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

N. I. Krasovska[A,B,C,D], **V. V. Stavytskyi**[C], **I. S. Nosulenko**[C], **O. Yu. Voskoboinik**[E], **S. I. Kovalenko***[A,F]

Zaporizhzhia State Medical University, Ukraine

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

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 the 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 medicinal 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(3*H*)-ylidene)hydrazides of mono-(di-)carboxylic acids, 2-R-[1,2,4]triazolo[1,5-*c*]quinazolines, 3-R-5-(2-aminophenyl)-1*H*-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]triazolo[1,5-*c*]quinazoline-4*a*(5*H*)-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 carboxylic-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(3*H*)-ylidene) hydrazides of dicarboxylic acids inhibit edema by 17.0–50.0 %, 2-carboxyalkyl-(phenyl-)-[1,2,4]triazolo[1,5-*c*]quinazolines – by 0.00–40.63 %, 2-(5-(2-aminophenyl)-1*H*-1,2,4-triazol-3-yl)alkyl-(phenyl-)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]triazolo[1,5-*c*]quinazoline-4*a*(5*H*)-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 conformation of the molecule and the nature of the "pharmacophore" moiety (carboxyalkyl residue length) at the corresponding positions of the heterocycle 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 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.).

Key words: quinazolines, triazoles, heterocyclic compounds, fused-ring, molecular docking simulation, computational prediction of drug-target interactions, anti-inflammatory agents.

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***E-mail:** kovalenko.si@zsmu.zp.ua

Карбоксилвмісні хіназоліни та споріднені гетероцикли як носії протизапальної активності

Н. І. Красовська, В. В. Ставицький, І. С. Носуленко, О. Ю. Воскобойнік, С. І. Коваленко

Лікарські засоби, які б поєднували у своїй структурі ароматичний і гетероциклічний фрагменти з «фармакофорною» карбоксильною групою, широко представлені на фармацевтичному ринку. Саме ця комбінація структурних елементів міститься в молекулах нестероїдних протизапальних засобів (НПЗЗ). Детальне вивчення механізмів дії НПЗЗ дало змогу пояснити ключову роль і вплив «фармакофорної» карбоксильної групи на активність, селективність і токсичність. Упровадження в медичну практику селективних

Ключові слова: хіназоліни, тріазоли, конденсовані гетероциклічні сполуки, моделювання молекулярного докінгу, обчислювальне прогнозування взаємодії ліки – біологічна мішень, протизапальні агенти.

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інгібіторів сприяло суттєвому зниженню ризику розвитку основних ускладнень (гастротоксичності), але не розв'язало проблему токсичності НПЗЗ. Отже, актуальним є пошук нових протизапальних засобів шляхом «біоізостеричних» замін ароматичних і гетероциклічних фрагментів відомих препаратів на інші структурні фрагменти з наявністю карбоксильної групи як носія фармакологічного ефекту.

Мета роботи – спрямований пошук нових протизапальних агентів серед карбоксилвмісних хіназолінів і споріднених гетероциклів, а також дослідження впливу карбоксильної групи на антиінфламаторну активність із використанням методології *in silico* та *in vivo*.

Матеріали та методи. Хіназолін-4(3*H*)-іліден)гідразиди моно-(ди-)карбонових кислот, 2-R-[1,2,4]тріазоло[1,5-*c*]хіназоліни, 3-R-5-(2-амінофеніл)-1*H*-1,2,4-тріазоли, 5-карбоксиалкіл[1,2,4]тріазоло[1,5-*c*]хіназоліни та 2-R-7-оксо-6,7-дигідропіроло[1,2-*a*][1,2,4]тріазоло[1,5-*c*]хіназолін-4*a*(5*H*)-карбонові кислоти дослідили на протизапальну активність. MarvinSketch 20.19.0, AutoDock Vina та AutoDockTools 1.5.6, Hy-perChem 7.5, Discovery Studio використали для *in silico* досліджень. Критерії «drug-like» оптимізували й оцінювали за допомогою електронного ресурсу. Прогнозування гострої токсичності, ембріотоксичності та мутагенності Еймса сполук здійснили, використавши програмне забезпечення TEST. Протизапальну активність синтезованих сполук оцінювали на білих щурах Wistar (масою 150–160 г) з ексудативною фазою гострого асептичного запалення («каррагінановий» тест). Флогоген (1 % водний розчин λ-каррагінану) вводили субплантально в дозі 0,1 мл у задню праву лапу щурів. Ліву лапу використовували як контроль. Внутрішньошлункове введення досліджуваних сполук як водного розчину або тонкодисперсної суспензії, стабілізованої твіном-80, у дозі 10 мг/кг здійснили з використанням атравматичного зонда за 1 годину до ін'єкції флогогену. Референтний препарат диклофенак натрію вводили внутрішньошлунково в рекомендованій дозі 8 мг/кг для доклінічних досліджень. Об'єм лап вимірювали до експерименту та через 4 години після ін'єкції флогогену. Активність сполук визначали за здатністю зменшувати набряк порівняно з контрольною групою, наводили у відсотках. Експерименти здійснили, дотримуючись біоетичних правил і норм.

Результати. За результатами молекулярного докінґу, критеріями «drug-like» та прогностичними параметрами токсичності теоретично обґрунтували пошук протизапальних агентів серед карбоксилвмісних хіназолінів і споріднених гетероциклів. Експериментальними методами *in vivo* («каррагінановий» тест) підтвердили наявність протизапальної активності та показали, що (хіназолін-4(3*H*)-іліден)гідразиди дикарбонових кислот пригнічують набряк на 17,0–50,0 %, 2-карбоксиалкіл-(феніл-)-[1,2,4]тріазоло[1,5-*c*]хіназоліни – на 0,00–40,63 %, 2-(5-(2-амінофеніл)-1*H*-1,2,4-тріазол-3-іл)алкіл-(феніл-)карбонові кислоти – на 2,43–49,65 %, 2-R-5-карбоксиалкіл[1,2,4]тріазоло[1,5-*c*]хіназоліни – на 0,47–22,93 % та 2-R-7-оксо-6,7-дигідропіроло[1,2-*a*][1,2,4]тріазоло[1,5-*c*] хіназолін-4*a*(5*H*)-карбонові кислоти – на 0,94–17,16 %. Серед них виявили сполуки, що за силою ефекту конкурують із препаратом порівняння диклофенаком натрію. SAR-аналіз показав: значущий вплив на протизапальну активність чинить і конформація молекули, і природа «фармакофорного» фрагмента (довжина карбоксиалкільного залишку) у відповідних положеннях базового гетероциклу. За даними візуалізації молекулярного докінґу, сполуки, що вивчали, мають інші фермент-лігандні взаємодії та, ймовірно, інший механізм дії.

Висновки. Дослідження на протизапальну активність у ряду карбоксилвмісних хіназолінів і споріднених гетероциклічних сполук дали змогу ввиявити сполуки, що конкурують із референс-препаратом диклофенаком натрію. Прогностичні значення афінності, розрахунки критеріїв «drug-like», параметрів токсичності методами *in silico* та візуалізації молекулярного докінґу сполук в активних центрах біомішеней показали перспективність цього класу для наступних досліджень. Наведені дані – теоретичне підґрунтя для продовження структурної модифікації для виявлення нових антифлогістиків і можливого механізму дії (інгібітори ліпооксигенази, фосфоліпази А тощо).

Ключевые слова: хиназолины, триазолы, конденсированые гетероциклические соединения, моделирование молекулярного докинга, вычислительное прогнозирование взаимодействий лекарство – биологическая мишень, противовоспалительные агенты.

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Карбоксилсодержащие хиназолины и родственные гетероциклы как носители противовоспалительной активности

Н. И. Красовская, В. В. Ставицкий, И. С. Носуленко, А. Ю. Воскобойник, С. И. Коваленко

Лекарственные средства, объединяющие в своей структуре ароматический и гетероциклический фрагменты с «фармакофорной» карбоксильной 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 влияние «фармакофорной» карбоксильной группы на активность, селективность и токсичность. Внедрение в медицинскую практику селективных ингибиторов привело к существенному снижению риска развития основных осложнений (гастротоксичности), но не решило проблему токсичности НПВС. Таким образом, актуален поиск новых противовоспалительных средств путем «биоизостерических» замен ароматических и гетероциклических фрагментов известных препаратов на другие структурные фрагменты с наличием карбоксильной группы как носителя фармакологического эффекта.

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

Материалы и методы. Хиназолин-4(3*H*)-илиден)гидразиды моно-(ди-)карбоновых кислот, 2-R-[1,2,4]триазоло[1,5-*c*]хиназолины, 3-R-5-(2-аминофенил)-1*H*-1,2,4-триазолы, 5-карбоксиалкил[1,2,4]триазоло[1,5-*c*]хиназолины и 2-R-7-оксо-6,7-дигидропирроло[1,2-*a*][1,2,4]триазоло[1,5-*c*]хиназолин-4а(5*H*)-карбоновые кислоты исследованы на противовоспалительную активность. MarvinSketch 20.19.0, AutoDock Vina и AutoDockTools 1.5.6, Hy-perChem 7.5, Discovery Studio использованы для *in silico* исследований. Критерии «drug-like» оптимизировали и оценивали с помощью электронного ресурса. Прогнозирование острой токсичности, эмбриотоксичности и мутагенности Эймса соединений проводили с использованием программного обеспечения TEST. Противовоспалительную активность синтезированных соединений оценивали на белых крысах Wistar (массой 150–160 г) с экссудативной фазой острого асептического воспаления («каррагинановый» тест). Флогоген (1 % водный раствор λ-каррагинана) вводили субплантально в дозе 0,1 мл в заднюю правую лапу крыс. Левую лапу использовали как контроль. Внутрижелудочное введение исследуемых соединений в виде водного раствора или тонкодисперсной суспензии, стабилизированной твином-80, в дозе 10 мг/кг проводили с использованием атравматичного зонда за 1 час до инъекции флогогена. Референтный препарат диклофенак натрия вводили внутрижелудочно в рекомендованной дозе 8 мг/кг для доклинических исследований. Объем лап измеряли до начала эксперимента

и через 4 часа после инъекции флогогена. Активность соединений определяли по их способности уменьшать отек по сравнению с контрольной группой, выражали в процентах. Эксперименты проведены с соблюдением биоэтических правил и норм.

Результаты. По результатам молекулярного докинга, критериям «drug-like» и прогностическим параметрам токсичности теоретически обоснован поиск противовоспалительных агентов среди карбоксилсодержащих хиназолинов и родственных гетероциклов. Экспериментальными методами *in vivo* («каррагинановый» тест) подтверждено наличие противовоспалительной активности и показано, что (хиназолин-4(3*H*)-илиден)гидразиды дикарбоновых кислот подавляют отек на 17,0–50,0 %, 2-карбоксиалкил-(фенил-)-[1,2,4]триазоло[1,5-*c*]хиназолин – на 0,00–40,63 %, 2-(5-(2-аминофенил)-1*H*-1,2,4-триазол-3-ил)алкил-(фенил-)карбоновые кислоты – на 2,43–49,65 %, 2-R-5-карбоксиалкил[1,2,4]триазоло[1,5-*c*]хиназолины – на 0,47–22,93 % и 2-R-7-оксо-6,7-дигидропирроло[1,2-*a*][1,2,4]триазоло[1,5-*c*]хиназолин-4а(5*H*)-карбоновые кислоты – на 0,94–17,16 %. Среди них обнаружены соединения, по силе эффекта конкурирующие с препаратом сравнения диклофенаком натрия. SAR-анализ показал, что существенное влияние на противовоспалительную активность оказывают и конформация молекулы, и природа «фармакофорного» фрагмента (длина карбоксиалкильной группы) в соответствующих положениях базового гетероцикла. По данным визуализации молекулярного докинга, исследуемые соединения имеют другие фермент-лигандные взаимодействия и, вероятно, другой механизм действия.

Выводы. Исследования на противовоспалительную активность в ряду карбоксилсодержащих хиназолинов и родственных гетероциклических соединений позволили ввыявить вещества, конкурирующие с референс-препаратом диклофенаком натрия. Прогностические значения аффинности, расчеты критериев «drug-like», параметров токсичности методами *in silico* и визуализации молекулярного докинга указанных соединений в активных центрах биомишени показали перспективность этого класса для дальнейших исследований. Представленные данные – теоретическая платформа для продолжения структурной модификации для выявления новых антифлогистиков и выяснения возможного механизма действия (ингибиторы липооксигеназы, фосфолипазы и т. д.).

Active pharmaceutical ingredients whose structure combines aromatic or heterocyclic fragments with pharmacophore carboxylic group are widespread on pharmaceutical market. Moreover, the above mentioned structural fragments present in molecules of nonsteroidal anti-inflammatory drugs (NSAIDs) [1]. NSAIDs are widely used for treatment of fever, pain, inflammation, rheumatoid arthritis and osteoarthritis. Despite some differences in chemical structure, members of abovementioned group of medications have the common mechanism of pharmacological effects. This mechanism is based on the effect of NSAIDs to suppress prostaglandin synthesis (PG) by inhibiting cyclooxygenases (COX) [2]. 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 [3–6]. It was found that there are similar interactions of carboxylic group with aminoacids moieties in active site of complexes of COX-1 and COX-2 with NSAIDs. For instance, carboxylic group of Diclofenac (DF) forms the hydrogen bonds with hydroxymethyl group of SER530 (2.53 Å) and with phenolic hydroxyl of Tyr385 (2.68 Å) of COX-1. The similar bonds are formed with hydroxymethyl group of SER530 (2.65 Å) and with phenolic hydroxyl of Tyr385 (2.73 Å) in Diclophenac – COX-2 complexes [3,4]. Thus, most of traditional NSAIDs, in whose molecules carboxylic group is present, form similar interactions with COX enzymes that cause the absence of selectivity of inhibitory activity. The nonselective inhibition of prostaglandin synthesis resulted in the wide spectrum of side effects including gastrointestinal tract complications, heart dysfunctions, kidney toxicity, hypertension, etc. [7,8].

The elaboration of modern conception of inflammation mechanism, evaluation of the important role of eicosanoid in the process of inflammation onset and development, estimation of structure of biological targets and known inhibitors, as well as, unprecedented development of machine learning, enhanced the role of artificial intelligence in NSAIDs design [9,10]. The abovementioned resulted in the introduction of selective COX-2-inhibitors to medicinal practice and a significant decrease of side effects and complication frequencies. However, the problem of NSAIDs toxicity has not been solved [11,12]. Thus, the search for the novel anti-inflammatory drugs using *in silico* methods and approaches that include structural modification of known NSAIDs by “bioisosteric” replacements of aromatic and heterocyclic fragments to other structural elements with carboxylic group as carrier of pharmacological effect is a current trend of medicinal chemistry.

Aim

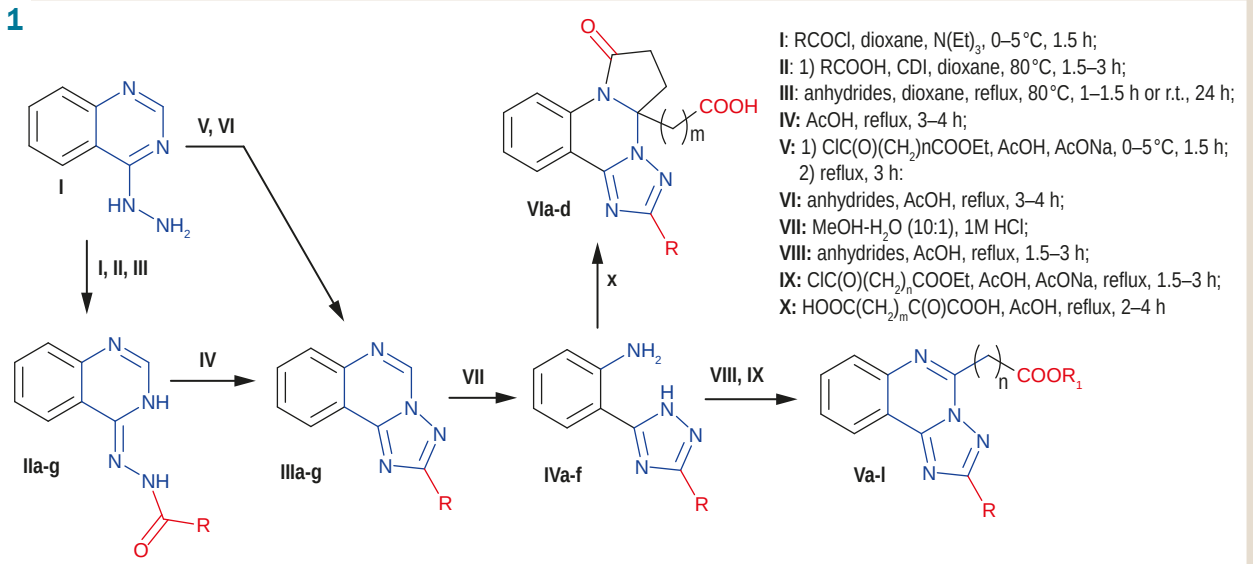
The aim of the present study is to purposefully search for anti-inflammatory agents among carboxyl-containing quinazolines and related heterocycles using *in silico* and *in vivo* methods, as well as the evaluation of carboxylic group effect on the level of anti-inflammatory activity.

Materials and methods

Quinazoline-4(3*H*)-ylidene)hydrazides of mono-(di-)carboxylic acids (**IIa–g**), 2-R-[1,2,4]triazolo[1,5-*c*]quinazolines (**IIIa–g**), 3-R-5-(2-aminophenyl)-1*H*-1,2,4-triazoles (**IVa–f**), 2-R-5-carboxylalkyl[1,2,4]triazolo[1,5-*c*]quinazolines (**Va–l**) and 2-R-7-oxo-6,7-dihydropyrrolo[1,2-*a*][1,2,4]triazolo[1,5-*c*]quinazoline-4*a*(5*H*)-carboxylic acids (**VIa–d**) were screened for anti-inflammatory activity. The synthesis and physicochemical 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 – 3N8Y), 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



IIa, IIIa, IVa R = -COOEt; **IIb**, **IIIb, IVc** R = -CH_2COOEt; **IIc, IIIc, IVd** R = -CH_2CH_2COOH; **IId, IIId, IVe** R = -$CH_2CH_2CH_2COOH$;
IIe, IIIe R = -$CH_2CH(Me)CH_2COOH$; **IIf, IIIf** R = -$CH_2CH(Cpn)CH_2COOH$; **IIg, IIIg, IVf** R = -C_6H_4COOEt-*p*; **IVb** R = -COOH;
Va R = Me, R_1 = H, n = 2; **Vb** R = -COOEt, R_1 = H, n = 2; **Vc** R = Bn, R_1 = H, n = 2; **Vd** R = Bn, R_1 = H, n = 3: **Ve** R = $PhCH_2CH_2$-, R1 = H, n = 3;
Vf R = Ph, R1 = Et, n = 0; **Vg** R = Ph, R_1 = Et, n = 1; **Vh** R = Ph, R_1-H, n = 2; **Vi** R = Ph, R_1 = H, n = 3; **Vj** R = 2-$MeOC_6H_4$-, R_1 = H, n = 2;
Vk R = 4-MeC_6H_4-, R1 = H, n = 2; **Vl** R = 4-MeC_6H_4-, R1 = H, m = 3; **VIa** R = Me, m = 0; **VIb** R = Ph, m = 0; **VIc** R = Me, m = 2; **VId** R = Ph, m = 2

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 (3N8Y); center_x = 3.86, center_y = 20.06, center_z = -9.06, size_x = 16, 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 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 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:

$$A, \% = 100\ \% - \left(\frac{Vpe - Vhe}{Vpc - V\,hc}\right) \times 100\ \%,$$

where A – antiexudative activity, %; Vpe – the volume of paw edema in the experiment; Vhe – the volume of healthy paw in the experiment; 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. Arithmetic 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].

Results

The study design implied the selection of basic molecules, namely quinazolin-4(3*H*)-ylidene)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(3*H*)-ylidene)hydrazides of carboxylic and dicarboxylic acids (**IIa–g**), 2-R-[1,2,4]triazolo[1,5-*c*]quinazolines (**IIIa–g**), 3-R-5-(2-aminophenyl)-1*H*-1,2,4-triazoles (**IVa–f**), 2-R-5-carboxylalkyl[1,2,4]triazolo[1,5-*c*]quinazolines (**Va–l**) and 2-R-7-oxo-6,7-dihydropyrrolo[1,2-*a*][1,2,4] triazolo[1,5-*c*]quinazoline-4a(5*H*)-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(3*H*)-ylidene) hydrazides of monocarboxylic acids, 2-alky-(benzyl-, phenethyl-, aryl-)-[1,2,4]triazolo[1,5-*c*]quinazolines were excluded from study considering their lower comparing with 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 satisfiying 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(3*H*)-ylidene)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**) inhibited the development of carrageenan-induced paw edema by 17–50 % in comparison with control group (*Fig. 2*).

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

Compd.	Affinity to COX-1 (3N8Y)	Affinity to COX-2 (3LN1)	Oral rat LD_{50}, mg/kg	Developmental toxicity
DF#	-7.6	-7.7	224.0	Category D***
IIa	-7.8	-8.2	585.7	Category C*
IIb	-7.9	-8.5	N/A	Category C*
IIc	-7.7	-8.7	N/A	Category C*
IId	-7.8	-9.0	N/A	Category C*
IIe	-8.4	-8.4	1675.9	Category C*
IIf	-8.3	-8.7	1015.6	Category C*
IIg	-8.3	-9.8	1464.2	Category C*
IIIa	-8.3	-8.7	N/A	Category C*
IIIb	-8.5	-8.5	N/A	Category C*
IIIc	-8.4	-9.2	844.7	Category C*
IIId	-8.4	-9.2	1209.5	Category C*
IIIe	-8.6	-8.8	1279.1	Category C*
IIIf	-9.1	-9.5	N/A	Category C*
IIIg	-8.7	-8.8	N/A	Category C*
IVa	-8.0	-7.9	N/A	Category C*
IVb	-7.8	-8.0	1854.8	Category C*
IVc	-7.7	-8.0	1644.8	Category C*
IVd	-7.7	-8.0	2048.7	Category C*
IVe	-7.6	-8.5	2191.9	Category C*
IVf	-8.0	-10.4	N/A	Category C*
Va	-7.7	-8.9	1641.2	Category C*
Vb	-8.0	-10.3	1022.2	Category C*
Vc	-8.4	-10.3	N/A	Category C*
Vd	-8.0	-10.8	1791.2	Category C*
Ve	-8.0	-10.5	1942.9	Category C*
Vf	-7.7	-10.2	N/A	Category B**
Vg	-7.4	-10.4	N/A	Category C*
Vh	-8.1	-10.6	1323.8	Category C*
Vi	-8.2	-10.0	1682.1	Category C*
Vj	-8.0	-10.2	2197.9	Category C*
Vk	-8.6	-9.9	1462.9	Category C*
Vl	-8.6	-10.2	1852.2	Category C*
VIa	-7.0	-7.7	1587.8	Category C*
VIb	-7.9	-7.3	N/A	Category C*
VIc	-7.5	-7.3	2650.6	Category C*
VId	-8.1	-7.3	N/A	Category C*

:** Category C – Possible developmental toxicant; *:** Category B – Non developmental toxicant; *****:** Category D – Developmental toxicant; **#:** Diclofenac.

At the same time, 3-R-5-(2-aminophenyl)-1*H*-1,2,4-triazoles (**IVa–f**), 2-R-5-carboxylalkyl[1,2,4]triazolo[1,5-*c*] quinazolines (**Va–l**) and 2-R-7-oxo-6,7-dihydropyrrolo[1,2-*a*] [1,2,4]triazolo[1,5-*c*]quinazoline-4*a*(5*H*)-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 (**IIe, IIIg, 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 **IIe, IIIg, 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

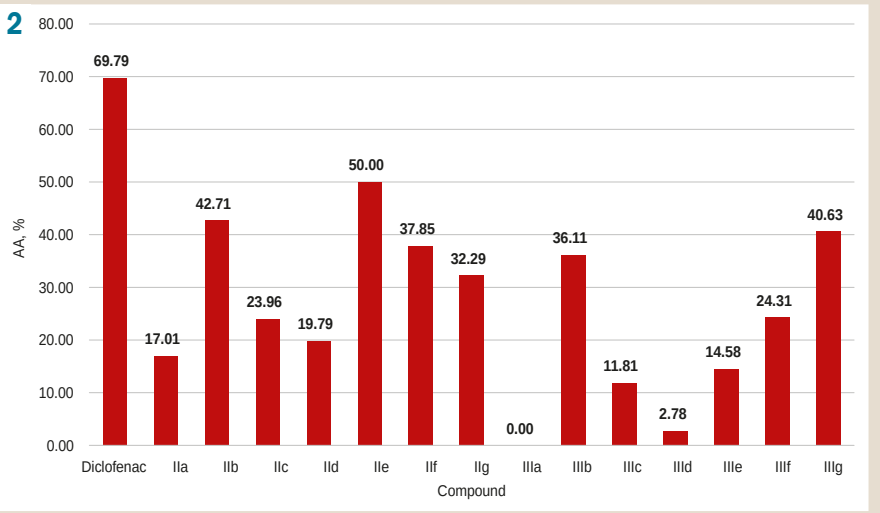


Fig. 2. Anti-inflammatory activity of quinazoline-4(3*H*)-ylidene)hydrazides of carboxylic and dicarboxylic acids (**IIa–g**) and 2-R-[1,2,4] triazolo[1,5-*c*]quinazolines (**IIIa–g**) (M ± m, n = 6).

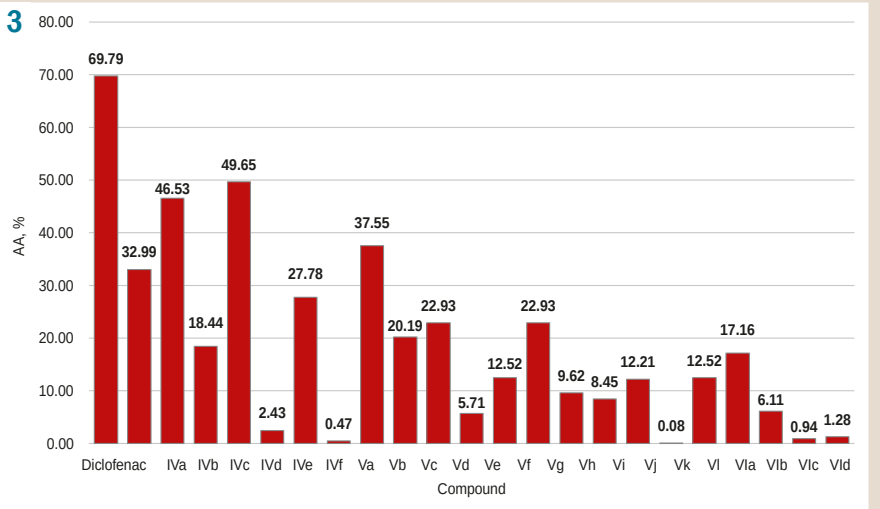


Fig. 3. Anti-inflammatory activity of 3-R-5-(2-aminophenyl)-1*H*-1,2,4-triazoles (**IVa–f**), 2-R-5-carboxylalkyl[1,2,4]triazolo[1,5-*c*] quinazolines (**Va–l**) and 2-R-7-oxo-6,7-dihydropyrrolo[1,2-*a*][1,2,4]triazolo[1,5-*c*] quinazoline-4*a*(5*H*)-carboxylic acids (**VIa–d**) (M ± m, n = 6).

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
DF#	4.57	49.33	19	296,15	3	2	4	238,73
IIa	1.01	96.45	19	260.25	7	2	4	224.84
IIb	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
IId	1.07	107.44	20	274.28	7	3	5	240.92
IIe	1.31	107.44	21	288.31	7	3	5	257.50
IIf	2.05	107.44	24	328.37	7	3	5	296.99
IIg	2.65	96.45	25	336.35	7	2	5	296.25
IIIa	1.48	69.40	18	242.24	6	0	3	206.26
IIIb	1.69	69.40	19	256.26	6	0	4	223.06
IIIc	1.97	80.39	18	242.24	6	1	3	205.53
IIId	1.24	80.39	19	256.26	6	1	4	222.33
IIIe	1.72	80.39	20	270.29	6	1	4	238.92
IIIf	2.45	80.39	23	310.36	6	1	4	278.40
IIIg	3.83	69.40	24	318.3	6	0	4	277.67
IVa	1.26	93.90	17	232.2	6	3	4	204.74
IVb	0.62	104.90	15	204.19	6	4	2	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

Cont. of table 2.

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
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	3.31	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
VIc	0.67	88.33	23	312.33	7	1	3	269.86
VId	2.70	88.33	28	374.40	7	1	4	324.71

#: Diclofenac.

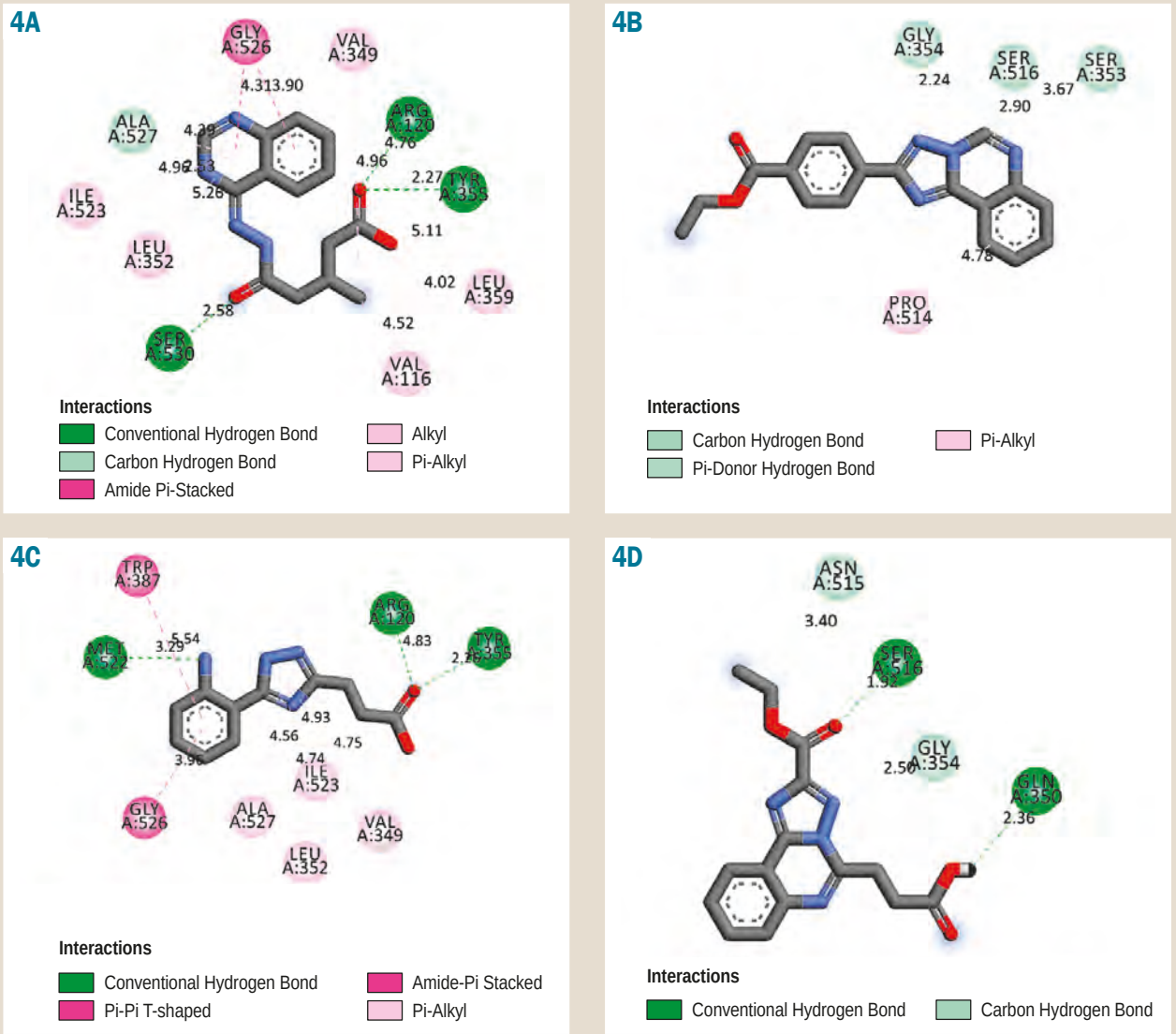


Fig. 4. Enzyme-ligand interactions for compounds **IIe** (A), **IIIg** (B), **IVd** (C), and **Vb** (D) with COX-1.

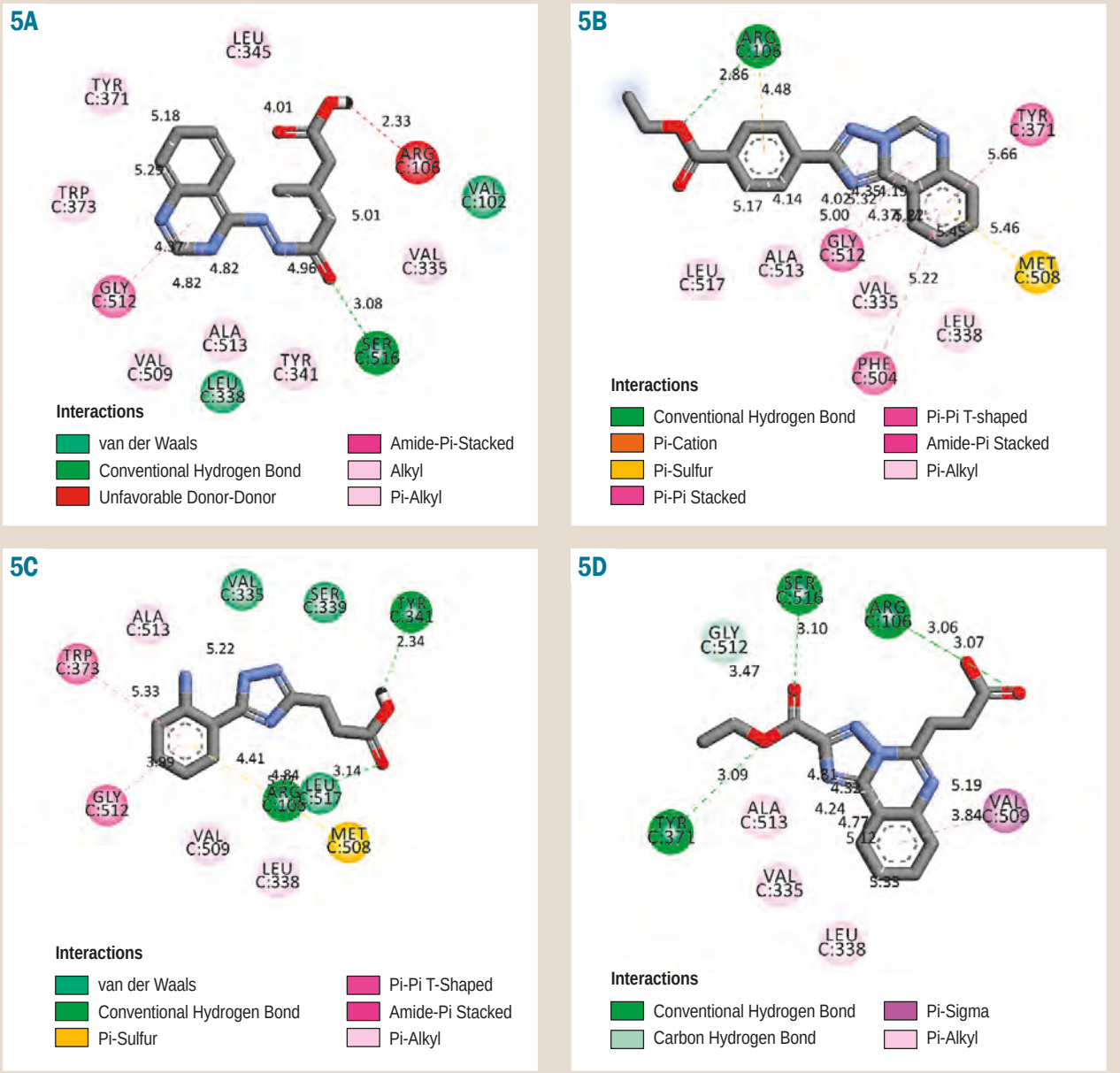


Fig. 5. Ligand-enzyme interactions of compounds **IIe** (A), **IIIg** (B), **IVd** (C) and **Vb** (D) with COX-2.

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(3*H*)-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 (**IIf**) acid and p-ethylbenzoate (**IIg**). At the same time, the formation of planar [1,2,4]triazolo[1,5-*c*]quinazoline 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 **IIIg**, 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)-1*H*-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 **II a** (AA = 17.01 %), **IIc** (AA = 23.96 %), **IIIa** (AA = 0.00 %) and **IIIc** (AA = 11.81 %). Additionally, it was found that prolongation of the hydrolytic degradation process for compound **IIIa** resulted the formation of 5-(2-aminophenyl)-1*H*-1,2,4-triazole-3-carboxylic acid (**IVb**), 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 2nd position (*Fig. 3*). The exception was compound **Vb**, that inhibited the development of the edema by 37.55 %. Above mentioned compound contains ethoxycarbonyl and carboxyethyl fragments in the 2nd and 5th positions correspondingly.

The formation of more complex heterocyclic system also did not lead to the increasing of anti-inflammatory activity. Thus, dihydropyrrolo[1,2-*a*][1,2,4]triazolo[1,5-*c*] quinazolines that contain carboxylic group (**VIa**, **VIb**) 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 (lipoxygenase inhibitors, phospholipase inhibitors, etc.).

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Information about authors:
Krasovska N. I., PhD student of the Department of Organic and Bioorganic Chemistry, Zaporizhzhia State Medical University, Ukraine.
ORCID ID: 0000-0001-5902-6596
Stavytskyi V. V., PhD, Assistant of the Department of Organic and Bioorganic Chemistry, Zaporizhzhia State Medical University, Ukraine.
ORCID ID: 0000-0001-5897-5845
Nosulenko I. S., PhD, Senior Lecturer of the Department of Pharmacognosy, Pharmacology and Botany, Zaporizhzhia State Medical University, Ukraine.
ORCID ID: 0000-0002-8725-7321

Voskoboinik O. Yu., PhD, DSc, Associate Professor of the Department of Organic and Bioorganic Chemistry, Zaporizhzhia State Medical University, Ukraine.
ORCID ID: 0000-0002-5790-3564
Kovalenko S. I., PhD, DSc, Professor, Head of the Department of Organic and Bioorganic Chemistry, Zaporizhzhia State Medical University, Ukraine.
ORCID ID: 0000-0001-8017-9108

Відомості про авторів:

Красовська Н. І., аспірантка каф. органічної та біоорганічної хімії, Запорізький державний медичний університет, Україна.
Ставицький В. В., д-р філософії, асистент каф. органічної та біоорганічної хімії, Запорізький державний медичний університет, Україна.
Носуленко І. С., канд. фарм. наук, старший викладач каф. фармакогнозії, фармакології та ботаніки, Запорізький державний медичний університет, Україна.
Воскобойнік О. Ю., д-р фарм. наук, доцент каф. органічної та біоорганічної хімії, Запорізький державний медичний університет, Україна.
Коваленко С. І., д-р фарм. наук, професор, зав. каф. органічної та біоорганічної хімії, Запорізький державний медичний університет, Україна.

Сведения об авторах:

Красовская Н. И., аспирант каф. органической и биоорганической химии, Запорожский государственный медицинский университет, Украина.
Ставицкий В. В., д-р философии, ассистент каф. органической и биоорганической химии, Запорожский государственный медицинский университет, Украина.
Носуленко И. С., канд. фарм. наук, старший преподаватель каф. фармакогнозии, фармакологии и ботаники, Запорожский государственный медицинский университет, Украина.
Воскобойник А. Ю., д-р фарм. наук, доцент каф. органической и биоорганической химии, Запорожский государственный медицинский университет, Украина.
Коваленко С. И., д-р фарм. наук, профессор, зав. каф. органической и биоорганической химии, Запорожский государственный медицинский университет, Украина.

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