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Antibacterial Activity and Molecular Docking Studies of Series Hydroxyxanthone

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ABSTRACT. Antibacterial assay of series hydroxyxanthone that was a synthesis via cyclization of phenol derivatives and acid derivatives with Eaton Reagent has been investigated through *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella typhimurium* by diffusion well method. The possess antibacterial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) has been reported by molecular docking. The result reveals that the 1,3,6-tryhydroxyxanthone were effective in inhibiting the growth of *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella typhimurium* in the inhibition zone of 30, 30, 35, than 34 mm respectively with MIC at 10%. There was a binding interaction between 1,3,6-tryhydroxyxanthone and the amino acid residues such as His38, His35, Val177, Lys150 and Met41 into methicillin-resistant *Staphylococcus aureus* (MRSA) (2x3f.pdb).

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital-acquired infections that are becoming increasingly difficult to combat because of emerging resistance to all current antibiotic classes [1] The infections caused by drug resistant *Staphylococcus aureus* in recent years have created an increase risk of death. This is due to failure to treat various infectious diseases, cancer chemotherapy, malaria treatment, surgery or various biological actions due to the resistance of active ingredients as antibacterial, even wider due to resistance antibiotics. Hydroxy xanthone compounds obtained in previous studies have various biological activities such as anticancer [2], antioxidants [3] and anti-malarial [4]. Based on in vitro activity data, this compound has the potential to be developed as an active ingredient in cancer chemotherapy drugs, antioxidants and malaria drugs. To maximize the function of these active ingredients, biological capability must also be complemented with activities as antibacterial, for this reason, an evaluation of antibacterial activity, especially xanthone derivative compounds, especially hydroxy xanthone, needs to be done. It is expected that the ability as an antibacterial will be able to increase the activity of hydroxy xanthone compounds as active ingredients of cancer chemotherapy, malaria drugs and antioxidant active ingredients.

Based on the results of the literature review, there are several xanthone derivatives, both synthesized and isolated, which have been reported to have good anti-bacterial activity, among others phenyl xanthone [5], tetraoxygenated xanthones [6], hybrid monoterpene tetrahydroxyxanthone and dihydroxyxanthone derivatives [7] isolated xanthones from the broth culture of Micrococcus sp. EG45 cultivated from the Red Sea sponge Spheciospongia vagabunda [8] phenyl oxygenated xanthone [9] amphiphilic xanthone [10]. The antibacterial activity in this study was carried out using a well diffusion method for 4 types of bacteria, *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella typhimurium* which belong to the group of gram negative and gram positive bacteria. This method, mainly simplicity and low cost, has contributed to its common use of the antimicrobial screening of plant extracts, essential oils and other drugs [11-13]. Herein, we report antibacterial

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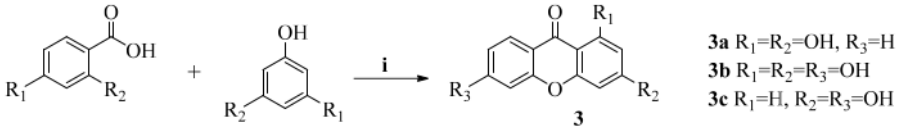
activities of hydroxy xanthone (3a-c) that has been synthetically before. Compounds with excellent in vitro antibacterial activity were chosen for molecular docking investigations into the resistant bacteria such as Methicillin-resistant *Staphylococcus aureus* (MRSA) (Pdb 2x3f).

MATERIAL AND METHODS

Experimental

Synthesis

The synthesis of hydroxyxanthone 3a-c were carried out by reacting the acid compound (a) (10.2 mmol) with phenol derivatives (b) (10 mmol) and Eaton's reagent (5 mL) in one pot reaction as method conducted by Yuanita et al., (2017;2018; 2019) [2-4]. As shown in Fig. 1.



1
1a R_1=OH, R_2 = H
1b R_1=R_2 = OH

2
2a R_1=OH, R_2 =H
2a R_1 = R_2 = OH

3

FIGURE 1. General procedure for synthesis of hydroxy xanthone: Reagents and conditions of synthesis: *(i)* Eaton Reagent, reflux 80 °C, 3hours

1,3-dihydroxy-9H-xanthen-9-one **3a** red solid (85.5%), m.p.: 223-223.8 °C. FTIR (KBr, ν; cm^{-1}): 3448 (OH), 1612 (C=O), 1458 (C-C aromatic), 1296 (C-O-C). ^{1}H-NMR (CD_3OD; 500 MHz) δ (ppm): 6.20 (1H, d, *J* = 2.00 Hz and *J* = 1.50 Hz), 6.36 (1H, dd, *J* = 8.00 Hz and *J* = 3.75 Hz), 7.49 (1H, dd, *J* = 8.00 Hz and *J* = 3.75 Hz), 7.40 (1H, dd, *J* = 8.50 Hz and *J* = 1.5 Hz), 7.74 (1H, dd, *J* = 8.50 Hz and *J* = 1.50Hz), MS (EI) *m/z*: 228 (M+1).
1,3,6-trihydroxy-9H-xanthen-9-one **3b** yellowish solid (81.96%), 322-323 °C (Dec.). FTIR (KBr, ν; cm^{-1}): 3163 (OH), 1612 (C=O), 1465 (C-C aromatic), 1296 (C-O). ^{1}H-NMR (DMSO-d_6; 500 MHz) δ (ppm): 8.75 (1H, dd, *J*=8.75 Hz and *J* = 2.10 Hz) 8.02- 8.04 (1H, dd, *J*=8.75 Hz and *J* = 2.10 Hz), 6.84 (1H, s, *J* = 2.10 Hz), 6.36 (1H, s, *J* 2.10 Hz), 6.20-6.22 (1H, s, *J* = 2.10 Hz). MS (EI) *m/z*: 244 (M+).
3,6-dihydroxy-9H-xanthen-9-one **3c** reddish solid (70.15 %), m.p.: 220-223 °C. FTIR (KBr, ν; cm^{-1}): 3248 (OH), 1620 (C=O), 1458 and 1512 (C-C aromatic), 1242 (C-O-C). ^{1}H-NMR (CD_3OD; 500 MHz) δ (ppm): 7.49 (2H, d, *J* = 8.7 Hz), 6.48 (2H, d, *J* = 8.7 Hz and 2.35 Hz), 6.43 (2H, d, *J* = 2.35 Hz). MS (EI) m/z: 228 (M+).

Antibacterial Activity

Well Diffusion Assay

The assay was conducted as described by Heatley, 1944 [14] and Perez et al. (1990)[15]. Briefly, microorganisms from growth on nutrient agar incubated at 37°C for 18 h were suspended in saline solution 0.85% sodium chloride and adjusted to a turbidity of 0.5 10^8cfu/ml (Mac Farland standard). The suspension was used to inoculate 90 mm diameter Petri plates with a sterile nontoxic cotton swab on a wooden applicator. Six millimeters diameter wells were punched in the agar and filled with 50 µl of 2000 µg/ml various hydroxy xanthone. The dissolution of the hydroxy xanthone was aided by 1% (v/v) DMSO which did not affect bacterial growth, according to our control experiments. Commercial antibiotics ciprofloxacin were used as positive reference standard to determine the sensitivity of the strains. Discs were directly placed onto the bacterial culture. Plates were incubated in air at 37°C for 24 h. Antibacterial activities were evaluated by measuring inhibition zone diameters (mm).

Determination of Minimum Inhibitory Concentration (MIC)

Determination of minimum inhibitory concentrations (MICs) by standard twofold micro broth dilution as described by NCCLS [16]. Series oh hydroxy xanthone was serially diluted in 96-wells Microtiter plate with Mueller Hinton broth to obtain a concentration ranging from 8 µg/ml to 4096 µg/ml. A standardized inoculum for each bacterial strain was prepared so as to give the inoculum size of approximately 5 × 105CFU/ml in each well. Microtiter plates were then kept at 37°C for an overnight incubation. Following incubation, the MIC was calculated as the lowest concentration of the extract inhibiting the visible growth of bacterial strain based on diameter inhibition (inhibition zone = 12).

Molecular Docking Study

The resistant bacteria such as Methicillin resistant *Staphylococcus aureus* (MRSA) (Pdb.2x3f) inhibition to be one of the strategies in treating antibiotic resistant. In this work, the protein crystal docking study of the active compound that obtained from the experiment result was performed to investigate its ability to inhibit MRSA, while comparing with native ligand penthonate synthase (2X3F.pdb). Docking simulations were performed in previous work [2-4] under the receptor–ligand interaction section in Discovery Studio 3.1 (Accelrys, Inc., San Diego, CA, USA). Other molecular modelling software used throughout this study was CHIMERA 1.9 and ChemOffice®2015.

RESULT AND DISCUSSION

For the first partway, The xanthone building block 2 was synthesized according to Grover, Shah, and Shah method and has reported Lim et al. (2012) [17] and The structures of the investigated compounds were confirmed by H NMR, mass spectrometry and FTIR previous reported [2-4]

Antibacterial Activity

This initial screening was conducted to determine the potential of synthesized compounds as antibacterial active ingredients. This stage is carried out by the well diffusion method on 4 types, namely bacteria, namely *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella typhimurium*. The inhibitory activity in the well method is characterized by the formation of a clear zone around the well which is 7 mm in diameter. The results are shown in Fig. 2.

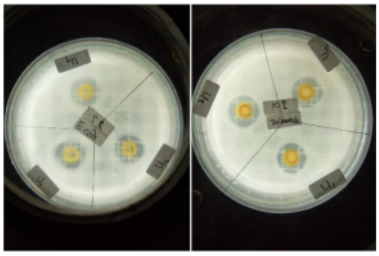


FIGURE 2. Nearest whole of hydroxy xanthone

Based on data from bacterial inhibition test results illustrate that compound 3a-c inhibits the growth of gram-positive bacteria and gram-negative bacteria with a diameter of 18-35 mm, primary screening indicated that all hydroxy xanthone series has broad spectrum to inhibiting the bacteria. According to CSLI the obstacle zones that are in the range ≥ 19 are sensitive categories [16]. A clear zone of resistance with a firm bactericidal edge diffuses into the surrounding area at the same speed and then kills the indicator bacteria, where the circle clearly shows the extent of diffusion of the component. While the zone of resistance is blurred, it may only be bacteriostatic, which will provide a blurred edge, because some indicator bacteria that do not die will remain alive despite very slow growth. Based on the inhibitory pattern and clear zone, which formed the best activity, namely for *Staphylococcus aureus*

and *Salmonella typhimurium* bacteria showed that compound 3b is good for treating diseases caused by these two types of bacteria. This shows that there is an effect of the addition of hydroxy groups to the clear zones formed. Besides the clear zone pattern, the activity formed is also measured based on the width of the zone or the diameter of the clear zone formed, as shown in the following Table 2.

TABLE 1. Diameter of inhibition zones (mm) hydroxy xanthone toward bacteria

Compounds	Diameter of Inhibition Zones (mm)			
	E. coli	*B. cereus*	*S. aureus*	*S. typhimurium*
1,3-dihydroxyxanthone (3a)	*20*	*20*	*20*	*30*
1,3,6-trihydroxyxanthone (3b)	*30*	*30*	*35*	*34*
3,6-dihydroxyxanthone (3c)	*30*	*18*	*25*	*30*

Determine minimum inhibitory concentration (MIC) by two folds micro-broth dilution method against respective susceptible bacterial species. MIC (Minimum Inhibitory Concentration) is the lowest concentration of antibiotics or antimicrobials that can inhibit the growth of certain microbes. MIC values are specific for each combination of antibiotics and microbes. The MIC of an antibiotic against microbes is used to determine the sensitivity of microbes to antibiotics. Based on the test results in various concentrations obtained a value of 10%, included in the intermediate category (CSLI category) [17]. Based on the clear zone produced and the MIC value it can be concluded that the hydroxy xanthone compound, specifically 1,3,6-trihydroxyxanthone, has the potential to be developed as an antibacterial active ingredient to support the treatment of infectious diseases in the category of sensitive inhibition and is included in the broad spectrum because it can provide good resistance to gram bacteria positive or gram negative reinforced by MIC values included in the intermediate category.

TABLE 2. MIC Hydroxyxanthones toward bacteria

Concentration	Compounds	Inhibition Zones			
		E. coli	*B. cereus*	*S. aureus*	*S. typhimurium*
5 %	1,3-dihydroxyxanthone (3a)	+	+	+	+
	1,3,6-trihydroxyxanthone (3b)	+	+	+	+
	3,6-dihydroxyxanthone (3c)	+	+	+	+
10 %	1,3-dihydroxyxanthone (3a)	+	+	+	+
	1,3,6-trihydroxyxanthone (3b)	-	-	-	-
	3,6-dihydroxyxanthone (3c)	-	--	-	-

Molecular Docking

Based on the clear zone and MIC values, it is obtained that the hydroxy xanthone compound has the potential to be developed into a broad-spectrum antibiotic material, that is, an antibiotic that kills gram-positive or gram-negative bacteria. Antibacterial actions generally fall within one of four mechanisms, three of which involve the inhibition or regulation of enzymes involved in cell wall biosynthesis, nucleic acid metabolism and repair, or protein synthesis, respectively. The fourth mechanism involves the disruption of membrane structure. Furthermore, to find out the mechanism of action of the action of hydroxy xanthone compounds carried out using molecular docking. Docking inhibition studies will be carried out on resistant bacteria represented by MRSA. Multi-drug resistant *Staphylococcus aureus* infections have created a critical need for the development of new classes of anti-bacterial. Discovery of new naturally derived antibacterial agents with a new mechanism of action remains a high priority globally, based on MRSA and VSE are very difficult to be paralyzed by antibiotics clinically. From Table 3 it can be seen that as a whole 1,3,6 trihydroxanthones compounds have the same binding interaction with His88, His35, Met41, Val177 and Lys150 as those formed by native ligand phenthoate synthase (2X3F.pdb) which is a native ligand which has interactions with MRSA amino acid receptors which mechanism inhibits DNA ligase. Interaction with amino acid residues of MRSA-resistant phenthoate synthase (2X3F.pdb) shows that 1,3,6-trihydroxy

compounds are also predicted to have antibacterial activity against *Staphylococcus aureus* bacteria that are already resistant.

TABLE 3. Energy, the distance hydrogen bonds and binding interaction of 1,3,6- trihydroxyxanthones and co-phenthoate synthase ligands of MRSA .

Compound	cDOCKER Energy (kcal/mol)	Binding Interaction (Amino acid residue)	Hydrogen bond distance (Å)
1,3,6 trihydroxanthones	-28,447	His38	4.72
		His35	6.10
		Met41	5.86
		Val177	3.52
		Lys150	4.41
Panthothenate synthetase	-55,1882	His38	2.90
		His35	5.38
		Met41	5.72
		Val177	2.15
		Lys150	4.87

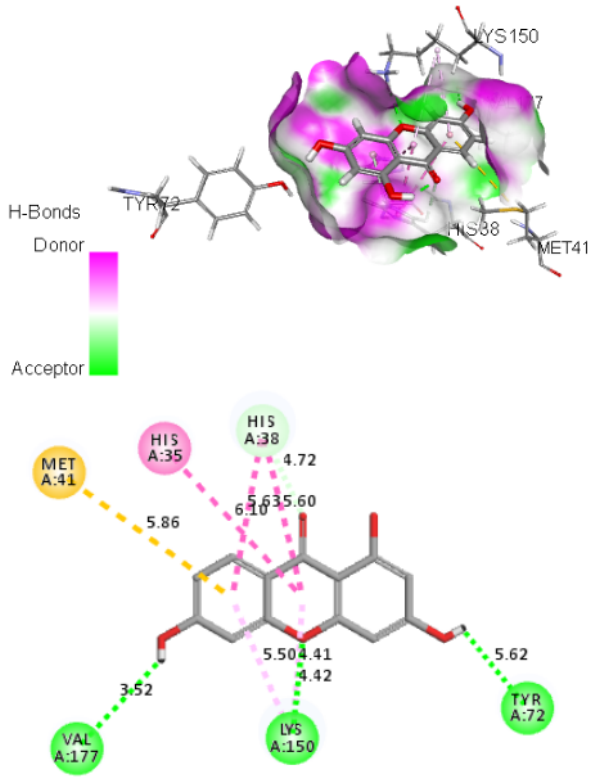


FIGURE 3. 2D and 3D predicted binding mode from docking simulation of 1.3.6-trihydroxyxanthone into the active site of MRSA (2X3F.pdb)

CONCLUSION

The 1,3,6-tryhydroxyxanthone were effective in inhibiting the growth of *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella typhimurium* in inhibition zones of 30, 30, 35, than 34 mm respectively with

MIC at 40 mm. Interactions with amino acid residues His88, His35, Met41, Val177 and Lys150 ligands of MRSA (2X3F.Pdb) indicate the 1,3,6-trihydroxyaxanthone compounds are also able to inhibit the growth of resistant bacteria *Staphylococcus aureus*.

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