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SYNTHESIS AND ANTIDEPRESSANT ACTIVITY OF 8-AMINO-SUBSTITUTED 1-BUTYL-3-METHYLXANTHINES CONTAINING A THIETANE RING

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A series of 8-amino-substituted 1-butyl-3-methylxanthines containing a thietane ring were obtained in 47 – 96% yields by reacting 7-(thietanyl-3)-, 7-(1-oxothietanyl-3)-, and 7-(1,1-dioxothietanyl-3)-8-bromo-1-butyl-3-methylxanthines with piperidine and morpholine. Reaction of 8-amino-substituted 1-butyl-3-methyl-7-(1,1-dioxothietanyl-3)xanthines with sodium ethoxide synthesized 8-amino-substituted 1-butyl-3-methylxanthines in 85 – 98% yields. The structures of the compounds were confirmed by IR and PMR spectroscopy. The synthesized compounds exhibited antidepressant activity.

Keywords: xanthines, thietanes, antidepressant activity.

Purine-type alkaloids used in medicine (caffeine, theophylline, theobromine) exhibit various types of activity including psychotropic [1]. New 8-amino-substituted 1-butyl-3-methylxanthines with a 7-thietane ring were synthesized by us to discover xanthine derivatives affecting the central nervous system and were tested for antidepressant activity.

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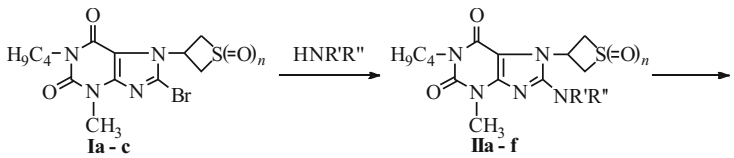
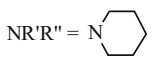
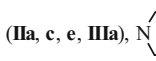
The starting compounds were 1-butyl-8-bromo-3-methyl-7-(thietanyl-3)xanthines (**Ia-c**), which were synthesized by the published methods [2 – 4].

8-Amino-substituted 1-butyl-3-methyl-7-(thietanyl-3)-xanthines (**IIa**, **b**) were produced by refluxing 8-bromo-7-(thietanyl-3)xanthine (**Ia**) with a three-fold molar excess of piperidine or morpholine in EtOH for 5 h. Analogously, 8-bromo-7-(1-oxo-thietanyl-3)xanthine (**Ib**) afforded 8-amino-substituted 1-butyl-3-methyl-7-(1-oxothietanyl-3)-xanthines (**IIc**, **d**). 8-Bromo-7-(1,1-dioxothietanyl-3)xanthine (**Ic**)



n = 0 (**Ia**, **IIa**, **b**), 1 (**Ib**, **IIc**, **d**), 2 (**Ic**, **IIe**, **f**);

NR'R" =  (**IIa**, **c**, **e**, **IIIa**),  (**IIb**, **d**, **f**, **IIIb**)

gave 8-amino-substituted 1-butyl-3-methyl-7-(1,1-dioxothietanyl-3)xanthines (**IIe**, **f**).

TABLE 1. Influence of Synthesized Compounds and Reference Drug on TST and FST Parameters

Compound	TST	FST	
	immobilization, c	immobilization, c	DI
Control	135.0 (145.0 – 107.0) $n = 13$	136.0 (164.0 – 128.0) $n = 14$	1.19 (1.55 – 0.93) $n = 14$
IIa	38.0* (91.0 – 21.0) $n = 7$	136.0 (145.0 – 126.0) $n = 6$	0.81* (0.85 – 0.72) $n = 6$
IIb	131.0 (161.0 – 92.0) $n = 6$	133.0 (145.0 – 118.0) $n = 6$	0.85 (1.21 – 0.65) $n = 7$
IIc	123.0 (167.0 – 28.0) $n = 7$	85.0* (125.0 – 75.0) $n = 6$	0.91 (1.18 – 0.81) $n = 7$
IId	90.0 (173.0 – 71.0) $n = 7$	112.0* (119.0 – 84.0) $n = 7$	1.04 (1.5 – 0.9) $n = 7$
IIe	152.0 (156.0 – 148.0) $n = 5$	138.5 (146.0 – 118.0) $n = 6$	0.79* (0.94 – 0.73) $n = 6$
IIf	168.0 (200.0 – 96.0) $n = 7$	156.0 (195.0 – 151.0) $n = 7$	0.88 (1.12 – 0.6) $n = 7$
IIIa	95.0 (141.0 – 55.0) $n = 7$	137.0 (142.0 – 134.0) $n = 6$	0.9* (0.97 – 0.71) $n = 7$
IIIb	160.5* (164.0 – 148.0) $n = 4$	109.0 (184.0 – 24.0) $n = 6$	0.91 (1.11 – 0.7) $n = 6$
H-criterion Kruskal—Wallis	H (8, N = 63) = 18.161 $p = 0.020$	H (8, N = 64) = 22.366 $p = 0.004$	H (8, N = 67) = 15.037 $p = 0.058$
Control	159.5 (189.5 – 143.0) $n = 12$	130.0 (142.5 – 100.5) $n = 8$	1.08 (1.11 – 0.86) $n = 7$
Fluoxetine	60.0 (121.0 – 40.0) $n = 5$	61.0* (87 – 30.5) $n = 8$	0.54* (0.69 – 0.45) $n = 8$
H-criterion Kruskal—Wallis	H (1, N = 17) = 3.211 $p = 0.073$	H (1, N = 16) = 8.040 $p = 0.004$	H (1, N = 15) = 8.385 $p = 0.003$

* Differences statistically significant vs. control ($p < 0.05$ for Mann—Whitney *U*-criterion). Tested compounds were administered at the minimal active dose of 2 mg/kg; data from two series of experiments (series 1, study of antidepressant activity of **IIa-IIIb**; series 2, study of antidepressant activity of fluoxetine) are given, statistically significant differences between medians of series 2 control groups were not found ($p > 0.05$ for Mann—Whitney *U*-criterion).

PMR spectra of **IIa-f** contained characteristic proton resonances for the thietane ring, 1-butyl substituent, xanthine methyl singlet, and amine radical protons. Spectra of 8-piperidinoxanthines **IIa**, **c**, **e** exhibited $(CH_2)_3$ resonances at 1.5 – 1.8 ppm and $N(CH_2)_2$ at 3.1 – 3.2. The $(CH_2)_3$ resonances overlapped methylene resonances of the 1-butyl substituent. Spectra of 8-mopholinoxanthines **IIb**, **d**, **f** contained $N(CH_2)_2$ and $O(CH_2)_2$ resonances at 3.1 – 3.2 and 3.8 – 3.9 ppm, respectively.

IR spectra of **IIc**, **d** had characteristic strong absorption bands for S=O stretching vibrations at 1055 – 1063 cm^{-1} that confirmed the thietane-oxide ring was retained. Spectra of **IIe**, **f** showed absorption bands for symmetric and asymmetric SO_2 stretching vibrations at 1133 – 1138 and 1309 – 1320 cm^{-1} and confirmed that the thietane-dioxide ring remained intact.

8-Amino-substituted 1-butyl-3-methylxanthines (**IIIa**, **b**) without the 7-thietane ring were prepared by reacting 8-amino-substituted 7-(1,1-dioxothietanyl-3)xanthines (**IIe**, **f**) with sodium ethoxide in refluxing EtOH for 0.5 h to eliminate the thietane-dioxide ring [4].

Elimination of the thietane-dioxide ring was confirmed by PMR spectra of **IIIa**, **b** where characteristic resonances of the 7-CH and two $S(CH)_2$ protons were missing and resonances of the 1-butyl substituent, xanthine methyl, and amine protons and a weak-field singlet for unsubstituted 7-NH at 11.43 – 11.70 ppm were observed.

IR spectra of **IIIa**, **b** had broad bands for 7-NH stretching vibrations at 3050 – 3300 cm^{-1}. Characteristic absorption bands of symmetric and asymmetric SO_2 stretching vibrations were missing, confirming that the structure was unsubstituted at the 7-position.

Screening of the synthesized compounds found that **IIa**, **c-e**, and **IIIa** possessed antidepressant-like activity of various strengths. Only **IIa** in the tail-suspension test (TST) caused a statistically significant reduction by 72% of the total time of immobilization (TTI) ($p = 0.004$) as compared with the controls, exceeding the strength of fluoxetine, which caused only a trend toward a decrease of the parameter by 62.4% ($p = 0.132$). Compounds **IIc**, **d** in the forced swim test (FST) caused statistically significant reductions of the TTI by 37.5% ($p = 0.010$) and 18% ($p = 0.027$), respectively. The depression index (DI) decreased statistically significantly in animals that received **IIa**, **e**, **IIIa** by 33% ($p = 0.014$), 42% ($p = 0.016$), and 25% ($p = 0.040$), respectively. Only animals that received fluoxetine showed statistically significant reductions simultaneously of TTI and DI by 53 and 50%, respectively. Compounds **IIf** and **IIIb** did not exhibit antidepressant properties. Moreover, **IIIb** gave a statistically significant increase by 19% ($p = 0.017$) of TTI in the TST as compared with the control (Table 1).

An analysis of the results led to the conclusion that the ability to manifest antidepressant properties of 8-amino-substituted 1-butyl-3-methylxanthines was probably not directly related to the presence of thietane/oxothietane/dioxothietane rings although they did affect the strength of the antidepres-

sant effect. For example, the activity of **IIIa** was like that of **IIa**. Both compounds caused a statistically significant reduction of DI, did not change FST TTI, and reduced TST TTI; **IIa**, statistically significantly by 72%; **IIIa**, at the clear trend level (by 30%). However, **IIa** with a thietane ring was the most active of all eight synthesized compounds whereas **IIc** (oxothietane) and **IIe** (dioxothietane) were inferior to **IIa**. Also, they exhibited antidepressant effects although for only one of the recorded parameters (TTI FST, **IIc**; DI, **IIe**). Replacing the 8-piperidine by morpholine decreased the antidepressant activity in the pairs **IIa-IIb**, **IIe-IIf**, **IIIa-IIIb** (statistically significant changes of the parameter marking antidepressant effects became statistically insignificant). The pair **IIc-IId** showed weakened antidepressant activity. Therefore, it seemed interesting to determine if the 7-thietane ring affected the toxicity of 8-amino-substituted 1-butyl-3-methylxanthines. As a result, the acute toxicities of the pair **IIa-IIIa** were determined. The LD_{50} with i.p. administration of both compounds allowed them to be assigned to hazard class IV (marginally toxic compounds) [5]. Fluoxetine with an analogous administration mode is moderately toxic (hazard class III) (Table 2).

EXPERIMENTAL CHEMICAL PART

IR spectra of compounds in KBr pellets were taken on an Infralum FT-02 instrument. PMR spectra were taken on a Bruker AM-300 instrument at operating frequency 300 MHz. The solvent was $CDCl_3$ with the solvent resonance as an internal standard.

Melting points of synthesized compounds were determined on an SMP-30 apparatus. The purity of compounds was determined by TLC on Silufol plates using BuOH–HOAc–H_2O (4:1:2, v/v/v). Spots were detected by I_2 vapor in a humid chamber. Table 3 presents the characteristics of the synthesized compounds. Elemental analyses of the synthesized compounds agreed with those calculated.

1-Butyl-3-methyl-7-(thietanyl-3)-8-piperidinoxanthine (IIa). A solution of **Ia** (1.87 g, 5 mmol) and piperidine (1.27 g, 15 mmol) in EtOH (40 mL) was refluxed for 5 h and cooled. The resulting precipitate was filtered off, rinsed with EtOH and H_2O, dried, and purified by crystallization from EtOH. IR spectrum (KBr), ν_{max}, cm^{-1}: 1609, 1648, 1653, 1698 (C=C, C=N, C=O). PMR spectrum ($CDCl_3$), δ, ppm: 0.95 (t, 3H, J 7.3 Hz, CH_3); 1.33 – 1.48 (m, 2H, CH_2); 1.60 – 1.82 ((m, 8H, CH_2 and $(CH_2)_3$); 3.09 – 3.16 ((m, 4H, $N(CH_2)_2$); 3.22 – 3.30 ((m, 2H, $S(CH)_2$); 3.52 (s, 3H, 3-CH_3); 4.01 – 4.10 ((m, 2H, 1-CH_2); 4.32 – 4.41 ((m, 2H, $S(CH)_2$); 5.40 – 5.55 ((m, 1H, 7-CH).

1-Butyl-3-methyl-8-morpholino-7-(thietanyl-3)xanthine (IIb) was prepared analogously to **IIa** using morpholine (1.30 g, 15 mmol) and purified by crystallization from EtOH. IR spectrum (KBr), ν_{max}, cm^{-1}: 1606, 1648, 1653, 1695 (C=C, C=N, C=O). PMR spectrum ($CDCl_3$), δ, ppm: 0.94 (t, 3H, J 7.3 Hz, CH_3); 1.32 – 1.46 ((m, 2H, CH_2); 1.58 – 1.70 ((m, 2H, CH_2); 3.15 – 3.20 ((m, 4H, $N(CH_2)_2$); 3.20 – 3.27 ((m, 2H, $S(CH)_2$); 3.51 (s, 3H, 3-CH_3); 3.84 – 3.89 ((m, 4H, $Î(CH_2)_2$); 4.01 – 4.08 ((m, 2H, 1-CH_2); 4.32 – 4.40 ((m, 2H, $S(CH)_2$); 5.45 – 5.59 ((m, 1H, 7-CH).

1-Butyl-3-methyl-8-piperidino-7-(1-oxothietanyl-3)-xanthine (IIc) was prepared analogously to **IIa** from **IIb**. The reaction mixture was cooled and evaporated under vacuum. The solid was treated with H_2O (40 mL). The precipitate was filtered off, rinsed with H_2O, and dried. IR spectrum (KBr), ν_{max}, cm^{-1}: 1063 (S=O), 1612, 1654, 1690 (C=C, C=N, C=O). PMR spectrum ($CDCl_3$), δ, ppm: 0.94 (t, 3H, J 7.3 Hz, CH_3); 1.32 – 1.44 ((m, 2H, CH_2); 1.56 – 1.78 ((m, 8H, CH_2 and $(CH_2)_3$); 3.10 – 3.16 ((m, 4H, $N(CH_2)_2$); 3.34 – 3.43 ((m, 2H, $S(CH)_2$); 3.51 (s, 3H, 3-CH_3); 3.94 – 4.02 ((m, 2H, 1-CH_2); 4.21 – 4.32 ((m, 2H, $S(CH)_2$); 5.99 – 6.12 ((m, 1H, 7-CH).

1-Butyl-3-methyl-8-morpholino-7-(1-oxothietanyl-3)-xanthine (IId) was prepared analogously to **IIa** using morpholine (1.30 g, 15 mmol). IR spectrum (KBr), ν_{max}, cm^{-1}: 1055 (S=O), 1605, 1650, 1693 (C=C, C=N, C=O). PMR spectrum ($CDCl_3$), δ, ppm: 0.93 (t, 3H, J 7.3 Hz, CH_3); 1.30 – 1.43 ((m, 2H, CH_2); 1.52 – 1.66 ((m, 2H, CH_2); 3.13 – 3.21 ((m, 4H, $N(CH_2)_2$); 3.32 – 3.42 ((m, 2H, $S(CH)_2$); 3.51 (s, 3H, 3-CH_3); 3.81 – 3.88 ((m, 4H, $O(CH_2)_2$); 3.93 – 4.01 ((m, 2H, 1-CH_2); 4.22 – 4.33 ((m, 2H, $S(CH)_2$); 6.06 – 6.21 ((m, 1H, 7-CH).

1-Butyl-3-methyl-8-piperidino-7-(1,1-dioxothietanyl-3)xanthine (IIe) was prepared analogously to **IIa** from **Ic**. IR spectrum (KBr), ν_{max}, cm^{-1}: 1133, 1309 (SO_2 str), 1611,

TABLE 2. Acute Toxicity of Synthesized Compounds and Reference Drug

Compound	LD_{50}, mg/kg	Toxicity class/degree
IIa	840	**IV**/Marginally toxic compound
IIIa	840	**IV**/Marginally toxic compound
Fluoxetine	87	**III**/Moderately toxic compound

TABLE 3. Characteristics of Synthesized Compounds

Compound	Yield, %	mp, °C	R_f	Empirical formula
IIa	87	112 – 114	0.77	$C_{18}H_{27}N_5O_2S$
IIb	96	169 – 171	0.71	$C_{17}H_{25}N_5O_3S$
IIc	78	162 – 164	0.72	$C_{18}H_{27}N_5O_3S$
IId	47	192 – 194	0.62	$C_{17}H_{25}N_5O_4S$
IIe	78	191 – 193	0.69	$C_{18}H_{27}N_5O_4S$
IIf	90	203 – 205	0.70	$C_{17}H_{25}N_5O_5S$
IIIa	85	231 – 233	0.81	$C_{15}H_{23}N_5O_2$
IIIb	98	263 – 265	0.72	$C_{14}H_{21}N_5O_3$

1655, 1694 (C=C, C=N, C=O). PMR spectrum ($CDCl_3$), δ, ppm: 0.95 (t, 3H, J 7.3 Hz, CH_3); 1.32 – 1.46 ((m, 2H, CH_2); 1.57 – 1.81 ((m, 8H, CH_2 and $(CH_2)_3$); 3.11 – 3.19 ((m, 4H, $N(CH_2)_2$); 3.53 (s, 3H, 3-CH_3); 4.00 – 4.08 ((m, 2H, 1-CH_2); 4.25 – 4.35 ((m, 2H, $S(CH)_2$); 5.06 – 5.26 ((m, 3H, $S(CH)_2$ and 7-CH).

1-Butyl-3-methyl-8-morpholino-7-(1,1-dioxothietanyl-3)xanthine (IIf) was prepared analogously to **IIa** from **Ic** using morpholine (1.30 g, 15 mmol). IR spectrum (KBr), ν_{max}, cm^{-1}: 1138, 1320 (SO_2 str), 1613, 1662, 1699 (C=C, C=N, C=O). PMR spectrum ($CDCl_3$), δ, ppm: 0.92 (t, 3H, J 7.3 Hz, CH_3); 1.29 – 1.42 ((m, 2H, CH_2); 1.55 – 1.66 ((m, 2H, CH_2); 3.15 – 3.24 ((m, 4H, $N(CH_2)_2$); 3.52 (s, 3H, 3-CH_3); 3.83 – 3.91 ((m, 4H, $O(CH_2)_2$); 3.97 – 4.06 ((m, 2H, 1-CH_2); 4.25 – 4.39 ((m, 2H, $S(CH)_2$); 5.12 – 5.27 ((m, 3H, $S(CH)_2$ and 7-CH).

1-Butyl-3-methyl-8-piperidinoxanthine (IIIa). Metallic Na (0.07 g, 3 mmol) was dissolved in absolute EtOH (20 mL). The resulting solution was treated with **IIe** (1.00 g, 2.5 mmol), refluxed for 0.5 h, cooled, and evaporated under vacuum. The solid was dissolved in H_2O (20 mL) and neutralized with dilute HCl to pH 3. The resulting precipitate was filtered off, rinsed with H_2O, dried, and purified by crystallization from EtOH. IR spectrum (KBr), ν_{max}, cm^{-1}: 1622, 1653, 1701 (C=C, C=N, C=O), 3050 – 3300 (N-H str). PMR spectrum ($CDCl_3$), δ, ppm: 0.94 (t, 3H, J 7.3 Hz, CH_3); 1.29 – 1.43 ((m, 2H, CH_2); 1.57 – 1.72 ((m, 8H, CH_2 and $(CH_2)_3$); 3.55 (s, 3H, 3-CH_3); 3.61 – 3.68 ((m, 4H, $N(CH_2)_2$); 3.94 – 4.02 ((m, 2H, 1-CH_2); 11.43 (s, 1H, 7-H).

1-Butyl-3-methyl-8-morpholinoxanthine (IIIb) was prepared analogously to **IIIa** from **IIf**. IR spectrum (KBr), ν_{max}, cm^{-1}: 1620, 1651, 1703 (C=C, C=N, C=O), 3050 – 3230 (N-H str). PMR spectrum ($CDCl_3$), δ, ppm: 0.95 (t, 3H, J 7.3 Hz, CH_3); 1.29 – 1.43 ((m, 2H, CH_2); 1.54 – 1.68 ((m, 2H, CH_2); 3.66 – 3.75 ((m, 4H, $N(CH_2)_2$); 3.55 (s, 3H, 3-CH_3); 3.77 – 3.86 ((m, 4H, $O(CH_2)_2$); 3.89 – 3.98 ((m, 2H, 1-CH_2), 11.70 (s, 1H, 7-H).

EXPERIMENTAL BIOLOGICAL PART

The experiments used laboratory male white mice (20 – 22 g). All animals were kept under standard vivarium conditions with free access to water and feed. The synthesized compounds were studied in the TST [6] and FST [7, 8], which were recommended for screening to evaluate the primary biological activity of antidepressants [9]. The TTI was evaluated in both tests. The DI, a biorhythmic parameter calculated as the ratio of the number of short immobilization periods to the number of active swimming periods, was also calculated in the FST [10]. Compounds were injected once i.p. at a dose of 2 mg/kg (minimal active dose determined in a separate series of experiments) 30 min before the tests. Fluoxetine (0.02 capsules, Lannacher Heilmittel, Austria; reference drug; capsule contents suspended with Tween-80) was injected once i.p. 30 min before the experiment at the optimal effective dose for animals of 10 mg/kg. Control animals received an equivalent volume of isotonic saline with Tween-80. Results were statistically processed using the Statistics 7.0 program suite. Variation series were described using the median (Me) and 25 and 75% percentiles (Per). Kruskal—Wallis *H*-criteria and Mann—Whitney *U*-criteria were calculated to compare groups. The critical significance level for statistical criteria was set to 0.05 [11]. Acute toxicity (LD_{50}) was determined using mature laboratory male mice and the Litchfield—Wilcoxon method as modified by Prozorovskii [12]. Compounds were suspended with Tween-80 *ex tempore* and injected once i.p. Animals were observed and deaths were counted for 14 d.

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