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Design, Synthesis and Analgesic Activity of 3-Chlorobenzoyl-N-(2-amino-4-chlorophenyl)anthranilic acid
Tanaya Jati Dharma Dewi, Siswandono Siswodihardjo, Juni Ekowati
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Design, Synthesis and Analgesic Activity of 3-Chlorobenzoyl- 
N-(2-amino-4-chlorophenyl) anthranilic acid

Tanaya Jati Dharma Dewi, Siswandono Siswodihardjo and Juni Ekowati

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Abstract

Anthranilic acid derivatives such as a mefenamic acid are well known as an analgesic drug. Modification of anthranilic acid derivate was designed to get better analgesic activity. Docking studies were performed using Molegro Virtual Docker 5.0 software with the protein target cycooxygenase-2 receptor (PDB ID: 5IKR). The synthesis was carried out by modification Scotten Baumann reaction via nucleophilic reaction of N-(2-amino-4-chlorophenyl) anthranilic acid and 3-chlorobenzoyl chloride. Synthesized compound was characterized by IR, $^1$H-NMR, $^{13}$C-NMR, Mass spectrometry. Biological activity for analgesic of the proposed compound by writhing test method on mice (Mus musculus). The result from docking studies revealed that rerank score of 3-chlorobenzoyl-N-(2-amino-4-chlorophenyl) anthranilic acid get lower than mefenamic acid which related its higher analgesic activity. 3-Chlorobenzoyl-N-(2-amino-4-chlorophenyl) anthranilic acid showed better pain inhibition activity compare mefenamic acid with ED$_{50}$ = 30.443 mg/kgBW. This result gave a development of anthranilic acid derivate to be potential analgesic drug candidate.

Keywords: Anthranilic acid derivative, docking, analgesic activity.

1. Introduction

The definition of pain according to the International Association for the Study of Pain (IASP) is a response and emotion that does not implicate in terms of actual or potential tissue damage or is terminologically described as a kind of damage. The cause of pain can be due to tissue damage or other pathophysiology, commonly occurs because of the influence of psychological [1]. Pain is an area widely used in drug development in the pharmaceutical industry. The development of drugs in analgesics and non-steroidal anti-inflammatory drugs is focused on the prevention of prostaglandin production [1].

Cyclooxygenase-2 (COX-2) is associated with an inflammatory response to fever and pain. COX-2 is expressed in the central nervous system and is associated with a central role directly to the central pain response. The mechanism of action of the cyclooxygenase is used as a potential basis for the development of new drugs. COX-2 inhibition of pain is a target of analgesic and anti-inflammatory drugs. Cyclooxygenase-2 enzyme work on the procession of release prostaglandin from arachidonic acid and its caused pain sensation.[2]

The N-arylthranilic acid derivative is a structural analog of salicylic acid derivatives. This derivative has an analgesic and anti-inflammatory activity. Its analgesic activity is proportional to pyrazolone derivatives and its anti-inflammatory activity is greater than that of salicylic acid derivatives [3]. The study of anthranilic derivatives has been modified and there has been an increase in analgesic and anti-inflammatory activity [4-6]. Anthranilic acid is a nitrogen analog of salicylic acid. N-Arylanthranilic acid derivatives are mainly used as an analgesic to reduce mild to moderate pain [7]. Examples of these derived drugs are mefenamic acid (Fig.1).
2. Material and Method

2.1 Docking

Structures were drawn experimentally by Chembiodraw v.12 and optimized for the 3D structure to minimized energy in ChemBio3D v12. docking of the compound on cyclooxygenase-2 protein (PDB ID: 5IKR) with mefenamic acid for native ligand was done using Molegro Virtual Docker 5.5. This step was obtained protein-ligand interaction which showed their minimum energy for interaction. The result of docking is got form rerank score, and lower rerank score is assumed that easier for interaction with a receptor that related with better activity. Preliminary test using ChembioDraw v.12 and ChemBio3D v12 to get physicochemical properties showed possessed CLogP value (lipophilic parameter) = -6.4; Etotol (electronic parameter) = -67.78; and CMR (steric parameter) = 10.60. Docking compound on the cyclooxygenase-2 enzyme (PDB ID: 5IKR) with mefenamic acid as a native ligand.

2.2 Synthesis

The reaction started with prepared cooled round flask in an ice cube for 15 minutes and stabilized the temperature in 0-5 °C, then mixture solution N-(2-amino-4-chlorophenyl)anthranilic acid in 40 ml tetrahydrofuran with pyridine. A separating funnel is mixed with 5 mmol of 3-chlorobenzoyl chloride with 15 ml of tetrahydrofuran. After that adding solution in tetrahydrofuran dropwise for 30 minutes, then reflux this reaction at 60 °C. The solvent was evaporated and washed product with sodium bicarbonate then recrystallized with methanol. The purity of the compound was measured by thin layer chromatography and melting point. Analysis structure of the synthesized compound was determined with ultraviolet (UV), infrared (IR), 1H-nuclear magnetic resonance (1H-NMR), 13C-nuclear magnetic resonance (13C-NMR) and a mass spectrometer to confirm the structure of 3-chlorobenzoyl-N-(2-amino-4-chlorophenyl) anthranilic acid.

2.3 Analgesic activity

The analgesic activity of the compound was performed by chemically induced pain inhibition (writhing test) method. Licensing for this study has approved by Airlangga University Animal Care and Use Committee written on the ethical clearance document no: 687-KE. Muscle contractions were induced on mice (Mus musculus) by intraperitoneal injection of 0.6% solution of acetic acid. Male mice aged 6-8 weeks previously adapted for 1 week before to use. Mice swayed overnight are not fed but given a drink. Six mice every group weighed 20-30 grams divided from the negative control group, positive control group and treatment group divided by three doses 25 mg/kg BW, 50 mg/kg BW and 100 mg/kg BW. The negative control group was given a mucilage CMC Na 0.6%. The positive control group was given a comparison of mefenamic acid.
3. Result
3.1 Docking Study Result

Figure 4: Docking of mefenamic acid on cyclooxygenase-2 Protein (PDB ID: 5IKR) as native ligand

Figure 5: Docking of 3-chlorobenzoyl-N-(2-amino-4-chlorophenyl) anthranilic acid on cyclooxygenase-2 Protein (PDB ID: 5IKR)

Figure 6: Interaction of mefenamic acid with cyclooxygenase-2 (PDB ID: 5IKR)

Figure 7: Interaction of 3-chlorobenzoyl- amino acid from N-(2-amino-4-chlorophenyl) anthranilic acid amino acid from cyclooxygenase-2

Table 1: Amino acid involved in interactions between mefenamic acid and 3-chlorobenzoyl-N-(2-amino-4-chlorophenyl) anthranilic acid on the COX-2 receptor

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rerank Score (kcal/mol)</th>
<th>Amino Acid</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mefenamic Acid</td>
<td>-83.203</td>
<td>Tyr 385</td>
<td>Hydrogen and Steric</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ser 530</td>
<td>Hydrogen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Met 522</td>
<td>Steric</td>
</tr>
<tr>
<td>3-Chlorobenzoyl-N-(2-amino-4-chlorophenyl)</td>
<td>-109.314</td>
<td>Tyr 355</td>
<td>Hydrogen and Steric</td>
</tr>
<tr>
<td>anthranilic acid</td>
<td></td>
<td>Ser 353</td>
<td>Hydrogen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leu 552</td>
<td>Hydrogen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arg 513</td>
<td>Hydrogen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>His 90</td>
<td>Steric</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ala 516</td>
<td>Steric</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Val 523</td>
<td>Steric</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phe 518</td>
<td>Steric</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ser 530</td>
<td>Steric</td>
</tr>
</tbody>
</table>
3.2 Spectral Analysis of Synthesized Compound

This was the result of structure confirmation of 3-chlorobenzoyl-N-(2-amino-4-chlorophenyl)anthranilic acid: pale yellow powder, yield 20.20%, melting point 181-182°C. UV spectrum, λmax (nm) in methanol 228, 238 and 338. IR spectrum, ν (cm⁻¹) in KBr pellet: 3466 (O-H carboxylic), 3234 (N-H secondary amine), 1699 (C=O amide), 1483-1594 (C=C aromatic), 1161-1335 (C-N amine). 1H-NMR spectrum, δ (ppm) in dimethyl sulfoxide (DMSO) D6: 13.01 (s, 1H, OH), 10.22 (s, 1H, NH secondary amine), 9.70 (s, 1H, NH amide) 7.86 (m, 1H, ArH), 7.83 (m, 1H, ArH), 7.55 (m, 1H, ArH), 7.54 (m, 1H, ArH), 7.53 (m, 1H, ArH), 7.52 (m, 1H, ArH), 7.50 (m, 1H, ArH), 7.34 (m, 1H, ArH), 7.30 (m, 1H, ArH), 7.02 (d, 1H, ArH), 6.74 (t, 1H, ArH). 13C-NMR spectrum, δ (ppm) in DMSO: D6: 170.29 (C=O carboxylic), 166.62 (C=O amide), 165.31 (C=N); 136.58; 135.68; 134.48; 133.70; 132.28; 132.16; 132.08; 130.91; 128.08; 127.70; 127.16; 127.02; 126.98; 126.97; 124.23; 118.15; 113.90 (Ar C), and HRMS: calculated for C20H13N2O5C12 [M-H]⁻ = 399.030, [M-H]⁺ found = 399.028.

3.3 Analgesic Activity using writhing test in mice (Mus musculus)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Doses</th>
<th>Percentage of Pain Inhibition</th>
<th>ED₅₀ (mg/kgBW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mefenamic Acid</td>
<td>25 mg/kgBW</td>
<td>42.27% ± 11.90</td>
<td>43.602</td>
</tr>
<tr>
<td></td>
<td>50 mg/kgBW</td>
<td>51.42% ± 10.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 mg/kgBW</td>
<td>62.14% ± 7.38</td>
<td></td>
</tr>
<tr>
<td>3-Chlorobenzoyl-N-(2-amino-4-chlorophenyl)-anthranilic Acid</td>
<td>25 mg/kgBW</td>
<td>40.37 % ± 18.42</td>
<td>30.443</td>
</tr>
<tr>
<td></td>
<td>50 mg/kgBW</td>
<td>65.93 ± 5.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 mg/kgBW</td>
<td>82.15 ± 4.39</td>
<td></td>
</tr>
</tbody>
</table>

4. Discussion

Table 2 showed that 3-chlorobenzoyl-N-(2-amino-4-chlorophenyl) anthranilic acid get lower ED₅₀ than mefenamic acid as a positive control. The result of the biological activity is aligned with docking result (Table 1) that suggest lower rerank score can be easier to interacting with the receptor. Structure modification by adding benzyol group gave the effect of biological activity. Benzyol group will increase the lipophilicity of the compounds, enhance the penetration through the membrane and increase drug-receptor interaction. By adding benzyol group is expected to increase biological activity[7].

Figure 6 and 7 showed that 3-chlorobenzoyl-N-(2-amino-4-chlorophenyl) anthranilic acid had more interactions with amino acid receptor than mefenamic acid. There were four hydrogen interactions and six steric interactions from 3-chlorobenzoyl-N-(2-amino-4-chlorophenyl) anthranilic acid interaction with receptor while mefenamic acid had two hydrogen interactions and two steric interactions. Chloro group from meta position at benzyol (Figure 7) also gave additional steric interaction. This differences number of interaction at amino acid receptor between 3-chlorobenzoyl-N-(2-amino-4-chlorophenyl) anthranilic acid and mefenamic acid can be a possible relation of increasing analgesic activity.

5. Conclusion

This study gets the conclusion that modified anthranilic acid derivate by adding benzyol and chloro group from the N-aryl anthranilic acid structure, get better analgesic activity than mefenamic acid using writhing test method on mice (Mus musculus). 3-Chlorobenzoyl-N-(2-amino-4-chlorophenyl)anthranilic acid can be developed as a further potential analgesic drug candidate.

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References


