Inhibitors for Attachment Protein BabA of *Helicobacter pylori*

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*Helicobacter pylori* causes gastric pathologies to human after attachment to gastric epithelial layer via BabA protein, which is considered as one of the most important virulence factors. The study aimed to find inhibitors to this protein using structure-based drug design (SBDD) approach on the protein 3D structure (pdb ID 4zho). Large number of molecules/ligands were obtained as the protein gets many binding pockets. Checking and filtering the compounds depending on different parameters such as types of toxicity, ADME (absorption, distribution, metabolism, and excretion) characters and others, only 6 molecules were obtained, these were docked with the protein, they gave reasonable binding affinity at root-mean-square deviation (RMSD) of zero which represented by mode 1 of results performed by AutoDock vina.

**Keywords:** *Helicobacter pylori*, BabA, Antiadhesive compounds, SBDD, Iraq

**INTRODUCTION**

*Helicobacter pylori* (*H. pylori*) specifically colonizes human stomach, can lead to different gastric pathologies. The bacterium equipped with an extraordinary large number of outer membrane proteins, and among the main features of the bacterium is the high genome plasticity, which is resulted from an elevated mutation rate and extensive exchange of genetic materials leading to free recombination of *H. pylori* genes (Wang et al. (2019); Suerbaum et al. (1998) Wang et al. 1999). The bacterium has been classified as class I carcinogen (IARC, 1994; Ansari and Yamaoka, 2017). It is prevalent in developing countries, where up to 80% of the mid-aged adults may be infected (De Falco et al. (2015). Many virulence factors are used by the bacterium such as outer membrane proteins (adhesins), which attach the bacterial cells to host surfaces, these adhesins play a vital role in pathogenesis, they can recognize the structures of glycans expressed in the gastric mucosa Huang et al. (2016). Among these adhesins is the blood group-binding antigen (BabA), which is one of the Hop family (known as HopS or OMP28), this protein can identify (MUC5AC) the difucosylated ABO/Lewis b (LeB) antigen found on red blood cells and gastrointestinal mucosa epithelial cells (Huang et al. 2016; Kusters et al. 2006; Odenbreit et al. 2009). BabA receptors also found on oral cavity and stomach (Ansari and Yamaoka, 2017), it is nearly 78kDa encoded by babA gene Kao et al. (2016). BabA and other adhesins are important to the bacterium, since any *H. pylori* cell that does not adhere to the epithelial cell/layer would be quickly removed from the epithelial layer and the mucus gel (Huang et al. 2016). In addition the BabA-LeB interaction which is resulted in adherence and initial colonization to the stomach surface, it also helps in anchoring and insertion of bacterial factors in host cell cytosol such as CagA and VacA (Kusters et al. 2006; Waskito et al. 2018; Sweeney and Guillemin, 2016). This leads to signaling process, since the injected type IV secretion system (TFSS) will result in induction of transcription of genes that enhance many associated activities related to pathogenicity Ishijima et al. (2011).

On the other hand, due to tremendous advances in 3D structure estimation of proteins, and the rapid growing field in the in-silico drug discovery, these approaches can be exploited to find out inhibitors/drugs for many virulence factors. In this aspect Structure-Based Drug Design (SBDD) which is an in-silico method can be used during the early stages of drug discovery or design Garyfallia et al. (2018).

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Choosing the target is quite essential for SBDD, the target should be essential and is a part of life cycle of the cell, targeting bacterial adherence can form of an effective nonantibiotic treatment (Cusumano et al. 2011; Spaulding et al. 2017), BabA represents a good and promising target as it is found at the out borders of cells and is unique for H. pylori. The aim of this work was to find chemical inhibitors to BabA of H. pylori for future development of anti-Helicobacter agents.

MATERIALS AND METHODS

Different databases and software were used, for different purposes:

DATABASES

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.

SOFTWARE

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 character of molecules.

T.E.S.T. software: https://www.epa.gov/
Toxicity-Estimation-Software tool-Test , to find out the safety of molecules.

PyRx software v.8: https://pyrx.sourceforge.io/
Used for molecular docking.

PyMOL software: https://pymol.org/2/
Used for docking visualization.

Discovery Studio Visualizer
Used for docking visualization.

RESULTS AND DISCUSSION

BabA is important for initial colonization of H. pylori (Waskito et al. 2018; Moonens et al. 2016; Prinz et al., 2001; Ilver, 1998), its binding affinities (Kd=10^-7-10^-12 M^-1), which means that it is greater than most carbohydrate binding proteins (Aspholm-Hurtig et al. 2004; Imberty et al. 2005). Adherence of the bacteria to cell receptors offers several advantages to the bacteria, such as protection from washing out during mucus shedding, getting nutrients from damaged host epithelial cells, promotes delivery of bacterial toxins and effector molecules such as CagA oncoprotein, VacA and others helping in development of pathogenicity and persistence infections (Kable et al. 2017; Odenbreit, 2005; Rhen et al. 2003; Aspholm et al. 2006). And since the transmission thought to take place through the oral route, then the possible initial attachment could be occurred with salivary proteins as it has been found that BabA mediates the attachment by binding with difucosylated glycans found on LeB antigens of salivary mucin MUC58 serving as a receptor of BabA (Walz et al. 2009; Prakobphol et al. 2005).

The drug target should be characterized with some criteria such as to be essential to the pathogen, in addition, it should be unique; this was explained by BLASTing the BabA sequence (pdb ID 4zh0) using BLAST algorithm and non-redundant database, the results showed that this protein is restricted to H. pylori. The retrieved crystal structure of BabA (pdb ID 4zh0) Hage et al. (2015) was found to be acceptable for drug design purposes. The recommended and acceptable resolution of crystal structure of protein is 2.5 ÅO, the resolution of 4zh0 is 1.91 ÅO, other criteria required are R factor which should be below 25%, this value for 4zh0 is 0.145, in addition, the free recommended values is below 28% and ideally below 25%, for the protein 4zh0 this value is 0.14, the other criteria concerning coordinate errors are low and satisfy the crystal structure to be drug target, finally 97.5% of the protein residues fall in favored region of Ramachandran plot (Anderson, 2003).

The target protein should be druggable with binding pockets, this was demonstrated using DoGsiteScorer (Volkamer et al. 2012), the results shown in Figure 1.

The protein shows different binding pockets with drug score extended from (0.24-0.8), this means that the protein can bind different ligands that complementary to its pockets which might lead to total modulation of its activity and death of the pathogen (Anderson, 2003; Volkamer et al. 2012; Yuan et al. 2013).

In this study SBDD was used which comprises several steps, the initial virtual screening was carried out using MTTOpenScreen server, this allows performing docking of ligands in suitable pocket using blind docking via AutoDock 4.2 and automated virtual screening with AutoDock vina (Labb’ et al. 2015; Lagarde et al. 2019).
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**Figure 1:** Pockets of BabA protein (pdb ID 4zh0) and druggability and providing starting collections, the web site has different databases to be screened. Such servers give the ligands with the best score concerning affinity and specificity, the resulted ligands then subjected for optimization for different aspects (Dhanik and Kavraki, 2012).

In this study the top 100 molecules of diverse-library were used, these were checked and filtered for different criteria, since the molecules with high score could fail in different assays or with other characters. BabA is related to stomach site, so it would be preferred to evaluate for orally bioavailability using Lipinski rule of five (Lipinski *et al.* 1997), in addition to check the ADME characters (Wanga *et al.* 2018), and different types of toxicity, synthetic accessibility of resulted ligands. The primary study checked mutagenicity and teratogenicity using T.E.S.T. software and LAZAR software, hREG was also estimated, only 6 molecules satisfied most criteria, shown in Table 1.

<table>
<thead>
<tr>
<th>Name</th>
<th>Volume Å³</th>
<th>Surface Å²</th>
<th>Drug Score</th>
<th>Simple Score</th>
<th>Additional information</th>
</tr>
</thead>
<tbody>
<tr>
<td>P_0</td>
<td>559.42</td>
<td>1900.91</td>
<td>0.1</td>
<td>0.31</td>
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</tr>
<tr>
<td>P_1</td>
<td>522.12</td>
<td>715.64</td>
<td>0.26</td>
<td>0.3</td>
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</tr>
<tr>
<td>P_10</td>
<td>170.64</td>
<td>148.59</td>
<td>0.36</td>
<td>0.0</td>
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<td>P_11</td>
<td>168.12</td>
<td>412.22</td>
<td>0.27</td>
<td>0.0</td>
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</tr>
<tr>
<td>P_12</td>
<td>164.43</td>
<td>220.87</td>
<td>0.28</td>
<td>0.01</td>
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</tr>
<tr>
<td>P_13</td>
<td>154.56</td>
<td>299.85</td>
<td>0.26</td>
<td>0.0</td>
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</tr>
<tr>
<td>P_14</td>
<td>145.62</td>
<td>315.35</td>
<td>0.38</td>
<td>0.0</td>
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</tr>
<tr>
<td>P_15</td>
<td>159.67</td>
<td>325.66</td>
<td>0.36</td>
<td>0.0</td>
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<tr>
<td>P_16</td>
<td>132.49</td>
<td>314.06</td>
<td>0.27</td>
<td>0.0</td>
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<tr>
<td>P_17</td>
<td>100.89</td>
<td>182.28</td>
<td>0.39</td>
<td>0.0</td>
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<tr>
<td>P_18</td>
<td>109.02</td>
<td>235.91</td>
<td>0.19</td>
<td>0.0</td>
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<tr>
<td>P_2</td>
<td>461.24</td>
<td>601.95</td>
<td>0.7</td>
<td>0.21</td>
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<tr>
<td>P_3</td>
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<td>719.15</td>
<td>0.78</td>
<td>0.18</td>
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<tr>
<td>P_4</td>
<td>298.18</td>
<td>345.78</td>
<td>0.63</td>
<td>0.06</td>
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<tr>
<td>P_5</td>
<td>250.02</td>
<td>465.76</td>
<td>0.5</td>
<td>0.03</td>
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<td>P_6</td>
<td>248.44</td>
<td>313.2</td>
<td>0.57</td>
<td>0.05</td>
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<tr>
<td>P_7</td>
<td>244.86</td>
<td>260.69</td>
<td>0.63</td>
<td>0.09</td>
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<td>P_8</td>
<td>235.93</td>
<td>321.88</td>
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<td>0.07</td>
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<td>P_9</td>
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<td>275.24</td>
<td>0.24</td>
<td>0.01</td>
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Table 1: Some characters of selected ligands

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<tr>
<th>Compound #</th>
<th>Mutagenicity</th>
<th>Teratogenicity</th>
<th>hERG</th>
<th>GI absorption</th>
<th>BBB</th>
<th>Pgp</th>
<th>bioavailability</th>
<th>Synthetic Accessibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>24827103</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>No</td>
<td>No</td>
<td>0.55</td>
<td>2.81</td>
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<tr>
<td>3712259</td>
<td>-</td>
<td>-</td>
<td>high</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>0.55</td>
<td>3.13</td>
</tr>
<tr>
<td>24833135</td>
<td>-</td>
<td>-</td>
<td>high</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>0.55</td>
<td>2.31</td>
</tr>
<tr>
<td>26535128</td>
<td>-</td>
<td>-</td>
<td>high</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>0.55</td>
<td>3.57</td>
</tr>
<tr>
<td>51087930</td>
<td>+</td>
<td>-</td>
<td>high</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>0.55</td>
<td>2.26</td>
</tr>
<tr>
<td>85149061</td>
<td>-</td>
<td>-</td>
<td>high</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>0.55</td>
<td>2.28</td>
</tr>
</tbody>
</table>

Other physico-chemical characters of the selected molecules are shown in Table 2.

Table 2: Physico-chemical properties of selected ligands

<table>
<thead>
<tr>
<th>Compound #</th>
<th>MW</th>
<th>nRot</th>
<th>LeadLike</th>
<th>HBA</th>
<th>HBD</th>
<th>logP</th>
<th>TPSA</th>
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<tbody>
<tr>
<td>24827103</td>
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<td>Yes</td>
<td>5</td>
<td>1</td>
<td>2.15</td>
<td>96.26</td>
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<td>51087930</td>
<td>265.6525</td>
<td>4</td>
<td>Yes</td>
<td>6</td>
<td>0</td>
<td>2.31</td>
<td>80.71</td>
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<tr>
<td>3712259</td>
<td>291.3256</td>
<td>4</td>
<td>Yes</td>
<td>6</td>
<td>1</td>
<td>0.56</td>
<td>103.34</td>
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<tr>
<td>24833135</td>
<td>253.3174</td>
<td>6</td>
<td>Yes</td>
<td>4</td>
<td>2</td>
<td>1.83</td>
<td>94.53</td>
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<tr>
<td>85149061</td>
<td>240.2988</td>
<td>8</td>
<td>Yes</td>
<td>5</td>
<td>1</td>
<td>1.42</td>
<td>73.56</td>
</tr>
</tbody>
</table>

Docking Studies

Molecular docking is a decisive step of SBDD, which helps in visualizing the interaction pattern and binding energy of receptor-ligand complexes, this could be carried out atom-by-atom or fragment-by-fragment (Wanga et al. 2018; Verlinde and Hol 1994). Therefore, it is considered as a key of success of placing/docking the ligand in the binding pocket of the target. Docking studies of this work used AutoDock vina implemented in PyRx package using Force-field based scoring function and the binding affinity (kcal/mol) is calculating through the sum of non-bound interactions such as electrostatics and van der Waals in addition to hydrogen bonds, solvents, and entropy contributions with each conformational states of ligands (Wanga et al. 2018; Huang and Zou, 2010; Kapetanovic ,2008; Carlson and Jorgensen, 1995). The binding affinity of selected ligands with BabA (pdb ID 4zh0) are shown in Table 3.

Table 3: Binding affinity of docked ligands. RMSD of zero

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Binding Affinity (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3712259</td>
<td>-5.5</td>
</tr>
<tr>
<td>24827103</td>
<td>-6.5</td>
</tr>
<tr>
<td>24833135</td>
<td>-5.4</td>
</tr>
<tr>
<td>26535128</td>
<td>-4.4</td>
</tr>
<tr>
<td>51087930</td>
<td>-5.8</td>
</tr>
<tr>
<td>85149061</td>
<td>-4.9</td>
</tr>
</tbody>
</table>

These values were for best mode at RMSD (Root-Mean Squared Deviation) values of zero, as in terms of docking accuracy, a threshold of 1.0-3.0 Å RMSD between the docked and X-ray pose has been generally considered to be a "successfully" docked structure (Halperin et al. 2002; Muegge and Rarey, 2001; Abagyan and Totrov, 2001; Taylor et al. 2002); the visualization of docked ligands are shown in Figure 2.
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Figure 2: Docking results of studied ligands with BabA protein (pdb ID 4zh0)

It is known that binding site can be active site as in enzymes or may be any other site such as communication site necessary in the metabolism, with concave surface on the protein that can accommodate drug molecules, this can induce conformational changes upon binding to a protein (Erickson et al. 2004; Anderson, 2003). So the potential drug target are not necessarily disease causing but must by definition be disease modifying as with BabA protein.

Generally, SBDD has emerged as promising tool for drug industry following by ligands optimization. On the other hand, suggesting anti-adhesive molecules cannot cure the acute infection, but might be used after eradication treatment to inhibit recurrence by preventing recolonization of the stomach, as they interact with surface protein of *H. pylori* and would work by clearing the bacterium out of the stomach through dislodging the bacterium off the stomach wall as in using BabA inhibitors. Such strategies are necessary to help in *H. pylori* eradication since BabA is unique in this bacterium, and the inhibitors will not affect normal flora Messing et al. (2014).
The suggested molecules are:

**IUPAC Recommended Name**

- **24827103**: N-[4-(pyridin-2-yl)-1,3-thiazol-2-yl]furan-2-carboxamide

- **51087930**: {1-[2-(4-chlorophenyl)-2-oxoethyl]-1H-imidazol-4-yl}dihydroxyaminyl

- **3712259**: 2-[[1-hydroxy-1,4-dihydropyridin-2-yl)sulfonyl]-N-(1-methyl-1H-pyrrole-2-carbonyl)acetamide

- **24833135**: (2R)-4-(methylsulfonyl)-2-(phenylformamido)butanoic acid

- **85149061**: 2-(4-amino-2-oxo-1,2-dihydropyrimidin-1-yl)-N-(3-methoxypropyl)acetamide

- **26535128**: propyl 2-(aminomethyl)-3-(((2R)-2-hydroxybutyl)[methyl]amino)prop-2-enoate

The chemical synthesis seems to be possible (see Table 2), the enhancement of molecule action could be improved through scaffold hopping when required. Most of the molecules are with limitation of Lipinski rule of five Wanga et al. (2018), which is necessary for oral drugs as in the case of *H. pylori* which resident in the stomach, except the molecule (# 26535128) with high rotatable bonds (8 rotatable bonds), this makes the molecule highly flexible and might bind to off-target, in addition this high number of bonds was found to decrease the docking accuracy (Verlinde and Hol 1994; Erickson et al. 2004).

Finally, inhibition of BabA might be possible and promising for future pharmaceutical and clinical treatment of *H. pylori*.

**REFERENCES**


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Accepted 16 May 2020


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