International Journal for Computational Biology (IJCB)
Vol.3, No.1, April 2014, Page: 31-38
ISSN: 2278-8115

Computer-aided Design of Organophosphorus Inhibitors of Urease

M. Padariya[1 2], U. Kalathiya[1 2 *], L. Berlicki [3], M. Baginski[2]
[1] Biotechnology (Bioinformatics) Department, Wroclaw University of Technology, Poland
[2] Department of Pharmaceutical Technology and Biochemistry, Gdansk University of Technology, Poland
[3]Department Bioorganic Chemistry, Wroclaw University of Technology, Poland

Article Info

Article history:

Received Oct 16th, 2013
Revised April 8th, 2014
Accepted April 9th, 2014

Keyword:

Diamidophosphate
Docking
Urease
Inhibitors
Scoring function

ABSTRACT

Based on the structure of the most potential inhibitor diamidophosphate, various novel groups of inhibitors were developed by knowledge-based design approach with covalent carbon-phosphorus or carbon-phosphorus-carbon bond to improve hydrolytic stability to inhibit the microbial ureases. Designed compounds were evaluated with 10 (LigScore1, LigScore2, PLP1, PLP2, JAIN, PMF, PMF04, LUDI_1, LUDI_2 and LUDI_3) different scoring functions implemented in Discovery Studio and conformation analysis by AutoDock package.

Corresponding Author:

Umesh Kalathiya,
Department of Pharmaceutical Technology and Biochemistry,
Gdansk University of Technology,
Poland
Email: umekalat@gmail.com

How to Cite:

M. Padariya *et. al.* Computer-aided design of Organophosphorus inhibitors of Urease. IJCB. 2014; Volume 3 (Issue 1): 31-38.

1. INTRODUCTION

Urease (urea amidohydrolase; E.C.3.5.1.5) is an enzyme that holds a salient place in the history of science (Figure 1). Urea was the first organic compound synthesized in laboratory [1], while urease derived from seeds of the Jack bean (JB) plant [2] is the first crystallized [3] and known as nickel containing enzyme [4]. It catalyzes urea hydrolysis in the nitrogen mineralization to form ammonia and carbamate, which later decomposes into ammonia and bicarbonate [5] [6]. First urease cleaved urea and produce one ammonia molecule and one of carbamate. Carbamate then decomposes in to ammonia and carbonic acid. Then, Carbonic acid equilibrates in water. As two molecules of ammonia become protonated to produce ammonium and hydroxide ions (Scheme 1). As a result there is a rise in the pH of the environment. Ammonia molecules thus formed are protonated by water at physiological pH, whereas the carbonic acid dissociates and cause an increase in pH [5] [6] [7] [8]. It involves environmental nitrogen transformations to provide nitrogen source to the organisms like algae, bacteria, fungi and plants [6]. But then, the reaction catalyzed by the dinuclear nickel active site of urease causes an aggregation of ammonia and a sharp pH increase, which has negative side effects in agriculture and health. For example, urease serves as a virulence factor in pathogens that are responsible for the development of diseases like kidney stones, pyelonephritis, and peptic ulcers [5] [6].

The precise information for the regions of the enzyme that is involved in the binding of inhibitors or substrates is the base in designing the efficient inhibitors which are capable to complement all the structural requirements for a close interaction. The urease active site (Figure 2) was found to contain pseudooctahedral, paramagnetic and bi-nuclear nickel ions in all the complexes of enzyme inhibitor analyzed so far [9] [10]. The

information of active site of urease was given through the resolved crystal structures for bacterial ureases from *Klebsiella aerogenes* [11] [12] and *Bacillus pasteurii* [9]. The active site was displayed to contain a binuclear nickel centre, in which the Ni-Ni distance was found 3.7Å in *Bacillus pasteurii.* In the center two nickel ions are bridged by a carbamylated lysine through its O-atoms, along Ni(1) further coordinated by two histidines with their N-atoms, and Ni(2) by two histidines also through N-atoms and furthermore by aspartic acid through its O-atom.

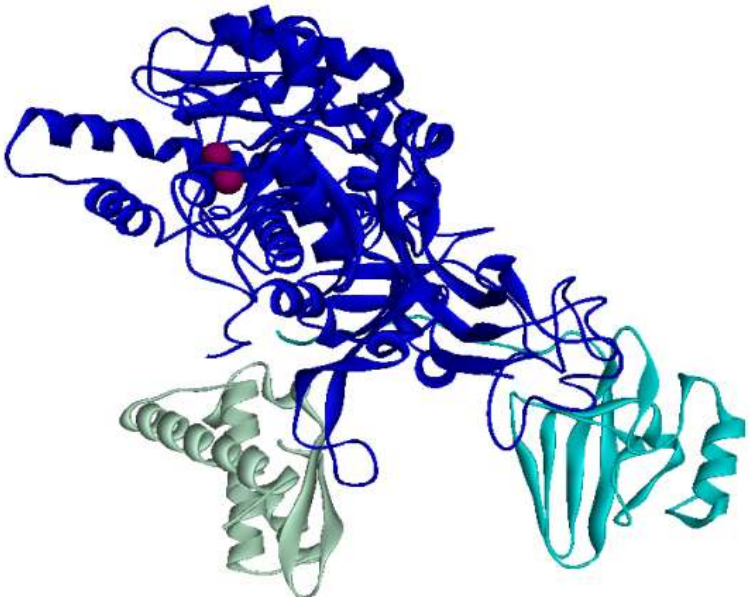


Figure 1. Ribbon diagram of the enzyme structure of *bacillus pasteurii* urease. The blue, light blue and light gray ribbons display α, β and γ subunits, respectively

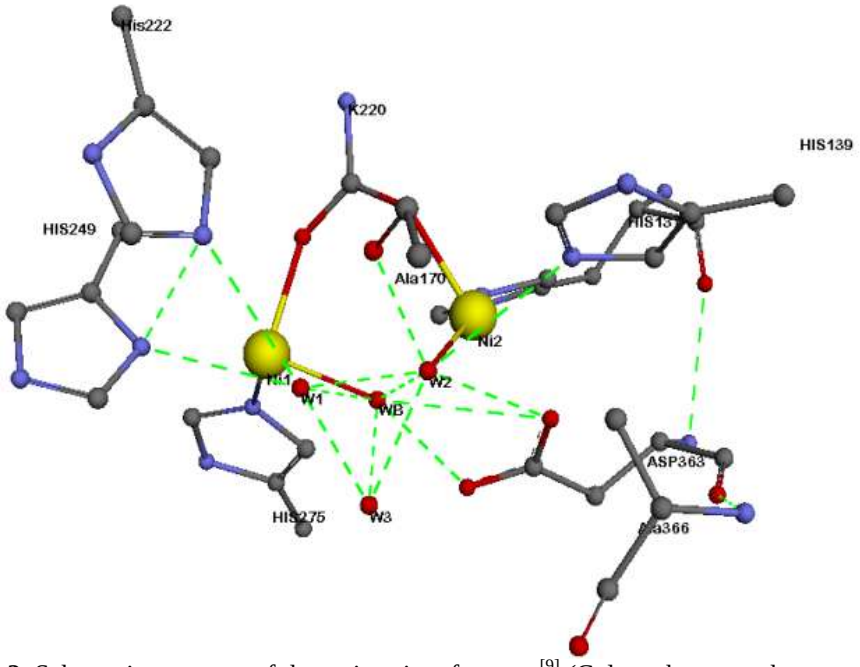


Figure 2. Schematic structure of the active site of urease [9] (Color scheme: carbon-grey; nitrogen-blue; oxygen-red; two nickel ions-yellow). Hydrogen bonds are shown as dashed lines. K=lysine.)

Along with, the Ni ions are bridged by a hydroxide ion (WB), which along with two terminal water molecules, W1 on Ni(1), W2 on Ni(2), and W3 located near the opening of the active site, which forms an H-bonded water tetrahedral cluster filling the active site cavity. It is the cluster (the carboxylate group of the carbamylated lysine and the hydroxide molecule) that urea replaces when binding to the active site for the reaction [11]. As a consequence of above ligations, Ni(1) is pentacoordinated and Ni(2) hexacoordinated, and

their coordination geometry is pseudo square pyramidal and pseudo octahedral, respectively. In another consideration, urease can severely decrease the efficiency of urea fertilizers to cause the release of large amounts of ammonia and further induce plant damage by ammonia toxicity and soil pH increase [11]. So, to control the rate of the enzymatic urea hydrolysis using urease inhibitors is an important goal. Large quantities of urea produced as a result of biological process. Each human being produces approximately 10 kg of urea per year. Spontaneous degradation of urea occurs with a half life of approximately 3.6 years [1], but in the presence of urease, the hydrolysis of urea is 104 times faster [7].

2. RESEARCH METHOD

The crystal structure of *Bacillus pasteurii* urease in complex with inhibitor DAP [13] retrieved from protein data bank [14] (PDB Code: 3UBP) was used as the starting point. To organize the active site of enzyme His137, His139, Ala170, Lys220, His222, His249, His275, Ala279, Gly280, Leu319, Cys322, His323, Ala366, Met367 and Asp363 amino acid residues and two Nickel ions (NI900,NI901) are selected [15] [16]. The structure of ligand (DAP) was appropriately modified by using Sketching module and fragment building tools in Discovery Studio 3.1 client program. In structure, missing bond orders, charges and angles were assigned and explicit hydrogens and hydrogen bonds were added.

2.1 Molecular modeling

From the created active site of enzyme, both Nickel ions and amino acids which are near and tightly bounded to them (His 137, His139, Lys220, His249, His275, Ala279 and Leu319) were applied to the fixed atom constraint tool of simulation [17] module of Discovery Studio 3.1 client program to limit the energy minimization. CHARMm [18] [19] force field was applied to the whole receptor-ligand complex. Apart from these, other part of active site was subjected to energy minimization using 1000 steps of 'smart minimizer', with none of the implicit solvent model and with a dielectric constant of 1.0, Nonbonded List Radius was set to 14 (nonbond higher cutoff distance 12, nonbond lower cutoff distance 10), With fixed minimization constraints and with Electrostatics spherical cutoff until either a RMS gradient of 0.1, or energy change and save result frequency of 0.0, was reached.

2.2 Ligand Fit docking and scoring

Ligand Fit, a modern docking program within Discovery Studio (Accelrys, San Diego, USA), was used for all runs. The Receptor-ligand complexes obtained after minimization were further used to determine the ligand binding affinity. The complex was splitted into the enzyme active site part and ligand part. By selecting only the active site part and using 'Define and Edit binding site from current selection tool' of 'Receptor-ligand interaction' module from the Discovery Studio 3.1 client, binding site sphere (of about 9.9 A°) was defined. After selecting both part (active site part and ligand part), docking studies were subjected to the 'score ligand poses' module of Discovery Studio 3.1 client and 10 scoring functions (LigScore1 [20], LigScore2 [20], PLP1 [21], PLP2 [21], JAIN [22], PMF [23], PMF04 [23], LUDI_1, LUDI_2, LUDI_3 [24] [25]) were implemented to evaluate the ligand binding affinity [26] [27] [28].

2.3 Molecular docking by AutoDock

Software AutoDock [29] [30] including a graphical user interface, MGL Tools [31] was utilized to generate grids, calculate dock score and assess the conformers. The structure of compounds were drawn using Discovery Studio client 3.1 and the energy minimization was implemented using CHARMm force field [18] [19] with smart minimize method to achieve a local minimum structure. These energy minimized structures are recognized for docking and the correlated pdbqt files were created in AutoDock. AutoDock needs the receptor and ligand coordinates in either Mol2 or PDB format. Other than the water molecules which are present in the active site (HOH 972, HOH 990, HOH 1043, HOH 1046, HOH 1167, HOH 1168 and HOH 1245), all other Nonpolar hydrogens were discarded from the receptor file (PDB [32] code: 2UBP) [9] and their partial charges were calculated to the parallel carbon atoms.

The receptor file was converted into the pdbqt file format containing the receptor atom coordinates, partial charges and solvation parameters. The ligand file was reformed into a pdbqt file and torsions were determined. The grid calculations were implemented and maps were calculated with the use of AutoGrid [33] program. The grid maps were centered on the ligand binding site in the dimension of 120×120×120 points (x, y, z). The grid spacing was 0.375 Å and other AutoDock parameters were used default for docking. All docking runs were carried out using the Lamarckian genetic algorithm [34] and the achieved dock scores were noted in Kcal/mol. The docking protocol applied in analysis involved of 200 independent runs of each ligand, utilizing

an initial population of 250 randomly distributed individuals, a maximum number of 75 × 10^6 energy evaluations, number of generation of 27 × 10^3, a mutation rate of 0.02, a crossover rate of 0.8 [35].

3. RESULTS AND ANALYSIS

Knowledge-based method is based on approved technique applied in the drug discovery, established as 'scaffold-hopping' [36] [37] where the objective is to design a new structure starting from a known active compound through the modification of the central core of the molecule [38]. Generally, basic or initial structure and analogue based design studies are done using Glide, Gold, Ligand Fit or Catalyst to establish a comparative model, then fragment-based and knowledge-based approaches are applied to design molecules of selective inhibitors [39] [40] [41] [42].

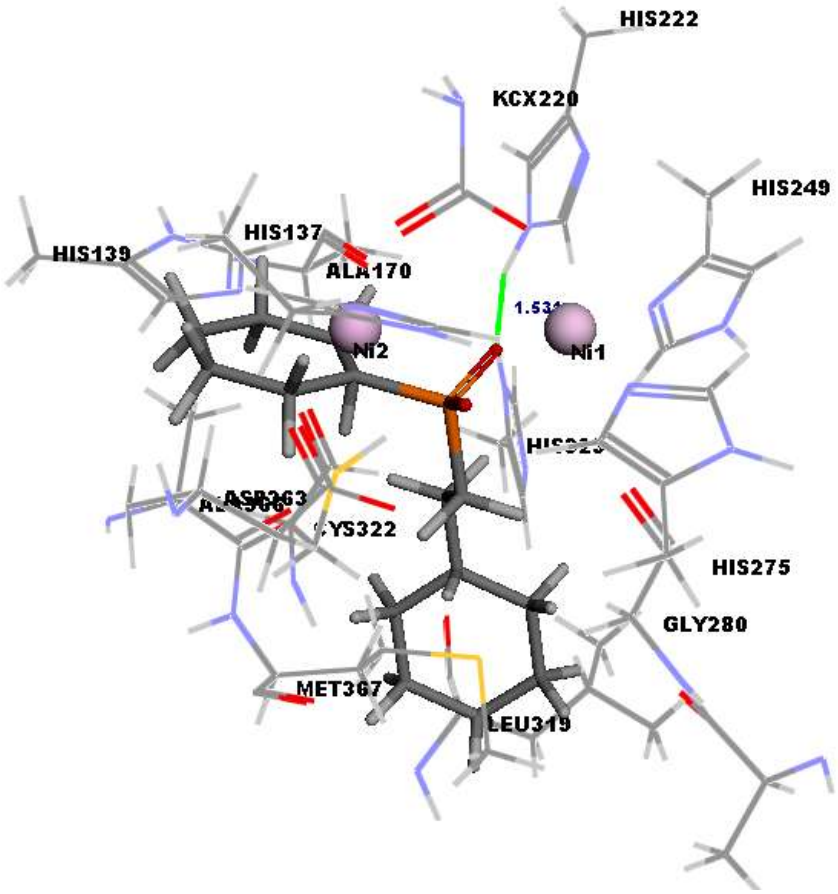
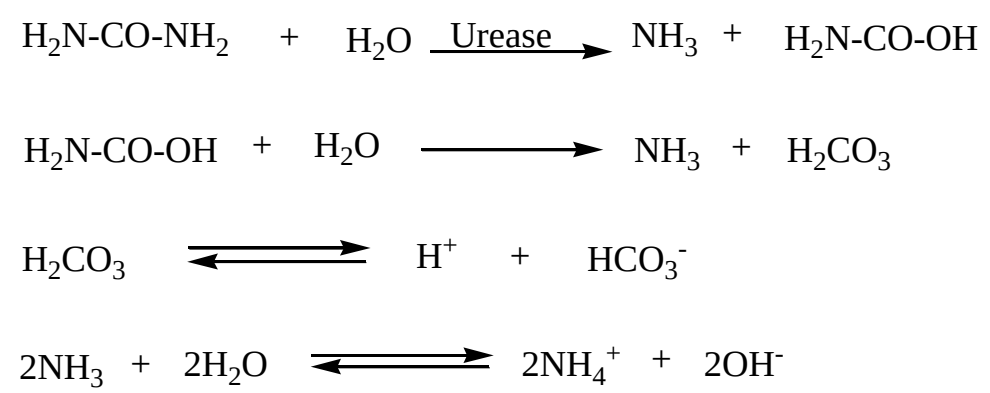
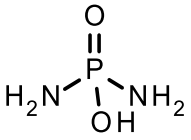


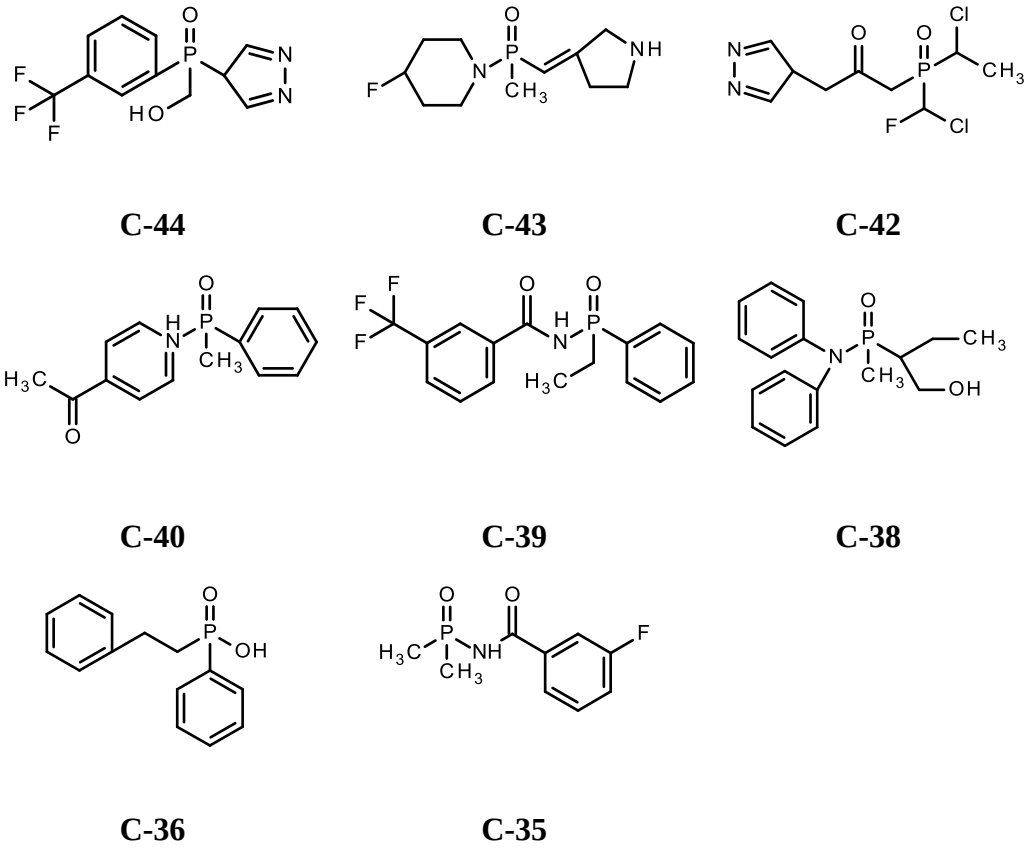
Figure 3. Modeled structure of inhibitor C-**36** to active site of *Bacillus pasteurii* urease. Hydrogen bonds are indicated as green lines. (Discovery Studio): the amino acid residue His222 of enzyme involved in hydrogen bond interaction with phosphinic acid group of inhibitor C-**36** with a distance of 1.5 Å.



Scheme 1. Reaction of urease.



Scheme 2. Chemical structure of DAP inhibitor of BPU.[43]



Scheme 3. Inhibitors constructed by using phenyl/aromatic/aliphatic rings with fluorine/chlorine substitution.

Table 1. Results of scoring functions of selected compounds and their binding energy with the enzyme.

compound no.	Ligscore 1	Ligscore 2	PLP1	PLP2	Jain	PMF	PMF04	Ludi-1	Ludi-2	Ludi-3	Mean Binding Energy
C-44	1.5	4.45	9.47	-27.6	3.44	144.7	109.19	457	431	742	-2.95
C-43	4.71	8.58	21.71	-9.21	7.6	65.7	29.96	586	543	651	-4.54
C-42	2.63	6.6	10.12	1.48	2.84	91.94	27.9	377	353	394	-5.58
C-40	1.04	2.28	-10.36	-25.86	7.34	108.82	63.99	540	501	745	-4.15
C-39	6.72	14.13	74.93	-19.49	8.74	14.96	3.72	794	657	854	-3.66
C-38	4.31	9.92	24.02	-7.95	8.53	132.92	51.38	476	466	606	-2.78
C-36	5.01	10.12	36.82	-6.37	7.65	35.03	-11.66	411	426	635	-4.99
C-35	2	5.08	-6.27	-21.48	3.87	123.48	76.25	459	431	529	-3.56

BPU is an heteropolymeric molecule $(\alpha\beta\gamma)^3$ with exact threefold symmetry and contains flexible subunit composition which depends on organism. The structure of the active site of urease is highly conserved which contains two nickel ions and has a comparably small volume. Urease inhibitors which are distinct in structure have been effectively identified. The most efficient urease inhibitors are Diamidophosphate (DAP) (Scheme 2) and its derivatives, which hydrolyze to the active molecule (DAP) in the active site [43].

DAP is a transition state analogue. It loses stability in aqueous environments because it contains hydrolyzable (especially at low pH) P-N bonds in its structure [44]. So the effort was done to modify the structure of this transition state analogue by using highly stable P-C or C-P-C bonds to improve its activity and stability against *Bacillus pasteurii* urease.

By using the knowledge-based design approach, 44 different compounds were designed to evaluate their potency against BPU. After designing the novel compounds using the knowledge of already synthesized urease inhibitors, they were energy minimized to the closest local minimum using the molecular mechanics CHARMm force field implemented in Discovery Studio. To study the interaction between the ligand and enzyme active site, all 44 compounds were docked in to the enzyme active site and were evaluated with 10 different scoring functions (Ligscore 1, Ligscore 2, PLP1, PLP2, Jain, PMF, PMF04, Ludi-1, Ludi-2, Ludi-3) of Discovery Studio package.

Automated docking was used to locate the appropriate binding orientations and conformations of different inhibitors in the BPU. To perform the task, genetic algorithm routine implemented in the program AutoDock was employed. Kollman charge, atomic solvation parameters and fragmental volumes were assigned to the protein using MGL Tools package. The program AutoGrid was used to generate the grid maps. Lamarckian genetic algorithm was applied for minimization using default parameters. The standard docking protocol was then applied by using AutoDock software package and binding free energies (ΔG_b, Kcal/mol) were obtained.

Inhibitor **C-44**, **C-43**, **C-42**, **C-40**, **C-39**, **C-38**, **C-36** and **C-35** (Table 1) (Scheme 3) are top ranked compounds according to evaluation of 10 scoring functions and binding free energy of AutoDock and these 8 compounds have also obtained highest number of conformations which were quite well overlaid. The inhibitors constructed by using phenyl/aromatic/aliphatic rings with fluorine/chlorine substitutions were obtained top ranked compounds in most of the scoring functions among 44 different structures.

4. CONCLUSION

By considering all scoring functions, inhibitor C-36 has achieved reliable scores values in all scoring functions (Figure 3). To sum up the attempt of designing organophosphorus compounds as *Bacillus pasteurii* urease inhibitors, many different compounds containing phosphate, phosphonate, phosphinate, phenyl/ cyclohexyl, pyrrole/cyclopentane in its structure were tested *in silico* based on knowledge of available inhibitors to improve its potency. From 8 of 10 scoring functions suggest that the compounds containing phenyl/ cyclohexyl/ pyrrole/ cyclopentane can be more potent structures as urease inhibitors than the compounds like phosphates/ phosphonates or phosphinates.

ACKNOWLEDGEMENTS

Authors would like to articulate sincere thanks to Prof. W. Andrzej Sokalski, for sharing his knowledge, and The Supercomputing and Networking Centre in Wroclaw, for providing their hardware and software resources on which molecular modeling was performed.

REFERENCES

[1] B. Zerner, "Recent advances in the chemistry of an old enzyme, urease", Bioorg. Chem, 19:116-131, (1991).
[2] N. E. Dixon, C. Gazzola, R. L. Blakeley and B. Zerner, "Jack bean urease (EC 3.5..1.5) a metalloenzyme. A simple biological role for nickel? " J. Am. Chem. Soc, 97:4131-4133, (1975).
[3] J. B. Sumner, "The isolation and crystallization of the enzyme urease", J. Biol. Chem, 69: 435-441, (1926).
[4] L. Alagna, S. S. Hasnain, B. Piggott and D. J. Williams, "The nickel ion environment in jack bean urease", Biochem. J., 220:591-595, (1984).
[5] H. L. Mobley, M. D. Island and R. P. Hausinger, "Molecular biology of microbial ureases", Microbiol. Rev, 59:451-480, (1995).
[6] H. L. T. Mobley and R. P. Hausinger, Microbiol. Rev, 53:85-108, (1989).
[7] R. P. Hausinger, "In Biochemistry of Nickel", Plenum Press New York, (1993).
[8] W. Li, M. X. Gao and D. D. Sheng, "Synthesis, temperature controlled structures and polyphenol oxidase activities of dinuclear manganese(II) complexes with N,N,N ',N '-tetrakis(2 '-benzimidazolylmethyl)-1,4-diethylene amino glycol ether", inorganica. chimica. acta, 387:181-185, (2012).
[9] A. Rauf, F. Ahmed and A. M. Qureshi, "Synthesis and Urease Inhibition Studies of Barbituric and Thiobarbituric Acid Derived Sulphonamides", J. Chin. Chem. Soc, 58:528-537, (2011).
[10] B. Zambelli, N. Cremades and P. Neyroz, "Insights in the (un)structural organization of Bacillus pasteurii UreG, an intrinsically disordered GTPase enzyme", Mol. Biosyst, 8:220-228, (2012).
[11] E. Jabri, M. B. Carr, R. P. Hausinger and P. A. Karplus, Science, 268:998–1004, (1995).
[12] R. P. Benjamin, M. R. Bill and A. E. Roitberg, "Wide-Open Flaps Are Key to Urease Activity", J. Am. Chem. Soc, 134:9934-9937, (2012).
[13] R. Wang, Y. Lu and S. Wang, "Comparative Evaluation of 11 Scoring Functions for Molecular Docking", J. Med. Chem, 46:2287-2303, (2003).
[14] H. J. Bohm, "The Development of a Simple Empirical Scoring Function to Estimate the Binding Constant for a Protein-Ligand Complex of Known Three-Dimensional Structure", J. Comput.-Aided Mol. Des, 8:243-256, (1994).
[15] Z. A. Ansari, M. Haque and S. H. Kee, "Urea Sensing Properties of Cu-Doped Titanate Nanostructures", Adv. Sci. Lett, 4:3451-3457, (2011).
[16] A. Balasubramanian and K. Ponuraj, Acta Crystallogr, F64:662-664, (2008).
[17] B. R. Brooks, C. L. Brooks, A. D. Mackerell, L. Nilsson, R. J. Petrella, B. Roux, Y. Won, G. Archontis, C. Bartels, S. Boresch, A. Caflisch, L. Caves, Q. Cui, A. R. Dinner, M. Feig, S. Fischer, J. Gao, M. Hodoscek, W. Im, K. Kuczera, T. Lazaridis, J. Ma, V. Ovchinnikov, E. Paci, R. W. Pastor, C. B. Post, J. Z. Pu, M. Schaefer, B. Tidor, R. M. Venable, H. L. Woodcock, X. Wu, W. Yang, D. M. York and M. Karplus, "The Biomolecular simulation Program", J. Comp. Chem, 30:1545-1615, (2009).
[18] B. R. Brooks, R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan and M. Karplus, "CHARMM: A Program for Macromolecular Energy, Minimization, and Dynamics Calculations", J. Comp. Chem, 4:187-217, (1983).
[19] R. Huey, G. M. Morris, A. J. Olson and D. S. Goodsell, "A semiempirical free energy force field with charge-based desolvation", J. Comput. Chem, 28:1145- 1152, (2007).
[20] A. Karmmer, P. D. Kirchhoff, X. Jiang, C. M. Venkatachalam and M. Waldman, "LigScore: a novel scoring function for predicting binding affinities", J. Mol. Graph. Model, 23:395-407, (2005).
[21] D. K. Gehlhaar, G. M. Verkhivker, P. A. Rejto, C. J. Sherman and D. B. Fogel, "Molecular recognition of the inhibitor AG-1343 by HIV-1 protease: conformationally flexible docking by evolutionary programming", Chemistry & Biology, 2:317-324, (1995).
[22] A. N. Jain, "Scoring non covalent proteinligand interactions: A continuous differentiable function tuned to compute binding affinities", J. Comput. Aided. Mol. Design, 10:427-440, (1996).
[23] I. Muegge, "PMF scoring revisited", J. Med. Chem, 49:5895-5902, (2006).
[24] H. J. Bohm, "Prediction of Binding Constants of Protein Ligands: A Fast Method for the Prioritization of Hits Obtained from De Novo Design or 3D Database Search Programs", J. Comput.-Aided Mol. Des, 12:309-323, (1998).

[25] H. J. Bohm, "The Development of a Simple Empirical Scoring Function to Estimate the Binding Constant for a Protein-Ligand Complex of Known Three-Dimensional Structure", J. Comput.-Aided Mol. Des, 8:243-256, (1994).
[26] R. Wang, Y. Lu and S. Wang, "Comparative Evaluation of 11 Scoring Functions for Molecular Docking", J. Med. Chem, 46:2287-2303, (2003).
[27] J. Koska, V. Z. Spassov, A. J. Maynard, L. Yan, N. Austin, P. K. Flook and C. M. Venkatachalam, "Fully Automated Molecular Mechanics Based Induced Fit Protein−Ligand Docking Method", J. Chem. Inf. Model, 48:1965-1973, (2008).
[28] I. Muegge and Y. C. Martin, "A general and fast scoring function for protein-ligand interactions: A simplified potential approach", J. Med. Chem, 42:791-804, (1999).
[29] G. M. Morris, D. S. Goodsell, M. E. Pique, W. L. Lindstrom, R. Huey, S. Forli, W. E. Hart, S. Halliday, R. Belew and A. J. Olson, "Automated Docking of Flexible Ligands to Flexible Receptors", user guide AutoDock, (2010).
[30] D. S. Goodsell, G. M. Morris and A. J. Olson, "Automated Docking of Flexible Ligands: Applications of autodock", J. Mol. Recognit, 9:1-5, (1996).
[31] G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell and A. J. Olson, "Autodock4 and AutoDockTools4: automated docking with selective receptor flexibility", J. Comput. Chem, 16:2785-91, (2009).
[32] H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov and P. E. Bourne, "The Protein Data Bank", Nucleic Acids Res, 28:235-42, (2000).
[33] R. Huey, D. S. Goodsell, G. M. Morris and A. J. Olson, "Grid-based hydrogen bond potentials with improved directionality", Lett. Drug. Des. Discov, 1:178-183, (2004).
[34] G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew and A. J. Olson, "Automated Docking Using a Lamarckian Genetic Algorithm and and Empirical Binding Free Energy Function", Comput. Chem, 19:1639-1662, (1998).
[35] D. K. Gehlhaar, D. Bouzida and P. A. Rejto, "In Rational Drug Design: Novel Methodology and Practical Applications", ACS-Washington, 292-311, (1999).
[36] H. J. Bohm, A. Flohr and M. Stahl, Drug Discov. Today. Technol, 1:217-224, (2004).
[37] H. Zhao, Drug Discov. Today, 12:149-155, (2007).
[38] P. Ertl, S. Jelfs, J. Muhlbacher, A. Schuffenhauer and P. Selzer, J. Med. Chem, 49:4568-4573, (2006).
[39] "Glide", Version 3.5 Schrodinger, L.L.C., New York, (2006).
[40] G. Jones, P. Willett and R. C. Glen, J. Mol. Biol, 245:43-53, (1995).
[41] "Cerius2", Version 4.11 Accelrys Inc, San Diego, CA, USA, (2007).
[42] "Catalyst", Version 4.11Accelrys Inc, San Diego, CA, USA, (2007).
[43] Z. Amtul, A. U. Rahman, R. A. Siddiqui and M. I. Choudhary, "Chemistry and mechanism of urease inhibition", Curr. Med. Chem, 14:1323-1348, (2002).
[44] S. Vassiliou, A. Grabowiecka, P. Kosikowska, A. Yiotakis, P. Kafarski and Ł. Berlicki, "Design, Synthesis,and Evaluation of Novel Organophosphorus Inhibitors of Bacterial Ureases", J. Med. Chem, 18:5736-5744, (2008).