Journal of Molecular Structure 1223 (2021) 129141

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstr

# Structural basis of binding and justification for the urease inhibitory activity of acetamide hybrids of N-substituted 1,3,4-oxadiazoles and piperidines

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ARTICLE INFO

*Article history:*
Received 12 May 2020
Revised 3 August 2020
Accepted 22 August 2020
Available online 30 August 2020

*Key words:*
Enzyme kinetics
Acetamide hybrids
Oxadiazole
Cytotoxicity
Phytotoxicity

ABSTRACT

In present, we have performed the Michaelis–Menten kinetics studies of urease inhibitors (**6a–o**), having basic skeleton of acetamide hybrids of N-substituted 1,3,4-oxadiazoles and piperidines. From the Lineweaver-Burk plot, Dixon plot and their secondary replots, it has been confirmed that all the compounds have inhibited the enzyme competitively with $K_i$ values of in range from 3.11 ± 0.2 to 5.20 ± 0.7 $\mu$M. Compound **6a** was found to have lowest $K_i$ among the series, while compounds **6d, 6e**, **6g** and **6i** were found subsequently the excellent $K_i$ values after **6a**. Molecular docking has supported their types of inhibitions and structure activity-relationship. Most frequently, the nitro group oxygen atoms were found in contact with nickel ions of the active site. Moreover, all the compounds were subjected to toxicity tests and were found nontoxic against human neutrophils and plants, respectively.



## 1. Introduction

Urease (urea amidohydrolase, EC 3.5.1.5) is a nickel dependent metalloenzyme, which enhances the hydrolysis of urea into ammonia and carbamate [1]. The hydrolysis of carbamate later produces carbon dioxide and ammonia at physiological pH [2]. Such an excess production of ammonia makes the environment basic, which favor the colonization of various bacterial species [3,4]. This rate of urease hydrolysis is approximately $10^{14}$ times to that of uncatalyzed reaction [5].

Urease exists in fungi, algae, yeast, bacteria, plants, animals and soil [6,7]. It plays a pivotal role in their growth by the degradation of urea and supply of nitrogen. Moreover, it participates in the metabolism of nitrogen within cattle and many other animals [8]. From medicinal point of view, the ureases from various bacteria (*H. pylori*) adversely affect the host and cause various diseases like gastric/peptic ulcer, gastric coma and stomach cancer [9–11]. In addition, it causes kidney stone formation, hepatic encephalopathy, hepatic coma, pyelonephritis and urinary catheter blockage [4,12,13].

The urea is considered to be the most prominent source of nitrogen (46%) in the field of agriculture [14]. It is supplied to the soil in the form of dehydrated granules, prills, and fluids in combination with $NH_4NO_3$. Adverse action of urease released by the soil microbes will loss a greater amount of urea (upto 30%) in the form of ammonia to elevate the surrounding pH which will suppress the growth of seeds and seedling germination as well as deprive plants from their nitrogenous nutrient [15]. It also causes an imbalance in the nitrogen cycle [7], eutrification which result in algae bloom to reduce fish and animal population and cause addition of greenhouse gasses in the environment [16].

During the last two decades, antibiotic combination of amoxicillin and clarithromycin along with omeprazole was used for the treatment of *H. pylori* eradication. However, in the recent years, the prominent drug resistance of *H. pylori* especially to clarithromycin along with increased side effects, these drugs has lost its medical importance [17,18]. Likewise the bismuth salt along with proton pump cell inhibitors and certain other antibiotics such as fluoroquinolones, aminopenicillins, and tetracyclines etc. efficacy have also reduced towards the treatment of *H. pylori* infections [17,18].

The most efficient way to control urease complications is to discover a variety of novel and safe natural or synthetic urease inhibitors in order to improve human, animal and agriculture life quality and to promote the fields of pharmaceutical, agricultural

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https://doi.org/10.1016/j.molstruc.2020.129141
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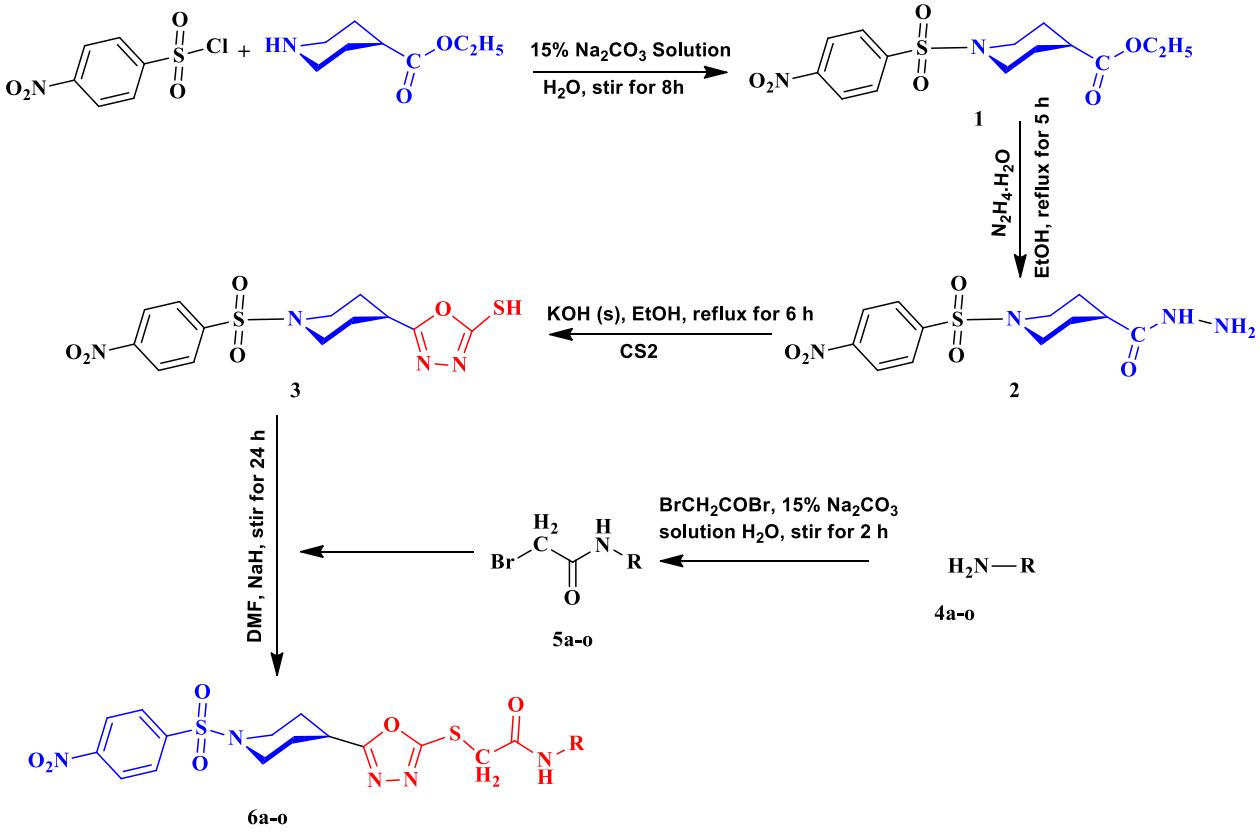



**Scheme 1.** Synthetic route of 2-(5-(1-(4-nitrophenylsulfonyl) piperidin-4-yl)−1,3,4-oxadiazol2-ylthio)-N-(substituted) acetamide (**6a–o**).

and environmental sciences. Certain synthetic compounds like hydroxyurea, flurofamide and hydroxamic acid have shown potential efficacy. However, some of these compounds were banned due to their toxic or unstable nature; for example, acetohydroxamic acid was found with teratogenic effect in rats [19]. Likewise, majority of the recognized inhibitors were redundant due to their harmful effects.

The identification of new natural and synthetic urease inhibitors is promising due to the involvement of urease in agriculture, health, environmental and economical problems. We have previously identified number of potent urease inhibitors which might use as alternate to standard thiourea [20,21]. In the same connection, we further try to discover effective inhibitors having considerably high efficacy against urease without any side effects.

In the present work, we have resynthesized acetamide hybrids of N-substituted 1,3,4-oxadiazoles and piperidines already identified urease inhibitors [22] and performed their detailed enzyme kinetics, in order to know about their mechanism of inhibition. Both oxadiazole and piperidines are active pharmacophores of many therapeutics compounds, such as antibacterial [23,24], antifungal [25,26], anticonvulsant, anti-enzymatic [27], and anticancer [28] agents. The combined effect of oxadiazole and piperidine compounds in one unit gave the best impetus for the search of highly active biological and pharmacological agents.

Therefore, due to the existence biological applications of 1,3,4-oxadiazoles and piperidine, we hereby report the synthesis, detail kinetics studies against urease and safety profile of hybrids N-(substituted)−2-(5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)−1,3,4-oxadiazol-2ylthio)acetamide having both 1,3,4-oxadiazoles and piperidine in one unit.

## 2. Materials and methods

### 2.1. General

All the reagents and chemicals used were of high analytical level. Deionized $H_2O$ was utilized for the preparation of various solutions at room temperature. Type X urease (Cat. No. U4002–50 KU), urea (Cat. No. U5378), phenol (Cat. No. 16,017) and dipotassium hydrogen phosphate (Cat. No. 16,788–57–1) were purchased from Sigma-Aldrich, USA. NaOCl (Cat. No. 230,394 M) and methanol (Cat. No. 20,864.320/0,823,601) were purchased from BDH Lab. Supplies, UAE. Sodium hydroxide (Cat. No. S41298–4 J) was purchased from Unichem, India. Thiourea (Cat. No. 1,079,791,000) was provided by Merck, Germany.

### 2.2. General procedure for the synthesis of target compounds (6a–p)

Equimolar of 4-nitrobenzene sulfonyl chloride (**a**) and ethyl isonipecotate (**b**) at 0.390 mol were react in the presence of sodium carbonate in aqueous medium to get ethyl 1-(4-nitrophenylsulfonyl)piperidin-4-carboxylate (**1**) at room temperature. The compound (**1**) (0.015 mol) was reacted with hydrazinium hydroxide (5 mL) in methanol to get 1-(4-nitrophenylsulfonyl)piperidine-4-carbohydrazide (**2**). The 5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)−1,3,4-oxadiazol-2-thiol (**3**) was synthesized by adding equimolar of KOH (0.0304 mol) and compound **2** (0.0304 mol) in 10 mL of ethanol followed by the addition of $CS_2$ (0.0608 mol) and refluxed for 6 h. Aralkyl/aryl amines (**4a–o**; 0.02 mol) were stirred with 2-bromoacetyl bromide (0.02 mol) in distilled water for 2 h to get N-(substituted)−2-bromoacetamides (**5a–o**). The targeted series of 2-(5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)−1,3,4-oxadiazol-2-ylthio)-N-(substituted) acetamide (**6a–o**) were obtained in the presence LiH and 10 mL of N,N-dimethyl form amide as reaction medium 24 h (Scheme 1).

### 2.3. Urease inhibition assay (Indophenol's method)

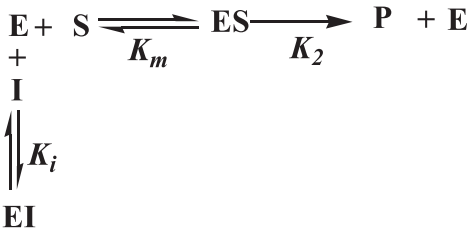
Initially, the reaction mixture consists of 25 $\mu$L (one unit per well) of Jack bean urease solution and 5.0 $\mu$L of a test compound (0.5 mM in solvent methanol) and was incubated at 30 °C for 15 min in 96-well plates. Later 55 $\mu$L of potassium phosphate

buffer (4 mM, pH 6.8) containing urea (100 mM) was added in each well and plate was incubated at 30 °C for additional 15 min. Activity of urease was determined by the measurement of the amount of $NH_3$ formed during urease catalysis as mentioned by Weatherburn [29]. After that, each well of the plate was inoculated with 45 $\mu$L of phenol reagent (1% w/v phenol + 0.005% w/v$Na_2$-nitroprusside) and 70 $\mu$L of alkali reagents (0.5% w/v sodium hydroxide+ 0.1% sodium hypochlorite). Finally, after 50 min the increase in absorbance was measured at 630 nm by the use of microplate readers (Molecular Devices., CA, USA). The entire reactions were performed (in triplicate) till to achieve a final volume 200 $\mu$L. Analysis of the results was carried out by the SoftMax Pro6.3 software (Molecular Device, USA). Thiourea was used as standard by applying this method. The percentage (%) inhibition was measured by using the formula;

$$\%\text{age inhibition} = 100\text{-}(OD_{\text{test well}}/OD_{\text{control}}) \times 100.$$

### 2.4. Determination of kinetic parameters

The $IC_{50}$ inhibitory potential of compounds is the concentration of the test compounds that blocked the urea (substrate) hydrolysis values represent by 50% was measured by monitoring the effects of their different concentrations (from high to low) in the assay. Such calculation was carried out by using the EZ-Fit Enzyme Kinetics Program (Perrella Scientific Inc., Amherst, USA). The ES represents the complex of Jack bean urease and urea, while P stand for the product obtained as a result of reaction. The Lineweaver-Burk plot was then applied to identify the type of inhibition, while dissociation and inhibition constants ($K_i$) values were calculated by applying the Dixon plots and their secondary replots [30]. Non-linear regression equation was applied to find out the $K_m$, $K_i$, and $V_{max}$ values. At first, the values of $1/V_{max app}$ were calculated at each intersection points of lines of every inhibitor concentration on y-axis of the Lineweaver-Burk plot and then replotted against various concentrations of inhibitor. In the subsequent proceeding, the slope of each line on the Lineweaver-Burk plot obtained as a result of inhibitor concentration was plotted against various concentrations of inhibitor. Competitive inhibition can be classically represented as;




**Scheme 2.** Classical representation of competitive inhibition.

### 2.5. Statistical analysis

Each experiment was performed in triplicate and results were expressed as a mean of three. SoftMax Pro 6.3 Software (Molecular Devices, CA, USA) was used for the analysis of these results. The graph plotting was carried out through GraFit program. By using the same program; we have obtained the values of correlation coefficients, intercepts, slopes, and their standard errors from linear regression analysis. The relationship for each and every line in all the graphs was more than 0.99.

### 2.6. Molecular docking

Molecular docking was performed for prediction the orientation of protein active site residues and potential inhibitors binding, which is important for structure-based drug designing. These results were then correlated with the experimental values. Molecular Operating Environment (MOE 2009–2010, Quebec, Canada) docking software was used to perform such studies (MOE. 2010). Ligands as well as the receptor protein (urease) preparation are the two basic steps prior to perform the docking.

(i) Ligands preparation

The 3D structures of all the ligands were sketched through ChemDraw Ultra 12.0 (Cambridge Soft-2001, Cambridge, USA) (Cambridge Soft. 2001) and saved in the mol format. The compounds scaffolds were 3D protonated by Protonate 3D Option in MOE software. The energies of identified ligands were minimized by applying the default parameters of energy minimization algorithm already adjusted in MOE (gradients: 0.05, force field: MMFF94x). The Database of compounds series was created in mdb file format.

(ii) Preparation of receptor protein

The 3D structure of urease (4UBP) having resolution of 1.55 Å, was retrieved from Protein Data Bank (PDB). $H_2O$ molecules from the receptor protein were extracted. Like ligands, Protonate 3D option was applied for the protonation of urease 3D structure. Similarly, the energy of urease protein was also minimized by applying the default parameters of energy minimization algorithm with gradients of 0.05 and force field Amber99, and saved in pdb file format. Later on, docking of all ligands was carried out in the binding pocket of urease by following the default parameters of MOE-Dock Program. In order to increase the accuracy of protocol, re-docking was repeated [31]. After complete docking of all the ligands, the most excellent 2D as well as 3D interaction images were chosen to elucidate their specific types of interactions and bond lengths respectively. All 3-D figures were made by discovery studio visualizer software [32].

### 2.7. Cytotoxicity evaluation

The cytotoxicity of various potential urease inhibitors were tested against neutrophil cell lines. The following steps were used for the evaluation of cytotoxicity.

**(i) *Isolation of human neutrophils***

Heparinized fresh venous blood was taken from the young and healthy volunteers in a city blood collection center. Isolation of neutrophils was carried out through Siddiqui et al., 1995 method [33]. Accordingly, mixing of whole blood was made with Ficoll-Paque (Pharmacia Biotech Amersham, Uppsala). When sedimentation occurred, the unnecessary red blood cells (RBCs) were layered in a buffy coat way on a 3.0 mL cushion of Ficoll. Centrifugation was then carried out for 30 min at a rate of 1500 rpm. Supernatant was discarded, while pellets were collected. Furthermore, it was mixed with 0.83% of hypotonic ammonium chloride solution in order to lyse the RBCs. Again, the solution was centrifuged and Modified Hank's Solution (MHS) was used for the washing of collected neutrophils. Later on, resuspension of neutrophil cells was performed in the same solution at the rate of $1 \times 10^7$ cells per mL.

**(ii) *Assay procedure for neutrophil based cytotoxicity***

Isolated human neutrophils ($1 \times 10^7$cells per mL) were treated first with compounds under trial for thirty minutes and then with 0.25 mM WST-1(Dojindo Laboratories Kumamoto, Japan) on shaking water bath at 37 °C [34]. Change in absorbance was calculated after incubation for 3 h, at 450 nm using 96-well plate reader (Spectra-MAX-340, Molecular Devices, CA, USA). Here the OD represents the mean of 5 investigational replicates. Percent (%) viability of cells was measured as:

$$\%\text{age viability of cells} = \{(\text{OD test compound} \times 100/\text{OD control}) \text{-}100\}\text{–}100$$

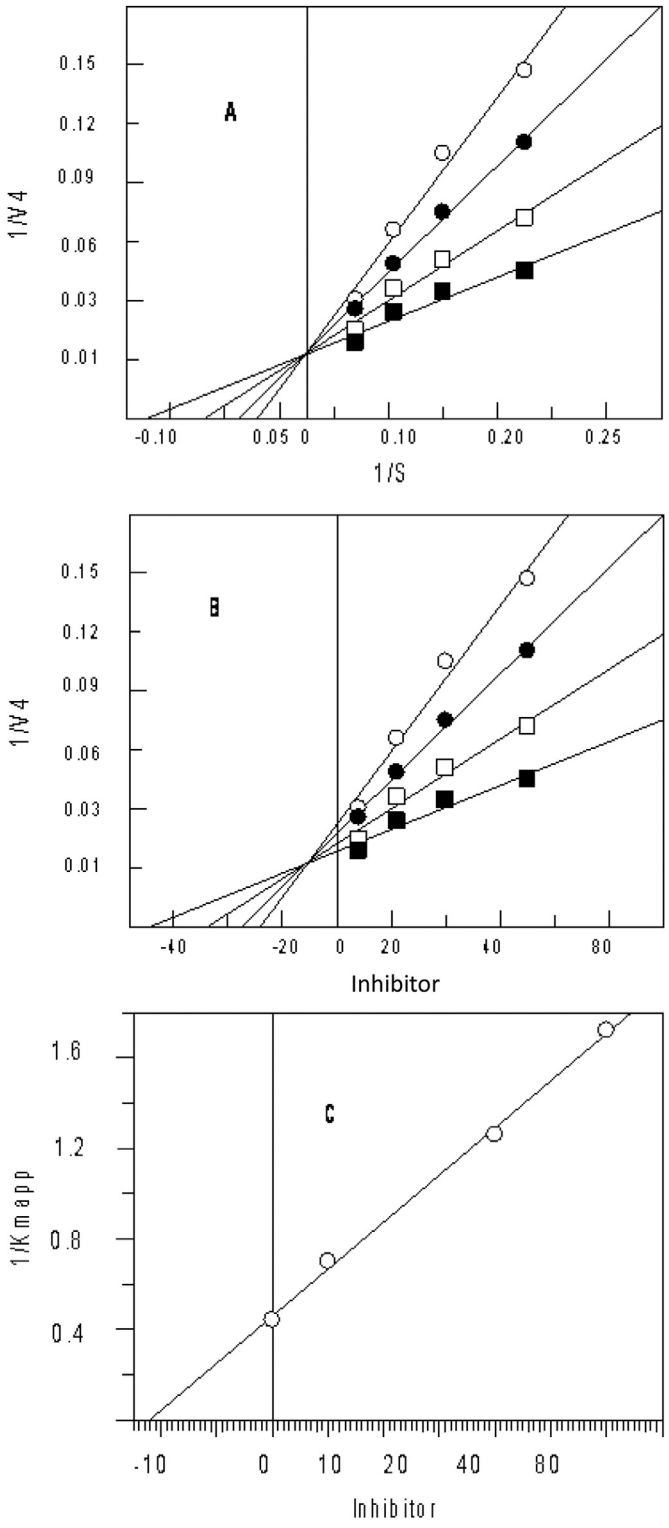



**Fig. 1.** Steady state inhibition of urease by acetamide hybrid compound No. **6o, A** is the Lineweaver–Burk plot of reciprocal of initial velocities versus reciprocal of four fixed Jack bean urease concentrations in absence (■) and presence of 12.5 $\mu$M (□), 25.0 $\mu$M (●), 50 $\mu$M (○) of compound No. **6o. B** is the Dixon plot of reciprocal of the initial velocities versus various concentrations of compound **15** at fixed urease concentrations, (■) 50 $\mu$M, (□) 25.0 $\mu$M, (●)12.5 $\mu$M and (○) 6.2 $\mu$M. **C** is the, 1/*Km*app versus various concentrations of compound No. **6o**.

### 2.8. Phytotoxicity evaluation

The urease inhibitors are used in agriculture to reduce the pH of soil and to control the loss of urea. Therefore, the identified inhibitors were checked for their phytotoxic effects according to the modified assay of McLaughlin et al., 1991 [35]. The identified inhibitors with their three different concentrations (i.e. 10, 100 and 1000 µg/mL in $CH_3OH$) were dissolved in sterilized E-medium. Later on, inhibitors having fixed concentrations (made by the dilution of stock solution) were taken in sterilized conical flasks and allowed their evaporation for overnight. In each of the flask, 20 mL of sterilized E-medium and 10 *Lemna aequinocitalis* Welv plants were inoculated, each having a rosette of 3 fronds. In some of flasks $CH_3OH$ and phosporoamide (commercially available urease inhibitors) were added which act as negative and positive control respectively. Such treatments were performed in triplicates and the flasks were kept in Fisons Fi-Totron 600H growth cabinet at 30 °C for a whole week having the environmental conditions (light intensity: 9000 lux, relative humidity: 56 ± 10 rh) at a short day span of 12 hrs. Growth rate of *Lemna aequinocitalis* Welv was measured by counting the number of fronds per dose present in flasks containing inhibitors, while the inhibition of growth was calculated with the reference to negative control.

## 3. Results and discussion

### 3.1. Chemistry

In the current study, we have synthesized a series of N-(substituted)−2-(5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)−1,3,4-oxadiazol-2-ylthio)acetamide having a wide range biological applications [23–28]. The synthesis of targeted compounds was initiated by the synthesis of sulfonamide through 4-nitrobenzene sulfonyl chloride and piperidine termed as compound 1, which was further modified into hydrazide the key product of this series. Hydrazide was converted into 1,3,4-oxadiazole and finally by the reaction 1,3,4-oxadiazole and a variety of acetamides to synthesize (**6a–o**) (Table 1). All the synthesized compounds were characterized by spectroscopic techniques like IR, EI-MS, $^1$H NMR and $^{13}$C NMR. The detail spectroscopic data were published in our previous article [22].

### 3.2. Biology

X-ray crystallography based resolved 3D structure identified two nickel ions in the active site of urease, which are coordinated by the nitrogen of imidazole ring, carboxylate group and water molecule. The architecture of the active site is almost similar in all ureases obtained from different origins and adopts tetrahedral geometry between two Ni atoms due to the presence of four $H_2O$ molecules that were observed in native *Bacillus pasteurii* (BP) and *Klebsiella aerogenes* (KA) ureases [36]. To understand about accurate mechanism concerning urease activity is basically the initiatives towards the effective drugs design in order to treat the urease related problems. It is essential to know clearly about the coordination mechanism of inhibitors with the active site of urease in order to understand about its inhibitory potential. Nearly from 100 years ago, the scientists are trying to know about the exact mechanism of action of urease but still it has been remained under investigation [37]. Several scientists have described about their mechanism of action, but the initial mechanism of urease was proposed by Zerner and his coworkers, who suggested that one nickel

**Table 1**
Chemical structures of different acetamide hybrid synthetic derivatives **6a-o**.

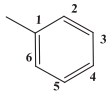
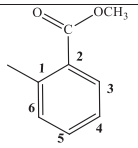
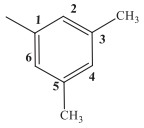
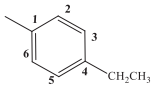
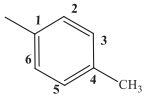
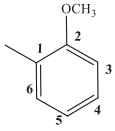
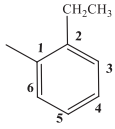
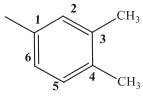
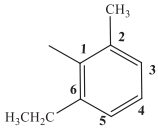
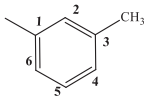
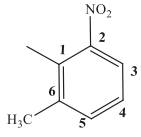
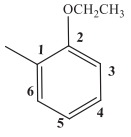
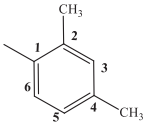
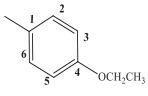
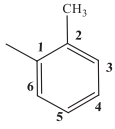
| Compounds | R | Molecular formula | Compounds | R | Molecular formula |
|---|---|---|---|---|---|
| **6a** |  | $C_{21}H_{21}N_5S_2O_6$ | **6i** |  | $C_{23}H_{23}N_5S_2O_8$ |
| **6b** |  | $C_{23}H_{25}N_5S_2O_6$ | **6j** |  | $C_{23}H_{25}N_5S_2O_6$ |
| **6c** |  | $C_{22}H_{23}N_5S_2O_6$ | **6k** |  | $C_{22}H_{23}N_5S_2O_7$ |
| **6d** |  | $C_{23}H_{25}N_5S_2O_6$ | **6l** |  | $C_{23}H_{25}N_5S_2O_6$ |
| **6e** |  | $C_{24}H_{27}N_5S_2O_6$ | **6m** |  | $C_{22}H_{23}N_5S_2O_6$ |
| **6f** |  | $C_{22}H_{22}N_6S_2O_8$ | **6n** |  | $C_{23}H_{25}N_5S_2O_7$ |
| **6g** |  | $C_{23}H_{25}N_5S_2O_6$ | **6o** |  | $C_{23}H_{25}N_5S_2O_7$ |
| **6h** |  | $C_{22}H_{23}N_5S_2O_6$ | | | |

**Table 2**
Inhibitory potency data of **6a-o** synthetic compounds against Jack bean urease.

| Enzyme | Compounds | $K_i$ ($\mu$M)± SEM | $K_m$ (mM) | $K_m$ (mM) *app* | $V_{max}$ ($\mu$mol / min)$^{-1}$ | $V_{maxapp}$ | Type of Inhibition |
|---|---|---|---|---|---|---|---|
| **Urease** | **6a** | 3.11 ± 0.2 | 2.5 | 8.4 | 105 | 104 | Competitive |
| | **6b** | 5.20 ± 0.7 | 2.5 | 8.0 | 105 | 104 | Competitive |
| | **6c** | 3.14 ± 1.1 | 2.5 | 8.5 | 105 | 106 | Competitive |
| | **6d** | 5.15 ± 0.5 | 2.5 | 6.4 | 105 | 105 | Competitive |
| | **6e** | 3.24 ± 0.3 | 2.5 | 8.6 | 105 | 107 | Competitive |
| | **6f** | 3.28 ± 0.4 | 2.5 | 9.7 | 105 | 102 | Competitive |
| | **6 g** | 3.23 ± 0.6 | 2.5 | 8.5 | 105 | 104 | Competitive |
| | **6h** | 3.16 ± 0.5 | 2.5 | 9.3 | 105 | 103 | Competitive |
| | **6i** | 3.17 ± 1.1 | 2.5 | 9.9 | 105 | 104 | Competitive |
| | **6j** | 3.25 ± 0.1 | 2.5 | 9.7 | 105 | 104 | Competitive |
| | **6k** | 4.11 ± 0.7 | 2.5 | 7.3 | 105 | 102 | Competitive |
| | **6l** | 4.14 ± 0.5 | 2.5 | 9.1 | 105 | 105 | Competitive |
| | **6m** | 3.12 ± 1.1 | 2.5 | 9.4 | 105 | 103 | Competitive |
| | **6n** | 3.32 ± 0.7 | 2.5 | 5.9 | 105 | 107 | Competitive |
| | **6o** | 11.2 ± 0.3 | 2.5 | 8.0 | 105 | 107 | Competitive |

$K_i$ (dissociation constant or inhibition constant) was determined from nonlinear regression analysis by Dixon plot and secondary.

**Table 3**
Viability results of human neutrophils ($1 \times 10^7$ cells per mL) against **6a-o** acetamide hybrids.

| Compounds | Conc. $\mu$g / mL | Viability [%] |
|---|---|---|
| **6a** | 200 | 76.45 ± 4.0 |
| **6b** | 200 | 92.86 ± 5.2 |
| **6c** | 200 | 86.41 ± 4.0 |
| **6d** | 200 | 78.35 ± 3.5 |
| **6e** | 200 | 68.12 ± 3.1 |
| **6f** | 200 | 99.94 ± 4.0 |
| **6 g** | 200 | 100 ± 1.0 |
| **6h** | 200 | 95.41 ± 5.2 |
| **6i** | 200 | 71.9 ± 4.4 |
| **6j** | 200 | 69.05 ± 4.9 |
| **6k** | 200 | 63.14 ± 2.2 |
| **6l** | 200 | 79.39 ± 2.6 |
| **6m** | 200 | 81.35 ± 4.2 |
| **6n** | 200 | 73.39 ± 3.9 |
| **6o** | 200 | 68.32 ± 1.2 |
| Acetohydroxamic acid | 200 | 88.04± 5.0 |

Mean ± SD of three experiments.

**Table 4**
Results of LemnaWelv Phytotoxicity assay of 6a-o acetamide hybrids.

| Compounds | Concentration of compounds ($\mu$g/ml) | | |
|---|---|---|---|
| | **1000** | **100** | **10** |
| **6a** | 80.0 | 58.3 | 17.5 |
| **6b** | 69.6 | 43.56 | 23.5 |
| **6c** | 87.55 | 78.54 | 27.8 |
| **6d** | 100 | 85.67 | 35.32 |
| **6e** | 64.5 | 42.3 | 23.2 |
| **6f** | 100 | 100 | 29.32 |
| **6 g** | 61.08 | 37.65 | 12.34 |
| **6h** | 70.6 | 23.99 | 14.00 |
| **6i** | 100 | 62.04 | 43.56 |
| **6j** | 89.7 | 74.05 | 28.12 |
| **6k** | 83.6 | 77.02 | 23.6 |
| **6l** | 32.5 | 17.0 | 7.7 |
| **6m** | 87.58 | 48.53 | 24.18 |
| **6n** | 79.2 | 60.36 | 24.01 |
| **6o** | 100 | 36.43 | 22.71 |
| Phosphoroamide | 81.5 | 70.3 | 19.0 |

activates the $H_2O$ molecules while the other Ni activates the substrate urea [38].

All the synthesized acetamide hybrids **(6a–o)** have inhibited the urease very effectively in a concentration dependent ways. We have determined their $K_i$ values, in order to know about their strength of inhibition. Three different methods were applied for the determination of $K_i$ values. The whole series of the compounds have inhibited the enzyme very strongly, showing low $K_i$ values of 3.11 ± 0.2 to 11.2 ± 0.3 $\mu$M. To know about the possible mode of inhibition of enzyme and binding moieties of the inhibitors with enzyme, we have determined their type of inhibition. In case of all the inhibitors, the $K_m$ values were increased, while $V_{max}$ values were remained fixed, which indicates competitive nature of inhibition. It has also been confirmed from the Lineweaver-Burk plots, Dixon plots and their secondary replots, that urease was inhibited at their active site as is clear from the Fig. 1 (A, B & C). In addition to this, $K_m$, $K_i$, $V_{max}$, $V_{max}$*app* and $K_m$*app* values as well as the type of inhibition of each compound are illustrated in Table 2.

The proposed structure activity relationship (SAR) showed that the activity of these compounds may be due the sulfonamide and nitro moieties, while substitution at R group have not significantly influence their activities, as all compounds have almost same *Ki* values (table 2).

Molecular docking was performed to confirm the accurate protein-ligand interactions geometries and also evaluated their predictive structure activity relationship (SAR). From this, we understand not only about the suitable configuations of the ligands obtained after adjustment in the active pocket of enzyme, but also supported us about the type of inhibition as well as about the strength of inhibition after measuring the bond lengths of interacted inhibitors. After docking repeatition for several turns, we have selected the images with best interactions and less docking scores for 3D configuration analysis. When measured their bondlengths, the most feasible images were selected for each compound. It was observed that all the bioassay identified inhibitors were supported strongly by the docking as they were mostly interacted with the Ni ions of the active site with more interactions at very short bond distances. Furthermore, it was also identified that terminal nitro group oxygen atoms of the main skeleton of ligands were in front line interactions with the Ni ions and have proved as potent inhibitors against urease. Some of the interaction images are listed below.

From the docking results, it has come to know that compound **6a** has made four interactions in the active site of urease enzyme. The first acidic interaction was made by the ligand through its sulfonyl oxygen with NH hydrogen of imidazole moiety of His323 showing bond length of 2.42 Å. The second metallic interaction was made between terminal nitro group oxygen of ligand and Ni ion of the receptor at a bond distance of 2.60 Å. The remaining two acidic as well as arene-cation interactions were established among sulfonyl oxygen, nitrophenyl ring and Arg339 amino acid residue

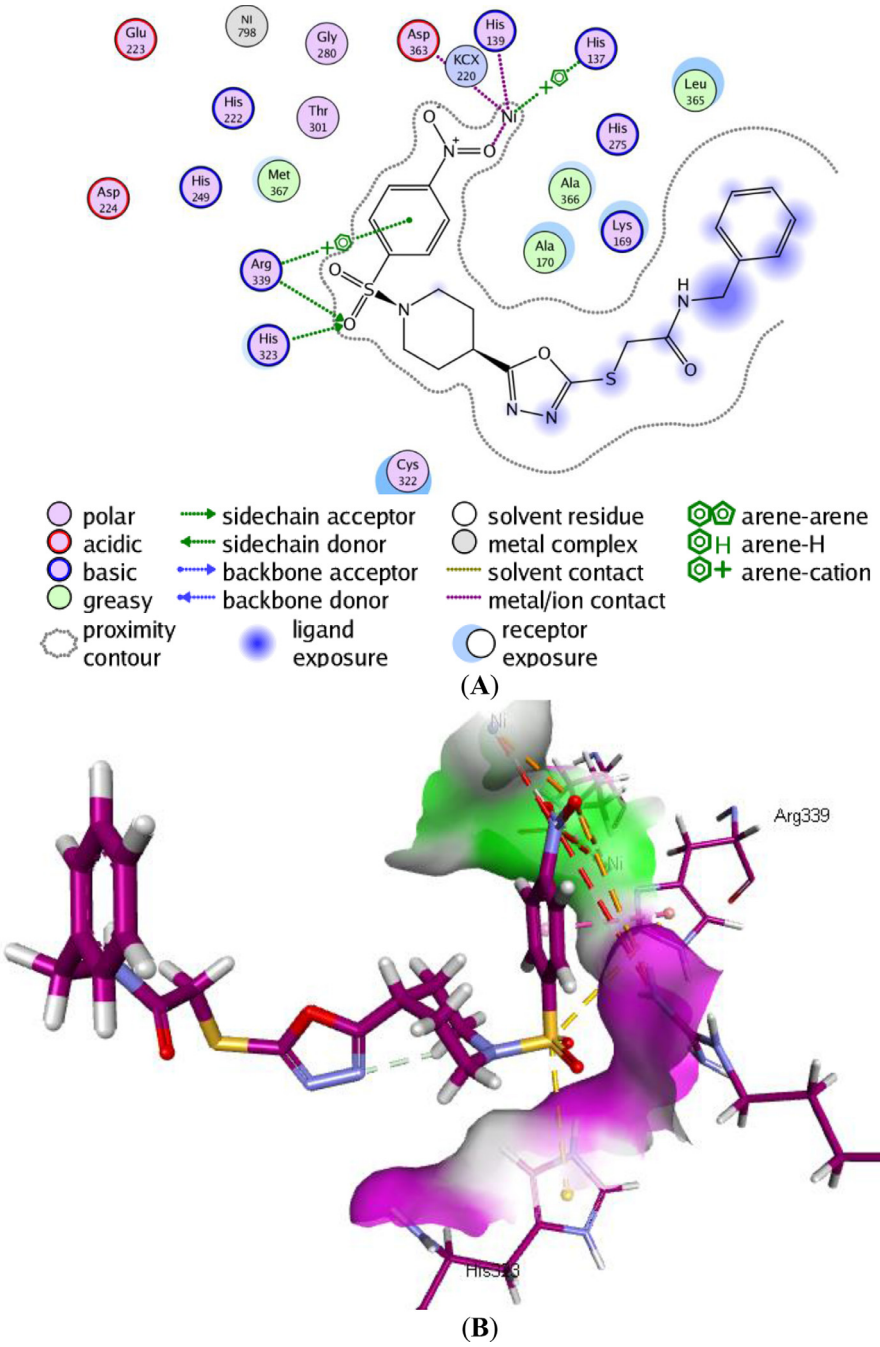
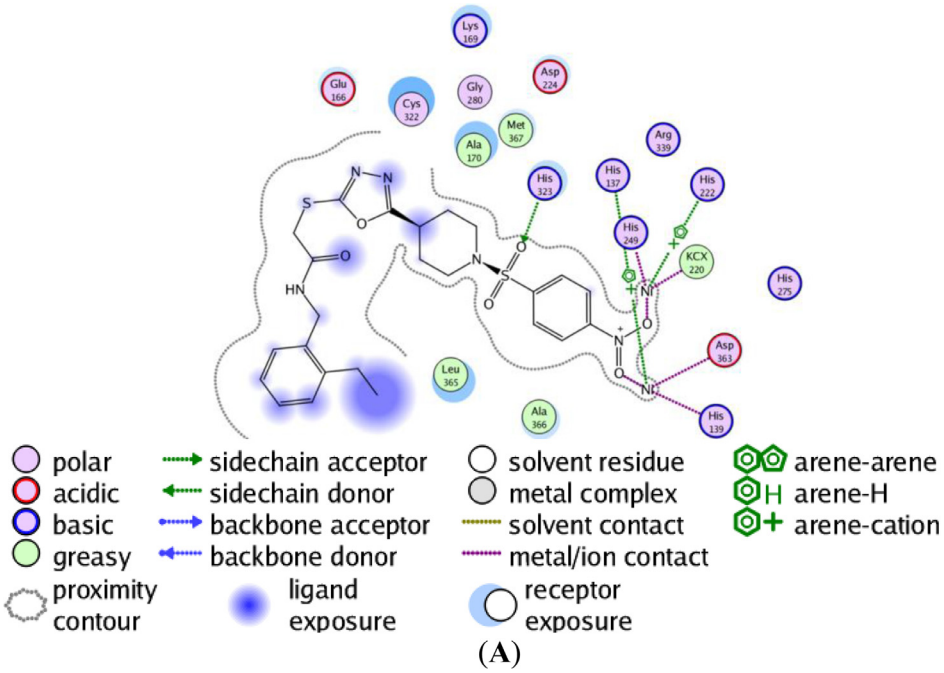



**Fig. 2.** The docked conformations of compound **6a** in 2D (A) and 3D (B) in the active site of urease. Compound is depicted in purple color thick stick, while protein is shown in ribbon with active site residues in thin stick.

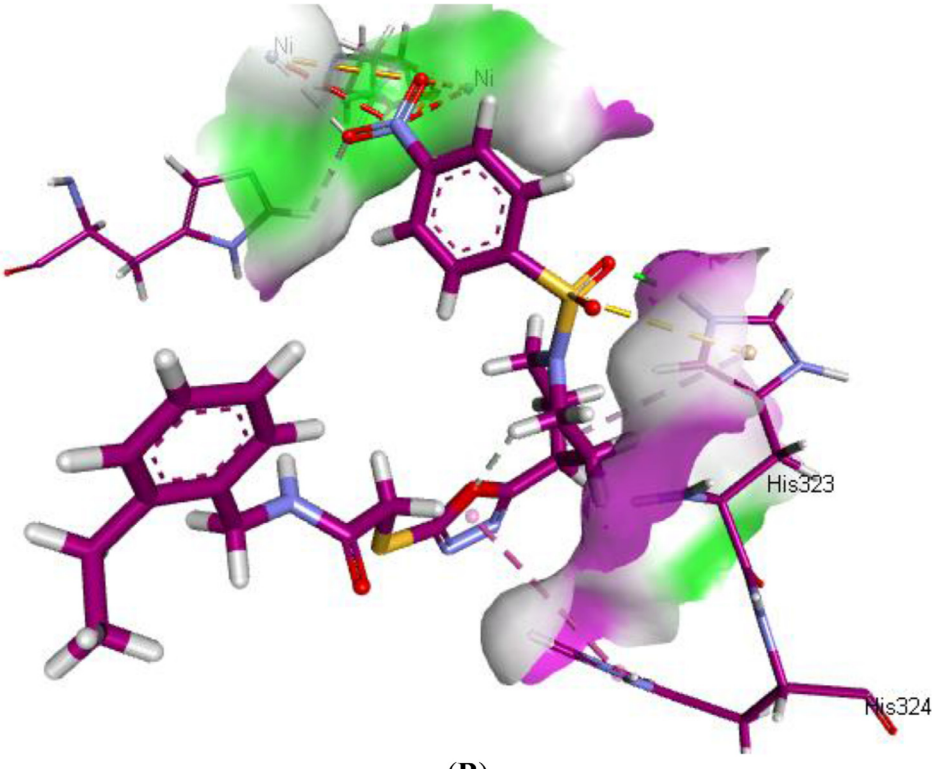
showing bond lengths of 2.70 and 2.74 Å, respectively as indicated in Fig. 2 (A & B).

Similarly, compound **6d** has inhibited the enzyme very strongly by making four interactions. The first hydrogen bond interaction existed between sulfonyl oxygen and NH group hydrogen of imidazole ring of His323 showing bond distance of 1.98 Å. The next two metallic interactions were found between two Ni ions of the urease and a couple of terminal nitro group oxygen atoms of the ligand indicating bond lengths of 2.59 and 2.85 Å, respectively. Somewhat interaction exhibited by the oxadiazole ring of compound with imidazole moiety of His324 at a distance of 4.50 Å, which is illustrated through Fig. 3 (A & B).

From the Fig. 4 (A & B), it is clear that compound **6e** has blocked very strongly the active pocket of urease by making five interactions. The NH proton of the imidazole ring of His323 and sulfonyl oxygen has made a acidic interaction with a bond length of 2.09 Å. Couples of the terminal oxygen atoms of the nitro group and Ni ions have established metallic interactions with bond lengths of 2.47 Å and 2.81 Å, respectively. His324 by itself has made an arene-cation as well as arene-arene interactions with the oxadiazole ring (bond length: 3.06 Å) and terminal phenyl moiety (average bond length: 4.52 Å), respectively. It has come to know from the Fig. 5 (A & B) that compound **6g** have inhibited the enzyme by showing three interactions. The first hydrogen bond




(A)




(B)

**Fig. 3.** The docked conformations of compound **6d** in 2D (A) and 3D (B) in the active site of urease. Compound is depicted in purple color thick stick, while protein is shown in ribbon with active site residues in thin stick.

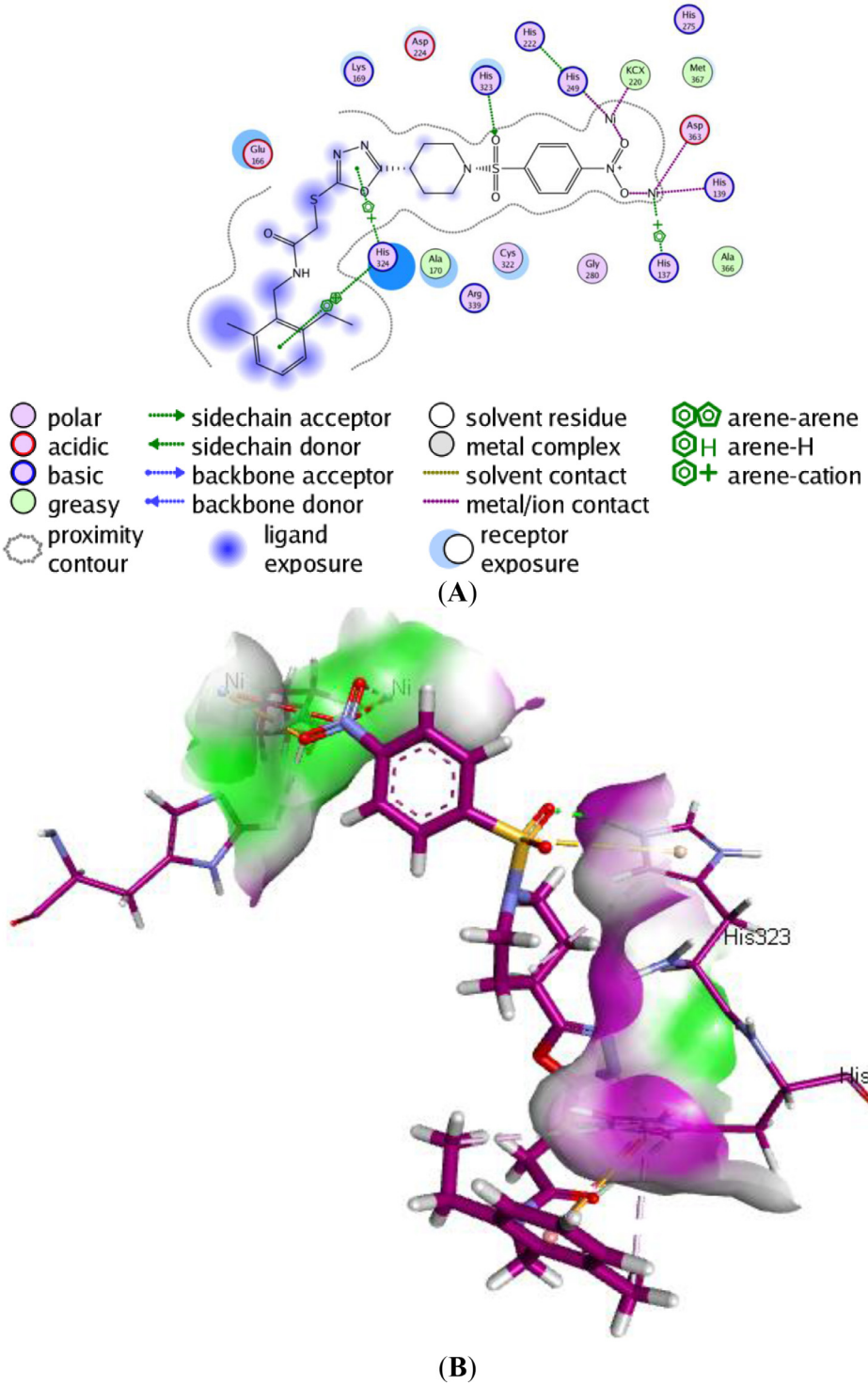



**Fig. 4.** The docked conformations of compound **6e** in 2D (A) and 3D (B) in the active site of urease. Compound is depicted in purple color thick stick, while protein is shown in ribbon with active site residues in thin stick.

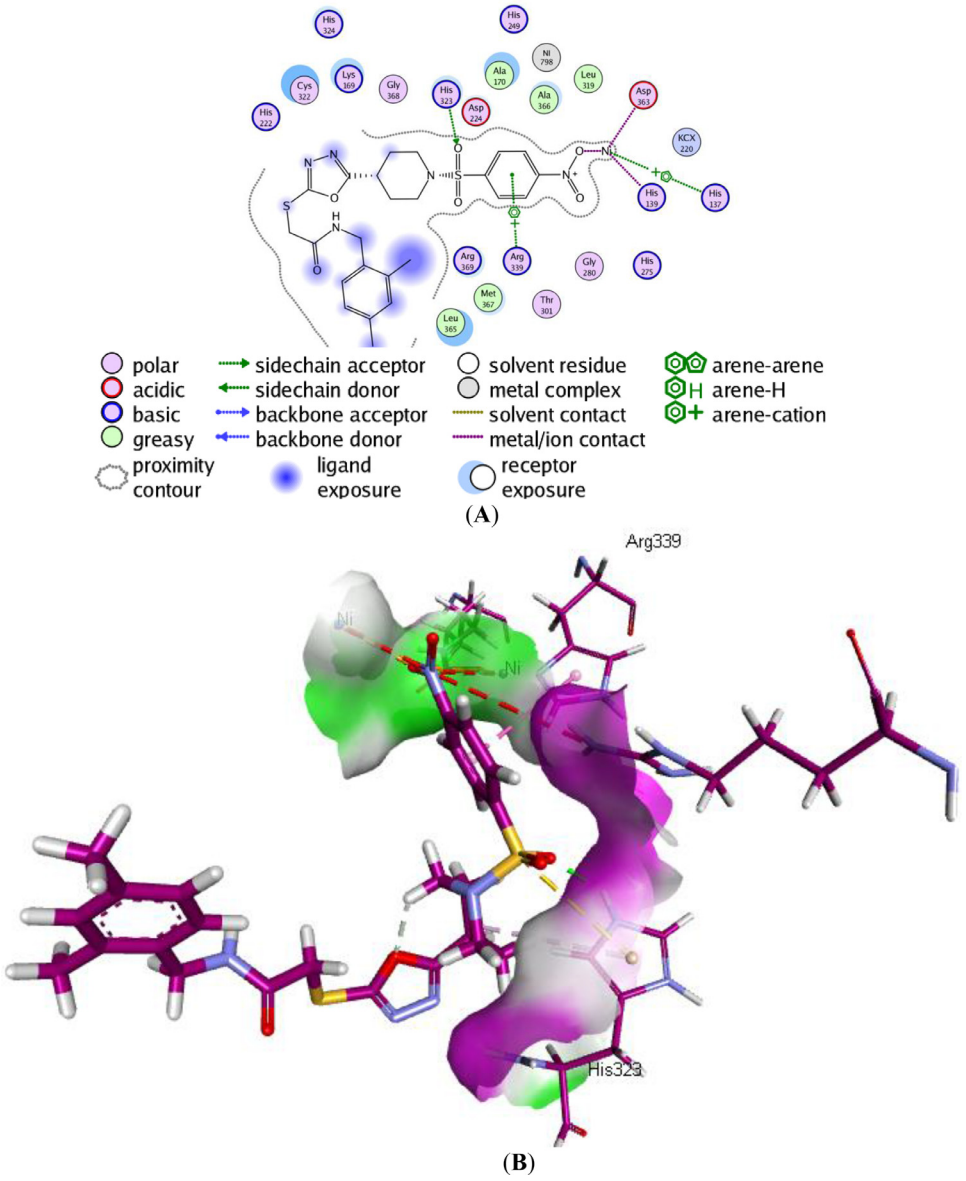



**Fig. 5.** The docked conformations of compound 6 g in 2D (A) and 3D (B) in the active site of urease. Compound is depicted in purple color thick stick, while protein is shown in ribbon with active site residues in thin stick.

formed between sulfonyl oxygen and proton of NH group in imidazole ring of His323 (bond length: 1.83 Å). In the same sequence, another metallic interaction has been found between Ni1 ion of the urease and nitro group oxygen of the ligand in the range of 2.56 Å. The $NH_2$ proton of Arg339 and benzyl moiety of compound also made an interaction of arene-cation with a bond length of 2.85 Å.

After docking, compound **6i** has given four interactions with a variety of amino acids of protein. Ala366 has made back bone donor interaction with NH hydrogen of the ligand (bond length: 2.06 Å), while His222 through its NH hydrogen of imidazole ring has given hydrogen bond with carbonyl oxygen (bond length: 2.36 Å). In addition, Lys169 and His324 have made arene-cation interactions with nitrophenyl and oxadiazole rings of the ligand in the range of 3.65 and 4.60 Å respectively (Fig. 6 A & B).

From the previous studies, we have understood that most of the identified urease inhibitors were toxic against animals, specifically for the human beings and were banned after some time. Therefore, it is must to know about the toxic nature of newly identified inhibiting compounds. In this regard, we have subjected all these synthesized compounds to human neutrophils by applying an already discussed standard assay and by using the positive control of most widely used urease inhibitor of acetohydroxamic acid. It has been known from the results that all **6a–o** inhibitors of the series are non toxic against human

Due to hyper activity, urease is also involved in a number of agricultural as well as neutrophil cells and can be proved as lead series of drugs for the urea related complications (Table 3) environmental problems. To control such complications, urease inhibitors were used. It has been known from the previous literature that most of the urease inhibitors have shown severe side effects against plants. To confirm such effects, we have checked all these compounds (**6a–o**) through standard LemnaWelv phytotoxicity assay. The results proved that mostly they were non phytotoxic against plants, except of the compound **6l** which is slightly phytotoxic against plants as indicated in Table 4.

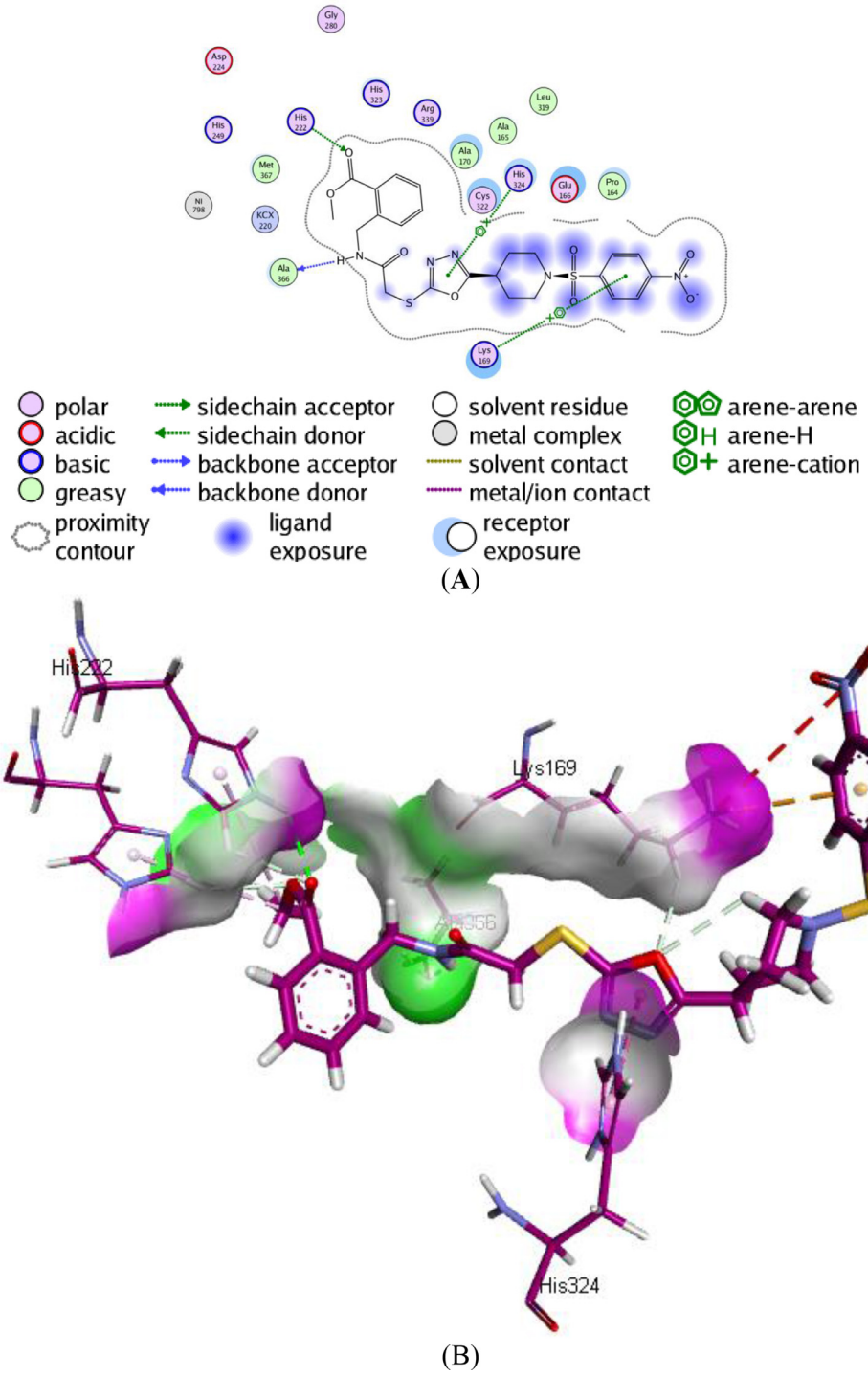



**Fig. 6.** The docked conformations of compound **6i** in 2D (A) and 3D (B) in the active site of urease. Compound is depicted in purple color thick stick, while protein is shown in ribbon with active site residues in thin stick.

## 4. Conclusion

Kinetics studies have confirmed their competitive type of inhibition. *In silico* studies have also shown close agreement with their competitive type of inhibition, as most of the compounds have interacted well with an individual or both the Ni ions of the active site, mostly through their nitro group oxygen atoms and rarely through their other functional groups. Moreover, they encompassed low cytotoxic effects against human neutrophils and non phytotoxic against plants. Shortly, it can be concluded that these compounds can serve lead drug candidates against urease related issues.

## Declaration of Competing Interest

The authors declare that they have no conflicts of interest concerning the publication of this paper.

## Acknowledgement

The authors are thankful to Higher Education Commission (HEC) of Pakistan for generous support.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2020.129141.

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