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Original Article

Molecular docking, synthesis, and antibacterial activity of the analogs of 1-allyl-3-benzoylthiourea

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Abstract

Background and purpose: The incidence of antibiotic resistance rapidly emerges over the globe. In the present study, the synthesis of thiourea derivatives as antibacterial agents and their biological evaluation are reported.

Experimental approach: Preliminary studies were done by molecular docking of four analogs of 1-allyl-3-benzoylthiourea, clorobiocin, and ciprofloxacin on the DNA gyrase subunit B receptor (PDB: 1KZN). The nucleophilic substitution reaction of benzoyl chloride analogs to the allylthiourea yielded four 1-allyl-3-benzoylthiourea analogs (Cpd 1-4). The reactions were done by a modified Schotten Baumann method. The *in vitro* antimicrobial activities were determined using the agar dilution method against methicillin-resistant *Staphylococcus aureus* (MRSA), *Salmonella* typhi, *Escherichia coli*, and *Pseudomonas aeruginosa.*

Findings/Results: The *in-silico* study showed that Cpd 1-4 possesses a good interaction on the DNA gyrase subunit B receptor compared to the ciprofloxacin. Cpd 3 had the best binding affinity with a rerank score of -91.2304. Although the candidate compounds showed unsatisfactory antibacterial activity, they indicated an increasing trend of growth inhibition along with the increment of concentration. Cpd 1 and 4 exhibited *in vitro* antibacterial activities against MRSA with a minimum inhibitory concentration value of 1000 µg/mL, better compared to the other compounds.

Conclusion and implication: Despite lacking antibacterial activity, all the synthesized compounds showed an increased trend of growth inhibition along with the increment of concentration. Therefore, additional development should be implemented to the compounds of interest in which optimization of lipophilicity and steric properties are suggested.

Keywords: Antibacterial; Molecular docking; Synthesis; Thiourea.

INTRODUCTION

The incidences of antibiotic resistance are increasing, and new types of resistance rapidly emerge over the globe. Inadequate infection prevention and inappropriate use of antibiotics make the incidence even more dangerous. In Indonesia and some other Southeast Asia countries access to antibiotics is considerably easy. Antibiotics are found to be available without prescription in some countries, even though it is against the regulation. This phenomenon leads to the increasing number of antibiotics inappropriate use which accelerates the incidence of antibiotic resistance (1,2). An improvement in antibiotics management and the discovery of new active substances will provide a solution to the problem.

Thiourea pharmacophore is well known to possess various biological activities on microbes such as antitubercular, antifungal, antiviral, and antibacterial (3-6). Numbers of thiourea-based derivatives molecules were synthesized and tested for their antibacterial activities.

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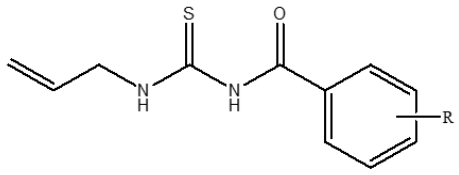

Compound	R
Cpd-1	-H
Cpd-2	-2-Cl
Cpd-3	-3-Cl
Cpd-4	-4-Cl

Fig. 1. The chemical structure of the candidate compounds.

The antibacterial activity of thiourea is affected by the capability of the C=S, C=O, and NH groups which are easily protonated under acidic conditions and can interact with the carboxyl and phosphate groups on the surface of the bacterial membrane, thus increasing their activity. A study reported that thiourea substituted with C10 and C12 alkyl chains provided good antibacterial activity, due to the increasing lipophilicity, thus providing a better disruption to the bacterial membrane (3). However, the longer the alkyl chains, causing lesser disruption to the bacterial membrane and the lower binding affinity with the target protein. Interestingly the N-aryl thiourea derivatives showed better antibacterial activity compared to alkyl substituents in the thiourea group (4).

The thiourea derivatives which are substituted with electron-withdrawing groups showed good antibacterial activity (5-8). The addition of chlorine atoms to the phenyl group of thiourea derivatives also showed good antibacterial activity (7). The substitution with dichloro and methoxy groups on aryl groups showed significant antibacterial activity (8). The presence of halogens showed higher activity compared to a methoxy group. Molecular docking studies also showed that substituents with light, nucleophilic, and polar characters such as CF_3, NO_2, NH, C=O, and C=S showed good interactions with target proteins so that they have good inhibitory activity. The presence of an electron-withdrawing atom on the benzene which binds to thiourea can increase the antibacterial activity, especially in *meta*- and *para*-position (5,6,9). The substituent position and the electronegativity of the atom are the main factors that determine the antibacterial activity potency; thus, we designed four thiourea-derived compounds to be studied as shown in Fig. 1.

The antibacterial mechanism of thiourea-derived analogs is proposed to be about the inhibition of topoisomerase II, DNA gyrase, and topoisomerase IV (4,5,10-12). Copper (II) complexes of 3-(trifluoromethyl)phenyl thiourea showed stronger inhibition activity towards *Staphylococcus aureus* DNA gyrase than the topoisomerase IV during the preliminary study (9). Studies of antimicrobial activity of novel thiourea derivatives tagged with thiadiazol, imidazole, and triazine moieties also exhibited excellent inhibitory activity against *Escherichia coli* DNA B gyrase in comparison with novobiocin (13). In this study we designed some thiourea derivative compounds by *in silico* molecular docking, using DNA B gyrase protein as the target receptor. Synthesis of thiourea derivatives was done by substituting benzoyl chloride analogs into thiourea structure. The synthesized compounds were tested for their antibacterial activity against both Gram-negative and Gram-positive bacteria.

MATERIALS AND METHODS

All reagents and solvents were purchased from Sigma-Aldrich (Singapore) and Merck (Germany). The synthesis reactions were evaluated by thin-layer chromatography using silica gel (F254 plate; Merck, Singapore). Melting points were determined in open capillaries using an electrothermal IA 9200 melting point apparatus. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained from NMR Jeol ECS 400 MHz spectrometer (USA) using deuterated chloroform ($CDCl_3$) as a solvent and tetramethylsilane as an internal standard. Mass spectra were detected on Agilent 1260 Infinity high-performance liquid chromatography (HPLC) and Agilent 6530 Accurate-Mass Q-TOF/MS (USA). Infrared spectra (KBr disk) were recorded with an 8400S/Shimadzu Fourier transform infrared (FT-IR) spectrophotometer (Japan). All microorganisms in the antibacterial

assay were obtained from the Microbiology Laboratory of RSUD, Dr. Saiful Anwar Hospital (Indonesia). Mannitol salt agar, Mueller-Hinton agar, Mueller-Hinton broth, and nutrient broth were purchased from Merck (Germany). Standard antibacterial drugs such as vancomycin (Fahrenheit, Indonesia), ciprofloxacin (Hexpharm Jaya, Indonesia), and gentamicin (Indofarma, Indonesia) were used for comparison.

Molecular docking studies

Preliminary studies were carried out by molecular docking studies to compare the energy of the interaction between four analogs of 1-allyl-3-benzoylthiourea to clorobiocin and ciprofloxacin in the DNA gyrase subunit B. The chemical structures of the ligand namely 1-allyl-3-benzoylthiourea (Cpd 1); 1-allyl-3-(2-chlorobenzoyl)thiourea (Cpd 2); 1-allyl-3-(3-chlorobenzoyl)thiourea (Cpd 3); and 1-allyl-3-(4-chlorobenzoyl)thiourea (Cpd 4) were drawn using ChemDraw Ultra 8.0 software, while energy minimization for the three-dimensional structure stabilization was performed using Chem3D Ultra 8.0. The three-dimensional structure of the target protein (PDB: 1KZN with a resolution of 2.30 Å) was downloaded from the protein data bank (https://www.rcsb.org/). The receptor is equipped with clorobiocin as the native ligand, which is suitable for the ligand-receptor interaction comparison. Later preparation of ligand and receptor was done using Molegro Virtual Docker 5.0, before the docking procedure. The quality of the ligand-receptor interaction was then evaluated using the rerank score provided by the software.

Synthesis

The synthesis was done by Schotten-Baumann reaction through nucleophilic substitution of benzoyl chloride analogs (benzoyl chloride; 2-chlorobenzoyl chloride; 3-chlorobenzoyl chloride; and 4-chlorobenzoylchloride to an allylthiourea, with tetrahydrofuran as the solvent, and triethylamine as the catalyst. The mixture of each benzoyl chloride analogs (0.02 mol) and tetrahydrofuran (15 mL) was added dropwise into the mixture of allylthiourea (0.024 mol), triethylamine (0.04 mol), and tetrahydrofuran (30 mL) in an ice bath for approximately 30 min. The final mixture was refluxed for 4 h, allowed to cool at room temperature then vacuum filtered using a Buchner funnel. The solvent was then evaporated using a rotary evaporator. The oil-like consistency product was solidified by saturated sodium carbonate (Merck, Singapore), then recrystallized by hot methanol-water (Cpd 1,2,4) and hot DMSO-water (Cpd 3).

Antibacterial activity

The antibacterial activity of the synthesized compounds was examined using the agar dilution method against four patient strains of methicillin-resistant *S. aureus* (MRSA) which were isolated from blood (11880), pus (16810 and 14599), and sputum (15621) as Gram-positive bacteria and three Gram-negative bacteria including two patient strains which were *Salmonella* Typhi blood isolate and *E. coli* urine isolate (1223), and one standard strain of *Pseudomonas aeruginosa* (ATCC 27853). Those bacteria are well known for causing the incident of resistance in Indonesia and were chosen based on their accessibility.

Bacterial suspension density was measured using a spectrophotometer at 620 nm. Suspensions were adjusted into 1.0×10^6 CFU/mL. The synthesized compounds and antibacterial standard compounds were dissolved and diluted in 0.4% Tween™ 80. The synthesized compounds were serially diluted from 1000 to 7.8125 µg/mL as final concentrations in a plate, while the antibacterial drugs were made into five concentration series based on the Clinical and Laboratory Standards Institute. Mueller-Hinton agar and Mannitol salt agar were used as media for Gram-negative and Gram-positive bacterial growth, respectively. We used 0.4% Tween™ 80 as the negative control, while gentamycin was used as the positive control for *S. aureus*, *E. coli*, and *P. aeruginosa* assay. Vancomycin and ciprofloxacin were used as the control for antibacterial assay in MRSA and *S. Typhi*, respectively. The minimum inhibitory concentration (MIC) then was determined by observing the lowest concentration with no bacterial growth. We also evaluated the growth of bacteria by visual interpretation and put a

bacterial growth score ranging from 0 (no bacterial growth) to 3 (high bacterial growth).

RESULTS

Molecular docking studies

All of the candidate compounds (Cpd 1-4) showed a good interaction on the DNA gyrase subunit B receptor (GDP: 1-KZN) when compared to the standard compounds, clorobiocin and ciprofloxacin. Clorobiocin is an aminocoumarin antibiotic that has a potent inhibitory effect on DNA gyrase and has been used as a potential template for drug development (14). Table 1 summarized the rerank scores of each tested compound and the docking conformation was shown in Fig. 2. The docking study represented that the compounds of interest showed a good affinity to the receptor.

The best rerank score was possessed by Cpd 3 (-91.2304), followed by Cpd 4 (-89.3188), Cpd 2 (-89.1417), and Cpd 1 (-85.9597). The rerank score of the reference clorobiocin was -130.551 and ciprofloxacin -97.0795. Based on the results, the rerank score of the candidate compounds was closer to the ciprofloxacin's standard rerank score than the clorobiocin. Ligand map interaction showed the presence of hydrogen bonding on Cpd 1 and Cpd 3 to Asn 46 of the receptor. The hydrogen bonding is also noticed in Cpd 2 and Cpd 4 to Thr 165. The Cpd 2 also showed interaction with Asp 73 of the receptor DNA gyrase subunit B.

Table 1. Rerank score and hydrogen bonds for four analogs of 1-allyl-3-benzoylthiourea (Cpd 1-4), clorobiocin and ciprofloxacin docked into DNA gyrase.

Number	Compound	Rerank score	Hydrogen bond
1	Clorobiocin	-130.551	Asn 46; Arg 136
2	Ciprofloxacin	-97.0795	Arg 136; Arg 76; Glu 50
3	Cpd 1	-85.9597	Asn 46
4	Cpd 2	-89.1417	Thr 165; Asp 73
5	Cpd 3	-91.2304	Asn 46
6	Cpd 4	-89.3188	Thr 165

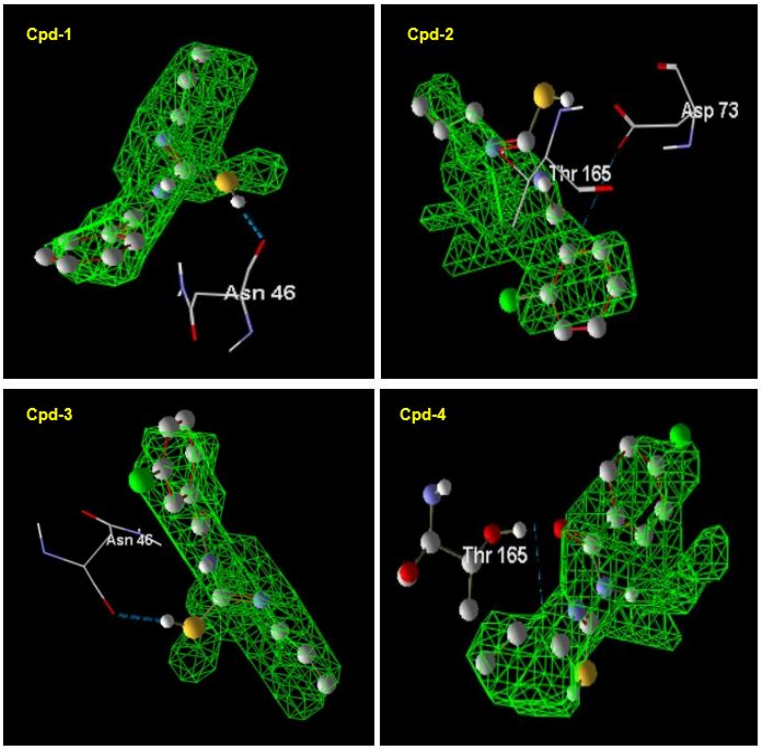

Fig. 2. Docked conformation of Cpd 1-4.

Synthesis

1-Allyl-3-benzoylthiourea (Cpd 1)

White crystals, yield: 11.99%. melting point (m.p): 68.5-69 °C, MS: m/z (%): 221.0744 (M+,100), 222.0779 (M+1) $C_{11}H_{11}ClN_2OS$ molecular weight (M.W.) 220.2908. FT-IR (KBr cm^{-1}): 3235.16 (N-H, str.), 3098.23-3053.87 (C-H aromatic, str.), 1676.79 (C=O amide), 1530.21 (C=C aromatic), 1269.84 (C-N). ^{1}H-NMR (400 MHz, $CDCl_3$) □ in ppm: 10.800 (1H, s, -N*H*), 9.023 (1H, s, -N*H*), 7.840-7.818 (2H, d, *J* = 7.2 Hz, Ar-*H*), 7.644-7.569 (1H, t, *J* = 7.6 Hz, Ar-*H*), 7.530-7.492 (2H, t, *J* = 7.6 Hz, Ar-*H*), 6.012-5.941 (1H, m, *J* = 17.2, 10.4, 6.4 Hz, vinylic *H*), 5.354-5.243 (2H, d, *J* = 17.2, 1.6, 1.2 Hz, vinylic *H*), 4.371-4.334 (2H, t, allylic *H*). ^{13}C-NMR (400 MHz, $CDCl_3$) □ in ppm: 180.090 (*C*=S), 166.904 (*C*=O), 131.884, 131.798, 129.271, 127.517 (aromatic *C*).

1-Allyl-3-(2-chlorobenzoyl)thiourea (Cpd 2)

Yellowish white crystals, yield: 14.41%. m.p: 148.5-150.3 °C, MS: m/z (%): 255.036 (M+,100), 256.0392 (M+1), 257.0336 (M+2), $C_{11}H_{11}ClN_2OS$ M.W. 254.7358. FT-IR (KBr cm^{-1}): 3246.74 (N-H, str.), 3109.80 (C-H aromatic, str.), 1690.29 (C=O amide), 1551.42-1476.21 (C=C aromatic), 1271.76 (C-N). ^{1}H-NMR (400 MHz, $CDCl_3$) □ in ppm: 10.580 (1H, s, -N*H*), 9.133 (1H, s, -N*H*), 7.689-7.667 (1H, q, *J* = 6.8 Hz, Ar-*H*), 7.484-7.458 (2H, m, Ar-*H*), 7.411-7.371 (1H, m, Ar-*H*), 6.018-5.921 (1H, m, *J* = 17.4, 10.8, 6.0 Hz, vinylic *H*), 5.360-5.250 (2H, m, *J* = 17.0, 1.2 Hz, vinylic *H*), 4.365-4.330 (2H, m, allylic *H*). ^{13}C-NMR (400 MHz, $CDCl_3$) □ in ppm: 179.547 (*C*=S), 166.065 (*C*=O), 133.276, 132.322, 131.779, 131.235, 131.006, 130.377 (aromatic *C*).

1-Allyl-3-(3-chlorobenzoyl)thiourea (Cpd 3)

White crystals, yield: 12.46%. m.p: 67.7-68.9 °C, MS: m/z (%): 253.0188 (M-, 100), 254.0209 (M-1), 255.0157 (M-2) $C_{11}H_{11}ClN_2OS$ M.W. 254.7358). FT-IR (KBr cm^{-1}): 3219.73 (N-H, str.), 3050.01 (C-H aromatic, str.), 1669.07 (C=O amide), 1537.92 (C=C aromatic), 1260.19-1184.97 (C-N). ^{1}H-NMR (400 MHz, $CDCl_3$) □ in ppm: 10.685 (1H, s, -N*H*), 9.049 (1H, s, -N*H*), 7.832-7.823 (1H, s, Ar-*H*), 7.698-7.673 (1H, d, *J* = 8.0 Hz, Ar-*H*), 7.594-7.565 (1H, d, *J* = 6.8, 0.8 Hz, Ar-*H*), 7.463-7.423 (1H, t, *J* = 8, 7.6 Hz, Ar-*H*), 6.000-5.904 (1H, m, *J* = 17.2, 10.4, 5.6 Hz, vinylic *H*), 5.345-5.241 (2H, q, *J* = 16.8, 0.8 Hz, vinylic *H*), 4.353-4.321 (2H, m, allylic *H*). ^{13}C-NMR (400 MHz, $CDCl_3$) □ in ppm: 179.814 (*C*=S), 165.617 (*C*=O), 135.612, 133.676, 133.609, 131.769, 130.501, 128.002 (Aromatic *C*).

1-Allyl-3-(4-chlorobenzoyl)thiourea (Cpd 4)

Yellowish-white crystals, yield: 19.962%. m.p: 105.9-106.9 °C, MS: m/z (%): 255.0365 (M+,100), 256.0393 (M+1), 257.0337 (M+2), $C_{11}H_{11}ClN_2OS$ M.W. 254.7358. FT-IR (KBr cm^{-1}): 3221.66 (N-H, str.), 3082.80 (C-H aromatic, str.), 1667.14 (C=O amide), 1547.57-1487.78 (C=C aromatic), 1264.05 (C-N). ^{1}H-NMR (400 MHz, $CDCl_3$) □ in ppm: 10.724 (1H, s, -N*H*), 8.988 (1H, s, -N*H*), 7.769 (2H, d, *J* = 8.8 Hz, Ar-*H*), 7.498-7.476 (2H, d, *J* = 8.8 Hz, Ar-*H*), 5.947-5.921 (1H, m, *J* = 16.8, 10.4, 6.0 Hz, vinylic *H*), 5.344-5.272 (2H, d, *J* = 16.8 Hz, vinylic *H*), 4.347-4.328 (2H, m, allylic *H*). ^{13}C-NMR (400 MHz, $CDCl_3$) □ in ppm: 179.890 (*C*=S), 165.827 (*C*=O), 131.788, 130.158, 129.614, 128.957 (aromatic *C*).

Antibacterial activity

The MIC values of the tested compounds are shown in Table 2. All substances did not show any inhibition activity at the highest series of concentrations (1000 µg/mL), except for Cpd 1 and Cpd 4 against MRSA sputum isolate 15621, where MIC values of 1000 µg/mL for both were obtained. Based on the categories set by previous methodologies, Cpd 1 and Cpd 4 indicated a weak effect against MRSA sputum isolate 15621 (15). However, this was not considered satisfactory, thus the next analysis was performed to describe antibacterial activity by the inhibition trend following the series of concentrations, shown in Table 3 and Fig. 3.

Table 2. MIC values (μg/mL) in the presence of thiourea analogs (Cpd 1-4) in multi-bacteria.

Bacteria	MIC value (μg/mL)			
	Cpd-1	**Cpd-2**	**Cpd-3**	**Cpd-4**
Gram-positive				
Methicillin-resistant *Staphylococcus aureus*				
Blood isolate (11880)	> 1000	> 1000	> 1000	> 1000
Pus isolate (16810)	> 1000	> 1000	> 1000	> 1000
Pus isolate (14599)	> 1000	> 1000	> 1000	> 1000
Sputum isolate (15621)	**1000**	> 1000	> 1000	**1000**
Gram-negative				
Salmonella Typhi blood isolate	> 1000	> 1000	> 1000	> 1000
Escherichia coli urine isolate (1223)	> 1000	> 1000	> 1000	> 1000
Pseudomonas aeruginosa ATCC 27853	> 1000	> 1000	> 1000	> 1000

MIC, Minimum inhibitory concentration.

Table 3. Antibacterial activity analysis by microbial growth scoring. 0: No growth; 1: low growth; 2: medium growth; and 3: high growth.

Compounds	Concentration (μg/mL)	Microbial growth scoring						
		Methicillin-resistant *Staphylococcus aureus*				*Salmonella* Typhi	*Escherichia coli*	*Pseudomonas aeruginosa*
		Blood isolate (11880)	Pus isolate		Sputum isolate (15621)	Blood isolate	Urine isolate (1223)	ATCC 27853
			16810	14599				
Cpd 1	7.8125	3	2	2	2	3	3	3
	15.625	3	2	3	2	3	3	3
	31.25	3	2	3	2	3	3	3
	62.5	3	2	2	2	3	3	3
	125	3	2	3	2	3	3	3
	250	3	2	2	2	2	2	3
	500	2	1	2	1	2	2	3
	1000	2	1	2	0	2	2	3
Cpd 2	7.8125	3	2	3	3	3	3	3
	15.625	3	3	3	3	3	3	3
	31.25	3	3	3	3	3	2	2
	62.5	3	3	3	3	3	3	2
	125	3	3	3	3	3	3	2
	250	3	3	3	3	2	3	2
	500	3	3	3	3	2	3	2
	1000	3	2	3	3	3	3	1
Cpd 3	7.8125	3	2	3	3	3	3	3
	15.625	3	2	3	3	3	3	2
	31.25	2	2	3	2	3	3	2
	62.5	3	2	3	2	3	3	2
	125	3	2	3	1	3	2	2
	250	2	2	2	2	2	3	2
	500	2	2	3	2	2	3	2
	1000	2	1	2	1	2	3	2
Cpd 4	7.8125	3	2	3	3	3	3	3
	15.625	2	2	3	3	3	3	3
	31.25	2	2	3	2	3	3	3
	62.5	1	2	3	2	3	2	3
	125	2	2	3	2	2	2	3
	250	3	2	3	1	2	2	2
	500	3	2	3	1	2	2	2
	1000	1	1	3	0	2	2	2

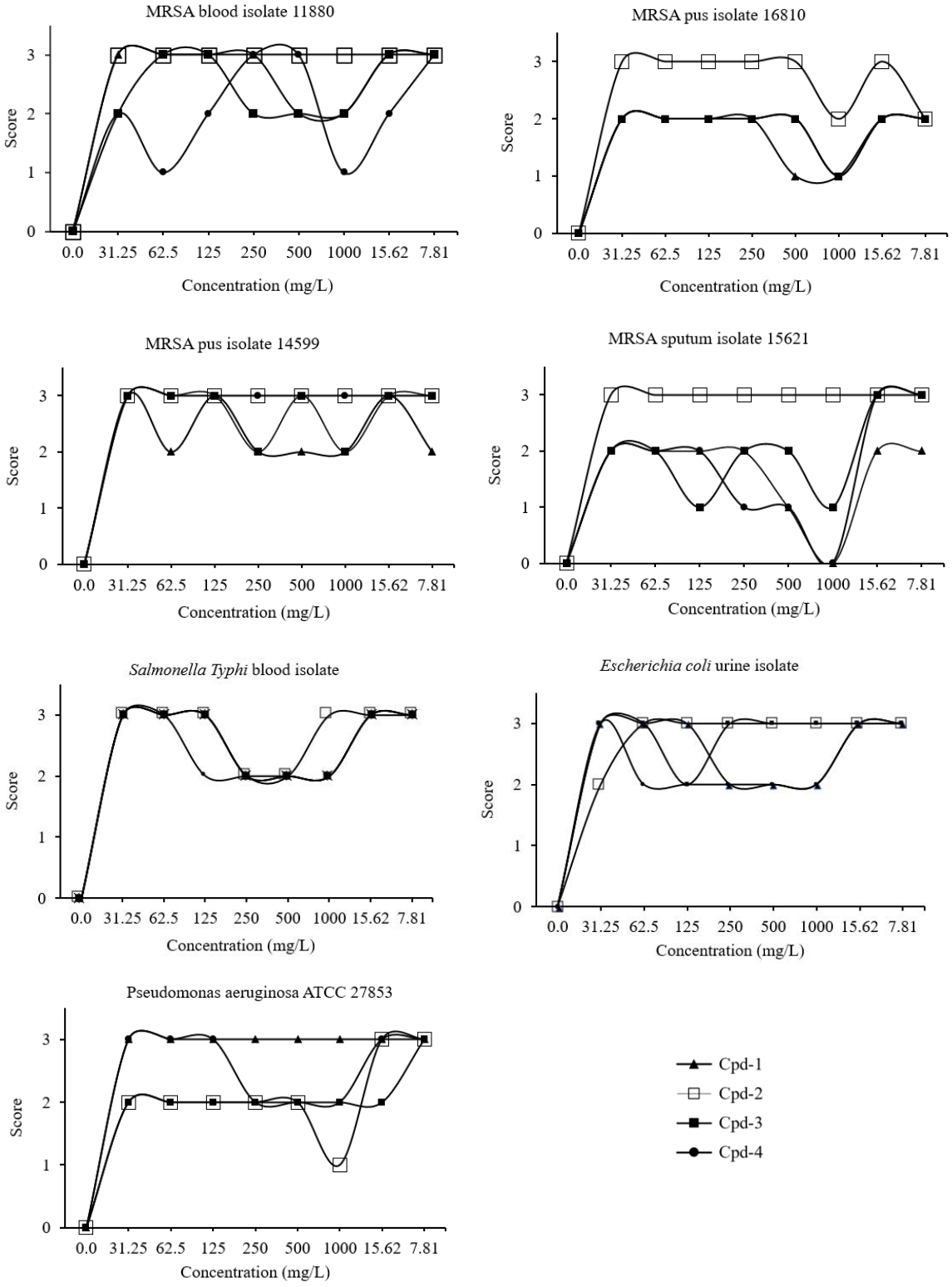

Fig. 3. Antibacterial activity of candidate compounds on selected microorganisms presented as concentration-response profiles.

DISCUSSION

The *in-silico* studies showed that all compounds have a good rerank score compared to ciprofloxacin. Rerank scores represent the affinity of the ligand to the receptor, which means that all four compounds have strong ligand-receptor interaction. These results come after the good electronegativity profile due to the chloro substitution which strengthens bonds to the receptor. A study of the structure-activity relationship (SAR) showed the ligand better biological activity by electron-withdrawing group substitution for they can improve the lipophilicity and hydrogen interaction to the receptor (16). The chloro group also took part in the hydrogen bonds formation and contributed to the bond strengthening, even ranked better than clorobiocin and tetracycline as standards (17).

However, all four candidate compounds showed unsatisfactory antibacterial activity, but nevertheless showed an increasing trend of growth inhibition along with the increment of concentration. It seems that the compound's lipophilicity leads to difficulty in membrane penetration. The longer alkyl chains or the more lipophilic a compound causes a decreased antibacterial activity due to the limited membrane partition. The limited partition coefficient makes it difficult for these compounds to penetrate the cell wall of the bacterial membrane.

It is implied that the lipophilicity of a compound is not sufficient to represent antibacterial activity, but more to a hydrophobic-hydrophilic balance (18). The same opinion was also conveyed, where compounds with low lipophilicity character showed higher antibacterial activity (19).

The partition coefficient or log P value indicates the lipophilicity of a compound, where the greater the value the more lipophilic the compound is. Table 4 summarized the log P values of all the candidate compounds based on the predictions of ChemDraw Ultra 8.0 and also the log P values of the standard compound ciprofloxacin based on PubChem. Ciprofloxacin, as a second generation of fluoroquinolone, was used as a standard antibiotic that exhibits notable antibacterial activity for both Gram-positive and Gram-negative bacteria. The primary mechanism of action is the inhibition of DNA gyrase, as well as the candidate compound (20).

The lipophilicity character of the candidate compounds was higher than that of the standard compounds. Ciprofloxacin could exhibit antibacterial activity with a log P value of 0.28 while the candidate compounds Cpd 1-4 showed higher log P values, 2.47 and 3.03. This may affect the absorption of compounds on the bacterial membrane prior to exhibiting inhibition activity.

The inhibition profile of the compounds on the MRSA showed different features. The MIC data values of compounds 1 and 4 against MRSA sputum isolate 15621 showed a MIC value of 1000 μg/mL, better compared to the other compounds. As a Gram-positive bacteria, MRSA has a lower lipid content on the cell wall than Gram-negative bacteria, so that active compound could be easier to penetrate the membrane. Although Gram-negatives had thin and single-layer peptidoglycan, the penetrating feature of the tested compounds is hampered due to the cell wall's high lipid and lipoprotein concentration (21).

The size and shape of the tested compounds also affect their interaction with the binding site. A bulky substituent experiences hindrance in its interaction with the binding site. On the other hand, it can also assist the ligand to orient optimally for maximum binding interaction. Molar refractivity is a measure of both the ease of polarization and the volume of a compound. Table 5 showed the prediction of ChemDraw Ultra 8.0 on calculated molar refractivity, where ciprofloxacin was bulkier than the candidate compound. The less bulky profile of the candidate compounds could be one of the reasons that affect the antibacterial activity related to the interaction with binding sites (22).

Table 4. LogP value of all four candidate compounds and ciprofloxacin.

No	Compound	LogP value
1	Ciprofloxacin	0.28
2	Cpd-1	2.47
3	Cpd-2	3.03
4	Cpd-3	3.03
5	Cpd-4	3.03

Table 5. CMR value of all four candidate compounds and ciprofloxacin.

No.	Compound	CMR value
1	Ciprofloxacin	8.7159
2	Cpd 1	6.6472
3	Cpd 2	7.1386
4	Cpd 3	7.1386
5	Cpd 4	7.1386

CMR, Calculated molar refractivity

CONCLUSION

All the synthesized compounds showed an increased trend of growth inhibition along with the increment of concentration, especially for Cpd 1 and Cpd 4 towards MRSA sputum isolate 15621. However, the MIC values of all tested compounds remained unsatisfactory. The membrane penetration profile of the compounds might be the reason for the lack of inhibition activities. The higher log P and the molar refractivity values of the compound can affect the ease of penetration of the active compound through the bacterial membrane. Lipophilicity and steric parameter should become the focus for further optimization of the lead compound design to produce better antibacterial activity.

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Conflict of interest statement

All authors declared no conflict of interest in this study.

Authors' contribution

A. F. Shalas contributed to molecular docking, compound synthesis, and structure elucidation; S. Winarsih and E. Wiloka conducted the antibacterial assay; B. R. Pratita Ihsan contributed to structuring elucidation and antibacterial assay; A. Kharismawati conducted molecular docking and compound synthesis; A. I. Firdaus synthesized the compounds. The finalized manuscript was approved by all authors.

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