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ORIGINAL ARTICLE

Analysis of the binding mode of the analogues of Emodin against β-hydroxyacyl-acyl carrier protein dehydratase from *Helicobacter pylori*

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ABSTRACT
Helicobacter pylori is (H.pylori) a gram negative bacterium inhabiting the human stomach. It has been consider as a class one carcinogen intensely concomitant with the improvement of peptic ulcer. Among diseases, Gastric adenocarcinoma, a well-known disease is the second leading cause of world's death rate, caused by colonization of H.pylori in gastric mucosa of 35 to 70 percent of the world population. The current triple therapy for the eradication of H.pylori has been concerned as an effective treatment for H.pylori infection. However, the overuse of the bacterial agents resulted in the antibiotic resistance strain that resist and lower the efficacy of the current therapy. Therefore, the developing of the new antibacterial agents that inhibit the new drug target has gained the attention of several research groups. FAS II, β-hydroxyacyl-ACP of (FabZ) is one of the important enzymes responsible for the elongation of both saturated and unsaturated fatty acids biosynthesis in the FAS II pathway. Structure-Based 3D Pharmacophore model were generated on the basis of Emodin interaction with FabZ. The analogous of Emodin were screened by using pharmacophore model. The resultant were then docked. Some of the analogous of the Emodin were found to have good docking score and binding affinity toward the FabZ. Furthermore the molecular dynamics simulation shows the stability of analogue in the active site. The present study provide lead compound that may be good inhibitors of FabZ.
Keywords: Emodin, Helicobacter pylori, FASII

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INTRODUCTION

Helicobacter pylori (H.pylori) is a gram negative bacterium inhabiting the human stomach and is a class one carcinogen intensely concomitant with the improvement of peptic ulcer [1]. It organizes risk factor for the cancer and severe gastric inflammation [2]. This aerophilic gram negative bacterium is etiologically tangled with gastric adenocarcinoma, primary gastric B-cell lymphoma and mucosa-associated lymphoid tissue (MALT) lymphoma [3, 4]. Gastric adenocarcinoma, a well-known disease is the second leading cause of world's death rate, caused by colonization of *H.pylori* in gastric mucosa of 35 to 70 percent of the world population [5, 6]. Peptic ulceration, gastric cancer and gastritis are several gastrointestinal illness of human caused by the *H.pylori* .

The current triple therapy for the eradication of *H.pylori* has been concerned as an effective treatment for *H.pylori* infection [7]. However, the overuse of the bacterial agents resulted in the antibiotic resistance strain that resist and lower the efficacy of the current therapy [8]. Therefore, the developing of the new antibacterial agents that inhibit the new drug target has gained the attention of several research groups. Type II fatty acid synthetic pathway (FAS II) has been used as a drug target due to the difference in the enzymes of *H.pylori* and human. The enzymes involved in the FAS II have been validated as antibacterial drug targets [9]. FASII, β-hydroxyacyl-ACP (FabZ) is one of the important enzymes responsible for the

elongation of both saturated and unsaturated fatty acids biosynthesis. FabZ has attracted the attention of many scientists as an essential target for the discovery of effective anti-bacterial compounds against pathogenic microbes [9].

Emodin, (3-methyl-1, 6, 8-trihydroxyanthraquinone), is the natural product isolated from the rhizomes of Rheum palmatum. Emodin is the constituent of the roots and bark of numerous different traditional Chinese medicine [10]. Emodin was found to have numerous pharmacological properties such as vasorelaxant activities [11], antiproliferation [12], anticancer [13] and anti-inflammatory [14]. In the previous study, Emodin was reported to have inhibitory activity against ***H.pylori*** and later the target was found to be FabZ [13, 15].

In the present study, we retrieved the analogous of the Emodin. All the compound were docked and found to have good docking score and binding affinity toward FabZ. Molecular Dynamics (MD) Simulation shows that all the compounds are stable in the active site. Our work is expected to have provided useful information for the optimization of Emodin inhibition mechanism against FabZ.

MATERIAL AND METHODS

Receptor protein preparation

The three-dimensional crystal structure of β hydroxyacyl-acyl carrier protein dehydratase (FabZ) complex with Emodin molecule was retrieved from protein databank (http://www.rcsb.org/) with PDB ID 3ED0 [15]. The crystal structure contains three dimers and water molecules. The entire crystallographic water molecules were removed for the crystal structure. The two dimers were deleted. The resultant structure with chain A and chain B complex with Emodin was subjected to energy minimization to remove any bad contacts using molecular operating environment (MOE) software.

Retrieval of Emodin analogues

The two dimensional structure of Emodin molecule was retrieved from PubChem database with accession number 3220. Similarity search was performed for Emodin molecule in the PubChem database to retrieve the related compounds and analogues. The search parameters were set at 90 % similarity. 906 molecules were retrieved from PubChem database and were converted to three dimensional structure using MOE.

Structure-Based 3D Pharmacophore Generation

The x-ray crystal structure of FabZ complex with Emodin (PDB ID 3ED0) was used as an input file for the pharmacophore modeling. The pharmacophore features were created based on the pharmacophore query. The important chemical features were identified based on the interaction of Emodin with active site residues. The features were used to construct the pharmacophore model. The final pharmacophore model consists of five features having hydrogen bond acceptors and hydrogen bond donors which were used for screening the retrieved compounds.

Molecular Docking

MOE-Dock program embedded in MOE2014 was used for docking. The crystal structure of the target protein was used for the docking study. Multiple conformations were generated for each compound by applying a preferred torsion angles to all rotatable bonds in each compound. Ten conformations were generated for each compound. The accepted conformations for each compound against receptor were scored using London dG scoring function which calculates the free energy for the binding of compound from a given conformation.

Binding Energy and Binding Affinity Calculations

To identify the most potential compound a, binding affinities of the complexes were calculated using Generalized-Born Volume Integral/Weighted Surface area method implemented in the MOE. Generalized Born interaction energy, such as Vander Waals, implicit solvent interaction energies and Coulomb electrostatic interaction, is non-bonded interaction energy between the receptor and the ligand molecule [16]. The binding affinity was calculated for each hit after energy minimization, and reported in unit of Kcal/Mol.

RESULT AND DISCUSSIONS

Pharmacophore modeling and database screening

An interesting application of pharmacophore modeling is to determine interaction points. These interaction points may enhance the binding affinity of compounds. A pharmacophore has modeled based on the crystal structure of FabZ and Emodin complex (PDB ID 3ED0). The active site of the FabZ-Emodin complex was carefully examined. As the Emodin has aromatic rings, most of the hydrophobic interactions were observed. The residues that are around 4 Å of the Emodin were defined as active site residues. The interaction analysis shows the important residues that help in binding of Emodin. The active site residues were defined as Phe59, Ile98, Tyr100, Phe101, Glu159 form chain 1 and His58, Ile64, Phe109, Pro112,

Ile111 and Arg110 from chain two because both chains are involved in the active site (Figure 1). While deriving pharmacophore model we aimed to keep a balance between complex pharmacophore which retrieve very potent compound but has only a very low hit rate and a relax pharmacophore which retrieve many compounds but with no activity. The aim of this pharmacophore modeling was to choose accurate lead compounds and to further search for the additional interactions with the active the site residues of FabZ. The pharmacophore model contains five features (Figure 2). The partial matching option in the pharmacophore modeling tool was set to four so, that the hit compounds match at least four features. The developed pharmacophore model was used to screen the 906 molecules retrieved from the pubchem database. As a result of screening, 490 compounds were retrieved as hits that match at least four features.

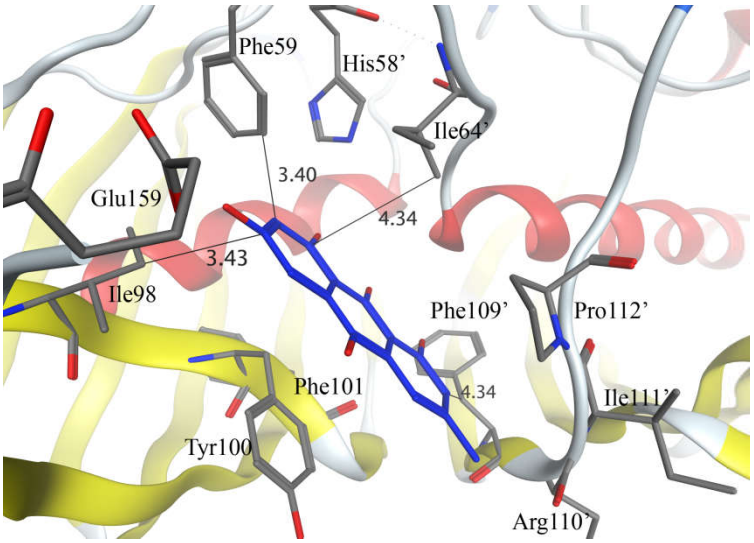


Figure 1: Binding mode of the Emodin. The Binding site residues were defined within the 4 angstrom of Emodin. The black line shows the hydrogen bond. The number with each line shows the distance of hydrogen bond.

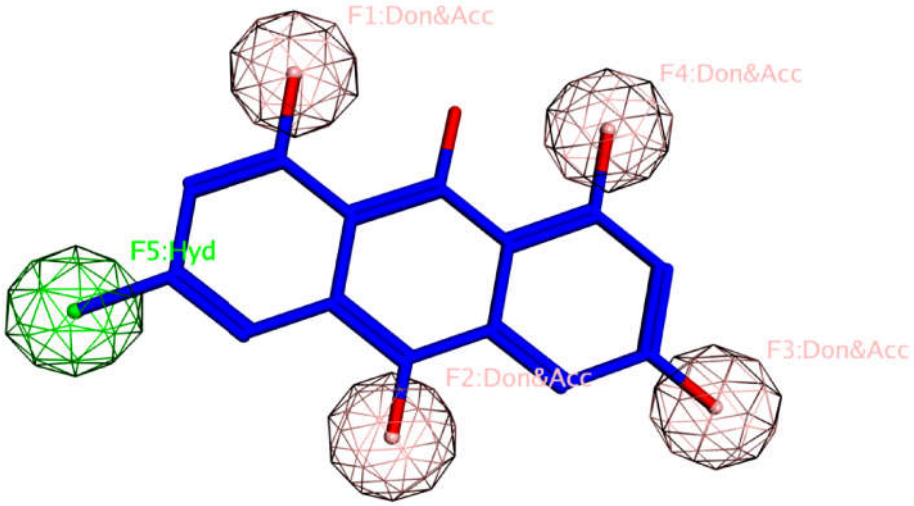


Figure 2: Two dimensional structure of Emodin. The Cage structure shows the pharmacophore features. The features with pink color shows both hydrogen bond donor and accepter properties while the feature with green color shows the hydrophobic feature.

Validation of docking protocol

Prior to docking, the docking protocol was validated. The x-ray crystal structure of β hydroxyacyl-acyl carrier protein dehydratase (FabZ) with PDB ID 3ED0 from *H.pylori* was used for the validation. The docking protocol was validated in such a way that Emodin was extracted from x-ray structure and then re-docked into the active site. The RMSD of 2 Å between crystal pose and docked pose of ligand is usually

considered as good docking protocol [17]. The same criterion was used for the current docking. The RMSD between crystal and docked pose were found to be 0.4 Å, suggesting a reasonable docking protocol (Figure 3).

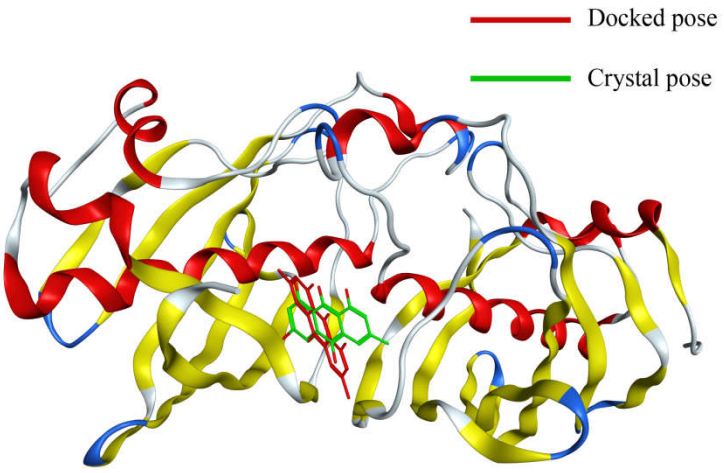


Figure 3: Validation of docking protocol. The Emodin with green color shows the crystal pose while the Emodin with red color shows the docked pose. The RMSD is 0.4 angstrom.

Molecular Docking and binding affinity calculation

To explore the binding interactions of the resultant 490 molecules, the 490 molecules were docked into the active site of FabZ. The Emodin was re-docked in order to validate the docking protocol in the previous section. The docking score of the Emodin were found to be -14.572. This docking score was used as cut off value to screen the best analogous of Emodin. As compared to the docking score of Emodin, 35 molecules were found to have better docking score than Emodin. The 35 molecules were then subjected to the binding affinity calculation. The binding affinity of Emodin was found to be 6.675 pKi. Five molecules were found to have better binding affinity than Emodin (Table 1).

Compound	PubChem ID	Docking Score (MOE)	Binding Affinity (pKi)
1	101419742	-19.2705	13.952
2	9872365	-19.2517	12.870
3	73071	-19.2428	12.803
4	12314053	-18.6606	12.861
5	9851005	-18.4850	13.593
6	EMODIN 3220	-14.5721	6.675

Table 1: The docking score and binding affinity of final five hit compounds along with their PubChem ID.

Binding Mode of the final compounds

All compounds were found to have good interaction with the active site residues. The compound 1 with PubChem ID 101419742 was found to fit well in the active site with good docking score -19.2705 kcal/mol and binding affinity 13.952 pKi. As compared to the Emodin, having docking score and binding affinity -14.5721 kcal/mol and 6.675 pKi respectively, the docking score of compound 1 is much better. Compound 1 makes 3 hydrogen bond with residues Glu60, Phe244 and Glu302 with hydrogen bond distance 3.06, 3.13 and 3.38 angstrom respectively (Figure 4). Furthermore numerous hydrophobic interaction were found with active site residues. The pharmacophore mapping shows that compound 1 mapped all five features, suggesting that it may be a good inhibitor of FabZ (Figure 5).

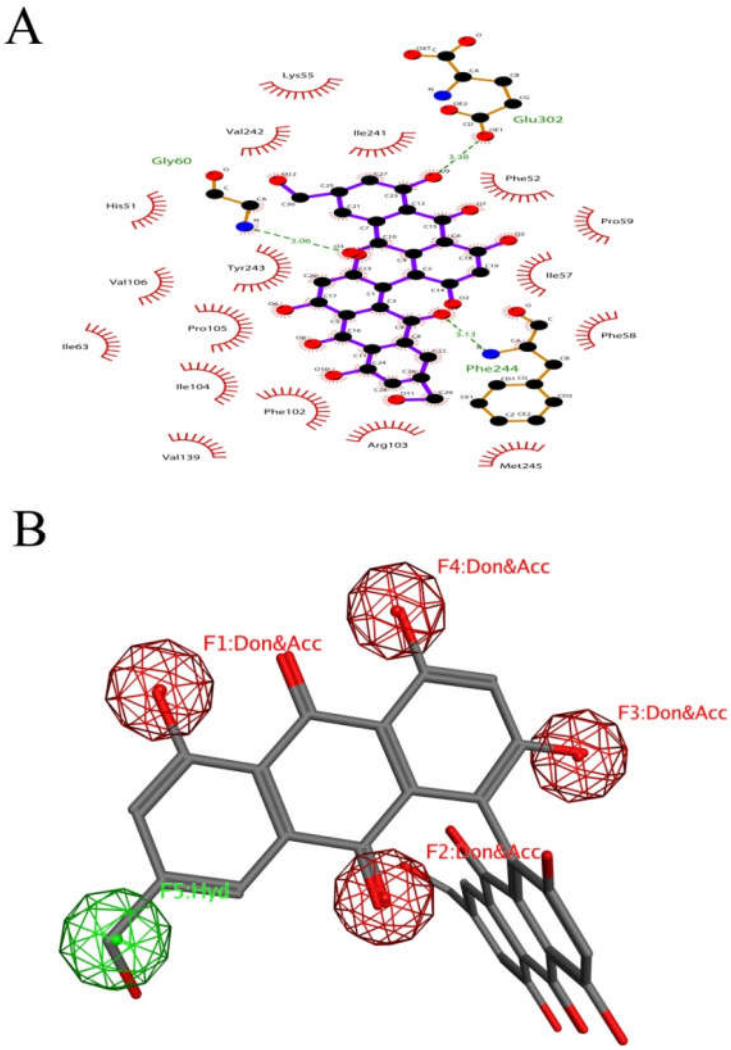
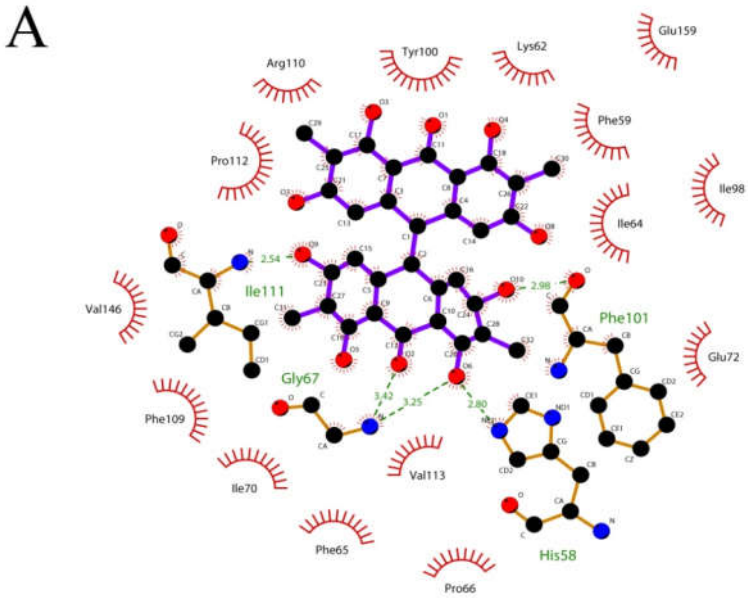


Figure 4: Binding mode of compound 1: (A) the two dimensional analysis of the compound 1 in the active site of FabZ. The dotted green lines shows the hydrogen bonds while the red arcs shows the hydrophobic interactions (B) compound 1 mapped all five features from F1 to F5.



B

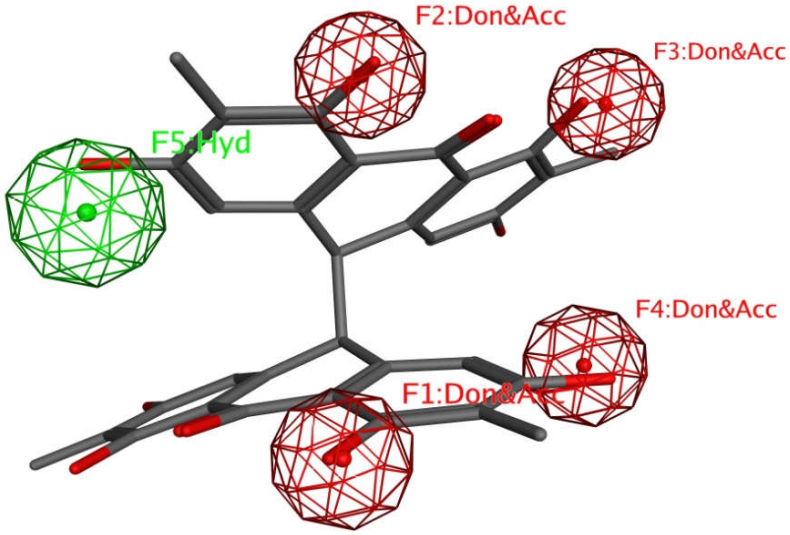
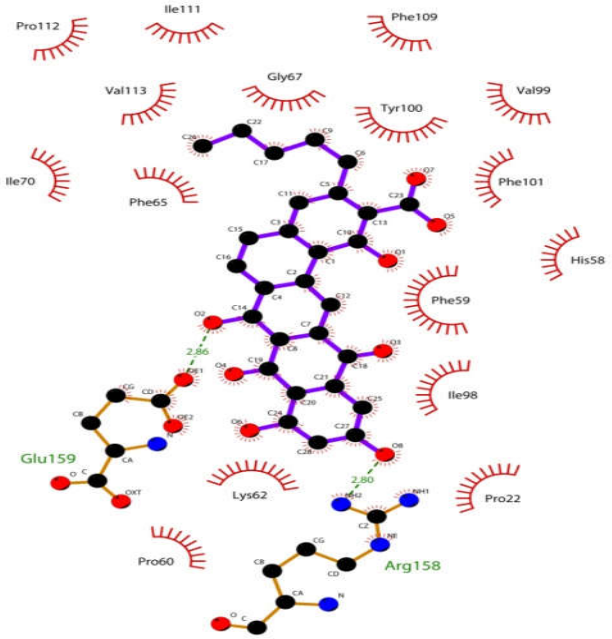


Figure 5: Binding mode of compound 2: (A) the two dimensional analysis of the compound 2 in the active site of FabZ. Compound 2 makes five hydrogen bonds. The dotted green lines shows the hydrogen bonds while the red arcs shows the hydrophobic interactions (B) compound 2 mapped only four features from F1 to F5.

Compound 2 with PubChem ID 9872365 fit well in the active site with docking score -19.2517 kcal/mol and binding affinity 12.870 pKi. It makes hydrogen bond with Gly67, His58, Phe101 and Ile111 with numerous hydrophobic interactions (Figure 6). The compound 2 mapped only 4 features (Figure 7).

A



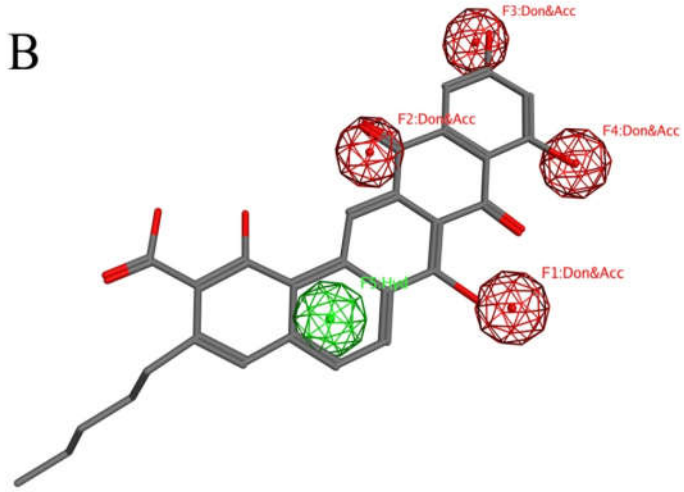


Figure 6: Binding mode of compound 3: (A) the two dimensional analysis of the compound 3 in the active site of FabZ. Compound 3 makes two hydrogen bonds. The dotted green lines shows the hydrogen bonds while the red arcs shows the hydrophobic interactions (B) compound 3 mapped all five features from F1 to F5.

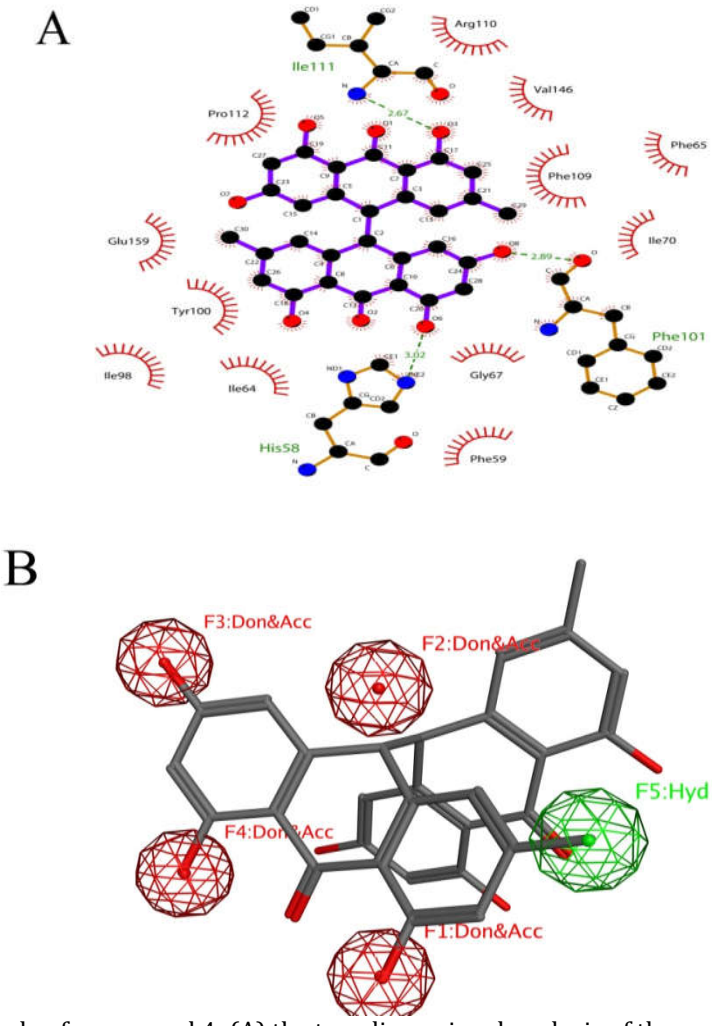


Figure 7: Binding mode of compound 4: (A) the two dimensional analysis of the compound 4 inthe active site of FabZ. Compound 4 makes three hydrogen bonds. The dotted green lines shows the hydrogen bonds while the red arcs shows the hydrophobic interactions (B) compound 4 mapped only four features from F1 to F5.

The docking score of compound 3 with PubChem ID 73071 was found to be -19.2428 kcal/mol. It fit well in the active site with binding affinity 12.803 pKi. The residues that make hydrogen bond interactions with compound 3 include Arg158 and Glu159 (Figure 8). The hydrogen bond distance between compound 1 and Glu158 is 2.80 angstrom while the distance with Arg159 is 2.86 angstrom. The pharmacophore mapping shows that compound 3 mapped five features.

Compound 4 with PubChem ID 12314053 was found to fit well in the active site with good docking score -18.6606 kcal/mol and binding affinity 12.861pKi Compound 4 makes 3 hydrogen bond with residues His58, Phe101 and Ile111. The hydrogen bond distance was found to be 3.02, 2.89 and 2.67 angstrom respectively. Furthermore numerous hydrophobic interactions were found with active site residues. The pharmacophore mapping shows that compound 1 mapped only four features, suggesting that it may be a good inhibitor of FabZ .

The docking score of compound 5 with PubChem ID 9851005 was found to be -18.4850 kcal/mol. It fit well in the active site with binding affinity 13.593 pKi. The residues that make hydrogen bond interactions with compound 5 include His23, Phe101, Val113, Ile111 and Glu159. The pharmacophore mapping shows that compound 5 mapped four features.

As compared to Emodin, all compounds have good docking score and binding affinity suggesting that it may be a good inhibitor of FabZ.

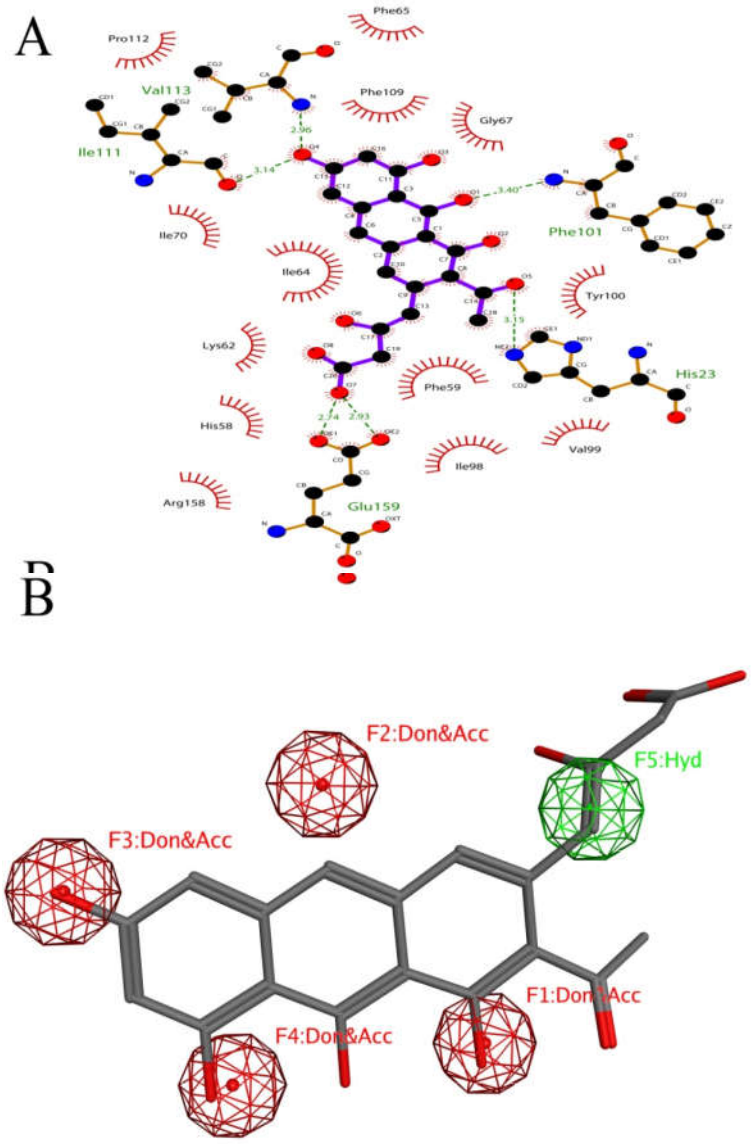


Figure 8: Binding mode of compound 5: (A) the two dimensional analysis of the compound 5 in the active site of FabZ. Compound 5 makes six hydrogen bonds. The dotted green lines shows the hydrogen bonds while the red arcs shows the hydrophobic interactions (B) compound 5 mapped only four features from F1 to F5.

Molecular dynamics simulation

The docking procedure was used and the position of each compound was found in the active site of FabZ. The docking results give only static interactions but in vivo the interaction process between ligand and target is dynamic in nature. Therefore, MD simulation was performed on each complex to check the stability of each compound in the active site of FabZ. The stability of each complex was checked in term of root mean square deviation (RMSD). Figure 9-13 shows the RMSD graph of each complex. As the RMSD value for all the complexes is below 2 Å, it means that FabZ suffered no significant structural changes during the 20 ns of MD simulation. The RMSD values increased up to 1.5 Å during the first 10 ns and then fluctuates around 1 Å for the rest of simulation. The RMSD graph shows that the all compounds are more stable.

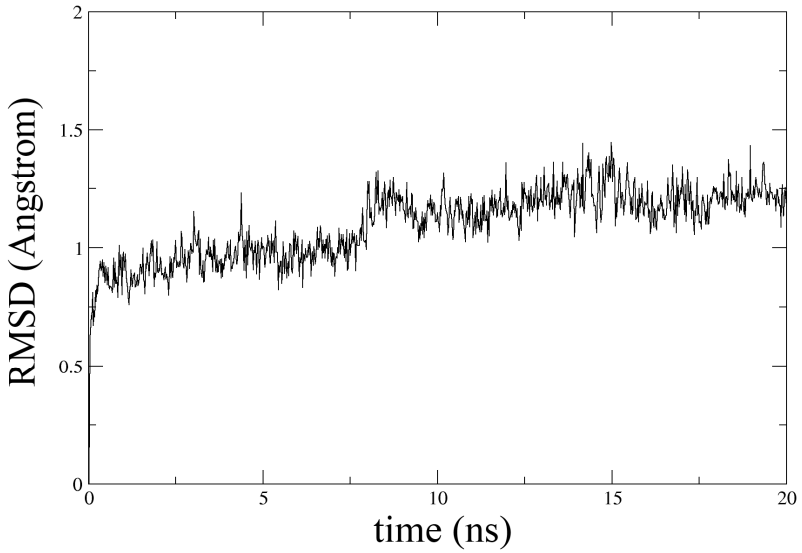


Figure 9: The RMSD graph of compound 10441542. The X-axis shows the length of MD simulation in nanosecond while the Y-Axis show the changes in structure by mean of root mean square deviation in angstrom

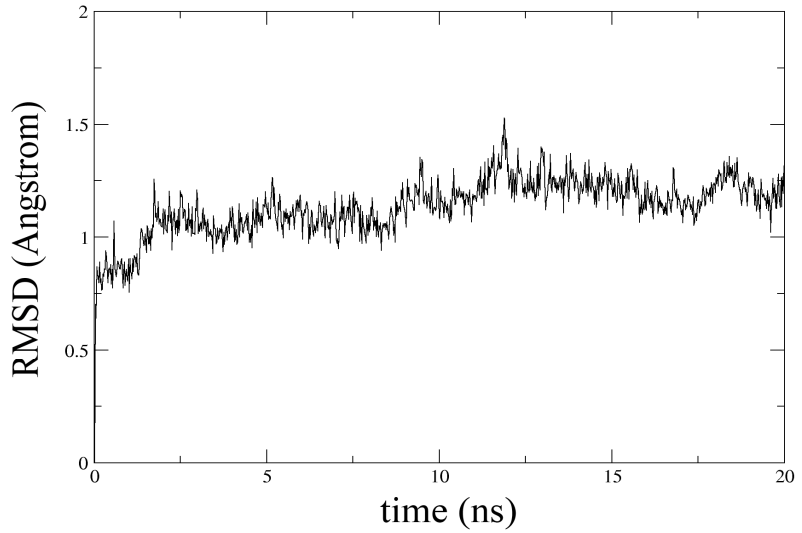


Figure 10: The RMSD graph of compound 11235803. The X-axis shows the length of MD simulation in nanosecond while the Y-Axis show the changes in structure by mean of root mean square deviation in angstrom

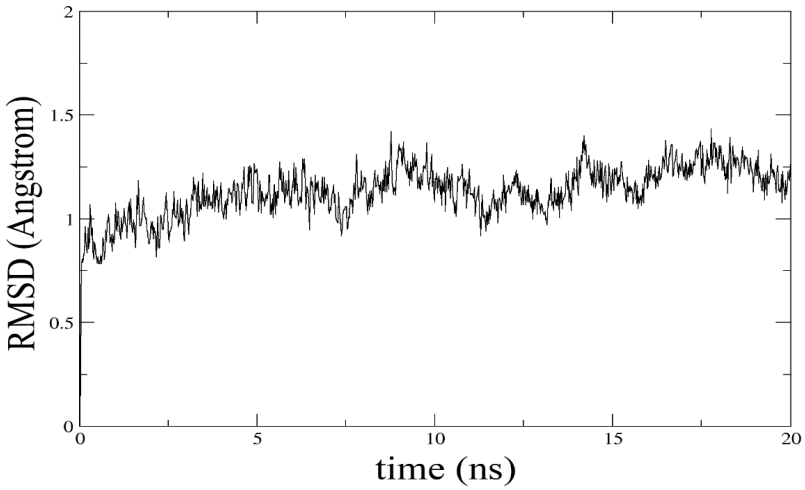


Figure 11: The RMSD graph of compound 12083893. The X-axis shows the length of MD simulation in nanosecond while the Y-Axis show the changes in structure by mean of root mean square deviation in angstrom

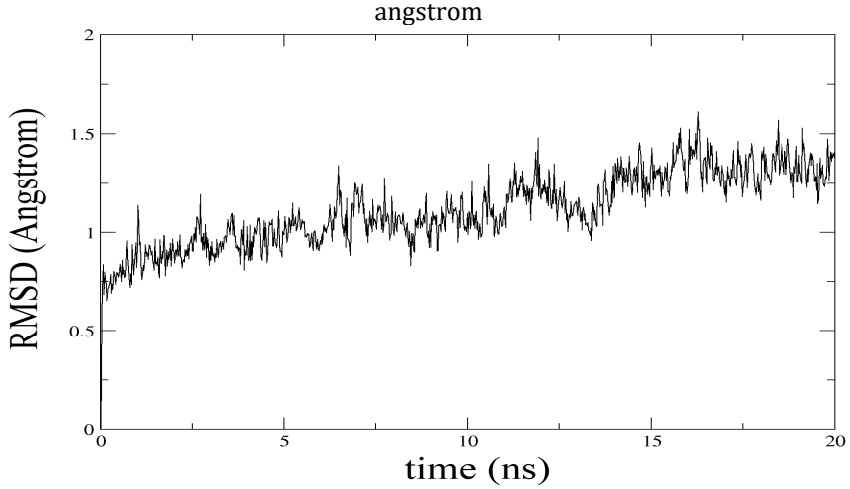


Figure 12: The RMSD graph of compound 12407020. The X-axis shows the length of MD simulation in nanosecond while the Y-Axis show the changes in structure by mean of root mean square deviation in angstrom

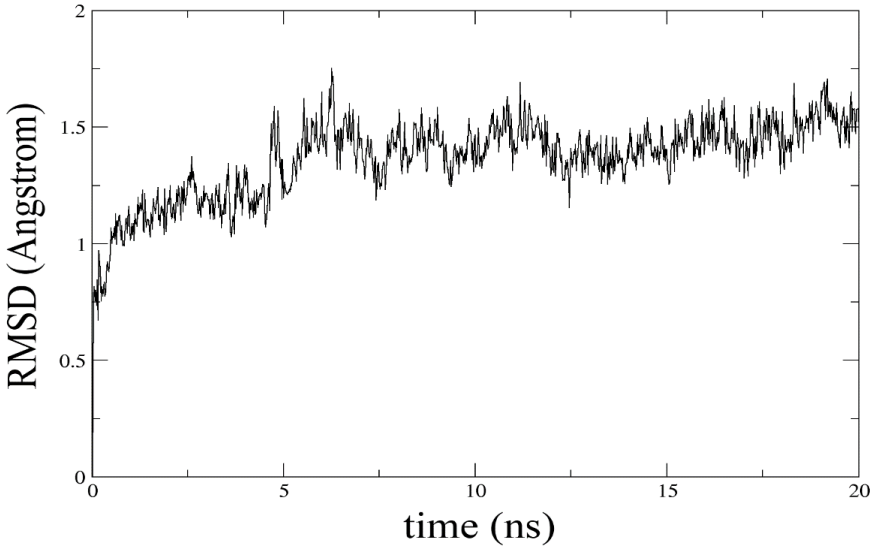


Figure 13: The RMSD graph of compound 12889810. The X-axis shows the length of MD simulation in nanosecond while the Y-Axis show the changes in structure by mean of root mean square deviation in angstrom

CONCLUSION

Type II fatty acid synthetic pathway (FAS II) has been used as a drug target due to the difference in the enzymes of *H.pylori* and human. The enzymes involved in the FAS II have been validated as antibacterial drug targets. FAS II, β-hydroxyacyl-ACP (FabZ) is one of the important enzymes responsible for the elongation of both saturated and unsaturated fatty acids biosynthesis. In the present study, five coumopunds were found to have good docking score and binding affinity than Emodin. Furthermore the MD simulation confirmed the stability of each coumpound in the active site of FabZ. This study is helpful in designing new inhibitors against FabZ.

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