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Research Article

APORPHINE ALKALOIDS AS INHIBITORS FOR MALONYL-COA: ACYL CARRIER PROTEIN TRANSACYLASE (MCAT) FROM *HELICOBACTER PYLORI*

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ABSTRACT

Helicobacter pylori, a pathogenic bacterium which colonizes in the human stomach leads to diseases associated with chronic gastritis and gastric cancer. The protein targets in the bacterium were screened and malonyl-coA: acyl carrier protein (MCAT) was found to be a critical protein target involved in fatty acid biosynthesis. Fatty acid biosynthesis is marked as a potential metabolic pathway for bacterial survival. Aporphine, an alkaloid, is assumed to possess inhibitory activity on MCAT. The aporphine compounds like coryturbine, boldine, dicentrine and glaucine were used for the molecular docking analysis with *H. pyroli* MCAT and also to characterize the binding affinity. Our report suggested that the aporphine alkaloids were lead candidates for the anti- *H.pylori* associated disease.

Keywords: *Helicobacter pyroli*, Aporphine, MCAT, Molecular docking, Binding energy.

INTRODUCTION

Helicobacter pylori, a gram-negative pathogenic bacterium, is a causative agent for peptic ulcer and gastric cancer[1]. *H. pylori* is one of the most common bacterial infections in the world[2]. *H. pylori* infection is spreading worldwide and is a reason for the increased cases of stomach carcinogenesis due to its unusual ability to survive in stomach under the low pH condition[3]. The infection acquisition rate of *H. pylori* is found to be more rapid in developing than developed countries[4]. The lack of effective therapy to eradicate the pathogenic *H. pylori* infection has led to combination therapies which include one proton pump inhibitor and two or three antibiotics[5]. The novel molecular target is necessary to develop new drugs against the pathogenic *H. pylori*[6]. The metabolic pathways involved in pathogenic bacterium are the key to identifying the novel molecular drug targets.

Fatty acid biosynthesis is found to be a critical metabolic pathway for the survival of the organism; since fatty acid is the major component of cell membranes and possesses biological function[7]. The key enzymes involved in type II fatty acid biosynthesis has been assumed to be attractive molecular targets for design of antibacterial agents[8]. The enzyme malonyl-CoA: acyl carrier protein transacylase (MCAT) belongs to EC 2.3.1.39 and is a vital enzyme within FAS II system. The catalysis step of transferring a malonyl moiety from malonyl-CoA to holo-ACP, forming malonyl-ACP which is the elongation substrate for the fatty acid biosynthesis pathway[9], thus making MCAT essential for the completion of fatty acid synthesis pathway in the organism[10].

MCAT consists of two functional domains with domain I containing a short four-stranded parallel b-sheet and 12 helices, and domain II containing a four-stranded anti-parallel b-sheet and two helices[12]. The structural classification of MCAT is depicted as a/b[13]. The key residues which could interact with the malonate carboxylate group[14] are GLN11, GLN63, SER97 and ARG117. The global increase in antibiotic-resistant infections has governed the need for a novel drug design for the pathogenic bacteria[15]. Corytuberine, a natural alkaloid with multi-pharmacological activity has been found to possess inhibitory activity against *H.pylori* MCAT[16].

MATERIALS & METHODS

Protein preparation

Experimental 3D structure of *H.pylori* MCAT (PDB code: 2H1Y) was used for the analysis. The monomer was composed of 14 helices and 10 sheets with alpha /beta classification. MCAT is divided into a large and a small sub domain. The large sub domain was made up of two noncontiguous segments MET1–ASN127 and VAL195–VAL309. The small sub domain residues LYS128–SER194 have a ferredoxin-like fold as observed in acylphosphatases[17].

Active site identification

The possible binding sites of *H. pylori* MCAT were searched using binding site prediction server Q-site finder (http://bmbpcu36.leeds.ac.uk/qsitefinder/)[18]. The prediction study revealed ctive site residues such as GLY9, GLN10, GLY11, GLN61 , HIS91 , SER92 , LEU93, ARG117, MET121, MET132, ASN157, VAL165, LEU191, MET193, VAL195 and SER197. The above identified active site was chosen as the most favorable site for docking studies.

Ligand preparation

Aporphine a naturally available alkaloid was selected for the molecular docking analysis of *H.pylori* MCAT. Four ligands were selected namely coryturbine, boldine, dicentrine and glaucine and were retrieved from pubchem database (http://pubchem.ncbi.nlm.nih.gov/)[19] with Lipinski's rule of five properties[20]. The ligand 2D & 3D drawing was done in ACD-Chemsketch[21]. The OPEN BABEL (www.vcclab.org/lab/babel/start.html) was used to convert mol format to PDB format.

Docking

Molecular docking was carried out by the software AutoDock 4.2[22] which offers the option of search algorithms to find active binding with efficiency. AutoTors was used to prepare ligand and defined the root for the flexible ligand. AutoGrid program constructs the grid for the search algorithm based on defined active sites. The grid size was set to 68× 58 ×64 points with a grid spacing of 0.375Å centered on the ligand in the active site. Autodock program was used to confirm the conformation of binding ligand with active sites of protein. Lamarckian genetic algorithm (LGA) was used for the algorithm. Ten runs were performed with maximum number of 2,500,000 and energy evaluations of 150 individuals were generated for the docking runs.

The best conformation was defined based on the least binding free energy of ligand with protein. The binding energy between a protein and a ligand was calculated using the following formula:

$$\Delta G_{binding} = \Delta G_{complex} - [\Delta G_{protein} + \Delta G_{ligand}]$$

Docking results were evaluated based on number of H- bond interactions between protein-ligand in docked complex. H-bond interactions were analyzed in Pymol software[23].

RESULTS & DISCUSSION

The 3D structure of *H.pyroli* MCAT showed that the protein was under classification of alpha/beta which contains 14 helices and 10 sheets. The protein structure was visualized in Rasmol (Fig 1) with alpha helices colored pink, betas sheets colored yellow and loops in white. The predicted binding sites, by Q-site finder for *H.pyroli* MCAT, were showed in binding cavity with blue color (Fig 2). The ligands of aporphine alkaloids was drawn in ACD- Chemsketch and converted in to PDB format. The 3D structure of ligands showed (Fig 3) carbon atom in grey, nitrogen atom in blue, oxygen atom in red and hydrogen atoms in white color.

The four ligands coryturbine, boldine, dicentrine and glaucine used for studies followed the rules of 'Lipinski's rule of 5' showed in (Table 1).

Fig. 1: **3D structure of *H pyroli* MCAT (2H1Y) visualized in Rasmol**

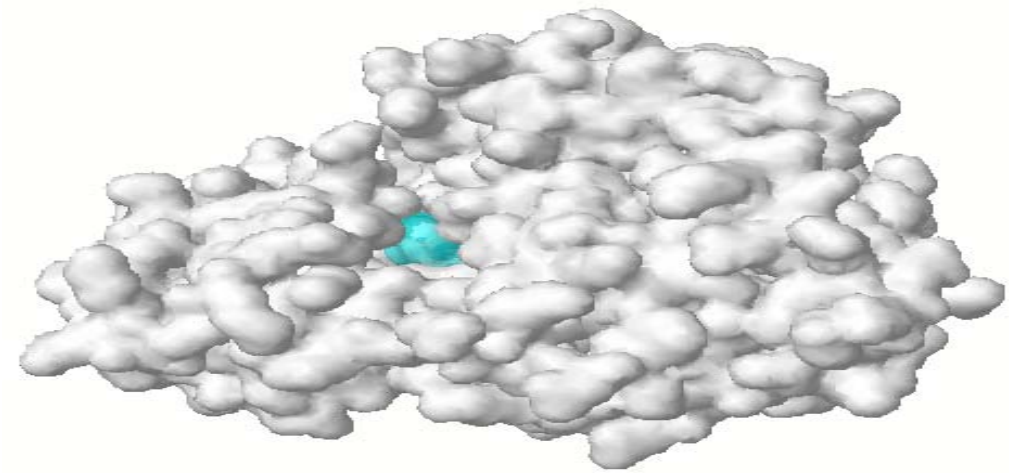


Fig. 2: **Predicted binding cavity of MCAT in Q-site finder**

Table 1: Lipinski's rule of 5 for ligands

S. No.	Aporphine Alkaloid	Molecular weight	X Log P	H-bond donor	H-bond acceptor
1	Coryturbine	327.37	2.2	2	5
2	Boldine	327.37	2.7	2	5
3	Dicentrine	339.38	3.2	0	5
4	Glaucine	355.42	3.5	0	5

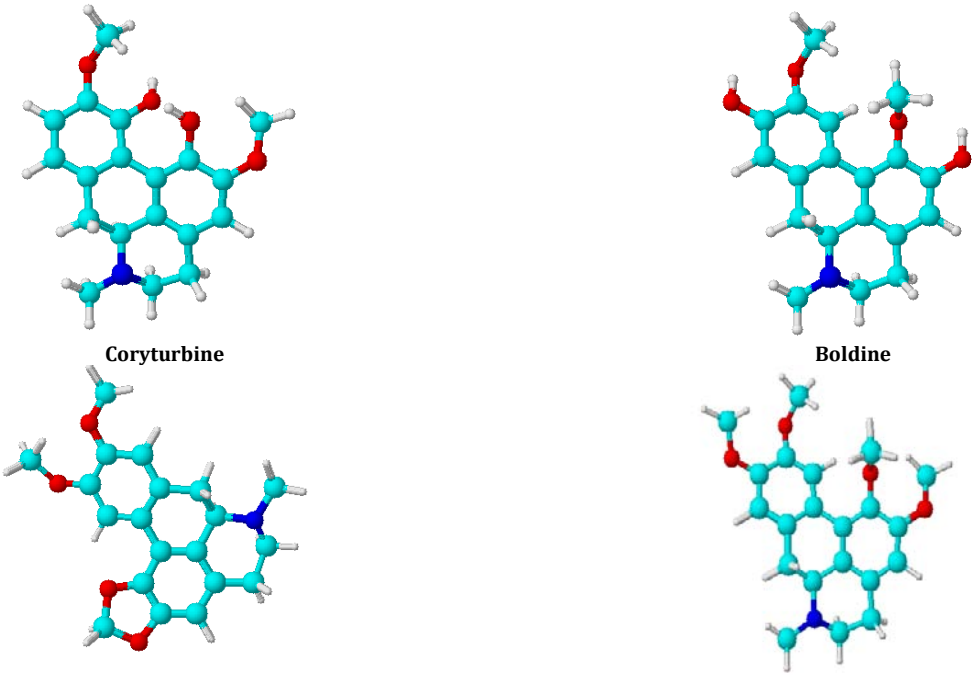
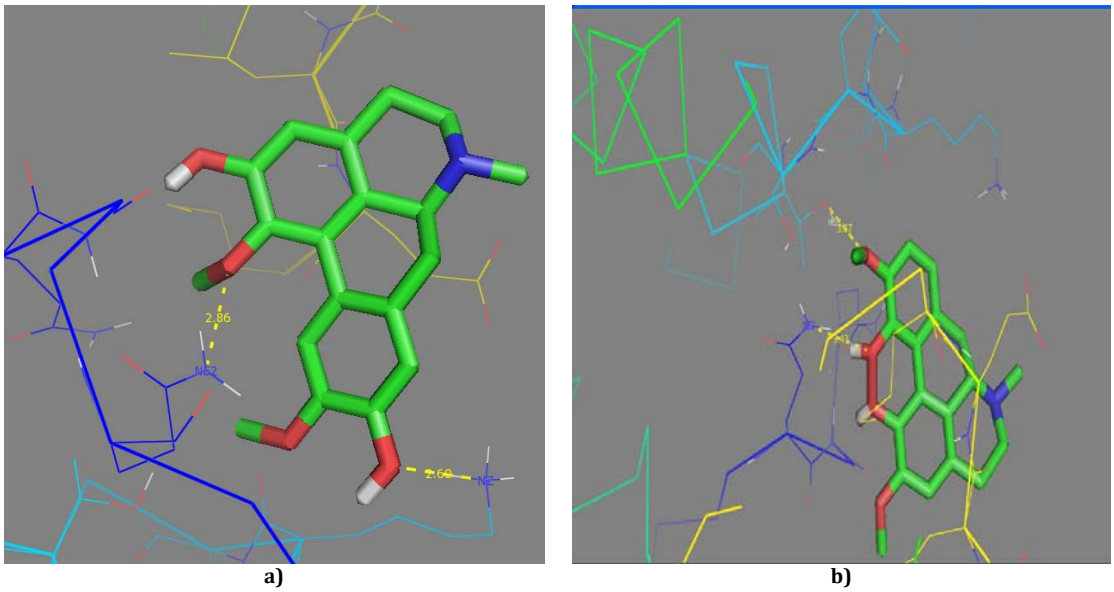


Fig. 3: **Chemical structures of MCAT inhibitors**

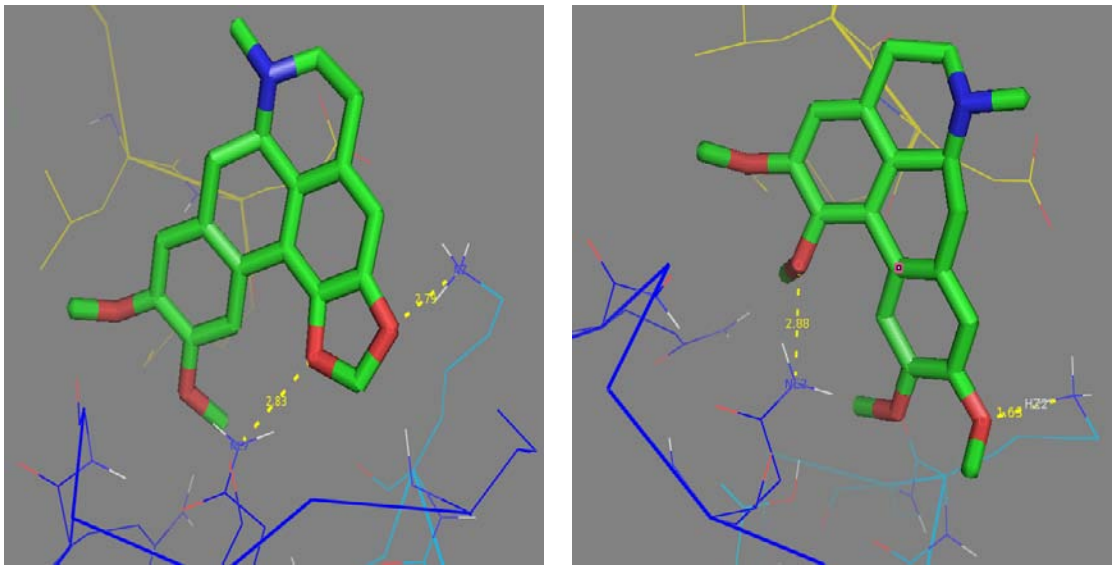
Molecular docking analysis using Autodock was a better method for identifying ligand-protein interactions. The ten docking conformations for each ligand were divided into groups according to a 1.0Å. The cluster conformation analysis was used to compare the RMSD of the lowest energy conformations and their RMSD to one another. The reliability of the docking analysis mainly depends on similarity of its final docked conformation with least binding energy.

The binding energy evaluation was a method to find the best binding mode of ligand with active sites of protein. The energy items calculated by AutoDock are characterized by intermolecular energy which consists of van der walls energy, hydrogen bonding energy, dissolution energy, electrostatic energy, internal energy of ligand, and torsional free energy. The first two of these when combined gives the docking energy while the first and third terms build up the binding energy. During all these interactions, the electrostatic interaction between ligands and receptor is the most important, because in most cases it can decide the binding strength and the location of ligand. The hydrophobic interaction of certain groups can affect the agonistic activity to a larger extent.

The ligand coryturbine which binds with active sites of *H. pylori* MCAT formed two hydrogen bonds. The binding free energy showed -7.18 Kcal/mol. The experimental critical residue GLN10 was allowed to interact with the ligand atoms to confirm the blocking of MCAT. The ligand boldine, dicentrine and glaucine bound with active sites of *H.pylori* MCAT and also formed two hydrogen bonds respectively. The binding free energy showed -5.95, 5.65 and 5.83 Kcal/mol respectively. When the residues GLN13 and LYS55 were allowed to interact with the ligand atoms to confirm the blocking of *H. pylori* MCAT, the predicted residues were found to be novel in the interaction with the ligands. Based on the amount of amino acids involved in the molecular docking interactions of four ligands, GLN13 and LYS55 were found to be critical. The mode of H- bond acceptor and donor showed mostly of NE2, HZ2 of H-bond donors and O of H-bond acceptors.



a) **b)**



c) d)

Fig. 4: **Calculated binding mode of aporphine alkaloids with active sites of *H.pylori* MCAT. a) Coryturbine b) Boldine c) Dicentrine d) Glaucine. Only selected residues are shown.**

H-bonds are shown as yellow dashes with bond lengths between two atoms

Table 2: Docking result for ligands

S. No.	Aporphine alkaloid	Binding energy Kcal/mol	H-bond interaction	Bond length Å
1	Coryturbine	-7.18	(THR60)HG1-----O (GLN10)NE2-----H	3.07, 3.43
2	Boldine	-5.95	(GLN13)NE2-----O (LYS55)HZ2-----O	2.86, 2.60
3	Dicentrine	-5.69	(GLN13)NE2-----O (LYS55)HZ2-----O	2.83, 2.79
4	Glaucine	-5.83	(GLN13)NE2-----O (LYS55)HZ2-----O	2.88, 1.63

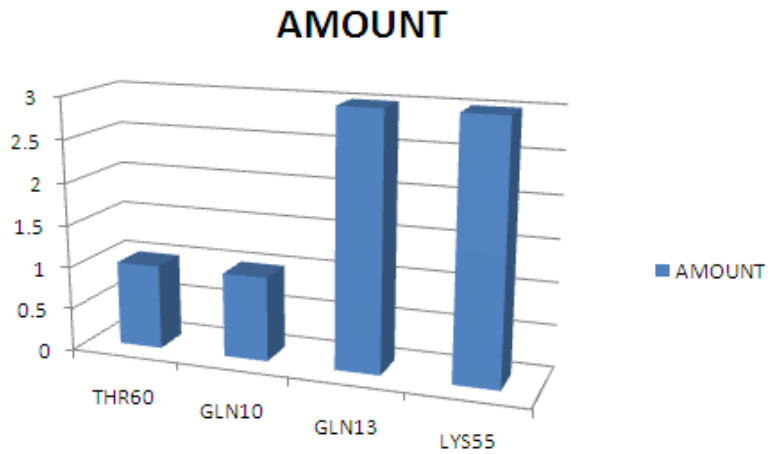


Fig. 5: **Statistic amounts of interacted amino acids**

CONCLUSION

In this study, we demonstrated the aporphine alkaloids inhibitory activity on *H. pylori* MCAT through computational approach. Experimental structure of MCAT improved the accuracy of the molecular analysis with ligands. The binding cavity from Q- site finder includes critical residues like GLN10, GLN13, and ARG117 which match with experimental data. Docking program using Autodock predicted the binding mode of the best docked ligand conformation with active sites of *H. pylori* MCAT. The binding energy of ligands confirmed the active reaction between ligand and protein to form a docked complex. Our results also showed that GLN13 and LYS55 were more critical in the ligand inhibitory activity on *H. pylori* MCAT. Among the four ligands, coryturbine docked well with active sites of *H. pylori* MCAT to block the enzyme. The computational approach on *H.pylori* MCAT with aporphine alkaloids showed coryturbine, boldine, dicentrine and glaucine as effective inhibitors for *H. pylori* MCAT. Our results conclude that aporphines alkaloids may be used as anti-*H. pylori* agents.

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