Pharmacy Education (2023) 23(4) 196 - 202
https://doi.org/10.46542/pe.2023.234.196202

ICMHS 2022 SPECIAL EDITION

RESEARCH ARTICLE

Investigating the anti-allergic activity of *Phyllanthus niruri* via MALT1 protease inhibition: An in silico approach

Honey Dzikri Marhaeny[1], Alma Nuril Aliyah[4], Andang Miatmoko[3], Junaidi Khotib[2]

[1] Doctoral Program of Pharmaceutical Science, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia
[2] Department of Pharmacy Practice, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia
[3] Department of Pharmaceutical Science, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia
[4] Master Program of Pharmaceutical Science, Faculty of Pharmacy, Airlangga University, Surabaya

Keywords
Anti-allergic agent
Immune system
In silico
MALT1 protease
Phyllantus niruri

Correspondence
Junaidi Khotib
Department of Pharmacy Practice
Faculty of Pharmacy
Airlangga University
Indonesia
junaidi-k@ff.unair.ac.id

Abstract

Background: Allergic inflammation is a condition caused by complex interactions between several inflammatory cells in the body. Mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) is a cysteine protease that bridges innate and adaptive immune responses in allergic inflammation. MALT1 protease inhibition is a potential strategy for controlling IgE-mediated allergic disease. *P. niruri* is a medicinal plant with anti-allergic properties. **Objective**: This study aimed to evaluate the anti-allergic activity of *P. niruri* secondary metabolites against MALT1 protease through in silico approach. **Method:** Physicochemical properties and drug-likeness evaluation were determined using SwissADME. The toxicity properties prediction was analysed using pkCSM. AutoDock Vina was used to evaluate the best binding energy of the compounds against the receptor (PDB ID: 3V4O and 4I1R). Visualisation was obtained using Biovia discovery studio visualiser. **Result:** Docking analysis showed that ten of 21 compounds have lower binding affinity than the native ligand and reference drugs at the 3V4O receptor. At the 4I1R receptor, only three compounds have a lower binding affinity than its native ligand, but only one compound has a lower binding affinity than all reference drugs. **Conclusion:** Several *P. niruri* secondary metabolites were potentially predicted to be developed as MALT1 protease inhibitory agents.

Introduction

The prevalence of allergic diseases has increased and reached up to 10% of the world's population (Wai *et al.,* 2021). Allergic inflammation can occur after persistent or repeated allergen exposure, leading to several other allergic diseases (Galli *et al.,* 2008). Therefore, therapeutic management for allergic inflammation is limited to temporary symptomatic treatment. In contrast, long-term treatment for the allergic disease can use allergen-specific immunotherapy (AIT) that induces immune tolerance to allergens (Pawankar, 2014). Nowadays, studies of AIT response have prompted the development of new therapeutic targets for treating allergic diseases. MALT1 (mucosa-associated lymphoid tissue lymphoma translocation protein 1) is an intracellular signalling protein crucial in innate and adaptive immunity. MALT1 contributes to late-phase hypersensitivity reactions by activating the NF-κB signalling pathway to upregulate IgE-mediated proinflammatory cytokines, leading to T-cell activation (Demeyer *et al.,* 2019). Thus, developing novel drugs targeting the MALT1 protease is a potential strategy for allergy treatment.

Phyllanthus niruri has been widely known in traditional medicine due to its various pharmacological activities, including anti-inflammation and anti-allergic (Marhaeny *et al.,* 2021). Recently, *P. niruri* was shown to have anti-asthmatic properties due to its role in reducing the number of mast cell degranulation (Mukherjee & Gupta, 2018). According to other studies,

hypophyllanthin found in *P. amarus* and also in *P. niruri* has anti-allergic activity by preventing H_1 receptor activation (Abd Rani *et al.,* 2021). Additionally, *P. niruri* secondary metabolites have been shown to inhibit TNF-α, IL-1, and IL-6 by inactivating NF-κB (Jantan *et al.,* 2019). Histamine and cytokines are inflammatory mediators released following mast cell degranulation (Marhaeny *et al.,* 2023).

Based on this evidence and the lack of safe and effective treatment for allergic inflammatory diseases, the development of novel anti-allergic agents derived from *P. niruri* is promising. Therefore, in order to evaluate the anti-allergy activity of *P. niruri* secondary metabolites against MALT1 protease, which regulates allergic inflammatory responses, in silico screening using molecular docking was performed.

Methods

Protein and ligand preparation

Protein structures targeted for docking were obtained from the RCSB PDB. The proteins used MALT1 protease with PDB ID: 3V4O (chain A) and 4I1R (chain A). Protein preparation was performed using autodock tools v1.5.7. Docking protocol validation was analysed using PyMOL software v4.6.0 (Schrödinger LLC). Secondary metabolites of *P. niruri* and reference drugs obtained from the Knapsack Family (KnapSack Family) and Pubchem. OpenBabel (online access) is used for ligand preparation. SwissADME was performed to evaluate the physicochemical properties and drugability of the compound. The pkCSM was used to analyse these compound's toxicity properties.

Virtual screening, molecular docking, and visualisation

Virtual screening was performed using an Intel Core i3-10110U a 2.59 GHz processor and 4.00 GB of RAM 64. bit operating system. The grid box for the 3V4O receptor was 18, Å×18, Å×18 Å, centred at -19.186, 5.408, 13.302, while 4I1R was 15, Å×15, Å×15Å, centred at -28.703, -16.188, 20.796. The docking protocol validity is accepted if the value of root means square deviation (RMSD) ≤ 2.0 Å. This study showed RMSD of the 3V4O receptor was 1.6 Å and 4I1R was 1.5Å. Molecular docking was performed using Cygwin command for running AutoDock Vina and visualised using Biovia discovery studio visualiser v21.1.0.20298 software (Dassault Systèmes, San Diego, California, USA).

Results

Physicochemical properties, drugability, and safety evaluation of *P. niruri* secondary metabolites and reference drugs The results showed that 8 of 21 *P. niruri* secondary metabolites unmeet Lipinski's rule (Table I). In drug discovery, violation of RO5 leads to poor membrane permeation and BA (Lipinski *et al.,* 2001). However, this rule specifically states that RO5 is applied only to compounds that penetrate the cell membrane through passive diffusion. In this case, the permeability of passively diffused compounds can be predicted based on the number of RBN. The lower RBN indicates a better ability to cross the membrane (Yang & Hinner, 2015; Benet *et al.,* 2016).

The LD_{50} parameter is used to assess the relative toxicity of different molecules, while hepatotoxicity is associated with impaired normal liver function. The analysis using pkCSM showed that all compounds predicted to have no toxic effect on the hepatotoxicity model except mepazine, MI-2, and loratadine– and acute toxicity tests, which indicated that these compounds could have a good safety (Table I).

Table I: Physicochemical properties, drugability, and safety prediction of the compounds

Compounds	Physicochemical properties				RBN	Drug-likeness evaluation			Toxicity prediction	
	MW (g/mol)	HBA	HBD	mLogP (Log $P_{o/w}$)		BA score	Violation	RO5	LD_{50} (mol/kg)	Hepato-toxicity
	≤ 500	≤ 10	≤ 5	≤ 4.15						
References										
Mepazine	310.46	1	0	4.31	2	0.55	1	Yes	2.82	Yes
MI-2	455.72	5	1	3.51	9	0.55	0	Yes	2.25	Yes
Methylprednisolone	374.47	5	3	1.52	2	0.55	0	Yes	2.14	No
Loratadine	382.88	3	10	3.72	3	0.55	0	Yes	2.85	Yes
***P. niruri* secondary metabolites**										
4-Methoxynor-securinine	233.26	4	0	1.10	1	0.55	0	Yes	2.19	No

Compounds	Physicochemical properties					Drug-likeness evaluation			Toxicity prediction	
	MW (g/mol)	HBA	HBD	mLogP (Log $P_{o/w}$)	RBN	BA score	Violation	RO5	LD_{50} (mol/kg)	Hepato-toxicity
	≤ 500	≤ 10	≤ 5	≤ 4.15						
Astragalin	448.38	11	7	-2.10	4	0.17	2	No	2.55	No
beta-Sitosterol	414.71	1	1	6.73	6	0.55	1	Yes	2.45	No
Eriodictin	434.39	10	6	-1.15	3	0.55	1	Yes	2.20	No
Fisetin 4'-glucoside	448.38	11	7	-2.10	4	0.17	2	No	2.23	No
Gallic acid	170.12	5	4	-0.16	1	0.56	0	Yes	2.48	No
Glucogallin	332.26	10	7	-2.29	4	0.55	1	Yes	2.42	No
Hinokinin	354.35	6	0	2.71	4	0.55	0	Yes	2.54	No
Hypophyllanthin	430.49	7	0	1.91	8	0.55	0	Yes	2.68	No
Kaempferol 4'-rhamnoside	432.38	10	6	-1.34	3	0.55	1	Yes	2.63	No
Lintetralin	400.46	6	0	2.23	7	0.55	0	Yes	2.61	No
Niranthin	432.51	7	0	1.91	12	0.55	0	Yes	2.60	No
Nirtetralin	430.49	7	0	1.91	8	0.55	0	Yes	2.62	No
Nirurin	664.65	15	9	-2.35	8	0.17	3	No	2.50	No
Pedunculagin	784.54	22	13	-2.60	0	0.17	3	No	2.51	No
Phyllanthin	418.52	6	0	2.43	13	0.55	0	Yes	2.47	No
Punigluconin	802.56	23	14	-2.97	9	0.11	3	No	2.60	No
Quercetin	302.24	7	5	-0.56	1	0.55	0	Yes	2.32	No
Quercetin-3-O-glucoside	464.38	12	8	-2.59	4	0.17	2	No	2.55	No
Quercitrin	448.38	11	7	-1.84	3	0.17	2	No	2.70	No
Rutin	610.52	16	10	-3.89	6	0.17	3	No	2.48	No

MW = Molecular weight, HBA = Hydrogen bond acceptors, HBD = Hydrogen bond donor, RBN = Rotatable bonds (RBN), BA = Bioavailability, RO5 = Rule of five, and LD_{50} = Lethal dose 50.

Docking analysis of P. niruri secondary metabolites against MALT1 protease receptor

The top ten molecular docking results of *P. niruri* secondary metabolites and four selected drug molecules (mepazine, MI-2, methylprednisolone, and loratadine) against 3V4O and 4I1R receptors are shown in Table II. The receptor-ligand interaction was determined by ΔG value, compared to the native ligand. The Ki parameter specifies the ligand concentration required to inhibit the protein target. The results showed that as many as ten of 21. *P. niruri* secondary metabolites had lower binding affinity than the native ligand (peptidic inhibitor Z-VRPR-fmk) and reference drugs, at the 3V4O receptor.

Meanwhile, at 4I1R, only three compounds showed a lower binding affinity than their native ligand (thioridazine). However, only hinokinin has a lower binding affinity than all reference drugs (Table II). In addition, a good linear correlation was also observed between ΔG and Ki parameters, leading to reliable molecular docking results. The receptor-ligand intermolecular interactions are shown in Figure 1 and Figure 2.

Table II: The top 10 hits of *P. niruri* secondary metabolites docking scores against MALT1 protease

Compounds	Reseptor 3V4O		Compounds	Reseptor 4I1R	
	ΔG (kcal/mol)	K_i (μM)		ΔG (kcal/mol)	K_i (μM)
References					
Peptidic inhibitor Z-VRPR-fmk	-7.7	2.6	Thioridazine	-8.0	1.3
MI-2	-7.2	5.2	Mepazine	-8.6	0.5
Methylprednisolone	-6.1	33.4	Methylprednisolone	-7.4	3.7
Loratadine	-7.5	3.1	Loratadine	-7.2	5.2
Top ten docking results of *P. niruri* secondary metabolites					
Astragalin	-9.1	0.2	Hinokinin	-8.8	0.3
Eriodictin	-8.7	0.4	Astragalin	-8.4	0.7
Punigluconin	-8.7	0.4	Eriodictin	-8.2	1.0

Compounds	Reseptor 3V4O		Compounds	Reseptor 4I1R	
	ΔG (kcal/mol)	K_i (μM)		ΔG (kcal/mol)	K_i (μM)
Kaempferol 4'-rhamnoside	-8.6	0.5	Kaempferol 4'-rhamnoside	-7.8	1.9
Hinokinin	-8.5	0.6	Rutin	-7.7	2.2
Rutin	-8.2	1.0	Lintetralin	-7.5	3.1
Fisetin 4'-glucoside	-8.1	1.1	Hypophyllanthin	-7.5	3.1
Quercitrin	-8.1	1.1	Nirurin	-7.3	4.4
Quercetin	-8.0	1.3	Fisetin 4'-glucoside	-7.2	5.2
Quercetin-3-O-glucoside	-7.9	1.6	4-Methoxynorsecurinine	-7.2	5.2

ΔG = binding affinity, K_i = inhibition constant

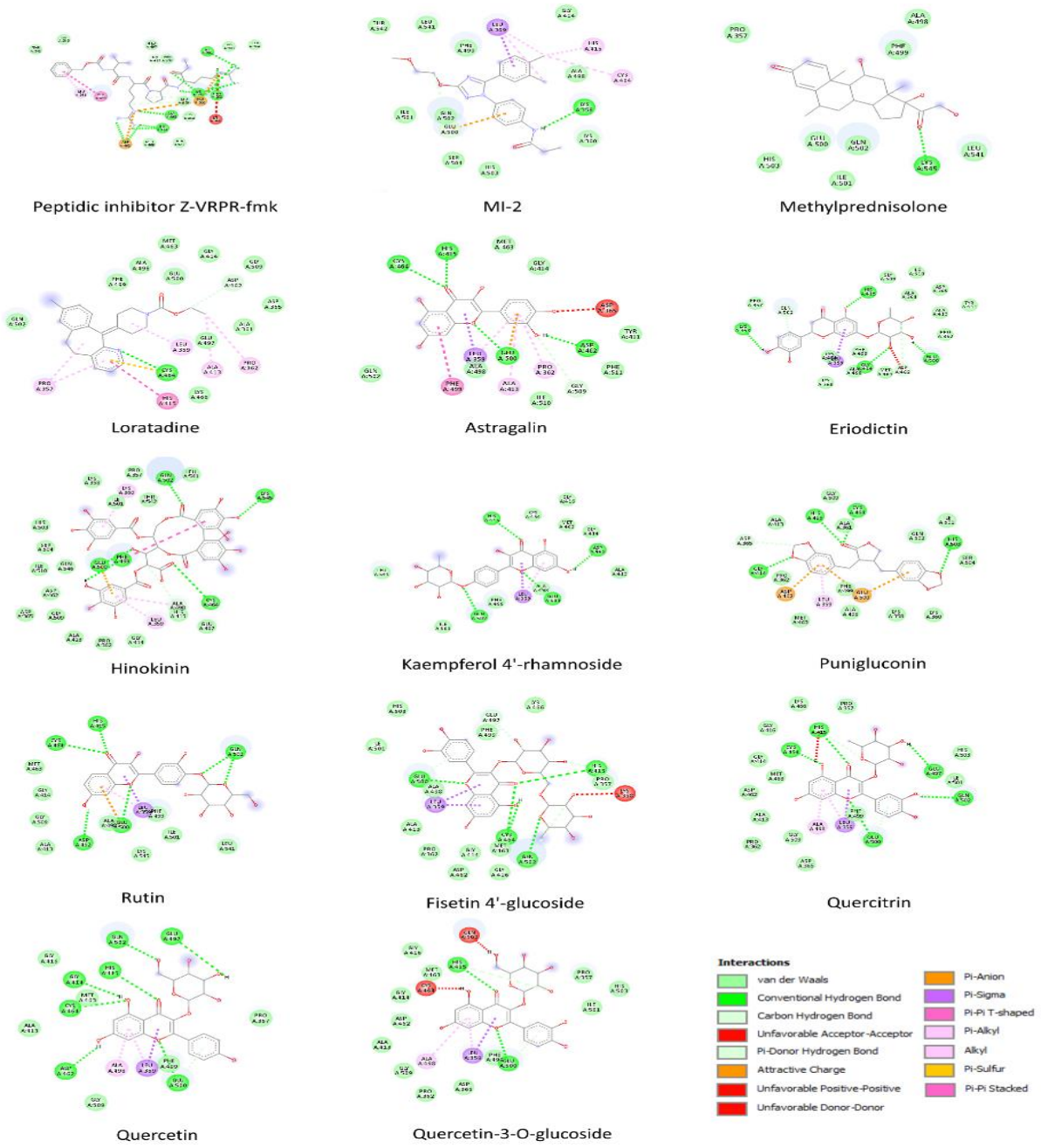

Figure 1: Intermolecular interaction of selected ligands docking toward 3V4O receptor

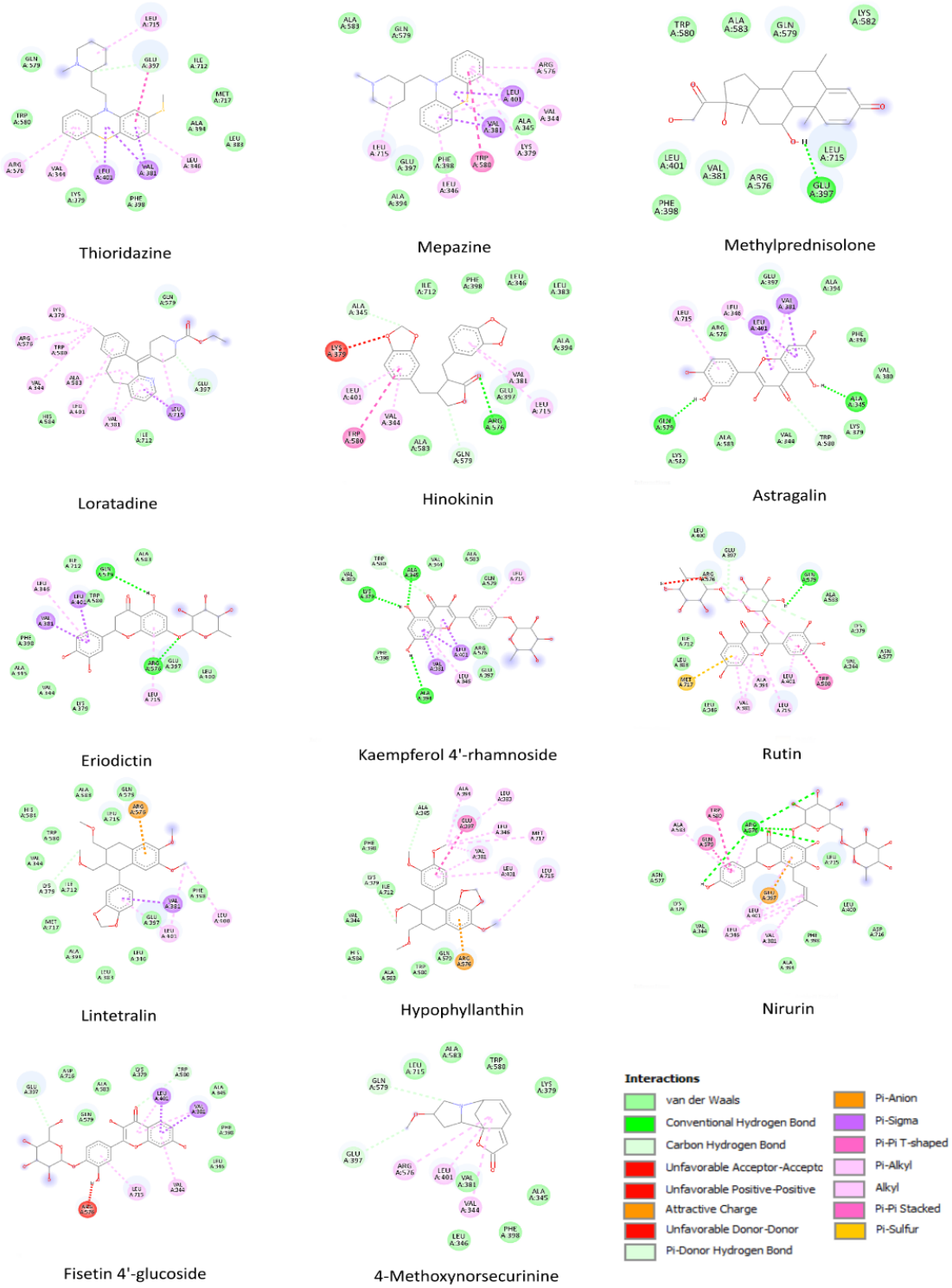

Figure 2: Intermolecular interaction of selected ligands docking toward 4I1R receptor

Discussion

The growing number of allergy cases dramatically established allergic diseases as a global health problem (Pawankar, 2014). Due to the limitations of current therapeutic management for allergies, the rapid discovery of anti-allergy agents is critical. Medicinal plants and their secondary metabolites are a starting point for drug discovery (Marhaeny *et al.,*

2021). Developing *P. niruri* as an anti-allergic agent candidate, in this case, represents a promising biomedical opportunity. *P. niruri*'s abundance in Indonesia is also lucrative in developing safe, effective, and reproducible anti-allergy agents (Paithankar *et al.,* 2011; Kartini *et al.,* 2021).

MALT1 protease has been proposed to be a promising target for allergy treatment due to its proteolytic activity in mediating IgE-dependent mast cell cytokine production and histamine-induced endothelial permeability (Alfano *et al.,* 2020). The discovery of an agent that inhibits MALT1 protease predicted can synergistically control IgE-mediated allergic disease.

This study evaluates the potential of P. niruri secondary metabolite in inhibiting MALT1 protease in the active (3V4O) and allosteric (4I1R) sites. The crystal structure of the 3V4O receptor is a complex formation between a protease (caspase domain) with an irreversible peptidic inhibitor (Z-VRPR-fmk inhibitor). The Z-VRPR inhibitor is a peptide derivative compound that covalently binds Cys464 from the MALT1 catalytic site. MI-2, the first small molecule inhibitor active site of MALT1 protease, was also reported to target the active catalytic site of MALT-1 irreversibly (Wiesman *et al.,* 2012; Zhang *et al.,* 2019; Hamp *et al.,* 2021).

Furthermore, the 4I1R receptor is a crystalline structure of an allosteric site in the interface between the caspase and the Ig3 domain of MALT1, which binds to the thioridazine complex. Therefore, inhibitors targeting this site, such as thioridazine and mepezine, can prevent the rearrangement of MALT1 from inactive to active state and irreversibly inhibit proteolytic cleavage of the substrate (Zhang *et al.,* 2019). The other two selected drugs, methylprednisolone and loratadine, are symptomatic therapeutic agents commonly used to treat allergies. Targeting both MALT1 protease sites results in a potent and selective inhibitory action in allergy therapy.

Based on the docking results, astragalin (ΔG = -9.1 kcal/mol and Ki = 0.2µM) and hinokinis (ΔG = -8.8 kcal/mol and Ki = 0.3µM) showed the most robust interactions among other compounds compared to the native ligands and reference drug, respectively, against 3V4O and 4I1R receptors. Interestingly, this study reported that hinokinin is the only compound with good inhibitory activity against both MALT1 protease binding sites compared to its native ligand and reference drugs.

Moreover, the drugability, including oral bioavailability, of *P. niruri* secondary metabolite was also investigated. The compound's bioavailability depends on its solubility and ability to membrane penetration, thus related to the compound's physicochemical properties. And then, the drugability of compounds can be easily predicted based on RO5 by Lipinski (Doak *et al.,* 2014; Wanat, 2020). In this study, 13 of 21 compounds represent good feasibility of oral bioavailability by satisfying Lipinski's rule. The toxic properties of *P. niruri* secondary metabolites were also predicted based on the LD_{50} and hepatotoxicity model to assess the safety of these compounds as drug candidates. Based on Table II, all of *P. niruri* secondary metabolites are predicted to be safe, while all the reference drugs –except methylprednisolone– show a toxic effect on the liver so it does not safe for long-term use. Therefore, several secondary metabolites of *P. niruri* could be further developed as candidates for MALT1 protease inhibitors.

Conclusion

This study concludes that astragalin, eriodictin, punigluconin, kaempferol 4'-rhamnoside, rutin, fisetin 4'-glucoside, quercitrin, quercetin, quercetin-3-O-glucoside, and, specially hinokinin were predicted to have strong anti-allergic activity thereby can be developed as a potent and selective MALT1 protease inhibitory agent. However, further in vitro and in vivo studies are also needed to support the development of novel agents for treating allergic inflammation by targeting the MALT1 protease.

Acknowledgement

The authors thank to Schrödinger Inc. for granting an academic licence.

Source of funding

Nil.

References

Abd Rani, N. Z., Lam, K. W., Jalil, J., Mohamad, H. F., Mat Ali, M. S., & Husain, K. (2021). Mechanistic studies of the antiallergic activity of Phyllanthus amarus Schum. & Thonn. and Its Compounds. *Molecules,* **26**(3), 695. https://doi.org/10.3390/molecules26030695

Alfano, D. N., Klei, L. R., Klei, H. B., Trotta, M., Gough, P. J., Foley, K. P., Bertin, J., Sumpter, T. L., Lucas, P. C., & McAllister-Lucas, L. M. (2020). MALT1 Protease Plays a Dual Role in the allergic response by acting in both mast cells and

endothelial cells. *Journal of immunology*, **204**(9), 2337–2348. https://doi.org/10.4049/jimmunol.1900281

Benet, L. Z., Hosey, C. M., Ursu, O., & Oprea, T. I. (2016). BDDCS, the rule of 5 and drugability. *Advanced drug delivery reviews,* **101**, 89–98. https://doi.org/10.1016/j.addr.2016.05.007

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports, **7**, 42717. Accessed on March 30, 2023.: http://www.swissadme.ch/,

Demeyer, A., Staal, J., & Beyaert, R. (2016). Targeting MALT1 proteolytic activity in immunity, inflammation and disease: good or bad? *Trends in molecular medicine,* **22**(2), 135–150. https://doi.org/10.1016/j.molmed.2015.12.004

Doak, B. C., Over, B., Giordanetto, F., & Kihlberg, J. (2014). Oral druggable space beyond the rule of 5: insights from drugs and clinical candidates. *Chemistry & biology,* **21**(9), 1115–1142.https://doi.org/10.1016/j.chembiol.2014.08.013

Galli, S. J., Tsai, M., & Piliponsky, A. M. (2008). The development of allergic inflammation. *Nature*, **454**(7203), 445–454. https://doi.org/10.1038/nature07204

Hamp, I., O'Neill, T. J., Plettenburg, O., & Krappmann, D. (2021). A patent review of MALT1 inhibitors (2013-present). *Expert opinion on therapeutic patents,* **31**(12), 1079–1096. https://doi.org/10.1080/13543776.2021.1951703

Jantan, I., Haque, M. A., Ilangkovan, M., & Arshad, L. (2019). An insight into the modulatory effects and mechanisms of action of Phyllanthus species and their bioactive metabolites on the immune system. *Frontiers in pharmacology,* **10**, Article 878. https://doi.org/10.3389/fphar.2019.00878

Kartini, K., Wulandari, W. A., Jayani, N. I. E., & Setiawan, F. (2021). TLC-based fingerprinting for Phyllanthus niruri from diverse geographical origins in East and Central Java Indonesia. IOP *Conference Series: Earth and Environmental Science*, **948**(1). https://doi.org/10.1088/1755-1315/948/1/012003

KnapSack Family. Accessed in: http://www.knapsackfamily.com/KNApSAcK_Family/, on March 30, 2023.

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced drug delivery reviews,* **46**(1-3), 3–26. https://doi.org/10.1016/s0169-409x(00)00129-0

Marhaeny, H. D., Widyawaruyanti, A., Widiandani, T., Fuad Hafid, A., & Wahyuni, T. S. (2021). Phyllanthin and hypophyllanthin, the isolated compounds of Phyllanthus niruri inhibit protein receptor of corona virus (COVID-19) through in silico approach. *Journal of basic and clinical physiology and pharmacology*, 32(4), 809–815. https://doi.org/10.1515/jbcpp-2020-0473

Marhaeny, H. D., Pratama, Y. A., Rohmah, L., Kasatu, S. M., Miatmoko, A., & Khotib, J. (2023) Development of gastro-food allergy model in shrimp allergen extract-induced sensitised mice promotes mast cell degranulation. *Journal of Public Health in Africa*, **14**(s1), 2512. https://doi.org/10.4081/jphia.2023.2512

Mukherjee, M., & Gupta, S. (2018). Evaluation of acute toxicity and anti-asthmatic activity of Phyllanthus niruri L. leaves extracts. *Asian Journal of Pharmacy and Pharmacology*, **4**(5), 615–619. https://doi.org/10.31024/ajpp.2018.4.5.11

National Center for Biotechnology Information - National Library Medicine: PubChem. Accessed on March 30, 2023. https://pubchem.ncbi.nlm.nih.gov/:

Pawankar R. (2014). Allergic diseases and asthma: A global public health concern and a call to action. *The World Allergy Organisation journal,* **7**(1), Article 12. https://doi.org/10.1186/1939-4551-7-12

Paithankhar, V. V., Raut, K. S., Charde, R. M., & Vyas, J. V. (2011). Phyllanthus niruri: A magic Herb. *Research in Pharmacy,* **1**(4), 1–9.

Pires, D. E. V., Blundell, T. L., & Ascher, D. B. (2015). pkCSM: predicting small-molecule pharmacokinetic properties using graph-based signatures. *Journal of Medicinal Chemistry*, **58**(9), 4066–4072. Accessed on March 30, 2023. https://biosig.lab.uq.edu.au/pkcsm/prediction

Wai, C., Leung, N., Leung, A., Wong, G., & Leung, T. F. (2021). Seafood allergy in Asia: Geographical specificity and Beyond. *Frontiers in allergy,* 2, Article 676903. https://doi.org/10.3389/falgy.2021.676903

Wanat K. (2020). Biological barriers, and the influence of protein binding on the passage of drugs across them. *Molecular biology reports,* **47**(4), 3221–3231. https://doi.org/10.1007/s11033-020-05361-2

Wiesmann, C., Leder, L., Blank, J., Bernardi, A., Melkko, S., Decock, A., D'Arcy, A., Villard, F., Erbel, P., Hughes, N., Freuler, F., Nikolay, R., Alves, J., Bornancin, F., & Renatus, M. (2012). Structural determinants of MALT1 protease activity. *Journal of molecular biology,* **419**(1-2), 4–21. https://doi.org/10.1016/j.jmb.2012.02.018

Yang, N. J., & Hinner, M. J. (2015). Getting across the cell membrane: an overview for small molecules, peptides, and proteins. *Methods in molecular biology,* **1266**, 29–53. https://doi.org/10.1007/978-1-4939-2272-7_3

Zhang, J., Ren, L., Wang, Y., & Fang, X. (2019). In silico study on identification of novel MALT1 allosteric inhibitors. RSC advances, **9**(67), 39338–39347. https://doi.org/10.1039/C9RA07036B