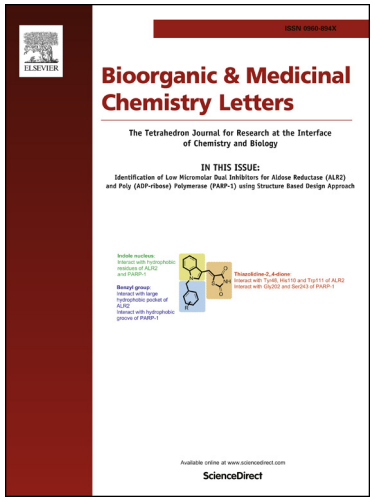
Accepted Manuscript



Synthesis and molecular docking study of some 5,6-dichloro-2-cyclopropyl-1*H*-benzimidazole derivatives bearing triazole, oxadiazole, and imine functionalities as potent inhibitors of urease

Emre Menteşe, Hakan Bektaş, Bahar Bilgin Sokmen, Mustafa Emirik, Demet Çakır, Bahittin Kahveci

PII: S0960-894X(17)30499-7
DOI: http://dx.doi.org/10.1016/j.bmcl.2017.05.019
Reference: BMCL 24964

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 10 March 2017
Revised Date: 4 May 2017
Accepted Date: 5 May 2017

Please cite this article as: Menteşe, E., Bektaş, H., Sokmen, B.B., Emirik, M., Çakır, D., Kahveci, B., Synthesis and molecular docking study of some 5,6-dichloro-2-cyclopropyl-1*H*-benzimidazole derivatives bearing triazole, oxadiazole, and imine functionalities as potent inhibitors of urease, *Bioorganic & Medicinal Chemistry Letters* (2017), doi: http://dx.doi.org/10.1016/j.bmcl.2017.05.019

Synthesis and molecular docking study of some 5,6-dichloro-2-cyclopropyl-1*H*-benzimidazole derivatives bearing triazole, oxadiazole, and imine functionalities as potent inhibitors of urease

Emre Menteşe[a,*], Hakan Bektaş[b], Bahar Bilgin Sokmen[b], Mustafa Emirik[a], Demet Çakır[b], Bahittin Kahveci[c]

[a]*Department of Chemistry,Art and Science Faculty, Recep Tayyip Erdogan University, Rize, Turkey*

[b]*Department of Chemistry, Faculty of Arts and Sciences, Giresun University, 28049, Giresun, Turkey;*

[c]*Department of Nutrition and Dietetics, Faculty of Health Sciences, Karadeniz Technical University, Trabzon, Turkey*

E-mail: emre.mentese@erdogan.edu.tr

Fax: +904642234019

ABSTRACT

A new series of benzimidazole compounds including hydrazinecarbothioamide, 1,2,4-triazole, 1,3,4-oxadiazole and imine function were synthesized starting from 5,6-dichloro-2-cyclopropyl-1H-benzimidazole. All of the benzimidazole derivatives exhibited good urease inhibitor activity. Compound **6a** proved to be the most potent showing an enzyme inhibitory activity with an IC_{50} = 0.06 µM. Molecular docking studies were also conducted on enzyme extracted from Jack bean urease to identify the binding mode of the newly synthesized compounds.

Keywords: Benzimidazole, 1,2,4-Triazole, Schiff base, Docking study, Antiurease activity.

The metalloenzyme urease (urea amidohydrolase EC 3.5.1.5) is a nickel-containing enzyme that catalyzes the hydrolysis of urea to ammonia and carbon dioxide. Urease, widely distributed in nature, and it is found in a variety of plants, fungi, algae and bacteria.[1,2] Urease is involved in the pathogenesis of hepatic encephalopathy, hepatic coma urolithiasis, pyelonephritis, ammonia and urinary catheter encrustation.[3] It is also a major cause of pathologies induced by *Helicobacter pylori* (*H. pylori*) as this allows bacteria to survive at the low pH of the stomach and hence plays an important role in producing peptic and gastric ulcers.[4] In the near past, a number of compounds have been proposed as urease inhibitors to reduce environmental problems and to enhance the uptake of urea nitrogen by plants and health problems.[5] Many urease inhibitors have been investigated in the past decades, such as imidazoles, phosphorodiamidates and hydroxamic acid derivatives, but most of these compounds are very unstable or toxic to allow their use *in vivo*. Therefore, the search is still on for novel urease inhibitors with promising levels of activity.[6]

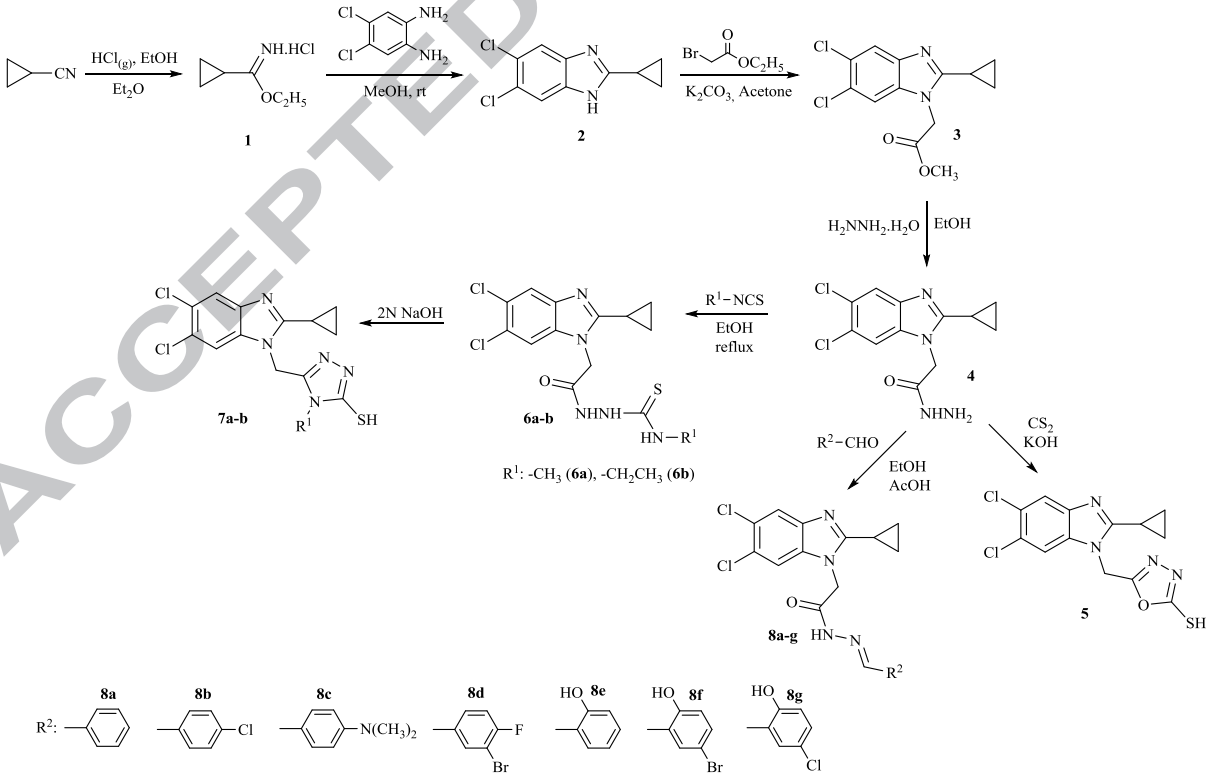
It is known that benzimidazoles are among the most important heterocyclic systems that show many different pharmaceutical properties. Recently, some of benzimidazole derivatives have been used as drugs in human and veterinary medicine.[7] In addition, many biological activities related to benzimidazole derivatives have been described: anthelmintic,[8] antiurease,[9] antimicrobial,[10] cytotoxic properties,[11] antitumor activity,[12,13] antibacterial activity,[14] anti-MRSA and anti-VRE agents,[15] anticancer agents,[16] anti-viralagents[17] and anti tubercular activity.[18] Moreover, previous studies show that 5,6-dichloro benzimidazole derivatives have some biological activities.[19-23]

Several literature reports of various heterocyclic compounds containing cyclopropyl substituent that are very important for their pharmacological effects. Introduction of cyclopropyl group can potentially increase inhibitory activity.[24-27] Also, literature survey reveals that Schiff bases are important in diverse field's chemistry has been because of their biological properties.[28] Furthermore, substituted acylhydrazide Schiff bases have shown a broad range of bioactivities like antibacterial and antiurease.[29,30]

Therefore, preparations of new benzimidazole derivatives containing different functional groups have received an increasing attention from synthetic chemists. In this study, some novel benzimidazole derivatives have been synthesized and their antiurease activities have been investigated with molecular docking study.

In the current study, we have synthesized some benzimidazole derivatives starting from iminoester hydrochloride and have been investigated for their antiurease activity. Starting material, iminoester

hydrochloride (**1**) was prepared according to the known method.[31] The reaction of compound **1** with 4,5-dichloro-*o*-phenylenediamine afforded 5,6-dichloro-2-cyclopropyl-1*H*-benzo[d]imidazole (**2**).[32,33] The reaction of compound **2** with ethyl bromoacetate in the presence of dry potassium carbonate yielded ethyl 2-(5,6-dichloro-2-cyclopropyl-1*H*-benzo[d]imidazol-1-yl)acetate **(3)**. The compound **4** was prepared by reacting **3** with hydrazine monohydrate in ethanolic solution. The ring closure reaction of **4** with carbon disulfide in presence of KOH resulted in the formation of compound **5**. Thiosemicarbazides were prepared by the nucleophilic addition of acid hydrazides (**5a-b**) to methyl isothiocyanate (for **6a**) or ethyl isothiocyanate (for **6b**). Intramolecular cyclization of thiosemicarbazides (**6a-b**) in the presence of 2N NaOH solution under reflux resulted in the formation of 1,2,4-triazole derivatives **7a-b**. We have also converted compound **4** to some schiff bases (**8a-g**) by the reaction of acetohydrazide derivative (**4**) with corresponding aldehydes. The synthetic path of the target compounds was illustrated in Scheme 1. The structures of the new compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR spectroscopy and mass spectra. For the spectroscopic investigations of newly synthesized compounds are accordance with the proposed structure.



Scheme 2. The synthetic path of the target compounds.

The inhibition effect of urease activity was shown in Table 1. All of the benzimidazole derivatives exhibited good urease inhibitor activity. Lower IC_{50} values indicate higher enzyme inhibitor activity. Compound **6a** which has methyl group on thiosemicarbazide part proved to be the most potent showing compound an enzyme inhibitory activity with an IC_{50} = 0.06 µM. Thiourea showed lower activity than compounds **2, 6a-b, 7a-b, 8b, 8d, 8e, 8f** bearing thiosemicarbazide, triazole and imine function at positionon-1 on the benzimidazole nucleus. It may be concluded from the results than benzimidazoles bearing open-chain thiosemicarbazide (**6a-b**), in general, are more potent that their mercapto-1,2,4-triazole (**7a-b**) counterparts.

Table 1. The antiurease activities and docking score of benzimidazole derivatives.

Compounds	Antiurease IC_{50} (µM)*	Docking Score
2	0.17±0.011	-6.424
3	1.12±0.08	-5.741
4	1.92±0.33	-6.353
5	1.34±0.28	-7.34
6a	0.05±0.002	-8.968
6b	0.28±0.006	-8.34
7a	0.17±0.072	-4.775
7b	0.38±0.083	-7.701
8a	2.07±0.31	-5.591
8b	0.29±0.030	-6.339
8c	1.49±0.29	-8.916
8d	0.06±0.008	-7.133
8e	0.22±0.089	-7.044
8f	0.14±0.034	-7.815
8g	0.15±0.019	-5.843
Thiourea	0.56±0.17	-6.11

*Values were the means of three replicates ± Standard deviation (SD).

Compound **8a** having phenyl ring with no substituent had the lowest antiurease activity among all the compounds tested and standards (IC_{50} = 2.07 µM). Compounds **8b**, **8d-g** containing halogen atoms and hydroxyl group on phenyl ring showed excellent inhibitor activity. Among the Schiff base derivatives, compound **8d** bearing a 3-flouro, and 4-bromo groups on phenyl ring was found to be the most active inhibitor when compared with the other Schiff bases. Compound **8e** contain hydroxyl group 2- position when compared with compounds **8f** and **8g** having hydroxyl group 2-position with bromo (**8f**) and chloro (**8g**) substituents respectively at phenyl part. The inhibitory potential of compounds **8f** and **8g** was found greater than **8e**. This indicates that halogen atoms on the phenyl ring enhance the activity. It is clear that electronic effect plays an important role in the inhibitor activity of compounds **8a-g**. We have demonstrated that the potency of the benzimidazole derivatives into inhibit urease.

In order to give an explanation and understanding of potent inhibitory activity, docking poses of the most potent compounds **6a** and **8d** at the binding site of Jack bean urease were illustrated. The docking scores of the studied compounds with 3LA4 were given in Table 1. The docking scores have a good agreement with the experimental results, in general. The newly synthesized benzimidazole derivatives have the capability to bind to active sites of urease enzyme. Thus, the hydrolysis of enzyme was stopped and activity of enzyme was inhibited.

According to the *in vitro* results, compound **8d** was the most active inhibitor of Jack bean urease. The binding model of compound **8d** was shown in Fig. 1. The docking studies showed that the best orientation of compound **6a** in the active pocket was formed by hydrogen bonding with the amino acid residues of Arg439, Asp494. Furthermore, phosphate, PO_4, group of the enzyme has an important contribution to binding energy with forming hydrogen bond and the salt bridge through the NH moiety of ligand. The amino acids Val591, Ile411, Ala436, Ala440, Met588 and Leu589 form hydrophobic interaction with compound **8d**. Fig. 2 represents the most interacting conformation of **8d** in the active side of the enzyme at the electrostatic surface.

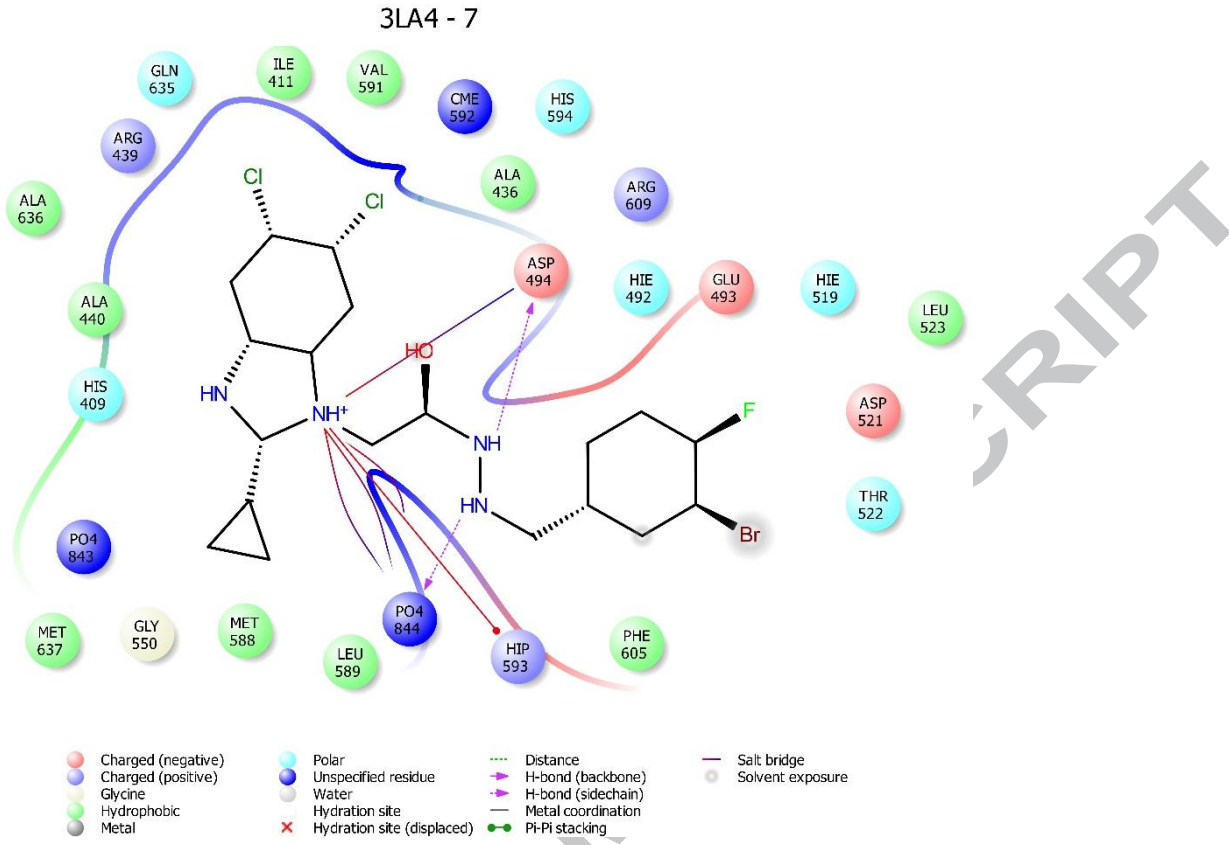


Fig.1. 2D interaction diagram of compound **8d** in the active pocket.

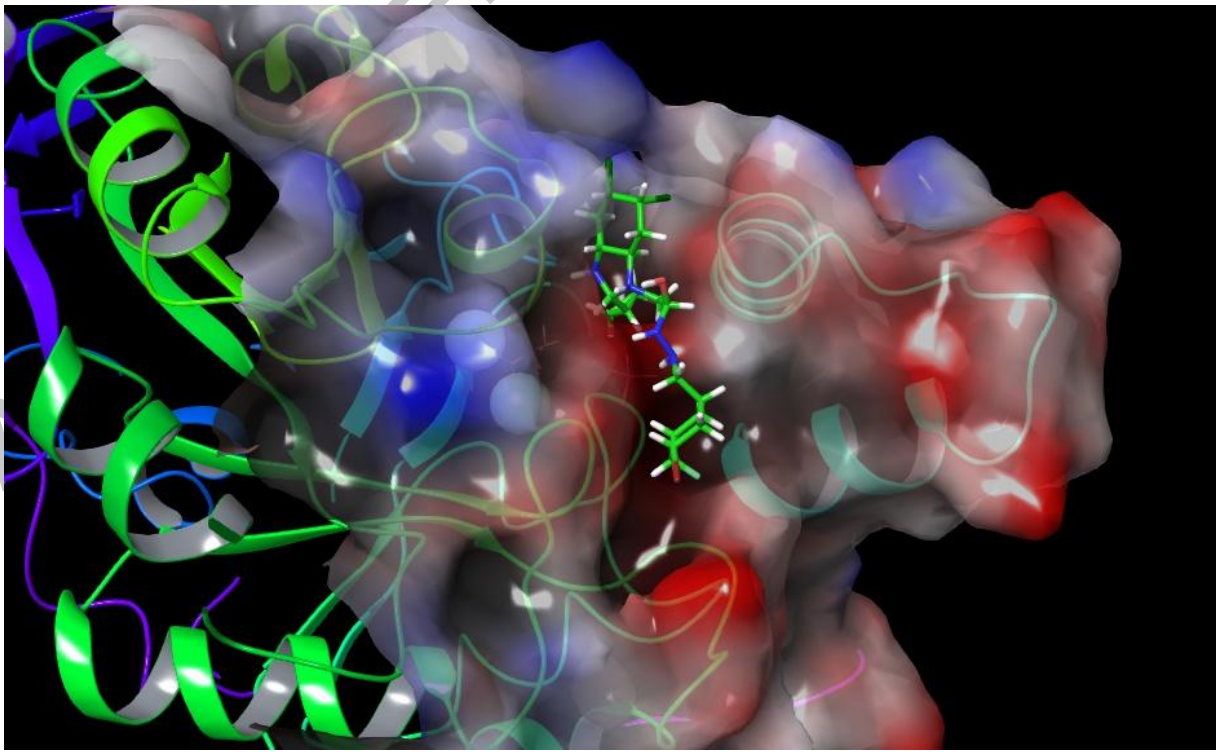


Fig. 2. The predicted binding mode of compound **8d** at electrostatic surface

Fig. 3 shows the docking pose of compound **6a**. The prominent interactions of compound **6a** with enzyme are three hydrogen bond interactions and salt bridge by means of PO_4 anion of enzyme. The docking study predicted that compound **6a** binds to enzyme at the binding site forming a hydrogen bond with Arg439 and Asp494 residue through the -NH and -OH groups of benzimidazole and other interactions involve the salt bridge with Asp494 and PO_4 groups of the enzyme. Van der Waals interactions were established with Ala636, Ala440, Ile411, Met637 and Leu523 amino acid residues in hydrophobic pocket. Figure 4 represents the most interacting conformation of **6a** in the active side of the enzyme at the electrostatic surface.

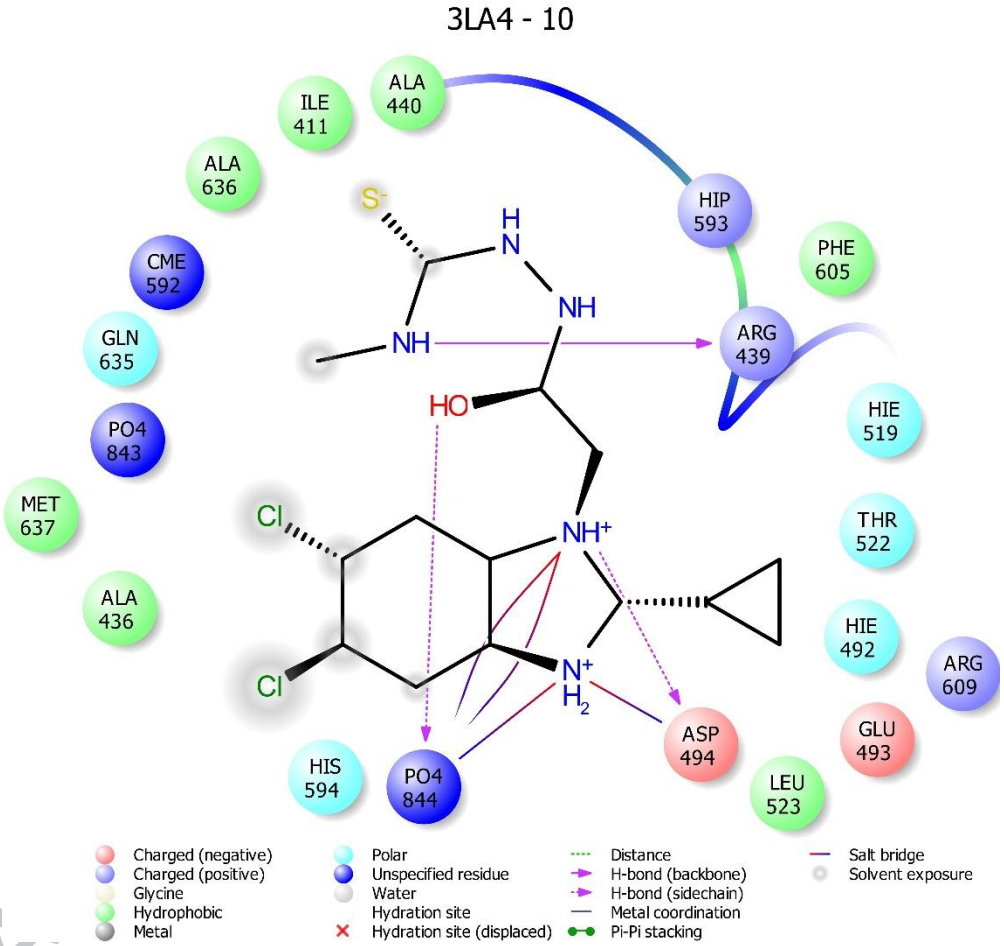


Fig. 3. 2D interaction diagram of compound **6a** in the active pocket.

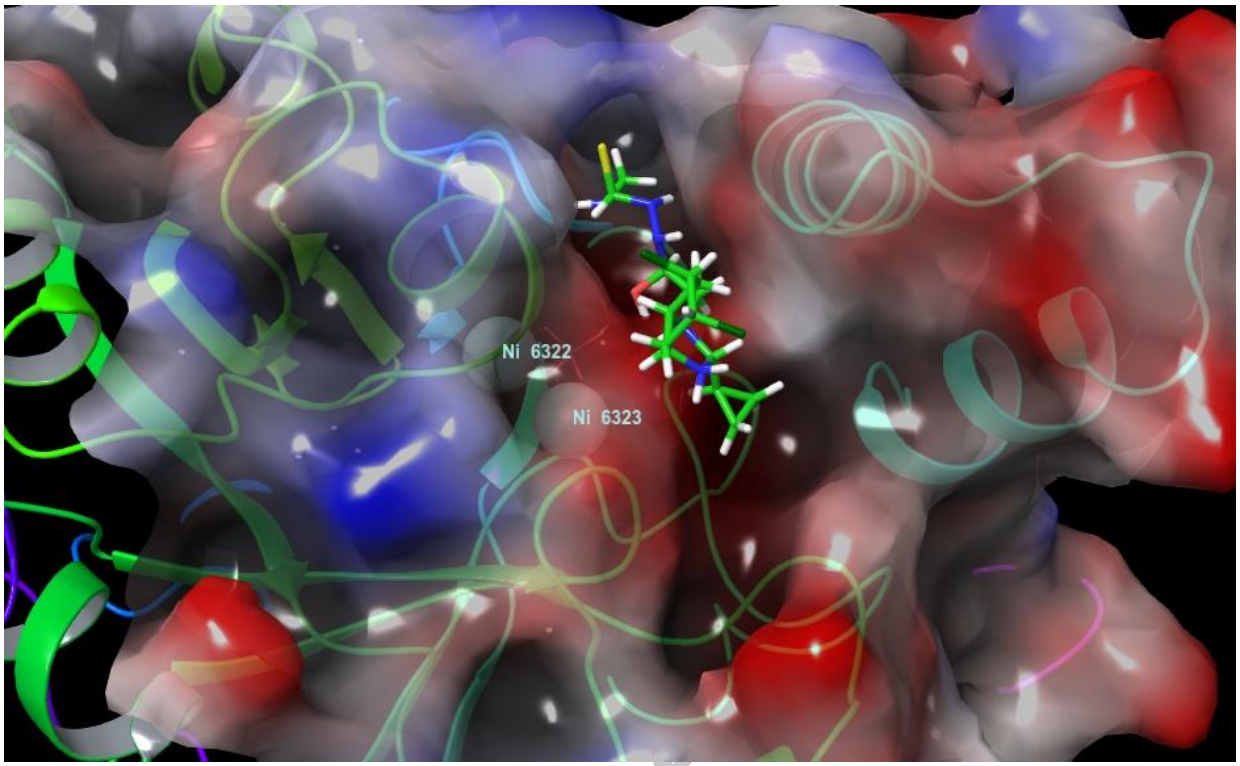


Fig. 4. The predicted binding mode of compound **6a** at electrostatic surface

In conclusion, a new series of benzimidazole compounds including hydrazinecarbothioamide, 1,2,4-triazole, 1,3,4-oxadiazole and imine function were synthesized and evaluated for their antiurease activities. All of the benzimidazole derivatives exhibited good urease inhibitor activity. Compound **6a** which has methyl group on thiosemicarbazide part proved to be the most potent showing compound an enzyme inhibitory activity with an IC_{50} = 0.06 µM. Among the Schiff base derivatives, containing halogen atoms on phenyl ring was found to be the most active inhibitor compared with other Schiff bases. The molecular docking results show that the H-bonding is more important than other factors in urease inhibition mechanism. PO_4^{3-} anion has a significant role as hydrogen bond acceptor in the active region of urease enzyme. The results could be inspiration for further structural modifications and investigations of potential antiurease activity within heterocyclic compounds.

Conflict of interest

The authors confirm that this article has no conflict of interest.

A. Supplementary data

References

1. Zerner B, *Bioorganic Chemistry* 1991;19:116-131.

2. Krajewska B. *Wiadomosci Chemiczne* 2002;56:223-253.

3. Mobley HLT, Island MD, Hausinger RP. *Microbiol. Rev.* 1995;59: 451-480.

4. Mobley HLT, Hausinger RP. *Microbiol. Rev.* 1989;53:85-108.

5. Amtul ZM. *Biochemical and Biophysical Research Communications* 2004;319:1053-1063.

6. Amtul Z, Atta-ur-Rahman SAR, Choudhary IM. *Curr. Med. Chem.* 2002;9:1323-1348.

7. Velik J, Baliharova V, Fink-Gremmels, J, Bull S, Lamka J, Skalova L. *Res.Vet. Sci.* 2004;76:95-98.

8. Stuchlíková L, Jirásko R, Skálová L, Pavlík F, Szotáková B, Holčapek M, Vaněk T, Podlipná R. *Chemosphere* 2016;157:10-17.

9. Baltas N, Yılmaz F, Menteşe E. *Hacettepe J. Biol. Chem.*, 2016;44:293-305.

10. Fang X, Jeyakkumar P, Avula SR, Zhou Q, Zhou C. *Bioorg. Med. Chem. Lett.* 2016;26: 2584-2588.

11. Marinelli M, Pellei M, Cimarelli C, Dias HVR, Marzano C, Tisato F, Porchia M, Gandin V, Santini, C. *J. Organomet. Chem.* 2016;806:45-53.

12. Castillo I, Suwalsky M, Gallardo MJ, Troncoso V, Sánchez-Eguía BN, Santiago-Osorio E, Aguiñiga I, González-Ugarte AK. *J. Inorg. Biochem.* 2016;156:98-104.

13. Kahveci B, Menteşe E, Özil M, Ülker S, Ertürk M. *Monatsh. Chem.* 2013;144:993-1001.

14. Strelciunaite V, Anusevicius K, Tumosiene I, Siugzdaite J, Jonuskiene I, Ramanauskaite I, Mickevicius V. *Heterocycles*, 2016;92:235-251.

15. Göker H, Karaaslan C, Puskullu MO, Yildiz S, Duydu Y, Ustundag A, Yalcin CO. *Chem. Biol. Drug. Des*. 2016;87:57-68.

16. Chhabra M, Sinha S, Banerjee S, Paira P. *Bioorg. Med. Chem. Lett.* 2016;26:213-217.

17. Eldebss TMA, Farag AM, Abdulla MM, Arafa RK. *Mini-Reviews in Med. Chem.* 2016;16:67-83.

18. Chandrasekera NS, Alling T, Bailey MA, Files M, Early JV, Ollinger J, Ovechkina Y, Masquelin T, Desai PV, Cramer JW, Hipskind PA, Odingo JO, Parish T. *J. Med. Chem.* 2015;18:7273-7285.

19. He Y, Yang J, Wu B, Risen L, Swayze EE. *Bioorg. Med. Chem. Lett.* 2004;14:1217-1220.

20. Ramla MM, Omar MA, Tokuda H, El-Diwani HI. *Bioorg. Med. Chem.* 2007;15:6489-6496.

21. Garuti L, Roberti M, Cermelli C. *Bioorg. Med. Chem. Lett.* 1999;9:2525-2530.

22. Raymond A, Guan J, Alford VC, Lanter JC, Allan GF, Sbriscia T, Linton O, Lundeen SG, Sui Z. *Bioorg. Med. Chem. Lett.* 2007;17:784-788.

23. Starčević K, Kralj M, Ester K, Sabol I, Grce M, Pavelić K, Karminski-Zamola G. *Bioorg. Med. Chem.* 2007;15:4419-4426.

24. Takai K, Chiyo N, Nakajima T, Nariai T, Ishikawa C, Nakatani S, Ikeno A, Yamamoto S, Sone T. *Bioorg. Med. Chem. Lett.* 2015;25:1705-1708.

25. Li H, Xue F, Kraus JM, Ji H, Labby KJ, Mataka J, Delker SL, Martásek P, Roman LJ, Poulos TL, Silverman RB. *Bioorg. Med. Chem.* 2013;21:1333-1343.

26. Wertmuth, C. G.; The Practice of medicinal chemistry, Academic Press: San Diego, 1996.

27. Byrtus H, Czopek A, Kamiriski K, Pawlowski M. *Bioorg. Med. Chem.* 2014;19:6149-6156.

28. Gumrukcuoglu N, Sokmen BB, Ugras S, Ugras HI, Yanardağ R. J. Enzym. Inh. Med. Chem. 28 (2013) 89-94.

29. Taha M, Ismail NH, Baharudin MS, Lalani S, Mehboob S, Khan KM, Yousuf S, Siddiqui S, Rahim F, Choudhary MI. *Med. Chem. Res.* 2015;24:1310-1324.

30. Taha N, Ismail NH, Khan A, Shah SAA, Anwar A, Halim SA, Fatmi QM, Imran S, Rahim F, Khan KM. *Bioorg. Med. Chem. Lett.* 2015;25:3285-3289.

31. Cloke JB, Knowles EC, Anderson RJ, *J. Am. Chem. Soc.* 1936;58:2547-2549.

32. Llona-Minguez S, Höglund A, Jacques SA, Johansson L, Calderón-Montaño JM, Claesson M, Loseva O, Valerie NCK, Lundbäck T, Piedrafita J, Maga G, Crespan E, Meijer L, Morón EB, Baranczewski P, Hagbjörk A, Svensson R, Wiita E, Almlöf I, Visnes T, Jeppsson F, Sigmundsson K, Jensen AJ, Artursson P, Jemth A, Stenmark P, Berglund UW, Scobieand M, Helleday T. *J. Med. Chem.* 2016;59:1140-1148.

33. Menteşe E, Bektaş H, Ülker S, Bekircan O, Kahveci B. *J. Enzyme Inh. Med. Chem.* 2014;29:64-68.