Synthesis, In Silico and Structural Insight of Flavonol Derivative Compounds as New Competitive Dengue NS2B/NS3 Protease Inhibitor

Neni FRIMAYANTI[1, *] (iD), Ihsan IKHTIARUDIN[1] (iD), Abdi Wira SEPTAMA[2] (iD), Adriani SUSANTY[1] (iD), Nia Daiatul ISROQ[1] (iD)

[1]   Sekolah Tinggi Ilmu Farmasi Riau, Jln Kamboja Panam Pekanbaru 28293 Indonesia
[2]   Research Center for Chemistry, Indonesian Institute of Sciences, Kawasan Puspitek Serpong, Tangerang Selatan, Banten 15314, Indonesia
*   Corresponding Author. E-mail: nenifrimayanti@gmail.com (N.F.); Tel. +62- 761-588007

ABSTRACT: Dengue virus (DENV) is a single-stranded RNA virus that belongs to the Flaviviridae family and the genus Flavivirus. Dengue virus infection can cause Dengue Hemorrhagic Fever (DD), it can lead to Dengue Hemorrhagic Fever (DHF). The main purpose of this study is to synthesis compound 2'-methoxy flavonol (**2F**) and its derivative (**TF2**) and also to predict the binding orientation of these molecules as inhibitor for dengue virus DENV-2 NS2B/NS3 serine protease. Synthesis was performed using stirring method, it was begun with compound 2'-hydroxy-2-methoxychalcone and hydrogen peroxide as starting material. 2'-methoxy flavonol (**2F**) was obtained in the form of a white powder with a yield of 71.85%. Furthermore, compound **2F** with 1-bromo-3-chloropropane reacted using reflux method, in order to obtain compound **TF2** in form of white crystals with yield of 82.32%. The molecular structure of synthesized compounds was confirmed using spectroscopic analysis i. e. UV, FT-IR, ^{1}H-NMR and ^{13}C-NMR. Based on the molecular docking and density functional theory (DFT), it was shown that compound **TF2** might be used as potential dengue DEN2 NS2B/NS3 serine protease inhibitor. This strategy is an early stage for discover new drugs for then it can be used as dengue virus inhibitors.

KEYWORDS Dengue; NS2B/NS3; Docking; ADME; Density functional theory

1. INTRODUCTION

Dengue virus (DENV) is a single-stranded RNA virus belonging to the family Flaviviridae; flavivirus genus [1]. This dengue virus (DENV) is a pathogen that can significantly cause clinical disease such as fever, dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [2]. Dengue fever is one of the infectious diseases that gets special attention from WHO (world health organization). According to WHO, there are around 390 million cases death due to dengue virus infection [3].

DENV serotype contained genomes that is encoded three structural proteins (C, prM, E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) [4]. From seven proteins, there are two components of serine-protease, namely NS2B and NS3 which can be used as anti-dengue targets. NS2B/NS3 play an important role for replication because it is required for the synthesis of polyprotein precursors prior to the assembly of a complex [5]. Thus, NS2B/NS3 is considered as significant target for the development of anti-dengue drugs [6]. Recently, there is no vaccines or specific treatment can be used to attack dengue virus infection. Therefore, It is necessary to develop an effective therapy strategies that can combine between high specificity with cheap costs in order to help patients enhance their quality of life.

Flavonoids are secondary metabolites that can be found in several plant species. This compound consisted of several types, such as aurons, flavones, flavanones, flavonols, etc. Some of these natural flavonoid compounds have been reported to have a potential as dengue antivirals. It was recently reported that panduratin A isolated from *Boesenbergia rotunda* (L) showed good inhibitory activity against DENV-2 NS3 serine protease [7]. In addition, there are also some reports reported that flavonoid synthesized was showed good inhibitory activity against DENV [2, 8, 9, 10]

In drug design process, computer-aided drug design can be used to forecast the conformation and orientation of a candidate medication's binding with its target, as well as to screen the most potent and important medicinal chemical with great efficiency [11]. To date, although there is a research on discover new dengue inhibitor using in silico tools but it have not been extensively. Thus in this research, we will explore the potential of synthesized compound (i.e. a methoxy and halogen substituted flavonol derivative by reacting 1-bromo-3-chloropropane as alkylation) against dengue virus NS2B/NS3 inhibitor using computer aided i.e. molecular docking, predict their toxicity using ADME tools, and density functional theory (DFT) calculation to predict the reactivity and also the efficiency of these synthesized compounds.

2. RESULTS AND DISCUSSION

2.1. Synthesis

In this work, a new halogenated flavonol derivative (TF2) was successfully synthesized and its potency as inhibitor for DEN-2 NS2B/NS3 protease was explored through in silico studies. The synthesis of TF2 was performed in two-step reaction. First, synthesis of flavonol 2F by stirring method at room temperature. Second, synthesis of TF2 under reflux condition, as depicted in Figure 1.
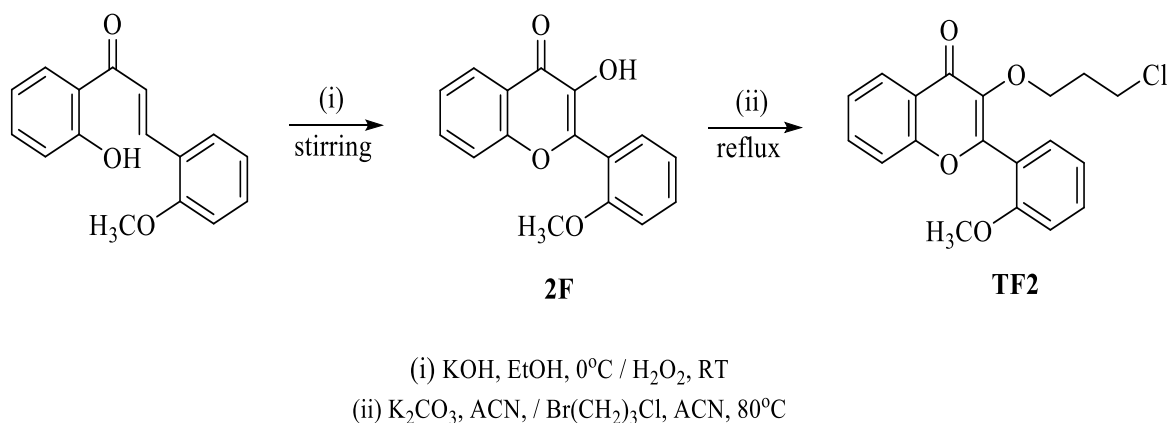

(i) KOH, EtOH, 0°C / H_2O_2, RT
(ii) K_2CO_3, ACN, / $Br(CH_2)_3Cl$, ACN, 80°C

Figure 1. The synthesis route of 2′-methoxyflavonol (**2F**) and its halogenated derivative (**TF2**)

The formation of compound 2F was began by the formation of epoxide (oxirane) as first intermediate. This intermediate was converted to flavanonol as second intermediate that will further oxidized to flavonol in the presence of hydrogen peroxide under basic condition. This reaction was called Algar-Flynn-Oyamada (AFO) reaction [12]. After the structure of product 2F was confirmed, then this compound was used as starting material for the synthesis of compound TF2. The synthesis reaction of TF2 was conducted through Wiliamson ether synthesis and carried out in two steps. In the first step, potassium carbonate activated compound 2F by abstracting the acidic 3-OH hydrogen to form an anion. In the second step, compound 2F in anion form acted as a nucleophile and attacked the electrophile carbon of 1-bromo-3-chloropropane. In this case, the more reactive electrophile is C with bound to Br, because Br is a better leaving group than Cl. This second step is SN_2 reaction, so Br group was substituted by formed anion in single step to form the product TF2.

The structures of both products 2F and TF2 were confirmed by spectroscopic analyses including UV, FT-IR, ^{1}H and ^{13}C NMR. The UV spectrum of compound 2F showed the maximum wavelengths at 332 nm (A = 0.229) and 238 nm (A = 0.414) that indicate the presence of absorption band I from the cinnamoyl conjugation system and absorption band II from benzoyl conjugation system, respectively. Then, the UV spectrum of compound TF2 showed the maximum wavelengths at 310 nm (A = 0.248) and 235 nm (A = 0.434) that also indicated the presence of band I and band II, respectively. In this case, the band I of compound TF2 was shifted as far as 22 nm to the lower wavelength. This hypsochromic shift indicate that the 3-OH group was substituted

[13] by a chloropropoxy group from 1-bromo-3-chloropropane. The presence of Cl group in compound TF2 was indicated by the appearance of absorption band at wavelength number of 754 cm^{-1} on the FT-IR spectrum [14].

The presence of 3-OH group in compound 2F was showed by the presence of broad band at wavelength number of 3284 cm^{-1} on the FT-IR spectrum [15]. This broad band is not observed in the FT-IR spectrum of compound TF2. This spectroscopic data showed that the free OH group in compound 2F was successfully substituted. In addition, the presence of 3-OH group in compound 2F was also supported by the presence of singlet signal at δ 6.44 ppm on ^{1}H NMR spectra [15]. As comparison, this signal is not observed in the ^{1}H NMR spectrum of compound TF2. The overlay of ^{1}H and ^{13}C NMR spectra of compounds 2F and TF2 were presented in Figure 2 and Figure 3, respectively.
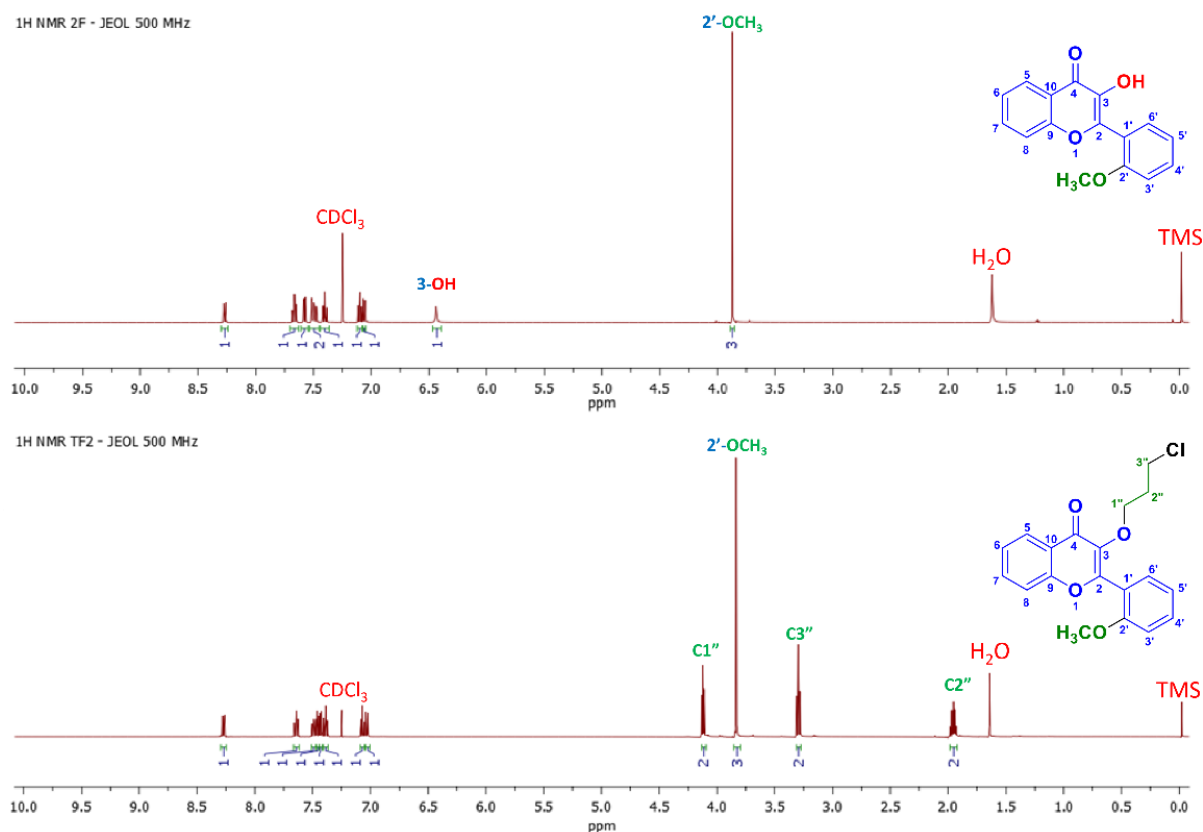

Figure 2. Overlay of ^{1}H NMR spectra of compounds 2F and TF2. The spectra were measured using a JEOL NMR spectrometer 500 MHz and CDCl$_3$ was used as solvent
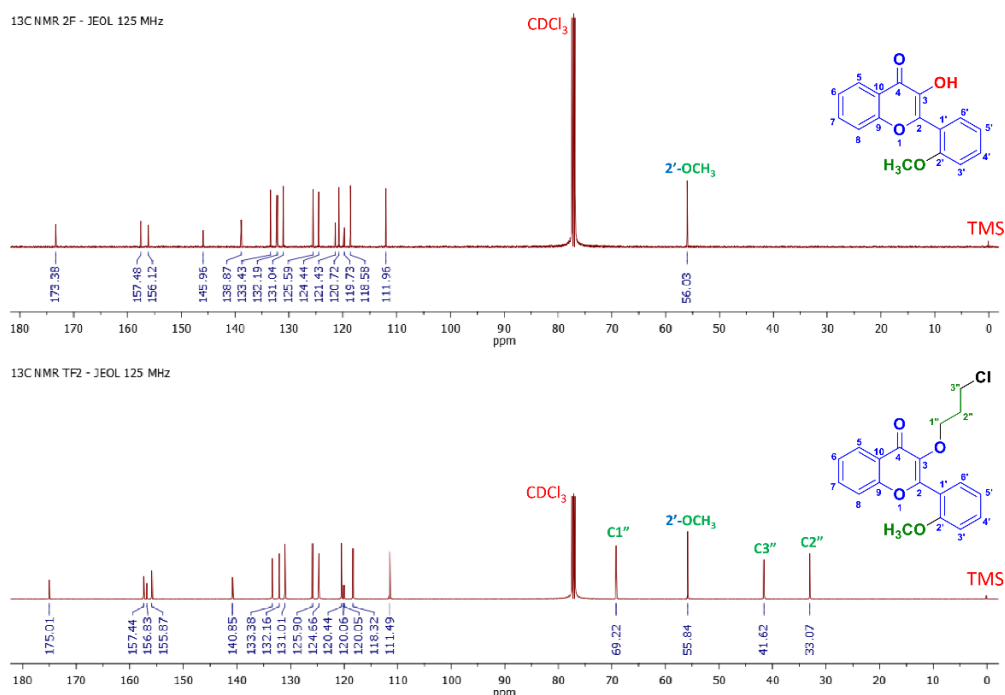
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Figure 3. Overlay of ^{13}C NMR spectra of compounds 2F and TF2. The spectra were measured using a JEOL NMR spectrometer 125 MHz and CDCl$_3$ was used as solvent

2.2. DFT Calculation

The DFT at B3LYP technique [17, 18] is used to optimize the gas phase structure of **2F** and **TF2** with the basis set of 6–31 G implemented by the Gaussian software package. The optimized molecular structure as well as the atom numbering is depicted in Figure 4.

The chemical stability of compounds are determined using highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) [19]. DFT/B3LYP/6-31G method is used to compute the energy gap and also the chemical reactivity descriptors, and the results are reported in Table 1. The HOMO, LUMO, and band gap energies of the compound **2F** and **TF2** are shown in Figure 5.

Table 1. Energy HOMO LUMO

No	Parameter	2F	TF2
1	Total energy	-917.81	-1495.35
2	RMS gradient Norm	0.0000105	0.000023
3	Dipole moment	6.56 Debye	6.25 Debye

Compound TF2 has an energy gap of 0.2714 eV in the current study. A narrow HOMO-LUMO energy gap indicated that the substance is chemically active, has low kinetic stability, and can be readily stimulated, promoting its biological activity. The capacity for electron donation is depicted by EHOMO, while the capacity for electron acceptance is depicted by ELUMO. This results was confirmed with the molecular docking results.
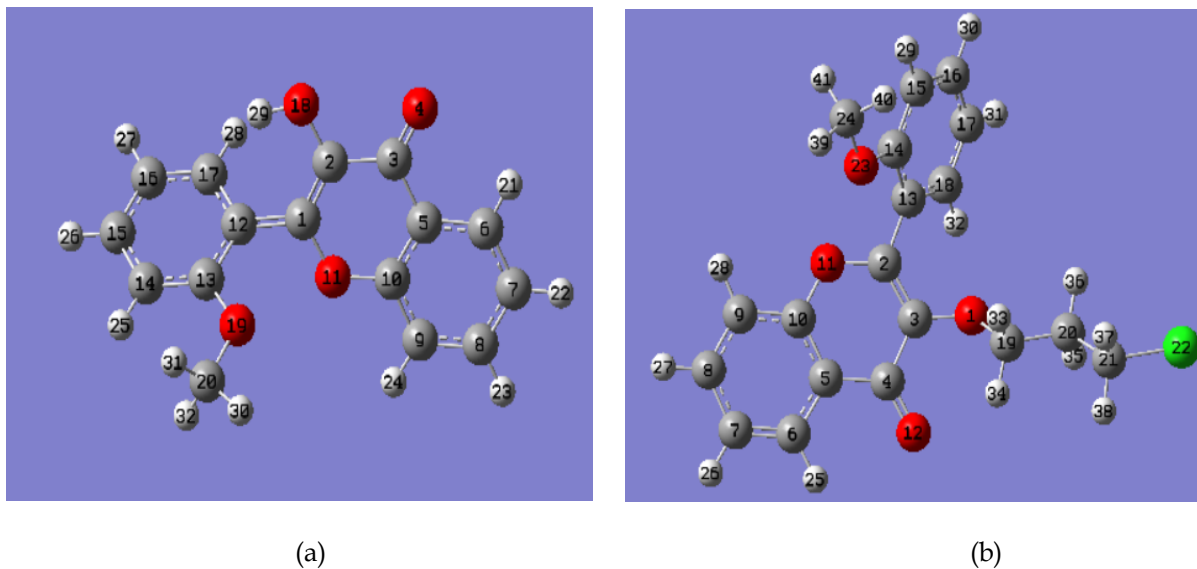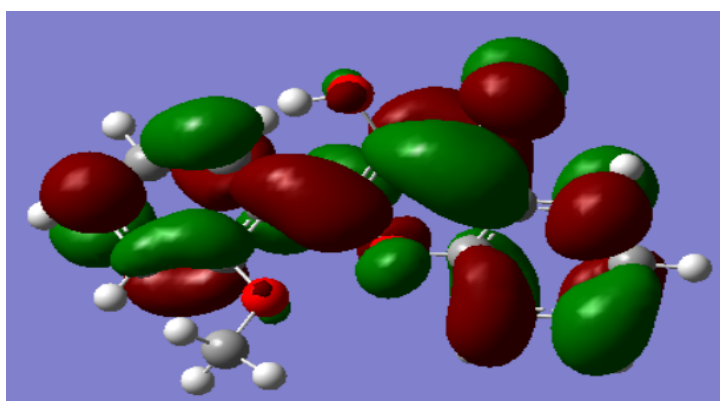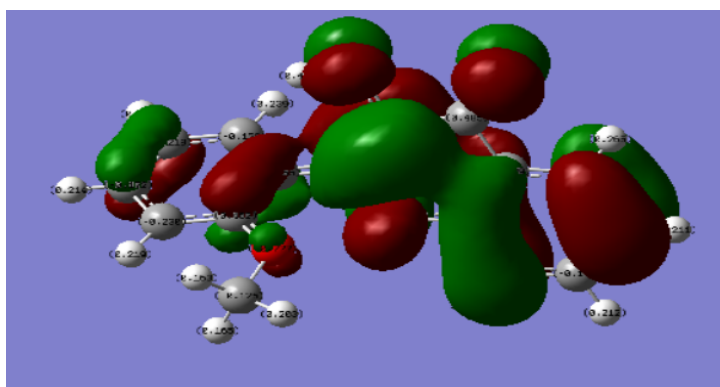
(a)

(b)

Figure 4. Optimized structure of compound (a) **2F** and (b) **TF2**

2F

$E_{LUMO} = -0.05311$ eV

$\Delta E_{gap} = 0.2630$ eV

$E_{HOMO} = -0.20$

TF2

E_{LUMO} = -0.0595 eV

ΔE_{gap} = 0.2794 eV
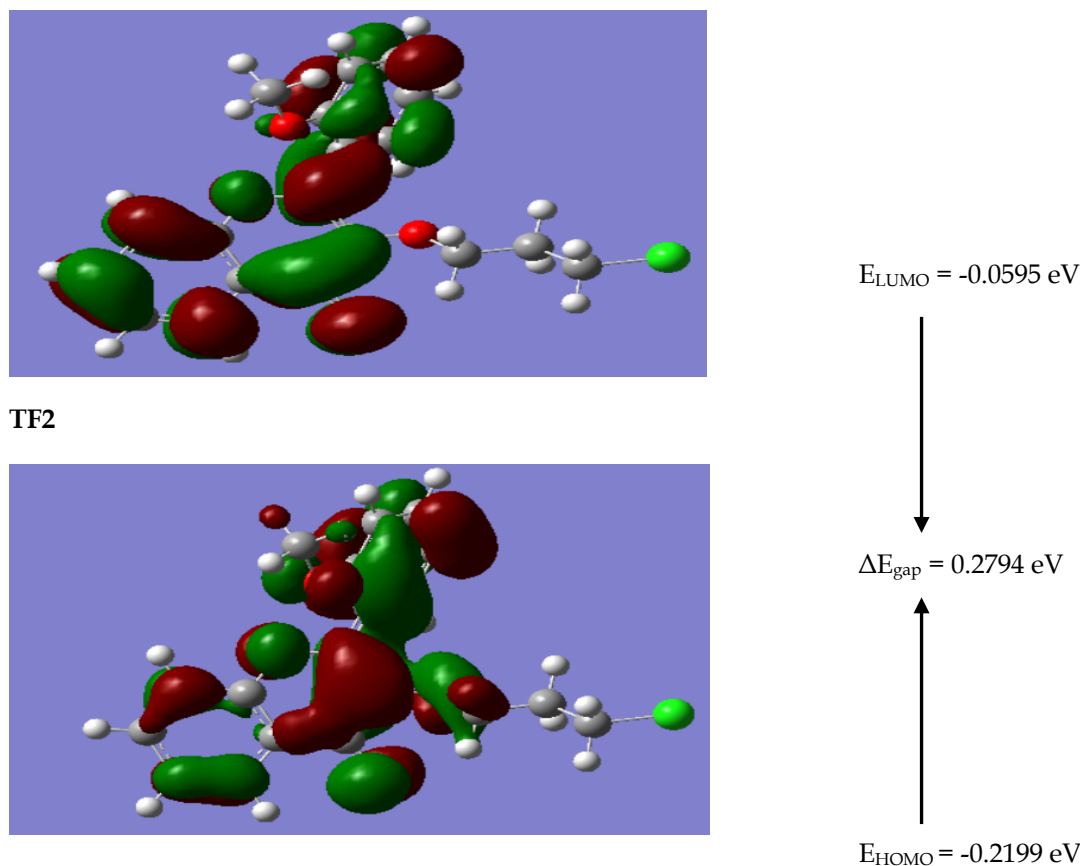
E_{HOMO} = -0.2199 eV

Figure 5. HUMO, LUMO and energy gap for compound **2F** and **TF2**

2.3. Molecular Docking

Molecular docking could obtain some parameters such as binding free energy (S) in kcal/mol and Root Mean Square Deviation (RMSD). Binding free energy (S) is energy that required for a ligand to bind to its protein (receptor). Smaller binding free energy indicated that interaction between ligand-protein is more stable [20]. RMSD value is a value that indicates that deviations or errors occur when docking. Smaller RMSD value indicated that smaller value of deviations or errors that occur when docking. The optimal complex ligand-protein poses were chosen based on the lowest binding free energy value as well as the lowest Root Mean Squared Deviation (RMSD) value [21]. In addition, hydrogen bonding and van der Waals interaction are also used as a supporting parameter to determine the stability of the ligand to the receptor [22].

Panduratin A as a positive control with binding free energy of -6.32 kcal/mol and an RMSD value of 1.10. In addition, panduratin A was able to form two hydrogen bonds with two amino acid residues in the active site of NS2B/NS3 serine protease. In this case, the hydrogen bond is formed between the O atom in the carbonyl group (C=O) of the panduratin A compound with His51 amino acid residue with a distance of 2.75Å. Hydrogen bonds are also formed between the H atoms in the hydroxy group of panduratin A with the amino acid residue Gly153 with a distance of 2.98Å. Panduratin A also binding via van der Waals interactions with amino acid residues such as Asp75, Ser135, Asn152, Val154, Ser163, Gly151, Tyr150, Ser131, Phe130 and Pro132 and also bind through other interactions with the amino acid residues Leu128 and Tyr161.

Based on the docking results in Table 2, **2F** was found the binding free energy value of -5.50 kcal/mol and an RMSD value of 1.62. It is seemed that **2F** is less negative than panduratin A. Thus, , it is indicated that compound **2F** more difficult to bind to the active site of NS2B/NS3 serine protease (2FOM). Based on the visualization of the docking results, it was observed that there was a hydrogen bond formed between the

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compound **2F** with the active site 2FOM, namely His51 amino acid residue with a distance of 2.96Å. In addition, compound **2F** can also bind to the active site of 2FOM through van der Waals interactions and other interactions with amino acid residues Gly153, Met49, Asp75, Ser135, Tyr161, Gly151, Ser163, Tyr150, Phe130, Pro132, Val52 and Leu128. Based on these interactions, it can be observed that compound **2F** can also form three amino acid residues in the catalytic triad and from the interactions that occur, it can be seen that twelve of the amino acid matches with panduratin A as a positive control. Spatial arrangement of compound **2F** with the protein is depicted in Figure 6.

Table 2. Docking results

| Compound	S (kcal/mol)	RMSD	Hydrogen bond	van der Walls	Other Interaction
2F	-5,50	1,62	**His51**	Gly153, Asn152, Val52, **Asp75,** Gly151, **Ser135,** Ser163, Tyr150, Tyr160, Phe130, Pro132, Met49	Leu128
TF2	-6,16	1,49	**His51**	Gly153, Asn152, Val154, **Asp75,** Gly151, **Ser135,** Tyr150, Pro132, Met49	Tyr161
Panduratin A	-6,32	1,10	**His51**, Gly153	Asn152, Val154, **Asp75,** Gly151, **Ser135,** Ser131, Ser163, Tyr150, Phe130, Pro132	Leu128, Tyr161
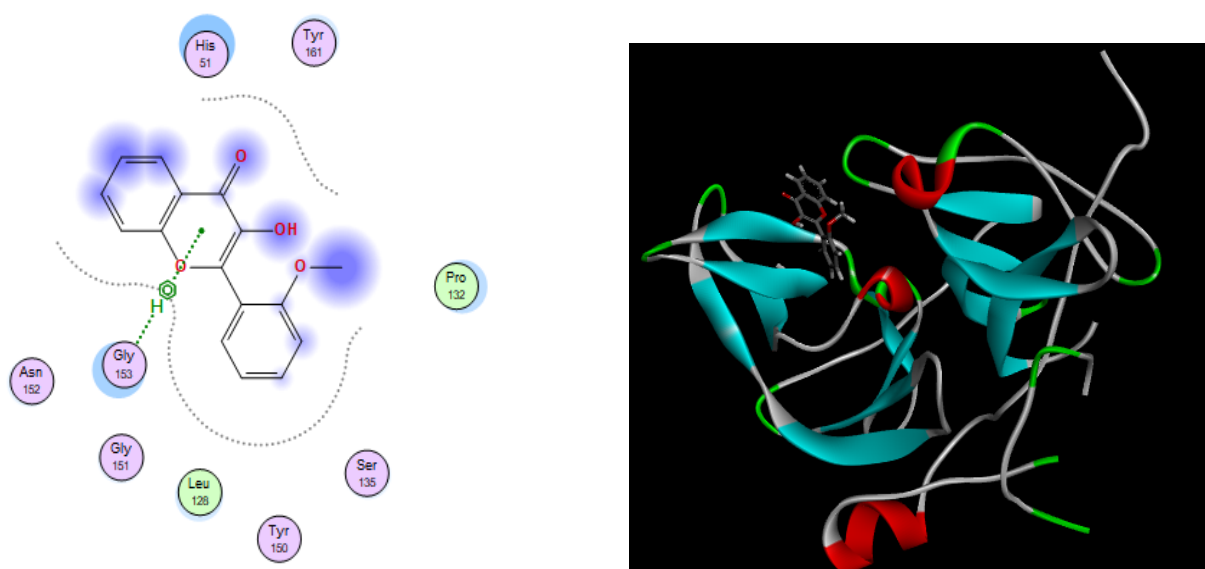
Figure 6. Spatial arrangement of compound **2F** with protein

Based on the docking results, it was found that the compound **TF2** has binding free energy value of -6.16 kcal/mol with RMSD value of 1.49. The binding free energy (S) of compound **TF2** was less negative than panduratin A (positive control). Furthermore, based on the docking results visualization, it was observed that there is a hydrogen bond formed between atom O and His51 amino acid residue. Interaction between this

ligand and amino acid was also observed in panduratin A-NS2B/NS3 serine protease complex [16]. Based on the 3D docking results visualization, it was detected that the hydrogen bond distance between compound **TF2** and His51 amino acid residue of 2.82 Å. Thus, it can cause the binding free energy value of compound **TF2** become more negative than compound **2F**. In addition, compound **TF2** also bind with 2FOM active site through van der Waals interactions and also other interactions with amino acid residues Gly153, Met49, Asp75, Ser135, Gly151, Asn152, Tyr150, Pro132, Val154, Leu128 and Tyr161. Based on these interactions, it seem that **TF2** compound performed interactions with the catalytic triad (i.e. three amino acid residues) and with eleven amino acid matches with panduratin A as a positive control. Figure 7 is presented the spatial arrangement of compound **TF2** with protein.
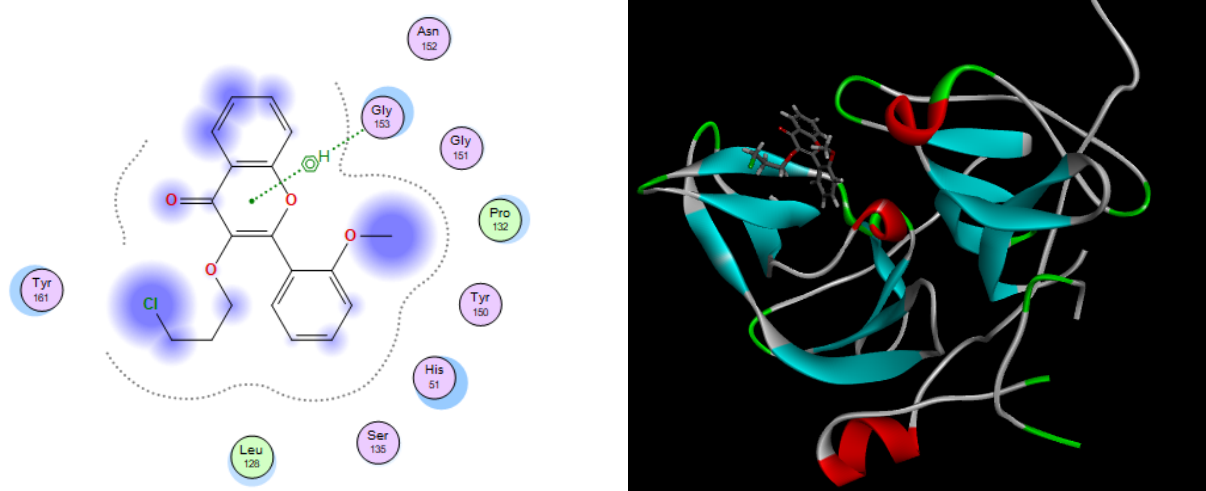

Figure 7. Spatial arrangement of compound **TF2** with the protein

Figure 8 shows the superimposition of these three compounds (*i.e.*, 2F, TF2 and panduratin A). three of these compounds were observed to take up similar poses with similar binding orientation around the active sites of the serine protease NS2B/NS3.

2.4. Adsorption, Distribution, Metabolism and Excretion (ADME) prediction

The process of absorption, distribution, metabolism, and excretion (ADME), also known as pharmacokinetic profiling in silico studies, is also known as pharmacokinetic profiling in silico studies. As a result, ADME was used to reduce risk and improve the screening process throughout the last stages of drug development [23].

Lipinski's rule of five can be used to calculate a compound's bioavailability. The maximum value for MW is 500, Log P is not to be exceeded at 5, the hydrogen bond donor is not to be exceeded at 5, and the hydrogen bond acceptor is not to be exceeded at 10 [24]. Lipinski's rule of five was used to assess the absorption rate of potentially active chemicals within the lipid bilayer in the human body. For compounds **2F** and **TF2**, the ADME profile yielded drug similarity properties according to Lipinski's rule of five, as shown in Table 3. The possibly active chemicals had reasonable medical characteristics based on the estimations. This proved that **TF2** is safe to use as potential inhibitor for dengue DEN2 NS2B/NS3.
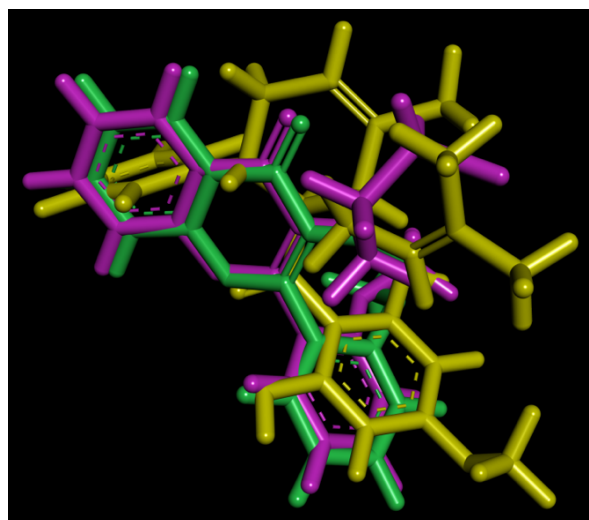
Figure 8 . Superimposition of 2F (green), TF2 (purple), and Panduratin A (yellow)

Table 3. ADME Results from http://www.swissadme.ch/index.php.

Parameter	2F	TF2
Mw (g/mol)	268.26	344.79
Consensus log Po/w	2.68	3.40
Hydrogen bond donor	1	0
Rotable bonds	2	6
Drug likeness (Lipinski)	yes	yes

4. CONCLUSION

The current study explored the effectiveness of halogen substituted flavonol derivative as dengue inhibitor. Based on the results of UV, FTIR, NMR, and GC-MS spectroscopic data analysis, the synthesized compounds matched the structure of the intended target molecule. The compounds TF2 was showed good potency as active dengue inhibitors NS2B/NS3 serine protease with the lowest binding free energy conformation and TF2 is also safe to use according to ADME profiles. This method is in the early stages of discovering new compounds that could be employed as dengue inhibitors NS2B/NS3 in the future.

5. MATERIALS AND METHODS

5.1. Materials

The materials used in this research such as 2'-hydroxy-2-methoxychalcone (STIFAR Riau), 1-bromo-3-chloropropane (Sigma-Aldrich), potassium hydroxide (Merck), potassium carbonate (Merck), sodium sulphate anhydrous (Merck), and some organic solvents such as absolute ethanol (Merck), acetonitrile (Merck), and chloroform (Merck).

5.1.1. Instruments

The instruments used in synthesis work including HPLC (Shimadzu Lc solution), spectrophotometer UV-Vis (Shimadzu UV-1800i), spectrophotometer FT-IR (Shimadzu, IR Spirit, A224158), NMR spectrometer (JEOL JNM-ECA 500), melting point apparatus (SMP-11), and UV lamp (Camag®). The instrument used in

DFT analysis and molecular docking studies is a personal computer (LG intel ® Core™ i7-8700 CPU 3.2 GHz, 16 GB RAM, 64 bit) completed with Molecular Operating Environment (MOE) 2020.0901 software package.

5.2. Methods

5.2.1. Synthesis of 3-hydroxy-2-(2-methoxyphenyl)-4H-chromen-4-one (2F)

The synthesis of compound 2F was performed by slightly modifying the previous methods. 2'-hydroxy-2-methoxychalcone (5 mmol, 1.27 g) was suspended in absolute ethanol (40 mL) using an ultrasonicator at room temperature. Potassium hydroxide solution (3N, 10 mL) was added to the suspension and the suspension was cooled in ice bath until temperature 0°C. Hydrogen peroxide (30%, 10 mL) was added to the cooled mixture, stirred at room temperature and the progress of reaction was monitored by TLC analysis. After the reaction was completed, hydrochloric acid (3N) was added to the mixture until pH 2 and the mixture was cooled in a refrigerator for 24 hours to maximize the precipitation. Then, the formed precipitate was filtered *in vacuo* using Buchner funnel, washed by cold distilled water and cold *n*-hexane, and allowed to dry in a desiccator. The crude product was recrystalized from ethanol to afford the pure product. The purity of recrystalized product was analysed by HPLC and the structure of product was confirmed by spectroscopic analyses, including UV, FT-IR, ^{1}H and ^{13}C NMR.

Compound 2F was obtained as white solid in 71.85% yield; m.p. 192-194°C; HPLC: t_R = 5.29 minutes; UV (in ethanol): λ_{max} = 238 and 332 nm; FTIR, v (cm^{-1}): 3284, 3008, 2943, 2841, 1608, 1571, 1230, 1205, 1132, 1096; ^{1}H NMR (in CDCl$_3$, 500 MHz), δ (ppm), J (Hz): 8.27 (dd, 1H, J_1 = 8.0, J_2 = 1.5); 7.67 (ddd, 1H, J_1 = 9.0, J_2 = 7.0, J_3 = 1.5); 7.58 (dd, 1H, J_1 = 7.5, J_2 = 2.0); 7.51 (dd, 1H, J_1 = 8.5, J_2 = 0.5); 7.49 (ddd, 1H, J_1 = 8.5, J_2 = 7.5, J_3 = 2.0); 7.40 (ddd, 1H, J_1 = 8.0, J_2 = 7.0, J_3 = 1.0); 7.10 (td, 1H, J_1 = J_2 = 7.5, J_3 = 2.0); 7.06 (dd, 1H, J_1 = 8.0, J_2 = 0.5); 6.44 (s, 1H); 3.87 (s, 3H). ^{13}C NMR (in CDCl$_3$, 125 MHz), δ (ppm): 173.38, 157.48, 156.12, 145.96, 138.87, 133.43, 132.19, 131.04, 125.59, 124.44, 121.43, 120.72, 119.73, 118.58, 111.96, 56.03.

5.2.2. Synthesis of 3-(3-chloropropoxy)-2-(2-methoxyphenyl)-4H-chromen-4-one (TF2)

2'methoxyflavonol (2.5 mmol, 0.67 g) was dissolved in acetonitrile (15 mL) and anhydrous potassium carbonate (5 mmol, 0.69 g) was added to the solution. The mixture was heated under reflux condition until temperature 80°C. Then, the solution of 1-bromo-3-chloropropane (7.5 mmol, 0.92 g) in acetonitrile (5 mL) was added dropwise to the hot mixture. The reflux was continued and TLC analysis was used to monitor the progress of reaction. After the reaction was completed, the hot mixture was allowed to reach room temperature and the solvent was removed by vacuum rotary evaporator. Then, the residue was dissolved in chloroform (30 mL) and poured to separatory funnel. This organic solution was washed by distilled water triplicate (3 x 30 mL) to remove the catalyst residue. The organic layer the bottom was separated and dried using sodium sulphate anhydrous and the solvent was removed. The crude product was recrystalized from ethanol to afford the pure product. The purity of recrystalized product was analysed by HPLC and the structure of product was confirmed by spectroscopic analyses, including UV, FT-IR, ^{1}H and 13C NMR.

Compound **TF2** was obtained as yellow solid in 82.32 % yield, m.p. 86-87°C. HPLC: t_R = 5.76 minutes. UV (in ethanol): λ_{max} = 235 and 310 nm. FT-IR: v (cm^{-1}) = 3010, 2958, 2879, 2839, 1633, 1610, 1463, 1185, 1138, 1113, 754. ^{1}H NMR (in CDCl$_3$, 500 MHz), δ (ppm), J (Hz): 8.27 (dd, 1H, J_1 = 8.0, J_2 = 1.5); 7.64 (ddd, 1H, J_1 = 9.0, J_2 = 7.0, J_3 = 2.0); 7.49 (ddd, 1H, J_1 = 8.5, J_2 = 7.5, J_3 = 1.5); 7.45 (dd, 1H, J_1 = 8.5, J_2 = 0.5 Hz); 7.43 (dd, 1H, J_1 = 7.5, J_2 = 1.5); 7.39 (ddd, 1H, J_1 = 8.0, J_2 = 7.0, J_3 = 0.5); 7.07 (td, 1H, J_1 = 7.5, J_2 = 7.5, J_3 = 0.5); 7.03 (d, 1H, J = 8.5); 4.12 (t, 2H, J_1 = 5.5, J_2 = 6.0); 3.83 (s, 3H); 3.29 (t, 2H, J_1 = J_2 = 6.5); 1.95 (quin, 2H, J_1 = J_4 = 6.5, J_2 = 5.5, J_3 = 6.0). ^{13}C NMR (in CDCl$_3$, 125 MHz), δ (ppm): 175.01, 157.44, 156.83, 155.87, 140.85, 133.38, 132.16, 131.01, 125.90, 124.66, 120.44, 120.06, 120.05, 118.32, 111,49, 69.22, 55.84, 41.62, 33.07.

5.2.3. DFT Calculation

To simulate molecules **2F** and **TF2**, the Gaussian software package was utilized. The Gaussian was used to optimize the molecular geometries of each molecule in the gas phase using density functional theory (DFT). The B3LYP was used to execute all of the quantum-chemical calculations, with the basis set of 6-31G.

5.2.4. Molecular Docking

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The molecular structure of ligands i. e. compound 2'-methoxy flavonol (2F), its derivative compound (TF2) and Panduratin A as positive control were sketched using Chemdraw Professional 15.0, then the 3D structure was further prepared using the Molecular Operating Environment (MOE) program 2020.0901 with MMFF94x force field and 0.0001 gradient. Figure 9 are depicted the molecular structure of ligands.

2'-methoxy flavonol (2F)   **TF2**   **Panduratin A**

Figure 9. Molecular structure of ligand
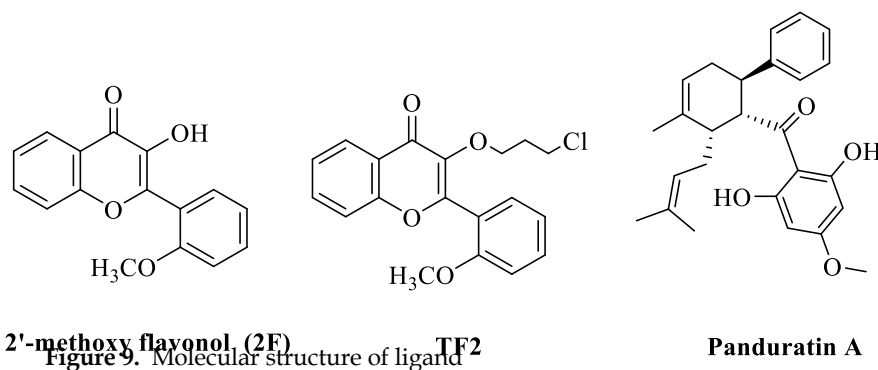
The protein structure used (PDB code: 2FOM) was downloaded from the website www.rcsb.org. The crystal structure of this protein was then prepared using the DSV 2020 (Biovia) and MOE 2020.0901. The 2FOM protein consisted of two chains, i.e. chain A and chain B. Furthermore, water molecules, native ligands, and ion Cl- from the protein were removed.

Then, the protein molecular structure was prepared using MOE 2020.0901 software package. Next, CHARMM27 was selected as a force field, the protein was prepared with parameter i. e. RMS gradient was set to 0.01 kcal/mol/A. Energy minimization is carried out on H atoms, alpha carbon, and also for backbone atoms [25]. The prepared structure is then saved in PDB format for then it can be used as a receptor for the docking process.

Prior to docking, the active site of the protein was determined using a site finder. Site 3 consisted of several amino acid residues (Leu128, Asp129, Phe130, Ser131, Pro132, Ser135, Tyr150, Gly151, Gly153) and site 13 which consists of several amino acid residues (His51, Lys74, Asp75, Gly151, Asn152, Gly153, Val154) is then set as a dummy atom to serve as the target side for the docking process. Then in the dock menu, the site is set as a dummy atom and the MDB file containing the prepared ligand structure is selected as the ligand. Next, the placement is set as a triangle, the refinement is set as rigid and the pose is set as 50 and 10, respectively.

5.2.5. Adsorption, Distribution, Metabolism and Excretion (ADME) prediction

To gain a better understanding of the physicochemical and pharmacokinetic features of drug candidates, as well as to anticipate the drug similarity, ADME profiles of compounds **2F, TF2** were determined. The ADME profiles were calculated in this investigation with the use of the SwissADME server (http://www.swissadme.ch/index.php).

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