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

It is known that carboxylic groups bonded to aryl or heteroaryl 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 biosisosteric 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-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)-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)-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazolin-5a(6H)-yl)carboxylic(propanoic) acids was conducted by esterification of the corresponding acids and tandem heterocyclization of 2-(6-R'-2,5-dihydro-5-oxo-1,2,4-triazino-3-yl)anilines with diethyl 4-oxo-heptanediode. The synthesis of amides was conducted by amination 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.

Conclusions. The methods for the synthesis of esters and amides of 2,8-dioxo-3-R-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazolin-5a(6H)-carboxylic(propanoic) acids have been developed. The abovementioned transformations were conducted by alcoholysis of generated in situ acyl halides and amination of N-acylimidazolides, respectively. The more efficient approach for the synthesis of the target esters via condensation of 2-(6-R'-2,5-dihydro-5-oxo-1,2,4-triazino-3-yl)anilines with diethyl 4-oxoheptanediode has been proposed. It has been found that the highest radical scavenging and LOX-inhibiting activities are characteristic for hetarylpropanoic acids that contain an electron withdrawing substituent 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-5a(6H)-carboxylic(propanoic) acid; esters; amides; synthesis; radical scavenging activity; LOX-inhibiting activity

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Висновки. Разработано методы синтеза эстеров и амидов 2,8-диоксо-3-R-7,8-диgidр-2H-пирроло[1,2-а][1,2,4]-триазино[2,3-с]хиназолин-5а(6Н)-карбоновых (пропановых) кислот. Вычислены значения энергетического выхода синтеза в situ ацилглукозидов и аминолизом N-ацилиазидолоидов. Более эффективным методом синтеза эстеров является комплексная гетероциклизация 2-(6-R-2,5-диgidр-5-оксо-1,2,4-триазино-3-ил)анилинов с дигидр-4-оксегетероциклатом синтезированы эфиры соответствующих кислот.

результаты и их обсуждение. Реакцией этерификации 3-(2,8-диоксо-3-R-7,8-диgidр-2H-пирроло[1,2-а][1,2,4]-триазино[2,3-с]хиназолин-5а(6Н)-ил) пропановых кислот или тандемной гетероциклизацией 2-(6-R-2,5-диgidр-5-оксо-1,2,4-триазино-3-ил)анилинов с дигидр-4-оксегетероциклатом синтезированы эфиры соответствующих кислот. Синтез амидов проведен аминолизом активированных кислот, где в качестве активирующей компоненты использован 1,1'-карбонилидимидазол. В рамках исследования проведено изучение ЛОГ-ингибиторного и антирадикального действия как одного из возможных механизмов фармакокинетической активности.

Эффекты и их обсуждение. Реакция этерификации 3-(2,8-диоксо-3-R-7,8-диgidр-2H-пирроло[1,2-а][1,2,4]-триазино[2,3-с]хиназолин-5а(6Н)-ил) пропановых кислот или тандемной гетероциклизацией 2-(6-R-2,5-диgidр-5-оксо-1,2,4-триазино-3-ил)анилинов с дигидр-4-оксегетероциклатом синтезированы эфиры соответствующих кислот. Синтез амидов проведен аминолизом активированных кислот, где в качестве активирующей компоненты использован 1,1'-карбонилдимидазол. В рамках исследования проведено изучение ЛОГ-ингибиторного и антирадикального действия как одного из возможных механизмов фармакокинетической активности.

Экспериментальная часть. Синтетические процедуры были выполнены согласно общепринятым подходам. Результаты и их обсуждение. Реакция этерификации 3-(2,8-диоксо-3-R-7,8-диgidр-2H-пирроло[1,2-а][1,2,4]-триазино[2,3-с]хиназолин-5а(6Н)-ил) пропановых кислот или тандемной гетероциклизации 2-(6-R-2,5-диgidр-5-оксо-1,2,4-триазино-3-ил)анилинов с дигидр-4-оксегетероциклатом синтезированы эфиры соответствующих кислот. Синтез амидов проведен аминолизом активированных кислот, где в качестве активирующей компоненты использован 1,1'-карбонилдимидазол. В рамках исследования проведено изучение ЛОГ-ингибиторного и антирадикального действия как одного из возможных механизмов фармакокинетической активности.

Выводы. Разработаны методы синтеза сложных эфиров и амидов 2,8-диоксо-3-R-7,8-диgidр-2H-пирроло[1,2-а][1,2,4]-триазино[2,3-с]хиназолин-5а(6Н)-карбоновых (пропановых) кислот. Изучение ЛОГ-ингибиторной и антирадикальной активности веществ было проведено с использованием соевой липооксигеназы и 1,1'-карбонилдимидазола. Выводы. Разработаны методы синтеза сложных эфиров и амидов 2,8-диоксо-3-R-7,8-диgidр-2H-пирроло[1,2-а][1,2,4]-триазино[2,3-с]хиназолин-5а(6Н)-карбоновых (пропановых) кислот. Изучение ЛОГ-ингибиторной и антирадикальной активности веществ было проведено с использованием соевой липооксигеназы и 1,1'-карбонилдимидазола.
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-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazolin-5a(6H)-carboxylic(-propanoic) acids with the promising anti-inflammatory activity. However, one of the possible approaches for the chemical modification of the abovementioned compounds, 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-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazolin-5a(6H)-carboxylic(-propanoic) acids with the promising anti-inflammatory activity. The attempt of the synthesis of amide via the method mentioned above required anhydrous dioxane and refluxing for 3–4 hours (Scheme). The yields of amides were in the range of 30–73%. The attempt of the synthesis of amide via the reaction of esters with amines was not successful.

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

1H 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 (CH2-fragment) and triplets at 1.16–1.04 ppm (CH3-group). Signals of the ethylene moiety in the ethoxycarbonylalkyl fragment of esters

Results and discussion

Previously described [8] substituted 2,8-dioxo-3-R1-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazolin-5a(6H)-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-R1-2,5-dihydro-5-oxo-1,2,4-triazino-3-yl)amines 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 4 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 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.

Scheme

$R^1 = $Me, Ph, 4-F-C3H7; $R^2 = $H, F; $R^3 = $Ph, 4-MeO-C2H5, 4-F-C3H7, 4-In-Ph, 4-MeO-Bn, 4-F-Bn, 4-CF3-Bn; $n = 0, 2$
3.1–3.6 were observed as series of multiplets overlapping on the equatorial and axial protons of the pyrrolyl cycle at 2.93–2.58 ppm (H-7) and 2.47–2.06 ppm (H-6). Such complex splitting is probably associated with the presence of an asymmetric carbon atom.

1H 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 (4.1–4.15) 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 1H NMR-spectra of amides 4.1–4.3 the signals of the pyrrole cycle protons (7, 6) were observed as a wide high-field multiplet in the range of 3.24–2.64 ppm. In 1H 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) and 2.69–2.41 ppm (H-6)). At the same time, in 1H 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 (–CH2CH2CONH–), and 2.18–2.10 ppm (–CH2CH2CONH–).

In the 1H 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 5a. Introduction of one or two fluorine atoms to the heterocyclic fragment of compounds 3.4–3.6 caused the additional splitting [11]. Besides, in 1H NMR-spectra of compounds 3 and 4 the signals were caused by the nature of substituents in positions 3 and 5a [11].

The 13C NMR-spectrum of compound 3.2 additionally proved its structure. The characteristic were the signals of a carbon atom of position 5a, 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 amides 4.

## Table

| 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                      |
| 2.2    | 10.68  | 6.44                      |
| 2.3    | 11.02  | 5.62                      |
| 2.4    | 10.85  | 5.25                      |
| 2.5    | 35.76  | 8.31                      |
| 2.6    | 36.44  | 7.38                      |
| 3.1    | 5.90   | 2.90                      |
| 3.2    | 1.97   | 0.27                      |
| 3.3    | 0.87   | 0.00                      |
| 3.4    | 3.39   | 1.61                      |
| 3.5    | 10.47  | 1.87                      |
| 3.6    | 17.71  | 2.26                      |
| 4.1    | 8.87   | 3.23                      |
| 4.2    | 14.68  | 10.48                     |
| 4.3    | 13.55  | 8.06                      |
| 4.4    | 3.71   | 2.26                      |
| 4.5    | 8.23   | 3.23                      |
| 4.6    | 5.00   | 4.19                      |
| 4.7    | 16.29  | 5.16                      |
| 4.8    | 43.31  | 13.71                     |
| 4.9    | 2.90   | 2.26                      |
| 4.10   | 4.84   | 1.94                      |
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. 1H NMR-spectra (400 MHz) and 13C NMR (101 MHz) were recorded using a Varian-Mercury 400 spectrometer with TMS as an internal standard in DMSO-d6 solution. LC-MS spectra were recorded using the chromatography/mass spectrometric system consisting of an Agilent 1100 Series high-performed liquid chromatography/mass spectrometric system (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).

1H NMR (400 MHz, DMSO-d6, δ, ppm: 8.30 (1H, d, J = 7.6 Hz, H-10), 7.70 (1H, t, J = 7.7 Hz, H-11), 7.41 (1H, t, J = 7.5 Hz, H-12), 7.26 (2H, dd, J = 11.5 Hz, J2 = 6.1 Hz, -OCH2CH3), 2.96 – 2.53 (4H, m, H, H7, 5 eq, 6 eq -CH2CH2COOC6H5), 2.43 – 2.07 (7H, m, H6, 6 eq -CH2CH2COOC6H5), 1.16 (3H, t, J = 7.0 Hz, -OCH2CH3). LC-MS: m/z = 369 [M+1].

**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-2H-pyrrolo[1,2-α][1,2,4]triazino[2,3-c]quinazolin-5a-(6H)-yl)propanoate 3.1.** Yield – 52.4% (method A), 77.0% (method B). M. p. 137 – 139°C. Anal. Calcd. for C21H15N6O4, %: C 61.95, H 5.47, N 15.21. Found, %: C 61.99, H 5.56, N 15.29. 1H NMR (400 MHz, DMSO-d6, δ, ppm: 8.08 (1H, d, J = 7.8 Hz, H-13), 8.04 (1H, d, J = 8.1 Hz, H-10), 7.70 (1H, t, J = 7.7 Hz, H-11), 7.59 – 7.19 (4H, m, H-12, Ar-H-2,6), 8.08 (1H, d, J = 8.0 Hz, H-10), 7.72 (1H, t, J = 7.3 Hz, H-11), 7.27 – 6.83 (7H, m, H11, Ar-H-2,6, 3-Ar-H-2,6). LC-MS: m/z = 369 [M+1].

**Ethyl 3-(2,8-dioxo-3-phenyl-7,8-dihydro-2H-pyrrolo[1,2-α][1,2,4]triazino[2,3-c]quinazolin-5a-(6H)-yl)propanoate 3.2.** Yield – 51.0% (method A), 79.3% (method B). M. p. 230 – 232°C. Anal. Calcd. for C21H15N6O4, %: C 66.97, H 5.15, N 13.02. Found, %: C 67.06, H 5.21, N 13.09. 1H NMR (400 MHz, DMSO-d6, 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, Ar-H-3,4,5), 4.09 – 3.62 (2H, m, –OCH2CH3), 3.23 – 1.90 (8H, m, H, H7, 5 eq, 6 eq -CH2CH2COOC6H5), 1.04 (3H, t, J = 7.1 Hz, -OCH2CH3). 11C NMR (101 MHz, DMSO-d6, 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-2H-pyrrolo[1,2-α][1,2,4]triazino[2,3-c]quinazolin-5a-(6H)-yl)propanoate 3.3.** Yield – 54.0% (method A), 72.8% (method B). M. p. 214 – 216°C. Anal. Calcd. for C21H15N6O4, %: C 68.63, H 5.97, N 11.86. Found, %: C 68.72, H 6.05, N, 11.92. 1H NMR (400 MHz, DMSO-d6, 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, -OCH2CH3), 2.93 – 2.58 (5H, m, H, H7, 5 eq, 6 eq -CH2CH2COOC6H5), 2.43 – 2.07 (4H, m, H, H6, 6 eq -CH2CH2COOC6H5), 1.30 (6H, d, J = 6.9 Hz, -CH2CH3), 1.05 (3H, t, J = 7.1 Hz, -OCH2CH3). LC-MS: m/z = 473 [M+1].

**Ethyl 3-(12-fluoro-2,8-dioxo-3-phenyl-7,8-dihydro-2H-pyrrolo[1,2-α][1,2,4]triazino[2,3-c]quinazolin-5a-(6H)-yl)propanoate 3.4.** Yield – 60.0% (method A), 73.6% (method B). M. p. 184 – 186°C. Anal. Calcd. for C24H24FN3O6, %: C 64.28, H 4.72, N 12.49.
Found, %: C 64.34, H 4.81, N 12.54. 1H 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, –OCH$_2$CH$_3$), 3.18–2.56 (4H, m, H-7 _eq_ –CH$_2$COOC$_2$H$_5$), 2.44–2.11 (4H, m, H-6 _eq_, 6 _ax_, –CH$_2$COOC$_2$H$_5$). 1.06 (3H, t, J = 7.1 Hz, –OCH$_3$). LC-MS: m/z = 450 [M+1].

Ethyl 3-(11,12-difluoro-2,8-dioxo-3-phenyl-7,8-di hydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazolin-5a(6H)-yl)propanoate 3.5. Yield – 33.0% (method B). M. p. 175–177 °C. Anal. Calcd. for C$_{28}$H$_{28}$N$_8$O$_7$: C 59.47, H 3.88, N 11.57. Found, %: C 59.47, H 3.88, N 11.50. 1H NMR (400 MHz, DMSO-d$_6$), δ, ppm: 8.20 (2H, d, J = 7.8 Hz, J = 6.0 Hz, Ar-H-2,6), 8.14 (1H, t, J = 9.4 Hz, H-13), 8.03 (1H, dd, J = 11.2 Hz, J = 7.1 Hz, H-10), 7.19 (2H, t, J = 8.6 Hz, Ar-H-3,5), 3.95–3.78 (2H, m, –OCH$_2$), 2.96–2.57 (4H, m, H-7 _eq_ –CH$_2$COOC$_2$H$_5$). 2.46–2.08 (4H, m, H-6 _eq_, 6 _ax_, –CH$_2$COOC$_2$H$_5$). 1.07 (3H, t, J = 7.1 Hz, –OCH$_3$). LC-MS: m/z = 467 [M+1].

**The general method for synthesis of N$^3$-R$^1$-3$^R$-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazolin-5a(6H)-yl]carboxamides 4.1–4.2 and N$^3$-R$^1$-3(3$^R$)-2,8-dioxo-7,8 dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazolin-5a(6H)-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-’carbonyldimidazole (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-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c] quinazolin-5a(6H)-yl]carboxamide 4.1.** Yield – 30.0%. M. p. 212–215 °C. Anal. Calcd. for C$_{28}$H$_{28}$FN$_8$O$_7$: C 67.35, H 4.19, N 14.55. Found, %: C 67.41, H 4.24, N 14.61. 1H 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$_5$ = 8.3 Hz, J$_5$ = 5.4 Hz, 5a-Bn-H-2,6), 6.97 (2H, t, J = 8.5 Hz, 5a-Bn-H-3,5), 6.23 (1H, t, J = 6.0 Hz, –NHCH$_2$), 4.18 (2H, d, J = 5.8 Hz, –NHCH$_2$). 3.24–2.64 (4H, m, H-7 _eq_, 7 _ax_, 6 _eq_, 6 _ax_). LC-MS: m/z = 482 [M+1].
3-(3-Methyl-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazolin-5a(6H)-yl)-N-phenylpropanamide 4.6. Yield – 68.0%. M. p. 264–265°C. Anal. Calcld. for C_{25}H_{22}N_{4}O_{9}: %: C 64.76, H 4.84, N 14.13. Found: %: C 64.78, H 4.51, N 14.19. 'H NMR (400 MHz, DMSO-d_{6}), δ, ppm: 9.63 (1H, s, NH), 8.24 (1H, dd, J = 8.0 Hz, J = 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, 5a-Ar-H-2,6), 7.16 (2H, t, J = 7.7 Hz, 5a-Ar-Ph-H-3,5), 6.92 (1H, t, J = 7.4 Hz, 5a-Ar-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, –CH<sub>2</sub>CH<sub>2</sub>CONH–). LC-MS: m/z = 416 [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<sub>max</sub> = 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 and keep at 30 min for 25°C. Then measure the absorbance (A<sub>λ</sub>) [17]. Simultaneously determine the absorbance of 2 mL of 0.1 mM DPPH solution in 2 mM of methanol (A<sub>DPPH</sub>). Calculate the antiradical activity (ARA) by the following formula: ARA, %: (A<sub>DPPH</sub> – A<sub>λ</sub>)×100 %/A<sub>DPPH</sub>. 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 2·10<sup>-5</sup> w/v solution of LOX in buffer and 40 µL of 100 µM of the compound studied solution (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<sub>control</sub> – A<sub>test compound</sub>)×100 %/A<sub>control</sub>.

**Conclusions**

The methods for the synthesis of esters and amides of 2,8-dioxo-3-R<sup>1</sup>-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazolin-5a(6H)-carboxylic (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<sup>1</sup>-2,5-dihydro-5-oxo-1,2,4-
tri azino-3-yl]anilines with diethyl 4-oxoheptanedi oate 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.

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