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Case Report

Effect of Zinc(II)-2,4,5-triphenyl-1*H*-imidazole Complex Against Replication DENV-2 in Vero Cell

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ABSTRACT

Dengue virus (DENV) is a significant pathogen emerging worldwide as a cause of infectious disease. DENVs are transmitted to humans through female mosquitoes from Aedes aegypti and Aedes albopictus species. Indonesia is one of the largest countries in the world in dengue endemic regions worldwide. Dengue fever was occurred for the first time as an outbreak in Surabaya and Jakarta in 1968. Many efforts have been made to prevent and treat DENV infections, and clinical trials of a number of vaccines are currently underway. Antiviral testing of DENV is an important alternative for drug characterization and development. Complex compounds are formed as a result of metal and organic complex reactions. Complex compounds can be used as an anti-inflammatory, antimicrobial antifungal, antibacterial, antivirus. The Zn^{2+} ion can be used as an antiviral candidate. The purpose of this project was investigated Zinc(II)-2,4,5-triphenyl-1H-imidazole antiviral compound to be further tested for inhibitory effect on the replication of DENV-2 in cell culture. DENV replication was measured by antiviral activity assay and cytotoxicity assay. The inhibitory activity of Zinc(II)-2,4,5-triphenyl-1H-imidazole complex compound was determined by Viral ToxGloTM Assay. The cytotoxicity of Zinc(II)-2,4,5-triphenyl-1H-imidazole complex compound was determined by CellTiter96® AQ_{uoeus} assay. The inhibitory concentration (IC_{50}) of Zinc(II)-2,4,5-triphenyl-1H-imidazole against dengue virus type-2 was 34.42 µg/ml. The cytotoxic concentration (CC_{50}) of compound against Vero cell was <100 µg/ml. The results of this study demonstrate the antidengue serotype 2 inhibitory activity of investigated Zinc(II)-2,4,5-triphenyl-1H-imidazole complex and its high toxicity in Vero cells. Further studies are not required before investigated Zinc(II)-2,4,5-triphenylimidazole can be applied in the treatment of DENV-2 infections.

Keywords: *Zinc (II), complex compound, cytotoxicity, inhibitory activity, DENV-2*

ABSTRAK

Virus Dengue (DENV) adalah patogen signifikan yang muncul di seluruh dunia sebagai penyebab penyakit menular. DENV ditransmisikan ke manusia melalui nyamuk betina dari spesies Aedes aegypti dan Aedes albopictus. Indonesia adalah salah satu negara terbesar di dunia di daerah endemik demam berdarah di seluruh dunia. Demam berdarah terjadi untuk pertama kalinya sebagai wabah di Surabaya dan Jakarta pada tahun 1968. Banyak upaya telah dilakukan untuk mencegah dan mengobati infeksi DENV, dan uji klinis sejumlah vaksin saat ini sedang berlangsung. Pengujian antivirus DENV adalah alternatif penting untuk karakterisasi dan pengembangan obat. Senyawa kompleks terbentuk sebagai hasil dari reaksi kompleks logam dan organik. Senyawa kompleks dapat digunakan sebagai anti-inflamasi, antimikroba antijamur, antibakteri, antivirus. Ion Zn^{2+} dapat digunakan sebagai kandidat antivirus. Tujuan dalam proyek ini adalah menyelidiki senyawa antivirus Zink(II)-2,4,5-trifenil-1H-imidazol yang diuji lebih lanjut untuk efek penghambatan pada replikasi DENV-2 dalam kultur sel. Replikasi DENV diukur dengan uji aktivitas antivirus dan uji sitotoksisitas. Aktivitas penghambatan senyawa kompleks Zinc(II)-2,4,5-triphenyl-1H-imidazol ditentukan dengan Viral ToxGloTM Assay. Sitotoksisitas senyawa kompleks Zinc(II)-2,4,5-triphenyl-

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1H-imidazol ditentukan dengan uji CellTiter96® AQuoeus. Konsentrasi penghambatan (IC_{50}) Zinc(II)-2,4,5-trifenil-1H-imidazol terhadap virus dengue tipe-2 adalah 34,42 µg/ml. Konsentrasi sitotoksik (CC_{50}) senyawa terhadap sel Vero adalah <100 µg/ml. Hasil penelitian ini menunjukkan aktivitas penghambatan serotipe 2 antidengue dari Zinc(II)-2,4,5-trifenil-1H-imidazol yang diteliti dan toksisitasnya yang tinggi dalam sel Vero. Studi lebih lanjut tidak diperlukan sebelum investigasi Zinc(II)-2,4,5-trifenil-1H-imidazol dapat diterapkan dalam pengobatan infeksi DENV-2.

Kata kunci: *Seng (II), senyawa kompleks, sitotoksisitas, aktivitas penghambatan, DENV-2*

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INTRODUCTION

Dengue virus (DENV) is a virus carried by the *flavivirus* genus of the family Flaviviridae. Dengue virus (DENV) consists of four serotypes which is dengue virus type 1, dengue virus type 2, dengue virus type 3, and dengue virus type 4. Dengue virus is transmitted to humans through female mosquitoes from *Aedes aegypti* and *Aedes albopictus* species. World Health Organization (WHO) reported 390 million dengue infections per year.[1] Indonesia is one of the largest countries in the world with dengue endemic areas. Surabaya and Jakarta were the cities where dengue disease was first reported in Indonesia in 1968.[2] Many studies have been conducted to overcome the threat of dengue virus infections, and clinical trials of a number of vaccines are currently on the way.[3] Antiviral testing on DENV is a very important method in the development and characterization of drugs. Supplementary to vaccines, inhibitors in each natural cycle of viral replication have the potential to cure dengue virus infection and indeed compounds such as RNA replication inhibitors have been tested as such.[4] However, there is no commercially available drug with antiviral activity for DENV.[5]

Ligand 2,3,5-triphenyl-1*H*-imidazole compound is a derivate of imidazole. Imidazole-containing drugs that have strong therapeutic properties have encouraged scientists to synthesize many novel chemotherapeutic agents consisting of these entities. N^5-(4-fluorophenyl)-N^4-(2-(pyridin-4-yl)benzyl)-1*H*-imidazole-4,5-dicarboxami-de, a derivate of imidazole, was reported anti-DENV activity.[6] 4-carbamoyl-5-(4,6-diamino-2,5-dihydro-1,3,5-triazin-2-yl) imidazole-1-β-D-ribofuranoside is examined for four different types of viruses from the flaviridae family *in vitro*, including hepatitis C virus (HCV), Japanese viral encephalitis (JEV), West Nile virus (WNV), and dengue virus (DENV) *in vitro* against NTPases/helicases. The compound showed activity highly active against WNV with IC_{50} was 23 µM.[7]

Complex compounds are formed as a result of metal and organic compound reactions. Complex compounds can be used as an anti-inflammatory[8], antimicrobial[9], antifungal, antibacterial[10], and antivirus[11]. Based on previous research, Copper(II)-imidazole derivatives can be used as antiDENV-2, can be used as low toxicity and potential as drug candidates. The compound exhibited adsorption inhibitory activity against DENV-2 at IC_{50} = 2.3 µg/ml.[12]

The Zn^{2+} ion can be used as an antiviral candidate.[13] Zn^{2+} ions can change the activity of various transcription factors and thus, patterns of cellular and viral gene expression.[14] Thus, the antiviral test of the compound Zinc(II)-2,4,5-triphenyl-1*H*-imidazole was investigated.

MATERIALS AND METHODS

Chemicals and Media

Chemical reagents used in this research were Zinc(II)-2,4,5-triphenyl-1*H*-imidazole complex compound, Minimum Essential Eagle Medium (Sigma-Aldrich, Germany), dengue virus serotype 2 Surabaya isolate (KT012509), Vero cells (African Green Monkey Kidney), Viral ToxGlo™ assay (Promega, USA), CellTiter96®

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AQ_{uoeus} One Solution Cell Proliferation Assay (Promega, USA).

Antiviral Activity Assay

Confluent monolayers of Vero cells were prepared on a 96-well plate (1×10^6 cells/10 ml) and counted using a hemocytometer, and the titer of DENV-2 (2×10^4 FFU/well) was expressed in Foci-Forming Units (FFU) after incubating at 37°C for 2 days. The concentrations of Zinc(II)-2,4,5-triphenyl-1*H*-imidazole were 50 µg/mL; 25 µg/mL; 12.5 µg/mL; 6.25 µg/mL; 3.13 µg/mL; 1.57 µg/mL; 0.78 µg/mL; and 0.39 µg/mL with addition 100 µL Viral ToxGlo™ Assay per well. The 50% inhibitory concentration (IC_{50}) of DENV-2 replication by each compound was further investigated by using GloMax® Discover System.

Cytotoxicity Assay

A cytotoxicity assay was performed using CellTiter96® AQ_{uoeus} One Solution Cell Proliferation reagent. The CellTiter96® Assay is a modification of the MTT assay method portrayed by Akter.[15] The concentrations of Zinc(II)-2,4,5-triphenyl-1*H*-imidazole were 100 µg/mL; 200 µg/mL; 400 µg/mL; 600 µg/mL; 800 µg/mL; and 1000 µg/mL. The medium was allowed to equilibrate for 1 hour; then 20µl/well of CellTiter 96® AQ_{ueous} One Solution Reagent was added. After 1 hour at 37°C in a humidified, 5% CO_2 atmosphere, the absorbance at 490nm was recorded using GloMax® Discover System.

Viral Detection by Reverse Transcriptase-Polymerase Chain Reaction

RNA replication was estimated using the Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). The purpose of this assay was to known RNA replication after treatment. Briefly, DENV-2 RNA was extracted from the DENV-2 infected cells and cell culture supernatant using RNA extraction kit by Qiagen, Germany. The two-step kit (Toyobo, Japan) was used for cDNA synthesis and Polymerase Chain Reaction (PCR) following the manufacturer's instructions. Primer oligonucleotide sequences were as follows by Bhatnagar et. al. 2012.[16] Amplification condition was 54 °C for one minute (annealing temperature) and the amplified product was the analyzed on 1.5% agarose gel.

RESULTS AND DISCUSSION

The cytotoxicity of Zinc(II)-2,4,5-triphenyl-1*H*-imidazole complex compound was determined by CellTiter96® AQ_{uoeus} assay and the recorded CC_{50} value is <100 µg/ml to Vero cells. When compared with a previous study, Copper(II) was found to be nontoxic to human erythrocyte cells to concentrations of 500 µg/ml.[17] CC_{50} is the cytotoxicity level of $[Cu(2,4,5\text{-triphenyl-1}H\text{-imidazole})_2]_n$ (compound) to cause death to 50% of Vero cells.[12] The toxicity value of Cobalt(II) complex with 2,4,5-triphenyl-1*H*-imidazole ligand was 362.24 mg/L, which was not toxic.[18] The toxicity value of 2-methyl-4,5-diphenyl-1*H*-Immidazole ligand compound was 192,3 µg/ml.[19] The toxicity of $[Mn(2\text{-}(4\text{-chlorophenyl})\text{-}4,5\text{-diphenyl-1}H\text{-imidazole})_2(H_2O)_2]\cdot 2H_2O$ was >200 µg/ml which had less toxicity.[20] Zinc(II)–2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxycromen-4-one complex compound defined cytotoxicity with CC_{50} at 3.59 µg/ml.[21] But, the metal-free imidazole more toxic for Vero cells (CC_{50} = 5.03 µg/ml).[22] Activity against HIV-1 strain IIIB and HIV-2 strain ROD in MT-4 cells (CC_{50}) by zinc(II) complexes with hexyl-Me_2-cyclam (HMC; 3,14-dimethyl-2,6,13,17-tetraazatricyclo(16.4.0.$0^{7,12}$)-docosane) were >372 µM and >372 µM with selectivity index >35 and >3. Activity against HIV-1 strain IIIB and HIV-2 strain ROD in MT-4 cells (CC_{50}) by Zn(II)–HMC diacetate were 110.67 ± 12.67 µM and 110.67 ± 12.67 µM with selectivity index 32 and <1.[23]

The complex stability is highly dependent on both the metallic ion and the ligands. As for the central ion (M^{2+}), Zn(II) more unstable than Cu(II), Mn(II), and Co(II). The Zn(II) complex has grater polarizability that that Cu(II), Mn(II), and Co(II) because it contains more *d*-electrons, and the Zn(II) complex produced more product ions soluble in water.[24] This effect causes Zn(II) to be more toxic, because Zn^{2+} in the medium are

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more numerous, so it damages the cell wall faster than complex compound that have high stability such as Cu(II), Mn(II), and Co(II).

The percentage inhibition of the development of dengue virus type-2 by the test sample of Zinc(II)-2,4,5-triphenyl-1*H*-imidazole complex compound was shown on figure 1. The IC_{50} value was determined from the concentration–response curve (Figure 1); the IC_{50} value was 34.42 µg/ml, R^2 was 0.9196. Based on the value of the IC_{50} Zinc(II)-2,4,5-triphenyl-1*H*-imidazole complex compound was a medium toxic compound.

Antiviral activity was also shown in Figure 2, these findings were corroborated by results obtained from RT-PCR which indicated significant reduction in the amount of DENV-2 genomic RNA levels. The highest percentage of viral inhibition was observed after treating the infected cells with 50 µg/ml.

Based on the previous study, $[Cu(2,4,5\text{-triphenyl-1}H\text{-imidazole})_2]_n$ complex compound exhibited adsorption inhibitory activity against DENV-2 at IC_{50} = 2.3 µg/ml. The inhibition at IC_{50} was not significantly high ($p<0.005$) compared to that of the metal-free imidazole (IC_{50} = 0.13 µg/ml).[12] The maximal inhibitory concentration (IC_{50}) of Copper(II)chloride Dihydrate against DENV-2 was 0.13 µg/ml.[22]

Activity against HIV-1 strain IIIB and HIV-2 strain ROD in MT-4 cells (IC_{50}) by zinc(II) complexes with hexyl-Me_2-cyclam (HMC; 3,14-dimethyl-2,6,13,17-tetraazatricyclo(16.4.0.$0^{7,12}$) docosane) were 10.51 ± 0.23 µM and 133.78 ± 14.10 µM. Activity against HIV-1 strain IIIB and HIV-2 strain ROD in MT-4 cells (IC_{50}) by Zn(II)–HMC diacetate were 3.50 ± 0.33 µM and >110.67 µM.[23] Anti-HIV-1 activity (IC_{50}) in C8166/IIIB, MT-4/GUN1 and PBLs/IIIB were 8.0 µg/ml, 3.5 µg/ml, and 9.3 µg/ml, respectively. The IC_{50} value of the Cobalt(II)–Morin complex for DENV-2 was 3.08 µg/ml.[25] MB21, a benzimidazole derivative, was found to be the most potential inhibitor of cloned proteases (IC_{50} = 5.95 µM).[26]

This study suggest that of Zinc(II)-2,4,5-triphenyl-1*H*-imidazole complex compound can't be an attractive antiviral option. It would be interesting to further investigate whether 2,4,5-triphenyl-1*H*-imidazole complex with other metal. The result of this study, Zinc(II)-2,4,5-triphenyl-1*H*-imidazole complex compound more toxic than Cu(II)-2,4,5-triphenyl-1*H*-imidazole, this is caused by the Zn (II) complex being unstable compared to the Cu (II) complex.

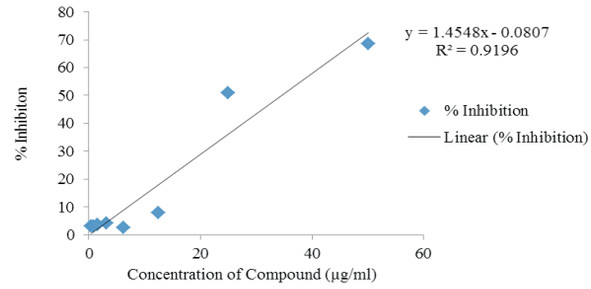

Figure 1. Inhibition of DENV-2, at variation concentrations of Zinc(II)-2,4,5-triphenyl-1*H*-imidazole complex compound

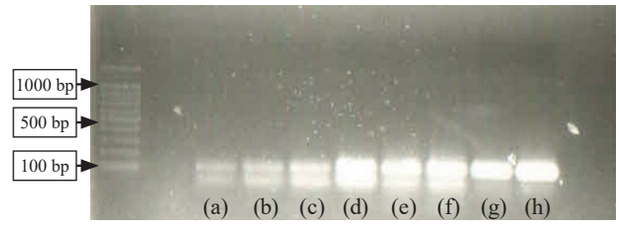

Figure 2. Electrophoresis on 1.5% agarose of RT-PCR after treatment, molecular weight marker (100 bp), (a) treatment with 50 µg/ml compound, (b) 25 µg/ml, (c) 12.5 µg/ml, (d) 6.25 µg/ml, (e) 3.13 µg/ml, (f) 1.57 µg/mL, (g) 0.78 µg/mL, and (h) 0.39 µg/mL

CONCLUSION

Further studies are not required before Zinc(II)-2,4,5-triphenyl-1*H*-imidazole can be applied in the medication of DENV-2 infections. This study did not show the potential of the Zinc(II)-2,4,5-triphenyl-1*H*-imidazole complex as a candidate for antiviral agents against DENV-2 because it was shown to be toxic to Vero cells.

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CONFLICT OF INTEREST

There is no conflict of interest of this paper.

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