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Synthesis and anticonvulsant activity of 6-methyl-2-thioxo-2, 3-dihydropyrimidin-4(1*H*)-one acetamides

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ABSTRACT

This research aimed at synthesizing new potential anticonvulsants in the series of 2-(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio-acetamides. An initial intermediate 6-methyl-2-thioxo-2,3-dihydro-pyrimidin-4(1*H*)-one was obtained by the reaction of thiourea with an acetoacetic ester in the presence of sodium methoxide. The target 2-(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl) thioacetamides were synthesized by alkylation of initial 6-methyl-2-thiopyrimidin-4-one with corresponding 2-chloroacetamides in Dimethylformamide (DMF) in the presence of potassium carbonate. The structure of compounds was confirmed by ^{1}H Nuclear magnetic resonance (NMR)-spectroscopy, LCMS, and elemental analysis. A screening of anticonvulsant activity of synthesized compounds was carried out using the pentylenetetrazole- and maximal electroshock-induced seizures models. In these studies, the highest anticonvulsant activity demonstrated a compound **5.5** 2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]-N-(4-bromophenyl)-acetamide, which decreased the lethality, the number and the severity of seizures, and increased their latent period. For these compound parameters of ED_{50}, acute (LD_{50}) and neurotoxicity (TD_{50}), as well as therapeutic (TI) and protective (PI) indexes were determined. Logical structure analysis of anticonvulsant activity screening revealed some patterns of "structure–activity" relationship.

INTRODUCTION

The medical use of 2-thiopyrimide derivatives began many decades ago. Thiobarbiturates exhibit psychotropic activity and are used as medicines for anesthesia (Russo and Bressolle, 1998). Propylthiouracil is used as an antithyroid drug for the treatment of hyperthyroidism (Azizi *et al*., 2017; Cooper, 2005). Recently, the range of pharmacological activity of 2-thiopyrimidines was significantly expanded. Thus, among them were found the reverse transcriptase inhibitors of human immunodeficiency virus type 1 (HIV-1) of non-nucleoside structure, and the effective concentration of which *in vitro* was in the nanomolar range (Mai *et al*., 2001; Nawrozkij *et al*., 2008). The inhibitors of herpes simplex virus (HSV) of type 1 and 2 (Shigeta *et al*., 2003), antagonists of P2Y12 receptors that possess an antiplatelet activity (Crepaldi *et al*., 2009), anticancer (Gorneva *et al*., 2005) and antibacterial agents (Basavaraja *et al*., 2010) which are 2-thiopyrimidine derivatives were also created.

High psychotropic activity of pyrimidines has attracted the interest of scientists, whose focus is on developing new pyrimidine containing drugs that affect the central nervous system. A successful strategy for the synthesis of new antiepileptic drugs was the introduction of the exocyclic sulfur atoms into molecules of compounds (Levin *et al*., 1986; Matias *et al*., 2017).

The positive experience of our own research concerning the synthesis of new anticonvulsants in the series of pyrimidin-4(*3H)*-one and 4-thione derivatives (Severina *et al*., 2013; 2015) and determination of the thioacetamide fragment (Saidov *et al*., 2014) effect on increase of antiepileptic activity prompted us to search for the new highly potent anticonvulsants among thioalkyl pyrimidine derivatives.

Therefore, this research aimed at synthesizing new potential anticonvulsants—S-alkylated derivatives of 6-methyl-2-thioxo-2,3 ethyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one.

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MATERIALS AND METHODS

Chemistry

All of the solvents and reagents were obtained from the commercial sources. The progress of the reactions was monitored by thin layer chromatography (TLC) using aluminum silica gel plates. The melting points (°C) were determined in a capillary using an electrothermal IA9100X1 (Bibby Scientific Limited, Staffordshire, UK) digital melting point apparatus. ^{1}H Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury-400 (Varian Inc., Palo Alto, CA) spectrometer (300 MHz) in hexadeuterodimethyl sulfoxide (DMSO-*d6*) using tetramethylsilane (TMS) as an internal standard (chemical shifts are in ppm). ^{13}C NMR spectra were recorded at Bruker Avance 400 (100.6 MHz). Chemical shifts were reported in ppm downfield from TMS as internal standards. The elemental analysis was performed on a Euro Vector EA-3000 (Eurovector SPA, Redavalle, Italy) microanalyzer. Elemental analyses were within ±0.4% of the theoretical values. LCMS was recorded with PE SCIEX API 150EX chromatograph.

6-Methyl-2-thioxo-2,3-dihydro-1H-pyrimidin-4-one ***3*** was obtained according to the method reported in the literature (Novikov *et al*., 2005).

General procedure of the synthesis of S-alkylated derivatives of 6-Methyl-2-thioxo-2,3-dihydro-1H-pyrimidin-4-one, 5.1-15

A mixture of 7, 34 mmol of 2-thiouracil **(3)** and 10, 85 mmol of potassium carbonate in 10 ml of Dimethylformamide (DMF) was stirred at 70°C–80°C for 1 hour, and the reaction mixture was cooled to room temperature; a solution of 7, 34 mmol of appropriate chloroacetanilide **(4)** in 10 ml of DMF was added, stirred for 5 hours, and left for 12 hours afterward. The reaction mixture was filtered and the filtrate evaporated in a vacuum; the residue was treated with 100 ml of cold water. The formed precipitate was filtered, air dried, and recrystallized from an acetone-DMF mixture.

2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]-N-phenylacetamide, 5.1

Yield: 82%, melting point (mp) 241°C –3°C; ^{1}H NMR (300 MHz, DMSO-d_6, δ (ppm)): 12.52 (1H, br. s, NH-3), 10.16 (1H, s, NHCO), 7.59–7.27 (4H, m, H-2′,3′,5′,6′), 7.05 (1H, t, *J* = 7.5, H-4′), 5.99 (1H, s, CH-5), 4.07 (2H, s, SCH_2), 2.13 (3H, s, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ 169.82 (C=O, amide), 166.39 (6-C=O), 165.22, 164.12 (C-S), 139.3, 129.14 (2C), 123.72, 119.54 (2C), 107.66 (5-CH, br), 35.61 (CH_2), 23.84 (CH_3). Found, *m*/*z*: 276.32 $[M+H]^+$. The Anal. Calcd. was for $C_{13}H_{13}N_3O_2S$: C, 56.71; N, 15.26; S, 11.65%; we found: C, 56.79; N, 15.31; and S, 11.68%.

2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]-N-(4-methylphenyl)acetamide, 5.2

Yield: 79%, mp 253°C –5°C; ^{1}H NMR (300 MHz, DMSO-d_6, δ (ppm)): 12.52 (1H, br. s, NH-3), 10.16 (1H, s, NHCO), 7.45 (2H, d, *J* = 8.4, H-2′,6′), 7.10 (2H, d, *J* = 8.4, H-3′,5′), 5.99 (1H, s, CH-5), 4.05 (2H, s, SCH_2), 2.24 (3H, s, CH_3), 2.13 (3H, s, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ 169.78 (C=O, amide), 167.38 (6-C=O), 166.46 (C-S), 165.33, 143.36, 136.82, 132.61, 129.48, 119.67 (2C, Ar), 107.13 (5-CH, br), 35.45 (CH_2), 23.75 (CH_3), 20.77 (CH_3). Found, *m*/*z*: 290.09 $[M+H]^+$. The Anal. Calcd. was for $C_{14}H_{15}N_3O_2S$: C, 58.11; N,14.52; S, 11,08; we found: C, 58.01; N, 14.57; and S, 11.05.

2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]-N-(3-methoxyphenyl)acetamide, 5.3

Yield: 76%, mp 192°C–4°C; ^{1}H NMR (300 MHz, DMSO-d_6, δ (ppm)): 12.52 (1H, br. s, NH-3), 9.99 (1H, s, NHCO), 7.28 (1H, s, H-2′), 7.12 (1H, t, *J* = 8.2, H-5′), 7.02 (1H, d, *J* = 7.5, H-6′), 6.52 (1H, d, *J* = 7.5, H-4′), 5.99 (1H, s, CH-5), 4.00 (2H, s. SCH_2), 3.75(3H, s, OCH_3), 2.19 (3H, s, OCH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ 166.46 (C=O, amide), 166.21 (6-C=O), 165.46, 164.12 (C-S), 159.92, 140.39, 129.89, 111.87, 109.21, 105.44, 107.13 (5-CH, br), 55.33 (OCH_3,) 35.62 (CH_2), 23.74 (CH_3). Found, *m*/*z*: 306.08 $[M+H]^+$. The Anal. Calcd. was for $C_{14}H_{15}N_3O_3S$: C, 55.07; N, 13.76; S, 10.04; we found: C, 55.25; N, 13.71; and S, 9.99.

2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]-N-(4-chlorophenyl)acetamide, 5.4

Yield: 76%, mp >282°C–4°C; ^{1}H NMR (300 MHz, DMSO-d_6, δ (ppm)): 12.48 (1H, br. s, NH-3), 10.22 (1H, s, NHCO), 7.56 (2H, d *J* = 8, H-3′,5′), 7.32 (2H, d, *J* = 8, H-2′,6′), 5.99 (1H, s, CH-5), 4.09 (2H, s. SCH_2), 2.45 (3H, s, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ 166.61 (C=O, amide), 166.43 (6-C=O), 165.54, 164.09 (C-S), 138.28, 129.09 (2C), 127.38, 121.25 (2C), 107.48 (5-CH, br), 35.59 (CH_2), 23.32 (CH_3). Found, *m*/*z*: 309.03 $[M+H]^+$. The Anal. Calcd. was for $C_{13}H_{12}ClN_3O_2S$: C, 50.40; N, 13.56; S, 10.35; we found: C, 50.32; N, 13.61; and S, 10.31.

2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]-N-(4-bromophenyl)acetamide, 5.5

Yield: 79%, mp >259°C –61°C; ^{1}H NMR (300 MHz, DMSO-d 6, δ (ppm)): 12.48 (1H, br. s, NH-3), 10.22 (1H, s, NHCO), 7.61 (2H, d, *J* = 7.9, H-3′,5′), 7.42 (2H, d, *J* = 7.9, H-2′,6′), 5.98 (1H, s, CH-5), 4.05 (2H, s. SCH_2), 2.15 (3H, s, CH_3). Found, *m*/*z*: 353.99 $[M+H]^+$. The Anal. Calcd. was for $C_{13}H_{12}BrN_3O_2S$: C, 44.08; N, 11.86; S, 9.05; we found: C, 43.95; N, 11.90; and S, 9.02.

2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]-N-(2,3-dichlorophenyl)acetamide, 5.6

Yield: 80%, mp 230°C–2°C; ^{1}H NMR (300 MHz, DMSO-d_6, δ (ppm)): 12.50 (1H, br. s, NH-3), 10.10 (1H, s, NHCO), 7.82 (1H, d, *J* = 8,2, H-4′), 7.41-7.28 (2H, m, H-5′, 6′), 6.01 (1H, s, CH-5), 4.12 (2H, s. SCH_2), 2.19 (3H, s, CH_3). Found, *m*/*z*: 344.21 $[M+H]^+$. The Anal. Calcd. was for $C_{13}H_{11}Cl_2N_3O_2S$: C, 45.36; N, 12.21; S, 9.32; we found: C, 45.29; N, 12.23; and S, 9.30.

2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]-N-(2,5-dimethylphenyl)acetamide, 5.7

Yield: 72%, mp >248°C –50°C; ^{1}H NMR (300 MHz, DMSO-d_6, δ (ppm)): 12.42 (1H, br. s, NH-3), 9.52 (1H, s, NHCO), 7.22 (1H, s, H-6'), 7.12 (1H, d, *J* = 8, H-4′), 6.89 (1H, d, *J* = 8, H-3′), 6.01 (1H, s, CH-5), 4.05 (2H, s, SCH_2), 2.29 (3H, s, CH_3), 2.15 (3H, s, CH_3); 2.09 (3H, s, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ 166.37 (C=O, amide), 166.21 (6-C=O), 165.34, 164.12 (C-S), 136.35, 135.2, 130.46, 128.87, 126.39, 125.62, 107.48 (5-CH, br), 34.75 (CH_2), 23.52 (CH_3-4), 20.95 (CH_3),

17.75 (CH_3). Found, *m/z*: 304.11 $[M+H]^+$. The Anal. Calcd. was for $C_{15}H_{17}N_3O_2S$: C, 59.38; N, 13.85; S, 10.57; we found: C, 59.19; N, 13.89; and S, 10.55.

2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]-N-(2,5-dimethoxyphenyl)acetamide, 5.8

Yield: 68%, mp 188°C–90°C; ^{1}H NMR (300 MHz, DMSO-d_6, δ (ppm)): 12.43 (1H, br. s, NH-3), 9.39 (1H, s, NHCO), 7.86 (1H, s, H-6′), 6.90 (1H, d, J = 7.9, H-4′), 6.56 (1H, d, J = 7.9, H-3′), 6.05 (1H, s, CH-5), 4.01 (2H, s, SCH_2), 3.75 (6H, s, $2CH_3$); 2.23 (3H, s, CH_3). Found, *m/z*: 336.11 $[M+H]^+$. The Anal. Calcd. was for $C_{15}H_{17}N_3O_4S$: C, 53.72; N, 12.53; S, 9.56; we found: C, 53.60; N, 12.58; and S, 9.53.

2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]-N-(2,4,6-trimethylphenyl)acetamide, 5.9

Yield: 69%, mp >271°C–3°C; ^{1}H NMR (300 MHz, DMSO-d_6, δ (ppm)): 12.33 (1H, br. s, NH-3), 9.39 (1H, s, NHCO), 6.85 (2H, s, H-3′,5′), 6.00 (1H, s, CH-5), 4.02 (2H, s, SCH_2), 2.22 (6H, s, $2CH_3$); 2.11 (6H, s, $2CH_3$). ^{13}C NMR (126 MHz, DMSO-d_6): δ 166.43 (C=O, amide), 166.09 (6-C=O), 165.43, 164.12 (C-S), 135.82, 135.26 (2C), 132.67, 128.66 (2C), 107.84 (5-CH, br), 34.32 (CH_2), 23.7 (CH_3-4), 20.92 (CH_3), 18.27 ($2CH_3$). Found, *m/z*: 318.12 $[M+H]^+$. The Anal. Calcd. was for $C_{16}H_{19}N_3O_2S$: C, 60.54; N, 13.24; S, 10.10; we found: C, 60.42; N, 13.19; and S, 10.05.

2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]-N-(2,4,6-trichlorophenyl)acetamide, 5.10

Yield: 76%, m.p. >258°C–60°C; ^{1}H NMR (300 MHz, DMSO-d_6, δ (ppm)): 12.50 (1H, br. s, NH-3), 10.01 (1H, s, NHCO), 7.69–7.51 (2H, m, Ar-H), 6.03 (1H, s, CH-5), 4.10 (2H, s, SCH_2), 2.22 (3H, s, CH_3). Found, *m/z*: 379.66 $[M+H]^+$. The Anal. Calcd. was for $C_{13}H_{10}Cl_3N_3O_2S$: C, 41.23; N, 11.10; S, 8.47; we found: C, 41.19; N, 11.06; and S, 8.44.

2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]-N-(2-methyl-3-chlorophenyl) acetamide, 5.11

Yield: 75%, mp >262°C; ^{1}H NMR (300 MHz, DMSO-d_6, δ (ppm)): 12.50 (1H, br. s, NH-3), 10.01 (1H, s, NHCO), 7.69–7.51 (3H, m, Ar-H), 6.01 (1H, s, CH-5), 4.09 (2H, s, SCH_2), 2.17 (3H, s, CH_3). Found, *m/z*: 324.05 $[M+H]^+$. The Anal. Calcd. was for $C_{14}H_{14}ClN_3O_2S$: C, 51.93; N, 12.98; S, 9.90; we found: C, 51.79; N, 12.93; and S, 9.87.

2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]-N-benzyl-acetamide, 5.12

Yield: 66%, mp 196°C –8°C; ^{1}H NMR (300 MHz, DMSO-d_6, δ (ppm)): 12.50 (1H, br. s, NH-3), 10.01 (1H, t, J = 5.1, $CH_2N\underline{H}CO$), 7.60–7.27 (4H, m, H-2′,3′,5′,6′), 7.05 (1H, t, J = 7.5, H-4'), 6.05 (1H, s, CH-5), 4.11 (2H, s. SCH_2), 4.01 (2H, d, J = 5.3, $NHC\underline{H}_2Ph$), 2.18 (3H, s, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ 167.58 (C=O, amide), 166.43 (6-C=O), 166.09, 165.43 (C-S), 139.61, 128.7 (2C), 127.59 (2C), 127.27, 107.84 (5-CH, br), 42.98 (CH_2), 34.27 (CH_2, amide), 23.83 (CH_3). Found, *m/z*: 290.09 $[M+H]^+$. The Anal. Calcd. was for $C_{14}H_{15}N_3O_2S$: C, 58.11; N, 14.52; S, 11.08; we found: C, 58.01; N, 14.48; and S, 11.04.

2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]-N-cyclohexlyl-acetamide, 5.13

Yield: 65%, mp 245°C–7°C; ^{1}H NMR (300 MHz, DMSO-d_6, δ (ppm)): 12.51 (1H, br. s, NH-3), 7.88 (1H, s, NHCO), 5.98 (1H, s, CH-5), 4.00 (2H, s. SCH_2), 2.21 (3H, s, CH_3) 1.90–1.18 (м, 11H, C_6H_{11}). ^{13}C NMR (126 MHz, DMSO-d_6): δ 167.58 (C=O, amide), 166.4 (6-C=O), 166.09, 165.43 (C-S), 107.65 (5-CH, br), 48.42, 36.66, 34.6, 32.74, 31.99, 25.55, 24.66, 23.53 (CH_3). Found, *m/z*: 282.19 $[M+H]^+$. The Anal. Calcd. was for $C_{13}H_{19}N_3O_2S$: C, 55.49; N, 10.68; S, 11.40; we found: C, 55.36; N, 10.65; and S, 11.36.

Ethyl 2-[2-(4-methyl-6-oxo-1,6-dihydropyrimidin-2-ylthio)-acetylamino]-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carboxylate, 5.14

Yield: 55%, mp 230°C –2°C; ^{1}H NMR (300 MHz, DMSO-d_6,, δ (ppm)): 12.61 (1H, br. s, NH-3), 11.29 (1H, s, NHCO), 6.03 (1H, s, CH-5), 4.35 (2H, q, $OC\underline{H}_2CH_3$), 4.10 (2H, s. SCH_2), 2.90-2.70 (2H, m, CH_2), 2.52–2.31 (4H, m, $2CH_2$), 2.12 (3H, s, CH_3) 1.20 (3H, t, J = 5.2, $OCH_2C\underline{H}_3$). ^{13}C NMR (126 MHz, DMSO-d_6): δ 167.66 (C=O amide), 166.10 (6-C=O), 166.05, 166.01, 164.12, 150.52, 141.37, 132.21, 108.54, 107.65 (5-CH, br), 60.81, 33.99 (CH_2), 30.37, 28.86, 27.7, 23.69 (CH_3) 14.42 (CH_3). Found, *m/z*: 393.48 $[M+H]^+$. The Anal. Calcd. was for $C_{17}H_{19}N_3O_4S_2$: C, 55.49; N, 10.68; S, 11.40; we found: C, 55.36; N, 10.72; and S, 11.35.

2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]-N-(4-phenoxy-phenyl)-acetamide, 5.15

Yield: 60%, mp 224°C–6°C; ^{1}H NMR (300 MHz, DMSO-d_6,, δ (ppm)): 12.45 (1H, br. s, NH-3), 10.08 (1H, s, NHCO), 7.75–7.55 (2H, m, Ar-H), 7.40–7.29 (2H, m, Ar-H), 7.10–6.91 (5H, m, Ar-H), 5.98 (1H, s, CH-5), 4.08 (2H, s. SCH_2), 2.21 (3H, s, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ 166.43 (C=O, amide), 166.09 (6-C=O), 165.43, 164.12 (C-S), 157.78, 152.44 (2C), 135.25, 130.43(2C), 123.49 (2C), 121.37, 119.94 (2C), 118.39, 107.83 (5-CH, br), 35.54 (CH_2), 23.91 (CH_3). Found, *m/z*: 368.43 $[M+H]^+$. The Anal. Calcd. was for $C_{19}H_{17}N_3O_3S$: C, 62.11; N, 11.44; S, 8.73; we found: C, 62.02; N, 11.40; and S, 8.69.

Anticonvulsant activity

Animals

The experiments were carried out on 95 adult male rats (130–150 g) and adult random-bred albino mice of either sex weighing 22–28 g. The animals were bred in the vivarium of National Pirogov Memorial Medical University, Vinnytsya housed in cages under standard conditions with a temperature of (22°C ± 1°C), relative humidity of (55% ± 15%) with free access to food and water and a 12-hour light/darkness cycle (8.00–20.00), respectively. All of the experiments were conducted in accordance with "Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes," with the procedures and requirements of the State Expert Center of the Ministry of Health of Ukraine and with the rules of European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986), resolution of the First National

Congress on Bioethics (Kyiv, 2001), with the Law of Ukraine National Congress on Bioethics (Kyiv, 2001) and with the Law of Ukraine №3447-IV "On Protection of Animals from Cruel Treatment" dated 02.21.2006.

Animals were randomly assigned to the experimental and control groups. The substances tested were dissolved in 1% starch gel and administered by oral gavage cannula at a volume of 0.5 ml/100 g body weight in rats and 0.2 ml/10 g body weight in mice 1 hour before seizure induction. Screening dose for testing compounds was 80 and 50 mg/kg. Referent compounds (phenobarbital, lamotrigine, and carbamazepine) were given the same manner in their median anticonvulsant doses 20, 20, and 15 mg/kg body weight, respectively (Metcalf *et al*., 2017). The determination of time for the experiment was based on the data concerning the peak of the anticonvulsant activity of the drug, described in the literature (Fisher, 1989; Löscher, 2011). Control rats received equivolume amounts of solvents. All seizures [both chemical (PTZ) and electrical (MES)] were induced between 9:00 and 11:00 to minimize possible inconsistencies arising from circadian rhythms (Löscher, 1996).

Pentylenetetrazole-induced seizures

Seizures in animals were modeled by a single subcutaneous pentylentetrazole injection (Sigma) at a dose of 80 mg/kg. The animals were administered intragastrically with a freshly prepared suspension of experimental compounds and reference drugs phenobarbital and lamotrigine (20 mg/kg). The anticonvulsant activity was assessed by the dynamic of the latent period, the intensity and duration of seizures in minutes, and the lethality rate of mice.

The intensity of seizures was evaluated using a 5-point scale, taking as a basis the following criteria (including the number of animals that died) (Gerald, 1973): 0—no seizure activity; 1—hyperkinesia; 2—trembling, twitching; 3—clonic seizures of upper limbs with the rise on their lower limbs; 4—pronounced tonic-clonic seizures, the animal's fall to the side, and the available phase of tonic extension; and 5—repeated tonic-clonic seizures, loss of posture, and death.

Anticonvulsant effect was considered an animal protection based on the clonic and tonic seizures and the lethality.

Maximal electroshock seizure testing in mice (MES)

The research was conducted on the non-linear mice of both sexes, weighing 25–28 grams. The animals were divided into four groups with 10 animals in each group. Group one served as the control group. Animals of other groups were receiving intragastrically compounds **(5.1-15)** (50 mg/kg), lamotrigine (20 mg/kg), or carbamazepine (15 mg/kg). The investigation of anticonvulsant activity was conducted 1 hour later after the administration of the experimental compounds. The MES test consisted of electrical stimulation of the cornea using 60 Hz of alternating current (150 mA), with a stimulus duration of 0.2 second, using a custom-build MES stimulator. The electrodes were soaked with a 0.9% sodium chloride solution. A number of animals with hind limb flexion-extension and mortality rate were estimated.

After estimation of the most active substance, its acute toxicity (LD_{50}), neurotoxicity (TD_{50}), and protective index (PI = LD_{50}/TD_{50}) were determined.

Neurotoxic activity

The possible neurotoxic effects in mice were quantified using a rotarod test. The test compound was administered intraperitoneally to animals at a dose rate of 10–200 mg/kg and the control mice received an equivalent amount of solvent. Mice were placed on a rotating knurled rod (12 rpm). Neurotoxic action was manifested in the form of violations of coordination of motion. Mice were considered impaired if they fell off the rotarod three times during the 1-minute observation period performed immediately prior to stimulation (rotarod failure). The mice of the control group were kept on the rods for several minutes. The value of TD_{50} was calculated by the probit analysis method.

Acute toxicity

During this experiment, we used the white nonlinear mice (24 ± 3g), which were divided into five groups. Animals of the experimental groups received a substance **5.5**, ranging in doses from 100 mg/kg to 400 mg/kg. The drug was administered in appropriate doses intragastrically, dissolving it in the required amount of 1% starch gel. Animal observations were carried out within 14 days; then, the number of dead animals in each group was noted and using the method implemented by Prozorovsky (2007), acute toxicity (LD_{50} and its confidence interval) was evaluated. We recorded the behavior and body weight of mice, the clinical signs of intoxication, the general condition of animals, the nature of motor activity, the characteristics of breathing, the condition of hair and skin, the presence of a vessel, the consumption of food and water, and also we noted the number of dead animals in each group and the value of LD_{50} was defined.

Statistical analysis

All values were expressed as the means ± S.E.M. The data were analyzed by ANOVA (analysis of variance) followed by Dunnett's test (Statistical package for social sciences, SPSS 16.0). An X2 analysis was performed to compare the neurotoxicity differences (number of failures per test). p values ≤ 0.05 was considered as significant.

RESULTS AND DISCUSSION

Chemistry

To establish the prospects of synthesis and optimization of further pharmacological screening, we performed a prediction of the biological activity that was planned for the synthesis of compounds using a PASS computer program (http://www.pharmaexpert.ru/passonline/). 6-Methyl-2-thioxo-2,3-dihydro-*1H*-pyrimidin-4-one acetamides were selected for synthesis. High psychotropic activities such as antiepileptic, anxiolytic, antidepressant, antineurotic, and anticonvulsant activities (Pa ≥ 0.50) were predicted for these compounds.

The synthesis of initial 6-methyl-2-thioxo-2,3-dihydro-1*H*-pyrimidin-4-one **3** consisted of condensation of the ethyl acetoacetate **1** with a 2.5-fold molar excess of thiourea **2** (Fig. 1). The reaction occurred successfully in refluxing absolute methanol

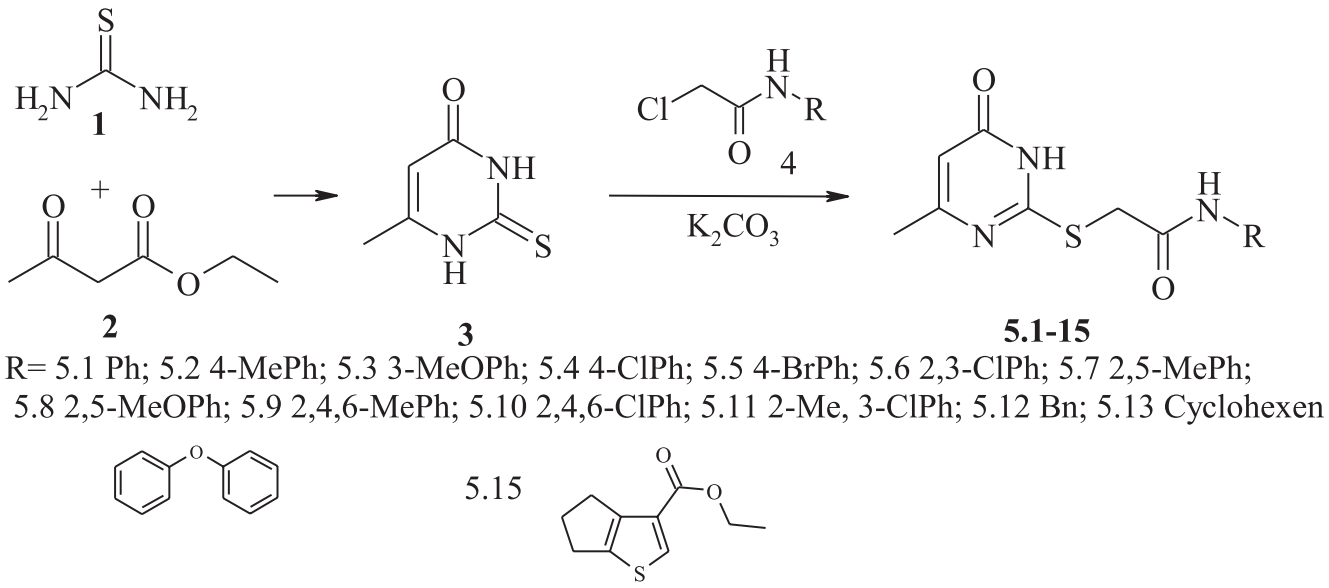

Figure 1. The synthesis of 2-[(1,6-dihydro-6-oxo-4-methyl-2-pyrimidinyl)thio]-N-acetamides.

with a 2.6–2.8-fold molar excess of sodium methylate as reported in known methods used (Novikov *et al*., 2005).

Alkylation of the resulting 6-methyl-2-thiopymidin-4-one **3** was carried out by an equimolar amount of N-arylsubstituted 2-chloroacetamides, 2-chloro-N-benzylacetamide, and ethyl 2-[(chloroacetyl)amino]-5,6-dihydro-4*H*-cyclopenta[b]thiophene-3-carboxylate according to known procedure of alkylation of thiopyrimidines (Gagnon *et al*., 2007; Rakhimov and Titova, 2007). The reaction was conducted in dimethylformamide solution in the presence of an excess of potassium carbonate and led to the formation of only 2-[(1,6-dihydro-6-oxo-4-methyl-2-pyrimidinyl) thio]-N-acetamides **5.1-15**. The yield of reaction products was 55%–82%.

Because the sulfur atom in the molecule of 4-methyl-2-thiopyrimidin-4-one ***3*** is the most nucleophilic, the alkylation reaction on this direction was expected and most likely.

In the literature (Danel, 1998), it was noted that the usage of primary alkyl halides as alkylating agents leads to the formation of a complex mixture consisting of S-mono, SN1- and SN3-disubstituted alkylation products. TLC, LCMS, and ^{1}H NMR spectroscopy data confirmed the formation of only S-derivatives which probably can be explained by using sizable alkylating agents that shielded Nitrogen atoms.

The ^{1}H NMR spectra of synthesized compounds **5.1–5.15** contain a wide singlet proton signal of the NH group in the third position of the pyrimidine cycle located at δ 12.52–12.43 ppm, a singlet signal of NHCO proton group of acetamide residue at δ 11.23–9.39 ppm, a signal of a methine proton at 5 position of the pyrimidine cycle at δ 6.05–5.98 ppm, and a singlet of a methylene SCH_2-group within a range 4.12–4.00 ppm. Protons of the phenyl radical resonate at δ 7.92–6.52 ppm and their multiplicity and intensity correspond to the nature and location of substituents.

^{13}C NMR spectra of all 2-[(1,6-dihydro-6-oxo-4-methyl-2-pyrimidinyl)thio]-N-acetamides **5.1-15** allow for reliably identifying the signals of only some carbon atoms: C=O amide, 6-C=O, C-S, 5-CH, 6-CH_3, CH_2, and CH_3. It is incorrect to relate all other signals to any specific carbon atom (especially in the aromatic region of the spectrum) without additional two-dimensional experiments. However, they also give useful information—at least, concerning the number of carbon atoms in the molecule. ^{13}C NMR spectra for compounds 5.5, 5.6, 5.8, and 5.10 were not recorded due to insufficient solubility.

Anticonvulsant activity

In the control group, pentylentetrazole administration led to the development of seizures in all animals. The duration of the latent period averaged 4.7 minutes and the duration of seizures —9.7 minutes (Table 1). The seizures which developed in this group of rats were accompanied by severe periodically repeated tonic-clonic convulsions. There was a clear phase of tonic extension (epistotonus). The lethality in this group of rats was 100%. Phenobarbital prevented the development of the seizures in all animals. At the same time, after administration of lamotrigine in rats, pentylentetrazole induced some manifestations of the seizures (convulsive twitching, jumps, and contractions of the upper limbs), but the duration of the latent period was statistically reliably lengthened (in 5.8 times), the degree of the seizure severity, and their total duration were significantly lower than in the control group. Lamotrigine prevented the lethality in 80% of animals.

All investigated compounds reduced the development of seizures in the chemoconvulsive seizure model (Table 1). After their administration, the extension of the latent period was observed in relation to the control group; however, compounds **5.4, 5.10, 5.12, 5.14**, and **5.15** slightly increased it (in 0.2–1.5 times).

The extension of the latent period of seizures, reduction of their intensity, and duration of seizures were most observed while administering the compounds **5.2, 5.5, 5.8, 5.13**, and **5.11.**

Compounds **5.5, 5.7, 5.8, 5.11**, and **5.13** prevented the lethality in 100% of rats similarly to phenobarbital. Compounds **5.1, 5.2, 5.3**, and **5.10** and lamotrigine reduced lethality, respectively,

Table 1. The effect of the synthesized compounds **5.1–5.15**, lamotrigine, and phenobarbital on seizures caused by administration of pentylentetrazole in rats.

Groups of animals	Number of rats in the group	Dose, mg/kg	Duration of the latent period, min	Duration of seizures, min	Lethality abs. units (%)	Intensity of seizures, (points)
Control	*n* =10	-	4.7 ± 0.30#/&	9.70±0.90#/&	10 (100%)	4.96
5.1	*n* = 5	80	11.2 ± 2.0*/#/&	5.6 ± 1.0*/#/&	2 (40%)	3.6
5.2	*n* = 5	80	14.4 ± 4.0*/#/&	7.6 ± 2.0#/&	2 (40%)	3.2
5.3	*n* = 5	80	1.8 ± 2.1*/#/&	15.0 ± 2.4*/#/&	2 (40%)	3.2
5.4	*n* = 5	80	5.0 ± 0.4#/&	9.2 ± 1.6#/&	5 (100%)	4.8
5.5	n = 5	80	27.0 ± 3.0*	1.0 ± 1.0*/#	0*	0.4
5.6	*n* = 5	80	7.6 ± 1.2*/#/&	14.8 ± 2.1*/#/&	3 (60%)	3.8
5.7	*n* = 5	80	8.4 ± 1.7*/#/&	11.4 ± 3.5#/&	0*	3.4
5.8	*n* = 5	80	16.2 ± 3.6*/#/&	4.4 ± 1.3*/#	0*	1.0
5.9	*n* = 5	80	13.8 ±2.2*/#/&	13.4 ± 1.8#/&	3 (60%)	2.6
5.10	*n* = 5	80	5.8 ± 0.9#/&	8.8 ± 2.2#/&	2 (40%)	3.2
5.11	*n* = 5	80	18.0 ± 5.1*/#	2.8 ± 1.2*/#	0*	1.8
5.12	*n* = 5	80	7.0 ± 0.3*/#/&	9.40 ±0.7*/#/&	5 (100%)	4.4
5.13	*n* = 5	80	12.6±1.4*/#/&	12.0 ±3.3#/&	0*	2.8
5.14	*n* = 5	80	11.8± 4.6#/&	7.2 ± 2.1#/&	3 (60%)	2.8
5.15	*n* = 5	80	5.0 ± 0.8#/&	10.4± 0.5#/&	5 (100%)	4.6
Phenobarbital	*n* = 5	20	30.0 ± 0.0*	0*	0*	0
Lamotrigine	*n* = 5	20	27.6 ± 0.8*	2.40±0.40*/#	1 (20%)	2.20

n—a number of animals in the group; *—statistically significant differences of obtained results in the experimental group ($p < 0.05$) compared with a control; #—statistically significant differences of obtained results in the experimental group ($p < 0.05$) compared with phenobarbital. &—statistically significant differences of obtained results in the experimental group ($p < 0.05$) compared with lamotrigine.

Table 2. The effect of the synthesized compounds (**5.5, 5.7, 5.8, 5.11**, and **5.13)**, carbamazepine, and lamotrigine on seizures caused by maximal electroshock in mice.

Experiment conditions	Number of animals in group	Dose, mg/kg	Number of mice with seizures	Duration of electroshock seizures (clonic seizures + tonic extension), sec
Control	10	-	9	47.3 ± 0.48
5.5	10	50	1	5.4 ± 0.31*# (-88.5%)
5.7	10	50	7	30.2±0.25*#&
5.8	10	50	5	10.1±1.36*#
5.11	10	50	5	12.4±2.15*#
5.13	10	50	6	18.2±0.18*#&
Lamotrigine	10	20	3	8.4 ± 0.39* (−82.3%)
Carbamazepine	10	15	2	6.2 ± 0.56* (−86.9%)

*—statistically significant differences of obtained results in the experimental group ($p < 0.05$) with a control; #—statistically significant differences of obtained results in the experimental group ($p < 0.05$) compared with carbamazepine; &—statistically significant differences of obtained results in the experimental group ($p < 0.05$) compared with lamotrigine.

in 80% and 60% of the experimental rats. 100% lethality of the experimental animals, similar to the control group, was observed while administering the compounds **5.4, 5.12**, and **5.15.**

According to our research, the most pronounced anticonvulsant activity was shown by compound **5.5**, which contained 4-bromophenyl radical. This compound reduced the duration of seizures in 2.4 times and the seizure severity in 5.5 times.

Structure-activity relationship (SAR) studies

Analysis of the results of screening studies of anticonvulsant activity of thiopyrimidine derivatives **5.1–5.15** on the model of pentylentetrazole seizures in rats allowed making some general conclusions about the influence of structural fragments on anticonvulsant activity of synthesized compounds.

It can be assumed that the introduction of the N-phenyl radical into the structure of compounds contributes to the increase of anticonvulsant activity, since the replacement of the N-phenyl radical with benzyl, 4-phenoxyphenyl, and cyclopentathiophene **(5.12, 5.14**, and **5.15)** leads to reduction of the latent period, prolongation of the duration of seizures, and an increase the percentage of lethality of the experimental animals (100%, 100%, and 60%, respectively).

Substituents in the phenyl radical also have a significant role in an anticonvulsant activity. 4-, 2,3- and 2,4,6-chloro-substituted derivatives **(5.4, 5.6**, and **5.10)** minimally extended the latent period, did not reduce the duration and severity of the seizures and did not prevent the lethality of animals, whereas, Me-, MeO- and, in particular, 4-Br-substituted derivatives improved this data.

The following pattern was observed: with the increase of the number of chlorine atoms, the lethality of the experimental animals decreased: **5.4, 5.6**, and **5.10** = 4-Cl, 2,3-diCl, 2,4,6-triCl = 100%, 60%, 40%. Such results were unexpected as there is a significant amount of literary data describing an increase of anticonvulsant activity when introducing chlorine atoms.

The next step in our research was focused on investigating the effects of the synthesize substances on the models of primary generalized seizures caused by maximal electroshock which is essential for identifying potential anticonvulsants.

Table 3. Characteristic of anticonvulsant activity of 2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl) thio]-N-(4-bromophenyl)acetamide **(5.5)** in intragastric administration.

Compound	LD_{50}, mg/kg	TD_{50}, mg/kg	Pentylenetetrazole (rats)			MES (mice)		
			ED_{50}, mg/kg	TI	PI	ED_{50}, mg/kg	TI	PI
5.5	258.0± (23.3)	105.9 ± 11.7	53.0± 0.33	9.96	1.98	12.5± 0.14	20.6	8.47

The results presented in Table 2 showed that the ability of the compounds to exhibit anticonvulsant effects on the MES model was different. The potent anticonvulsant activity in this study showed compounds **5.8** and **5.11**, but the highest anticonvulsant activity among the experimental compounds was shown by compound **5.5**. Thus, after its administration, the number of dead animals was 10% versus 90% in control, and the total duration of seizure was 88.5% less ($p < 0.05$) than in animals without pharmacological correction. Due to the ability to prevent seizures, this compound was not statistically different from carbamazepine and prevailed lamotrigine taken in their median effective doses.

Based on the data obtained in this study, the dose-dependent parameters (ED_{50}) in the pentylenetetrazole-induced seizures model, MES test, acute (LD_{50}) and neurotoxicity (TD_{50}) indexes, as well as the therapeutic (TI) and protective (PI) indexes were established for the lead compound **5.5** (Table 3).

CONCLUSIONS

Based on the PASS prediction, the synthesis of potential anticonvulsants in a series of S-alkylated derivatives of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one was designed. By the interaction of thiourea and acetoacetate in a medium of sodium methylate, 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one was obtained.

It was found that as the result of alkylation of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one by N-arylsubstituted 2-chloroacetamides, 2-chloro-N-benzylacetamide, and ethyl 2-[(chloroacetyl)amino]-5,6-dihydro-*4H*-cyclopenta[b]thiophene-3-carboxylate in DMF medium in the presence of potassium carbonate, only monosubstituted S-acetamide derivatives of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(*1H*)-one were formed.

Using the models of pentylenetrazole-induced seizures and maximal electroshock test, a screening study of the anticonvulsant activity of synthesized compounds was carried out. A lead compound- 2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]-N-(4-bromophenyl)acetamide was revealed. For these compound parameters of ED_{50}, acute (LD_{50}) and neurotoxicity (TD_{50}) as well as TI and PI indexes were determined. Some relationships "structure-anticonvulsant activity" were established.

CONFLICTS OF INTEREST

The authors have declared there is no conflicts of interest.

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