IDENTIFICATION OF NOVEL INHIBITORS

AGAINST *Helicobacter pylori and Campylobacter jejuni*

Submitted by-

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Under the supervision of

Dr. Saurabh Bansal

Submitted in partial fulfillment of the
requirement for degree of Bachelor of Technology

Department Biotechnology

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

CANDIDATE'S DECLARATION

This is to declare that the work carried throughout in this project entitled **"Identification of novel inhibitors against *Helicobacter pylori* and *Campylobacter jejuni"*** for the partial fulfilment of the requirement for the award of degree of Bachelor of Technology in Biotechnology is an original and authentic record, carried out over a period from July 2018 to May 2019 under the guidance of Dr. Saurabh Bansal (Assistant Professor, Department of Biotechnology and Bioinformatics).

This work has not been submitted for the award of any other degree or diploma.

Bhushan Kumar, 151802

This is to certify that the above statement made by the candidate is true to the best of my knowledge.

Supervisor Name: Dr. Saurabh Bansal

(Assistant Professor, Department of Biotechnology and Bioinformatics)

Signature:

CERTIFICATE

This is to certify that the above statement made by the candidate is true to the best of my knowledge. The project titled "**Identification of novel inhibitors against *Helicobacter pylori* and *Campylobacter jejuni***" submitted by **Mr. Bhushan Kumar** in partial fulfilment for the reward of degree of **Bachelor of Technology in Biotechnology** from Jaypee University of Information Technology, Solan has been carried out under my supervision.

Signature of Supervisor

Name of Supervisor: Dr. Saurabh Bansal

Date:

CHAPTERS

ACKNOWLEDGEMENT

At first I would like to thank Dr. Sudhir Syal, HOD, Department of Biotechnology and Bioinformatics for giving me this opportunity to work on a research project and providing all the necessary requirements for its successful completion.

I would like to express a sincere gratitude to my support system throughout this journey without whom this wouldn't be possible; my research guide Dr. Saurabh Bansal, Assistant Professor, Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat. His constant motivation encouraged me to work out all the problems that came along the way. I highly appreciate and value his efforts throughout the tenure of this project work.

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ABSTRACT

This project work seeks to identify new inhibitor molecules which are capable of inhibiting the pathogenic activity of two closely related microbes, namely *Helicobacter pylori* and *Campylobacter pylori*. For the same, on the basis of literature survey and computational approach, the possible target i.e. Outer Membrane Phospholipase A (OMPLA) for both the pathogens were identified. Through virtually screening of the potential inhibitors against target i.e. OMPLA were selected on the basis of having minimum binding energy and maximum stability. The current study suggested that Aristolochic acid and Bromophenacyl bromide might be the potential drug molecules against both the pathogens by virtue of their inhibiting capacity of OMPLA.

INTRODUCTION

Both microbes *Helicobacter pylori* and *Campylobacter jejuni* are mainly gram-negative bacteria in nature and are specifically microaerophilic. They are the pathogens responsible for causing gastro-intestinal infections in humans. *H. pylori* targets the gastrodudenal region in the body while *C. jejuni* is responsible for targeting the intestinal mucosa.

Although both the bacteria have slightly different form of expression, *H. pylori* leading to gastric disease prompting gastritis, peptic ulcers and in the long run gastric malignant growth (1) and *C. jejuni* causing intense contamination initiating diarrhoeal sickness, the endotoxins present in both the bacterial species are responsible for leading to their pathogenesis (2). In the interior section of bacteria the presence of endotoxin, that of *H. pylori* has fundamentally lower level of endotoxicity the reason being the underphosphorylation and underacylation of the lipid A part which is responsible for connecting with resistant receptors (3). The low level of immunological expression by endotoxin helps in long term onset of *H. pylori* contamination and in this manner the disease chronicity.

Also, both *H. pylori* and *C. jejuni* display a very close relationship in similarity between the saccharide parts of their endotoxins which can initiate the activation of antibodies which are automatically reactive in nature; *H. pylori* communicates mimicry of Lewis and some ABO blood bunch antigens, *C. jejuni* mimicry of gangliosides (2). The former has been embroiled in impacting the improvement of aggravation and gastric decay (a forerunner of gastric disease), the last is key to the advancement of the neurological issue Guillain-Barré disorder (4).

Since both these microbes share similar characteristics, it becomes of great interest to target them by developing a common methodology to end their pathogenic activity. The work that follows in this project involves virtual screening of novel inhibitor molecules efficient enough to target both of these pathogens at once.

Structure-based medication disclosure (SBDD) is turning into a basic instrument in helping quick and cost-productive lead revelation and enhancement (5). The utilization of balanced, structure-based medication configuration has turned out to be more productive than the customary method for medication revelation since it plans to comprehend the sub-atomic

premise of an ailment and uses the learning of the three-dimensional structure of the organic focus all the while (5).

The recognizable proof of lead mixes indicating pharmacological action against an organic target and the dynamic enhancement of the pharmacological properties and power of these mixes are the central purposes of beginning time tranquilize disclosure (5).

The objective structure can be obtained from exploratory information (X-beam, NMR or neutron dissipating spectroscopy), homology displaying, or from Molecular Dynamics (MD) recreations. There are various key issues that ought to be analyzed while considering a natural focus for SBVS; for instance, the druggability of the receptor, the decision of restricting site, the determination of the most pertinent protein structure, consolidating receptor adaptability, appropriate task of Protonation states, and thought of water atoms in a coupling site, to give some examples.

Bioinformatics tools provide a great insight into understanding the mechanism and pathway of different proteins and their interactions with each other and other molecules without actual analysis in wet lab. Data and vital information obtained using these tools assists any form of research as a prerequisite blue print to be followed.

Several database libraries available online like PDB, i-screen, drug bank as well as several softwares act as useful tools in seeking information to identify or design new compounds for human welfare.

A set methodology was followed in pursuit of finding the most appropriate inhibitors capable of targeting the OMPLA1 enzyme found in both *Helicobacter pylori* as well as *Campylobacter jejuni*, details of which are elaborated later (Fig 1).

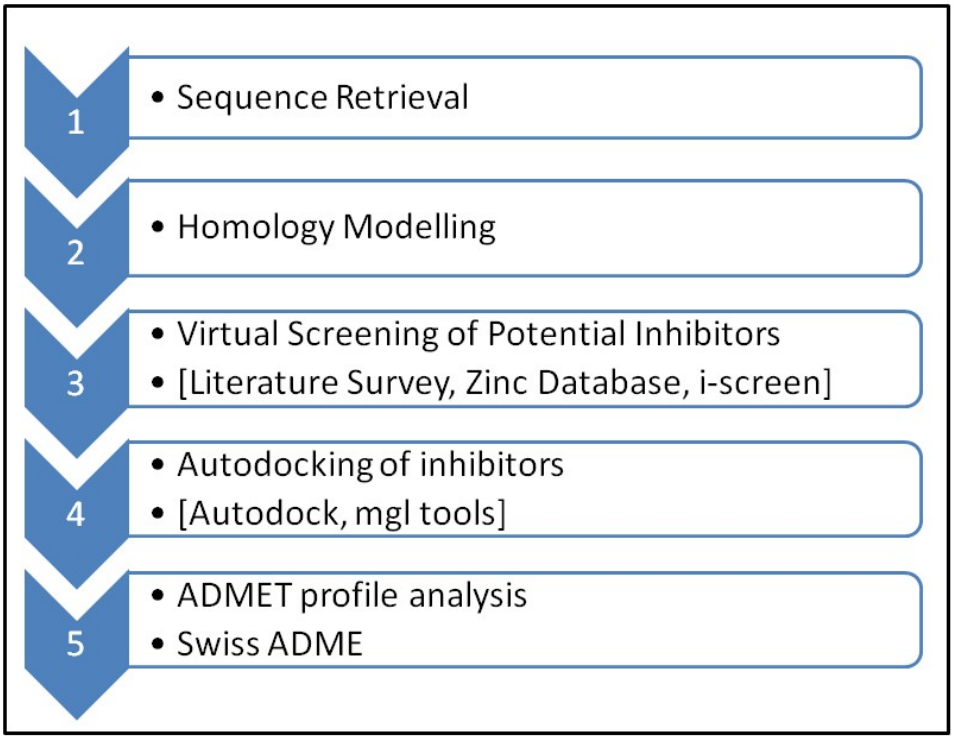

Fig. 1. Workflow for the conducted study

Each step acts as a progressive indicator of the expected outcome and everything that is followed in the procedure is inter related with each other.

The interior present toxins in both these microbes play an essential role in leading to their pathogenic activity. Even though both of their microaerophilic digestion systems are comparable more severe activity is observed in case of *C. jejuni* causing chronic infection (1), while *H. pylori* incessantly colonizes the gastric mucosa of the stomach or gastric Metaplasia in the duodenum (2). *H. pylori* is a profoundly common bacterial microbe, which usually shows its effect at a late onset at nearly 50 years of age colonising the Gastroduodenal region (6).

Contamination once settled can continue forever whenever left untreated and is related with a functioning irritation in the gastric mucosa, named gastritis (6). Despite the fact that disease result is different, including the improvement and repeat of gastritis just as gastric and duodenal ulcers, a few people with long haul disease create atrophic gastritis, which can have an immune system contributory foundation, and which is a forerunner state in gastric malignant growth improvement (2).

REVIEW OF LITERATURE

Selection of Target Microbes:

Seeing the correlation between pathogenesis caused by *H. pylori* and *C. jejuni*, it was decided to make a combined effort to target a common pathway to inhibit their activity using the same inhibitor. It is of keen interest to first understand the structure and function of both these microbes and correlate between the two.

Helicobacter pylori:

Helicobacter pylori are gram negative bacteria which are usually found in the stomach. It is a major pathogen for causing gastritis and is also associated in development of peptic ulcers. Barry Marshall and Robin Warren first found out in 1982 that an individual having chronic gastritis had an underlying bacterial reason responsible (7). It is likewise connected to the onset of duodenal ulcers and stomach malignant growth. Nonetheless, over 82% of the total population with the presence of this bacterium is usually asymptomatic, and it might assume a critical job in the normal stomach biology (8).

Over half of the total populace has *H. pylori* present in the upper region of gastrointestinal tract. Disease is more typical in creating nations than Western nations. *H. pylori*'s helical shape (from which the class name infers) is thought to have developed to infiltrate the mucoid coating of the stomach.

The overwhelming morphological type of this class is bended to winding; in any case, a few members have either a short or decreased pole shape. All Helicobacters are motile by methods for flagella (1). These fundamental attributes of morphology and motility are believed to be profitable to these living beings because of their limitation in the mucous layer of the gastrointestinal tracts of people and an assortment of creatures.

H. pylori is a S-formed bacterium with 1 to 3 turns, 0.5 ×5 μm long, with a tuft of 5 to 7 polar sheathed flagella under ideal environment conditions (9). This morphology has been associated with most extreme in vitro motility. Most of the *Helicobacter* forms have this essential morphology of S shape with polar, sheathed flagella, however varieties in shape and size are observed (9). Be that as it may, other variation frames are found in various different species (Fig. 2).

Thin sectioned areas of this bacterium uncover the average cell divider details of a bacterium that is gram-negative in nature comprising of external and inward, or plasma, layers isolated by the periplasm of around 30 nm thickness. The thick cytoplasm contains nucleoid material and ribosomes. Examination of the peptidoglycan from *H. pylori* uncovered it had a remarkable muropeptide creation, being less unpredictable basically than that seen in other gram-negative microscopic organisms (10). In most of *Helicobacter* species inspected, an electron-lucent region is situated in the terminal areas. Related with this district and situated close to the flagella inclusion site is a "polar film". This is an extra electron-thick band 6 to 8 nm thick found 20 nm underneath the plasmic layer yet connected to it.

It has been observed that *H. pylori* is typically situated inside the mucous layer which is pretty thick in nature surrounded epithelial cells of the gastrointestinal tract (10). A debilitating of the mucous obstruction by *H. pylori*, driving at times to its breakdown, has been proposed as *H. pylori* has a gene that is practically indistinguishable to *Vibrio cholera*e having the mucinase gene (11). Such mucinase action might be in charge of the disintegration of the net-like structure of the bodily fluid and the differently measured cavern like clear territories encompassing *H. pylori* as seen in vivo with electron minute procedures. In any case, it can be thought about in vitro propose that the loss of gel structure may likewise emerge from high nearby pH produced by the urease movement of *H. pylori* instead of by mucolytic action.

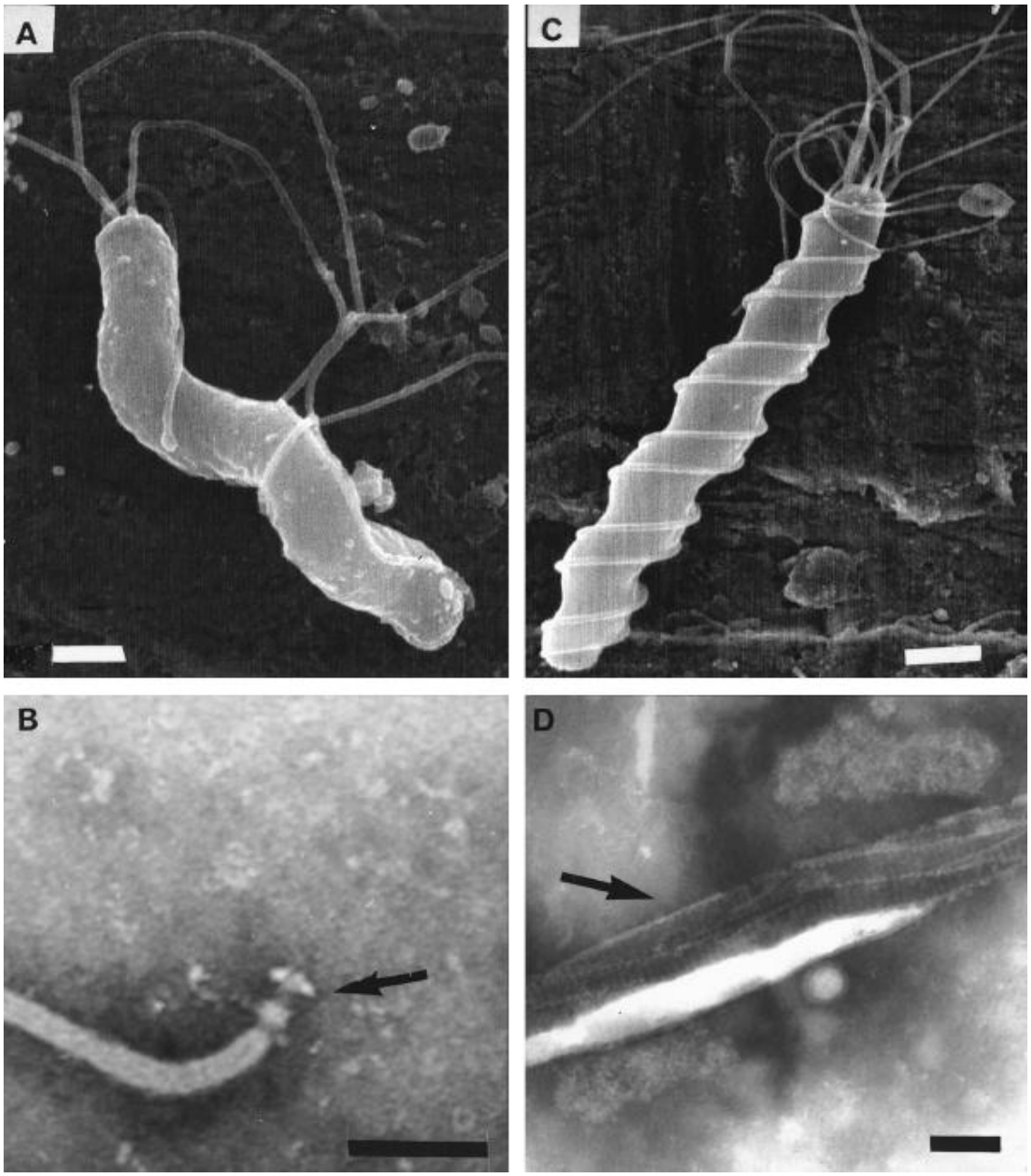

Fig. 2. Morphology of *Helicobacter pylori*

Campylobacter jejuni:

*Campylobacte*r *jejuni* is the main bacterial reason for foodborne diseases around the world (12). In any case the comprehension of its cell cycle is poor. The plausible *C. jejuni* starting point of replication (oriC) – a key component for commencement of chromosome replication, which is likewise critical for chromosome structure, upkeep and elements has been distinguished.

Campylobacter jejuni requires having centralisation of oxygen with variable amount of 3-6% oxygen and 3-9% of Carbon dioxide which make it microaerophilic. Campylobacter *jejuni* is the main source causing usually the disease of sustenance borne prevalent in the America, where it has been approximated a number of 15 cases for a populace of 100000 people (13). But a multiple number of cases end up unnoticed or being reported. Therefore, it is imperative to succession its genome to decide its job in these sustenance borne diseases and devise compelling medications. *Campylobacter jejuni* is available in abnormal states in loose bowels movement of contaminated people just as creature dung. This microbe can be secluded from people and other organisms. Disconnection requires extraordinary anti-infection containing media and a domain that contains ideal dimension of oxygen for microaerophilic living beings (2).

Clinical proof suggests that the site of *Campylobacter* disease is by all accounts the ileum and jejunum in the small digestive organs as opposed to in the internal organs. Amid the pathogenesis procedure, the living being initially starts by infiltrating the gastrointestinal bodily fluid utilizing its motility and winding shape. The microscopic organisms at that point holds fast to the gut Enterocytes where the flagellum has been appeared to discharge *Campylobacter* obtrusive antigens (Coa) and cytolethal distending poisons (CdtA,B,C) dependable in host cell apoptosis. When disease has happened, the immunoglobulin, explicitly IgA, rises and crosses the gut divider. This causes immobilization and supplement is actuated (14).

The genetic material of *Campylobacter jejuni* has been repoted to consist of 1,616,554 nucleotides in form of a circular DNA (15). The total gene count and the translated proteins are 1707 and 1653 in number respectively. The GC nucleotide proportion in the total sequence is suggested to be about 30%(15). To achieve glycosylation N- binding protein is

arranged in a eukaryotic-style network. The progression of *Campylobacter jejuni* inhibiting a system is different from individual to individual. The dissemination of variable successions demonstrated that they can utilize the acceptor electron present at the ending position to bind with oxygen. This speaks to the assorted life hotspots for *Campylobacter jejuni*. As indicated by MLST type, the relationship of clonal complex and dispersion of the qualities is solid. In any case, these dispersion of qualities demonstrated no proof for their host inclinations (16). In this manner, across the board flat quality exchange between clonal edifices isn't bolstered.

It shares a structure similarity with most of the other bacteria gram negative in nature. There is presence of a single flagellum at either or both the ends. The cell membrane is segmented into outer and inner forms that flank the Periplasm in between them. Lipopolysaccharides are the major component of the outer membrane which are Endotoxic in nature (17). The outer surface of membrane has membrane proteins attached on it which play the role of antigenic molecules (Fig. 3).

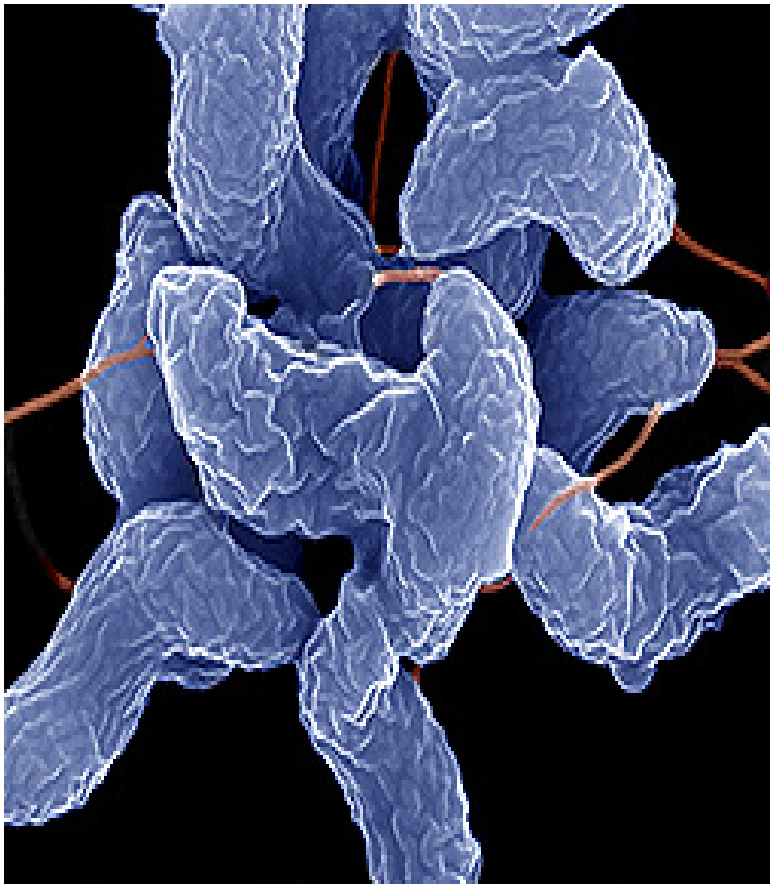

Fig. 3 *Campylobacter jejuni*

Lipolytic Enzymes:

Lipolytic enzymes have common ability to hydrolyse multiple ester linkages making a diverse family of Phospholipases. From nutrient digestion to bioactive molecule formation Phospholipases serve many roles. Thus variability in their function is indicative of their critical importance in survival (18).

The division of Phospholipases has been characterised on their Phospholipid attack site namely plaA, plaB, plaC and plaD indicating the target bond present in molecules (Fig 4). Pla1 form an expansive group of 1-acyl hydrolysis (19). It has been reported that this enzyme plays a crucial role as a pathogenic factor originating an different bacteria and fungi.

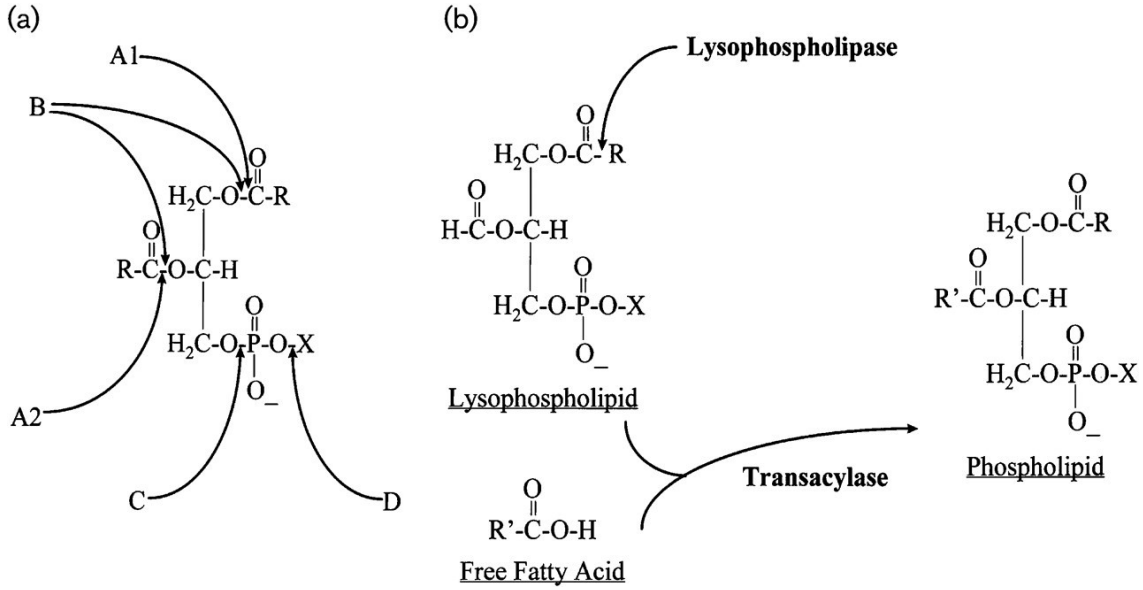

Fig. 4. Sites of action of different phospholipases. (a) A1 and A2 PLA1 and PLA2 respectively; B, PLB; C, PLC; D, PLD (b) Lyso-PL and Lyso-PL Transacylase (18)

Outer Membrane Phospholipase A1 has an expansive specificity with an outer membrane of bacteria. In gram negative bacteria it is one of the few enzymes present there. Ser164 has been proposed as the active site of this protein. The expression of this enzyme has been studied through *E. coli* in most of the cases (20). It plays a role in secretion of bacteriocins in *E. coli* which is initiated by protein lysemis. The accumulation of lysophospholipids and fatty acids occurs in the outer membrane which is dependent on phospholipase. The permeability of outer membrane gets increased by these reactions products allowing for bacteriocins to

secrete. The structure of this enzyme is made of 12 stranded antiparallel beta barrels with 1 flat and 1 convex side (21). Reversible dimerisation regulates the enzyme activity.

This enzyme is strictly dependent on calcium and displays variable specificity. It has been reported that this enzyme is necessary for growth of bacteria in the natural environment.

Functional Activity of OMPLA

Enzymatic activity of OMPLA cannot be accurately explained by regulation of genes, the protein that is expressed correctly gets fit in the outer membrane which is made of inner and outer leaf lets having phospholipids and lipopolysaccharide respectively (22). The necessity of entering the cell envelop requires use of temperature shock, lysis or secretion of scoliosis to fully express the activity of enzyme. Melittin or cardiotoxin are the kinds of enzymes which can be activated by entry of some special peptides.

Structural analysis

Since the structure of *E. coli* ompla has been studied, it has been found that its 12 β-stranded structure is similar to folding pattern of other beta barrel outer membrane proteins (OMPs) having 8-22 strands (19). After the depiction of crystal structure of monomeric and dimeric enzymes, it was found that a folding into 12 stranded anti-parallel beta-parallel with 1 convex and flat side of ompla monomers. Periplasmic turns along with the loops extending from extracellular parts are responsible for joining the beta strands together (20). The region where the protein binds is an outer surface hydrophobic in nature. The active site is present at the interface between hydrophilic and hydrophobic leaflet of the outer membrane.

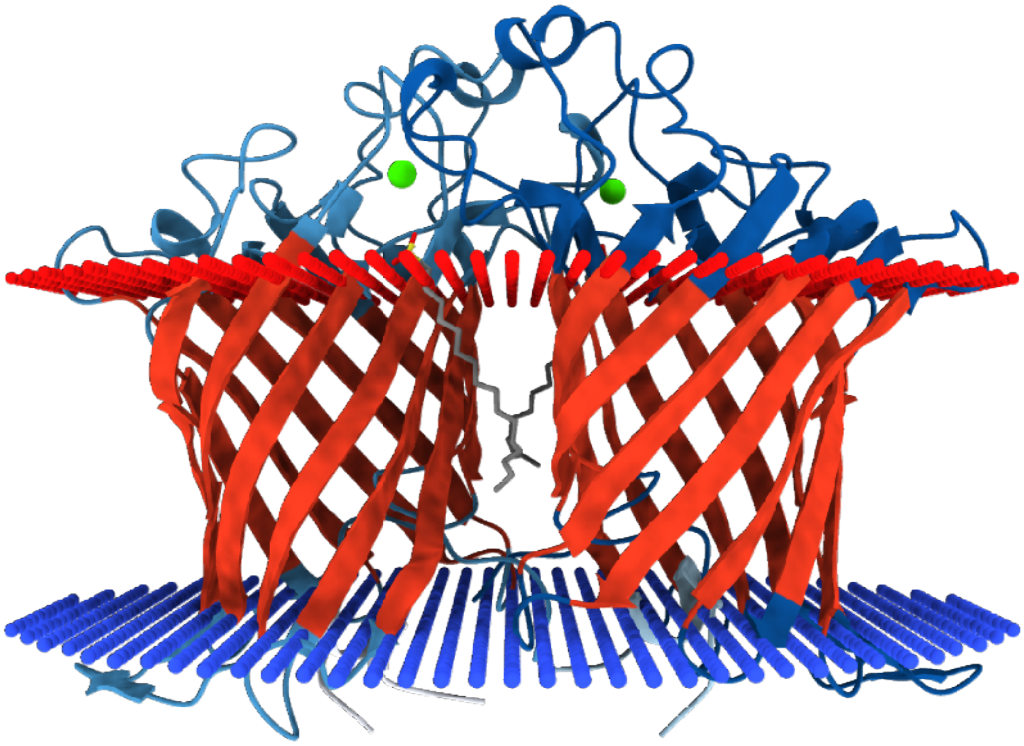

Fig.5. 3D structure for the OMPLA molecule of *E. coli*

E. coli OMPLA has been used as a reference molecule in the work that has been carried out in this project for understanding and studying the properties associated with *H. pylori* and *C. jejuni*.

Molecular Analysis of OMPLA groupings encoded by the pldA gene in Gram-negative microscopic organisms:

The analysis of the anticipated essential structures of the pldA gene in *S. typhimurium*, *Klebsiella pneumoniae* and *Proteus vulgaris* with that of the *E. coli* pldA gene has been done previously (22). Hence these microbes serve an important role in understanding how it works. This arrangement examination revealed a great occurrence of similarity pattern, showing 79% of the amino corrosive build ups being indistinguishable in each of the four proteins. Moreover, Enzymatically dynamic genes were brought out when these pldA genes were cloned using the *E. coli* (22). In an ensuing distribution Brok et al. an entire sequencing of genome was carried out by screening databases, which uncovered the nearness of proteins with homology to OMPLA in a number of essential bacterial species including our targets namely *H. pylori*, *C. jejuni*, *Yersinia pestis*, *Neisseria meningitidis* and *N. gonorrhoeae* (23). Simultaneously examination of OMPLA amino acids in these microorganisms uncovered a crucial evidence stating that about 28 amino acid sequences are totally conserved (22).

The experiment also provided subsidiary information about dynamic site deposits His-142 and Ser-144 claimed as the best sequence regions and furthermore the Asn-145 and Gly-146 responsible for Oxyanion development. Moreover, the synergist calcium site is conserved among all OMPLA conformations that were studied, as Ser-152, ligates the calcium particle with its hydroxyl gathering (19). Interestingly, the L3L4 calcium site isn't completely monitored, as Asp-149 is supplanted by an arginine residues in *Neisseria* species, and by a Glycine residue in the *Helicobacter* strains. The second calcium Ligand, Asp-184, is monitored in all with the exception of in *Helicobacter* strains, where it is supplemented by a serine residue (23).

Relationship between bacterial Phospholipases and pathogenesis

Despite the fact that there is significance of Phospholipases present in bacteria responsible for pathogenic activity, the very precise component of specific Phospholipase activity in vivo is still not completely predicted. Cytolytic movement is usually connected with the lethal nature of Phospholipase coming about because of the gathering of layer destabilizing items or by the broad pulverization of film phospholipids.(24) The cause of Cytolysis can be stated to occur by a functioning bacterial Phospholipase with wide explicitness or working together with host Degradative proteins prompted by the bacterial Phospholipase. The property of Cytolitic activity of the Phospholipase is mainly reliant on its capacity to connect legitimately with and hydrolyse phospholipids in the film. Regularly, for comfort the Lytic action is estimated as haemolysis, paying little heed to the probability of a specific pathogen causing fundamental contamination or a bacterial Phospholipase entering the circulation system (24). Despite the fact that Phospholipases C (PLCs) are the best-contemplated bacterial Phospholipases as harmfulness factors, different examinations have demonstrated that numerous bacterial external layer or emitted Phospholipases are Cytolytic and in this manner in charge of tissue harm amid disease.

Phospholipase A as a destructive determinant in Gram-negative microscopic organism *Helicobacter pylori*:

H. pylori has a few distinctive Phospholipase activities, for example, PLA 1, PLA 2 and PLC, which are connected to the debasement of the Phospholipid segments of the mucosal hindrance. Notwithstanding immediate interruption of the mucosal obstruction, Phospholipases are likewise ready to start creation of Leukotrienes and different Eicosanoids, changed over from Arachidonic corrosive, which could likewise influence film penetrability and mucous release (25).

The *H. pylori* genome arrangements uncovered the closeness of an open perusing outline with the OMPLA enzyme found in *E. coli*; it was affirmed that this gene encodes a functioning Phospholipase with Lecithinase, PLA 2, and haemolytic exercises (26). Scientists developed a pldA strain in *H. pylori* that was isogenic, which demonstrated a stamped decrease in PLA 2 and haemolytic exercises and, in contrast to the wild-type, it proved unfit to inhibit the activity in mice in a course of about 7 weeks. In spite of the fact that the capacity of the freak to stick to human gastric Adenocarcinoma cells was unaffected, it induced a critical resistant reaction, showing a job for PLA in colonization of the gastric mucosa and conceivable tissue harm after colonization. These discoveries were affirmed by Xerry and Owen; they demonstrated that transformations in qualities encoding the urease (ureB) and Phospholipase (pldA) catalysts did not influence adherence of the microscopic organisms to gastric epithelial cell lines (27). Nonetheless, it cannot be stated with confidence that the adherence to gastric epithelial cells can cause the strain to effectively colonize in vivo.

In an examination to decide the level of variety in the PLA gene in *H. pylori* confines, found that the pldA quality is a profoundly rationed component of the *H. pylori* genome regardless of the geological inception of the confine (28). The examination additionally researched connections between pldA genotype and clinical infection seriousness, just as with variety in cagA status and vacA genotypes. It was presumed that pldA is a moderated highlight of the *H. pylori* genome, with disengages of the equivalent PCR-RFLP genotype having a far

reaching topographical dissemination. The examination additionally upheld the hypothesis that the job for PLA movement in *H. pylori* is in colonization and diligence instead of in chronicity of disease (28).

H. pylori can immediately and reversibly change its film lipid, under somewhat acidic conditions, from the 'typical' L-variation, with under 2% lysophospholipids, to the 'lyso' S-variation, with over 50% lysophospholipids in the layer (29). The 'lyso' variation is increasingly haemolytic, with improved proclivity for epithelial cells and expanded arrival of urease and VacA poison. The erasure of the pldA quality in the S-variation re-established the ordinary phenotype, recommending that the L-to S-change is because of the actuation of OMPLA (29). In an augmentation of this investigation demonstrated that the adjustment in film lipid synthesis is in reality because of stage variety in the pldA gene. They demonstrated that an adjustment in the C-tract length of this quality outcomes in reversible casing shifts, interpretation of a full-length or truncated pldA, and the generation of dynamic or inert OMPLA. Moreover, development at low pH brought about a choice of the best-adjusted (S) phenotype, with a practically total change of the typical L-variation (C7, OMPLA 'off') to the lyso S-variation (C8, OMPLA 'on'). The watched distinction in province morphology although well observed was not made clear by a LPS variety; presuming ultimately that the pldA gene could be responsible to add for survival at acidic pH conditions for *H. pylori* (30). All the more as of late, research was carried to find the centrality of stage variety in the pldA quality of *H. pylori* and its relationship with peptic ulcer sickness. The investigation recommended that OMPLA action is a destructiveness factor in *H. pylori*, as separates with high OMPLA movement are altogether connected with patients experiencing peptic ulcer. The examination additionally recommended a two-advance guideline of OMPLA in *H. pylori*: (I) stage variety in the Homopolymorphic tract of the pldA gene from 'off' to 'on' and (ii) protein level guideline among the pldA 'on' bacterial cells (30). It was explored that 31 stage variable qualities in *H. pylori* following murine gastric colonization for as long as one year in three irrelevant *H. pylori* strains. They reasoned that qualities which are destined to be related with prompt specialty fitting, including the pldA gene and LPS biosynthesis qualities, changed more quickly than other surface-protein qualities which might be levelled out of the versatile insusceptible framework. It is these discoveries that have enabled the rightful function for these genes in deciding entry site for colonization of *H. pylori* (31). Besides, it was studied about as an underlying mucosal boundary breaker used by the pH-enacted

Phosoholipase A 2 in *H. pylori*. This also proved to be the major reason for its pathogenic activity leading to formation of peptic ulcers It was also demonstrated experimentally that the gastric juice of people infected with infection by *H. pylori* contained essentially more amount of PLA 2 and Lyso-PC fixations.

Phospholipase A as a destructive determinant in Gram-negative microscopic organism *Campylobacter jejuni*:

OMPLA is a key enzyme for study since it is recognised as a haemolytic factor in *C. coli* (32). pldA gene upstream of an operon encoding an enterochelin transport framework in *C. coli*. The pldA gene is responsible for expression of a protein weighing 35kDa showing noteworthy closeness to the OMPLA enzyme found in *E. coli*. The recombinant protein has been observed to show calcium-subordinate Lysophospholipase and Phospholipase activity in vitro. pldA gene arrangement was additionally revealed for *C. jejuni*, encoding a putative PLA protein with critical closeness to PLA in *C. coli* (21). The cell-related PLA was separated and mostly cleansed and was observed to be a warmth stable, calcium-subordinate PLA with both haemolytic and Cytolytic consequences for various sorts of erythrocytes and on tissue culture cell lines individually. The arrangement of the pldA gene in *C. concisus* strain RCH3 (GenBank Accession No. AJ786391) was identified and the deciphered 297 amino corrosive succession for this quality item (Q6A175) has an anticipated atomic mass of 35·076 kDa.

An impact seek performed on Q6A175 demonstrated that it has 97% similarity to the amino corrosive grouping for PLA in C. coli (O32349) and 76% similarity to PLA of *C. jejuni* (Q9PMU8), while it has just 34% similarity to the OMPLA grouping of E. coli (Q8CVJ6). For the the OMPLA having a monomeric based structural arrangement, closeness has been observed in *C. consisus* and *C. coli* (32). The β-barrel structure of OMPLA in *Campylobacter* spp. has a huge likeness to the structure of OMPLA protein particles of other enteric Gram-negative microscopic organisms.

Virtual Screening

To be able to screen and verify the right inhibitors to target the enzyme, Autodock acts as a primary virtual tool to assist in the job. It is an application of mechanized docking devices. It is intended to foresee how little atoms, for example, substrates or medication hopefuls, attach to a receptor whose 3D structure is known.

Current disseminations of AutoDock comprise of two ages of programming: AutoDock 4 and AutoDock Vina (33).

AutoDock 4 really comprises of two fundamental projects: autodock plays out the docking of the ligand to a lot of lattices depicting the objective protein; autogrid pre-ascertains these frameworks.

Notwithstanding utilizing them for docking, the nuclear fondness matrices can be envisioned. This can help, for instance, to control natural engineered physicists' configuration better folios.

OBJECTIVES

The prime objective of this entire project was to identify novel inhibitors against OMPLA enzyme present in *H. pylori* and *C. jejuni* respectively using virtual screening methods.

Sub objectives:

1. Modelling of OMPLA enzyme crystal structure in *H. pylori* and *C. jejuni.*
2. Finding inhibitors through literature survey and homology modelling.
3. Autodocking to screen potential inhibitors with low Binding Energy.
4. ADMET analysis using Swiss ADME.

MATERIAL AND METHODS

Selection of Target Enzyme-

To check the possibility of OMPLA as drug target molecule, firstly the OMPLA sequences of *H. pylori* and *C. jejuni* and the sequences of human Phospholipases were retrieved from Uniprot in Fasta format. The sequence length for *C. jejuni* was found to be 356 AA long while for *H. pylori* it was 324 AA long. The sequences obtained were further analyzed by BLAST to compare their sequence similarity with human Phospholipases as well as among themselves.

Structure Homology Modelling-

The OMPLA enzyme has not been extensively studied in *H. pylori* and *C. jejuni* before which posed another challenge, the absence of availability of a crystal structure. To tackle this, modelling of structure was required to be done for both the bacterial enzymes.

Phyre 2.0, an online modelling tool was used to model the structures. A key aid that proved to be of great importance was the thoroughly studied E. coli OMPLA enzyme of which crystal structure is already known. This served as a reference point in our structural modelling and comparative analysis as well.

Virtual Screening of compounds-

Several potential inhibitors were identified through extensive literature survey and the screening of compound database using modelled structure of OMPLA. Autodock was then

further used to see the binding energy and thus screen the best of compounds that could be potential drug molecules.

Following steps were incorporated in autodocking-

1. Selection of our primary enzyme
2. For the purpose of autodocking, it needs to be free of charges therefore first Hydrogens are added, only polar ones.
3. Then selecting charge, Kolman charges are selected.
4. The atoms are assigned to ad4 type
5. After this Hydrogens are again added but merging the non polar ones.
6. The total charge should be checked on residues and file should be saved in pdbq.
7. Now the Ligand preparation takes place.
8. After inserting the file, addition of all Hydrogens takes place.
9. Charges are computed to Gasteiger charges.
10. Atoms are assigned to ad4 type.
11. The total charge should be show zero.
12. The output file should be saved as pdbqt.
13. The last step is of grid preparation.
14. Set map type, choose the ligand file and select grid box.
15. Using the scale adjust the box according to active site coordinates.
16. Close saving the current.
17. Then select grid output and save gpf format.
18. Select Docking, macromolecule and set rigid file name

19. Choose ligand and go to docking search parameter and select genetic algorithm.

20. Perform 50 GA runs.

21. For the output select Lamarcian and save as dock.dpf

22. Then select Run- Run Autogrid- browse- grid.gpf

23. Then go to analyse-docking-open-dock.dlg

24. Then analyse conformation select play ranked.

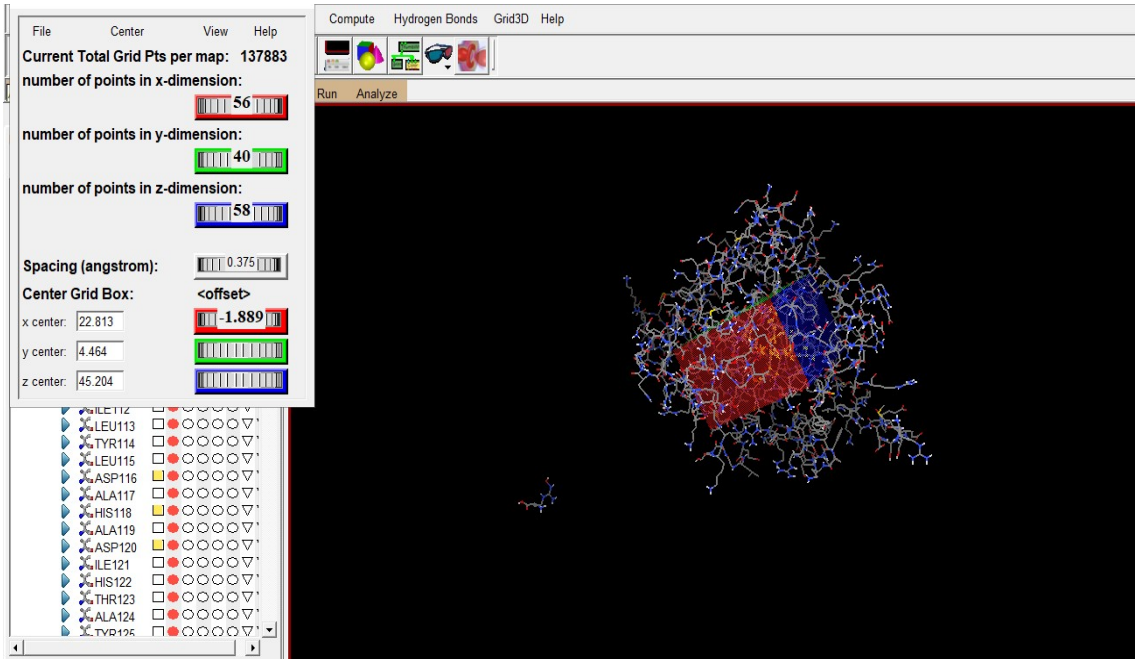

Fig. 6. Grid Formation while Autodocking

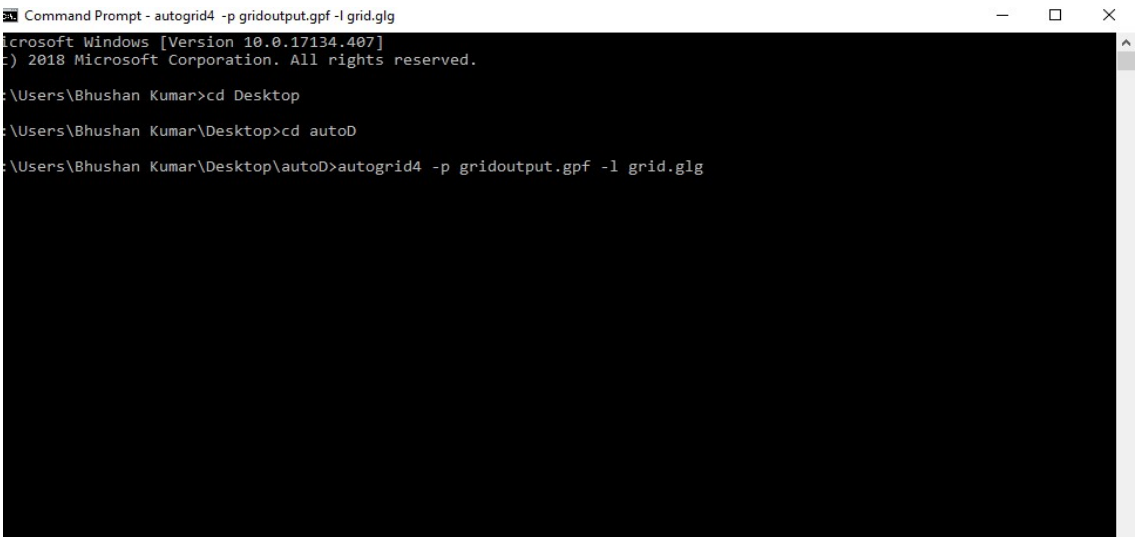

Fig.7. Command prompt displaying final docking command

ADMET profiling

This analysis is based on Lipinski's rule of five to check for the likeness of drug. It stands for Absorption, distribution, metabolism, excretion and toxicity. These parameters define the pharmacokinetics of the drug in human body and every drug must pass these parameters to undergo commercialisation.

The ADMET profiling of selected potential drug molecules were carried out using Swiss ADME (34).

RESULT AND DISCUSSION

BLAST results for OMPLA enzyme verification-

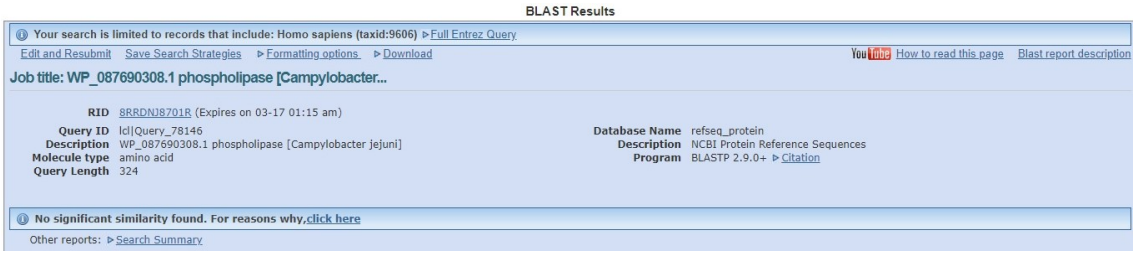

Fig. 8. Blast result: Human phospholipase with *C. jejuni* OMPLA

(No significant similarity < 18%)

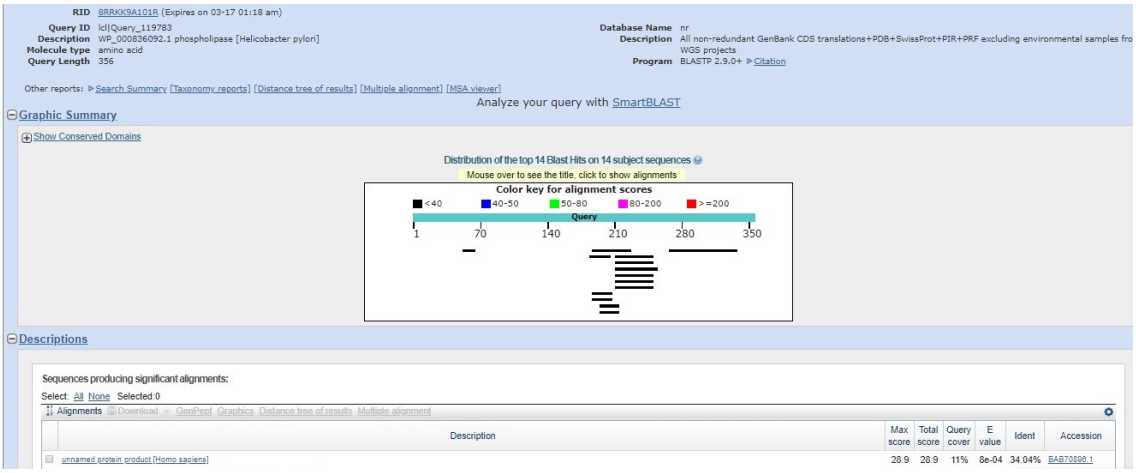

Fig. 9. Blast result: Human phospholipase with *H. pylori* OMPLA

(Very less similarity of 27.9%)

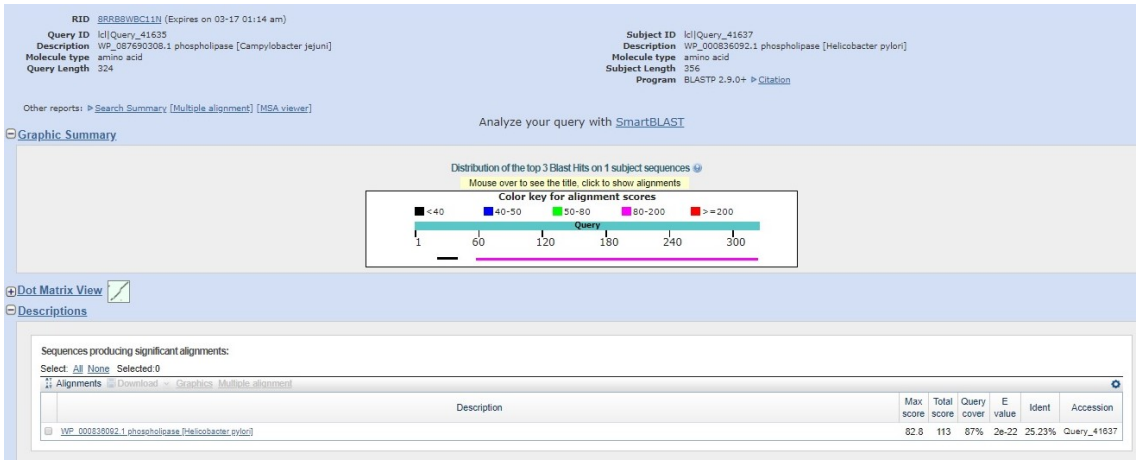

Fig. 10. Blast result: OMPLA of *C. jejuni* with *H. pylori*

(Showing high similarity of 82.6%)

Homology Modelling-

Using the Phyre 2.0 server following results were obtained-

For *C. jejuni* (INTENSIVE MODE)

Confidence in the model: 239 residues (74%) modelled at >90% accuracy

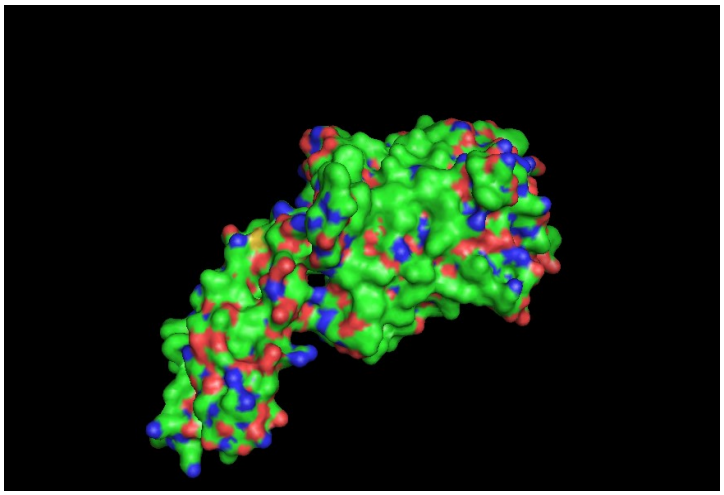

Fig. 11. Space filling Modelled Structure of *C. jejuni*

For H. *pylori* (INTENSIVE MODE)

Confidence in the model: 252 residues (71%) modelled at >90% accuracy

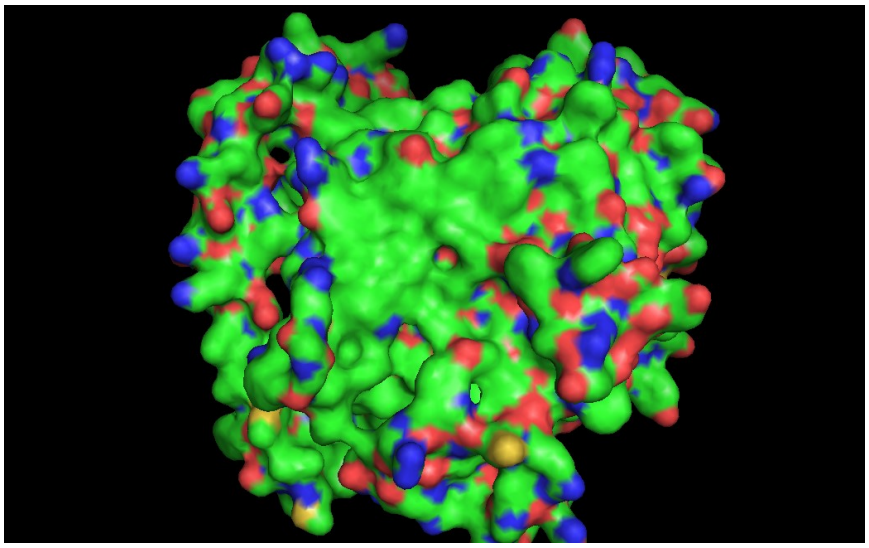

Fig. 12. Space filling Modelled Structure of *H. pylori*

Autodocking

20 inhibitors were selected through extensive structure based homology and from literature survey. A key aid that proved to be of great importance was the thoroughly studied OMPLA enzyme in *E. coli* whose crystal structure is already proved. It served as a reference point to identify more inhibitors on basis of structure based similarity.

Since no information was available about specific conserved residues available on the enzyme, docking was carried out with the complete sequence.

The results for top 5 hits are given in the table below-

Inhibitor	**Binding Energy (kcal/mol)**		**Internal Energy (kcal/mol)**	
	H. pylori	***C. jejuni***	***H. pylori***	***C. jejuni***
Aristolochic acid	-6.83	-6.32	-8.02	-7.51
Bromophenacyl bromide	-5.76	-4.85	-6.36	-5.44
2, 4-di aminobutyric acid	-5.32	-4.24	-5.80	-4.94
N- hydoxindospicine	-5.07	-4.11	-5.36	-4.33
2-sulfonamidoethyl cysteine	-4.86	-3.87	-5.13	-4.03

Another two potential inhibitors which had shown great efficiency against OMPLA of *E. coli* were docked besides the above mentioned ones, but due to their structure complexity and a long sequence length, the software could not render final results for them. These compounds are Hexadecanesulfonyl fluoride and 1- Hexadecanosulfonic acid.

Binding Energy results reveal two compounds Aristolochic acid and Bromophenacy bromide as the best applicable inhibitors to target the OMPLA molecule.

Their docked results with details are given below-

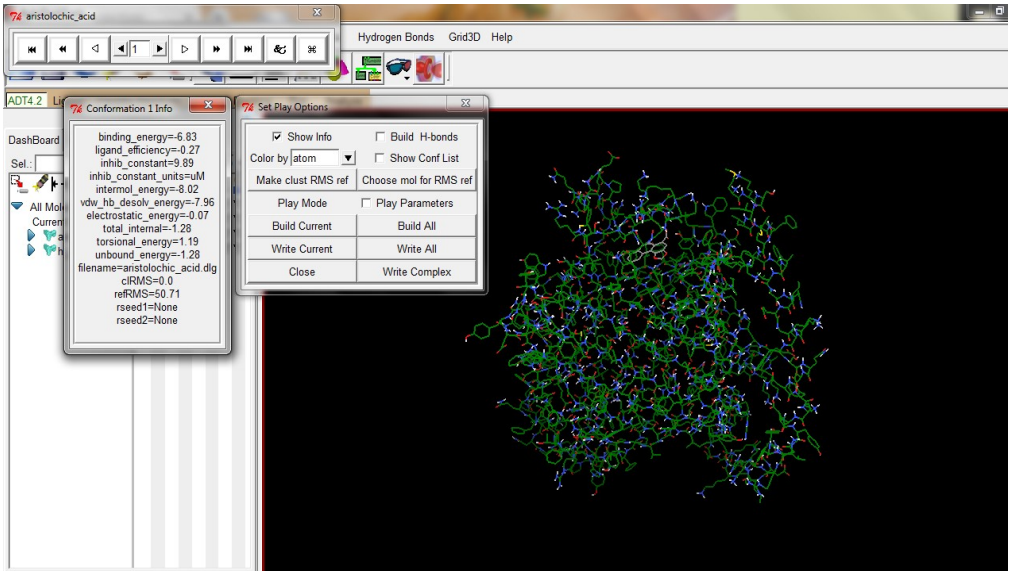

Fig. 13. Docked result of Aristolochic acid with OMPLA of *H. pylori*

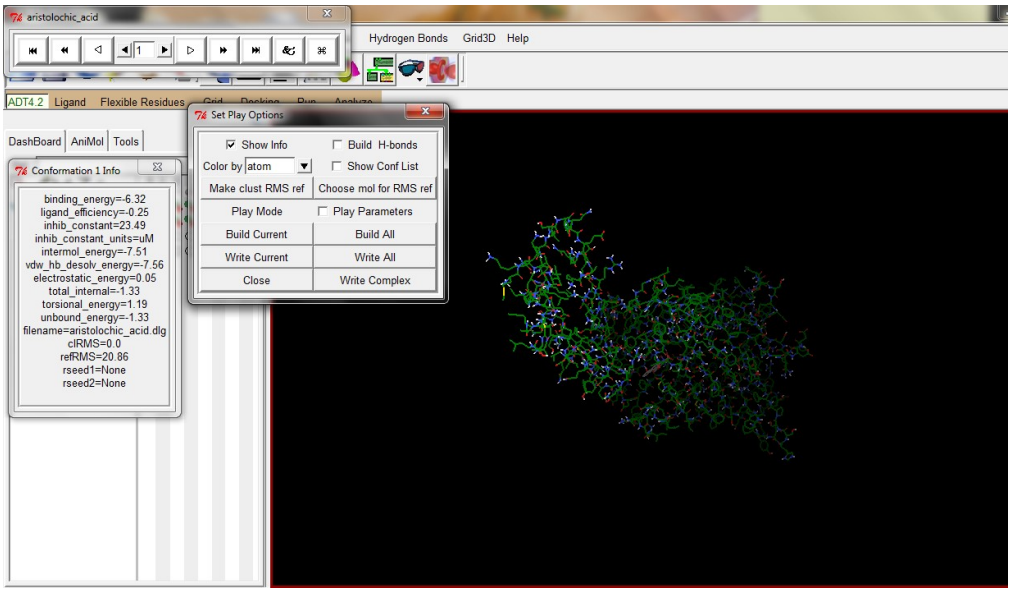

Fig. 14.Docked result of Aristolochic acid with OMPLA of *C. jejuni*

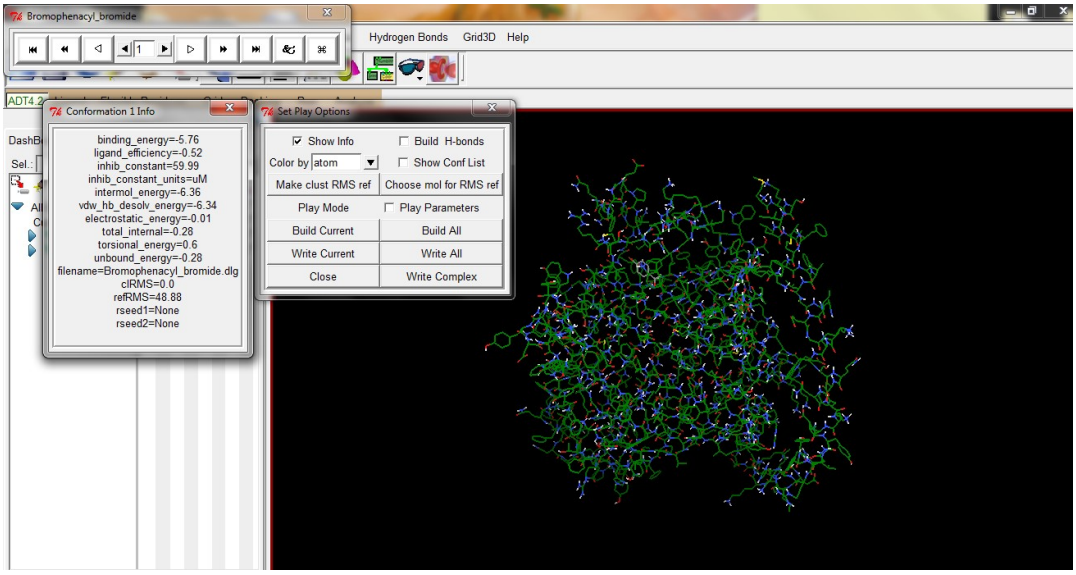

Fig. 15. Docked result of Bromophenacyl bromide with OMPLA of *H. pylori*

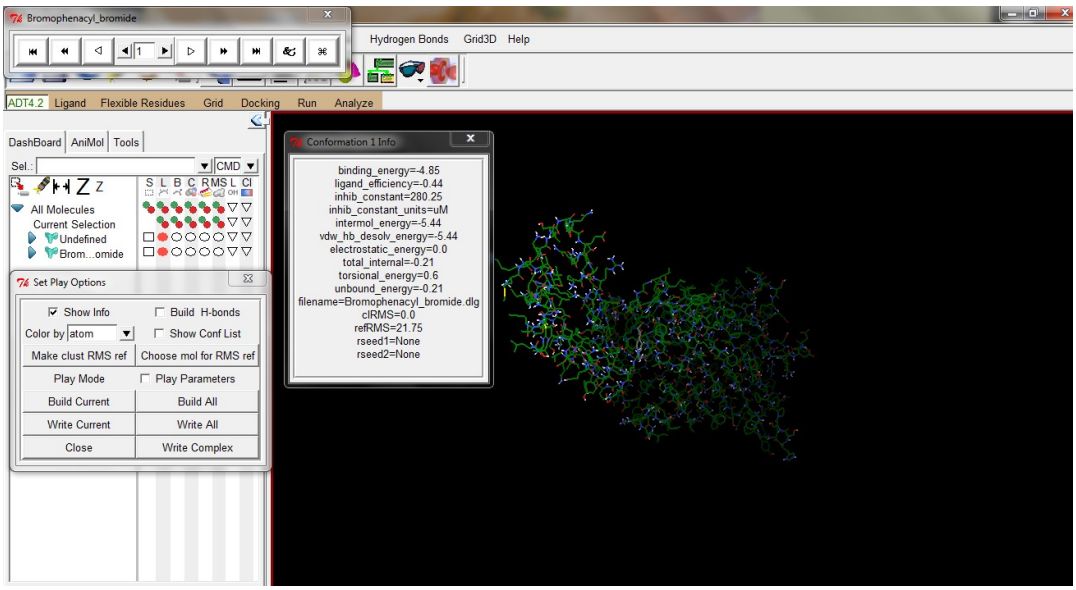

Fig. 16. Docked result of Bromophenacyl bromide with OMPLA of *C. jejuni*

ADMET Analysis

SwissADME was used as the main tool for carrying out the ADMET analysis. It required acquiring of SMILES format of compounds given below-

CCCCCCCCCCCCCCCCS(=O)(=O)OCC(N)C(O)=O

COc1cccc3c1cc([N+]([O-])=O)c4c(cc2OCOc2c34)C(O)=O

C1=CC(=CC(=C1)Br)C(=O)CBr

CCCCCCCCCCCCCCCCS(=O)(=O)F

The results are as follow for the final four compounds-

Molecule	1-Hexadecanosulfonyl-O-L-Serine	Aristolochic acid	3-Bromophenacyl bromide	Hexadecanesulfonyl Fluoride
Canonical SMILES	CCCCCCCCCCCCCCCS(=O)(=O)OCC(	COc1cccc2c1cc([N+	BrCC(=O)c1cccc(c1)Br	CCCCCCCCCCCCCCCS(=O)(=O)F
Formula	C19H39NO5S	C17H11NO7	C8H6Br2O	C16H33FO2S
MW	393.58	341.27	277.94	308.5
#Heavy atoms	26	25	11	20
#Aromatic heavy atoms	0	14	6	0
Fraction Csp3	0.95	0.12	0.12	1
#Rotatable bonds	19	3	2	15
#H-bond acceptors	6	7	1	3
#H-bond donors	2	1	0	0
MR	107.98	89.79	52.21	88.04
TPSA	115.07	110.81	17.07	42.52
iLOGP	4.01	1.96	2.09	4.21
XLOGP3	3.81	3.56	3.26	7.89
WLOGP	5.31	3.34	3.03	7.27
MLOGP	0.74	1.35	2.96	4.21
Silicos-IT Log P	4.16	1.14	3.37	5.54
Consensus Log P	3.6	2.27	2.94	5.82
ESOL Log S	-3.43	-4.42	-3.89	-5.73
ESOL Solubility (mg/ml)	1.47E-01	1.31E-02	3.59E-02	5.70E-04
ESOL Solubility (mol/l)	3.75E-04	3.85E-05	1.29E-04	1.85E-06
ESOL Class	Soluble	Moderately solubl	Soluble	Moderately soluble
Ali Log S	-5.92	-5.57	-3.29	-8.63
Ali Solubility (mg/ml)	4.72E-04	9.13E-04	1.42E-01	7.21E-07
Ali Solubility (mol/l)	1.20E-06	2.68E-06	5.10E-04	2.34E-09

Ali Solubility (mol/l)	1.20E-06	2.68E-06	5.10E-04	2.34E-09
Ali Class	Moderately soluble	Moderately solubl	Soluble	Poorly soluble
Silicos-IT LogSw	-5.4	-4.35	-4.45	-6.53
Silicos-IT Solubility (mg/ml)	1.57E-03	1.52E-02	9.79E-03	9.13E-05
Silicos-IT Solubility (mol/l)	3.98E-06	4.47E-05	3.52E-05	2.96E-07
Silicos-IT class	Moderately soluble	Moderately solubl	Moderately soluble	Poorly soluble
GI absorption	Low	High	High	Low
BBB permeant	No	No	Yes	No
Pgp substrate	Yes	No	No	No
CYP1A2 inhibitor	No	Yes	Yes	Yes
CYP2C19 inhibitor	Yes	Yes	No	No
CYP2C9 inhibitor	Yes	Yes	No	Yes
CYP2D6 inhibitor	No	No	No	No
CYP3A4 inhibitor	No	No	No	No
log Kp (cm/s)	-6	-5.85	-5.68	-2.58
Lipinski #violations	0	0	0	1
Ghose #violations	0	0	1	1
Veber #violations	1	0	0	1
Egan #violations	0	0	0	1
Muegge #violations	1	0	1	1
Bioavailability Score	0.55	0.56	0.55	0.55
PAINS #alerts	0	0	0	0
Brenk #alerts	1	3	1	0
Leadlikeness #violations	3	1	0	2
Synthetic Accessibility	5.15	2.77	1.84	3.73

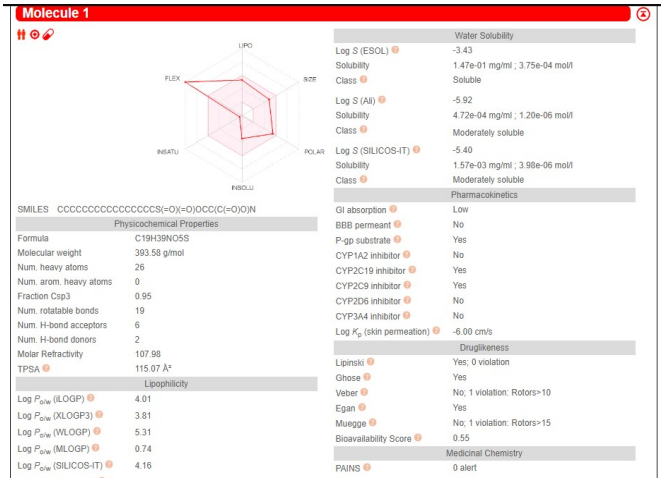

Fig. 17. 1-Hexadecanosulfonyl-O-L-Serine

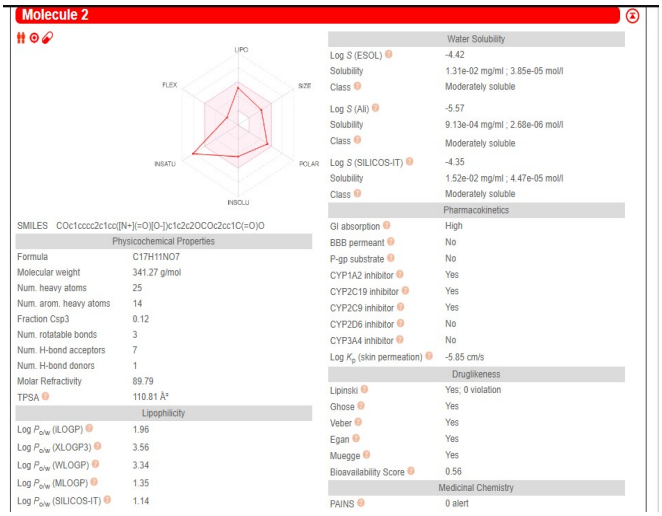

Fig. 18. Aristolochic acid

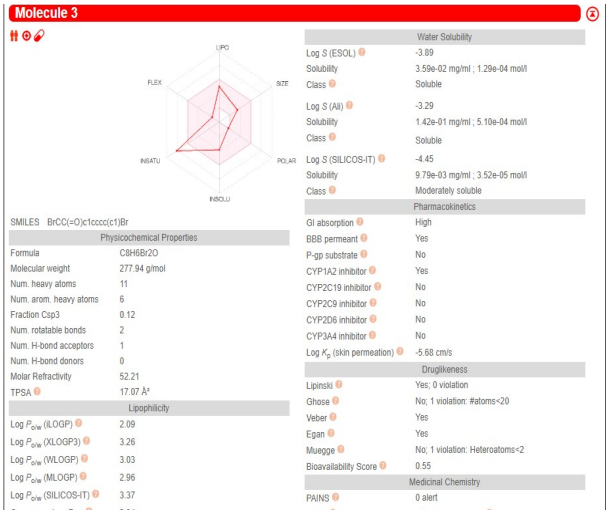

Fig. 19. BromophEnacyl bromide

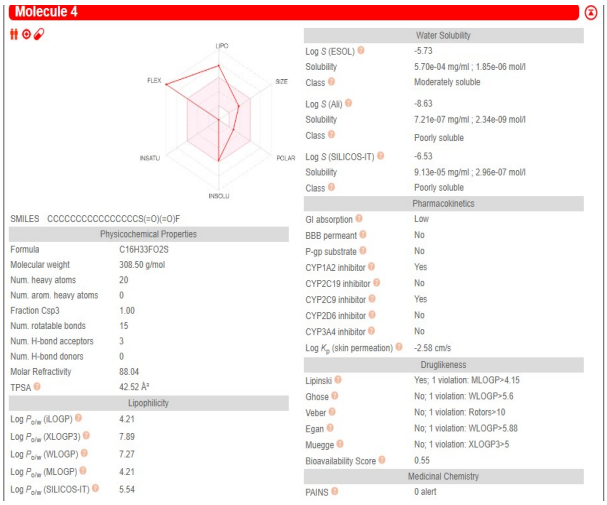

Fig. 20. Hexadecanesulfonyl Fluoride

SwissADME results for each compound predicting necessary parameters on being subjected to a body.

CONCLUSION

After carrying out virtual screening for novel inhibitors which are efficient against OMPLA in both the bacteria, the results show that Aristolochic acid and Bromophenacyl bromide are the two most energy efficient and site specific compounds giving positive result on binding. These molecules show strong potential to inhibit the activity of OMPLA enzyme both in *H. pylori* as well as *C. jejuni.*

These results can be further strengthened by carrying out GROMACS analysis to predict the nature of compound precisely in the human body environment.

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