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Insight into the *in silico* Study and Biological Evaluation of Curcumin Analogue Compounds as New Potential Inhibitors for Dengue DEN2 NS2B/NS3 Serine Protease

(Kajian *in silico* dan Penilaian Biologi Sebatian Analog Kurkumin sebagai Perencat Baharu yang Berpotensi untuk Serin Protease Denggi DEN2 NS2B/NS3)

NENI FRIMAYANTI[1,*], NOVAL HERFINDO[1], SITI AISYAH[2] & ENI NUR RAHMAWATY[2]

[1]*Sekolah Tinggi Ilmu Farmasi Riau, Pekanbaru, Indonesia*

[2]*Department of Chemistry, Faculty of Mathematic and Natural Products, Universitas Riau, Kampus Bina Widya KM. 12,5, Simpang Baru, Kec. Tampan, Kota Pekanbaru, Riau 28293*

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ABSTRACT

Dengue is an infectious disease caused by a virus and it is a rapidly emerging pandemic disease in many parts of the world. However, to date, one licensed tetravalent Dengvaxia vaccine based on a yellow fever virus vaccine variant has been reported. *In silico* and biological assay were performed to twenty two curcumin analogue compounds with DEN2 NS2B/NS3 serine protease as target. The main purpose of this study were to predict and estimate the binding interaction and also the ability of curcumin analogue compounds to be potential inhibitors for DEN2 NS2B/NS3. Computational pipeline such as molecular docking and molecular dynamic were constructed to get insight into potential inhibitor for DEN2 NS2B/NS3. Biological assay was performed to validate *in silico* results. Docking results reported that compounds **3**, **10**, and **13** have the lowest binding free energy value of -15.2 kcal/mol, -13.66 kcal/mol and -13.68 kcal/mol, respectively. All these three compounds were also able to interacts with Lys74 (i.e., allosteric site of serine protease) through hydrogen bonding, these binding is keep maintain during the molecular dynamic simulation. Among all of the compounds tested on their percent inhibition against DEN2 NS2B/NS3, compounds **3**, **10**, and **13** exhibited the best of percent inhibition. Thus, three of these compounds can be used as potential candidate for the next stage in the drug discovery process.

Keywords: ADME; curcumin; dengue DEN2 NS2B/NS3; docking; molecular dynamic

ABSTRAK

Denggi ialah penyakit berjangkit yang disebabkan oleh virus dan ia merupakan penyakit pandemik yang cepat muncul di seluruh bahagian dunia. Namun setakat ini, hanya satu vaksin tetravalen yang berlesen, Dengvaxia, yang berdasarkan varian vaksin virus demam kuning yang telah dilaporkan. *In silico* dan ujian biologi telah dilakukan kepada dua puluh dua sebatian analog kurkumin dengan DEN2 NS2B/NS3 serine protease sebagai sasaran. Tujuan utama kajian ini adalah untuk meramal dan menganggar interaksi pengikatan dan juga keupayaan sebatian analog kurkumin menjadi perencat yang berpotensi untuk DEN2 NS2B/NS3. Saluran paip pengiraan seperti dok molekul dan dinamik molekul telah dibina untuk mendapatkan pandangan tentang potensi perencat untuk DEN2 NS2B/NS3. Ujian biologi dilakukan untuk mengesahkan keputusan *in silico*. Keputusan dok melaporkan bahawa sebatian **3**, **10** dan **13** mempunyai nilai tenaga bebas pengikatan terendah masing-masing -15.2 kcal/mol, -13.66 kcal/mol dan -13.68 kcal/mol. Ketiga-tiga sebatian ini juga dapat berinteraksi dengan Lys74 (iaitu tapak alosterik protease serin) melalui ikatan hidrogen, pengikatan ini dikekalkan semasa simulasi dinamik molekul. Antara semua sebatian yang diuji pada perencatan peratus mereka terhadap DEN2 NS2B/NS3, sebatian **3**, **10** dan **13** menunjukkan perencatan peratus terbaik. Oleh itu, tiga daripada sebatian ini boleh digunakan sebagai calon berpotensi untuk peringkat seterusnya dalam proses penemuan dadah.

Kata kunci: ADME; denggi DEN2 NS2B/NS3; dinamik molekul; dok; kurkumin

INTRODUCTION

Dengue is a serious re-emerging vector-borne viral infectious disease, with a substantial increase in the number of dengue epidemics in the past 10 years. It is reported to be endemic in over 100 countries, with current estimates of between 50 and 100 million cases of dengue fever per annum worldwide (Brady et al. 2016). Dengue infection is caused by the dengue virus, a member of the Flaviviridae family. There are four serotypes of dengue viruses (DEN1, DEN2, DEN3 and DEN4), with the most prevalent being dengue virus type 2 (DEN2). Currently, one licensed tetravalent vaccine, Dengvaxia, which is based on a yellow fever virus vaccine variant, has been reported. Clinical studies have shown that the protective efficacy of this vaccine varies across different dengue virus serotypes, with rates of 61.2%, 81.9%, and 90% for serotypes 1, 3, and 4, respectively (Shukla et al. 2020). However, the vaccine does not provide protection against dengue virus serotype 2. A major disadvantage of this vaccine is the potential danger it poses to individuals who have not previously contracted dengue fever.

Dengue fever (DF) is a major health concern. Because of this, there are many studies on antivirals, and information about this topic has also grown every year. Natural compounds that have shown potential as anti-dengue drugs are curcuminoid group (Osman et al. 2017). Curcuminoids are diarylheptanoid derivatives with turmeric (*Curcuma longa*) as the main pigment. One of the curcuminoid compounds is curcumin, which is known to have anticancer activity (Handler et al. 2007). Curcumin is the main pigment found in the Curcuma turmeric plant longa. Curcuminoids are generally used as additives (coloring food). In addition, curcumin has a broad range of biological and pharmacological properties, including antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, and antifibrotic properties (Achmad et al. 2007). The double bonds in curcumin's core chain, diketone group, and hydroxy phenolic group are linked to its pharmacological effects (Liang et al. 2008).

In silico studies are crucial for drug design (Frimayanti et al. 2020a). Molecular docking and molecular dynamics can provide information regarding binding orientation and estimate the activity of drug candidates. Recently, there have been few reports (Norshidah et al. 2023; Roney et al. 2023) on curcumin compounds for the discovery of new drugs against dengue DEN2 NS2B/NS3 using *in silico* tools. In this study, we identify novel potential candidates against DEN2 NS2B/NS3 through *in silico* studies, and subsequently validate the findings through biological activity assessments.

MATERIALS AND METHODS

MOLECULAR DOCKING

Molecular docking was performed using the MOE 2022.0901 software package (Chemical Computing Group). Ligands and proteins were prepared before constructing molecular docking. In this study, 22 curcumin analog compounds were synthesized by our research group (Eryanti et al. 2015, 2014; Zamri et al. 2019) and used as ligands, and panduratin A was used as a positive control. The molecular structures of the ligands and the positive control are depicted in Table 1. For ligand preparation, the molecular structure of these ligands was sketched using ChemDraw and then copied into the MOE 2022.0901 (Chemical Computing Group) software package to create a database and then saved in mdb format.

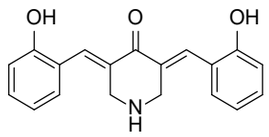
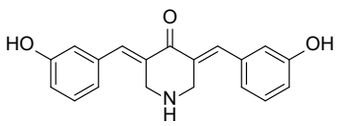
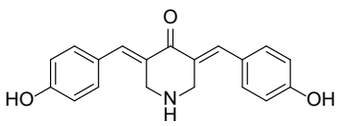
The protein structure was retrieved from the www.rcsb.org website with the PDB code 2FOM. The crystal structure of this protein was created using MOE 2022.0901 and DSV 2020 (Biovia). Chains A and B comprise two chains that make up the 2FOM protein. In addition, the water molecules, natural ligands, and Cl ions of the protein were eliminated. The molecular structure of the proteins was determined using the MOE 2022.0901 software package. The protein was then constructed using the parameter, i.e., the RMS gradient, set to 0.01 kcal/mol/A, with CHARMM27 as the force field. Alpha carbon, H, and backbone atoms are all subject to energy minimization (Frimayanti et al. 2011). Subsequently, the constructed structure was saved in PDB format so that it could later be used as a docking receptor.

The active site of the protein was identified before docking using a site finder. Site 13 contained several amino acid residues, including Leu128, Asp129, Phe130, Ser131, Pro132, Ser135, Tyr150, Gly151, and Gly153. Site 13 also contained several residues, including His51, Lys74, Asp75, Gly151, Asn152, Gly153, and Val154, which were set as dummy atoms to act as the target side for the docking process. The site was then set to a dummy atom in the dock menu, and the MDB file containing the ready-made ligand structure was selected as the ligand. Next, the refinement was set to be rigid, the pose was set to 50 and 10, and the placement was set as a triangle. Furthermore, a docking process could be performed.

MOLECULAR DYNAMIC

Molecular dynamics simulation (MD) is the next step in an *in silico* study. A preliminary MD study was developed using Nanoscale Molecular Dynamic program v2.9 with CHARMM27 (Chemistry at Harvard Macromolecular Mechanics) as the best selected force field. Modelled protein was achieved using TIP3P with a 2.5 Å water layer for each direction of the coordinated structure.

TABLE 1. Molecular structure of ligands and positive control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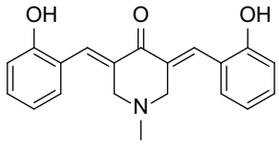	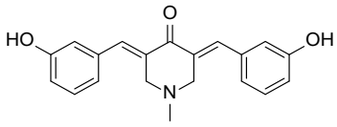	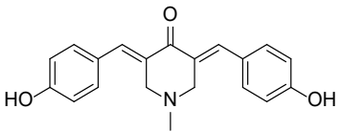
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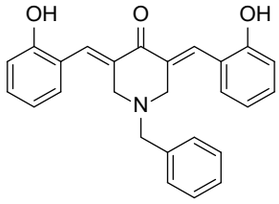	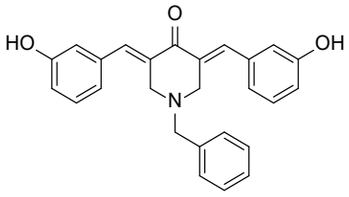	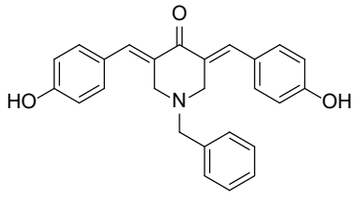
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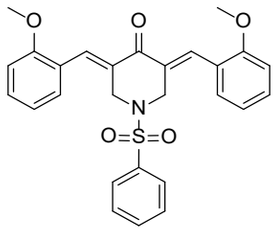	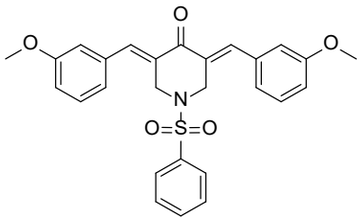	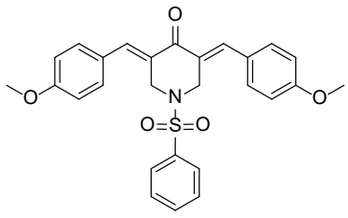
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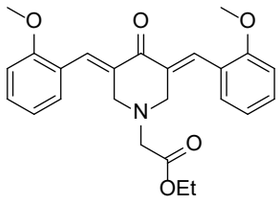	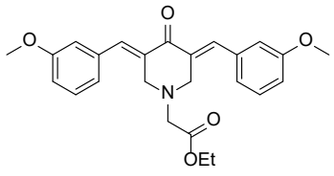	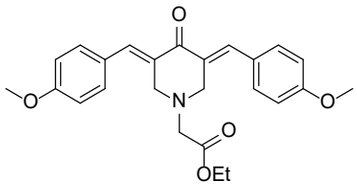
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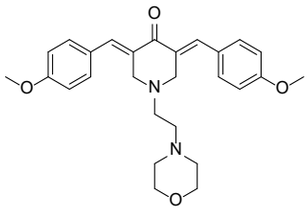	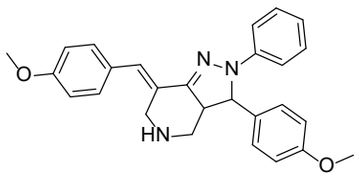	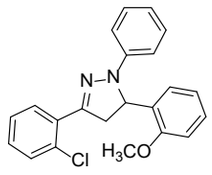
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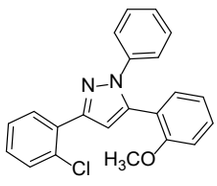	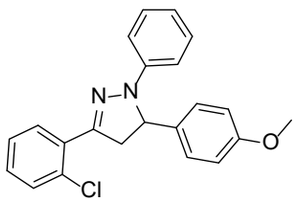	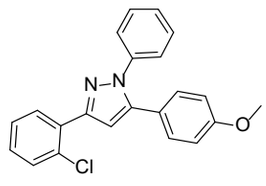
P-1	Pi-3	P-2

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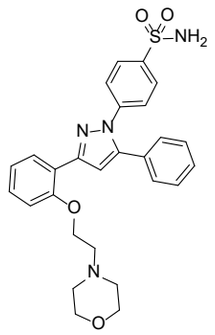

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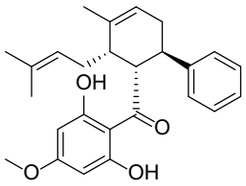


(2,6-dihydroxy-4-methoxyphenyl)((1R,2R,3S)-4-methyl-3-(3-methylbut-2-en-1-yl)-1,2,3,6-tetrahydro-(1,1'-biphenyl)-2-yl)methanone

Panduratin A

The heat of the system was prepared using a canonical ensemble (NVT) with temperature variation from 0 to 300 K over 100 ps. An isothermal isobaric ensemble (NPT) with periodic boundary conditions was used to conduct the MD simulation on a time scale of 50 ns for each system. The coordinates during the sampling process were recorded every 0.1 ps. The binding free energy were further calculated based on the best conformation from this simulation and decomposition process was also determined (Milne, Nicklaus & Wang 1998).

ADME PROFILING

ADME analysis was designed to examine the pharmacokinetics of a certain molecule that might be used as a medication. SwissADME (http://www.swissadme.ch/index.php) was used to make ADME forecasts online.

BIOLOGICAL ACTIVITY ASSAY

EXPRESSION AND PURIFICATION OF NS2B-NS3

Escherichia coli BL21 (DE3) was used to express the recombinant protein pET14-NS2B-NS3. The cells were inoculated in LB medium containing 100 g/mL ampicillin, and *E. coli* BL21 (DE3) with pET14-NS2B-NS3 expression vectors was allowed to develop overnight at 37 °C. 10 mL of the culture was incubated with 1 L of LB medium at 37 °C with shaking until the absorbance at 600 nm reached 0.8. The cells were subsequently stimulated with 0.5 mM IPTG and harvested the next day by centrifuging at 12.000 rpm for 10 minutes at 4 °C. The cells were lysed using a sonicator and the protein was extracted through ultracentrifugation. To purify the His-tagged fusion protein at 4 °C, the protein solution was filtered through a membrane filter (0.22 M), and the supernatant was then applied to a Ni-NTA agarose column that was charged with Ni^{2+}. Buffer A (Tris-HCl pH 8.5, 20 mM Imidazole, and 0.5% glycerol) was used to wash the protein-containing column, and Buffer B (Tris-HCl pH 8.5, 0.5% glycerol, and 250 mM imidazole) was used to elute the sample.

DENV2 NS2B-NS3 PROTEASE INHIBITION ASSAY

The bioassay used in this study was previously published by Rothan et al. (2012a). Reaction mixtures (100 µL) were prepared consisting of 10 mM fluorogenic peptide substrate (Boc-Gly-Arg-Arg-MCA), 0.5 µM recombinant DENV2 NS2B-NS3, buffered at pH 8.5 with 200 mM Tris-HCl. Three types of reactions were performed: buffer only, buffer with enzyme, and buffer with enzyme and 200 ppm plant extract (i.e., panduratin A). The drug inhibitors were pre-incubated with NS2B/NS3pro at 37 °C for 10 min prior to addition of the fluorogenic peptide substrate.

The assay mixtures were incubated for another 1 hour at the same temperature. The positive control (100 L of 200 mM Tris-HCl alone) and the blank control (100 M substrate BOC-GRR-MCA and 0.5 M NS2B/NS3pro in 200 mM Tris-HCl) were run concurrently to validate the assay conditions. There were four replicates for each experiment (n = 4). The percentage of inhibitory activity of the peptide inhibitors was then calculated using the fluorescence signals. The assay was performed on a Tecan M1000 PRO microplate reader in a black 96-well flat-bottom plate format. The fluorescence reading at an emission wavelength of 410-460 nm following excitation at 365 nm was measured using a microplate reader.

RESULTS AND DISCUSSION

MOLECULAR DOCKING

Generally, molecular docking can be used as a tool to predict the binding orientation and best conformation of complex protein ligands. Docking was performed on 22 of these curcumin analog compounds, and the docking results are presented in Table 2. According to the docking results, the best conformation of the complex protein-ligand was selected based on parameters such as the lowest value of binding free energy, the root mean square deviation (RMSD) less than 2, and the existence of hydrogen bonding with the active site or allosteric site (Frimayanti et al. 2020b; Zhong et al. 2013).

TABLE 2. Docking results

Compound	Binding free energy (Kcal/mol)	RMSD	Hydrogen bonding
1	-8.60	0.00	Asn152, Asn167
2	-9.20	0.00	Gly87
3	**-15.20**	**0.00**	**Lys74, Asn162**
4	-10.12	0.00	Lys74
5	-13.06	0.00	Trp83, Trp89
6	-13.05	0.00	Arg55, His60, Lys61
7	-11.12	0.00	Thr120, Asn152
8	-9.23	0.00	Lys74, Glu88
9	-8.78	0.00	Lys73, Gly87, Asn152
10	**-13.66**	**0.00**	**Lys74**
11	-10.05	0.00	-
12	-9.89	0.00	Arg54
13	**-13.68**	**0.00**	**-**
14	-8.97	0.00	His51
15	-7.90	0.00	-
16	-7.56	0.00	Gly153
Pi-1	-8.95	0.00	Gly153
Pi-2	-9.23	0.00	Lys74, Trp83
P-1	-9.12	0.00	Lys74, Trp83
Pi-3	-8.56	0.00	Trp83
P-2	-10.23	0.00	-
P-3	-9.23	0.00	-
Panduratin A	-16.32	0.00	His51, Gly153

Based on the docking results, three compounds (compounds **3**, **10**, and **13**) were estimated to be active DEN2 NS2B/NS3 inhibitors. Compound **3** exhibited two hydrogen bonds with Lys74 (i.e., the allosteric site) and Asn162; this compound was also able to form hydrophobic interactions with Lys73. In addition, compound **3** had a binding free energy value of -15.2 kcal/mol with an RMSD value of 0.000. This may cause the compound to be identified as an active compound.

As shown in Table 2, the docking results showed that the RMSD for all curcumin analogue compounds was zero. Compound **10** has the lowest binding free energy of -13.66 kcal/mol and can explore one hydrogen bond with Lys74 (i.e., an allosteric site) with an RMSD less than 2. A stable interaction with the protein serine protease was estimated as an active compound. However, another compound, compound **14**, interacted with one of the active sites, His51. Unfortunately, this compound has a higher binding free energy of -8.97 kcal/mol, thus it cannot be considered as an active compound.

Compound **13** was considered a potentially active compound with a binding free energy value of -13.68 kcal/mol and the RMSD value was less than 2. No hydrogen bonding with the protein was observed for this compound, however, compound **13** was able to interact with one of the active sites, His51, through hydrophobic interactions. Figure 1 shows the spatial arrangement of compounds **3**, **10**, and **13**.

Panduratin A was used as positive control has binding free energy of -16.32 kcal/mol and an RMSD value of 0.00. In the active site of NS2B/NS3 serine protease, panduratin A was also able to create two hydrogen bonds with two amino acid residues. In this instance, a hydrogen bond is created at a distance of 2.75 Å between the O atom in the carbonyl group (C=O) of the panduratin A molecule and the His51 amino acid residue. Additionally, hydrogen bonds are created at a distance of 2.98 Å between the panduratin A hydroxy group and the amino acid residue Gly153 (Frimayanti et al. 2023a, 2023b).

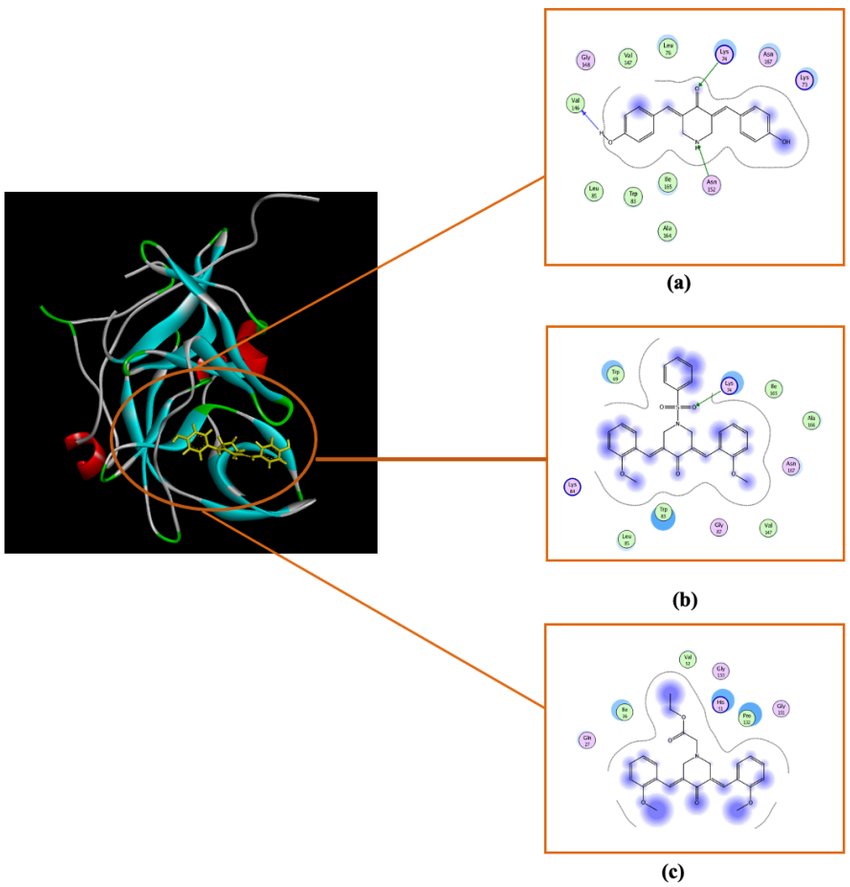


FIGURE 1. Spatial arrangement of compounds (a) **3** (b) **10** and (c) **13**

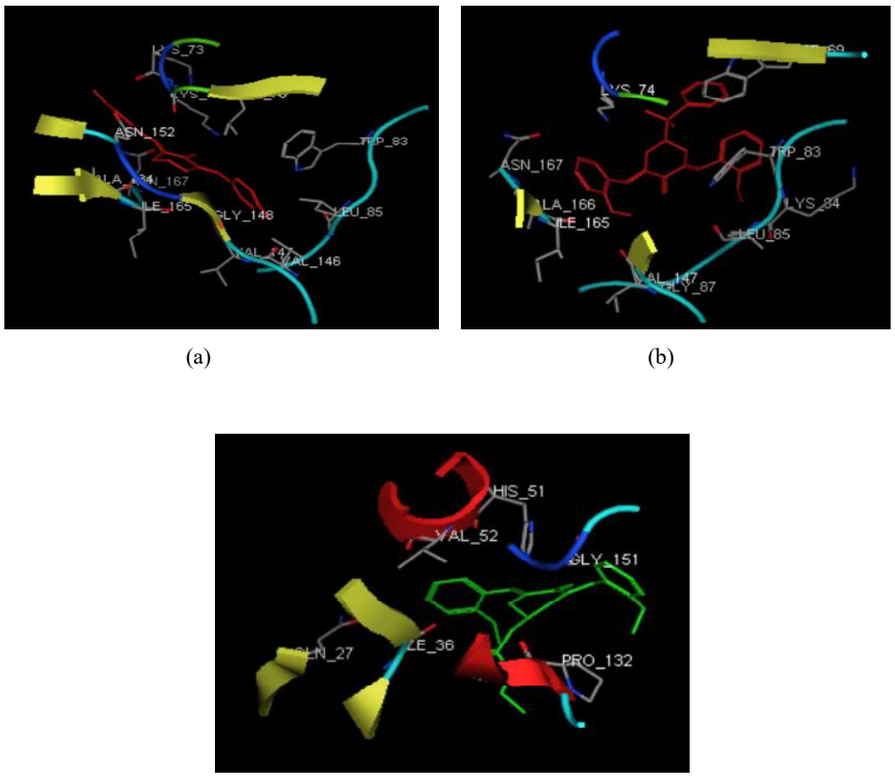
MOLECULAR DYNAMIC

MD simulations were performed to check the stability or flexibility of the complex protein ligands with variations in temperature, pressure, or volume. MD simulation was applied to ensure the orientation of binding interactions between the ligands and the protein (Al-Karmalawy et al. 2021). In addition, MD can also provide an overall impression of the estimated active curcumin analog compounds. MD simulation was run for 300 ns and initiated using the high stability with minimum energy at a temperature of 300 K to determine the affinity of the ligand to the binding site.

MD simulation results were investigated by examining the stability and efficiency of hydrogen binding in three potentially active compounds: **3**, **10**, and **13**. Generally, compounds **3**, **10**, and **13** showed the ability to maintain the binding interaction with the same residue before and after the MD simulation. Furthermore, the hydrogen bond was maintained when the distance was less than 2.9 Å. In different cases with other compounds, it seemed to lose their activity because the presence of some interactions between the ligand and receptor was not maintained, and they were also unable to maintain the existence of hydrogen bonds. The visualization of the MD simulation for compounds **3, 10**, and **13** is shown in Figure 2.

Based on the MD simulation, it was assumed that compounds **3, 10**, and **13** could maintain the hydrogen bond. The hydrogen bond distance over the simulation time was important for elucidating the affinity of the potential compound. Thus, three of these compounds can be used as potentially active DEN2 NS2B/NS3 inhibitors. Table 3 presents the interactions with the amino acids before and after the simulations.

The complex dynamic behaviour was evaluated using root mean square deviation (RMSD) and root mean square fluctuation (RMSF). RMSD was used to assess the conformational stability of the complex, structural, and dynamic measures. If the RMSD value of a protein is larger, it is less stable. This calculation led to the conclusion that complexes **3**, **10**, and **13** proteins oscillated between 50 and 100 ns, with an average RMSD of 0.36 nm. The average RMSD of this complex compound-protein combination varied somewhat before 50 ns but remained consistent throughout the simulation length. The typical RMSD for these compounds was approximately 0.36 nm. Figure 3 shows the RMSD values for three of these compounds.



(a) (b)

(c)

FIGURE 2. Visualization of MD simulation for compounds (a) **3** (b) **10** and (c) **13**

ADME PROFILING

One of the important processes for maximizing the screening and testing of drug candidates and lowering the risk of late stages of drug development is Absorption, Distribution, Metabolism, and Excretion (ADME). In this study, Lipinski's rule of five was used to predict the properties of the drug-like molecules.

The degree of absorption or permeability of prospective chemicals to pass through the lipid bilayer in the human body was calculated using Lipinski's rule of five (Lipinski et al. 2012). If a drug complies with the Lipinski rule (maximum MW 500, log P not larger than 5, hydrogen bond donors not more than 5, and hydrogen bond acceptors not more than 10), it can be anticipated that it will have high bioavailability. Compounds **3**, **10**, and **13** adhered to Lipinski rule-based drug candidate standards based on ADME calculations. The log P values for each of these compounds were 2.28, 4.93, and 3.50, respectively.

Log P is a partition coefficient that influences drug transfer throughout the pharmacokinetic phase (drug solubility in water or fat). As a result, it can be used to pinpoint the location of a drug's action. The chemical will be too soluble in water if the log P value is too low, yet it could not be able to cross the lipid barrier. In contrast, the molecule dissolves in fat if the log P value is too high and may occasionally fail to reach the target (Khelfaoui, Harkati & Saleh 2021; Kiat et al. 2006).

New drug discovery is important for predicting the predisposition of molecules that will cause significant drug interactions through CYP inhibition as well as for determining which isoforms are affected. Based on ADME calculations using SwissADME, three compounds (**3, 10**, and **13**) showed potential to inhibit CYP2C9, CYP2D6, and CYP3A4. However, these compounds did not have the potential to inhibit CYP1A2 and CYP2C19. Overall, the ADME profiles of these three compounds showed reasonable medicinal properties. The findings demonstrated that compounds **3, 10** and **13** followed the Lipinski criteria, and also showed potential to inhibit CYP2C9, CYP2D6, and CYP3A4. There is no alert was obtained in PAIN for these three compounds, it indicated that there are no frequently reproducing structurally promiscuous moieties. In addition, compounds **3, 10**, and **13** also complies the rule of five (RO5), this compound is easily absorbed and has good permeability and may not dramatically effect on the structural moiety of this potential candidate (Syahri et al. 2023). Table 4 presents the ADME profiles of the selected compounds.

TABLE 3. Interaction of ligands with amino acid

Compound	Docking results	MD results	Distance of H bond
3	Lys73, Lys74	Lys73, Lys74	2.90 Å
10	Lys74	Lys74	2.90 Å
13	His51	His51	2.90 Å

TABLE 4. ADME profiling

Profiles	3	10	13
MW	323.39	475.56	421.49
Log concentration ($P_{o/w}$)	2.28	4.93	3.50
Num. H-bond donors	3	0	0
Num. H-bond acceptors	4	6	6
Rotable bonds	2	6	8
Druglikeness (Lipinski)	Yes	Yes	yes
Water solubility (mg/mL)	2.93e-02 (moderately soluble)	-	2.27e-0.5 (moderately soluble)
Bioavailability score	0.55	0.55	0.55
CYP1A2 inhibitor	Yes	Yes	No
CYP2C19 inhibitor	No	No	Yes
CYP2C9 inhibitor	No	No	Yes
CYP2D6	Yes	Yes	Yes
CYP3A4	No	No	yes

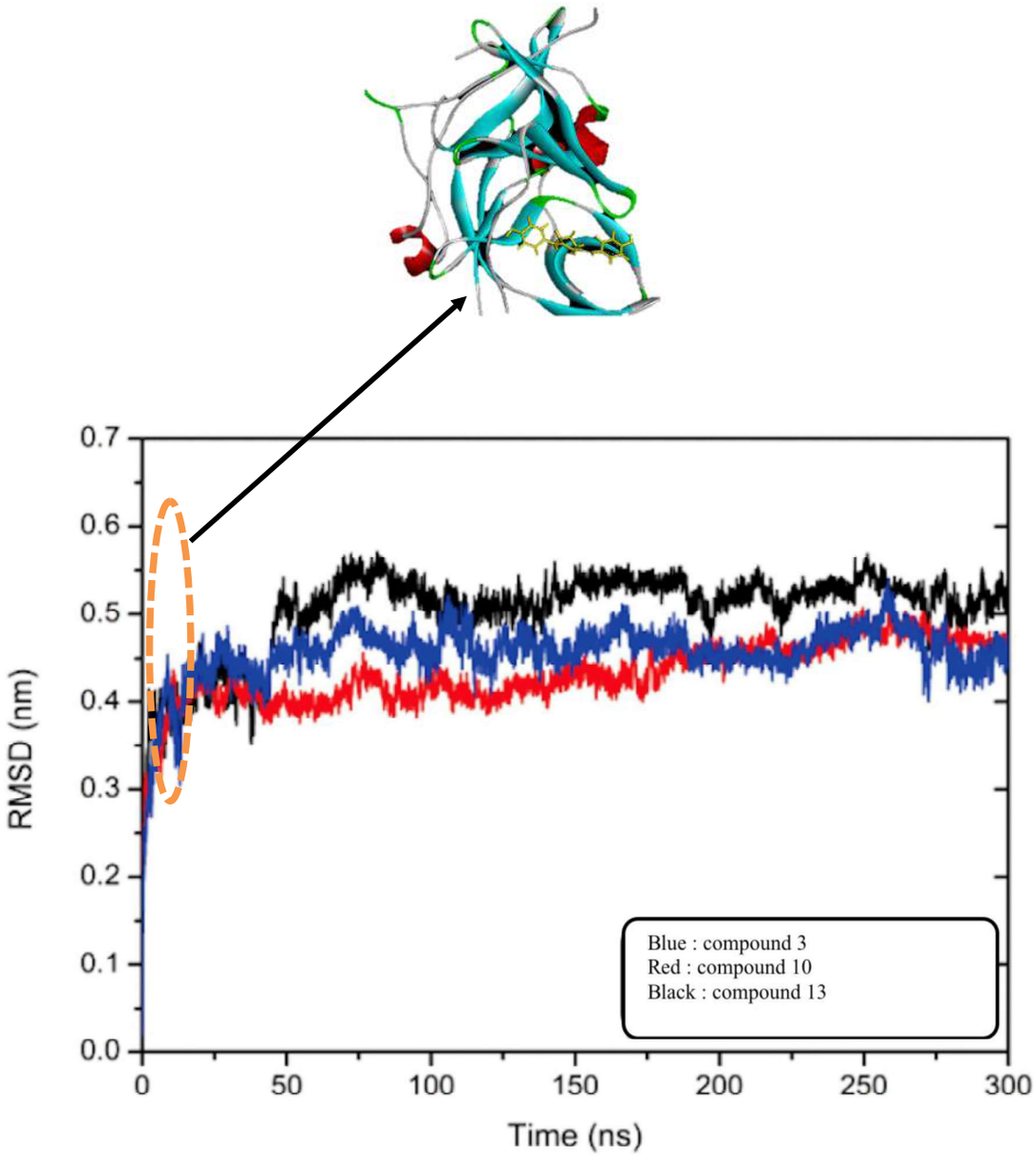


FIGURE 3. Root mean square deviation (RMSD) for compounds 3, 10, and 13

DEN2 NS2B/NS3 PROTEASE INHIBITION ASSAY

Panduratin A was reported to be an active compound that inhibits DENV2 NS2B/NS3 serine protease with a Ki value of 25 µM and percentage inhibition of 90.4% (Kiat et al. 2006). According to panduratin A Ki value and percent inhibition, among the 22 curcumin analogue compounds, only these three compounds, i.e., **3**, **10**, and **13** exhibited significant inhibition activity (>60% inhibition) towards DEN2 NS2B/NS3 (Hariono et al. 2019; Nogrady & Donal 2005). The other compounds exhibited negligible inhibition of the protease activity (Muhamad et al. 2010; Rothan et al. 2012b). Table 5 and Figure 4 show the percent inhibition of all curcumin analog compounds. The protease results confirmed that these three compounds were present *in silico*. Furthermore, all three compounds seemed to have high potency to become DEN2 NS2B/NS3 inhibitors.

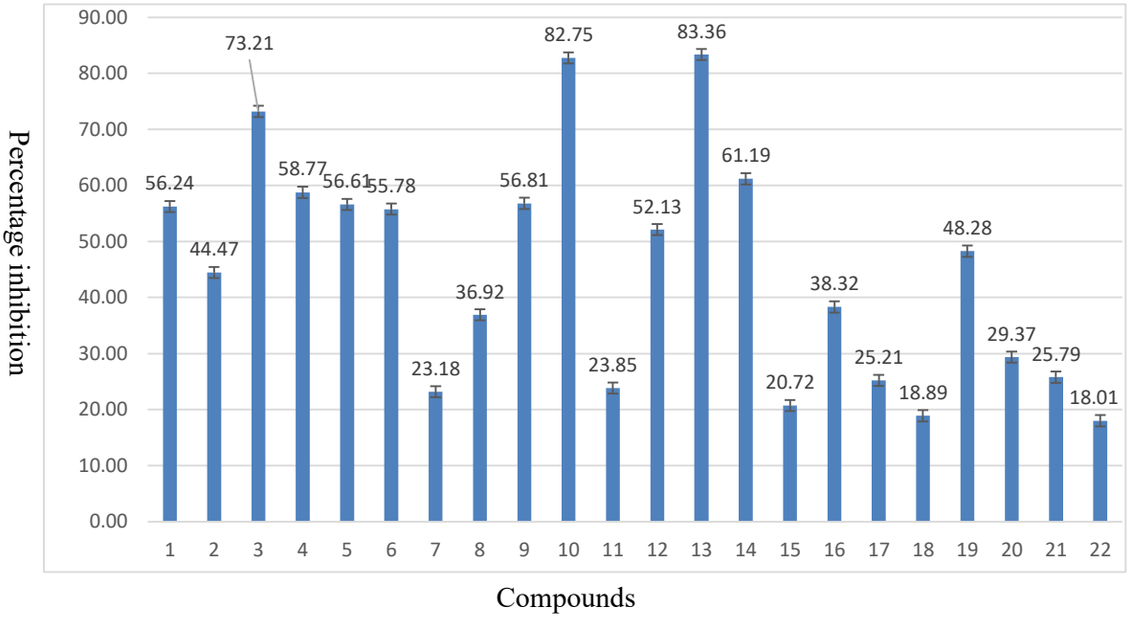


FIGURE 4. Graph for biological assay results

TABLE 5. Percent inhibition of curcumin anologue compounds

Compound	% Inhibition	Compound	% Inhibition
1	56.40	2	44.14
3	**73.34**	4	58.77
5	56.81	6	55.78
7	23.18	8	36.92
9	56.81	**10**	**82.76**
11	23.18	12	52.13
13	**83.36**	14	61.91
15	21.05	16	38.09
Pi-1	25.21	Pi-2	18.89
P-1	48.28	Pi-3	29.37
P2	25.79	P3	18.01
Panduratin A	90.40		

CONCLUSION

In silico studies can be used to predict and estimate the potential of curcumin analog compounds against DEN2 NS2B/NS3. According to docking and MD simulation results it was found that compounds **3**, **10**, and **13** has huge potential to become DEN2 NS2B/NS3 inhibitor with the binding free energy of -15.2 kcal/mol, -13.66 kcal/mol and -13.68 kcal/mol, respectively. The *in silico* results were consistent with those of the protease inhibition assay. Hence, compounds **3**, **10**, and **13** were selected as references for the next stage of drug discovery.

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REFERENCES

Achmad, S.A., Hakim, E.H., Makmur, L., Syah, Y.M., Juliawaty, L.D. & Mujahidin, D. 2007. *Ilmu Kimia dan Kegunaan Tumbuh-tumbuan Obat Indonesia.* Bandung: ITB.

Al-Karmalawy, A.A., Dahab, M.A., Metwaly, A.M., Elhady, S.S., Elkaeed, E.B., Eissa, I.H. & Darwish, K.M. 2021. Molecular docking and dynamics simulation revealed the potential inhibitory activity of ACEIs against SARS-CoV-2 targeting the *h*ACE2 receptor. *Front Chem.* 9: 661230. doi: 10.3389/fchem.2021.661230

Brady, O.J., Gething, P.W., Bhatt, S., Messina, J.P., Brownstein, J.S., Hoen, A.G., Moyes, C.L., Farlow, A.W., Scott, T.W. & Hay, S.I. 2016. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. *PLoS Negl. Trop. Dis.* 6(8): e1760. doi: 10.1371/journal.pntd.0001760

Eryanti, Y., Herlina, T., Zamri, A., Shiono, Y., Awang, K., Halim, S.N.A. & Supratman, U. 2015. N-benzyl-(3E,5E)-3,5-bis(2-hydroxybenzylidene)-4-piperidone. *Molbank.* M852. https://doi.org/10.3390/M852

Eryanti, Y., Herlina, T., Zamri, A., Halim, S.N.A., Shiono, Y., Syah, Y.M., Awang. K. & Supratman, U. 2014. 3,5-Bis(2-hydroxybenzylidene)piperidin-4-one. *Molbank.* M825. https://doi.org/10.3390/M825

Frimayanti, N., Yaeghoobi, M., Namavar, H., Ikhtiarudin, I. & Afzali, M. 2020a. *In silico* studies and biological evaluation of chalcone-based 1,5-benzothiazepines as new potential H1N1 neuraminidase inhibitors. *J. Appl. Pharm. Sci.* 10(10): 086-094. Doi: 10.7324/JAPS.2020.1010010

Frimayanti, N., Ikhtiarudin, I., Dona, R., Agustini, T.T., Murdiya, F. & Zamri, A. 2020b. Computational approach to drug discovery: Search for chalcone analogues as the potential candidates for anti colorectal cancer (HT29). *Walailak. J. Sci. Technol.* 12(2): 64-74. https://doi.org/10.48048/wjst.2020.5910

Frimayanti, N., Ikhtiarudin, I., Septama, A.W., Susanty, A. & Isroq, N.D. 2023a. Synthesis, *in silico* and structural insight of flavonol derivative compounds as new competitive dengue NS2B/NS3 protease inhibitor. *Journal of Research in Pharmacy* 27(3): 1157-1169. http://dx.doi.org/10.29228/jrp.406

Frimayanti, N., Ikhtiarudin, I., Dona, R., Putri, I. & Septama, A.W. 2023b. Synthesis of 3'-methoxy flavonol and its derivatives as potential inhibitors for Dengue NS2B/NS3 and molecular insight into binding interaction. *Pharmacy Education* 23(2): 231-243. https://doi.org/10.46542/pe.2023.232.231243

Frimayanti, N., Chee, C.F., Zain, S.M. & Rahman, N.A. 2011. Design of new competitive dengue Ns2b/Ns3 protease inhibitors - A computational approach. *International Journal of Molecular Sciences* 12(2): 1089-1100. https://doi.org/10.3390/ijms12021089

Handler, N., Jaeger, W., Puschacher, H., Leisser, K. & Erker T. 2007. Synthesis of novel curcumin analogues and their evaluation as selective cyclooxygenase-1 (COX-1) inhibitors. *Chem. Pharm. Bull. (Tokyo)* 55(1): 64-71. doi: 10.1248/cpb.55.64

Hariono, M., Choi, S.B., Roslim, R.F., Nawi, M.S., Tan, M.L., Kamarulzaman, E.E., Mohamed, N., Yusof, R., Othman, S., Abd Rahman, N., Othman, R. & Wahab, H.A. 2019. Thioguanine-based DENV-2 NS2B/NS3 protease inhibitors: Virtual screening, synthesis, biological evaluation and molecular modelling. *PLoS ONE* 14(1): e0210869. doi:10.1371/journal.pone.0210869

Khelfaoui, H., Harkati, D. & Saleh, B.A. 2021. Molecular docking, molecular dynamics simulations and reactivity, studies on approved drugs library targeting ACE2 and SARS-CoV-2 binding with ACE2. *J. Biomol. Struct. Dyn.* 39(18): 7246-7262. doi: 10.1080/07391102.2020.1803967

Kiat, T.S., Pippen, R., Yusof, R., Ibrahim, H., Khalid, N. & Rahman, N.A. 2006. Inhibitory activity of cyclohexenyl chalcone derivatives and flavonoids of fingerroot, *Boesenbergia rotunda* (L.), towards dengue-2 virus NS3 protease. *Bioorg. Med. Chem. Lett.* 16(12): 3337-3340. doi:10.1016/j.bmcl.2005.12.075

Liang, G., Yang, S., Jiang, L., Zhao, Y., Shao, L., Xiao, J., Ye, F., Li, Y. & Li, X. 2008. Synthesis and anti-bacterial properties of mono-carbonyl analogues of curcumin. *Chem Pharm Bull (Tokyo)* 56(2): 162-167. doi:10.1248/cpb.56.162

Lipinski, C.A., Lombardo, F., Dominy, B.W. & Feeney, P.J. 2012. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews* 64: 4-17. https://doi.org/10.1016/s0169-409x(00)00129-0

Muhamad, M., Kee, L.Y., Rahman, N.A. & Yusof, R. 2010. Antiviral actions of flavanoid-derived compounds on dengue virus type-2. *Int. J. Biol. Sci.* 6(3): 294-302. doi:10.7150/ijbs.6.294

Milne, G.W., Nicklaus, M.C. & Wang, S. 1998. Pharmacophores in drug design and discovery. *SAR QSAR Environ. Res.* 9(1-2): 23-38. doi: 10.1080/10629369808039147

Nogrady, T. & Donal, F.W. 2005. *Medicinal Chemistry: A Molecular and Biochemical Approach*. New York: Oxford University Press.

Norshidah, H., Leow, C.H., Ezleen, K.E., Wahab, H.A., Vignesh, R., Rasul, A. & Lai, N.S. 2023. Assessing the potential of NS2B/NS3 protease inhibitors biomarker in curbing dengue virus infections: *In silico* vs. *In vitro* approach. *Front Cell Infect. Microbiol*. 13: 1061937. doi:10.3389/fcimb.2023.1061937. PMID: 36864886

Osman, H., Idris, N.H., Kamarulzaman, E.E., Wahab, H.A. & Hassan, M.Z. 2017. 3,5-Bis(arylidene)-4-piperidones as potential dengue protease inhibitors. *Acta Pharm. Sin. B.* 7(4): 479-484. doi: 10.1016/j.apsb.2017.04.009

Roney, M., Huq, A.K.M., Moyeenul, A., Mohd, F.F., Mohd, T. & Saiful, N. 2023. *In-silico* design of curcumin analogs as potential inhibitors of dengue virus NS2B/NS3 protease. *Journal of Computational Biophysics and Chemistry* 22(06): 645-653. https://doi.org/10.1142/S2737416523500321

Rothan, H.A., Abdulrahman, A.Y., Sasikumer, P.G., Othman, S., Rahman, N.A. & Yusof, R. 2012a. Protegrin-1 inhibits dengue NS2B-NS3 serine protease and viral replication in MK2 cells. *J. Biomed. Biotechnol.* 2012: 251482. doi: 10.1155/2012/251482

Rothan, H.A., Han, H.C., Ramasamy, T.S., Othman, S., Rahman, N.A. & Yusof, R. 2012b. Inhibition of dengue NS2B-NS3 protease and viral replication in Vero cells by recombinant retrocyclin-1. *BMC Infect Dis.* 12: 314. doi: 10.1186/1471-2334-12-314

Syahri, J., Hilma, R., Nurlaili, Sari, M.K., Frimayanti, N., Ali, A.H. & Latip, J. 2023. Synthesis, antimalarial activities of secondary amine-substituted eugenol compounds against *Plasmodium falciparum* and *in silico* molecular docking analysis. *Sains Malaysiana* 52(12): 3463-3474. DOI: 10.17576/jsm-2023-5212-09

Shukla, R., Ramasamy, V., Shanmugam, R.K., Ahuja, R. & Khanna, N. 2020. Antibody-dependent enhancement: A challenge for developing a safe dengue vaccine. *Front. Cell. Infect. Microbiol.* 10: 572681. doi:10.3389/fcimb.2020.572681

Zamri, A., Teruna, H.Y., Rahmawati, E.N., Frimayanti, N. & Ikhtiarudin, I. 2019. Synthesis and *in silico* studies of a benzenesulfonyl curcumin analogue as a new anti dengue virus type 2 (DEN2) NS2B/NS3. *Indonesian Journal of Pharmacy* 30(2): 84-90. |DOI:10.14499/indonesianjpharm30iss2

Zhong, H., Huang, W., He, G., Peng, C., Wu, F. & Ouyang, L. 2013. Molecular dynamics simulation of tryptophan hydroxylase-1: Binding modes and free energy analysis to phenylalanine derivative inhibitors. *Int. J. Mol. Sci.* 14(5): 9947-9962. doi: 10.3390/ijms14059947

*Corresponding author; email: nenifrimayanti@gmail.com