Food constituents for inhibition of BabA of Helicobacter pylori

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ABSTRACT

Helicobacter pylori causes several gastric pathogeneses to human, nowadays the bacterium developed incredible drug and antibiotic resistance. The bacterium starts its activities by attachment to gastric epithelia via BabA as the main player in the virulence factors, among them the bact, and persistence of bacterium in the oral cavity and stomach continuous changes as in the appearance of antibiotic resistance. The recognition, adhesion, and persistence of bacterium in the stomach is mainly mediated by several OMPs (hop) group including blood group antigen-binding adhesion, one of the most important is BabA which is also known as HopS 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22. This protein mediates binding to fucosylated Lewis b (Leb) blood group antigens and facilitates the injection of some virulence factors such as CagA and VacA 17,22,22. In addition, BabA binding contributes to gene mutations through the formation of double-stranded DNA breaks in host cell lines 23. This might lead to the suggestion that BabA and other binding proteins such as SabA are involved in carcinogenesis 24. This protein was reported to be necessary for H. pylori replication and nutrient acquisition 25,26, it has been estimated in population with the highest incidence of gastric cancer worldwide as in East Asia 22.
On the other side, foods have been used for a long time as anti-Helicobacter. Food constituents work in different mechanisms to inhibit H. pylori infection, therefore, they can be considered as alternatives to prevent and manage H. pylori infection. To explore that computational aid drug design (CADD) can play an important role in drug design and discovery using different approaches, among these Structure-Based Drug Design (SBDD), since a lot of 3D structures are now available. SBDD is the most powerful and efficient process for accelerating drug discovery as it is specific and cost-effective, so it has emerged as a promising tool for the drug industry to design and optimizes ligands/drugs and become as an integral part of most drug discovery.

This study aims to use BabA crystal structure (pdb ID 4zh0) to screen food database and drug database using SBDD approach in an attempt to find out new inhibitors or drugs from food constituents to inhibit the attachment of H. pylori.

MATERIALS AND METHODS

Diferent databases and software were used, for different purposes:

- **Databases**
  - NCBI: https://www.ncbi.nlm.nih.gov/
  - Used for retrieve sequences and BLASTing
  - PDB database: https://www.rcsb.org/
  - Used to find out the pdb structure of BabA (4zh0) protein
  - Uniprot database: https://www.uniprot.org/
  - To find out some information about the target (BabA).
  - Zinc database: http://zinc.docking.org/
  - Used to download different chemical formats, and information about compounds.
  - MarvinSketch: https://chemaxon.com/products/marvin
  - Used for chemical format manipulation, and finding some molecule descriptors.
  - Online SMILES Translator and Structure File Generator: https://cactus.nci.nih.gov/translate/
  - Used to get SMILES format of some molecules.
  - Swiss ADME: http://www.swissadme.ch/
  - Used for finding pharmacokinetic characters of molecules.

Table 1: Molecules found from drug database screening food database

| Molecule # | Molecule Name | ZINC766 | Comments |
|------------|---------------|---------|----------|
| 81         | 2_3_4_5_6_Penta_O_acetyl_D_glucose | ZINC000003861047 |
| 86         | N2_N2_Dimethylguanosine | ZINC000005115341 |
| 91         | 5_Methylthiodenosine | ZINC000005929300 |
| 97         | Glycerol_5_hydroxydecanoate | ZINC000002557905 |
| 101        | Monoisopropyl citrate | ZINC000002528012 |

RESULTS AND DISCUSSION

**Target importance and characterization:**

The choice of a drug target is primarily made on a biological and biochemical basis, it should have well-defined binding pockets and the goal is total inhibition leading to death and eradication of the pathogens. The target chosen in this study BabA (pdb ID 4zh0) is druggable and got the most criteria to be a good drug target, it has been characterized in a previous study. It has been used in the SBDD process which had been carried out in multistep relies on the knowledge of the 3D of the protein. The steps involve protein preparation, binding site identification, ligand library preparation, docking, and scoring function, so current SBDD methods consider the key features of binding cavities of a therapeutic target to design efficient ligands. Many tools can perform such tasks, among them MTTOpenScreen which use AutoDock4.2 and automated virtual screening with AutoDock Vina and provides valuable starting collections using pdb structure to screen several databases, it has been used to find and identifying ligands for human acetylcholinesterase (Alzheimer’s disease). In this study, hundreds of molecules resulted in a screening food database and a drug database. Once small molecules have been identifying as potentially binding to the target, it must be evaluated before proceeding to further steps, since even molecule with high scoring could fail in some aspects of characterizations or in vivo and in vitro assays. In the case of H. pylori, the rule of 5 should be considered. The resulted molecules were subjected to different filtration steps. First of all, molecules with rotatable bonds more than 7 were omitted since such molecules could bind to off-targets. Their mutagenicity, teratogenicity, and carcinogenicity were estimated. Such a survey resulted in two molecules from the drug database and 5 molecules from the food database. Table 1 shows the molecules.
Other properties such as bioavailability and ADME (Absorption, Distribution, Metabolism, Excretion) i.e. Pharmacokinetics and Druglikeness and Medicinal Chemistry characters and hERG activity were estimated in addition to synthetic accessibility, as shown in Table 2.

### Table 2: Some characters of suggested molecules

| Molecule # | RTs | GI absorption | BBB permeant | P-gp substrate | Druglikeness/ Lipinski | PAINS | Synthetic accessibility | Bioavailability |
|------------|-----|----------------|--------------|----------------|-------------------------|-------|-------------------------|-----------------|
| Rivoglitazone | 6 | High | NO | NO | 0 violation | NO | 3.54 | 0.55 |
| Tiaprinol | 3 | High | NO | NO | 0 violation | NO | 3.45 | 0.55 |
| 81 | 6 | High | NO | NO | 1 violation: NorO>10 | NO | 4.79 | 0.55 |
| 86 | 3 | High | NO | NO | 0 violation | NO | 4.10 | 0.55 |
| 91 | 3 | Low | NO | NO | 0 violation | NO | 4.12 | 0.55 |
| 97 | 5 | High | NO | NO | 0 violation | NO | 3.61 | 0.55 |
| 101 | 7 | High | NO | NO | 0 violation | NO | 3.04 | 0.56 |

From the results it is obvious that chemical synthesis is possible, however, optimization of these molecules, if required, can be done using the scaffold hopping approach. It has been suggested that the inhibition could be active site as in enzymes, but inhibitor could affect the assembly sites with other macromolecules or communication sites or proteins with other function as for BabA, so the potential drug targets are not necessarily disease-causing but could sharing in disease development. Therefore, the anti-adhesion would be able to clear *H. pylori* out of the stomach wall through dislodging off the bacterium, since that BabA is unique to *H. pylori* without affecting the other good bacteria of normal flora.

**Docking studies**

Docking is the critical step in SBDD, which helps in visualizing the interaction patterns and binding energy of protein/receptor-ligand complexes, and gives insight into the interactions at the atomic level, offering the opportunity to fully characterize the binding site of each molecule. The obtained molecules were docked with BabA using PyRx package v.8, the top affinities results with RMSD value of zero are shown in Table 3.

### Table 3: Binding affinity of docked molecules

| Molecule # | Comments | Binding affinity kcal/mol |
|------------|----------|--------------------------|
| Rivoglitazone | Drug database | -6.9 |
| Tiaprinol | Drug database | -5.9 |
| 81 | ZINC000003861047 | -6.8 |
| 86 | ZINC000005115341 | -5.7 |
| 91 | ZINC000005929300 | -6.0 |
| 97 | ZINC000002557905 | -5.2 |
| 101 | ZINC000002528012 | -5.2 |

It is known that docking accuracy can be evaluated by values of RMSD with a threshold of 1.0-3.0 Å between the docked ligand and X-ray pose which is generally considered to be successful. However, this could depend on the resolution of the X-ray structure of the protein. The docking depends on a variety of interactions such as electrostatics, potential hydrogen bond donors and acceptors, hydrophobic patches, van der Waals and also affected by neighboring patches near the ligand-binding sites. Different types of interactions were observed between the selected molecules and BabA protein as shown in the following figure (Figure 1).
Figure 1: Attachments and types of molecular interaction with BabA (4zh0)
From the results above, it can be noticed that most of the molecules have an aromatic ring(s) (5 out of 7) which is in agreement with the fact that 75% of marketed drugs contain one or more aromatic rings.\(^\text{47-49}\). van der Waals forces are the most frequent interactions, these are formed between atoms, molecules, and ions when they are sufficiently close to each other, although they are weak but they are abundant and have additive action with no directional characteristics.\(^\text{47}\). The docking interactions involve H-bonds for some molecules which are stronger than van der Waals and are mainly electrostatic forces between an electronegative atom and partial positive hydrogen atom, they are long-lived and strong forces. The other interactions are of π type, π-sigma considered as the strongest interaction are found in molecule \(8\).

The anti-adhesion molecule could be direct or indirect as found for the extract of Okra fruits, it acts by changing the binding capacity of the bacterial adhesion to Leb by interaction with surface structures in the vicinity of BabA\(^\text{48}\), this supported by Kaelin\(^\text{49}\) that the loss of function in one molecule often correlated with a gain of function in another.

Generally, it has been reported of antibiotic resistance of \(H.\) pylori is increasing worldwide\(^\text{50}\) which led to a low eradication rate and redistricted the application of triple therapy, this means that the novel diet-based therapeutics should be used when conventional antibiotic therapies failed and this received considerable attention.\(^\text{27,51}\)

CONCLUSION

BabA is one of the significant protein involving in many inflammatory processes in addition to its role in the attachment for persistent colonization\(^\text{7}\). \(H.\) pylori eradication might be impossible on these days due to antibiotic resistance, and alternatives must be used, foods and natural products offer such alternative, especially those work early at the begging of infection processes as for the activity of BabA and other adhesins. The anti-adhesion molecules are safe and representing interesting tools for future medicinal developments since they interact with surface proteins of pathogens.

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