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Short communication

Synthesis and molecular modelling studies of novel sulphonamide derivatives as dengue virus 2 protease inhibitors

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ABSTRACT

Development of antivirals for dengue is now based on rational approach targeting the enzymes involved in its life cycle. Among the targets available for inhibition of dengue virus, non-structural protein NS2B–NS3 protease is considered as a promising target for the development of anti-dengue agents. In the current study we have synthesized a series of 4-(1,3-dioxo-2,3-dihydro-1H-isoindol-2-yl)benzene-1-sulphonamide derivatives and screened for DENV2 protease activity. Compounds **16** and **19** showed IC_{50} of DENV2 Protease activity with 48.2 and 121.9 μM respectively. Molecular docking and molecular dynamic simulation studies were carried out to know the binding mode responsible for the activity. MD simulations revealed that, NS2B/NS3 protease was more stable when it binds with the active compound. Structure optimization of the lead compounds **16** and **19** and their co-crystallization studies are underway.

1. Introduction

Dengue is the oldest viral infection reported in Chinese medical encyclopedia during the Jin-dynasty (265–420 AD) as "water poison" associated with flying insects [1]. First confirmed case of dengue case reported in 1789 and is by Benjamin Rush, who coined the term "break bone fever" because of the symptoms of myalgia and arthralgia [2]. Dengue is widespread mosquito-borne disease, which is rapidly spreading throughout the globe where the mosquito vector *Aedes aegypti* is found [3]. Dengue infection is caused by dengue virus (DENV), which belongs to the family Flaviviridae [4] and is one of the major emerging pathogens for which there is neither a vaccine nor any antiviral therapy. Among all viral hemorrhagic fevers, dengue accounts maximum [5]. There are four serotypes in Dengue virus, DENV1–DENV4 [6]. According to World Health Organization (WHO) statistics 2012, 2.5 billion people are at risk for dengue infection throughout the world [7]. A recent study estimates about 390 million dengue infections takes per year, in which 96 million manifest some level of clinical or subclinical severity [8]. Another study estimates that 3900 million in 128 countries are at risk with dengue virus [9]. Dengue virus has RNA as genetic material. Upon infection, the genomic RNA of the virus is translated by the host cell machinery into a single polyprotein, which is subsequently cleaved and processed into ten distinct structural (C, prM and E) and nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) proteins [6]. Dengue Virus encodes for a two component protease NS2B–NS3 which is responsible for the processing at the junctions of NS2A/NS2B, NS2B/NS3, NS3/NS4A and NS4B/NS5, as well as at internal sites within C, 2A, NS3, NS4A [6,10,11]. This makes the NS2B–NS3 protease an ideal target for drug design against dengue infection.

Non-peptidic small molecule inhibitors are targeted successfully against HCV and HIV viral proteases and their resistant strains [12–14]. Moreover, small molecule inhibitors promote large conformational changes in the dengue virus NS2B–NS3 protease [15] for example, 8-hydroxy quinoline derivatives [16], anthracene based inhibitors [17], α-keto amides [18], aryl cyanoacryl amides [19], 1,2-benzisothiazole-3(2H)-one, 1,3,4-oxadiazole hybrid derivatives [20], benz[d]isothiazol-3(2H)-one derivatives [21] and cyclohexenylchalcone derivatives from natural product [22]. They were reported to inhibit DENV protease.

High throughput virtual screening (HTVS) protocol was found very successful for getting leads in drug discovery [23]. In an attempt to obtain selective inhibitors of the DENV NS2B/NS3 protease, HTVS was performed. Whole Zinc 8 database was filtered for drug like molecules and resultant dataset was docked to DENV2 NS2B/NS3 protease (pdb code: 2FOM [24]). Top hundred

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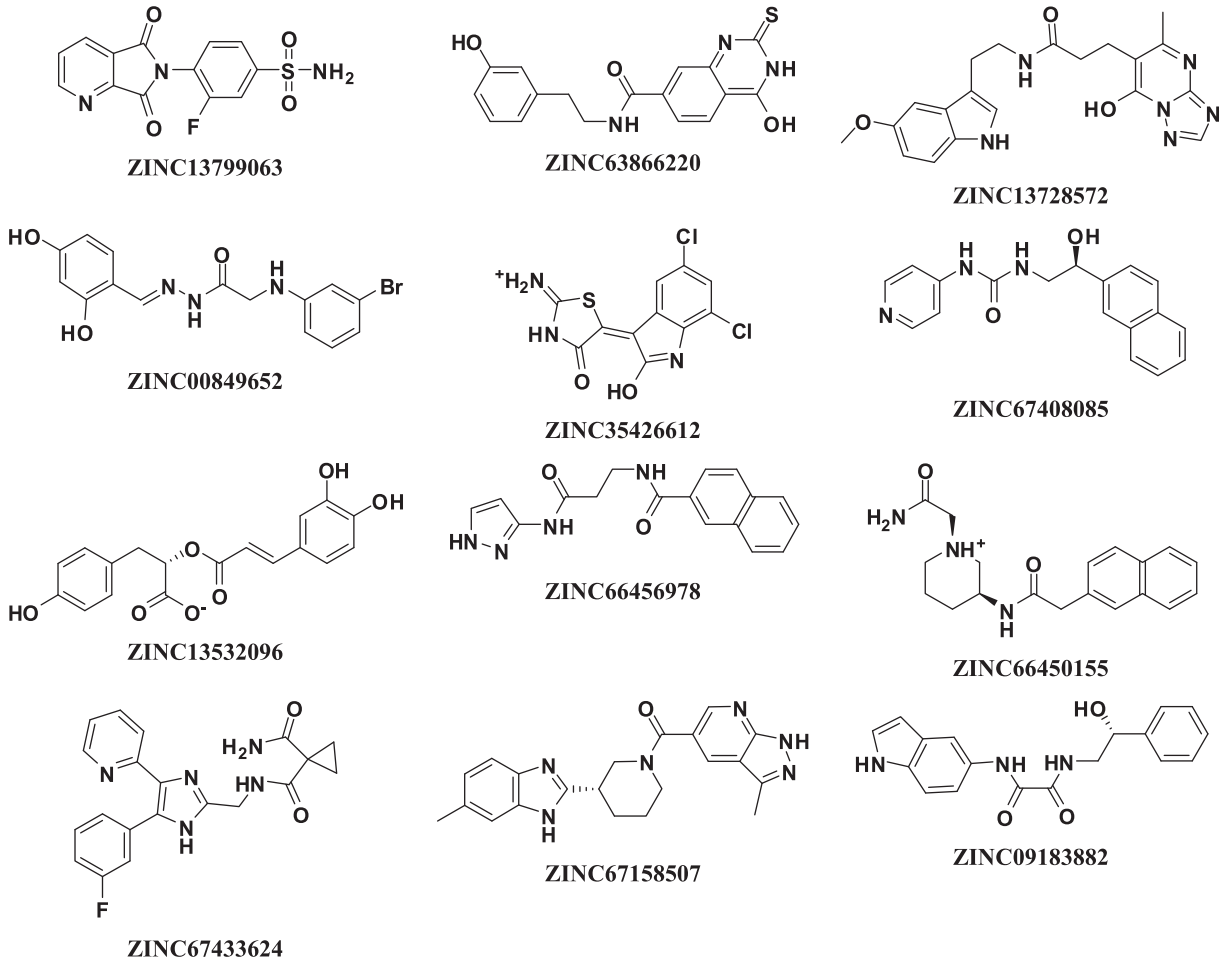
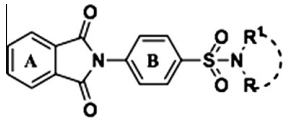
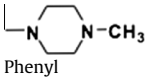


Fig. 1. Best scaffolds as inhibitors against DENV2 protease.

Table 1
DENV protease activity of Compounds **1**–**20**.



1-18

Compound code	R	R^1	IC_{50} (μM)
1	H	H	>100
2	Methyl	Methyl	>100
3	Ethyl	Ethyl	>100
4	Isopropyl	H	>100
5			>100
6			>100
7	Phenyl	H	>100
8	2-Hydroxy Phenyl	H	>100
9	3-Methoxy Phenyl	H	>100
10	4-Methoxy Phenyl	H	>100
11	3-Chloro Phenyl	H	>100
12	4-Chloro Phenyl	H	>100
13	2-Methyl phenyl	H	>100
14	3-Methyl phenyl	H	>100
15	4-Methyl phenyl	H	>100
16	4-Ethyl phenyl	H	48.2
17	4-Nitro phenyl	H	>100
18	Phenyl-4-carboxylic acid	H	>100
19	1-Napthyl	H	121.9
20	Phenyl ethyl	H	>100

molecules were manually analyzed to pick different scaffolds (Fig. 1) that makes a diverse set. Among the top 4-(5,7-dioxo-5H-pyrrolo[3,4-b]pyridin-6(7H)-yl)-2-fluorobenzenesulphonamide has created interest, because it has phthalimide (1,3-dioxoisoindolin-2-yl) kind of scaffold bonded to sulphonamide group. Many sulphonamides were reported as protease inhibitor for HIV [25] and HCV [26]. Moreover, sulphonamides mimic peptide bond by increasing the stability towards protease catalyzed degradation [27]. Apart from it, phthalimide derivatives are reported to have antiviral activities [28], antimycobacterial activities [29] and they are very recently reported to have anticancer activity [30]. This created enthusiasm to synthesize few phthalimide–sulphonamides hybrid analogues. We synthesized few 4-(1,3-dioxoisoindolin-2-yl) benzenesulphonamide derivatives (see Table 1) and investigated for anti DENV2 protease activity and two compounds were found to have DENV2 protease inhibitory activity at lower micro molar concentration. In order to understand the interaction of active compounds (**16** and **19**) with the DENV2 protease, protein structure was modelled against DENV3 X-ray crystal structure (PDB: 3U1I). This has provided much clear picture of DENV2 protease in its catalytically competent form.

2. Experimental section

2.1. Materials and methods

Chemicals and solvents were of reagent grade and purchased from Sigma–Aldrich/Merck/CDH/Rankem. Completions of reaction were monitored on TLC plates (Merck™, KGaA, Germany). Melting