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SYNTHESIS AND *IN VITRO* STUDY OF THE ANTICOAGULATION AND ANTIPLATELET ACTIVITY OF ABIETIC AND MALEOPIMARIC ACID AMIDES

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The main goal of applying antiplatelet drugs is a reduced probability of thrombosis so that active substances that are superior to reference drugs in terms of the level and spectrum of their antiplatelet activity are being sought. Several amides of abietic and maleopimaric acids were synthesized and their anticoagulation and antiplatelet activity studied using the Born method and clotting tests. The synthesized amides of the diterpenic acids had different effects on the plasma component of the hemostasis system, which manifested as a change in the internal blood coagulation pathway, i.e., the activated partial thromboplastin time, without affecting the fibrinogen concentration and prothrombin time. The *N*-phenylamide and *N*-(*p*-fluoro)phenylamide of abietic and maleopimaric acids and the *N*-morpholylamide of maleopimaric acid exhibited antiplatelet activity comparable to the widely used antiplatelet agent and cyclooxygenase-1 inhibitor acetylsalicylic acid and more effectively inhibited the platelet release reaction (lag period in collagen-induced platelet aggregation).

Keywords: diterpenoids, abietic acid, maleopimaric acid, anticoagulant activity, antiplatelet activity.

Diterpenic acids occur in resins of many conifer species and have recently been used as scaffolds for synthesizing biologically active compounds via targeted modification [1]. The main resinous acids of the abietane series include abietic (AA) and levopimaric acids (LPA). The biological activity and polyfunctional properties in addition to the availability are important considerations for the synthesis of new abietic acid derivatives. The anti-inflammatory [2], antiplatelet [3], and antiviral activities of AA are well known, while insecticidal, acaricidal, fungicidal, and growth-regulating activities were reported for its sodium and ammonium salts [4]. AA was used to synthesize (+)-3-deoxyaphidicolin, a selective inhibitor of eukaryotic DNA α-polymerase [5], and biologically active natural warbuganal and its 4α-methoxycarbonyl analog, which had unique structures and possessed insecticidal, growth-regulating, cytotoxic, antimicrobial, and other valuable properties [6]. AA inhibits human 5-lipoxygenase and can be used to treat several diseases, including allergy, asthma, arthritis, and psoriasis [3] and to regulate lipid metabolism and atherosclerosis [7].

The diene adduct of LPA and AA with maleic anhydride, i.e., maleopimaric acid (MPA), is a convenient substrate for chemical transformations [1] and is widely used in industrial chemistry to manufacture typographic dyes, alkyd resins, and lubricants and to produce paper [8, 9]. Its derivatives exhibit anti-inflammatory [10], anticancer [11], antiviral [12], and antitumor activity [13].

The present article reports data on the synthesis of a series of amides of AA and MPA and *in vitro* studies of their anticoagulant and antiplatelet activities.

The target amides of AA and MPA were synthesized in two steps from the corresponding acids, which were isolated from pine (*Pinus silvestris*) sap by known methods [14]. The obtained acids were converted to acid chlorides **I** and **II** by treatment with thionyl chloride or oxalyl chloride, which re-

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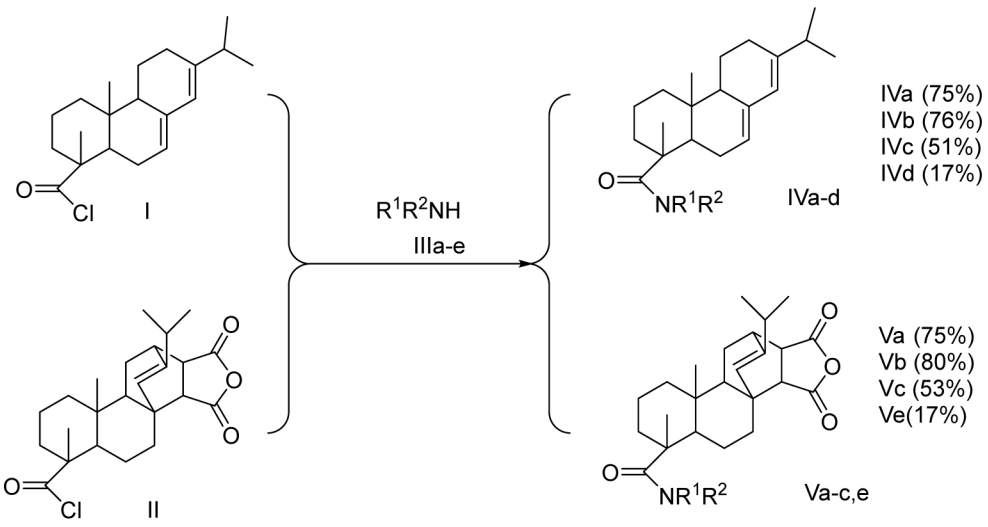
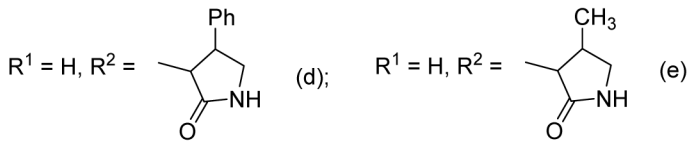


R^1 = H, R^2 = Ph (a); R^1 = H, R^2 = 4-F-C_6H_4 (b); R^1+R^2 = $CH_2CH_2OCH_2CH_2$ (c);



Reagents and conditions: CH_2Cl_2, Et_3N, 20-23ºC, 24 h

acted smoothly under mild conditions in CH_2Cl_2 at room temperature in the presence of Et_3N with various amines [aniline (**IIIa**), *p*-fluoroaniline (**IIIb**), morpholine (**IIIc**), 3-amino-4-phenylpyrrolidone (**IIId**), and 3-amino-4-methylpyrrolidone (**IIIe**)] according to the following scheme:

The structures of amides **IVa-d** and **Va-c**, **-e**, which were isolated as light-yellow crystals, were confirmed by IR spectroscopy, mass spectrometry, and PMR and ^{13}C NMR spectroscopy. PMR spectra of amides **IVa-d** and **Va-c**, **-e** showed resonances for diterpene protons with chemical shifts close to those of the starting resinous acids and characteristic NH resonances as broad singlets in the range 6.11 – 8.32 ppm. IR spectra of the synthesized amides had distinct absorption bands in the range 1620 – 1665 cm^{-1} that were characteristic of O=C–N< stretching vibrations.

EXPERIMENTAL CHEMICAL PART

PMR and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS internal standard on a Bruker AM 300 spectrometer at operating frequency 300 (^1H) and 75.5 MHz (^{13}C). IR spectra were recorded from KBr pellets or the pure compounds on an IR Prestige-21 instrument (Fourier Transform Spectrophotometer, Shimadzu). Mass spectra were measured in a Shimadzu LCMS 2010 EV mass spectrometer in APCI mode. Elemental analysis used a Euro EA 3000 CHNS analyzer. The course of reactions and purity of products were monitored using TLC on Sorbfil PTSKh-AF-A plates and $CHCl_3$–MeOH (10:1). Melting points were determined on a Boetius apparatus.

Molecular ions were determined by LC-MS on a Shimadzu LCMS-2010 liquid-chromatograph–mass spectrometer in APCI mode for samples in MeCN solutions.

Column chromatography (CC) used silica gel (SG) 50/150 μm (Sorbpolimer).

All solvents and reagents used in the experiments were purified and had characteristics that agreed with literature data.

General method for synthesizing amides of diterpenic acids. A solution of MPA or AA chloride (1 mmol) in CH_2Cl_2 (10 mL) at room temperature was stirred vigorously and treated dropwise with a solution of Et_3N (0.1 g, 1 mmol) and the appropriate amine **IIIa-e** (1 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred for 3 h and washed with HCl solution (5%), H_2O, NaOH solution (5%), and H_2O again. The organic layer was dried over Na_2SO_4 and filtered. The solvent was evaporated from the filtrate. The obtained amides were recrystallized from hexane–EtOH.

Abietic acid *N*-phenylamide (IVa). Yield 75%, mp 87 – 88°C. PMR spectrum ($CDCl_3$, δ, ppm): 0.86 (s, 3H, 20-Me), 1.00 (d, J 4.0 Hz, 3H, 16-H), 1.02 (d, J 3.2 Hz, 3H, 17-H), 1.27 (s, 3H, 19-Me), 1.19 – 2.42 (m, 15H, 1-H, 2-H, 3-H, 11-H, 12-H), 5.39 (s, 1H, 7-H), 5.77 (s, 1H, 14-H), 7.11 (s, 1H, NH), 7.28 – 7.34 (m, 2H, Ph), 7.49 (s, 1H, Ph),

7.52 – 7.54 (m, 2H, Ph). ^{13}C NMR spectrum ($CDCl_3$, δ_C, ppm): 14.0 (C-20), 16.8 (C-19), 18.0 (C-2), 20.9 (C-16), 21.4 (C-17), 22.5 (C-11), 25.6 (C-6), 27.4 (C-12), 34.5 (C-10), 34.9 (C-15), 37.2 (C-3), 38.3(C-1), 44.9 (C-5), 46.3 (C-4), 50.9 (C-9), 120.2 (C-4′, Ph), 120.5 (C-7), 122.4 (C-14), 124.1 (C-2′, C-6′, Ph), 128.8 (C-3′, C-5′, Ph), 135.8 (C-8), 138.0 (C-1′, Ph), 145.1 (C-13), 177.9 (C-18). Found, %: C 82.70; H 9.32; N 3.73. $C_{26}H_{35}NO$. Calc., %: C 82.71; H 9.34; N 3.71.

Abietic acid *N*-(*p*-fluorophenyl)amide (IVb). Yield 76%, mp 66 – 67°C. PMR spectrum ($CDCl_3$, δ, ppm): 0.86 (s, 3H, 20-Me), 1.00 (d, J 4.0 Hz 3H, 16-H), 1.02 (d, J 3.2 Hz, 3H,17-H), 1.27 (s, 3H, 19-Me), 1.19 – 2.42 (m, 15H, 1-H, 2-H, 3-H, 11-H, 12-H), 5.39 (s, 1H, 7-H), 5.77 (s, 1H, 14-H), 7.11 (s, 1H, NH), 7.10 – 7.14 (m, 2H, Ph), 7.55 – 7.59 (m, 2H, Ph). ^{13}C NMR spectrum ($CDCl_3$, δ_C, ppm): 14.0 (C-20), 16.8 (C-19), 18.0 (C-2), 20.9 (C-16), 21.4 (C-17), 22.5 (C-11), 25.6 (C-6), 27.4 (C-12), 34.5 (C-10), 34.9 (C-15), 37.2 (C-3), 38.3(C-1), 44.9 (C-5), 46.3 (C-4), 50.9 (C-9), 115.7 (C-4′, Ph), 120.6 (C-2′, C-6′, Ph), 120.5 (C-7), 122.4 (C-14), 134.1 (C-3′, C-5′, Ph), 135.8 (C-8), 145.1 (C-13), 162.9 (C-1′, Ph), 177.9 (C-18). Found, %: C 78.91; H 8.64; N 3.53. $C_{26}H_{34}FNO$. Calc., %: C 78.95; H 8.66; N 3.54.

Abietic acid *N*-morpholylamide (IVc). Yield 51%, amorphous compound. PMR spectrum ($CDCl_3$, δ, ppm): 0.86 (s, 3H, 20-Me), 1.00 (d, J 4.0 Hz 3H, 16-H), 1.02 (d, 3H, 17-H), 1.27 (s, 3H, 19-Me), 1.19 – 2.42 (m, 15H, 1-H, 2-H, 3-H, 11-H, 12-H), 5.39 (s, 1H, 7-H), 5.77 (s, 1H, 14-H), 3.47 (t, 4H, 2′-H, 6′-H), 3.61 (t, 4H, 3′-H, 5′-H). ^{13}C NMR spectrum ($CDCl_3$, δ_C, ppm): 14.0 (C-20), 16.8 (C-19), 18.0 (C-2), 20.9 (C-16), 21.4 (C-17), 22.5 (C-11), 25.6 (C-6), 27.4 (C-12), 34.5 (C-10), 34.9 (C-15), 37.2 (C-3), 38.3(C-1), 44.9 (C-5), 46.3 (C-4), 48.6 (C-2′, C-6′), 50.9 (C-9), 66.2 (C-3′, C-5′), 120.5 (C-7), 122.4 (C-14), 135.8 (C-8), 145.1 (C-13), 177.9 (C-18). Found, %: C 77.52; H 10.05; N 3.71. $C_{24}H_{37}NO_2$. Calc., %: C 77.58; H 10.04; N 3.77.

Abietic acid *N*-(4-phenyl-2-oxopyrrolidin-3-yl)amide (IVd). Yield 17%, mp 103 – 104°C. PMR spectrum ($CDCl_3$, δ, ppm): 0.86 (s, 3H, 20-Me), 1.00 (d, J 4.0 Hz 3H,16-H), 1.02 (d, J 3.2 Hz, 3H,17-H), 1.27 (s, 3H, 19-Me), 1.19 – 2.42 (m, 15H, 1-H, 2-H, 3-H, 11-H, 12-H), 3.56 and 3.81 (both t, 1H each, J 7 Hz, 3′-H), 4.01 (q, J 7 Hz, 1H, 4′-H), 4.71 (d, J 7 Hz, 1H, 5′-H), 5.39 (s, 1H, 7-H), 5.77 (s, 1H, 14-H), 6.64 and 8.32 (both s, 1H each, NH), 7.19 – 7.28 (m, 5H, Ph). ^{13}C NMR spectrum ($CDCl_3$, δ_C, ppm): 14.0 (C-20), 16.8 (C-19), 18.0 (C-2), 20.9 (C-16), 21.4 (C-17), 22.5 (C-11), 25.6 (C-6), 27.4 (C-12), 34.5 (C-10), 34.9 (C-15), 35.4 (C-4′), 37.2 (C-3), 37.3 (C-3′), 38.3(C-1), 44.9 (C-5), 46.3 (C-4), 50.9 (C-9), 70.5 (C-5′), 126.1 (C-4″, Ph), 120.5 (C-7), 122.4 (C-14), 125.9 (C-2″, C-6″, Ph), 128.4 (C-3″, C-5″, Ph), 135.8 (C-8), 148.4 (C-1″, Ph), 145.1 (C-13), 177.9 (C-18), 179.1 (C-1′). Found, %: C 78.18; H 8.85; N 6.10. $C_{30}H_{40}N_2O_2$. Calc., %: C 78.22; H 8.75; N 6.08.

Maleopimaric acid *N*-phenylamide (Va). Yield 83%, mp 113 – 114°C. PMR spectrum ($CDCl_3$, δ, ppm): 0.60 (s, 3H, 17-H), 1.00 (d, 3H, 15-H, J 6.9 Hz), 0.96 (d, 3H, 16-H, J 6.4 Hz), 1.17 (s, 3H, 18-H), 1.21 – 1.91 (m, 13H, 4-H, 5-H, 5a-H, 7-H, 8-H, 9-H, 10-H), 2.26 (quint, 1H, 14-H, J 6.7 Hz), 2.54 (dt 1H, 9b-H, J 3.0, 13.8 Hz), 2.73 (d, 1H, 3a-H, J 8.7 Hz), 3.09 (dd, 1H, 11-H, J 8.6, 3.0 Hz), 3.98 (d, 1H, 11a-H, J 9 Hz), 5.54 (s, 1H, 13-H), 7.1 – 7.5 (m, 5H, Ph), 6.8 (br.s 1H, NH). ^{13}C NMR spectrum ($CDCl_3$, δ_C, ppm): 14.28 (C-17), 15.56 (C-18), 17.02 (C-8), 19.96 (C-16), 20.57 (C-15), 21.56 (C-5), 27.23 (C-10), 32.77 (C-14), 34.83 (C-4), 35.68 (C-11), 36.64 (C-7), 36.94 (C-9a), 38.04 (C-9), 40.48 (C-3b), 45.67 (C-11a), 46.87 (C-6), 49.38 (C-5a), 53.07 (C-3a), 53.28 (C-9b), 66.32 (C-21), 124.77 (C-13), 128.49 (C-2′, C-6′), 128.06 (C-4′), 127.73 (C-3′, C-5′), 136.30 (C-1′), 148.31 (C-12), 171.0 (C-1), 172.78 (C-3), 176.69 (C-19). Found, %: C 75.75; H 7.83 N, 2.92. $C_{30}H_{37}NO_4$ Calc., %: C 75.76; H 7.84; N, 2.94.

Maleopimaric acid *N*-(*p*-fluorophenyl)amide (Vb). Yield 76%, amorphous compound. PMR spectrum ($CDCl_3$, δ, ppm): 0.60 (s, 3H, 17-H), 1.00 (d, 3H, 15-H, J 6.9 Hz), 0.96 (d, 3H, 16-H, J 6.4 Hz), 1.17 (s, 3H, 18-H), 1.21 – 1.91 (m, 13H, 4-H, 5-H, 5a-H, 7-H, 8-H, 9-H, 10-H), 2.26 (quint, 1H, 14-H, J 6.7 Hz), 2.54 (dt, 1H, H-9b, J 3.0, 13.8 Hz), 2.73 (d, 1H, 3a-H, J 8.7 Hz), 3.09 (dd, 1H, 11-H, J 8.6, 3.0 Hz), 3.98 (d, 1H, 11a-H, J 9 Hz), 5.54 (s, 1H, 13-H) 7.1 – 7.5 (m, 4H, C_6H_4), 6.8 (br.s, 1H, NH). ^{13}C NMR spectrum ($CDCl_3$, δ_C, ppm): 14.28 (C-17), 15.56 (C-18), 17.02 (C-8), 19.96 (C-16), 20.57 (C-15), 21.56 (C-5), 27.23 (C-10), 32.77 (C-14), 34.83 (C-4), 35.68 (C-11), 36.64 (C-7), 36.94 (C-9a), 38.04 (C-9), 40.48 (C-3b), 45.67 (C-11a), 46.87 (C-6), 49.38 (C-5a), 53.07 (C-3a), 53.28 (C-9b), 124.77 (C-13), 128.49 (C-2′, C-6′), 128.06 (C-4′), 127.73 (C-3′, C-5′), 136.30 (C-1′), 148.31 (C-12), 171.00 (C-1), 172.78 (C-3), 177.20 (C-19). Found, %: C 73.02; H 7.33; F, 3.88; N, 2.82. $C_{30}H_{36}FNO_4$. Calc., %: C 73.00; H 7.35; F, 3.85; N, 2.84.

Maleopimaric acid *N*-morpholylamide (Vc). Yield 54%, mp 79 – 80°C. PMR spectrum ($CDCl_3$, δ, ppm): 0.60 (s, 3H, 17-H), 1.00 (d, 3H, 15-H, J 6.9 Hz), 0.96 (d, 3H, 16-H, J 6.4 Hz), 1.17 (s, 3H, 18-H), 1.21 – 1.91 (m, 13H, 4-H, 5-H, 5a-H, 7-H, 8-H, 9-H, 10-H), 2.26 (quint, 1H, 14-H, J 6.7 Hz), 2.54 (dt, 1H, 9b-H, J 3.0, 13.8 Hz), 2.73 (d, 1H, 3a-H, J 8.7 Hz), 3.09 (dd, 1H, 11-H, J 8.6, 3.0 Hz), 3.98 (d, 1H, 11a-H, J 9 Hz), 3.55 – 3.86 (m, 8H, 2′-H, 3′-H, 5′-H, 6′-H), 5.54 (s, 1H, 13-H). ^{13}C NMR spectrum ($CDCl_3$, δ_C, ppm): 14.28 (C-17), 15.56 (C-18), 17.02 (C-8), 19.96 (C-16), 20.57 (C-15), 21.56 (C-5), 27.23 (C-10), 32.77 (C-14), 34.83 (C-4), 35.68 (C-11), 36.64 (C-7), 36.94 (C-9a), 38.04 (C-9), 40.48 (C-3b), 45.67 (C-11a), 46.53 (C-3′, C-5′), 46.87 (C-6), 49.38 (C-5a), 53.07 (C-3a), 53.28 (C-9b), 66.91 (C-2′, C-6′), 124.77 (C-13), 148.31 (C-12), 171.00 (C-1), 172.78 (C-3), 177.5 (C-19). Found, %: C 71.68; H 8.33; N, 2.90. $C_{28}H_{39}NO_5$. Calc., %: C 71.61; H 8.37; N, 2.98.

Maleopimaric acid *N*-(4-methyl-2-oxopyrrolidin-3-yl)amide (Ve). Yield 70%, mp 162 – 163°C. PMR spectrum ($CDCl_3$, δ, ppm): 0.60 (s, 3H, 17-H), 1.00 (d, 3H, 15-H, J 6.9 Hz), 0.96 (d, 3H, 16-H, J 6.4 Hz), 1.17 (s, 3H, 18-H), 1.21 – 1.91 (m, 13H, 4-H, 5-H, 5a-H, 7-H, 8-H, 9-H, 10-H), 1.38 (s, 3H, CH_3), 2.26 (quint, 1H, 14-H, J 6.7 Hz), 2.54 (dt, 1H, 9b-H, J 3.0, 13.8 Hz), 2.57 – 2.63 (m, 1H, 4′-H), 3.34 – 3.50 (m, 2H, 5′-H), 2.73 (d, 1H, 3a-H, J 8.7 Hz), 3.09 (dd, 1H, 11-H, J 8.6, 3.0 Hz), 3.98 (d, 1H, 11a-H, J 9 Hz), 4.18 – 4.23 (m, 1H, 3′-H), 5.54 (s, 1H, 13-H), 6.3 (br.s, 1H, NH), 6.5 (br.s, 1H, NH). ^{13}C NMR spectrum ($CDCl_3$, δ_C, ppm): 14.28 (C-17), 15.56 (C-18), 16.71 (C-20), 17.02 (C-8), 19.96 (C-16), 20.57 (C-15), 21.56 (C-5), 27.23 (C-10), 32.77 (C-14), 34.83 (C-4), 35.68 (C-11), 35.77 (C-4′), 36.64 (C-7), 36.94 (C-9a), 38.04 (C-9), 40.48 (C-3b), 45.67 (C-11a), 46.52 (C-5′), 46.87 (C-6), 49.38 (C-5a), 53.07 (C-3a), 53.28 (C-9b), 63.92 (C-3′), 124.77 (C-13), 148.31 (C-12), 171.00 (C-1), 172.78 (C-3), 176.03 (C-2′), 178.20 (C-19). Found, %: C 70.18; H 8.13 N, 5.68. $C_{29}H_{40}N_2O_5$. Calc., %: C 70.13; H 8.12; N, 5.64.

The antiplatelet activities of the diterpenic acid amides were evaluated using *in vitro* experimental methods and a peripheral blood cell model.

EXPERIMENTAL BIOLOGICAL PART

The experiments were conducted in compliance with requirements of Good Laboratory Practice Rules of the Eurasian Economic Union on Drug Circulation.

Antiplatelet and anticoagulant activities were evaluated under *in vitro* conditions on blood samples from 27 healthy male donors aged 18 – 24 years. The study was approved by the Ethics Committee of Bashkir State Medical University, Ministry of Health of Russia (protocol No. 1 of Feb. 20, 2019). Informed consent was obtained from all study participants for collection of their blood.

The effects of the compounds on platelet aggregation were studied by the Born method [15] on an AT-02 aggregometer (Medtekh NPF, Russia). The antiplatelet activities of the studied compounds and reference drugs were evaluated at a final concentration of $2 \cdot 10 - {}^3$ M after incubation for 5 min. The aggregation inductors were adenosine diphosphate (ADP) at a concentration of 20 ìg/mL and collagen at a concentration of 5 mg/mL (Tekhnologiya-Standart, Russia). The effects of the compounds on the maximum aggregation amplitude (MA), aggregation rate, and time to reach MA with platelet aggregation induced by ADP were studied. The latent period of aggregation in a collagen-induced platelet aggregation test was evaluated and corresponded to platelet release. The reference drug was acetylsalicylic acid (powder substance, Shandong Xinhua Pharmaceutical Co., Ltd., China) [16].

Anticoagulant activity was determined by clotting tests [16] on a Solar CGL 2110 turbidimetric hemocoagulometer (Solar ZAO, Belarus). The final concentration of the studied compounds and reference drug was $5 \cdot 10 - {}^4$ g/mL. The activated partial thromboplastin time (APTT), prothrombin time (PT), and fibrinogen concentration were studied according to A. Clauss. The reference drug was heparin sodium (heparin sodium, 5000 IU/mL, solution for injection, 1-mL ampuls, Sintez OAO, Russia).

Statistical analysis used Statistica 10.0 software (StatSoft Inc., USA). The normalcy of distributions was checked using the Shapiro–Wilk criterion. The median and 25 and 75 percentiles and minimum and maximum values were calculated to describe the variational series. One-factor dispersion analysis (if the data set obeyed normal distribution laws and the dispersions of all sets were equal; F-criterion) or the Kruskal–Wallis test (if the data set did not obey normal dis-

TABLE 1. Effect of Newly Synthesized Compounds and Reference Drug on Platelet Aggregation and Plasma Coagulation, Me (0.25 – 0.75)

Compound	Change of latent period, % of control	Change of maximum amplitude, % of control	Change of aggregation rate, % of control	APTT lengthening, % of control
IVa	–9.3 (8.4 – 11.5)$^{*,\#}$	–16.9 (14.3 – 17.5)**	–15.4 (12.3 – 19.1)$^{*,\#}$	4.3 (3.7 – 6.5)
IVb	–11.2 (9.3 – 12.4)$^{*,\#}$	–15.9 (15.3 – 18.4)**	–27.3 (25.4 – 30.5)**	5.3 (4.7 – 8.2)*
IVc	+3.6 (2.7 – 5.2)$^{\#}$	–4.5 (3.7 – 5.9)$^{*,\#\#}$	–19.4 (15.7 – 21.3)$^{*,\#}$	6.5 (5.8 – 7.9)*
IVd	+1.6 (1.3 – 2.5)$^{\#\#}$	–8.4 (7.6 – 9.1)$^{*,\#}$	+16.4 (13.2 – 18.6)**	9.7 (7.4 – 11.2)*
Va	–12.1 (11.7 – 14.7)$^{*,\#}$	–14.1 (12.1 – 15.2)**	–20.1 (16.9 – 24.5)$^{**,\#}$	8.4 (6.1 – 10.2)*
Vb	–13.4 (11.8 – 17.3)$^{*,\#}$	–12.4 (10.5 – 14.5)**	–10.3 (6.8 – 11.9)*	4.7 (3.2 – 6.4)*
Vc	–10.2 (9.6 – 16.3)$^{*,\#}$	–9.2 (7.1 – 12.4)*	–13.8 (11.1 – 15.6)*	7.5 (6.1 – 8.9)*
Ve	+4.5 (3.7 – 5.4)$^{\#\#}$	–2.5 (1.4 – 5.2)$^{\#\#}$	+1.5 (0.8 – 1.9)$^{*,\#\#}$	9.4 (8.8 – 10.5)*
Acetylsalicylic acid	–2.1 (1.1 – 2.6)	–13.7 (10.8 – 16.4)*	–10.5 (7.6 – 12.3)*	-
Heparin sodium	–	–	–	20.3 (19.7 – 21.4)*

$^{*}p \le 0.05$, $^{**}p \le 0.001$ vs. the control; $^{\#}p \le 0.05$, $^{\#\#}p \le 0.001$ vs. acetylsalicylic acid; $n = 6$.

tribution laws; A-criterion) was used. The critical significance level of p for statistical criteria was taken as 0.05.

RESULTS AND DISCUSSION

The study results established that the series of compounds exhibited antiplatelet activity at the level of acetylsalicylic acid for the maximum amplitude (Table 1). However, compounds **IVc**, **IVd**, and **Ve**, in contrast to acetylsalicylic acid, statistically significantly increased the lag period, which corresponded to the platelet release response. The platelet aggregation rate, like the effect of acetylsalicylic acid, decreased for the new derivatives except for **IVd** and **Ve** (heterocyclic amides containing a 2-oxopyrrolidine fragment). This parameter decreased most effectively for **IVa-c** and **Va**.

It is noteworthy that all compounds caused hypocoagulation, increasing the APTT by 4.3 – 9.7% as compared to the control and did not affect the fibrinogen concentration and prothrombin time. The studied compounds were significantly less potent than heparin, which increased the APTT by 20.3%.

Thus, compounds exhibiting antiplatelet and anticoagulant properties were identified among the synthesized AA and MPA amides and were interesting for further testing.

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Conflict of interest

We declare no conflict of interest requiring disclosure in this article.

Contributions of authors

All authors contributed equally to the research. RMS developed the concept of the scientific work, reviewed publications relevant to the article, and wrote the article text; NSKh, YuGB, ZAV, and EVK conducted the experiments, processed the material, and wrote the text; GZR analyzed the literature, wrote the article, formalized the reference list, and statistically processed the results; SAM consulted on issues with conducting separate stages of the research; SSZ consulted on planning issues, methodology, and performance of the research; AVS consulted on issues with conducting separate stages of the research.

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