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Design, Synthesis and Analgesic Activity of 3-Chlorobenzoyl-N-(2-amino-4-chlorophenyl)anthranilic acid

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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

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Airlangga, Jl. Dharmawangsa Dalam, Surabaya, Indonesia

QR Code

***Correspondence Info:**
Tanaya Jati Dharma Dewi,
Department of Pharmaceutical Chemistry,
Faculty of Pharmacy, University of Airlangga,
Jl. Dharmawangsa Dalam, Surabaya, Indonesia

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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 cyclooxygenase-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 enzym work on the procession of release prostaglandin from arachidonic acid and its caused pain sensation.[2]

The *N*-arylanthranilic 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).

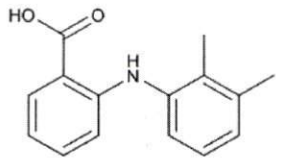


Figure1: Structure of Mefenamic Acid

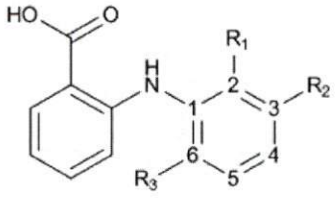


Figure 2: Structure of *N*-Aryl-anthranilic acid derivatives

Rational drug design develops potential compound using the computational method. A computational approach which is done simulation in silico or also known as Computer Aided Drug Design (CADD). The designed compound is changed in 3-dimensional form and is seen as how it interacts with the receptor [8]. In silico study was by docking structure using Molegro Virtual Docker to get rerank score. Rerank score (RS) was used as the score prediction platform. Lower RS compound has stable drug-receptor bond interaction, so it can be predicted that compound has higher biology activity [9].

In this research, we have modified anthranilic acid derivate as a potential analgesic.The *N*-arylanthranilic pharmacophore approach was modified from an *N*-(2-amino-4-chlorophenyl) anthranilic acid compound reacted with a benzoyl chloride derivative, in which the addition of substituents to the position of C 2 atoms of the benzene ring. In addition, the addition of the benzoyl group will increase the lipophilicity of the compounds (Fig. 2) so that it is expected to facilitate the penetration of the membrane so that it is aligned with the increase of analgesic activity [7].

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; Etotal (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), ^{1}H-nuclear magnetic resonance (^{1}H-NMR), ^{13}C-nuclear magnetic resonance (^{13}C-NMR) and a mass spectrometer to confirm the structure of 3-chlorobenzoyl-*N*-(2-amino-4-chlorophenyl) anthranilic acid.

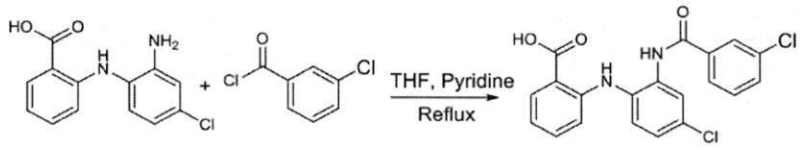


Figure 3: Synthesis of 3-Chlorobenzoyl-*N*-(2-amino-4-chlorophenyl) anthranilic Acid

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 mices every group weighed 20-30 grams divided from the negative control group, positive control group and treatment group divided by three doses 25 mg/kgBW, 50 mg/kgBW 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

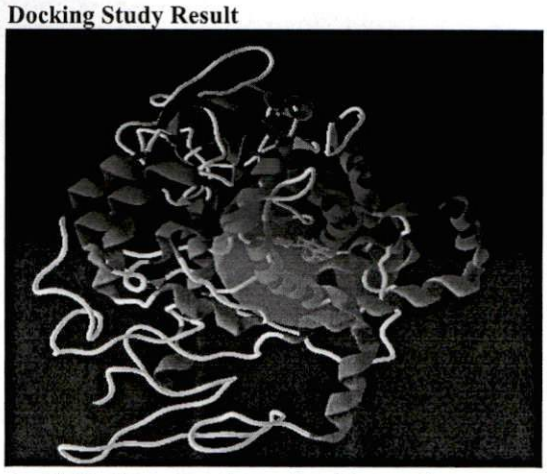


Figure 4: Docking of mefenamic acid on cyclooxygenase-2 Protein (PDB ID: 5IKR) as native ligand

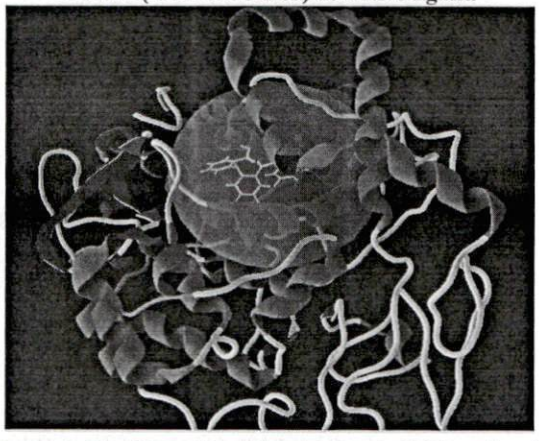


Figure 5: Docking of 3-chlorobenzoyl- *N*-(2-amino-4-chlorophenyl) anthranilic acid on cyclooxygenase-2 Protein (PDB ID: 5IKR)

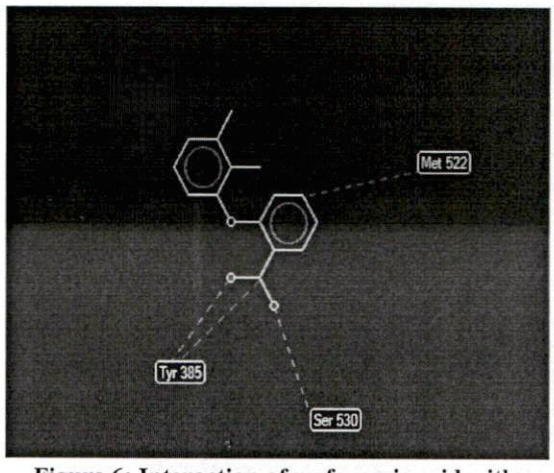


Figure 6: Interaction of mefenamic acid with cyclooxygenase-2 (PDB ID: 5IKR)

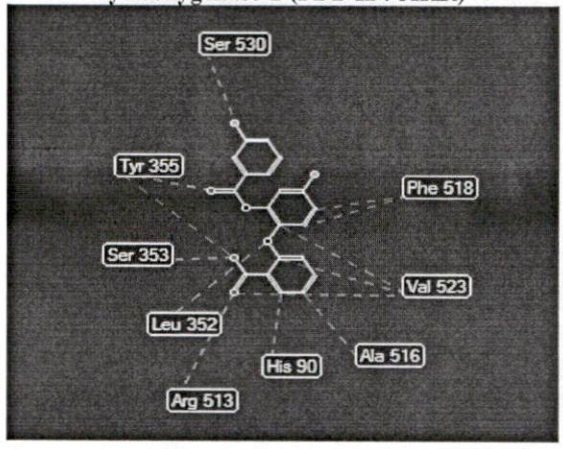
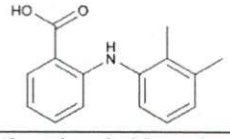
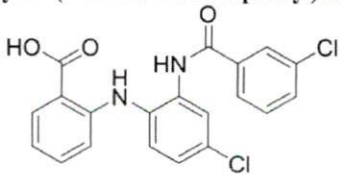


Figure 7: Interaction of 3-chlorobenzoyl- amino acid from *N*-(2-amino-4-chlorophenyl) anthranilic acid amino acid from cyclooxygenase-2

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

Compound	Rerank Score (kcal/mol)	Amino Acid	Interaction
Mefenamic Acid 	-83.203	Tyr 385 Ser 530 Met 522	Hydrogen and Steric Hydrogen Steric
3-Chlorobenzoyl-N-(2-amino-4-chlorophenyl) anthranilic acid 	-109.314	Tyr 355 Ser 353 Leu 352 Arg 513 His 90 Ala 516 Val 523 Phe 518 Ser 530	Hydrogen and Steric Hydrogen Hydrogen Hydrogen Steric Steric Steric Steric Steric

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 carbocylic), 3234 (N-H secondary amine), 1699 (C=O amide), 1483-1594 (C=C aromatic), 1161-1335 (C-N amine). ^{1}H-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). ^{13}C-NMR spectrum, δ (ppm) in DMSO D_6: 170.29 (C=O carboxyclic), 166.62 (C=O amide), 165.31 (C-N); 146.99 (C-N); 136.58; 135.68; 13.,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 $C_{20}H_{13}N_2O_3Cl_2$ $[M-H]^+$ = 399.030, $[M-H]^+$ found = 399.028.

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

Table 2: Analgesic Activity Report

Compound	Doses	Percentage of Pain Inhibition	ED_{50} (mg/kgBW)
Mefenamic Acid	25 mg/kgBW	42.27% ± 11.90	43.602
	50 mg/kgBW	51.42% ± 10.95	
	100 mg/kgBW	62.14% ± 7.38	
3-Chlorobenzoyl-N-(2-amino-4-chlorophenyl)-anthranilic Acid	25 mg/kgBW	40.37 % ± 18.42	30.443
	50 mg/kgBW	65.93 ±5.17	
	100 mg/kgBW	82.15 ±4.39	

4. Discussion

Table 2 showed that 3-chlorobenzoyl-*N*-(2-amino-4-chlorophenyl) anthranilic acid get lower ED_{50} 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 benzoyl group gave the effect of biological activity. Benzoyl group will increase the lipophilicity of the compounds, enhance the penetration through the membrane and increase drug-receptor interaction. By adding benzoyl 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 benzoyl (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 benzoyl 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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