UNIVERSITI TEKNOLOGI MARA

ANTIVIRAL EFFECT AND MECHANISM OF ACTION OF NOVEL N-SUBSTITUTED 5-(PHENYLAMINO)URACIL DERIVATIVES AGAINST DENGUE AND CHIKUNGUNYA VIRUSES

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Dengue virus (DENV) and Chikungunya virus (CHIKV) infections are considered the most important and serious arbovirus infections in Malaysia. DENV infection, complicated with haemorrhage and dengue shock syndrome, is associated with severe morbidity and high mortality, whereas CHIKV infection is commonly complicated with chronic arthritis contributing to disability of the patients. Newly synthesized 5-(phenylamino)uracil derivatives belong to non-nucleoside analogues with the addition of uracil, previously have shown antiviral activity and pharmacokinetic properties of antiviral drugs. Some of 5-(phenylamino)uracil compounds have shown activity against HIV, Hepatitis C and some other RNA viruses, however, the antiviral activity of newly synthesized N-substituted 5-(phenylamino)uracil compounds against DENV and CHIKV remains unknown. This study was aimed to investigate the potential antiviral effect and possible mechanism of antiviral action of novel N-substituted 5-(phenylamino)uracil derivatives against DENV and CHIKV. As the tested compounds are newly synthesized, firstly, the search for the appropriate solvent was performed and solubility of 17 compounds in selected solvents was determined prior to cytotoxicity study on Vero 76 cells. As the compounds are highly lipophilic, only 11 compounds were solubilized in 1% DMSO and the CC_{50} for each of those compounds was identified. Primary screening of 11 selected compounds with the dose ranged from 1.25 to 100 uM was performed on DENV2 and CHIKV with a MOI of 1 on Vero 76 cells. Compounds which showed inhibitory effects against tested viruses were subjected to time-of-addition assay, anti-entry assay, prophylactic assay and anti-adsorption assay to indicate the exact phase of viral life cycle affected. The proteins from infected and treated, as well as the control groups were subjected to 2D gel electrophoresis for determination of the differentially expressed proteins and mass spectrometry analysis was carried out for proteins identification. Proteins potentially involved in the mechanism of antiviral action of novel N-substituted 5-(phenylamino)uracil derivatives were subjected to gene expression study. The results showed that none of the 11 tested N-substituted 5-(phenylamino)uracil derivatives showed antiviral activity against DENV2. However, compounds Z214 and Z364 were found to produce significant virus inhibitory effect against CHIKV. Time-of-addition assay showed significant inhibitory effect of both compounds against CHIKV occurred at 4 to 6 hours post-infection which corresponds to the later stage of CHIKV life cycle. Proteomic analysis showed identification of few proteins possibly involved in the mechanism of antiviral action of Z214 and Z364. Annexin A2(AnxA2) and peroxiredoxin-1(Prx1) were significantly upregulated in the treated groups compared to virus control group and were selected as promising targets for the antiviral action of novel N-substituted 5-(phenylamino)uracil derivatives. Real time PCR revealed increased gene expression of both AnxA2 and Prx1 within 24 hours of treatment with Z214 and Z364 compounds. In conclusion, Z214 and Z364 compounds produced anti-CHIKV effect at post-infection stage and the mechanism of their anti-CHIKV action was most likely associated with modulation of the host proteins. Thus, the upregulation of AnxA2 and Prx1 most likely promote cell defence mechanism by neutralizing reactive oxygen species produced by virus to the host cells hence, interfering with CHIKV replication process leading to maintaining the survival ability of host cells in the presence of virus infection.

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