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SYNTHESIS AND ANTIAGGREGANT ACTIVITY OF 2-[3-METHYL-1-ETHYLXANTHINYL-8-THIO]ACETIC ACID SALTS CONTAINING A THIETANE RING

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Reaction of 7-(thietanyl-3)-, 7-(1-oxothietanyl-3)-, and 7-(1,1-dioxothietanyl-3)-8-bromo-3-methyl-1-ethylxanthines with thioglycolic acid produced 2-[3-methyl-1-ethylxanthinyl-8-thio]acetic acids containing a thietane ring with yields of 76 – 93%. Interaction of these acids with bases (sodium hydroxide, morpholine, hexamethyleneimine) produced 2-[3-methyl-1-ethylxanthinyl-8-thio]acetic acid salts containing a thietane ring, with yields of 44 – 96%. The structures of these compounds were confirmed by IR and NMR spectroscopy data. The compounds synthesized here had potentially high antiaggregatory activity.

Keywords: xanthines, thietanes, antiaggregatory activity

The xanthine derivative pentoxifylline is used for correction of impairments to the rheological properties of the blood, as it increases erythrocyte elasticity and suppresses erythrocyte and platelet aggregation [1]. Antiaggregatory properties in relation to erythrocytes comparable with those of pentoxifylline are found with the 8-substituted 7-(thietanyl-3)- [2] and 7-(1-oxothietanyl-3)-1-alkyl-3-methylxanthines [3], as described by ourselves. With the aim of seeking xanthine derivatives affecting the adhesive-aggregatory function of platelets, we have synthesized 2-[3-methyl-1-ethylxanthinyl-8-thio]acetic acid salts containing a thietane ring at position 7.

Reaction of 8-bromo-3-methyl-1-ethylxanthines (Ia-c), containing a thietane ring, with thioglycolic acid in the presence of potassium hydroxide with boiling for 1 – 1.5 h in ethanol or aqueous medium produced 2-[3-methyl-7-(thietanyl-3)-, 2-[3-methyl-7-(1-oxothietanyl-3)-, and 2-[3-methyl-7-(1,1-dioxothietanyl-3)-1-ethylxanthinyl-8-thio]acetic acids (IIa-c) with yields of 76 – 93%. Interaction of acid IIa with sodium hydroxide, morpholine, or hexamethyleneimine in acetone produced 2-[3-methyl-7-(thietanyl-3)-1-ethylxanthinyl-8-thio]acetic acid salts (IIIa-c) with yields of 77 – 91%. Similarly, acid IIb produced 2-[3-methyl-7-(1-oxothietanyl-3)-1-ethylxanthinyl-8-thio]acetic acid salts (IIId-f) with yields of 44 – 96% and acid IIc produced 2-[methyl-7-(1,1-dioxothietanyl-3)-1-ethylxanthinyl-8-thio]-acetic acid salts (IIIg-i) with yields of 82 – 89%.

The presence of a thioglycolic acid residue in acids IIa-c was confirmed by the presence of absorption bands in the IR spectra corresponding to valent oscillations of bound and free O-H bonds at 2300 – 3550 cm^{-1}. Formation of acids IIa-c was also confirmed by ^1H NMR spectra where, apart from the characteristic signals of thietane ring and alkyl substituent protons, a singlet of protons from the SCH_2 group of the thioglycolic acid residue was seen at about 4.1 ppm.

The absence of absorption bands for valent oscillations of the O-H bond in the IR spectra of compounds IIIa, d, g confirmed the formation of the sodium salts. The spectra of the morpholine IIIb, d, h and hexamethyleneimine IIIc, f, i salts showed absorption bands for valent oscillations of the N^+-H bond at 2230 – 3150 cm^{-1}. Furthermore, the spectra of salts IIId-f contained an intense band corresponding to valent oscillations of the S=O bond at 1029 – 1050 cm^{-1} and the spectra of salts IIIg-i contained absorption bands for the symmetrical and asymmetrical valent oscillations of the SO_2 group at 1128 – 1149 and 1311 – 1319 cm^{-1}.

The ^1H NMR spectra of salts IIIb, e, h contained typical signals from protons in the thietane ring, alkyl substituents,

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and the thioglycolic acid residue, along with signals from $N(CH_2)_2$ and $O(CH_2)_2$ group protons at 2.9 – 3.2 and 3.7 – 3.9 ppm respectively.

Among the newly synthesized 2-[3-methyl-1-ethylxanthinyl-8-thio]acetic acid salts containing a thietane ring, significant antiaggregatory activity was seen with compounds IIIf and IIIh (Table 1). Compounds IIIf and IIIh inhibited platelet aggregation induced by adenosine diphosphate (ADP) by about 20% relative to controls, which was greater than the antiaggregatory activities of most reference agents but less than those of pentoxifylline and acetylsalicylic acid. However, the salts whose synthesis is first reported here dif-

TABLE 1. Effects of Newly Synthesized Compounds and Reference Agents on ADP- and Collagen-Induced Platelet Aggregation, Me(25 – 75)*

Compound	ADP-induced platelet aggregation (% inhibition compared with control level)	*p*	Collagen-induced platelet aggregation (% inhibition compared with control level)	*p*
IIIa	2.8 (0.9 – 4.3)	$p_1 = 0.001$ $p_2 = 0.0006$ $p_3 = 0.000007$ $p_4 = 0.0007$	3.9 (3.1 – 6.2)	$p_1 = 0.5$ $p_2 = 0.2$
IIIb	6.8 (2.7 – 11.2)	$p_1 = 0.5$ $p_2 = 0.004$ $p_3 = 0.00002$ $p_4 = 0.0001$	2.3 (1.1 – 4.2)	$p_1 = 0.3$ $p_2 = 0.004$
IIIc	2.3 (1.1 – 4.2)	$p_1 = 0.07$ $p_2 = 0.0008$ $p_3 = 0.000009$ $p_4 = 0.0007$	3.6 (2.7 – 4.5)	$p_1 = 0.03$ $p_2 = 0.8$
IIId	3.2 (1.2 – 5.1)	$p_1 = 0.0003$ $p_2 = 0.006$ $p_3 = 0.00008$ $p_4 = 0.0003$	4.7 (2.7 – 5.8)	$p_1 = 0.4$ $p_2 = 0.8$
IIIe	14.3 (9.5 – 17.3)	$p_1 < 0.0001$ $p_2 = 0.3$ $p_3 = 0.007$ $p_4 = 0.4$	3.6 (1.3 – 5.2)	$p_1 = 0.7$ $p_2 = 0.4$
IIIf	19.3 (16.7 – 24.9)	$p_1 < 0.0001$ $p_2 = 0.002$ $p_3 = 0.0005$ $p_4 = 0.003$	3.6 (2.1 – 4.8)	$p_1 = 0.5$ $p_2 = 0.6$
IIIg	10.1 (7.2 – 13.3)	$p_1 = 0.03$ $p_2 = 0.006$ $p_3 = 0.00003$ $p_4 = 0.0005$	6.4 (4.2 – 7.9)	$p_1 = 0.002$ $p_2 = 0.4$
IIIh	20.4 (17.5 – 24.6)	$p_1 < 0.0001$ $p_2 = 0.002$ $p_3 = 0.0008$ $p_4 = 0.003$	4.6 (3.1 – 5.4)	$p_1 = 0.2$ $p_2 = 0.7$
IIIi	6.2 (5.2 – 8.6)	$p_1 = 0.6$ $p_2 = 0.002$ $p_3 = 0.000005$ $p_4 = 0.0004$	7.3 (5.4 – 8.7)	$p_1 = 0.0003$ $p_2 = 0.02$
Euphylline	7.4 (5.6 – 9.3)	-	2.5 (0.8 – 4.2)	-
Caffeine sodium benzoate	14.7 (10.3 – 17.9)	-	5.3 (3.9 – 7.2)	-
Pentoxifylline	48.4 (42.7 – 56.5)	-	0.0 (0.0 – 0.0)	-
Acetylsalicylic acid	13.7 (10.8 – 16.4)	-	0.0 (0.0 – 0.0)	-

* Data were described using the numerical characteristics of variables: median (Me), 25% and 75%; the level of statistical significance of differences compared with euphylline (p_1), caffeine sodium benzoate (p_2), pentoxifylline (p_3), acetylsalicylic acid (p_4), $n = 7$.

fered from pentoxifylline and acetylsalicylic acid in having different levels of antiaggregatory activity in relation to collagen-induced platelet aggregation. The maximum level of inhibition of the collagen-induced functional activity of platelets was by about 7% relative controls, obtained with compounds IIIg and IIIi. Thus, the group of newly synthesized 2-[3-methyl-1-ethylxanthinyl-8-thio]acetic acid salts, containing a thietane ring, included compounds with a potentially wide spectrum of antiaggregatory activity as compared with therapeutic agents in current use.

EXPERIMENTAL CHEMICAL SECTION

The IR spectra of compounds in potassium bromide tablets were taken on an Infralyum FT-02 instrument; ^{1}H NMR spectra were taken on a Bruker AM-300 instrument with a working frequency of 300 MHz. Solvents were deuterated chloroform and dimethylsulfoxide and the internal standard consisted of signals from the solvents.

The identities of the compounds synthesized here were verified by thin layer chromatography on Sorbfil plates using a solvent system consisting of chloroform and ethanol (1:3, v/v). Spots were detected with iodine vapor in a moist chamber. Elemental analysis data for the compounds synthesized here corresponded to calculated values. The properties of the newly synthesized compounds are given in Table 2.

Compound Ia, c were prepared as described in [4] and compound Ib as described in [3].

2-[3-methyl-7-(thietanyl-3)-1-ethylxanthinyl-8-thio]-acetic acid (IIa). A solution of 1.68 g (30 mmol) of potassium hydroxide in 10 ml of water was supplemented with a solution of 1.84 g (20 mmol) of thioglycolic acid in 100 ml of ethanol and 3.44 g (10 mmol) of compounds Ia. The reaction mix was boiled for 1.5 h. It was then cooled, evaporated in vacuo to half the volume, and filtered; the filtrate was acidified with dilute hydrochloric acid to pH 2. The resulting precipitate was collected by filtration, washed with water, and dried. Compounds were purified by crystallization from a mixture of ethanol and water (1:1 v/v). The IR spectrum (KBr), ν_{max}, cm^{-1}, was: 1648, 1698, 1742 (C=C, C=N, C=O), 2400 – 3300, 3380 – 3550 (O-H). The ^{1}H NMR spectrum ($CDCl_3$), δ, ppm, was: 1.25 (t, 3H, J 7.0 Hz, CH_3); 3.27 – 3.36 (m, 2H, $S(CH)_2$); 3.51 (s, 3H, 3-CH_3); 4.06 (s, 2H, 8-SCH_2); 4.12 (q, 2H, J 7.0 Hz, 1-CH_2); 4.30 – 4.39 (m, 2H, $S(CH)_2$); 5.83 – 5.97 (m, 1H, 7-CH).

2-[3-Methyl-7-(1-oxothietanyl-3)-1-ethylxanthinyl-8-thio]-acetic acid (IIb). A solution of 0.84 g (15 mmol) of potassium hydroxide in 50 ml of water was supplemented with 0.92 g (10 mmol) of thioglycolic acid and 1.94 g (5 mmol) of compound Ib. The reaction mix was boiled for 1 h. It was then cooled and acidified with dilute hydrochloric acid to pH 2. The resulting precipitate was collected by filtration, washed with water, and dried. Substance was purified by dissolving in a minimal volume of 1% potassium hydroxide solution and reprecipitation with dilute hydrochloric acid. The IR spectrum (KBr), ν_{max}, cm^{-1}, was: 1032 (S=O), 1636, 1651, 1684, 1727 (C=C, C=N, C=O), 2300 – 3020 (O-H). The ^{1}H NMR spectrum (DMSO-d_6), δ, ppm, was: 1.13 (t, 3H, 6.9 Hz, CH_3); 3.40 (s, 3H, 3-CH_3); 3.41 – 3.53 (m, 2H, $S(CH)_2$); 3.93 (q, 2H, J 6.9 Hz, 1-CH_2); 4.09 (s, 2H, 8-SCH_2); 4.07 – 4.20 (m, 4H, $S(CH)_2$ and 8-SCH_2); 6.25 – 6.39 (m, 1H, 7-CH).

2-[3-Methyl-7-(1,1-dioxothietanyl-3)-1-ethylxanthinyl-8-thio]acetic acid (IIc). This was prepared by the same method as compound IIb, from compound Ic. Product was purified by recrystallization from water. The IR spectrum (KBr), ν_{max}, cm^{-1}, was: 1134, 1312 (SO_2 val.), 1651, 1659, 1672, 1694, 1725 (C=C, C=N, C=O), 2380 – 3030 (O-H). The ^{1}H NMR spectrum ($CDCl_3$), δ, ppm, was: 1.26 (t, 3H, J 7.0 Hz, CH_3); 3.55 (s, 3H, 3-CH_3); 4.09 (s, 2H, 8-SCH_2); 4.06 – 4.18 (m, 4H, 8-SCH_2 and 1-CH_2); 4.35 – 4.44 (m, 2H, $S(CH)_2$); 5.17 – 5.28 (m, 2H, $S(CH)_2$); 5.53 – 5.66 (m, 1H, 7-CH).

2-[3-Methyl-7-(thietanyl-3)-1-ethylxanthinyl-8-thio]-acetic acid sodium salt (IIIa). A solution of 0.14 g (3.6 mmol) of sodium hydroxide in 1.5 ml of water was supplemented with 1.07 g (3 mmol) of acid IIa and 50 ml of acetone and heated to boiling. The reaction was cooled and the resulting precipitate was collected by filtration, washed with acetone, and dried. Substance was purified by recrystallization from a mixture of acetone and water (1:1 v/v). The IR spectrum, (KBr), ν_{max}, cm^{-1}, was: 1607, 1657, 1695 (C=C, C=N, C=O).

2-[3-Methyl-7-(thietanyl-3)-1-ethylxanthinyl-8-thio]-acetic acid morpholine salt (IIIb). A hot solution of 1.07 g (3 mmol) of acid IIa in 50 ml of acetone was supplemented with 0.31 g (3.9 mmol) of morpholine. The reaction was cooled and the resulting precipitate was collected by filtration, washed with acetone, and dried. Compound was purified by crystallization from a mixture of acetone and dioxane

TABLE 2. Properties of Compounds Synthesized Here

Compound	Yield, %	T_m, °C	Molecular formula
IIa	93	180 – 182	$C_{13}H_{16}N_4O_4S_2$
IIb	76	241 – 243	$C_{13}H_{16}N_4O_5S_2$
IIc	78	201 – 203	$C_{13}H_{16}N_4O_6S_2$
IIIa	91	> 275 dif.	$C_{13}H_{15}N_4NaO_4S_2$
IIIb	77	170 – 172	$C_{17}H_{25}N_5O_5S_2$
IIIc	86	196 – 198	$C_{19}H_{29}N_5O_4S_2$
IIId	96	> 350 dif.	$C_{13}H_{15}N_4NaO_5S_2$
IIIe	80	236 – 238	$C_{17}H_{25}N_5O_6S_2$
IIIf	44	209 – 211	$C_{19}H_{29}N_5O_5S_2$
IIIg	89	> 245 dif.	$C_{13}H_{15}N_4NaO_6S_2$
IIIh	82	205 – 207	$C_{17}H_{25}N_5O_7S_2$
IIIi	84	207 – 209	$C_{19}H_{29}N_5O_6S_2$

(3:1 v/v). The IR spectrum (KBr), ν_{max}, cm^{-1}, was: 1602, 1656, 1695 (C=C, C=N, C=O), 2250 – 3150 (N^+H_2). The ^1H NMR spectrum ($CDCl_3$), δ, ppm, was: 1.26 (t, 3H, J 6.8 Hz, CH_3); 3.07 – 3.17 (broad s, 4H, $N(CH_2)_2$); 3.26 – 3.35 (m, 2H, $S(CH)_2$); 3.53 (s, 3H, 3-CH_3); 3.86 – 3.96 (broad s, 4H, $O(CH_2)_2$); 4.12 (s, 2H, 8-SCH_2); 4.02 – 4.18 (m, 4H, 1-CH_2 and 8-SCH_2); 4.32 – 4.43 (m, 2H, $S(CH)_2$); 5.89 – 6.03 (m, 1H, 7-CH).

2-[3-Methyl-7-(thietanyl-3)-1-ethylxanthinyl-8-thio]-acetic acid hexamethyleneimine salt (IIIc). This was prepared by a method analogous to that used for compound IIIb, using 0.39 g (3.9 mmol) of hexamethyleneimine. Compound was purified by crystallization from a mixture of acetone and dioxane (3:1 v/v). The IR spectrum (KBr), ν_{max}, cm^{-1}, was: 1605, 1661, 1695 (C=C, C=N, C=O), 2380 – 3150 (N^+H_2).

2-[3-Methyl-7-(thietanyl-3)-1-ethylxanthinyl-8-thio]-acetic acid sodium salt (IIId). A solution of 0.26 g (6.5 mmol) of sodium hydroxide in 3 ml of water was supplemented with 1.81 g (5 mmol) of acid IIb and 80 ml of acetone and the reaction was heated to boiling. The reaction was cooled and the resulting precipitate was collected by filtration, washed with acetone, and dried. Compound was purified by dissolution in a minimal volume of water and reprecipitation with acetone. The IR spectrum (KBr), ν_{max}, cm^{-1}, was: 1050 (S=O), 1600, 1650, 1700 (C=C, C=N, C=O).

2-[3-Methyl-7-(1-oxothietanyl-3)-1-ethylxanthinyl-8-thio]acetic acid morpholine salt (IIIe). A solution of 0.57 g (6.5 mmol) of morpholine in 5 ml of water was supplemented with 1.81 g (5 mmol) of acid IIb and 80 ml of acetone and heated to boiling. The reaction was cooled and the resulting precipitate was collected by filtration, washed with acetone, and dried. Compound was purified by crystallization from a mixture of acetone and dioxane (1:1 v/v). The IR spectrum (KBr), ν_{max}, cm^{-1}, was: 1029 (S=O), 1655, 1702 (C=C, C=N, C=O), 2400 – 3100 (N^+H_2). The ^1H NMR spectrum ($CDCl_3$), δ, ppm, was: 1.24 (t, 3H, J 7.0 Hz, CH_3); 3.12 – 3.18 (m, 4H, $N(CH_2)_2$); 3.41 – 3.51 (m, 2H, $S(CH)_2$); 3.54 (s, 3H, 3-CH_3); 3.88 – 3.94 (m, 4H, $O(CH_2)_2$); 4.03 (s, 2H, 8-SCH_2); 4.02 – 4.12 (m, 4H, 8-SCH_2 and 1-CH_2); 4.28 – 4.38 (m, 2H, $S(CH)_2$); 6.40 – 6.53 (m, 1H, 7-CH); 7.28 (s, 2H, N^+H_2).

2-[3-Methyl-7-(1-oxothietanyl-3)-1-ethylxanthinyl-8-thio]acetic acid hexamethyleneimine salt (IIIf). A solution of 0.64 g (6.5 mmol) of hexamethyleneimine in 4 ml of water a supplemented with 1.81 g (5 mmol) of acid IIb and 100 ml of acetone and heated to boiling. The reaction was cooled and the resulting precipitate was collected by filtration, washed with acetone, and dried. Compound was purified by crystallization from a mixture of acetone and dioxane (1:1 v/v). The IR spectrum (KBr), ν_{max}, cm^{-1}, was: 1035 (S=O), 1654, 1691 (C=C, C=N, C=O), 2400 – 3100 (N^+H_2).

2-[3-Methyl-7-(1,1-dioxothietanyl-3)-1-ethylxanthinyl-8-thio]acetic acid sodium salt (IIIg). A solution of 0.26 g (6.5 mmol) of sodium hydroxide in four drops of water was supplemented with 1.94 g (5 mmol) of acid IIc and 50 ml of acetone and heated to boiling. The reaction was cooled and the resulting precipitate was collected by filtration, washed with acetone, and dried. Compound was purified by redissolution in a minimal volume of DMSO with warming and reprecipitation from acetone. The IR spectrum (KBr), ν_{max}, cm^{-1}, was: 1136, 1313 (SO_2 val.), 1618, 1662, 1699 (C=C, C=N, C=O).

2-[3-Methyl-7-(1,1-dioxothietanyl-3)-1-ethylxanthinyl-8-thio]acetic acid morpholine salt (IIIg). This was prepared using the same method as compound IIIb, from acid IIc. Compound was purified by recrystallization from dioxane. The IR spectrum (KBr), ν_{max}, cm^{-1}, was: 1128, 1311 (SO_2 val.), 1634, 1660, 1698 (C=C, C=N, C=O), 2230 – 3100 (N^+H_2). The ^1H NMR spectrum (DMSO-d_6), δ, ppm, was: 1.14 (t, 3H, J 7.0 Hz, CH_3); 2.91 – 3.00 (m, 4H, $N(CH_2)_2$); 3.41 (s, 3H, 3-CH_3); 3.65 – 3.72 (m, 4H, $O(CH_2)_2$); 3.89 (s, 2H, 8-SCH_2); 3.94 (q, 2H, J 7.0 Hz, 1-CH_2); 4.50 – 4.60 (m, 2H, $S(CH)_2$); 5.01 – 5.11 (m, 2H, $S(CH)_2$); 5.51 – 5.64 (m, 1H, 7-CH).

2-[3-Methyl-7-(1,1-dioxothietanyl-3)-1-ethylxanthinyl-8-thio]acetic acid hexamethyleneimine salt (IIIi). This compound was prepared using the same method used for compound IIIb, from acid IIc and 0.39 g (3.9 mmol) of hexamethyleneimine. Compound was purified by recrystallization from dioxane. The IR spectrum (KBr), ν_{max}, cm^{-1}, was: 1149, 1319 (SO_2 val.), 1602, 1661, 1696 (C=C, C=N, C=O), 2380 – 3100 (N^+H_2).

EXPERIMENTAL BIOLOGICAL SECTION

The antiaggregatory action of the newly synthesized compounds and reference agents was assessed *in vitro* using human donor blood on a Thromlite-1006A aggregometer, using the Born method [5]. Experimental studies were performed using blood from healthy male donors aged 18 – 24 years. Blood was collected from the cubital vein using a BD Vacutainer® system (Dickinson and Company, USA). The stabilizer for venous blood was 3.8% sodium citrate solution at a ratio of 9:1. Platelet aggregation was induced with ADP at a concentration of 20 ìg/ml and collagen at a concentration of 5 mg/ml (Tekhnologiya-Standart, Barnaul).

Reference agents were pentoxifylline, caffeine sodium benzoate, and euphylline (Dal'khimfarm, Russia), as well as acetylsalicylic acid (Shandong Xinhua Pharmaceutical Co. Ltd, China).

Study results were processed in Statistica 10.0 (StatSoft Inc., USA). Testing for normal data distributions was with the Shapiro-Wilks test. Data are presented as medians with 25^{th} and 75^{th} centiles. Analysis of variance was performed using the Kruskal-Wallis test. The critical level of significance *p* was taken as 0.05.

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