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ANTIHYPOXIC ACTIVITY OF SEVERAL SCAFFOLD DERIVATIVES OF QUINOPIMARIC ACID

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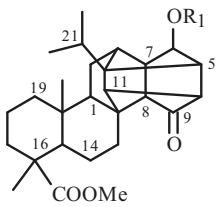
*The antihypoxic activity of a series of quinopimaric acid scaffold derivatives was studied. It was shown that compounds **3**, **5**, and **8** increased the lifespan of mice in various hypoxia models.*

Keywords: normobaric, hemic, histotoxic hypoxia, quinopimaric acid scaffold derivatives.

The brain is exceedingly sensitive to various types of injury. Hypoxia, ischemia, and neurointoxication impair brain functions and decrease the quality of life [1]. The creation of preparations with high antihypoxic activity and other useful effects (nootropic, anti-amnestic, etc.) is advisable for effective therapy of brain pathologies [2]. Adamantane derivatives, e.g., amantadine and memantine preparations, are divided into a separate group of drugs used to treat neurodegenerative diseases [3–7]. Quinopimaric acid scaffold derivatives are pentacyclododecanes and structural analogs of adamantane. In this respect, it seemed interesting to study the adamantane structural analogs, i.e., quinopimaric acid scaffold derivatives produced by photolysis of diene adducts of levopimaric acid and *p*-benzoquinones, for antihypoxic activity.

Pentacycloun- and -dodecane scaffold derivatives are known to have neurophysiological activity. Quinopimaric acid scaffold derivatives are so-called birdcages. Therefore, we synthesized quinopimaric acid scaffold derivatives **1**–**8** in order to discover compounds with antihypoxic activity.



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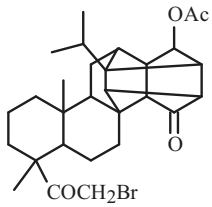

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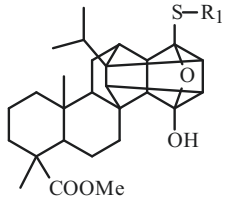

4 - 8

1: $R_1 = CO(CH_2)_2CONHC_{18}H_{37}$; **3:** $R_1 = COCF_3$; **4:** $R_1 = CH_2CH_2OH$; **5:** $R_1 = CH_2COOH$
6: R_1 = 4-(4-SH-Ph)-O-Ph; **7:** $R_1 = C_6H_{13}$; **8:** R_1 = Bn

4′-{[(16*R*,20*R*)-4-Isopropyl-16,20-dimethyl-16-methoxycarbonyl-9-oxoheptacyclo[10.8.0.0^{3,7}.0^{4,11}.0^{5,10}.0^{8,12}.0^{15,20}]eicos-6-yl]oxy}-4′-oxobutanoic acid (**1**) was prepared in two steps via the reaction of the methyl ether of the ketoalcohol [8] with succinic anhydride in refluxing Py followed by the reaction of the hemisuccinate with octadecylamine in dioxane with microwave irradiation. Methyl (16*R*,20*R*)-4-isopropyl-16,20-dimethyl-9-oxo-6-[(2′,2′,2′-trifluoroacetyl)oxy] heptacyclo[10.8.0.0^{3,7}.0^{4,11}.0^{5,10}.0^{8,12}.0^{15,20}]eicosane-16-carboxylate (**3**) was prepared via the reaction of the ketoalcohol [8] with trifluoroacetic anhydride. The syntheses of ketobromide derivative **2** and sulfanyl-substituted *oxa*-birdcages **4**–**8** were reported [9, 10].

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TABLE 1. Effect of Quinopimaric Acid Scaffold Derivatives **1**–**8** on Resistance of Mice to Acute Hypoxia (n = 8)

Compound*	Normobaric with hypercapnia		Hemic		Histotoxic	
	1	2	1	2	1	2
Control	37.0 ± 2.92	–	21.8 ± 1.60	–	10.7 ± 0.59	–
1	36.9 ± 1.09	–0.2	24.3 ± 3.48	11.3	14.5 ± 1.84	35.7
2	41.8 ± 3.70	12.9	21.2 ± 1.53	–2.9	11.9 ± 1.25	11.4
3	50.1 ± 1.79**	35.5	26.9 ± 1.53**	23.4	11.0 ± 0.74	2.6
4	43.7 ± 1.28	18.1	22.3 ± 1.94	2.5	11.1 ± 1.09	4.2
5	42.6 ± 2.44	15.1	21.1 ± 2.12	–3.2	13.6 ± 0.93**	27.5
6	37.6 ± 0.61	1.7	18.0 ± 1.85	–17.3	10.6 ± 1.02	–0.5
7	38.2 ± 0.86	3.2	26.6 ± 3.94	22.0	11.4 ± 0.94	6.7
8	45.2 ± 1.26**	22.2	29.0 ± 1.09**	33.2	12.1 ± 1.05	13.2

Lifespan (min) (1) and increase of lifespan (%) (2).
*At a dose of 50 mg/kg; n is the number of animals per group; **statistically significant relative to the control, $P < 0.05$.

Antihypoxic activity of **1**–**8** was studied using three hypoxia models, i.e., normobaric, hemic, and histotoxic. Table 1 presents the experimental results. Screening of the antihypoxic properties of the eight quinopimaric acid scaffold derivatives found that the compounds had different antihypoxic effects depending on the hypoxia model. Thus, compounds **3** and **8** exhibited a distinct protective effect in the normobaric hypoxia model by increasing the lifespan relative to the control by 35.5 ($P < 0.01$) and 22.2% ($P < 0.05$). Compounds **2**, **4**, and **5** showed a tendency to increase the lifespan of the mice under hypoxia by 12.9, 18.1, and 15.1%, respectively (results not statistically significant). The other compounds did not possess noticeable antihypoxic effects.

Compounds **3** and **8** in the acute hemic and normobaric hypoxia models increased the resistance of mice to the action of a methemoglobin former by 23.4 ($P < 0.05$) and 33.2% ($P < 0.05$), respectively. Compound **7** increased the lifespan by 22.0% ($P < 0.05$). Compounds **1** and **4** exhibited weak antihypoxic activity. The other compounds (**2**, **5**, **6**) decreased the resistance of the laboratory animals to sodium nitrite.

Conversely, compounds **1** and **5** showed an antihypoxic effect in the acute histotoxic hypoxia model and increased the lifespan under tissue hypoxia conditions by 35.7 ($P > 0.05$) and 27.5% ($P < 0.02$), respectively. Compounds **2** and **8** increased the lifespan of the animals by an average of 12% ($P > 0.05$). The other compounds exhibited only an insignificant antihypoxic effect (**3**, **4**, **7**) or were completely inactive (**6**).

Thus, screening of eight quinopimaric acid scaffold derivatives for antihypoxic activity discovered two compounds (**3** and **8**) that exhibited antihypoxic activity at a dose of 50 mg/kg in two hypoxia models (normobaric and hemic). Compounds **1** and **5** at the same dose exhibited antihypoxic activity in the histotoxic hypoxia model.

EXPERIMENTAL

^{13}C NMR spectra were recorded from solutions (10–20%) in deuterated solvents with the solvent resonance or TMS as an internal standard on Bruker AM-300 (75.47 MHz) and Avance III 500 (125.75 MHz) instruments. Chemical shifts were given on the δ scale. Two-dimensional correlation spectra were recorded using the standard library of instrument pulse sequences. IR spectra were recorded from thin layers or nujol mulls on a Shimadzu instrument. Elemental analyses were performed on a Euro EA3000 analyzer. Optical rotation angles were measured on a PerkinElmer 341 polarimeter (λ 589 nm) at 20°C. Melting points were uncorrected and were determined on a Boetius apparatus. NMR and IR spectra were recorded on equipment of the Khimiya CCU, UfIC, RAS.

A laboratory system with a Discover focused microwave source in the standard configuration and patented software was used to perform reactions with microwave irradiation. The experiments were carried out in hermetically sealed 10-mm vials. The irradiation frequency was 2455 MHz; input power, 300 ± 10% W.

The course of reactions was monitored by TLC on Sorbfil PTSKh-AF-A plates. Compounds were detected by spraying with H_2SO_4 solution (5%) followed by heating to 100–120°C. The eluents were solvent systems $CHCl_3$–MeOH (50:1, 10:1, 5:1). Column chromatography used standard silica gel 60 (0.063–0.2 mm, 70–230 mesh, Macherey-Nagel, Germany); flash chromatography, standard silica gel 60 (0.04–0.063 mm, 230–400 mesh, Macherey-Nagel, Germany).

Ketobromide derivative **2** and sulfanyl-substituted *oxa*-birdcages **4**–**8** were synthesized as before [9, 10]. Their physical and spectral characteristics agreed with those in the literature.

Methyl (16*R*,20*R*)-4-Isopropyl-16,20-dimethyl-6-{[4′-(octadecylamino)-4′-oxobutanoyl]oxy}-9-oxoheptacyclo[10.8.0^{3,7}.0^{4,11}.0^{5,10}.0^{8,12}.0^{15,20}]eicosane-16-carboxylate (1). *a*. A mixture of ketoalcohol [8] (0.7 g, 1.64 mmol) and succinic anhydride (8.5 mmol) was refluxed in anhydrous Py (25 mL) with 4-Å molecular sieves. The course of the reaction was monitored by TLC. When the reaction was finished, the mixture was poured into H_2O (200 mL) and extracted with $CHCl_3$. The organic phase was washed with HCl (5%) and H_2O, dried over Na_2SO_4, and evaporated at reduced pressure. The product was purified by column chromatography over silica gel using $CHCl_3$–MeOH (20:1). $C_{32}H_{44}O_7$. Yield of hemisuccinate derivative, 87%, mp 70–73°C, $[\alpha]_D^{20}$ +58° (*c* 2.05, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1744, 1722, 1460, 1377, 1244, 1165, 1103, 1026, 754. ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 15.21 (q, Me), 16.51 (q, Me), 16.83 (q, Me), 17.09 (t, C-18), 17.47 (t, C-14), 18.41 (q, Me), 21.01 (t, C-2), 26.48 (d, C-21), 28.62 t, 28.89 t ($2CH_2$), 33.52 (t, C-13), 34.92 (d, C-10), 36.58 (s, C-20), 37.46 (t, C-17), 37.79 (s, C-12), 38.27 (t, C-19), 38.99 (d, C-5), 40.39 (d, C-7), 42.46 (d, C-11), 43.69 (d, C-3), 45.37 (d, C-15), 47.00 (s, C-16), 49.47 (d, C-1), 50.61 (s, C-4), 51.82 (q, COOMe), 57.11 (d, C-8), 75.54 (d, C-6), 170.91 (s, OCO), 177.14 (s, COOH), 178.97 (s, COOMe), 216.91 s (C=O).

b. A mixture of the hemisuccinate derivative (0.34 g, 0.65 mmol) and octadecylamine (0.17 g, 0.65 mmol) in anhydrous dioxane was irradiated by microwaves for 90 min at a maximum temperature of 153°C and pressure 67 psi. The course of the reaction was monitored by TLC. When the reaction was finished, the mixture was evaporated at reduced pressure. The product was purified by flash chromatography over silica gel using $CHCl_3$–MeOH (10:1). $C_{49}H_{79}NO_6$. Yield 75%, mp 75°C, $[\alpha]_D^{20}$ +64° (*c* 2.0, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1746, 1726, 1464, 1377, 1245, 1166, 1103. ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 14.14 (q, Me), 15.35 (q, Me), 16.67 (q, Me), 17.09 (q, Me), 17.25 (t, C-18), 17.72 (t, C-14), 18.61 (q, Me), 21.17 (t, C-2), 22.70 (t, C-17″), 26.65 (d, C-21), 26.73 (t, C-3″), 28.32 (t, C-2″), 29.27, 29.39, 29.62, 29.69, 29.77, 30.96, 31.94, 32.36 (t, $15CH_2$), 33.72 (t, C-13), 35.08 (d, C-10), 36.73 (t, C-17), 37.60 (s, C-20), 37.97 (t, C-19), 38.39 (s, C-12), 39.09 (d, C-5), 39.57 (t, NHCH″$_2$), 40.53 (d, C-7), 42.58 (d, C-11), 43.86 (d, C-3), 45.57 (d, C-15), 47.14 (s, C-16), 49.63 (d, C-1), 50.75 (s, C-4), 51.93 (q, COOMe), 57.31 (d, C-8), 75.02 (d, C-6), 172.59 (s, OCO), 178.82 (s, CONH), 179.01 (s, COOMe), 216.54 (s, C=O).

Methyl (16*R*,20*R*)-4-Isopropyl-16,20-dimethyl-9-oxo-6-[(2′,2′,2′-trifluoroacetyl)oxy]heptacyclo[10.8.0^{3,7}.0^{4,11}.0^{5,10}.0^{8,12}.0^{15,20}]eicosane-16-carboxylate (3). A mixture of ketoalcohol [8] (0.8 g, 1.88 mmol), trifluoroacetic anhydride (0.52 mL, 3.75 mmol), and Et_3N (0.52 mL, 3.75 mmol) in CH_2Cl_2 (2.5 mL) was held at –5°C for 24 h and washed with saturated $NaHCO_3$ solution and H_2O. The organic layer was dried over Na_2SO_4 and evaporated at reduced pressure. The residue was purified by flash chromatography over silica gel ($CHCl_3$–MeOH, 200:1). $C_{29}H_{37}F_3O_5$. Yield 96%, mp 155–156°C, $[\alpha]_D^{20}$ +64.5 ± 0.5° (*c* 2.05, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1777, 1743, 1723, 1464, 1377, 1355, 1257, 1219, 1189, 1173, 1169. ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 15.31 (q, Me), 16.58 (q, 2Me), 17.17 (t, C-18), 17.40 (t, C-14), 18.45 (q, Me), 21.07 (t, C-2), 26.47 (d, C-21), 33.48 (t, C-13), 34.98 (d, C-10), 36.69 (s, C-20), 37.55 (t, C-17), 37.88 (t, C-19), 38.39 (s, C-12), 39.16 (d, C-5), 40.16 (d, C-7), 42.49 (d, C-11), 43.57 (d, C-3), 45.31 (d, C-15), 47.08 (s, C-16), 49.54 (d, C-1), 50.82 (s, C-4), 51.89 (q, COOMe), 56.86 (d, C-8), 79.44 (d, C-6), 112.39, 116.19, 128.77, 130.68 (s, CF_3), 156.00, 156.57 (s, $COCF_3$), 178.97 (s, COO), 215.61 (s, C-9=O).

Antihypoxic activity of **1**–**8** was studied using three hypoxia models and 216 laboratory white male mice (18–23 g). Normobaric hypoxia with hypercapnia was produced by placing animals of the same weight into hermetically sealed jars (250 cm^3) [11]. Acute hemic hypoxia was induced by injecting s.c. the methemoglobin former sodium nitrite at a dose of 240 mg/kg; acute histotoxic (tissue) hypoxia, by injecting i.p. sodium nitroprusside at a dose of 25 mg/kg [12, 13]. The lifespans (reserve times) of the animals under hypoxia were recorded. The percent increase of the reserve time relative to the control was calculated. All tested compounds were administered orally at a dose of 50 mg/kg (according to hepatoprotective doses obtained earlier) 1 h before the start of the experiment. Control animals received equal volumes of distilled H_2O. Results were processed statistically using the Student *t*-criterion [14]. Effects were considered statistically significant for $p \le 0.05$.

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