Chapter VI

*In silico* study of curcumin inhibitory potential in comparison to conventional drugs against CagA oncoprotein of *Helicobacter pylori*

Introduction

About fifty percent population of the world are infected with *Helicobacter pylori*, having with severe gastritis, peptic ulcer, and after prolongation finally developed into cancer (Peek et al., 2002). The most extensive virulent factors harbour the cag pathogenicity island (cag-PAI), and its 40-kb DNA fragments encodes the cytotoxin CagA and a type IV secretion system (cag-T4SS). The virulent factor CagA is exceptionally found in *Helicobacter pylori*. The pathogenic activity of CagA oncoprotein has been established at the time of cytotoxin expression in developing gastric polyps and adenocarcinoma in transgenic mice (Ohnishi et al., 2008). Firstly, *Helicobacter pylori* adhesions with the gastric epithelial cells, then transports CagA protein through route the cag-T4SS, and needle-like molecular appendage spanning both bacterial membranes prolonged by a large extracellular pilus (Odenbreit et al., 2000; Rohde et al., 2003). The transportation of CagA is mediated by other protein, like CagL, CagY, and CagA of the *Helicobacter pylori*, which decorate cag-T4SS pilus and use α5β1 integrin at focal adhesions as a receptor for CagA delivery (Jiménez-Soto et al., 2009; Terradot and Waksman, 2011). Once injected inside the membrane, CagA localises at the inner leaflet of the plasma membrane and activates itself with multifaceted attack on host cell signaling. At adherence junctions, CagA forms complexes with E-cadherin a cytoskeleton proteins (Murata-Kamiya et al., 2007; Oliveira et al., 2009). After screening of soluble fragment throughout (Angelini et al., 2009) and analysis in vivo proteolysis data, CagA has been shown as protein consisting of two functional domain (Odenbreit et al., 2001). Much attention has been paid toward the biological function of the C-terminal domain (residues 885-1,186). In fact Pro-Ile-Tyr-Ala (EPIYA) motifs have been tyrosine phosphorylated by Src and Abl kinases in eukaryotic cells (Kaplan-Turkoz et al., 2012). In later stage of
infection, phosphorylated CagA binds and activates Src homology 2 domain phosphatase (SHP2) via SH2 domains, leading to dephosphorylation and inactivation of Src family kinases, resulting in morphological transformation and dramatic cytoskeleton rearrangement (Hatakeyama, 2004). Other pathogenic activity of CagA are phosphorylation independent. In particular, specific sequences MKI located in the CagA C terminus check the Par1b/MARK2 kinase activity to mimic the enzyme’s natural substrate (Nesić et al., 2010). The inhibition of the Par1b/MARK2 perturbed atypical PKC signaling, that disrupts the tight junction as well as loss the cell polarity (Saadat et al., 2007). This has been indicated that the N-terminal portion of CagA (residues 1-884, CagA1-884) interacts with several intracellular factors, such as apoptosis-stimulating protein of p53-2 (ASPP2) (Buti et al., 2011), RUNX3 (Tsang et al., 2010), TAK1 and TRAF6 (Lamb et al., 2009), and thus plays an important role in the development of gastric cancer. CagA is a remarkable versatile oncoprotein of H. pylori which interact with a plethora of host signaling factors for promoting gastric cancer. The structure of CagA1-884 has clear definition of the domain organization, N-terminal region of CagA covering about two-thirds of the CagA polypeptide chain. The flexible nature of N-terminal portion of CagA interact with intracellular factors, whereas C-terminal region of CagA was found in disordered. Nevertheless, both in vitro and in vivo proteolysis studies showed that the protein is rather unstable and heavily prone to degradation (Angelini et al., 2009). This intrinsic flexibility indicate an important character of CagA for interactions with drugs like curcumin.

Several drugs have been recommended to Helicobacter pylori infected patients, of them clarithromycin, amoxicillin, metronidazole, and pantoprazole, are currently usedas sequential therapy, concomitant therapy, and triple therapy (Hsu et al., 2014). The limitations of the current therapy are the lack of therapeutic compliance, producing side effects and discomfort the multiple doses (Armuzz et al., 2001, Patel and Patel, 2007) and these factors may also lead to the development of antibiotic resistance (Armuzz et al., 2001). The degradation of antimicrobial agents such as amoxicillin and clarithromycin also occurred in stomach by the presence of gastric acid (Bardonnet et al., 2008).
On the above circumstances, if it is used in higher doses may reflect an increase of gastrointestinal side effects, like diarrhoea, nausea, vomiting, bloating, and abdominal pain (Armuzz et al., 2001). Another important reason is the antibiotic resistance that \textit{H. pylori} has been developing, for instance the resistance to metronidazole has reached around 40\% in developed countries and exceeded to 90\% in developing countries (Obonyo et al., 2012). The rate of resistance of \textit{H. pylori} to conventional drugs is increasing due to continuously use of antibiotics worldwide for example CagA+ \textit{H. pylori} resistance to metronidazole and clarithromycin. Simultaneously, multi drug resistant (MDR) strains that are becoming resistant to multidrug like amoxicillin, metronidazole, clarithromycin, and pantoprazole (Boyanova et al., 2008; Wueppenhorst et al., 2009). In view of the incomplete cure achieved with conventional therapy due to development of resistant strains. Other reasons regarding its uses are undesirable side effects (Myllyluoma et al., 2005), noncompliance among the patients (Broutet et al., 2003), the cost of the antibiotic regimens (Wong et al., 2003), and other factors contributing to ineffectiveness. Therefore, there is an urgent need to develop new treatment strategies for \textit{H. pylori} infection by finding the possible alternative drugs.

With reference to above, curcumin (diferuloylmethane), a yellow pigment is a major component of turmeric (\textit{Curcuma longa}) and is commonly used as a spice and food-coloring agent. The typical structural features of curcumin consist two o-methoxy phenol units, two enol moieties and a 1, 3- diketone-1, 3- keto-enol system that suppose to act as key factor for the designing and development of new and improved anti-inflammatory agents (Bukhari et al., 2013, 2014). Many in vitro and in vivo studies using animal models and human, found that curcumin was extremely safe even at higher doses (Anand et al., 2008). Potentiality of curcumin can open a new alternative in development of modern medicines for the treatment of various ailments (Chattopadhyay et al., 2004). In order to improve the pharmacological properties of curcumin, it was conjugated with various functional groups. For instance, curcumin-amino acids conjugates were also synthesised using different substitution schemes, that were also recognised as an antioxidant, antimicrobial,
antiviral, antiproliferative, and proteasome inhibition activities (Singh et al., 2010).

It has been reported that even low concentrations of curcumin is capable to downregulate *H. pylori*-induced AID (activation-induced cytidine deaminase) expression due to inhibition of NF-kβ in *H. pylori*-infected MKN-45 gastric cancer cells (Zaidi et al., 2009). Further, it has also been described that the elevated levels of MMP-3 and -9 in gastric tissues of mice or cultured cells infected with *H. pylori* strains (either cag +ve or cag-ve) inhibited by curcumin treatment. Curcumin mediated downregulation of MMP-3 and -9 levels in *H. pylori*-infected mice and cultured cells indicated immense therapeutic potential against Hp associated gastrointestinal diseases (Kundu et al., 2011). Another pathogens for example *Vibrio vulnificus*, a halophilic estuarine bacterium, causing fatal septicemia and necrotic wound infections (Oliver, 2005) had been also inhibited by curcumin. It also inhibits the cytotoxicity and bacteriostatic activity significantly in a dose-dependent manner in *V. Vulnificus* (Na et al., 2011) and also responsible for reduction of MDR (multi drug resistant) *Pseudomonas aeruginosa* isolates causing pneumonia (Negi et al., 2014).

In present scenario, computer-aided drug design is becoming one of the most effective methods for developing new drugs (Ma et al., 2014) and also widely applied to pharmacology hypothesis development and testing. Computational study includes database searching, quantitative structure-activity relationships, similarity searching, pharmacophore identification, computational modelling, and docking. Such methods have been frequently used in the discovery and optimization of novel molecules with affinity to a target, and the clarification of absorption, distribution, metabolism, excretion, and toxicity properties (Ekins et al., 2007). Presently, curcumin have been the subjects of computational studies, predominantly with the purpose of unravelling its exclusive structural characters and for taking advantage in getting information for further molecular design. Here, we have performed computational study to investigate the inhibitory potential of curcumin against *Helicobacter pylori* targeting CagA oncoprotein and compared with conventional drugs (clarithromycin, amoxicillin, metronidazole, and pantoprazole).
Material and Methods

Selection of compounds and proteins

The traditional Indian medicine curcumin (PubChem id: CID_969516) and synthetic medicines (Clarithromycin PubChem id: CID_84029, Amoxicillin PubChem id: CID_33613, Pantoprazole PubChem id: CID_4679, Metronidazole PubChem id: CID_4173) were downloaded in SDF (standard data file) file from the database PubChem (www.pubchem.ncbi.nlm.nih.gov) for computational study. For molecular docking analysis, the SDF file of each compound was converted into PDB (protein data bank) and analysed 3D-structure of compounds using software UCSF Chimera (Saha et al., 2013) (Fig. 16).

The crystal structure of cytotoxine associated gene A protein of \textit{H. pylori} 26695 has been reported in the literature which possess different confirmations (Kaplan-Turkoz et al., 2012). The protein structures file of CagA (PDB ID: 4VDZ) containing resolution 3.19 Å was retrieved from PDB (protein data bank) (www.rcsb.org). Amino acids length was 1,186 (CagA1-884 N-terminal, CagA885-1,186 C-terminal), and its mass was 120 kDa (Fig. 17).

ADME and Druglikeness Analysis

Molecular properties such as membrane permeability and bioavailability of leading compound are always associated with some basic molecular descriptors such as logP (partition coefficient), molecular weight (MW), or number of hydrogen bond acceptors and donors in a molecules (Ertl et al., 2000). These molecular properties were used in formulating “rule of five” (Lipinski et al., 1997). Lipinski’s rule states, that most molecules with good membrane permeability have MW ≤ 500, hydrogen bond donors ≤ 5 and acceptors ≤ 10. Therefore, Lipinski’s Rule of Five was used to test the bioavailability characteristics such as absorption, distribution, metabolism, and elimination (ADME) of the lead compounds. In the present study, these molecular properties and Druglikeness score for each leading compounds were estimated by using Molinspiration tool (Sirajuddin et al., 2015).

Molecular Docking of compounds
The molecular docking experiment was accomplished to investigate the binding mode of leading compounds (curcumin, clarithromycin, amoxicillin, pantoprazole, and metronidazole) with CagA protein, especially the binding sites of the receptor. The conservative residues and active sites in CagA oncoprotein was identified and calculated by Bayesian method using ConSurf web server (Landau et al., 2005).

Docking study of curcumin and conventional drugs was performed using PatchDock online server (Ramakrishna et al., 2013). The Clustering RMSD was 4.0 Å and the complex type was set to default. The number of solutions with their score, area, and six dimensional transformation space were obtained. PatchDock provided results which were ranked according to a geometric shape complementarily score after molecular shape representation and surface patch matching. These results were used for further refinement and re-scoring of 1000 top scoring complexes using FireDock (Andrusier et al., 2007). The top ten scoring molecules of each crystal structure were refined automatically by FireDock and represented the energy involvement in complex formation as global binding energy, attractive van-der Wall, and hydrogen bond energies. Each complexes of FireDock was ranked on the basis of minimum global binding energy. As next step, Chimera 1.8.1. (Saha et al., 2013) for surface view and Discovery Studio 4.0 Client (Talambedu et al., 2014) for determining the mode of interaction between the receptor and ligands were used.

**Results**

*Analysis of physicochemical pharmacokinetics properties*

ADME properties of lead compounds were estimated by using online data server Molinspiration. The obtained results were based on Lipinski’s five rule which showed that all leading molecules except clarithromycin (MW > 500, hydrogen bond acceptors > 10) have zero violations of Rule of 5 and suggests the molecules (curcumin, amoxicillin, pantoprazole, and metronidazole) have good bioavailability (Table 8).

The druglikeness score predicted the compounds properties in formulation as drug was assigned with the combination of GPCR, ion channel modulator, kinase inhibitor, nuclear receptor ligands, protease inhibitor, and enzyme
inhibitor. The obtained results of druglikeness score reveal that curcumin and conventional drugs had followed the druglikeness score (>0.50) (Table 9).

**Docking results of lead compounds**

Our present study undertook molecular docking of curcumin and conventional drugs against CagA oncoprotein to explore curcumin inhibitory potency in comparison to conventional drugs. The molecular docking results revealed that the curcumin interacted with C-terminal of CagA oncoprotein similar to conventional drugs.

*Molecular Docking of curcumin*

The surface view of complex revealed that, the binding pocket of CagA (residues 640-653) provided the accommodation of curcumin structure (Fig. 18a). Fig. 18(b) represents binding mode of curcumin with residues (LYS640, LYS644, SER641, and ALA653) of CagA.

*Molecular docking of conventional drugs*

*Clarithromycin*

The structure of clarithromycin bound in deep pocket of CagA protein formed by residues 406-500 (Fig. 18c). In the clarithromycin-CagA complex, the residues of helix (GLU406, GLU422, LYS499, and HIS500) and β-turn (ASN411) of N-terminal CagA protein played role in interaction (Fig. 18d). The residue GLU422 from helical structure was conserved in case of clarithromycin interaction.

*Amoxicillin*

Amoxicillin adopted in binding pockets of CagA (residues 382-440) as shown in Fig. 18(e). The structure of amoxicillin made extensive interactions with four helical residues (GLU397, GLU429, LYS401, and TYR440), two β-strand (LYS382 and GLU383), and one β-turn (GLN385) (Fig. 18f). The two helical residues (GLU429 and TYR440), one β-strand (LYS382) and one β-turn (GLN385) were found to be conserved at N-terminal binding sites of CagA.

*Pantoprazole*
Fig. 18(g) represented the surface view of Cag-amoxicillin complex, the binding pockets for pantoprazole accommodation was made by residues (383-432). Pantoprazole acts on different active sites of CagA oncoprotein and form complex, which had five H-bond and six hydrophobic interactions [Fig. (18h)].

Metronidazole

The residues (382-440) of CagA protein synthesised binding pockets for adopting metronidazole structure (Fig. 18i). In silico study showed that the three helical residues (LYS401, ASP432 and TYR440) and one (LYS382) from β-strand of N-terminal CagA protein made extensive interaction with atoms of metronidazole (Fig. 18j).

**Determination of binding energy in complex**

The docking analysis estimated global binding energy (-36.37 kcal/mol) of curcumin and conventional drugs: clarithromycin (51.06 kcal/mol), amoxicillin (-34.78 kcal/mol), pantoprazole (-34.08 kcal/mol), and metronidazole (-25.12 kcal/mol). The attractive van der Waals energy was determined as -20.16, -35.79, -23.09, -16.69, and -9.66 kcal/mol for curcumin, clarithromycin, amoxicillin, pantoprazole, and metronidazole, respectively. The highest H-bond (-10.73 kcal/mol) for clarithromycin, and metronidazole had lowest (-3.29 kcal/mol) (Fig. 19).

**Discussion**

*Determination of ADME and Druglikeness Score*

The clinical trial of many drug compounds are generally failed due to no interactive relation in the potency against the intended drug target. It was noticed that pharmacokinetics of compounds were directly blamed to more than half of clinical trials. Curcumin is stable at acidic condition and its slow degradation occurs at pH 1-6 (Wang et al., 1997) and normally encountered in stomach, the site of *H. pylori* colonisation (Morris et al., 1991). Therefore pharmacokinetic properties of curcumin has been evaluated to reduce the problems arising due to interactive potency of conventional drugs against drug target. Hence, in this study, we have examined the overall drug likeness score
for curcumin and compared with that of conventional drugs are being used against *H. pylori* infections targeting CagA oncoprotein.

Analysis of ADME (absorption, distribution, metabolism, and elimination) property for compounds have been crucial in drug development process. Hence, the molecular properties and bioactivity of curcumin and conventional drugs were determined by using online data server Molinspiration and obtained logP values along with other physiochemical properties i.e. molecular mass, the number of hydrogen bond acceptors and donors. Molecules violating more than one of the Lipinski’s rule may have problems with bioavailability. Our results showed that all leading compounds except clarithromycin (MW > 500, hydrogen bond acceptors > 10) have zero violations of Rule of 5 and indicated that the compounds (curcumin, amoxicillin, pantoprazole, and metronidazole) have good bioavailability (Table 8). To violate the Lipinski’s rule by clarithromycin implies that either there may be exception similar to drugs such as atorvastatin and cyclosporin or some structural modification should be carried out to improve its bioavailability (Khan et al., 2013).

As a general rule, larger is the bioactivity score, higher is the probability of the particular molecule to be active (Khan et al., 2013). Therefore, a molecule having bioactivity score more than 0.00 is most likely to possess considerable biological activities, while values -0.50 to 0.00 are expected to be moderately active and if score is less than -0.50 presumed to be inactive (Verma et al., 2012). On the basis of above the obtained values of druglikeness score showed that curcumin exhibited good drug likeness score (>0.50) as compared with other standard drugs like clarithromycin, amoxicillin, pantoprazole, and metronidazole (Table 9).

**Docking study**

Subsequently, the lead molecule curcumin and conventional drugs (clarithromycin, amoxicillin, pantoprazole, and metronidazole) with good ADME and druglikeness score properties has been considered for molecular docking to investigate the interactive potency with oncoprotein CagA of *H. pylori*. Docking is a process in which small molecules and the active site of biological macromolecule fit together in three-dimensional space and made
important contribution with a proceedings role in discovery of drugs for many years (Karthick et al., 2014). The docking study was performed to identify the interactive residues of CagA oncprotein with each leading compounds.

In the present study, initially we undertook molecular docking of a curcumin and standard drugs with CagA protein to explore potent and efficient anti-
_Helicobacter pylori_ molecule. Hydrogen bond and hydrophobic interactions proved to be essential to support the drug-receptor interaction and provide stability by the surface visualisation of each complex by Chimera 1.8.1. (Saha et al., 2013) and the mode of interaction by Discovery Studio 4.0 Client (Talambedu et al., 2014) after docking of each compounds with CagA were analysed.

**Molecular docking analysis of Curcumin**

Figure 18(a) and (b) displayed the pockets and interactive mode of CagA oncprotein for curcumin, respectively. The N-domain of CagA oncprotein interacts with curcumin, that plays important role in development of gastric cancer.In this complex, curcumin showed H-bond as well as hydrophobic interaction with LYS640/644 residues. The connecting of C-atom of curcumin with atom N (LYS640) and O (SER641) through hydrogen bond made extensive interaction with CagA oncprotein. The atom N of ALA653 from helical structure showed H-bond interaction with O5 of curcumin. The O3 of ligand interacted with O (LYS644) via H-bond interaction. The residue LYS644 and ALA653 of CagA were conserved in curcumin binding pockets. The docking results showed that the interactive properties of curcumin with CagA residues could inhibit the tumor formation activity of CagA protein of _H. pylori_ and supported in vitro study, which advocated as the curcumin inhibits the growth of _H. pylori_ cagA+ strains, and this could be one of the mechanisms by which curcumin exerts its chemopreventative effects (Mahady et al., 2002).

**Molecular docking analysis of conventional drugs**

Many reports described that the conventional drugs were ineffective to cure _H. pylori_ infected patients due to development of resistance in _H. pylori_. Therefore, in this study, molecular docking of some conventional drugs
(clarithromycin, amoxicillin, pantoprazole, and metronidazole) were established to examine interactive potency with CagA oncoprotein and compared with interactive potential of curcumin.

Molecular docking analysis of Clarithromycin

Fig. 18(c) showed the pocket of CagA for clarithromycin adoption and explained the geometric fitness of particular ligand. Subsequently, stable interaction of clarithromycin with CagA residues explained in Fig. 18(d). The structural complex showed that the active site OE2 (GLU406) of N-terminal binds to atoms O7, C35, and N1 of clarithromycin by H-bond, while interaction of common atom OE2 (GLU422) with two different atoms (C35 and N1) of clarithromycin has been contributed by two H-bond. The active site OD1 of ASN411 from β-turn made stable H-bond interaction with O3, and C34 of clarithromycin. The interaction of amide nitrogen (NZ) of LYS499 also enhanced the strength of the complex by forming H-bond with O3 of ligand. The clarithromycin atom (C32) has attributed in formation of complex with amide nitrogen (NE2) of HIS500. The result of molecular docking analysis indicated that the H-bond interaction between atoms of clarithromycin and residues of N-terminal might have played effective role in changing of CagA confirmation and interrupted oncogenic activity. Earlier, it has been also reported experimentally that the CagA+ H. pylori was highly sensitive to clarithromycin that supported our findings (Saruç et al., 2001).

Molecular docking analysis of Amoxicillin

The geometrical fit of amoxicillin in pockets of CagA oncoprotein indicated for stable binding (Fig. 18e). It has been observed that O (GLU383), O (GLU397), and OE1 (GLU429) have interacted with atoms of N3, O2, O2 of amoxicillin, respectively by H-bond (Fig. 18f). Whereas other residues of CagA protein revealed H-bond interaction with atoms of amoxicillin namely N (LYS401) with O2, and OH (TYR440) with O1. Despite of H-bond interaction, the hydrophobic interactions also contributed for stable binding of amoxicillin with LYS382 and NE2 (GLN385). Such interactions of amoxicillin might have perturbed the oncogenic activities of CagA, according to earlier study which
explained that the implication of amoxicillin can sensitised the CagA+ *H. pylori* (Saruç et al., 2001).

*Docking analysis of Pantoprazole*

The binding pockets of CagA oncoprotein for pantoprazole provided insights of stable binding (Fig. 18g). The residue GLU383 from β-strand showed that its three atoms (OE2, OE1, O) interacted with F1, F2, F2 of pantoprazole respectively via H-bonds. The atom C13 of pantoprazole involved for stable binding with O (GLU397) through H-bond interaction. An atom of helical residue O (LYS425) interacted with atom C14 of pantoprazole by H-bond in complex formation. The other active sites of CagA N-terminal in which four helical residues (OE2:GLU397, OD1:ASP432, OE1:GLU429, and ND2:ASN400) and one (NE2:GLU385) from β-turn made strength in complex by hydrophobic interactions with ligand. Among interactive residues, GLN385, ASP384, LYS382, and GLU429 were conserved at binding sites for pantoprazole. These extensive interactions provided stability of pantoprazole for interfering with native confirmation of CagA protein (Fig. 18h), that may be one of reason for growth inhibition of CagA+ *Helicobacter pylori*. In an experiment, Hsu et al. (2014) have also demonstrated the inhibition of CagA+ *H. pylori* growth by application of pantoprazole.

*Docking analysis of Metronidazole*

Earlier, it has been reported that the CagA+ *H. pylori* strain was highly sensitive to metronidazole (Yue et al., 2014). Our docking results explained the binding interaction of metronidazole with CagA oncoprotein represented as pockets (Fig. 18i) and interactive mode (Fig. 3j). The common atom O3 of metronidazole interacted with amide nitrogen NZ (LYS401) and OH of conservative residue TYR440 by H-bond, whereas OD1 of ASP432 made H-bond interaction with C4 of metronidazole. The metronidazole also involved in hydrophobic interaction with conservative residue LYS382 from β-strand. The interaction of metronidazole with different active sites of N-terminal CagA protein have evidenced as an inhibitory agent of *Helicobacter pylori*.

*Analysis of binding energy in complexes*
The global energy, VDW (van der Waals), and H-bond contributed for stable binding of ligands with protein structure (Fig. 19). The global energy of each complexes was considered to be related to free binding energy and its higher negative value indicated higher free binding energy toward higher interaction probability (Vařecha et al., 2009). Based on ensemble docking analysis, it is worth noting that the obtained pharmacological activity of curcumin against H. pylori infection targeting Cag oncoprotein was found encouragingly similar to conventional drugs. The docking analysis revealed that the estimated global binding energy (-36.37 kcal/mol) of curcumin was higher than amoxicillin (-34.78 kcal/mol), pantoprazole (-34.08 kcal/mol), and metronidazole (-25.12 kcal/mol) whereas it had lower than the highest binding energy (-51.06 kcal/mol) of clarithromycin. The attractive van der Waals energy contribution to the total \( \Delta G \) binding energy were estimated: -20.16, -35.79, -23.09, -16.69, and -9.66 kcal/mol for curcumin, clarithromycin, amoxicillin, pantoprazole, and metronidazole, respectively and revealed that negative values of van der Waals interactions were highest in clarithromycin (-35.79 kcal/mol) and lowest in metronidazole (-9.66 kcal/mol) that was also responsible for binding with CagA protein. Whereas, H-bond highly contributed to the global energy for stable binding of the ligands with protein as shown in Fig. 4. The highest H-bond (-10.73 kcal/mol) for clarithromycin, and metronidazole had lowest (-3.29 kcal/mol). Here, three different types of energy were contributed to lead compound for strong interaction with oncoprotein (CagA), furthermore H-bond in the protein ligand complex structures contributed to same trends of global energy and could also be validated the stability of leading compounds as global energy.

Present study showed that the all leading compounds have positive interactive properties with CagA protein and suggested to be potential inhibitor of CagA+ H. pylori. This finding can support with the concomitant therapy (pantoprazole, clarithromycin, amoxicillin and metronidazole for 7 days) with 100% eradication rate for the treatment of H. pylori strains (Hsu et al., 2014). The main limitation of such therapy are incidence of side effect and adverse results lead to the development of resistance in H. pylori has become challenge to cure patients. On the other hand, António et al. (2015) have reported that the
nutritional approach of curcumin may play promising role in prevention of \textit{H. pylori}. Findings of our computational approaches based on Lipinski’s rule, druglikeness score as well as molecular docking supported the previous reports, and suggested that curcumin could be one of the potential drugs, in case conventional drugs are not effective against \textit{H. pylori}. The main limitations with the built structure of leading compounds were used for prediction of pharmacological activities against built model CagA oncoprotein, though predicted activities of such compounds by in silico methods might be less reliable in vivo study. However, our computational approaches concluded that naturally produced curcumin may play similar pharmacological role to conventional drugs against CagA+ \textit{H. pylori} infection, hence curcumin could be alternative of that drugs being used in prevention of oncogenic activity of CagA protein in \textit{H. pylori} infected patients.
Fig. 16. 3D-Structure of curcumin and conventional drugs

Table 8. In silico determination of physicochemical pharmacokinetics for leading compounds by using online server Molinspiration

<table>
<thead>
<tr>
<th>Details</th>
<th>Curcumin</th>
<th>Clarithromycin</th>
<th>Amoxicillin</th>
<th>Pantoprazole</th>
<th>Metronidazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octanol-water coefficient partition</td>
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<td>2.897</td>
<td>-1.352</td>
<td>1.95</td>
<td>-0.468</td>
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<tr>
<td>Polar surface area</td>
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<td>182.93</td>
<td>132.957</td>
<td>86.348</td>
<td>83.878</td>
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<tr>
<td>Number of nonhydrogen atoms</td>
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<td>52</td>
<td>25</td>
<td>26</td>
<td>12</td>
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<tr>
<td>Molecular weight</td>
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<td>747.964</td>
<td>365.411</td>
<td>383.376</td>
<td>171.156</td>
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<tr>
<td>Number of hydrogen-bond acceptors (O and N atoms)</td>
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<td>8</td>
<td>7</td>
<td>6</td>
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<tr>
<td>Number of hydrogen-bond donor (OH and NH atoms)</td>
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<td>Number of Rule of 5 violations</td>
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<tr>
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<td>8</td>
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<td>Molecular volume</td>
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<td>306.886</td>
<td>305.361</td>
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Table 9. Estimation of Druglikeness scores of leading compounds by using online server Molinspiration

<table>
<thead>
<tr>
<th>Properties</th>
<th>Curcumin</th>
<th>Clarithromycin</th>
<th>Amoxicillin</th>
<th>Pantoprazole</th>
<th>Metronidazole</th>
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</thead>
<tbody>
<tr>
<td>GPCR</td>
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<td>-0.65</td>
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<td>Enzyme inhibitor</td>
<td>0.08</td>
<td>-0.79</td>
<td>0.27</td>
<td>0.37</td>
<td>-0.32</td>
</tr>
</tbody>
</table>
Fig. 17. Crystal structure of CagA oncoprotein of *Helicobacter pylori* 26695

Sequences of CagA oncoprotein (PDB ID: 4VDZ)

MTNETDQTRTPQDSGAFIKNVGAASDQTKPIVDKNDRDQRQAQDPSIQGRLYENKIKPDPDKKAEFLKSAKQSFAGIIIIGNQRTSFKFMGVTDESLDERQAEKNGGPVGDWLIDLFSTFNSKSSDKEANIEQEPYHIQPQAPATTTDQKLPLLREMLRDEGNYSKFTLGDMEMDQEGVADJPNKYNFQNLIEINNALSYYLYSHMNGIEPKVSVLIAANGGFQGDKHWDNMATVGYKQDDQNNVAYLINVHMKNSSGLV1AGGEKGNPSFYLYIKEDQLTGSRALSQEEIRNQVFMEFLAQNNKLNLN.ISEKEKFKQNEIEFDQSDKAYLaLGNDRIAFVSKKDTHKSAITEFNNGLDLYTLKDYGGKAKADKADREKVITLQSGKLHDGVMVVDYNKTYMNASKPNKGVGTNGVSHLEAGFNNVATNLPLDLNPLALTSMFVRNLNLENKLTAQGLSLEQANKLIDFLGSSNKLGAFLNFKVAAEKSTNGYDEVKKAQKDLKSLMRKREHLEKEV

- Red colour for conserved residues
Fig. 18. Visualisation of the geometric preferences of CagA oncoprotein in complexation with ligands molecules represents surface view (a, c, e, g, and i) as well as binding mode (b, d, f, h, and j) of ligands with CagA structural protein of Helicobacter pylori. The ligand and interacting residues are shown in stick and line representation respectively.

Curcumin

![Curcumin Image](image1)

Clarithromycin

![Clarithromycin Image](image2)
Amoxicillin

Pantoprazole
Metronidazole

Fig. 19. Inherent free binding energy (kcal/mol) attributes representing the complex formation by interaction of leading compounds with CagA protein of *Helicobacter pylori*.