UDC 615.31:615.276:547.792'741'856.1'213-32.057.04

V. Stavytskyi [a], O. Voskoboinik [a], M. Kazunin [a], I. Nosulenko [a], S. Shishkina [b], S. Kovalenko [a]

SUBSTITUTED PYRROLO[1,2-*a*][1,2,4]TRIAZOLO-([1,2,4]TRIAZINO-)[*C*]QUINAZOLINE-4*a*(5*a*)-PROPANOIC ACIDS: SYNTHESIS, SPECTRAL CHARACTERISTICS AND ANTI-INFLAMMATORY ACTIVITY

[a] Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine
[b] SSI «Institute for Single Crystals», National Academy of Sciences of Ukraine, Kharkiv, Ukraine

The heterocyclization of 3-(2-aminophenyl)-6-(4-isopropylphenyl)-1,2,4-triazin-5(2*H*)-one with 4-oxoheptanedioic acid was studied in this work. It was shown that this reaction yielded partial hydrogenated [1,2,4]triazino[2,3-*c*]quinazoline, pyrrolo[1,2-*a*][1,2,4]-triazino[2,3-*c*]quinazoline-5*a*(6*H*)-yl)propanoic acid or their mixture depending on the reaction condition (the nature of a solvent, the temperature, and the process duration). It was found that the refluxing of initial compounds in acetic acid was the most suitable in the case of the synthesis of 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)propanoic acid. The found optimal conditions were used for the condensation of 2-azaheterylanilines with 4-oxoheptanedioic acid. These conditions created the basis for preparative methods for the synthesis of tetracyclic derivatives series. The structure and purity of the obtained compounds were determined by a complex of appropriate physicochemical methods, including ^{1}H and ^{13}C NMR, IR, chromatography-mass, mass-spectroscopies and X-ray diffraction analysis. The features of the fragmentation of different heterocycles molecular ions under electronic ionization were discussed. It was established that the destruction of pyrrolidone cycle caused higher fragmentation of aromatic pyrrolo[1,2-*a*][1,2,4]triaz>lo[1,5-*c*]quinazoline system. It was found that the introduction of carboxyethyl fragment into the pyrrolo[1,2-*a*][1,2,4]triazino-)[2,3-*c*]quinazoline system was favorable for the formation of anti-inflammatory agents. Pharmacological studies revealed that only substituted pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazoline-5*a*(6*H*)-propanoic acids showed high anti-inflammatory activity. The series of the obtained compounds demonstrated an anti-inflammatory activity that was comparable to or higher than the pharmacological effect of the reference compound «diclofenac». The essential role of the presence of fluorine atom in the anti-inflammatory activity of the synthesized compounds was discussed.

Keywords: 3-(2-aminophenyl)-6-R-1,2,4-triazin-5(2*H*)-ones, 2-(3-R-1*H*-1,2,4-triazol-5-yl)anilines, heterocyclization, pyrrolo[1,2-*a*][1,2,4]triazolo-([1,2,4]triazino-)[*c*]quinazoline-4*a*(5*a*)-propanoic acids, spectral data, anti-inflammatory activity.

DOI: 10.32434/0321-4095-2020-128-1-61-70

Introduction

The combination of a carboxylic group with an aromatic and heterocyclic moiety is an effective strategy for the creation of novel biologically active compounds [1]. Thus, carboxylic group is a common «pharmacophore» with a high ability for chemical modification that allows affecting the pharmacodynamics, pharmacokinetics and technological characteristics [2]. At the same time, an aromatic or heterocyclic fragment may be a carrier of biological activity, «spacer» or «linker» group [3]. Moreover, the introduction of a carboxylic or carboxyalkyl group into an aromatic or heterocyclic fragment allowed forming a series of effective anti-inflammatory drugs (propionic acid derivatives, acetic acid derivatives, enolic acid (oxicam) derivatives, anthranilic acid derivatives, etc.) [4]. It should be noted that the combination of a carboxylic group with condensed quinazoline moiety is one of the most promising way to realize the above-mentioned

strategy. Our previous studies showed the prospects of search for anti-inflammatory agents among condensed [5] and spiro-condensed [7] quinazoline derivatives.

Thus, the present study was aimed to design the methods of preparation of substituted pyrrolo[1,2-*a*]-[1,2,4]triazolo-([1,2,4]triazino-)[*c*]quinazoline-4*a*(5*a*)-propanoic acids and evaluate their structure features and anti-inflammatory activity.

Experimental

Melting points were determined in open capillary tubes in a «Stuart SMP30» apparatus and were uncorrected. The elemental analyses (C, H, and N) were performed using an «ELEMENTAR vario EL cube» analyzer. IR spectra (4000–600 cm^{-1}) were recorded by means a Bruker ALPHA FT-IR spectrometer using a module ATR eco ZnSe. ^1H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz) were recorded by using 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 a chromatography/mass spectrometric system which consists of high performance liquid chromatograph «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) were recorded using a Varian 1200 L instrument at 70 eV (Varian, USA). The purity of all obtained compounds was checked by ^1H NMR and LC-MS.

Substances 1a–m were synthesized according to the procedures reported elsewhere [7,8]. Other starting materials and solvents were obtained from commercially available sources and used without additional purification.

Synthesis of 3,3'-(3-(4-isopropylphenyl)-2-oxo-6,7-dihydro-2H-[1,2,4]triazino[2,3-c]quinazoline-6,6-diyl)dipropionic acid (2a)

1.74 g (10 mM) of 4-oxoheptanedioic acid and 1–2 drops of concentrated sulfuric acid (or 1–2 crystals of p-toluenesulfonic acid) were added to the suspension of 1.53 g (10 mM) 3-(2-aminophenyl)-6-(4-isopropylphenyl)-1,2,4-triazin-5(2*H*)-one (1a) in 10 mL of propanol-2. The formed mixture was refluxed for 30 min. After the completion of the reaction, the solvent was evaporated under vacuum, then 5 mL of cooled methanol was added to residue and the mixture was shaken. The formed precipitate was filtered off, washed by diethyl ether and dried. White crystals were obtained. Yield: 47.0%; m.p.: 228–230^{0}C; ^1H NMR δ (ppm): 8.88 (s, 1H, 7-NH), 8.65 (d, *J*=8.0 Hz, 1H. H-8), 8.23 (d, *J*=8.0 Hz, 2H, 3-Ar H-2,6), 8.00 (t, *J*=7.6 Hz, 1H, H-9), 7.91 (d, *J*=8.1 Hz, 1H, H-11), 7.79 (t, *J*=7.6 Hz, 1H, H-10), 7.36 (d, *J*=8.0 Hz, 2H, 3-Ar H-3,5), 3.09–2.94 (m, 5H, $(CH_2CH_2COOH)_2$, $CH(CH_3)_2$), 1.32 (m, *J*=7.0 Hz, 6H, $CH(CH_3)_2$), 1.27–0.97 (m, 4H, $(CH_2CH_2COOH)_2$). LC-MS: *m/z*=463 [M+1]; Anal. Calcd. for $C_{25}H_{26}N_4O_5$: C 64.92; H 5.67; N 12.11; Found: C 64.96; H 5.71; N 12.19.

Synthesis of substituted pyrrolo[1,2-a][1,2,4]-triazino-)[2,3-c]quinazoline-5a(6H)-propanoic acids (3a–j)

1.74 g (10 мМ) of 4-oxoheptanedioic acid was added to the suspension of 10 мМ of corresponding substituted 3-(2-aminophenyl)-6-R-1,2,4-triazin-5(2*H*)-one (1a-j) in 10 mL of glacial acetic acid. The formed mixture was refluxed for 6 h. Then solvent was evaporated under vacuum, 15 mL of methanol was added to the formed residue. The formed precipitate was filtered off, washed by diethyl ether and dried. The obtained compounds may be purified be re-crystallization from methanol.

*3-(3-(4-isopropylphenyl)-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolin-5a(6*H*)-yl)propanoic acid (3a)*

White crystals; Yield: 89.5%; m.p.: 136–138^{0}C; IR (cm^{-1}): 3744, 2968, 1730, 1658, 1591, 1547, 1502, 1484, 1339, 1175, 1066, 845, and 754; ^1H NMR δ (ppm): 12.04 (s, 1H, COOH), 8.30 (d, *J*=7.7 Hz, 1H, H-13), 8.15-8.06 (m, 3H, H-10, 3-Ph H-2,6), 7.72 (t, *J*=7.7 Hz, 1H, H-11), 7.44 (t, *J*=7.6 Hz, 1H, H-12), 7.30 (d, *J*=8.1 Hz, 2H, 3-Ph H-3,5), 3.04–2.56 (m, 5H, H-7,7', CH_2CH_2COOH, $CH(CH_3)_2$), 2.41–2.10 (m, 4H, H-6,6', CH_2CH_2COOH), 1.30 (d, *J*=6.8 Hz, 6H, $CH(CH_3)_2$); LC-MS, m/z=445.0 [M+l]; Anal. Calcd. for $C_{25}H_{24}N_4O_4$: C 67.55; H 5.44; N 12.60; Found: C 67.61; H 5.51; N 12.69.

*3-(3-methyl-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolin-5a(6*H*)-yl)propanoic acid (3b)*

White crystals; Yield: 78.8%; m.p. 228–230^{0}C; IR (cm^{-1}): 3744, 2987, 2311, 1730, 1693, 1643, 1590, 1503, 1478, 1405, 1338, 1217, 1066, 879, 778, and 692; ^1H NMR δ (ppm): 12.06 (s, 1H, COOH), 8.26 (d, *J*=7.7 Hz, 1H, H-13), 8.07 (d, *J*=8.1 Hz, 1H, H-10), 7.69 (t, *J*=7.5 Hz, 1H, H-11), 7.40 (t, *J*=7.5 Hz, 1H, H-12), 2.95–2.52 (m, 4H, H-7,7', CH_2CH_2COOH), 2.38–1.91 (m, 7H, H-6,6', CH_2CH_2COOH, CH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ (ppm) 173.6, 172.9, 162.6, 152.5, 152.2, 134.7, 134.5, 127.6, 126.2, 121.9, 119.6, 83.6 (C-5*a*), 32.8, 29.9, 28.3, 28.2, 17.9; LC-MS, m/z=341.0 [M+l]; Anal. Calcd. for $C_{17}H_{16}N_4O_4$: C 60.00; H 4.74; N 16.46; Found: C 60.09; H 4.82; N 16.51.

*3-(2,8-dioxo-3-phenyl-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolin-5a(6*H*)-yl)propanoic acid (3c)*

White crystals; Yield: 51.5%; m.p.: 231–233°C; IR (cm^{-1}): 2988, 2311, 1724, 1642, 1586, 1536, 1501, 1483, 1418, 1339, 1188, 1075, 884, 811, 776, 749, and 690; ^{1}H NMR δ (ppm): 12.09 (s, 1H, COOH), 8.31 (d, *J*=7.7 Hz, 1H, H-13), 8.19 (d, *J*=6.3 Hz, 2H, 3-Ph H-2,6), 8.10 (d, *J*=8.1 Hz, 1H, H-10), 7.73 (t, *J*=7.6 Hz, 1H, H-11), 7.53–7.38 (m, 4H, H-12, 3-Ph H-3,4,5), 3.19–2.56 (m, 4H, H-7, 7', CH_2CH_2COOH), 2.46–2.07 (m, 4H, H-6,6', CH_2CH_2COOH); ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) 173.6, 172.9, 161.5, 151.4, 147.7, 134.8, 134.7, 133.0, 130.9, 129.3, 128.6, 127.7, 126.4, 122.1, 119.4, 84.2 (C-5*a*), 32.8, 30.0, 28.3, 28.1; EI-MS (m/z ($I\%_{rel}$): 329 (9.6), 227 (36.7), 226 (100.0), 200 (9.3), 199 (9.9), 198 (39.1), 172 (6.3), 171 (6.7), 157 (11.4), 155 (33.8), 145 (6.2), 143 (6.7), 129 (16.6), 117 (5.1), 105 (11.9), 103 (28.8), 102 (24.3), 90 (9.6), 89 (12.1), 77 (19.1), 75 (14.2), 63 (12.8), 55 (12.0), 52 (5.2), 51 (12.2), 49 (5.7), 44 (5.1); LC-MS, m/z=403.0 [M+l]; Anal. Calcd. for $C_{22}H_{18}N_4O_4$: C 65.66; H 4.51; N 13.92; Found: C 65.71; H 4.59; N 14.02.

*3-(11-fluoro-2,8-dioxo-3-phenyl-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*] quinazolin-5a(6*H*)-yl)propanoic acid (3d)*

White crystals; Yield: 69.5%; m.p.: 202–204°C; ^{1}H NMR δ (ppm): 12.10 (s, 1H, COOH), 8.43–8.25 (m, 1H, H-13), 8.17 (d, *J*=6.6 Hz, 2H, 3-Ph H-2,6), 7.88 (d, *J*=10.7 Hz, 1H, H-10), 7.46 (d, *J*=6.8 Hz, 3H, 3-Ph H-3,4,5), 7.21 (t, *J*=7.6 Hz, 1H, H-12), 2.99–2.57 (m, 4H, H-7,7', CH_2CH_2COOH), 2.44–2.08 (m, 4H, H-6,6', CH_2CH_2COOH); LC-MS, m/z=421.0 [M+l]; Anal. Calcd. for $C_{22}H_{17}FN_4O_4$: C 62.85; H 4.08; N 13.33; Found: C 62.90; H 4.16; N 13.41.

*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-5a(6*H*)-yl)propanoic acid (3e)*

White crystals; Yield: 86.0%; m.p.: 143–146°C; ^{1}H NMR δ (ppm): 12.08 (s, 1H, COOH), 8.19 (d, *J*=6.6 Hz, 2H, 3-Ph H-2,6), 8.10 (dd, *J*=8.8, 4.6 Hz, 1H, H-13), 7.97 (dd, *J*=8.5, 2.6 Hz, 1H, H-10), 7.62–7.39 (m, 4H, H-11, 3-Ph H-3,4,5), 2.91–2.56 (m, 4H, H-7,7', CH_2CH_2COOH), 2.43–2.07 (m, 4H H-6,6', CH_2CH_2COOH); LC-MS, m/z=421.0 [M+l]; Anal. Calcd. for $C_{22}H_{17}FN_4O_4$: C 62.85; H 4.08; N 13.33; Found: C 62.93; H 4.13; N 13.40.

*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-5a(6*H*)-yl)propanoic acid (3f)*

White crystals; Yield: 35.9%; m.p.: 139–141°C; ^{1}H NMR δ (ppm): 12.11 (s, 1H, COOH), 8.23–8.08 (m, 3H, H-13, 3-Ph H-2,6), 8.04 (dd, *J*=11.2, 7.1 Hz, 1H, H-10), 7.58–7.36 (m, 3H, 3-Ph H-3,4,5), 2.94–2.56 (m, 4H, H-7,7', CH_2CH_2COOH), 2.43–2.16 (m, 4H, H-6,6', CH_2CH_2COOH); LC-MS, m/z=439.0 [M+l]; Anal. Calcd. for $C_{22}H_{16}F_2N_4O_4$: C 60.28; H 3.68; N 12.78; Found: C 60.34; H 3.73; N 12.84.

*3-(3-(4-fluorophenyl)-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]quinazolin-5a(6*H*)-yl)propanoic acid (3g)*

White crystals; Yield: 90.6%; m.p.: 193–195°C; ^{1}H NMR δ (ppm): 12.03 (s, 1H, COOH), 8.32–8.26 (m, 3H, H-13, 3-Ph H-2,6), 8.09 (d, *J*=8.1 Hz, 1H, H-10), 7.72 (t, *J*=7.7 Hz, 1H, H-11), 7.44 (t, *J*=7.5 Hz, 1H, H-12), 7.20 (t, *J*=8.6 Hz, 2H, 3-Ph H-3,5), 2.95–2.57 (m, 4H, CH_2CH_2COOH, H-7,7'), 2.43–2.09 (m, 4H, H-6,6', CH_2CH_2COOH); ^{13}C NMR δ (ppm): 173.6, 172.9, 163.9 (d, *J*=248.8 Hz), 161.4, 151.4, 146.7, 134.9, 134.6, 131.8 (d, *J*=8.7 Hz), 129.5 (d, *J*=2.8 Hz), 127.8, 126.4, 122.1, 119.3, 115.7 (d, *J*=21.4 Hz), 84.2 (C-5*a*), 32.8, 30.0, 28.3, 28.1; LC-MS, m/z= =421.0 [M+l]; Anal. Calcd. for $C_{22}H_{17}FN_4O_4$: C 62.85; H 4.08; N 13.33; Found: C 62.81; H 4.00; N 13.29.

*3-(11-fluoro-3-(4-fluorophenyl)-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]-quinazolin-5a(6*H*)-yl)propanoic acid (3h)*

White crystals; Yield: 62.2%; m.p.: 246–248°C; ^{1}H NMR δ (ppm): 12.06 (s, 1H, COOH), 8.42–8.21 (m, 3H, H-13, 3-Ar H-2,6), 7.93–7.83 (m, 1H, H-12), 7.30–7.12 (m, 3H, H-10, 3-Ar H-3,5), 2.95–2.79 (m, 2H, H-6,6'), 2.79–2.59 (m, 2H, CH_2CH_2COOH), 2.42–2.31 (m, 2H, H-7,7'), 2.31–2.09 (m, 2H, CH_2CH_2COOH); LC-MS, m/z=438.0 [M+l]; Anal. Calcd. for $C_{22}H_{16}F_2N_4O_4$: C 60.28; H 3.68; N 12.78; Found: C 60.35; H 3.74; N 12.75.

*3-(12-fluoro-3-(4-fluorophenyl)-2,8-dioxo-7,8-dihydro-2*H*-pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]-quinazolin-5a(6*H*)-yl)propanoic acid (3i)*

White crystals; Yield: 81.6%; m.p.: 281–283°C, IR (cm^{-1}): 3737, 2988, 1703, 1630, 1545, 1488, 1371, 1230, 1161, 1066, 894, 849, and 824; ^{1}H NMR δ (ppm): 12.08 (s, 1H, COOH), 8.35–8.23 (m, 2H, 3-Ph H-2,6), 8.09 (dd, *J*=8.8, 4.5 Hz, 1H, H-13), 7.95 (dd, *J*=8.5, 2.2 Hz, 1H, H-10), 7.52 (td, *J*=8.7, 2.3 Hz, 1H, H-11), 7.20 (t, *J*=8.6 Hz, 2H, 3-Ph H-3,5), 2.90–2.55 (m, 4H, H-7,7', CH_2CH_2COOH), 2.44–2.03 (m, 4H, H-6,6', CH_2CH_2COOH); ^{13}C NMR δ (ppm): 173.6, 173.0, 163.9 (d, *J*=249.1 Hz), 161.3, 159.7 (d, *J*=243.7 Hz), 150.6 (d, *J*=2.3 Hz), 146.9, 131.8 (d, *J*=8.8 Hz), 131.2 (d, *J*=1.6 Hz), 129.3 (d, *J*=2.9 Hz), 124.7 (d, *J*=8.9 Hz), 122.1

(d, *J*=22.8 Hz), 121.4 (d, *J*=8.5 Hz), 115.7 (d, *J*=21.5 Hz), 113.6 (d, *J*=24.7 Hz), 84.5 (C-5*a*), 32.9, 29.9, 28.3, 28.1; LC-MS, m/z=439.0 [M+l]; Anal. Calcd. for $C_{22}H_{16}F_2N_4O_4$: C 60.28; H 3.68; N 12.78; Found: C 60.33; H 3.74; N 12.85.

*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-5a(6*H*)-yl)propanoic acid (3j)*

White crystals; Yield: 22.2%; m.p.: 268–270^{0}C; ^{1}H NMR δ (ppm): 12.09 (s, 1H, COOH), 8.28 (dd, *J*=7.9, 5.8 Hz, 2H, 3-Ph H-2,6), 8.15 (t, *J*=9.4 Hz, 1H, H-13), 8.04 (dd, *J*=11.1, 7.2 Hz, 1H, H-10), 7.20 (t, *J*=8.6 Hz, 2H, 3-Ph H-3,5), 2.94–2.58 (m, 4H, H-7,7', CH_2CH_2COOH), 2.43–2.12 (m, 4H, H-6,6', CH_2CH_2COOH); LC-MS, m/z=457.0 [M+l]; Anal. Calcd. for $C_{22}H_{15}F_3N_4O_4$: C 57.90; H 3.31; N 12.28; Found: C 57.98; H 3.38; N 12.33.

Synthesis of substituted pyrrolo[1,2-a][1,2,4]-triazolo[1,5-c]quinazoline-4a(5H)-propanoic acids (3k–m)

1.74 g (10 mM) of 4-oxoheptanedioic acid and 1–2 drops of concentrated sulfuric acid (or 1–2 crystals of *p*-toluenesulfonic acid) were added to the suspension of corresponding substituted 2-(3-R-1*H*-1,2,4-triazol-5-yl)aniline in 10 mL of acetic acid (methanol, propanol-2 or dioxane). The formed mixture was refluxed for 2–6 hours. After the completion of the reaction, the solvent was evaporated under vacuum, 15 mL of diethyl ether (acetone) was added to the formed residue and obtained mixture was shaken. The formed precipitate was filtered off, washed by ether and dried. The obtained compounds may be additionally purified by re-crystallization from methanol.

*3-(2-methyl-7-oxo-6,7-dihydropyrrolo[1,2-*a*][1,2,4]-triazolo[1,5-*c*]quinazolin-4a(5*H*)-yl)-propanoic acid (3k)*

Yield: 65.0%; m.p. 225–228^{0}C; IR (cm^{-1}): 3737, 2987, 2311, 1711, 1512, 1484, 1338, 1213, 1066, 871, 752, 710, and 668; ^{1}H NMR, δ (ppm): 12.01 (s, 1H, COOH), 7.99 (d, *J*=8.1 Hz, 1H, H-12), 7.91 (d, *J*=7.7 Hz, 1H, H-9), 7.54 (t, *J*=7.9 Hz, 1H, H-10), 7.37 (t, *J*=7.6 Hz, 1H, H-11), 2.97–2.47 (m, 4H, H-6,6', CH_2CH_2COOH), 2.37 (s, 3H, CH_3), 2.25–1.89 (m, 4H, H-5,5', CH_2CH_2COOH); ^{13}C NMR, δ (ppm): 173.6, 173.3, 161.0, 148.1, 132.6, 131.5, 126.5, 124.6, 123.2, 117.1 (12a-C), 80.9 (4a-C), 34.1, 29.8, 28.5, 28.5, 14.3; LC-MS m/z=313 [M+1]; Anal. Calcd. for $C_{16}H_{16}N_4O_3$: C 61.53; H 5.16; N 17.94; Found: C 61.59; H 5.21; N 17.99.

*3-(7-oxo-2-phenyl-6,7-dihydropyrrolo[1,2-*a*][1,2,4]-triazolo[1,5-*c*]quinazolin-4a(5*H*)-yl)-propanoic acid (3l)*

Yield 79.1%; m.p. 232–235^{0}C; IR (cm^{-1}): 3732, 2977, 2311, 1732, 1493, 1445, 1338, 1214, 750, 719, and 693; ^{1}H NMR δ (ppm): 8.12–8.02 (m, 4H, H-12,9, 2-Ar H-2,6), 7.58 (t, *J*=7.9 Hz, 1H, H-10), 7.49–7.36 (m, 4H, H-11, Ar H-3,4,5), 3.07–2.79 (m, 3H, H-6, CH_2CH_2COOH), 2.70–2.57 (m, 2H, H-6',5), 2.42 (t, *J*=6.6 Hz, 2H, CH_2CH_2COOH), 2.15–2.06 (m, 1H, H-5'); ^{13}C NMR δ (ppm): 173.6, 173.3, 162.0, 148.9, 132.8, 131.8, 131.0, 130.0, 129.3, 126.6, 126.6, 124.9, 123.4, 117.0, 81.3 (C-4*a*), 37.1, 34.1, 29.9, 28.6; EI-MS (m/z ($I\%_{rel}$)): 301 (38.2), 273 (5.1), 262 (14.7), 260 (48.9), 259 (28.1), 248 (26.4), 246 (100.0), 245 (36.2), 143 (10.8), 129 (19.6), 127 (5.1), 116 (13.7), 109 (6.5), 104 (13.7), 103 (18.3), 102 (36.9), 90 (25.4), 88 (30.3), 86 (11.1), 77 (32.1), 76 (18.5), 75 (24.7), 64 (9.4), 63 (31.3), 62 (24.1), 54 (6.5), 52 (5.2), 51 (27.1), 45 (6.7), 44 (8.7), 42 (8.6), 41 (5.4), 39 (18.9); LC-MS, m/z: 374, Anal. Calcd. for $C_{21}H_{18}N_4O_3$: C 67.37; H 4.85; N 14.96; Found: C 67.35; H 4.87; N 14.99.

*3-(2-(4-isopropylphenyl)-7-oxo-6,7-dihydro-pyrrolo[1,2-*a*][1,2,4]triazolo[1,5-*c*]quinazolin-4a(5*H*)-yl)propanoic acid (3m)*

Yield 70.0%; m.p. 230–232^{0}C; IR (cm^{-1}): 3856, 2987, 2377, 1729, 1470, 1337, 1214, 851, 756, 723, and 668; ^{1}H NMR δ (ppm): 8.08 (d, *J*=7.6 Hz, 1H, H-9), 8.05–7.97 (m, 3H, H-12, 2-Ar H-2,6), 7.58 (t, *J*=7.9 Hz, 1H, H-11), 7.42 (t, *J*=7.7 Hz, 1H, H-10), 7.29 (d, *J*=7.9 Hz, 2H, 2-Ar H-3,5), 2.92–2.82 (m, 4H, H-5, $CH(CH_3)_2$, CH_2CH_2COOH), 2.68–2.56 (m, 2H, H-6',5'), 2.39–2.05 (m, 3H, H-6, CH_2CH_2COOH), 1.33–1.27 (m, 6H, $CH(CH_3)_2$); LC-MS, m/z: 416.18 Anal. Calcd. for $C_{24}H_{24}N_4O_3$: C 69.21; H 5.81; N 13.45; Found: C 69.17; H 5.84; N 13.47.

X-Ray diffraction study of 3-(2-methyl-7-oxo-6,7-dihydropyrrolo[1,2-a][1,2,4]triazolo[1,5-c]-quinazolin-4a(5H)-yl)propanoic acid (3k)

Crystals of 3k are triclinic, $C_{16}H_{16}N_4O_3$, at 20^{0}C, a=7.995(1) Å, b=9.413(1) Å, c=10.635(1) Å, α= =76.15(1)^0, β=71.63(1)^0, γ=71.41(1)^0, V=711.4(1) Å^3, M_r=312.33, Z=2, space group $P\bar{1}$, d_{calc}=1.458 g/cm^3, μ (MoK_α)=0.104 mm^{-1}, F(000)=328. The unit cell parameters and intensities of 8051 reflection (2497 independent, R_{int}=0.0546) were measured by using a «Xcalibur-3» diffractometer (MoK_α radiation, CCD-detector, graphite monochromator, w-scanning, $2\theta_{max}$=50^0).

The structure was solved by direct method using SHELXTL program [9]. The positions of hydrogen atoms were located from electronic density difference maps and refined using a «riding» model with U_{iso}=nU_{eq} (n=1.5 for methyl groups and n=1.2 for other hydrogen atoms) of the carrier atom. The

hydrogen atom of the hydroxyl group is refined within isotropic approximation. The structure was refined in anisotropic approximation for non-hydrogen atoms using least mean square method with 2487 reflections up to wR_2=0.166 (R_1=0.052 using 1755 reflections with $F>4\sigma(F)$, S=0.913). The final atomic coordinates, and crystallographic data for molecule 3k have been deposited to with the Cambridge Crystallographic Data Centre, 12 Union Road, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk) and are available on request quoting the deposition numbers CCDC 1970508).

Anti-inflammatory activity

The evaluation of anti-inflammatory activity of the synthesized compounds was conducted on 90 Wistar white rats (with a weight of 150–160 g), obtained from the nursery of the «Institute of Pharmacology and Toxicology of Ukraine» (Kyiv, Ukraine). All experimental procedures and treatment were carried out according to the European Convention and «Regulations on the use of animals in biomedical research» [10].

In order to estimate anti-inflammatory activity, the screening of the synthesized compounds began with the study of their effect on exudative phase of acute aseptic inflammation (a «formalin» test) [11]. Phlogogen (1% aqueous solution of formaldehyde) 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 the studied compounds was conducted using atraumatic probe as water solution or finely dispersed suspension stabilized by Tween-80 in a dose of 10 mg/kg 1 hour before the injection of phlogogen. The reference drug «Diclofenac sodium» was administered intragastrically in a recommended dose of 8 mg/kg for pre-clinical studies. The measurement of paws volume was conducted before the experiment and in 3 hours after injection of phlogogen using the described methods.

The activity of these substances was determined by their ability to reduce the swelling as compared with the 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 by the following equation:

$$A = 100\% - \frac{(V_{se} - V_{he})}{(V_{sc} - V_{hc})},$$

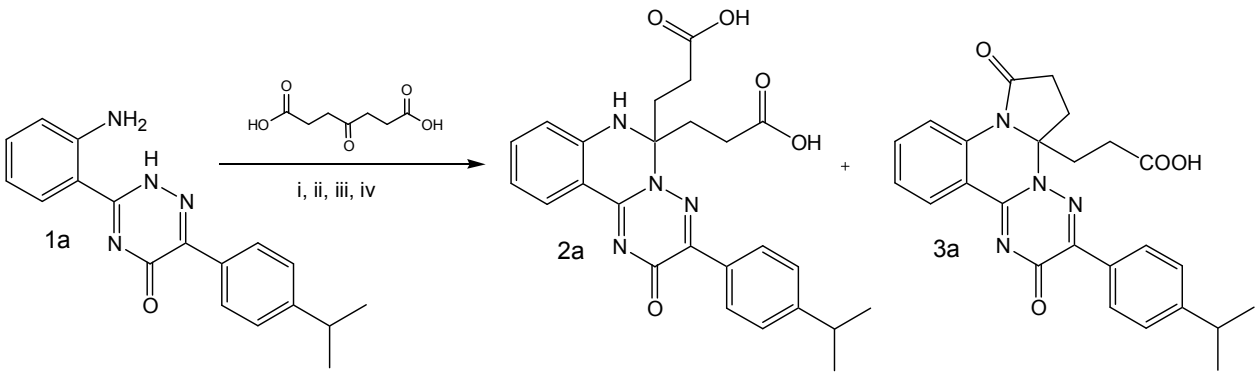
where A is the antiexudative activity, %; V_{se} is the volume of swollen paw in the experiment; V_{he} is the volume of healthy paw in the experiment; V_{sc} is the volume of swollen paw in control; and V_{hc} is the volume of healthy paw in control.

Statistical data processing was performed using a license program «STATISTICAT for Windows 6.0» (StatSoftInc., No. AXXR712D833214FAN5) as well as «SPSS 16.0» and «Microsoft Office Excell 2003». 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 $p<0.05$ [12].

Results and discussion

Considering the proposed strategy of the search for anti-inflammatory compounds, 2-azahetery-lanilines were used as initial compounds for the synthesis of quinazoline condensed derivatives. The above-mentioned transformation may be conducted *via* tandem cyclocondensation that yielded polycondensed heterocyclic systems [9,10]. The interaction between 3-(2-aminophenyl)-6-(4-isopropylphenyl)-1,2,4-triazin-5(2*H*)-one (1a) and 4-oxoheptanedioic acid was used as a model reaction to study the influence of conditions (the solvent nature, temperature, catalyst, and duration) on the heterocyclization process (Scheme 1, Table 1). The nature and content of the products in the reaction mixture were estimated using LC-MS method. It was shown that the refluxing of equimolar quantities of compound 1a with 4-oxoheptanedioic acid in methanol for 3 h resulted in the formation of compound 2a. It should be noted that the conversion of compound 1a in polycondesed derivative 2a did not exceed 30% irrespective of the reaction duration and catalyst nature. The initial compound 1a was the main component of the reaction mixture after completing the process, which may be associated with its low solubility in methanol.

The almost total (96%) conversion of compound 1a into product 2a was observed in case of the refluxing of initial reagents in propanol-2 in the presence of acidic catalyst during 2 h (Table 1). The prolongation of reaction duration up to 3 h resulted in the formation of a mixture of products 2a and 3a with the components ratio of 76%:24%, respectively. The reaction in dioxane during 2 h also was not selective and yielded a mixture of the products 2a and 3a (with a ratio of 30%:70%, respectively). The reaction between initial compounds in acetic acid was more selective. Thus, the total conversion of compound 1a into product 3a occurred after the refluxing of initial compounds in the above-



i: MeOH, H_2SO_4 or p-TosH; ii: i-PrOH, H_2SO_4 or p-TosH;
iii: dioxane, H_2SO_4 or p-TosH; iv AcOH

Scheme 1

Table 1

The features of the interaction between 3-(2-aminophenyl)-6-(4-isopropylphenyl)-1,2,4-triazin-5(2*H*)-one (1a) and 4-oxoheptanedioic acid

Conditions (solvent, catalyst, duration)	Contents of the components in the mixture after completing the reaction, 1a:2a:3a (%)
methanol, H_2SO_4 or TosOH, 3 h	70:30:0
propanol-2, H_2SO_4 or TosOH, 2 h	4:96:0
dioxane, H_2SO_4 or TosOH, 2 h	0:30:70
acetic acid, 2 h	0:0:100

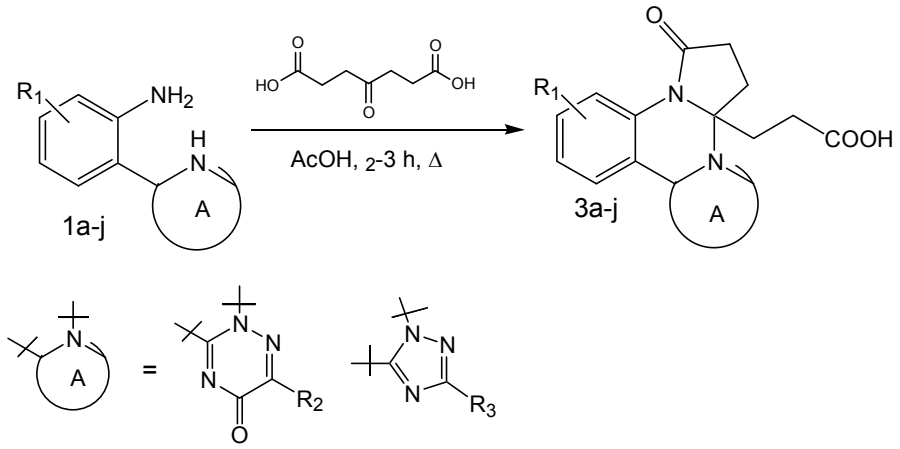
mentioned solvent during 2 h (Table 1). It was considered that increasing the solvent boiling point and, consequently, removing water from the reaction mixture had a positive effect on the formation of compound 3a.

The reactivity of 2-(3-R-1*H*-1,2,4-triazol-5-yl)anilines toward 4-oxoheptanedicarboxylic acid was different. Thus, the mixture of compounds 2m and 3m (at the ratio of 20%:80%, respectively) was formed as a result of the refluxing of compound 1m with 4-oxoheptanedicarboxylic acid in methanol or propanol-2 during 2 h (Scheme 2). This was caused by a higher solubility of the initial compound 1m-l in alcohols. The total conversion of the initial compound 1m into polycondensed derivative 3m was observed in case of the refluxing in acetic acid with water removal.

The found optimal conditions was used for the synthesis of pyrrolo[1,2-*a*][1,2,4]triazolo-([1,2,4]triazino-)[*c*]quinazoline-4*a*(5*a*)-propanoic acids 3 as a promising anti-inflammatory agents (Scheme 2). The initial compounds that contain a fluorine atom were used among others to obtain the products with a high anti-inflammatory activity. Thus, the series of fluorine-containing polycondensed derivatives were synthesized with satisfactory yields.

The structure and purity of the synthesized compounds were proved by a complex of appropriate physicochemical methods. Thus, the signals of proton in the 7th position and multiplets of carboxyethyl moieties in ^{1}H NMR spectra of compound 2a were characteristic and were observed as a singlet at the 8.88 ppm and at 3.09–2.94 ppm and at 1.27–0.97 ppm, respectively. At the same time, the signals of pyrrolidine cycle protons and carboxyethyl moiety at angular carbon atom of compounds 3a-m in ^{1}H NMR were registered. The above-mentioned signals were observed as series of multiplets at 3.19–2.52 ppm and 2.44–1.61 ppm, wich can be explained by the presence of asymmetric carbon atom. The signal of carboxylic group proton was observed in the spectra of the most compounds as a singlet at 12.11–12.03 ppm. The signals of heterocyclic fragment benzene ring protons were registered as AB, ABC or ABCD-systems, wherein the position and multiplicity were affected by the nature of substituents. Moreover, the signals associated with the substituents in positions 2 (of triazole cycle) or 3 (of triazine cycle) were registered in ^{1}H NMR spectra of the compounds 3.

The formation of tetracyclic system was additionally proved by ^{13}C NMR spectra of the compounds 3. Thus, the signals of deshielded carbon atoms of carboxylic group and the 2nd position were



R_1 = H, F; R2=Me, Ph, 4-*i*-PrC_6H_4; 4-FC_6H_4; R_3=Me, Ph, 4-*i*-PrC_6H_4

Scheme 2

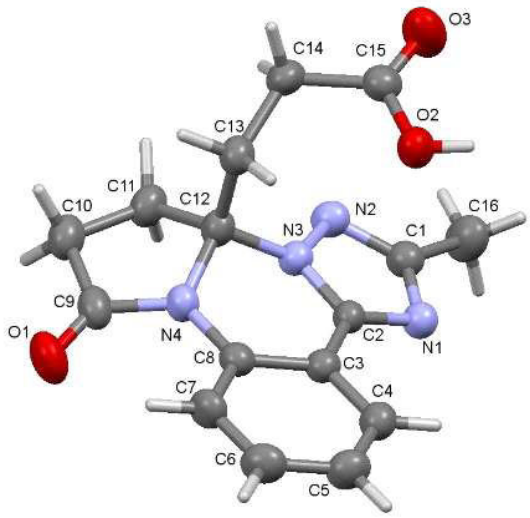
characteristic of pyrrolo[1,2-*a*][1,2,4]]triazino[2,3-*c*]quinazoline derivatives (3b, 3c, 3g, 3i) and were observed at 173.6 ppm and 165.2–11.2 ppm, respectively. The formation of pyrrole ring was proved by the signals of carbon atoms in the 8th, 7th, 6th and 5*a* positions at 173.2–172.9 ppm, 30.0–29.9 ppm, 32.9–32.8 ppm and 84.5–83.6 ppm, respectively. The signals of carboxyethyl moiety of sp^3-hybridized carbon atoms were registered at 32.9–28.3 and 30.0–28.2 ppm, correspondingly. At the same time, the signals of carbon atoms in pyrrolidone cycle condensed with more aromatic [1,2,4]-triazolo-[1,5-*c*]quinazoline systems (compounds 3k, 3l) were diamagnetically shifted. This effect was more pronounced for the signals of carbons atoms in the positions 4a, 5 and 6. The positions of other signals corresponded to the proposed structures.

The mass-spectrum (EI) of the compounds 3c and 3l were characterized by the absence of molecular ions signals. The initial fragmentation of the molecular ion of compound 3c was caused by the elimination of carboxyethyl fragment and the formation of ion $F_1[M\text{-}CH_2CH_2COOH]^{\cdot+}$ with m/z=329 (I_{rel}=9.6%). The fragmentation of F_1 was associated with the degradation of triazine fragment: m/z=260 (I_{rel}=100.0%) and m/z=198 (I_{rel}=39.1%). The 2-oxo-5-imino-3.3a,4,5-tetrahydropyrrolo-[1,2-a]quinazolinium cation ($[C_{11}H_8N_3O]^{\cdot+}$, m/z= =198 (I_{rel}=39.1%)) subsequently eliminated radicals CO, CH_2CO, CH_2CH_2CO, what resulted in the formation of ions with *m/z* 171, 155 and 143. The above-mentioned signals corresponded to the fragmentation of pyrrolidone cycle and additionally proved the formation of tetracyclic system.

The mass-spectrum of the compound 3l was also characterized by the absence of a molecular ion signal and the presence of a signal from an ion that was formed as a result of the elimination of carboxyethyl fragment ($F_1[M\text{-}CH_2CH_2COOH]^{\cdot+}$ m/z 301 (I_{rel}=38.2%). At the same time, the fragmentation of F_1 ion had a different direction as compared with the compound 3c. Thus, the fragmentation of F_1 for the compound 3l was caused by the destruction of pyrrolidone $[F_1\text{-}CH_2CO]^{\cdot+}$ and $[F_1\text{-}CH_2CH_2CO]^{\cdot+}$ what resulted in the formation of ions F_2 with m/z 260 (I_{rel}=48.9%) and F_3 with m/z 246 (I_{rel}=100.0%). This feature was due to a higher aromaticity of [1,2,4]triazolo[1,5-c]quinazoline system. The fragmentation of the formed 2-phenyl-[1,2,4]-triazolo[1,5-*c*]quinazolinium cation (F_3) was caused by the cleavage of N_1–C_2 and N_3–N_4 bonds which yielded ion F4 (m/z 143 ((I_{rel}=10.8%).

Taking into account some ambiguity of a tandem heterocyclization reaction consisted in the possibility to form different structural isomers, we confirmed the structure of the compound 3k using X-ray diffraction method (Figure). It was shown that tetrahydropyrimidine cycle adopts a non-symmetrical half-chair conformation (the puckering parameters are as follows [14]: S=0.55, Θ=42.7^0, Ψ=47.6^0). The deviations of the N_4 and C_{12} atoms from the mean square plane of the remaining cyclic atoms are 0.13 Å and −0.40 Å, respectively. Pyrrolidine cycle adopts an envelope conformation where C11 atom deviates by 0.44 Å from the other atoms of this cycle. The carboxyl group of the substituent at the C12 atom is located almost orthogonally towards the C12–C13 bond (the C12–C13–C14–N1 torsion angle is −83.3(2)^0) and is slightly turned to the C13–C14 bond (the C13–C14–C15–O3 torsion

angle is −15.0(3)^0). Such a position of the carboxylic fragment is stabilized by the intramolecular stacking interaction with the triazole cycle (the distance between C15 atom and triazole cycle π-system is 3.01 Å) causing the appearance of the H(14a)...H(11b) intramolecular short contact (the distance is 2.30 Å as compared to the van der Waals radii sum [15] equal to 2.34 Å). In the crystal phase, molecules 3k form centrosymmetric dimer due to the O(2)−H(2)...N(1)' intermolecular hydrogen bond (1−x, −y, 1−z; H...N 1.94 Å O−H...N 162^0).



The molecular structure of the compound 3k according to the X-ray diffraction study. Thermal ellipsoids of atoms are shown at 50% probability level

Anti-inflammatory activity of the synthesized compounds

The results of pharmacological study showed that most of the obtained compounds demonstrated anti-inflammatory activity (Table 2). The compounds 3b, 3d, 3g and 3j were identified as the most active anti-inflammatory agents. Thus, the activity of these compounds exceeded the pharmacological effect of the reference-compound «Diclofenac» by 33.1%, 10,8%, 32.0% and 28.1%, correspondingly. The SAR-analysis showed that a high anti-inflammatory activity was observed for pyrrolo[1,2-*a*][1,2,4]-triazino[2,3-*c*]quinazoline derivatives (compounds 3a-j) which can be explained by their structural similarity to the known anti-inflammatory agents [4−6]. At the same time, the compound 2a as well as substituted pyrrolo[1,2-*a*][1,2,4]triazolo[2,3-*c*]-quinazolines (3k-m) showed only a low activity. It should be mentioned that nature of substituent in position 3 of pyrrolo[1,2-*a*][1,2,4]triazino[2,3-*c*]-quinazoline derivatives significantly affects the anti-inflammatory activity. Thus, the replacement of methyl group (compound 3b) with phenyl moiety (3c) resulted in a loss of the anti-inflammatory activity. At the same time, the introduction of fluorine atom into phenyl substituent in the 3rd position (3g) significantly increased the pharmacological activity level. The introduction of additional fluorine atoms into positions 11 (3d) and 11, 12 (3j) was also reasonable approach for purposeful search of anti-inflammatory agents.

Table 2

Anti-inflammatory activity of synthesized compounds (M±m, n=6)

No.	Compound	Dose, mg/kg	AA, %×
1	control	–	–
2	diclofenac	10.0	50.93
3	2a		29.35
4	3b		84.05
5	3c		32.33
6	3d		61.73
7	3e		42.69
8	3f		43.19
9	3g		82.98
10	3i		50.78
11	3j		79.05
12	3k		41.22
13	3l		36.71
14	3m		39.21

Conclusions

The interaction between 2-azaheterylanilines and 4-oxoheptanedioic acids resulted in the formation of condensed quinazoline derivatives. The nature of a heterocyclic fragment in molecules of initial compounds, the nature of solvent and the process duration significantly affect the structure of the products. The refluxing of initial compounds mixture in acetic acid during 2 h was optimal for the synthesis of tetracyclic derivatives 3. The obtained compounds revealed promising anti-inflammatory activity that was comparable to or higher than pharmacological effect of the reference drug «Diclofenac». The presence of pyrrolidone and triazine systems was essential for revealing anti-inflammatory activity. The introduction of fluorine atom allowed increasing the pharmacological effect of the synthesized compounds.

Acknowledgements

The work was performed with the financial

support of «Enamine Company» (Kyiv, Ukraine).

REFERENCES

1. *Krogsgaard-Larsen P., Liljefors T., Madsen U.* Textbook of drug design and discovery. – Washington: Taylor & Francis, 2002. – 652 p.

2. *Ballatore C., Huryn D.M., Smith A.B.* Carboxylic acid (bio)isosteres in drug design //. ChemMedChem. – 2013. – Vol.8. – P.385-395.

3. *Erlanson D.A.* Introduction to fragment-based drug discovery // Fragment-based drug discovery and X-ray crystallography. Topics in current chemistry. – 2011. – Vol.317. – P.1-32.

4. *Vane J.R.* The mechanism of action of anti-inflammatory drugs // Advances in eicosanoid research. Ernst Schering research foundation workshop 31. – 2000. – P.1-23.

5. *Purposeful* search and characteristic of anti-inflammatory activity of sodium (3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)alkylcarboxylates and their halogen containing analogues / Yakubovska V.V., Seredinska N.M., Voskoboynik O.Yu., Stepanyuk G.I., Kovalenko S.I. // Aktualni Pytannya Farmatsevtychnoyi i Medychnoyi Nauky ta Praktyky. – 2016. – No. 1(20). – P.60-66.

6. *Design*, synthesis and anti-inflammatory activity of derivatives 10-R-3-aryl-6,7-dihydro-2*H*-[1,2,4] triazino[2,3-*c*]-quinazolin-2-ones of spiro-fused cyclic frameworks / Kolomoets O., Voskoboynik O., Antypenko O., Berest G., Nosulenko I., Palchikov V., Karpenko O., Kovalenko S. // Acta Chim. Slov. – 2017. – Vol.64. – No. 4. – P.902-910.

7. *Hydrazinolysis* of 3-R-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones. Synthetic and theoretical aspects / Sergeieva T.Y., Voskoboynik O.Y., Okovytyy S.I., Kovalenko S.I., Shishkina S.V., Shishkin O.V., Leszczynski J. // J. Phys. Chem. A. – 2014. – Vol.118. – P.1895-1905.

8. *Hydrolytic* cleavage of the pyrimidine ring in 2-aryl-[1,2,4]triazole[1,5-*c*]quinazolines: physico-chemical properties and the hypoglycemic activity of the compounds synthesized / Kholodnyak S.V., Schabelnyk K.P., Shernova G.A., Sergeieva T.Yu., Ivchuk V.V., Voskoboynik O.Yu., Kovalenko S.I., Trzhetsinskiy S.D., Okovytyy S.I., Shishkina S.V. // News Pharm. – 2015. – No. 3(83). – P.9-17.

9. *Sheldrick G.M.* A short history of SHELX // Acta Crystallogr., Sect. A: Found. Crystallogr. – 2008. – Vol.64. – P.112-122.

10. *European* convention for the protection of vertebrate animal used for experimental and other scientific purposes. – Strasbourg: Council of Europe, 1986. – 53 p.

11. *Fehrenbacher J.C., Vasko M.R., Duarte D.B.* Models of inflammation: Carrageenan- or complete Freund's Adjuvant (CFA)-induced edema and hypersensitivity in the rat // Curr. Protoc. Pharmacol. – 2012. – Vol.56. – P.5.4.1-5.4.4.

12. *Lapach S.N., Chubenko A.V., Babich P.N. Statistical methods in biomedical research using EXCEL.* – Kiev: Morion, 2000. – 408 p.

13. *Synthesis*, anticancer and FGFR1 inhibitory activity of isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline derivatives / Voskoboynik O.Yu., Starosyla S.A., Protopopov M.V., Volynets H.P., Shyshkina S.V., Yarmoliuk S.M., Kovalenko S.I. // Med. Clin. Chem. – 2016. – Vol.18. – No. 1(66). – P.5-18.

14. *Zefirov N.S., Palyulin V.A., Dashevskaya E.E.* Stereochemical studies. XXXIV. Quantitative description of ring puckering via torsional angles. The case of six-membered rings // J. Phys. Org. Chem. – 1990. – Vol.3. – No. 3. – P.147-158.

15. *Zefirov Yu.V.* Sokrashchennye mezhmolekularnye kontakty i spetsificheskie vzaimodeistviya v molekularnykh kristallakh // Kristallographiya. – 1997. – Vol.42. – P.936-958.

Received 02.12.2019

ЗАМІЩЕНІ ПІРОЛО[1,2-*a*][1,2,4]ТРИАЗОЛО-([1,2,4]ТРИАЗИНО-)[*C*]ХІНАЗОЛІН-4*a*(5*a*)ПРОПАНОВІ КИСЛОТИ: СИНТЕЗ, СПЕКТРАЛЬНІ ХАРАКТЕРИСТИКИ ТА ПРОТИЗАПАЛЬНА АКТИВНІСТЬ

В. Ставицький, О. Воскобойник, М. Казунін, І. Носуленко, С. Шишкіна, С. Коваленко

*В даній роботі наведені результати дослідження гетероциклізації 3-(2-амінофеніл)-6-(4-ізопропілфеніл)-1,2,4-триазин-5(2*H*)-ону з 4-оксогептандіовою кислотою. Показано, що в залежності від умов перебігу (температурний режим, природа розчинника та каталізатора, тривалість процесу) в результаті реакції утворюються частково гідрований [1,2,4]триазино[2,3-*c*]хіназолін, піроло[1,2-*a*][1,2,4]триазино[2,3-*c*]хіназолін-5*a*(6*H*)-іл)пропанова кислота або їх суміш. Встановлено, що кип'ятіння зазначених вихідних сполук в оцтовій кислоті є оптимальними умовами для синтезу 3-(3-(4-ізопропілфеніл)-2,8-діоксо-7,8-дігідро-2*H*-піроло[1,2-*a*][1,2,4]триазино[2,3-*c*]-хіназолін-5*a*(6*H*)-іл)пропанової кислоти. Виявлені оптимальні умови були використані для здійснення конденсації 2-азагетериланілінів з 4-оксогептандіовою кислотою та слугували препаративним методом для одержання серії тетрациклічних похідних. Структура та чистота синтезованих сполук була підтверджена за допомогою комплексу фізико-хімічних методів дослідження, який серед іншого охоплював ІЧ-, ^{1}H, ^{13}C ЯМР-, хромато-мас-, мас-спектрометрію та рентгеноструктурне дослідження. Показані особливості фрагментації молекулярних іонів одержаних гетероциклічних похідних в умовах електронної іонізації. Встановлено, що більш ароматична піроло[1,2-*a*][1,2,4]триазоло[1,5-*c*]хіназолінова система зазнає фрагментації за піролідоновим циклом. Виявлено, що введення карбоксиетильного фрагменту до піроло[1,2-*a*][1,2,4]триазино[2,3-*c*]хіназолінової системи є виправданим в контексті конструювання протизапальних агентів. Результати фармакологічних досліджень показали, що тільки заміщеним піроло[1,2-*a*][1,2,4]триазино[2,3-*c*]хіназолін-5*a*(6*H*)-пропановим кислотам притаманна висока протизапальна дія. Низка одержаних сполук проявляють протизапальну дію, яка за рівнем була вищою у порівняні з фармакологічною активністю препарату порівняння «Диклофенак». Показано, що наявність атомів фтору в молекулі синтезованих сполук є критичним для прояву зазначеної дії.*

Ключові слова: 3-(2-амінофеніл)-6-R-1,2,4-триазин-5(2*H*)-они, 2-(3-R-1*H*-1,2,4-триазол-5-іл)аніліни, гетероциклізація, піроло[1,2-*a*][1,2,4]триазоло-([1,2,4]триазино-)[*c*]-

хіназолін-4а(5а)пропанові кислоти, спектральні характеристики, протизапальна активність.

SUBSTITUTED PYRROLO[1,2-*a*][1,2,4]TRIAZOLO-([1,2,4]TRIAZINO-)[*C*]QUINAZOLINE-4*a*(5*a*)-PROPANOIC ACIDS: SYNTHESIS, SPECTRAL CHARACTERISTICS AND ANTI-INFLAMMATORY ACTIVITY

***V. Stavytskyi* [a], *O. Voskoboinik* [a], *M. Kazunin* [a], *I. Nosulenko* [a], *S. Shishkina* [b], *S. Kovalenko* [a, *]**

[a] **Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine**

[b] **SSI «Institute for Single Crystals», National Academy of Sciences of Ukraine, Kharkiv, Ukraine**

[*] **e-mail: kovalenkosergiy@gmail.com**

The heterocyclization of 3-(2-aminophenyl)-6-(4-isopropylphenyl)-1,2,4-triazin-5(2H)-one with 4-oxoheptanedioic acid was studied in this work. It was shown that this reaction yielded partial hydrogenated [1,2,4]triazino[2,3-c]quinazoline, pyrrolo-[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)-yl)propanoic acid or their mixture depending on the reaction condition (the nature of a solvent, the temperature, and the process duration). It was found that the refluxing of initial compounds in acetic acid was the most suitable in the case of the synthesis of 3-(3-(4-isopropylphenyl)-2,8-dioxo-7,8-dihydro-2H-pyrrolo[1,2-a][1,2,4]triazino[2,3-c]-quinazolin-5a(6H)-yl)propanoic acid. The found optimal conditions were used for the condensation of 2-azaheterylanilines with 4-oxo-heptanedioic acid. These conditions created the basis for preparative methods for the synthesis of tetracyclic derivatives series. The structure and purity of the obtained compounds were determined by a complex of appropriate physicochemical methods, including ^{1}H and ^{13}C NMR, IR, chromatography-mass, mass-spectroscopies and X-ray diffraction analysis. The features of the fragmentation of different heterocycles molecular ions under electronic ionization were discussed. It was established that the destruction of pyrrolidone cycle caused higher fragmentation of aromatic pyrrolo[1,2-a][1,2,4]triaz>lo[1,5-c]-quinazoline system. It was found that the introduction of carboxyethyl fragment into the pyrrolo[1,2-a][1,2,4]triazino-)[2,3-c]quinazoline system was favorable for the formation of anti-inflammatory agents. Pharmacological studies revealed that only substituted pyrrolo-[1,2-a][1,2,4]triazino[2,3-c]quinazoline-5a(6H)-propanoic acids showed high anti-inflammatory activity. The series of the obtained compounds demonstrated an anti-inflammatory activity that was comparable to or higher than the pharmacological effect of the reference compound «diclofenac». The essential role of the presence of fluorine atom in the anti-inflammatory activity of the synthesized compounds was discussed.

Keywords: 3-(2-aminophenyl)-6-R-1,2,4-triazin-5(2H)-ones; 2-(3-R-1*H*-1,2,4-triazol-5-yl)anilines; heterocyclization; pyrrolo[1,2-a][1,2,4]triazolo-([1,2,4]triazino-)[c]quinazoline-4a(5a)-propanoic acids; spectral data; anti-inflammatory activity.

REFERENCES

1. Krogsgaard-Larsen P., Liljefors T., Madsen U., *Textbook of drug design and discovery, 3rd Edition*. Taylor & Francis, 2002.

2. Ballatore C., Huryn D.M., Smith A.B. Carboxylic acid (bio)isosteres in drug design. *ChemMedChem*, 2013, vol. 8, pp. 385-395.

3. Erlanson D.A. Introduction to fragment-based drug discovery. In: Davies T., Hyvonen M. (eds.). *Fragment-based drug discovery and X-ray crystallography. Topics in current chemistry*, 2011, vol. 317, pp. 1-32.

4. Vane J.R. The mechanism of action of anti-inflammatory drugs. In: Serhan C.N., Perez H.D. (eds.). *Advances in eicosanoid research. Ernst Schering research foundation workshop 31*, Springer-Verlag, Berlin, 2000, pp. 1-23.

5. Yakubovska V.V., Seredinska N.M., Voskoboynik O.Yu., Stepanyuk G.I., Kovalenko S.I. Purposeful search and characteristic of anti-inflammatory activity of sodium (3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)alkylcarboxylates and their halogen containing analogues. *Aktualni Pytannya Farmatsevtychnoyi i Medychnoyi Nauky ta Praktyky*, 2016, no. 1(20), pp. 60-66. (*in Ukrainian*).

6. Kolomoets O., Voskoboynik O., Antypenko O., Berest G., Nosulenko I., Palchikov V., Karpenko O., Kovalenko S. Design, synthesis and anti-inflammatory activity of derivatives 10-R-3-aryl-6,7-dihydro-2H-[1,2,4] triazino[2,3-c]quinazolin-2-ones of spiro-fused cyclic frameworks. *Acta Chimica Slovenica*, 2017, vol. 64, no. 4, pp. 902-910.

7. 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. *The Journal of Physical Chemistry A*, 2014, vol. 118, pp. 1895-1905.

8. Kholodnyak S.V., Schabelnyk K.P., Shernova G.A., Sergeieva T.Yu., Ivchuk V.V., Voskoboynik O.Yu., Kovalenko S.I., Trzhetsinskiy S.D., Okovytyy S.I., Shishkina S.V. Hydrolytic cleavage of the pyrimidine ring in 2-aryl-[1,2,4]triazole[1,5-c]-quinazolines: physico-chemical properties and the hypoglycemic activity of the compounds synthesized. *News of Pharmacy*, 2015, no. 3(83), pp. 9-17.

9. Sheldrick G.M. A short history of SHELX. *Acta Crystallographica, Section A: Foundations of Crystallography*, 2008, vol. 64, pp. 112-122.

10. *European convention for the protection of vertebrate animal used for experimental and other scientific purposes.* Council of Europe, Strasbourg, 1986. 53 p.

11. Fehrenbacher J.C., Vasko M.R., Duarte D.B. Models of inflammation: Carrageenan- or complete Freund's Adjuvant (CFA)-induced edema and hypersensitivity in the rat. *Current Protocols in Pharmacology*, 2012, vol. 56, pp. 5.4.1-5.4.4.

12. Lapach S.N., Chubenko A.V., Babich P.N., Statistical methods in biomedical research using EXCEL. Morion Publishers, Kiev, 2000. 408 p. *(in Russian)*.

13. Voskoboynik O.Yu., Starosyla S.A., Protopopov M.V., Volynets H.P., Shyshkina S.V., Yarmoliuk S.M., Kovalenko S.I. Synthesis, anticancer and FGFR1 inhibitory activity of isoindolo[2,1-a][1,2,4]triazino[2,3-c]quinazoline derivatives. *Medical and Clinical Chemistry*, 2016, vol. 18, no. 1(66), pp. 5-18. Available from: https://doi.org/10.11603/mcch.2410-681X.2016.v0.i1.6123.

14. Zefirov N.S., Palyulin V.A., Dashevskaya E.E. Stereochemical studies. XXXIV. Quantitative description of ring puckering via torsional angles. The case of six-membered rings. *Journal of Physical Organic Chemistry*, 1990, vol. 3, no. 3, pp. 147-158.

15. Zefirov Yu.V. Sokrashchennye mezhmolekularnye kontakty i spetsificheskie vzaimodeistviya v molekularnykh kristallakh [Reduced intermolecular contacts and specific interactions in molecular crystals]. *Kristallographiya*, 1997, vol. 42, pp. 936-958. *(in Russian)*.