

EXPLORING 3D MOLECULAR STUDIES OF DIKETOPIPERAZINE ANALOGUES ON *Staphylococcus aureus* DEHYDROSQUALENE SYNTHASE USING GLIDE-XP¹

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ABSTRACT

There is a strong correlation between 3D molecular docking result with dehydrosqualene synthase protein and antibacterial activity against *Staphylococcus aureus* (*S. aureus*) of the pyrazoline analogues. The enzyme has been known as important protein for the synthesis of staphyloxanthin in *S. aureus*. Diketopiperazine analogues have similar structure to pyrazoline. Glide-XP, Schrodinger application that seeks for molecular docking screening between ligand and protein target is designed for speed, efficiency, and accuracy to conduct discovery efforts. The research report the three-dimension molecular studies diketopiperazine analogues for their antibacterial activity on dehydrosqualene synthase of *S. aureus* using Glide-XP. Analogues compound of diketopiperazine and curcumin has been calculated their geometry optimization using Gaussian-Density Functional Theory method. These 3D-optimized ligands along with reference ligands obtained from bindingDB database, MIMICs fingerprint shape screening and the compound from previous research were performed on dehydrosqualene synthase (2ZCO) for their docking score. The lowest values docking score were analyzed with multiple linear regressions. The results suggest that the diketopiperazine framework is a prospective template for modification and optimization to accomplish better potency of antibacterial activity in laboratory testing.

Keywords: diketopiperazine, Glide-XP, docking score, *Staphylococcus aureus*, multiple linear regression.

ABSTRAK

Terdapat korelasi positif antara hasil molecular docking ligan-protein dehidroskualen sintase dan aktivitas antibakteri terhadap *Staphylococcus aureus* (*S. aureus*) analog pirazolon. Protein enzim ini merupakan komponen utama dalam biosintesis stafiloksantin pada *S. aureus*. Analog diketopiperazin memiliki kemiripan struktur dengan pirazolon. Glide-XP merupakan aplikasi dalam Schrodinger yang digunakan untuk skrining molecular docking antara ligan dan protein dan didesain lebih cepat, efisien dan akurat untuk memenuhi kebutuhan dalam penemuan senyawa baru. Penelitian ini melaporkan hasil studi molekular-3D analog diketopiperazin untuk aktivitas antibakteri terhadap enzim dehidroskualen sintase *S. aureus* menggunakan Glide-XP. Molekul analog diketopiperazin dan kurkumin telah dilakukan optimasi geometri menggunakan metode Gaussian-Density Functional Theory (DFT). Semua molekul yang telah dioptimasi bersama dengan ligan pembanding yang diperoleh dari database BindingDB, database MIMICs dengan pendekatan fingerprint shape dan molekul-molekul dari penelitian terdahulu dilakukan molecular docking pada protein dehidroskualen sintase untuk memperoleh docking score. Nilai docking score terendah dianalisis dengan multiple linear regression dan hasilnya menyatakan bahwa inti diketopiperazin merupakan template yang prospektif untuk modifikasi dan optimasi dalam memperoleh potensi antibakteri yang lebih baik di penguujian laboratorium.

Kata Kunci: diketopiperazine, Glide-XP, docking score, *Staphylococcus aureus*, multiple linear regression.

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INTRODUCTION

Health problem in Indonesia increase rapidly, especially the use of antibiotics that cause higher resistance accident related to higher morbidity, mortality, and medical cost. Irrational use of antibiotic is the primary source of antibiotic resistance. The habit can develop and create bacterial strains resistant

to antibacterial agents faster. *Staphylococcus aureus* is one of the most important pathogens that predominantly produce post operative and community-acquired infections, with methicillin-resistant *S. aureus* causing a serious health threat (Bhatia et al, 2010; Triyana, 2009). The microbial have been resistant to penicillin, oxacillin, and other beta-

lactam antibiotics. In Asia, ciprofloxacin resistant *S. aureus* reached 37%, methicillin (MRSA) in Taiwan 60%, China 20%, Hong Kong 70%, Philippines 5%, Singapore 60%, and Korea over 70%. Sensitivity pattern of MRSA in Indonesia against meropenem slightly decreased, as well as to other antibiotics ranged from 1-60% (Mardiastuti et al, 2007; Cha et al, 2005).

Other research state that patients of three referral Hospital East Java and Bali (Indonesia) have been known to 3% among 2500 patients carried MRSA isolates (Santosaningih et al, 2008 in Radiono, 2011). And the bacteria of *S. aureus* has rate of resistance to trimethoprim/sulfamethoxazole and tetracycline in Surabaya was higher than in Semarang (Lestari et al, 2008). Staphyloxanthin, the golden carotenoid pigment of *S. aureus* as a virulence factor, can react with, and thus deactivate the reactive oxygen species (ROS) and host neutrophil-based killing produced during the inflammatory response. The process makes *S. aureus* resistant to innate immune clearance (Bhatia et al, 2010; Song et al, 2009; Liu et al, 2008; Liu et al, 2005). Structure of dehydrosqualene synthase that responsible for bacterial survival during infections has been identified and studied as protein model to developing potent and selective inhibitors of an important virulence factor in *S. aureus* by doing 3D molecular docking of ligand-receptor (Bhatia et al, 2010; Parameshwar et al, 2009; Song et al, 2009).

There is a strong correlation between 3D molecular docking result with dehydrosqualene synthase protein and antibacterial activity against *S. aureus* of the pyrazoline analogues. The results indicate that addition of groups which increase hydrophobic and steric interactions of pyrazoline will promote the activity of the new pyrazoline analogues against MRSA bacteria. Diketopiperazine analogues have similar structure to pyrazoline. One of them has been tested its antibacterial activity against *S. aureus* (will published as Santoso et al, 2012; Bhatia et al, 2010). Diketopiperazine are analogues compounds of curcumin as first lead compound. Curcumin derivatives and analogues have been developed and studied for its biological activity. Its antibacterial activity is very weak and functional group of phenolic has the responsibility for it (Naz et al, 2010; Sunilson et al, 2009; Rai et al, 2008; Cikrikci et al, 2008). Past research has proven that diketopiperazine analogues has antimicrobial activity (Shani et al, 2011; Villemin and Alloum, 1990).

Molecular modeling techniques have shown its success in helping to accelerate the

process of screening and discovery of potential compounds ready to market. Initially, AutoDock has started the method followed various other software either free or licensed. Glide-XP is an improved-module in the Schrodinger Maestro which has been proven helpful Astra Zeneca and Wyeth Pharmaceuticals to develop their new product (Waszkowycz et al, 2011; Cross et al, 2009; Trylska et al, 2007; Friesner et al, 2006; Xiaoqi and Zhehao, 2006; Krovat et al, 2005; Kubinyi, 1998). Santoso (2010) has investigated molecular docking result of diketopiperazine analogues on tubulin, the limiting factor in the cycle process of breast cancer in cell line T47D using AutoDock and Glide-XP (Santoso, 2011^a; Da'i et al, 2007). The results confirm that the molecular docking capabilities and performance of Glide-XP slightly different and better than AutoDock. Other previous studies of 3D-molecular screening of diketopiperazine have been carried out using AutoDock-Vina compared to fingerprint shape of MIMICs database (Santoso, 2011^b). The aim of the study is to explore 3D-molecular binding modes of diketopiperazine analogues on dehydrosqualene synthase of *S. aureus* using Glide-XP.

MATERIAL AND METHODS

Appliances: personal computer with specifications: 1) the Gaussian-DFT calculations using Intel Core2Quad processor 2.4 GHz, NVidia 7300GT 256MB with 4GB RAM and 2) on molecular screening using intel i5 QuadCore processor 2.4 GHz, ATI Mobility Radeon HD 545v 512MB with 4GB RAM and intel i7 QuadCore processor 3.4 GHz, ATI Radeon HD 6670 1GB with 8GB RAM.

Tested ligands: 3D geometry optimized molecules of diketopiperazine analogues, curcumin analogues, curcumin, were obtained with Gaussian-Density Functional Theory (B3LYP) calculation.

Protein structure and preparation: 3D X-ray crystallized structure of the C (30) carotenoid dehydrosqualene synthase from *Staphylococcus aureus* (PDB: 2ZCO, resolution = 1.58 Å) was downloaded from the Protein Data Bank (Dutta et al, 2009). The downloaded protein has single chain A with 284 residues and contains 627 water molecules of crystallization. The protein structure was prepared using the protein preparation module of Schrödinger software. The final modeled protein was taken as receptor protein and found the most suitable-binding site using sitemap script of Schrödinger.

Reference ligands: a set of MIMICs reference compounds obtained by online

screening of the 3D-MIMICs database in the following procedure: target protein (crystal structure of dehydrosqualene synthase obtained from www.pdb.org) is uploaded into a database, amino acids (residue) of the binding site pocket were selected by referring to research of Bhatia et al (2010), then fingerprint- based filtering of shaped similarity scoring method was selected and submitted to obtain a maximum of 200 reference compounds (generated by using Rotate v1.0). All downloadable reference compounds serve as inputs in next step of molecular screening using Glide-XP. Other reference compounds are acquired from bindingDB database with source of dehydrosqualene synthase (optimized with Vconf v2.0) and tested compounds of Bhatia et al (2010) research which have optimized by LigPrep.

Molecular screening procedure: all the selected compounds that have been 3D-optimized geometry along with the reference ligands were docked for finding their docking score on dehydrosqualene synthase (2ZCO) using Glide-XP. These was done by doing protein preparation wizard for 2ZCO, making grid box area for molecular interaction, importing all the compounds in Maestro

workspace table, and docking them on grid box with Glide-XP to get docking score value. The results were analyzed with Glide-XP and Canvas.

RESULTS AND DISCUSSION

Geometry Optimization

All ligands structures have performed geometry optimization to obtain 3D molecular using one of these methods: DFT calculation with B3LYP/6-31(g) algorithm, Rotate ver. 1.0, Vconf v2.0, and LigPrep. The results of geometry optimization of diketopiperazine analogues, reference ligand obtained from MIMICs and bindingdb database, and Bhatia's molecules showed in the top two of each groups (Figure 1). The numbers of ligands involved are 388 molecules, with details as follows: 61 molecules are analogues of diketopiperazine, 200 molecules of MIMICs database, 57 molecules from bindingdb database, and 70 molecules generated from Bhatia research data. Selected molecules in Figure 1 were the best two of each group for docking score result using Glide-XP and 3D-position both when combined together. Figure 1 described their docked conformation virtually coincides.

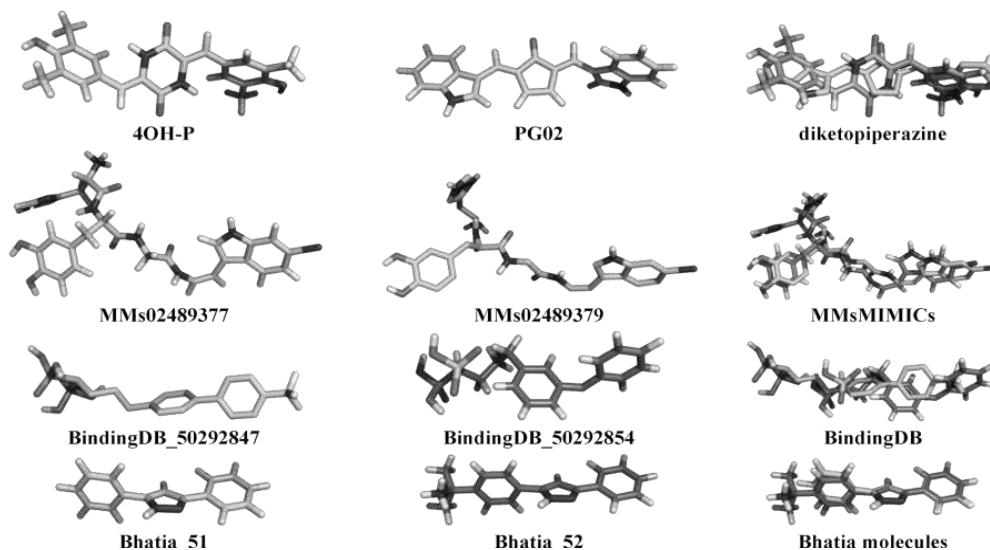


Figure 1- Structure of 3D-optimized geometry of top two of each groups: diketopiperazine analogues, reference ligand obtained from MIMICs and bindingdb database, and Bhatia's molecules (reproduced by PyMol).

Protein Target Site Map

Protein target dehydrosqualene synthase (2ZCO obtained from www.pdb.org) were prepared using Maestro: Protein Preparation Wizard with default parameter. As can be seen in Figure 2, the crystal of dehydrosqualene synthase consists of a single chain and conformation stability of the position of amino acid residue which result of protein preparation presented in the Ramachandran plot. Almost all amino acid residues are in yellow and red areas on the

Ramachandran Plot.

Bhatia et al (2010) states that there are multiple binding sites pocket on the target protein of dehydrosqualene synthase (2ZCO). The results of site mapping using Maestro, 2ZCO has 4 positions binding site pocket as shown in Figure 2 with the biggest volume site location in sitemap_site_1. Previous research for searching 2ZCO site map have been done using Q-Sitefinder online and gained the biggest binding site pocket volume in the same location (Santoso, 2011b).

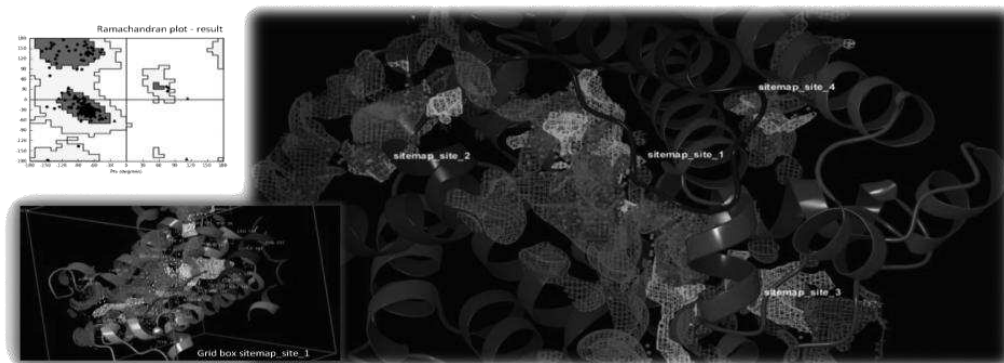


Figure 2- Result of binding site mapping of dehydrosqualene synthase (2ZCO) using Maestro.

Molecular screening

Molecular docking was performed by using a maximized grid box with center of point of sitemap_site_1 (Figure 2) because crystal structure of dehydrosqualene synthase is not obtained in conjunction with specific ligands that can serve as a molecular template (Figure 2). Results of binding energy selected ligands represented by GScore (GlideScore or Docking Score in GlideXP) are shown in Figure 3. The highest GScore is given by molecules of the bindingdb and MIMICs, then Bhatia et al (2010) molecules, and the lowest GScore belong to diketopiperazine analogues. The result showed.

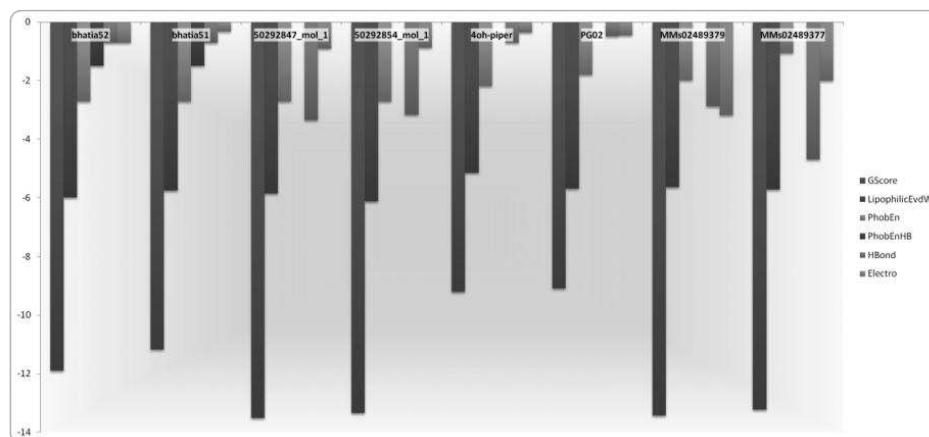


Figure 3- GScore (kcal/mol) of selected ligands of 3D-molecular screening using GlideXP on *Staphylococcus aureus* dehydrosqualene synthase protein (2ZCO), diketopiperazine analogues = 4OH-piper and PG02; with reference ligands from Bhatia et al research molecules, bindingdb and MIMICs database.

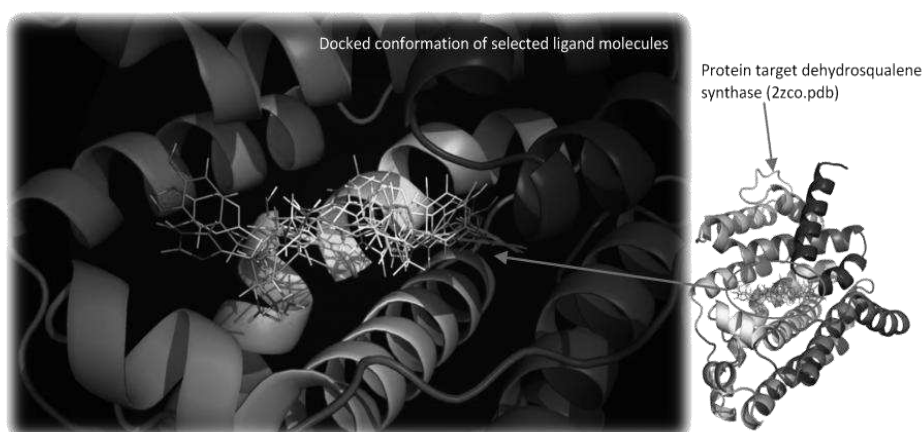


Figure 4- Secondary structure (cartoon) representation of the active site of dehydrosqualene synthase protein with docked conformation of selected ligand molecules using GlideXP (images is reproduced by PyMol).

that the GScore value is influenced by LipophilicEvdW (hydrophobic grid potential and fraction of the total protein-ligand Van der Waals energy), PhobEn (hydrophobic enclosure reward), PhobEnHB (reward for

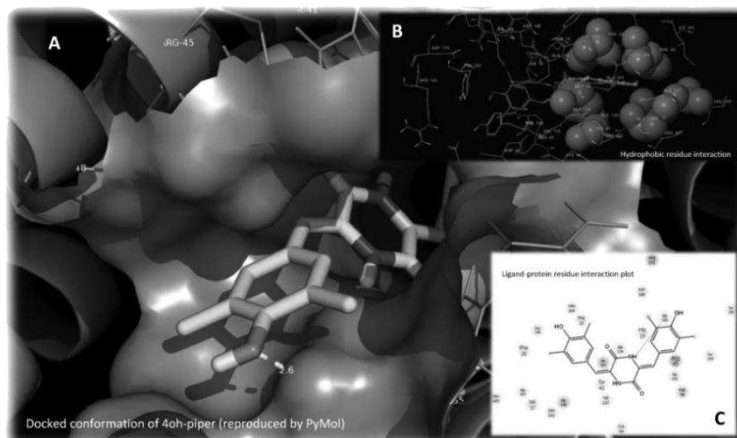
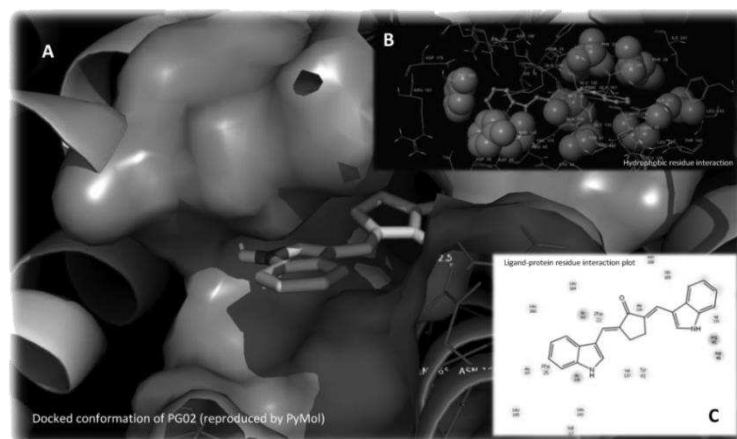
hydrophobically packed H-Bond), and HBond (hydrogen-bonding). The Electro (electrostatic rewards) value is not proportional to GScore thus its contribution is excluded. Figure 5-12 show the docked conformation of each

Table 1. Dehydrosqualene synthase protein residues interact with selected molecules using GlideXP

Serial No.	Ligands	Interacting residues of dehydrosqualene synthase	Number of Hydrophobic residue interaction
1	Binding_DB50292847	His18, Phe22, Phe26, Tyr41, Arg45, Asp48, Tyr129, Val133, Ala134, Gly135, Val137, Gly138, Leu141, Leu145, Ala157, Leu160, Gly161, Leu164, Gln165, Asn168, Ile241	11
2	MMs02489379	His18, Phe26, Tyr41, Cys44, Arg45, Asp48, Val111, Asp114, Tyr129, Val133, Ala134, Val137, Leu141, Leu145, Ala157, Leu160, Gly161, Leu164, Gln165, Asn168, Ile169, Arg171, Asp172, Glu175, Asp176, Arg181, Tyr183, Ile241, Arg265	16
3	BindingDB_50292854	His18, Phe22, Phe26, Tyr41, Arg45, Asp48, Val133, Ala134, Val137, Gly138, Leu141, Thr142, Leu145, Ala157, Leu160, Gly161, Leu164, Gln165, Asn168, Phe233, Ile241, Ala244, Tyr248	15
4	MMs02489377	His18, Phe22, Phe26, Val37, Tyr41, Cys44, Arg45, Asp48, Asp49, Leu107, Val111, Asp114, Tyr129, Val133, Ala134, Val137, Leu141, Leu160, Gly161, Leu164, Gln165, Asn168, Ile169, Arg171, Asp172, Asp176, Asn179, Arg181, Tyr183, Arg265	15
5	Bhatia_52	His18, Phe22, Phe26, Arg45, Asp48, Val133, Ala134, Gly135, Val137, Gly138, Leu141, Thr142, Leu145, Ala157, Leu160, Gly161, Leu164, Gln165, Asn168, Phe233, Ile241, Ala244, Tyr248	15
6	Bhatia_51	His18, Phe22, Phe26, Tyr41, Ala134, Gly135, Val137, Gly138, Leu141, Thr142, Leu145, Ala157, Leu160, Gly161, Leu164, Gln165, Asn168, Phe233, Ile241, Ala244	13
7	4oh-piper	His18, Phe22, Phe26, Val37, Tyr41, Cys44, Arg45, Asp48, Tyr129, Val133, Ala134, Val137, Gly138, Leu141, Leu145, Ala157, Leu160, Gly161, Leu164, Gln165, Asn168, Arg171, Tyr183	15
8	PG02	Phe22, Phe26, Val37, Tyr41, Arg45, Asp48, Val133, Ala134, Val137, Gly138, Leu141, Leu145, Ala157, Leu160, Gly161, Leu164, Gln165, Asn168	12

selected ligands at the active site cavity of dehydrosqualene synthase protein (2ZCO) based on binding energy (GScore) which obtained by molecular docking using GlideXP-Maestro. Each image shows the interaction of the ligand with a hydrophobic residue, and all

amino acid residues of the protein synthase dehydrosqualene. Molecule PG02 does not have hydrogen bonding interactions with amino acid residues of protein target. Molecule of MMs02489379 and MMs02489377 have the most number of hydrogen bonding and

**Figure 5.** Docked conformation of 4oh-piper view, 5.b with hydrophobic residue interaction, and 5.c with interacting amino acids residue of dehydrosqualene synthase protein at the active site cavity.**Figure 6.** Docked conformation of PG02 view, 6.b with hydrophobic residue interaction, and 6.c with interacting amino acids residue of dehydrosqualene synthase protein at the active site cavity.

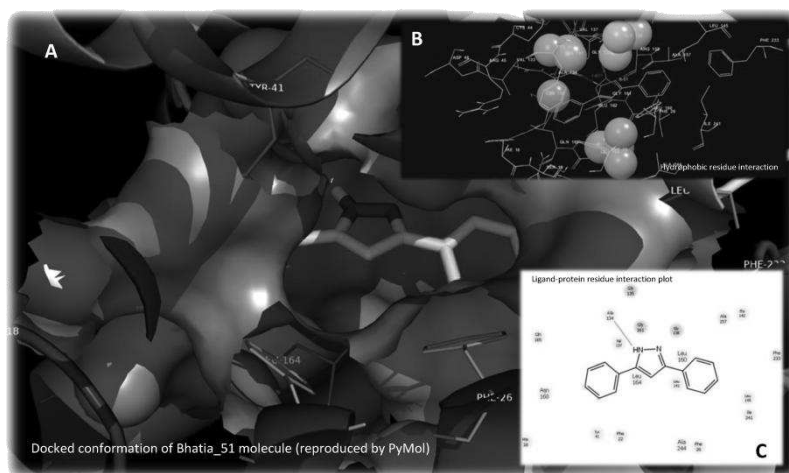


Figure 7. Docked conformation of Bhatia_51 molecule view, 7.b with hydrophobic residue interaction, and 7.c with interacting amino acids residue of dehydrosqualene synthase protein at the active site cavity.

amino acid residue interaction compared with other ligands. They also have a value of GScore less than -10 kcal/mol. This is due all the reference ligand obtained from the MM5MIMICs database using the screening

method of fingerprint-based filtering of shaped similarity scoring. The method willselect ligands from database that have a high compatibility with the binding site pocket of protein target based on shaped similarity.

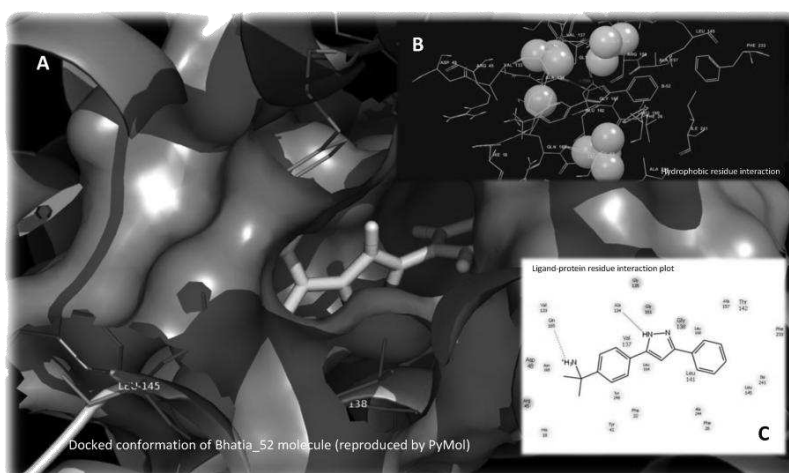


Figure 8. Docked conformation of Bhatia_52 molecule view, 8.b with hydrophobic residue interaction, and 8.c with interacting amino acids residue of dehydrosqualene synthase protein at the active site cavity.

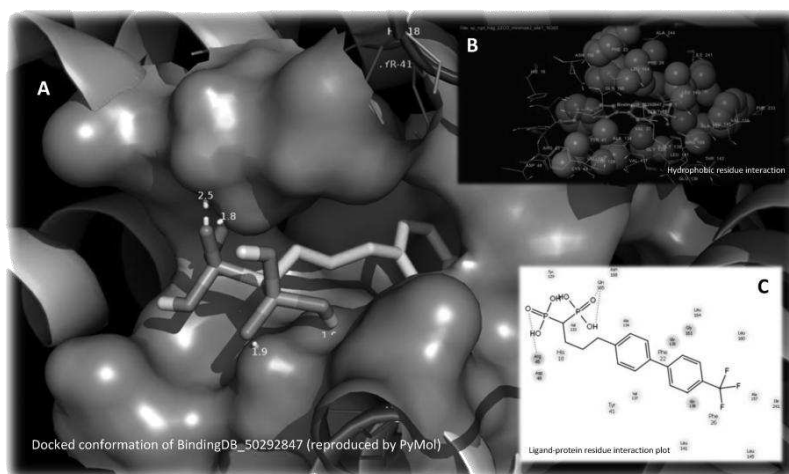


Figure 9. Docked conformation of BindingDB_50292847 molecule view, 9.b with hydrophobic residue interaction, and 9.c with interacting amino acids residue of dehydrosqualene synthase protein at the active site cavity.

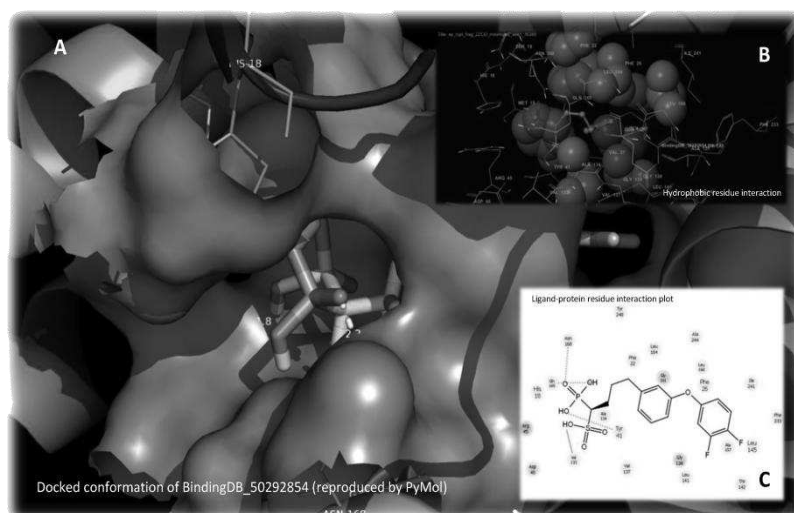


Figure 10. Docked conformation of BindingDB_50292854 molecule view, 10.b with hydrophobic residue interaction, and 10.c with interacting amino acids residue of dehydrosqualene synthase protein at the active site cavity. Three-dimensional of docked conformation of eight selected molecules with dehydrosqualene synthase protein was shown in Figure 4 using GlideXP. It is indicate that sitemap_site_1 as main binding site pocket for dehydrosqualene synthase. In group of diketopiperazine analogues, molecule of 4oh-piper has the strongest predicted binding affinity that showed by its lowest GScore then PG02 and van-piper (data not shown). Molecule of van-piper has antibacterial activity against *S. aureus* and 4oh-piper has antibacterial activity against *Bacillus subtilis* (will published as Santoso et al, 2012; Santoso et al, 2011). Molecule of PG02 is predicted to have antimicrobial activity also against *S.aureus* and *B. subtilis*. Table 1 present dehydrosqualene synthase protein residues which interact with selected molecules using

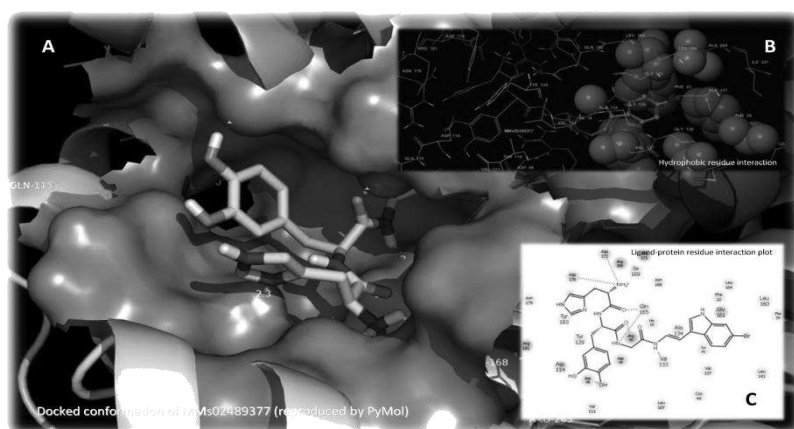


Figure 11. Docked conformation of MMs02489377 molecule view, 11.b with hydrophobic residue interaction, and 11.c with interacting amino acids residue of dehydrosqualene synthase protein at the active site cavity.

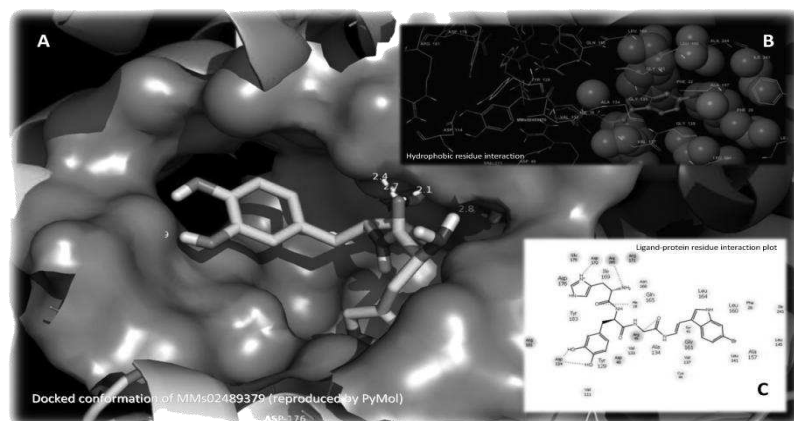


Figure 12. Docked conformation of MMs02489379 molecule view, 12.b with hydrophobic residue interaction, and 12.c with interacting amino acids residue of dehydrosqualene synthase protein at the active site cavity.

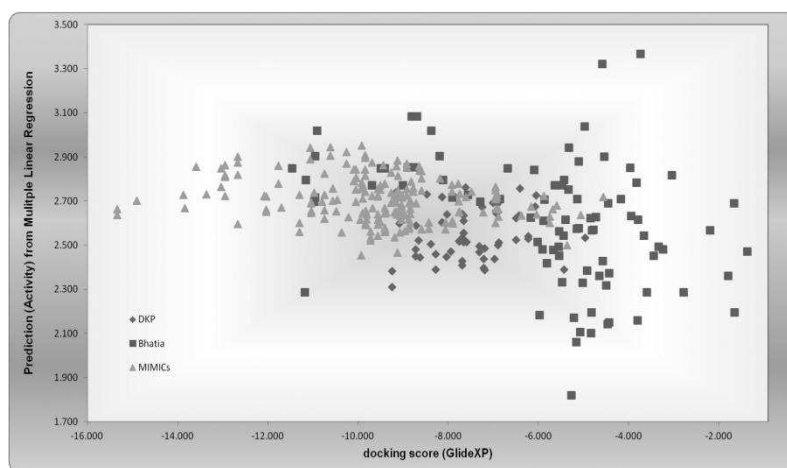


Figure 13. Distribution of docking scores (GScore) value with dehydrosqualene synthase protein versus Prediction activity value (based on Multiple Linear Regression) of selected group of ligand (diketopiperazine derivatives, Bhatia research molecule, and MIMICs molecule).

GlideXP, and number of hydrophobic residues interaction.

These results are consistent with the primary QSAR descriptor of pyrazoline analogues interactions with dehydrosqualene synthase protein on Bhatia et al (2010) research but in contrast with previous research of molecular docking using AutoDock-Vina (Santoso, 2011^b). Figure 13 confirms that the diketopiperazine analogues still have a chance to be developed and further tested for antibacterial activity. The additions of functional groups containing halogens are expected to increase the antibacterial activity of diketopiperazine analogues.

CONCLUSION

The results suggest that the diketopiperazine framework is a prospective template for modification and optimization to accomplish better potency of antibacterial activity in laboratory testing.

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