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SYNTHESIS AND COMPARATIVE INOTROPIC EFFECTS OF SEVERAL ISOQUINOLINE ALKALOIDS

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Compounds **3a** [1-(3′,4′-methylenedioxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline] and **3b** [1-(6′-bromo-3′,4′-dimethoxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline] demonstrated dose-dependent (5-75 μM) negative inotropic effects. Compound **5** [1,3-*bis*-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)propane] demonstrated a two-phase inotropic effect. The experimental results and a literature analysis suggested that the negative inotropic effects of the tested compounds (**3a**, **3b**, **5**) could be due to their influences on the $[Ca^{2+}]_i$ concentration through blocking of cardiomyocyte Na^+- and Ca^{2+}-channels. The positive inotropic effect of free base **5** could be related to modulation of cardiomyocyte muscarinic receptor activity.

Keywords: papillary muscle, positive inotropic effect, isoquinoline alkaloids.

Natural isoquinolines and many of their derivatives possess broad spectra of pharmacological activity [1 – 8]. Biotesting of several isoquinoline derivatives revealed that they had physiological effects on the cardiovascular system [9 – 15]. Therefore, the synthesis of new isoquinoline derivatives (Scheme 1) and the study of their mechanism of cardiotropic action on the contractile activity of rat papillary muscle are highly interesting.

The target compounds were prepared using Pictet–Spengler and Bischler–Napieralski reactions. The amine component was homoveratrylamine (**1**), condensation of which with aldehydes **2a** and **2b** gave Schiff bases that formed isoquinolines (**3a** and **3b**) after cyclization in CF_3COOH [16]. The reaction of **1** with glutaric acid followed by Bischler—Napieralski cyclization of the diamide (**4**) and reduction of the 3,4-dihydroisoquinoline synthesized *bis*-tetrahydroisoquinoline **5** [17]. The structures of the synthesized compounds were confirmed using IR and PMR spectroscopy.

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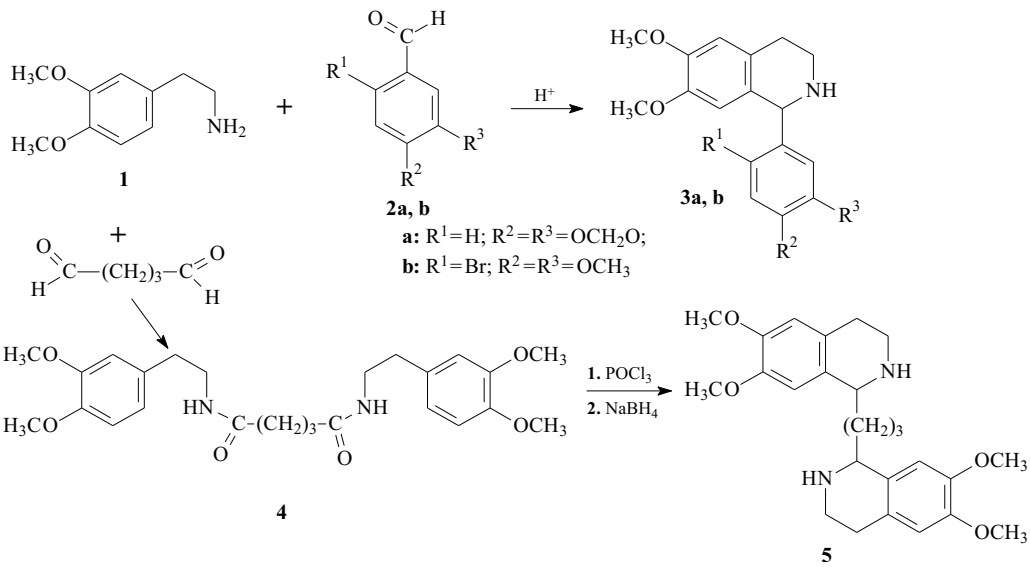
EXPERIMENTAL CHEMICAL PART

PMR spectra were recorded on Tesla BS-567A (100 MHz) and Varian UNITY-400+ spectrometers ($CDCl_3$ and DMSO-d_6 solvents, HMDS internal standard). R_f values were determined on LS 5/40 silica gel plates with elution by $CHCl_3$—MeOH (4:1). Melting points of all synthesized compounds were determined on a Boetius melting-point apparatus.

General method for preparing substituted 1-aryltetrahydroisoquinolines. A solution of 3,4-dimethoxyphenylethylamine (**1**, 1.66 g, 0.009 mol) in C_6H_6 (30 mL) was treated with a substituted benzaldehyde (**2**, 0.009 mol), refluxed with a Dean—Stark trap until H_2O evolution ceased (1 – 2 h), cooled, and made basic to pH 9 – 10 using NH_4OH. The amine was exhaustively extracted by $CHCl_3$.

1-(3′,4′-Methylenedioxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (3a), $C_{18}H_{19}O_4N$, was prepared from the amine (1.66 g, 0.009 mol) and 3,4-methylenedioxybenzaldehyde (1.37 g, 0.009 mol). Yield 2.06 (72%), mp 254 – 257°C, Me_2CO, R_f 0.5. PMR (400 MHz, $CDCl_3$), δ, ppm: 2.66 (1H, dt, J 4.7, 15.9 Hz, H_a-4), 2.85 (1H, ddd, J 4.6,

Scheme 1



8.4, 12.1 Hz, H_e-4), 2.96 (1H, ddd, J 5.4, 8.4, 15.8 Hz, H_a-3), 3.15 (1H, dt, J 5.2, 12.1 Hz, H_e-3), 3.61* (3H, s, OCH_3-7), 3.80* (3H, s, OCH_3-6), 4.90 (1H, s, H-1), 5.87 (2H, dd, J 1.4, 2.3 Hz, 3′-OCH_2O-4′), 6.20 (1H, s, H-8), 6.55 (1H, s, H-5), 6.64 (1H, d, J 1.4 Hz, H-2′), 6.67 (1H, dd, J 1.5, 7.9 Hz, H-6′), 6.69 (1H, d, J 7.9 Hz, H-5′). ^{13}C NMR spectrum, δ, ppm: 29.51 (C-4), 42.09 (C-3), 56.05 (6-OCH_3), 56.12 (7-OCH_3), 61.42 (C-1), 101.16 (C-7′), 108.06 (C-5′), 109.35 (C-2′), 111.17 (C-8), 111.64 (C-5), 122.39 (C-6′), 127.85 (C-1′), 130.14 (C-8a), 139.24 (C-4a), 146.99 (C-6), 147.29 (C-7), 147.87 (C-3′), 147.91 (C-4′).

***N,N′*-(3,4-Dimethoxy-β-phenylethyl)glutardiamide (4)**, $C_{25}H_{34}N_2O_6$. A mixture of **1** (5 g, 0.027 mol) and glutaric acid (2 g, 0.011 mol) was dissolved in MeOH (5 mL). Then, the salt was heated at 178°C on an oil bath for 2 h. The reaction mixture was dissolved in $CHCl_3$ (100 mL) and treated with HCl solution (3%), NaOH solution (2%), and H_2O until neutral. The $CHCl_3$ was distilled off. The solid was crystallized from Me_2CO to afford crystals that were filtered off. Yield 79% (5 g), mp 132 – 135°C (Me_2CO), R_f 0.76. IR spectrum (KBr), ν, cm^{-1}: 3290 (NH), 2931 (Ar-CH), 1638 (N-C=O), 1591, 1551, 1519 (Ar-H). PMR spectrum (400 MHz, $CDCl_3$, δ, ppm: 1.82 (2H, t, J 6.9 Hz, H-2′); 2.10 (4H, t, J 7 Hz, H-1′,3′); 2.70 (4H, t, J 7 Hz, H-α); 3.41 (4H, q, J 6.2 Hz, H-β); 5.69 (2H, t, NH); 6.64 (2H, d, J 2 Hz, H-2); 6.66 (2H, dd, J 2 Hz, 8.6, H-6); 6.74 (2H, d, J 8.6 Hz, H-5).

1,3-*bis*-(6,7-Dimethoxy-3,4-dihydroisoquinolin-1-yl)propane. A mixture of diamide **4** (0.5 g, 0.6 mmol) in $POCl_3$ (1.5 mL) was refluxed on a water bath for 6 h to afford the dihydroisoquinoline (0.4 g, 87%). $C_{25}H_{32}N_2O_4$. PMR spectrum (400 MHz, $CDCl_3$, δ, ppm: 2.02 (2H, q, J 7.2 Hz, CH_2); 2.57 (4H, t, J 7.3 Hz, $2CH_2$); 2.81 (4H, t, J 7.5 Hz, H-4, 4′); 3.57 (4H, t, J 7.5 Hz, H-3.3′); 3.84 (6H, s, OCH_3); 3.85 (6H, s, OCH_3); 6.62 (2H, s, H-8.8′); 7.00 (2H, s, H-5.5′).

1,3-*bis*-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)propane (5). Yield 5.84% (0.33 g), $C_{25}H_{34}N_2O_4$, mp 99 – 101°C (Me_2CO), R_f 0.35. IR spectrum, ν, cm^{-1}: 3378, 2917, 1612, 1520, 1468, 1257, 1223. PMR spectrum (400 MHz, $CDCl_3$, δ, ppm: 1.57 (4H, q, J 7.4, H-1′,2′); 1.83 (2H, m, CH_2), 2.63 (4H, dt, J 6 Hz, H-4.4′); 3.86 (2H, dd, J 3.5, 8.5 Hz, H-1, 1′); 6.50 (2H, s, H-8, 8′); 6.55 (2H, s, H-5, 5′).

EXPERIMENTAL BIOLOGICAL PART

The experiments were conducted according to the Council of International Organizations for Medical Sciences (CIOMS) *International Guiding Principles for Biomedical Research Involving Animals* of 1985. Experiments were performed on prepared papillary muscle that was isolated from the right cardiac ventricle of mature laboratory white rate (150 – 200 g) and placed into a special chamber perfused with Krebs—Henseleit buffer (pH 7.4). The solutions were oxygenated with carbogen (O_2 95%, CO_2 5%) at 35 ± 0.5°C. Prepared muscle was clamped in the experimental chamber with one end connected to the rod of an F30 stress sensor (Germany). The muscle was stimulated using Pt electrodes and an ESL-2 stimulator (Russia) with rectangular pulses at 0.5 – 3 Hz for 5 – 10 ms at amplitude 20% greater than threshold. The muscle was stabilized for 60 min, after which the length at which it developed the maximal isometric stress (L_{max}) was found. All experiments were performed under these conditions. The stress sensor signal was fed into an amplifier (TAM-A, Hugo Sachs Elektronik, Germany) and recorded using a TZ 4620 recorder (Czech Rep.). Results were statistically processed using the OriginPro 7.5 statistical program suite (OriginLab Corp., USA). The paired Student

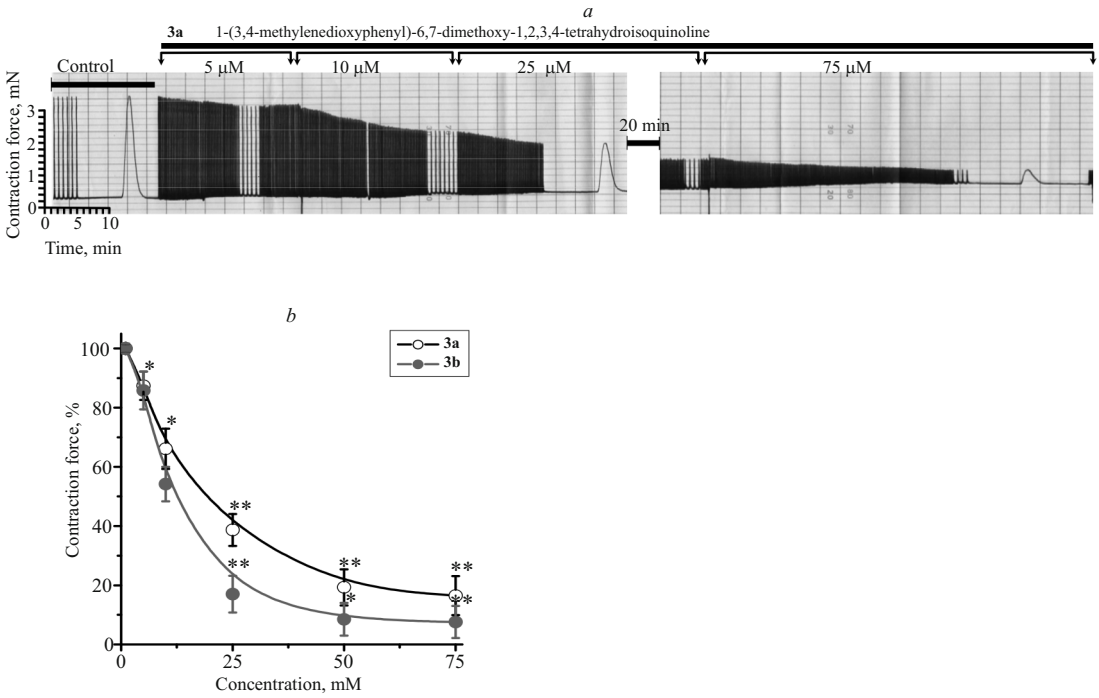


Fig. 1. Negative inotropic effects of alkaloids **3a** [1-(3′,4′-methylenedioxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline] and **3b** [1-(6′-bromo-3′,4′-dimethoxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline] on contractile activity of rat heart papillary muscle: negative trace of contractions of papillary muscle at stimulation frequency 0.5 Hz (*a*); concentration-dependent inotropic effect of studied alkaloids (*b*). Along the ordinate, muscle contraction force expressed in percent of maximal; along the abscissa, solution concentration of **3a** and **3b**. * $p < 0.05$; ** $p < 0.01$ ($n = 3 – 5$).

t-criterion was used to find the statistical significance of changes before and after administration of the alkaloid. Differences were considered statistically significant for $p < 0.05$ and 0.01.

The experimental results showed that isoquinoline alkaloids **3a** and **3b** had dose-dependent (5 – 75 μM) effects on the contractile activity of rat papillary muscle starting at 5 μM. Compounds **3a** and **3b** suppressed the contraction force by 12.6 ± 2.8 and 14.2 ± 3.4%, respectively, as compared to the control ($n = 3 – 5$). The degrees of suppression reached maxima at 75 μM of 83.5 ± 6.6 and 92.4 ± 5.4%, respectively, as compared to the control (Fig. 1). Also, compound **5** affected specifically the contractile activity of rat heart papillary muscle. Compound **5** at all used stimulation frequencies (0.1 – 3 Hz) and a concentration of 75 μM initially increased the muscle contraction force by 27.3 ± 4.7% relative to the control.

The positive inotropic effect (PIE) of **5** changed to negative (NIE) after 1 – 1.5 min of incubation. The contraction force decreased by 85.4 ± 7.1% as compared to the control ($p < 0.05$) (Fig. 2).

Under these conditions, the EC_{50} (concentration causing 50% suppression of contraction force) for **5** was 24.6 μM. Reduction of the intracellular $[Ca^{2+}]_i$ in cardiomyocytes is one reason for NIEs of most pharmacological agents. Several of these compounds decrease $[Ca^{2+}]_i$ by inhibiting its entry into cardiomyocytes from the extracellular milieu; others, by modifying Ca^{2+}-transporter systems of intracellular Ca^{2+}-depots. Therefore, it could be proposed that the NIE of the studied alkaloids was due to reduction of $[Ca^{2+}]_i$ by modifying Ca^{2+} transport through the sarcolemma. Data indicating that the NIE of isoquinoline alkaloids is related to blocking of Na^+-, K^+-, and Ca^{2+}-channels of cardiomyocytes were published. For example, the isoquinoline alkaloid dauricine, which possesses significant antiarrhythmic activity, blocks Na^+-, K^+-, and Ca^{2+}-channels of cardiomyocytes and increases the duration of the myocardium action potential. Modulation of the duration of the refractory myocardium action potential resulting from modification of ion channels was found experimentally to underly the antiarrhythmic activity of several isoquinoline derivatives demonstrating NIEs [18]. Research on several isoquinolone alkaloids, in particular berberine, found that they blocked L-type and T-type Ca^{2+}-channels of cardiomyocytes [19] whereas others, e.g., cycleanine, blocked only myocardium L-type Ca^{2+}-channels and smooth-muscle cells of isolated rabbit aorta [20]. Several studies found that isoquinolone alkaloids such as berberine blocked L-type and T-type cardiomyocyte Ca^{2+}-channels [19] whereas cycleanine blocked more effectively myocardium Ca^{2+}-channels and isolated rabbit aorta smooth muscle

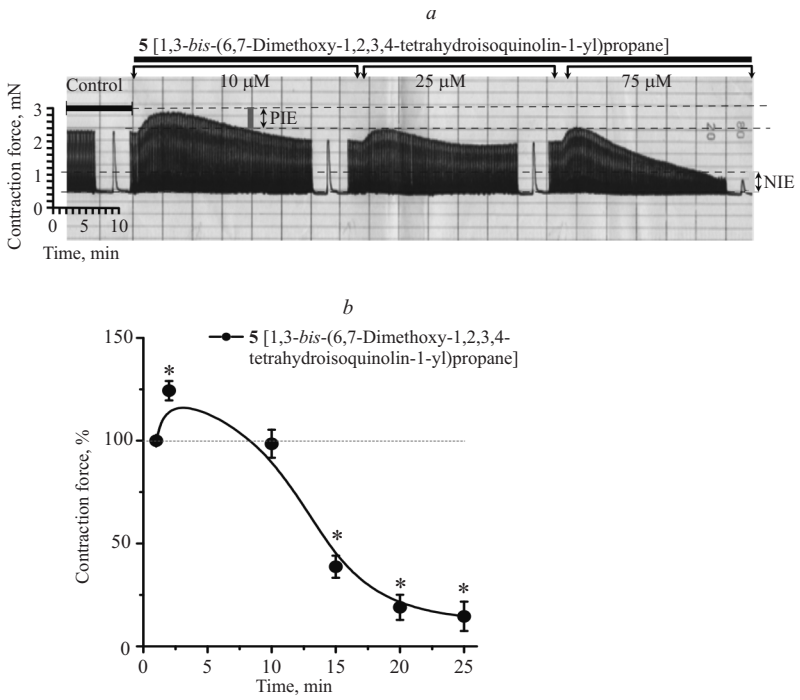


Fig. 2. Two-phase inotropic effect of **5** [1,3-*bis*-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)propane] on contractile activity of rat heart papillary muscle: original trace of contractions of papillary muscle at stimulation frequency 1 Hz (*a*); time-dependent two-phase inotropic effect of **5** (*b*). Along the ordinate, muscle contraction force expressed in percent of maximal; along the abscissa, concentration incubation time, min. * $p < 0.05$ vs. the control ($n = 4$).

cells, in contrast with nifedipine, which blocked potential-dependent L-type Ca^{2+}-channels [20].

Thus, the NIEs of **3a**, **3b**, and **5** could be assumed to be due to their influence on $[Ca^{2+}]_i$ via blocking of cardiomyocyte Na^+-, K^+-, and Ca^{2+}-channels, which determined their contractile activity. Synthetic isoquinoline-alkaloid derivatives CSH109 and CSH118 increased dose-dependently rat myocardium contraction force with the latter also increasing the myocardium action potential duration. The PIE of CSH109 was proposed to be related to increased $[Ca^{2+}]_{in}$ via activation of cardiomyocyte β-adrenoreceptors [21]. β-Adrenergic stimulation is known to have a PIE on mammal myocardium that is caused, among others, by an increase of cardiomyocyte current ICa^{2+}_L. Activation of cardiomyocyte β-adrenoreceptors is related to the β-receptor of transmembrane adenylate cyclase that stimulates G_s-protein, thereby increasing cAMP. In turn, cAMP activates protein kinase A, which phosphorylates cellular proteins, including L-type Ca^{2+}-channels. Phosphorylation of Ca^{2+}-channels increases intracellular current ICa^{2+}_L, which increases Ca^{2+} release from sarcoplasmic reticulum by ryanodine receptors (RyR) and increases myocardium contractions (PIE). Therefore, the role of cardiomyocyte β-adrenoreceptors on the PIE of **5** was studied in the next series of experiments.

It is noteworthy that the PIE of the tested alkaloid did not change if the β-adrenoreceptor blocker propranolol (10 μM) was used. Previously, a PIE was reported for the isoquinoline alkaloid BIIA (3-benzylamino-5,6-dihydro-8,9-dimethoxyimidazo[5,1-*a*]isoquinoline hydrochloride) and was associated with blocking of cardiomyocyte Na^+/K^+-ATPase activity without influencing cardiomyocyte α and β-adrenoreceptors [22]. BIIA (5 – 100 μM) competed with the cardiac glycoside ouabain for blocking of cardiomyocyte Na^+/K^+-ATPase activity. The antiarrhythmic effect of this alkaloid may be related to its influence on the duration of the cardiomyocyte action potential [23]. The antiarrhythmic activity of the isoquinoline alkaloid tetradrine was demonstrated to be due to blocking of Ca^{2+}-channels via modulation of cardiomyocyte muscarinic receptor (MR) activity [13]. Therefore, we surmised that the PIE caused by **5** might be due to its influence on cardiomyocyte MR functioning. The influence of atropine, a MR blocker, on the effects caused by **5** was studied to check this hypothesis. The experiments demonstrated that the PIE of **5** decreased significantly if MRs were blocked by atropine (5 μM).

cAMP-dependent activation through G-protein with stimulation of cardiomyocyte MRs is known to occur with phosphorylation of cardiomyocyte enzyme systems that regulate contraction [23]. Thus, the Ca^{2+}-sensitivity of myofilaments is increased by phosphorylation of myosin so that the contraction force increases (PIE) [24].

A comparison of the present results with the literature suggested that the PIE of free base **5** and its effect on cardiomyocyte MR activity were consistent.

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