Design, synthesis, anti-inflammatory assessment and molecular docking study of novel ketorolac analogues as potent anti-inflammatory agents targeting cyclooxygenase-2 enzyme

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Abstract
Ketorolac is one of the most widely used NSAIDs in the world, it is used for many types of acute pain including and not limited to surgeries and traumas management. In this research, novel Ketorolac derivatives were synthesized, their pharmacological activities were assayed by in vivo and in silico methods. The results revealed that some products were superior over ketorolac regarding binding to receptor’s active sites which was reflected their analgesic activity.

Keywords: cyclooxygenase-2 enzyme, anti-inflammatory; anti-inflammatory

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Introduction
NSAIDs are one of the most commonly prescribed classes of medication for pain and inflammation (1). They are responsible for approximately 5-10% of all medications prescribed each year (2). The prevalence of NSAID use in patients over 65 years old is as high as 96% in the general practice setting (3). Approximately 7.3% of elderly patients over 60 years old filled at least one NSAID prescription in one year period (4). In addition to their anti-inflammatory effect, NSAIDs have antipyretic and analgesic properties. These medications inhibit Cyclooxygenases (COXs) enzymes, which are rate-determining enzymes for prostaglandins and other prostanoids synthesis, such as thromboxanes.

A common definition of acute pain is “the normal, predicted physiological response to an adverse chemical, thermal or mechanical stimulus, associated with surgery, trauma and acute illness”(5). Yet patients’ attitudes, beliefs, and personalities also strongly affect their immediate experience of acute pain. Over 50 years ago, Beecher found soldiers requested less analgesic medication than civilians with comparable injuries. He observed that the injured soldier expected evacuation and safe recuperation, but the civilian expected loss of wages and social hardship. Beecher set up placebo trials (6), trials of clinical analgesics (7), and investigated how personality and culture shape the experience of acute pain (8). Accordingly, experimental clinical pain research may produce results that are not duplicated in clinical settings, where anxiety, sleep disruption, and illness burden are present (9).

Acute pain should, therefore, be viewed as the initiation phase of an extensive, persistent nociceptive and behavioral cascade triggered by tissue injury (10). This cascade (11) has the potential to span orders of magnitude of space and time but generally subsides within weeks. If suppression of pain responses was not mobilized along with processes of pain amplification, any minor injury could progress to chronic pain (unfortunately some do). An individual’s responses for months after transient injury may be determined by processes that occur within the first day. As with other complex dynamic systems, (12) small differences in the initial state of the host and in the
intensity, quality, and meaning of the nociceptive stimulus can produce major differences in the detailed manner in which this process unfolds.

Ketorolac was first introduced in 1990 in the US. Within 3 years following the launch of ketorolac, 16 million patients had received the drug with 97 mortalities reported, nearly half of which were related to gastrointestinal complications. Table I summarizes the findings of the Council of International Organizations of Medical Sciences for adverse events reported for ketorolac \(^{(13)}\). By 1992, the European Union member states were divided over the risk-benefit ratio of ketorolac, and 5 countries (The Netherlands, Greece, Portugal, Germany, and France) had instituted a ban on its use \(^{(14)}\). Because of the number of adverse events reported, the significant regulatory review began in a number of countries following the release of ketorolac. This review quickly led to a revision of labeling, dosage recommendations and prescribing practices \(^{(15, 16)}\). Most of the reports of serious toxicity with ketorolac occurred at high dosages and in patients in whom drug therapy would not currently be recommended and predated the manufacturer’s revised dosage guidelines. Happily, these revised recommendations have resulted in fewer adverse effects.

**Table (1).** Summary of ketorolac adverse events by body system, March 1990 to June 1994\(^{[1]}\)

<table>
<thead>
<tr>
<th>Body system</th>
<th>Number of events</th>
<th>Proportion of total events %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal</td>
<td>533</td>
<td>27.9</td>
</tr>
<tr>
<td>General/Non specific</td>
<td>366</td>
<td>19.2</td>
</tr>
<tr>
<td>Haematological</td>
<td>277</td>
<td>14.5</td>
</tr>
<tr>
<td>Urinary</td>
<td>238</td>
<td>12.5</td>
</tr>
<tr>
<td>Respiratory</td>
<td>123</td>
<td>6.4</td>
</tr>
<tr>
<td>Nervous system</td>
<td>101</td>
<td>5.3</td>
</tr>
<tr>
<td>Psychiatric</td>
<td>63</td>
<td>3.3</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>41</td>
<td>2.1</td>
</tr>
<tr>
<td>Integumenary</td>
<td>39</td>
<td>2</td>
</tr>
<tr>
<td>Metabolic and endocrine</td>
<td>37</td>
<td>1.9</td>
</tr>
<tr>
<td>Injection site</td>
<td>32</td>
<td>1.7</td>
</tr>
<tr>
<td>Hepatobiliary</td>
<td>31</td>
<td>1.6</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>11</td>
<td>0.6</td>
</tr>
<tr>
<td>Reproductive</td>
<td>9</td>
<td>0.5</td>
</tr>
<tr>
<td>Neoplasia</td>
<td>1901</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Scheme 1: Overall scheme of reagents and reaction conditions: (i) SOCl₂, reflux, 2.5 h; (ii) THF, triethyl amine, amine, 72 C, 6 h; (iii) HetArCHO, C₂H₅OH, AcOH, reflux, 4 h. Molecule 1 is Ketorolac.

Materials and Methods

General procedure for the synthesis of ketorolac amide derivatives [(S)-5-benzoyl-N-aryl-2, 3-dihydro-1H-pyrrolizine-1-carboxamide]

A mixture of (5.5 mmoles of Ketorolac: 1 in Scheme 1) and (6.0 mmoles of thionyl chloride) was refluxed on an oil bath for 2.5 h. The excess of thionyl chloride was removed under vacuum in order to produce ketorolac acyl chloride which is obtained as an orange oil. Then, (5.5 mmoles of ketorolac acyl chloride) was dissolved in tetrahydrofuran (25 mL), (5.5 mmoles of Triethylamine) and (5.5 mmoles of amine derivatives or hydrazide) were added into this solution and then the solution was subjected to increased temperature to 72 C for a period of time of 6 h. Then the mixture was filtered and the solvent was evaporated. The residue was chromatographed by TLC using the mobile phase (ethyl acetate: hexane as 1:1). The solvent was evaporated under reduced pressure to obtain ketorolac amide derivatives (Scheme 1).

General procedure for the synthesis of ketorolac acyl hydrazine derivatives[(S, E)-5-benzoyl-N’-ethylidene-2, 3-dihydro-1H-pyrrolizine-1-carbohydrazide derivatives]
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(5.5 mmoles of Ketorolac acyl chloride) was dissolved in (25 mL of tetrahydrofuran), then (5.5 mmoles of Triethylamine) and (5.5 mmoles of hydrazine hydrate) were added into this solution which was heated at 72 C for 7 h. The reaction mixture was poured into the cold water. Then the precipitate was filtered and washed with water. The crude ketorolac hydrazide was refluxed with a mixture of heteroaryl aldehydes and glacial acetic acid in ethanol for 5 h. Then the reaction mixture was cooled and the precipitate was filtered. The crude product was recrystallized from ethanol yielded ketorolac acyl hydrazone derivatives (Scheme 1).

Computational Methods:
The computational approach adopted in this work is outlined in (Figure 2-1). CCDC GOLD Suite (v. 5.7.3) was used to perform the molecular docking studies for the compounds. CCDC Hermes visualizer software (v. 1.10.3) was used to visualize: the protein, ligands, hydrogen bonding interactions, short contacts, and bond length calculation. The chemical structures of our ligands were drawn using the ChemBioOffice (v. 19.1) software.

Preparation of ligands and protein receptor:
The crystal structures of the enzyme COX 2 [PDB ID: 3LN1] were downloaded from the Protein Data Bank (PDB), and their missing atoms were added with the assistance of Swiss PDB Viewer (SPDBV) (v. 3.7). The crystal structures of our downloaded proteins were prepared by removing all water molecules and by adding hydrogen atoms to get a correct ionization and tautomeric states of amino acid residues. CheBio3D (v.19.1) was used to minimize the energy for our synthesized ligands by applying the MM2 force field.

Docking procedures:
The full license version of Genetic Optimization for Ligand Docking (GOLD) (v. 5.6.2) was used for molecular docking (17,18). The Hermes visualizer software in the GOLD Suite was used to set up the receptors for the docking process additionally. The binding site used for GOLD docking was defined as all the protein residues within the ten A° of the reference ligands that exist in the downloaded protein structure complexes. COX-2 protein (3LN1) were downloaded from the PDB website to perform the process of docking.

The cavity and the active site were determined by using CCDC Superstar. The reference ligand of the protein was used to determine the radius (10 A°) of the active site. Chemscore kinase has been used as a configuration template. ChemPLP was used for the scoring function. The values of all parameters used during the process of docking were kept the default, and all solutions are scored according to Piecewise Linear Potential (CHEMPLP) fitness function. According to CHEMPLP, the steric complementary between protein and ligand is calculated while the distance and angle-dependent hydrogen are considered. The results of docking, i.e., the binding mode, docked pose, and binding free energy was studied to evaluate the interaction between the amino acids residues of the proteins COX-2 and our synthesized ligands.

Hot Plate Test
The hot plate test has been described previously by Baker et al. in 2002 (19). Rats were placed on an aluminum hot plate kept at a temperature of 55± 0.5 °C for a maximum time of 30 s. The temperature of the plate was monitored at all times. Animals were divided into ten groups (n = 6 for each group). Group 1 served as the placebo, group 2 served as ketorolac, group (3 – 10) received a fixed dose of our synthesized ligands listed in table II. The reaction time was determined when animals licked their forepaws and/or hind paws and jumped before and 15, 30, 45, 60, 90, 120, and 180 min after the IV injection of each synthesized ligand. After each measurement, the plate was wiped with a damp cloth to remove traces of urine and feces.

Anti-inflammatory Test
Animals
Albino rats of either sex weighing (250 ± 50 gm) were supplied by Biotechnology Research Center, Al-Nahrain University, and were housed under standardized conditions in the Biotechnology Research Center, Al-Nahrain University animal house. Commercial chaw was used to feed the animals and they had free access to water ad libitum. Animals were brought to the laboratory, one hour before the experiment, animals were divided into nine groups (six rats per group) as follow: Group A: injected with the vehicle (dimethyl sulfoxide 10% v/v) and served as a control group. Group B: injected with Ketorolac as reference substance with a dose of 50mg/kg (19), dissolved in dimethyl sulfoxide 10% (v/v). Group C-I: injected with the tested compounds (2a-3d) by doses that are determined below. (Dissolved in dimethyl sulfoxide 10% v/v).
Calculations for Dose Determination \(^{(20)}\)
Dose of reference compound / Mwt. of reference
Compound = Dose of tested compound / Mwt. of tested compound.

### Table (2): Compounds with their molecular weight and dose.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Molecular weight g/mol</th>
<th>Dose mg / kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketorolac</td>
<td>255.3</td>
<td>2</td>
</tr>
<tr>
<td>2a</td>
<td>342.4</td>
<td>2.7</td>
</tr>
<tr>
<td>2b</td>
<td>356.4</td>
<td>2.8</td>
</tr>
<tr>
<td>2c</td>
<td>370.5</td>
<td>2.9</td>
</tr>
<tr>
<td>2d</td>
<td>374.4</td>
<td>2.9</td>
</tr>
<tr>
<td>3a</td>
<td>347.3</td>
<td>2.7</td>
</tr>
<tr>
<td>3b</td>
<td>363.4</td>
<td>2.8</td>
</tr>
<tr>
<td>3c</td>
<td>396.5</td>
<td>3.1</td>
</tr>
<tr>
<td>3d</td>
<td>530.6</td>
<td>4.1</td>
</tr>
</tbody>
</table>

**Experimental Design**
Egg albumin was used to induce rat paw edema as an acute inflammatory model for studying the activity of the final compound \(^{(21)}\). 0.05mL of undiluted ovalbumin was subcutaneously injected into the rats’ planter side of the hind paw; preceded by a half-hour of intraperitoneal injection of the drugs or their vehicle. Electronic vera used for measuring paw thickness at 7 time periods (0, 30, 60, 120, 180, 240, and 300 minutes) after the Compounds injection \(^{(22)}\).

**Statistical Analysis**
The mean ± SEM was used to report data of this work then student t-test (Two-Sample Assuming Equal Variances) used to calculate data statistical significance between means. Analysis of variance (Two factors without Replication) is used to compare between different groups. P-value < 0.05 was assumed significant.
Results

2a: 5-benzoyl-N-(2, 2-dimethoxyethyl)-2, 3-dihydro-1H-pyrrolizine-1-carboxamide
Oily product (61%) IR (KBr) \(v\,(\text{cm}^{-1})\): 1239.54 (C-O-CH\(_3\)), 1645.21 (C=O amide), 1703.32 (C=O), 1598.41 (Aromatic), 3303.05 (NH). \(^1\)H NMR (400 MHz, CDCl\(_3\)): 1.96, 2.23 (d of t, 2H, CH2 of pyrrolidine), 3.5 (s, 6H dimethoxy), 3.61 (d, 2H, CH2 of carboxamide), 3.73 (t, 1H, CH of pyrrolidine), 3.81, 3.92 (m, 2H, CH2 of Pyrrolidine), 5.75 (t, 1H, CH bridge of dimethoxy), 6.05 (d, 1H of CH of pyrrol), 7.5 (d, 1H of CH of pyrrol), 7.45-7.8 (m, 5H aromatic H).

2b: 5-benzoyl-N-(2, 2-dimethoxyethyl)-N-methyl-2, 3-dihydro-1H-pyrrolizine-1-carboxamide
Oily product (52%) IR (KBr) \(v\,(\text{cm}^{-1})\): 1237.22 (C-O-CH\(_3\)), 1648.09 (C=O amide), 1699.12 (C=O), 1594.15 (Aromatic). \(^1\)H NMR (400 MHz, CDCl\(_3\)): 1.91, 2.26 (d of t, 2H, CH2 of pyrrolidine), 2.89 (s of aminomethyl 3H), 3.56 (s, 6H dimethoxy), 3.64 (d, 2H, CH2 of carboxamide), 3.77 (t, 1H, CH of pyrrolidine), 3.83, 3.88 (m, 2H, CH2 of Pyrrolidine), 5.79 (t, 1H, CH bridge of dimethoxy), 6.11 (d, 1H of CH of pyrrol), 7.44 (d, 1H of CH of pyrrol), 7.47-7.84 (m, 5H aromatic H).

2c: 5-benzoyl-N-(4, 4-dimethoxybutyl)-2, 3-dihydro-1H-pyrrolizine-1-carboxamide
Oily product (49%) IR (KBr) \(v\,(\text{cm}^{-1})\): 1240.88 (C-O-CH\(_3\)), 1642.07 (C=O amide), 1705.76 (C=O), 1594.73 (Aromatic), 3301.85 (NH). \(^1\)H NMR (400 MHz, CDCl\(_3\)): 1.47 (d of d, 4H CH2-CH2), 1.95-2.27 (d of t, 2H, CH2 of pyrrolidine), 3.54 (d, 2H, CH2 of carboxamide), 3.77 (t, 1H, CH of pyrrolidine), 3.83, 3.88 (m, 2H, CH2 of Pyrrolidine), 5.71 (t, 1H, CH bridge of dimethoxy), 6.09 (d, 1H of CH of pyrrol), 7.53 (d, 1H of CH of pyrrol), 7.44-7.83 (m, 5H aromatic H).

2d: 5-benzoyl-N'-isonicotinoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxyhydrazide
Oily product (53%) IR (KBr) \(v\,(\text{cm}^{-1})\): 1234.80 (C-O-CH\(_3\)), 1644.44 (C=O amide), 1665.72 (C=O amide), 1705.39 (C=O), 1605.73 (Aromatic), 3311.08 (NH). \(^1\)H NMR (400 MHz, CDCl\(_3\)): 1.93-2.27 (d of t, 2H, CH2 of pyrrolidine), 3.57 (s, 6H dimethoxy), 3.76 (t, 1H, CH of pyrrolidine), 3.85-3.96 (m, 2H, CH2 of Pyrrolidine), 5.79...
(t, 1H, CH bridge of dimethoxy), 6.02 (d, 1H of CH of pyrrol), 7.56 (d, 1H of CH of pyrrol), 7.47-8.32 (m, 9H aromatic and pyridine H), 9.05 (s, 2H of Hydrazine).

3a: 5-benzoyl-N’-(furan-2-ylmethylene)-2, 3-dihydro-1H-pyrrolizine-1-carbohydrazide
Yellow Crystals (79%). m.p.: 149–152 °C. IR (KBr) v (cm⁻¹): 1645.21 (C=O amide), 1651.46 (C=C), 1671.39 (C=N), 1703.32 (C=O), 1598.41 (Aromatic), 3303.05 (NH). ¹H NMR (400 MHz, d₆-DMSO): 1.92-2.29 (d of t, 2H, CH₂ of pyrrolidine), 3.63 (2H, CH₂ of carboxamide), 3.78 (t, 1H, CH of pyrrolidine), 3.77-3.89 (m, 2H, CH₂ of Pyrrolidine), 6.09 (d, 1H of CH of pyrrol), 6.6-6.9 (m, 3H of furan), 7.56 (d, 1H of CH of pyrrol), 7.49-7.82 (m, 5H aromatic H), 8.35 (s, 1H of CH of methanimine), 10.49 (s, 1H of NH).

3b: 5-benzoyl-N’-(thiophen-2-ylmethylene)-2, 3-dihydro-1H-pyrrolizine-1-carbohydrazide
Brown Crystals (62%). m.p.: 149–152 °C. IR (KBr) v (cm⁻¹): 1640.81 (C=O amide), 1648.73 (C=C), 1667.08 (C=N), 1698.24 (C=O), 1602.16 (Aromatic), 3303.05 (NH). ¹H NMR (400 MHz, d₆-DMSO): 1.87-2.21 (d of t, 2H, CH₂ of pyrrolidine), 3.69 (2H, CH₂ of carboxamide), 3.8 (t, 1H, CH of pyrrolidine), 3.72-3.82 (m, 2H, CH₂ of Pyrrolidine), 6.11 (d, 1H of CH of pyrrol), 6.63-6.86 (m, 3H of thiol), 7.54 (d, 1H of CH of pyrrol), 7.53-7.88 (m, 5H aromatic H), 8.39 (s, 1H of CH of methanimine), 10.53 (s, 1H of NH).

3c: N’-((1H-indol-2-yl) methylene)-5-benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carbohydrazide
White Crystals (72%). m.p.: 149–152 °C. IR (KBr) v (cm⁻¹): 1645.38 (C=O amide), 1653.78 (C=C), 1672.44 (C=N), 1689.11 (Aromatic), 3308.01 (NH). ¹H NMR (400 MHz, d₆-DMSO): 1.91-2.32 (d of t, 2H, CH₂ of pyrrolidine), 3.66 (2H, CH₂ of carboxamide), 3.82 (t, 1H, CH of pyrrolidine), 4.2 (t, 2H of CH₂ attached to carbazole), 6.09 (d, 1H of CH of pyrrol), 7.48-7.83 (m, 12H of three aromatic rings H), 8.34 (s, 1H of CH of methanimine), 10.54 (s, 1H of NH), 11.21 (s, 1H of NH of indole).

3d: 5-benzoyl-N’-((9-hexyl-9H-carbazol-2-yl) methylene)-2, 3-dihydro-1H-pyrrolizine-1-carbohydrazide
Pale yellow Crystals (77%). m.p.: 149–152 °C. IR (KBr) v (cm⁻¹): 1628.03 (C=O amide), 1648.15 (C=C), 1666.68 (C=N), 1696.78 (C=O), 1603.43 (Aromatic), 3302.87 (NH). ¹H NMR (400 MHz, d₆-DMSO): 0.91 (t, 3H of CH₃), 1.25-1.5 (m, 8H of (CH₂)₄), 1.86-2.33 (d of t, 2H, CH₂ of pyrrolidine), 3.57 (d, 2H, CH₂ of carboxamide), 3.8 (t, 1H, CH of pyrrolidine), 3.75-3.86 (m, 2H, CH₂ of Pyrrolidine), 4.2 (t, 2H of CH₂ attached to carbazole), 6.09 (d, 1H of CH of pyrrol), 7.55 (d, 1H of CH of pyrrol), 7.48-7.83 (m, 12H of three aromatic rings H), 8.34 (s, 1H of CH of methanimine), 10.56 (s, 1H of NH).

Molecular Docking Results
Table (3): molecular docking results of the synthesized compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>COX-2 Binding Energy(PLP Fitness)/Kcal/Mol</th>
<th>Amino Acids Included in H-bonding</th>
<th>Amino Acids Included in Hydrophobic Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketorolac</td>
<td>55.03</td>
<td>ARG 106, TYR 341</td>
<td>ARG 106 (4), SER 516, VAL 335 (4), PHE 504 (5), VAL 509.</td>
</tr>
<tr>
<td>2A</td>
<td>61.33</td>
<td>SER 516, ARG 106</td>
<td>LEU 517, VAL 335 (3), TRP 373 (2), VAL 102 (3), ARG 106 (7), TYR 341 (2)</td>
</tr>
<tr>
<td>2B</td>
<td>70.54</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2D</td>
<td>73.33</td>
<td>GLM 178 (2), LEU 338 (2)</td>
<td>PHE 504 (5), ALA 502 (6)</td>
</tr>
<tr>
<td>3A</td>
<td>64.1</td>
<td>SER 339, HIS 75</td>
<td>SER 339, ALA 502 (3), GLN 178, PHE 504 (6), VAL 509 (2)</td>
</tr>
<tr>
<td>3B</td>
<td>60</td>
<td>HIS 75</td>
<td>VAL 509 (7), PHE 504 (6), GLN 178, LEU 338, SER 339.</td>
</tr>
<tr>
<td>3C</td>
<td>60</td>
<td>VAL 568, GLN 178</td>
<td>VAL 568 (2), GLN 178 (5), HIS 75</td>
</tr>
<tr>
<td>3D</td>
<td>97.27</td>
<td>PHE 504</td>
<td>LEU 338 (2), GLM 178 (4), HIS 75, PHE 504 (6)</td>
</tr>
</tbody>
</table>

3d pose of compound.

Table (4): The anti-inflammatory effect of control, Ketorolac and compounds 2a-3d on egg-white induced paw edema in rats.
*significantly different compared to control (P<0.05). Data are expressed in mm paw thickness as mean ± SEM. n= number of animals. Time (0) is the time of i.p. injection of mefenamic acid and dimethyl sulfoxide. Time (30) is the time of injection of egg white (induction of paw edema).
Discussion
Ketorolac is a very dangerous NSAID because of its severe side effects related to GI irritations, in this work we tried to decrease these side effects and enhance the anti-inflammatory activity of the synthesized ligands.

The most widely utilized crucial test to investigate novel anti-inflammatory compounds measures the capability of the compound to diminish local edema which stimulated in the rat paw by injecting an irritant agent \( \text{(23)} \). There are many different irritants available that have been used widely in the induction of paw edema such as egg-white, dextran and carrageenan solution; the last one has been expansively examined in the measurement of the anti-inflammatory effect of NSAIDs containing numerous chemical mediators including histamine, bradykinin, prostaglandins, and serotonin \( \text{(24)} \). Compound 2a, 2d, and 3d showed good agreement results between docking and hot plate test, these compounds showed the best analgesics activities in comparison with ketorolac and other synthesized ligands.

Compound 3d showed best anti-inflammatory activity in paw edema reduction and this is a good agreement with the docking results with the COX-2 enzyme (the plp fitness was 97) this mean the compound has a good fitting with the enzyme cavity and has an H bonding with PHE 504 and short contacts with LEU 338 (2), GLM 178 (4), HIS 75, PHE 504 \( \text{(6)} \) all these amino forms the active site of the COX-2 enzyme. The second and third ligands which have good anti-inflammatory activities were 2D and 2B respectively GOLD is a “genetic algorithm for docking flexible ligands into protein binding sites” \( \text{(25)} \). GOLD has been broadly tested and has shown excellent performance for pose prediction and excellent results for virtual screening \( \text{(26, 27)} \). It is supplied as part of the GOLD Suite, which includes additional software components, Hermes, Mercury, Isostar and Conquest, and GoldMine, etc. There is an excellent agreement between our docked results and experimental results (Invivo study).

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