Anti-inflammatory, analgesic evaluation and molecular docking studies of o-benzoyl benzoic acid based 1,3,4-oxadiazole analogues

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ABSTRACT

A novel series of o-benzoyl benzoic acid based 1,3,4-oxadiazoles were prepared and subjected to anti-inflammatory, analgesic activity as well as molecular docking studies to target cyclooxygenase-2 enzyme. 1,3,4-Oxadiazole derivatives were screened for anti-inflammatory activity in carrageenan-induced rat paw edema and analgesic activity by tail immersion method. In synthesized compounds, the free carboxylic group, which is responsible for gastric side effects, was derivatized by heterocyclic 1,3,4-oxadiazole bioactive core, which showed good interaction with COX-2 receptor with good docking score. Among all the synthesized compound having m-methoxy-p-hydroxy have emerged out as potential COX-2 inhibitor.

**Key Words:** 1,3,4-Oxadiazole, anti-inflammatory activity, analgesic activity, COX-2 inhibitor
1. Introduction
Non-steroidal anti-inflammatory drugs (NSAIDs) form an imperative class of commonly used therapeutic agents due to their anti-inflammatory, analgesic and antipyretic effects. The biological activity of NSAIDs is associated to suppression of prostaglandin biosynthesis by inhibiting the enzyme cyclooxygenase (COX). COX is an endogenous enzyme which catalyzes the conversion of arachidonic acid into prostaglandins. The isoforms (COX-1 and COX-2) catalyze the same biochemical transformation but are subject to a dissimilar expression regulation. COX-1 is responsible for the physiological function of prostaglandins (PGs) like maintenance of the integrity of the gastric mucosa and provides adequate vascular homeostasis whereas COX-2 is expressed only after an inflammatory stimulus [1].

NSAIDs are commonly associated with one noticeable side effect i.e., formation of gastric ulcers and various others like gastric upset, irritation and ulceration. 1,3,4-oxadiazole nucleus possess a diversity of useful biological effects such as anti-edema and anti-inflammatory activities. 1,3,4-Oxadiazoles have anti-inflammatory activity by virtue of dual mechanism i.e., inhibiting both COX/LOs to reduce gastric ulceration [2]. The reported literature confirms that gastrointestinal side effects of NSAIDs such as irritation, GI bleeding etc. are due to the presence of a free carboxylic group in drug [3-6]. Our studies and studies of other researchers [6] have shown that derivatization of the carboxylate function of some NSAIDs resulted in an increased anti-inflammatory activity with a reduced ulcerogenic effect. Hence, by including the oxadiazolyl moiety, expecting to get better anti-inflammatory compounds.

2. Material and Method
2.1 Preparation of target compounds

Target compounds were prepared previously in our laboratory [7](Figure 1). The aromatic esters were prepared by Fischer esterification. Further these esters were subjected to formation of aryl hydrazide in presence of hydrazine hydrate and cyclized in presence of bromine, acetic acid and sodium acetate by stirring for 2-3 hours. Similarly compounds (13b-e) were prepared. The physical properties and spectral characterization were already discussed [7].

2.2 Chemicals and instruments

All chemicals and reagents used in the estimation were of analytical grade. Carrageenan was purchased from sigma chemicals. A digital plethysmometer (model pth-7070, sr.no.pt
070509, Medicad system) was used for measuring paw volume (ml) of rats edema. Molecular docking studies were carried out on Molecular Virtual Docker 5.0.0.

![Molecular structure]

Figure 1: o-Benzoyl benzoic acid based substituted 1,3,4-oxadiazole analogues (13a-e)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>13a</td>
<td>[苯基]</td>
</tr>
<tr>
<td>13b</td>
<td>[苯基]Cl</td>
</tr>
<tr>
<td>13c</td>
<td>[苯基]NO2</td>
</tr>
<tr>
<td>13d</td>
<td>[苯基]Me</td>
</tr>
<tr>
<td>13e</td>
<td>[苯基]OMe</td>
</tr>
</tbody>
</table>

### 2.3 Animals

Albino rats of either sex, having weight around 180-200g were selected for both anti-inflammatory and analgesic activity respectively. The animals were kept in polypropylene cages (3 in each cage) at an ambient temperature of 20±2°C and 55-65% relative humidity. A 12-12 hrs light and dark schedule was maintained in the animal house. The rats had free access to water and fed. The experimental protocol was approved by Institutional Animals Ethical Committee (MMCP/IEC/10/16) and animal care was done as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India.

### 2.4 Acute toxicity studies
It has been fond from literature survey that 1,3,4-oxadiazole derivatives in a dose of 1000 mg/kg produces mortality (LD_{50}) in rats. So on tenth of the LD_{50} i.e., 100 mg/kg(ED_{50}) was selected as a dose for anti-inflammatory and analgesic activity [8].

2.5 In vivo anti-inflammatory activity

In carrageenan model albino rats of all groups were treated with subcutaneous injection of 0.1 ml of 1% w/v solution of carrageenan into the sub plantar region of the right hind paw. The paw was marked with permanent marker at the planter region where paw volume was to be measured. The diclofenac sodium (10mg/kg) and test compounds (100mg/kg) were suspended in 0.3% sodium carboxy methyl cellulose. The test compounds, vehicle (control) were administered p.o with the help of gastric cannula half an hour after the injection of carrageenan in sub planter region of right paw. Mean normal paw volume was measured 30 min. prior to carrageenan injection by using plethysmometer. Mean increase in the paw volume for control group (after carrageenan injection) and test group was measured at 1hr, 2hr and 3hr[9]. Percent inhibition of inflammation after test/standard was calculated using the formula 1.

\[
\% \text{ inhibition} = \frac{V_c - V_t}{V_c} \times 100
\]  
(1)

Where \( V_t \) is the of paw volume (ml) of test/standard compound at corresponding time and \( V_c \) is paw volume (ml) of control.

2.6 Analgesic activity

Tail immersion method is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice. Albino rats were divided in twenty six groups each containing six animals. The tail of mice was immersed (1-2 cm) in warm water kept constant at 55ºC. The reaction time was recorded through stop watch (the reaction time is the time taken by the rat to flick their tails). The latent period of the tail flick response will determine before and 15, 30, 60 and 120 min. after drug administration [10].

2.7 Statistical analysis

The results were expressed as mean±SEM for six animals in each group for anti-inflammatory and analgesic activity. All the grouped data was statistically evaluated. Hypothesis testing method included one way analysis of variance (ANOVA) followed by Dunnett’s comparison test. P-values of less than 0.05 were considered to indicate statistical significance.
2.8 Molecular docking studies

Comparative docking of set of ligands with specific proteins involves methodology with Easy user interface and their respective scoring function provided by Molegro Virtual Docker. Molecular docking studies were performed to target COX-2. The compound which have shown potent anti-inflammatory and analgesic activity (13e) were subjected to molecular docking to target COX-2 (Pdb-1CX2)[11,12].

Steps in methodology:
1) Importing a protein file and ligand file and preparation of ligands.
2) Protein preparation and detecting cavities of protein molecules.
3) Executing a docking set up through docking wizard panel.
4) Poses of protein-ligand complex obtained after docking process with their specific mol dock scores displayed in output file.

3. Results
3.1 In vivo anti-inflammatory activity

1,3,4-Oxadiazole of o-benzoyl benzoic acid series compounds were evaluated for anti-inflammatory activity in carrageenan induced rat paw edema. Among all the compounds, 13e have shown maximum activity at 100mg/kg (Table 1).

3.2 Analgesic activity

1,3,4-Oxadiazole compounds of o-benzoyl benzoic acid series were evaluated for analgesic activity by tail immersion method. Among all the compounds, 13e have shown maximum analgesic activity at 100mg/kg (Table 2).

Table-1: In vivo anti-inflammatory activity of synthesized 1,3,4-oxadiazole analogues

<table>
<thead>
<tr>
<th>Groups</th>
<th>Change in Paw edema volume (ml)</th>
<th>1 h</th>
<th>Percentage Inhibition</th>
<th>2 h</th>
<th>Percentage Inhibition</th>
<th>3 h</th>
<th>Percentage Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.43±0.05</td>
<td>-</td>
<td>0.72±0.06</td>
<td>-</td>
<td>0.98±0.09</td>
<td>-</td>
</tr>
<tr>
<td>Diclofenac sodium (10mg/kg)</td>
<td></td>
<td>0.25±0.03</td>
<td>57.13</td>
<td>0.30±0.02</td>
<td>58.33</td>
<td>0.26±0.01</td>
<td>73.46</td>
</tr>
<tr>
<td>13a(100mg/kg)</td>
<td></td>
<td>0.37±0.04</td>
<td>13.9</td>
<td>0.57±0.02</td>
<td>20.83</td>
<td>0.71±0.07</td>
<td>27.55</td>
</tr>
<tr>
<td>13b(100mg/kg)</td>
<td></td>
<td>0.29±0.05*</td>
<td>32.55</td>
<td>0.38±0.03*</td>
<td>47.23</td>
<td>0.38±0.04*</td>
<td>59.22</td>
</tr>
</tbody>
</table>
13c (100mg/kg)  0.30±0.08  30.22  0.42±0.06*  41.67  0.39±0.07*  58.20
13d (100mg/kg)  0.36±0.04  16.2  0.54±0.01  25.00  0.65±0.03  33.67
13e (100mg/kg)  0.27±0.04*  37.2  0.37±0.04*  48.62  0.30±0.04*  70.40

All values are expressed as Means ±S.E.M, (n=6), *P<0.05 vs control.

Table-2: Analgesic activity of activity of synthesized 1,3,4-oxadiazole analogues

<table>
<thead>
<tr>
<th>Groups</th>
<th>Reaction time (Sec.)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(After 30 min)</td>
<td>(After 60 min)</td>
<td>(After 120 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.43 ± 1.7</td>
<td>5.29 ± 0.9</td>
<td>6.30 ± 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diclofenac sodium (10mg/kg)</td>
<td>5.07 ± 1.6</td>
<td>10.60± 2.0</td>
<td>18.83± 2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13a (100mg/kg)</td>
<td>4.93 ± 2.4</td>
<td>6.59±0.04</td>
<td>8.43±2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13b (100mg/kg)</td>
<td>5.03 ± 0.9</td>
<td>8.05±0.03</td>
<td>10.42±3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13c (100mg/kg)</td>
<td>5.07 ± 1.2</td>
<td>7.49±0.04</td>
<td>9.38±1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13d (100mg/kg)</td>
<td>5.73 ± 0.8</td>
<td>7.81±0.02</td>
<td>9.40 ±2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13e (100mg/kg)</td>
<td>5.10 ± 1.0</td>
<td>10.76±0.06*</td>
<td>15.28±2.6*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed as Means ±S.E.M, (n=6), *P<0.05 vs control.

3.3 Molecular docking studies

1,3,4-Oxadiazole compounds were subjected for molecular docking to target COX-2. Compound 13e have shown good interaction with COX-2. The interaction of ligand (13e), Diclofenac sodium, and selective cyclooxygenase inhibitor-558 with receptor in terms of docking score (binding energy) was depicted in Table 3 (Figure 2 and 3).

Table-3: Ligand-receptor interaction of target compound and standard drugs

<table>
<thead>
<tr>
<th>Compound</th>
<th>Docking score</th>
<th>Distance</th>
<th>Amino acid</th>
<th>Group involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>13e</td>
<td>-98.534</td>
<td>2.69</td>
<td>Thr 476</td>
<td>Nitrogen of oxadiazole ring</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.41</td>
<td>Nitrogen of oxadiazole ring</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.11</td>
<td>Oxygen of oxadiazole ring</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.92</td>
<td>Nitrogen of oxadiazole ring</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.99</td>
<td>Nitrogen of oxadiazole ring</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.17</td>
<td>Carbonyl group of diphenylmethanone</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>-58.259</td>
<td>2.69</td>
<td>Lys 492</td>
<td>-O- of nitro group</td>
</tr>
</tbody>
</table>
Fig-2: Binding mode of 13e (13e as docking view and 13e’ as interaction view) with COX-2, where blue/green lines and red lines represent hydrogen bonding and favourable steric interactions respectively.
4. Discussion

Gastric ulceration is the main side effect associated with NSAIDs which is due to presence of free acid functionality in structure of molecule. This free acidic group was masked by introduction of 1,3,4-oxadiazole nucleus. A series of 1,3,4-oxadiazole derivatives which were previously prepared in our laboratory, subjected to anti-inflammatory, analgesic and molecular docking studies to target COX-2. From all compounds 13ehaving m-methoxy and p-hydroxywere found to be potent in both analgesic and anti-inflammatory which is comparable to standard.
Also the presence of para and ortho substituted halogens (-Cl) in the compound results in enhanced biological activities. This is attributed due to their good lipophilicity that could facilitate the penetration or passage of these compounds across the biological membrane easily.

The docking score of 13e was much more than standard drug diclofenac sodium and SC-S58 (selective COX-2 inhibitor). The docking score is representative of binding energy of ligand to receptor. The replacement of free carboxylic group by oxadiazole nucleus in target compound 13e enhanced the interaction by formation of numerous hydrogen bonds with COX-2. These results could be used for development of novel, potent and safe COX-2 inhibitors.

Results also revealed that the hydrogen bond distance is important in docking studies. The distance more than 3.2Å indicates weak hydrogen bond bonding between ligand and receptor, 2.6Å-3.2Å represents good hydrogen bonding.

5. Conclusion

Compounds having 1,3,4-oxadiazole nucleus instead of a free carboxylic group are lacking of gastrointestinal side effects. The compound 13e have presented good interaction with COX-2 enzyme and thus emerged out as potential lead for the development of novel anti-inflammatory agent with good effectiveness and fewer side effects.

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References


