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Synthesis, preliminary pharmacological evaluation, molecular docking, and ADME studies of new 4-thiazolidinone derivatives bearing ketoprofen moiety targeting cyclooxygenase enzyme

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Abstract

Thiazolidinone, a saturated form of thiazole with a carbonyl group on fourth carbon, has been considered as a magic moiety (wonder nucleus) that possesses almost all types of biological activities. A new series of 4-thiazolidinones bearing ketoprofen moiety had been designed, then synthesized by reacting Schiff-base with chloroacetic acid and sodium acetate in ethanol according to Baldwin rules for ring closure and finally evaluated as a potent cyclooxygenase-2 (COX-2) inhibitors.

Characterization and identification of the synthesized compounds were established by the determination of ^{1}H-NMR spectra,^{13}C-NMR, FT-IR spectroscopy, and physical properties.

These newly synthesized compounds have been evaluated in vivo for their anti-inflammatory efficiency and In silico selectivity toward COX-2 through molecular docking by using GOLD suite v.5.6.2. All the tested compounds via molecular docking showed anti-inflammatory activity and some of them have significant activity when compared with diclofenac, and ketoprofen as referenced drugs because of having hydrogen bonding interaction toward the key amino acids within COX isozymes Tyr355, and Met522, and all these results were compatible with the study of in vivo acute anti-inflammatory activities for tested compounds.

Also, ADME studies had been accomplished to predict which compounds are a candidate to be taken orally, absorption sites, bioavailabilities, topological polar surface area(TPSA), and also drug-likeness. The ADME results reported that all the synthesized compounds can be absorbed from GIT.

The objective of this work is to synthesize and initial pharmacological assessment of new derivatives of ketoprofen by studying their interactions with COX-1 and COX-2 by docking and to determine some relationships between their structures and biological activity. Incorporating of the 4-thiazolidinone nucleus into ketoprofen moiety to increase the selectivity toward COX-2. Comparing the *In silico* results with *In vivo* results by using egg white to induce acute inflammation. The anti-inflammatory assessment was done for six final compounds.

Keywords ketoprofen, docking, ADME, GOLD, Lipinski rule

1. Introduction

Cyclooxygenase (COX) enzymes have an important role during the synthesis of prostaglandins. Therefore, three isoenzymes of COX enzymes have been discovered; COX-1, COX-2, and COX-3. The constitutional COX-1 isoenzyme that can be found throughout the body, in which it has the responsibility for the protection of the gastrointestinal tract, whereas COX-2 has an inducible isoenzyme responsibility for inflammation [(1,2)]. The COX-3 is a splice variant/isoenzyme of COX-1, encoded by the same gene [(3)]. It was suggested that COX-3 The importance of COX-3 is that it could explain the pharmacological actions of drugs such as acetaminophen and other antipyretic analgesics which are weak inhibitors of COX-1 and COX-2 but penetrate easily into the central nervous system [(4)]. The discovery of the important role of COX-2 isoenzyme has led researchers to study the potency of involvement of these enzymes in pain [(5)]. Studies have been carried out for obtaining some selectivity between COX-1 and COX-2 enzymes. Although COX-1 isoenzyme plays an important role in the protection of the gastrointestinal system and COX-2 is important for pain, the selectivity for inhibition of COX-2 is extremely significant. Since, the development of non-steroid anti-inflammatory drugs (NSAIDs), has the attention of researchers to obtain potent and selective COX-2 inhibitors [(6)]. The COX-2 is the readily inducible kind of enzyme

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and is usually related to many pathological conditions, COX-2 is readily found in the heart [(7)], spinal cord [(8)], vascular endothelium, brain, kidney, bone, and female reproductive system and is also involved in certain physiological processes [(9,10)]. Moreover, it is stimulated by inflammatory stimuli such as bacterial endotoxin and cytokines [(11,12)]. For example, up-regulation of COX-2 in arthritic joints provides the classical symptoms of rheumatoid arthritis (RA). High levels of COX-2 have also been marked in diseases like Alzheimer's disease (AD), systemic lupus erythematous, colon, breast, and pancreatic cancer, although diabetic neuropathy and premature labor [(9,11)].

Ketoprofen is one of the propionic acid classes of nonsteroidal anti-inflammatory drugs (NSAID) with analgesic and antipyretic effects. It acts by inhibiting the body's production of prostaglandin[(13)].

To increase the suppressive effect of ketoprofen on the COX-2 enzyme, some ketoprofen derivatives were synthesized by adding new functional groups to the ketoprofen scaffold. For this purpose, medicinal chemists use different tools to optimize the potency, unwanted property, and selectivity of a given lead drug structure toward a given targeted COX-2 enzyme.

Several biological active molecules contain various heteroatoms like nitrogen, sulfur, and oxygen, always have the attention of chemist over the years mainly due to their biological importance. Thiazolidinones are thiazolidine derivatives, which have an atom of sulfur at position 1, an atom of nitrogen at position 3, and also a carbonyl group at position 2, 4, or 5 [(14)].

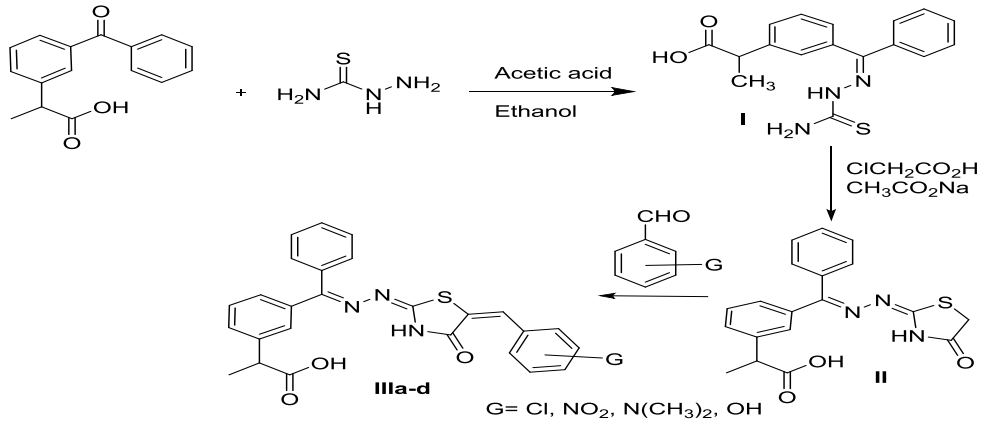
However, its derivatives belong to the most frequently studied moieties and its presence in penicillin was the first recognition of its occurrence in nature.

The 4-thiazolidinone scaffold is very and has featured in several clinically used drugs. They have found uses as antibacterial [(15)], antitubercular [(16)], antiviral agents [(17),] and anti-inflammatory [(18)] agents, especially as anti-HIV agents [(19)].

These newly synthesized compounds may demonstrate potent anti-inflammatory agents and show higher selectivity with COX-2 enzyme because of their larger size than their parent ketoprofen compound, however, the fact of presence the side pocket close to the base of the active site of COX-2 enzyme makes its site 20% larger than that of COX-1, therefore, the active center of COX-2 can accommodate larger structures than those that are capable of fitting the active site of COX-1[(20)].

2. Materials and Methods

All reagents and anhydrous solvents were of analar type and generally used as received from the commercial suppliers (Merck, Germany, Reidel-De Haen, Germany, Sigma-Aldrich, Germany, and BDH, England). Ketoprofen was supplied by the GK Bio-Technology Company, China. Melting points were determined by capillary method on Bamstead/Electrothermal 9100 an Electric melting point apparatus (England). The identification of compounds was done using a UV IR spectrum and was recorded on an FTIR-spectrophotometer FT-IR-6100 TypeA as KBr disks. ^{1}H-NMR determined by H-NMR device Instrument Model: Bruker 300 MHz-Avanc III. ^{13}C-NMR determined by C-NMR device Instrument Model: Bruker 75.65 MHz-Avanc II



Experimental: Synthesis of Ketoprofen thiosemicarbazone derivative, compound(I):

Ketoprofen thiosemicarbazone was synthesized by mixing ketoprofen (0.254g, 0.001mole) and thiosemicarbazide (0.091g, 0.001mole) both dissolved in methanol (10 mL) with one drop of concentrated hydrochloric acid and bringing the reaction mixture to a reflux on the water bath for two hours. A white-colored microcrystalline product

separated when the mixture was allowed to cool, filtered, and then washed with ether and dried in a vacuum[21].

Synthesis_of_2-(3-((E)-(((E)-4-oxothiazolidin-2-ylidene)hydrazono)(phenyl)methyl)phenyl)propanoic acid, compound (II):

A mixture consists of compound I (3.27g, 0.01mole) in absolute ethanol (10mL) that contains chloroacetic acid (0.01mole) with fused sodium acetate (0.03mole) had been refluxed for 6 hrs. The product obtained was then poured onto ice-water (100mL) and the resulted precipitate had been filtered off, then washed with water and dried, finally recrystallized from ethanol. [22].

General procedure for the synthesis of compounds (III_{a-d}):

To a solution of compound II (0.01 mole) and anhydrous sodium acetate (0.015 moles) in the solvent glacial acetic acid (10 mL) had been added the benzaldehyde (0.01 mole). The mixture was then refluxed for 6h with continuous stirring. The mixture was left for cooling, then poured onto crushed ice with stirring. The separated solid had been filtered, then washed with water and dried, finally recrystallized from ethanol [23].

Characterization of prepared compounds

Z)-2-(3-((2-carbamothioylhydrazono)(phenyl)methyl)phenyl)propanoic acid(I):Off-white crystals (84% yield);mp 166-168ºC; IR (KBr) v (cm^{-1}): 3429 (NH2- C=S), 3159 (NH), 1732 (C=O), 1589 (C=N), 927 (C=S); ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 7.25 (s, 1H, SH), δ 8.40 (d, 2H, $\underline{NH_2}$-C=S), δ 8.44 (s, 1H, $\underline{NH}$-C=S), δ 12.39 (br. s, 1H, OH); ^{13}C-NMR (DMSO-d_6, 75.65 MHz): δ 174.39 (1C, C=N), δ 175.50 (1C, C=O), δ 178.35 (1C, C=S).

2-(3-((E)-(((E)-4-oxothiazolidin-2-ylidene)hydrazono)(phenyl)methyl)phenyl)propanoic acid (II): Off-white crystals (88% yield); mp 91-92ºC; IR (KBr) v (cm-1): 3452 (NH), 1728 (C=O carboxylic acid), 1653 (C=O amide), 1589 (C=N); ^{1}H-NMR (DMSO-d6, 300 MHz): δ 3.58-3.62 (doublet of doublet, 2H, S-CH2-C=O), δ 8.44 (s, 1H, NH-C=O), δ 12.39 (br. s, 1H, OH); ^{13}C-NMR (DMSO-d6, 75.65 MHz): δ 33.35 (C-S), δ 161.65 (1C, C=N of ring), δ 166.47 (1C, C=N), δ 166.66 (1C, C=O of 4-thiazolidinone), δ 174.55 (1C, C=O of carboxylic acid).

2-(3-((E)-(((E)-5-((E)-4-chlorobenzylidene)-4-oxothiazolidin-2-ylidene)hydrazono)(phenyl)methyl)phenyl)propanoic acid (III_a): Yellow powder (67% yield); mp 188-189ºC; IR (KBr) v (cm-1): 3452 (NH), 1728 (C=O carboxylic acid), 1654 (C=O amide), 1589 (C=N); ^{1}H-NMR (DMSO-d6, 300 MHz): δ 7.79 (s, 1H, CH vinyl group), δ 8.69 (s, 1H, NH-C=O), δ 12.01 (br. s, 1H, OH); ^{13}C-NMR (DMSO-d6, 75.65 MHz): δ 33.35 (C-S), δ 141.24 (1C , C=C vinyl group), δ 161.83 (1C, C=N of ring), δ 166.30 (1C, C=N), δ 174.27 (1C, C=O of 4-thiazolidinone), δ 174.61 (1C, C=O of carboxylic acid).

2-(3-((E)-(((E)-5-((E)-4-nitrobenzylidene)-4-oxothiazolidin-2-ylidene)hydrazono)(phenyl)methyl)phenyl)propanoic acid (III_b): Orange crystals (75% yield); mp 106-108 ºC; IR (KBr) v (cm-1): 3452 (NH), 1730 (C=O carboxylic acid), 1654 (C=O amide), 1527 (C=N); ^{1}H-NMR (DMSO-d6, 300 MHz): δ 7.79 (s, 1H, CH vinyl group), δ 8.69 (s, 1H, NH-C=O), δ 12.01 (br. s, 1H, OH); ^{13}C-NMR (DMSO-d6, 75.65 MHz): δ 33.27 (C-S), δ 141.24 (1C , C=C vinyl group), δ 161.83 (1C, C=N of ring), δ 166.30 (1C, C=N), δ 174.27 (1C, C=O of 4-thiazolidinone), δ 174.61 (1C, C=O of carboxylic acid).

2-(3-((E)-(((E)-5-((E)-4-hydroxybenzylidene)-4-oxothiazolidin-2-ylidene)hydrazono)(phenyl)methyl)phenyl)propanoic acid (III_c): Green-yellowish crystals (60% yield); mp 80-81ºC; IR (KBr) v (cm-1): 3452 (NH), 1730 (C=O carboxylic acid), 1654 (C=O amide), 1527 (C=N); ^{1}H-NMR (DMSO-d6, 300 MHz): δ 5.92 (s,1H,OH phenol), δ 7.40 (s, 1H, CH vinyl group), δ 8.67 (s, 1H, NH-C=O), δ 12.01 (br. s, 1H, OH); ^{13}C-NMR (DMSO-d6, 75.65 MHz): δ 33.27 (C-S), δ 141.24 (1C , C=C vinyl group), δ 161.83 (1C, C=N of ring), δ 166.30 (1C, C=N), δ 174.27 (1C, C=O of 4-thiazolidinone), δ 174.61 (1C, C=O of carboxylic acid).

2-(3-((E)-(((E)-5-((E)-4-(dimethylamino)benzylidene)-4-oxothiazolidin-2-ylidene)hydrazono)(phenyl)methyl)phenyl)propanoic acid (III_d): Red crystals (73% yield); mp 96-97ºC; IR (KBr) v (cm-1): 3452 (NH), 1728 (C=O carboxylic acid), 1654 (C=O amide), 1554 (C=N); ^{1}H-NMR (DMSO-d6, 300 MHz): δ 3.08 (s,6H, N-$(CH_3)_2$), δ 7.46 (s, 1H, CH vinyl group), δ 8.63 (s, 1H, NH-C=O), δ 9.27 (br. s, 1H, OH); ^{13}C-NMR (DMSO-d6, 75.65 MHz): δ 33.27 (C-S), δ 52.40 (2C,$(CH_3)_2$ of N$(CH_3)_2$), δ 142.43 (1C , C=C vinyl group), δ 162.79 (1C, C=N of ring), δ 166.94 (1C, C=N), δ 174.35 (1C, C=O of 4-thiazolidinone), δ 174.39 (1C, C=O of carboxylic acid).

Computational Method

CCDC GOLD Suite (*v. 5.6.2*) had been utilized for achieving the study of molecular docking for all the tested ligands. CCDC Hermes visualizer software (*v. 1.9.2*) had been utilized for visualizing: the protein used, tested ligands, hydrogen bonding interactions, short contacts, and bond length calculations. These ligands' chemical structures had been drawn with ChemBioOffice (*v. 17.1*) software.

The pharmacokinetic profiles, i.e., ADME of the newly synthesized compounds were reported with the help of the swiss ADME server [24].

ADME procedures:

All the synthesized ligands (III_b-III_f) had been drawn by Chem Sketch (*v.12*), then converted to SMILE name using Swiss ADME tool that estimates the physicochemical parameters and pharmacokinetic characteristics. BOILED EGG was used to predict the lipophilicity and polarity report for the small molecule [25].

Preparation of the tested ligands and protein receptor:

The crystalic structures for the enzyme COX-1 [PDB ID: 3N8Z] and COX-2 [PDBID: 4M11] had been obtained by the Protein Data Bank (PDB), and then their missing atoms had been inserted with the assistance of Swiss PDB Viewer (SPDBV) (*v. 3.7*). The crystalic structures for our COX proteins were prepared by deleting all the molecules of water, then the addition of hydrogen atoms to achieve the correct ionization and tautomeric states of amino acid residues. CheBio3D (*v. 17.1*) had been utilized for minimizing the energy of our synthesized ligands via applying the MM2 force field.

Docking procedures:

The full license version of Genetic Optimization for Ligand Docking (GOLD) (*v. 5.6.2)* had been utilized for the molecular docking [26,27]. The Hermes visualizer software is used in the GOLD Suite for setting up the receptors for the docking process. The binding sites utilized in GOLD docking were defined that all the protein residues inside the 10 A^o of the referenced ligands that hold in the COX protein structure complexes. Five COX-2 proteins had been downloaded from the PDB website (1pxx, 4m11, 3LN1, 3KK6, and 5kIR) for performing the molecular docking process [28]. Therefore, 4m11 had been selected for the docking process of the tested compounds.

The cavity and the active sites had been demonstrated by using CCDC Superstar. The reference ligand of the used protein had been applied for the determination of the radius (10 A^o) of the active sites. Chemscore kinase had been utilized as a configuration template. ChemPLP was applied for the scoring function. The parameter values that were used during the process of docking were kept the default, and all the solutions are scored referring to Piecewise Linear Potential (CHEMPLP) fitness function. Referring to the CHEMPLP, the steric complementary between protein and ligand was determined while the distance and angle-dependent hydrogen are assessed. The docking results, i.e., the binding mode, docked pose, and binding free energy was determined for estimating the interaction between the amino acid residues of the COX-1 and COX-2 proteins and our synthesized compounds.

Results and Discussion - The first step of the reaction exhibit the removal of a proton from NH by sodium acetate resulted in the conversion of the resulting intermediate to partially or totally to thiol form.

Second The second step represents a nucleophilic attack by thiol on a carbon atom that bears a good leaving group (CH-Cl) will result in the formation of a new S-C bond. This step is followed by a nucleophilic attack by NH_2 on the carbon atom of the carbonyl group resulted in the formation of a five-member heterocyclic ring. The carbonyl group at position 4 of the heterocyclic ring may be formed by losing one molecule of water.

Thiosemicarbazone and benzaldehyde derivatives involve the following steps:

Step 1: Formation of enolate ion (acid-base reaction): This is an acid-base reaction. Hydroxide functions as a base and removes an acidic α-hydrogen giving a reactive enolate.

Step 2: Alkoxide formation (nucleophilic addition): The nucleophilic enolate attacks the carbonyl carbon of benzaldehyde in a nucleophilic addition process giving an intermediate alkoxide.

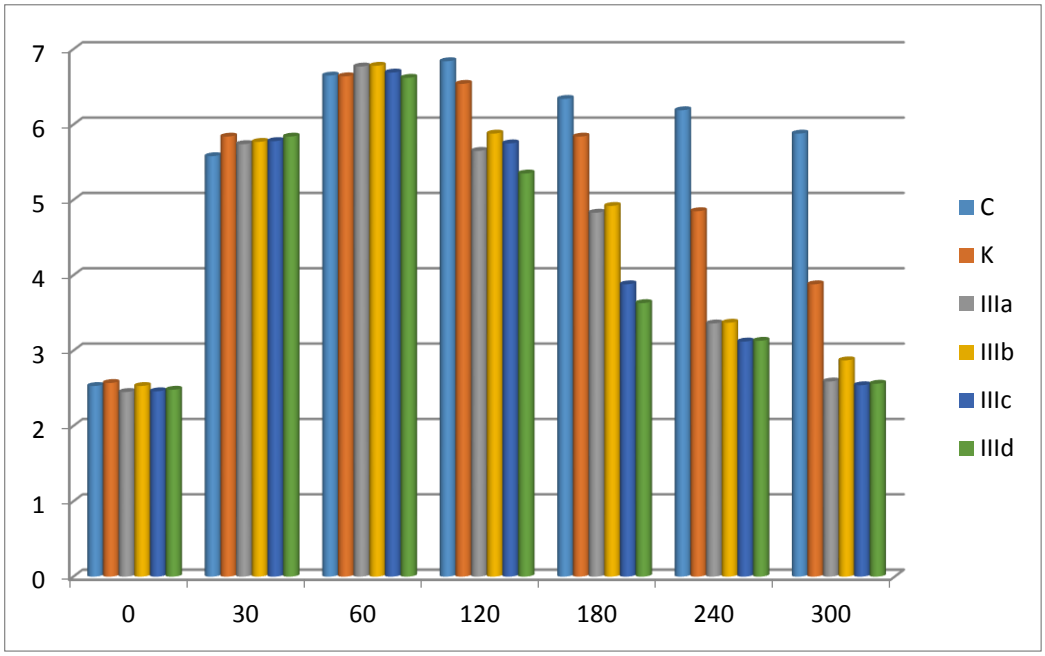
Step 3: Protonation of alkoxide: The alkoxide deprotonates a water molecule producing a hydroxide ion and a β-hydroxy ketone, the aldol product.

Step 4: Dehydration: The hydroxide acts as a base and removes an acidic β-hydrogen giving the reactive enolates. The electrons associated with a negative charge of the enolate are used to form a carbon-carbon double bond (C=C) and displace a leaving group, regenerating the hydroxide giving the final product, the conjugated ketone.

Many irritant agents have been used in the paw-edema method such as dextran, egg-white, and carrageenan solution. The intraplantar injection of egg-white into the rat's hind paw induces progressive edema. To assess the validity of the method (paw-edema) used for the evaluation of newly synthesized anti-inflammatory compounds, ketoprofen was used as a reference compound of known anti-inflammatory activity profile, the results are shown in Table 1 and Fig. 2. Non-identical superscripts (III_a, III_b, III_c, and III_d). Numbers are stated in mm paw width as mean ± SEM. *n* = number of rats. Time (0) is the time of i.p. injection of tested compounds. Time (30) is the time of egg-white injection.

Table 1. The anti-inflammatory action of synthesized compounds (III_a, III_b, III_c, and III_d), ketoprofen, and control on egg-white induced paw edema in rats

Compounds	Time(min) Versus Paw thick. in (mm)						
	0	30	60	120	180	240	300
control	2.53±0.06	5.85±0.04	6.65±0.07	6.84±0.04	6.34±0.04	6.19±0.05	5.88±0.04
ketoprofen	2.57±0.04	5.84±0.08	6.64±0.05	6.54±0.05*[a]	5.84±0.05*[a]	4.85±0.03*[a]	3.88±0.06*[a]
III_a	2.45±0.03	5.74±0.06	6.77±0.04	5.65±0.05*[b]	4.83±0.05*[b]	3.36±0.03	2.59±0.05*[b]
III_b	2.53±0.05	5.77±0.02	6.78±0.03	5.88±0.03*[b]	4.92±0.04*[b]	3.37±0.02	2.87±0.04*[c]
III_c	2.46±0.04	5.78±0.05	6.69±0.06	5.75±0.04*[b]	3.88±0.07*[c]	3.12±0.02*[c]	2.54±0.06*[b]
III_d	2.48±0.03	5.84±0.04	6.62±0.03	5.35±0.04*[c]	3.63±0.06*[c]	3.13±0.04*[c]	2.56±0.03*[b]



(Figure 1)Effect of propylene glycol, ketoprofen, compounds (III_a, III_b, III_c, and III_d) on egg-white provoked paw edema in rats.

Molecular Modeling

GOLD is considered as a "genetic algorithm to dock flexible ligands into protein binding sites" [(29)].GOLD was widely verified and was showed superb
rendering for pose predictions and excellent docking results for the virtual screening [(30)]. It was provided in a part of the GOLD Suite, which has additional software components, such as GoldMine, Hermes, Isostar and Conquest, and Mercury, etc.….

Energy minimization of ligands and proteins can fix the twisted geometries by shifting atoms to exhibit internal constraints. After the energy minimization, the geometry is fixed which means minimum energy has been reached.

To expect the energy of selectivity and binding for the synthesized compounds to COX -2, docking studies were carried out with the help of GOLD Suite software for studying the molecular interactions involved between the synthesized compounds (III_a to III_d) and active binding sites of the targeted protein. The Coxs inhibition action of the compounds III_a-III_d, diclofenac, and ketoprofen had been ranked based on their PLP fitness that involved at the active sites during the complex formation. The docking PLP fitness of all tested compounds on COX 2 was found in the range of **65.58** to **84.98**, respectively table **(2)**.

There was an excellent consistency between our docking results and the
experimental results (*In vivo* study). Ensemble docking is so important because it decreases the risk of accidentally choosing of inappropriate protein model, improving in pose expectations, virtual screening improvements, and ensure that the docking process is true, this is why we started ensemble docking in the first step by involving five different COX-2 proteins.

Docking analysis reported that Arg120, Tyr355, Ser530, Val116, Tyr385,
Gly526, Val523, Trp387, Ala527, Leu531, Leu534, Leu345, Leu539, Val89, Val349, listed in the table **(3-17)** of this enzyme, involved interaction through hydrogen bonding and also short contacts with our tested ligands library.

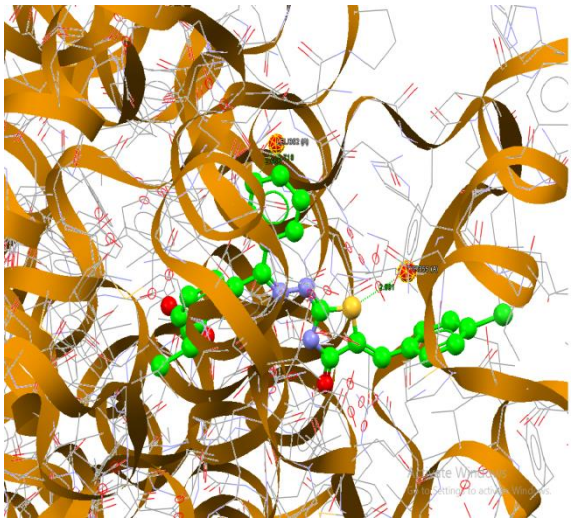
The distance of these short contacts and hydrogen bonds between a specific protein atom and our synthesized ligands is reported by GOLD and all bonds length less than 3A^{o} [31].

The short contacts can be defined as other interaction forces like van der
Waals, electrostatic, steric, pi-pi stacking, dipole-dipole, and others.
All the synthesized ligands having very well docking results with
Coxs, fitting in the COX-2 active site as shown in **(figures 2to 9)** COX- 1 results reported lower binding energy because COX-2 active site is larger than COX-1 active site, and the synthesized ligands have a big structure that makes difficult insertion within COX-1 enzyme pocket.

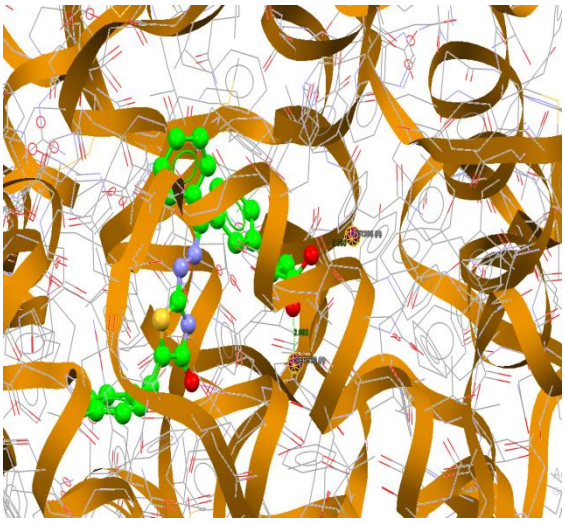
Compound III$_d$ show the best docked PLP fitness, that was 84.98 within
COX-2, there was one H-bond contact with Met522 and short contact with Tyr385 as in **(figure 8)**. Little hydrophobic contacts give low biological activity, when the number of hydrophobic contacts increases, the biological activity will also increase because these contacts will outweigh the H-bonding contacts and these bonds play a major role in the binding of a ligand to an active site [32].

(Table 2): The binding energies for ketoprofen derivatives and references . NSAIDs docking with COX-2**.

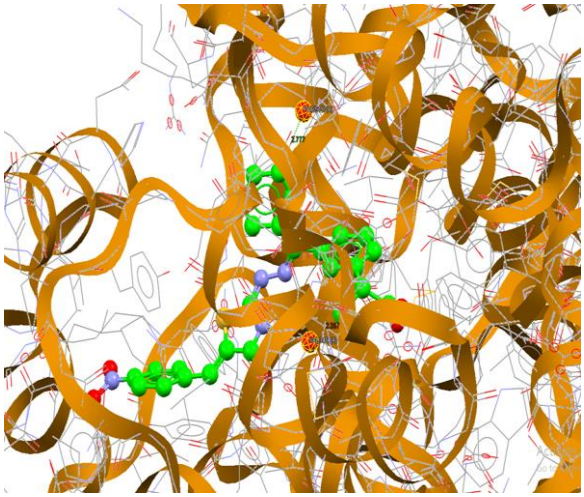
Compounds	COX-2 Binding Energy (PLP Fitness)	RMSD value	Amino Acids Included in H-bonding	Amino Acids Included in Hydrophobic Interactions
III$_a$	78.17	2.5	Tyr385&Ser530	Leu352&Tyr355
III$_b$	65.58	2.5	Ser530	Phe512&Val349
III$_c$	84.98	2.7	Met522	Tyr385
III$_d$	84.51	2.6	Tyr385&Ser530	Leu352,Tyr355&Leu93
Diclofenac	71.7		Ser530 &Tyr385	Ala527, Val349, Gly526 &Trp387
Ketoprofen	67.5	1	Tyr355&Met522	Gly526



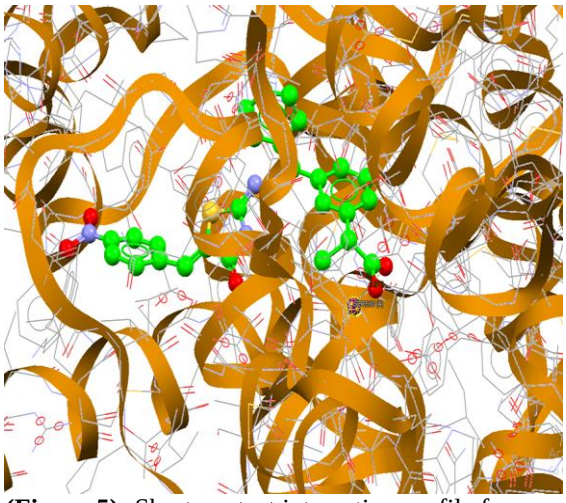
(Figure 2): Hydrogen bond interaction profile for compound III$_a$. The interaction between compound III$_a$ and amino acid residues Tyr385&Ser530. [III$_b$: Ball and stick style, amino acid residues in Ball and stick style, and the active site pocket in purple color].



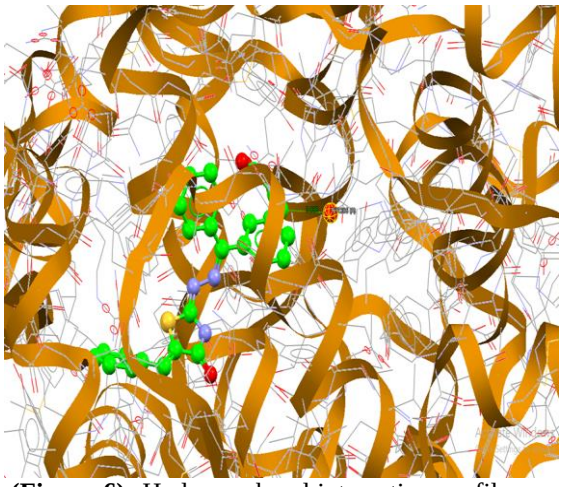
(Figure 3): Short contact interaction profile for compound III$_a$. The interaction between compound III$_a$ and amino acid residues Leu352&Tyr355. [III$_b$: Ball and stick style, amino acid residues in Ball and stick style, and the active site pocket in red color].



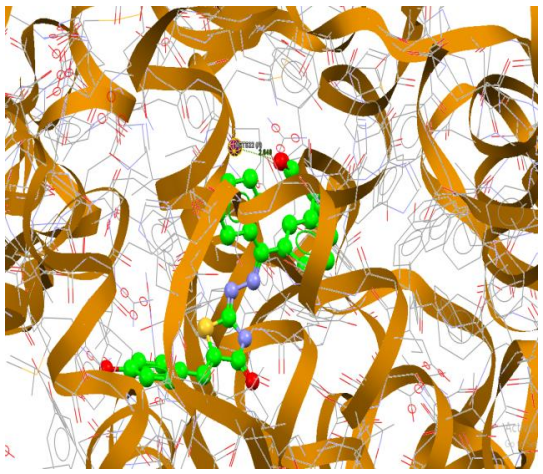
(Figure 4): Hydrogen bond interaction profile for compound III_b. The interaction between compound III_b and amino acid residues Ser530. [III_c: Ball and stick style, amino acid residues in Ball and stick style, and the active site pocket in purple color].



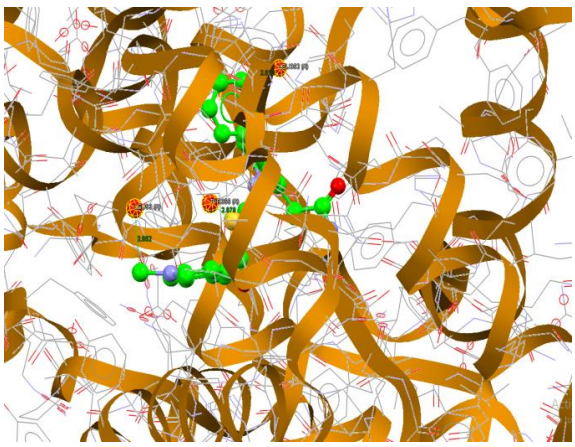
(Figure 5): Short contact interaction profile for compound III_b. The interaction between compound III_b and amino acid residues Phe512&Val349. [III_c: Ball and stick style, amino acid residues in Ball and stick style, and the active site pocket in red color].

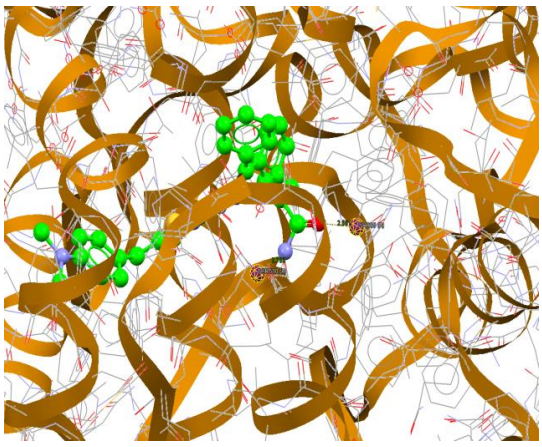


(Figure 6): Hydrogen bond interaction profile for compound III_c. The interaction between compound III_c and amino acid residues Met522. [III_d: Ball and stick style, amino acid residues in Ball and stick style, and the active site pocket in purple color].



(Figure 7): Short contact interaction profile for the compound III_c. The interaction between compound III_c and amino acid residues Tyr385. [III_d: Ball and stick style, amino acid residues in Ball and stick style, and the active site pocket in red color].





(Figure 8): Hydrogen bond interaction profile for the compound III_d. The interaction between compound III_d and amino acid residues Tyr385&Ser530. [III_f: Ball and stick style, amino acid residues in Ball and stick style, and the active site pocket in purple color].

ADME Studies

The ADME studies results for our synthesized analogs had been reported by the Swiss ADME server to demonstrate which is the safer and potent drug candidate(s), for excluding the tested compounds that may fail in the next stages of the drug development because of the uncomplimentary ADME results.

We exposed all the synthesized compounds to ADME (adsorption, distribution, metabolism, excretion) method. Briefly, Lipinski rule related to the oral administration of the drugs that should have ≤ 5 hydrogen bonds donor, ≤ 10 hydrogen bond acceptor, LogP ≤ 5 and molecular weight (M.Wt.) ≤ 500 to be given orally[33].

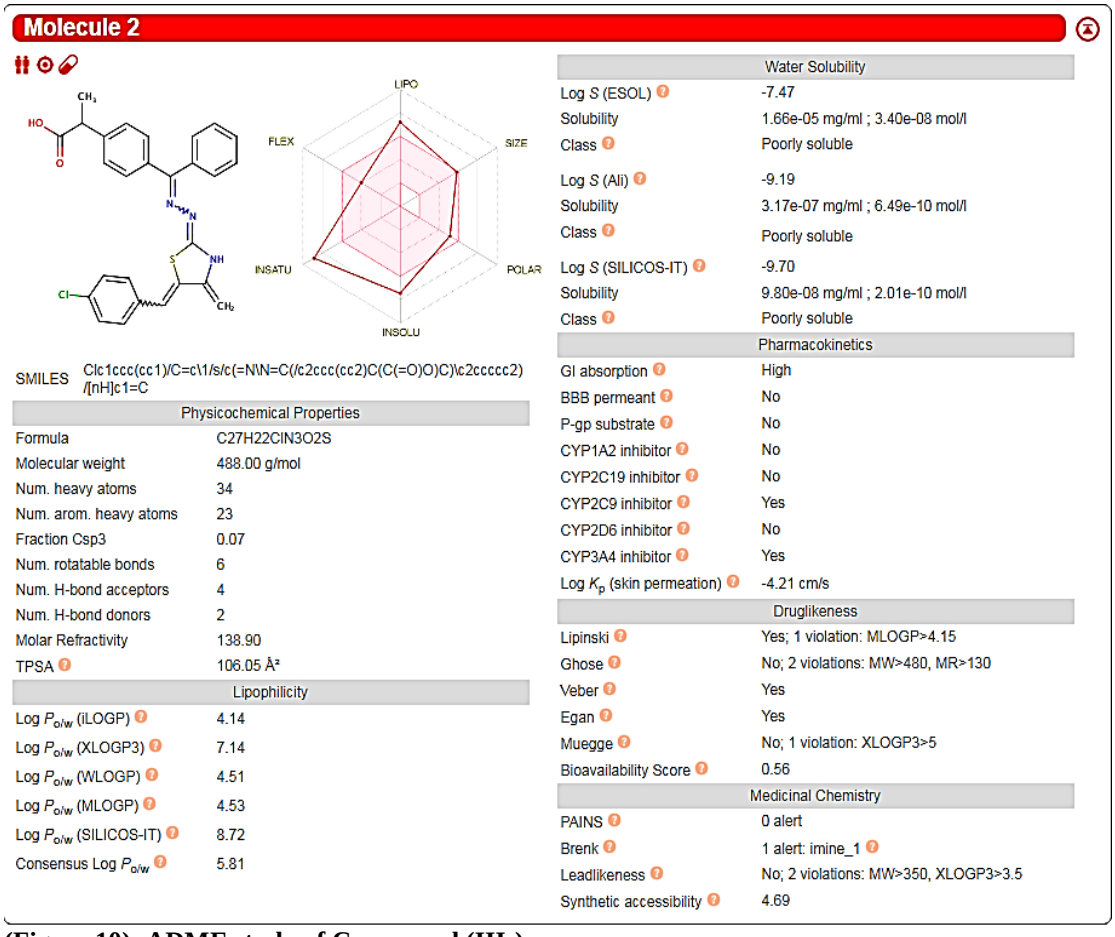
Also, the topological polar surface area (TPSA) was calculated, because it considers as a very important characteristic that was associated with the bioavailability of the drugs.

(Figure 9): Short contact interaction profile for the compound III_d. The interaction between compound III_d and amino acid residues Leu352, Tyr355 & Leu93. [III_f: Ball and stick style, amino acid residues in Ball and stick style, and the active site pocket in red color].

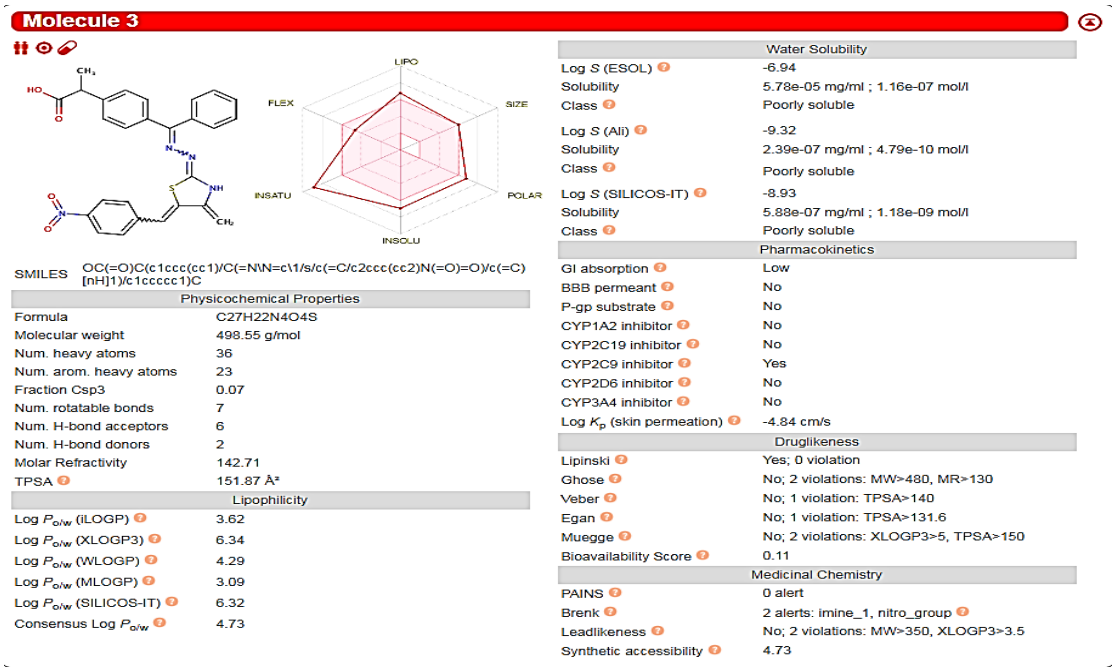
As a result, the passively absorbing molecules within a TPSA >140 A° are considered to have lower oral bioavailability [34]. Our study results reported that all synthesized compounds had TPSA < 140, that are in the range (106-126) and the bioavailability results were 0.55 for all ligands this means that all ligands can reach the systemic circulation.

Compounds III_a, III_b, III_c, and III_d fulfilled the Lipinski rule, (Figures11to 14) respectively. In addition, they also fulfilled the topological description and fingerprints of molecular drug-likeness structure keys such as LogP and Log S.

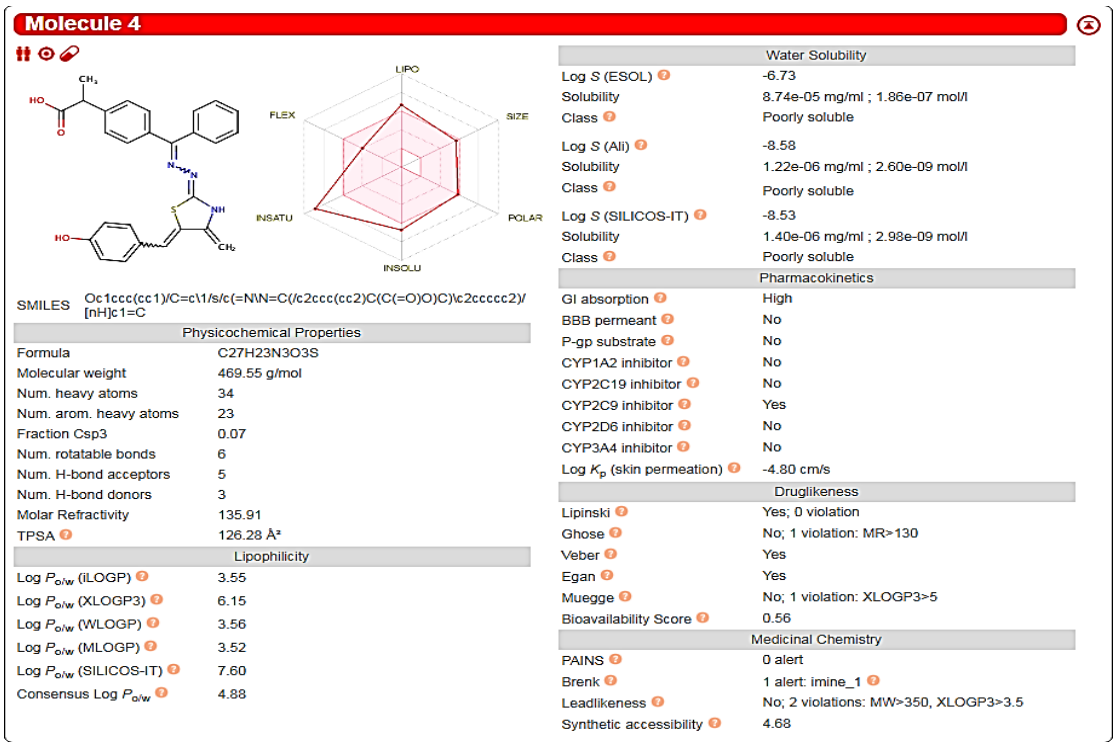
The GI absorption result score is the amount of absorption of a molecule by the intestine after the oral administration. The absorption would be maximum if the result was high. For our study, the absorption of GI for all synthesized ligands was higher than expected to be well absorbed by the intestine.



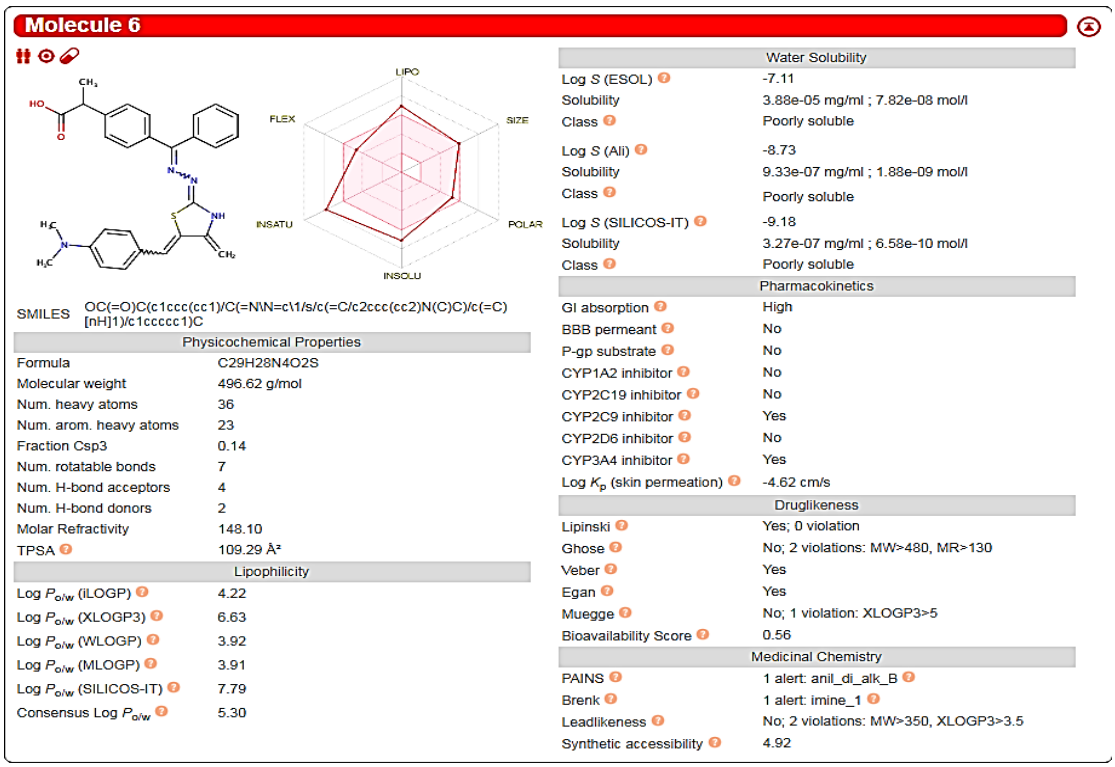
(Figure 10): ADME study of Compound (III_a)



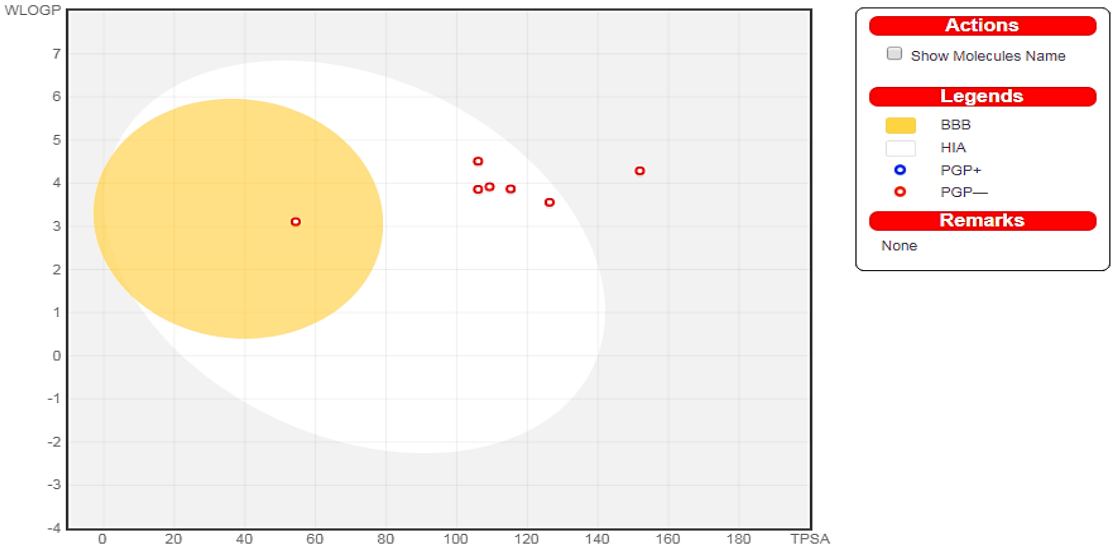
(Figure 11): ADME study of Compound (III_b)



(Figure 12): ADME study of Compound (III_c)



(Figure 13): ADME study of Compound (III_d)



(Figure 14): BOILED EGG – for ketoprofen and final compounds.
Yellow ovule (yolk): are molecules predicted to passively permeate through blood-brain barriers.
White ovule (white): are molecules predicted to passively absorbed by the GIT.
PGP+: Blue dots are for molecules predicted to be effluated from the CNS by the P-glycoprotein.
PGP-: Red dots are for molecules predicted not to be effluated from the CNS by the P-glycoprotein

antiinflammatory efficacy and In silico selectivity toward COX-2 through molecular docking by using GOLD suite v.5.6.2.The study of Docking reported a perfect consistency with In vivo study of compounds (III_a, III_b, III_c, and III_d). The Preliminary study of anti-inflammatory efficiency reported that compounds (III_d) have a higher anti-inflammatory impact than all the other compounds.

Conclusion

The anti-inflammatory evaluation of the final products indicates that the insertion of 4-thiazolidinone pharmacophore into ketoprofen enhanced its anti-inflammatory activity. The study of ADME reported that compounds III_a, III_c, and III_d fulfilled the Lipinski rule and all the synthesized compounds that were absorbed by GIT and had been evaluated in vivo for their

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