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Molecular Docking and Pharmacokinetic prediction of Novel Thiadiazole-Based Anti-inflammatory Derivatives

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Abstract**—***The most commonly prescribed pharmaceuticals worldwide for the management of pain and inflammation are non-steroidal anti-inflammatory medicines (NSAIDs), but they cause GIT side effects such as gastric irritation and sometimes bleeding in chronic uses. Modifying popular non-selective NSAIDs is one of the primary approaches used to create new medications. Derivatization of the carboxylate function group of typical traditional NSAIDs such as acetylsalicylic acid and diflunisal had been found in several trials to offer the dual benefits of lowering gastrointestinal side effects and increasing anti-inflammatory activity. The four novel thiadiazol derivatives nonsteroidal anti-inflammatory compounds had been designed and synthesized in order to increase anti-inflammatory activity and decrease gastrointestinal side effect, that confirmed by docking of the target compounds to cox-1 and cox-2 enzymes. 2–Amino–5 Methyl–1,3,4–thiadiazole, 2–Amino–5 Methylsulfany–1,3,4–thiadiazole moieties were conjugated with acetylsalicylic acid and diflunisal to produce compounds (1-4) successfully via a synthetic pathway involving anhydride formation, nucleophilic acyl substitution, and alkaline hydrolysis, then confirmed by IR and 1H-NMR spectroscopy. Followed by docking to cox-1 & 2 enzymes as a target receptors by using software effective MOE program, then a pharmacokinetic predictable study perfomed by using SwissADME (PreADMET web tool). Molecular docking studies revealed that all synthesized compounds exhibited good binding affinity for the COX-2 active site. Furthermore, in silico pharmacokinetic profiles for compounds (1-4) were predicted using the PreADMET web tool. The results indicated a favorable pharmacokinetic profile for the three of compounds (2,3,4), they showed a high potential for oral bioavailability and weak blood-brain barrier (BBB) penetration. Compound 3 characterized by weak BBB penetration, high human intestinal absorption, good permeability via intestinal barrier, low plasma protein binding, and no P-glycoprotein (P-gp) inhibition, the higher binding affinity, and the binding mode similar to selective COX-2 inhibitors, and slightly inhibiting cox-1 enzyme so that, Compound 3 a promising as preferential selective anti-inflammatory candidate drug.*

Keywords**—** *NSAIDs, Acetylsalicylic acid, Diflunisal, Molecular docking, Swiss ADME.*

I. INTRODUCTION

Non-steroidal anti-inflammatory drugs NSAID's (NSAID's) are among one of the most widely prescribed and form cornerstone of the symptomatic treatment of fever, pain, and inflammation [1]. NSAID's effect on prostaglandin synthesis pathways by inhibiting of two cyclooxygenase enzymes with differences in selectivity [2-3].

Two COX isoforms, COX-1 and COX-2, have been described in human. COX-2 and COX-1 isoforms share approximately 65% similarity, however, the most significant variation between them, which allows for its selective inhibition, is the substitution of isoleucine at site 523 in COX-1 with valine in COX-2 which permit inhibitors better access into its side-pocket [4-6]. COX-1, expressed constitutively in most cells have housekeeping functions, such as gastric epithelial cytoprotective and homeostasis. In other hand, COX-2 is inducible which is activated in inflammatory cells by an inflammatory stimulus and is involved in the inflammatory processes [7].

NSAIDs can be classified by their selectivity for these isoforms into three groups; traditional NSAID's, Oxicam's and COXIB's. Traditional NSAID's inhibit both COX-1 and COX-2, which can lead to gastro-intestinal (GI) adverse events [2,8]. In an effort to minimize the gastrointestinal toxicity, through new group that known as preferential selective cox inhibitors, and COXIB's are developed, However, COXIBs have shown cardiac adverse events such as arterial hypertension, myocardial infarction, stroke, and heart failure [9]. As result, there is a pressing need to develop new anti-inflammatory to attenuate the safety and tolerability concerns and optimize the selectivity of NSAIDs. One strategy is based on derivatization of the carboxylate group of traditional NSAIDS such as aspirin and diflunisal to form hybrid molecules by using DCC as coupling agent that conjugated with suitable heterocyclic ring to form preferential selective cox inhibitors that characterized by less GIT side effects and more anti-inflammatory activity [10].

The molecular hybridization is a powerful strategy of rational design of new drugs [11]. hybrid NSAID conjugates incorporating traditional NSAIDs and 1,3,4-thiadiazole demonstrate minimal adverse events as result of masking of carboxylic group which is partly responsible for the direct and less indirect gastric toxicity [10,12]. 1,3,4-thiadiazole is an important heterocyclic ring system that exhibit a range of pharmacological activities, including Anti-inflammatory [13], Antiviral [14], Antihyperglycemic [15], Antimicrobial [16], Antioxidant [17], and. Several studies have focused on synthesizing thiadiazole-based COX-2 inhibitors and have concluded that 1,3,4-thiadiazole is COX pharmacophore

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scaffold, so hybrid traditional NSAID's with thiadiazole could optimize anti-inflammatory activity [18].

The sope of this work, to design and synthesis of derivatives of hiadiazole that conjugated with acetylsalicylic acid and diflunisal to change their effect from nonselective COX inhibitor to preferential selective COX inhibitors, such as aceclofenac and their in-silico properties [19].

II. Chemical and Method

Chemicals: Diflunisal powder, was kind gift from Ram Pharmaceutical Industry Jordan.

Acetylsalicylic acid crystalline powder was purchased from Modern Yemeni Pharma.

2–Amino–5 Methyl–1,3,4–thiadiazole and 2–Amino–5 Methylsulfany–1,3,4–thiadiazole were purchased from Aldrich Chem. Co. Germany.

N,N–Dicyclohexylcarbodiimide (DCC) was obtained from Acros USA.

Dicholromethane AR 99.5% was from chem.–Supply PTV, Italy.

Equipments: Melting points were determined by using a calibrated STUART SMP11 (U.K.) melting apparatus.

Infrared (IR) Spectra, were recorded using KBr disc, by using Shimadzu FT– (8101 IR) Spectroscopy (Japan).

Nuclear Magnetic Resonance (^{1}HNMR) Spectra on Mercury 300 MHz ^{1}HNMR spectrometer (δ PPM), Aldenmark.

Chemical Synthesis of: 2-hydroxy-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzamide (1).

Acetylsalicylic acid (l0 g, 55.5 mmol) was dissolved in 150 ml methylene chloride, dicyclohexylcarbodiimide (5.72 g, 27.7 mmol) was added. The reaction mixture was continuously stirred at room temperature for 3 hr, a white precipitate of dicyclohexylurea was formed and removed by filtration, the solvent was evaporated under vacuum, and an oily product was formed to yield anhydride compound A1.

Compound A1 (5 g, 14.60 mmol) , 2–amino–5–methyl–1,3,4–thiadiazole (1.681 g, 14.60 mmol), zinc dust (0.013 g), glacial acetic acid (1.4 ml, 24.481 mmol), and dioxane (50 ml) were placed in 100 ml round bottom flask, equipped with reflux condenser, boiling stones were added. The reaction mixture was refluxed for about 2hr with continuous stirring, and the reaction was checked by TLC to make sure that completion of reaction. The solvent was evaporated under vacuum, and the residue was dissolved in chloroform, washed, with $NaHCO_3$ (10%, 3X), Hcl (1N, 3X), and 3 times with distilled water, filtered over anhydrous sodium sulphate. The filtrate was evaporated and the residue was redissolved in ethyl acetate and filtered. The recrystallization was carried out by adding petroleum ether (60–80 ^{o}C) on the filtrate until turbidity occurred and kept in cold place over night. Then the mixture was filtered while it is cold and the precipitate was collected to give compound A2.

Compound A2, (10 mmol) was dissolved in minimum volume of ethanol (95%). The solution was cooled to 18 °C, and then sodium hydroxide (6 ml, 12 mmol, 2N) was added drop wise, with continuous stirring, over a period of about 30minutes. Stirring was continued at 18 °C, during which the reaction mixture was checked by TLC, until the disappearance of methylester of compound (A2), that indicating a complete alkaline hydrolysis. Then the reaction mixture was acidified with Hcl (6 ml, 12 mmol, 2N), excess of cold water was added and the crude phenolic compound was precipitated. TLC showed a single spot [20]. The recrystallization was carried out by using ethanol and water to liberate target compound 1, the percent yield 54.5%.

Identification of compound (1): Milting pint (MP) was `263–266 ^{0}C.

FT-IR spectra (KBr, Cm-1) a strong stretching vibration in region (3250 cm–^{1} represent (N – H). Slightly broad phenolic (OH) stretching vibration in region (3600–3350 cm^{-1}). A bands in regions (1200, 1325 cm^{-1}) representing phenolic (C – O) bending vibration. The presence of strong sharp band in region (1675 cm^{-1}) representing the carbonyl (C = O) of secondary amide group. The presence of small sharp band in region (2900 cm^{-1}) representing stretching vibration of hydrogen (C – H) methyl group.

^{1}H–NMR Spectroscopy, (DMSO-d6) δ ppm spectral, Phenyl: 7.93 (1H, d, proton) at 6 position,) 7.47 (1H, m, proton) at 3 position 7.3 (2H, m, proton at 4 and 5 position. Aliphatic: amide, 4.12 (1H, bs) exchangeable with D_2O, 2.5(3H, s of CH3).

Chemical synthesis of: 2-hydroxy-N-[5-(methylsulfanyl)-1,3,4-thiadiazol-2-yl] benzamide (2).

Compound A2, (5 g, 14.6 mmol), 2–amino-5-(methylthio)–1,3,4–thiadiazolyl (1.895 g , 14.60 mmol), zinc dust (0.014 g), glacial acetic acid (1.4 ml, 24.481 mmol), dioxane (50 mL), then follows as described before in synthesis of compound 1 to give Compound 2, the percent yield 59.4%.

Identification of compound (2): Milting pint (MP) was `272–276 ^{0}C. FT-IR spectra (KBr, Cm-1) The presence of strong sharp band in region (1675 cm^{-1}) representing the carbonyl (C = O) of secondary amide group. A strong stretching vibration in region (3250 cm–^{1} represent (N – H). Slightly broad phenolic (OH) stretching vibration in region (3600–3350 cm^{-1}). A bands in regions (1200, 1325 cm^{-1}) representing phenolic (C – O) bending vibration.

^{1}H–NMR Spectroscopy, (DMSO-d6). in δ (PPM; Aromatic: 7.93 (1H, d, proton) at 6 position 7.47 (1H, m, proton) at 3 position 7.3 (2H, m, proton at 4 and 5 position. Amide COH 4.14 (1H, bs) exchangeable with D_2O, 2.41 (3H, s of SCH3).

Chemical synthesis of: 4'-difluoro-4-hydroxy-N-(5-methyl-1,3,4-thiadiazol-2-yl)-[1,1'-biphenyl]-3-carboxamide (3).

To a dry Diflunisal (l0 g, 40 mmol), excess acetic anhydride (25 ml, 262 mmol), and 5 drops of conc. sulfuric acid was added drop wise, and mixed the contents by rotating the conical flask, warmed on water bath about 50–60 ^{o}C, with stirring for 30 min.

The reaction mixture was allowed to cool with occasional stirring, then cold distilled water was added the precipitate formed, filtered washed by cold distilled water several times, the crude product was collected. And recrystallized from ethanol 95%, the precipitate was collected and dried to give Compound A3.

Compound A3, (10 g, 34.22 mmol) was dissolved in (160 ml) methylene chloride, dicyclohexylcarbodiimide (3.53 g, 17.11

mmol) was added. The reaction mixture was, stirred at room temperature for about 3hr. A white precipitate of dicyclohexylurea was formed and removed by filtration. The solvent was evaporated under vacuum, a solid product was obtained to yield compound A4.

Compound A4, (5 g, 8.82 mmol), 2–amino–5–methyl–1,3,4– thiadiazole (1.016 g, 8.82 mmol), zinc dust (0.008 g), glacial acetic acid (0.85 ml, 14.864 mmol), dioxane (60 ml. The reaction mixture was refluxed for about 2hr, with stirring, the reaction was checked by TLC to make sure that completion of the reaction, The solvent was evaporated under vacuum, and the residue was dissolved in ethyl acetate, washed with, $NaHCO_3$ (10%, 3X), Hcl (1N, 3X), 3 times with distilled water, filtered over anhydrous sodium sulphate. The filtrate was evaporated and the residue was redissolved in ethyl acetate and filtered. The crystallization was carried out by adding petroleum ether (60–80°C) on the filtrate until turbidity occurred and kept in cold place over night. Then the mixture was filtered while it is cold and the precipitate was collected to give compound A5.

Compound A5, (10 mmol) was dissolved in minimum volume of ethanol (95%). The solution was cooled to 18 °C, and then sodium hydroxide (6 ml, 12 mmol, 2N) was added drop wise, with continuous stirring, about 30min. Stirring was continued at 18°C, during which the reaction mixture was checked by TLC, until the disappearance of methyl ester of compound A4, that indicating a complete alkaline hydrolysis. Then the reaction mixture was acidified with HCl (6 ml, 12 mmol, 2N), excess of cold water was added and the crude phenolic compound was precipitate [21,22]. TLC showed a single spot. The recrystallization was carried out by using ethanol to liberate compound3, percent yield **%**51.6 .

Identification of compound (3): MP was 289-291^{0}C. FT-IR spectra (KBr, Cm-1): a phenolic (OH) stretching vibration in region (3600–3350 cm^{-1}). A strong stretching vibration in region (3250 cm–^{1} represnt (N – H). A bands in regions (1200, 1325 cm^{-1}) representing phenolic (C – O) bending vibration.The presence of small sharp band in region (2900 cm^{-1}) representing stretching vibration of hydrogen (C – H) methyl group . The presence of strong sharp band in region (1675 cm^{-1}) representing the carbonyl (C = O) of secondary amide group.

^{1}HNMR (DMSO-d6) δ ppm, difluorophenyl: 8.1 (1H, s, proton) at 3–position 7.66 (1H, d, proton), at 5–position 7.2 (1H, d, proton) at 6–position. Phenyl: 7.65 (1H, s, proton) at position–6 7.32 (1H, d, proton), at position–3 ,7.18 (1H, d, proton) at position–4. amide -CON 3.19 (1H, bs) exchangeable with D2O, CH3 2.6 (3H, s).

Synthesis of: 4'-difluoro-4-hydroxy-N-[5-(methylsulfanyl)-1,3,4-thiadiazol-2-yl]-[1,1'-biphenyl]-3-carboxamide (4).

Compound A4, (5 g, 8.82 mmol), 2–amino-5-(methylsulfanyl)–1,3,4–thiadiazole (1.298 g , 8.82 mmol), zinc dust (0.008 g), glacial acetic acid (0.85 mL, 14.864 mmol), dioxane (60 mL), then follow as described before in compound 3 to give Compound 4, Percent yield 57%.

Identification of compound ([4): MP. Was 281–282 C. FT-IR (KBr, Cm-1) 3500 (OH), 3150 (NH, amide), 3050(CH, ArH), 1650(C=O), 1610, 1540, 1450 (C=C,Ar).

^{1}H–NMR (DMSO-d6) δ ppm: 2.5 (s, 3H, SCH3, thiadiazolyl), 5.65 (br, 2H, OH, CONH, exchangeable with D2O), 7.45, 7.33 (m, 3H, ArH), 7.50 7.69 (m, 2H, ArH2F2), 8.1, (s, 1H, ArHF2)

Methods of Molecular Docking and ADME

Chemdraw; A program for drawing chemical structures. It is also useful for obtaining basic information about the drawn structures, such as the name according to the International Union of Pure and Applied Chemistry (IUPAC), the molecular formula, and the molecular weight [23].

MOE (Molecular Operating Environment): An application developed by the Chemical Computing Group. It enables the performance of various computational studies, such as molecular docking, pharmacophore modeling, and 3D quantitative structure-activity relationship (3D-QSAR) studies [24].

Docking of Celecoxib or Diclofenac onto active site of COX-1 and COX-2 Enzymes

A molecular docking study was performed for celecoxib and diclofenac onto the active site of cyclooxygenase enzymes 1 and 2 (COX-1 and COX-2) as target receptors.

Ligand Drawing and Preparation.

The structures of celecoxib and diclofenac are drawn using Chemdraw and saved in MDL MOLFILE format (*.mol), adding protons and energy minimization of the molecule using the [25].

Receptor Preparation.

The three-dimensional structures of the studied biological targets are obtained from the Protein Data Bank (PDB). Then 3D protonation, correction of bonds and charges, identification and correction of errors in residues, followed by energy minimization of the corrected protein using the Amber99 forcefield [26].

The molecular docking study is performed on the following receptors for celecoxib, diclofenac, and for target compounds (1,2,3, and 4) [27,28].

Cyclooxygenase-1 (COX-1) receptor: The crystal structure 3KK6.

Cyclooxygenase-2 (COX-2) receptor: The crystal structure 3LN1.

Validation

The reliability of the applied method is validated by re-docking the ligand present in the crystal structure. The Root Mean Square Deviation (RMSD) between the docking-predicted pose and the crystallographic pose of the ligand is determined. An RMSD value of less than 2.0 Å was considered acceptable.

SwissADME

The pharmacokinetic prediction and drug-likeness properties of the investigated compounds were evaluated using the Swiss ADME web tool (http://www.swissadme.ch).

SwissADME is an indispensable tool in early drug discovery due to its ability to provide a rapid, comprehensive, and cost-effective in silico profile of compound libraries. Its key benefits include:

Computational Efficiency: It allows for the high-throughput screening of thousands of compounds in minutes, enabling the prioritization of the most promising candidates for costly and time-consuming in vitro and in vivo experiments.

Comprehensive Profiling: It integrates the prediction of fundamental physicochemical properties, pharmacokinetics (absorption, distribution, metabolism, excretion), and drug-likeness into a single platform.

Intuitive Visualization: It presents complex data through easily interpretable graphics, such as the BOILED-Egg plot, which facilitates quick decision-making.

The Topological Polar Surface Area (TPSA) polar atoms (oxygen, nitrogen, and attached hydrogens).

intestinal absorption and blood-brain barrier; compounds with a TPSA > 140 Å² are typically associated with poor intestinal absorption.

Lipinski's Rule of Five: Flags compounds that violate more than one of the following: molecular weight (MW) ≤ 500, Log P ≤ 5, number of hydrogen bond donors (HBD) ≤ 5, and number of hydrogen bond acceptors (HBA) ≤ 10 ,[29].

PreADMET

Predictions of key absorption, distribution, metabolism, excretion, and toxicity.

permeability properties for the investigated compounds were performed using the preADMET (https://preadmet.bmdrc.kr/) web-based tool. This in silico method is widely used in early drug discovery to estimate the pharmacokinetic profile of molecules prior to costly laboratory experimentation. The chemical structures of the compounds were input, and the program's algorithms were used to calculate the following critical parameters:

Human Intestinal Absorption (HIA) [30] .

The absorption of a compound in the human intestines can be predicted by calculating the HIA%, which is the percentage of the compound absorbed in the human intestine at pH 7.4. Compounds are generally classified as:

poor absorption (HIA% = 0-20%), moderate absorption (HIA% = 20-70%), good absorption (HIA% = 70-100%).

Plasma Protein Binding (PPB) [31].

Compounds are classified based on their PPB% value as either highly plasma protein-bound (PPB > 90%) or weakly plasma protein-bound (PPB% < 90%).

Blood-Brain Barrier Penetration (BBB) [32].

A drug's ability to cross the blood-brain barrier (BBB) allows it to reach the central nervous system (CNS). It is measured by the following equation:

$$BBB = [Brain] / [Blood].$$

TABLE 1. Compounds can be classified according to their BBB permeability.

Classification	BB (Cbrain/Cblood)	logBB
High Penetration to CNS	more than 2.0	more than 0.3
Middle Penetration to CNS	2.0 ~ 0.1	0.3 ~-1.0
Low Penetration to CNS	less than 0.1	less than -1.0

Intestinal Permeability

Permeability across the gastrointestinal tract, most notably the Caco-2 cell permeability assay and the MDCK cell permeability assay. Caco-2 cells are colon adenocarcinoma cells that contain various transporters for drug transfer across the intestinal epithelium. Classification (PCaco-2 (nm/sec) Low permeability less than 4, Middle permeability 4 ~ 70, High permeability more than 70 [33].

Glycoprotein Inhibition (P-gp)

P-glycoprotein (P-gp) is a protein found in the cell membranes of many body cells. It functions as an efflux pump, transporting foreign compounds out of the cell. It is responsible for cancer cell resistance to chemotherapeutic drugs and is also known as Multi-Drug Resistance Protein (MDR). The PreADMET program predicts whether a compound is a P-gp substrate or inhibitor [34].

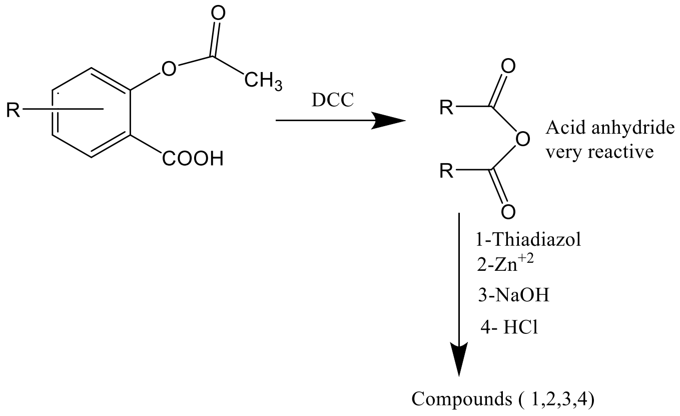
III. Result and Discussion

Chemistry

In this study, a novel 1,3,4–thiadiazols derivatives compounds 1-4 were designed and synthesized, and their predictable efficacy and pharmacokinetic behavior were caried out.

Compounds 1,(2-hydroxy-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzamide) and 2,(2-hydroxy-N-[5-(methylsulfanyl)-1,3,4-thiadiazol-2-yl]benzamide) were synthesized from acetylsalicylic acid via the formation of anhydride of methyl ester of acetylsalicylic in the presence of a coupling agent DCC, followed by the nucleophilic acyl substitution of the anhydride with the amino group of 1,3,4–thiadiazole using zinc as a catalyst in an acidic medium. The final step involved alkaline hydrolysis to obtain compounds 1 &2.

Compounds 3,(4'-difluoro-4-hydroxy-N-(5-methyl-1,3,4-thiadiazol-2-yl)-[1,1'-biphenyl]-3-carboxamide) and 4 (4'-difluoro-4-hydroxy-N-[5-(methylsulfanyl)-1,3,4-thiadiazol-2-yl]-[1,1'-biphenyl]-3-carboxamide) were synthesized from Diflunisal via the protection of the phenolic group of Diflunisal, formation of Diflunisal Anhydride in the presence of a coupling agent, followed by nucleophilic acyl substitution of the anhydride with the amino group of 1,3,4–thiadiazole using zinc as a catalyst in an acidic medium [35]. The final step involved deprotection of the phenol by alkaline hydrolysis as shown in scheme1.



Scheme 1, general pathway of chemical synthesis of compounds (1,2,3,4)

Results of Molecular Docking

Computational study of binding mode inside the active site of COX-2 enzyme

The investigation of potential anti-inflammatory of the synthesized compound 1-4, a three-dimensional structure of the compounds was docked into the active site of the target protein using the docking software's molecular operating environment (MOE) . The crystal structures of COX-2 were retrieved from the protein data bank (PDB) using the code (PDB id), which is 3LN1. The dominant orientation was opted on the bases of the S-score and the interaction in the active site of the protein.

For the purpose of validation, before carrying out the docking studies, the molecular docking protocol was validated by redocking of the co-crystallized Celecoxib back into the same active site of COX-2 and found to follow the same interaction pattern and results. the root-mean-square deviation (RMSD) value of co-crystallized Celecoxib was found 0.849 Å (≤2 Å), which reflects a good agreement between the experimental and predicted binding modes. the sulfonamide moiety in celecoxib shows a hydrogen bonding interaction with the important residues of Ser399, Leu338, Gln178 and Arg499, while Ala513 displayed a π-alkyl interaction in the active site of the target protein. Ser399, Leu338, and Arg499 constitute the side pocket of the COX-2 enzyme, and this pocket play a crucial role in enhancing selectivity towards COX-2 [36,37]. The interaction with Ala513 further increases the selectivity of celecoxib for COX-2 [38]. Figure 1A.

In other hand, the carboxylic acid group of diclofenac interacted with the COX-2 active site by forming hydrogen bonds with Met508 and Val509. Additionally, the chlorine atom substituted on the aromatic ring formed a hydrogen bond with Ser516. The hydrogen bond formed between diclofenac and Ser516 is one of the essential and fundamental interactions for its anti-inflammatory activity against COX-2 [39] Figure 1B.

The docking studies of tested compounds (1-4) showed docking scores (ranging from −6.16 to −8.04) that reflect a good binding of these compounds with the COX-2 enzyme. Based on 2D plot of the interactions of compounds 1-4 (figure 2A-D), it is very important to note that all the derivatives in 1,3,4-thiadiazole exhibited a high degree of selectivity, where 1,3,4-thiadiazole ring (COX-2 pharmacophore) explored a pi hydrogen interaction between the and Ser 339 (except compound 4 which bind with
Thr 79).

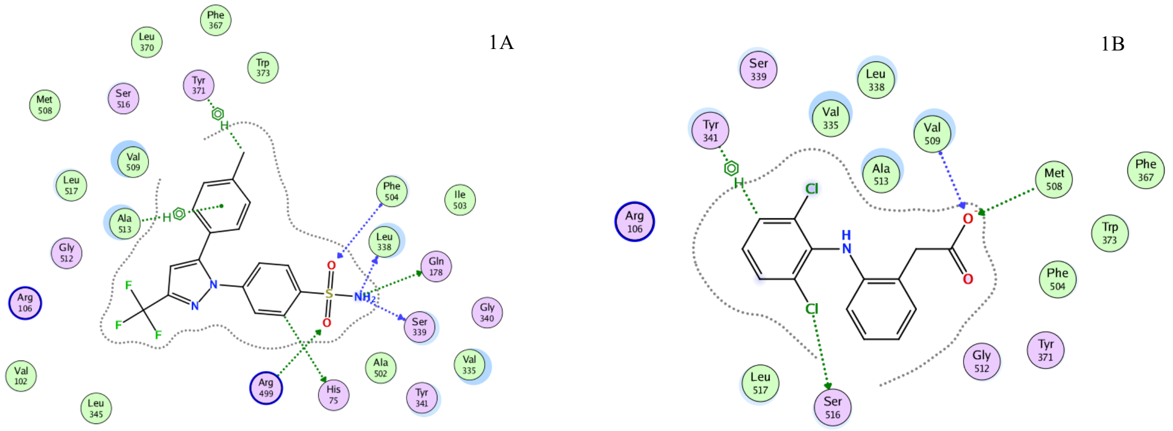
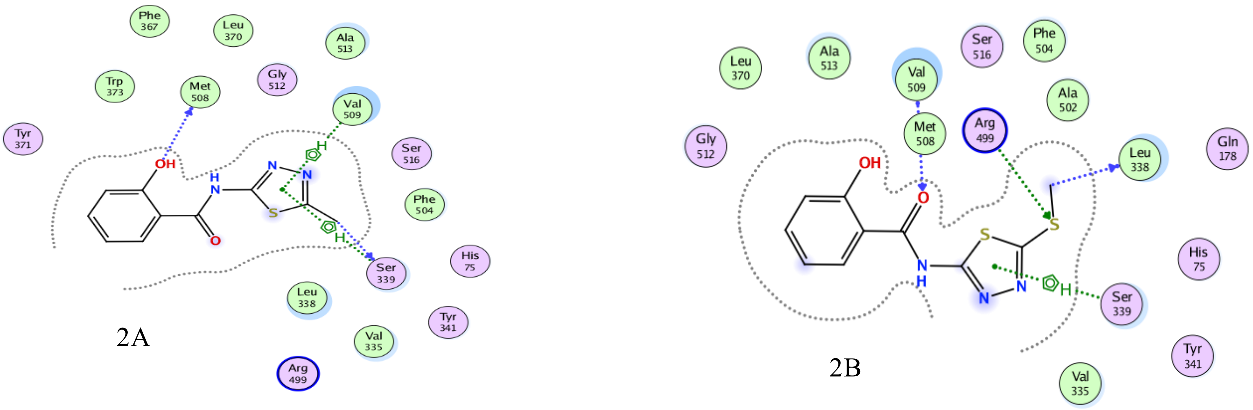
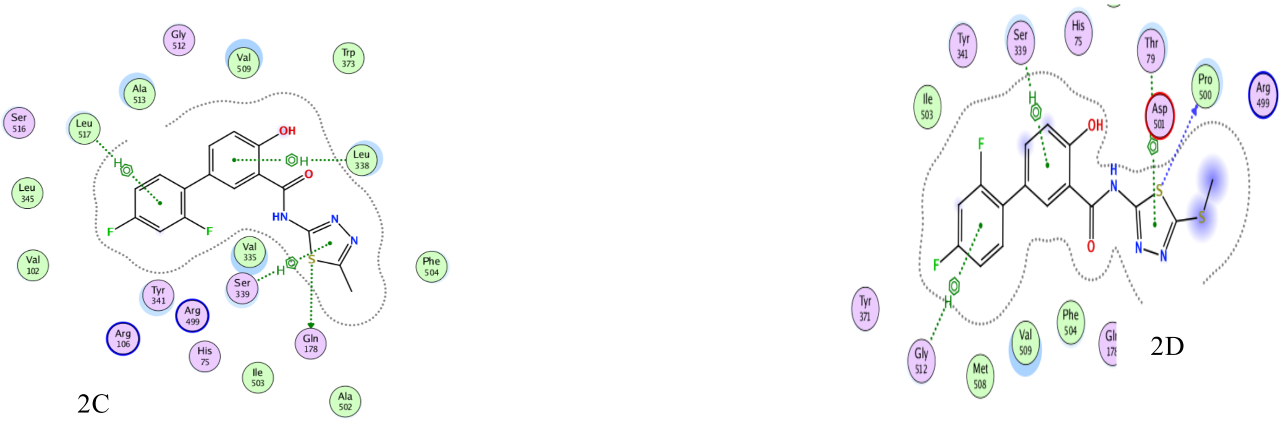


Figure 1: The two-dimensional (2D) representation of the binding interactions formed by celecoxib (1A) and diclofenac (1B) with 3LN1 active site of cox-2.





2C

2D

Figure 2: The two-dimensional (2D) representation of the binding interactions formed by compounds 1-4 with 3LN1 active site

According the table 1, compound 3 showed high affinity forward cox-2. By closer observation of the binding mode, compound 3 showed two pi hydrogen interaction, the first is between alcoholic phenyl ring with Leu 338, and the second was between fluorine phenyl ring and Leu 517. Moreover, the hydrogen bond interaction was observed between Sulphur atom of 1,3,4-thiadiazole ring and Gln 178. In fact, the interactions with Leu338 and Ser339, which were as a part of the polar side pocket of the COX-2 active site, are essential for the selective inhibition of COX-2 [36,37].

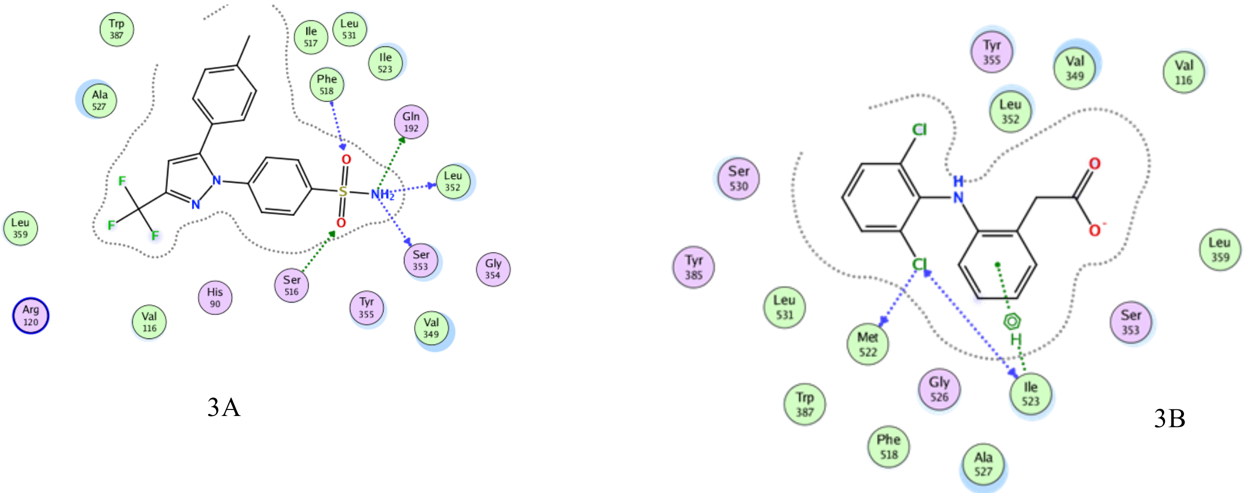
TABLE 2. The docking scores of compounds 1-4. And diclofenac and celecoxib as reference agents

Compounds	Cox-2 enzyme (3LN1) score affinity	Cox-1 enzyme (3KK6) score affinity
Compound 1	-6.68	-6.84
Compound 2	-6.82	-7.60
Compound 3	-8.04	-8.43
Compound 4	-6.16	-8.07
Celecoxib	-9.578	-9.52
Diclofenac	-6.94	-6.77

Computational study of binding mode inside the active site of COX-1 enzyme

In our current study, we have tried to examine and clarify a binding mode of compounds 1-4 towards COX-1 by using Crystal Structure of cox-1 (pdb;3KK6). Initially, all stereochemical conformations of celecoxib, ligand of the 3KK6 crystal structure, were docked. The Root Mean Square Deviation (RMSD) value for the best pose within the active site of the COX-1 enzyme was 1.1384 Å (≤2 Å) which reflects a good agreement between the experimental and predicted binding modes.

Co-crystallized ligand, celecoxib, interacted with the COX-1 active site by forming hydrogen bonds with Gln192, Ser353, Phe518, and Leu352. Our study results are consistent with the study by Remon et al, which showed that celecoxib and other coxibs bind tightly to a to one monomer of COX-1, and this mode of binding does not affect the normal catalytic processing of arachidonic acid by the second. The mode of binding depends on an extended rotamer conformation allowing access to the otherwise inaccessible hydrophobic side pocket comprised of residues Leu352, Ser353, Ile517, and Phe518 [40] figure 3A



3A

3B

Figure 3: The two-dimensional (2D) representation of the binding interactions formed by celecoxib (3A) and diclofenac (3B) with 3KK6 active site of cox-1.

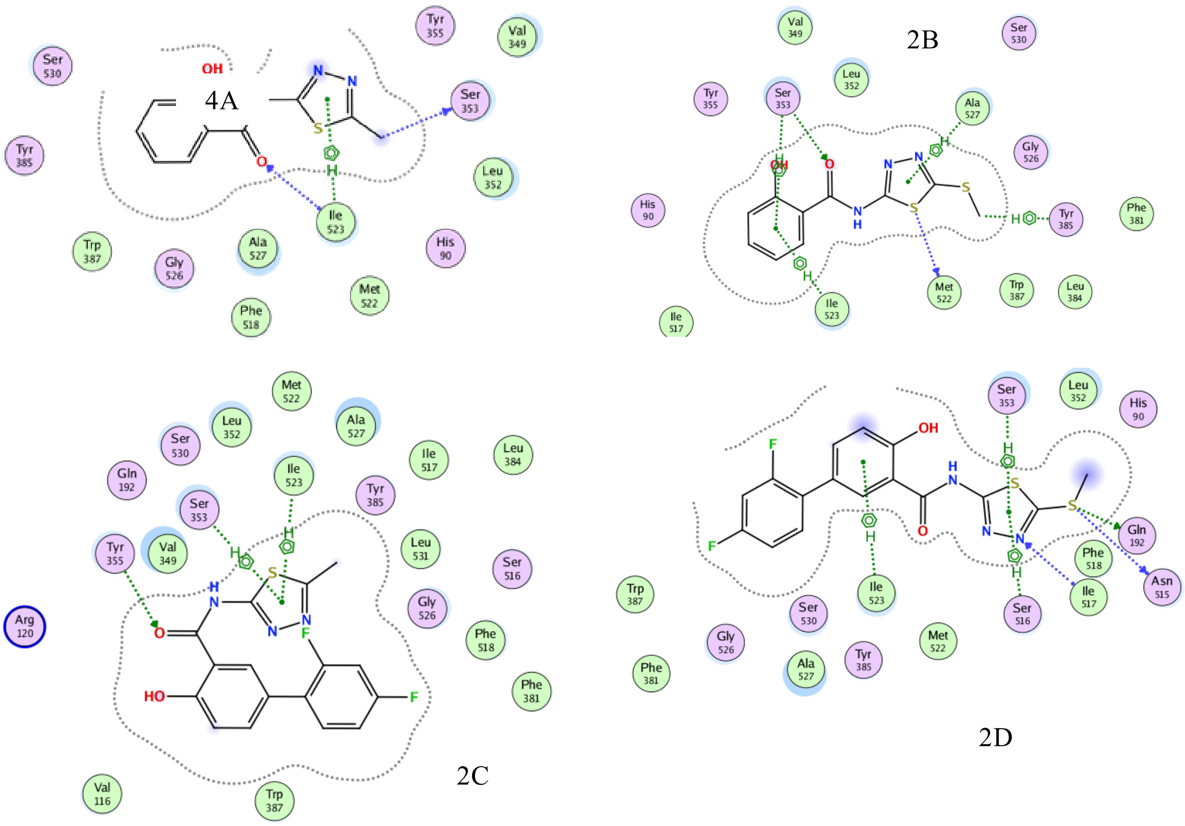


Figure 4: the two-dimensional (2D) representation of the binding interactions formed by compounds 4(A-D) with 3KK6 active site of cox-1.

TABLE 3. Drug-likeness descriptors and physicochemical properties of the designed compounds

Compounds	nROT	Mw	nHA	Nhd	MR	TPSA	LogS	CLogp
1	3	235.26	4	2	61.11	103.35	-3.93	1.6
2	4	267.33	4	2	67.87	128.65	-4.93	1.91
3	4	347.34	6	2	86.46	103.35	-6.84	3.69
4	5	379.4	6	2	93.22	128.65	-7.83	4.09

Results of docking of 1,3,4-thiadiazole derivatives on Cyclooxygenase-1 (COX-1) receptor by using the crystal structure 3KK6 are consistent with previous findings, where the compounds 1-4 have shown docking scores (ranging from −6.84 to −8.43), and the compound 3 showed the highest docking score (−8.43) by forming two pi hydrogen interaction between 1,3,4-thiadiazole and Ile523 and Ser353. Moreover, it was observed hydrogen bond interaction between Tyr355 and carbonyl of amide group. These results confirm that compound 4 is a selective COX-2 and exhibits the same binding mode to that of celecoxib with both cyclooxygenase enzymes (COX1 & COX2) Figure 4A-D.

Drug-Likeness and Physicochemical Properties prediction

The physicochemical characteristics are essential for analyzing the ligand's therapeutic and pharmacological effects. The SwissADME tool online was used to calculate the molecular properties of synthesized compounds like TPSA, number of rotatable bonds (n ROT), molecular weight (MW), Molar reflectivity (MV), number of hydrogen bond donor (HBD) and number of hydrogen bond Accepter (HBA), [29,41]. Results of prediction drug-likeness descriptors and physicochemical properties by swissadme have shown in table 2.

The compounds studied showed a number of hydrogen bond acceptors and hydrogen bond donors less than ten and five, respectively. Also, the number of rotatable bonds in each molecule was less than 10, The molar refractivity ranges from 61.11 to 93.22. Topological polar surface area (TPSA) is an essential descriptor of the transport of drug molecule across the biological barriers, and bioavailability of orally administered drugs. In our assessment, the TPSA values vary within small boundaries between 103.35-128.6 A°.

The Log Po/w calculated by SwissADME is an average of iLOGP, XLOGP3, WLOGP, MLOGP, and SILICOS-IT, referred to as the consensus Log Po/w. Log Po/w is the Log of the octanol/water partition coefficient. A higher log Po/w value indicates higher lipophilicity, Log Po/w values of compounds 1-4 ranged from 1.6 to 4.09.

The compounds 1-4 are compliant with many widely acceptable drug likeness rules; Lipinski, Muegge, Ghose, Veber, and Egan.

The boiled-egg graph for Compounds 1-4 is shown below. All compounds showed high gastrointestinal (GI) absorption, except for Compound 4, which is predicted to have poor intestinal absorption. Furthermore, none of the compounds are predicted to cross the blood-brain barrier (BBB). Finally, all derivatives are predicted not to be P-glycoprotein substrates.

ADME prediction

The PreADMET results were analyzed to evaluate the compounds' pharmacokinetic properties, Table 3 shows the values of these constants. HIA property is the determinant for those drugs that purport oral administration. All the compounds expressed greater than 70% HIA values indicating good permeation across the membrane [42].

TABLE 4. ADME constants for the synthesized compound

ADME descriptors	Ref. values	Compound 1	Compound 2	Compound 3	Compound 4
BBB (C brain/C blood)	Low <0.1 Moderate 0.1-2.0 High >2.0	0.3280 Moderate	0.1388 Moderate	0.0662 Low	0.0406 Low
HIA (Human Intestinal Absorption) %	Poor (0-20%) Moderate (20-70%) High (70-100%)	87.87 High	88.59 High	94.74 High	95.78 High
P_{Caco-2} (nm/second)	Low < 4.0 Moderate 4-70 High > 70	1.257 Low	19.557 Moderate	1.261 Low	19.899 Moderate
Plasma Protein Binding (PPB%)	Low <90% High >90%	52.515 Low	73.410 Low	87.764 Low	91.254 High
Pgp inhibition	Non/inhibitor	Non	Non	Non	Inhibitor

The in vitro Caco-2 cell permeability is an important parameter to assess intestinal absorption of the drug since Caco-2 cells are derived from human colon adenocarcinoma, possessing transports via the intestinal epithelium. The results indicated that compounds 1-4 exhibited low-moderate permeation [30].

The blood–brain barrier (BBB) penetration is symbolized as BB=(Brain)/(Blood). Compounds 1 and 2 exhibited moderate blood-brain barrier (BBB) permeability, with predicted BBB ratios ranging between 0.1 and 2. In contrast, Compounds 3 and 4 were predicted to have weak BBB penetration [30,43].

The percentage of drug bind to plasma protein is another remarkable factor, the percent of drug bound with plasma proteins was estimated and almost all the compounds (except 4) was predicted to possess weak binding [30].

P-glycoprotein (P-gp) is a protein found in the cell membranes of many body cells. It functions as an efflux pump, transporting foreign compounds out of the cell. In our assessment, compound 4 was predicted to act as a P-glycoprotein (P-gp) inhibitor. This activity suggests a potential for drug-drug interactions by altering the pharmacokinetics of co-administered P-gp substrates [34].

IV. CONCLUSION

The designed and synthesized of compounds (1,2,3, 4) as thiadiazol derivatives to achieve optimal anti-inflammatory activity and less GIT side effects, and identified by IR and 1HNMR spectroscopy had been caried out.

A molecular docking study was performed for these compounds and compared with reference agents' diclofenac and celecoxib by using Molecular Operating Environment (MOE)program, showed good binding affinity for the active sites of COX-1 and COX-2 enzymes. Compound 3 showed a higher binding affinity compared to the other three compounds, with a binding affinity greater than diclofenac and close to that of celecoxib. A study of the binding modes of compounds 1-4 revealed that compounds 3 and 4 adopt the binding mode responsible for the potency and selectivity of diaryl heterocyclic inhibitors such as celecoxib. In contrast, compound 2 binds to COX-2 similarly to diclofenac. Whereas compound1 showed equal activity against cox-2 and cox-1 enzymes.

The physicochemical properties and pharmacokinetic parameters of compounds (1,2,3,4) were predicted using the SwissADME web tool. The results indicate that all compounds generally exhibit favorable drug-like properties. A key unified feature is their inability to cross the blood-brain barrier and that they are not substrates for P-glycoprotein. Most compounds also show a satisfied potential oral bioavailability, excepted compound 4, in which predicted to have poor intestinal absorption.

The pharmacokinetic profiles of tested compounds were predicted using the PreADMET web tool. The results indicate a generally favorable pharmacokinetic profile for the majority of the compounds. Compound 3 shows a distinct profile, in which characterized by weak BBB penetration, high human intestinal absorption, good permeability to across the intestinal barrier, low plasma protein binding, and no P-glycoprotein (P-gp) inhibition. The unique result of compound 3 showed a higher binding mode affinity similar to selective COX-2 inhibitors, and distinct pharmacokinetic profile make compound 3 a promising anti-inflammatory candidate as preferential selective cox-2 inhibitor.

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