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RESEARCH ARTICLE

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

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**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 3V40 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.

**Keywords**

Anti-allergic agent
Immune system
In silico
MALT1 protease
*Phyllanthus niruri*

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**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$_2$ 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-kB (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 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 (KnappSack 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.

**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 ROS leads to poor membrane permeation and BA (Lipinski et al., 2001). However, this rule specifically states that ROS 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**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>MW (g/mol) ≤ 500</th>
<th>Physicochemical properties</th>
<th>mLogP (Log P&lt;sub{o/w}&lt;/sub&gt; ≤ 4.15)</th>
<th>Drug-likeness evaluation</th>
<th>Toxicity prediction</th>
<th>Hepatotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>RBN</td>
<td>BA score</td>
<td>Violation</td>
<td>ROS</td>
</tr>
<tr>
<td>Mepazine</td>
<td>310.46</td>
<td></td>
<td>4.31</td>
<td>2</td>
<td>0.55</td>
<td>1</td>
</tr>
<tr>
<td>MI-2</td>
<td>455.72</td>
<td></td>
<td>3.51</td>
<td>9</td>
<td>0.55</td>
<td>0</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>374.47</td>
<td></td>
<td>1.52</td>
<td>2</td>
<td>0.55</td>
<td>0</td>
</tr>
<tr>
<td>Loratadine</td>
<td>382.88</td>
<td></td>
<td>3.72</td>
<td>3</td>
<td>0.55</td>
<td>0</td>
</tr>
<tr>
<td>4-Methoxyxor-securinine</td>
<td>233.26</td>
<td></td>
<td>1.10</td>
<td>1</td>
<td>0.55</td>
<td>0</td>
</tr>
</tbody>
</table>

**References**

- Abd Rani et al., 2021
- Jantan et al., 2019
- Yang & Hinner, 2015
- Benet et al., 2016
- Marhaeny et al., 2019
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 $\Delta G$ value, compared to the native ligand. The $K_i$ 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 $\Delta G$ and $K_i$ 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

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Receptor 3V4O</th>
<th>Compounds</th>
<th>Receptor 4I1R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Delta G$ (kcal/mol)</td>
<td>$K_i$ ($\mu$M)</td>
<td>$\Delta G$ (kcal/mol)</td>
</tr>
<tr>
<td>Peptidic inhibitor Z-VRPR-fmk</td>
<td>-7.7</td>
<td>2.6</td>
<td>-8.0</td>
</tr>
<tr>
<td>MI-2</td>
<td>-7.2</td>
<td>5.2</td>
<td>-8.6</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>-6.1</td>
<td>33.4</td>
<td>-7.4</td>
</tr>
<tr>
<td>Loratadine</td>
<td>-7.5</td>
<td>3.1</td>
<td>-7.2</td>
</tr>
<tr>
<td>References</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Top ten docking results of P. niruri secondary metabolites

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$\Delta G$ (kcal/mol)</th>
<th>$K_i$ ($\mu$M)</th>
<th>Compounds</th>
<th>$\Delta G$ (kcal/mol)</th>
<th>$K_i$ ($\mu$M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astragalin</td>
<td>-9.1</td>
<td>0.2</td>
<td>Hinokinin</td>
<td>-8.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Eriodictin</td>
<td>-8.7</td>
<td>0.4</td>
<td>Astragalin</td>
<td>-8.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Punigluconin</td>
<td>-8.7</td>
<td>0.4</td>
<td>Eriodictin</td>
<td>-8.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Compounds</td>
<td>Reseptor 3V4O</td>
<td>Compounds</td>
<td>Reseptor 4I1R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------</td>
<td>--------------------</td>
<td>---------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ΔG (kcal/mol)</td>
<td>Ki (µM)</td>
<td>ΔG (kcal/mol)</td>
<td>Ki (µM)</td>
<td></td>
</tr>
<tr>
<td>Kaempferol 4’-rhamnoside</td>
<td>-8.6</td>
<td>0.5</td>
<td>Kaempferol 4’-rhamnoside</td>
<td>-7.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Hinokinin</td>
<td>-8.5</td>
<td>0.6</td>
<td>Rutin</td>
<td>-7.7</td>
<td>2.2</td>
</tr>
<tr>
<td>Rutin</td>
<td>-8.2</td>
<td>1.0</td>
<td>Lintetralin</td>
<td>-7.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Fisetin 4’-glucoside</td>
<td>-8.1</td>
<td>1.1</td>
<td>Hypophyllanthin</td>
<td>-7.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Quercetin</td>
<td>-8.1</td>
<td>1.1</td>
<td>Nirurin</td>
<td>-7.3</td>
<td>4.4</td>
</tr>
<tr>
<td>Quercetin</td>
<td>-8.0</td>
<td>1.3</td>
<td>Fisetin 4’-glucoside</td>
<td>-7.2</td>
<td>5.2</td>
</tr>
<tr>
<td>Quercetin-3-O-glucoside</td>
<td>-7.9</td>
<td>1.6</td>
<td>4-Methoxynorsecurinine</td>
<td>-7.2</td>
<td>5.2</td>
</tr>
</tbody>
</table>

ΔG = binding affinity, Ki = inhibition constant

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**Figure 1:** Intermolecular interaction of selected ligands docking toward 3V4O receptor
Marhaeny et al.  

Anti-allergic activity of Phyllanthus niruri via MALT1 protease inhibition

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.,

Figure 2: Intermolecular interaction of selected ligands docking toward 4I1R receptor
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 thioridine complex. Therefore, inhibitors targeting this site, such as thioridine 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 hinokinin (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 ROS 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 LD₅₀ 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, quercitin, 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.

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**References**


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


