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Synthesis, docking study, and pharmacological evaluation of S-acetamide derivatives of 4,6-dimethyl-2-thiopyrimidine as anticonvulsant agents

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ABSTRACT

The aim of this study is the direct synthesis of new (4,6-dimethylpyrimidin-2-yl)thio-N-acetamides derivatives as possible anticonvulsants. The interaction of thiourea with acetylacetone in sodium ethoxide resulted in the scaffold of 4,6-dimethyl-2-thiopyrimidine. Thioacetamide derivatives were synthesized by alkylation of 4,6-dimethyl-2-thiopyrimidine with comparable α-chloroacetamides in the Dimethylformamide (DMF) environment and in the presence of K_2CO_3. The methods of ^1H and ^{13}C Nuclear magnetic resonance (NMR) spectroscopy, Liquid chromatography–mass spectrometry (LS/MS), and elemental analysis established the structure of the synthesized compounds. The affinity of the studied compounds with anticonvulsant biotargets— Type-A γ-aminobutyric acid receptor (GABAAR) and the gamma-aminobutyric acid-aminotransferase enzyme—was carried out using the molecular-docking method. The highest affinity was predicted for the compound having 4-bromophenyl substituent: −7.0 (GABAA) and −8.0 (GABAAT) kcal/mol. Nevertheless, all the studied compounds conceded to the reference ligands—phenobarbital (−7.6 kcal/mol) and vigabatrin (−9.0 kcal/mol). The model of pentylenetetrazole-induced seizures in rats has shown that the studied compounds have moderate anticonvulsant activity. 4-Bromophenyl acetamide has also shown the most pronounced activity: the substance statistically significantly extended the latency period and reduced the duration of seizures by 3.4 and 2.2 times, respectively; moreover, it reduced lethality of the laboratory animals by 80% and by 2.5 times severity of seizures. Correspondence between the docking results and *in vivo* studies, using PTZ-induced seizures, as well as some parameters of "structure-anticonvulsant activity" correlation, was determined.

INTRODUCTION

Epilepsy is one of the most serious brain diseases having uncontrolled seizures and attacks of impaired movement and sensory, autonomic, and mental functions, resulting from excessive neuronal discharges (Yuen *et al*., 2018). According to the WHO, more than 65 million people suffer from epilepsy in the world, and it takes the leading position among psychoneurological diseases (Moshé *et al*., 2015). In developed countries, from 1 to 18 new cases of the disease per 1,000 people are annually registered, and in developing countries to 30 cases (Johnson, 2019; Vogt *et al*., 2017). Despite the large number of works on epilepsy pharmacotherapy, a relatively wide range of antiepileptic drugs (AEDs) with different mechanisms of action, including prolonged action, and satisfactory control of seizures is only achieved in 65%–70% of patients (Gesche *et al*., 2019). In some patients, AEDs cause an increase in the frequency of attacks and the appearance of a large number of side effects, the transformation of attacks, and the deterioration of encephalographic indicators (Janmohamed, 2020; Vossler *et al*., 2018). Therefore, despite the availability of both well-known and successful developments of new AEDs in recent decades, the search for new compounds that are promising for the treatment of seizures and epilepsy in preclinical and clinical studies has improved efficacy and tolerability (Bialer and White, 2010).

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A targeted search for new Active pharmaceutical ingredients (APIs) affecting the central nervous system (CNS), particularly pyrimidine derivatives, remains relevant for the scientists worldwide (Kumar *et al*., 2015). Phenobarbitale (PHB), having a GABAergic mechanism of pharmacological activity (Vossler *et al*., 2018), is the first pyrimidine-structure anticonvulsant which has been widely used for treatment of resistant epilepsy till today (Zhang *et al*., 2019). An inhibitory neurotransmitter gamma-aminobutyric acid (GABA) modulates the neurological function of the CNS and affects specific processes such as anxiety, cognition, sedation/sleep, and convulsions. GABA is the most important inhibitory neurotransmitter that regulates the central genesis muscle tone due to its ability to activate ionotropic and metabotropic G-protein-coupled receptors: $GABA_A$, Type-C γ-aminobutyric acid receptor ($GABA_C$), and Type-B γ-aminobutyric acid receptor ($GABA_B$), respectively (Sahu *et al*., 2018). Scientists agree that GABAergic inhibition is the main mechanism affecting neuronal networks and preventing the formation and distribution of paroxysmal brain activities (Trevelyan and Schevon, 2013). GABAergic dysfunctions, innate or acquired, can lead to epilepsy (Lerche *et al*., 2013). Normalization or enhancement of GABAergic inhibition due to allosteric modulation of GABA receptors, GABA neuronal reuptake blockade, and GABA degradation inhibition remains the main direction for pharmacological correction of epilepsy and convulsions (Khazipov, 2016).

Our previous studies identified a number of substances with pronounced anticonvulsant properties and a sufficient profile among pyrimidine derivatives (Severina *et al*., 2019), its annelated (El Kayal *et al*., 2019) and condensed derivatives (Severina *et al*., 2017). In each of the study groups, promising anticonvulsants were found and significant patterns of "structure-anticonvulsant activity" were established. However, the search for the "ideal" anticonvulsant is ongoing and, in this study, we decided to synthesize the structural analogues of the previously synthesized thiopyrimidine-4(3*H*)-one acetamides (Severina *et al*., 2019), by changing the carbonyl group at the fourth position of the pyrimidine cycle to the hydrophobic methyl group (Fig. 1).

The hydrogen-bonding site, which is a carbonyl group when binding to biotarget, is undoubtedly one of the key factors for the maximal affinity in receptor–ligand interaction. However, the nature of the bonds is quite diverse, and the introduction of a hydrophobic domain, such as a methyl group, can enhance hydrophobic interaction with the active receptor site and create a more stable conformation. In addition, modification of the carbonyl group to a methyl usually leads to an increase of the lipophilicity of the molecule, which can improve the permeability through the blood–brain barrier, which directly affects the expression of activity (Lipinski, 2016).

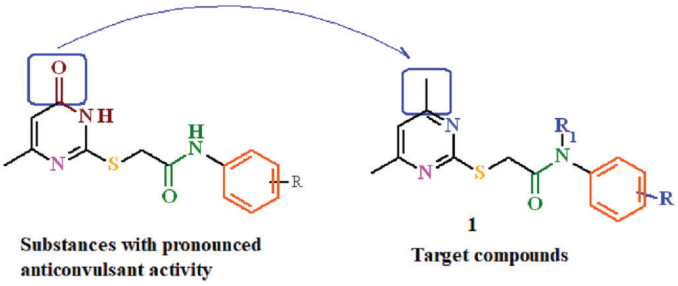

Figure 1. Directions for modifications and pharmacophore fragments of the study compounds.

The aim of this study is to synthesize 4,6-dimethyl-2-thiopyrimidineS-acetamide derivatives, to carry out docking research, and to calculate the Type-A γ-aminobutyric acid receptor (GABAAR) and γ-Aminobutyrate aminotransferase (GABAAT) affinities with the further pharmacological screening of anticonvulsant activity using a pentylenetetrazole model of seizures.

MATERIALS AND METHODS

Chemistry

To carry out research, purified analytical reagents were used (Sigma-Aldrich, |St. Louis, MO|). The required reagents were purified using standard techniques. Thin layer chromatography (TLC) method on aluminum silica gel plates was used to estimate the reactions' progress. The electrothermal digital melting point apparatus IA9100X1 (Bibby Scientific Limited, Staffordshire, UK) was used for the determination of the melting points (°C) in a capillary. To record 1H Nuclear magnetic resonance (NMR) spectra in the hexa deutero dimethylsulfoxide-d6 (DMSO-d_6) environment, while tetramethylsilane (TMS) was used as an internal standard, spectrometer Varian Mercury 400 (300 MHz) (Varian Inc., USA) was used. Bruker Avance 400 (126 MHz, DMSO-d_6) equipment was used to register ^{13}C NMR spectra. Nuclear frequency resonances (ppm) were described according to internal standards (TMS). Euro Vector EA-3000 microanalyzer (Eurovector SPA, Italy) was used for elemental analysis. The deviation of the obtained results did not exceed ± 0.4%, relative to the known target values. PE SCIEX API 150EX chromatograph was used for LC/MS analysis.

To synthesize 4,6-dimethyl-2-thiopyrimidine, a traditional method described as early by Hunt *et al*. (1959) was used. A thiourea (100 mmol) was suspended in a solution of acetylacetone (120 mmol) in 250 ml of ethanol. The obtained mixture was stirred after adding 25 ml of concentrated hydrochloric acid and left for 2 hours heating under reflux to complete the reaction. As soon as the solution was cooled down, yellow needle-like crystals of 2-thio-4,6-dimethyl-pyrimidine hydrochloride were formed. After the residue was dissolved in hot water (50°C), 1M NaOH was added to the obtained solution for its pH neutralization (optimal pH value is 10.5). The mixture was put into the refrigerator for 10 hours. Then, the vacuum-filtration method was used to separate 4,6-methyl-2-pyrimidinethiol after its crystallization from 50% aqueous ethanol.

Method of the Synthesis of 2-((4,6-Dimethylpyrimidin-2-yl) thio)-N-acetamides 1a–k

About 10 ml of Dimethylformamide (DMF) was used to dissolve 4,6-dimethyl-2-thiopyrimidine (10 mmol) and potassium carbonate (20 mmol) mixture. The solution was stirred for 60 minutes after its temperature reached 70°C–80°C. A mixture of α-chloroacetanilide (10 mmol) and 10 ml of DMF was put into the

flask with the reaction solution (as soon as it cooled down) and was left for 5 hours with stirring. The obtained mixture was vacuum-evaporated after filtration. When the residue was formed, it was washed with 100 ml of cold water, filtered, air dried, and then subjected to recrystallization using a mixture of acetone and DMF.

2-((4,6-Dimethylpyrimidin-2-yl)thio)-N-phenylacetamide 1a

Yield: 89.0% white crystals; mp = 145–7°C; ^{1}H NMR: 9.10 (1H, s, NHCO), 7.58–7.25 (4H, m, H-2′,3′,5′,6′), 7.05 (1H, t, *J*=8, H-4′), 6.88 (1H, s, CH-5), 4.01 (2H, s. SCH_2), 2.45 (3H, s, CH_3), 2.27 (3H, s, CH_3). LC/MS: *m/z* =274.10 [M+1]. Anal. calcd. for $C_{14}H_{15}N_3OS$: C, 61.52; H, 5.53; N, 15.37; S, 11.73. Found: C, 61.49; H, 5.50; N, 15.39; S, 11.70.

N-(2-methylphenyl)-2-((4,6-dimethylpyrimidin-2-yl)thio) acetamide 1b

Yield: 78.0% white crystals; mp = 153–5°C; ^{1}H NMR: 9.18 (1H, s, NHCO), 7.46 (1H, d, *J*=8, H-3′),6.94-6.87 (4H, m, H-4′,5′,6′; CH-5), 3.90 (2H, s, SCH_2), 2.50 (6H, s, $2CH_3$), 2.40 (3H, s, CH_3). LC/MS: *m/z* = 288.11 [M+1]. Anal. calcd. for $C_{15}H_{17}N_3OS$: C, 62.69; H, 5.96; N, 14.62; S, 11.16. Found: C, 62.49; H, 5.94; N, 14.66; S, 11.13.

N-(2,4-dimethylphenyl)-2-((4,6-dimethylpyrimidin-2-yl)thio) acetamide 1c

Yield: 79.0% white crystals; mp = 134–6°C; ^{1}H NMR: 9.89 (1H, s, NHCO), 7.36 (1H, d, *J*=8, H-6′), 7.00 (1H, s, H-3′), 6.90–6.81 (2H, d, H-5′, CH-5), 3.91 (2H, s, SCH_2), 2.87 (3H, s, CH_3), 2.41 (3H, s, CH_3), 2.27 (3H, s, CH_3),2.10 (3H, s, CH_3). ^{13}C NMR: δ 169.82 (C=O), 167.46 (2C), 166.83 (C-S), 134.73, 134.08, 132.07, 131.25, 126.91, 125.38, 116.58 (C-5), 35.28 (CH_2), 23.83 ($2CH_3$), 20.94 (CH_3), 18.11 ($CH_2\underline{C}H_3$). LC/MS: *m/z* = 302.13[M+1]. Anal. calcd. for $C_{16}H_{19}N_3OS$: C, 63.76; H, 6.35; N, 13.94; S, 10.64. Found: C, 63.55; H, 6.33; N, 13.97; S, 10.60.

N-(4-chlorophenyl)-2-((4,6-dimethylpyrimidin-2-yl)thio) acetamide 1d

Yield: 91.0% white crystals; mp = 154–6°C; ^{1}H NMR: 9.75 (1H, s, NHCO), 7.47 (2H, d, *J*=8, H-3′, 5′), 6.98 (2H, d, *J*=8, H-2′,6′), 6.80 (1H, s, CH-5), 3.92 (2H, s, SCH_2), 2.80 (3H, s, CH_3), 2.40 (3H, s, CH_3). LC/MS: *m/z* = 308.06 [M+1]. Anal. calcd. for $C_{14}H_{14}ClN_3OS$: C, 54.63; H, 4.58; N, 13.65; S, 10.42. Found: C, 54.58; H, 4.56; N, 13.68; S, 10.40.

N-(4-bromophenyl)-2-((4,6-dimethylpyrimidin-2-yl)thio) acetamide 1e

Yield: 80.0% yellow crystals; mp = 158–60°C; ^{1}H NMR: 9.70 (1H, s, NHCO), 7.47 (2H, d, *J*=7.2, H-3′, 5′),6.98 (2H, d, *J*=7.2, H-2′,6′), 6.84 (1H, s, CH-5), 3.98 (2H, s, SCH_2), 2.78 (3H, s, CH_3), 2.42 (3H, s, CH_3). LC/MS: *m/z* = 352.01 [M+1]. Anal. calcd. for $C_{14}H_{14}BrN_3OS$: C, 47.74; H, 4.01; N, 11.93; S, 9.10. Found: C, 47.68; H, 3.99; N, 11.97; S, 9.08;.

N-(2-methoxyphenyl)-2-((4,6-dimethylpyrimidin-2-yl)thio) acetamide 1f

Yield: 86.0% white crystals; mp = 112–4°C; ^{1}H NMR: 9.15(1H, s, NHCO), 8.23 (1H, d, *J*=8.2, H-3′),7.13-7.00 (3H, m, H-4′,5′,6′), 6.88 (1H, s, CH-5), 3.97 (2H, s, SCH_2), 3.75 (3H, s, OCH_3), 2.58 (3H, s, CH_3), 2.45 (3H, s, CH_3). LC/MS: *m/z* = 304.11 [M+1]. Anal. calcd. for $C_{15}H_{17}N_3O_2S$: C, 59.39; H, 5.65; N, 13.85; S, 10.57. Found: C, 59.20; H, 5.63; N, 13.89; S, 10.53.

N-(4-ethoxyphenyl)-2-((4,6-dimethylpyrimidin-2-yl)thio) acetamide 1g

Yield: 88.0% white crystals; mp = 138–40°C; ^{1}H NMR: 9.75 (1H, s, NHCO), 7.42 (2H, d, *J*=8.2, H-3′, 5′),6.77 (2H, d,*J*=8.2, H-2′,6′), 6.81 (1H, s, CH-5), 3.97 (2H, q, *J*=7.2, $O\underline{CH_2}CH_3$). 3.88 (2H, s, SCH_2), 2.80 (3H, s, CH_3), 2.40 (3H, s, CH_3),1.39 (3H, t, *J*=6.2, $OCH_2\underline{CH_3}$). ^{13}C NMR: *δ* 169.88(C=O), 167.41(2C), 166.43(C-S), 155.00, 132.58, 121.15 (2C), 116.50 (C-5), 114.86 (2C), 63.55 ($\underline{C}H_2CH_3$), 35.85 (CH_2), 23.81 ($2\underline{C}H_3$), 15.16 ($CH_2\underline{C}H_3$). LC/MS: *m/z* = 318.12 [M+1]. Anal. calcd. for $C_{16}H_{19}N_3O_2S$: C, 60.55; H, 6.03; N, 13.24; S, 10.10. Found: C, 60.32; H, 6.02; N, 13.26; S, 10.05.

Methyl 4-(2-((4,6-dimethylpyrimidin-2-yl)thio)acetamido) benzoate 1h

Yield: 72.0% white crystals; mp = 164–6 °C; ^{1}H NMR: 12.54 (1H, s, NHCO), 7.89 (2H, d, *J*=7.2, H-3′,5′),7.66 (2H, d, *J*=7.2, H-2′,6′), 6.77 (1H, s, CH-5), 3.94(2H, s, SCH_2),3.85 (3H, s, OCH_3), 2.60 (3H, s, CH_3), 2.39 (3H, s, CH_3). LC/MS: *m/z* = 332.10 [M+1]. Anal. calcd. for $C_{16}H_{17}N_3O_3S$: C, 57.99; H, 5.17; N, 12.68; S, 9.67. Found: C, 57.87; H, 5.15; N, 12.70; S, 9.65.

2-((4,6-dimethylpyrimidin-2-yl)thio)-N-isopropyl-N-phenylacetamide 1i

Yield: 78.0% white crystals; mp = 126–8°C; ^{1}H NMR: 7.41-7.32 (4H, m, H-2′,3′,5′,6′), 7.26 (1H, t, *J*=6.8, H-4′), 6.67 (1H, s, CH-5), 4.85-4.79 (1H, m, CH), 3.97 (2H, s, SCH_2),2.32 (6H, s, $2CH_3$), 1.18 (3H, s. CH_3), 1.06 (3H, s, CH_3). LC/MS: *m/z* = 316.14 [M+1]. Anal. Calcd. for $C_{17}H_{21}N_3OS$: C, 64.73; H, 6.71; N, 13.32; S, 10.16. Found: C, 64.68; H, 6.70; N, 13.34; S, 10.14.

N-cyclohexyl-2-((4,6-dimethylpyrimidin-2-yl)thio)acetamide 1j

Yield: 81.0% white crystals; mp = 123–5 °C; ^{1}H NMR: 7.32 (1H, d, *J*=6, NHCO), 6.78 (1H, s, CH-5), 3.64 (2H, s, SCH_2), 2.39 (6H, s, $2CH_3$), 1.80-1.10 (м, 11H, C_6H_{11}).^{13}C NMR: δ 170.04 (C=O), 167.24 (2C), 166.72 (C-S), 116.43, 48.31, 34.97 (CH_2), 32.81, 25.66(2C), 24.94 (2C), 23.80 ($2CH_3$). LC/MS: *m/z* = 280.14 [M+1]. Anal. calcd. for $C_{14}H_{21}N_3OS$: C, 60.18; H, 7.58; N, 15.04; S, 11.47. Found: C, 60.15; H, 7.56; N, 15.08; S, 11.45.

N-(2,3-dihydrobenzo[b][1,4]dioxin-5-yl)-2-((4,6-dimethylpyrimidin-2-yl)thio)acetamide 1k

Yield: 68.0% white crystals; mp = 135–7°C; ^{1}H NMR: 9.62 (1H, s, NHCO), 7.14 (1H, d, *J*=7.2, H-2′), 6.88 (1H, t, *J*=6, H-3′), 6.77 (1H, s, CH-5), 6.65 (1H, d, *J*=7.2, H-4′), 4.21 (4H, s, $2CH_2$),3.86 (2H, s, SCH_2),2.39 (6H, s, $2CH_3$).^{13}C NMR: δ 169.78(C=O), 167.38 (2C), 166.46 (C-S), 143.36, 139.71, 133.15, 117.13, 116.39, 112.81, 108.7, 64.62, 64.36 (2C, benzodioxane), 35.86 (CH_2), 23.67 ($2\underline{C}H_3$) LC/MS: *m/z* = 332.10 [M+1]. Anal. calcd. for $C_{16}H_{17}N_3O_3S$: C, 57.99; H, 5.17; N, 12.68; S, 9.67. Found: C, 57.89; H, 5.15; N, 12.70; S, 9.65.

Molecular-Docking Study

Flexible molecular docking, as a main approach of the search for molecules having affinity to specific biological targets, was used for this study. The Protein Data Bank was used to select specific macromolecules, i.e., GABAAR (PDB ID 4COF) and GABAAT (PDB ID 10HW) (Protein Data Bank). The IsisDraw 2.4 software was used for depicting the ligand structures, which were saved as .mol files. At the next stage, the Chem3D software was used to optimize the given molecules by the MM2 molecular mechanical algorithm, with the results saved in .pdb format. Using AutoDockTools 1.5.6, the latter were converted into File format .pdbqt (PDBQT), and the number of active torsions was set as default (Trott and Olson, 2010). PDB files of macromolecules were downloaded from the Protein Data Bank. Water and ligand were removed from the crystals by means of the Discovery Studio Visualizer 2017/R2 software tool. The structures of the obtained proteins were saved in .pdb format. Then, in the AutoDockTools 1.5.6, polar hydrogen atoms were added and saved as PDBQT. Molecular docking was carried out using AutoDock Vina, and the Discovery Studio Visualizer 2017/R2 was used to visualize the obtained results.

Anticonvulsant activity

Animals

A total of 50 adult male rats (130–150 g) were used for this study. The animals from National Pirogov Memorial Medical University (Vinnytsya, Ukraine) vivarium were used for this study. Standard housing conditions for the lab animals were designed in accordance with the "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" and with the Law of Ukraine №3447-IV "On Protection of Animals from Cruel Treatment" dated February 2, 2006. The optimal temperature (22 ± 1°C) and humidity (55 ± 15%) levels were set. The animals were allowed to drink and eat whenever they wanted. The groups of the animals were kept under the standard 24-hours cycle consisting of 12 hours of light (8.00–20.00) and 12 hours of darkness.

Animals were divided into experimental and control groups at random. The probe through the oral cannula was used for intragastric administration of the test substances in a volume of 0.5 ml per 100 g body weight of the animal after its dissolution in 1% starch gel. About 80 mg/kg dose was chosen for the screening. The mean doses (20 mg/kg body weight) of the reference drugs PHB and lamotrigine were also administered intragastrically. The experimental duration was calculated according to the data about the maximum level of antiseizure effect of the studied drug according to the corresponding literature data (Vogel, 2008). In the control group of animals, an equivalent amount of solvent was administered. Pentylentetrazol-induced seizures were caused in the period 9:00–11:00, in order to minimize the influence of circadian rhythms.

Pentylenetetrazole-induced seizures

To start a convulsive attack, 80 mg/kg of pentylenetetrazole (Sigma, USA) was used for a single subcutaneous injection to the laboratory animals. The animals were given experimental compounds in the form of suspension and reference drugs. PHB (PHB IC, InterChem, Ukraine) and lamotrigine (20 mg/kg) (Lamictal, GlaxoSmithKline, Poland) were used as the reference drugs. To analyze the anticonvulsant activity of the studied compounds, the following markers were used: the latent phase duration, the severity of seizures, duration of convulsive attack, and the lethality in the groups of the laboratory animals. The 5-point scale was used to estimate the severity of seizures, as described in Gerald and Riffee's (1973) study. Anticonvulsant effect was considered as the protection of animals from the beginning of both clonic and tonic seizures, as well as lethality decrease in the studied groups.

Statistical analysis

The obtained results were calculated as the mean values ± standard error. One-way analysis of variance using Dunnett's multiplicity method was selected for comparison between groups and estimation of the observed effect (SPSS Statistics, version 16.0, Chicago, IL). A p-value ≤ 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

To replace the carbonyl group with the methyl group in the structure of the pyrimidine cycle as the starting reagent, instead of the acetoacetic ester we used acetylacetone. The latter was condensed with thiourea under boiling in absolute ethanol in the presence of ethoxide solution (Scheme 1).

The choice of alkylating agents was based on the literature and own research findings on the effect of substituent in the amide moiety on anticonvulsant activity (Matias *et al*., 2017; Severina *et al*., 2019). As can be seen from Scheme 1, the modification of the scaffold 4,6-dimethyl-2-thiopyrimidine occurred by introducing an acetamide moiety with various biologically active substituents: aryl, cyclohexyl, and 2,3-dihydro-1,4-benzodioxin.

We conducted a reaction of the interaction of 4,6-dimethyl-2-thiopyrimidine with the corresponding acetamides in dimethylformamide under common conditions of thiopyrimidines S-alkylation, by adding excess solution of potassium carbonate and heating to a temperature of 70–80°C (Kigundi *et al*., 2007). The synthesized compounds **1a–k** were white crystals with good solubility in organic liquids (2-propanol, dioxane, and dimethyl formamide) and poor solubility in water. The chemical structure of the synthesized substances and their individuality were proved by TLC, LC/MS, ^{1}H and ^{13}C NMR spectroscopy, and elemental analysis.

^{1}H NMR spectra presented all the relevant proton signals. The singlet peak of the proton of the NHCO group of the acetamide residue reflects at the site δ 9.89–9.10 ppm. It should be noted that the chemical shift of the NH amide residue into the weak field (12.54 ppm) of compound **1h** contains a strong electron-withdrawing substituent in the aryl moiety—COOMe. The NH proton of compound **1j** with the cyclohexyl substituent resonates in the form of a doublet and in a stronger field (7.32 ppm). The protons of the methylene SCH_2 group of the synthesized compounds **1a–k** shifted slightly to the strong field (4.00–3.67

ppm), compared to the similar signals synthesized by us earlier (4-oxo-6-methyl-2-pyrimidinyl)thio-N-acetamides (4.12–4.00 ppm) (Severina *et al*., 2019) due to the absence of electron-withdrawing effects of the carbonyl group at the fourth position of the pyrimidine cycle. The singlet signal of the proton in the fifth position of the pyrimidine cycle was observed at 6.88–6.67 ppm, and the compounds **1b, c** were overlaid with the aromatic proton signals. In addition, signals from two methyl groups, aryl and alkyl protons, which resonated in characteristic regions were recorded.

In the ^{13}C NMR spectra of **1c, g, j, k** compounds, typical are the following: the signal of a carbon atom of the C-S group, which gives a resonant signal in a weak field (166.83–166.43 ppm) and the signal of the carbon atom of the NHC=O group (170.4–170.0 ppm), aromatic fragments (167.47–108.7 ppm), and aliphatic groups (64.62–15.16 ppm), the position and number of which completely correspond to the structure of the compounds.

The search for new AEDs is based on a strategy for the integrated use of screening models of seizures with different pathogenesis (Löscher, 2017), the gold standard among which is models of pentylenetetrazole and maximal electroshock (MES) seizures in rats and mice. High animal mortality in the screening experiment is one of the factors that limits the effective search for anticonvulsants. Modern target-based virtual screening (Palestro *et al*., 2018), based on scientific knowledge of molecular changes that generate epileptic seizures, molecular alterations that generate epileptic seizures, mechanisms of anticonvulsant action, structure of target proteins, and amyloid proteins of the receptor sites, as well as the arsenal of techniques to carry out analysis and evaluate the receptor-ligand affinity, allows maximizing the rationalization of the search for new AEDs, computing the possible mechanism of biochemical action, and choosing the correct model for pharmacological screening.

The synthesized compounds **1a–k** are structural analogues of S-acetamides derivatives of 6-methyl-2-thiopyrimidin-4(1*H*)-one, which exhibited activity on the Pentylenetetrazole (PTZ) model of seizures in rats. The anticonvulsive action of PTZ is a result of the suppression of the GABA fragment of the benzodiazepine receptor complex. The effect is also due to reduction of GABAergic inhibition in the CNS. Therefore, to predict the effect of **1a–k** compounds, specifically on GABAergic system, we examined their affinity with the GABA allosteric site of $GABA_AR$ (PDB 4COF) receptor (Miller and Aricescu, 2014) and the GABA-aminotransferase enzyme (PDB 1OHW) (Storici, 1999). Docking in the active sites of the receptor and enzyme was carried out in comparison to the native ligands—PHB and vigabatrin (VGN), respectively. Linking energy (scoring function) became a quantification feature. A 3D visualization is shown in Figure 2; for example, the bromo-substituted acetamide derivative **1e** and the binding energies of all ligands are presented in Table 1.

The results of the docking are somewhat surprising to us, since the separation energy of the studied ligands, corresponding to the affinity of the substances to the selected targets, significantly covers the binding energy of the reference compounds (Table 1). At the same time, our assumption that the introduction of the methyl group at the fourth position of the pyrimidine cycle will increase the hydrophobic interaction was confirmed and well-illustrated by the example of ligand 1e: with the $GABA_A$ receptor, the methyl group forms three

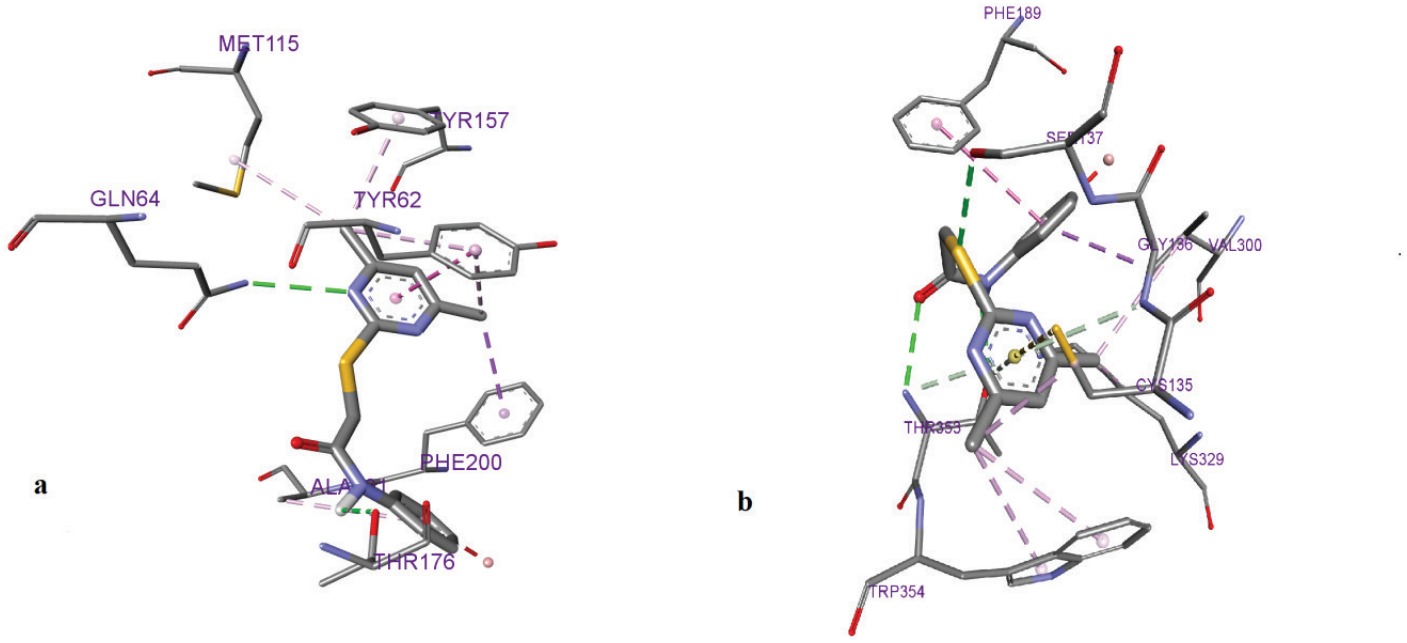

Figure 2. 3D interaction between $GABA_AR$ (ACOF) (a) and $GABA_{AT}$ (10HW) (b) and ligand **1e**: hydrogen bonds are indicated by green dotted lines, hydrophobic interactions—purple dotted lines.

hydrophobic bonds with aromatic rings of tyrosine residues (Tyr62, 157) and methionine (Met 115), and $GABA_{AT}$ was also observed to have a connection with the mercapto group of cysteine (Cys135) and two bonds with the indole fragment of tryptophan (Trp354).

Not the best values of scoring functions can be explained by the shallow and somewhat one-sided immersion of the ligands in the hydrophobic pocket of the $GABA_A$ receptor and the minor involvement in the interaction of the acetamide moiety: in the **1e** ligand, this is one hydrophilic and hydrophobic bond with tryptophan residues (Thr176) and alanine (Ala201), respectively.

The predicted affinity of the studied **1a–k** ligands with GABA aminotransferase is also inferior to that of the native ligand VGN. The lowest binding energy was demonstrated by compound **1e**, forming a fairly stable conformation due to hydrophobic interaction with valine (Val300), cysteine (Cys135), phenylalanine (Phe189), lysine (Lys329), and tryptophan (Trp335), and hydrophilic with tryptophan (Trp33554) and serine (Ser137).

Therefore, in accordance with uncertain rates in the docking results, for the further PTZ-induced screening model, 6 compounds out of 11 were chosen. The selected substances demonstrated the best values of scoring functions with both biotargets.

In the control group of animals, the introduction of pentylenetetrazole caused convulsions (Table 2), together with strong tonic–clonic seizures with a pronounced phase of tonic extension and 100% lethality. Reference drug PHB essentially prevented the epileptic syndrome development in all animals. At the same time, the lamotrigine effect limited the protection of the animals from pentylenetetrazole chemotoxicity and some symptoms of epileptic condition were withdrawn; for example, convulsive spasms, jumps, and forelimbs tonic contractions. Lamotrigine statistically remarkably extended the duration of the latent period by 5.8 times and significantly reduced the severity of convulsions and the epileptic attack period, compared to controls, and only 20% of the animals of the studied groups died.

Pharmacological screening in this model of convulsions resulted in that none of the tested compounds showed statistically significant anticonvulsant action on the integral protective index parameter and reduced lethality in test groups, in contrast to the control group, and they were inferior to the reference remedies for all epileptic syndrome indexes (Table 2).

As with the thiopyrimidine-4(3*H*)-one acetamides (Severina *et al*., 2019), the compound with the 4-bromophenyl radical **1e** was the most active: it statistically significantly prolonged the latency period and the duration of the seizure by 3.4 and 2.2 times, respectively, and by 80% reduced the lethality of animals; the severity of seizure was 2.0 points against 4.96 in the control group. However, the anticonvulsant action of N-(4-bromophenyl)-2-((4,6-dimethylpyrimidin-2-yl)thio)acetamide **1e** was significantly weaker than that of its structural analogue: N-(4-bromophenyl)-2-[(4-methyl-6-oxo-1*H*-pyrimidin-2-yl) thio]acetamide. Therefore, the carbonyl group's replacement at the fourth position of the pyrimidine cycle by the methyl group is an inappropriate modification and leads to a decrease in the anticonvulsant activity demonstrated in the entire group of tested compounds. Compound **1h** was found to be completely indifferent to pentylenetetrazole seizures and had no effect on any of their progression, with 100% animal mortality.

Table 1. Binding energy (kcal/mol) ligands 1a–k with active sites of $GABA_A$ and $GABA_{AT}$.

Targets	Ligands											PHB	VGN
	1a	1b	1c	1d	1e	1f	1g	1h	1i	1j	1k		
$GABA_AR$	−5.5	−5.7	−6.5	−6.8	−7.0	−5.9	−4.9	−6.0	−5.0	−5.8	−6.9	−7.6	–
$GABA_{AT}$	−6.0	−5.5	−7.0	−7.4	−8.0	−6.2	−5.8	−5.8	−6.8	−6.9	−7.0	–	−9.0

Table 2. Influence of the acetamides 1 on the pentylenetetrazole-induced seizures in rats.

Groups of animals	Number of rats in the group	Dose, mg/kg	Duration of the latent period, min	Duration of seizures, minute	Lethality abs. units (%)	Severity of seizures, (points)
Control	$n = 10$	–	4.7 ± 0.30	9.70 ± 0.90	10 (100%)	4.96
1c	$n = 5$	80	9.4 ± 1.6[a]	14.0 ± 2.4	2 (40%)	3.4
1d	$n = 5$	80	12.0 ± 1.6[a]	15.4 ± 3.0	2 (40%)	3.4
1e	$n = 5$	80	16.2 ± 3.6[a]	4.4 ± 1.3[a]	1 (20%)	2.0
1h	$n = 5$	80	5.6 ± 0.4[a]	8.4 ± 1.2	5 (100%)	4.6
1j	$n = 5$	80	9.8 ± 1.2[a]	7.4± 2.1	3 (60%)	2.8
1k	$n = 5$	80	8.6 ± 0.5[a]	15.0 ± 3.7[a]	3 (60%)	3.6
PHB	$n = 5$	20	30.0 ± 0.0[a]	0[a]	0[a]	0
Lamotrigine	$n = 5$	20	27.6 ± 0.8[a]	2.40 ± 0.40[a]	1 (20%)	2.20

n = number of animals in the group.

[a]- compared to control group, $p < 0.05$.

CONCLUSION

New S-acetamide derivatives of 4,6-dimethyl-2-thiopyrimidine were synthesized and their structure was proved. The binding energy for the studied compounds with the active sites of GABAA receptor and the GABA-aminotransferase enzyme was worse compared to the reference ligands— PHB and VGN. The molecular-docking parameters correlated with *in vivo* study results on the model of PTZ-induced seizures in rats. All the studied compounds showed moderate activity and conceded to the reference drugs PHB and lamotrigine. N-(4-Bromophenyl)-2-(4,6-dimethylpyrimidin-2-yl)thio-acetamide showed the most pronounced activity, according to *in silico* and *in vivo* studies. The obtained results allow recommending the used docking methodology as a tool for PTZ-induced pharmacological screening optimization. It was determined that the carbonyl group substituted by the methyl group in the fourth pyrimidine cycle position leads to a decrease in anticonvulsant activity.

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AUTHORS' CONTRIBUTIONS

Hanna I. Severina played a leading role in conducting the experiment, interpreting the data, and writing this article. Olha O. Skupa suggested the idea of this article, participated in the experiment, and made substantial contributions to the design of this article. Natalya I. Voloshchuk played a significant role in conducting the pharmacological experiment and interpreting the pharmacological research data. Victoriya A. Georgiyants made a significant contribution to the interpretation of the data, writing, and final editing of this article.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest related to the publication of this paper.

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