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Original Article

In-silico Design of Covalently Bonding Catechol based Urease Inhibitors as Potential Candidates for Treatment of *Helicobacter pylori* Infection

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ABSTRACT

Design and synthesis of novel urease inhibitors are gaining importance now days with specific context as a remedy to *Helicobacter pylori* infection. Toxicity and hydrolytic profile of urease inhibitors are deciding factors for success of *in vivo* and clinical trials. An attempt is made to design covalently bound inhibitors through C-S bond between aromatic ring carbon and sulfur (SG) atom of Cys321 in *H.* pylori urease, 1e9y. Catechol and p-benzoquinone are known to form such covalent linkage. The catechol ring substituted with an alkyl chain ending with a functional group with potentiality to bind to Ni (II) atoms are designed *in silico*. These designed inhibitors at one end form covalent linkage at Cys321 at the mouth of the active site while link to Ni (II) deep down the site on the other end. These types of molecules with both ends sticky may serve as highly potent inhibitors of urease hence potent drug candidates against *H. pylori* infection. The docking scores and acceptable ADMET parameters are also considered in the design. The compounds may serve as novel covalent inhibitors with high specificity, high potency and low toxicity.

Key words: Urease inhibitors, covalently bound ligands, Helicobacter pylori, Catechol.

1. INTRODUCTION

Importance of the role of urease in microbial pathogenicity in general and *Helicobacter pylori* (*H. Pylori*) induced gastro-duodenal infection in particular is well established [1-7]. Urease inhibitors may serve as a drug candidate for remedy to urease related pathogenic conditions. In spite of the discovery of a large and diverse set of urease inhibitors,

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the search for a novel one is still open as equally large numbers of inhibitors fail to succeed in clinical trials due to toxicity and bioavailability [8]. Polyphenolic compounds are promising inhibitors of urease. Catechol and p-benzonequinol derivatives have been reported be potent inhibitors of urease [9-11]. Crystal structures of catechol and p-benzoquinone show that they are covalently bound to Cys322 in *Sporosarcina* (former *Bacillus*) *pasteurii* urea (SPU) through S-bridge [10, 11]. A free radical mechanism induced by dissolved O_2 is proposed, which is supported with QM calculations [10]. Binding compounds to Cys322 prevents movement of flap stops closing and opening of the catalytic cavity, hence inhibiting the enzyme function.

There is another moiety deep inside the cavity – two Ni(II) ions chelating to the amino acid residues. Urea binds to the Ni (II) dimer and is hydrolysed by enzyme catalysis. The metal ions and its surrounding residues are targets for inhibition. In the present work we design a catechol based molecule which contains a Ni(II) binding functional group attached to catechol ring such that catechol covalently binds to Cys at the mouth of the cavity and the tail binds to the Ni(II) deep inside. The compounds with sticky head and tail are expected to provide novel inhibitors of urease with high potency and specificity.

The active site

The structural coordinates of *H. pylori* urease in complex with acetohydroxamic acid with a resolution of 3Å was obtainedfrom the Protein Data Bank (*http://www.rcsb.org/pdb/*) (PDB ID 1E9Y). The chain B contains the active site for the binding and hydrolysis of urea. The two Ni^{+2}ions in the active site are bridged by carboxylate group of a carbamylated lysine (KCX219). The Ni3001 is co-ordinated by His248 and His274. The Ni3002 is co-ordinated by His136, His138 and Asp362. The acetohydroxamic acid (HAE800) co-ordinates to both the Ni^{+2} [12].

The binding site residues of active site for HAE ligand were identified by using "make a binding site from the ligand" module of ArgusLab 4.0.1 (*http://www.arguslab.com*). 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.

2. MATERIALS AND METHODS

The Target and the Active Site

The file containing the 3D structural coordinates of *H. pylori* urease in complex with acetohydroxamic acid (AHA, crystal structure symbol HAE) with a resolution of 3Å was selected from the Protein Data Bank (*http://www.rcsb.org/pdb/*) (PDB ID 1E9Y). The chain B contains the active site for the binding and hydrolysis of urea. The active site contains two Ni^{+2} bridged by carboxylate group of a carbamylated lysine (KCX219). The Ni3001 is co-ordinated by His248 and His274. The Ni3002 is co-ordinated by His136, His138 and Asp362. The acetohydroxamic acid (HAE800) co-ordinates to both the Ni^{+2} [12].The binding site residues of active site for HAE ligand were identified by using "make a binding site from the ligand" module of ArgusLab 4.0.1 (*http://www.arguslab.com*). 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.

Design of Urease Inhibitors

Catechol is taken as a base structure. Alkyl chains ending with carboxylic, thiocarboxylic, sulfanyl, or phenol are substituted in catechol ring at position 4 (Fig. 1). The 7 molecular models are built using the "Build" module of HyperChem 8.0 Pro. The ligand structures were geometrically optimized applying molecular mechanics and 1000 steps of steepest descend algorithm with Mm+ force field. The model number 2 was taken from one of the best catechol derivative inhibitor (IC50 1.5 µM) for comparison [9].

Docking

AutoDock with general Lamarckian algorithm in YASARA was used for docking of designed compounds to urease active site [13].YASARA Structure provides a tuned derivative of the AutoDock, originally developed by Scripps Research Institute [14]. Provision of simulation cell around the active site, flexibility of ligand as well as some receptor residues in the active site is there inthe YASARA version of AutoDock. Semi-empirical QM calculations employing AM1-BCC (Austin Method 1 Bond Charge Correction) [15] and general AMBER force field (GAFF) parameters [16] to assign high quality Restrained Electrostatic Potential (RESP)-like AutoSMILES charges are generated. These charges are further tuned to the AutoDock scoring function.

A simulation cell of dimensions 25x25x25 Å including the Ni(II) ions and other active site residues was defined. The active site residues, Thr 170 His 221 Asp 223 Trp 224 His 248 Cys 321 His 322 Arg 338 Asp 362 Met 366, were assigned flexibility.

Ligand Efficiency

Ligand Efficiency (LE) is a parameter for comparing molecules according to their average binding energy per heavy atom [17]

LE = (1.37/HA)*pIC50 OR LE = (1.37/HA)* pKd…… (1)

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

MolSoft Drug-likeness Score

The drug-likeness score of compounds were obtained through MolSoft online server (*http://www.molsoft.com/mprop*). Drug-likeness score is computed from different molecular properties, i.e. molecular weight, number of hydrogen bond donors (HBD), number of hydrogen bond acceptors (HBA), polar surface area (PSA), MolLogP, MolLogS, and number of stereo centers. The

score lies between -6.0 to 6.0. The curves for abundance of drug-like molecules show a peak at score 1.0.

ProTox II

ProTox-II is a freely available web server (http://tox.charite.de/protox_II), which predicts toxicities of small molecules [18]. Various models of toxicity prediction are taken into account namely oral toxicity, hepatotoxicity, mutagenicity, carcinogenicity, cytotoxicity, immunotoxicity along with the metabolic pathways. Targets in the toxicological pathways are also identified, which are inhibited by the compounds [18]. The toxicity is summarized in terms of LD50 value (mg/kg body weight).

Bioactivity Prediction

Urease inhibitor and anti-Helicobacter activity of the compounds were predicted by PASS (Prediction of Activity Spectra for Substances). It is an online tool that predicts probabilities of more than 4000 types of biological activity (http://www.pharmaexpert.ru/passonline/predict.php) [19, 20].Structural formula of a compound was fed as an input to get the predicted probabilities of bioactivities.

Molecular Model Building

Molecular drawing 2D, 3D, and structural modifications were done using HyperChem 8.0 Pro (http://www.hyper.com). Geometry optimization is achieved applying molecular mechanic force field, Mm+, running 1000 cycles of steepest descend algorithm.

Structure Visualization

Molecular structures are visualized using RasMol2.7.5 (http://www.openrasmol.org) and Biovia Discovery Studio Visualizer 16.1.0 tools. DS Visualizer is available from (https://www.3dsbiovia.com/products/collaborative-science/biovia-discovery-studio/visualization-download.php).

3. RESULTS AND DISCUSSION

Properties of Ligands

The ligand binding properties namely, binding energy, ligand efficiency and dissociation constant obtained as AutoDock output are presented in Table 1. Compounds 2, 4, 5, and 7 have binding energy < -8.0 and pKd> 6.0. However, compound 2 has toxic activity (Table 1). Ligand efficiency of compounds 5 and 7 are high. Drug-likeness scores obtained by MolSoft online tool is 0.34 for compound 5, and 0.33 for compound 7. Binding energy value of compound 5 (-11.02 kcal/mol) tops the list. Therefore, further study was carried out with the compound 5 (3-(3,4-dihydroxyphenyl) propanoic acid), commonly known as dihydrocaffeic acid (DHCA).

Bioactivity of Ligands

The bioactivities of the ligands namely, urease inhibitor and Anti-*Helicobacter pylori* predicted by PASS are presented in Table 2. The probability that DHCA acts as urease inhibitor is 0.606 (Table 2). Probability of its Anti-*Helicobacter pylori*activity is 0.274. Urease inhibitor and Anti-*Helicobacter pylori* activity probability of the compound 7 is 0.313 and 0.203, respectively.

Table 1: Ligand binding, toxicity and drug-likeness properties of the compounds

Comp	SMILES	BE	LE	pK_d	ProToxLD50	Toxic activity	Drug-likeness
1	Oc1ccc(cc1O)CCc1ccc(cc1)S	-7.01	0.412	5.137	2000	ER,ER-LBD,MMP	0.47
2	Oc1ccc(cc1O)CCc1ccc(cc1)O	-8.43	0.496	6.176	2000	ER,ER-LBD,MMP	0.47
3	Oc1cc(ccc1O)/C=C/C(=S)O	-6.78	0.522	4.971	980	Carcino,Immunotoxicity,AhR,ER-LBD	-0.32
4	Oc1cc(ccc1O)CCC(=S)O	-8.89	0.684	6.516	2000	Inactive	0.25
5	Oc1cc(ccc1O)CCC(=O)O	-11.02	0.848	8.077	2000	Inactive	0.34
6	Oc1cc(ccc1O)/C=C/C(=O)O	-7.02	0.540	5.145	2980	Carcino,AR	-0.02
7	c1cc(c(cc1CCS)O)O	-8.88	0.807	6.511	2820	Inactive	0.33

BE: binding energy in kcal/mol, LE: ligand efficiency. pK_d:negative logarithmto the base 10 of dissociation constant in M, LD50: 50% lethal dose in mg/kg body weight, ER: estrogen receptor alpha, ER_LBD: estrogen receptor ligand binding domain, MMP: mitochondrial membrane potential, AR: androgen receptor, AhR: aryl hydrocarbon receptor.

Table 2: Predicted Bioactivity of catechol derivatives by PASS

Compound number and name	Activity	Pa	Pi
1. 1-(3,4-dihydroxyphenyl)-2-(4-sulfanylphenyl)ethane	Urease inhibitor	0.398	0.019
	Anti-Hp	-	-
2. 1-(3,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)ethane	Urease inhibitor	0.537	0.005
	Anti-Hp	0.286	0.36
3.(E)-3-(3,4-dihydroxyphenyl)prop-2-enethioic O-acid	Urease inhibitor	0.533	0.007
	Anti-Hp	0.256	0.056
4.3-(3,4-dihydroxyphenyl)propanethioic O-acid	Urease inhibitor	0.551	0.006
	Anti-Hp	0.405	0.009
5.3-(3,4-dihydroxyphenyl)propanoic acid	Urease inhibitor	0.606	0.004
	Anti-Hp	0.274	0.043
6.(E)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid	Urease inhibitor	0.554	0.006
	Anti-Hp	0.391	0.010
7.4-(2-sulfanylethyl)benzene-1,2-diol	Urease inhibitor	0.313	0.037
	Anti- Hp	0.203	0.036

Pa: probability of activity, Pi: probability of inactivity, Hp: *Helicobacter pylori*

DHCA: The Lead

DHCA emerges as the best candidate as urease inhibitor as well as pro-drug. The orientation and interactions of DHCA with the active site residues of urease are depicted in fig. 2A and 2B, respectively. The carboxylic group is close to both the Ni3001 and Ni3002 (1e9y chain B) at a distance of 1.94 and 2.19 Å. The carbon C6 of the catechol ring of DHCA is at a distance of 4.57 Å from the S (SG) of cys321. C6 and SG form a covalent bond through free radical mechanism in presence of dissolve oxygen [10]. The distance between these atoms before the bond formation in QM calculation is

4.28 Å [10]. The distance values are comparable in both the cases. The interaction shows a bridge H-bond between O of Ala169 and OH (2.73 Å) of carboxylic group of DHCA and carboxyl O of KCX219 (1.77 Å). Other hydrogen bonds are O of carboxylic group of DHCA and H(ND) of His248 (2.06), O of hydroxy at C2 of DHCA and H(ND) of His221 (2.21 Å), H(OH) at C2 of DHCA and OE2 of Glu222 (2.04 Å). O of the carboxyl group of DHCA form metal coordination (1.94 Å).

Covalent Bond Formation

Covalent bond formation between C6 (DHCA) and SG (Cys321) is accomplished *in-silico* followed by geometry optimization. The orientation of covalently bonded DHCA in the active site is depicted in fig. 3A and 3B. The carboxylic group of DHCA moves slightly away from Ni atoms (O of carboxyl of DHCA and Ni3001 metal coordinate bond, 2.50 Å). H-bonds are also reformed. O of carboxylic group of DHCA forms H-bond with H(ND) of His221 (1.70 Å). HO of both hydroxyl group of catechol ring form H-bond with O of carboxylic group of Asp223. C6-S and CB-S bond lengths are 1.89Å.

The design principle of orientation of catechol based ligands is validated *in silico* by results of docking process.

DHCA: The Metabolite

DHCA is a known metabolite in human after the intake of caffoeylquinic acids present in coffee beans, artichoke leaves, and *Hibiscus subdariffa leaves* [21]. Its concentration in plasma peaks at 4-5 hrs after the intake of caffoeylquinic acids containing diet [21]. DHCA, Chlorogenic acid, and caffeic acids are reported as an effective antioxidant inhuman [22, 23].

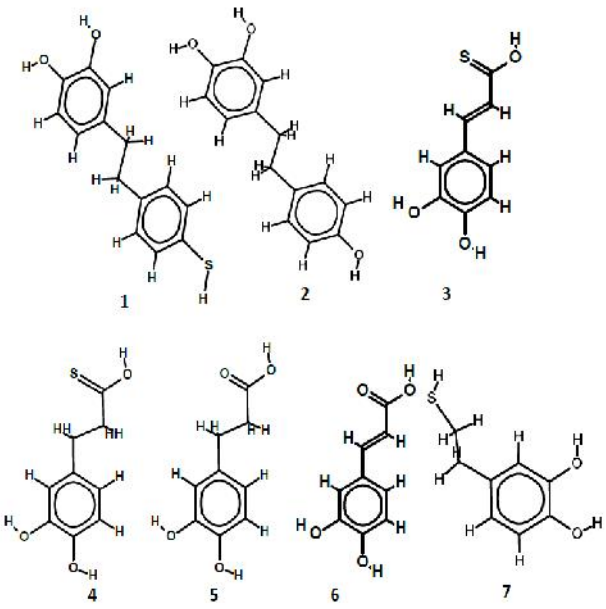
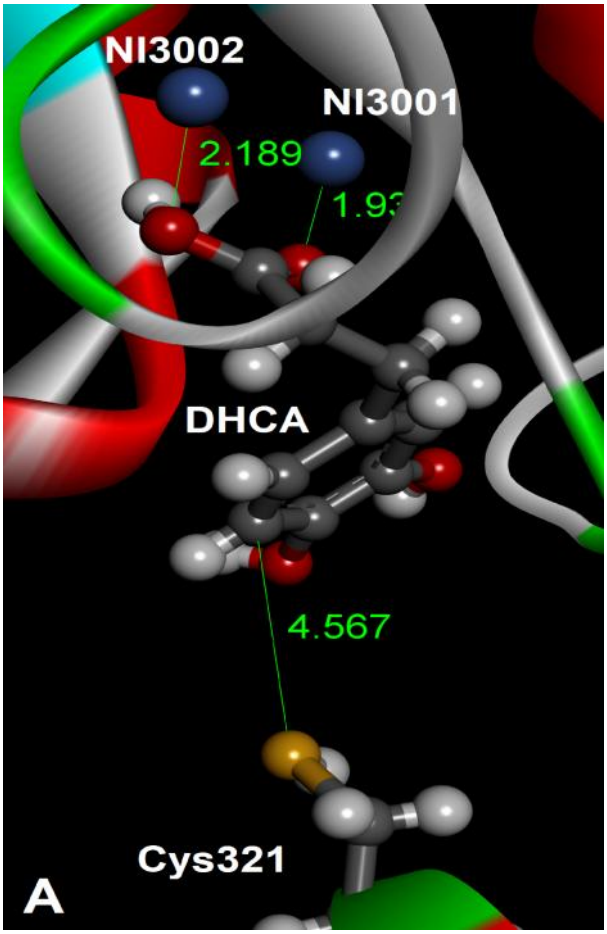


Fig 1: The structural representation of designed ligand data set compounds. For details of the compound please refer to Table 2.



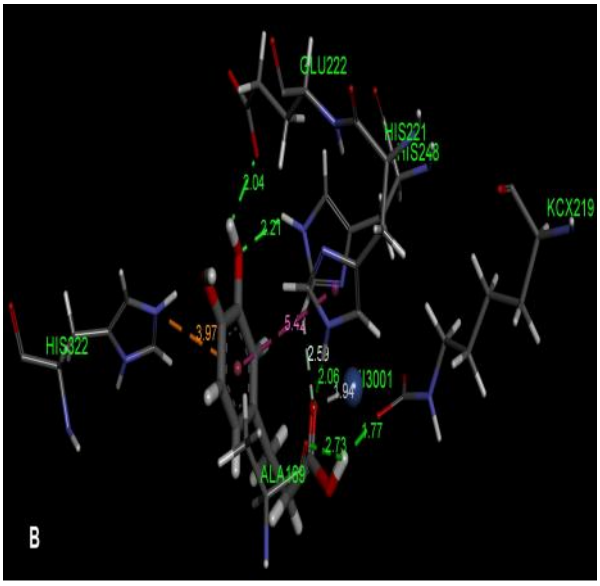
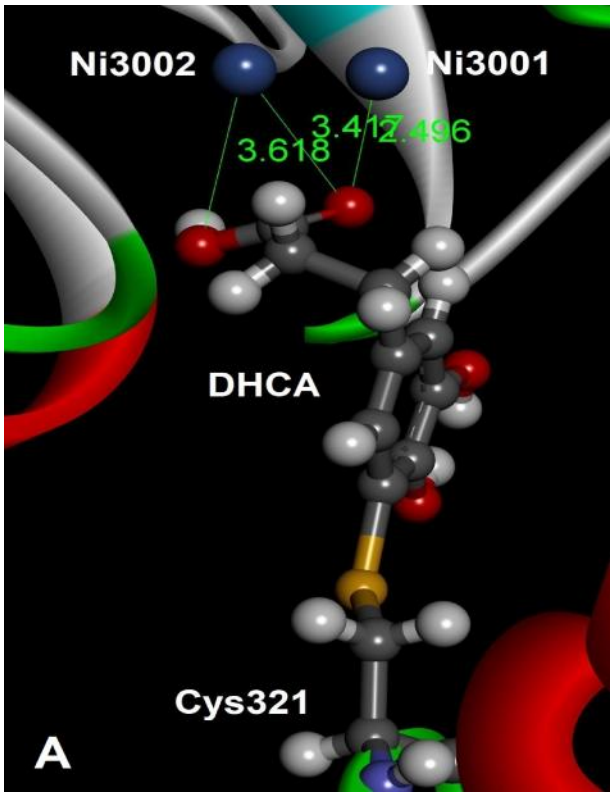


Fig 2. A: Depiction of the orientation of the docked dihydrocaffeic acid (DHCA) in the active site cavity of urease (PDB ID 1e9y Chain B). B: Interactions of DHCA with the active site residues. The figures are prepared applying Biovia Discovery Studio visualizer.



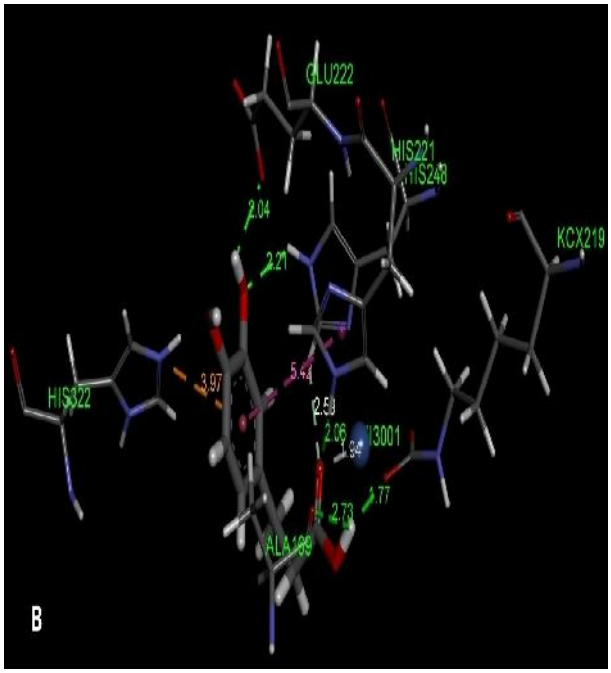


Fig 3. A: Depiction of the orientation of the covalently bonded dihydrocaffeic acid (DHCA) to the active site residue Cys321 of urease (PDB ID 1e9y Chain B). B: Interactions of covalently bound DHCA to Cys321 with the active site residues. The figures are prepared applying Biovia Discovery Studio visualizer

4. CONCLUSION

The design of the ligands in the present work was inspired by the knowledge of binding site and its covalent nature of binding through the work of Mazzei *et al*. (2016) [10]. The design principle of the attachment of a tail piece with a metal coordinating group at position 4 of the catechol ring was successful at least at the theoretical level to achieve a novel class of inhibitors, which bind both at the mouth of the active site cavity as well as deep inside the cavity with covalent and metal coordinate bonds. This may impart a tight binding inhibitor with high potency and specificity. The work would be further carried out to establish the experimental validity of the findings. There is scope to design novel molecules with novel tail ending groups and benzoquinone base in place of catechol. The work opens up a new avenue in the search of novel urease inhibitors.

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