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ORIGINAL ARTICLE

Synthesis, characterization, molecular docking studies of new alkyl derivatives of 5-(2-bromo-4-fluorophenyl)-4-ethyl-4*H*--1,2,4-triazole-3-thiol

Syntéza, charakterizace, studie molekulárního dokování nových alkylových derivátů 5-(2-brom-4-fluorfenyl)-4-ethyl--4*H*-1,2,4-triazol-3-thiolu

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Summary

The main goal of this article is to present the results of the synthesis of new alkyl derivatives of 5-(2-bromo-4-fluorophenyl)-4-ethyl-4*H*-1,2,4-triazole-3-thiol and molecular docking studies against COX-1 and COX-2. Previous studies have established a wide range of biological activity of 1,2,4-triazole derivatives. Therefore, it was essential to determine how a new series of 1,2,4-triazole derivatives would provide potential anti-inflammatory activity. To reach the goal, raw alkyl derivatives of 5-(2-bromo-4-fluorophenyl)-4-ethyl-4*H*-1,2,4-triazole-3-thiols (**2a-2i**) from 5-(2-bromo-4-fluorophenyl)-4-ethyl-4*H*-1,2,4-triazole-3-thiol (**1e**) were obtained. The structure of the synthesized compounds was confirmed by ^{1}H-NMR elemental analyses. The individuality and purity of compounds were confirmed by the method of liquid chromatography-mass spectrometry. These compounds have a relatively simple synthesis scheme, which gives them an advantage in creating a potential drug, and the appearance of alkyl radicals in the molecule should positively affect pharmacokinetic indicators, stability, selectivity, and bioavailability. An *in silico* study was conducted for the synthesized compounds, namely molecular docking, in relation to the interaction with COX-1 and COX-2. Based on the selectivity indexes of binding modes observed for the selected compounds (**2e, 2g**) with active COX-1 centers, it was found that compounds can reliably exhibit their anti-inflammatory effect through the prostaglandin biosynthesis pathway, inhibiting COX-1 instead of COX-2. The effect of hydrophobic interactions of alkyl groups of 1,2,4-triazole derivatives on changes in affinity and selectivity to COX-1 or COX-2 has also been proven. Therefore, derivatives of 1,2,4 are promising candidates for improvement, further study, and future development of new, more powerful anti-inflammatory drugs for therapeutic use.

Key words: 1,2,4-triazole • synthesis • molecular docking • anti-inflammatory activity • *in silico*

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Souhrn

Hlavním cílem tohoto článku je prezentovat výsledky syntézy nových alkylových derivátů 5-(2-brom-4-fluorfenyl)-4-ethyl-4*H*-1,2,4-triazol-3-thiolu a molekulárního dokování studie proti COX-1 a COX-2. Předchozí studie prokázaly široký rozsah biologické aktivity 1,2,4-triazolových derivátů. Proto bylo důležité zjistit, jak nová řada 1,2,4-triazolových derivátů poskytne potenciální protizánětlivou aktivitu. K dosažení cíle byly připraveny alkylové deriváty 5-(2-brom-4-fluorfenyl)-4-ethyl-4*H*--1,2,4-triazol-3-thiolů (**2a-2i**) z 5-(2- Byl získán brom-

4-fluorfenyl)-4-ethyl-4*H*-l,2,4-triazol-3-thiol (**1e**). Struktura syntetizovaných sloučenin byla potvrzena ^{1}H-NMR elementární analýzou. Totožnost a čistota sloučenin byla potvrzena metodou kapalinové chromatografie-hmotnostní spektrometrie. Tyto sloučeniny mají relativně jednoduché schéma syntézy, což jim dává výhodu v procesu tvorby potenciálního léčiva a výskyt alkylových radikálů v molekule by měl mít pozitivní vliv na farmakokinetické ukazatele, stabilitu, selektivitu a biologickou dostupnost. U syntetizovaných sloučenin byla provedena studie *in silico*, konkrétně molekulární dokování týkající se interakce s COX-1 a COX-2. Na základě indexů selektivity vazebných režimů pozorovaných pro vybrané sloučeniny (**2e, 2g**) s aktivními místy COX-1 bylo zjištěno, že sloučeniny mohou pravděpodobně uplatňovat svůj protizánětlivý účinek cestou biosyntézy prostaglandinů, inhibicí COX -1 místo COX-2. Rovněž byl prokázán vliv hydrofobních interakcí alkylových skupin 1,2,4-triazolových derivátů na změnu afinity a selektivity k COX-1 nebo COX-2. Proto jsou deriváty 1,2,4 slibnými kandidáty na zlepšení, další studium a budoucí vývoj nových, účinnějších protizánětlivých léčiv pro terapeutické použití.
Klíčová slova: 1,2,4-triazol • syntéza • molekulární dokování • protizánětlivá aktivita • *in silico*

Introduction

Inflammation is a complex biological response to harmful stimuli, such as pathogens, irritants, or tissue damage, and is a contributing factor to the development of a number of chronic diseases. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat inflammation and pain, but long-term use of such drugs has been associated with serious gastrointestinal side effects, such as ulcers, bleeding, and perforation, which limits their therapeutic efficacy[1)].

COX enzymes are responsible for the production of pro-inflammatory prostaglandins and are the main target of NSAIDs. In particular, selective COX-2 inhibitors have been developed to reduce gastrointestinal side effects associated with non-selective COX inhibitors[2, 3)]. Unfortunately, many of these medications are also associated with an increased risk of cardiovascular events[4)]. In addition, recent studies have shown that selective COX-2 inhibitors may contribute to certain cancers' development[5)]. Thus, there is an urgent need to develop new selective drugs with better safety profiles that can effectively inhibit both COX-1 and COX-2 without causing serious side effects[6–8)].

The scientific literature contains large amounts of data describing the synthesis of [9–12)], biological[13, 14)], pharmacological[15–20)], and other types of activities of [21–25)] derivatives of 1,2,4-triazole. On the basis of these heterocycles, powerful and harmless potential drug candidates are created and modern drugs are registered[9, 13, 14, 21, 24)]. This is due to the fact that usually the compounds containing the 1,2,4-triazole nucleus in their structure are endowed with high biological activity[13, 16, 19, 20)] and low toxicity[13, 14, 24)]. In this respect, 1,2,4-triazole derivatives have shown promising anti-inflammatory activity due to their ability to inhibit cyclooxygenase (COX) enzymes in combination with low toxicity[26–30)]. The binding of arachidonic acid, a natural substrate, to COX-1/2, is significantly affected by hydrophobic interactions of the aliphatic chain[31)]. As a result, developing new analogues of 1,2,4-triazole with various aliphatic substitutions is crucial for evaluating their effect on COX-1/2 binding affinity.

Materials and methods

The melting points data for synthesized compounds were obtained by the open capillary method with a MPA100 (OptiMelt, USA) device with a range of temperature measurements of 30–400 °C and 1 °C resolution. The elemental analysis of synthesized compounds was provided by an Elementar Vario L cube analyzer (Carbon, Hydrogen, Nitrogen, Sulfur (II)).

The ^{1}H NMR spectra were obtained by a Varian MR-400 spectrometer with 400 MHz dimension and hexadeuterodimethyl sulfoxide (DMSO-d_6) as the solvent with next analyzation by the ADVASP Analyzer program. The individuality and purity of the compounds were carried out using an Agilent 1260 Infinity HPLC System (Agilent Technologies, Germany) and an Agilent 6120 single quadrupole mass spectrometer with ionization in electrospray (ESI). All chemicals were obtained from UKRORGSYNTEZ Ltd. (Kyiv, Ukraine) with documental approval of their purity and quality.

Molecular docking

Ligand structures were drawn using Marvinsketch software and converted to SDF format using OpenBabel. The ligands were additionally subjected

Table 1. Data from protocols used for molecular docking verification

Receptor	Ligand	Coordinates of the center Grid Box	Size Grid Box	RMSD
COX-1 (PDB: 1EQG)	Ibuprofen	26.71 x 34.01 y 200.28 z	20 x 20 y 20 z	0.35
COX-2 (PDB: :1CX2)	SC–588/ bromocelecoxib	24.39 x 21.70 y 16.26 z	22 x 22 y 22 z	0.83

to energy minimization using Chimera. X-ray crystal structures of COX-1/2 cyclohexogenases (PDB ID: 1EGQ/1CX2) with co-crystallized ligands ibuprofen and SC-558 (bromocelecoxib), respectively, obtained from Protein Data Bank (PDB). Polar hydrogen atoms and the combined charges of Coleman atoms were then added to the resulting protein structures to prepare them for docking studies.

The DockingPie Vina[32] plugin in PyMOL was used to perform the study of protein and ligand docking and their conversion to pdbqt format. To confirm the docking protocol, the data of which are shown in Table 1, it was validated.

Previous studies show that the RMSD values, which represent the difference between the calculated and crystallographic conformations of the ligand complex, should not exceed 2.0 Å[33, 34]. Re-docking allowed us to obtain similarities in the overlap of crystallographic postures (orientation + conformation, blue) and calculated (yellow) postures, which illustrates the low RMSD value (Fig. 1).

PyMOL v. 2.5 and Discovery Studio Visualizer[35] were used to create shapes of receptor-ligand complexes). The predicted inhibitory constant (*pKi*) was estimated using the following standardized equation[36]:

$$pKi = 10^{\left[\frac{connectionenergyestimation}{1.336}\right]}$$

Synthesis and structural characterization

The initial compound 5-(2-bromo-4-fluorophenyl)-4--ethyl-4*H*-1,2,4-triazole-3-thiol (**1e**) was synthesized by known methods[11, 16, 18, 19], 2-bromo-4-fluorobenzoic acid (**1a**, CAS#1006-41-3) was used as the starting substance, from which isopropyl 2-bromo-4--fluorobenzoate (**1b**) and the corresponding 2-bromo-4-fluorobenzohydrazide (**1c**) were synthesized. Subsequently, 2-(2-bromo-4-fluorobenzoyl)-*N*-ethyl--hydrazine-1-carbothioamide (**1d**) was obtained by interacting the 1c compound in an alcoholic medium with ethyl isothiocyanate. 5-(2-Bromo-4-fluorophenyl)--4-ethyl-4*H*-1,2,4-triazole-3-thiol (**1e**) was obtained in two stages. In the first stage, the **1d** compound was boiled in excess alkali, and in the second stage, the hydrochloric acid reaction mixture was neutralized with filtration of the resulting precipitate (Fig. 2)

Further synthesis was carried out by reacting the initial thiol 1e with the relevant quantity of halohenalkans (iodomethane, bromoethane, 1-bromopropane, 1-bromobutane, 1-bromopentane, 1-bromhexane, 1-bromoheptane, 1-bromooctane, 1-bromononane) in i-propanol medium and the presence of equivalent potassium hydroxide (Fig. 3).

The structure of the synthesized compounds (**1e, 2a-i**) was confirmed by: 1H-NMR (Nuclear Magnetic Resonance Spectroscopy), EA (Elemental analyses). Individuality and purity of compounds were confirmed by the method of LC/MS (liquid chromatography-mass spectrometry) (Figs. 4 and 5).

Analyzing the obtained nuclear magnetic resonance spectra, the structure of the synthesized compounds was confirmed. The first sign of the alkylation reaction of the initial thiol was the absence of an SH group signal indicating the formation of an alkyl derivative. Sets of signal protons of S-alkyl fragments were fixed in the corresponding magnetic field, and their parameters coincided with the literature data. For example, proton signals of the methyl group are expressed at 2.75 ppm as a singlet. Elongation of the alkyl chain provokes a shift in proton signals towards a stronger field (+ I – and + M-effects). Thus, the proton signals of the methyl fragment (2a-2i) gradually changed to 0.85 ppm. Proton signals of the methylene fragment were observed in a strong field in the form of triplets (3.12–3.23 ppm) or multiplets (1.21–1.43 ppm, 1.63–1.80 ppm). Signals in the form of doublets (7.27–7.81 ppm) are generated in the proton absorption region of the aromatic fragment.

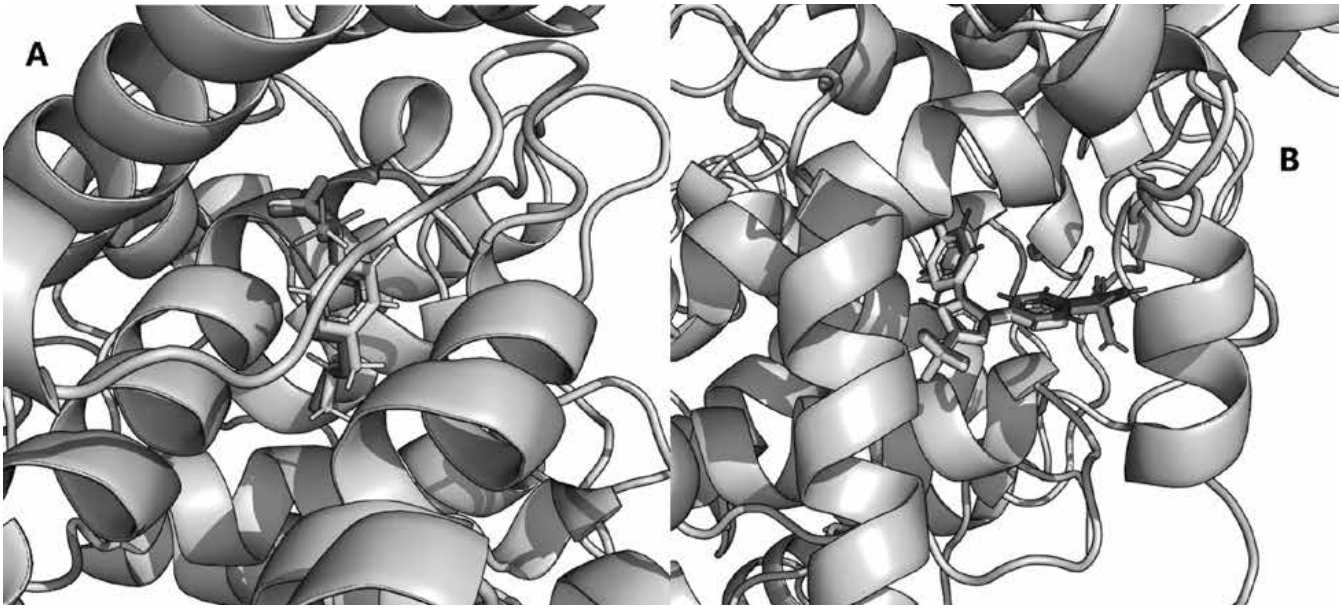

Fig. 1. Receptors in complexes with calculated and experimental ligand conformation: ibuprofen with COX-1(A), bromocelecoxib with COX-2 (B)

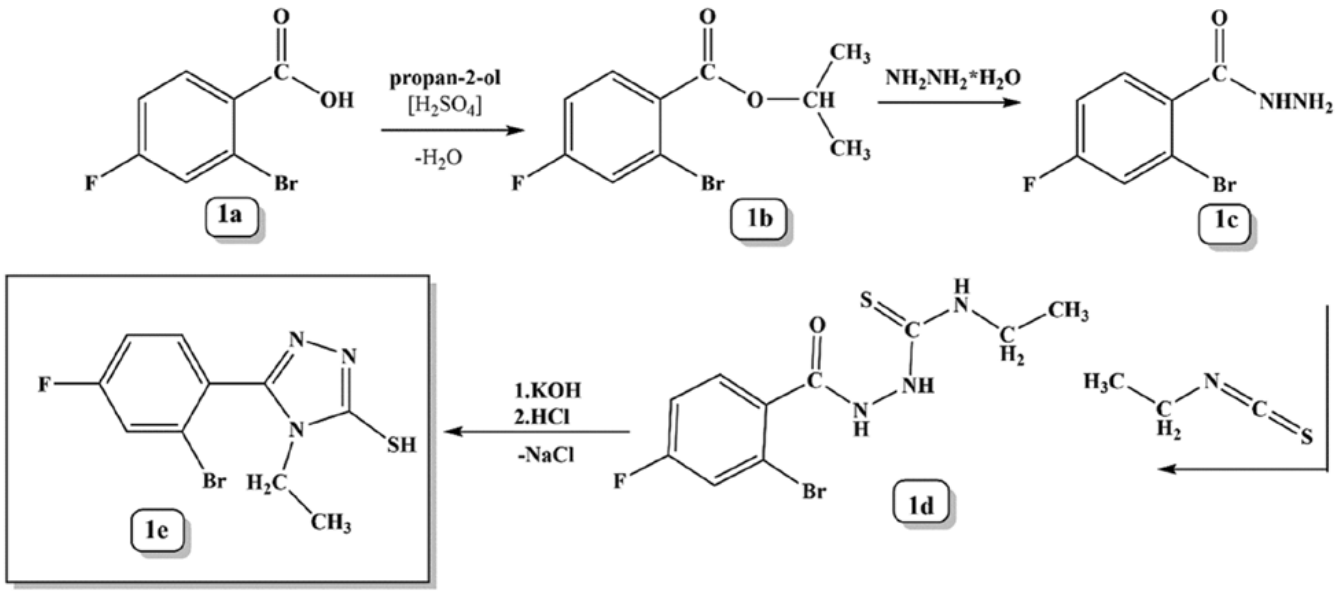

*Fig. 2. Scheme of synthesis 5-(2-bromo-4-fluorophenyl)-4-ethyl-4H-1,2,4-triazole-3-thiol (**1e**)*

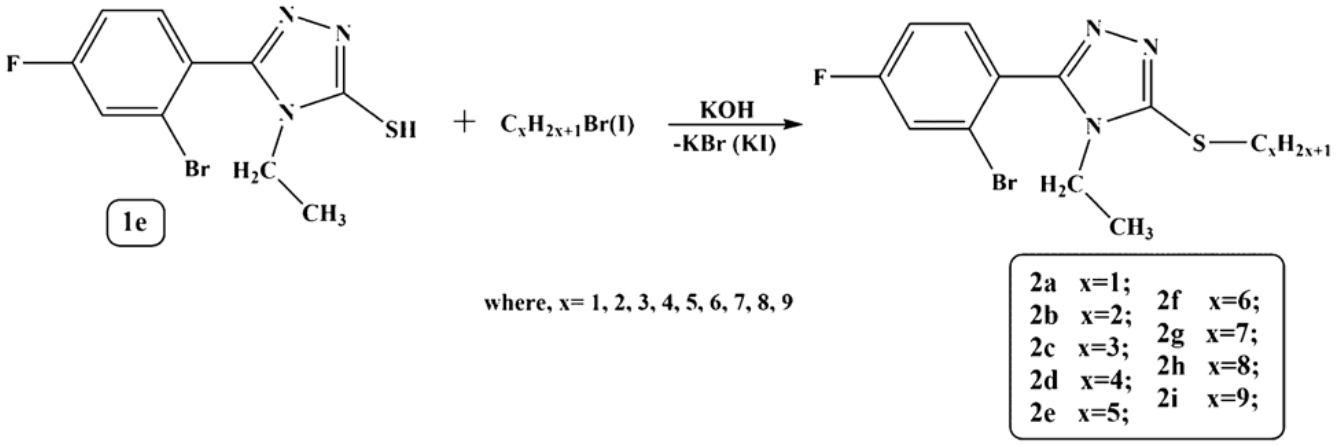

*Fig. 3. Scheme of synthesis of alkyl derivatives of 5-(2-bromo-4-fluorophenyl)-4-ethyl-4H-1,2,4-triazole-3-thiol (**2a-2i**)*

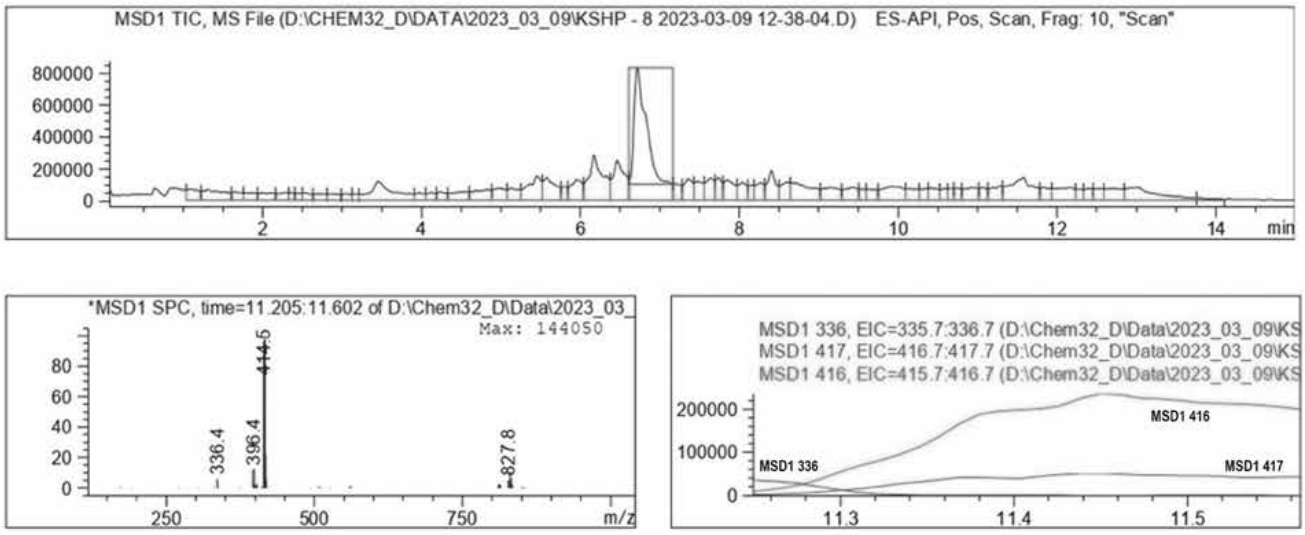

*Fig. 4. LC/MS-spectrum of the 3-(2-bromo-4-fluorophenyl)-4-ethyl-5-(octylthio)-4H-1,2,4-triazole **2h***

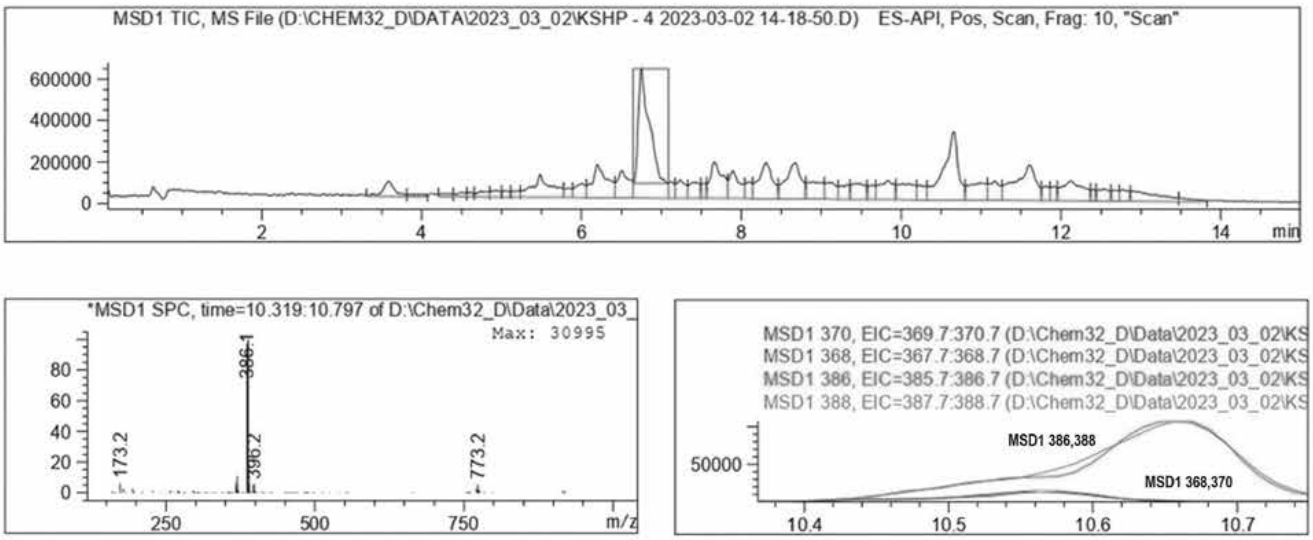

Fig. 5. LC/MS-spectrum 3-(2-bromo-4-fluorophenyl)-4-ethyl-5-(hexylthio)-4H-1,2,4-triazole ***2f***

Synthesis of isopropyl 2-bromo-4-fluorobenzoate 1b. To 0.01 mol of the acid **1a** in 50 ml i-propanol was added 0.001 mol acid sulfuric. The reaction mixture was heated during 6 h, cooled, and neutralized by a water solution of sodium carbonate to pH = 7. The obtained ether was retrieved from the solution by chloroform which was evaporated. The synthesized compound is a white crystalline substance. The ether was recrystallized from propane-2-ol for analysis.

Isopropyl 2-bromo-4-fluorobenzoate ***1b***: White residue; yield 87 %; m. p. 258–260 °C.; ^{1}H NMR (400 Mz, $DMSOd_6$) δ ppm: 7.86 (d, ^{1}H, H-6, 2-Br-4-F-C_6H_3), 7.58 (d, ^{1}H, H-3, 2-Br-4-F-C_6H_3), 7.23 (d, ^{1}H, H-5, 2-Br-4-F-C_6H_3), 5.22–5.13 (m, ^{1}H, O-C*H*-$(CH_3)_2$), 1.31–1.26 (m, ^{6}H, O-CH-$(CH_3)_2$). ESI-MS: m/z = 262 [M+H]+, Anal. Calcd. For $C_{10}H_{10}BrFO_2$: C, 46.00; H, 3.86. Found: C, 45.55; H, 3.95.

Synthesis of 2-bromo-4-fluorobenzohydrazide 1c. To 0.01 mol of the ether **1b** in 50 ml i-propanol was added 1,2 ml hydrazine hydrate solution (60%). The reaction mixture was heated for 2h, cooled, and evaporated. The synthesized compound is a white crystalline substance. The hydrazide was recrystallized from propane-2-ol for analysis.

2-Bromo-4-fluorobenzohydrazide ***1c***. White residue; yield 81 %; m. p. 272–274 °C.; ^{1}H NMR (400 Mz, $DMSOd_6$) δ ppm: 9.77 (t, ^{1}H, N*H*-NH_2), 7.80 (d, ^{1}H, H-6, 2-Br-4-F--C_6H_3), 7.60 (d, ^{1}H, H-3, 2-Br-4-F-C_6H_3), 7.29 (d, ^{1}H, H-5, 2-Br-4-F-C_6H_3), 4.61 (d, ^{2}H, NH-NH_2). ESI-MS: m/z = 234 [M+H]+, Anal. Calcd. For $C_7H_6BrFN_2O$: C, 36.08; H, 2.60; N, 12.02. Found: C, 35.55; H, 2.95; N, 12.19.

Synthesis of 2-(2-bromo-4-fluorobenzoyl)-*N*--ethylhydrazine-1-carbothioamide 1d. To 0.01 mol of the hydrazide 1c in 50 ml i-propanol was added 0.01 mol ethyl isothiocyanate. The reaction mixture was heated to full solving of reagents and left to cool at room temperature to form the residue, which was filtered. The synthesized compound is a white crystalline substance. The carbothioamide was recrystallized from propane-2-ol for analysis.

2-(2-bromo-4-fluorobenzoyl)-N-ethylhydrazine-1-carbothioamide ***1d***. White residue; yield 78 %; m. p. 242–244 °C.; ^{1}H NMR (400 Mz, $DMSOd_6$) δ ppm: 10.44 (d, ^{1}H, NH-NH), 9.40 (d, ^{1}H, NH-NH), 7.79 (d, ^{1}H, H-6, 2-Br-4-F-C_6H_3), 7.63 (d, ^{1}H, H-3, 2-Br-4-F-C_6H_3), 7.33–7.21 (m, ^{2}H, H-5, 2-Br-4-F-C_6H_3, S = C-NH-CH_2-CH_3), 3.65–3.55 (m, ^{2}H, S = C-NH-CH_2-C*H*$_3$), 1.17 (t, ^{3}H, S = C-NH-C*H*$_2$-CH_3). ESIMS: m/z = 321 [M+H]+, Anal. Calcd. For $C_{10}H_{11}BrFN_3OS$: C, 37.51; H, 3.46; N, 13.12; S, 10.01. Found: C, 37.01; H, 3.40; N, 13.31; S, 9.81.

Synthesis of 5-(2-bromo-4-fluorophenyl)-4-ethyl--4*H*-1,2,4-triazole-3-thiol 1e. To 0.01 mol of carbothioamide 1d in 50 ml distilled water was added 0.02 mol sodium hydroxide. The reaction mixture was heated for 4h, cooled, and neutralized by hydrochloric acid to pH = 7. The forming residue was filtered and dried on air. The synthesized compound is a white crystalline substance that was recrystallized from propane-2-ol for analysis.

5-(2-bromo-4-fluorophenyl)-4-ethyl-4H-1,2,4-triazole-3-thiol ***1e***. White residue; yield 76 %; m. p. 180–182 °C; ^{1}H NMR (400 Mz, $DMSOd_6$) δ ppm: 7.81 (d, ^{1}H, H-6, 2-Br-4-F-C_6H_3), 7.65 (d, ^{1}H, H-3, 2-Br-4-F-C_6H_3), 7.24 (d, 1H, H-5, 2-Br-4-F-C6H3), 6.64 (s, ^{1}H, SH), 4.32 (t, ^{2}H, N-C*H*$_2$-CH_3), 1.46 (t, ^{3}H, N-CH_2-C*H*$_3$). ESI-MS: m/z = 303 [M+H]+, Anal. Calcd. For $C_{10}H_9BrFN_3S$: C, 39.75; H, 3.00; N, 13.91; S, 10.61. Found: C, 40.21; H, 3.09; N, 13.80; S, 10.55.

General synthesis of the alkyl derivatives of 5-(2-bromo-4-fluorophenyl)-4-ethyl-4*H*-1,2,4-triazole-3-thiol (2a-2i). To 0.01 mol of the thiol 1e solution in 50 ml i-propanol was added 0.01 mol of potassium hydroxide which was previously dissolved in a minimal amount of distilled water. The reaction mixture was heated until the thiol dissolved. After the 0.01 mol of appropriate halohenalkan (iodomethane, bromoethane, 1-bromopropane, 1-bromobutane, 1-bromopenta-

ne, 1-bromhexane, 1-bromoheptane, 1-bromooctane, 1-bromononane) was added and continued heating to boiling on water to pH = 7. The obtained solutions were filtered, and the filtrates were evaporated. The synthesized compounds are white crystalline substances (**2a, 2b**), yellow (**2c, 2d, 2e, 2g, 2h**), and orange (**2f, 2i**) color. The compounds **2a-2i** were recrystallized from propane-2-ol for analysis.

• *3-(2-bromo-4-fluorophenyl)-4-ethyl-5-(methylthio)--4H-1,2,4-triazole* ***2a***: White residue; yield 81 %; m. p. 156–158 °C.; ^{1}H NMR (400 Mz, DMSOd_6) δ ppm: 7.86 (d, ^{1}H, H-6, 2-Br-4-F-C_6H_3), 7.58 (d, ^{1}H, H-3, 2-Br-4-F-C_6H_3), 7.23 (d, 1H, H-5, 2-Br-4-F-C_6H_3), 5.22-5.13 (m, 1H, O-CH--$(CH_3)_2$), 1.31–1.26 (m, ^{6}H, O-C*H*-$(CH_3)_2$); ESI-MS: m/z = 317 [M+H]+; Anal. Calcd. For $C_{11}H_{11}BrFN_3S$: C, 41.79; H, 3.51; N, 13.29; S, 10.14. Found: C, 41.31; H, 3.58; N, 13.17; S, 10.01.

• *3-(2-bromo-4-fluorophenyl)-4-ethyl-5-(ethylthio)-4H--1,2,4-triazole* ***2b***: White residue; yield 72 %; m. p. 120–118 °C.; ^{1}H NMR (400 Mz, DMSOd_6) δ ppm: 7.82 (d, 1H, H-6, 2-Br-4-F-C_6H_3), 7.67 (d, ^{1}H, H-3, 2-Br-4-F-C_6H_3), 7.25 (d, ^{1}H, H-5, 2-Br-4-F-C_6H_3), 4.34 (t, ^{2}H, N-CH_2-CH_3), 3.15 (t, ^{2}H, S-CH_2-CH_3), 1.41 (t, ^{3}H, N-CH_2-CH_3), 1.36 (t, ^{2}H, S-CH_2-CH_3), ESI-MS: m/z = 331 [M+H]+; Anal. Calcd. For $C_{12}H_{13}BrFN_3S$: C, 43.65; H, 3.97; N, 12.73; S, 9.71. Found: C, 43.99; H, 3.94; N, 12.65; S, 9.79.

• *3-(2-bromo-4-fluorophenyl)-4-ethyl-5-(propylthio)--4H-1,2,4-triazole* ***2c***: Yellow residue; yield 68 %; m. p. 109–107 °C; ^{1}H NMR (400 Mz, DMSOd_6) δ ppm: 7.81 (d, ^{1}H, H-6, 2-Br-4-F-C_6H_3), 7.68 (d, ^{1}H, H-3, 2-Br-4-F-C_6H_3), 7.27 (d, ^{1}H, H-5, 2-Br-4-F-C_6H_3), 4.30 (t, ^{2}H, N-CH_2-CH_3), 3.10 (t, ^{2}H, S-CH_2-CH_2-CH_3), 1.81–1.71 (m, ^{2}H, S-CH_2-CH_2--CH_3), 1.42 (t, 3H, N-CH_2-CH_3), 1.05 (t, ^{3}H, S-$(CH_2)_2$-CH_3); ESI-MS: m/z = 344 [M+H]+; Anal. Calcd. For $C_{13}H_{15}BrFN_3S$: C, 45.36; H, 4.39; N, 12.21; S, 9.31. Found: C, 45.89; H, 4.36; N, 12.29; S, 9.26.

• *3-(2-bromo-4-fluorophenyl)-5-(butylthio)-4-ethyl-4H--1,2,4-triazole* ***2d***: Yellow residue; yield 63 %; m. p. 113–115 °C; ^{1}H NMR (400 Mz, DMSOd_6) δ ppm: 7.79 (d, ^{1}H, H-6, 2-Br-4-F-C_6H_3), 7.70 (d, ^{1}H, H-3, 2-Br-4-F-C_6H_3), 7.26 (d, ^{1}H, H-5, 2-Br-4-F-C_6H_3), 4.34 (t, ^{2}H, N-CH_2-CH_3), 3.15 (t, ^{2}H, S-CH_2-$(CH_2)_2$-CH_3), 1.71–1.62 (m, ^{2}H, S-CH_2-CH_2-CH_2--CH_3), 1.47–1.33 (m, ^{5}H, S-$(CH_2)_2$-CH_2-CH_3, N-CH_2-CH_3), 0.89 (t, ^{3}H, S-$(CH_2)_3$-CH_3); ESI-MS: m/z = 358 [M+H]+; Anal. Calcd. For C14H17BrFN3S: C, 46.93; H, 4.78; N, 11.73; S, 8.90. Found: C, 46.23; H, 4.76; N, 11.65; S, 8.81.

• *3-(2-bromo-4-fluorophenyl)-4-ethyl-5-(pentylthio)--4H-1,2,4-triazole* ***2e***: Yellow residue; yield 64 %; m. p. 103–105 °C; ^{1}H NMR (400 Mz, DMSOd_6) δ ppm: 7.80 (d, ^{1}H, H-6, 2-Br-4-F-C_6H_3), 7.68 (d, ^{1}H, H-3, 2-Br-4-F-C_6H_3), 7.27 (d, ^{1}H, H-5, 2-Br-4-F-C_6H_3), 4.35 (t, ^{2}H, N-CH_2-CH_3), 3.12 (t, ^{2}H, S-CH_2-$(CH_2)_3$-CH_3), 1.78–1.65 (m, ^{2}H, S-CH_2--CH_2-(CH_2)$_2$-CH_3), 1.46–1.31 (m, ^{7}H, S-$(CH_2)_2$-(CH_2)$_2$-CH_3, N-CH_2-CH_3), 0.95–0.83 (t, ^{3}H, S-$(CH_2)_4$-CH_3); ESI-MS: m/z = 372 [M+H]+; Anal. Calcd. For $C_{15}H_{19}BrFN_3$: C, 48.39; H, 5.14; N, 11.29; S, 8.61. Found: C, 48.65; H, 5.17; N, 11.37; S, 8.55.

• *3-(2-bromo-4-fluorophenyl)-4-ethyl-5-(hexylthio)-4H--1,2,4-triazole* ***2f***: Orange residue; yield 67 %; m. p. 118–120 °C; ^{1}H NMR (400 Mz, DMSOd_6) δ ppm: 7.82 (d, ^{1}H, H-6, 2-Br-4-F-C_6H_3), 7.65 (d, ^{1}H, H-3, 2-Br-4-F-C_6H_3), 7.25 (d, ^{1}H, H-5, 2-Br-4-F-C_6H_3), 4.34 (t, ^{2}H, N-CH_2-CH_3), 3.11 (t, ^{2}H, S-CH_2-$(CH_2)_4$-CH_3), 1.72–1.64 (m, ^{2}H, S-CH2-CH_2--$(CH_2)_3$-CH_3), 1.48 (t, ^{3}H, N-CH_2-CH_3), 1.39-1.25 (m, ^{6}H, S-$(CH_2)_2$-(CH_2)$_3$-CH_3,) 0.91–0.82 (t, ^{3}H, S-$(CH_2)_5$-CH_3); ESI--MS: m/z = 386 [M+H]+; Anal. Calcd. For $C_{16}H_{21}BrFN_3S$: C, 49.74; H, 5.48; N, 10.88; S, 8.30. Found: C, 49.41; H, 5.46; N, 10.81; S, 8.36.

• *3-(2-bromo-4-fluorophenyl)-4-ethyl-5-(heptylthio)--4H-1,2,4-triazole* ***2g***: Yellow residue; yield 71 %; m. p. 93–95 °C; ^{1}H NMR (400 Mz, DMSOd_6) δ ppm: 7.79 (d, ^{1}H, H-6, 2-Br-4-F-C_6H_3), 7.68 (d, ^{1}H, H-3, 2-Br-4-F-C_6H_3), 7.27 (d, ^{1}H, H-5, 2-Br-4-F-C_6H_3), 4.31 (t, ^{2}H, N-CH_2-CH_3), 3.12 (t, ^{2}H, S-CH_2-$(CH_2)_5$-CH_3), 1.73–1.65 (m, ^{2}H, S-CH_2-CH_2--$(CH_2)_4$-CH_3), 1.45–1.39 (m, ^{5}H, N-CH_2-CH_3, S-$(CH_2)_2$--CH_2-$(CH_2)_3$-CH_3), 1.30–1.23 (m, ^{6}H, S-$(CH_2)_3$-(CH_2)$_3$-CH_3) 0.95–0.81 (t, ^{3}H, S-$(CH_2)_6$-CH_3); ESI-MS: m/z = 401 [M+H]+; Anal. Calcd. For $C_{17}H_{23}BrFN_3S$: C, 51.00; H, 5.79; N, 10.50; S, 8.01. Found: C, 51.25; H, 5.75; N, 10.54; S, 8.05.

• *3-(2-bromo-4-fluorophenyl)-4-ethyl-5-(octylthio)-4H--1,2,4-triazole* ***2h***: Yellow residue; yield 74 %; m. p. 87–89 °C; ^{1}H NMR (400 Mz, DMSOd_6) δ ppm: 7.81 (d, ^{1}H, H-6, 2-Br-4-F-C_6H_3), 7.66 (d, ^{1}H, H-3, 2-Br-4-F-C_6H_3), 7.28 (d, ^{1}H, H-5, 2-Br-4-F-C_6H_3), 4.34 (t, ^{2}H, N-CH_2-CH_3), 3.10 (t, ^{2}H, S-CH_2-$(CH_2)_6$-CH_3), 1.71–1.64 (m, ^{2}H, S-CH_2-CH_2--$(CH_2)_5$-CH_3), 1.46 (t, ^{3}H, N-CH_2-CH_3) 1.37–1.31 (m, ^{4}H, S-$(CH_2)_2$-C(H$_2$)$_2$-(CH_2)$_3$-CH_3), 1.29–1.23 (m, ^{6}H, S-$(CH_2)_4$--(CH_2)$_3$-CH_3) 0.95–0.81 (t, ^{3}H, S-$(CH_2)_7$-CH_3); ESI-MS: m/z = 414 [M+H]+; Anal. Calcd. For $C_{18}H_{25}BrFN_3S$: C, 52.17; H, 6.08; N, 10.14; S, 7.74. Found: C, 52.44; H, 6.04; N, 10.20; S, 7.76.

• *3-(2-bromo-4-fluorophenyl)-4-ethyl-5-(nonylthio)--4H-1,2,4-triazole* ***2i***: Orange residue; yield 65 %; m. p. 78–80 °C; ^{1}H NMR (400 Mz, DMSOd_6) δ ppm: 7.80 (d, ^{1}H, H-6, 2-Br-4-F-C_6H_3), 7.69 (d, ^{1}H, H-3, 2-Br-4-F-C_6H_3), 7.26 (d, ^{1}H, H-5, 2-Br-4-F-C_6H_3), 4.31 (t, ^{2}H, N-CH_2-CH_3), 3.12 (t, ^{2}H, S-CH_2-$(CH_2)_7$-CH_3), 1.69–1.63 (m, ^{2}H, S-CH_2--CH_2-$(CH_2)_6$-CH_3), 1.44 (t, ^{3}H, N-CH_2-CH_3) 1.36–1.30 (m, ^{2}H, S-$(CH_2)_2$-CH_2-$(CH_2)_5$-CH_3), 1.26–1.16 (m, ^{10}H, S-$(CH_2)_3$--(CH_2)$_5$-CH_3), 0.93–0.81 (t, ^{3}H, S-$(CH_2)_8$-CH_3); ESI-MS: m/z = 429 [M+H]+; Anal. Calcd. For $C_{19}H_{27}BrFN_3S$: C, 53.27; H, 6.35; N, 9.81; S, 7.40. Found: C, 53.49; H, 6.31; N, 9.79; S, 7.48.

Results and discussion

Uptake, distribution, metabolism, and elimination

ADME properties (uptake, distribution, metabolism, and elimination) were predicted using Lipinsky's rule of five36) in the ADMETlab and SWISSADME Web Services (Table 2). According to Lipinski's rule of five, a molecule violates this rule if it has more than 5 hydrogen bond donors, a molecular weight of more than 500, a log p value greater than 5, and the sum of N and O atoms greater than 10, resulting in poor absorption or penetration of the drug. A lipophilicity logP value of less than 5.0 implies a good distribution

*Table 2. Drug similarity and bioavailability profile of 5-(2-bromo-4-fluorophenyl)-4-ethyl-4H-1,2,4-triazole-3-thiol (**1e**) and alkyl derivatives of 5-(2-bromo-4-fluorophenyl)-4-ethyl-4H-1,2,4–triazole-3-thiol (**2a–2i**)*

Compounds	LogS	LogD	Consensus Log P	nHA	nHD	TPSA	MW
1e	–3.187	2.825	2.93	3	1	69.51	302.17
2a	–4.335	3.202	3.29	3	0	56.01	316.19
2b	–4.496	3.487	3.58	3	0	56.01	330.22
2c	–5.044	3.78	3.95	3	0	56.01	344.25
2d	–5.436	3.997	4.29	3	0	56.01	358.27
2e	–5.932	4.164	4.64	3	0	56.01	372.3
2f	–6.255	4.301	4.98	3	0	56.01	386.33
2g	–6.543	4.407	5.35	3	0	56.01	400.35
2h	–6.725	4.503	5.72	3	0	56.01	414.38
2i	–6.889	4.597	6.07	3	0	56.01	428.41

*Table 3. Pharmacokinetic profile of uptake and metabolism of the studied 5-(2-bromo-4-fluorophenyl)-4-ethyl-4H-1,2,4-triazole-3-thiol (**1e**) and alkyl derivatives of 5-(2-bromo-4-fluorophenyl)-4-ethyl-4H-1,2,4-triazole-3-thiol (**2a–2i**)*

Compounds	Blood-brain barrier	P-glycoprotein substrate	Inhibitor CYP1A2	Inhibitor CYP2C19	Inhibitor CYP2C9	Inhibitor CYP2D6	Inhibitor CYP3A4
1e	+	–	+	+	–	–	–
2a	+	–	+	+	+	–	–
2b	+	–	+	+	+	–	–
2c	+	–	+	+	+	+	–
2d	+	–	+	+	+	+	–
2e	+	–	+	+	+	+	–
2f	–	–	+	+	+	+	–
2g	–	–	–	+	+	+	–
2h	–	–	–	+	+	+	–
2i	–	–	–	–	+	+	+

coefficient. The water solubility value of logS suggests that molecules in the range of –5.0 to 0.5 have drug-like properties. Higher logD and logP values, as well as fewer hydrogen bonds, predict greater bioavailability of drugs. The topological polar surface (TPSA) area should not exceed 140 Å^2, and exceeding this indicator is associated with low penetration through the blood-brain barrier and poor membrane permeability. In addition, the hydrogen bond donor and acceptor sum must be less than or equal to 12.

Compounds **1e, 2a, 2b, 2c, 2d, 2e,** and **2f** have demonstrated properties that indicate their potential use as therapeutic agents. Their molecular weight was less than 500 Daltons, and they showed fewer than five hydrogen bond donors and fewer than ten acceptors. The Consensus LogP values also corresponded to Lipinsky's rule. The polar surface area of all the compounds studied ranged from 56.01 Å^2 and 69.51 Å^2, respectively, which indicates acceptable membrane permeability[37].

When analyzing the uptake and distribution parameters, it was found that **1e-2e** ligands have a positive rate of passing the blood-brain barrier (Table 3). None of the ligands are a substrate of P-glycoprotein, but all of them can act as inhibitors of at least one of the main cytochromes of P450.

Molecular docking

Molecular docking studies allow us to understand the efficiency of ligand binding to target cyclooxygenases and evaluate their selectivity. The results of the study, including binding affinity, inhibition constants, and selectivity indices, are presented in Table 4. Comparing the affinity values of the studied compounds, we can state a certain pattern: with an increase in the hydrocarbon chain, the selectivity of COX-2 with respect to COX-1 decreases. The optimal hydrocarbon radical was the C_5–C_7 range for COX-1 cyclooxygenase. Docking showed that a number of compounds (**1e-2i**) have a potential to bind to COX-1/2, and their values range from –6.672 to –7.843 kcal/mol. Compounds **2e** and **2g** showed binding energies of –7.843 and –7.796 kcal/mol and predicted inhibition constants of 0.135 and 0.146 μm, respectively, indicating a higher

Table 4. Results of the study of the attachment of synthesized compounds to COX-1 (pdb: 1EQG) and COX-2 (pdb: 1CX2) compared to re–attached native ligands (ibuprofen and SC-588/ bromocelecoxib)

Compounds	COX-1		COX-2		Selectivity index (COX-1 pKi /COX-2 pKi)	Selectivity index (COX-2 pKi /COX-1 pKi)
	Affinity (kcal/ mole)	Predicted pKi value (μM)	Affinity (kcal/ mole)	Predicted pKi value(μM)		
1e	–7.429	0.275	–7.203	0.406	0.677	1.476
2a	–7.231	0.387	–7.538	0.228	1.697	0.589
2b	–7.257	0.370	–7.702	0.172	2.153	0.464
2c	–7.497	0.245	–7.907	0.121	2.027	0.493
2d	–7.651	0.188	–7.988	0.105	1.787	0.559
2e	–7.843	0.135	–6.907	0.676	0.199	5.019
2f	–7.585	0.210	–7.524	0.233	0.900	1.111
2g	–7.796	0.146	–6.672	1.014	0.144	6.939
2h	–7.500	0.243	–6.793	0.823	0.296	3.382
2i	–7.504	0.242	–6.952	0.626	0.386	2.589
Ibuprofen	–7.782	0.150	–7.757	0.156	0.958	1.044
bromocelecoxib	–4.182	74.090	–10.99	< 0.001	125545.860	< 0.001

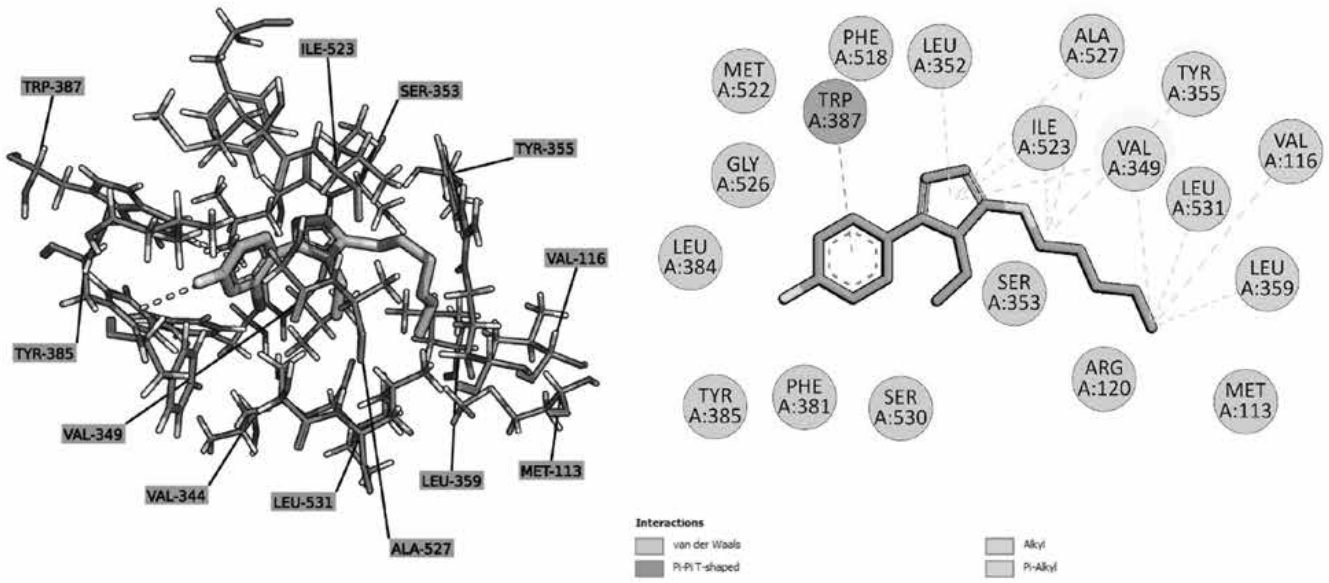

Fig. 6. Graphical representation of the binding position and interaction of 2e with COX-1 cyclooxygenase

affinity for the enzyme compared to ibuprofen. In addition, the pKi ratio values showed that compounds **2e** and **2g** had a higher potential selectivity to COX-1 than ibuprofen. On the other hand, none of the compounds studied showed a COX-2 binding energy greater than that of the native SC-588 ligand. Substance **2e** had a pKi = 5.019 coefficient, which indicates that 2e is 5 times higher than the potential selectivity for COX-1 inhibition compared to COX-2. There was also a noticeable increase in the pKi ratio for **2g**, which indicates a relatively higher COX-1 selectivity index. Undoubtedly, the celecoxib derivative was the most selective for COX-2, confirming practical use and consistent with previous studies[38].

Accordingly, compounds **2e** and **2g** were selected for further study as potential scaffolds with high COX-1 binding selectivity and for demonstrating the 2D and 3D modes of binding to the enzyme.

Unlike ibuprofen, which uses the nitro group to form a pair of hydrogen bonds with AGR120 (SER35339), compound **2e** achieves a higher affinity value of –7.843 kcal/mol by forming alkyl hydrophobic bonds between the amino acids ILE523, ALA527, VAL349, TYR355, LEU531, VAL116, LEU359 and the aliphatic substituent and π-alkyl contacts between residues leu352, Le523, ala527, val349 and the aromatic system of 1,2,4-triazole. There was also a stabilizing π-π t-like interaction between the π-electron cloud of the aromatic ring of 4-fluorophenyl and the aromatic ring of indole TPR387 (Fig. 6).

For the **2g** compound, a similar binding profile was recorded as for **2e**, with a binding energy of –7.796 kcal/mol.

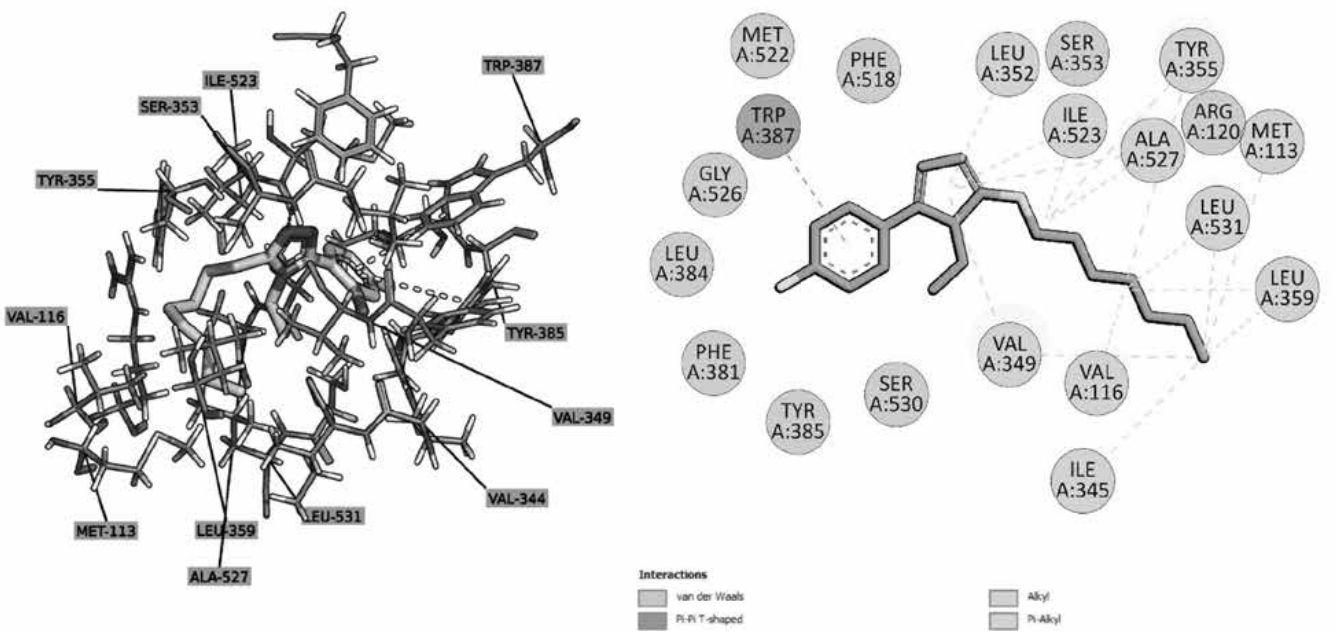

Fig. 7. 2D and 3D interactions of the studied 2g ligand with COX-1

The difference was the presence of alkyl hydrophobic contacts between ILE345, MET113 residues, and the heptyl radical. In total, there were 18 hydrophobic interactions of the alkyl-π, π-π, and alkyl types (4.01–6.09 Å) with ILE523, ALA527, VAL349, TYR355, LEU531, VAL116, LEU359, LEU352, ILE345, MET113, and TPR387 (Fig. 7).

Conclusion

As a result of the study, 5-(2-bromo-4-fluorophenyl)-4--ethyl-*4H*-1,2,4-triazole-3-thiol (1e) and alkyl derivatives of 5-(2-bromo-4-fluorophenyl)-4-ethyl-4*H*-1,2,4-triazole-3-thiol (**2a-2i**) were synthesized. The structure of the synthesized compounds was confirmed by using ^{1}H-NMR, elemental analyses, and by the method of LC/MS. For the synthesized compounds, *in silico* studies were performed, namely molecular docking in relation to the interaction with COX-1 and COX-2. The compounds (**2e, 2g**) show a higher affinity or preference for binding to COX-1 over COX-2, which indicates their potential to selectively target COX-1 and inhibit its activity, thereby reducing the production of inflammatory prostaglandins. However, it is important to acknowledge the limitations of our study. First, we focused on the in-silico molecular docking approach and some aspects of metabolism and pharmacokinetics, which provide valuable information on ligand binding and the predicted bioactivity of compounds. However, future studies should include in vitro and in vivo experiments to confirm the inhibitory activity and evaluate the pharmacological properties of these compounds.

The effect of hydrophobic interactions of alkyl groups of 1,2,4-triazole derivatives on changes in affinity and selectivity to COX-1 or COX-2 has also been proven. Furthermore, the structure-activity relationship analysis revealed that increasing the hydrocarbon chain length led to decreased selectivity for COX-2 inhibition. This observation suggests that further exploration of different substitutions and modifications in the 1,2,4-triazole scaffold is necessary to enhance selectivity while maintaining potent anti-inflammatory activity. Certain chemical simulations, along with pharmacophore search, are also needed to improve the pharmacokinetic properties and increase the probability of binding to the ARG120 residue, which is considered critical for the selectivity of the COX-1 enzyme[40]. To overcome the limitations, comprehensive in vitro studies using human cell-based assays or tissue samples are used to evaluate the efficacy and safety profiles of compounds **2e** and **2g**. Additionally, performing structure-activity relationship studies focusing on optimizing selectivity for COX-1 inhibition without compromising potency against COX-2 would provide valuable information on the potential of 1,2,4-triazole derivatives as anti-inflammatory agents. Thus, the derivatives of 1,2,4-triazole are promising candidates for improvement, further study, and future development of new, more powerful anti-inflammatory drugs for therapeutic use.

Conflict of interests: none.

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