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RESEARCH ARTICLE

Substituted 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 Acids and Their Salts – a Promising Class of Anti-inflammatory Agents

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Abstract: ***Background:*** Fragment-based drug design as well as *in silico* pre-screening of affinity to biological targets are among the most effective methods of medicinal chemistry. Abovementioned approaches were used for purposeful search of anti-inflammatory agents among quinazoline condensed derivatives.

Objective: Purposeful synthesis of novel 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 acids and their salts as promising anti-inflammatory agents, evaluation of their structure by physicochemical methods and establishing of their anti-inflammatory activity.

Method: The structures of target compounds were proposed due to principles of fragment-based drug design. The features of the synthesized compounds structures were evaluated by IR-, NMR spectroscopy and chromatography-mass spectrometry and were discussed in detail. Probable molecular mechanisms of activity were predicted due to molecular docking. The anti-inflammatory activity was determined by their ability to reduce the formalin- and carrageenan-induced paw edema in rats.

Results: It was found, that condensation of 3-(2-aminophenyl)-6-R-1,2,4-triazin-5(2*H*)ones with 2-oxoglutaric acid yielded 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 acids which may be considered as a promising anti-inflammatory agents. *In silico* study showed, that obtained compounds revealed affinity to the molecular targets and corresponded to the «drug-like» criteria. Additionally docking study allowed to estimate the nature of interactions between synthesized compounds and molecular targets. The *in vivo* experiments showed that obtained compounds demonstrated the significant anti-inflammatory activity comparable or higher than activity of the reference drug «Diclofenac».

Conclusion: The developed and implemented search strategy of the anti-inflammatory agents was justified. 3-R-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazoline5*a*(6*H*)carboxylic acids possessed the mentioned activity and additional introduction of fluorine atoms in position 11 or 12 of the heterocyclic system led to amplification of anti-inflammatory activity.

Keywords: anti-inflammatory activity, drug design, 3-(2-aminophenyl)-6-R-1,2,4-triazin-5(2*H*)ones, pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolines, molecular docking, SAR.

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1. INTRODUCTION

The inflammatory process is the important pathogenetic component of many diseases of various etiologies. Its correction is one of the most significant problems of the modern medicine. Pharmacocorrection of conditions accompanied by an inflammatory process involves the usage of glucocorticoids and nonsteroidal anti-inflammatory drugs (NSAIDs), and in cases of acute pain syndrome - opioid and non-opioid analgesics [1]. It should be noted, that in most cases, NSAIDs are the drug of choice. Although their usage is often accompanied by side effects such as gastrotoxicity, hepatotoxicity and nephrotoxicity. These effects are common to all representative of this drug class due to particularities of mechanism of action [2-4]. It should be noted, that despite a rather long history of effective antiflogistics search, Diclofenac sodium remained to be the «gold standard» of anti-inflammatory therapy [5]. This fact in most cases was associated with the permanence of the anti-inflammatory agents search strategy. Such, creation of substances that could inhibit the activity of cyclooxygenase, one of the key enzymes in the development of the inflammation process, was the base of the mentioned strategy.

The implementation of modern methods in medical chemistry, as well as the results of a more detailed study of the mechanisms of the inflammatory process have significantly changed approaches of innovative drugs creation [6, 7] and resulted the appearance of alternative molecular targets for potential anti-inflammatory agents (lipoxygenases, matrix metalloproteinases, PPAR-receptors, protein kinases C, phospholipase A2 etc.) [1]. Undoubtedly, this led the first experimental «non-classical» anti-inflammatory agents to be found [8].

Given the above, the objective of the present work was to develop a strategy for directed search of compounds with anti-inflammatory activity among substituted 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 acids and their salts. The design of the structure of these molecules (Fig. **1**) was carried out by combining in one molecule fragments, found in the well-known drugs. Namely, Ketorolac (COX1/COX2 inhibitor) [9], Licofelon (5-LOX/COX inhibitor) [8] and promising triazino[2,3-*c*]quinazolines derivatives [10, 11] which have expressed anti-inflammatory activity.

2. MATERIALS AND METHODS

2.1. Chemistry

Melting points were determined in open capillary tubes in a «Mettler Toledo MP 50» apparatus and were uncorrected. The elemental analyses (C, H, N) were performed using the ELEMENTAR vario EL cube analyzer (USA). Analyses were indicated by the symbols of the elements or functions within ±0.3% of the theoretical values. IR spectra (4000–600 cm^{-1}) were recorded on a Bruker ALPHA FT-IR spectrometer (Bruker Bioscience, Germany) using a module for measuring attenuated total reflection (ATR). ^{1}H NMR spectra (400 MHz) and $_{13}C$ NMR spectra (100 MHz) were recorded on a Varian-Mercury 400 (Varian Inc., Palo Alto, CA, USA) spectrometers with TMS as internal standard in DMSO-d_6 solution. LC-MS were recorded using chromatography/mass spectrometric system which consists of high performance liquid chromatography «Agilent 1100 Series» (Agilent, Palo Alto, CA, USA) equipped with diode-matrix and mass-selective detector «Agilent LC/MSD SL» (atmospheric pressure chemical ionization – APCI). Electron impact mass spectra (EI-MS)

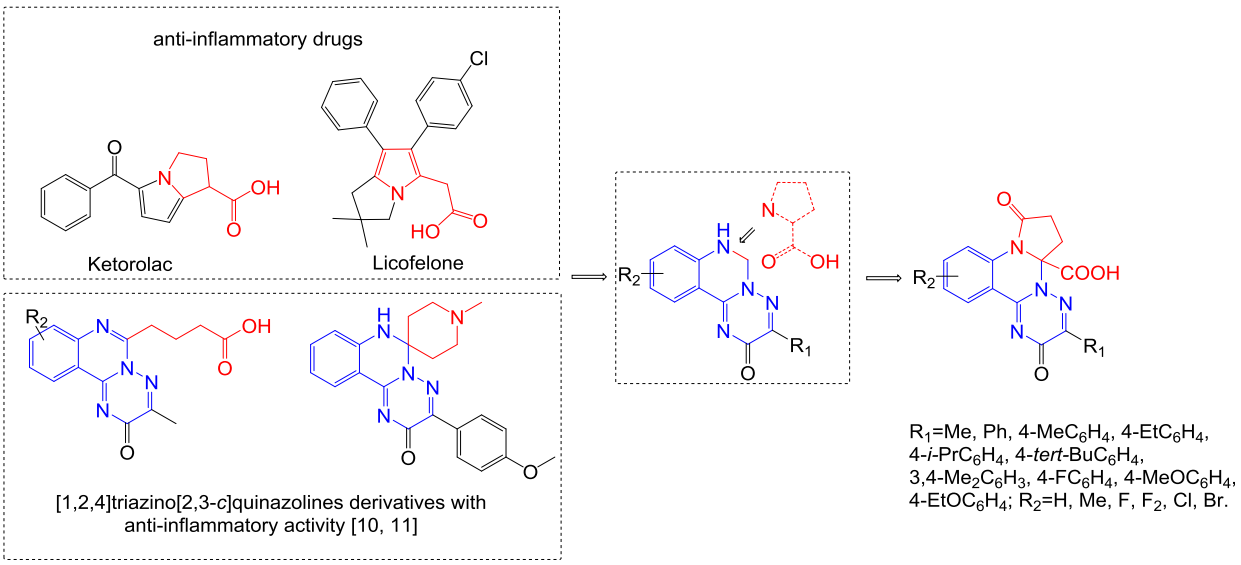

Fig. (1). The search strategy for compounds with anti-inflammatory activity, that include different «pharmacophore» fragments combination in one molecule.

were recorded on a Varian 1200 L instrument at 70 eV (Varian, USA). The purity of all obtained compounds was checked by ^{1}H-NMR and LC-MS.

Substances **1.1-1.24** were synthesized according to the reported procedures [12]. Other starting materials and solvents were obtained from commercially available sources and were used without additional purification.

2.2. 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 Diclofenac (PDB ID - 3N8Y), COX-2 in combination with Celecoxib (PDB ID - 3LN1) and phospholipase A2 (PLA2) in combination with Licofelone (PDB ID - 1ZYX) [13]. The choice of biological targets was due to the literature about the mechanism of anti-inflammatory drugs activity [1].

Ligand preparation. Substances were drawn using MarvinSketch 19.24 and saved in mol format [14]. 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 highly parameterized. Using AutoDockTools-1.5.6 pdb-files were converted into PDBQT, number of active torsions was set as default [15].

Protein preparation. PDB files were downloaded from the protein data bank. Discovery Studio v 19.1.0.18287 was used to delete water molecules and ligands. Structures of proteins were saved as pdb-files [16]. 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 [15]. For visualization Discovery Studio v 19.1.0.18287 was used.

2.3. Lipinski's rule of five.

Drug-like characteristics were evaluated and optimized using an electronic resource [17].

2.4. Anti-inflammatory activity.

Evaluation of anti-inflammatory activity of the synthesized compounds was conducted on 90 Wistar white rats (weight 150-160 g), obtained from the nursery «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» [18].

Screening of the synthesized compounds with estimated anti-inflammatory activity began with the study of their effect on exudative phase of acute aseptic inflammation («formalin» and «carrageenan» test) [19]. Phlogogen (1% aqueous solution of formaldehyde and 1% aqueous solution of λ-carrageenan) was subplantally injected in a dose of 0.1 ml in the rats' back right paw. The left one was used as a control. Intragastric administration of studied compounds was conducted using atraumatic probe as a water solution or as a dispersed suspension stabilized by Tween-80 1 hour before the injection of phlogogen. The compounds **2** were administrated in a dose of 25 mg/kg for carrageenan model and 10 mg/kg for formaline model, compounds 3 in dose 25 mg/kg for both models. 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 in 4 («carrageenan» test) or 3 («formalin» 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:

$$A = 100\% - \frac{(Vpe - Vhe)}{Vpc - Vhc}$$

where A - antiexudative activity, %; V_{pe} - the volume of paw edema in the experiment; V_{he} - the volume of healthy paw in the experiment; V_{pc} - the volume of paw edema in control; V_{hc} - the volume of healthy paw in control.

Statistical data processing was performed using a license program «STATISTICA® forWindows 10.0» (StatSoftInc., № AXXR712D833214FAN5) 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 $p<0.05$ [20].

2.5. The general procedure for the synthesis of substituted 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 acids (2.1-2.24).

To the suspension of 10 mM of substituted 3-(2-aminophenyl)-6-R-1,2,4-triazin-5(2*H*)one (**1.1-1.24**) in 20 ml of glacial acetic acid 1.74 g (10 mM) of 2-oxopentanedioic acid was added. The formed mixture was refluxed for 6 hours. The solvent was evaporated under vacuum. 10 ml of methanol was added to the residue and the formed

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mixture was shacked. The formed precipitate was filtered, washed by ether and dried.

3-Methyl-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4] triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.1**).

Yield: 83.3%; m.p. 232-234°C; IR (cm^{-1}): 2925, 2851, 1730, 1681, 1601, 1573, 1555, 1502, 1469, 1453, 1443, 1416, 1374, 1338, 1292, 1242, 1203, 1184, 1166, 1141, 1103, 1090, 1002, 969, 886, 823, 806, 771, 753, 718, 687, 656, 645, 617; ^{1}H NMR δ 13.39 (s, 1H, COOH), 8.31-8.14 (m, 2H, H-10,13), 7.72 (t, *J* = 7.1 Hz, 1H, H-11), 7.41 (t, *J* = 7.3 Hz, 1H, H-12), 3.10-2.68 (m, 4H, H-6,6`,7,7`), 2.30 (s, 3H, CH_3); ^{13}C NMR δ 171.7 (C-8), 169.2 (COOH), 161.4 (C-2), 152.7 (C-3), 151.6 (C-13b), 134.1 (C-11), 127.2 (C-9a), 125.3 (C-13), 119.4 (C-12), 118.0 (C-10), 95.5 (C-13a), 81.1 (C-5a), 29.7 (C-7), 27.0 (C-6), 17.1 (CH_3); EI-MS, m/z ($I\%_{rel.}$): 268 (9.2), 267 (57.4), 227 (27.6), 226 (100), 225 (6.6), 199 (13.9), 198 (43.1), 197 (7.5), 172 (13.2), 171 (9.7), 155 (43.7), 144 (5.1), 143 (9.5), 116 (7.7), 103 (6.3), 102 (31.8), 76 (8), 75 (9.8), 64 (6.2), 63 (5), 55 (11.3), 54 (6.5), 52 (5.2), 51 (6.4), 45 (5.7), 44 (10.2), 42 (8.6), 41 (5.4); LC-MS, *m/z* = 313 [M+l]; Anal. Calcd. for $C_{15}H_{12}N_4O_4$: C, 57.69; H, 3.87; N, 17.94; Found: C, 57.71; H, 3.91; N, 17.98.

2,8-Dioxo-3-phenyl-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.2**).

Yield: 95.3%; m.p. 243-245°C; IR (cm^{-1}): 3015, 2847, 1738, 1701, 1615, 1577, 1563, 1511, 1472, 1465, 1422, 1386, 1343, 1301, 1268, 1236, 1191, 1177, 1141, 1115, 1099, 1012, 977, 871, 823, 803, 763, 753, 731, 677, 656, 635, 621; ^{1}H NMR δ 8.32 (d, *J* = 7.7 Hz, 1H, H-13), 8.27 (d, *J* = 8.2 Hz, 1H, H-10), 8.22 (d, *J* = 6.3 Hz, 2H, 3-Ph H-2,6), 7.73 (t, *J* = 7.6 Hz, 1H, H-11), 7.54-7.34 (m, 4H, H-12, 3-Ph H-3,4,5), 3.25-3.11 (m, 1H, H-6), 3.01-2.86 (m, 2H, H-6,7), 2.83-2.70 (m, 1H, H-7); ^{13}C NMR δ 173.2 (C-8), 169.9 (COOH), 161.2 (C-2), 152.4 (C-3), 147.7 (C-13b), 135.4 (C-11), 134.9 (C-9a), 132.5 (3-Ar C-1), 131.2 (3-Ar C-4), 129.2 (3-Ar C-2,6), 128.7 (3-Ar C-3,5), 127.9 (C-13), 126.6 (C-12), 120.3 (C-10), 118.3 (C-13a), 82.4 (C-5a), 30.4 (C-7), 27.2 (C-6); EI-MS, m/z ($I\%_{rel.}$): 330 (19.2), 228 (26.9), 227 (100.0), 226 (25.3), 199 (72.3), 185 (57.9), 171 (78.3), 155 (58.1), 143 (19.5), 129 (49.7), 118 (12.3), 117 (10.8), 116 (8.7), 102 (40.8), 89 (23.8), 76 (37.3), 63 (11.2), 51 (8.5), 44 (8.3), 41 (5.4); LC-MS, *m/z* = 375 [M+l]; Anal. Calcd. for $C_{20}H_{14}N_4O_4$: C, 64.17; H, 3.77; N, 14.97; Found: C, 64.21; H, 3.80; N, 15.02.

10-Methyl-2,8-dioxo-3-phenyl-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.3**).

Yield: 83.0%; m.p. 245-246°C; ^{1}H NMR δ 8.21 (d, *J* = 7.0 Hz, 2H, 3-Ph H-2,6), 8.13 (d, *J* = 7.5 Hz, 1H, H-13), 7.59 (d, *J* = 7.3 Hz, 1H, H-11), 7.49-7.40 (m, 4H, H-12, 3-Ph H-3,4,5), 3.72-3.53 (m, 1H, H-6), 2.97-2.67 (m, 3H, H-6,7,7`), 2.41 (s, 3H, CH_3); ^{13}C NMR δ 174.0 (C-8), 169.5 (COOH), 161.5 (C-2), 153.0 (C-3), 147.1 (C-13b), 136.7 (C-11), 134.8 (C-9a), 133.5 (C-10), 132.8 (3-Ar C-1), 131.1 (3-Ar C-4), 129.3 (3-Ar C-2,6), 128.7 (3-Ar C-3,5), 128.4 (C-13), 125.2 (C-12), 122.4 (C-13a), 84.4 (C-5a), 28.8 (C-7), 25.7 (C-6), 18.5 (CH_3); LC-MS, *m/z* = 389 [M+l]; Anal. Calcd. for $C_{21}H_{16}N_4O_4$: C, 64.94; H, 4.15; N, 14.43; Found: C, 64.89; H, 4.08; N, 14.39.

11-Fluoro-2,8-dioxo-3-phenyl-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.4**).

Yield: 88.2%; m.p. 229-232°C; ^{1}H NMR δ 8.37 (t, *J* = 7.4 Hz, 1H, H-13), 8.20 (d, *J* = 7.3 Hz, 2H, 3-Ph H-2,6), 8.05 (d, *J* = 9.2 Hz, 1H, H-10), 7.54-7.40 (m, 3H, 3-Ph H-3,4,5), 7.21 (t, *J* = 7.3 Hz, 1H, H-12), 3.24-3.09 (m, 1H, H-6), 3.02-2.86 (m, 2H, H-6,7), 2.85-2.72 (m, 1H, H-7); LC-MS, m/z = 393 [M+l]; Calcd. for $C_{20}H_{13}FN_4O_4$: C, 61.23; H, 3.34; N, 14.28 Found: C, 61.25; H, 3.37; N, 14.31.

12-Methyl-2,8-dioxo-3-phenyl-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.5**).

Yield: 99.2%; m.p. 260-262°C; ^{1}H NMR δ 8.20 (d, *J* =7.3 Hz, 2H, 3-Ph H-2,6), 8.17-8.07 (m, 2H, H-10,13), 7.52 (d, *J* = 8.6 Hz, 1H, H-11), 7.49-7.37 (m, 3H, 3-Ph H-3,4,5), 3.21-3.09 (m, 1H, H-6), 2.98-2.83 (m, 2H, H-6,7), 2.81-2.66 (m, 1H, H-7), 2.46 (s, 3H, CH_3); ^{13}C NMR δ 173.1 (C-8), 170.1 (COOH), 161.2 (C-2), 152.4 (C-3), 147.6 (C-13b), 136.2 (C-9a), 136.2 (C-11), 132.7 (C-12), 132.6 (3-Ar C-1), 131.2 (3-Ar C-4), 129.2 (3-Ar C-2,6), 128.7 (3-Ar C-3,5), 127.7 (C-13), 120.2 (C-10), 118.2 (C-13a), 82.5 (C-5a), 30.4 (C-7), 27.2 (C-6), 20.97 (CH_3); LC-MS, *m/z* = 389 [M+l]; Anal. Calcd. for $C_{21}H_{16}N_4O_4$: C, 64.94; H, 4.15; N, 14.43; Found: C, 64.89; H, 4.09; N, 14.38.

12-Fluoro-2,8-dioxo-3-phenyl-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.6**).

Yield: 88.2%; m.p. 229-232°C; ^{1}H NMR δ 8.37 (t, *J* = 7.6 Hz, 1H, H-13), 8.20 (d, *J* = 7.3 Hz, 2H, 3-Ph H-2,6), 8.05 (d, *J* = 9.2 Hz, 1H, H-10), 7.54-7.40 (m, 3H, 3-Ph H-3,4,5), 7.21 (t, *J* = 7.3 Hz, 1H, H-11), 3.24-3.09 (m, 1H, H-6), 3.02-2.86 (m, 2H, H-6,7), 2.85-2.72 (m, 1H, H-7); LC-MS, *m/z* = 393 [M+l]; Anal. Calcd. for $C_{20}H_{13}FN_4O_4$: C, 61.23; H, 3.34; N, 14.28; Found: C, 61.28; H, 3.39; N, 14.35.

12-Chloro-2,8-dioxo-3-phenyl-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.7**).

Yield: 94.4%; m.p. 248-250°C; ^{1}H NMR δ 8.30-8.16 (m, 4H, H-10,13, 3-Ph H-2,6), 7.71 (d, *J* = 8.5 Hz, 1H, H-11), 7.51-7.36 (m, 3H, 3-Ph H-3,4,5), 3.20-3.11 (m, 1H, H-6), 3.01-2.86 (m, 2H, H-6,7), 2.82-2.71 (m, 1H, H-7); LC-MS, *m/z* = 409 [M+l]; Anal. Calcd. for $C_{20}H_{13}ClN_4O_4$: C, 58.76; H, 3.21; N, 13.71; Found: C, 58.81; H, 3.27; N, 13.77.

12-Bromo-2,8-dioxo-3-phenyl-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.8**).

Yield: 94.4%; m.p. 248-250°C; ^{1}H NMR δ 8.31-8.15 (m, 4H, H-10,13, 3-Ph H-2,6), 7.61 (d, *J* = 8.5 Hz, 1H, H-11), 7.51-7.36 (m, 3H, 3-Ph H-3,4,5), 3.20-3.11 (m, 1H, H-6), 3.01-2.86 (m, 2H, H-6,7), 2.82-2.64 (m, 1H, H-7); LC-MS, *m/z* = 453 [M+l]; Anal. Calcd. for $C_{20}H_{13}BrN_4O_4$: C, 53.00; H, 2.89; N, 12.36; Found: C, 53.06; H, 3.06; N, 13.42.

11,12-Difluoro-2,8-dioxo-3-phenyl-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.9**).

Yield: 88.2%; m.p. 229-232°C; ^{1}H NMR δ 8.37 (t, *J* = 7.4 Hz, 1H, H-13), 8.20 (d, *J* = 7.3 Hz, 2H, 3-Ph H-2,6), 8.05 (d, *J* = 9.2 Hz, 1H, H-10), 7.54-7.40 (m, 3H, 3-Ph H-3,4,5), 7.21 (t, *J* = 7.3 Hz, 1H, H-12), 3.24-3.09 (m, 1H, H-6), 3.02-2.86 (m, 2H, H-6,7), 2.85-2.72 (m, 1H, H-7); LC-MS, *m/z* = 411 [M+l]; Anal. Calcd. for $C_{20}H_{12}F_2N_4O_4$: C, 58.54; H, 2.95; N, 13.65; Found: C, 58.62; H, 3.07; N, 13.76.

2,8-Dioxo-3-(4-tolyl)-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.10**).

Yield: 91.6%; m.p. 248-252°C; ^{1}H NMR δ 8.31 (d, *J* = 7.8 Hz, 1H, H-13), 8.27 (d, *J* = 8.1 Hz, 1H, H-10), 8.13 (d, *J* = 7.7 Hz, 2H, 3-Ph H-2,6), 7.73 (t, *J* = 7.5 Hz, 1H, H-11), 7.43 (t, *J* = 7.4 Hz, 1H, H-12), 7.24 (d, *J* = 7.7 Hz, 2H, 3-Ph H-3,5), 3.20-3.10 (m, 1H, H-6), 3.01-2.83 (m, 2H, H-6,7), 2.82-2.70 (m, 1H, H-7), 2.42 (s, 3H, CH_3); LC-MS, *m/z* = 389 [M+l]; Anal. Calcd. for $C_{21}H_{16}N_4O_4$: C, 64.94; H, 4.15; N, 14.43; Found: C, 64.98; H, 4.19; N, 14.49.

3-(4-Ethylphenyl)-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.11**).

Yield: 88.7%; m.p. 248-252°C; ^{1}H NMR δ 8.30 (d, *J* = 7.8 Hz, 1H, H-13), 8.27 (d, *J* = 8.2 Hz, 1H, H-10), 8.15 (d, *J* = 7.9 Hz, 2H, 3-Ph H-2,6), 7.72 (t, *J* = 7.6 Hz, 1H, H-11), 7.42 (t, *J* = 7.5 Hz, 1H, H-12), 7.26 (d, *J* = 7.9 Hz, 2H, 3-Ph H-3,5), 3.23-3.09 (m, 1H, H-6), 3.01-2.84 (m, 2H, H-6,7), 2.84-2.74 (m, 1H, H-7), 2.70 (dd, *J* = 15.0, 7.4 Hz, 2H, -C$\underline{H}_2$CH$_3$), 1.28 (t, *J* = 7.5 Hz, 3H, -CH$_2$C$\underline{H}_3$); LC-MS, *m/z* = 403 [M+l]; Anal. Calcd. for $C_{22}H_{18}N_4O_4$: C, 65.66; H, 4.51; N, 13.92; Found: C, 65.70; H, 4.56; N, 13.98.

3-(4-(i-Propyl)phenyl)-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.12**).

Yield: 92.9%; m.p. 243-248°C; ^{1}H NMR δ 8.30 (d, *J* = 7.7 Hz, 1H, H-13), 8.26 (d, *J* = 7.9 Hz, 1H, H-10), 8.15 (d, *J* = 7.7 Hz, 2H, 3-Ph H-2,6), 7.76 (t, *J* = 7.2 Hz, 1H, H-11), 7.45 (t, *J* = 7.0 Hz, 1H, H-12), 7.33 (d, *J* = 7.5 Hz, 1H, 3-Ph H-3,5), 3.28-3.11 (m, 1H, H-6), 3.07-2.85 (m, 3H, H-6,7, -C$\underline{H}$(CH$_3$)$_2$), 2.80 (m, 1H, H-7), 1.30 (d, *J* = 6.4 Hz, 6H, -CH(C$\underline{H}_3$)$_2$); ^{13}C NMR δ 173.2 (C-8), 170.00 (COOH), 161.3 (C-2), 152.3 (C-3), 151.9 (3-Ar C-4), 147.7 (C-13b), 135.5 (C-11), 134.9 (C-9a), 130.1 (3-Ar C-1), 129.3 (3-Ar C-2,6), 127.8 (C-13), 126.7 (3-Ar C-3,5), 126.5 (C-2), 120.3 (C-10), 118.4 (C-13a), 82.4 (C-5a), 33.9 (-$\underline{C}$H(CH$_3$)$_2$) 30.4 (C-7), 27.3 (C-6), 24.1 (CH(-$\underline{C}$H$_3$)$_2$); LC-MS, *m/z* = 417 [M+l]; Anal. Calcd. for $C_{23}H_{20}N_4O_4$: C, 66.34; H, 4.84; N, 13.45; Found: C, 66.38; H, 4.89; N, 13.47.

3-(4-(t-Butyl)phenyl)-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.13**).

Yield: 81.8%; m.p. 241-242°C; ^{1}H NMR δ 8.31 (d, *J* = 7.8 Hz, 1H, H-13), 8.27 (d, *J* = 8.2 Hz, 1H, H-10), 8.16 (d, *J* = 8.1 Hz, 2H, 3-Ph H-2,6), 7.72 (t, *J* = 7.6 Hz, 1H, H-11), 7.51-7.35 (m, 3H, H-12, 3-Ph H-3,5), 3.29-3.06 (m, 1H, H-6), 3.02-2.84 (m, 2H, H-6,7), 2.84-2.70 (m, 1H, H-7), 1.37 (s, 9H, -C(C$\underline{H}_3$)$_3$); ^{13}C NMR δ 173.2 (C-8), 170.0 (COOH), 161.3 (C-2), 154.1 (3-Ar C-4), 152.3 (C-2), 147.7 (C-13b), 135.4 (C-11), 134.9 (C-9a), 129.8 (3-Ar C-1), 129.1 (3-Ar C-2,6), 127.8 (C-13), 126.6 (C-12), 125.6 (3-Ar C-3,5), 120.3 (C-10), 118.4 (C-13a), 82.4 (C-5a), 35.1 (-$\underline{C}$(CH$_3$)$_3$), 31.4 (-C(-$\underline{C}$H$_3$)$_3$), 30.4 (C-7), 27.3 (C-6); LC-MS, *m/z* = 431 [M+l]; Anal. Calcd. for $C_{24}H_{22}N_4O_4$: C, 66.97; H, 5.15; N, 13.02; Found: C, 67.03; H, 5.18; N, 13.07.

3-(3,4-Dimethylphenyl)-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.14**).

Yield: 93.1%; m.p. 245-246°C; ^{1}H NMR δ 8.30 (d, *J* = 7.8 Hz, 1H, H-13), 8.27 (d, *J* = 8.3 Hz, 1H, H-10), 7.96 (s, 1H, 3-Ph H-2), 7.94 (d, *J* = 8.6 Hz, 1H, 3-Ph H-6), 7.72 (t, *J* = 7.5 Hz, 1H, H-11), 7.42 (t, *J* = 7.3 Hz, 1H, H-12), 7.16 (d, *J* = 7.6 Hz, 1H, 3 Ph H-6), 3.14 (m, 1H, H-6), 3.02-2.83 (m, 2H, H-6,7), 2.83-2.70 (m, 1H, H-7), 2.32 (s, 3H, 4-CH$_3$), 2.29 (s, 3H, 3-CH$_3$); LC-MS, m/z = 403 [M+l]; Anal. Calcd. for $C_{22}H_{18}N_4O_4$: C, 65.66; H, 4.51; N, 13.92; Found: C, 65.67; H, 4.53; N, 13.93.

3-(4-Fluorophenyl)-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.15**).

Yield: 91.8%; m.p. 253-254°C; ^{1}H NMR δ 8.36-8.28 (m, 3H, H-13, 3-Ph H-2,6), 8.26 (d, *J* = 8.3 Hz, 1H, H-10), 7.73 (t, *J* = 7.6 Hz, 1H, H-11), 7.42 (*t*, *J* = 7.5 Hz, 1H, H-12), 7.19 (t, *J* = 8.1 Hz, 2H, 3-Ph H-3,5), 3.25-3.14 (m, 1H, H-6), 3.01-2.84 (m, 2H, H-6,7), 2.84-2.71 (m, 1H, H-7); ^{13}C NMR δ 173.2 (C-8), 170.0 (COOH), 164.1 (d, *J* = 249.3 Hz, 3-Ar C-4), 161.2 (C-2), 152.4 (C-3), 146.6 (C-13b), 135.5 (C-11), 135.00 (C-9a), 131.7 (d, *J* = 8.7 Hz, 3-Ar C-2,6), 128.9 (d, *J* = 2.8 Hz, 3-Ar C-1), 127.9 (C-13), 126.6 (C-12), 120.3 (C-10), 118.3 (C-13a), 115.8 (d, *J* = 21.6 Hz, 3-Ar C-3,5), 82.5 (C-5a), 30.4 (C-7), 27.2 (C-6); LC-MS, *m/z* = 393 [M+l]; Anal. Calcd. for $C_{20}H_{13}FN_4O_4$: C, 61.23; H, 3.34; N, 14.28; Found: C, 61.29; H, 3.38; N, 14.37.

11-Fluoro-3-(4-fluorophenyl)-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.16**).

Yield: 83.3%; m.p. 237-240°C; ^{1}H NMR δ 8.36 (dd, *J* = 8.2, 6.6 Hz, 1H, H-13), 8.31 (dd, *J* = 7.6, 5.9 Hz, 2H, 3-Ph H-2,6), 8.04 (d, *J* = 9.2 Hz, 1H, H-10), 7.27-7.13 (m, 3H, H-12, 3-Ph H-3,5), 3.26-3.09 (m, 1H, H-6), 3.04-2.86 (m, 2H, H-6,7), 2.86-2.72 (m, 1H, H-7); ^{13}C NMR δ 173.4 (C-8), 169.7 (COOH), 166.1 (d, *J* = 253.2 Hz, C-11), 164.1 (d, *J* = 249.4 Hz, 3-Ar C-4), 161.0 (C-2), 151.7 (C-3), 146.7 (C-13b), 136.8 (d, *J* = 12.7 Hz, C-9a), 131.7 (d, *J* = 8.7 Hz, 3-Ar C-2,6), 130.9 (d, *J* = 10.7 Hz, C-13), 129.8-127.9 (m, 3-Ar C-1), 115.8 (d, *J* = 21.7 Hz, 3-Ar C-3,5), 114.8-114.6 (m, C-13a), 114.3 (d, *J* = 22.9 Hz, C-12), 107.2 (d, *J* = 27.7 Hz, C-13), 82.4 (C-5a), 30.4 (C-7), 27.3 (C-6); LC-MS, *m/z* = 411 [M+l]; Anal. Calcd. for $C_{20}H_{12}F_2N_4O_4$: C, 58.54; H, 2.95; N, 13.65; Found: C, 58.58; H, 3.02; N, 13.71.

12-Fluoro-3-(4-fluorophenyl)-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.17**).

Yield: 91.1%; m.p. 248-250°C; ^{1}H NMR δ 8.31 (dd, *J* = 7.8, 6.0 Hz, 2H, 3-Ph H-2,6), 8.27 (dd, *J* = 9.0, 4.7 Hz, 1H, H-13), 8.01-7.79 (m, 1H, H-10), 7.53 (t, *J* = 7.0 Hz, 1H, H-11), 7.20 (t, *J* = 8.5 Hz, 2H, 3-Ph H-3,5), 3.28-3.10 (m, 1H, H-6), 3.04-2.85 (m, 2H, H-7,7`), 2.85-2.69 (m, 1H, H-6); LC-MS, *m/z* = 411 [M+l]; Anal. Calcd. For $C_{20}H_{12}F_2N_4O_4$: C, 58.54; H, 2.95; N, 13.65; Found: C, 58.59; H, 3.01; N, 13.72.

11,12-Difluoro-3-(4-fluorophenyl)-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.18**).

Yield: 57.6%; m.p. 238-241°C; ^{1}H NMR δ 12.06 (s, 1H, COOH), 8.35-8.27 (t, *J* = 7.6 Hz, 2H, 3-Ph H-2,6), 8.24-8.08 (m, 2H, H-10,13), 7.20 (t, *J* = 7.8 Hz, 2H, 3-Ph H-3,5), 3.23-3.07 (m, 1H, H-6), 3.01-2.87 (m, 2H, H-6,7), 2.86-2.71 (m, 1H, H-7); LC-MS, *m/z* = 428 [M+l]; Anal. Calcd. for $C_{20}H_{11}F_3N_4O_4$: C, 56.08; H, 2.59; N, 13.08; Found: C, 56.13; H, 2.64; N, 13.14.

3-(4-Methoxyphenyl)-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.19**).

Yield: 83.0%; m.p. 247-248°C; ^{1}H NMR δ 8.31 (d, *J* = 7.6 Hz, 1H, H-13), 8.29-8.18 (m, 3H, H-10, 3-Ph H-2,6), 7.72 (t, *J* = 7.6 Hz, 1H, H-11), 7.43 (t, *J* = 7.6 Hz, 1H, H-12), 6.97 (d, *J* = 7.2 Hz, 2H, 3-Ph H-3,5), 3.87 (s, 3H, OCH_3), 3.25-3.09 (m, 1H, H-6), 2.99-2.86 (m, 2H, H-6,7), 2.86-2.70 (m, 1H, H-7); LC-MS, *m/z* = 405 [M+l]; Anal. Calcd. for $C_{21}H_{16}N_4O_5$: C, 62.37; H, 3.99; N, 13.86; Found: C, 62.41; H, 4.06; N, 13.91.

3-(4-Methoxyphenyl)-10-methyl-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.20**).

Yield: 48.8%; m.p. 253-255°C; ^{1}H NMR δ 8.24 (d, *J* = 8.6 Hz, 2H, 3-Ph H-2,6), 8.10 (d, *J* = 7.4 Hz, 1H, H-13), 7.56 (d, *J* = 7.2 Hz, 1H, H-11), 7.43 (t, *J* = 7.6 Hz, 1H, H-12), 6.95 (d, *J* = 8.6 Hz, 2H, 3-Ph H-3,5), 3.85 (s, 3H, OCH_3), 3.72-3.44 (m, 1H, H-6), 3.09-2.63 (m, 3H, H-6,7,7`), 2.39 (s, 3H, CH_3); LC-MS, *m/z* = 419 [M+l]; Anal. Calcd. for $C_{22}H_{18}N_4O_5$: C, 63.15; H, 4.34; N, 13.39; Found: C, 63.21; H, 4.39; N, 13.43.

12-Chloro-3-(4-methoxyphenyl)-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.21**).

Yield: 79.9%; m.p. 225-227°C; ^{1}H NMR δ 11.87 (s, 1H, COOH), 8.28-8.20 (m, 4H, H-10,13, 3-Ph H-2,6), 7.70 (d, *J* = 8.8 Hz, 1H, H-11), 6.95 (d, *J* = 8.5 Hz, 2H, 3-Ph H-3,5), 3.85 (s, 3H, OCH_3), 3.22-3.05 (m, 1H, H-6), 3.04-2.85 (m, 2H, H-6,7), 2.85-2.68 (m, 1H, H-7); LC-MS, *m/z* = 439 [M+l]; Anal. Calcd. for $C_{21}H_{15}ClN_4O_5$: C, 57.48; H, 3.45; N, 12.77; Found: C, 57.53; H, 3.49; N, 12.81.

12-Bromo-3-(4-methoxyphenyl)-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.22**).

Yield: 99.4%; m.p. 230-232°C; ^{1}H NMR δ 11.90 (s, 1H, COOH), 8.36 (s, 1H, H-13), 8.24 (d, *J* = 8.5 Hz, 2H, 3-Ph H-2,6), 8.19 (d, *J* = 8.7 Hz, 1H, H-10), 7.83 (d, *J* = 8.7 Hz, 1H, H-11), 6.95 (d, *J* = 8.4 Hz, 2H, 3-Ph H-3,5), 3.85 (s, 1H, OCH_3), 3.25-3.05 (m, 1H, H-6), 3.00-2.85 (m, 2H, H-6,7), 2.82-2.70 (m, 1H, H-7); LC-MS, *m/z* = 484 [M+l]; Anal. Calcd. for $C_{21}H_{15}BrN_4O_5$: C, 52.19; H, 3.13; N, 11.59; Found: C, 52.22; H, 3.17; N, 11.64.

3-(4-Ethoxyphenyl)-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.23***).

Yield: 76.3%; m.p. 252-254°C; ^{1}H NMR δ 8.30 (d, *J* = 7.8 Hz, 1H, H-13), 8.28-8.15 (m, 3H, H-10, 3-Ph H-2,6), 7.72 (t, *J* = 7.4 Hz, 1H, H-11), 7.42 (t, *J* = 7.3 Hz, 1H, H-12), 6.93 (d, *J* = 7.3 Hz, 2H, 3-Ph H-3,5), 4.10 (d, *J* = 6.7 Hz, 2H, $O\underline{CH_2}CH_3$), 3.26-3.10 (m, 1H, H-6), 3.02-2.85 (m, 2H, H-6,7), 2.84-2.68 (m, 1H, H-7), 1.43 (t, *J* = 6.7 Hz, 3H, -$OCH_2C\underline{H_3}$); LC-MS, m/z = 419 [M+l]; Anal. Calcd. for $C_{22}H_{18}N_4O_5$: C, 63.15; H, 4.34; N, 13.39; Found: C, 63.19; H, 4.37; N, 13.43.

2,8-Dioxo-3-(thienyl-2-yl)-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)carboxylic acid (**2.24**)

Yield: 93.9%; m.p. 259-260°C; ^{1}H NMR δ 8.32 (d, *J* = 7.8 Hz, 1H, H-13), 8.30-8.23 (m, 2H, H-10, 3-thienyl H-3), 7.73 (t, *J* = 7.7 Hz, 1H, H-H-11), 7.69 (d, *J* = 4.7 Hz, 1H, 3-thienyl H-5), 7.43 (t, *J* = 7.5 Hz, 1H, H-12), 7.18 (t, *J* = 7.6 Hz, 1H, 3-thienyl H-4), 3.22-3.07 (m, 1H, H-6), 3.02-2.86 (m, 2H, H-6,7), 2.77 (m, 1H, H-7); ^{13}C NMR δ 173.2 (C-8), 169.9 (COOH), 160.0 (C-2), 152.0 (C-3), 143.8 (C-13b), 135.4 (C-11), 134.8 (C-9a), 133.9 (thienyl C-2), 132.9 (thienyl C-5), 131.6 (thienyl C-3), 128.4 (thienyl C-4), 127.8 (C-13), 126.5 (C-12), 120.3 (C-10), 118.3 (C-13a), 82.5 (C-5a), 30.4 (C-7), 27.2 (C-6); LC-MS, *m/z* = 381 [M+l]; Anal. Calcd. for $C_{18}H_{12}N_4O_4S$: C, 56.84; H, 3.18; N, 14.73; S, 8.43; Found: C, 56.87; H, 3.23; N, 14.78; S, 8.46.

Synthesized compounds (**2.1-2.24**) are white or light-yellow crystal substances, soluble in DMF, DMSO, dioxane and alcohols, insoluble in water.

2.6. The general procedure for the synthesis of potassium 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*)carboxylats (3.1).

10 mM of corresponding acid (**2.1**) and 0.56 g (10 mM) of potassium hydroxide in 15 ml of water was refluxed until precipitate dissolved. The formed solution was cooled and filtered. The water was evaporated under vacuum. The obtained residue was crystallized from ethanol.

Potassium 3-methyl-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)-carboxylate (**3.1**).

Yield: 88.6%; m.p. > 280°C; IR (cm^{-1}): 1719, 1630, 1505, 1484, 1350, 1238, 778, 648; Anal. Calcd. for $C_{15}H_{11}KN_4O_4$: C, 51.42; H, 3.16; N, 15.99; Found: C, 51.63; H, 3.32; N, 16.17.

2.7. The general procedure for the synthesis of ammonium 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*)carboxylats (3.2-3.4).

To the suspension of 10 mM of corresponding carboxylic acid (**2.1**, **2.2**) in 10 ml of ethyl alcohol 10 mM of corresponding amine was added (monoethanolamine, morpholine and piperidine). The formed mixture was refluxed until the precipitate dissolved. The formed solution was cooled and filtered. The water was evaporated under vacuum. The obtained residue was crystallized from ethanol.

2-Hydroxyethan-1-aminium 3-methyl-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)-carboxylate (**3.2**).

Yield: 78.4%; m.p. > 280°C; IR (cm^{-1}): 1711, 1621, 1587, 1502, 1481, 1346, 1234, 821, 772, 759, 649; Anal. Calcd. for $C_{17}H_{19}N_5O_5$: C, 54.69; H, 5.13; N, 18.76; Found: C, 54.84; H, 5.23; N, 18.88.

Morpholin-4-ium 2,8-dioxo-3-phenyl-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)-carboxylate (**3.3**).

Yield: 74.3%; m.p. > 280°C; IR (cm^{-1}): 1712, 1642, 1505, 1475, 1341, 1236, 1183, 1108, 1032, 877, 775, 754, 723, 648; Anal. Calcd. for $C_{24}H_{23}N_5O_5$: C, 62.47; H, 5.02; N, 15.18; Found: 62.56; H, 5.22; N, 15.27.

Piperidin-1-ium 3-methyl-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)-carboxylate (**3.4**).

Yield: 72.1%; m.p. > 280°C; IR (cm^{-1}): 1714, 1645, 1592, 1505, 1483, 1351, 1227, 772, 756, 719; Anal. Calcd. for $C_{20}H_{23}N_5O_4$: C, 60.44; H, 5.83; N, 17.62; Found: C, 60.65; H, 6.01; N, 17.82.

Synthesized compounds (**3.1**-**3.4**) are white crystal substances, soluble in water, ethanol, slightly soluble in dioxane, DMF, DMSO.

3. RESULTS AND DISCUSSION

3.1. Molecular docking.

At the first stage of the study the combinatorial library was created and analyzed using molecular docking [21, 22]. That was done, considering the important role of eicosanoids as mediators of inflammation. Besides important fact is that the enzymes involved in eicosanoids biosynthesis are the main molecular targets. So, their inhibitors are the basis for the development of anti-inflammatory drugs. Cyclooxygenases (COX-1 and COX-2) [1] and phospholipase A2 were selected as the targets for molecular docking among other enzymes [23].

The results of molecular docking showed, that substituted pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolines exhibited significant affinity towards molecular targets (Table **1**). It should be noted, that the indicated affinity was slightly higher for COX-1 and phospholipase A2. It is important to note, that halogen atom in the pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolines, especially fluorine, led to increased affinity for enzymes (compounds **2.4**, **2.6**, **2.9**, **2.16-2.18**). This could be explained due to the fact, that halogens could form additional donor-acceptor bonds with substrates [24].

Table 1. The results of synthesized compounds' molecular docking.

Compd.	Affinity (kcal/mol) to COX-1 (PDB ID - 3N8Y)	Affinity (kcal/mol) to COX-2 (PDB ID 3LN1)	Affinity (kcal/mol) to PLA2 (PDB ID 1ZYX)
Diclofenac	-8.5	–	–
Celecoxib	–	-10.1	–
Licofelone	–	–	-7.9
2.1	-7.1	-6.7	-7.7
2.2	-8.4	-7.5	-9.0
2.3	-8.8	-7.4	-8.9
2.4	-9.2	-7.5	-8.9
2.5	-8.8	-7.1	-8.6
2.6	-8.1	-7.2	-8.7
2.7	-8.1	-7.2	-9.2
2.8	-8.7	-6.9	-9.3
2.9	-8.6	-7.1	-9.0
2.10	-8.5	-7.5	-9.0
2.11	-7.8	-7.6	-8.8
2.12	-8.6	-7.9	-9.0
2.13	-8.1	-7.5	-9.3

(Table 1) contd….

Compd.	Affinity (kcal/mol) to COX-1 (PDB ID - 3N8Y)	Affinity (kcal/mol) to COX-2 (PDB ID 3LN1)	Affinity (kcal/mol) to PLA2 (PDB ID 1ZYX)
2.14	-7.9	-7.5	-8.5
2.15	-8.3	-7.4	-8.5
2.16	-9.0	-7.8	-9.3
2.17	-9.9	-7.2	-9.3
2.18	-8.9	-7.8	-9.3
2.19	-8.9	-7.5	-8.5
2.20	-8.3	-7.8	-9.2
2.21	-8.5	-7.7	-8.2
2.22	-8.9	-7.5	-8.6
2.23	-8.5	-7.5	-8.6
2.24	-8.1	-6.9	-8.1

These facts, as well as the structural features of pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolines, served as the basis for a more detailed determination of the main types of interactions with amino acid residues of enzymes.

Analysis of the main types of interactions of compounds **2** and standard drugs with amino acid residues of enzymes showed a large number of hydrogen bonds, halogen and hydrophobic interactions (Table **2**).

Table 2. The main types of interactions of the most active compounds and pharmacological standards with amino acid residues of enzymes, according to the docking studies.

Compd.	COX-1, PDB ID - 3N8Y	COX-2, PDB ID - 3LN1	PLA2, PDB ID - 1ZYX
2.2	GLN192[a], PRO514[b].	ALA513[b], SER516[a], GLY512[a], VAL335[b], VAL509[b], ALA513[b], LEU517[b], ALA513[b], ALA502[b].	GLY30[a], PHE5[b], CYS45[b], LYS69[b].
2.4	GLN192[a], GLN351[a], GLY354[a], PRO514[c], ASN515[c].	HIS337[a], HIS337[a], GLY340[a], ASN567[c], VAL568[c], GLN178[b], GLY179[b], ALA502[b].	HIS48[a], TRP31[a], TYR22[b], SER23[b], ALA18[b].
2.6	GLN351[a].	HIS337[a], GLN178[a], GLY340[a], ALA502[b].	TRP31[a], ASP49[c], PHE5[b], TYR52[b], ALA18[b].
2.12	GLN192[a], GLN351[a], GLN192[b], GLY193[b], PRO514[b], PRO514[b], HIS90[b], HIS95[b].	HIS337[a], ASN567[a], ASN567[a], ALA502[b], HIS75[b], HIS75[b].	LEU2[b], TYR52[b], ALA18[b], LEU2[b], LEU2[b], ILE19[b], PHE5[b].
2.16	GLN192[a], GLN351[a], GLN192[a], GLN351[a], PRO514[c], ASN515[c].	HIS337[a], HIS75[a], GLN178[a], GLY340[a], PRO500[c], ASN567[c], ALA502[b].	GLY30[a], ASP49[a], SER23[a], SER23[c], TYR52[b], GLY30[b], TRP31[b], PRO68[b].
2.17	GLN351[a], GLN351[a], HIS90[c], PRO514[c], ASN515[c], SER516[a], HIS581[b].	HIS337[a], HIS337[a], HIS75[a], GLN178[a], GLY340[a], HIS75[c], PRO500[c], ASN567[c], VAL568[c].	TYR28[a], GLY30[a], ASP49[c], TYR52[c], TYR52[b], GLY30[b], TRP31[b], GLY30[b], TRP31[b].
2.18	GLN351[a], HIS581[a], GLN351[a], GLN351[a], HIS90[c], PRO514[c], ASN515[c], VAL582[c].	HIS337[a], HIS75[a], ALA502[b].	TYR28[a], GLY30[a],ASP49[c], TYR52[c], TYR52[b], GLY30[b], GLY30[b].
Diclofenac	TYR385[a], SER530[a], ALA527[b], LEU352[b], ALA527[b], ALA527[b], LEU352[b], ILE523[b], VAL349[b], LEU531[b], VAL349[b], ILE523[b].	–	–
Celecoxib	–	ARG106[a], ARG106[c], ARG499[a], GLN178[a], LEU338[a], SER339[a], VAL335[b], SER339[b], VAL509[b], VAL509[b], LEU370[b], VAL335[b], LEU345[b], LEU517[b], TYR371[b], TRP373[b], ALA513[b], ALA513[b].	–
Licofelone	–	–	GLY3[a], TYR28[a], ASP49[a], CYS29[a], ASP49[c], TRP31[b], TRP31[b], LEU2[b], LYS69[b].

a – hydrogen, b – hydrophobic, c – halogen (F)

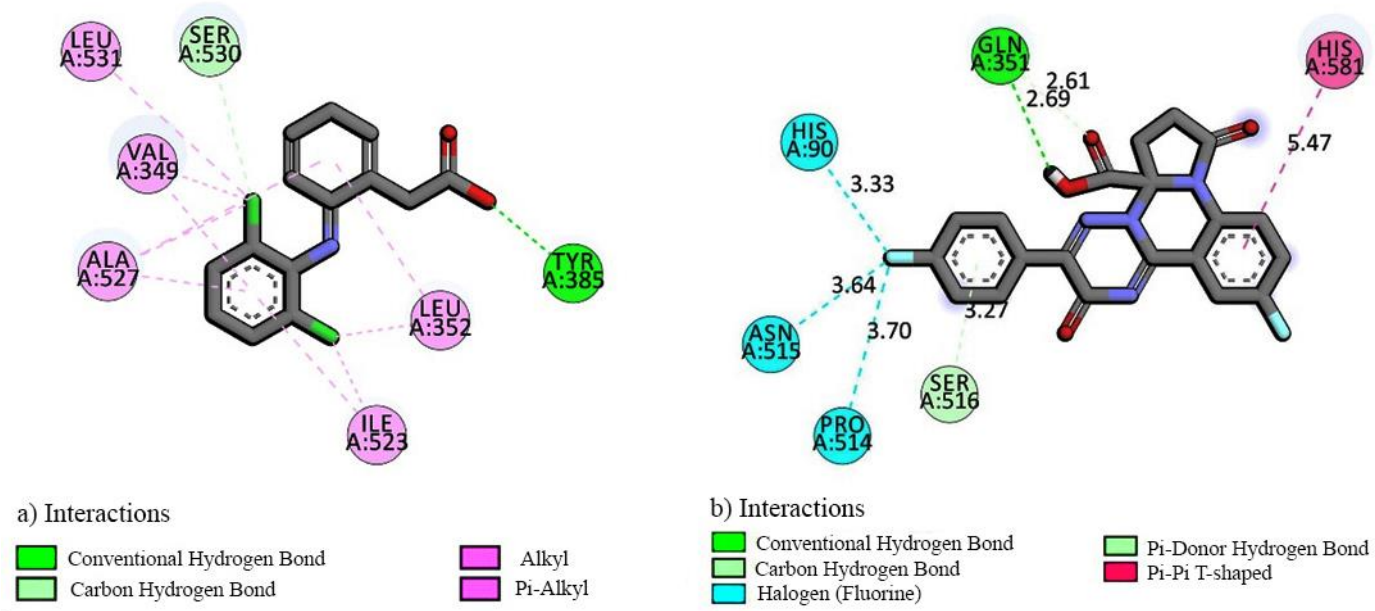


Fig. (2). Visualization of affinity according to the docking. a) Diclofenac with COX-1, b) compound **2.17** with COX-1.

Visualization of the structure **2.17** with the active site of COX-1 (Fig. **2**) allowed to establish, that structure has similar interactions to those, that exist between Diclofenac and this site. It was characterized by three hydrogen bonds with the following amino acid residues: GLN351 (2.61Å, 2.69Å) and SER516 (3.27Å). In addition, a number of halogen HIS90 (3.33Å), PRO514 (3.70Å), ASN515 (3.64Å) and hydrophobic interaction HIS581 (5.47Å) were present.

Structure **2.17** also has the highest affinity to PLA2. Visualization of the structure with active site of PLA2 (Fig. **3**) showed, that it was characterized by two hydrogen bonds with amino acid residues: TYR28 (2.87Å), GLY30 (1.89Å), hydrophobic interactions with TYR52 (5.42Å), GLY30 (3.75Å, 4.49Å), TRP31 (3.76Å, 4.49Å) and quite strong halogen interactions with ASP49 (3.64Å) and TYR52 (3.49Å). It is interesting, that similar interactions also exist in complex of Lycofelon with the PLA2 site.

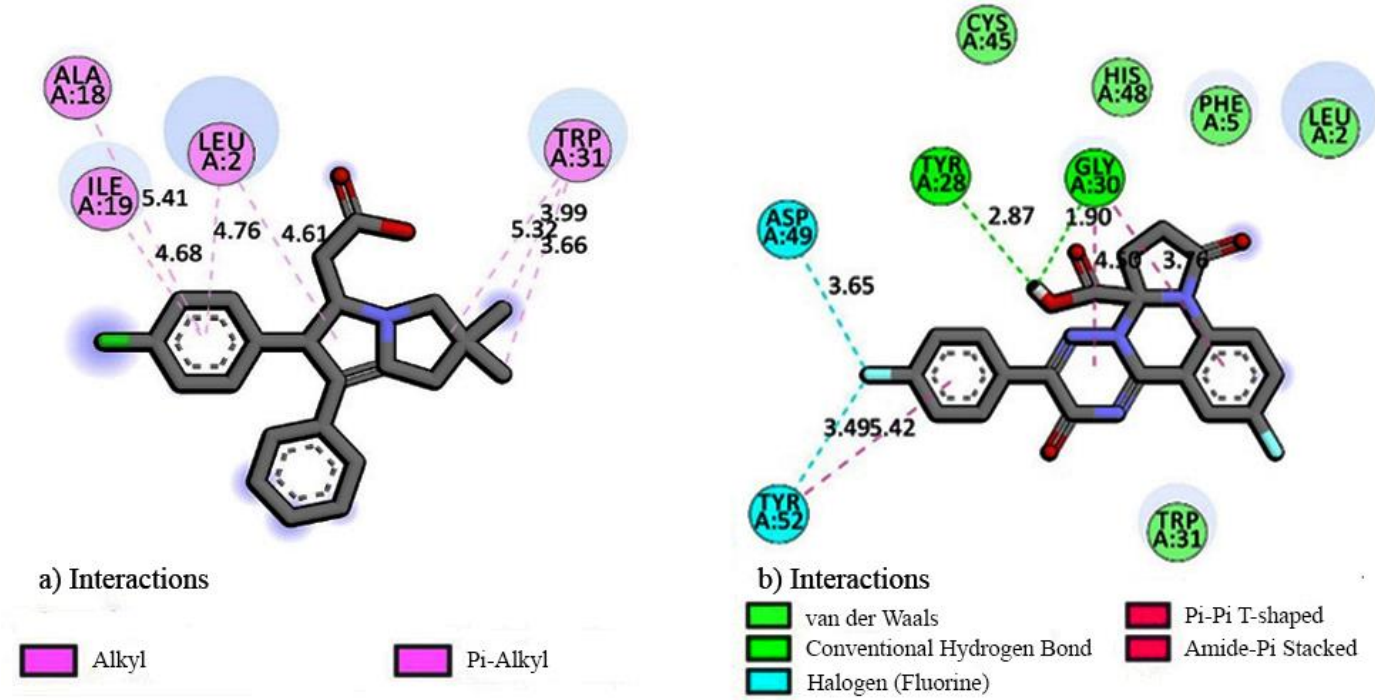


Fig. (3). Visualization of affinity according to the docking a) Licofelone with PLA2, b) compound **2.17** with PLA2.

3.2. Assessment and optimization of drug-like characteristics.

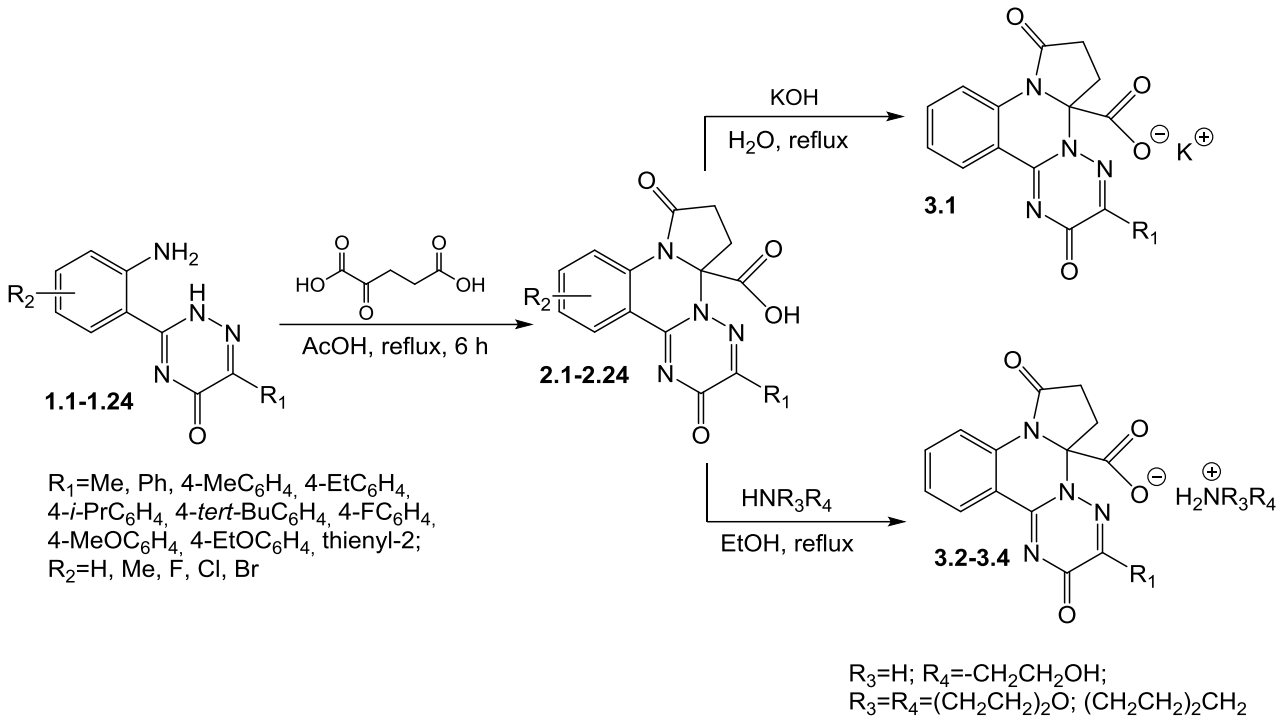
High affinity for these enzymes is not always the main factor for revealing anti-inflammatory activity. Also, it could be associated with the influence of additional factors and the characteristics of the body's metabolism. Given the above, calculations of the «druglike» criteria (Table **3**) were carried out. An analysis of the results indicated, that the substituted pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolines **(2.1-2.24)** have no deviations from Lipinski's rules (LogP ≤ 5; molecular weight ≤ 500; the ability to be a proton acceptor ≤ 10; the ability to be a proton donor ≤ 5; rotation of connections ≤ 8), as well as the Diclofenac and Celecoxib. Whereas, Licofelone corresponds the criteria of «Lipinski's rule of five» not by all indicators (LogP). This is an important argument for further experimental chemical and biological research.

Table 3. The value of the druglike criteria according to the «Lipinski's rule of five».

Compd.	Log P	Molecular polar surface area, Å	Number of non-hydrogens	Molecular weight	Number of hydrogen bond acceptors (groups N and O)	Number of hydrogen bond donors (groups NH and OH)	Number of rotatable bonds	Molecular volume, Å^3
Diclofenac	4.57	49.33	19	296.15	3	2	4	238.78
Celecoxib	3.61	77.99	26	381.38	5	2	4	298.65
Licofelone	6.15	42.23	27	379.89	3	1	4	341.95
2.1	0.02	105.40	23	312.29	8	1	1	255.24
2.2	1.47	105.40	28	374.36	8	1	2	310.09
2.3	1.87	105.40	29	388.38	8	1	2	326.65
2.4	1.63	105.40	29	392.35	8	1	2	315.02
2.5	1.89	105.40	29	388.38	8	1	2	326.65
2.6	1.61	105.40	29	392.35	8	1	2	315.02
2.7	2.12	105.40	29	408.80	8	1	2	323.63
2.8	2.25	105.40	29	453.25	8	1	2	327.98
2.9	1.70	105.40	30	410.34	8	1	2	319.95
2.10	1.92	105.40	29	388.38	8	1	2	326.65
2.11	2.38	105.40	30	402.41	8	1	3	343.45
2.12	2.98	105.40	31	416.44	8	1	3	360.04
2.13	3.17	105.40	32	430.46	8	1	3	376.28
2.14	2.29	105.40	30	402.41	8	1	2	343.21
2.15	1.63	105.40	29	392.35	8	1	2	315.02
2.16	1.77	105.40	30	410.34	8	1	2	319.95
2.17	1.77	105.40	30	410.34	8	1	2	319.95
2.18	1.86	105.40	31	428.33	8	1	2	324.88
2.19	1.52	114.63	30	404.38	9	1	3	335.64
2.20	1.92	114.63	31	418.41	9	1	3	352.20
2.21	2.18	114.63	31	438.83	9	1	3	349.17
2.22	2.31	114.63	31	483.28	9	1	3	353.52
2.23	1.90	114.63	31	418.41	9	1	4	352.44
2.24	1.25	105.40	27	380.38	8	1	2	300.80

3.3. Chemistry.

Substituted 3-(2-aminophenyl)-6-R-1,2,4-triazin-5(2*H*)ones (**1.1-1.24**) were used as the starting compound to form a combinatorial library of potential anti-inflammatory agents [12]. These compounds, like the classic 1,5-binucleophiles interacted with 2-oxopentanedioic acid and made it possible annulation with formation of the triazine cycle of the quinazoline and pyrrolidine systems. Probably, this process proceeded as a tandem reaction and involved cyclization along Ad_N- mechanism through the Schiff bases. Furhter nucleophilic substitution involving a nitrogen atom (position 7) with formation of the corresponding pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolines occurred (compounds **2.1-2.24**, Scheme **1**). It was found, that optimal conditions for abovementioned reaction were refluxing for 6 hours in acetic acid. As a result, the target compounds were formed with high yields **2.1-2.24**. Considering the low solubility of the compounds in water, several salts were synthesized to improve the pharmaco-technological properties **3.1-3.4** (Scheme **1**).



Scheme 1. Synthetic approaches for 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 acids and their salts.

The complex of physicochemical studies indicated compounds' **2.1**-**2.24** formation. Thus, quasimolecular ion was detected [M+1], in the chromatography mass spectrometry spectra of compounds **2.1**-**2.24**, which corresponded to the estimated mass. Mass spectra of acids **2.1**, **2.2** were characterized by the formation of ions F_1 $[M - CO_2]^+$ with *m/z* 268 (9.2%) and F_2 $[M - CO_2]^+$ 330 (19.2%), respectively. In addition, compound **2.1** was characterized by an ion F_3 $[M - COOH]^+$ with *m/z* 267 (57.4%). Subsequently, the F_1 and F_2 ions fragmentated by bonds C(2)–N(3) and N(4)–N(5) with the ions F_4 $[F_1\text{-}CH_3CN]^+$ formation with *m/z* 227 (27.6%) and F_5 $[F_1\text{-}C_6H_5CN]^+$ with *m/z* 227 (100%), respectively. Whereas, the most intensive signal in the mass spectrum of compound **2.1** was determined by similar fragmentation F_3 ($[F_3\text{-}CH_3CN]^+$ with *m/z* 226 (100%). The specified destruction of the triazine cycle was characteristic for the triazino[2,3-*c*]quinazolines and was described previously [25]. Stepwise elimination from ions F_4 and F_5 moieties of CO and the formation of ions with m/z 199, 198, 172 and 171 further confirmed the presence of the oxopyrrole cycle in the structure of compounds **2.1** and **2.2**. Thus, the given mass spectra confirmed the progress of the tandem reaction and the formation of the original heterocyclic system.

The structure of compounds **2.1**-**2.24** was also confirmed by ^{1}H NMR spectra data. Such characteristic series of proton signals of pyrrolidine and triazinoquinazoline cycles were registered. The signals of the protons of the pyrrolidine cycle resonated in the form of sequentially located multiplet H-6_{axial}. at the 3.29-3.10 ppm, common multiplets of H-$6_{equatorial.}$ and H-7_{axial}. at the 3.07-2.83 ppm and multiplets of H-$7_{equatorial}$. at the 2.86-2.68 ppm. Compound **2.3** was the exception with the methyl group in position 10, in the ^{1}H NMR spectrum of which H-6_{axial}. was registered as a multiplet at the 3.72-3.44 ppm, a H-$6_{equatorial.}$, H-$7_{axial.}$, H-$7_{equatorial}$. – joint multiplet at the 3.09-2.63 ppm. Then, in case of compounds **2.1** (methyl group at position 3) these protons were registered in the form of a wide multiplet at the 3.10-2.68 ppm. The complex splitting of above-mentioned signals could be the explained by the presence of an asymmetric Carbon atom in the molecule.

Signals of the protons of the triazinoquinazoline cycle (H-10, H-11, H-12 and H-13) were registered as AB, ABC or ABCD-systems in which position and multiplicity of signals were caused by nature of substituents. It is important, that the observed proton signals had a significant paramagnetic shift compared to the initial compounds **1.1**-**1.24** [12, 25]. The carboxyl group protons in ^{1}H NMR spectra in most cases were not registered due to exchange processes (**2.2**-**2.17**, **2.19**, **2.20**, **2.23** and **2.24**) or were registered in a low field at the 13.39 ppm (**2.1**), 12.06 ppm (**2.18**), 11.87 ppm (**2.21**) and 11.90 ppm (**2.22**). In addition, the ^{1}H NMR spectra of compounds **2.1**-**2.24** were characterized by the signals associated with substituents in position 3, 10, and 12, which had classical multiplicity and chemical shifts [26].

The formation of a heterocyclic system was confirmed by the ^{13}C NMR spectra of the compounds **2.1**, **2.3**, **2.5**, **2.12**, **2.13**, **2.15**, **2.16** and **2.24**. Thus, the deshielded signals of the sp^2-hybridized carbon atoms of position 8 were registered at the 174.0-171.7 ppm, signals of the sp^3-hybridized carbon atoms – at the 84.4-81.1 ppm (C-5a), 30.4-28.8 ppm (C-7) and 27.3-25.7 ppm. (C-6). This undoubtedly confirmed the presence of a pyrrolidine fragment in the molecule. The characteristic signal of the Carbon atom of COOH-group was detected at the 170.1-169.2 ppm.

A comparative analysis of the IR spectra of acids **2.1** and **2.2** and salts **3.1**-**3.4** showed, that salts did not have a

stretching band of OH-groups at the 3015-1925 cm^{-1}. In addition, the vibration bands of ν_{COO}-group at the 1701-1681 cm^{-1} (**2.1** and **2.2**) had a significant bathochromic shift (by 55-60 cm^{-1}), which confirmed the formation of an ionic bond (**3.1**-**3.4**).

3.5. Anti-inflammatory activity.

The results of pharmacological studies showed, that the synthesized compounds exhibited anti-inflammatory activity (Table **4**). 3-Methyl-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 acid (**2.1)** suppressed inflammation in both the carrageenan and formalin models by 57.90 and 53.76%, respectively. The replacement of methyl (compound **2.1**) by the phenyl (**2.2**) fragment at the third position of the heterocycle resulted 17.1% reduction in anti-inflammatory activity in the carrageenan model. Whereas, the anti-exudative activity on the «formalin» test increased by 16.9%. This fact is probably related with different mechanisms inflammation progress [1]. Further modification of compound **2.2**, namely, the replacement of phenyl by 4-isopropylphenyl (**2.12**) fragment in the heterocycle led to a significant loss of activity (Table **4**). Whereas compound **2.15** with the 4-fluorophenyl

Table 4. The anti-inflammatory activity of the synthesized compounds.

Compd.	Carrageenan test		Formalin test	
	Dose, mg/kg	AA, %×	Dose, mg/kg	AA, %×
2.1	25.0	57.90	10.0	53.76
2.2	25.0	40.81	10.0	70.18
2.4	25.0	75.77	10.0	59.52
2.6	25.0	64.72	10.0	49.42
2.9	25.0	54.92	10.0	46.74
2.12	25.0	44.66	10.0	34.60
2.15	25.0	63.02	10.0	64.30
2.16	25.0	74.60	10.0	60.31
2.17	25.0	69.23	10.0	64.38
2.18	25.0	51.37	10.0	46.21
3.1	25.0	65.11	25.0	61.09
3.2	25.0	64.47	25.0	60.37
3.3	25.0	63.30	25.0	59.28
3.4	25.0	57.62	25.0	53.61
Diclofenac sodium	8.0	61.09	8.0	50.92

fragment at the third position of the heterocycle exhibited activity at the reference drug level. As it was expected, the introduction of additional fluorine atom to positions 11 (**2.4**) and 12 (**2.6**) led to a significant increase of activity [27]. Their activity exceeded Diclofenac sodium by 14.7% and 3.6%, respectively. At the same time, the 11,12-difluoro-substituted analogue (compound **2.9**) exhibited less anti-inflammatory activity, than the previous ones. Also interesting was the modification associated with the additional introduction of fluorine into the compound **2.15**. Fluorine-containing analogues **2.16** (position 11) and **2.17** (position 12) exhibited high anti-inflammatory activity, which exceeded Diclofenac sodium activity by 8.2-13.5%. As for compound **2.18**, which is a trifluoro-containing analogue, like the previous compound **2.9**, it had significantly less activity. It was found, that increased solubility of acids **2.1** and **2.2** by formation of appropriate salts **3.1**-**3.4** also led to a slight increase in the activity (Table **4**). Higher anti-inflammatory activity was characteristic for potassium (**3.1**) and monoethanolammonium (**3.2**) salts. So, improving of the hydrophilicity of the synthesized acids was justified for further development of the strategy search of anti-inflammatory agents.

CONCLUSION

Thus, the developed and implemented search strategy of the anti-inflammatory agents was justified. 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 acids possessed the mentioned activity and additional introduction of fluorine atoms in position 11 or 12 to the heterocycle led to increasing of anti-inflammatory activity. Research in this area is ongoing.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All experimental procedures and treatment were carried out according to the European Convention and «Regulations on the use of animals in biomedical research» (European convention for the protection of vertebrate animal used for experimental and other scientific purposes. – Council of Europe, Strasbourg, 1986).

HUMAN AND ANIMAL RIGHTS

No humans were used in this study. All the reported experiments on animals were performed in accordance with the Guide for the Care and Use of Laboratory Animals, 8th Edition, published by the National Academy of Sciences, The National Academies Press, Washington DC, USA.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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