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SEARCH FOR NEW DRUGS

SYNTHESIS AND ANTIPLATELET AND ANTICOAGULANT ACTIVITY OF THIETANE-CONTAINING 2-(5-BROMO-2,4-DIHYDRO-3-OXO-1,2,4-TRIAZOLYL-4)ACETATE SALTS

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Sulfone **II** was synthesized via oxidation of ethyl 2-[5-bromo-2,4-dihydro-3-oxo-2-(thietanyl-3)-1,2,4-triazolyl-4]acetate (**I**) by H_2O_2. Hydrolysis of esters **I** and **II** synthesized 2-[5-bromo-2,4-dihydro-3-oxo-2-(thietanyl-3)-1,2,4-triazolyl-4]- and 2-[5-bromo-2,4-dihydro-3-oxo-2-(1,1-dioxothietanyl-3)-1,2,4-triazolyl-4]acetic acids **III** and **IV**, respectively. Water-soluble salts **V** and **VI** were prepared by reacting acids **III** and **IV** with alkali-metal hydroxides and amines. The structures of the synthesized compounds were confirmed IR and NMR spectroscopic data. The antiplatelet and anticoagulant activity of the synthesized compounds was studied *in vitro* based on predictions of the PASS computer program. Compounds **III** and **VIb**, which showed the absence of predicted toxic risks and were superior to the reference drug in the collagen-induced aggregation test, had the most pronounced antiplatelet activity (comparable to that of acetylsalicylic acid) in the ADP-induced aggregation test. The anticoagulant activity of the compounds was significantly inferior to that of heparin sodium. All synthesized compounds satisfied Lipinski's rule-of-5.

Keywords: 2,4-dihydro-1,2,4-triazol-3-one, thietane, antiplatelet activity, anticoagulant activity, Lipinski's rule-of-5.

Thrombohemorrhagic disorders are currently the most common types of human pathologies [1]. Thrombi and associated complications are some of the causes of invalidism and lethality among the total causes of death of the geriatric population [2]. Therapy of clots has become especially critical because of emergent coronavirus infections [3, 4]. Antiplatelet drugs such as ticlodipine, clopidogrel, flurbiprofen, ozagrel, lotrafiban, and acetylsalicylic acid are currently used to treat and prevent thrombi. However, several side effects can be associated with their use [5 – 7]. Therefore, the development of new domestic drugs capable of correcting the hemostasis system is crucial. Previous research on the synthesis of 1-thietanyl-1,2,4-triazole derivatives demonstrated that they were promising for preparing new biologically active compounds with antiplatelet activity [8, 9]. Therefore, the aim of the present work was to synthesize new thietane-containing 2-(5-bromo-2,4-dihydro-3-oxo-1,2,4-triazolyl-4)acetic acid derivatives and to assess preliminarily their antiplatelet and anticoagulant activity.

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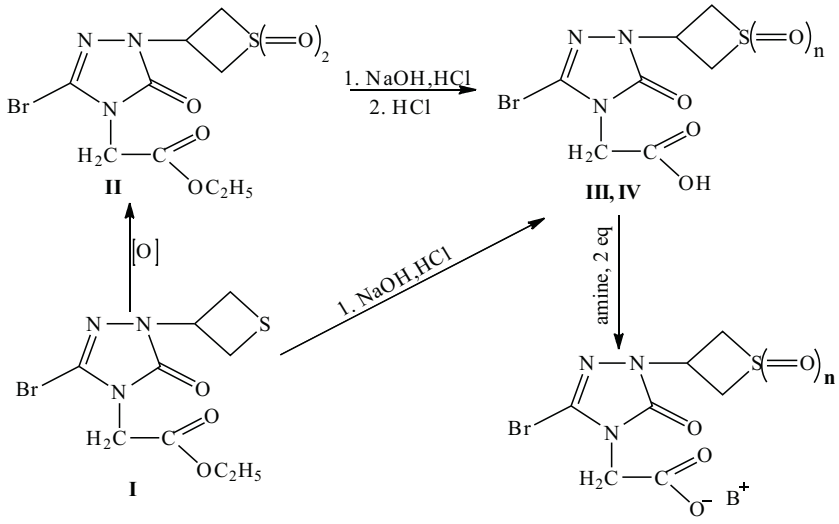
EXPERIMENTAL CHEMICAL PART

The target compounds were synthesized according to Scheme 1.

PMR spectra were recorded in $CDCl_3$ and DMSO-d_6 on a Bruker AM-300 instrument at operating frequency 300 MHz for ^1H. The internal standards were solvent resonances at 2.50 ppm (DMSO-d_6) and 7.26 ppm [$CD(H)Cl_3$]. IR spectra were taken from KBr pellets on an Infralum FT-02

Scheme



n = 0 (**Va-e**); 2 (**Via-e**)
B = K+ (**Va**, **VIa**), Na^+ (**Vb**, **VIb**), $H_3N^+C(CH_2OH)_3$ (**Vc**, **Vic**), $H_3N^+CH_2CH_2OH$ (**Vd**), $N_2N^+(C_2H_4OH)_2$ (**Ve**), $H_3N^+CH_2C_6H_5$ (**VId**), $H_3N^+C_6H_{11}$ (**VIe**)

instrument. Melting points were measured on an SMP30 apparatus. The purity of compounds was confirmed by TLC on Sorbfil PTSKh-P-A-UF plates using $CHCl_3$–EtOH (9:1, v/v) and hexane–EtOH (5:5). Spots were detected by I_2 vapor in a humid chamber. Elemental analyses were performed on a Hekatech Euro3000 CHNS analyzer. Analyses for C, H, N, and S agreed with the calculated values. Table 1 presents the characteristics of the synthesized compounds and spectral data.

Ethyl 2-[5-bromo-2,4-dihydro-2-(1, 1-dioxothietanyl-3)-3-oxo-1,2,4-triazolyl-4]acetate (II). Compound **1** (4.42 g, 14 mmol) in glacial HOAc (135 mL) was treated with H_2O_2 (37%, 12.86 g, 140 mmol). The mixture was refluxed for 1 h, cooled to room temperature, and neutralized to pH 7.0 with NH_4OH solution. The resulting precipitate was filtered off, rinsed with H_2O, and dried.

2-[5-Bromo-2,4-dihydro-3-oxo-2-(thietanyl-3)-1,2,4-triazolyl-4]acetic acid (III). Ester **I** (10.74 g, 33 mmol) was added to a solution of NaOH (2.64 g, 66 mmol) in H_2O (265 mL). The mixture was stirred at room temperature for 1 d. The unreacted precipitate was filtered off. The filtrate was treated with HCl to pH 3.0 – 4.0. The resulting precipitate was filtered off, rinsed with H_2O, and dried.

2-[5-Bromo-2,4-dihydro-2-(1, 1,-dioxothietanyl-3)-3-oxo-1,2,4-triazolyl-4]acetic acid (IV). A. Ester **II** (0.50 g, 14 mmol) was added to a solution of NaOH (0.22 g, 56 mmol) in H_2O (20 mL). The mixture was stirred at room temperature for 2 d. The unreacted precipitate was filtered off. The filtrate was treated with HCl to pH 3.0 – 4.0. The resulting precipitate was filtered off, rinsed with H_2O, and dried. B. Ester **II** (2.72 g, 7 mmol) was treated with H_2O (20 mL) and conc. HCl (10 mL). The mixture was refluxed for 2 h and cooled. The resulting precipitate was filtered off, rinsed with H_2O, and dried.

General method for synthesizing salts Va, b and VIa, b. A solution of KOH or NaOH (3.3 mmol) in *i*-PrOH (35 mL) and H_2O (1 mL) was treated with acid **III** or **IV** (3 mmol). The mixture was refluxed for 10 min and cooled. The resulting precipitate was filtered off, rinsed with *i*-PrOH, dried, and purified by crystallization from *i*-PrOH.

General method for synthesizing salts Vc-e and VIc-e. Acid **III** or **IV** (3 mmol) was treated with *i*-PrOH (15 mL), heated until dissolved, and treated with an amine (6 mmol). The mixture was refluxed for 10 min and cooled. The resulting precipitate was filtered off, rinsed with *i*-PrOH, dried, and purified by crystallization from *i*-PrOH.

Toxicity and drug-likeness of the synthesized compounds were predicted by the Osiris DataWarrior program [10]. The biological activity of the synthesized compounds was predicted from the chemical structural formula using the online version of the PASS computer program [11].

EXPERIMENTAL BIOLOGICAL PART

Experiments were conducted according to Good Laboratory Practice Rules of the Eurasian Economic Union on Circulation of Medicines [12].

Antiplatelet and anticoagulant activity were assessed *in vitro* using isolated blood samples from 25 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 (No. 2 of Oct. 17, 2012). Informed consent was obtained from all study participants before blood collection.

TABLE 1. Characteristics of Synthesized **II-VI**

Compound	mp, °C	R_f	Yield, %	Spectral data
II	117 – 119	0.84*	80	IR spectrum, ν_{max}, cm^{-1}: 1139, 1189 (C-N), 1524 (C=N), 1717 (C=O), 1153 and 1314 (SO_2). PMR spectrum ($CDCl_3$), δ, ppm: 1.30 (3H, t, J 7.1 Hz, $C\underline{H}_3CH_2$), 4.26 (2H, q, J 7.1Hz, $CH_3C\underline{H}_2$), 4.40 (2H, s, CH_2CO), 4.41 – 4.52 (2H, m, $S(CH)_2$), 4.63 – 4.71 (2H, m, $S(CH)_2$), 5.05 – 5.15 (1H, m, NCH).
III	191 – 193	0.30*	58	IR spectrum, ν_{max}, cm^{-1}: 1175, 1192 (C-N), 1521 (C=N), 1690 (C=O), 1755 (C=O), 3105 (OH). PMR spectrum (DMSO-d_6), δ, ppm: 3.27 – 3.33 (2H, m, $S(CH)_2$), 3.75 – 3.81 (2H, m, $S(CH)_2$), 4.36 (2H, s, CH_2CO), 5.38 – 5.44 (1H, m, NCH).
IV	201 – 203	0.63**	A. 31 B. 77	IR spectrum, ν_{max}, cm^{-1}: 1106, 1220 (C-N), 1145 and 1334 (SO_2), 1530 (C=N), 1714 (C=O), 1736 (C=O), 3051 (OH). PMR spectrum (DMSO-d_6), δ, ppm: 4.39 (2H, s, CH_2CO), 4.51 – 4.56 (2H, m, $S(CH)_2$), 4.67 – 4.75 (2H, m, $S(CH)_2$), 5.06 – 5.12 (1H, m, NCH).
Va	194 – 196	0**	64	IR spectrum, ν_{max}, cm^{-1}: 1175, 1192 (C-N), 1385 and 1524 (COO^-), 1614, 1637 (C=N), 1686 (C=O). PMR spectrum (DMSO-d_6), δ, ppm: 3.24 – 3.30 (2H, m, $S(CH)_2$), 3.74 – 3.80 (2H, m, $S(CH)_2$), 3.76 (2H, s, CH_2CO), 5.31 – 5.43 (1H, m, NCH).
Vb	282 – 284	0**	56	IR spectrum, ν_{max}, cm^{-1}: 1206, 1173 (C-N), 1389, 1526 (COO^-), 1633 (C=N), 1683 (C=O). PMR spectrum (DMSO-d_6), δ, ppm: 3.24 – 3.30 (2H, m, $S(CH)_2$), 3.75 – 3.78 (2H, m, $S(CH)_2$), 3.79 (2H, s, CH_2CO), 5.35 – 5.40 (1H, m, NCH).
Vc	110 – 112	0**	95	IR spectrum, ν_{max}, cm^{-1}: 1184, 1220 (C-N), 1390, 1531 (COO^-), 1606 (C=N), 1709 (C=O), 2848 – 2948 (N^+-H), 3264 – 3350 (O-H). PMR spectrum (DMSO-d_6), δ, ppm: 3.25 – 3.31 (2H, m, $S(CH)_2$), 3.36 (6H, s, $3CH_2$), 3.75 – 3.81 (2H, m, $S(CH)_2$), 3.87 (2H, s, CH_2CO), 5.35 – 5.41 (1H, m, NCH).
Vd	120 – 121	0**	63	IR spectrum, ν_{max}, cm^{-1}: 1184, 1069 (C-N), 1379, 1594 (COO^-), 1524 (C=N), 1700 (C=O); 2822 – 3155 (N+-H, O-H). PMR spectrum (DMSO-d_6), δ, ppm: 2.82 (2H, t, J 5.3Hz, NCH_2), 3.25 – 3.31 (2H, m, $S(CH)_2$), 3.56 (2H, t, J 5.3Hz, OCH_2), 3.75 – 3.81 (2H, m, $S(CH)_2$), 3.86 (2H, s, CH_2CO), 5.35 – 5.44 (1H, m, NCH), 7.99 (3H, s, N^+-H).
Ve	103 – 104	0**	55	IR spectrum, ν_{max}, cm^{-1}: 1184, 1064 (C-N), 1393, 1526 (COO^-), 1596, 1637 (C=N), 1675 (C=O), 2816 – 3078 (N^+-H), 3422 (O-H). PMR spectrum (DMSO-d_6), δ, ppm: 2.95 (4H, t, J 5.3Hz, $N(CH_2)_2$), 3.25 – 3.31 (2H, m, $S(CH)_2$), 3.62 (4H, t, J 5.3Hz, $2C\underline{H}_2OH$), 3.75 – 3.81 (2H, m, $S(CH)_2$), 3.91 (2H, s, CH_2CO), 5.36 – 5.44 (1H, m, NCH).
VIa	228 – 230	0**	77	IR spectrum, ν_{max}, cm^{-1}: 1220 (C-N), 1136, 1320 (SO_2), 1383, 1532 (COO^-), 1598.1618 (C=N), 1697 (C=O). PMR spectrum (DMSO-d_6), δ, ppm: 3.75 (2H, s, CH_2CO), 4.47 – 4.53 (2H, m, $S(CH)_2$), 4.64 – 4.72 (2H, m, $S(CH)_2$), 5.02 – 5.08 (1H, m, NCH).
VIb	205 – 207	0**	58	IR spectrum, ν_{max}, cm^{-1}: 1106, 1223 (C-N), 1139, 1320 (SO_2), 1321, 1542 (COO^-), 1544 (C=N), 1722 (C=O). PMR spectrum (DMSO-d_6), δ, ppm: 3.84 (2H, s, CH_2CO), 4.47 – 4.53 (2H, m, $S(CH)_2$), 4.65 – 4.75 (2H, m, $S(CH)_2$), 5.00 – 5.10 (1H, m, NCH).
VIc	154 – 156	0**	52	IR spectrum, ν_{max}, cm^{-1}: 1058, 1195 (C-N), 1136, 1309 (SO_2), 1311, 1534 (COO^-), 1628, 1598 (C=N), 1703 (C=O), 2894 – 2977 (N^+-H), 3345 (O-H). PMR spectrum (DMSO-d_6), δ, ppm: 3.34 (6H, s, $3CH_2$), 3.88 (2H, s, CH_2CO), 4.47 – 4.54 (2H, m, $S(CH)_2$), 4.64 – 4.72 (2H, m, $S(CH)_2$), 5.02 – 5.06 (1H, m, NCH).
VId	197 – 199	0**	88	IR spectrum, ν_{max}, cm^{-1}: 1064, 1178 (C-N), 1139, 1323 (SO_2), 1319, 1536 (COO^-), 1588.1655 (C=N), 1700 (C=O), 2947 – 3158 (N^+-H, O-H). PMR spectrum (DMSO-d_6), δ, ppm: 3.89 (2H, s, CH_2CO), 3.97 (2H, s, NCH_2), 4.47 – 4.53 (2H, m, $S(CH)_2$), 4.65 – 4.73 (2H, m, $S(CH)_2$), 5.03 – 5.11 (1H, m, NCH), 7.34 – 7.45 (5H, m, C_6H_5).
VIe	191 – 193	0**	72	IR spectrum, ν_{max}, cm^{-1}: 1064, 1186 (C-N), 1142, 1319 (SO_2), 1327, 1573 (COO^-), 1632 (C=N), 1720(C=O), 2855 – 2947 (N^+-H). PMR spectrum (DMSO-d_6), δ, ppm: 1.02 – 1.22 (5H, m, CH_{2amine}), 1.54 – 1.86 (5H, m, $(CH)_{2amine}$), 2.88 m (1H, CH_{amine}), 3.84 (2H, s, CH_2CO), 4.46 – 4.53 (2H, m, $S(CH)_2$), 4.65 – 4.73 (2H, m, $S(CH)_2$), 5.02 – 5.27 (1H, m, NCH).

* Chromatography using hexane–EtOH (5:5);
** Chromatography using $CHCl_3$–EtOH (9:1).

The effect of the compounds on platelet aggregation was studied using the Born method [13] on an AT-02 aggregometer (Medtekh NPF, Russia). Antiplatelet activity of the tested compounds and reference drugs was assessed at a final concentration of 1×10^{-3} M. The aggregation inductors were adenosine diphosphate (ADP) at a concentration of 20 μg/mL and collagen at a concentration of 5 mg/mL (Tekhnologiya-Standart, Russia). The reference drugs were pentoxifylline (Pentoxifylline, 20 mg/mL solution for injection, 5-mL ampuls; JSC Dalkhimfarm, Russia) and acetylsalicylic acid (substance-powder; Shandong Xinhua Pharmaceutical Co. Ltd., China).

Anticoagulant activity was determined in clotting tests [14] on a Solar CGL 2110 turbidimetric hemocoagulometer (CSC SOLAR, Belarus). The final concentration of the tested compounds and reference drug was 5×10^{-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; JSC Sintez, Russia).

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 results was found to differ from normal. Therefore, nonparametric methods were used for further work. The obtained results were given as medians and 25 and 75 percentiles. Dispersion analysis used the Kruskal–Wallis criterion. The critical significance level p for statistical criteria was taken as 0.05 [15].

RESULTS AND DISCUSSION

Starting ethyl 2-[5-bromo-2,4-dihydro-3-oxo-2-(thietanyl-3)-1,2,4-triazolyl-4]acetate (**I**) was synthesized by the literature method [16]. Oxidation of **I** by a 10-fold molar excess of H_2O_2 in glacial HOAc with heating produced ethyl 2[(5-bromo-2,4-dihydro-3-oxo-2-(1,1-dioxothietanyl-3)-1,2,4-triazolyl-4]acetate (**II**) in 80% yield (Scheme 1). The PMR spectrum of sulfone **II** showed weak-field shifts of 1.2 and 0.7 ppm for the multiplets of the two $S(CH)_2$ groups of the thietane dioxide ring and a strong-field shift by 0.4 ppm for the multiplet of the NCH proton relative to the analogous resonances of the unoxidized thietane ring.

Acid **III** was prepared in 58% yield (Scheme 1) via alkaline hydrolysis of ester **I** by a two-fold molar excess of aqueous NaOH at room temperature. Hydrolysis of ester **II** under analogous conditions led to the formation of acid **IV** in 31% yield (Scheme 1). The yield of **IV** was increased to 77% by acid hydrolysis of **II** using HCl solution and heating. The formation of acids **III** and **IV** was confirmed by the appearance in their IR spectra of absorption bands for O–H stretching vibrations at 3051 – 3105 cm^{-1} (Table 1). PMR spectra of acids **III** and **IV** lacked resonances for ethoxy protons.

Salts of K (**Va** and **VIa**) and Na (**Vb** and **VIb**) were synthesized in 58 – 77% yields (Scheme 1) by reacting acids **III** and **IV** with a slight excess of KOH or NaOH in *i*-PrOH. Alkylammonium salts **Vc-e** and **VIc-e** were synthesized in 83 – 95% yields (Scheme 1) by heating acids **III** and **IV** with a two-fold molar excess of the amines in *i*-PrOH. Formation of salts **Va-e** and **VIa-e** was confirmed by the presence in their IR spectra (Table 1) of absorption bands for stretching vibrations of carboxylate ions at 1319 – 1390 and

TABLE 2. Biological Activity of **II-VI** Predicted by the PASS Online Computer Program

Compound	Probability of biological activity (Pi)				
	hematopoiesis inhibition	platelet adhesion inhibition	platelet activity suppression	fibrinogen receptor blockage	P2T-purinergic receptor blockage
II	0.365	0	0.171	0.589	0
III	0.308	0.379	0.118	0.448	0.068
IV	0.279	0.412	0.181	0.553	0
Va	0.340	0.381	0.130	0.483	0.100
Vb	0.345	0.388	0.132	0.492	0.108
Vc	0.380	0.408	0.151	0.460	0.160
Vd	0.388	0.411	0.148	0.458	0.120
Ve	0.385	0.400	0.140	0.452	0.134
VIa	0.219	0.328	0	0.371	0
VIb	0.220	0.330	0	0.360	0
VIc	0.200	0.315	0	0.365	0
VId	0.242	0.320	0	0.377	0
VIe	0.230	0.332	0	0.360	0

TABLE 3. Effect of **II-VI** and Reference Drugs on Platelet Aggregation and Plasma Hemostasis, Me (0.25 – 0.75)

Compound	ADP-induced change of platelet aggregation, % vs. control	Collagen-induced change of platelet aggregation, % vs. control	APTT increase, % vs. control
II	– 6.4 (4.9 – 8.3)$^{*,\dagger\dagger,\#}$	– 4.5 (3.8 – 6.5)$^{*,\dagger\dagger,\#\#}$	6.4 (4.9 – 7.6)*
III	**– 14.3 (11.2 – 16.7)***,††	– 7.8 (6.2 – 9.1)$^{*,\dagger\dagger,\#\#}$	2.3 (1.8 – 3.4)
IV	– 11.4 (10.1 – 13.7)*, †	– 10.4 (9.1 – 12.3)$^{*,\dagger\dagger,\#\#}$	3.1 (2.5 – 4.1)
Va	– 11.5 (9.4 – 12.2)*,††	– 10.3 (8.7 – 11.2)$^{*,\dagger\dagger,\#\#}$	5.3 (4.8 – 6.7)*
Vb	– 8.8 (7.2 – 10.4)$^{*,\dagger\dagger,\#}$	– 8.6 (7.5 – 9.3)$^{*,\dagger\dagger,\#\#}$	6.2 (5.1 – 7.3)*
Vc	– 7.4 (5.9 – 9.2)$^{*,\dagger\dagger,\#}$	– 8.2 (7.6 – 9.5)$^{*,\dagger\dagger,\#\#}$	4.7 (3.5 – 6.2)*
Vd	– 6.5 (5.8 – 7.7)$^{*,\dagger\dagger,\#}$	– 7.3 (6.9 – 8.7)$^{*,\dagger\dagger,\#\#}$	8.3 (7.1 – 9.4)*
Ve	– 7.8 (6.3 – 8.9)$^{*,\dagger\dagger,\#}$	– 8.1 (6.9 – 9.1)$^{*,\dagger\dagger,\#\#}$	8.5 (6.2 – 9.7)*
VIa	– 9.4 (8.5 – 11.7)*,††	– 2.3 (1.4 – 3.7)$^{\dagger\dagger,\#\#}$	5.6 (4.9 – 6.7)*
VIb	**– 15.3 (14.8 – 17.1)****,††	**– 16.3 (15.1 – 17.4)**$^{**,\dagger\dagger,\#\#}$	9.2 (8.3 – 11.1)*
VIñ	– 10.3 (8.6 – 12.1)*,††	– 11.3 (9.5 – 12.7)$^{*,\dagger\dagger,\#\#}$	6.7 (5.3 – 7.5)*
VId	– 8.3 (6.5 – 9.7)$^{*,\dagger\dagger,\#}$	– 7.5 (5.9 – 8.3)$^{*,\dagger\dagger,\#\#}$	9.3 (8.1 – 10.6)*
VIe	– 9.3 (7.7 – 11.5)$^{*,\dagger\dagger,\#}$	– 6.3 (5.7 – 9.2)$^{*,\dagger\dagger,\#\#}$	8.2 (7.2 – 10.1)*
Acetylsalicylic acid	– 13.7 (10.8 – 16.4)*,††	0.0 (0.0 – 0.0)	-
Pentoxifylline	– 48.4 (42.7 – 56.5)$^{**,\#\#}$	0.0 (0.0 – 0.0)	-
Heparin sodium	-	-	54.7 (47.7 – 60.2)**

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

1526 – 1594 cm^{-1}. IR spectra of alkylammonium salts **Vc-e** and **VIc-e** showed absorption bands for stretching vibrations of N^{+}–H groups in the range 2816 – 3078 cm^{-1}. Resonances for protons of the corresponding amines in PMR spectra of **Va-e** confirmed that salts **Vc-e** and **VIc-e** formed. For example, spectra of **Vc** and **VIc** showed a 6H singlet at ~3.3 ppm that belonged to the protons of the 3 CH_2 groups of Tris-amine.

The biological activity of the synthesized compounds predicted by the PASS program (Table 2) showed that **II-VI** with probability Pi = 0.2 – 0.4 could inhibit adhesion of platelets and hematopoiesis and act as platelet antagonists (Pi ~ 0.1), fibrinogen receptors (Pi ~ 0.4 – 0.6), and purine P2T-receptors (Pi ~ 0.1). Therefore, antiplatelet and anticoagulant activity of the synthesized compounds was studied in *in vitro* experiments.

Acid **III** (–14.3%, $p \le 0.05$) and its K salt **Va** (–11.5%, $p \le 0.05$) among thietanyl derivatives **III** and **V** showed statistically significant antiplatelet effects that were comparable to that of acetylsalicylic acid in the ADP-induced platelet aggregation test. The effect decreased slightly to –11.4% for acid **IV** and to –9.4% for K salt **VIa** if the oxidation state of the S atom was increased to the sulfone. The Na salt **VIb** (–15.3%, $p \le 0.05$) showed an antiplatelet effect comparable to that of acetylsalicylic acid. However, the antiplatelet effect of **III**, **Va**, and **VIb** was significantly (by 3.2 – 4.2 times) inferior to that of pentoxifylline (Table 3).

TABLE 4. Predicted Toxicity, Drug-Likeness, and Lipinski's Rule-of-5 Agreement of Synthesized Compounds in Osiris DataWarrior Program

Compound	Toxic risk*	log*P*	Mol weight	TPSA, Å^2	nOH	nOHNH	Drug-likeness
II	-	– 1.13	382.2	121.80	9	0	– 14.79
III	-	– 0.27	293.0	98.51	6	1	– 2.68
IV	-	– 1.96	354.1	132.80	9	1	– 6.65
Va	-	– 2.35	332.2	101.34	6	0	– 7.02
VIa	-	– 4.04	392.2	135.63	9	0	– 5.53
Vb	-	– 2.26	316.1	101.34	6	0	– 7.21
VIb	-	– 4.05	376.1	135.60	9	0	– 5.54
Vñ	-	– 0.24	415.3	98.51	6	1	– 2.05
VIc	-	– 1.36	447.2	115.73	8	1	– 5.84
Vd	-	– 0.27	355.2	98.51	6	1	– 2.68
VId	-	– 1.37	433.3	116.63	8	1	– 5.90
Vf	-	– 0.48	399.2	98.51	6	1	– 3.80
VIf	-	– 1.36	425.3	115.8	8	1	– 5.88

* Toxic risks: mutagenicity, oncogenicity, irritation, effect on reproductive function. log*P*, lipophilicity coefficient; nOH, number of H acceptors; nOHNH, number of H donors; TPSA, topological polar surface area.

All compounds except for **VIa** exhibited antiplatelet activity from –4.5 to –16.3% ($p \le 0.05$) in the collagen-induced aggregation test. Pentoxifylline and acetylsalicylic acid did not exhibit biological activity in this test.

Ester **II** and salts **V** and **VI** caused significant hypocoagulation, increasing the APTT by 4.7 – 9.3% ($p \le 0.05$) as compared to the control and did not affect the PT and fibrinogen concentration. The effects of the tested compounds were significantly inferior to that of heparin sodium, which increased the APTT by 54.7%.

The new compounds were analyzed for agreement with Lipinski's rule-of-5 [17, 18], toxic risks, and the drug-likeness parameter (similarity to a drug) using the Osiris DataWarrior program to discover compounds that could lead to potential drugs (drug candidates) after *in vivo* testing (Table 4).

A calculation of the toxic risks showed that mutagenic, oncogenic, and irritation properties and a negative effect on reproductive functioning were not predicted for the synthesized compounds.

The calculated physicochemical parameters of **II-VI** were found to satisfy Lipinski's rule-of-5. The molecular mass of the synthesized compounds was less than 447.2 g/mol. The lipophilicity coefficient fell in the range from –4.05 to –0.24. The number of H acceptors was <9; H donors, 1. The topological polar surface area was 98.51 – 135.63 Å^2, which suggested that the synthesized compounds had good penetrating power through cell membranes. The drug-likeness parameter lay in the range from –14.79 to –2.05, which confirmed the structures of the synthesized compounds were novel.

Thus, the antiplatelet activity in the ADP-induced aggregation test was greatest for **III** and **VIb** and comparable to that of acetylsalicylic acid. These compounds typically lacked toxic risks and were superior to the reference drugs in the collagen-induced aggregation test. The calculated drug-likeness parameter led to the conclusion that the search for new compounds with antiplatelet activity among this class of compounds was promising.

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