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Identification of Novel Urease Inhibitors by High-Throughput Virtual and in Vitro Screening

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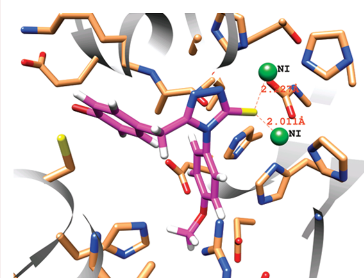
ABSTRACT Ureases are important in both agriculture and human health. Bacterial ureases are directly involved in many farm-field problems and pathological conditions. Here, we report a structure-based virtual screening of an in-house compound bank of about 6000 molecular entities by computational docking and binding free energy calculations followed by in vitro screening. Applied protocol leads to the identification of novel urease inhibitors, which can serve as starting points for structural optimization.

KEYWORDS Urease inhibitors, high-throughput screening, in vitro screening, computational docking, binding free energy calculations



Urease (EC 3.5.1.5; urea amidohydrolase) is a metal-containing enzyme that catalyzes the hydrolysis of urea into ammonia and carbon dioxide.[1] It is present in a wide variety of organisms, including plants, algae, fungi, and bacteria.[2−4] Urease-producing bacteria have a negative effect on human health. They are directly involved in urinary tract infections.[5] Urease is known to be a major cause of pathologies induced by *Helicobacter pylori*, as it allows the bacteria to survive at the low pH of stomach during colonization, which leads to gastric and peptic ulcers and, in some cases, may lead to cancer.[6] In agriculture, high urease activity causes significant environmental and economic problems by releasing abnormally large amounts of ammonia into the atmosphere during urea fertilization.[3,5,7] Besides the importance of urease in making nitrogen available to plants and the impact of microbial ureases in agriculture and medicine, the structure and catalytic mechanism of this enzyme are of academic interest, because of its large enhancement (10^{14}-fold) of the rate of urea hydrolysis and the presence of active site nickel, which is unique among hydrolytic enzymes.[8]

The structure, number, and type of subunits, molecular weight, and amino acid sequence of urease depend on its origin. The urease from jack bean (*Canavalia ensiformis*) is a homohexameric molecule ($\alpha6$), whereas the bacterial ureases are heteropolymeric and contain three subunits, α, β, and γ. Despite the difference in the molecular structure, the amino acid sequences of the active site are largely similar in all known ureases, with same catalytic mechanism. The active sites are always located in α subunits and contain the binuclear nickel center, in which the Ni−Ni distances range between 3.5 and 3.7 Å.[9,10] The studies on novel urease inhibitors are essential for the possible development of a highly needed therapy for urease-mediated bacterial infections.[2,3,5,6]

Over the past decade, high-throughput virtual screening (HTVS) has emerged as an attractive and a valuable alternative to high-throughput screening (HTS). The protocol of structure-based virtual screening (SBVS) can be built up in many ways. Two conditions, however, are essential for SBVS: a target structure and a database of ligands. In our case, the target structure was *Bacillus pasteurii* (BP) urease, in complex with acetohydroxamic acid (PDB code 4UBP),[11] which was retrieved from the Protein Data Bank.

The current status of urease inhibitors is quite limited. All available urease inhibitors are not satisfactorily substantial to the binding cavity and hence not able to optimally interact with the crucial amino acid residues and the metal ions present in the urease active site. Therefore, a search of new and more effective urease inhibitors to diversify the current urease inhibitors is essential.

Recently, we have developed an in-house bank of over 6000 compounds of synthetic or natural origins. These structurally characterized compounds fulfill the druglike criteria, according to Lipinski's rule of five, with the exceptions of a few naturally occurring compounds. The compounds were prepared for docking in two steps. First, the "washing" module of Molecular Operating Environment (MOE)[12] was applied to generate physiologically relevant protonation states of compounds. Second, three-dimensional (3D) coordinates were generated by Concord implemented in Sybyl 7.3.[13]

Computational docking methods GOLD[14] and MOE-Dock[12] were implemented. The protein structure was prepared by

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deletion of water molecules, 3D protonation, and energy minimization by using the MOE. Energy minimization was carried out by implementing the MMFF94x force field and distance-dependent dielectric potential with the rms gradient of 0.05. During minimizations, all heavy atoms in the protein were kept fixed. Histidine residues were considered as neutral according to Musiani et al.[15]

Docking calculations were performed by using default parameters (unless explicitly mentioned) with GOLD scores and London dG scores as scoring functions for GOLD and MOE-Dock, respectively. In both cases, an active site region was defined by a sphere of radius 10 Å around the ligand in the complex structure of BP urease.

First, structures of 6000 in-house compounds were analyzed to check the redundancy. Consequently, the repeated compounds were removed from the database. Then, it was ensured that each class of compounds was represented at least once. This selection procedure made certain the diversity of the database.

To test the efficiency of applied docking protocol, three urease inhibitors (i.e., biscoumarin, imidazol, and hydroxamic acid derivatives) were included in a randomly selected 3000 compounds (decoy set) as positive controls. Two separate docking runs were carried out, one with MOE-Dock and the other with the GOLD docking program. There are three possible scoring functions in MOE-Dock, while there are two with GOLD.

In addition to scoring functions, there were several other parameters that needed to be set for attaining appropriate binding poses and rankings. For example, we saw that out of a few available placement methods in MOE-Dock, only Triangle Matcher was quite satisfactory in reproducing the binding mode of one of the known inhibitors (acetohydroxamic acid), which was retrieved from PDB ID 4UBP.

Similarly, each scoring function, implemented in docking programs, was evaluated to observe the ability of placing the correct docking pose in the top rankings. The London dG scoring function implemented in MOE-Dock was able to identify all three known inhibitors within the top 1 % of the whole decoy set tested. With the GOLD docking, a gold fitness function was found to produce similar enrichments like London dG in MOE-Dock.

After setting the docking and scoring protocol, a SBVS experiment was set up against urease using compounds from the in-house database. Each molecule of the database was docked, and the top 10 conformations of each compound were retained for further analysis.

In the first postdocking filtering strategy, 200 top-ranked compounds were selected by both GOLD and MOE-Dock scoring functions. There were few identical compounds ranked identically in the top 200 list by both scoring functions.

To filter out further, another selection criterion was applied. A visual inspection of all selected compounds was performed to filter out similar compounds, hence improving the diversity in the selected compounds. We also analyzed the docked orientation of each selected compound inside the binding site of BP urease enzyme. Docked conformation analysis helped to select only those compounds that were able to depict key interactions with two nickel ions and additionally with the crucial active site residues.[15,16] Specifically, coordination with nickel ions and hydrogen bonding with His323, His324, Arg339, Gly280, Cys322, Ala366, and Ala170 were considered. In addition, special attention was given to the fact that compounds showed some enhancement in the potency with respect to urease inhibition when possessing a petite hydrophobic group. It is because of that that the active site of urease is located within the cavity or the crevice in the internal territory and is surrounded by hydrophobic amino acid residues of the urease enzyme. The hydrophobic character of compounds may play a role in the random walk process of the compounds to the active site and in the hydrophobic binding, near the active site of urease.[17] After the aforementioned selection criteria were applied, 14 structurally distinct compounds were shortlisted and submitted for in vitro screening against urease inhibition by the standard mechanism-based in vitro urease assay. In this assay, reaction mixtures comprised of 25 μL of enzyme (jack bean urease) solution and 55 μL of buffer containing 100 mM urea were incubated with 5 μL of test compounds (0.5 mM concentration) at 30 °C for 15 min in 96-well plates. The urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn.[18] Percentage inhibitions were calculated from the formula $100 - (OD_{testwell}/OD_{control}) \times 100$. Thiourea was used as the standard inhibitor of urease. The results are reported in Table 1.

Table 1. Initial Hit IDs with Their Respective Scores (i.e., Gold Fitness Scores and London dG Scores) and Their Inhibitory Activities against Urease Enzyme

compd ID	Gold fitness score	London dG score	IC_{50} ± SEM[a] (μM)
AAB429	56.28	−13.4425	110.0 ± 3.06
AAB539	41.38	−11.0070	238.50 ± 2.04
AAD355	55.93	−15.6482	31.20 ± 0.47
8d	60.59	−10.8738	34.10 ± 1.24
14i	47.20	−09.0359	150.23 ± 2.10
thiourea	ND[b]	ND[b]	21.0 ± 0.01

[a] SEM, standard error of the mean. [b] ND, not determined.

All of the 14 hit compounds (Table S1 in the Supporting Information) were experimentally tested against urease enzyme by using a standard urease inhibition assay. Five compounds among 14 hits showed urease inhibitory activities with IC_{50} values ranging between 34.10 ± 1.24 and 238.16 ± 2.04 μM, as shown in Table 1. The observed activities for compounds AAB429,[19] AAB539,[20] AAD355,[21] **8d**, and **14i** were 110.0 ± 3.06, 238.16 ± 2.04, 31.2 ± 0.47, 34.10 ± 1.24, and 150.23 ± 5.65 μM, respectively.

From the binding mode of compound AAB429, it was observed that hydroxyl oxygen on the purine ring of this compound coordinates with both nickel ions, like the hydroxyl oxygen in the acetohydroxamic acid. Similarly, one of the nitrogen atoms in purine ring coordinates with one of the nickel ions, reminiscent of the carbonyl oxygens in the acetohydroxamic acid (Figure S1a in the Supporting Information). This compound mimicked the binding mode of acetohydroxamic acid. In addition, there are three hydrogen bonds formed between compound AAB429 and important active site residues, that is, Asp224, His323, and Cys322.

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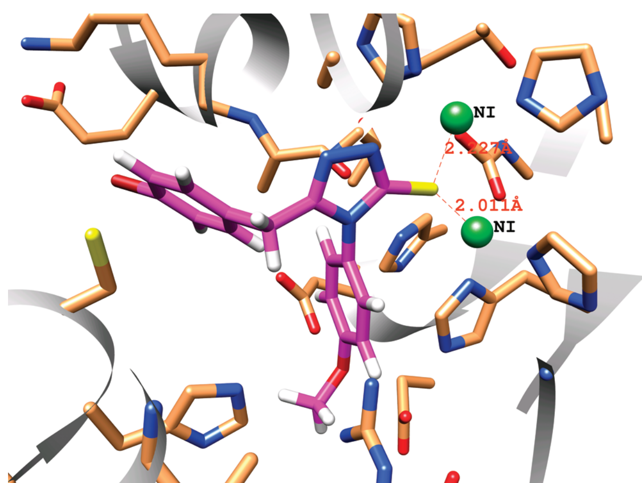


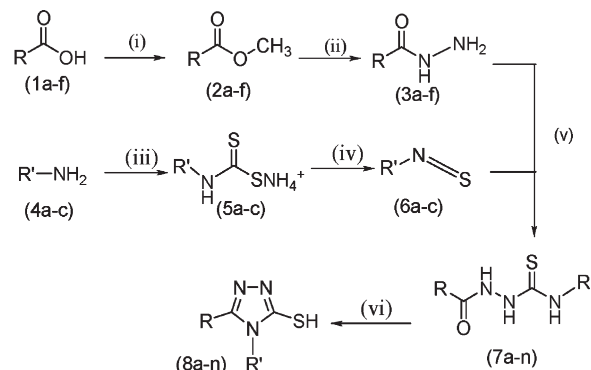
Figure 1. Interactions of compound **8d** (magenta) with the nickel ions and key residues of BP urease.

Just like compound AAB429, the docked conformation of compound AAD355 (Figure S1b in the Supporting Information) also coordinates with the Ni atom and forms four hydrogen bonds with important active site residues. The hydrogen bonding was observed between the hydroxyl and carbonyl oxygens of the compounds and the binding site residues Arg339, His323, Kcx220 (modified lysine), and Ala170. Both compounds have distinct features in the ring substituent near the Ni atoms. Those features are responsible for the difference in the activity. For example, with the compound AAD355, the aromatic ring has three hydroxyl substituents; one interacts with the Ni atom, whereas the other two form hydrogen bonds with the Ala170 and Arg339. This extra stability due to additional hydrogen bonding plays a significant role in enhanced bioactivity of compound AAD355. In effect, the extra hydrogen bonding in AAD355 renders the interaction of this compound with the crucial Ni atom more favorably than the AAB429.

In the binding mode of compound AAB539, the arene−cation interaction between the phenyl ring and one of the nickel atoms was observed. The hydrogen bond between the carbonyl oxygen and the binding site residue Arg339 was observed. These weak interactions to the binding pocket of urease might be one of the reasons for the least activity exhibited by this compound than its other counterparts, that is, AAB429 and AAD355 (Figure S1c in the Supporting Information).

From the docking conformation of compound **8d** (Figure 1), it was observed that the sulfur moiety of the triazole ring of the compound coordinates with both of the nickel atoms in the active site. Similarly, in compound **14i**, the carbonyl oxygen coordinates with nickel atoms, bridging them and also mediating few hydrogen bonds with the active site residues. Unexpectedly, this compound showed less activity. Although the reason remains unclear, however, a possible explanation for this discrepancy is the requirement of the carboxylate group at the position where it is interacting with the Ni ion and the compound **14i** lacks this feature (Figure S3 in Supporting Information).

Scheme 1. Synthesis of 4,5-Disubstituted 2,4-Dihydro-3*H*-1,2,4-triazole-3-thione **8a−n**[a]



[a] Reagents and conditions: (i) Dry methanol, concentrated H_2SO_4, reflux ∼10−12 h. (ii) Dry methanol, $NH_2-NH_2 \cdot H_2O$ (80%), reflux ∼8−10 h. (iii) Pure CS_2, 33% NH_4OH, dry ethanol, stir at room temperature ∼10−14 h. (iv) $Pb(NO_3)_2$, stir at room temperature overnight. (v) Dry ethanol, reflux ∼10−15 h. (vi) 4 N NaOH, reflux 15−18 h, neutralize with 4 N HCl.

compd	R	R′
8a	2-(Br)C_6H_4-CH_2	4-(CH_3)C_6H_4
8b	2-(Br)C_6H_4-CH_2	4-(OCH_3)C_6H_4
8c	4-(Br)C_6H_4-CH_2	4-(CH_3)C_6H_4
8d	4-(Br)C_6H_4-CH_2	4-(OCH_3)C_6H_4
8e	3-(Br)C_6H_4-CH_2	4-(Br)C_6H_4-CH_2
8f	3-(Br)C_6H_4-CH_2	4-(CH_3)C_6H_4
8g	2-(Br)C_6H_4-CH_2	3-(OCH_3)C_6H_4
8h	3-(Br)C_6H_4-CH_2	3-(OCH_3)C_6H_4
8i	4-(Br)C_6H_4-CH_2	3-(OCH_3)C_6H_4
8j	3,4-(OCH_3)$_2$$C_6H_3$	4-(CH_3)C_6H_4
8k	2,4-(Cl)$_2$$C_6H_3$-$CH_2$	4-(OCH_3)C_6H_4
8l	2,4-(Cl)$_2$$C_6H_3$-$CH_2$	4-(CH_3)C_6H_4
8m	3-(OH)-4-(OCH_3)C_6H_3	4-(CH_3)C_6H_4
8n	2,4-(Cl)$_2$$C_6H_3$-$CH_2$	3-(OCH_3)C_6H_4

Three compounds (i.e., AAB429, AAB539, and AAD355) out of five hits were of commercial origin. We were not able to further investigate them due to inaccessibility at the moment of the present study. We are in the process of procuring them from commercial sources. The remaining two compounds (i.e., **8d** and **14i**) were part of the in-house-synthesized compounds and were thus further investigated.[22,23]

To optimize the ligand interactions with the binding site, we decided to synthesize different derivatives of compounds **8d** and **14i**. For compound **8d**, 13 analogues (series A) were synthesized, whereas for the second hit **14i**, 21 different derivatives (series B) were synthesized (Table S2 in Supporting Information). Scheme 1 presents the general synthesis of the compounds in series A, whereas Schemes 2 and 3

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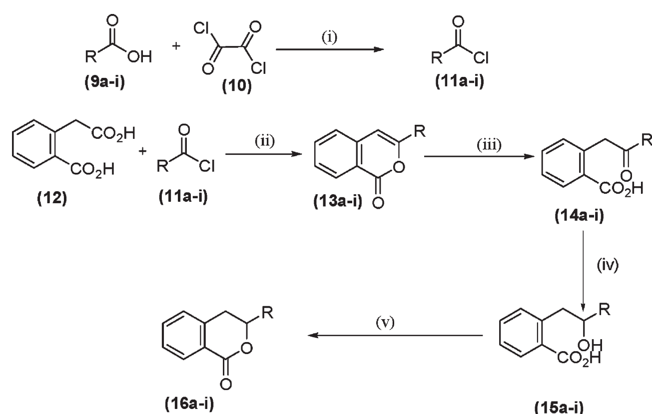
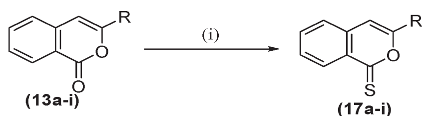
Scheme 2. Synthesis of 3-(Halobenzyl)isocoumarins **13a–i**, Their Respective Keto Acids **14a–i**, and 3,4-Dihydroisocoumarins **16a–i**[a]



[a] Reagents and conditions: (i) Dry benzene, stirring. (ii) Heat at 200 °C for 4 h. (iii) 5% KOH/ethanol, reflux 5 h. (iv) $NaBH_4$/1% NaOH, overnight stirring. (v) Ac_2O, reflux for 1 h.

Scheme 3. Synthesis of 3-(Halobenzyl)-1-thioisocoumarins **17a–i**[a]



[a] Reagents and conditions: (i) Lawesso's reagent, dry toluene, reflux for 8 h. Key: **a**, 2-(Br)-C_6H_4-CH_2; **b**, 3-(Br)-C_6H_4-CH_2; **c**, 4-(Br)-C_6H_4-CH_2; **d**, 2-(Cl)-C_6H_4-CH_2; **e**, 3-(Cl)-C_6H_4-CH_2; **f**, 4-(Cl)-C_6H_4-CH_2; **g**, 2-(F)-C_6H_4-CH_2; **h**, 3-(F)-C_6H_4-CH_2; and **i**, 4-(F)-C_6H_4-CH_2

describe the synthesis of compounds included in series B. The detailed procedure of synthesis in Schemes 1–3 can be found in the Supporting Information.

All synthetic compounds were then submitted for biological screening against urease inhibition through the standard urease assay[18] (Table S2 in Supporting Information). To analyze the binding modes, we used GOLD and MOE-Dock programs, and to calculate the binding energies, we used the AutoDock method, which is famous for its quality predictions of binding energy calculations.

Compounds of series A and B showed interesting biological results. As expected, the compounds of A series are more active than the series B. In the case of series A, most of the analogues coordinate with nickel ions by a thiol moiety of triazole, along with forming hydrogen bonds with important active site residues. In series B, the carboxylate group of keto acids coordinates with one of the nickel ions, whereas in isocoumarins, the arene–cation interaction between the phenyl ring and the nickel ion was observed. Other moieties in these compounds showed hydrogen bonding with crucial active site residues.

From the docking results, the similar docking poses were observed for the synthesized derivatives. Docking analysis identified the essential thiol group in series A, at the position of interaction with the Ni ion, as a key factor for the activity, since it mimicks the role of the hydroxyl group, as present in one of the most active hits (i.e., AAD355).

In series B, there are two types of compounds; one is isocoumarin, while the other is keto acid. The isocoumarins in series B showed a strong interaction between their carbonyl oxygen and the His and Arg residues of the active site. This strong interaction could be the basis of the activity of this class of compounds. The docking analysis of keto acids resulted in an interesting interaction of these compounds with the binding pocket of urease. Like acetohydroxamic acid, most of these compounds interact with urease in a famous bidentate fashion with the nickel ions. The carboxylate oxygens of these compounds bridge the two nickel ions.[11] With the docking analysis, we found that more favorable interactions point to keto acids than the isocoumarin. This might be one of the reasons of turning keto acids into more actives than the isocoumarins (Figures S2a,b in the Supporting Information). An acceptable trend between calculated binding energies and experimental activities within the individual series was observed for synthesized compounds (Table S2 in Supporting Information).

Furthermore, an attempt was made to develop a trivial structure–activity relationship for the synthesized compounds. The compounds **8a–n** in the A series and **13a–f**, **i**, **14a–c**,**e–i**, **16a**,**c**,**e**,**f**,**i**, and **17a**,**d** in series B were screened for their urease inhibitory activities, and results are presented in Table S2 in Supporting Information. In the A series, the tested compounds **8h** and **8f** were found to be more potent than the standard, and compounds **8b**, **8c**, and **8n** exhibited an activity comparable with the standard, whereas compounds **8a**, **8g**, **8j**, and **8l** exhibited very good activities. Compounds **8e**, **8i**, and **8k** were only moderate. The most active compounds **8h** and **8f** both have a bromine atom as a substituent at the *meta* position of the benzyl part, whereas they have methoxy or methyl substituents at *para* or *meta* positions in the phenyl part. Compounds **8b** and **8c** have a bromine atom with substituents at *ortho* and *para* positions in the benzyl part and methoxy or methyl with substituents at the *para* position of the phenyl part, whereas **8n** has chlorine substituents at *ortho* and *para* positions in the benzyl part and a methoxy substituent at the *meta* position in the phenyl part exhibited urease inhibition comparable with the standard. These results revealed that a bromine substituent at the *meta* position in the benzyl part and methoxy or methyl at *para* or *meta* positions in the phenyl part play an important role in the enhancement of activity. The compounds **8h** and **8f** may serve as lead compounds for further investigation. In the B series, the tested compounds **13e**, **14b**, and **14e** exhibited moderate urease inhibition activity, whereas the compounds **13a–d**,**f**, **i**, **14a**,**c**,**f–i**, **16c**,**e**,**f**, and **17a**,**d** exhibited a low activity. Compounds **16a**, **16i**, and **17d** exhibited no activity. Among the isocoumarins (**13a–f**,**i**), **13e** exhibited more potent urease inhibition as compared to others. It might be due to a chlorine substituent at the *meta* position in the benzyl part of **13e**. The rest of the compounds have chlorine either at *ortho* (**13d**) or *para* (**13f**) positions. Similarly, a bromine or fluorine substituent at *ortho* (**13a** and **13g**), *meta* (**13b** and **13h**), and *para* (**13c** and **13i**) positions in the benzyl part is not required for optimum activity. In keto acids **14a–c**,**e–i**, compounds **14b** and **14e** are more active with bromine and

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chlorine substituents at the *meta* position in the benzyl part as compared to *ortho* (**14a** and **14d**) and *para* (**14c** and **14f**) positions. Dihydroisocoumarins **16c**,**e**,**f** and thioisocoumarins **17a**,**d** exhibited low activity, whereas **16a**,**i** and **17d** were inactive. These results revealed that chloro and bromo substitutions at the *meta* position in the benzyl part play an important role in the enhancement of the activity. Compound **14e** may serve as a lead compound for further investigation.

Despite identifying compounds with good inhibitory activities, we still observed some compounds with no activity (IC_{50} > 1000 μM in Table S2 in Supporting Information). The reasons for lack of activity of these compounds are still under investigation.

In summary, we have carried out a virtual screening of an in-house database with the aim to find novel urease inhibitors. Initially, we identified five novel compounds as urease inhibitors. Further investigation of two classes of inhibitors was performed. The biological activities and binding energies of these compounds were carried out against the urease. From this study, 34 new inhibitors were identified with acceptable correlation between biological activities and binding energies. The binding modes of the synthesized compounds were analyzed with the docking programs and were found to be consistent, as reported in earlier studies.[15]

Moreover, we also succeeded in identifying some compounds showing inhibitory potency better than the standard thiourea. Future studies will lead to a better understanding of structure and activity relationships of these compounds. The results presented so far led us to conclude that these compounds can be used as starting points in lead optimization stages.

SUPPORTING INFORMATION AVAILABLE Structures of the initial hit compounds, three commercially available hits and their diagrams showing interactions with urease receptor, graphical illustration of the inhibitory activities at various concentrations of the most active compound, and compound spectral information and complete synthesis schemes. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions: Molecular modeling was done by A.W. In vitro testing was done by A. K.

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REFERENCES

(1) Dixon, N. E.; Gazzola, T. C. Letter: Jack bean urease (EC 3.5.1.5). A metalloenzyme. A simple biological role for nickel? *J. Am. Chem. Soc.* **1975,** *97*, 4131–4133.
(2) Blakeley, R. L.; Zerner, B. Jack bean urease: The first nickel enzyme. *J. Mol. Catal.* **1984,** *23*, 263–292.
(3) Mobley, H. L.; Hausinger, R. P. Microbial ureases: Significance, regulation, and molecular characterization. *Microbiol. Mol. Biol. Rev.* **1989,** *53*, 85–108.
(4) Reuter, H. D.; Reuter, H. D. Allium sativum and Allium ursinum part 2: Pharmacology and medicinal application. *Phytomedicine* **1995,** *2*, 73–91.
(5) Burne, R. A.; Chen, Y. Y. M. Bacterial ureases in infectious diseases. *Microb. Infect.* **2000,** *2*, 533–542.
(6) Mobley, H. L.; Island, M. D.; Hausinger, R. P. Molecular biology of microbial ureases. *Microbiol. Rev.* **1995,** *59*, 451–480.
(7) Brodzik, R.; Koprowski, H.; Yusibov, V.; Sirko, A. Production of urease from *Helicobacter pylori* in transgenic tobacco plants. *Cell Mol. Biol. Lett.* **2000,** *5*, 357–366.
(8) Estiu, G.; Suárez, D.; Merz, K. M. Quantum mechanical and molecular dynamics simulations of ureases and Zn β-lactamases. *J. Comput. Chem.* **2006,** *27*, 1240.
(9) Ciurli, S.; Benini, S.; Rypniewski, W. R.; Wilson, K. S.; Miletti, S.; Mangani, S. Structural properties of the nickel ions in urease: Novel insights into the catalytic and inhibition mechanisms. *Coord. Chem. Rev.* **1999,** *190*, 331–355.
(10) Dixon, N. E.; Blakeley, R. L.; Zerner, B. Jack bean urease (EC 3.5.1.5). I. A simple dry ashing procedure for the microdetermination of trace metals in proteins. The nickel content of urease. *Can. J. Biochem.* **1980,** *58*, 469–473.
(11) Benini, S.; Rypniewski, W. R.; Wilson, K. S.; Miletti, S.; Ciurli, S.; Mangani, S. The complex of *Bacillus pasteurii* urease with acetohydroxamate anion from X-ray data at 1.55 Å resolution. *J. Biol. Inorg. Chem.* **2000,** *5*, 110–118.
(12) *Molecular Operating Environment MOE 2008.10*; C.C.G.I.M.: Quebec, Canada, 2008.
(13) *SYBYL Software Package*, version 6.9; Triops Associates Inc.: St. Louis, MO, 2006.
(14) *GOLD*, Version 4.0; Astex Technology: Cambridge, United Kingdom, 2001.
(15) Musiani, F.; Arnofi, E.; Casadio, R.; Ciurli, S. Structure-based computational study of the catalytic and inhibition mechanisms of urease. *J. Biol. Inorg. Chem.* **2001,** *6*, 300–314.
(16) Amtul, Z.; Siddiqui, R. A.; Choudhary, M. I. Chemistry and mechanism of urease inhibition. *Curr. Med. Chem.* **2002,** *9*, 1323–1348.
(17) Tanaka, T.; Kawase, M.; Tani, S. Alpha-hydroxyketones as inhibitors of urease. *Bioorg. Med. Chem.* **2004,** *12*, 501–505.
(18) Weatherburn, M. W. Enzymic method for urea in urine. *Anal. Chem.* **1967,** *39*, 971–974.
(19) Catalog No. 102049, Lot No. 3478E; M. B., LLC: Aurora, OH.
(20) Lot and Filling Code 1108805; Fluka Chemie GmbH: Buchs, Germany.
(21) Tlegenoy, R. T. K. S. U., 742012, 24-microaion, 5-1, Nukus City, Uzbekistan.
(22) Amtul, Z.; Rasheed, M.; Choudhary, M. I.; Rosanna, S.; Khan, K. M. Kinetics of novel competitive inhibitors of urease enzymes by a focused library of oxadiazoles/thiadiazoles and triazoles. *Biochem. Biophys. Res. Commun.* **2004,** *319*, 1053–1063.
(23) Serwar, M.; Akhtar, T.; Hameed, S.; Khan, K. M. Synthesis, urease inhibition and antimicrobial activities of some chiral 5-aryl-4-(1-phenylpropyl)-2*H*-1,2,4-triazole-3(4*H*)-thiones. *ARKIVOC* **2009,** *7*, 210–221.

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