

**DESIGN AND SYNTHESIS OF A POTENTIAL
INHIBITOR FOR DEN2 NS2B/NS3 SERINE PROTEASE**

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**FACULTY OF SCIENCE
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KUALA LUMPUR**

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ABSTRACT

This work involves searching and designing of inhibitors for DEN2 NS2B/NS3 serine protease. It comprises three phases: modeling, synthesis and screening. Homology model construction of DEN2 NS2B/NS3 serine protease was carried out using HCV NS3/NS4A as a template. The model was then evaluated using server-based structural verification from UCLA-DOE Institute for Genomics and Proteomics server (<http://nihserver.mbi.ucla.edu/SAVES/>) and PROCHECK, VERIFY3D and ERRAT. The results revealed the homology model have reasonable protein fold compared to the crystal structure of DEN2 NS3 without the cofactor of NS2B bound within. The work then continued with *in silico* protein-ligand docking experiment using AUTODOCK 3.05, where the homology model was used as the macromolecule and the ligands were the competitive inhibitor (4-hydroxypanduratin A, panduratin A and ethyl 3-(4-(hydroxymethyl)-2-methoxy-5-nitrophenoxy)propanoate). The docking results suggested several putative binding informations for each of the ligand tested, when the detail binding interactions between the enzyme and the ligands were carried out. Based on these informations, a novel ligand was designed with better *in silico* binding energy. This ligand was then synthesised in convergent approach by employing 1,4-dihydropyridine synthesis, Michael addition and Grignard reaction as the key steps. The screening was performed using the synthesised product on the DEN2 NS2B/NS3 serine protease recombinant and the result seemed to indicate the compound to exhibit uncompetitive inhibition mode.

ABSTRAK

Kajian ini melibatkan pencarian dan rekaan inhibitor untuk enzim serine DEN2 NS2B/NS3. Ianya terdiri daripada tiga fasa: pemodelan, sintesis dan biocerakinan. Pembinaan model homologi enzim serine DEN2 NS2B/NS3 dilakukan dengan menggunakan struktur kristal HCV NS3/NS4A. Kemudian, model ini dinilai dengan menggunakan algoritma pengesahan struktur dari laman web UCLA-DOE Institut Genomik dan Proteomik (<http://nihserver.mbi.ucla.edu/SAVES/>, 16 April 2005). Dalam laman web ini, program PROCHECK, VERIFY3D dan ERRAT telah digunakan. Keputusan kajian menunjukkan model homologi yang dibina mempunyai struktur protein yang logikal berbanding dengan struktur kristal NS3 DEN2 tanpa kofaktor NS2B. Kajian ini dilanjutkan dengan *in silico protein-ligand docking* dengan menggunakan perisian AUTODOCK 3.05, di mana model homologi digunakan sebagai makromolekul dan ligan yang digunakan adalah inhibitor kompetitif (4-hidroksipanduratin A, panduratin A dan etil-3-(4-(hidroksimetil)-2-metoksi-5-nitrofinoksi)propanoat. Keputusan doking bagi kesemua ligan yang diuji memberi maklumat tentang mod interaksi antara enzim dan ligan. Berdasarkan maklumat tersebut, satu ligan baru direkapipta yang dijangka akan mempunyai keaktifan biologi yang lebih kuat akibat dari sudut tenaga pengikatannya yang lebih baik. Ligan ini kemudian disintesis cara konvergen dengan menggunakan langkah-langkah sintesis 1,4-dihidropiridin, penambahan Michael dan tindakbalas Grignard sebagai langkah utama. Biocerakinan dilakukan ke atas enzim serin DEN2 NS2B/NS3 dengan menggunakan ligan yang telah disintesis tersebut. Keputusan menunjukkan ligan ini tidak mempamerkan mekanisme inhibisi kompetitif seperti yang dijangkakan.

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CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iii
ACKNOWLEDGEMENTS	iv
CONTENTS	v
LIST OF FIGURES	x
LIST OF SCHEMES	xiii
LIST OF TABLES	xv
ABBREVIATIONS	xvi
APPENDICES	
CHAPTER 1 INTRODUCTION	1-17
1.1 Dengue Fever (DF) and Dengue Haemorrhagic Fever (DHF)	1
1.1.1 Symptoms and prevalence	1
1.1.2 Diagnosis and treatment	2
1.2 Dengue Virus, the Genome and Lifecycles	3
1.2.1 Transmission	3
1.2.2 Polyprotein processing	4
1.3 Serine Proteases	8
1.3.1 Dengue Virus NS2B/NS3 Serine Protease	8
1.3.2 Mechanism of action	12
1.4 Approaches towards Dengue Virus Inhibition	13
1.4.1 Attenuated vaccine	13
1.4.2 Therapeutic agents: virus inhibitor	14

1.4.3	Dengue Virus NS2B/NS3 Serine Protease inhibitor	14
1.5	Aims and Objectives	17
CHAPTER 2 HOMOLOGY, DOCKING AND NEW LIGAND DESIGN		18-60
OF DEN2 NS2B/NS3 SERINE PROTEASE INHIBITION		
2.1	Molecular Modelling in Drug Design	18
2.2	Homology Modelling	20
2.2.1	Target-template selection	21
2.2.2	Target-template alignment	22
2.2.3	Model construction	23
2.2.4	Model evaluations	23
2.3	Molecular Docking	25
2.3.1	Introduction	25
2.3.2	AUTODOCK	29
2.3.3	Searching methods for AUTODOCK	30
2.3.4	Scoring function of AUTODOCK	32
2.3.5	Programs in AUTODOCK	33
2.4	Materials and Methods	35
2.4.1	Homology model of DEN2 NS2B/3 Serine Protease	35
2.4.2	Comparison of the homology model with crystal structures of and DEN2 NS3 and HCV NS3/4A	36
2.4.3	Docking experiment using homology model	36
2.4.4	Design of the new ligand from the docked bioactive molecules	39
2.5	Homology Model of DEN2 NS2B/NS3 Serine Protease	40
2.5.1	Results	40
2.5.1.1	Homology model building and model evaluation	40

2.5.2	Discussions	43
2.5.2.1	Comparison of the homology model with crystal structures of and DEN2 NS3 and HCV NS3/4A	43
2.6	Molecular Docking Studies	48
2.6.1	Results	48
2.6.1.1	Inhibition of bioactive compounds towards DEN2 NS2B/NS3	48
2.6.1.2	Active site docking	49
2.6.2	Discussions	51
2.6.2.1	Interactions between inhibitors and residues in DEN2 NS2B/NS3	51
2.6.2.2	New ligand design strategy	57
2.6.2.3	Virtual screening of newly designed ligand	59
CHAPTER 3 SYNTHESIS OF THE DESIGNED MOLECULE		61-97
3.1	Retrosynthetic Analysis	61
3.2	Chemistry of Pyridinyl, Dihydropyridinyl and Piperidinyl Ring Synthesis: Synthesis of Dihydropyridines	63
3.3	1, 4-Michael Addition	65
3.4	Weinreb Amide	67
3.5	Materials and Methods	69
3.5.1	Materials and instruments used	69
3.5.2	Synthesis of Ethyl Nicotinate	72
3.5.3	Synthesis of diethyl 4-phenylpyridine-1,3(4H)-dicarboxylate	73
3.5.4	Synthesis of ethyl (1-phenoxy carbonyl-4-phenylpyridinyl)- 3(4H)-carboxylate	74

3.5.5	Synthesis of ethyl (1-tert-butoxycarbonyl-4-phenylpyridinyl)- 3(4H)-carboxylate	75
3.5.6	Synthesis of ethyl (1-tert-butoxycarbonyl-2-butyl-4-phenyl)- 3,4-dihydropyridinyl-3-carboxylate	76
3.5.7	Synthesis of ethyl (1-tert-butoxycarbonyl-2-butyl-4-phenyl)- piperidinyl-3-carboxylate	77
3.5.8	Synthesis of (1-tert-butoxycarbonyl-2-butyl-4-phenyl)- piperidinyl-3-carboxylic acid	78
3.5.9	Synthesis of 1-tert-butoxycarbonyl-2-butyl-3- (methoxy(methyl)carbamoyl)-4-phenylpiperidine	79
3.5.10	Synthesis of 1-tert-butoxycarbonyl-2-butyl-3-(benzo-1,3- dioxol-4-carbonyl)-4-phenylpiperidine	80
3.5.11	Synthesis of 1-tert-butoxycarbonyl-2-butyl-3-hydroxymethyl-4- phenylpiperidine	81
3.5.12	Synthesis of 1-tert-butoxycarbonyl-2-butyl-3-formyl-4- phenylpiperidine	82
3.5.13	Synthesis of 1-tert-butoxycarbonyl-3-(benzo-1,3-dioxol-4- carbonyl)-2-butyl-4-phenylpiperidine	83
3.5.14	Synthesis of (2-butyl-4-phenylpiperidin-3-yl)(2,3- dihydroxyphenyl)methanone	84
3.6	Results and Discussions	86
3.6.1	Synthesis setup for the designed ligand: (2-butyl-4- phenylpiperidin-3-yl)(2,3-dihydroxyphenyl)methanone	86
3.6.2	Stereochemical Control of the Proposed Synthesis	96

CHAPTER 4 INHIBITION STUDY OF THE DESIGNED AND	98-118
 SYNTHESISED COMPOUND AGAINST DEN2	
 NS2B/NS3 SERINE PROTEASE	
4.1 Introduction	98
4.2 Cell Cytopathic Effect	99
4.3 Analysis of Enzyme Kinetics Data	100
4.4 Materials and Methods	105
4.4.1 Materials	105
4.4.2 Instrument used for Analysis and Bioassay	105
4.4.3 Expression and Purification of DEN2 NS2B/NS3 serine protease complex	105
4.4.4 DEN2 NS2B/NS3 Inhibition assay using fluorogenic peptides	108
4.4.5 Determination of K_i for the synthesised compound	109
4.5 Results	110
4.5.1 Cytopathic effect study of the compound 14	110
4.5.2 <i>In vitro</i> kinetic assay of CP14	112
4.5.3 Lineweaver-Burk plot of the inhibition assays	114
4.5.4 Effect of CP14 against DEN2 Viral Replication	115
4.6 Discussions	116
CHAPTER 5 GENERAL DISCUSSIONS	119-122
CHAPTER 6 CONCLUSIONS	123
REFERENCES	124-134

LIST OF FIGURES

Figures		Page
1.1	World distribution of Dengue in year 2008	2
1.2	Structural and non-structural polyprotein assembly of DEN2 virus	5
1.3	Proteolytic process at the catalytic triad of serine proteas	13
1.4	Small peptide substrate	15
1.5	Structures of the compounds with terminal guanidiny group that have potential inhibition activity against DEN2 NS2B-NS3 serine protease	16
2.1	Structure of Taxol®	18
2.2	The protocol of Lamarckian Genetic Algorithm (LGA) search method	32
2.3	Work flow of homology model construction for 3D structure of DEN2 NS2B/NS3 serine protease	36
2.4	Workflow of performing docking experiment using AUTODOCK 3.05	38
2.5	Ramachandran plot of built homology model of DEN2 NS2B/NS3 complex	41
2.6	VERIFY 3D plot of DEN2 NS2B/NS3 homology model	42
2.7	ERRAT analysis of DEN2 NS2B/NS3 homology model	43
2.8	Structures of flavivirus serine proteases	46
2.9	Spatial arrangement of catalytic triad	47
2.10	Structure of the selected competitive inhibitors	49
2.11	Connolly surface representations of the active site of DEN2 NS2B/NS3 protease with the bound ligands	50

2.12	Hydrogen bond analysis of the docked ligands	52
2.13	Van der Waals interactions and hydrophobic interactions between the docked ligands (1, 2 and 3) and the DEN2 NS2B/NS3 serine protease protein model	54
2.14	Molecular orientation of the docked ligand at the catalytic triad of DEN2 NS2B/NS3	56
2.15	Superimposition of the best docked conformer of the three competitive inhibitor	58
2.16	Superimposition of the best docked conformer of the three competitive inhibitor ligands	59
2.17	Binding interactions illustration between the newly designed ligand and the homology model of DEN2 NS2B/NS3 serine protease	60
4.1	Lineweaver-Burk plot of $1/v$ versus $1/[S]$ to evaluate K_m and V_{max}	102
4.2	Lineweaver-Burk plot of different inhibitor	104
4.3	Workflow of harvesting and purification of DEN2 NS2B/NS3 serine protease complex	107
4.4	HepG2 cell morphology	110
4.5	Percent inhibition of CP14 on various DEN2 virus titre in HepG2 cells	111
4.6	Plot of intensity versus concentration of fluorogenic moiety of the peptide substrate, AMC	112
4.7	Curves with different concentration of CP14, $[I]$, of enzyme velocity versus substrate concentrations	113
4.8	Lineweaver-Burk plot of CP14 with the different concentration of inhibitor	114

4.9	RT-PCR of DENV2 serine protease from HepG2 cell in the presence of CP14	115
4.10	Plausible bindings suggested by AUTODOCK3.05	118

LIST OF SCHEMES

Scheme		Page
3.1	Retrosynthesis analysis of the targeted compound	62
3.2	Hantzsch dihydropyridine synthesis	63
3.3	Hilgeroth's synthesis of dihydropyridine as the precursor of cubanes	64
3.4	Synthesis of inhibitors of 2,3-oxidosqualene-lanosterol cyclase using 1,4-Michael addition as a key step	66
3.5	Proposed reaction using 1,4-Michael addition as the key step to incorporate the butyl moiety to the dihydropyridine	67
3.6	General example of ketone synthesis from Weinreb amide using Grignard reagent	67
3.7	Structure of the target compound	86
3.8	Esterification of nicotinic acid	86
3.9	1,4-nucleophilic addition of the phenyl moiety to ethyl nicotinate activated by ethyl chloroformate	87
3.10	Proposed reaction mechanism related to the deprotection of the dihydropyridine 4 and the rearomatisation	88
3.11	1,4-nucleophilic addition of the phenyl moiety to ethyl nicotinate activated by phenyl chloroformate	89
3.12	Functional group interconversion from phenyl carbamate to t-butyl carbamate followed by 1,4-Michael addition of butyl moiety insertion	89
3.13	Reduction of 6 using 10% palladium on activated carbon	90
3.14	Partial synthesis of designed ligand, with 3 moieties attached	91
3.15	Functional group interconversions from ethyl ester to Weinreb	92

amide

3.16	Reaction of Weinreb amide to make ketone by Grignard reagent	92
3.17	Revised route from Weinreb amide 9 to furnish the targeted product	93
3.18	Revised route to synthesise aldehyde 12 from ester 7	94
3.19	Different routes to synthesise target molecule 14	95
3.20	NOE on the compound 6	97

LIST OF TABLES

Table		Page
2.1	Structural verification (PROCHECK, VERIFY3D, ERRAT) and comparison between structure of HCV NS3/NS4A crystal, homology model of DEN2 NS2B/NS3 and DEN2 NS3 crystal	47
2.2	K_i values of the found competitive inhibitors	49
2.3	Energies (in kcal/mol) calculated using AUTODOCK 3.05	51
2.4	Residues in the active site of DEN2 NS2B/NS3 that are involved in hydrogen bonding with the various ligands	53
2.5	Residues in the active site of DEN2 NS2B/NS3 that are involved in Van der Waals interaction	55
3.1	List of molecules that were used and synthesised in this work	69
3.2	Percent yield of the targeted product from 3 different route of synthesis	96
4.1	Comparison of the binding site, V_{max} and K_m among different type of inhibitor	103

ABBREVIATIONS

%	Percent
φ	Psi
ϕ	Phi
π	Pi
[E]	Enzyme Concentration
[ES]	Enzyme-Substrate Concentration
[I]	Inhibitor Concentration
[P]	Product Concentration
[S]	Substrate Concentration
μg	Microgram
μl	Microlitre
^{13}C	Carbon 13
1d	One Dimentional
^1H	Proton
3d	Three Dimentional
Å	Angstrom
Ala	Alanine
AMBER	Assisted Model Building with Energy Refinement
AMC	Aminomethylcoumrin
Arg	Arginine
Asn	Asparagine
Asp	Aspartic Acid
BCl_3	Boron Trichloride

BHK	Baby Hamster Kidney Fibroblast cells
BOC	Butoxycarbonyl
bp	Base Pair
brd	Broad Doublet
brs	Broad Singlet
C	Capsid
C6/36	Larval Tissue
CaH ₂	Calcium Hydride
CH ₂ Cl ₂	Dichloromethane
CHARMM	Chemistry at Harvard Molecular Mechanics
cm ³	Cubic Centimetre
CuCN	Copper(I) Cyanide
CuI	Copper(I) Iodide
d	Doublet
dd	Doublet of Doublet
dddd	Doublet of Doublet of Doublet of Doublet
DEN2	Dengue Virus Type 2
DENV	Dengue Virus
DF	Dengue Fever
DHF	Dengue Haemorrhagic Fever
DMAP	1,4-Dimethylaminopyridine
DME	Dimethyl Ether
DMP	Dess-Martin Periodinane
DSS	Dengue Shock Syndrome
dt	Doublet of Triplet
E	Envelope

eg	Examples
EI	Electron Impact
eqv.	Equivalent
ER	Endoplasmic Reticulum
<i>et al.</i>	And Others
Et ₂ O	Diethyl Ether
EtOAc	Ethyl Acetate
EtOCOCl	Ethyl Chloroformate
EtOH	Ethanol
EtOH	Ethanol
g	Gram
GA	Genetic Algorithm
GI	Gastrointestinal
Gln	Glutamine
Glu	Glutamic Acid
Gly	Glycine
H ₂	Hydrogen
H ₂ O	Water
H ₂ SO ₄	Sulphuric Acid
HCl	Hydrochloric Acid
HCV	Hepatitis C Virus
HeLa	Henrietta Lacks
HepG2	Liver Hepatocellular Cells
hex	Hexane
His	Histidine

HIV	Human Immunodeficiency Virus
HIV-1	Human Immunodeficiency Virus Type 1
HOAc	Acetic Acid
HRMS	High Resolution Mass Spectrometry
id	Identity
Ile	Isoleucine
Inc.	Incorporated
<i>i</i> -PrMgCl	Isopropyl Magnesium Chloride
IUPAC	International Union of Pure and Applied Chemistry
kcal	kilo Calorie
kD	kilo Dalton
K_i	Inhibition Constant
K_m	Michaelis-Menten Constant
LGA	Larmackian Genetic Algorithm
LiAlH_4	Lithium Aluminium Hydride
LRMS	Low Resolution Mass Spectrometry
LS	Local Search
Lys	Lysine
M	Molar
m	Multiplet
m/z	Mass-to-charge Ratio
MCA	4-methyl-coumaryl-7-amides
MeOH	Methanol
MeOH	Methanol
Mg	Magnesium
MgSO_4	Magnesium Sulphate

min	Minute
ml	Millilitre
mm	Millimetre
mm ³	Cubic millilitre
mM	Millimolar
mmol	Millmole
MNTD	Minimum Non-Toxic Dose
mol	Mole
Na ₂ CO ₃	Sodium Carbonate
Na ₂ S ₂ O ₃	Sodium Thiosulphate
Na ₂ SO ₄	Sodium Sulphate
NaOH	Sodium Hydroxide
n-BuLi	n-Buthyllithium
NH ₃	Ammonia
NH ₃	Ammonia
NH ₄ Cl	Ammonium Chloride
NHMe(OMe).HCl	N,O-dimethylhydroxylamine hydrochloride
Ni ²⁺	Nickel (II) ion
nm	Nanometre
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
NTA	Nitrilotriacetic Acid
NTPase	Nucleoside Triphosphatase
°C	Celsius
OD	Optical Density

OPLS	Optimized Potentials for Liquid Simulations
ORF	Open Reading Frame
PAGE	Polyacrylamide Gel Electrophoresis
Pd	Palladium
Pd/C	Palladium on Activated Carbon
pdb	Protein Data Bank
Phe	Phenylalanine
PhMgCl	Phenyl Magnesium Chloride
PhOCOCl	Phenyl Chloroformate
Pro	Proline
PyBrOP	Bromo- <i>tris</i> -pyrrolidino-phosphonium hexafluorophosphate
q	Quartet
ref.	Reference
RMSD	Root Mean Square Deviation
RNA	Ribonucleic Acid
rpm	Rotation Per Minute
rt	Room Temperature
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
s	Singlet
SA	Simulated Annealing
SAR	Structure-Activity Relationship
SBDD	Structural-Based Drug Design
SDS	Sodium Dodecyl Sulfate
Ser	Serine
t	Triplet
TBE	Tick-Borne Encephalitis

t-BOC	<i>tert</i> -Butoxycarbonyl
t-BuOK	Potassium <i>tert</i> -Butoxide
TCID	Tissue Culture Infective Dose
TCID	Tissue Culture Infective Dose
THF	Tetrahydrofuran
Thr	Threonine
TLC	Thin Layer Chromatography
TMEDA	Tetramethylethylenediamine
Tris	Tris(hydroxymethyl)aminomethane
Tyr	Tyrosine
UCLA	University of California, Los Angeles
US	United States
UV	Ultraviolet
v	Enzyme Velocity
Val	Valine
V _{max}	Maximum Enzyme Velocity
WHO	World Health Organisation
ZnSO ₄	Zinc(II) Sulphate
α	Alpha
β	Beta
γ	Gamma
μm	Micrometer
μM	Micromolar