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RESEARCH ARTICLE

# Synthesis of 3'-methoxy flavonol and its derivatives as potential inhibitors for Dengue NS2B/NS3 and molecular insight into binding interaction

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**Keywords**
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## Abstract

**Background:** Infection with the Dengue virus can result in dengue fever (DD), which can progress to dengue hemorrhagic fever (DHF). **Objective:** In this work, molecule 3'-methoxy flavonol (3F) and its derivatives (T3F) were synthesized because they may be used to develop dengue antiviral DENV-2 NS2B/NS3 serine proteases. **Method:** Compound 2'-hydroxy-3-methoxy chalcone and hydrogen peroxide are used in the stirrer method to synthesize compound 3F with a yield of 33.24%, and yellow crystals of the 3F compound were produced. **Results:** The substance T3F was produced as white crystals with a yield of 69.42% by reacting compound 3F with ethyl chloroacetate using the reflux technique. Compound 3F exhibited absorption at maximal wavelengths of 212 nm, 244 nm, and 344 nm according to UV-Vis spectroscopy. UV-Vis spectroscopy of compound T3F revealed absorption at maximal wavelengths of 212 nm, 243 nm, and 320 nm. While a C=O ester group was replaced in the T3F synthesis step, the FT-IR spectroscopy of the 3F molecule revealed the existence of an OH group. **Conclusion:** Based on docking results, compound T3F has a binding free energy value that is extremely similar to that of panduratin A. Thus, it revealed that the T3F molecule has the potential to be an inhibitor of the NS2B/NS3 Protease virus DENV-2.

## Introduction

Dengue virus is a positive single-stranded RNA virus transmitted by mosquitoes from the Flaviviridae family; genus Flavivirus. Infection with this virus can cause Dengue Fever (DD) which can lead to Dengue Hemorrhagic Fever (DHF) (WHO, 2011). This disease is an infectious disease that has received special attention from WHO. Recently, there are 390 million dengue virus infections have been reported worldwide (Bhatt *et al.,* 2013).

There are five serotypes of the dengue virus that have been reported (DENV-1, DENV-2, DENV-3, DENV-4, and DENV-5). Among the dengue virus serotypes, DENV-2 is the most dangerous serotype with a fairly high prevalence circulating in Southeast Asian countries (Wang *et al.*, 2016). There was a significant correlation between DENV-2 infection and more severe dengue disease. A number of studies have revealed that non-structural serine protease 3 (NS3) is required for viral polyprotein maturation. The binding of serine protease NS3 to cofactor NS2B will form the protease complex NS2B-NS3 (Chambers *et al.,* 1993) which is required to cleave the viral precursor polyprotein needed in the DENV-2 virus replication process. Therefore, interference with the function of the NS2B-NS3 protease complex by an inhibitor can inhibit the viral replication process (Rothan *et al.,* 2012). Thus, it can be used as a promising target for the development of dengue antivirals (Sampath & Padmanabhan, 2009).

Flavonoids are secondary metabolite compounds that can be found in several plant species. These compounds consisted of several types, such as aurons, flavones, flavanones, flavonols, etc. Some of these natural flavonoid compounds have been reported to have the potential as dengue antivirals. Based on research conducted by Zandi and researchers, stated that quercetin compounds (flavonols) showed an $IC_{50}$ value of 35.7 µg/mL against the dengue virus after the virus adsorbed into the cells. Other flavonol compounds such as fisetin have also been reported to interfere with DENV-2 replication by binding directly to viral RNA and forming flavonoid-RNA complexes or affecting RNA polymerase (Zandi *et al.,* 2011). In addition, several methoxy-substituted synthetic flavonoids such as 5-hydroxy-7-methoxy-6-methyl flavanone were also reported to have good dengue DENV-2 antiviral activity, with an $EC_{50}$ value of 12.31 ± 1.64 µM.

Another flavonoid compound that has been reported to have dengue antiviral properties is panduratin A. Panduratin A is a cyclohexenyl chalcone derivative isolated from Boesenbergia rotunda and it is often used as a positive control in research seeking dengue antivirals (Abdurrahman *et al.,* 2018). Based on the literature, the antiviral activity of dengue panduratin A is associated with its ability to inhibit the NS3 protease virus DENV-2 (Tan *et al.,* 2015). In the structure of panduratin A, there are carbonyl, hydroxy, methoxy and alkyl groups originating from isoprene units.

The main purpose of this study is to synthesise flavonol compound derivatives from chalcone with carbonyl, hydroxy and methoxy groups in its structure. Then, an alkyl ester group was added from ethyl chloroacetate to obtain a flavonol derivative which is expected to have potential as an NS2B/NS3 protease inhibitor of the DENV-2 virus. In this study, molecular docking was used to estimate the potential bioactivity of this synthesized compound.

## Methods

### *General synthesis procedure*

The synthesis route of compounds 3F and T3F was depicted in Figure 1.

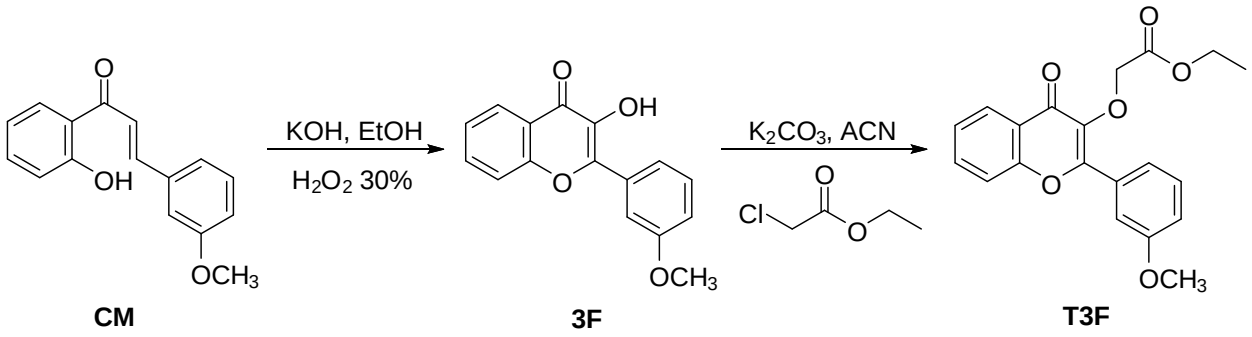



**Figure 1: Synthesis route of compounds 3F and T3F**

### *General synthesis procedure of 3-hydroxy-2-(3-methoxyphenyl)-4H-chromen-4-one (3F)*

Synthesis of compound 3F was performed by slight modification from the previously described method (Dias *et al.,* 2013). As much as five mmol (1.2715 g) of chalcone analogue (CM) was suspended in 40 mL of absolute ethanol using an ultrasonicator and ten mL of potassium hydroxide 3N was added to the suspension. The mixture was cooled on the ice bath to afford a temperature of 0 °C. Then, ten mL of hydrogen peroxide 30% was added to the mixture, and the mixture was stirred on the magnetic stirrer. The progress of the reaction was monitored by TLC. After the reaction was completed, hydrochloric acid solution 3N was added to the reaction mixture to afford a pH of two, then the mixture was cooled in a refrigerator for 24 hours. The formed precipitate was filtered *in vacuo* and washed with distilled water and cold *n*-hexane. The obtained crude product was dried in a desiccator and it was recrystallized in hot ethanol to afford the pure product.

Compound 3F was obtained as a yellow crystal, % yield = 33.2%, m.p. = 130-131°C. UV (EtOH, 5 ppm) $\lambda_{max}$ (nm) = 244 and 344. FT-IR $\nu$ ($cm^{-1}$) = 3253, 3007, 2972, 2941, 2901, 2832, 1608, 1564, 1476, 1278, 1195, 1115. $^1H$ NMR ($CDCl_3$, Agilent 500 MHz): 8.24 (*dd*, 1H, *J* = 8.0, 1.5), 7.85 (*dd*, 1H, *J* = 7.5, 0.5), 7.82 (*t*, 1H, *J* = 2.3), 7.70 (*ddd*, 1H, *J* = 8.5, 7.0, 1.5), 7.59 (*d*, 1H, *J* = 8.5), 7.44 (*t*, 1H, *J* = 8.3), 7.41 (*t*, 1H, *J* = 7.8), 7.04 (*s*, 1H), 7.02 (*dd*, 1H, *J* = 8.3, 2.8).

***General synthesis procedure of ethyl 2-((2-(3-methoxyphenyl)-4-oxo-4H-chromen-3-yl)oxy)acetate (T3F)***

As much as 2.5 mmol (0.6706 g) of compound 3F was dissolved in 15 mL of acetonitrile and five mmol (0.6910 g) of anhydrous potassium carbonate was added to the solution. The mixture was heated to afford a temperature of 80°C and a solution of 7.5 mmol (0.9191 g) of ethyl chloroacetate in five mL of acetonitrile was added dropwise. The mixture was refluxed and the progress of the reaction was monitored by TLC. After the reaction was completed, the mixture was cooled to rise the room temperature and the solvent was evaporated *in vacuo* to afford a solid residue. The residue was dissolved in 30 mL of chloroform, transferred to a separatory funnel, and washed with distilled water (3 x 30 mL). The organic layer was collected and dried with anhydrous sodium sulfate and the solvent was evaporated to afford a solid crude product and it was recrystallized in ethanol to afford the pure product.

Compound T3F was obtained as white solid, % yield = 69.4 %, m.p. = 64-65°C. UV (EtOH, 4.5 ppm) $\lambda_{max}$ (nm) = 243 and 320. FT-IR $\nu$ ($cm^{-1}$) = 3062, 2971, 2930, 2865, 1763, 1628, 1602, 1471, 1263, 1191, 1143, 1057. $^1$H NMR ($CDCl_3$, Agilent 500 MHz): 8.23 (*dd*, 1H, *J* = 8.0, 2.0), 7.77 (*dd*, 1H, *J* = 2.5, 1.5), 7.75 (*ddd*, 1H, *J* = 8.0, 1.5, 1.0), 7.68 (*ddd*, 1H, *J* = 8.6, 7.0, 2.0), 7.54 (*dd*, 1H, *J* = 8.5, 0.5), 7.42 (*t*, 1H, *J* = 8.0), 7.40 (*ddd*, 1H, *J* = 8.0, 7.0, 0.5), 7.04 (*ddd*, 1H, *J* = 8.3, 2.5, 1.0), 4.86 (*s*, 2H), 4.17 (*q*, 2H, *J* = 6.6), 3.88 (*s*, 3H), 1.21 (*t*, 3H, *J* = 7.25).

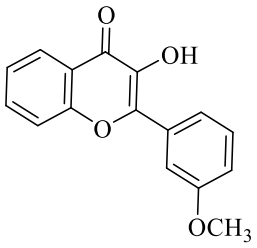
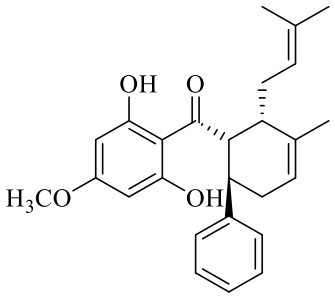
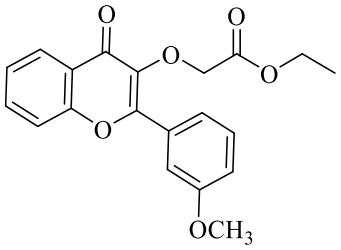
***Melting point measurement, HPLC analysis and structure characterization of compounds 3F and T3F***

The melting points of compounds 3F and T3F were measured using SMP-11. Both compounds were also analysed using reverse-phase HPLC with gradient elution of acetonitrile and water by following the previously described method (Ikhtiarudin *et al.*, 2017). The structures of 3F and T3F were characterized by spectroscopic analyses including UV, FT-IR, and NMR. The measurements of UV and FT-IR spectra were conducted in Sekolah Tinggi Ilmu Farmasi Riau and the measurements of $^1$H and $^{13}$C NMR spectra were performed in Lembaga Ilmu Pengetahuan Indonesia (LIPI), now known as Badan Riset dan Inovasi Nasional (BRIN), Serpong, Tangerang, Indonesia.

***Molecular Docking***

The molecular structure of the 3'-methoxy flavonol compound (3F), its derivative compound (T3F) and Panduratin A as positive control were sketched using ChemDraw 2015 application. The 3D structure of each ligand was prepared using MOE 2020.0901 (Chemical computing grup) with a force field of MMFF94x and a gradient of 0.0001. Then all the structures were saved as a database of ligands in *mdb format. the molecular structures for this ligand are listed in Table I.

**Table I: Molecular structure of ligand**

| No | Structure | Positive control | structure |
|---|---|---|---|
| 3F |  | Panduratin A |  |
| T3F |  | | |

The crystal structure of dengue Virus NS2B/NS3 serine protease was retrieved from the rcsb.org website using PDB ID 2FOM. The protein consists of two chains, i.e. chain A and chain B, respectively. The DSV application was then used to eliminate water molecules, and initial (innate) ligands, and remove ion $Cl^-$ from the protein. Energy minimization of this protein, H atoms, alpha carbon atoms, and backbone atoms was carried out using the force field of CHARMM27 (RMS gradient was set to 0.01 kcal/mol/A) in MOE 2020.0901 software package (Frimayanti *et al.,* 2011).

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 consists of several amino acid residues (His51, Lys74, Asp75, Gly151, Asn152, Gly153, Val154) were then set as a dummy atom to serve as the target site for the docking process. Then on 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. Furthermore, the docking process can be performed.

## Result

### *Synthesis*

The synthesis of compound **3F** has been reported in previous work (Dias *et al*., 2013). In this work, we have performed slight modifications for the synthesis method of compound **3F**, but the result seems to be similar to the previous result. The comparison of the synthesis method and the result for the synthesis of compound **3F** between this work and previously reported work were displayed in Table II. On the other hand, the synthesis of compound **T3F** has not been previously reported.

The proposed interpretation for $^1H$ and $^{13}C$ NMR spectra of compounds 3F and T3F were presented in Table III. Overall, all spectroscopic data agreed that the obtained products have a structure that is following the desired product structure.

### *Docking*

The molecular docking of two of these compounds is presented in Table IV. The spatial arrangement of panduratin A with protein is depicted in Figure 2. Figure 3 is depicted the spatial arrangement of compound 3F. The spatial arrangement for compound T3F is presented in Figure 4.

**Table II: The comparison of the synthesis method and result for the synthesis of compound 3**

| Comparison | Previous work (Dias *et al*., 2013) | This work |
|---|---|---|
| Base solution was used | NaOH 4M | KOH 3N |
| Solvent for reaction | methanol | ethanol |
| Synthesis method and temperature used | Stirred at 55 °C | Cooled to 0 °C, then stirred at room temperature |
| pH of the reaction mixture after the reaction was completed | 2 | 2 |
| Product colour | Beige solid | Yellow crystal |
| Percentage of yield | 39.2 % | 33.2 % |
| Melting point | 133-135 °C | 130-131 °C |

**Table III: The proposed interpretation for $^{1}H$ and $^{13}C$ NMR spectra of compounds 3F and T3**

| Atomic Numbering | δH (ppm), multiplicity, integration (H), *J* (Hz) | | δC (ppm) | |
|---|---|---|---|---|
| | Compound 3F | Compound T3F | Compound 3F | Compound T3F |
| 1 | - | - | - | - |
| 2 | - | - | 144.72 | 155.23 |
| 3 | - | - | 138.64 | 139.64 |
| 3-OH | 7.04 (*s*, 1H) | - | - | - |
| 4 | - | - | 173.59 | 174.70 |
| 5 | 8.24 (*dd*, 1H, *J* = 8.0, 1.5) | 8.23 (*dd*, 1H, *J* = 8.0, 2.0) | 125.54 | 125.83 |
| 6 | 7.41 (*t*, 1H, *J* = 7.8) | 7.40 (*ddd*, 1H, *J* = 8.0, 7.0, 0.5) | 124.64 | 124.91 |
| 7 | 7.70 (*ddd*, 1H, *J* = 8.5, 7.0, 1.5) | 7.68 (*ddd*, 1H, *J* = 8.6, 7.0, 2.0) | 133.80 | 133.72 |
| 8 | 7.59 (*d*, 1H, *J* = 8.5) | 7.54 (*dd*, 1H, *J* = 8.5, 0.5) | 118.41 | 118.12 |
| 9 | - | - | 155.48 | 155.20 |
| 10 | - | - | 120.67 | 124.05 |
| 1’ | - | - | 132.39 | 132.05 |
| 2’ | 7.82 (*t*, 1H, *J* = 2.3) | 7.77 (*dd*, 1H, *J* = 2.5, 1.5) | 113.28 | 114.36 |
| 3’ | - | - | 159.71 | 159.50 |
| 3’-O$\underline{C}H_3$ | 3.89 (s, 3H) | 3.88 (*s*, 3H) | 55.51 | 55.56 |
| 4’ | 7.02 (*dd*, 1H, *J* = 8.3, 2.8), | 7.04 (*ddd*, 1H, *J* = 8.3, 2.5, 1.0) | 116.04 | 116.94 |
| 5’ | 7.44 (*t*, 1H, *J* = 8.3), | 7.42 (*t*, 1H, *J* = 8.0) | 129.74 | 129.52 |
| 6’ | 7.85 (*dd*, 1H, *J* = 7.5, 0.5) | 7.75 (*ddd*, 1H, *J* = 8.0, 1.5, 1.0) | 120.33 | 121.33 |
| 1” | - | - | - | 169.06 |
| 2” | - | 4.86 (*s*, 2H) | - | 68.39 |
| 1’’’ | - | 4.17 (*q*, 2H, *J* = 6.6) | - | 61.10 |
| 2’’’ | - | 1.21 (*t*, 3H, *J* = 7.25) | - | 14.18 |

**Table IV: Docking results**

| Compound | Binding free energy (kcal/mol) | RMSD | Hydrogen bond | Van der Waals | Another interaction | Factor of binding |
|---|---|---|---|---|---|---|
| Panduratin A | -6.87 | 1.63 | His51 (2,85 Å), Ser135 (2,77 Å) | Tyr150, Ser131, Gly151, Asn152, Met49, Asp75, Gly153, Tyr161, Arg54 | Phe130, Pro132, Val52, Val154, Leu128 | |
| 3F | -5.61 | 0.82 | Gly153 (2,32 Å | Lys74, Asp75, Asn152, Met49, Tyr161, Phe130, Tyr150, Pro132, Ser135, Gly151 | His51, Leu128 | 12 |
| T3F | -6.39 | 0.91 | Gly153 (2,14 Å) | Asn152, Met49, Val154, Asp75, Ser131, Gly151, Tyr150, Ser135 | His51, Leu128, Pro132, Tyr161, Phe130 | 14 |

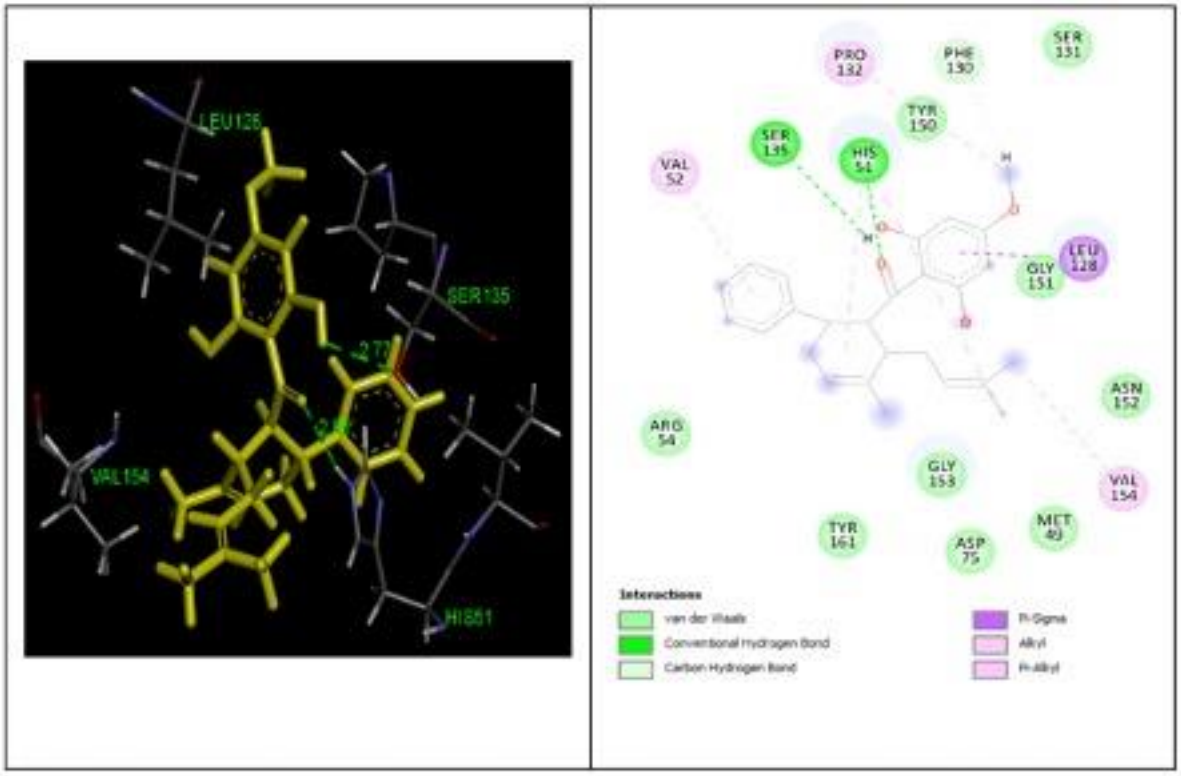



**Figure 2: Spatial arrangement of panduratin A**

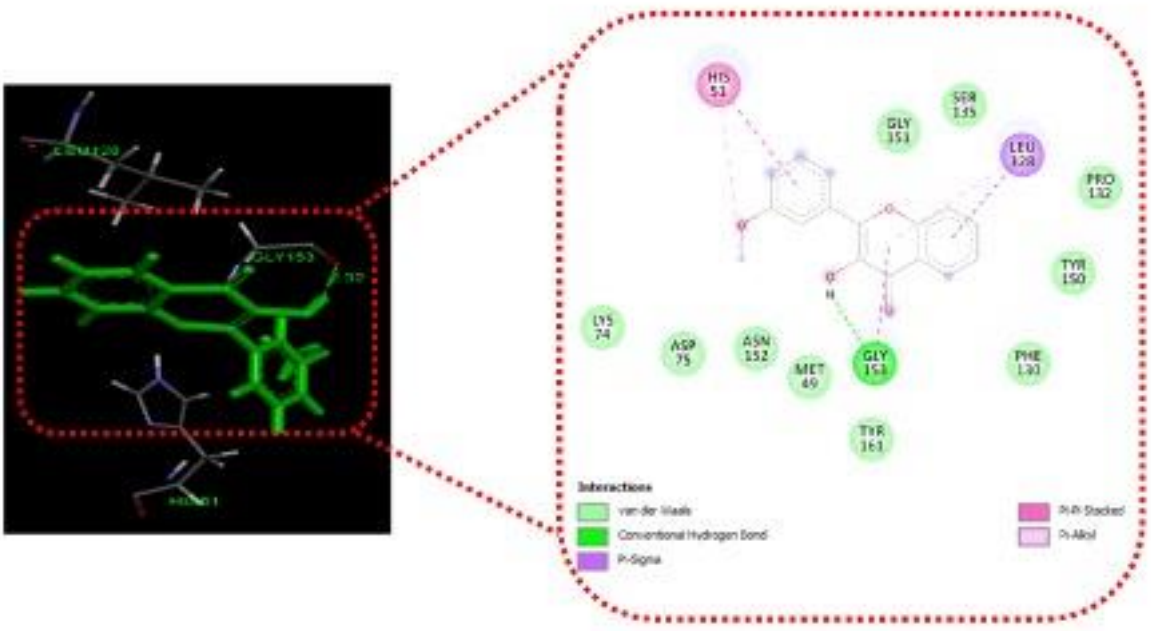



**Figure 3: Spatial arrangement of compound 3F**

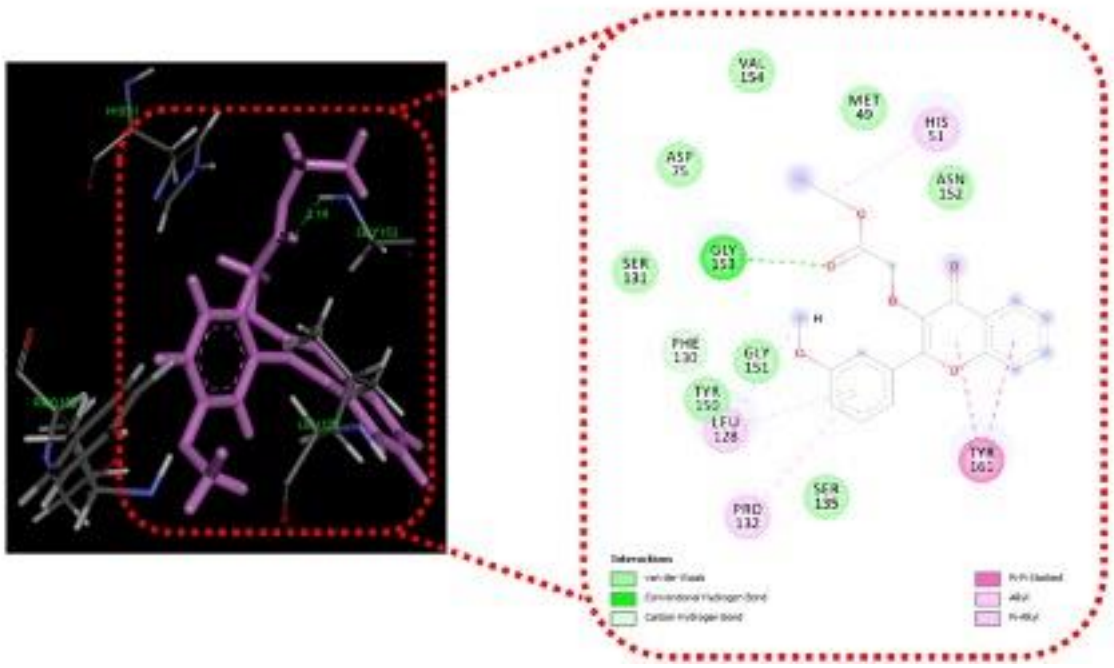



**Figure 4: Spatial arrangement of compound T3F**

## Discussion

### *Synthesis*

In this work, we reported the synthesis of a new ethyl ester compound, 3-methoxyflavonol derivative (T3F) using 2'-hydroxy-3-methoxychalcone (CM) as starting material. The reaction was performed in two-step reactions. The first is the synthesis of 3-methoxy flavonol (3F) by stirring method via Algar-Flynn-Oyamada (AFO) reaction and the second is the synthesis of compound T3F under reflux condition via Williamson ether synthesis. The reaction mechanism of the first step begins with the grabbing of acidic hydrogen on the hydroxyl group of CM by potassium hydroxide to form a phenoxide anion. Then, the addition of hydrogen peroxide caused the oxidation of α, β double bond of CM to form an oxirane ring as an intermediate product. The phenoxide anion act as a nucleophile and attack the β carbon on the oxirane ring to form flavanonol which will be further oxidized to form flavonol 3F. In this step, we obtained a 33.2% yield of yellow crystal of compound 3F. Then, the reaction mechanism of the second step begins with the capturing of acidic hydrogen on the hydroxyl group of 3F by potassium carbonate to form an alkoxide anion. Then, the anion act as a nucleophile and attack the electrophilic carbon C-Cl of ethyl chloroacetate to afford compound T3F. In this step, we obtained a 69.4% yield of white solid of compound T3F. Both synthesized compounds 3F and T3F have sharp melting points (≤ 2°C) and based on the HPLC analysis, they show a single peak which indicated that both products have good purity.

The structures of both synthesized products 3F and T3F were characterized using spectroscopic analyses, including UV, FT-IR, and NMR. The measurements of UV spectra were carried out to identify the presence of chromophores or a typical type of conjugation system in the molecular structure of products. The UV spectrum of 3F in ethanol shows absorption at maximum wavelengths of 212, 244, and 344 nm, with an absorbance of 0.671, 0.411, and 0.335, respectively. Based on the literature, the UV spectrum of a flavone and flavonol exhibited two major absorption peaks in the region of 240 – 400 nm. These two peaks are commonly referred to as band I (usually 300 – 380 nm) and band II (usually 240 – 280 nm) (Mabry *et al*., 1997). In this case, the band I on the UV spectra of compound 3F appears at a wavelength of 344 nm due to the conjugation system of 3'-methoxycinnamoyl (B ring), while band II appears at a wavelength of 244 nm due to the conjugation system of benzoyl (A ring). The absorption of the cinnamoyl system appears at a larger wavelength because its conjugation system is longer than the benzoyl system. The longer the conjugation system, the less energy is required for the electronic transition, so the absorption will appear at a larger wavelength. In this study, we observed that the substitution of the OH group with an ethyl ester moiety caused a hyperchromic shift of band I which initially appeared at 344 nm, and then shifted to 320 nm on the UV spectra of compound T3F. This result was also

supported by the theory which states that band I of flavonols with 3-OH substituted will appear at a lower wavelength (328 - 357 nm) than flavonols with free 3-OH (352 – 385 nm) (Mabry *et al*., 1997). In this case, the band I on UV spectra of compound T3F appears at a wavelength of 320 nm with an absorbance of 0.188 and band II appears at a wavelength of 243 nm with an absorbance of 0.246.The measurement of the FT-IR spectrum was carried out to identify the presence of certain functional groups or bonds present in the molecular structure of the synthesized products. The FTIR spectrum of compound 3F shows a wide absorption band at a wave number of 3253 $cm^{-1}$. The absorption is due to the hydroxy group attached to the C ring of 3F. Then, the absorption band at a wave number of 3007 $cm^{-1}$ shows the vibration of the aromatic C-H bond and the absorption band at a wave number of 2972-2832 $cm^{-1}$ shows the stretching vibration of the aliphatic C-H bond of the methoxy group. Furthermore, the sharp absorption band with strong intensity at wave number 1608 $cm^{-1}$ shows the vibration of the conjugated ketone C=O group and several absorption bands that appear at wave number 1564-1476 $cm^{-1}$ indicate the presence of aromatic C=C bonds. In addition, several absorption bands that appear at wave numbers 1278-1115 $cm^{-1}$ indicate the presence of C-O ether and alcohol bonds in the flavonol structure (Nandiyanto *et al*., 2019). In this study, we observed that the substitution of the OH group with an ethyl ester moiety caused a loss of absorption band around 3252 $cm^{-1}$ on the UV spectra of compound T3F. This indicated that the hydroxyl group was successfully substituted into ethyl ester moiety. In addition, the presence of a new absorption band at a wave number of 1764 $cm^{-1}$ is due to the presence of the ester carbonyl group on T3F.

Measurements of NMR spectra were carried out to determine the number and chemical environment type of protons and carbons that are present in both synthesized products. The $^{1}H$ NMR spectra showed that both products 3F and T3F had the number of protons corresponding to the desired product structures, 12 protons for compound 3F and 18 protons for compound T3F. The $^{1}H$ NMR spectrum of compound 3F showed a characteristic chemical shift when compared to the spectrum of the starting material (CM). Based on the literature, the hydroxyl group (2'-OH) of compound CM in $CDCl_3$ always give a typical singlet signal at a chemical shift of 12-13 ppm (Zamri *et al*., 2016). Thus, the absence of this signal in the $^{1}H$ NMR spectrum of the product 3F indicated that the 2'-OH group successfully undergo cyclization to form a flavonoid heterocyclic ring (Zamri *et al*., 2016). In addition, another peculiarity is the appearance of a single peak at a chemical shift of 7.04 ppm with 1H integration which indicated the presence of a proton from the hydroxyl group (3-OH) attached to the C3 carbon of chromen ring. Based on our previous work, the singlet signal of 3-OH on chromen ring of some substituted flavonols in $CDCl_3$ appeared around 6.56 – 7.10 ppm (Ikhtiarudin *et al*., 2017) and around 6.97 ppm. The absence of this signal on the $^{1}H$ NMR spectrum of product T3F indicates that the 3-OH group was successfully substituted by ethyl ester moiety. Based on Table X, the presence of this moiety also caused the emergence of several new aliphatic proton signals in the $^{1}H$ NMR spectrum of compound T3F at a chemical shift of 4.86 ppm ($CO\text{-}CH_2\text{-}O$-), 4.17 ppm ($\text{-}O\text{-}CH_2\text{-}CH_3$), and 1.21 ppm ($\text{-}O\text{-}CH_2\text{-}CH_3$). Furthermore, the $^{13}C$ NMR spectra also showed that both products 3F and T3F had the number of carbons corresponding to the desired product structures, 16 carbons for compound 3F and 20 carbons for compound T3F. We observed the presence of four new signals on the $^{13}C$ NMR spectrum of T3F when compared to 3F. Three new carbon signals appeared in the aliphatic carbon area, around 68.39 - 14.18 ppm, while another signal appeared at 169.06 ppm which indicates the presence of the ester carbonyl group on T3F.

***Molecular docking***

Based on the docking results as presented in Table IV, panduratin A as a positive control has a binding free energy value of -6.87 kcal/mol and an RMSD value of 1.63. Panduratin A was able to form two hydrogen bonds with two amino acid residues on the active site of the NS2B/NS3 serine protease. In this case, the hydrogen bond is formed between atom O in the carbonyl group (C=O) of the panduratin A compound with His51 amino acid residue with a distance of 2.85Å. A hydrogen bond is also formed between the H atom in the hydroxy group of the panduratin A with the amino acid residue Ser135 with a distance of 2.77 Å (Frimayanti *et al.,* 2011; Kee *et al.,* 2007). Panduratin A has also constructed interaction through van der Waals interactions with amino acid residues Asp75. These three amino acid residues His51, Ser135, and Asp75 are important amino acid residues in the catalytic triad found on the active site of NS2B/NS3 serine protease. The existence of binding with these three amino acid residues can contribute to a molecule inhibiting the catalytic activity of NS2B/ NS3. In this case, binding with one of the three amino acid residues in the catalytic triad is important because these three amino acid residues are involved in the breaking down of polyproteins that are required for viral replication.

Based on the docking results, molecule panduratin A can also bind through van der Waals interactions with other amino acid residues such as Tyr150, Ser131, Gly151, Asn152, Met49, Asp75, Gly153, Tyr161, and

Arg54. Then there are also several other types of interactions with the amino acid residues Phe130, Pro132, Val52, Val154, and Leu128. In addition, the docking results for panduratin A have an RMSD value of 2, thus, the docking method can be said to be valid (Prieto *et al.,* 2018). The smaller RMSD value indicated a smaller deviation value or errors that occur when docking.

Based on docking results, it shows that compound **3F** has a binding free energy value of -5.61 kcal/mol and an RMSD value of 0.82. Binding free energy is a parameter of conformational stability between the ligand and the receptor. More negative binding free energy of a molecule, made this molecule become more stable and the reaction proceeds spontaneously. Furthermore, the negative binding free energy value indicated that it becomes easier for a ligand to bind to the active site of the protein. In this case, the binding free energy value of the **3F** compound was less negative compared to panduratin A as a positive control. This indicated that compound **3F** is more difficult to bind with the active site of NS2B/NS3 serine protease (2FOM) compared to panduratin A. Based on the visualization of the docking results, it was observed that hydrogen bonds were formed between the H atoms in the hydroxy group of the compound **3F** and the active site 2FOM, namely the amino acid residue Gly153 with a bond distance of 2.32 Å. (Brinkworth *et al.,* 1999).

Compound 3F also binds with the active site through van der Waals interactions with amino acid residues Lys74, Asp75, Asn152, Met49, Tyr161, Phe130, Tyr150, Pro132, Ser135, and Gly151. In addition, other interactions were also formed with the amino acid residues of His51 and Leu128. Based on these interactions, it can be observed that compound 3F can also form with all three amino acid residues in the catalytic triad. In addition, compound 3F has a factor of binding of 12 (a measure of the probability that a tested compound will bind to the same amino acid residues to which the positive control compound.

Based on the docking results, it was found that compound T3F has a binding free energy value of -6.39 kcal/mol with an RMSD value of 0.91. The binding free energy value of compound T3F is very close to the binding free energy value of panduratin A as a positive control. Based on the visualization of the docking results, it was observed that hydrogen bonds were formed between atom O in the C=O ester group and the amino acid residue Gly153. Based on the literature, the interaction of ligands with amino acid residues is one of the most important interactions (Brinkworth *et al.*, 1999). In addition, interactions with these residues were also observed in the panduratin A-NS2B/NS3 protease complex which has been previously reported by researchers (Frimayanti *et al.,* 2011). Based on the 3D visualization of the docking results, it can be observed that the distance of the hydrogen bond formed between the T3F compound and this residue is 2.14 Å. This value is closer than the distance of the hydrogen bond between the 3F compound and the residue. This may cause the binding free energy value of the T3F compound to be more negative than the binding free energy value of the 3F compound.

In addition, compound T3F can also interact through van der Waals interactions with amino acid residues Asn152, Met49, Val154, Asp75, Ser131, Gly151, Tyr150, and Ser135. Compound T3F also interacted with amino acid residues His51, Leu128, Pro132, Tyr161, and Phe130 through several other types of interactions. Based on these interactions, it can be observed that this compound can also be bound to the three amino acid residues in the catalytic triad, but with different types of interactions. Factor binding of compound T3F is also higher than compound, it may cause that this compound was predicted as a potential candidate for inhibitor DEN2 NS2B/NS3.

Furthermore, superimposition analysis was carried out to ascertain whether the binding orientation of 3F, T3F ligands and panduratin A simultaneously. It seems that three of them have the same orientation to bind with the protein.

## Conclusion

3'-methoxy flavonol (3F) can be synthesized through the Flynn-Algar-Oyamada reaction using the initial compound 2'-hydroxy-3-methoxy chalcone and hydrogen peroxide in alkaline conditions using a stirrer method. Furthermore, the derivative of 3'-methoxy flavonol (T3F) can be synthesized through the Williamson ether synthesis reaction using the initial compound 3F and ethyl chloroacetate in alkaline conditions with the reflux method. 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. Furthermore, the results of the molecular docking study showed that the T3F compound has the potential to act as an NS2B/NS3 Protease DENV-2 inhibitor. Thus, this compound was chosen as the reference for the next stage in the drug design.

## Acknowledgement

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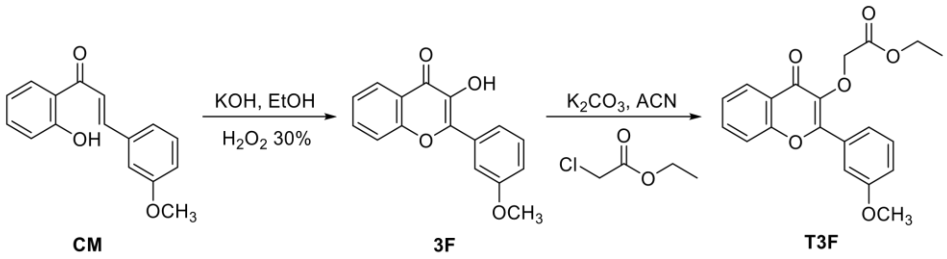
**Appendix A: Data Analysis**

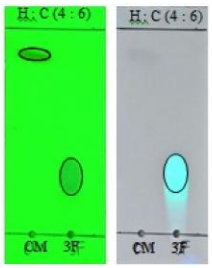
**Physical properties and TLC profiles of 3F and T3F**

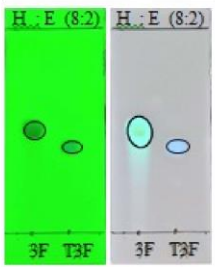



Yellow crystal
33,24 %
mp. 130-131 °C

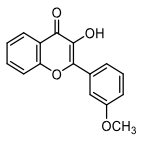
White solid
69,42 %
mp. 64-65 °C

CM = 2'-hydroxy-3-methoxychalcone
3F = 3-methoxyflavonol
T3F = ethyl ester of 3-methoxyflavonol
H = *n*-hexane
C = chloroform
E = ethyl acetate

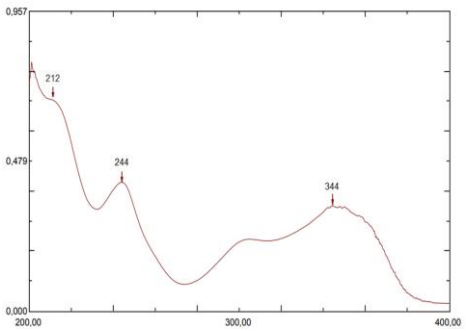


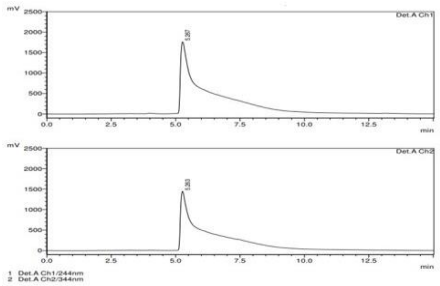



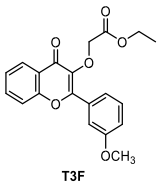

**UV Spectra and HPLC chromatogram of Compounds 3F and T3F**



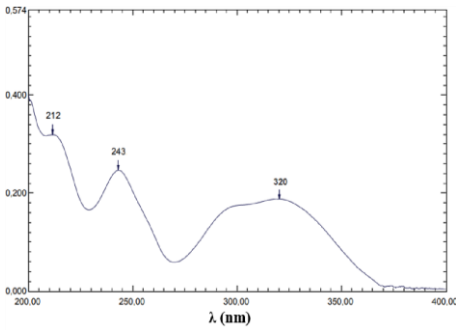
3F

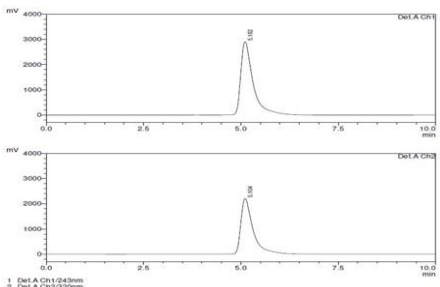


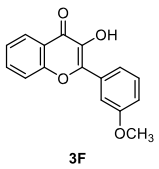


T3F

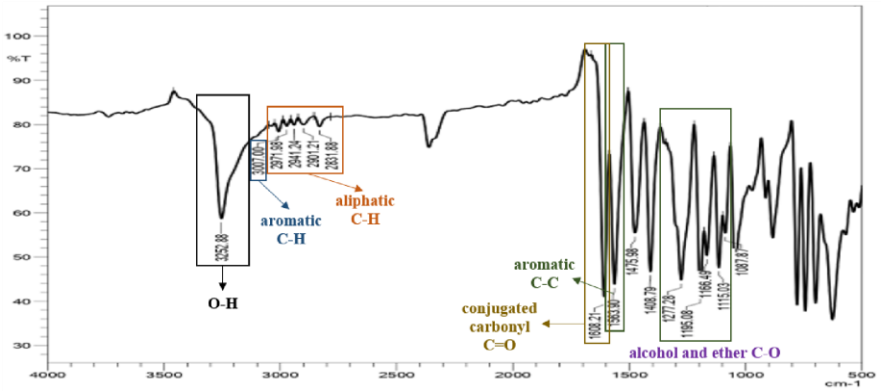


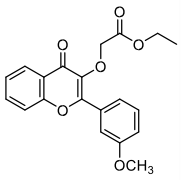



**FTIR Spectra of Compounds 3F and T3F**

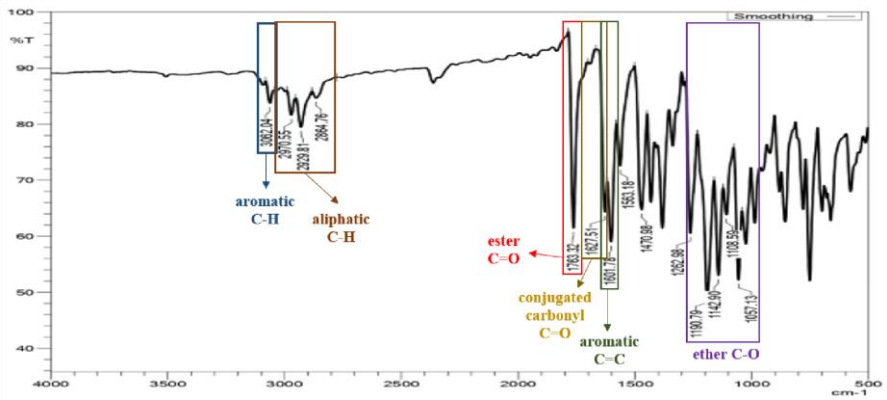



3F

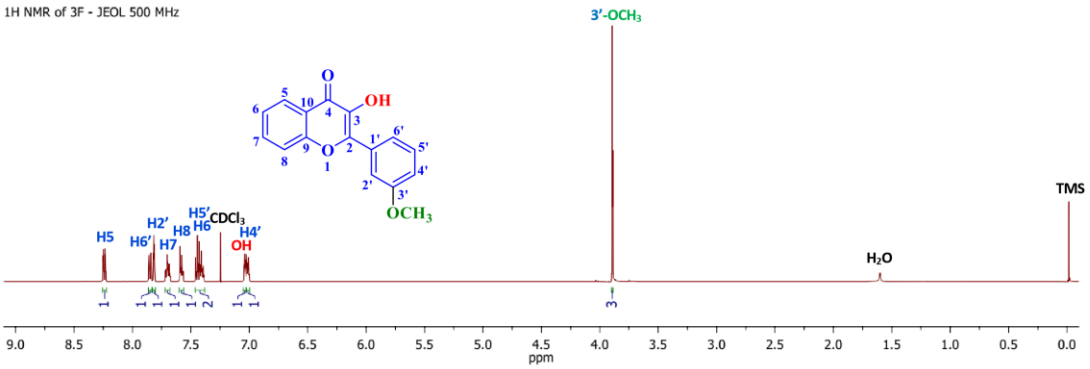


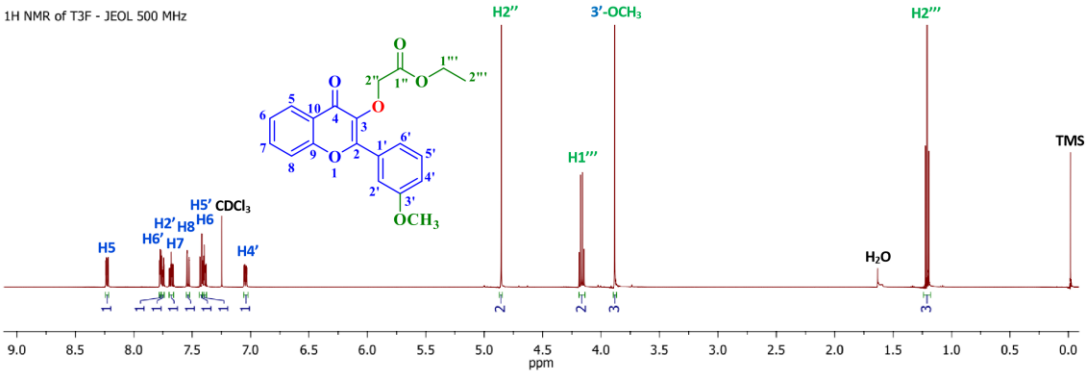




T3F

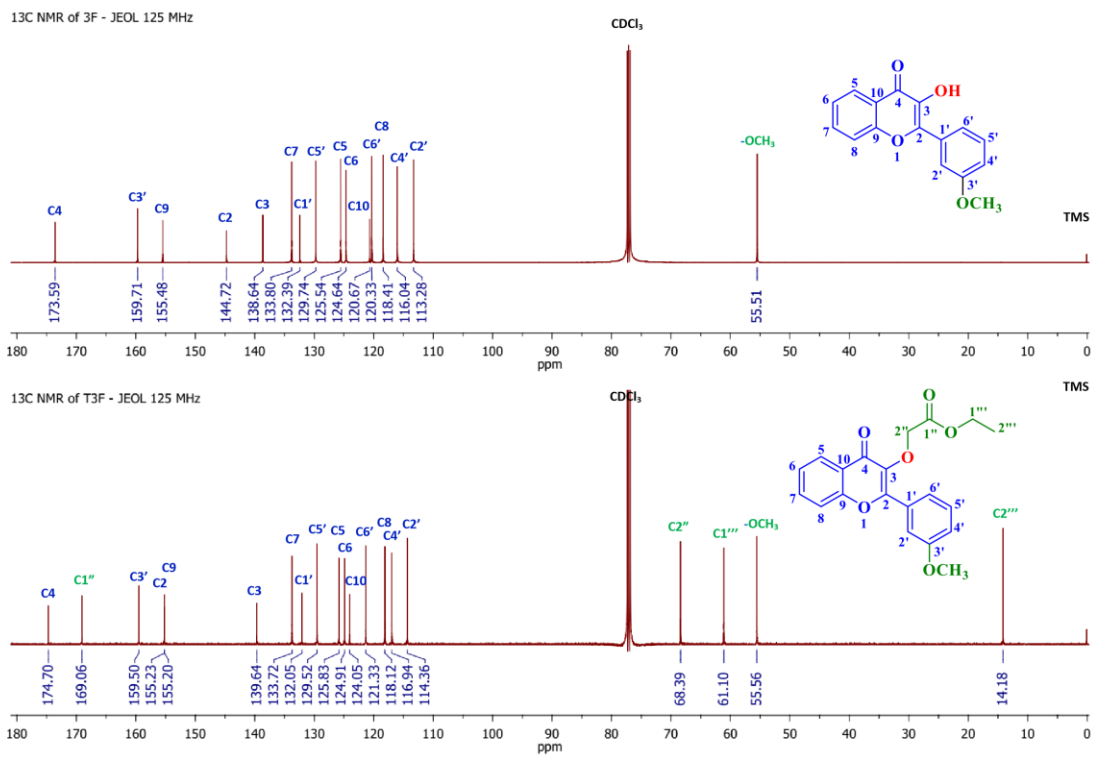



**$^1$H NMR Spectra of Compounds 3F and T3F**



**$^{13}$C NMR Spectra of Compounds 3F and T3F**




**$^{13}$C NMR Spectra of Compounds 3F and T3F (aromatic area)**