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

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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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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Viral haemorrhagic fevers (VHFs) are a group of acute viral infections which caused by enveloped, single-stranded RNA viruses and present with generalised symptoms such as fever, malaise, headache, myalgia, vomiting, diarrhoea, rash and bleeding manifestations in severe condition (Bray, 2005). Arthropods such as ticks and mosquitoes serve as vectors for virus transmission from animal host to human. Therefore, most VHFs are geographically restricted to the areas where their hosts and vector species live. Human cases or outbreaks of haemorrhagic fevers caused by these viruses occur sporadically and unpredictable (Ftika & Maltezou, 2013). Among all VHFs, dengue fever (DF) and chikungunya fever (CF) are considered as serious virus-related infections in South East Asian (SEA) region with severe morbidity and mortality reported in the last two decades (Yoshikawa, 2010). Both viruses are arthropod-borne viruses and are transmitted by the same mosquitoes which are *Aedes albopictus* and *Aedes aegyptii*. The spread of these mosquitoes to a new geographical area has contributed to the spread of the diseases to many new countries in Mediterranean and Europe of which dengue and chikungunya infections were never heard of before (Jing & Wang, 2019). The recent climate change around the world and the impact of globalization of human and vectors have introduced these viruses to other new regions, in which have raised a great concern among health care practitioners as it may cause an epidemic in a new area with economic implications (Lee & Farlow, 2019).

The early clinical manifestation of both infections is similar and difficult to be differentiated especially in the absence of diagnostic test availability (Avarado et al., 2019). There is no specific antiviral treatment available for these viral infections to date. The recent advances in medical sciences have contributed to the understanding of pathogenesis as well as viruses' structures and their functions in virus replication. These valuable knowledges have given a direction to the researchers in searching for targets for developing effective antiviral therapy. Despite many attempts in the past in evaluating potential natural and synthetic compounds with some promising results *in vitro* and *in vivo* studies, none of the tested compounds have been developed as a clinical