DESIGN, SYNTHESIS, MOLECULAR DOCKING AND ANTICONVULSANT EVALUATION OF 6-METHYL-2-ARYLAMINOPYRIMIDIN-4(3*H*)-ONE

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Мета. Синтез 2-аміноарильних похідних 6-метил-піримідин-4(3Н)-ону, віртуальний мішень-орієнтований скринінг з наступним вивченням протисудомної активності та встановлення закономірностей «структура-активність».

Матеріали та методи. У роботі використовували стандартні методи органічного синтезу, будову синтезованих сполук доведено елементним аналізом, ¹Н ЯМР-спектроскопію, хромато-мас-спектрометрію. Молекулярний докінг проведено за допомогою AutoDockTools-1.5.6 та AutoDock Vina. Протисудомну активність вивчено на пентилентетразоловій моделі судом у щурів.

Результати. Метилюванням 6-метил-піримідин-4(3Н)-ону диметилсульфатом або метилйодидом одержано 2-тіометильне похідне. Нагріванням останнього з ароматичними амінами при 140 °C одержані цільові 2-аміноарильних 6-метил-піримідин-4(3Н)-они. Перспективність скринінгу синтезованих сполук на пентилентетразоловій моделі судом та вибір об'єктів здійснено за результатами енергії зв'язування та оцінки конформації в активних сайтах ГАМКА рецептору та ГАМК-АТ. Досліджувані речовини не продемонстрували протисудомної активності: лише 2 сполуки виявили тенденція до прояву активності за критерієм інтегрального захисного показника – зменшення летальності порівняно з контролем, попереджуючи летальність у 100 та 80 % тварин, відповідно. Співставлення з попередніми результатами активності 2-тіоацетанілідних похідних дозволило довести позитивну роль тіоацетамідного та фенільного фрагментів, а також 4-Br, 4-MeO радикалів в прояві протисудомної активності та збільшення летальності при наявності атомів Cl.

Висновки. Здійснено синтез та доведено будову 2-аміноарильних похідних 6-метил-піримідин-4(3Н)-он. На пентилентетразоловій моделі судом у щурів досліджувані сполуки не продемонстрували протисудомної активності. Однак одержані результати дозволили встановити ряд структурних фрагментів, що впливають на протисудомну активність. Визначено позитивну кореляцію між результатами in vivo досліджень на моделі пентилентетразолових судом та результатами докінгу в активні сайти ГАМКА та ензиму ГАМК_{АТ}

Ключові слова: ситнтез, піримідин, ацетаміди, докінг, ГАМК, протисудомна активність

1. Introduction

To date, epilepsy remains one of the most common neurological diseases [1]. It has a chronic course and in the case of irrational treatment leads to mental, severe cognitive and behavioral disorders, personality disorders, etc. [2]. Studies have consistently demonstrated comorbid associations of epilepsy with numerous psychiatric and somatic conditions [3]. These include not only structural and functional CNS disorders such as stroke, dementia and migraines, but also heart disease, hypertension, chronic obstructive pulmonary disease, etc.

Despite the slight progress in understanding some aspects of the pathophysiology of epileptogenesis [4, 5], the development of a number of new anti-epileptic drugs (AEDs) and established mechanisms of anticonvulsant action [6, 7], the spread of refractory forms of epilepsy [8, 7] and the development of a wide range of side effects in the treatment of existing AEDs [9] remains a major problem today. That is why design of new AEDs with a maximum security profile remains a pressing issue. Priority methods for implementing modern "drug-design" are structural variations of known AEDs and selective target-oriented screening [10]. One such drug is phenobarbital (Fig. 1), which is still used for the treatment of convulsive status epilepticus in infants and children [11] and is the drug of choice for refractory forms of epilepsy [12]. The introduction of a hydrophobic methyl group into the structure of phenobarbital resulted in methylphenobarbital (mephobarbital), a drug with distinct anticonvulsant properties, which is effective in resistant forms of epilepsy due to the fact that it is not a substrate for P-glycoprotein [13].
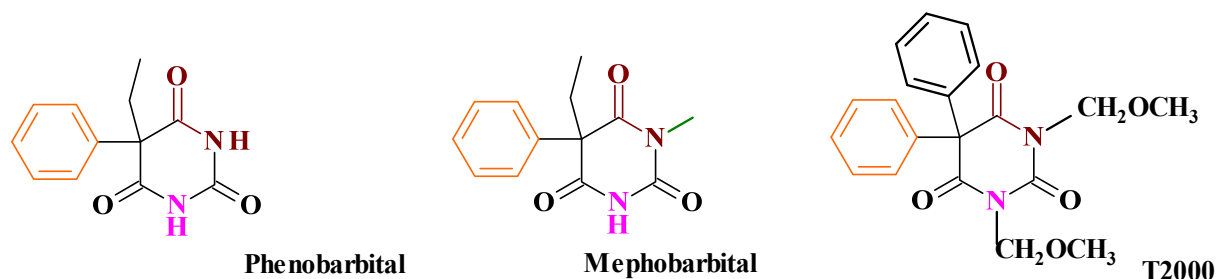
Fig. 1. Anticonvulsant drugs containing pyrimidine cycle and its pharmacophoric fragments

Another structural analogue of phenobarbital is 1,3-dimethoxymethyl-5,5-diphenyl-barbituric acid (T2000) [14]. The drug has undergone the first phase of clinical trials as a treatment for epilepsy and essential tremor in Parkinson's disease. In addition, our studies have identified a number of substances with significant anticonvulsant properties among pyrimidine-4(3*H*)-one derivatives [15] and its annelated derivatives [16].

The aim of this study was to synthesize 2-aminoaryl derivatives of 6-methyl-pyrimidin-4(3*H*)-one, a virtual target-oriented screening followed by a study of anticonvulsant activity in the rat pentylenetetrazole seizures model and to discuss "structure-activity" patterns.

2. Planning (methodology) of research

The presented study is based on the modification of the "pyrimidine" matrix - the main pharmacophore fragment of phenobarbital and includes all stages of the concept of modern "drug-design".

1. Design of target molecules on the basis of logical and structural analysis of the literature data on the "structure-activity" interaction and the basic principles of the pharmacophore concept.

The positive effect of acetamide and phenyl fragments on anticonvulsant activity has been previously evaluated (Fig. 2) [15, 17]. Continuing research on the establishment of pharmacophore anticonvulsant fragments into the structure of 6-methyl-pyrimidin-4(3*H*)-one, a 2-aminoaryl moiety is introduced.
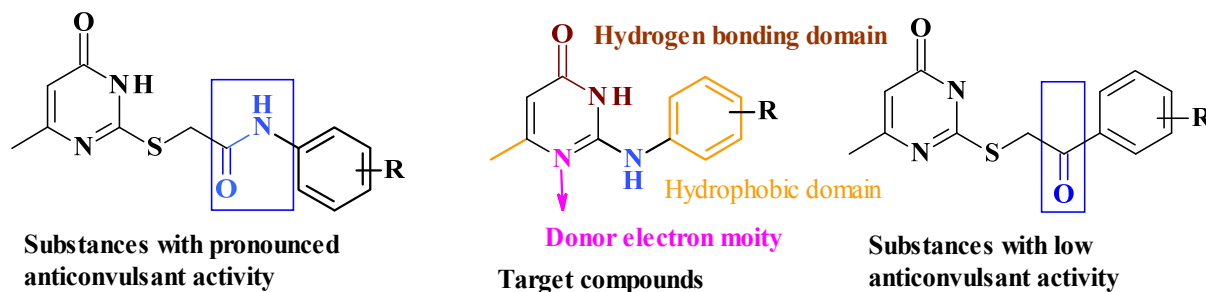

Fig. 2. Modification direction and pharmacophore fragments of the studied compounds

Comparison with the preliminary results of 2-thioacetanilide derivatives will allow to evaluate the role of thioacetate and aminophenyl moiety in the manifestation of anticonvulsant activity. In addition, such modification is quite promising in terms of the ability to interact with the active site of the receptor, since the target substances remain lipophilic domains in the form of an aryl ring and methyl group, a hydrogen bond domain to further stabilize the ligand-receptor conformation, as well as the donor electrons to form covalent bonds.

2. Synthesis and structure determination of new 2-aminoaryl derivatives of 6-methyl-pyrimidin-4(3*H*)-one.

3. Virtual target-oriented screening of investigated ligands using flexible molecular docking tools. Evaluation of the affinity of the ligands to the active sites of GABA$_A$ and GABA$_{AT}$ will allow us to select the most

promising compounds for investigation on the PTZ seizures model.

4. Pharmacological screening on a PTZ seizures model in rats.

5. SAR analysis of the results.

3. Materials and methods

All solvents and reagents were obtained from commercial sources. The melting points (°C) were determined in a capillary using an electrothermal IA9100X1 (Bibby Scientific Limited, Staffordshire, UK) digital melting point apparatus. 1H NMR spectra were recorded on a Bruker Varian Mercury instrument (Varian Inc., Palo Alto, CA, USA) (400 MHz) in DMSO-d6 internal TMS standard. Chromatography-mass spectrometric analysis was performed on a PE SCIEX API

150EX chromatograph. Elemental analysis was performed on a Euro Vector EA-3000 microanalyzer (Eurovector SPA, Redavalle, Italy). The content of the elements was within ± 0.4 % of theoretical values.

6-Methyl-2-thiopyrimidin-4(3H)-one (1) was obtained earlier [15].

Synthesis of 6-methyl-2-methylthiopyrimidin-4(3H)-one (2).

A. A mixture of 6-methyl-2-thiopyrimidin-4 (3H)-one (3) (1.42 g, 10 mmol), dimethyl sulfate (1.94 ml, 10 mmol), potassium carbonate (2.76 g, 20 mmol), ethanol (50 ml) is stirred for two hours at room temperature. The reaction time was determined by thin layer chromatography, the solvent system chloroform-butanol (9: 1), the developer - iodine vapour. After the reaction, the ethanol was evaporated in vacuum. The residue was transferred to cold water (50 ml) acidified with hydrochloric acid. The precipitate is filtered off and dried. Further reactions can be used without further purification. If necessary, recrystallized from ethanol. The melting point 217–220 °C corresponds to the literature [18]. Yield 94 %.

B. To a freshly prepared solution of sodium methylate (0.62 g, 27 mmol sodium / 100 ml anhydrous methanol) was added 6-methyl-2-thiopyrimidin-4(3H)-one (1) (3.50 g, 24.6 mmol) and heated the reaction mixture to 40 °C, then methyl iodide (6.53 ml, 24.6 mmol) was added. The mixture was refluxed for 3 hours, naturally cooled and left for 12 hours. The precipitate was washed with methanol and dried. The yield was 92 %, melting point 217–220 °C corresponds to the literature.

General method of synthesis of 6-methyl-2-arylaminopyrimidin-4(3H)-one (3.1-3.9).

A mixture of 0.01 mol (1.56 g) of 6-methyl-2-methylthiopyrimidin-4(3H)-one (2) and 0.01 of the corresponding arylamine was heated to 140 °C until methanethiol had ceased to secrete. The resulting residue is gradually triturated with ethanol and the formed precipitate is filtered off. Wash twice with 20 ml ethanol, dry and crystallize from dimethylformamide. Compound **3.1** has been described in the literature [19].

Molecular docking

Flexible molecular docking was performed using AutoDock Vina. The ability of the docking algorithm used to reproduce the experimental data was evaluated by reference to the native ligand annealing. As biological targets were used macromolecules from Protein Data Bank (PDB) [20]: GABA$_A$R (PDB ID – 4COF), GABA$_{AT}$ (PDB ID -1OHW).

Preparation of ligands

Structures 3.1-3.9 were obtained using MarvinSketch 18.23 and saved in mol format. Optimized by Chem3D using molecular-mechanical MM2 algorithm and saved as pdb files. Using AutoDockTools-1.5.6, the pdb files have been converted to PDBQT [21].

Preparation of proteins

PDB files are downloaded from the PDV protein database. Discovery Studio Visualizer 2017 / R2 was used to remove water and ligand molecules from the crystal. Protein structures were saved as pdb files. In AutoDockTools-1.5.6, polar hydrogen is added to the protein structure and stored as PDBQT. Grid boxes were installed on native ligands. AutoDock Vina was used for docking [21]. Discovery Studio V17.2.0.16349 was visualized and analysed for the obtained docking results.

Anticonvulsant activity.
Animals

Anticonvulsant activity was studied in adult Wistar rats of two genders (150-170 g) obtained from the vivarium of the "Institute of Pharmacology and Toxicology of AMS of Ukraine". The animals were kept at the vivarium of National Pirogov Memorial Medical University, Vinnytsya. All animals were acclimatized for a period of ten days before the start of the experiments. During the experiments, the animals were in vivarium at t = 19–24 °C, humidity not more than 60 %, natural "day/night" light mode in polypropylene cages on a standard diet with free access to water and food [22]. The studies were conducted in accordance with the principles of Directive 210/63 / EU of the European Parliament and the Council of the EU "On the protection of animals used for scientific purposes" (Brussels, 2010) and "General ethical principles for animal experimentation" (Kyiv, 2001), Law of Ukraine "On the Protection of Animals from Cruelty" No. 3477-IV of February 21, 2006, as amended, and the Order of the Ministry of Youth and Sports of Ukraine "On Approval of the Procedure for Scientific Experiments with Animals in Scientific Institutions" No. 249 of March 1, 2012.

Pentylenetetrazole seizures

Screening studies of potential anticonvulsants were performed within the international outsourcing program – Antiepileptic Drug Development Program [23]. The test compounds were administered at a dose of 80 mg/kg intramuscularly for 30 min before the introduction of pentylenetetrazole. The convulsive condition in animals was simulated by single subcutaneous administration of pentylenetetrazole ("Sigma", USA) at a dose of 80 mg/kg. After introduction of the convulsant, each animal was placed in a separate plastic cylindrical box with a diameter of 20 cm and a height of 35 cm. The animals were continuously monitored for 60 minutes. The manifestation of anticonvulsant action was evaluated by the dynamics of the latent, the nature and duration of the seizure in minutes, as well as the mortality rate. The severity of seizures was determined in points [24].

Statistical analysis

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

4. Result

The original 6-methyl-2-thiopyrimidin-4(3*H*)-one (1) was previously obtained by condensation of thiourea and acetoacetic ester in the medium of ethyl alcohol, respectively, in the presence of sodium hydroxide (Fig. 3) [15, 25].
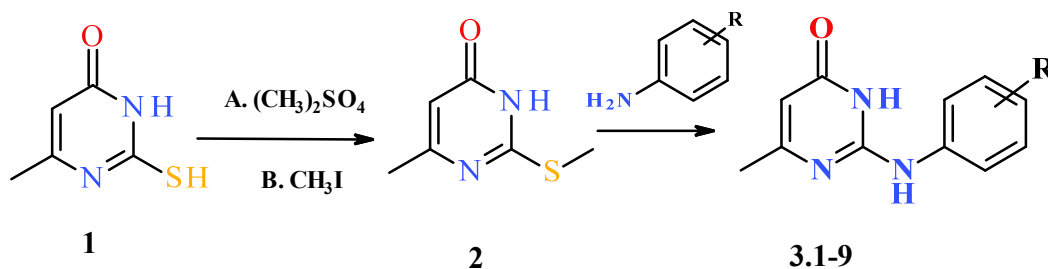
R= **3.1** H; **3.2** 4-Me; **3.3** 4-Et; **3.4** 4-Br; **3.5** 3-CF$_3$; **3.6** 3-OMe; **3.7** 4-OMe; **3.8** 3,4-diCl; **3.9** 3,4-Me

Fig. 3. The synthesis of 6-methyl-2-arylaminopyrimidin-4(3*H*)-one.

Methylation of 6-methyl-2-thiopyrimidin-4(3H)-one (**1**) was performed in two ways: using dimethyl sulfate (A) and methyl iodide (B), medium K_2CO_3 / EtOH and MeONa / MeOH, respectively. The yields of the reaction products were high in both cases, allowing the use of both reagents for alkylation of the thio group. By heating the resulting 6-methyl-2-methylthio-pyrimidin-4(3H)-one (**2**) with aromatic amines at 140 °C, the target 2-arylaminopyrimidines (**3.1-3.9**) were obtained. The choice of substituents in the phenyl radical was based on their own research findings on their effect on anticonvulsant activity [15, 17].

Synthesized compounds (**3.1-3.9**) are white crystalline substances with clear melting points, insoluble in water, readily soluble in dioxane and dimethylformamide. The structure of the synthesized compounds was proved by the method of 1H NMR spectroscopy, molecular masses and individuality – chromatomass spectrometry, composition – elemental analysis (Tables 1, 2).

Table 1

Physico-chemical characteristics of the synthesized of 2-arylamino-6-methylpyrimidin-4(3*H*)-one (**3.1-3.9**)

№ п/п	R	Yield %	MP ˚C	Gross formula	М$_M$	Calculated, % Found, %			LC/MS m/z, %
						C	H	N	
3.1*	H	80	255-7	C$_{11}$H$_{11}$N$_3$O	201.22	65.71 / 65.43	5.51 / 5.2	20.90 / 20.82	202.09 (96)
3.2	4-Me	78	225-7	C$_{12}$H$_{13}$N$_3$O	215.26	66.96 / 66.78	6.09 / 6.07	19.52 / 19.59	216.1 (96)
3.3	4-Et	77	207-9	C$_{13}$H$_{15}$N$_3$O	229.12	68.10 / 67.97	6.59 / 6.56	18.33 / 18.37	230.1 (97)
3.4	4-Br	81	248-50	C$_{11}$H$_{10}$BrN$_3$O	280.13	47.17 / 47.01	3.60 / 3.58	15.00 / 15.04	281.0 (96)
3.5	3-CF$_3$	66	240-2	C$_{12}$H$_{10}$F$_3$N$_3$O	269.23	53.54 / 53.47	3.74 / 3.72	15.61 / 15.60	270.0 (95)
3.6	3-OMe	81	194-6	C$_{12}$H$_{13}$N$_3$O$_2$	231.26	62.33 / 62.27	5.67 / 5.65	18.17 / 18.20	232.1 (96)
3.7	4-OMe	85	220-2	C$_{12}$H$_{13}$N$_3$O$_2$	231.26	62.33 / 62.30	5.67 / 5.66	18.17 / 18.18	-
3.8	3,4-diCl	84	255-7	C$_{11}$H$_9$Cl$_2$N$_3$O	270.11	48.91 / 48.82	3.36 / 3.35	15.56 / 15.58	271.2 (97)
3.9	2,3-Me	77	234-6	C$_{13}$H$_{15}$N$_3$O	229.28	68.10 / 67.96	6.59 / 6.57	18.33 / 18.37	-

*Melting point and the IR spectrum of compound **3.1** as described in the literature [Abdel-Samei]*

The character of the 1H NMR spectra of the synthesized compounds (**3.1-3.9**) fully corresponds to their structure: the singlet signal of the region 11.35–10.35 ppm is characteristic of the NHC = O proton in the third position of the pyrimidine cycle (lactam form); the proton of the NH group is fixed at the site of 8.95–8.22 ppm; the singlet signal of the methin proton at the 5 position of the pyrimidine cycle resonates in the region of 5.80–5.52 ppm; three protons of the methyl radical resonate as a singlet about 2.20–2.10 ppm; the character of the aromatic proton signals is characteristic of the location of the substituents in the phenyl ring.

Table 2

^{1}H NMR spectra of the synthesized 2-arylamino-6-methylpyrimidin-4(3*H*)-one (**3.1-3.9**)

Com-pound	NHCO, c, 1H	NH, c 1H,	Ar-H	CH-5, c, 1H	CH$_3$ c, 2H	Signals of other protons
3.1	10.51	8.95	7.66, 2H, d, *J*=8,1, H-2',6' 7.30, 2H, t, *J*=7.5, H-3',5' 7.01, 1H, t, *J*=7.2, H-4'	5,71	2.13	–
3.2	10.52	8.81	7.43, 2H, d, *J*=8.2, H-3',5' 7.09, 2H, d, *J*=8.2, H-2',6'	5,68	2.13	2.23. 1H, c, CH$_3$
3.3	10.35	8.39	7.47 2H, d, *J*=8.4, H-3',5' 7.09 2H, d, *J*=8.4, H-2',6'	5.52	2.14	2.62 q, 2H, CH$_2$ 1.22 t, 3H, CH$_3$
3.4	10.52	8.82	7.63 2H, d, *J*=8, H-3',5' 7.10 2H, d, *J*=8, H-2',6'	5.72	2.15	–
3.5	11.24	8.94	7.62-7.50 m, 2H, H-2`,4` 7.27-7.20 m, 2H, H-5`,6`	5.80	2.20	–
3.6	10.50	8.82	7.42 1H, s, H-2' 7.21 1H, t, *J*=8.2, H-5' 7.12 1H, d, *J*=7.5, H-4' 6.69 1H, d, *J*=7.5, H-6'	5.72	2.12	3.75 3H, s, OCH$_3$
3.7	10.25	8.22	7.48 2H, d *J*=8, H-3',5' 6,85 2H, d, *J*=8, H-2',6'	5.55	2.12	3.75 3H, s, OCH$_3$
3.8	10.60	8.88	8.02 1H, s, H-2' 7.52 1H, d, *J*=7.5, H-5' 7.32 1H, d, *J*=7.5, H-6'	5.65	2.20	–
3.9	10.52	8.81	7.52 1H, *J*=8, H-4' 7.02 1H, t, J=7.8, H-5' 6.90 1H, d, *J*=8, H-6'	5.55	2.10	2.30, 3H, s, CH$_3$ 2.19, 3H, s, CH$_3$

The appropriateness of the anticonvulsant activity study was further evaluated using molecular docking tools in biotargeting. The synthesized compounds are structural analogues of substances that exhibit activity on the pentylenetetrazole seizures model. Mechanism of convulsive action of PTZ is caused by blocking of chlorine ionophore of GABA$_A$-benzdiazepine complex [10]. The effect of arylamines (**3.1-3.9**) on the GABA-ergic system is predicted by their affinity for the allosteric site of the GABA$_A$ receptor (PDB 4COF) [26] and the GABA aminotransferase enzyme GABA$_{AT}$ (PDB-1OHW) [27]. The ability of the docking algorithm to reproduce the experimental data was confirmed by the estimation of the affinity and conformation of the native ligands of benzamidine (BA), a new GABA$_A$R agonist, and vigabatrin (VGN) with active GABA$_A$R and GABAAT sites, respectively (Figs. 4–6). Another reference ligand was phenobarbital (PHB) as an analogue in structure with GABAergic mechanism of action. Binding energy (scoring function) became a quantitative characteristic (Table 3). 3D visualization of the reference and the studied conformations on the example of 4-methylphenyl derivative (**4.2**) is presented in Fig. 4, 5: hydrogen bonds are indicated by green dotted lines, hydrophobic interactions – purple dotted lines.

Table 3

Binding energy (kcal/mol) ligands **3.1-3.9** with active site of GABA$_A$ and GABA$_{AT}$

Targets	Ligands									PHB	VGN	BA
	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9			
GABA$_A$R	–6.5	*–8.0*	–6.5	–6.8	–5.4	–5.9	*–7.1*	–7.0	–5.4	–7.2	–	–8.5
GABA$_{AT}$	–6.0	*–6.4*	–6.0	–6.4	–6.4	–6.4	*–7.2*	–6.8	–6.7	–8.5	–8.4	–

The binding energies of all the synthesized ligands, although illustrating the affinity for GABA biomass, are higher than the binding energies of the comparison drugs (Table 3). The best scoring function with both targets was calculated for the 4-methoxy substituted derivative (**3.7**): -7.2 against 8.5 kcal / mol in benzamidine in the case of GABA$_A$R and -7.2 against -8.4 kcal / mol in vigabatrin when docked in GABA=. The lowest binding energies are predicted for the CF3 substituted derivative (**3.5**). A detailed comparative analysis of the conformations of the ligands (**3.1-3.9**) in the active site GABA$_A$R showed that immersion in the pocket occurs aryl fragment (Fig. 4d), which reacts hydrophobically with tyrosine residues (Tyr62A, 205B) and phenylalanine (Phe200B). These residues in the experiment interact with the native ligand (Fig. 4).
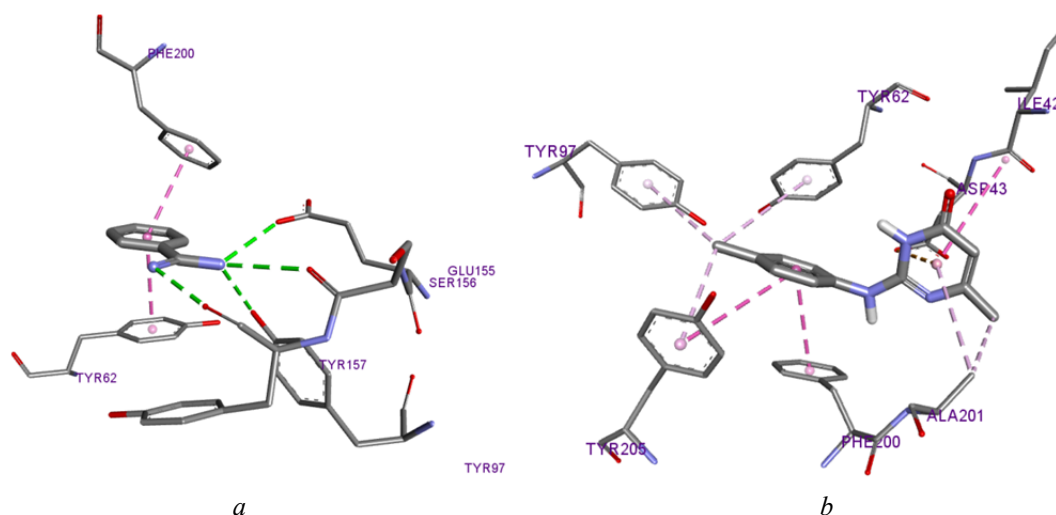
a   *b*

Fig. 4. 3D interaction between GABA$_A$: *a* – with BA; *b* – with ligand **3.2**

The pyrimidine moiety remains in the input portion of the hydrophobic pocket, and the protein moieties asparagine (Asp43A), isoleucine (Ile42), alanine (Ala201) are not positioned in the experiment as amino acids of the active site. At the simultaneous placement in the active site of BA, PHB and the inves-tigated ligands (**3.1-3.9**) (Fig. 5a) it is evident that the pyrimidine moiety (yellow molecule) is only partially immersed in the active site and does not overlap even with the pyrimidine cycle of phenobarbital, which also occupies a slightly "shallower" position compared to benzamidine.
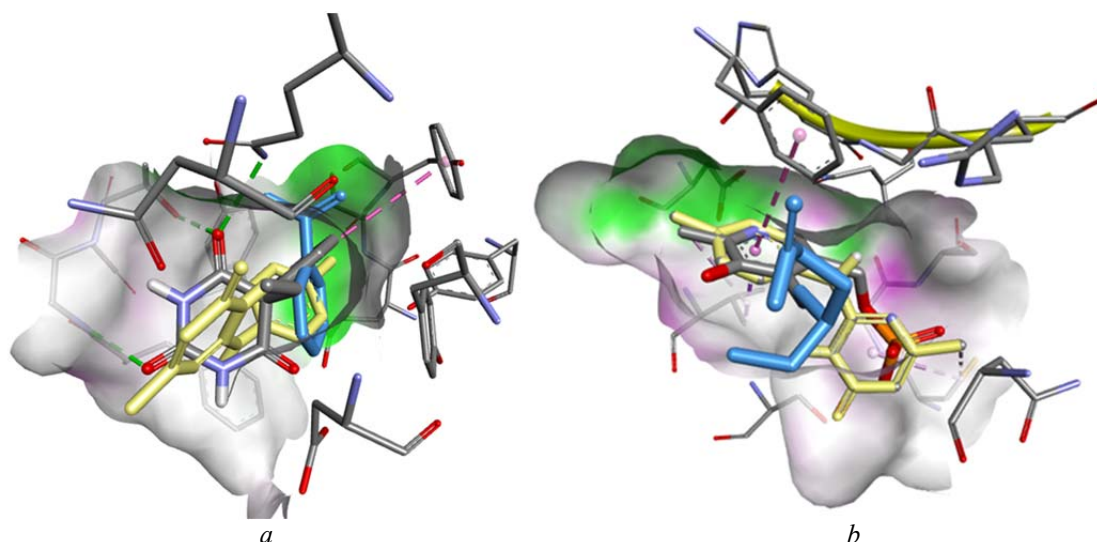

a   *b*

Fig. 5. 3D joint conformation: *a* – BA (blue), PHB (gray) and ligand **3.2** (yellow) in the active site of GABA$_A$R; *b* – VGB (blue), ligand 3.2 (yellow) and PLP (gray) in the active site of GABA$_{AT}$

In a detailed analysis of the position of the ligands (**3.1-3.9**) in the active site of GABA-AT, it should be noted that only two amino acid residues interacting with the vigabatrin – valin (Val300) and phenylalanine (Phe189) – are involved in the hydrophobic interaction. Other interactions occur with amino acids, which in the experiment capture the cofactor GABA-AT – pyridoxal-5-phosphate (PLP) – threonine (Thr353) and cysteine (Cys135). At the simultaneous placement in the active site of VGN, PLP and the investigated ligands (**3.1-3.9**) (Fig. 4b), it is evident that the pyrimidine cycle is immersed in the hydrophobic pocket much deeper than the vigabatrin and overlaps with the pyrimidine cycle of PLP. This conforma-tional arrangement in the active sites cannot be considered optimal, but does not exclude the possible agonistic effect on GABA$_A$ and inhibitor one on GABA-AT.
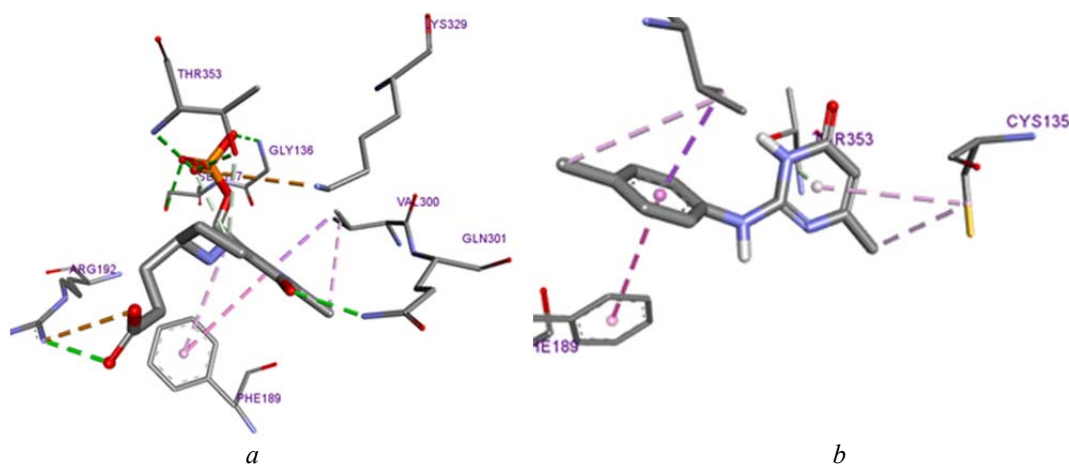
a   *b*

Fig. 6. 3D interaction between GABA$_{AT}$: *a* – with VGN and PLP: *b* – with ligand **3.2**

Thus, given the ambiguous molecular docking rates for screening for anticonvulsant activity on the PTZ model, we selected only 4 compounds out of 9, which showed relatively better docking results with both biotargets. In addition, compound 2 was explored to better understand the effect of substituents on activity.

All of the studied phenylamines (3) did not show a significant anticonvulsant effect: seizures occurred on the background of PTZ and tested compounds administration, and significant mortality was observed (Table 1). In all animals of the control group, PTZ administration induced a convulsive condition (Table 4), which was accompanied by pronounced tonic-clinical convulsions and 100 % mortality. Phenobarbital significantly prevented the lethality and development of convulsive syndrome, while lamotrigine partially protected the animals from the chemotoxic effect of pentylenetetrazole, leaving convulsive twitching, jumps and tonic contractions of the forelimbs. Lamotrigine significantly prolonged the latency period 5.8 times, decreased the severity and duration of seizures relative to control, and the mortality rate was 20 % of animals.

According to the screening results, only 4-methoxyphenylamine (**3.7**) according to the criterion of the integral protective indicator - a decrease in mortality compared to control statistically significantly prevented mortality in 100 % of animals. However, it was inferior to the comparison drugs in all indicators of the convulsive syndrome (Table 4). 4-bromophenylamine (**3.4**) also reduced the mortality rate by up to 20 % and extended the latency period twice, reduced the intensity and severity of seizures.

Table 4

Influence of the 2-arylamino-6-methylpyrimidin-4(3*H*)-one on the pentylenetetrazole-induced seizures in rats

Groups of animals	Number of rats	Dosemg/kg	Duration of the latent period, min	Duration of seizures, min	Lethality abs. units (%)	Intensity of seizures, (points)
Control	10	–	4.7±0.30	9.70±0.90	10 (100 %)	4,96
2	5	80	9,0± 1,5*	18,6±3,7*	5 (100 %)	4,9
3.1	5	80	8,8±1,1*	12,6±2,9	3 (60 %)	4,4
3.4	5	80	10.2±3.6*	4.4±1.3*	1 (20 %)	2,0
3.7	5	80	12,8±1,7*	8,2±1,2*	0*	2,4
3.8	5	80	6,0±0,7	7,0±1,0*	5 (100 %)	4,6
PNB	5	20	30.0±0.0*	0*	0*	0
Lamotrigine	5	20	27.6±0.8*	2.40±0.40*	1 (20 %)	2.20

*Footnotes: * – compared to control group indicators p<0.05*

5. Discussion

The complete reproduction of the conditions of the previous pharmacological experiment allows us to compare the results and discuss some regularities of the influence of molecule fragments on anticonvulsant activity. As with the acetanilide derivatives of thiopyrimidine-4(3*H*)-one [15], among phenylamines (**3**), the best performance also showed 4-Br and 4-OMe substituted derivatives, however, they have significantly lower rates of protection against PTZ seizure, which in turn allows us to predict the positive effect of thioacetamide. Once again, the positive effect of the phenyl moiety itself became apparent, as compound 2 proved to be completely indifferent to PTZ seizure protection. The lack of seizure protection and 100 % lethality of animals using 2,4-dichlorophenylamine (**3.8**) is completely correlated with the results regarding the negative effect of chlorine atoms in the phenyl radical and the increase in mortality of the experimental animals in the presence of chlorine atoms in the phenyl radical [15, 17].

A positive correlation was found between *in vivo* studies on PTZ seizure model and scoring functions and conformational placement of ligands in the active

GABA$_A$ site and the GABA$_{AT}$ enzyme, which substantiates the feasibility of target-based virtual screening to streamline pharmacological screening.

6. Conclusions
6-methyl-2-arylaminopyrimidin-4 (3H) -one was synthesized as structural analogues of phenobarbital and promising anticonvulsants. According to the results of the docking of the investigated ligands into active sites of GABA$_A$ receptor and GABA$_{AT}$ in comparison with benzamidine, phenobarbital and vigabatrin compounds for pharmacological screening were selected. Only 2 compounds tended to have an anticonvulsant effect on the PTZ seizures model in rats, preventing mortality in 100 % of cases and prolonging the latency period. The established relationship between the conformational placement of ligands in active GABA$_A$ receptor sites and GABA$_{AT}$ and screening data allows us to recommend the presented docking methodology as a tool for streamlining

screening on the PTZ seizures model. The positive role of thioacetamide and phenyl fragments, as well as 4-Br, 4-MeO radicals in the manifestation of anticonvulsant activity, was proved.

Study Limitations. Using a different docking methodology or other computer programs for docking data of scoring functions may be different.

Prospects for further research. According to current approaches to the search for new antiepileptic drugs [28] despite the lack of significant results on the PTZ seizures model for this group of compounds screening for the maximum electroshock (MES) model and the psychomotor (6 Hz) model of seizures in mice is recommended as this techniques characterize different mechanisms of anticonvulsant action.

Conflict of interests
There are no conflicts of interest regarding this study.

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