″)); 33.21 (C(3′), C(3″)); 38.45 (C(6′), C(6″)); 44.63, 44.92 (C(2′), C(2″), C(4′), C(4″)); 47.72 (C(1′), C(1″)); 49.77 (C(7′), C(7″)); 110.66, 118.16 (C(4), C(7)); 120.49 (C(10)); 121.12, 121.63 (C(5), C(6)); 123.55 (C(11), C(15)); 131.08 (C(12), C(14)); 134.90, 143.79 (C(8), C(9)); 152.28 (C(13)); 157.13 (C(2)).

**4-(1*H*-Benzo[*d*]imidazol-2-yl)-2,6-di-{1,7,7-trimethylbicyclo[2.2.1]hept-*exo*-2-yl}phenol (8) (racemate).** A light brown powder, m.p. 238—242 °C. The yield was 64%. Found (%): C, 82.00; H, 8.56; N, 5.73. $C_{33}H_{42}N_2O$. Calculated (%): C, 82.11; H, 8.77; N, 5.80. IR, ν/cm$^{-1}$: 3601, 2949, 2875, 1620, 1535, 1458, 1431, 1399, 1365, 1321, 1271, 1184, 742. $^1$H NMR (DMSO-$d_6$), δ: 0.71 (s, 6 H, C(10′)$H_3$, C(10″)$H_3$); 0.80 (s, 6 H, C(8′)$H_3$, C(8″)$H_3$); 0.88 (s, 6 H, C(9′)$H_3$, C(9″)$H_3$); 1.25—1.42 (m, 2 H, C(5′)H, C(5″)H); 1.52—1.66 (m, 6 H, C(3′)H, C(3″)H, C(6′)$H_2$, C(6″)$H_2$); 1.78—1.86 (m, 4 H, C(5′)H, C(5″)H, H(4′), H(4″)); 2.23—2.41 (m, 2 H, C(3′)H, C(3″)H); 3.27—2.42 (m, 2 H, H(2′), H(2″), overlapped by the signal of HOD); 7.06—7.20 (m, 2 H, H(5), H(6)); 7.47, 7.61 (both d, 1 H each, H(4), H(7), $J$ = 6.8 Hz, $J$ = 6.8 Hz); 7.98 (s, 2 H, H(11), H(15)); 8.22 (s, 1 H, OH); 12.67 (s, 1 H, NH). $^{13}$C NMR (DMSO-$d_6$), δ: 12.24 (C(10′), C(10″)); 20.16, 21.27 (C(8′), C(8″), C(9′), C(9″)); 27.07 (C(5′), C(5″)); 33.65 (C(3′), C(3″)); 38.61 (C(6′), C(6″)); 44.59, 44.96 (C(2′), C(2″), C(4′), C(4″)); 47.72 (C(1′), C(1″)); 49.28 (C(7′), C(7″)); 110.69, 118.17 (C(4), C(7)); 120.14 (C(10)); 121.13, 121.62 (C(5), C(6)); 123.74 (C(11), C(15)); 130.74 (C(12), C(14)); 134.90, 143.81 (C(8), C(9)); 152.41 (C(13)); 156.77 (C(2)).

**4-(1*H*-Benzo[*d*]imidazol-2-yl)-2-*tert*-butyl-6-{1,7,7-trimethylbicyclo[2.2.1]hept-*exo*-2-yl}phenol (9).** A light beige powder, m.p. 278—280 °C (with decomp.). The yield was 50%. Found (%): C, 80.23; H, 8.37; N, 6.70. $C_{27}H_{34}N_2O$. Calculated (%): C, 80.55; H, 8.51; N, 6.96. IR, ν/cm$^{-1}$: 3631, 3597, 2953, 1620, 1535, 1456, 1425, 1394, 1361, 1323, 1271, 1190, 742. $^1$H NMR (DMSO-$d_6$), δ: 0.73 (s, 3 H, C(10′)$H_3$); 0.79 (s, 3 H, C(8′)$H_3$); 0.83 (s, 3 H, C(9′)$H_3$); 1.31—1.87 (m, 6 H, C(3′)H, H(4′), C(5′)$H_2$, C(6′)$H_2$); 1.44 (s, 9 H, C($CH_3$)$_3$); 2.33—2.46 (m, 1 H, C(3′)H); 3.30—3.41 (m, 1 H, H(2′), overlapped by the signal of HOD); 7.10—7,18 (m, 2 H, H(5), H(6)); 7.48 (d, 1 H, H(4), H(7), $J$ = 7.4 Hz, $J$ = 7.4 Hz); 7.92, 7.99 (both s, 1 H each, H(11), H(15)); 8.11 (s, 1 H, OH); 12.71 (s, 1 H, NH). $^{13}$C NMR (DMSO-$d_6$), δ: 11.87 (C(10′); 20.01, 21.26 (C(8′), C(9′)); 27.17 (C(5′)); 29.71 (C($\underline{C}H_3$)$_3$); 33.23 (C(3′)); 34.63 ($\underline{C}$($CH_3$)$_3$); 38.16 (C(6′)); 44.10 (C(2′)); 44.96 (C(4′)); 47.91 (C(1′)); 49.33 (C(7′)); 110.75, 118.20 (C(4), C(7)); 120.69 (C(10)); 121.16, 121.67 (C(5), C(6)); 122.55, 123.91 (C(11), C(15)); 131.60, 134.93, 138.05, 143.83 (C(8), C(9), C(12), C(14)); 152.42 (C(13)); 156.45 (C(2)).

**4-(1*H*-Benzo[*d*]imidazol-2-yl)-2-methyl-6-{1,7,7-trimethylbicyclo[2.2.1]hept-*exo*-2-yl}phenol (10).** A light beige powder, m.p. 256—258 °C (with decomp.). The yield was 86%. Found (%): C. 79.38; H, 7.86; N, 7.41. $C_{24}H_{28}N_2O$. Calculated (%): C, 79.96; H, 7.83; N, 7.77. IR, ν/cm$^{-1}$: 3599, 3572, 2949, 1602, 1539, 1452, 1433, 1392, 1327, 1271, 1192, 744. $^1$H NMR (DMSO-$d_6$), δ: 0.75 (s, 3 H, C(10′)$H_3$); 0.81 (s, 3 H, C(8′)$H_3$); 0.90 (s, 3 H, C(9′)); 1.30—1,39 (m, 1 H, C(5′)H); 1.48—1.60 (m, 3 H, C(3′)H, C(6′)$H_2$); 1.76—1.90 (m, 2 H, C(5′)H, H(4′)); 2.24—2.38 (m, 1 H, C(3′)H); 2.28 (s, 3 H, C(14)$CH_3$); 3.26—3.41 (m, 1 H, H(2′), overlapped by the signal of HOD); 7.06—7.20 (m, 2 H, H(5), H(6)); 7.46—7.57 (m, 2 H, H(4), H(7)); 7.77, 7.96 (both s, 1 H each, H(11), H(15)); 8.62 (s, 1 H, OH); 12.62 (s, 1 H, NH). $^{13}$C NMR (DMSO-$d_6$), δ: 12.08 (C(10′)); 17.03 (C(14)$\underline{C}H_3$); 20.06, 21.22 (C(8′), C(9′)); 27.04 (C(5′)); 33.29 (C(3′)); 38.94 (C(6′)); 44.68, 44.95 (C(2′), C(4′)); 47.57 (C(1′)); 49.35 (C(7′)); 110.70, 118.07 (C(4), C(7)); 120.50 C(10); 121.23, 121.59 (C(5), C(6)); 123.82, 126.41 (C(11), C(15)); 130.66, 136.22, 143.80 (C(8), C(9), C(12), C(14)); 152.09 (C(13)); 155.92 (C(2)).

**4-(1*H*-Benzo[*d*]imidazol-2-yl)-2,6-di-*tert*-butylphenol (11).**[22] The yield was 70%. $^1$H NMR (DMSO-$d_6$), δ: 1.46 (s, 18 H); 7.08—7.21 (m, 2 H); 7.39—7.70 (m, 2 H); 7.96 (s, 2 H); 12.70 (s, 1 H).

**4-(1*H*-Benzo[*d*]imidazol-2-yl)phenol (12).**[23] The yield was 54%. $^1$H NMR (DMSO-$d_6$), δ: 6.92 (d, 2 H, $J$ = 8.4 Hz); 7.05—7.24 (m, 2 H); 7.39—7.70 (m, 2 H); 8.01 (d, 2 H, $J$ = 8.4 Hz); 9.99 (s, 1 H); 12.66 (s, 1 H).

**Antioxidant activity** of compounds was evaluated by their ability to inhibit the LPO processes in the substrate obtained from the brain of laboratory mice.[34,35] The brain was homogenized (10%) in physiological solution (0.9% aqueous NaCl, pH 7.4) and centrifuged for 10 min. Then the supernatant (S1) was collected, which contained water, proteins, DNA, RNA, and lipids. The test compounds were added to the supernatant as solutions in acetone (a final concentrations of 10 and 100 μmol $L^{-1}$). After 30 min, LPO was initiated by the addition of freshly prepared $FeCl_2$ and ascorbic acid,[36] the test samples were incubated for 1 h at 37 °C with slow stirring. The TBA-RS content was determined on a Thermo Spectronic Genesys 20 spectrophotometer at λ = 532 nm; the extinction coefficient ε used for calculations was equal to $1.56 \cdot 10^5$ L $mol^{-1}$ $cm^{-1}$ (see Refs 35, 37, and 38).

**Radical scavenging activity** of the compounds was evaluated by their ability to react with DPPH.[39] The test compounds were added to a solution of DPPH in MeOH (a final concentration 100 μmol $L^{-1}$) with stirring, 30 min later the optical density of the solution was measured at λ = 517 nm on a Thermo Spectronic Genesys 20 spectrophotometer.

**Study of hemolytic activity (cytotoxicity), membrane-protective, and antioxidant properties.** A suspension of the red blood cells of laboratory mice in phosphate-buffered saline (pH 7.4) was used for evaluation. The toxicity of the compounds was evaluated in the *in vitro* model by their ability to induce hemolysis of erythrocytes. Solutions of the compounds in acetone were added to a suspension of red blood cells at a final concentration of 10 μmol $L^{-1}$ and incubated for 5 h at 37 °C in a thermostated shaker.

Membrane-protective and antioxidant activities were determined by the degree of inhibition of induced hemolysis, inhibition of accumulation of secondary LPO products, and oxidation of oxyhemoglobin (oxyHb) in erythrocytes. For this purpose, hemolysis was initiated with a solution of $H_2O_2$ (0.006%) 30 min after the addition of solutions of test compounds to the suspension of red blood cells (final concentrations 1 and 0.1 μmol $L^{-1}$). The reaction mixture was incubated in a thermostated shaker with slow stirring at 37 °C for 5 h. Every 60 min, an aliquot was collected from the incubated medium, centrifuged for 5 min (1600 g), the degree of hemolysis was determined by the hemoglobin content in the supernatant on a Thermo Spectronic Genesys spectrophotometer 20 at λ = 524 nm.[40] The percentage of hemolysis was calculated relatively to the complete hemolysis of the sample. The content of TBA-RS was determined spectrophotometrically as described above. The accumulation of oxidation products of hemoglobin was evaluated by the analysis of the absorption spectrum in the range λ = 540—640 nm. The contents of oxyHb and methemoglobin (metHb) were calculated taking into account the corresponding extinction coefficients.[41] To evaluate the concentration of heme degradation products formed in the oxidation of membrane-bound hemoglobin by active forms of oxygen, we used $I_{fl}$ at a maximum of λ = 472 nm (a Fluorat-02-Panorama spectrofluorometer, excitation at λ = 321 nm, emission at λ = 400—600 nm, the spectrum recording step 2 nm).[42—44] Each experiment was repeated 4—6 times. Statistical data processing was performed using Microsoft Office Excel 2007, 2010 and Statistica 6.0 software packages.

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