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MOLECULAR DOCKING STUDIES FOR A NEW 1,2,4-TRIAZOLE DERIVATIVES WITH A 2-BROMO-5-METHOXYPHENYL FRAGMENT

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Introduction. In contemporary medicinal chemistry and pharmacology, the discovery of novel, highly effective, and safe anti-inflammatory agents remains a paramount challenge. This issue acquires particular urgency under the conditions of prolonged martial law, where military medicine daily encounters a vast number of combat-related injuries, wounds, and concussions accompanied by massive inflammatory processes and intense acute pain syndromes. Existing non-steroidal anti-inflammatory drugs do not always meet clinical needs due to severe adverse effects, particularly ulcerogenicity, which limits their application in field conditions and during medical evacuation. Therefore, the development of new selective cyclooxygenase-2 (COX-2) inhibitors that combine high efficacy with low toxicity is critically needed. Among the diversity of molecular frameworks, 1,2,4-triazole derivatives attract significant attention from researchers. Thanks to their unique electronic structure and ability to form various non-covalent bonds with biological targets, they demonstrate a broad spectrum of pharmacological activities, including potent anti-inflammatory effects. The integration of specific pharmacophore groups into the 1,2,4-triazole structure opens new avenues for creating innovative molecules with an optimized biological profile.

Objective. In light of the aforementioned, the primary objective of this research is the targeted design and synthesis of novel 5-(2-bromo-5-methoxyphenyl)-4-R-1,2,4-triazole-3-thiols, alongside their acetic acid derivatives and corresponding esters, followed by an *in silico* evaluation of their anti-inflammatory potential through molecular docking simulations with the cyclooxygenase-2 (COX-2) enzyme.

Materials and Methods. Conventional organic synthesis protocols were meticulously employed to obtain the target heterocyclic molecules (27 compounds in total). This involved the cyclization of intermediate thiosemicarbazides in an alkaline medium and subsequent esterification utilizing two distinct procedures (Methods A and B). The chemical structures, purity, and individuality of all synthesized derivatives were rigorously validated using ¹H NMR spectroscopy (Varian MR-400), liquid chromatography-mass spectrometry (Agilent 1260 Infinity HPLC/MS), and elemental analysis. Computational molecular docking of the ligands with the COX-2 enzyme was performed utilizing the AutoDockTools software package. Spatial configuration optimization was achieved using HyperChem 8 (applying MM+ and PM3 algorithms), while the crystallographic target protein structures were retrieved from the Protein Data Bank repository and refined using Discovery Studio 4.0 to isolate the active site.

Results. Spectral analysis data completely confirmed the successful formation of the target structures: the protons of the 2-bromo-5-methoxyphenyl substituents were recorded in the characteristic aromatic zone (7.71-6.91 ppm), sulfhydryl functionality was verified by a distinct singlet, and the formation of esters was confirmed by the presence of expected alcohol residues. Computational analysis of the 27 novel compounds highlighted their significant binding affinity towards the active site of the COX-2 enzyme. The predicted binding mode of the reference drug celecoxib (binding free energy -13.4 kcal/mol) was highly consistent with previously reported experimental data, thus confirming the validity of the applied molecular model. Among the evaluated series, molecules 4a, 5e, and 5j displayed superior binding profiles. Notably, compound 5j demonstrated the highest interaction energy (-12.2 kcal/mol) by establishing robust, multifunctional interactions (including Pi-Sigma, Pi-Sulfur, hydrogen bonds, and Alkyl interactions) with key amino acid residues of the enzyme, such as TYR A:356, SER A:354, LEU A:532, ARG A:121, and ALA A:528.

Conclusions. As a result of the conducted comprehensive study, a new series of 5-(2-bromo-5-methoxyphenyl)-4-R-1,2,4-triazole derivatives was successfully synthesized and structurally elucidated using modern instrumental techniques. The calculated binding free energies for the investigated ligands ranged between -3.2 and -12.2 kcal/mol, values that align remarkably closely with those of the reference COX-2 inhibitor, celecoxib. These findings strongly suggest that the COX-2-mediated mechanism is a highly probable pathway for the anti-inflammatory action of the studied compounds. The identified lead scaffolds represent highly promising candidates for subsequent *in vivo* pharmacological evaluation, aiming to develop new-generation domestic anti-inflammatory drugs capable of addressing the urgent needs of military pharmacy and treating victims of combat-related injuries.

Keywords: 1,2,4-Triazole, synthesis, molecular docking, *in silico*, anti-inflammatory activity, military medicine.

ДОСЛІДЖЕННЯ МОЛЕКУЛЯРНОГО ДОКІНГУ НОВИХ ПОХІДНИХ 1,2,4-ТРИАЗОЛУ З 2-БРОМО-5-МЕТОКСИФЕНІЛЬНИМ ФРАГМЕНТОМ

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Вступ. У сучасній медичній хімії та фармакології пошук нових, високоефективних та безпечних протизапальних засобів залишається одним із найважливіших завдань. Особливої гостроти ця проблема набуває в умовах тривалого воєнного стану, коли військова медицина щодня стикається з величезною кількістю бойових травм, поранень та контузій, що супроводжуються масивними запальними процесами та інтенсивним гострим больовим синдромом. Існуючі нестероїдні протизапальні препарати не завжди задовольняють клінічні потреби через наявність серйозних побічних ефектів, зокрема ульцерогенної дії, що обмежує їх застосування у польових умовах та на етапах медичної евакуації. Саме тому розробка нових селективних інгібіторів циклооксигенази-2 (ЦОГ-2), які б поєднували високу ефективність із низькою токсичністю, є критично необхідною. Серед розмаїття молекулярних каркасів особливу увагу науковців привертають похідні 1,2,4-триазолу. Завдяки своїй унікальній електронній будові та здатності утворювати різноманітні нековалентні зв'язки з біологічними мішенями, вони демонструють широкий спектр фармакологічної активності, включаючи потужну протизапальну дію. Інтеграція в структуру 1,2,4-триазолу специфічних фармакофорних груп відкриває нові можливості для створення інноваційних молекул із оптимізованим профілем дії.

Мета. З огляду на вищезазначене, метою цього дослідження є цілеспрямований дизайн та синтез нових похідних 5-(2-бромо-5-метоксибеніл)-4-*R*-1,2,4-триазол-3-тіолів, їх похідних оцтової кислоти та відповідних естерів, з подальшою *in silico* оцінкою їхнього протизапального потенціалу шляхом симуляції молекулярного докінгу з ферментом циклооксигеназою-2 (ЦОГ-2).

Матеріали і методи. Для отримання цільових гетероциклічних молекул (загалом 27 нових сполук) застосовували класичні протоколи органічного синтезу, що включали реакції циклізації проміжних тіосемікарбазидів у лужному середовищі та подальшу етерифікацію двома різними методами (А та В). Хімічні структури, чистоту та індивідуальність усіх синтезованих похідних було ретельно підтверджено за допомогою ¹H ЯМР-спектроскопії (Varian MR-400), рідинної хромато-мас-спектрометрії (Agilent 1260 Infinity HPLC/MS) та елементного аналізу. Обчислювальний молекулярний докінг лігандів із ЦОГ-2 виконували за допомогою програмного пакета AutoDockTools. Просторова оптимізація молекул здійснювалася у середовищі HyperChem 8 (із застосуванням алгоритмів MM+ та PM3), а кристалографічні структури цільових ферментів були завантажені з репозиторію Protein Data Bank та підготовлені за допомогою Discovery Studio 4.0.

Результати. Дані спектрального аналізу повністю підтвердили утворення цільових структур: протони 2-бромо-5-метоксибенільних замісників надійно фіксувалися в ароматичній зоні спектра (7.71-6.91 ppm), утворення тіолів підтверджувалося синглетом SH-групи, а формування естерів – наявністю характерних залишків спиртів. Обчислювальний аналіз 27 нових сполук виявив їхню високу спорідненість до активного центру ферменту ЦОГ-2. Прогнозований механізм зв'язування препарату порівняння целекоксибу (вільна енергія -13.4 ккал/моль) відповідав експериментальним даним, що підтверджує валідність використаної докінг-моделі. Серед усього досліджуваного ряду молекули 4a, 5e та 5j продемонстрували найкращі профілі зв'язування. Зокрема, сполука 5j показала найвищу енергію взаємодії (-12.2 ккал/моль) за рахунок формування стабільних багатофункціональних зв'язків (Pi-Sigma, Pi-Sulfur, водневих зв'язків та Alkyl-взаємодій) з ключовими амінокислотними залишками ферменту, такими як TYR A:356, SER A:354, LEU A:532, ARG A:121 та ALA A:528.

Висновки. У результаті проведеного комплексного дослідження було успішно синтезовано новий ряд похідних 5-(2-бромо-5-метоксибеніл)-4-*R*-1,2,4-триазолу, структуру яких доведено сучасними інструментальними методами. Розраховані вільні енергії зв'язування для досліджуваних лігандів знаходилися в діапазоні від -3.2 до -12.2 ккал/моль, що надзвичайно близько до значень референтного протизапального препарату целекоксибу. Отримані результати переконливо свідчать про те, що одним із ймовірних механізмів протизапальної дії синтезованих сполук є безпосередня взаємодія з активним центром ЦОГ-2. Виявлені сполуки-лідери є високopersпективними кандидатами для подальших *in vivo* фармакологічних досліджень з метою створення вітчизняних протизапальних препаратів нового покоління, здатних вирішувати актуальні завдання військової медицини та допомагати постраждалим із бойовими травмами.

Ключові слова: 1,2,4-триазол, синтез, молекулярний докінг, *in silico*, протизапальна активність, військова медицина.

Introduction. In recent years, the imperative to discover novel bioactive compounds with therapeutic potential has fueled extensive research in medicinal chemistry, particularly in the pursuit of potent anti-inflammatory agents that can address the critical and urgent needs of military medicine [1-3]. During armed conflicts, the prompt management of acute inflammation and severe pain associated with combat-related trauma, gunshot wounds, and blast injuries is vital for preserving the lives and combat readiness of service members. From the vast spectrum of molecular architectures investigated for these demanding therapeutic applications, 1,2,4-triazole-based compounds [4-6] have gained prominence as high-potential leads, attributed to their multifaceted chemical and biological attributes that make them highly suitable for extreme clinical conditions [7-12]. In the context of this study, the integration of a 2-bromo-5-methoxyphenyl fragment into 1,2,4-triazole derivatives may introduce a distinctive molecular architecture, holding significant promise for enhanced bioactivity in the treatment of military casualties [6, 13, 31].

Molecular docking studies have become indispensable tools in the rational design of pharmaceutical agents [14-16], offering invaluable insights into the intricate binding interactions between ligands and target proteins [17,18]. In the fast-paced environment of military medical research, where the rapid development and deployment of effective treatments is crucial, such computational methods are highly advantageous. By simulating and analyzing the binding of molecules to their respective biological targets, molecular docking aids in predicting the potential efficacy of compounds, guiding subsequent synthetic efforts toward optimized drug candidates capable of acting effectively in tactical medical scenarios and field hospitals [19].

The central focus of our investigation centers around molecular docking studies targeting the anti-inflammatory activity of newly formulated 1,2,4-triazole derivatives featuring a 2-bromo-5-methoxyphenyl fragment. Given the escalating demand for effective anti-inflammatory agents in battlefield medicine, the exploration of compounds exhibiting potential in mitigating severe combat-induced inflammatory disorders assumes paramount importance for military healthcare systems [20, 21]. Such agents are essential for inclusion in tactical first-aid kits to prevent the systemic inflammatory response syndrome often seen in wounded personnel.

This study is based on a comprehensive examination of derivatives of 1,2,4-triazole using a big array of scientific data [6]. In this scientific

domain, a considerable number of significant pharmacological [7-10, 13] and biological [4, 11, 22] properties of 1,2,4-triazole derivatives have been identified, which are highly relevant for creating robust field medications. Innovative drugs are developed and potential candidates for pharmaceuticals are selected based on these properties to ensure maximum efficacy and stability under extreme conditions [23, 24]. Scientists comprehensively study the properties of compounds containing this heterocyclic system and note its powerful potential to address complex clinical and military medical challenges [4-13, 20-24, 31].

Therefore, considering our previous work [5, 6, 20, 23, 24] and the results presented by scientific teams from all over the world [6, 25-28], our goal is to reveal the binding mechanisms and predict their affinity to key inflammatory targets associated with battle trauma. The results of this study have the potential to identify compounds with potent anti-inflammatory activity, thereby significantly contributing to the development of next-generation anti-inflammatory agents specifically tailored for the urgent needs of military medicine and the effective rehabilitation of wounded soldiers.

Material and methods. Chemistry. All starting materials and chemical reagents utilized in the current study were sourced from UOS Ltd (Ukraine), with their quality and purity verified by the manufacturer's documentation. The synthesized compounds' melting points were determined by Melting Point Apparatus 100 device (Stanford Research Systems, USA), covering a temperature range of 30-400°C with a resolution of 1°C and were uncorrected. Elemental analysis of the synthesized compounds, involving Carbon, Hydrogen, Nitrogen, and Sulfur (II), was conducted using the Elementar Vario L cube analyzer (Germany). ¹H NMR spectras data were acquired using a Varian MR-400 device operating at 400 MHz, and Hexadeuterodimethyl sulfoxide (DMSO-_d₆) was employed as the solvent. The spectra were further analyzed using the ADVASP program.

The purity of the synthesized products were evaluated using an Agilent 1260 Infinity HPLC system (Agilent Technologies, Germany) integrated with an Agilent 6120 single quadrupole mass detector. Ionization was achieved through an electrospray ionization (ESI) source.

Molecular docking. Computational docking simulations between the 3D ligand models and the target receptors were performed utilizing the AutoDockTools software package. The crystallographic structures of the enzymes serving as models were retrieved from the Protein Data

Bank (PDB) repository. The docking workflow was executed in three distinct, consecutive phases:

Phase 1. Ligand Setup: Two-dimensional structural frameworks of the targeted molecules were generated and saved in *.mol format utilizing the MarvinSketch 6.3.0 environment.

Phase 2. 3D Optimization: Spatial configurations of the compounds were established using HyperChem 8, where geometry optimization was achieved through a combination of MM+ molecular mechanics and PM3 quantum mechanical refinement, employing the Polak-Ribiere minimization algorithm. Resulting coordinates were archived in *.pdb format before being converted into *.pdbqt files via the AutoDockTools-1.5.6 utility.

Phase 3. Receptor Refinement: Target protein structures were prepared by stripping away co-crystallized ligands and solvent (water) molecules using Discovery Studio 4.0 to isolate the active site. The processed enzyme templates were stored as *.pdb files and subsequently transformed into *.pdbqt format using AutoDockTools-1.5.6. **Execution and Analysis:** The AutoDock Vina engine was deployed for the primary docking simulations. Following the runs, molecular interactions were visualized and interpreted through the graphical tools in Discovery Studio 4.0 [29, 30].

Results Discussion. The initial structures 5-(2-bromo-5-methoxyphenyl)-4-R-1,2,4-triazole-3-thiol (3a, 3b, 3c, 3d) were synthesized using known methods [8, 10, 13]. The starting material was 2-bromo-5-methoxybenzohydrazide (1a, CAS#112584-40-4), from which a 2-(2-bromo-5-methoxybenzoyl)hydrazine-1-carbothioamide (2a) was synthesized under condition (a). Intermediate compounds 2-(2-bromo-5-methoxybenzoyl)-N-R-hydrazine-1-carbothioamide (2b, 2c, 2d) were obtained by reacting hydrazide 1a with isothiocyanates in an ethanol medium under condition (b). Further synthesis of 5-(2-bromo-5-methoxyphenyl)-4-R-1,2,4-triazole-3-thiols (3a, 3b, 3c, 3d) was achieved by cyclization of compounds 2a and 2b, 2c, 2d. The cyclization process was initiated by boiling the intermediate precursors 2a-2d in an alkaline medium (excess alkali). Subsequently, the reaction was quenched by neutralizing the mixture with hydrochloric acid, leading to the formation of a solid residue which was then isolated through filtration (following conditions (c) and (d) as shown in Fig. 1).

The target compounds 5a-5t were obtained by two methods. In Method A, acids 4a-4d were

synthesized from thiols 3a-3d under condition (e) and then boiled with the corresponding alcohols with the addition of sulfuric acid as a catalyst (condition (g)). In Method B, the starting compounds 3a-3d were reacted with the corresponding chloroacetic acid esters (condition (f)). Comparison of the products synthesized via Methods A and B revealed identical melting points with no observed depression upon mixing, confirming the structural consistency of the compounds (Fig. 1).

Structural elucidation of the newly synthesized derivatives was achieved through ¹H NMR spectroscopy and elemental analysis (EA). Furthermore, the chemical purity of the products were verified using liquid chromatography-mass spectrometry (LC/MS). (Fig. 2).

The study of ¹H NMR spectra revealed and helped to analyze synthesized structure. The protons of the 2-bromo-5-methoxyphenyl substituent were observed in the characteristic "aromatic zone" as three distinct doublets in the ranges of 7.71-7.60 ppm, 7.47-7.36 ppm, and 6.98-6.91 ppm. The methoxy group appeared as a singlet at 3.83-3.80 ppm. Regarding the N₄-substituent of the triazole core, the NH proton signal was observed as a distinctive singlet in the deshielded (downfield) region, appearing at 11.29 ppm for the thiol derivatives. In contrast, for the corresponding acetic acid and ester analogs, this resonance underwent an upfield shift to the 8.30-8.35 ppm range. The methyl group at the 4th position was recorded as a singlet in the range of 3.75-3.67 ppm, while the ethyl fragment (N-CH₂) resonated at 4.39-4.29 ppm as a multiplet, and the CH₃ group appeared as a triplet at 1.46-1.42 ppm. Protons associated with the phenyl ring were detected as a multiplet within the 7.36-7.45 ppm range; these signals coincided with the resonance patterns of the 2-bromo-5-methoxyphenyl moiety in the aromatic zone. Verification of the sulfhydryl (SH) functionality was established by a distinct singlet appearing between 9.93 and 9.97 ppm. Spectral evaluation of the acid derivatives revealed a characteristic carboxyl group resonance, manifesting as a singlet in the 11.37-11.41 ppm range. The presence of the methylene group (S-CH₂) was recorded as a singlet in the range of 4.10-4.04 ppm. The formation of esters was confirmed by the presence of alcohol residues, which formed the expected triplets in the range of 1.30-1.20 ppm for methyl and ethyl groups. As for propyl, isopropyl, and butyl residues, they mostly appeared as multiplets in the range of 4.25-4.05 ppm. (Fig. 3).

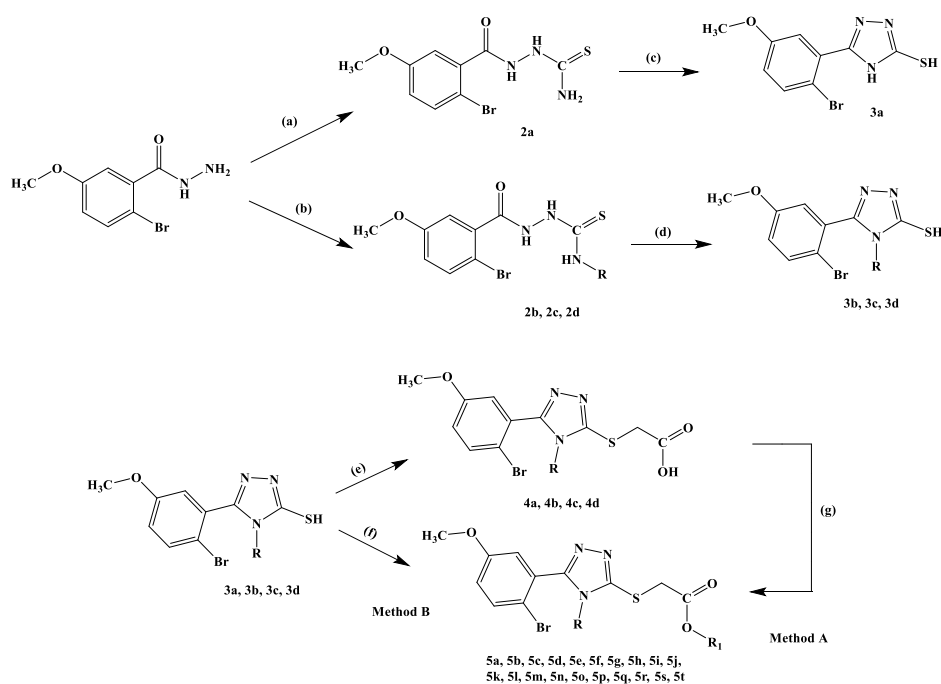


Figure 1. Scheme of synthesis 5-(2-bromo-5-methoxyphenyl)-4-R-1,2,4-triazole-3-thiol (**3a**, **3b**, **3c**, **3d**) and ethers of 2-((5-(2-bromo-5-methoxyphenyl)-4-R-4H-1,2,4-triazol-3-yl)thio)acetic acid (**5a**, **5b**, **5c**, **5d**, **5e**, **5f**, **5g**, **5h**, **5i**, **5j**, **5k**, **5l**, **5m**, **5n**, **5o**, **5p**, **5q**, **5r**, **5s**, **5t**)

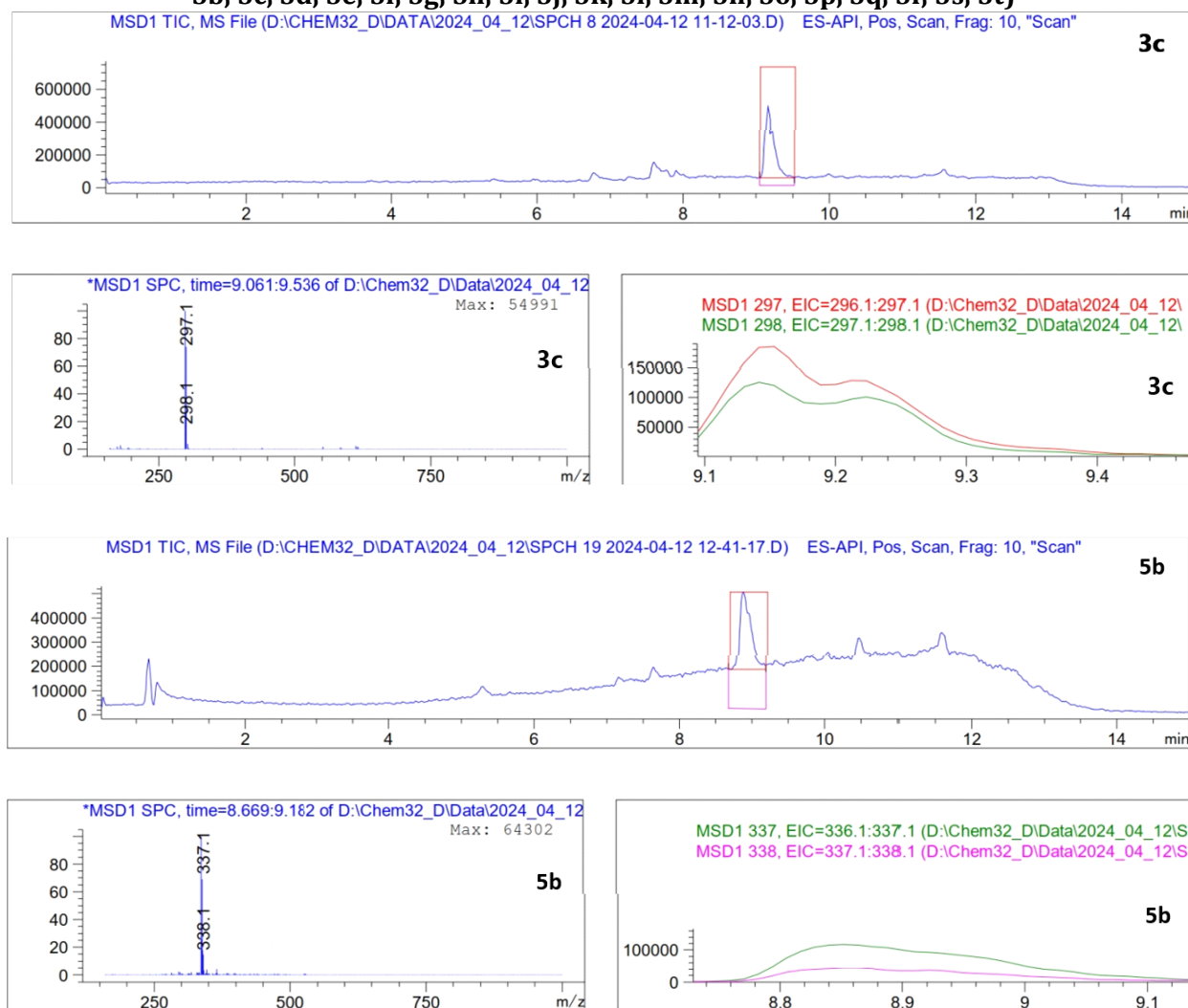


Figure 2. LC/MS-spectrum of the 5-(2-bromo-5-methoxyphenyl)-4-ethyl-4H-1,2,4-triazole-3-thiol (**3c**) and ethyl 2-((5-(2-bromo-5-methoxyphenyl)-4-methyl-4H-1,2,4-triazol-3-yl)thio)acetate (**5b**)

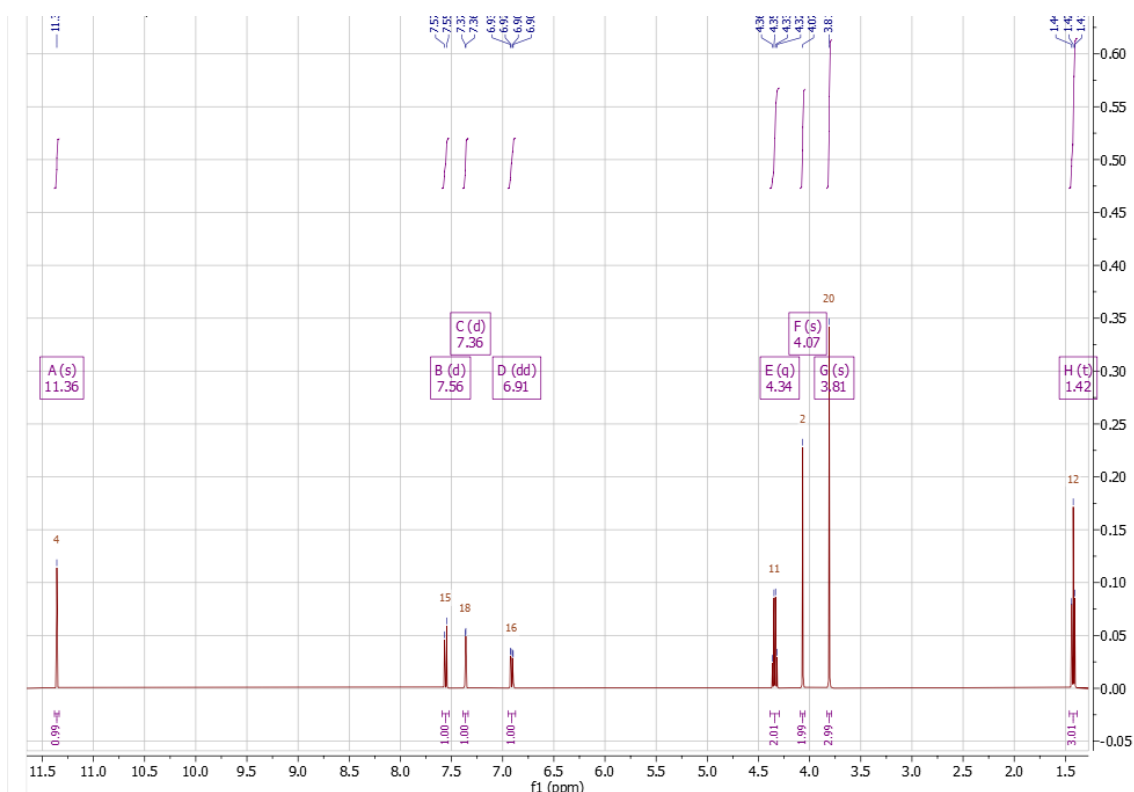


Figure 3. ^1H NMR spectrum of the compound 2-((5-(2-bromo-5-methoxyphenyl)-4-ethyl-4H-1,2,4-triazol-3-yl)thio)acetic acid (**4c**)

General procedure for the synthesis of the 2-(2-bromo-5-methoxybenzoyl)hydrazine-1-carbothioamide (2a)

To a solution of 0.2 mol potassium thiocyanate in 30 mL of water, 0.1 mol of 2-bromo-5-methoxybenzohydrazide (**1a**, CAS#112584-40-4) was added. Upon reaching the boiling point, the solution was treated with 0.2 mol of hydrochloric acid, added dropwise in several portions, followed by sustained refluxing for a 4-hour duration. Once the mixture was allowed to reach ambient temperature, the resulting solid precipitate was collected via filtration.

General procedure for the synthesis of the compounds 2-(2-bromo-5-methoxybenzoyl)-N-R-hydrazine-1-carbothioamide (2b, 2c, 2d)

To a solution of 0.1 mol 2-bromo-5-methoxybenzohydrazide (**1a**, CAS#112584-40-4) in 50 mL of propan-2-ol, 0.1 mol of methyl isothiocyanate (ethyl isothiocyanate, phenyl isothiocyanate) was added, and the mixture is left for 24 hours. The precipitate was filtered. The synthesized compounds were recrystallized from butan-1-ol (**2b**, **2c**) and water (**2d**) for analysis.

General procedure for the synthesis of the 5-(2-bromo-5-methoxyphenyl)-4-R-1,2,4-triazole-3-thiol (3a, 3b, 3c, 3d)

To 0.1 mol of compounds **2a**, **2b**, **2c**, and **2d**, were added 0.2 mol of potassium hydroxide in 30

mL of water. The solutions were heated on a water bath for 3 hours, then cooled, filtered, and neutralized with hydrochloric acid (**3a**, **3b**) or acetic acid (**3c**, **3d**) to pH= 7. The precipitates were filtered and washed with a water. The synthesized compounds are crystalline substances, which were recrystallized from methanol (**3a**), propan-2-ol (**3b**), and butan-1-ol (**3c**, **3d**) for analysis.

5-(2-bromo-5-methoxyphenyl)-4H-1,2,4-triazole-3-thiol (3a)

White precipitate; yield 83 %; m. p. 259-261 °C.; ^1H NMR, δ (ppm): δ 11.29 (s, 1H, NH), 9.96 (s, 1H, SH), 7.57 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.47 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.98 (d, J = 8.5, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 3.81 (s, 3H, O-CH₃). ESI-MS: m/z = 286 [M+H]⁺, Anal. Calcd. For C₉H₈BrN₃OS: C, 37.78; H, 2.82; N, 14.69; S, 11.20. Found: C, 37.68; H, 2.83; N, 14.72; S, 11.17.

5-(2-bromo-5-methoxyphenyl)-4-methyl-4H-1,2,4-triazole-3-thiol (3b)

White precipitate; yield 79 %; m. p. 243-245 °C.; ^1H NMR, δ (ppm): 9.93 (s, 1H, SH), 7.56 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.39 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.89 (d, J = 8.5, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 3.79 (s, 3H, O-CH₃), 3.75 (s, 3H, N-CH₃). ESI-MS: m/z = 301 [M+H]⁺, Anal. Calcd. For C₁₀H₁₀BrN₃OS: C, 40.01; H, 3.36; N, 14.69; S, 11.17.

14.00; S, 10.68. Found: C, 40.11; H, 3.35; N, 14.03; S, 10.65.

5-(2-bromo-5-methoxyphenyl)-4-ethyl-4H-1,2,4-triazole-3-thiol (3c)

White precipitate; yield 81 %; m. p. 251-253 °C.; ¹H NMR, δ (ppm): 9.95 (s, 1H, SH), 7.53 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.36 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.91 (d, J = 8.5, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.35-4.30 (m, 2H, N-CH₂-CH₃), 3.81 (s, 3H, O-CH₃), 1.46 (t, J = 5.5 Hz, 3H, N-CH₂-CH₃). ESI-MS: m/z = 315 [M+H]⁺, Anal. Calcd. For C₁₁H₁₂BrN₃OS: C, 42.05; H, 3.85; N, 13.37; S, 10.20. Found: C, 42.15; H, 3.86; N, 13.33; S, 10.17.

5-(2-bromo-5-methoxyphenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (3d)

White precipitate; yield 83 %; m. p. 270-272 °C.; ¹H NMR, δ (ppm): 9.37 (s, 1H, SH), 7.62 - 7.52 (m, 2H, 3-H 2-Br-5-O-CH₃-C₆H₄, 4-H C₆H₅), 7.50 - 7.45 (m, 2H, 2,6-H C₆H₅), 7.41 - 7.34 (m, 3H, 3,5-H C₆H₅, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.92 (d, J = 8.5, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 3.79 (s, 3H, O-CH₃). ESI-MS: m/z = 363 [M+H]⁺, Anal. Calcd. For C₁₅H₁₂BrN₃OS: C, 49.74; H, 3.34; N, 11.60; S, 8.85. Found: C, 49.61; H, 3.35; N, 11.57; S, 8.87.

General procedure for the synthesis of the compounds 2-((5-(2-bromo-5-methoxyphenyl)-4-R-1,2,4-triazol-3-yl)thio)acetic acid (4a, 4b, 4c, 4d)

In a 50 mL heat-resistant flask, 0.01 mol of the corresponding thiol **3a**, **3b**, **3c**, or **3d** in 30 mL of propan-2-ol was added along with 0.01 mol of KOH. The mixture was heated on a water bath with a reflux condenser until the precipitate dissolved, and then 0.01 mol of chloroacetic acid was added gradually. Boiling was continued for approximately 3 hours. The solution was filtered hot, and the filtrate was left at room temperature until the solvent evaporated and target compound precipitates formed. The synthesized compounds are crystalline substances, practically insoluble in water but soluble in organic solvents and alkaline solutions. For analysis, the compounds are recrystallized from ethanol (**4a-4d**).

2-((5-(2-bromo-5-methoxyphenyl)-4H-1,2,4-triazol-3-yl)thio)acetic acid (4a)

White precipitate; yield 74 %; m. p. 248-250 °C.; ¹H NMR, δ (ppm): 11.41 (s, 1H, OH), 8.31 (s, 1H, NH), 7.61 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.45 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.98 (d, J = 8.5, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.13 (s, 2H, S-CH₂-), 3.80 (s, 3H, O-CH₃). ESI-MS: m/z = 345 [M+H]⁺, Anal. Calcd. For C₁₁H₁₀BrN₃O₃S: C, 38.39; H, 2.93; N, 12.21; S, 9.31. Found: C, 38.29; H, 2.94; N, 12.18; S, 9.33.

2-((5-(2-bromo-5-methoxyphenyl)-4-

methyl-4H-1,2,4-triazol-3-yl)thio)acetic acid (4b)

White precipitate; yield 77 %; m. p. 239-241 °C.; ¹H NMR, δ (ppm): 11.36 (s, 1H, OH), 7.59 (d, J = 8.6 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.39 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.96 (d, J = 8.5, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.08 (s, 2H, S-CH₂-), 3.81 (s, 3H, O-CH₃), 3.72 (s, 3H, N-CH₃). ESI-MS: m/z = 359 [M+H]⁺, Anal. Calcd. For C₁₂H₁₂BrN₃O₃S: C, 40.24; H, 3.38; N, 11.73; S, 8.95. Found: C, 40.34; H, 3.39; N, 11.70; S, 8.93.

2-((5-(2-bromo-5-methoxyphenyl)-4-ethyl-4H-1,2,4-triazol-3-yl)thio)acetic acid (4c)

White precipitate; yield 81 %; m. p. 222-223 °C.; ¹H NMR, δ (ppm): 11.38 (s, 1H, OH), 7.56 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.36 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.91 (d, J = 8.5, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.37-4.31 (m, 2H, N-CH₂-CH₃), 4.07 (s, 2H, S-CH₂-), 3.79 (s, 3H, O-CH₃), 1.42 (t, J = 6.0 Hz, 3H, N-CH₂-CH₃). ESI-MS: m/z = 373 [M+H]⁺, Anal. Calcd. For C₁₃H₁₄BrN₃O₃S: C, 41.95; H, 3.79; N, 11.29; S, 8.61. Found: C, 41.86; H, 3.80; N, 11.32; S, 8.59.

2-((5-(2-bromo-5-methoxyphenyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetic acid (4d)

White precipitate; yield 85 %; m. p. 272-274 °C.; ¹H NMR, δ (ppm): 11.37 (s, 1H, OH), 7.65 - 7.50 (m, 2H, 3-H 2-Br-5-O-CH₃-C₆H₄, 4-H C₆H₅), 7.46 - 7.40 (m, 3H, 2,6-H C₆H₅, 6-H 2-Br-5-O-CH₃-C₆H₄), 7.35 - 7.31 (m, 2H, 3,5-H C₆H₅), 6.92 (d, J = 8.6, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.04 (s, 1H, S-CH₂-), 3.81 (s, 2H, O-CH₃). ESI-MS: m/z = 421 [M+H]⁺, Anal. Calcd. For C₁₇H₁₄BrN₃O₃S: C, 48.58; H, 3.36; N, 10.00; S, 7.63. Found: C, 48.70; H, 3.35; N, 10.02; S, 7.61.

General procedure for the synthesis of the ethers of 2-((5-(2-bromo-5-methoxyphenyl)-4-R-4H-1,2,4-triazol-3-yl)thio)acetic acid (5a, 5b, 5c, 5d, 5e, 5f, 5g, 5h, 5i, 5j, 5k, 5l, 5m, 5n, 5o, 5p, 5q, 5r, 5s, 5t)

Method A: In a 100 mL heat-resistant flask, 0.01 mol of the starting 2-((5-(2-bromo-5-methoxyphenyl)-4-R-4H-1,2,4-triazol-3-yl)thio)acetic acid (**4a**, **4b**, **4c**, **4d**) in 30 mL of the corresponding alcohol (methanol, ethanol, 1-propanol, 2-propanol, and 1-butanol) was added along with 0.5 mL of concentrated sulfuric acid. The mixture was boiled on a water bath equipped with a reflux condenser for 10 hours. The solvent was evaporated, and the residue was neutralized with a sodium bicarbonate solution, filtered, and dried.

Method B: In a 50 mL heat-resistant flask, 0.01 mol of the corresponding 5-(2-bromo-5-methoxyphenyl)-4-methyl-4H-1,2,4-triazole-3-

thiol (**3a**, **3b**, **3c**, **3d**) in 30 mL of propan-2-ol was added with 0.01 mol of NaOH and 0.01 mol of the corresponding chloroacetic acid ester (methyl, ethyl, 1-propyl, 2-propyl, and 1-butyl). The mixture was boiled until neutral for 3 hours. Subsequently, the solution was filtered, the solvent was evaporated, and the target compounds were obtained as precipitates.

The synthesized compounds are crystalline substances, which were purified for further analysis by recrystallization from propan-2-ol (**5a**–**5t**).

Methyl 2-((5-(2-bromo-5-methoxyphenyl)-4H-1,2,4-triazol-3-yl)thio)acetate (5a)

White precipitate; yield 70 %; m. p. 238–240 °C.; ¹H NMR, δ (ppm): δ 8.32 (s, 1H, NH), 7.59 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.47 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.98 (dd, J = 8.5, 2.8 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.11 (s, 2H, S-CH₂-), 3.81 (s, 3H, O-CH₃), 3.72 (s, 3H, COO-CH₃). ESI-MS: m/z = 359 [M+H]⁺, Anal. Calcd. For C₁₂H₁₂BrN₃O₃S: C, 40.24; H, 3.38; N, 11.73; S, 8.95. Found: C, 40.35; H, 3.39; N, 11.70; S, 8.93.

Methyl 2-((5-(2-bromo-5-methoxyphenyl)-4-methyl-4H-1,2,4-triazol-3-yl)thio)acetate (5b)

White precipitate; yield 67 %; m. p. 248–251 °C.; ¹H NMR, δ (ppm): δ 7.57 (d, J = 8.6 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.39 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.89 (d, J = 8.5 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.08 (s, 2H, S-CH₂-), 3.79 (s, 3H, O-CH₃), 3.71 (s, 3H, COO-CH₃), 3.67 (s, 3H, N-CH₃). ESI-MS: m/z = 373 [M+H]⁺, Anal. Calcd. For C₁₃H₁₄BrN₃O₃S: C, 41.95; H, 3.79; N, 11.29; S, 8.61. Found: C, 41.85; H, 3.80; N, 11.32; S, 8.59.

Methyl 2-((5-(2-bromo-5-methoxyphenyl)-4-ethyl-4H-1,2,4-triazol-3-yl)thio)acetate (5c)

White precipitate; yield 71 %; m. p. 231–233 °C.; ¹H NMR, δ (ppm): δ 7.55 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.36 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.91 (d, J = 8.5 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.39–4.33 (m, 2H, N-CH₂-CH₃), 4.07 (s, 2H, S-CH₂-), 3.81 (s, 3H, O-CH₃), 3.72 (s, 3H, COO-CH₃), 1.42 (t, J = 6.0 Hz, 3H, N-CH₂-CH₃). ESI-MS: m/z = 386 [M+H]⁺, Anal. Calcd. For C₁₄H₁₆BrN₃O₃S: C, 43.53; H, 4.18; N, 10.88; S, 8.30. Found: C, 43.41; H, 4.19; N, 10.85; S, 8.27.

Methyl 2-((5-(2-bromo-5-methoxyphenyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetate (5d)

White precipitate; yield 80 %; m. p. 257–259 °C.; ¹H NMR, δ (ppm): δ 7.65 (d, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.58–7.53 (m, 1H, 4-H C₆H₅), 7.46–7.36 (m, 5H, 2,3,5,6-H C₆H₅, 6-H 2-Br-5-O-CH₃-C₆H₄),

6.93 (d, J = 8.6 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.09 (s, 1H, S-CH₂), 3.81 (s, 1H, O-CH₃), 3.70 (s, 3H, COO-CH₃). ESI-MS: m/z = 435 [M+H]⁺, Anal. Calcd. For C₁₈H₁₆BrN₃O₃S: C, 49.78; H, 3.71; N, 9.68; S, 7.38. Found: C, 49.91; H, 3.72; N, 9.65; S, 7.36.

Ethyl 2-((5-(2-bromo-5-methoxyphenyl)-4H-1,2,4-triazol-3-yl)thio)acetate (5e)

White precipitate; yield 68 %; m. p. 220–221 °C.; ¹H NMR, δ (ppm): δ 8.32 (s, 1H, NH), 7.61 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.45 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.97 (dd, J = 8.5, 2.8 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.21–4.16 (m, 3H, COO-CH₂-CH₃), 4.12 (s, 2H, S-CH₂-), 3.82 (s, 3H, O-CH₃), 1.23 (t, J = 6.6 Hz, 3H, COO-CH₂-CH₃). ESI-MS: m/z = 373 [M+H]⁺, Anal. Calcd. For C₁₃H₁₄BrN₃O₃S: C, 41.95; H, 3.79; N, 11.29; S, 8.61. Found: C, 41.84; H, 3.80; N, 11.26; S, 8.64.

Ethyl 2-((5-(2-bromo-5-methoxyphenyl)-4-methyl-4H-1,2,4-triazol-3-yl)thio)acetate (5f)

White precipitate; yield 73 %; m. p. 235–237 °C.; ¹H NMR, δ (ppm): δ 7.58 (d, J = 8.6 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.39 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.89 (d, J = 8.5 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.19–4.16 (m, 3H, COO-CH₂-CH₃), 4.07 (s, 1H, S-CH₂-), 3.80 (s, 3H, O-CH₃), 3.72 (s, 2H, N-CH₃), 1.25 (t, J = 6.6 Hz, 2H, COO-CH₂-CH₃). ESI-MS: m/z = 387 [M+H]⁺, Anal. Calcd. For C₁₄H₁₆BrN₃O₃S: C, 43.53; H, 4.18; N, 10.88; S, 8.30. Found: C, 43.42; H, 4.19; N, 10.85; S, 8.28.

Ethyl 2-((5-(2-bromo-5-methoxyphenyl)-4-ethyl-4H-1,2,4-triazol-3-yl)thio)acetate (5g)

White precipitate; yield 64 %; m. p. 224–227 °C.; ¹H NMR, δ (ppm): δ 7.56 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.36 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.91 (d, J = 8.5 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.36–4.31 (m, 2H, N-CH₂-CH₃), 4.21–4.17 (m, 2H, COO-CH₂-CH₃), 4.09 (s, 2H, S-CH₂-), 3.78 (s, 3H, O-CH₃), 1.42 (t, J = 6.0 Hz, 3H, N-CH₂-CH₃), 1.23 (t, J = 6.6 Hz, 3H, COO-CH₂-CH₃). ESI-MS: m/z = 401 [M+H]⁺, Anal. Calcd. For C₁₅H₁₈BrN₃O₃S: C, 45.01; H, 4.53; N, 10.50; S, 8.01. Found: C, 45.13; H, 4.52; N, 10.47; S, 8.83.

Ethyl 2-((5-(2-bromo-5-methoxyphenyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetate (5h)

White precipitate; yield 74 %; m. p. 244–247 °C.; ¹H NMR, δ (ppm): δ 7.71 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.67–7.53 (m, 1H, 4-H C₆H₅), 7.45–7.36 (m, 5H, 2,3,5,6-H C₆H₅, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.89 (d, J = 8.5 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.19–4.15 (m, 3H, COO-CH₂-CH₃), 4.08 (s, 2H, S-CH₂-), 3.81 (s, 3H, O-CH₃), 1.21 (t, J = 6.6 Hz, 3H, COO-CH₂-CH₃). ESI-MS: m/z = 449 [M+H]⁺, Anal. Calcd. For C₁₉H₁₈BrN₃O₃S: C, 50.90; H, 4.05; N, 9.37; S, 7.15. Found: C, 50.76; H, 4.04; N, 9.39; S, 7.17.

Propyl 2-((5-(2-bromo-5-methoxyphenyl)-4H-1,2,4-triazol-3-yl)thio)acetate (5i)

White precipitate; yield 70 %; m. p. 211-213 °C.; ^1H NMR, δ (ppm): δ 8.32 (s, 1H, NH), 7.63 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.47 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.98 (d, J = 8.5 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.14 - 4.06 (m, 4H, S-CH₂-, COO-CH₂-CH₂-CH₃), 3.80 (s, 3H, O-CH₃), 1.70 - 1.63 (m, 2H, COO-CH₂-CH₂-CH₃), 0.94 (t, J = 7.9 Hz, 3H, COO-(CH₂)₂-CH₃). ESI-MS: m/z = 387 [M+H]⁺, Anal. Calcd. For C₁₄H₁₆BrN₃O₃S: C, 43.53; H, 4.18; N, 10.88; S, 8.30. Found: C, 43.64; H, 4.19; N, 10.90; S, 8.32.

Propyl 2-((5-(2-bromo-5-methoxyphenyl)-4-methyl-4H-1,2,4-triazol-3-yl)thio)acetate (5j)

White precipitate; yield 82 %; m. p. 231-232 °C.; ^1H NMR, δ (ppm): δ 7.59 (d, J = 8.6 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.39 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.91 (d, J = 8.5 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.13 - 4.05 (m, 4H, S-CH₂-, COO-CH₂-CH₂-CH₃), 3.82 (s, 3H, O-CH₃), 3.72 (s, 3H, N-CH₃), 1.75 - 1.69 (m, 2H, COO-CH₂-CH₂-CH₃), 0.92 (t, J = 7.9 Hz, 3H, COO-(CH₂)₂-CH₃). ESI-MS: m/z = 401 [M+H]⁺, Anal. Calcd. For C₁₅H₁₈BrN₃O₃S: C, 45.01; H, 4.53; N, 10.50; S, 8.01. Found: C, 45.13; H, 4.52; N, 10.52; S, 8.03.

Propyl 2-((5-(2-bromo-5-methoxyphenyl)-4-ethyl-4H-1,2,4-triazol-3-yl)thio)acetate (5k)

White precipitate; yield 70 %; m. p. 228-230 °C.; ^1H NMR, δ (ppm): δ 7.56 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.36 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.93 (d, J = 8.5 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.34-4.29 (m, 2H, N-CH₂-CH₃), 4.13 - 4.05 (m, 4H, S-CH₂-, COO-CH₂-CH₂-CH₃), 3.81 (s, 3H, O-CH₃), 1.72 - 1.65 (m, 2H, COO-CH₂-CH₂-CH₃), 1.44 (t, J = 6.0 Hz, 3H, N-CH₂-CH₃), 0.94 (t, J = 7.9 Hz, 3H, COO-(CH₂)₂-CH₃). ESI-MS: m/z = 415 [M+H]⁺, Anal. Calcd. For C₁₆H₂₀BrN₃O₃S: C, 46.38; H, 4.87; N, 10.14; S, 7.74. Found: C, 46.50; H, 4.88; N, 10.11; S, 7.72.

Propyl 2-((5-(2-bromo-5-methoxyphenyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetate (5l)

White precipitate; yield 77 %; m. p. 218-220 °C.; ^1H NMR, δ (ppm): δ 7.68 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.63 - 7.57 (m, 1H, 4-H C₆H₅), 7.47 - 7.67 (m, 5H, 2,3,5,6-H C₆H₅, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.90 (d, J = 8.5 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.15 - 4.06 (m, 4H, S-CH₂-, COO-CH₂-CH₂-CH₃), 3.83 (s, 3H, O-CH₃), 1.70 - 1.64 (m, 2H, COO-CH₂-CH₂-CH₃), 0.92 (t, J = 7.9 Hz, 3H, COO-(CH₂)₂-CH₃). ESI-MS: m/z = 463 [M+H]⁺, Anal.

Calcd. For C₂₀H₂₀BrN₃O₃S: C, 51.95; H, 4.36; N, 9.09; S, 6.93. Found: C, 51.80; H, 4.35; N, 9.11; S, 6.95.

Isopropyl 2-((5-(2-bromo-5-methoxyphenyl)-4H-1,2,4-triazol-3-yl)thio)acetate (5m)

White precipitate; yield 72 %; m. p. 217-219 °C.; ^1H NMR, δ (ppm): δ 8.30 (s, 1H, NH), 7.61 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.45 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.98 (d, J = 8.5 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.95 (sept, J = 5.9 Hz, 1H, CH-isopropyl), 4.14 (s, 2H, S-CH₂-), 3.79 (s, 3H, O-CH₃), 1.21 (d, J = 5.9 Hz, 6H, (CH₃)₂-isopropyl). ESI-MS: m/z = 387 [M+H]⁺, Anal. Calcd. For C₁₄H₁₆BrN₃O₃S: C, 43.53; H, 4.18; N, 10.88; S, 8.30. Found: C, 43.41; H, 4.19; N, 10.85; S, 8.33.

Isopropyl 2-((5-(2-bromo-5-methoxyphenyl)-4-methyl-4H-1,2,4-triazol-3-yl)thio)acetate (5n)

White precipitate; yield 85 %; m. p. 223-225 °C.; ^1H NMR, δ (ppm): δ 7.59 (d, J = 8.6 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.39 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.93 (d, J = 8.5 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.93 (sept, J = 5.9 Hz, 1H, CH-isopropyl), 4.07 (s, 1H, S-CH₂-), 3.81 (s, 3H, O-CH₃), 3.72 (s, 3H, N-CH₃), 1.19 (d, J = 5.9 Hz, 6H, (CH₃)₂-isopropyl). ESI-MS: m/z = 401 [M+H]⁺, Anal. Calcd. For C₁₅H₁₈BrN₃O₃S: C, 45.01; H, 4.53; N, 10.50; S, 8.01. Found: C, 44.89; H, 4.54; N, 10.52; S, 8.03.

Isopropyl 2-((5-(2-bromo-5-methoxyphenyl)-4-ethyl-4H-1,2,4-triazol-3-yl)thio)acetate (5o)

White precipitate; yield 71 %; m. p. 236-238 °C.; ^1H NMR, δ (ppm): δ 7.56 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.36 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.91 (d, J = 8.5 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.93 (sept, J = 5.9 Hz, 1H, CH-isopropyl), 4.36-4.31 (m, 2H, N-CH₂-CH₃), 4.08 (s, 2H, S-CH₂-), 3.82 (s, 3H, O-CH₃), 1.42 (t, J = 6.0 Hz, 3H, N-CH₂-CH₃), 1.20 (d, J = 5.9 Hz, 6H, (CH₃)₂-isopropyl). ESI-MS: m/z = 415 [M+H]⁺, Anal. Calcd. For C₁₆H₂₀BrN₃O₃S: C, 46.38; H, 4.87; N, 10.14; S, 7.74. Found: C, 46.50; H, 4.88; N, 10.11; S, 7.72.

Isopropyl 2-((5-(2-bromo-5-methoxyphenyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetate (5p)

White precipitate; yield 76 %; m. p. 242-245 °C.; ^1H NMR, δ (ppm): δ 7.67 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.65 - 7.51 (m, 1H, 4-H C₆H₅), 7.46 - 7.35 (m, 5H, 2,3,5,6-H C₆H₅, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.90 (d, J = 8.5 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.94 (sept, J = 5.9 Hz, 1H, CH-isopropyl), 4.09 (s, 2H, S-CH₂-), 3.81 (s, 3H, O-CH₃), 1.22 (d, J = 5.9 Hz, 6H, (CH₃)₂-isopropyl). ESI-MS: m/z = 463 [M+H]⁺, Anal. Calcd. For C₂₀H₂₀BrN₃O₃S:

C, 51.95; H, 4.36; N, 9.09; S, 6.93. Found: C, 51.83; H, 4.37; N, 9.12; S, 6.91.

Butyl 2-((5-(2-bromo-5-methoxyphenyl)-4H-1,2,4-triazol-3-yl)thio)acetate (5q)

White precipitate; yield 67 %; m. p. 204-206 °C.; ¹H NMR, δ (ppm): δ 8.31 (s, 1H, NH), 7.61 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.47 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.98 (d, J = 8.5 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.14 - 4.04 (m, 4H, S-CH₂, COO-CH₂-(CH₂)₂-CH₃), 3.79 (s, 3H, O-CH₃), 1.64 - 1.57 (m, 2H, COO-CH₂-CH₂-CH₃), 1.40 - 1.34 (m, 2H, COO-(CH₂)₂-CH₂-CH₃), 0.92 (t, J = 7.0 Hz, 3H, COO-(CH₂)₃-CH₃). ESI-MS: m/z = 401 [M+H]⁺, Anal. Calcd. For C₁₅H₁₈BrN₃O₃S: C, 45.01; H, 4.53; N, 10.50; S, 8.01. Found: C, 45.13; H, 4.54; N, 10.46; S, 8.03.

Butyl 2-((5-(2-bromo-5-methoxyphenyl)-4-methyl-4H-1,2,4-triazol-3-yl)thio)acetate (5r)

White precipitate; yield 69 %; m. p. 217-218 °C.; ¹H NMR, δ (ppm): δ 7.59 (d, J = 8.6 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.39 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.93 (d, J = 8.5 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.12 - 4.04 (m, 4H, S-CH₂, COO-CH₂-(CH₂)₂-CH₃), 3.82 (s, 3H, O-CH₃), 3.72 (s, 3H, N-CH₃), 1.60 - 1.52 (m, 2H, COO-CH₂-CH₂-CH₃), 1.36 - 1.29 (m, 2H, COO-(CH₂)₂-CH₂-CH₃), 0.89 (t, J = 7.0 Hz, 3H, COO-(CH₂)₃-CH₃). ESI-MS: m/z = 415 [M+H]⁺, Anal. Calcd. For C₁₆H₂₀BrN₃O₃S: C, 46.38; H, 4.87; N, 10.14; S, 7.74. Found: C, 46.50; H, 4.88; N, 10.11; S, 7.72.

Butyl 2-((5-(2-bromo-5-methoxyphenyl)-4-ethyl-4H-1,2,4-triazol-3-yl)thio)acetate (5s)

White precipitate; yield 82 %; m. p. 211-213 °C.; ¹H NMR, δ (ppm): δ 7.56 (d, J = 8.5 Hz, 1H, 3-H 2-Br-5-O-CH₃-C₆H₄), 7.36 (d, J = 2.7 Hz, 1H, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.91 (d, J = 8.5 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.34 - 4.28 (m, 2H, N-CH₂-CH₃), 4.15 - 4.06 (m, 4H, S-CH₂, COO-CH₂-(CH₂)₂-CH₃), 3.79 (s, 3H, O-CH₃), 1.64 - 1.54 (m, 2H, COO-CH₂-CH₂-CH₃), 1.46 - 1.29 (m, 5H, N-CH₂-CH₃, COO-(CH₂)₂-CH₂-CH₃), 0.91 (t, J = 7.0 Hz, 3H, COO-(CH₂)₃-CH₃). ESI-MS: m/z = 429 [M+H]⁺, Anal. Calcd. For C₁₇H₂₂BrN₃O₃S: C, 47.67; H, 5.18; N, 9.81; S, 7.48. Found: C, 47.55; H, 5.19; N, 9.78; S, 7.50.

Butyl 2-((5-(2-bromo-5-methoxyphenyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetate (5t)

White precipitate; yield 70 %; m. p. 230-232 °C.; ¹H NMR, δ (ppm): δ 7.65 - 7.50 (m, 2H, 3-H 2-Br-5-O-CH₃-C₆H₄, 4-H C₆H₅), 7.42 - 7.31 (m, 5H, 2,3,5,6-H C₆H₅, 6-H 2-Br-5-O-CH₃-C₆H₄), 6.88 (d, J = 8.5 Hz, 1H, 4-H 2-Br-5-O-CH₃-C₆H₄), 4.12 - 4.04 (m, 4H, S-CH₂, COO-CH₂-(CH₂)₂-CH₃), 3.81 (s, 1H,

O-CH₃), 1.69 - 1.60 (m, 2H, COO-CH₂-CH₂-CH₃), 1.42 - 1.36 (m, 2H, COO-(CH₂)₂-CH₂-CH₃), 0.88 (t, J = 7.0 Hz, 3H, COO-(CH₂)₃-CH₃). ESI-MS: m/z = 477 [M+H]⁺, Anal. Calcd. For C₂₁H₂₂BrN₃O₃S: C, 52.95; H, 4.66; N, 8.82; S, 6.73. Found: C, 52.83; H, 4.65; N, 8.85; S, 6.75.

Molecular docking

Based on the computational docking analysis, the most energetically stable conformation of Celecoxib within the COX-2 active site was found to be in high agreement with previously reported experimental findings. The binding free energy of the "Celecoxib-COX-2" complex, according to the conducted molecular modeling, is (-13.4) kcal/mol. Based on the molecular modeling data, the binding active site of the Celecoxib molecule includes the residues S354, Q193, and F519, where the nitrogen hydrogen of the guanidine fragment forms a hydrogen bond with the oxygen of the aminosulfonyl fragment of Celecoxib. Additionally, V350, L360, and L532 are involved in Pi-Alkyl interactions with the CF₃ fragment, while V524 and G527 form Pi-Sigma and Amide-Pi Stacked interactions with the aromatic rings.

Accordingly, compounds 4a, 5e, and 5j were chosen for further investigation as promising scaffolds with high selectivity for binding to COX-2, and to demonstrate their 2D binding mode with the enzyme. For example, compound 4a exhibited a binding free energy of -8.5 kcal/mol and showed interactions with the enzyme via V524, L353 (Alkyl and Pi-Alkyl interactions with the aromatic ring of the substituted phenyl radical), A528, and Y356, which in turn were involved in Pi-Sigma and Pi-Sulfur interactions with the 1,2,4-triazole core (Fig. 4).

The analysis of compound 5e revealed the presence of similar amino acid residues, but with a different type of interaction. A528 formed a Pi-Sigma bond with the aromatic rings of the 1,2,4-triazole and substituted phenyl radical. Y356 interacted with the sulfur atom via a Pi-Sulfur interaction, and S354 formed a Carbon Hydrogen Bond and Pi-Donor Hydrogen Bond with the methoxy group of the aromatic fragment at the 5-position (Fig. 4).

Regarding compound 5j, which demonstrated the highest interaction energy of -12.2 kcal/mol, the presence of multifunctional interactions was noted.

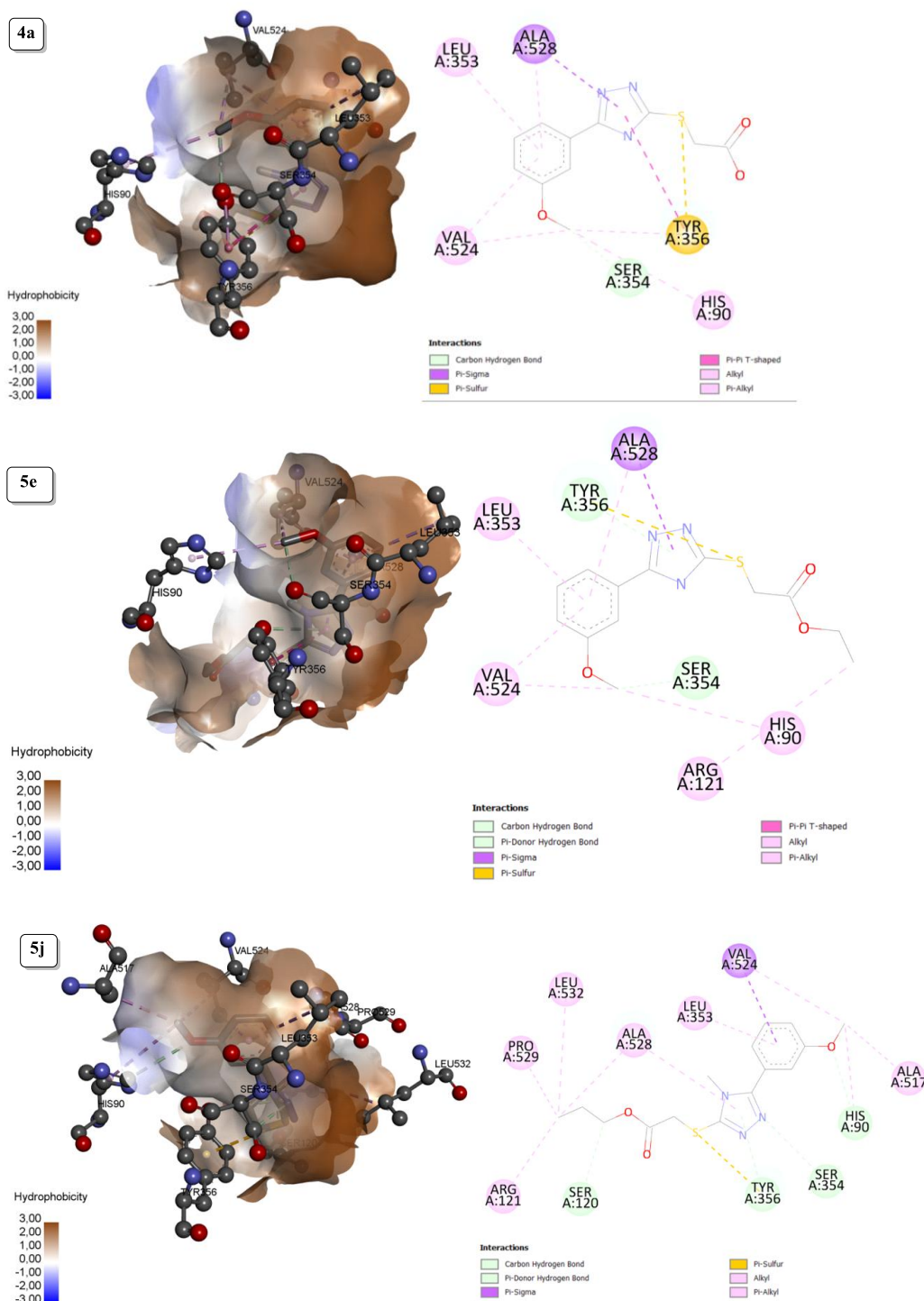


Figure 4. Visualization scheme for binding compound **4a**, **5e** and **5j** to COX-2

Specifically, R121, P529, L532, and A528 showed Alkyl and Pi-Alkyl interactions, as well as Carbon Hydrogen Bond and Pi-Donor Hydrogen Bond with the propyl residue of the ester group of S120, Y356 formed a Pi-Sulfur interaction with the sulfur atom and a Pi-Donor Hydrogen Bond with the nitrogen atom of the 1,2,4-triazole fragment. Additionally, S354 formed a Pi-Donor Hydrogen Bond with the nitrogen atom of the triazole fragment (Fig. 4).

The interaction of L353 was of the Alkyl Pi-Alkyl type, while V524 exhibited a Pi-Sigma interaction with the aromatic ring of the 2-bromo-5-methoxyphenyl substituent. The methoxy group showed interactions via Carbon Hydrogen Bond, Pi-Donor Hydrogen Bond, and Alkyl Pi-Alkyl with H90 and A517, respectively. The obtained results are presented in Table 1.

Table 1

Binding with key amino acids of the synthesized derivatives of 5-(2-bromo-5-methoxyphenyl)-4-R-1,2,4-triazole-3-thiols

№	Compound	Interaction Energy	Amino Acid Residues	Type of Interaction
1	3a	-6.6	V524, L353, A517, Y356, H90, A528, S354	C-H, Pi-D, Pi-Sig, Pi-S, Alk, Pi-Alk
2	4a	-8.5	L353, A528, V524, Y356, S354, H90	C-H, Pi-Sig, Pi-S, Pi-Pi T, Alk, Pi-Alk
3	5a	-6.9	S354, Y356, A528, E525, H90, L353, A517, V524	C-H, Pi-D, Pi-Sig, Pi-S, Pi-Pi T, Alk, Pi-Alk
4	5e	-8.7	A528, L353, Y356, V524, S354, H90, R121	C-H, Pi-D, Pi-Sig, Pi-S, Pi-Pi T, Alk, Pi-Alk
5	5i	-5.4	A528, W388, V524, Y356, Y386, L353, V117, L93, V89, F358	Pi-D, Pi-Pi T, Alk, Pi-Alk
6	5m	-8.2	A528, R121, L353, Y356, V524, S354, H90	C-H, Pi-D, Pi-Sig, Pi-S, Pi-Pi T, Alk, Pi-Alk
7	5q	-7.2	Y116, V524, V117, V89, L353, A517, Y356, H90, S354, A528	C-H, Pi-D, Pi-Sig, Pi-S, Pi-Pi T, Alk, Pi-Alk
8	3b	-7.2	L353, V524, W388, Y386, V350, M523, F519	Conv. H, C-H, Pi-Sig, Pi-S, Alk, Pi-Alk
9	4b	-5.6	V117, Y356, A528, S354, E525, V89, P86	Conv. H, C-H, Pi-An, Pi-D, Pi-Pi T, Alk, Pi-Alk
10	5b	-5.8	V117, Y356, A528, E525, V89, P86	C-H, Pi-An, Pi-D, Pi-Pi T, Alk, Pi-Alk
11	5f	-5.6	V117, A528, Y356, Y349, E525, L353, V89, P86, V350	C-H, Pi-An, Pi-D, Pi-Pi T, Alk, Pi-Alk
12	5j	-12.2	V524, L532, P529, L353, A528, A517, H90, R121, S120, S354, Y356	C-H, Pi-D, Pi-Sig, Pi-S, Alk, Pi-Alk
13	5n	-6.7	L353, V89, V524, A517, H90, F519, Y356, S354, A528	C-H, Pi-D, Pi-Sig, Alk, Pi-Alk, Pi-S
14	5r	-6.6	V524, V89, L93, H90, L353, Y356, A517, A528, S354	C-H, Pi-D, Pi-Sig, Pi-S, Alk, Pi-Alk
15	4c	-6.1	V117, L532, A528, S120, V350	C-H, Alk, Pi-Alk
16	5c	-6.5	S354, L353, V350, V524, R514, F519, A517, H90	C-H, Pi-Sig, Alk, Pi-Alk
17	5g	-6.0	V89, E525, R121, Y356, A528	C-H, Pi-An, Pi-D, Pi-S, Pi-Pi T, Alk, Pi-Alk
18	5k	-6.2	E525, V524, R121, A528, Y356	C-H, Pi-An, Pi-D, Pi-S, Pi-Pi T, Alk, Pi-Alk
19	5o	-5.0	A528, Y356, Y386, V117, L93	Pi-D, Pi-Pi T, Alk, Pi-Alk
20	5s	-4.8	A528, Y356, Y386, V117, L93	Pi-D, Pi-Pi T, Alk, Pi-Alk
21	3d	-4.8	V117, V89, P86, E525, V524, Y356, A528	Pi-An, Pi-D, Pi-Sig, Pi-S, Pi-Pi T, Pi-Alk
22	4d	-4.7	V117, V89, P86, E525, Y356, V524, A528, S120	C-H, Pi-An, Pi-Sig, Pi-Pi T, Pi-Alk
23	5d	-3.4	V350, V117, L532, V89, A528, S120, Y356, V524, E525	C-H, Pi-An, Pi-D, Pi-Sig, Pi-Pi T, Pi-Alk
24	5h	-3.5	V524, A528, S120, Y116, F519, L353, V117, L93, Y356	Pi-D, Pi-Pi T, Alk, Pi-Alk
25	5e	-6.7	P86, V89, E525, A528, L353, F519, S354, V524, Y356, R121, V117	C-H, Pi-An, Pi-D, Pi-Sig, Pi-S, Alk, Pi-Alk
26	5p	-6.7	L532, V350, V117, Y356, V89, V524, P529, L93, A528, R121, S120	Pi-D, Pi-Sig, Conv. H, Alk, Pi-Alk
27	5t	-7.0	P86, L124, V89, R121, E525, V117, L353, V524, F519, Y356, A528	C-H, Pi-An, Pi-D, Pi-Sig, Pi-S, Alk, Pi-Alk
28	Celecoxib	-13.4	Y386, L385, L532, W388, G527, L353, A528, S354, L360, V350, V524, F519, Q193, R514, H90	Conv. H, C-H, Pi-Sig, Amide-Pi, Alk, Pi-Alk

Note: Interaction types are abbreviated as follows: Conv. H – Conventional Hydrogen Bond; C-H – Carbon Hydrogen Bond; Pi-D – Pi-Donor Hydrogen Bond; Pi-Sig – Pi-Sigma; Pi-S – Pi-Sulfur; Pi-An – Pi-Anion; Pi-Pi T – Pi-Pi T-shaped; Amide-Pi – Amide-Pi Stacked; Alk – Alkyl; Pi-Alk – Pi-Alkyl. Amino acid residues are listed using single-letter codes and their positions within Chain A (e.g., V524 denotes VAL A:524)

Conclusions. As a result of the conducted study, which was driven by the urgent need to develop novel therapeutics for the challenges of military medicine, a new series of 5-(2-bromo-5-methoxyphenyl)-4-R-1,2,4-triazole-3-thiols (3a, 3b, 3c, 3d), 2-((5-(2-bromo-5-methoxyphenyl)-4-R-1,2,4-triazol-3-yl)thio)acetic acids (4a, 4b, 4c, 4d), and the ethers of 2-((5-(2-bromo-5-methoxyphenyl)-4-R-4H-1,2,4-triazol-3-yl)thio)acetic acids (5a, 5b, 5c, 5d, 5e, 5f, 5g, 5h, 5i, 5j, 5k, 5l, 5m, 5n, 5o, 5p, 5q, 5r, 5s, 5t) were successfully synthesized. The chemical structures of all the obtained compounds were rigorously confirmed using ^1H -NMR spectroscopy, Elemental Analyses (EA), and LC/MS methods to ensure their high purity and stability, parameters that are essential for pharmacological agents intended for field deployment. *In silico* studies, specifically molecular docking, were comprehensively carried out for the obtained compounds with respect to their interactions with COX-1 and COX-2 enzymes, aiming to evaluate their potential as robust anti-inflammatory agents for combat casualties.

The complex formation between the macromolecule COX-2 - a critical target in the management of severe trauma-induced inflammation - and ligands based on the triazole scaffold of 5-(2-bromo-5-methoxyphenyl)-4-R-1,2,4-triazole-3-thiol derivatives was deeply studied. Binding energy calculations were performed, and the differences between ligand atoms and the amino acid residues of the protein,

as well as between key atoms of the ligand fragments, were determined. The nearest surroundings of the ligands by amino acid residues were characterized, and the preferred types of interactions between the ligands and COX-2 were analyzed, providing a solid structural basis for designing drugs capable of mitigating acute pain and swelling in wounded personnel.

The intermolecular interactions of 5-(2-bromo-5-methoxyphenyl)-4-R-1,2,4-triazole-3-thiol derivatives with COX-2 are characterized by binding energies ranging from -3.2 to -12.2 kcal/mol, which is highly comparable to the binding energy of the reference drug celecoxib (-13.4 kcal/mol). Ligands forming triazole fragments are capable of strongly interacting with the active site of COX-2, represented by the residues L532, L353, A528, S354, and H90. This firmly suggests that one of the mechanisms of the anti-inflammatory action of the studied compounds is their direct interaction with COX-2. A series of the most promising compounds has been selected as lead candidates for further *in vivo* investigation of anti-inflammatory activity. These subsequent studies will focus on validating their efficacy and safety profiles, with the ultimate goal of developing potent, next-generation therapeutic agents tailored to address the acute medical needs of military pharmacy and to improve clinical outcomes for patients with combat-related injuries.

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