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RESEARCH ARTICLE

Synthesis and molecular docking of thiourea derivatives as antibacterial agents targeting enzymes involved in biosynthesis of bacterial cell wall

Suzana[1,3], Nuzul Wahyuning Diyah[1,3], Rini Susanti[1], Fika Amalia Najati[2], Juni Ekowati[1,3], Tutuk Budiati[1]

[1] Department of Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2] Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[3] Research Group of Drug Development, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

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Correspondence
Suzana
Department of Pharmaceutical Science
Faculty of Pharmacy
Universitas Airlangga
Surabaya
Indonesia
suzana@ff.unair.ac.id

Abstract

Background: New antibacterials are needed due to the increasing resistance of bacteria to existing antibiotics. Thiourea derivative compounds (benzoylthiourea and 1,3-dibenzoylthiourea) contain aromatic groups, thio groups (C=S), and amide groups (H_2N-C=O), which are commonly found in the class of antibacterial drugs. Molecular docking can be used to predict their antibacterial activity. **Objective:** This study aimed to synthesise thiourea derivatives and predict their antibacterial activity by *in silico* method. **Methods**: Synthesis was performed using nucleophilic substitution reactions. The synthesised compounds were identified using UV-Vis, FT-IR, and ^{1}H-NMR. Molecular docking was conducted using the MOE program ver 2022.02. **Results:** Benzoylthiourea (BTU) and 1,3-dibenzoylthiourea (DBTU) compounds were obtained with yields of 36.55% and 12.68%, respectively. The melting point 171-173°C for BTU and 202-204°C for DBTU. Molecular docking results showed higher binding affinity of DBTU against PBP2a (docking score < -5.75 kcal/mol) and FaBH (docking score <-4.7935 kcal/mol) compared to the corresponding native ligands, while the two compounds had lower affinity for the muramyl ligase. **Conclusion:** BTU and DBTU can be synthesised by nucleophilic substitution reactions. DBTU is predicted to exhibit antibacterial activity against Methicilin Resistant *Staphylococcus aureus* (MRSA) and *Mycobacterium tuberculosis*.

Introduction

Infectious diseases caused by bacteria are widely prevalent among the population and represent one of the dominant illnesses in Indonesia (Ministry of Health of the Republic of Indonesia, 2011). Research in the United States has shown that antibacterial resistance results in more than 2,499,442 people experiencing illnesses and 23,000 deaths annually, and these occurrences are increasing each year (Romero *et al.,* 2015). Pathogenic bacteria resistant to specific antibacterial drugs, such as the methicillin-resistant Staphylococcus aureus (MRSA), are also on the rise. Antibacterial drugs used in treatment work through various mechanisms, including inhibiting the enzymes involved in the biosynthesis of peptidoglycan present on the cell membrane of gram-positive bacteria. Over the past decade, drug resistance has become a significant challenge in managing infectious diseases (Saha & Sarkar, 2021). The incidence of bacterial resistance to multiple drugs simultaneously (multi-drug resistance) has also been reported (Saha & Sarkar, 2021; Catalano *et al.*, 2022; Pulingam *et al*., 2022). Therefore, there is an ongoing need to discover new antibacterial agents in response to bacterial resistance to currently used drugs, which can be achieved through combinations of natural substances and chemical synthesis approaches (Fernandes *et al.,* 2017).

Enzymes involved in peptidoglycan biosynthesis are attractive potential targets in efforts to inhibit the growth of gram-positive pathogenic bacteria. Inhibiting these enzymes can disrupt peptidoglycan synthesis, leading to bacterial cell deformation and ultimately inhibiting growth and replication (George *et al.,* 2014). Bacterial resistance to penicillin and β-lactam antibiotics occurs through the production of β-lactamase, which destroys the β-lactam ring in the structure of the antibiotics, while methicillin resistance is caused by mutation of the transpeptidase enzyme (Penicillin Binding Protein= PBP) to PBP2a so that methicillin cannot bind to the enzyme. PBP2a takes over the function of Staphylococcal PBP in cell wall synthesis (Carretto *et al*., 2018). Recently, PBP2a has become a target for antibacterial drugs aimed at resistant bacteria (Catalano *et al*., 2022). Enzymes other than PBP2a that contribute to peptidoglycan biosynthesis, such as muramyl ligase, can also be a target for new antibacterial compounds (Pulingam *et al*., 2022).

Peptidoglycan biosynthesis is a multi-step process consisting of three main stages: 1) formation of UDP-N-acetylmuramic acid (UDP MurNAc) from N-acetylglucosamine (GlcNAc), 2) addition of short polypeptide chain to UDP MurNAc, and 3) addition of a second GlcNAc to the disaccharide-pentapeptide chain followed by the transport of this unit through the cytoplasmic membrane and their incorporation into the peptidoglycan layer which keeps growing. The four main enzymes of Mur ubiquitin ligase (MurC, MurD, MurE, and MurF) play an important role in the second stage (Rani *et al*., 2018). Inhibition of muramyl ligase will result in inhibition of the peptidoglycan biosynthesis so that muramyl ligases are targets for antibacterial compounds.

In the case of *Mycobacterium tuberculosis* (MTB), mycolic acids are the key constituents of mycobacterial cell walls, which protect the bacteria from antibiotic susceptibility, helping to subvert and escape from the host immune system. The β-ketoacyl-acyl carrier protein synthase III (FabH) of *Mycobacterium tuberculosis*, which is the key regulatory enzyme of the mycolic acid pathway, can be explored as a potential drug target to kill MTB (Kumar *et al*., 2022).

Thiourea derivative compounds are widely used due to their diverse biological activities, including anticancer (Xiong *et al.*, 2008; Samir *et al.*, 2020) and antibacterial properties (George *et al.,* 2014b). The development of thiourea derivatives has garnered attention in the quest for new effective antibacterial compounds. Advances in computational technology have enabled us to perform molecular docking simulations that depict the virtual interactions between candidate compounds and target enzymes. The ability of thiourea derivatives to interact specifically and effectively with biological targets makes them intriguing candidates as antibacterial agents (Wei *et al.*, 2022). This method allows researchers to predict potential interactions between compounds and target enzymes, providing valuable guidance in the design of more potent compounds. This study aimed to synthesise thiourea derivatives and predict their antibacterial activity by *in silico* method.

Methods

Design

In this study, three enzymes were used as targets to inspect the potential of thiourea derivatives against MRSA, Gram-negative *E. coli*, and weak Gram-positive MTB. The synthesised thiourea derivative compounds contain aromatic groups, thio groups (C=S), and amides (H_2N-C=O), which are commonly found in the class of antibacterial drugs (Merkl *et al.*, 2010; Lucio *et al.,* 2018; Ajani *et al.,* 2019; Carmen *et al.*, 2020). The synthesis reaction employed a nucleophilic substitution reaction (Figure 1). The synthesis was carried out using the non-volatile organic solvent toluene (Solomons, 2011).

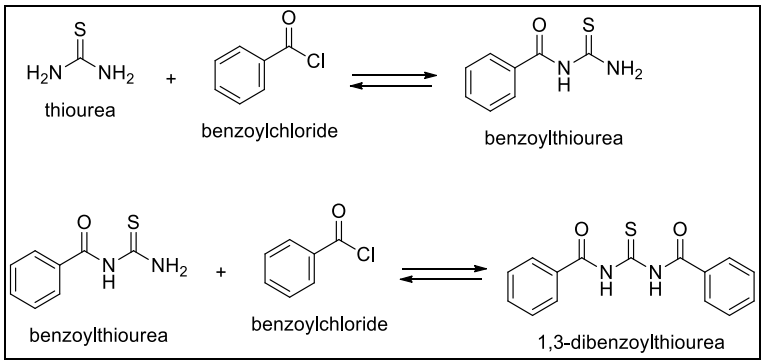

Figure 1: Synthesis of benzoylthiourea and 1, 3-dibenzoylthiourea

Materials

The substances employed in this investigation were utilised in their pro analysis. purity grade. These substances include thiourea, benzoyl chloride, KBr, chloroform, 96% ethanol, ethyl acetate, hexane, acetone, and silica gel 60 GF254. Study of molecular docking using Molecular Operating Environment (MOE) program ver 2022.02.

Instrumentation

The equipment includes an electrothermal melting point apparatus, a Buck Scientific IR Spectroscopy M 500, a HEWLETT PACKARD 8452A UV-Vis Spectroscopy instrument, and a JEOL ECS-400 ^{1}H-NMR spectrometer.

Molecular docking

The benzoyl-thiourea and 1,3-dibenzoylthiourea compounds were subjected to molecular docking using the MOE program ver 2022.02 with three different receptors: PDB.4CJN, PDB.2Y1O, and PDB.2QO0 (El Maatougui *et al.*, 2016; Basu *et al.*, 2017). Enzymes are involved in the biosynthesis of bacterial cell walls as target receptors. Before the docking process, the compound structures were generated using the ChemDraw Ultra 3D software, optimised for geometry using the MMFF94 method, and saved in Sybyl mol2 format.

Thiourea derivative synthesis

Benzoylthioureasynthesis

Thiourea (50 mmol) was added to benzoyl chloride (23.5 mmol) diluted with toluene solvent at room temperature. After the homogeneous mixture was heated with a reflux condenser (temperature 80-85°C) for three hours, the reaction mixture was cooled and filtered, and a saturated $NaHCO_3$ solution was added. After filtration, the product was washed and recrystallized with 70% ethanol. Purity testing and identification were performed (Furnish *et al.*, 1989; McMurry, 2008).

1,3-dibenzoylthiourea synthesis

The method is similar to benzoyl-thiourea synthesis but with a reactant molar ratio of 1:3 and heating at 105-110°C (Furnish *et al.*, 1989; McMurry, 2008).

Results

Characterisation of benzoyl-thiourea

White needle crystals were obtained, m.p.171-173°C; yield 36.55%; UV-Vis (Et-OH), nm: 235 and 281. IR (KBr in cm^{-1}): 1682 (-C=O amide), 1535 (-C=C- aromatic), 1238 (C=S), 3308 and 3225 (-NH_2), 3159 (-NH-). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.45-7.88 ppm (m, 5H aromatic ring, C_6H_5-), 9.10 ppm (s, 2H,-NH_2), 10.01 ppm (s, 1H, -NH-) (Silverstein *et al.,* 2004; Pavia *et al*., 2009).

Characterisation of 1,3-dibenzoylthiourea

White needle crystals were obtained, yield: 12.06%; melting point (m.p.):202-204°C. UV-Vis (Et-OH), nm: 241. IR (KBr in cm^{-1}): 1753 (-C=O amide), 1529 (-C=C- aromatic), 1284 (C=S), 3267 (-NH-). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.53-8.05 ppm (m, 5H aromatic ring, C_6H_5-), 10.01 ppm (s, 1H, -NH-) (Silverstein *et al.,* 2004; Pavia *et al*., 2009).

The results of the physicochemical properties test of benzoyl thiourea (BTU) and 1, 3-dibenzoyl thiourea (DBTU) compounds indicated an increase in the Log P value of the 1, 3-dibenzoyl thiourea compound due to the addition of the benzoyl group. The benzoyl group contains a benzene ring, which is non-polar (Table I). The docking results of thiourea derivative molecules with several target receptors (4CJN receptor, 2Y10 receptor, and 2QO0 receptor) are depicted in Table II.

The docking results and interactions of thiourea derivative compounds with the 4CJN receptor are shown in Figures 2 and 3.

Table I: The physicochemical properties of thiourea derivatives

Compound	Log P	MR (cm^3/mol)	tPSA (Å^2)	HBA	HBD	Rots bonds
Benzoylthiourea	1.12	49.49	87.21	1	2	3
1,3-Dibenzoylthiourea	2.95	79.08	90.29	2	2	6

HBA= number of hydrogen bond acceptors; HBD= number of hydrogen bond donors; Rot bonds= number of rotatable bonds

Table II: The docking result of ligands against three enzymes involved in the biosynthesis of bacterial cell walls as target receptors

Compound	Receptor					
	PBP2a (PDB. 4CJN)		Muramyl ligase (PDB. 2Y1O)		FabH (PDB. 2QO0)	
	Energy (S) (kcal/mol)	Type of bond & amino acid	Energy (S) (kcal/mol)	Type of bond & amino acid	Energy (S) (kcal/mol)	Type of bond & amino acid
Benzoylthiourea	-5.0525	Hydrophobic interactions: Lys273, Tyr272 H-bond: Asp295	-5.3394	2 H-bonds: Arg302, Lys319	-4.6498	3 H-bonds: Trp195, Phe84, Gln86
1,3-dibenzoylthiourea	-6.1912	Hydrophobic interactions: Lys273, Tyr272 H-bond: Asp295	-5.6923	Hydrophobic interactions: Asn421. 2 H-bonds: Ile133, Lys319	-6.0002	Hydrophobic interactions: Trp195, His83. H-bonds: Trp195
QLN (native ligand)	-5.7574	Hydrophobic interactions: Glu145, Ile144 No H-bonds				
T26 (native ligand)			-8.3837	Hydrophobic interactions: Arg37, Asn421 4 H-bonds: Asn421, His183, Lys319, Ser415		
D1T (native ligand)					-4.7935	H-bond: Phe84
Oxacillin (reference drug)	-5.3715	H-bond: Lys273				
Isoniazid (INH) (reference drug)					-4.7835	Hydrophobic interactions: Thr145. H-bonds: Asn81

Notes: QLN, T26, and DIT were inhibitors of the corresponding enzymes as reference ligands. Oxacillin and INH were reference drugs

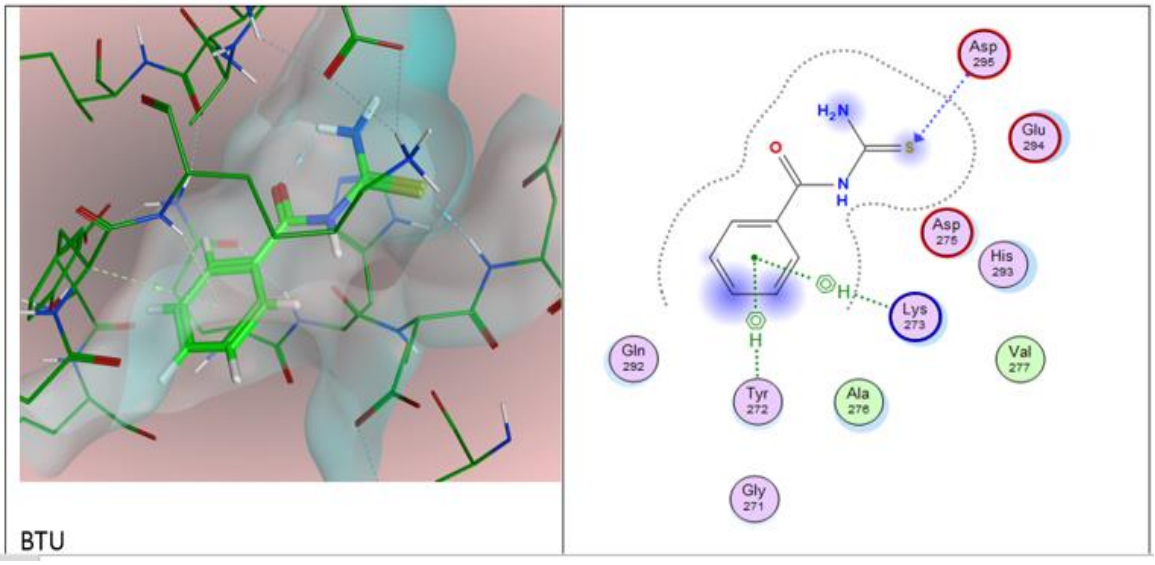

Figure 2: The results of the docking of the compound Benzoylthiourea (BTU) with the receptor 4CJN

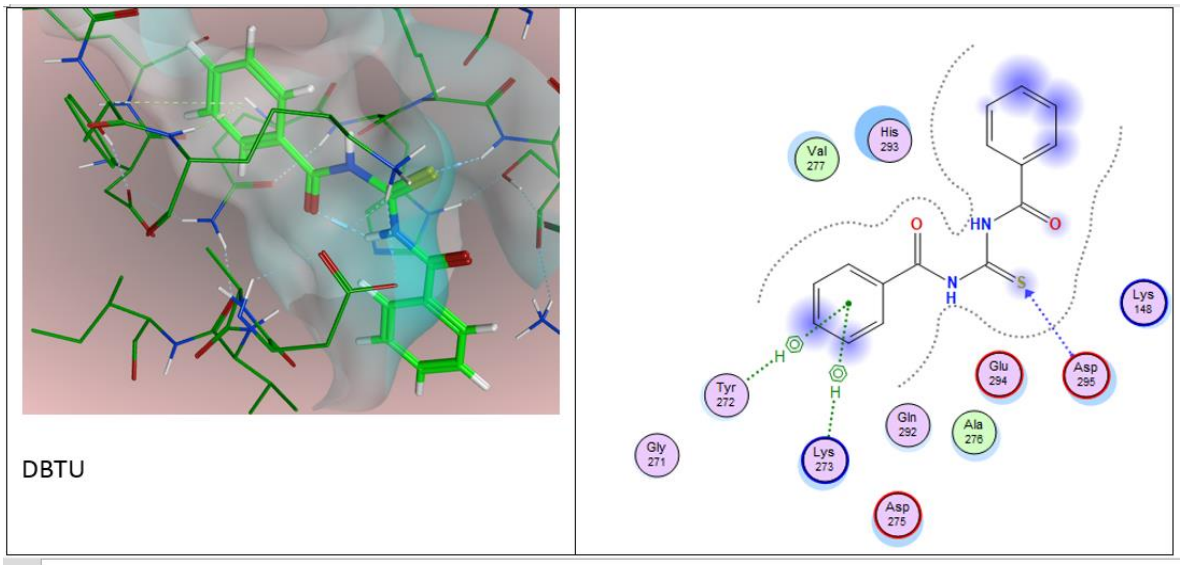

Figure 3: The results of docking of the compound 1, 3-Dibenzoylthiourea (DBTU) with the receptor 4CJN

Discussion

The synthesis process of BTU involves heating a mixture of thiourea and benzoyl chloride in toluene solvent at a temperature range of 80-85°C for a duration of three hours (Elkanzi *et al*., 2022).The choice of these specific temperature and duration conditions s was powered by the need to optimise the synthesis results while minimising the occurrence of substitution reactions involving other NH_2 groups. Toluene was selected as the solvent due to its high boiling point (110°C).

The synthesis of DBTU, on the other hand, entailed an acylation reaction that affects both amino groups (-NH_2) of thiourea. The presence of a benzoyl group already attached to one of the amino groups of thiourea impeded the access of the next benzoyl group to the second amino group, mainly due to a steric hindrance. To overcome this challenge, an excess amount of benzoyl chloride reactant was added, thereby favouring the reaction towards completion. The heating temperature for the synthesis of DBTU was increased to a range of 105-110°C. Additionally, a solution of NaOH was introduced to neutralise any HCl and benzoic acid by-products.

The outcome of the BTU reaction produced a suspension that still contained unreacted thiourea. The remaining thiourea was isolated, washed, and subsequently treated with a saturated sodium bicarbonate solution to neutralise HCl and benzoic acid. The sodium bicarbonate solution reacted with HCl and benzoic acid, forming soluble salts. After washing to remove any residual sodium bicarbonate solution, purification is accomplished through recrystallization, utilising ethanol as the solvent.

Docking studies were conducted on BTU and DBTU with three target enzymes involved in the synthesis of the bacterial cell wall, namely PBP2a from MRSA (PDB. 4CJN), muramyl ligase from *E. coli* (PDB. 2Y1O), and FaBH from *M. tuberculosis* (PDB.2QO0). The docking results showed that DBTU had a higher binding affinity compared to BTU and the native ligand QLN for PBP2a. Similarly, DBTU exhibited a higher binding affinity compared to BTU and D1T (native ligand) for the FaBH enzyme, but the affinities of both BTU and DBTU were higher compared to the native ligand T26 for muramyl ligase. Molecular docking results showed higher binding affinity of DBTU against PBP2a (docking score < -5.7574 kcal/mol) and FaBH (docking score < -4.7935 kcal/mol) compared to the corresponding native ligands.

Molecular docking analysis yielded a lower docking score for DBTU when compared to the native ligand and BTU. This suggests that DBTU was predicted to possess antibacterial activity against *Staphylococcus aureus* MRSA and *M. tuberculosis*. The docking results revealed interactions between DBTU and the receptor. Specifically, the sulfur atom within the thio group formed hydrogen bonds with Asp296, while the benzene ring engages in hydrophobic interactions with Tys272 and Lys273. The inclusion of an additional benzene ring enhanced the interactions with the receptor (Figure 3). Furthermore, with a Log P value of 2.95, DBTU exhibits a higher Log P value compared to BTU, indicating its greater ability to penetrate cell membranes (Table I).

Another study reported the bacteriostatic activities of bis(thiourea) derivatives against *E. coli* (ATCC 25922)

via turbidimetric kinetic method (Halim & Ngaini, 2016). The compounds consisted of two folds of N-H, C=O, and C=S and long alkyl chain substituents. Bis(thiourea) derivatives with alkyl (n=10 and n=12) displayed excellent activity against *E. coli*. The trend was observed in binding affinity to the active site of enoyl acyl carrier protein reductase (FabI), which demonstrated binding free energy around −5.3 kcal/mol (Halim & Ngaini, 2016).

In this study, DBTU and BU were less potent against muramyl ligase from *E. coli*. Meanwhile, DBTU showed higher affinity as an inhibitor of FabH (β-ketoacyl-acyl carrier protein synthase III) from MTB.

Conclusion

The percentages of obtained yields for BTU and DBTU were 36.55% and 12.68%, respectively. It was proposed that DBTU would exhibit antibacterial properties against both MRSA and *M. tuberculosis.*

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