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# Design of an inhibitor of *Helicobacter pylori* Cholesteryl-α-Glucoside Transferase critical for bacterial colonization

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## Abstract

### Background

50% of the world's population is surviving as a host for *Helicobacter pylori*, gastric-cancer causing bacteria, in the gastric region of digestive tract. It has a remarkable capacity to colonize the host stomach for entire lifetime despite development of immune response.

### Methods

In this study, we have performed the virtual screening analysis of protein-inhibitor binding between the Glycosyl Transferase enzymes of *Helicobacter pylori* (CapJ or HP0421) and its library of inhibitors in the known substrate binding pockets. We have docked our library of ligands consisting of cholesterol backbone with CapJ protein and identified several ligands' interacting amino acid residues present in active site pocket of the protein.

### Results

In most of the cases the ligands show an interaction with the same pocket's residues of the enzyme. In most of the cases, ligands interacted with the same amino acids present in the active site pocket of the enzyme. Top three (03) hits have been filtered out from the whole data set, which may act as most potent inhibitors for the enzyme-substrate reaction. Top three (03) hits have been filtered out from the

whole data set, based on their binding affinity with the protein, which may act as most potent inhibitors for the enzyme-substrate reaction.

**Conclusions**

This study describes a new possibility by which colonization of *Helicobacter pylori* can be limited. The reported evidence suggests that comprehensive knowledge and wet lab validation of these inhibitors are needed to be developed for eradication of this disease.

## Introduction

A variety of microorganisms inhabit as commensal bacteria or parasitic pathogens in humans. These microbes enter the gastrointestinal (GI) tract, but due to acidic nature of the stomach, most of them are unable to colonize successfully[1]. Notably, the high prevalence of *Helicobacter pylori* (*H. pylori*) in the GI tract of humans all over the world makes us believe, also in the light of a plethora of available studies that it may have definite mechanisms to survive this acidic environment and innate immune response[1].

Today, over half of the world's population is infected with the Gram-negative pathogen, *H. pylori*[1]. Various kinds of gastric diseases, such as peptic ulcer, gastric adenocarcinoma, and gastric lymphoma are some of the severe consequences of this infection[1]. CapJ catalyzes the conversion of cholesterol to α-glucosyl cholesterol at the time of incorporation of it in the cell membrane of *H. pylori*, as *H. pylori* does not synthesize it and rather extracts it from the host[2-4]. This glucosyl cholesterol, in turn, plays a vital role in immune evasion[3, 4].

In this study, we studied the molecular interaction of a library of ligands with CapJ protein by using an *in silico* approach. We have also examined the hydrogen bonding and hydrophobic interactions among these protein-ligand complexes in substrate binding pockets of the proteins which further confirmed our findings. Our study revealed that these amino acid residues present in substrate binding pockets possess high binding affinity and strong protein-ligand interactions in docked complexes. The interaction of protein and ligand in substrate binding pockets present on the protein may lead to the inhibition of the normal activity of CapJ. We believe that this study will assist in the efficient designing of therapeutic intervention against *H. pylori.*

## Methods

**Retrieval of the three-dimensional structure of CapJ and preparation of a library of ligands of our interest**

The Protein Data Bank (PDB) structure of CapJ was modeled using i-Tasser program[5, 6]. The literature survey about crystal structure of catalytic domain of CapJ helped us to identify substrate-binding pocket[7]. The library of ligands containing the cholesterol backbone was made using the PubChem download service[8]. Conversion into an executable PDB file format, interactive visualization, and analysis of molecular structures was undertaken using UCSF Chimera[9] and PyMol[10,11,12].

**Protein-ligand docking**

Protein-ligand docking was performed by the Autodock and Autodock Vina wizard tools of the PyRx software. It was a multi-step process in which .pdbqt files of protein and ligands were generated using MGL (Molecular Graphics Laboratory) tools[13]. To perform precise docking of a library of ligands with target protein the values of X, Y and Z axis were taken from the amino acids present in the substrate-binding pockets. The diameter of the grid box was set according to the size of the ligands. The docking was performed in click prompt using Autodock Vina wizard. AutoDock Vina is the combination of multiple things such as treating docking as a stochastic global optimization of the scoring function, pre-calculating grid maps and some other implementation tricks, such as pre-calculating the interaction between every atom type pair at every distance. It also uses the same type of structure format (PDBQT) for maximum compatibility with auxiliary software[14].

Three hundred and eighty-six different molecules (library) (1095 States) were used as ligand and CapJ was used as a receptor in this study. Based on the structural and functional aspects of each protein-ligand complex, three best-docked complexes were obtained/identified, and structures were visualized using UCSF Chimera molecular graphics program[15]. Protein-ligand interactions were plotted using the LIGPLOT program of LIGPLUS software using default parameters[16]. It is used to determine hydrogen bonds and hydrophobic interactions. LIGPLOT program generated two-dimensional plots representing hydrogen bonds and hydrophobic interactions between the interacting amino acid residues[16].

## Results and Discussion

We have carried out *in silico* study to investigate and validate the screening result of inhibitor binding in substrate binding pockets of receptor protein, CapJ. The details of the protein are shown in Table 1 and further details of the top three hits of molecular docking analysis are represented in Table 2.

**Identification of substrate binding pockets in CapJ**

The substrate-binding regions were already reported; therefore, the residues of interest were noted down and the average value of x y and z center was calculated to perform precise docking of a library (ligands) using Autodock Vina. We had given the click prompt which possesses the values of x, y and

z center along with the defined boundary size of the grid box. Grid box parameters had been provided by us. To perform docking, .pdbqt files were generated for both; i.e. library of ligands and receptor using MGL tools. After the completion of all basic requirements of docking i.e. defining grid box values and sizes, defining exhaustiveness, we performed protein-ligand docking and the best-suited models having maximum negative binding affinity according to the involvement of substrate binding pocket were selected for further analysis. Empirical methods such as docking usually use scoring function to measure the likelihood of ligand to bind to the protein target. *Autodock vina* uses several terms to be included in its own scoring function: gaussian, repulsion, hydrophobic, hydrogen bonding, and the number of rotatable bonds. The interaction of most ligands with their binding sites can be characterized in terms of a binding affinity. In general, high-affinity ligand binding results from greater intermolecular force between the ligand and its receptor while low-affinity ligand binding involves less intermolecular force between the ligand and its receptor. Looking at the binding affinity values of the top three (03) ligands which are <0 Kcal/mol (Kilo-Calorie/Mol) and considering the thermodynamic property for a reaction to take place, we can conclude that the formation of the top three (03) complexes are energetically feasible. Proteins may undergo large conformational changes during the function performance, with these properties which will be defined by energetics and from the way in which stabilizing interactions are defined by the structure. Therefore, we may say that interactions, and so the energetics (binding affinity) will guide the stability of those complexes. The complete list of protein-ligand interaction affinities has been provided as Supplementary Table1. The finally screened docked complexes were analyzed using the LigPlot program and then the model showing the involvement of the maximum hydrogen bonding and hydrophobic interaction among the atoms of ligand and protein were selected and the binding affinity in Kcal/mol was noted down (Table 2). Lower the binding affinity, stronger is the binding between protein and ligand. The locations of these pockets are shown in Table 2. In this study, we observed that the binding of ligands specifically happened in the substrate-binding pockets (Fig. 1-3 A, B and C). The final docked models of CapJ and 3 different ligands in cartoon structures are depicted in Fig. 1-3. The interacting binding partners and the hydrogen bond interaction in between them are represented in green dotted sticks with respective bond lengths, whereas yellow color sticks represent amino acid residues of CapJ (Fig. 1-3 D).

We have also identified several other residues in target CapJ which are involved in strong hydrophobic interactions with ligand and shown in Table 2. We have selected all the pairs of interactive amino acid residues of the target CapJ with ligands in the docked complex and shown in cartoon structures Fig. 1-3 (A, B and C) where blue/pink/green color sphere represents CapJ (protein) and green/yellow/red color stick/sphere represent ligands respectively. In most of the cases as many as 20 amino acid residues participate in the interaction with ligands. This *in silico* analysis revealed a strong physical

interaction between the ligands and the functional sites of CapJ where the substrate-binding site is located.

Molecular docking simulation of all cholesterol derivative ligands exhibited a strong binding with CapJ, but 24-Methylencholesterol proves to be the best anti-pathogenic drug that can be developed. Principally all the ligands were docked in the same pocket (same binding site) as that of cholesterol, the natural ligand of CapJ, which indicates that these all follow the same biochemical pathway not as an agonist but as an antagonist. Although, wet lab validation is required to support these findings, they hold potential to open a new horizon in the field of drug discovery against *H. pylori*.

## Epilogue and the way forward

Gastric and duodenal ulcers are the two main diseases that are caused by *H. pylori*[17,18]. In the last few decades, the use of antibiotics has lowered the incidence of *H. pylori* infection. However, the emergence of drug-resistant *H. pylori* strains has complicated the possibility of the eradication of these bacteria[1]. It is, therefore, possible to direct the efforts at the discovery of new molecules and drugs that bind to critical choke point enzymes of the pathogen. In the metabolic networks, such choke points are crucial junction points. Disruption of any of the choke point enzymes may lead to the inability to produce or consume specific metabolites, which may cause clearance of the microbial pathogen from the host [19].

Apart from its mechanisms for avoiding innate and adaptive immune responses, the helical shape of the bacilli also acts as a protective feature of *H. pylori*. It mechanically facilitates the bacilli to penetrate and sail the viscous mucus layer of the stomach and duodenum[20]. Another strategy adopted by *H. pylori* for immune evasion is comprised of a system that ensures glucosylation of exogenous cholesterol[21]. Therefore, the constituent proteins and enzymes of the cholesterol biosynthesis and modification pathways are significant in the maintenance of the cell wall integrity of the bacterium. Cholesterol backbone is the core structure in all the screened inhibitors in this study and being the ubiquitous component of the cell membrane in most of the cases, cholesterol has played an important role in drug delivery earlier[22]. Cholesterol proved to be an attractive scaffold in the drug/gene delivery moieties, however in the current study, we propose potent inhibitors against cholesteryl-α-glucoside transferase (encoded by *HP0421*)[22]. In the field of chemical glycobiology and drug discovery, the finding of small molecule inhibitor for cholesteryl-α-glucoside transferase is of great scientific interest[23]. In light of the mechanistic similarities between many bacterial glycosyl transferases from pathogens, it is very much possible that this strategy will represent a general way for the development of potent therapeutics against a broad range of pathogenic bacteria[24].

The current work reveals the site of binding for the potent drug candidates. Docking studies were performed to better rationalize the inhibition patterns. So, deciphering the mode of action and detailed

understanding of the inhibitor-enzyme interactions will be the next steps to give this study a practical implication. Regarding the downstream exploitation of the screened hit molecules, we may not state much, as the knowledge about those molecules is extremely limited till date. Therefore, that proves to be a limitation for this study, but at the same time, the novelty of those moieties as potent inhibitors against cholesteryl-α-glucoside transferase tends to open a wide door for a novel therapeutic finding against the bacteria.

Therefore, further progress in this direction will give rise to the possibility that the perception of cholesteryl-α-glucoside transferase as a difficult target for drug discovery will change. But the enzyme's own conformational movement and the conformational flexibility of the inhibitor itself stand as a barrier in its actual materializing as a useable drug target. Experimental validation of these results *in vitro*/*in vivo* will open a horizon of information that might enable us to create a more potent drug for the effective control and possible elimination of the bacterium.

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**Figure legends**

Fig. 1-3 (A) The cartoon representation of ligands docked in substrate binding pocket of CapJ; (B) and (C) Enlarged view of substrate binding pocket of receptor-ligand complex showing ligand binding site; (D) Ligplot image showing protein-ligand interactions [Amino acid residues highlighted in yellow sticks (receptor) and blue (Ligand) color respectively].

**Table 1: Receptor protein and their pocket residues**

| Protein | Amino acid present in substrate binding pocket | Reference |
|---|---|---|
| CapJ | Val 208, Gly 209, Arg 210, Glu 214, Lys 215, Val 263, Glu 285, Ser 286, Glu 287, Ala 288, Ile 289, Ala 290, Glu 293 | Lee et al., 2011 |

Table 2: List of top 3 ligands along with identification

| Ligand | Pubchem CID | Amino acids present in binding pocket | Amino acids involved in interaction | Binding affinity (Kcal/mol) |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| 24-Methylencholesterol | 53477901 | Asn 15, Gly 16, Thr 17, His 120, Leu 121, Val 208, Gly 209, Arg 210, Lys 238, Phe 262, Val 263, Asn 264, Ser 265, Leu 268, His 280, Glu 285, Ser 286, Ile 289, Ala 290, Glu 293 | Val 263 | -6.0 |
| Epoxycholesterol | 13247303 | Asn 15, Gly 16, Thr 17, Leu 121, Val 208, Gly 209, Arg 210, Lys 238, Phe 262, Val 263, Asn 264, Ser 265, Leu 268, His 280, Glu 285, Ser 286, Ile 289, Ala 290, Glu 293 | Val 263 | -5.3 |
| 5,6 beta-epoxy-5alpha-cholestan-3beta-ol | 440665 | Asn 15, Gly 16, His 120, Val 208, Gly 209, Arg 210, Lys 238, Phe 262, Val 263, Asn 264, Ser 265, Leu 268, His 280, Glu 285, Glu 287, Ala | Val 263 and Asn 264 | -5.3 |

| | | | | |
|---|---|---|---|---|
| | | 288, Ile 289, Ala 290 | | |

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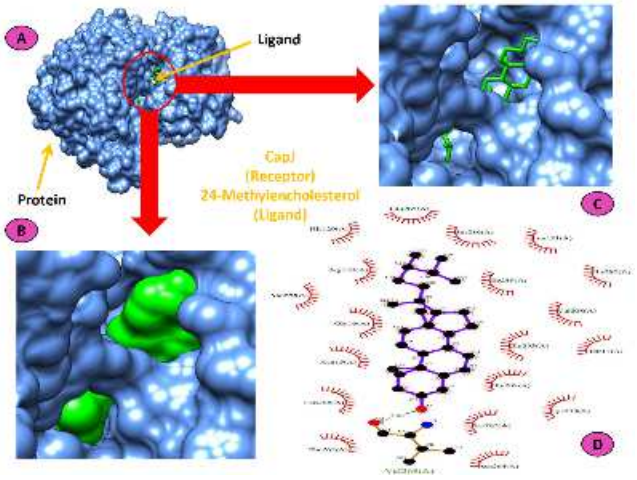


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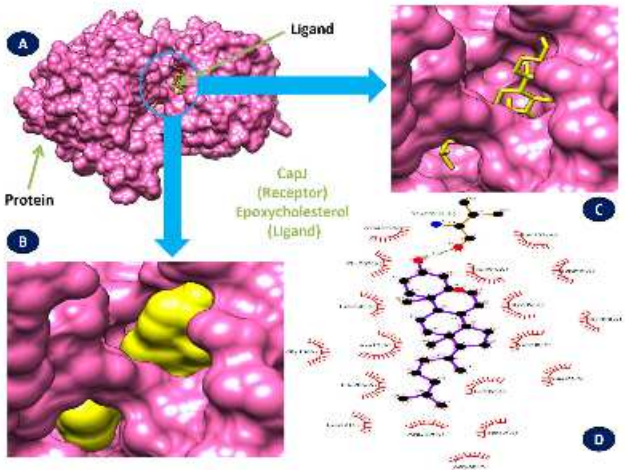


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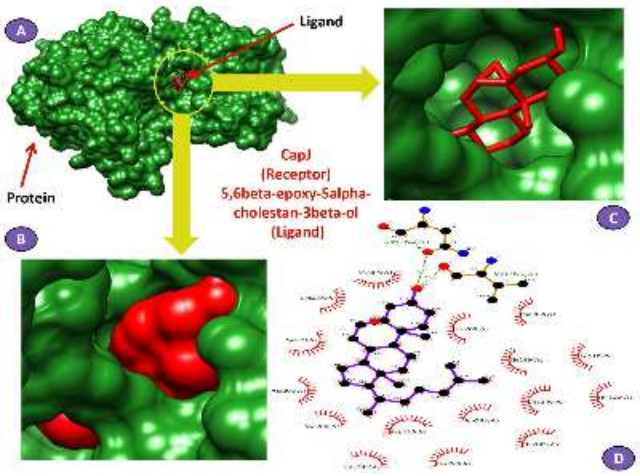


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