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Research Report

# ANTI-DENGUE TYPE 2 VIRUS ACTIVITIES OF ZINC (II) COMPLEX COMPOUNDS WITH 2-(2,4-DIHYDROXYPHENYL)-3,5,7-TRIHYDROXYCROMEN-4-ONE LIGANDS IN VERO CELLS

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***ABSTRACT***

*Dengue virus (DENV) is a disease that is transmitted through Aedes aegypti and Aedes albopictus mosquitoes, and is spread in tropical and sub-tropical regions. Now, dengue or antiviral vaccines for humans do not yet exist, but there are great efforts to achieve this goal. Complex compounds are reported to fungicidal, bactericidal and antiviral activity. Antiviral activity against DENV is an important alternative to the characterization and development of drugs candidate. The purpose of this study was to study zinc(II) compounds with 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxycromen-4-one ligand on DENV-2 replication in Vero cells. Vero cell lines (African green monkey kidney) was used in this study, maintained and propagated in Minimum Essential Eagle Medium containing 10% fetal bovine serum at 37°C in 5% $CO_2$. The activity of dengue virus was carried out by enzyme-immunosorbent assay (ELISA) method and CellTiter96® Non-Radioactive Proliferation. The value of activity inhibition ($IC_{50}$) of complex compounds with variations of mol metal: ligand 1:2, 1:3, and 1:4 against dengue virus type 2 (DENV2) was 2.44 µg/ml, 2.75 µg/ml, respectively and 2.00 µg/ml, also the toxicity value ($CC_{50}$) of complex compounds with variation mol metal: ligand 1:4 for Vero cells is 3.59 µg/ml. The results of this study were indicate that these properties have been shown to inhibit anti-dengue type 2 virus (DENV-2), but are also toxic in Vero cells. Including previous study about complex compound interaction with dengue virus type 2 activity, Zn(II) more reactive compound then Cu(II), and Co(II). The comparison with Cu(II) complex compound, it has been revealed that Co(II) and Zn(II) is more toxic, was found to be nontoxic to human erythrocyte cells even at a concentration of 500 µg/ml.*

***Keywords:*** *Anti-DENV2, Complex Compounds, Zinc(II), 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxycromen-4-one*

***ABSTRAK***

*Virus Dengue (DENV) adalah penyakit yang ditularkan melalui nyamuk Aedes aegypti dan Aedes albopictus, serta didistribusikan di daerah tropis dan sub-tropis. Kini, vaksin dengue atau antivirus untuk manusia belum disetujui secara klinis, meski telah ada upaya besar untuk mencapai tujuan ini. Senyawa kompleks dilaporkan menunjukkan aktivitas fungisida, bakterisida, dan antivirus. Aktivitas antivirus melawan DENV merupakan alternatif penting untuk karakterisasi dan pengembangan obat-obatan. Tujuan dalam penelitian ini adalah untuk investigasi aktivitas senyawa kompleks logam seng(II) dengan ligan 2-(2,4-dihidroksifenil)-3,5,7-trihidroksikromen-4-on terhadap replikasi DENV-2 pada sel Vero. Sel Vero (African green monkey kidney) yang digunakan dalam penelitian ini, dipelihara dan diperbanyak dalam Medium Essential Eagle yang mengandung 10% serum janin sapi pada 37°C dalam 5% CO2. Aktivitas senyawa kompleks virus dengue dilakukan dengan metode enzyme-immunosorbent assay (ELISA) dan toksititas dengan metode CellTiter96® Non-Radioactive Proliferation. Nilai penghambatan aktivitas ($IC_{50}$) senyawa kompleks dengan perbandingan mol logam:ligan 1:2, 1:3, dan 1:4 terhadap virus dengue tipe 2 (DENV2) masing-masing adalah 2.44 µg/ml, 2.75 µg/ml, dan 2.00 µg/ml, serta nilai toksisitas ($CC_{50}$) senyawa kompleks dengan perbandingan mol logam:ligan 1:4 untuk sel Vero adalah 3,59 µg/ml. Hasil penelitian ini menunjukkan bahwa senyawa kompleks tersebut menunjukkan aktivitas penghambatan anti-dengue virus tipe 2 (DENV-2), tetapi juga bersifat toksik pada sel Vero. Termasuk penelitian sebelumnya tentang interaksi senyawa kompleks dengan aktivitas virus dengue tipe 2, Zn (II) lebih*

*reaktif senyawa kemudian Cu (II), dan Co (II). Perbandingan dengan kompleks Cu (II), telah diketahui bahwa Co (II) dan Zn (II) lebih toksik, ditemukan tidak beracun pada sel eritrosit manusia bahkan pada konsentrasi 500 µg / ml.*

***Kata kunci:*** *Anti-DENV2, Senyawa Kompleks, Seng(II), 2-(2,4-dihidroksifenil)-3,5,7-trihidroksikromen-4-on*

## INTRODUCTION

A major public health concern worldwide in recent years is a most prevalent mosquito-borne viral pathogen dengue virus (DENV), was transmitted through *Aedes aegypti* and *Aedes albopictus* mosquitoes, and is spread in tropical and sub-tropical regions.[1,2,3] Presently around the world dengue is endemic in 112 countries.[4] The incidence of DENV has increased approximately 30-fold over the past 50 years.[5] Dengue virus, the causal agent of dengue, has been shown to induce apoptosis in vitro and in vivo.[6,7] The mechanisms that trigger the apoptotic cellular responses, however, have not been thoroughly investigated.[8]

Micronutrient homeostasis is a key factor in maintaining a healthy immune system. Trace element zinc is a critical cofactor for many proteins involved in cellular processes like differentiation, proliferation and apoptosis that zinc is a nutritionally fundamental trace element and is second most abundant trace metal in the human body after iron.[9] In previous study, $Zn^{2+}$ was suggested that DENV-2 infection of Vero cells and Human breast adenocarcinoma cell line resulted in the induction of apoptosis.[8,10]

The complex compound from metal and organic compound reaction can be used an anti-DENV-2, especially Cu(II) with 2,4,5-triphenylimidazole exhibited adsorption inhibitory activity at $IC_{50}$ = 2.3 µg/ml.[11] A significant inhibitory activity to that of the complex Co(II) with 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxycromen -4-one ligand was reported against the tested pathogenic DENV-2 in Vero cells 3.08 µg/ml.[12]

In the present study, the inhibitory activity of Zinc(II) with 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxycromen-4-one ligand against the replication of DENV-2 in Vero cells was investigated.

## MATERIAL AND METHODS

### Chemicals and Medium

The chemical reagents used in this research were the Zinc(II)–2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxycromen -4-one complex compound, dimethyl sulfoxide by Merck 99.98%, Germany, Minimum Essential Eagle Medium by Sigma-Aldrich (Germany), dengue virus type 2 Surabaya Isolate (KT012513), Vero cell by African green monkey kidney, CellTiter96® Non-Radioactive Proliferation reagent by Promega (USA), and DENV antibody (4G2) for enzyme-linked immunosorbent assay (ELISA).

### Vero Cells Preparation

Vero cell lines (African green monkey kidney) was used in this study, maintained and propagated in Minimum Essential Eagle Medium containing 10% fetal bovine serum. Cultured Vero cell lines were incubated at 37 °C, respectively in 5% $CO_2$. Confluent monolayer of Vero cells were detached with trypsin-EDTA and incubate cells at 37 °C for 5 minutes. Add Minimum Essential Eagle Medium containing 10% fetal bovine serum, pipetting gently to break up any clumps of cells and counted using a Hemocytometer. Add cells in 96-well plate with $1 \times 10^6$ cells/10 ml and incubated in 37°C incubator with 5 $CO_2$. Monitor cells daily or every other day, cells reach a >90 % confluent monolayer.[13,14]

### Anti-dengue Type 2 Virus Assay

Confluent monolayers of Vero cells were prepared on a 96-well plate ($1 \times 10^6$ cells/10 ml), and the titer of DENV-2 ($2 \times 10^4$ FFU/well). The 50% inhibitory concentration ($IC_{50}$) was calculated as follows: $IC_{50}$ = (NC − AC) 100/ NC after incubating at 37°C for 2 days in 5% $CO_2$, where NC is the mean of the absorbance of negative controls and AC is the absorbance of the compound tested. The DENV-2 inhibition of replication by compound was further investigated by using quantitative ELISA at 415 nm.[15]

### Cytotoxicity assay

The dye of CellTiter96® Non-Radioactive Proliferation reagent by Promega is a modification of MTT assay method by Mosmann. The assay is suitable to use in adherent and suspension cells. Assay is very sensitive, it can detect 1,000 cells/well of a plate reader. Vero cells ($1 \times 10^5$ cells/ml) were seeded in plate at 37°C in 5% $CO_2$ overnight. A total of 100 µl of serial delusion compound were incubated with Vero cells for 24 h. A total of 100 µl of Cell Proliferation Reagent was added into each well, incubated for 4 hour at 37°C. The plate was read at 570 nm using ELISA reader (iMark™ Microplate Absorbance Reader).

## RESULT AND DISCUSSION

### Anti-dengue Type 2 Virus Activity

Metal complex compounds are a promising class of drug leads, and the associated studies have attracted more attention. However, the systematic basic research of metal complex compounds is lagging behind, partly because very few efforts have been made to establish a set activity screening and subsequent evaluation system for the comprehensive investigations on the structure and activity relationship of metal complex compounds.

The in vitro anti-dengue virus activity of the Zinc(II)–2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxycromen-4-one complex compound was tested against one type dengue virus (dengue virus type 2) by enzyme-linked immunosorbent

assay (ELISA).[15] The susceptibility of the strains toward the present compounds was judged measuring the size of inhibition. Zinc(II)– 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxycromen-4-one complex compound was further studied for their inhibitory effect on replication of the dengue virus type 2 in Vero cells. The $IC_{50}$ (inhibitory concentration 50) was determined from the dose response curve with variations of mol metal:ligand 1:2 (Figure 1), 1:3 (Figure 2), and 1:4 (Figure 3).

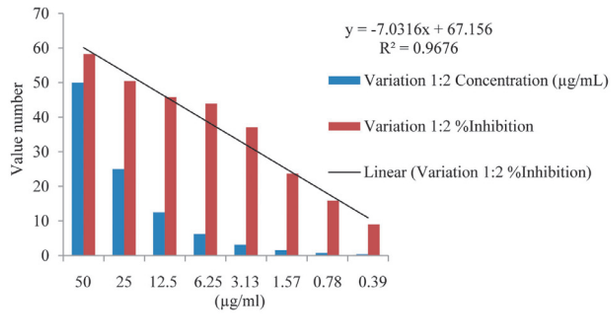


**Figure 1.** Inhibition curve of dengue virus type 2 at several concentrations of Zn(II) complex with variations of mol metal:ligand 1:2

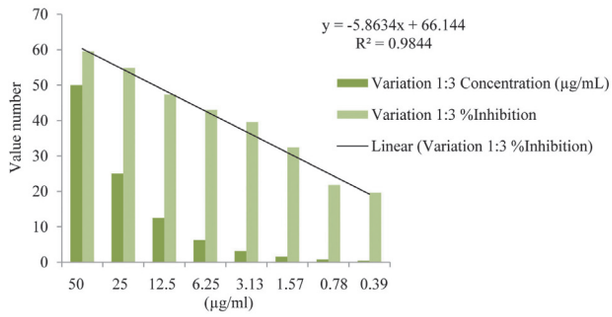


**Figure 2.** Inhibition curve of dengue virus type 2 at several concentrations of Zn(II) complex with variations of mol metal:ligand 1:3

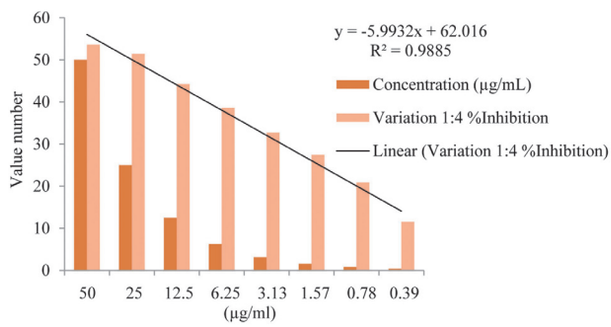


**Figure 3.** Inhibition curve of dengue virus type 2 at several concentrations of Zn(II) complex with variations of mol metal:ligand 1:4

The $IC_{50}$ value with variations of mol metal:ligand 1:2, 1:3, and 1:4 against dengue virus type 2 was 2.44 µg/ml, 2.75 µg/ml, respectively and 2.00 µg/ml. The comparison of the complex compounds and the known anti-dengue virus type 2 activity showed that the 1:4 variation mol metal:ligand was more effective than 1:2 and 1:3. The bulky 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxycromen -4-one ligand on binding to the metal cation reduces the polarity of the metal ion due to the ligand orbital overlap with the metal orbitals, resulting in a delocalization of positive charge.[16] This increases the lipophilic character of the metal favors its permeation through the lipoid layer of the virus membranes.[17] The effects of other compounds against cellular RNA polymerases and formation of the complex with RNA have reported suggesting that compound could also affect the similar replication enzymes.[18]

Previous research was reported anti-dengue type 2 activity, especially Cu(II) with 2,4,5-triphenylimidazole exhibited adsorption inhibitory activity at $IC_{50}$ = 2.3 µg/ml.[11] A significant inhibitory activity to that of the complex Co(II) with 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxycromen -4-one ligand was reported against the tested pathogenic dengue virus type 2 in Vero cells 3.08 µg/ml. The comparison of the other complex, Zn(II) complex compound more significant activity for inhibit dengue virus type 2 replication then Cu(II) complex, and Co(II) complex. As for the central ion ($M^{2+}$), when chelated with ligand to form the complex, it has the following order in stability: $Zn^{2+} > Cu^{2+} > Co^{2+}$. Besides that, Cu(II) free ligand more reactive to dengue virus type 2 up to 0.13 µg/ml because $Cu^{2+}$ has stronger oxidative activity[19] and react with cysteine residues on the surface of the protease.[18] At molecular scale such complex compound interact directly with proteins and DNA, leading to dysfunction and cleavage of the structure of macromolecular.[20]

**Cytotoxicity Activity**

This compound was tested for cytotoxicity by modification of MTT assay method by Mosmann assay on Vero cell lines, Zinc(II)–2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxycromen-4-one complex compound showed cytotoxicity with $CC_{50}$ at 3.59 µg/ml. The $CC_{50}$ value was found to increase with an increasing concentration of the test compound, as shown in Figure 4.

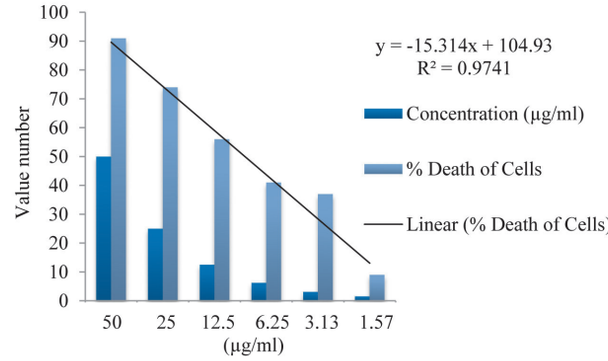


**Figure 4.** Cytotoxicity of Zn(II) complex curve for Vero cell lines at several concentrations

Previous research was reported cytotoxicity other complex compounds to Vero cell lines, Cu(II) with 2,4,5-triphenylimidazole at CC50 = 44.74 µg/ml11 and the complex Co(II) with 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxycromen-4-one ligand at 3.36 µg/ml.12 The comparison with Cu(II) complex compound, it has been revealed that Co(II) and Zn(II) is more toxic, was found to be lower toxic to human MCF7 cell proliferation.[21]

## CONCLUSION

Metal complex compounds are a promising class of drug leads, and the associated studies have attracted more attention. Including previous study about complex compound interaction with dengue virus type 2 activity, Zn(II) more reactive compound then Cu(II), and Co(II).

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