Synthesis and biological activity of isoalantolactone—tryptamine conjugates

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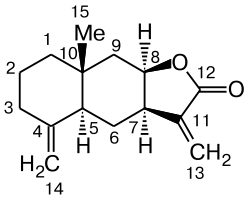
Previously unknown adducts of substituted tryptamines with sesquiterpene lactones, *viz.*, isoalantolactone and its epoxy derivative, were synthesized by the Michael reaction. The compounds obtained were tested for various types of biological activity.

Key words: isoalantolactone, sesquiterpenes, lactones, tryptamines, Michael reaction, biologically active compounds.

Natural unsaturated γ-lactones found among secondary metabolites of the most part of angiosperm plants have a wide spectrum of biological activity, for example, antitumor,[1] antiinflammatory, antibacterial, and antiprotozoal properties.[2] The structure—biological activity relationship has recently been studied for this series.[3] The modification of natural γ-lactones can provide novel drugs and solve the problem of their poor solubility in water and polar solvents. To study possibilities of this modification, we chose easily accessible objects: isoalantolactone (**1**) isolated[4] from elecampane roots (*Inula helenium* L.: Asteraceae) and its epoxide **2**. The latter has been synthesized previously[5] by the selective oxidation of the exocyclic double bond of the perhydronaphthalene fragment in lactone **1**.*



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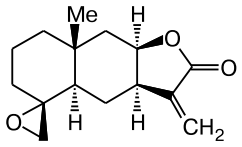

2

It should be mentioned that the biological properties of isoalantolactone **1** are well studied: it is used as an antiinflammatory and antiulcerogenic agents[6] and has a high cytotoxic activity.[7,8]

The lactone cycle of compounds **1** and **2** contains the exocyclic double bond activated by the carbonyl group. One of the routes for modifying such derivatives seems to be the Michael reaction, *viz.*, addition of nucleophiles to electron-deficient alkenes.[9] We used substituted tryptamines (3-(2-aminoethyl)indoles) to introduce an additional pharmacophoric fragment as an N-nucleophile into the lactone molecule. The tryptamines are classified as physiologically active indole alkaloids and are intensively studied as neuromodulators.[10]

The reactions of lactones **1** and **2** with unsubstituted (**3a**) and substituted (**3b—j**) tryptamines occur on storage of the reactants in methanol at ambient temperature for several hours. The reaction is highly stereoselective: only one stereoisomer of the corresponding 13-tryptamine derivative of 11,13-dihydroisoalantolactone **4** or **5** is formed (Scheme 1).

The synthesis of adducts **4a**,**b** and **5a**,**b** has been described earlier.[11] The hitherto unknown adducts of isoalantolactone **2** with other tryptamine derivatives **3c—j** were synthesized to expand the scope of studied compounds with the aim to study the structure—biological activity relationship (see Scheme 1). Compounds **3c—j** contain various substituents in the benzene ring (Me, Bu^n, Bu^s, Cl, CF_3) and/or methyl group in position 2 of the pyrrole cycle. The duration of the reaction between lactones **1** or **2** and tryptamines **3** was almost independent of the nature of substituents in their molecules. Preparative HPLC was used for the isolation and purification of the obtained products **4c—j**.

An analysis of the ^{1}H NMR spectra combined with ^{1}H-^{1}H COSY experiments proved the structures of the synthesized compounds.

* Hereinafter traditional names and numeration of atoms in lactones are used.

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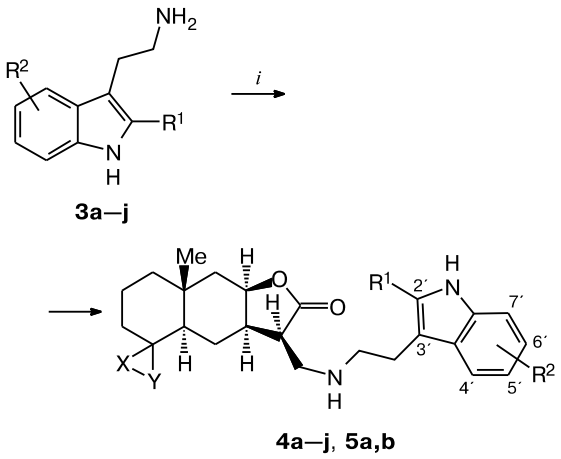
Scheme 1



i. **1** or **2**, MeOH, 20 °C.

X + Y = CH_2 (**1**, **4**), OCH_2 (**2**, **5**); R^1 = H, R^2 = H (**a**), 5-MeO (**b**), 5-Bu^n (**e**); R^1 = Me, R^2 = 5-BnO (**c**), 5-MeO (**d**), 7-CF_3 (**f**), H (**g**), 5-Bu^s (**h**), 5-Cl (**i**), 5,7-Me_2 (**j**).

As should be expected, the ^{1}H NMR spectra of derivatives **4c—j** exhibit signals from the protons of the tryptamine moiety (ethylene bridge and heteroaromatic protons in the low field) and signals from the protons at the C(11) and C(13) atoms at δ 2.8—3.0, whereas the signals characteristic of the exocyclic $=CH_2$ group (doublets at δ 5.56 and 6.08) are absent.[5] For example, the ^{1}H NMR spectrum of compound **4b** contains pronounced signals from the protons at the C(13) atom as a doublet of doublets at δ 2.83 and 3.08 with a high geminal spin-spin coupling constant (interaction with each other, J_1 = = 11.74 Hz) and vicinal coupling constants for the interaction with the proton at the C(11) atom (J_2 = 7.34 and 7.04 Hz). The signal from the H(11) proton (δ 2.34) is a doublet of doublet of doublet of doublets in which a far-range constant of interaction with the axial proton at the C(9) atom and with the proton at the C(3) atom can be observed. The spectrum also exhibits signals from the protons of the tryptamine fragments $NC\underline{H}_2CH_2$ at δ 3.0—3.05 and $NCH_2C\underline{H}_2$ at δ 2.98, as well as the well resolved signals from the heteroaromatic protons of tryptamine: the signals from the protons at the C(2′) and C(4′) atoms as a doublet of doublets at δ 7.05 (J = 2.35 Hz), at the C(6′) atom as a doublet of doublets at δ 6.86 (J_1 = 8.51 Hz, J_2 = 2.35 Hz), at the C(7′) atom as a doublet of doublets at δ 7.25 (J = 8.80 Hz), and the broadened signal from the indole NH group (δ 7.95), which confirms the structure proposed for compound **4b**.

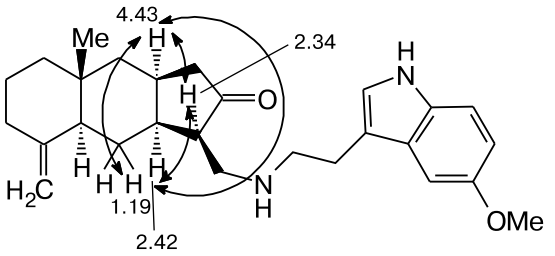


Fig. 1. Main correlations in the 2D ^{1}H-^{1}H NOESY NMR spectrum of compound **4b**. Chemical shifts are given in the δ scale.

Table 1. Effect of compounds **1**, **2**, **4a—c**,**e**,**g—i**, and **5a** on the peroxide oxidation of lipids from the rat brain homogenate initiated by the Fe^{3+} ion and *tert*-butyl hydroperoxide and their Fe^{2+}-chelating and antiradical activity

Compound	Inhibition of POL		Fe^{2+}-chelating activity[a,b] (%)	Antiradical activity (DPPH test)[a] (%)
	Inductor of Fe^{3+}, IC_{50}/μmol L^{-1}	Inductor of tBHP[a] (%)		
1	Prooxidant	—[c]	—[c]	—[c]
2	Prooxidant	—[c]	—[c]	—[c]
4a	31.2±11.0	—[c]	85.5±4.3 (30.6±3.2)[b]	—[c]
4b	13.9±1.1	—[c]	81.5±2.6 (40.2±2.9)[b]	—[c]
4c	4.2±2.4	39.8±4.7	25.4±5.1	35.3±0.9
4e	3.9±0.4	32.5±3.1	34.7±0.7	28.2±0.7
4g	5.0±1.0	22.4±5.4	42.7±3.1	14.7±2.9
4h	4.3±0.4	46.9±6.4	32.4±2.5	19.4±0.4
4i	7.7±0.6	31.4±2.5	39.7±0.9	<10
5a	31.9±10.1	—[c]	59.8±4.3 (83.02±4.3)[b]	—[c]

[a] The concentration of the compound is 100 μmol L^{-1}.
[b] The effective concentration EC_{50} (μmol L^{-1}) is given in parentheses.
[c] Under the experimental conditions, the corresponding activity was not observed.

Stereochemistry of the new asymmetric center at the C(11) carbon atom was determined from the data of 2D ^{1}H-^{1}H COSY and ^{1}H-^{1}H NOESY NMR experiments. The main correlations in the NOESY spectrum of the alicyclic fragment are presented in Fig. 1 for compound **4b** as an example. The intense cross-peak between the H(7) (δ_H 2.42, td, $J_1 = J_2 = 6.16$ Hz, $J_3 = 4.11$ Hz) and H(8) protons (δ_H 4.43) in the NOESY spectrum indicates that the five-membered lactone ring is *cis*-fused to the cyclohexane ring. The NOESY spectrum exhibits the pronounced cross-peaks between the H(8), H(7), and H(11) protons, indicating α-orientation of the C(11)—C(13)

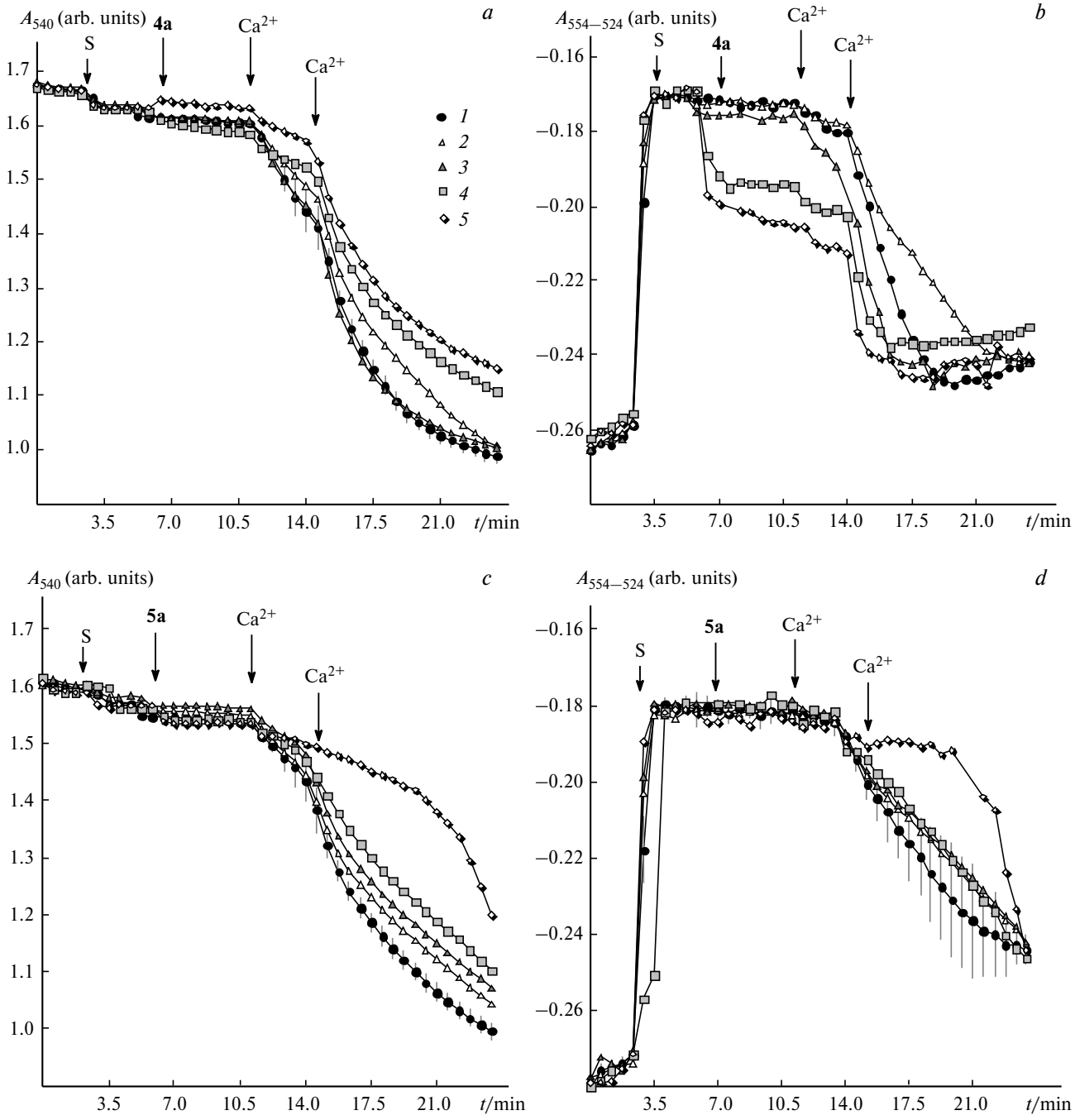


Fig. 2. Effect of adducts **4a** (*a*, *b*) and **5a** (*c*, *d*) on the nonspecific permeability (*a*, *c*) and membrane potential (*b*, *d*) of mitochondria. The moments of addition of the substrate (S), adducts, and calcium ions are shown by arrows. Concentrations of added adducts **4a** and **5a**: 0 (*1*), 1 (*2*), 10 (*3*), 50 (*4*), and 100 μmol L^{-1} (*5*). Concentration of $CaCl_2$: 6.25 nmol mL^{-1} (first addition) and 12.5 nmol mL^{-1} (second addition).

bond in the addition product of tryptamine to the lactone molecule. This stereoconfiguration is also confirmed by the NOE correlation in the spectrum between the β-H(5) proton (δ_H 1.73, br.d, J = 12.32 Hz), axial H(6) proton, and β-proton at C(11).

The effect of the starting compounds **1**, **2**, and **3a** and obtained products **4a—c**,**e**,**g—j** and **5a** on the peroxide oxidation of lipids (POL) of the rat brain homogenate initiated by the Fe^{3+} ion or *tert*-butyl hydroperoxide (tBHP) was also studied (Table 1).

The starting unsaturated γ-lactones, *viz.*, isoalantolactone (**1**) and its epoxidated derivative **2**, exhibit no antioxidant activity; moreover, isoalantolactone and epoxy isoalantolactone enhance the intensity of the Fe^{3+}-induced POL, *i.e.*, they are prooxidants. Tryptamine **3a** does not exert antioxidant activity under our experimental conditions. It has earlier been shown[12] that tryptamine **3a** does not almost exhibit antioxidant activity: it is active only in millimolar concentrations (IC_{50} = 0.84 mmol L^{-1}). The more unexpected is the fact that the studied tryptamine adducts **4** and **5** efficiently suppress the Fe^{3+}-induced POL (see Table 1), and for compounds **4c**,**e**,**g—i** the activity is comparable with that of the known antioxidant ionol.[13] All adducts are chelating agents for the iron ion, although no direct correlation is observed between the antioxidant activity and ability to bind Fe^{2+} ions. The tryptamine derivatives of lactones are considerably less active towards the tBHP-induced POL. The antioxidant effect was revealed only for adducts **4c**,**e**,**g—i** (as well as for unsubstituted tryptamine **3a**) in the concentration 100 μmol L^{-1}. These compounds also exert antiradical activity in the tests with the model stable radical diphenylpicrylhydrazyl (DPPH).

The study of the effect of the synthesized tryptamine adducts on the functional characteristics of mitochondria (Fig. 2) revealed that epoxy isoalantolactone **2** and its derivatives exerted no effect on the membrane potential of mitochondria, whereas isoalantolactone **1** and its tryptamine adducts induce the dose-dependent depolarization of mitochondria and, correspondingly, prevent the potential-dependent incorporation of calcium ions into mitochondria. Evidently, it is this phenomenon that is responsible for the suppression of mitochondria "swelling" in the presence of isoalantolactone or its tryptamine adducts upon the addition of calcium ions to a suspension of mitochondria, which is a characteristic of the appearance of nonspecific permeability of mitochondria. The typical curves showing the effect of compounds **4a** and **5a** on the membrane potential of mitochondria are presented in Figs 2, *b* and *d*; other adducts **4** and **5** have similar dependences. The suppression of "swelling" of mitochondria upon the addition of exogenic calcium in the presence of isoalantolactone and its analogs, which correlates with a decrease in the potential of mitochondria, does not qualitatively differ from that presented in Fig. 2, *a* for adduct **4a**. In the series of tryptamine derivatives of isoalantolactone, the suppression of calcium-induced "swelling" of mitochondria was observed only for compound **5a** (see Fig. 2, *c*). The data obtained suggest that the further search for compounds increasing stability of mitochondria to the induction of nonspecific permeability of mitochondria in the series of the tryptamine adducts of epoxy isoalantolactone and isoalantolactone (to a less extent) can reveal leader compounds, potential neuroprotectors.

Thus, the obtained tryptamine derivatives of isoalantolactones are interesting as efficient antioxidants, chelating agents for the iron ion, and mitoprotectors. The use of these compounds seems promising for the development of novel neuroprotectors and, possibly, cytoprotectors of a wide range of effect.

Experimental

High-resolution mass spectra were recorded on a Thermo Fisher Exactive mass spectrometer with an ORBITRAP mass analyzer, the orthogonal ion injection, and an electrospray ionization. Solutions of the starting compounds in acetonitrile with the concentration ~10^{-5} mol L^{-1} were taken for ionization, and the obtained values of m/z corresponded to the peak of the protonated molecular ion. Specific rotation was measured on a Model 341 polarimeter (Perkin—Elmer), the values of rotation were expressed in deg mL g^{-1} dm^{-1}, and the solution concentrations were expressed in g (100 mL)$^{-1}$. IR spectra were recorded on a Bruker ZFS-113 instrument in KBr pellets. The ^{1}H NMR spectra of compounds **4c—j** were measured on a Bruker DPX 200 (200.13 MHz) instrument and those of compound **4b** were obtained on a Bruker AVANCE III instrument (500.13 MHz) in $CDCl_3$, the signals are given in the δ scale relative to the signal from the residual protons of the solvent (δ 7.25). The ^{1}H-^{1}H COSY and ^{1}H-^{1}H NOESY experiments were carried out on a Bruker AVANCE III instrument (500.13 MHz).

The reaction course and purity of the synthesized compounds were monitored by TLC on the Silufol UV-254 plates (eluent benzene—ethyl acetate, 3 : 2) and GLC (chromatograph Chrom 5, column 3600×3 mm packed with Inerton Super 0.125—0.160 mm with 5% XE-60, flame-ionization detector, detector and evaporator temperature 250 °C, temperature-programmed thermostat from 75 to 225 °C). Individual components were isolated by semipreparative HPLC (chromatograph Turbo LC 200 (Perkin—Elmer), UV detection (λ = 254 nm); analytical column 4×100 mm with Kromasil C18 (5 μm); preparative column 10×250 mm with Kromasil C18 (5 μm)); gradient elution was used (eluent A: 0.1% trifluoroacetic acid in distilled water (pH 2.0), eluent B: acetonitrile) with the elution rate 1 and 4 mL min^{-1} for the analytical and preparative columns, respectively.

Reagents from Aldrich, freshly distilled solvents, and pure-grade reagents were used. Isoalantolactone **1** was isolated from roots of *Inula helenium* L. (family Asteraceae); separation was carried out on a column impregnated with silver nitrate as described,[5] and the purity of compounds was monitored by GLC.

Reaction of (epoxy)isoalantolactone with tryptamines (general procedure). A mixture of lactone **1** or **2** (1 mmol) and the corre-

sponding tryptamine **3** (1.1 mmol) was dissolved in methanol with stirring, and the mixture was kept at ambient temperature. After completion of the reaction (TLC monitoring), the solvent was evaporated *in vacuo*, the residue was dissolved in acetonitrile and purified by HPLC. Compounds **4a** and **5a**,**b** have been described earlier.[11]

(11*R*)-13-(5-Methoxytryptamino)-11,13-dihydroisoalantolactone ((3*R*,3a*R*,4a*R*,8a*R*,9a*R*)-3-{[2-(5-methoxy-1*H*-indol-3-yl)ethylamino]methyl}-8a-methyl-5-methylidenedecahydronaphtho[2,3-*b*]furan-2-one) (4b). The physicochemical characteristics of the compound are given in Ref. 11. ^{1}H NMR, δ: 0.77 (s, 3 H, H(15)); 1.18 (m, 1 H, H_a(6)); 1.24 (m, 1 H, H_a(1)); 1.44 (dd, 1H, H_a(9), J_1 = 15.55 Hz, J_2 = 4.40 Hz); 1.47—1.55 (m, 2 H, H(2)); 1.58 (m, 2 H, H_b(1), H_b(6)); 1.73 (br.d, 1 H, H(5), J = 12.32 Hz); 1.98 (m, 1 H, H_a(3)); 2.15 (dd, 1 H, H_b(9), J_1 = = 15.55 Hz, J_2 = 2.05 Hz); 2.34 (dddd, 1 H, H(11), J_1 = 2.35 Hz, J_2 = 4.11 Hz, J_3 = 6.16 Hz, J_4 = 12.73 Hz); 2.42 (td, 1 H, H(7), J_1 = J_2 = 6.16 Hz, J_3 = 4.11 Hz); 2.83 (dd, 1 H, H_a(13), J_1 = = 11.74 Hz, J_2 = 7.34 Hz); 2.92 (q, 1 H, H_b(3), J_1 = J_2 = J_3 = = 6.75 Hz); 2.98 (q, 2 H, $NCH_2C\underline{H}_2$, J_1 = J_2 = J_3 = 2.93 Hz); 3.0—3.05 (m, 2 H, $NHC\underline{H}_2CH_2$); 3.08 (dd, 1 H, H_b(13), J_1 = 11.74 Hz, J_2 = 7.04 Hz); 3.87 (s, 3 H, OMe); 4.40 (d, 1 H, H_a(14), J = 1.17 Hz); 4.43 (m, 1 H, H(8)); 4.73 (d, 1 H, H_b(14), J = 1.47 Hz); 6.86 (dd, 1 H, H(6′), J_1 = 8.51 Hz, J_2 = 2.35 Hz); 7.05 (s, 1 H, H(2′), 7.06 (s, 1 H, H(4′)); 7.25 (d, 1 H, H(7′), J = 8.80 Hz); 7.95 (br.s, 1 H, NH).

(11*R*)-13-(5-Benzyloxy-2-methyltryptamino)-11,13-dihydroisoalantolactone ((3*R*,3a*R*,4a*R*,8a*R*,9a*R*)-3-{[2-(5-benzyloxy-2-methyl-1*H*-indol-3-yl)ethylamino]methyl}-8a-methyl-5-methylidenedecahydronaphtho[2,3-*b*]furan-2-one) (4c). The yield was 46%, m.p. 102—104 °C, $[\alpha]_D^{20}$ +32° (*c* 0.1; $CHCl_3$). MS (ESI), *m/z*: 513.3117 $[M + H]^+$; calculated for $C_{33}H_{40}N_2O_3$, $[M + H]^+$, *m/z*: 513.3112. IR, ν/cm^{-1}: 1762 (OC=O), 3471 (NH). ^{1}H NMR, δ: 0.77 (s, 3 H, H(15)); 1.19—1.31 (m, 2 H, H(2)); 1.44—1.88 (m, 7 H, H(1), H(3), H(6), H_a(9)); 1.84—2.06 (m, 1 H, H(5)); 2.17 (dd, 1 H, H_b(9), J_1 = 1.5 Hz, J_2 = 15.5 Hz); 2.41 (s, 3 H, C(2′)Me); 2.56—2.75 (m, 3 H, H(7), H(13)); 2.81 (t, 2 H, $NCH_2C\underline{H}_2$, J = 7.4 Hz); 2.91 (t, 2 H, $NC\underline{H}_2CH_2$, J = 7.4 Hz); 3.09 (dt, 1 H, H(11), J_1 = 5.5 Hz, J_2 = 10.9 Hz); 4.44 (d, 1 H, H_a(14), J = 1.4 Hz); 4.50 (m, 1 H, H(8)); 4.80 (d, 1 H, H_b(14), J = 1.4 Hz); 5.14 (br.s, 2 H, OCH_2); 6.88 (dd, 1 H, H(6′), J_1 = 1.9 Hz, J_2 = 8.4 Hz); 7.12 (d, 1 H, H(4′), J = 1.9 Hz); 7.19 (d, 1 H, H(7′), J = 8.4 Hz); 7.26—7.61 (m, 5 H, Ar); 7.82 (br.s, 1 H, NH).

(11*R*)-13-(5-Methoxy-2-methyltryptamino)-11,13-dihydroisoalantolactone ((3*R*,3a*R*,4a*R*,8a*R*,9a*R*)-3-{[2-(2-methyl-5-methoxy-1*H*-indol-3-yl)ethylamino]methyl}-8a-methyl-5-methylidenedecahydronaphtho[2,3-*b*]furan-2-one) (4d). The yield was 76%, m.p. 111—112 °C, $[\alpha]_D^{20}$ +75° (*c* 0.1; $CHCl_3$). MS (ESI), *m/z*: 437.2779 $[M + H]^+$, calculated for $C_{27}H_{36}N_2O_3$, $[M + H]^+$, *m/z*: 437.2799. IR, ν/cm^{-1}: 1762 (OC=O), 3470 (NH). ^{1}H NMR, δ: 0.73 (s, 3 H, H(15)); 1.19—1.31 (m, 2 H, H(2)); 1.44—1.88 (m, 7 H, H(1), H(3), H(6), H_a(9)); 1.84—2.06 (m, 1 H, H(5)); 2.08 (dd, 1 H, H_b(9), J_1 = 2.1 Hz, J_2 = 15.3 Hz); 2.66—2.75 (m, 3 H, H(7), H(13)); 2.32 (s, 3 H, C(2′)Me); 2.84 (t, 2 H, $NCH_2C\underline{H}_2$, J = 6.5 Hz); 2.98 (m, 1 H, H(11)); 3.02 (t, 2 H, $NC\underline{H}_2CH_2$, J = 6.5 Hz); 3.79 (s, 3 H, OMe); 4.34 (d, 1 H, H_a(14), J = 1.4 Hz); 4.38 (m, 1 H, H(8)); 4.70 (d, 1 H, H_b(14), J = 1.4 Hz); 6.70 (dd, 1 H, H(6′), J_1 = 2.3 Hz, J_2 = 8.6 Hz); 6.92 (d, 1 H, H(4′), J = 2.1 Hz); 7.08 (d, 1 H, H(7′), J = 8.6 Hz); 7.68 (br.s, 1 H, NH).

(11*R*)-13-(5-Butyltryptamino)-11,13-dihydroisoalantolactone ((3*R*,3a*R*,4a*R*,8a*R*,9a*R*)-3-{[2-(5-*n*-butyl-1*H*-indol-3-yl)ethylamino]methyl}-8a-methyl-5-methylidenedecahydronaphtho[2,3-*b*]furan-2-one) (4e). The yield was 96%, m.p. 89 °C, $[\alpha]_D^{20}$ +65° (*c* 0.1; $CHCl_3$). MS (ESI), *m/z*: 449.3167 $[M + H]^+$, calculated for $C_{29}H_{40}N_2O_2$, $[M + H]^+$, *m/z*: 449.3163. IR, ν/cm^{-1}: 1762 (OC=O), 3480 (NH). ^{1}H NMR, δ: 0.73 (s, 3 H, H(15)); 0.89 (t, 3 H, $(CH_2)_3C\underline{H}_3$, J = 7.0 Hz); 0.98—1.47 (m, 7 H, H(1), H(2), H(6), H_a(9)); 1.47—1.74 (m, 5 H, H(5), $CH_2(C\underline{H}_2)_2CH_3$); 1.97—2.01 (m, 2 H, H(3)); 2.08 (dd, 1 H, H_b(9), J_1 = 1.2 Hz, J_2 = 16.0 Hz); 2.26 (m, 1 H, H(7)); 2.28 (br.d, 2 H, H(13), J = 11.0 Hz); 2.70 (dt, 2 H, $C\underline{H}_2(CH_2)_2Me$, J_1 = 7.6 Hz, J_2 = 14.9 Hz); 2.89 (t, 2 H, $NCH_2C\underline{H}_2$, J = 6.1 Hz); 3.00 (t, 2 H, $NC\underline{H}_2CH_2$, J = 6.1 Hz); 3.09 (dd, 1 H, H(11), J_1 = 5.3 Hz, J_2 = 11.0 Hz); 4.36 (d, 1 H, H_a(14), J = 1.4 Hz); 4.40 (m, 1 H, H(8)); 4.71 (d, 1 H, H_b(14), J = 1.4 Hz); 6.95 (br.s, 1 H, H(4′)); 6.97 (d, 1 H, H(6′), J = 6.5 Hz); 7.19 (d, 1 H, H(7′), J = 6.5 Hz); 7.35 (s, 1 H, H(2′)); 8.06 (br.s, 1 H, NH).

(11*R*)-13-(7-Trifluoromethyl-2-methyltryptamino)-11,13-dihydroisoalantolactone ((3*R*,3a*R*,4a*R*,8a*R*,9a*R*)-3-{[2-(7-trifluoromethyl-2-methyl-1*H*-indol-3-yl)ethylamino]methyl}-8a-methyl-5-methylidenedecahydronaphtho[2,3-*b*]furan-2-one) (4f). The yield was 39%, m.p. 85—86 °C, $[\alpha]_D^{20}$ +27° (*c* 0.1; $CHCl_3$). MS (ESI), *m/z*: 475.2571 $[M + H]^+$, calculated for $C_{27}H_{33}F_3N_2O_2$, $[M + H]^+$, *m/z*: 475.2567. IR, ν/cm^{-1}: 1090 (CF), 1762 (OC=O), 3471 (NH). ^{1}H NMR, δ: 0.76 (s, 3 H, H(15)); 1.12—1.31 (m, 2 H, H(2)); 1.34—1.88 (m, 7 H, H(1), H(3), H(6), H_a(9)); 2.02 (m, 1 H, H(5)); 2.12 (dd, 1 H, H_b(9), J_1 = 2.2 Hz, J_2 = 14.7 Hz); 2.37 (m, 1 H, H(7)); 2.42 (s, 3 H, C(2′)Me); 2.67—2.91 (m, 2 H, H(13)); 2.84 (t, 2 H, $NCH_2C\underline{H}_2$, J = 5.7 Hz); 2.98 (t, 2 H, $NC\underline{H}_2CH_2$, J = 5.7 Hz); 3.04 (m, 1 H, H(11)); 4.37 (d, 1 H, H_a(14), J = 1.3 Hz); 4.42 (m, 1 H, H(8)); 4.73 (d, 1 H, H_b(14), J = 1.3 Hz); 7.10 (t, 1 H, H(5′), J = 7.4 Hz); 7.33 (d, 1 H, H(6′), J = 7.4 Hz); 7.65 (d, 1 H, H(4′), J = 7.4 Hz); 8.21 (br.s, 1 H, NH).

(11*R*)-13-(2-Methyltryptamino)-11,13-dihydroisoalantolactone ((3*R*,3a*R*,4a*R*,8a*R*,9a*R*)-3-{[2-(2-methyl-1*H*-indol-3-yl)ethylamino]methyl}-8a-methyl-5-methylidenedecahydronaphtho[2,3-*b*]furan-2-one) (4g). The yield was 83%, m.p. 83—85 °C, $[\alpha]_D^{20}$ +98° (*c* 0.1; $CHCl_3$). MS (ESI), *m/z*: 407.2697 $[M + H]^+$, calculated for $C_{26}H_{34}N_2O_2$, $[M + H]^+$, *m/z*: 407.2693. IR, ν/cm^{-1}: 1760 (OC=O), 3480 (NH). ^{1}H NMR, δ: 0.81 (s, 3 H, H(15)); 1.05—1.35 (m, 2 H, H(2)); 1.34—1.87 (m, 7 H, H(1), H(3), H(6), H_a(9)); 2.00 (m, 1 H, H(5)); 2.12 (d, 1 H, H_b(9), J = 15.5 Hz); 2.25—2.39 (m, 3 H, H(7), H(13)); 2.41 (s, 3 H, C(2′)Me); 2.89 (t, 2 H, $NCH_2C\underline{H}_2$, J = 6.1 Hz); 2.98 (t, 2 H, $NC\underline{H}_2CH_2$, J = 6.1 Hz); 3.09 (dd, 1 H, H(11), J_1 = 2.7 Hz, J_2 = 10.0 Hz); 4.44 (d, 1 H, H_a(14), J = 1.4 Hz); 4.49 (m, 1 H, H(8)); 4.80 (d, 1 H, H_b(14), J = 1.4 Hz); 7.12 (dt, 2 H, H(5′), H(6′), J_1 = 7.6 Hz, J_2 = 10.2 Hz); 7.28 (d, 1 H, H(7′), J = 7.6 Hz); 7.54 (d, 1 H, H(4′), J = 7.6 Hz); 8.14 (br.s, 1 H, NH).

(11*R*)-13-[5-(But-2-yl)-2-methyltryptamino]-11,13-dihydroisoalantolactone ((3*R*,3a*R*,4a*R*,8a*R*,9a*R*)-3-{2-[5-(but-2-yl)-2-methyl-1*H*-indol-3-yl)ethylamino)-8a-methyl-5-methylidenedecahydronaphtho[2,3-*b*]furan-2-one) (4h). The yield was 83%, m.p. 83—85 °C, $[\alpha]_D^{20}$ +38° (*c* 0.1; $CHCl_3$). MS (ESI), *m/z*: 463.3325 $[M + H]^+$; calculated for $C_{30}H_{42}N_2O_2$, $[M + H]^+$, *m/z*: 463.3319. IR, ν/cm^{-1}: 1761 (OC=O), 3470 (NH). ^{1}H NMR, δ: 0.73 (s, 3 H, H(15)); 0.78 (t, 3 H, $CH_2C\underline{H}_3$, J = 7.2 Hz); 1.22

(d, 3 H, CHC$\underline{H}_3$, J = 6.8 Hz); 1.27—1.98 (m, 10 H, H(1), H(2), H(3), H(5), H(6), H_a(9)); 1.61 (dq, 2 H, CHC$\underline{H}_2$CH$_3$, J_1 = 7.2 Hz, J_2 = 14.5 Hz); 2.07 (dd, 1 H, H_b(9), J_1 = 2.1 Hz, J_2 = 7.5 Hz); 2.25 (m, 1 H, H(7)); 2.32 (s, 3 H, C(2′)Me); 2.60 (dd, 1 H, C$\underline{H}$Me, J_1 = 6.8 Hz, J_2 = 12.9 Hz); 2.62—2.75 (m, 2 H, H(13)); 2.83 (t, 2 H, NCH$_2$C$\underline{H}_2$, J = 7.2 Hz); 2.89 (t, 2 H, NC$\underline{H}_2$CH$_2$, J = 7.2 Hz); 3.00 (m, H(11), 1 H); 4.34 (d, 1 H, H_a(14), J = 1.4 Hz); 4.41 (m, 1 H, H(8)); 4.70 (d, 1 H, H_b(14), J = 1.4 Hz); 6.89 (d, 1 H, H(6′), J = 4.0 Hz); 7.12 (d, 1 H, H(7′), J = 4.0 Hz); 7.24 (s, 1 H, H(4′)); 7.68 (br.s, 1 H, NH).

(11*R*)-13-(5-Chloro-2-methyltryptamino)-11,13-dihydroisoalantolactone ((3*R*,3a*R*,4a*R*,8a*R*,9a*R*)-3-{[2-(5-chloro-2-methyl-1*H*-indol-3-yl)ethylamino]methyl}-8a-methyl-5-methylidenedecahydronaphtho[2,3-*b*]furan-2-one) (4i). The yield was 64%, m.p. 95—96 °C, $[\alpha]_D^{20}$ +53° (*c* 0.1; $CHCl_3$). MS (ESI), *m/z*: 441.2312 $[M + H]^+$; calculated for $C_{26}H_{33}ClN_2O_2$, $[M + H]^+$, *m/z*: 441.2303. IR, ν/cm^{-1}: 850 (CCl), 1762 (OC=O), 3472 (NH). ^{1}H NMR, δ: 0.81 (s, 3 H, H(15)); 1.10—1.32 (m, 2 H, H(2)); 1.37—1.89 (m, 7 H, H(1), H(3), H(6), H_a(9)); 1.84—2.06 (m, 1 H, H(5)); 2.17 (d, 1 H, H_b(9), J = 16.0 Hz); 2.40 (s, 3 H, C(2′)Me); 2.66—2.75 (m, 3 H, H(7), H(13)); 2.83 (t, 2 H, NCH$_2$C$\underline{H}_2$, J = 6.4 Hz); 2.94 (m, 1 H, H(11)); 3.05 (t, 2 H, NC$\underline{H}_2$CH$_2$, J = 6.4 Hz); 4.44 (d, 1 H, H_a(14), J = 1.3 Hz); 4.48 (m, 1 H, H(8)); 4.79 (d, 1 H, H_b(14), J = 1.32 Hz); 7.07 (d, 1 H, H(6′), J = 8.4 Hz); 7.18 (d, 1 H, H(7′), J = 8.4 Hz); 7.49 (br.s, 1 H, H(4′)), 8.16 (br.s, 1 H, NH).

(11*R*)-13-(2,5,7-Trimethyltryptamino)-11,13-dihydroisoalantolactone ((3*R*,3a*R*,4a*R*,8a*R*,9a*R*)-3-{[2-(2,5,7-trimethyl-1*H*-indol-3-yl)ethylamino]methyl}-8a-methyl-5-methylidenedecahydronaphtho[2,3-*b*]furan-2-one) (4j). The yield was 70%, m.p. 138—140 °C, $[\alpha]_D^{20}$ +110° (*c* 0.1; $CHCl_3$). MS (ESI), *m/z*: 435.3021 $[M + H]^+$; calculated for $C_{28}H_{38}N_2O_2$, $[M + H]^+$, *m/z*: 435.3006. IR, ν/cm^{-1}: 1762 (OC=O), 3475 (NH). ^{1}H NMR, δ: 0.76 (s, 3 H, H(15)); 1.19—1.31 (m, 2 H, H(2)); 1.34—1.92 (m, 7 H, H(1), H(3), H(6), H_a(9)); 1.94—2.06 (m, 1 H, H(5)); 2.11 (dd, 1 H, H_b(9), J_1 = 1.5 Hz, J_2 = 14.5 Hz); 2.38 (s, 9 H, C(2′)Me, C(5′)Me, C(7′)Me); 2.82 (t, 2 H, NCH$_2$C$\underline{H}_2$, J = 6.0 Hz); 2.83—2.90 (m, 3 H, H(7), H(13)); 2.94 (t, 2 H, NC$\underline{H}_2$CH$_2$, J = 6.0 Hz); 3.04 (dd, 1 H, H(11), J_1 = 5.3 Hz, J_2 = 10.4 Hz); 4.37 (d, 1 H, H_a(14), J = 1.3 Hz); 4.43 (m, 1 H, H(8)); 4.74 (d, 1 H, H_b(14), J = 1.3 Hz); 6.73 (br.s, 1 H, H(6′)); 7.13 (br.s, 1 H, H(4′)); 7.65 (br.s, 1 H, NH).

Effect of compounds 4 and 5 on the peroxide oxidation of lipids in the rat brain homogenates. Males of white nonlinear rats (300—400 g) were used in experiments. The animals had free access to food and water. The animals were pre-narcotized with carbon dioxide and decapitated with a guillotine. The withdrawn brain was homogenized in cold in a solution containing 120 mmol L^{-1} KCl—20 mmol L^{-1} Hepes (pH 7.6). To obtain the subcellular fraction, the brain homogenate was centrifuged at 1500 *g*, and the supernatant was tested. The prepared brain homogenate was used in experiment on the same day. The protein in the rat brain homogenate was determined using the biuretic method.[14]

The POL intensity of the rat brain homogenate was determined by the modified TBARS assay test using Fe^{3+} ions ($Fe(NH_4)(SO_4)_2$, C = 0.5 mmol L^{-1}) or *tert*-butyl hydroperoxide as an inductor.[15]

Chelating activity of compounds 4 and 5 towards Fe^{2+} ions was determined by a standard procedure using ferrozine.[16] The substances under study are not bound with ferrozine, which was confirmed by the UV spectra of ferrozine in the pure form and in the presence of the studied substance recorded in the 300—800 nm range.

Radical-binding activity of compounds 4 and 5 were determined using the model stable radical diphenylpicrylhydrazyl (DPPH) by a known procedure.[17]

Effect of compounds 4 and 5 on the functional characteristics of mitochondria. Rat brain mitochondria were isolated by a standard method of differential centrifugation. The effect of compounds **4** and **5** on the functional characteristics of mitochondria were examined by two parameters: a change in the membrane potential and calcium-induced "swelling" of mitochondria caused by a change in the mitochondrial shape due to opening of special pores of nonspecific permeability. Mitochondrial "swelling" was measured as a decrease in the absorbance (A_{540}) of a suspension of mitochondria after the addition of calcium ions (Fig. 2, *a*, *c*). The membrane potential of mitochondria was measured with the potential-dependent indicator safranine[18] (Fig. 2, *b*, *d*) in the presence of the inhibitor of complex **I** in the respiratory chain of rotenone mitochondria (1 μmol L^{-1}) after the addition of the substrate of complex **II** succinate (0.5 mmol L^{-1}). The opening of special mitochondrial pores was induced by to successive additions of calcium ions: 6.25 and 12.5 nmol mL^{-1} $CaCl_2$.

The statistical processing of the results was performed using the Excel-5 statistical program package.

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