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Original Research Article

In-silico Evaluation of Hexagamavunon Analogs for Antibacterial Activity Against *Helicobacter pylori*

Nurul Jannah[1]* and Rahmat A Hi Wahid[1]

[1]*Department of Pharmacy, Faculty of Science and Technology, Universitas PGRI Yogyakarta, Bantul, Yogyakarta, Indonesia*

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ABSTRACT

Helicobacter pylori (*H. pylori*) infection has been associated with gastric cancer. Antibiotic resistance has reached dangerous levels. Therefore, finding new anti-effective drugs against *H. pylori* is crucial. This study evaluated the potential of hexagamavunon (HGV) analogs as anti-*H. pylori* drugs through molecular docking. The AutoDock Vina program was used in the molecular tethering process. The ligands (HGV analogs), were docked to the shikimate kinase enzyme (PDB ID: 3N2E) and urease (PDB ID: 1E9Y) as the targets in inhibiting *H. pylori*. The parameter observed was the ligands' binding energy (kcal/mol) compared to native ligands. The results of molecular docking of the shikimate kinase enzyme showed that the binding energies of A6 (-10.7), A7 (-9.9), and A11 (-9.9) were lower compared with native ligand binding energy (-9.8). Also, the binding energy of the urease enzyme with A6 (-7.5), A7 (-8.1), and A11 (-7.7) was lower than the binding energy of the urease with native ligand (-3.4). Low binding energy correlated with the strength of the bonds between ligands and receptors. HGV analogs, A6, A7, and A11, have higher anti-*H. pylori* potential than other analogs because they have the lowest binding energies. Further *in vitro* research is needed to evaluate the potential of HGV analogs as anti-*H. Pylori* agents.

Keywords: AutoDock Vina; Binding energy; Molecular docking; Shikimate kinase; Urease

Introduction

Helicobacter pylori (*H. pylori*), a gram-negative bacteria, is one of the *Helicobacter* gastric species.[1] *H. pylori* can infect humans or primates, and it causes chronic gastritis, peptic ulcer, and gastric mucosa-associated lymphoid tissue (MALT).[2,3] WHO classifies *H. pylori* infection as the leading cause of gastric cancer.[4,5] Improper and ineffective treatment of *H. pylori* infection increases cancer risk.[6] Antibacterial resistance used in *H. pylori* treatment has increased, causing a decrease in the effectiveness of therapy.[4,7,8] The first-line treatment of *H. pylori* infection is the Proton Pump Inhibitors (PPIs), combined with clarithromycin and amoxicillin or metronidazole for 10-14 days.[9–11] Inhibiting the synthesis of DNA, proteins, and cell walls is the mechanism of antibiotics used as the primary therapy.[1] The shikimate kinase (SK) enzyme and the urease enzyme are the targets of new anti-*H. Pylori* agents. SK enzymes play an essential role in synthesising amino acids in *H. pylori*.[12–17] Urease enzyme functions as a defence against *H. pylori* bacteria when living in gastric fluid with low acidity (pH).[18]

Curcumin, a compound extracted from *Curcuma longa*, has been investigated for its activity. Curcumin and its derivates have antioxidant, anti-inflammatory, anti-cholesterol, antiviral, anticancer, and chemopreventive activities.[19–21] Curcumin has shown inhibitory activity against various types of bacteria, including anti-*H. pylori*.[22–27] The modified structure of curcumin has been synthesized and studied as a mono-carbonyl analog. Modification of the curcumin structure aims to improve its stability and solubility. Introducing different substituents in the phenyl ring of the mono-carbonyl analogs aims to reduce the hydrolytic effect on the molecule.[28,29]

*Corresponding author. E mail: nurul@upy.ac.id
Tel: +62-812-1526-2576

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Hexagamavunon (HGV) is a mono-carbonyl analog of curcumin. HGV and its analog have been reported to have activities against gram-positive and gram-negative bacteria, aerobic and anaerobic bacteria, and even have activity against *Mycobacterium tuberculosis*. HGV antibacterial activity studies have been carried out both *in-silico* and *in-vitro*.[30–34]

In silico studies, known as molecular docking, are carried out by docking a candidate drug molecule with a target receptor to determine drug-protein interactions. Drug-protein interactions can be used as a basis for predicting the candidate molecular activity and molecular affinity.[35,36] AutoDock Vina is one of the programs used in molecular docking. The advantage of this program is its high speed and accuracy.[37,38] This research explores molecular docking in determining the activity of HGV analogs as anti-*H. pylori* bacteria by blocking shikimate kinase and urease enzymes.

Materials and Methods

Instruments

The molecular docking used a computer with Intel Celeron N3150 1,60 GHz, ram 2GB, and a Windows 8 operating system. The molecular docking procedure was performed using AutoDock Vina software. AutoDock Tools 1.5.6., Open Babel, Marvin Sketch, and Discovery Studio (DS) Visualizer 2016 were used as supporting software.

Materials

Curcumin analog (Table 1):
A0: 2,6-bis(4-hydroxybenzylidene)cyclohexan-1-one
A1: 2,6-bis(4-hydroxy-3-methoxybenzylidene)cyclohexan-1-one
A2: 2,6-di-benzylidenecyclohexan-1-one
A4: 2,6-bis(4-methoxybenzylidene)cyclohexan-1-one
A6: 2,6-bis(4-(tert-butyl)benzylidene)cyclohexan-1-one
A7: 2,6-bis(4-(trifluoromethyl)benzylidene)cyclohexan-1-one
A8: 2,6-bis(4-(dimethylamino)benzylidene)cyclohexan-1-one
A9: 2,6-bis(3,4-dichlorobenzylidene)cyclohexan-1-one
A10: 2,6-bis(3-chlorobenzylidene)cyclohexan-1-one

A11: 2,6-bis(4-hydroxy-3,5-dimethylbenzylidene)cyclohexan-1-one
A15: 2,6-bis(4-hydroxy-3,5-dimethoxybenzylidene)cyclohexan-1-one
A16: 2,6-bis(3,5-dichloro-4-hydroxybenzylidene)cyclohexan-1-one
Shikimate kinase enzym (PDB ID: 3N2E) (Figure 1a), urease enzym (PDB ID: 1E9Y) (Figure 1b).

Protein and Ligand Preparation
Shikimate kinase (PDB ID: 3N2E) and urease (PDB ID: 1E9Y) proteins were obtained from the Protein Data Bank website (http://www.rcsb.org). The proteins were prepared (separated from the native ligand and other residues) using DS Visualizer software. The protein was saved in .pdb format. The 3N2E and 1E9Y protein contains three (A, B, C) chains and two (A, B) chains, respectively. The B chain from each protein was used for protein preparation. Native ligand (NL) was separated from the protein and saved in .pdb form. Ligand (A0 – A16) structures were drawn with Marvin Sketch and saved in .pdb form.

Validation of Molecular Docking
The validation procedure was performed to ensure the accuracy of the molecular docking procedure with AutoDock Vina. NL was re-docked to the binding site pocket of its protein. The parameter of the validation procedure was the root mean square deviation (RMSD) in amstrong (Å).

Molecular Docking and Analysis
Ligands (A0 – A16) were docked into the protein's binding pocket. The parameter of the docking procedure was binding energy (kcal/mol) between ligand and protein. The ligand's binding energy was compared with NL's binding energy.

Visualization of Protein-ligand Interaction
The interaction of ligand and protein was observed with DS Visualizer software. This interaction was studied to understand the binding of compounds with amino acid residues of the protein. The output of the docking process was in .pdb format for easy visualization.

Results and Discussion

Protein and Ligand Preparation
Protein preparation was processed by separating the protein from the native ligand and other residues. The B chain from each protein was used as a target in the docking process (Figure 2). The NL of 3N2E is 7-amino-4-hydroxy-3-[(E)-(5-hydroxy-7-sulfonaphthalen-2-yl)diazenyl]naphthalene-2-sulfonic acid (Figure 3a) while the NL of 1E9Y is acetohydroxamic acid (Figure 3b). The structure of HGV and its analog were acquired from previous studies.[22]

Validation of Molecular Docking
NL was re-docked to the binding site pocket of its protein in the validation procedure with RMSD as the parameter. The RMSD of SK and urease was 1.787 Å & 1.689 Å, respectively. SK and urease can be used in molecular docking procedures because of the value of their RMSD < 2 Å.[39] In the molecular docking process, ligand and control were docked to the binding site of SK and urease.

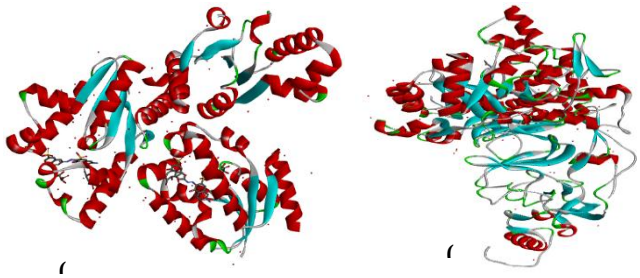
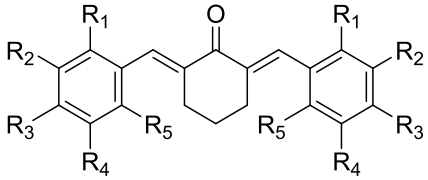


Figure 1: Crystal structure of *Helicobacter pylori* (a) shikimate kinase in complex with NSC162535 (PDB ID: 3N2E) (b) urease in complex with acetohydroxamic acid (PDB ID: 1E9Y)

Table 1: Structure of HGV analog[22]



Compounds	R_1	R_2	R_3	R_4	R_5
A0	H	H	OH	H	H
A1	H	OCH_3	OH	H	H
A2	H	H	H	H	H
4	H	H	OCH_3	H	H
A6	H	H	*t*-C_4H_9	H	H
A7	H	H	CF_3	H	H
A8	H	H	$N(CH_3)_2$	H	H
A9	H	Cl	Cl	H	H
A10	H	Cl	H	H	H
A11	H	CH_3	OH	CH_3	H
A15	H	OCH_3	OH	OCH_3	H
A16	H	Cl	OH	Cl	H

Molecular docking

Determining whether a compound has potential as a drug molecule starts with knowing it meets Lipinski's rule of five. These rules evaluate the physical, chemical, and similarity of compounds with drugs, which can be used to predict their pharmacological and biological activities. Lipinski's rule of five states that an orally active molecule should not violate more than one of the following criteria (1) has a molecular weight of less than 500 Da, (2) logP value of less than 5, (3) the number of hydrogen bond donors is less than 5, and (4) the number of hydrogen bond acceptors less than 10.[40,41] Previously, all compounds have been selected based on Lipinski's rule of five (RO5). The ligand used in this study meets at least three rules of RO5, while NL of SK only meets two (Table 2). Previous research has confirmed that if a compound does not have more than two RO5, it will affect the solubility and permeability of the molecule.[42]

The first-line treatment of *H. pylori* infection is the Proton Pump Inhibitor (PPI) group, combined with antibiotics for 10-14 days.[9–11] Broad-spectrum first and second-line antibiotics in *H. pylori* therapy have a mechanism of action inhibiting DNA synthesis and cell replication, inhibiting protein, and cell wall synthesis. Future perspective of anti-*H. pylori* drugs are to inhibit virulence factors, metabolism routes, and pH control pathways.[1,10,12]

The shikimate pathway plays a role in the biosynthetic of aromatic amino acids present in bacteria, fungi, and plants but not in mammals. This pathway consists of seven steps, where a different enzyme catalyzes each step. The enzymes that play a role in this pathway are 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase, 3-dehydrogenate synthase, 3-dehydroquinate dehydratase/shikimate dehydrogenase, shikimate kinase, 5- enolpyruvylshikimate-3-phosphate synthase, and chorismate synthase.[17,43] Shikimate kinase is an enzyme that catalyzes the phosphorylation of shikimic acid, the fifth step of the shikimate pathway. This enzyme has become a new target of broad-spectrum anti-H pylori drugs.[1] The result of molecular docking show that the binding energy of SK with ligands A6, A7, and A10 is lower than the binding energy of NL (Table 3). The binding energy of ligands A6, A7, and A10 are -10.7 kcal/mol; -9.9 kcal/mol; and -9.9 kcal/mol, respectively, while the binding energy of NL is -9.8 kcal/mol.

Urease is an enzyme that is responsible for the ability of *H. pylori* to survive in conditions of gastric fluid with low acidity (pH). *H. pylori* produce large amounts of urease in the cytoplasm and on the cell surface. Urease plays a vital role in the hydrolysis reaction of urease to ammonia. The presence of ammonia will neutralize the acidity of the gastric fluid. This enzyme becomes the new target of new broad-spectrum anti-*H. pylori*.[1,14,18,44] The urease binding energy with all ligands is lower than all the NL (Table 3). The ligands with the lowest binding energy to urease are A6, A7, and A10, which are -7.5 kcal/mol, -8.1 kcal/mol, and -7.7 kcal/mol.

In molecular docking, the parameters observed are the binding energy between the ligand and the target protein. This bond energy shows the stability of the complex between the ligand and protein. Low binding energy values indicate that the ligand-protein complex formed is more stable. The interaction of ligands and proteins, in the form of hydrogen bonds and Van der Waals, has a role in determining the value of binding energy.[45–50] In this study, A6, A7, and A11 ligands possess more potential to block SK enzymes than other ligands, while all ligands show the potential to inhibit the urease enzyme.

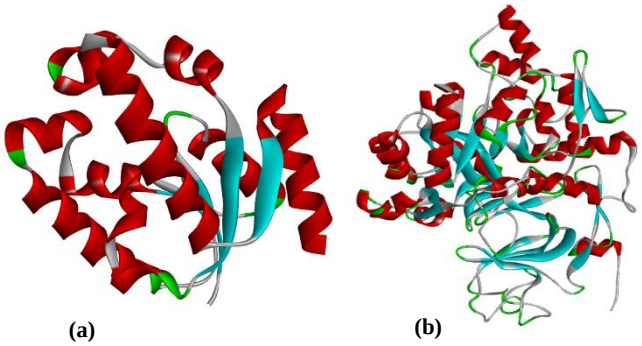
Table 2: The RO5 of ligands

Ligand	Molecular Formula	Molecular Weight (<500 Da)	LogP (<5)	H-Bond Donor (<5)	H-Bond Acceptor (<10)	Violation	Meet RO5
A0	$C_{20}H_{18}O_3$	306.3551	4.99	2	3	0	Yes
A1	$C_{22}H_{22}O_5$	366.4071	4.67	2	5	0	Yes
A2	$C_{20}H_{18}O$	274.3563	5.6	0	1	1	Yes
A4	$C_{22}H_{22}O_3$	334.4083	5.28	0	3	1	Yes
A6	$C_{28}H_{34}O$	386.569	8.69	0	1	1	Yes
A7	$C_{22}H_{16}F_6O$	401.3523	7.35	0	7	1	Yes
A8	$C_{24}H_{28}N_2O$	360.4919	5.81	0	3	1	Yes
A9	$C_{20}H_{14}Cl_4O$	412.137	8.01	0	5	1	Yes
A10	$C_{20}H_{16}Cl_2O$	343.246	6.81	0	3	1	Yes
A11	$C_{24}H_{26}O_3$	362.4614	7.04	2	3	1	Yes
A15	$C_{24}H_{26}O_7$	426.459	4.36	2	7	0	Yes
A16	$C_{20}H_{14}Cl_4O_3$	444.135	7.41	2	7	1	Yes

Table 3: Molecular docking result of shikimate kinase and urease

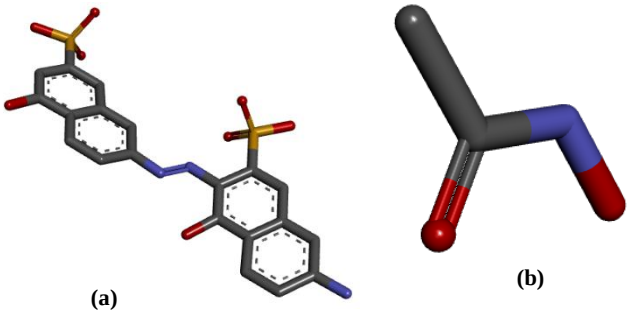
Ligand	Shikimate Kinase (SK)			Urease		
	RMSD (Å)	Binding Energy (kcal/mol)		RMSD (Å)	Binding Energy (kcal/mol)	
NL	1.787	-9.8		1.689	-3.4	
A0	1.390	-7.4		0.675	-6.5	
A1	1.203	-7.3		1.333	-7.4	
A2	1.535	-7.4		1.752	-6.5	
A4	0.566	-8.1		0.903	-6.7	
A6	0.128	-10.7		1.338	-7.5	
A7	0.910	-9.9		0.254	-8.1	
A8	1.129	-7.9		0.334	-7.0	
A9	1.594	-8.9		1.395	-7.4	

A10	1.160	-8.5	1.136	-6.9
A11	0.875	-9.9	1.381	-7.7
A15	0.263	-8.3	1.497	-7.0
A16	0.267	-9.0	1.866	-7.1



(a) **(b)**

Figure 2: Structure of B chain of (a) shikimate kinase (b) urease



(a) **(b)**

Figure 3: Structure of (a) 7-amino-4-hydroxy-3-[(E)-(5-hydroxy-7-sulfonaphthalen-2-yl)diazenyl]naphthalene-2-sulfonic acid (b) acetohydroxamic acid

Interaction of Protein-ligand

The ligand-protein interactions show that A6, A7, and A11 form bonds with the amino acid residue of SK, which is the binding site of the native ligand (Table 4). Ligands A6 and A11 interact with 5 of the same amino acids, ARG116, ARG132, MET10, PHE48, and PHE9, while A7 interacts with 3 of the same amino acids, ARG116, PHE48, and PHE9. Ligands A7 and A11 form 2 hydrogen bonds with SK proteins with a distance of 3.75 & 3.31 Å (A7) and 2.86 & 3.25 Å (A11). A6 ligands form 3 hydrogen bonds at 2.85, 4.12, and 3.28 Å.

The results of ligand-protein interactions on urease showed no similarity between amino acids bound to the original ligand and the test ligand (Table 4). Native ligands form bonds with two amino acids (ALA365; ASP362) and three nickel metals. A6 ligand interacts with four amino acid residues and forms one hydrogen bond with a distance of 3.14 Å. A7 ligands interact with nine amino acid residues of urease, four forming hydrogen bonds with a range of 3.15, 3.92, 3.17 and 3.20 Å. A11 ligands interact with seven amino acid residues, and three form hydrogen bonds with a distance of 4.15, 3.23, and 3.29 Å.

Interactions between proteins and ligands occur in various types of bonds. Bonds that have a significant role in determining the binding energy of ligand-protein are the hydrogen and Van der Waals bonds. The number and distance of hydrogen and Van der Waals bonds determine the low strength of the ligand-protein binding energy.[45–51] In SK interactions with ligands, A6 shows the lowest binding energy because it has more hydrogen bonds and shorter distances than other ligands.

Conclusion

This paper reported the HGV analog employed in the *in silico* investigation of SK and urease enzymes in *H. pylori* bacteria. Analogs A6, A7, and A11 showed the highest potential to inhibit *H. pylori* bacteria by inhibiting the SK and urease enzymes from the molecular docking results. However, further *in vitro* studies are necessary to validate the potential of HGV analogs as anti-*H. Pylori* drugs.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

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Table 4: Interaction of ligand-protein of shikimate kinase and urease

Ligand	Amino acid involved in the interaction	
	Shikimate Kinase (SK)	**Urese**
NL	ARG116; ARG132; ARG57; GLY80; MET10; PHE48; PHE9	ALA365; ASP362; HIS248; NI3001; NI3001; NI3002
A0	ARG45; ASP33; LYS111; LYS14; MET10; PHE48; SER15; VAL44	ALA185; MET182; PRO142; TYR189
A1	ARG116; LYS115; PHE48; PRO117	ALA563 ; ASN309; ASN558; GLN378; GLN564; GLU371; SER151; SER567; VAL444; VAL560
A2	ARG116; MET10; PHE48; VAL44	ALA563; GLN378; GLU371; GLN564; THR307; VAL560

Ligand	Amino acid involved in the interaction	
	Shikimate Kinase (SK)	**Urese**
A4	ASP33; GLY80; LYS115; MET10; PHE48	ALA563; ARG375; GLU371; SER151; THR307; VAL560
A6	ARG116; ARG132; LEU119; LYS115; MET10; PHE48; PHE9	ALA563; GLU371; PHE569; VAL369
A7	ALA125; ARG116; ARG45; ILE105; LEU119; PHE101; PHE129; PHE48; PHE9	ALA557; ALA563; ASN309; ASN558; GLN378; GLU371; LYS559; SER151; VAL560
A8	ARG45; LYS115; MET10; PHE48; VAL44	ALA563; ARG368; GLN378; GLU371; PHE569
A9	ARG116; ILE105; LEU104; LEU108; LEU119; MET10; PHE48; PHE9; VAL44	ALA563; GLN378; GLU371; VAL560
A10	ARG116; ARG132; GLY80; LYS115; MET10; MET84; PHE48; PHE9; TYR136; VAL83	ALA563; GLN378; GLU371; LYS445; LYS559; SER567; VAL560
A11	ARG116; ARG132; LEU104; LEU108; LEU119; LEU128; MET10; PHE48; PHE9	ALA563; GLN378; GLU371; GLY370; SER151; VAL444; VAL560
A15	ARG116; ARG45; ARG57; GLU53; GLY80; GLY81; LYS115; LYS115; MET10; PHE48	ALA563; ASN309; GLN564; GLU371 ; PHE569; SER151
A16	ARG116; ARG57; ASP33; GLY80; LYS115; MET10; PHE48; VAL44	ALA563; GLU371; LEU562; LYS445; PRO305; SER151; VAL560

References

1. Debraekeleer A, Remaut H. Future perspective for potential *Helicobacter pylori* eradication therapies. Future Microbiol. 2018;13(6):671–87.
2. Burkitt MD, Duckworth CA, Williams JM, Pritchard DM. *Helicobacter pylori*-induced gastric pathology: Insights from *in vivo* and *ex vivo* models. DMM Dis Model Mech. 2017;10(2):89–104.
3. Diaconu S, Predescu A, Moldoveanu A, Pop CS, Fierbinţeanu-Braticevici C. *Helicobacter pylori* infection: old and new. J Med Life [Internet]. 2017;10(2):112–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28616085%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5467250
4. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in *Helicobacter pylori*: A Systematic Review and Meta-analysis in World Health Organization Regions. Gastroenterology [Internet]. 2018;155(5):1372-1382.e17. Available from: https://doi.org/10.1053/j.gastro.2018.07.007
5. Moss SF. The Clinical Evidence Linking *Helicobacter pylori* to Gastric Cancer. Cmgh. 2017;3(2):183–91.
6. Lee YC, Chiang TH, Chou CK, Tu YK, Liao WC, Wu MS, Graham DY. Association between *Helicobacter pylori* Eradication and Gastric Cancer Incidence: A Systematic Review and Meta-analysis. Gastroenterology. 2016;150(5):1113–24.
7. Kuo YT, Liou JM, El-Omar EM, Wu JY, Leow AHR, Goh KL, Das R, Lu H, Lin JT, Tu YK, Yamaoka Y, Wu MS. Primary antibiotic resistance in *Helicobacter pylori* in the Asia-Pacific region: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2017;2(10):707–15.
8. Bilgilier C, Stadlmann A, Makristathis A, Thannesberger J, Kastner MT, Knoflach P, Steiner, P, Schöniger-Hekele M. Högenauer C, Blesl A, Datz C, Huber-Schönauer U, Schöfl R, Wewalka F, Püspök A, Mitrovits N, Leiner J, Tilg H, Effenberger M, Moser M, Siebert F, Hinterberger I, Wurzer H, Stupnicki T, Watzinger N, Gombotz G, Hubmann, Klimpel S, Biowski-Frotz S, Schrutka-Kölbl C, Graziadei I, Ludwiczek O, Kundi M, Hirschl AM, Steininger C. Prospective multicentre clinical study on inter- and intrapatient genetic variability for antimicrobial resistance of *Helicobacter pylori*. Clin Microbiol Infect. 2018;24(3):267–72.
9. Chey WD, Leontiadis GI, Howden CW, Moss SF. ACG Clinical Guideline: Treatment of *Helicobacter pylori* Infection. Am J Gastroenterol. 2017;112(2):212–38.
10. Talebi Bezmin Abadi A, Yamaoka Y. *Helicobacter pylori* therapy and clinical perspective. J Glob Antimicrob Resist. 2018;14:111–7.
11. Peng C, Hu Y, Ge Z-M, Zou Q-M, Lyu N-H. Diagnosis and treatment of *Helicobacter pylori* infections in children and elderly populations. Chronic Dis Transl Med. 2019;5(4):243–51.
12. Baltas N, Karaoglu SA, Tarakci C, Kolayli S. Effect of propolis in gastric disorders: inhibition studies on the growth of *Helicobacter pylori* and production of its urease. J Enzyme Inhib Med Chem. 2016;31:46–50.
13. Savoldl A, Carrara E, Graham D, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in Helicobcater pylori: Revisón sistemática y Metaanálisis en las Regiones de la OMS. Gastroenterology. 2018;155(5):1372–82.
14. Zhou JT, Li CL, Tan LH, Xu YF, Liu YH, Mo ZZ, Dou YX, Su R, Su ZR, Hunag P, Xie JH. Inhibition of *Helicobacter pylori* and its associated urease by Palmatine: Investigation on the potential mechanism. PLoS One. 2017;12(1):1–15.
15. Prado V, Lence E, Maneiro M, Vázquez-Ucha JC, Beceiro A, Thompson P, Hawkins AR, González-Bello C. Targeting the Motion of Shikimate Kinase: Development of Competitive Inhibitors that Stabilize an Inactive Open Conformation of the Enzyme. J Med Chem. 2016;59(11):5471–87.
16. Tarsia C, Danielli A, Florini F, Cinelli P, Ciurli S, Zambelli B. Targeting *Helicobacter pylori* urease activity and maturation: In-cell high-throughput approach for drug discovery. Biochim Biophys Acta - Gen Subj. 2018;1862(10):2245–53.
17. Francenia Santos-Sánchez N, Salas-Coronado R, Hernández-Carlos B, Villanueva-Cañongo C. Shikimic Acid Pathway in Biosynthesis of Phenolic Compounds. Plant Physiol Asp Phenolic Compd. 2019;1–15.

18. Ansari S, Yamaoka Y. Survival of *Helicobacter pylori* in gastric acidic territory. *Helicobacter*. 2017;22(4):1–13.
19. Golonko A, Lewandowska H, Świsłocka R, Jasińska UT, Priebe W, Lewandowski W. Curcumin as tyrosine kinase inhibitor in cancer treatment. Eur J Med Chem. 2019;181.
20. Meiyanto E, Septisetyani EP, Larasati YA, Kawaichi M. Curcumin analog pentagamavunon-1 (PGV-1) sensitizes widr cells to 5-fluorouracil through inhibition of NF-κB activation. Asian Pacific J Cancer Prev. 2018;19(1):49–56.
21. Kaladhar D, Banjara T, Kant S, Tiwari Mishra S, Kumari Dupplala S. In Silico Screening of Compounds From Turmeric (Curcuma Longa L.) Against Cancer Causing Proteins. Int J Curr Trends Eng Technol www.ijctet.org [Internet]. 2018;01:2395–3152. Available from: www.ijctet.org,
22. Wijianto B, Purnomo H, Nurrochmad A. Qsar And Synthesis Of Curcumin Analogues As Antibacterial. 2018;17(8):72–82.
23. Vetvicka V, Vetvickova J, Fernandez-Botran R. Effects of curcumin on *Helicobacter pylori* infection. Ann Transl Med. 2016;4(24):1–7.
24. Ranjbar R, Mohammadi A. Synergistic effects of combined curcumin and antibiotic in ameliorating an animal model of *Helicobacter pylori* infection. Biomed Res. 2018;29(8):1702–7.
25. Banuppriya G, Sribalan R, Padmini V. Evaluation of Antioxidant, Anti-Inflammatory, Antibacterial Activity and In Silico Molecular Docking Study of Pyrazole Curcumin Bisacetamide Analogs. ChemistrySelect. 2017;2(28):9168–73.
26. Shrivash MK, Mishra S, UpmaNarain, Pandey J, Misra K. In-silico designing, chemical synthesis, characterization and in-vitro assessment of antibacterial properties of some analogues of curcumin. Microb Pathog [Internet]. 2018;123(May):89–97. Available from: https://doi.org/10.1016/j.micpath.2018.06.030
27. Zorofchian Moghadamtousi S, Abdul Kadir H, Hassandarvish P, Tajik H, Abubakar S, Zandi K. A review on antibacterial, antiviral, and antifungal activity of curcumin. Biomed Res Int. 2014;2014.
28. Khor PY, Aluwi MFFM, Rullah K, Lam KW. Insights on the synthesis of asymmetric curcumin derivatives and their biological activities. Eur J Med Chem. 2019;183.
29. Noureddin, Sawsan A., El-Shishtawy RM, Al-Footy KO. Curcumin analogues and their hybrid molecules as multifunctional drugs. Eur J Med Chem. 2019;182.
30. Wijianto B, Ritmaleni, Purnomo H, Nurrochmad A. in Silico and in Vitro Assay of Hgv Analogue As Antibacterial. Int J Pharm Pharm Sci. 2019;11(3):78–85.
31. Safitri CINH, Ritmaleni, Rintiswati N, Sardjiman, Kaneko T. Evaluation of benzylidene-acetone analogues of curcumin as antituberculosis. Asian J Pharm Clin Res. 2018;11(4):226–30.
32. Safitri CINH, Rintiswati N, Kaneko T. Antimycobacterial Activity of Benzylidene Acetone Analogues on Curcumin Against Resistant And Sensitive Mycobacterium Tuberculosis. IOSR J Dent Med Sci. 2017;16(12):21–6.
33. Rahmania TA, Ritmaleni R, Setyowati EP. *In silico* and *in vitro* assay of Hexagamavunon-6 analogs, Dibenzilyden-N-Methyl-4-piperidone as antibacterial agents. J Appl Pharm Sci. 2020;10(3):39–43.
34. Wardani AK, Ritmaleni, Setyowati EP. Molecular Docking Studies Of HGV-6 Analogue As A Potential PBP-1A Inhibitor. Int J Pharm Pharm Sci. 2020;12(4):8–12.
35. Du B-X, Qin Y, Jiang Y-F, Xu Y, Yiu S-M, Yu H, Shin J-Y. Compound–protein interaction prediction by deep learning: Databases, descriptors and models. Drug Discov Today. 2022;27(5):1350–66.
36. Wang M, Liu Y, Liu Y, Xia Z. MOFs and PDA-supported immobilization of BSA in open tubular affinity capillary electrochromatography: Prediction and study on drug-protein interactions. Talanta. 2022;237.
37. Vieira TF, Sousa SF. Comparing AutoDock and Vina in ligand/decoy discrimination for virtual screening. Appl Sci (S. 2019;9(21):1-18.
38. Yasman S, Yanuar A, Tamimi Z, Riadhi SR. In Silico Analysis of Sea Cucumber Bioactive Compounds as Anti-Breast Cancer Mechanism Using AutoDock Vina. Iran J Pharm Sci. 2020;16(1):1–8.
39. Sokalingam S, Munussami G, Kim JR, Lee SG. Validation on the molecular docking efficiency of lipocalin family of proteins. J Ind Eng Chem. 2018;67:293–300.
40. Chagas CM, Moss S, Alisaraie L. Drug metabolites and their effects on the development of adverse reactions: Revisiting Lipinski's Rule of Five. Int J Pharm. 2018;549(1–2):133–49.
41. Huang H, Chu CL, Chen L, Shui D. Evaluation of potential inhibitors of squalene synthase based on virtual screening and *in vitro* studies. Comput Biol Chem. 2019;80:390–7.
42. Benet LZ, Hosey CM, Ursu O, Oprea TuI. BDDCS, the Rule of 5 and Drugability. Adv Drug Deliv Rev. 2016;101:89–98.
43. Goyal M, Chauhan S, Goyal P, Prabha J. Structural modeling of shikimate pathway enzymes for herbicide and drug development : A review. J Entomol Zool Stud. 2018;6(2):785–90.
44. Graham DY, Miftahussurur M. *Helicobacter pylori* urease for diagnosis of *Helicobacter pylori* infection: A mini review. J Adv Res. 2018;13:51–7.
45. de Souza AS, Pacheco BDC, Pinheiro S, Muri EMF, Dias LRS, Lima CHS, Garrett R, de Moraes MBM, de Souza BEG, Puzer L. 3-Acyltetramic acids as a novel class of inhibitors for human kallikreins 5 and 7. Bioorganic Med Chem Lett [Internet]. 2019;29(9):1094–8. Available from: https://doi.org/10.1016/j.bmcl.2019.02.031
46. Al-Karmalawy AA, Dahab MA, Metwaly AM, Elhady SS, Elkaeed EB, Eissa IH, Darwish KM. Molecular Docking and Dynamics Simulation Revealed the Potential Inhibitory Activity of ACEIs Against SARS-CoV-2 Targeting the hACE2 Receptor. Front Chem. 2021;9(May).
47. Rahayu S, Prasetyawan S, Suprihatin T, Ciptadi G. *In-silico* study of Marselia crenata compounds as activator Keap1/Nrf2 pathway in ovarian function. IOP Conf Ser Earth Environ Sci. 2021;743(1).
48. Kanagavalli U, Deboral E, Lakshmipriya MD, Sadiq AM, Priya AM. In silico Molecular Docking of Anthraquinone Identified from Boerhavia diffusa Linn against Bax and Bcl-2 Gene. J Pharm Res Int. 2021;33:352–9.
49. Singh R, Bhardwaj VK, Sharma J, Purohit R, Kumar S. In-silico evaluation of bioactive compounds from tea as potential SARS-CoV-2 nonstructural protein 16 inhibitors. J Tradit Complement Med [Internet]. 2022;12(1):35–43. Available from: https://doi.org/10.1016/j.jtcme.2021.05.005
50. Martiz RM, Patil SM, Abdulaziz M, Babalghith A, Al-Areefi M, Al-Ghorbani M, Kumar JM, Prasad A, Nagalingaswamy NPM, Ramu R. Defining the Role of Isoeugenol from Ocimum tenuiflorum against Diabetes Mellitus-Linked Alzheimer's Disease through Network Pharmacology and Computational Methods. Molecules. 2022;27(8):1-21.
51. Sundari S, Mas'ud A, Sari DRT. Molecular Docking Discovered Potential of Cyclooxygenase – 2 Inhibitor Activity of Oily Compounds of Walnuts. Trop J Nat Prod Res. 2022;6(12):1947–52.