

**DESIGN AND CHARACTERIZATION OF
THERAPEUTIC PEPTIDES TARGETING THE
RECEPTOR BINDING DOMAIN OF THE SPIKE
GLYCOPROTEIN FROM SARS-COV-2**



HO CHIAN

UMMS
UNIVERSITI MALAYSIA SABAH

**FACULTY OF SCIENCE AND NATURAL RESOURCES
UNIVERSITI MALAYSIA SABAH
2023**

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THERAPEUTIC PEPTIDES TARGETING THE
RECEPTOR BINDING DOMAIN OF THE SPIKE
GLYCOPROTEIN FROM SARS-COV-2**

HO CHIAN



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**THESIS SUBMITTED IN FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE**

**FACULTY OF SCIENCE AND NATURAL RESOURCES
UNIVERSITI MALAYSIA SABAH
2023**

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/

TIDAK TERHAD


Disahkan Oleh,

Ho Chian

HO CHIAN
MS2011004T

 ANITA BINTI ARSAD
PUSTAKAWAN KANAN
UNIVERSITI MALAYSIA SABAH

(Tandatangan Pustakawan)



(Prof. Dr. Lee Ping Chin)
Penyelia

Tarikh : 16 August 2023

DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, equations, summaries, and references, which have been duly acknowledged.

16 May 2023

Ho Chian

Ho Chian
MS2011004T



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CERTIFICATION

NAME : **HO CHIAN**
MATRIC NO. : **MS2011004T**
TITLE : **DESIGN AND CHARACTERIZATION OF THERAPEUTIC PEPTIDES TARGETING THE RECEPTOR BINDING DOMAIN OF THE SPIKE GLYCOPROTEIN FROM SARS-COV-2**
DEGREE : **MASTER OF SCIENCE**
FIELD : **BIOTECHNOLOGY**
VIVA DATE : **16 MAY 2023**



SUPERVISOR
Prof. Dr. Lee Ping Chin

CERTIFIED BY:

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Signature

A handwritten signature in black ink, appearing to read 'Lee Ping Chin', written over a horizontal line.

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16 May 2023

ABSTRACT

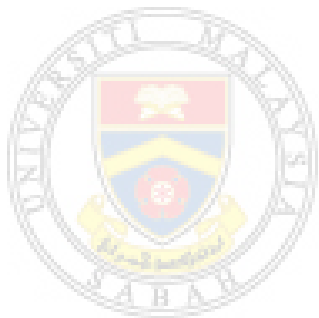
Coronavirus Disease 2019 (COVID-19), a global pandemic which first emerged in Wuhan City, Hubei Province, China, in December 2019, was caused by a novel *Betacoronavirus*, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). To date, there are only two Food and Drug Administration (FDA)-approved therapeutic drugs to counter COVID-19. Hence, development for COVID-19 treatment needs to be broadened. This study aims to explore an alternative treatment for COVID-19 using therapeutic peptides. This study hypothesised that therapeutic peptides that can bind with the receptor binding domain (RBD) of spike glycoprotein (S protein) of SARS-CoV-2 might inhibit the entry of the virus into host cells and prevent infection. Bioinformatics tools include PyMOL, UCSF chimera, PEP-FOLD3, HADDOCK (High Ambiguity Driven protein-protein DOCKing), and molecular dynamics simulation (MDS) are first utilised for peptide design. PyMOL and UCSF chimera were used to determine the amino acid residues involved in the binding interface. A total of 291 peptides were designed, and PEP-FOLD3 was used to generate the peptide models. Docking analysis on the peptides and RBD of S protein was performed via HADDOCK. The peptide-RBD complexes of peptides 65, 66, and 189 showed the highest HADDOCK score and hence were chosen for 200 ns of MDS analysis. MDS results showed that all three peptide-RBD complexes were compact and stable, and their interaction energy being -480.38 ± 118.61 kJ/mol, -410.92 ± 126.31 kJ/mol, and -338.49 ± 97.88 kJ/mol, respectively. The peptides were then subjected to a binding affinity test and competitive assay via ELISA (enzyme-linked immunosorbent assay). For the binding affinity test, ELISA was performed using the hemagglutinin (HA)-tagged peptides in a series of concentrations bound to RBD of S protein on a 96-well plate. A competitive assay was performed using His-tagged ACE2 protein incubated with the peptides. Peptide 65 showed the best result with bioinformatics analysis and binding affinity test with a K_D of 43.26 μ M. However, the results of the competitive assay showed that binding of peptide 65 to S protein is weak compared to the ACE2 protein. Bioinformatics tools can be utilised for preliminary analysis of the peptides designed and enables choosing of peptides with the best results for further laboratory testing. Peptide 65 can be further modified to improve its inhibiting ability and further develop into therapeutic drugs to counter COVID-19.

ABSTRAK

REKAAN DAN PENCIRIAN PEPTIDA TERAPEUTIK YANG MENSASARKAN DOMAIN PENGIKAT RESEPTOR BAGI GLIKOPROTEIN SPIKE DARIPADA SARS-CoV-2

Penyakit koronavirus 2019 (COVID-19), pandemik global yang pertama kali muncul di bandar Wuhan, Provinsi Hubei, China pada Disember 2019, disebabkan oleh sejenis Betacoronavirus baru, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Sehingga kini, hanya terdapat dua ubat terapeutik yang diluluskan oleh Food and Drug Administration (FDA) untuk melawan COVID-19. Oleh itu, pembangunan untuk rawatan COVID-19 perlu diperluaskan. Kajian ini bertujuan untuk meneroka rawatan alternatif untuk COVID-19 dengan penggunaan peptida terapeutik. Adalah dihipotesiskan bahawa peptida terapeutik yang boleh mengikat dengan domain pengikat reseptor (RBD) spike glikoprotein (protein S) SARS-CoV-2 mungkin dapat menghalang kemasukan virus ke dalam sel perumah dan mencegah jangkitan. Alat bioinformatik seperti PyMOL, UCSF chimera, PEP-FOLD3, HADDOCK (High Ambiguity Driven protein-protein DOCKing), dan simulasi dinamik molekul digunakan terlebih dahulu untuk merekakan peptide-peptida. PyMOL dan UCSF chimera digunakan untuk menentukan residu asid amino yang terlibat antara muka pengikat. Sebanyak 291 peptida telah direka dan PEP-FOLD3 digunakan untuk menjana model peptida. Analisis dok pada peptida dan RBD protein S dilakukan melalui HADDOCK. Kompleks peptida-RBD bagi peptida 65, 66, dan 189 menunjukkan skor HADDOCK tertinggi dan telah dipilih untuk 200 ns analisis MDS. Keputusan MDS menunjukkan bahawa ketiga-tiga kompleks peptida-RBD adalah padat dan stabil, dan tenaga interaksi masing-masing ialah -480.38 ± 118.61 kJ/mol, -410.92 ± 126.31 kJ/mol, dan -338.49 ± 97.88 kJ/mol. Peptida-peptida ini kemudiannya ditujukan kepada ujian afiniti mengikat dan ujian kompetitif melalui ELISA (ujian imunosorben berpaut enzim). Untuk ujian afiniti mengikat, peptida tag dengan hemagglutinin (HA) dalam satu siri kepekatan diikat pada RBD protein S pada plat 96-telaga. Untuk ujian kompetitif, kehadiran protein ACE2 dengan tag His dieram dengan peptide dalam satu siri kepekatan. Peptida 65 menunjukkan keputusan terbaik dengan analisis bioinformatik serta ujian afiniti mengikat dengan K_D 43.26 μ M. Walau bagaimanapun, keputusan ujian kompetitif menunjukkan bahawa pengikatan peptida 65 dengan protein S adalah lemah berbanding dengan protein

ACE2. Alat bioinformatik boleh digunakan untuk analisis awal peptida yang direka dan membolehkan pemilihan peptida dengan hasil terbaik untuk ujian makmal selanjutnya. Peptide 65 boleh diubah suai lagi untuk meningkatkan keupayaan perencatannya supaya ia dapat dihasilkan dengan lebih lanjut lagi untuk menjadi ubat terapeutik untuk melawan COVID-19.



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LIST OF CONTENTS

	Page
TITLE	i
DECLARATION	ii
CERTIFICATION	iii
ACKNOWLEDGEMENT	iv
ABSTRACT	v
<i>ABSTRAK</i>	vi
LIST OF CONTENTS	viii
LIST OF TABLES	xi
LIST OF FIGURES	Xiii
LIST OF ABBREVIATIONS	xvii
LIST OF APPENDICES	xxi
CHAPTER 1: INTRODUCTION	
1.1 Background of The Research	1
1.2 Problem Statement	3
1.4 Research Questions	4
1.5 Objectives of The Study	4
1.6 Hypotheses	4
CHAPTER 2: LITERATURE REVIEW	
2.1 Coronavirus	6
2.1.1 Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	6
2.1.2 Viral Entry into Host Cell, Viral Genome Replication and Translation	12
2.2 Clinical Manifestation of COVID-19 and Impact on Health	15
2.3 Current Treatment for COVID-19 and Ongoing Trials for COVID-19 Therapeutic Drugs	17
2.3.1 Ongoing Trials for COVID-19 Therapeutic Drugs by ACTIV	23
2.4 Peptide Drugs as A Potential Area for Drug Development	27

2.4.1	Computational-based studies on peptides targeting RBD of S Protein	27
2.4.2	Laboratory-based studies on peptides targeting RBD of S Protein	33
2.5	Bioinformatics Tools for Peptide Design and Analysis	39
2.5.1	PyMOL and UCSF Chimera	39
2.5.2	PEP-FOLD3	40
2.5.3	HADDOCK	42
2.5.4	Molecular Dynamics Simulation	43
2.6	Enzyme Linked Immunosorbent Assay (ELISA)	44

CHAPTER 3: METHODOLOGY

3.1	Data Mining and Structural Analysis	47
3.2	Peptides Design, Molecular Docking, Physicochemical and ADMET Analysis	48
3.3	Molecular Dynamics Simulation	53
3.4	ELISA	54

CHAPTER 4: RESULTS

4.1	Structural Analysis by PyMOL and UCSF Chimera	63
4.2	Peptides Design, Molecular Docking, Physicochemical and ADMET Analysis	68
4.2.1	Results for Peptides Design and Molecular Docking	68
4.2.2	Physicochemical and ADMET Analysis	87
4.3	Molecular Dynamics Simulation	90
4.4	ELISA for Binding Affinity (K_D) and Inhibition (IC_{50}) Test	102

CHAPTER 5: DISCUSSION

5.1	Structural Analysis by PyMOL and UCSF Chimera	109
5.2	Peptides Design and Molecular Docking	111
5.2.1	Peptides Design	111
5.2.2	RBD and Peptides Refinement Before Molecular Docking	112
5.2.3	Peptides Selection by Peptide Conformation and Molecular Docking	113

5.2.4	Physicochemical and ADMET Analysis	114
5.3	PCA and Molecular Dynamics Simulation	121
5.4	The Binding Constants and The Inhibition Properties of The Peptides	124
5.4.1	The Binding Constant (K_D) of The Peptides	124
5.4.2	The Inhibiting Properties of The Peptides	127
5.5	Further Improvement Suggested for Peptides Targeting Spike Glycoprotein of SARS-CoV-2	129
5.6	Limitation of The Study and Future Research for Peptide 65 on SARS-CoV-2 Variants	133

CHAPTER 6: CONCLUSION

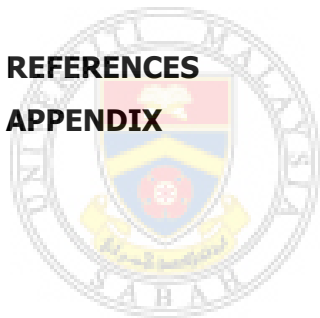
6.1	Conclusion	134
6.2	Future Research	135

REFERENCES

136

APPENDIX

158



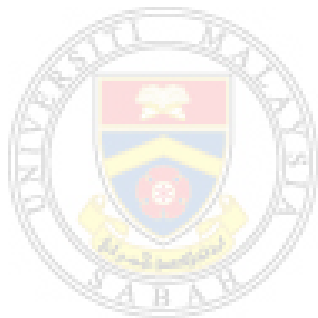
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LIST OF TABLES

	Page
Table 2.1 : Mutations Recorded in The S Protein of SARS-CoV-2 Variants and Their Association with the Binding Affinity	10
Table 2.2 : FDA Approved and FDA EUA Approved Drugs	21
Table 2.3 : Therapeutic Drugs Currently under ACTIV Partnership	25
Table 2.4 : Peptides or Mini Proteins (<i>In Silico</i>) Targeting RBD of SARS-CoV-2 S Protein	31
Table 2.5 : Peptides or Mini Proteins (Laboratory-Based) Targeting RBD of SARS-CoV-2 S Protein	36
Table 3.1 : Plate Preparation for The Direct Peptide-RBD Interaction Assay	58
Table 3.2 : Plate Preparation for The Competitive Peptide-RBD Interaction Assay	61
Table 4.1 : Key Residues Involved in The Binding Interface of RBD and ACE2	63
Table 4.2 : The 18 Peptides with HADDOCK Score $> -125.0 \pm 0.0$ and Reason being Excluded or Chosen for MDS	73
Table 4.3 : The Designed peptides, HADDOCK Results, and ToxinPred Results for Peptides 65, 66, and 189	77
Table 4.4 : Key Residues Involved in The Binding Interface of Peptide 65-RBD Complex	78
Table 4.5 : Key Residues Involved in The Binding Interface of Peptide 66- RBD Complex	81
Table 4.6 : Key Residues Involved in The Binding Interface of Peptide 189-RBD Complex	84
Table 4.7 : The Results of Physicochemical Analysis for The Peptides	89
Table 4.8 : The Results of ADMET Analysis for The Peptides	90
Table 4.9 : Results for PCA and Cosine Content Analysis	94
Table 4.10 : The MDS Results for The Peptide-RBD Complexes	102
Table 4.11 : The Direct Peptide-RBD Interaction Results for Peptide 65	103
Table 4.12 : The Direct Peptide-RBD Interaction Results for Peptide 66	105
Table 4.13 : The Direct Peptide-RBD Interaction Results for Peptide 189	105

Table 4.14 : The Competitive peptide-RBD Interaction Results for 107 Peptide 65

Table 4.15 : The *In Silico* and *In Vitro* Results for Peptides 65, 66, and 108 189



UMS
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LIST OF FIGURES

	Page
Figure 2.1 : The Phylogenetic Relationships of SARS-CoV-2 Variants	11
Figure 2.2 : The Structural of S Protein. A. Linear Diagram of S Protein. SS (Signal Sequence), NTD (N-terminal Domain), RBD (Receptor Binding Domain), SD1/SD2 (Subdomain 1/Subdomain 2), FP (Fusion Peptide), HR1 (Heptad Repeat 1), CH (Central Helix), HR2 (Heptad Repeat 2), TM (Transmembrane Domain), and CT (Cytoplasm Terminal). B. The Trimeric S protein. C. The S Protein Monomer	12
Figure 2.3 : The RBD-ACE2 Receptor Complex and Interacting Residues. Green: RBD of S protein, Purple: ACE2 receptor	13
Figure 3.1 : The PEP-FOLD3 Webserver for Peptide Model Generation	49
Figure 3.2 : Flow Chart Detailing The Design and Selection of The Peptides	52
Figure 3.3 : An Overview on The Principle of The SARS-CoV-2 Spike-ACE2 Interaction Inhibitor Screening Assay Kit	55
Figure 3.4 : Workflow for The Direct Peptide-RBD Interaction Assay	59
Figure 3.5 : Workflow for The Competitive Peptide-RBD Interaction Assay	62
Figure 4.1 : The RBD-ACE2 Complex. Green: ACE2, Yellow: ACE2 Active Residues, Red: RBD Active Residues, Cyan: RBD of S Protein	65
Figure 4.2 : Binding Residues of RBD-ACE2 Complex. Yellow: ACE2 Active Residues, Red: RBD Active Residues	66
Figure 4.3 : Active Residues at Binding Interface of The RBD-ACE2 Complex. Green: ACE2, Yellow: ACE2 Active Residues, Red: RBD Active Residues, Cyan: RBD of S Protein	66
Figure 4.4 : The 2D Interaction Map of RBD-ACE2 Complex Generated by LigPlot. Black Horizontal Dashed Line: Interface of S Protein and ACE2 Receptor. Green and Blue: Residues Forming Hydrogen Bonds, Black: Residues Forming Hydrophobic Contacts. Green Lines: Hydrogen Bonds, Red	67

Lines: Hydrophobic Contacts

- Figure 4.5 : PROCHECK Analysis for Peptide 65. (a): PROCHECK Summary. (b): Ramachandran Plot Showing Residues in The Most Favoured Region (Red); Additional Allowed Regions (Yellow); Generously Allowed Regions (Light Yellow); and Disallowed Regions (White). 69
- Figure 4.6 : PROCHECK Analysis for Peptide 66. (a): PROCHECK Summary. (b): Ramachandran Plot Showing Residues in The Most Favoured Region (Red); Additional Allowed Regions (Yellow); Generously Allowed Regions (Light Yellow); and Disallowed Regions (White). 70
- Figure 4.7 : PROCHECK Analysis for Peptide 189. (a): PROCHECK Summary. (b): Ramachandran Plot Showing Residues in The Most Favoured Region (Red); Additional Allowed Regions (Yellow); Generously Allowed Regions (Light Yellow); and Disallowed Regions (White). 71
- Figure 4.8 : The Models of The 18 Peptides 75
- Figure 4.9 : The Peptide 65-RBD Complex. Blue: Peptide 65, Green: RBD of S Protein 79
- Figure 4.10 : The Binding Residues at The Binding Interface of Peptide 65-RBD Complex. Blue: Peptide 65, Green: RBD of S Protein 79
- Figure 4.11 : The 2D Interaction Map of Peptide 65-RBD Complex Generated by LigPlot. Black Horizontal Dashed Line: Interface of RBD and Peptide 65. Green and Blue: Residues Forming Hydrogen Bonds, Black: Residues Forming Hydrophobic Contacts. Green Dashed Lines: Hydrogen Bonds, Red Dashed Lines: Hydrophobic Contacts 80
- Figure 4.12 : The Peptide 66-RBD Complex. Blue: Peptide 66, Green: RBD of S Protein 82
- Figure 4.13 : The Binding Residues at The Binding Interface of Peptide 66-RBD Complex. Blue: Peptide 66, Green: RBD of S Protein 82
- Figure 4.14 : The 2D Interaction Map of Peptide 66-RBD Complex 83

	Generated by LigPlot. Black Horizontal Dashed Line: Interface of RBD and Peptide 66. Green and Blue: Residues Forming Hydrogen Bonds, Black: Residues Forming Hydrophobic Contacts. Green Dashed Lines: Hydrogen Bonds, Red Dashed Lines: Hydrophobic Contacts	
Figure 4.15	: The Peptide 189-RBD Complex. Blue: Peptide 189, Green: RBD of S Protein	85
Figure 4.16	: The Binding Residues at The Binding Interface of Peptide 189-RBD Complex. Blue: Peptide 189, Green: RBD of S Protein	85
Figure 4.17	: The 2D Interaction Map of Peptide 189-RBD Complex Generated by LigPlot. Black Horizontal Dashed Line: Interface of RBD and Peptide 189. Green and Blue: Residues Forming Hydrogen Bonds, Black: Residues Forming Hydrophobic Contacts. Green Dashed Lines: Hydrogen Bonds, Red Dashed Lines: Hydrophobic Contacts	86
Figure 4.18	: Graph of Eigenvectors Versus Eigenvalues for Peptide 65-RBD Complex	92
Figure 4.19	: Graph of Eigenvectors Versus Eigenvalues for Peptide 66-RBD Complex	93
Figure 4.20	: Graph of Eigenvectors Versus Eigenvalues for Peptide 189-RBD Complex	93
Figure 4.21	: Radius of Gyration (Rg) for Peptide 65-RBD Complex Versus Time (ns)	95
Figure 4.22	: RMSD for Peptide 65-RBD Complex Versus Time (ns)	96
Figure 4.23	: RMSF of RBD for Peptide 65-RBD Complex	96
Figure 4.24	: RMSF of Peptide 65 for Peptide 65-RBD Complex	97
Figure 4.25	: Radius of Gyration (Rg) for Peptide 66-RBD Complex Versus Time (ns)	98
Figure 4.26	: RMSD for Peptide 66-RBD Complex Versus Time (ns)	98
Figure 4.27	: RMSF of RBD for Peptide 66-RBD Complex	99
Figure 4.28	: RMSF of Peptide 66 for Peptide 66-RBD Complex	99
Figure 4.29	: Radius of Gyration (Rg) for Peptide 189-RBD Complex	100

	Versus Time (ns)	
Figure 4.30	: RMSD for Peptide 189-RBD Complex Versus Time (ns)	100
Figure 4.31	: RMSF of RBD for Peptide 189-RBD Complex	101
Figure 4.32	: RMSF of Peptide 189 for Peptide 189-RBD Complex	101
Figure 4.33	: Graph of Corrected Mean Absorbance Versus Log Peptide 65 Concentration (Log μM)	103
Figure 4.34	: Graph of % Binding Versus Log Peptide 65 Concentration (Log μM).	104
Figure 4.35	: Graph of Corrected Mean Absorbance Versus Peptide 66 Concentration (μM)	105
Figure 4.36	: Graph of Mean Absorbance for Positive Control, Negative Control and Peptide 189 Concentration (200.00, 100.00, 50.00, 25.00, 12.50, 6.25, and 3.13 μM)	106
Figure 4.37	: Graph of Mean Absorbance Versus Spike Glycoprotein S1 RBD and ACE2 Protein Concentration (nM)	107



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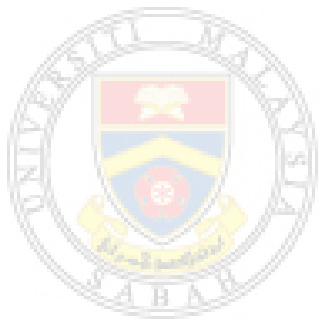
LIST OF ABBREVIATIONS

3CL^{pro}	-	3C-like Protease
AA	-	Amino Acid
ACE2	-	Angiotensin-converting Enzyme 2
ACTIV	-	Accelerating COVID-19 Therapeutic Interventions and Vaccines
ADMET	-	Absorption, Distribution, Metabolism, Excretion, and Toxicity
AIR	-	Ambiguous Interaction Restraint
AKI	-	Acute Kidney Injury
ALP	-	Alkaline Phosphatase
ARDS	-	Acute Respiratory Distress Syndrome
ATP	-	Adenosine Triphosphate
AVPdb	-	Antiviral Peptide Database
BBB	-	Blood Brain Barrier
BC	-	Binet-Cauchy
BLI	-	Bio-layer Interferometry
CAPRI	-	Critical Assessment of Predicted Interactions
CDC	-	Centers for Disease Control and Prevention
CH	-	Central Helix
CNS	-	Central Nervous System
COVID-19	-	Coronavirus Disease 2019
CoVs	-	Coronaviruses
CT	-	Cytoplasmic Terminal
E	-	Envelope
ECMO	-	Extracorporeal Membrane Oxygenation
ELISA	-	Enzyme-Linked Immunosorbent Assay
EMA	-	European Medicines Agency
EUA	-	Emergency Use Authorization
Fc	-	Fragment Crystallizable
FCC	-	Fraction of Common Contact
FDA	-	Food and Drug Administration
FNIH	-	Foundation for The NIH
FP	-	Fusion Peptide
FS	-	Femtosecond

Fu	-	Fraction Unbound
GDT	-	Global Distance Test
GIT	-	Gastrointestinal Tract
GLP-1	-	Glucagon-like Peptide-1
GM-CSF	-	Granulocyte-macrophage Colony-stimulating Factor
GnRH	-	Gonadotropin-releasing Hormone
GRAVY	-	Grand Average of Hydropathicity Index
GROMACS	-	Groningen MACHine for Chemical Simulation
HA	-	Hemagglutinin
HADDOCK	-	High Ambiguity Driven Protein-protein DOCKing
hERG	-	Human Ether-à-go-go-related Gene
HIA	-	Human Intestinal Absorption
HR	-	Heptad Repeat
HRP	-	Horseradish Peroxidase
IC₅₀	-	Half Maximal Inhibitory Concentration
IFN	-	Interferon
IL	-	Interleukin
IL-1RA	-	Interleukin-1 Receptor Antagonist
i-RMSD	-	Interface RMSD
JAK	-	Janus Kinase
K_D	-	Dissociation Constant
LC₅₀	-	Lethal Concentration Causing 50% Death
LD₅₀	-	Lethal Dose Causing 50% Death
LJ	-	Lennard-Jones
LOAEL	-	Lowest Observed Adverse Effect
LRA	-	Ligand-receptor Interaction Assay
LSPR	-	Localised Surface Plasmon Resonance
M	-	Membrane
M^{pro}	-	Main Protease
MCP-1	-	Macrophage Inflammatory Protein 1
MDS	-	Molecular Dynamics Simulation
MERS	-	Middle East Respiratory Syndrome
MM-GBSA	-	Molecular Mechanics Generalised Born Surface Area
MRTD	-	Maximum Recommended Tolerated Dose

N	-	Nucleocapsid
NIH	-	National Institutes of Health
NS	-	Nanosecond
NSP	-	Non-structural Proteins
NTD	-	N-terminal Domain
OCT2	-	Organic Cation Transporter 2
PaO₂	-	Arterial Partial Pressure of Oxygen
PCA	-	Principal Component Analysis
PEG	-	Polyethylene Glycol
pI	-	Isoelectric Point
PL^{pro}	-	Papain-like Protease
PNPP	-	P-nitrophenyl Phosphate Disodium Salt
PRODIGY	-	Protein Binding Energy Prediction
PTH1	-	Parathyroid Hormone 1
RBD	-	Receptor Binding Domain
RdRp	-	RNA-dependent RNA Polymerase
R_g	-	Radius of Gyration
RMSD	-	Root Mean Square Deviation
RMSF	-	Root Mean Square Fluctuation
RTC	-	Replication and Transcription Complex
S	-	Spike Glycoprotein
SA	-	Structural Alphabet
SARS	-	Severe Acute Respiratory Syndrome
SARS-CoV-2	-	Severe Acute Respiratory Syndrome Coronavirus 2
SD1/SD2	-	Subdomain 1/Subdomain 2
sOPEP	-	Optimised Potential for Efficient Structure Prediction
SpO₂	-	Oxygen Saturation
SS	-	Signal Sequence
ssRNA	-	Single-stranded Positive-sense RNA
SuPAR	-	Soluble Urokinase Plasminogen Activator Receptor
SVM	-	Support Vector Machine
TM	-	Transmembrane Domain
TMB	-	3,3',5,5'-tetramethylbenzidine
TMPRSS2	-	Transmembrane Protease Serine 2

TNF-α	-	Tumour Necrosis Factor- α
UK	-	United Kingdom
VBM	-	Variant Being Monitored
vdW	-	van der Waals
VDss	-	Volume of Distribution
VOC	-	Variant of Concern
VOHC	-	Variant of High Consequence
VOI	-	Variant of Interest
WHO	-	World Health Organization



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LIST OF APPENDIX

	Page
Appendix A : Table A.1: The 291 Peptides Designed with Their Respective HADDOCK Scores	138



CHAPTER 1

INTRODUCTION

1.1 Background of The Research

The outbreak of coronavirus disease 2019 (COVID-19), first emerged in Wuhan city, Hubei Province, China in December 2019, was caused by a novel *Betacoronavirus*, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (Wu *et al.*, 2020). On March 11, 2020, the disease was declared a global pandemic by the World Health Organization (WHO) (Cucinotta and Vanelli, 2020). As of 18 June 2023, over 768 million confirmed COVID-19 cases and 6.9 million deaths had been reported worldwide. This global pandemic has caused devastating social and economic disruption. To date, the number of positive cases as well as the number of deaths, has decreased globally, compared to previous years. However, WHO also noted that due to a reduction in testing and reporting, the number of recorded cases did not represent an accurate infection rate (COVID-19 Weekly Epidemiological Update on COVID-19-22 June 2023, 2023).

SARS-CoV-2 S protein and the host cell angiotensin-converting enzyme 2 (ACE2) receptor are the key components for infection of COVID-19. The presence of the ACE2 receptor in the vital organs, especially the lungs, enables viral infection in the host (Xu *et al.*, 2020; Jain, 2020). The treatment for COVID-19 is associated with the stages of the disease (Gandhi, 2021). For this purpose, the United States Food and Drug Administration (FDA) has approved two drugs for the treatment of COVID-19, the antiviral drug Veklury (remdesivir) in the early stages, and the immune modulator Olumiant (baricitinib), in the later stages (Coronavirus (COVID-19) | Drugs, 2022).