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A New Method for Obtaining Carboxylic Derivatives of Oxazolo[5,4-b]pyridine Based on 3-Aminopyridine-2(1*H*)-ones

Current methods for synthesis of oxazolo[5,4-b]- and oxazolo[4,5-b]pyridines have several limitations, such as severe reaction conditions, lengthy reaction times, low yields and concurrent formation of side reaction products. This article presents the results of study focused on a one-step method for the synthesis of new derivatives of oxazolo[5,4-b]pyridine incorporating an aliphatic carboxylic group as a linker. During the investigation of acylation reactions of 3-aminopyridine-2(1H)-ones with cyclic anhydrides of dicarboxylic acids (succinic, maleic and glutaric), it was found that the monoamides formed at the initial stage undergo intramolecular cyclization yielding derivatives of oxazolo[5,4-b]pyridine. Subsequently, the reaction conditions were studied and optimized to achieve the target compounds with high yield and purity. The potential anti-inflammatory activity of the obtained derivatives of oxazolo[5,4-b]pyridine was evaluated by molecular docking method using AutoDock Vina software. Compounds 11-14b exhibited higher binding affinity with the selected target protein Prostaglandin synthase-2 (1CX2) compared to the reference anti-inflammatory drug diclofenac. Thus, taking into account the results of in silico analyses, the newly synthesized oxazolo[5,4-b]pyridine derivatives based on 3-aminopyridine-2(1*H*)-ones are promising candidates for further investigation of their potential anti-inflammatory activity through in vivo methods.

Keywords: 3-aminopyridin-2(1*H*)-ones, oxazolo[5,4-b]pyridines, oxazolo[4,5-b]pyridines, intramolecular heterocyclization, biological activity, anti-inflammatory activity, molecular docking.

Introduction

Over the past decade, the interest of scientists in oxazolo[5,4-b]- and oxazolo[4,5-b]pyridines has increased due to their application in various areas of chemistry and a wide range of biological activities, including antimicrobial, anticancer, anti-inflammatory, analgesic, herbicidal, antioxidant, anticoagulant, and antidiabetic activities (Fig. 1) [1–9]. Recently, modulators of calcium channel activity have been discovered within this class [10]. Some derivatives of oxazolopyridines exhibit activity comparable to phenylbutazone or indomethacin, but without causing gastrointestinal irritation, which is commonly associated with many acidic anti-inflammatory compounds [11]. Preclinical studies on human and animal cell lines have shown that oxazolopyridines are generally non-toxic [12]. Furthermore, these compounds meet the criteria for potential drug candidates due to their lack of asymmetric carbon atoms and low molecular weight, which adhere to Lipinski's rule of five [13].

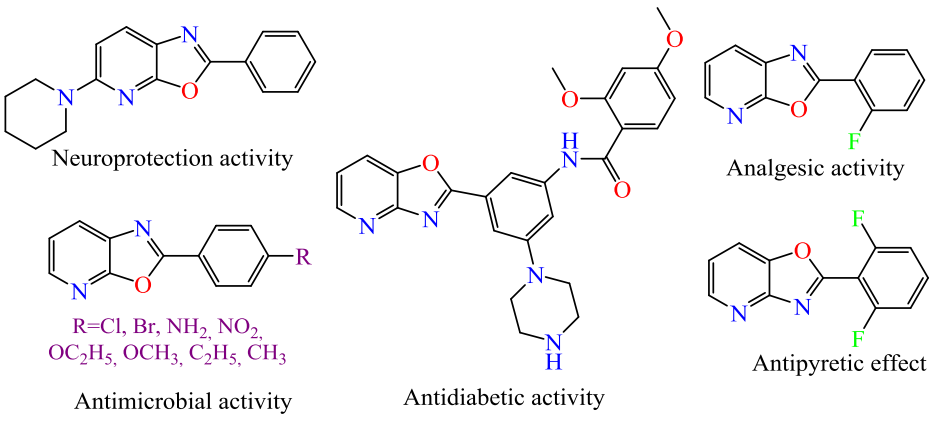
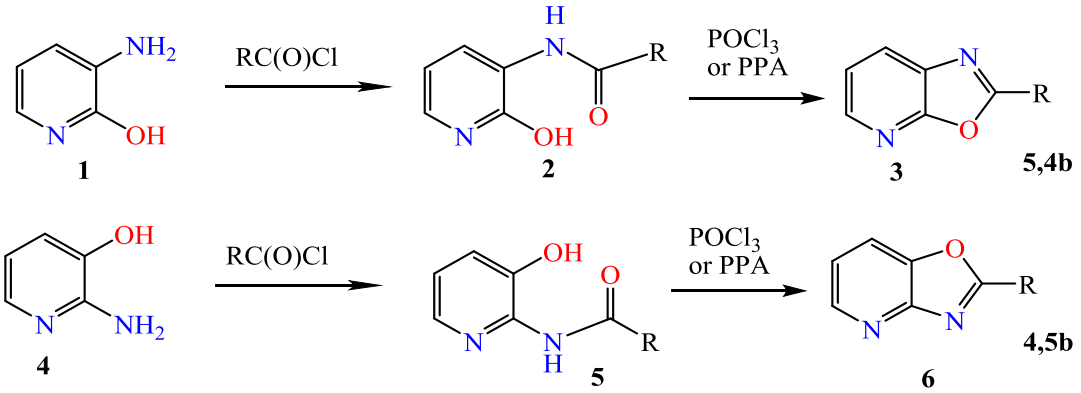


Figure 1. Examples of biologically active oxazolopyridines

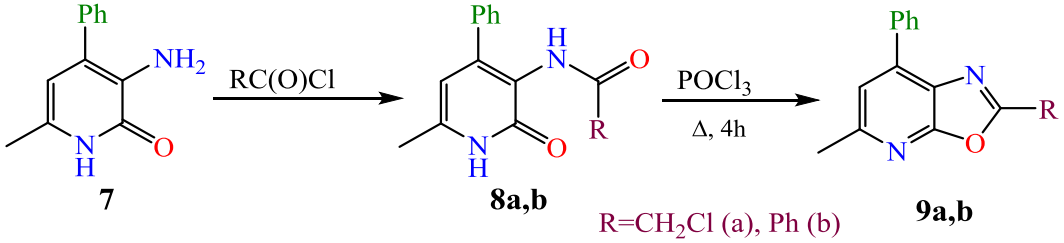
However, only a limited number of synthetic strategies exist for the synthesis of 2-substituted oxazolo[5,4-b]- and oxazolo[4,5-b]pyridines [14–19]. One such approach involves the reaction of halogenated aminopyridine derivatives with trimethylsilyl polyphosphate ether or polyphosphoric acid [4]. Another commonly employed method entails the condensation of 2- or 3-aminohydroxypyridines with carboxylic acid derivatives under acidic conditions using such agents as boric acid, aromatic carboxylic acids and polyphosphoric acid at elevated temperatures [20] (Scheme 1).



Scheme 1. Formation of oxazolo[5,4-b]- and oxazolo[4,5-b]pyridines derivatives **3,6**

Mainly, oxazolo[5,4-b]pyridine **3** obtained based on unsubstituted N-(2-hydroxypyridin-3-yl)benzamides **2** are described in the literature, particularly for amides of aromatic, rather than aliphatic acids [21]. Examples of 5,7-disubstituted and 2-alkyl-substituted oxazolo[5,4-b]pyridines syntheses are presented in the literature only in isolated cases [22–24].

Continuing our study on the modification of 3-amino-pyridin-2(1*H*)-ones, derivatives of which exhibit high antiradical, neurotropic, antidiabetic, hemorheological, and cytoprotective activity [25–30], we obtained corresponding oxazolo[5,4-b]pyridines using a general Scheme 2, by reacting phosphorus oxychloride with previously obtained chloroacetamide **8a** or benzamide **8b**.



Scheme 2. Intramolecular cyclization of chloroacetamide **8a** or benzamide **8b** under the action of $POCl_3$ into the corresponding oxazolo[5,4-b]pyridines **9a, b**

Additionally, the obtained 2-(chloromethyl)oxazolo[5,4-b]pyridine **9a** served as a very effective synthon for obtaining various N-substituted derivatives through the reaction of nucleophilic substitution of the chlorine atom with various N-nucleophiles, including natural alkaloids [31].

However, existing synthetic methods for the production of oxazolo[5,4-b]- and oxazolo[4,5-b]pyridines have a number of limitations, including harsh reaction conditions, long reaction time, low yields of target products and simultaneous formation of by-products. Therefore, the development of new methods for obtaining oxazolo[5,4-b]pyridines and/or optimization of existing methods is an urgent task.

Experimental

Materials

The obtained compounds were analyzed on an Agilent 1260 Infinity II chromatograph connected to an Agilent 6545 LC/Q-TOF high-resolution mass spectrometer equipped with an AJS ESI dual ion source operating in positive ion mode. Mass spectra with LC/MS precision were obtained in the range of 100–1000 m/z, at a scan rate of 1.5 spectra per second.

The chromatographic separation was carried out using ZORBAX RRHD Eclipse Plus C18 columns (2.1×50 mm, particle size 1.8 μm). The column temperature was kept at 35 °C during the analysis. The mobile phase consisted of eluents A and B. For positive ionization mode, eluent A was a 0.1 % formic acid solution in deionized water, and eluent B was a 0.1 % formic acid solution in acetonitrile. The chromatographic separation was achieved with the following elution gradient: 0–10 min with 95 % A, 10–13 min with 100 % B, and 13–15 min with 95 % A. The mobile phase flow rate was maintained at 400 μL/min throughout the analysis. A sample injection volume of 1 μL was used in all experiments. The sample was prepared by dissolving the entire sample (in 1000 μL) in methanol for HPLC analysis. Sample dilution was performed immediately before analysis.

The recorded data were processed using Agilent MassHunter 10.0 software.

The ^{1}H and ^{13}C NMR spectra in DMSO-d6 solutions were recorded on Bruker AVANCE 500 (500 MHz, 125 MHz) and Magritek spinsolve 80 carbon ultra (81 MHz, 20 MHz) spectrometers.

Melting points of synthetic compounds were determined on a Stuart SMP10 hot bench. All reactions were monitored by thin-layer chromatography (TLC) and identified by UV or iodine vapor.

3-Aminopyridine-2(1*H*)-ones were prepared according to a similar literature procedure [30].

Synthesis and Spectral Analysis of Synthesized Compounds

The synthesis of **oxazolo[5,4-b]pyridine derivatives 11-14a-c** involved heating a mixture of 1 mmol of 3-amino-pyridin-2-(1*H*)-one and 5 mmol of the corresponding anhydrides (succinic, maleic, glutaric, phthalic anhydrides) in 5 mL of acetic acid at reflux temperature with vigorous stirring for 10 hours. The mixture was then cooled and poured into 25 mL of water. The resulting precipitates were filtered and recrystallized from a mixture of hexane, 2-propanol, and dichloromethane.

3-(5,7-Dimethyloxazolo[5,4-b]pyridin-2-yl)propanoic acid 11a. Yield: 0.116g (53 %), grey crystals, M.p.: 270–272°C.

^{1}H NMR spectrum (500 MHz, DMSO-d6), δ ppm: 1.89 (s., 3H, CH_3); 2.16 (s., 3H, CH_3); 2.81 (d., 4H, CH_2-CH_2); 5.98 (s., 1H, H-6); 11.85 (br.s., 1H, OH).

^{13}C NMR spectrum (125 MHz, DMSO-d6), δ ppm: 17.2 (CH_3); 18.3 (CH_3); 28.4 (2C CH_2-CH_2); 106.3 (C-6); 118.0; 145.2; 150.2; 158.8; 176.5 (2C).

HRMS m/z: calcd for $C_{11}H_{13}N_2O_3^+$ $[M + H]^+$: 221.0921; found: 221.0935.

3-(5-methyl-7-phenyloxazolo[5,4-b]pyridin-2-yl)propanoic acid 11b. Yield: 0.182 g (64 %), grey powder, M.p.: 280–281°C.

^{1}H NMR spectrum (500 MHz, DMSO-d6), δ ppm: 2.25 (s., 3H, CH_3); 2.57 (d.d., 2H, CH_2CO); 2.76 (d.d., 2H, CH_2); 6.13 (s., 1H, H-6); 7.21 (m., 2H, H-2,6 Ph); 7.40 (m., 3H, H-3,4,5 Ph); 12.20 (br.s., 1H, OH).

^{13}C NMR spectrum (125 MHz, DMSO-d6), δ ppm: 18.6 (CH_3); 28.2 (2C CH_2-CH_2); 105.6 (C-6); 116.6; 126.8 (2C Ph); 128.6 (2C Ph); 130.0 (C Ph); 136.1; 146.6; 152.3; 159.2; 176.8 (2C).

HRMS m/z: calcd for $C_{16}H_{15}N_2O_3^+$ $[M + H]^+$: 283.1077; found: 283.1075.

3-(5-methyl-7-(thiophen-2-yl)oxazolo[5,4-b]pyridin-2-yl)propanoic acid 11c. Yield: 0.165 g (57 %), grey powder, M.p.: 327–329°C.

^{1}H NMR spectrum (500 MHz, DMSO-d6), δ ppm: 2.25 (s., 3H, CH_3); 2.84-2.92 (m., 4H, CH_2-CH_2); 6.57 (s., 1H, H-6); 7.17 (d.d., ^{3}J=5.1 Hz, ^{4}J=3.9 Hz, 1H, H-4 thiophen); 7.63 (d.d., ^{3}J=3.9 Hz, ^{4}J=1.0 Hz, 1H, H-3 thiophen); 7.74 (d.d., ^{3}J=5.1 Hz, ^{4}J=1.0 Hz, 1H, H-5 thiophen); 12.05 (br.s., 1H, OH).

^{13}C NMR spectrum (20 MHz, DMSO-d6), δ ppm: 18.6 (CH_3); 28.7 (2C CH_2-CH_2); 103.0 (C-6); 114.2; 127.8 (C thiophen); 129.7 (C thiophen); 130.4 (C thiophen); 135.6; 143.3; 145.9; 159.3; 176.9 (2C).

HRMS m/z: calcd for $C_{14}H_{13}N_2O_3S^+$ $[M + H]^+$: 289.0641; found: 289.0648.

(E)-3-(5,7-dimethyloxazolo[5,4-b]pyridin-2-yl)acrylic acid 12a. Yield: 0.111g (51 %), white crystals, M.p.: 215-218°C.

^{1}H NMR spectrum (80 MHz, DMSO-d6), δ ppm: 1.93 (s., 3H, CH_3); 2.17 (s., 3H, CH_3); 6.00 (s, 1H, H-6); 7.18 (s., 2H, 2-CH=CH); 11.86 (s., 1H, OH).

^{13}C NMR spectrum (125 MHz, DMSO-d6), δ ppm: 17.3 (CH_3); 18.3 (CH_3); 106.4 (C-6); 135.1 (4C); 145.4; 151.4; 159.3; 170.0.

HRMS m/z: calcd for $C_{11}H_{11}N_2O_3^+$ $[M + H]^+$: 219.0764; found: 219.0768.

(E)-3-(5-methyl-7-phenyloxazolo[5,4-b]pyridin-2-yl)acrylic acid 12b. Yield: 0.216g (77 %), white crystals, M.p.: 282–284°C.

^{1}H NMR spectrum (500 MHz, DMSO-d6), δ ppm: 2.26 (s., 3H, CH_3); 6.16 (s., 1H, H-6); 7.07 (s., 2H, H-2,6 Ph); 7.21 (d.d., *J*=7.5, 2.0, 2H, CH=CH); 7.36-7.39 (m., 3H, H-3,4,5 Ph); 12.23 (br.s., 1H, OH).

^{13}C NMR spectrum (125 MHz, DMSO-d6), δ ppm: 18.6 (CH_3); 105.8 (C-6); 115.4; 126.9 (2C Ph); 128.6 (2C Ph); 129.0; 135.3 (CH=CH); 136.2; 164.8; 153.7; 159.9; 170.4 (2C).

HRMS m/z: calcd for $C_{16}H_{13}N_2O_3^+$ $[M + H]^+$: 281.0921; found: 281.0919.

(E)-3-(5-methyl-7-(thiophen-2-yl)oxazolo[5,4-b]pyridin-2-yl)acrylic acid 12c. Yield: 0.152g (53 %), beige crystals, M.p.: 315-316°C.

^{1}H NMR spectrum (80 MHz, DMSO-d6), δ ppm: 2.26 (s., 3H, CH_3); 6.62 (s., 1H, H-6); 7.18 (t., 1H, *J*=5.1 Hz, H-4 thiophen); 7.30 (s., 2H, 2-CH=CH); 7.67-7.76 (m., 2H, H-3,5 thiophen); 12.05 (s., 1H, OH).

^{13}C NMR spectrum (21 MHz, DMSO-d6), δ ppm: 18.6 (CH_3); 102.8 (C-6); 127.8 (1C thiophen); 129.9 (1C thiophen); 130.8 (1C thiophen); 135.4; 135.9 (4C); 144.5; 146.2; 159.9; 170.5.

HRMS m/z: calcd for $C_{14}H_{11}N_2O_3S^+$ $[M + H]^+$: 287.0485; found: 287.0490.

4-(5,7-Dimethyloxazolo[5,4-b]pyridin-2-yl)butanoic acid 13a. Yield: 0.110g (47 %), white crystals, M.p.: 236–239°C.

^{1}H NMR spectrum (80 MHz, DMSO-d6), δ ppm: 1.82 (s., 3H, CH_3); 1.90-1.98 (m., 2H, 3-CH_2); 2.13 (s., 3H, CH_3); 2.70 (t., *J*=5.7 Hz, 4H, 4,2-CH_2); 5.91 (s., 1H, H-6); 11.67 (s., 1H, OH).

^{13}C NMR spectrum (21 MHz, DMSO-d6), δ ppm: 17.1 (CH_3); 18.2 (CH_3); 32.2 (3C 2,3,4-CH_2); 106.2 (C-6); 121.4; 143.9; 148.7; 159.1; 171.9 (2C).

HRMS m/z: calcd for $C_{12}H_{15}N_2O_3^+$ $[M + H]^+$: 235.1077; found: 235.1081.

4-(5-Methyl-7-phenyloxazolo[5,4-b]pyridin-2-yl)butanoic acid 13b. Yield: 0.166 g (56 %), white crystals, M.p.: 312–314°C.

^{1}H NMR spectrum (80 MHz, DMSO-d6), δ ppm: 1.43-1.87 (m., 2H, 3-CH_2); 2.23 (s., 3H, CH_3); 2.49-2.64 (m., 4H, 4,2-CH_2); 6.05 (s., 1H, H-6); 7.17 (d., *J*=3.1 Hz, 2H, H-2,6 Ph); 7.35 (br.s., 3H, H-3,4,5 Ph); 12.00 (br.s., 1H, OH).

^{13}C NMR spectrum (21 MHz, DMSO-d6), δ ppm: 18.4 (CH_3); 32.2 (3C 2,3,4-CH_2); 105.4 (C-6); 120.2; 126.7 (2C Ph); 128.4 (2C Ph); 128.6; 136.5; 145.1; 150.8; 159.3; 172.2 (2C).

HRMS m/z: calcd for $C_{17}H_{17}N_2O_3^+$ $[M + H]^+$: 297.1234; found: 297.1230.

4-(5-Methyl-7-(thiophen-2-yl)oxazolo[5,4-b]pyridin-2-yl)butanoic acid 13c. Yield: 0.157 g (52 %), white crystals, M.p.: 299–302°C.

^{1}H NMR spectrum (80 MHz, DMSO-d6), δ ppm: 1.90-2.06 (m., 2H, 3-CH_2); 2.22 (s., 3H, CH_3); 2.73 (t., *J*=6.0 Hz, 4H, 4,2-CH_2); 6.49 (s., 1H, H-6); 7.14 (d.d., ^3J=5.0 Hz, ^4J=3.8 Hz, 1H, H-4 thiophen); 7.55-7.60 (m., 1H, H-3 thiophen); 7.67-7.73 (m., 1H, H-5 thiophen); 12.85 (br.s., 1H, OH).

^{13}C NMR spectrum (21 MHz, DMSO-d6), δ ppm: 17.1 (CH_3); 32.9 (3C 2,3,4-CH_2); 106.2 (C-6); 118.9; 127.9 (C thiophen); 129.6 (C thiophen); 130.3 (C thiophen); 136.6; 142.4; 144.9; 159.9; 172.7 (2C).

HRMS m/z: calcd for $C_{15}H_{15}N_2O_3S^+$ $[M + H]^+$: 303.0798; found: 303.0801.

2-(5,7-Dimethyloxazolo[5,4-b]pyridin-2-yl)benzoic acid 14a. Yield: 0.161 g (60 %), light brown crystals, M.p.: 302–305°C.

^{1}H NMR spectrum (80 MHz, DMSO-d6), δ ppm: 2.00 (s., 3H, CH_3); 2.20 (s., 3H, CH_3); 6.05 (s., 1H, H-6); 7.92 (br.s., 4H, H-3,4,5,6 Ar); 11.96 (br.s., 1H, OH).

^{13}C NMR spectrum (21 MHz, DMSO-d6), δ ppm: 17.5 (CH_3); 18.4 (CH_3); 106.5 (C-6); 117.2; 123.5 (3C); 131.6; 134.8 (3C); 145.6; 151.4; 159.3; 166.9.

HRMS m/z: calcd for $C_{15}H_{13}N_2O_3^+$ $[M + H]^+$: 269.0921; found: 269.0930.

2-(5-Methyl-7-phenyloxazolo[5,4-b]pyridin-2-yl)benzoic acid 14b. Yield: 0.225 g (68 %), light brown powder, M.p.: 312–314°C.

^{1}H NMR spectrum (80 MHz, DMSO-d6), δ ppm: 2.29 (s., 3H, CH_3); 6.21 (s., 1H, H-7); 7.30 (br.s., 5H, H-2,3,4,5,6 Ph); 7.88 (br.s., 4H, H-3',4',5',6' Ar); 12.31 (br.s., 1H, OH).

^{13}C NMR spectrum (21 MHz, DMSO-d6), δ ppm: 18.6 (CH_3); 105.8 (C-6); 115.8; 123.6 (3C); 126.8 (3C); 128.6 (3C); 128.9; 131.2; 135.0; 136.2; 146.9; 153.5; 159.6; 167.4.

HRMS m/z: calcd for $C_{20}H_{15}N_2O_3^+$ $[M + H]^+$: 331.1077; found: 331.1085.

2-(5-Methyl-7-(thiophen-2-yl)oxazolo[5,4-b]pyridin-2-yl)benzoic acid 14c. Yield: 0.209g (62 %), gray crystals, M.p.: 341–343°C.

^{1}H NMR spectrum (80 MHz, DMSO-d6), δ ppm: 2.29 (s., 3H, CH_3); 6.67 (s., 1H, H-6); 7.14 (d.d., ^3J=5.0 Hz, ^4J=3.8 Hz, 1H, H-4 thiophen); 7.60-7.69 (m., 2H, H-3,5 tiophene); 7.97 (br.s., 4H, H-3,4,5,6 Ar); 12.45 (br.s., 1H, OH).

^{13}C NMR spectrum (21 MHz, DMSO-d6), δ ppm: 18.7 (CH_3); 102.9 (C-6); 113.5; 123.8; 127.8; 128.4; 130.01; 130.8; 130.9; 131.7; 132.8; 135.3; 135.5; 144.5; 146.4; 159.9; 167.5; 168.7.

HRMS m/z: calcd for $C_{18}H_{13}N_2O_3S^+$ $[M + H]^+$: 337.0641; found: 337.0638.

Molecular Docking

Molecular docking is a method used to predict the optimal position of a ligand relative to a protein receptor to form a stable complex [32]. This method takes into account scoring functions and allows the estimation of binding strength or affinity between a ligand and a protein. Molecular docking is commonly used to predict how potential drug compounds may bind to target proteins, allowing their effectiveness and binding strength to be assessed. This technique is essential for the design and development of pharmaceuticals [33].

The main goal of molecular docking is computer modeling of the molecular identification process and achieving optimal conformation with minimal free energy of the entire system. The discovery of a new drug is a complex task, and modern approaches are mainly based on the *in silico* approach. The use of computer technologies in the discovery and development of drug compounds is becoming increasingly popular and recognized. Therefore, molecular docking plays a key role in the search for new pharmacologically active compounds in medical science, and its utility is leveraged in the field of structure-based drug design and biochemical investigations.

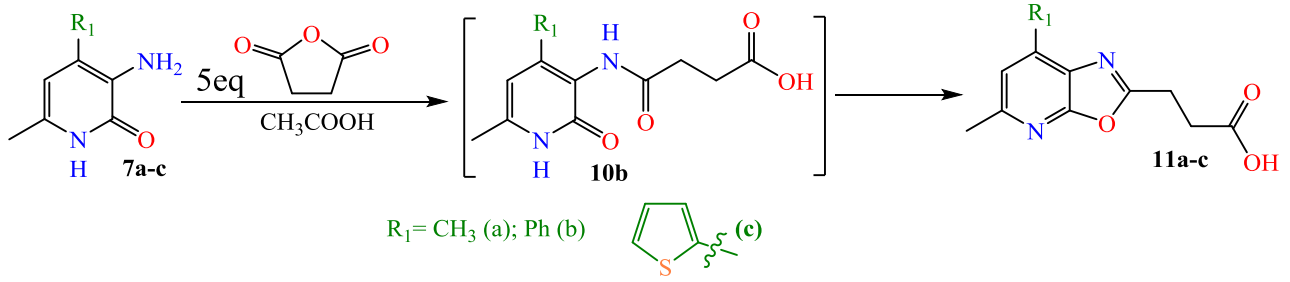
The docking procedure was performed using AutoDock Vina software [34]. Ligand molecules were designed using ChemBio3D Ultra 14.0 software, and 3D protein structures were obtained from the Protein Data Bank (RCSB) [35]. Before docking, the protein structures underwent preparation steps, including removing native ligands and water molecules, addition of polar hydrogen atoms, and conversion of the structures to.pdbqt format using AutoDock MGL software package [36]. For the enzyme COX-1 (PDB: 1EQG), the active site grid coordinates (X=29.29, Y=34.10, Z=199.90; 19.61 × 15.10 × 18.40 $Å^3$) were specified, and for the enzyme COX-2 (PDB: 1CX2), the active site grid coordinates (X=24.25, Y=20.06, Z=16.66; 17.26×14.75×15.26 $Å^3$) were provided. The ligands interactions within the binding sites were examined using Discovery Studio Visualizer software [37].

Results and Discussion

Chemistry

Cyclic anhydrides of dicarboxylic acids were chosen to study new cases of the cyclization reaction of amide derivatives of 3-aminopyridin-2(1H)-ones into the corresponding oxazolo[5,4-b]pyridines. It was assumed that monoamides containing a carboxylic linker fragment could be synthesized through the acylation of 3-aminopyridine-2(1H)-ones 7a-c with cyclic anhydrides of dicarboxylic acids (Scheme 4). Activation of the carboxyl group followed by aminolysis of 3-aminopyridine-2(1H)-one may lead to the formation of diamides from the respective acids, which can efficiently cyclize to produce the corresponding bisderivatives of oxazolo[5,4-b]pyridine.

For this purpose, we carried out the reaction of 3-aminopyridones **7a-c** with succinic anhydride according to Scheme 3. The reaction was carried out by refluxing in acetic acid using a slight excess (1.5 equiv.) of succinic anhydride. Analysis of the reaction mixture by thin-layer chromatography showed the presence of unreacted starting 3-aminopyridine-2(1*H*)-one. To increase the conversion of the starting 3-aminopyridine-2(1*H*)-one into the desired product, we gradually added an additional excess of succinic anhydride to the reaction mixture. The optimal amount of succinic anhydride added to the reaction until the complete disappearance of the starting 3-aminopyridine-2(1*H*)-one was 5 equiv.



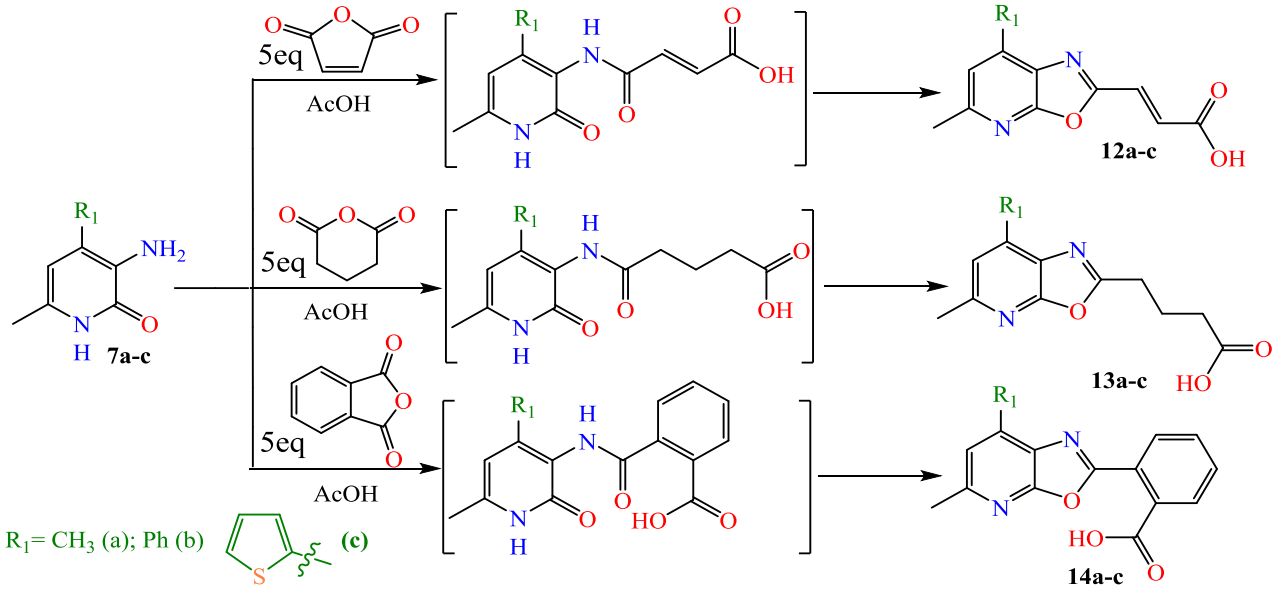
Scheme 3. Cyclization reaction of 3-aminopyridones **7a-c** with 5 equiv of succinic anhydride into oxazolo[5,4-b]pyridine **11a-c**

Analysis of the isolated product of the reaction of aminopyridone **11b** with succinic anhydride by high-resolution mass spectrometry showed that the target product had a molecular ion peak not at [M+] = 300.3140, as expected for the monoamide **10b**, but at [M+] = 282.3573, i.e., 18 a.m.u. lower, indicating the

loss of one water molecule. Based on this, we hypothesized that under the excess anhydride conditions, the monoamide part underwent cyclization to form the corresponding oxazolopyridine **11b**.

The formation of oxazolopyridine **11b** was unambiguously confirmed by ^{1}H and ^{13}C NMR spectral analysis, which showed the absence of the amide NH proton singlet in the spectrum. A slight shift was observed for the H-5 proton, registering from 5.98 ppm in the starting 3-aminopyridone **11a** to 6.13 ppm, indirectly confirming the aromatization of the condensed pyridine ring. The spectrum also showed characteristic multiplets as two doublets of doublets of two aliphatic methylene groups at 2.57 ppm and 2.76 ppm. The acidic proton of the carboxyl OH group appeared as a singlet at 12.20 ppm. Similar reactions took place between 3-aminopyridones **11a-c** and maleic and glutaric anhydrides.

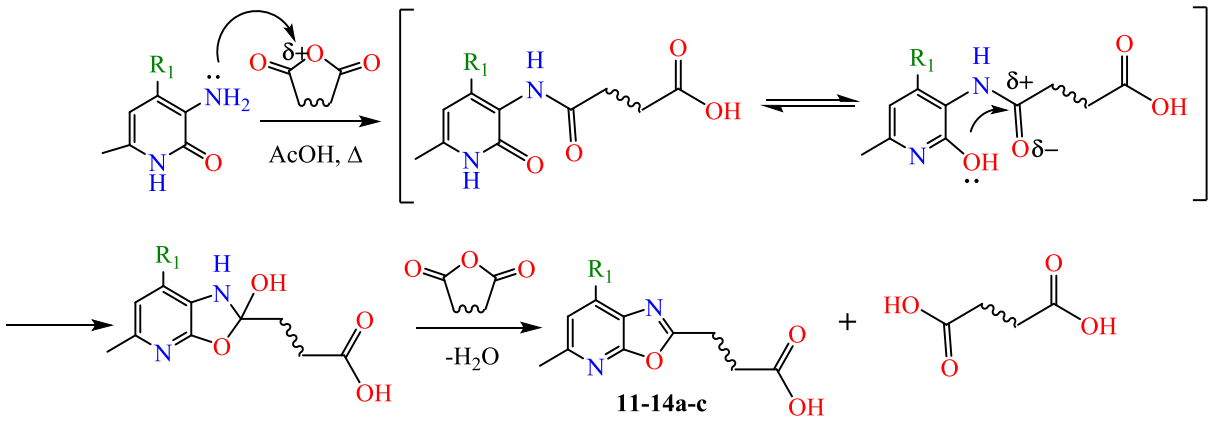
In order to expand the arsenal of new derivatives of oxazolo[5,4-b]pyridine and verify their synthesis using cyclic anhydrides of dicarboxylic acids, we carried out a similar intramolecular heterocyclization of 3-aminopyridine-2(1*H*)-ones with a five-fold excess of maleic and glutaric anhydrides (Scheme 4).



Scheme 4. Cyclization reaction of 3-aminopyridones **7a-c** with 5 equiv. of maleic or glutaric anhydride into the corresponding oxazolo[5,4-b]pyridine **12-14a-c**

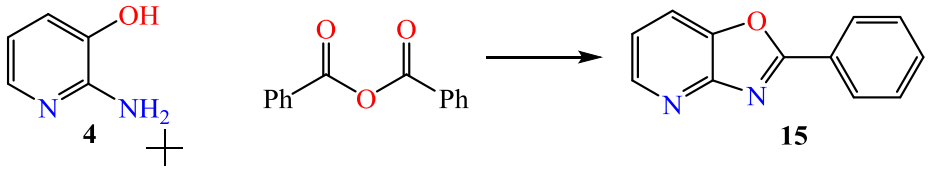
The structure of all obtained compounds **11-14a-c** was confirmed by ^{1}H and ^{13}C NMR spectroscopy and high-resolution mass spectrometry.

Apparently, the monoamide formed at the first stage under the excess anhydride, which acts as an effective dehydrating agent in this case, undergoes intramolecular cyclization as follows (Scheme 5). In this case, intramolecular nucleophilic addition of the hydroxyl group of the lactim form of pyridone to the amide carbonyl with subsequent elimination of water occurs.



Scheme 5. The proposed reaction mechanism

It is worth noting that only one example of a similar method for obtaining unsubstituted oxazolo[4,5-b]pyridine **15** is given in the literature, based on the cyclization reaction of 2-amino-3-hydroxypyridine with benzoic anhydride (Scheme 6) [38, 32].



Scheme 6. Cyclization reaction of 2-amino-3-hydroxypyridine with benzoic anhydride into oxazolo[4,5-b]pyridine **15**

Thus, the reaction we discovered between 3-aminopyridones and dicarboxylic acid anhydrides leads to a one-step cyclization into oxazolo[5,4-b]pyridines **11-13a-c** with an acidic carboxylic linker. This transformation not only improves the water solubility of the compounds for bioassays but also facilitates additional structural modifications.

Molecular Docking

The literature contains data confirming the anti-inflammatory activity of oxazolopyridines [11]. Therefore, molecular docking was used to evaluate presumed anti-inflammatory activity and understand the molecular interactions between synthesized ligand molecules and target proteins.

The enzyme COX-1 (PDB: 1EQG) [39] and enzyme COX-2 (PDB: 1CX2) [40] were chosen as target proteins and Diclofenac was chosen as a well known anti-inflammatory reference drug. Cyclooxygenases (COX), also known as prostaglandin-endoperoxide synthases, are pivotal enzymes that are essential for the biosynthesis of prostaglandins, critical molecules involved in regulating inflammation, pain, and fever. The body houses two primary isoforms of these enzymes — cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). COX-1 is primarily responsible for the production of key signaling molecules such as prostaglandins, prostacyclin, and thromboxanes, contributing to processes related to pain sensitivity, blood clotting, and gastric mucosal protection [41]. Conversely, COX-2 is primarily involved in transmitting pain signals during inflammatory conditions and plays a significant role in prostaglandin synthesis within inflammatory cells and the central nervous system [42].

The docking results showed that for the studied structures, the binding affinity with the selected protein receptors was slightly higher than the binding affinity of these proteins with diclofenac, chosen as a reference drug (Table 1).

Table 1

Binding affinity (kcal/mol) of oxazolo[5,4-b]pyridine derivatives 12-14a-c and diclofenac in the active site of COX-1 (PDB: 1EQG) and COX-2 (PDB: 1CX2) proteins

Ligand / Receptor	**Diclofenac**	**11a**	**11b**	**11c**	**12a**	**12b**	**12c**	**13a**	**13b**	**13c**	**14a**	**14b**	**14c**
1EQG	–8.4	–7.5	–7.9	–7.4	–7.3	–6.3	–5.6	–7.5	–7.9	–7.3	–9.1	–6.6	–6.4
1CX2	–8.1	–7.7	–9.4	–8.7	–8.2	–9.7	–9.2	–8.0	–9.6	–8.8	–9.1	–9.8	–9.0

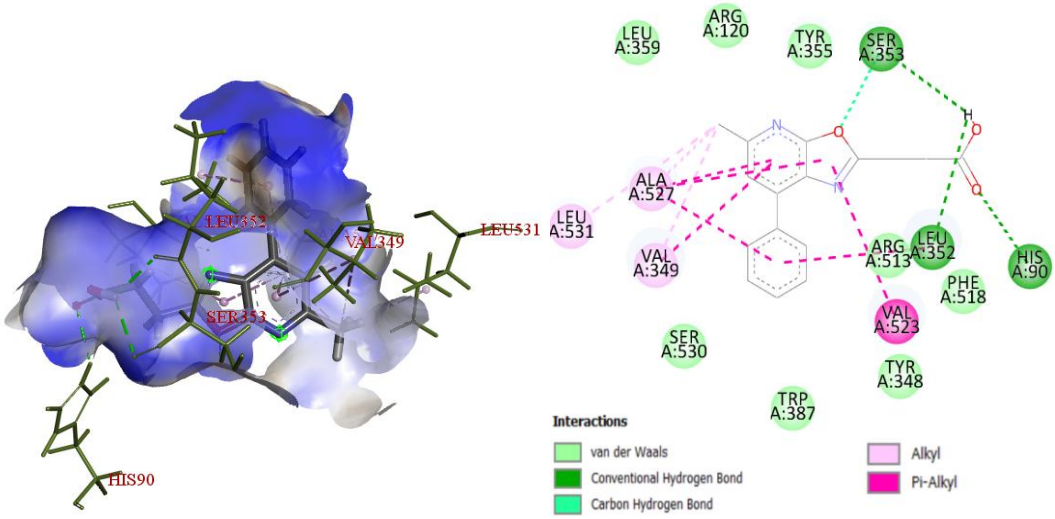
As can be seen in Table 1, compounds **11–14b** exhibited the highest binding energy values with the 1CX2 receptor protein, so we further described its interaction in more detail (Table 2).

The interaction of compound **11b** with the enzyme cyclooxygenase-2 (COX-2) (PDB: 1CX2) has a higher binding affinity of –9.4 kcal/mol. This is explained by the formation of one carbon-hydrogen bond and three hydrogen bonds between the oxygen atom of the oxazole ring and the hydrogen atom of the carboxyl group oxygen of compound **11b** and the amino acid residues LEU352, HIS90, SER353, respectively. The oxazolopyridine and phenyl rings also form six π-Alkyl interactions with amino acids VAL523, LEU352, ALA527, and VAL349. Additionally, compound 2 forms van der Waals interactions with residual amino acids SER530, TRP387, TYR348, LEU359, TYR355, ARG120, ARG513, PHE518 (Fig. 2).

Table 2

Basic hydrogen bonds and acid interactions of oxazolo[5,4-b]pyridine derivatives 11-14и with the COX-2 (PDB: 1CX2) protein

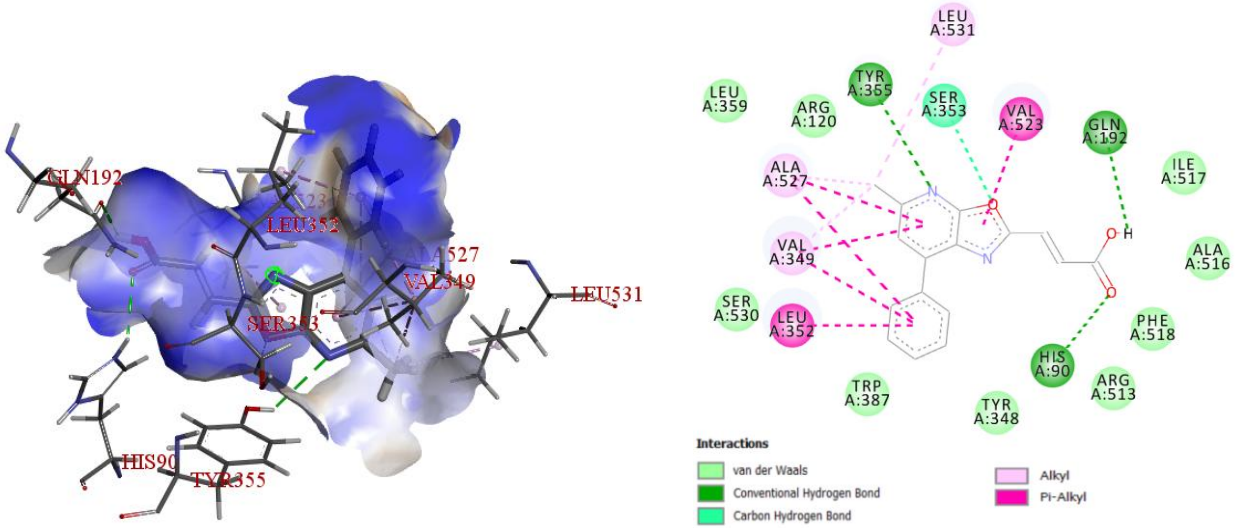
Compound	Receptor	H-Hydrogen bonds	Residual Amino acid Interactions	
			Amide-Pi Stacked / Pi-Sulfur/ Pis interactions/ Pi-Pi Stacked/Pi-Anion/ Pi-Pi T-shaped/Pi-Alkyl/	Van-der Walls interactions
11b	1CX2	SER353, LEU352, HIS90	VAL523, VAL349, ALA527, LEU531, LEU352	SER530, TRP387, TYR348, LEU359, TYR355, ARG120, ARG513, PHE518
12b		TYR355, GLN192, HIS90, SER353	LEU352, VAL349, ALA527, VAL523, LEU531	LEU359, SER530, TRP387, TYR348, ARG513, PHE518, ALA516, ILE517, ARG120
13b		TYR355, HIS90, SER353	LEU352, VAL349, ALA527, VAL523, LEU531	LEU359, ARG120, SER530, TRP387, TYR348, ARG513, PHE518
14b		SER530	LEU352, VAL349, ALA527, VAL523, LEU531, HIS90, YR355, ARG120, TRP387, GLY526	ARG513, SER353, VAL116, LEU359, MET522, LEU384, TYR385, PHE518, TYR348



3D docking model; (b) 2D docking model

Figure 2. Complex of **11b** with cyclooxygenase-2 (COX-2)

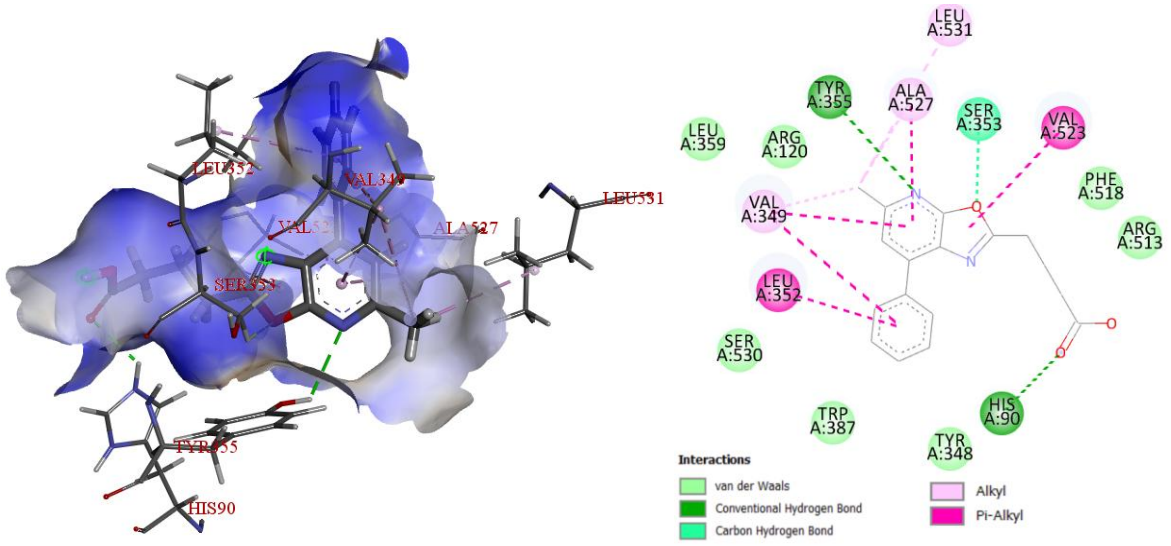
Compound **12b** has a binding affinity of –9.7 kcal/mol with cyclooxygenase-2 (COX-2) (PDB: 1CX2) due to the formation of six π-alkyl interactions of the oxazolopyridine and phenyl rings with the amino acids VAL349, LEU352, ALA527 and VAL523. Additionally, a carbon-hydrogen bond is formed between the amino acid residue SER 353 and the oxygen atom. Also, three hydrogen bonds are established between the nitrogen atom of the pyridine ring and the oxygen and hydrogen atoms of the carbonyl group with the amino acid residues GLN192, TYR355, HIS90, respectively. Compound **12b** forms van der Waals interactions with the residual amino acids LEU359, SER 530, TRP387, TYR348, ARG513, PHE518, ALA516, ILE517, ARG120 (Fig. 3).



(*a*) 3D docking model; (*b*) 2D docking model

Figure 3. Complex of **12b** with cyclooxygenase-2 (COX-2)

It was found that compound **13b** has a docking score of –9.6 kcal/mol with cyclooxygenase-2 (COX-2) (PDB: 1CX2) due to the formation four π-Alkyl interactions observed between the phenyl and oxozolopyridine rings with amino acid residues LEU352, VAL349, ALA527, and VAL523, respectively. Additionally, there is one carbon-hydrogen bond interaction between the oxygen of the oxazole ring and the amino acid residue SER353. There are also two hydrogen bonds between the nitrogen atom of the pyridine ring and the amino acid TYR355, as well as between the oxygen atom of the carbonyl group and the amino acid residue HIS90. Compound **13b** also forms van der Waals interactions with residual amino acids LEU359, SER530, TRP387, TYR348, ARG513, PHE518, ALA516, ARG120 (Fig. 4).

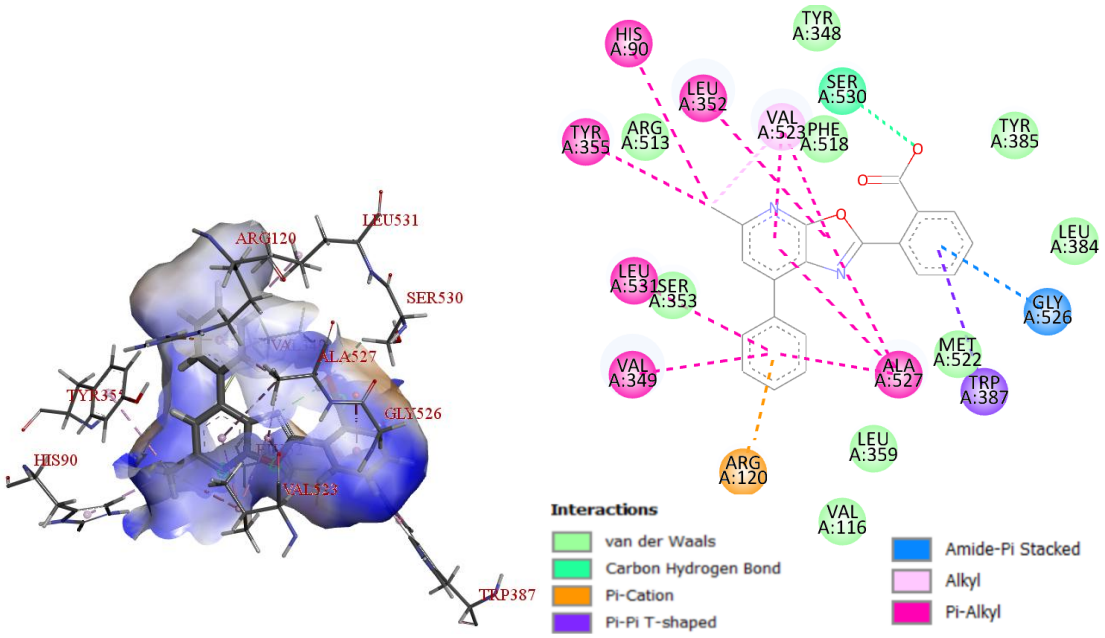


(*a*) 3D docking model; (*b*) 2D docking model

Figure 4. Complex of **13b** with cyclooxygenase-2 (COX-2)

In conclusion, compound **14b** has a binding affinity of -9.8 kcal/mol with cyclooxygenase-2 (COX-2) (PDB: 1CX2) due to the formation of a single carbon-hydrogen bond between the oxygen atom of the carbonyl group and the amino acid residue SER530. There are also eight π-alkyl interactions observed between the phenyl and oxozolopyridine rings with amino acid residues LEU351, VAL349, ALA527, LEU352, HIS90 and TYR355, respectively. Moreover, the benzoyl ring forms one π-π T-shaped and one amide-π stacked interaction with the amino acid residues TRP387 and GLY526, respectively. Compound **14b** also

forms van der Waals interactions with the residual amino acids ARG513, SER 53, VOL 116, LEU359, MET522, LEU384, TYR385, PHE518, TYR348.



(*a*) 3D docking model; (*b*) 2D docking model

Figure 5. Complex of **14b** with cyclooxygenase-2 (COX-2)

It was found that the presence of a phenyl substituent at the 4th pyridone position in compounds **11–14b** increases their affinity to the selected receptors compared to other derivatives.

Thus, taking into account computer modeling, the newly synthesized oxazolo[5,4-b]pyridine derivatives based on 3-aminopyridin-2(1*H*)-ones are very promising for further study of their potential anti-inflammatory activity.

Conclusions

Consequently, our acylation reaction of 3-aminopyridones with dicarboxylic acid anhydrides showed that the monoamides formed at the first stage of the reaction undergo intramolecular cyclization to form oxazolo[5,4-b]pyridines **11–14a-c**. The presence of carboxylic acid linkers in structures **11–14a-c** not only increases water solubility of the compounds for further bioassays, but will also allow various modifications of the structure. In addition, the potential fluorescence of the condensed oxazolo[5,4-b]pyridine fragment [43] combined with the carboxylic acid linker will allow these derivatives to be used as possible biomarkers. The possible anti-inflammatory activity of the 12 newly obtained oxazolo[5,4-b]pyridine derivatives was evaluated using molecular docking with the AutoDock Vina program. Some compounds (**11–14b**) showed higher binding affinity to the target protein (1CX2) compared to well-known anti-inflammatory drug diclofenac. Molecular studies have revealed that the presence of a phenyl substituent at the 4th position of oxazolo[5,4-b]pyridine promotes a stronger interaction of compounds **11–14b** with the target protein. Therefore, the synthesized oxazolo[5,4-b]pyridines have high potential and prospects for further investigation their anti-inflammatory activity in vivo.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Irina Valerievna Palamarchuk** investigation, formal analysis, data curation and writing — original draft preparation; **Ivan Vyacheslavovich Kulakov** conceptualization, methodology, validation, writing — review and editing and supervision.

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Conflicts of Interest

The authors declare no conflict of interest.

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