Carboxyl-containing quinazolines and related heterocycles as carriers of anti-inflammatory activity

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Abstract

Active pharmaceutical ingredients whose structure combines aromatic or heterocyclic fragments with pharmacophore carboxylic group are widespread on pharmaceutical market. The isolation of COX-NSAIDs complexes and following X-ray studies allowed to explain the key role of pharmacophore carboxylic group in the formation of enzyme-ligand interactions and the effect of its presence on the activity and selectivity. The introduction of selective COX-2-inhibitors to medicinal practice resulted in a significant decrease of side effects and complication frequencies. However, the problem of NSAIDs toxicity has not been solved. Thus, the search for novel anti-inflammatory drugs using in silico methods and approaches including structural modification of known NSAIDs by "bioisosteric" replacements of aromatic and heterocyclic fragments with other structural elements with carboxylic group as the carrier of pharmacological effect, is a current trend of medical chemistry.

The aim of present study is to purposefully search for anti-inflammatory agents among carboxyl-containing quinazolines and related heterocycles using in silico and in vivo methods, as well as to evaluate carboxylic group effect on the level of anti-inflammatory activity.

Materials and methods. Quinazoline-4(3H)-yldene/hydrazides of mono-(di-)carboxylic acids, 2-R-{1,2,4}triazolo[1,5-c]quinazolines, 3-R-5-(2-aminophenyl)-1H-1,2,4-triazoles, 5-carboxyalkyl[1,2,4]triazolo[1,5-c]quinazolines and 2-R-7-oxo-6,7-dihydropyrrolo[1,2-a][1,2,4]triazol[1,5-c]quinazoline-4a(5H)-carboxylic acids were screened for their anti-inflammatory activity. MarvinSketch 20.19.0, AutoDock Vina and AutoDockTools 1.5.6, HyperChem 7.5, Discovery Studio were used for in silico research. "Drug-like" characteristics were evaluated using an online service. Prediction of toxicity and Ames mutagenicity of the studied compounds were performed in silico using Test software. Evaluation of the anti-inflammatory activity of the synthesized compounds was carried out on white Wistar rats (150–160 g of weight) using carrageenan induced paw edema model. Phlogogen (1 % aqueous solution of λ-carrageenan) was subplantarly injected in the dose of 0.1 ml in the rats' hind right paw. The left one was used as a control. The studied compounds were intragastrically administered with atraumatic probe as water solution or finely dispersed suspension stabilized by Tween-80 in the dose of 10 mg/kg 1 hour before the injection of phlogogen. The reference drug Diclofenac sodium was administered intragastrically in a recommended for pre-clinical studies dose of 8 mg/kg. The paw volume was measured before the experiment and in 4 hours after phlogogen injection. The activity of these substances was determined by their ability to reduce the swelling compared with control group and was expressed in percentage. The experiments were carried out with respect to Bioethical rules and norms.

Results. The search for anti-inflammatory agents among carboxyl-containing quinazolines and related heterocycles was theoretically substantiated using results of molecular docking, druglike criteria calculations and predicted parameters of toxicity. Experimental in vivo methods ("carrageenan" test) confirmed the anti-inflammatory activity of studied compounds and showed that (quinazoline-4(3H)-yldiene) hydrazides of dicarboxylic acids inhibit edema by 17.0–50.0 %, 2-carboxyalkyl-(phenyl-)1H-1,2,4-triazoles, 5-carboxyalkyl[1,2,4]triazolo[1,5-c]quinazolines – by 0.00–40.63 %, 2-(2-aminophenyl)-1H-1,2,4-triazol-3-ylidene)carboxylic acids – by 2.43–49.65 %, 2-R-5-carboxyalkyl[1,2,4]triazolo[1,5-c]quinazolines – by 0.47–22.93 % and 2-R-7-oxo-6,7-dihydropyrrolo[1,2-a][1,2,4]triazol[1,5-c]quinazoline-4a(5H)-carboxylic acids – by 0.94–17.16 %. Among them, there are compounds that compete with the reference drug “Diclofenac sodium”. The SAR analysis showed that both configuration of the molecule and the nature of the "pharmacophore" moiety (carboxyalkyl residue length) at the corresponding positions of the hormone have a significant effect on the anti-inflammatory activity. It was shown that the test compounds, according to molecular docking visualization data, have other enzyme-ligand interactions and probably a different mechanism of activity.

Conclusions. The predicted affinity values, calculated “drug-like” criteria and toxicity parameters, visualization of the docking of studied molecules in active site of biological targets as well as experimental studies results showed that investigated compounds are promising in scope of purposeful search for anti-inflammatory drugs. The conducted in vivo screening of anti-inflammatory activity among carboxy-containing quinazolines and related heterocyclic compounds allowed to detect series of substances that by the level of anti-inflammatory activity compete with reference-compound "Diclofenac sodium" on the carrageenan-induced paw edema model. Presented data may be considered as a theoretical basis for further structural modification of studied compounds aimed on elaboration of novel anti-inflammatory agents and evaluation of their activity mechanism (lipoxygenase inhibitors, phospholipase inhibitors, etc.).

Key words: quinazolines, triazoles, heterocyclic compounds, fused-heterocyclic systems, pharmacophore, in silico methods, as well as to evaluate carboxylic group effect on the level of anti-inflammatory activity.

Materials and methods. Quinazoline-4(3H)-yldene/hydrazides of mono-(di-)carboxylic acids, 2-R-{1,2,4}triazolo[1,5-c]quinazolines, 3-R-5-(2-aminophenyl)-1H-1,2,4-triazoles, 5-carboxyalkyl[1,2,4]triazolo[1,5-c]quinazolines and 2-R-7-oxo-6,7-dihydropyrrolo[1,2-a][1,2,4]triazol[1,5-c]quinazoline-4a(5H)-carboxylic acids were screened for their anti-inflammatory activity. MarvinSketch 20.19.0, AutoDock Vina and AutoDockTools 1.5.6, HyperChem 7.5, Discovery Studio were used for in silico research. "Drug-like" characteristics were evaluated using an online service. Prediction of toxicity and Ames mutagenicity of the studied compounds were performed in silico using Test software. Evaluation of the anti-inflammatory activity of the synthesized compounds was carried out on white Wistar rats (150–160 g of weight) using carrageenan induced paw edema model. Phlogogen (1 % aqueous solution of λ-carrageenan) was subplantarly injected in the dose of 0.1 ml in the rats' hind right paw. The left one was used as a control. The studied compounds were intragastrically administered with atraumatic probe as water solution or finely dispersed suspension stabilized by Tween-80 in the dose of 10 mg/kg 1 hour before the injection of phlogogen. The reference drug Diclofenac sodium was administered intragastrically in a recommended for pre-clinical studies dose of 8 mg/kg. The paw volume was measured before the experiment and in 4 hours after phlogogen injection. The activity of these substances was determined by their ability to reduce the swelling compared with control group and was expressed in percentage. The experiments were carried out with respect to Bioethical rules and norms.

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Conclusions. The predicted affinity values, calculated “drug-like” criteria and toxicity parameters, visualization of the docking of studied molecules in active site of biological targets as well as experimental studies results showed that investigated compounds are promising in scope of purposeful search for anti-inflammatory drugs. The conducted in vivo screening of anti-inflammatory activity among carboxyl-containing quinazolines and related heterocyclic compounds allowed to detect series of substances that by the level of anti-inflammatory activity compete with reference-compound "Diclofenac sodium" on the carrageenan-induced paw edema model. Presented data may be considered as a theoretical basis for further structural modification of studied compounds aimed on elaboration of novel anti-inflammatory agents and evaluation of their activity mechanism (lipoxygenase inhibitors, phospholipase inhibitors, etc.).
ініціаторів сприяло суттєвому зниженню ризику розвитку основних ускладнень (гастроскопічності), але не роз'яснило проблему токсичності НПЗЗ. Отже, актуальним є пошук нових протизапальних засобів шляхом «біозестеричних» замін ароматичних і гетероцикличних фрагментів відомих препаратів на інші структурні фрагменти з наявністю карбооксиду як носія фармакохімічного ефекту.

Мета роботи – спрямований пошук протизапальних агентів серед карбоксилових хіназолів і споріднених гетероциклів, а також дослідження впливу карбооксиду групи на антиінфамматорну активність із використанням методології in silico та in vivo.

Матеріали та методи. Хіназолін-4(3H)-іліден)гідразид моно-(ди-)карбових кислот, 2-R-[1,2,4]триазоло[1,5-c]хіназолини, 3-R-5-(2-амінофеніл)-1H-1,2,4-триазол, 5-карбоксилалін[1,2,4]триазоло[1,5-c]хіназолини та 2-R-7-оксо-6,7-дигідропірроло[1,2-a] [1,2,4]триазоло[1,5-c]хіназоліни 4a(5H)-карбонові кислоти досліджували на протизапальну активність. МарвинСкетч 20.19.0, AutoDock Vina та AutoDockTools 1.5.6, HyperChem 7.5, Discovery Studio використовували для визначення гострої токсичності, ембріотоксичності та мутагенності.

Закінчення молекулярного докінґу в активних центрах біомішеней показали перспективність цього класу для наступних досліджень. Наведені результати, а також дослідження впливу карбооксиду на антиінфамматорну активність із використанням методології in silico і in vivo, відповідають останнім науковим дослідженням.

Висновки. Дослідження на протизапальну активність у ряду карбоксилових хіназолів і споріднених гетероциклів показали, що вони конкурують з референс-препаратом диклофенаком натрію.

Закінчення молекулярного докінґу, що вони вивчали, мала інші фермент-лігандні взаємодії і, вірогідно, інший механізм дії.

Як контроль. Внутрішньошлункове введення досліджуваних сполук як водного розчину або тонкодисперсної суспензії, стабілізованої твіном-80, у дозі 10 мг/кг здійснили за 1 годину навіть з контрастної групи, наводили у відсотках. Експерименти здійснили, дотримуючись біоетичних правил і норм.

Лекарственные средства, объединяя в своей структуре ароматический и гетероциклический фрагменты с “фармакофорной” карбооксидной группой, широко представлены на фармацевтическом рынке. Именно указанная комбинация структурных элементов содержится в молекулах нестероидных противовоспалительных средств (НПВС). Детальное изучение механизма действия НПВС позволило объяснить ключевую роль и значение “фармакофорной” карбооксидной группы на активность, селективность и токсичность. Введение в медицинскую практику селективных ингибиторов привело к существенному снижению риска развития основных осложнений (гастроскопия), но не решило проблему токсичности НПВС. Таким образом, актуален поиск новых противовоспалительных средств путем “біозестеричности” замен ароматических и гетероциклических фрагментов известных препаратов на другие структурные фрагменты с наличием карбооксидной группы как носителя фармакологического эффекта.

Резюме. Мета роботи – направленный поиск новых противовоспалительных агентов среди карбоксилсодержащих хиназолинов и родственных гетероциклов. В рамках изучения механизма действия (биоизостерные замены ароматических и гетероциклических фрагментов известных препаратов на новые структурные фрагменты с наличием карбооксидной группы) ведется поиск новых противовоспалительных агентов с “фармакофорной” карбооксидной группой на основе изучения биоизостерических замен ароматических и гетероциклических фрагментов известных препаратов на другие структурные фрагменты с наличием карбооксидной группы как носителя фармакологического эффекта.

Висновки. Дослідження на протизапальну активність у ряду карбоксилових хіназолів і споріднених гетероциклів показали, що вони конкурують з референс-препаратом диклофенаком натрію. Прогностичні значення адорінності, розрахунки критеріїв “drug-like”, параметри токсичності методами in silico та візуалізації молекулярного докінґу в активних центрах біомішеней показали перспективність цього класу для наступних досліджень. Наведені дані – теоретичне підґрунтя для проведення структурної модифікації для виявлення нових антифламматорів і можливого механізму дії (ініціатори ліпідного генезу, фосфоліпаз А ттп.)

Карбоксилсодержащие хиназолины и родственные гетероциклы как носители противовоспалительной активности

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Лекарственные средства, объединяющи в своей структуре ароматический и гетероциклический фрагменты с “фармакофорной” карбооксидной группой, широко представлены на фармацевтическом рынке. Именно указанная комбинация структурных элементов содержится в молекулах нестероидных противовоспалительных средств (НПВС). Детальное изучение механизма действия НПВС позволило объяснить ключевую роль и значение “фармакофорной” карбооксидной группы на активность, селективность и токсичность. Введение в медицинскую практику селективных ингибиторов привело к существенному снижению риска развития основных осложнений (гастроскопия), но не решило проблему токсичности НПВС. Таким образом, актуален поиск новых противовоспалительных средств путем “біозестеричности” замен ароматических и гетероциклических фрагментов известных препаратов на другие структурные фрагменты с наличием карбооксидной группы как носителя фармакологического эффекта.

Цель работы – направленный поиск новых противовоспалительных агентов среди карбоксилсодержащих хиназолинов и родственных гетероциклов, а также исследование влияния карбооксидной группы на антифламматорную активность с использованием методологий in silico и in vivo.

Материалы и методы. Хиназолин-4(3H)-иліден)гідразид моно-(ди-)карбоновых кислот, 2-R-[1,2,4]триазоло[1,5-c]хиназолини, 3-R-5-(2-амінофеніл)-1H-1,2,4-триазол, 5-карбоксилалін[1,2,4]триазоло[1,5-c]хиназолини та 2-R-7-оксо-6,7-дигідропірроло[1,2-a] [1,2,4]триазоло[1,5-c]хиназоліни 4a(5H)-карбонові кислоти исследованы на противовоспалительную активность. MarvinSketch 20.19.0, AutoDock Vina и AutoDockTools 1.5.6, HyperChem 7.5, Discovery Studio использованы для визуализации молекулярного докинга в активных центрах биомишеней показали перспективность этого класса для наступных исследований. Наведенные данные – теоретическое подгрунтя для проведения структурной модификации для выявления новых антифламматоров и мозгового механизма дії (ініціатори ліпідного генезу, фосфоліпаз А ттп.).
и через 4 часа после инъекции флогена. Активность соединений определяли по их способности уменьшать отек по сравнению с контрольной группой, выражали в процентах. Эксперименты проведены с соблюдением биотических правил и норм.

**Results.** By the results of molecular docking, the criteria «drug-like» and prognostic parameters toxicity of theoretically predicted were shown by modern concepts of inflammation pathogenesis. However, the problem of NSAIDs toxicity has not been solved [11,12]. Thus, the search for novel anti-inflammatory drugs using in silico methods and approaches that include structural modification of known NSAIDs by "biosimilar" replacement of aromatic and heterocyclic fragments to other structural elements with carboxylic group as a carrier of pharmacological effect is a current trend of medicinal chemistry.

**Aim**

The aim of the present study is to purposefully search for anti-inflammatory agents among carboxyl-containing quinazolines and related heterocycles using in silico methods, as well as the evaluation of carboxylic group effect on the level of anti-inflammatory activity.

**Materials and methods**

Quinazoline-4(3H)-ylidenehydrazides of mono-(di-)carboxylic acids (Iia–g), 2-R-[1,2,4]triazolo[1,5-c]quinazolines (Ila–g), 3-R-5-(2-aminophenyl)-1H-1,2,4-triazoles (Iva–f), 2-R-5-carboxyalkyl[1,2,4]triazolo[1,5-c]quinazolines (Vla–d) and 2-R-7-oxo-6,7-dihydropryrolo[1,2-a][1,2,4]triazolo[1,5-c]quinazoline-4a(5H)-carboxylic acids (Vla–d) were screened for anti-inflammatory activity. The synthesis and physico-chemical data of the tested compounds was previously described [13–16].

**Molecular docking.** Research was conducted by flexible molecular docking as an approach of finding molecules with affinity to a specific biological target. Macromolecules from Protein Data Bank (PDB) were used as biological targets, namely COX-1 enzyme in complex with DF (PDB ID – 3NY), COX-2 in combination with DF (PDB ID – 1PXX) [17]. The choice of biological targets was due to the literature about the mechanism of anti-inflammatory drugs activity [2].

**Ligand preparation.** Substances were drawn using MarvinSketch 20.19.0 and saved in mol format [18]. After that they were optimized by program Chem3D, using molecular mechanical MM2 algorithm and saved as PDB files. Molecular mechanics was used to produce more realistic geometry values for most organic molecules, owing to the fact of being...
Fig. 1. Research design and approaches to the carboxyl-containing quinazolines and related heterocycles synthesis.

highly parameterized. Using AutoDockTools-1.5.6, PDB files were converted into PDBQT, number of active torsions was set as default [19].

Protein preparation. PDB files were downloaded from the protein data bank. Discovery Studio was used to delete water molecules and ligands. Structures of proteins were saved as PDB files [20]. In AutoDockTools-1.5.6, polar hydrogens were added and saved as PDBQT. Grid box was set as following: center_x = 18.37, center_y = -52.30, center_z = 53.95, size_x = 18, size_y = 16, size_z = 16 for COX-2 (3LN1); center_x = 32.98, center_y = -44.49, center_z = -3.76, size_x = 16, size_y = 16, size_z = 16 for COX-1 (3NY8); center_x = 3.86, center_y = 20.06, center_z = -9.06, size_x = 18, size_y = 18, size_z = 18 for PLA2 (1ZYX). Vina was used to carry docking [19]. For visualization, Discovery Studio v 19.1.0.18287 was used.

In Silico Prediction. “Drug-like” characteristics were evaluated using an electronic resource [21]. Acute toxicity of the studied compounds was predicted in silico using TEST software [22,23].

Anti-inflammatory activity. Anti-inflammatory activity of the synthesized compounds was evaluated on 228 Wistar white rats (150–160 g of weight), obtained from the breeding station of “Institute of Pharmacology and Toxicology of Ukraine” (Kyiv). All experimental procedures and treatment were carried out according to the European Convention and “Regulations on the use of animals in biomedical research” [24]. Screening of the synthesized compounds with estimated anti-inflammatory activity began with the study of their effect on exudative phase of acute aseptic inflammation (“carrageenan” test) [25]. Phlogogen (1 % aqueous solution of λ-carrageenan) was subplantarily injected in the dose of 0.1 ml in the rats’ hind right paw. The left one was used as a control. The studied compounds were intragastrically administered with atraumatic probe as water solution or finely dispersed suspension stabilized by Tween-80 in a dose of 10 mg/kg, 1 hour before the injection of phlogogen. The reference drug Diclofenac sodium was administered intragastrically in a recommended dose of 8 mg/kg for pre-clinical studies. Measurement of paws volume was conducted before the experiment and 4 (“carrageenan” test) hours after injection of phlogogen using the described methods. The activity of these substances was determined by their ability to reduce the swelling compared with control group and was expressed in percentage. It showed how the substance inhibited phlogogen swelling in relation to control swelling where the value was taken as 100 %.

The activity of the studied compounds was calculated as following:

\[ A, \% = 100 \% \times \left( \frac{V_{pe} - V_{he}}{V_{pc} - V_{hc}} \right) \times 100 \% \]

where A – antiexudative activity, %; Vpe – the volume of paw edema in the experiment; Vhc – the volume of healthy paw in control; Vpc – the volume of paw edema in control; Vhc – the volume of healthy paw in control.

Data were statistically processed with the licensed program Statistica for Windows 13 (StatSoft Inc., No. JPZ804I382130ARCN10-J) and “SPSS 16.0”, Microsoft Office Excel 360. The results were presented as mean ± standard error of the mean. Arithmetical mean and standard error of the mean were calculated for each of the studied parameters. During verification of statistical hypothesis, null hypothesis was declined if statistical criterion was \( P < 0.05 \) [26].

Zaporozhye medical journal. Volume 24. No. 1, January – February 2022
Results

The study design implied the selection of basic molecules, namely quinazolin-4-(3H)-yldiene/hydrazides of mono-(di-) carboxylic acids (II) that were used as basis for construction of the virtual library of potential anti-inflammatory agents. For evaluation of promising structural modification routes, the literature data as well as our own “structure – biological activity” data were used [27–36] (Fig. 1). It should be mentioned that selected heteroaromatic basic molecules have ample opportunities for structural modification by the heterocyclization and nucleophilic degradation reactions that additionally allow to introduce various pharmacophore groups that are associated with anti-inflammatory activity (primarily carboxylic group).

The general methods for the synthesis of the target quinazoline-4(3H)-yldiene/ hydrazides of carboxylic and dicarboxylic acids (IIa–g), 2-R[1,2,4]triazolo[1,5-c]quinazolines (IIIa–g), 3-R-S-(2-amino phenyl)-1H-1,2,4-triazoles (IVa–f), 2-R-S-carboxyalkyl[1,2,4]triazolo[1,5-c]quinazolines (Va–I) and 2-R-7-oxo-6,7-dihydropyrrrole[1,2-a][1,2,4] triazolo[1,5-c]quinazoline-4a(5H)-carboxylic acids (Via–d) are presented in Fig. 1.

Considering the prospects of aforementioned class of the compounds and ample opportunities for their chemical modification, the in silico screening aimed at the estimation of promising objects for in vivo studies was conducted. Thus, docking studies to COX-1 and COX-2, as key enzymes of inflammation process developing, calculation of physicochemical properties, “drug-like” criteria, and toxicity parameters were performed for more than 100 candidate compounds using appropriate software and services [21–23]. The analysis of molecular docking results showed that calculated affinity of the most of the studied compounds to key enzymes of the inflammation were higher or comparable with reference compound. It was found that the highest affinity to enzymes were characteristic for compounds II–VI that contain the carboxylic groups. Quinazoline-4(3H)-yldiene) hydrazides of monocarboxylic acids, 2-alky-(benzyl-, aryl-)-[1,2,4]triazolo[1,5-c]quinazolines were excluded from study considering their lower comparing reference compound affinity values. Besides, studied compounds have satisfactory toxicity measures, most of them refer to non-toxic compounds (LD$_{50}$ = 585.7–2650.6 mg/kg) (Table 1).

Results of calculation revealed that proposed compounds have the satisfying value of “drug-like” criteria (Table 2). Thus, for all studied compounds logP values were less than 5, molecular weight was less than 500; molecules contain no more than 10 nitrogen and oxygen atoms, less than 5 atoms – donors of hydrogen bonds, and no more than 8 rotatable bonds. The accordance to listed above parameters indicates the ability of compounds to ligand-enzyme interaction on binding site of the molecular target. Obtained data allowed to distinguish the narrower range of compounds for further synthetic and biological studies and revealed that chemical modification of carboxyl-containing heterocyclic compounds is reasonable in scope of purposeful search for agents with anti-inflammatory activity.

The in vivo studies of anti-inflammatory activity revealed that quinazolin-4(3H)-yldiene/ hydrazides of carboxylic and dicarboxylic acids (IIa–g) and products of their cyclization, namely 2-R[1,2,4]triazolo[1,5-c]quinazolines (IIIa–g) inhibit the development of carrageenan-induced paw edema by 17–50 % in comparison with control group (Fig. 2).

At the same time, 3-R-S-(2-amino-phenyl)-1H-1,2,4-triazoles (IVa–f), 2-R-S-carboxyalkyl[1,2,4]triazolo[1,5-c] quinazolines (Va–I) and 2-R-7-oxo-6,7-dihydropyrrrole[1,2-a][1,2,4] triazolo[1,5-c]quinazoline-4a(5H)-carboxylic acids (Via–d), that were obtained as result of further modification, inhibited paw edema by 0.94–49.65 % (Fig. 3).

The visualization of molecular docking results obtained for most active compounds (Ille, Illg, Ivd, Vb) was conducted for more detailed understanding of “structure – anti-inflammatory activity” correlations and the creation of theoretic background for further purposeful search for anti-inflammatory agents. Visualization of compounds Ille, Illg, Ivd, Vb docking to COX-1 revealed that abovementioned compounds take the position that is different from that of “Sodium Diclofenac” in active site of the enzyme, and as a consequence form interaction with alternative amino-acids moieties (Fig. 4). Visualization of compound

**Table 1. Results of molecular docking and probable toxicometric parameters of compounds according to the Test data**

| Compd. | Affinity to COX-1 (DN1Y) | Affinity to COX-2 (LL1H) | Oral rat LD$_{50}$ (mg/kg) | Developmental toxicity |
|--------|--------------------------|--------------------------|---------------------------|-----------------------|
| IVa    | -8.0                     | -10.4                    | 1641.2                    | Category C*           |
| IVb    | -8.0                     | -10.3                    | 1022.2                    | Category C*           |
| IVc    | -8.4                     | -10.3                    | N/A                       | Category C*           |
| IVd    | -8.0                     | -10.5                    | 1942.9                    | Category C*           |
| IVe    | -7.7                     | -10.2                    | N/A                       | Category C*           |
| IVf    | -7.4                     | -10.4                    | N/A                       | Category C*           |
| IVg    | -8.1                     | -10.5                    | 1323.8                    | Category C*           |
| IVh    | -8.2                     | -10.0                    | 1682.1                    | Category C*           |
| IVi    | -8.0                     | -10.2                    | 2197.9                    | Category C*           |
| IVj    | -8.6                     | -9.9                     | 1462.9                    | Category C*           |
| IVk    | -8.6                     | -10.2                    | 1852.2                    | Category C*           |
| IVl    | -7.0                     | -7.7                     | 1587.8                    | Category C*           |
| IVm    | -7.9                     | -7.3                     | N/A                       | Category C*           |
| IVn    | -7.5                     | -7.3                     | 2565.0                    | Category C*           |
| IVo    | -8.1                     | -7.3                     | N/A                       | Category C*           |

*: Category C – Possible developmental toxicant; **: Category B – Non developmental toxicant; ***: Category D – Developmental toxicant; : Diclofenac.
Table 2. “Drug-like” calculated parameters

| Compd. | Log P | Molecular polar surface area, Å | Number of non-hydrogens | Molecular volume, Å³ | Number of hydrogen bond acceptors (groups N and O) | Number of hydrogen bond donors (groups NH and OH) | Number of rotatable bonds | Molecular volume |
|--------|-------|---------------------------------|--------------------------|----------------------|-----------------------------------------------|-----------------------------------------------|-------------------------|-----------------|
| DF²⃣ | 4.57  | 49.33                           | 19                       | 296.15               | 3                                             | 2                                             | 4                       | 238.73         |
| Ila    | 1.01  | 96.45                           | 19                       | 260.25               | 7                                             | 2                                             | 4                       | 224.84         |
| Ilb    | 0.98  | 96.45                           | 20                       | 274.28               | 7                                             | 2                                             | 5                       | 241.64         |
| IIC    | 0.56  | 107.44                          | 19                       | 260.25               | 7                                             | 3                                             | 4                       | 224.12         |
| Ild    | 1.07  | 107.44                          | 20                       | 274.28               | 7                                             | 3                                             | 5                       | 240.92         |
| Ilf    | 1.51  | 107.44                          | 21                       | 288.31               | 7                                             | 3                                             | 5                       | 257.50         |
| Ilg    | 2.05  | 107.44                          | 24                       | 328.37               | 7                                             | 3                                             | 5                       | 296.99         |
| Ila    | 1.45  | 69.40                           | 18                       | 242.24               | 6                                             | 2                                             | 5                       | 226.25         |
| Ilb    | 1.69  | 69.40                           | 19                       | 256.26               | 6                                             | 0                                             | 4                       | 223.06         |
| Ilc    | 1.97  | 80.39                           | 18                       | 242.24               | 6                                             | 1                                             | 4                       | 222.33         |
| Ild    | 1.24  | 80.39                           | 19                       | 256.26               | 6                                             | 1                                             | 4                       | 238.92         |
| Ilf    | 1.72  | 80.39                           | 20                       | 270.29               | 6                                             | 1                                             | 4                       | 278.40         |
| IIG    | 2.45  | 80.39                           | 23                       | 310.36               | 6                                             | 1                                             | 4                       | 277.67         |
| IVA    | 3.83  | 69.40                           | 24                       | 318.3                | 6                                             | 0                                             | 4                       | 204.74         |
| IVb    | 1.26  | 93.90                           | 17                       | 232.2                | 6                                             | 3                                             | 4                       | 170.41         |
| IVc    | 1.47  | 93.90                           | 18                       | 246.27               | 6                                             | 3                                             | 5                       | 221.55         |
| IVd    | 0.74  | 104.90                          | 17                       | 232.24               | 6                                             | 4                                             | 4                       | 204.02         |
| IVe    | 1.01  | 104.90                          | 18                       | 246.27               | 6                                             | 4                                             | 5                       | 220.82         |
| IVF    | 3.60  | 93.90                           | 23                       | 308.34               | 6                                             | 3                                             | 5                       | 276.15         |

Fig. 2. Anti-inflammatory activity of quinazoline-4(3H)-ylidene/hydrazides of carboxylic and dicarboxylic acids (IIa–g) and 2-R-[1,2,4]triazolo[1,5-c]quinazolines (IIIa–g) (M ± m, n = 6).

Fig. 3. Anti-inflammatory activity of 3-R-S-(2-aminoophenyl)-1H-1,2,4-triazoles (IVA–f), 2-R-5-carboxylalkyl-[1,2,4]triazolo[1,5-c]quinazolines (IVA–I) and 2-R-7-oxo-6,7-dihydropyrrolo[1,2-a][1,2,4]triazolo[1,5-c]quinazoline-4a(3H)-carboxylic acids (IVA–d) (M ± m, n = 6).
### Cont. of table 2.

| Compd. | Log P | Molecular polar surface area, Å² | Number of non-hydrogens | Molecular volume, Å³ | Number of hydrogen bond acceptors (groups N and O) | Number of hydrogen bond donors (groups NH and OH) | Number of rotatable bonds | Molecular volume |
|--------|-------|--------------------------------|-------------------------|----------------------|-----------------------------------------------|---------------------------------|------------------|------------------|
| Va     | 1.06  | 80.39                          | 19                      | 256.26               | 6                                             | 1                             | 3                | 222.09          |
| Vb     | 1.57  | 69.40                          | 19                      | 256.26               | 6                                             | 0                             | 3                | 222.82          |
| Vc     | 3.52  | 80.39                          | 25                      | 332.36               | 6                                             | 1                             | 4                | 293.50          |
| Vd     | 3.81  | 80.39                          | 26                      | 346.39               | 6                                             | 1                             | 5                | 310.30          |
| Ve     | 3.33  | 80.39                          | 27                      | 360.42               | 6                                             | 1                             | 7                | 327.34          |
| Vf     | 3.60  | 69.40                          | 24                      | 318.34               | 6                                             | 0                             | 4                | 277.67          |
| Vg     | 3.81  | 69.40                          | 25                      | 332.36               | 6                                             | 0                             | 5                | 294.47          |
| Vh     | 3.09  | 80.39                          | 24                      | 318.34               | 6                                             | 1                             | 4                | 276.94          |
| Vi     | 3.36  | 80.39                          | 25                      | 332.36               | 6                                             | 1                             | 5                | 293.74          |
| Vj     | 3.10  | 89.62                          | 26                      | 348.36               | 7                                             | 1                             | 5                | 302.48          |
| Vk     | 3.04  | 80.39                          | 25                      | 332.36               | 6                                             | 1                             | 5                | 293.74          |
| Vl     | 3.11  | 80.39                          | 26                      | 346.39               | 6                                             | 1                             | 6                | 310.54          |
| VIa    | 0.13  | 88.33                          | 21                      | 284.27               | 7                                             | 1                             | 1                | 236.26          |
| VIb    | 2.16  | 88.33                          | 26                      | 346.35               | 7                                             | 1                             | 2                | 291.11          |
| Vlc    | 0.67  | 88.33                          | 23                      | 312.33               | 7                                             | 1                             | 3                | 269.86          |
| Vld    | 2.70  | 88.33                          | 28                      | 374.40               | 7                                             | 1                             | 4                | 324.71          |

# Diclofenac.

**Fig. 4.** Enzyme-ligand interactions for compounds IIe (А), IIlg (В), IVd (С), and Vb (D) with COX-1.
IIe with active site of COX-1 showed the presence of conventional hydrogen bond that formed as the result of interaction of carboxylic and hydrazide group with amino acids SER A:530 (2.58 Å), TYR A:355 (2.27 Å) and ARG A:120 (4.76 Å) correspondingly (Fig. 4A). At the same time, conformationally more rigid compound IIIg interacts with active site of enzyme in its lipophilic part via p-donor interaction of GLY A:354 (2.24 Å), SER A:516 (2.90 Å) and SER A:353 (3.67 Å) with triazinoquinazoline cycle (Fig. 4B). Small and hydrophilic molecule of compound IVd forms conventional hydrogen bonds between ARG A:120 (4.83 Å), TYR A:355 (2.26 Å) and carboxylic group as well as between MET A:522 (3.29 Å) and aminophenyl fragment (Fig. 4C). Visualization of docking study of compound Vb that contains as carboxylic so ester groups (2nd and 5th positions) allowed to evaluate that molecule is located in hydrophilic part of the active site of enzyme and form conventional hydrogen bond between SER A:516 (1.92 Å) and GLN A:350 (2.36 Å) (Fig. 4D).

Visualization of compounds IIe, IIIg, IVd, Vb docking to COX-2 revealed the patterns that are similar to the described above. Thus, compounds IIe, IIIg, IVd, Vb take the position which is different from Sodium Diclofenac in the active site of enzyme, as well as form alternative interactions with aminoacid moieties of protein molecule (Fig. 5).

Carboxylic group of compound IIe does not form any interactions with amino acid moieties of enzyme, but there is conventional hydrogen bond between hydrazide group and SER A:516 (3.08 Å) (Fig. 5A). Compound IIIg, despite the location in hydrophilic part, forms conventional hydrogen bond of carboxylic group with ARG C:106 (2.85 Å) (Fig. 5B).

Visualization of compound IVd interaction with active site of COX-2 (Fig. 5C) allowed to evaluate the position,
similar to previous compounds, in active site of enzyme and the presence of conventional hydrogen bond between carboxylic group and TYR C:341 (2.34 Å). It should be mentioned that compound Vb form more conventional hydrogen bonds comparing to the listed above compounds. It may be explained by the presence of both carboxylic and ester groups in same molecule, aforementioned fragments form interactions with SER C:516 (3.10 Å), Tyr C:371 (3.09 Å) and ARG C:106 (3.06 Å).

**Discussion**

As expected, among quinazolin-4(3H)-ylidene)hydrazides of carboxylic and dicarboxylic acids (II), the highest activity was characteristic for compounds that contain “classic” pharmacophore fragments: ethylacetate (IIb), propanoic acid (IIc), b-methylbutanoic acid (IIe), b-(cyclopropyl-1,1¢)butanoic (III) acid and p-ethylbenzoate (IIg). At the same time, the formation of planar [1,2,4]triazolo[1,5-c]quinazoline line cycle (III) resulted the loss of anti-inflammatory activity (AA = 0.00–40.63 %). It should be mentioned that high anti-inflammatory activity was detected only for compounds IIIb and IIlg, which also contain pharmacophore ethylacetate (AA = 36.11 %) and ethylbenzoate (AA = 40.63 %) fragments in position 2 of the cycle.

Nucleophilic degradation of 2-R-[1,2,4]triazolo[1,5-c]quinazoline (III) that yielded more conformationally flexible 2-(5-(2-aminophenyl)-1H-1,2,4-triazol-3-yl)alkyl-(phenyl-)carboxylic acids (IV) did not lead to the significant increasing of anti-inflammatory activity (AA = 2.43–49.65 %, Fig. 3). The exceptions were compounds with ethylcarboxylate (IVA) and propanoic acids (IVc) fragments in molecule that inhibited carrageenan-induced paw edema by 32.99 % and 49.65 % correspondingly. The aforementioned compounds favorably compare with substances Ilia (AA = 17.01 %), IIlc (AA = 23.96 %), IIIa (AA = 0.00 %) and IIlc (AA = 11.81 %). Additionally, it was found that prolongation of the hydrolytic degradation process for compound IIla resulted the formation of 5-(2-aminophenyl)-1H-1,2,4-triazole-3-carboxylic acid (Vb), that inhibited the carrageenan – induced paw edema by 46.53 % and exceeded the activity of compound IVA on 13.54 %.

Reconstruction of [1,2,4]triazolo[1,5-c]quinazoline cycle with additional introduction of carboxyalkyl group to position 5 (V) caused the significant loss of anti-inflammatory activity (AA = 0.47–22.93 %) independently determined by the substituent in the 2° position (Fig. 3). The exception was compound Vb, that inhibited the development of the edema by 37.55 %. Above mentioned compound contains ethoxycarboxyl and carboxylethyl fragments in the 2° and 5° positions correspondingly.

The formation of more complex heterocyclic system also did not lead to the increasing of anti-inflammatory activity. Thus, dihydropyrolo[1,2-a][1,2,4]triazolo[1,5-c]quinazolines that contain carboxylic group (Via, Vb) or propanoic acid moiety (Vic, VId) in angular position 4a were not effective and reduce paw edema on 0.94–17.16 % (Fig. 3). Therefore, anti-inflammatory activity of the studied compounds significantly depends on molecule conformation, the nature of pharmacophore, its position in heterocyclic fragment, and length of linker alkyl fragment that effects on the lipophilicity of molecule.

The conducted visualization of molecular docking results proved our assumption about dependence of anti-inflammatory activity level on spatial location of molecule in active center (i. e. conformation) and lipophilicity (the length of carboxyalkyl fragment). Thereby, studied compounds take the position that differs from that of the classic COX-inhibitor (Sodium Diclofenac) in active site, and therefore form alternative enzyme-ligand interactions between carboxylic group and amino-acid moieties of protein. It should be mentioned that in some cases studied compounds do not form the abovementioned type of interaction (Figs. 4, 5). Although studied compounds are promising anti-inflammatory agents, they cannot be referred to classic COX inhibitors and require the further investigations of mechanism of action (PLA-inhibiting activity, LOX-inhibiting activity, etc.) and feasibilities of structural optimization.

**Conclusions**

1. The predicted affinity values, calculated “drug-like” criteria and toxicity parameters, visualization of the docking in active site of biological targets as well as experimental studies results showed that investigated compounds are promising in scope of purposeful search for anti-inflammatory drugs.

2. The conducted in vivo screening of anti-inflammatory activity among carboxyl-containing quinazolines and related heterocyclic compounds allowed to detect series of substances that by the level of anti-inflammatory activity compete with reference-compound “Sodium diclofenac” on the carrageenan-induced paw edema model.

3. Presented data may be considered as theoretical basis for further structural modification of studied compounds aimed at the elaboration of novel anti-inflammatory agents and the evaluation of their activity mechanism (lipooxygenase inhibitors, phospholipase inhibitors, etc.).

**Funding**

This research was a part of the scientific project of Zaporizhzhia State Medical University “Directed search for biologically active substances among annulated quinazoline and pteridine derivatives”, state registration No. 0117U006961.

**Conflicts of interest:** authors have no conflict of interest to declare.

**Конфлікт інтересів:** відсутній.

Надійшла до редакції / Received: 06.09.2021
Після допрацювання / Revised: 12.10.2021
Прийнято до друку / Accepted: 24.10.2021

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