European Journal of Medicinal Chemistry 180 (2019) 134–142

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

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

Wassim Mokhamad El Kayal [a], Sergiy Yu Shtrygol [a], Sergiy V. Zalevskyi [a], Amjad abu Shark [a], Vadim V. Tsyvunin [a], Sergiy M. Kovalenko [b, c], Natalya D. Bunyatyan [c, d], Lina O. Perekhoda [a], Hanna I. Severina [a], Victoriya A. Georgiyants [a, *]

[a] National University of Pharmacy, 53, Pushkinskaya st., Kharkiv, 61002, Ukraine
[b] V.N.Karazin Kharkiv National University, 4 Svobody sq., Kharkiv, 61077, Ukraine
[c] Federal State Autonomous Educational Institution of Higher Education I.M. Sechenov First Moscow State Medical University, 8 Trubeckaya, Moscow, 119991, Russia
[d] Federal State Budgetary Institution "Scientific Centre for Expert Evaluation of Medicinal Products", Petrovsky boulevard 8. bld. 2, Moscow, 127051, Russia

ARTICLE INFO

Article history:
Received 17 April 2019
Received in revised form
26 June 2019
Accepted 28 June 2019
Available online 2 July 2019

Keywords:
Synthesis
2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)acetamide
Molecular docking
Anticonvulsant activity

ABSTRACT

In order to expand the arsenal of biologically active substances of anticonvulsive action by the interaction of 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)acetic acid with the corresponding amines in the presence of *N,N′*-carbonyldiimidazole in the dioxane medium, a systematic series of 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)-*N*-R-acetamides was obtained. A novel approach to synthesis of the key intermediate - 2-(2,4-dioxo-1,4-dihydro-quinazolin-3(2H)-yl)acetic acid was developed. The structure and purity of the resulting substances was confirmed by elemental analysis, ^{1}H NMR, ^{13}C NMR spectroscopy and LC/MS. Based on the results of docking studies using SCIGRESS software, selected compounds with the best affinity for anticonvulsant protein biomes (PDB codes: 4COF, 3F8E and 1 EOU) are promising for experimental studies of anticonvulsant activity. A comparative analysis of the results of molecular docking and *in vivo* results suggests that there is a positive correlation between scoring protein inhibition and experimental data. Pharmacological studies have revealed the leader compound 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)-*N*-[(2,4-dichlorophenyl)methyl]acet-amide, which improved all the experimental convulsive syndrome rates in mice without motor coordination impairment and may be recommended for further research. The lowest values of the scoring function of the ligand-peptide interaction are obtained for the synthesized compound and carbonic anhydrase II (gene name CA2) (PDB code 1 EOU), so its inhibition is proposed by us as the most probable mechanism of the anticonvulsive effect of the leader compound.

© 2019 Elsevier Masson SAS. All rights reserved.

1. Introduction

Epilepsy takes one of the leading places among neurological diseases, which greatly affects life quality and life expectancy. According to Lancet [1], mortality from epilepsy today is 0.22% of global mortality. Disability-adjusted life years for epilepsy is 0.5% of total, and this disease is in the top 5 of neurological diseases for years of healthy life lost as a result of disability [1]. That is why the expansion of the range of antiepileptic drugs is necessary for improving this situation. In world neurological practice as in Ukraine, anticonvulsants of different generations and different chemical structures are used [2,3]. Unfortunately, at the present time, there are no drugs that are equally effective at different forms and degrees of convulsive disorders [4,5]. It should be noted that despite the emergence of new modern anticonvulsants, the first generation drugs – phenobarbital (I), phenytoin (II), which have certain significant disadvantages – remain in demand in the treatment of convulsive conditions. New anticonvulsant drugs: stiripentol (III), pregabalin (IV), zonisamide (V), lamotrigine (VI), tiagabine (VII), topiramate (VIII) – do not give a long-term therapeutic result, are dose-dependent and toxic (Fig. 1) [6–9].

* Corresponding author.
E-mail address: vgeor@nuph.edu.ua (V.A. Georgiyants).

https://doi.org/10.1016/j.ejmech.2019.06.085
0223-5234/© 2019 Elsevier Masson SAS. All rights reserved.

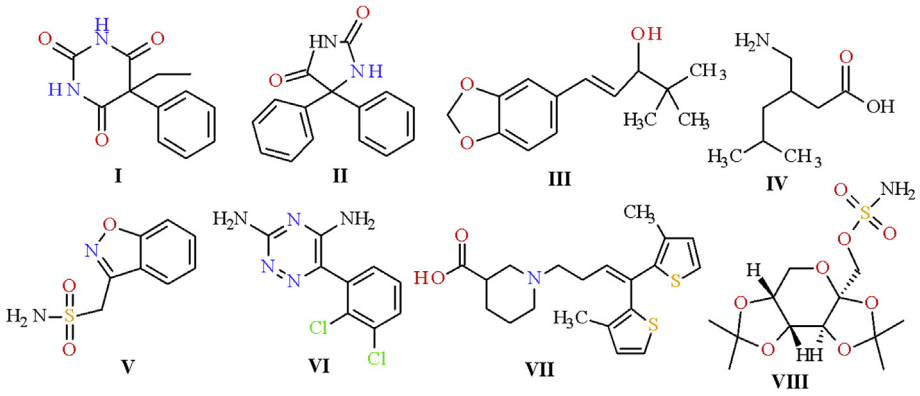


Fig. 1. Chemical structures of known anticonvulsants.

One of the most promising directions in modern medical chemistry used for purposeful synthesis of potential new APIs is the structural modification of known drugs in order to improve their pharmacological properties. In view of this our attention is attracted by the heterocyclic system of pyrimidine as the basis of the most "old" anticonvulsant – phenobarbital. By modification of its structure we have already received substances with promising anticonvulsant properties [10]. Continuing the research on the modification of the pyrimidine ring, we drew attention to derivatives of quinazoline, which actively search for potential anticonvulsants [11], and compound (IX) has previously been used in medical practice, called methaqualone, as a hypnotic and anticonvulsant drug [12]. The versatility of synthetic approaches to the production and chemical modification of quinazoline derivatives, the presence of anticonvulsant activity in the spectrum of their pharmacological activity is an indisputable justification for further studies of this group of compounds [13–15]. We decided to modify the structure of methaqualone, replacing the aryl substituent on the acetic acid residue, thus combining quinazoline scaffold with one of the major neurotropic mediators, glycine. To block the hydrophilic carboxyl group, its amination was planned using amines of different nature: alkyl, aryl-alkyl and aryl (Fig. 2).

2. Materials and methods

Reagents manufactured by Sigma-Aldrich, USA were used in this work. The required reagents were purified using standard techniques. Control of the reactions was carried out using thin-layer chromatography (eluent-ethyl acetate-hexane 1 : 2) on the plates "Sorbfil UV-254". Manifestations of chromatograms were carried out in the UV rays of the "Chromatographic UVF 254/365 Illuminator" (mode 254 nm). The melting points were determined using the Kofler block. Elemental analysis was determined by the Dumas method. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on the Varian Gemini 400 MHz spectrometers and the Varian Gemini 100 MHz spectrometers. The solvent was dimethyl sulfoxide (DMSO-d_6). Chemical shifts are shown on a scale (m.ch.). LC/MS spectra were recorded with a PE SCIEX API 150EX liquid chromatograph equipped with a UV detector (λ_{max} 215 and 254 nm) and using a Luna-C18 column, Phenomenex (100 × 4 mm). Elution was started with water and stopped with acetonitrile/water (95:5, v/v) using a linear gradient at a flow rate of 0.15 mL/min and an analysis cycle time of 25 min.

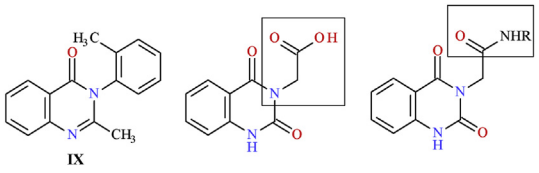


Fig. 2. Modification of the structure of methaqualone in the target compounds.

The docking simulations were performed with the SCIGRESS software package (Fujitsu, Fukuoka, Japan (license 742F6852C191)). Docking studies were conducted with the methods of a fast and quantum molecular docking in order to search the affinity of synthesized chemical structures to targets and to determine the possible mechanisms of their anticonvulsive effect. The stages of molecular docking with the help of the SCIGRESS software package were as follows:

2.1. The choice of bio-targets and determination of their active sites

In the present study, to carry out the docking studies the proteins type-A γ-ami-nobutyric acid receptors (GABAARs) (code 4COF), carbonic anhydrase II (gene name CA2) (codes 3F8E and 1OEU) domains were chosen as a targets for anticonvulsant activity. The structure of proteins of the Human $GABA_A$ Receptor (code 4COF) and carbonic anhydrase II (gene name CA_2) (code 3F8E and 1OEU) were chosen from Protein Data Bank (PDB) [16].

Type-A γ-aminobutyric acid receptors (GABAAR) are the principal mediators of rapid inhibitory synaptic transmission in the human brain [17]. Because the reversal potential for chloride in most neurons is close to or more negative than the resting membrane potential, activation of $GABA_A$ receptors tends to stabilize or hyper polarize the resting potential, and can make it more difficult for excitatory neurotransmitters to depolarize the neuron and generate an action potential. The net effect is typically inhibitory, reducing the activity of the neuron. A decline in GABAAR signalling triggers hyperactive neurological disorders such as epilepsy.

The carbonic anhydrase inhibition has demonstrated interesting pharmacologic applications such as anticonvulsant agents. These enzymes have shown a potential target for designing anticonvulsant drugs with a novel mechanism of action. The enzymes play an important role in the anion exchange processes [18]. Carbonic anhydrase II (gene name CA_2), is one of fourteen forms of human α carbonic anhydrases, which catalyses reversible hydration of carbon dioxide. Inhibition of carbonic anhydrase in the brain leads to the accumulation of CO_2 in the brain and inhibition of excessive paroxysmal discharges of the neurons, which causes the antiepileptic activity of the drug [19–21]. The protein type-A γ-aminobutyric acid receptors, carbonic anhydrase II (gene name CA2) have been modeled using the electron crystallographic structure at 3,5 Å resolution and were downloaded from the RCSB Protein Data Bank

(PDB ID: 4COF, 3F8E and 1OEU respectively) (www.pdb.org). Water molecules were removed and hydrogens were added to crystal structure of protein before docking. After assigning charge and protonation state final refinement (energy minimization) was done using MM3 force field runs.

2.2. The 3D-optimization of all structures

All chemical structures were generated using ISIS DRAW 4.0 software using standard bond, lengths and angles. All structures were designed using the ISIS DRAW 4.0 software and saved as.mol. files. Then these structures were imported into the SCIGRESS software and saved in.csf format. The next step was the optimization of all structures by the MM3 molecular mechanics method.

2.3. The fast dock and fast quantum dock into active site

The docking study was performed using Scigress Explorer 7.7 installed in a single machine running on a 3.4 GHz Intel Core 2 Duo Processor with 1 GB RAM and 160 GB Hard Disk with Windows XP as the Operating System. Fast docking method, in which receptor is rigid and ligands are flexible, was adopted and binding energy values were compared with each other. The main achievement of our approach is a combination of fast search with a special account for overlooked physical interactions (the implementation of sophisticated quantum methods). At the end of the docking study, the minimum Consensus scores for the best ligand position for each of ligand was obtained. Energy minimization or complex optimization was done for molecular docking calculations and to optimize geometries within the binding site. Complex optimization gave ligands with minimum energy pose within the active site cavity of the protein. The geometry of the molecules was optimized using MO-G computational application that computes and minimizes an energy related to the heat of molecule formation. The augmented Molecular Mechanics (MM3) parameter was used for optimizing the molecules up to its lowest stable energy state. This energy minimization was done until the energy change was less than 0.001 kcal/mol or the molecules were updated almost 500 times. The valency and hydrogen bonding of the ligands, as well as target proteins were subsequently corrected using the Workspace module. Hydrogen atoms were added to protein targets for correct ionization. For automated docking of ligands into the active sites we used the genetic algorithm with a fast and simplified Potential of Mean Force (PMF) scoring scheme. PMF uses types of atoms, which are similar to the empirical force fields used in mechanics and dynamics. The minimization is performed by the Fast-Dock engine, which uses a Lamarkian genetic algorithm (LGA), so that individuals adapt to the surrounding environment (Population size: 500; Crossover: 0.2; Elitism: 50; Max Generations: 50 000; Mutation rate: 0.0; Convergence 0.1; Max iterations: 1000; Rate: 0.01). Visual analysis of the complexes of compounds from the active site of proteine (PDB ID: 4COF, 3F8E and 1OEU) was performed using Discovery Studio Visualizer 4.0 program.

Pharmacological screening for anticonvulsant activity was performed on a baseline model of convulsions in mice [22,23]. Corazole (pentylenetetrazole) is a classic proconvulsant, the effect of which is caused by inhibition of the GABA site of the benzodiazepine receptor complex and the decrease in the intensity of GABA-ergic inhibitory processes in the central nervous system. 72 adult random-bred albino mice of either sex weighing 18–25 g were used. Animals were kept in conditions in accordance with the hygiene rules and principles of the EU Directive 2010/10/63 EU for animal experiments. Mice were randomly divided into 12 groups (each was formed from animals of either sex): (1) control (n = 12) – a model of convulsions induced by pentylenetrazole, (Sigma, USA) at a dose of 90 mg/kg subcutaneously in form of aqueous solution; (2) a reference drug group (n = 6) – sodium valproate (Depakin, Sanofi-Aventis, France) at a dose of 300 mg/kg intragastrically 30 min prior to the administration of pentylenetetrazole; (3–12) groups of experimental animals (n = 5–9), which were administered the test compounds at a dose of 100 mg/kg into the stomach in the form of a suspension on twin-80 30 min before the pentylenetetrazole. The observation lasted 60 min. Latency of the convulsions, the number of clonic-tonic convulsions in one mouse, % of animals with clonic and tonic convulsions, the duration of the convulsive period (from the first to the last attack) and the lifetime of the animals before death (in mice with lethal outcome) were calculated. The severity of seizures was evaluated according to a scale ranging from 1 to 6: 1 – trembling; 2 – circus movement; 3 – clonic seizures; 4 – clonic-tonic seizures with a lateral position; 5 – tonic extension; 6 – tonic extension leading to the animal's death [24].

To evaluate ***the influence of compounds on motor coordination*** the rotarod test was used [22,23]. 72 adult random-bred albino mice of either sex (weighing 18–22 g) were randomly divided into 12 groups (each was formed from animals of either sex): (1) control – intact mice (n = 6); (2) a reference drug group (n = 6) – sodium valproate (Depakin, Sanofi-Aventis, France) at a dose of 300 mg/kg intragastrically 30 min prior to the test; (3–12) groups of experimental animals (n = 6), which were administered the compounds at a dose of 100 mg/kg into the stomach in the form of a suspension on twin-80 30 min before the test. Number of mice that fall from the rotating rod before 1, 2, 3 and 5 min has been determined.

Study of acute toxicity of a leader compound. To determine acute toxicity using the express method by T. Pastushenko [25] the leader compound as a suspension on tween-80 was administered into the mice stomach (3 animals per dose) once in a wide range of doses (1000, 3000, 5000 mg/kg dose which are respectively 10, 30 and 50 times higher than the ED_{50}). The volume of the suspension was 40 ml/kg, corresponding to 0.8 ml per mouse weighing 20 g. The animals were observed for 14 days, assessing their behaviour and physical condition and overall survival after 30 min, 1 h, 3 h, 6 h, 12 h, 24 h, 7 days and 14 days.

Statistical analysis. STATISTICA 8.0 for Windows has been used. Statistically significant intergroup differences were evaluated by parametric Student's t-test for normal distribution and non-parametric Mann-Whitney *U* test in case of his absence [26,27]. Quantitative data were presented as mean ± standard error of mean (SEM), and for results in the alternative form (death – survival) – as the % of the observed effect in the group. In this case, for the statistical significance determination the Fisher's angular transformation was used. Differences were considered statistically significant as $p < 0.05$.

3. Results and discussions

The study described in the article was aimed at a targeted search for substances with anticonvulsant activity. At the same time, for the synthesis of potential APIs, it was necessary to develop an approach that allows to synthesize the aim products with good yields and acceptable purity. Authors [28] have used for synthesis of analogous substances the approach described by Bogert and Scatchard [29] via preparation benzoyleneurea, allowed to synthesize anilide (compound ***7h***) with a yield 19%. There are data in the literature about other approaches and attempts to introduce amino acid residues into the quinazoline fragment. 'Direct Amide Cyclization' to Peptides Containing an Anthranilic Acid Residue was used by Philipova et al. [30] and other authors [31]; the interaction of methyl 2-isocyanatobenzoate with glycine described in literature too [32]. However, the methods described do not allow to obtain

key intermediate -2-(2,4-dioxo-1,4-dihydro-quinazolin-3(2*H*)-yl) acetic acid with appropriate yield. Therefore, we decided to develop a different approach for the synthesis of planned substances in order to increase the yields of both key intermediate and aim products.

Previously, we proposed an approach to the synthesis of combinatorial libraries of condensed heterocycles containing the 2-mercapto-4-(3*H*)-pyrimidinone fragment [33], which consists in the cyclization of substituted 2-(methylcarboxy)benzene-isothiocyanates with primary amines. This method advantageously differs from the traditional synthesis of such compounds by cyclization of substituted methyl anthranilates with isothiocyanates, since it allows the use as primary amines of compounds such as amino acids for which it is not possible to obtain isothiocyanates, therefore the traditional pathway of synthesis is unacceptable.

A starting material for the synthesis of a 2-(2,4-dioxo-1,4-dihydroquinazolin-3 (2*H*)-yl)-*N*-R-acetamide ***7a-7n*** systematic series was used 2-(2,4-dioxo-1,4-dihydro-quinazolin-3(2H)-yl)acetic acid ***4*** which was prepared by oxidation of 2-(4-oxo-2-thioxo-1,4-dihydroquinazolin-3(2*H*)-yl) acetic acid ***3*** hydrogen peroxide heating to 70 °C for 30 min 2-(4-Oxo-2-thioxo-1,4-dihydroquinazolin-3(2*H*)-yl) acetic acid ***3*** was synthesized by adding methyl 2-isothiocyanatobenzoate ***1*** glycine ***2*** in the presence of triethylamine in a medium of 2-propanol during boiling for 30 min (Scheme 1).

New derivatives of 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2H)-yl) acetamide ***7a-7n*** were prepared by sequential interaction of 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl) acetic acid ***4*** with *N*,*N*′-carbonyldiimidazole ***5*** and the corresponding amines ***6*** in dioxane (Scheme 2).

2-(2,4-Dioxo-1,4-dihydroquinazolin-3(2*H*)-yl) acetic acid **4**, in reaction with *N*,*N*′-carbonyldiimidazole, forms an appropriate acylimidazole. The carbon dioxide released during the reaction is a reaction catalyst for amines [34,35], with the resultant target 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)-*N*-R-acetamides.

Synthesized substances ***7a-7n*** – white crystalline substances, easily soluble in 2-propanol, dioxane, dimethylformamide, not soluble in water.

The structure and purity of the compounds obtained were confirmed by elemental analysis, ^{1}H NMR, ^{13}C NMR spectroscopy and LC/MS. The reaction was monitored by thin layer chromatography.

One of the reasons for the effective search for anticonvulsants is the high death rate of animals in a screening experiment [36]. As a result, in order to pre-evaluate the anticonvulsant potential of substances *in silico* methods, which allow us to select not only the most promising substances, but also to predict the mechanism of action and to correctly select the screening model, are prevalent [37,38]. The most commonly used method for such purposes is molecular docking.

Molecular docking gives possibility to reduce efforts and time due to carrying out the procedure that is similar to high-performance biological screening. Knowing the structure of the target (receptor or enzyme) and the structure of the ligand it is possible to explain the mechanism of interaction at the molecular level and calculate the strength of binding between them (affinity) [39]. The aim of the docking is the search of the most suitable positions and orientations of the ligands in the ligand binding centre of the receptor or enzyme, as well as identification of factors that may lead to improvement of the ligand-receptor interaction.

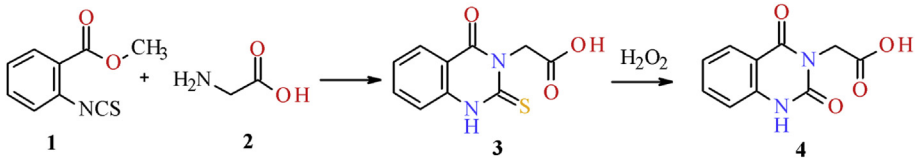
The target protein structures of 4COF, 3F8E and 1EOU were docked with new quinazoline derivatives, which provided excellent results as were seen by the least values of the binding energy in Table 1. The scores of docking results are based on the free binding energies and the hydrogen bond interactions involved in the binding mode. The obtained docking scores of results of synthesized derivatives are presented in Table 1.

The results of docking studies have shown that utilization of benzyl, phenyl, phenoxymethyl, 2,4-dihalogenbenzyl residue for the structure optimization of quinazoline scaffolds is effective approach in novel anticonvulsant agents design and may be taken as the variant of hybrid pharmacophore approach.

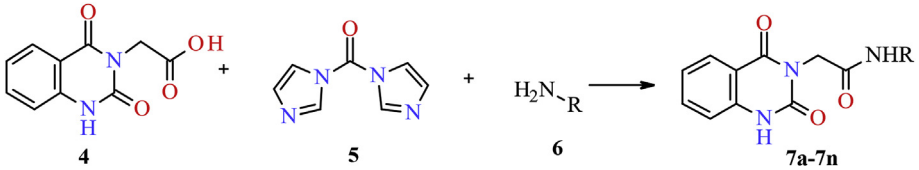
Based on the results of docking studies, 10 compounds with the highest affinity for anticonvulsant biotargets - proteins (PDB codes: 4COF, 3F8E and 1 EOU) were selected and are promising for experimental studies of anticonvulsant activity.

Molecule ***7m*** showed better binding energies (the highest negative dock score −117) to the protein 1 EOU than the others. The stability of this complex is due mainly to the energetically favourable geometric location of the ligands in the active centres of these receptors, the formation of hydrogen bonds between them, intermolecular electrostatic and donor-receptor interactions. This means that it can fit well in the receptor cavity (1 EOU), forming an energetically most stable drug receptor complex, thus inhibition of this protein is proposed by us as the most probable mechanism for the implementation of the anticonvulsant effect (Fig. 3).

Molecule ***7m*** form enzyme-ligand complex with 1 EOU protein due to the favourable acceptor and hydrogen bond between the Oxygen atoms of the quinazoline fragment, and amide fragment with the amino acid moieties of Asn 67, His64, Thr199, Thr198. The 2,4-dichlorophenyl ring of ***7m*** forms a hydrophobic interaction (π-π interaction) with Asn 67, His 64 and almost occupies the same



Scheme 1. Synthesis of 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2H)-yl)acetic acid 4.



Scheme 2. Synthesis of 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-R-acetamides 7a-7n.

Table 1
Compound Dock scoring functions.

Com-pound	1 EOU	4COF	3F8E
	The scores of Fast Dock		
7a	−84.7	−68.5	−28.1
7b	−94.8	−64.8	−28.9
7c	−96.9	−75.0	−26.6
7d	−96.8	−68.3	−29.2
7e	−93.6	−64.6	−21.4
7f	−93.3	−66.7	−30.5
7g	−100.7	−70.4	−21.4
7h	−110.8	−71.6	−33.5
7i	−107.5	−72.6	−33.8
7j	−106.9	−66.9	−48.6
7k	−108.8	−75.6	−54.3
7l	−104.0	−72.2	−35.9
7m	−117.6	−82.9	−55.3
7n	−115.5	−83.0	−32.8

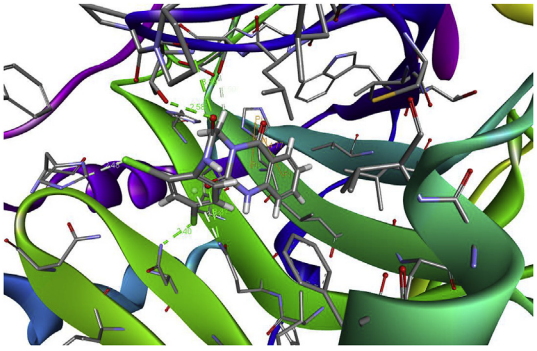


Fig. 3. Complex of the ligand ***7m*** with the binding site of protein (PDB ID: 1EOU).

hydrophobic pocket. Pi-Cationic and Pi-anionic interactions are formed between the phenyl fragments of the molecules and the moietiy of Gln 92. π-H interactions arise with the participation of aromatic and quinazoline fragments of molecules with amino acid moieties of His64. Additional stabilization of complexes occurs as result of π-π interaction of the quinazoline fragments of the molecules with amino acid moiety His119 (Fig. 3).

The following colour codes have been employed: red, green and light blue represent oxygen, chlorine and nitrogen atoms respectively; ashen gray forms the carbon backbone. Active site amino acid residues are represented as sticks coloured according to residue type (Sequence protocol-Karplus and Schultz Flexibility).

The variation of binding energy of the stable enzyme-ligand complexes is in good agreement with the observed anticonvulsant activities of 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)-*N*-R-acetamides.

Analysis of the results of pharmacological screening for 10 selected compounds shows that four compounds caused statistically significant anticonvulsant effect according to the criterion of the integral protective index – reduction of lethality compared with control (Table 2). They can be considered highly effective anticonvulsants (see Table 3).

In addition, the aim of this study is to investigate the influence of alkyl chain length and electronic effect of the electron donating- and withdrawing groups at phenyl ring in amide fragment of 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)-*N*-R-acetamides on the anticonvulsant activity and hence deduce the structure activity relationship.

The presence of an aromatic ring in the structure of synthesized compounds ***7h-7n*** increases the lipophilicity of molecules and thus facilitates their penetration through the blood-brain barrier. Obviously, therefore, all these compounds have the best anticonvulsant activity. The enhancement of activity is also facilitated by the presence of electron-acceptor substituents in the 2-position of the aromatic nucleus. The simultaneous presence of two chlorine atoms in the 2 and 4 positions of the benzyl substituent brings the compound ***7m*** into absolute leaders. Compound ***7m*** provided a complete protective effect lethality 0% vs. 91.7% in control, $p < 0.01$) and not inferior to the standard anticonvulsant medicine sodium valproate without any indication of positive effect on the course of the convulsions. Satisfactory indices were in compounds ***7m.***

Absolute leader-compound ***7m*** improved all the experimental convulsive syndrome indicators. It was 3.5 times longer ($p < 0.05$), lengthened the latency of the convulsions, contributed to the maximum reduction in the number of clonic and tonic paroxysms (severity ≥ 3 points) to 1.00 ± 0.32 versus 3.58 ± 0.42 in the control ($p < 0.05$) per animal. For animals with clonic (severity of 3 points) and tonic ($\geq$4 points) attacks – 80% vs. 100% ($p < 0.05$) and up to 40% vs. 100% ($p < 0.01$). The median severity of the convulsions against the background of compound 7 (13) decreased by 2.1 times and was 2.80 ± 0.73 points against 5.83 ± 0.17 range in the control ($p < 0.01$). The duration of the convulsive period decreased by 26.7

Table 2
Influence of the investigated substances on the pentylenetetrazole-induced seizures on mice (M ± m).

Group of animals	n	Dose, mg/kg	Latency, min	Number of clonic-tonic convulsions in 1 mouse	% of mice with convulsions		Severity of the convulsions, points	Duration of the convulsive period, min	Lifetime of the animal to death, min	Lethality, %
					clonic	tonic				
Control	12	–	5.79 ± 0.58	3.58 ± 0.42	100	100	5.83 ± 0.17	12.00 ± 1.34	17.45 ± 1.85	91.7
Sodium valproate	6	300	26.65 ± 10.61**	2.00 ± 0.82*	66.7**	50 **	2.83 ± 0.98**	7.64 ± 3.62	20.90	16.7**
7c	5	100	7.50 ± 3.20	1.40 ± 0.24**	100#	100#	5.00 ± 0.45*	2.20 ± 1.26**	6.89 ± 0.70*	40.0*
7f	5	100	6.60 ± 3.69	2.20 ± 0.58	100#	80 *	5.00 ± 0.63	9.80 ± 7.83	16.95 ± 12.89	60.0
7g	5	100	3.80 ± 0.55	3.20 ± 0.97	100#	100#	5.60 ± 0.40	13.11 ± 6.49	11.53 ± 5.60	80.0#
7h	9	100	10.57 ± 3.32	1.22 ± 0.22**	100##	55.5**	4.22 ± 0.46**	1.24 ± 1.06**#	7.14 ± 3.45*	33.3**
7i	5	100	4.96 ± 1.37	3.00 ± 0.55	100#	100#	5.60 ± 0.40	14.05 ± 2.98	17.50 ± 3.82	80.0#
7j	5	100	11.43 ± 4.06	1.80 ± 0.37*	100#	100#	5.60 ± 0.40	9.98 ± 5.44	17.79 ± 2.93	80.0#
7k	5	100	15.51 ± 11.13	1.20 ± 0.37**	80 *	80 *	4.00 ± 1.10 *	3.05 ± 2.76 **	12.16 ± 6.42	40.0 *
7l	5	100	4.82 ± 1.30	2.00 ± 0.32*	100#	100#	6.00 ± 0.00#	4.54 ± 1.89 **	9.36 ± 2.06*	100##
7m	5	100	20.38 ± 10.00*	1.00 ± 0.32**	80 *	40 **	2.80 ± 0.73 **	0.45 ± 0.38 **	–	0 **
7n	5	100	15.14 ± 11.31	2.40 ± 0.93	80 *	80 *	4.40 ± 1.17	7.25 ± 2.93	12.45 ± 5.91	60.0

Note. Statistically significant differences:
1. compared with control group indicators: * – $p < 0.05$; ** – $p < 0.01$.
2. compared with indicators of sodium valproate group: # – $p < 0.05$; ## – $p < 0.01$.

Table 3
Influence of the investigated substances on motor coordination (rotarod test).

Group of animals	n	Number of mice that fall from the rod, amount/percentage			
		before 1 min	before 2 min	before 3 min	before 5 min
Control	6	1/17%	2/33%	2/33%	3/50%
Sodium valproate	6	3/50%	4/67%	5/83% *	6/100% *
7c	6	2/33%	3/50%	3/50%	3/50%#
7f	6	2/33%	3/50%	3/50%	4/67%#
7g	6	1/17%	2/33%	3/50%	3/50%#
7h	6	1/17%	2/33%	3/50%	4/67%#
7i	6	1/17%	2/33%	3/50%	3/50%#
7j	6	2/33%	3/50%	3/50%	4/67%#
7k	6	1/17%	2/33%	3/50%	3/50%#
7l	6	3/50%	4/67%	4/67%	5/83%
7m	6	1/17%	3/50%	3/50%	4/67%#
7n	6	3/50%	4/67%	4/67%	5/83%

Note. Statistically significant differences:
1. compared with control group indicator: * – $p < 0.05$.
2. compared with indicator of sodium valproate group: # – $p < 0.05$.

times and was 0.45 ± 0.38 min versus 12.00 ± 1.34 min, ($p < 0.01$) in the control.

Two other "hit compounds" ***7h*** and ***7k***, in addition to decreasing the lethality, are improved by 5 indicators of convulsive syndrome, and compound ***7c*** – 4 indicators (Table 2). However, all of them, unlike compound ***7m***, only tendentiously increased the latency of the convulsions.

An additional advantage of compound ***7h*** is a significant decrease in the number of the most dangerous tonic convulsions (the latter only occurred in 55.5%, $p < 0.01$ vs. control, against the background of compound ***7k*** – 80%, $p < 0.05$ vs. control). Compound ***7c*** did not affect this indicator.

Three compounds (***7f, 7g*** and ***7 n***) showed a weak anticonvulsant effect. By decreasing the lethality rate by 60–80%, they improved only 1–2 indicators of the severity of the convulsive syndrome. In particular, compound ***7g*** reduced the total number of attacks per animal, compound ***7f*** reduced only the number of tonic attacks, and compound ***7n*** reduced the number of both clonic and tonic attacks per animal (Table 2).

Two compounds ***7g*** and ***7i*** were indifferent to the pentylenetetrazole convulsions, since they did not have the desired effect on any of their rates of lethality at 80% and yielded to this indicator of sodium valproate group (Table 2).

One compound ***7l*** revealed moderate proconvulsive properties: the lethality of animals at the background of its application was equal to 100%, while the time of death decreased by 1.9 times compared to control ($p < 0.05$).

The results obtained confirm the results of molecular docking and show GABA-positive properties of compounds ***7m, 7h***, ***7c*** and ***7k***, since the convulsive action of pentylenetetrazole is due to depressant effects on GABA-ergic inhibitory processes.

Analysis of the results of rotarod test for 10 selected compounds (Tabl. 3) shows that there are no substances impairing motor coordination of mice among 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2H)-yl)acetamide derivatives studied. However, sodium valproate revealed mild motor impairment, significantly increasing the number of mice that fell down from the rotating rod before 3 and 5 min.

In the study of acute toxicity, the median death dose (LD50) of leader compound ***7m*** could not be established, since administration of any study dose (including a maximum dose of 5000 mg/kg) was not accompanied by death of animals for 14 days. The assessment of the general condition of the animals did not reveal manifestations of intoxication, such as lateral position, blepharoptosis, hypersalivation, diarrhoea, etc. In addition, normal coordination of the movements and tone of the skeletal muscles was maintained. When the test compound was administered at doses of 3000 and 5000 mg/kg in 30 min and 1 h, a temporary decrease in motor activity of mice was detected due to overloading of the volume of the administered solution of compound ***7m***.

Consequently, compound ***7m*** is classified for toxicity class V according to Hodge and Sterner [[40]] as practically non-toxic substance ($LD_{50} > 5000$ mg/kg).

3.1. General method of synthesis of 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-R-acetamides 7a -7n

Glycine (0.1 mol) 7.5 g dissolved in a mixture of 150 ml of water and 14 ml (0.1 mol) of triethylamine. The resulting solution is added with stirring to a warm solution of 19.2 ml (0.1 mol) of methyl ester of 2-isothiocyanatobenzoic acid in 200 ml of 2-propanol. The solution was boiled for 30 min and acidified with hydrochloric acid (10–12.5 ml) to pH = 2. The formed precipitate was filtered, washed with water and boiled under stirring in 200 ml of acetone. After cooling, the precipitate is filtered off and washed twice with 20 ml of acetone. To a solution (0.3 mol) of sodium hydroxide in 400 ml of water at a temperature of 50 °C and stirring, add 2-(4-oxo-2-sulfanilidene-1,2,3,4-tetrahydroxy-quinazolin-3-yl) acetic acid (0.1 mol). Then, at intensive stirring at 5 °C, hydrogen peroxide (35 ml of 50% (1 mol) diluted 1 : 1 with water) is carefully added (drops or small portions of 5 ml) at a rate such that the temperature of the reaction mixture does not exceed 70 °C. The reaction mixture is stirred for 30 min. After cooling to room temperature, add 9 ml of acetic acid. The formed precipitate is filtered, washed with 100 ml of water. Recrystallize from a mixture of 50 ml of DMF and 200 ml of ethanol. To a suspension of (0.1 mol) of 2-(2,4-dioxo-1,2,3,4-tetrahydroxyquinazolin-3-yl) acetic acid in 200 ml of anhydrous dioxane is added (0.12 mol) of *N,N′*- carbonyldiimidazole. The mixture is boiled for 2–4 h, controlling the reaction through thin-layer chromatography (eluent-ethyl acetate-hexane 1 : 2). After passing the reaction, add (0.12 mol) of the corresponding amine and boil for 2 more hours, controlling the passage of the TLC reaction (eluent - ethyl acetate-hexane 1 : 2). After cooling, brew 500 ml of water. The formed precipitate is filtered off, washed with 50 ml of 2-propanol and recrystallized from a mixture of 50 ml of DMF and 200 ml of 2-propanol.

*3.1.1. 2-(2,4-Dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-methylacetamide **7a***

80% yield, T. melt. = 292–294 °C (dimethylformamide-2-

propanole 1:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.6 (d, 3H, −NH−CH$_3$ J = 6,4 Hz), 5.0 (s, 2H, -CH$_2$-CO-), 7.32 (t, 1H, Ar−H, J = 6 Hz), 7.4 (d, 1H, Ar−H, J = 8 Hz), 7.73 (t, 1H, Ar−H, J = 6 Hz), 7.92 (d, 1H, Ar−H, J = 8 Hz), 7.96 (t, 1H, −CO−NH, J = 4 Hz), 13.0 (s, 1H, NH (het)). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.08, 161.89, 150.11, 139.56, 135.08, 127.40, 122.54, 115.19, 113.85, 42.64, 25.52. 55.50% C, 4.50% H, 18.00% N, Calculated for $C_{11}H_{11}N_3O_3$ Found,%: C 56.65, H 4.75, N 18.02, LC-MC m/z: 234.0 [(M+H)$^+$].

3.1.2. 2-(2,4-Dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-ethylacetamide **7b**

84% yield, T. melt. = 268−270 °C (dimethylformamide-2-propanole 1:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.00 (t, 3H, −CH$_2$-CH$_3$, J = 8 Hz), 3.05 (quintet, 2H, -CH$_2$-CH$_3$), 4.45 (s, 2H, -CH$_2$-CO-), 7.19 (t, 2H, Ar−H, J = 9.3 Hz), 7.65 (t, 1H, Ar−H, J = 8 Hz), 7.9 (d, 1H, Ar−H, J = 9.3 Hz), 8.03 (t, 1H, −CO−NH, J = 6.4 Hz), 11.5 (s, 1H, NH (het)). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.29, 161.87, 150.10, 139.53, 135.06, 127.40, 122.53, 115.19, 113.82, 42.58, 33.35, 14.71. 58.05% C, 5.17% H, 17.29% N Calculated for $C_{12}H_{13}N_3O_3$ Found,%: C 58.29, H 5.30, N 17.09, LC-MC m/z: 248.0 [(M+H)$^+$].

3.1.3. 2-(2,4-Dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-propylacetamide **7c**

82% yield, T. melt. = 312−314 °C (dimethylformamide-2-propanole 1:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.8 (t, 3H, −CH$_2$-CH$_3$ J = 9.3 Hz), 1.35 (kv, 2H, -CH$_2$-CH$_3$ J = 6.4 Hz), 3.0 (kv, 2H, −NH−CH$_2$- J = 2.6 Hz), 4.45 (s, 2H, -CH$_2$-CO), 7.18 (t, 2H, Ar−H, J = 7 Hz), 7.63 (t, 1H, Ar−H, J = 7 Hz), 7.9 (d, 1H, Ar−H, J = 7 Hz), 8.05 (t, 1H, −CO−NH, J = 4 Hz), 11.4 (s, 1H, NH (het)). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.48, 161.87, 150.11, 139.54, 135.06, 127.41, 122.53, 115.18, 111.83, 42.58, 38.75, 22.36, 11.37. 59.46% C, 5.65% H, 16.03% N Calculated for $C_{13}H_{15}N_3O_3$ Found,%: C 59.76, H 5.79, N 16.08, LC-MC m/z: 262.0 [(M+H)$^+$].

3.1.4. 2-(2,4-Dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-butylacetamide **7d**

75% yield, T. melt. = 292−294 °C (dimethylformamide-2-propanole 1:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.8 (t, 3H, −CH$_2$-CH$_3$ J = 8 Hz), 1.15−1.4 (m, 4H, -CH$_2$-CH$_2$-CH$_3$), 3.0 (kv, 2H, −NH−CH$_2$- J = 6.4 Hz), 4.41 (s, 2H, -CH$_2$-CO), 7.18 (t, 2H, Ar−H, J = 8 Hz), 7.65 (t, 1H, Ar−H, J = 7.6 Hz), 7.89 (d, 1H, Ar−H, J = 7.6 Hz), 8.0 (t, 1H, −CO−NH, J = 5 Hz), 11.4 (s, 1H, NH (het)). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.41, 161.87, 150.10, 139.54, 135.08, 127.42, 122.54, 115.19, 113.84, 42.52, 38.32, 31.24, 19.51, 13.69. 61.02% C, 6.17% H, 15.03% N Calculated for $C_{14}H_{17}N_3O_3$ Found,%: C 61.08, H 6.22, N 15.26, LC-MC m/z: 276.2 [(M+H)$^+$].

3.1.5. 2-(2,4-Dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-(2-methylpropyl)acetamide **7e**

82% yield, T. melt. = 270−272 °C (dimethylformamide-2-propanole 1:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.8 (d, 6H, 2 × CH$_3$ J = 6.4 Hz), 1.6−1.7 (m, 1H, -CH-(CH$_3$)$_2$), 2.85 (t, 2H, −NH−CH$_2$- J = 6.4 Hz), 4.45 (s, 2H, -CH$_2$-CO), 7.19 (t, 2H, Ar−H, J = 6 Hz), 7.63 (t, 1H, Ar−H, J = 6 Hz), 7.88 (d, 1H, Ar−H, J = 6 Hz), 8.05 (t, 1H, −CO−NH, J = 4 Hz), 11.4 (s, 1H, NH (het)). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.55, 161.88, 150.12, 139.54, 135.07, 127.42, 122.54, 115.19, 113.85, 46.16, 42.58, 28.13, 20.10. 61.17% C, 6.36% H, 15.06% N Calculated for $C_{14}H_{17}N_3O_3$ Found,%: C 61.08, H 6.22, N 15.26, LC-MC m/z: 276.0 [(M+H)$^+$].

3.1.6. 2-(2,4-Dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-(prop-2-en-1-yl)acetamide **7f**

80% yield, T. melt. = 272−274 °C (dimethylformamide-2-propanole 1:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.65 (t, 2H, −NH−CH$_2$- J = 5 Hz), 4.48 (s, 2H, -CH$_2$-CO-), 5.05 and 5.13 (dd, 2H, −CH=CH$_2$- J = 12 Hz, J = 16 Hz), 7.18 (t, 2H, Ar−H, J = 10 Hz), 7.65 (t, 1H, Ar−H, J = 10 Hz), 7.9 (d, 1H, Ar−H, J = 10 Hz), 8.25 (t, 1H, −CO−NH-, J = 4 Hz), 11.4 (s, 1H, NH (het)). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.55, 161.91, 150.12, 139.56, 135.09, 127.42, 122.55, 115.20, 115.12, 113.87, 42.63, 40.92. 60.11% C, 5.12% H, 16.36% N Calculated for $C_{13}H_{13}N_3O_3$ Found,%: C 60.23, H 5.05, N 16.21, LC-MC m/z: 260.1 [(M+H)$^+$].

3.1.7. 2-(2,4-Dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-cyclohexylacetamide **7g**

86% yield, T. melt. = 308−310 °C (dimethylformamide-2-propanole 1:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.0−1,25 (m, 5H, cyclohexyl), 1.5 (d, 1H, cyclohexyl, J = 13.3 Hz), 1.7 (t, 4H, cyclohexyl, J = 20 Hz), 3.4−3.55 (m, 1H, -CH- (cyclohexyl)), 4.45 (s, 2H, -CH$_2$-CO-), 7.19 (t, 2H, Ar−H, J = 8 Hz), 7.64 (t, 1H, −CO−NH-, J = 8 Hz), 7.9 (t, 2H, Ar−H, J = 10 Hz), 11.4 (s, 1H, NH (het)). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.48, 161.83, 150.10, 139.50, 135.09, 127.42, 122.56, 115.19, 113.77, 47.80, 42.48, 32.47, 25.25, 24.57. 63.63% C, 6.18% H, 13.82% N Calculated for $C_{16}H_{19}N_3O_3$ Found,%: C 63.77, H 6.36, N 13.94, LC-MC m/z: 302.2 [(M+H)$^+$].

3.1.8. 2-(2,4-Dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-phenylacetamide **7h**

75% yield, T. melt. = 294−296 °C (dimethylformamide-2-propanole 1:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.26 (s, 2H, -CH$_2$-CO), 7.02 (t, 1H, Ar−H, J = 8 Hz), 7.28 (t, 2H, Ar−H, J = 8 Hz), 7.35 (t, 1H, Ar−H, J = 8 Hz), 7.42 (d, 1H, Ar−H, J = 8 Hz), 7.53 (d, 2H, Ar−H, J = 8 Hz), 7.77 (t, 1H, Ar−H, J = 8 Hz), 7.97 (d, 1H, Ar−H, J = 8 Hz), 8.44 (t, 1H, −CO−NH-, J = 6 Hz), 11.55 (s, 1H, NH (het)). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.50, 161.89, 150.12, 139.52, 138.80, 135.29, 128.82, 127.71, 127.47, 126.22, 122.73, 115.30, 113.67, 43.12. 61.55% C, 4.20% H, 13.20% N Calculated for $C_{16}H_{13}N_3O_3$ Found,%: C 61.72, H 4.21, N 13.50, LC-MC m/z: 296.0 [(M+H)$^+$].

3.1.9. 2-(2,4-Dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-benzylacetamide **7i**

84% yield, T. melt. = 294−296 °C (dimethylformamide-2-propanole 1:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.25 (d, 2H, −NH−CH$_2$- J = 5.7 Hz), 4.5 (s, 2H, -CH$_2$-CO), 7.15−7.32 (m, 7H, Ar−H), 7.64 (t, 1H, Ar−H, J = 10 Hz), 7.9 (d, 1H, Ar−H, J = 10 Hz), 8.58 (t, 1H, −CO−NH, J = 8 Hz), 11.5 (s, 1H, NH (het)). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.85, 161.95, 150.15, 139.57, 139.28, 135.11, 128.27, 127.44, 127.11, 126.77, 122.56, 115.21, 113.89, 42.74, 42.12. 66.12% C, 4.78% H, 13.36% N Calculated for $C_{17}H_{15}N_3O_3$ Found,%: C 66.01, H 4.89, N 13.58, LC-MC m/z: 310.0 [(M+H)$^+$].

3.1.10. 2-(2,4-Dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-(2-phenylethyl)acetamide **7j**

69% yield, T. melt. = 300−302 °C (dimethylformamide-2-propanole 1:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.7 (t, 2H, -CH$_2$-C$_6$H$_5$ J = 8 Hz), 3.25 (s, 2H, −NH−CH$_2$-), 4.45 (s, 2H, -CH$_2$-CO-), 7.15−7.30 (m, 7H, Ar−H), 7.64 (t, 1H, Ar−H, J = 8 Hz), 7.92 (d, 1H, Ar−H, J = 8 Hz), 8.18 (t, 1H, −CO−NH, J = 5.3 Hz), 11.5 (s, 1H, NH (het)). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.69, 161.88, 150.12, 139.55, 139.44, 135.24, 128.68, 128.34, 127.42, 126.10, 122.70, 122.53, 115.29, 113.83, 42.63, 41.73, 35.16. 66.63% C, 5.22% H, 13.08% N Calculated for $C_{18}H_{17}N_3O_3$ Found,%: C 66.86, H 5.30, N 13.00, LC-MC m/z: 324.2 [(M+H)$^+$].

3.1.11. 2-(2,4-Dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-(3-phenylpropyl)acetamide **7k**

82% yield, T. melt. = 315−317 °C (dimethylformamide-2-propanole 1:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.7 (kvin, 2H, -CH$_2$-C$_6$H$_5$), 2.45−2.6 (m, 2H, −CH$_2$-CH$_2$-CH$_2$-), 3.05 (kv, 2H, −NH−CH$_2$-, J = 5.3 Hz), 5.0 (s, 2H, -CH$_2$-CO-), 7.1−7.2 (m, 3H, Ar−H),

7.24 (t, 2H, Ar–H, $J = 9.3$ Hz), 7.32 (t, 1H, Ar–H, $J = 9.3$ Hz), 7.39 (d, 1H, Ar–H, $J = 10.6$ Hz), 7.73 (t, 1H, Ar–H, $J = 10.6$ Hz), 7.93 (d, 1H, Ar–H, $J = 10.6$ Hz), 8.07 (t, 1H, –CO–NH-, $J = 6.4$ Hz), 12.95 (s, 1H, NH (het)). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.61, 161.90, 150.13, 141.77, 139.55, 135.06, 128.32, 128.27, 127.41, 125.72, 122.62, 122.53, 115.20, 113.85, 42.67, 38.28, 32.45, 30.96. 63.00% C, 6.10% H, 17.00% N Calculated for $C_{19}H_{19}N_3O_3$ Found,%: C 63.68, H 6.12, N 17.09, LC-MC m/z: 338.2 [$(M+H)^+$].

3.1.12. 2-(2,4-Dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-[(4-chlorophenyl)methyl] acetamide **7l**

88% yield, T. melt. = 300–302 °C (dimethylformamide-2-propanole 1:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.3 (d, 2H, –NH–CH$_2$-, $J = 5$ Hz), 5.05 (s, 2H, -CH$_2$-CO), 7.26 (d, 2H, Ar–H, $J = 8.9$ Hz), 7.34 (d, 3H, Ar–H, $J = 8$ Hz), 7.4 (d, 1H, Ar–H, $J = 8.9$ Hz), 7.73 (t, 1H, Ar–H, $J = 8$ Hz), 7.94 (d, 1H, Ar–H, $J = 8$ Hz), 8.6 (t, 1H, –CO–NH-, $J = 5$ Hz), 13.0 (s, 1H, NH (het)). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.03, 161.95, 150.15, 139.56, 138.38, 135.09, 131.37, 128.96, 128.19, 127.43, 122.56, 115.22, 113.87, 42.78, 41.49. 66.00% C, 5.15% H, 13.20% N, 9.8% Cl Calculated for $C_{17}H_{14}ClN_3O_3$ Found,%: C 66.01, H 5.30, N 13.00, O 17.84, 9.85 Cl, LC-MC m/z: 344.1 [$(M+H)^+$].

3.1.13. 2-(2,4-Dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-[(2,4-dichlorophenyl)methyl]-acetamide **7m**

78% yield, T. melt. = 310–312 °C (dimethylformamide-2-propanole 1:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.3 (d, 2H, –NH–CH$_2$-, $J = 6.4$ Hz), 5.2 (s, 2H, -CH$_2$-CO-), 7.35 (t, 1H, Ar–H, $J = 8$ Hz), 7. 4 (t, 3H, Ar–H, $J = 4.8$ Hz), 7.58 (s, 1H, Ar–H), 7.75 (t, 1H, Ar–H, $J = 8$ Hz), 7.95 (d, 1H, Ar–H, $J = 8$ Hz), 8.65 (t, 1H, –CO–NH, $J = 4.8$ Hz), 13.0 (s, 1H, NH (het)). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.27, 161.96, 150.15, 139.55, 135.44, 135.17, 132.86, 132.26, 130.05, 128.56, 127.45, 127.28, 122.61, 115.24, 113.84, 42.75, 41.93. 58.00% C, 6.12% H, 15.20% N, 17.90% Cl Calculated for $C_{17}H_{13}Cl_2N_3O_3$ Found,%: C 58.30, H 6.20, N 15.26, 17.98 Cl, LC-MC m/z: 378.0 [$(M+H)^+$].

3.1.14. 2-(2,4-Dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-[(2-methoxyphenyl)methyl] acetamide **7n**

85% yield, T. melt. = 292–294 °C (dimethylformamide-2-propanole 1:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.7 (s, 3H, -OCH$_3$), 4.2 (d, 2H, –NH–CH$_2$-, $J = 6.4$ Hz), 5.05 (s, 2H, -CH$_2$-CO-), 6.84 (d, 2H, Ar–H, $J = 8$ Hz), 7.17 (d, 2H, Ar–H, $J = 8$ Hz), 7.34 (t, 1H, Ar–H, $J = 8$ Hz), 7.4 (d, 1H, Ar–H, $J = 8$ Hz), 7.74 (t, 1H, Ar–H, $J = 8$ Hz), 7.95 (d, 1H, Ar–H, $J = 8$ Hz), 8.5 (t, 1H, –CO–NH-, $J = 6$ Hz), 12.95 (s, 1H, NH (het)). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.91, 161.95, 156.60, 150.17, 139.56, 135.09, 128.03, 127.62, 127.43, 126.61, 122.55, 120.13, 115.20, 113.87, 110.43, 55.34, 42.72, 37.24. 60.00% C, 4.55% H, 11.60% N Calculated for $C_{18}H_{17}N_3O_4$ Found,%: C 60.83, H 4.82, N 11.82, LC-MC m/z: 340.2 [$(M+H)^+$].

4. Conclusions

A synthesis of new derivatives of 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2H)-yl) acetamide ***7a-7n*** was performed with good yields by amination of 2-(2,4-dioxo-1,4-dihydro-quinazolin-3(2H)-yl)acetic acid ***4*** in anhydrous dioxane at CDI presence. A novel approach for synthesis key intermediate **4** has been developed. It was prepared by oxidation of previously synthesized 2-(4-oxo-2-thioxo-1,4-dihydroquinazolin-3(2*H*)-yl) acetic acid **3** by hydrogen peroxide at 70 °C. 2-(4-Oxo-2-thioxo-1,4-dihydroquinazolin-3(2*H*)-yl) acetic acid was synthesized by interaction of methyl 2-isothiocyanatobenzoate ***1*** with glycine ***2*** in the presence of triethylamine in 2-propanol. The structure and purity of the all resulting substances were confirmed by elemental analysis, ^{1}H NMR, ^{13}C NMR spectroscopy and LC/MS.

Docking studies were performed to estimate anticonvulsant potential and to select more prospective substances for pharmacological investigations. Among 10 compounds selected four compounds ***7c, 7h, 7k, 7m*** caused statistically significant anticonvulsant effect on pentyltetrazole seizures at animals. Compound ***7m*** provided a complete protective effect lethality and found to be superior to sodium valproate. Experimental studies have found that it is a non-toxic substance, devoid of motor coordination impairment. Taken together our docking results and *in vivo* results show that there is a positive correlation between the dock scores and the inhibition of protein carbonic anhydrase II (gene name CA2) (PDB code 1 EOU).

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgments

The authors gratefully acknowledge the financial support from the National University of Pharmacy.

References

[1] GBD 2015 Neurological Disorders Collaborator Group, Global, regional, and national burden of neurological disorders during 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015, Lancet Neurol. 16 (11) (2017) 877–897. https://doi.org/10.1016/S1474-4422(17)30338-1.
[2] D.G. Vossler, M. Weingarten, B.E. Gidal, Summary of antiepileptic drugs Available in the United States of America: working toward a world without epilepsy, Am. Epilepsy Soc. Treat. Comm. Epilepsy Curr. 18 (4 suppl 1) (2018) 1–26. PMCID: PMC6130739, https://doi.org/10.5698/1535-7597.18.4s1.1.
[3] A.A. Kotvitska, I.O. Lobova, Marketing researches of market on medications for neuroprotective action in Ukraine, Acta Fac. Pharm. Univ. Comen. LX 1 (2013) 15–20. https://doi.org/10.2478/afpuc-2013-0012.
[4] L. Emily, M.D. Johnson, Seizures and epilepsy, Med. Clin. N. Am. 103 (2) (2019) 309–324. https://doi.org/10.1016/j.mcna.2018.10.002.
[5] A.C. Marino, G.J. Yang, E. Tyrtova, Wu Kun, P. Hitten, F.Z. Pue, D.D. Spencer, S.K. Bandt, Resting state connectivity in neocortical epilepsy: the epilepsy network as a patient-specific biomarker, Clin. Neurophysiol. 130 (2) (2019) 280–288. https://doi.org/10.1016/j.clinph.2018.11.016.
[6] C.L. Faingold, Anticonvulsant drugs as neuronal network-modifying agents, Encycl. Basic Epilepsy Res. 1 (2009) 50–58. https://doi.org/10.1016/B978-012373961-2.00074-6.
[7] H. Kaur, B. Kumar, B. Medhi, Antiepileptic drugs in development pipeline: a recent update, Neurol. Sci. 4 (2016) 42–51. https://doi.org/10.1016/j.ensci.2016.06.003.
[8] H. Stefan, T. Feuerstein, Novel anticonvulsant drugs, Pharmacol. Therapeut. 113 (2007) 165–183. https://doi.org/10.1016/j.pharmthera.2006.07.005.
[9] C.C. Hansen, H. Ljung, E. Brodtkorb, A. Reimers, Mechanisms underlying aggressive behavior induced by antiepileptic drugs: focus on topiramate, levetiracetam, and perampanel, Behav. Neurol. (2018) 2064027, 2018, https://doi.org/10.1155/2018/2064027.
[10] H. Severina, O. Skupa, A. Khairulin, N. Voloshchuk, V. Georgiyants, Synthesis and anticonvulsant activity of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one acetamides, J. Appl. Pharm. Sci. 9 (02) (2019) 12–19. https://doi.org/10.7324/JAPS.2019.90202.
[11] N. Siddqui, J.M. Akhtar, R. Ali, B. Azad, An updated review: emerging anticonvulsants, Int. J. Pharm. Biosci. Arch. 1 (2010) 404–415.
[12] G. Mc Carthy, B. Myers, N. Siegfried, Treatment for methaqualone dependence in adults, Cochrane Database Syst. Rev. 2 (2005), https://doi.org/10.1002/14651858.CD004146.pub2. PMID 15846700.
[13] P. Ilangovan, S. Ganguly, V. Pandit, Design and synthesis of novel quinazolinone derivatives as broad spectrum anticonvulsant and antimicrobial agent, J. Pharm. Res. 3 (2010) 703–706.
[14] V.G. Ugale, S.B. Bari, Quinazolines: new horizons in anticonvulsant therapy, Eur. J. Med. Chem. 80 (10) (2014) 447–501. https://doi.org/10.1016/j.ejmech.2014.04.072.
[15] M.K. Ibrahim, K. El-Adl, A.A. Al-Karmalawy, Design, synthesis, molecular docking and anticonvulsant evaluation of novel 6-iodo-2-phenyl-3-substituted-quinazoli-4(3H)-ones, Bull. Fac. Pharm. Cairo Univ. 53 (2015) 101–116.
[16] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, Ph E. Bourne, The protein data bank, Nucleic Acids Res. 28 (1) (2000) 235–242. https://doi.org/10.1093/nar/28.1.235.
[17] P.S. Miller, A.R. Aricescu, Crystal structure of a human gabaa receptor, Nature 512 (2014) 270–275. PubMed: 24909990 S, https://doi.org/10.1038/nature13293.
[18] C.T. Supuran, Carbonic anhydrase inhibitors and activators for novel

therapeutic applications, Future Med. Chem. 3 (9) (2011) 1165–1180. https://doi.org/10.4155/fmc.11.69.
[19] R. Meleddu, S. Distinto, F. Cottiglia, R. Angius, M. Gaspari, D. Taverna, C. Melis, A. Angeli, G. Bianco, S. Deplano, Tuning the dual inhibition of carbonic anhydrase and cyclooxygenase by dihydrothiazole benzensulfonamides, ACS Med. Chem. Lett. 9 (10) (2018) 1045–1050. https://doi.org/10.1021/acsmedchemlett.8b00352.
[20] M.O. Karataş, H. Uslu, S. Sarı, M.A. Alagöz, A. Karakurt, B. Alıcı, C. Bilen, E. Yavuz, N. Gencer, O. Arslan, Coumarin or benzoxazinone based novel carbonic anhydrase inhibitors: synthesis, molecular docking and anticonvulsant studies, J. Enzym. Inhib. Med. Chem. 31 (2016) 760–772. https://doi.org/10.3109/14756366.2015.1063624.
[21] R. Recacha, M.J. Costanzo, B.E. Maryanoff, D. Chattopadhyay, Crystal structure of human carbonic anhydrase II complexed with anticonvulsant sugar sulfamate, Molecules 22 (2017) 437–441. https://doi.org/10.2210/pdb1EOU/pdb.
[22] H.G. Vogel, Drug Discovery and Evaluation: Pharmacological Assays, Chapter E: Psychotropic and neurotropic activity, 2008, pp. 459–493.
[23] А.Н. Миронова, Н.Д. Бунятян, А.Н. Васильева, Руководство по проведению доклинических исследований лекарственных средств. Часть первая, 2012, p. 944.
[24] С.Ю. Штыголь, Модуляция фармакологических эффектов при различных солевых реЖимах: Монография, 2007, p. 360.
[25] Т.В. Пастушенко, П.Б. Маруший, А.А. Жуков, Экспресс-метод определения средне смертельных доз химических веществ, Гигиена и санитария. 6 (1985) 46–49.
[26] M.P. Fay, M.A. Proschan, Wilcoxon–Mann–Whitney or t-test? On assumptions for hypothesis tests and multiple interpretations of decision rules, Stat. Surv. 4 (2010) 1–39. https://doi.org/10.1214/09-SS051.
[27] Ei Kasuya, Mann–Whitney U test when variances are unequal, Anim. Behav. 61 (6) (2001) 1247–1249. https://doi.org/10.1006/anbe.2001.1691.
[28] B. Danielsson, L. Kronberg, F. Ljungner, Studies on the medicinal chemistry of oxoquinazolines. I. N-(Arylcarbamoylmethyl)- and N-(4-t-amino-2-butynyl) derivatives of 1,2,3,4-tetrahydro-2,4-dioxoquinazoline, Acta Pharm. Suec. 5 (2) (1968) 77–84.
[29] M. Bogert, G. Scatchard, Researches on quinazolines. XXXIV. The synthesis of certain nitro and amino benzoylene ureas and some compounds related thereto, J. Am. Chem. Soc. 31 (1919) 2052.
[30] I. Philipova, A. Linden, H. Heinz, Application of the "direct amide cyclization" to peptides containing an anthranilic acid residue, Helv. Chim. Acta 88 (7) (2005) 1711–1733.
[31] E.P. Papadopoulos, Reactions of o-aminonitriles with isocyanates. 2. A facile synthesis of imidazo[1,2c]quinazoline-2,5-(3H,6H)dione, J. Heterocycl. Chem. 18 (3) (1981) 515–518. https://doi.org/10.1002/jhet.5570180315.
[32] P. Canonne, M. Akssira, A. Dahdouh, H. Kasmi, M. Boumzebra, Synthesis of chiral 3-substituted 2,4(*1H,3H*)-Quinazolinediones, Heterocycles 36 (6) (1993) 1305–1314, https://doi.org/10.3987/COM-92-6296.
[33] A.V. Ivachtchenko, S.M. Kovalenko, O.G. Drushlyak, Synthesis of substitu-ted 4-Oxo-2-thioxo-1,2,3,4-tetrahydroquinazolines and 4-Oxo-3,4-dihydroquinazoline-2-thioles, J. Comb. Chem. 5 (6) (2003) 775–788. https://doi.org/10.1021/cc020097g.
[34] D. Kim Kee Soo Lee Kwan, Efficient synthesis of primary amides from carboxylic acids using N,N′-Carbonyldiimidazole and ammonium acetate in ionic liquid, Synth. Commun. 41 (23) (2011) 3497–3500. https://doi.org/10.1080/00397911.2010.518331.
[35] N. Usachova, G. Leitis, A. Jirgensons, I. Kalvinsh, Synthesis of hydroxamic acids by activation of carboxylic acids with N,N′-Carbonyldiimidazole: exploring the efficiency of the method, Synth. Commun. 40 (6) (2010) 927–935. https://doi.org/10.1080/00397910903026723.
[36] S.F. Suat, B. Kaynak, S. Dalkara, Synthesis and anticonvulsant screening of 1,2,4-triazole derivatives, Pharmacol. Rep. 70 (16) (2018) 1116–1123. https://doi.org/10.1016/j.pharep.2018.06.007.
[37] P.H. Palestro, N. Enrique, S. Goicoechea, M.L. Villalba, L.L. Sabatier, P. Martin, V. Milesi, L.E. Bruno Blanch, L. Gavernet, Searching for new leads to treat epilepsy: target-based virtual screening for the Discovery of anticonvulsant agents, J. Chem. Inf. Model. 58 (7) (2018) 1331–1342. https://doi.org/10.1021/acs.jcim.7b00721.
[38] M.K. Ibrahim, Kh Ahmed, A. El-Adl, Al-Karmalawy, Design, synthesis, molecular docking and anticonvulsant evaluation of novel 6-iodo-2-phenyl-3-substituted-quinazolin-4(3H)-ones, Bull. Fac. Pharm. Cairo Univ. 53 (2) (2015) 101–116. https://doi.org/10.1016/j.bfopcu.2015.05.001.
[39] YuS. Prokopenko, L.O. Perekhoda, V.A. Georgiyants, Docking studies of biologically active substances from plant extracts with anticonvulsant activity, J. Appl. Pharm. Sci. 9 (01) (2019) 66–72. https://doi.org/10.7324/JAPS.2019.90110.
[40] H.C. Hodge, L.H. Sterner, Tabulation of toxicity classes, Am. Ind. Hyg. Ass. Quart. 10 (4) (1943) 93.