Synthesis and Molecular Docking Study of 1-(3-Chloropropyl)-3,5-Bis((E)-4-Methoxybenzylidene)Piperidin-4-One as Dengue Virus Type 2 (DEN2) NS2B/NS3 Protease Inhibitor Candidate

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Abstract

Curcumin is a secondary metabolite compound that has diverse biological activities. However, it is easily hydrolyzed at physiological pH due to the presence of the β-diketone group. Therefore, the replacement of the β-diketone group with mono ketone is expected to overcome this issue. We hereby report the synthesis of mono ketone curcumin derivatives from piperidone by two-steps reactions. The synthesis of curcumin derivate 3 was carried out by Claisen-Schmidt condensation between 4-piperidone and 4-methoxybenzaldehyde using alkaline catalyst. The synthesized curcumin derivate 3 was then reacted with the 1-bromo-3-chloropropane to produce curcumin derivate 5, 1-(3-chloropropyl)-3.5-bis((E)-4-methoxybenzylidene)piperidin-4-one, with 72% yield. The calculated docking scores, the curcumin derivate 5 possessed a better affinity for receptors than the standard panduratin A. The curcumin derivate 5 has a lower docking score of -6.40 kcal/mol compared to panduratin A with value of -5.18 kcal/mol and also had strong binding interactions to DEN2 NS2B/NS3 protease. Thus, this compound is a promising candidate as a new anti-dengue agent.

Keywords: curcumin, dengue protease inhibitor, molecular docking, DEN2 NS2B/NS3.

Introduction

Dengue virus infection is a major health problem in 112 tropical and subtropical countries in Southeast Asia, the Western Pacific, Central America and South America, and nearly 50 million new cases occur worldwide each year. The number cases in Indonesia is rapidly increasing due to several reasons such as population and lack of public awareness in healthy living.

There are four serotypes of dengue virus (DEN1, DEN2, DEN3 and DEN4), whereas dengue virus type 2 (DEN2) is the most prevalent type. The protease complex, consisting of non-structural protein 3 (NS3) and its cofactor (NS2B). The NS3 serine protease is responsible for proteolytic processing of the viral polyprotein. The binding of the NS3 serine protease to an NS2B cofactor will form NS2B-NS3 protease complex that is required for DEN2 viral replication. Thus, this protease may be a potential target for dengue antiviral drugs by blocking the association of NS3 protease with its protein cofactor NS2B.

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One of natural compound that has potential as an anti-dengue virus is the curcuminoid group. Curcuminoids include diarylheptanoid derivatives and are the main pigment of turmeric (Curcuma longa) and various types of genus Curcuma.

Curcumin has traditionally been used in the treatment of various diseases such as cough, diabetes, hepatitis, anti-inflammatory, rheumatism, sinusitis, antioxidants, anti-inflammatory, and antibacterial. Curcumin is also known to have antiviral activity. Furthermore, in vivo study showed that curcumin extract at doses of 0.147 mg/ml has antiviral effect against DEN2 and reduce viremia period.

Apart from the diverse biological activities, the bioavailability of curcumin is very low due its rapid hydrolysis to become inactive metabolites and excreted from the body. This is due to the presence of unstable beta-diketone groups at physiological pH. Therefore, synthesis of curcumin derivatives for replacement of the beta-diketones with mono ketone is expected to overcome this.

In previous study, several mono ketone curcumin derivatives from acetone, cyclopentanone and cyclohexanone have been synthesized to increase their stability, activity and selectivity. Recently, researchers have put their interest to curcumin derivatives from piperidone due to their broad structure variations and spectrum of bioactivity, including as antiviral. However, curcumin derivatives with the modification of amine group in 4-piperidon as anti-dengue agent only few has been reported.

Accordingly, our effort was to synthesis curcumin derivatives from N-substituted piperidone by using 1-bromo-3-chloropropane as alkylating agent to the amine group and determined for their affinity to inhibit the dengue virus type 2 against NS2B/NS3 protease through molecular docking studies.

**Material and Methods**

**Materials**

UV-Visible spectrophotometer (Genesys 10S UV-VIS v4.002 2L9N175013), IR spectrophotometer (Shimadzu FTIR, IR Prestige-21), HPLC (Shimadzu UFLC Solution), NMR spectroscopy (Agilent 500 MHz), and glassware commonly used in synthesis laboratories. The starting materials used in this study were synthesis reagent grade (≥98%) includes 4-piperidone monohydrate hydrochloride (Sigma-Aldrich), 4-methoxybenzaldehyde (Sigma-Aldrich), 1-bromo-3-chloropropane (Sigma-Aldrich), cesium carbonate (Sigma-Aldrich), absolute ethanol (Merck), sodium hydroxide (Merck), hydrochloric acid 37% (Merck), universal indicator (Merck), GF254 TLC plates (Merck), aqua DM, absolute ethanol (Merck), and organic solvents.

**Synthesis Procedure**

**Synthesis of 3,5-bis((E)-4-methoxy benzylidine) piperidine-4-one (3):**

The synthesis of curcumin derive 3 has been carried out by modifying the previous method. Compound 4-piperidone monohydrate hydrochloride (5 mmol) and 4-methoxybenzaldehyde (10 mmol) were dissolved in 10 ml of absolute ethanol and 10 ml of 1N NaOH was added. Then the mixture was refluxed for 5 hours at 80 °C. Reaction was monitored using TLC plate per 30 minutes. After the completion of the reaction, the mixture was neutralized using 1N HCl. The mixture was allowed to sit overnight until a precipitate was formed. The precipitate was filtered and washed using n-hexane and aqua DM then air dried at room temperature. The obtained product was recrystallized with methanol to obtain curcumin derivate 3.
**Synthesis of 1-(3-chloropropyl)-3,5-bis((E)-4-methoxybenzylidine) piperidine-4-one (5):**

Curcumin derivate 3 (1 mmol) was dissolved in 20 ml of acetonitrile, then cesium carbonate (2 mmol) and 1-bromo-3-chloropropane (1.5 mmol) were added, respectively. Then the mixture was refluxed for 24 hours at 80 °C. Reaction was monitored using TLC plate per 6 hours. After the completion of the reaction, the mixed reaction was cooled and concentrated using a rotary evaporator to produce a crude product. The crude product was partitioned using ethyl acetate:water system (3 x 15 ml). The organic layer was taken, dried with anhydrous sodium sulfate and evaporated using a rotary evaporator. The obtained product was recrystallized with methanol to obtain curcumin derivate 5.26

**Molecular Docking**

The docking study was carried out using Molecular Operating Environment 2019.0101 from Chemical Computing Group Inc., (https://www.chemcomp.com).27 Molecular docking simulation was performed using ASUS E202S Netbook with Intel Celeron N3060 1.6 GHz and 2GB RAM. The crystal structure of DEN2 NS2B/NS3 Protease (PDB ID: 2FOM) was downloaded from Protein Data Bank (https://www.rcsb.org/structure/2FOM). The receptor protein is prepared using a structure preparation wizard. Then, the protein was energy minimized by using AMBER14:EHT force field. Ligand compounds, curcumin derivate 5 was prepared using the same method, Panduratin A (PubChem ID: 6483648) was used as positive control. The docking site was set up using the site finder feature from MOE around His51, Asp75, and Ser135 amino acids residues. The ligand was synthesized using the initial scoring methodology (London dG) and the final scoring methodology (GBVI/WSA) by placement using the Triangle Matcher protocol and post-placement refinement was Rigid Receptor. The lowest bond energy conformation was taken and visualized using the BIOVIA Discovery Visualizer 2019 for further analysis.

**Results and Discussion**

The synthesis of targeted curcumin compound was carried out through two-steps reactions as shown in Figure 1. The first step was the synthesis of curcumin derivate 3 through aldol condensation between 4-piperidone monohydrate hydrochloride 1 and 4-methoxybenzaldehyde 2. The reaction...
is initiated by the attack of the hydroxide ion of strong base catalyst which acts as a nucleophile to the acidic Hα of the ketone compound to form the enolate ion. Enolate ions that are formed will attack the aldehydes which act as electrophiles to form β-hydroxy ketone as intermediate compounds, followed by dehydration to form curcumin derivate 3. The second step, the curcumin derivate 3 was reacted with a compound 4 to obtain curcumin derivate 5 with 72% yield. This reaction occurs between the amine group (NH) of 4-piperidone ring with 1-bromo-3-chloropropane as alkylating agent. This alkylation is a simple substitution reaction, where the nitrogen atom in the amine group acts as a nucleophile, the atomic carbon (CX) in the alkylating agent as an electrophile and Cs₂CO₃ as a catalyst. The amine group that has been activated by the catalyst will attack the C-Br carbon, followed by the release of the halogen atom to obtain the final product. The UV spectrum of curcumin derivate 5 showed absorption at wavelength of 244 and 365 nm which indicated an electronic transition from orbitals π → π* of the conjugated framework of curcumin derivate molecule with π electrons are delocalized through the whole molecule. The FTIR spectrum showed transmittance at 2995 and 2960 cm⁻¹ revealed the presence of aromatic C-H bonds from phenyl rings and vibrations of the aliphatic C-H bond of the methoxy group and the piperidone ring, respectively. In addition, the presence of transmittance at 1666 and 1597 cm⁻¹ indicated of the C=O and C=C bonds from an enone group of curcumin, and C-O bond vibration from the methoxy group at 1258 cm⁻¹. The absences of N-H bond in the spectrum of curcumin derivate 5, indicated the substitution reaction of N-H group with compound 4 was successfully occured and the final product was formed.

| Position | δ_C (ppm) | δ_H (ppm) (Multiplicity, J) |
|----------|------------|-----------------------------|
| -OCH₃     | 55.5       | 3.85 (s, 6H)                |
| 2         | 55.2       | 3.83 (s, 2H)                |
| 3         | 131.4      | -                           |
| 4         | 187.3      | -                           |
| 5         | 131.4      | -                           |
| 6         | 55.2       | 3.83 (s, 2H)                |
| Cβ        | 136.3      | 7.7 (s, 2H)                 |
| 1’        | 128.1      | -                           |
| 2’        | 132.5      | 7.38 (d, J = 8.8 Hz, 4H)    |
| 3’        | 114.3      | 6.96 (d, J = 8.8 Hz, 4H)    |
| 4’        | 160.4      | -                           |
| 5’        | 114.3      | 9.96 (d, J = 8.8 Hz, 4H)    |
| 6’        | 132.5      | 7.38 (d, J = 8.8 Hz, 4H)    |
| 1”        | 43.1       | 2.71 (t, J = 6.9 Hz, 2H)    |
| 2”        | 30.4       | 1.91 (p, J = 6.6 Hz, 2H)    |
| 3”        | 54.3       | 3.54 (t, J = 6.4 Hz, 2H)    |
Furthermore, NMR dan HRMS spectra confirmed the curcumin derivate 5 chemical structure. $^1$H NMR spectrum showed the presence of two set of aromatics with two substitutions and methoxy groups. In addition of 2 set of methylenes at $\delta_H$ 3.83 (s, 2H) corresponding to piperidone unit and proton $\beta$ resonance at $\delta_H$ 7.77 (s, 2H). The presence of three methylenes at $\delta_H$ 2.71(t, $J=6.9$ Hz, 2H), 1.91 (p, $J=6.6$ Hz, 2H), and 3.54 (t, $J=6.4$ Hz, 2H) assigned as chloropropane unit. $^{13}$C NMR spectrum revealed carbonyl ketone at $\delta_C$ 187.3 and methoxy unit at $\delta_C$ 55.5 (Table 1). HRMS spectrum showed [M+H] of 412.1678 corresponding to a molecular formula of $C_{24}H_{28}NO_3Cl$ (calcd. 412.1679 for $C_{24}H_{27}NO_3Cl$).

Molecular docking simulation of curcumin derivate 5 was performed to identify plausible binding modes to DEN2 NS2B/NS3 protease. Panduratin A was also docked as comparison, panduratin A isolated from Boesenbergia rotunda (L.) showed good inhibitory activities towards dengue-2 virus. The molecular docking result of curcumin derivate 5 to DEN2 NS2B/NS3 protease showed that this compound had a strong bond to the catalytic triad of the DENV protease enzyme (His51, Asp75, and Ser135). Panduratin A exhibited hydrophilic interactions through hydrogen bonds with Asp75 and Gly151, and hydrophobic interactions with catalytic triads, through π stack interactions with His51 (Figure 2A). The curcumin derivate 5 demonstrated hydrophilic interaction through cationic interaction with Asp75 and through hydrogen bonding with Arg54 and Thr120, as well as hydrophobic interactions through van der Waals forces with Gly153 (Figure 2B). These amino acid residues are known to be essential in antagonistic activity against dengue virus. Calculated docking scores of the curcumin derivate 5 and panduratin A were -6.40 kcal/mol and -5.18 kcal/mol, respectively (Table 2). A lower docking score of the curcumin

| No | Compound | S (score) (kcal/mol) | Interactive AA (Distance in Å) |
|----|----------|----------------------|-------------------------------|
|    |          |                      | Hydrophilic                   | Hydrophobic                   |
| 1  | Panduratin A | -5.18                | Asp75 (2.15)                  | His51 (5.46)                  |
|    |           |                      | Gly151 (3.08)                 | Val72 (2.76)                  |
|    |           |                      |                                | Leu128 (5.41)                 |
|    |           |                      |                                | Pro132 (4.85)                 |
|    |           |                      |                                | Val155 (4.62)                 |
|    |           |                      |                                | Tyr161 (5.14)                 |
| 2  | Curcumin Derivate 5 | -6.40                | Arg54 (2.46)                  | Val72 (2.93)                  |
|    |           |                      | Asp75 (4.72)                  | Lys73 (2.88)                  |
|    |           |                      | Thr120 (2.44)                 | Asn152 (2.42)                 |
|    |           |                      |                                | Gly153 (2.35)                 |
|    |           |                      |                                | Val154 (4.83)                 |

Table 2. Molecular Docking Result Against the DEN2 NS2B/NS3 Protease

1. H NMR spectrum showed the presence of two set of aromatics with two substitutions and methoxy groups.
2. Panduratin A, isolated from Boesenbergia rotunda (L.), showed good inhibitory activities towards dengue-2 virus.
dervate 5 compared to panduratin A and some strong interactions to the essential amino acid residues at the active site of the protease, presumably the curcumin derivate 5 showed good inhibitory activity against DEN2 NS2B/NS3 protease.

**Conclusion**
The curcumin derivate 5 was successfully synthesized by two-steps reactions with 72% yield. Chemical structure of curcumin derivate 5 was confirmed by FTIR, NMR, and HRMS spectroscopy analysis. Molecular docking result showed that the compound has better affinity to the DEN2 NS2B/NS3 protease than panduratin A as positive control. The curcumin derivate 5 also showed strong interactions to Arg54, Asp75, and Thr120 which is essential in antagonistic activity NS2B/NS3 protease. Thus, it can be assumed that the compound is a potential candidate as a new agent in inhibiting the type 2 dengue virus.

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**Figure 2. 2D and 3D Visualization of the Binding Mode of Panduratin A (A) and Curcumin Derivate 5 (B) Compound to the NS2B/NS3 Protease**
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Conflict of Interest
None declared.

References
1. Gurugama P, Garg P, Perera J, Wijewickrama A, Seneviratne SL. Dengue viral infections. Indian Journal of Dermatology. 2010;55(1):68-78. doi:10.4103/0019-5154.60357
2. Murray NEA, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. Clinical Epidemiology. 2013;5:299-309. doi:10.2147/CLEP.S34440
3. Carnec X, Meertens L, Dejarnac O, Pararera-Lecoin M, Hafirassou ML, Kitaura J, Ramdasi R, Schwartz O, Amara A. The Phosphatidylserine and Phosphatidylethanolamine Receptor CD300a Binds Dengue Virus and Enhances Infection. Journal of Virology. 2015;90(1):92-102. doi:10.1128/JVI.01849-15
4. Almuhanna R, Alobudi A, Alazdi S, Alghamdi H, Hindi M, Ghanim A, Fakieh E, Alharbi A, Jumbi R, Banjar A. Knowledge, awareness and attitude towards dengue fever outbreaks in the summer. International Journal of Advances in Medicine. 2018;5(4). doi:10.18203/2349-3933.ijam20182957
5. Frimayanti N, Chee CF, Zain SM, Rahman NA. Design of new competitive dengue NS2B/NS3 protease inhibitors-a computational approach. International Journal of Molecular Sciences. 2011;12(2):1089-1100. doi:10.3390/ijms12021089
6. Wu D, Mao F, Ye Y, Li J, Xu C, Luo X, Chen J, Shen X. Policresulen, a novel NS2B/NS3 protease inhibitor, effectively inhibits the replication of DENV2 virus in BHK-21 cells. Acta Pharmacologica Sinica. 2015;36(9):1126-1136. doi:10.1038/aps.2015.56
7. Chambers TJ, Nestorowicz A, Amberg SM, Rice CM. Mutagenesis of the yellow fever virus NS2B protein: effects on proteolytic processing, NS2B-NS3 complex formation, and viral replication. Journal of Virology. 1993;67(11):6797-6807.
8. Powers CN, Setzer WN. An In-Silico Investigation of Phytochemicals as Antiviral Agents Against Dengue Fever. Combinatorial Chemistry & High Throughput Screening. 2016;19(7):516-536. doi:10.2174/1386207319666160506123715
9. Kim HY, Park EJ, Joe E, Jou I. Curcumin Suppresses Janus Kinase-STAT Inflammatory Signaling through Activation of Src Homology 2 Domain-Containing Tyrosine Phosphatase 2 in Brain Microglia. The Journal of Immunology. 2003;171(11):6072 LP - 6079. doi:10.4049/jimmunol.171.11.6072
10. Suzuki M, Nakamura T, Iyoki S, Fujiwara A, Watanabe Y, Mohri K, Isobe K, Ono K, Yano S. Elucidation of Anti-allergic Activities of Curcumin-Related Compounds with a Special Reference to Their Anti-oxidative Activities. Biological and Pharmaceutical Bulletin. 2005;28(8):1438-1443. doi:10.1248/bpb.28.1438
11. Fadus MC, Lau C, Bikhchandani J, Lynch HT. Curcumin: An age-old anti-inflammatory and anti-neoplastic agent. Journal of Traditional and Complementary Medicine. 2016;7(3):339-346 doi:10.1016/j.jtcme.2016.08.002
12. Chattopadhyay I, Biswas K,
Bandyopadhyay U, Banerjee RK. Turmeric and curcumin: Biological actions and medicinal applications. *Current Science.* 2004;87(1):44-53.

13. Mathew D, Hsu W-L. Antiviral potential of curcumin. *Journal of Functional Foods.* 2018;40:692-699. doi:https://doi.org/10.1016/j.jff.2017.12.017

14. Anand P, Thomas SG, Kunnumakkara AB, et al. Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. *Biochemical Pharmacology.* 2008;76(11):1590-1611. doi:https://doi.org/10.1016/j.bcp.2008.08.008

15. Mounce BC, Cesaro T, Carrau L, Vallet T, Vignuzzi M. Curcumin inhibits Zika and chikungunya virus infection by inhibiting cell binding. *Antiviral Research.* 2017;142:148-157. doi:https://doi.org/10.1016/j.antiviral.2017.03.014

16. Marbawati D, Umniyati SR. Effects of Curcumin and Pentagamavunon-0 Against Dengue-2 Virus Infection in Vero Cells; an in Vitro Study. *Procedia Environmental Sciences.* 2015. doi:10.1016/j.proenv.2015.01.033

17. Tan S, Pippen R, Yusof R, Rahman N, Ibrahim H, Khalid N. Screening of selected zingiberaceae extracts for dengue-2 virus protease inhibitory activities. *Sunway Academic Journal.* 2006;3:1-7.

18. Ichsyani M, Ridhany a, Risanti M, Desti H, eria R, Putri DH, Sudiro TM, Dewi BE. Antiviral effects of Curcuma longa L. against dengue virus in vitro and in vivo. *Earth and Environmental Science.* 2017;1-10. doi:10.1088/1755-1315/101/1/012005

19. Eryanti Y, Nurulita Y, Hendra R., Y, Syahri J, Zamri A. Synthesizing Derivatives From Cyclopentanone Analogue Curcumin and Their Toxic, Antioxidant and Anti-Inflammatory Activities. *MAKARA of Science Series.* 2012;15(2):117-123. doi:10.7454/mss.v15i2.1060

20. Adams BK, Ferstl EM, Davis MC, Herold M, K urtkaya S, Camalier RF, Hollingshead MG, Kaur G, Sausville EA, Rickles FR, Synder JP, Liotta CD, Shoji M. Synthesis and biological evaluation of novel curcumin analogs as anti-cancer and anti-angiogenesis agents. *Bioorganic & Medicinal Chemistry.* 2004;12(14):3871-3883. doi:https://doi.org/10.1016/j.bmc.2004.05.006

21. Da’i M, Meiyanto E, Supardjan, Jenie UA, Kawaichi M. Potensi Antiproliferative Analog Kurkumin Pentagamavunon Terhadap Sel Kanker Payudara T47D. *Artocarpus.* 2007;7(1):14-20.

22. Hossain M, Das S, Das U, Doroudi A, Zhu J, Dimmock JR. Novel hybrid molecules of 3,5-bis(benzylidene)-4-piperidones and dichloroacetic acid which demonstrate potent tumour-selective cytotoxicity. *Bioorganic and Medicinal Chemistry Letters.* 2020;30(3):126878. doi:https://doi.org/10.1016/j.bmcl.2019.126878

23. Eryanti Y, Zamri A, Frimayanti N, et al. Synthesis, structure-activity relationship, docking and molecular dynamic simulation of curcumin analogues against HL-60 for antican cer agents (leukemia). *Oriental Journal of Chemistry.* 2017;33(5):2164-2172. doi:10.13005/ojc/330503

24. Osman H, Idris NH, Kamarulzaman EE, Wahab HA, Hassan MZ. 3,5-Bis(arylidene)-4-piperidones as potential dengue protease inhibitors. *Acta Pharmaceutica Sinica B.* 2017;7(4):479-484. doi:10.1016/j.apsb.2017.04.009

25. Eryanti Y, Herlina T, Zamri A, et al. 3,5-Bis(2-hydroxybenzylidene)piperidin-4-one. *MolBank.* 2014(2):3-5. doi:10.3390/M825

26. Zamri A, Teruna HY, Rahmawati EN, Frimayanti N, Ikhtiarudin I. Synthesis and in silico studies of a benzenesulfonyl curcumin analogue as a new anti dengue virus type 2 (DEN2) NS2B/
27. Herfindo N, Prasetiawati R, Sialagan D, Frimayanti N, Zamri A. Synthesis, Antiproliferative Activity and Molecular Docking Studies of 1,3,5-Triaryl Pyrazole Compound as Estrogen α Receptor Inhibitor Targeting MCF-7 Cell Line. *Molekul*. 2020;15(1):18-25. doi:https://doi.org/10.20884/1.jm.2020.15.1.585

28. Noble CG, Seh CC, Chao AT, Shi PY. Ligand-bound structures of the dengue virus protease reveal the active conformation. *Journal of Virology*. 2012;86(1):438-446. doi:10.1128/JVI.06225-11