Heterochelates of metals as an effective anti - Urease agents couple with their docking studies

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Abstract: The current article discusses the activities of several synthesized metal heterochelates in in-vitro as anti-ulcer agents followed by their docking study. For this purpose, two important ligands like 8-hydroxyquinoline and *DL*-methionine were used in synthesis of heterochelates of metal including Cr (III), Mn (II), Fe (III), Co (II), Ni (II), Cu (II), Zn (II), Cd (II) and Pb (II). It was observed that these complexes showed excellent urease inhibition activities in which thiourea was the standard having IC_{50} value 21.6 ± 0.12µM. The Cu (II) complex showed potent inhibitory activity (22.6 ± 0.72 µM) when compared with the standard thiourea (21.6±0.12µM) among the nine synthesized complexes while Mn (II), Fe (III), Cd (II) and Pb (II) also showed better inhibitory activities. The urease inhibitory activities of hetercochelates also tested and validated by docking analysis.

Keywords: Urease, inhibition activity, complexes, docking.

INTRODUCTION

Urease is referred to as a Ni-containing metalloenzyme used to catalyze the hydrolysis of urea into ammonia (NH_3) and carbon dioxide (CO_2). It is naturally present in plant seeds (jack beans, soybeans, etc.), animal tissues and soil microorganisms. Hydrolysis of urea is a slow reaction while in the presence of urease enzyme, the rate is increased by 10^{14} times than nonenzymatic hydrolysis (Mobley *et al*., 1995). Urease mechanism is responsible for the development of many diseases in humans like ulcer, urolithiasis, pyelonephritis, ammonia and hepatic encephalopathy, hepatic coma, and urinary catheter encrustation (Bremner *et al*., 1989, Arshad *et al*., 2018, Kajiwara 2003). A Gram-negative bacteria, Helicobacter pylori, produces urease enzyme which causes peptic ulcer and stones by using urease mechanism (Bremner *et al*., 1989). In soil, urease enzyme is produced by some microorganism (genus *Proteus*, *Klebsiella*, *Bacillus* and *Sporosarcina*) (Phang *et al*., 2018); hydrolyzed product of urea is further converted into ammonium salt which is a basic nutrient for the plants. However, the excessive urease activity increases the production of ammonia which increases the ammonia toxicity in the environment as well as increases the consumption of urea (Sahrawat 1980, Bremner *et al*., 1989). Urease inhibitors are used to reduce the activity of urease enzyme by decreasing the hydrolysis of urea. This inhibition results in the decrement of *H. Pylori* survival inside the mucosa of the stomach. Discovery of new urease inhibitors is the active area of research to overcome all losses and health problems. A number of urease inhibitors have been reported which includes heavy metal ions (Habala *et al*., 2018), hydroxamate derivatives (Font *et al*. 2008), phosphoramidites, urea derivatives, polyphenol (Zizian *et al*., 2012), thiols (Benini *et al*. 1998), boric acid (Krajewska *et al*., 1997). heterocyclic compounds (Karaali *et al*., 2018), schif base (Sangeeta *et al*., 2018). and phosphate (Krajewska and Ciurli., 2005). However, metal ion and their complexes with biologically active ligands are widely used to inhibit urease. Literature proved that heterochelates of many metals are known for biologically active nature for pathogenic microorganisms (Thakkar 2000) and play a significant part in the activation of enzymes. Usually, it has been seen that the complexes which have oxine ligand as a primary ligand are biologically active (Thakkar and Howard-Lock 1987). It was also reported that metal complexes of amino acids as ligands have significant biological and enzymatic properties (Perrin and Agarwal 1973). The aims and objectives of the current investigation are to search for a new horizon in the synthesis of metal complexes with their activities for controlling the significant diseases. For this purpose, synthesized complexes used in-vitro to monitor the urease activity. The chemical docking studies

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of new complexes were also discussed in the relevant section of this article.

MATERIALS AND METHODS

Inhibition activity and docking of Heterochelates

All solvents and chemicals used as received. Heterochelates (1-9) synthesized by using Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb metal with L_1 (8-Hydroxyquinoline) and L_2 (DL-Methionine). Synthesis and characterizations of several hetrochelates conducted by the method described by (Patel *et al*., 2012) and their structure reported in the (fig. 1).

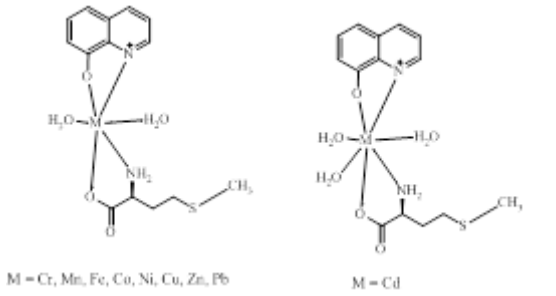

Fig. 1: The structure of synthesized metals complexes

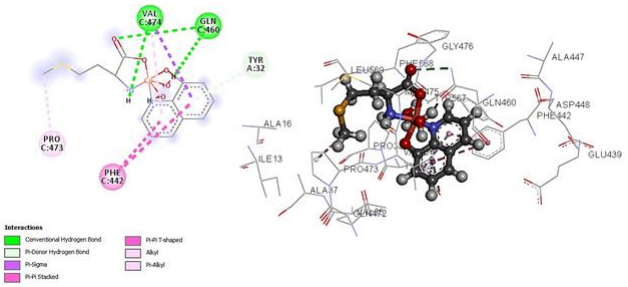

Fig. 2: 2D and 3D depiction of docking of (6) in active site of *Bacillus pasteurii* urease.

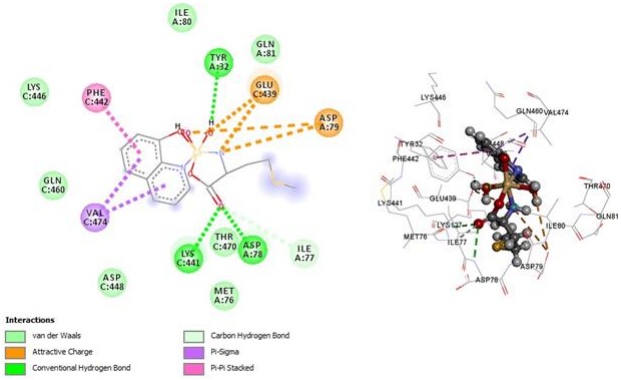

Fig. 3: 2D and 3D depiction of docking of (8) in active site of *Bacillus pasteurii* urease.

Urease inhibition activity

The urease inhibitory activity was resoluted by a modified indophenol method described by (Lateef *et al*., 2012) in triplicates which established quantity of ammonia produced during the reaction, results an increase in absorbance after 50 min which was recorded at 630 nm on the micro plate reader. The thiourea was used as standard in this assay while inhibitions percentage determined through following formula:

$$\text{Inhibition (\%)} = \frac{(\text{OD control} - \text{OD test comp})}{\text{OD control}} \text{ X } 100$$

The IC_{50} values were then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc. Amherst, MA, USA).

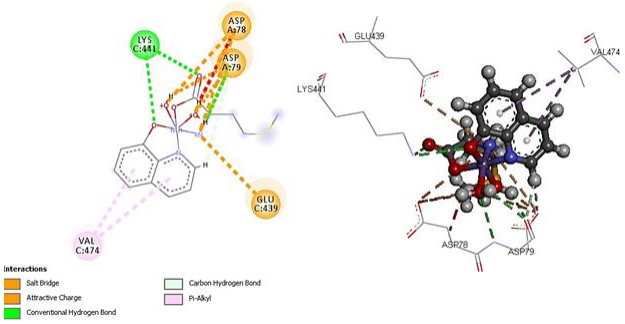

Fig. 4: 2D and 3D depiction of docking of (2) in active site of *Bacillus pasteurii* urease.

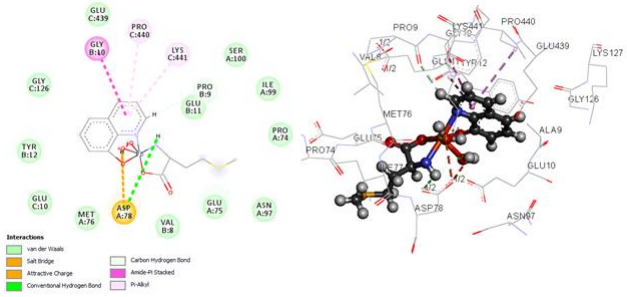

Fig. 5: 2D and 3D depiction of docking of (9) in active site of *Bacillus pasteurii* urease.

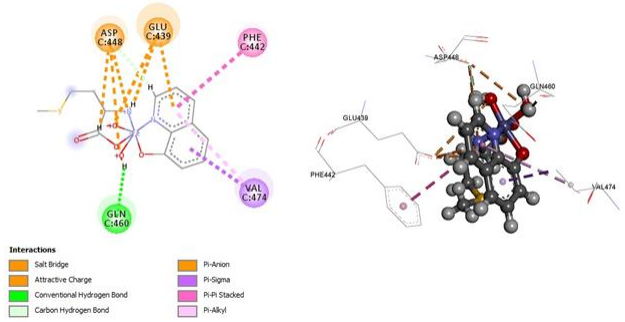

Fig. 6: 2D and 3D depiction of docking of (3) in active site of *Bacillus pasteurii* urease.

Molecular docking study

Autodock (Morris *et al*., 2009) was used to perform the docking simulations employing the 3D structure of *Bacillus pasteurii* urease (PDB code: 4ubp). The Protein Data Bank was used to retrieve the "pdb" file (www.pdb.org). After the removal of all the het-atoms and the ligand, the Autodock tools were used to convert the protein to pdbqt format (1.5.6) (Morris *et al*., 2009). For the preparation of ligand, the Marvin sketch (5.8.3)

Table 1: Urease inhibition activity of Ligands and Compounds 1-9

Compound	Molecular formula	Mol. wt. (g/mol)	Urease Inhibition IC50 (µM)
L1			75.86 ± 0.82
L2			49.9 ± 0.20
1	$C_{14}H_{20}CrN_2O_5S$	380.05	> 200 µM
2	$C_{14}H_{16}MnN_2O_3S$	347.29	57.4 ± 0.44
3	$C_{14}H_{16}FeN_2O_3S$	348.20	69.0 ± 0.34
4	$C_{14}H_{16}CoN_2O_3S$	351.29	> 200 µM
5	$C_{14}H_{16}NiN_2O_3S$	351.05	> 200 µM
6	$C_{14}H_{16}CuN_2O_3S$	355.90	22.6 ± 0.72
7	$C_{14}H_{16}ZnN_2O_3S$	357.73	> 200 µM
8	$C_{14}H_{21}CdN_2O_6S$	458.81	53.7 ± 0.34
9	$C_{14}H_{20}N_2O_5PbS$	535.58	64.5 ± 0.82
Thiourea			21.6 ± 0.12

Table 2: Binding energies of synthesized compounds with bacillus pasteurii urease

Compound	Urease Inhibition IC50 (µM)	Binding affinity (kcal/mol)
2	57.4 ± 0.44	-7.0
3	69.0 ± 0.34	-6.7
6	22.6 ± 0.72	-8.2
8	53.7 ± 0.34	-7.2
9	64.5 ± 0.82	-9.7

was used for two-dimensional chemical structures of ligands (http://www.chemaxon.com) and these structures were then converted to 3D format by the Open Babel (ver 2.3.1) (O'Boyle *et al*., 2011). Finally, Autodock Tools were used to prepare the final pdbqt format of ligands. The following parameters were used to perform the docking simulation: size x=82.6443; size y= 86.0453.2962; size z=67.2583; center x=15.5310; center y = 94.9548; center z=74.2453.

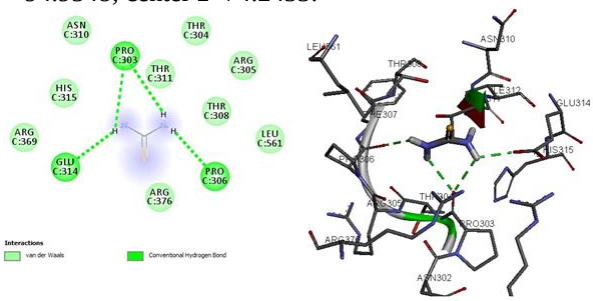

Fig. 7: 2D and 3D depiction of docking of in active site of *Bacillus pasteurii* urease.

RESULTS

The molecular formula of all synthesized complexes (1-9) using two lignads were reported in the table 1 which were subjected to urease activity followed by in- silico or docking studies, while their results are expressed in the form of IC_{50} values (table 1). The IC_{50} values of both ligands compared with a standard Thiourea (IC_{50} =21.6 ± 0.12), displayed urease inhibition activity. The superior activities of synthesized complexes were observed when compared with the standard Thiourea, where compounds 2, 3, 8 and 9 were found to be active except compounds 1 and 7 (IC_{50}>200 µM). While compound 6 containing Cu metal (IC_{50} =22.6 ± 0.72) exhibited significant inhibitory potential.

The interaction patterns of the synthesized compounds with urease enzyme were investigated through molecular docking studies. The Auto Dock tool was employed in order to calculate the preeminent conformational place of the synthesized compounds against *Bacillus pasteurii* urease (PDB code: 4ubp). Only those compounds were analyzed, which were already active against urease in-vitro activity. The analysis of the generated docked complexes was done by the minimum energy values (kcal/mol) and bonding interaction pattern (hydrogen/hydrophobic). Docking results revealed that the compound (6) showed the lowest energy value (-8.2 kcal/mol). Docked complex of compound (3) has predicted -6.7 kcal/mol energy value, which was comparatively higher than other synthesized compounds as shown in Table 2.

DISCUSSION

According to the result of urease inhibition activity, the compound 6 containing copper (II) ion when coordinated with the active site of nickel-dependent enzyme urease, fallouts in the alteration of the active site (Qiu *et al*. 2013, Sun *et al*. 2012, Mumtaz *et al*. 2018). The strong inhibitory effect related to ureolytic activity through synthesized copper complex may be explained as i) might be due to the strong Lewis acid properties of Copper (II) ions (Li Y-G *et al*., 2007), ii) its binding to histidine residues in proteins, iii) polymerization of

protein iv), and blocking of the thiol group (Follmer ***et al***., 2005). It has also been observed that compounds containing O and N donor site displayed greater interaction and formation of H-bonding with the urease enzyme (Rauf *et al*., 2011, Ghous *et al*., 2010, Akhtar *et al*., 2011).

The following interaction through analysis of docked complexes were observed, which were hydrogen bonding, Pi-Alkyl, Pi-Sulfur, Pi-anion, Pi-sigma, and Van der Waals. Docking analysis showed that all the docked compounds interacted with active binding region of *Bacillus pasteurii* urease (Kafarski *et al*., 2018 29). Different interactions in docked complexes and the 2D graphical depictions are presented in Fig. (2-6). Docking results of the standard drug (thiourea) showed that it was strongly bound to the active site of the urease enzyme and formed three conventional hydrogen bonds with PRO C:303, PRO C:306 and GLU C:314. Van-der-Waal interactions were also shown by thiourea with the urease structure. These interactions bind thiourea with the *Bacillus pasteurii* urease, as a result thiourea acts as a good inhibitor of urease (fig. 7).

Higher anti-urease activity of (6) against urease obtained from experimental results is also elucidated from docking results as (6) strongly binds to *Bacillus pasteurii* urease and form multiple conventional hydrogen bonds with GLN C:460, and VAL C:474 along with other Pi-Pi, Pi-alkyl and alkyl interactions with different other amino acid groups (as shown in Fig. 2). Docking results showed that the binding energy value of (6) is lower than all other compounds and this data is in good agreement with the experimental data. In case of (8), it showed relatively higher binding energy (-7.2 kcal/mol) than (6) and formed hydrogen bonds with TYR A: 32 and LSS C441 along with some attractive interactions with other amino acid residues of the urease enzyme (Fig. 3). Similar interaction patterns were also shown by compounds (2, 3 and 9).

CONCLUSION

The inhibitory activity of metal compounds has been a subject of interest in the current study. The IC_{50} of all hetero-chelates were compared with the standard. Moreover, it was found that the copper compound exhibited significant urease inhibition activities. The docking study of these heterochelates also showed that the compound containing copper as its center metal found to have greater potential in the inhibitory the effect of urease enzyme as compare to all synthesized hetero-chelates. Due to its highest activity it is recommended as future potent anti-ulcer agents.

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