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Original Article

IN SILICO AND *IN VITRO* ASSAY OF HGV ANALOGUE AS ANTIBACTERIAL

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ABSTRACT

Objective: The objective of this research was to design a new analogue compound, hexagamavunon (HGV).

Methods: New design of analogue compound, HGV, was performed by QSAR study using BuildQSAR program. In this QSAR study, parameterized model (PM3) method using the Polak-Ribière algorithm was applied to calculate the optimal geometric structures of the used compounds. The new analogue compound, HGV had been synthesized using aldol condensation reaction. The assay of antibacterial activities was performed using the dilution method. Molecular operating environment (MOE) program was used for protocol docking.

Results: The results of QSAR study reveal the good relationship of antibacterial activities. The *in vitro* antibacterial activities of 2,6-bis((E)-3,5-dibromo-4-hydroxybenzylidene) cyclohexan-1-one (A113) indicates the good potential to against *S. aureus, B. subtilis* and *E. coli* with IC_{50} 27.3 µg/ml, 30.9 µg/ml, 32 µg/ml respectively. This is in accordance with the *in silico* evaluation showing that 2,6-bis((E)-3,5-dibromo-4-hydroxybenzylidene) cyclohexan-1-one has lower docking score than both amoxicillin and cefoxitin do as the native ligand of receptor 3MZE.

Conclusion: Based on *in silico* and *in vitro* assay, 2,6-bis((E)-3,5-dibromo-4-hydroxybenzylidene) cyclohexan-1-one (A113) has good antibacterial activities against *S. aureus, B. subtilis,* and *E. coli.*

Keywords: *In silico, In vitro,* 2,6-bis((E)-3,5-dibromo-4-hydroxybenzylidene)cyclohexan-1-one, Antibacterial

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INTRODUCTION

Heksagamavunon (HGV), pentagamavunon (PGV) and gamavuton (GVT) are mono-ketone analogue compounds which have been successfully synthesized and patented. Basically, they are curcumin analogues [1] that have biological activities including anti-inflammatory, antioxidant, and antibacterial [1, 2]. The basic differences of the three compounds refer to the carbonyl ketone group that connects two rings of benzene aromatic (fig. 1). The analogue compounds were synthesized using aldol condensation [1, 3].

Bacteria are dangerous microorganisms, some bacteria cause health problems such as typhoid [4], common cold [5], syphilis [6], and more. *S. aureus, B. subtilis and E. coli* are Gram-positive and Gram-negative bacteria respectively that commonly lead to some diseases such as pneumonia, meningitis, and diarrhea [7]. In addition, they are organism model that is frequently used in antibiotic screening studies [8]. The bacteria are less pathogenic so that they are saved for laboratory experiments.

The drug design for finding new types of medicine can be done using computational chemistry method. This was applied to redesign the molecules of lead compounds for better biological activities and less side effects. Considering time and cost efficiency, quantitative structure-activity relationship (QSAR) is a computational method which can be mainly implemented in finding new drugs. Moreover, it is possible to implement to avoid trial and error with considerable significance or credibility level [9]. QSAR study revealed the quantitative relationship of microscopic (molecular structure) and macroscopic (biological activity) of a molecule. The study on analogue compounds, HGV, PGV, and GVT, was performed for antioxidant activities [10]; it is possible to conduct to predict antibacterial activities.

The objective of this research was to design new analogue compounds involving HGV, PGV, and GVT that are potential to be used as antibacterial. They were synthesized and evaluated for their antibacterial activities against *S. aureus, B. subtilis* and *E. coli* using the liquid dilution method [11, 12].

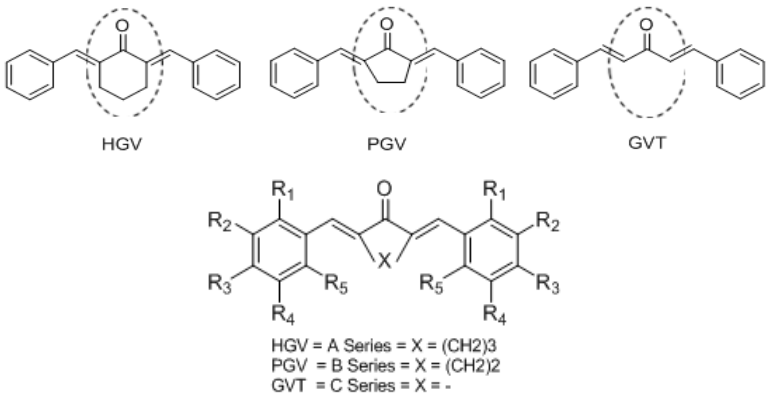

Fig. 1: Basic framework of HGV, PGV, and GVT analogues

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MATERIALS AND METHODS

Data set *QSAR*

In QSAR study, the data set used involve nine compounds of HGV, PGV and GVT analogue (unpublished Ritmaleni's collection) with their antibacterial activities against *S. aureus*, ten compounds of HGV, PGV and GVT analogue (unpublished Ritmaleni's collection) with their antibacterial activities against *B. subtilis*, and nine analogue compounds of HGV, PGV and GVT analogue (unpublished Ritmaleni's collection) with their antibacterial activities against *E. coli* (table 1). The *in vitro* antibacterial activities were interpreted as inhibitory concentration value [IC_{50} (µg/ml)] which is logarithm-transformed (log IC_{50}).

Table 1: HGV, PGV, GVT analogues used as data set for QSAR

Comp.	Substitute					*S. aureus* Log IC50	*B. subtilis* Log IC50	*E. coli* log IC50
	R1	R2	R3	R4	R5			
A103	H	OC2H5	OH	H	H	1.43	-	2.22
C113	H	Br	OH	Br	H	-	2.07	-
B113	H	Br	OH	Br	H	-	1.92	1.71
A114	H	Br	OH	H	H	1.91	-	-
A101	H	OCH3	OCH3	OCH3	H	3.02	2.71	-
B101	H	OCH3	OCH3	OCH3	H	2.66	2.65	-
C101	H	OCH3	OCH3	OCH3	H	2.66	2.7	2.79
B115	H	Br	H	H	OCH3	2.83	2.74	-
C115	H	Br	H	H	OCH3	-	-	-
A145	H	H	H	H	OC2H5	-	-	2.55
B145	H	H	H	H	OC2H5	2.78	2.66	2.6
C145	H	H	H	H	OC2H5	-	-	-
A150	Cl	H	H	H	Cl	3.05	-	2.6
B150	Cl	H	H	H	Cl	-	-	2.71
C150	Cl	H	H	H	Cl	2.39	2.65	-
A143	Cl	H	H	H	F	-	2.41	2.37
C143	Cl	H	H	H	F	-	2.26	2.23

Instrumentation

In the QSAR study, a PC with the processor of Intel Core i3-6006U 2.0 GHz, 4GB, Windows 7 Operating System was used. All calculations of quantum mechanics, atomic descriptors and molecular descriptors applied HyperChem 7.5 program. The QSAR model was generated using MLR analysis with BuildQSAR program. Protocol docking was performed with molecular operating environment (MOE) version 2018.0101.

BUCHI Melting Point B-540 with temperature gradient at 5 °C/min was used for melting point test. The purity of compounds was measured using GC17A MSQP 5000 Shimadzu. JEOL 500 MHz spectrophotometer was used to measure ^{13}C NMR Spectrum. All of the starting materials were obtained from Sigma Aldrich. The solvent materials used are Synthesis grade and Pro analysis.

Procedure

QSAR study

Geometric optimization

Each leading compound used in the QSAR study (table 1) was transformed into a two-dimensional structure (2D) (fig. 1) with HyperChem program. Atom H was added to complete the structure to transform it into the three-dimensional structure (3D). Its geometric structure was then optimized with PM3 semi-empirical method using the Polak-Ribière algorithm [10].

Descriptor calculation

The single point calculation was conducted for the optimized three-dimensional structures using HyperChem program. This was to obtain the electronic parameters such as atom C's net charge, total energy (E total), binding energy (BE) and heat of formation (HF); lipophilicity parameters; and steric parameters (molecule size) of each molecule using the HyperChem program [10].

Statistical analysis

All of the descriptors were analyzed with multilinear regression (MLR) in order to find the antibacterial activities using BuildQSAR program. The best equation models resulted were selected, considering its best statistical parameters, and were used to predict its antibacterial activities. The best statistical parameters involve the highest R (regression), the largest F ratio, the smallest s value, the smallest PRESS statistic (the predicted residual sum of squares), and the lowest Q2. The selected equations were validated using the available leave-one-out cross-validation (LOOCV) method of BuildQSAR program.

Docking studies

Preparation of ligand structures

A113 (2,6-bis((E)-3,5-dibromo-4-hydroxybenzylidene) cyclohexan-1-one) and cefoxitin, the reference molecules of the docking simulations, were built in the two-dimensional model in the form of MOE database. The determination of conformation was then performed on the database using import conformation. Furthermore, its minimal energy was calculated.

Docking simulations

The 3D Penicillin-Binding Protein 5 (PBP5) was obtained from Protein Data Bank (https://www. rcsb. org/; PDB ID code: 3MZE) [13]. Protein contains a reference molecule that is cefoxitin (3a) as the native ligand. The protein binding sites were managed using modules of Ligand Atoms Selection of MOE, and the cefoxitin binding sites were used in crystal structure as a reference. The sequence order of the binding sites was set using MOE program. Hydrogen was added to complete the protein structure using selected Protonate 3D of MOE program; only ligand and

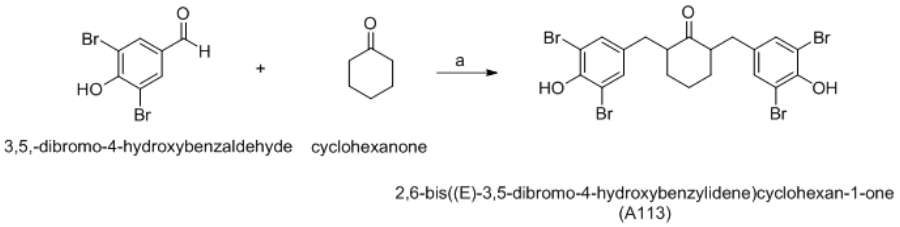
protein chain A of the crystal structure was used. The protein was then aligned using Align module of MOE. For the docking simulation process, the placement was managed on triangular matcher, rescoring was managed on London dG parameter, and the conformation number was managed in 10 poses; conformation was selected as the forcefield of MOE and was used to generate 10 poses. As the results of docking run, the output file is in mdb form with the scoring of some conformations. All of the docking conformations were analyzed, and the best value with the precise pose was selected for the further interactional study.

General procedure for synthesis of A113 (2,6-bis((E)-3,5-dibromo-4-hydroxybenzylidene) cyclohexan-1-one)

3.572 mol 3,5,-dibromo-4-dihydroxybenzaldehyde, 1.786 mol cyclohexanone, 2.0 ml THF and 0.2 ml of concentrated hydrochloric acid were stirred for two hours at 50 °C, and the stirring was continued for 8 h at room temperature. After set aside for 3 d, the mixture was treated with cold ethanol-water (1:1), filtered and the residue was dried. TLC, melting point, was used to confirm the purity of the compound.

2,6-bis((E)-3,5-dibromo-4-hydroxybenzylidene) cyclohexan-1-one (A113)

Reddish yellow powder, yield 34.84 %, mp>300 °C; IR γ (cm^{-1}) (KBr): 3485.75–OH stretch non bonded, 2928.87 =C-H stretch aromatic, 1597.95 C=O stretch α β, α' β'-unsat, 1475.35 C=C stretch aromatic, 1165.12 C-O stretch. ^{13}C-NMR (500 MHz, Asetone-d6) δ (ppm): 206.258 (C=O ketone), 188.875 (C-OH), 151.987 (C-C aromatic, ketone), 137.550 (C=C aliphatic), 133.941 (C-Br). ^{1}HNMR (300 MHz, Asetone-d6) δ: 8.98 (s, 1H, OH), 7.73 (s, 1H, Ar-H), 7.531 (s, 1H, aliphatic), 2.97-2.94 (4H, ketone-H), 2.05-2.04 (2H, ketone-H).



Scheme 1: Reagent and conditions of synthesis: (a) THF and HCl; 8 h

***In vitro* antibacterial activity assay**

The antibacterial activities against ATCC 6538P, *B. subtilis* ATCC 6633 and *E. coli* ATCC 25922 were tested using liquid dilution method. For *in vitro* assay, A113 was prepared. BHI media (50 µl) was added into microplate 96 well; experimental compound (50 µl) was added into 1A until 1E column. The obtained concentrations of the dilution method is 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml, 0.063 mg/ml, and 0.031 mg/ml respectively. Selected bacteria (50 µl) was added into each well. Solvent control, compound control, and media control were prepared in the same microplate. The incubation was conducted for 24 h at 37 °C. It was then measured using microplate reader (595 nm). This method was used to determine the inhibitory concentration of the investigated compounds which showed inhibitory effect on the growth of the tested bacteria. This concentration was considered as inhibitory concentration (IC_{50}).

RESULTS AND DISCUSSION

Selection of the best model

The new compound design of HGV, PGV, and GVT analogues as antibacterial was conducted based on QSAR study using BuildQSAR. The best equation of QSAR was selected for predicting the antibacterial activities against *S. aureus, B. subtilis* and *E. coli*. The selection of descriptors was conducted using systematic search (SS) method. QSAR model consists of one to five variables; it must have correlation criteria, r>0.8 and forbid cross-correlation (Rij)>0.6. BuildQSAR was validated using leave-one-out cross-validation.

X1 and X5 were found on the selected variables; it shows the number of selected variables. In addition, the values of R, s, F, Q2, SPress, SDep were identified. The selection was based on the best statistical parameters including the highest R (regression), the largest F ratio, the smallest s value, the smallest PRESS statistic (the predicted residual sum of squares), and the lowest Q2. The calculation of MLR of the descriptors of *S. aureus, B. subtilis* and *E. coli* using BuildQSAR provides 4 QSAR models as shown in table 2, table 3, and table 4.

Basically, the best model selected was based on the number of variables and statistical parameters of the model [14]. The results indicate that all models reveal the linear correlation of biological activities and descriptors as indicated by the R-value of each model.

The QSAR equation model (1) of each table was selected for predicting the antibacterial activities (log IC_{50}) against *S. aureus* (table 2), *B. subtilis* (table 3), and *E. coli* (table 4); it is the best QSAR model. The equation (1) of each table was selected as it has the most number of variable, the highest R (regression), the largest F (Fisher) coefficient, and the smallest PRESS statistic (the predicted residual sum of squares). Considering the smallest value, the equation was considered as a model closed to the experimental results.

Table 2: Statistical model QSAR and parameter statistical of MLR result against *S. aureus*

No.	Descriptor	n	m	R	s	F	p	Q2	SPress	SDep
1.	qC11, qC15, qC16, qC17, Volum	9	5	0.998	0.052	171.136	0.0007	0.951	0.194	0.119
	Equation	Log IC_{50} =-7.5725(±2.9753) qC11-6.0978(±0.7519) qC15-7.9387(±1.3551) qC16-8.3101(±1.7492) qC17+0.0061(±0.0010) Volum-5.5336(±1.3713)								
2.	qC4, qC5, qC14, Mass	9	4	0.986	0.125	36.048	0.0021	0.864	0.281	0.198
	Equation	Log IC_{50} =+17.9885(±4.6196) qC4+5.5044(±2.0227) qC5+4.9255(±1.6793) qC14+0.0057(±0.0035) Mass+3.5267(±1.3433)								
3.	qC4, Mass, Surface Area (SA)	9	3	0.979	0.138	38.698	0.0007	0.867	0.248	0.196
	Equation	Log IC_{50} =+18.1619(±4.7645) qC4+0.0080(±0.0035) Mass+0.0064(±0.0020) SA-1.9560(±1.6218)								
4.	qC1, E Hydration	9	2	0.931	0.227	19.506	0.0024	0.685	0.348	0.301
	Equation	Log IC_{50} =+4.0200(±2.6086) qC1+0.1221(±0.0491) E Hydra+3.3811(±0.3852)								

n = number of data, m = number of variable, R= correlation coefficient, s = standard error, F = Fisher coefficient, SPress = Predictive Error Sum of Squares.

The best equation model generated from QSAR study of S. *aureus* is Log IC_{50} =-7.5725 (±2.9753) qC11-6.0978 (±0.7519) qC15-7.9387 (±1.3551) qC16-8.3101 (±1.7492) qC17+0.0061 (±0.0010) Volume-5.5336 (±1.3713). The equation was used to design new HGV, PGV and GVT analog with antibacterial activities against *S. aureus*. Based on the model, the five variables (qC11, qC15, qC16, qC17, Volume) have significant effects on IC_{50} value. The model has R correlation (0.988), Fisher coefficient (171.1336), s value (0.052) and PRESS statistic (0.194), showing that the model is closed to the experimental results.

Table 3: Statistical model QSAR and parameter statistical of MLR result against *B. subtilis*

No.	Descriptor	n	m	R	s	F	p	Q2	SPress	SDep
1.	qC3, qC7, qC14, E Homo, E Lumo	10	5	0.999	0.022	314.092	<0.0001	0.985	0.054	0.036
	Equation	Log IC_{50} =-2.1710(±0.2592) qC3-10.9207(±3.0630) qC7+1.9909(±0.3572) qC14+1.1712(±0.2004) E Homo-0.1840(±0.1227) E Lumo+13.1381(±1.6947)								
2.	qC3, qC7, qC14, E Homo	10	4	0.993	0.046	91.018	0.0001	0.946	0.093	0.069
	Equation	Log IC_{50} =-2.2830(±0.4747) qC3-7.3596(±3.7010) qC7+2.3556(±0.5006) qC14+0.9164(±0.2034) E Homo+11.0401(±1.8293)								
3.	qC14, EHomo, EHydration	10	3	0.973	0.084	35.699	0.0003	0.872	0.130	0.106
	Equation	Log IC_{50} =+2.2158(±0.8318) qC14+0.7866(±0.3162) E Homo+0.0650(±0.0194) E hydr+10.2998(±2.9280)								
4.	qC2, qC3	10	2	0.881	0.159	12.138	0.0053	0.625	0.206	0.182
	Equation	Log IC_{50} =+3.4866(±1.8956) qC2-1.7717(±1.3208) qC3+2.8625(±0.2275)								

n = number of data, m = number of variable, R= correlation coefficient, s = standard error, F = Fisher coefficient, SPress = Predictive Error Sum of Squares

Table 4: Statistical model QSAR and parameter statistical of MLR result against *E. coli*

No.	Descriptor	n	m	R	s	F	p	Q2	SPress	SDep
1.	qC2, qC11, qC12, E Hydration, Mass	9	5	1.000	0.008	2954.907	<0.0001	0.981	0.074	0.045
	Equation	Log IC_{50} =+7.5805(±0.4244) qC2+6.7449(±0.5069) qC11+1.6726(±0.5181) qC12+0.1204(±0.0067) E hydr+0.0031(±0.0003) Mass+2.7435(±0.0978)								
2.	qC2, qC13, E Hydration, Surface Area (SA)	10	4	0.993	0.046	91.018	0.0001	0.946	0.093	0.069
	Equation	Log IC_{50} =+3.8354(±0.4729) qC2-0.6987(±0.2637) qC13+0.0850(±0.0064) E hydr+0.0021(±0.0004) SA+1.9206(±0.2315)								
3.	qC4, qC16, Surface Area (SA)	9	3	0.994	0.047	132.012	<0.0001	0.944	0.100	0.079
	Equation	Log IC_{50} =+8.6541 (±1.3551) qC4+2.8786 (±0.5855) qC16+0.0016 (±0.0008) SA+2.9920 (±0.4357)								
4.	qC4, qC16	9	2	0.955	0.114	30.878	0.0007	0.708	0.208	0.180
	Equation	Log IC_{50} =+7.2988(±2.7772) qC4+3.3001(±1.2774) qC16+3.8190(±0.4727)								

n = number of data, m = number of variable, R= correlation coefficient, s = standard error, F = Fisher coefficient, SPress = Predictive Error Sum of Squares

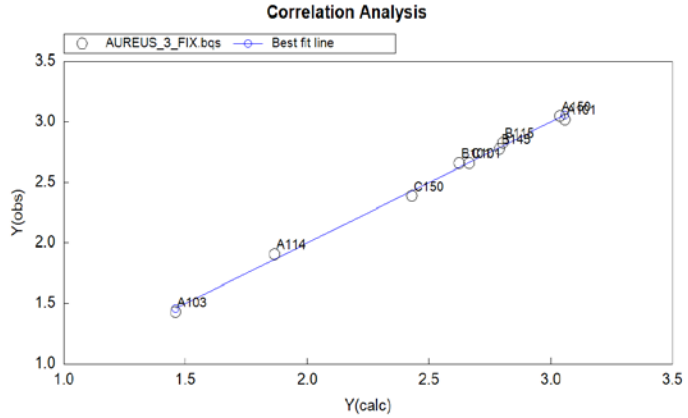
Table 5: The comparison between calculated and experimental antibacterial activity (log IC_{50}) against *S. a ureus, B. subtilis* and *E. coli* by BuildQSAR

No.	Bacteria	Comp	Log IC_{50} experimental	Log IC_{50} calculation
1.	*S. aureus*	A103	1.43	1.47
		A114	1.91	1.88
		A101	3.02	3.08
		B101	2.66	2.64
		C101	2.66	2.68
		B115	2.83	2.82
		B145	2.78	2.80
		A150	3.05	3.05
		C150	2.39	2.44
2.	*B. subtilis*	A143	2.41	2.41
		C143	2.26	2.27
		A113	2.07	2.09
		B113	1.92	1.90
		A101	2.71	2.72
		B101	2.65	2.65
		C101	2.70	2.70
		B115	2.74	2.72
		B145	2.66	2.67
		C150	2.65	2.65
3.	*E. coli*	A143	2.37	2.38
		C143	2.23	2.25
		B113	1.71	1.74
		A103	2.22	2.24
		A145	2.55	2.57
		A150	2.60	2.62
		C101	2.79	2.80
		B145	2.60	2.62
		B150	2.71	2.74

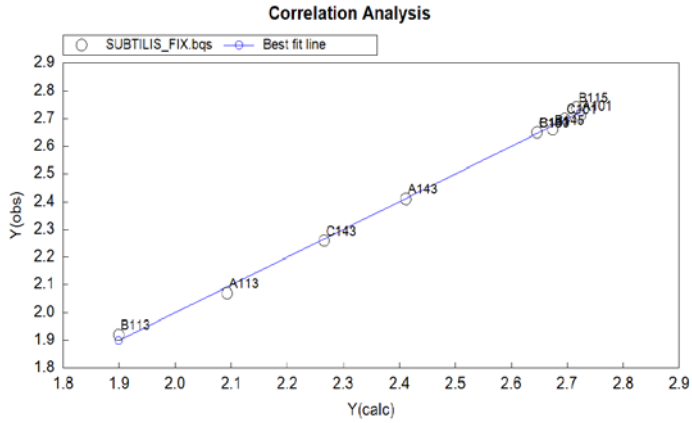
The best equation model generated from QSAR study of *B. subtilis* is Log IC_{50} =-2.1710 (±0.2592) qC3-10.9207 (±3.0630) qC7+1.9909 (±0.3572) qC14+1.1712 (±0.2004) E Homo-0.1840 (±0.1227) E Lumo+13.1381 (±1.6947). The equation was used to design new HGV, PGV and GVT analog with antibacterial activities against *B. subtilis*. Based on the model, the five variables (qC3, qC7, qC14, EHomo, ELumo) have significant effects on IC_{50} value. The model has R correlation (0.999), Fisher coefficient (314.092), s value (0.022) and PRESS statistic (0.054), showing that the model is closed to the experimental results.

The best equation model generated from QSAR study of *E. coli* is Log IC_{50} =+7.5805 (±0.4244) qC2+6.7449 (±0.5069) qC11+1.6726 (±0.5181) qC12+0.1204 (±0.0067) E hydr+0.0031 (±0.0003) Mass+2.7435 (±0.0978). The equation was used to design new HGV, PGV and GVT analog with antibacterial activities against *E. coli*. Based on the model, the five variables (qC2, qC11, qC12, E Hydration, Mass) have significant effects on IC_{50} value. The model has R correlation (1,000), Fisher coefficient (2954.907), s value (0.008) and PRESS statistic (0.074), showing that the model is closed to the experimental results.

The prediction of antibacterial activities against *S. aureus, B. subtilis,* and *E. coli* of 9 to 10 test set which was resulted from the best equation was compared and plotted with experimental data using linear regression calculation to identify the correlation of each equation as shown in table 5 and fig. 2.



(a)



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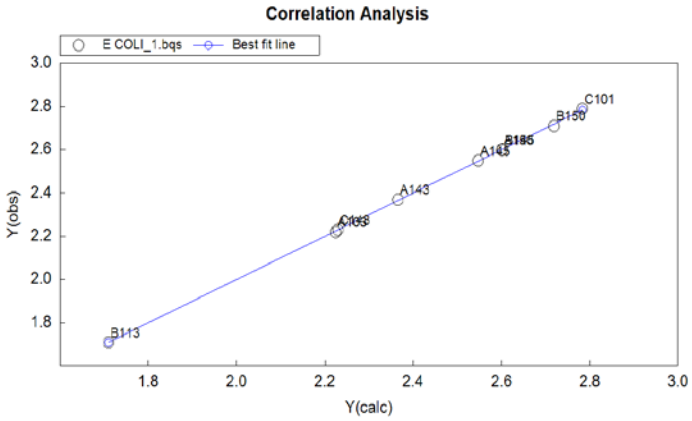


(C)

Fig. 2: The correlation analysis of result MLR statistical between calculation (Log IC_{50}) and experimental (Log IC_{50}) (a) *S. aureus*, (b) *B. subtilis*, (c) *E. Coli*

The best QSAR equation model was used as the guideline of predicting the antibacterial activities against *S. aureus, B. subtilis* and *E. coli* in designing the ratio of HGV, PGV, and GVT analog. The substituents of R1 to R5 were substituted into a new molecule as the descriptor that will influence the antibacterial activities.

Based on the equation 1 in table 2, to generate the best antibacterial activities against *S. aureus* the charge of atom C11, C15, C16 and C17 must be positive for small Log $IC_{50.}$ The positive charge of atom C15, C16 and C17 can be generated by adding electronegative substituents such as hydroxyl and halogen. To influence the positive charge of atom C11, either hydroxyl or halogen was added to the nearest or neighbouring atom C.

Based on the equation 1 in table 3, to generate the best antibacterial activities against *B. subtilis* the charge of atom C3, C7, and C14 must be positive for small Log $IC_{50.}$ The positive charge of atom C3 and C14 can be generated by adding electronegative substituents such as hydroxyl and halogen. To influence the positive charge of atom C7, either hydroxyl or halogen was added to the nearest or neighboring atom C.

Based on the equation 1 in table 4, to generate the best antibacterial activities against *E. coli.* The charge of atom C2, C11 and C12 must be negative for small Log $IC_{50.}$ The negative charge of atom C2 can be generated by adding electronegative substituents such as–OH and-OR. To influence the negative charge of atom C11 and C12, either hydroxyl or halogen was added to atom C13 and C17 due to its resonance effect that can make the charge of atom C9 and C14 more negative. The numbering structure of atom C in the basic structure is shown in fig. 3.

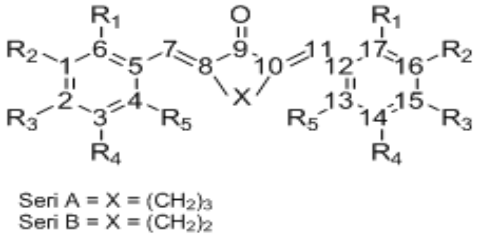


Fig. 3: The numbering structure of the C atom in HGV, PGV, GVT analogues basic structure

New analogue of HGV, PGV, and GVT with better antibacterial activities against *S. aureus, B. subtilis,* and *E. coli* is revealed from the value of IC_{50} as shown in table 6, table 7, and table 8. A113 was selected for synthesis as its compound design has the potential of antibacterial activities against *S. aureus, B. subtilis* and *E. coli.*

Table 6: Design new HGV, PGV and GVT analogues and its predicted antibacterial activity against *S. aureus* calculated using the best QSAR model

No.	Comp	Substitute					Log IC_{50} predict
		R1	R2	R3	R4	R5	
1	A113	H	Br	OH	Br	H	1.84
2	C113	H	Br	OH	Br	H	1.72

Table 7: Design new HGV, PGV and GVT analogues and its predicted antibacterial activity against *B. subtilis* calculated using the best QSAR model

No.	Comp	Substitute					Log IC_{50} predict
		R1	R2	R3	R4	R5	
5	A113	H	Br	OH	Br	H	1.79

Table 8: Design new HGV, PGV and GVT analogues and its predicted antibacterial activity against *E. coli* calculated using the best QSAR model

No.	Comp	Substitute					Log IC_{50} predict
		R1	R2	R3	R4	R5	
1	A113	H	Br	OH	Br	H	1.89
2	C113	H	Br	OH	Br	H	1.54

Docking studies of 2,6-bis((E)-3,5-dibromo-4-hydroxy-benzylidene) cyclohexan-1-one (A113)

In this research, *in silico* evaluation was performed for A113 as an antibacterial. A113 was docked into the β-lactam receptor, according to the formation stages of peptidoglycan, the basic component of D-Alanil-D-Alanine formation. The transport enzyme of D-Alanil-D-Alanin is D-Alanil-D-Alanine decarboxylase (DACA). The enzyme is provided by protein data bank with PDB ID code: 3MZE. Protein contains a reference molecule that is cefoxitin as native ligand [13].

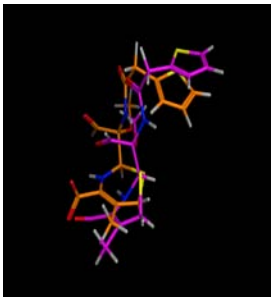


Fig. 4: Alignment of native ligand (purple) and docked ligand (yellow)

The validation of molecular docking protocol was performed to ensure that the receptors are proper to use in the molecular docking process. Based on the validation process, the obtained root mean square deviation (RMSD) is 1.7753 Å. It is less than 2 Å indicating that the docking protocol with receptor PDB 3MZE is valid and feasible for the advanced docking process (fig. 4) [15].

Based on the docking results, conformations and the best favorable docking poses with the maximum number of interactions were analyzed; they were considered as negative s by MOE software. Furthermore, the best docking pose of 10 conformations of each compound was analyzed for further investigation of any interactions resulted in using active residual sites. The lowest binding energy (s) with the most interaction number is compounded with high potential of antibacterial agents [17]. The docking results reveal proper binding poses with a strong interaction of active residual sites of the protein. The 2D-protein ligand interaction was visualized using MOE and shown in table 9.

The docking result of A113 and amoxicillin using receptor PDB ID 3MZE showed docking score (s) of 13.2599. It is much lower than the docking score of cefoxitin, a native ligand, (-10.8382) and amoxicillin (-11.5230). The docking score of amoxicillin is higher than of A113 and lower than of cefoxitin as the antive ligand. This means that A113 is more active as antibacterial than amoxicillin. Therefore, A113 was considered as the potential antibacterial

compound. The lower docking score with proper pose results in better stability levels of ligand and receptor [16]. The lower binding energy (s), the bigger possibility to accept the experimental compounds as medicine [17-20].

Table 9: Docking results with protein PDB ID 3MZE

Compound	S	Rmsd_refine	E_conf	E_place	E_score1	E_refine	No. of Conf
A113	-13.2599	3.31144	23.9537	-42.0713	-10.8615	-35.9689	10
Amoxicillin	-11.5230	0.8853	53.8637	-67.6785	-11.1618	-32.3243	10
Cefoxitin	-10.8382	2.6991	65.8911	-74.7419	-11.2002	-40.5919	10

S-The final score, rmsd_refine-The root mean square deviation between the pose before refinement and the pose after refinement, E_conf-The energy of the conformer. E_place-Score from the placement stage, E_score1-Score from the rescoring stage(s), E_refine-Score from the refinement stage and No. of conf-number of conformations generated by ligand.

The best docking pose and ligand interaction were selected from each compound of A113, amoxicillin, and cefoxitin; they formed a cluster in active site gap of the receptors as shown in fig. 5, fig. 6, and fig. 7.

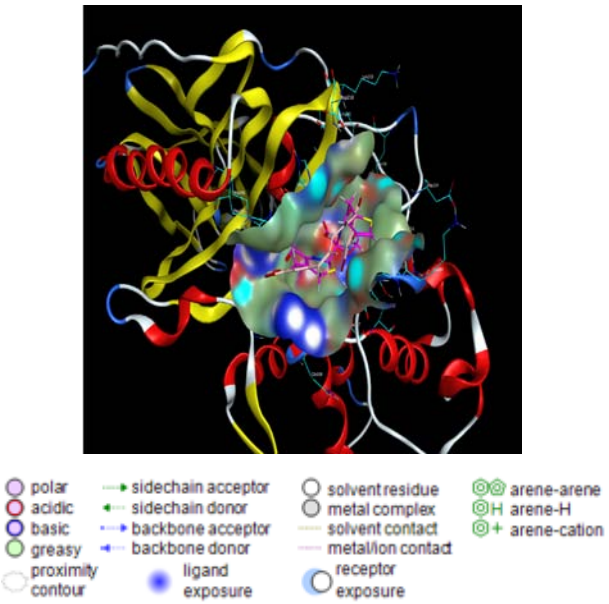


Fig. 5: Binding surface and ligand interaction of compound A113 with 3MZE

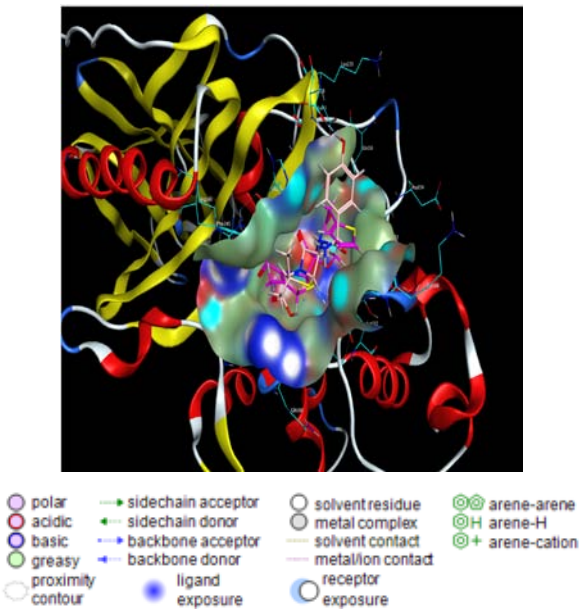


Fig. 6: Binding surface and ligand interaction of compound amoxicillin with 3MZE

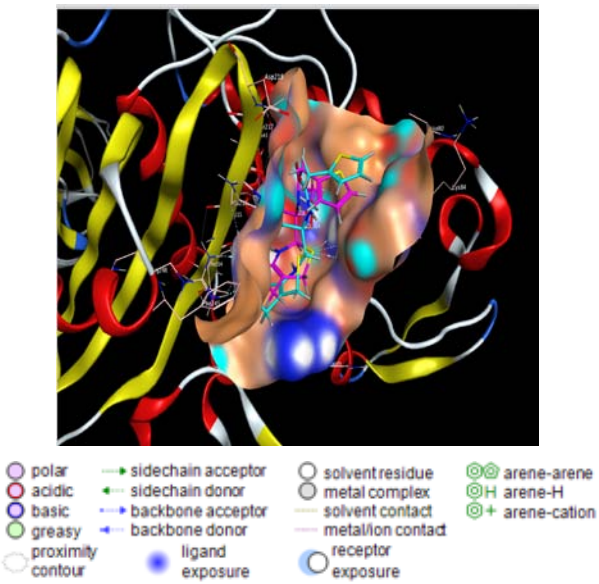


Fig. 7: Binding surface and ligand interaction of compound cefoxitin with 3MZE

The docking results reveal that O substituent (C=O, O-H) of A113 compound has interaction with Asn A112, Asn B112 (same as amoxicillin) and Asp A11, Asp B11 residue through sidechain acceptor and sidechain donor. Besides that, the aromatic ring of compound A113 has an interaction with Leu A153, B153 residue through the arene-H bond. In amoxicillin, atom O of the β-lactam ring has interaction on Asn A112 and B112 residue through sidechain acceptor; whereas, the S group of an aromatic ring has interaction with Ser B44, Ser A110, and B110 residue through sidechain acceptor. In cefoxitin, atom O (C=O) has interaction with His A216, B126 and Ser A44, B44 residue through backbone acceptor. Atom S has an interaction with Ser A86, B86, A87, B87 residue through backbone acceptor (fig. 5, 6, and 7). The confirmation energy of A113 compound, amoxicillin and cefoxitin is 23.9537, 53.8637, and 65.8911 respectively, indicating the active compounds of the confirmation energy.

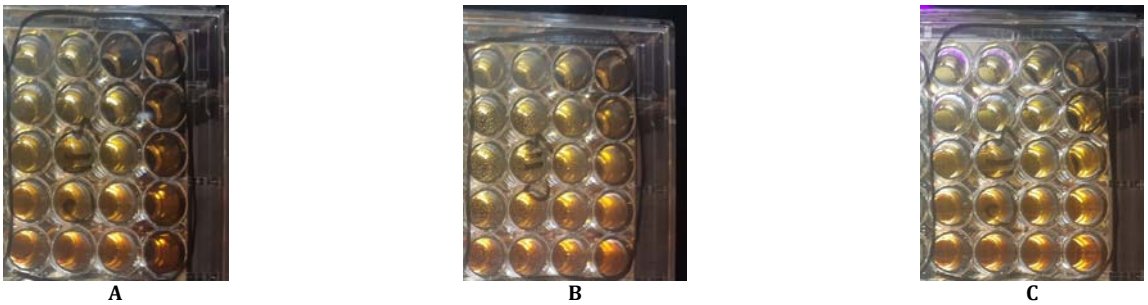
In vitro evaluation of 2,6-bis((E)-3,5-dibromo-4-hydroxy-benzylidene) cyclohexan-1-one (A113)

The *in vitro* evaluation of antibacterial activities was performed using dilution method on some bacteria species represented by Gram-positive and Gram-negative bacteria. Table 10 and fig. 8 show good inhibitory activities 2,6-bis((E)-3,5-dibromo-4-hydroxy-benzylidene) cyclohexan-1-one (A113) against *S. aureus, B. subtilis* and *E. coli*. The Inhibitory Concentration 50 (IC_{50}) A113 againts *S. aureus, B. subtilis,* and *E. coli* is 27.3 µg/ml, 30.9 µg/ml, 32 µg/ml respectively. A113 is a potential compound for advanced study.

Atom Br and OH of A113 is essential for its antibacterial activities. It is shown by the conducted docking study. However, some previous studies reveal that OH group has also essential roles to inhibit the bacterial activities.

Table 10: IC_{50} value of A113 against *S. aureus, B. subtilis* and *E. coli*

Bacteria	IC_{50} (µg/ml)
S. aureus	27.3
B. subtilis	30.9
E. coli	32



A **B** **C**

Fig. 8: The result dilution test of A113 against *S. aureus* (A), *E. coli* (B), *B. subtilis* (C)

CONCLUSION

The results of the research reveal that compound 2,6-bis((E)-3,5-dibromo-4-hydroxybenzylidene)cyclohexan-1-one (A113), the result of QSAR study using BuildQSAR, is a new design of GVT analog proven to have antibacterial activities against *S. aureus, B. subtilis* dan *E. coli*. A113 is potential to be used as drug with antibacterial activities through interaction and PBP5 inhibition confirmed by docking studies.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

The authors confirm that this article content has no conflicts of interest.

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