UNIVERSITI TEKNOLOGI MARA

OVEREXPRESSION OF HOST FURIN PROTEASE AND INHIBITORY ACTIVITIES OF SYNTHETIC CHALCONES- AND AZEPINES- DERIVATIVE COMPOUNDS TOWARD DENGUE VIRUS TYPE-2

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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

The outbreak of dengue disease continues to occur despite extensive measures of vector control. Meanwhile, progress in the vaccine development is often affected by various challenges thus delaying in the production of an effective vaccine across all four dengue serotypes. Thus, efforts in combating dengue are being channelled to other alternatives such as dengue antivirals. The search for this therapeutics has given rise to various screening methods to test for potential inhibitory activities of purified as well as synthetic compounds. Therefore, one of the aims of this study is to produce a furin recombinant protein. This current study also aims to determine the inhibitory effect of the synthetic chalcones- and azepines-derivatives toward dengue virus infection *in vitro*. To achieve the first objective, the furin gene isolated from HepG2 cells inoculated with dengue virus type-2 (DENV2) was cloned and overexpressed in the *E.coli*. The protein lysates of the overexpressed protein were purified using Ni^{2+} (resin) affinity chromatography and its concentration was measured by Bradford assay. The purified furin was confirmed by SDS-PAGE and Western blot analysis. The result showed that the furin is expressed at 60 kDa and was positive toward Rabbit Monoclonal Anti-Furin antibody. Subsequently, two groups of synthetic chalcones (2446DA and 20H46DA) and azepines (MA13, MA15 and MA16) derivatives were measured for their inhibitory activities toward dengue infection by cytotoxicity assay, plaque assay, indirect immunostaining, *in vitro* inhibition assay and fluorescence scanning microscopy. The cytotoxicity assay results showed that the concentration below maximum non-toxic dose (MNTD) for both 2446DA and 20H46DA in HepG2 cells was 15 µg/mL. The same value was obtained for MA15 and MA16. However, MA13 was observed to be less toxic compared to all test compounds with MNTD of 30 µg/mL. The plaque forming unit per ml (pfu/ml) was reduced prominently by 10 to 1000 fold when the infected BHK21 cells were treated with the highest non-toxic concentration compared to lowest non-toxic concentration. The indirect immunostaining results showed a similar trend of virus particles reduction on infected HepG2 cells in both the chalcones- and azepines-derivatives when treated at simultaneous- and post- conditions. However, the azepines MA13 exhibited the most potent activity towards DENV2 whereby total inhibition of virus particles was observed during simultaneous-treatment condition. The *in vitro* inhibition assay results showed that at concentration below MNTD, all compounds exhibited inhibitory activity against DENV2 in a dose dependent manner, indicated by the absence of cytopathic effects. The inhibitory potency strength exhibited was between 73% to 100% against both 1000 $TCID_{50}$ ($p>0.05$) and 100 $TCID_{50}$ ($p<0.05$). Results of the fluorescence scanning microscopy showed that all cytoskeletal changes induced by DENV2 infection were managed by the inhibitory activity of chalcones- and azepines-derivatives in BHK21 cells. In conclusion, we proposed that the purified furin host protease to have high substrate specificity and highly potential to be developed as screening tool for anti-furin compounds. More importantly, the synthetic chalcones- and azepines-derivatives are suitable candidates for the development of therapeutics against dengue infection.

TABLE OF CONTENTS

CHAPTER ONE
INTRODUCTION

1.1 GENERAL BACKGROUND

Dengue is defined from a Swahili phrase ‘Kadinga pepo’ describing an illness of sudden cramp-like seizure caused by an evil spirit or plague (Gupta et al. 2012) which currently refers to the disease known as dengue fever. In the past decades, dengue has become one of the most significant global health threats with an alarming increase of incidences each year. According to World Health Organization, 40% of the world population is at risk for dengue disease with 50 to 100 millions cases reported annually (Cecilia, 2014). Among these dengue cases, about 250,000 patients were diagnosed of more severe disease presentation such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which often lead to fatality (Garcia et al. 2009).

Currently, dengue diseases are endemic in more than 100 countries, including Africa, America, South East Asia, Eastern Mediterranean and the Western Pacific. The Southest Asia and Western Pacific are the most seriously affected countries imposing major health and economic burden through these regions (Cheah et al. 2014). Meanwhile in Malaysia, incidences of dengue diseases have been persistent for more than ten decades with the first case reported in the year of 1902 in Penang. Five years ago a number of 29,183 cases with 67 deaths have been reported (Hamid, 2010). At present, the incidence rate of dengue infection have been estimated to be approximately 64 per 100,000 of the population (Aziz et al. 2006; Chew et al. 2012). For instance in the year 2012, there was 1466 of dengue incidences reported, whereby 91.5% of the cases were of dengue fever while 8.5% was dengue hemorrhagic fever (Nizal et al. 2012). As of June 2015, more than 53823 cases of dengue fever, including 158 deaths, have been reported in Malaysia which indicate an increase by 34% compared to the number of cases reported within the same period in the year 2014 (WHO, 2015a).