# COMPUTATIONAL DESIGN AND SYNTHESIS OF POTENTIAL NS2B-NS3 DENGUE PROTEASE INHIBITORS

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# COMPUTATIONAL DESIGN AND SYNTHESIS OF POTENTIAL NS2B-NS3 DENGUE PROTEASE INHIBITORS

**by**

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## LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| Å | Angstrom |
| ARG | Arginine |
| ASN | Asparagine |
| ASP | Aspartic acid |
| L-BFGS | Limited-memory Broyden–Fletcher–Goldfarb–Shanno algorithm |
| CYS | Cysteine |
| DENV | Dengue virus |
| DIC | Disseminated Intravascular Coagulation |
| DF | Dengue fever |
| DHF | Dengue Haemorrhagic Fever |
| DSS | Dengue shock syndrome |
| EA | Ethyl acetate |
| $EC_{50}$ | $EC_{50}$ (half-maximal effective concentration) is a measure of the potency of a substance in producing a particular effect or response |
| EF | Enrichment Factor |
| FEB | Free energy of binding |
| FTIR | Fourier Transform Infrared Spectroscopy |
| FGA | Genetic algorithm |
| GLN | Glutamine |
| GPF | Grid parameter file |
| GLU | Glutamic acid |
| GLY | Glycine |
| HB | Hydrogen bond |
| HEX | Hexane |
| HIS | Histidine |
| HIV | Human Immunodeficiency Virus |
| HBA | Hydrogen bond acceptor |
| HBD | Hydrogen bond donor |
| HTS | High Throughput Screening |
| $IC_{50}$ | Concentration of the compounds necessary to inhibit 50% of the control values. |
| ILE | Isoleucine |
| Ki | Inhibition constant |

| | |
|---|---|
| LEU | Leucine |
| LCMS | Liquid Chromatography Mass Spectrophotometer |
| LGA | Lamarckian genetic algorithm |
| LYS | Lysine |
| NMR | Nuclear Magnetic Resonance |
| MD | Molecular Dynamics |
| MET | Methionine |
| MM/PBSA | Molecular Mechanics/Poisson-Boltzmann Surface Area |
| [M-H]- | Mass-to-charge ratio |
| µM | micromolar |
| nM | nanomolar |
| PRO | Proline |
| PHE | Phenylalanine |
| RER | Rough Endoplasmic Reticulum |
| RNA | ribonucleic acid |
| RMSD | Root mean square deviation |
| ROC | Receiver operating characteristic curve |
| SARS-COV-2 | SARS-CoV-2 papain-like protease |
| SER | Serine |
| SI | Selectivity index |
| SLR | Systematic Literature Review |
| THR | Thrionine |
| TLC | Thin layer chromatography |
| TRP | Tryptophan |
| TYR | Tyrosine |
| VAL | Valine |
| vdW | van der Waals |
| W | Watt |
| WHO | World Health Organization (WHO) |
| WNV | West Nile Virus |

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## ABSTRAK

Virus Denggi Serotip 2 (DENV2), salah satu jenis virus denggi yang kerap dijumpai di Asia Tenggara, kini merupakan isu kesihatan awam yang besar. Pada minggu ke-6 tahun 2024, Malaysia melaporkan lebih daripada 3,631 kes denggi dan 10 kematian disebabkan oleh DENV2, menandakan peningkatan sebanyak 68.75 % dari tempoh yang sama pada tahun 2023, merujuk kepada laporan Pertubuhan Kesihatan Sedunia (WHO). Walaupun jumlah bilangan kes yang tinggi, masih tiada ubat yang terbukti secara klinikal untuk menghalang replikasi virus ini, terutamanya disebabkan oleh terhadnya struktur kristal 3D protein-protein yang boleh disasarkan untuk pengaktifan ubat daripada DENV2 dan virus West Nile (WNV) termasuk NS2B-NS3 protease. Untuk menangani masalah ini, pendokkan dan model farmakofor telah digunakan dengan ligan dari DENV2 dan virus West Nile. Analisis Literatur Secara Sistematik (SLR) mengenal pasti 9 sebatian aktif daripada 2 jenis *in vitro* anti-DENV2 NS2B-NS3 protease iaitu 21 kelas sebatian aktif mensasarkan enzim dan 9 kelas sebatian aktif mensasarkan sel. 2 model daripada 9 model farmakofor dikenalpasti sebagai model farmakofor terbaik berdasarkan nilai Luas di Bawah Lengkung (AUC) dan Faktor Pengayaan (EF) yang tinggi, yang hampir mencapai 1 dan 100%, dan kemudiannya digunakan sebagai model untuk pengesahan farmakofor. Penjajaran skor kesesuaian farmakofor dengan afiniti pengikatan yang baik menunjukkan interaksi yang baik dengan residu asid amino, termasuk SER83, TYR161, dan VAL155. Dalam kajian ini, 11 sebatian telah disintesis, termasuk 4 kalkon, 4 flavon, dan 3 biflavonoid.

Struktur setiap sebatian yang disintesis ditentukan melalui analisis spektral menggunakan Kromatografi Lapisan Nipis (TLC), Spektroskopi Transformasian Fourier Inframerah (FTIR), Spektroskopi Resonans Magnet Nukleus Proton ($^1$H NMR) dan Kromatografi Cecair Spektrometri Jisim (LCMS). Daripada 11 sebatian yang disintesis, empat sebatian kalkon ini (CD-OH, CA-F, CB-OH, CC-OH) menunjukkan aktiviti perencat enzim yang baik dengan nilai $IC_{50}$ yang sebanding dengan kawalan positif quercetin dan ginkgetin. Sebatian CD-OH (struktur baru) dengan nilai $IC_{50}$ sebanyak 114.03 µM berbanding Quercetin (116.73 µM) dan ginkgetin (126.74 µM). Kalkon (CA-F, CB-OH, dan CC-OH) mempunyai nilai $IC_{50}$ masing-masing sebanyak 124.80 µM, 141.26 µM dan 146.30 µM. Penemuan ini menunjukkan potensi aktiviti penghalang potensi sebatian berdasarkan biflavonoid dengan keberkesanan 36.07- 48.29 % terhadap protease DENV NS2B-NS3, dan boleh digunakan sebagai asas dalam usaha penyelidikan dan pembangunan pada masa depan dalam bidang ini.

**COMPUTATIONAL DESIGN AND SYNTHESIS OF POTENTIAL NS2B-NS3 DENGUE PROTEASE INHIBITORS**

## ABSTRACT

DENV2, the type of dengue virus commonly found in Southeast Asia, is a major public health concern. In week 6 of 2024, Malaysia reported with 3,631 dengue cases and 10 deaths due to DENV2, marking a 68.75 % increase from the same period in 2023, as reported by the World Health Organization (WHO). Despite the high number of cases, there is currently no clinically proven drug available to inhibit the virus replication, primarily due to the limited availability of 3D crystal structures of targetable protein-protein interactions for drug activation, including the NS2B-NS3 protease from DENV2 and West Nile virus (WNV). To address this issue, docking and pharmacophore modelling have been used with the ligands from DENV2 NS2B-NS3 protease and West Nile Virus. The Systematic Literature Review (SLR) analysis identified 9 active compounds from 2 types of *in vitro* DENV NS2B-NS3 protease assays i.e. 21 compound classes from cell-free-based assays and 9 compound classes from cell-based assays. 2 of the 9 scaffolds' molecules were identified as the best pharmacophore model based on their high Area Under the Curve (AUC) and Enrichment Factor (EF) values, which were close to 1 and 100% respectively, and were further used as a model for pharmacophore validation. The pharmacophore fit score alignment with good binding affinity showed good interaction with amino acid residues, including SER83, TYR161, and VAL155. In this study, 11 compounds were synthesized, including 4 chalcones, 4 flavones, and 3 biflavonoids. The structures of each synthesized compound were determined through spectral analysis using Thin Layer Chromatography (TLC), $^{1}$H Nuclear Magnetic Resonance (NMR)

Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR) and Liquid Chromatography Mass Spectrometry (LCMS) data analysis. Out of the 11 synthesized compounds, four chalcone compounds (CD-OH, CA-F, CB-OH, CC-OH) showed good enzyme inhibitory activity with $IC_{50}$ values comparable to the positive controls quercetin and ginkgetin. The compound CD-OH (a new structure) had an $IC_{50}$ value of 114.03 µM compared to quercetin (116.73 µM) and ginkgetin (126.74 µM). The other chalcones (CA-F, CB-OH, and CC-OH) had $IC_{50}$ values of 124.80 µM, 141.26 µM and 146.30 µM respectively. These findings provide valuable insights into the potential inhibitory activity of biflavonoid-based compounds with 36.07-48.29 % efficiency against the target DENV NS2B-NS3 protease and can serve as a basis for further research and development efforts in this field.

# CHAPTER 1

# INTRODUCTION

## 1.1 Background

Dengue, a mosquito-borne viral disease caused by the dengue virus (DENV), is widespread and endemic in tropical and subtropical climates (Tomlinson & Watowich, 2012; Yao et al., 2019). In recent years, DENV infections have been responsible for 390 million infections per year throughout the world (Balasubramanian et al., 2019; Wahid et al., 2022). DENV, a mosquito borne Flaviviridae family member, which comprises of five different serotypes (DENV 1, 2, 3, 4 and 5), has been responsible in the increase of dengue infection by 30 folds in the last fifty years (Ryan et al., 2021) and thousands of deaths (Lin et al., 2016). DENV2 is the most common serotype that causes significant epidemics in Malaysia (Yenamandra et al., 2021) and other Southeast Asian countries and has resulted in high economic burden to the countries. A 2020 study found that the economic burden of eight major diseases, including infectious diseases, in low- and middle-income countries was substantial (Armitage, 2021). The annual healthcare cost of non-communicable diseases in Malaysia exceeds RM 9.65 billion as reported by the World Health Organization (WHO) in November 2023.

DENV, an arbovirus has a very complex life cycle as it requires more than one host to complete it, thus presenting a challenge in monitoring the spread of the arbovirus. *Aedes aegypti*, *Aedes polynesiensis*, and *Aedes albopictus*, are the main transmitting vectors of DENV where *A. aegypti* is deemed the main causative agent for the infection (Bellone et al., 2020; Uno & Ross, 2018).The virus consists of a single, positive-strand RNA genome of ~10,700 nucleotides that is packed by three structural proteins (capsid, C; membrane-associated, M and envelope, E) and a lipid bilayer. The

Ribonucleic acid (RNA) of dengue virus encodes a 5'-untranslated region (UTR), a single open reading frame (ORF) and 3'-UTR (Murugesan & Manoharan, 2019).

DENV can potentially cause acute systemic illnesses and other serious health complications in humans and typically the symptoms of infections appear after five to eight days following the bite by an infected mosquito (Bonheur et al., 2021). Dengue complications vary from dengue fever (DF), dengue haemorrhagic fever (DHF), or dengue shock syndrome (DSS) (Lima et al., 2015; Qamar et al., 2019). DF vary between being asymptomatic to mild fever. Patients with dengue fever who experience persistent illness despite their fever subsiding may be at higher risk of developing DHF (Ng et al., 2016). DHF is characterised by the symptoms of DF, but might lead to epistaxis haemorrhage, circulatory failure, pleural effusions, ascites, glomeruli injury and pericarditis (Guzman et al., 2008). Insufficient or improper treatment of DHF often leads to an intense shock leading to DSS. DSS might manifest thrombocytopenia ($<100\times10^{9}$ $L^{-1}$), encephalopathy, encephalitis, hepatic failure, myocarditis, and disseminated intravascular coagulation (DIC) leading to immense haemorrhage (Pérez et al., 1998). Figure 1.1 and Table 1.1 show respectively the WHO classification of symptomatic dengue infection and the classification of DHF.

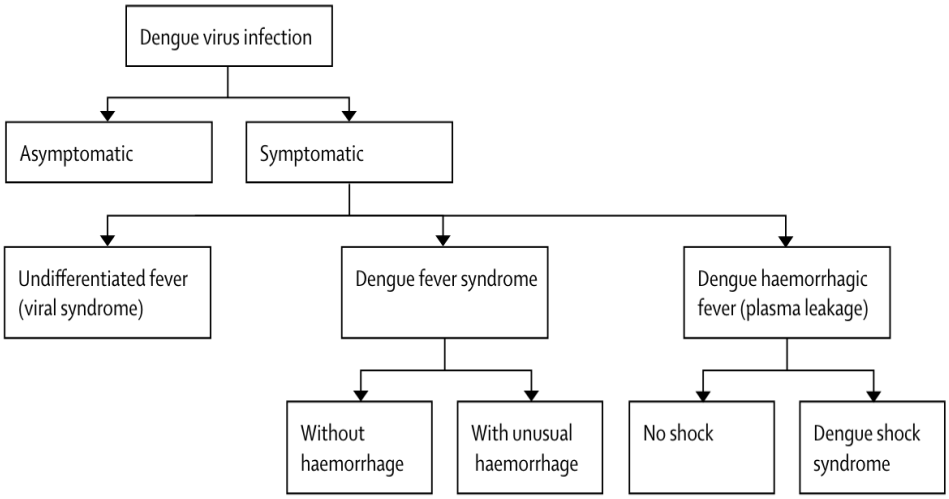


Figure 1.1 WHO classification of symptomatic dengue infection taken from (Deen et al., 2006)

To date, there is no specific treatment available for dengue until (Wahid et al., 2022). Various therapeutic options have been assessed in clinical trials with minimal success over the last 60 years. The so-called "critical period" (the danger period) of dengue is when the patient might undergo sudden deterioration, which is relatively short (between 48–72 hours). The chance of a fatal outcome can be minimized if the patient is appropriately managed during this phase (Rajapakse et al., 2012). Generally, bed rest and hydration are recommended for patients with mild dengue. In severely affected dengue patients, dengue treatment relies solely on a supportive therapy which attempts to minimise pro-inflammatory responses induced by the infections. World Health Organization (WHO) recommends the use of paracetamol to control pain and intravenous crystalloids and colloid solutions for patients experiencing dengue shock syndrome. A vaccine called Dengvaxia is currently available for people who have had dengue at least once and live in places where the disease is common (Flasche et al.,

2016). However, the effectiveness of the vaccine is still controversial (Lasco & Yu, 2021).

Table 1.1 WHO classification of dengue haemorrhagic fever. Source: (Bandyopadhyay et al., 2006)

| Grade | Clinical description |
|---|---|
| I | Fever with non-specific constitutional symptoms and the only haemorrhagic manifestations being a positive tourniquet test |
| II | Symptoms same as for Grade I, but accompanied by more extensive haemorrhagic manifestations |
| III | Signs of circulatory failure or hypertension |
| IV | Profound shock with pulse and blood pressure being undetectable |

## 1.2 Problem Statement

Dengue is a fast-emerging pandemic-prone viral disease in many parts of our globe, including in the Southeast Asian Region. Globally, about 100-400 million cases and up to 25,000 deaths due to DENV infections occur annually, according to WHO on March 2023. However, until today, there are no specific effective treatments for dengue (Andrei et al., 2023).

In recent years, many research activities have been directed towards the discovery of anti-dengue chemotherapeutic agents especially those targeting the dengue viral protease enzyme. Affinity values in the lower micromolar range have been observed for small-molecule, structurally diverse protease inhibitors. However, peptide-based, substrate-derived molecules are frequently having poor drug-likeness. With a few exceptions, most of the described anti-dengue cellular activity has been

patchy and insufficient for future research. Using several structural approaches, recent progress has been made in the elucidation of inhibitor binding. This will ideally lead to more rational attempts to identify multiple lead compounds that will be successful in cellular assays, animal models, and eventually in patients (Nitsche, 2018). There has been slow progress in identifying drug-like compounds that block the protease and serve as a starting point for lead optimization (Steuer et al., 2011). This study thus aims to contribute to the progress in the discovery of potential treatments for dengue targeting DENV protease.

## 1.3 Objectives of the study

The main goal of this study is to develop and evaluate a series of novel and effective anti-DENV protease agents based on bioflavonoid scaffold through a comprehensive approach. Thus, the specific objectives of the study are:

1. To develop a computational model of DENV2 NS2B-NS3 inhibitors through a comprehensive evaluation of systematic literature review.
2. To design a series of bioflavonoid compounds based on the model developed in **(1)** with a focus on their molecular interactions with target protease DENV NS2B-NS3.
3. To synthesize a series of bioflavonoid compounds designed in **(2)**.
4. To evaluate the inhibitory activities of the synthesized compounds in **(3)** against NS2B-NS3 protease.

# CHAPTER 2 LITERATURE REVIEW

## 2.1 The structure and genome of Dengue Virus

Dengue virus is a member of the Flavivirus genus. The viral genome consists of a negative strand RNA, which serves as a template for the amplification of additional positive strand genomic RNA (Figure 2.1). A correlation between the RNA structural elements required for polymerase activity *in vitro* and viral RNA replication in infected cells has been observed (Gebhard et al., 2011). The virus enters the host cell by receptor mediated endocytosis. The DENV genome is 11 kb long, has a type 1 cap(m) structure at the 5' end, and a tail at the 3' end. The N-terminal of the polyprotein encodes the three structural proteins (C-prM-E), followed by at least seven non-structural proteins (NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5). After translation of the RNA, virus-induced hypertrophy of intracellular membranes occurs (Gebhard et al., 2011).

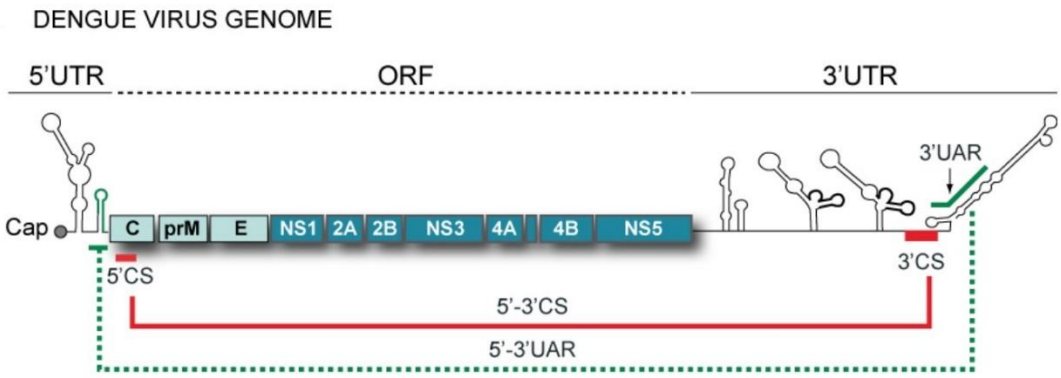


Figure 2.1 Schematic representation of the Dengue virus (DENV) genome (Gebhard et al., 2011)

## 2.2 Life cycle of dengue virus and role of important proteins

The virus attaches to the target cell's surface receptor and enters the cell. After internalisation by receptor-mediated endocytosis, the virus-receptor complex forms an endosomal vesicle (Figure 2.2). Endosomal membrane and viral membrane fusion occurs as a result of the acidic endosomal environment, and this is followed by the release of the RNA genome. The rough endoplasmic reticulum (RER) processes and amplifies the translated genome once the released RNA leaves it. The flavivirus replicates on the RER's surface. The replication complex is made up of host proteins and non-structural proteins of DENV. On the other side of the ER membrane, NS1 dimers in the ER lumen and assists other replication complex components in the replication process. In the endoplasmic reticulum lumen, viruses are further matured and assembled. The trans-golgi network is where an immature virus particle goes to continue maturing. Finally, mature virus particles exit the cell and enter the extracellular space (Madhry et al., 2021).

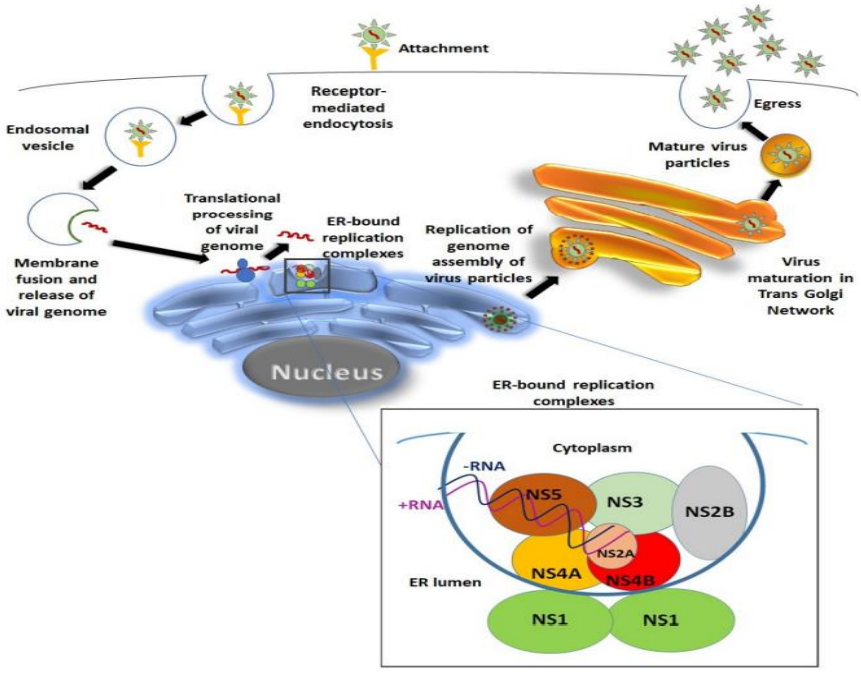


Figure 2.2 Life cycle of dengue virus (Madhry et al., 2021)

## 2.3 DENV NS2B-NS3 protease

The DENV NS2B-NS3 protease has been proposed as the main target for the development of anti-dengue drugs. A single polyprotein precursor included inside the flavivirus genome must be cleaved numerous times by host and viral proteases in order to construct the constituent proteins that make up an infectious virion (Bharadwaj et al., 2019; Brecher et al., 2017; Constant et al., 2018; Kouretova et al., 2017; Kühl et al., 2020; Millies et al., 2019; Yao et al., 2019). The NS2B-NS3 protease processes 8 of the 13 viral polyprotein cleavage sites to allow viral maturation (Lin et al., 2016).

DENV NS2B-NS3 protease complex has been considered as a target for designing specific antivirals due to its importance in viral replication, its high degree of conservation (Montalvo et al., 2016) and considered to be amenable to antiviral inhibition (Nguyen et al., 2013). Due to the great degree of structural similarity and substrate-recognition profile shared by flaviviral proteases, developing flaviviral protease inhibitors which are effective against DENV NS2B-NS3 may also be more feasible (Nitsche, 2018).

## 2.4 Proteolytic mechanism of NS2B-NS3 protease

DENV NS2B-NS3 protease is a serine protease. These proteases belong to the group of proteolytic enzymes known as reactive serine residue-containing enzymes. Serine proteases make up more than one-third of all proteolytic enzymes. They have a catalytic triad made up of residues of histidine, serine, and aspartic acid. As seen in Figure 2.3, the shape of the active site and the accompanying proteolytic activity are significantly influenced by the hydrogen bonding interactions of the catalytic residues. A highly reactive oxyanion species is produced by the hydrogen bonding mechanism on serine, which attacks the carbonyl group of the peptide bond in the substrate. As a

result, a tetrahedral intermediate is formed, with the carbonyl carbon of the substrate peptide switching from sp2 to sp3. This intermediate then undergoes rearrangement to liberate the N-terminal peptide while leaving the C-terminal connected to Serine. The C-terminal peptide is released from serine through further hydrolysis caused by a water molecule close to the active site which then regenerates the catalytic triad. The residues close to the active site made into pockets (P10, P1, P2, P3, P4) that can recognise and fit the amino acids (S1', S1, S2, S3, S4) close to the cleavage site (Timiri et al., 2016).

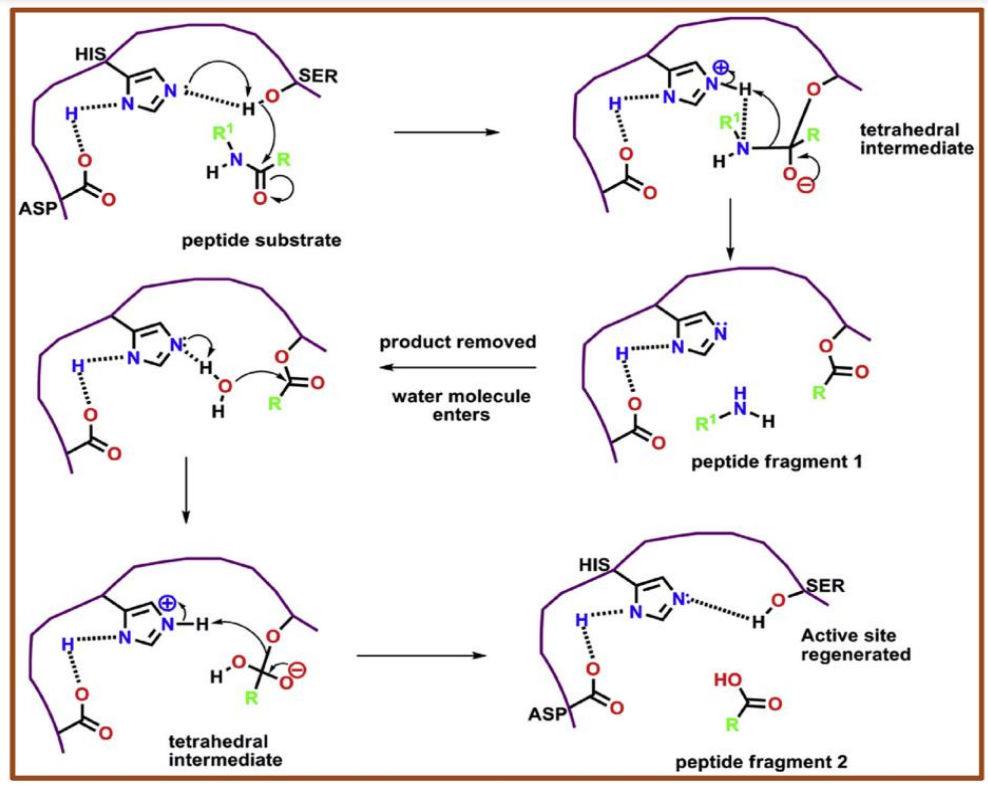


Figure 2.3 Mechanism of cleavage of a peptide by serine protease having catalytic triad (Timiri et al., 2016)

## 2.5 NS2B-NS3 Protease inhibitors

### 2.5.1 Quercetin

Quercetin (Figure 2.4) has been found to be a potent inhibitor of the Dengue virus protease, with an inhibitory concentration ($IC_{50}$) of 35.7 µg/mL (Zandi et al., 2011). It has also been studied for its antiviral effects against other viruses, such as Human Immunodeficiency Virus (HIV) and Hepatitis C Virus (HCV) (Biancatelli et al., 2020), as well as DENV2 strains (Cano et al., 2021) as well as for its potential role in the prevention and treatment of COVID-19. The $IC_{50}$ value of 73 µM against 3CLpro (also known as Mpro) of SARS-CoV-2 has been observed (Gasmi et al., 2022). However, it is important to note that quercetin has significant cytotoxicity (Garmendia et al., 2022).

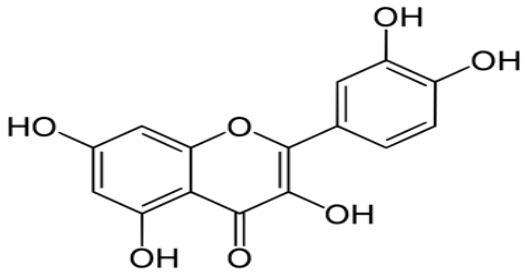


Figure 2.4 Quercetin

### 2.5.2 Thioguanine Derivatives

Hariono et al. (2019) found a small molecule that inhibited DENV2 NS2-NS3B Protease from the National Cancer Institute database by virtual screening. D0713 ($IC_{50}$ = 62 µM), one of the hits, was derivatized into 21 compounds and tested for DENV2 NS2B-NS3 protease inhibitory activity. Compounds 18 and 21 exhibited the highest potency, with $IC_{50}$ values of 0.38 µM and 16 µM, respectively. The interaction

mechanism of these compounds with the protease was investigated using molecular dynamics (MD) and Molecular Mechanics Poisson-Boltzmann Surface Area (MM/PBSA) free energy of binding calculations. The computed free energy of binding for 18 by MM/PBSA is (-16.10 kcal/mol), which is significantly less than the known inhibitor panduratin A (-11.27 kcal/mol), correlating well with the experimental observation. Additionally, molecular dynamics simulations revealed that both compound 18 and 21 binds at the active site and are stabilised by hydrogen bonds formed with Asn174 (Hariono et al., 2019).

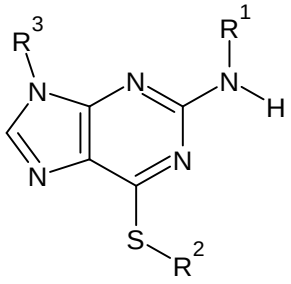


Figure 2.5 Thioguanine derivatives

### 2.5.3 Imine Derivatives

Frimayanti et al. (2017) performed molecular docking studies on a series of imine derivatives (Figure 2.6), and the information obtained from the docking studies was used to investigate the binding modes of these imine derivatives with DENV2 NS2B-NS3 serine protease. Frimayanti et al. (2017) performed molecular docking studies on a series of imine derivatives. A series of imines were synthesized, and then a bioassay investigation was carried out to determine the inhibitory actions of these compounds. MY8 to be the best inhibitor amongst the nine ligands tested with $IC_{50}$ of 174.6 µmol/L, followed by MY4 with $IC_{50}$ of 193.2 µmol/L, MY10 ($IC_{50}$ of 244.9

μmol/L). The findings revealed that compound MY8 and MY4 have the ability to block the DENV2 NS2B-NS3 proteolytic activity in a dose-dependent manner (Frimayanti et al., 2017).

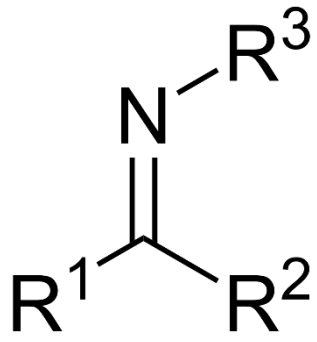


Figure 2.6 Imine derivatives

### 2.5.4 Thiazolidinedione-based peptide hybrids

The synthesis, characterization, biological evaluation, and docking studies of new peptide-hybrids based on 2,4-thiazolidinedione (Figure 2.7) (Nitsche et al., 2013) scaffolds having non-polar groups have been carried out by Lima et al., (2015). The most promising molecule has an $IC_{50}$ of 0.46 μM against West Niles Virus protease, which indicates a seventyfold increase in activity when compared to previously reported compounds. Observations from both experimental and docking studies support the concept that a non-polar group in the scaffold is critical for obtaining interactions between inhibitors and a hydrophobic pocket in the substrate recognition domain of the DENV and West Niles Virus (WNV) NS2B-NS3 serine protease (Lima et al., 2015).

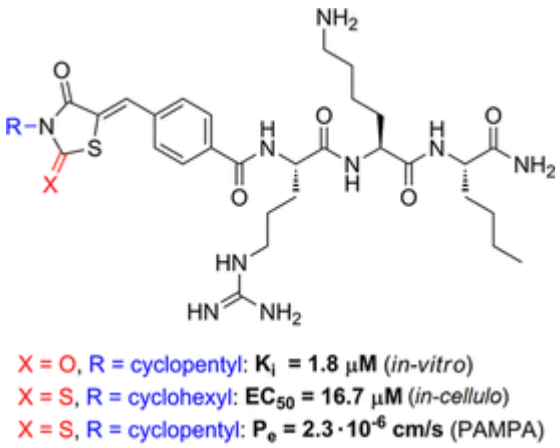


Figure 2.7 Thiazolidinedione-based peptide hybrids

### 2.5.5 Sulphonamide derivatives

Timiri et al. (2015) synthesized and evaluated a series of 4-(1,3-dioxo-2,3-dihydro-1H-isoindol-2-yl)benzene-1-sulphonamide derivatives (Figure 2.8) (Apaydın & Török, 2019) for DENV2 protease activity. Compounds 16 and 19 had 48.2 and 121.9 µM $IC_{50}$ for DENV2 Protease activity, respectively. To determine the binding mode responsible for the action, researchers used molecular docking and MD simulations. MD simulations revealed that when NS2B-NS3 protease binds to the active molecule, it becomes more stable. The lead compounds 16 and 19 are undergoing structure optimization and co-crystallization experiments (Timiri et al., 2015).

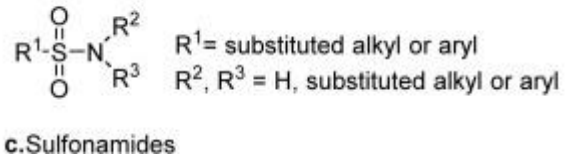


Figure 2.8 Sulphonamide derivatives

### 2.5.6 Thiadiazoloacrylamide Derivatives

A novel family of thiadiazoloacrylamide compounds (Figure 2.9) with significant inhibitory activity against the DENV2 NS2B-NS3 protease has been discovered and modified. Thiadiazolopyrimidinone 1 was discovered as a novel chemical structure in a commercial compound library against DENV2 NS2B-NS3. Similar compounds with a thiadiazoloacrylamide core were identified that would be more active. The nitrile group in the linker section was discovered to be an important component for the inhibitory activity in a series of synthesized analogues. Based on *in vitro* DENV2 NS2B-NS3 protease tests, the best of them compound 8b had an $IC_{50}$ of 2.24 μM (Liu et al., 2014).

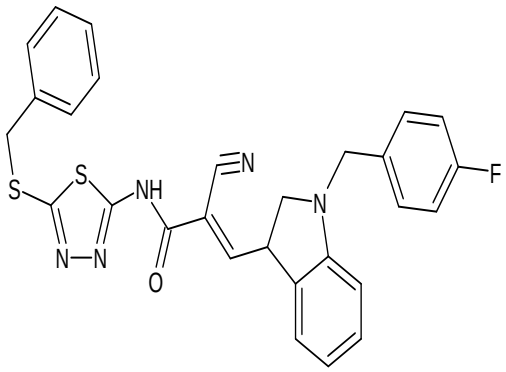


Figure 2.9 Thiadiazoloacrylamide Derivatives

### 2.5.7 1,2-benzisothiazol-3(2H)-one-1,3,4-oxadiazole derivatives

Previously, 1,2-benzisothiazol-3(2H)-ones and 1,3,4-oxadiazoles drew interest as antibacterial and antifungal agents. Lai et al. (2013) previously synthesised and tested functionalized 1,2-benzisothiazol-3(2H)-one-1,3,4-oxadiazole hybrid derivatives (Figure 2.10) against Dengue and WNV proteases. Ten of the twenty-four substances inhibited DENV2 and WNV proteases by more than 50%. The $IC_{50}$ values of compound

7n against DENV2 and WNV NS2B-NS3 were reported to be 3.75 ± 0.06 and 4.22 ± 0.07 μM, respectively. The kinetics data support a competitive mode of inhibition by compound 7n. Molecular modelling was used to study this potential chemical binding mechanism. A hit-to-lead optimization strategy using iterative structure-activity relationship investigations, *in vitro* screening, and X-ray crystallography was well demonstrated (Lai et al., 2013).

Compounds containing the 8-hydroxyquinoline (8-HQ) scaffold were found to be inhibitors of WNV serine protease in a previous investigation. In this investigation, the potencies of several compounds including (8-HQ)-aminobenzothiazole derivatives for DENV2 protease inhibition *in vitro. In vitro* protease studies revealed that analogues 1-4 with a 2-aminothiazole or 2-aminobenzothiazole scaffold had sub-micromolar potencies $IC_{50}$. The most powerful 8-HQ-aminobenzothiazole inhibitor (compound 1), with an $IC_{50}$ value of 0.91 ± 0.05μM, was found to have a kinetic constant $K_i$ of 2.36 ± 0.13μM. This drug inhibits DENV2 NS2B-NS3 protease via a competitive inhibition mechanism (Lai et al., 2013).

Two targeted libraries based on the benz[d]isothiazol-3(2H)-one scaffold were synthesized and tested against the NS2B-NS3 proteases of Dengue virus and West Nile virus. In the absence and presence of additional detergent, some compounds (4l, 7j-n) showed notable inhibitory effects against DENV NS2B-NS3 protease. These compounds might be a good starting point for a hit-to-lead optimization strategy (Tiew et al., 2012).

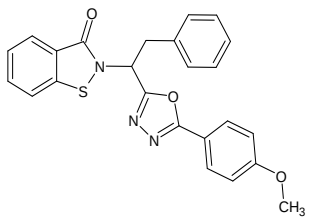


Figure 2.10 1,2-benzisothiazol-3(2H)-one-1,3,4-oxadiazole derivatives

### 2.5.8 Arylcyanoacrylamides derivative

Nitsche et al. (2011) created the 3-aryl-2-cyanoacrylamide (Figure 2.11) scaffold as a key pharmacophore for inhibitors of the DENV serine proteases NS2B-NS3. To investigate the structure-activity connections in greater depth, 86 analogues were synthesized. As a result, it was discovered that the electron density of the aryl moiety and the central double bond have a significant impact on the activity of the compounds, whereas the influence of amide residue substituents is less significant. With a $K_i$ value of 35.7 µM for the Dengue virus protease and 44.6 µM for the West Nile virus protease, the para-hydroxy substituted analogue was determined to be the most effective inhibitor in this series. The aprotinin competition assay shows that the inhibitor molecule interacts directly with the active centre of the DENV. In a counterscreen using thrombin, the target selectivity was determined to be 2.8:1 in favour of DENV protease and 2.3:1 in favour of WNV protease, respectively (Nitsche et al., 2011).

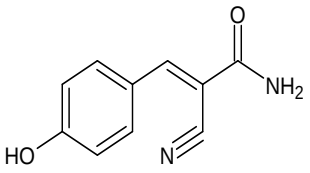


Figure 2.11 Arylcyanoacrylamides derivative

### 2.5.9 Benz[d]isothiazol-3(2H)-one derivatives

Several novel N-substituted 1,2-benzisothiazol-3(2H)-ones (BITs) (Figure 2.12) (Tiew et al., 2012) were synthesised and tested for anti-dengue protease inhibition using a simple synthetic approach. In contrast to traditional multistep synthesis, this study used a two-step, one-pot reaction technique to produce structurally varied BITs with good yields. The online Swiss Absorption, Distribution, Metabolism, and Elimination (SwissADME) model was used to prescreen all of the synthesized compounds for drug-like qualities, showing their favourable pharmacological features. The inhibitory activity of the synthesized BITs was tested against the recombinant dengue virus serotype-2 DENV2 NS2B-NS3 protease. Several BITs bind to the protease in the area of the catalytic triad with $IC_{50}$ values in the micromolar range of 2 to 8.25 μM, according to dose-response tests and computer docking simulations. The DENV2 infection experiment revealed that two BITs, 2-(2-chlorophenyl)benzo[d] and 2-(2-chlorophenyl)benzo[d] 2-isothiazol-3(2H)-one and 2-isothiazol-3(2H)-one (2,6-dichlorophenyl) benzo[d] DENV reproduction and virus infectivity could be inhibited by isothiazol-3(2H)-one (Batool et al., 2021).

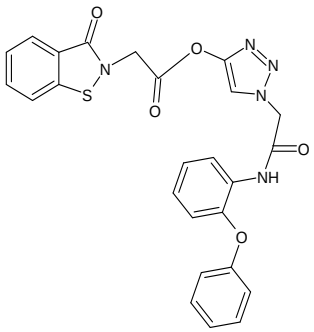


Figure 2.12 Benz[d]isothiazol-3(2H)-one derivatives

### 2.5.10 Phenylglycine derivatives

Kühl et al. (2020) also present the design, synthesis, and *in vitro* and cellular evaluation of a novel chemotype of powerful non-peptidic dengue protease inhibitors derived from 4-benzyloxyphenylglycine. The activity of the compounds in a cellular context was determined using a newly developed luciferase based DENV2 protease reporter system in HeLa cells (DENV2proHeLa). Virus titer reduction experiments supported the DENV2proHeLa system's specificity and selectivity. In cell-based experiments, the compounds have inhibitory potencies ranging from $EC_{50}$ values between 0.49 and 3 μM are selective against other serine proteases, and have negligible cytotoxicity (Kühl et al., 2020).

According to another study by Kouretova et al., (2017), decarboxylated P1-trans(4-guanidino)cyclohexylamide was replaced with an arginyl-amide moiety in a second series. With an inhibition constant of 0.11 μM, the compound 4-(guanidinomethyl)-phenylacetyl-Lys-Lys-Arg-$NH_2$ inhibits West Niles Virus 's NS2B-NS3 protease. The potency of published multibasic furin inhibitors was also examined because of their similar substrate specificity. The chimeric inhibitors were then further modified to increase their efficacy against the WNV and DENV proteases. The furin inhibitors, which lowered virus titers up to 10,000-fold in cell culture, showed a substantial suppression of West Nile Virus and DENV propagation. These findings show that powerful furin inhibitors can stop DENV and West Nile Virus from replicating (Kouretova et al., 2017).

The development of synthesized inhibitors including benzyl ethers of 4-hydroxyphenylglycine (Figure 2.13) (Kühl et al., 2020) as non-natural peptidic building blocks generated via a copper-complex intermediate, as well as comprehensive biological testing. A three-step optimization technique that started with fragment

growth of the C-terminal 4-hydroxyphenylglycine to the benzyloxy ether, then C- and N-terminal optimization, and finally fragment merging resulted in compounds with low nanomolar *in vitro* affinities. At the DENV2 and West Nile Virus proteases, the most promising derivative had Ki values of 12 nM and 39 nM, respectively. In cell-based studies of virus replication, some of the newly identified protease inhibitors resulted in a significant reduction of dengue and WNV titers, with an $EC_{50}$ value of 3.4 µM for DENV2 and 15.5 µM for WNV for the most potent analogue (Behnam et al., 2015).

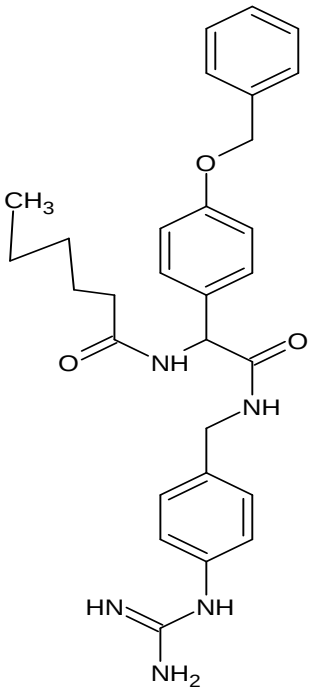


Figure 2.13 Phenylglycine derivatives

### 2.5.11 Peptide-lactam derivative

Drazic et al. (2020) also explored at producing di- and tripeptides with lactam electrophilic warheads (Figure 2.14) as DENV and West Nile virus NS2B-NS3 protease inhibitors. In biochemical assays, compound 11d containing a (3S) lactam moiety had an $IC_{50}$ value of 2.5 μM. In cellular experiments with DENV2 infected human hepatocarcinoma cells (Huh-7), the compound 11d had an $EC_{50}$ value of 4.1 μM. Dengue protease activity was generally higher than West Nile virus protease activity. The chemicals did not effect on the off-target proteins thrombin and trypsin. Tripeptide lactam inhibitors interact with the protease in two unique binding mechanisms, according to liquid chromatography-mass spectrometry investigations. Only one binding mode results in a covalent but reversible engagement of the lactam ring with the catalytic serine, followed by the release of the inhibitor with the lactam ring opened. The alternative binding mode causes the peptide backbone to be cleaved. This is the first experimental proof that benzyloxyphenylglycine, found in flaviviral protease inhibitors, is located at the enzyme's primary site (Dražić et al., 2020).

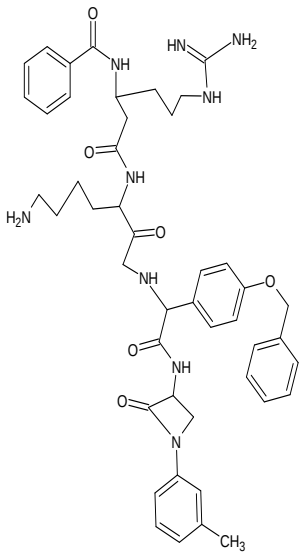


Figure 2.14 Peptide-lactam derivative

### 2.5.12 Curcumin derivatives

In a recent high-throughput screening (HTS) study, Balasubramanian et al. (2019) discovered curcumin as a DENV2 NS2B-NS3 protease inhibitor. Four curcumin analogues (curcuminoids) (Figure 2.15) were synthesized and examined for *in vitro* protease inhibition and replication inhibition using cell-based assays. Curcumin showed activity with $IC_{50}$ value range from 36.23 µM to 66.01 µM, according to the findings. However, the analogues inhibited DENV infectivity more effectively in plaque experiments, indicating that these compounds seem to be acting on host cell processes/pathways that the virus relies on to replicate and produce new virions. Curcuminoids' suppression of genes involved in lipid biosynthesis and actin polymerization is likely to be implicated in their mode of action in DENV2-infected cells, according to further research. When compared to the original curcumin, three of the curcumin derivatives have high selectivity indices (SI >10) (Balasubramanian et al., 2019).

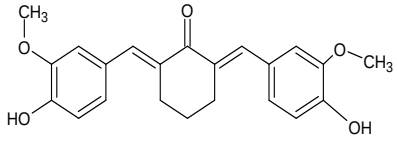


Figure 2.15 Curcumin derivatives

### 2.5.13 Pyrrolidine and imidazolidinone derivative

The DENV2 NS2B-NS3 protease and wild-type DENV2 virus were tested using a series of fused ring derivatives of pyrrolidine and imidazolidinone (Figure 2.16) that were developed, synthesized, described, and assessed. In a viral replication assay, the linear dipeptide compound 1 and the non-peptidic fused ring compound 2 had similar

effects against DENV2 NS2B-NS3 protease and wild-type DENV2 virus. Compound 2, with an $IC_{50}$ value of 1.2 ± 0.4 μM, showed comparable inhibitory activity to DENV2 NS2B-NS3 protease as compound 1, which also had an of $IC_{50}$ value of 1.2 ± 0.4 μM. A substituent and its stereochemistry at the C-3 position, substitution (X) at N-2 arene, and a linker (Y) between C-3 position and its attached arene are all important for the fused-ring scaffold of pyrrolidino [1,2-c] imidazolidinone to block the active site of NS2B-NS3 protease, according to the preliminary SAR. The identification of non-peptidic, effective NS2B-NS3 protease inhibitors to limit dengue virus infections will be aided by this potential structural core (Weng et al., 2017).

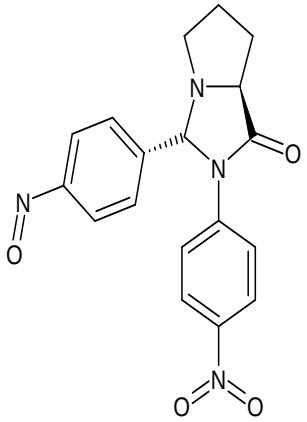


Figure 2.16 Pyrrolidine and imidazolidinone derivative

### 2.5.14 Phenylalanine and phenylglycine derivatives

The synthesis and *in vitro* characterization of strong peptidic inhibitors of dengue virus protease that contain phenylalanine and phenylglycine derivatives (Figure 2.17) as arginine-mimicking groups with regulated basicity were reported by Weigel et al., (2015). The most promising inhibitors were those containing (4-amidino)-l-phenylalanine, which had $IC_{50}$ value range from 0.44-0.89 μM for dengue virus

protease. The kind and position of the substituents on the phenylglycine and phenylalanine side chains have a big impact on the inhibitory action and selectivity against other proteases against dengue virus protease. Furthermore, the non-natural, basic amino acids reported here could be useful in the development of new peptidic and peptidomimetic medicines, such as blood clotting inhibitors (Weigel et al., 2015).

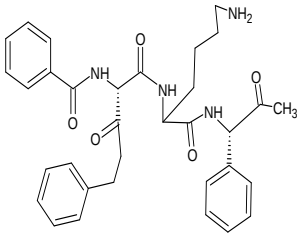


Figure 2.17 Phenylalanine and phenylglycine derivatives

### 2.5.15 Benzothiazole derivatives

Diaryl (thio)ethers (Figure 2.18) have been found as possibilities for a novel class of protease inhibitors by Wu et al., (2015). They showed that the synthesised benzothiazole derivatives demonstrated 50 percent inhibitory concentrations ($IC_{50}$) in the low micromolar range selectively and noncompetitively inhibit the serotype 2 and 3 dengue virus protease inhibitor in the enzyme inhibition study. All compounds had no effect on HCV or HIV1 replication, hence inhibition on DENV might be specific. In cellular assay, the compounds were seen inhibit to DENV protease with $IC_{50}$ in the low micromolar to submicromolar range. Furthermore, at submicromolar concentrations, these new protease inhibitors impede viral propagation (Wu et al., 2015).

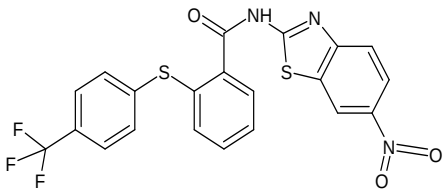


Figure 2.18 Benzothiazole derivatives

### 2.5.16 Quinoline derivative

Deng et al., 2012, reported a quinoline derivative (1) which was active against the NS2B-NS3 protease through virtual screening ($IC_{50}$ = 13.12 ± 1.03 μM). Compound (1) was further derivatized into 21 novel quinoline-containing compounds (Figure 2.19) were further developed and synthesised compound (23, 24). Protease inhibition studies revealed that 12 compounds containing the novel scaffold are NS2B-NS3 protease inhibitors. Additionally, luciferase reporter replicon-based assays identified 17 novel compounds as NS2B-NS3 protease inhibitors with $IC_{50}$ values ranging from 7.46 μM ± 1.15 to 48.59 μM ± 3.46. Among these, 8 compounds belonging to two separate scaffolds showed some activity against DENV. These unique chemical entities could be used as lead structures for developing DENV treatments.

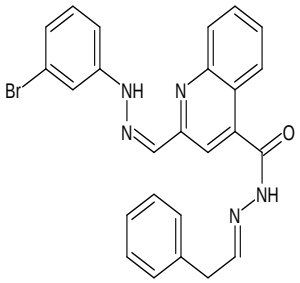


Figure 2.19 Quinoline derivative