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# Synthesis, characterization, molecular docking, ADMET prediction, and anti-inflammatory activity of some Schiff bases derived from salicylaldehyde as a potential cyclooxygenase inhibitor

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## Abstract:

A series of Schiff base-bearing salicylaldehyde moiety compounds (1-4) had been designed, synthesized, subjected to insilico ADMET prediction, molecular docking, characterization by FT-IR, and CHNS analysis techniques, and finally to their Anti-inflammatory profile using cyclooxygenase fluorescence inhibitor screening assay methods along with standard drugs, celecoxib, and diclofenac. The ADMET studies were used to predict which compounds would be suitable for oral administration, as well as absorption sites, bioavailability, TPSA, and drug likeness. According to the results of ADME data, all of the produced chemicals can be absorbed through the GIT and have passed Lipinski's rule of five. Through molecular docking with PyRx 0.8, these synthesized compounds were tested insilico selectivity toward COX-1 and COX-2 and in vitro for their anti-inflammatory efficacy . In vitro testing demonstrated that all of the produced compounds had significantly stronger activity against the COX-2 enzyme than COX-1. Among these, compound 1 displayed the most potent inhibitory activity with an $IC_{50}$ value of 0.19 µM compared to standard drug celecoxib ($IC_{50}$ = 0.29 µM). The most active derivative compound1 was oriented towards the active site and occupied the target enzyme based on the docking investigation against COX-1 and COX-2. In addition, insilico investigations found that COX-2 has a higher inhibitory activity than COX-1

**Keywords** Anti-inflammatory agent, Cyclooxygenase inhibitor, Insilico ADMET, Molecular docking, Salicylaldehyde, Schiff base.

## Introduction:

Inflammation is a common and very important pathological process that can cause tissue or cell damage characterized by redness, edema, fever, heat, pain, and loss of function [1,2]. As a result, inflammatory processes are involved in a variety of diseases such as atherosclerosis, Alzheimer's disease, Parkinson's disease, cancer, asthma, arthritis, etc. [3,4]. Although nonsteroidal anti-inflammatory drugs (NSAIDs) are currently among the most commonly prescribed drugs, they are attributed to their wide range of medical indications as they can be used as analgesic, anti-inflammatory, anti-rheumatic and antipyretic agents [5]. In addition, NSAIDs' pharmacological effect is linked to the inhibition of PG synthesis from arachidonic acid via inhibition of the crucial regulating enzyme cyclooxygenase COX. At least two isoforms of cyclooxygenase have been identified: COX-1, or prostaglandin H1 synthase, and COX-2, or prostaglandin H2 synthase [6].COX-1 is expressed in most tissues, regulates physiological processes such as gastric cryoprotection, renal function, and platelet aggregation, and is stimulated by growth factors and hormones, while COX-2 mainly supplies prostaglandin E2 (PGE2) and prostacyclin (PGI2). Many NSAIDs interact with both COX-1 and COX-2 isoforms and non-selectively inhibit their enzymatic activity, resulting in a reduction in the production of the prostaglandins PGE2 and PGI2 [7,8]. NSAIDs, despite their widespread usage, have been linked to significant adverse effects such as gastrointestinal (GI) problems, hypertension, edema, kidney disease, and heart disease risk [9]. Because of the difference in expression profiles between COX-1 and COX-2, a hypothesis was advanced in the 1990s that selective inhibitors(celecoxib)of COX-2 would share the beneficial anti-inflammatory properties of traditional

NSAIDs but lack the gastric toxicity [10]. Therefore, it is crucial to synthesize and develop novel and more potent anti-inflammatory drugs with no or fewer side effects. Schiff bases are aldehyde or ketone-like compounds in which the carbonyl group is replaced by an imine or azomethine group(C=N), when reacted with any primary amine under certain conditions [11]. Schiff bases are among the most commonly used organic compounds [12,13]. In recent years, Schiff bases have been widely used to formulate various types of drugs due to their diverse biological activities including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic properties [11,14-17]. Numerous studies have reported that Schiff's bases of substituted salicylaldehyde (2-hydroxybenzaldehyde) are well-known antimicrobial agents, analgesic, and anti-inflammatory in free form or as ligands in metal complexes [18-20]. In the present study, both experimental and computational approaches are used to discover novel anti-inflammatory drug candidates.

## Experimental Section:

### Chemistry

### Materials

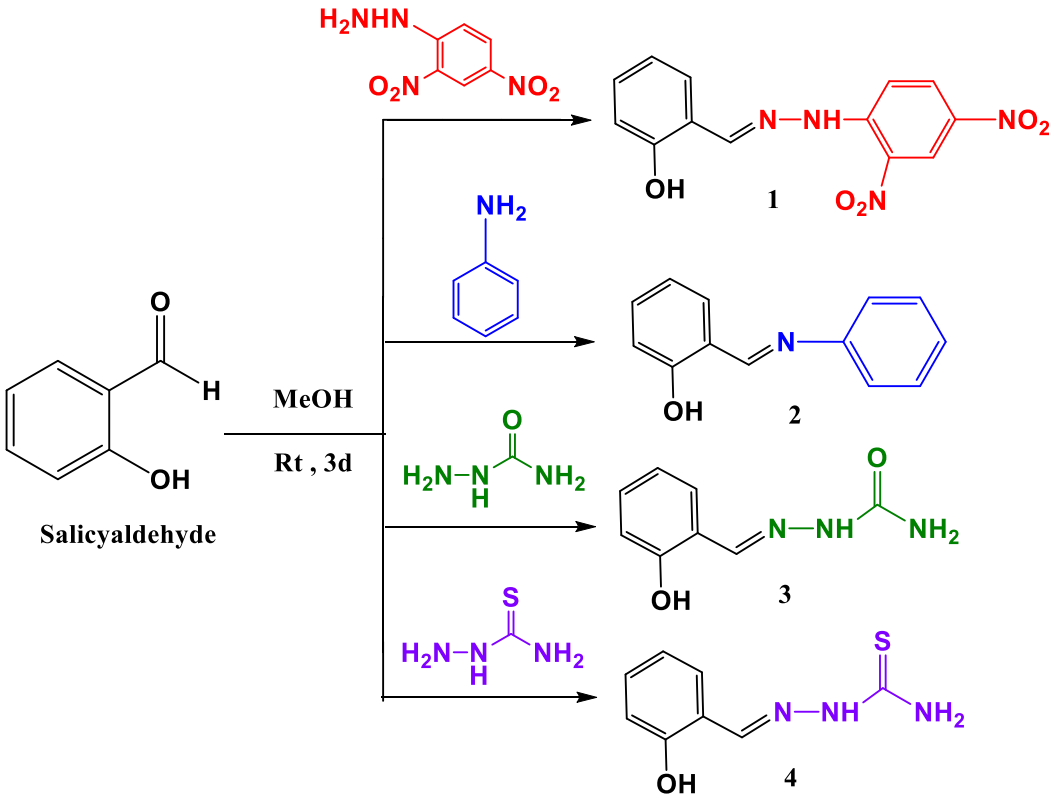
Salicyaldehyde, 2,4-dinitrophenylhydrazine aniline, semicarbazide, thiosemicarbazide, methanol, chloroform, and ethyl acetate were used purchased from Sigma Aldrich and Merck.

### Instrumentation

Synthesized compounds were purified by recrystallization in appropriate solvents and examined through thin layer chromatography (Merck Silica gel 60 F254) and UV light (320 nm). The melting points were determined in open capillary tubes using the Stuart/SMP3 melting point equipment version 5.0. Infrared spectra were recorded without a KBr disk using Thermo Scientific™ Nicolet™ iS™10 FT-IR Spectrophotometer in Pioneer company for pharmaceutical industry-Sulaimani-Iraq. CHNS-elemental analysis was used to characterize the produced substances (CHNS-O Elemental Analyzer Vario EL, ELEMENTAR, Hanau-Germany) in the College of Pharmacy-Hamedan University of Medical Sciences- Hamedan-Iran.

### Chemical synthesis:

The synthesis of Schiff base derivatives was carried out using the methods illustrated in Scheme 1.




**Scheme 1. Synthetic route to Schiff base derivatives (1-4)**

**General Procedure for Schiff bases derivatives synthesis (1-4):**

Equimolar amounts of salicylaldehyde and the primary amine (2,4-dinitrophenylhydrazine, aniline, semicarbazide, thiosemicarbazone) were dissolved in methanol 10 mL and agitated for 10 minutes at room temperature to yield a transparent solution. After approximately three days of standing. TLC was used to monitor the reaction, then filtering was used to collect the precipitate. The precipitate was recrystallized from methanol, washed three times with methanol, and dried in a vacuum desiccator containing anhydrous $CaCl_2$. Table 1 shows the physicochemical parameters of the compounds (1-4) [21].

**2-((2-(2,dinitrophenyl)hydrazineylidene)methyl) phenol, compound (1):**

Orange powder 71% yield; mp 253–255°C; IR (without KBr) v $cm^{-1}$: 3269.65 (O-H phenol), 3087.72 (C-H aromatic), 1608 (C=N),1585.18(C=C aromatic). CHNS elemental analysis calculated for $C_{13}H_{10}N_4O_5$: C, 51.66%; H, 3.34%; N, 18.54%. Found: C, 51.72%; H, 3.37%; N, 18.52%.

**2-((phenylimino) methyl) phenol, compound (2):**

Orange powder 87% yield; mp 56-58°C; IR (without KBr) v $cm^{-1}$: 3054.85 (O-H phenol), 2980.96 (C-H aromatic), 1614.12(C=N), 1588.69& 1570.17 (C=C aromatic). CHNS elemental analysis calculated for $C_{13}H_{11}NO$: C,79.17%; H,5.62%; N,7.10%. Found: C, 79.36%; H, 5.57%; N, 7.13%.

**2-(2-hydroxybenzylidene)hydrazine-1-carboxamide ,compound (3):**

Yellow powder 64% yield; mp 205-207°C; IR (without KBr) v $cm^{-1}$: 3492.31 (O-H phenol), 3145.41 ($NH_2$), 3054.09 (C-H aromatic), 1682.55 (C=O amide), 1621 (C=N), 1584.41& 1520.74 (C=C aromatic). CHNS elemental analysis calculated for $C_8H_9N_3O_2$: C,53.63%; H, 5.06%; N, 23.45%. Found: C,53.79%; H, 4.95%; N, 23.49%.

**2-(2-hydroxybenzylidene)hydrazine-1-carbothioamide, compound (4):**

Yellow powder78% yield; mp 230-232°C; IR (without KBr) v $cm^{-1}$: 3269.49 (O-H phenol), 2920.91 (C-H aromatic), 1618.35 (C=N), 1545.50 & 1525.72 (C=C aromatic) ,1345.74 (C=S). CHNS elemental analysis calculated for $C_8H_9N_3OS$: C,49.22%; H, 4.65%; N, 21.52%, S,16.42%. Found:C,49.24%; H,4.28%; N,21.95%, S,16.37%.

**Table 1. Physical properties of compounds (1-4).**

| Comp. | Chemical formula | Colour | Solubility | M.P(°C) | % Yield | $R_f$ value |
|---|---|---|---|---|---|---|
| **1** | $C_{13}H_{10}N_4O_5$ | Orange powder | MeOH | 253-255 | 71 | 0.6 |
| **2** | $C_{13}H_{11}NO$ | Orange powder | MeOH | 56-58 | 87 | 0.7 |
| **3** | $C_8H_9N_3O_2$ | Yellow powder | MeOH | 205-207 | 64 | 0.7 |
| **4** | $C_8H_9N_3OS$ | Yellow powder | MeOH | 230-232 | 78 | 0.5 |

Solvent system: ethyl acetate: chloroform (2:8)

**Computational Studies:**

**In silico ADMET properties**

In-silico prediction of ADME properties of the Schiff base derivatives was carried out using the SWISS-ADME online server [22]. while toxicity was predicted using the organ toxicity and endpoint toxicity model of the ProTox-II software [23]. Several critical parameters were predicted, including molecular weight, topological polar surface area (TPSA), number of hydrogen bond donors (HBD), and number of hydrogen bond acceptors (HBA). Organ toxicity is classified in the category: of hepatotoxicity. The endpoints of toxicity are classified: carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity. The chemical structure of the designed compounds (1-4) was drawn by using theMarvinSketch19.9 application[24] and then converted to the SMILE name. The smile notation for all synthesized compounds was considered as the starting point and input to the SWISS_ADME and ProTox-II webserver and thereby ADME and toxicity predictions were carried out.

**Molecular Docking study**

**Ligand Preparation**

MarvinSketch19.9 [24] was used to draw the compounds (1-4). They were sketched primarily as 2D structures and then converted to 3D format (pdb) using the same program. Ligand energy was minimized by applying the MMFF94 (Merck Molecular Force Field 94) force field algorithm [25], and the minimized structures were converted into PDBQT format by using PyRx 0.8 [26] before performing molecular docking analysis.

**Preparation of receptors**

The Protein Data Bank [27] was used to get the crystal structures of the cyclooxygenase receptor, the COX-1 enzyme (PDBID:3N8Z), and the COX-2 receptor (PDB ID:1PXX) Fig.1. The Discoverystudio2021 client [28] was used to remove the water molecules, heteroatoms, and co-

crystallized ligands. Autodock-Tool-1.5.6 [29] was used to add the polar hydrogens and Kollman charges. PyRx was used to convert the PDB files to PDBQT format.

**Docking study**

The docking tool PyRx (Python Prescription 0.8) was used to dock compounds (1-4) into the previously synthesized proteins COX-1 (ID:3N8Z) and COX-2 (ID:1PXX) [30]. The binding sites were chosen based on the target protein's co-crystallized ligands. PyRx affinity scores (in kcal/mol) for each chemical were obtained and rated using the free energy binding theory (more negative value means greater binding affinity). DS Visualizer and PyMOL Molecular Graphics System (Version 2.3.2 Schrödinger) were used to visualize docked conformations (poses) and receptor-ligand interactions at the molecular level.

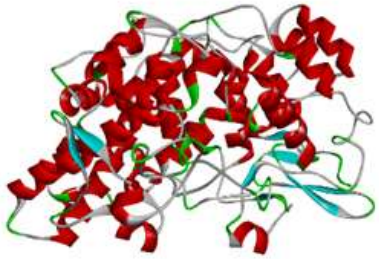


**Figure 1. The crystal structure of COX-2 receptor (PDB ID:1PXX)**

**Anti-Inflammatory Studies**

**In vitro COX-1 and COX-2 inhibition assay**

The assay for COX-1 and COX-2 enzyme inhibitory activity of the synthesized compounds (1-4) was performed based on a slightly modified protocol published by Kezhal M. et al. has been reported [31]. Cayman's COX fluorescent inhibitor screening assay (Catalog number 700100, Cayman Chemical, Ann Arbor, MI, USA), provides a convenient fluorescent-based method for screening both ovine COX-1and human recombinant COX-2 for isozyme-specific inhibitors. All reagents and solutions were prepared according to the protocols established by Cayman Chemical for the COX-1 and COX-2 inhibition assays.

The solvent for the stock solutions for the test samples was dimethyl sulfoxide (DMSO). 10 µL of various concentrations of test sample solutions 0.01, 0.1, 1, 10, 50, and 100 µM were added to a series of supplied reaction buffer solutions (960 mL 0.1 M Tris-HCl pH 8.0 with 5 mM EDTA and 2 mM phenol) with either COX-I or COX2 enzyme 10 µL in the presence of heme 10 µL and 10 µL of fluorometric substrate ADHP (10-acetyl-3,7-dihydroxyphenoxazine) after a 5 min incubation at 37 °C, Added 10 µL of arachidonic acid 100 µM solution and stopped the COX reaction after 2 minutes with 50 µLof 1 M HCl. When PGG2 and ADHP react, the extremely fluorescent chemical resorufin is formed. With an excitation wavelength of 535 nm and an emission wavelength of 590 nm, the fluorescence of produced resorufin can be detected. The intensity of this fluorescence depends on the amount of resorufin present in the well throughout the incubation period and is proportional to the amount of PGG2 present in the well. From the concentration-inhibition-response curve, the concentration of the test chemical causing 50% inhibition $IC_{50}$, µM was calculated. The in vitro tests were carried out three times.

## Results and Discussions:

**Chemistry:**

By condensing salicylaldehyde with various substituted primary amine, acceptable yields from the derivatized compounds (1-4) reaching 64-87% were obtained after recrystallization. All compounds have been fully characterized by using FT-IR and elemental analysis (C.H.N-S). The FT-IR spectrum showed O-H stretching for the phenol group is observed at 3492.31- 3054.85 $cm^{-1}$ for compounds 1,2,3 and 4. N-H stretching of $NH_2$ frequency at 3145.41 $cm^{-1}$ for compound 3. The C-H stretching of aromatic ring and alkene is obtained at 3087.72 cm-1 for compound 1, 2980.96 $cm^{-1}$ for compound 2, 3054.09 $cm^{-1}$ for compound 3, and 2920.91cm-1 for compound 4. The appearance of a new band at 1608 $cm^{-1}$ which is C=N stretching of Schiff base for

compound 1, 1614.12 $cm^{-1}$ for compound 2, 1621 $cm^{-1}$ for compound 3, and 1618.35 $cm^{-1}$ for compound 4. The C=C stretching of aromatic ring frequency at 1588.69- 1520.74 $cm^{-1}$ for compounds (1-4). Elemental analysis (C.H.N-S) of compounds (1-4) mentioned in the method.

## Computational Studies:

### Analysis of ADMET properties:

One of the most important aspects of the drug discovery/development process is predicting absorption, distribution, metabolism, and excretion (ADME) characteristics prior to experimental trials[32]. The bioactivity score of the synthesized compounds was calculated using the SwissADME webserver. SwissADME's freely available web server (http://www.swissadme.ch/) was used to determine ADME, pharmacokinetic parameters, and drug-like features by entering chemical structure followed by SMILES. Table 2, shows the pharmacokinetic and physicochemical data determined, including cLogP (partition coefficient), compound weight, heavy atoms, hydrogen donors, hydrogen acceptors, rotatable bonds, and TPSA values.

**Table 2. *In silico* computed ADME characteristics of the synthesized Schiff base derivatives**

| | Physicochemical parameters | | | | | | | | Absorption Distribution | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Entry** | **MW[m] (≤ 500)** | **TPSA[t] (Å²)** | **HBA[a] (≤ 10)** | **HBD[d] (≤ 5)** | **RB[r] (≤ 5)** | **cLogP[c] (≤ 5)** | **B.S[b]** | **Lipinski's violation** | **GIA[g]** | **BBB[i]** | **Pgp[p]** |
| **1** | 302.24 | 136.26 | 6 | 2 | 5 | 0.98 | 0.55 | 0 | High | No | No |
| **2** | 197.23 | 32.59 | 2 | 1 | 2 | 2.87 | 0.55 | 0 | High | Yes | No |
| **3** | 179.18 | 87.71 | 3 | 3 | 3 | 0.49 | 0.55 | 0 | High | No | No |
| **4** | 195.24 | 102.73 | 2 | 3 | 3 | 1.15 | 0.55 | 0 | High | No | No |

m Molecular weight, t Topological polar surface area, a Number of hydrogen bond acceptor, d Number of hydrogen bond donor, r Number of rotatable bonds, c Consensus of calculated lipophilicity, b bioavailability score, g gastrointestinal absorption, iblood brain barrier, p P-glycoprotein substrate

According to Lipinski's criteria, molecules intended to be taken orally should contain no more than one violation of the following rules: (i) a maximum of 5 hydrogen donors (ii) a maximum of ten hydrogen-bond acceptors. (iii) The molecular weights of the molecules should not exceed 500 m/z. (iv) The lipophilicity (cLogP) should not exceed 5. The compounds do not function as therapeutic candidates if they exhibit more than one harm based on the above criteria [33]. From Table 2, all compounds (1-4) show no Lipinskis injury and all compounds synthesized have less than ten hydrogen bond acceptors and less than 5 hydrogen bond donors. In addition, the molecule produced has a molecular weight of less than 500 m/z. All of the compounds show a decent drug-like profile based on the parameters given above. The sum of the surface areas of all polar atoms or molecules, usually hydrogen, oxygen, and nitrogen, is known as the topological molecular surface area (TPSA). The TPSA calculation is required to estimate the drug's ability to enter cells. A TPSA value of less than 140 $Å^2$ indicates that the molecules have good drug transport properties across cell membranes [34]. These values vary from 32.59 $Å^2$ to 136.26 $Å^2$ for synthesized compounds. These numbers show that all compounds are able to penetrate cell membranes. According to Lipinski's rule, molecules with a higher number of rotatable bonds have a more flexible structure and are better for interaction. Compound 2 has two rotatable bonds, while compounds 3 and compound 4 have three, and compound 1 has five. This demonstrates that the produced compounds, particularly compound1 with its five rotatable bonds, have a high ability to interact with living cells. The most crucial criterion is bioavailability, and bioactivity score values larger than zero imply that the chemical is very drug-like [35]. Bioactivity levels of 0.55 are found in all synthesized derivatives. The two most significant pharmacokinetic activities to be investigated at various phases of drug development are gastrointestinal (GI) absorption and blood-brain barrier (BBB) characteristics of a molecule. The permeability of the synthesized compounds (1-4) identified by the BOILED-Egg method may be assessed using gastrointestinal absorption and blood-brain barrier tests, as illustrated in Fig. 2. The BOILED Egg model (Brain or IntestinaL EstimateD Penetration) is offered as a precise predicting model based on the lipophilicity index (WLOGP) and polarity of the produced chemical. The white area suggests that the compounds are likely to be

passively absorbed by the gastrointestinal system, while the yellow area indicates that the compounds can cross through the blood-brain barrier (BBB) and get access to the central nervous system. The gray areas reflect substances that are not expected to be well absorbed or permeate the BBB. The blue dot indicates that the molecule is projected to be a P-glycoprotein substrate (PGP+), whereas the red dot indicates that the molecule is likely to be a P-glycoprotein non-substrate (PGP-) [36]. As shown in Fig. 2, all of the synthesized derivatives (1-4) are well absorbed in the gastrointestinal system, but they are unable to pass through the BBB, with the exception of compound 2, which shows that the compound has access to the central nervous system (CNS) and can thus be used to treat CNS inflammation. The formulas (percent of Abs) = 109-0.345 x TPSA and (percent of Abs) = 109-0.345 x TPSA and (percent of Abs) = 109-0.345 x The component compound 1 has a 61.99 percent absorption rate, while the compounds 2, 3, and 4 have absorption rates of 97.75 percent, 79 percent, and 73.55 percent, respectively[37].

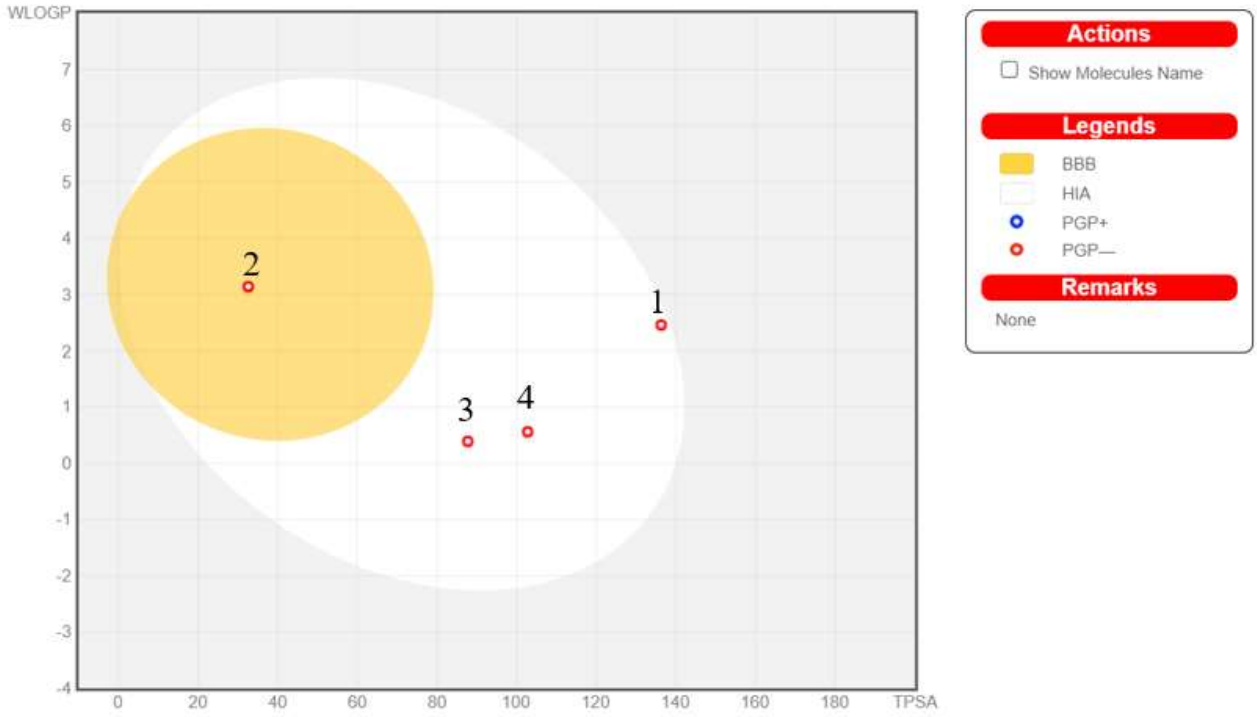



**Figure 2. BOILED egg plot of synthesized Schiff base derivatives (1-4) from the SwissADME web tool.**

**Toxicity Prediction results:**

The endpoints toxicity and organ toxicity were calculated using the online software ProTox-II. All compounds have no carcinogenic profile and no toxicity found in the hepatotoxicity and AMES toxicity assessment, Table 3.

**Table 3. Toxicity Prediction of Compounds (1-4)**

| Entry | Organ Toxicity | Toxicity - endpoints | | | |
|---|---|---|---|---|---|
| | Hepatotoxicity | Carcinogenicity | Immunotoxicity | Mutagenicity | Cytotoxicity |
| **1** | No | No | No | No | No |
| **2** | No | No | No | No | No |
| **3** | No | No | No | No | No |
| **4** | No | No | No | No | No |

**Molecular Docking Study**

Recently synthesized compounds (1-4) docked to two receptors including cyclooxygenase-1 (COX-1) enzyme (3N8Z.pdb) and cyclooxygenase-2 protein (COX-2) (1PXX.pdb). All compounds had docking scores between -9.3 and -6.7 kcal/mol Tables 4 and 5. These compounds have a higher potential to form hydrogen bonds and hydrophobic interactions with THR206, ASN382, HIS386, TYR385, HIS388, ALA202, LEU390, and TRP387 were common interacting residues. It is worth mentioning, that compound 1 was the most active compound for each protein (Tables 4 and 5). For this reason, compound 1 was selected for further analysis.

The potential energy of all molecules docked with the COX-1 enzyme ranged from -8.5 to -6.7 kcal/mol. Compound1, which had the greatest docking score of -8.5 kcal/mol, has a strong inhibitory effect against the target receptor, as indicated in Table 4. The illustration of the molecular docking for the interactions of compound 1 and receptor was presented in Fig. 3 A, B, and C. The analysis of the best-docked pose of compound1 showed that the amino acid residues including THR206, THR212, and ASN382 had formed hydrogen-bonding interactions. Additionally, HIS207 and HIS386 generated two interactions including hydrogen bonding and unfavorable acceptor-acceptor interaction. Hydrophobic interactions have been observed, including amideπ-stacked and π-alkyl with ALA202. Furthermore, interactions between HIS207 and HIS386 residues were discovered using the π-cation interaction type. Other, interactions involving THR206, HIS207, HIS386, and HIS388 residues via acceptor-acceptor and positive-positive interaction types were also observed.

**Table 4. Compounds (1-4) docking scores and contact modes with the cyclooxygenase-1 (COX-1) receptor (PDB ID: 3N8Z)**

| Entry | Score Kcal/mol | Interactions | | |
|---|---|---|---|---|
| | | **H bond** | **Hydrophobic** | **Other** |
| **1** | **-8.5** | THR206, THR212, ASN382 | Amide-π Stacked and π-Alkyl: ALA202 | **π-Cation: HIS207, HIS386 Acceptor-Acceptor: THR206 Positive-positive: HIS207, HIS386, HIS388** |
| **2** | **-7.8** | TYR385 | Amide-π Stacked and π-Alkyl ALA202 π-π Stacked: PHE210 | **π-Cation: HIS386 Acceptor-Acceptor: THR206 Positive-Positive: HIS207, HIS388,** |
| **3** | **-6.9** | ALA202, THR206, TYR385, HIS388 | Amide-π Stacked: TRP387 π-Alkyl: ALA199, LEU390 | **π-Sulfur: MET391** |
| **4** | **-6.7** | THR206, ASN382, THR385, HIS386 | Amide-π Stacked and π-Alkyl: ALA202 | **Positive-Positive: HIS207** |

H bond hydrogen bonding

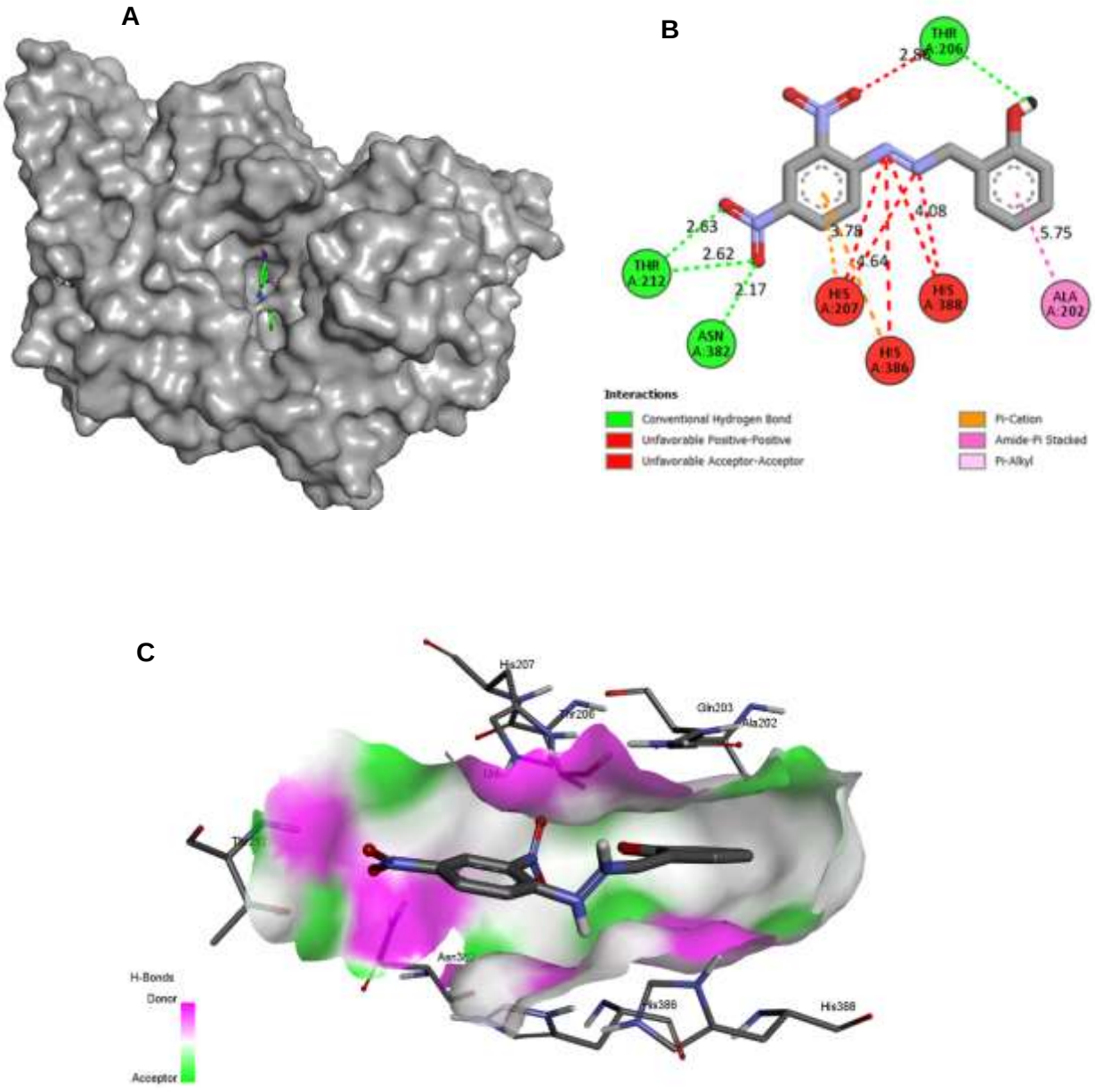


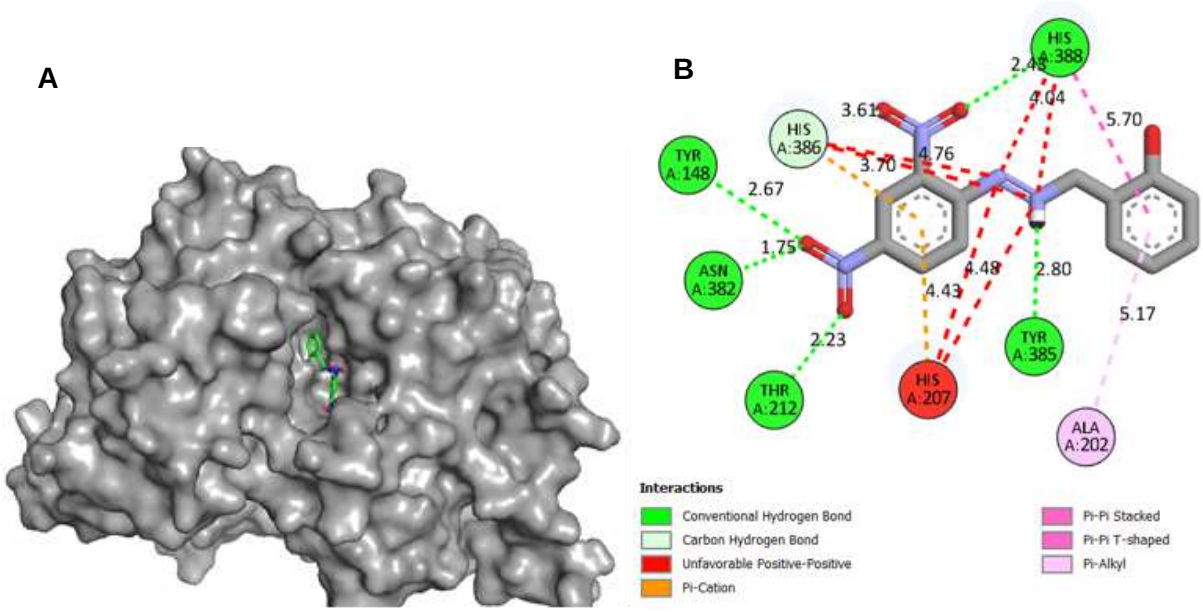

**Figure 3. Visualization of binding interactions of C1 with COX-1 residues (PDB ID: 3N8Z). (A) The surface of COX-1protein with compound1 at target site (B) Compound 1's 3-dimensional -molecular structure interaction The carbon atoms are represented by gray, oxygen by red, and nitrogen by pale blue in the scaffold (C) Hydrogen bonding interaction of compound 1, the green color represents the hydrogen bond acceptor and purple of hydrogen bond donor.**

The compounds (1-4) were docked to the active site of the COX-2 target, and the results showed that all compounds had potential energy of -9.3 to -6.8 kcal/mol and could inhibit target receptors. The docking score for compound1 was found to be -9.3 kcal/mol (Table 5). The molecular interactions of compound1 in the active site led to the formation of six H-bonds with amino acid residues, as shown in Fig.4A, B, and C. These are TYR148, THR212, ASN382, TYR385, HIS386, and HIS388. In addition, three hydrophobic interactions were observed, including π-alkyl, π-π -T-shaped, and π-π -stacked with ALA202, HIS388, and HIS386 amino acid residues. Other interactions involving HIS207, HIS386, and HIS388 residues via π-Cation and positive-positive interaction types were also observed.

**Table 5. Compounds (1-4) docking scores and modes of interaction with the cyclooxygenase-2 (COX-2) receptor (PDB-ID: 1PXX)**

| Entry | Score Kcal/mol | Interactions | | |
|---|---|---|---|---|
| | | H bond | Hydrophobic | Other |
| **1** | **-9.3** | TYR148, THR212, ASN382, TYR385, HIS386, HIS388 | π-Alkyl: ALA202<br>π-π T-shaped: HIS388<br>π-π Stacked: HIS386 | **π-Cation: HIS207, HIS386**<br>**Positive-positive: HIS207, HIS386, HIS388** |
| **2** | **-7.6** | SER530 | π-Sigma: VAL349, ALA527<br>π-Alkyl: LEU531<br>π-π T-shaped: TRP387 | **π-Sulfur: MET522** |
| **3** | **-6.8** | THR206, PHE210, TRP387 | π-π T-shaped: HIS388<br>π-Alkyl: LEU390 | **Positive-Positive: HIS207, HIS386** |
| **4** | **-6.9** | THR206, ASN382, HIS386, TYR385 | π-π T-shaped: HIS388<br>Amide-π Stacked: TRP387<br>π-Alkyl: LEU390 | **Positive-Positive HIS207:** |

H bond hydrogen bonding



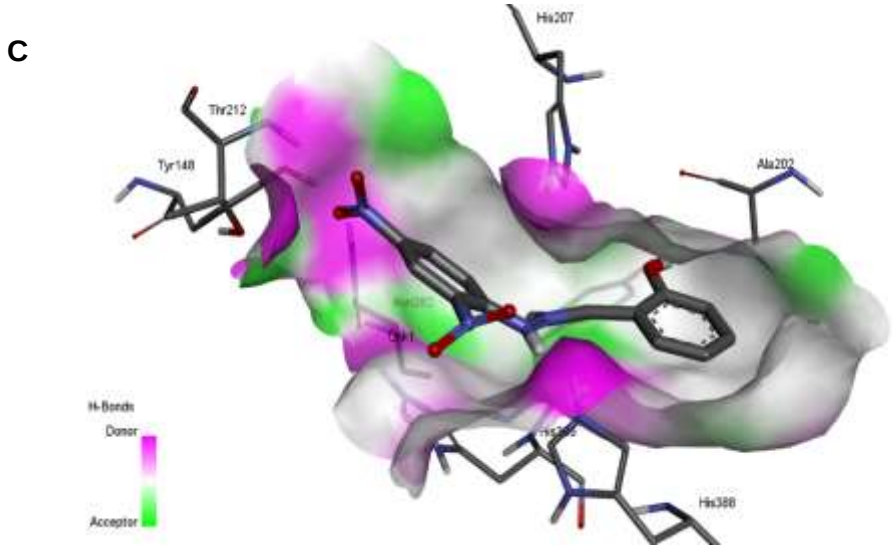



**Figure 4. Visualization of binding interactions of compound 1 with COX-2 residues (PDB ID: 1PXX). (A) The surface of COX-2 protein with compound 1 at target site (B) Compound 1's 3-dimensional - molecular structure interaction The carbon atoms are represented by gray, oxygen by red, and nitrogen by pale blue in the scaffold(C) Hydrogen bonding interaction of compound 1, the green color represents the hydrogen bond acceptor and purple of hydrogen bond donor.**

## Anti-Inflammatory Studies

### In vitro COX inhibition

The in vitro anti-inflammatory activity of the synthesized Schiff base derivatives (1-4) was tested using ovine COX-1 and human recombinant COX-2 (for isozyme-specific inhibitors), Celecoxib, and Diclofenac were also utilized as reference drugs, the results are listed in Table 6. The results showed that the reference drugs; celecoxib and diclofenac exhibited COX-1inhibitory activity with $IC_{50}$ 15.8 and 0.21 μM respectively and COX-2 inhibitory activity with $IC_{50}$ 0.29 and 3.8 μM respectively. Also, the results showed COX-1 inhibitory activities of compounds 1, 2, 3, and 4 with $IC_{50}$ 0.98, 4.58, 11.23, and 17.86μM respectively. Compound 1 was the most effective COX-1 inhibitor in this study, with an $IC_{50}$ of 0.98 M, which was 15 times greater than celecoxib's $IC_{50}$ 15.8 M. In comparison to the inhibition of COX-1 ($IC_{50}$ > 0.98M), those compounds showed potent inhibition of COX-2 with 0.19,1.98, 10, and 8 M, respectively. Compound 1 was also found to be a more effective inhibitor of COX-2 than celecoxib $IC_{50}$ 0.29 M, with a greater activity $IC_{50}$ 0.19 M. The effects of compound 1 substituents at the 2 and 4-positions of a dinitro group boosted both COX-1 and COX-2 inhibitory action, with the latter having higher efficacy, resulting in a COX-2-selective inhibitor (SI = 5.157)[38]. In addition, the results showed that the highest COX-2 selectivity index S.I. of all compounds(1-4) is equal to 5.157,2.313,1.123, and 2.23 respectively which is higher than Diclofenac (S.I. = 0.055).

**Table 6. $IC_{50}$ Values for Cyclooxygenase-1 and Cyclooxygenase -2 Enzymes**

| Compounds | $IC_{50}$ (μM) [i] | | COX-1 selectivity index (COX-2/COX-1) | COX-2 Selectivity Index (COX-1/COX-2) |
|---|---|---|---|---|
| | COX-1 | COX-2 | | |
| **1** | 0.98±0.17 | 0.19±0.011 | 0.194 | 5.157 |
| **2** | 4.58 ± 0.89 | 1.98±0.17 | 0.432 | 2.313 |
| **3** | 11.23±1.78 | 10 ± 0.99 | 0.890 | 1.123 |
| **4** | 17.86±1.56 | 8 ±1.05 | 0.447 | 2.23 |
| **Celecoxib** | 15.8±3.08 | 0.29±0.011 | 0.018 | 54.48 |
| **Diclofenac** | 0.21±0.008 | 3.8±0.026 | 18.09 | 0.055 |

i The chemical concentration necessary to produce 50% inhibition of COX-1or COX-2 for means of two measurements and deviation from the mean is 10% of the mean value is known as the $IC_{50}$ value. Data are presented as mean ± SD (n = 3).

## Conclusion:

To summarize, created a series of Schiff bases derived from salicylaldehyde as a cyclooxygenase inhibitor, estimated ADMET parameters in silico, and synthesized them. IR and CHNS elemental analyses were used to describe the title compounds. Compound 1 outperformed the conventional medications celecoxib and diclofenac in vitro in inhibiting COX-1 and COX-2. The findings revealed that the dinitro substitution of compound 1 was critical for cyclooxygenase inhibition. Furthermore, adding $NH_2$ groups to compounds 3 and 4 reduced the inhibitory activity of cyclooxygenase. According to the ADME analysis, all produced compounds satisfied the Lipinski criterion and were absorbed by GIT. Through molecular docking studies, it was found that the most active molecule, compound1, fits into the target enzyme. The results indicated that compound 1 could be a lead molecule because this ligand showed the best computational and experimental results. The cyclooxygenase inhibitory activity of these compounds could be further improved to find a promising therapeutic candidate for the treatment of inflammatory diseases.

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## Authors' declaration:

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine. Besides, the Figures and images, which are not mine, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Sulaimani.

## References:

1. Gandhimathi R, Sangeetha M.Synthesis, Characterization, Molecular Docking of Sulphamethoxazole Schiff Base Metal Complexes and Its Antibacterial, Anti-Inflammatory, and Anti Depressant Activity. Indian J Forensic Med Toxicol. 2022; 16(1): 47-57. https://doi.org/10.37506/ijfmt.v16i1.17413.
2. Hsin-Tzu Liu , Yu-Ning Lin , Ming-Cheng Tsai , Ya-Chi Wu, Ming-Chung Lee. Baicalein Exerts Therapeutic Effects against Endotoxin-Induced Depression-like Behavior in Mice by Decreasing Inflammatory Cytokines and Increasing Brain-Derived Neurotrophic Factor Levels. Antioxidants .2022; 11(947): 1-14. https://doi.org/10.3390/antiox11050947 .
3. Nesrin M. Morsy, Ashraf S. Hassan, Taghrid S. Hafez, Mohamed R. H. Mahran, Inass A. Sadawe, Abdul M. Gbaj Synthesis, antitumor activity, enzyme assay, DNA binding and molecular docking of Bis-Schiff bases of pyrazole. J Iran Chem Soc. 2021; 18: 47–59. https://doi.org/10.1007/s13738-020-02004-y .
4. Yang Xu Chen, Yanli Li, Shaofen Guo, Zhen Wang, ,Xiuling Yu. Advances in Pharmacological Activitiesof TerpenoidsWenqiang. Nat Prod Commun. 2020; 15(3). https://doi.org/10.1177/1934578X20903555 .
5. Deema A Al-Turki, Mohamed A Al-Omar, Laila A Abou-zeid, Ihsan A Shehata, Mohammed S Al-Awady. Design, synthesis, molecular modeling and biological evaluation of novel diaryl heterocyclic analogs as potential selective cyclooxygenase-2 (COX-2) inhibitors. Saudi Pharm J. 2017; 25(1): 59-69. https://doi.org/10.1016/j.jsps.2015.07.001 .
6. Efraı´n Polo-Cuadrad, Karen Acosta-Quirog, Cristian Rojas-Pen, Yeray A. Rodriguez-Nun˜ez , Yorley Duarte, Iva´n Brito. et al. Molecular modeling and structural analysis of some tetrahydroindazole and cyclopentanepyrazole derivatives as COX-2 inhibitors. Arab J Chem. 2021; 15: 1-13. https://doi.org/10.1016/j.arabjc.2021.103540 .
7. Punsa Tobwor, Pacharawan Deenarn, Thapanee Pruksatraku, Surasak Jiemsup, Suganya Yongkiettrakul, Vanicha Vichai, *et al*. Biochemical characterization of the cyclooxygenase enzyme in penaeid shrimp. PLOS One. 2021; 16(4): 1-24. https://doi.org/10.1371/journal.pone.0250276 .
8. Zhiran Ju, Menglan Li, Junde Xu, Daniel C. Howell, Zhiyun Li, Fen-Er Chen. Recent development on COX-2 inhibitors as promising anti-inflammatory agents: The past 10 years. Acta Pharm Sin B. 2022; 12(6): 2790-2807. https://doi.org/10.1016/j.apsb.2022.01.002
9. Haider Al-Waeli, Ana Paula Reboucas, Alaa Mansour, Martin Morris, Faleh Tamimi, Belinda Nicolau. Non-steroidal anti-inflammatory drugs and bone healing in animal models—a systematic review and meta-analysis. Syst Rev. 2021; 10(201): 1-20. https://doi.org/10.1186/s13643-021-01690-w .
10. Chitra Rawat, Samiksha Kuka, Ujjwal Ranjan Dahiya , Ritushree Kukreti. Cyclooxygenase-2 (COX-2) inhibitors: future therapeutic strategies for epilepsy management. J Neuroinflammation. 2019; 16(197): 1-15. https://doi.org/10.1186/s12974-019-1592-3 .
11. Jessica Ceramella,Domenico Iacopetta, Alessia Catalano, Francesca Cirillo,Rosamaria Lappano , Maria Stefania Sinicropi. A Review on the Antimicrobial Activity of Schiff Bases: Data Collection and Recent Studies. Antibiotics (Basel). 2022; 11(2): 1-23. https://doi.org/10.3390/antibiotics11020191 .
12. Domenico Iacopetta , Jessica Ceramella , Alessia Catalano , Carmela Saturnino ,Maria Grazia Bonomo , Carlo Franchini ,Maria Stefania Sinicropi. Schiff

Bases: Interesting Scaffolds with Promising Antitumoral Properties. Appl Sci. 2021; 11 (1877): 1-20. https://doi.org/10.3390/app11041877 .

13. Edyta Raczuk, Barbara Dmochowska, Justyna Samaszko-Fiertek, Janusz Madaj Different Schiff Bases—Structure, Importance and Classificatio. Molecules. 2022; 27(3): 787. https://doi.org/10.3390/molecules27030787
14. Shorouk S Mukhtar, Ashraf S Hassan, Nesrin M Morsy, Taghrid S Hafez, Fatma M Saleh ,Hamdi M. Hassaneen. Design, synthesis, molecular prediction, and biological evaluation of pyrazole-azomethine conjugates as antimicrobial agents. Synth. Commun. 2021; 51(10): 1564-1580. https://doi.org/10.1080/00397911.2021.1894338.
15. Uba Bala .Synthesis, Characterization and Antimicrobial Activities of Schiff Base Complexes of Co (II) and Cu (II) Derived from Salicyldehyde and Diphenylamine. Sch Int J Chem Mater Sci. 2022; 5(1): 6-10. https://doi.org/10.36348/sijcms.2022.v05i01.002
16. Hassan M A Al-Redha ,Safaa H Ali, Saad S Mohammed. Syntheses, Structures and Biological Activity of Some Schiff Base Metal Complexes. Baghdad Sci J. 2022; 19(2): 704-7015. http://dx.doi.org/10.21123/bsj.2022.19.3.0704.
17. Sahar S Hassan, Nidhal M Hassan, Shaymaa Rajab Baqer, Asmaa M Saleh. Biological Evaluation and Theoretical Study of Bi-dentate Ligand for Amoxicillin Derivativewith Some Metal Ions. Baghdad Sci J. 2021; 18(4): 1269-1278. http://dx.doi.org/10.21123/bsj.2021.18.4.1269
18. Ngozi P Ebosie, Martin Oonwu, Gerald O. Onyedika, Fidelis C. Onwumere. Biological and analytical applications of Schiff base metal complexes derived from salicylidene-4-aminoantipyrine and its derivatives: a review. J Iran Chem Soc. 2021; 18: 3145–3175. https://doi.org/10.1007/s13738-021-02265-1.
19. Ahmed M, Mohammed A, Majid A, Mohd N, Amani S. Synthesis, analgesic, anti-inflammatory, and anti-ulcerogenic activities of certain novel Schiff's bases as fenamate isosteres. Bioorg Med Chem Lett. 2015; 25(2): 179-183. https://doi.org/10.1016/j.bmcl.2014.11.088.
20. Martin Krátký, Magdaléna Dzurková, Janoušek Jiˇrí, Klára Koneˇcná , František Trejtnar , Jiˇrina Stolaˇríková, et al . Sulfadiazine Salicylaldehyde-Based Schiff Bases: Synthesis, Antimicrobial Activity, and Cytotoxicity. Molecules. 2017; 22(9): 1573. https://doi.org/10.3390/molecules22091573.
21. Lei Shi, Hui-Ming Ge, Shu-Hua Tan, Huan-Qiu Li, Yong-Chun Song, Hai-Liang. *et al*. Synthesis and antimicrobial activities of Schiff bases derived from 5-chloro-salicylaldehyde. Eur J Med Chem. 2007; 42(4): 558-64. https://doi.org/10.1016/j.ejmech.2006.11.010 .
22. Aso Hameed Hasan, Sankaranarayanan Murugesan, Syazwani Itri Amran, Subhash Chander, Mohammed M Alanazi, Taibi Ben Hadda .et al. Novel thiophene Chalcones-Coumarin as acetylcholinesterase inhibitors: Design, synthesis, biological evaluation, molecular docking, ADMET prediction and molecular dynamics simulation. Bioorg Chem. 2021; 119: 1-12. https://doi.org/10.1016/j.bioorg.2021.105572.
23. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017; 7(1): 42717. https://doi.org/10.1038/srep42717
24. Kumar V, Roy K. Development of a simple, interpretable and easily transferable QSAR model for quick screening antiviral databases in search of novel 3C-like protease (3CLpro) enzyme inhibitors against SARS-CoV diseases. SAR QSAR Environ Res. 2020; 31(7): 511-526. https://doi.org/10.1080/1062936X.2020.1776388
25. Malgorzata N Drwal , Priyanka Banerjee , Mathias Dunkel , Martin R Wettig , Robert Preissner. ProTox: a web server for the in-silico prediction of rodent oral toxicity. Nucleic Acids Res. 2014; 42(1): W53–W58. https://doi.org/10.1093/nar/gku401.
26. Mubarak A Alamri, Mohammed A. Alamri. Pharmacophore and docking-based sequential virtual screening for the identification of novel Sigma 1 receptor ligands. Bioinformation.2019; 15(18): 586-595. https://doi.org/10.6026/97320630015579.
27. Helen M Berman, John Westbrook, Zukang Feng, Gary Gilliland, Bhat T N, Helge Weissig ,et al. The Protein Data Bank. Nucleic Acids Res .2000; 28(1): 235-42. https://doi.org/10.1093/nar/28.1.
28. Absarul Haque , Ghazanfar Ali Baig , Abdulelah Saleh Alshawli , Khalid Hussain Wali Sait, Bilal Bin Hafeez, Manish Kumar Tripathi. et al. Interaction Analysis of MRP1 with Anticancer Drugs Used in Ovarian Cancer: In Silico Approach .Life. 2022; 12(2): 1-12. https://doi.org/10.3390/life12030383
29. Narmin H. Amin Hussen. Docking Study of Naringin Binding with COVID-19 Main Protease Enzyme. Iraqi J Pharm Sci. 2020; 29(2): 231-238. https://doi.org/10.31351/vol29iss2pp231-238.
30. Trott O , Olson A. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010; 31(2): 455-461. https://doi.org/10.1002/jcc.21334.
31. Kezhal M. Salih, Dana Ameen, Aras Najmaddin Hamad, Aryan Rizgar Ganjo,Sarbast Muhammed. Synthesis and pharmacological profile of some new 2-substituted-2, 3-dihydro-1H-perimidine. Zanco J Med Sci. 2020; 24 (1): 68-79. https://doi.org/10.15218/zjms.2020.010 .
32. Rotondo E, Pietropaolo R, Tresoldi G, .Faraone F, Cusmano F. Mechanism of formation of Schiff base complexes. Part. I. Reaction of Ni (bis-salicylaldehyde) with primary amines.Inorganica Chim. Acta. 1976; 17:181-191
33. Christopher A Lipinski. Lead-and drug-like compounds: the rule-of-five revolution. Drug Discov Today Technol. 2004; 1(4): 337–341. https://doi.org/10.1016/j.ddtec.2004.11.007 .
34. Aso Hameed Hasan, Narmin Hamaamin Hussen,

Sonam Shakya, Joazaizulfazli Jamalis, Mohammad Rizki Fadhil Pratama,et al. In silico discovery of multi-targeting inhibitors for the COVID-19 treatment by molecular docking, molecular dynamics simulation studies, and ADMET predictions. Struct Chem. 2022; 1-21. https://doi.org/10.1007/s11224-022-01996-y

35. Lakshmipraba J, Arunachalam S, Solomon R, Venuvanalingam P, Riyasdeen A, Dhivya R, et al.Surfactant-copper (II) Schiff base complexes: synthesis, structural investigation, DNA interaction, docking studies, and cytotoxic activity. J Biomol Struct Dyn. 2015; 33(4): 877–891. https://doi.org/10.1080/07391102.2014.918523
36. Antoine Daina, Vincent Zoete. A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules. Chem Med Chem. 2016; 11(11): 1117–1121. https://doi.org/10.1002/cmdc.201600182 .
37. Zhao Y H, Abraham M, Le J, Hersey A, Luscombe C, Beck G, et al. Rate-limited steps of human oral absorption and QSAR studies. Pharm Res. 2002; 19(10): 1446–1457. https://doi.org/10.1023/A:1020444330011
38. Alaa A –M Abdel-Aziz, Adel S El-Azab, Nawaf A AlSaif, Mohammed M Alanazi, Manal A El-Gendy, Ahmad J Obaidullah, ,etal. J Enzyme Inhib Med Chem.2020;35(1): 610-621. https://doi.org/10.1080/14756366.2020.1722120

# التوليف والتوصيف والالتحام الجزيئي وتنبؤ ADMET والنشاط المضاد للالتهابات لبعض قواعد شيف المشتقة من الساليسيل ألدهيد كإمكانات محتمل لانزيم الأكسدة الحلقية

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**الخلاصة:**

تم تصميم سلسلة من مركبات مجموعة الساليسيل ألدهيد الحاملة لقاعدة شيف (1-4) ، وتوليفها ، وتعريضها لتنبؤ Insilco ADMET ، والالتحام الجزيئي ، والتوصيف بواسطة تقنيات تحليل FT-IR ، و CHNS ، وأخيراً إلى ملفها المضاد للالتهابات باستخدام طرق فحص مثبطات إنزيمات الأكسدة الحلقية الفلورية جنبًا إلى جنب مع الأدوية القياسية ، والسيليكوكسيب ، والديكلوفيناك. تم استخدام نتائج دراسات ADMET للتنبؤ بالمركبات التي ستكون مناسبة للإعطاء عن طريق الفم ، بالإضافة إلى مواقع الامتصاص ، والتوافر البيولوجي ، و TPSA ، ومثال العقاقير. وفقًا لبيانات ADME ، يمكن امتصاص جميع المواد الكيميائية المنتجة من خلال الجهاز الهضمي. من خلال الالتحام الجزيئي باستخدام PyRx 0.8 ، تم اختبار هذه المركبات المركبة في المختبر لفعاليتها المضادة للالتهابات وانتقائية Insilco تجاه COX-1 و COX-2. أظهر الاختبار في المختبر أن جميع المركبات المنتجة كان لها نشاط أقوى بكثير ضد إنزيم COX-2 من COX-1. وبنائا على ذلك، أظهر المركب 1 النشاط المثبط الأكثر فاعلية مع قيمة $IC_{50}$ 0.19 ميكرومتر مقارنة بالعقار القياسي celecoxib ($IC_{50}$ = 0.29 ميكرومتر). تم توجيه المركب المشتق الأكثر نشاطًا نحو الموقع النشط واحتلال الإنزيم المستهدف بناءً على تحقيق الالتحام ضد COX-1 و COX-2. بالإضافة إلى ذلك ، وجدت التحقيقات في السيليكو أن COX-2 له نشاط تثبيط أعلى من COX-1

**الكلمات المفتاحية:** عامل مضاد للالتهابات، مثبط انزيمات الأكسدة الحلقية، الالتحام الجزيئي، إنسيلكو أدميت (ADMET)، ساليسيل ألدهيد، قاعدة شيف.