

ORIGINAL ARTICLE

Promising anticonvulsant N-[(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1*H*-quinazolin-3-yl) acetamide: dose-dependent study and evaluation of anticonvulsant action spectrum *in vivo* and *in silico*

Slibné antikonvulzivum N-[(2,4-dichlorfenyl) methyl]-2-(2,4-dioxo-1*H*-chinazolin-3-yl) acetamid: studium působení v závislosti na dávce a hodnocení spektra antikonvulzivního účinku *in vivo* a *in silico*

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Summary

The anticonvulsant spectrum of the original promising anticonvulsant N-[(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1*H*-quinazolin-3-yl) acetamide was studied. The compound had a pronounced anticonvulsant effect, significantly reducing the mortality of mice in models of seizures induced by pentylenetetrazole, picrotoxin, strychnine, and caffeine. In the thiosemicarbazide-induced seizure model, the test compound did not reduce mortality. The obtained results indicated that the mechanism of anticonvulsant action involved GABA-ergic (effective in models of pentylenetetrazole and picrotoxin-induced seizures), glycinergic (efficiency in the strychnine model of paroxysms), and adenosinergic (effectiveness in the model of caffeine-

induced seizures). Molecular docking of a promising anticonvulsant to anticonvulsant biotargets follow the mechanisms of chemo-induced seizures, namely GABA, glycine, and adenosine receptors type A₂A, GABA_{AT}, and BCAT enzymes. The conformity between *in vivo* and *in silico* studies results was revealed.

Key words: quinazoline • acetamide • dose • anticonvulsant effect • GABA receptor • A₂A receptor • Gly receptor • docking

Souhrn

Bylo studováno antikonvulzivní spektrum původního slibného antikonvulziva N-[(2,4-dichlorfenyl)methyl]-2-(2,4-dioxo-1*H*-chinazolin-3-yl) acetamidu. Sloučenina vykazovala výrazný antikonvulzivní účinek a významně snižovala mortalitu myší na modelech záchvatů vyvolaných pentylenetetrazolem, pikrotoxinem, strychninem a kofeinem. Na modelu záchvatů vyvolaných thiosemikarbazidem testovaná sloučenina mortalitu nesnížila. Získané výsledky naznačily, že mechanismus antikonvulzivního účinku zahrnuje GABA-ergní (účinnost v modelech záchvatů vyvolaných pentylenetetrazolem a pikrotoxinem), glycinergní (účinnost v modelu paroxysmů vyvolaných strychninem) a adenosynergí (účinnost v modelu záchvatů vyvolaných kofeinem). Molekulární dokování slibného antikonvulziva k antikonvulzivním biologickým cílům bylo v souladu s mechanismy chemoindukovaných záchvatů, konkrétně GABA, glycine a adenosinových receptorů typu A₂A, GABA_{AT} a enzymů BCAT. Byla zjištěna shoda mezi výsledky studií *in vivo* a *in silico*.

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Klíčová slova: chinazolin • acetamid • dávka • antikonvulzivní účinek • GABA receptor • A_{2A} receptor • Gly receptor • dokování

Introduction

Effective and safe treatment of epilepsy remains a problematic issue in modern medicine, as a significant proportion of patients – up to 30% according to the latest data – have drug resistance^{1, 2)}. On the one hand, ways to solve this problem are using adjuvants that modulate antiepileptic drugs (AEDs) and, on the other, innovative AEDs development.

Quinazolines are one of the promising classes of compounds for potential anticonvulsants creation. In previous studies, we found that among the 20 original acetamide derivatives of 2,4-dioxo- and 4-oxo-2-thioxo-quinazoline synthesized at the National University of Pharmacy, N-[(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1H-quinazolin-3-yl) acetamide occurred to be the most effective anticonvulsant (Fig. 1), on the base screening seizure model caused by pentylenetetrazole (PTZ). A dose of 100 mg/kg provides an apparent protective effect, which is not inferior to sodium valproate at 300 mg/kg^{3, 4)}.

This compound has been shown to have an acceptable acute toxicity level (Class V – virtually non-toxic), exhibits anxiolytic and antidepressant properties, and does not impair muscle tone and coordination⁵⁾. This indicates the feasibility of in-depth studies of its primary pharmacodynamics – anticonvulsant action, in particular its dose-dependence and spectrum, as well as possible mechanisms.

The aim of the study is to determine the dependence of the anticonvulsant action of N-[(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1H-quinazolin-3-yl) acetamide on doses using PTZ-induced seizures in mice, as well as to study the spectrum of anticonvulsant activity in seizure models induced by picrotoxin, strychnine, caffeine, thiosemicarbazide, and maximal electroshock. Moreover, the study focused on predicting the affinity for anticonvulsant biotargets by molecular docking and determining the accordance of results *in silico* and *in vivo* studies.

Experimental part

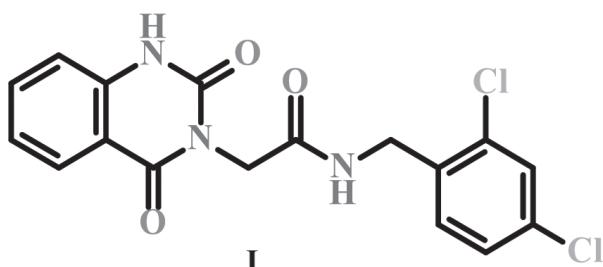


Fig. 1. Chemical structure of N-[(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1H-quinazolin-3-yl) acetamide

Materials

The dose-dependent effect of N-[(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1H-quinazolin-3-yl)acetamide (laboratory name quinazoline derivative I) was determined in the basic model of PTZ-induced seizures. Quinazoline derivative I was synthesized at the Department of Pharmaceutical Chemistry of the National University of Pharmacy (NUPh), Kharkiv, Ukraine.

The quinazoline derivative I was administered intragastrically (IG) at doses of 50 mg/kg, 100 mg/kg, and 150 mg/kg as a Tween® 80 stabilized thin suspension in a volume of 0.1 ml per 100 g weight. The dose range of quinazoline derivative I was chosen based on screening results in the PTZ-induced seizure model³⁾, according to which strong anticonvulsant properties were detected at 100 mg/kg. PTZ (Corazol, Sigma, USA) at a dose of 90 mg/kg subcutaneously (SC), picrotoxin (Sigma, USA) at a dose of 3 mg/kg SC, strychnine (Sigma, USA) at a dose of 1.2 mg/kg SC, caffeine-sodium benzoate (Darnitsa, Ukraine) at a dose of 650 mg/kg intraperitoneally (IP), thiosemicarbazide (Sigma, USA) at a dose of 25 mg/kg IP in the form of aqueous solutions were used as convulsants with different mechanisms of action. A maximal electroshock seizure (MES) model was also used, for which the mice were exposed to a current of 50 mA and a frequency of 50 Hz for 0.2 s using corneal electrodes^{6–8)}. The minimum dose of quinazoline derivative I was used in these experiments (100 mg/kg IG), which had the most pronounced anticonvulsant effect in PTZ-induced seizures.

Reference drugs and substances:

- Sodium valproate (Depakin, Sanofi-Aventis, France) at a dose of 300 mg/kg IG in models of PTZ, picrotoxin, thiosemicarbazide seizures, and MES;
- Aqueous glycine solution (Sigma, USA) 50 mg/kg IP as a specific agonist in the strychnine-induced seizure model;
- Inosine (Riboxin, Arterium, Ukraine) 1000 mg/kg IP as an agonist of adenosine receptors in the model of caffeine-induced seizures^{8–10)}.

Methods

• *In vivo* study

Study design

After chemoconvulsant administration, the animals were immediately placed into separate transparent plastic cylindrical containers (diameter – 15 cm; height – 30 cm) and continuously monitored for 60 min (240 min in the model of thiosemicarbazide-induced seizures since their course is long with slow development due to a gradual decrease in the content of GABA in the brain). The following indicators evaluated the effectiveness of anticonvulsant drugs and their combinations: latency period of first convulsions (latency), the number of clonic-tonic seizures per 1 mouse, percentage of animals in the group separately with clonic and tonic convulsi-

ons, the severity of seizures – in points (1 point – single tremors, 2 points – "manege" running or "kangaroo" position, 3 points – clonic convulsions without lateral position, 4 points – clonic-tonic convulsions with lateral position, 5 points – a tonic extension of hind limbs, and 6 points – tonic extension, which led to the death of the animal), duration of convulsive period (period of seizures), the life expectancy of animals to death (time to death), and lethality^{8–10}. If seizures were not observed for 1 h, the latency was considered to be 60 min (240 min in the model of thiosemicarbazide-induced seizures). The percentage of mice with clonic and tonic seizures, the severity of seizure scores, the duration of the seizure period, the time spent in the lateral position, and the lifetime were determined in the MES model.

Experimental animals and grouping

The experiments were performed at the Educational and Scientific Institute of Applied Pharmacy of the National University of Pharmacy (Kharkiv, Ukraine) following the EU Directive 2010/10/63 EU on animal experiments with bioethical principles. The experiments were carried out in accordance with the Animal (Scientific Procedures) Act 1986 and the principles and requirements of the EU Directive 2010/63/EU. (2010) on the protection of animals used for scientific purposes. The experiments were conducted on 118 random-bred male albino mice weighing 20–25 g, which were kept on a standard vivarium diet with free access to water under controlled conditions (20–24 °C and 50% relative humidity in a well-ventilated room with a 12 h light/dark cycle with free access to food and water). The animals were randomly divided into groups for research

on different pathochemical mechanisms of seizures (Table 1).

Statistical analysis

Statistical analysis was conducted using the program Statistica 10.0. The results were expressed as mean ± standard error of the mean. The level of statistical significance was considered as $p < 0.05$. Statistical differences between groups were analyzed using a non-parametric Kruskal-Wallis H-test. Fisher's angular transformation was used for the results in the alternative form (lethality, percentage of mice with clonic and tonic convulsions). Quantitative data are given as means with standard errors of the mean ($M \pm m$) and medians with 25% and 75% percentiles ($Me [Q25; Q75]$).

• Molecular docking

AutoDock Vina and AutoDockTools 1.5.6¹¹ were used for docking. ASUS VivoBook X530UN S15 hardware with 64-bit Intel Core i7 8550U 8th generation operating system and Windows 10. Bio-target macromolecules used with Protein Data Bank¹². PDB ID: GABA_A – 4COF and 6HUP; GABA_{AT} – 10HW; hBCATc – 2COI; GlyR – 5VDH; A₂A – 3EML. The design of ligand structures was performed using the program BIOVIA Draw 2017R2 and stored in mol format. The structures were optimized by Chem3D using the molecular mechanical algorithm MM2, saved in .pdb format, and converted to .pdbqt¹¹ using AutoDockTools-1.5.6. Discovery Studio Visualizer 2017/R2 was used to remove solvent and native protein-ligand. The prepared macromolecule was saved in .pdb format. In AutoDockTools-1.5.6, polar hydrogen atoms were added to the protein structure

Table 1. Mice grouping to study the anticonvulsant activity of N-[(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1H-quinazolin-3-yl) acetamide under different models of seizures

Convulsant, dose, route of administration	Groups, impact, number of animals			
	Group 1 (control)	Tested compound (TC), reference drug (RD), dose, route and time of administration before the convulsant		
		Group 2, n = 6	Group 3, n = 6	Group 4, n = 6
Pentylenetetrazole 90 mg/kg SC (the anticonvulsant effect dose-dependence)	Purified water IG (0.1 ml per 10 g) 30 minutes before the convulsant, n = 6	TC 50 mg/kg IG 30 min	TC 100 mg/kg IG 30 min	TC 150 mg/kg IG 30 min
Picrotoxin 3 mg/kg SC		RD sodium valproate 300 mg/kg IG 30 min	–	–
Strychnine 1.2 mg/kg SC		RD glycine 50 mg/kg IP 30 min	–	–
Caffeine 650 mg/kg IP		RD inosine 1000 mg/kg IP 30 min	–	–
Thiosemicarbazide 25 mg/kg IP		RD sodium valproate 300 mg/kg IG 30 min	–	–
Maximal electroshock seizure (MES)		Purified water IG (0.1 ml per 10 g) 30 minutes before MES test, n = 10	RD sodium valproate 300 mg/kg IG 30 min	–

IG – intragastrically, SC – subcutaneously, IP – intraperitoneally

and saved in .pdbqt format. The size of the Grid box and its center was determined by the native ligand: PDB ID 4COF: $x = -3.45$, $y = -31.27$, $z = 118$; size $x = 30$, $y = 32$, $z = 84$; PDB ID 6HUP: $x = 117.44$, $y = 157.46$, $z = 110.46$; size $x = 26$, $y = 28$, $z = 20$; PDB ID 10HW: $x = 9.75$, $y = -0.87$, $z = 20.85$; size $x = 28$, $y = 24$, $z = 28$; PDB ID 5VDH: $x = 480$, $y = 87.4$, $z = 446.16$; size $x = 28$, $y = 24$, $z = 28$; PDB ID 3EML: $x = -9.06$, $y = -7.14$, $z = 55.86$; size $x = 12$, $y = 10$, $z = 16$; PDB ID 2COI: $x = -23.53$, $y = 4.14$, $z = -12.64$; size $x = 32$, $y = 26$, $z = 24$.

Validation of the used docking methodology was performed by redocking the native ligand and comparing its conformational arrangement with the experimentally determined one. The value of the standard deviation (RMSD) between the two conformations did not exceed $< 2 \text{ \AA}$, which confirms the validity of the methodology. The standard deviation calculation during the method validation was carried out using the online resource ProFit Results. Visualization and analysis of the obtained docking results were performed by Discovery Studio V17.2.0.16349.

Results

In vivo study

Dose-dependent study

The results of the dose-dependence study of the anticonvulsant effect of quinazoline derivative I in the basic model of PTZ-induced seizures are shown in Table 2. In the group of control pathology, there were typical severe seizures with tonic extension, which averaged 10 min and led to the death of all animals. The significant anticonvulsant effect was caused by

the lowest tested dose of quinazoline derivative I – 50 mg/kg: the latency of seizures tended to increase, 2 out of 6 animals survived the most dangerous tonic extension, and mortality decreased by 33% ($p < 0.05$). Increasing the dose to 100 mg/kg significantly enhanced the protective effect: the latency of seizures increased more than 2 times ($p < 0.05$), and their severity decreased by almost 2 points ($p < 0.01$) due to the reduction of tonic seizures ($p < 0.05$), and mortality decreased by 83% ($p < 0.01$), which corresponds to the results of the previous studies³. Features of the quinazoline derivative I anticonvulsant effect at a dose of 100 mg/kg deserve special attention. An increased period of seizures ($p < 0.05$ when compared with a dose of 50 mg/kg) may give a false impression of a worsening of the convulsive syndrome course. But the studied compound in a dose of 100 mg/kg, on the contrary, renders a pronounced anticonvulsant effect. Under the influence of a dose of 100 mg/kg, only 1 animal out of 6 died (17% vs. 67%, respectively, $p < 0.05$). In the 5 surviving animals, less severe convulsions still continued, while in the control and in the 50 mg/kg dose group, most of the mice had already died. That is why prolonging the convulsive period is not an unfavorable sign in this case. This corresponds to a significant increase in the latent period of the first attacks, a significant decrease in animals with lethal tonic extension, a decrease in the severity of seizures, and a significant decrease in lethality. A further increase in dose to 150 mg/kg reduced the severity and number of seizures, but there were no statistically significant differences in seizure rate and mortality at 100 mg/kg. Thus, a 100 mg/kg dose was selected as effective for further studies.

Table 2. Dose-dependent anticonvulsant action of N-[(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1H-quinazolin-3-yl) acetamide study in the model of pentylenetetrazole seizures in mice ($M \pm m$, $Me[Q25; Q75]$)

Group of animals	n	Dose, mg/kg	Latency, min	Number of clonic-tonic seizures in 1 mouse	% of mice with convulsions		Severity of seizures, points	Period of seizures, min	Time to death, min	Lethality, %
					clonic	tonic				
Control	6	–	2.92 ± 0.44 2.79 [2; 352]	2.50 ± 0.22 2.50 [2; 3]	100	100	6.00 ± 0.00 6 [6; 6]	7.04 ± 0.72 7.48 [5.75; 7.98]	9.96 ± 0.99 9.59 [9; 11.02]	100
Tested compound	6	50	6.21 ± 2.17 5.31 [1.57; 9.67]	2.33 ± 0.33 2.50 [2; 3]	100	100	5.33 ± 0.42 6 [4; 6]	4.01 ± 1.35 4.95 [1.1; 6.28]	9.10 ± 0.90 9.38 [7.66; 1.54]	67*
	6	100	$7.07 \pm 1.61^*$ 5.42 [4.1; 11.55]	1.83 ± 0.40 1.5 [1; 3]	100	67**	$4.17 \pm 0.48^{**}$ 4 [3; 5]	10.09 ± 3.26 13.31 [0.15; 15.07]	16.00^{\wedge} 16.00 [16; 16]	17**#
	6	150	13.27 ± 9.35 4.25 [3.40; 5.22]	$1.17 \pm 0.31^*$ 1.00 [1; 2]	83	67**	$3.50 \pm 0.81^*$ 4 [3; 4]	$2.91 \pm 1.58^*$ 0.9 [0.15; 6.75]	5.05^{\wedge} 5.05 [5.05; 5.05]	17**#

* $p < 0.05$ when compared with control, ** $p < 0.01$ when compared with control

$p < 0.05$ when compared with N-[(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1H-quinazolin-3-yl) acetamide in a dose of 50 mg/kg

^ time to death in 100 mg/kg and 150 mg/kg is the value of one surviving mouse

Picrotoxin-induced seizures

In the model of picrotoxin-induced seizures, the pathogenesis of which is based on the blockade of chlorine channels GABA/barbiturate/benzodiazepine receptor complex¹³⁾, the test compound showed pronounced

anticonvulsant properties, almost not inferior to sodium valproate (Table 3).

Strychnine-induced seizures

In a model of strychnine seizures, having a mechanism

Table 3. Study of anticonvulsant effect of *N*-(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1*H*-quinazolin-3-yl) acetamide in the different chemoinduced seizure models in mice ($M \pm m$, Me[Q25; Q75])

Group of animals	n	Dose, mg/kg	Latency, min	Number of clonic-tonic seizures in 1 mouse	% of mice with convulsions		Severity of seizures, points	Period of seizures, min	Time to death, min	Let-hality, %
					clonic	tonic				
Picrotoxin – induced seizures										
Control	6	–	11.76 ± 1.61 9.84 [9.4; 16.2]	4.67 ± 0.88 4.5 [3; 6]	100	100	5.67 ± 0.33 6.00 [6; 6]	9.28 ± 2.44 8.47 [6.33; 14.55]	21.67 ± 2.49 24.67 [17.17; 25.55]	83
Tested compound	6	100	33.61 ± 8.67* 26.6 [15.15; 60]	1.33 ± 0.49** 1.5 [0; 2]	67*	17**	2.67 ± 0.95* 3.00 [0; 4]	8.33 ± 3.92 5.84 [0; 16.70]	35.67 35.67 [35.67; 35.67]	17**
Sodium valproate	6	300	36.67 ± 7.72* 29.76 [20.55; 60]	0.83 ± 0.31** 1.00 [0; 1]	67*	17**	2.17 ± 0.70** 3.00 [0; 3]	0.62 ± 0.46 0.15 [0; 0.5]	–	0**
Strychnine – induced seizures										
Control	6	–	6.62 ± 0.89 6.96 [4.6; 8.63]	1.00 ± 0.00 1.00 [1; 1]	100	100	6.00 ± 0.00 6.00 [6; 6]	0.32 ± 0.07 0.25 [0.20; 0.40]	6.94 ± 0.94 7.21 [4.80; 9.03]	100
Tested compound	6	100	26.16 ± 10.72* 10.29 [9.15; 60]	0.67 ± 0.21 1.00 [0; 1]	67*	67*	4.00 ± 1.26 6.00 [0; 6]	0.28 ± 0.09 0.36 [0.00; 0.45]	9.65 ± 0.87 9.69 [8.54; 10.76]	67*
Glycine	6	50	10.27 ± 2.73 8.82 [5.50; 9.80]	1.00 ± 0.00 1.00 [1; 1]	100	83	5.17 ± 0.54 6.00 [4; 6]	0.48 ± 0.12 0.42 [0.35; 0.50]	8.20 ± 0.86 8.75 [6.35; 9.58]	83
Caffeine – induced seizures										
Control	6	–	4.81 ± 0.63 4.37 [3.50; 5.67]	5.83 ± 1.40 4.50 [4; 9]	100	100	6.00 ± 0.00 6.00 [6; 6]	23.24 ± 6.37 23.31 [11.85; 34.80]	28.04 ± 6.24 27.87 [19.33; 39.40]	100
Tested compound	6	100	21.70 ± 9.31* 9.30 [6.7; 39.77]	2.00 ± 0.73* 1.50 [1; 3]	83	67*	4.33 ± 0.99 5.50 [3; 6]	8.64 ± 4.06 7.06 [0.10; 14.20]	25.61 ± 2.24 23.85 [22.92; 30.05]	50**
Inosine	6	1000	19.44 ± 8.44* 10.89 [7.20; 21.45]	3.50 ± 1.09 4 [1; 5]	83	50**	3.67 ± 0.92 3.50 [3; 6]	23.40 ± 10.03 19.22 [0.15; 48.92]	29.69 ± 10.37 29.68 [19.32; 40.05]	33**
Thiosemicarbazide-induced seizures										
Control	6	–	72.80 ± 2.85 73.20 [65.8; 77.3]	3.17 ± 0.75 3.50 [1; 5]	100	100	6.00 ± 0.00 6.00 [6; 6]	29.32 ± 13.47 16.40 [0.9; 68.75]	102.11 ± 13.30 82.7 [81.33; 143.6]	100
Tested compound	6	100	79.79 ± 6.88 85.25 [73.52; 92.8]	1.83 ± 0.17 2.00 [2; 2]	100	100	6.00 ± 0.00 6.00 [6; 6]	34.02 ± 7.83 35.39 [30.35; 44.18]	113.81 ± 5.16 115.83 [107.05; 123.5]	100#
Sodium valproate	6	300	144.58 ± 13.50* 104.30 [92.90; 113.70]	1.83 ± 0.60 1.50 [1; 3]	83	83	4.83 ± 0.98 6.00 [5; 6]	16.21 ± 9.95 1.05 [0.55; 41.45]	109.48 ± 8.63 105.32 [98.03; 120.92]	67*

* p < 0.05 when compared with control, ** p < 0.01 when compared with control

p < 0.05 when compared with the corresponding reference drug

for reduction of glycinergic inhibition¹⁴⁾, quinazoline derivative I showed a strong protective effect. The latency increased by an average of 4 times ($p < 0.05$), both clonic and tonic seizures were reduced ($p < 0.05$), and mortality was significantly reduced by 33% ($p < 0.05$). Glycine in this model only tended to improve the rate of seizures and insignificantly reduced mortality by 17% (Table 3).

Caffeine-induced seizures

In a model of caffeine seizures that occur due to adenosinergic inhibition¹⁵⁾, the test compound caused a pronounced protective effect. The latency of seizures increased on average 4.5 times ($p < 0.05$), and the number of paroxysms decreased by almost three times ($p < 0.05$) due to tonic seizures, which reduced mortality by 50% ($p < 0.01$). The reference drug inosine reduced mortality by 67% ($p < 0.01$) without significant differences in seizures under the influence of quinazoline derivative I (Table 3).

Thiosemicarbazide-induced seizures

In a model of seizures caused by thiosemicarbazide, which inhibits glutamate decarboxylase by decreasing GABA and glutamate accumulation¹⁶⁾, the quinazoline derivative I showed no protective effect on the effect, contrary to sodium valproate, which almost doubled the latency ($p < 0.05$) and reduced mortality by 33% (Table 3).

MES seizure test

In the model of electro-induced seizures (Table 4), quinazoline derivative I significantly reduced seizure period ($p < 0.05$) and tended to mortality from 50% to 33%, contrary to sodium valproate, which provides a complete protective effect (mortality 0%).

In silico study

To predict the GABA-ergic mechanism of anticonvulsant action, the interaction of ligand I with active

sites of diazepam¹³⁾ and benzamidine¹⁷⁾ – positive allosteric modulators of the GABA_A receptor, and with the site of vigabatrin – an irreversible selective inhibitor of GABA-aminotransferase¹⁸⁾ were studied. Quantitative characteristics of affinity-binding energy (kcal/mol) and types of interaction with amino acid residues of the active site are given in 5.

The quinazoline derivative I showed an affinity for all GABAergic targets at a level above the values of reference ligands (Table 5). Visualization of compatible conformations with respect to native ligands allowed a detailed determination of the probability of exposure to the receptor (Fig. 2). In the benzamidine site, the GABA_A receptor (4COF) ligand I is fixed only at the entrance to the hydrophobic pocket with the participation of amino acid residues (Fig. 2a), which are not experimentally identified as the active site (Ala201 (2),

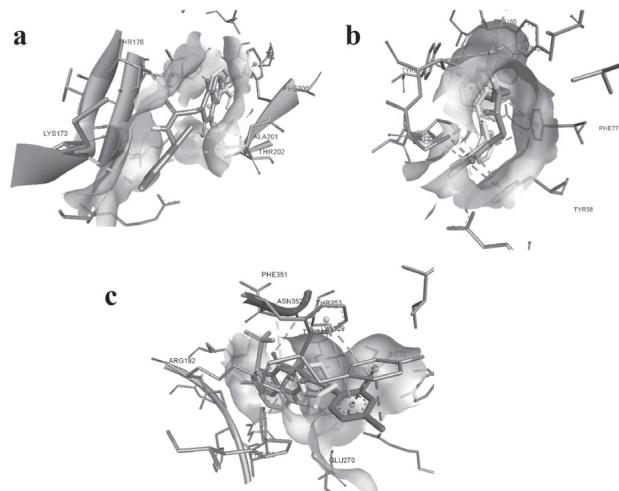


Fig. 2. 3D conformation of N-[(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1H-quinazolin-3-yl) acetamide (gray) **a** – with benzamidine and **b** – with diazepam (yellow molecules) in active GABA_A receptor sites, **c** – N-[(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1H-quinazolin-3-yl) acetamide (purple), vigabatrin (yellow) and PLP (blue) in the active site of the enzyme GABA_{AT}

Table 4. Anticonvulsant effect of N-[(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1H-quinazolin-3-yl) acetamide and sodium valproate in the MES test in mice ($M \pm m$, Me[Q25; Q75])

Group of animals	n	Dose, mg/kg	% of mice with convulsions		Severity of seizures, points	Period of seizures, sec	Recovery Period (Lateral Position), sec	Time to death, sec	Lethality, %
			clonic	tonic					
Control	10	–	100	100	5.40 ± 0.22 5.5 [5; 6]	29.40 ± 2.52 29.5 [22; 34]	70.20 ± 28.38 39 [33; 70]	29.40 ± 3.40 30 [24; 34]	50
Tested compound	6	100	100	100 [#]	5.33 ± 0.21 5 [5; 6]	20.67 ± 0.67 ^{*#} 20.5 [19; 22]	91.00 ± 6.93 91 [79; 103]	20.00 ± 1.00 20 [19; 21]	33 [#]
Sodium valproate	6	300	100	67 ^{**}	3.83 ± 0.31 4 [3; 4]	4.17 ± 1.97 [*] 2 [2; 3]	14.33 ± 12.13 10 [2; 36]	–	0 ^{**}

* $p < 0.05$ when compared with control, ** $p < 0.01$ when compared with control

[#] $p < 0.05$ when compared with the sodium valproate

Lys173*, Asn41*). At the site of the GABA_{AT} inhibitor (1OHW), the deep longitudinal placement of ligand I in the hydrophobic pocket is prevented due to the fact that it reaches the fixation site of the enzyme cofactor – pyridoxal-5-phosphate (PLP). The best affinity and conformational position of the quinazoline derivative I was predicted to the benzodiazepine site of the GABA_A receptor (6HUP): –10.6 vs. –9.9 kcal/mol in diazepam (Table 5). Stabilization of all molecule fragments by hydrophobic bonds, particularly the stacking interaction of the quinazoline ring with the hydroxyphenyl tyrosine moiety (Tyr58) and the tetrahedral network of bonds of the dichlorobenzyl moiety with tyrosine (Tyr210), suggests the possibility of a stable ligand receptor.

To confirm the glycinergic properties, the test ligand (quinazoline derivative I) was docked to the active site of the positive allosteric glycine receptor modulator (GlyR) – AM-3607 (5TIN)¹⁹. The low scoring function was predicted for the derivative I (Table 5), with the possibility of 10 hydrophobic bonds, including Pi-Pi stacking and T-like interactions with hydroxyphenyl tyrosine (Tyr161) and benzene ring phenylalanine (Phe32). The formation of a halogen bond with arginine carbonyl (Arg27) and additional fixation of the distal zone of the ligand due to the hydrogen bond between the amide and carboxyl groups of aspartic acid (Asp165). Complete immersion of quinazoline derivative I in the

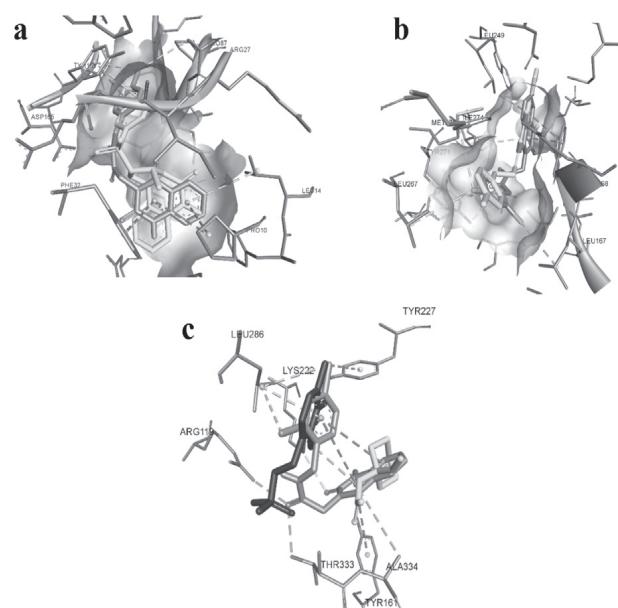


Fig. 3. Conformation visualization: **a** – N-[2,4-dichlorophenyl] methyl]-2-(2,4-dioxo-1H-quinazolin-3-yl) acetamide (blue) and positive allosteric modulator AM-3607 (yellow) at the Gly receptor site, **b** – N-[2,4-dichlorophenyl] methyl]-2-(2,4-dioxo-1H-quinazolin-3-yl) acetamide (gray) with ZM241385 (yellow) at the A₂A receptor site, **c** – gabapentin (yellow), ligand derivative I (blue) and PLP (purple) on the BCAT site

Table 5. Docking results of N-[2,4-dichlorophenyl] methyl]-2-(2,4-dioxo-1H-quinazolin-3-yl) acetamide and reference ligands in active sites of anticonvulsant biotargets

Target	Binding energy, kcal/mol	Hydrophobic interaction	Hydrogen bonds	Others	Reference ligand, kcal/mol
GABAergic biotargets					
GABA _A -site positive allosteric modulation	–8.7	TYR62, PHE200(2), ALA201(2)*, LYS173*	THR176, THR202, ASN41*	–	–8.5 benzamidine
GABA _A -benzodiazepine-site	–10.6	PHE77 (3), PHE100, HIS102	TYR58, HIS102 (2), AGN60, SER205	–	–9.9 diazepam
GABA _{AT}	–10.1	VAL300, TYR348(2)*, PHE189(2), PHE351(2)	ARG192, THR353, ASN352	LYS329, GLU270	–8.4 vigabatrin
Adenosinergic inhibition biotargets					
A ₂ A	–9.2	LEU249, PHE168 (2), TYR271, LEU267*, MET270, LEU167, ILE274	TYR271	–	–8.5 ZM241385
Glycenergic inhibition biotargets					
GlyR	–10.8	TYR161(3), PHE32, PRO87, TYR161, PRO10(2), LEU14, LEU83(2)	ASP165*	ARG27 Halogen	–11.4 AM-3607
Antiglutamate mechanism					
hBCATc	–7.4	Val175, Tyr90, Phe95, Tyr227	Arg119, Tyr193, Ala334, Thr333, Thr260, Ser331	Lys 222 (Pi-Cation)	–7.6 gabapentin

* Amino acid residues that are not an experimentally determined active site.

hydrophobic pocket with longitudinal fixation in space relative to the reference ligand (Fig. 3a) indicates a high probability of positive allosteric modulation of the glycine receptor.

To determine the possible antagonism of the quinazoline derivative I to caffeine, docking was performed at the site of a highly selective inhibitor of human adenosine receptors type A_{2A} (3EML) – ZM241385²⁰. A high level of affinity was predicted: –9.2 kcal/mol in the quinazoline derivative I against –8.5 kcal/mol in the reference drug. The compatible conformation demonstrates the identity of the spatial arrangement and superimposition of structural fragments of the two ligands (Fig. 3b). A network of hydrophobic bonds, including three strong Pi-Pi stacking interactions with phenylalanine (Phe168) and tyrosine (Tyr271) (Table 5), confirms the high affinity of the quinazoline derivative I for adenosine receptors with possible antagonism to caffeine.

The average level of affinity of the quinazoline derivative I was predicted to the site of gabapentin (Table 5) – an inhibitor of amino acid aminotransferase with branched chain BCAT (2COI)²¹. Immersion in a small hydrophobic pocket is impossible because the quinazoline derivative I reaches the fixation site of the PLP co-factor and is superimposed on it (Fig. 3c).

Discussion

The results of the given studies indicate that the acetamide derivative of quinazoline I has a completely wide range of anticonvulsant activity. A number of mechanisms may be involved in the anticonvulsant effect: GABA-ergic (effective in PTZ- and picrotoxin-induced seizure models), glycinergic (efficacy in the strychnine model), and adenosinergic (efficacy in caffeine-induced seizures). Moderate efficacy in the electro-induced seizure model indicates the possible involvement of inhibitory effects on sodium channels as part of the mechanism of anticonvulsant action. The lack of a protective effect on thiosemicarbazide-induced seizures suggests a lack of pronounced antiglutameric properties of the test compound.

It is important to compare the obtained results of an *in vivo* experiment with *in silico* evaluation of the affinity of quinazoline derivative I to the receptors of neurotransmitter systems to determine their role in the mechanisms of action in models of the convulsive syndrome.

The docking results indicate a high probability of allosteric modulation of the GABA_A receptor through the benzodiazepine site. It is unlikely that through the benzimidine site, it is also impossible to inhibit GABA_{AT}. Positive allosteric modulation by classical benzodiazepines leads to conformational changes in the structure of the GABA_A receptor and prevents not only the fixation of PTZ but also picrotoxin. Therefore, the results of the *in vivo* experiment on the PTZ and picrotoxin model of seizures are absolutely comparable with the

results of *in silico* studies and prove the GABA-ergic mechanism of anticonvulsant activity of quinazoline derivative I. Due to strychnine being a potent, selective antagonist of postsynaptic glycine receptors, *in silico* and *in vivo* correspondence of seizures in the strychnine model with seizures is quite obvious.

The results of docking at the site of ZM241385 demonstrate a high affinity of the quinazoline derivative I for adenosine receptors and possible antagonism to caffeine. It confirms the adenosinergic mechanism of anticonvulsant action of quinazoline derivative I, determined *in vivo* in a model of caffeine seizures.

Inhibition of BCAT affects leucine transport and increases the activity of glutamate decarboxylase, which in turn is inhibited by thiosemicarbazide in the seizure model²². Implementation of the anticonvulsant effect due to BCAT inhibition is unlikely, which correlates with the results of *in vivo* experiment in a model of thiosemicarbazide-induced seizures.

According to the literature data, substances with an inhibitory effect on voltage-dependent Nav and Cav T-type ion channels show significant effectiveness in the model of electroinductive seizures. For example, topiramate enhances the rapid inactivation of sodium channels, while lacosamide enhances the slow inactivation of sodium channels²³. However, reliable prediction of affinity for Nav and Cav T-type receptors is currently not possible due to the lack of crystallized in conformation with anticonvulsant drugs macromolecule ion channels in Protein Data Bank.

For a deeper understanding of the action mechanisms, the results of the quinazoline derivative I anticonvulsant effect studies under the thiosemicarbazide model should be compared with its impact on the neuroactive amino acids content in the brain of mice during the first thiosemicarbazide-induced seizure attack²⁴. As shown in the cited study, thiosemicarbazide led to a significant decrease in the cerebral GABA content against the background of an elevated glutamate level and a decrease in the content of glycine with an unchanged level of aspartate. The quinazoline derivative I provided a beneficial effect on the balance of inhibitory and excitatory amino acids in the brain, increasing the content of GABA (moderately) and glycine (very significantly) and moderately reducing the level of glutamate and aspartate. According to the ability to normalize GABA and glutamate levels, the studied compound was somewhat inferior to sodium valproate, while it influenced no less effectively the content of glycine and aspartate. In addition, the quinazoline derivative I counteracted oxidative stress in the brain, significantly reducing the content of 8-iso-prostane, which was increased under the influence of thiosemicarbazide. In terms of antioxidant properties, the test compound was somewhat inferior to the reference drug sodium valproate²⁴. Therefore, the original quinazoline derivative I at a dose of 100 mg/kg intragastrically increases the reduced level of inhibitory amino acids and decreases the content of excit-

atory amino acids in the mouse brain at the peak of thiosemicarbazide-induced seizures. Besides, the test compound inhibits oxidative stress judging by reducing the cerebral content of 8-isoprostanate. Although in the current study, the test compound did not affect the clinical course of thiosemicarbazide seizures, these first neurochemical results elucidate the amino acid link of the mechanism of action. The ability to improve the balance of cerebral inhibitory and excitatory amino acids may partially explain the anticonvulsant effects of the quinazoline derivative I, especially under the models of pentylenetetrazol-, picrotoxin-, and strychnine-induced seizures. Reduction of oxidative stress can have a non-specific value, useful in seizures of various geneses.

An in-depth study of the neurochemical mechanism will be the next step in our in-depth studies of the anticonvulsant action of N-[(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1*H*-quinazolin-3-yl) acetamide. In the future, an *in silico* study is also planned on the possible glutamatergic mechanism of anticonvulsant action by modulating metabotropic and ionotropic glutamate receptors to predict activity in kindling seizure models, and effects on voltage-gated potassium ion channels and type II carbonic anhydrase to predict activity in psychomotor 6Hz seizures.

Conclusions

This study presents the research results of the dose-dependent effect of N-[(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1*H*-quinazolin-3-yl) acetamide and the evaluation of the anticonvulsant spectrum *in vivo* and *in silico*. In the basic model of pentylenetetrazole-induced seizures in mice, it was found that N-[(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1*H*-quinazolin-3-yl) acetamide administered intragastrically has an anticonvulsant effect in a fairly wide range of doses (50–150 mg/kg). The maximum protective effect was observed in a dose of 100 mg/kg. N-[(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1*H*-quinazolin-3-yl) acetamide (100 mg/kg) has shown a wide range of anticonvulsant activity, significantly reducing the severity of seizures and mortality in models of seizures caused by pentylenetetrazole, picrotoxin, strychnine, caffeine, and has a moderate effect on models of electro-induced seizures. The course of seizures caused by thiosemicarbazide was not affected by the test compound. The affinity of the test substance for anticonvulsant biotargets was determined using molecular docking. It provided a mechanism of anticonvulsant action and correlated *in vivo* and *in silico* results: antagonism with pentylenetetrazole and picrotoxin – stimulation of GABAergic mechanisms due to positive allosteric receptor mode; caffeine antagonism – modulation of the adenosinergic link due to competitive inhibition of A_{2A} receptors; activity on strychnine-induced seizures – a glycinergic mechanism through stimulation of Gly-receptors.

The obtained results substantiated the expediency of further in-depth research on the anticonvulsant properties of N-[(2,4-dichlorophenyl) methyl]-2-(2,4-dioxo-1*H*-quinazolin-3-yl) acetamide with the evaluation of neurochemical mechanisms.

Conflicts of interest: none.

References

1. **Chen Z., Brodie, M. J., Liew D., Kwan, P.** Treatment outcomes in patients with newly diagnosed epilepsy treated with established and new antiepileptic Drugs: a 30-year longitudinal cohort study. *JAMA Neurol.* 2018; 75: 279–286.
2. **Bigelow M. D., Kouzani A. Z.** Neural stimulation systems for the control of refractory epilepsy: a review. *J. Neuroeng. Rehabil.* 2019; 16(1): 126.
3. **El Kayal W. M., Mokhamad W., Shtrygol S. Yu., Zalevskyi S. V., Abu Shark A., Tsyvunin V. V., Kovalenko S. M., Bunyatyan N. D., Perekhoda L. O., Severina H. I., Georgiyants V. A.** Synthesis, *in vivo* and *in silico* anticonvulsant activity studies of new derivatives of 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)acetamide. *Eur. J. Med. Chem.* 2019; 180(15): 134–142.
4. **Bunyatyan N. D., Severina H. I., El Kayal W. M., Zalevskyi S. V., Shtrygol S. Yu., Abu Shark A., Tsyvunin V. V., Kompantsev D. V., Shevchenko A. M., Kovalenko S. N., Georgiyants V. A., Ogay M. A., Khadzhieva Z. J.** Synthesis and anticonvulsant activity of new 2-(4-oxo-2-thioxo-1,4-dihydro-3(2*H*)quinazolinyl)acetamides. *Pharm. Chem. J.* 2020; 54(1): 1–8.
5. **Zalevskyi S. V., Shtrygol'S. Yu., Shtrygol'D. V.** Psychotropic properties and acute toxicity of 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)-N-[(2,4-dichlorophenyl)methyl]-acetamide – a promising anticonvulsant. *Pharm. & Drug Toxic.* 2021; 15(6): 363–371.
6. **Golovenko M. Ya., Gromov L. O.** Preclinical study of the specific activity of potential anticonvulsants: Guidelines. K.: DC of the Ministry of Health of Ukraine 2003.
7. **Hock F. J.** Drug Discovery and Evaluation: Pharmacological Assays. Switzerland: Springer International Publishing 2016.
8. **Mishchenko M. V., Shtrygol' S. Yu.** The range of anticonvulsant action and acute toxicity of 5-[(Z)-(4-nitrobenzylidene)]-2-(thiazol-2-ylimino)-4-thiazolidinone. *Pharm. & Drug Toxic.* 2020; 14(6): 389–396.
9. **Kavraiskyi, D. P., Shtrygol' S. Yu., Georgiyants V. A., Severina H. I.** Experimental determination of the spectrum of anticonvulsant action of new pyrazole [3,4-d]pyrimidine-4-one derivatives. *ScienceRise: Pharm. Sci.* 2016; 1: 10–17.
10. **Tsyvunin V., Shtrygol' S., Shtrygol' D.** Digoxin enhances the effect of antiepileptic drugs with different mechanism of action in the pentylenetetrazole-induced seizures in mice. *Epilepsy Res.* 2020; 167: 106465.
11. **Trott O., Olson A. J.** AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 2010; 31: 455–461.

12. Protein Data Bank. URL: <http://www.rcsb.org/pdb/home/home.do> (Date of access: 04.02.2021).

13. **Masiulis S., Desai R., Uchański T., et al.** GABA receptor signaling mechanisms revealed by structural pharmacology. *Nature* 2019; 565: 454–459.

14. **Golovenko M. Ya., Larionov V. B., Reder A. S., Valivodz' I. P.** An effector analysis of the interaction of propoxazepam with antagonists of GABA and glycine receptors. *Neurochem. J.* 2017; 11(4): 302–308.

15. **van Koert R. R., Bauer P. R., Schuitema I., Sander J. W., Visser G. H.** Caffeine and seizures: A systematic review and quantitative analysis. *Epilepsy Behav.* 2018; 80: 37–47.

16. **Golovenko M. Ya., Reder A. S., Larionov V. B., Valivodz' I. P.** The effect of propoxazepam on development of thiosemicarbazide-induced GABA-deficient seizures in mice. *Clin. Pharm.* 2017; 21(2): 34–40.

17. **Miller P. S., Aricescu A. R.** Crystal structure of a human GABA receptor. *Nature* 2014; 512(7514): 270–275.

18. **Storici P., Capitani G., de Biase D., Moser M., John R. A., Jansonius J. N., Schirmer T.** Crystal structure of GABA aminotransferase, a target for antiepileptic drug therapy. *Biochem.* 1999; 38(27): 8628–8634.

19. **Huang X., Shaffer P. L., Ayube S., Bregman H., Chen H., Lehto S. G., Gingras J.** Crystal structures of human glycine receptor $\alpha 3$ bound to a novel class of analgesic potentiators. *Nat. Struct. Mol. Biol.* 2017; 24(2): 108–113.

20. **Sun B., Bachhawat P., Chu M. L.-H., Wood M., Ceska T., Sands Z. A., Kobilka B. K.** Crystal structure of the adenosine A2A receptor bound to an antagonist reveals a potential allosteric pocket. *Proc. Natl. Acad. Sci.* 2017; 114: 2066–2071.

21. **Goto M., Miyahara I., Hirotsu K.** Structural Determinants for Branched-chain Aminotransferase Isozyme-specific Inhibition by the Anticonvulsant Drug Gabapentin. *J. Biolog. Chem.* 2005; 280: 37246–37256.

22. **Severina H. I., Skupa O. O., Voloshchuk N. I., Saidov N., Bunyatyan V. A., Kovalenko S. M., Georgiyants V. A.** Molecular docking, ADMET study and *in vivo* pharmacological research of N-(3,4-dimethoxyphenyl)-2-{[2-methyl-6-(pyridine-2-yl)-pyrimidin-4-yl]thio} acetamide as a promising anticonvulsant. *Res. Results in Pharm.* 2020; 6(2): 27–41.

23. **Santos R., Ursu O., Gaulton A.** A comprehensive map of molecular drug targets. *Nat. Rev. Drug. Discov.* 2017; 16(1): 19–34.

24. **Shtrygol' S. Yu., Zalevskyi S. V., Gorbach T. V.** Promising anticonvulsant 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-[(2,4-dichlorophenyl)methyl]-acetamide: the influence on the content of neuroactive amino acids and 8-isoprostanone in the mouse brain under the model of thiosemicarbazide-induced seizures. *Pharm. & Drug Toxic.* 2022; 16(1): 19–26.