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SEARCH FOR NEW DRUGS

SYNTHESIS AND ANTIDEPRESSANT PROPERTIES OF 2-[3-METHYL-7-(THIETANYL-3)-1-ETHYLXANTHINYL-8-THIO]ACETIC ACID HYDRAZIDES

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2-[3-Methyl-7-(thietanyl-3)-1-ethylxanthinyl-8-thio]acetic acid hydrazide was synthesized in two steps by our improved method. The structures of the synthesized compounds were confirmed by IR and NMR spectroscopic data. The acute toxicity was studied. The LD_{50} values were 700 mg/kg for outbred albino mice. Tail-suspension tests (TST) and forced swimming tests (FST) found that 2-[3-methyl-7-(thietanyl-3)-1-ethylxanthinyl-8-thio]acetic acid hydrazide exhibited antidepressant activity that was most pronounced at a dose of 12 mg/kg.

Keywords: xanthines, thietanes, hydrazides, acute toxicity, antidepressant effect, mice.

Xanthine derivatives are used as psychostimulants, bronchodilators, and antiplatelet drugs [1 – 3]. New xanthine derivatives, i.e., xanthinylthioacetic acid hydrazides, were synthesized at the Department of Pharmaceutical Chemistry, BSMU, in order to produce new biologically active compounds [2]. 2-[3-Methyl-7-(thietanyl-3)-1-ethylxanthinyl-8-thio]acetic acid hydrazide (**III**) was the most interesting of them.

The goals of the present work were to synthesize **III** and determine its acute toxicity and antidepressant properties.

Compound **III** was synthesized by our improved method (Scheme 1).

Starting **I** was esterified by EtOH in the presence of $SOCl_2$ in 86% yield to produce ethyl ester **II**. Reaction of **II** with hydrazine hydrate (60%) in EtOH synthesized hydrazide **III** in 85% yield.

EXPERIMENTAL CHEMICAL PART

IR spectra were taken from KBr pellets on an Infralum FT-02 instrument. PMR and ^{13}C NMR spectra were taken from $CDCl_3$ solutions with solvent resonances as internal standards on a Bruker AM-300 instrument at 300 (^1H) and 75 MHz (^{13}C).

The purity of the synthesized compounds was determined by TLC on Sorbfil plates using $CHCl_3$—EtOH (1:3, v/v). Spots were detected by I_2 vapor in a humid chamber. Elemental analyses of the synthesized compounds agreed with those calculated.

[(1-Ethyl-3-methyl-2, 6-dioxo-7-thietan-3-yl-2, 3, 6, 7-tetrahydro-1*H*-purin-8-yl)thio]acetic acid (1) was synthesized by the literature method [3].

Ethyl [(1-ethyl-3-methyl-2, 6-dioxo-7-thietan-3-yl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)thio]acetate (II). Thionylchloride (0.60 g, 5 mmol) and acid **I** (1.78 g, 5 mmol) were added to EtOH (40 mL). The mixture was refluxed for 1 h and cooled. The resulting precipitate was filtered off, washed with H_2O, and dried to afford **II** (1.65 g, 86%), mp 103 – 105°C (EtOH). $C_{15}H_{20}N_4O_4S_2$. IR spectrum (KBr), ν_{max}, cm^{-1}: 1609, 1651, 1698, 1742 (C=C, C=N, C=O). PMR ($CDCl_3$), δ, ppm: 1.19 – 1.31 (m, 6H, 1-CCH_3 and $OCCH_3$); 3.25 – 3.36 (m, 2H, $S(CH)_2$); 3.49 (s, 3H, 3-CH_3); 4.01 – 4.14 (m, 4H, SCH_2 and 1-CH_2); 4.20 (q, 2H, J 7.1 Hz,

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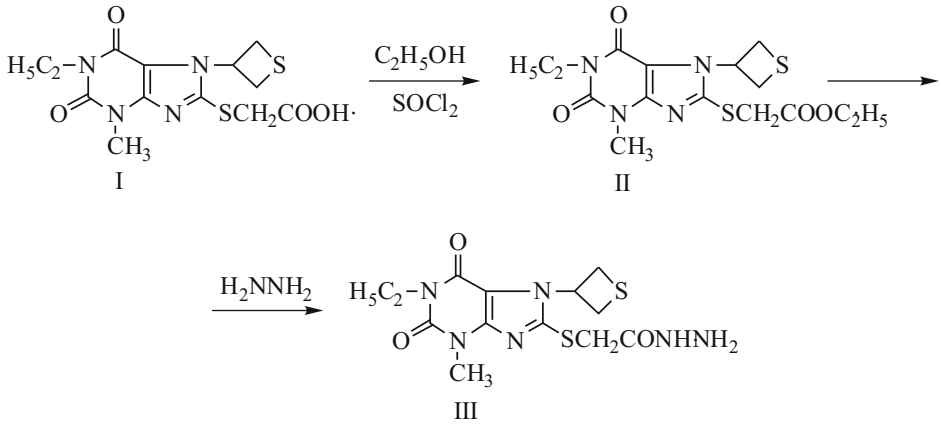
Scheme 1



OCH$_2$); 4.30 – 4.41 (m, 2H, S(CH)$_2$); 5.83 – 5.97 (m, ^{1}H, 7-CH). ^{13}C NMR (CDCl$_3$),δ, ppm: 13.28 (1-CH$_3$); 14.16 (8-CH$_3$); 29.64 (3-CH$_3$); 35.06 [S(CH$_2$)$_2$ and SCH$_2$]; 36.92 (1-CH$_2$); 51.87 (7-CH); 62.17 (OCH$_2$); 108.90 (C^5); 148.82 (C^4); 149.13 (C^2); 150.89 (C^6); 153.92 (C^8); 168.04 (8-CO). The given constants and spectral characteristics agreed with the literature [2].

2-[(1-Ethyl-3-methyl-2, 6-dioxo-7-thietan-3-yl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)thio]acetohydrazide (III). A solution of **II** (1.92 g, 5 mmol) and hydrazine hydrate solution (60%, 1.25 g, 15 mmol) in EtOH (60 mL) was refluxed for 15 min, evaporated in vacuo, and treated with H$_2$O (40 mL). The resulting precipitate was filtered off, rinsed with H$_2$O, and dried to afford **III** (1.57 g, 85%), mp 181 – 183°C (PrOH). $C_{13}H_{18}N_6O_3S_2$. IR spectrum, ν_{max}, cm^{-1}: 1645, 1657, 1665, 1696, 1699 (C=C, C=N, C=O, N–H); 3100 – 3380 (N-H). PMR (CDCl$_3$),δ, ppm: 1.24 (t, 3H, J 6.8 Hz, CH$_3$); 3.24 – 3.35 (m, 2H, S(CH)$_2$); 3.53 (s, 3H, 3-CH$_3$); 3.82 – 3.98 (br.s, 4H, SCH$_2$ and NH$_2$); 4.12 (q, 2H, J 6.8 Hz, 1-CH$_2$); 4.28 – 4.39 (m, 2H, S(CH)$_2$); 5.74 – 5.88 (m, 1H, 7-CH); 8.24 – 8.35 (br.s, 1H, NH). ^{13}C NMR (CDCl$_3$), δ, ppm: 13.16 (CH$_3$); 29.72 (3-CH$_3$); 33.88 (SCH$_2$); 34.82 [S(CH)$_2$]; 36.97 (1-CH$_2$); 51.94 (7-CH); 109.10 (C^5); 148.88 (C^4); 149.01 (C^2); 150.69 (C^6); 153.79 (C^8); 168.43 (8-CO). The given constants and spectral characteristics agreed with the literature [2].

EXPERIMENTAL BIOLOGICAL PART

Tests were carried out on 182 outbred male mice (20 – 23 g) from the SUE Immunopreparat (Ufa). Animals were maintained under standard vivarium conditions with natural lighting and free access to water and a full ration (GOST R 50258-92).

All requirements of the *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* (Strasbourg, 1986) and the Federal Law of the Russian Federation "On the protection of animals from cruel treatment" of Jan. 1, 1997, were strictly observed during the experiments. Tests were conducted from 12:00 to 18:00 in consideration of biological rhythms described previously [4, 5].

Test compound was injected i.p. as a suspension 30 min before the experiment. The control group received an equal volume of isotonic saline. Acute toxicity of **III** was determined from a single i.p. injection at doses from 100 to 1000 mg/kg. Control and test groups were observed continuously for the first day and then once per day for a period of 14 d. The intoxication syndrome considered the overall condition, behavioral reactions, onset time and nature of convulsions, and times of deaths of animals. Then, the fraction of dead animals (%) was found as a function of tested dose. LC$_{50}$ values were calculated by the Litchfield–Wilcoxon method [6].

Two classical tests, tail-suspension test (TST) and forced swimming test (FST) as modified by Shchetinin were used to study antidepressant activity [7 – 9]. Hydrazide **III** was injected at doses of 35, 23, 12, 7.8, 3.7, 1.85, and 0.97 mg/kg (1/20, 1/30, 1/60, 1/90, 1/180, 1/720 of LD$_{50}$, respectively). The positive control was fluoxetine at a dose of 10 mg/kg (Fluoxetine Lannacher, 0.02 capsules, Lannacher Heilmittel).

The antidepressant effect was characterized by assessing the immobilization time (IM TST, IM FST) and depression index (DI FST). Animal behavior was assessed visually using the BrainTest program developed at the Department of Pharmacology No. 1 within a clinical pharmacology course of BSMU [10].

The open-field (OF) test was used to differentiate reliably antidepressant activity from psychostimulant activity [11, 12]. Individual behavior parameters were recorded for 3 min. Conclusions were drawn about the psychostimulant or psychosedative activity using the increase and decrease of locomotor and orienting-exploratory activity.

Results were analyzed statistically using Statistica 6.1 software, Mann–Whitney non-parametric *U*-test, and the Kruskal–Wallis *H*-criterion. Differences were considered significant for $p < 0.05$ [13].

RESULTS AND DISCUSSION

A unique clinical intoxication syndrome was noted after a single i.p. injection of hydrazide **III** to test animals at doses from 100 to 1000 mg/kg. Animals died in the first hour after injection of the studied compound. Initially, the intoxication consisted of increased locomotor activity, grooming activities, vertical tail position, and increased heart and breathing rates, and concluded with death from clonic-tonic convulsions. Signs of poisoning disappeared by the end of the first day in living animals. The LD_{50} value of **III** was 700 mg/kg. Thus, the compound had class 4 toxicity, i.e., was slightly toxic, according to the Sidorov classification of toxicity upon s.c. and i.p. injection.

TABLE 1. Effect of Hydrazide **III** on Immobilization Time and Depression Index (DI) of Mice

Group	Immobilization		DI (FST), Me [25 %; 75 %]
	(TST), Me [25 %; 75 %]	(FST), Me [25 %; 75 %]	
Control *n* = 30	110 [67; 131]	195 [175; 217]	0.97 [0.8; 1.4]
Fluoxetine *n* = 16 (10 mg/kg)	157 [57; 183]	133 [71; 195]	0.63 * [0.34; 0.69]
Hydrazide **III** (35 mg/kg) *n* = 16	106 [56; 119]	168 [127; 202]	0.69 * [0.57; 0.81]
Hydrazide **III** (23 mg/kg) *n* = 16	68 [43; 154]	165 [154; 198]	0.75* [0.5; 1]
Hydrazide **III** (12 mg/kg) *n* = 16	33* [13; 68]	181 [158; 214]	0.41* [0.23; 0.58]
Hydrazide **III** (7.8-mg/kg) *n* = 16	136 [108; 137]	174 [148; 200]	0.55* [0.47; 0.67]
Hydrazide **III** (3.7 mg/kg) *n* = 16	148 [98; 158]	178 [167, 206]	0.58 * [0.47; 0.79]
Hydrazide **III** (1.85 mg/kg) *n* = 16	68 [63; 111]	172 * [160, 191]	0.67 * [0.54; 0.83]
Hydrazide **III** (0.97 mg/kg) *n* = 16	50* [40; 54]	151* [117; 165]	0.56* [0.45; 0.68]

* Differences significant vs. the control ($p < 0.05$ for Mann—Whitney *U*-test).

The TST (Table 1) found that groups of animals receiving a single injection of **III** at doses of 12 and 0.97 mg/kg had immobilization times reduced statistically significantly by 70 and 54% compared with the control.

The FST (Table 1) found that **III** also reduced statistically significantly DI values at all studied doses (35, 23, 12, 7.8, 3.7, 1.85, and 0.97 mg/kg) by 28, 23, 57, 43, 40, 31, and 43%, respectively; and immobilization times at doses of 1.85 and 0.97 mg/kg by 23 and 12%, respectively, compared with the control.

Hydrazide **III** at a dose of 12 mg/kg exhibited the maximum antidepressant activity, reducing DI by 57% compared with the control. However, fluoxetine reduced the DI by 32%. It should also be noted that hydrazide **III**, even at the minimal studied dose (0.97 mg/kg), reduced DI by 43% compared with the control. This could indicate that the molecule is highly active.

The OF test parameters (Table 2) for individual behavior of test animals remained at the control level. The results led to the conclusion that the reduced immobilization time was due not to psychostimulant but antidepressant activity of **III**.

Thus, **III** was synthesized in two steps using our improved method. The LD_{50} of **III** was 700 mg/kg in laboratory

TABLE 2. Effect of Hydrazide **III** on Individual Mouse Behavior Parameters

Group	Locomotor activity	Entries into center	Orienting–exploratory activity
Control *n* = 30	78 [68; 90]	13 [10; 18]	32 [24; 43]
Hydrazide **III** (35 mg/kg) *n* = 16	74 [60; 104]	7.5 [5.5; 12]	29 [20; 34]
Hydrazide **III** (23 mg/kg) *n* = 16	84 [69; 87]	10 [5; 14]	28 [22; 33]
Hydrazide **III** (12 mg/kg) *n* = 16	88 [75; 92]	16 [10; 17]	36 [27; 35]
Hydrazide **III** (7.8 mg/kg) *n* = 16	72 [58; 82]	12 [3; 16]	28 [48; 40]
Hydrazide **III** (3.7 mg/kg) *n* = 16	65 [41; 93]	14 [5; 20]	32 [22; 45]
Hydrazide **III** (1.85 mg/kg) *n* = 16	88 [59; 102]	11 [7; 13]	33 [17; 37]
Hydrazide **III** (0.97 mg/kg) *n* = 16	62 [43; 78]	10 [4; 13]	22 [15; 29]

* Differences significant vs. the control ($p < 005$ for Mann–Whitney *U*-test).

white mice. Therefore, the compound was slightly toxic. Antidepressant activity of **III** that was comparable with the reference drug fluoxetine was found in the TST and FST.

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