Brief Communications

Synthesis and antiplatelet activity of 2-substituted imidazolines

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2-Substituted 4,5-dihydro-1*H*-imidazoles were synthesized by the condensation of aromatic carboxylic acids with ethylenediamine in the presence of KU-2/8 cation-exchange resin as a catalyst. Subsequent alkylation of the resulting compounds with 2-chloromethyl-*gem*-dichlorocyclopropane or 2-bromomethyl-1,3-dioxolane gave previously unknown substituted imidazolines containing carbo- and heterocyclic fragments. The synthesized 2-substituted 4,5-dihydro-1*H*-imidazoles were found to possess the antiplatelet activity at the level of acetylsalicylic acid.

Key words: carboxylic acids, ethylenediamine, imidazolines, condensation, alkylation, antiplatelet activity.

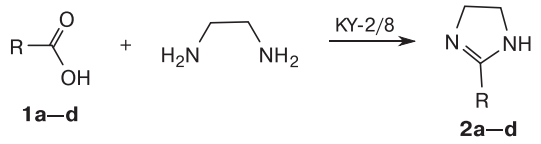
Imidazoline ring is a structural fragment of a number of natural[1,2] and synthetic[3—7] biologically active substances. These compounds exhibit high antifungal,[8] antitumor,[9—15] hypotensive,[16] and antiglycemic[17] activity, and are also efficient in the treatment of Parkinson′s[18] and Alzheimer′s diseases.[19,20]

In the present work, we obtained new 4,5-dihydro-1*H*-imidazole derivatives, which contain *gem*-dichlorocyclopropane and 1,3-dioxolane fragments, and for the first time studied their anticoagulant and antiplatelet activity.

The starting compounds for the synthesis of the target products were benzoic (**1a**), salicylic (**1b**), phenoxyacetic (**1c**), and 2,4-dichlorophenoxyacetic (**1d**) acids. 2-Alkylimidazolines **2a—d** were synthesized by the condensation of carboxylic acids **1a—d** with ethylenediamine in the presence of KU-2/8 cation-exchange resin. The reaction was conducted at 130 °C for 12 h (Scheme 1). The target 2-substituted imidazolines **2a—d** were isolated in 82—86% yields. It was established that the carboxylic acid structure has practically no effect on the course of the reaction.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* Vol. 72, No. 7, pp. 1711—1716, July, 2023.
1066-5285/23/7207-1711

Scheme 1

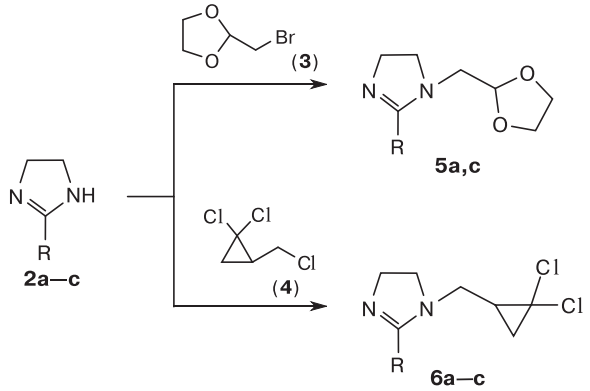


1, 2: R = Ph (**a**), 2-HOC_6H_4 (**b**), $PhOCH_2$ (**c**), 2,4-$Cl_2C_6H_3OCH_2$ (**d**)
Reaction conditions: *p*-xylene, 130 °C, 12 h.

The structure of compounds **2a—d** was confirmed by IR spectroscopy, ^1H and ^{13}C NMR spectroscopy, and gas chromatography-mass spectrometry (GC/MS). The IR spectra of 2-substituted dihydroimidazoles **2a—d** exhibit absorption bands of the C=N bond characteristic of the imidazoline ring. The position of the absorption bands characteristic of the stretching vibrations of this bond changes depending on the nature of the substituent at position 2 of the heterocycle. Thus, in the case of compounds **2a—d**, the absorption bands of the C=N bond of imidazolidine are shifted to the region of lower wavenumbers (1612—1600 cm^{-1}) as compared to 2-alkyl-substituted imidazolines (1654—1639 cm^{-1}).[21] The IR spectra of compounds **2a—d** also exhibit characteristic of imidazolines absorption bands of the stretching vibrations of the N—H bond in the region of 3300—3310 cm^{-1} and of the C=CH bonds of the aromatic ring in the regions of 1495 and 1605 cm^{-1}.

The alkylation of imidazolines **2a—c** with 2-bromomethyl-1,3-dioxolane **3** and 2-chloromethyl-*gem*-dichlorocyclopropane **4** (DMSO, 75 °C, 10 h) gave derivatives **5a,c** and **6a—c** in 40—60% yields (Scheme 2). It should be noted that under the chosen conditions, the alkylation of compound **2b** containing a 2-hydroxyphenyl moiety with bromodioxolane **3** did not lead to the expected tertiary amine. We explain this by the formation of a strong intramolecular hydrogen bond between the hydroxy group and the nitrogen atom of imidazoline **2b**.[22]

Scheme 2



2, 5, 6: R = Ph (**a**), 2-HOC_6H_4 (**b**), $PhOCH_2$ (**c**)
Reaction conditions: DMSO, 75 °C, 10 h.

It is known that arterial hypertension is one of the main risk factors for the development of atherosclerosis, coronary heart disease and leads to cardiovascular complications such as myocardial infarction, cerebral stroke, and chronic heart failure, as a result of thrombosis. At the same time, it is known that 2-phenyl-4,5-dihydro-1*H*-imidazole (**2a**) and 2-phenoxymethyl-4,5-dihydro-1*H*-imidazole (**2c**)[23,24] exhibit high hypotensive (antihypertensive) activity. In this connection, in this work we studied the antiplatelet and anticoagulant activity of the synthesized 4,5-dihydro-1*H*-imidazoles **2a—c**, **5a**, and **6a** *in vitro*.

Measurement of anticoagulant activity was carried out using generally accepted clotting tests. Activated partial thromboplastin time (APTT), prothrombin time (PT), and fibrinogen concentration were studied by the Clauss method based on the measurement of the time required for the formation of the fibrin polymer insoluble in diluted blood plasma after the addition of a large amount of thrombin.[25] It was found that compounds **2a—c**, **5a**, and **6a** do not exhibit anticoagulant activity and at a concentration of $5 \cdot 10^{-4}$ g mL^{-1} do not affect the index of fibrinogen concentration and PT and do not change the indicator of the intrinsic pathway of blood coagulation APTT.

The study of the effect on platelet aggregation was carried out following the Born method.[26] The antiplatelet activity of the studied compounds and reference drugs was determined at the final concentration of 10^{-3} mol L^{-1}. The aggregation inducers were adenosine diphosphate (ADP) at a concentration of 20 μg mL^{-1} and collagen at a concentration of 5 мg mL^{-1}. The maximum amplitude (MA) of aggregation, the rate of aggregation, the time to reach the maximum amplitude (MA) and disaggregation in the presence of test compounds at ADP-induced platelet aggregation were evaluated. In the collagen-induced platelet aggregation, we assessed the latency period during which phospholipase C was activated (which led to the formation of second messengers, resulting in the secretion of platelet granules and synthesis of thromboxane A2) (Table 1).

The results obtained (see Table1) indicate that, in contrast to acetylsalicylic acid, compounds **2a—c**,

Table 1. Effect of compounds **2a—c**, **5a**, and **6a** on indicators of ADP- and collagen-indused platelet aggregation (median (Me) 0.25—0.75)

Compound	Change in latency period (in % to control)	Change in MA (in % to control)	Change in rate of aggregation (in % to control)	Time to reach MA (in % to control)
2a	+9.2(8.1—12.3)[a,b]	−15.6(12.4—17.9)[a,c]	−15.8(12.6—18.4)[a,b]	+13.5 (11.9—15.6)[a,b]
2b	+5.2(4.3—8.6)[a,b]	−18.3(15.7—20.4)[a,c]	−14.7(11.5—16.4)[a,b]	+17.6(15.4—20.2)[a,b]
2c	+5.4(4.3—9.2)[a,b]	−15.3(11.5—16.7)[a,c]	−8.4(6.2—11.3)[a,b]	+19.3(17.2—23.2)[a,c]
5a	+11.8(9.2—12.7)[a,b]	−15.7(13.2—16.9)[a,c]	−9.3(7.3—11.4)[a,b]	+14.6(12.3—16.3)[a,c]
6a	+4.1(2.7—5.1)[b]	−14.3(10.2—15.6)[a,b]	−16.5(14.3—18.5)[a,b]	+18.5(15.6—21.4)[a,c]
Acetylsalicylic acid	−2.1(1.1—2.6)[b]	−13.7(10.8—16.4)[a,b]	−10.5(7.6—12.3)[a,b]	+10.5(8.7—13.4)[a,b]

Note. The latency period is given for collagen-induced platelet aggregation, the remaining parameters are given for ADP-induced platelet aggregation.
[a] $p \leqslant 0.05$ in comparison with control.
[b] $p \leqslant 0.001$ in comparison with acetylsalicylic acid, $n = 6$.
[c] $p \leqslant 0.05$ in comparison with acetylsalicylic acid.

5a, and **6a** statistically increase the latency period, which corresponds to the reaction of platelets release at the collagen-induced platelet aggregation. At the same time, at the ADP-induced platelet aggregation, imidazolines **2a—c**, **5a**, and **6a** reduce the MA of platelet aggregation and are not inferior in their activity to the widely used drug, acetylsalicylic acid. It was found that the introduction of the pharmacophore *gem*-dichlorocyclopropane and 1,3-dioxolane fragments into the molecule of imidazoline **2a** (compounds **5a** and **6a**) has virtually no effect on the antiplatelet activity of substituted imidazolines (see Table 1). All the obtained compounds are promising for further biological studies *in vivo*, as well as for the investigation of hypotensive activity, acute toxicity, and calculation of therapeutic index.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-300 spectrometer (300.13 and 75.47 MHz, respectively), using Me_4Si as an internal standard. IR spectra were recorded on an IR Prestige 21 Shimadzu instrument for neat samples. GLC studies were carried out on a Chrom-5 instrument (column length of 1.2 m, stationary phase silicone SE-30 (5%) on Chromaton N-AW-DMCS (0.16—0.20 mm), working temperature of 50—300 °C), using helium as a carrier gas. Gas chromatography-mass spectrometry (GC/MS) was carried out on a Kristall-5000 M instrument with a Finnigan DSQ II mass spectrometric detector at an ionizing voltage of 70 eV. The ion source temperature was 200 °C. Separation of components was carried out on a Thermo Scientific TRACE® TR-5MS column (the length of 30 m, the inner diameter of 0.25 mm, the thickness of the stationary liquid phase of 0.25 μm), the flow rate through the column was 0.7 mL min^{-1}, helium was a carrier gas.

The course of the reactions and the purity of the obtained compounds were monitored by TLC on Sorbfil silica gel plates (Russia), using petroleum ether—ethyl acetate as an eluent. Lancaster silica gel (70—230 mesh, UK) was used for column chromatography.

The solvents used in the work (toluene, hexane, ethyl acetate, methyl *tert*-butyl ether) were purified according to the standard procedures.[27,28] The starting 2-bromomethyl-1,3-dioxolane (**3**) and 2-chloromethyl-*gem*-dichlorocyclopropane (**4**) were obtained by known procedures.[29]

Synthesis of 2-substituted imidazolines 2a—d (general procedure). A mixture of carboxylic acid **1a—d** (8 mmol), ethylenediamine (2.1 mL, 32 mmol), and KU-2/8 cation-exchange resin (30% by weight of the starting reagents) in *p*-xylene (15 mL) was refluxed for 12 h with a Dean—Stark trap. After the reaction completion, the catalyst was filtered off, the solvent was evaporated under reduced pressure, the product was isolated by column chromatography (eluent petroleum ether—ethyl acetate, 10 : 1 → 7 : 3).

2-Phenyl-4,5-dihydro-1*H*-imidazole (2a). The yield was 88%. An oil. IR, ν/cm^{-1}: 3200 (NH), 1600 (C=N), 1510 (C—N), 1495, 860 (C—H). ^{1}H NMR ($CDCl_3$), δ: 3.79 (s, 4 H, C(4)H$_2$, C(5)H$_2$); 6.48 (br.s, 1 H, NH); 7.45—7.80 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$), δ: 46.8 (2 CH$_2$), 117.8 (2 CH), 128.3 (Ph, 2 CH), 131.0 (CH), 133.0 (Ph, C), 166.1 (C). MS, *m*/*z* (I_{rel} (%)): 56, 80, 106, 119, 146 [M]$^+$.

2-(4,5-Dihydro-1*H*-imidazol-2-yl)phenol (2b). The yield was 86%. An oil. IR, ν/cm^{-1}: 3362 (NH), 3202 (OH), 1609 (C=N), 1530 (C—N), 1472, 900 (C—H). ^{1}H NMR ($CDCl_3$), δ: 3.79 (s, 4 H, C(4)H$_2$, C(5)H$_2$); 6.48 (br.s, 1 H, NH); 6.90—7.04 (m, 3 H); 7.30—7.40 (m, 1 H); 10.27

(br.s, 1 H, OH). ^{13}C NMR ($CDCl_3$), δ: 46.8 (2 CH_2), 110.4 (C_{Ar}), 117.8 ($C_{Ar}H$), 121.4 ($C_{Ar}H$), 128.6 ($C_{Ar}H$), 132.4 ($C_{Ar}H$), 161.6 ($C_{Ar}H$), 166.1 (C). MS, *m/z* (I_{rel} (%)): 56, 80, 108, 146, 162 $[M]^+$.

2-Phenoxymethyl-4,5-dihydro-1*H*-imidazole (2c). The yield was 86%. An oil. IR, ν/cm^{-1}: 3210 (NH), 1605 (C=N), 1543 (C—N), 1475, 860 (C—H). ^{1}H NMR ($CDCl_3$), δ: 3.60 (s, 4 H, C(4)H_2, C(5)H_2); 4.93 (s, 2 H, OCH_2); 6.90—7.00 (m, 3 H, Ph); 7.30—7.40 (m, 2 H, Ph); 8.10 (br.s, 1 H, NH). ^{13}C NMR ($CDCl_3$), δ: 51.2 (2 CH_2), 76.7 (OCH_2), 114.3 ($C_{Ph}H$), 121.0 ($C_{Ph}H$), 129.7 ($C_{Ph}H$), 158.1 (C_{Ph}), 166.0 (C). MS, *m/z* (I_{rel} (%)): 56, 80, 108, 133, 148, 176 $[M]^+$.

2-(2,4-Dichlorophenoxymethyl)-4,5-dihydro-1*H*-imidazole (2d). The yield was 95%. An oil. IR, ν/cm^{-1}: 3234 (NH), 1609 (C=N), 1510 (C—N), 1475, 800 (C—H). ^{1}H NMR ($CDCl_3$), δ: 3.60 (s, 4 H, C(4)H_2, C(5)H_2); 4.93 (s, 2 H, OCH_2); 7.12 (d, 1 H, $C_{Ar}H$, J = 7.5 Hz); 7.29 (dd, 1 H, $C_{Ar}H$, J_3 = 7.5 Hz, J_2 = 1.5 Hz); 7.45 (s, 1 H, $C_{Ar}H$); 8.10 (br.s, 1 H, NH). ^{13}C NMR ($CDCl_3$), δ: 51.2 (2 CH_2), 117.1 (C_{Ar}), 121.5 ($C_{Ar}H$), 128.0 (C_{Ar}), 129.0 ($C_{Ar}H$), 131.4 ($C_{Ar}H$, CH), 152.7 (C_{Ar}, CH), 166.0 (C). MS, *m/z* (I_{rel} (%)): 56, 80, 108, 120, 148, 159, 245 $[M]^+$.

***N*- Alkylation of secondary amines (general procedure).** A mixture of a secondary amine (3 mmol) (2-phenyl-4,5-dihydro-1*H*-imidazole (**2a**) (438 mg) or 2-(4,5-dihydro-1*H*-imidazol-2-yl)phenol (**2b**) (486 mg) or 2-phenoxymethyl-4,5-dihydro-1*H*-imidazole (**2c**) (528 mg)), a corresponding haloalkyl derivative (1 mmol) (2-bromomethyl-1,3-dioxolane (**3**) (167 mg) or 2-chloromethyl-*gem*-dichlorocyclopropane (**4**) (159.5 mg)), and DMSO (20 mL) was vigorously stirred at 75 °C for 10 h. The reaction mixture was cooled and extracted with methyl *tert*-butyl ether (2×20 mL). The organic layers were washed with a 20% aqueous solution of NaOH, then water until neutrality and dried with K_2CO_3. The solvent was evaporated *in vacuo*, the products were isolated by column chromatography (eluent petroleum ether—ethyl acetate, 10 : 1 → 7 : 3).

1-[(1,3-Dioxolan-2-yl)methyl]-2-phenyl-4,5-dihydro-1*H*-imidazole (5a). The yield was 30%. An oil. IR, ν/cm^{-1}: 1600 (C=N), 1510 (C—N), 1040 (C—O—C), 1495, 860 (C—H). ^{1}H NMR ($CDCl_3$), δ: 3.60—3.66 (m, 2 H, C(1′)H_2); 3.70—3.72 (m, 2 H, C(5)H_2); 3.85—3.88 (m, 2 H, C(4″)H_2, C(5″)H_2); 3.98—4.10 (m, 2 H, C(4″)H_2, C(5″)H_2); 5.51 (t, 1 H, C(2″)H, J = 7.1 Hz); 5.95—6.00 (m, 2 H, C(4)H_2); 7.45—7.80 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$), δ: 48.6 (CH_2) 49.7 (CH_2), 54.3 (CH_2), 64.4 (2 CH_2), 104.7 (CH), 125.1 (2 $C_{Ph}H$), 128.38 (2 $C_{Ph}H$), 131.0 ($C_{Ph}H$), 133.0 (C_{Ph}), 157.9 (C). MS, *m/z* (I_{rel} (%)): 50, 56, 77, 108, 232 $[M]^+$.

1-[(1,3-Dioxolan-2-yl)methyl]-2-phenoxymethyl-4,5-dihydro-1*H*-imidazole (5c). The yield was 35%. An oil. IR, ν/cm^{-1}: 1605 (C=N), 1543 (C—N), 1043 (C—O—C), 1475, 860 (C—H). ^{1}H NMR ($CDCl_3$), δ: 3.62—3.65 (m, 2 H, C(1′)H_2); 3.70—3.72 (m, 2 H, C(5)H_2); 3.73—3.75 (m, 2 H, C(4)H_2); 3.86—3.89 (m, 2 H, C(4″)H_2, C(5″)H_2); 3.97—4.00 (m, 2 H, C(4″)H_2, C(5″)H_2); 4.87 (s, 2 H, OCH_2); 5.51 (t, 1 H, C(2″)H, J = 7.1 Hz); 7.05—7.35 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$), δ: 45.3 (CH_2) 46.4 (CH_2), 51.0 (CH_2), 64.4 (2 CH_2), 104.7 (CH), 121.3 ($C_{Ph}H$), 121.7 (2 $C_{Ph}H$), 130.1 (2 $C_{Ph}H$), 151.8 (C_{Ph}), 157.8 (C). MS, *m/z* (I_{rel} (%)): 56, 90, 125, 248 $[M]^+$.

1-[(2,2-Dichlorocyclopropyl)methyl]-2-phenyl-4,5-dihydro-1*H*-imidazole (6a). The yield was 60%. An oil. IR, ν/cm^{-1}: 3080 (cyclopropane), 1605 (C=N), 1543 (C—N), 1475, 860 (C—H), 700 (C—Cl). ^{1}H NMR ($CDCl_3$), δ: 1.30—1.37 (m, 1 H, C(3″)H_2); 1.60—1.65 (m, 1 H, C(3″)H_2); 2.20—2.25 (m, 1 H, C(1″)H); 3.10—3.15 (m, 1 H, C(1′)H_2); 3.40—3.45 (m, 1 H, C(1′)H_2); 3.70—3.72 (m, 2 H, C(5)H_2); 5.95—6.00 (m, 2 H, C(4)H_2); 7.45—7.80 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$), δ: 20.1 (CH_2), 24.3 (CH), 45.8 (CH_2), 48.6 (CH_2), 54.3 (CH_2), 56.7 (C), 125.1 (2 $C_{Ph}H$), 128.38 (2 $C_{Ph}H$), 131.0 ($C_{Ph}H$), 133.0 (C_{Ph}), 157.9 (C). MS, *m/z* (I_{rel} (%)): 55, 81, 108, 124, 269 $[M]^+$.

2-{1-[(2,2-Dichlorocyclopropyl)methyl]-4,5-dihydro-1*H*-imidazol-2-yl}phenol (6b). The yield was 40%. An oil. IR, ν/cm^{-1}: 3040 (cyclopropane), 3200 (OH), 1609 (C=N), 1530 (C—N), 1472, 900 (C—H), 700 (C—Cl). ^{1}H NMR ($CDCl_3$), δ: 1.30—1.37 (m, 1 H, C(3″)H_2); 1.60—1.65 (m, 1 H, C(3″)H_2); 2.20—2.25 (m, 1 H, C(1″)H), 3.10—3.15 (m, 1 H, C(1′)H_2); 3.40—3.45 (m, 1 H, C(1′)H_2), 3.70—3.72 (m, 2 H, C(5)H_2); 5.95—6.00 (m, 2 H, C(4)H_2); 7.00—7.30 (m, 5 H, Ar); 10.27 (br.s, 1 H, OH). ^{13}C NMR ($CDCl_3$), δ: 20.0 (CH_2), 24.3 (CH), 45.8 (CH_2), 48.6 (CH_2), 54.3 (CH_2), 56.7 (C), 112.4 (C_{Ar}), 117.8 ($C_{Ar}H$), 121.4 ($C_{Ar}H$), 128.6 ($C_{Ar}H$), 132.4 ($C_{Ar}H$), 158.6 ($C_{Ar}H$), 157.9 (C). MS, *m/z* (I_{rel} (%)): 55, 90, 122, 139, 285 $[M]^+$.

1-[(2,2-Dichlorocyclopropyl)methyl]-2-phenoxymethyl-4,5-dihydro-1*H*-imidazole (6c). The yield was 50%. An oil. IR, ν/cm^{-1}: 3080 (cyclopropane), 1605 (C=N), 1543 (C—N), 1475, 860 (C—H), 705 (C—Cl). ^{1}H NMR ($CDCl_3$), δ: 1.33—1.39 (m, 1 H, C(3″)H_2); 1.58—1.63 (m, 1 H, C(3″)H_2); 2.22—2.25 (m, 1 H, C(1″)H); 3.09—3.14 (m, 1 H, C(1′)H_2); 3.40—3.45 (m, 1 H, C(1′)H_2); 3.70—3.72 (m, 2 H, C(5)H_2); 4.88 (s, 2 H, OCH_2), 5.95—6.00 (m, 2 H, C(4)H_2); 7.31—7.55 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$), δ: 20.1 (CH_2), 24.3 (CH), 42.5 (CH_2), 45.3 (CH_2), 51.0 (CH_2), 56.8 (C), 121.3 ($C_{Ph}H$), 121.7 (2 $C_{Ph}H$), 130.1 (2 $C_{Ph}H$), 151.8 (C_{Ph}), 157.8 (C). MS, *m/z* (I_{rel} (%)): 55, 81, 90, 175, 229, 259, 285 $[M]^+$.

Biological studies under *in vitro* conditions were performed on the blood of healthy male donors at the age of 18—24 years. The total number of donors was 27 people. Blood sampling for the study of compounds in relation to the hemostasis system was carried out from the cubital vein using BD Vacutainer® vacuum blood sampling systems (Becton Dickinson and Company, USA). A 3.8%

solution of sodium citrate was used as a venous blood stabilizer in a ratio of 9 : 1. All tests were performed on platelet-rich and platelet-poor plasma. Platelet-rich plasma samples were obtained by centrifugation of citrated blood at 1000 rpm for 10 min, platelet-free plasma at 3000 rpm for 20 min.

Anticoagulant activity. A solution of test compounds in DMSO (10 μL) at a concentration of $5 \cdot 10^{-4}$ g mL^{-1} was introduced into a cuvette with platelet-poor plasma with constant stirring (the volume of the solvent did not exceed 5% of the plasma volume) and this was incubated at 37 °C for 5 min. Next, the anticoagulant activity of the studied compounds was determined *in vitro* by standard clotting assay on a Solar CGL turbodimetric hemocoagulometer (Belarus). Activated partial thromboplastin time (APTT), prothrombin time (PT), and fibrinogen concentration were determined following the Clauss method.[25]

Antiplatelet activity. A solution of test compounds in DMSO (10 μL) at a concentration of 10^{-3} g mL^{-1} was introduced into a cuvette with platelet-poor plasma with constant stirring and this was incubated at 37 °C for 5 min. Then, the effect of compounds **2a—c**, **5a**, and **6a** on platelet aggregation was studied following the Born method.[26] Adenosine diphosphate (ADP) at a concentration of 20 μg mL^{-1} and collagen at a concentration of 5 мg mL^{-1} (Tekhnologiya-Standart (Russia)) were used as inducers of aggregation. The effect of compounds on the maximum aggregation amplitude (MA), the rate of aggregation, and the time to reach MA at ADP-induced platelet aggregation were studied. In the test of collagen-induced platelet aggregation, the latency period was evaluated, which corresponds to the reaction of the release of platelets. The research results are shown in Table 1.

The results were processed using the statistical package Statistica 10.0 (StatSoft, Inc, USA). Checking for the normality of the distribution of actual data was performed using the Shapiro—Wilk test. Data are presented as median, 25th and 75th percentiles. Dispersion analysis was performed using the Kruskal—Wallis test. The critical level of significance p for statistical criteria was taken equal to 0.05.

The work was carried out within the framework of the Russian state assignment of the Ministry of Education and Science of the Russian Federation in the field of scientific activity (number for publications FEUR — 2022-0007 "Petrochemical reagents, oils and materials for thermal power industry") and with the financial support of the Russian Science Foundation (Project "Development of agents for the correction of depression in cerebrovascular accident") and FGBOU VO BGMU of the Ministry of Health of Russia as part of the program of strategic and industry leadership Priority 2030.

Biological experiments were performed in accordance with the requirements of the Rules of Good Laboratory Practice of the Eurasian Economic Union in the field of drug circulation. The evaluation of antiplatelet and anticoagulant activity was carried out under *in vitro* conditions on isolated blood portions of 27 healthy male donors aged 18—24 years. The study was approved by the ethics committee of the FGBOU VO Bashkir State Medical University of the Ministry of Health of Russia (No. 1 dated 20.02.2019) and complies with the ethical standards of the institutional national research ethics committee and the 1964 Declaration of Helsinki and its subsequent amendments. Informed consent was obtained from all study participants prior to blood sampling.

The authors declare no competing interests.

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Received November 1, 2022;
in revised form December 12, 2022;
accepted December 15, 2022