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Research Article

Synthesis, Antimicrobial Activity and Molecular Docking Study of Monocarbonyl Curcumin Analogue D125, D144, D156

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ABSTRACT

A novel curcumin analogue D125, D144 and D156 were developed to be potent antimicrobial against Gram positif and negative. Our work coupled both the tetrahydro-4H-thiopyran-4-one and new benzaldehyde derivatives by using Aldol condensation protocol under acidic condition. Three series of the curcumin analogue were synthesized and characterized by IR, ^{1}HNMR, ^{13}CNMR and Mass spectrometer analysis then evaluated for *in vitro* antimicrobial activities. All the compounds were subjected to molecular docking studies for the inhibition of the cell wall bacteria, inside the active site of peptidyl transferase center of *E. coli* ribosome (PDB ID: 2EX8). Antimicrobial activities were tested using the serial difusion method. It was observed the most exhibited activity against the cell wall division is D156 with docking score (S) -11.7859. The test compound D144 exhibited moderate activities compared to the derivates compound whereas showed weak activities than HGV-6, D154 not exhibited activities compared to the derivates compound and HGV-6, D156 were more active than to the derivates compound and HGV-6. The trisubstituted D156 showed strongest antibacterial effects than the parent compounds HGV-6 and their derivates in Gram positive and negative but no activities against tested fungal strains.

INTRODUCTION

Curcumin 1,7-*bis*-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, a compound isolated from Curcuma longa L is commonly used as a dietary pigment and spice recently. Curcumin is a phenolic compound with a variety of ancient pharmaceutical applications on diseases, including external/internal wounds, liver diseases (particularly jaundice), blood purification, microbial effects and inflamed [1–6]. This review focuses on the antibacterial activities of curcumin against staphylococcus aureus has been reported [7, 8].

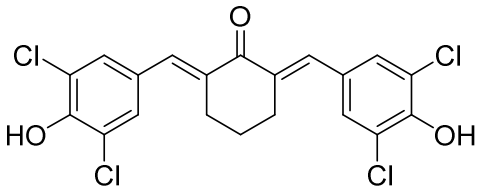

Figure 1: Chemical Structure of HGV-6

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The challenge of new antimicrobial development is one of the subject of an ongoing research that could overcome the resistance problem [7–10]. One of curcumin analogue compound has potent antimicrobial activity, i.e HGV-6 with the main scaffold of cyclohexanone and chloro substituents on C3 and 5, hydroxy C4 atoms. At a concentration of 1000 µg/ml, HGV-6 has the best inhibitory activity from the other series, ie with an inhibitory diameter of 11.5 mm against *S. aureus* bacteria, 16.5 mm against *B. subtilis, K. pneumoniae* at 11.4 mm, *C. albicans* at 13.0 mm, but did not inhibit *E. coli* growth [11].

Pyrazoles, isoxazole and diazepine are five-membered heterocyclic compounds and have played as an promising antibacterial activity. Heterocyclic compounds considered one of the vital classes of organic compounds with various bioactivities ranging from antibacterial to anticancer [12–14]. Diazepines and benzodiazepines have various therapeutic applications. Many members of the diazepine family are widely used as anticonvulsants, anti-anxiolytics, analgesics, sedatives,

antidepressants and hypnotic agents [15]. Moreover of imidazoles and nitroimidazoles, found that compounds 5a–d exhibited promising antibacterial activities while showing weak non-nucleosidal reverse transcriptase activity [16]. These literature findings have led us to synthesize the proposed group of curcumin based heterocycles and screen them against representative panel of Gram-positive and Gram-negative bacteria [17].

In the present work, we have studied influenced of hetero atoms and substituent groups employed curcumin analogues. These compounds were also designed to examine the role of different substitutes in the benzene ring and the influence of the space structure of the linking c-strain. It is hoped that continued research will lead to development of new lead compounds from curcumin as antibacterial agents and extrapolated agents for bacteria infected diseases.

MATERIAL

Chemicals and Reagents

All reagents used were of analytical grade 2-chlorobenzaldehyde, 4-chlorobenzaldehyde, 3,5-dichloro-4-hydroxy benzaldehyde and tetrahydro-4H-thiopyran-4-one were purchased from Aldrich, U.S.A. Other reagents were purchased from Merck Chemical Reagent Co., Ltd., China. All new compounds purity was checked by thin-layer chromatography (TLC) and ^{1}H-NMR. All reactions were monitored by TLC on pre-coated Silica Gel F254 plates (purchased from Qingdao Marine Chemical Factory, China) with detection by UV. Synthetic routes are presented in Schemes 1–2. Melting points were determined on a Buchi melting point B540.

Instrumentation

The proposed work was carried out on a ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL ECZ-500R spectrophotometer. All ^{1}H-NMR experiments were reported in δ units, parts per million (ppm) downfield from tetramethylsilane (TMS) as the internal reference. Electron-impact ionization mass spectra in positive mode were recorded on GC17A MSQP 5000 Shimadzu spectrometer. Infrared spectra were recorded in KBr on a Thermo Nicolet IS10 FT-IR spectrophotometer.

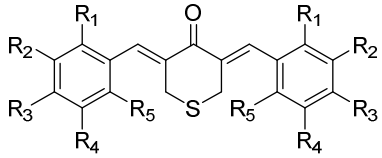
Selection of Solvents

On the basis of solubility study acetic acid was selected as the solvent for dissolving aldehyde and tetrahydro-4H-thiopyran-4-one.

METHODS AND RESULTS

General Procedure of Synthesis

To a solution D125, D144, D154 of 2 mmol substituted benzaldehyde in acetic acid 100% was added 1 mmol ketone but D156 2 mmol. The solution was stirred at room temperature for 8 hours, up to homogenous solution and monitored with TLC. When the reaction is complete, the residue was collected and thrown away. The powder was washed with water and dried in vaccum. The yellow solid was obtained and purified by recristalization with acetone as the eluent to afford compounds D125, D144 and D156.



Compound	R1	R2	R3	R4	R5
D125	Cl	-	-	-	-
D144	-	-	Cl	-	-
D156	-	Cl	OH	Cl	-

Figure 2: Structure of Monocarbonyl Analogue Curcumin

Characterization of Synthesis

3,5-bis-(2´-chlorobenzylidene)-tetrahydro-4H-thiopyran-4-one (D125)

M.p. 228.7-230.6°C, yield: 53, 16 %, yellow crystalline solid. IR spectrum: 2723, 49 (α.β unsaturated =C-H str), 1604,77 (α.β unsaturated –C=C stretching). NMR (500 MHz, DMSO-d) δ 8.031 (s, 1H), 7.495 (dd, J = 1.5, 8.5 Hz, 1H), 7.305 (dd, J = 1.5, 7.5 Hz, 1H), 7.169 (dd, J = 8 Hz, 1H), 7.081 (dd, J = 7.5, 7.5 Hz, 1H), 4.345 (s, 2H), ^{13}C NMR (125 MHz, common NMR solvents) δ 182.406, 158.083, 135.113, 132.003, 130.179, 127.645, 122.222, 120.437, 111.59, 55.703, Mass spectrum: m/z M^{+} 360.1 (100 %), 325, 279, 257, 228 , 215, 200, 181, 169, 147, 125, 115, 101, 89, 63, 39.

3,5-bis-(4´-chlorobenzylidene)-tetrahydro-4H-thiopyran-4-one (D144)

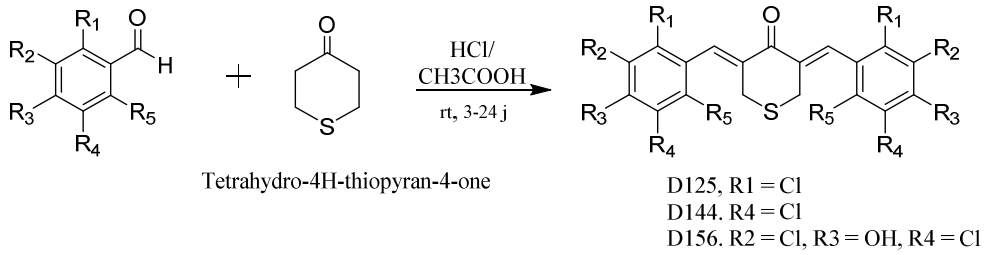
M.p. 164.8-164.9°C, yield: 46.91%, yellow crystalline solid. IR spectrum: 2854, 6 (α.β unsaturated =C-H str), 1581,63 (α,β unsaturated –C=C stretching). ^{1}H NMR (500 MHz, DMSO-d) δ 7.643 (s, 1H), 7.556 (d, J = 8Hz, 4H), 4.009 (s, 2H), ^{13}C NMR (125 MHz, common NMR solvents) δ197.218, 126.994, 126.274, 126.082, 125.938, 123.750, 120.746, 21.630, Mass spectrum: m/z

M^+ 360.1 (100 %), 360, 332, 325, 297, 285, 263, 251, 228, 215, 202, 181, 152; 150, 116, 115, 89, 63, 45, 39.

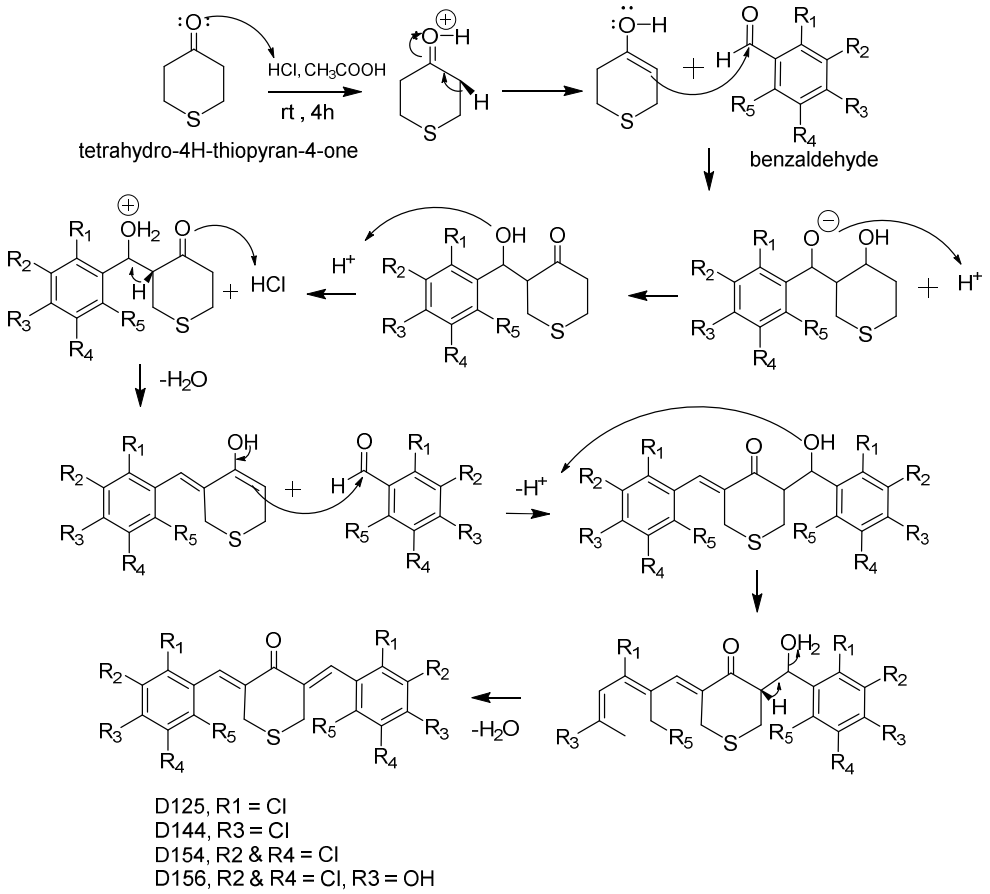
3,5-bis-(3´,5´-dichloro-4-hydroxybenzylidene)-tetrahydro-4H-thiopyran-4-one (D156)

M.p. 164.7-164.9°C, yield: 38.76%, brown crystalline solid. IR spectrum: 2854,65 (α.β unsaturated =C-H str), 1597,06 (α,β unsaturated –C=C stretching).^{1}H NMR (500 MHz, DMSO-d) δ 9.343 (s, 1H), 7.525 (s, 2H), 4.032 (s, 1H), 2.931 (s, 2H), ^{13}C NMR (125 MHz, common NMR solvents) δ 188.030, 150.585, 135.391, 133.912, 131.225, 129.324, 122.941, 30.592, Mass spectrum: m/z M^+ 462 (100 %), 462; 452; 427; 399; 364; 328; 298; 259; 231; 227; 200; 165; 138; 136; 101; 75; 45.

Synthesis, *In Vitro* and *In Silico* Antimicrobial Studies of Curcumin Analogue



Scheme 1: Synthesis of Curcumin Analogue D125, D144, D156



Scheme 2: Mechanism of Curcumin Analogue D125, D144, D156

Antimicrobial Evaluation

The antibacterial bioactivities of the prepared curcumin analogue were screened using the agar diffusion method. Seven different bacteria were selected *Staphylococcus aureus* (ATCC 25923), *Bacilus Subtilis* (ATCC 6633), *Streptococcus mutans* (ATCC 20175), *Enterococcus faecalis* (ATCC 29212), *Klebsiella pneuumoniae*, *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and fungal strains *Candida albicans* (ATCC 10231). In this method, 10 ml Mu¨ller–Hinton for bacteria medium was

spread on Petri plates, and these plates were spread with 24 h cultured 108 colony-forming units (CFU)/ml of microbial strains. Wells of 6 mm diameter were made in the culture medium. Target compounds (50 µl) were added to the paper discs [18] In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface. The petri dishes are incubated at 37°C for 24 h [19]. Amoxicilin (32,25 µg/ml each) were taken as reference drugs for antibacterial activity, and nistatin for antifungal activity. A positive control was kept for reference. The activity of the target compounds was measured based on the inhibition zone formed around the well. The assay was carried out in triplicate. The diameter of the zone of inhibition was measured in millimeters. Results of the initial antimicrobial activity evaluation of the synthesized compounds are presented in Table 1. Based on the result of the compounds D125, D144, D156 were selected for discs diffusion tests, the results of which are shown in Table 1, 2 and 3.

Compounds D144 showed good activity against Gram positive only in *E. faecalis* up to 125 µg/ml but not in fungal. For another Gram positive bacteria compounds D144 were active but only at high concentrations of 1000 µg/ml and 500 µg/ml. Among all target compounds it is possible the influence of the S (sulfur) group on the cyclic ring and the electrophilicity of the compound which increases with the spread of the positive charge with the resonance occurring from the carbonyl group to the Cl atom at the para position where the positive atomic charge increases the area of the electrophilicity of the compound. Bactericidal showed the highest activity.

Table 1: Antimicrobial activity of curcumin analogue D125

Concentration (µg/ml)	Zone of Inhibition (mm)				Zone of Inhibition (mm)				*n*
	Gram positive				Gram negative			Fungi	
	S. aureus	*B. subtilis*	*E. faecalis*	*S. mutans*	*E. coli*	*K. Pneumonia*	*P. aeruginosa*	*C. albicans*	
1000	0	0	0	0	0	0	0	0	3
500	0	0	0	0	0	0	0	0	3
250	0	0	0	0	0	0	0	0	3
125	0	0	0	0	0	0	0	0	3
62,5	0	0	0	0	0	0	0	0	3
31,25	0	0	0	0	0	0	0	0	3
DMSO	0	0	0	0	0	0	0	0	3
Amox 31,25	14,13,14[a]	9,10,9[a]	15,15, 14[b]	19,20,20[b]	17,18,17[b]	13,13,12[b]	0,0,0	-	3
Nistatin 31,25	-	-	-	-	-	-	-	12,11,12[a]	3

* Bactericidal, •Bacteriostatic, n: replication

Table 2: Antimicrobial activity of curcumin analogue D144

Concentration (µg/ml)	Zone of Inhibition (mm)				Zone of Inhibition (mm)				*n*
	Gram positive				Gram negative			Fungi	
	S. aureus	*B. subtilis*	*E. faecalis*	*S. mutans*	*E. coli*	*K. Pneumonia*	*P. aeruginosa*	*C. albicans*	
1000	13;13;13[a]	12;12;12[b]	13;13;12[b]	11,12,12[b]	7;7;9[b]	11;11;10[b]	0	0	3
500	13;12;13[a]	0	13;13;13[b]	7,7,8[b]	0	7,7,6[b]	0	0	3
250	10;10;9[a]	0	12;12;11[b]	0	0	0	0	0	3
125	0	0	11;11;12[b]	0	0	0	0	0	3
62,5	0	0	0	0	0	0	0	0	3
31,25	0	0	0	0	0	0	0	0	3
DMSO	0	0	0	0	0	0	0	0	3
Amox 31,25	13,13,14[a]	10,9,9[a]	15,15,14[b]	10,12,12[b]	15,13,15[b]	11,10,11[b]	0,0,0	-	3
Nistatin 31,25	-	-	-	-	-	-	-	11,11,12[b]	3

[a] Bactericidal, [b]Bacteriostatic, n: replication

Table 3: Antimicrobial activity of curcumin analogue D156

Concentration (µg/ml)	Zone of inhibition (mm)				Zone of inhibition (mm)				*n*
	Gram positive				Gram negative			Fungi	
	S. aureus	*B. subtilis*	*E. faecalis*	*S. mutans*	*E. coli*	*K. Pneumonia*	*P. aeruginosa*	*C.albicans*	
1000	13;13;13[a]	10,11,10[a]	13;13;12[b]	11;11;10[b]	13;14;13[b]	13;14;14[b]	0	0	3
500	13;12;13[a]	9,10,9[a]	13;13;13[b]	8;8;7[b]	12;12;10[b]	9;9;9[b]	0	0	3
250	9;9;9[a]	8,8,9[a]	11;12;11[b]	6;6;7[b]	10;11;10[b]	0	0	0	3
125	8;9;9[a]	6,7,7[a]	11;11;12[b]	0	11;11;10[b]	0	0	0	3
62,5	0	0	0	0	0	0	0	0	3
31,25	0	0	0	0	0	0	0	0	3
DMSO	0	0	0	0	0	0	0	0	3
Amox 31,25	17,16,16[a]	19,21,20[a]	15,15,16[b]	10;10;9[b]	17,16,16[b]	13,13,15[b]	0	-	3
Nistatin 31,25	-	-	-	-	-	-	-	12,12,11[a]	3

[a] Bactericidal, [b]Bacteriostatic, n: replication

Table 4: Antimicrobial activity of curcumin analogue HGV-6

Concentration (µg/ml)	Zone of inhibition (mm)				Zone of inhibition (mm)				*n*
	Gram positive				Gram negative			Fungi	
	S. aureus	*B. subtilis*	*E. faecalis*	*S. mutans*	*E. coli*	*K. Pneumonia*	*P. aeruginosa*	*C. albicans*	
1000	8[a]	10[a]	13[b]	9[b]	0	12[b]	11[b]	0	1
500	8[a]	8[a]	10[b]	9[b]	0	9[b]	8[b]	0	1
250	9[a]	8[a]	10[b]	7[b]	0	9[b]	8[b]	0	1
125	9[a]	8[a]	8[b]	6[b]	0	7[b]	7[b]	0	1
62,5	0	0	0	0	0	0	0	0	1
31,25	0	0	0	0	0	0	0	0	1
DMSO	0	0	0	0	0	0	0	0	1
Amox 31,25	11[a]	9[a]	15[b]	20[b]	15[b]	10[b]	0	-	1
Nistatin 31,25	-	-	-	-	-	-	-	22[a]	1

[a] Bactericidal, [b]Bacteriostatic, n: replication

D156 showed very strong antibacterial activity compared to the D125, D144 and HGV-6. The presence of heterocyclic groups increases antibacterial activity [20, 21] especially against *E. faecalis* and *E. coli*, which might be due to presence of benzen ring-activating hydroxyl group. The chemical attack generally takes place at the carbonyl group or at the heteroatom or at the substituted alkyl group. OH substituents in phenolic groups such as benzene increasing antimicrobial activity. Most of the compounds were active against *K. pneumoniae*, with compound D156 showing the highest activity in 1000 µg/ml (14 mm). The Gram-negative bacterium *E. coli* showed large inhibition zone (11 mm) at 125 ppm for the applied standard drug. The antifungal activity of all target molecules is nothing. Compound D125, D144, D156 with ring-activating hydroxyl group showed not active on antifungal activity against *Candida albicans*.

In Silico Docking Studies

Possible docking modes of the curcumin analogue D125, D144, D156 with (Escherichia coli) were studied using Moe 2015.1 software [22]. The target molecules were designed using molinspiration online software and used to analyze the binding affinity with the bacterial proteins. Crystal structures of all the bacterial proteins were downloaded from the Protein Data Bank website (http://www.rcsb.org) in PDB format. Hydrogen is added to complete the structure of the protein using the 3D Protonate program on MOE, only ligands are selected and protein chains from the crystal structure are used. The protein is then aligned using the Align MOE module. For the docking simulation process, the placement is set on the triangular matcher, rescoring is set with the London dG parameter, and the confirmation number is set at 10 poses; Selected conformations with rmsd values <2Å are used for molecular docking. The

results of the docking file output in mdb form with some conformation compared to the results positive control of amoxicillin. All docking conformations were analyzed, and the best value with the right pose was chosen for further interaction studies [23]. MoeDock was run several times to obtain various docked conformations and used to analyze the predicted docking score and binding energy. The best conformation of ligand was generated and the result using Moe is docking score. Docking to 2ex8 (Penicilin G) were carried out using Moe 2015.1 software. The binding energies of the target compounds with all microorganisms are expressed in kcal/mol in Table 5.

Table 5: Docking result D125, D144, D156 and Penicilin-G

Compound	S	Rmsd_refine	E_conf	E_place	E_score1	E_refine	No. of Conf
D125	-9.183	1.746	39.058	-34.298	-8.246	-28.927	10
D144	-8.796	1.525	13.046	-49.083	-8.718	-25.463	10
D156	-11.785	1.156	24.786	-67.399	-11.117	-29.572	10
HGV-6	-11.675	1.100	43.549	-61.321	-10.740	-31.141	10
Penicilin-G	-11,904	3.845	-18.215	-46.847	-10.486	-37.535	10

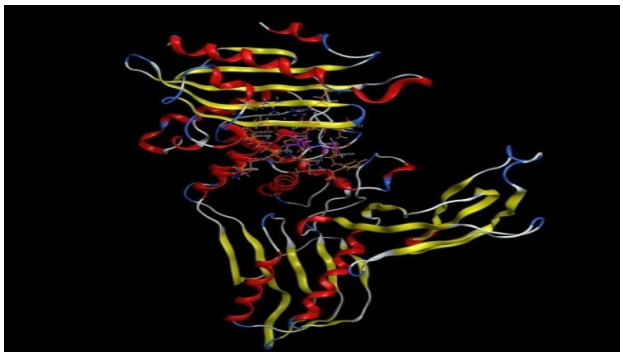


Figure 3: Protein PBP-4 (PDB; 2EX8)

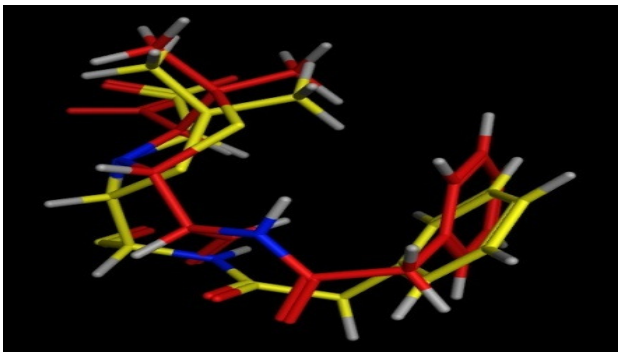


Figure 4: Alignment *native ligand* (red) and *docked ligand* (yellow) of Penicilin-G

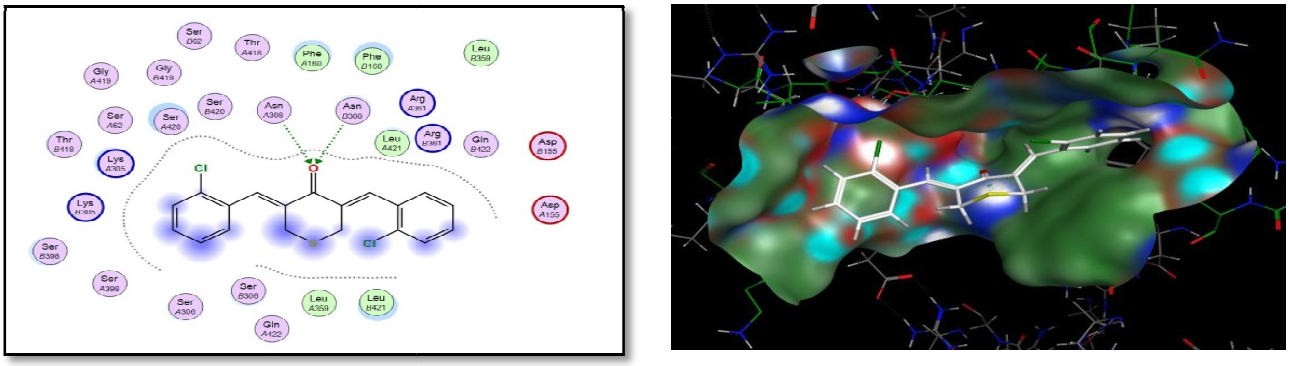


Figure 5: Interaction of D125 and Protein Target (2ex8)

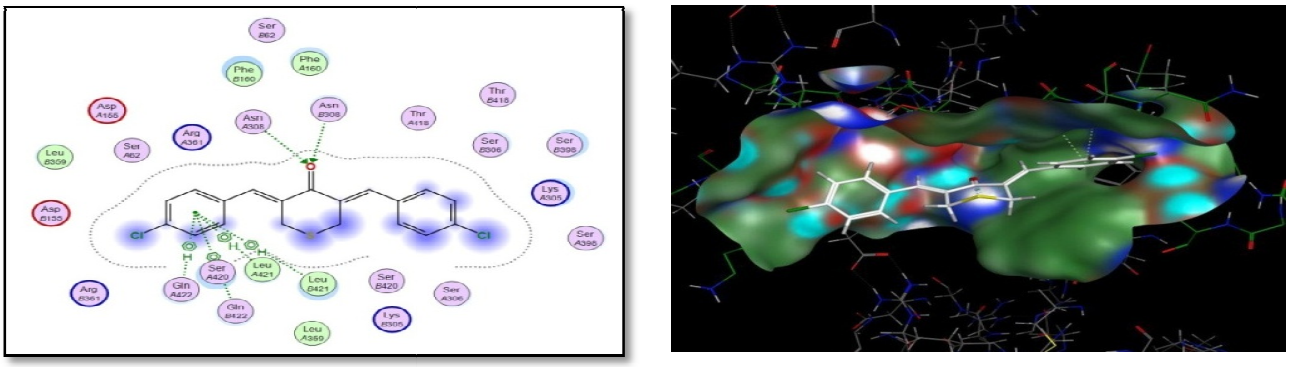


Figure 6: Interaction of D144 and Target Protein (2ex8)

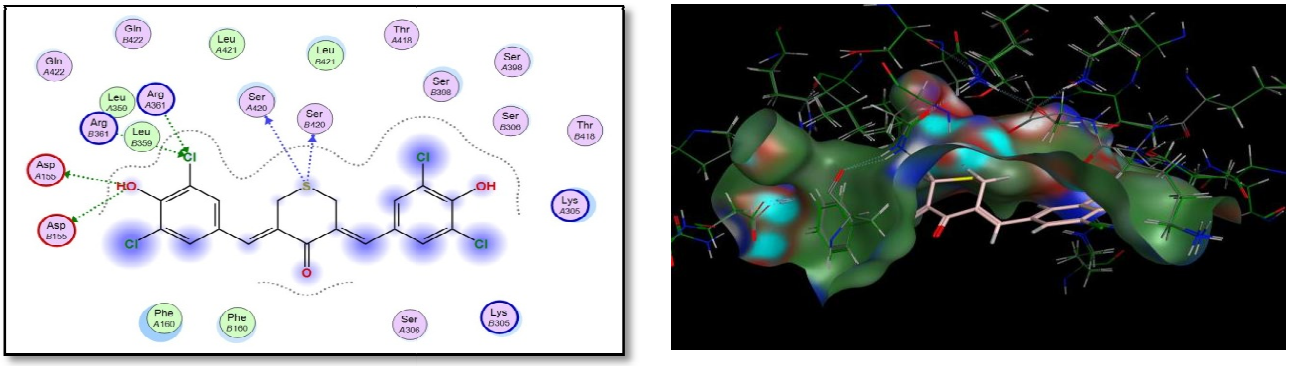


Figure 7: Interaction of D156 and Protein Target (2ex8)

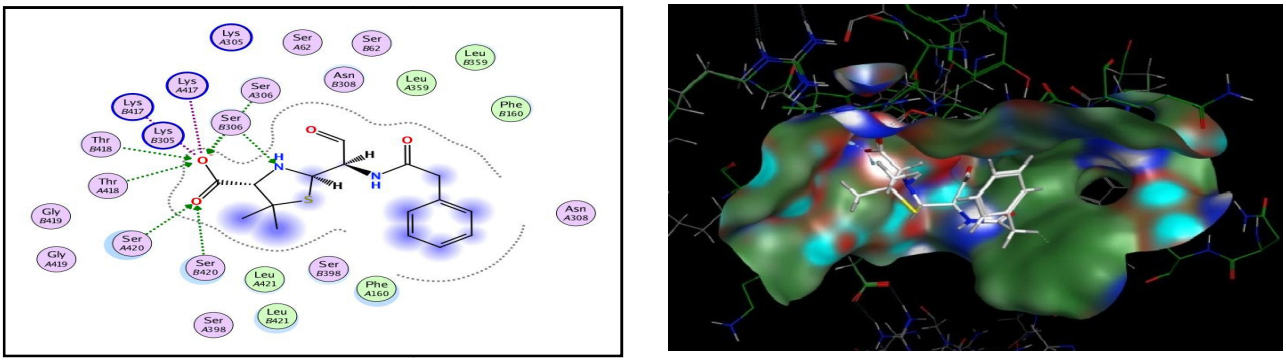


Figure 8: Interaction Penicilin-G and Protein Target (2ex8)

The best docking poses and ligand interactions are selected from each molecule D125, D144, D156, HGV-6 and penicilin-G orming a cluster in the receptor active site gap as shown in Fig. 3 and 4. Docking results showed that the substituents O (C = O, O-H), Cl, phi bonding (C = C) aromatic in D125, D144, D156 interaction with amino acids in the target protein.

In compound D125 only C = O interaction with Asn A308, B308 through H-acceptor (side chain acceptor) (Fig. 5).

D144 C = O showed amino acid that interacted with Asn 308, B 308 through H-acceptor (side chain acceptor) and interacting with Gln A422, B422 as through the pi-H (arene-H) bonding (Fig. 6).

D156 -S interacted with Ser A420, B420 via H-donor, C-Cl interacted with Arg A361, B361 through H-acceptor, -OH interacted with Asp A155, B155 through H-donor (Fig. 7).

In Penicilin-G, O (C-O) carboxilic acid interacted with the Ser A306, Thr A418, Thr B418. C=O carbonyl interacted with Ser A420, Ser B420. N with the Ser B306 residues through the H-acceptor (Fig. 8).

DISCUSSION

The 3,5-bis-(4′-chlorobenzylidene)-tetrahydro-4H-thiopyran-4-one (D144) and 3,5-bis-(3′,5′-dichloro-4-hydroxybenzylidene)-tetrahydro-4H-thiopyran-4-one (D156) showed remarkable antimicrobial activity but not in fungal. Compound D125 had no antimicrobial activities whereas D144, D156 had better antimicrobial activity in Gram positive *B. subtilis, E. faecalis, S. aureus, S. mutans* and Gram negative bacteria *E. coli, K. pneumoniae*, but not in fungal. Each compound had a different active site depends on the substituents, heteroatom groups, and molecular geometries**.** D156 had the highest antimicrobial activity in Gram positive bacteria up to 125 µg/ml and in Gram negative bacteria up to 125 µg/ml. Antimicrobial activity of HGV-6 is lower than D156 to a concentration of 125 µg/ml. We reason that the increase in antibacterial activity might be due to their electrophile compound.

Based on the results of molecular docking, conformation and mooring positions that best suit the maximum number of interactions had been analyzed, indicated by the S value in the MOE software. The best docking pose of 10 conformations of each compound was analyzed for interaction research produced using active residue sites. The lowest binding energy with the

highest number of interactions had the highest potential as an antibacterial agent [24].

Native ligand of Penicilin-G showed important amino acids that O (C-O) carboxilic acid interacted with the Ser A306, Thr A418, Thr B418. C=O carbonyl interacted with Ser A420, Ser B420, Atom N with the Ser B306 residues through the H-acceptor (Fig. 6).

The steric factor of D125 at ortho position of the Cl group is greater because the sp3 hybridized Cl atom has a tetrahedral form in which a single C-Cl bond can be a shaft of rotation of the free electron pair Cl for resonance, so that if the P orbital position of the free electron pair is not aligned (coplanar) with orbitals P on the C sp2 atom, the resonance does not occur. Therefore, the electrophilicity of the compound is reduced (Fig. 3).

D144 C = O showed amino acid that interacted with Asn A308, B308 through H-acceptor (side chain acceptor) and interacting with Gln A422, B422, LeuA421, B421 as through the pi-H (arene-H) bonding. Steric effect of Cl in meta position may be unfavorable, and much more interaction indicated that compound had potential as an antibacterial (Fig. 4).

D156 showed similar interaction with native ligand since the structure of test compound had an interaction involving -S interacted with Ser A420, B420 via H-donor, C-Cl interacted with Arg A361, B361 through H-acceptor, -OH interacted with Asp A155, B155 through H-donor. Theoretically, chlorine atom would deactivate the benzene ring but in silico illustrated that electronegative atom was the center of action other than carbonyl since it seemed that some amino acids could interact with it. OH group electronically influences, which induces the bonding electrons to the O in the OH group, so easily released as an electrophile as (H^+).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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