Project on

"Determination of the G protein of Nipah virus and its ligands as a potential new Anti-Nipah viral drug"

Submitted to

Department of Pharmacy

Faculty of Allied Health Science

Daffodil international university

Submitted by

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Batch 20th DSC

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Submission Date: 24th November 2022

Approval

This project paper, entitled **"Determination of the G protein of Nipah virus and its ligands as a potential new Anti-Nipah viral drug"** submitted to the **Department of Pharmacy, Faculty of Allied Health Sciences**, Daffodil International University, has been recognized as acceptable for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm.) and approved as to its style and contents.

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Certificate

This is to certify that the results of the investigation that are embodied in this project are original and have not been submitted before in substance for any degree of this University. The entire present work submitted as a project work for the partial fulfillment of the degree of Bachelor of pharmacy, is based on the result of author's (Id: 183-29-151) own investigation.

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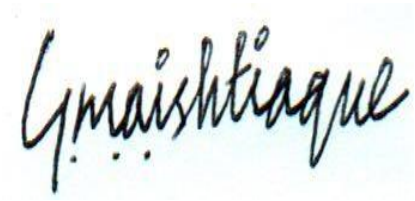

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Declaration

I, Md. Ripon Mridha , Id: 183-29-151, **Department of Pharmacy**, Daffodil International University, under the supervision of Mr. Galib Muhammad Abrar Ishtiaque, Lecturer, **Department of Pharmacy**, **Faculty of Allied Health Sciences**, hereby affirm that the work presented herein, entitled "Determination of the G protein of Nipah virus and its ligands as a potential new Anti-Nipah viral drug" represents my independent and thoughtful efforts toward completion of the requirements for the Bachelor of Pharmacy degree (B. Pharm.). I hereby claim that the content and ideas included in this work are mine. Also, I swear that I haven't turned in this project, or any portion of it, anywhere else to get my bachelor's or any other degree.

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Dedication

I would like to thank my parents and teachers for always loving me and supporting me through this process. And also, thanks to all of my friends who assisted me with this project.

Acknowledgement

The first thing I want to do is offer thanks to Allah, the Almighty, for blessing me with the chance to study this topic and the skills to accomplish my project. I owe a lot of thanks to Mr. Galib Muhammad Abrar Ishtiaque, Lecturer, in the **Department of Pharmacy**. He was my project supervisor and gave me a lot of helpful advice and supervision throughout the course.

Thank you for the chance to collaborate on this project, Professor Dr. Muniruddin Ahamed, Head of the **Department of Pharmacy** at Daffodil International University. I'd also like to thank the other faculty and staff at DIU's Pharmacy Department for being helpful and let the instructors there know how much I appreciate them.

Md. Ripon Mridha

Author

Abstract

The aim of this study was to determine the full-length 3D structure of the Glyco (G) protein of Nipah virus, followed by the De Novo drug design, which will generate ligands as prospective Nipah virus inhibitor. Due to the unavailability of the complete structure of the Nipah virus in RCSB-PDB, Homology modelling was adopted by using the sequence from Uni Prot KB. BLAST assisted in the search of templates with the highest sequence similarity and coverage. After the joining of loops with the functional domains through ab-initio modelling, the full sequence of the Glyco (G) protein of Nipah virus was submitted in the i-TASSER server, which further predicted five models. Ramachandran assessment helped in the validation of those models. From the i-TASSER model, joining of the loops with the functional domains were cut off using UCSF-Chimera software. These loops and fragments were joined with the help of a tool that is in house developed. Once energy minimization has been carried out in the Swiss PDB viewer, CASTp server provided the determination of ligand binding pockets. After pocket determination, e-LEA3D server helped in the design of ligand molecules that will bind to those pockets. The pharmacokinetic properties of each of those ligands were further assessed in the Mobyle@RPBS web portal so that prospective Nipah virus inhibitor drugs can be developed in future.

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CHAPTER 1

INTRODUCTION

1.1 Nipah Virus

In contrast to other priority diseases designated by the WHO, the Nipah virus, which is strongly linked to the Hendra virus and is a species of the Paramyxoviridae family, is an emerging pathogen that is indigenous to Southeast Asia. For the first time, the Nipah virus was recognized in 1999 as a serious human pandemic that affected 283 people and killed 109 people in Malaysia (1). Even though there haven't been any more instances in Malaysia since then, Bangladesh and India have periodically seen outbreaks. Since 2001, the Nipah virus has infected hundreds of individuals, with a 75% average mortality rate (2). It is possible for zoonotic and human transmission of the Nipah virus through its host reservoir, which has a broad geographic spread. Nipah virus has been identified by the WHO as a high-priority virus because of its limitations in terms of prevention and treatment. There aren't many validated and controlled diagnostic studies for the Nipah virus, though. Since there are now no approved medicines or vaccinations on the market, there is only supportive therapy available for treating the Nipah virus (3).

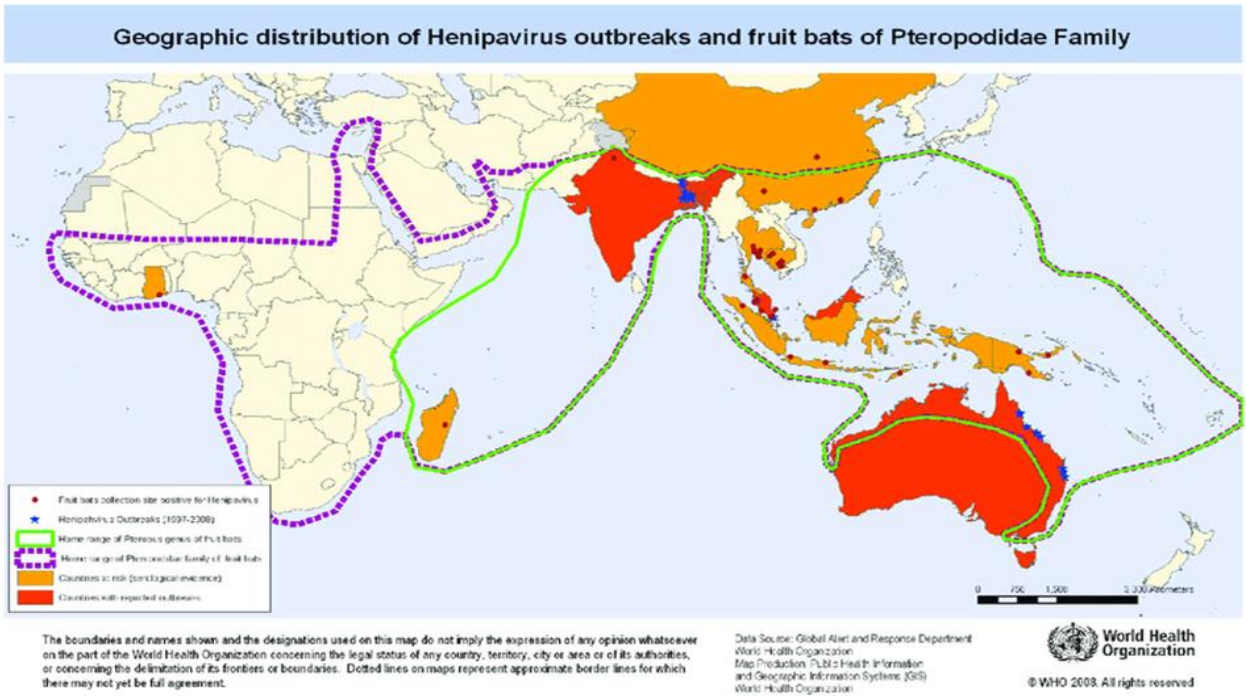


Figure 1.1.1: Geographic distribution of Henipavirus outbreaks and fruit bats of Pteropopodidae Family.

92 people were discovered to have confirmed and probable cases of Nipah virus infection in studies on the disease conducted in Bangladesh between 2001 and 2004. Out of the 92 patients, 67 passed away, for a 73% mortality rate (4). Seven outbreaks of the Nipah virus infection were identified in Bangladesh between the years 2001 and 2007(5).

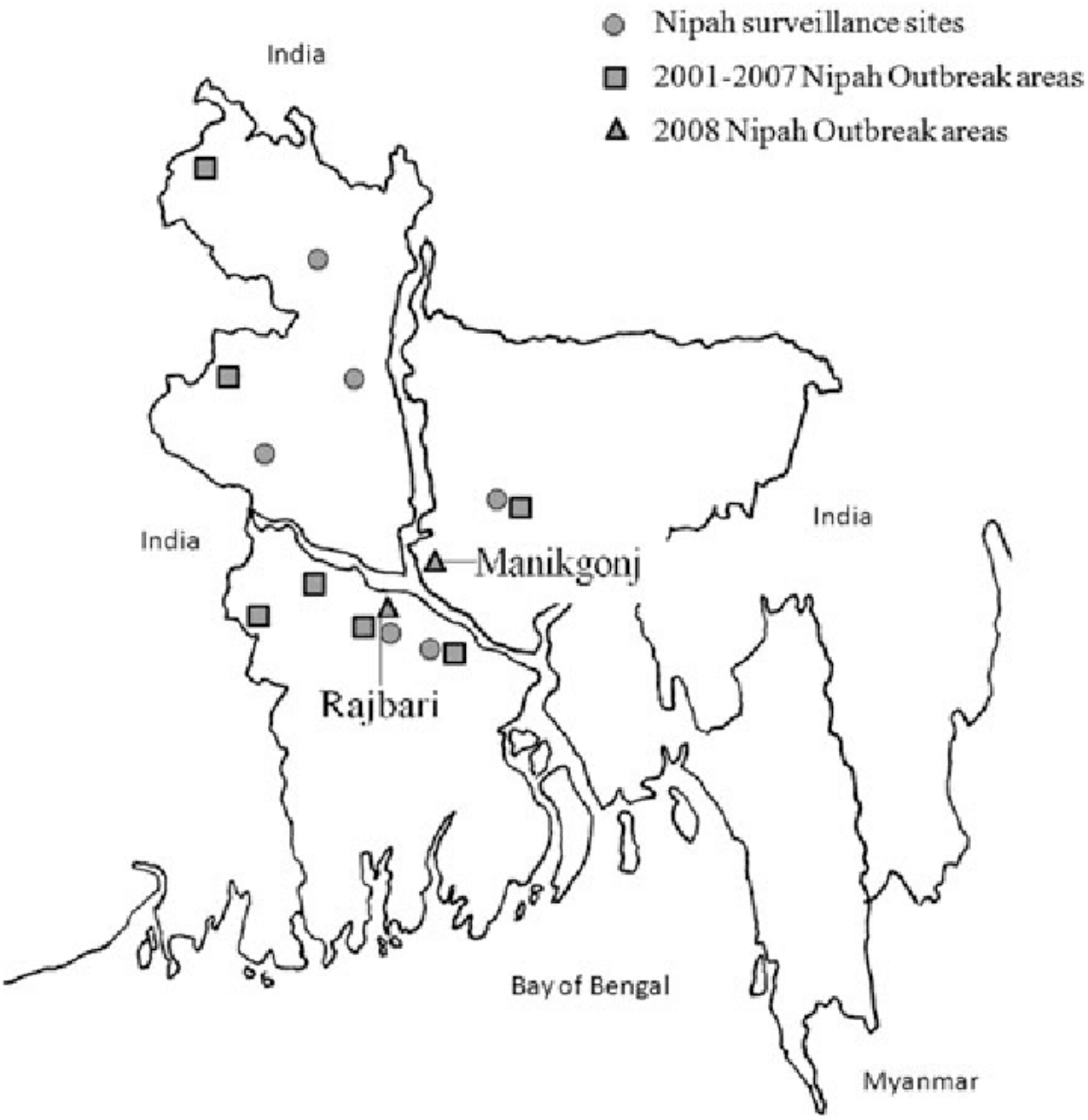


Figure 1.1.2: Date and location of Nipah outbreaks in Bangladesh

In the World Health Organization's (WHO) surveillance and outbreak alert report, it was noted that better nursing and treatment facilities, along with public awareness campaigns, had not been able to lower the morbidity and mortality rates of Nipah virus infection, particularly during the winter and spring, which is thought to be the bats' breeding season and when they are most likely to spread the virus (6).

Table 1.1: Chronology of Nipah virus outbreaks in Bangladesh

Table: Yearly distribution of Nipah cases in Bangladesh 2001 – 2020

Year	Total no of NiV cases in a Year	Total number of Death due to NiV in a year	Percentage of Death from NiV (%)
2001	13	9	69.23
2002	0	8	0
2003	12	0	0
2004	67	50	74.63
2005	13	11	84.62
2006	0	0	0
2007	18	9	50.00
2008	11	9	81.82
2009	4	0	0
2010	18	16	88.89
2011	42	36	85.71
2012	18	13	72.22
2013	26	22	84.62
2014	38	15	39.47
2015	18	11	61.11
2016	0	0	0
2017	3	2	66.67
2018	4	3	75.00
2019	8	7	87.50
2020	6	4	66.66
Total	**319**	**225**	**70.53**

1.2 Mode of transmission

Transmission in the Malaysia-Singapore outbreak occurred mostly through contact with pigs, but in India and Bangladesh, t Person-to-person and bat-to-person transmission are related terms. Most typically, bats in Bangladesh transmit the Nipah virus to humans via three distinct paths. The consumption of date palm sap is one of the most popular pathways. The other modes of transmission involve direct contact with diseased bats and contamination by domestic animals (7).

1.3 Pathology

In 32 fatalities of the Malaysian epidemic, autopsy (29 complete, 3 limited to the brain) revealed pathological lesions largely in the brain, with diffuse microinfarction as a result of vasculitis-induced thrombosis and direct neuronal involvement. The heart, kidneys, and respiratory system all had similar vasculitic lesions. Everybody had the Nipah virus (serology or immunohistochemistry). The Nipah virus appears to be particularly harmful to small and medium-sized blood vessels, resulting in multinucleated endothelial syncytia and fibrinolytic necrosis (8).

1.4 Symptoms

Human encephalitis caused by the Nipah virus is severe and is characterized by vasculitis and necrosis of the central nervous system (CNS). The incubation period for the Nipah virus is typically 4–14 days. Nipah virus primarily affects the central nervous system (CNS) by infection of endothelial, vascular, and parenchymal cells, with enhanced viral replication in neuronal bodies (9). The early stages of Nipah virus infection frequently present as fever encephalitis or pneumonia and can be challenging to distinguish from other febrile illnesses. Depending on the intensity, patients may also have a fever, malaise, headache, myalgia, nausea, vomiting, vertigo, and disorientation. The prognosis for encephalitis is poor, with mortality occurring six days following the beginning of symptoms (10).

1.5 Prevention

Nipah virus is regarded as a Biosafety Level-4 agent because of the enhanced pathogenicity linked to Henipavirus (11). Nipah virus treatment is limited to care and assistance because there is no medicine or vaccination available. There is evidence that ribavirin reduces mortality (12), However, its entire efficacy against the Nipah virus infection has not yet been shown (13).

1.6 Treatment

There is presently no treatment for the Nipah virus. Consult your doctor right once if you experience any flu-like symptoms, and depending on how bad they are, they could send you to an infectious disease specialist. The major goal of treatment is to control symptoms like fever and, if present, any neurological signs. The only thing that can help a patient with Nipah virus illness is intensive support care (14).

1.7 Morphology

Like other paramyxoviruses, Nipah viruses are pleomorphic, spherical to filamentous, and range in size from 40 nm to 1,900 nm. They consist of a single layer of 17 surface projections that are 1 nm long (15).

1.8 Genetic Diversity

The Nipah virus, which is known to harm people, has two important genetic lineages (16):

i. Nipah Virus- Malaysia (NiV-MY)
ii. Nipah Virus- Bangladesh (NiV-BD)

1.9 Genome Size and structure

The Bangladesh Nipah virus has 18,252 nucleotides in its genome, compared to 18,246 in the Malaysia Nipah virus. The possible role of this increase in viral pathogenicity and interhost transmission of this genome size is yet unknown (17).

1.10 Sequence of Nipah virus polyprotein

MPAENKKVRFENTTSDKGKIPSKVIKSYYGTMDIKKINEGLLDSKILSAFNTVIALLGSIVI
IVMNIMIIQNYTRSTDNQAVIKDALQGIQQQIKGLADKIGTEIGPKVSLIDTSSTITIPANIG
LLGSKISQSTASINENVNEKCKFTLPPLKIHECNISCPNPLPFREYRPQTEGVSNLVGLPNN
ICLQKTSNQILKPKLISYTLPVVGQSGTCITDPLLAMDEGYFAYSHLERIGSCSRGVSKQR
IIGVGEVLDRGDEVPSLFMTNVWTPPNPNTVYHCSAVYNNEFYYVLCAVSTVGDPILNS
TYWSGSLMMTRLAVKPKSNGGGYNQHQLALRSIEKGRYDKVMPYGPSGIKQGDTLYF
PAVGFLVRTEFKYNDSNCPITKCQYSKPENCRLSMGIRPNSHYILRSGLLKYNLSDGENP
KVVFIEISDQRLSIGSPSKIYDSLGQPVFYQASFSWDTMIKFGDVLTVNPLVVNWRNNTV
ISRPGQSQCPRFNTCPEICWEGVYNDAFLIDRINWISAGVFLDSNQTAENPVFTVFKDNEI
LYRAQLASEDTNAQKTITNCFLLKNKIWCISLVEIYDTGDNVIRPKLFAVKIPEQCT

1.11 Subcellular Location

The Glycoprotein (G) of the Nipah virus has two subcellular locations:

1. Topological domain
   I. Topological domain (Intravirion)

Position: 1-49

Amino acid sequence:

MPAENKKVRFENTTSDKGKIPSKVIKSYYGTMDIKKINEGLLDSKILSA

II. Topological domain (Virion Surface)

Position:71-602

Amino acid sequence:

QNYTRSTDNQAVIKDALQGIQQQIKGLADKIGTEIGPKVSLIDTSSTITIPANIGLLGSKISQ
STASINENVNEKCKFTLPPLKIHECNISCPNPLPFREYRPQTEGVSNLVGLPNNICLQKTSN
QILKPKLISYTLPVVGQSGTCITDPLLAMDEGYFAYSHLERIGSCSRGVSKQRIIGVGEVL
DRGDEVPSLFMTNVWTPPNPNTVYHCSAVYNNEFYYVLCAVSTVGDPILNSTYWSGSL
MMTRLAVKPKSNGGGYNQHQLALRSIEKGRYDKVMPYGPSGIKQGDTLYFPAVGFLV
RTEFKYNDSNCPITKCQYSKPENCRLSMGIRPNSHYILRSGLLKYNLSDGENPKVVFIEIS
DQRLSIGSPSKIYDSLGQPVFYQASFSWDTMIKFGDVLTVNPLVVNWRNNTVISRPGQSQ
CPRFNTCPEICWEGVYNDAFLIDRINWISAGVFLDSNQTAENPVFTVFKDNEILYRAQLA
SEDTNAQKTITNCFLLKNKIWCISLVEIYDTGDNVIRPKLFAVKIPEQCT

2.Transmembrane (Helical)

Position:50-70

Amino acid sequence:

FNTVIALLGSIVIIVMNIMII

CHAPTER 2

PURPOSE OF THE STUDY

2. Purpose of the Study

To determine the G protein of Nipah Virus in an in-silico approach and identify top 21 ligands that can be used as a potential new anti-Nipah viral drug.

This study will illustrate the structure of the G protein in silico which will further identify the possible targets for an Anti-Nipah viral drug.

CHAPTER 3

MATERIALS & METHODS

3.Materials and Methods

In this research, the following materials have been used:

i. Protein Data Bank (RCSB-PDB)
ii. UniProt Knowledgebase (UniProt KB)
iii. Iterative Threading ASSEmbly Refinement (i-TASSER)
iv. Ramachandran Plot Assessment (RAMPAGE)
v. UCSF Chimera (version 1.13.1)
vi. Swiss PDB Viewer
vii. Computed Atlas of Surface Topography of Proteins (CASTp)
viii. e-LEA3D web server
ix. Mobyle RPBS web portal

The below-mentioned methods were used in this research:

i. ab-initio modelling
ii. Ramachandran plot analysis
iii. Structure energy minimization
iv. Determination of ligand binding pocket
v. Ligand design
vi. Determination of the pharmacokinetic property of ligand

CHAPTER 4

PROCEDURE & RESULTS

4. Procedure & Results

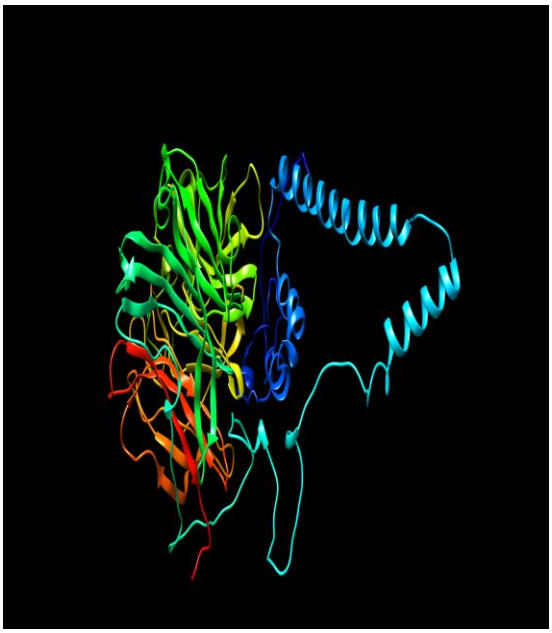
4.1 Methods for Molecular Modelling of Nipah Virus

A well-known and publicly accessible database called UniProt contains the functional details and amino acid sequences of every protein discovered as a result of genome sequencing initiatives. It has become possible to preserve a significant quantity of knowledge on the biological activity of proteins with the assistance of research literature. This database may be used to determine a protein's target sequence.

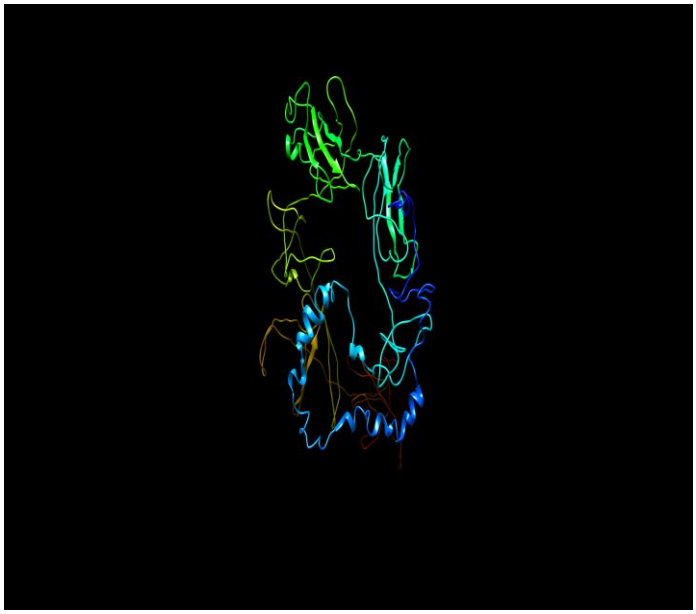
4.1.2 ab-initio Modelling

4.1.2.1 *i*-TASSER Modeling

Ab-initio modeling's i-TASSER server was visited at the initial stage. The full-length sequence of the Nipah virus' Glyco (G) protein was entered into the i-TASSER server, which resulted in the prediction of five full-length structures for the Glyco (G) protein. The ribbon structures of all five anticipated models, as determined by the UCSF Chimera program, are shown below:

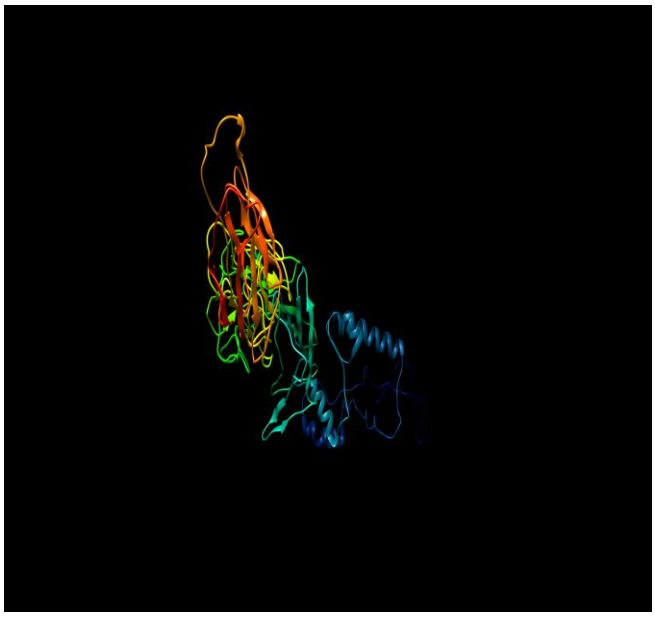


Model 1



Model 2

Model 3



Model 4

Model 5

Figure 4.1.2.1: Ribbon structures of the predicted models from the i-TASSER server

4.1.2.2 Ramachandran Plot Analysis

The Ramachandran plot analysis is completed for each of the model structures by submitting the five projected models from the i-TASSER server and gaining access to the RAMPAGE server. Ramachandran plot analysis will provide us with the preferred region (FR), permitted region (AR), and outlier region for every model structure (OR).

Table 4.1.2.2: Ramachandran plot assessment of all 5 predicted models

Model Number	Favoured Region **(FR)**	Allowed Region **(AR)**	Outlier Region **(OR)**	FR+AR	Inference
Model-1	87.786%	8.969%	3.244%	96.755%	Good
Model-2	79.962%	13.550%	6.489%	93.512%	Bad
Model-3	85.687%	10.878%	3.435%	96.565%	Medium
Model-4	80.688%	13.002%	6.310%	93.69%	Bad
Model-5	83.779%	11.069%	5.153%	94.848%	Bad

The aforementioned result makes it easy to forecast that model 1 has a good interference since it has the largest preferred region+allowed region and the least amount of outlier region.

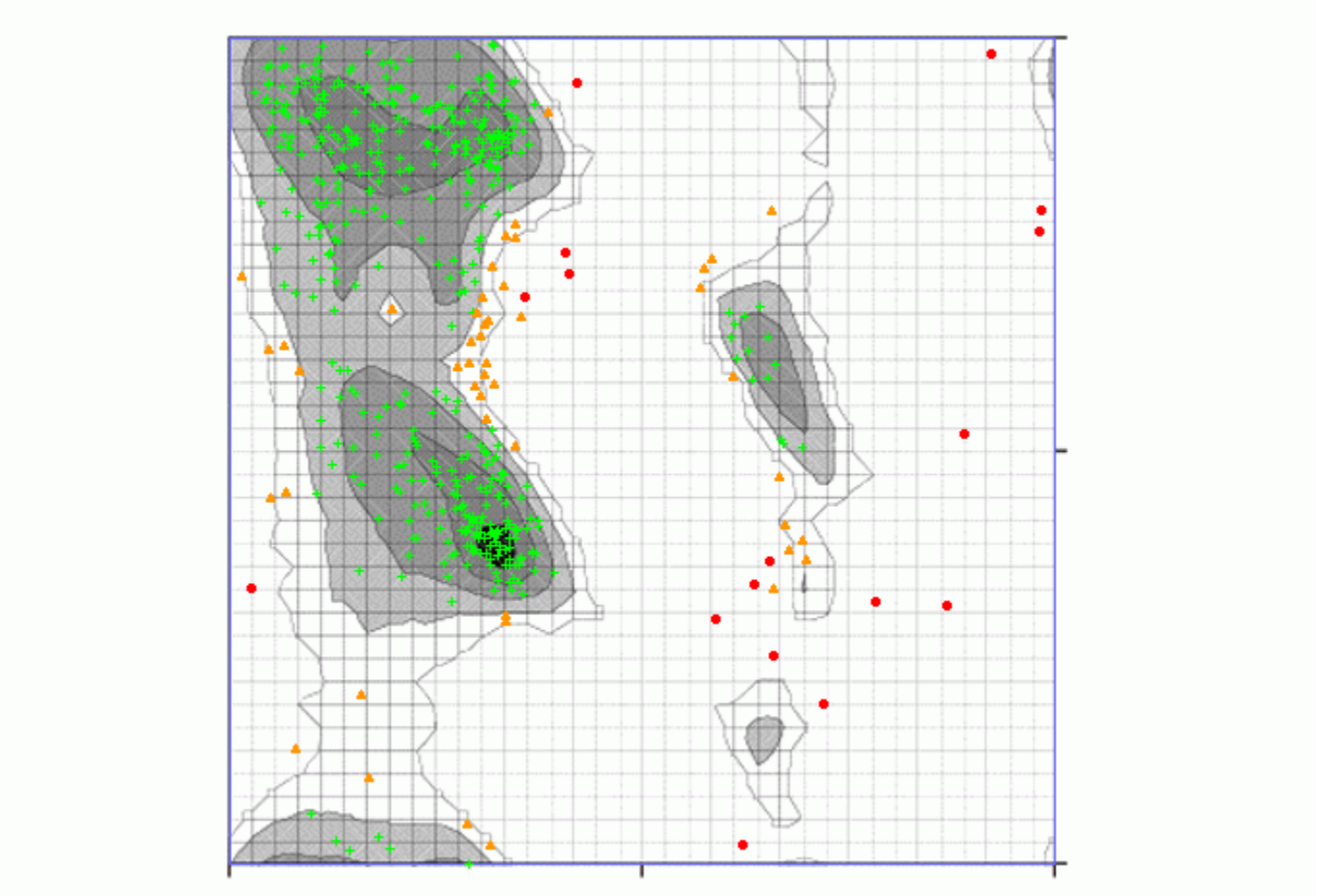


Figure 4.1.2.2: Ramachandran plot analysis report of i-TASSER predicted model 1

4.2 Structure Energy Minimization use of swiss -PDB viewer

Swiss-PdbViewer is a program that offers a user-friendly interface that permits the simultaneous analysis of many proteins. To compare active sites or other important components and determine structural alignments, the proteins can be overlaid.

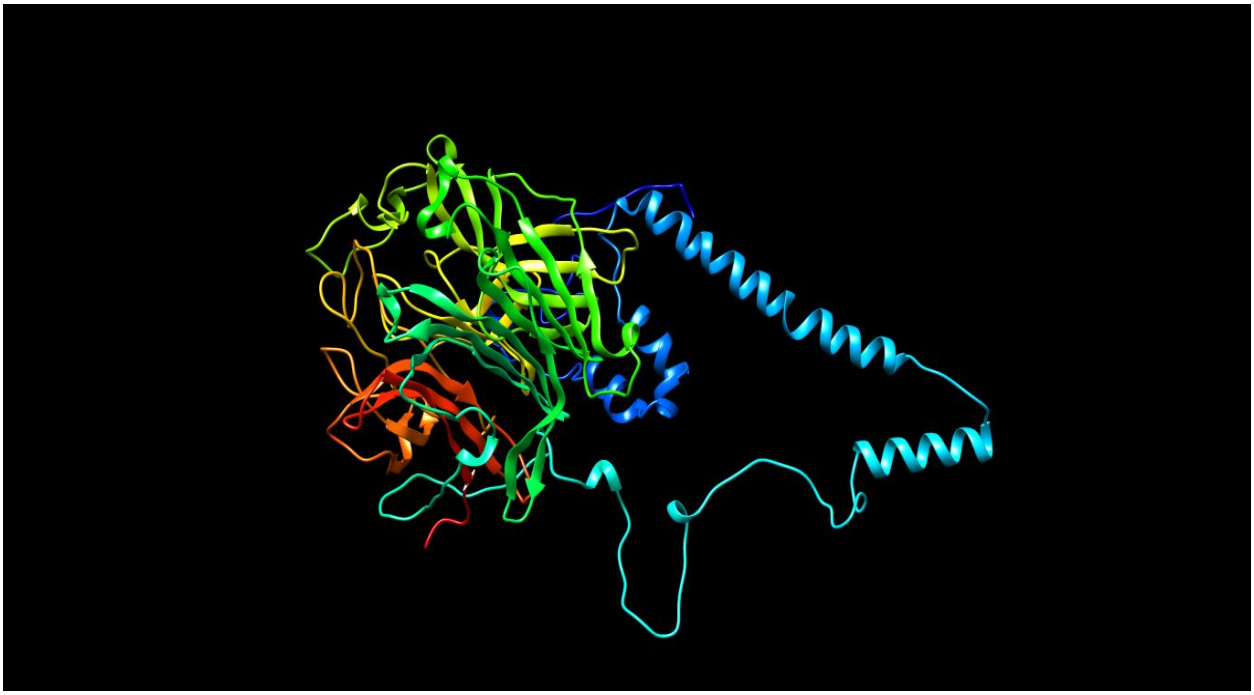


Figure 4.2: Full-length structure of the Glyco(G) Protein of Nipah Virus after energy minimization.

4.3 Ligand Binding Pocket Determination

The ligand binding pockets were identified when the energy was reduced and the full-length structure of the Glyco (G) protein of the Nipah virus was uploaded in the PDB format to the CASTp server. Only the pockets with an MS volume of less than 1000 but more than 50 and several apertures equal to 1 are selected for further experimentation once the CASTp server identifies all the pockets. Then, they are arranged in order of MS pocket area value, highest to lowest. This selection of 11 pockets was created. The binding site coordinates: x, y, and z are identified for each of the 11 pockets listed below using the UCSF Chimera Program: -

Table 4.3: Binding site coordinate (x, y, z) determination of 11 pockets

ID	MS Volume	Pocket MS Area	# Openings	Mouth MS Area	MS circumference sum	X	Y	Z
4	607.2	164.1	1	301.2	94.6	86.662	63.982	45.698
5	259.0	182.1	1	65.2	36.0	106.148	103.604	103.928
8	204.8	222.8	1	35.6	25.2	92.579	111.494	100.683
17	74.2	92.0	1	8.5	10.8	78.966	69.732	102.317
19	69.0	82.2	1	15.6	14.7	75.774	105.331	79.862
18	67.9	79.5	1	15.1	14.4	106.735	87.432	81.253
11	66.2	56.8	1	38.5	24.8	76.517	90.507	80.983
20	63.2	83.2	1	18.1	17.2	67.820	87.016	109.163
10	60.3	45.2	1	44.5	28.3	96.733	82.669	117.680
21	54.9	71.8	1	12.8	12.9	97.842	106.981	109.019
38	52.2	87.2	1	9.5	12.8	101.051	112.725	100.733

4.4 Ligand Design

The ligands that will bind to the binding sites of the pockets, which were earlier identified using the CASTp web server, are designed using the e-LEA3D server. The procedures listed below produce a ligand molecule for each of the Nipah virus' Glyco (G) protein's energy-minimized structures: -

i. You may access the e-LEA3D web server by going to http://chemoinfo.ipmc.cnrs.fr

ii. Click "Enter" after selecting "Drug design or Screen."

iii. Upload the protein's energy-minimized structure in PDB format,

iv. provide the binding site coordinates (x, y, and z).

v. Enter 3.0 for "Binding site radius" and 1.0 for "Weight in the final score," appropriately.

vi. Then choose "Submit"

vii. Enter your email address and choose "De-novo Drug Design" on the next screen.

Viii. Select the "Stem Drug" option, and then click "submit" to finish the application

The findings for all 11 pockets will then be sent to the specified email address via the e-LEA3D server. It will create 21 ligands for each pocket, which will then be arranged again in decreasing order according to each ligand's best energy score and binding affinity percentage (%).

Below are the best energy scores and percentage scores for each ligand's binding affinity for each of the 11 pockets:

Table 4.4.1: Ligand Design of Pocket ID 38 & 10

Pocket id 38			**Pocket id 10**		
Ligands (Generation Number)	Score of Binding Affinity (%)	Best Score of Energy	**Ligands (Generation Number)**	Score of Binding Affinity (%)	Best Score of Energy
Gen08	47.47	-71.200	Gen18	87.99	-131.990
Gen05	47.43	-71.140	Gen20	83.7	-125.550
Gen07	47.4	-71.100	Gen19	81.45	-122.170
Gen04	47.39	-71.080	Gen16	79.55	-119.320
Gen06	47.35	-71.02	Gen17	78.41	-117.620
Gen19	47.29	-70.940	Gen14	76.47	-114.7
Gen09	47.28	-70.920	Gen15	76.07	-114.1
Gen20	47.2	-70.800	Gen13	75.73	-113.59
Gen03	46.5	-69.75	Gen11	70.68	-106.020
Gen14	46.31	-69.47	Gen12	70.08	-105.120
Gen15	46.17	-69.250	Gen08	63.28	-94.920
Gen16	46.16	-69.24	Gen09	63.25	-94.880
Gen17	46.16	-69.25	Gen07	63.21	-94.810
Gen18	46.13	-69.19	Gen10	62.25	-93.370
Gen13	45.79	-68.69	Gen06	59.54	-89.310
Gen12	45.66	-68.490	Gen05	57.19	-85.790
Gen10	44.36	-66.54	Gen04	53.41	-80.12
Gen11	44.32	-66.48	Gen03	51.26	-76.890
Gen02	43.68	-65.520	Gen02	49.25	-73.870
Gen01	42.6	-63.900	Gen00	46.36	-69.540
Gen00	42.55	-63.830	Gen01	46.31	-69.47

Table 4.4.2: Ligand Design of Pocket id 17 & 8

Pocket id 17			Pocket id 8		
Ligands (Generation Number)	Score of Binding Affinity (%)	Best Score of Energy	**Ligands (Generation Number)**	Score of Binding Affinity (%)	Best Score of Energy
Gen19	95.41	-143.12	Gen18	84.79	-127.190
Gen20	89.92	-134.880	Gen14	84.18	-126.270
Gen15	89.63	89.63	Gen17	83.81	-125.72
Gen18	87.24	-130.860	Gen20	83.78	-125.670
Gen14	86.33	-129.490	Gen19	83.63	-125.440
Gen17	85.51	-128.26	Gen12	83.33	-124.99
Gen16	84.51	-126.77	Gen16	83.17	-124.760
Gen12	84.41	-126.61	Gen15	81.27	-121.900
Gen09	82.45	-123.670	Gen13	81.19	-121.790
Gen13	80.31	-120.47	Gen11	74.47	-111.7
Gen11	79.69	-119.53	Gen10	71.27	-106.900
Gen10	75.43	-113.140	Gen09	70.56	-105.84
Gen08	73.75	-110.630	Gen08	68.91	-103.360
Gen07	72.76	-109.140	Gen06	68.63	-102.950
Gen04	61.8	-92.7	Gen07	67.52	-101.280
Gen05	61.8	-92.7	Gen04	60.97	-91.460
Gen06	61.74	-92.610	Gen05	60.97	-91.46
Gen03	54.27	-81.410	Gen03	58.25	-87.380
Gen02	50.55	-75.82	Gen02	57.29	-85.93
Gen01	48.08	-72.120	Gen01	56.37	-84.560
Gen00	47.52	-71.280	Gen00	56.27	-84.41

Table 4.4.3: Ligand Design Pocket id 20 & 18

Pocket id 20			Pocket id 18		
Ligands (Generation Number)	Score of Binding Affinity (%)	Best Score of Energy	**Ligands (Generation Number)**	Score of Binding Affinity (%)	Best Score of Energy
Gen18	82.37	-123.560	Gen15	82.14	-123.210
Gen14	80.8	-121.200	Gen20	82.1	-123.17
Gen15	79.67	-119.51	Gen16	82.1	-123.150
Gen17	77.8	-116.700	Gen14	81.37	-122.050
Gen16	77.61	-116.420	Gen19	79.2	-118.800
Gen20	77.59	-116.390	Gen18	78.87	-118.3
Gen19	76.79	-115.190	Gen13	78.61	-117.92
Gen13	74.06	-111.09	Gen17	76.79	-115.19
Gen12	73.93	-110.89	Gen11	76.66	-114.990
Gen11	65.85	-98.78	Gen10	75.57	-113.360
Gen10	65.83	-98.74	Gen09	73.15	-109.72
Gen09	63.43	-95.15	Gen12	72.94	-109.410
Gen08	63.15	-94.720	Gen08	70.29	-105.430
Gen07	58.24	-87.36	Gen07	69.41	-104.11
Gen05	53.5	-80.250	Gen06	67.22	-100.830
Gen06	53.5	-80.250	Gen05	63.87	-95.800
Gen04	53.49	-80.240	Gen04	59.49	-89.230
Gen03	52.4	-78.600	Gen03	54.69	-82.030
Gen02	51.45	-77.170	Gen01	50.44	-75.660
Gen00	46.36	-69.540	Gen02	50.3	-75.450
Gen01	46.32	-69.480	Gen00	50.04	-75.060

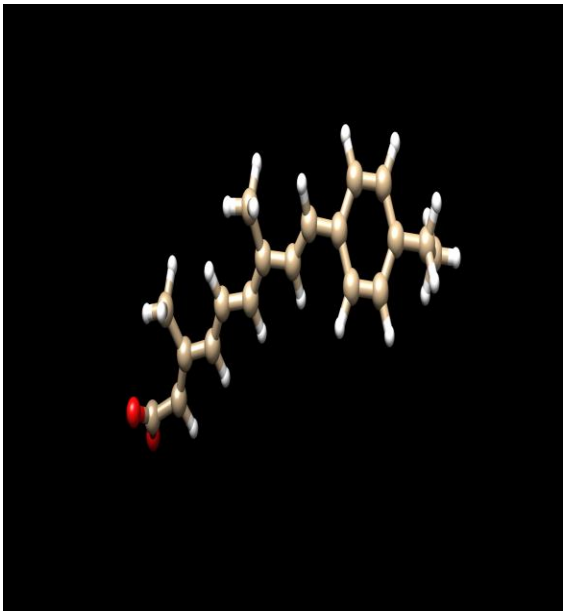
Table 4.4.4: Ligand Design Pocket id 4 & 5

Pocket id 4			Pocket 5		
Ligands (Generation Number)	Score of Binding Affinity (%)	Best Score of Energy	**Ligands (Generation Number)**	Score of Binding Affinity (%)	Best Score of Energy
Gen19	51.01	-76.51	Gen05	49.87	-74.800
Gen17	51	-76.500	Gen07	49.87	-74.800
Gen18	50.98	-76.470	Gen08	49.87	-74.8
Gen20	50.97	-76.460	Gen10	49.87	-74.800
Gen16	50.13	-75.200	Gen11	49.87	-74.800
Gen15	48.59	-72.880	Gen12	49.87	49.87
Gen13	48.58	-72.870	Gen13	49.87	-74.800
Gen10	48.57	-72.850	Gen14	49.87	-74.800
Gen12	48.57	-72.860	Gen15	49.87	-74.800
Gen14	48.57	-72.850	Gen18	49.87	-74.800
Gen11	48.55	-72.830	Gen19	49.87	-74.810
Gen06	47.32	-70.980	Gen20	49.87	-74.800
Gen09	47.31	-70.96	Gen06	49.86	-74.79
Gen07	47.29	-70.940	Gen17	49.86	-74.790
Gen05	47.21	-70.810	Gen16	49.85	-74.770
Gen08	47.19	-70.78	Gen03	48.57	-72.85
Gen04	46.31	-69.460	Gen01	48.51	-72.76
Gen03	46.29	-69.430	Gen04	48.49	-72.730
Gen01	46.27	-69.400	Gen02	48.39	-72.590
Gen02	46.27	-69.400	Gen00	46.96	-70.440
Gen00	46.2	-69.300	Gen09	-74.8	-74.810

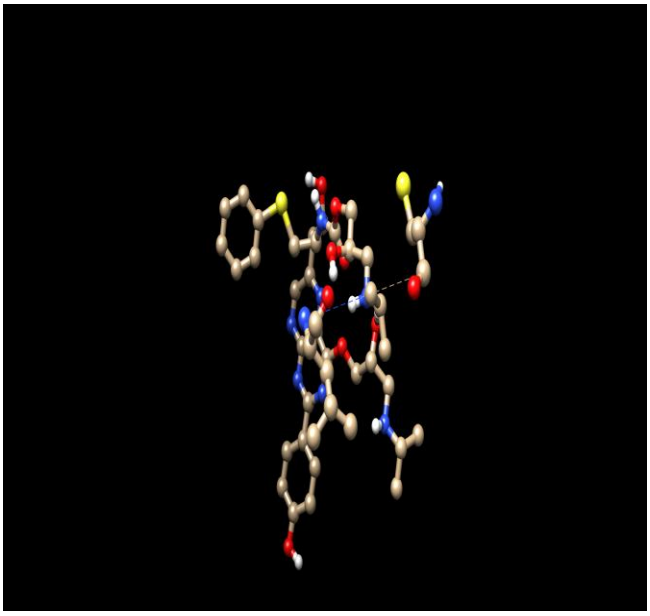
Table 4.4.5: Ligand Design Pocket id 11 & 19

Pocket id 11			Pocket id 19		
Ligands (Generation Number)	Score of Binding Affinity(%)	Best Score of Energy	Ligands (Generation Number)	Score of Binding Affinity(%)	Best Score of Energy
Gen19	71.73	-107.59	Gen14	94.94	-142.410
Gen13	71.7	-107.550	Gen15	94.8	-142.200
Gen14	71.7	-107.550	Gen10	93.56	-140.340
Gen18	71.69	-107.54	Gen18	93.21	-139.82
Gen15	71.67	-107.500	Gen12	92.17	-138.250
Gen17	71.67	-107.500	Gen13	91.33	-136.99
Gen20	71.59	-107.390	Gen19	91.29	-136.94
Gen16	71.57	-107.36	Gen16	90.81	-136.210
Gen12	69.29	-103.93	Gen11	90.4	-135.6
Gen08	64.89	-97.330	Gen09	90.33	-135.490
Gen09	64.85	-97.280	Gen17	89.48	-134.22
Gen06	64.81	-97.210	Gen20	87.23	-130.84
Gen11	64.81	-97.210	Gen08	86.55	-129.82
Gen10	64.77	-97.150	Gen06	84.07	-126.1
Gen07	64.71	-97.07	Gen07	75.85	-113.77
Gen04	55.62	-83.43	Gen05	74.27	-111.410
Gen05	55.3	-82.95	Gen03	62.15	-93.220
Gen02	53.49	-80.24	Gen04	60.83	-91.240
Gen03	53.49	-80.23	Gen02	53.84	-80.760
Gen01	51.6	-77.400	Gen01	53.32	-79.980
Gen00	48.9	-73.350	Gen00	53.28	-79.920

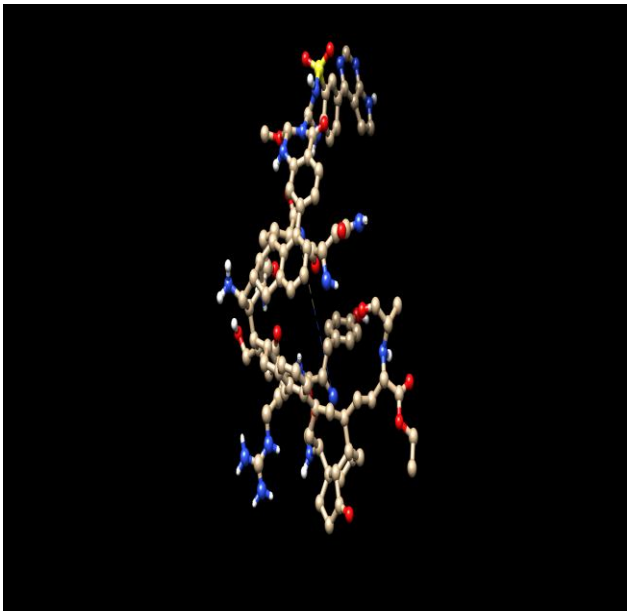
The structure of the ligand molecules in each pocket with the highest percentage of binding affinity and the best energy score is shown below:



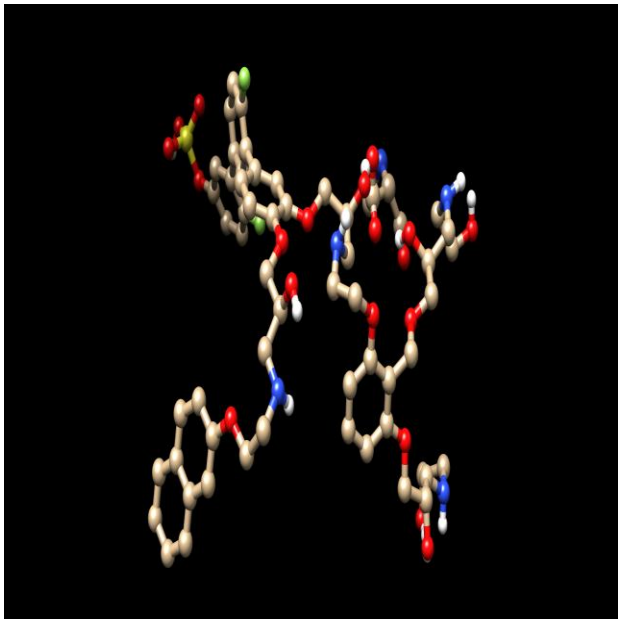
Pocket id 38 Generation 8



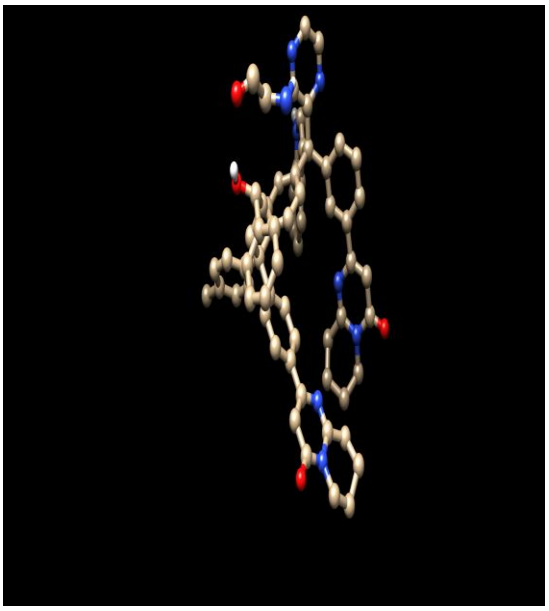
Pocket id 8 Generation 14



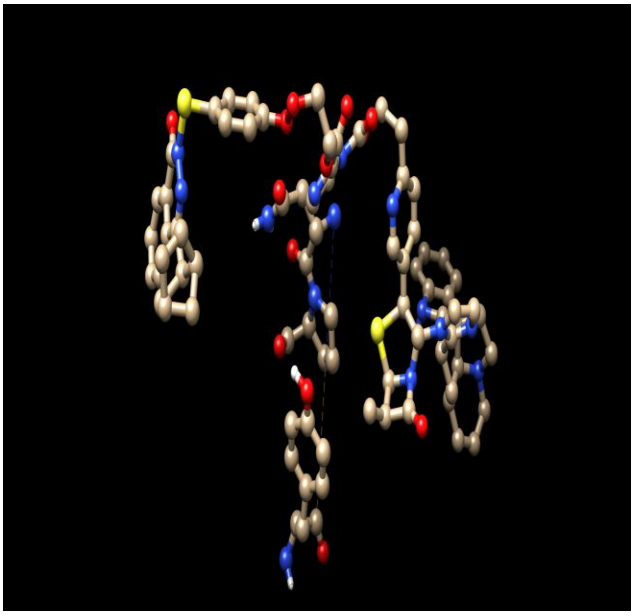
Pocket id 20 Generation 14



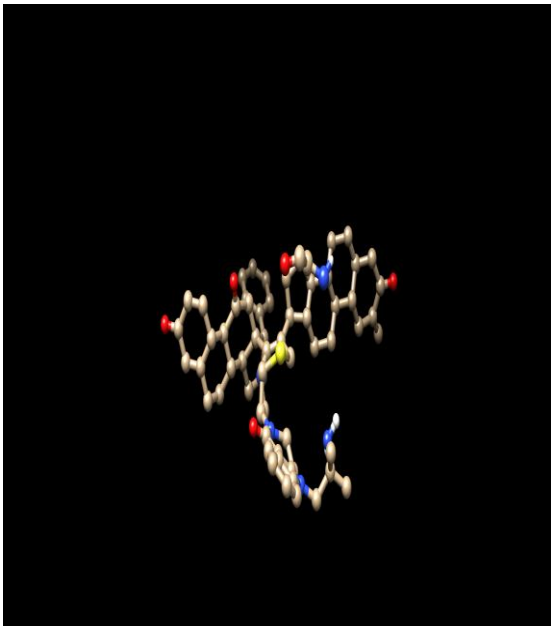
Pocket id 17 Generation 19



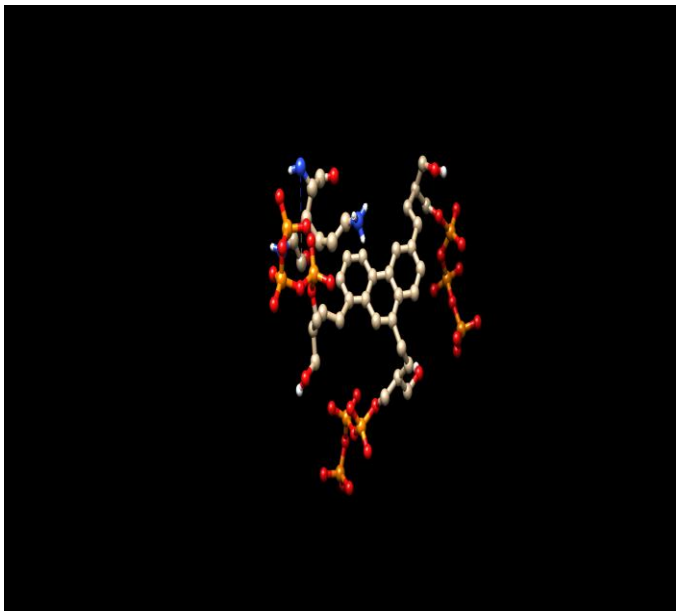
Pocket id 10 Generation 18



Pocket id 18 Generation 19



Pocket id 11 Generation 19



Pocket id 19 Generation 14

Figure 4.4: 3D Structure of Ligand Molecules

4.5 Ligand Pharmacokinetic Property Determination

The Mobyle RPBS online site may be used to determine the pharmacokinetic characteristics of ligand molecules once they have been created using the e-LEA3D server. The following measures were taken to evaluate each ligand molecule's pharmacokinetic profile for the glyco (G) protein pockets: -

1. Visit the RPBS web portal at http://mobyle.rpbs.univ-paris-diderot.fr/cgibin/portal.py#welcome
2. Go to the 'Programs' menu and select 'Drugs'
3. Then select the 'FAF-Drugs4' option and the following 'FAF-Drugs4' option as well
4. In the 'Demonstration mode' menu, choose 'No' to test the service with server sample data
5. In the 'Input data' window, select upload and choose the sdf file of the ligand molecules of each pocket
6. In the 'logP method' menu, select 'XLOGP3' for logP computation program
7. In the 'Filtering options' window-

    - Select 'No' for In house [*] and published physchem filters [+]
    - Select 'No' for PPIHitProfiler (Sperandio et. al.)
    - Select 'No' to Filter undesirable substructures moieties
    - Select 'Yes' to Retrieve covalent inhibitors
    - Select 'Yes' for Filter Pan Assay Interference Compounds (PAINS) Filter A
    - Select 'Yes' for Filter Pan Assay Interference Compounds (PAINS) Filter B
    - Select 'Yes' for Filter Pan Assay Interference Compounds (PAINS) Filter C
    - Choose 'regular' option for Lilly MedChem Rules (only detection, no triage)
    - Scroll up on top of the web page and to submit, click 'Run'

Following submission, we will get the pharmacokinetic characteristics of each ligand for each pocket, which are listed below:

Table 4.5.1: Pharmacokinetic Properties of Pocket ID

Ligands (Generation Number)	ID	MW	logP	logD	logSw	tPSA	Rotatable Bonds	Rigid Bonds	Flexibility
Gen 8	ID38	282.38	5.85	2.77	-4.98	40.13	6	11	0.35
Gen 18	ID 10	972.18	9.48	9.95	-11.3	131.58	13	70	0.16
Gen 19	ID 17	1111.21	0.74	-5.43	-4.93	318.01	37	37	0.5
Gen 14	ID 8	709.86	2.61	-1.11	-4.73	238.56	20	25	0.44
Gen 14	ID 20	1295.59	9.1	6.62	-12.6	299.16	17	86	0.17
Gen 19	ID 18	1123.31	8.34	5.78	-11.1	241.86	17	77	0.18
Gen 19	ID 11	983.31	8.86	6.1	-11.2	149.7	6	76	0.07

Table 4.5.1: Pharmacokinetic Properties of Pocket ID(Continued)

Ligands (Generation Number)	ID	HB Donnors	HB Acceptors	HBD_HBA	Rings	Max Size Ring	Charge	Total Charge	Heavy Atoms
Gen 8	ID38	1	2	3	1	6	1	-1	21
Gen 18	ID 10	2	10	12	9	10	0	0	74
Gen 19	ID 17	11	20	31	5	10	5	3	78
Gen 14	ID 8	7	14	21	3	10	2	2	50
Gen 14	ID 20	9	20	29	6	16	1	1	94
Gen 19	ID 18	1	18	19	9	19	1	1	81
Gen 19	ID 11	2	9	11	6	17	1	1	72

Table 4.5.1: Pharmacokinetic Properties of Pocket ID(Continued)

Ligands (Generation Number)	ID	Carbon Atoms	Hetero Atoms	Ratio H/C	Lipinski Violations	Solubility (mg/l)	SolubilityForecast Index	Oral Bioavailability (VEBER)	Oral Bioavailability (EGAN)
Gen 8	ID38	19	2	0.11	1	1934.31	Good Solubility	Good	Good
Gen 18	ID 10	64	10	0.16	2	11.98	Reduced Solubility	Low	Good
Gen 19	ID 17	55	23	0.42	3	8062.21	Good Solubility	Low	Good
Gen 14	ID 8	35	15	0.43	3	6245.26	Good Solubility	Low	Good
Gen 14	ID 20	73	21	0.29	4	4.29	Reduced Solubility	Low	Low
Gen 19	ID 18	61	20	0.33	3	16.33	Reduced Solubility	Low	Low
Gen 19	ID 11	62	10	0.16	2	13.44	Reduced Solubility	Good	Low

Table 4.5.1: Pharmacokinetic Properties of Pocket ID(Continued)

Ligands (Generation Number)	ID	Traffic Lights	4_400	3_75	Phospholipidosis	Fsp3	StereoCenters	PPI_Friendly	Status
Gen 8	ID38	2	good	bad	NonInducer	0.21	0	Not Computed	Accepted
Gen 18	ID 10	7	bad	warning	NonInducer	0.22	3	Not Computed	Accepted
Gen 19	ID 17	6	good	good	NonInducer	0.38	4	Not Computed	Accepted
Gen 14	ID 8	6	good	good	Inducer	0.46	4	Not Computed	Accepted
Gen 14	ID 20	8	bad	warning	NonInducer	0.52	18	Not Computed	Accepted
Gen 19	ID 18	8	bad	warning	NonInducer	0.38	13	Not Computed	Accepted
Gen 19	ID 11	6	bad	warning	Inducer	0.56	15	Not Computed	Accepted

According to the results of the FAF-Drugs4 filtering, any ligands with an Accepted status may be synthesized in the lab and, after undergoing several laboratory tests (from preclinical to phase 4 of a clinical trial), can be selected as an anti-Nipah drug.

CHAPTER 5

CONCLUSION

5.Conclusion

X-ray crystallography is still regarded as a costly and time-consuming method for determining protein structure, despite its excellent precision. As a result, comparative modeling enables us to forecast the structure and extends the field of potential proteins and antiviral medications. After the SWISS-MODEL web server successfully generated the 3D homology models of the glyco (G) protein of the Nipah virus, Ramachandran analysis was used to validate the models. Diagram creation and modification were aided using UCSF Chimera. The CASTp web server was used to help locate the inhibitory sites for the glyco (G) protein of the Nipah virus. The creation of a Nipah virus inhibitor may be possible in the future based on this work when we molecularly dock the structures in the pockets to determine the best-suited structure.

CHAPTER 6

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6. References

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