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Pharmacophore Modeling of Furanosyl Borate Diester: An Attempt to Combat Antibiotic Resistance Induced *Helicobacter pylori* Quorum Sensing Mediated Peptic and Duodenal Ulcer

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Abstract

Helicobacter pylori is a gram-negative, microaerophilic bacterium found usually in the stomach of a person with chronic gastritis and gastric ulcers. More than 50% of the world's population harbor *H. pylori* in their upper gastrointestinal tract. About 85% of people infected with *H. pylori* never experience symptoms or complications. Individuals with chronic gastritis and infected with *H. pylori* have a 10 to 20% lifetime risk of developing peptic ulcers, MALT lymphoma, the pathogenesis of gastric cancer and several extra-gastric diseases. No vaccines are developed yet and the bacterial antibiotic resistance has been a growing concern. *Helicobacter pylori* produce virulence and antibiotic resistance through quorum sensing mechanisms by generating AI-2. Inhibition of quorum sensing would be a novel approach for the effective treatment of antibiotic-resistant strains of *H. pylori*. Chemical nature of AI-2 is furanosyl borate diester which is generated from 4,5-dihydroxy 2,3-pentanedione (DPD). But there are no synthetic congeners of AI-2 and DPD compounds tested against *H. pylori* till date. Therefore, it is the aim of the present study to design some potent AI-2 and DPD compounds under the framework of pharmacophore modeling.

Keywords: *Helicobacter pylori;* Quorum sensing; Peptic and duodenal ulcer; Autoinducer-2; 4,5-dihydroxy-2,3-pentanedione; Pharmacophore modeling

Introduction

Since long antiquity, spiral-shaped bacteria was found in the human stomach lining in the year 1875 [1,2]. The Italian researcher Giulio Bizzozero described similarly shaped bacteria living in an acidic environment of the stomach of dogs in 1893 [3]. The bacteria were also being observed in 1979 by Robin Warren, who was unable to culture the same. In collaboration with Warren, Barry Marshall attempted repeatedly and finally got success to colonies the bacteria in 1982 [4]. *Campylobacter pylori* were discovered as *Helicobacter pylori* which is a gram-negative, helix-shaped bacterium having a dimension of about 3 μm long with a diameter of 0.5 μm. It is a very popular bacteria present in the gastrointestinal tract can attack the internal liner membranes of the G.I. tract in case of chronic gastritis patients [5]. In October 1987, a group of scientists attempted to conduct Annual International Workshop on *Helicobacter* and Related Bacteria and European Consensus on the management of *H. pylori* in collaboration with European *Helicobacter* Study Group (EHSG), an international multidisciplinary research group and the only institution focused on *H. pylori* [6-8]. In 1987, the Sydney gastroenterologist Thomas Borody invented the first triple therapy for the treatment of duodenal ulcers [9,10]. In 1994, the National Institutes of Health stated most recurrent duodenal and gastric ulcers were caused by *H. pylori* and recommended antibiotics be included in the treatment regimen [11].

Chronic *H. pylori* infection is majorly responsible for producing stomach and duodenal ulcers and further, it may lead to cancer [12]. In recognition of their discovery, Marshall and Warren were awarded the Nobel Prize in Physiology or Medicine in 2005. Several samples were taken for biopsies. Signs of inflammation were always present in the gastric mucosa close to where the bacteria were seen. It was now firmly established that *H. pylori* cause more than 90% of duodenal ulcers and up to 80% of gastric ulcers [13]. *H. pylori* have many dangerous components such as flagella, urease lipopolysaccharide, vacuolating cytotoxin A (VacA), CagA, etc which are responsible for damaging the mucosal layer (Figure 1). Flagella help in chemotaxis motility and drilling into the G.I. mucosal layer. Urease can produce ammonia which neutralizes gastric environment. Lipopolysaccharides adhere to the host cell. It produces mucinase, protease, and lipase which can damage the gastric mucosal layer. It also provides vacuolating toxin (Vac A) which is toxic to the gastric mucosal system. It has pilli-like injector device (Type IV secretion system) which injects effectors responsible for producing IL-8 and Cag A that stimulate the inflammatory response of host stomach's epithelial cells and cause mucosal damage.

Further, the Cag A protein is translocated into the host cell membrane where it was shown to be tyrosine phosphorylated and stimulate the overexpression of eukaryotic signal transduction pathways. That may contribute to the pathogenesis of the gastric malignancies such as mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma worldwide [14-16].

Current therapy still relies on a different combination of known antibiotics and anti-secretory agents. A standard triple therapy consisting of two antibiotics such as clarithromycin and amoxicillin and a proton-pump inhibitor such as (omeprazole, pantoprazole or rabeprazole) is proposed as the first-line regimen. Amoxicillin could be replaced

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with metronidazole for people who are allergic to penicillin. Bismuth-containing quadruple treatment, sequential treatment or a non-bismuth quadruple treatment (concomitant) are also an alternative therapy. Levofloxacin containing triple treatment are also recommended as rescue treatment for infection of *H. pylori* after the defeat of first-line therapy. The rapid acquisition of antibiotic resistance reduces the effectiveness of any regimens involving these remedies [17].

It was observed that *H. pylori* opt quorum sensing mechanism for producing antibiotic resistance. *Helicobacter pylori* is a Gram-negative gastric pathogen. Gram-negative bacteria release extra-cellular signaling molecules (also termed as auto-inducers) to perform intercellular communication. It is generally assumed that auto-inducers are employed to regulate aspects of bacterial behavior in response to cell population density so-called quorum sensing. This includes changes in the expression of genes crucial for bacterial survival or virulence. *H. pylori* can produce autoinducer-2 (AI-2) which is a quorum-sensing signal molecule. It is produced by the LuxS protein that accumulates in the bacterial environment in a density-dependent manner (Figure 2). Through this AI-2 mediated quorum sensing mechanism, *H. pylori* can produce virulence and antibiotic resistance because the antibiotic is unable to dissolve the micro dense barricade of bacterial QS molecules. This barricade is also called as biofilm which is very crucial for the environmental survival and also for the successful infection [18,19]. The results of scanning electron microscopy revealed that the bacterial strain endowed with a strong ability of biofilm production on the gastro mucosal epithelial layer.

Rader and co-workers experimented that *H. pylori* perceive LuxS-produced AI-2 mediated quorum sensing as a chemo-repellent *via* the chemoreceptor TlpB [20]. However, deletion of the *luxS* gene in mutants lacks any of three key flagellar regulatory genes such as the sensor kinase *flgS*, the flagellar sigma factor *fliA*, and its anti-sigma factor, *flgM*. Therefore, the regulation of flagellar morphology is mediated by this extracellular signal molecule which may cause flagellar gene expression in *H. pylori* [21].

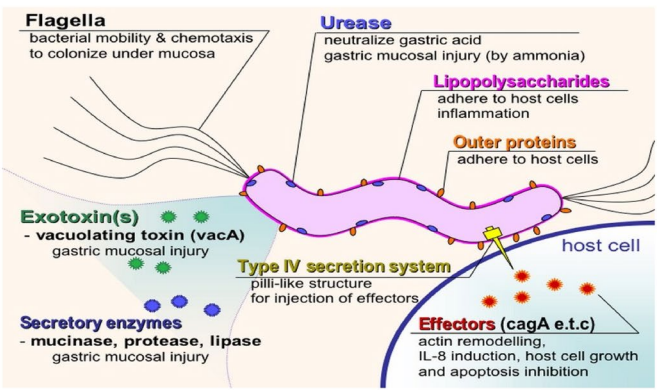

Figure 1: *H. pylori* bacterial components.

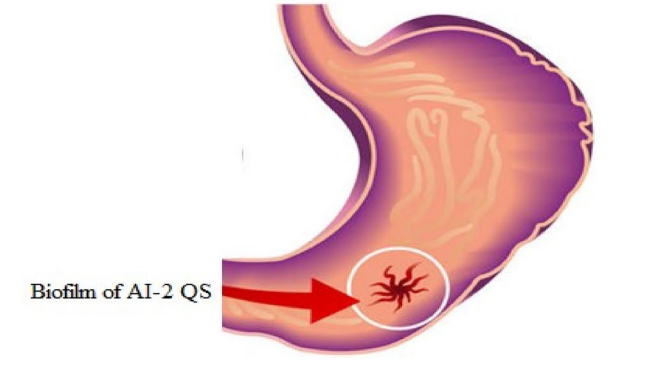

Figure 2: *H. pylori* biofilm produced by quorum sensing.

Therefore, it has been assumed that AI-2 congeneric compounds could be designed and used along with the triple therapy to inhibit quorum sensing.

Methods of Pharmacophore Design

Chemically AI-2 is furanosyl borate diester. *H. pylori* synthesize AI-2 from 4,5-dihydroxy 2,3- pentadione (DPD) and homocysteine. DPD forms a cyclic molecule which acts as AI-2 precursor. Addition of naturally occurring borate to an AI-2 precursor generates active AI-2. A number of microorganisms potentially generate boron for the induction of the QS mechanism [22,23]. AI-2 has an affinity to LsrR (LuxS regulated transcriptional regulator) target for producing quorum sensing mechanism and gene expression (Figure 3) [24].

It is assumed that congeneric AI-2 and DPD synthetic compounds may inhibit LuxS. But there is no synthetic AI-2 and DPD congeneric molecules derivatized and tested against *H. pylori*. It could be done using pharmacophore modeling. Therefore, it is our target to design pharmacophores of AI-2 and DPD to focus crucial features responsible for the design and synthesis of congeneric AI-2 and DPD compounds.

The structure of furanosyl borate diester and 4,5 dihydroxy 2,3 pentadione (DPD) derivatives were drawn using 2D ChemDraw. The drawn structures were then converted into 3D modules and the geometries were fully optimized using MM2 force field considering the default conversion procedure implemented in ChemDraw 3D ultra [25]. Pharmacophore models of furanosyl borate diester (AI-2) and 4,5-dihydroxy 2,3-pentanedione (DPD) derivatives were generated by using Portable InteLigand LigandScout 2.02 [26]. A pharmacophore is a structure which is based on the measurement of three-dimensional structural features necessary to be cleft in the target cavity. IUPAC defines a pharmacophore to be an ensemble of steric and electronic features that is necessary to ensure the optimal supra-molecular interactions with a specific biological target and to trigger (or block) its biological response. Common 3D features include hydrophobic, charged, or hydrogen bond features and may include additional information, such as the direction of hydrogen bonds. A pharmacophore model explains how structurally diverse ligands can bind to a common receptor site. Furthermore, pharmacophore models can be used to identify through de novo design or virtual screening novel ligands that will bind to the same receptor. It helps to identify the mode of binding of structurally diverse compounds bearing common binding mode towards the receptor site [27].

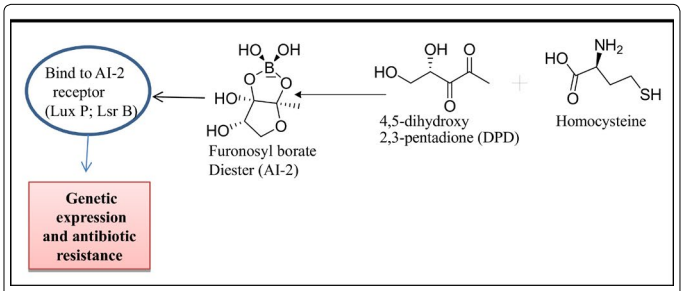

Figure 3: Biosynthesis of AI-2 QS molecule [24].

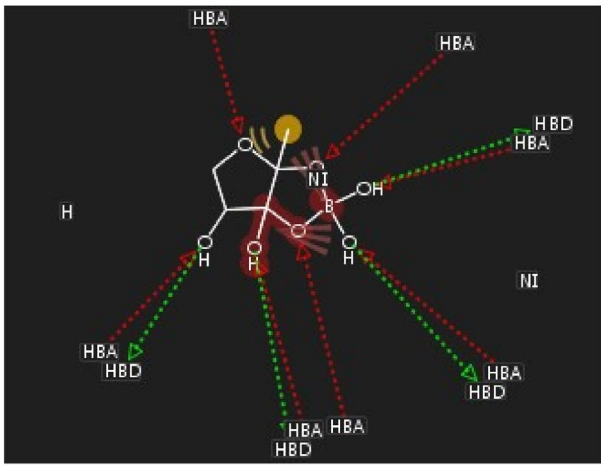

Figure 4: Pharmacophore model of AI-2.

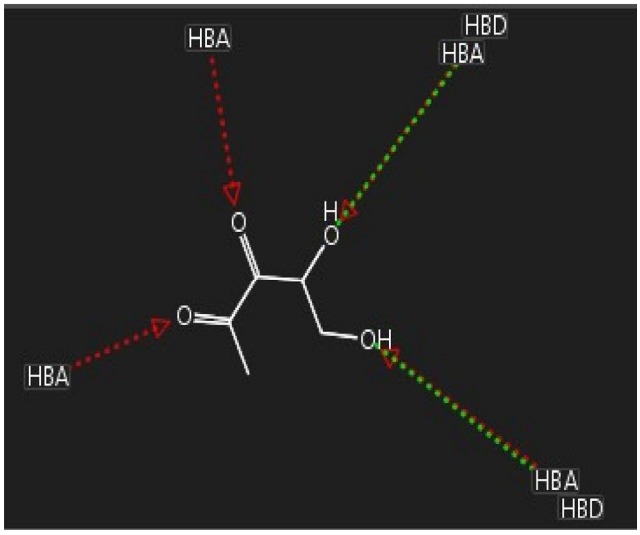

Figure 5: Pharmacophore model of DPD.

Results and Discussion

The identified maximum number of features which are common to all active compounds but excluded from the inactive compounds within the conformationally allowable region of space is helpful for lead optimization through the virtual screening of structurally diverse hit compounds [28,29]. In the present study, pharmacophores (Figures 4 and 5) of AI-2 and DPD have been generated by LigandSCOUT software which predicted HBAs (red arrow), HBDs (green arrow) and negative ionization.

Pharmacophore model of AI-2 molecule shows that the -OH groups produce hydrogen bond interaction. O atoms of furan can produce hydrogen bond acceptor (HBA). -OH groups of Dioxane can produce hydrogen bond interaction. Boron atom produces negative ionization. Methyl group produces hydrophobicity. The fused angle containing O-C-OH produces negative ionization. A pharmacophoric feature of DPD has been given as follows: 2,3 dione produce hydrogen bond accepting properties, 4,5 Dihydroxy produce both hydrogen bond acceptor and hydrogen bond donor properties

Conclusion

Eradication of *H. pylori* is important not only for the treatment of gastric/duodenal ulcer but also for the treatment and prevention of *H. pylori*-associated diseases such as gastric cancer, as well as for inhibiting the spread of this microorganism. Combination therapy including AI-2 inhibitor could be designed for the complete eradication of *H. pylori*. Synthetic AI-2 congeneric molecules could be designed through pharmacophore modeling approach. These derivatives compounds may act as competitive inhibitors to inhibit AI-2 mediated quorum sensing. -OH groups of AI-2 and DPD could be substituted by -SH to produce congeneric molecules of AI-2 and DPD because -SH is a bioisostere of the same which may also produce hydrogen bond interaction. This proposed molecule could be synthesized and tested to combat antibiotic resistance induced *Helicobacter pylori* quorum sensing mediated peptic and duodenal ulcer.

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