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Synthesis and Anti-inflammatory Activity of Some New 6-Aryltriazolo[3,4-*b*][1,3,4]thiadiazole Derivatives

Abstract

A series of 6-aryl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives has been synthesized. Their anti-inflammatory activity has been studied *in vivo* in a carrageenan model of the paw inflammatory edema in rats. 3-(2-Fluorophenyl)-6-phenyl-[1,2,4]triazolo-[3,4-*b*][1,3,4]thiadiazole (**3c**) and 3-(2-fluorophenyl)-6-(4-methoxy-phenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (**3d**) have been identified as hit compounds with the anti-exudative activity. The crucial role of the fluorine atom in the anti-inflammatory activity has been determined, which value considerably correlates with the calculated values of lipophilicity and solubility.

Keywords: [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole; anti-inflammatory activity; cyclooxygenase; docking

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Синтез та протизапальна активність деяких нових похідних 6-арил[1,2,4]триазоло[3,4-*b*][1,3,4]тіадіазолу

Анотація

Синтезовано ряд похідних 6-арил[1,2,4]триазоло[3,4-*b*][1,3,4]тіадіазолу. Для цих сполук досліджено *in vivo* протизапальну активність на карагеніновій моделі запального набряку лапи щура. 3-(2-Фторофеніл)-6-феніл-[1,2,4]триазоло[3,4-*b*]-[1,3,4]тіадіазол (**3c**) та 3-(2-фторофеніл)-6-(4-метоксифеніл)[1,2,4]триазоло[3,4-*b*][1,3,4]тіадіазол (**3d**) було ідентифіковано як сполуки-хіти з антиексудативною активністю. Виявлено важливу роль атома Фтору в протизапальній активності, величина якої корелює з розрахованими значеннями ліпофільності та розчинності.

Ключові слова: [1,2,4]триазоло[3,4-*b*][1,3,4]тіадіазол; протизапальна активність; циклооксигеназа; докінг

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■ Introduction

Inflammation is a universal physiological response to injury that can be caused by infectious, allergic, toxic, physical, and neurogenic factors. It is one of the most common diseases that can be fatal [1]. Nonsteroidal anti-inflammatory drugs (NSAIDs) represent a class of important therapeutic agents. They are used as anti-inflammatory, antipyretic, and analgesic medicines. However, NSAIDs may cause serious side effects, including gastrointestinal bleeding, peptic ulcer disease, hypertension, edema, and kidney disease [2]. Some NSAIDs have also been associated with an increased risk of myocardial infarction [3]. Therefore, novel anti-inflammatory and analgesic compounds with improved safety profiles still need to be invented.

Currently, the synthesis and biological activities of various 1,2,4-triazole and 1,3,4-thiadiazole derivatives and their *N*-bridged heterocyclic analogs are the subject of intensive research [4–7]. 1,2,4-Triazole and 1,3,4-thiadiazole rings are known to be unique pharmacophores in several drugs and natural products. The favorable properties of triazole and thiadiazole rings, including

moderate dipole properties, hydrogen bonding ability, optimal lipophilicity and rigidity, are responsible for their biological activities [4–7]. Thiadiazole is the bioisostere of pyrimidine and oxadiazole [7].

[1,2,4]Triazolo[3,4-*b*][1,3,4]thiadiazoles, including 1,2,4-triazole and 1,3,4-thiadiazole rings, are attractive heterocyclic compounds for medical chemists. They are important analogs of bioactive compounds with diverse pharmacological effects, including the anti-inflammatory one [8–18]. Among [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives, some selective inhibitors of the cyclooxygenase COX-2 isoform were found [18, 19].

The aim of this work was to synthesize new [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole derivatives and study their anti-exudative potential.

■ Results and discussion

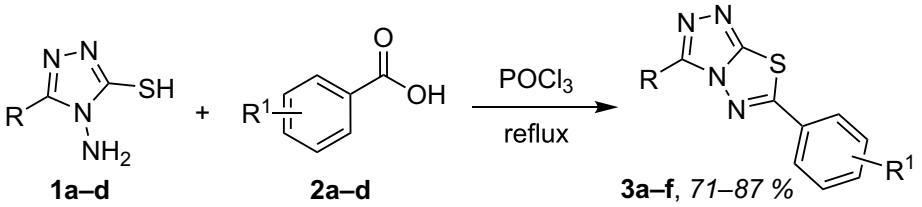
Synthesis of [1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazole targeted derivatives

The synthesis of the targeted [1,2,4]triazolo-[3,4-b][1,3,4]thiadiazole derivatives was carried out by the reaction of 1-amino-4*H*-[1,2,4]triazol-3-thiols **1a–c** with commercially available benzoic acid **2a** and its substituted derivatives **2b–d** (**Scheme**). The reaction was performed in $POCl_3$ under reflux. The resulting compounds are light yellow substances that are highly soluble in DMFA, DMSO, and dioxane, moderately soluble in ethanol and acetic acid, and insoluble in water and non-polar solvents.

The anti-inflammatory activity of compounds **3a–f** synthesized was evaluated by the carrageenan-induced paw edema method [19]. The compounds were tested in the dose of 50 mg kg^{-1}, and the activity was compared to the reference drugs – Ibuprofen, Diclofenac, and Ketorolac in the mean therapeutic dose of 10 mg kg^{-1} (**Table 1**).

Compounds **3c** and **3d** were identified as the most active among the substances studied, and their anti-inflammatory effect was equal to or higher than that of the reference drugs. A significant effect was also observed in the case of compound **3b**. All active compounds possess a fluorine atom in the aromatic cycle in position 6 of the [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole moieties.

Compounds containing heterocyclic rings substituted with fluorinated phenyl moiety are



1a: R = C_6H_5; **1b**: R = 2-F-C_6H_4; **1c**: R = 4-Cl-$C_6H_4CH_2$; **1d**: R = 4-MeO-$C_6H_4CH_2$

2a: R^1 = H; **2b**: R^1 = 4-CF_3; **2c**: R^1 = 4-MeO; **2d**: R^1 = 3,4-*di*-MeO

3a: R = C_6H_5, R^1 = H; **3b**: R = C_6H_5, R^1 = 4-CF_3; **3c**: R = 2-F-C_6H_4, R^1 = H;

3d: R = 2-F-C_6H_4, R^1 = 4-MeO; **3e**: R = 4-Cl-$C_6H_4CH_2$, R^1 = H;

3f: R = 4-MeO-$C_6H_4CH_2$, R^1 = 3,4-*di*-MeO

Scheme. The synthesis of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles **3a–f**

Table 1. The anti-inflammatory activity of compounds **3a–f** synthesized compared to Ibuprofen, Diclofenac and Ketorolac

Compound	Paw edema volume, (mL) ± SEM*	Inhibition, %	Activity relative to Ibuprofen, %	Activity relative to Diclofenac, %	Activity relative to Ketorolac, %
Control	2.20 ± 0.05	-	-	-	-
3a	1.73 ± 0.04	21.4	53.5	49.1	55.4
3b	1.48 ± 0.05	32.7	81.8	75.0	84.7
3c	1.24 ± 0.03	43.6	109.0	100.0	113.0
3d	1.27 ± 0.05	42.3	105.8	97.0	109.6
3e	1.64 ± 0.02	25.5	63.8	58.5	66.1
3f	1.94 ± 0.03	11.8	29.5	27.1	30.6
Ibuprofen	1.32 ± 0.03	40.0	100	-	-
Diclofenac	1.24± 0.03	43.6	-	100	-
Ketorolac	1.35± 0.04	38.6	-	-	100

Note: *SEM denotes the standard error of the mean

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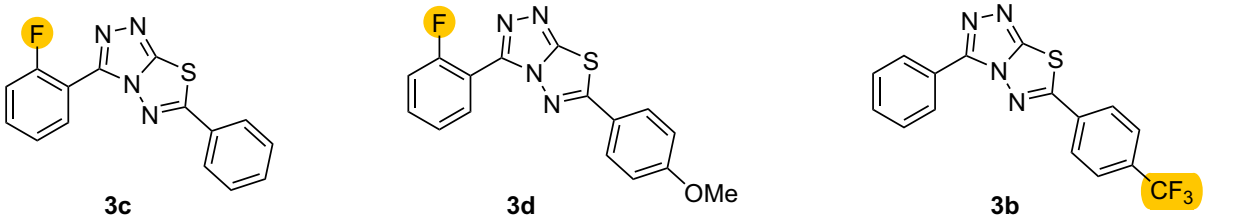
well-recognized anti-inflammatory and analgesic agents [20–24]. There are many reports that the incorporation of fluorine into a molecule results in increased binding affinity to the target protein [25–29]. In addition, an increase in the rate of absorption and transport of the drug *in vivo* was observed [27–29].

The consensus values for lipophilicity and water solubility [30] calculated using the SwissADME web resource [31] correlate with the anti-inflammatory activity of highly active fluorinated compounds (**Figure 1**). An increase in lipophilicity and a decrease in solubility lead to the loss of the anti-inflammatory effect. It should be noted that fluoro-substituted derivatives are also among the anti-inflammatory drugs available in the pharmaceutical market (Celecoxib, Fluproquazone).

Cyclooxygenase (COX) enzymes are involved in the synthesis of various prostanoids, which participate in physiological and pathological mechanisms of inflammation. There are two isoforms of COX enzymes: COX-1 and COX-2. COX-1 is a regulatory enzyme with physiological and homeostatic functions, whereas COX-2 stimulates the synthesis of prostaglandins, which cause pathological conditions, such as inflammation. Side effects of NSAIDs are caused by the inhibition of the physiological function of COX-1, while selective COX-2 inhibitors cause the anti-inflammatory activity without the risk of side effects. Compounds **3b–d** were docked [32, 33] into the binding site pockets of COX-1 and COX-2. The molecular docking studies were conducted using Autodock Vina [32] for docking simulations and a Discovery Studio Visualizer [33] for visualization and interpretation. The protein structures were retrieved from the Protein Data Bank (PDB): Cyclooxygenase 2 (PDB ID 1PXX) and Cyclooxygenase 1 (PDB ID 1HT5). Proteins were prepared by removing water molecules, adding hydrogen atoms, assigning partial charges, and converting the protein to the PDBQT format using AutoDock Tools. Ligands were drawn using ChemDraw and prepared by adding hydrogens, assigning charges, and optimizing their geometry through the energy minimization using Open Babel, then converted to the PDBQT format. AutoDock Vina was then utilized to perform the docking simulation, with a grid box size of 20 Å defined to encompass the protein's active site and an exhaustiveness value of 8 to ensure a thorough search. A Discovery Studio Visualizer was used to visualize and analyze the docked complexes, identifying key interactions.

These compounds exhibited stronger interactions at the COX-2 binding site than the COX-1 active site pocket. Binding scores and information about amino acids involved in interactions of the docked compounds **3b–d** on the active sites of COX-1 and COX-2 enzymes are provided in **Table 2** and visualized in **Figures 2–4**.

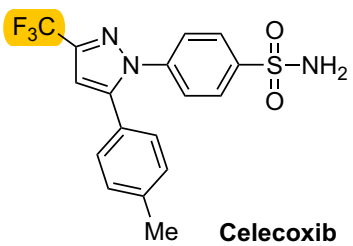
Compounds **3b–d** are characterized by Pi-Sigma and Pi-Alkyl interactions of amino acid fragments, which contain alkyl residues with aromatic cycles or a heterocyclic system (**Table 2**). In addition, an important role in bonding is played by Pi-Cation, Pi-Sulfur, Pi-Pi Stacked, Pi-Pi T-shaped, and various hydrogen bonds. (Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Donor Hydrogen Bond) (**Table 2**).



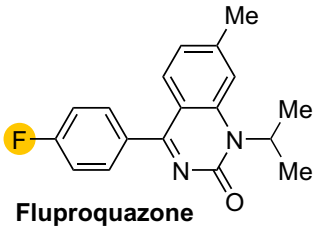
Activity relative to ibuprofen = 109.0 %
Consensus Log $P_{o/w}$ = 3.66
Water solubility = 8.01e-03 mg mL^{-1}

Activity relative to ibuprofen = 105.8 %
Consensus Log $P_{o/w}$ = 3.59
Water solubility = 7.93e-03 mg mL^{-1}

Activity relative to ibuprofen = 81.8 %
Consensus Log $P_{o/w}$ = 4.32
Water solubility = 2.08e-03 mg mL^{-1}



Celecoxib



Fluproquazone

Figure 1. Bioactive fluorinated compounds **3b–d** and anti-inflammatory drugs

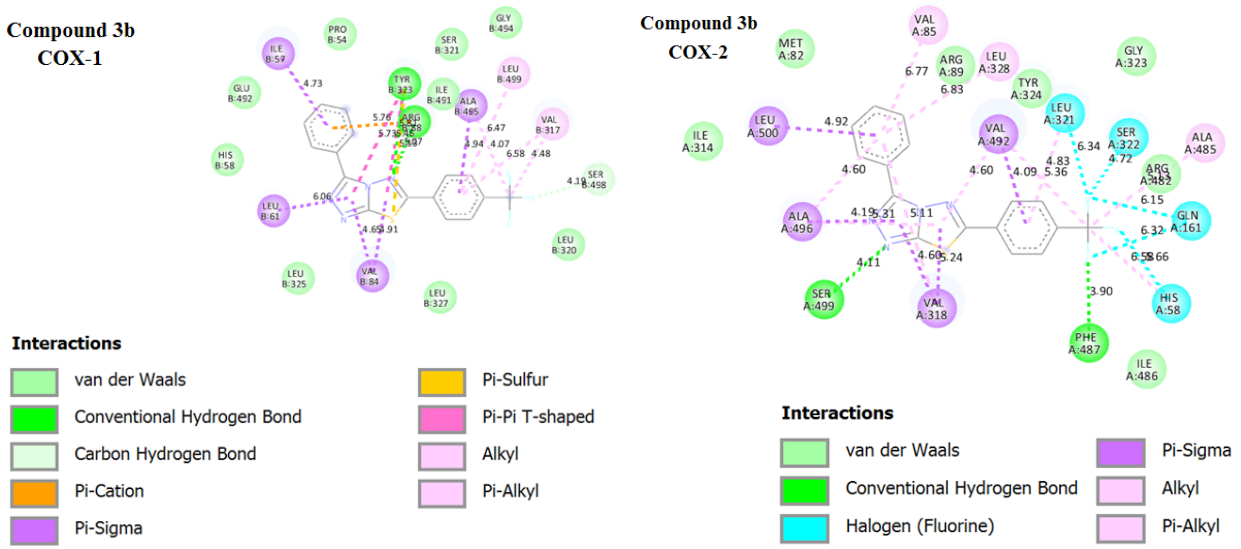


Figure 2. The binding mode of compound **3b** into the binding sites of COX-1 and COX-2

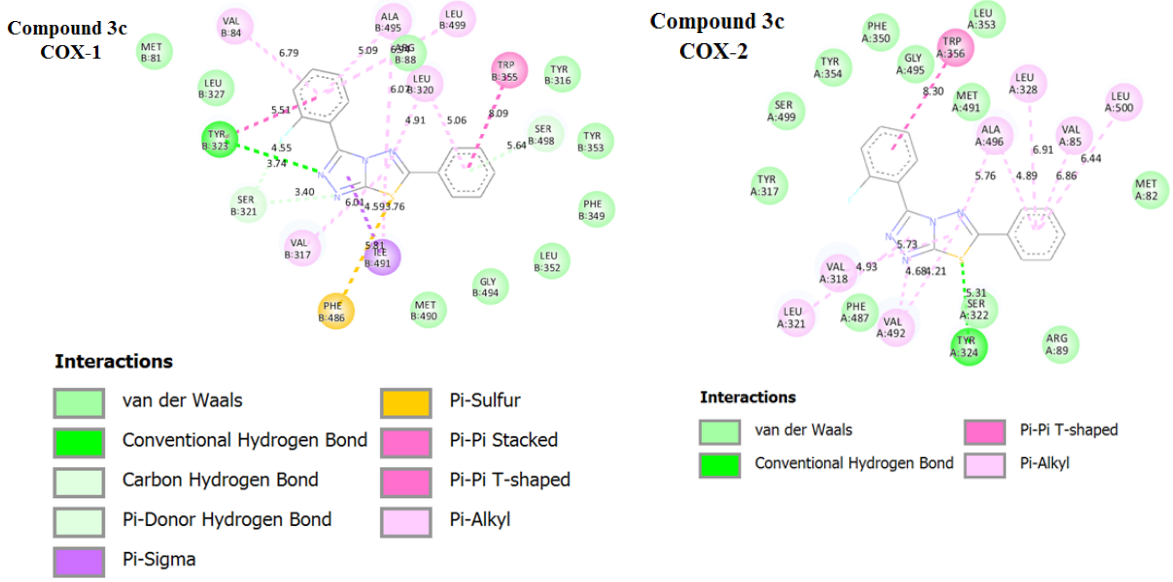


Figure 3. The binding mode of compound **3c** into the binding sites of COX-1 and COX-2

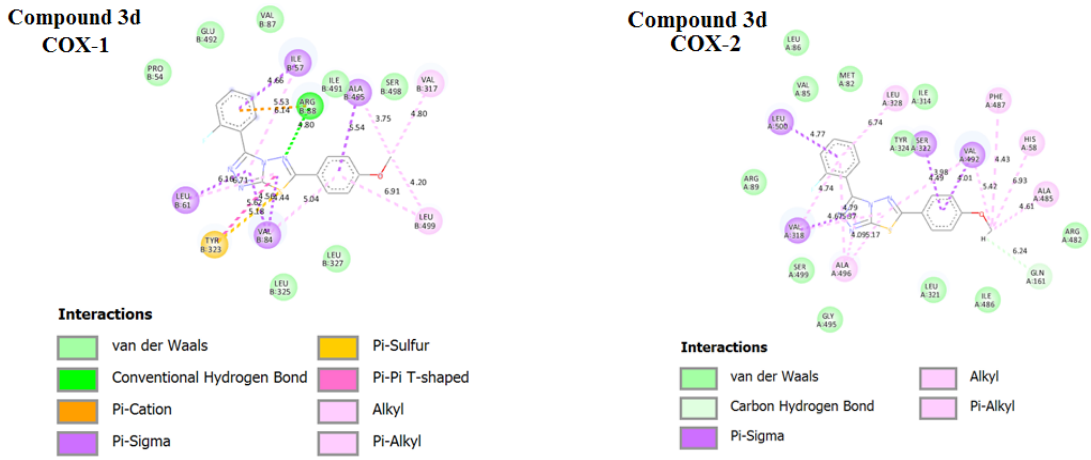


Figure 4. The binding mode of compound **3d** into the binding sites of COX-1 and COX-2

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Table 2. Binding scores and amino acids involved in interactions of the docked compounds **3b-d** on the active sites of COX-1 and COX-2 enzymes*

Compound	Binding energy, kcal mol^{-1}		Interacting residues			
			Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Donor Hydrogen Bond	Pi-Cation, Pi-Sulfur	Pi-Pi Stacked, Pi-Pi T-shaped	Pi-Alkyl, Pi-Sigma
3b	COX-1	-7.4	Arg B:88 Tyr B:323 Ser B:498	Arg B:88 Tyr B:323	Tyr B:323	Leu B:499 Val B:317 Ile B:57 Leu B:61 Val B:84 Ala B: 495
	COX-2	-9.6	Ser A:499 Phe A:487	–	–	Ala A:496 Val A:85 Val A:318 Val A:492 Leu A:328 Leu A:321 Ala A:485 His A:58 Leu A:500 Ala A:496 Val A:318 Val A:492
3c	COX-1	-7.5	Tyr B:323 Ser B:321 Ser B:498	Phe B:486	Tyr B:323 Trp B:355	Val B:84 Val B:317 Ala B: 495 Leu B:320 Leu B:499 Ile B:491
	COX-2	-8.8	Tyr A:324		Trp A:356	Leu A:321 Val A:318 Val A:492 Ala A:496 Leu A:328 Val A:85 Leu A:500
3d	COX-1	-6.6	Arg B:88	Tyr B:323	Tyr B:323	Leu B:61 Ile B:57 Val B:84 Leu B:499 Val B:317 Leu B:61 Ile B:57 Val B:84 Ala B:495
	COX-2	-8.9	Gln A:161	–	–	Val A:318 Ala A:496 Leu A:328 Val A:492 Phe A:487 His A:58 Ala A:485 Leu A:500 Val A:318 Ser A:322 Val A:492

Note: *Colors of the amino acids involved in the interactions correspond to those shown in **Figures 2-4**

The calculated binding energy of compounds **3b–d** is similar to the binding energy for such non-steroidal anti-inflammatory drugs as Ibuprofen, Diclofenac, Ketorolac, and Celecoxib. The selectivity of binding to cyclooxygenases was not observed (**Table 3**).

■ Conclusions

In order to develop new effective anti-inflammatory agents, a series of [1,2,4]triazolo[3,4-*b*]-[1,3,4]thiadiazoles has been synthesized. Among them, 3-(2-fluorophenyl)-6-phenyl-[1,2,4]triazolo-[3,4-*b*][1,3,4]thiadiazole (**3c**) and 3-(2-fluorophenyl)-6-(4-methoxy-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazole (**3d**) have shown the best activity.

■ Experimental part

The ^{1}H NMR spectra presented in this work were obtained on a Varian instrument at an operating frequency of 400 MHz with DMSO-d_6 as a solvent and tetramethylsilane as an internal standard. Elemental analyses were performed using a Carlo Erba 1106 instrument. Melting points were determined on a Boetius melting point apparatus.

4-Amino-5-(4-R-benzyl)-4*H*-[1,2,4]triazol-3-thiols **1a,b** were obtained according to the procedure described in [34].

The general procedure for the synthesis of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives 3a–f

The corresponding 4-amino-5-R-4*H*-[1,2,4]triazol-3-thiol **1a–d** (5 mmol) and benzoic acids **2a–d** (5 mmol) were dissolved in $POCl_3$ (10 mL). The reaction mixture was refluxed for 8 h, cooled to room temperature, and poured into a mixture of NaOH (20 g), water (50 mL), and ice (50 g). In 1 hour, the precipitate was filtered off and recrystallized from an ethanol-DMFA mixture giving light yellow powders.

3,6-Diphenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**3a**)

Yield – 75 %. M. p. 159–160 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 7.81–7.48 (6H, m, ArH), 8.07 (2H, d, J = 7.1 Hz, ArH), 8.32 (2H, d, J = 7.3 Hz, ArH). Anal. Calcd for $C_{15}H_{10}N_4S$, %: C 64.73, H 3.62, N 20.13. Found, %: C 64.77, H 3.72, N 20.20.

3-Phenyl-6-(4-trifluoromethylphenyl)-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazole (**3b**)

Yield – 87 %. M. p. 188–200 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 7.82–7.47 (3H, m, ArH), 8.02 (2H, d, J = 8.2 Hz, ArH), 8.26–8,34 (4H, m, ArH). Anal. Calcd for $C_{16}H_9F_3N_4S$, %: C 55.49, H 2.62, N 16.18. Found, %: C 55.62, H 2.73, N 16.22.

3-(2-Fluorophenyl)-6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**3c**)

Yield – 84%. M. p. 175–176 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 7.53–7.44 (2H, m, ArH), 7.61 (2H, t, J = 7.7 Hz, ArH), 7.71–7.64 (2H, m, ArH), 7.97 (2H, d, J = 8.0 Hz, ArH), 8.07 (1H, t, J = 7.5 Hz, ArH). Anal. Calcd for $C_{15}H_9FN_4S$, %: C 60.80, H 3.06, N 18.91. Found, %: C 60.75, H 2.99, N 18.70.

3-(2-Fluorophenyl)-6-(4-methoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**3d**)

Yield – 81 %. M. p. 181–183 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.85 (3H, s, CH_3O), 7.14 (2H, d, J = 7.4 Hz, ArH), 7.56–7.41 (2H, m, ArH), 7.67 (1H, d, J = 6.2 Hz, ArH), 7.92 (2H, d, J = 7.3 Hz, ArH), 8.06 (1H, t, J = 7.5 Hz, ArH). Anal. Calcd for $C_{16}H_{11}FN_4OS$, %: C 58.89, H 3.40, N 17.17. Found, %: 58.95, H 3.45, N 17.10.

3-(4-Chlorobenzyl)-6-phenyl-[1,2,4]triazolo-[3,4-b][1,3,4]thiadiazole (**3e**)

Yield – 71 %. M. p. 192–193 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 4.47 (2H, s, CH_2), 7.40 (4H, s, ArH), 7.60–7.72 (2H, m, ArH), 7.93 (2H, d, J = 6.7 Hz, ArH). Anal. Calcd for $C_{16}H_{11}ClN_4S$, %: C 58.81, H 3.39, N 17.14. Found, %: 58.69, H 3.44, N 17.21.

6-(3,4-Dimethoxyphenyl)-3-(4-methoxybenzyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**3f**)

Yield – 72 %. M. p. 201–202 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.70 (3H, s, CH_3O), 3.85 (3H, s, CH_3O), 3.86 (3H, s, CH_3O), 4.36 (2H, s), 6.88 (2H, d, J = 8.6 Hz, ArH), 7.14 (1H, d, J = 8.5 Hz, ArH), 7.30 (2H, d, J = 8.5 Hz, ArH), 7.37 (1H, d, J = 1.9 Hz, ArH), 7.48 (1H, dd, J = 8.4, 2.0 Hz, ArH). Anal. Calcd for $C_{19}H_{18}N_4O_3S$, %: C 59.67, H 4.74, N 14.65. Found, %: 59.79, H 4.61, N 14.54.

Table 3. The binding energy, kcal mol^{-1} of the docked compounds **3b–d** and Ibuprofen, Diclofenac, Ketorolac, and Celecoxib on the active sites of COX-1 and COX-2 enzymes

Target protein	Compound						
	3b	**3c**	**3d**	Ibuprofen	Diclofenac	Ketorolac	Celecoxib
COX-1	-7.4	-7.5	-6.6	-7.6	-7.7	-8.6	-5.2
COX-2	-9.6	-8.8	-8.9	-7.4	-8.4	-8.7	-10.2

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The method for studying the anti-inflammatory activity *in vivo* [19]

All *in vivo* procedures on animals comply with the standards of the European Convention for the Protection of Vertebrate Animals Used for Research and Scientific Purposes (Strasbourg, 1985), the Council Directive 2010/63/EU and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" as amended by 440-IX of 14.01.2020.

We conducted a study of the anti-inflammatory activity using the method of carrageenan-induced paw edema in rats. White Wistar rats weighing 180-250 g were used for this study. The laboratory animals were divided into 10 groups. Each group consisted of 5 rats. To test the anti-inflammatory activity of 6 compounds synthesized and 3 reference drugs (Ibuprofen, Diclofenac and Ketorolac), in total, 9 experimental groups were used, and the 10th test group was the control group.

The compounds studied (50 mg/kg of the body weight) and Ibuprofen, Diclofenac, Ketorolac in the mean therapeutic dose (10 mg/kg of the body weight) were dissolved in DMSO and administered intraperitoneally. Only DMSO was administered to the animals from the control group. In 1 hour, a generalized edema was induced by injecting 0.1 mL of 2% carrageenan solution under aseptic conditions under the aponeurosis of the rat hindlimb sole. The inflammatory response was determined by the change in the limb volume using the oncometric method at the beginning of the experiment and 4 hours after administration of the phlogogenic agent. The inhibition of the inflammatory response was calculated as a percentage of the reduction in the paw volume using the following formula:

$$\% \text{ Inhibition} = \frac{V_{control} - V}{V_{control}} \times 100\ \%$$

where $V_{control}$ is the increase in the paw volume in the control group of animals;

V is the increase in the paw volume in animals injected with the test substances.

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