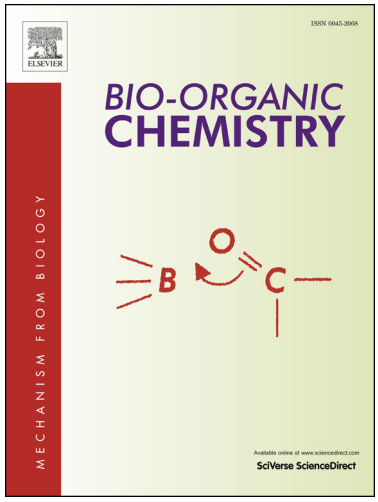

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PII: S0045-2068(15)30029-8
DOI: http://dx.doi.org/10.1016/j.bioorg.2015.10.005
Reference: YBIOO 1852

To appear in: *Bioorganic Chemistry*

Received Date: 7 September 2015
Revised Date: 19 October 2015
Accepted Date: 20 October 2015

Please cite this article as: F. Rahim, K. Zaman, H. Ullah, M. Taha, A. Wadood, M.T. Javed, W. Rehman, M. Ashraf, R. Uddin, I. uddin, H. Asghar, A.A. Khan, K.M. Khan, Synthesis of 4-Thiazolidinone Analogs as Potent *in Vitro* Anti-urease Agents, *Bioorganic Chemistry* (2015), doi: http://dx.doi.org/10.1016/j.bioorg.2015.10.005

Synthesis of 4-Thiazolidinone Analogs as Potent *in Vitro* Anti-urease Agents

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Abstract:

4-Thiazolidinone analogs **1-20** were synthesized, characterized by ^{1}HNMR and EI-MS and investigated for urease inhibitory activity. All twenty (20) analogs exhibited varied degree of urease inhibitory potential with IC_{50} values 1.73-69.65 μM, if compared with standard thiourea having IC_{50} value of 21.25 ± 0.15 μM. Among the series, eight derivatives **3**, **6**, **8**, **10**, **15**, **17**, **19**, and **20** showed outstanding urease inhibitory potential with IC_{50} values of 9.34 ± 0.02, 14.62 ± 0.03, 8.43 ± 0.01, 7.3 ± 0.04, 2.31 ± 0.002, 5.75 ± 0.003, 8.81 ± 0.005, and 1.73 ± 0.001 μM, respectively, which is better than the standard thiourea. The remaining analogs showed good to excellent urease inhibition. The binding interactions of these compounds were confirmed through molecular docking studies.

Keywords: 4-Thiazolidinone, Synthesis, Urease inhibition, Docking studies.

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1.0. Introduction:

Urease enzymes catalyze the hydrolysis of urea into carbamate and ammonia [1-3]. The carbamate decomposes readily into another molecule of ammonia. These reactions cause substantial rise in pH which may cause adverse effects of urease activity on agriculture, animal and human health [4]. Clinically this enzyme is used as indicative to fix manifestation of pathogen in urinary and gastrointestinal tracts. The bacterial urease results in stomach ulcer, infectious stones, peptic ulcer and damaging infections both in animals and human [5]. It also causes the pathogenesis of pyelonephritis, hepatic coma urolithiasis, hepatic encephalopathy, ammonia and urinary catheter encrustation [2]. Lot of microorganisms uses this enzyme to give cradle of nitrogen for growth. In germination process this enzyme has key role in metabolism of plants [2, 6].

Heterocyclic compounds are important for medicinal chemists [7]. Thiazole analogs have medical claims such as bacteriostatic, antibiotics [8], diuretics [9], local anesthetics [10, 11], anti-inflammatory [12], analgesics [13, 14], and anti-HIV [15, 16].

4-Thiazolidinones are the derivatives of thiazole that have been extensively studied and found it to be a part of vitamin-B, Penicillins and antibacterial thiazoles. Reduced thiazole occurs as structural units in compounds of biological importance that serves in the study of polypeptide and proteins [17]. 4-Thiazolidinones have been reported to possess a varied range of biological activities including antitubercular [18], antibacterial [19], antitumor [20], antihistaminic [21], anti-inflammatory [22] and anticonvulsant activity [23].

Our research group is continuously doing effort in search of biologically potent scaffolds [24], which are easy to synthesize and have no tedious chemistry. Previously our group has reported thiobarbituric acid analogs as potent urease inhibitors [25]. In a continued effort in search for biologically active molecules, here we are going to report synthesis of 4-thiazolidinone derivatives and their urease inhibition activity that has not been published earlier.

2. Results and Discussion:

2.1. Chemistry

Synthesis of 4-thiazolidinone analogs was done in two steps [26]. First, substituted benzaldehyde/ acetophenone (1 mmol) was reacted with thiosemicarbazide and 2-3ml of HCl in ethanol and reflux for 3-4 h. The color change showed reaction completion which was monitored by TLC. On completion, reaction mixture was filtered, washed with *n*-hexane to get Schiff base intermediate.

In step-2, Schiff base intermediate product was reacted with chloro acetic acid (1 mmol) and sodium acetate (1 mmol) in acetic acid and reflux for 6 h. Reaction completion was monitored by TLC. On completion, mixture was filtered, washed with *n*-hexane/ethanol to obtain 4-thiazolidinone analogs.

Insert Scheme-1 Here

Insert Table-1 Here

2.2. Biological Activity

The urease inhibitory effects of newly synthesized 4-thiazolidinone analogs were measured, for *Bacillus pasteurii* the results are summarized in Table-2 and compared with urease well known inhibitor thiourea (IC_{50} value 21.25 ± 0.15 μM) as reference compound. Out of these twenty analogs, eight analogs **3**, **6**, **8**, **10**, **15**, **17**, **19**, and **20** showed outstanding urease inhibitory potential with IC_{50} values 9.34 ± 0.02, 14.62 ± 0.03, 8.43 ± 0.01, 7.3 ± 0.04, 2.31 ± 0.002, 5.75 ± 0.003, 8.81 ± 0.005, and 1.73 ± 0.001 μM respectively, which were many folds better than the standard. The remaining analogs also showed good to excellent urease inhibition. Structure-activity relationship suggested that the urease activity of a particular molecule is seemingly directed by the substitution pattern present at aromatic residues. In particular, the analog **20** having methoxy group at 2,5 position and bromine at 4-position on phenyl part was an excellent urease inhibitor with IC_{50} value 1.73 ± 0.001 which was better than the standard inhibitor thiourea. Compound **20** was when compared with analogs **7** and **9** having two methoxy groups with bromo and hydroxyl group respectively at phenyl part. The inhibitory potential of

compound **20** was greater than **7** which were greater than **9**. The difference in potential was seem to be due difference in position of substituents. The compound **15** was found to be the second most active analog among the series having Br at position-2 while nitro at position-4 on the phenyl part. If we comparing compound **15** with analog **1** and **2** having only nitro group on phenyl ring, the compound **15** showed greater potential that is mainly because of extra bromo group. Similarly analog **17** the third most active compound among the series having methoxy group at position-3, while ethoxy at position-4 on the phenyl part. The greater potential of analog **17** then other disubstituted (EDG) analogs like **4** and **18** was mainly because of position difference of substituents. Similar affect was also observed in other analogs. In conclusion we observed here that both electrons donating as well as electron withdrawing groups on phenyl ring play role in the inhibition but the electron donating groups are superior up to some extent. The binding interactions were confirmed through molecular docking studies.

Insert Table-2 Here

Molecular docking

In order to explore the binding mode of newly synthesized 4-thiazolidinone derivatives in the active site of *Bacillus pasteurii* urease (PDB Code: 4UBP) [32] docking study was carried out using MOE (Molecular Operating Environment) software package. The docking results showed that all the compounds well accommodated in the binding pocket of *Bacillus pasteurii* urease. The docked conformation of the most active compound **20,** showed that the carbonyl oxygen of thiazolidin ring of the compound coordinates with both nickel ions like the hydroxyl oxygen in the acetohydroxamate (a potent urease inhibitor) [32]. In addition to nickel chelation, two hydrogen bonds and several hydrophobic interactions between the active site residues and the compound were also observed (**Figure 1a**). The carbonyl oxygen of thiazolidin ring and nitrogen atom of imino moiety established hydrogen bonds with active site residues His222 and His323 respectively. If we compare the structures of compound **20**, **4**, **7** and **9** all the compounds have about similar structures the only difference in their structures is the presence or absence of bromine moiety and the position of methoxy moiety. The biological activities of these compounds showed that bromine moiety and the position of methoxy play a key role in the inhibitory activities of these compounds (**Table 1**). For example in case of compound **18**, although the compound have methoxy moieties but bromine is not present and the activity of this

compound is less as compare to compound **20**. The binding mode of compound **18** showed poor interaction as compare to compound **20**. As shown in Figure **1b**, although compound **18** established coordinate bond with nickel ions but only one hydrogen bond was observed whereas in case of compound **20** two hydrogen bonds were observed. Furthermore, the distance between the nickel ions and carbonyl oxygen is more as compare to compound **20** (**Figure 1a and 1b**).

Insert Figure-1 Here

Similarly, the positions of methoxy moiety play a key role in the biological activities as well as binding interactions. The docking results showed that the compounds having adjacent methoxy moiety showed poor interactions with active site residues of urease and less biological activities (compound **4** and **7**). About similar experimental and docking results were observed for compounds **15** and **2**. In these compounds the bromine play the same role as in the above mentioned compounds. When bromine is present, for example compound **15**, good biological activity and strong bonding network was observed (**Table 1** and **Figure 2a**). But when bromine is absent from the structure (compound **2**), lower biological activity and poor interactions were observed (**Tabel 1** and **Figure 2b**).

Insert Figure-2 Here

In case of compounds having hydroxy phenyl ring in their structures (compound **3**, **5** and **6**), it was observed that the presence and absence of chlorine moiety and the position of hydroxyl group play a pivotal role in the biological activities as well as the binding interactions of these compounds. For example when chlorine is present, compound **3**, the inhibitory activity and binding interactions are good (**Table 1**). But when chlorine is absent from the structure (compound **6**) both biological activity and binding interactions were poor (**Figure 3a**). Like bromine and chlorine a key role was observed for benzoyloxy-4-methylbenzine and indole moieties. For example when these moieties present (compound **11**, **12** and **18**) lower biological activities as well as poor interactions were observed for these compounds (**Table 1** and **Figure 3b**). These lower activities and poor interactions might be due to the steric clash produce by these bulky hydrophobic groups. The docking conformation of compound **12** showed that although the carbonyl oxygen of the thiazolidin ring coordinate with the nickel ions of the

enzyme but the distance is more as compare to compound lacking benxoyl-4-methylbenzine ring (**Figure 3b**).

Insert Figure-3 Here

Overall the docking results showed that bromine and chlorine moieties play a key role in the biological activities of these compounds whereas the presences of bulky hydrophobic groups are detrimental for the biological activities and binding interactions.

3.0. Conclusion

In conclusion we have synthesized twenty analogs of 4-thiazolidinone and evaluated for urease inhibition. All compounds showed varied degree of urease inhibition ranging in between 1.73 ± 0.001 to 69.65 ± 0.12 μM when compared with the standard inhibitor thiourea having IC_{50} value 21.25 ± 0.15 μM. Among the series, analogs **3**, **6**, **8**, **10**, **15**, **17**, **19**, and **20** showed outstanding urease inhibitory potential with IC_{50} values 9.34 ± 0.02, 14.62 ± 0.03, 8.43 ± 0.01, 7.3 ± 0.04, 2.31 ± 0.002, 5.75 ± 0.003, 8.81 ± 0.005 and 1.73 ± 0.001 respectively which is better than the standard thiourea. The remaining analogs also showed good to excellent urease inhibition.

4. Experimental Section

4.1. General Methods

The NMR spectra of concerned analogs of 4-thiazolidinone were recorded by on Avance Av-300, 500 MHz NMR spectrometers in which DMSO-d_6/Acetone-d_6 were used as a solvent. Chemical shift values of spectra are assigned in δ (ppm). Tetramethylsilane act as internal standard. Jeol JMS-600H instrument were used to obtained Electron Ionization Mass Spectra. The probability of reactions was checked by TLC on precoated silica gel aluminum plates (kieselgel 60,254, E. Merck, Germany). UV lamp at range of 254 and 365nm were used to observe the spots on TLC plate.

*4.2. Methodology for synthesis of 4-thiazolidinone **(1-20)***

Synthesis of 4-thiazolidinone analogs was done in two steps [26]. First, substituted benzaldehyde/ acetophenone (1 mmol) was reacted with thiosemicarbazide and 2-3ml of HCl in

ethanol and reflux for 3-4 h. The color change showed reaction completion which was monitored by TLC. On completion, reaction mixture was filtered, washed with *n*-hexane to get Schiff base intermediate.

In step-2, Schiff base intermediate product was reacted with chloro acetic acid (1 mmol) and sodium acetate (1 mmol) in acetic acid and reflux for 6 h. Reaction completion was monitored by TLC. On completion, mixture was filtered, washed with *n*-hexane/ethanol to obtain 4-thiazolidinone analogs.

4.2.1. (E)-2-((E)-(2-Nitrobenzylidene) hydrazono) thiazolidin-4-one ***(1)***

Yield: 81%, ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 11.2 (s, 1H, NH), 8.6 (s, 1H, HC=N), 8.0 (dd, J = 1, J = 8 Hz, 1H, H-3), 7.9 (dd, J = 1.5 Hz, $J_{6,4}$ = 8 Hz, 1H, H-6), , 7.88 (dt, J = 0.5 Hz, J = 7.5 Hz, 1H, H-4), 7.6 (dt, J = 1.5 Hz, J = 8.5 Hz, 1H, H-5), 3.9 (s, 2H, CH_2). EI-MS: *m/z* (rel. int. %): 264 (M^+, 100), 219 (56), 157 (77), 101 (69) 77 (81).

4.2.2. (E)-2((E)-(4-Nitrobenzylidene) hydrazono) thiazolidine-4-one ***(2)***

Yield: 70%, ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 11.2 (s, 1H, NH), 8.5 (s 1H, CH=N), 8.3 (d, $J_=$ 8.5 Hz, 2H, H-3/5), 7.9 (d, $J_=$ 8.3 Hz, 2H, H-2/6) 3.9 (s, 2H, CH_2). EI-MS: *m/z* (rel. int. %): 264 (M^+, 100), 219 (56), 157 (77), 101 (69) 77 (81).

4.2.3. (E)-2-((E)-(3-Chloro-4-hydroxybenzylideno) thiazolidin-4-one ***(3)***

Yield: 72%, ^{1}H-NMR: (300 MHz, Acetone-d_6): δ 11.2 (s, 1H, NH), 8.2 (s, 1H, HC=N), 7.8 (d, $J_{2,6}$= 2.1 Hz, 1H, H-2), 7.6 (dd, $J_=$ 1.8 Hz, $J_{6,5}$ = 8.4 Hz, 1H, H-6). 7.0 (d, $J_=$ 8.4 Hz, 1H, H-5), 3.8 (s, 2H, CH_2). EI-MS: *m/z* (rel. int. %): 271 (M^+ +2, 30), 269 (M^+, 100), 105 (52), 140 (64), 154 (76) 77 (47).

4.2.4. (E)-2-((E)-(2, 3-Dimethoxybenzylidene) hydrazono) thiazolidin-4-one) ***(4)***

Yield: 64%,.^{1}H-NMR: (500 MHz, DMSO-d_6): δ 11.2 (s, 1H, NH), 8.5 (s, 1H, HC=N), 7.4 (dd, $J_{6,4}$ = 3 Hz, J = 6.5 Hz, 1H, H-6), 7.1 (m, 2H, H-4/5), 3.8 (s, 2H, CH_2), 3.7 (s, 6H, OMe). EI-MS: *m/z* (rel. int. %): 279 (M^+, 100), 263 (37), 157 (44), 101 (39) 77 (57).

4.2.5. (E)-2-((E)-(3, 5-Dichloro-2-hydroxybenzylidene) hydrazono) thiazolidin-4 one ***(5)***

Yield: 81%, ^{1}H-NMR: (300 MHz, Acetone-d_6): δ 11.2 (s, 1H, NH), 8.5 (s, 1H, HC=N), 7.9 (s, 1H, H-4), 7.4 (d, $J_{6,4}$ = 2.4 Hz, 1H, H-6), 3.8 (s, 2H, CH_2). EI-MS: *m/z* (rel. int. %) 307 (M^+ +2, 8), 305 (M^+, 18), 263 (34), 187 (55), 124 (120), 97 (48).

4.2.6. (E)-2((E)-(4-Hydroxybenzylidin) hydrazono) thiazolidine-4-one ***(6)***

Yield: 68%, ^{1}H-NMR (300 MHz, Acetone-d_6), δ 10.2 (s, 1H, NH), 8.0 (s, 1H, HC=N), 7.6, (d, J = 8.7 Hz, 2H, H-3/5), 6.8 (d, J = 8.4 Hz, 2H, H-2/6), 3.7 (s, 2H, CH_2). EI-MS: *m/z* (rel. int. %) 235 (M^+, 100), 195 (22), 135 (14), 120 (92), 106 (51).

4.2.7. (E)-2((E)-(3-Bromo-4, 5-dimethoxybenzylidene) hydrazono) thiazolidine 4-one ***(7)***

Yield: 65%, ^{1}H-NMR (300 MHz, Acetone-d_6): δ 10.4 (s, 1H, NH), 8.2 (s, 1H, HC=N), 8.0 (s, 1H, H-2), 7.9 (d, J = 3.9 Hz, 1H, H-6), 3.9 (s, 6H, 2xOMe), 3.8 (s, 2H, CH_2). EI-MS: *m/z* (re lint %) 358 (M^+ +2, 83), 356 (M^+, 85), 278 (2), 228 (20), 169 (10), 78 (29).

4.2.8. (E)-2((E)-(3-nitrobenzylidene) hydazono) thiazolidin-4-one ***(8)***

Yield: 75%, ^{1}H-NMR(300 MHz, Acetone-d_6), δ 10.4 (s,1H, NH), 8.6 (s, 1H, HC=N), 8.2 (s, 1H, H-2), 8.2 (d, J = 8.1 Hz, 1H, H-6), 7.7 (t, J = 8.1 Hz, 2H, H-4/5), 3.8 (s, 2H, CH_2). EI-MS: *m/z* (rel int%) 224 (M^+, 69), 118 (58), 89 (53), 76 (100), 60 (71).

4.2.9. (E)-2-((E)-(4-Hyrazoxy-3, 5-dimethoxybenzylidene) hydrazono) thiazolidin-4-one ***(9)***

Yield: 70%, ^{1}H-NMR (300 MHz, Acetone-d_6), δ 10.4 (s, 1H, NH), 8.2 (s, 1H, HC=N), 8.0 (s, 1H, OH), 7.12 (d, J = 4.8Hz, 2H, H-2/6), 3.8 (s, 2H, CH_2), 3.5 (s, 6H, 2 x OMe). EI-MS: *m/z* (rel int%) 295 (M^+, 95), 166 (29), 151 (13), 95 (27), 68 (18).

4.2.10. (E)-2-((E)-(4-(Benzyloxy)-2-methoxybenzylidene) hydrazono) thiazolidin-4-one ***(10)***

Yield: 68%, (^{1}H-NMR (300 MHz, Acetone-d_6), δ 10.2 (s, 1H, NH), 8.2 (s, 1H, HC=N), 7.55 (d, $J_{3,5}$ =1.8 Hz, 1H, H-3), 7.50 (m, 3H, H-5'/6'/2'), 7.3 (m, 3H, H-3'/4'/6), 7.1 (dd, J = 1.8 Hz, J = 8.4 Hz, 1H, H-5), 5.1 (s, 2H, OCH_2). 3.87 (s, 2H, CH_2), 3.85 (s, 3H, OMe). EI-MS: *m/z* (rel. int. %) 355 (M^+, 13), 263 (23) 163 (7) 135 (9).

4.2.11. 4-((E)-((E)-(4-Oxothiazolidin-2-ylidene) hydrazono) methyl) benzaldehyde ***(11)***

Yield: 60%, (^{1}H-NMR (300 MHz, Acetone-d_6), δ 10.2 (s, 1H, NH), 8.2 (s, 1H, HC=N), 8.1 (s, 1H, CHO aldehydic), 7.9 (d, J = 8.7 Hz, 2H, H-2/3) 7.5 (d, J = 8.4Hz, 2H, H-5/6), 3.8 (s, 2H, CH_2). EI-MS: *m/z* (rel. int. %) 247 (M^+, 20), 129 (25), 178 (78), 115 (36), 76 (46).

4.2.12. (Z)-2-((Z)-(4-(Dimethyl amino) benzylodine) hydrazono) thiazolidine-4-one ***(12)***

Yield: 63%, (^{1}H-NMR (300 MHz, Acetone-d_6), δ 10.3 (s, 1H, NH), 8.2 (s, 1H, HC=N), 7.9, (d, J = 7.2 Hz, 2H, H-3/5), 6.9 (d, J = 7.4 Hz, 1H, H-2), 3.7 (s, 2H, CH_2), 3.06 (s, 6H, 2 x OMe). EI-MS: *m/z* (rel. int. %) 262 (M^+, 100), 219 (67), 157 (47), 115 (21), 77 (58).

4.2.13. (E)-2-((E)-(4-(Benzyloxy) benzylidene) hydrazono) thiazolidine-4-one ***(13)***

Yield: 68%, ^{1}H-NMR (300 MHz, Acetone-d_6) δ 10.1 (s, 1H, NH), 8.0 (s, 1H, HC=N), 7.5 (dd, J = 1.6 Hz, J = 7.4 Hz, 2H, H-2/6), 7.4 (m, 3H, H-5'/6'/2'), 7.2 (m, 2H, H-3'/4'), 7.0 (dd, J = 1.8 Hz, J = 8.4 Hz, 2H, H-3/5), 5.1 (s, 2H, OCH_2). 3.9 (s, 2H, CH_2). EI-MS: *m/z* (rel. int. %) 325 (M^+, 66), 263 (75), 219 (45), 101 (100), 77 (81).

4.2.14. (2Z)-2, 2' (Z)-(1, 4-Phenylenebis (methane-1-yl-ylidene)) bis (hydrazine-2, 1-diylidene)) dithiazolidin-4-one ***(14)***

Yield: 55%, ^{1}H-NMR (300 MHz, Acetone-d_6), δ 10.2 (s, 2H, 2 x NH), 8.0 (s, 2H, 2 x HC=N'), 7.41 (dd, J = 1.6 Hz, J = 8.1 Hz, 2H, H-3/5), 7.40 (dd, J = 1.6 Hz, $J_{2,\,3/6,\,5}$ = 8.0 Hz, 2H, H-3/5), 3.8 (s, 4H, 2-CH_2). EI-MS: *m/z* (rel. int. %) 360 (M^+, 72), 273 (61), 233 (41), 157 (36), 101 (100).

4.2.15. (Z)-2-((Z)-(1-(2-Bromo-4-nitrophenyl) ethylidene) hydrazono) thiazolidine-4-one ***(15)***

Yield: 58%, (^{1}H-NMR (300 MHz, Acetone-d_6), δ 10.1 (s, 1H, NH), 8.2 (s, 1H, H-3), 8.1 (d, $J_{5,\,6}$ = 8.1 Hz, 1H, H-5), 7.9 (d, $J_{6,\,5}$ = 8.0 Hz, 1H, H-6), 3.7 (s, 2H, CH_2), 2.7 (s, 3H, CH_3). EI-MS: *m/z* (rel. int. %) 359 (M^+ +2, 98), 357 (M^+, 100), 270 (51), 146 (44), 101 (378), 77 (19).

4.2.16 (E)-2-(E)-((1H-Indole-3-yl) methylene) hydrazono) thiazolidine-4-one ***(16)***

Yield: 58%, ^{1}H-NMR (300 MHz, Acetone-d_6), δ 10.5 (s, 1H, NH), 10.3 (s, 1H, NH), 8.1 (s, 1H, HC=N), 7.5 (d, J = 7.2 Hz, 2H, H-4/7), 7.3 (s, 1H, H-2) 7.1 (m, 2H, H-5/6) 3.8 (s, 2H, CH_2). EI-MS: *m/z* (rel. int. %) 258 (M^+, 100), 157 (76), 116 (35), 101 (56), 70 (18).

4.2.17. (Z)-2-((Z)-(4-Ethoxy-3-methoxybenzoylidine) hydrazono) thiazolidine-4-one ***(17)***

Yield: 77%, ^{1}H-NMR (300 MHz, Acetone-d_6), δ 10.1 (s,1H, NH), 8.1 (s, 1H, HC=N), 7.2 (s, 1H, H-2), 7.1 (m, 2H, H-5/6) 3.7 (m, 2H, OCH_2), 3.6 (s, 3H, OMe) 1.34 (t, 3H, CH_3). EI-MS: *m/z* (rel. int. %) 293 (M^+, 75), 219 (92), 157 (38), 101 (100), 77 (23).

4.2.18. (Z)-2-((Z)-(2, 4-Dimethoxybenzoylidine) hydrazono) thiazolidine-4-one ***(18)***

Yield: 56%, ^{1}H-NMR (300 MHz, Acetone-d_6), δ 10.1 (s,1H, NH), 8.2 (s, 1H, HC=N), 7.6 (m, 2H, H-5/6) 7.3 (s, 1H, H-3), 3.8 (s, 2H, CH_2), 3.6 (s, 6H-OMe).EI-MS: *m/z* (rel. int. %) 279 (M^+, 100), 219 (51), 100 (31), 77 (56), 77 (37).

4.2.19. (Z)-2-((Z)-(Napthalen-1-ylmethylene) hydrazono) thiazolodin-4-one ***(19)***

Yield: 56%, ^{1}H-NMR (300 MHz, Acetone-d_6), δ 10.4 (s,1H, NH), 8.1 (s, 1H, HC=N),7.5 (m, 2H, H-2/4), 7.4 (d, J = 7.2 Hz, 1H, H-8), 7.3 (m, 4H, H-5/67/3), 3.8 (s, 2H, CH_2). EI-MS: *m/z* (rel. int. %) 289 (M^+, 60), 157 (80), 127 (100), 101 (33), 70 (16).

4.2.20. (Z)-2-((Z)-(4-Bromo-2, 5-dimethoxybenzoylidine) hydrazono)thiazolidine-4-one ***(20)***

Yield: 45%, ^{1}H-NMR (300 MHz, Acetone-d_6), δ 10.4 (s,1H, NH), 8.2 (s, 1H, HC=N), 7.4 (s, 1H, H-3) 7.2 (s, 1H, H-6), 3.84 (s, 2H, CH_2), 3.83 (s, 6H, 2xOMe); EI-MS: *m/z* (rel. int. %) 360 (M^+ +2, 99), 358 (M^+, 100), 328 (52), 219 (37), 157 (28), 77 (62).

4.3. Urease inhibition Assay

This assay was modified from Berthelot assay and was employed for the determination of urease activity [27]. The assay is based on the hydrolysis of urea into ammonia which reacts with phenol-hypochlorite to form light blue colored complex measured at 625 nm. A total volume of 85 µl assay mixture contained 10 µl of 50 mM phosphate buffer, pH 7.0, 10 µl of sample solution and 25 µl of *Bacillus pasteurii* urease (Sigma) solution (0.015 units). The contents were pre-incubated at 37ºC for 10 min. Then, 40 µl of urea stock solution (20 mM) was added to each well and incubation continued at 37ºC for further 10 min and preread at 625nm using the 96-well plate reader Synergy HT (Biotek Inc.). Phenol hypochlorite (115 µl) reagent was added in each well (freshly prepared by mixing 45 µl phenol reagent with 70 µl of alkali reagent). For color development, incubation was done at 37oC for another 10 min. Absorbance was again measured at 625 nm. The percentage enzyme inhibition was calculated by the following formula:

$$\text{Inhibition (\%)} = 100 - [(\text{Abs. of test sample} / \text{Abs. of control}) \times 100]$$

IC50 values (concentration at which enzyme inhibition is 50%) of active compounds were determined by measuring activities at further dilutions and the data was computed by using EZ-

Fit Enzyme software (Perrella Inc, USA). The absorbance units of uninhibited enzyme were between 1.0-1.2.

Molecular docking

The three dimensional (3D) X-ray structure of BP urease with the resolution 1.55 Å was retrieved from the protein data bank (http://www.rscb.org./pdb; code 4UBP) [28]. All the water molecules were removed from the structure and hydrogen atoms were added. This structure was then energy minimized with amber99 force field in the MOE Software packages (http://www.chempcomp.com). The three dimensional structure of the compounds were modeled using Builder software implemented on MOE. All the structures were then energy minimized using mmff94 force field in MOE prior to molecular docking studies.

Acknowledgements: This work was economically sustained by the Higher Education Commission (HEC), Pakistan, Project No. 20-1910 under the National Research Program for Universities.

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Captions

Scheme-1: Synthesis of 4-thiazolidinone analogs **1-20**

Table-1: Different substituent 4-thiazolidinone analogs **1-20**

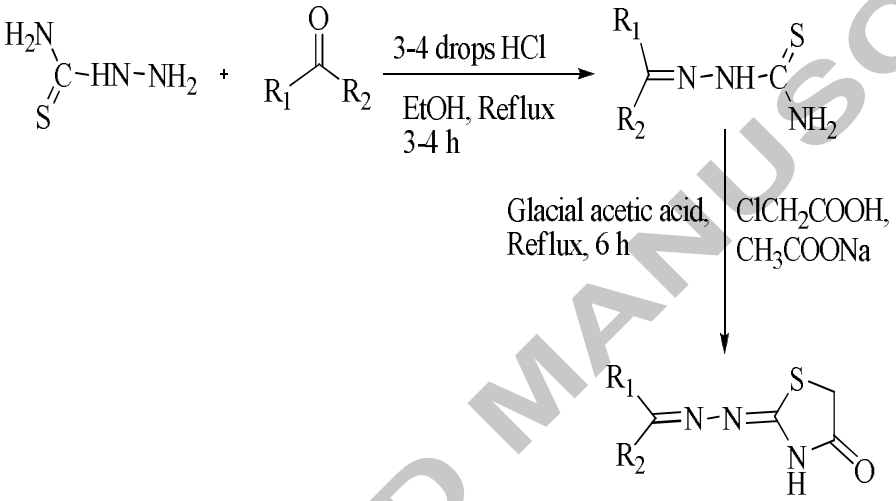
Table-2: Urease inhibitory activities and molecular docking score of compounds **1-20**.

Figure-1: Docking conformation of compounds (**a**) **20** and (**b**) **18** in the active site of urease enzyme.

Figure-2: Docking conformation of compounds (**a**) **15** and (**b**) **2** in the active site of urease enzyme.

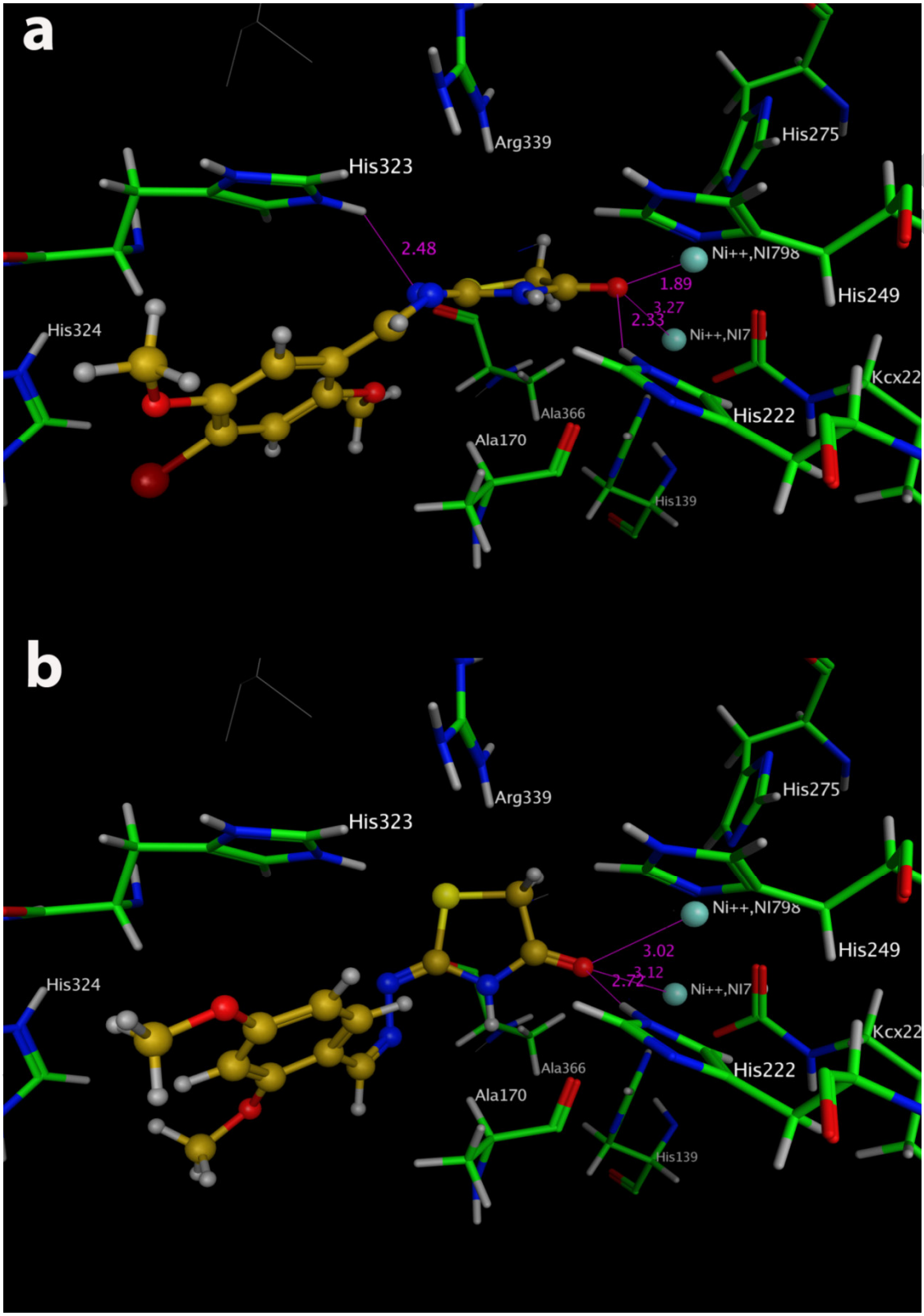
Figure-3: Docking conformation of compounds (**a**) **6** and (**b**) **12** in the active site of urease enzyme.

Scheme-1:



1-20

Figure-1: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Arg339
His275
His323
2.48
Ni++,NI798
1.89
3.27
2.33
His249
Ni++,NI7
His324
Kcx22
Ala366
His222
Ala170
His139
b
Arg339
His275
His323
Ni++,NI798
3.02
3.12
His249
His324
Ni++,NI7
Kcx22
Ala366
His222
Ala170
His139

Figure-2

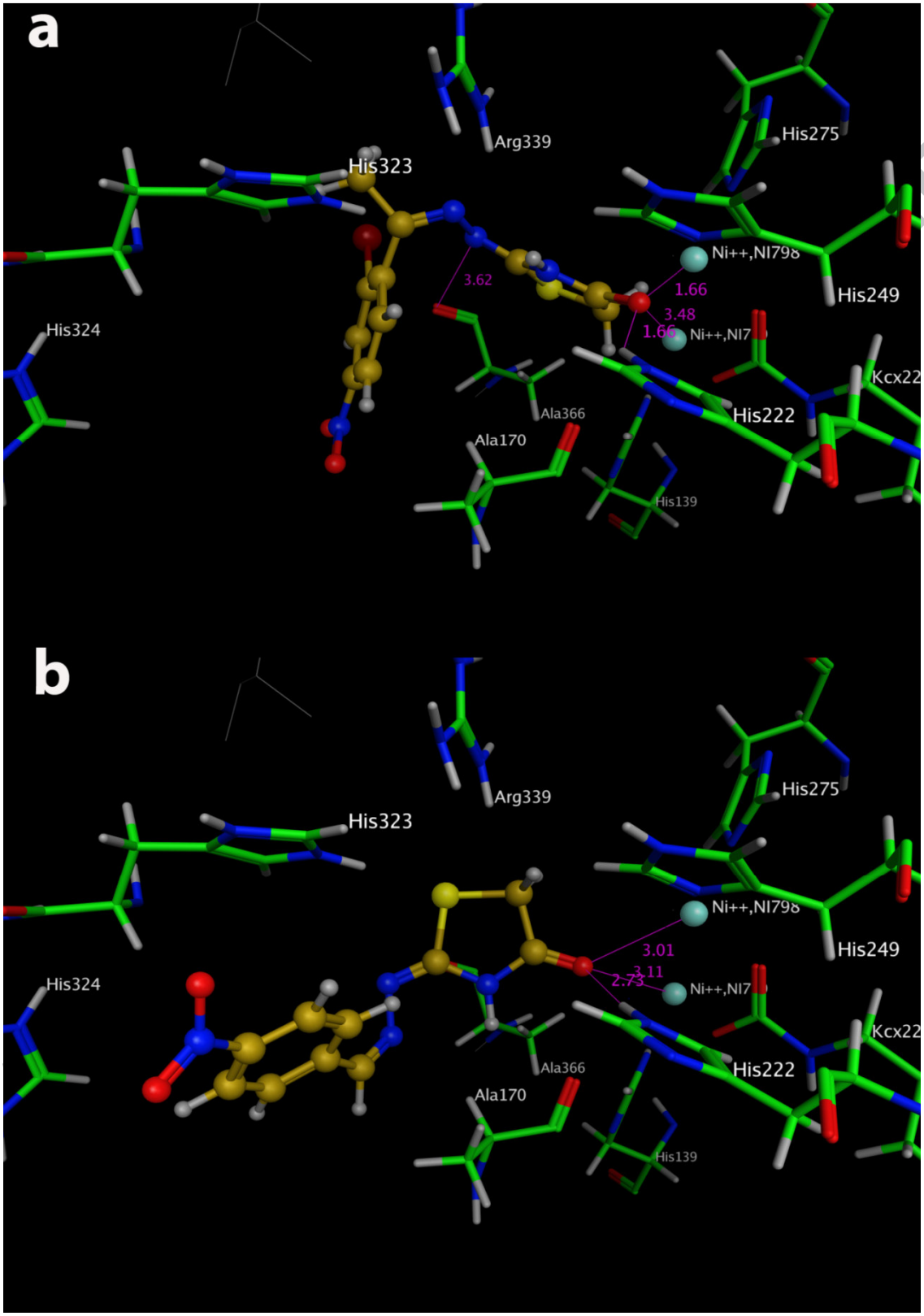


Figure-3:

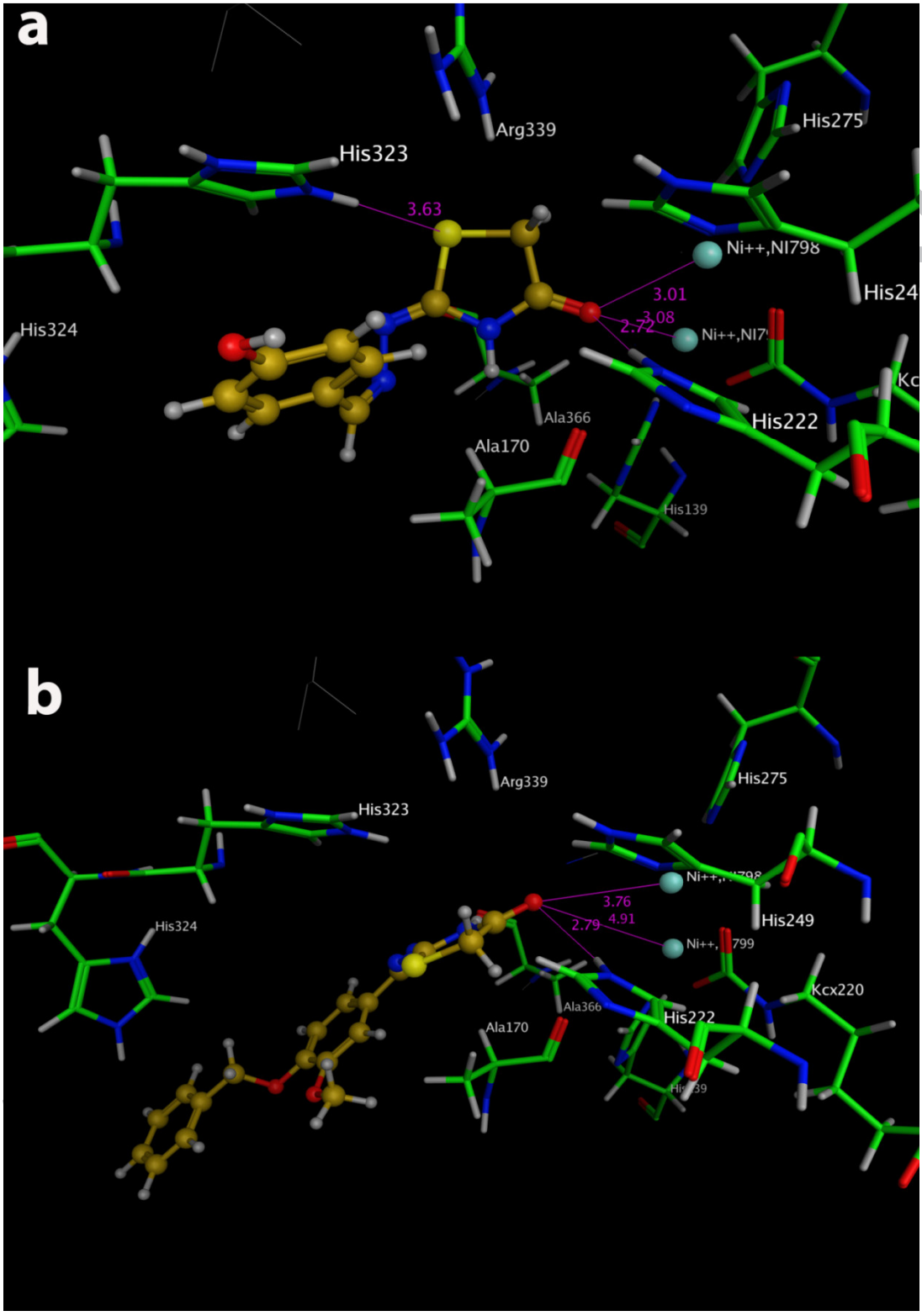
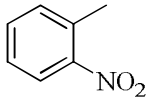
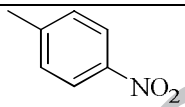
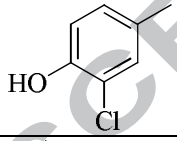
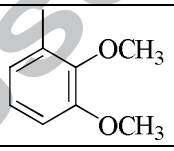
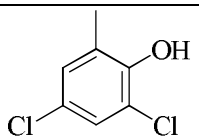
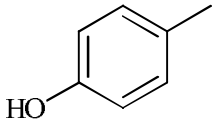
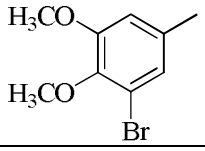
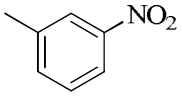
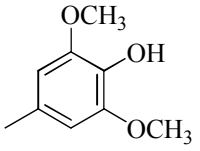
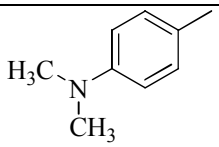
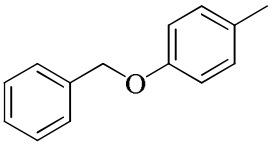

a
Arg339
His323
His275
3.63
Ni++,NI798
3.01
3.08
2.72
His24
His324
Ala366
His222
Ala170
His139
b
Arg339
His275
His323
3.76
4.91
2.79
His249
His324
Kcx220
Ala366
His222
Ala170

Table-1: Different substituent of 4-thiazolidinone analogs **1-20**

S.No	R_1	R_2
1	H	
2	H	
3	H	
4	H	
5	H	
6	H	
7	H	
8	H	
9	H	
10	H	
11	H	

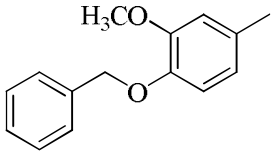
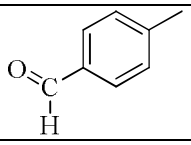
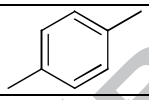
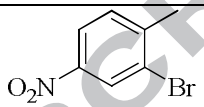
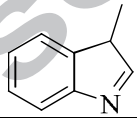
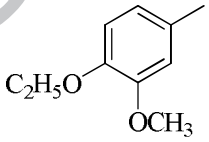
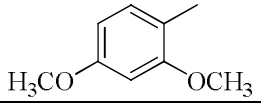
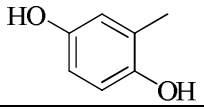
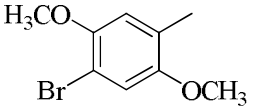
12	H	
13	H	
14	H	
15	CH_3	
16	H	
17	H	
18	H	
19	H	
20	H	

Table-2: Urease inhibitory activities and molecular docking score of compounds **1-20**.

Compound No.	IC_{50}(µM)	Docking Score
1	47.52±0.06	-10.9123
2	26.91±0.05	-10.8194
3	**9.34±0.02**	-15.2562
4	52.53±0.08	-10.6082
5	26.71±0.06	-10.1501
6	**14.62±0.03**	-11.0493

7	36.64±0.06	-9.1254
8	**8.43±0.01**	-17.5732
9	37.91±0.07	-10.4323
10	**7.3±0.04**	-20.8895
11	48.54±0.08	-8.3454
12	68.92±0.02	-8.3095
13	39.21±0.09	-7.5423
14	43.93±0.08	-7.1573
15	**2.31±0.002**	-21.1516
16	51.42±0.09	-7.0901
17	**5.75±0.003**	-21.0824
18	69.65±0.12	-6.9867
19	**8.81±0.005**	**-14.2858**
20	**1.73±0.001**	-23.8513
Thiourea	21.25±0.15	

Synthesis of 4-Thiazolidinone Analogs as Potent *in Vitro* Anti-urease Agents

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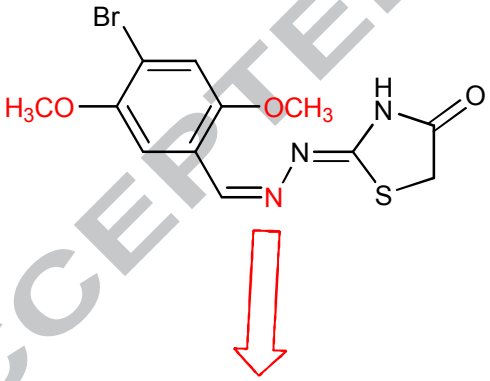
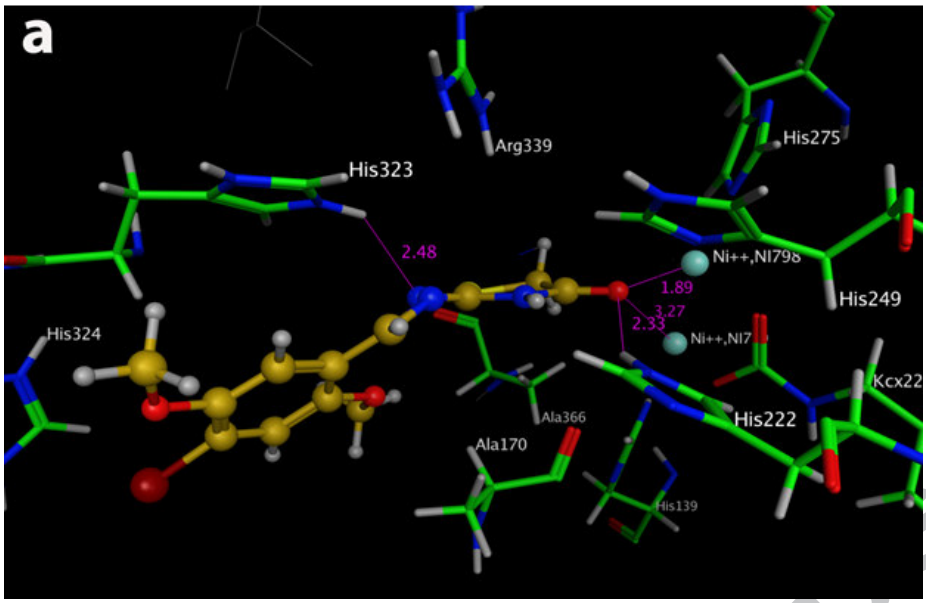
[f] *HEJ,Research Institute of Chemistry, ICCBS, University of Karachi, Karachi-75270, Pakistan*



Compound 20

(IC_{50} = 1.73 ± 1.001 *u*M)
Potent Inhibitor of Urease

Standard Inhibitor Thiourea
(IC_{50} = 21.25 ± 0.15 *u*M)



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