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SYNTHESIS, CHARACTERIZATION, AND IN-SILICO STUDIES OF CINNAMIC ACID DERIVATIVES TOWARDS DENGUE VIRUS

(Sintesis, Pencirian dan Kajian In-Siliko Sebatian Terbitan Asid Sinamik Terhadap Virus Denggi)

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Abstract

The dengue virus (DENV) has posed a serious global threat to human health for the past few decades. However, there are still no clinically approved antiviral drug available for the treatment of DENV. Cinnamic acid and its derivatives have attracted great attention due to their broad range of pharmacological properties. The present study aimed to synthesize and investigate the affinity of cinnamic acid derivatives against DENV. Six cinnamic acid derivatives (AC1-AC6) were synthesized by the reaction of substituted cinnamoyl chloride with the corresponding alcohol and amine. The structures of the compounds were confirmed by using ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) and mass spectrometry. The synthesized compounds were then simulated for molecular docking to investigate their binding affinity to the protein target of DENV-2 NS2B/NS3 protease. The in-silico study reveals that the compound AC5 has the highest binding affinity and fit into the allosteric pocket of DENV-2 NS2B/NS3 serine protease with van der Waals interaction, C-H bonding and a few pi interactions such as π-cation, π-lone pair, π-π T-shaped as well as π-alkyl interaction.

Keywords: cinnamic acid, synthesis, anti-dengue virus, docking

Abstrak

Virus denggi (DENV) memberikan ancaman sejagat yang serius dalam kesihatan manusia sejak beberapa dekad yang lalu. Walau bagaimanapun, masih belum ada ubat anti-virus yang diluluskan secara klinikal bagi rawatan DENV. Asid sinamik dan terbitannya telah menarik banyak perhatian oleh kerana sifat farmakologi yang mempunyai julat yang luas. Kajian ini bertujuan untuk mensintesis dan menyiasat afiniti terbitan asid sinamik terhadap DENV. Enam terbitan asid sinamik (**AC1-AC6**) telah disintesis dengan tindakbalas penukargantian sinnamoil klorida dengan alkohol dan amina. Struktur sebatian telah disahkan dengan menggunakan ^{1}H dan ^{13}C Resonans Magnet Nukleus (NMR) dan spektrometer jisim. Sebatian yang telah disintesis kemudian disimulasikan dengan mengedok molekul untuk mengkaji sifat afiniti dengan protein sasaran iaitu protease DENV-2

NS2B / NS3. Kajian in-siliko mendedahkan bahawa sebatian **AC5** mempunyai afiniti pengikatan tertinggi dan dapat memasuki poket alosterik DENV-2 NS2B / NS3 protease serin dengan interaksi van der Waals, ikatan C-H dan beberapa interaksi pi seperti π-cation, π -lone pair, π-π berbentuk T dan juga interaksi π-alkil.

Kata kunci: asid sinamik, sintesis, anti-virus denggi, mengedok

Introduction

Dengue virus (DENV), the most significant virus in the *Flaviviridae* family with the highest morbidity and mortality rates, has been a serious global threat in the past few decades. The latest statistics from the World Health Organization (WHO) has shown an increasing trend of DENV infection throughout Malaysia with 127,407 reported cases in 2019. Despite this alarming issue, there is currently no effective vaccine nor antiviral treatment available for dengue patients. Prevention against DENV infection largely depends on controlling the mosquito vectors as well as on the use of the natural remedies. This underscores an urgent need to discover and develop a novel antiviral agent that is safe and effective in controlling dengue infection.

Cinnamic acid has gained a lot of interest due to its simple structure with great potential of pharmacological properties. It is a group of aromatic carboxylic acids, appearing naturally in the plant kingdom. Cinnamic acids occur in all green plants as well as the reproductive organs of flowering plants [1]. Cinnamic acids with varied substitution on the aryl ring, and their esters have been identified in natural bee products including honey and propolis [2]. It is also readily available from coffee beans, tea, cocoa, apples, tomatoes, and cereals [3]. It is a safe and extensive source of material due to its natural and low toxicity properties.

Cinnamic acid and its related molecules have a variety of biological activities, such as anticancer, antimalarial, antimicrobial and antioxidant [4,5]. Recently, cinnamic acid was reported to be an effective antiviral drug against Zika virus (ZIKV) [6]. It was found that cinnamic acid possessed anti-ZIKV properties against the post-entry stage of the ZIKV replication cycle, and inhibited RNA-dependent RNA polymerase (RdRp) activity. Additionally, cinnamic acid was also reported to have an excellent activity against *Tobacco mosaic virus* (TMV) in which most target compounds in this research exhibited good anti-plant virus activities [7]. One of the cinnamic acid derivatives also significantly inhibits hepatitis C virus (HCV) replication via the induction of oxidative stress [8].

Despite their rich medicinal tradition and remarkable biological activities, cinnamic acid derivatives and their potentials remained underutilized for several decades. Currently, the antiviral activity of cinnamic acid related molecules against dengue virus infection has never been experimentally addressed despite of its well-known therapeutic potential. In this study, six cinnamic acid derivatives have been synthesized and simulated for molecular docking to investigate their binding interactions to the protein target of DENV-2 NS2B/NS3 protease. Overall, this study demonstrated that cinnamic acid would be an excellent potential candidate for the development of antiviral drugs against DENV.

Materials and Methods

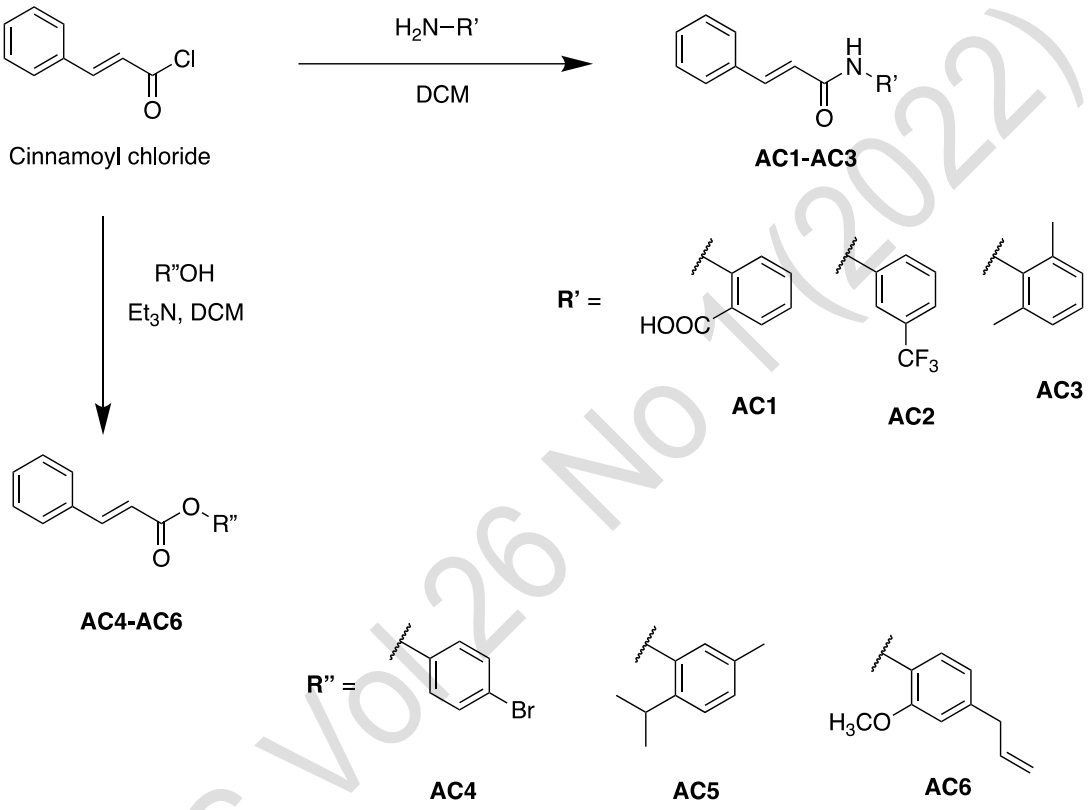
Chemicals and instrumentations

Ferulic acid, cinnamoyl chloride, anthranilic acid, 3-aminobenzotrifluoride, 2,6-dimethylaniline, 4-bromo benzyl alcohol, thymol, eugenol, anhydrous dichloromethane (DCM), triethylamine, hydrochloric acid (HCl), sodium bicarbonate ($NaHCO_3$), magnesium sulphate (Mg_2SO_4), hexane, ethyl acetate and acetone were purchased from standard commercial suppliers such as Sigma-Aldrich, Merck, Acrós Organics, Fisher Scientific, HmbG® Chemicals and R & M Chemical. All chemicals and solvents used in this study were of reagent grade (AR) and were used without further purification. Column chromatography was performed using silica gel 60 (230-400 mesh, Merck). Analytical thin-layer chromatography (TLC) was done on precoated silica gel 60 (F254, Merck) plates and visualized under UV 254 nm without treatment.

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were determined by Bruker Avance II 400 spectrometer in deuterated dimethyl sulfoxide (DMSO-d_6). The chemical shifts were reported in a ppm scale. The synthesized compounds were dissolved in dichloromethane (DCM) and analysed using Perkin Elmer GC-MS (Clarus 500 Chromatography/Mass Spectrometry).

Chemistry

The synthetic route of the synthesized compounds is described in Scheme 1.



Scheme 1. The synthetic work of cinnamic acid amide and ester derivatives

Synthesis of cinnamoyl amide derivatives (AC1-AC3)

Cinnamoyl chloride (0.40 g, 2.4 mmol) in anhydrous DCM (5 mL) was added dropwise at 0 ℃ to a stirred solution of 3.6 mmol of amine (anthranilic acid, 3-aminobenzotrifluoride and 2,6-dimethylaniline) in anhydrous DCM (10 mL). The mixture was stirred at 0℃ for 30 min and then at RT for 3 hours. After completion of the reaction, 10 mL of DCM was added. The solution was washed 3 times with 1 N HCl and 2 times with 1 N $NaHCO_3$. The separated organic layer was dried over Mg_2SO_4 and the solvent was evaporated completely to obtain a residue. The residue was purified by silica gel column chromatography to afford the desired products of **AC1**-**AC3** by using a solvent system of hexane and ethyl acetate.

2-cinnamamidobenzoic acid (AC1)

Colorless powder (0.34 g, 53 %), **AC1** was prepared from the reaction of cinnamoyl chloride (0.40 g, 2.4 mmol) with anthranilic acid (0.49 g, 3.6 mmol) in the same manner as described in Scheme 1, ^{1}H NMR (400MHz, DMSO-d_6): δ_H ppm, 11.38 (1H, s, NH) , 8.61 (1H, d, *J* = 7Hz, H-ar), 8.02 (1H, d, *J* = 6Hz, H-

ar), 7.74 (1H, t, *J* = 7Hz, H-ar), 7.66 (1H, d, *J* = 7Hz, CH), 7.62 (1H, t, *J* = 9Hz, H-ar), 7.44 (4H, m, H-ar), 7.19 (1H, t, *J* = 14Hz, H-ar), 6.89 (1H,d, *J* = 15Hz, CH); ^{13}C NMR (100MHz, DMSO-d_6): δ_C ppm, 117.5, 120.9, 123.4, 128.6, 129.4, 130.5, 131.6, 134.4, 134.8, 141.26 (CH-aromatic), 141.7 & 122.9 (C=C), 169.9 (C=O). HRESIMS (positive mode) m/z calculated for $[C_{16}H_{13}NO_3]^+$: 267, $[M+H]^+$; found: 268.2639.

N-(3-(trifluoromethyl)phenyl)cinnamamide (AC2)

Colorless powder (0.42 g, 61 %), **AC2** was prepared from the reaction of cinnamoyl chloride (0.40 g, 2.4 mmol) with 3-aminobenzotrifluoride (0.58 g, 3.6 mmol) in the same manner as described in Scheme 1, ^{1}H NMR (400MHz, DMSO-d_6): δ_H ppm, 10.57 (1H, s, NH) , 8.22 (1H, s, H-ar), 7.87 (1H, d, *J* = 8Hz, H-ar), 7.66 (2H, m, H-ar), 7.62 (1H, d, *J* = 4Hz, CH), 7.58 (1H, t, *J* = 8Hz, H-ar), 7.45 (4H, m, H-ar), 6.82 (1H, d, *J* = 16Hz, CH); ^{13}C NMR (100MHz, DMSO-d6): δ_C ppm, 115.7, 120.1, 123.2, 125.9, 128.3, 129.8, 1130.1, 130.5, 134.9, 140.4 (CH-aromatic), 141.4 & 122.1 (C=C), 164.5 (C=O). HRESIMS (positive mode) m/z calculated for $[C_{16}H_{12}NOF_3]^+$: 291, $[M+H]^+$; found: 292.0951.

N-(2,6-dimethylphenyl)cinnamamide (AC3)

Colorless powder (0.28 g, 46 %), **AC3** was prepared from the reaction of cinnamoyl chloride (0.40 g, 2.4 mmol) with 2,6-dimethylaniline (0.44 g, 3.6 mmol) in the same manner as described in Scheme 1, ^{1}H NMR (400MHz, DMSO-d_6): δ_H ppm, 9.52 (1H, s, NH) , 7.64 (2H, d, *J* = 7.0 Hz, H-aromatic), 7.57 (1H, d, *J* = 16.0 Hz, CH), 7.45 (3H, m, H-aromatic), 7.09 (3H, s, H-aromatic), 6.89 (1H, d, *J* = 16.0 Hz, CH), 2.00 (6H, s, CH_3); ^{13}C NMR (100MHz, DMSO-d_6): δ_C ppm, 18.6 (CH_3), 126.9, 128.1, 129.4, 130.1, 135.2, 135.5 (CH-aromatic), 163.9 (C=O), 140.1 & 122.2 (C=C). HRESIMS (positive mode) m/z calculated for $[C_{17}H_{17}NO]^+$: 251, $[M+H]^+$; found: 252.1391.

Synthesis of cinnamoyl ester derivatives (AC4-AC6)

Triethylamine (3 eq) was added into a stirred solution of 1 eq of alcohol (4-bromobenzyl alcohol, thymol, and eugenol) in anhydrous DCM (20 ml). The mixture was magnetically stirred for 0.5h at 0-5°C. Then, cinnamoyl chloride (1 eq) was slowly added to the mixture within a 15 min period. The reaction mixture was continued stirring for 0.5h at 0-5°C before being slowly removed to room temperature. Then, the stirring continued for another 24 h. The completion of the reaction was monitored by TLC. The solvent was evaporated by using a rotary evaporator and the compound was purified via column chromatography. Hexane/ethyl acetate was used as the eluent to afford the title ester products of **AC4**-**AC6**.

4-bromophenyl cinnamate (AC4)

Colorless powder (0.32 g, 43 %), **AC4** was prepared from the reaction of cinnamoyl chloride (0.40 g, 2.4 mmol) with 4-bromobenzyl alcohol (0.44 g, 2.4 mmol) in the same manner as described in Scheme 1, ^{1}H NMR (400MHz, DMSO-d_6): δ_H ppm, 7.69 (2H, m, H-aromatic) , 7.61 (1H, d, *J* = 16.0 Hz, H-aromatic), 7.52 (1H, d, *J* = 8.4 Hz, H-aromatic), 7.42 (3H, m, H-aromatic), 7.28 (1H, d, *J* = 8.4 Hz, CH), 6.55 (1H, d, *J* = 16.0 Hz, CH), 4.46 (1H, s, H-aromatic), 2.51 (1H, s, H-aromatic); ^{13}C NMR (100MHz, DMSO-d_6): δ_C ppm, 128.6, 129.0, 129.3, 130.7, 131.3, 134.7 (CH-aromatic). 142.4 &119.6 (C=C), 144.4 (C-O), 168.0 (C=O). HRESIMS (positive mode) m/z calculated for $[C_{15}H_{11}O_2Br]^+$: 303, $[M+H]^+$; found: 304.3010.

2-isopropyl-5-methylphenyl cinnamate (AC5)

Colorless powder (0.24 g, 35 %), **AC5** was prepared from the reaction of cinnamoyl chloride (0.40 g, 2.4 mmol) with thymol (0.36 g, 2.4 mmol) in the same manner as described in Scheme 1, ^{1}H NMR (400MHz, DMSO-d_6): δ_H ppm, 9.11 (1H, s, H-aromatic) , 7.92 (1H, d, *J* = 16.0 Hz, CH), 7.83 (3H, m, H-aromatic), 7.27-7.05 (2H, d, *J* = 8 Hz, H-aromatic), 6.97-6.61 (2H, m, H-aromatic), 6.56 (1H, d, *J* = 7.6 Hz, CH), 3.42 (3H, s, CH_3), 1.17 (6H, m, CH_3); ^{13}C NMR (100MHz, DMSO-d_6): δ_C ppm, 27.1-21.1 (CH_3), 123.2, 126.0, 126.8, 127.4, 129.1, 129.4, 135.7, 136.6, 137.2 (CH-aromatic), 146.8 & 116.0 (C=C), 148.1 (C-O), 165.6 (C=O). HRESIMS (positive mode) m/z calculated for $[C_{19}H_{20}O_2]^+$: 280, $[M+H]^+$; found: 281.1537.

4-allyl-2-methoxyphenyl cinnamate (AC6)

Colorless powder (0.33 g, 43 %), **AC6** was prepared from the reaction of cinnamoyl chloride (0.40 g, 2.4

mmol) with eugenol (0.39 g, 2.4 mmol) in the same manner as described in Scheme 1, ^{1}H NMR (400MHz, DMSO-d_6): δ_H ppm, 8.72 (1H, s, H-aromatic) , 7.54 (2H, m, H-aromatic), 7.48 (1H, d, *J* = 4.0 Hz, CH), 7.35 (3H, m, H-aromatic), 6.71 (2H, m, H-aromatic), 6.56 (1H, d, *J* = 4.0 Hz, CH), 3.74 (3H, s, CH_3) 3.25 (2H, d, *J* = 8.0 Hz, CH_2); ^{13}C NMR (100MHz, DMSO-d_6): δ_C ppm, 23.4 (CH_3), 115.9, 116.0, 123.3, 126.2, 148.3, 149.5 (CH-aromatic), 144.9 & 116.0 (C=C), 148.3 (C-O), 168.4 (C=O). HRESIMS (positive mode) m/z calculated for $[C_{18}H_{18}O_3]^+$: 282, $[M+H]^+$; found: 282.0887. Structures of synthesized cinnamic acid derivatives are shown in Figure 1.

Computational study

The synthesized derivatives (**AC1**-**AC6**) were simulated for molecular docking to investigate their binding affinity to the protein target of DENV type 2 (DENV-2) NS2B/NS3 protease, with Ser135, His51 and Asp75 as their catalytic triad amino acid residues. The virtual screening was carried out on the homology model of the DENV non-structural protein, NS2B/NS3pro developed by Wichapong et al. [16]. The DENV-2 NS2B/NS3pro model was built based on the DENV-2 complex cofactor-protease using the crystal structure of NS2B/NS3pro West Nile Virus (WNV) as the template. The protein structure was prepared as a macromolecule prior to docking using AutoDock version 1.5.6 package (www.autodock.scrips.edu). Briefly, the protein preparation was done by removing the native ligand, tetrapeptide inhibitor (Bz-Nle-Lys-Ar-H) and water molecules, the addition of polar hydrogen and Kollmann charges.

The 3D structures of all cinnamic acid derivatives were constructed and energetically optimized using ChemDraw Professional 16.0. The minimized structures were saved in sdf format before converted into pdb format using OpenBabel-3.1.1 software. The validation of the docking protocol was done by re-docking the inhibitor tetrapeptide (Bz-Nle-Lys-Ar-H) with the RMSD value not greater than 2.0 Å. The ligands were prepared by merging non-polar hydrogen and assigned a Gasteiger charge. The center of the grid box was employed around the protease active site at 23.038, 43.372, -0.316 in x, y, and z coordinates, respectively, with a box size of 60 × 60 × 60 dimensions and grid spacing 0.375 Å. The docking of ligands was run with the Lamarckian Genetics Algorithm (GA) search program applied to generate 100 runs. The binding modes of compounds were analyzed using Discovery Studio Client 2020 (www.accelrys.com). The identification of hit compound was identified based on the conformations with the ones of lowest free binding energy and of the most populated cluster.

Preparation of enzyme and ligand for docking

The structure of available DENV2, which is a complex of NS2B-NS3 protease (PDB code: 2FOM), is devoid of any ligand. Thus, to get an insight into the binding cavity we used coordinates of co-crystallized ligand from West Nile virus (PDB code: 2FP7) belonging to the same family as that of DENV and that shares the structural similarity of the catalytic triad. It is available in complex with the inhibitor tetrapeptide Bz-Nle-Lys-Arg-Arg-H. The structure of DENV2 complexed with the inhibitor tetrapeptide Bz-Nle- Lys-Arg-Arg-H was constructed by homology modelling method.

Results and Discussion

Chemistry

Six cinnamic acid derivatives (Figure 1) were successfully synthesized in moderate yield (35-61%) by modification of established methods [9]. The synthesis of the target compounds was straightforward, and the general synthetic pathway is illustrated in Scheme 1. Cinnamoyl chloride was reacted with various amines; anthranilic acid, 3-aminobenzotrifluoride and 2,6-dimethylaniline to produce the titled compounds of cinnamic acid amides (**AC1**, **AC2** and **AC3)**. For the synthesis of cinnamic acid ester derivatives, different alcohols; 4-bromobenzyl alcohol, thymol and eugenol were reacted with cinnamoyl chloride in DCM to afford the title compounds **AC4**, **AC5** and **AC6**. To optimize the reaction conditions, the polarity of the solvent medium from polar to non-polar was adjusted using DMSO, ethanol and tetrahydrofuran (THF) in the reaction of substituted cinnamoyl chloride with the corresponding alcohol and amine.

^{1}H and ^{13}C nuclear magnetic resonance (NMR) analysis confirmed the identity of all synthesized cinnamic acid derivatives. The presence of NH resonance peaks as observed in all three ^{1}H NMR spectra of **AC1**-**AC3** prove that the cinnamoyl amide derivatives were successfully synthesized. NH resonance which appears as singlet is found to be at the most downfield region (δ_H 11.38-9.52 ppm) for **AC1** to **AC3**. There is also a high-intensity singlet resonance in the upfield region at δ_H 2.17 ppm representing two methyl moieties presented in the **AC3** compound. For cinnamoyl ester derivatives, in the range of δ_H 7.41-7.70 ppm, multiple peaks can be observed in **AC4** indicating the presence of aromatic groups. Meanwhile, for **AC6** the peaks are in the range of δ_H 6.55-6.70 ppm. There are other high-intensity singlet resonances at δ_H 1.13-2.29 ppm and at δ_H 3.73 ppm respectively, representing methyl moieties presented in both compounds **AC5** and **AC6**. The alkene groups for all three compounds of **AC4**-**AC6** can be observed in the peak range of δ_H 6.51-6.57 ppm.

The resonance of the C=O group was clearly observed for all synthesized compounds at δ 169.9-163.8 ppm. The alkene group is also presented in all compounds which are in the range of δ 146.8-116.0 ppm. For the aromatic ring, the resonance carbon was found at the range between δ 129.8-123.3 ppm. Furthermore, the resonance for CH_3 moieties can also be observed for the compounds of **AC3**, **AC5** and **AC6** which are in the region δ 27.1-18.7 ppm.

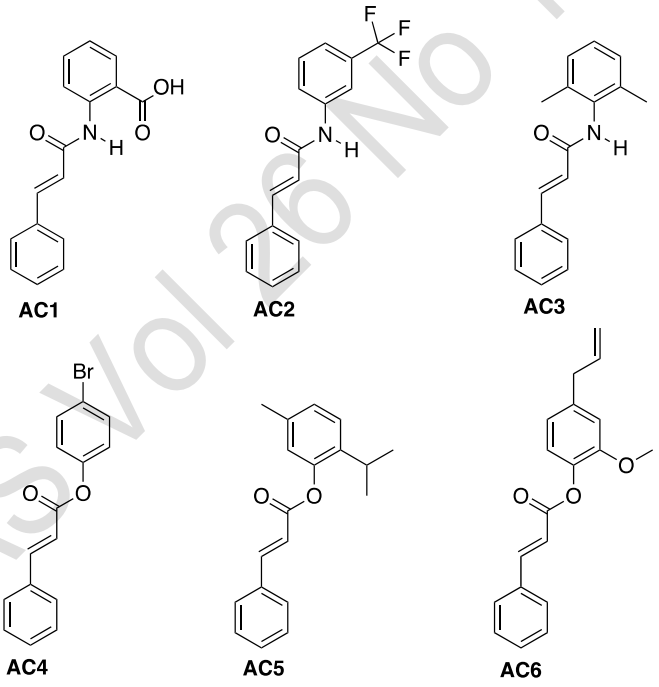


Figure 1. Structure of synthesized cinnamic acid derivatives

Molecular docking

The in-silico studies were conducted to investigate the binding interactions of the synthesized compounds **AC1**-**AC6** towards DENV-2 NS2B/NS3 protease and search for the best orientation of ligand-protease complex with the lowest free energy of binding (FEB). This step serves as a good tool to predict and match the desired binding site, understanding possible conformation of the compounds and further clarifies the binding interactions in the binding pocket [10].

DENV is a single-stranded RNA genome that encodes a single open reading frame (5-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3) with three structural and seven non-structural proteins [11]. The N-terminal of NS3 is a trypsin-like serine protease, which when combined with its co-factor can cleave the viral polyprotein [12]. Disruption of NS2B-NS3 protease inhibits viral replication [13]. This poses NS2B-NS3 protease of DENV-2 as a promising target for antiviral drug design [14].

The three catalytic triad amino acid residues were located in NS3 serine proteases namely His51, Asp75 and Ser135. The NS2B protease acted as an NS3 serine protease co-factor for an optimal catalytic activity [15]. The homology protein crystal structure generated by Wichapong et al., (2010) was used due to the lack of DENV-2 NS2B/NS3 serine protease inhibitor-bound 3D structures. Moreover, some of the protease database structures were found to have missing amino acid residues [16].

The Lamarckian genetic algorithm was employed in AutoDock version 1.5.6 to determine the binding modes and compounds conformation towards DENV-2 NS2B/NS3 protease homology protein crystal structure. In the preparation for docking simulation, only protease was retained as a part of the macromolecule file, while the Bz-Nle-Lys-Arg-Arg-H was extracted and saved as a positive control ligand. The ligand was extracted and re-docked into the binding site to evaluate the success of the docking method in reproducing the experimentally known complex.

The covalent map parameter was used during positive control docking simulation to constrain the molecular geometry of the peptide inhibitor. After that, all the synthesized compounds were docked with Wichapong et al., [16] homology crystal structure using the same parameter. The re-docked was carried out successfully with the root mean square deviation (RMSD) value of 1.23 Å. Henever et al. stated that the programs that able to poses the RMSD value in the range of 1.5-2 Å, depending on the ligand size, are considered to have performed successfully [17].

The synthesized compounds, **AC1**-**AC6** fit into the allosteric pocket of NS2B/NS3 serine protease. The free energy of binding (FEB) obtained was tabulated in Table 1. Figure 2 shows the overlay docked conformation of the cinnamic acid derivatives between the Wichapong et al. [16] homology model and Bz-Nle-Lys-Arg-Arg-H referenced ligand. The image was generated using Discovery Studio Visualizer 4.5.

The detailed ligand-binding site interaction in 2D structural views of all the compounds are shown in Figure 3 below. Compound **AC5** shows the lowest free energy of binding (-6.53 kcal mol^{-1}) indicating the highest binding affinity towards NS2B/NS3 protease of DENV-2 compared to the other five compounds [18]. Figure 3 also shows the poses and non-covalent interactions involved between the ligands and the protease-binding site. This compound showed a few van der Waals interaction, one C-H bonding interaction, one π-cation interaction, one π-lone pair interaction, one π-π T-shaped interaction, and two π-alkyl interactions with the amino acid residue in the DENV-2 NS2B/NS3 protease.

The presence of the aromatic group in the synthesized cinnamic acid derivatives enhances the possibilities of van der Waals interaction. Hence, we can see this interaction in all the synthesized compounds of **AC1**-**AC6**. Individually, van der Waals interaction was also observed in compound **AC5**, involving Ser135 (one of the catalytic triads) and other residues namely Asp129, Ser131, Gly151 and Thr134. Pi interactions such as π-π T-shaped interaction and π-lone pair interaction were also observed between the ester moiety of the synthesized compound with Tyr161 and Phe130, respectively. Meanwhile, another aromatic ring at cinnamic moiety of compound **AC5** appeared to have a π-cation interaction with the His51 (from the catalytic triad) and π-alkyl interactions with Val52. The π-cation interaction with the His51 suggested that the DENV-2 NS2B/NS3 replication was affected as His51 is one of the catalytic triads of NS2B required for replication activities [19].

Table 1. The free energy of binding (FEB) of compounds **AC1-AC6**

Compound	**FEB (kcal mol^{-1})**
Positive Control (peptide inhibitor)	-6.58
AC1	-6.11
AC2	-6.36
AC3	-6.45
AC4	-6.33
AC5	-6.53
AC6	-6.50

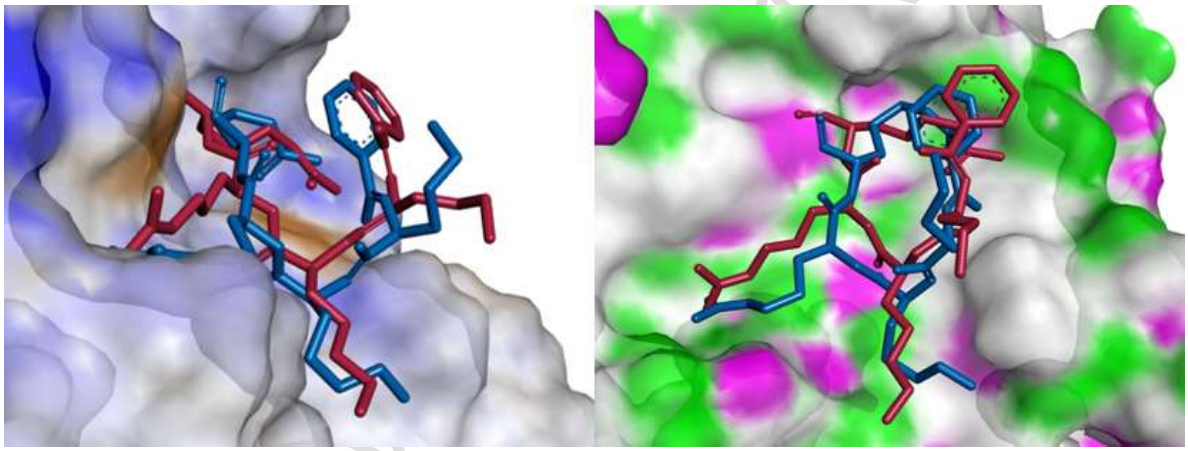


Figure 2. The overlay docked conformation of the cinnamic acid between the Wichapong et al. [16] homology model and Bz-Nle-Lys-Arg-Arg-H referenced ligand (red: original ligand conformation, blue: docked ligand conformation).

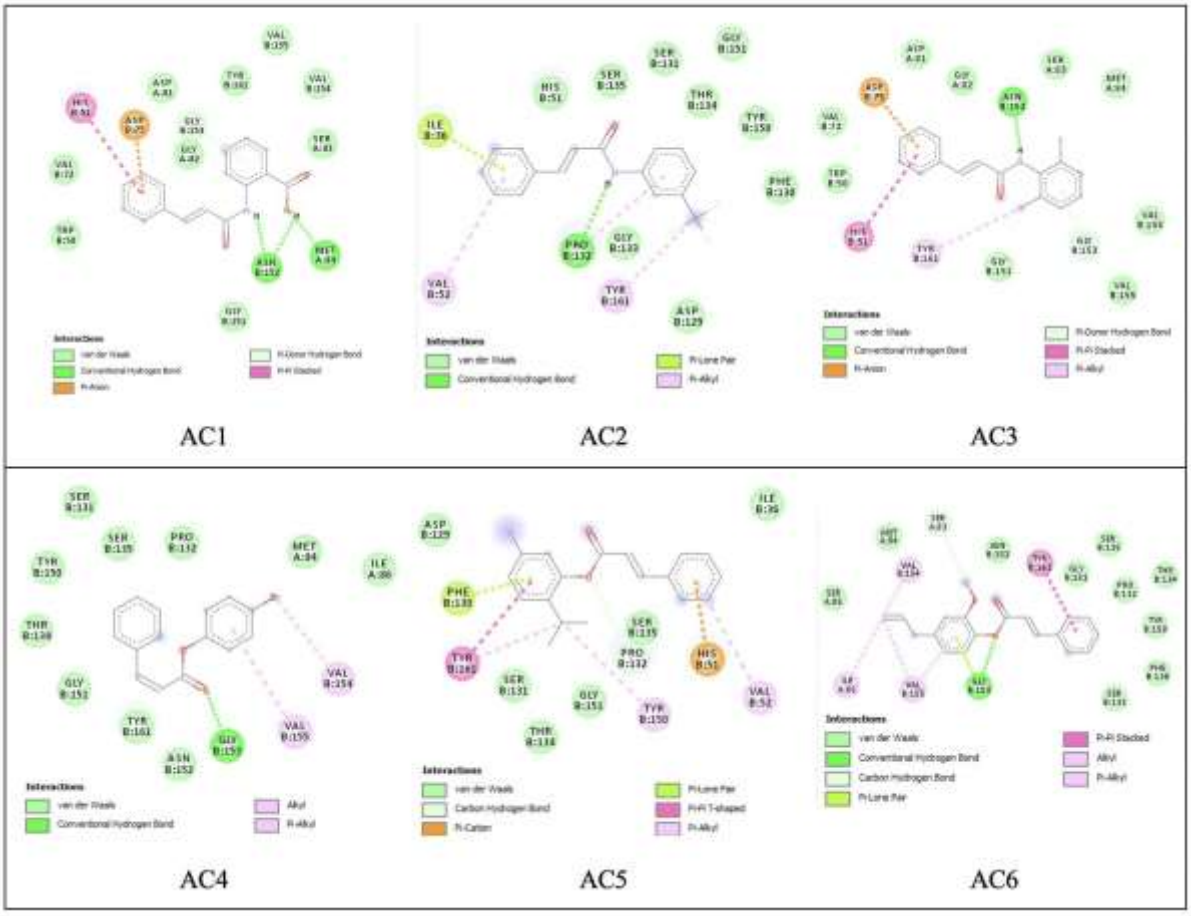


Figure 3. The 2D structural views of ligand-binding site interactions for synthesized compounds AC1-AC5

Conclusion

The present work has demonstrated that six cinnamic acid derivatives were successfully synthesized by the reaction of cinnamoyl chloride with various amines and alcohols. All synthesized compounds were characterized by ^{1}H and ^{13}C-NMR, and mass spectrometry. The significant molecular interaction between the synthesized compounds and DENV2 NS2B/NS3 protease were revealed by molecular studies indicating their potential activity as a protease inhibitor. The compound **AC5** is predicted to have a higher DENV-2 NS2B/NS3 protease inhibitor as it has the highest binding affinity, with a significant van der Waals force and pi interactions between the synthesized compound and the amino acid residues. Further verification studies such as *in-vitro*, toxicity analysis, and *in-vivo* analysis are required for identifying the possibilities of the cinnamic acid derivatives to be a lead molecule for anti-dengue drug development.

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