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Research Article

In silico Screening of ZINC Database for Discovery of Novel Urease Inhibitors as a Remedy to Gastro-duodenal Ulcer Caused by *Helicobacter pylori*

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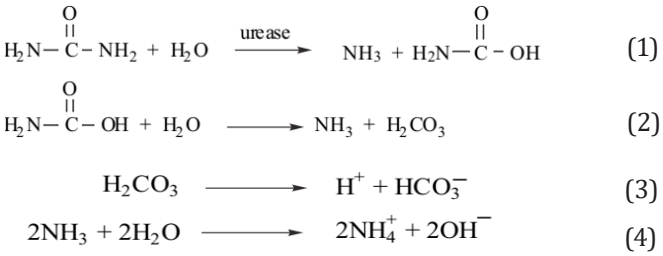
ABSTRACT

Design and synthesis of novel urease inhibitors taking center stage nowadays with specific attention as a remedy to *Helicobacter pylori* infection. Several inhibitors fail *in-vivo* and clinical trials owing to the toxicity and hydrolytic profile. In the present study, we are making an attempt to screen a large small-molecule database, ZINC, for a potential urease inhibitor. The structure-based drug discovery approach has been adopted with acceptable absorption distribution metabolism excretion (ADMET) parameters so that the lead molecules may have fair chances of passing *in vitro* and *in-vivo* trials. The lead molecule in our study, with ID ZINC90446454, is a urea derivative and predicted to be nontoxic. It comes out to be a promising drug candidate with pKd value 7.83, LE 0.429, and LD_{50} value 10100 mg/kg body weight. Its sulfanyl derivative, with predicted high LD_{50} (10100 mg/kg body weight), exhibits the feasibility of a disulfide covalent bond with Cys321 in the active site. The derivative may serve as a novel covalent inhibitor with high specificity, high potency and low toxicity. The derivative, in the future, may be a successful drug candidate for *H. pylori*-induced gastro-duodenal ulcer.

INTRODUCTION

Helicobacter pylori (H. pylori) is known to form a colony in the human stomach inducing gastro-duodenal ulcer. [1-2] *H. pylori* is considered as the most common infectious agent related to stomach cancer. [3] The chronic inflammation of normal gastric mucosa by the bacterium leads to atrophic gastritis, which subsequently leads to intestinal dysplasia and metaplasia. The condition further worsens to the development of carcinoma.[4] The bacterium is capable of surviving and growing in the acidic medium of the stomach because of the production of ammonia as a result of catalytic hydrolysis of urea by urease.[5-6] The catalytic reaction involving hydrolysis of urea has been proposed as follows.[7-9]



(1)

(2)

(3)

(4)

Ammonia being basic, protects the organism against the low pH of the stomach. Experimentally it is found out that a mutant of *H. pylori* lacking in urease is incapable of forming colonies. Hence, specific urease inhibition has been considered as a measure of the strategic elimination of the organism.[10] The people of the developing countries are

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suffering more from the infections caused by *H. pylori*. It is also reported that in certain areas of the world about 50% of the population are infected with *H. pylori*. [5-6] The present regimen for the treatment is a triple therapy which comprises of a proton pump inhibitor along with any two from the following antibiotics amoxicillin (AMX), clarithromycin (CLA), metronidazole (MNZ), and tetracycline (TET).[6] The antibiotic drugs are associated with various side effects, and also the prolonged use of antibiotics develops resistance in the bacterium. The treatment of gastroduodenal ulcers with these drugs has limitations. [11] Therefore, an alternative line of treatment against *H. pylori* is being explored. Urease is a crucial survival factor for the bacterium, is expected to be a potential target for drug development. The absence of urease in an essential function of human is an added advantage in selecting urease as the target. Hence inhibitors of urease may serve as potential anti-*H. pylori* drug candidates. Several small molecules have been reported to be potent inhibitors of the urease. However, many of them have failed as drug candidates due to toxicity or their inefficiency *in vivo*. Hydrolysis of inhibitors may be a significant factor in its failure *in vivo*. There is a need to search for molecules that should satisfy the inhibitory property, low toxicity, and sustainability against hydrolysis. In the present work, we have attempted to screen a large small-molecule database, ZINC, for a urease inhibitor with acceptable ADMET parameters so that the lead molecules may have fair chances of passing *in vitro* and *in-vivo* trials.

Materials and Methods

Selection and Preparation of *H. pylori* Urease

The coordinate file of x-ray crystallographic structure of *H. pylori* urease in complex with acetohydroxamic acid with a resolution of 3Å was obtained from the Protein Data Bank (PDB ID 1E9Y). The enzyme contains two chains, i.e., chain A and chain B. The chain A contains 238 amino acids, and chain B has 569 amino acids. Chain B contains the active site for the binding and hydrolysis of urea. The active site comprises of two Ni^{+2} separated by a distance of 3.02Å. The two Ni^{+2} are coordinated by carboxylate bridge of a carbamylated lysine (KCX219). His248 and His274 coordinate the Ni3001. The Ni3002 is coordinated by His136, His138, and Asp362. The acetohydroxamic acid (HAE800) coordinates to both the Ni^{+2}.[12]

The chain A of 1e9y was deleted from the coordinate file of 1e9y and saved as 1e9yB. The binding site residues of the active site for HAE ligand were identified by using "make a binding site from the ligand" module of ArgusLab 4.0.1. All water molecules were also removed. The residues identified to be present in the binding site are Asp165, Asn168, His221, Glu222, Asp223, Thr251, Cys321, His322, and Arg338.

Docking with ZINC Database

The active site of urease (1e9yB.pdb) was docked with ZINC database using i-Dock online docking server. i-Dock uses the algorithm of scoring function of AutoDock Vina. Small molecules from ZINC database[13] with the following filtering parameters were screened by i-Dock: (a) Molecular weight (g/mol): [220- 500] (b) Partition coefficient xlogP: [1-3] (c) Rotatable bonds: [4-6] (d) Hydrogen bond donors: [2-4] (e) Hydrogen bond acceptors: [4-6] (f) Net charge: [0-0] (g) Apolar desolvation (kcal/mol): [0-10] (h) Polar desolvation (kcal/mol): [-40-0] (i) Polar surface area tPSA (Å2): [60-80]. From the huge ZINC database 1, 58,417 compounds pass through the above filter. The receptor was kept rigid along with the binding site residues within a cubic box of 20 Å with center at coordinate (128, 129, 87), to confine the conformational space to a definite cavity of the receptor.[14] Binding free energy calculation was done as a sum of electrostatic energy, internal energy due to flexible conformational changes, Van der Waals energy, and translational and rotational energy. The more negative the free energy of a ligand-receptor complex, the more stable the complex is. The top 100 ligands were selected from the list. The output from i-Dock is saved as hits.csv and hits.pdbqt. The hits.csv contains binding free energy (iDock score), ligand efficiency, RF-score (binding affinity prediction by random forest method), hydrogen bonds, molecular properties, list of vendors of hit compounds in a tabular format. The hits.pdbqt stores predicted conformations of the hit compounds in pdbqt format.

Lead Identification

The top 100 hit molecules are subjected for screening to check its LipE and ligand efficiency dependent lipophilicity (LELP) ADMET and drug likeness through FAF drugs4 and FAF QED, toxicity screening through ProToxII, and drug-likeness score by MolSosft.

LELP Scoring

From the result of i-dock score two important parameters were calculated, LipE and LELP, those were used for lead identification. If LLE or LipE scoring is >3 and LELP scoring is <10 then the molecule is treated as good lead-like. If LipE is >5 and LELP is within 0 to 7.5 then the molecule is considered as a good clinical drug-like compound. [15] Parameters used for the calculation of LipE and LELP are:

- Ligand Efficiency (LE): LE is proposed to be a parameter, which compares the average binding energy per atom of the molecules [15]

  LE = (1.37/HA)*pIC50 or LE = (1.37/HA)*pKd (5)

  where, HA: The number of heavy atoms, pIC50: the negative logarithm to the base 10 of the half-maximal inhibitory concentration, pKd: negative logarithm to the base 10 of dissociation constant.

LipE or LLE: Lipophilic ligand efficiency is calculated as the difference between pIC50 and lipophilicity (xLogP)

and is an estimate of the specificity of a molecule in binding to the target relative to partitioning into 1-octanol: water.[16]

LipE = pIC50 – xLogP or LipE = pKd – xLogP (6)

LELP: it is called as ligand efficiency dependent lipophilicity is defined as the ratio of xlogP and LE. [17]

LELP = xLogP/LE (7)

Drug Likeness

The prediction for drug-likeness of compounds was made by an online tool FAF-drugs4 (Free ADME-TOX filtering tool 4) developed by Molecules Therapeutic *in silico* (MTI) of University of Paris, France[18] In house Drug-like soft and PAINS A, B, and C[19,20] filters were selected to predict the properties of the compounds and to categorize them into accepted, intermediate, or rejected.

FAF QED (Quantitative Estimation of Drug Likeness)

FAF quantitative estimation of drug-likeness (FAF QED) is an online tool for quantitative estimation of the compound to be drug like in a scale 0–1.[21] For the computation of FAF QED eight physicochemical parameters are considered, namely, molecular weight (MW), octanol-water partition coefficient (logP), the number of hydrogen bond donors and acceptors (HBD and HBA), polar surface area of the molecule (PSA), the number of rotatable bonds (ROTB), the number of aromatic rings (AROM), and already published and known 113 structural alerts (ALERT).

Toxicity Screening Through ProTox-II

ProTox-II is a virtual lab for the prediction of toxicities of small molecules.[22] It is a freely available web server for computing toxicity of small molecules. It can be accessed free online (http://tox.charite.de/protox_II). The webserver enables screening and prediction of toxicity of a molecule at a broad range of toxicity endpoints. Many models of toxicity prediction are taken into account like oral toxicity, hepatotoxicity, mutagenicity, carcinogenicity, cytotoxicity, and immune-toxicity along with the metabolic pathways which are inhibited by the molecule and more specifically the specific target which is inhibited by the molecule in a toxicological pathway.[22] The toxicity is defined in terms of LD_{50} value (mg/kg body weight). The LD_{50} is the oral dose at which death of 50% of test subjects occurs upon intake of a compound. The LD_{50} values are classified into the following six classes:

- Fatal ($LD_{50} \leq 5$)
- Fatal ($5 < LD_{50} \leq 50$)
- Toxic ($50 < LD_{50} \leq 300$)
- Harmful ($300 < LD_{50} \leq 2000$)
- Probably harmful ($2000 < LD_{50} \leq 5000$)
- Non-toxic ($LD_{50} > 5000$)

Lead Optimization

Lead molecule was structurally modified to obtain lead derivatives (LD) using build module of HyperChem Pro 8.0. The derivatives were geometrically optimized by steepest descent method (1000 step) applying Mm+ force field using HyperChem 8.0.

Results

Lead Identification

Top 100 hits in order of idock score were considered for lead identification. The hit molecules were subjected to FAF4 Drugs analysis (supplementary file SF1.xls). All the ligands were found acceptable as a drug like molecules. FAF QED yielded quantitative drug-like property in the scale 0 (most unfavorable) – 1 (most favorable) (supplementary file SF2.xls). The LE, LipE, LELP, ProTox II LD50 and MolSoft Drug-like Scores were estimated (supplementary file SF3.xls) and only ligands with toxicity class 5 and 6 are presented in the Table 1. These are the leads. The ZINC90446454 was selected for further study as it had the lowest toxicity and the best drug-like score reflected by LipE, LELP, QED and MolSoft score (Table 1).

Lead Optimization

The lead ZINC90446454 (1-[(1*R*)-1-benzyl-2-(7-chloro-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-oxoethyl]urea) is a urea derivative (Fig. 1). It is not reported so far as the urease inhibitor as per the best of our knowledge. The interactions of the ligand in the active site of 1e9yB are depicted in Fig. 2a, b. Cys321, H322, and Arg333 are important interactive residues, which sit in the flap region and monitor inhibition. The Cl in isoquinoline ring at position 7 in the lead was substituted by Br and F to obtain the derivatives LD1 and LD2, respectively. It is observed that H12 at position 4 of the isoquinoline ring is close to

Table 1: Lead molecules selected on the basis of dock score, toxicity, and drug-like score

ZINC ID	*Idock score (kcal/mol)*	*pKd*	*HA*	*MW*	*xLogP*	*LE*	*LipE*	*LELP*	*QEDw*	*Pro tox II class*	*LD_{50} mg/kg body weight*	*Mol soft sore*
47874303	-8.507	6.670	24	353.756	2.75	0.381	3.920	7.223	0.885	5	4640	1.47
70702792	-8.422	6.592	25	368.277	2.81	0.361	3.782	7.779	0.831	5	2250	-0.34
65653330	-8.419	7.236	26	354.385	1.99	0.381	5.246	5.219	0.751	5	2200	1.06
90446454	-8.213	7.830	25	357.841	2.56	0.429	5.270	5.966	0.882	6	10100	1.01

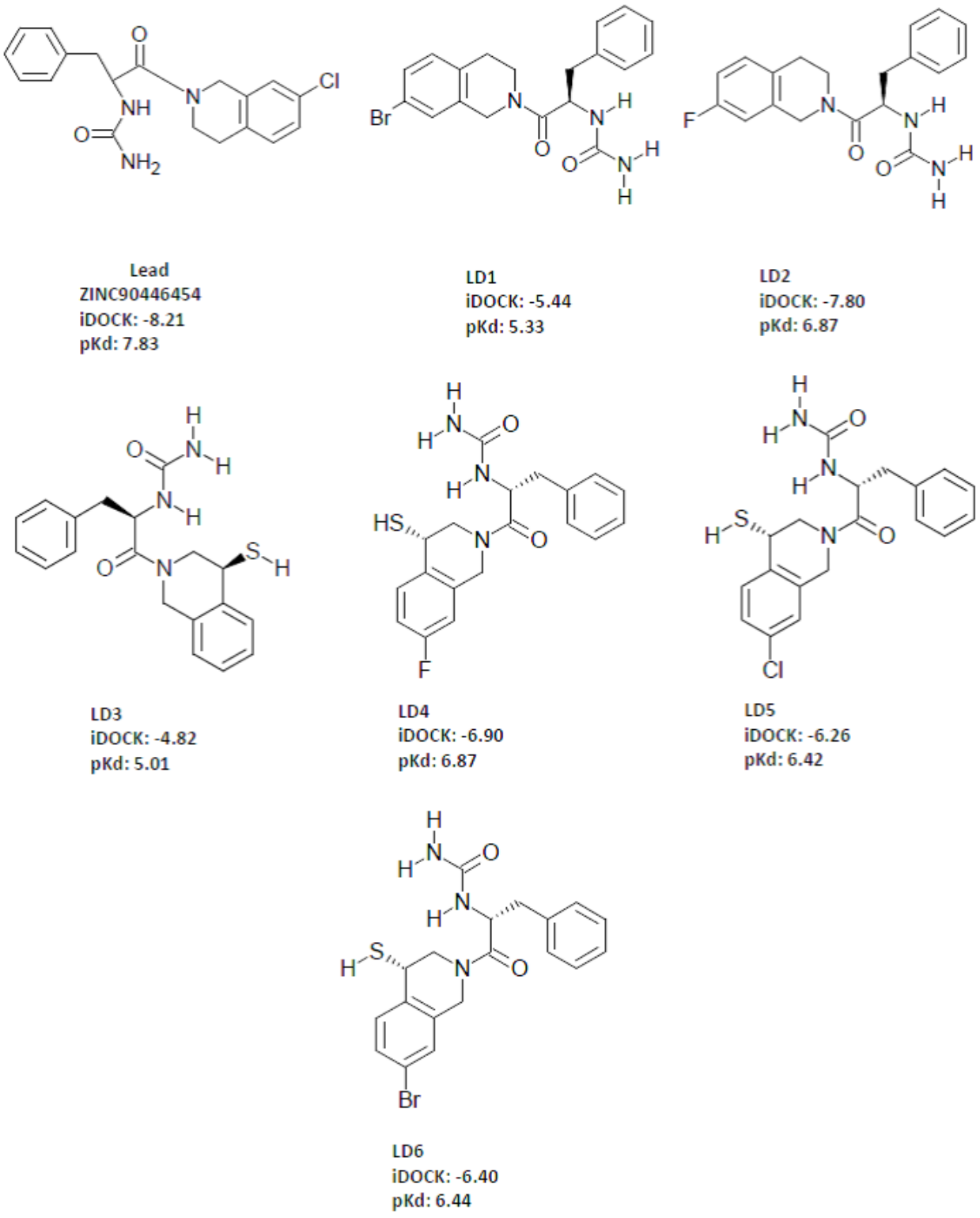

Fig. 1: Structural 2D representation of the lead molecule (ZINC90446454) along with the lead derivatives, LD1 to LD6. iDock score and binding affinity (pKd) values are mentioned below each structure

SG of Cys321 of the enzyme (2.80 Å). The hydrogen may be substituted with SH group to create an opportunity of forming disulfide linkage between SG of Cys321 and S of the ligand. The covalent ligand binding may improve the inhibitory potency of the ligand. Derivative with SH substitution and Cl deletion in ZINC90446454 was built

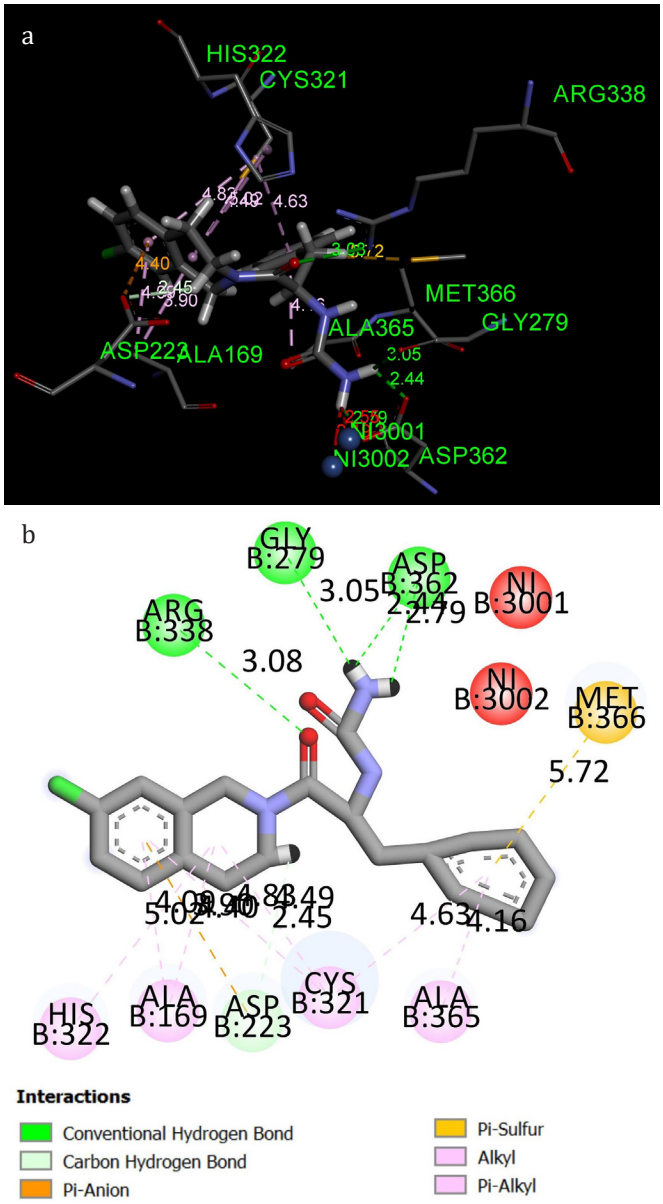

Fig. 2: Interaction of the ligand, ZINC90446454, at the active site of the enzyme 1e9yB, in 3D with atomic details (a) and 2D at residue level only (b). Biovia Discovery Studio 2016 visualizer has been used to prepare the figures.

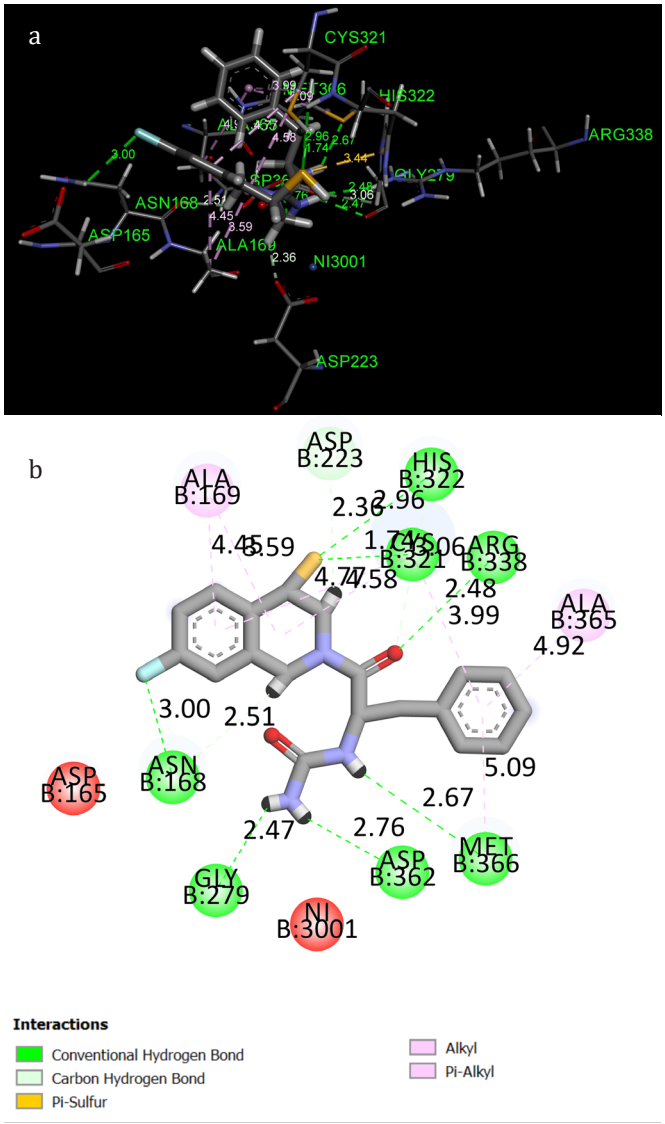

Fig. 3: Shows interactions of the lead LD4 at the active site of enzyme 1e9yB, in 3D with atomic details (a) and 2D at residue level only (b). Biovia Discovery Studio 2016 visualizer has been used to prepare the figures.

(LD3). F, Cl, and Br substituents in LD3 were built as LD4, LD5 and LD6 respectively (Fig. 1). The iDock score and pKd values are mentioned below each molecular diagram. LD4 (1-{(1*R*)-1-benzyl-2-[(4*S*)-7-fluoro-4-sulfanyl-3,4-dihydroisoquinolin-2(1*H*)-yl]-2-oxoethyl}urea) may be having the highest potency among all the LDs. The binding interactions of LD4 with active site of enzyme are depicted in Figs. 3 a, b.

The Covalent Ligand Binding

The covalent binding of the ligand with the receptor was created *in silico*. The optimized disulfide bond and ligand interactions are presented in Fig. 4a, b. The S-S bond length was measured to be 2.07 Å.

Discussion

A large number of compounds of different classes have been reported as a urease inhibitor with the potentiality to be used as drug against *H. pylori*. However, equally, a huge number of them are not found suitable as drug candidates due to toxicity and hydrolytic instability during pharmacokinetics.[23] We made an attempt to explore ZINC database containing 3D structures of over 230 million commercially available compounds with an aim to fish an inhibitor of urease with low toxicity and high efficiency.

A Promising Candidate

The lead molecule ZINC90446454 is a promising drug candidate with value 7.83, LE 0.429, and LD_{50} value 10100 mg/kg body weight. ProTox predictions show no toxic

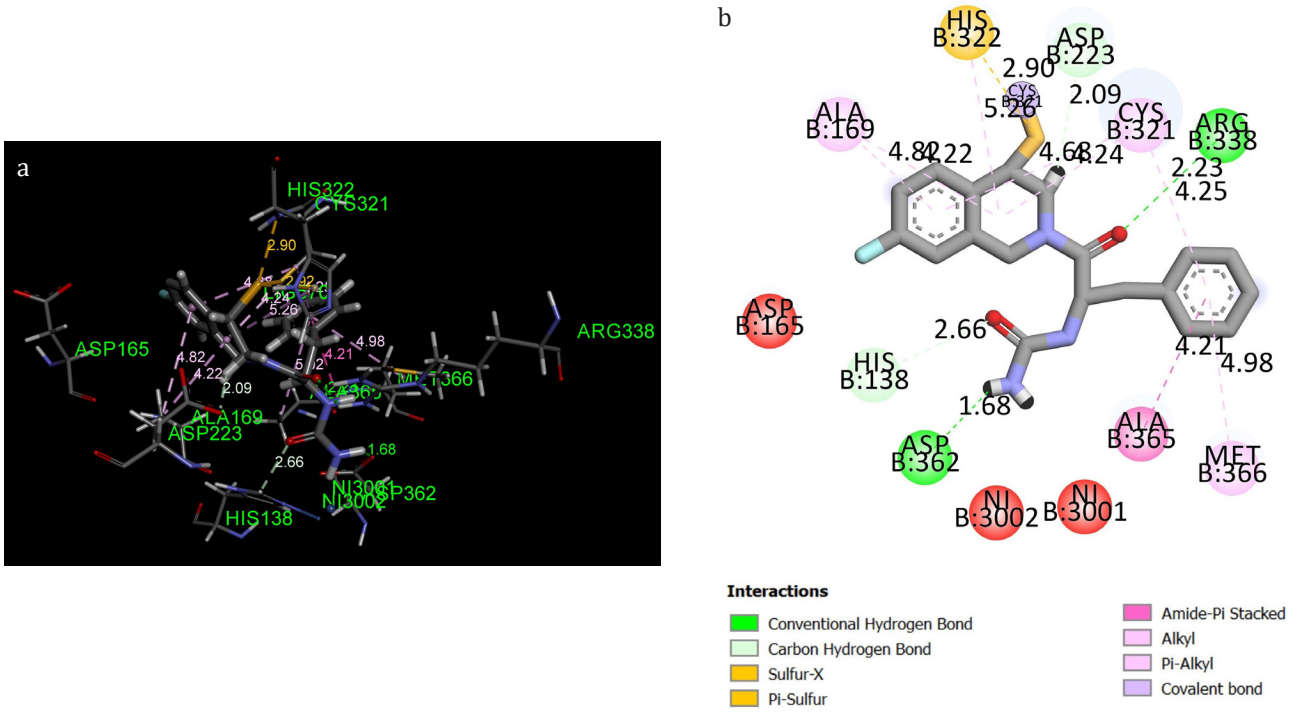

Fig. 4: Covalent binding of lead derivative 1-{(1*R*)-1-benzyl-2-[(4*S*)-7-fluoro-4-sulfanyl-3,4-dihydroisoquinolin-2(1*H*)-yl]-2-oxoethyl}urea to the active site of the enzyme 1e9yB showing disulphide bond formation with Cys321, in 3D with atomic details
(a) and 2D at residue level only
(b) Biovia Discovery Studio 2016 visualizer has been used to prepare the figures.

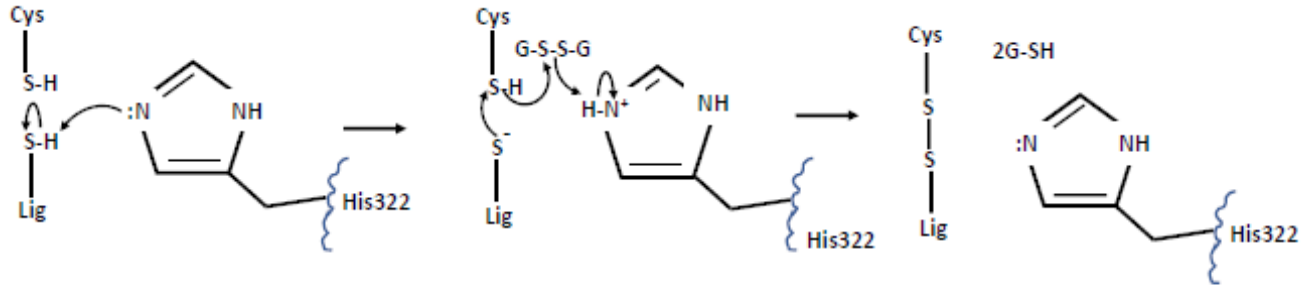

Fig. 5: Proposed mechanism showing His322 mediated nucleophilic attack on SG of Cys321 by sulfanyl group to form disulphide bond in presence of oxidizing agent namely, glutathione disulfide

reactions. It is a urea derivative. A standard approach in drug design is to use a non-active substrate derivative as a successful inhibitor due to pharmacophoric similarity. The present lead is a urea derivative, but still a novel candidate as it has not been reported as urease inhibitor to the best of our knowledge.

A Covalently Bound Inhibitor

A lead derivative 1-{(1*R*)-1-benzyl-2-[(4*S*)-7-fluoro-4-sulfanyl-3,4-dihydroisoquinolin-2(1*H*)-yl]-2-oxoethyl} urea, has a potency to covalently bind to the active site through disulfide bond with Cys321 (Fig. 4a, b). The sulfanyl group is a neighbor to His322 (S12 – NE2: 3.30 Å). His322 may catalyse the formation of a disulfide bond by a mechanism proposed earlier.[24] The NE2 of His322 may act as a base and accept hydrogen from the sulfanyl group forming a negatively charged sulfide group. Nucleophilic attack on SG of Cys321 results in a disulfide bond. Removal of hydride in the presence of ambient oxidant in biosystems, namely, glutathione, hydrogen peroxide, has been depicted in Fig. 5. Designing covalently bound inhibitors are getting increasing attention due to their high potency, low toxicity, and increased stability of the drug-enzyme complex.[25-26]

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