Scientific paper

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

Oleksandra Kolomoets,[1] Oleksii Voskoboynik,[1] Oleksii Antypenko,[1] Galyna Berest,[1] Inna Nosulenko,[1] Vitaliy Palchikov,[2] Olexandr Karpenko[3] and Sergiy Kovalenko[1,*]

[1] *Organic and Bioorganic Chemistry Department, Zaporizhzhya State Medical University, 26, Mayakovsky Ave., Zaporizhzhya, 69035, Ukraine*

[2] *Chemistry Department, Oles Honchar Dnipropetrovsk National University, 72, Gagarina Ave., Dnipropetrovsk 49050, Ukraine*

[3] *Enamine Ltd., Oleksandra Matrosova 23, Kyiv, 01103, Ukraine*

** Corresponding author: E-mail: kovalenkosergiy@gmail.com*

Received: 24-05-2017

Abstract

Present work is devoted to the purposeful search of novel promising anti-inflammatory agents among the insufficiently known 3'-R-10'-R_1-spiro[hetaryl-3(4),6'-[1,2,4]triazino[2,3-*c*]quinazolin]-2'(7'*H*)-ones. The virtual combinatorial library of previously unknown spiro-condensed derivatives of [1,2,4]triazino[2,3-*c*]quinazolines was formed and promising COX-2 inhibitors were identified by molecular docking method. Potential anti-inflammatory agents were synthesized by [5+1]-cyclocondensation of substituted 3-(2-aminophenyl)-6-R-1,2,4-triazin-5(2*H*)-ones with heterocyclic ketones. The structures of synthsized compounds were verified by complex of physicochemical methods and spectral characteristics features were discussed. Obtained compounds were studied for anti-inflammatory activity using formalin induced paw edema model and highly active compounds were identified. Conducted SAR-analysis showed that combination of triazino[2,3-*c*] quinazoline moiety with spiro-condensed fragments is a reasonable approach for creating novel anti-inflammatory agents.

Keywords: 6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones, spiro-fused cycle, synthesis, molecular docking, anti-inflammatory activity

1. Introduction

Inflammation is a chain of complex metabolic and morphological changes, aimed to restore the functions of the damaged tissues or organ in general. Despite the fact that the mentioned process is a natural response to a variety of factors, its role in the pathological states requires the development of drugs for pharmacotherapeutic correction. Thus, the use of drugs such as NSAIDs, may correct as certain stages of inflammation, so exclude the process in general.

At the first stages of the antiphlogistics chemistry formation, the carboxylic acids of various nature (aspirin, diclofenac, ibuprofen and others) were considered as privileged objects of studies. Whereas recently, the majority of studies focus on substances with a heterocyclic fragment.[1] This signifies considerable side effects of NSAIDs of first generation (COX-1), namely their negative impact on the gastrointestinal tract (gastrotoxicity). Nowadays, new classes of NSAIDs are found, which to some extent do not have mentioned side effect: selective COX-2 inhibitors (nimesulide, meloxicam, piroxicam, lornoxicam) and highly selective (specific) COX-2 inhibitors (celecoxib, rofecoxib, parecoxib, etoricoxib etc.).[2]

The current strategy of creating anti-inflammatory drugs is inextricably associated with further study of

mechanism of inflammation. Advances in molecular biology in the last decades allowed to characterize every stage of this process and made it possible to form a number of approaches of the creation of this drug group.[3,4] Thus, the main trends in the creation of innovational drugs include the development of C5a receptor antagonists, inhibitors of interleukin converting enzyme and tumor necrosis factor inhibitors, p38 MAP kinase inhibitors, inhibitors of matrix metalloproteinase, etc. Undoubtedly, the mentioned strategy with the use of *de novo* methodology (molecular docking) and X-ray analysis of the macromolecules active-site has significantly changed the direction of the synthetic work aimed at creating the drugs for correcting the inflammation. In particular, innovative anti-inflammatory drug of dual inhibition of COX-2/5-LOX – 2-(2,2-dimethyl-6,7-diphenyl-2,3-dihydro-1*H*-pyrrolizin-5-yl)acetic acid (Licofelone)[5] was found. Moreover, new classes of biologically active substances with the mentioned type of activity were found among triazoles, imidazoles, thiazolidines, 2*H*-benzo[*e*][1,2]thiazine-1,1-dioxide, quinolines, quinazolines and other.[1,6–11] Therefore, rational design based on structural similarity to innovative new structures of NSAIDs using the *de novo* methodology and traditional pharmacological screening is important and justified.

So, purposeful search of selective anti-inflammatory agents among original derivatives of 10-R-3-aryl-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones of spiro-fused cyclic frameworks was the aim of this work. That is based on rational design, namely structural similarity to a number of innovative and well-known drugs and forecasting the likely biological effects (COX inhibitors) using methods of computer modeling and *in vivo* tests.

2. Experimental Section

2. 1. Chemistry

General Methods

Melting points were determined in open capillary tubes in a «Stuart SMP30» apparatus and were uncorrected. Elemental analyses (C, H, N) were performed at the ELEMENTAR vario EL Cube analyzer (USA) and were within ± 0.3% from the theoretical values. IR spectra (4000–600 cm^{-1}) were recorded on a Bruker ALPHA FT-IR spectrometer (Bruker Bioscience, Germany) using a module ATR eco ZnSe. ^{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) spectrometer with TMS as internal standard in DMSO-d_6 solution. LC-MS were recorded using chromatography/mass spectrometric system which consists of a 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 on a Varian 1200 L instrument at 70 eV (Varian Inc., Palo Alto, CA, USA).

Substances **1.1**–**1.5** 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) and COX-2 in association with celecoxib (PDB ID – 3LN1).[13] The choice of biological targets was due to the literature on the mechanism of action of anti-inflammatory drugs.[1]

Ligand preparation. Substances were drawn using MarvinSketch 6.3.0 and were saved in mol format.[14] After, they were optimized by program Chem3D using molecular mechanical MM2 algorithm and saved as pdb files. Molecular mechanics has been used to produce more realistic geometry values for the majority of organic molecules owing to the fact of being highly parameterized. Using AutoDockTools-1.5.6 pdb files were converted to PDBQT, number of active torsions was set as default.[15]

Protein preparation. Pdb files were downloaded from the protein data bank. Discovery Studio 4.0 was used to delete water molecules and ligand from crystal. Proteins were saved as pdb files. 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 = –52.296, center_z = 53.949, size_x = 18, size_y = 16, size_z = 16 for COX-2 (3LN1); center_x = 32.978, center_y = –44.488, center_z = –3.76, size_x = 16, size_y = 16, size_z = 16 for COX-1 (3N8Y). Vina was used to carry docking.[15] For visualization Discovery Studio 4.0 was used.

2. 3. Pharmacology

2. 3. 1. Anti-inflammatory Activity

Evaluation of anti-inflammatory activity of the synthesized compounds was performed on 84 Wistar white rats of 150–160 g, obtained from the nursery «Institute of Pharmacology and Toxicology of Ukraine» (Kyiv). All experimental procedures and treatments were carried out according to the European Convention and «Regulations on the use of animals in biomedical research».[16] Screening of synthesized compounds with estimated anti-inflammatory activity began with the study of their effect on exudative phase of acute aseptic inflammation (the formalin test). Phlogogen (1% aqueous solution of formaline)[17] was subplantally administered at a dose of 0.1 mL in the back right paw of the rat, the left served as control. The studied compounds stabilized by Tween-80 were intragastric ad-

ministered in a dose of 10 mg/kg 1 h before the administration of phlogogen. Reference drug diclofenac sodium was intragastrically administered to rats at a recommended dose for pre-clinical studies of 8 mg/kg. Measuring paws volume was conducted before the experiment and 3 h after the administration of phlogogen using the described[18] methods.

The activity of these substances was determined by their ability to reduce the extension of swelling compared with control and expressed as a percentage showing how the substance inhibited formalin swelling in relation to control swelling where the value was taken as 100%. The activity of the studied compounds was calculated as follows:

$$A = 100\% - \frac{(Vse - Vhe)}{Vsc - Vhc} \quad (1)$$

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

Statistical data processing was performed using a license program «STATISTICA® for Windows 6.0» (StatSoftInc., № AXXR712D833214FAN5) and «SPSS 16.0», «Microsoft Office Excell 2003». The results are 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 is $p < 0.05$.[19]

General Procedure for the Synthesis of 3'-R-10′-R[1]-Spiro[hetaryl-3(4),6'-[1,2,4]triazino[2,3-*c*]quinazolin]-2'(7'*H*)-ones 2.1–2.11

To a solution of 0.01 M of compounds **1.1**–**1.5** in 20 mL of glacial acid was added 0.01 M of appropriate heterocyclic carbonyl compound (tetrahydro-4*H*-pyran-4-one, dihydrothiophen-3(2*H*)-one, dihydro-2*H*-thiopyran-3(4*H*) -one, 1-methylpiperidin-4-one, dihydrothiophen-3(2*H*) -one 1,1-dioxide, dihydro-2*H*-thiopyran-3 (4*H*)-one 1,1 -dioxide). The reaction mixture was refluxed for 3 h. The solvent was removed by vacuum distillation, the residue was triturated in methanol, the precipitate was filtered and dried. If necessary, crystallized from dioxane.

3'-Phenyl-2,3,5,6-tetrahydrospiro[pyran-4,6'-[1,2,4]triazino[2,3-c]quinazolin]-2'(7'H)-one (2.1). Yield: 3.8 g (92.4%); pale yellow crystals; m.p. >300; IR: 3256, 2952, 2856, 1638, 1624, 1611, 1592, 1551, 1514, 1498, 1479, 1435, 1417, 1394, 1334, 1299, 1261, 1214, 1177, 1160, 1150, 1136, 1107, 1097, 1080, 1041, 1027, 1004, 975, 951, 927, 874, 858, 836, 815, 778, 757, 709, 697, 675, 634, 620 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.18 (d, J = 8.4 Hz, 2H, H-2, 6 Ph), 8.02 (d, J = 7.8 Hz, 1H, H-11), 7.55–7.29 (m, 5H, H-9, H-3, 4, 5 Ph, NH), 7.04 (d, J = 8.1 Hz, 1H, H-8), 6.88 (t, J = 7.5 Hz, 1H, H-10), 3.90–3.80 (m, 2H, H-2, 2′,6, 6′ pyrane), 2.56–2.51 (m, 2H, H-3, 5 pyrane), 1.99–1.99 (m, 2H, H-3′, 5′ pyrane); ^{13}C NMR (DMSO-d_6) δ 34.19, 63.26, 75.98, 113.70, 116.81, 118.37, 119.81, 127.36, 128.62, 129.10, 130.27, 130.69, 133.37, 135.46, 144.56, 146.29, 147.28, 161.00. LC-MS: m/z = 347 [M+1]. Anal. Calcd. for $C_{20}H_{18}N_4O_2$: C, 69.35; H, 5.24; N, 16.17. Found: C, 69.38; H, 5.29; N, 16.28.

1-Methyl-3'-phenylspiro[piperidine-4,6'-[1,2,4]triazino[2,3-c]quinazolin]-2'(7'H)-one (2.2). Yield: 2.68 g (74.7%); pale yellow crystals; m.p. 269–271 °C; IR: 3274, 1700, 1637, 1623, 1611, 1594, 1550, 1516, 1498, 1484, 1457, 1436, 1416, 1365, 1336, 1266, 1222, 1202, 1186, 1157, 1139, 1108, 1080, 1064, 1031, 1016, 995, 951, 929, 882, 853, 816, 769, 748, 710, 694, 665, 636 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.21 (d, J = 8.0 Hz, 2H, H-2,6 Ph), 8.02 (d, J = 7.7 Hz, 1H, H-11), 7.54–7.37 (m, 3H, H-9; H-3, 5 Ph), 7.28 (s, 1H, NH), 7.07 (d, J = 7.8 Hz, 1H, H-8), 6.89 (t, J = 7.8 Hz, 1H, H-10), 2.71 (br.s, 2H, H-2, 6 piperidine), 2.51 (m, 2H, H-2′, 6′ piperidine), 2.29 (s, 3H, CH_3), 2.08 (d, J = 7.3 Hz, 2H, H-3, 5 piperidine), 1.92 (m, 2H, H-3′, 5′ piperidine); ^{13}C NMR (DMSO-d_6) δ 33.26, 45.79, 50.84, 76.54, 113.61, 116.77, 119.58, 127.28, 128.59, 129.03, 130.66, 133.36, 135.37, 144.57, 147.12, 152.35, 154.20, 156.50, 161.11. LC-MS: m/z = 360 [M+1]. Anal. Calcd. for $C_{21}H_{21}N_5O$: C, 70.17; H, 5.89; N, 19.48. Found: C, 70.21; H, 5.93; N, 19.53.

1-Methyl-3'-(4-tert-butylphenyl)-spiro[piperidine-4,6'-[1,2,4]triazino[2,3-c]quinazolin]-2'(7'H)-one (2.3). Yield: 3.30 g (79.4%); pale yellow crystals; m.p. 255–257 °C; ^1H NMR (DMSO-d_6) δ 8.13 (d, 2H, H-2, 6 Ph), 8.01 (d, 1H, H-11), 7.53–7.35 (m, 3H, H-9, H-3, 5 Ph), 7.30 (s, 1H, NH), 7.08 (d, 1H, H-8), 6.89 (t, 1H, H-10), 2.85–2.68 (m, 2H, H-2, 6 piperidine), 2.61–2.42 (m, 2H, H-2′, 6′ piperidine), 2.33 (s, 3H, -CH_3), 2.21–2.02 (m, 2H, H-3, 5 piperidine), 2.00–1.81 (m, 2H, H-3′, 5′ piperidine), 1.37 (s, 9H, $C(CH_3)_3$); LC-MS: m/z = 416 [M+1]. Anal. Calcd. for $C_{25}H_{29}N_5O$: C, 72.26; H, 7.03; N, 16.85. Found: C, 72.30; H, 7.11; N, 16.89.

3'-(4-Fluorophenyl)-1-methylspiro[piperidine-4,6'-[1,2,4]triazino[2,3-c]quinazolin]-2'(7'H)-one (2.4). Yield: 3.3 g (88.3%); pale yellow crystals; m.p. 260–262 °C; ^1H NMR (DMSO-d_6) δ 8.32 (t, J = 5.8 Hz, 2H, H-2, 6 Ph), 8.03 (d, J = 7.7 Hz, 1H, H-11), 7.42 (t, J = 7.4 Hz, 1H, H-9), 7.29-7.16 (m, 3H, NH, H-3, 5 Ph), 7.09 (d, J = 8.0 Hz, 1H, H-8), 6.90 (t, J = 7.3 Hz, 1H, H-10), 2.74 (m, 2H, H-2, 6 piperidine), 2.55 (m, 2H, H-2′, 6′ piperidine), 2.32 (s, 3H, -CH_3), 2.09 (m, 1H, H-3, 5 piperidine), 1.92 (m, 2H, H-3′, 5′ piperidine); ^{13}C NMR (DMSO-d_6) δ 33.15, 45.70, 50.78, 76.55, 113.57, 115.60 (d, J = 21.5 Hz), 116.78, 119.59, 127.30, 129.80 (d, J = 2.8 Hz), 131.49 (d, J = 8.6 Hz), 135.40, 144.56, 146.06, 152.36, 152.49, 161.10, 163.74 (d, J = 248.3 Hz), 172.54. LC-MS: m/z = 378 [M+1]; Anal. Calcd. for $C_{21}H_{20}FN_5O$: C, 66.83; H, 5.34; N, 18.56; Found: C, 66.89; H, 5.41; N, 18.64.

3'-(4-Methoxyphenyl)-1-methylspiro[piperidine-4,6'-[1,2,4]triazino[2,3-c]quinazolin]-2'(7'H)-one (2.5). Yield: 3.68 g (94.6%); pale yellow crystals; m.p. 270–272 °C; ^{1}H NMR (DMSO-d_6) δ 8.25 (d, J = 8.6 Hz, 2H, H-2, 6 Ph), 8.01 (d, J = 7.4 Hz, 1H, H-11), 7.40 (t, J = 6.9 Hz, 1H, H-9), 7.23 (s, 1H, NH), 7.08 (d, J = 8.0 Hz, 2H, H-8), 6.97 (d, J = 8.7 Hz, 2H, H-3, 5 Ph), 6.89 (t, J = 7.3 Hz, 1H, H-10), 3.86 (s, 3H, CH_3), 2.73 (br.s, 2H, H-2, 6 piperidine), 2.51 (brs, 2H, H-2′, 6′ piperidine), 2.31 (s, 3H, -CH_3), 2.09 (m, 1H, H-3, 5 piperidine), 1.92 (brs, 2H, H-3′, 5′ piperidine); ^{13}C NMR (DMSO-d_6) δ 33.24, 45.78, 50.84, 55.76, 76.43, 113.70, 114.06, 114.27, 116.72, 119.53, 125.65, 127.18, 130.69, 131.65, 144.46, 146.56, 151.98, 161.21, 161.42; LC-MS: m/z = 390 [M+1]. Anal. Calcd. for $C_{22}H_{23}N_5O_2$: C, 67.85; H, 5.95; N, 17.98. Found: C, 67.91; H, 6.02; N, 18.02.

10'-Bromo-1-methyl-3'-phenylspiro[piperidine-4,6'-[1,2,4]triazino[2,3-c]quinazolin]-2'(7'H)-one (2.6). Yield: 3.8 g (86.6%); pale yellow crystals; m.p. 270–272 °C; ^{1}H NMR (DMSO-d_6) δ 8.21 (d, J = 7.8 Hz, 2H, H-2, 6 Ph), 8.11 (s, 1H, H-11), 7.56–7.33 (m, 5H, H-9; NH; H-3, 4, 5 Ph), 7.05 (d, J = 8.4 Hz, 1H, H-8), 2.73 (m, 2H, H-2, 6 piperidine), 2.51 (m, 2H, H-2′, 6′ piperidine), 2.30 (s, 3H, -CH_3), 2.14–1.99 (m, 2H, H-3, 5 piperidine), 1.92 (m, 2H, H-3′, 5′ piperidine); EI-MS, m/z (I_{rel}, %) = 292 (6.2), 290 (5.9), 278 (7.5), 276 (6.5), 266 (5), 265 (18), 264 (6.3), 263 (19), 223 (12.1), 155 (5.4), 116 (8.9), 115 (5.3), 109 (11.1), 104 (20.3), 103 (71.9), 102 (6.4), 77 (14.8), 76 (22.6), 75 (5.8), 72 (32.2), 71 (32.5), 70 (100), 69 (6.1), 68 (8.1), 63 (12.3), 58 (17), 57 (41.7), 56 (20.8), 54 (10.3), 53 (6.8), 45 (11.2); LC-MS: m/z = 440 [M+2]. Anal. Calcd. for $C_{21}H_{20}BrN_5O$: C, 57.54; H, 4.60; N, 15.98. Found: C, 57.50; H, 4.57; N, 15.93.

3'-Phenyl-2,3,5,6-tetrahydrospiro[thiopyran-4,6'-[1,2,4]triazino[2,3-c]quinazolin]-2'(7'H)-one (2.7). Yield: 3.5 g (89.0%); pale yellow crystals; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 8.19 (d, J = 7.6 Hz, 1H, H-2, 6 Ph), 8.02 (d, J = 8.0 Hz, 1H, H-11), 7.51–7.43 (m, 3H, H-3, 4, 5 Ph), 7.40 (t, 1H, H-9), 7.26 (s, 1H, NH), 7.10 (d, J = 7.9 Hz, 1H, H-8), 6.89 (t, J = 7.3 Hz, 1H, H-10), 3.25–3.12 (m, 2H, H-2, 6 thiopyran), 2.52 (m, 6H, H-2′, 3, 3′, 5, 5′, 6′ thiopyran); ^{13}C NMR (DMSO-d_6) δ 23.71, 35.25, 77.54, 113.41, 116.63, 119.74, 127.24, 128.61, 129.08, 130.71, 133.32, 135.51, 144.36, 147.29, 152.07, 161.05; LC-MS: m/z = 363[M+1]. Anal. Calcd. for $C_{20}H_{18}N_4OS$: C, 66.28; H, 5.01; N, 15.46. Found: C, 66.31; H, 5.09; N, 15.49.

3'-Phenyl-4,5-dihydro-2H-spiro[thiophene-3,6'-[1,2,4]triazino[2,3-c]quinazolin]-2'(7'H)-one (2.8). Yield: 2.8 g (81.6%); m.p. 263–265 °C; pale yellow crystals; IR: 3299, 3076, 2926, 1714, 1640, 1626, 1615, 1598, 1551, 1503, 1486, 1440, 1411, 1338, 1312, 1271, 1249, 1221, 1191, 1170, 1154, 1107, 1080, 1028, 992, 948, 926, 867, 815, 776, 750, 692 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.17 (d, J = 4.9 Hz, 2H, H-2,6 Ph), 8.03 (d, J = 7.6 Hz, 1H, H-11), 7.66 (s, 1H, NH), 7.44–7.34 (m, 3H, H-9, H-3,5 Ph), 6.95 (d, J = 7.9 Hz, 1H, H-8), 6.88 (t, J = 7.9 Hz, 1H, H-10), 3.51 (d, J = 11.6 Hz, 1H, H-2 thiophene), 3.13 (d, J = 11.2 Hz, 1H, H-2′ thiophene), 3.10–2.92 (m, 2H, H-5, 5′ thiophene), 2.62–2.41 (m, 2H, H-4, 4′ thiophene); ^{13}C NMR (DMSO-d_6) δ 27.85, 39.14, 39.41, 87.60, 113.17, 116.05, 119.72, 127.49, 128.61, 129.16, 130.69, 133.29, 135.48, 145.53, 147.02, 152.34, 161.22. LC-MS: m/z = 349 [M+1]. Anal. Calcd. for $C_{19}H_{16}N_4OS$: C, 65.50; H, 4.63; N, 16.08. Found: C, 65.61; H, 4.75; N, 16.12.

3'-Phenyl-5,6-dihydro-2H,4H-spiro[thiopyran-3,6'-[1,2,4]triazino[2,3-c]quinazolin]-2'(7'H)-one (2.9). Yield: 3.3 g (91.8%); pale yellow crystals; m.p. 274–276 °C; IR: 3272, 2891, 1641, 1626, 1609, 1594, 1553, 1515, 1499, 1483, 1442, 1414, 1333, 1312, 1298, 1273, 1249, 1188, 1153, 1103, 1076, 1030, 1012, 980, 950, 923, 872, 859, 825, 814, 787, 774, 749, 694, 670 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.18 (d, J = 5.3 Hz, 2H, H-2, 6 Ph), 8.00 (d, J = 7.7 Hz, 1H, H-11), 7.52–7.29 (m, 5H, H-9, H-3, 4, 5 Ph, NH), 7.17 (d, J = 8.0 Hz, 1H, H-8), 6.86 (t, J = 7.2 Hz, 1H, H-10), 3.38 (d, J = 13.4 Hz, 1H, H-2 thiopyran), 2.92 (d, J = 13.2 Hz, 1H, H-2` thiopyran), 2.78–2.59 (m, 1H, H-6 thiopyran), 2.55–2.05 (m, 5H, H-4, 4′, 5, 5′, 6′ thiopyran); ^{13}C NMR (DMSO-d_6) δ 24.37, 27.02, 33.55, 34.76, 75.81, 112.99, 116.56, 116.62, 119.51, 127.27, 128.55, 129.20, 130.68, 133.32, 135.46, 144.45, 144.51, 152.17, 161.04. LC-MS: m/z = 363 [M+1]. Anal. Calcd. for $C_{20}H_{18}N_4OS$: C, 66.28; H, 5.01; N, 15.46. Found: C, 66.30; H, 5.03; N, 15.49.

3'-Phenyl-4,5-dihydro-2H-spiro[thiophene-3,6'-[1,2,4]triazino[2,3-c]quinazolin]-2'(7'H)-one 1,1-dioxide (2.10). Yield: 2.1 g (56.1%); pale yellow crystals; m.p. 266–269 °C; ^{1}H NMR (DMSO-d_6) δ 8.25 (d, J = 5.3 Hz, 1H, H-2, 6 Ph), 8.04 (d, J = 7.5 Hz, 1H, H-11), 7.97 (s, 1H, NH), 7.49–7.41 (m, 4H, H-9, H-3, 4, 5 Ph), 7.04–6.84 (m, J = 7.7 Hz, 2H, H-8, 10), 4.24 (d, J = 14.9 Hz, 1H, H-2 thiophene), 3.50 (d, J = 15.0 Hz, 1H, H-2′ thiophene), 3.47–3.34 (m, 2H, H-5, 5′ thiophene), 3.30–3.03 (m, 1H, H-4 thiophene), 2.74–2.55 (m, 1H, H-4′ thiophene); LC-MS: m/z = 381 [M+1]. Anal. Calcd. for $C_{19}H_{16}N_4O_3S$: C, 59.99; H, 4.24; N, 14.73. Found: C, 60.03; H, 4.27; N, 14.77.

3'-Phenyl-5,6-dihydro-2H,4H-spiro[thiopyran-3,6'-[1,2,4]triazino[2,3-c]quinazolin]-2'(7'H)-one1,1-dioxide (2.11). Yield: 4.5 g (91.8%); pale yellow crystals; m.p. 296–299 °C; ^{1}H NMR (DMSO-d_6) δ 8.25 (d, J = 5.1 Hz, 2H, H-2, 6 Ph), 8.05 (d, J = 7.7 Hz, 1H, H-11), 7.46 (m, 4H, H-9, H-3, 4, 5 Ph), 7.14 (s, 1H, NH), 7.06 (d, J = 8.0 Hz, 1H, H-8), 6.96 (t, J = 7.3 Hz, 1H, H-10), 3.97 (d, J = 13.9 Hz, 1H, H-2 thiopyran), 3.53 (d, J = 15.4 Hz, 1H,H-2′ thiopyran), 3.50–3.35 (m, 1H, H-6 thiopyran), 3.23–2.91 (m, 1H, H-6′ thiopyran), 2.90–2.63 (m, 1H, H-4 thiopyran), 2.34 (m, 3H, H-4′, 5, 5′ thiopyran); ^{13}C NMR (DMSO-d_6) δ 18.09, 31.64, 50.06, 54.57, 77.78, 113.38, 116.95, 120.21, 127.32, 128.50, 129.38, 130.80, 133.18, 135.61, 143.78, 147.26, 151.84, 161.03; LC-MS: m/z = 395 [M+1]. Anal. Calcd. for

$C_{20}H_{18}N_4O_3S$: C, 60.90; H, 4.60; N, 14.20. Found: C, 60.93; H, 4.64; N, 14.26.

2. Results and Discussion

Considering the roles of cyclooxygenases (COX-1 and COX-2) as important pharmacological targets and their inhibitors are the basis for the developing of anti-inflammatory drugs, in the first phase of the study the virtual base (80 compounds) of 3'-R-10'-R^1-spiro [hetaryl-3(4),6'-[1,2,4]triazino[2,3-*c*]quinazolin]-2'(7'*H*)-ones was analyzed using molecular docking. Virtual structural modification of studied compounds, which have heterocyclic fragments at spiro position (position 6) was held by the 3^d position and at positions 8, 9, 10 and 11 of the aromatic ring. Complexes of COX-1 and COX-2 were downloaded from the Protein Data Bank, to determine the affinity (Table 1), http://www.rcsb.org/pdb/home/home.do. As a reference known selective COX-1 and COX-2 inhibitors were used, such as diclofenac sodium and celecoxib. Results of the studies showed that the structures analyzed have a higher affinity to COX-2 and much lower to COX-1. Among 80 tested compound, 7 with the highest affinity, according to the docking, are presented in the Table 1. Compound **2.10** revealed the highest affinity, yet still lower than celecoxib.

The visualization of the interaction of the structures with the active site of COX-2 (Fig. 1) showed, that compound **2.10** revealed the highest affinity (11.5 kcal/mol). There were four hydrogen bonds with the following amino acid residues D:TYR341 (3.08 Å), D:HIS75 (3.37 Å), D:GLY512 (3.33 Å), D:SER516 (3.63 Å), and besides π-cation electrostatic interaction with D:ARG106 (4.86 Å), π-sigma hydrophobic interactions with D:VAL335 (3.76 Å), D:VAL509 (3.40 Å, 3.65 Å), D:ALA513 (3.82 Å), π-sulfur interaction with D:TRP373 (5.93 Å), π-π stacked hydrophobic interaction with D:HIS75 (5.26 Å) and π-alkyl hydrophobic interactions with D:LEU517 (5.14 Å), D:ARG499 (5.27 Å), D:ALA502 (4.31 Å). Analyzing the complex of celecoxib and compound **2.10** with COX-2, similar interactions can be traced, namely with such amino acids D:ARG106, D:ARG499, D:VAL335, D:VAL509, D:LEU517, D:TRP373, D:ALA513. This may indicate that stated class of compound might have the ability to inhibit the COX-2 as celecoxib does.

The second stage, namely the synthesis of the corresponding spiro-derivatives **2** provided the interaction of substituted 6-R^1-3-(2-aminophenyl)-1,2,4-triazine-5(2*H*)-ones **1** and the corresponding heterocyclic ketones (Scheme 1). The heterocyclization was carried out by known method,[20] namely the refluxing in glacial acid.

Synthesized spiro-derivatives **2** are pale yellow crystalline substances, soluble in DMF, slightly soluble in dioxane, insoluble in alcohols and water. The compounds' structures were established with elemental analysis, LC-MS-data, IR and NMR spectra. Thus, triazino[2,3-*c*]quinazoline fragment of compounds **2** in ^{1}H NMR spectra has the appropriate chemical shifts and multiplicity: 8.11–8.00 ppm (H-11, d), 7.42–7.40 ppm (H-9, t), 7.17–6.95 ppm (H-8, d), and 6.96–6.86 ppm (H-10, t). It is important to note, that H-9 in most cases resonated together with the aromatic protons of substituent at the position 3 and NH proton of position 7 as multiplets. Whereas, protons H-11, H-8 and H-10 have

Table 1. Results of molecular docking of the most active structures

Comp.	Affinity (kcal/mol) to COX-1	Affinity (kcal/mol) to COX-2	Types of interactions with amino acid residues of COX-2
2.1	–7.4	–10.1	D:ARG106[b], D:VAL509[c], D:ALA513[c], D:HIS75[c], D:VAL335[c], D:LEU517[c], D:ARG499[c], D:ALA502[c].
2.2	–3.6	–10.3	D:ARG106[b], D:VAL509[c], D:ALA513[c], D:HIS75[c], D:VAL335[c], D:LEU517[c], D:ARG499[c], D:ALA502[c].
2.7	–5.2	–9.0	D:ARG106[b], D:VAL509[c], D:ALA513[c], D:TRP373[d], D:HIS75[c], D:LEU338[c], D:PHE504[c], D:VAL335[c], D:LEU517[c], D:ALA502[c].
2.8	–3.3	–10.4	D:HIS75[a], D:VAL335[c], D:VAL509[c], D:ALA513[c], D:TRP373[d], D:HIS75[c], D:LEU338[c], D:PHE504[c], D:LEU517[c], D:ARG499[c], D:ALA502[c].
2.9	–1.3	–10.6	D:TYR341[a], D:HIS75[a], D:VAL509[c], D:ALA513[c], D:SER339[c], D:VAL335[c], D:LEU338[c], D:LEU517[c], D:ALA502[c].
2.10	–1.4	–11.5	D:TYR341[a], D:HIS75[a], D:GLY512[a], D:SER516[a], D:ARG106[b], D:VAL335[c], D:VAL509[c], D:ALA513[c], D:TRP373[d], D:HIS75[c], D:LEU517[c], D:ARG499[c], D:ALA502[c].
2.11	–0.4	–10.9	D:TYR341[a], D:HIS75[a], D:SER516[c], D:VAL509[c], D:ALA513[c], D:LEU338[c], D:SER339[c], D:VAL335[c], D:LEU517[c], D:ALA502[c].
Celecoxib	–	–12.1	D:ARG106[a], D:ARG499[a], D:GLN178[a], D:LEU338[a], D:SER339[a], D:VAL335[c], D:CEL682[c], D:VAL509[c], D:LEU370[c], D:VAL335[c], D:LEU345[c], D:LEU517[c], D:TYR371[c], D:TRP373[c], D:ALA513[c].

a – hydrogen, b – electrostatic, c – hydrophobic, d – others.

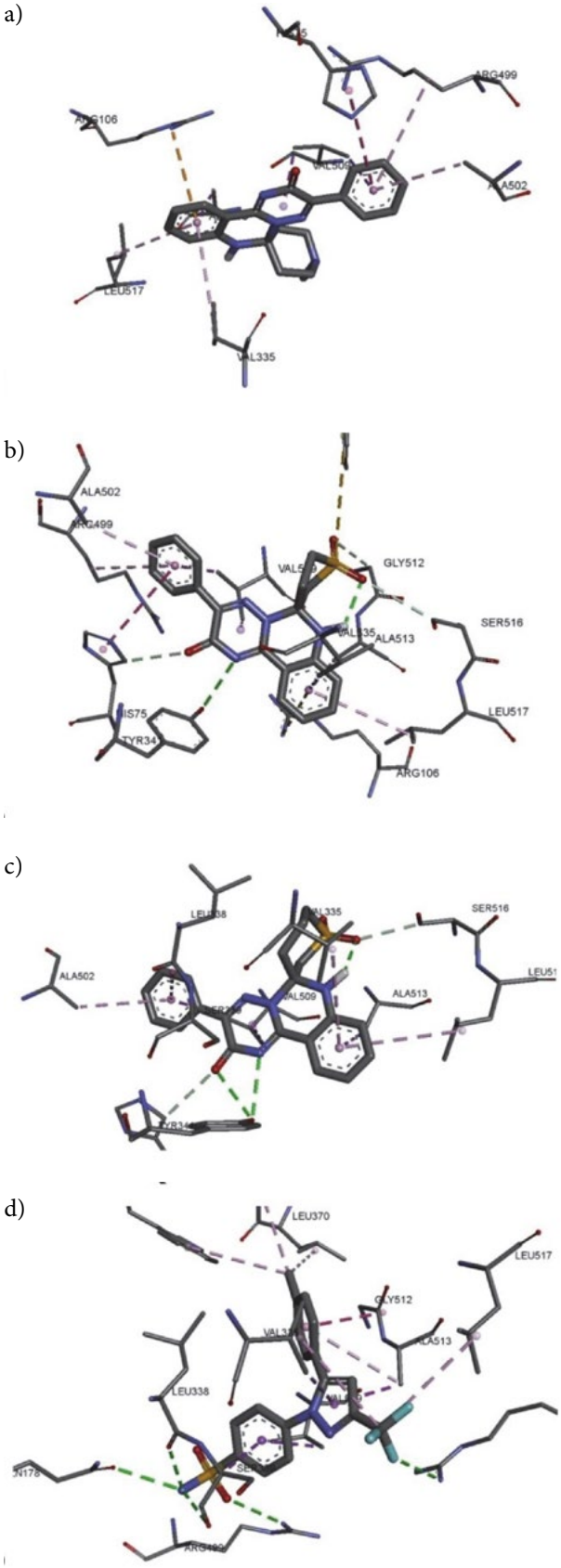


Figure 1. Interaction of compound **2.2** (a), **2.10** (b), **2.11** (**c**), celecoxib (**d**) with COX-2, PDB ID: 3LN1

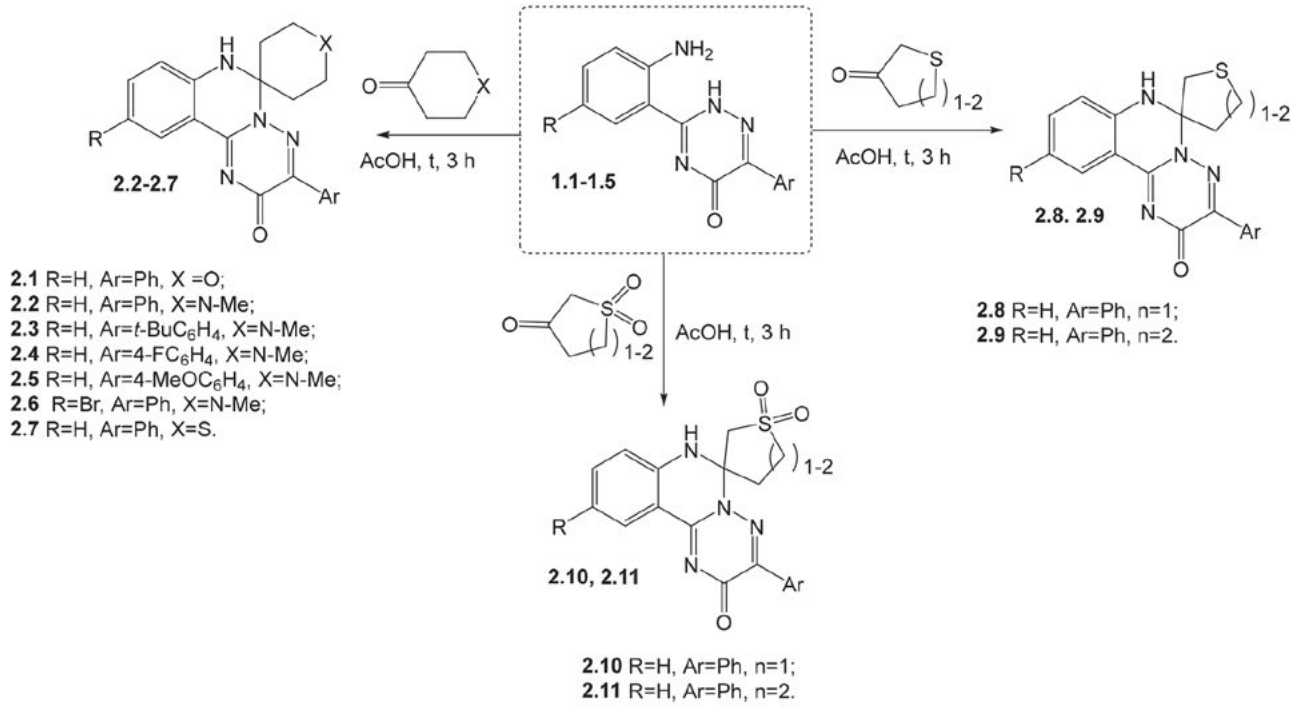
classical multiplicity and *J*-coupling (*J* = 8.05–7.3 Hz).[21] Phenyl moiety in position 3 of ^{1}H NMR spectrum resonated as two proton singlets, H-2,6 at 8.25–8.17 ppm and three proton multiplets of H-3,4,5 at 7.56–7.35 ppm. Signals of *p*-substituted phenyl derivatives **2** were registered as A_2B_2-systems, as two doublets H-2,6 and H-3,5.

The characteristic singlets of NH protons (position 7) in ^{1}H NMR spectra of compounds **2** were observed at 7.97–7.14 ppm and their chemical shift depended on the electronic effects of spiro moiety at position 6. Thus, the signal of NH proton of compound **2.1** with spiropyran cycle were detected at 7.30 ppm, with spiropiperidine (**2.2**–**2.6**) at 7.33–7.23 ppm, with spirothiopyran (**2.7**, **2.9**) at 7.29–7.26 ppm and with spirothiophene (**2.8**) cycle at 7.66 ppm. In ^{1}H NMR spectra of compound **2.11** (oxidized analogue of compound **2.9**) signal of NH proton were shifted to a higher field (7.14 ppm). Whereas, the stated proton of compound **2.10** resonated at a lower field, namely at 7.97 ppm, compared to compound **2.8**, what is likely due to the formation of hydrogen bond between nearby structural fragments.

In the ^{1}H NMR spectra of compounds **2.1**–**2.11** proton signals of position 6 of spiro moiety were recorded as a complex set of multiplets in aliphatic part of spectrum due to the presence of magnetic nonequivalent axial and equatorial protons.[21] Whereas, ^{1}H NMR spectra of compounds **2.8**, **2.9** have characteristic features, which were associated with the presence in the molecule of a center of asymmetry. Thus, in the spectra of mentioned compounds protons of methylene group of thiophene at position 2 or thiopyran were registered as doublets at 3.13 ppm H_{ax} (*J* = 11.2 Hz), 2.92 ppm (*J* = 13.2 Hz) and H_{eq} at 3.51 ppm (*J* = 11.6 Hz), 3.38 ppm (*J* = 13.4 Hz), respectively. A similar pattern of signals was characteristic for their dioxo analogues (compounds **2.10**, **2.11**).

In the ^{13}C NMR spectra of compounds **2** characteristic signal of sp^3-hybridized C in spiro position (position 6) was observed at 87.60–75.81 ppm, and its chemical shift was determined by electron withdrawing effect of the spiro cycle. In addition, further structure confirmation was made by the corresponding ^{13}C signals of hydrogenated pyran cycle (compound **2.1**) at 34.19 and 63.26 ppm, *N*-methylpiperidine cycle (**2.2**, **2.4**, **2.5**) at 33.26–33.15 ppm, 45.79–45.70 ppm and 50.84–50.78 ppm and hydrogenated thiopyrane cycle (**2.7**) at 23.71 ppm and 35.28 ppm at 4,6′-spiro position. While other electronic environments of carbon atoms of hydrogenated thiophene (**2.8**) and thiopyran (**2.9**) cycle at 3,6′-spiro position caused a series of four signals at a high field. A similar pattern of signals was characteristic for dioxo analogue compound **2.11**.

Mass spectrometric investigation showed, that compound **2.6** was characterized by a low-intensity molecular ion, that formed high-intensity fragmentation ions F_1 $[C_6H_5CH{=}N]^+$ (*m/z* 103, 71.9%), F_2 $[CH_2N(Me)CH_2{=}CH_2]^+$ (*m/z* 70, 100%) and F_3 $[C_{10}H_6BrN_3O]^+$ (*m/z* 265/263, 18.0/19.0%). The last fraagmented ion described



Scheme 1. The heterocyclization of 6-R[1]-3-(2-aminophenyl)-1,2,4-triazine-5(2*H*)-ones

the presence of bromine in the molecule and its isotopic profile. Further carbon-carbon bond breaking and hydrogen rearrangements in F_2 created intense ions $[CH_2NHCH_2{=}CH_2]^+$ (*m/z* 57, 41.7%) and $[CH_2NCH_3]^+$ (*m/z* 45, 11.2%), indicating the presence of *N*-methylpiperidine fragment in the molecule.[22]

3. Pharmacology

Formalin acute inflammation is characterized by a powerful inflammatory response, which in 3 h of the experiment can be verified by significant swelling of the paw in the control group of animals (average increase in volume of the paw is 47.38%). Administration of compounds **2** to the animals with experimental pathology led to a decrease of exudative reactions and most of compounds exhibited anti-inflammatory action comparable (compounds **2.2**, **2.6**, **2.7**, **2.10**) or higher (**2.3**–**2.5**, **2.8**, **2.11**) than the effect of the reference diclofenac sodium (Table 2). SAR-analysis (influence of substituents at positions 3 and 6) showed, that 3'-phenyl-2,3,5,6-tetrahydrospiro[pyran-4,6'-[1,2,4]triazino[2,3-*c*]quinazolin]-2'(7'*H*)-one (**2.1**) showed moderate anti-inflammatory effect (higher in the

Table 2. Anti-inflammatory activity of synthesized compounds under formalin induced inflammation model

Comp.	Dosage, mg/kg	Increase of the paw volume in 3 h, %	Anti-inflammatory activity, %
Experimental pathology	–	47.38	0
2.1	10.0	24.22	39.39
2.2	10.0	26.28	44.44
2.3	10.0	15.43	69.19
2.4	10.0	20.47	56.57
2.5	10.0	6.74	85.86
2.6	10.0	24.75	48.48
2.7	10.0	22.60	44.44
2.8	10.0	21.61	55.56
2.9	10.0	33.45	29.29
2.10	10.0	23.91	49.49
2.11	10.0	20.04	60.61
Diclofenac sodium	8.0	26.58	45.45

control group to 39.4%). Replacing the hydrogenated spiropyran (**2.1**) by 1-methyl-piperidine (**2.2**) fragment at the spiro position 6 increased activity to 44.44% compared with the control. The intensity of anti-inflammatory activity of compound **2.2** is comparable with diclofenac sodium. The position of sulfur in spirocycle of the isomer hydrogenated spirothiopyranes (**2.7**, **2.9**) determined the anti-inflammatory activity. Thus, the compound **2.7** with 4,6′-spiro position of thiopyran towards to triazinoquinazoline cycle exhibited anti-inflammatory activity at the level of diclofenac sodium. Relocation of sulfur in the thiopyran cycle (3,6′-spiro position, compound **2.9**) led to a significant decrease of activity, whereas contraction of thiopyran cycle by a homologous unit (**2.8**) in contrast increased activity to 11.11% (compared with diclofenac). The structural similarity of compounds **2.10** and **2.11** to the celecoxib-like drugs, as we consider led to high anti-inflammatory activity.

Modification of position 3 substituent of triazinoquinazoline cycle *via* replacing of phenyl substituent by the 4-*tert*-butylphenyl (**2.3**), 4-fluorophenyl (**2.4**) or 4-methoxyphenyl (**2.5**) was substantiated and led to increasing of anti-inflammatory activity at 11.12-40.21%, compared with the reference drug (Tab. 2). While, the introduction of additional bromine (**2.6**) at the 10th position of compound **2.2** led to a loss of activity compared to the compounds **2.3**-**2.5**. Such, this way of modification of the molecule was not promising.

4. Conclusions

Based on the methodology of purposeful search of NSAIDs an effective method for the synthesis of 6-spirofused 10-R-3-aryl-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones (a promising class of anti-inflammatory agents) was proposed. Structural features of the synthesized compounds, as well as their ^{1}H, ^{13}C NMR spectroscopy and mass spectrometry data were discussed. It was established that compounds **2.3**–**2.5**, **2.8** and **2.10** under formaline-induced paw edema model revealed the activity higher comparing to the reference drug – diclofenac sodium. SAR analysis showed that combination of triazine[2,3-*c*]quinazoline cycle with other heterocyclic fragments is reasonable in scope of novel NSAIDs creation and calls for further research.

5. References

1. J. I. Levin, S. Laufer, Anti-Inflammatory drug discovery. *Royal Society of Chemistry Series* No. 26, Cambridge, **2012**, 528 p. **DOI:**10.1039/9781849735346.
2. G. A. FitzGerald, C. Patrono, *N. Engl. J. Med.* **2001**, *345(6),* 433–442. **DOI:**10.1056/NEJM200108093450607.
3. R. G. Kulkarni, G. Achaiah, G. N. R. Sastry, *Curr. Pharm. Des.* **2006**, *12*, 2437–2454. **DOI:**10.2174/138161206777698945.
4. B. Botz, K. Bölcskei, Z. Helyes, Challenges to develop novel anti-inflammatory and analgesic drugs, *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.* **2017**, *9(3)*, 1427. **DOI:**10.1002/wnan.1427.
5. S. K. Kulkarni, V. P. Singh, *Curr. Top. Med. Chem.* **2007**, *7*, 251–263. **DOI:**10.2174/156802607779941305.
6. R. Paprocka, M. Wiese, A. Eljaszewicz, A. Helmin-Basa, A. Gzella, B. Modzelewska-Banachiewicz, J. Michalkiewicz, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 2664–2667. **DOI:**10.1016/j.bmcl.2015.04.079.
7. A. Puratchikody, M. Doble, *Bioorg. Med. Chem.***2007**, *15*, 1083–1090. **DOI:**10.1016/j.bmc.2006.10.025.
8. C. D. Barros, A. A. Amato, T. B. de Oliveira, K. B. R. Iannini, A. L. da Silva, T. G. da Silva, E. S. Leite, M. Z. Hernandes, M. do C. A. de Lima, S. L. Galdino, F. de A. R. Neves, I. da R. Pitta, *Bioorg. Med. Chem.***2010**, *18*, 3805–3811. **DOI:**https://doi.org/10.1016/j.bmc.2010.04.045.
9. Y.-L. Chen, Y.-L. Zhao, C.-M. Lu, C.-C. Tzeng, J.-P. Wang, *Bioorg. Med. Chem.***2006**, *14*, 4373–4378. **DOI:**10.1016/j.bmc.2006.02.039.
10. X. Wen, S.-B. Wang, D.-C. Liu, G.-H. Gong, Z.-S. Quan, *Med. Chem. Res.* **2015**, *24*, 2591–2603. **DOI:**10.1007/s00044-015-1323-y.
11. M. A. Hussein, *Med. Chem. Res.* **2013**, *22*, 4641–4653. **DOI:**10.1007/s00044-013-0468-9.
12. T. Yu. Sergeieva, O. Yu. Voskoboynik, S. I. Okovytyy, S. I. Kovalenko, S. V. Shishkina, O. V. Shishkin, J. Leszczynski, *J. Phys. Chem. A* **2014**, *118*, 1895–1905. **DOI:**10.1021/jp4052616.
13. Protein Data Bank, pdb. Retrieved from http://www.pdb.org
14. MarvinSketch version: 6.3.0, **2015**, ChemAxon (http://www.chemaxon.com).
15. O. Trott, A. J. Olson, *J. Comput. Chem.* **2010**, *31*, 455–461. **DOI:**10.1002/jcc.21334.
16. European convention for the protection of vertebrate animal used for experimental and other scientific purposes. Council of Europe, Strasbourg, **1986**.
17. J. D. J. Liégeois, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1999**, *359*, 220–227. **DOI:**10.1007/PL00005345.
18. N. Shejawal, S. Menon, S. Shailajan, *Hum. Exp. Toxicol.* **2014**, *33*, 123–129. **DOI:**10.1177/0960327113482594.
19. M. Pagano, K. Gauvreau, Principles of Biostatistics, Kimberlee Gauvreau Statistics Series, Duxbury Press, **1994**, 524 p.
20. S. V. Kholodnyak, K. P. Schabelnyk, O. Yu. Voskoboynik, O. M. Antypenko, S. I. Kovalenko, V. O. Palchykov, S. I. Okovyty, S. V. Shishkina, *J. Org. Pharm. Chem.* **2016**, *14,3(55)*, 24–31.
21. E. Breitmaier. Structure Elucidation by NMR in Organic Chemistry: A Practical Guide, Third Edition, **2002**, Wiley, 270 p.
22. C. Dass. Fundamentals of contemporary mass spectrometry, **2007**, John Wiley & Sons, Inc., 608 p.

Povzetek

Naše raziskave smo usmerili v načrtno iskanje novih, obetavnih antiinflamatornih spojin, zlasti med relativno neznanimi 3'-R-10'-R_1-spiro[heteroaril-3(4),6'-[1,2,4]triazino[2,3-*c*]kinazolin]-2'(7'*H*)-oni. Pripravili smo virtualno kombinatorialno knjižnico doslej neznanih spiro-pripojenih derivatov [1,2,4]triazino[2,3-*c*]kinazolinov ter s pomočjo metod molekulskega sidranja identificirali najbolj obetavne inhibitorje COX-2. Nato smo te potencialne spojine, ki bi lahko imele protivnetni učinek, sintetizirali s pomočjo [5+1] ciklokondenzacij substituiranih 3-(2-aminofenil)-6-R-1,2,4-triazin-5(2*H*)-onov s heterocikličnimi ketoni. Strukture pripravljenih spojin smo določili na osnovi fizikalno-kemijskih metod in spektroskopskih značilnosti. Za dobljene spojine smo določili antiinflamatorno aktivnost z uporabo modela s formalinom induciranega edema šape testnih živali. Na ta način smo identificirali najbolj aktivne spojine. Izvedena analiza primerjave aktivnosti od strukture spojin (SAR analiza) je pokazala, da je kombinacija triazino[2,3-*c*]kinazolinskega in spiro-pripojenega fragmetna smiselen pristop k pripravi še novih antiinflamatornih zdravil.