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Synthesis and Activity Test of 1-Allyl-3-(4-tertiary-Butylbenzoyl) Thioureaas a Candidate of an Analgesic Drug

Rais Razak[1]*, Siswandono[2], Juni Ekowati[2]
[1]Faculty of Pharmacy, Muslim University of Indonesia, Makassar, Indonesia
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: raiz.razak@umi.ac.id

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Abstract

Background*: Urea derivatives showed good analgesic activity compared to diclofenac sodium. The addition of the allyl group to the thiourea and 4-tertiary-butylbenzoyl chlorideis expected to provide a better analgesic effect.* ***Objective****: The research aimed to synthesize 1-allyl-3-(4-tertiary-Butylbenzoyl) Thiourea and determine its analgesic activity in mice (Mus musculus).* ***Methods****: The synthesis was carried out by a modified Schotten-Baumann reaction, via nucleophilic substitution reaction of allylthiourea on 4-tertiary-butylbenzoyl chloride. A writhing test was performed to observe analgesic activity in the test compound. Confirmation of the structure of pure 1-allyl-3-(4-tertiary-Butylbenzoyl) Thiourea was obtained through UV, IR, ^{1}H-NMR, and ^{13}C-NMR data.* ***Results****: The compound showed better pain inhibition activity compared to diclofenac sodium, with ED_{50}19,018 mg/kg BW.* ***Conclusion****: The compound 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea showed better analgesic activity than diclofenac sodium.*

Keywords*: 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea, drug candidate, potential analgesic, urea derivatives*

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INTRODUCTION

Pain is the most individualized common symptom of a disease characterized by a feeling of sensory and emotional discomfort as a signal of or potentially causing tissue damage (HCANJ, 2017). Pain management aims to increase the effectiveness of treatment, either reducing pain intensity or duration, improving quality of life, and preventing the risk of side effects (Cregg *et al*., 2013).

Non-steroid anti-inflammatory drugs (NSAIDs) are widely used to treat mild to moderate inflammation and pains (Sun *et al*., 2018). Although relatively safe, the use of this drug is also often associated with the overuse of medication (Auriel *et al*., 2014). NSAIDs work by inhibiting the cyclooxygenase enzyme, which converts arachidonic acid into prostaglandin in the inflammatory process (Somakala & Amir, 2017). NSAIDs can offer pain inhibiting effects without the severe side effects of sedation and respiratory depression common with opioid use (Bader *et al*., 2011). According to the National Health Service (NHS), although NSAIDs are often prescribed, not everyone is suitable for this drug, especially at severe pain sensation, which causes side effects (National Health Service, 2019).

One of the NSAIDs that is often used is diclofenac sodium. However, this drug has a short half-life and has some side effects, such as gastric ulcers, gastric irritation, and bleeding, thus limiting its use as a pain agent (Aielo *et al*., 2014). Therefore, the discovery of new drugs for pain treatment is an opportunity to develop a drug.

Urea derivatives have biological activity as antiviral, antibacterial, anti-Human Immunodeficiency Virus (HIV), analgesic, and anti-inflammation (Alagarsamy *et al*., 2013). The chlor and nitro substituents at the para position added to a benzoyl thiourea derivative were able to increase analgesic and anti-inflammatory activity, where the influence of the chlorsubstituent was superior to that of the nitrosubstituent. Compounds 4-nitrobenzoylthiourea and 4-chlorobenzoylthiourea showed strong analgesic activity with lower anti-inflammatory activity compared to diclofenac sodium (Budiati *et al*., 2010).

In-silico identification has become a popular approach in computer-aided drug discovery. In new drug development, in-silico screening is carried out to find the ranking or screening value of a compound based on the data structure using one or more computational procedures (Budiati et al., 2010). This approach can narrow down the search of a potential lead compound from a massive number of compound databases to select potential hits by using high-throughput molecular docking; or elucidate the mechanistic interaction of potential hits, which helps rationalise or optimise bioactivity (Yap *et al*., 2019). It has many advantages; among other things, it can reduce excessive use of tools and materials and save on trial costs (Dona *et al*., 2019). The addition of the allyl group to the thiourea is expected to provide a better analgesic effect.

MATERIALS AND METHODS

Materials

*Allylthiourea*p.a 98% (Aldrich), 4-tertiary-butylbenzoyl chloridep.s (Aldrich), and diclofenac sodium (Dexa Medica),Tetrahydrofuran p.a (Merck), Ethyl acetate p.a (Merck), Methanol p.a (Merck), n-hexane p.a (Merck), Triethylamine p.a (Merck), Chloroform p.a (Merck).

Tools

Computer Intel core i7 Memory 8 GB, Chemdraw Ultra 8.0, Chem3D Ultra 8.0, and Molegro Virtual Docker 5.0 programs are used as devices for *in-silico* testing. HEWLET PACKARD 8452A Diode Array Spectrophotometer, PERKIN ELMER Spectrum One FTIR Spectrophotometer, and BRUKER BioSpinAvance III NMR Spectrometer are used as structural identification tools.

Method

In-silico procedures

In-silico test of 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea against the cyclooxygenase (COX-2) receptor (pdb: 1PXX) was carried out using Chembio Draw and Molegro Virtual Docker (MVD) computer programs. The 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea is docked with the receptor in the cavity position. The results obtained are the energy of the interaction between the test compound and the COX-2 receptor, in the form of a Rank Score (RS). *In-silico* testing is carried out as a basis for synthesizing the compound 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea

Synthesis procedure

In a 250 mL round bottom flask, dissolve 0.0172 mol N-allylthiourea with 50 mL of tetrahydrofuran (THF) and 0.028 mol of triethylamine. Add a solution of 0.0143 mol benzoyl chloride derivative in 15 mL of THF gradually in the ice bath while stirring the mixture using a magnetic stirrer for 0.5 hours. Then the mixture is refluxed over a water bath and tested using thin-layer chromatography (TLC) every hour until it produces one spot on the TLC plate, indicating that the target compound has been formed. If there are still two stains, the reaction is considered

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incomplete. When the reaction is deemed to be complete, the mixture is washed three times with saturated sodium carbonate solution, then the mixture is filtered over a Buechner funnel and recrystallized with methanol.

Animals

Male white mice aged 6-8 weeks were kept in cages at room temperature, maintained under a 12-hour light-dark cycle. All the animals were given an adaptation period of at two weeks and allowed free access to food and water *ad libitum*. The research was implemented after getting ethical clearance no. 512-KE Ethics Committee, Faculty of Veterinary Medicine Universitas Airlangga

Analgesic activity test

The experimental animals were divided into three test groups consisting of five experimental animals and given intraperitoneal injection (i.p). The division of the group included a negative control group that was given 1% CMC-Na suspension with a dose of 10 mL/kg BW, a positive control group was given a suspension of diclofenac sodium in 1% CMC-Na suspension at a dose of 12.5 mg/kg BW, and the test group given the test compound suspension in 1% CMC-Na suspension, at a dose of 6.25 mg/Kg BW; 12.5 mg/Kg BW; and 25 mg/Kg BW.

The experimental animals were observed in each group for 30 minutes to calculate the percentage of pain. The formula of pain resistance is calculated by comparing the number of writhes as the effect of pain resistance between the control and the test animals (Jakaria *et al*., 2015). The reduction in the amount of writhing in the test animals compared to the control group is evidence of an analgesic effect (Abdulmalik *et al*., 2011).

Researchers also count the median effective dose (ED) of 50%, the dose that produces 50% of the maximum response (Koyagura *et al*., 2015).

$$\%\ Inhibition = \frac{Mean\ number\ of\ writhes\ (control) - Mean\ number\ of\ writhes\ (test)}{Mean\ number\ of\ writhes\ (control)}\ x\ 100$$

After all the writhes data of the mice were obtained, then the ED50 value of each test compound was determined using probit analysis on SPSS 22.

RESULTS AND DISCUSSION

This study will compare the compound 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea with benzoylthiourea. Benzoylthiourea was chosen as a comparison because it is a urea derivative tested for its activity as an analgesic and anti-inflammatory (Budiati *et al*., 2010).

The RS value is a value that reflects the bond energy required to form a bond between a ligand and its receptor. This value predicts the activity of the test compound when it binds to the receptor. The smaller the RS value, the more stable the resulting bond, and the greater the predicted activity (Suhud, 2015). The amino acids involved in the interaction of 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea and benzoyltiourea at the COX-2 receptor can be seen in Table 1.

Table 1. Amino acids involved in the interaction between 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea and benzoyltiourea at the COX-2 receptor

Compound	Amino Acid	Type of Interaction
a	*Val 3523*	Steric
	Gln 3192	Steric
	Ala 3516	Steric
	Leu 3352	Steric
	Phe 3518	Steric
b	*Gly 3256*	Hydrogen
	Ser 3530	Steric

Based on docking data using MVD in Figure 1, the RS value of both 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea and benzoylthiourea were -95,9587 and -67.5824 respectively. It was predicted that 1-allyl-3-(4-tertiary-butylbenzoyl) thioureahas better analgesic activity than benzoylthiourea. Theoretically, when calculated using the Chemdraw Ultra 8.0 and Chem3D Ultra 8.0 computer program, the1-allyl-3-(4-tertiary-butylbenzoyl) thioureahas a lipophilic parameter value (Clog P) 3.3871 and a steric parameter value (CMR) of 7.2461 and (Etot) -16.0039 kcal/mol. While benzoylthiourea has a lipophilic parameter value (Clog P) of 0.566 and a steric parameter value (CMR) of 5.2812 (Etot) of -46.7383 kcal/mol.

The synthesis design in this study was to carry out the nucleophilic acyl substitution reaction in one of the amine groups in allythiourea with the benzoyl chloride substituted for the tertiarybutyl group in a THF solvent in the alkaline atmosphere with triethylamine as a catalyst. The primary amine group on the number three N atom of the allylthiourea compound acts as a nucleophile that attacks the C carbonyl atom on the benzoyl chloride substituted for the tertiarybutyl group because the C atom lacks electrons and is more positive, as shown in Figure 2. This reaction is expected to increase the interaction of pain receptors.

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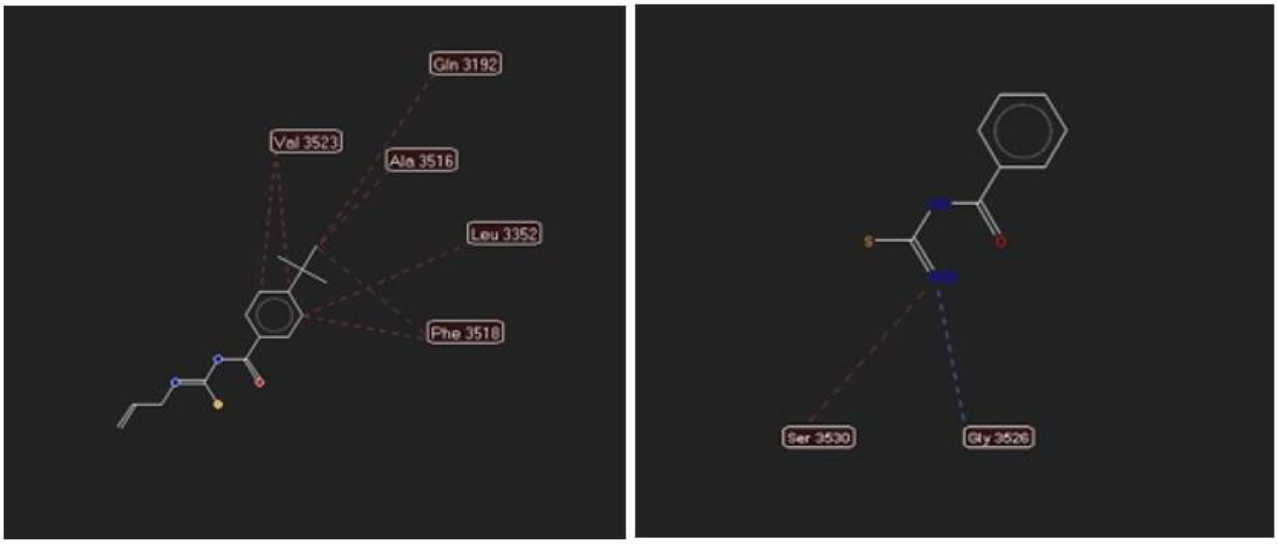

Figure 1. Docking result of (a) 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea and (b) Benzoyltiourea on 1PXX receptor

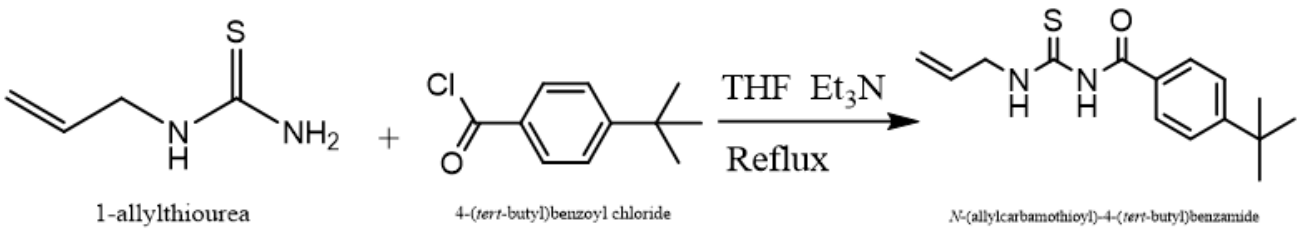

Figure 2. Synthesis of 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea

The compounds synthesized on the TLC chromatogram showed a single stain, and the melting point showed a range of less than 1 – 2ºC. The results of the synthesis obtained colorless crystals as much as 25.3%. mp 150-151 0C. TLC Rf: 0.78. Determination of the synthesized structure was carried out using ultra violet, infrared, and magnetic resonance; this was done to confirm the compound obtained with the desired target compound.

Table 2 shows IR data ν cm-1: 849 (Para substituted benzene); 1169 (-C=S); 1268 (-C-N); 1547 (-C=C aromatic); 1669 (-C=O amide); 3429 (-OH); 3244 (-NH).The interpretation of the IR spectrum for the compound 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea in Figure 3 shows an absorption band at 1669 cm^{-1} which indicates the presence of the –C=O amide group; absorption of 1169 cm-1 indicates the presence of a –C=S group; absorption of 1268 cm^{-1} indicates the presence of –C-N group; 1428-1547 cm^{-1} indicates the presence of –C=C- aromatic group; and the absorption of 3429 cm^{-1} indicates the presence of –O-H groups. In addition, the absorption at 846 cm^{-1} indicates a para substitution pattern in the aromatic ring.

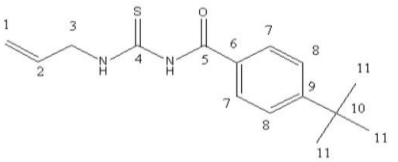

Figure 3. Structure of 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea

Table 2. IR data of 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea

Wave number (cm^{-1})	Type of vibration
849	Para disubtituted benzene
1169	-C=S
1268	-C-N
1547	Aromatic -C=C
1669	Amide -C=O
3429	-OH
3244	-NH

In the ^{1}HNMR spectrum (Table 3), the compound 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea is a compound that has the same symmetrical plane as the last peak at 1.37 ppm and contained a singlet with an integration indicating 9 H atoms of methyl at the tertierbutyl position of the substitution on the aromatic ring.

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Table 3. ^{13}C NMR and ^{1}H-NMR data of 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea

Position	δ_H ppm	δ_C ppm
(1)	5.22 (*dt, ^2J = 1.2 ,^3J = 10.4)*; 5.30 (*dt ,^2J* = 1.6, *^3J = 17.2*)	117.8
(2)	5.90 (*m*)	131.9
(3)	4.32 (*t)*	48.1
(4)	-	180.1
(5)	-	166.8
(6)	-	128.8
(7)	7.74 (*d,^3J* = 8.8)	127.8
(8)	7.48 (*d, ^3J* = 8.4)	127.4
(9)	-	157.6
(10)		35.2
(11)	1.37 (*s,^3J* = 6.8)	31.1
NH-C(5)	10.83 (*s*)	
NH-C(3)	9.4 *(s*)	

*$CDCl_3$ peak was reference at 7.24 ppm for ^{1}H NMR and 77 ppm for ^{13}C NMR; coupling constant (*J*) are reported in Hz

Table 4. The results of the analgesic activity of the compound1-allyl-3-(4-tertiary-butylbenzoyl) thiourea and diclofenac sodium

Compound	Doses	Mean percentage of pain inhibition	SD
Diclofenac sodium	12.5 mg/Kg BW	33.22%	8.26
1-allyl-3-(4-tertiary-butylbenzoyl) thiourea	6.25 mg/Kg BW	38.41%	9.20
	12.5 mg/Kg BW	48.10%	9.15
	25 mg/Kg BW	53.29%	6.36

In the ^{13}CNMR spectrum (Table 3) of the compound 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea, two peaks in the distant chemical shift can be seen at 166.8 ppm representing the carbon atom –C=O and at 180.1 ppm representing the carbon atom – C = S; this is because the carbon atom is bonded to another atom with a large electronegativity, namely -O and -S atoms. In the aromatic ring shift area, there are four peaks, namely at 127.8 ppm, at 127.4 ppm indicating two pairs of equivalent C atoms, at 128.8 ppm a C atom attached to a carbonyl group; and 157.6 ppm are -C-C atoms bonded to tertierbutyl on para substituents while the 35.2 ppm peak represents one -C-C atom and the last at 31.1 ppm there are three C atoms of three methyl groups.

Furthermore, the analgesic activity test was carried out using a writhing test on mice (*Mus musculus*) (Table 4). This method tests the sensation of pain felt by rats by observing the amount of writhing. Philosophically, pain cannot be directly monitored in animals but can only be estimated by examining their response to nociceptive stimuli (electrical, thermal, mechanical, or chemical), but chemical stimulation is the closest approach to clinical pain (Lee Bars *et al*., 2001). Therefore, the writhing test was chosen in this analgesic activity test by giving 1% acetic acid suspension in 1% CMC-Na at a dose of 10 mL/kg BW as pain induction. The diclofenac sodium was used as a comparison because it is one of the most widely used NSAIDs in pain management and anti-inflammatory. The analgesic activity of the test compound was inferred from a decrease in the writhings frequency (Gawade, 2012).

Better analgesic activity is shown by the compound1-allyl-3-(4-tertiary-butylbenzoyl) thiourea. Structural modification by adding Allyl groups and tertiarybutyl substitution to benzoyl ring can better affect analgesic activity (Shalas *et al*., 2016).

CONCLUSION

Based on this study, 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea was successfully synthesized by the modified Schotten-Baumann reaction and it showed better analgesic activity than 12.5 mg diclofenac sodium. This compound needs further investigation as a potential analgesic drug candidate.

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AUTHOR CONTRIBUTIONS

Conceptualization, S.S.; Methodology, S.S.; Software, R.R.; Validation, J.E.; Formal Analysis, R.R.; Investigation, R.R.; Resources, R.R.; Data Curation, J.E.; Writing - Original Draft, R.R.; Writing - Review & Editing, J.E..; Visualization, R.R.; Supervision, S.S.; Project Administration, S.S.; Funding Acquisition, R.R.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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