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Molecular Modeling and docking of Wheat Hydroquinone Glucosyl transferase by using Hydroquinone, Phenyl phosphorodiamate and n-(n butyl) Phosphorothiocic Triamide as Inhibitors

Tayyaba Huma*, Arooma Maryam & Tahir ul qamar

Department of Bioinformatics and Biotechnology, Government College University (GCUF), 38000, Faisalabad, Punjab, Pakistan; Tayyaba Huma – Email: tayyabashahbaz@gmail.com; Phone: +92300-6606933; *Corresponding author

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Abstract:
In agriculture high urease activity during urea fertilization causes substantial environmental and economical problems by releasing abnormally large amount of ammonia into the atmosphere which leads to plant damage as well as ammonia toxicity. All over the world, urea is the most widely applied nitrogen fertilizer. Due to the action of enzyme urease; urea nitrogen is lost as volatile ammonia. For efficient use of nitrogen fertilizer, urease inhibitor along with the urea fertilizer is one of the best promising strategies. Urease inhibitors also provide an insight in understanding the mechanism of enzyme catalyzed reaction, the role of various amino acids in catalytic activity present at the active site of enzyme and the importance of nickel to this metallo enzyme. By keeping it in view, the present study was designed to dock three urease inhibitors namely Hydroquinone (HQ), Phenyl Phosphorodiamate (PPD) and N-(n-butyl) Phosphorothiocic triamide (NBPT) against Hydroquinone glucosyltransferase using molecular docking approach. The 3D structure of Hydroquinone glucosyltransferase was predicted using homology modeling approach and quality of the structure was assured using Ramachandran plot. This study revealed important interactions among the urease inhibitors and Hydroquinone glucosyltransferase. Thus, it can be inferred that these inhibitors may serve as future anti toxic constituent against plant toxins.

Keywords:
Soil Urease activity, Hydroquinone glucosyltransferase, Molecular docking and Homology modeling

Background:
Urea produced in large quantity as a product of catabolism of nitrogen-containing compounds **[1].** Urea accounts for over 50 percent of all nitrogen based fertilizer which shows that it is the most widely applied nitrogen fertilizer in the world. Reasons of its high preference in the field area are its high nutritive content, solubility and ease handling during application **[2, 3, 4].** Urea decomposes with a half-life of ca. 3.6 years, and without an efficient degradation process, it would quickly accumulate and cause severe environmental problems **[5].** Urease (urea amidohydrolases, EC 3.5.1.5) is a thiol-rich and nickel-dependent metalloenzyme that can catalyze the hydrolysis of urea, thereby producing ammonia and carba-mate **[6].** Urease can be synthesized by several organisms, including plants, bacteria, algae, fungi, and invertebrates. Ions and the sulfhydryl group, especially the multiple cysteinyl residues in the active

site of the enzyme, are essential for the catalytic activity of all ureases **[7].** Urea is rapidly hydrolyzed by urease enzymes to form ammonia which leads to accumulation of excess ammonia. Access amount of ammonia is harmful for the plant and leads to toxicity in them and posed significant environmental and economic problems **[2].** One of the promising strategies to prevent toxicity to plants and to inhibit ammonia volatilization upon application of urea is to apply urease inhibitors along with urea **[8, 9].** Soil inhibitors possess extensive application in agriculture, clinical science and understanding enzyme kinetics. Different compounds like, Hydroquinone (HQ), Phenyl Phosphorodiamate (PPD) and N-(n-butyl) Phosphorothiocic triamide (NBPT) reported as widely used urease inhibitors but all these inhibitors work by inhibiting the activity of soil urease and block the formation of ammonia during urea hydrolysis. They reduces the loss of nitrogen from the urea fertilizers in the form of volatile ammonia which further helps to improve the utility of urea based fertilizers **[10].** PPD is reported as most effective among others urease inhibitors in retarding the hydrolysis of urea **[11, 12, 13, 14].** While Bremner and Chai (1986, 1989) stated that NBPT is more efficient than PPD in delaying urea hydrolysis and decreasing ammonia volatilization. In the same way, HQ is normally preferred as a urease inhibitor in the field because of its cost effectiveness **[15].**

In the present study computational tools related to protein modelling and docking were applied to find out the best possible structure of Hydroquinone glucosyltransferase and its mode of interaction with three urease inhibitors HQ, PPD NBPT. The objective of this study was to find out the potent residues from the active site of Hydroquinone glucosyltransferase through which urease inhibitors occupy the catalytic site and blocks its activity. This study will in turn help to find out the mechanism of inhibition of Hydroquinone glucosyltransferase upon binding of inhibitors.

Methodology:

Preparation of protein structure

Amino acid sequence of wheat Hydroquinone glucosyltransferase was retrieved from Uniprot database (Uniprot Id: D8LAL9) **[16].** This amino acid sequence was further used in 3D modeling approach. MODELLER 9.10 tool a python based protein modeling software was utilized to determine the 3D structure of Hydroquinone glucosyltransferase **[17].** Appropriate template structures were selected from NCBI Blastp server on the basis of lowest e value and highest similarity. Two structures 2VCE and 2VCH were selected as templates against the target protein. After predicting the 3D structure, its quality and reliability was checked using different validation softwares. Model predicted by Modeller 9.10 was evaluated in PDBsum database **[18]** in order to calculate the Z-score and Ramachandran plot of our desired model.

Preparation of inhibitors

Hydroquinone (HQ), Phenyl Phosphorodiamate (PPD) and N-(n-butyl) Phosphorothiocic triamide (NBPT) were selected as potent inhibitors on the basis of the literature review and their three dimensional structures were downloaded in .sdf format from Pubchem **[19].** Pubchem Ids of Hydroquinone (HQ), Phenyl Phosphorodiamate (PPD) and N-(n-butyl) Phosphorothiocic triamide (NBPT) are CID 785, CID 8194 and CID 93502 respectively.

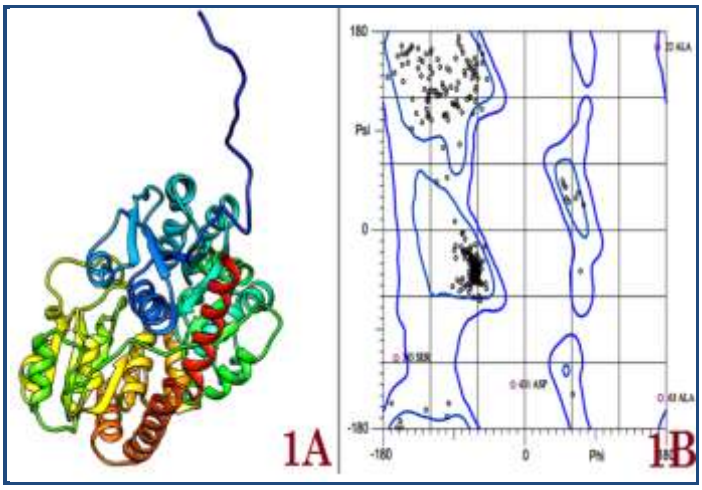

Figure 1: A) 3D structure of Hydroquinone glucosyltransferase; **B)** Ramachandran plot showing 90.3% residues in most favourable regions.

Molecuar docking

Molecular docking was performed between the wheat Hydroquinone glucosyltransferase and three wheat inhibitors (Hydroquinone, Phenyl phosphorodiamidate (PPD) and N-(n-butyl) phosphorothioic trimaide (NBPT) using the MOE software **[20].** The metal ions in the structure were deleted. After this polar hydrogen and partial charges were added to the model of soil urease and in the last step energy minimization was performed to prepare the structure for docking. Sdf format of inhibitors were converted into a 3D PDB file and optimization steps were performed. After optimization "inhibitors" database was created. Active site Finder tool of MOE was applied to find and calculate active sites in the receptor molecule from the 3D atomic coordinates of the receptor. By default, all calculated sites were appeared as selected. Active site of the receptor appeared at the top of the list with highest number of receptor residues was selected. After selecting one site, particular site is isolated from rest of the structure and secondary structures were removed. In the next step Gaussian Contact surface was drawn around the binding site of soil urease and saved the specific file in .moe format. MOE docking program with default parameters was used to bind the selected ligands with receptor protein and to find the correct conformation (with the rotation of bonds, structure of molecule is not rigid) of the ligand so as to obtain minimum energy structure. After docking, best conformations were analyzed for hydrogen bonding/π-π interactions.

Results & Discussion:

Homology modeling

Protein sequence of Hydroquinone glucosyltransferase was retrieved from Uniprot database using D8LAL9. A BLASTp search was performed against PDB database to find the best matching template. PDB ID: 2VCE with 96% similarity was selected for homology modeling. Modeller v9.10 was used to predict the 3D structure of Hydroquinone glucosyltransferase. Best Structure was selected on the basis of the minimum DOPE score. The 3D structure of the protein is shown in **(Figure 1A)**. The model was used to determine the Ramachandran plot. The

results of the Ramachandran plot showed that it has 90.3% residues in favorable regions **(Figure 1B).** According to Ramachandran plot statistics, model was found to be of a good quality as it has more than 90% amino residues in the most favored regions and only 0.5% residues were in disallowed region. Therefore it can be inferred that the predicted model is a good quality model.

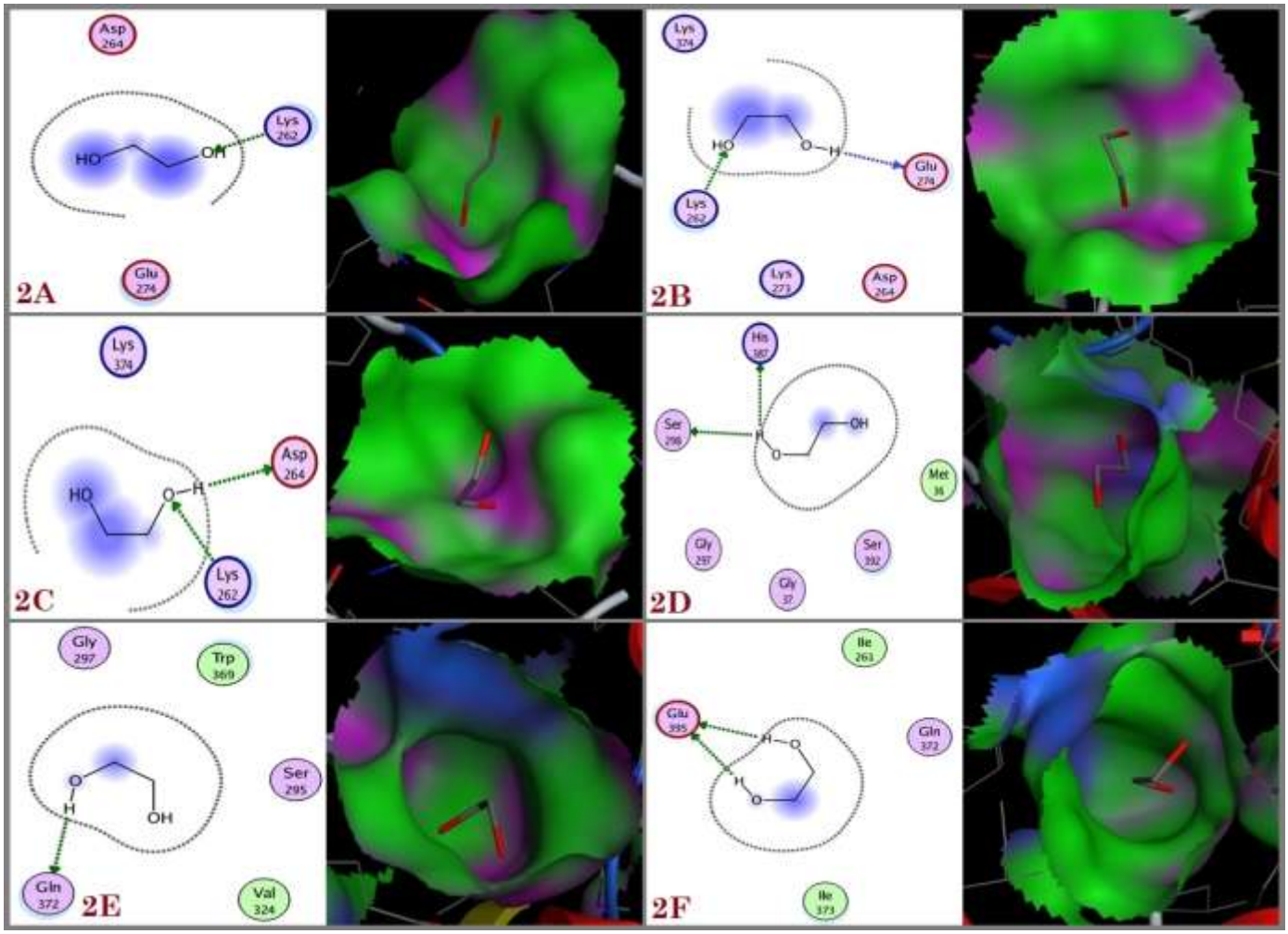

Figure 2: A) Interaction of Lys262 from Hydroquinone glucosyltransferase with Hydroquinone; **B)** Interaction of Lys262 and Glu274 from Hydroquinone glucosyltransferase with Hydroquinone**; C)** Interaction of Lys262 and Asp264 from Hydroquinone glucosyltransferase with Hydroquinone; **D)** Interaction of active site residues (Ser298, His387) of wheat Hydroquinone glucosyltransferase and Hydroquinone; **E)** Interaction of Gln372 from Hydroquinone glucosyltransferase with Hydroquinone; **F)** Interaction of Glu395 from Hydroquinone glucosyltransferase with Hydroquinone.

Docking analysis

Docking with Hydroquinone

All ligands were docked with the active site of NS3/4A protease enzyme and top ranked conformations of each ligand were saved in a separate database. After post dock analysis, it was observed that active site of wheat Hydroquinone glucosyltransferase that lies close to the Hydroquinone inhibitor are Met36, Gly37, Ile261, Lys262, Lys273, Glu274, Ser295, Gly297, Ser298, Gln322, Val324, Trp369, Gln372, Ile 373, Lys374, His387, Ser392 and Glu395. Among these eighteen residues of wheat Hydroquinone glucosyltransferase that lies close to the Hydroquinone only seven active site residues were observed as directly interacting residues. Lys262, a basic amino acid from the active site of wheat Hydroquinone glucosyltransferase interacts with the hydroxyl group of hydroquinone and behaves as a side chain donor molecule. Strength of chemical bond between this active side residue and inhibitor is 87% **Figure 2A**. Lys262 and Glu274 were also observed as strongly interacting residue. Glu274 is acting as an acidic backbone acceptor residue.

The strength of chemical bond between the active site residue of Hydroquinone glucosyltransferase and Hydroquinone (inhibitor) is 82%. In **Figure 2B** along with Lys262, Glu274 was also observed as strongly interacting residue. Glu274 is acting as an acidic backbone acceptor residue. The strength of chemical bond between the active site residue of Hydroquinone glucosyltransferase and Hydroquinone (inhibitor) is 82%. In other docking results Asp264 was also observed as an interacting residue from the active site of Hydroquinone glucosyltransferase. It binds to the hydroxyl group of Hydroquinone inhibitor and donates hydrogen ion from its side chain group given in **Figure 2C.** Chemical bond between Hydroquinone glucosyltransferase and Hydroquinone inhibitor has moderate bond strength of 59%. Hydroquinone inhibitor

also binds to the Ser298 and His387 residues with the bond strength of 98% and 27% as shown in **Figure 2D**. In the interaction diagram given in **Figure 2D** strongly bound Ser298 being a polar residue behave as a side chain acceptor and binds with the hydroxyl group of Hydroquinone and makes a hydrogen bond on the expense of a water molecule. His 387 is a basic amino acid which acts as side chain acceptor molecule like Ser298 and binds to hydroxyl group of Hydroquinone with weak bond strength. Gln372, Glu395 were also bound by Hydroquinone inhibitor. During interaction Gln372 behaves as a side chain donor residue that binds to the hydroxyl group of inhibitor and protonate it. The polar bond between the Gln372 (active site residue) and Hydroquinone inhibitor is having a low bond strength (39%) shown in **Figure 2E**. While in **Figure 2F** stacking interaction with varied bond strength was observed between Glu395 and inhibitor molecule. In the docking complex between Hydroquinone glucosyltransferase with Hydroquinone (inhibitor) given in **Figure 2F** Glu395 is interacting as a side chain donor molecule for hydroxyl group of Hydroquinone. From the docking analysis it is hypothesized that Lys262, Asp264, Glu274, Ser298, Gln372, His387 and Glu395 are the residues that make Hydroquinone a potent inhibitor of Hydroquinone glucosyltransferase. Through different kinds of chemical interactions with these above mentioned residues Hydroquinone occupies the active site of enzyme and may inhibit or lower its catalytic efficiency. HQ is normally preferred as a urease inhibitor in the field because of its cost effectiveness **[15].** In a study Zhengping *et al* (2007) reported that HQ decreased gaseous nitrogen loss by decreasing the activity of the denitrifiers in the soil. The inhibitory effect was increased by adding increasing amounts of HQ. Because denitrification is stimulated by readily decomposable organic matter, the retardation seems to be a short-term effect. The other urease inhibitors, PPDA and NBPT, had no significant influence on the denitrification process when they were applied at the rate of 4 mg per kilogram of soil.

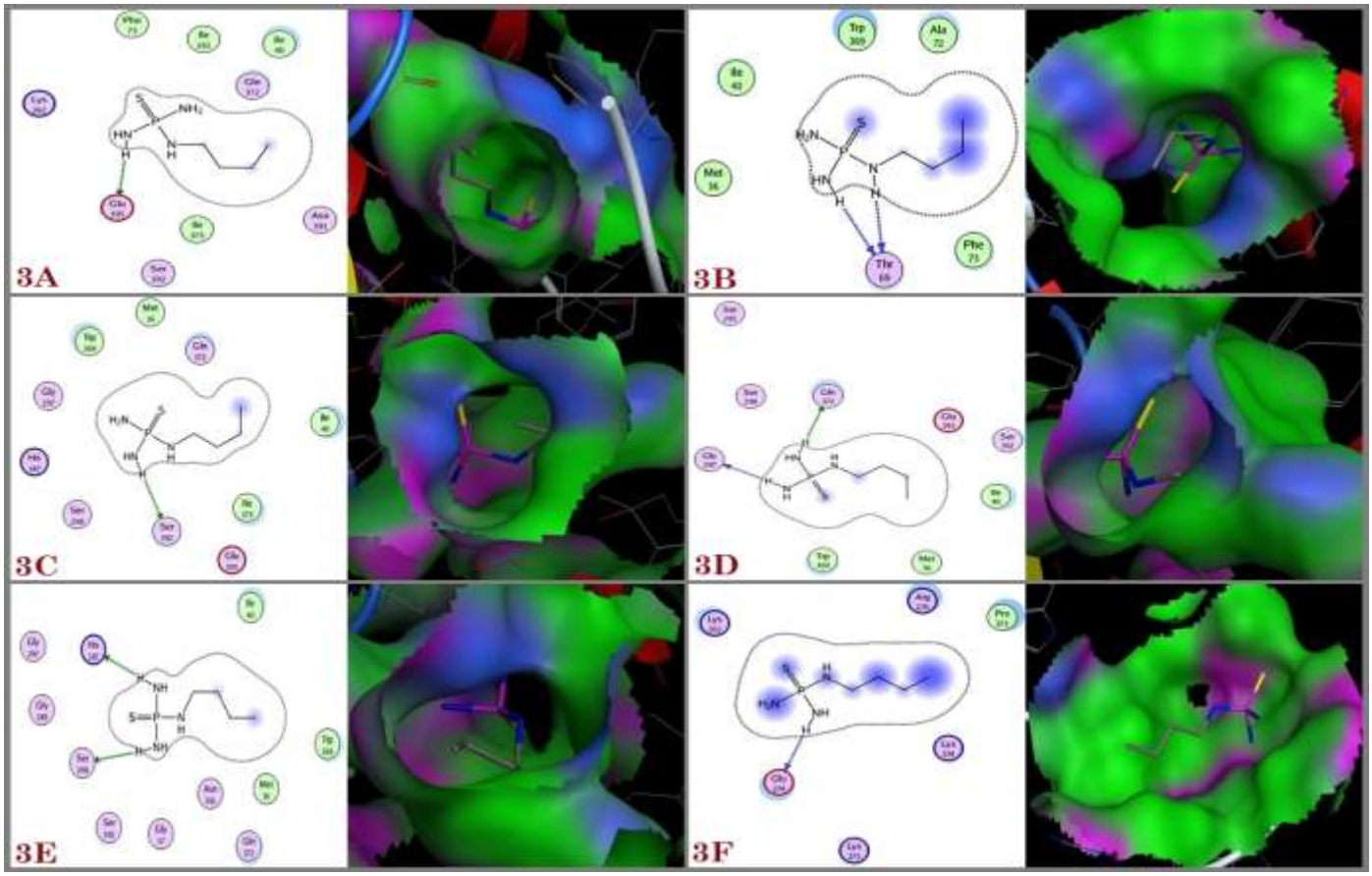

Figure 3: A) Interaction of Glu395 from Hydroquinone glucosyltransferase with N-(n-butyl) Phosphorothiocic triamide; **B)** Interaction of Thr69 from Hydroquinone glucosyltransferase with N-(n-butyl) Phosphorothiocic triamide; **C)** Interaction of Ser392 from Hydroquinone glucosyltransferase with N-(n-butyl) Phosphorothiocic triamide; **D)** Interaction of Gln372 and Gly297 from Hydroquinone glucosyltransferase with N-(n-butyl) Phosphorothiocic triamide; **E)** Interaction of Ser298 and His387 from Hydroquinone glucosyltransferase with N-(n-butyl) Phosphorothiocic triamide; **F)** Interaction Glu274 from Hydroquinone glucosyltransferase with N-(n-butyl) Phosphorothiocic triamide.

Docking with N-(n-butyl) Phosphorothiocic triamide

In the docking analysis between wheat Hydroquinone glucosyltransferase and its N-(n-butyl) Phosphorothiocic triamide inhibitor, it was observed that active site of wheat Hydroquinone glucosyltransferase that lies close to the this inhibitor are Met36, Ile40, Thr69, Ala72, Phe73, Ile261, Lys262, Lys273, Arg276, Glu274, Ser295, Gly297, Ser298, Gln322, Val324, Trp369, Pro371, Gln372, Ile 373, Lys374, His387, Asn391, Ser392 and Glu395. Out of these twenty four residues only eight residues are directly interacting with its N-(n-butyl) Phosphorothiocic triamide inhibitor. Most of residues that are in close proximity to the inhibitor are hydrophobic in nature. In

the docking results given in **Figure 3A** it was observed that Glu395 is interacting with the -NH_2 group of the inhibitor molecule with bond strength of 61%. In this chemical interaction active site residue Glu395 is acting as a side chain donor molecule and it is an acidic amino residue.Threonine residue being a polar residue was also found to be an interacting residue in the **Figure 3B**. Thr69 is acting as backbone donor molecule for one of the amino group (NH_2) of N-(n-butyl) Phosphorothiocic triamide inhibitor. In another docking result shown in **Figure 3C** Ser392 being a polar residue binds with the amino group of the inhibitor and acts as a side chain donor residue.Amongst the active site residues Ser 298, Gly297, Gln372 and His387 also bind N-(n-butyl) Phosphorothiocic triamide inhibitor molecule shown in **Figure 3D** & **Figure 3E.** Gly297, Ser298 and Gln372 are polar residues that bind both the amino groups of NBPT molecule. In the interaction diagram given in **Figure 3D** Gln372 is behaving as a side chain acceptor while Gly297 is acting as backbone donor molecule for amino group of the inhibitor molecule.In **Figure 3E** Ser298 is acting as a side chain donor residue and His387 is a basic amino residue and interacting diagram shows that it is a backbone donor molecule for one of the amino group of inhibitor. Docking results of NBPT and wheat Hydroquinone glucosyltransferase suggests that glutamic residue at position 274 is acting as an acidic backbone donor residue and interacts with amino group of the NBPT. The strength of chemical bond between the active site residue of Hydroquinone glucosyltransferase and NBPT (inhibitor) is 47%. In a study reported by Bremner & Chai (1986, 1989) have also proved that NBPT is more efficient than PPD in delaying urea hydrolysis and decreasing ammonia volatilization. NBPT significantly decreased ammonia volatilization and reduced losses from urea by 42-55%. NBPT+DCD seemed to increase ammonia losses compared to NBPT alone.

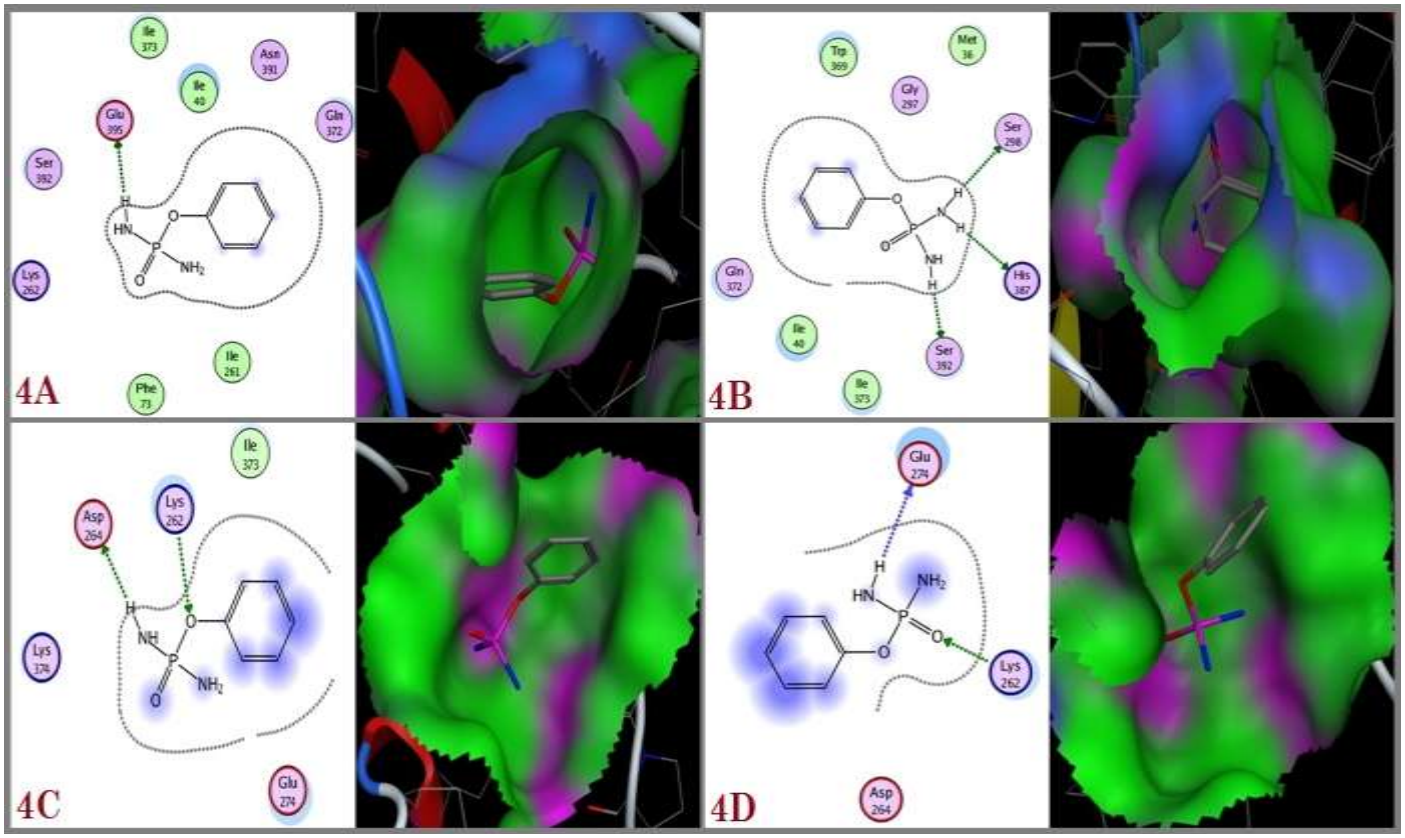

Figure 4: A) Interaction of Glu395 from Hydroquinone glucosyltransferase with Phenyl Phosphorodiamate (PPD) inhibitor; **B)** Interaction of Ser298, His387 and Ser392 from Hydroquinone glucosyltransferase with Phenyl Phosphorodiamate (PPD) inhibitor; **C)** Interaction Lys262 and Asp264 from Hydroquinone glucosyltransferase with Phenyl Phosphorodiamate (PPD) inhibitor; **D)** Interaction Lys262 and Glu274 from Hydroquinone glucosyltransferase with Phenyl Phosphorodiamate (PPD) inhibitor.

Docking with Phenyl Phosphorodiamate (PPD)

In the docking analysis between wheat Hydroquinone glucosyltransferase and Phenyl Phosphorodiamate (PPD) inhibitor, it was observed that active site of wheat Hydroquinone glucosyltransferase that lies close to the this inhibitor are Met36, Ile40, Phe73, Ile261, Lys262, Ser298, Gln372, Ile 373, Lys374, His387, Asn391, Ser392 and Glu395.Out of these thirteen residues only eight residues are directly interacting with the PPD inhibitor molecule. In **Figure 4A** docking complex and interaction diagrams of Hydroquinone glucosyltransferase with Phenyl Phosphorodiamate (PPD) inhibitor shows that Glu395 is directly interacting with (-NH_2) group of inhibitor with bond strength of 59%.It is an acidic residue that is behaving as a side chain donor residue for the inhibitor molecule. In other docking results of PPD complex with Hydroquinone glucosyltransferase Ser298, His387 and Ser 392 were observed as interacting residues from the active site of enzymes. Serine residues at position 298 and 392 are polar residues and both are acting as side chain donor residues and occupy both the amino groups of inhibitor shown in **Figure 4B**. Three other residues through which inhibitor molecule bind to the active site of the enzyme are Lys262, Asp264 and Glu274

shown in **Figure 4C** & **Figure 4D.** In docking results it was observed that the oxygen molecule (i.e. present between the benzene ring and amino side group) is interacting with Lys262. Lys262 is a basic amino residue that showed varied bond strength in both the figures but behaves as side chain acceptor molecule. While Asp264 is an acidic amino residue that is acting on amino group of inhibitor molecule and behave as side chain donor residue given in **Figure 4C.** Glutamic residue at position 274 also interacts with the amino group of PPD inhibitor molecule with weak bond strength of 11%. It is acting as a backbone donor residue for the inhibitor molecule. PPD is reported as most effective among others urease inhibitors in retarding the hydrolysis of urea **[11, 12, 13, 14].** Wang *et al*. (1991) stated that in the presence of urease inhibitors such as hydroquinone (HQ), phenyl phosphorodiamidate and N-(*n*-butyl) phosphorothioic triamide, the urea-15N recovery in an alkaline soil was increased by 5–30% of the amount applied as fertilizer 15N, and the effect depended on the inhibitor and soil type.

Conclusion:

From the docking analysis it was observed that Lys262, Glu274, Ser298, His387 and Glu395 were occupied by all the three inhibitors. Due to the interaction of these residues with all the wheat inhibitors it is concluded that these residues are important residues that might be involved in the activity of the Hydroquinone glucosyltransferase. Activity of Hydroquinone glucosyltransferase lowered or inactivated by the chemical interaction of the inhibitors with the active site residues. Asp264 was observed as a common interacting active site residue in HQ and PPD while Ser392 was common active site residue that has shown similar behavior in binding NBPT and PPD. Therefore, the development of urease inhibitors would lead to a reduction of environmental pollution, to enhance efficiency of nitrogen uptake by plants and to improved therapeutic strategies for treatment of infections due to ureolytic bacteria. Structure-based design of urease inhibitors would require knowledge of the enzyme mechanism at the molecular level.

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