Non-steroidal anti-inflammatory drugs (NSAIDs) are a common and diverse group of compounds that are mainly used to treat patients suffering from pain and inflammation (chronic pain, osteoarthritis, rheumatoid arthritis, postoperative surgical conditions, etc.) [1, 2]. In addition, this group of drugs is used as analgesics and antipyretics. Aryl carboxylic acids and their derivatives, which were among the first to be introduced into medical practice, are still important among NSAIDs [3]. In addition to the well-known salicylic and acetylsalicylic acids, this group of anti-inflammatory agents includes salts of salicylic acid, salsalate and diflunisal. Recently, representatives of anthranilic (2-aminobenzoic) acid derivatives have also been widely used, namely fenamates (mefenamic, meclofenamic, flufenamic and tolfenamic acids).

Despite the interclass chemical diversity of NSAIDs, their pharmacological action is based on the inhibition of cyclooxygenase (COX)/prostaglandin-endoperoxide synthase (PGHS-1 and PGHS-2), of regulatory enzymes involved in the biosynthesis of prostaglandins (PG) – mediators of inflammation. However, due to non-selective inhibition of COX-1 and COX-2, the group of NSAIDs, despite its high efficiency, has significant side effects (complications of the gastrointestinal tract, cardiovascular system, liver, kidneys, etc.) [4]. With this in mind, medical chemists still pay considerable attention to the design and synthesis of NSAIDs [5]. Undoubtedly, these studies are devoted firstly to the modification of the carboxyl group to esters; secondly, its «bioisosteric» replacement by other structural fragments; thirdly, the search for new compounds with anti-inflammatory activity among heterocyclic compounds. These modifications among NSAIDs are carried out to increase their selectivity for biological targets, search for compounds that would affect other biological targets (phospholipase, lipoxygenase), solubility, redox processes, etc. [5]. Given the above facts, the creation of hybrid molecules that would combine in one structure a fragment with anti-inflammatory activity (phenylcarboxyl group) and a quinazoline heterocycle, which is also characterized by this activity [6-8], is a promising and relevant direction. Moreover, molecular pharmacologists have explained the key role of the «pharmacophore» carboxyl group in the formation of enzyme-ligand interactions and the effect of its presence on activity and selectivity [9-12].

Therefore, the aim of the present study is to develop methods for the synthesis of [(1,2,4]triazolo[1,5-c]quinazolin-2-yl)benzoic acids as potential anti-inflammatory agents.
Materials and methods

Melting points were determined in open capillary tubes in a «Stuart SMP30» apparatus and were uncorrected. The elemental analyses (C, H, N) were performed using the «ELEMENTAR vario EL cube» analyzer. $^1$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). The purity of all obtained compounds was checked by $^1$H-NMR and LC-MS.

Synthetic studies were conducted according to general approaches to the search for potential biologically active substances, using reagents from Merck (Darmstadt, Germany), Sigma-Aldrich (Missouri, USA) and Enamine (Kyiv, Ukraine).

Benzendicarboxylic acids monoesters (monoethyl isophthalate, monoethyl terephthalate, monobutyl phthalate) and 4-hydrazinoquinazoline (1.1) for the synthetic part of the work were obtained by known methods with constants that correspond to the literature [13, 14].

Methods for the synthesis of alkyl 2(3-, 4-)(2-(quinazolin-4(3H)-ylidene)hydrazine-1-carbonyl)benzoates (2). To a solution of 0.01 M monoesters of benzendicarboxylic acids (monoethyl isophthalate, monoethyl terephthalate, monobutyl phthalate) in 10 ml of anhydrous dioxane was added 1.95 g (0.011 M) of $\text{N,N}^\prime$-carbonyldiimidazole (CDI) and heated up to 60 °C on a water bath for 1 h, protecting from moisture with a calcium chloride tube. 1.60 g (0.01 M) of 4-hydrazinoquinazoline (1.1) was then added to the reaction mixture with stirring and refluxed for 1.5–2 hours. The mixture was poured into water, adjusted with acetic acid to pH 6–7. The formed precipitate was filtered off and dried. If necessary, crystallized from dioxane.

Butyl 2-(2-(quinazoline-4(3H)-ylidene)hydrazine-1-carbonyl)benzoate (2.1). Yield: 46.7%; m.p. 146–148 °C, $^1$H NMR, δ: 12.05 (br.s, 1H, quin. 3-NH), 11.12 (br.s, 1H, -CONH), 8.95–6.67 (m, 9H, quin. H-2, 5, 6, 7, 8, Ar H-3, 4, 5, 6), 4.19 (q, $J = 6.3$ Hz, 2H, OCH$_2$CH$_2$CH$_2$CH$_3$), 1.74–1.50 (m, 2H, OCH$_2$CH$_2$CH$_2$CH$_3$), 1.45–1.24 (m, 2H, OCH$_2$CH$_2$CH$_2$CH$_3$), 0.88 (t, $J = 7.4$ Hz, 3H, OCH$_2$CH$_2$CH$_2$CH$_3$); LC-MS, m/z = 365 (M+H); Calculated for: C$_{20}$H$_{20}$N$_4$O$_3$: C, 65.92; H, 5.53; N, 15.38; Found: C, 65.98; H, 5.57; N, 15.41.

Ethyl 3-(2-(quinazoline-4(3H)-ylidene)hydrazine-1-carbonyl)benzoate (2.2). Yield: 93.7%; m.p. 187–189 °C, $^1$H NMR, δ: 11.80 (br.s, 1H, quin. 3-NH), 10.86 (br.s, 1H, CONH), 8.47 (s, 1H, quin. H-2), 8.28–7.97 (m, 3H, Ar H-2,3,6), 7.79–6.94 (m, 5H, quin. H-5, 6, 7, 8, Ar H-4), 4.35 (q, $J = 7.2$ Hz, 2H, -CH$_2$CH$_3$), 1.33 (t, $J = 7.2$ Hz, 3H, -CH$_2$CH$_3$); LC-MS, m/z = 337 (M+H); Calculated for: C$_{18}$H$_{16}$N$_4$O$_3$: C, 64.28; H, 4.79; N, 16.66; Found: C, 64.33; H, 4.83; N, 16.71.

Ethyl 4-(2-(quinazoline-4(3H)-ylidene)hydrazine-1-carbonyl)benzoate (2.3). Yield: 67.9%; m.p. 193–195 °C, $^1$H NMR, δ: 11.85 (br.s, 1H, 3-NH), 10.83 (br.s, 1H, CONH), 8.86–6.39 (m, 9H, quin. H-2, 5, 6, 7, 8, Ar H-2, 3, 5, 6), 4.34 (q, $J = 7.0$ Hz, 2H, -CH$_2$CH$_3$), 1.33 (t, $J = 6.9$ Hz, 3H, -CH$_2$CH$_3$); LC-MS, m/z = 337 (M+H); Calculated for: C$_{18}$H$_{16}$N$_4$O$_3$: C, 64.28; H, 4.79; N, 16.66; Found: C, 64.25; H, 4.73; N, 16.61.

Methods for the synthesis of esters 2(3-, 4-)[1,2,4]triazolo[1,5-c]quinazolin-2-yl) hydrazine-1-carbonyl)benzoates (3). 0.01 Mol of the corresponding monoesters of 2 (3-, 4-)-(2-(quinazolin-4(3H)-ylidene) hydrazine-1-carbonyl)benzoates (2) were refluxed in glacial acetic acid for 6 hours. The solvent was distilled off under vacuum, the residue was washed with aqueous methanol and filtered. Dry, if necessary crystallized from ethanol.
Ethyl 3-[1,2,4]triazolo[1,5-c]quinazolin-2-yl)benzoate (3.1). Yield: 75.5%; m.p. 186–188 °C; H NMR, δ: 9.63 (s, 1H, H-5), 8.75 (s, 1H, 2-Ar H-2), 8.47 (s, 2H, H-10, 2-Ar H-6), 8.05 (s, 2H, 2-Ar H-4,5), 7.92 (d, 1H, H-7), 7.81 (1H, H-9), 7.69 (t, 1H, H-8), 4.37 (d, 2H, OCH2CH3), 1.38 (t, 3H, OCH2CH3); LC-MS, m/z = 319 (M+H); Calculated for: C18H14N2O2: C, 67.92; H, 4.43; N, 17.60; Found: C, 67.97; H, 4.47; N, 17.65.

Ethyl 4-[1,2,4]triazolo[1,5-c]quinazolin-2-yl)benzoate (3.2). Yield: 67.9%; m.p. 193–195 °C; H NMR, δ: 9.68 (s, 1H, H-5), 8.53 (d, J = 7.8 Hz, 1H, H-10), 8.39 (d, J = 8.2 Hz, 2H, Ar H-2,6), 8.14 (d, J = 8.3 Hz, 1H, H-7), 7.96 (t, J = 7.4 Hz, 1H, H-8), 7.85 (d, J = 7.4 Hz, 1H, H-9), 4.35 (q, J = 7.0 Hz, 2H, CH2), 1.35 (t, J = 7.0 Hz, 3H, CH3); LC-MS, m/z = 319 (M+H); Calculated for: C18H14N2O2: C, 67.92; H, 4.43; N, 17.60; Found: C, 67.95; H, 4.48; N, 17.63.

Methods for the synthesis of 4-(5-(2-aminophenyl)-1H-1,2,4-triazol-3-yl)benzoic acid (4.2) and its ethyl ester (4.1). To 1.59 g (0.005 M) of ethyl 4-[1,2,4]triazolo[1,5-c]quinazolin-2-yl)benzoate (6.3) 50 ml of 70% alcohol and 5 ml of hydrochloric acid or 10 ml of hydrochloric acid or 10% sodium hydroxide solution were added and refluxed for 4 hours. Mixture was cooled down, in the case of acid hydrolysis sodium acetate solution was added (in case of alkaline hydrolysis mixture was acidified to pH 5–6). The formed precipitates were filtered off and dried. Crystallized from ethanol.

Ethyl 4-(5-(2-aminophenyl)-1H-1,2,4-triazol-3-yl)benzoate (4.1). Yield: 97.2%; m.p. 207–209 °C; 1H NMR, δ: 14.46 (br.s, 1H, triazole NH), 8.21 (d, J = 7.6 Hz, 2H, 3-Ar H-2,6), 8.08 (d, J = 8.0 Hz, 2H, 3-Ar H-3,5), 7.81 (s, 1H, 5-Ar H-6), 7.19–7.05 (m, 1H, 5-Ar H-4), 6.84 (d, J = 8.1 Hz, 1H, 5-Ar H-3), 6.71 (t, J = 7.4 Hz, 1H, H-9), 6.45–4.17 (m, 2H, CH2CH3), 1.34 (t, J = 7.0 Hz, 3H, CH3). 13C NMR (126 MHz, DMSO), δ: 165.39 (COOCH2CH3), 162.15, 129.73, 126.06, 116.17, 115.24, 60.88 (COOCH2CH3), 14.19 (COOCH2CH3); LC-MS, m/z = 309 (M+H); Calculated for: C17H16N4O2: C, 66.22; H, 5.23; N, 18.17; Found: C, 66.27; H, 5.29; N, 18.22.

4-(5-(2-aminophenyl)-1H-1,2,4-triazol-3-yl)benzoic acid (4.2). Yield: 73.9%; m.p. > 300 °C; 1H NMR, δ: 14.30 (br.s, 1H, triazole NH), 8.21 (d, J = 7.6 Hz, 2H, 3-Ar H-2,6), 8.08 (d, J = 7.9 Hz, 2H, 3-Ar H-3,5), 7.83 (d, J = 8.1 Hz, 1H, 5-Ar H-6), 7.17 (t, 1H, 5-Ar H-4), 6.85 (d, J = 8.5 Hz, 1H, 5-Ar H-3), 6.64 (t, J = 7.3 Hz, 1H, 5-Ar H-5); LC-MS, m/z = 281 (M+H); Calculated for: C15H12N4O2: C, 64.28; H, 4.32; N, 19.99; Found: C, 66.27; H, 5.29; N, 18.22.

Method for the synthesis of (quinazolin-4(3H)-ylidene)hydrazineylidene)methyl)benzoic acids (5). To a suspension of 1.6 g (0.01 M) of 4-hydrazinoquinazoline (1.1) in 10 ml of dioxane 0.11 M of the corresponding formylbenzoic acid was add. The suspension was refluxed for 1.5–2 hours, cooled and poured into water. The formed precipitates were filtered and dried. Crystallized from ethanol.

2-(Quinazolin-4(3H)-ylidene)hydrazineylidene)methyl)benzoic acid (5.1). Yield: 86.3%; m.p. 254–256 °C; 1H NMR, δ: 13.25 (br.s, 1H, COOH), 11.72 (br.s, 1H, NH), 9.19 (s, 1H, -NH-C=NH), 8.48 (d, 1H, H-3 Ar), 8.25 (d, 1H, H-5), 7.90 (t, 1H, H-5 Ar); 7.89 (s, 1H, H-2), 7.14 (m, 2H, H-7, H-6 Ar), 7.54 (m, 2H, H-8, H-4 Ar), 7.44 (t, 1H, H-6); LC-MS, m/z = 293 (M+H); Calculated for: C15H12N4O2: C, 65.79; H, 4.48; N, 19.21.

3-(Quinazolin-4(3H)-ylidene)hydrazineylidene)methyl)benzoic acid (5.2). Yield: 97.3%; m.p. 248–250 °C; 1H NMR, δ: 13.16 (br.s, 1H, COOH), 11.76 (br.s, 1H, 3-NH), 8.61 (s, 1H, Ar H-2), 8.48 (s, 1H, CH=N-N), 8.27 (d, J = 6.3 Hz, 1H, Ar H-4), 8.21 (d, J = 7.1 Hz, 1H, quin H-5), 8.00 (d, J = 7.6 Hz, 1H, Ar H-6), 7.89 (s, 1H, quin H-2), 7.66 (t, 1H, quin H-6), 7.60 (t, J = 7.7 Hz, 1H, Ar H-5), 7.51 (d, J = 7.3 Hz, 1H, quin H-8), 7.44 (t, J = 6.8 Hz, 1H, quin H-7); LC-MS, m/z = 293 (M+H); Calculated for: C16H12N4O2: C, 65.75; H, 4.14; N, 19.17; Found: C, 65.79; H, 4.48; N, 19.21.
4-((Quinazolin-4(3H)-ylidene)hydrazineylidene)methyl)benzoic acid (5.3). Yield: 86.9%; m.p. 262–264 °C; 1H NMR, δ: 12.71 (s, 1H, COOH), 11.61 (s, 1H, 3-NH), 8.50 (s, 1H, N=CH-), 8.24 (s, 1H, quin. H-2), 8.06–7.91 (m, 4H, Ar H-2, 3, 5, 6), 7.83 (d, 1H, quin. H-5), 7.61 (t, 1H, quin. H-7), 7.48 (d, 1H, quin. H-8), 7.38 (t, 1H, quin. H-6); LC-MS, m/z = 293 (M+H);
Calculated for: C_{16}H_{12}N_{4}O_{2}: C, 65.75; H, 4.14; N, 19.17; Found: C, 65.71; H, 4.09; N, 19.11.

The general method for the synthesis of [1,2,4]triazolo[1,5-c]quinazolin-2-yl)benzoic acids (6). 1.46 g (5 mmol) of the corresponding quinazolin-4(3H)-ylidene)hydrazinyldiene) methyl)benzoic acids (5) and 1.23 g (15 mmol) of anhydrous sodium acetate were added to 40 ml of glacial acetic acid with constant stirring. A solution of 0.8 g of bromine (5 mmol) in 10 ml of glacial acetic acid was then added dropwise to the starting compounds. Continue stirring for 3 hours, after which the mixture was poured into cold water. The formed precipitate was filtered off and dried. Crystallized from ethanol or dioxane.

3-([1,2,4]Triazolo[1,5-c]quinazolin-2-yl)benzoic acid (6.1). Yield: 72.4%; m.p. 242–244 °C; 1H NMR, δ: 13.20 (br.s, 1H, COOH), 9.67 (d, J = 1.9 Hz, 1H, H-5), 8.85 (s, 1H, 2-Ar H-2), 8.54 (d, J = 8.1 Hz, 1H, 2-Ar H-4), 8.48 (d, J = 7.9 Hz, 1H, H-10), 8.18–8.03 (m, 2H, H-7, 2-Ar H-6), 7.95 (t, J = 7.8 Hz, 1H, H-8), 7.85 (t, J = 7.7 Hz, 1H, H-9), 7.72 (t, J = 7.8 Hz, 1H, 2-Ar H-5). LC-MS, m/z = 291 (M+H); Calculated for: C_{16}H_{10}N_{4}O_{2}: C, 66.20; H, 3.47; N, 19.30; Found: C, 66.23; H, 3.52; N, 19.33.

4-([1,2,4]triazolo[1,5-c]quinazolin-2-yl)benzoic acid (6.2). Yield: 68.6%; m.p. 248–250 °C; 1H NMR, δ: 13.21 (br.s, 1H, COOH), 9.66 (s, 1H, H-5), 8.50 (d, J = 8.1 Hz, 1H, H-10), 8.36 (d, J = 8.0 Hz, 2H, 2-Ar H-2,6), 8.12 (d, J = 8.1 Hz, 2H, 2-Ar H-3,5), 8.07 (d, J = 8.2 Hz, 1H, H-7), 7.94 (t, J = 7.7 Hz, 1H, H-9), 7.84 (t, 1H, H-8). LC-MS, m/z = 291 (M+H); Calculated for: C_{16}H_{10}N_{4}O_{2}: C, 66.20; H, 3.47; N, 19.30; Found: C, 66.19; H, 3.46; N, 19.27.

Anti-inflammatory activity. Evaluation of anti-inflammatory activity of the synthesized compounds was conducted on 84 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» [15]. Screening of the synthesized compounds with estimated anti-inflammatory activity began with the study of their effect on exudative phase of acute aseptic inflammation («carrageenan» test) [16]. Phlogogen (1% aqueous solution of λ-carrageenan) was subplantally injected in a dose of 0.1 ml in the rats’ hind 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. Measurement of paws volume was conducted before the experiment and in 4 («carrageenan» 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:

\[
A, \% = 100\% - \left( \frac{V_{pe} - V_{he}}{V_{pc} - V_{hc}} \times 100\% \right),
\]

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® for Windows 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 \( p < 0.05 \) [17].

**Results and discussion**

We have previously shown that the interaction of 4-hydrazinoquinazoline (1.1) with anhydrides alkanedicarboxylic acids under extreme conditions leads to the formation of the corresponding \([1,2,4]\text{triazolo}[1,5-c]\text{quinazolin-2-yl})alkane-carboxylic acids [18]. An attempt to synthesize these heterocycles with cycloalkane(aryl-)carboxyl groups at 2\textsuperscript{nd} position using aromatic or alicyclic anhydrides (endic, phthalic anhydride and its hydrogenated analogues) in this reaction leads to the formation of the corresponding cyclic 4-imidoaminoquinazolines [19].

To study the possibility of the heterocyclization in another direction, namely with the formation of triazoloquinazoline systems with a carboxyl group, considering the purpose of the work, we have developed a number of original approaches. The first approach was to form monoesters of \((2-(quinazolin-4(3\text{H})-ylidene)hydrazine-1-carbonyl)benzoic acids (2)\) by interreacting of 4-hydrazinoquinazoline (1.1) with benzenedicarboxylic acid monoesters in the carbonyldiimidazole synthesis (Fig. 1).

**Fig. 1. Approaches to synthesis of \([1,2,4]\text{triazolo}[1,5-c]\text{quinazolin-2-yl})benzoic acids**

Heterocyclization of esters (2) in acetic acid leads to the formation of esters of \([1,2,4]\text{triazolo}[1,5-c]\text{quinazolin-2-yl})benzoic acids (3.1, 3.2) with satisfactory yields (Fig. 1). An exception is compound (2.1) with an o-carboxyl group, which under these conditions forms a mixture of reaction products and can be explained by the peculiarity of the formation of these heterocycles. Namely, the annealing of the triazole ring to the quinazoline cycle under acid catalysis is accompanied by Dimroth rearrangement as a result of which \([1,2,4]\text{triazolo}[4,3-c]\text{quinazolines through the opening of the pyrimidine cycle form the corresponding [1,5-c]-series [20]. Thus, in the case of compound (2.1), several alternative products (A, B1, B2 and C) are possible, which are found in chromatogram and \(^1\text{H} \text{NMR spectra (Fig. 2).}}

[48]
An attempt to obtain 4-[(1,2,4]triazolo[1,5-c]quinazolin-2-yl]benzoic acid (6) by hydrolysis of compound (3) was also unsuccessful due to the additional hydrolytic cleavage of the pyrimidine cycle and the formation of compounds (4) (Fig. 1).

Another approach to the formation of triazolo[c]quinazoline systems is the oxidative heterocyclization of 4-(benzylidene)hydrazineylidene)-3,4-dihydro-quinazolines [21]. The method we have used showed that treatment of compounds (5) with bromine in glacial acetic acid leads to the formation of [1,2,4]triazolo[1,5-c]quinazolin-2-yl]benzoic acids (6.1, 6.2, Fig. 1). The probable mechanism of this reaction is realized through the stage of electrophilic addition of bromine on the azomethine fragment, formation of carbocation with subsequent nucleophilic attack of the endocyclic atom of the Nitrogen cycle with the formation of \([1,2,4]triazolo[4,3-c]quinazolines\). It is important, that \([1,2,4]triazolo[4,3-c]quinazolines\) were isomerized to the corresponding \([1,5-c]\)-series under these conditions. However, as in the above case, compound (5.1) forms a mixture of products, which is also, in our opinion, complicated by Dimroth rearrangement (Fig. 2).

Structure and individuality of synthesized compounds was confirmed by elemental analysis, chromato-mass and \(^1\)H NMR spectrometric. A quasimolecular ion [M+1] was registered in the chromato-mass spectra substances, which confirms their structure and individuality.

\(^1\)H NMR spectra also indicate their unambiguous formation. Thus, the \(^1\)H NMR spectra of compounds (2) were characterized by signals of exchange singlet protons of the endocyclic 3-NH- and -\(\text{NHCO}\) groups at 12.05–11.80 ppm and 11.12–10.83 ppm, respectively. The aromatic protons of the quinazoline ring and the phenyl substituent of compounds (2) were in most cases manifested in the form of multiplet signals, which were associated with tautomeric transitions in the molecule (hydrazide-hydrazone tautomerism). Signals with characteristic splitting and chemical shift were manifested in the strong magnetic field of the spectra of compounds (2) [22]. Exchangeable protons of -COOH and protons of endocyclic 3-NH groups were observed at 13.25–12.71 ppm and 11.76–11.61 ppm, respectively in \(^1\)H NMR spectra of compounds (5). The characteristic azomethine proton (-N=CH-) and the proton of 2\(^{nd}\) position of the heterocycle of compounds (5) resonate as singlets at 9.19–8.48 ppm and 8.61–7.89 ppm, respectively. Aromatic protons have «classical» chemical shifts and multiplicity, which is characteristic for these systems [18, 22].

In favor of the heterocyclization and oxidative cyclization of compounds (2) and (5), unambiguously indicate the \(^1\)H NMR spectra of compounds (3) and (6). Thus, the signals of singlet protons at the 5\(^{th}\) position of the heterocycle in these compounds was characteristic and resonates at 9.68–9.63 ppm, indicating the course of Dimroth rearrangements [18]. Regarding the reaction of nucleophilic degradation of the pyrimidine cycle of compounds (3), the uniqueness of its course indicates the absence in compounds (4) of the proton...
at the 5th position and the appearance of broad one-proton singlet protons of the triazole cycle at 14.46–14.30 ppm and NH₂ groups at 6.71 ppm [24]. The latter is not registered in compound (4.2), due to tautomeration and interaction with the COOH group of the molecule. Additionally, this confirms the 13C NMR spectrum of compound (4.2), in which the required number of chemical shifts of the carbon atom was observed.

Studies on anti-inflammatory activity have shown that (quinazoline-4(3H)-ylidene) hydrazides of dicarboxylic acids (2) inhibit the development of carrageenan edema by 34.44–32.29% compared to control, inferior to diclofenac sodium (AA = 69.79%, table). In this case, there is a certain dependence, namely the decrease in activity in the series \( p > o > m \)-position of the ester group. The corresponding hydrazones (5) were low-activity compounds (AA = 5.63–14.86%). They were characterized by a similar dependence of the decrease in activity in the series \( p > o > m \)-position of the carboxyl group.

| № | Compd.                  | The healthy paw volume, ml*       | Edema paw volume on 4th h of exp., ml* | AA, % |
|---|-------------------------|-----------------------------------|---------------------------------------|-------|
| 1 | Control                 | 1.410 ± 0.021                     | 2.370 ± 0.042                         | –     |
| 2 | Diclofenac sodium       | 1.553 ± 0.041                     | 1.843 ± 0.046                         | 69.79 |
| 3 | 2.1                     | 1.390 ± 0.033                     | 2.186 ± 0.133                         | 17.08 |
| 4 | 2.2                     | 1.280 ± 0.047                     | 2.207 ± 0.108                         | 3.44  |
| 5 | 2.3                     | 1.506 ± 0.042                     | 2.156 ± 0.076                         | 32.29 |
| 6 | 3.1                     | 1.563 ± 0.066                     | 2.11 ± 0.114                          | 20.67 |
| 7 | 3.2                     | 1.273 ± 0.025                     | 2.076 ± 0.097                         | 16.35 |
| 8 | 4.1                     | 1.740 ± 0.037                     | 2.430 ± 0.055                         | 27.78 |
| 9 | 4.2                     | 1.370 ± 0.066                     | 2.186 ± 0.133                         | 15.00 |
| 10| 5.1                     | 1.357 ± 0.062                     | 2.216 ± 0.026                         | 10.52 |
| 11| 5.2                     | 1.310 ± 0.056                     | 2.216 ± 0.026                         | 5.63  |
| 12| 5.3                     | 1.386 ± 0.045                     | 2.206 ± 0.098                         | 14.86 |
| 13| 6.1                     | 1.623 ± 0.044                     | 2.226 ± 0.075                         | 37.15 |
| 14| 6.2                     | 1.370 ± 0.060                     | 2.100 ± 0.058                         | 23.96 |

Note: significant changes in control (\( p < 0.05 \)); \( n \) is the number of animals in the group.

The formation of planar \([1,2,4]\)triazolo\([1,5-c]\)quinazoline cycle (3) leads to loss of anti-inflammatory activity of the corresponding esters (AA = 16.35–20.67%). Importantly, higher anti-inflammatory activity was observed for compound (3.1), in which the ester group is in the \( m \)-position. Compound (6.1) with a carboxyl group in the \( m \)-position (AA = 37.15%) turned to be also the most active, that is speaking about \([1,2,4]\)triazolo\([1,5-c]\)quinazolin-2-yl)benzoic acids (6). Replacing of the substituent to the \( p \)-position leads to the loss of activity (AA = 23.96%). Nucleophilic degradation of the triazolo\([1,5-c]\)quinazoline cycle showed that ethyl 4-(5-(2-aminophenyl)-1H-1,2,4-triazol-3-yl)benzoate (4.1) was more active (AA = 27.78%). However, the corresponding acid was an inactive compound (AA = 15.06%).

The results of studies confirmed the presence of anti-inflammatory activity in carboxy-containing quinazolines and \([1,2,4]\)triazolo\([1,5-c]\)quinazolines and shows prospects for their further structural modification. Interesting objects in this regard are \([1,2,4]\)triazolo\([1,5-c]\)quinazolin-2-yl)benzoic acids for which it is easy to further functionalize the carboxyl group and 4-(5-(2-aminophenyl))-1H-1,2,4-triazol-3-yl)benzoic acid, which as 1,5-NCCCN-binucleophiles, can undergo a 5+1-heterocyclization with various electrophiles. This makes it possible to expand the combinatorial chemical library with targeted anti-inflammatory activity.
Conclusion

1. Methods for the synthesis of alkyl 2(3-,4-)-(2-(quinazolin-4(3H)-ylidene)hydrazine-1-carbonyl)benzoates and (quinazolin-4(3H)-ylidene)hydrazineylidine)methyl)benzoic acid were developed and factors limiting the course of the heterocyclization and oxidative cyclization in the synthesis of [1,2,4]triazolo[1,5-c]quinazolin-2-yl)benzoic acid and their esters were established. It was shown that (quinazolin-4(3H)-ylidene)hydrazide(hydrazone) with o-carboxyphenyl group are the exception. In these reactions mentioned group under the conditions of Dimroth rearrangement of [1,2,4]triazolo[4,3-c]quinazolines in the corresponding [1,5-c]-series, form alternative reaction products due to approximate nucleophilic centers. It was found, that the hydrolysis of ester of 4-([1,2,4]triazolo[1,5-c]quinazolin-2-yl)benzoic acid is not a preparative method for the synthesis of the corresponding acid, due to additional hydrolytic cleavage of the pyrimidine cycle and the formation of 4-(5-(2-aminophenyl)-1H-1,2,4-triazol-3-yl)benzoic acid.

2. 1H NMR spectra were studied and regularities of splitting of characteristic protons in synthesized compounds were established.

3. Moderate anti-inflammatory activity in carboxy-containing quinazolines and [1,2,4]triazolo[1,5-c]quinazolines was revealed and prospects for their further structural modification were shown.

Funding. This research was a part of the scientific project «Directed search for biologically active substances among annulated quinazoline and pteridine derivatives» (problem «Pharmacy», state registration №0117U 006961, period of study 2017–2022). The authors gratefully acknowledge «Enamine Ltd.» (Kiev, Ukraine) for financial support of this work.

The authors would like to thank all the brave defenders of Ukraine who made the finalization of this article possible.

References

1. Anti-Inflammatory Drug Discovery. Edited by J. I. Levin., S. Laufer. RSC Drug Discovery Series N 26. – Cambridge: Royal Society of Chemistry, 2012. – 528 p. https://doi.org/10.1039/9781849735346
2. Lou Y., Zhu J. Carboxylic Acid Nonsteroidal Anti-Inflammatory Drugs (NSAIDs). Bioactive Carboxylic Compound Classes // Pharmaceuticals and Agrochemicals. – 2016. – P. 221–236. https://doi.org/10.1002/9783527693931.ch16
3. Kotvitska A. A., Kostiuk V. H. Marketynovi doslidzhennia farmatsevtychnoho rynku nesteroidnykh protyzapalnykh likarskykh zasobiv // Farmats. chasopys. – 2016. – № 2. – S. 48–53. https://doi.org/10.11063/2312-0967.2016.2.6651
4. Bindu S., Mazumder S., Bandypadhyay U. Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: a current perspective // Biochem. Pharmacol. – 2020. – V. 180. – P. 114147. https://doi.org/10.1016/j.bcp.2020.114147
5. Ajmal M. Qandil. Prodrugs of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), More Than Meets the Eye: A Critical Review // Int. J. Mol. Sci. – 2012. – V. 13. – P. 17244–17274. https://doi.org/10.3390/ijms131217244
6. Quinazolinone and Quinazoline Derivatives. Acad. Ed. Ali Gamal Al-kaf. – Sana’a University, 2020. – 126 p. https://doi.org/10.5772/intechopen.85315
7. Chandrika P. M., A. Rao R. R., Narsaiah B., Raju M. B. Quinazoline derivatives with potent anti-inflammatory and anti-allergic activities // Int. J. Chem. Sci. – 2008. – V. 6 (3). – P. 1119–1146. – URL: https://www.tsjournals.com/articles/quinazoline-derivatives-with-potent-antiinflammatory-and-antiallergic-activities.pdf
8. Krasovska N. I., Starvytskiy V. V., Nosulenko I. S. et al. Carboxyl-containing quinazolines and related heterocycles as carriers of anti-inflammatory activity // Zaporozhye Med. J. – 2022. – V. 24, N 1. – P. 91–101. https://doi.org/10.14739/2310-1210.2022.1.241286
9. Siddhu R. S., Lee J. Y., Yuan C., Smith W. L. Comparison of Cyclooxygenase-1 Crystal Structures: Cross-talk Between Monomers Comprising Cyclooxygenase-1 Homodimers. // Biochem. – 2010. – V. 49. – P. 7069–7079. https://doi.org/10.1021/bi1003298
10. Rowlinson S. W., Kiefer J. R., Prusakiewicz J. J. et al. Novel Mechanism of Cyclooxygenase-2 Inhibition Involving Interactions with Ser-530 and Tyr-385 // J. Biol. Chem. – 2003. – V. 278, N 46 (14). – P. 45763–45769. https://doi.org/10.1074/jbc.M305481200
11. Selinsky B. S., Gupta K., Sharkey C. T., Loll P. J. Structural analysis of NSAID binding by prostaglandin H2 synthase: time-dependent and time-independent inhibitors elicit identical enzyme conformations // Biochem. – 2001. – V. 40, N 17. – P. 5172–5180. https://doi.org/10.1021/bi010045s

12. Kurumbail R. G., Stevens A. M., Gierse J. K. et al. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents // Nature. – 1996. – V. 384, N 19 (26). – P. 644–648. https://doi.org/10.1038/384644a0

13. Chemet E., Bernardi D., Comel A., Kirsch G. Preparation of Monoalkyl Terephthalates; An Overview // Synthetic Communications. – 2007. – V. 37, N 3. – P. 483–490. https://doi.org/10.1080/00397910601039226

14. Armarego W. L. F. The Chemistry of Heterocyclic Compounds. Fused Pyrimidines, Part I: «Quinazolines», Brown D. J., ed.; V. 24/1, Interscience Publishers: N.-Y.–London–Sydney, 1967. – URL: https://vdoc.pub/download/chemistry-of-heterocyclic-compounds-fused-pyrimidines-part-i-quinazolines-volume-24-7870hdpq490

15. European convention for the protection of vertebrate animal used for experimental and other scientific purposes, Council of Europe, Strasbourg, 1986. – URL: https://rm.coe.int/168007a6b7

16. 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. – V. 56 (1). – UNIT. 5.4.1-5.4.7

17. Lapach S. N., Chubenko A. V., Babich P. N. Statistical methods in biomedical research using EXCEL. – Kyiv: Morion, 2001. – 408 p. – URL: https://www.scirp.org/(S(351jmbntvnsjt1aadkposzje))/reference/Papers.aspx?ReferenceID=1723702

18. Kovalenko S. I., Antypenko L. M., Bilyi A. K. et al. Synthesis and Anticancer Activity of 2-(Alkyl-,Alkaryl-,Aryl-,Hetaryl-)[1,2,4]triazolo[1,5-c]quinazolines // Scientia Pharmaceutica. – 2013 – V. 81 (2). – P. 359–391. https://doi.org/10.3797/scipharm.1205.11210-08

19. Karpenko O. V., Kovalenko S. I. Heterotolksyzhitazii na osnovi 4-hidrazynokhinazolinu ta anhidrydv dykarbonovykh kyslot // Visnyk NU «Lvivska politekhnika». Khimiia, tekhnolohiia rechovyn ta yikh zastosuvannia. – 2006. – № 553. – S. 77–85. – Rezhym dostupu: http://www.disslib.org/syntez-anelovanykh-dykarbonovyh-kyslot

20. Potts K. T., Brugel E. G. 1,2,4-Triazoles. XXIV. Isomerization of 5-Triazolo[4,3-c]quinazoline Derivatives // J. Org. Chem. – 1970. – V. 35, N 10. – P. 3448–3451. https://doi.org/10.1021/jo00835a058

21. Gibson M. S. Hydrazones – IV: The bromination of benzylidene 2-pyridylhydrazone // Tetrahedron. – 1963. – V. 19, N 11. – P. 1587–1589. https://doi.org/10.1016/S0040-4020(01)99232-4

22. Brevetmaier E. Structure elucidation by NMR in organic chemistry: a practical guide, third edition. – Wiley, 2002. – 270 p. ISBN: 978-0-470-85007-7

23. Kholodynyak S. V., Schabelnyk K. P., Zhernova G. O. et al. Hydrolytic cleavage of pyrimidine ring in 2-aryl-[1,2,4]triazolo[1,5-c]quinazolines: physico-chemical properties and hypoglycemial activity of the synthesized compounds // News of pharmacy. – 2015. – V. 3, N 83. – Р. 9–17. https://doi.org/10.24959/nphj.15.2054

Список використаної літератури

1. Anti-Inflammatory Drug Discovery. Edited by J. I. Levin., S. Laufer. RSC Drug Discovery Series N 26. – Cambridge: Royal Society of Chemistry, 2012. – 528 p. https://doi.org/10.1039/9781849735346

2. Lou Y., Zhu J. Carboxylic Acid Nonsteroidal Anti-Inflammatory Drugs (NSAIDs). Bioactive Carboxyl-containing quinazolines and related heterocycles as carriers of anti-inflammatory activity // Zaporozhye Med. J. – 2012. – V. 24, N 1. – P. 91–101. https://doi.org/10.14739/2310-1210.2022.1.241286

3. Selinsky B. S., Gupta K., Sharkey C. T., Loll P. J. Structural analysis of NSAID binding by prostaglandin H2 synthase: time-dependent and time-independent inhibitors elicit identical enzyme conformations // Biochem. – 2001. – V. 40, N 17. – P. 5172–5180. https://doi.org/10.1021/bi010045s

4. Kovalenko S. I., Antypenko L. M., Bilyi A. K. et al. Synthesis and Anticancer Activity of 2-(Alkyl-,Alkaryl-,Aryl-,Hetaryl-)[1,2,4]triazolo[1,5-c]quinazolines // Scientia Pharmaceutica. – 2013 – V. 81 (2). – P. 359–391. https://doi.org/10.3797/scipharm.1205.11210-08

5. Gibson M. S. Hydrazones – IV: The bromination of benzylidene 2-pyridylhydrazone // Tetrahedron. – 1963. – V. 19, N 11. – P. 1587–1589. https://doi.org/10.1016/S0040-4020(01)99232-4

6. Brevetmaier E. Structure elucidation by NMR in organic chemistry: a practical guide, third edition. – Wiley, 2002. – 270 p. ISBN: 978-0-470-85007-7

7. Kholodynyak S. V., Schabelnyk K. P., Zhernova G. O. et al. Hydrolytic cleavage of pyrimidine ring in 2-aryl-[1,2,4]triazolo[1,5-c]quinazolines: physico-chemical properties and hypoglycemial activity of the synthesized compounds // News of pharmacy. – 2015. – V. 3, N 83. – Р. 9–17. https://doi.org/10.24959/nphj.15.2054
The aim of the present study is to develop methods for the synthesis of [1,2,4]triazolo[1,5-c]quinazolin-2-yl)benzoic acids, their esters and dimroth rearrangement. Spectral characteristics, anti-inflammatory activity and possibilities and limitations of synthesis of [1,2,4]triazolo[1,5-c]quinazolines: physical-chemical properties and hypoglycemia activity of the synthesized compounds // News of pharmacy. – 2015. – V. 3, N 83. – P. 9–17. https://doi.org/10.24959/nphj.15.2054

N. I. Krasovska (https://orcid.org/0000-0001-5902-6596)
Zaporizhzhia State Medical University

APPROACHES TO SYNTHESIS OF ([1,2,4]TRIAZOLO[1,5-c]QUINAZOLIN-2-YL)BENZOIC ACIDS AS POTENTIAL ANTI-INFLAMMATORYS

Key words: heterocyclization, oxidative cyclization, ([1,2,4]triazolo[1,5-c]-quinazolin-2-yl)benzoic acids, Dimroth rearrangement, spectral characteristics, anti-inflammatory activity

ABSTRACT

Despite their high efficacy NSAIDs have significant side effects due to non-selective inhibition of COX-1 and COX-2. Due to this, medical chemists still pay considerable attention to their design and synthesis, in particular the creation of hybrid molecules that combine in their structure a fragment with anti-inflammatory activity and the quinazoline heterocycle.

The aim of the present study is to develop methods for the synthesis of [1,2,4]triazolo[1,5-c]-quinazolin-2-yl)benzoic acids as potential anti-inflammatory agents.

Quinazolin-4-(H)-ylidene)hydrazides (hydrazones) of benzenedicarboxylic acids, their esters, products of their heterocyclization and nucleophilic degradation were the subjects of the study. The structure of the synthesized compounds was confirmed by elemental analysis and NMR spectroscopy. Anti-inflammatory activity was studied in a model of acute aseptic inflammation («carrageenan test») in rats.

Possibilities and limitations of synthesis of [1,2,4]triazolo[1,5-c]-quinazolin-2-yl)benzoic acids and their esters via heterocyclization of the corresponding hydrazides and hydrazones oxidative cyclization are
shown. It was found that the hydrolysis of 4-(1,2,4]triazolo[1,5-c]quinazolin-2-yl)benzoic acid esters is not a preparative method for the synthesis of the target acids, due to the hydrolytic cleavage of the pyrimidine cycle. Compounds 3, 4 and 6 with moderate anti-inflammatory activity have been identified, which can be used for further structural modification.

Conclusions. It was found that quinazolin-4(3H)-ylidene)hydrazides (hydrazones) of benzenedicarboxylic acids and their esters under the conditions of heterocyclization and oxidative cyclization form [1,2,4]triazolo[1,5-c]quinazolin-2-yl)benzoic acids and their esters. The synthesized compounds are carriers of anti-inflammatory activity and promising for further research.

Н. І. Красовська (https://orcid.org/0000-0001-5902-6596)
Запорізький державний медичний університет

ПІДХОДИ ДО СИНТЕЗУ ([1,2,4]ТРИАЗОЛО[1,5-c]ХІНАЗОЛІН-2-ІЛ)БЕНЗОЗІНИХ КИСЛОТ ЯК ПОТЕНЦІЙНИХ ПРОТИЗАПАЛЬНИХ АГЕНТІВ

Ключові слова: гетероциклізація, окиснювальна циклізація, ([1,2,4]триазоло[1,5-c]хіназолін-2-іл) бензойні кислоти, перегрупування Дімрота, спектральні характеристики, протизапальна активність

А Н О Т А Ц І Я

Нестероїдні протизапальні препарати, незважаючи на високу ефективність, за рахунок неселективного інгібіювання ЦОГ-1 та ЦОГ-2 мають значні побічні ефекти. З огляду на це медичні хіміки і на сьогодні приділяють значну увагу їх дизайну, зокрема створенню гібридних молекул, які б поєднували в одній структурі фрагмент із протизапальною активністю та хіназоліновий гетероцикл.

Метою представленого дослідження є розроблення методів синтезу ([1,2,4]триазоло[1,5-c] хіназолін-2-іл)бензозіних кислот як потенційних протизапальних агентів.

Об’єктом дослідження були хіназолін-4(3H)-іліден)гідразиди(гідразони) бензендикарбонових кислот та їх естери, продукти їх гетероциклізації та нуклеофільної деградації. Будова синтезованих сполук підтверджена даними елементного аналізу і ЯМР-спектроскопії. Протизапальну активність вивчили на моделі гострого асептичного запалення («карагенановий тест») у шурах.

Показана можливість та обмеження синтезу [1,2,4]триазоло[1,5-c]хіназолін-2-іл)бензозіних кислот та їх естерів на основі реакції гетероциклізації та окиснюваної циклізації відповідних гідразидів та гідразонів. Встановлено, що гідроліза естерів 4-([1,2,4]триазоло[1,5-c]хіназолін-2-іл)бензозіної кислоти не є препаративним методом синтезу відповідних кислот, унаслідок гідролітичного розщеплення піримідинового циклу. Виявлено сполуки 3, 4 та 6 із помірною протизапальною активністю, які у майбутньому можуть бути викорisteni для подальшої структурної модифікації.

Встановлено, що хіназолін-4(3H)-іліден)гідразиди(гідразони) бензендикарбонових кислот та їх естерів за умов гетероциклізації та окиснюваної циклізації формулюють [1,2,4]триазоло[1,5-c]хіназолін-2-іл) бензозіні кислоти та їх естери. Синтезовані сполуки є носіями протизапальної активності і перспективними для подальших досліджень.

Електронна адреса для листування з автором: natamala1111@gmail.com
(Красовська Н. І.);
kovalenkosergiy@gmail.com
(Коваленко С. І.)