DOI 10.1007/s11094-022-02636-9
Pharmaceutical Chemistry Journal, Vol. 56, No. 3, June, 2022 (Russian Original Vol. 56, No. 3, March, 2022)

SYNTHESIS AND BIOLOGICAL ACTIVITY OF 5-ALKOXY- AND 5-AMINO-SUBSTITUTED 3-BROMO-4-NITRO-1-(THIETAN-3-YL)-1*H*-PYRAZOLES

F. A. Khaliullin,[1,*] E. E. Klen,[1] V. N. Pavlov,[1] A. V. Samorodov,[1] S. O. Shepilova,[1] N. N. Makarova,[1] S. N. Nurlanova,[1] and T. N. Abzalilov[1]

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 56, No. 3, pp. 15 – 20, March, 2022.

Original article submitted November 14, 2021.

5-Alkoxy- and 5-amino-substituted 3-bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazoles were synthesized by reactions of 3,5-dibromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazole with sodium alcoholates and primary amines. The structures of the synthesized compounds were established by IR, PMR, and ^{13}C NMR spectroscopy. *In vitro* studies of the antiplatelet, anticoagulant, and antioxidant activity revealed promising compounds, including 3-bromo-5-methoxy-4-nitro-1-(thietan-3-yl)-1*H*-pyrazole with antioxidant action and 2-{[3-bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazol-5-yl]amino}ethan-1-ol with an antiplatelet effect. Results of *in silico* calculations predicted the absence of toxic risks (mutagenicity, oncogenicity, reproductive toxicity, local irritation) and acceptable bioavailability (in terms of the topological polar surface area).

Keywords: thietane, pyrazole, amines, alcoholates, antiplatelet activity, anticoagulant activity, antioxidant activity, Lipinsky's "rule of five."

Our previous studies showed that thietane-containing xanthines [1, 2] and triazoles [3] affected the hemostasis system and possessed antioxidant activity [4]. Pyrazole derivatives are interesting in the search for biologically active thietanylazoles. The pyrazole moiety is present in drugs with antitumor, anti-inflammatory, antidiabetic, and other types of activity, including anticoagulant [5-9] (Fig. 1). The goal of the present research was to synthesize new 5-alkoxy- and 5-amino-substituted 3-bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazoles and to evaluate their antiplatelet, anticoagulant, and antioxidant activity.

Starting 3,5-dibromo-4-nitro-(thietan-3-yl)-1*H*-pyrazole (**I**) was prepared by reacting 3,5-dibromo-4-nitropyrazole with 2-chloromethylthiirane by the literature method [10]. Reactions of **I** with sodium alcoholates were performed in the corresponding alcohol at room temperature for 2-3 h. 5-Alkoxy-substituted 3-bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazoles **IIa-e** were synthesized in 36-84% yields (Scheme 1).

[Scheme 1, R. p. 15]

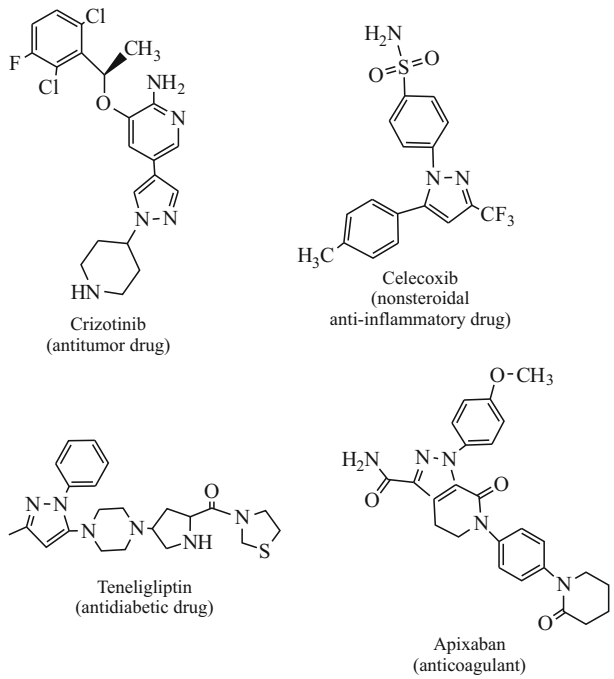

Fig. 1. Medicinal pyrazole derivatives.

[1] Bashkir State Medical University, Ministry of Health of Russia, 3 Lenina St., Ufa, Bashkortostan, 450008 Russia.
[*] e-mail: ferkat@mail.ru

Pyrazole **I** was reacted with primary aliphatic amines under reflux in EtOH for 1-3 h. 5-Amino-substituted 3-bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazoles **IIIa-d** were obtained in 54-82% yields (Scheme 1).

EXPERIMENTAL CHEMICAL PART

IR spectra were recorded in KBr pellets on an Infralyum-FT-02 spectrometer. Melting points were measured on an SMP 30 apparatus. PMR and ^{13}C NMR spectra were recorded at 298 K on a Bruker Avance III spectrometer at operating frequency 500.13 MHz (^{1}H) and 125.47 MHz (^{13}C) using a 5-mm PABBO probe with Z-gradient. Chemical shifts in PMR and ^{13}C NMR spectra were given vs. residual solvent resonances ($CDCl_3$, 7.26 ppm for ^{1}H, 77.0 ppm for ^{13}C). ^{13}C NMR spectra were interpreted based on DEPT-90 and DEPT-135 experiments. Elemental analyses for C, H, N, and S agreed with the calculated values. The purity of products was confirmed by TLC on Sorbfil PTSKh-P-A-UF plates using hexane–$CHCl_3$ (1:4, v/v) for **II** and $CHCl_3$–MeOH–NH_4OH (9:1:0.1, v/v) for **III**. Spots were detected by UV light and in a chamber with I_2 vapor.

General synthetic method for 5-alkoxy-substituted 3-bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazoles (IIa-c). The appropriate alcohol (12 mL) was treated with metallic Na (0.04 g, 1.65 mmol), stirred until gas evolution stopped, treated with pyrazole **I** (0.51 g, 1.5 mmol), stirred at room temperature for 2-3 h, and treated with H_2O (20 mL). The resulting precipitate was filtered off, rinsed with H_2O, and dried. The reaction mixture for **IIe** was evaporated under vacuum and then worked up analogously.

3-Bromo-5-methoxy-4-nitro-1-(thietan-3-yl)-1*H*-pyrazole (IIa). Yield 0.43 g (84.0%). mp 70 – 71°C (EtOH). R_f 0.17. IR spectrum, ν, cm^{-1}: 1047, 1401, 1502, 1567 (C-O, C-N, C=C, C=N), 1349, 1463 (NO_2). PMR spectrum, δ, ppm: 3.23 – 3.27 (m, 2H, $S(CH)_2$), 4.04 – 4.07 (m, 2H, $S(CH)_2$), 4.20 (s, 3H, OCH_3), 5.59 – 5.66 (m, 1H, NCH). ^{13}C NMR spectrum, δ, ppm: 33.2 ($S(CH_2)_2$), 52.4 (NCH), 63.9 (OCH_3), 122.8 (C_3), 149.8 (C_5). $C_7H_8BrN_3O_3S$.

3-Bromo-4-nitro-1-(thietan-3-yl)-5-ethoxy-1*H*-pyrazole (IIb). Yield 0.36 g (78.3%). mp 96 – 97°C (EtOH). R_f 0.29. IR spectrum, ν, cm^{-1}: 1038, 1400, 1495, 1556 (C-O, C-N, C=C, C=N), 1337, 1456 (NO_2). PMR spectrum, δ, ppm: 1.48 (t, 3H, CH_3, J 7.1 Hz), 3.22 – 3.25 (m, 2H, $S(CH)_2$), 4.04 – 4.08 (m, 2H, $S(CH)_2$), 4.47 (q, 2H, OCH_2, J 7.1 Hz), 5.60 – 5.67 (m, 1H, NCH). ^{13}C NMR spectrum, δ, ppm: 15.4 (CH_3), 33.3 ($S(CH_2)_2$), 52.2 (NCH), 73.8 (ÎCH$_2$), 122.9 (C_3), 148.9 (C_5). $C_8H_{10}BrN_3O_3S$.

3-Bromo-4-nitro-5-*n*-propoxy-1-(thietan-3-yl)-1*H*-pyrazole (IIc). Yield 0.27 g (56.3%). mp 67 – 69°C (EtOH — H_2O, 1:3). R_f 0.19. IR spectrum, ν, cm^{-1}: 1042, 1402, 1502, 1569 (C-O, C-N, C=C, C=N), 1346, 1455 (NO_2). PMR spectrum, δ, ppm: 1.06 (t, 3H, CH_3, J 7.4 Hz), 1.83 – 1.89 (m, 2H, CH_2), 3.22 – 3.25 (m, 2H, $S(CH)_2$), 4.04 – 4.10 (m, 2H, $S(CH)_2$), 4.34 (t, 2H, OCH_2, J 6.7 Hz), 5.59 – 5.66 (m, 1H, NCH). ^{13}C NMR spectrum, δ, ppm: 10.0 (CH_3), 23.2 (CH_2), 33.2 ($S(CH_2)_2$), 52.1 (NCH), 79.2 (OCH_2), 120.4 (C_4), 122.9 (C_3), 149.2 (C_5). $C_9H_{12}BrN_3O_3S$.

3-Bromo-5-isopropoxy-4-nitro-1-(thietan-3-yl)-1*H*-pyrazole (IId). Yield 0.24 g (50%). mp 141 – 143°C (EtOH). R_f 0.33. IR spectrum, ν, cm^{-1}: 1047, 1405, 1488, 1558 (C-O, C-N, C=C, C=N), 1346; 1457 (NO_2). PMR spectrum, δ, ppm: 1.41 (d, 6H, $(CH_3)_2$, J 6.2 Hz), 3.19 – 3.23 (m, 2H, $S(CH)_2$), 4.04 – 4.08 (m, 2H, $S(CH)_2$), 4.92 – 4.97 (m, 1H, OCH), 5.61 – 5.68 (m, 1H, NCH). ^{13}C NMR spectrum, δ, ppm: 22.4 ($(CH_3)_2$), 33.3 ($S(CH_2)_2$), 51.8 (NCH), 82.1 (OCH), 123.2 (C_3), 148.2 (C_5). $C_9H_{12}BrN_3O_3S$.

3-Bromo-5-*n*-butoxy-4-nitro-1-(thietan-3-yl)-1*H*-pyrazole (IIe). Yield 0.18 g (36%). mp 74 – 76°C (hexane). R_f 0.21. IR spectrum, ν, cm^{-1}: 1038, 1409, 1497, 1558 (C-O, C-N, C=C, C=N), 1342, 1461 (NO_2). PMR spectrum, δ, ppm: 1.00 (t, 3H, CH_3, J 7.4 Hz), 1.46 – 1.53 (m, 2H, CH_2CH_3), 1.79 – 1.84 (m, 2H, CH_2), 3.21 – 3.25 (m, 2H, $S(CH)_2$), 4.04 – 4.08 (m, 2H, $S(CH)_2$), 4.38 (t, 2H, OCH_2, J 6.7 Hz), 5.58 – 5.65 (m, 1H, NCH). ^{13}C NMR spectrum, δ, ppm: 13.6 (CH_3), 18.7 (CH_2), 31.7 (CH_2), 33.2 ($S(CH_2)_2$), 52.1 (NCH), 77.6 (OCH_2), 120.4 (C4), 122.9 (C_3), 149.2 (C_5). $C_{10}H_{14}BrN_3O_3S$.

General synthetic method for 5-amino-substituted-3-bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazoles (IIIa-d). A solution of pyrazole **I** (1.73 g, 5 mmol) in EtOH (50 mL) was treated with an amine (15 mmol, 25 mmol for **IIIb**), refluxed for 1 – 3 h, and cooled. The resulting precipitate was filtered off, rinsed with H_2O, and dried. The reaction mixture was evaporated under vacuum to one half the volume for **IIc**, cooled, treated with H_2O (20 mL), and then worked up analogously.

Yield 1.33 g (82.3%). mp 147 – 148°C (EtOH — H_2O, 1:3). R_f 0.45. IR spectrum, ν, cm^{-1}: 1041, 1405, 1526, 1604 (C-O, C-N, C=C, C=N), 1329, 1475 (NO_2), 3240 (N-H), 3536 (O-H). PMR spectrum, δ, ppm: 3.24 – 3.28 (m, 2H, $S(CH)_2$), 3.54 – 3.56 (m, 2H, NCH_2), 3.87 – 3.93 (m, 2H, OCH_2), 4.15 – 4.19 (m, 2H, $S(CH)_2$), 5.61 – 5.68 (m, 1H, NCH), 6.98 (br.c, 1H, NH). ^{13}C NMR spectrum, δ, ppm: 33.9 ($S(CH_2)_2$), 48.8 ($NHCH_2$), 54.7 (NCH), 61.5 (OCH_2), 114.3 (C_3), 147.2 (C_5). $C_8H_{11}BrN_4O_3S$.

2-{[3-Bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazol-5-yl]-amino}ethan-1-ol (IIIa). Yield 1.33 g (82.3%). mp 147 – 148°C (EtOH — H_2O, 1:3). R_f 0.45. IR spectrum, ν, cm^{-1}: 1041, 1405, 1526, 1604 (C-O, C-N, C=C, C=N), 1329, 1475 (NO_2), 3240 (N-H), 3536 (O-H). PMR spectrum, δ, ppm: 3.24 – 3.28 (m, 2H, $S(CH)_2$), 3.54 – 3.56 (m, 2H, NCH_2), 3.87 – 3.93 (m, 2H, OCH_2), 4.15 – 4.19 (m, 2H, $S(CH)_2$), 5.61 – 5.68 (m, 1H, NCH), 6.98 (br.c, 1H, NH). ^{13}C NMR spectrum, δ, ppm: 33.9 ($S(CH_2)_2$), 48.8 ($NHCH_2$), 54.7 (NCH), 61.5 (OCH_2), 114.3 (C_3), 147.2 (C_5). $C_8H_{11}BrN_4O_3S$.

***N*^{1}-[3-Bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazol-5-yl]-ethane-1,2-diamine (IIIb).** Yield 0.87 g (54%). mp 118 – 119°C (EtOH). R_f 0.35. IR spectrum, ν, cm^{-1}: 1041, 1405, 1511, 1620 (C-N, C=C, C=N), 1329, 1474 (NO_2), 3226 – 3443 (N-H). PMR spectrum, δ, ppm: 1.33 (br.c, 2H, NH_2), 3.01 – 3.03 (m, 2H, CH_2NH_2), 3.22 – 3.26 (m, 2H, $S(CH)_2$), 3.42 – 3.46 (m, 2H, $NHCH_2$), 4.14 – 4.18 (m, 2H, $S(CH)_2$), 5.61 – 5.68 (m, 1H, NCH), 7.28 (br.c, 1H, NH). ^{13}C NMR spectrum, δ, ppm: 34.0 ($S(CH_2)_2$), 41.3 (NH_2CH_2), 49.0 ($NHCH_2$), 54.8 (NCH), 118.3 (C_4), 123.3 (C_3), 147.2 (C_5). $C_8H_{12}BrN_5O_2S$.

***N*^{1}-[3-Bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazol-5-yl]-propane-1,2-diamine (IIIc).** Yield 1.27 g (75.5%). mp 142 – 143°C (EtOH). R_f 0.39. IR spectrum, ν, cm^{-1}: 1045, 1402, 1497, 1606 (C-N, C=C, C=N), 1321, 1477 (NO_2), 3281 – 3381 (N-H). PMR spectrum, δ, ppm: 1.19 (d, 3H, CH_3, J 6.1 Hz), 1.38 (br.c, 2H, NH_2), 3.14 – 3.40 (m, 5H, $S(CH)_2$, CH_2, CH), 4.14 – 4.19 (m, 2H, $S(CH)_2$), 5.59 – 5.66 (m, 1H, NCH), 7.35 (br.c, 1H, NH). ^{13}C NMR spectrum, δ, ppm: 22.0 (CH_3) 34.0 ($S(CH_2)_2$), 46.7 (CH), 54.2 ($NHCH_2$), 54.8 (NCH), 123.3 (C_3), 147.3 (C_5). $C_9H_{14}BrN_5O_2S$.

***N*^{1}-[3-Bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazol-5-yl]-*N*^{2}, *N*^{2}-dimethylethane-1,2-diamine (IIId).** Yield 1.32 g (75.4%). mp 147 – 148°C (*i*-PrOH). R_f 0.67. IR spectrum, ν, cm^{-1}: 1042, 1400, 1514, 1606 (C-N, C=C, C=N), 1333, 1474 (NO_2), 3263 (N-H). PMR spectrum, δ, ppm: 2.29 (s, 6H, $N(CH_3)_2$), 2.57 (t, 2H, $CH_2N(CH_3)_2$, J 5.9 Hz), 3.22 – 3.25 (m, 2H, $S(CH)_2$), 3.42 – 3.46 (m, 2H, $NHCH_2$), 4.16 – 4.19 (m, 2H, $S(CH)_2$), 5.55 – 5.62 (m, 1H, NCH), 7.52 (br.c, 1H, NH). ^{13}C NMR spectrum, δ, ppm: 34.1 ($S(CH_2)_2$), 44.2 (CH_2), 44.9 (($CH_3)_2$), 57.7 ($NHCH_2$), 54.7 (NCH), 123.3 (C_3), 147.2 (C_5). $C_{10}H_{16}BrN_5O_2S$.

EXPERIMENTAL BIOLOGICAL PART

Experiments were conducted in compliance with *Good Laboratory Practice Rules of the Eurasian Economic Union in the Field of Medicinal Products in Circulation* [12].

Antiplatelet and anticoagulant activities were determined *in vitro* using blood from healthy male donors (18 – 24 years old). The studies were approved by the Ethics Committee of Bashkir State Medical University, Ministry of Health of Russia (Protocol No. 2, Oct. 17, 2012). Informed consent was obtained from all study participants before collecting blood.

The effects of the compounds on platelet aggregation were studied using the Born method [13] on an AT-02 aggregometer (NPF Medtekh, Russia). Antiplatelet activity of the tested compounds and reference drugs was assessed at a final concentration of $1\cdot10^{-3}$ M. The aggregation indicators were adenosine diphosphate (ADP) at a concentration of 20 ìg/mL and collagen at a concentration of 5 mg/mL (Tekhnologiya-Standart, Russia). The assessed parameters were the latent period in the collagen-induced aggregation test, maximum amplitude, aggregation rate, and time to reach the maximum amplitude in the ADP-induced aggregation test. The reference drugs were pentoxifylline (20 mg/mL solution for injection, 5-mL ampuls, OAO Dalkhimfarm, Russia) and acetylsalicylic acid (substance powder, Shandong Xinhua Pharmaceutical Co., Ltd., China).

Anticoagulant activity was determined using clotting tests [14] on a Solar CGL 2110 turbidimetric hemocoagulometer (ZAO SOLAR, Belarus). The final concentration of the tested compounds and reference drug was $5\cdot10^{-4}$ g/mL. The assessed parameters were the activated partial thromboplastin time (APTT), prothrombin time, and fibrinogen concentration according to Clauss. The reference drug was heparin sodium (5000 IU/mL solution for injection, 1-mL ampuls, OAO Sintez, Russia).

Antioxidant properties of the compounds were determined *in vitro* by recording chemiluminescence on a KhLM-003 chemiluminometer (Russia). Three model systems in which the formation of reactive oxygen species (ROS) (model I), lipid peroxidation (LPO) (model II), and ROS generation by phagocytes (model III) were initiated were used [15]. The assessed parameters were the emission light sum and maximum flash. The tested compounds and reference drug ascorbic acid (substance powder, Hebei Welcome Pharmaceutical Co., Ltd., China) were added to a final concentration of $1\cdot10^{-3}$ M.

Statistical analysis used the Statistica 10.0 software (StatSoft Inc., USA). A check for normal distributions of actual data used the Shapiro–Wilk criterion. The distribution of the obtained data was found to differ from normal. Therefore, further work used nonparametric methods. Data were presented as medians and 25 and 75 percentiles. Dispersion analysis used the Kruskal–Wallis criterion. The critical significance level p was taken as 0.05 [16].

RESULTS AND DISCUSSION

The structures of **IIa-e** and **IIIa-d** were confirmed by IR, PMR, and ^{13}C NMR spectra. NMR spectra contained resonances for the thietane ring in the characteristic regions [11] and resonances of the 5-substituents. For example, the PMR spectrum of **IIa** exhibited a singlet at 4.20 ppm (OCH_3); of **IIId**, a singlet at 2.29 [$N(CH_3)_2$], a triplet at 2.57, and a multiplet in the range 3.42 – 3.46 corresponding to two NCH_2 groups. ^{13}C NMR spectra of **II** and **III** showed the resonance of C5 at 147 – 149 ppm and a weak-field shift of ~30 ppm relative to the analogous resonance of starting **I**. This confirmed that the pyrazole 5-Br atom had been displaced [10].

The study of the antiplatelet activity of the synthesized compounds found that **IIc**, **IIe**, and **IIIa-d** increased the latent period in the collagen-induced aggregation test. However, they were considerably inferior to the reference drug pentoxifylline. The other studied compounds and acetylsalicylic acid did not affect this parameter (Table 1).

Compounds **IIa**, **IIc**, **IIIa**, and **IIIb** in the ADP-induced platelet aggregation test decreased the maximum amplitude as compared to acetylsalicylic acid. Compounds **IIa**, **IIc-e**, and **IIId** caused a decrease in the platelet aggregation rate that was comparable to that of acetylsalicylic acid while 5-aminopyrazoles **IIIa** and **IIIc** were superior to acetylsalicylic acid. Compounds **II** and **III** increased the time to reach the maximum amplitude of aggregation as compared to the control group (except for **IIIc**) while 5-alkoxypyrazoles **IIa-e** increased this parameter as compared to acetylsalicylic acid and 5-aminopyrazoles **IIIa** and **IIIb** were superior to acetylsalicylic acid. The parameters of compounds **II** and **III** were inferior to those of pentoxifylline (Table 1).

Compounds **II** and **III** caused significant hypocoagulation, increasing the APTT by 3.9 – 11.2% as compared to the control, and did not affect the prothrombin time and

TABLE 1. Effect of **IIa-e**, **IIIa-d**, and Reference Drugs on Platelet Aggregation and Coagulation Parameters, Me (25%–75%), $n = 6$

Compound	Latent period, % of control	Maximum amplitude, % of control	Aggregation rate, % of control	Time to reach maximum amplitude, % of control	APTT, % of control
	Collagen-induced aggregation	ADP-induced aggregation			
IIa	–3.3 (3.1 – 5.2)†	–9.3 (7.2 – 11.7)*,†	–7.9 (6.1 – 11.2)*,†	+11.3 (9.7 – 13.8)*,†	+5.3 (4.8 – 6.9)*,§
IIb	+3.8 (2.9 – 4.1)$^{\dagger,\#}$	–5.1 (4.7 – 8.5)$^{*,\dagger,\#}$	–2.5 (1.2 – 3.7)$^{\dagger,\#}$	+7.4 (5.9 – 9.7)*,†	+11.2 (10.5 – 14.3)*,§
IIc	+6.2 (5.1 – 7.7)$^{*,\dagger,\#}$	–11.3 (8.6 – 13.2)*,†	–12.5 (10.2 – 15.7)*,†	+12.5 (10.7 – 14.3)*,†	+7.1 (6.4 – 8.9)*,§
IId	–3.4 (2.6 – 4.1)†	–6.9 (4.9 – 8.3)$^{*,\dagger,\#}$	–10.3 (8.7 – 12.1)*,†	+13.1 (11.2 – 15.7)*,†	+9.7 (7.4 – 12.7)*,§
IIe	+8.3 (6.3 – 10.5)$^{*,\dagger,\#}$	–8.4 (6.3 – 10.4)$^{*,\dagger,\#}$	–11.9 (10.3 – 13.4)*,†	+9.8 (8.3 – 12.1)*,†	+4.7 (3.2 – 6.1)*,§
IIIa	+9.4 (8.3 – 11.7)$^{*,\dagger,\#}$	–15.7 (12.3 – 17.8)*,†	–15.9 (12.6 – 19.7)$^{*,\dagger,\#}$	+13.4 (11.9 – 15.7)$^{*,\dagger,\#}$	+4.1 (3.2 – 7.6)*,§
IIIb	+10.6 (7.8 – 13.3)$^{*,\dagger,\#}$	–12.1 (10.4 – 15.7)*,†	–4.6 (2.5 – 6.2)$^{\dagger,\#}$	+20.1 (17.6 – 22.4)$^{*,\dagger,\#}$	+9.2 (8.4 – 11.4)*,§
IIIc	+14.7 (10.8 – 18.7)$^{*,\dagger,\#}$	–1.9 (0.5 – 2.3)$^{\dagger,\#}$	–13.5 (11.2 – 17.7)$^{*,\dagger,\#}$	+4.9 (2.3 – 7.5)$^{\dagger,\#}$	+4.1 (3.6 – 5.7)*,§
IIId	+13.5 (11.2 – 15.9)$^{*,\dagger,\#}$	–5.4 (3.8 – 9.3)$^{*,\dagger,\#}$	–9.5 (7.6 – 10.4)*,†	+6.3 (4.5 – 8.7)$^{*,\dagger,\#}$	+3.9 (3.1 – 4.5)*,§
Pentoxifylline	+32.4 (28.7 – 35.6)*	–48.4 (42.7 – 56.5)**	–34.9 (28.7 – 39.6)**	+32.1 (27.6 – 32.4)**	–
Acetylsalicylic acid	–2.1 (1.1 – 2.6)†	–13.7 (10.8 – 16.4)*	–10.5 (7.6 – 12.3)*	+10.5 (8.7 – 13.4)*	–
Heparin sodium	–	–	–	–	+20.3 (19.7 – 21.4)*

$^{*}p \le 0.05$; $^{**}p \le 0.001$ vs. control; $^{\dagger}p \le 0.05$ vs. pentoxifylline; $^{\#}p \le 0.05$ vs. acetylsalicylic acid; $^{\S}p \le 0.05$ vs. heparin sodium.

TABLE 2. Effect of **IIa**, **IIc**, **IIIa**, **IIId**, and Reference Drug on Chemiluminescence Parameters, Me (25%–75%), $n = 6$

No.	Compound	Model	Emission light sum, % of control	Maximum flash, % of control
1	**IIa**	I	–88.2 (85.1 – 93.5)**	–17.4 (15.7 – 20.8)*,†
		II	–24.5 (21.5 – 29.3)**,†	–14.6 (12.5 – 17.6)*,†
		III	–16.4 (13.3 – 17.8)*,†	–11.6 (9.3 – 16.5)*,†
2	**IIc**	I	–44.2 (39.6 – 46.7)**,†	–45.1 (42.7 – 51.6)**,†
		II	–11.7 (10.5 – 14.8)*,†	–37.6 (34.6 – 41.3)**,†
		III	–16.2 (14.3 – 19.5)**,†	–24.5 (22.1 – 24.9)**,†
3	**IIIa**	I	–33.9 (28.7 – 35.9)**,†	–16.8 (13.1 – 18.7)*,†
		II	–15.4 (12.7 – 18.3)*,†	–13.6 (10.1 – 17.5)*,†
		III	–9.4 (7.8 – 12.3)*,†	–12.7 (11.8 – 15.9)*,†
4	**IIId**	I	–30.5 (28.3 – 32.5)*,†	–14.9 (12.7 – 16.8)*,†
		II	–11.4 (10.2 – 15.3)*,†	–12.5 (10.3 – 14.8)*,†
		III	–10.4 (9.6 – 12.6)*,†	–13.9 (12.1 – 17.5)*,†
5	Ascorbic acid	I	–84.5 (79.3 – 87.1)**	–91.7 (82.3 – 95.2)**
		II	–78.1 (70.4 – 82.4)**	–86.8 (80.3 – 92.1)**
		III	+73.1 (66.7 – 75.2)**	+98.7 (94.8 – 100.3)**

$^{*}p \pm 0.05$; $^{**}p \pm 0.001$ vs. control; $^{\dagger}p \pm 0.05$ vs. ascorbic acid.

TABLE 3. Agreement with Lipinsky's "Rule of Five" and "Drug-likeness" of Synthesized Compounds

Compound	Molecular mass	log *P*	Number of H acceptors	Number of H donors	TPSA, Å^2	Drug-likeness
IIa	294	0.31	6	0	98.17	–5.65
IIb	308	0.71	6	0	98.17	–7.48
IIc	322	1.17	6	0	98.17	–6.45
IId	322	1.07	6	0	98.17	–6.46
IIe	336	1.62	6	0	98.17	–11.29
IIIa	323	–0.50	7	2	121.2	–5.18
IIIb	322	–2.62	7	2	126.99	–7.95
IIIc	336	–2.26	7	2	126.99	–6.42
IIId	350	–1.37	7	1	104.21	–4.42

fibrinogen concentration. The anticoagulant activities of the tested compounds were inferior to that of heparin, which increased the APTT by 20.3% (Table 1).

Compounds **IIa**, **IIc**, **IIIa**, and **IIId** diminished ROS generation in models I and III both according to the emission light sum and maximum flash as compared to the control. 5-Alkoxypyrazole **IIa** caused a reduction of ROS generation in model I according to the emission light sum that was comparable to that of ascorbic acid. It is noteworthy that the tested compounds in model III exhibited antioxidant activity while ascorbic acid acted as a prooxidant (Table 2).

The synthesized compounds were analyzed using the Osiris DataWarrior program [19] to determine toxic risks, the "drug-likeness" parameter, and agreement with Lipinsky's "rule of five" [17, 18].

Toxic risks such as a negative influence on reproductive function and mutagenic and oncogenic properties were not predicted for the synthesized compounds although 4-butoxypyrazole **IIe** and 5-aminopyrazole **IIIc** could cause irritation.

The calculated physicochemical parameters of **II** and **III** were found to satisfy Lipinsky's rule of five (Table 3). The molecular masses of the synthesized compounds were less than 350 g/mol. The lipophilicity coefficient (log P) fell in the range from –2.62 to +1.62. The number of hydrogen (H) acceptors was ?7; H donors, ?2. The topological polar surface area (TPSA) ranged from 98.17 to 126.99 Å^2 and suggested that the synthesized compounds had good permeability through cell membranes. The drug-likeness parameter in the range from –11.29 to –4.42 confirmed that the tested compounds had novel structures.

Thus, the pharmacological screening results and toxic risks and physicochemical properties calculated *in silico* established that the 5-alkoxy- and 5-amino-substituted 3-bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazoles formed a promising class of compounds for discovering antiplatelet, anticoagulant, and antioxidant drugs. 5-Methoxypyrazole **IIa** (antioxidant) and 5-aminopyrazole **IIIa** (antiaggregant), which satisfied Lipinsky's rule of five and were characterized by novel structures and a lack of toxic risks, were the most active. This indicated that research on this series of compounds should continue.

REFERENCES

1. E. E. Klen, V. A. Nebogatova, L. I. Bashirova, et al., *Vopr. Biol., Med. Farm. Khim.*, **23**(1), 23 – 29 (2020).
2. K. G. Gurevich, A. L. Urakov, E. E. Klen, et al., *Khim.-farm. Zh.*, **54**(3), 3 – 10 (2020); *Pharm. Chem. J.*, **54**, 213 – 219 (2020).
3. A. V. Samorodov, F. Kh. Kamilov, E. E. Klen, et al., *FASEB J.*, **31**, No. S1, 674.14 (2017).
4. K. G. Gurevich, A. L. Urakov, G. A. Rozit, et al., *Khim.-farm. Zh.*, **55**(5), 3 – 8 (2021); *Pharm. Chem. J.*, **55**, 417 – 422 (2021).
5. K. Karrouchi, S. Radi, and Y. Ramli, *Molecules*, **23**(1), 134 (2018).
6. M. Proietti, I. Romanazzi, G. F. Romiti, and A. Farcomeni, *Stroke*, **49**(1), 98 – 106 (2018).
7. D. F. Heigener and M. Reck, "Critozinib," in: *Small Molecules in Oncology*, 3rd Ed., U. M. Martens (ed.), *Recent Results in Cancer Research*, Vol. 211, Springer International Publishing AG, part of Springer Nature, (2018), pp. 57 – 65.
8. M. Krasselt and C. Baerwald, *Expert Opin. Pharmacother.*, **20**(14), 1689 – 1702 (2019).
9. S. Kimura, T. Inoguchi, T. Yamasaki, et al., *Metab., Clin. Exp.*, **65**(3), 138 – 145 (2016).
10. F. A. Khaliullin, E. E. Klen, N. N. Makarova, et al., *Khim. Geterotsikl. Soedin.*, **56**(9), 1 – 5 (2020); *Chem. Heterocycl. Compd.*, **56**, 1213 – 1217 (2020).
11. S. Lesniak, W. J. Kinart, and J. Lewkowski, *Comprehensive Heterocyclic Chemistry III*, Vol. 2, Elsevier (2008), pp. 389 – 428.
12. *Good Laboratory Practice Rules of the Eurasian Economic Union in the Field of Medicinal Products in Circulation* [in Russian], Approved by EEC Committee Decision No. 81, Nov. 3, 2016.
13. G. G. V. Born, *Nature*, **194**, 927 – 929 (1962).
14. A. N. Mironov (ed.), *Handbook for Preclinical Drug Studies* [in Russian], Vol. 1, Grif i K, Moscow (2013).
15. R. R. Farkhutdinov and S. I. Tevdoradze, *Procedures for Studying Chemiluminescence of Biological Material on an KhL-003 Chemiluminometer. Methods for Assessing Antioxidant Activity of Biologically Active Substances* [in Russian], RUDN, Moscow (2005), pp. 147 – 154.
16. R. Kh. Khafiz'yanova, I. M. Burykin, and G. N. Aleeva, *Mathematical Statistics in Experimental and Clinical Pharmacology* [in Russian], Meditsina, Kazan' (2006).
17. C. A. Lipinsky, *J. Pharmacol. Toxicol. Methods*, **44**(1), 235 – 249 (2000).
18. C. A. Lipinsky, F. Lombardo, B. W. Dominy, et al., *Adv. Drug. Deliv. Rev.*, **46**(1) – 3, 3 – 26 (2001).
19. T. Sander, J. Freyss, M. von Korff, et al., *J. Chem. Inf. Model.*, **55**(2), 460 – 473 (2015).