UDC 547.792'856'213-326/-327.057:615.015.4 https://doi.org/10.24959/ophcj.20.192826

V. V. Stavytskyi, I. S. Nosulenko, K. I. Kandybey, O. Yu. Voskoboinik, S. I. Kovalenko

Zaporizhzhia State Medical University, Ukraine
26, Mayakovsky Ave., Zaporizhzhia, 69035, Ukraine. E-mail: kovalenko.si@zsmu.zp.ua

Esters and amides of 3-R-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolin-5*a*(6*H*)-carboxylic(-propanoic) acids: synthesis and biological activity

It is known that carboxyl groups bonded to aryl or hetaryl moieties play a role of the “pharmacophore” fragment in most NSAID molecules. It should be mentioned that the carboxyl group may cause the appearance of toxic effects and is characterized by unsatisfactory pharmacokinetic properties. The structural modification of the carboxyl group, including its bioisosteric replacement, is among the most widely used approaches in medicinal chemistry to improve pharmacodynamic, pharmacokinetic and technological characteristics.

Aim. To develop the synthetic procedures for functional derivatives of 3-R-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*]-[1,2,4]triazino[2,3-*c*]quinazoline-5*a*(6*H*)-carboxylic(-propanoic) acids, study the effect of the carboxyl group chemical modification on the LOX-inhibiting and antiradical activity as a possible mechanism of the pharmacological activity.

Results and discussion. The synthesis of esters of 3-(2,8-dioxo-3-R^1-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]-triazino[2,3-*c*]quinazolin-5*a*(6*H*)-yl)carboxylic(propanoic) acids was conducted by esterification of the corresponding acids and tandem heterocyclization of 2-(6-R^1-2,5-dihydro-5-oxo-1,2,4-triazino-3-yl)anilines with diethyl 4-oxoheptanedioate. The synthesis of amides was conducted by aminolysis of *N*-acylimidazolides generated *in situ*. The antiradical and LOX-inhibiting activities of the compounds obtained were studied as possible mechanisms of the anti-inflammatory activity. The series of the compounds revealed the LOX-inhibiting activity that was comparable with the effect of the reference compound – nordihydroguaiaretic acid.

Experimental part. The synthetic procedures were conducted according to the commonly used methods. The purity and the structure of the compounds obtained were proven by modern physicochemical methods (^{1}H and ^{13}C NMR-spectroscopy, LC-MS-spectrometry). The antiradical activity was measured by the ability to scavenge a DPPH-radical. The study of the LOX-inhibiting activity was performed using soybean LOX as an enzyme and linolenic acid as a substrate.

Conclusions. The methods for the synthesis of esters and amides of 2,8-dioxo-3-R^1-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolin-5*a*(6*H*)-carboxilic(propanoic) acids have been developed. The above-mentioned transformations were conducted by alcoholysis of generated *in situ* acyl halides and aminolysis of *N*-acylimidazolides, respectively. The more efficient approach for the synthesis of the target esters *via* condensation of 2-(6-R^1-2,5-dihydro-5-oxo-1,2,4-triazino-3-yl)anilines with diethyl 4-oxoheptanedioate has been proposed. It has been found that the highest radical scavenging and LOX-inhibiting activities are characteristic for hetarylpropanoic acids that contain electron withdrawing substituents in position 3, as well as fluorine atoms in positions 11 and 12. The chemical modification of the carboxylic group in most cases results in a decrease or the loss of the activity.

Key words: pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolin-5*a*(6*H*)-carboxylic(-propanoic) acid; esters; amides; synthesis; radical scavenging activity; LOX-inhibiting activity

В. В. Ставицький, І. С. Носуленко, К. І. Кандибей, О. Ю. Воскобойник, С. І. Коваленко
Запорізький державний медичний університет, Україна

Естери та аміди 3-R-2,8-діоксо-7,8-дигідро-2*H*-піроло[1,2-*a*][1,2,4]триазино[2,3-*c*]-хіназолін-5*a*(6*H*)-карбонових(-пропанових) кислот: синтез та біологічна активність

Відомо, що карбоксильні групи, пов’язані з арильним або гетарильним фрагментами, відіграють роль «фармакофору» у молекулах більшості НПЗЗ. Слід зазначити, що карбоксильна група може спричинити появу токсичної дії і характеризується незадовільними фармакокінетичними властивостями. Структурна модифікація карбоксильної групи, включаючи її біоізостеричну заміну, є однією з найбільш широко застосовуваних підходів медичної хімії для вдосконалення фармакодинамічних, фармакокінетичних та технологічних характеристик.

Мета. Розробити методи синтезу ряду функціональних похідних 3-R-2,8-діоксо-7,8-дигідро-2*H*-піроло[1,2-*a*]-[1,2,4]триазино[2,3-*c*]хіназолін-5*a*(6*H*)-карбонових(-пропанових) кислот, вивчити вплив структурної модифікації карбоксильної групи на ЛОГ-інгібуючу та антирадикальну дію як одного з можливих механізмів фармакологічної активності.

Результати та їх обговорення. Реакцією естерифікації 3-(2,8-діоксо-3-R^1-7,8-дигідро-2*H*-піроло[1,2-*a*]-[1,2,4]триазино[2,3-*c*]хіназолін-5*a*(6*H*)-іл)пропанових кислот або тандемною гетероциклізацією 2-(6-R^1-2,5-дигідро-5-оксо-1,2,4-триазин-3-іл)анілінів з діетил-4-оксогептандіонатом синтезовано естери відповідних кислот. Синтез амідів проведено амінолізом активованих кислот, де як активуючу компоненту використано 1,1’-карбонілдіімідазол. У рамках дослідження проведено вивчення ЛОГ-інгібуючої і антирадикальної дії як одного з можливих механізмів протизапальної активності. Виявлено ряд сполук, які проявляють ЛОГ-інгібуючу активність на рівні фармакологічного стандарту нордигідроваяретової кислоти.

Експериментальна частина. Синтетичні процедури були виконані згідно з загальноприйнятими підходами. Чистоту і структуру сполук встановлено за допомогою сучасних фізико-хімічних методів (^{1}H і ^{13}C ЯМР-спектроскопія, ВЕРХ-МС-спектрометрія). Антирадикальну активність встановлювали за здатністю синтезованих сполук інгібувати DPPH-радикал. Вивчення ЛОГ-інгібуючої активності сполук було проведено з використанням соєвої ліпооксигенази як ферменту і ліноленової кислоти як субстрату.

Висновки. Розроблено методи синтезу естерів і амідів 2,8-діоксо-3-R[1]-7,8-дигідро-2*H*-піроло[1,2-*a*]-[1,2,4]триазино[2,3-*c*]хіназолін-5*a*(6*H*)-карбонових (пропанових) кислот. Вищезазначені перетворення проведено алкоголізом утворених *in situ* ацилгалогенідів та амінолізом *N*-ацилімідазолідів. Більш ефективним методом синтезу естерів виявилася тандемна гетероциклізація 2-(6-R[1]-2,5-дигідро-5-оксо-1,2,4-триазин-3-іл)анілінів з діетил-4-оксогептандіонатом. Встановлено, що найбільш висока ЛОГ-інгібуюча і антирадикальна активність характерні для гетарилпропанових кислот, які мають електроноакцепторні замісники у положенні 3, а також атоми флуору у положеннях 11 та 12. Модифікація карбоксильної групи відповідних кислот у більшості випадків призводить до зниження або втрати активності.

Ключові слова: піроло[1,2-*a*][1,2,4]триазино[2,3-*c*]хіназолін-5*a*(6*H*)-карбонові(-пропанові) кислоти; естери; аміди; синтез; антирадикальна та ЛОГ-інгібуюча активність

В. В. Ставицкий, И. С. Носуленко, К. И. Кандыбей, А. Ю. Воскобойник, С. И. Коваленко
Запорожский государственный медицинский университет, Украина

Эфиры и амиды 3-R-2,8-диоксо-7,8-дигидро-2*H*-пирроло[1,2-*a*]-[1,2,4]триазино[2,3-*c*]хиназолин-5*a*(6*H*)-карбоновых(-пропановых) кислот: синтез и биологическая активность

Известно, что карбоксильные группы, связанные с арильным или гетарильным фрагментами, играют роль «фармакофора» в молекулах большинства НПВС. Следует отметить, что карбоксильная группа может привести к появлению токсического действия и характеризуется неудовлетворительными фармакокинетическими свойствами. Структурная модификация карбоксильной группы, включая ее биоизостерическую замену, является одним из наиболее широко применяемых подходов медицинской химии для совершенствования фармакодинамических, фармакокинетических и технологических характеристик.

Цель. Разработать методы синтеза ряда функциональных производных 3-R-2,8-диоксо-7,8-дигидро-2*H*-пирроло[1,2-*a*][1,2,4]триазино[2,3-*c*]хиназолин-5a(6*H*)-карбоновых(-пропановых) кислот, изучить влияние структурной модификации карбоксильной группы на ЛОГ-ингибирующее и антирадикальное действие как одного из возможных механизмов фармакологической активности.

Результаты и их обсуждение. Реакцией этерификации 3-(2,8-диоксо-3-R[1]-7,8-дигидро-2*H*-пирроло[1,2-*a*]-[1,2,4]триазино[2,3-*c*]хиназолин-5a(6*H*)-ил)пропановых кислот или тандемной гетероциклизацией 2-(6-R[1]-2,5-дигидро-5-оксо-1,2,4-триазино-3-ил)анилинов с диэтил-4-оксогептандионатом синтезированы эфиры соответствующих кислот. Синтез амидов проведен аминолизом активированных кислот, где в качестве активирующей компоненты использован 1,1'-карбонилдиимидазол. В рамках исследования проведено изучение ЛОГ-ингибирующего и антирадикального действия как одного из возможных механизмов противовоспалительной активности. Выявлен ряд соединений, которые проявляют ЛОГ-ингибирующую активность на уровне стандарта нордигидрогваяретовой кислоты.

Экспериментальная часть. Синтетические процедуры были выполнены согласно общепринятым подходам. Чистота и структура соединений установлены с помощью современных физико-химических методов (^{1}H и ^{13}C ЯМР-спектроскопия, ВЭЖХ-МС-спектрометрия). Антирадикальную активность устанавливали путем измерения способности синтезированных соединений связывать DPPH-радикал. Изучение ЛОГ-ингибирующей активности веществ было проведено с использованием соевой липооксигеназы в качестве фермента и линоленовой кислоты в качестве субстрата.

Выводы. Разработаны методы синтеза сложных эфиров и амидов 2,8-диоксо-3-R[1]-7,8-дигидро-2*H*-пирроло[1,2-*a*][1,2,4]триазино[2,3-*c*]хиназолин-5a(6*H*)-карбоновых(-пропановых) кислот. Указанные превращения проведены алкоголизом образованных *in situ* ацилгалогенидов и аминолизом *N*-ацилимидазолидов. Более эффективным методом синтеза сложных эфиров оказалась тандемная гетероциклизация 2-(6-R[1]-2,5-дигидро-5-оксо-1,2,4-триазино-3-ил)анилинов с диэтил-4-оксогептандионатом. Установлено, что наиболее высокая ЛОГ-ингибирующая и антирадикальная активность характерны для гетарилпропановых кислот, которые имеют электроноакцепторные заместители в положении 3, а также атомы фтора в положениях 11 и 12. Модификация карбоксильной группы соответствующих кислот в большинстве случаев приводит к снижению или потере активности.

Ключевые слова: пирроло[1,2-*a*][1,2,4]триазино[2,3-*c*]хиназолин-5a(6*H*)-карбоновые(-пропановые) кислоты; сложные эфиры; амиды; синтез; антирадикальная и ЛОГ-ингибирующая активность

It is well known that most of anti-inflammatory drugs used in medical practice belong to NSAIDs (nonsteroidal anti-inflammatory drugs). The mechanism of the NSAIDs activity is associated with the cyclooxygenase activity inhibition and, as a result, a decrease in prostaglandin production. Even currently, therapy by NSAIDs is often accompanied with side effects, including gastrointestinal bleeding and thrombocyte aggregation suppression [1–3]. The more detailed study of the cyclooxygenase nature allowed to evaluate the existing several isoforms (COX-1 and COX-2) revealing mechanisms of occurrence of side effects during the long-term therapy of inflammatory processes [3]. Thus, the early generation of anti-inflammatory agents inhibits both COX-1 and COX-2 and belongs to non-selective NSAIDs. These agents control the inflammation process, but also cause the appearance of side effects. It is known that carboxyl groups bonded to aryl or hetaryl moieties play a role of the "pharmacophore" fragment in most NSAID molecules [4, 5]. It should be mentioned that the carboxyl group may cause the appearance of toxic effects and is characterized by unsatisfactory pharmacokinetic properties. The structural modification of the carboxyl group, including its bioisosteric replacement, is among the most widely used approaches in medicinal che-

mistry to improve pharmacodynamic, pharmacokinetic and technological characteristics [4, 5]. Implementation of this approach allowed creating highly selective NSAIDs. The pharmacological effects of the abovementioned group of agents are caused by specific inhibition of COX-2. Thus, the use of highly selective NSAIDs is accompanied with a lower risk of gastrointestinal bleeding and other unfavorable reactions. However, side effects of this type of medicines were also described [6, 7]. Thus, the search of effective and safe NSAIDs among novel heterocyclic derivatives using the up-to-date "drug design" methodology is among urgent problems of medicinal chemistry.

Recently, the pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]-quinazoline heterocyclic system was described as a promising "scaffold" for construction of novel bioactive compounds [8]. Additionally, the concept of the purposeful structural optimization of the abovementioned heterocyclic system previously developed [9] allowed us to synthesize the novel 3-R-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]-quinazolin-5*a*(6*H*)-carboxylic(-propanoic) acids with the promising anti-inflammatory activity. However, one of the possible approaches for the chemical modification of the abovementioned acids, namely functionalization of carboxylic group, has not been described.

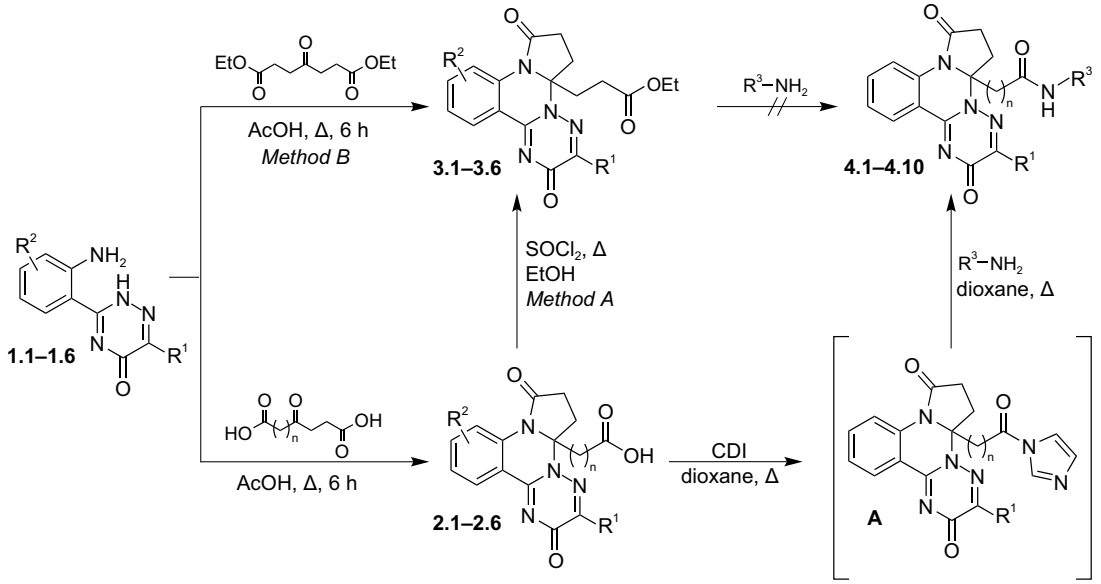
Hence, the present study aimed at the development of the synthetic procedures towards functional derivatives of 3-R-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo-[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazoline-5*a*(6*H*)-carboxylic(-propanoic) acids, as well as the study of the effect of the carboxyl group chemical modification on the LOX-inhibiting and antiradical activity as a possible mechanism of the pharmacological activity.

Results and discussion

Previously described [8] substituted 2,8-dioxo-3-R^1-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino-[2,3-*c*]quinazolin-5*a*(6*H*)-carboxylic(propanoic) acids **2** were used as substrates for the chemical modification of the carboxyl group. It was found that abovementioned compounds under esterification conditions (Scheme, Method A) resulted in esters **3** with the yields of 33–60%. Tandem heterocyclization of 2-(6-R^1-2,5-dihydro-5-oxo-1,2,4-triazino-3-yl)anilines **1** with diethyl 4-oxoheptanedioate in glacial acetic acid (Scheme, Method B) was shown to be more efficient approach to obtain compounds **3** (yields of 74–87%). The synthesis of amides **4** was conducted by the known method [10], namely by aminolysis of N-acylimidazolides obtained *in situ*. It was found that the abovementioned intermediates **A** were synthetically available and revealed high reactivity towards benzyl-(aryl-)amines. The conversion of acids **2** in amides **4** *via* the method mentioned above requires anhydrous dioxane and refluxing for 3–4 hours (Scheme). The yields of amides **4** were in the range of 30–73%. The attempt of the synthesis of amide **4** *via* the reaction of esters **3** with amines was not successful.

Elemental analysis, ^{1}H, ^{13}C NMR and LS-MS data proved the structure and purity of the substances synthesized.

^{1}H NMR-spectra of compounds **3** were characterized by the signals of ethoxy-group registered as the AM-system formed by doublet (**3.1**), doublet of doublets, multiplets (**3.2, 3.4–3.6**) or quintet (**3.3**) at 4.09–3.62 ppm (CH_2-fragment) and triplets at 1.16–1.04 ppm (CH_3-group). Signals of the ethylene moiety in the ethoxycarbonylalkyl fragment of esters



R^1 = Me, Ph, 4-F-C_6H_4, 4-*i*Pr-Ph;
R^2 = H, F;
R^3 = Ph, 4-MeO-C_6H_4, 4-F-C_6H_4, 4-*i*Pr-Ph, 4-MeO-Bn, 4-F-Bn, 4-CF_3-Bn;
n = 0, 2

Scheme

3.1–3.6 were observed as series of multiplets overlapping on the equatorial and axial protons of the pyrrole cycle at 2.93–2.58 ppm (H-7_{eq}, 7_{ax}, $-CH_2C\underline{H}_2COOC_2H_5$) and 2.47–2.06 ppm (H-$6_{eq}$, 6_{ax}, $-C\underline{H}_2CH_2COOC_2H_5$). Such complex splitting is probably associated with the presence of an asymmetric carbon atom.

^{1}H NMR-spectra of compounds **4** were characterized by the signals of amide NH-protons. The abovementioned signals were registered as triplets at 8.09–6.23 ppm (**4.1–4.5**) or singlet at 9.71–9.49 ppm (**4.6–4.10**). Additionally, the signals of the methylene group protons were observed as two-proton doublets at 4.29–4.09 ppm. As it was expected, in ^{1}H NMR-spectra of amides **4.1–4.3** the signals of the pyrrole cycle protons (7_{eq}, 7_{ax}, 6_{eq}, 6_{ax}) were observed as a wide high-field multiplet in the range of 3.24–2.64 ppm. In ^{1}H NMR-spectra of amides **4.4–4.10** the pattern of the abovementioned protons signals was similar to the spectra of the corresponding esters **3** (two sequential multiplets at 2.95–2.70 ppm (H-7_{eq}, 7_{ax}) and 2.69–2.41 ppm (H-6_{eq}, 6_{ax}). At the same time, in ^{1}H NMR-spectra of compounds **4.4–4.10** the signals of the exocyclic ethylene fragment protons were shifted to the low field compared to esters **3**. The abovementioned signals were observed as series of multiplets at 2.38–2.23 ppm ($-CH_2C\underline{H}_2CONH-$), and 2.18–2.10 ppm ($-C\underline{H}_2CH_2CONH-$).

In the ^{1}H NMR-spectra of compounds **3.1–3.3**, **4.1–4.10** the signals of the benzene fragment protons were observed as the ABCD-system consisting of a doublet of H-13 at 8.31–8.22 ppm, a doublet of H-10 at 8.23–8.02 ppm, a triplet of H-11 at 7.73–7.59 ppm and a triplet H-12 at 7.47–7.31 ppm. The signals of proton in position 12 in most cases form multiplets with signals of aromatic protons of substituents in positions 3 and 5*a*. Introduction of one or two fluorine atoms to the heterocyclic fragment of compounds **3.4–3.6** caused the additional splitting [11]. Besides, in ^{1}H NMR-spectra of compounds **3** and **4** the signals were caused by the nature of substituents in positions 3 and 5*a* [11].

The ^{13}C NMR-spectrum of compound **3.2** additionally proved its structure. The characteristic were the signals of a carbon atom of position 5*a*, cyclic and exocyclic ethylene fragments. The abovementioned signals were registered at 31.8, 29.5, 27.7, 27.7 and 83.5 ppm, respectively.

The antiradical and LOX-inhibiting activities of the compounds obtained were studied as possible mechanisms of the anti-inflammatory activity [12]. It was found that compounds **2**, **3** and **4** revealed the antiradical activity (ARA = 0.87–43.6%) in the concentration of 10^{-3} M. The SAR-analysis conducted showed that introduction of electron-withdrawing substituents to position 3, as well as introduction of fluorine atoms in positions 11 and 12 increased the DPPH-scavenging activity of hetarylpropanoic acids **2.1–2.6.** All compounds exhibited lower antiradical activity in the concentration of 10^{-4} M, but the "structure – antiradical activity" relationship was preserved. Hetarylcarboxylic acids **2.7** and **2.8** were characterized by a moderate antiradical activity.

Moreover, the antiradical activity of compounds **2.1–2.6** correlated with their LOX-inhibiting activity (Table). Thus, the highest LOX-inhibiting activity (16.7–31.02%) was characteristic for compounds **2.4–2.6** containing fluorine atoms in their structures [13, 14]. At the same time, acids **2.7** and **2.8** did not reveal the LOX-inhibiting activity.

The chemical modification of acids **2.1–2.6** by the carboxylic group esterification (compounds **3.1–3.6**) resulted in decreasing of the radical scavenging (0.87–17.11%) and the LOX-inhibiting activity (3.32–15.78%). Thus, fluorine-containing compound **3.5** revealed the highest LOX-inhibiting activity among esters **3**. Compounds **4.2**, **4.7** and **4.8** (Table) exhibited the highest radical scavenging activity among ami-

Table

The antiradical and LOX-inhibitory activity of the compounds synthesized

Compd.	ARA, %		LOX-inhibiting activity, %
	10^{-3} M	10^{-4} M	
Ascorbic acid	94.80	82.36	–
NDGA	–	–	32.14
2.1	6.94	4.86	19.18
2.2	10.68	6.44	7.83
2.3	11.02	5.62	7.47
2.4	10.85	5.25	16.73
2.5	35.76	8.31	20.41
2.6	36.44	7.38	31.02
3.1	5.90	2.90	7.44
3.2	1.97	0.27	3.32
3.3	0.87	0.00	6.41
3.4	3.39	1.61	0.00
3.5	10.47	1.87	15.87
3.6	17.71	2.26	0.00
4.1	8.87	3.23	0.00
4.2	14.68	10.48	0.00
4.3	13.55	8.06	0.00
4.4	3.71	2.26	17.17
4.5	8.23	3.23	0.00
4.6	5.00	4.19	0.00
4.7	16.29	5.16	0.00
4.8	43.31	13.71	0.00
4.9	2.90	2.26	0.00
4.10	4.84	1.94	0.00

des **4** obtained. It is interesting to note that the abovementioned compounds contain the 4-methoxybenzyl (**4.2**) or 4-methoxyphenyl (**4.4**, **4.8**) fragment. Esters **3** and amides **4** did not reveal the LOX-inhibiting activity.

Hence, the study conducted allowed us to detect the classes of effective anti-inflammatory agents, as well as to propose the effective approaches for constructing novel anti-inflammatory agents. It should be noted that the lipophilic, but not active esters obtained can not be considered as non-promising bioactive agents due to the possibility of their biotransformation in active metabolites.

Experimental part

Melting points were determined in open capillary tubes in a Stuart SMP30 apparatus and were uncorrected. The elemental analyses (C, H, N) were performed using an ELEMENTAR vario EL cube analyzer. ^{1}H NMR-spectra (400 MHz) and ^{13}C NMR (101 MHz) were recorded using a Varian-Mercury 400 spectrometer with TMS as an internal standard in DMSO-d_6 solution. LC-MS spectra were recorded using the chromatography/mass spectrometric system consisting of an Agilent 1100 Series high-performed liquid chromatograph equipped with an Agilent LC/MSD SL diode-matrix and mass-selective detector (atmospheric pressure chemical ionization – APCI). The ionization mode was a concurrent scanning of positive and negative ions in the mass range of 80–1000 m/z. The synthetic studies were conducted according to the general approach to the search of potential biologically active substances using reagents of companies Sigma-Aldrich (Missouri, USA) and Enamine (Kyiv, Ukraine).

2-(6-R^1-2,5-dihydro-5-oxo-1,2,4-triazino-3-yl)anilines **1.1–1.6**, 3-(2,8-dioxo-3-R^1-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolin-5*a*(6*H*)-yl)propanoic acids **2.1–2.6** and 2,8-dioxo-3-R^1-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]-quinazoline-5*a*(6*H*)-carboxylic acids **2.7** and **2.8** were synthesized according to the known methods and their constants corresponded to the article [8, 15].

The general method for the synthesis of ethyl 3-(3-R^1-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*]-[1,2,4]triazino[2,3-*c*]quinazolin-5*a*(6*H*)-yl)propanoates 3.1–3.6:

Method A. To 10 mmol of the corresponding acid **2.1–2.6** in 20 mL of ethanol add 1.29 g (11 mmol) of thionyl chloride and 1 drop of DMF. Heat the resulting mixture on a water bath for 6 h. Then cool the reaction mixture and pour into 5 mL of the saturated sodium bicarbonate solution. Filter the precipitate formed and dry. If it is necessary, the compounds obtained can be recrystallized from ethanol.

Method B. To the suspension of 10 mmol of the corresponding anilines **1.1–1.6** in glacial acetic acid add 2.30 g (10 mmol) of diethyl 4-oxoheptanedioate. Reflux the resulting mixture for 6 h. Evaporate the solvent under vacuum, add 15 mL of methanol to the residue formed. Filter the precipitate formed, wash by diethyl ether and dry. The compounds obtained can be purified by recrystallization from methanol.

Ethyl 3-(3-methyl-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolin-5*a*-(6*H*)-yl)propanoate 3.1. Yield – 52.4% (method A), 77.0% (method B). M. p. 137–139°C. Anal. Calcd. for $C_{19}H_{20}N_4O_4$, %: C 61.95, H 5.47, N 15.21. Found, %: C 61.99, H 5.56, N 15.29. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 8.26 (1H, d, *J* = 7.8 Hz, H-13), 8.06 (1H, d, *J* = 8.1 Hz, H-10), 7.70 (1H, t, *J* = 7.7 Hz, H-11), 7.41 (1H, t, *J* = 7.5 Hz, H-12), 3.93 (2H, dd, J_1 = 11.5 Hz, J_2 = 6.1 Hz, –OC$\underline{H}_2$CH$_3$), 2.96–2.53 (4H, m, H-7$_{eq}$, 7$_{ax}$, –CH$_2$C$\underline{H}_2$COOC$_2$H$_5$), 2.43–2.07 (7H, m, H-6$_{eq}$, 6$_{ax}$, –C$\underline{H}_2$CH$_2$–COOC$_2$H$_5$, CH$_3$), 1.16 (3H, t, *J* = 7.0 Hz, –OCH$_2$C$\underline{H}_3$). LC-MS: m/z = 369 [M+1].

Ethyl 3-(2,8-dioxo-3-phenyl-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolin-5*a*-(6*H*)-yl)propanoate 3.2. Yield – 51.0% (method A), 79.3% (method B). M. p. 230–232°C. Anal. Calcd. for $C_{24}H_{22}N_4O_4$, %: C 66.97, H 5.15, N 13.02. Found, %: C 67.06, H 5.21, N 13.09. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 8.30 (1H, d, *J* = 7.6 Hz, H-13), 8.20 (2H, d, *J* = 6.3 Hz, Ar-H-2,6), 8.08 (1H, d, *J* = 8.0 Hz, H-10), 7.72 (1H, t, *J* = 7.3 Hz, H-11), 7.59–7.18 (4H, m, H-12, Ar-H-3,4,5), 4.09–3.62 (2H, m, –OC$\underline{H}_2$CH$_3$), 3.23–1.90 (8H, m, H-7$_{eq}$, 7$_{ax}$, 6$_{eq}$, 6$_{ax}$, –C$\underline{H}_2$C$\underline{H}_2$COOC$_2$H$_5$), 1.04 (3H, t, *J* = 7.1 Hz, –OCH$_2$C$\underline{H}_3$). ^{13}C NMR (101 MHz, DMSO-d_6) δ, ppm: 13.7, 27.7, 27.7, 29.5, 31.8, 60.2, 83.5, 118.6, 121.4, 125.8, 127.2, 128.0, 128.6, 130.4, 132.3, 134.1, 134.3, 146.8, 150.8, 160.8, 171.5, 172.3. LC-MS: m/z = 431 [M+1].

Ethyl 3-(3-(4-isopropylphenyl)-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]-quinazolin-5*a*(6*H*)-yl)propanoate 3.3. Yield – 54.0% (method A), 82.3% (method B). M. p. 214–216°C. Anal. Calcd. for $C_{27}H_{28}N_4O_4$, %: C 68.63, H 5.97, N 11.86. Found, %: C 68.72, H 6.05, N, 11.92. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 8.31 (1H, d, *J* = 7.8 Hz, H-13), 8.14 (2H, d, *J* = 8.0 Hz, 3-Ar-H-2,6), 8.09 (1H, d, *J* = 8.2 Hz, H-10), 7.73 (1H, t, *J* = 7.8 Hz, H-11), 7.44 (1H, t, *J* = 7.6 Hz, H-12), 7.30 (2H, d, *J* = 8.0 Hz, 3-Ar-H-3,5), 3.85 (2H, q, *J* = 7.0 Hz, –OC$\underline{H}_2$CH$_3$), 2.93–2.58 (5H, m, H-7$_{eq}$, 7$_{ax}$, –CH$_2$C$\underline{H}_2$COOC$_2$H$_5$, –C$\underline{H}$(CH$_3$)$_2$), 2.47–2.06 (4H, m, H-6$_{eq}$, 6$_{ax}$, –C$\underline{H}_2$CH$_2$COOC$_2$H$_5$), 1.30 (6H, d, *J* = 6.9 Hz, –CH(C$\underline{H}_3$)$_2$), 1.05 (3H, t, *J* = 7.1 Hz, –OCH$_2$C$\underline{H}_3$). LC-MS: m/z = 473 [M+1].

Ethyl 3-(12-fluoro-2,8-dioxo-3-phenyl-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]-quinazolin-5*a*(6*H*)-yl)propanoate 3.4. Yield – 60.0% (method A), 73.6% (method B). M. p. 184–186°C. Anal. Calcd. for $C_{24}H_{21}FN_4O_4$, %: C 64.28, H 4.72, N 12.49.

Found, %: C 64.34, H 4.81, N, 12.54. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 8.21 (2H, d, *J* = 6.6 Hz, Ph-H-2,6), 8.10 (1H, m, H-13), 7.98 (1H, m, H-10), 7.60–7.43 (4H, m, H-11, Ar-H-3,4,5), 4.01–3.66 (2H, m, –OC$\underline{H}_2$CH$_3$), 3.18–2.56 (4H, m, H-7$_{eq}$, 7$_{ax}$, –CH$_2$C$\underline{H}_2$COOC$_2$H$_5$), 2.44–2.11 (4H, m, H-6$_{eq}$, 6$_{ax}$, –C$\underline{H}_2$CH$_2$COOC$_2$H$_5$), 1.06 (3H, t, *J* = 7.1 Hz, –OCH$_2$C$\underline{H}_3$). LC-MS: m/z = 450 [M+1].

Ethyl 3-(11,12-difluoro-2,8-dioxo-3-phenyl-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]-quinazolin-5*a*(6*H*)-yl)propanoate 3.5. Yield – 33.0% (method A), 87.0% (method B). M. p. 299–301°C. Anal. Calcd. for $C_{24}H_{19}F_2N_4O_4$, %: C 61.80, H 4.32, N 12.01. Found, %: C 61.89, H 4.41, N 12.11. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 8.30 (2H, dd, J_1 = 7.8 Hz, J_2 = 6.0 Hz, Ar-H-2,6), 8.14 (1H, t, *J* = 9.4 Hz, H-13), 8.03 (1H, dd, J_1 = 11.2 Hz, J_2 = 7.1 Hz, H-10), 7.19 (2H, t, *J* = 8.6 Hz, Ar-H-3,5), 3.95–3.78 (2H, m, –OC$\underline{H}_2$CH$_3$), 2.96–2.57 (4H, m, H-7$_{eq}$, 7$_{ax}$, –CH$_2$C$\underline{H}_2$COOC$_2$H$_5$), 2.46–2.08 (4H, m, H-6$_{eq}$, 6$_{ax}$, –C$\underline{H}_2$CH$_2$COOC$_2$H$_5$), 1.07 (3H, t, *J* = 7.1 Hz, –OCH$_2$C$\underline{H}_3$). LC-MS: m/z = 467 [M+1].

Ethyl 3-(11,12-difluoro-3-(4-fluorophenyl)-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino-[2,3-*c*]quinazolin-5*a*(6*H*)-yl)propanoate 3.6. Yield – 35.0% (method A), 74.3% (method B). M. p. 175–177°C. Anal. Calcd. for $C_{24}H_{19}F_3N_4O_4$, %: C 59.51, H 3.95, N 11.57. Found, %: C 59.47, H 3.88, N 11.50. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 8.30 (2H, dd, J_1 = 7.8 Hz, J_2 = 6.0 Hz, Ar-H-2, 6), 8.14 (1H, t, *J* = 9.4 Hz, H-13), 8.03 (1H, dd, J_1 = 11.2 Hz, J_2 = 7.1 Hz, H-10), 7.19 (2H, t, *J* = 8.6 Hz, Ar-H-3,5), 3.95–3.78 (2H, m, –OC$\underline{H}_2$CH$_3$), 2.96–2.57 (4H, m, H-7$_{eq}$, 7$_{ax}$, –CH$_2$C$\underline{H}_2$COOC$_2$H$_5$), 2.46–2.08 (4H, m, H-6$_{eq}$, 6$_{ax}$, –C$\underline{H}_2$CH$_2$COOC$_2$H$_5$), 1.07 (3H, t, *J* = 7.1 Hz, –OCH$_2$C$\underline{H}_3$). LC-MS: m/z = 485 [M+1].

The general method for the synthesis of N-R^3-3-R^1-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]-triazino[2,3-*c*]quinazoline-5*a*(6*H*)-yl-carboxamides 4.1–4.2 and N-R^3-3-(3-R^1-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino-[2,3-*c*]-quinazoline-5*a*(6*H*)-yl)propanamides 4.3–4.10. To the suspension of 10 mmol of the corresponding acid **2.7–2.8, 2.1, 2.2** in 20 mL of anhydrous dioxane add 1.78 g (11 mmol) of 1,1'-carbonyldiimidazole (CDI). Heat the resulting mixture at 80°C for 1 h (until the complete evolution of carbon dioxide). Then add 10 mmol of the corresponding amine and heat the mixture obtained for 3–4 h. Cool the reaction mixture and pour into water. Filter the precipitate formed and dry. The compounds obtained can be purified by recrystallization from dioxane.

***N*-(4-Fluorobenzyl)-2,8-dioxo-3-phenyl-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]-quinazoline-5*a*(6*H*)-carboxamide 4.1.** Yield – 30.0%. M. p. 212–215°C. Anal. Calcd. for $C_{27}H_{20}FN_5O_3$, %: C 67.35, H 4.19, N 14.55. Found, %: C 67.41, H 4.24, N 14.61. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 8.28 (1H, d, *J* = 7.8 Hz, H-13), 8.23 (1H, d, *J* = 8.2 Hz, H-10), 8.18 (2H, d, *J* = 6.0 Hz, Ar-H-2,6), 7.70 (1H, t, *J* = 7.9 Hz, H-11), 7.47–7.37 (4H, m, H-12, Ar-H-3,4,5), 7.23 (2H, dd, J_1 = 8.3 Hz, J_2 = 5.4 Hz, 5*a*-Bn-H-2,6), 6.97 (2H, t, *J* = 8.5 Hz, 5*a*-Bn-H-3,5), 6.23 (1H, t, *J* = 6.0 Hz, –N$\underline{H}$CH$_2$–), 4.18 (2H, d, *J* = 5.8 Hz, –NHC$\underline{H}_2$–), 3.24–2.64 (4H, m, H-7$_{eq}$, 7$_{ax}$, 6$_{eq}$, 6$_{ax}$). LC-MS: m/z = 482 [M+1].

***N*-(4-Methoxybenzyl)-2,8-dioxo-3-phenyl-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]-quinazoline-5*a*(6*H*)-carboxamide 4.2.** Yield – 56.2%. M. p. 228–231°C. Anal. Calcd. for $C_{28}H_{23}N_5O_4$, %: C 68.14, H 4.70, N 14.19. Found, %: C 68.17, H 4.75, N 14.15. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 8.29–8.21 (2H, m, H-10,13), 8.18 (2H, d, *J* = 7.3 Hz, Ar-H-2,6), 7.68 (1H, t, *J* = 7.8 Hz, H-11), 7.49–7.34 (4H, m, H-12, Ar-H-3,4,5), 7.21 (2H, d, *J* = 8.2 Hz, 5*a*-Bn-H-2,6), 7.12 (2H, d, *J* = 8.2 Hz, 5*a*-Bn-H-3,5), 6.77 (1H, t, *J* = 4.5 Hz, –N$\underline{H}$CH$_2$–), 4.12 (2H, d, *J* = 4.5 Hz, –NHC$\underline{H}_2$–), 3.73 (3H, s, –OC$\underline{H}_3$), 3.17–2.60 (4H, m, H-7$_{eq}$, 7$_{ax}$, H-6$_{eq}$, 6$_{ax}$). LC-MS: m/z = 494 [M+1].

2,8-Dioxo-3-phenyl-*N*-(4-(trifluoromethyl)-benzyl)-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]-triazino[2,3-*c*]quinazoline-5*a*(6*H*)-carboxamide 4.3. Yield – 55.4%. M. p. 171–174°C. Anal. Calcd. for $C_{28}H_{20}F_3N_5O_3$, %: C 63.28, H 3.79, N 13.18. Found, %: C 63.33, H 3.87, N 13.25. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 8.26 (2H, d, *J* = 8.0 Hz, Ar-H-2,6), 8.23–8.08 (2H, m, H-10,13), 7.67 (1H, t, *J* = 7.8 Hz, H-11), 7.54 (2H, d, *J* = 7.8 Hz, 5*a*-Bn-H-3,5), 7.47–7.30 (6H, m, H-12, Ar-H-3,4,5, 5*a*-Bn-H-2,6), 6.47 (1H, t, *J* = 5.2 Hz, –N$\underline{H}$CH$_2$), 4.29 (2H, d, *J* = 5.4 Hz, –NHC$\underline{H}_2$–), 3.14–2.60 (4H, m, H-7$_{eq}$, 7$_{ax}$, H-6$_{eq}$, 6$_{ax}$). LC-MS: m/z = 532 [M+1].

3-(3-Methyl-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolin-5*a*(6*H*)-yl)-*N*-(4-(trifluoromethyl)benzyl)propanamide 4.4. Yield – 75.3%. M. p. 144–147°C. Anal. Calcd. for $C_{25}H_{22}F_3N_5O_3$, %: C 60.36, H 4.46, N 14.08. Found, %: C 60.42, H 4.54, N 14.18. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 8.22 (1H, d, *J* = 7.4 Hz, H-13), 8.03 (1H, d, *J* = 8.2 Hz, H-10), 7.66 (1H, t, *J* = 7.7 Hz, H-11), 7.56–7.49 (2H, m, 5*a*-Bn-H-3,5), 7.39–7.24 (3H, m, H-12, 5*a*-Bn-H-3,5), 6.87 (1H, br s, –N$\underline{H}$CH$_2$–), 4.23 (2H, d, *J* = 4.6 Hz, –NHC$\underline{H}_2$–), 2.95–2.72 (2H, m, H-7$_{eq}$, 7$_{ax}$), 2.67–2.41 (2H, m, H-6$_{eq}$, 6$_{ax}$), 2.22 (3H, s, CH$_3$), 2.20–2.01 (4H, m, –C$\underline{H}_2$C$\underline{H}_2$CONH–). LC-MS: m/z = 498 [M+1].

***N*-(4-Fluorobenzyl)-3-(3-methyl-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]-quinazolin-5*a*(6*H*)-yl)propanamide 4.5.** Yield – 68%. M. p. 200–203°C. Anal. Calcd. for $C_{24}H_{22}FN_5O_3$, %: C 64.42, H 4.96, N 15.65. Found, %: C 64.45, H 4.91, N 15.68. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 8.22 (1H, d, *J* = 7.8 Hz, H-13), 8.09 (1H, t, *J* = 5.6 Hz, –N$\underline{H}$CH$_2$–), 8.03 (1H, d, *J* = 8.0, H-10), 7.66 (1H, t, *J* = 7.5 Hz, H-11), 7.37 (1H, t, *J* = 7.6 Hz, H-12), 7.17 (2H, dd, J_1 = 8.1 Hz, J_2 = 5.7 Hz, 5*a*-Bn-H-2,6), 6.95 (2H, t, *J* = 8.7 Hz, 5*a*-Bn-H-3,5), 4.09 (2H, d, *J* = 4.4 Hz, –NHC$\underline{H}_2$–), 2.91–2.70 (2H, m, H-7$_{eq}$, 7$_{ax}$), 2.68–2.49 (2H, m, H-6$_{eq}$, 6$_{ax}$), 2.38–2.23 (2H, m, –CH$_2$C$\underline{H}_2$CONH–), 2.22 (3H, s, CH$_3$), 2.18–2.00 (2H, m, –C$\underline{H}_2$CH$_2$CONH–). LC-MS: m/z = 448 [M+1].

3-(3-Methyl-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolin-5*a*(6*H*)-yl)-*N*-phenylpropanamide 4.6. Yield – 68.0%. M. p. 264–267°C. Anal. Calcd. for $C_{23}H_{21}N_5O_3$, %: C 66.49, H 5.10, N 16.86. Found, %: C 66.56, H 5.17, N 16.94. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 9.63 (1H, s, –NH–), 8.24 (1H, dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, H-13), 8.03 (1H, d, J = 8.2 Hz, H-10), 7.70–7.59 (1H, m, H-11), 7.39 (3H, m, H-12, 5*a*-Ph-H-2,6), 7.16 (2H, t, J = 7.7 Hz, 5*a*-Ph-H-3,5), 6.92 (1H, t, J = 7.4 Hz, 5*a*-Ph-H-4), 2.95–2.76 (2H, m, H-7_{eq}, 7_{ax}), 2.69–2.52 (2H, m, H-6_{eq}, 6_{ax}), 2.46 (3H, s, CH_3), 2.38–2.05 (4H, m, –C<u>H</u>$_2$C<u>H</u>$_2$CONH–). LC-MS: m/z = 416 [M+1].

***N*-(4-Methoxyphenyl)-3-(3-methyl-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino-[2,3-*c*]quinazolin-5*a*(6*H*)-yl)propanamide 4.7.** Yield – 67.7%. M. p. 242–245°C. Anal. Calcd. for $C_{24}H_{23}N_5O_4$, %: C 64.71, H 5.20, N 15.72. Found, %: C 64.78, H 5.29, N 15.81. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 9.49 (1H, s, –N<u>H</u>–), 8.28–8.20 (1H, m, H-13), 8.03 (1H, d, J = 8.2 Hz, H-10), 7.64 (1H, t, J = 7.5 Hz, H-11), 7.37 (1H, t, J = 7.7 Hz, H-12), 7.30 (2H, d, J = 8.9 Hz, 5*a*-Ar-H-2,6), 6.74–6.66 (2H, d, J = 8.9 Hz, 5*a*-Ar-H-3,5), 3.69 (3H, s, –OCH_3), 2.95–2.76 (2H, m, H-7_{eq}, 7_{ax}), 2.69–2.48 (2H, m, H-6_{eq}, 6_{ax}), 2.35–2.20 (2H, m, –CH_2–C<u>H</u>$_2$CONH–), 2.17 (3H, s, 3-CH_3), 2.14–1.97 (2H, m, –C<u>H</u>$_2$CH_2CONH–). LC-MS: m/z = 446 [M+1].

3-(2,8-Dioxo-3-phenyl-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolin-5*a*-(6*H*)-yl)-*N*-(4-methoxyphenyl)propanamide 4.8. Yield – 66.1%. M. p. 210–213°C. Anal. Calcd. for $C_{29}H_{25}N_5O_4$, %: C 68.63, H 4.97, N 13.80. Found, %: C 68.67, H 4.95, N 13.83. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 9.50 (1H, s, –NH–), 8.27 (1H, d, J = 8.3 Hz, H-13), 8.19–8.09 (2H, m, Ar-H-2,6), 8.06 (1H, d, J = 8.0 Hz, H-10), 7.67 (1H, t, J = 8.0 Hz, H-11), 7.48–7.32 (4H, m, H-12, Ar-H-3,4,5), 7.26 (2H, d, J = 9.0 Hz, 5*a*-Ar-H-2,6), 6.68 (2H, d, J = 9.0 Hz, 5*a*-Ar-H-3,5), 3.68 (3H, s, –OCH_3), 3.13–1.86 (8H, m, H-7_{eq}, 7_{ax}, 6_{eq}, 6_{ax}, –C<u>H</u>$_2$C<u>H</u>$_2$CONH–). LC-MS: m/z = 508 [M+1].

***N*-(4-Fluorophenyl)-3-(3-methyl-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino-[2,3-*c*]quinazolin-5*a*(6*H*)-yl)propanamide 4.9.** Yield – 67.7%. M. p. 265–268°C. Anal. Calcd. for $C_{23}H_{20}FN_5O_3$, %: C 63.73, H 4.65, N 16.16. Found, %: C 63.79, H 4.72, N 16.24. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 9.71 (1H, s, –NH–), 8.24 (1H, d, J = 7.6 Hz, H-13), 8.02 (1H, d, J = 8.1 Hz, H-10), 7.64 (1H, t, J = 7.7 Hz, H-11), 7.42 (2H, dd, J_1 = 8.8 Hz, J_2 = 5.0 Hz, 5*a*-Ar-H-2,6), 7.36 (1H, t, J = 7.6 Hz, H-12), 6.91 (2H, t, J = 8.7 Hz, 5*a*-Ar-H-3,5), 2.95–2.73 (2H, m, H-7_{eq}, 7_{ax}), 2.69–2.50 (2H, m, H-6_{eq}, 6_{ax}), 2.37–2.19 (2H, m, –CH_2C<u>H</u>$_2$CONH–), 2.16 (3H, s, CH_3), 2.10 (2H, m, –C<u>H</u>$_2$CH_2CONH–). LC-MS: m/z = 434 [M+1].

3-(2,8-Dioxo-3-phenyl-7,8-dihydro-2*H*-pyrrolo-[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolin-5*a*(6*H*)-yl)-*N*-(4-fluorophenyl)propanamide 4.10. Yield – 77.3%. M. p. 270–273°C. Anal. Calcd. for $C_{28}H_{22}FN_5O_3$, %: C 67.87, H 4.48, N 14.13. Found, %: C 67.94, H 4.51, N 14.19. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 9.69 (1H, s, NH), 8.29 (1H, d, J = 7.9 Hz, H-13), 8.13 (2H, d, J = 6.9 Hz, 3-Ar-H-2,6), 8.05 (1H, d, J = 8.2 Hz, H-10), 7.67 (1H, t, J = 7.4 Hz, H-11), 7.47–7.21 (6H, m, 3-Ar-H-3,4,5, H-12, 5a-Ar-H-2,6), 6.87 (2H, t, J = 8.7 Hz, 5*a*-Ar-H-3,5), 3.18–2.03 (8H, m, H-7_{eq}, 7_{ax}, 6_{eq}, 6_{ax}, –C<u>H</u>$_2$C<u>H</u>$_2$CONH–). LC-MS: m/z = 496 [M+1].

Antiradical activity. The *in vitro* research of the antiradical activity was based on the interaction of the compounds synthesized with 2,2-diphenyl-1-picrylhydrazyl (DPPH) [16]. DPPH is a stable free radical, and its alcohol solutions are colored in an intense purple color (λ_{max} = 517 nm). DPPH interacts with compounds that are able to bind free radicals yielding the products, which are yellow colored, and does not absorb the light at the wavelength specified above.

Research methodology. Dissolve the compounds in DMSO to obtain 1 mM solution. Mix 2 mL of this solution with 2 mL of 0.1 mM DPPH methanol solution and incubate for 30 min at 25°C. Then measure the absorbance (A_d) [17]. Simultaneously determine the absorbance of 2 mL of 0.1 mM DPPH solution in 2 mM of methanol (A_{DPPH}). Calculate the antiradical activity (ARA) by the following formula: ARA, % = $(A_{DPPH} - A_d)\times100\%/A_{DPPH}$. In the case of a negative meaning ARA in % is estimated as 0.

Weighing of reagents and the compounds synthesized were conducted on ANG200C electronic scales (Axis, Gdansk, Poland), and the absorbance was measured by a ULAB 108UV spectrophotometer (Ulab, Shanghai, China).

The *in vitro* study of soybean LOX inhibition. The *in vitro* study was evaluated as it was reported previously [18, 19]. To 3.88 mL of borate buffer add 40 μL of $2\cdot10^{-5}$ w/v solution of LOX in buffer and 40 μL of 100 μM of the compound solution studied (or nordihydroguaiaretic acid (NDGA)). Shake the resulting mixture and incubate at ambient temperature for 5 min. After incubation add 40 μL of 0.01 M solution of sodium linolenate. The intensity of absorbance at 234 nm is recovered after 20 min of the incubation at ambient temperature. Calculate the results by the formula: LOX inhibiting activity, % = $(A_{control} - A_{test\ compound})\times100\%/A_{control}$.

Conclusions

The methods for the synthesis of esters and amides of 2,8-dioxo-3-R^1-7,8-dihydro-2*H*-pyrrolo[1,2-*a*]-[1,2,4]triazino[2,3-*c*]quinazolin-5*a*(6*H*)-carboxilic (propanoic) acids have been developed. The above-mentioned transformations were conducted by alcoholysis of generated *in situ* acyl halides and aminolysis of *N*-acylimidazolides. The more efficient alternative approach for the synthesis of the target esters *via* condensation of 2-(6-R^1-2,5-dihydro-5-oxo-1,2,4-

triazino-3-yl)anilines with diethyl 4-oxoheptanedioate has been proposed. It has been found that the highest radical scavenging and LOX-inhibiting activities are characteristic for hetarylpropanoic acids that contain electron withdrawing substituents in position 3, as well as fluorine atoms in positions 11 and 12. The chemical modification of the carboxylic group in most cases results in a decrease or the loss of the activity.

Conflict of interests: authors have no conflict of interests to declare.

References

1. Claria, J. Cyclooxygenase-2 Biology. *Current Pharmaceutical Design* **2003**, 9 (27), 2177-2190. https://doi.org/10.2174/1381612033454054.
2. Iezzi, A.; Ferri, C.; Mezzetti, A.; Cipollone, F. COX-2: Friend or Foe? *Current Pharmaceutical Design* **2007,** *13* (16), 1715-1721. http://dx.doi.org/10.2174/138161207780831293.
3. Rao, P.; Knaus, E. E. Evolution of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs): Cyclooxygenase (COX) Inhibition and Beyond. *Journal of Pharmacy & Pharmaceutical Sciences* **2008,** *11* (2), 81s-110s. http://dx.doi.org/10.18433/j3t886.
4. Lassalas, P.; Gay, B.; Lasfargeas, C.; James, M. J.; Tran, V.; Vijayendran, K. G.; Brunden, K. R.; Kozlowski, M. C.; Thomas, C. J.; Smith, A. B.; Huryn, D. M.; Ballatore, C. Structure Property Relationships of Carboxylic Acid Isosteres. *J. Med. Chem.* **2016,** *59* (7), 3183-3203. http://dx.doi.org/10.1021/acs.jmedchem.5b01963.
5. Ballatore, C.; Huryn, D. M.; Smith III, A. B. Carboxylic Acid (Bio)Isosteres in Drug Design. *ChemMedChem* **2013,** *8* (3), 385-395. http://dx.doi.org/10.1002/cmdc.201200585.
6. Szabó, G.; Fischer, J.; Kis-Varga, Á.; Gyires, K. New Celecoxib Derivatives as Anti-Inflammatory Agents. *J. Med. Chem.* **2008,** *51* (1), 142-147. http://dx.doi.org/10.1021/jm070821f.
7. Biava, M.; Porretta, G. C.; Poce, G.; Battilocchio, C.; Alfonso, S.; Rovini, M.; Valenti, S.; Giorgi, G.; Calderone, V.; Martelli, A.; Testai, L.; Sautebin, L.; Rossi, A.; Papa, G.; Ghelardini, C.; Di Cesare Mannelli, L.; Giordani, A.; Anzellotti, P.; Bruno, A.; Patrignani, P.; Anzini, M. Novel Analgesic/Anti-Inflammatory Agents: Diarylpyrrole Acetic Esters Endowed with Nitric Oxide Releasing Properties. *J. Med. Chem.* **2011,** *54* (22), 7759-7771. http://dx.doi.org/10.1021/jm200715n.
8. Stavytskyi, V.; Voskoboinik, O.; Antypenko, O.; Krasovska, N.; Shabelnyk, K.; Konovalova, I.; Shishkyna, S.; Kholodniak, S.; Kovalenko, S. Tandem heterocyclization of 2-(azolyl-(azinyl-))anilines as an efficient method for preparation of substituted pyrrolo[1,2-*a*]azolo-(azino-)[*c*]quinazolines. *J. Heterocycl. Chem. n/a* (n/a). http://dx.doi.org/10.1002/jhet.3862.
9. Ставицький, В. В.; Воскобойник, О. Ю.; Носуленко, І. С.; Клімова, О. О.; Брашко, О. А.; Коваленко, С. І. Заміщені 3-R-7,8-дигідро-2*H*-піроло[1,2-*a*][1,2,4]триазино[2,3-*c*]хіназолін-5а(6*H*)-алкілкарбонові кислоти – перспективний клас малотоксичних протизапальних агентів. *Фармацевтичний часопис* **2019,** (3), 5-12. http://dx.doi.org/10.11603/2312-0967.2019.3.10468.
10. Staab, H. A. New Methods of Preparative Organic Chemistry IV. Syntheses Using Heterocyclic Amides (Azolides). *Angew. Chem., Int. Ed. Engl.* **1962,** *1* (7), 351-367. http://dx.doi.org/10.1002/anie.196203511.
11. Breitmaier, E. *Structure Elucidation by NMR in Organic Chemistry: A Practical Guide*. 3rd ed.; John Willey & Sons Ltd: Chichester, 2002; p 272.
12. *Anti-Inflammatory Drug Discovery*; Levin, J. I., Laufer, S., Eds.; Drug Discovery; The Royal Society of Chemistry, 2012. https://doi.org/10.1039/9781849735346.
13. Auffinger, P.; Hays, F. A.; Westhof, E.; Ho, P. S. Halogen bonds in biological molecules. *Proceedings of the National Academy of Sciences* **2004**, *101* (48), 16789-16794. http://dx.doi.org/10.1073/pnas.0407607101.
14. Stavytskyi, V. V.; Nosulenko, I. S.; Portna, O. O.; Shvets, V. M.; Voskoboynik, O. Yu.; Kovalenko, S. I. Substituted pyrrolo[1,2-*a*][1,2,4]triazolo-(triazino-)[*c*]quinazolines – a promising class of lipoxygenase inhibitors. *Актуальні питання фармацевтичної та медичної науки та практики* **2020**, 1 (32), 213-221. https://doi.org.10.14739/2409-2932.2020.1.184170.
15. Sergeieva, T. Y.; Voskoboynik, O. Y.; Okovytyy, S. I.; Kovalenko, S. I.; Shishkina, S. V.; Shishkin, O. V.; Leszczynski, J. Hydrazinolysis of 3-R-[1,2,4]Triazino[2,3-*c*]quinazolin-2-ones. Synthetic and Theoretical Aspects. *J. Phys. Chem. A* **2014,** *118* (10), 1895-1905. http://dx.doi.org/10.1021/jp4052616.
16. Kedare, S. B.; Singh, R. P. Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology* **2011,** *48* (4), 412-422. http://dx.doi.org/10.1007/s13197-011-0251-1.
17. Szabo, M. R.; Idițoiu, C.; Chambre, D.; Lupea, A. X. Improved DPPH determination for antioxidant activity spectrophotometric assay. *Chemical Papers* **2007,** *61* (3), 214-216. http://dx.doi.org/10.2478/s11696-007-0022-7.
18. Pontiki, E.; Hadjipavlou-Litina, D. Synthesis and pharmacochemical evaluation of novel aryl-acetic acid inhibitors of lipoxygenase, antioxidants, and anti-inflammatory agents. *Bioorg. Med. Chem.* **2007,** *15* (17), 5819-5827. http://dx.doi.org/10.1016/j.bmc.2007.06.001.
19. Korobko, D.; Hadjipavlou-Litina, D. J.; Logoyda, L. Antioxidant and anti-inflammatory properties of a series of new 7,8-disubstituted theophylline containing a pyrazole ring. *Asian Journal of Pharmaceutical and Clinical Research* **2018,** *11* (6). http://dx.doi.org/10.22159/ajpcr.2018.v11i6.25990.

Received: 10. 01. 2019
Revised: 04. 02. 2020
Accepted: 27. 02. 2020

Acknowledgements
The work was performed with the technical support of Enamine Ltd (Kyiv, Ukraine).
The research was carried out according to the budget theme of the Ministry of Public Health of Ukraine "Purposeful search for anti-inflammatory agents among condensed and spiro-condensed quinazoline derivatives" (the state registration No. 0118U004370; the research period 2018–2020).