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INTERACTIONS OF UREASE WITH FLAVONE: A THEORETICAL PERSPECTIVE

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Abstract: This research investigates the molecular level interactions between jack bean urease enzyme and flavone as an inhibitor. It is important to understand how flavone interacts with the active site of urease to inhibit the enzyme's activity. In this work, the interaction between urease enzyme and flavone inhibitor was examined using semiempirical quantum mechanical theoretical investigations. Between the two nickels (labelled Ni88 and Ni89), flavone showed a tendency to attach to Ni89, where the structure of the inhibitor was distorted to create more positive interactions with the surrounding atoms. The overall interactions, based on the Wiberg Bond Order, show that flavone is able to form stable complexes with urease. It was also found that the interaction energy of -1.52 eV and -1.87 eV for flavone as an inhibitor are weaker than urea as an adsorbate. Furthermore, the interaction is analysed not to be that of a covalent nature. Hence flavone's potential as urease inhibitor is proven theoretically.

Keywords: Flavone, urease, enzyme, inhibitor, semiempirical.

Introduction

Nitrogen is an essential element for the growth of pathogenic bacteria and plants which is catalysed by the urease enzyme from urea (Dimkpa *et al.*, 2020; Matczuk & Siczek, 2021). Urease is a large multimeric enzyme, belonging to the family of amido-hydrolases. Urease enzymes are present in fungi, bacteria, and plants (Kappaun *et al.*, 2018; Proshlyakov *et al.*, 2021). Urease catalyses the hydrolysis of urea into ammonia (NH_3) and carbamate, as the reaction in Figure 1 shows. It plays an important role in the nitrogen cycle, as it supplies nitrogen in the form of ammonia for seed germination and the growth of microorganisms. Despite its significance, hydrolysis may also have negative consequences. The presence of excess ammonia and carbamate can result in adverse effects, such as infectious stones, stomach ulcers, and peptic ulcers. Additionally, it contributes to the development of hepatic encephalopathy, pyelonephritis, hepatic coma, urolithiasis, central nervous system disorders, and urinary catheter encrustation (Mobley & Hausinger, 1989; Collins & D'Orazio, 1993; Karplus *et al.*, 1997; Almeida *et al.*, 2021). This enzyme is a crucial indicator of bacterial infection. In agriculture, if the hydrolysis is too rapid, it can result in the unproductive loss of nitrogen through ammonia volatilisation. In contrast, ammonia toxicity, alkalinity, and accumulated nitrite can cause plant damage by affecting seed germination, seedling growth, and early plant growth in soil, causing severe environmental and economic issues (Sahrawat, 1980; Mulvaney & Bremner, 1981; Bremner & Krogmeier, 1989; Zulkifli *et al.*, 2022).

Due to its damaging behaviour, studies to find the most suitable inhibitor for the urease enzyme were performed (Awllia *et al.*, 2016; Rana *et al.*, 2021; Song *et al.*, 2022). It has been shown that flavonoids, a large group of polyphenolic compounds that naturally occur in fruits, vegetables, nuts, seeds, flowers, and bark, can act as a potent inhibitor against the urease enzyme (Xiao *et al.*, 2013; Jan & Abbas, 2018; He *et al.*, 2022; Tavares *et al.*, 2022). This report is on the interaction between flavone (a flavonoid) with the active site of the jack bean

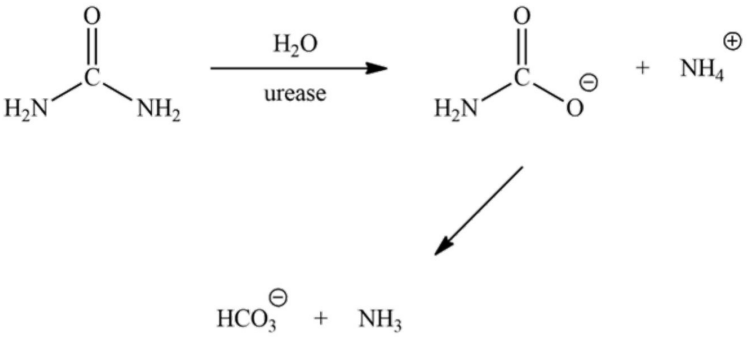


Figure 1: Hydrolysis of urea catalysed by urease enzyme

urease enzyme. The investigation is carried out to gain more knowledge and improve the grasp of what could be the most suitable urease inhibitor. Previous researchers tested nine naturally occurring flavonoids for urease inhibition in this matter (Balasubramanian & Ponnuraj, 2010; Liu *et al.*, 2020). In general, the hydroxy (OH) groups in flavonoids are thought to be primarily responsible for their urease inhibiting effect. Furthermore, OH groups can generate electrostatic interactions (hydrogen bonding) with charged active site residues such as histidine, arginine, and aspartic acid. These results lead to the concept that various interactions of a specific group of flavonoids would improve enzyme inhibition.

Materials and Methods

Flavone is the simplest member of its class of flavonoids (Panche *et al.*, 2016), bearing an oxo substituent at position 4, as shown in Figure 2. For this purpose, we truncated the urease from jack bean to a manageable structure (Jabri *et al.*, 1995; Balasubramanian & Ponnuraj, 2010; Mazzei *et al.*, 2020), as shown in Figure 3. The structure of flavone and urease were retrieved from PubChem and Protein Bank Database (PDB) respectively (Berman, 2000; Kim *et al.*, 2021). Urease has nickel as its active site. For the purpose of our investigations, flavone was positioned near Ni88 (labelled as P1) and near Ni89 (labelled as P2). The arrangements are shown in Figure 4. In Figure 4, the atoms that are allowed to move are represented by spheres, while those fixed atoms are in tubes. To find the most probable arrangements of flavone in the two P1 and P2 systems, geometry optimisations were performed using the xTB method, as implemented with Grimme's GFN2-xTB program, interfaced to G09 with Tian Lu's script and programs (Lu & Chen, 2012; Bannwarth *et al.*, 2019; Lu, 2020).

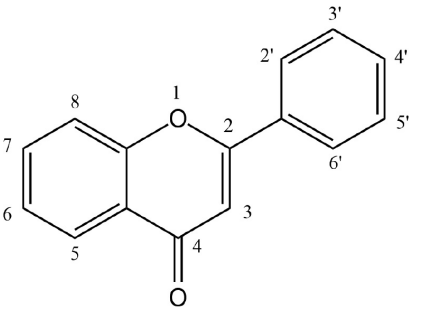


Figure 2: Structure of flavone

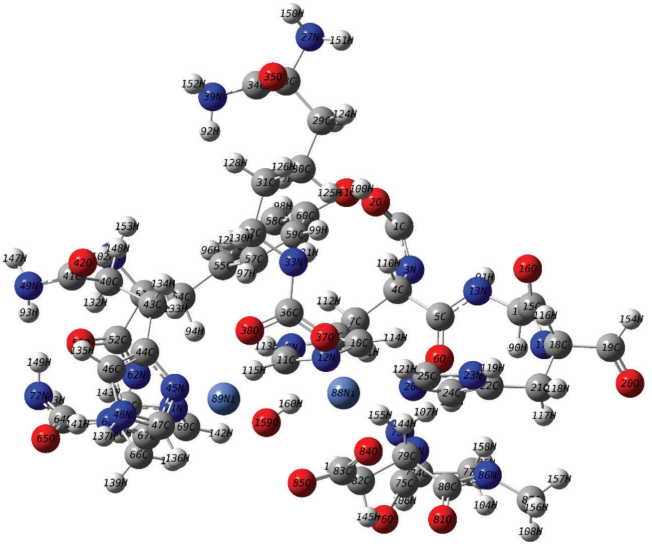
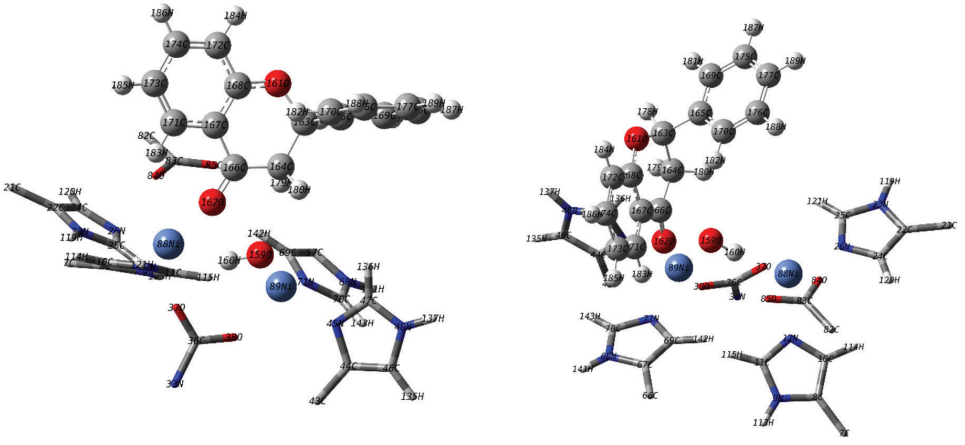


Figure 3: The truncated structure of urease (PDB code: 3LA4). The elements in the structure are labelled



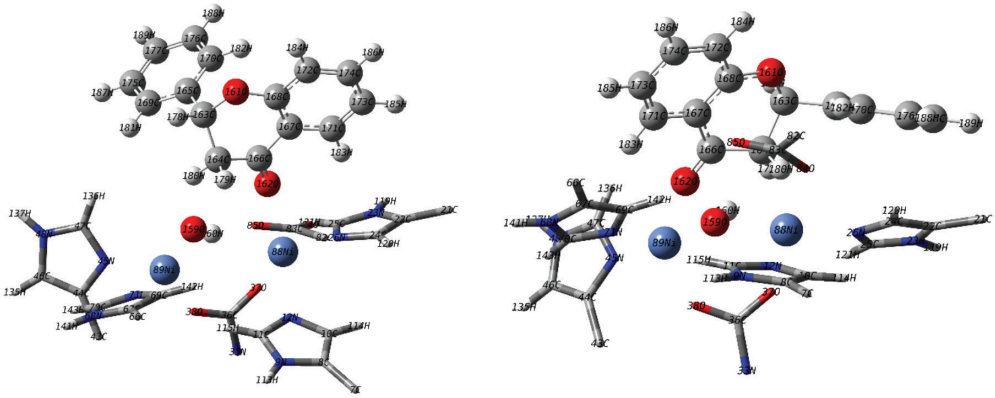
(a) (b)

Figure 4: The initial arrangements for (a) P1 and (b) P2. P1 is the system where the oxygen on the inhibitor (O162) is being positioned near Ni88 while for P2, the same oxygen is positioned near Ni89. The "ball and bond" type represents the inhibitor, hydroxide, and nickel ions while the "tube" type represents the other residues. For simplicity, some parts of the residues are not shown

Results and Discussion

The final arrangement of flavone and urease, as obtained from the xTB program, is shown in Figure 5. The optimised geometries show that the phenyl ring on flavone was distorted from the initial position. For P1, the phenyl moved away from the active site, while for P2, it moved towards the active site.

As a comparison of the stability between the two P1 and P2 systems, the total energy and interaction energy are calculated and tabulated in Table 1. The total energy E_t of P2 is more than P1, hence P2 is found to be stable compared to P1. The interaction energy of P2 is also higher than the one in P1. Without the inhibitor, Ni88



(a) (b)

Figure 5: Geometries after optimisation, (a) P1 and (b) P2. Notice the changes to the phenyl ring in flavanone after optimisation (compare to Figure 3)

and Ni89 are positively charged, with values of 0.023e and 0.052e, respectively. With the inhibitor, changes occurred to the charges and the interactions. The related values are tabulated in Table 2, while the optimised geometry is shown in Figure 5.

For P1, the interaction between Ni88 and the neighbouring atoms, as listed in Table 2, through the Wiberg Bond Order (WBO) analysis, shows that existing bond orders were lower with the existence of an inhibitor. Thus, with new interactions being created between Ni88 and the inhibitor through O162, it indirectly weakens the interactions between Ni88 and atoms in the residues. However, the total WBO for Ni88 increases from 2.936 to 3.050 with the insertion of the inhibitor. The magnitude of changes in WBO before and after the inclusion of inhibitor is between 0.031 to 0.071. For Ni89, there are no significant changes (the magnitude of changes before and after the inclusion of inhibitor ranges between 0.002 to 0.029) to the contributing WBO from the neighbouring atoms. This can be attributed to the position of O162 (from inhibitor) that is aligned nearer to Ni88. The total WBO for Ni89 before (3.155) and after inclusion (3.135) of the inhibitor further strengthens the point of discussion for the interaction of Ni89. Except for the hydroxide, there is no direct bonding for other atoms in the vicinity of active sites, as the WBO are all lower than 1. All the neighbours also possessed negative charges (as shown in Table 1), while the active sites are positive: Ni88 has a charge of 0.125e (increased from 0.023e) while Ni89 has 0.032e (down from 0.052e). In this situation, it is expected that the interaction involving nickels might be electrostatic.

Table 1: The stability and the interaction energy E_{int} of the systems P1 and P2

System	Energy (au)			E_{int} (eV)
	Total, E_t	Urease, E_a	Inhibitor, E_i	
P1	-327.05360190	-280.63906	-46.35867	-1.52038
P2	-327.08062354	-280.65632	-46.35564	-1.86833

Table 2: Properties of selected atoms in the vicinity of active sites for initial location P1. The values in brackets are before the addition of the inhibitor

Atoms	Nearest Atoms	Properties from Nearest Atoms	
		WBO	Charge
Ni88	N26	0.608 (0.684)	-0.039 (-0.014)
	O84	0.574 (0.612)	-0.369 (-0.342)
	N12	0.424 (0.495)	-0.122 (-0.106)
	O37	0.395 (0.426)	-0.403 (-0.388)
	O162	0.373	-0.273
Ni89	O159	1.203 (1.205)	-0.419 (-0.425)
	O38	0.562 (0.553)	-0.362 (-0.358)
	N45	0.491 (0.520)	-0.109 (-0.096)
	N71	0.533 (0.517)	-0.127 (-0.132)

Table 3: Properties of selected atoms in the vicinity of active sites for initial location P2

Atoms	Nearest Atoms	Properties from Nearest Atoms	
		WBO	Charge
Ni88	N26	0.594 (0.684)	-0.054 (-0.014)
	O84	0.541 (0.612)	-0.374 (-0.342)
	N12	0.442 (0.495)	-0.098 (-0.106)
	O37	0.445 (0.426)	-0.385 (-0.388)
	O159	0.511	-0.415
Ni89	O159	0.790 (1.205)	-0.415 (-0.425)
	O162	0.329	-0.337
	O38	0.566 (0.553)	-0.345 (-0.358)
	N45	0.515 (0.520)	-0.107 (-0.096)
	N71	0.521 (0.517)	-0.108 (-0.132)

For the initial position P2, the inhibitor after optimising the geometry is curled towards the residue's alanine and histidine, as shown in Figure 5. The related values for atoms in the vicinity of the active sites are tabulated in Table 3. As in the situation in P1, the inclusion of flavone weakens the interactions involving Ni88, and has not much effect on the interactions of Ni89 with the existing atoms.

Electron localisation function (ELF) analysis was performed using Multiwfn to further study the bonding involved in the P1 and P2 systems (Lu & Chen, 2012). Figure 6 shows the isosurface map of the ELF. Since the ELF maps of P1 and P2 are similar, only P1 is shown. From Figure 6, the oxygens, and nitrogens in the vicinity of nickels are still having lone pairs to them, instead of showing the bonding (orange lobes) to the nickels. Hence, the interactions of nickels with the surrounding atoms are not covalent in nature.

Conclusion

The interaction of inhibitor with urease enzyme was studied. It was found that flavone is able to interact favourably with the active sites of urease, the nickels. The interaction energy of -1.52 eV and -1.87 eV is weaker than urea as adsorbate, and the interaction is analysed to be not covalent. Hence, flavone's potential as a urease inhibitor is proven theoretically.

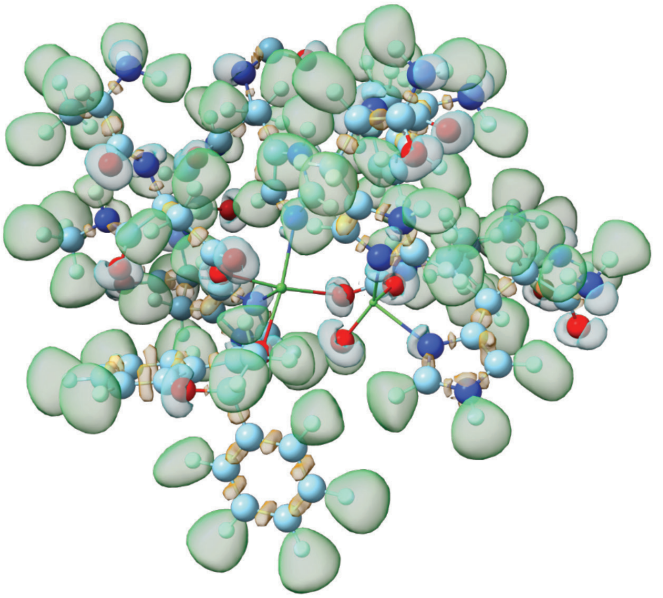


Figure 6: ELF map of P1. The isosurface has a value of 0.83. The colour scheme used: Cyan for lone pair domains; dark green for hydrogen-related domains; and orange for bonding domains between heavy atoms

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References

Almeida, C. G. M., Costa-Higuchi, K., Piovesan, A. R., Moro, C. F., Venturin, G. T., Greggio, S., Costa-Ferro, Z. S., Salamoni, S. D., Peigneur, S., Tytgat, J., de Lima, M. E., Silva, C. N. d., Vinadé, L., Rowan, E. G., DaCosta, J. C., Dal Belo, C. A., & Carlini, C. R. (2021). Neurotoxic and convulsant effects induced by jack bean ureases on the mammalian nervous system. *Toxicology*, *454*, 152737.

Awllia, J., Al-Ghamdi, M., Huwait, E., Javaid, S., Rasheed, S., & Iqbal Choudhary, M. (2016). Flavonoids as natural inhibitors of jack bean urease enzyme. *Letters in Drug Design & Discovery*, *13*(3), 243-249.

Balasubramanian, A., & Ponnuraj, K. (2010). Crystal structure of the first plant urease from jack bean: 83 years of journey from its first crystal to molecular structure. *Journal of Molecular Biology*, *400*(3), 274-283.

Bannwarth, C., Ehlert, S., & Grimme, S. (2019). GFN2-xTB - an accurate and broadly parametrized self-consistent tight-binding quantum chemical method with multipole electrostatics and density-dependent dispersion contributions. *Journal of Chemical Theory and Computation*, *15*(3), 1652-1671.

Berman, H. M. (2000). The protein data bank. *Nucleic Acids Research*, *28*(1), 235-242.

Bremner, J. M., & Krogmeier, M. J. (1989). Evidence that the adverse effect of urea fertilizer on seed germination in soil is due to ammonia formed through hydrolysis of urea by soil urease. *Proceedings of the National Academy of Sciences*, *86*(21), 8185-8188.

Collins, C. M., & D'Orazio, S. E. F. (1993). Bacterial ureases: Structure, regulation of expression and role in pathogenesis. *Molecular Microbiology*, *9*(5), 907-913.

Dimkpa, C. O., Fugice, J., Singh, U., & Lewis, T. D. (2020). Development of fertilizers for enhanced nitrogen use efficiency – Trends and perspectives. *Science of the Total Environment*, *731*, 139113.

He, Y., Zhang, X., Li, M., Zheng, N., Zhao, S., & Wang, J. (2022). Coptisine: A natural plant inhibitor of ruminal bacterial urease screened by molecular docking. *Science of the Total Environment*, *808*, 151946.

Jabri, E., Carr, M. B., Hausinger, R. P., & Karplus, P. A. (1995). The crystal structure of urease from klebsiella aerogenes. *Science*, *268*(5213), 998-1004.

Jan, S., & Abbas, N. (2018). Chapter 4 - Chemistry of himalayan phytochemicals. In S. Jan & N. Abbas (Eds.), *Himalayan Phytochemicals* (pp. 121-166). Elsevier.

Kappaun, K., Piovesan, A. R., Carlini, C. R., & Ligabue-Braun, R. (2018). Ureases: Historical aspects, catalytic, and non-catalytic properties – A review. *Journal of Advanced Research*, *13*, 3-17.

Karplus, P. A., Pearson, M. A., & Hausinger, R. P. (1997). 70 years of crystalline urease: What have we learned? *Accounts of Chemical Research*, *30*(8), 330-337.

Kim, S., Chen, J., Cheng, T. J., Gindulyte, A., He, J., He, S. Q., Li, Q. L., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J., & Bolton, E. E. (2021). PubChem in 2021: New data content and improved web interfaces. *Nucleic Acid Research*, *49*(1), 1388-1395.

Liu, H., Wang, Y., Lv, M., Luo, Y., Liu, B.-M., Huang, Y., Wang, M., & Wang, J. (2020). Flavonoid analogues as urease inhibitors: Synthesis, biological evaluation, molecular docking studies, and in-silico ADME evaluation. *Bioorganic Chemistry*, *105*, 104370.

Lu, T. (2020). *Gau_xtb: A gaussian interface for xtb code*. http://sobereva.com/soft/gau_xtb

Lu, T., & Chen, F. (2012). Multiwfn: A multifunctional wavefunction analyzer. *Journal of Computational Chemistry*, *33*(5), 580-592.

Matczuk, D., & Siczek, A. (2021). Effectiveness of the use of urease inhibitors in agriculture: A review. *International Agrophysics*, *35*(2), 197-208.

Mazzei, L., Musiani, F., & Ciurli, S. (2020). The structure-based reaction mechanism of urease, a nickel dependent enzyme: Tale of a long debate. *Journal of Biological Inorganic Chemistry*, *25*(6), 829-845.

Mobley, H. L., & Hausinger, R. P. (1989). Microbial ureases: significance, regulation, and molecular characterization. *Microbiological Reviews*, *53*(1), 85-108.

Mulvaney, R., & Bremner, J. (1981). Control of urea transformations in soils. In *Soil biochemistry* (1st ed., pp. 153-196). Marcel Dekker, Inc.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science*, *5*, E47.

Proshlyakov, D. A., Farrugia, M. A., Proshlyakov, Y. D., & Hausinger, R. P. (2021). Iron-containing ureases. *Coordination Chemistry Reviews*, *448*, 214190.

Rana, M. A., Mahmood, R., & Ali, S. (2021). Soil urease inhibition by various plant extracts. *PLOS ONE*, *16*(10), e0258568.

Sahrawat, K. L. (1980). Control of urea hydrolysis and nitrification in soil by chemicals—Prospects and problems. *Plant and Soil*, *57*(2), 335-352.

Song, W.-Q., Liu, M.-L., Li, S.-Y., & Xiao, Z.-P. (2022). Recent efforts in the discovery of urease inhibitor identifications. *Current Topics in Medicinal Chemistry*, *22*(2), 95-107.

Tavares, M. C., Olímpio, C. R. S. D. G., de Fátima, Â., Modolo, L. V., de Oliveira, J. A. C., da Silva-Filho, C. J. A., Barbosa, F. G., Mafezoli, J., & Santos, J. C. C. (2022). Interaction and inhibition mechanism of urease in vitro and soil system by a natural

benzylisothiocyanate isolated from moringa oleifera. *Industrial Crops and Products*, *178*, 114580.

Xiao, Z. P., Peng, Z. Y., Dong, J. J., He, J., Ouyang, H., Feng, Y. T., Lu, C. L., Lin, W. Q., Wang, J. X., & Xiang, Y. P. (2013). Synthesis, structure–activity relationship analysis and kinetics study of reductive derivatives of flavonoids as helicobacter pylori urease inhibitors. *European Journal of Medicinal Chemistry*, *63*, 685-695.

Zulkifli, M., Abu Hasan, H., Sheikh Abdullah, S. R., & Muhamad, M. H. (2022). A review of ammonia removal using a biofilm-based reactor and its challenges. *Journal of Environmental Management*, *315*, 115162.