DOI 10.1007/s11094-018-1738-0
Pharmaceutical Chemistry Journal, Vol. 51, No. 12, March, 2018 (Russian Original Vol. 51, No. 12, December, 2017)

SEARCH FOR NEW DRUGS

SYNTHESIS AND ANTIDEPRESSANT PROPERTIES OF 3-METHYL-7-(1,1-DIOXOTHIETAN-3-YL)-8-CYCLOHEXYLAMINO-1-ETHYL-1*H*-PURINE-2,6(3*H*,7*H*)-DIONE

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Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 51, No. 12, pp. 3 – 6, December, 2017.

Original article submitted June 28, 2017.

3-Methyl-7-(1,1-dioxothietan-3-yl)-8-cyclohexylamino-1-ethyl-1*H*-purine-2,6(3*H*,7*H*)-dione was synthesized via the reaction of 8-bromo-3-methyl-7-(1,1-dioxothietan-3-yl)-1-ethyl-1*H*-purine-2,6(3*H*,7*H*)-dione and cyclohexylamine and exhibited antidepressant activity that was most pronounced at a dose of 1.6 mg/kg.

Keywords: xanthines, thietanes, synthesis, antidepressant activity.

Most pharmacological effects of purine-type alkaloids are associated with adenosine-receptor antagonism. Caffeine weakens or eliminates the regulatory effect of adenosine, improves functioning of higher brain sections, relieves fatigue and insomnia, and increases mental capacity and functioning of brain stem sections that regulate respiration and arterial pressure [1]. A dioxothietane ring was introduced into the 1*H*-purine-2,6(3*H*,7*H*)-dione 7-position to synthesize new compounds designed to affect the CNS. Several of the derivatives exhibited high antidepressant activity [2]. Cyclohexylamine was chosen as the substituent in the 8-position because 8-cycloalkylamino-1*H*-purine-2,6(3*H*,7*H*)-diones were strong and selective adenosine-receptor antagonists [3].

The goals of the present work were to synthesize and study the antidepressant activity of the new 1*H*-purine-2,6(3*H*,7*H*)-dione derivative 3-methyl-7-(1,1-dioxothietan-3-yl)-8-cyclohexylamino-1-ethyl-1*H*-purine-2,6(3*H*,7*H*)-dione.

The new 1*H*-purine-2,6(3*H*,7*H*)-dione derivative was synthesized by reacting available 8-bromo-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (**I**) with 2-(chloromethyl)thiirane in H_2O in the presence of an equimolar amount of KOH. A thiirane–thietane rearrangement produced 8-bromo-3-methyl-7-(thietan-3-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (**II**). Alkylation of **II** by EtI in DMF in the presence of an equimolar amount of KOH synthesized 8-bromo-3-methyl-7-(thietan-3-yl)-1-ethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (**III**).

8-Bromo-3-methyl-7-(1,1-dioxothietan-3-yl)-1-ethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (**IV**) was prepared via oxidation of **III** by a 10-fold molar excess of H_2O_2 in glacial HOAc. 3-Methyl-7-(1,1-dioxothietan-3-yl)-8-cyclohexylamino-1-ethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (**V**) was synthesized by reacting **IV** with a 3-fold molar excess of cyclohexylamine in DMF.

Nucleophilic substitution of the Br atom was confirmed by resonances for cyclohexylamine protons in the PMR spectrum of **V**. The spectrum contained resonances for the $(CH_2)_5$ protons that overlapped with those for CH_3 protons of the 1-ethyl substituent in the range 1.10 – 2.15 ppm; a multiplet for the NCH proton in the range 3.42 – 3.48 ppm; and a doublet for the NH proton at 5.61 ppm. The spectrum exhibited characteristic resonances for dioxothietane protons as two false triplets in the ranges 4.52 – 4.63 and 4.90 – 5.02 ppm that corresponded to two $S(CH)_2$ groups and a multiplet in the range 5.78 – 5.92 ppm that corresponded to the 7-CH group. The spectrum also contained resonances for the 1-ethyl substituent and a singlet for the 3-methyl of the 1*H*-purine-2,6(3*H*,7*H*)-dione.

The IR spectrum of **V** showed absorption bands for N–H stretching vibrations in the range 3360 – 3400 cm^{-1}, which

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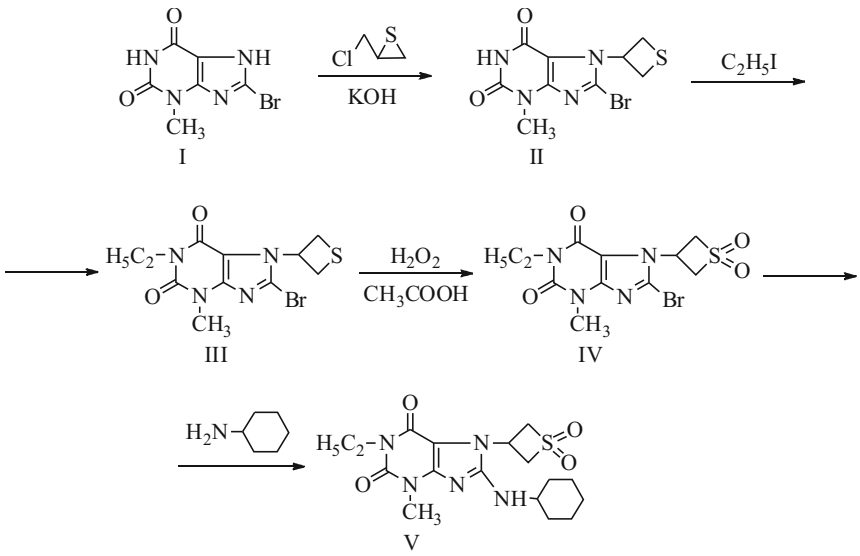

also confirmed that the cyclohexylamine was present. Strong absorption bands for symmetric and asymmetric stretching vibrations of SO_2 bonds at 1139 and 1313 cm^{-1} indicated that the dioxothietane ring was intact.

EXPERIMENTAL CHEMICAL PART

IR spectra of the compounds in KBr pellets were taken on an InfraLUM FT-02 spectrometer. PMR spectra of $CDCl_3$ solutions with solvent resonances as internal standards were recorded on a Bruker AM-300 spectrometer at operating frequency 300 MHz.

Melting points of the synthesized compounds were measured on an SMP-30 apparatus. The purity of the compounds was confirmed by TLC on Sorbfil plates using $CHCl_3$—EtOH (1:3 v/v) or *n*-BuOH—HOAc—H_2O (4:1:2, v/v/v). Spots were detected by I_2 vapor in a humid chamber. Elemental analyses of the synthesized compounds agreed with those calculated.

Compound **II** was prepared by the literature method [4]; **III** and **IV**, as before [5]. 2-(Chloromethyl)thiirane was synthesized by the published method [6].

3-Methyl-7-(1,1-dioxothietan-3-yl)-8-cyclohexylamino-1-ethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (V). A solution of **IV** (1.13 g, 3 mmol) and cyclohexylamine (0.89 g, 9 mmol) in DMF (25 mL) was refluxed for 1 h, cooled, and diluted with H_2O until a precipitate formed. The resulting precipitate was filtered off, rinsed with H_2O, and dried to afford **V** (0.60 g, 50%), mp = 228 – 230°C (EtOH). $C_{17}H_{25}N_5O_4S$. IR spectrum (KBr), ν_{max}, cm^{-1}: 1139, 1313 (SO_2 str), 1616, 1647, 1694 (C=C, C=N, C=O), 3360 – 3400 (N-H str). PMR spectrum ^1H ($CDCl_3$), δ, ppm: 1,10 – 2,15 (m, 13H, CH_3 and $(CH_2)_2$); 3,54 (s, 3H, 3-CH_3); 3,71 – 3,87 (m, 1H, CH); 4,08 (q, 2H, J 6,9 Hz, 1-CH_2); 4,52 – 4,63 (m, 2H, $S(CH)_2$); 4,90 – 5,02 (m, 2H, $S(CH)_2$); 5,61 (d, 1H, J 6,9 Hz, 8-NH); 5,78 – 5,92 (m, 1H, 7-CH).

TABLE 1. Effect of **V** on Immobilization Time and Index of Depression in Mice in the TST and FST Tests with a Single Injection

Group	Immobilization (TST), Me [25%, 75%]	Immobilization (FST), Me [25%, 75%]	ID (FST), Me [25%, 75%]
Control *n* = 23	74 [72, 83]	193 [180, 210]	1.0 [1.0, 1, 2]
Fluoxetine *n* = 14	106 [73, 123]	165* [103, 201]	0.6* [0, 4, 0, 8]
V, 56 mg/kg, *n* = 8	115 [70, 130]	198 [127, 213]	0.7* [0, 5, 0, 8]
V, 37.5 mg/kg, *n* = 8	116 [62, 163]	207 [191, 228]	0.9 [0, 7, 1, 1]
V, 22.5 mg/kg, *n* = 8	72 [30, 94]	204 [184, 215]	0.9 [0, 7, 1, 1]
V, 12.5 mg/kg, *n* = 8	108 [85, 137]	115* [108, 160]	0.7 [0, 7, 0, 9]
V, 6.2 mg/kg, *n* = 8	126* [91, 181]	198 [133, 211]	0.6* [0, 4, 0, 8]
V, 3.1 mg/kg, *n* = 8	105 [67, 126]	179 [100, 195]	0.6* [0, 4, 0, 6]
V, 1.6 mg/kg, *n* = 8	120 [75, 153]	164 [133, 205]	0.4* [0, 4, 0, 5]

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

TABLE 2. Effect of **V** on Behavior Parameters of Individual Mice in the Open Field Test

Group	LA	R	Vs	Ss	C	OEA
Control $n = 9$	78 [70, 86]	15 [14, 19]	0 [0, 0]	7 [5, 14]	9 [7, 10]	28 [22, 37]
V, 56 mg/kg, $n = 8$	67 [46, 5, 74]	17 [8, 5, 20, 5]	0 [0, 1]	10 [6, 5, 13]	7 [6, 5, 10, 5]	28.5 [17, 5, 31]
V, 37.5 mg/kg, $n = 8$	74 [64, 76]	17 [13, 23]	2 [0, 5]	20 [14, 23]	15 [13, 19]	41 [37, 47]
V, 22.5 mg/kg, $n = 8$	73 [63, 5, 76]	14 [8, 5, 18]	0 [0, 0]	7 [5, 5, 12]	10 [7, 11, 5]	25.5 [14, 5, 28, 5]
V, 12.5 mg/kg, $n = 8$	69 [58, 5, 75]	13 [11, 16]	0 [0, 0]	8 [5, 5, 11, 5]	5.5 [4, 7, 5]	22.5 [17, 5, 26, 5]
V, 6.2 mg/kg, $n = 8$	65 [62, 72]	18 [12, 21]	0 [0, 0]	8 [4, 15]	8 [5, 9]	31 [19, 39]
V, 3.1 mg/kg, $n = 8$	59.5 [44, 87]	19 [16, 19]	0 [0, 0]	9 [6, 12]	8 [7, 9]	25 [22, 31]
V, 1.6 mg/kg, $n = 8$	78.5 [68, 85]	17.5 [16, 21]	0 [0, 0]	11 [7, 17]	8 [8, 8]	33 [25, 36]

Note: LA is locomotor activity; R, rearing; Vs, vertical standing; Ss, standing with support; C, entry into the center; OEA, orientating-exploratory activity.

EXPERIMENTAL BIOLOGICAL PART

Tests used 120 laboratory male mice (20 – 23 g) bred at GUP Immunopreparat (Ufa). Animals were kept under standard vivarium conditions with natural lighting and free access to water and full-ration feed according to 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); RF Federal Law dated Jan. 1, 1997, On the Protection of Animals from Cruelty; Ministry of Health and Social Development of the RF Order No. 708n dated August 23, 2010, On Approval of Good Laboratory Practices; and GOST 51000.3-96, General Requirements for Testing Laboratories.

The acute toxicity of synthesized **V** was determined from a single i.p. injection in the dose range 100 – 1,500 mg/kg. Control and test groups were observed continuously for 1 d and then once per day for 14 d. The general condition, behavioral reactions, convulsion appearance time and nature, and lifespan were considered in recording the toxicity pattern. Then, the fraction of dead animals (%) as a function of test compound dose was found. The LD_{50} value was calculated using the Litchfield—Wilcoxon method [7].

The antidepressant properties of **V** were studied in tail-suspension tests (TSTs) and forced swimming tests (FSTs) [8 – 11]. The antidepressant effect was assessed from the immobilization time (IM TST, IM FST) and the index of depression (ID FST). The behavior of the animals was assessed visually using the BrainTest program that was developed at the Department of Pharmacology No. 1 with a BSMU clinical pharmacology course [12].

The effect of **V** on the individual behavior of the mice was determined in the open-field test [13].

Tests were performed considering biorhythms as described before [14].

Antidepressant activity was studied by i.p. injection of **V** as a suspension at doses of 1/20, 1/30, 1/50, 1/90, 1/180, 1/360, and 1/720 of the LD_{50} value (56, 37.5, 22.5, 12.5, 6.2, 3.1, and 1.6 mg/kg, respectively). Each dose was tested in 8 – 23 laboratory animals. The suspension was prepared using Tween-80 stabilizer. Control animals received equal volumes of normal saline (0.9%) with Tween-80. The positive control drug was fluoxetine at a dose of 10 mg/kg.

Statistical analysis used the Statistica 6.1 program and the nonparametric Mann—Whitney *U*-criterion and Kruskal—Wallis *H*-criterion. Differences were considered statistically significant for $p < 0.05$ [15].

The acute toxicity studies established that the LD_{50} of **V** was 1,123 mg/kg, i.e., it had low toxicity according to the Sidorov classification.

The immobilization time of control mice was 74 sec in the TST (Table 1) after a single i.p. injection. The immobilization time increased statistically significantly by 1.7 times compared to the control in the test group of mice with **V** at a dose of 6.2 mg/kg. A tendency of the immobilization time to increase was noted in all other groups except that receiving **V** at a dose of 22.5 mg/kg.

The immobilization time and ID of the control group were 193 sec and 1.0, respectively, in the FST. The reference drug fluoxetine diminished these parameters statistically significantly by 14.5 and 40%. The immobilization time of the test animals remained at the control level after injection of **V**. This parameter decreased statistically significantly to 115 sec only at a dose of 12.5 mg/kg. However, **V** affected the ID over a broad dose range. The ID decreased by 30, 40, 40, and 60% relative to the control in test animals that received a single injection of **V** at doses of 56, 6.2, 3.1 and 1.6 mg/kg, respectively.

The locomotor and orienting-exploratory activities and anxiety-related emotional behavior after injection of **V** did not differ from those of the control group. This indicated that **V** lacked sedative and psychostimulatory activity.

Thus, 3-methyl-7-(1,1-dioxothietan-3-yl)-8-cyclohexylamino-1-ethyl-1*H*-purine-2,6(3*H*,7*H*)-dione exhibited antidepressant activity that was most pronounced at a dose of

1.6 mg/kg of body mass, had low toxicity, and did not affect anxiety-related emotional behavior and locomotor and orienting-exploratory activity of laboratory animals.

REFERENCES

1. M. D. Mashkovskii, *Drugs* [in Russian], Novaya Volna, Umerenkov (2014).
2. O. A. Ivanova, I. L. Nikitina, et al., RU Pat. 2,424,799, Jul. 27, 2011; *Byull. Izobret.*, No. 21 (2011); http: // www1.fips.ru / Archive / PAT / 2011FULL / 2011.07.27 / DOC / RUNWC1 / 000 / 000 / 002 / 424 / 799 / document.pdf
3. T. Katsushima, L. Nieves, and J. Wells, *J. Med. Chem.*, **33**(7), 1906 – 1910 (1990).
4. F. A. Khaliullin, V. A. Kataev, and Yu. V. Strokin, *Khim. Geterotsikl. Soedin.*, **4**, 516 – 518 (1991).
5. F. A. Khaliullin, Yu. V. Shabalina, and R. M. Sharafutdinov, *Zh. Org. Khim.*, **46**(5), 698 – 701 (2010).
6. C. C. J. Culvenor, W. Davies, and K. H. Pausacker, *J. Chem. Soc.*, 1050 – 1052 (1946).
7. M. A. Belen'kii, *Elements of Quantitative Assessment of Pharmacological Effects* [in Russian], Medgiz, Leningrad (1963).
8. V. V. Bagmetova, I. N. Tyurenkov, Yu. V. Chernysheva, and D. D. Borodin, *Neuroscience for Medicine and Psychology. Collection of Scientific Articles of the IXth International Interdisciplinary Congress* [in Russian], Moscow (2013), pp. 70 – 71.
9. E. V. Shchetinin, V. A. Baturin, E. B. Arushanyan, et al., *Zh. Vyssh. Nervn. Deyat. im. I. P. Pavlova*, **5**, 958 – 964 (1989).
10. L. Steru, R. Chermat, B. Thierry, et al., *Psychopharmacology*, Berlin (1985).
11. L. A. Valeeva, G. G. Davlyatova, Yu. V. Shabalina, et al., *Khim.-farm. Zh.*, **50**(6), 8 – 11 (2016); *Pharm. Chem. J.*, **50**(6), 358 – 361 (2016).
12. R. A. Gabidullin, I. L. Nikitina, O. A. Ivanova, and E. K. Alekhin, RU Pat. Appl. No. 2008610170 (2009).
13. R. U. Khabriev, *Handbook for Experimental (Preclinical) Trials of New Drugs* [in Russian], Meditsina, Moscow (2005).
14. L. A. Valeeva and O. Yu. Godorazhi, *Med. Vestn. Bashkortostana*, **4**(2), 186 – 188 (2009).
15. S. Glantz, *Primer of Biostatistics*, McGraw-Hill, New York (1997) [Russian translation, Praktika, Moscow (1998)].