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# A Novel Series of Pyrazole and Thiadiazole Derivatives Bearing Nabumetone Moiety: Design, Molecular Docking Study, Synthesis, Characterization, and Preliminary Pharmacological Evaluation.

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## Abstract

A series of new pyrazole and thiadiazole compounds including the Nabumetone moiety were designed, synthesized, and subjected to assessment for their anti-inflammatory potential against cyclooxygenase enzyme-2. Following an *In silico* experiments involving molecular docking analysis, the most promising compound set was synthesized and further described. After the prediction of their suspected activity by molecular docking study using Cambridge Crystallographic Data Centre software tool (GOLD), Compounds were tested in vivo as anti-inflammatory agents using egg white procedure. Hydrogen bonding interaction with key amino acids in COX-2 isozymes, including Arginine120, Tyrosine355, and Serine530, gave the compounds investigated in molecular docking much higher activity than the reference drugs naproxen, diclofenac, and 6MNA. The data obtained from docking studies were highly correlated with that obtained from the in vivo assay where the newly synthesized compounds were tested in real as anti-inflammatory agents using the egg white procedure. Compounds **3c, 4c, 5c** showed a PLP fitness 91.35, 89.66 and 92.09 respectively, which are the best scores from all synthesized compounds. This research offered helpful direction for the identification of novel thiadiazole anti-inflammatory compounds.

**Keywords: ADME, Docking, GOLD, COX-2, Nabumetone.**

## Introduction

Inflammation, the body's main defensive mechanism, is essential for defending living things where prostaglandin (PG) levels are elevated in relation to both inflammation and pain. Non-steroidal anti-inflammatory medicines (NSAIDs) are a broad category of medications that have historically been used to treat pain and inflammation. These medications function by preventing the synthesis of prostaglandins. In 1971, John R. Vane discovered that the particular molecular target for NSAIDs was cyclooxygenase enzymes (COX) [(1-3)].According to certain research, altering the way that standard non-steroidal anti-inflammatory medications (NSAIDs) operate as carboxylates might boost their anti-inflammatory benefits while lowering the likelihood of gastrointestinal adverse effects. A hydrophobic channel makes up the cyclooxygenase (COX) binding site. The main amino acids present in COX-1 and COX-2's active sites are shown in Figure. 1. Both COX enzymes include Glu524 and Arg120 as charged residues. [(4,5)]. Analysis of X-ray data reveals apparent interaction between Arg120, located in the center of the Cyclooxygenase channel, and the carboxyl group of arachidonic acid (AA) and common NSAIDs in Cyclooxygenase 1. All selective COX-2 inhibitors to far lack a carboxylic moiety, which has sparked curiosity about the residue's role in the enzyme. Aspirin also acetylates Ser530, a crucial residue in the channel's upper region. Located at the top of the channel between the COX active site and the peroxidase (POX) site, Tyr385 is involved in hydroperoxidase activity. The selectivity of certain COX-2 inhibitors may be explained by the differences between the COX active sites of COX-1 and COX-2 [(6)]. Similarities exist between the COX-2 enzyme and COX-1, despite three amino acid changes. Particularly, Arginine 513 in COX-1 has been changed to Histidine, Isoleucine 523 is replaced by the smaller Valine 523 in COX-2, and Isoleucine 434 in COX-1 is substituted with Valine in COX-2 (permitting the relocation of Phenylalanine 518). These modifications caused the polar hydrophilic side pocket of the Cyclooxygenase -2 active site to

enlarge relative to the Cyclooxygenase -1 active site. This implies that certain molecules may fit in the COX-2 active site even if they are too large for the COX-1 active site. The 22 amino acids that make up the COX-2 active site are separated into three discrete regions, or "cavities:" cavity A (the aromatic area), cavity B (the aliphatic region), and cavity C (the selective region).” Figure. 1”[7].

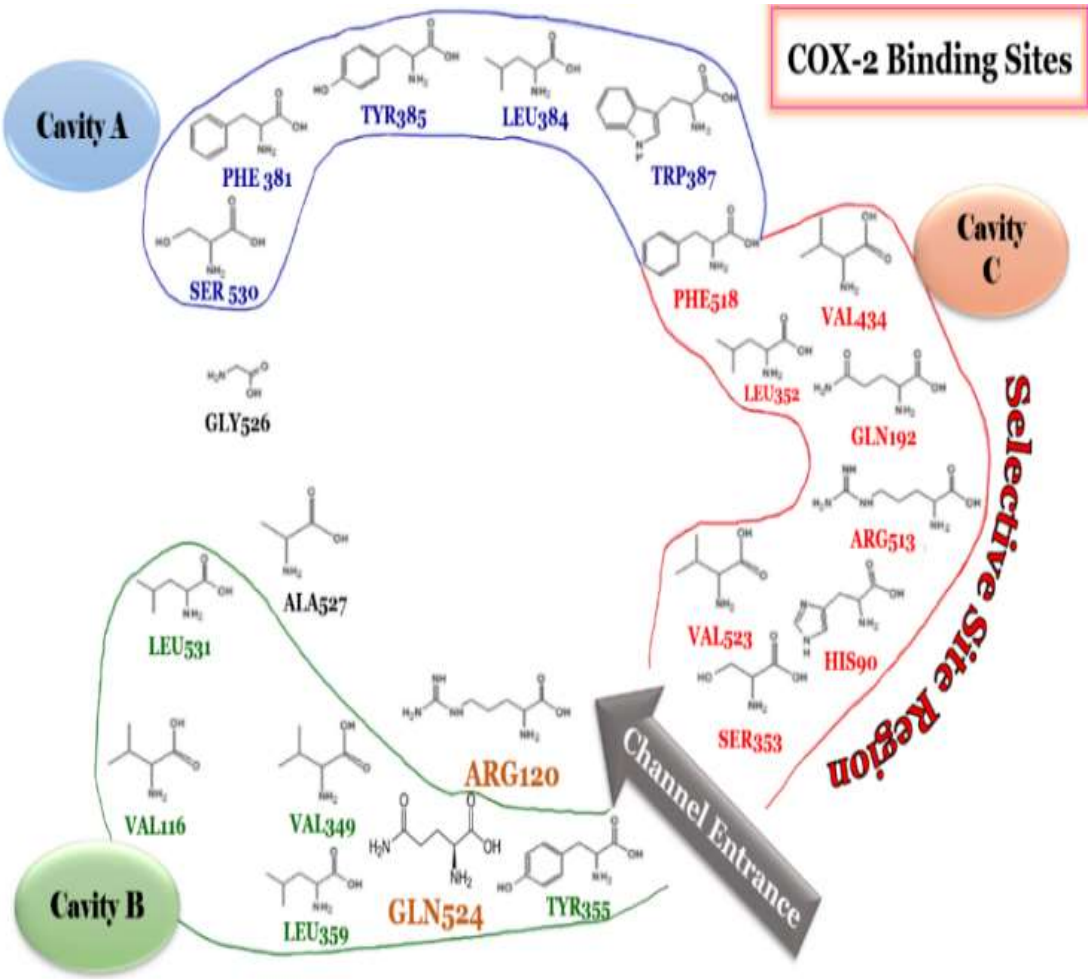



**Figure 1. The amino acid composition of the COX-2 active site [7].**

In 1985, Smith-Kline Beecham released Nabumetone, also known as 4-(6-methoxy-2-naphthalenyl)-2-butanone, a novel nonacidic substance having anti-inflammatory, analgesic, and antipyretic effects. Subsequently, Bencard, Fujisawa, Uriach, and Ambelette were granted licenses to manufacture and sell this drug. Its effectiveness surpasses that of naproxen and indomethacin and even exceeds that of aspirin [8].The primary therapeutic effect of Nabumetone is attributed to its active metabolite, 6-methoxy-2-naphthylacetic acid (6MNA). In comparison, Nabumetone itself exhibits weak inhibition of COX-2 byproducts, particularly prostaglandins. It is believed to have potentially lower nephrotoxicity than indomethacin [9].Pyrazole derivatives are highly potent compounds with diverse biological activities, making them a prominent class of substances. In recent times, numerous drugs have been synthesized based on pyrazole derivatives [10]. Noteworthy examples include celecoxib, which exhibits anti-inflammatory properties and acts as a COX-2 inhibitor; rimonabant, utilized for obesity treatment by functioning as a cannabinoid receptor antagonist; fomepizole, an inhibitor of alcohol dehydrogenase; and sildenafil, a phosphodiesterase inhibitor. Many drugs are available as examples of NSAIDs with pyrazole moiety, including Ramifenazone, and Famprofazone [11]. ligands containing pyrazole carboxamides, pyrazole-1-carbothioamide and 2,4-dinitro phenyl groups pharmacophore at the position 1 of pyrazole ring showed a good anti-inflammatory activity against secreted phospholipase A2 (sPLA2) [12-14]. Ligands containing pyrazolyl methylene hydrazine carboxamide pharmacophore have anti-inflammatory activity and safer ulcerogenic profile and great bioavailability this compound showed a good *In silico* and *In vitro* study [15].The 1,3,4-thiadiazole ring possesses unique characteristics

such as weak basicity, high aromaticity, and stability in acidic conditions but susceptibility to ring cleavage in basic solutions. Additionally, the ring demonstrates electron deficiency due to the presence of nitrogen atoms, making it resistant to electrophilic substitution but susceptible to nucleophilic attack. However, when substitutions occur at the 2' or 5' positions of the ring, it becomes highly reactive, leading to the formation of diverse derivatives [16].Due to their unique characteristics, 1,3,4-thiadiazole derivatives are widely used in the fields of pharmaceuticals, agriculture, and materials chemistry. In particular, the pharmacological actions of these compounds span a wide range, from antibacterial to antioxidant to anti-inflammatory to anticonvulsant to antidepressant to anxiolytic to antihypertensive to anticancer. Acetazolamide is a pharmacological agent that belongs to the class of carbonic anhydrase inhibitors. It is commonly prescribed for the treatment of various medical conditions such as glaucoma, high-altitude illness, epileptic seizures, idiopathic intracranial hypertension, hemiplegic migraine, cystinuria, obstructive sleep apnea, and congenital myasthenic syndromes. [17].

## Materials and Methods

***Chemistry***

Sigma-Aldrich, Reidel-De Haen, and Merck (all of Germany) and BDH (of England) were used as sources for the high-purity reagents and anhydrous solvents used in this work. The Shanghai Renyoung Company, supplied the Nabumetone. The compounds' melting points were calculated using the capillary technique and an electric melting point device from the English company Bamstead/Electrothermal 9100 (Model) Electric. Ascending thin layer chromatography (TLC) on 0.2 mm thick DC-Kartan SI alumina plates was used to examine the reaction's purity and track its development. The compounds were identified using infrared spectroscopy recordings made on an FT-IR spectrophotometer FT-IR-6100 TypeA using KBr discs. The $^1$H-NMR experiment employed 400 MHz Bruker equipment.

***General procedure for the synthesis of Schiff base 2- (4- (6- methoxy naphthalene- 2-yl) butan-2-ylidene) hydrazine -1- carboxamide [2a]***

A round-bottomed flask was filled with a mixture of Nabumetone (0.228 g, 1 mmol), hydrazine carboxamide (0.091g, 1 mmol), methanol : diethylether (5:2), and four drops of glacial acetic acid. The mixture was continuously stirred for 72 hours. Absolute ethanol : ether (5:2) was added after one hour of constant stirring. The solid product formed was filtered off and washed with ethanol, ether and dried. Then the product was recrystallized from ethyl acetate to collect the white crystals [18].

***General procedure for the synthesis of Schiff base 2- (4- (6- methoxy naphthalene- 2 -yl) butan-2-ylidene) hydrazine - 1 - carbothioamide [2b]***

Compounds were combined in a round-bottom flask and swirled constantly for 72 hours: Nabumetone (0.228 g, 1 mmol), hydrazinecarbothioamide (0.091 g, 1 mmol), methanol:ether (5:2), and four drops of glacial acetic acid. During the last stirring hour, absolute ethanol:ether (5:2) was added while the mixture was continuously stirred. After the solid was separated, it was filtered, washed with ethanol and ether, and finally dried. It was then recrystallized from ethyl acetate to remove the white crystals [19, 20].

***General procedure for the synthesis of Schiff base 1-(2,4-dinitrophenyl)-2-(4-(6-methoxynaphthalen-2-yl)butan-2-ylidene)hydrazine [2c]***

Beaker (1) had 5 mL of ethanol added to 1.5 mL of D.W., while beaker (2) had 1 mL of $H_2SO_4$ added to 0.1 mmol of 2,4-dinitrophenylhydrazine and was stirred for 5 minutes before being added to beaker (1). Next, Nabumetone (0.228 g, 1 mmol) was added, followed by 3 mL of water and 10 minutes of stirring. The final product was washed with ether and an orange precipitate developed after it was filtered and rinsed again with cold water to neutralize the pH [21].

***General procedure for the synthesis of pyrazole-4-carbaldehyde derivatives[3a-c] Vilsmeier – Haack reagent: A solution of compounds [2a-c]***

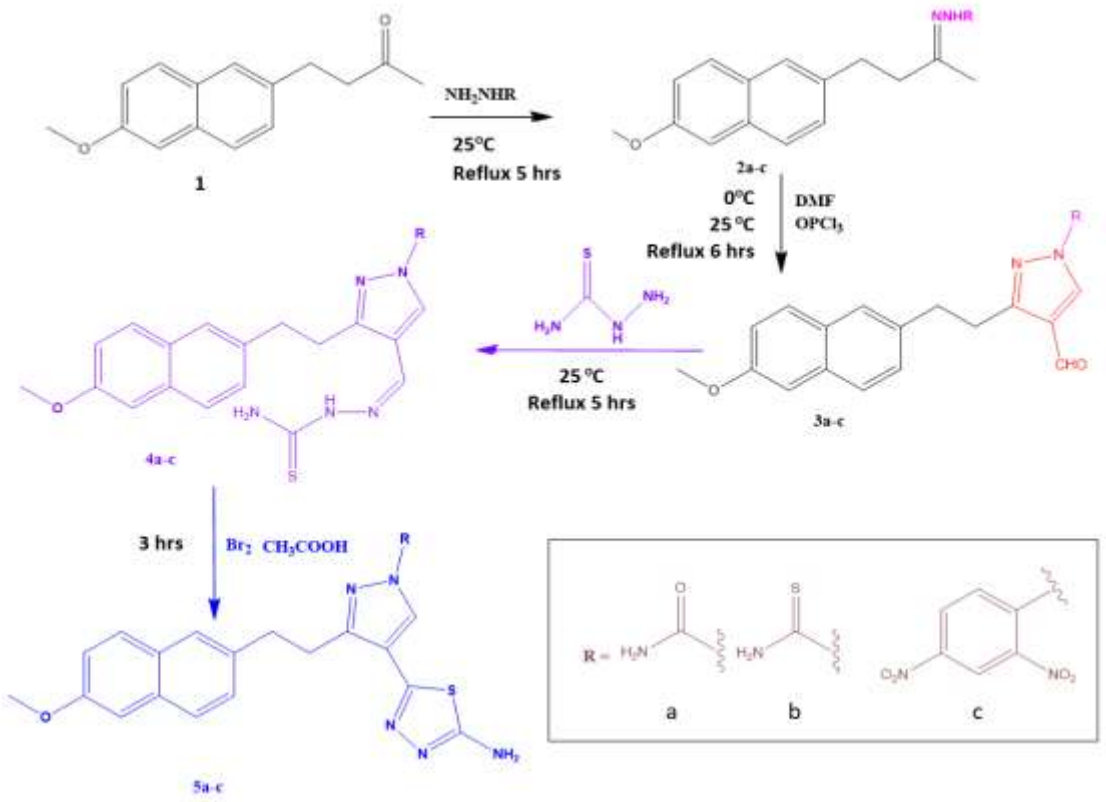
(1 mmol) in 0.4 mL DMF was added dropwise to DMF (0.25 mL) at 0ºC, followed by the addition of $POCl_3$ (0.25 mL) at 0 ºC. The liquid was cooled to room temperature using 100 g of ice after being agitated at 0 ºC for 3 hours and then refluxed for 6 hours. The final combination was alkaline (pH = 9-10) by the addition of 30% aq NaOH. The ultimate outcome was achieved after the product was washed and filtered many times with water [22].

***General procedure for the synthesis of pyrazole-4-Schiff base derivatives [4a-c] In a round-bottomed flask, we mixed 1 mmol of pyrazole-4-carbaldehyde derivatives [3a-c]***

with hydrazinecarbothioamide [b] (0.091 g, 1 mmol), 5 mL of methanol, and 4 drops of glacial acetic acid and stirred the mixture at reflux for 5 hours. After an hour of stirring, 5 mL of methanol was added while the mixture was still being stirred. We removed the solid by filtering, then washed it in methanol and ether, and finally dried it. After that, ethyl acetate was used to recrystallize the product [18].

***General procedure for the synthesis of (pyrazol-4-yl)-1,3,4-oxadiazol-2-amine [5a-c]***

Using a magnetic stirrer, 1 mmol of compounds (4a-c) were dissolved in 0.8 mL of glacial acetic acid with the addition of 2 drops of bromine solution. After 2 to 3 hours of stirring at room temperature, the reaction mixture was dumped into a bowl of crushed ice and given a good stir. The final products were obtained after the solid was filtered, rinsed with water, and dried [23].




**Scheme 1. Synthesis of the designed compounds**

***(E)- 2- (4 - ( 6 - methoxynaphthalen-2-yl)butan-2-ylidene) hydrazine-1-carboxamide (2a),***
white powder (82% yield); mp 196–197°C; IR (KBr) v ($cm^{-1}$): 1261 (C-O-$CH_3$), 1465 (C-N), 1671 (C=N), 1698 (C=O), and 3303, 3322, 3480 (For NH & $NH_2$). $^1$H-NMR (DMSO-*d6*, 400 MHz): δ 1.85 (s, 3H, N=C-C$\underline{H}_3$), 2.54 (t, 2H, $CH_2$–C$\underline{H}_2$), 2.95 (t, 2H, C$\underline{H}_2$–$CH_2$), 3.87 (s, 3H, O–$CH_3$), 6.25 (s, 2H, N$\underline{H}_2$), 7.12-7.97 (m, 6H, aromatic H), and 9.00 (s, 1H, N$\underline{H}$)
***(E)-2- (4- (6-methoxy naphthalen-2-yl) butan-2-ylidene) hydrazine-1-carbothioamide (2b),***
white powder (82% yield); mp 194–195°C; IR (KBr) v ($cm^{-1}$): 1271 (C-O-$CH_3$), 1298 (C=S), 1424 (C-N), 1585 (C=N), and, 3391, 3510 (For NH & $NH_2$). $^1$H-NMR (DMSO-*d6*, 400 MHz): δ 1.96 (s, 3H, N=C-C$\underline{H}_3$), 2.89 (t, 2H, $CH_2$–C$\underline{H}_2$), 2.97 (t, 2H, C$\underline{H}_2$–$CH_2$), 3.88 (s, 3H, O–$CH_3$), 7.13 (s, 2H, N$\underline{H}_2$), 7.30-8.10 (m, 6H, aromatic H), and 10.00 (s, 1H, N$\underline{H}$)
***(E )-1- (2,4-dinitrophenyl) -2- (4- (6-methoxy naphthalen-2-yl) butan-2- ylidene) hydrazine (2c),***
orange powder (86% yield); mp 165–170°C; IR (KBr) v ($cm^{-1}$): 1264 (C-O-$CH_3$), 1403 (C-N), 1628 (C=N), and, 3301 (NH). ;$^1$H-NMR (DMSO-*d6*, 400 MHz): δ 1.91 (s, 3H, N=C-CH3), 2.63 (t, 2H, $CH_2$–C$\underline{H}_2$), 2.86 (t, 2H, C$\underline{H}_2$–$CH_2$), 3.87 (s, 3H, O–C$\underline{H}_3$), 7.12-8.23 (m, 9H, aromatic H), and 10.12 (s, 1H, NH)
***4 – formyl -3- (2 -( 6-methoxy naphthalen-2-yl) ethyl) -1H - pyrazole-1- carboxamide (3a),*** off white powder (68% yield); mp 181–183°C; IR (KBr) v ($cm^{-1}$): 1262 (C-O-$CH_3$), 1410 (C-N), 1631 (C=N), 1698 (C=O amide), 1715 (CHO), and 3506, 3520 ($NH_2$). $^1$H-NMR (DMSO-*d6*, 400 MHz): 2.53 (t, 2H, $CH_2$–C$\underline{H}_2$), 2.89 (t, 2H, C$\underline{H}_2$–$CH_2$), 3.40 (s, 3H, O–C$\underline{H}_3$), 6.41 (s, 2H, $NH_2$), 7.13-8.18 (m, 7H, aromatic & diazole H), and 9.98 (s, 1H, CHO).
***4-formyl-3-(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazole-1-carbothioamide (3b),*** off white powder (62% yield); mp 185–187°C; IR (KBr) v ($cm^{-1}$): 1243 (C-O-CH3), 1391 (C-N), 1564 (C=N), 1714 (CHO), and 3501, 3525 ($NH_2$). $^1$H-NMR (DMSO-*d6*, 400 MHz): 2.61 (t, 2H, $CH_2$–C$\underline{H}_2$), 2.86 (t, 2H, C$\underline{H}_2$–$CH_2$), 3.77 (s, 3H, O–$CH_3$), 6.45 (s, 2H, $NH_2$), 7.19-8.17 (m, 7H, aromatic & diazole H), and 9.96 (s, 1H, CHO).
***1-(2,4-dinitrophenyl)-3-(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazole-4-carbaldehyde (3c),*** brown powder (92% yield); mp 155–157°C; IR (KBr) v ($cm^{-1}$): 1229 (C-O-CH3), 1388 (C-N), 1538 (N-O), 1618(C=N), and 1718 (CHO). $^1$H-NMR (DMSO-*d6*, 400 MHz): 2.94 (t, 2H, $CH_2$–C$\underline{H}_2$), 3.10 (t, 2H, C$\underline{H}_2$–$CH_2$), 3.87 (s, 3H, O–C$\underline{H}_3$), 7.09-8.76 (m, 10H, aromatic H and diazole), and 9.98 (s, 1H, CHO).
***(Z) -4 -(((hydrazine carbonothioyl) imino) methyl)-3- (2- (6- methoxy naphthalen -2-yl) ethyl)-1H-pyrazole-1-carboxamide (4a),*** white crystals (68% yield); mp 200–202°C; IR (KBr) v ($cm^{-1}$): 1222 (C-O-CH3), 1358 (C-N), 1625 (C=N), 1656 C=O pyrazole carboxamide, and, 3311 and 3401 (For NH & NH2). $^1$H-NMR (DMSO-*d6*, 400 MHz): 2.93 (t, 2H, $\underline{CH_2}$–$CH_2$), 3.16 (t, 2H, $CH_2$–C$\underline{H}_2$), 3.84 (s, 3H,

O–$CH_3$), 6.35 (s, 2H, $NH_2$ thioamide), 6.38 (s, 2H, $NH_2$, carboxamide), 7.07-8.02 (m, 7H, Aromatic and pyrazole), 8.39 (s, 1H, C<u>H</u>=N), 10.12 (s, 1H, N<u>H</u>-N=CH).

***(Z)-4-(((hydrazinecarbonothioyl)imino)methyl)-3-(2- (6- methoxynaphthalen -2 -yl )ethyl)-1H-pyrazole-1-carbothioamide (4b),***

white crystals (63% yield); mp 192-194 °C; IR (KBr) v ($cm^{-1}$): 1319 (C-O-CH3), 1389 (C-N), 1583 (C=N), and, 3310, 3445 and 3451 (For NH & NH2). $^1$H-NMR (DMSO-*d6*, 400 MHz): 2.91 (t, 2H, <u>$CH_2$</u>–$CH_2$), 3.12 (t, 2H, $CH_2$–C<u>$H_2$</u>), 3.79 (s, 3H, O–$CH_3$), 6.26 (s, 2H, $NH_2$ thioamide), 6.79 (s, 2H, $NH_2$, carbothioamide), 7.03-7.89 (m, 7H, Aromatic), 8.34 (s, 1H, C<u>H</u>=N imine group), 10.12 (s, 1H, N<u>H</u>-N=CH methylenehydrazine group).

***(Z)- N- (( 1- (2 ,4- dinitrophenyl )-3-( 2-(6-methoxynaphthalen -2-yl )ethyl) -1H-pyrazol-4-yl)methylene)hydrazinecarbothioamide (4c),***

Brown precipitate (77% yield); mp 171-173 ºC °C; IR (KBr) v ($cm^{-1}$): 1319 (C-O-CH3), 1382 (C-N), 1424 (C=N), and, 3354, 3410 (For NH & NH2). $^1$H-NMR (DMSO-*d6*, 400 MHz): 2.84 (t, 2H, <u>$CH_2$</u>–$CH_2$), 3.25 (t, 2H, $CH_2$–C<u>$H_2$</u>), 3.91 (s, 3H, O–$CH_3$), 6.23 (s, 2H, $NH_2$ thioamide), 7.12-7.99 (m, 10H, Aromatic and pyrazole), 8.49 (s, 1H, C<u>H</u>=N imine group), 10.19 (s, 1H, N<u>H</u>-N=CH methylenehydrazine group).

***4 - (5 – amino -1 , 3 ,4 – thiadiazol – 2 -yl) -3- (2-(6- methoxy naphthalen-2-yl) ethyl) -1H-pyrazole-1-carboxamide (5a),***

Off white crystals (64% yield); mp 214–218°C; IR (KBr) v ($cm^{-1}$): 1229 (C-O-$CH_3$), 1401(C-N), 1609(C=N), 1653 (C=O) and 3281, 3349 ($NH_2$) $^1$H-NMR (DMSO-*d6*, 400 MHz): 2.64 (t, 2H, <u>$CH_2$</u>–$CH_2$), 3.26 (t, 2H, $CH_2$–C<u>$H_2$</u>), 3.86 (s, 3H, O–$CH_3$), 6.16 (s, 2H, $NH_2$ thiadiazole), 6.19 (s, 2H, $NH_2$ carboxamide), 7.26-7.73 (m, 6H, Aromatic), 8.09 (s, 1H, C<u>H</u> of pyrazole).

***4 - (5 – amino -1 , 3 , 4- thiadiazol – 2 - yl) -3 -(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazole-1-carbothioamide (5b),***

Off white crystals (71% yield); mp 209–212°C; IR (KBr) v ($cm^{-1}$): 1234 (C-O-$CH_3$), 1328 (C=S), 1377 (C-N), 1514 (C=N), and 3419 ($NH_2$). $^1$H-NMR (DMSO-*d6*, 400 MHz): 2.94 (t, 2H, <u>$CH_2$</u>–$CH_2$), 3.15 (t, 2H, $CH_2$–C<u>$H_2$</u>), 3.95 (s, 3H, O–$CH_3$), 5.92 (s, 2H, $NH_2$ thiadiazole), 6.99 (s, 2H, $NH_2$ thioamide), 7.23-7.82 (m, 6H, Aromatic), 8.19 (s, 1H, C<u>H</u> of pyrazole).

***5 - ( 1- ( 2 , 4 – dinitrophenyl ) – 3 - ( 2 - ( 6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazol-4-yl)-1,3,4-thiadiazol-2-amine (5c),***

Deep brown precipitate (79% yield); mp 187–191°C; IR (KBr) v ($cm^{-1}$): 1321 (C-O-$CH_3$), 1399 (C-N), 1512 (C=N), and 3316 ($NH_2$). $^1$H-NMR (DMSO-*d6*, 400 MHz): 2.96 (t, 2H, <u>$CH_2$</u>–$CH_2$), 3.11 (t, 2H, $CH_2$–C<u>$H_2$</u>), 3.95 (s, 3H, O–$CH_3$), 6.16 (s, 2H, $NH_2$ thiadiazole), 7.16-8.07 (m, 9H, Aromatic and pyrazole), 8.91 (s, 1H, C<u>H</u> of pyrazole).

***Computational method***

"Figure. 2" illustrates the methods used in this study. The fully licenced CCDC genetic optimization for ligand docking (GOLD) Suite (v. 2022.1) was used to carry out ligand docking investigations on the drugs. The protein and ligands were visualized, as well as hydrogen bonding interactions, short contacts, and bond lengths, using the CCDC Hermes visualizer program (version 2022.1). Using the ChemBioOffice program (version 20.1), the ligands' chemical structures were produced. The Swiss ADME server (http://www.swissadme.ch/) was used to estimate the pharmacokinetic profile, including absorption, distribution, metabolism, and excretion (ADME), of the synthesized ligands [24].

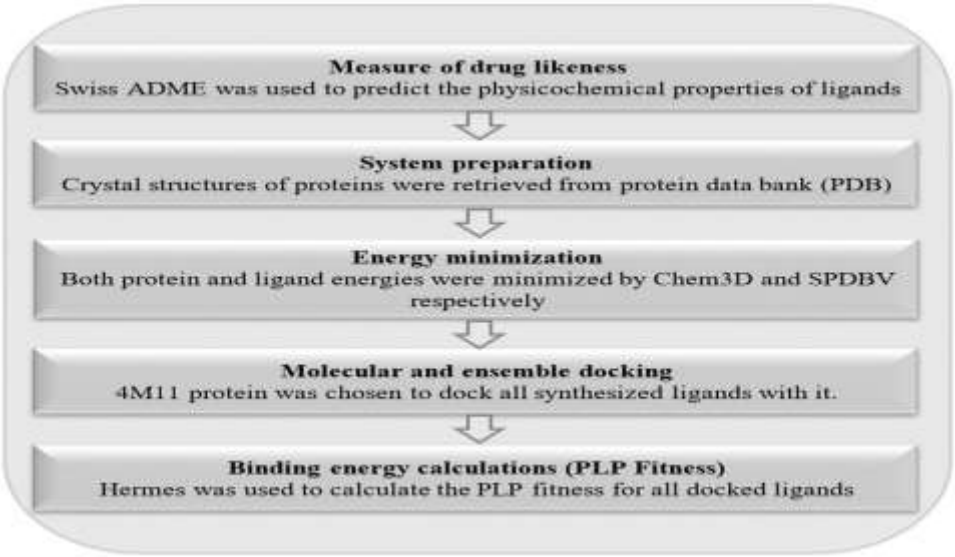



**Figure. 2 The computational protocol employed in this study [7].**

***ADME procedure***

The ligands (1-5c) were created using ChemSketch (v. 14) and converted into SMILE format using the Swiss ADME tool. This tool was used to predict the physicochemical descriptors and pharmacokinetic properties of the ligands. Additionally, the lipophilicity and polarity of the small molecules were computed using the BOILED-EGG software [25].

***ligands and protein receptor preparation***

The Protein Data Bank was used to get the crystal structures of the COX 2 enzyme (4M11) and the COX 1 enzyme (3N8Z). Swiss Protein Data Bank Viewer (v.3.8) was used to supplement the structures with any missing atoms. In addition, the tautomeric states and ionization of the amino acid residues were corrected by eliminating all water molecules and replacing them with hydrogen atoms. MM2 force field and CheBio3D (version 21.0.0) were used to find the lowest possible energy for our synthesized compounds [26].

***Docking procedures***

The molecular docking was performed using GOLD (v. 2022.1), a fully licensed version of the program [27, 28]. Hermes, a visualizer program included in the GOLD Suite, was used to prepare the receptors for docking. Protein residues within a 10-radius of the reference ligands acquired from the downloaded protein structure complexes were considered to be part of the binding region for GOLD ensemble docking (GED). Five different COX-2 proteins (1pxx, 4m11, 3LN1, 3KK6, and 5kIR) were used in the docking procedure; 4m11 was chosen for the docking research of the compounds [29]. Using CCDC Superstar software, we were able to locate the cavity and active site. To guarantee that the active site radius was in agreement with the size of the reference ligand in the protein structure, it was fixed at 10. The configuration template was ChemScore kinase, and the scoring function was the ChemPiecewise linear potential (CHEMPLP). During docking, we utilized the default settings for all parameters, and the CHEMPLP fitness function was used to evaluate each solution. Our synthesized ligands were docked with the COX-2 and COX-1 proteins, and the docked pose, binding mode, and binding free energy were evaluated to determine the degree of interaction between the amino acid residues of the proteins and the ligands [30].

***Anti-inflammatory Study***

The National Center for Drug Control and Research donated albino rats (155±10 g) that were maintained in the animal house at the Baghdad College of Medical Sciences / Pharmacy Department. Animals were fed commercial chaw and allowed unrestricted access to water. There was a total of eight groups created, each including six albino rats.(Group 1) Six control rats were injected intraperitoneally with a vehicle (propylene glycol, 50% v/v).

(Group 2) Nabumetone, the standard reference drug, was administered into a second set of six rats at a dosage of 15 mg/kg (i.p.) in propylene glycol 50% (v/v).

Six rats per group (3–8) were intraperitoneally injected with the compounds of interest (4a–5c). The solvent utilized was propylene glycol.

## Results and Discussion

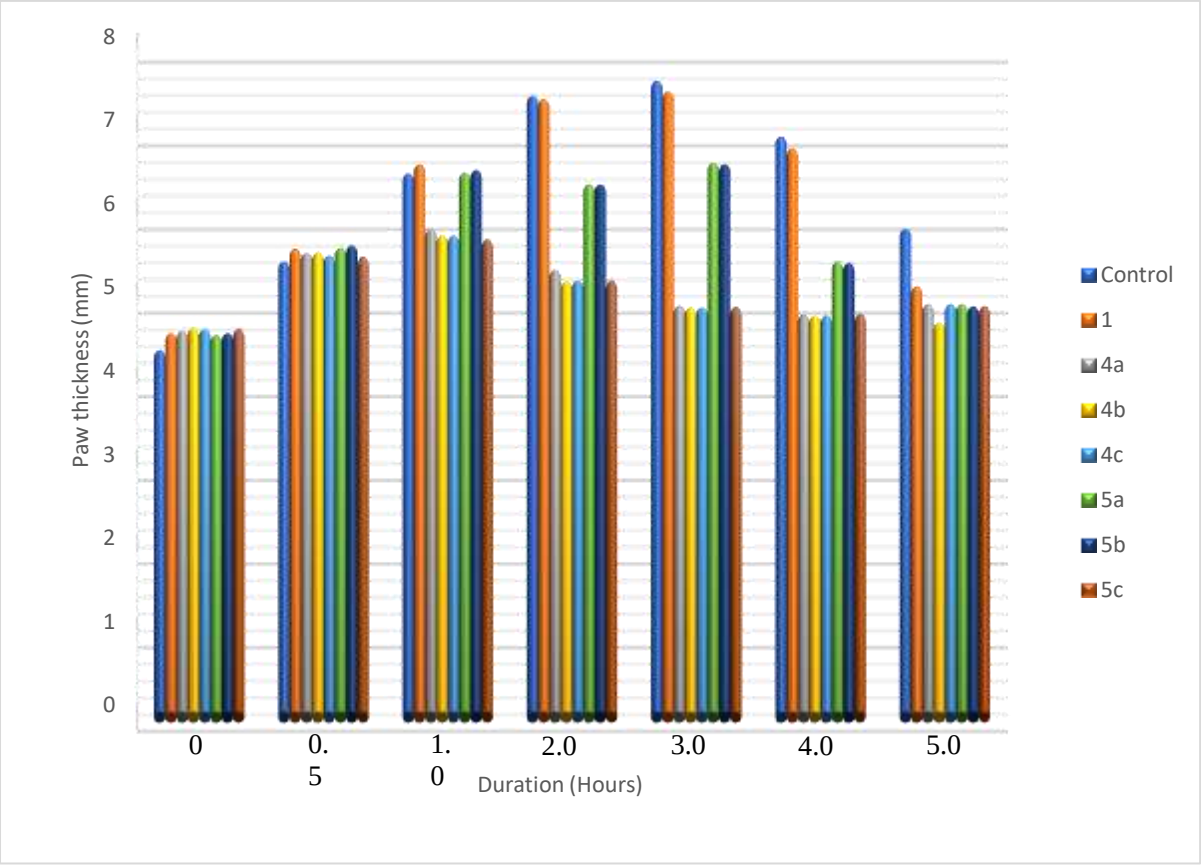
***Anti-inflammatory***

The paw-edema technique has utilized a variety of irritating substances. such as carrageenan solution, dextran, and egg white. The rat's hind paw injected with egg white interplanetary causes a persistent edema. In order to validate the evaluation method for newly synthesized anti-inflammatory compounds, Nabumetone, a reference compound with a known anti-inflammatory activity profile, was used. The results of this evaluation can be found in “Table 1” and “Figure.3”. Significantly different outcomes among the various tested agents were indicated by non-identical superscripts (a, b, and c) with a significance level of $p < 0.05$. Numbers are presented as mean SEM in mm of paw width. Rats are counted as n. The time of testing compounds i.p. injection is time (0). The injection of egg whites occurs at time [30].

***Molecular Modeling***

GOLD, which stands for "genetic algorithm for docking flexible ligands into protein binding sites," is a widely validated software tool [31]. It has demonstrated excellent performance in pose prediction and virtual screening [32]. The GOLD Suite includes additional software components, such as Isostar, Conquest, Hermes, GoldMine. and Mercury,Energy minimization is performed for both the ligands and proteins to correct any biased geometries and relieve internal constraints. Through energy minimization, the geometry is optimized to achieve a state of minimal energy.To evaluate the selectivity and binding energies of the synthesized compounds (1-5c) for both COX-1 and COX-2 enzymes, docking studies were performed using the GOLD Suite software. The primary goal of these studies was to identify the molecular interactions between the synthesized materials and the active binding sites of the protein targets. PLP fitness ratings, which reflect complex formation at the active sites, and subsequent COX-2 enzyme inhibition were used to rank compounds 1-5c, 6MNA, diclofenac, and naproxen. COX-1 PLP fitness values were between 49.36 and 72.47, while COX-2 PLP values were between 62.35 and 92.09 “Table 2”.The docked results exhibited excellent agreement with the experimental results from in vivo studies. Ensemble docking was employed as an essential step, utilizing five different COX-2

proteins. This approach reduces the risk of selecting an unsuitable protein model and improves pose prediction, enrichments in virtual screening, and ensures the accuracy of the docking process.The results of the docking study demonstrated that the synthesized compounds interact with the enzyme through hydrogen bonds and short interactions involving specific amino acid residues. These residues, namely Val89, Val116, Arg120, Leu345, Val349, Tyr355, Tyr385, Trp387, Val523, Gly526, Ala527, Ser530, Leu531, Leu534, and Leu539, are visually depicted in Figure. 1 and their corresponding identities are provided in "Table 2".The GOLD program is used for the computation of proximity distances and hydrogen bonding interactions between designated protein atoms and the ligands that we have produced. Bonds having lengths that are less than 3Å are regarded as being of interest. Short-range interactions comprise a diverse array of interacting forces, including as van der Waals, electrostatic, steric, pi-pi stacking, dipole-dipole, and other similar forces [33].The synthesized ligands provide encouraging COX-2 enzyme docking findings., fitting well into the active site of COX-2 as shown in "Table 3". The binding energy for COX-1 is lower compared to COX-2 due to the larger size of the COX-2 active site, making it challenging for the synthesized compounds with larger structures to insert into the COX-1 enzyme pocket.Compounds 3c and 4c exhibit hydrogen bond interactions with the amino acids Arg120 and Tyr355, which are both necessary for the binding of the five NSAIDs (Ibuprofen, Naproxen, Indomethacin, Flurbiprofen, and Des-methylflurbiprofen) that have been authorized for treatment of inflammatory diseases. With Ser530, the binding site for lumiracoxib, tolfenamic acid, and diclofenac, compounds 3c and 5c form hydrogen bonds.The best docking score (PLP Fitness) has been recorded for compound 3c, which was 91.35, also all compounds with di nitrobenzene derivative has been shown a good binding affinity for the receptor, this is because the conformation of these compounds fitted with the active site of the COX-2 receptor and bind to the Arg120 and tyr355.




*Significantly different compared with control agent ($p < 0.05$).

**Figure. 3 Effect of propyleneglycol, Nabumetone, compounds (4a, 4b, 4c, 5a, 5b, and 5c) on egg-white provoked paw edema in rats.**

**Table 1. Using an egg-white produced paw edema model in rats, the anti-inflammatory effects of the synthesized compounds (4a-5c) were compared to those of Nabumetone and a control group.**

| Compounds | | Time (min) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **0** | **30** | **60** | **120** | **180** | **240** | **300** |
| Paw Thickness (mm) / n=6 | **Control** | 4.36 ±0.03 | 5.42 ±0.02 | 6.47 ±0.03 | 7.40 ±0.04 | 7.58 ±0.05 | 6.91 ±0.04 | 5.81 ±0.03 |
| | **1** | 4.56 ±0.01 | 5.57 ±0.02 | 6.58 ±0.04 | 7.36 ±0.03 | 7.45 ±0.05 | 6.77 ±0.04 | 5.12 ±0.03 |
| | **4a** | 4.59 ±0.05 | 5.52 ±0.06 | 5.81 ±0.05*[a] | 5.32 ±0.05*[a] | 4.89 ±0.03*[a] | 4.79 ±0.06*[a] | 4.91 ±0.04*[b] |
| | **4b** | 4.63±0.03 | 5.53 ±0.06 | 5.73 ±0.05*[a] | 5.18 ±0.09*[a] | 4.87 ±0.03*[a] | 4.77 ±0.05*[a] | 4.69 ±0.03*[a] |
| | **4c** | 4.61 ±0.03 | 5.49 ±0.05 | 5.73 ±0.06*[a] | 5.19 ±0.04*[a] | 4.87 ± 0.03*[a] | 4.77 ±0.06*[a] | 4.91 ±0.03*[b] |
| | **5a** | 4.54±0.02 | 5.58 ±0.03 | 6.48 ±0.04 | 6.34 ±0.05*[c] | 6.60 ±0.03* | 5.42 ±0.03*[c] | 4.91 ±0.02*[b] |
| | **5b** | 4.56±0.02 | 5.61 ±0.03 | 6.51 ±0.02 | 6.34 ±0.04*[c] | 6.58 ±0.04* | 5.40 ±0.01*[c] | 4.88 ±0.04*[b] |
| | **5c** | 4.62 ±0.04 | 5.48 ±0.03 | 5.69 ±0.05* | 5.20 ±0.06*[a] | 4.88 ±0.06*[a] | 4.80 ±0.04*[a] | 4.89 ±0.05*[b] |

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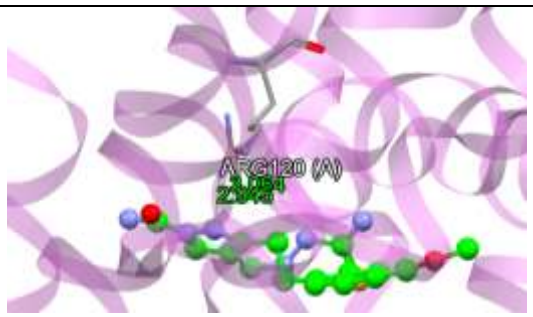
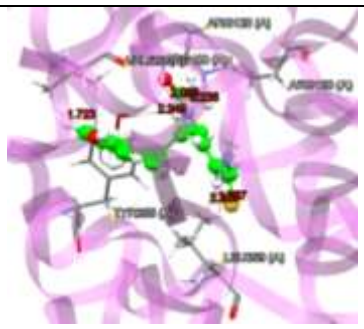
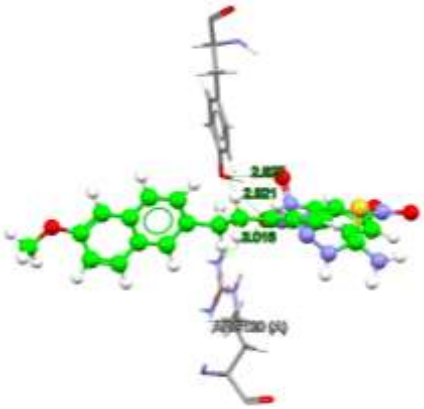
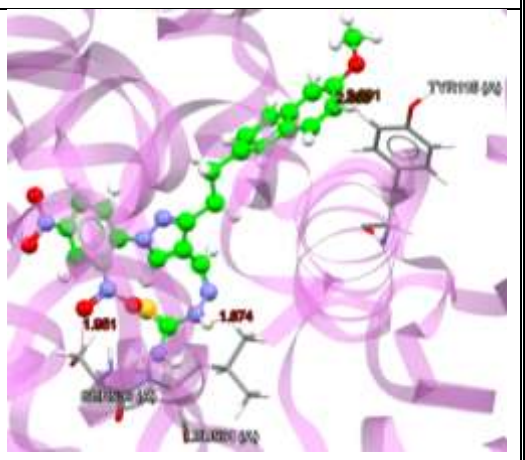
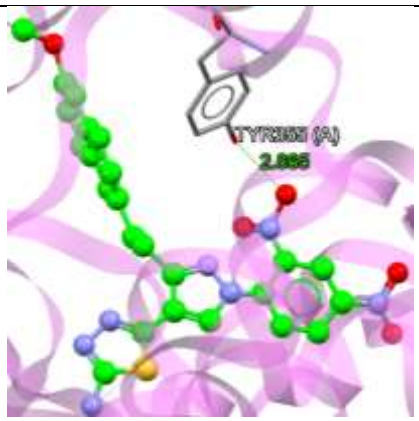
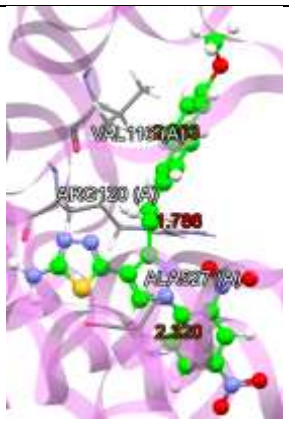
**Table 2. The present table investigates the binding energies of Nabumetone derivatives and reference nonsteroidal anti-inflammatory drugs (NSAIDs) when docked with cyclooxygenase-2 (COX-2) and cyclooxygenase-1 (COX-1).**

| Compounds | COX-2 Binding Energy (PLP Fitness) | Amino Acids Included in H-bonding | Amino Acids Included in Hydrophobic Interactions | COX-1 Binding Energy (PLP Fitness) |
|---|---|---|---|---|
| 1 | 60.22 | Tyr355, Arg120 | Gly526, Val523, Trp387, Arg120, Tyr355 | 67.36 |
| 4a | 85.06 | Arg120 | Val 523, Arg120, Leu359, tyr355 | 60.08 |
| 4b | 84.56 | Arg120, Ala 527 | Leu359, Val349, Val116, Ala527, Arg120, Tyr355, Trp387, Leu384, Gly526, Val523 | 59.87 |
| **4c** | **89.66** | Ser530, Tyr115 | Leu531, Ser530, Tyr115, | 51.79 |
| 5a | 74.32 | Tyr355, Arg120 | Val116, Ala527, Arg120, Tyr355, Gly526, Leu384, Trp387, | 60.38 |
| 5b | 72.47 | Arg120 | Ile345, Leu531, Val349, Ala527, Val523, Trp387 | 61.89 |
| **5c** | **92.09** | Tyr355 | Val116, Ala527, Tyr355, Arg120 | 49.32 |
| Diclofenac | 71.7 | Ser530, Tyr385 | Ala527, Val349, Gly526, Trp387 | 68.60 |
| Naproxen | 74.23 | Arg120, Tyr355 | Ser530, Ala527, Gly526, Val349, Leu352, Val523 | 63.12 |

**Table 3. 3D structure of some synthesized compounds* binding to active amino acids.**

| | H-Bond | Short Contact |
|---|---|---|
| **4a** |  |  |
| **4c** |  |  |
| **5c** |  |  |

* Compounds in ball and stick style and amino acid residues in capped stick style.

## Conclusions

The final products were successfully synthesized, characterized, and evaluated for their biological activity as anti-inflammatory candidates. FT-IR spectroscopy, and 1H-NMR spectroscopy were all used to identify and describe the synthesized compounds. Docking studies exhibited excellent agreement with the results from in vivo experiments. A preliminary study on the anti-inflammatory activity indicated that cCompound (5c) showed significantly greater anti-inflammatory effects compared to other compounds and standards. The synthesized compounds exhibited significantly reduced side effects in comparison to the reference drugs.

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## Ethics Statements

This study was approved by the scientific and ethical committees of the College of Pharmacy University of Mustansiriyah.

## Conflict of interest

The authors declare that there is no conflict of interest.

## Author contributions

The authors contribute in practical procedures, biostatistics sand writing of this study.

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# سلسلة جديدة من مشتقات البايرازول و الثياديازول تحمل جزيئة النابيوميتون: التصميم ودراسة الالتحام الجزيئي والتوليف والتوصيف والتقييم الدوائي الأولي.

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**الخلاصة**

تم تصميم لسلسلة من مركبات البيرازول والثياديازول الجديدة بما في ذلك جزء نابوميتون ، وتم إنتاجها وإخضاعها لتقييم قدرتها المضادة للالتهابات ضد إنزيم السيكلواوكسيجينيز- ٢. بعد اختبارها بجهاز الكومبيوتر الذي يتضمن تحليل الالتحام الجزيئي ، حيث تم تصنيع أكثر مجموعة المركبات الواعدة و تشخيصها. بعد التنبؤ بنشاطها من خلال دراسة الالتحام الجزيئي باستخدام أداة برنامج Cambridge Crystallographic Data Centre (GOLD)، قد تمت دراسة الفعالية الحيوية لها كعوامل مضادة للالتهابات باستخدام إجراء بياض البيض أيضا عن طريق الحاسوب. أعطى ارتباط الهيدروجين مع الأحماض الأمينية الرئيسية في إنزيمات السيكلواوكسيجينيز- ٢ ، بما في ذلك ارجنين ١٢٠ و تايروسين ٣٥٥ و سيرين ٥٣٠ ، المركبات التي تم فحصها في الالتحام الجزيئي نشاطًا أعلى بكثير من الأدوية المرجعية نابروكسين و الدايكلوفيناك و ٦ .MNAكانت البيانات التي تم الحصول عليها من دراسات الالتحام مرتبطة بشكل كبير بتلك التي تم الحصول عليها من الاختبار في الجسم الحي حيث تم اختبار المركبات المصنعة حديثًا بشكل حقيقي كعوامل مضادة للالتهابات باستخدام إجراء بياض البيض. أظهرت المركبات ٣ gc و ٤ gc و ٥ cلياقة 91.35 PLP و ٨٩,٦٦ و ٩٢,٠٩ على التوالي ، وهي أفضل الدرجات من جميع المركبات المركبة. قدم هذا البحث توجيهًا مفيدًا لتحديد مركبات البيرازول والثياديازول الجديدة المضادة للالتهابات .

**الكلمات المفتاحية: الدوائية، الالتحام الجزيئي، GOLD، السيكلواوكسيجينيز، نابوميتون.**