Project On

Determination of P 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

In the partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy

Submitted By

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Batch: 20th
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Approval

This project paper, entitled **"Determination of the P 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: 183-29-147) own investigation.

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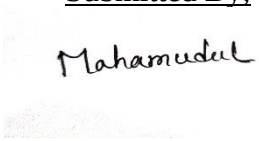
Declaration

I am Md. Mahamudul Hasan, ID: 183-29-147, 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 affirms that the work presented herein, entitled "Determination of the P 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.

Submitted By,



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My parents,
Teachers
And
All those people who have been supportive towards me throughout my life.

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I would want to express my sincere gratitude to Allah, the All-Powerful, for giving me the ability to complete my challenging task and the chance to focus on this topic.

I owe a great deal of gratitude to my project supervisor, Mr.Galib Muhammad Abrar Ishtiaque, Lecturer in the Department of Pharmacy at Daffodil International University, for his excellent leadership, steady oversight, provision of key information regarding the task, and assistance in its completion. I would like to express my humble regards to Dr. Muniruddin Ahmed, Professor, and Head, of the Department of Pharmacy, at Daffodil International University. I also wish to offer my respect to all of the teachers of the Pharmacy Department, at Daffodil International University, and thankful to other members for their excellent cooperation with us.

Last but not least, I would want to thank my parents and other family members for their great support and encouragement in helping me finish this project.

ABSTRACT

The primary objective of this study was to discover the major (P) protein of the Nipah virus's full-length 3D structure. This was done in preparation for a De Novo drug design that would provide potential Nipah virus inhibitor ligands. The Nipah virus' full structure was not included in the RCSB-PDB, thus homology modeling was used in conjunction with the Uni Prot KB sequence. The search for templates with the greatest sequence similarity and coverage was aided by BLAST. The whole sequence of the large (P) protein of the Nipah virus was entered into the i-TASSER server after loops and functional domains were joined by ab-initio modeling, which further predicted five models. The Ramachandran analysis was useful in validating such models. Using UCSF-Chimera software, the linking of the loops with the functional domains from the i-TASSER model was removed. With the use of a custom tool created in-house, these loops and pieces were connected. After the Swiss Pdb viewer server has completed its energy minimization, the CASTp server has delivered the identification of ligand binding pockets. Following the identification of the pockets, the e-LEA3D server assisted in creating the ligand molecules that would bind to those pockets. To facilitate the development of potential Nipah virus inhibitor medications in the future, the pharmacokinetic characteristics of each of those ligands were further evaluated on the Mobyle@RPBS website.

Key words: Nipah Virus, P Protein, De Novo Drug Design, Ab-initio Modelling, Nipah virus inhibitors.

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Chapter One : Introduction

1. Introduction

1.1.Nipah Virus

Unlike other priority diseases designated by the WHO, the Nipah virus, which is closely linked to the Hendra virus and belongs to the Paramyxoviridae family, is an emerging pathogen that is confined to Southeast Asia. The Nipah virus was recognized as a serious human pandemic in Malaysia for the first time in 1999, affecting 283 people and resulting in 109 deaths (1). Although there have been no other instances in Malaysia since then, outbreaks have happened on occasion in India and Bangladesh. Since 2001, the Nipah virus has infected hundreds of individuals, with a 75% mortality rate (2). Nipah virus's host reservoir has a large geographical range and the potential for zoonotic and human transmission. The WHO has designated the Nipah virus as a high-priority infection because of its limitations in prevention and treatment. However, there have been few validated and controlled diagnostic studies for the Nipah virus. The availability of Nipah virus treatment is still unknown, since there are no recognized medicines or vaccinations on the market, leaving only supportive care(3

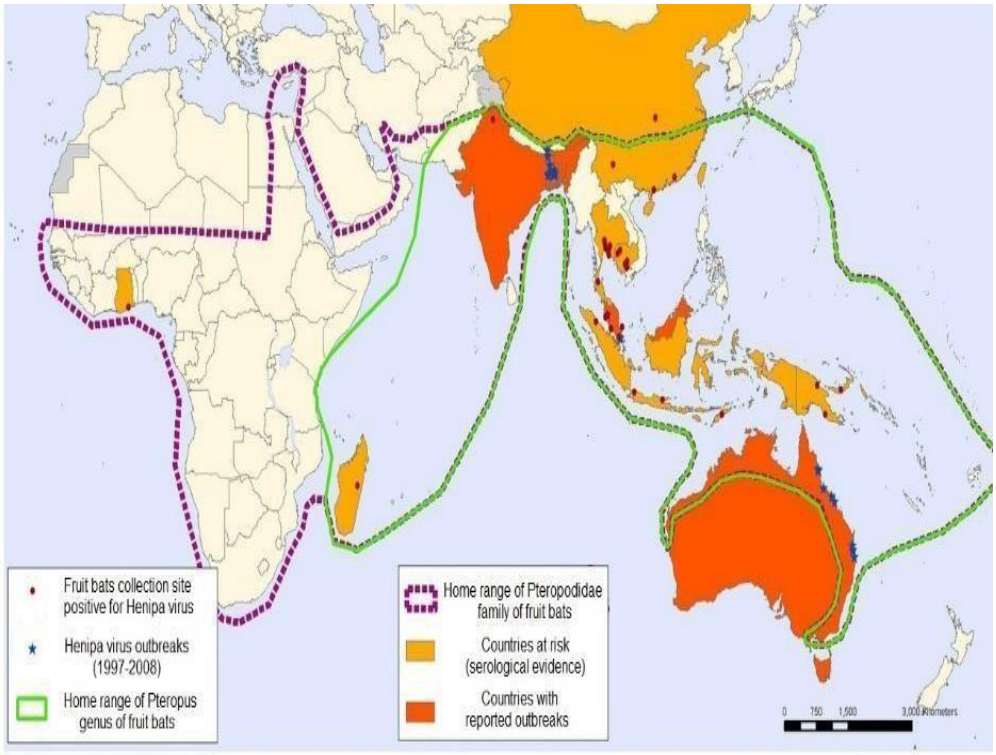
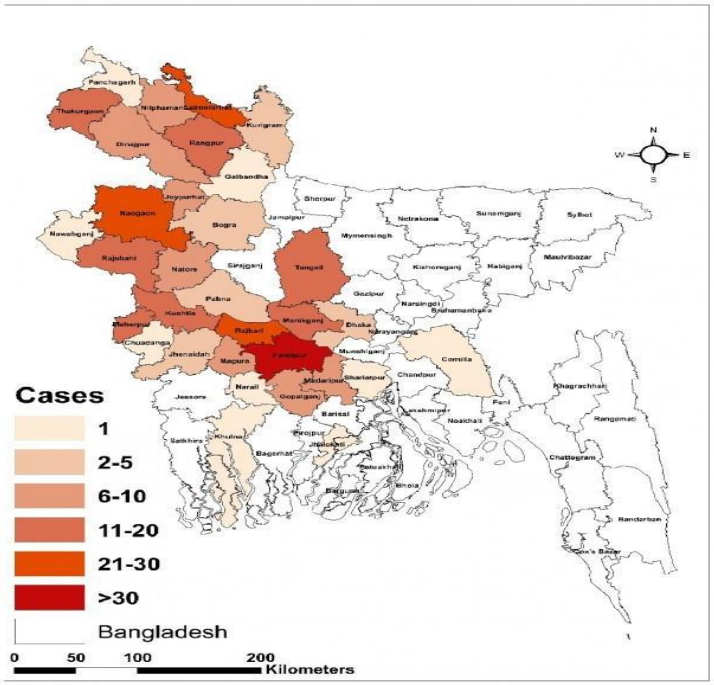


Figure 1.1.1: Geographic distribution of Henipavirus outbreaks and fruit bats of Pteropopodidae Family.

Nipah virus infection was studied between 2001 and 2004 in Bangladesh, where 92 individuals had confirmed or very probable cases of the illness. A 73% death rate was achieved with 67 patients out of 92 participants (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



It was noted in the World Health Organization's (WHO) surveillance and outbreak alert report that, despite having better nursing and treatment facilities and public awareness campaigns, the morbidity and mortality rates of Nipah virus infection did not decrease (Table 1.1), especially during the winter and spring, which is thought to be the bats' breeding season, which is believed to be the main carrier of the virus (6).

Table 1.1: Chronology of Nipah outbreaks in Bangladesh

TIMELINE	NO. OF CASES	NO. OF DEATHS	RATE OF MORTALITY
2001	13	9	69%
2002	0	0	0
2003	12	8	67%
2004	67	50	74.63%
2005	13	11	84%
2006	0	0	0.00%
2007	18	9	50%
2008	11	9	81%
2009	4	0	0.00%
2010	18	16	88.89%
2011	42	36	85.71%
2012	18	13	72.22%
2013	26	22	84.62%
2014	38	15	39%
2015	18	11	61%
2016	0	0	0.00%
2017	3	2	66.67%
2018	4	3	75%
2019	8	7	87.50%
2020	6	4	66.67%
2021	2	0	0.00
TOTAL	321	225	70.9%

1.2 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 typically 4–14 days. Nipah virus primarily affects the central nervous system (CNS) by infection of endothelial, vascular, and parenchymal cells, with increased viral replication in neuronal bodies (9). The early stages of Nipah virus infection often present as fever encephalitis or pneumonia and may be difficult 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).

1.3 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 since there is no medicine or vaccination available. There is evidence that ribavirin reduces mortality (12). yet the Nipah virus infection has not yet been completely shown to be resistant to it (13)

1.4 Treatment

There is presently no treatment for the Nipah virus. Consult your doctor right once if you have 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 sole treatment for a Nipah virus-infected patient is intensive supportive care (14).

1.5 Morphology

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

1.6 Genetic Diversity

The Nipah virus is known to have two important genetic lineages that are known to be disease-causing in humans (16).

i. Nipah Virus- Malaysia (NiV-MY)

ii. Nipah Virus –Bangladesh(Niv-BD)

1.7 Genome Size and Structure

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

1.8 Sequence of Nipah Virus Polyprotein

The large (P) protein of Nipah virus has a molecular mass of **53,898**Dalton and consists of **507** amino acids (Retrieved from: *https://www.uniprot.org/uniprotkb/A0A2Z5VFT7*). The amino acid sequence of large (P) protein of Nipah virus is as follows:

MAEEQARHVKNGLECIRALKAEPIGSLAVEEAMAAWSEISDNPGQDRATCKEEEAG
SSGLSKPCLSAIGSTEGGAPRIRGQGSGESDDDAETLGIPSRNLQASSTGLQCYHVYD
HSGEAVKGIQDADSIMVQSGLDGDSTLSGGDDESENSDVDLGEPDTEGYAITDRGSA
PISMGFRASDVETAEGGEIHELLKLQSRGNNFPKLGKTLNVPPPPNPSRASTSETPIKK
GTDARLASFGTEIASLLTGGATQCARKSPSEPSGPGAPAGNVPECVSNAALIQEWTPE
SGTTISPRSQNNEEGGDYYDDELFSDVQDIKTALAKIHEDNQKIISKLESLLLLKGEVE
SIKKQINRQNISISTLEGHLSSIMIAIPGLGKDPNDPTADVELNPDLKPIIGRDSGRALAE
VLKKPVASRQLQGMTNGRTSSRGQLLKEFQLKPIGKKVSSAVGFVPDTGPASRSVIR
SIIKSSRLEEDRKRYLMTLLDDIKGANDLAKFHQMLMKIIMK

1.9 Functional Domains

1. The large (P) protein of Nipah virus has functional domains:

(1) Paramyxo_P_V_N domain

Position: 4-312
Amino Acid Sequence:

EQARHVKNGLECIRALKAEPIGSLAVEEAMAAWSEISDNPGQDRATCKEEEAGSSGL
SKPCLSAIGSTEGGAPRIRGQGSGESDDDAETLGIPSRNLQASSTGLQCYHVYDHSGE
AVKGIQDADSIMVQSGLDGDSTLSGGDDESENSDVDLGEPDTEGYAITDRGSAPISM
GFRASDVETAEGGEIHELLKLQSRGNNFPKLGKTLNVPPPPNPSRASTSETPIKKGTD
ARLASFGTEIASLLTGGATQCARKSPSEPSGPGAPAGNVPECVSNAALIQEWTPESGT
TISPRSQNNEEGGDYYDDELF

Chapter Two: Purpose Of The Study

2.Purpose of the study.

To determine the P protein of Nipah Virus in an in silico approach and identify ligands that can be used as a potential new anti-nipah viral drug.
This study will illustrate the structure of the P protein in silico which will further identify the possible targets for an Anti-Nipah viral drug.

Chapter Three: Materials and 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. Normal Mode Analysis, Deformation, and Refinement (NOMAD-Ref)

viii. Computed Atlas of Surface Topography of Proteins (CASTp)

ix. e-LEA3D web server

x. Mobyle RPBS web portal

xi. The below-mentioned methods were used in this research:

xii. ab-initio modelling

xiii. Ramachandran plot analysis

xiv. Structure energy minimization

xv. Determination of ligand binding pocket

xvi. Ligand design

xvii. Determination of the pharmacokinetic property of ligand

Chapter Four: Procedure & Results

4.1Methods for Molecular Modelling of Nipah Virus

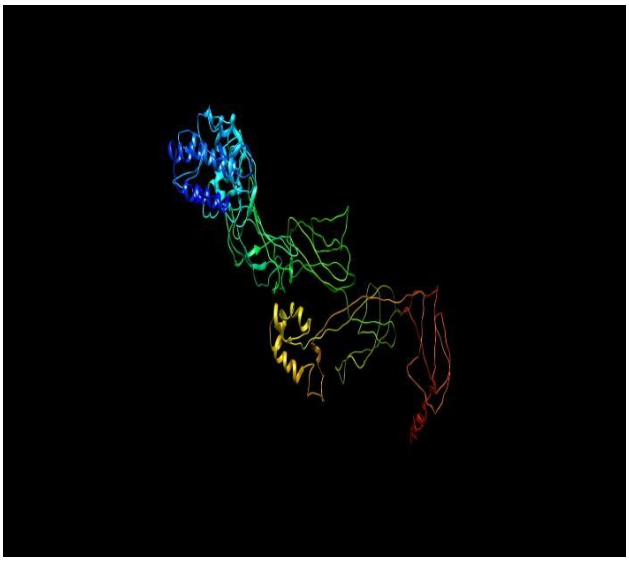
A well-known and publicly accessible database called UniProt contains the functional details and amino acid sequences of every protein discovered as a result of genome sequencing initiatives. It has become possible to preserve a significant quantity of knowledge on the biological activity of proteins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

4.2 ab-initio Modelling

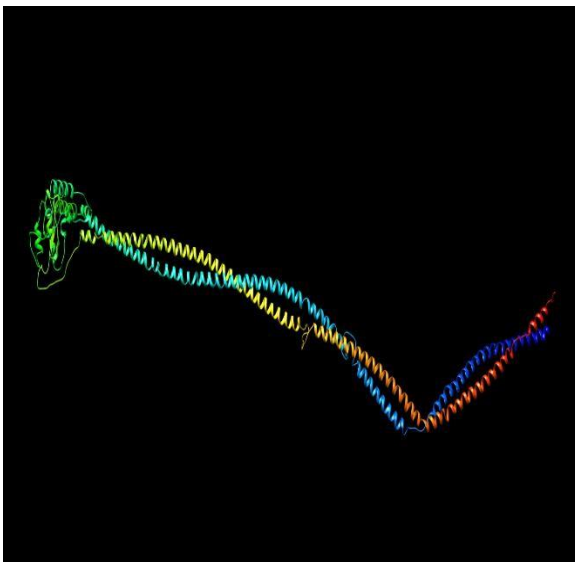
4.1.2i-TASSER Modeling

In the first step of ab-initio modelling, i-TASSER server was accessed first. In the i-TASSER server, the full-length sequence of large (L) protein of Nipah virus was submitted which led to the prediction of 5 full-length structure of the large (L) protein of Nipah virus.

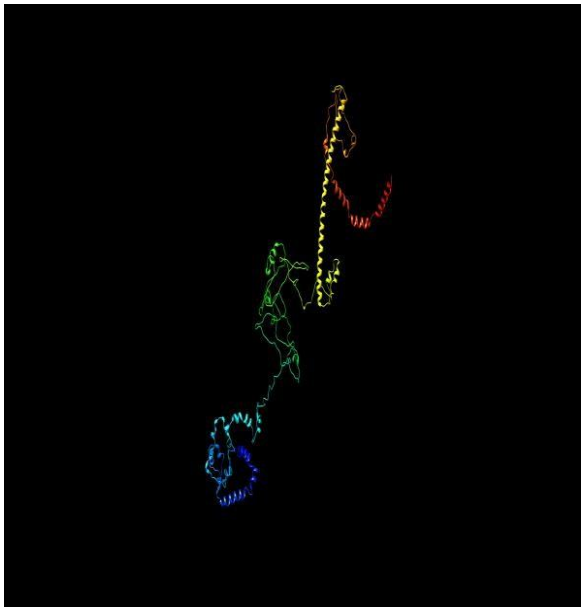
By using the UCSF Chimera software, the ribbon structure of all five predicted models are given below-



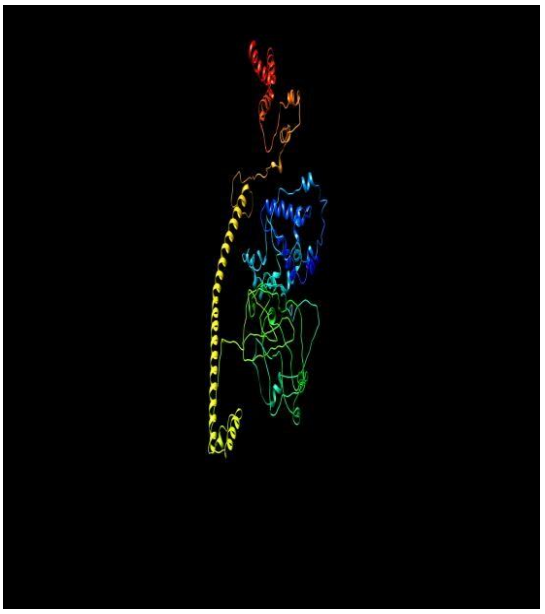
MODEL 1



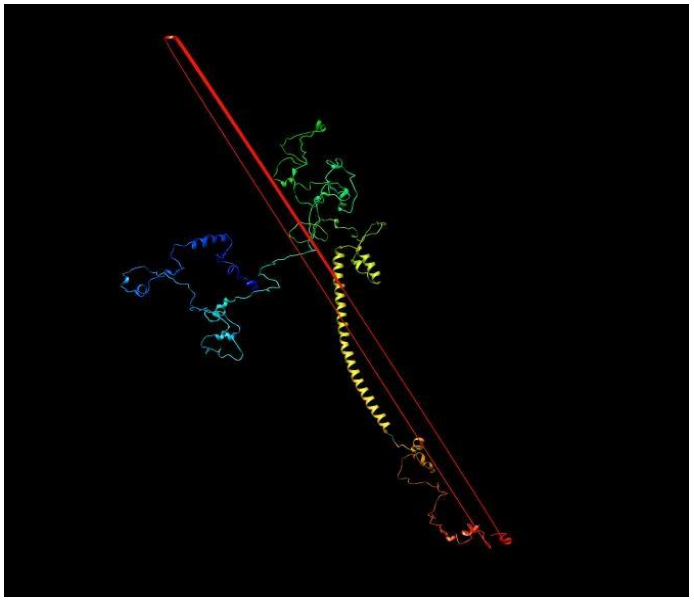
MODEL 2



MODEL 3



MODEL 4



MODEL 5

4.1.1Ramachandran 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 4.2.2: Ramachandran plot assessment of all 5 predicted models.

Model Number	Favoured Region (FR)	Allowed Region (AR)	Outlier Region (OR)	FR+AR	Inference
Model 1	74.384%	20.361%	5.255%	94.745%	Bad
Model 2	91.790%	6.897%	1.314%	98.687%	Good
Model 3	77.011%	18.719%	4.269%	95.73%	Medium
Model 4	74.384%	20.525%	5.090%	94.909	Bad
Model 5	66.174%	24.130%	9.688%	90.312	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.

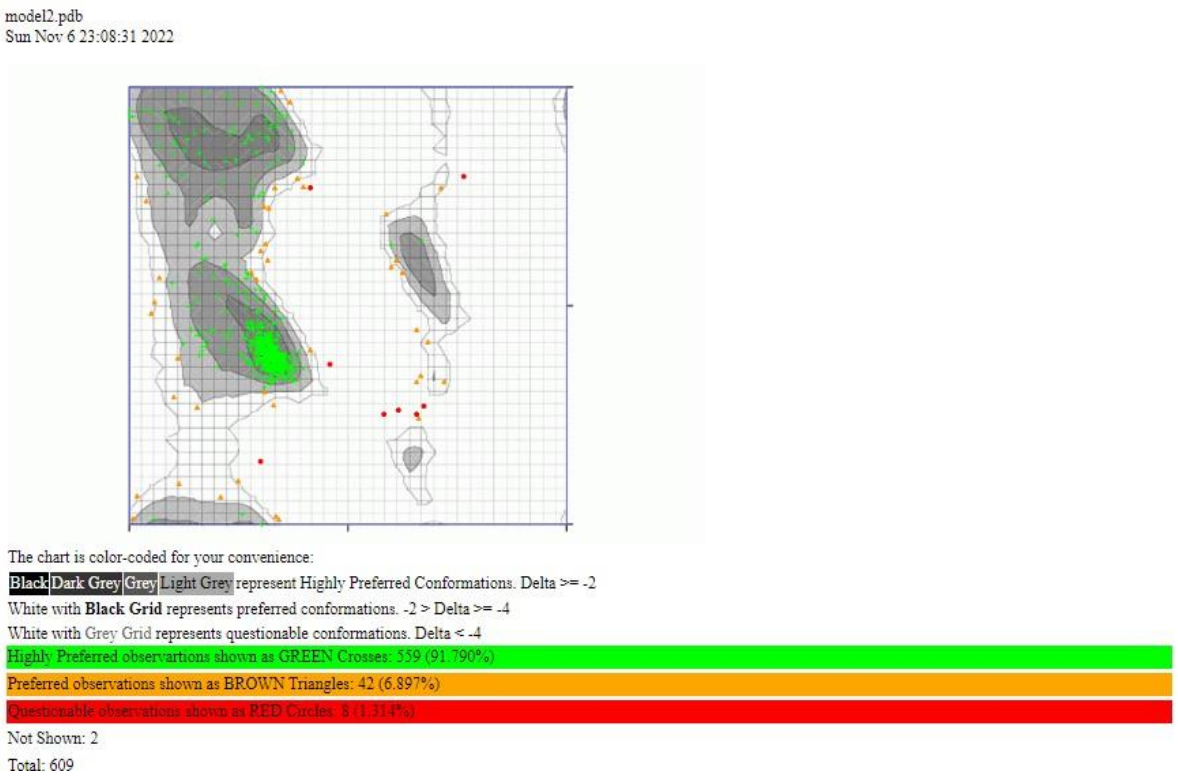


Figure 4.2.3: Ramachandran plot analysis report of i-TASSER predicted model 2.

4.2: Swiss Pdb viewer Energy Minimization.

An program called Swiss-PdbViewer (also known as DeepView) has a user-friendly interface that enables simultaneous analysis of many proteins. To compare active sites or other important components and determine structural alignments, the proteins may be overlaid. Thanks to the user-friendly visual and menu interface, it is simple to access information on amino acid mutations, H-bonds, angles, and distances between atoms.

Since 1994, Nicolas Guex has been developing Swiss-PdbViewer (also known as DeepView). The automated homology modeling server SWISS-MODEL, which was created by the Swiss Institute of Bioinformatics (SIB) at the Structural Bioinformatics Group at the Biozentrum in Basel, was originally closely related to Swiss-PdbViewer. Nevertheless, the SWISS-MODEL online interface has matured to the point that it can currently be used directly for complex modeling. The direct interface with Swiss-PdbViewer is no longer maintained since it is too difficult to maintain.

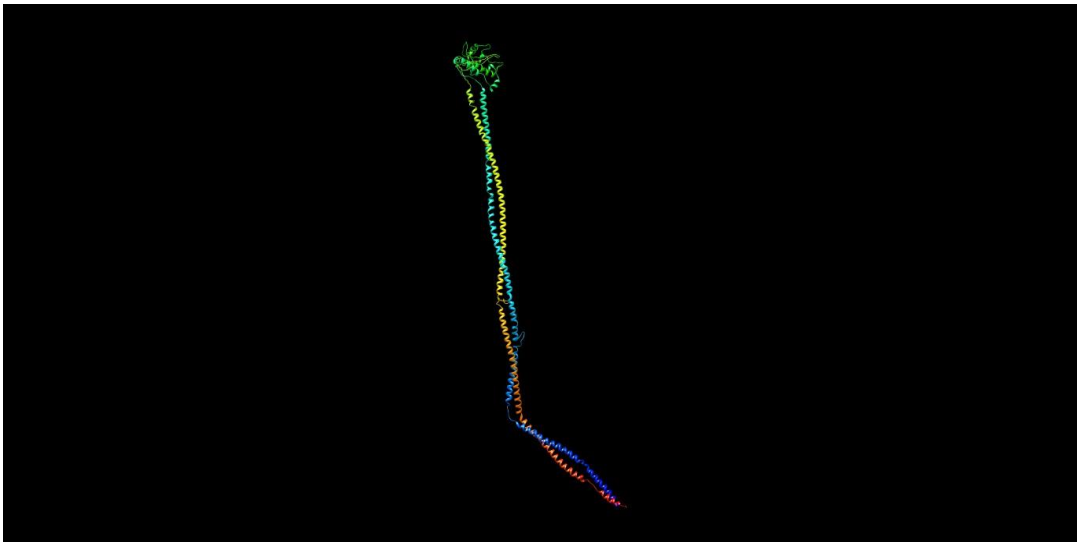


Figure:4.2:4 Swiss Pdb viewer Energy Minimization.

4.3 Ligand Binding Pocket Determination

Once energy is minimized and the full-length structure of the large (P) protein of Nipah virus is submitted in the PDB format to the CASTp server, ligand binding pockets were determined.

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After CASTp server determines all the pockets, only those pockets that have an MS volume of less than 1071 but more than 50, with a number of openings equal to 1 are chosen for further experiment. Then they are sorted from the highest to the lowest value of MS pocket area. A total of 15 pockets were shortlisted like this.

ID	MS VOLUME	Poket Ms Area	number of opening	Mouth Ms Area	Ms Circumference	XS	Y	Z
10	51	56.5	1	26.4	21.2	210.84	298.069	224.259
24	51.7	61.9	1	16.3	15.4	199.427	327.587	223.321
17	55.1	61.7	1	23.6	18.6	305.507	33.369	232.304
16	55.5	63.8	1	13	13.5	197.161	362.648	231.395
22	56	69.3	1	13.3	13.2	227.587	133.447	224.817
4	58.5	22.2	1	84.7	36.1	192.303	346.553	225.408
13	63.6	69.1	1	21	17	220.108	200.032	235.689
23	66.8	93.5	1	18.5	16.2	278.059	57.521	226.452
14	67.7	73.6	1	22.3	18.3	199.173	338.675	223.575
8	70.5	69.5	1	31.1	22.2	186.612	367.302	197.13
19	76.9	108.5	1	10.2	12.5	270.401	63.749	225.638
5	113.2	91.9	1	45.5	28	296.622	45.289	232.321
3	161.7	130.9	1	70.1	40.2	229.771	94.212	221.311
7	177.2	227.2	1	19.2	18.4	188.897	374.297	208.35
2	252.4	172.2	1	86.3	46.6	197.05	369.44	224.588

4.3.1 Ligand Binding Pocket Determination4 Ligand Design

4.4: Ligand Design

e-LEA3D server is used to design the ligands that will bind to the binding sites of the pockets, which was previously determined using the CASTp web server. Ligand molecule is generated for each of the energy minimized structures of the large (L) protein of the Nipah virus by following the below steps –

1. e-LEA3D web server is accessed by visiting
2.S elect ‘Drug design or Screen’ and click ‘Enter’
3.Upload the energy-minimized structure of the protein in PDB format
4. Binding site coordinates (x, y, z) are given as well
5.Input the value of ‘Binding site radius’ and ‘Weight in final score’ as 3.0 and respectively
6.Click ‘Submit’
7.In the following page, input the email address, select ‘De-novo Drug Design’

The findings for all 15 pockets will then be sent to the specified email address via the e-LEA3D server. It will produce 11 ligands for each pocket, which will then be arranged once again in decreasing order according to each ligand's best energy score and binding affinity percentage (%).
The best energy score and percentage score of binding affinity for each of the 15 ID is shown below

Table 4.4.1: Ligand design of ID 02

Generation number	Score of Binding Affinity (%)	Best Score of Energy
Gen16	75.42	-113.13
Gen17	74.38	-111.570
Gen15	74.15	-111.230
Gen12	73.95	-110.930
Gen14	73.88	-110.820
Gen13	73.72	-110.58
Gen18	73.63	-110.44
Gen08	73.61	-110.420
Gen05	73.48	-110.22
Gen09	73.29	-109.940
Gen11	73.21	-109.810
Gen10	71.8	-107.700
Gen06	71.65	-107.480
Gen07	71.64	-107.46
Gen04	65.61	-98.410
Gen03	65.02	-97.53
Gen01	60.03	-90.050

Table 4.4.2: Ligand design of Pocket ID 24 ,14

Generation number	Score of Binding Affinity (%)	Best Score of Energy
Gen13	83.53	-125.29
Gen17	80.93	-121.39
Gen11	80.45	-120.67
Gen08	80.26	-120.39
Gen14	79.81	-119.710
Gen09	77.93	-116.900
Gen16	77.22	-115.830
Gen19	76.79	-115.180
Gen18	76.69	-115.03
Gen10	76.46	-114.690
Gen15	75.69	-113.530
Gen12	75.37	-113.05
Gen06	73.93	-110.890
Gen07	69.49	-104.240
Gen04	62.86	-94.290
Gen05	60.12	-90.180
Gen02	59.29	-88.94
Gen03	57.9	-86.85
Gen01	55.73	-83.600
Gen00	44.85	-67.280

Generation number	Score of Binding Affinity (%)	Best Score of Energy
Gen18	83.8	-125.700
Gen13	83.73	-125.6
Gen15	82.27	-124.400
Gen17	81.98	-122.970
Gen16	78.93	-118.390
Gen12	78.7	-118.05
Gen11	77.55	-116.32
Gen10	77.21	-115.82
Gen14	77.14	-115.710
Gen09	77.11	-115.67
Gen08	75.92	-113.88
Gen06	75.02	-112.530
Gen19	74.84	-112.26
Gen07	74.56	-111.84
Gen04	67.75	-101.62
Gen05	67.16	-100.74
Gen03	65.58	-98.37
Gen00	63.68	-95.52
Gen01	62.04	-93.06
Gen02	61.89	-92.83

Table 4.4.3: Ligand design of Pocket ID 23,17

Generation number	Score of Binding Affinity (%)	Best Score of Energy
Gen19	95.47	-143.2
Gen17	84.53	-126.800
Gen16	80.15	-120.22
Gen18	79.73	-119.590
Gen15	76.76	-115.140
Gen14	74.14	-111.21
Gen13	73.01	-109.510
Gen11	69.46	-104.190
Gen08	69.01	-103.510
Gen10	67.7	-101.55
Gen12	67.59	-101.39
Gen09	65.27	-97.9
Gen07	60.19	-90.29
Gen06	57.43	-86.15
Gen05	55.85	-83.780
Gen04	54.78	-82.17
Gen03	52.97	-79.46
Gen01	52.96	-79.44
Gen02	52.14	-78.21
Gen00	49.66	-74.490

Generation number	Score of Binding Affinity (%)	Best Sco of Energ
Gen19	91.32	-136.98
Gen13	90.42	-135.63
Gen17	89.81	-134.72
Gen18	89	-133.5
Gen15	88.85	-133.28
Gen16	86.74	-130.11
Gen14	85.97	-128.96
Gen11	84.93	-127.39
Gen10	84.43	-126.65
Gen12	83.04	-124.56
Gen09	77.85	-116.78
Gen08	73.32	-109.98
Gen07	71.81	-107.71
Gen06	63.45	-95.170
Gen05	62.23	-93.34
Gen04	60.15	-90.23
Gen03	58.91	-88.360
Gen02	55.52	-83.280
Gen01	53.28	-79.92
Gen00	52.49	-78.74

Table 4.4.4: Ligand design of Pocket ID 10,3

Generation number	Score of Binding Affinity (%)	Best Score of Energy
ff Gen12	69.4	-104.100
Gen11	68.8	-103.200
Gen09	68.57	-102.85
Gen19	68.33	-102.5
Gen18	68.06	-102.090
Gen17	67.54	-101.310
Gen14	66.99	-100.480
Gen08	66.05	-99.08
Gen15	65.82	-98.730
Gen16	65.79	-98.68
Gen10	65.73	-98.6
Gen13	64.01	-96.01
Gen07	63.43	-95.14
Gen05	62.76	-94.14
Gen06	62.03	-93.04
Gen04	60.37	-90.55
Gen03	58.09	-87.14
Gen02	56.06	-84.090
Gen01	52.83	-79.24
Gen00	46.69	-70.04

Generation number	Score of Binding Affinity (%)	Best Score of Energy
Gen18	86.12	-129.18
Gen16	83.6	-125.400
Gen19	83.29	-124.930
Gen10	83.22	-124.830
Gen17	82.57	-123.860
Gen11	81.59	-122.39
Gen14	81.53	-122.29
Gen13	81.49	-122.240
Gen09	81.46	-122.190
Gen08	81.03	-121.550
Gen15	80.35	-120.520
Gen05	80.09	-120.14
Gen12	79.17	-118.76
Gen06	78.77	-118.15
Gen04	78.35	-117.530
Gen07	77.21	-115.81
Gen03	74.68	-112.020
Gen00	71.05	-106.58
Gen02	68.61	-102.92
Gen01	68.51	-102.76

Table 4.4.5: Ligand design of Pocket ID 13,4

Generation number	Score of Binding Affinity (%)	Best Score of Energy
Gen08	80.85	-121.280
Gen18	72.9	-109.35
Gen16	71.38	-107.070
Gen13	70.03	-105.04
Gen10	69.89	-104.83
Gen19	68.99	-103.48
Gen14	68.85	-103.270
Gen11	67.2	-100.8
Gen17	66.88	-100.320
Gen15	66.68	-100.020
Gen09	66.59	-99.89
Gen07	65.39	-98.09
Gen12	65.21	-97.820
Gen06	64.66	-96.99
Gen03	57.72	-86.580
Gen05	57.65	-86.47
Gen04	57	-85.5
Gen02	55.64	-83.460
Gen01	47.78	-71.670
Gen00	39.08	-58.62

Generation number	Score of Binding Affinity (%)	Best Score of Energy
Gen08	80.85	-121.280
Gen18	72.9	-109.35
Gen16	71.38	-107.070
Gen13	70.03	-105.04
Gen10	69.89	-104.83
Gen19	68.99	-103.48
Gen14	68.85	-103.270
Gen11	67.2	-100.8
Gen17	66.88	-100.320
Gen15	66.68	-100.020
Gen09	66.59	-99.89
Gen07	65.39	-98.09
Gen12	65.21	-97.820
Gen06	64.66	-96.99
Gen03	57.72	-86.580
Gen05	57.65	-86.47
Gen04	57	-85.5
Gen02	55.64	-83.460
Gen01	47.78	-71.670
Gen00	39.08	-58.62

Table 4.4.6: Ligand design of Pocket ID 22,16

Generation number	Score of Binding Affinity (%)	Best Score of Energy
Gen18	97.97	-146.95
Gen14	96.37	-144.56
Gen12	96.23	-144.35
Gen13	95.55	-143.33
Gen17	95.45	-143.18
Gen19	95.4	-143.1
Gen16	93.58	-140.37
Gen15	92.39	-138.58
Gen11	85.97	-128.96
Gen08	85.45	-128.17
Gen09	85.21	-127.820
Gen10	85.13	-127.7
Gen07	85.09	-127.63
Gen06	83.64	-125.46
Gen05	79.75	-119.620
Gen04	73.69	-110.54
Gen03	71.29	-106.94
Gen02	66.96	-100.440
Gen00	66.91	-100.37
Gen01	66.15	-99.22

Generation number	Score of Binding Affinity (%)	Best Score of Energy
Gen14	79.11	-118.66
Gen10	78.78	-118.17
Gen19	76.98	-115.470
Gen16	76.43	-114.64
Gen13	76.27	-114.41
Gen15	75.61	-113.42
Gen12	75.52	-113.28
Gen17	75.25	-112.88
Gen18	74.73	-112.1
Gen09	74.09	-111.140
Gen11	72.85	-109.27
Gen08	68.45	-102.68
Gen07	64.97	-97.46
Gen06	58.53	-87.800
Gen05	54.75	-82.120
Gen02	48.89	-73.330
Gen03	48.89	-73.330
Gen04	48.89	-73.33
Gen01	48.63	-72.950
Gen00	48.07	-72.1

Table 4.4.7: Ligand design of ID 8,7

Generation number	Score of Binding Affinity (%)	Best Score of Energy
Gen15	81.88	-122.82
Gen19	79.86	-119.79
Gen18	79.56	-119.34
Gen17	78.83	-118.240
Gen16	78.72	-118.080
Gen13	77.16	-115.74
Gen14	76.91	-115.36
Gen12	72.61	-108.92
Gen11	65.59	-98.39
Gen10	64.33	-96.49
Gen09	60.98	-91.47
Gen06	60.82	-91.23
Gen07	60.77	-91.16
Gen08	60.36	-90.540
Gen05	58.52	-87.78
Gen04	56.73	-85.1
Gen03	54.03	-81.05
Gen01	51.24	-76.86
Gen02	51.14	-76.710
Gen00	39.58	-59.370

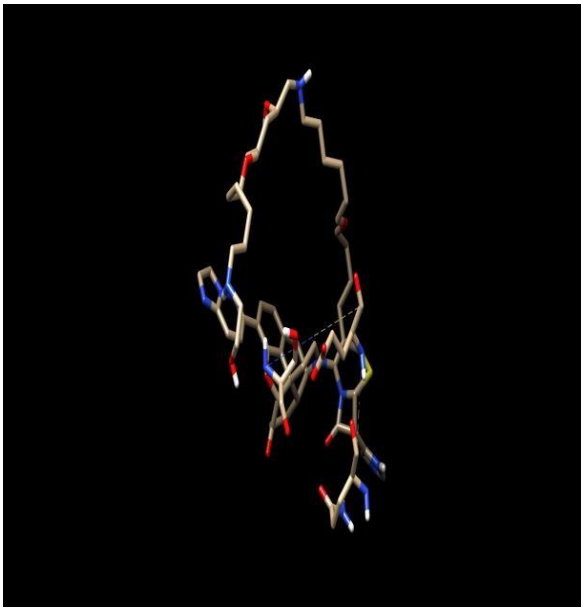
Generation number	Score of Binding Affinity (%)	Best Score of Energy
Gen17	78.13	-117.2
Gen19	76.95	-115.43
Gen18	76.65	-114.98
Gen16	76.62	-114.93
Gen15	72.87	-109.3
Gen12	71.88	-107.82
Gen14	69.3	-103.95
Gen13	67.29	-100.9
Gen11	60.26	-90.39
Gen09	60.18	90.27
Gen07	58.51	-87.77
Gen08	58.06	-87.09
Gen06	58.04	-87.06
Gen05	57.5	-86.250
Gen03	57.39	-86.090
Gen10	55.73	-83.600
Gen04	53.87	-80.8
Gen02	51.23	-76.850
Gen01	51.01	-76.520
Gen00	49.83	-74.750

Table 4.4.8: Ligand design of ID 19,5

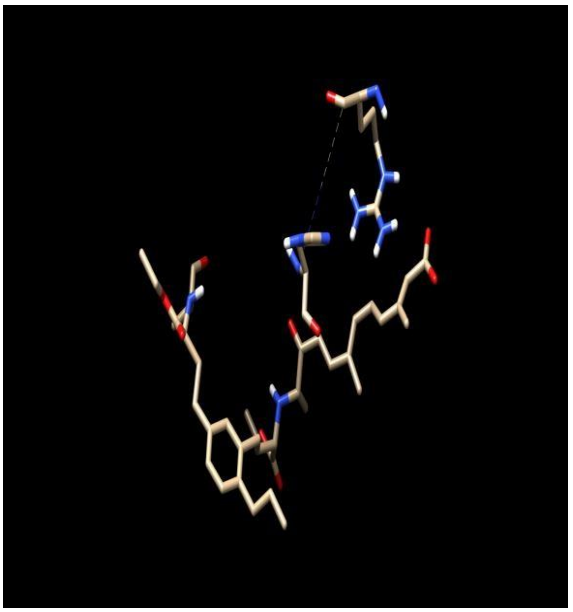
Generation number	Score of Binding Affinity (%)	Best Score of Energy
Gen15	81.21	-121.81
Gen18	79.48	-119.220
Gen14	78.26	-117.39
Gen19	78.16	-117.24
Gen13	78.1	-117.15
Gen17	77.69	-116.53
Gen08	76.79	-115.18
Gen11	74.67	-112
Gen16	74.1	-111.15
Gen06	73.69	-110.53
Gen12	73.2	-109.8
Gen10	71.45	-107.17
Gen07	69.75	-104.63
Gen09	69.41	-104.12
Gen05	64.91	-97.370
Gen03	64.41	-96.62
Gen04	61.53	-92.29
Gen01	55.52	-83.280
Gen02	55.4	-83.100
Gen00	54.17	-81.250

Gen15	81.21	**-121.810**
Gen18	79.48	-119.22
Gen14	78.26	-117.39
Gen19	78.16	-117.24
Gen13	78.1	-117.15
Gen17	77.69	-116.53
Gen08	76.79	-115.18
Gen11	74.67	-112.0
Gen16	74.1	-111.15
Gen06	73.69	-110.53
Gen12	73.2	-109.80
Gen10	71.45	-107.17
Gen07	69.75	-104.63
Gen09	69.41	-104.12
Gen05	64.91	-97.37
Gen03	64.41	-96.620
Gen04	61.53	-92.29
Gen01	55.52	-83.28
Gen02	55.4	-83.100
Gen00	54.17	-81.25

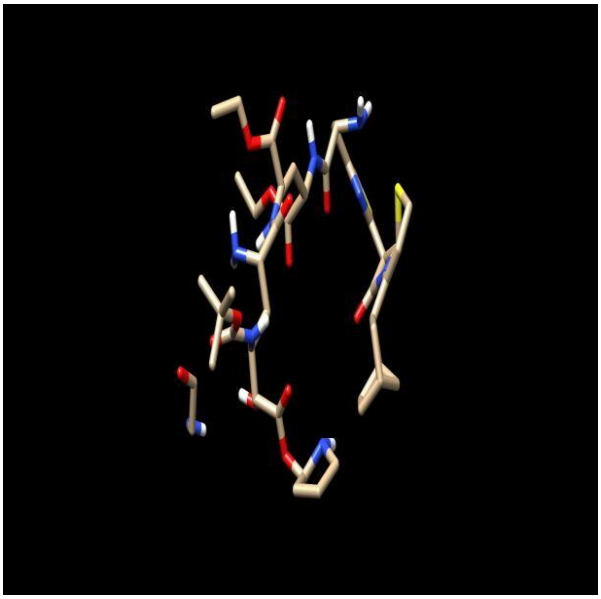
The Structures of the ligand molecules for each poket with the highest percentage of binding affinity and the highest energy score and listed below-



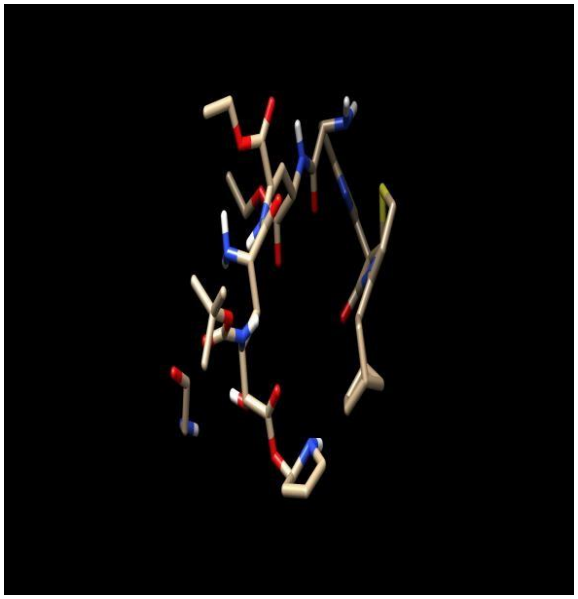
ID 3 Generation 18



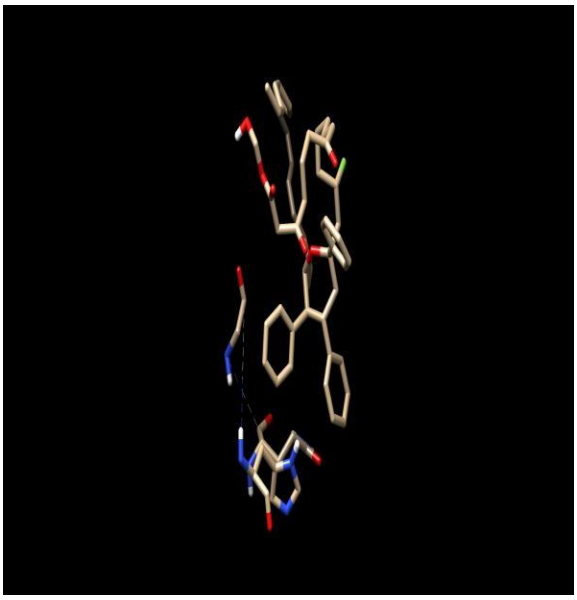
ID 4 Generation 8



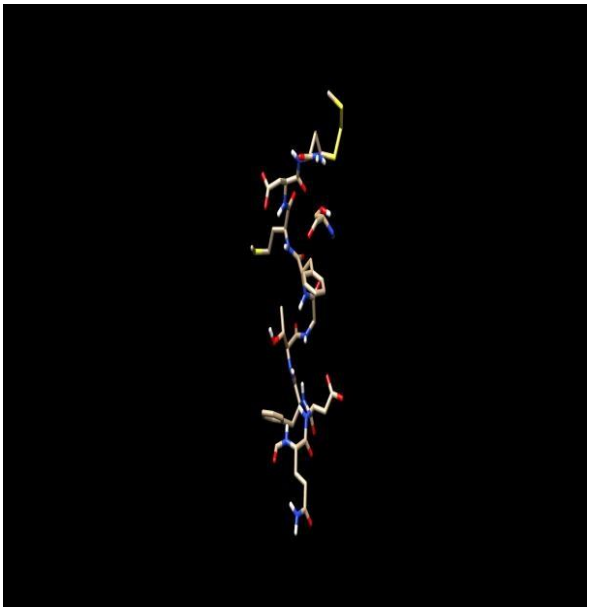
ID 8 Generation 15



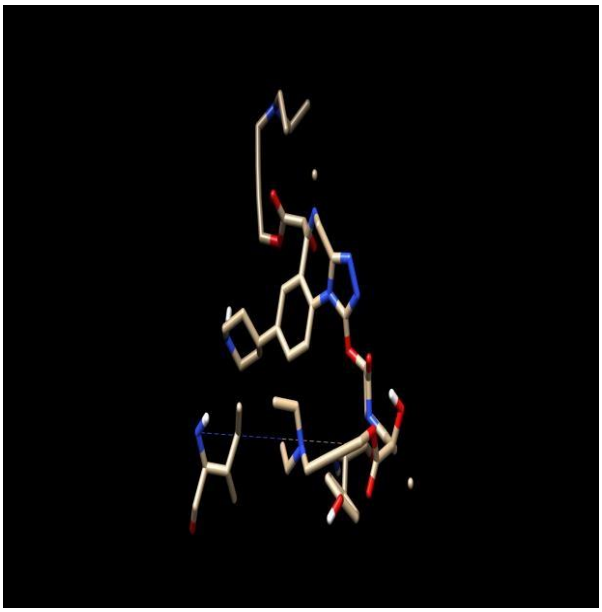
ID 10 Generation 12



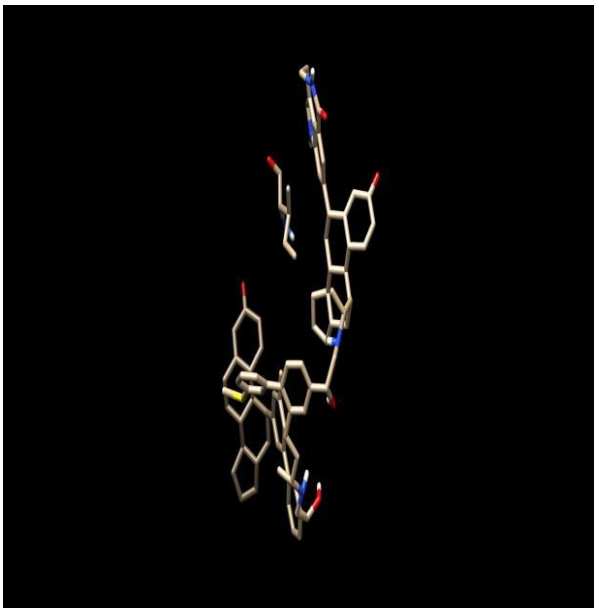
ID 13 Generation 18



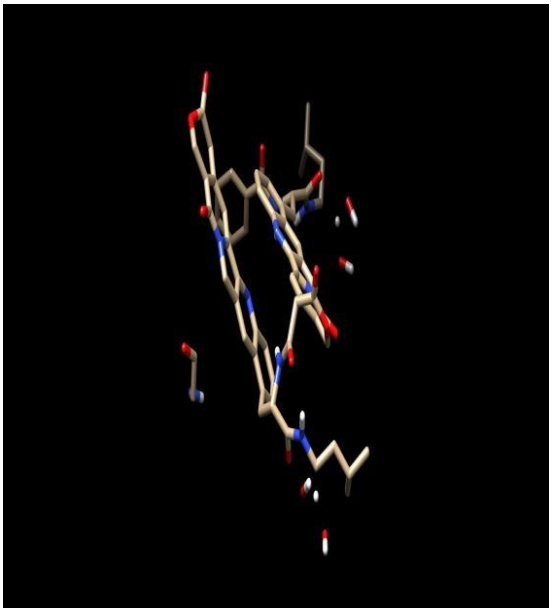
ID 16 Generation 14



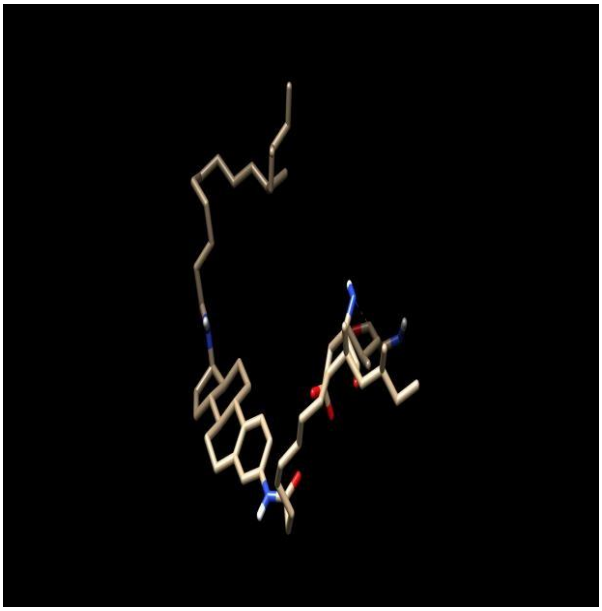
ID 22 Generation 18



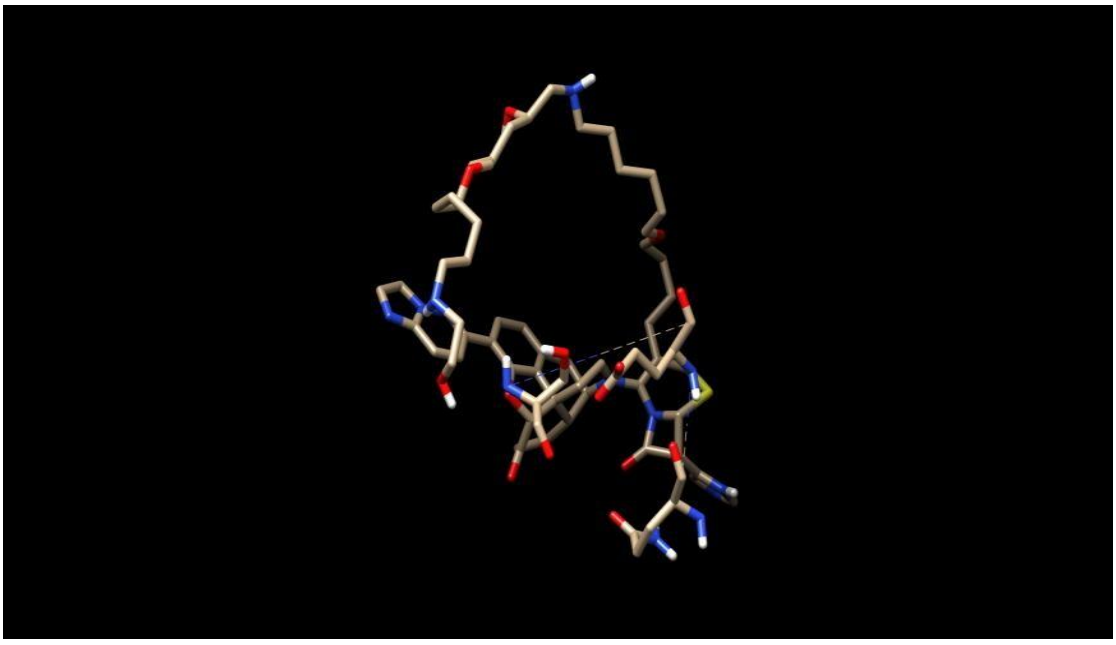
ID 23 Generation 19



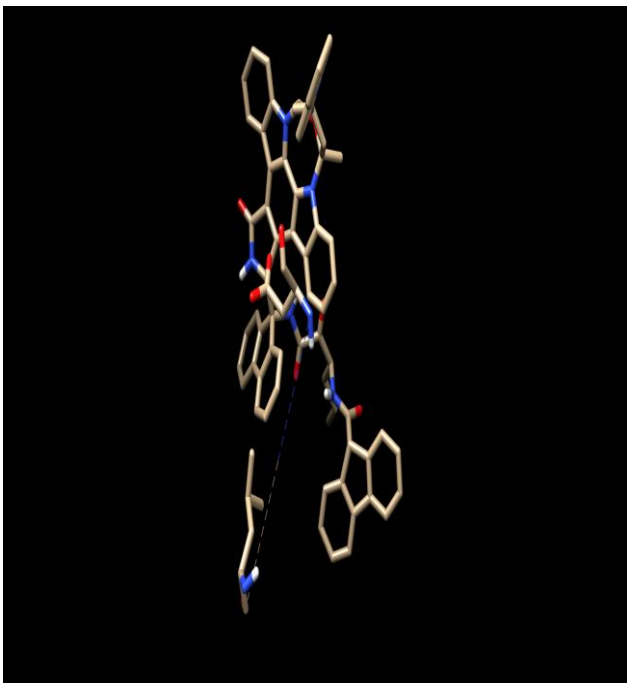
ID 02 Generation 16



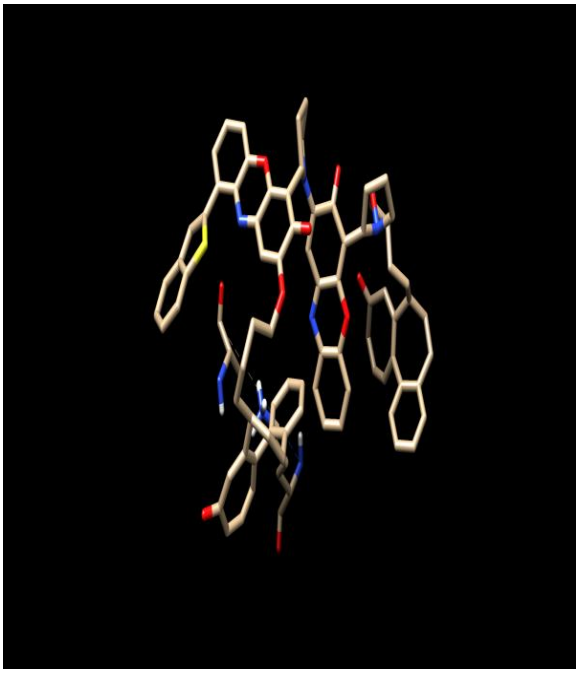
ID14 Generation 18



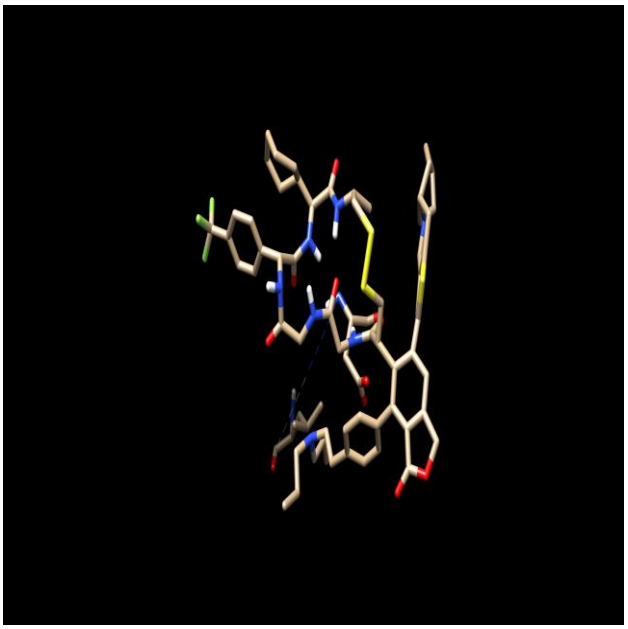
ID 24 Generation 13



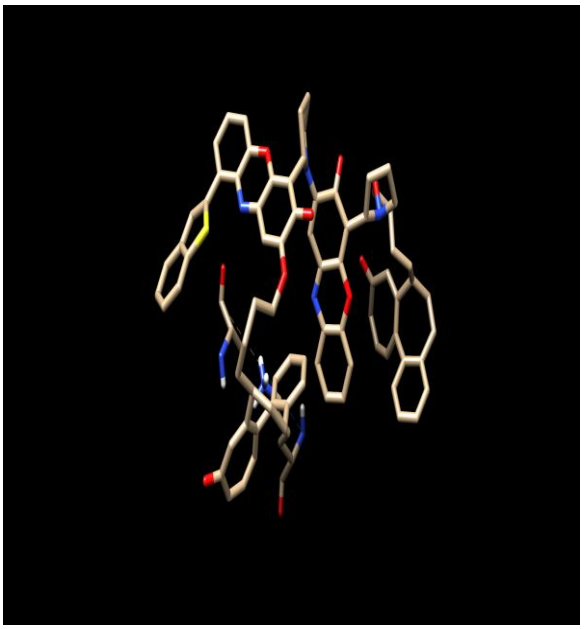
ID 7 Generation 19



ID 5 Generation 19



ID 19 Generation 15



ID 17 Generation 19

4.5 : Ligand Pharmacokinetic Property

DeterminationThe Mobyle RPBS online site may be used to evaluate 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 large (P) protein pockets

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

- Select 'No' for In house [*] and published physchem filters [+]
- Select 'No' for PPIHitProfiler (Sperandio et. al.)
- Select 'No' for Filter undesirable substructures moieties
- Select 'Yes' for 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'

Table 4.5.1: Pharmacokinetic Property of all pokets

ID Number	Genation Number	MW	LOgP	LOGD	logsw	tpsa	Rotable Bond	Rigid Bond
2	**Gene16**	**775.18**	**4.82**	**-3.21**	**-4.91**	**183.82**	**42**	**9**
14	**Gene15**	**1008.32**	**4.45**	**-1.51**	**-6.95**	**227.36**	**31**	**48**
23	**Gene19**	**1261.74**	**15.66**	**11.35**	**-16.4**	**178.01**	**19**	**82**
14	**Gen19**	**733.2**	**16.54**	**14.95**	**-13.12**	**58.2**	**26**	**24**
23	**Gen19**	**1261.74**	**15.66**	**11.35**	**-16.4**	**178.01**	**19**	**82**
17	**Gene19**	**704.08**	**10.69**	**4.45**	**-9.31**	**74.36**	**26**	**22**
10	**Gen 12**	**972.2**	**-0.71**	**-2.12**	**-3.44**	**296.96**	**32**	**34**
3	**Gen 13**	**775.18**	**4.82**	**-3.21**	**-4.91**	**183.82**	**42**	**9**
10	**Gen 12**	**924.13**	**-0.22**	**-1.37**	**-3.45**	**237.93**	**32**	**34**
13	**Gen 18**	**762.99**	**11.64**	**10.97**	**-11.85**	**89.9**	**2**	**55**
4	**Gen8**	**785.02**	**8.04**	**7.29**	**-8.06**	**150.93**	**27**	**21**
22	**Gen18**	**982.91**	**2.77**	**-0.08**	**-6.62**	**219.23**	**18**	**45**
7	Gen 19	1153.32	11.26	12.44	-13.7	171.88	9	93
5	Gen 20	1060.33	9.99	9.99	-10.83	227.38	31	45
19	Gen 15	1186.52	10.62	4.51	-13.06	259.52	14	70

Table 4.5.2: Pharmacokinetic Property of all pokets(Continued)

ID Number	Genation Number	Flexibility	HB Donors	HB Acceptors	HBD_HBA	Rings	Max Size Ring
2	**Gene16**	**0.82**	**6**	**11**	**17**	**1**	**7**
14	**Gene15**	**0.39**	**5**	**16**	**21**	**4**	**20**
23	**Gene19**	**0.19**	**6**	**9**	**15**	**8**	**17**
14	**Gen19**	**0.52**	**2**	**4**	**6**	**1**	**17**
23	**Gen19**	**0.19**	**6**	**9**	**15**	**8**	**17**
17	**Gene19**	**0.54**	**3**	**6**	**9**	**3**	**9**
10	**Gen 12**	**0.48**	**7**	**16**	**23**	**5**	**6**
3	**Gen 13**	**0.82**	**6**	**11**	**17**	**1**	**7**
10	**Gen 12**	**0.48**	**6**	**15**	**21**	**5**	**6**

13	Gen 18	0.04	1	6	7	3	40
4	Gen8	0.56	3	10	13	2	6
22	Gen18	0.29	2	19	21	3	14
7	Gen 19	0.09	3	14	17	6	30
5	Gen 20	0.41	5	16	21	6	6
19	Gene 15	0.17	5	14	19	7	20

Table 4.5.3: Pharmacokinetic Property of all pokets(Continued)

ID Number	Genation Number	Charge	Total charge	Heavy Atoms	Carbon Atoms	Hetero Atoms	Ratio H/C
2	Gene16	3	3	53	41	12	0.29
14	Gene15	3	3	72	55	17	0.31
23	Gene19	2	2	93	83	10	0.12
14	Gen19	0	0	53	49	4	0.08
23	Gen19	2	2	93	83	10	0.12
17	Gene19	2	2	51	45	6	0.13
10	Gen 12	5	1	69	52	17	0.33
3	Gen 13	3	3	53	41	12	0.29
10	Gen 12	5	1	67	52	15	0.29
13	Gen 18	0	0	56	49	7	0.14
4	Gen8	1	-1	57	47	10	0.21
22	Gen18	3	3	69	48	21	0.44
7	Gen 19	3	3	69	48	21	0.44
5	Gen 20	0	0	87	73	14	0.19
19	Gene15	0	0	78	62	16	0.26
7	Gen 19	2	2	82	62	20	0.32

Table 4.5.4: Pharmacokinetic Property of all pokets(Continued)

ID Number	Genation Number	Lipinski Violations	Solubility(mg/dl)	Solubility(Forcast index)	Oral Bioavailability(VERBER)
2	Gene16	3	5710.99	Good Solubility	Low
14	Gene15	2	968.14	Good Solubility	Low
23	Gene19	3	0.09	Reduced Solubility	Low
14	Gen19	2	1.47	Reduced Solubility	Good
23	Gen19	3	0.09	Reduced Solubility	Low
17	Gene19	2	63.51	Reduced Solubility	Good
10	Gen 12	3	31266.45	Good Solubility	Low
3	Gen 13	3	5710.99	Good Solubility	Low
10	Gen 12	3	29348.74	Good Solubility	Low
13	Gen 18	2	5.45	Reduced Solubility	Good
4	Gen8	2	247.09	Reduced Solubility	Low
22	Gen18	2	1304.65	Good Solubility	Low
5	Gen 20	2	1304.65	Good Solubility	Low
19	Gene15	3	1.3	Reduced Solubility	Good
7	Gen 19	3	20.92	Reduced Solubility	Low

Table 4.5.5: Pharmacokinetic Property of all pokets(Continued)

ID Number	Genation Number	Traffic Lights	Oral Bioavailability(EGAN)	4_400	3_75
2	Gene16	Good	7	bad	warning
14	Gene15	Good	7	bad	warning
23	Gene19	Low	8	bad	warning
14	Gen19	Good	6	bad	bad
23	Gen19	Low	8	bad	warning
17	Gene19	Good	6	bad	bad
10	Gen 12	Good	6	good	good
3	Gen 13	Good	7	bad	warning
10	Gen 12	Good	6	good	good
13	Gen 18	Good	4	bad	warning
4	Gen8	Low	8	bad	warning
22	Gen18	Good	6	good	good
5	Gen 20	Low	7	bad	warning
19	Gen 15	Low	8	bad	warning
7	Gen 19	Low	8	bad	warning

Table 4.5.6: Pharmacokinetic Property of all pokets(Continued)

ID Number	Genation Number	Phospholipidosis	Fsp3	Stereo Centers	PPI_Friendly
2	Gene16	NonInducer	0.98	4	Not Computed
14	Gene15	NonInducer	0.65	7	Not Computed
23	Gene19	Inducer	0.48	17	Not Computed
14	Gen19	NonInducer	0.84	9	Not Computed
23	Gen19	Inducer	0.48	17	Not Computed
17	Gene19	Inducer	0.69	6	Not Computed
10	Gen 12	NonInducer	0.5	9	Not Computed
3	Gen 13	NonInducer	0.98	4	Not Computed
10	Gen 12	NonInducer	0.5	8	Not Computed
13	Gen 18	NonInducer	0.41	5	Not Computed
4	Gen8	NonInducer	0.47	4	Not Computed
22	Gen18	Inducer	0.44	2	Not Computed
5	Gen 20	NonInducer	0.34	14	Not Computed
19	Gen 15	Inducer	0.42	4	Not Computed
7	Gen 19	Inducer	0.55	5	Not Computed

Table 4.5.7: Pharmacokinetic Property of all pokets(Continued)

ID Number	Genation Number	Status
2	Gene16	Accepted
14	Gene15	Accepted
23	Gene19	Accepted
14	Gen19	Accepted
23	Gen19	Accepted
17	Gene19	Accepted
10	Gen 12	Accepted
3	Gen 13	Accepted
10	Gen 12	Accepted
13	Gen 18	Accepted
4	Gen8	Accepted
22	Gen18	Accepted
5	Gen 20	Accepted
19	Gen 15	Accepted
7	Gen 19	Accepted

Finally, all of the ligand molecules have been approved after the successful screening of all the attributes from the aforementioned table

Chapter Five: 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 expands the field of potential proteins and antiviral medications. After the SWISS-MODEL online server successfully generated the 3D homology models of the major (P) 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 (P) 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 Six: References

6.References

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