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Antineuroinflammatory Properties of Compounds from Ethyl Acetate Fraction of *Marsilea crenata* C. Presl. Against Toll-Like Receptor 2 (3A7B) *In Silico*

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Abstract

Parkinson's disease (PD) can be triggered by overactive TLR2 due to α-synuclein abnormalities and aggregation. *Marsilea crenata* C. Presl. leaves inhibit neuroinflammatory progression. This study aimed to predict the antineuroinflammatory activity of M. crenata leaves with TLR2 (ID 3A7B) in an *in silico* study. The list of chemicals was collected through metabolite profiling with UPLC-QToF MS/MS, then analyzed for physicochemical properties using SwissADME and toxicity using the ProTox II online program. This analysis confirmed the molecule's safety for therapeutic use. ChemDraw 12.0 was used to build metabolite-profiled compounds. Avogadro 1.2.0 was utilized to optimize geometry, while PyRx 0.8 was used for AutoDock Vina molecular docking. Agonist-TLR2 interactions were examined using docking results from Biovia Discovery Studio 2021. Tethering is valid; the program can be used because the RMSD is less than 2. The results showed that 6 of the 84 metabolite-profiled compounds were antagonistic to 3A7B and shared similar pharmacophore distances and amino acid linkages with N-acetyl-D-glucosamine, a native ligand of 3A7B. By binding to TLR2, the compounds from the ethyl acetate fraction of *M. crenata* leaves may potentially inhibit PD progression.

***Keywords**: Marsilea crenata C. Presl., Parkinson disease, neuroinflammation, in silico, 3A7B*

Background

Neuroinflammation is a natural response of the central nervous system (CNS) to neurotoxic chemicals in order to protect neural tissue. On the other hand, conditions that cause inflammation in the central nervous system (CNS) for a long time can kill hippocampal neuronal cells and reduce cognitive function, which can lead to neurodegenerative diseases (Cherry *et al*., 2014; Chen *et al*., 2016; Mizuno, 2015; Ma'arif *et al*., 2022).

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Parkinson's disease (PD) is a neurological illness with a significant prevalence, particularly among geriatrics aged 65 to 70 years (Balestrino & Schapira, 2020; Ma'arif *et al*., 2021a), marked by tremors, bradykinesia, melancholy, anxiety, sleep disturbances, and dementia (Syamsudin, 2015). The accumulation of lewy bodies caused by aberrant α-synuclein aggregation is the primary etiology of Parkinson's disease. α-synuclein can promote neuronal cell degeneration and apoptosis as a result of the activation of tool-like receptor 2 (TLR2) on microglia cells, which leads to increased production of proinflammatory cytokines and neuroinflammation. TLR2 is made by microglia cells and helps them recognize pathogens, such as chemicals that are harmful to neurons (Cario, 2008; Borrelo *et al*., 2011).

Semanggi (*Marsilea crenata* Presl.) is a plant that is used as a particular cuisine for the local community in Surabaya, East Java. In a previous study, *M. crenata* Presl. was shown to inhibit neuroinflammation progression via the estrogen-receptor (ER) dependent pathway, specifically by decreasing the expression of major histocompatibility complex II (MHCII) and increasing the expression of arginase 1 (Arg1), both of which were caused by phytoestrogen compounds in *M. crenata* Presl. leaves binding with ER (Ma'arif *et al*., 2020a; 2020b; 2021b, 2022c; 2022d), This plant also has the effect of increasing locomotor activity in the rotenone-induced parkinsonian zebrafish (Ma'arif *et al*., 2022e; 2022f).

The goal of this work is to build on prior research that was used *in silico* to anticipate the antineuroinflammatory action of *M. crenata* Presl. leave on the TLR2 activation inhibitory pathway (ID 3A7B). *In silico* research is a computational simulation method that predicts the activity of substances in new drug discovery attempts by employing specific software and web technologies. *In silico* molecular docking is used to connect small compounds or ligands to macromolecules or receptors (Prieto-Martnez *et al*., 2018; Makatita *et al*., 2020).

Materials and Methods

Material

The ingredients in this study were 84 compounds from previous studies (Ma'arif, 2020) on the metabolite profiling of the ethyl acetate fraction of *M. crenata* Presl. leaves using the UPLC-QToF-MS/MS method and TLR2 receptors with ID 3A7B downloaded from www.rcsb.org containing the native ligand N-acetyl-D-glucosamine. This natural ligand stops microglia cells from becoming active, which is what causes neuroinflammation (Hwang *et al*., 2010).

Physicochemical examination

Compounds identified through metabolite profiling were structured into a simplified molecular-input line-entry system (SMILES) using ChemDraw Ultra 12.0, and the SMILES form was employed so that the compounds could be examined for their physicochemical qualities in the IUPAC name format (Sliwoski *et al*., 2014). The format is then copied one at a time onto the SwissADME webtool (http://www.swissadme.ch) and run to find the topological polar surface area (TPSA), molecular weight, log P, HBA, HBD, and the statement "Yes" or "No" in meeting Lipinski's five law requirements (Muslikh *et al*., 2022).

Toxicity testing

A toxicity study was performed by entering the SMILES format into the ProTox II online program (http://tox.charite.de/protox_II/) to predict compound toxicity (LD_{50}) based on the globally harmonized system (GHS).

Preparation of the sample

The Biovia Discovery Studio 2021 program was used to extract receptors from macromolecules and natural ligands. Using ChemDraw Ultra 12.0, the metabolite profiles of the ethyl acetate fraction of *M. crenata* Presl. leaves were used to make 84 compounds with a 3D structure.

Molecular docking

Compounds that satisfy Lipinski's five law parameters and are non-toxic are geometrically optimized using Avogadro 1.0.1 and the MMFF94 technique. Internal validation of the receptor and native ligand was performed first using AutoDock vina (PyRx 0.8) to determine the root mean square deviation (RMSD), with an RMSD value of less than 2 indicating that the application is suitable for use (Riwanti *et al*., 2021). Each drug was docked to the 3A7B receptor using AutoDock Vina (PyRx 0.8), and the interaction was visualized using Biovia Discover Studio 2021 to determine the distance between the pharmacophore and the bound amino acids.

Result and Discussion

A total of 84 compounds from metabolite profiling with UPLC-QToF-MS/MS were screened using SwissADME to see pharmacokinetic and pharmacodynamic properties. The results showed that there were 74 compounds that stated "Yes" in fulfilling Lipinski's five law parameters of molecular weight <500 g/mol, HBD <5, HBA <10, and log p <5, which indicate the compound can be accepted by the body (Ma'arif *et al*., 2021c). **Table 1** A molecular weight of less than 500 g/mol suggests that the molecule is capable of penetrating biological membranes. The log P value shows the compound's capacity to dissolve in the liquid membrane. The hydrogen bonding capacity of the H-acceptor and H-donor is shown, and the higher the value, the more energy is required for the absorption process (Lipinski *et al*., 1997). The TPSA value is a measure that shows a chemical's capacity to cross the blood-brain barrier when the compound is aimed at the central nervous system (Martin, 2005; Villa *et al*., 2016; Ma'arif *et al*., 2022b).

Toxicity studies were performed on 74 substances that matched the Lipinski's five law parameters. This test was performed to determine the capacity of hazardous chemicals in the ethyl acetate fraction of *M. crenata* Presl. leaves to be absorbed by the body. The goal of determining the LD_{50} value is to find a single dose of the test compound that may kill 50% of the experimental animals in one administration so that the potential toxicity of the compound can be determined (Nurmianti & Gusmawarni, 2020). The GHS classification of toxicity levels into six classes. The six toxicity classes are as follows: class I ($LD_{50} \leq 5$ mg/kg) is fatal if swallowed, class II ($5 < LD_{50} \leq 50$ mg/kg) is toxic if swallowed, class III ($50 < LD_{50} \leq 300$ mg/kg) is toxic if swallowed, class IV ($300 < LD_{50} \leq 2000$ mg/kg) is harmful if swallowed, class V ($2000 < LD_{50} \leq 5000$ mg/kg) may be harmful if swallowed, and class VI ($LD_{50} > 5000$ mg/kg) (Muslikh *et al*., 2023). The bigger the LD_{50} number, the less dangerous the compound is, and vice versa, the greater the value indicated by the LD_{50}, the safer the substance is for the body. The results of this toxicity test show 64 substances with low toxicity in classes 4 and 5 (Supandi *et al*., 2018), as shown in **Table 1**.

Table 1. Pharmacokinetic and pharmacodynamic analysis from ethyl acetate fraction of *M. crenata* Presl. leaves antagonistic to 3A7B

No.	Compounds	Parameters of Lipinski's Five Laws				Lipinski's Five Laws	TPSA (Å²)	LD_{50}
		Molecular Weight ≤ 500 g/mol	HBA ≤ 10	HBD ≤ 5	Log P ≤ 5			
1.	Valinol	103.16	2	2	0.28	Yes	46.25	V
2.	3,3'-[(1E)-3-Ethyl-1-triazene-1,3-diyl]bis(4-methoxy-1,2,5-oxadiazole)	269.22	10	0	1.38	Yes	124.26	IV
3.	11-Aminoundecanoic acid	201.31	3	2	1.62	Yes	63.32	VI
4.	Diethofencarb	267.32	4	1	2.75	Yes	56.79	V
5.	N,N'-1,4-Phenylenediacetamide	192.21	2	2	0.91	Yes	58.20	IV
6.	2,2'-[(6-Amino-5-nitro-2,4-pyrimidinediyl)diimino]diethanol	258.23	6	5	-1.04	Yes	162.14	V
7.	Phenprobamate	179.22	2	1	1.80	Yes	52.32	IV
8.	N-(3-Oxododecanoyl)-L-homoserine	315.41	5	3	2.19	Yes	103.70	V

No.	Compounds	Parameters of Lipinski's Five Laws				Lipinski's Five Laws	TPSA (Å²)	LD_{50}
		Molecular Weight ≤ 500 g/mol	HBA ≤ 10	HBD ≤ 5	Log P ≤ 5			
9.	3-(4-Carbamimidoyl-1-piperazinyl)-2-hydroxy-1-propanesulfonic acid	266.32	6	4	-2.17	Yes	139.33	IV
10.	N,N,N'-Trimethyl-6-[3-(methylsulfanyl)-1H-1,2,4-triazol-1-yl]-1,3,5-triazine-2,4-diamine	266.33	5	1	0.75	Yes	109.95	IV
11.	Dopamantine	315.41	3	3	3.03	Yes	69.56	IV
12.	DMCM	314.34	5	1	2.82	Yes	73.44	IV
13.	Serratidine	261.36	3	1	1.72	Yes	40.54	IV
14.	Propazone	129.11	3	1	0.22	Yes	55.40	V
15.	N-Ethyl-N-(2-thienylmethyl)-L-methioninamide hydrochloride	308.89	2	1	1.96	Yes	99.87	IV
16.	2-Methyl-2-propanyl 4-oxo-1-piperidinecarboxylate	199.25	3	0	1.19	Yes	46.61	IV
17.	1-(Isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]-2-propanol (2E)-2-butenedioate (1:1)	383.44	8	4	1.49	Yes	125.32	IV
18.	Perlolyrine	264.28	3	2	2.55	Yes	62.05	IV
19.	Tetradecasphinganine	245.40	3	3	2.92	Yes	66.48	V
20.	C16 phytosphingosine	289.45	4	4	2.87	Yes	86.71	V
21.	(1-Oxo-4-phenyl-2(1H)-phthalazinyl)aceticacid	280.28	4	1	2.19	Yes	72.19	IV
22.	1,1'-(Decylimino)di(2-propanol)	273.45	3	2	3.58	Yes	43.70	IV
23.	Phytosphingosine	317.51	4	4	3.60	Yes	86.71	V
24.	(2S,3R)-2-Amino-1,3-heptadecanediol	287.48	3	3	4.08	Yes	66.48	V
25.	Pentadecylamine	227.43	1	1	4.21	Yes	26.02	IV
26.	Safingol	301.51	3	3	4.43	Yes	66.48	V
27.	3-(Hexadecylamino)-1,2-propanediol	315.53	3	3	4.81	Yes	52.49	V
28.	N'-[4,6-Di(4-morpholinyl)-1,3,5-triazin-2-yl]-N'-methyl-2-(4-morpholinyl)propanehydrazide	436.51	8	1	-0.13	Yes	108.42	IV
29.	Gloeolactone	292.41	3	0	4.09	Yes	38.83	IV
30.	Dioctyl phthalate	390.56	4	0	6.30	Yes	52.60	IV
31.	Erucamide	337.58	1	1	6.76	Yes	43.09	IV
32.	(2E,4E)-N-Isobutyl-2,4-icosadienamide	363.62	1	1	7.27	Yes	29.10	V
33.	1-Hydroxy-2,2,6,6-tetramethyl-4-piperidinyl stearate	439.71	4	1	7.37	Yes	49.77	IV
34.	2-Deoxy-2-(diethylamino)hexopyranose	235.28	6	4	-0.94	Yes	93.39	IV
35.	3,4,5-Tri(1,2,4-triazin-3-yl)-2-pyridinecarboxamide	359.31	11	1	-0.67	Yes	171.99	IV
36.	Dimethylsulfoxide (DMSO)	78.13	1	0	0.05	Yes	36.28	VI
37.	Picolinamide	122.12	2	1	0.31	Yes	55.98	IV
38.	Chlorogenic acid	354.31	9	6	-0.39	Yes	164.75	V
39.	Clovamide	359.33	7	6	1.10	Yes	147.32	IV
40.	Deoxyclovamide	343.33	6	5	1.59	Yes	127.09	IV
41.	3,4,5-Trimethoxy-N-(4-pyridinylmethyl)benzamide	302.33	5	1	1.79	Yes	69.68	IV
42.	7-methoxy-4-(aminomethyl)coumarin	205.21	4	1	1.31	Yes	65.46	IV
43.	6-(4-Chlorophenyl)-3-[4-(4-chlorophenyl)-4-oxo-1-phenylbutyl]-3,4-dihydro-2H-pyran-2-one	465.37	3	0	6.24	Yes	43.47	IV
44.	Kaempferol	286.24	6	4	1.58	Yes	111.13	V
45.	1,3,5-Trinitro-2,4,6-tripropoxybenzene	387.34	9	0	1.41	Yes	165.15	IV
46.	2-Chloro-4-(1H-imidazol-1-yl)-6-(1-piperidinyl)-1,3,5-triazine	264.71	4	0	1.67	Yes	59.73	IV
47.	Alifedrine	289.41	3	2	2.98	Yes	49.33	IV
48.	Piperidolate	323.43	3	0	3.75	Yes	29.54	IV
49.	Aspirin Arginine	354.36	8	6	-1.03	Yes	188.82	VI
50.	2,2-Dimethyl-4,13-dioxo-3,8,11,17,20- pentaoxa-5,14-diazadocosan-22-oic acid	408.44	9	3	0.37	Yes	141.65	V
51.	Jasmolone	180.24	2	1	1.89	Yes	37.30	V
52.	CB-13	368.47	2	0	6.08	Yes	26.30	VI
53.	Asparaginylcysteinylthreonine	336.36	7	6	-2.78	Yes	223.64	V

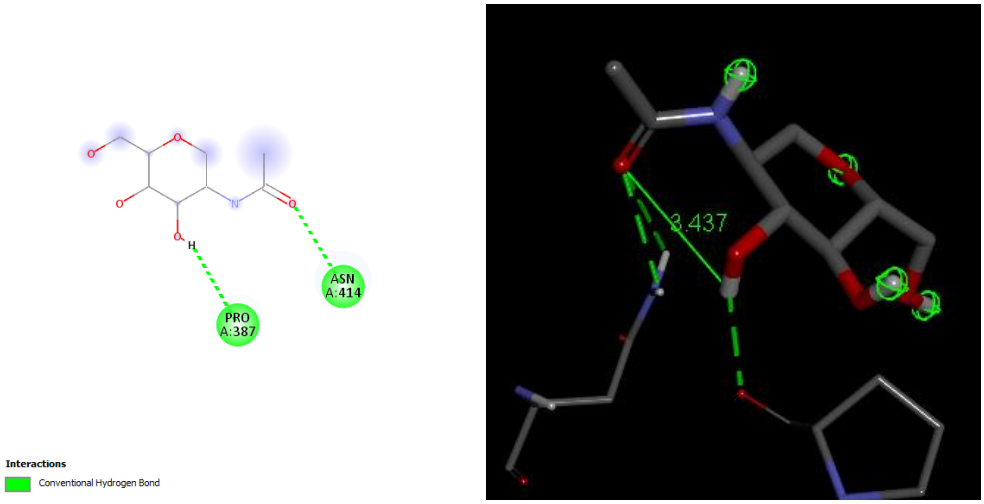
No.	Compounds	Parameters of Lipinski's Five Laws				Lipinski's Five Laws	TPSA (Å²)	LD_{50}
		Molecular Weight ≤ 500 g/mol	HBA ≤ 10	HBD ≤ 5	Log P ≤ 5			
54.	L-γ-Glutamyl-L-cysteinylglycinamide	306.34	7	6	-1.38	Yes	181.59	IV
55.	8-[(4-Benzyl-1-piperazinyl)methyl]-1,3-dimethyl-7-(1-naphthylmethyl)-3,7- dihydro-1H-purine-2,6-dione	508.61	5	0	3.14	Yes	68.30	IV
56.	4-(Dodecylamino)-1-oxaspiro[4.5]dec-3-en-2-one	335.52	2	1	5.48	Yes	38.33	V
57.	(4E)-2-[2-(1H-[1,3]Oxazolo[3,4-c][1,3]oxazol-7a(7H)-ylmethoxy)-2-oxoethyl]-4-icosenoic acid	495.69	7	1	5.45	Yes	85.30	VI
58.	(3R,4S,6S,9R,11R,12R,13S,14R)-6-{[(2S,3R,4S,6S)-4-(Dimethylamino)-3- hydroxy-6-methyltetrahydro-2H-pyran-2-yl]oxy}-14-ethyl-4,12,13-trihydroxy- 3,9,11,13-tetramethyloxacyclotetradecane-2,10-dione	531.68	10	4	1.30	Yes	145.99	IV
59.	8-Methyl-3-[({2-methyl-1-[1-(2-methyl-2-butanyl)-1H-tetrazol-5-yl]propyl}[2-(4-morpholinyl)ethyl]amino)methyl]-2(1H)- quinolinone	495.66	7	1	3.41	Yes	92.17	V
60.	(2R)-2-{(4R,5R)-2,2-Dimethyl-5-[(1E)-1-tetradecen-1-yl]-1,3-dioxolan-4-yl}-2-({[(2-methyl-2-propanyl)oxy]carbonyl}amino)ethyl acetate	497.71	6	1	6.29	Yes	83.09	IV
61.	Octadecyl 2-acetamido-2-deoxy-β-D-glucopyranoside	473.69	6	4	4.70	Yes	108.25	V
62.	Ethyl (3R)-3-(6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)-3-(pentadecylamino)propanoate	499.72	7	1	5.91	Yes	72.25	IV
63.	Trigonosin B	606.66	10	4	2.53	Yes	147.44	V
64.	Pheophorbide A	594.70	6	4	4.61	Yes	136.64	V

The RMSD of the validation technique using receptor and native ligand binding using AutoDock Vina (PyRx 0.8) was 1.761 Å. RMSD of less than 2 implies that the application is appropriate for molecular anchoring techniques that provide near-experimental outcomes (Nursamsiar *et al*., 2020; Ma'arif *et al*., 2021d). The receptor 3A7B and 64 metabolite-profiled molecules were then re-tethered with AutoDock Vina (PyRx 0.8). In addition, Biovia Discovery Studio 2021 was used to determine the amino acids produced, the pharmacophore distance, and the type of bond. The results of molecular docking showed that six molecules were bad for 3A7B. They are all listed in **Table 2**.

Table 2. Pharmacokinetic and pharmacodynamic analysis from ethyl acetate fraction of *M. crenata* Presl. leaves antagonistic to 3A7B

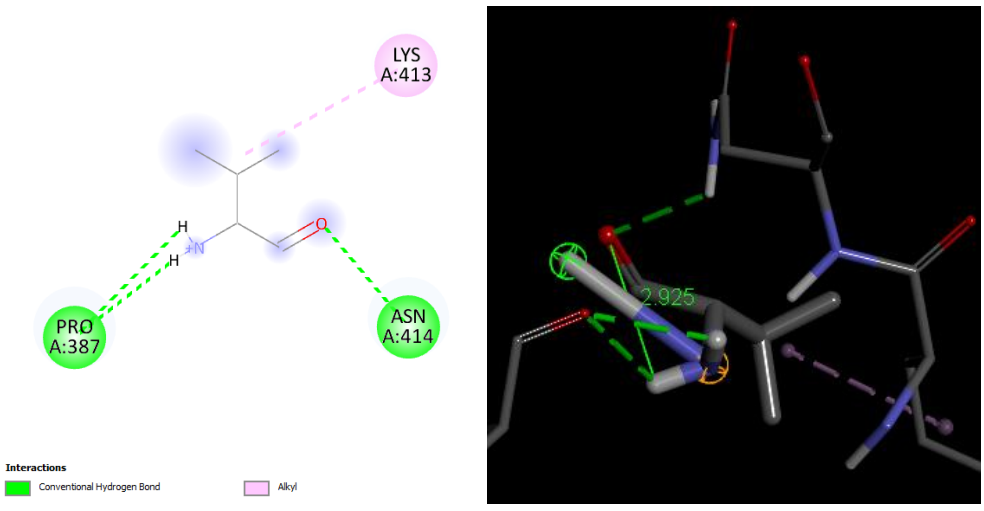
No	Compounds	Binding Affinity (kcal/mol)	Amino Acids (Types of Bonds)	Pharmacophore Distance (Å)
1.	*Native Ligand* N-Acetil-D-Glucosamine	-2.4	Pro387 (Hydrogen) Asn414 (Hydrogen)	3.437
2.	Valinol	-2	Pro387 (Hydrogen) Asn414 (Hydrogen)	2.925
3.	3,3'-[(1E)-3-Ethyl-1-triazene-1,3-diyl]bis(4-methoxy-1,2,5-oxadiazole)	-2.27	Pro387 (Hydrogen) Asn414 (Pi-Hydrogen Donor)	2.536
4.	Phenprobamate	-2.37	Pro387 (Hydrogen) Asn414 (Hydrogen)	2.575
5.	Propazone	-2.3	Pro387 (Hydrogen) Asn414 (Hydrogen)	2.640
6.	Tetradecasphinganine	-1.33	Pro387 (Hydrogen) Asn414 (Hydrogen)	3.781
7.	2-Deoxy-2-(diethylamino)hexopyranose	-1.8	Pro387 (Carbon Hydrogen) Asn414 (Hydrogen)	3.597

The native ligand N-Acetyl-D-Glucosamine is antagonistic and inhibits TLR2 activation by binding to amino acids in the form of Pro 387 and Asn 414 and a pharmacophore distance of 3.437 Å, each of which is in the form of hydrogen bonds. Compounds are said to be antagonists if they bind to the same amino acid as the native ligand (**Figure 1**). The more similar the amino acids bound by the compounds in *M. crenata* Presl. leaves are to native ligands, the more similar the types of interactions that occur (Ekins *et al*., 2007; Suhud *et al*., 2015). Also, the similarity of the pharmacophore distance determines how similar the activities are (source). The resulting binding affinity shows a negative value, which explains the strong and stable bond formed (Syahputra *et al*., 2014). The results of the analysis of agonist compounds in Biovia Discovery Studio 2021 can be seen in **Figures 2** to **7**.



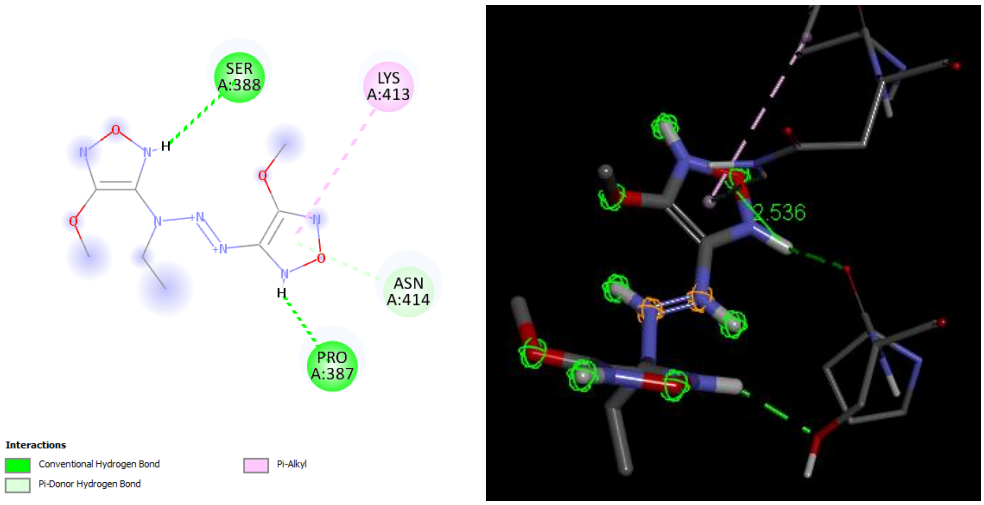
A **B**

Figure 1. Visualization of N-acetyl-D-glucosamine native ligand with TLR2. A: 2D; B: 3D



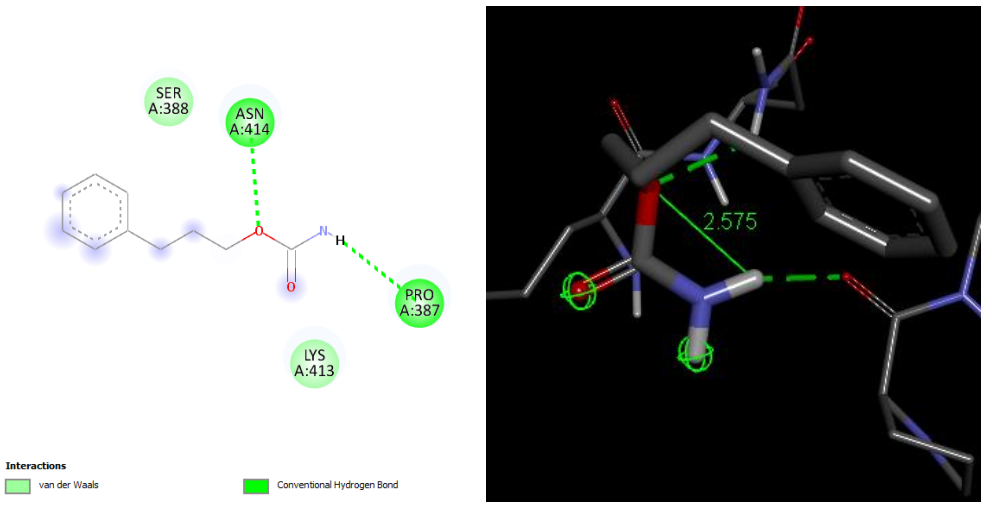
A **B**

Figure 2. Visualization of valinol with TLR2. A: 2D; B: 3D



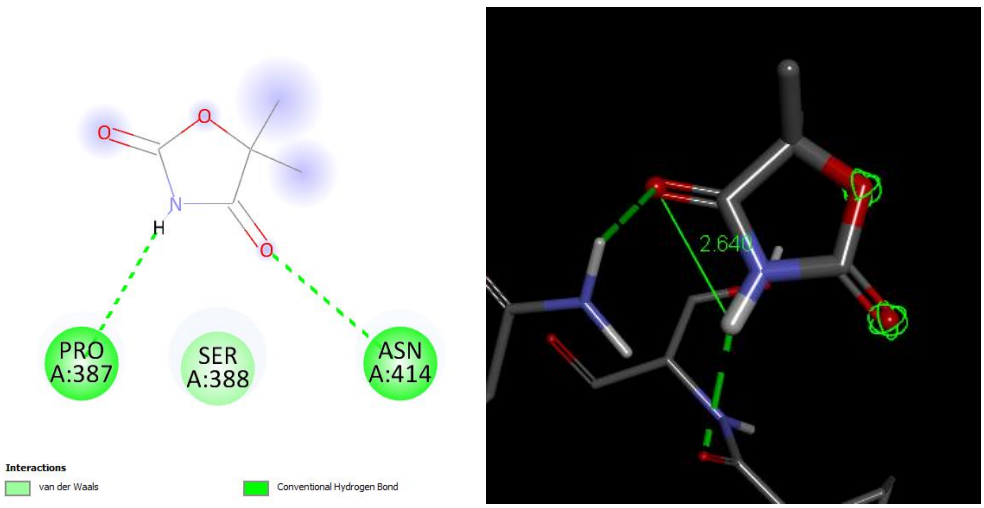
A **B**

Figure 3. Visualization of 3,3'-[(1E)-3-ethyl-1-triazene-1,3-diyl]bis(4-methoxy-1,2,5-oxadiazole) compounds with TLR2. A: 2D; B: 3D



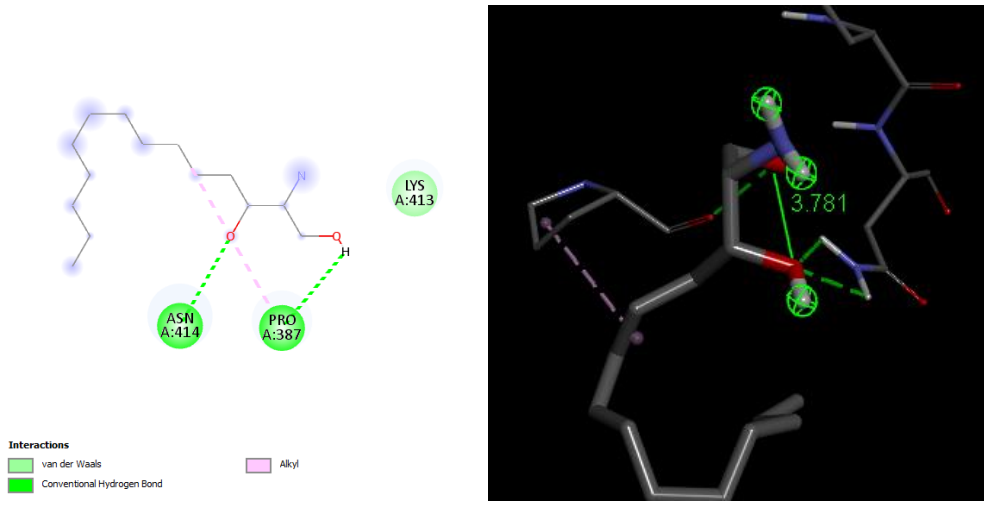
A **B**

Figure 4. Visualization of phenprobamate with TLR2. A: 2D; B: 3D



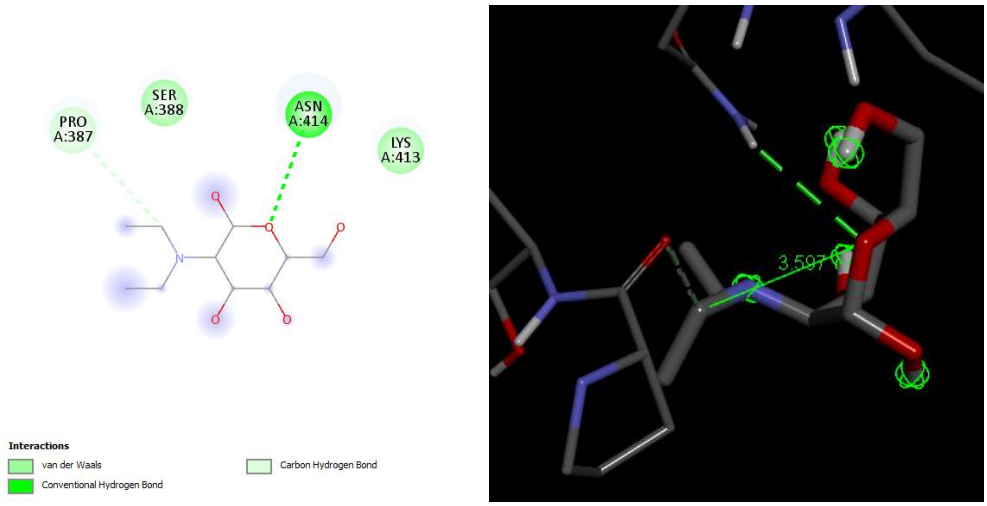
A **B**

Figure 5. Visualization of propazone with TLR2. A: 2D; B: 3D



A **B**

Figure 6. Visualization of tetradecasphanganine with TLR2. A: 2D; B: 3D



A **B**

Figure 7. Visualization of 2-deoxy-2-(diethylamino)hexopyranose with TLR2. A: 2D; B: 3D

Compounds that match Lipinski's five law requirements, have a high potential as antineuroinflammatory agents. Six chemicals are "yes" and have low toxicity: valinol; 3,3'-[(1E)-3-ethyl-1-triazene-1,3-diyl]bis(4-methoxy-1,2,5-oxadiazole); phenprobamate; propazone; tetradecasphanganine; and 2-deoxy-2-(diethylamino)hexopyranose. These chemicals are expected to be powerful enough to bind TLR2, hence suppressing their activity. Blocking the TLR2 pathway could be the basis of future treatments for neuroinflammation (Dzamko *et al*., 2017).

The abnormal aggregation of α-synuclein is harmful to dopaminergic neuron cells and plays a significant role in the etiology of Parkinson's disease (Yulianti *et al*., 2015). TLR2 is involved in the stimulation of the inflammatory response in microglia cells as a result of α-synuclein abnormalities in Parkinson's disease (Dzamko *et al*., 2017). Gene mutations can result in α-synuclein disorders such aggregation and degeneration, which are the major components of Lewy bodies (Lewis & Spillane, 2018). Because excessive activation leads the generation of proinflammatory cytokines, microglia cells will be the first to respond to this α-synuclein abnormalities (Gelosa *et al*., 2017). *Marsilea crenata* Presl. is an alternative for treating inflammation in Parkinson's disease sufferers, which primarily affects the elderly. *Marsilea crenata* Presl. leaves anti-neuroinflammatory activity was tested *in silico* using the molecular docking approach. Molecular docking is beneficial for predicting the conformation of the ligand when it binds to the target and looking for connections between receptors and ligands (Ferreira *et*

al., 2015). When the ligand (drug) comes in contact with the receptor, the shape of the macromolecule's changes, which leads to a biological response (Siswandono, 2015).
Pathogens or anomalies that attack the body will be met with self-defense by the immune system, in this example by the TLR. Over-activation of TLRs, on the other hand, can alter brain homeostasis due to excessive synthesis of proinflammatory cytokines, resulting in a variety of illnesses (Gao *et al*., 2017). TLR2 activation enhances NF-κB translocation from the cytoplasm to the nucleus, where it binds to DNA and initiates the transcription process. This causes microglia cells to respond to M1 polarity circumstances and activates proinflammatory molecules (Penn, 2002; Engler-Chiurazzi *et al*., 2017). Compounds from *M. crenata* Presl. suppress TLR activation, causing microglia activity to shift from M_1 polarity to M_2 polarity, transforming it into an antineuroinflammatory drug (Cui *et al*., 2013). Furthermore, suppressing TLR2 activation can diminish α-synuclein aggregation. As a result, *M. crenata* Presl. can act as a neuroprotector, reducing neuroinflammation and improving cognitive performance (Villa *et al*., 2016; Kwon *et al*., 2019).

Conclusion

The results showed that 6 of the 84 metabolite-profiled compounds were antagonistic to 3A7B and shared similar pharmacophore distances and amino acid linkages with N-acetyl-D-glucosamine, a native ligand of 3A7B. By binding to TLR2, the compounds from the ethyl acetate fraction of *M. crenata* leaves may have the potential to inhibit PD progression with an anti-inflammatory mechanism.

References

Balestrino, R. & Schapira, A. H. V. (2020). Parkinson Disease. *European Journal of Neurology*, **27**(1), 27-42. https://doi.org/10.1111/ene.14108

Borrello, S., Nicolò, C., Delogu, G., Pandolfi, F., & Ria, F. (2011). TLR2: A Crossroads Between Infections and Autoimmunity? *International Journal of Immunopathology and Pharmacology*, **24**(3), 549–556. https://doi.org/10.1177/039463201102400301

Cario, E. (2008). Barrier-Protective Function of Intestinal Epithelial Toll-Like Receptor 2. *Mucosal Immunology*, **1**, S62–S66. https://doi.org/10.1038/mi.2008.47

Chen, W. W., Zhang, X., & Huang, W. J. (2016). Role of Neuroinflammation in Neurodegenerative Diseases (Review). *Molecular Medicine Reports*, **13**(4), 3391–3396. https://doi.org/10.3892/mmr.2016.4948

Cherry, J., Olschowka, J., & O'Banion, K. (2014). Neuroinflammation and M2 Microglia: The Good, The Bad, and The Inflamed. *Journal of Neuroinflammation*, **11**, 98. https://doi.org/10.1186/1742-2094-11-98

Cui, L., Zahedi, P., Saraceno, J., Bristow, R., Jaffray, D., & Allen, C. (2013). Neoplastic Cell Response to Tiopronin-Coated Gold Nanoparticles. *Nanomedicine: Nanotechnology, Biology, and Medicine*, **9**(2), 264-273. https://doi.org/10.1016/j.nano.2012.05.016

Dzamko, N., Gysbers, A., Perera, G., Bahar, A., Shankar, A., Gao, J., *et al*. (2017). Toll-Like Receptor 2 is Increased in Neurons in Parkinson's Disease Brain and May Contribute to Alpha-Synuclein Pathology. *Acta Neuropathologica*, **133**(2), 303–319. https://doi.org/10.1007/s00401-016-1648-8

Ekins, S., Mestres, J., & Testa, B. (2007). In silico Pharmacology for Drug Discovery: Applications to Targets and Beyond. *British Journal of Pharmacology*, **152**(1), 21-37. https://doi.org/10.1038/sj.bjp.0707306

Engler-Chiurazzi, E. B., Brown, C. M., Povroznik, J. M., & Simpkins, J. W. (2017). Estrogens as Neuroprotectants: Estrogenic Actions in the Context of Cognitive Aging and Brain Injury. *Progress in Neurobiology*, **157**, 188-211. https://doi.org/10.1016/j.pneurobio.2015.12.008

Ferreira, L., Santos, R., Glaucius, O., & Andricopulo, A. (2015). Molecular Docking and Structure-Based Drug Design Strategies. *Molecules*, **20**(7), 13384-13421. https://doi.org/10.3390/molecules200713384

Gao, W., Xiong, Y., Li, Q., & Yang, H. (2017). Inhibition of Toll-Like Receptor Signaling as a Promising Therapy for Inflammatory Diseases: A Journey from Molecular to Nano Therapeutics. *Frontiers in Physiology*, **8**, 508. https://doi.org/10.3389/fphys.2017.00508

Gelosa, P., Colazzo, F., Tremoli, E., Sironi, L., & Castiglioni, L. (2017). Cysteinyl Leukotrienes as Potential Pharmacological Targets for Cerebral Diseases. *Mediators of Inflammation*, 2017, 3454212. https://doi.org/10.1155/2017/3454212

Hwang, S. Y., Shin, J. H., Hwang, J. S., Kim, S. Y., Shin, J. A., Oh, E. S., *et al*. (2010). Glucosamine Exerts A Neuroprotective Effect Via Suppression of Inflammation in Rat Brain Ischemia/Reperfusion Injury. *Glia*, **58**(15), 1881–1892. https://doi.org/10.1002/glia.21058

Kwon, S., Iba, M., Masliah, E., & Kim, C. (2019). Targeting Microglial and Neuronal Toll-like Receptor 2 in Synucleinopathies. *Experimental Neurobiology*, **28**(5), 547–553. https://doi.org/10.5607/en.2019.28.5.547

Lewis, P. A. & Spillane J. E. (2018). *Neurodegenerative Disease 1st edition*. Cambridge: Academic Press.

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (1997). 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

Ma'arif, B., Mirza, D. M., Suryadinata, A., Muchlisin, M. A., & Agil, M. (2019). Metabolite Profiling of 96% Ethanol Extract from Marsilea crenata Presl. Leaves Using UPLC-QToF-MS/MS and Anti-Neuroinflammatory Predicition Activity with Molecular Docking. *Journal of Tropical Pharmacy and Chemistry*, **4**(6), 261-270. https://doi.org/10.25026/jtpc.v4i6.213

Ma'arif, B. (2020). Aktivitas Antineuroinflamasi Ekstrak dan Fraksi Daun Semanggi (M. crenata Presl.) terhadap sel Mikroglia HMC3. *Disertasi*. Surabaya: Universitas Airlangga.

Ma'arif, B., Mirza, D. M., Hasanah, M., Laswati, H., & Agil, M. (2020a). Antineuroinflammation Activity Of N-Butanol Fraction of Marsilea crenata Presl. in Microglia HMC3 Cell Line. *Journal of Basic and Clinical Physiology and Pharmacology*, **30**(6), 20190255. https://doi.org/10.1515/jbcpp-2019-0255

Ma'arif, B., Agil, M., & Laswati, H. (2020b). The Enhancement of Arg1 and Activated ERβ Expression in Microglia HMC3 by Induction of 96% Ethanol Extract of Marsilea crenata Presl. Leaves. *Journal of Basic and Clinical Physiology and Pharmacology*, **30**(6), 20190284. https://doi.org/10.1515/jbcpp-2019-0284

Ma'arif, B., Muslikh, F. A., Fihuda, D. A. P., Syarifuddin, S., & Fauziyah, B. (2021a). Prediction of Compounds from 96% Ethanol Extract of Marsilea crenata Presl. Leaves in Increasing Estrogen Receptor-α Activation. *Proceedings of International Pharmacy Ulul Albab Conference and Seminar (PLANAR)*, **1**, 67-76. https://doi.org/10.18860/planar.v1i0.1461

Ma'arif, B., Muslikh, F. A., Anggraini, W., Taek, M. M., Laswati, H., & Agil, M. (2021b). In vitro Anti-Neuroinflammatory Effect of Genistein (4', 5, 7-trihydroxyisoflavone) on Microglia HMC3 Cell Line, and In Silico Evaluation of its Interaction with Estrogen Receptor-β. *International Journal of Applied Pharmaceutics*, **13**(4), 183-187. https://doi.org/10.22159/IJAP.2021.V13S4.43855

Ma'arif, B., Aminullah, M., Saidah, N. L., Muslikh, F. A., Rahmawati, A., Indrawijaya, Y. Y. A., *et al*. (2021). Prediction of antiosteoporosis Activity of Thirty-Nine Phytoestrogen Compounds in Estrogen Receptor-Dependent Manner Through In Silico Approach. *Tropical Journal of Natural Product Research*, **5**(10), 1727-1734. http://dx.doi.org/10.26538/tjnpr/v5i10.6

Ma'arif, B., Muslikh, F. A., Najib, L. A., Atmaja, R. R. D., & Dianti, M. R. (2021d). In Silico Antiosteoporosis Activity of 96% Ethanol Extract of Chrysophyllum cainito L. Leaves. *Proceedings of International Pharmacy Ulul Albab Conference and Seminar (PLANAR)*, **1**, 61-66. https://doi.org/10.18860/planar.v1i0.1460

Ma'arif, B., Fihuda, D. A. P., Muslikh, F. A., Syarifuddin, S., Fauziyah, B., Sari, D. P., *et al*. (2022a). Studi In Silico Penghambatan Aktivasi TLR2 Ekstrak Etanol Daun Semanggi (Marsilea crenata Presl.). *Jurnal Tumbuhan Obat Indonesia*, **15**(1), 31-40. https://doi.org/10.22435/jtoi.v15i1.5792

Ma'arif, B., Muslikh, F. A., Amalia, D., Mahardiani, A., Muchlasi, L. A., Riwanti, P., *et al*. (2022b). Metabolite Profiling of the Environmental-Controlled Growth of Marsilea crenata Presl. and Its In Vitro and In Silico Antineuroinflammatory Properties. *Borneo Journal of Pharmacy*, **5**(3):209-228. https://doi.org/10.33084/bjop.v5i3.3262

Ma'arif, B., Muslikh, F. A., Guhir, A. M., Fitri, H., Najib, L. A., Salmasfattah, N., *et al*. (2022d). Efek Penurunan Ekspresi MHCII Pada Sel Mikroglia HMC3 Teraktivasi M1 Polarity Oleh Fraksi n-Heksana dan Etil Asetat Daun Semanggi (Marsilea crenata Presl.). *Journal Pharmasci (Journal of Pharmacy and Science)*, **7**(1), 35-41. https://doi.org/10.53342/pharmasci.v7i1.271

Ma'arif, B., Suryanto, S., Muslikh, F. A., Suryadinata, A., & Fauziyah, B. (2022c). Systematic Review: Anti-Osteoporosis Potential Activities Of Phytoestrogen Compounds In Chrysophyllum cainito L., Elaeis guineensis Jacq., Lannea acida Rich., Marsilea crenata Presl., and Medicago sativa L. *Jurnal Farmasi Sains dan Komunitas (Journal of Pharmaceutical Sciences and Community)*, **19**(1), 41-52. https://doi.org/10.24071/jpsc.003166

Ma'arif, B., Maimunah, S., Muslikh, F. A., Saidah, N. L., Fihuda, D. A., Khotimah, H., *et al*. (2022e). Efek Ekstrak Daun Marsilea crenata Presl. pada Aktivitas Lokomotor Ikan Zebra. *FARMASIS: Jurnal Sains Farmasi*, **3**(1), 18-24. https://doi.org/10.36456/farmasis.v3i1.5389

Ma'arif, B., Muslikh, F. A., Fihuda, D. A. P., Khotimah, H., Taek, M. M., & Agil, M. (2022f). The Effect of Ethanol Extract of Marsilea crenata Presley Leaves on Rotenone-Induced Zebrafish

Locomotor Activity. *Jurnal Farmasi Sains dan Komunitas (Journal of Pharmaceutical Sciences and Community)*, **19**(2), 87-92. https://doi.org/10.24071/jpsc.004576

Makatita, F. A., Wardhani, R., & Nuraini. (2020). Riset In Silico dalam Pengembangan Sains di Bidang Pendidikan, Studi Kasus: Analisis Potensi Cendana Sebagai Agen Anti-Aging', *Jurnal Sosial, Budaya dan Sains (ABDI)*, **2**(1), 33–39.

Martin Y. C. (2005). A Bioavailability Score. *Journal of Medicinal Chemistry*, **48**(9), 3164–3170. https://doi.org/10.1021/jm0492002

Mizuno, T. (2015) Neuron-Microglia Interaction in Neuroinflammation. *Clinical and Experimental Neuroimmunology*, **6**(3), 225–231. https://doi.org/10.1111/cen3.12228

Muslikh, F. A., Samudra, R. R., Ma'arif, B., Ulhaq, Z. S., Hardjono, S., & Agil, M. (2022a). In Silico Molecular Docking and ADMET Analysis for Drug Development of Phytoestrogens Compound with Its Evaluation of Neurodegenerative Diseases. *Borneo Journal of Pharmacy*, **5**(4):357-366. https://doi.org/10.33084/bjop.v5i4.3801

Muslikh, F. A., Samudra, R. R., & Ma'arif, B. (2023). Prediksi Senyawa Fraksi Etil Asetat Daun Semanggi (Marsilea crenata Presl.) Sebagai Agen Antineuroinflamasi (agonis ERα). *JIKSN: Jurnal Ilmu Kesehatan dan Sains Nusantara*, **1**(1), 10-21.

Nurmianti, L. & Gusmarwani, S. R. (2020). Penentuan Lethal Dose 50% (LD50) Pestisida Nabati dari Campuran Buah Bintaro, Sereh, Bawang Putih, Lengkuas. *Jurnal Inovasi Proses*, **5**(1), 22-26.

Nursamsiar, N., Mangande, M. M., Awaluddin, A., Nur, S., & Asnawi, A. (2020). In Silico Study of Aglycon Curculigoside A and Its Derivatives as α-Amilase Inhibitors. *Indonesian Journal of Pharmaceutical Science and Technology*, **7**(1), 29-37. https://doi.org/10.24198/ijpst.v7i1.23062

Penn, D. J. (2002). *Encyclopedia of Life Sciences: Major Histocompatibility Complex (MHC)*. London, UK: Macmillan Publishers Ltd.

Prieto-Martínez, F. D., Arciniega, M., & Medina-Franco, J. L. (2018). Acoplamiento Molecular: Avances Recientes y Retos. *TIP Revista Especializada En Ciencias Químico-Biológicas*, **21**(Suppl 1), e20180143. https://doi.org/10.22201/fesz.23958723e.2018.0.143

Riwanti, P., Arifin, M. S., Muslikh, F. A., Amalia, D., Abada, I., Aditama, A. P., *et al*. (2021). Effect of Chrysophyllum cainito L. leaves on bone formation in vivo and in silico. *Tropical Journal of Natural Product Research*, **5**(2):260-264. http://dx.doi.org/10.26538/tjnpr/v5i2.8

Sliwoski, G., Kothiwale, S., Meiler, J., & Lowe, E. W. Jr. (2014). Computational Methods in Drug Discovery. *Pharmacological Reviews*, **66**(1), 334-395. https://doi.org/10.1124/pr.112.007336

Siswandono. (2015). *Kimia Medisinal Jilid Satu (2nd edition)*. Surabaya: Universitas Airlangga.

Suhud, F. (2015). Uji Aktivitas In-silico Senyawa Baru 1-Benzil-3-benzoilurea Induk dan Tersubstitusi sebagai Agen Antiproliferatif. *Jurnal Farmasi Indonesia*, **7**(4), 242-251.

Supandi, Yeni, & Merdekawati, F. (2018). In Silico Study of Pyrazolylaminoquinazoline Toxicity by Lazar, Protox and Admet Predictor. *Journal of Applied Pharmaceutical Science*, **8**(9), 119-129. http://dx.doi.org/10.7324/JAPS.2018.8918

Syahputra, G., Ambarsari, L., & Sumaryada, T. (2014). Simulasi Docking Kurkumin Enol, Bismetoksikurkumin dan Analognya sebagai Inhibitor Enzim 12-Lipoksigenase. *Jurnal Biofisika*, **10**(1), 55–67.

Syamsudin, T. (2015). Penyakit Parkinson. In: Syamsuddin, T., Subagya., & Akbar, M. (editor). *Buku Panduan Tatalaksana Penyakit Parkinson dan Gangguan Gerak Lainnya. Kelompok Studi Movement Disorder*. Jakarta: Perhimpunan Dokter Spesialis Saraf Indonesia. 9-31.

Villa, A., Vegeto, E., Poletti, A., & Maggi, A. (2016). Estrogens, Neuroinflammation, and Neurodegeneration. *Endocrine Reviews*, **37**(4), 372–402. https://doi.org/10.1210/er.2016-1007

Yulianti, A. B., Sumarsono, S. H., Ridwan, A., & Yusuf, A. T. (2015). Hubungan Reactive Oxygen Species (Ros), Superoxide Dismutase (Sod) dengan Protein α-Sinuklein-Larut Air pada Batang Otak Tikus yang Diinduksi Rotenon. *Global Medical and Health Communication*, **3**(2), 83-92. https://doi.org/10.29313/gmhc.v3i2.1508