Molecular Docking Study of 5-substituted-8-methyl-2H-pyrido [1, 2-a] pyrimidine-2, 4 (3H) – diones As Inhibitors of *Basilluspasteurii* urease

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ABSTRACT:

Urease is a nickel dependent amido hydrolase which help in the catalysis of urea breakdown into ammonia and carbon dioxide. Ureases are acknowledged to be a causal agent of many diseases in humans and animals. High urease activity in agriculture during urea fertilization causes significant environmental and economic problems by releasing large amount of ammonia into the atmosphere. Molecular docking of some reported compounds was performed via MOE-Dock to predict their binding modes. The docking result showed good correlation between experimental activities and docking scores. From the binding modes of the compounds it was observed that most active compounds established coordination with nickel atoms whereas less active compounds were unable to make coordination bond with nickel ions. These finding may be exploited to design new and potent urease inhibitors.

Key words: Molecular docking, MOE-Dock, Urease inhibitor, Bacillus pasteurii.

1. Introduction

Urease is a nickel dependent amido hydrolase which help in the catalysis of urea breakdown into ammonia and carbon dioxide With carbamate as intermediate molecule [1]. Major pathologies are caused by ureases induced by *Helicobacter pylori* (HP), as it allows the HP to survive on low pH of stomach, thus allowing them to inhabit bacteria in the mucus layer[2.3]. Ureases are acknowledged to be causal agent of many diseases in animals and humans. Ureases are known to be involved in the development of contagious stones as well as in the pathogenesis of pyelonephritis, ammonia and hepatic encephalopathy, urinary catheter encrustation and hepatic coma. Urilithiasis mainly in dogs and cats are caused by the secretion of urease by *Helicobacter pylori* [4-5].

Ureases from Jack bean (JB) [6,7], *Klebsiella aerogenes* (KA) [8] and *Basillus pasteurii*(BP) [9] have been broadly studied. It has been confirmed by Stoichiometric and spectroscopic investigation of thiol bond that nickel per active site is present in all these ureases [10,11]. Structural differences have been seen between the enzymes obtain from plants and micro-organisms. KA ureases have three subunits α, β and γ, in $\alpha\beta_2\gamma_2$ form, [12,13], BP urease is in heteropolymeric form having α, β and γ subunits and JB urease is present in hexamer form having identical subunits [14].

Urease inhibitors identified up-to-date is rather limited and incapable to interact with key amino acid residue and metal ion of active site. In the present study binding modes of some known urease inhibitors were explore via molecular docking. Good correlation was found between docking scores and biological activities of compounds. These results might be useful in the designing of new and potential urease inhibitors.

2.1. Material and methods

The molecular docking study was performed on Intel xenon server system having 3.8GB RAM with the open Suse 11.4 (X 86_64) operating platform. The MOE-Dock was used as docking software implemented in MOE (Molecular Operating Environment) [15]. MOE is a software system designed by the Chemical Computing Group to support Chemoinformatics, Molecular Modeling, Bioinformatics, Virtual Screening, Structure-based design and can be used to build new applications based on

SVL (Scientific Vector Language). LigPlot implemented in MOE was used to visualize the interaction between urease and ligands.

2.2. Ligand preparation

The compounds included in our study were all collected from reported literature [16]. All these compounds were modeled using Builder tool implemented in MOE. All the compounds were energy minimized via MOE using default parameters [17].

2.3. Protein preparation

The protein structure of *Bacillus pasteurii* complex with acetohydroxamic acid was retrieved from protein data bank (PDB ID 4UBP) [18]. All water molecules were removed from the protein and then 3D protonation and energy minimization was performed via MOE with default parameters.

2.4. Molecular docking

To explore the binding modes of ligands, molecular docking was carried out using MOE-dock with most of the default parameters [19, 20]. For each ligand 30 conformations were generated. The top ranked conformation of each ligand was used for detailed study of binding mode.

3.1. Result and discussion

To check the validation of our docking tool, the co-crystallized ligand (acetohydroxamic acid) was removed and redocked within the binding cavity of *Bacillus pasteurii* urease and the docked pose was compared to the crystal structure conformation by calculating RMSD values (**Figure 1**). RMSD values smaller than 2.0 A^o indicate that the docking protocol is capable of accurately predicting the binding orientation of the co-crystallized ligand. The calculated RMSD was found to be 0.3964 A^0 that showed that docking protocol could be used for the prediction of binding modes of other compounds.

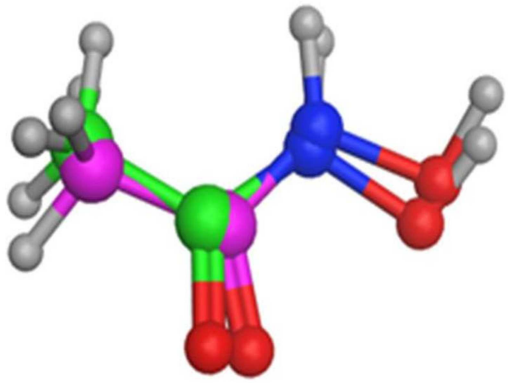


Figure 1; Root Mean Square Deviation of Co-Crystallized ligand (Greenish) and native ligand (Purple).

3.2. Correlation between docking scores and biological activities

Good correlation between docking scores and biological activities was observed as shown in **Table 1**. The results produce good coefficient result (r^2 = 0.572) between docking score and IC_{50} values after the removal of three compounds **6**, **8** and **17** as shown in **Figure 2**.

From the docking conformation of compound **9**, the most active compound in the series showed that sulfur group of 2-amino thiophenol of PPMDO interact with active site residues, Arg 339, Gly 223 and Asp 224. Compound **2** also have good activity at the concentration of 0.01 µM as shown in **Table 1**. Pyrimidine-dione ring of the compound showed arene-hydrogen interaction with His 323. The amine group (NH) made a back bone hydrogen donor interaction with Ala 170. Carbonyl oxygen of acetophenone moiety interacts with the metal Ni 798 which is an important structural feature of the target protein as shown in **Figure 3** (a).

Binding mode of compound **14,** the third most active at 0.01 µM (Table 1), showed that oxygen atoms of nitro group of 4-nitrobenzoic hydrazide substituent of PPMDO coordinate with the Ni 798, Ni 799. A hydrogen bonding interaction was found between acidic Asp 224 and Nitrogen of hydrazide moiety. An arene-hydrogen interaction was also observed between pyrimidine-dione ring of the compound and His 323 as shown in **Figure 3(b)**.

Compounds **9, 2** and **14** docking scores showed good correlation to that of experimental activities. These three compounds are more potent than other compounds because of the presence of SH group, carbonyl oxygen and of nitro group respectively. All these groups are metal chelating groups and interact with the important structural metallic feature of target protein that may enhance the inhibition.

Compound **6** the moderate active compound (Table 1) also showed three interactions with the binding side residues of target protein as shown in **Figure 3(c).** Two arene-hydrogen interactions were observed between the constituent rings of PPMDO and His 323. A back bone hydrogen interaction was found between Lys 169 and amino (NH) group of the compound. The lower activity of this compound may be

attributed to the absence of metal chelating group.

Compounds **8** and **10** are showing weak activities as given in **Table 1.** In compound **8** the aromatic ring of fluorobenzene interacts with Ala 170 and the adjacent amino group (NH) made interaction with Ala 366 as shown in **Figure 3(d).** While in compound **10** carbonyl oxygen of PPMDO ring interacts with that of His 323.

The reason for the low activity of compound **8** and **10** as compared to that of most active compounds is may be the presence of less active groups, fluorobenzene and methylbenzene respectively, lake of metal chelating groups and lower number of interactions to that of important active site residues. Other compounds as given in **Table 1** showing lower activity shares the same reason.

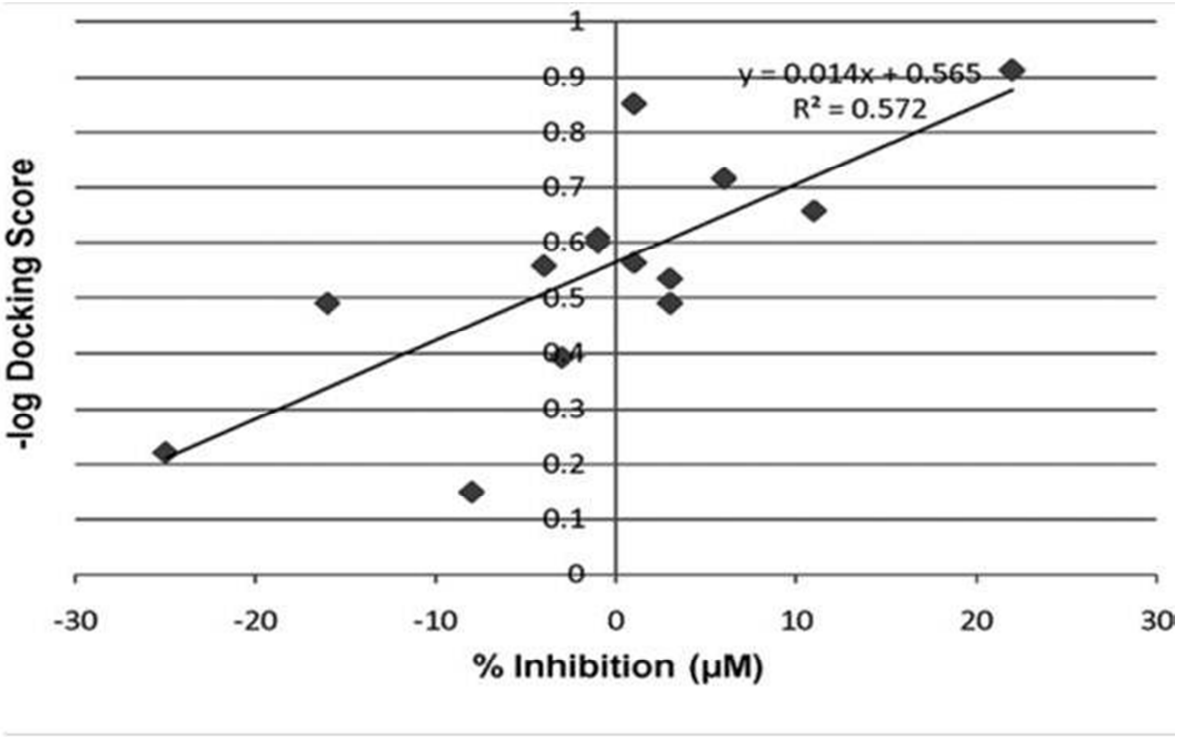


Figure2; Correlation graph between docking predicting activity and % inhibition.

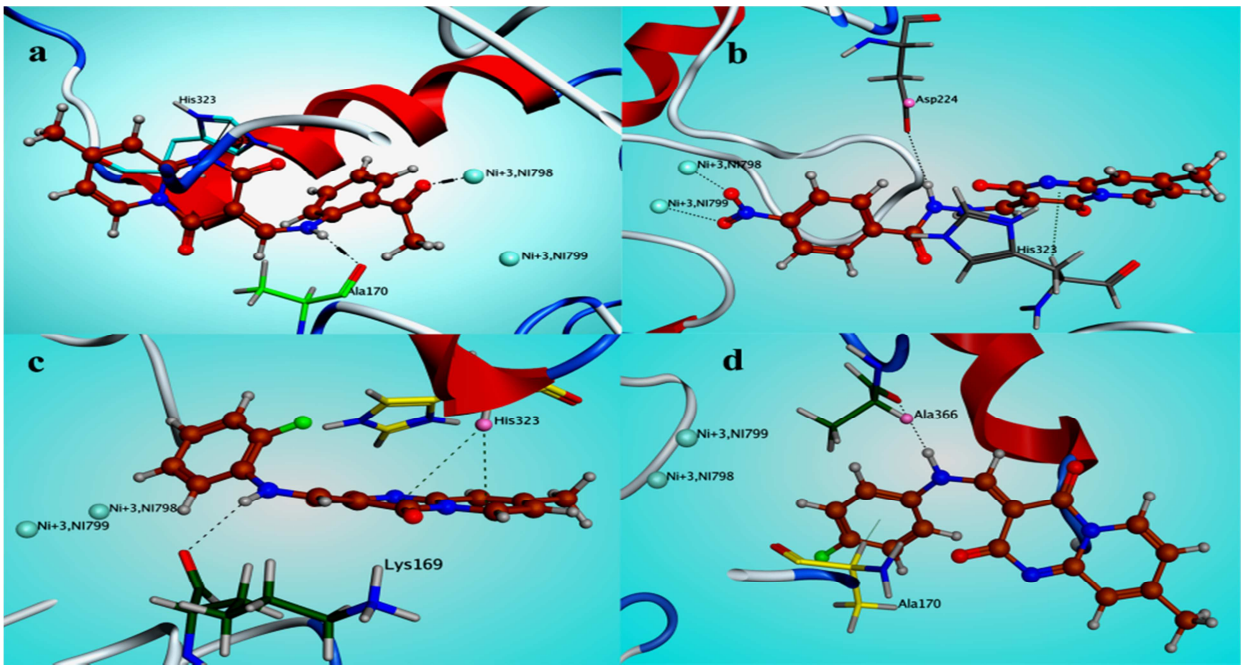
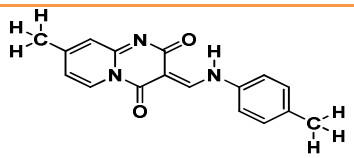
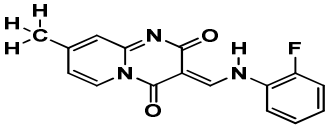
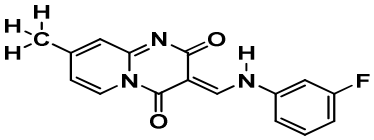
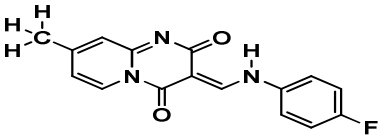
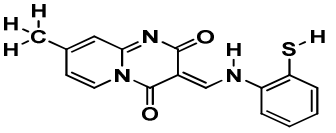
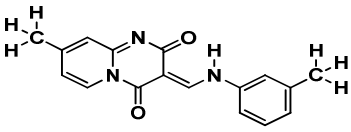
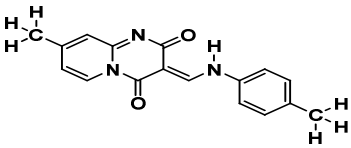
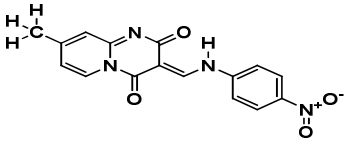
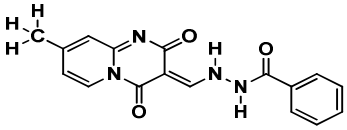
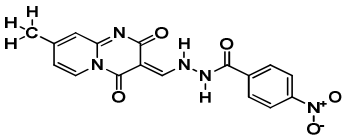
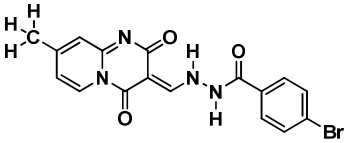
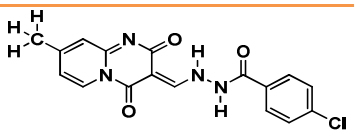
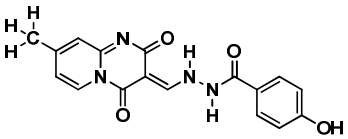


Figure 3; 3D Pictures of the docked conformations of most active compounds showing their interaction with active residues (a) binding mode of compound 2 (b) binding mode of Compound 14 (c) binding mode of compound 6 (d) binding mode of compound 8.

Table 1: Docked compounds structures with their respective docking score and their inhibitory activities against urease enzyme.

S.no	Structures	S. scores	% inhibition at 0.01 µM
1		0.2730	1
2		-4.5470	11
3		-4.0738	-1
4		-2.4688	-3

5		-3.0988	-16
6		0.0513	3
7		-0.6005	-25
8		0.9927	1
9		-8.1905	22
10		7.1140	1
11		-3.0965	3
12		-3.4246	3
13		-3.9923	-1
14		-5.2060	6
15		0.7073	-8

16		0.2764	-4
17		1.0122	-5

4. Conclusion

Molecular docking was performed to recognize the binding interactions. We analyze the interaction of ligands to that of our target protein. Docking results indicate good correlation between activities of active compounds and docking scores. This study tells us the importance of utility of computational tools in correlating experimental results with docking scores. We suggest that compound 9, 2 and 14 shows good interactions with active site residues, might be good candidate for discovery of urease based disorders.

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