

CHAPTER 1

GENERAL INTRODUCTION

Dengue virus belongs to the *Flaviviridae* family and is a widespread human pathogen that can cause diseases ranging from a harmless flu-like illness to a severe haemorrhagic fever with high mortality, especially in children (Kautner *et al.*, 1997). Dengue infections place some 2.5 billion people (or 40% of the world population) at risk, especially in the tropical and subtropical regions (WHO, 2009). Management of dengue fever is largely supportive and its severe haemorrhagic manifestation may require blood transfusions.

The most prevalent of the four dengue serotypes is Dengue virus type 2 (DEN-2) which contains a single-stranded RNA of positive polarity. The RNA genome codes for a single polyprotein precursor of 3 391 amino acids, comprising three structural and seven non-structural proteins, arranged in the order C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5 (Irie *et al.*, 1989). Flavivirus replication is dependent upon the correct cleavage of this polypeptide and requires both host cell proteases and a virus-encoded, two-component protease, NS2B-NS3 (Falgout *et al.*, 1991; Yusof *et al.*, 2000). Hence, the NS2B-NS3 protease complex serves to be a target for the development of antiviral drugs.

Although there have been several efforts by many research groups (Diamond *et al.*, 2002; Hrobowski *et al.*, 2005; Putnak *et al.*, 2005; Tan *et al.*, 2006; Whitby *et al.*, 2005; Yin *et al.*, 2006a), no vaccines or antiviral drugs are currently available against dengue virus infections (Ray & Shi, 2006). In the recent years, dengue fever has been on the rise globally, with dengue infection incidence reported to increase 30-fold in the last 50 years (WHO, 2009). Thus, there is an immense continuous interest in developing new antiviral therapeutic agents to fight this disease.

The search for antiviral therapeutic agents from natural resources is a common practice in drug discovery research. This approach has never ceased to furnish investigators with new and interesting findings. For example, in recent studies several compounds isolated from *Boesenbergia rotunda* (L.) Mansf. Kulturpfl. was reported to exhibit competitive and non-competitive inhibitory activities against the ability of the DEN-2 protease to cleave fluorogenic peptide substrates (Tan, 2005; Tan *et al.*, 2006). This plant, belonging to the ginger family (Zingiberaceae), is a common spice within the Southeast Asian region, especially in Thailand, Malaysia and Indonesia. *B. rotunda* has been reported for its traditional medicinal application mainly for problems and diseases of women, such as its use as post-partum protective medicine, treatment for rheumatism and tonic/lotion (Ibrahim & Rahman, 1989). Scientific reports on the anti-inflammatory and anti-HIV activities of extracts from this plant have also been published (Tewtrakul *et al.*, 2003a; Tewtrakul *et al.*, 2003b; Tuchinda *et al.*, 2002).

1.1 Aim and objectives

The general aim of this work is to study and understand interactions between inhibitors and the protease and understand its mechanism of action. The main method employed to achieve this aim involves structural study of the protein-ligand complex through protein crystallization as well as computational modelling.

The objectives of the present study are:

- i. to perform secondary structure prediction on DEN-2 NS3 using computational tools,
- ii. to overexpress and purify DEN-2 active protease (NS2B-NS3),
- iii. to perform crystallization experiments on the purified NS2B-NS3 and obtain its three-dimensional structure,

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- iv. to carry out automated docking experiments of non-competitive inhibitors into the DEN-2 NS2B-NS3 using computational techniques and analyse the interactions involved, and
- v. to perform automated docking and hybrid quantum mechanic / molecular mechanic (QM/MM) calculations, and study the mode of interactions between the competitive inhibitors and the active site of DEN-2 protease.