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# SEARCH FOR NEW DRUGS

## SYNTHESIS, ANTIAGGREGANT, AND ANTIOXIDANT ACTIVITY OF 2-{[1-ISO-BUTYL-3-METHYL-7-(THIETANYL-3)XANTHIN-8-YL]THIO}ACETIC ACID SALTS

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8-Bromo-1-*iso*-butyl-3-methyl-7-(thietanyl-3)xanthine (**II**) was synthesized by alkylation of 8-bromo-3-methyl-7-(thietanyl-3)xanthine (**I**) with isobutyl bromide. Nucleophilic substitution of **II** with thioglycolic acid gave 2{[1-*iso*-butyl-3-methyl-7-(thietanyl-3)xanthin-8-yl]thio}acetic acid (**III**). A series of water-soluble salts of 2{[1-*iso*-butyl-3-methyl-7-(thietanyl-3)xanthin-8-yl]thio}acetic acid (**IVa-h**) were synthesized by reaction of **III** with organic and inorganic bases. Compounds possessing antiaggregant activity on the level of acetylsalicylic acid were found among salts **IV**. All obtained compounds except for **IVa** and **IVc** suppressed lipid peroxidation less than ascorbic acid. However, all synthesized compounds suppressed generation of reactive oxygen species by phagocytes, in contrast to ascorbic acid.

**Keywords:** thietanes, xanthines, antiaggregant activity, antioxidant activity.

Natural methylxanthines are nonspecific phosphodiesterase inhibitors [1] and adenosine receptor antagonists [2]; possess cardiotonic, bronchodilator, and diuretic activity; and stimulate the central nervous system [3, 4].

The search for biologically active compounds, development of new ones, and optimization of known synthetic methods for xanthine derivatives are driven by the capability of xanthine for monosubstitution at several positions, di- and tri-substitution in several combinations, and the enormous potential for pharmacological activity [5 – 7]. Synthetic derivatives of xanthine with antidiabetic [8 – 10], antidepressant [11, 12], and anti-inflammatory activity [13] that affect blood rheological properties [4, 4 – 18] are known. The biological activity of 3-*iso*-butyl-1-methylxanthine and its binding mechanism to various types of phosphodiesterase are known [19 – 22]. Xanthine derivatives that exhibit antioxidant activity were synthesized [23 – 25].

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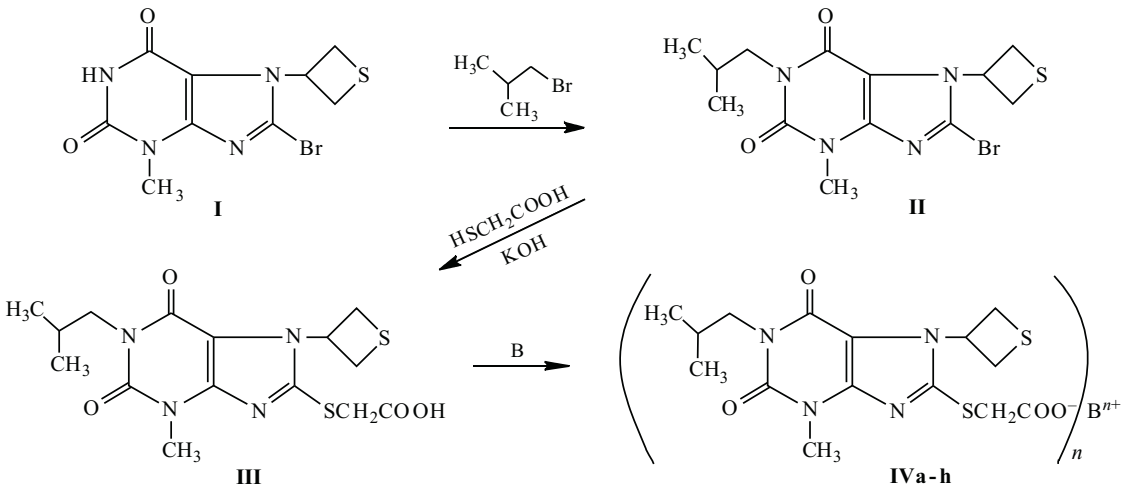
The goals of the present research were to synthesize salts of 2-{[1-*iso*-butyl-3-methyl-7-(thietanyl-3)xanthin-8-yl]-thio}acetic acid and to study their antiaggregant and antioxidant activity.

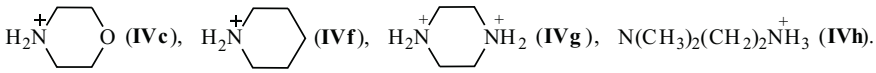
The 1-*iso*-butyl substituent was introduced by reacting 8-bromo-3-methyl-7-(thietanyl-3)xanthine (**I**) with *iso*-butyl bromide in the presence of KOH in DMF to produce 8-bromo-1-*iso*-butyl-3-methyl-7-(thietanyl-3)xanthine (**II**) in 62% yield. The reaction of **II** with thioglycolic acid and KOH in $H_2O$–EtOH (1:1, v/v) preserved the thietane ring and substituted the Br atom to form 2-{[1-*iso*-butyl-3-methyl-7-(thietanyl-3)xanthin-8-yl]thio}acetic acid (**III**) in 91% yield. Salts of 2-{[1-*iso*-butyl-3-methyl-7-(thietanyl-3)xanthin-8-yl]thio}-acetic acid (**IVa-h**) were prepared by reacting acid **III** with various bases in $Me_2CO$, MeOH, or $Et_2O$ in 56 – 79% yield. The reaction of acid **III** with piperazine hexahydrate led to di(2-{[1-*iso*-butyl-3-methyl-7-(thietanyl-3)xanthin-8-yl]thio}-acetate) (**IVg**).

The PMR spectrum of **II** showed resonances for the thietane ring and xanthine methyl, protons of the *iso*-butyl substituent as a doublet $(CH_3)_2$ at 0.91 ppm with SSCC 6.7 Hz, a multiplet for the CH proton at 2.12 – 2.20 ppm, and



$n = 1$ (**IVa-f,h**), $n = 2$ (**IVg**)

$B^{n+} = K^+$ (**IVa**), $H_3N^+C(CH_2OH)_3$ (**IVb**), $H_3N^+CH_2CH_2OH$ (**IVc**), $H_2N^+(CH_2CH_2OH)_2$ (**IVd**),



(**IVc**), (**IVf**), (**IVg**), $N(CH_3)_2(CH_2)_2\overset{+}{N}H_3$ (**IVh**).

a doublet for the 1-$CH_2$ group at 3.89 ppm with SSCC 7.5 Hz. The $^{13}C$ NMR spectrum showed resonances for the *iso*-butyl C atoms at 20.06 ppm [$(CH_3)_2$], 27.12 (CH), and 48.56 (1-$CH_2$).

The PMR spectrum of **III** had resonances for the thietane ring, the alkyl substituents, and an 8-$SCH_2$ singlet at 4.05 ppm. The presence of thioglycolic acid was also confirmed by resonances in the $^{13}C$ NMR spectrum for the 8-$SCH_2$ at 34.86 ppm and C=O at 171.15 ppm.

PMR and $^{13}C$ NMR spectra of salts **IVb-h** showed resonances for the acid **III** and for the protonated amines. For example, the PMR spectrum of monoethanolammonium salt **IVa** exhibited triplets for the $NCH_2$ group at 2.81 ppm and the $OCH_2$ group at 3.55 ppm with SSCC 4.9 Hz. The structure of piperazinium salt **IVg**, which contained two **III** acids, was confirmed by the intensity ratio of 8:4 for the two $N(CH_2)_2$ singlets at 2.88 ppm and the 8-$SCH_2$ resonance at 3.95 ppm.

The IR spectrum of potassium salt **IVa** lacked absorption for characteristic O–H stretching vibrations in the range 2500 – 3350 $cm^{-1}$ for **III**. Protonation of the $NH_2$ group of *N*,*N*-dimethylethylenediamine was confirmed by the lack of absorption for stretching vibrations of unprotonated N–H bonds in the IR spectrum, in which absorption for $N^+$–H vibrations in the range 2300 – 3100 $cm^{-1}$ was observed, and the position of the resonances for unprotonated $N(CH_3)_2$ at 2.13 ppm in the PMR spectrum of **IVh**.

**TABLE 1.** Yields and Characteristics of Synthesized Compounds

| Compound | Yield, % | mp, °C (solvent) | Empirical formula |
|---|---|---|---|
| **II** | 62 | 138.0 – 139.0 (hexane) | $C_{13}H_{17}BrN_4O_2S$ |
| **III** | 91 | 185.0 – 186.0 (EtOAc) | $C_{15}H_{22}N_4O_4S_2$ |
| **IVa** | 79 | 230.7 – 231.5 (*n*-PrOH) | $C_{15}H_{19}KN_4O_4S_2$ |
| **IVb** | 70 | 100 (dec.) ($Me_2CO$) | $C_{19}H_{31}N_5O_7S_2$ |
| **IVc** | 75 | 161.2 – 162.2 (MeCN) | $C_{17}H_{27}N_5O_5S_2$ |
| **IV**d | 56 | 129.5 – 130.5 ($Me_2CO$) | $C_{17}H_{27}N_5O_6S_2$ |
| **IVe** | 75 | 157.0 – 158.5 ($Me_2CO$) | $C_{19}H_{29}N_5O_5S_2$ |
| **IVf** | 78 | 177.2 – 178.4 ($Me_2CO$) | $C_{20}H_{31}N_5O_4S_2$ |
| **IVg** | 67 | 197.5 – 199.5 (dioxane) | $C_{19}H_{30}N_6O_4S_2$ |
| **IVh** | 66 | 148.5 – 150.0 (MeCN) | $C_{19}H_{33}N_6O_4S_2$ |

## EXPERIMENTAL CHEMICAL PART

IR spectra were taken from KBr pellets on an Infralum FT-02 spectrophotometer. PMR spectra were recorded in $CDCl_3$ and DMSO with solvent resonances as internal standards on a Bruker AV-500 instrument at operating frequency 500 MHz; $^{13}C$ NMR spectra, at 125 MHz. The purity of synthesized compounds was determined by TLC on Sorbfil plates using *n*-BuOH–HOAc–$H_2O$ (4:1:2, v/v/v) (**II** and **III**) or $CHCl_3$–EtOH (1:2, v/v) (**IVa-h**). Spots were detected by $I_2$ vapor in a moist chamber. Melting points were measured on an SMP 30 apparatus. Elemental analyses of the synthesized compounds agreed with those calculated. Table 1 lists the characteristics of the synthesized compounds.

Compound **I** was synthesized by the literature method [26].

**8-Bromo-1-*iso*-butyl-3-methyl-7-(thietanyl-3)xanthine (II).** A mixture of **I** (6.34 g, 20 mmol) in DMF (160 mL) was treated with a solution of KOH (1.34 g, 24 mmol) in $H_2O$

(8 mL) and *iso*-butyl bromide (3.28 g, 24 mmol), stirred at 60°C for 6 h, cooled, and treated with $H_2O$ (160 mL). The resulting precipitate was filtered off, rinsed with $H_2O$, and dried.

**2-{[1-*iso*-Butyl-3-methyl-7-(thietanyl-3)xanthin-8-yl]-thio}acetic acid (III).** A solution of KOH (2.52 g, 45 mmol) in an $H_2O$–EtOH mixture (150 mL, 1:1, v/v) was treated with thioglycolic acid (2.76 g, 30 mmol) and **II** (5.60 g, 15 mmol), refluxed for 1.5 h, cooled, and acidified with dilute HCl to pH = 3.0. The resulting precipitate was filtered off, rinsed with $H_2O$, and dried.

**General method for synthesizing K, Tris-ammonium, monoethanolammonium, diethanolammonium, morpholinium, and piperidinium salts of 2-{[1-*iso*-butyl-3-methyl-7-(thietanyl-3)xanthin-8-yl]thio}acetic acid (IVa-e).** Acid **III** (1.15 g, 3 mmol) was dissolved with heating in $Me_2CO$ (50 mL) and cooled. The solution was treated with the appropriate base (3.6 mmol). The resulting precipitate was filtered off, rinsed with $Me_2CO$, and dried. The K (**IVa**) and Tris-ammonium (**IVb**) salts were prepared by dissolving the base beforehand in $H_2O$ (0.3 mL).

**Piperazinium di(2-{[1-*iso*-butyl-3-methyl-7-(thietanyl-3)xanthin-8-yl]thio}acetate) (IVg).** A solution of **III** (1.15 g, 3 mmol) in MeOH (25 mL) was treated with piperazine hexahydrate (1.16 g, 6 mmol). The mixture was evaporated *in vacuo*, cooled, and treated with $Me_2CO$ (25 mL). The precipitate was filtered off, rinsed with $Me_2CO$, and dried.

***N*,*N*-Dimethylethylenediammonium salt of 2-{[1-*iso*-butyl-3-methyl-7-(thietanyl-3)xanthin-8-yl]thio}acetic acid (IVh).** Acid **III** (0.58 g, 1.5 mmol) was dissolved under reflux in $Et_2O$ (80 mL). The solution was cooled and treated with *N*,*N*-dimethylethylenediamine (0.13 g, 1.5 mmol). The

**TABLE 2.** IR Spectra of Synthesized Compounds, ($\nu_{max}$, $cm^{-1}$

| Compound | (C=C, C=N, C=O) | ($N^+H$, O-H) |
|---|---|---|
| **II** | 1605, 1661, 1702 | – |
| **III** | 1645, 1703, 1743 | 2500 – 3350 |
| **IVa** | 1615, 1660, 1700 | – |
| **IVb** | 1578, 1666, 1703 | 2700 – 3550 |
| **IVc** | 1582, 1660, 1701 | 2400 – 3450 |
| **IVd** | 1595, 1656, 1702 | 2300 – 3600 |
| **IVe** | 1606, 1660, 1701 | 2300 – 3250 |
| **IVf** | 1586, 1663, 1702 | 2300 – 3150 |
| **IVg** | 1600, 1664, 1702 | 2300 – 3100 |
| **IVh** | 1605, 1655, 1700 | 2300 – 3100 |

**TABLE 3.** PMR Spectra of Synthesized Compounds, δ, ppm

| Compound | 1-*iso*-$C_4H_9$ | | | 3-$CH_3$ 3H, s | 7-(thietanyl-3) | | | 8-$SCH_2$ 2H, s | Other protons |
|---|---|---|---|---|---|---|---|---|---|
| | $(CH_3)_2$ 6H, d | CH 1H, m | $CH_2$ 2H, d | | $S(CH)_2$ 2H, m | $S(CH)_2$ 2H, m | 7-CH 1H, m | | |
| **II** | 0.91 | 2.12 – 2.20 | 3.89 | 3.51 | 3.26 – 3.30 | 4.34 – 4.38 | 5.94 – 6.01 | - | - |
| **III** | 0.90 | 2.11 – 2.19 | 3.82 | 3.48 | 3.27 – 3.30 | 4.29 – 4.33 | 5.85 – 5.93 | 4.05 | - |
| **IVa** | 0.84 | 1.99 – 2.08 | 3.73 | 3.37 | 3.23 – 3.31 | 4.16 – 4.24 | 5.83 – 5.91 | 3.77 | - |
| **IVb** | 0.84 | 2.00 – 2.07 | 3.70 | 3.37 | 3.27 – 3.31 | 4.17 – 4.21 | 5.83 – 5.91 | 3.86 | 3.42 (s, 6H, $(CH_2)_3$) |
| **IVc** | 0.82 | 1.98 – 2.06 | 3.70 | 3.36 | 3.26 – 3.30 | 4.16 – 4.20 | 5.82 – 5.89 | 3.84 | 2.81 (t, 2H, J 4.9 Hz, $NCH_2$), 3.55 (t, 2H, J 4.9 Hz, $OCH_2$) |
| **IVd** | 0.86 | 2.07 – 2.15 | 3.82 | 3.45 | 3.22 – 3.26 | 4.27 – 4.31 | 5.86 – 5.93 | 4.01 | 3.13 (t, 4H, J 4.45 Hz, $N(CH_2)_2$), 3.86 (t, 4H, J 4.5 Hz, $O(CH_2)_2$) |
| **IVe** | 0.87 | 2.08 – 2.16 | * | 3.46 | 3.22 – 3.26 | 4.28 – 4.32 | 5.84 – 5.92 | 4.05 | 3.11 (t, 4H, J 4.6 Hz, $N(CH_2)_2$), 3.80 – 3.89 (m, 6H, 1-$CH_2$, $O(CH_2)_2$) |
| **IVf** | 0.88 | 2.10 – 2.18 | 3.85 | 3.47 | 3.22 – 3.26 | 4.31 – 4.35 | 5.90 – 5.97 | 4.07 | 1.58 – 1.80 (m, 6H, $(CH_2)_3$), 3.02 (t, 4H, J 5.5 Hz, $N(CH_2)_2$) |
| **IVg**** | 0.84 | 2.00 – 2.08 | 3.73 | 3.37 | 3.28 – 3.32 | 4.17 – 4.21 | 5.83 – 5.90 | 3.95 | 2.88 (s, 8H, $2N(CH_2)_2$) |
| **IVh** | 0.84 | 2.00 – 2.08 | 3.72 | 3.37 | 3.27 – 3.31 | 4.18 – 4.22 | 5.84 – 5.91 | 3.83 | 2.13 (s, 6H, $N(CH_3)_2$), 2.39 (t, 2H, J 6.2 Hz, $NCH_2$), 2.82 (t, 2H, J 6.2 Hz, $N^+CH_2$) |

* Resonances of $CH_2$ protons of 1-*iso*-butyl substituent overlap morpholine $O(CH_2)_2$ protons.
** Intensity of protons twice that shown in table except for piperazine protons.

resulting precipitate was filtered off, rinsed with $Et_2O$, and dried.

Tables 2 – 4 list the spectral data of the synthesized compounds.

## EXPERIMENTAL BIOLOGICAL PART

Synthesized salts **IVa-h** and starting compounds **II** and **III** were tested for antiaggregant and antioxidant activity. The effects of the new compounds at a concentration of 2 mM on platelet aggregation were tested *in vitro* using the Born method on an AT-02 aggregometer (NPF Medtekh, Russia) [27]. The biological studies used blood from healthy male donors aged from 18 to 24 years. Blood was collected from a cubital vein using a BD Vacutainer® vacuum kit (Dickinson and Co., USA). A solution (3.8%) of sodium citrate was used in a 9:1 ratio as a stabilizer of the venous blood. Adenosine diphosphate (ADP) at a concentration of 20 μg/mL and collagen at a concentration of 5 mg/mL (Tekhnologiya-Standart, Barnaul, Russia) were used to induce platelet aggregation.

The effects of the synthesized compounds on lipid peroxidation (LPO) were determined using lipoprotein complexes from egg yolk (model **I**). The effects of the new compounds on generation of reactive oxygen species (ROS) by phagocytes were studied using whole heparinized blood (50 IU heparin per mL of blood) from healthy volunteers. The intensity of ROS generation by phagocytes was determined by recording the level of luminol-dependent chemiluminescence. Phagocyte oxygen bursts were caused by adding a suspension (1%) of zymosan followed by incubation for 5 min at 37°C (model **II**) [28].

The reference drugs were pentoxifylline [3,7-dimethyl-1-(5-oxohexyl)xanthine, Dalkhimfarm Co. Russia] and acetylsalicylic (2-acetyloxybenzoic acid) and ascorbic acids (Pharmaceutical Plant Shandong Xinhua Pharmaceutical Co., Ltd., China).

**TABLE 4.** $^{13}C$ NMR Spectra of Synthesized Compounds, d, ppm

| Compound | 1-*iso*-$C_4H_9$ | | | 3-$CH_3$ | 7-(thietanyl-3) | | 8-$SCH_2$ | C=O | Xanthine C atoms | Other C atoms |
|---|---|---|---|---|---|---|---|---|---|---|
| | $(CH_3)_2$ | CH | 1-$CH_2$ | | 7-CH | $S(CH_2)_2$ | | | | |
| **II** | 20.06 | 27.12 | 48.56 | 29.94 | 52.97 | 34.92 | - | - | 109.25, 126.82, 149.09, 151.09, 154.25 | - |
| **III** | 20.05 | 27.17 | 48.59 | 29.95 | 52.01 | 34.92 | 34.86 | 171.15 | 108.99, 148.78, 149.02, 151.43, 154.26 | - |
| **IVa** | 20.48 | 27.22 | 48.01 | 29.95 | 51.61 | 35.31 | 41.78 | 167.77 | 107.65, 149.43, 151.18, 153.38, 154.13 | - |
| **IVb** | 20.48 | 27.22 | 48.04 | 29.98 | 51.69 | 35.31 | 40.00 | 170.51 | 107.85, 149.36, 151.12, 152.19, 154.13 | 60.08 ($(CH_2)_3$), 61.10 (NC) |
| **IVc** | 20.46 | 27.22 | 48.01 | 29.95 | 51.66 | 35.30 | 40.28 | 170.12 | 107.81, 149.34, 151.09, 152.30, 154.10 | 41.75 ($NCH_2$), 58.19 ($OCH_2$) |
| **IVd** | 20.06 | 27.16 | 48.37 | 29.76 | 51.66 | 35.07 | 38.86 | 174.12 | 108.46, 149.16, 150.48, 151.35, 154.28 | 50.03 ($N(CH_2)_2$), 57.18 ($O(CH_2)_2$) |
| **IVe** | 20.06 | 27.16 | 48.37 | 29.76 | 51.68 | 35.05 | 37.88 | 173.07 | 108.53, 149.15, 150.11, 151.34, 154.36 | 43.11 ($N(CH_2)_2$), 63.86 ($O(CH_2)_2$) |
| **IVf** | 20.04 | 27.17 | 48.33 | 29.69 | 51.62 | 35.04 | 39.06 | 173.00 | 108.31, 149.24, 151.07, 151.39, 154.33 | 22.56 ($(CH_2)_2$ $\underline{CH_2}$), 22.61 ($(\underline{CH_2})_2$ $CH_2$), 44.25 ($N(CH_2)_2$) |
| **IVg** | 20.00 | 26.74 | 47.59 | 29.49 | 51.30 | 34.81 | 37.96 | 169.21 | 107.60, 148.83, 150.63, 150.86, 153.70 | 42.68 ($N(CH_2)_2$) |
| **IVh** | 20.48 | 27.23 | 48.03 | 29.95 | 51.69 | 35.31 | 40.58 | 169.23 | 107.79, 149.37, 151.14, 152.55, 154.14 | 36.81 ($NCH_2$), 45.41 ($N(CH_3)_2$), 56.52 ($N^+CH_2$) |

**TABLE 5.** Effect of Synthesized Compounds and Reference Drug on Platelet Aggregation Parameters, Me (0.25 – 0.75)

| Compound | Latent period, % vs. control | Maximum amplitude, % vs. control | Aggregation rate, % vs. control |
|---|---|---|---|
| **II** | + 4.0 (3.9 – 5.6)$^{\dagger\dagger,\#}$ | – 4.4 (1.1 – 5.7)$^{\dagger\dagger,\#\#}$ | – 7.5 (5.9 – 8.3)$^{*,\dagger\dagger}$ |
| **III** | + 13.0 (12.1 – 14.6)$^{**,\dagger\dagger,\#\#}$ | – 12.8 (10.4 – 14.7)$^{*,\dagger\dagger}$ | – 12.6 (9.6 – 13.6)$^{*,\dagger\dagger}$ |
| **IVa** | + 3.6 (3.1 – 4.2) | – 7.6 (7.3 – 9.2) | + 9.4 (8.7 – 10.4) |
| **IVb** | – 2.3 (1.7 – 8.9)$^{\dagger\dagger}$ | – 9.4 (7.9 – 12.5)$^{*,\dagger\dagger}$ | – 7.4 (6.3 – 8.5)$^{*,\#,\dagger\dagger}$ |
| **IVc** | – 4.7 (3.8 – 5.6)$^{\dagger\dagger}$ | – 7.2 (7.4 – 9.1)$^{*,\dagger\dagger,\#}$ | – 18.9 (15.6 – 20.4)$^{*,\#,\dagger\dagger}$ |
| **IVd** | – 3.2 (2.6 – 4.1)$^{\dagger\dagger}$ | – 7.7 (5.1 – 8.6)$^{*,\dagger\dagger,\#}$ | + 0.9 (0.4 – 1.3)$^{\#\#,\dagger\dagger}$ |
| **IVe** | + 10.6 (8.9 – 11.3)$^{*,\dagger\dagger,\#\#}$ | – 11.8 (9.4 – 13.1)$^{*,\dagger\dagger}$ | – 9.7 (7.8 – 10.4)$^{*,\dagger\dagger}$ |
| **IVf** | + 10.0 (9.5 – 13.4)$^{*,\dagger\dagger,\#\#}$ | – 11.6 (8.3 – 14.2)$^{*,\dagger\dagger}$ | – 10.8 (10.1 – 12.3)$^{*,\dagger\dagger}$ |
| **IVg** | – 3.3 (2.7 – 4.3)$^{\dagger\dagger}$ | – 6.4 (4.3 – 8.3)$^{*,\dagger\dagger,\#}$ | – 4.2 (3.5 – 7.6)$^{\#,\dagger\dagger}$ |
| **IVh** | + 11.8 (9.7 – 12.3) | – 10.7 (8.5 – 12.3) | – 0.2 (0.1 – 0.7) |
| Acetylsalicylic acid | – 2.1 (1.1 – 2.6)$^{\dagger\dagger}$ | – 13.7 (10.8 – 16.4)$^{**,\dagger\dagger}$ | – 10.5 (7.6 – 12.3)$^{*,\dagger\dagger}$ |
| Pentoxifylline | + 32.4 (28.7 – 35.6)$^{**,\#\#}$ | – 48.4 (42.7 – 56.5)$^{**,\#\#}$ | – 34.9 (28.7 – 39.6)$^{**,\#\#}$ |

**Note**: Latent period given for collagen-induced platelet aggregation, other parameters for ADP-induced platelet aggregation.
$^{*}p \le 0.05$, $^{**}p \le 0.001$ vs. control; $^{\dagger}p \le 0.05$, $^{\dagger\dagger}p \le 0.001$ vs pentoxifylline; $^{\#}p \le 0.05$, $^{\#\#}p \le 0.001$ vs. reference acetylsalicylic acid; $n = 6$.

**TABLE 6.** Chemiluminescence Parameters in Model Lipid Peroxidation Systems (I) and Blood Macrophage Activity (II) with Added Test Compounds, % vs. Control

| Compound | Model | Light sum | Flash |
|---|---|---|---|
| **II** | I | – 11.5 (10.7 – 13.5)$^{*}$ | – 9.4 (9.3 – 10.1)$^{*}$ |
| | II | – 30.8 (27.5 – 33.1)$^{\#}$ | – 28.4 (25.1 – 30.6)$^{\#}$ |
| **III** | I | – 16.5 (15.3 – 17.8)$^{*}$ | – 8.1 (8.0 – 8.7)$^{*}$ |
| | II | – 17.6 (12.9 – 19.5)$^{\#}$ | – 10.3 (9.4 – 12.7)$^{\#}$ |
| **IVa** | I | + 12.4 (10.4 – 15.7)$^{*}$ | + 37.5 (35.2 – 40.4)$^{*}$ |
| | II | – 36.3 (34.1 – 40.5)$^{\#}$ | – 39.7 (36.2 – 44.1)$^{\#}$ |
| **IVb** | I | – 42.9 (40.7 – 45.4)$^{*}$ | – 13.7 (12.8 – 15.6)$^{*}$ |
| | II | – 50.8 (47.8 – 53.4)$^{\#}$ | – 59.9 (57.1 – 62.4)$^{\#}$ |
| **IVc** | I | + 7.9 (7.3 – 9.2)$^{*}$ | + 37.5 (35.2 – 40.4)$^{*}$ |
| | II | – 32.3 (31.8 – 36.2)$^{\#}$ | – 40.4 (37.7 – 45.2)$^{\#}$ |
| **IVd** | I | – 49.9 (47.6 – 50.8)$^{*}$ | – 28.2 (27.6 – 30.4)$^{*}$ |
| | II | – 46.6 (43.5 – 50.2)$^{\#}$ | – 56.1 (53.2 – 57.8)$^{\#}$ |
| **IVe** | I | – 24.3 (22.9 – 25.7)$^{*}$ | – 8.9 (8.7 – 9.6)$^{*}$ |
| | II | – 15.7 (14.2 – 19.3)$^{\#}$ | – 10.2 (9.4 – 13.5)$^{\#}$ |
| **IVf** | I | – 42.3 (39.8 – 46.9)$^{*}$ | – 18.7 (16.4 – 20.7)$^{*}$ |
| | II | – 45.1 (41.9 – 48.2)$^{\#}$ | – 53.1 (50.5 – 56.3)$^{\#}$ |
| **IVg** | I | – 23.8 (22.7 – 24.5)$^{*}$ | – 9.7 (9.5 – 10.4)$^{*}$ |
| | II | – 23.2 (22.1 – 25.7)$^{\#}$ | – 17.5 (16.3 – 19.2)$^{\#}$ |
| **IVh** | I | – 37.4 (32.6 – 40.1)$^{*}$ | – 30.1 (24.1 – 37.2)$^{*}$ |
| | II | – 46.6 (43.5 – 50.2)$^{\#}$ | – 43.4 (39.6 – 47.1)$^{\#}$ |
| Ascorbic acid | I | – 78.1 (70.4 – 82.4) | – 86.8 (80.3 – 92.1) |
| | II | + 73.1 (66.7 – 75.2) | + 98.7 (94.8 – 100.3) |

**Note**: Values given as differences in % between test and control group values; median and interquartal interval given for results of six measurements; $p \le 0.05$ vs. control for all parameters; $^{*}p \le 0.05$, $^{\#}p \le 0.05$, statistically significant differences from ascorbic acid for models I and II, respectively.

Results for biological activity were processed using Statistica 10.0 software (StatSoft Inc., USA). A check for normal distribution of actual data was made using the Shapiro–Wilk criterion. Data were presented as medians and 25- and 75-percentiles. Dispersion analysis used the Kruskal–Wallis criterion. The critical significance level $p$ was set to 0.05 for statistical criteria.

Table 5 presents the antiaggregant activity parameters for the new compounds.

The research results established that **III**, **IVc**, **IVe**, and **IVf** suppressed platelet aggregation (maximum amplitude and/or aggregation rate) on the level of acetylsalicylic acid. Compounds **III**, **IVe**, and **IVf** lengthened the latent period for collagen-induced platelet aggregation more effectively than acetylsalicylic acid.

Table 6 presents results for the antioxidant activity of the compounds.

All compounds except for **IVa** and **IVc** were found to suppress LPO. However, they were inferior to ascorbic acid with respect to this parameter. All synthesized compounds suppressed generation of ROS by phagocytes, in contrast to ascorbic acid.

Thus, compounds exhibiting antiaggregant activity on the level of acetylsalicylic acid combined with an antioxidant effect that were manifested as reduced LPO and inhibition of ROS generation by phagocytes were discovered.

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