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SYNTHESIS AND EVALUATION OF THE ANTIDEPRESSANT ACTIVITY OF 5-AMINE-SUBSTITUTED 3-BROMO-1-(THIETAN-3-YL)-4-NITRO-1*H*-PYRAZOLES

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5-Amino-substituted 3-bromo-1-(thietan-3-yl)-4-nitro-1*H*-pyrazoles were synthesized by reacting 3,5-dibromo-1-(thietan-3-yl)-4-nitro-1*H*-pyrazole with aliphatic, aromatic, and heterocyclic amines. The structures of the synthesized compounds were confirmed by IR, PMR, and ^{13}C NMR spectroscopy and GC-MS data. According to *in silico* calculations, they were predicted to have no toxic risks (mutagenicity, tumorigenicity, reproductive effects, irritating effects), low toxicity (class IV-V), and acceptable oral bioavailability. The compounds exhibited varying degrees of antidepressant activity after a single intraperitoneal administration to male mice. The most significant effects were typical of 5-piperazinopyrazoles **IIh** and **IIg**, while **IIh** exhibited psychosedative properties and **IIg** had a psychoactivating effect.

Keywords: thietane, pyrazole, amines, antidepressant activity, DataWarrior, Swiss ADME, GUSAR-online.

Depression is one of the leading global problems of public health [1]. However, a significant patient population does not achieve remission and convalescence because existing antidepressants are not effective enough. Therefore, the discovery of more efficacious antidepressants, including those directed at new therapeutic targets, is an important direction in neuropharmacology [2]. New antidepressants are being rapidly developed. For example, the USA Food and Drug Administration (FDA) in 2023 alone approved three new antidepressants: Spravato® (esketamine), the S-enantiomer of ketamine, for treating therapy-resistant depression [3, 4]; Zurzuvae® (zuranolone), a synthetic analog of the neurosteroid allopregnanolone, for treating postpartum depression [5, 6]; and Exxua (gepirone hydrochloride), a 5-HT_{1a}-receptor agonist, for therapy of major depressive disorder [7]. Of these, only gepirone hydrochloride is a first-in-class drug, belonging to the azapirone class that includes buspirone (anxiolytic with antidepressant activity [8]).

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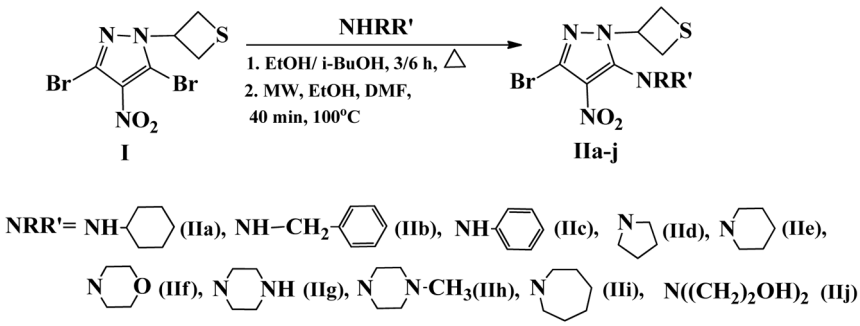
Research conducted by our group on the development of first-in-class thietanylazole antidepressants discovered several promising compounds [9-11], including 1-(1,1-dioxothietan-3-yl)-1*H*-pyrazoles [12]. The pyrazole ring is a preferred structure in medicinal chemistry and is often incorporated into psychotropics (mepiprazole [13], fezolamine [14], zaleplon [15], etc.). In continuation of the search for compounds with antidepressant activity among thietane-containing pyrazoles and studies on the effect of oxidation of the thietane-ring S atom on the antidepressant effect, we synthesized 5-amino-substituted 3-bromo-1-(thietan-3-yl)-4-nitro-1*H*-pyrazoles.

The starting compound was 3,5-dibromo-4-nitro-(thietan-3-yl)-1*H*-pyrazole (**I**) [16], which was reacted with a 3-fold molar excess of primary aliphatic and heterocyclic amines in refluxing EtOH for 3 h. A 5.2-fold molar excess of the amine was used for piperazine. The reaction with aniline was conducted in *i*-BuOH for 6 h. The synthesis with diethanolamine required microwave activation. The 5-amino-substituted 3-bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazoles (**IIa-j**) were obtained in 66-95% yields (Scheme 1).

Scheme 1



Synthesis of 5-amino-substituted 3-bromo-1-(thietan-3-yl)-4-nitro-1*H*-pyrazoles **IIa**-**j**.

EXPERIMENTAL CHEMICAL PART

IR spectra were recorded in KBr pellets on an InfraLUM FT-02 FTIR spectrometer (Russia). PMR and ^{13}C NMR spectra were recorded at 298 K on a Bruker Avance III pulsed spectrometer (USA) at operating frequency 500.13 MHz (^{1}H) and 125.47 MHz (^{13}C) using a 5-mm Z-gradient PABBO probe. Chemical shifts in PMR and ^{13}C NMR spectra were given vs. residual solvent ($CDCl_3$) resonances (7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C). ^{13}C NMR spectra were edited using DEPT-90 and DEPT-135 experiments. Resonances were assigned and structures of **IIa**,**f** were elucidated using homo- and heteronuclear $^{1}H(^{1}H$ NOESY, $^{1}H(^{13}C$ HMBC, and $^{1}H(^{15}N$ HMBC two-dimensional correlation spectra recorded using standard modes of multi-pulse sequences in the instrument software. Chemical shifts in ^{15}N NMR spectra were given vs. liquid NH_3 external standard.

Elemental analyses for C. H, N, and S used a Hekatech Euro3000 analyzer (Germany) and agreed with those calculated. The course of reactions and purity of obtained compounds were monitored by TLC on Sorbfil PTSKh-P-A-UF plates using $CHCl_3$(MeOH(NH_4OH (9:1:0.1 v/v). Spots were detected in UV light and a chamber with I_2 vapor.

GC-MS of the compounds were recorded on a GC with an Agilent MDS 5977B mass-selective detector (USA). The carrier gas was He (1.0 mL/min). Electron-impact ionization was used (70 eV). The range of scanned masses was 40-500 *m*/*z*. Experimental results were recorded and processed using Qualitative Analysis 10.0 software.

Microwave syntheses used a CEM Discover SP monomodal microwave system (USA) at operating frequency 2.45 GHz. Reactions were performed in a 35-mL reaction vessel with a special cover. The reaction temperature was controlled by an embedded IR sensor on the outer surface of the reaction vessel.

Commercially available reagents were used in the work.

General method for synthesizing 5-amino-substituted 3-bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazoles (IIa-i). A solution of pyrazole **I** (0.86 g, 2.5 mmol) in EtOH (35 mL) was treated with an amine (7.5 mmol; 13 mmol for **IIg**), refluxed for 3 h (6 h in *i*-BuOH for **IIc**), and cooled. Water (30 mL) was added for **IIe**,**h**,**i**. The resulting precipitate was filtered off, rinsed with H_2O, and dried. The reaction mixture was evaporated at reduced pressure to dryness for **IIa**,**g** and cooled. The oily residue was treated with H_2O (20 mL, **IIa**) or *i*-PrOH (35 mL, **IIg**). The resulting precipitate was filtered off, rinsed with H_2O, and dried.

3-Bromo-4-nitro-1-(thietan-3-yl)-*N*-cyclohexyl-1*H*-pyrazol-5-amine (IIa). Yield 0.76 g (89%), yellowish crystals, mp 94 – 95°C (EtOH). R_f 0.88. IR spectrum (ν_{max}, cm^{-1}): 1239, 1479, 1591 (C-N, C=N, C=C), 1329, 1527 (NO_2), 2828 – 2977 (CH), 3325 (NH). PMR spectrum (δ, ppm): 1.28 – 1.42 (m, 4H, $2CH_2$), 1.62 – 1.65 (m, 2H, CH_2), 1.77 – 1.81 (m, 2H, CH_2), 1.96 – 1.99 (m, 2H, CH_2), 3.23 – 3.27 (m, 2H, $S(CH)_2$), 3.36 – 3.38 (m, 1H, CH), 4.15 – 4.19 (m, 2H, $S(CH)_2$), 5.41 – 5.48 (m, 1H, NCH), 6.59 (d, 1H, J 8.8 Hz, NH). NMR spectrum ^{13}C (δ, ppm): 24.4 (CH_2), 25.1 ($2CH_2$), 33.8 ($S(CH_2)_2$), 34.01 ($2CH_2$), 55.0 (NCH), 56.6 (CH), 123.3 (C^3), 146.3 (C^5). NMR spectrum ^{15}N (δ, ppm): 197.9 (N^1), 278.8 (N^2). Mass spectrum, *m*/*z* (I_{rel}, %): 360.05 [M] (6.1), 330.00 (13.4), 289.98 (22.5), 281.03 (12.2), 206.99 (13.8), 73.00 (100), 55.05 (25.9), 41.04 (15.7). $C_{12}H_{17}BrN_4O_2S$. Calc.: *M* = 360.03.

***N*-Benzyl-3-bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazol-5-amine (IIb).** Yield 0.70 g (81%), white crystals, mp 136 – 137°C (EtOH). R_f 0.84. IR spectrum (ν_{max}, cm^{-1}): 1236, 1480, 1603 (C-N, C=N, C=C), 1330, 1533 (NO_2), 2877 – 3028 (CH), 3331 (NH). PMR spectrum (δ, ppm): 2.99 – 3.02 (m, 2H, $S(CH)_2$), 4.06 – 4.10 (m, 2H, $S(CH)_2$), 4.64 (d, 2H, J 6.4 Hz, $NHCH_2$), 5.46 – 5.52 (m, 1H, NCH), 7.30 – 7.45 (m, 5H, H_{ar}). NMR spectrum ^{13}C (δ, ppm): 34.0 ($S(CH_2)_2$), 50.1 ($NHCH_2$), 55.2 (NCH), 123.5 (C_{pyr}), 126.6 (CH_{ar}), 128.7 (CH_{ar}), 129.5 (CH_{ar}), 136.2 (C_{ar}), 146.3 (C_{pyr}). Mass spectrum, *m*/*z* (I_{rel}, %): 367.90 [M] (0.8), 335.96 (23.8), 295.95 (25.9), 280.97 (10.2), 207.01 (15.6), 105.02 (41.0), 91.04 (100.0), 73.00 (73.5), 43.98 (17.4). $C_{13}H_{13}BrN_4O_2S$. Calc.: *M* = 367.99.

3-Bromo-4-nitro-*N*-phenyl-1-(thietan-3-yl)-1*H*-pyrazol-5-amine (IIc). Yield 0.71 g (83%), yellowish crystals,

mp 163 – 164°C (*i*-BuOH). R_f 0.74. IR spectrum (ν_{max}, cm^{-1}): 1247, 1474, 1496 (C-N, C=N, C=C), 1330, 1569 (NO_2), 3286 (NH). PMR spectrum (δ, ppm): 2.95 – 2.99 (m, 2H, $S(CH)_2$), 3.98 – 4.02 (m, 2H, $S(CH)_2$), 5.03 – 5.10 (m, 1H, NCH), 7.01 (d, 2H, J 7.93 Hz, H_{ar}), 7.27 – 7.29 (m, 1H, H_{ar}), 7.41 – 7.43 (m, 2H, H_{ar}), 8.06 (br.s, 1H, NH). NMR spectrum ^{13}C (δ, ppm): 33.1 ($S(CH_2)_2$), 54.6 (NCH), 121.5 (CH_{ar}), 123.2 (C_{pyr}), 126.3 (CH_{ar}), 130.2 (CH_{ar}), 139.3 (C_{ar}), 139.2 (CH_{ar}), 142.6 (C_{pyr}). Mass spectrum, *m*/*z* (I_{rel}, %): 353.93 [M] (26.0), 323.96 (24.7), 281.94 (81.1), 273.94 (16.7), 247.93 (8.06), 206.99 (31.3), 195.04 (14.0), 156.01 (8.2), 144.00 (8.03), 77.02 (39.2), 73.00 (100.0), 51 (8.5), 43.98 (32.0). $C_{12}H_{17}BrN_4O_2S$. Calc.: *M* = 353.98.

3-Bromo-4-nitro-5-(pyrrolidin-1-yl)-1-(thietan-3-yl)-1*H*-pyrazole (IId). Yield 0.76 g (91%), yellow crystals, mp 114 – 115°C (*i*-PrOH). R_f 0.86. IR spectrum (ν_{max}, cm^{-1}): 1258, 1436, 1494 (C-N, C=N, C=C), 1337, 1549 (NO_2), 2856 – 2973 (CH). PMR spectrum (δ, ppm): 2.05 – 2.10 (m, 4H, $2CH_2$), 3.22 – 3.26 (m, 6H, $N(CH_2)_2$, $S(CH)_2$), 4.07 – 4.11 (m, 2H, $S(CH)_2$), 5.72 – 5.79 (m, 1H, NCH). NMR spectrum ^{13}C (δ, ppm): 26.3 ($2CH_2$), 33.7 ($S(CH_2)_2$), 52.0 ($N(CH_2)_2$), 52.7 (NCH), 123.5 (C_{pyr}), 146.1 (C_{pyr}). Mass spectrum, *m*/*z* (I_{rel}, %): 331.90 [M] (1.3), 316.96 (61.5), 242.96 (14.2), 219.05 (17.6), 73 (100.0), 44.99 (20.0). $C_{10}H_{13}BrN_4O_2S$. Calc.: *M* = 331.99.

4-[3-Bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazol-5-yl]-piperidine (IIe). Yield 0.65 g (75%), yellowish crystals, mp 133 – 134°C (*i*-PrOH). R_f 0.88. IR spectrum (ν_{max}, cm^{-1}): 1251, 1428, 1497 (C-N, C=N, C=C), 1335, 1538 (NO_2), 2853 – 2939 (CH). PMR spectrum (δ, ppm): 1.67 – 1.75 (m, 6H, $(CH_2)_3$), 3.01 – 3.11 (m, 4H, $N(CH_2)_2$), 3.22 – 3.26 (m, 2H, $S(CH)_2$), 4.07 – 4.10 (m, 2H, $S(CH)_2$), 5.73 – 5.80 (m, 1H, NCH). NMR spectrum ^{13}C (δ, ppm): 23.5 (CH_2), 26.1 ($2CH_2$), 33.6 ($S(CH_2)_2$), 50.8 ($N(CH_2)_2$), 52.5 (NCH), 123.2 (C_{pyr}), 147.7 (C_{pyr}). Mass spectrum, *m*/*z* (I_{rel}, %): 346.00 [M] (1.3), 330.96 (48.9), 313.96 (17.9), 256.98 (12.8), 233.06 (15.9), 73.01 (100.0), 45.00 (18.0). $C_{11}H_{15}BrN_4O_2S$. Calc.: *M* = 346.00.

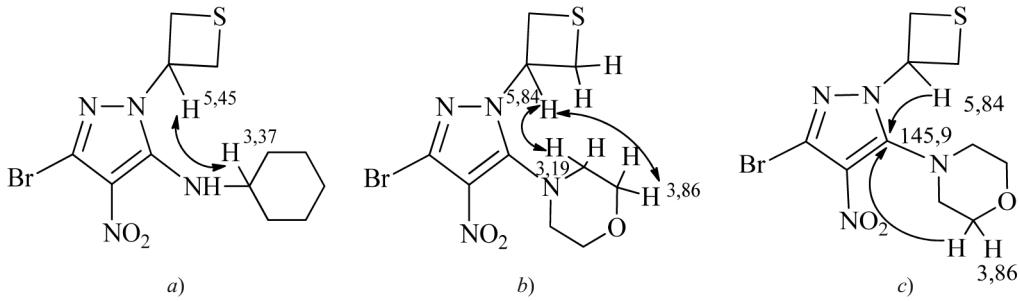
4-[3-Bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazol-5-yl]-morpholine (IIf). Yield 0.74 g (85%), yellowish crystals, mp 156 – 157°C (EtOH). R_f 0.87. IR spectrum (ν_{max}, cm^{-1}):1258, 1427, 1495 (C-N, C=N, C=C), 1345, 1540 (NO_2), 2858 – 2967 (CH). PMR spectrum (δ, ppm): 3.07 – 3.14 (m, 4H, $N(CH_2)_2$), 3.21 – 3.25 (m, 2H, $S(CH)_2$), 3.85 – 3.87 (m, 4H, $O(CH_2)_2$), 4.07 – 4.10 (m, 2H, $S(CH)_2$), 5.81 – 5.88 (m, 1H, NCH). NMR spectrum ^{13}C (δ, ppm): 33.6 ($S(CH_2)_2$), 49.5 ($N(CH_2)$, 52.5 (NCH), 67.0 ($O(CH_2)_2$), 123.5 (C^3), 127.4 (C^4), 145.9 (C^5). NMR spectrum ^{15}N (δ, ppm): 38.14 ($N^{4\prime}$), 219.28 (N^1), 286.29 (N^2). Mass spectrum, *m*/*z* (I_{rel}, %): 347.96 [M] (3.13), 332.96 (18.7), 286.96 (9.5), 252.06 (11.7), 73.01 (100.0), 44.99 (17.7). $C_{10}H_{13}BrN_4O_3S$. Calc.: *M* = 347.99.

1-[3-Bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazol-5-yl]-piperazine (IIg). Yield 0.83 g (95%), yellowish crystals, mp 116 – 117°C (*i*-PrOH). R_f 0.38. IR spectrum (ν_{max}, cm^{-1}): 1254, 1430, 1493 (C-N, C=N, C=C), 1331, 1534 (NO_2), 2853 – 2940 (CH), 3314 (NH). PMR spectrum (δ, ppm): 2.89 – 3.13 (m, 8H, $2N(CH_2)_2$), 3.21 – 3.26 (m, 2H, $S(CH)_2$), 4.06 – 4.12 (m, 2H, $S(CH)_2$), 5.80 – 5.85 (m, 1H, NCH), 7.25 – 7.28 (m, 1H, NH). NMR spectrum ^{13}C (δ, ppm): 33.5 ($S(CH_2)_2$), 46.2 ($NH(CH_2)_2$), 50.7 ($N(CH_2)_2$), 52.5 (NCH), 123.3 (C_{pyr}), 146.2 (C_{pyr}). Mass spectrum, *m*/*z* (I_{rel}, %): 346.80 [M] (1.5), 316.96 (51.4), 307.02 (12.4), 281.08 (17.6), 276.92 (18.6), 243.91 (19.0), 208.00 (11.6), 72.99 (100.0), 56.00 (30.4), 43.98 (52.3). $C_{10}H_{14}BrN_5O_2S$. Calc.: *M* = 347.01.

1-[3-Bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazol-5-yl]-4-methylpiperazine (IIh). Yield 0.72 g (80%), yellowish crystals, mp 118 – 119°C (*i*-PrOH). R_f 0.66. IR spectrum (ν_{max}, cm^{-1}):1254, 1434, 1498 (C-N, C=N, C=C), 1345, 1538 (NO_2), 2796 – 2938 (CH). PMR spectrum (δ, ppm): 2.38 (s, 3H, CH_3), 2.48 – 2.68 (m, 4H, $N(CH_2)_2$), 2.97 – 3.19 (m, 4H, $N(CH_2)_2$), 3.22 – 3.25 (m, 2H, $S(CH)_2$), 4.06 – 4.10 (m, 2H, $S(CH)_2$), 5.80 – 5.88 (m, 1H, NCH). NMR spectrum ^{13}C (δ, ppm): 33.5 ($S(CH_2)_2$), 46.3 (CH_3), 49.5 ($N(CH_2)_2$), 52.5 (NCH), 55.1 ($N(CH_2)_2$), 123.3 (C_{pyr}), 146.5 (C_{pyr}). Mass spectrum, *m*/*z* (I_{rel}, %): 361.02 [M] (5.1), 343.99 (14.6), 331.03 (22.9), 315.96 (20.4), 257.96 (10.6), 86.05 (40.6), 73.01 (100.0), 70.07 (48.4), 57.06 (17.1), 43.04 (42.3). $C_{11}H_{16}BrN_5O_2S$. Calc.: *M* = 361.02.

1-[3-Bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazol-5-yl]-hexamethyleneimine (IIi). Yield 0.77 g (82%), yellowish crystals, mp 106 – 108°C (*i*-PrOH). R_f 0.88. IR spectrum (ν_{max}, cm^{-1}):1259, 1432, 1494 (C-N, C=N, C=C), 1343, 1541 (NO_2), 2847 – 2942 (CH). PMR spectrum (δ, ppm): 1.75 – 1.80 (m, 8H, $(CH_2)_4$), 3.14 – 3.18 (m, 4H, $N(CH_2)_2$), 3.22 – 3.26 (m, 2H, $S(CH)_2$), 4.08 – 4.11 (m, 2H, $S(CH)_2$), 5.80 – 5.88 (m, 1H, NCH). NMR spectrum ^{13}C (δ, ppm): 27.6 ($2CH_2$), 29.9 ($2CH_2$), 33.6 ($S(CH_2)_2$), 52.1 (NCH), 53.8 ($N(CH_2)_2$), 123.3 (C_{pyr}), 149.0 (C_{pyr}). Mass spectrum, *m*/*z* (I_{rel}, %): 360.00 [M] (0.2), 345.00 (38.5), 328.00 (16.9), 247.08 (22.8), 96.07 (15.0), 73.01 (100.0), 55.05 (11.9), 44.99 (17.2). $C_{12}H_{17}BrN_4O_2S$. Calc.: *M* = 360.03.

2-{[3-Bromo-4-nitro-1-(thietan-3-yl)-1*H*-pyrazol-5-yl]-(2-hydroxyethyl)amino}ethanol (IIj). Thietanylpyrazole **I** (0.86 g, 2.5 mmol) in DMF (5 mL) and EtOH (5 mL) in a reaction tube was treated with diethanolamine (1.96 g, 18.6 mmol). The tube was placed into an SEM Discover microwave oven for 40 min at 100°C (microwave radiation power 100 W, dynamic regime). The reaction mixture was cooled and treated with H_2O (30 mL). The resulting precipitate was filtered off, rinsed with H_2O, and dried. Yield 0.56 g (66%), yellowish crystals, mp 148 – 149°C (EtOH(H_2O, 1:1 v/v). R_f 0.21. IR spectrum (ν_{max}, cm^{-1}): 1171 (C-O), 1243, 1442, 1490 (C-N, C=N, C=C), 1336, 1547 (NO_2), 2901 – 3008 (CH), 3080 – 3416 (NH, OH). PMR spectrum (δ, ppm): 3.13 – 3.15 (m, 4H, $N(CH_2)_2$), 3.27 – 3.31 (m, 2H, $S(CH)_2$), 3.39 – 3.41 (m, 4H, 2ÎCH$_2$), 3.80 – 3.84 (m, 2H,



a) *b*) *c*)

Fig. 1. Main correlations in NOESY $^{1}H–^{1}H$ spectra of **IIa, f** (*a*, *b*) and $^{1}H–^{13}C$ HMBC spectrum of **IIf** (*c*).

$S(CH)_2$), 4.58 (t, 2H, J 4.9 Hz, 2OH), 6.12 – 6.19 (m, 1H, NCH). NMR spectrum ^{13}C (δ, ppm): 33.6 ($S(CH_2)_2$), 52.1 (NCH), 56.3 ($N(CH_2)_2$), 60.0 ($O(CH_2)_2$), 123.2 (C_{pyr}), 126.8 (C_{pyr}), 148.1 (C_{pyr}). Mass spectrum, *m/z* (I_{rel}, %): 365.99 [M] (0.1), 355 (8.1), 280.99 (41.1), 207 (100.0), 190.94 (11.3), 95.99 (5.69), 72.98 (23.8), 43.97 (71.8). $C_{10}H_{15}BrN_4O_4S$. Calc.: *M* = 366.00.

EXPERIMENTAL BIOLOGICAL PART

Computer prediction methods. Toxic risks: mutagenicity, oncogenicity, irritating action, and effect on reproductive function; physicochemical properties; and agreement with Lipinsky [18] and Weber rules [19] (molecular mass, lipophilicity coefficient, number of H-bond donors, number of H-bond acceptors, number of rotating bonds, topological polar surface area) were determined using DataWarrior V5.5.0 [20] and SwissADME web resources [21]. Acute toxicity was predicted using GUSAR Online web resource [22].

Antidepressant activity. The antidepressant activity of synthesized **IIa-j** was evaluated using outbred male white mice in the basic behavioral tail-suspension test (TST) [23], modified forced-swimming test (FST) [24], and open-field test (OF) [25]. Experimental animals (20 – 24 g) were housed under standard vivarium conditions on a balanced ration (GOST R 50258(92) with access *ad libitum* to water and feed with a 12-h day/night regime. All studies were conducted in compliance with requirements of the *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* (ETS No. 123, 1986) [26] and Good Laboratory Practice Rules of the Eurasian Economic Union on Drug Circulation (Decision No. 81 of the EEC Council of Nov. 3, 2016 "On approval of Good Laboratory Practice Rules of the Eurasian Economic Union on Drug Circulation") [27].

Four experimental series were conducted. Animals in each series of experiments were distributed arbitrarily into groups of six animals each: control (1), amitriptyline (10 mg/kg) (2), and tested compounds (3-n). The compounds were suspended *ex tempore* in normal saline with Tween-80

TABLE 1. Calculation of Pharmacological Properties of Synthesized Compounds **IIa-j**

Compound	**IIa**	**IIb**	**IIc**	**IId**	**IIe**	**IIf**	**IIg**	**IIh**	**IIi**	**IIj**
MM (g/mol)	361.26	369.24	355.21	333.20	347.22	349.21	348.22	362.25	361.26	367.22
MlogP	1.58	1.44	2.86	2.31	1.21	0.05	–0.10	0.16	2.85	–0.09
TPSA, Å^2	100.97	100.97	100.97	92.18	92.18	101.41	104.21	95.42	92.18	132.64
HBA	3	3	3	3	3	4	4	4	3	5
HBD	1	1	1	0	0	0	1	0	0	2
RB	4	5	4	3	3	3	3	3	3	7
Lipinsky rule	+	+	+	+	+	+	+	+	+	+
Weber rule	+	+	+	+	+	+	+	+	+	+
DL	–10.81	–5.22	–5.62	–4.36	–5.84	–5.03	–3.50	0.02	–8.35	–4.11
Toxic risks*	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)
LD_{50} (mg/kg)/Sidorov toxicity class	696.2*/4	465.7/4	465.7/4	518.7*/4	475.3*/4	569.6*/4	249.6*/4	143.5*/4	465.7*/4	1018.0*/5

Note: MM, molecular mass; MlogP, lipophilicity coefficient; TPSA, topological polar surface area; HBA, number of H-bond acceptors; HBD, number of H-bond donors; DL, drug-likeness parameter; RB, number of rotating bonds; toxic risks: mutagenicity, oncogenicity, local-irritating effect, influence on reproductive function. * Compound located outside applicability region of models.

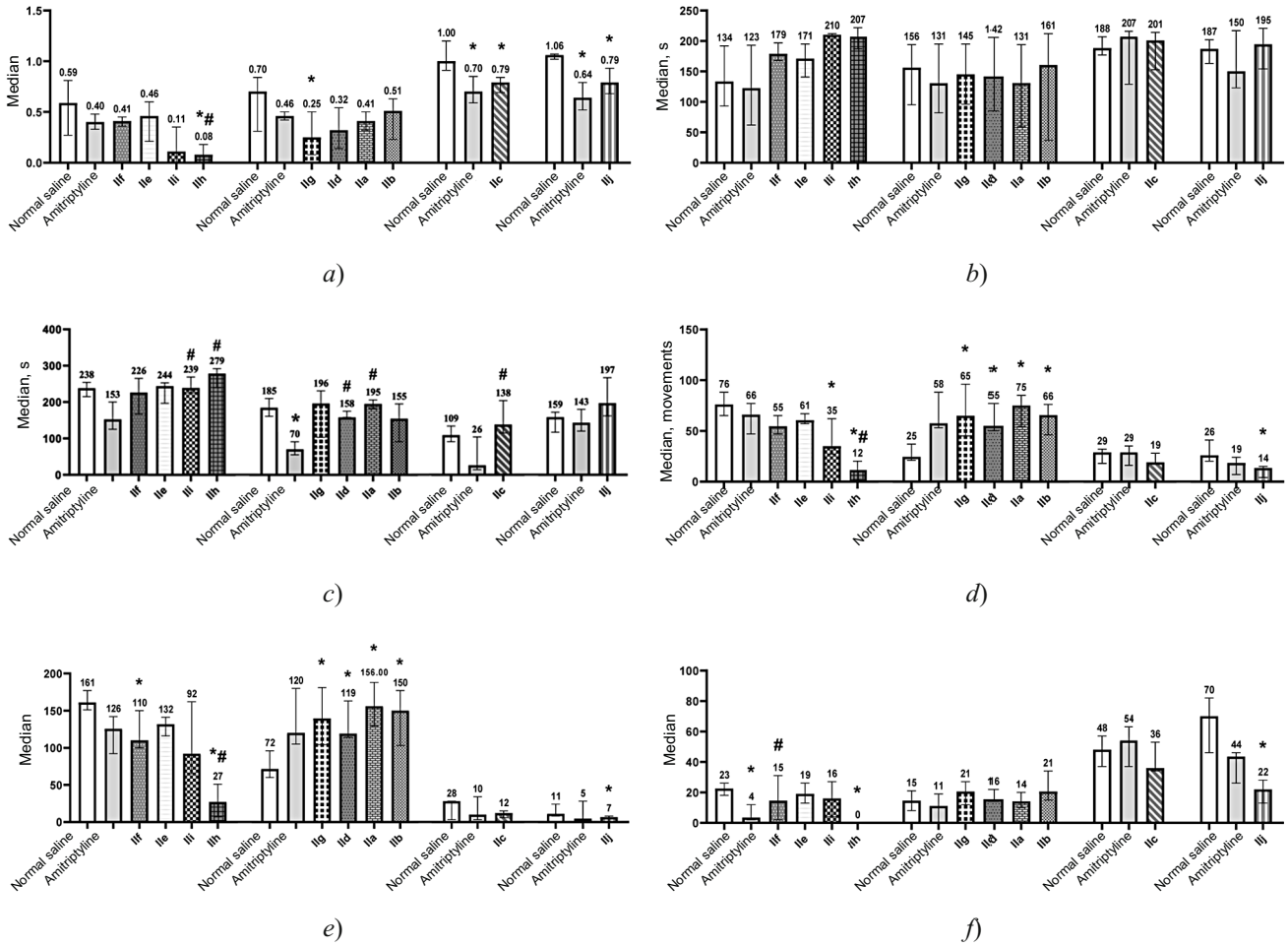


Fig. 2. Influence of **IIa-j** on main screening parameters of forced-swimming, tail-suspension, and open-field tests after a single administration to male mice: FST ID (*a*), FST DIm (*b*), TST DIm (*c*), number of movements (*d*), OF orientational-exploratory activity (*e*), OF emotional anxiety (*f*). The plots show medians and interquartile intervals; $^{*}p < 0.05$ for Mann(Whitney *U*-criterion vs. the control group; $^{\#}p < 0.05$ for Mann(Whitney *U*-criterion vs. amitriptyline.

(1 – 2 drops, Polysorbate Lauropan T/80, Italy) and administered at doses equimolar to amitriptyline (10 mg/kg) once intraperitoneally 30 min before testing (**IIa**, 13.3; **IIb**, 13.3; **IIc**, 12.8; **IId**, 12.0; **IIe**, 12.5; **IIf**, 12.6; **IIg**, 12.6; **IIh**, 13.1; **IIi**, 13.0; and **IIj**, 13.2 mg/kg). The reference drug amitriptyline (10 mg/kg, Amitriptyline, solution for intravenous and intramuscular injection 10 mg/mL; Moscow Endocrine Plant, RF) was administered analogously. Control mice received once an equivalent volume of normal saline with Tween-80. The behavior of the animals in the tests was recorded by a video camera and analyzed using the Brain Test program [28]. The total duration of immobilization (DIm) was measured in the TST; the duration of active and passive swimming (DIm), in the FST. The index of depression (ID, ratio of the number of short periods of immobilization of duration sec to the number of periods of active swimming) was also calculated. In the OF test, the number of behavioral patterns (grooming, standing, standing with support, minking, sniffing, moving, moving in place, sitting) was recorded. The parameters emotional anxiety (EA, sum of patterns sniffing, moving, and minking) and orientational-exploratory activity (OEA, sum of patterns moving in place, standing, and standing with support) were calculated.

Results were statistically analyzed using the Statistica 13.3 program (TIBCO Software Inc., USA). The normalcy of distributions (Shapiro(Wilk criterion) was evaluated. The basic parameters of descriptive statistics (median, interquartile interval, minimum-maximum, mean-square deviation, outliers) were calculated. Dispersions of sets were compared using the Kruskal(Wallis criterion (H-criterion). The Mann(Whitney criterion was used for post hoc analysis (paired comparison of groups). The statistical significance level was taken as 0.05 [29 – 31].

RESULTS AND DISCUSSION

The compositions and structures of synthesized **IIa-j** were confirmed using IR, PMR, and ^{13}C NMR spectroscopy and GC-MS.

PMR spectra of **IIa-j** contained resonances for the thietane ring in the characteristic regions [17] and for protons of substituents in the 5-position. For example, spectra of **IIa, c** showed the NH proton resonance at 6.6 – 8.1 ppm. Protons of the phenyl substituent in **IIc** appeared at weak field as a doublet at 7.01 ppm and two multiplets in the ranges 7.27 – 7.29 and 7.41 – 7.43 ppm. PMR spectra of **IIa** contained resonances for the cyclohexane-ring protons as five multiplets in the characteristic regions. ^{13}C NMR spectra of **IIa-j** showed resonances for the pyrazole C^5 C atom in the range 142.6 – 149.0 ppm that were shifted to weak field by ~25 ppm as compared to the analogous resonance of starting **I**. This confirmed that the pyrazole C^5 Br atom was replaced.

Two-dimensional HMBC and NOESY spectra of **IIa, f** were taken to establish unambiguously their structures. 2D NOESY ^{1}H(^{1}H spectra of **IIa, f** showed correlations between the thietane-ring NCH protons with the cyclohexylamine CH protons (Fig. 1*a*) and morpholine $N(CH_2)_2$- and $O(CH_2)_2$-groups (Fig. 1*b*), respectively. The 2D HMBC ^{1}H(^{13}C spectrum of **IIf** had correlations of the resonances for the morpholine $O(CH_2)_2$-protons and the thietane-ring NCH group with the resonance of the pyrazole-ring C^5 C atom (Fig. 1*c*). These results indicated that the pyrazole C^5 position of **I** reacted to form the 5-aminothietanylpyrazoles.

The acute toxicity after intraperitoneal injection to rats was calculated using the GUSAR Online web resource and showed that **IIa-i** had class 4 toxicity (marginally toxic substances); **IIj**, class 5 (practically nontoxic) according to the Sidorov toxicity classification of substances [32] (Table 1).

The pharmaceutical potential of the new compounds was assessed by analyzing the toxic risks using the DataWarrior program and the Lipinsky rule-of-five and the Weber rule using the SwissADME web resource (Table 1). The toxic risks predicted by the calculations demonstrated a lack of potential adverse effects for **IIa-j** on reproductive functions, mutagenic and oncogenic properties, and local-irritating action. The physicochemical parameters calculated for **IIa-j** satisfied the Lipinsky rule-of-five and the Weber rule. The molecular mass (MM) of the synthesized compounds was <369 g/mol. The lipophilicity coefficient (MlogP) spanned the interval from (0.10 to +2.86. The number of H-bond acceptors (HBA) was ≤5; of H-bond donors (HBD), ≤2. The number of rotating bonds (RB) was ≤7. The topological polar surface area (TPSA) was in the range 92.18 – 132.64 Å^2. The drug-likeness parameter (DL) ranged from (10.81 to 0.02, confirming the novelty of the structures of the synthesized compounds (Table 1).

Series 1. Screening for antidepressant activity found that **IIe, f**,**h, i** reduced the FST ID by 13 – 78% and did not change the DIm in the TST and FST. The greatest statistically significant effect was observed in the group of mice that received **IIh**, the FST ID of which decreased by 88% ($p = 0.041$) vs. the control group and by 54% vs. the amitriptyline group ($p = 0.008$) (Fig. 2*a*). Compound **IIh** in the OF test significantly decreased the number of movements by 85% ($p = 0.002$), reduced the OEA by 83% ($p = 0.002$), and eliminated EA ($p = 0.002$) of the mice (Fig. 2, d-f). This indicated that the molecule had a strong sedative effect. Compound **IIi** exhibited similar activity, decreasing the ID in the FST by 81% vs. the control ($p = 0.132$) and by 48% vs. amitriptyline ($p = 0.064$) and statistically significantly lowering the number of movements by 54% ($p = 0.04$). It caused a distinct tendency to reduce the OEA and EA ($p > 0.05$).

Compound **IIf** significantly decreased the OEA by 32% ($p = 0.041$) (Fig. 2*d*, 2e). The reference drug amitriptyline (10 mg/kg) reduced the ID by 33% ($p = 0.484$) vs. the control group and did not affect the DIm in the FST and TST (Fig. 2, a-c).

Series 2. Amitriptyline (10 mg/kg) reduced the ID by 35% ($p = 0.485$) as compared to the control group and did not affect the FST DIm (Fig. 2*a*, 2b). Amitriptyline significantly reduced the TST DIm by 62% ($p = 0.005$) vs. the control (Fig. 2*c*). Compound **IIg** significantly reduced ID by 64% ($p = 0.041$) and did not change DIm in the FST and TST (Fig. 2, a-c). The whole series of 5-amino-substituted thietanylpyrazole derivatives in the OF test significantly changed the number of movements by 225 – 306% ($p < 0.05$) and OEA by 166 – 218% ($p < 0.05$) vs. the control (Fig. 2*d*, 2e).

Series 3. Compound **IIc**, like amitriptyline, significantly reduced the ID by 21% ($p = 0.006$), less than the amitriptyline ID of 30% ($p = 0.004$), did not change the DIm in the FST and TST, and did not affect the OF patterns (Fig. 2, a-f).

Series 4. Compound **IIj** significantly reduced the ID by 25% ($p = 0.004$), less than the amitriptyline ID of 39% ($p = 0.004$), and, like amitriptyline, did not change the DIm in the FST and TST (Fig. 2, a-c). Compound **IIj** in the OF test significantly decreased the number of movements by 48% ($p = 1.000$) and reduced the OEA and EA by 69% ($p = 0.429$) and 41% ($p = 0.667$), respectively (Fig. 2, d-f).

The results presented above and those evaluating 5-amino-substituted 3-bromo-4-nitro-1-(1,1-dioxothietan-3-yl)-1*H*-pyrazoles that were published earlier [12] were compared to evaluate the possible influence of the degree of oxidation of the thietane-ring S atom on the antidepressant activity. The comparison showed that oxidation of the thietane ring of the thietane-1,1-dioxide reduced the antidepressant activity for 5-piperazino-, 5-pyrrolidino-, and 5-hexamethyleneiminopyrazoles.

It is noteworthy that the structure of the 5-amine was important for manifestation of psychosedative/psychoactivating activity in addition to the antidepressant activity. For example, addition of a methyl substituent (**IIh**) to a piperazine ring (**IIg**) inverted the OEA effect by sharply decreasing it (**IIh**) (Fig. 2*e*).

The series of **IIa-c** contained a carbocyclic NH group. Replacing a cyclohexane ring (**IIa**) by benzene (**IIc**) did not affect appearance of antidepressant properties but did invert the activating effect to sedative, which appeared as horizon-

tal locomotor activity reduced by 4.4 times (Fig. 2*d*). Introduction of a methylene linker (**IIb**) between the NH and benzene ring increased horizontal locomotor activity and exchanged sedative by activating activity (Fig. 2*d*).

Thus, the results led to the conclusion that all studied compounds with a single intraperitoneal injection to outbred male mice at equimolar doses exhibited antidepressant activity to various degrees that was combined with either psychosedative or psychoactivating activity. The 5-piperazinopyrazoles **IIh, g** exhibited the greatest antidepressant activity and significantly reduced the ID (by 64 – 87% vs. the control). Compound **IIh** was characterized by the ability to decrease horizontal locomotor activity and OEA and EA of male mice; **IIg**, to increase the horizontal locomotor activity and OEA.

Conflict of interest

We declare no conflict of interest in the article.

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Contributions of authors

EEK and ILN proposed the research, directed the work, analyzed and interpreted the results, critically analyzed the results, and developed the article; SOSh, EAN, and GGG performed the research, collected data, analyzed and interpreted the results, and prepared the table and figures; FAKh, AVS, and VNP consulted and critically reviewed significant intellectual content.

Permission of ethics committee for animal experiments

The research was approved by the local ethics committee of BSMU, Ministry of Health of Russia (Protocol No. 9 of Oct. 28, 2020).

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