PHYTOCHEMICALS FROM INDIAN POLYHERBAL MIXTURE AS DRUG CANDIDATE FOR OMICRON: SARS-CoV-2 VARIANT SPIKE PROTEIN

Submitted in partial fulfilment of the requirements for the award of

Bachelor of Technology degree in

Biotechnology

BY

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DEPARTMENT OF BIOTECHNOLOGY
B. TECH(BIOTECHNOLOGY) IV YEAR

SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited with Grade “A” by NAAC
JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Elanthendral Gopalsami (38230701)** who have done the Project work entitled **Phytochemical from Indian polyherbal mixture as drug candidates for Omicron: SARS CoV-2 variant spike protein** under my supervision from **06/07/2021** to **28/03/2022**

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Submitted for Viva voce Examination held on _____

Internal Examiner **External Examiner**

DECLARATION

I **Elanthendral Gopalsami** hereby declare that the project entitled **Phytochemical from Indian polyherbal mixture as drug candidates for Omicron: SARS CoV-2 variant spike protein** done by me under the guidance of **Dr. Inbathamizh L.** (Internal & External) at **Sathyabama Institute of Science and Technology** is submitted in partial fulfilment of the requirements for the award of **Bachelor of Technology degree** in **Biotechnology**

Date: 21/04/2022

Place: Sathyabama Institute of Science and Technology

SIGNATURE OF CANDIDATE

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ABSTRACT

The omicron variant, first identified in south Africa is the most contagious variant of concern so far evolved from SARS CoV-2. It first developed from immunocompromised patients. It contains numerous mutations especially in its Receptor binding domain, that plays a vital role in interacting with the human ACE-2 receptor. These mutations increase its binding affinity and immune invasion. The development of treatment against this virus is extremely urgent and ultimately necessary. Anti-viral and monoclonal antibodies have been used so far to tackle the pandemic; however, effectiveness depends on the individual's response to them. Herbal plants contain abundant phytochemicals and is used for almost all health problems across the world, especially in India and China. There are no side effects caused as most herbal plants are non-toxic. In this study, we use compounds isolated through GC-MS from a polyherbal mixture made of Coleus amboinicus (Mexican mint/karpooravalli), Citrus limon, Curcuma longa (Turmeric/Manjal), Leucas aspera (Thumbai), Mentha piperita (Peppermint/Pudina), Ocimum basilicum (thiruneetru pacchilai), Ocimum gratissimum (Thulasi), Vitex negundo (Nocchi), Allium sativum (Garlic) and perform in-silico analysis against Spike protein from the Omicron variant. Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4- [(2-methoxyethoxy)methoxy] - 5, 9 - dimethyl-, Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy] - 2 - methyl-,1,2-Phenylene bis(mesitylsulfonate), Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-, Phthalimide, N-(1-hydroxy-2-propyl)-, 2(1H)-Naphthalenone, octahydro-, trans-, t-Butyl 1-thio-.alpha.-D-glucopyranoside and 8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl- show higher hydrogen bond formation and minimal binding energy. These compounds were chosen as the potential drug candidates. Further in process, these ligands can should be subjected to in-vitro studies to evaluate accuracy of the study performed for drug development process.

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CHAPTER 1

INTRODUCTION

Coronaviruses belong to the Coronaviridae family and the order Nidovirales. They contain positive RNA about 32kb encased within a lipid membrane [1]. Coronaviruses are the largest in the virus family that can be phylogenetically classified into α, β, γ and δ. The α and β types can infect humans [1]. SARS CoV-2 emerged at the end of 2019 in Wuhan, China and then on, variants of the Wuhan virus have evolved. SARS CoV-2 omicron variant (B.1.1.529) was first detected in Botswana and South Africa on November 2021. It is believed that the variant emerged from immunocompromised individuals [2]. This variant has been termed as the variant of concern (VOC) by World Health Organisation (WHO). It contains multiple mutations at the spike protein that is the key player in spread of disease. The viral surface proteins ingrained on the lipid envelope are termed spike proteins [1]. The viral RNA is contained within the a nucleocapsid [3]. Once the virus enters the host cell, it replicates in the cytoplasm of the host [4]. The RNA is single stranded positive type. Generally, all Coronaviruses contain different structural proteins such as spike (S), membrane (M), envelope (E), nucleocapsid (N) and hemmagglutin-esterase (HE) [4]. The spike protein assists the virus to enter the host cell [5]. It is a homotrimer and is present in multiple copies on the membrane. This organisation gives the virus its crown like feature [5]. This S protein is a class I fusion protein that aids in receptor attachment. Each monomer can be cleaved by protease in host into S1 and S2 domains [5][6]. S1 aids in receptor binding functions and S2 gives structural support to the protein [5]. The S1 domain is made of five stranded β antiparallel sheets connected with α helices [7]. The receptor binding region lies within the S1 domain in the C-terminal for SARS-CoV species [5]. This receptor binding domain (RBD) binds to the Angiotensin-converting enzyme-2 on the host surface [8]. 17 amino acid residues bind from the S1 domain to 20 amino acid residues of ACE-2[8]. It is this RBD that is targeted for treatment or neutralisation with antibodies from vaccines [9]. There are approximately 30 mutations found in the spike protein [10]. Almost half of it is located in the RBD [7] most of which are in the ACE-2 binding interface [11]. The RBD of omicron was analysed as ARG319 to PHE541[12]. It may be found in

standing or lying position. The standing position indicates receptor binding position while the lying down is used for immune evasion [13]. The mutations in the variants of SARS CoV-2 may have effects in their pathogenicity. For instance, mutation D614G increases the stability of replication in the lungs of the patient and escalates transmission rate of Omicron [8]. Mutation in residue 484 and 452 boosts the RBD binding strength to ACE-2 and bypassing antibodies [14][15]. The binding affinity may increase to 1000 folds [7]. Substitution at residue 417 enhances immune evasion by inducing structure changes [15]. Other mutation may alter their conformation and extremity of disease [2]. These mutations cause faster spread of the disease [6].

Coronaviruses can affect the nervous, respiratory and gastrointestinal systems. A person can become infected through exposure with infected objects by touching them, through inhalation or by coming in close proximity with an infected person [1]. Some of the symptoms of the infection are fever, shortness of breath, sore throat and cough [1]. People who are infected are usually quarantined and treatment is based on severity their condition [1]. The Omicron variant is highly contagious and has the ability to reduce the effect of vaccines. Reinfection of the disease and transmissibility between humans are also high in contrast to other variants [2][17]. In most cases, symptoms are not severe and there is no need for oxygen support [15]. Drug discovery and development can be a tedious and challenging process because response to treatment differs between individuals [18]. SARS CoV-2 treatment can be classified into 2 groups. The first kind of treatment acts on human immune system and the second type acts on the virus [1]. Currently antivirals and monoclonal antibodies are being used depending on the patient's need. Some of them have been provided in table 1.1.

Table 1.1: Current treatment methods used for SARS CoV-2 and its variants.

Antivirals [6]		Monoclonal Antibodies[6]	
Remdesivir	Blocks viral replication by inhibition of RNA	Cocktail of Bamlanivimab and etesevimab	Increase the neutralisation rate and blocks the

	dependant RNA polymerase		binding of RBD and ACE-2.
Molnupiravir	Increases the frequency of viral RNA mutations impairing its replication	Cocktail of casirivimab and imdevimab	Reduces severity of infection.
Nirmatrelvir	Inhibits SARS CoV-2 protease that is vital in replication process.		

Using medicinal herbs can be a better choice. Traditionally people around the world have been using herbal ingredients to treat numerous kinds of health issues. Herbal treatment is sustainable as it can be sourced easily and is safe to use. Most of them are non-toxic, hence, no side-effects [19]. Plants contain powerful phytochemicals that works against countless diseases [18] The herbal medicine used against SARS-CoV-2 and its variants should be able to obstruct virus replication and combat symptoms related to the infection [1]. Artemisia annua contains anti-SARS CoV-2 properties and hence has been used in Traditional Chinese medicine [20]. Traditional Indian medicine houses a huge variety of herbs that are used over centuries to combat various health problems and diseases[21] In siddha, kabasura kudineer has been used by many to increase immunity against Coronavirus infection [22].

The present study focuses on a novel polyherbal formulation using Coleus amboinicus (Mexican mint/karpooravalli), Citrus limon, Curcuma longa (Turmeric/Manjal), Leucas aspera (Thumbai), Mentha piperita (Peppermint/Pudina), Ocimum basilicum (thiruneetru pacchilai), Ocimum gratissimum (Thulasi), Vitex negundo (Nocchi), Allium sativum (Garlic). These herbs are used frequently in an Indian household due to their abundant health benefits. The extract from these herbs are subjected to GC-MS for phytochemical analysis. Then using in-silico methods, drug-like compounds are docked with the spike protein of Omicron variant to find the potential drug candidates.

CHAPTER 2

LITERATURE SURVEY

Phytochemicals have been well documented for their medicinal uses for various diseases such as diabetes, cancer, skin diseases such as dermatophytosis. These phytochemicals have the potential to work against SARS CoV-2 and its variants

2.1 MEDICINAL PLANTS IN SARS CoV-2

Withania somnifera (Ashwagandha), *Tinospora cordifolia* (Giloy), *Ocimum sanctum* (Tulsi) [23]*, Tinospora cordifolia*) [24] and *Azadirachta indica* (Neem) [25] were all evaluated to contain phytochemicals with the potential to inhibit the spike protein

2.2 THERAPEUTIC BENEFITS OF MEDICINAL PLANTS

The polyherbal mixture used in this study are *Coleus amboinicus* (Mexican mint/karpooravalli*), Citrus limon, Curcuma longa* (Turmeric/Manjal), Leucas aspera (Thumbai), *Mentha piperita* (Peppermint/Pudina), *Ocimum basilicum* (thiruneetru pacchilai), *Ocimum gratissimum* (Thulasi), *Vitex negundo* (Nocchi), *Allium sativum* (Garlic). Some of the medicinal properties are described below in Table 2.1.

Table 2.1: Current treatment methods used for SARS CoV-2 and its variants.

Name of species	Properties
Leucas aspera	Anti-microbial, hepatoprotective activity, Analgesic property, cytotoxic effects, central nervous system depressant activity, anti-inflammatory effects [26].
Allium sativum (Garlic/Poondu)	Treats respiratory problems, against bronchitis. Anti-bacterial properties, against tuberculosis [27].
Ocimum gratissimum	Analgesic activity, immunostimulatory properties, antimicrobial, anti-inflammatory effects [28].

Ocimum basilicum	Anti-microbial, antioxidant properties [29].
Curcuma longa	Antioxidant, hepatoprotective, anti-microbial [30][31]
Vitex negundo	Promotes and regulates apoptosis, anti-microbial [32].
Coleus amboinicus	Anti-fungal, treats, infections related to respiratory system used for chronic diseases, anti-inflammatory, anti-tumor [33].
Mentha piperita	Antibacterial, antiparasitic, antiviral, analgesic, Radioprotective [33].
Citrus limon	Anti-parasitic, anti-allergic, hepatoregenerating effect. Anti-viral, positive effects on the respiratory, gastrointestinal, skeletal and nervous system [34].

2.3 PHYTOCHEMICAL REVIEW

Secondary metabolism synthesizes phytochemicals and many other toxins for plant growth. All herbal plants contain carbohydrate, phenolics and alkaloids in general. Vitex negundo, contains many polyphenolic compounds, terpenoids, glycosidic iridoids and alkaloids. Leucas aspera contains terpenoids, flavonoids and Tanninc [35]. Garlic contains saponin, Tannins, Phenolics and Cardiac glycosides [36].

2.4 MEDICINAL PLANT AGAINST SARS COV-2

Table 2.2: Previous studies conducted by different authors, the targets and ligands

Plant	Target	Phytochemicals
Azadirachta indica (Neem)	Proteins M, E	7-Deacetyl-7-Benzoylgedunin, Nimbolin A, 24-Methylenecycloartanol [25].

Tinospora cordifolia (giloy)	$3CL^{pro}$	Berberine [37].
Melissa officinalis	Proteins S, M^{pro}	Luteolin-7-glucoside-3′-glucuronide, Melitric acid-A, Quadranoside-III [38]

CHAPTER 3

AIM AND SCOPE OF PRESENT INVESTIGATION

3.1 AIM

The aim of this study thus, is to find potential drug targets from polyherbal plant extracts that are potential against the spike protein of SARS-CoV-2 Omicron variant (B.1.1.529). By inhibiting the spike protein, there are possibilities to prevent binding of this protein to the ACE-2. We chose 9 herbal plants to find the therapeutic natural compound that has drug-like properties which could act against the spike protein. The results from this study, can help to develop an effective treatment against SARS CoV-2 Omicron variant.

3.2 OBJECTIVES

- Collection and extraction of plant and its extracts
- Analyse the phytochemicals present through GC-MS
- Perform toxicity studies to select drug-like phytochemicals
- To carry out molecular docking for the phytochemicals and Spike protein of SARS CoV-2 Omicron variant
- Analyse Docking results for potential drug candidate for future in-vivo studies.

3.3 SCOPE

The scope of this study is to predict potential drug candidates against SARS CoV-2. The drug candidates have the potential for a breakthrough in the research community in finding treatment for the viral infection.

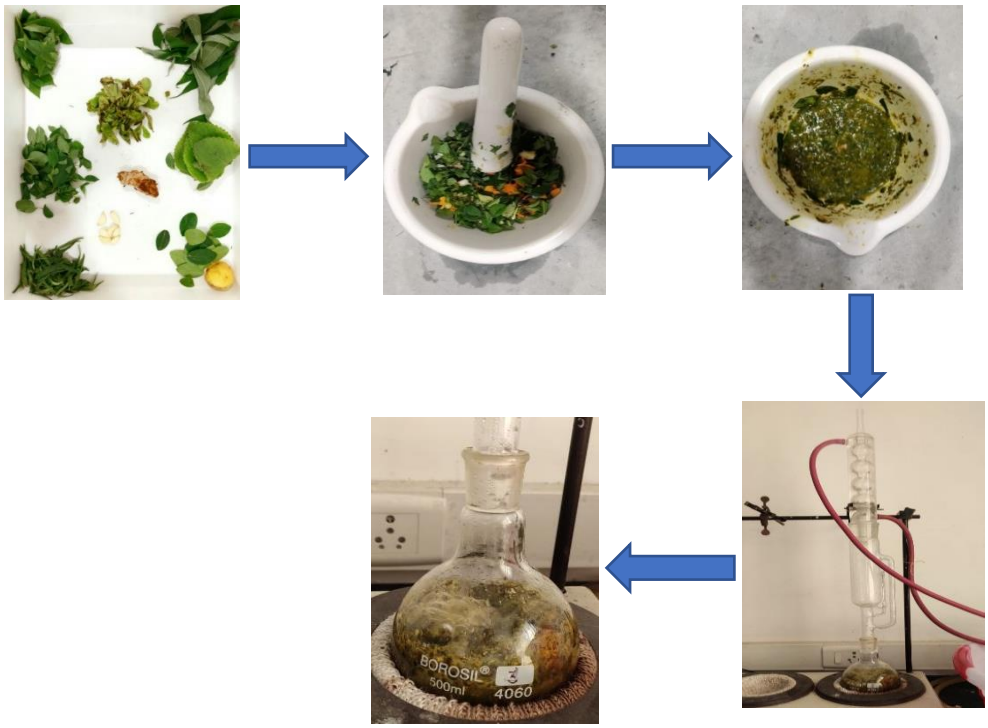
CHAPTER 4

MATERIALS AND METHODS

4.1 SELECTION AND EXTRACTION OF PLANT SAMPLES

Coleus amboinicus (Mexican mint/karpooravalli), *Citrus limon*, *Curcuma longa* (Turmeric/Manjal), *Leucas aspera* (Thumbai), *Mentha piperita* (Peppermint/Pudina), *Ocimum basilicum* (thiruneetru pacchilai), *Ocimum gratissimum* (Thulasi), *Vitex negundo* (Nocchi), *Allium sativum* (Garlic/Poondu) were used in the experiment. The leaves of *Coleus amboinicus, Citrus limon, Leucas aspera, Mentha piperita, Ocimum basilicum, Ocimum gratissimum, Vitex negundo* were used while, the bulbs and roots were used from *Allium sativum and Curcuma longa* respectively. The samples were washed first with tap water and then with distilled water. After left to dry out 5g of each sample were ground together using a mortar and pestle into a paste. The ground paste was diluted with 100ml of water. This mixture was transferred to the Soxhlet apparatus for extraction of volatile compounds at 60 degrees Celsius. The compounds extracted were subjected to GC-MS analysis.

Fig 4.1: Extraction process of polyherbal mixture.



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4.2 PREPARATION OF PROTEIN

The crystal structure of SARS-CoV-2 S Omicron Spike B.1.1.529 (PDB id: 7QO7) was downloaded from RCSB PDB (Protein Data Bank). Discovery 2021 client software (https://discover.3ds.com/discovery-studio-visualizer-download) was used to remove unnecessary multiple ligands and chains from the protein. The sequence length of the protein downloaded was 1285. Only the A chain was used for this experiment as the protein is a homotrimer. Chain A contained 1101 residues. For further analysis, the Receptor binding domain of the Omicron variant was retrieved from literature data [39]. Then the A chain was further removed of all other amino acids except residues 319-541 using discovery studio 2021 client software. The files were saved as .pdb files. Fig4.2 to Fig 4.4 below displays the Raw protein structure downloaded from PDB, processed chain A and Receptor binding domain respectively.

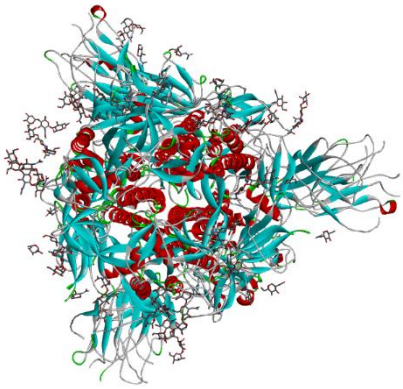


Fig 4.2

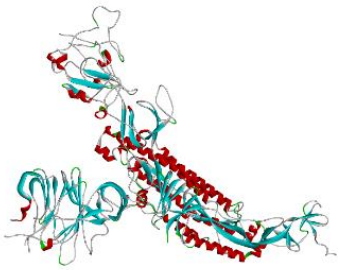


Fig 4.3

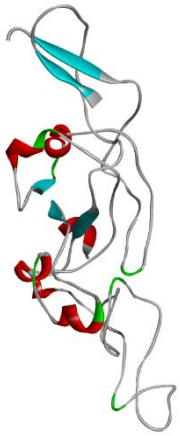
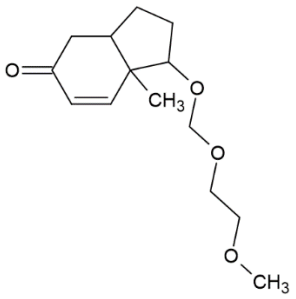


Fig 4.4

4.3 PREPARATION OF LIGANDS

The chemical compounds extracted from GC-MS results were listed for toxicity and Drug-likeness using SWISS-adme and pk-CSM. The canonical structure of these drug-like compounds was retrieved from PubChem and Drugbank. The 2D structures were drawn, converted to 3D structures and 3D structure was optimised using chemsketch tool and saved as .sdf files. Open babel GUI was used to convert .sdf to .pdb files and saved. Bicyclo[4.3.0]non-2-en-4-one, 9-[(2-methoxyethoxy) is displayed as 2D and optimized 3D in Fig 4.5 and 4.6.



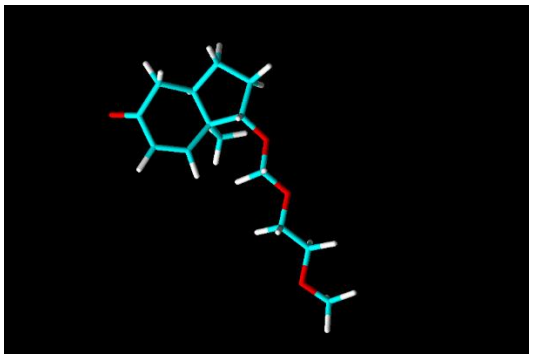
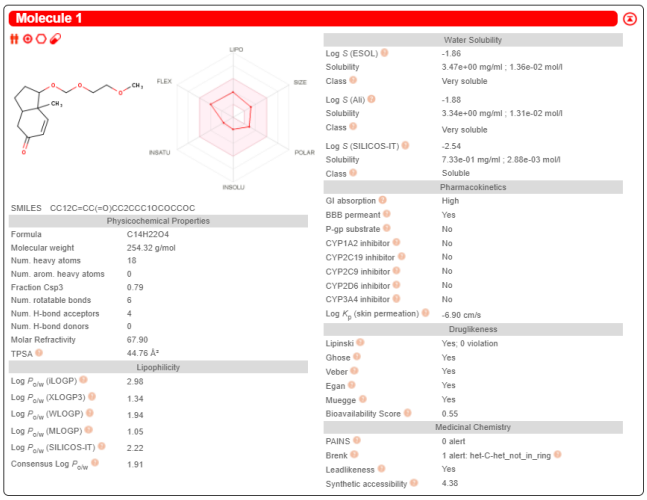
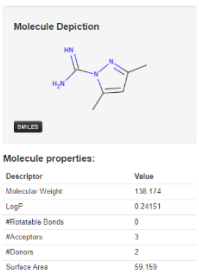


Fig 4.5 **Fig 4.6**

4.4 ADMET ANALYSIS

SwissADME and pk-CSM were used to eliminate the compounds according to the Lipinski rule of 5. For a compound to be suitable as a drug candidate, the molecular mass should be lower than 500kda, Hydrogen bond donors (HBD) below five. Hydrogen bond acceptor, below 10 and Log P lesser than 5 [41]. Molar refractivity should be between 40-130 and rotatable bonds should be less than 10. Fig 4.7 and 4.8 shows the swissADME and pk-CSM analysis.





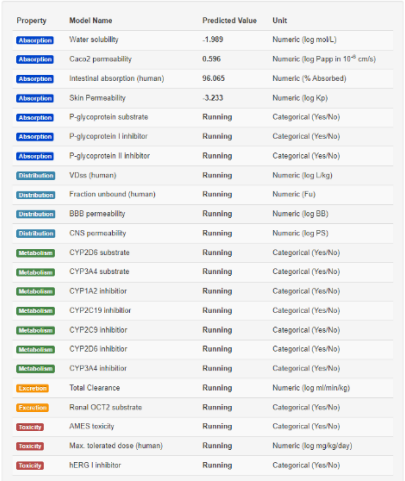


Fig 4.7 **Fig 4.8**

4.5 DOCKING PROCESS

The main objective of molecular docking is to analyse the protein-ligand interactions to predict the ligand activity. Molecular docking was done using autodock 1.5.6 (https://ccsb.scripps.edu/mgltools/1-5-6/) . Polar hydrogen bonds and kollman charges were added to the protein. The ligand was then docked with

the protein and the results were obtained. The number points in the X, Y, Z was 126. The spacing was kept at 1.000. The center grid box was placed at X= 180.597, Y=203.419 and Z=191.73, with offset values for Z set at 18.000. For this further analysis experiment the number points in the X, Y, Z was 126. The spacing was kept at 0.675. The center grid box was placed at X= 211.759, Y=186.017 and Z=137.034. The results were analysed of best conformation that contained the highest number of hydrogen bonds. The conformation with the least binding energy as well as the poses with the highest number of hydrogen bonds were assessed. Interaction poses with both hydrogen bonds and least binding energy was chosen as the optimum pose. The amino acids that were involved in the hydrogen bond was taken note. The analysis of the protein and ligand structure after docking was done using discovery studio 2021 client software and pymol which the 2D and 3D structures being obtained. Fig 4.9 and 4.10 shows the grid box values

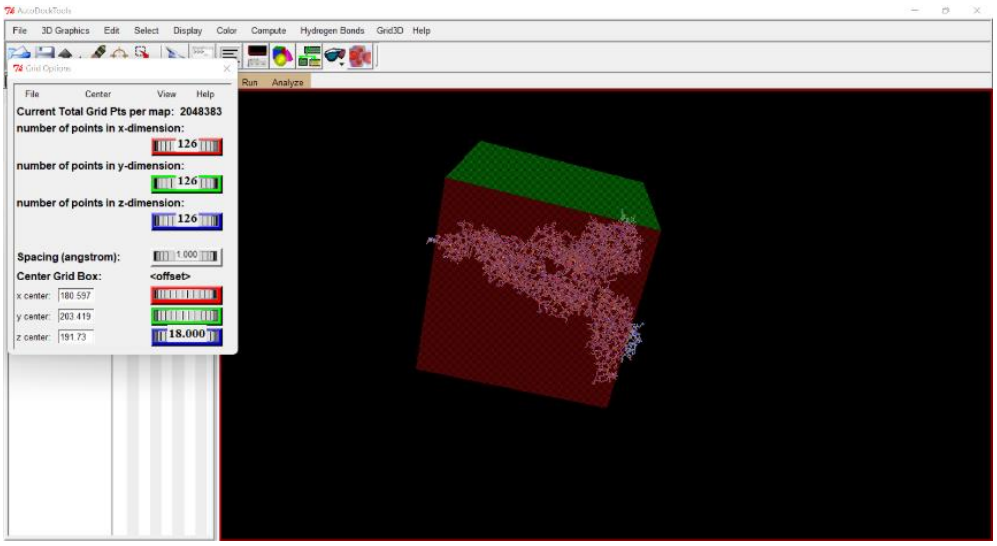


Fig 4.9

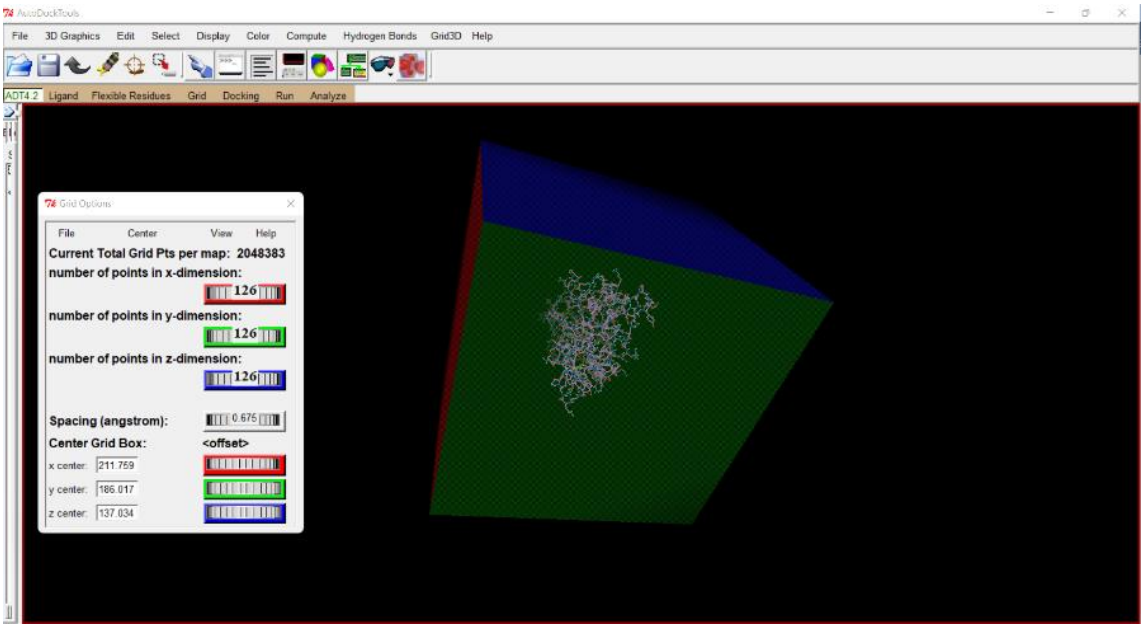

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Fig 4.10

CHAPTER 5

RESULTS AND DISCUSSION

5.1 COMPARISON OF SARS CoV-2

Firstly, the SARS CoV-2 and SARS CoV-2 omicron variant was subjected to sequence identity test using EMBL-EBI. The FASTA sequences of Omicron variant 7QO7 and SARS CoV-2 7LRT were used in the process. Both the sequences were 89.5% identical. Total of 102 gaps were found. Total of 34 mutation were spotted. The data is provided below as Fig 5.1

Fig 5.1

5.2 GC-MS RESULTS

A total of 154 Compounds were obtained from the GC-MS analysis. The results of the GC-MS analysis is represented by Fig 5.2. GC-MS provides clear greater separation of phytochemicals [41]. All of the compounds were put through ADMET (absorption, distribution, mechanism, excretion and toxicity) test. This test aids in the discovery of new drug candidates that are least or non-toxic with desired ADME properties [42]. The list of the compounds is provided below in Tables 5.1, 5.2 and 5.3. The RT value indicates the time taken for a solute to pass through the chromatography column. This is calculated from the time of injection to time of detection. The area of the peak reflects the amount of each analyte present.

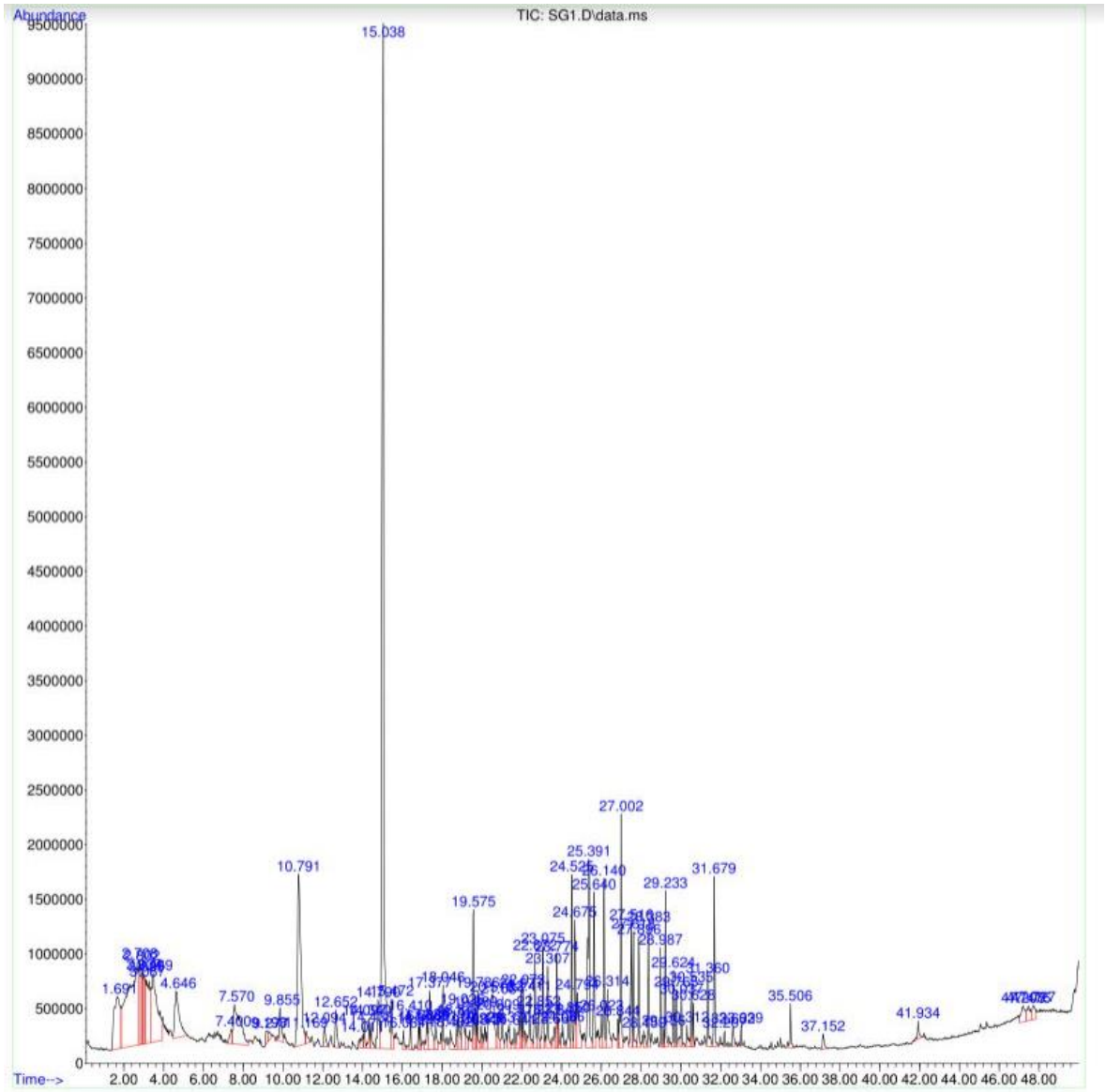


Fig 5.2

Table 5.1: List of compounds obtained from GC-MS 1-58

S.No	Compound Name	RT (min)	Area %	S.No	Compound Name	RT (min)	Area %
1.	Propanoic acid, 2-chloro-, methyl ester	1.682	2.79	30.	2,4-Diaminophenol	10.795	5.03
2.	Carbonochloridic acid, 4-nitrophenyl ester	1.682	2.79		4H-Pyran-4-one, 3,5-dimethyl-	11.167	0.25

				31.			
3.	Bis(2-chloroethyl) sulfone	1.682	2.79	32.	5-Methyl-2-pyrazinylmethanol	11.167	0.25
4.	Ethane, 1,1-bis(ethylthio)-	2.718	8.19	33.	6-Methyl-2-pyrazinylmethanol	11.167	0.25
5.	Cyclopentanecarboxylic acid, 4,4-dimethyl-3-trimethylsilylmethylene-, methyl ester	2.718	8.19	34.	Dimethyl dl-malate	12.099	0.32
6.	Germacyclopent-3-ene, 1,1,3,4-tetramethyl-	2.801	1.66	35.	3-Acetoxy-3-hydroxypropionic acid, methyl ester	12.099	0.32
7.	Trimethylsilyl ethaneperoxoate	2.925	0.68	36.	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	12.659	0.58
8.	Bicyclo[4.3.0]nonan-4-one, 9-(2-methoxyethoxymethoxy)-1-methyl-	3.029	1.01	37.	2,4-Difluoroanisole	12.659	0.58
9.	Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4-[(2-methoxyethoxy)methoxy]-5,9-dimethyl-	3.091	2.81	. 38	1,2,4,5-Tetrazine-3,6-diamine,1,4-dioxide	12.659	0.58
10.	Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4-[(2-methoxyethoxy)methoxy]-5-dimethyl-	3.091	2.81	39.	Cyclohexanone,3-ethyl-3,5,5-trimethyl-	14.005	0.25
11.	Spiro[3,5-dioxatricyclo[6.3.0.0(2,7)]undecan-6-one-4,2'-cyclohexane], 1'-	3.091	2.81		Cyclohexanol, 5-methyl-2-(1-methylethyl)-, sulfite (2:1), [1R-	14.005	0.25

	isopropyl-2,4'-dimethyl-9,11-bis(2-met			40.	[1.alpha.(1R*,2S*,5R*),2.beta.,5.alpha.]]-		
12.	1,4-Eicosanediol	3.463	3.61	41.	Cyclohexanecarboxylic acid, 4-butyl-, 2,3-dicyano-4-(pentyloxy)phenyl ester	14.005	0.25
13.	Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy]-2-methyl-	3.463	3.61	42.	2-Tetradecene, (E)-	14.088	0.33
14.	Methoxydi(tert-butyl)silane	3.463	3.61	43.	3-Tetradecene, (Z)-	14.088	0.33
15.	1H-Pyrazole-1-carboximidamide, 3,5-dimethyl-	4.644	1.95	44.	2-Pentene-1,4-dione, 1-(1,2,2-trimethylcyclopentyl)	14.44	0.34
16.	3-Furaldehyde	4.644	1.95	45.	Cyclohexane, (1,2-dimethylpropyl)-	14.44	0.34
17.	Furfural	4.644	1.95	46.	Cyclohexane, 1-ethyl-2,4-dimethyl-	14.44	0.34
18.	6,6-Dimethyl-1,5-diazabicyclo[3.1.0]hexane	7.398	0.28	47.	1H-Inden-5-ol, 2,3-dihydro-	14.792	0.84
19.	N-(2-Isopropoxyphenyl)-2-thiophenecarboxamide	7.398	0.28	48.	Benzaldehyde, 4-ethyl-	14.792	0.84
20.	2-Thiophenecarboxylic acid hydrazide	7.398	0.28	49.	Benzaldehyde, 3,4-dimethyl-	14.792	0.84
21.	2-Furancarboxaldehyde, 5-methyl-	7.564	2.43	50.	5-Hydroxymethylfurfural	15.04	16.48

22.	7-Diethoxymethylbicyclo[3.2.0]heptan-2-one	9.2	0.2	51.	2-Fluorobenzyl alcohol	15.04	16.48
23.	Dibutyl 2,2'-(2,2'-oxybis(ethane-2,1-diyl)bis(oxy))diacetate	9.2	0.2	52.	Cyclohexane, (4-methylpentyl)-	15.475	0.69
24.	Pyrazolidin-3-one, 2-(4-methylbenzoyl)-1-phenyl-	9.283	0.19	53.	Cyclohexane, hexyl-	15.475	0.69
25.	1,2-Phenylene bis(mesitylsulfonate)	9.283	0.19	54.	Undecane, 2,4-dimethyl-	16.055	0.19
26.	o-Cymene	9.283	0.19	55.	Tetradecane, 4-ethyl-	16.055	0.19
27.	Benzeneacetaldehyde	9.863	0.7	56.	Sulfurous acid, hexyl octyl ester	16.055	0.19
28.	2,5-Furandicarboxaldehyde	10.795	5.03	57.	Tridecane, 1-iodo-	16.407	0.35
29.	Orcinol	10.795	5.03	58.	Nonadecane	16.407	0.35

Table 5.2: List of compounds obtained from GCMS 59-120

S. No	Compound Name	RT (min)	Area %	S.No	Compound Name	RT (min)	Area %
59.	Dodecane, 4,6-dimethyl-	16.407	0.35	90.	Benzene, 1-chloro-2-methoxy-	19.679	0.39
60.	3,4-Difluorobenzoic acid, 3-pentadecyl ester	16.821	0.22	91.	Dodecane	19.783	0.54
61.	3,4-Difluorobenzoic acid, 4-pentadecyl ester	16.821	0.22	92.	9-Eicosene, (E)-	19.969	0.25
62.	2,4-Difluorobenzoic acid, 4-pentadecyl ester	16.821	0.22	93.	1,1,3,6-tetramethyl-2-(3,6,10,13,14-pentamethyl-3-ethyl-pentadecyl)cyclohexane	19.969	0.25
63.	2-Undecanone	16.904	0.23	94.	Hexadecane	20.156	0.35
64.	4-Heptenal	17.235	0.4	95.	Tetradecane	20.156	0.35
65.	3H-Pyrazol-3-one, 2,4-dihydro-4,4,5-trimethyl-	17.235	0.4	96.	Boric acid, ethyl-, didecyl ester	20.156	0.35

66.	Phthalic anhydride	17.38	1.26	97.	6,8-Dioxa-3-thiabicyclo(3,2,1)octane 3,3-dioxide	20.528	2.54
67.	1,2-Benzenedicarboxylic acid	17.38	1.26	98.	t-Butyl 1-thio-.alpha.-D-glucopyranoside	20.528	2.54
68.	Dodecane, 2,6,10-trimethyl-	17.691	0.4	99.	Lethane	20.528	2.54
69.	Tetratetracontane	17.691	0.4	100.	2(1H)-Naphthalenone, octahydro-, trans-	20.818	0.51
70.	Benzenemethanol, 3-fluoro-	17.691	0.4	101.	Decalin, anti-1-methyl-, cis-	20.818	0.51
71.	Phthalic acid, monoamide, N-ethyl-N-(3-methylphenyl)-, pentyl ester	18.043	0.61	102.	Decalin, syn-1-methyl-, cis-	20.818	0.51
72.	Phthalic acid, propyl 2-tert-butyl-6-methylphenyl ester	18.043	0.61	103.	Cyclohexane, octyl-	21.025	0.56
73.	Phthalic acid, monoamide, N-ethyl-N-(3-methylphenyl)-, octyl ester	18.043	0.61	104.	1-Heneicosyl formate	21.647	0.22
74.	Cyclooctacosane	18.437	0.34	105.	2-Butenedioic acid (Z)-, monododecyl ester	21.647	0.22
75.	Cyclohexane, 1,2,4,5-tetraethyl-	18.437	0.34	106.	2-Bromo dodecane	21.978	0.57
76.	N-Isopropoxy-2-carbomenthyloxyaziridine	18.437	0.34	107.	Pentadecane, 2,6,10-trimethyl-	21.978	0.57
77.	Methoxy(methyl)chlorosilane	18.747	0.28	108.	Docosane	21.978	0.57
78.	Urea, N-(4-hydroxy-2-methylcyclohexyl)-N'-(4-hydroxyphenyl)-	18.747	0.28	109.	2-Acetylthiazole	22.081	0.51
79.	3-Pyridinol, 6-methyl-	18.747	0.28	110.	Propanamide, N-methyl-	22.081	0.51
80.	1-Docosanol, methyl ether	18.851	0.5	111.	Phenol, 2,5-bis(1,1-dimethylethyl)-	22.413	0.69
81.	Cyclotetradecane	18.851	0.5	112.	1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol	22.661	0.91
82.	2-Cyclohexylpiperidine	19.016	0.74	113.	8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-	22.661	0.91
83.	DL-Proline, 5-oxo-, methyl ester	19.182	0.37	114.	Pyrrole, 4-ethyl-2-methyl-	22.661	0.91
84.	beta.-(3,4-Dichlorophenyl)ethylamine, N-fluoroacetyl-N-(2-pyrrolidinoethyl)-	19.182	0.37	115.	Thiocyanic acid, 1H-indol-3-yl ester	22.848	0.49
85.	LISDEXAMFETAMINE	19.182	0.37	116.	Glycine, N,N-bis(trimethylsilyl)-, trimethylsilyl ester	22.848	0.49
86.	1-Nonadecene	19.576	1.2	117	Phthalimide, N-(1-hydroxy-2-propyl)-	22.848	0.49

87.	1-Tetradecene	19.576	1.2	118.	Benzene, (1-butylhexyl)-	23.076	1.04
88.	1H-Pyrazole-4-carbothioamide, 5-amino-	19.679	0.39	119.	Benzene, (1-butyloctyl)-	23.076	1.04
89.	Benzene, 1-chloro-4-methoxy-	19.679	0.39	120.	Benzene, (1-propylheptyl)-	23.303	0.8

Table 5.3: List of compounds obtained from GCMS 121-154

S. No	Compound Name	RT (min)	Area %	S. No	Compound Name	RT (min)	Area %
121.	Benzene, (1-propylnonyl)-	23.303	0.8	139.	Benzene, (1-methyldecyl)-	24.67	1.36
122.	Methoxyacetic acid, tetradecyl ester	23.655	0.33	140.	Tetracosane, 1-bromo-	24.794	1.22
123.	Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-	23.655	0.33	141.	Dodecane, 2,6,11-trimethyl-	24.794	1.22
124.	Sulfurous acid, octadecyl 2-propyl ester	23.655	0.33	142.	Pentadecane, 2,6,10,14-tetramethyl-	24.794	1.22
125.	Benzene, (1-ethyloctyl)-	23.78	0.72	143.	Benzene, (1-butylheptyl)-	25.395	2.98
126.	Benzene, (1-ethylundecyl)-	23.78	0.72	144.	Benzene, (1-propyloctyl)-	25.644	1.43
127.	Benzene, (1-ethyldecyl)-	23.78	0.72	145.	2-Cyclohexylnonadecane	26.016	0.4
128.	Dodecane, 1-fluoro-	23.842	0.29	146.	Cyclohexane, decyl-	26.016	0.4
129.	Triacontane, 1,30-dibromo-	23.842	0.29	147.	Heptylcyclohexane	26.016	0.4
130.	5-Eicosene, (E)-	23.842	0.29	148.	Benzene, (1-ethylnonyl)-	26.141	1.46
131.	Dodecane, 1,1-dimethoxy-	24.07	0.36	149.	8-Pentadecanone	26.306	0.92
132.	Undecanal dimethyl acetal	24.07	0.36	150.	Heptadecane, 9-octyl-	26.845	0.27
133.	Hexadecane, 1,1-dimethoxy-	24.07	0.36	151.	Benzene, (1-methylundecyl)-	27.01	2.05

134.	Diethyl Phthalate	24.36	0.46	152.	Benzene, (1-pentylheptyl)-	27.507	1.13
135.	1-Octadecene	24.525	1.34	153.	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	28.149	0.24
136.	7-Hexadecene, (Z)-	24.525	1.34	154.	Nonahexacontanoic acid	29.144	0.14
137.	E-15-Heptadecenal	24.525	1.34	155.			
138.	Benzene, (1-methylnonyl)-	24.67	1.36	156.			

Toxicity studies is an important factor in drug discovery process. Toxicity determination is necessary to identify adverse effects of compounds on humans and animals. Compounds can be subjected to in-vitro or in-silico toxicity tests. In-silico tests can be used as a preliminary evaluation of drug candidates. In-silico tests may use algorithms or different software. Computational toxicity evaluation minimize animal testing and reduce the cost and labour [43]. In this study the compounds were put through toxicity test using the Osiris Property Explorer. Among these 107 were non-toxic, which are listed in Table 5.4, 5.5 and 5.6.

Table 5.4: List of compounds found non-toxic 1-36

S. No	Name of Compound	S. No	Name of Compound
1	Germacyclopent-3-ene, 1,1,3,4-tetramethyl-	19	4H-Pyran-4-one, 3,5-dimethyl-
2	Bicyclo[4.3.0]nonan-4-one, 9-(2-methoxyethoxymethoxy)-1-methyl-	20	5-Methyl-2-pyrazinylmethanol
3	Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4-[(2-methoxyethoxy)methoxy]-5,9-dimethyl-	21	6-Methyl-2-pyrazinylmethanol
4	Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4-[(2-methoxyethoxy)methoxy]-5-dimethyl-	22	Dimethyl dl-malate

5	Spiro[3,5-dioxatricyclo[6.3.0.0(2,7)]undecan-6-one-4,2'-cyclohexane], 1'-isopropyl-2,4'-dimethyl-9,11-bis(2-met	23	3-Acetoxy-3-hydroxypropionic acid, methyl ester
6	1,4-Eicosanediol	24	1,2,4,5-Tetrazine-3,6-diamine, 1,4-dioxide
7	Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy]-2-methyl-	25	Cyclohexanone, 3-ethyl-3,5,5-trimethyl-
8	1H-Pyrazole-1-carboximidamide, 3,5-dimethyl-	26	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, sulfite (2:1), [1R-[1.alpha.(1R*,2S*,5R*),2.beta.,5.alpha.]]-
9	6,6-Dimethyl-1,5-diazabicyclo[3.1.0]hexane	27	Cyclohexanecarboxylic acid, 4-butyl-, 2,3-dicyano-4-(pentyloxy)phenyl ester
10	N-(2-Isopropoxyphenyl)-2-thiophenecarboxamide	28	3-Tetradecene, (Z)-
11	7-Diethoxymethylbicyclo[3.2.0]heptan-2-one	29	Cyclohexane, (1,2-dimethylpropyl)-
12	Dibutyl 2,2'-(2,2'-oxybis(ethane-2,1-diyl)bis(oxy))diacetate	30	Cyclohexane, 1-ethyl-2,4-dimethyl-
13	Pyrazolidin-3-one, 2-(4-methylbenzoyl)-1-phenyl-	31	Benzaldehyde, 3,4-dimethyl-
14	1,2-Phenylene bis(mesitylsulfonate)	32	Cyclohexane, (4-methylpentyl)-
15	o-Cymene	33	Undecane, 2,4-dimethyl-
16	Tetradecane, 4-ethyl-	34	3,4-Difluorobenzoic acid, 4-pentadecyl ester
17	Sulfurous acid, hexyl octyl ester	35	3,4-Difluorobenzoic acid, 3-pentadecyl ester
18	Dodecane, 4,6-dimethyl-	36	2,4-Difluorobenzoic acid, 4-pentadecyl ester

Table 5.5: List of compounds found non-toxic 37-94

S.No	Name of Compound	S.No	Name of Compound
37	3H-Pyrazol-3-one, 2,4-dihydro-4,4,5-trimethyl-	66	Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-
38	Tetratetracontane	67	Sulfurous acid, octadecyl 2-propyl ester

39	Cyclooctacosane	68	Benzene, (1-ethyloctyl)-
40	Cyclohexane, 1,2,4,5-tetraethyl-	69	Benzene, (1-ethylundecyl)-
41	1-Docosanol, methyl ether	70	Benzene, (1-ethyldecyl)-
42	Cyclotetradecane	71	5-Eicosene, (E)-
43	2-Cyclohexylpiperidine	72	Dodecane, 1,1-dimethoxy-
44	beta.-(3,4-Dichlorophenyl)ethylamine, N-fluoroacetyl-N-(2-pyrrolidinoethyl)-	73	Undecanal dimethyl acetal
45	1H-Pyrazole-4-carbothioamide, 5-amino-	74	Hexadecane, 1,1-dimethoxy-
46	Benzene, 1-chloro-4-methoxy-	75	7-Hexadecene, (Z)-
47	9-Eicosene, (E)-	76	Benzene, (1-methylnonyl)-
48	1,1,3,6-tetramethyl-2-(3,6,10,13,14-pentamethyl-3-ethyl-pentadecyl)cyclohexane	77	Benzene, (1-methyldecyl)-
49	Boric acid, ethyl-, didecyl ester	78	Dodecane, 2,6,11-trimethyl-
50	6,8-Dioxa-3-thiabicyclo(3,2,1)octane 3,3-dioxide	79	Benzene, (1-butylheptyl)-
51	t-Butyl 1-thio-.alpha.-D-glucopyranoside	80	Benzene, (1-propyloctyl)-
52	2(1H)-Naphthalenone, octahydro-, trans-	81	2-Cyclohexylnonadecane
53	Decalin, anti-1-methyl-, cis-	82	Cyclohexane, decyl-
54	Cyclohexane, octyl-	83	Benzene, (1-ethylnonyl)-
55	1-Heneicosyl formate	84	8-Pentadecanone
56	Pentadecane, 2,6,10-trimethyl-	85	Heptacosane
57	1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol	86	Heptadecane, 9-octyl-
58	8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-	87	Benzene, (1-methylundecyl)-
59	Pyrrole, 4-ethyl-2-methyl-	88	Benzene, (1-pentylheptyl)-
60	Phthalimide, N-(1-hydroxy-2-propyl)-	89	Nonahexacontanoic acid
61	Benzene, (1-butylhexyl)-	90	Tritetracontane
62	Benzene, (1-butyloctyl)-	91	Benzene, (1-pentyloctyl)-
63	Benzene, (1-propylheptyl)-	92	Benzene, (1-hexylheptyl)-
64	Benzene, (1-propylnonyl)-	93	Benzene, (1-butylnonyl)-
65	Methoxyacetic acid, tetradecyl ester	94	Benzene, (1-propylheptadecyl)-

Table 5.6: List of compounds found non-toxic 95-107

S. No	Name of Compound	S. No	Name of Compound
95	Benzene, (1-propyldecyl)-	102	Heptadecanoic acid, 16-methyl-, methyl ester
96	Hexadecane, 2,6,10,14-tetramethyl-	103	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
97	Propiohydrazide, 2,2-dimethyl-N2-(1-methyl-3-oxo-3-phenylpropylideno)-	104	Glycerol 1-palmitate
98	Pentadecanoic acid, 14-methyl-, methyl ester	105	Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-
99	2H-1-Benzopyran-2-one, 4,7-dimethoxy-	106	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-
100	Z-5-Nonadecene	107	4-Methyl-2-trimethylsilyloxy-acetophenone
101	Methyl stearate		

The compounds listed above were screened for ADME properties to check compounds with drug like properties. SwissADME and pk-CSM were used in the process. The Lipinski rule describes in the methods section was followed. Out of the 107 compounds 24 compounds passed the Lipinski's rule of 5 and showed drug-like properties. These compounds have been listed in Table 5.7 below.

Table 5.7: List of compounds found to have drug-like properties 1-24

S.No	Name of Compound	S.No	
1	Bicyclo[4.3.0]nonan-4-one, 9-(2-methoxyethoxymethoxy)-1-methyl-	15	beta. -(3,4-Dichlorophenyl) ethylamine, N-fluoroacetyl-N-(2-pyrrolidinoethyl)-
2	Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4-[(2-methoxyethoxy)methoxy] - 5, 9 - dimethyl-	16	t-Butyl 1-thio-.alpha.-D-glucopyranoside
3	Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy] - 2 - methyl-	17	2(1H)-Naphthalenone, octahydro-, trans-
4	1H-Pyrazole-1-carboximidamide, 3,5-dimethyl-	18	1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol

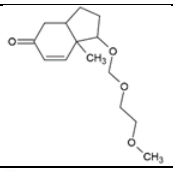
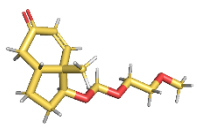
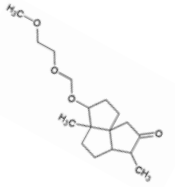
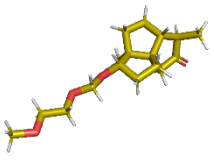
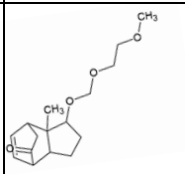
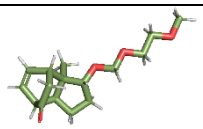
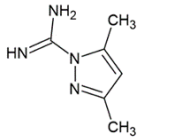
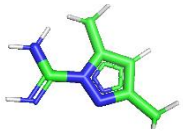
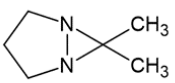
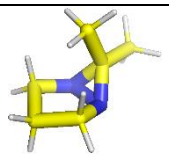
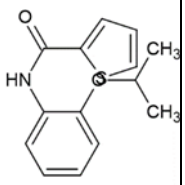
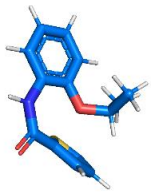
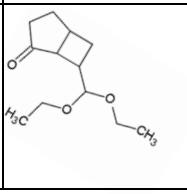
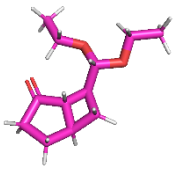
5	6,6-Dimethyl-1,5-diazabicyclo [3.1.0] hexane	19	8-Azabicyclo [3.2.1] oct-6-en-3-one, 8-methyl-
6	N-(2-Isopropoxyphenyl)-2-thiophenecarboxamide	20	Phthalimide, N-(1-hydroxy-2-propyl)-
7	7-Diethoxymethylbicyclo[3.2.0]heptan-2-one	21	Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-
8	Pyrazolidin-3-one, 2-(4-methylbenzoyl)-1-phenyl-	22	Propiohydrazide, 2,2-dimethyl-N2-(1-methyl-3-oxo-3-phenylpropylideno)-
9	1,2-Phenylene bis(mesitylsulfonate)	23	2H-1-Benzopyran-2-one, 4,7-dimethoxy-
10	Cyclohexanone, 3-ethyl-3,5,5-trimethyl-	24	Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-
11	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, sulfite (2:1), [1R-[1.alpha.(1R*,2S*,5R*),2.beta.,5.alpha.]]-		
12	Benzaldehyde, 3,4-dimethyl-		
13	3H-Pyrazol-3-one, 2,4-dihydro-4,4,5-trimethyl-		
14	2-Cyclohexylpiperidine		

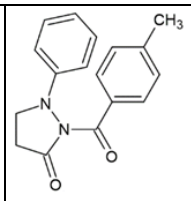
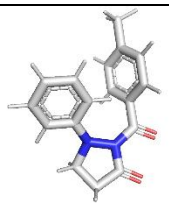
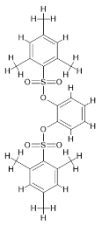
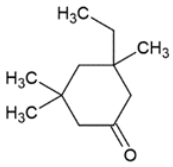
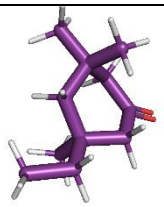
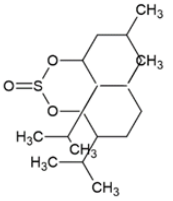
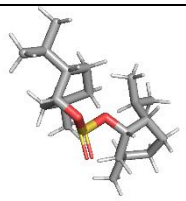
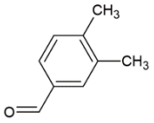
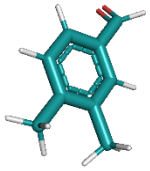
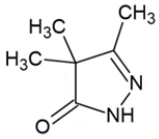
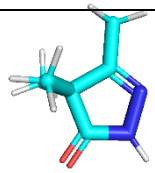
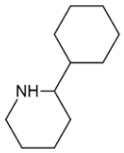
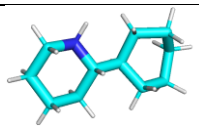
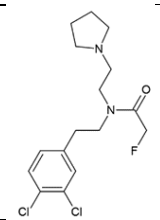
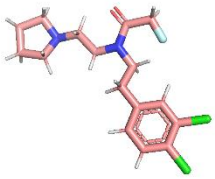
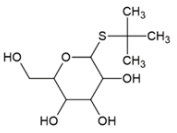
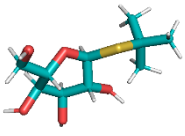
Table 5.8: Drug-like properties of the compounds

S.No	Name of Compound	Molecular weight (<500kDa)	H-bond donor (<5)	H bond acceptor (<10)	Log *P*	No. of Rotatable bonds
1.	Bicyclo[4.3.0]non-2-en-4-one, 9-[(2-methoxyethoxy) methoxy]-1-methyl	254.326	0	4	1.937	6
2.	Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4- [(2-methoxyethoxy)methoxy] - 5, 9 - dimethyl-	296.407	0	4	2.798	6
3.	Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy]-2-methyl-	280.364	0	4	2.183	6
4.	1H-Pyrazole-1-carboximidamide, 3,5-dimethyl-	138.174	2	3	0.242	0
5.	6,6-Dimethyl-1,5-diazabicyclo [3.1.0]hexane	112.176	0	2	0.659	0

6.	N-(2-Isopropoxyphenyl)-2-thiophenecarboxamide	261.346	1	3	3.788	4
7.	7-Diethoxymethylbicyclo [3.2.0]heptan-2-one	212.289	0	3	2.001	5
8.	Pyrazolidin-3-one, 2-(4-methylbenzoyl)-1-phenyl-	280.327	0	3	2.789	2
9.	1,2-Phenylene bis(mesitylsulfonate)	474.600	0	6	5.072	6
10.	Cyclohexanone, 3-ethyl-3,5,5-trimethyl-	168.280	0	1	3.182	1
11.	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, sulfite (2:1), [1R-[1.alpha.(1R*,2S*,5R*),2.beta.,5.alpha.]]-	358.588	0	3	5.520	6
12.	Benzaldehyde, 3,4-dimethyl-	134.178	0	1	2.116	1
13.	3H-Pyrazol-3-one, 2,4-dihydro-4,4,5-trimethyl-	126.159	1	2	0.518	0
14.	2-Cyclohexylpiperidine	167.296	1	1	2.709	1
15.	beta.-(3,4-Dichlorophenyl)ethylamine, N-fluoroacetyl-N-(2-pyrrolidinoethyl)-	347.261	0	2	3.430	7
16.	t-Butyl 1-thio-.alpha.-D-glucopyranoside	252.332	4	6	-0.682	2
17.	2(1H)-Naphthalenone, octahydro-, trans-	152.237	0	1	2.546	0
18.	1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol	212.29	1	3	2.045	3
19.	8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-	137.182	0	2	0.588	0
20.	Phthalimide, N-(1-hydroxy-2-propyl)-	205.213	1	3	0.663	2
21.	Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-	273.315	2	4	2.580	3
22.	Propiohydrazide, 2,2-dimethyl-N2-(1-methyl-3-oxo-3-phenylpropylideno)-	260.337	1	3	2.798	4
23.	2H-1-Benzopyran-2-one, 4,7-dimethoxy-	206.197	0	4	1.810	2
24.	Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-	222.332	0	3	2.040	7

Table 5.9: outlines the chemical structures of the drug-like ompounds

S.No	Name of compound	Pubchem ID	Canonical Structure	2D Structure	Optimized 3D Structure
1.	Bicyclo[4.3.0]non-2-en-4-one, 9-[(2-methoxyethoxy) methoxy]-1-methyl	547949	CC12C=CC(=O)C C1CCC2OCOCCOC		
2.	Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4-[(2-methoxyethoxy)methoxy] - 5, 9 - dimethyl-	560626	CC1C2CCC3(C2(CCC3OCOCCOC)CC1=O)C		
3.	Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy]-2-methyl-	560624	CC12C(CCC1OCOCCOC)C3C=CC2CC3=O		
4.	1H-Pyrazole-1-carboximidamide, 3,5-dimethyl-	97525	CC1=CC(=NN1C(=N)N)C		
5.	6,6-Dimethyl-1,5-diazabicyclo[3.1.0]hexane	573988	CC1(N2N1CCC2)C		
6.	N-(2-Isopropoxyphenyl)-2-thiophenecarboxamide	573781	CC(C)OC1=CC=CC=C1NC(=O)C2=CC=CS2		
7.	7-Diethoxymethylbicyclo[3.2.0]heptan-2-one	568494	CCOC(C1CC2C1C(=O)CC2)OCC		

8.	Pyrazolidin-3-one, 2-(4-methylbenzoyl)-1-phenyl-	576835	CC1=CC=C(C=C1)C(=O)N2C(=O)CCN2C3=CC=CC=C3		
9.	1,2-Phenylene bis(mesitylsulfonate)	576714	CC1=CC(=C(C(=C1)C)S(=O)(=O)OC2=CC=CC=C2OS(=O)(=O)C3=C(C=C(C=C3C)C)C)C		
10.	Cyclohexanone, 3-ethyl-3,5,5-trimethyl-	557975	CCC1(CC(=O)CC(C1)(C)C)C		
11.	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, sulfite (2:1), [1R-[1.alpha.(1R*,2S*,5R*),2.beta.,5.alpha.]]-	558254	CC1CCC(C(C1)OS(=O)OC2CC(CCC2C(C)C)C)C(C)C		
12.	Benzaldehyde, 3,4-dimethyl-	22278	CC1=C(C=C(C=C1)C=O)C		
13.	3H-Pyrazol-3-one, 2,4-dihydro-4,4,5-trimethyl-	76665	CC1=NNC(=O)C1(C)C		
14.	2-Cyclohexylpiperidine	92439	C1CCC(CC1)C2CCCCN2		
15.	beta.-(3,4-Dichlorophenyl)ethylamine, N-fluoroacetyl-N-(2-pyrrolidinoethyl)-	558426	C1CCN(C1)CCN(CCC2=CC(=C(C=C2)Cl)Cl)C(=O)CF		
16.	t-Butyl 1-thio-.alpha.-D-glucopyranoside	91691351	CC(C)(C)SC1C(C(C(O1)CO)O)O)O		

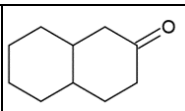
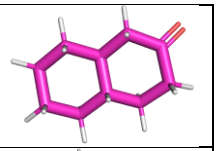
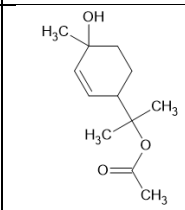
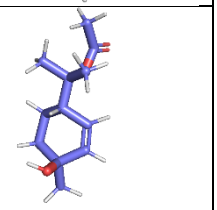
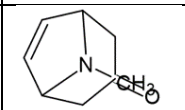
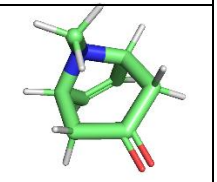
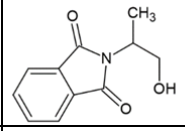
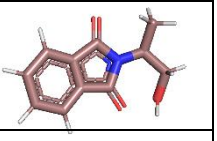
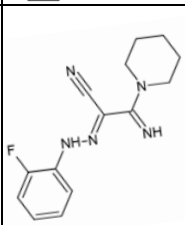
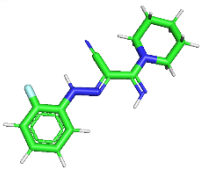
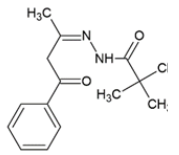
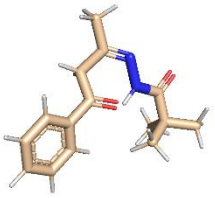
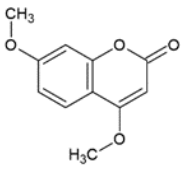
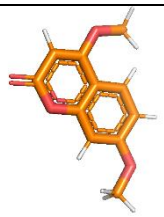
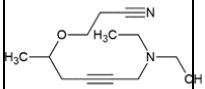
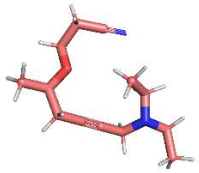
17.	2(1H)-Naphthalenone, octahydro-, trans-	100328	C1CCC2CC(=O)CCC2C1		
18.	1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol	249906236	CC1(O)C=CC(CC1)C(C)(C)OC(C)=O		
19.	8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-	565060	CN1C2CC(=O)CC1C=C2		
20.	Phthalimide, N-(1-hydroxy-2-propyl)-	600020	CC(CO)N1C(=O)C2=CC=CC=C2C1=O		
21.	Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-	6401026	C1CCN(CC1)C(=N)C(=NNC2=CC=CC=C2F)C#N		
22.	Propiohydrazide, 2,2-dimethyl-N2-(1-methyl-3-oxo-3-phenylpropylideno)-	9602535	CC(=NNC(=O)C(C)(C)C)CC(=O)C1=CC=CC=C1		
23.	2H-1-Benzopyran-2-one, 4,7-dimethoxy-	609860	COC1=CC2=C(C=C1)C(=CC(=O)O2)OC		
24.	Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-	610055	CCN(CC)CC#CCC(C)OCCC#N		

Table 5.10: Describes the Lipinski violation, GI absorption and Blood-brain-barrier of the drug-like compounds

S. No	Name of Compound	Lipinski	GI Absorption	BBB penetrability
1.	Bicyclo[4.3.0]non-2-en-4-one, 9-[(2-methoxyethoxy) methoxy]-1-methyl	0 violation	High	Yes
2.	Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4-[(2-methoxyethoxy)methoxy] - 5, 9 - dimethyl-	0 violation	High	Yes
3.	Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy]-2-methyl-	0 violation	High	Yes
4.	1H-Pyrazole-1-carboximidamide, 3,5-dimethyl-	0 violation	High	No
5.	6,6-Dimethyl-1,5-diazabicyclo[3.1.0]hexane	0 violation	Low	No
6.	N-(2-Isopropoxyphenyl)-2-thiophenecarboxamide	0 violation	High	Yes
7.	7-Diethoxymethylbicyclo[3.2.0]heptan-2-one	0 violation	High	Yes
8.	Pyrazolidin-3-one, 2-(4-methylbenzoyl)-1-phenyl-	0 violation	High	Yes
9.	1,2-Phenylene bis(mesitylsulfonate)	1 violation: log P>4.15	Low	No
10.	Cyclohexanone, 3-ethyl-3,5,5-trimethyl-	0 violation	High	Yes
11.	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, sulfite (2:1), [1R-[1.alpha.(1R*,2S*,5R*),2.beta.,5.alpha.]]-	1 violation: log P>4.15	High	No
12.	Benzaldehyde, 3,4-dimethyl-	0 violation	High	Yes
13.	3H-Pyrazol-3-one, 2,4-dihydro-4,4,5-trimethyl-	0 violation	High	No

14.	2-Cyclohexylpiperidine	0 violation	High	Yes
15.	beta.-(3,4-Dichlorophenyl)ethylamine, N-fluoroacetyl-N-(2-pyrrolidinoethyl)-	0 violation	High	Yes
16.	t-Butyl 1-thio-.alpha.-D-glucopyranoside	0 violation	High	Yes
17.	2(1H)-Naphthalenone, octahydro-, trans-	0 violation	High	Yes
18.	1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol	0 violation	High	Yes
19.	8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-	0 violation	High	No
20.	Phthalimide, N-(1-hydroxy-2-propyl)-	0 violation	High	Yes
21.	Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-	0 violation	High	Yes
22.	Propiohydrazide, 2,2-dimethyl-N2-(1-methyl-3-oxo-3-phenylpropylideno)-	0 violation	High	Yes
23.	2H-1-Benzopyran-2-one, 4,7-dimethoxy-	0 violation	High	Yes
24.	Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-	0 violation	High	Yes

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5.3. DOCKING RESULTS

5.3.1. RESULTS FOR CHAIN A DOCKING

Tables 5.11 to 5.34 shows the results of the docking between spike protein 7QO7 Chain A and the 24 drug-like ligands

Table 5.11:

7QO7 Chain A		Bicyclo[4.3.0]nonan-4-one, 9-(2-methoxyethoxymethoxy)-1-methyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
LYS41	NZ(Donor)	O	2.87	-4.81
ARG44	N (Donor)	O	2.88	

Table 5.12:

7QO7 Chain A		Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4- [(2-methoxyethoxy)methoxy] - 5, 9 - dimethyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
LYS525	N(Donor)	O	3.32	-4.26
LYS525	C(Donor)	O	3.51	
PHE326	O	C (Donor)	3.27	
PRO327	O	C (Donor)	3.17	
PRO327	O	C (Donor)	3.19	

Table 5.13:

7QO7 Chain A		Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy]-2-methyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
LYS41	N(Donor)	O	3.04	-4.38

Table 5.14:

7QO7 Chain A		1H-Pyrazole-1-carboximidamide, 3,5-dimethyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
GLU1108	O	H(Donor)	2.42	-4.47
GLU1108	O	H(Donor)	2.15	
VAL101	O	H (Donor)	2.39	

Table 5.15:

7QO7 Chain A			

Residues	Atoms	6,6-Dimethyl-1,5-diazabicyclo[3.1.0]hexane	Distance (Angstrom)	Binding energy
There is no hydrogen bond interaction present				

Table 5.16:

7QO7 Chain A		N-(2-Isopropoxyphenyl)-2-thiophenecarboxamide	Distance (Angstrom)	Binding energy
Residues	Atoms			
TYR793	O	H (Donor)	2.16	-4.40
PHE895	N(Donor)	O	3.16	

Table 5.17:

7QO7 Chain A		7-Diethoxymethylbicyclo [3.2.0]heptan-2-one	Distance (Angstrom)	Binding energy
Residues	Atoms			
ALA27	N(Donor)	O	2.58	-4.27

Table 5.18:

7QO7 Chain A		Pyrazolidin-3-one, 2-(4-methylbenzoyl)-1-phenyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
PHE895	N(Donor)	O	3.21	-5.49
PHE895	N(Donor)	O	2.93	

Table 5.19:

7QO7 Chain A		1,2-Phenylene bis(mesitylsulfonate)	Distance (Angstrom)	Binding energy
Residues	Atoms			
There is no hydrogen bond interaction present				

Table 5.20:

7QO7 Chain A		Cyclohexanone, 3-ethyl-3,5,5-trimethyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
LEU174	N(Donor)	O	3.10	-5.69

Table 5.21:

7QO7 Chain A		Cyclohexanol, 5-methyl-2-(1-methylethyl)-, sulfite (2:1), [1R-[1.alpha.(1R*,2S*,5R*), 2.beta.,5.alpha.]]-	Distance (Angstrom)	Binding energy
Residues	Atoms			

PHE562	N(Donor)	O	3.23	-5.26
PHE562	N(Donor)	O	3.05	

Table 5.22:

7QO7 Chain A		Benzaldehyde, 3,4-dimethyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
GLY70	N(Donor)	O	3.01	-4.90
SER69	CA(Donor)	O	3.24	

Table 5.23:

7QO7 Chain A		3H-Pyrazol-3-one, 2,4-dihydro-4,4,5-trimethyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
TRP255	O(Donor)	H	2.02	-4.49
GLY70	N(Donor)	O	3.38	
SER69	CA(Donor)	O	3.16	

Table 5.24:

7QO7 Chain A		2-Cyclohexylpiperidine	Distance (Angstrom)	Binding energy
Residues	Atoms			
GLN1110	OE(Donor)	H	2.24	-5.31
GLU1108	C	O(Donor)	3.27	

Table 5.25:

7QO7 Chain A		beta.-(3,4-Dichlorophenyl)ethylamine, N-fluoroacetyl-N-(2-pyrrolidinoethyl)-	Distance (Angstrom)	Binding energy
Residues	Atoms			
ASN916	ND2(Donor)	O	3.20	-3.44
GLU915	OE	C(Donor)	2.92	
GLU915	OE	C(Donor)	3.63	

Table 5.26:

7QO7 Chain A		t-Butyl 1-thio-.alpha.-D-glucopyranoside	Distance (Angstrom)	Binding energy
Residues	Atoms			
ILE785	O	H(Donor)	2.23	-3.17
ILE785	O	H(Donor)	2.33	
LYS787	O	H(Donor)	2.03	

Table 5.27:

7QO7 Chain A		2(1H)-Naphthalenone, octahydro-, trans-	Distance (Angstrom)	Binding energy
Residues	Atoms			
VAL632	N(Donor)	O	2.93	-6.14

Table 5.28:

7QO7 Chain A		1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol	Distance (Angstrom)	Binding energy
Residues	Atoms			
ALA519	O(Donor)	H	2.13	-4.85

Table 5.29:

7QO7 Chain A		8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
CYS522	N(Donor)	O	3.11	-4.59

Table 5.30:

7QO7 Chain A		Phthalimide, N-(1-hydroxy-2-propyl)-	Distance (Angstrom)	Binding energy
Residues	Atoms			
TYR793	O	H(Donor)	2.10	-4.81
PHE794	CA(Donor)	O	3.54	

Table 5.31:

7QO7 Chain A		Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-	Distance (Angstrom)	Binding energy
Residues	Atoms			
ILE329	O	H(Donor)	2.51	-5.54
ILE329	O	C(Donor)	3.79	
THR520	O	H(Donor)	1.98	

Table 5.32:

7QO7 Chain A		Propiohydrazide, 2,2-dimethyl-N2-(1-methyl-3-oxo-3-phenylpropylideno)-	Distance (Angstrom)	Binding energy
Residues	Atoms			
PHE895	N(Donor)	N	3.09	-5.29

Table 5.33:

7QO7 Chain A		2H-1-Benzopyran-2-one, 4,7-dimethoxy-	Distance (Angstrom)	Binding energy
Residues	Atoms			
There is no hydrogen bond interaction present				

Table 5.34:

7QO7 Chain A		Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-	Distance (Angstrom)	Binding energy
Residues	Atoms			
THR427	OG(Donor)	N	3.20	-3.14
THR427	N(Donor)	N	2.85	
ASP425	O	C(Donor)	2.90	

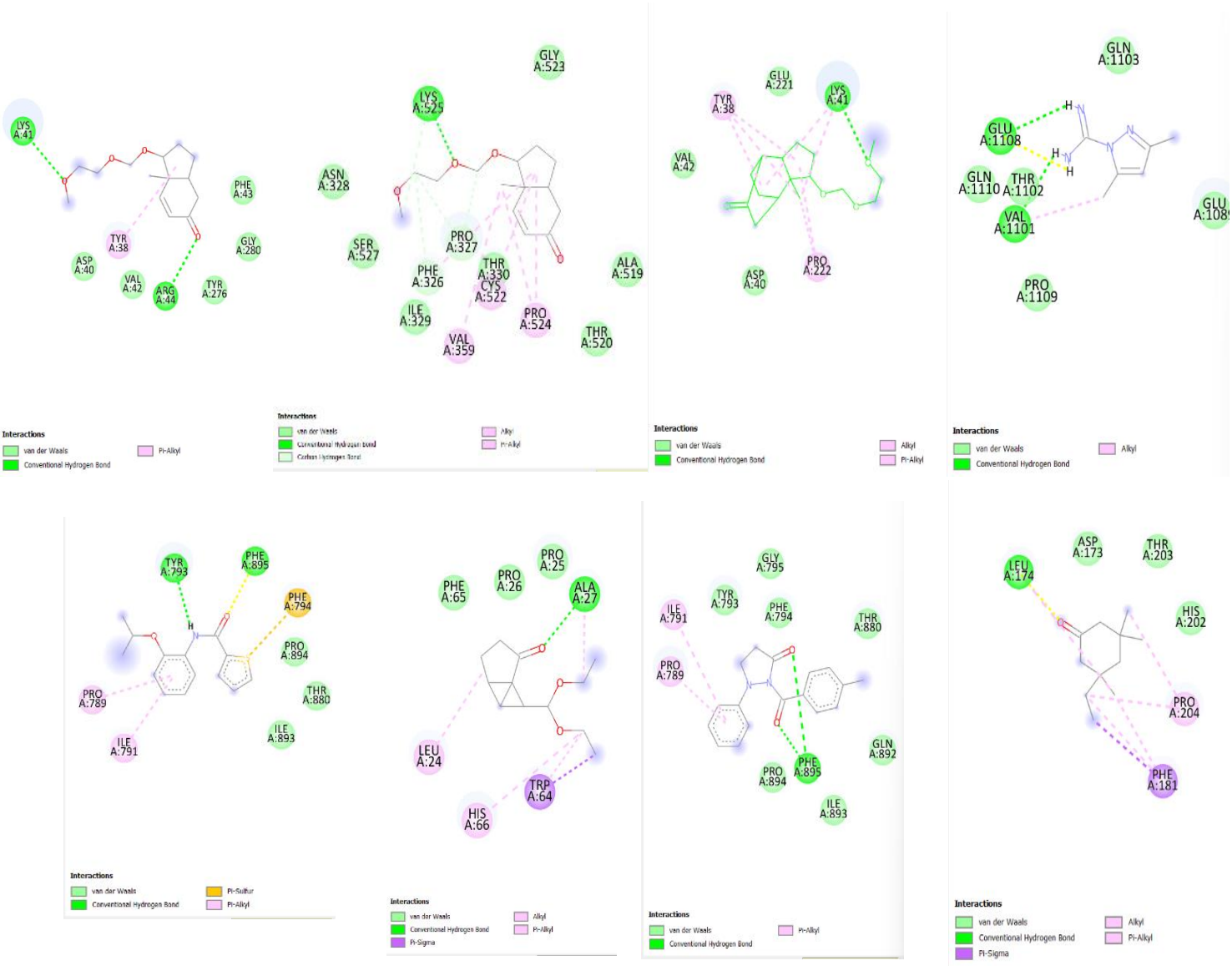

Fig 5.3: From left to right the interaction of 1) *Bicyclo[4.3.0]non-2-en-4-one, 9-[(2-methoxyethoxy) methoxy]-1-methyl,* **2)** *Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4- [(2-methoxyethoxy)methoxy] - 5, 9 - dimethyl-,***3)** *Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy]-2-methyl-,* **4)** *1H-Pyrazole-1-carboximidamide, 3,5-dimethyl-,* **5)** *N-(2-Isopropoxyphenyl)-2-thiophenecarboxamide,* **6)** *7-Diethoxymethylbicyclo[3.2.0]heptan-2-one,* **7)** *Pyrazolidin-3-one, 2-(4-methylbenzoyl)-1-phenyl-,* **8)** *Cyclohexanone, 3-ethyl-3,5,5-trimethyl-* **with spike protein 7QO7 Chain A**

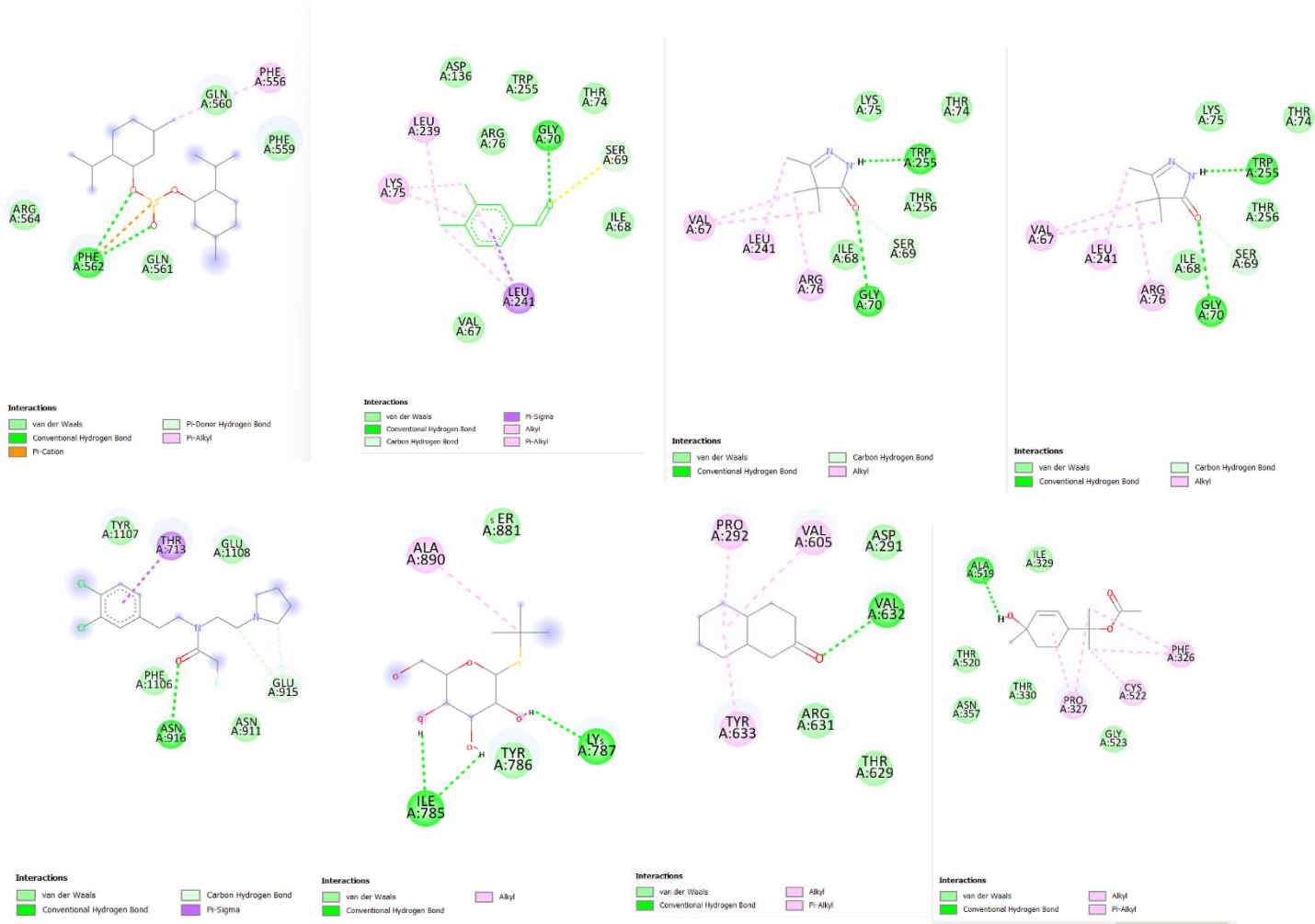

Fig 5.4: From left to right the interaction of 1) *Cyclohexanol, 5-methyl-2-(1-methylethyl)-, sulfite (2:1), [1R-[1.alpha.(1R*,2S*,5R*),2.beta.,5.alpha.]],* **2)** *Benzaldehyde, 3,4-dimethyl-,* **3)** *3H-Pyrazol-3-one, 2,4-dihydro-4,4,5-trimethyl-,* **4)** *2-Cyclohexylpiperidine,* **5)** *beta.-(3,4-Dichlorophenyl)ethylamine, N-fluoroacetyl-N-(2-pyrrolidinoethyl)-,* **6)** *t-Butyl 1-thio-.alpha.-D-glucopyranoside,* **7)** *2(1H)-Naphthalenone, octahydro-, trans-,* **8)** *1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol* **with spike protein 7QO7 Chain A**

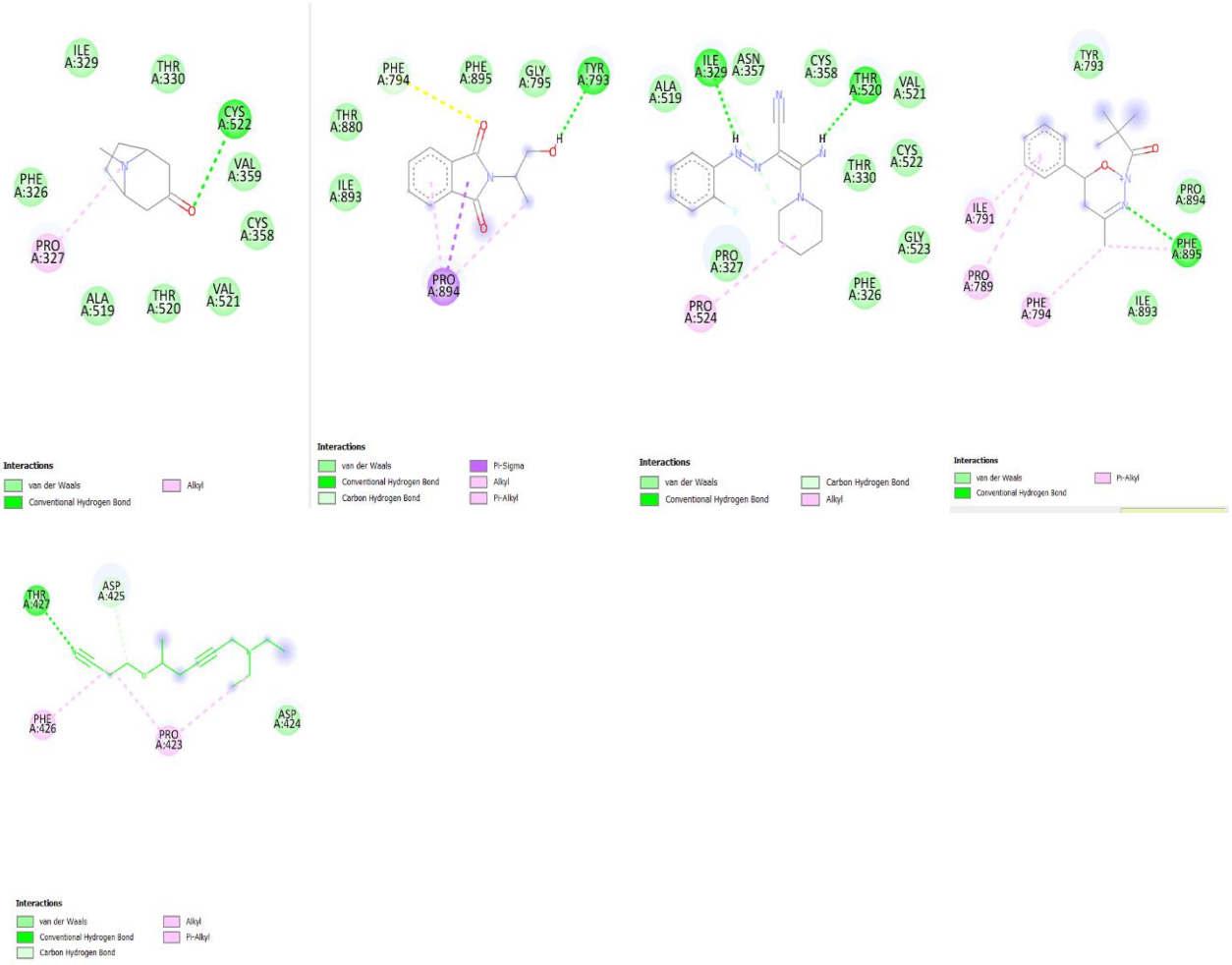

Fig 5.4: From left to right the interaction of 1)*8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-***, 2)** *Phthalimide, N-(1-hydroxy-2-propyl)-***, 3)** *Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-***, 4)** *Propiohydrazide, 2,2-dimethyl-N2-(1-methyl-3-oxo-3-phenylpropylideno).* **5)** *Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-* **with spike protein 7QO7 Chain A**

5.3.2. RESULTS FOR RECEPTOR BINDING DOMAIN

Tables 5.35 to 5.58 shows the results of the docking between Receptor binding protein of spike protein 7QO7 Chain A and the 24 drug-like ligands

Table 5.35:

7QO7 Receptor Binding Domain		Bicyclo[4.3.0]nonan-4-one, 9-(2-methoxyethoxymethoxy)-1-methyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
LYS525	N(Donor)	O	3.32	-5.80
LYS525	C (Donor)	O	3.51	
PHE326	O	C (Donor)	3.27	
PRO327	O	C (Donor)	3.17	
PRO327	O	C (Donor)	3.19	

Table 5.36:

7QO7 Receptor Binding Domain		Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4- [(2-methoxyethoxy)methoxy] - 5, 9 - dimethyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
THR520	O	C (Donor)	3.65	-6.41
THR520	O	C (Donor)	3.00	
THR330	CA (Donor)	O	3.42	

Table 5.37:

7QO7 Receptor Binding Domain		Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy]-2-methyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
SER527	N(Donor)	O	3.16	-6.35
PHE326	O	C (Donor)	3.60	
LYS525	O	C (Donor)	3.49	
PRO327	O	C (Donor)	3.16	
THR330	CB	O (Donor)	3.70	

Table 5.38:

7QO7 Receptor Binding Domain		1H-Pyrazole-1-carboximidamide, 3,5-dimethyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
PHE335	O	H (Donor)	2.38	-4.77

Table 5.39:

7QO7 Receptor Binding Domain		6,6-Dimethyl-1,5-diazabicyclo[3.1.0]hexane	Distance (Angstrom)	Binding energy
Residues	Atoms			
There is no hydrogen bond interaction present				

Table 5.40:

7QO7 Receptor Binding Domain		N-(2-Isopropoxyphenyl)-2-thiophenecarboxamide	Distance (Angstrom)	Binding energy
Residues	Atoms			
LYS534	N(Donor)	O	3.02	-5.21
ASN533	N(Donor)	O	3.04	
VAL531	O	H (Donor)	1.90	

Table 5.41:

7QO7 Receptor Binding Domain		7-Diethoxymethylbicyclo[3.2.0]heptan-2-one	Distance (Angstrom)	Binding energy
Residues	Atoms			
TYR362	OH(Donor)	O	2.52	-5.17

Table 5.42:

7QO7 Receptor Binding Domain		Pyrazolidin-3-one, 2-(4-methylbenzoyl)-1-phenyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
There were no hydrogen bonds observed in the interaction				

Table 5.43:

7QO7 Receptor Binding Domain		1,2-Phenylene bis(mesitylsulfonate)	Distance (Angstrom)	Binding energy
Residues	Atoms			
ASN533	N(Donor)	O	2.82	-6.30
LYS534	N(Donor)	O	2.87	

Table 5.44:

7QO7 Receptor Binding Domain		Cyclohexanone, 3-ethyl-3,5,5-trimethyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
CYS522	N(Donor)	O	2.69	-5.96

Table 5.45:

7QO7 Receptor Binding Domain		Cyclohexanol, 5-methyl-2-(1-methylethyl)-, sulfite (2:1), [1R-[1.alpha.(1R*,2S*,5R*),2.beta.,5.alpha.]]-	Distance (Angstrom)	Binding energy
Residues	Atoms			
ARG343	O	O (Donor)	2.88	-7.86
ALA345	N	O (Donor)	2.99	

Table 5.46:

7QO7 Receptor Binding Domain		Benzaldehyde, 3,4-dimethyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
LYS525	N(Donor)	O	3.17	-5.09

PRO327	O	C (Donor)	3.42	

Table 5.47:

7QO7 Receptor Binding Domain		3H-Pyrazol-3-one, 2,4-dihydro-4,4,5-trimethyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
LYS457	O	H (Donor)	1.70	-4.47
ALA472	N(Donor)	N	2.80	
LYS457	CE(Donor)	O	3.11	

Table 5.48:

7QO7 Receptor Binding Domain		2-Cyclohexylpiperidine	Distance (Angstrom)	Binding energy
Residues	Atoms			
CYS522	O(Donor)	H	2.21	-5.81

Table 5.49:

7QO7 Receptor Binding Domain		beta.-(3,4-Dichlorophenyl)ethylamine, N-fluoroacetyl-N-(2-pyrrolidinoethyl)-	Distance (Angstrom)	Binding energy
Residues	Atoms			
There were no hydrogen bonds observed in the interaction.				

Table 5.50:

7QO7 Receptor Binding Domain		t-Butyl 1-thio-.alpha.-D-glucopyranoside	Distance (Angstrom)	Binding energy
Residues	Atoms			
THR520	O	H (Donor)	2.11	-4.07
CYS522	SG	H (Donor)	2.59	
ALA519	O	H (Donor)	1.88	

Table 5.51:

7QO7 Receptor Binding Domain	2(1H)-Naphthalenone, octahydro-, trans-	Distance (Angstrom)	Binding energy

Residues	Atoms			
LYS525	N (Donor)	O	3.17	-6.40

Table 5.52:

7QO7 Receptor Binding Domain		1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol	Distance (Angstrom)	Binding energy
Residues	Atoms			
ILE329	O	H (Donor)	1.92	-5.82
PRO524	CA(Donor)	O	3.71	

Table 5.53:

7QO7 Receptor Binding Domain		8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-	Distance (Angstrom)	Binding energy
Residues	Atoms			
CYS522	N(Donor)	O	3.10	-4.88
CYS522	N(Donor)	O	3.10	
CYS358	O	O (Donor)	2.70	
THR520	O	O (Donor)	2.73	
ILE329	O	C (Donor)	3.20	
ILE329	O	C (Donor)	3.20	

Table 5.54:

7QO7 Receptor Binding Domain		Phthalimide, N-(1-hydroxy-2-propyl)-	Distance (Angstrom)	Binding energy
Residues	Atoms			
LYS525	N (Donor)	O	2.76	-5.80
LYS525	O	H (Donor)	1.81	
SER527	CB(Donor)	O	3.24	
PHE326	O	C (Donor)	3.62	

Table 5.55:

7QO7 Receptor Binding Domain		Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-	Distance (Angstrom)	Binding energy
Residues	Atoms			
THR330	OG1	H (Donor)	2.49	-6.23
CYS522	O	H (Donor)	1.98	
ILE329	O	C(Donor)	3.75	

Table 5.56:

7QO7 Receptor Binding Domain		Propiohydrazide, 2,2-dimethyl-N2-(1-methyl-3-oxo-3-phenylpropylideno)-	Distance (Angstrom)	Binding energy
Residues	Atoms			
There were no hydrogen bonds observed in the interaction.				

Table 5.57:

7QO7 Receptor Binding Domain		2H-1-Benzopyran-2-one, 4,7-dimethoxy-	Distance (Angstrom)	Binding energy
Residues	Atoms			
CYS522	N (Donor)	O	3.04	-5.46
THR330	OG1(Donor)	O	3.38	
ALA519	O	C(Donor)	3.51	
PRO327	O	C(Donor)	3.49	

Table 5.58:

7QO7 Receptor Binding Domain		Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-	Distance (Angstrom)	Binding energy
Residues	Atoms			
LYS525	O	C (Donor)	3.30	-3.00

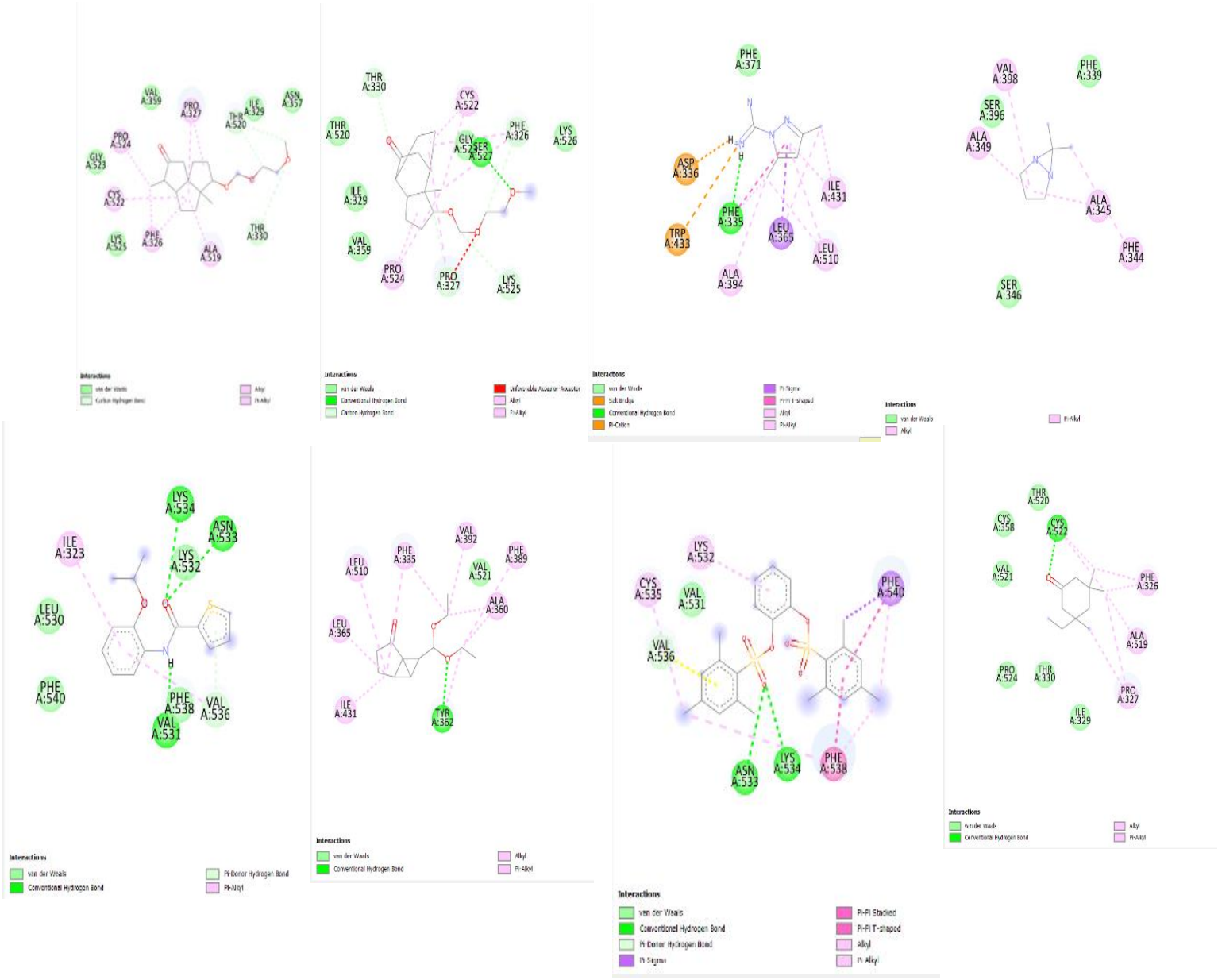

Fig 5.5: From left to right the interaction of 1) *Bicyclo[4.3.0]non-2-en-4-one, 9-[(2-methoxyethoxy) methoxy]-1-methyl,* **2)** *Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4- [(2-methoxyethoxy)methoxy] - 5, 9 - dimethyl-,***3)** *Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy]-2-methyl-,* **4)** *1H-Pyrazole-1-carboximidamide, 3,5-dimethyl-,* **5)** *N-(2-Isopropoxyphenyl)-2-thiophenecarboxamide,* ***6)*** *7-Diethoxymethylbicyclo[3.2.0]heptan-2-one,* ***7)*** *1,2-Phenylene bis(mesitylsulfonate)-,* ***8)*** *Cyclohexanone, 3-ethyl-3,5,5-trimethyl-* **with spike protein 7QO7 recepor binding domain.**

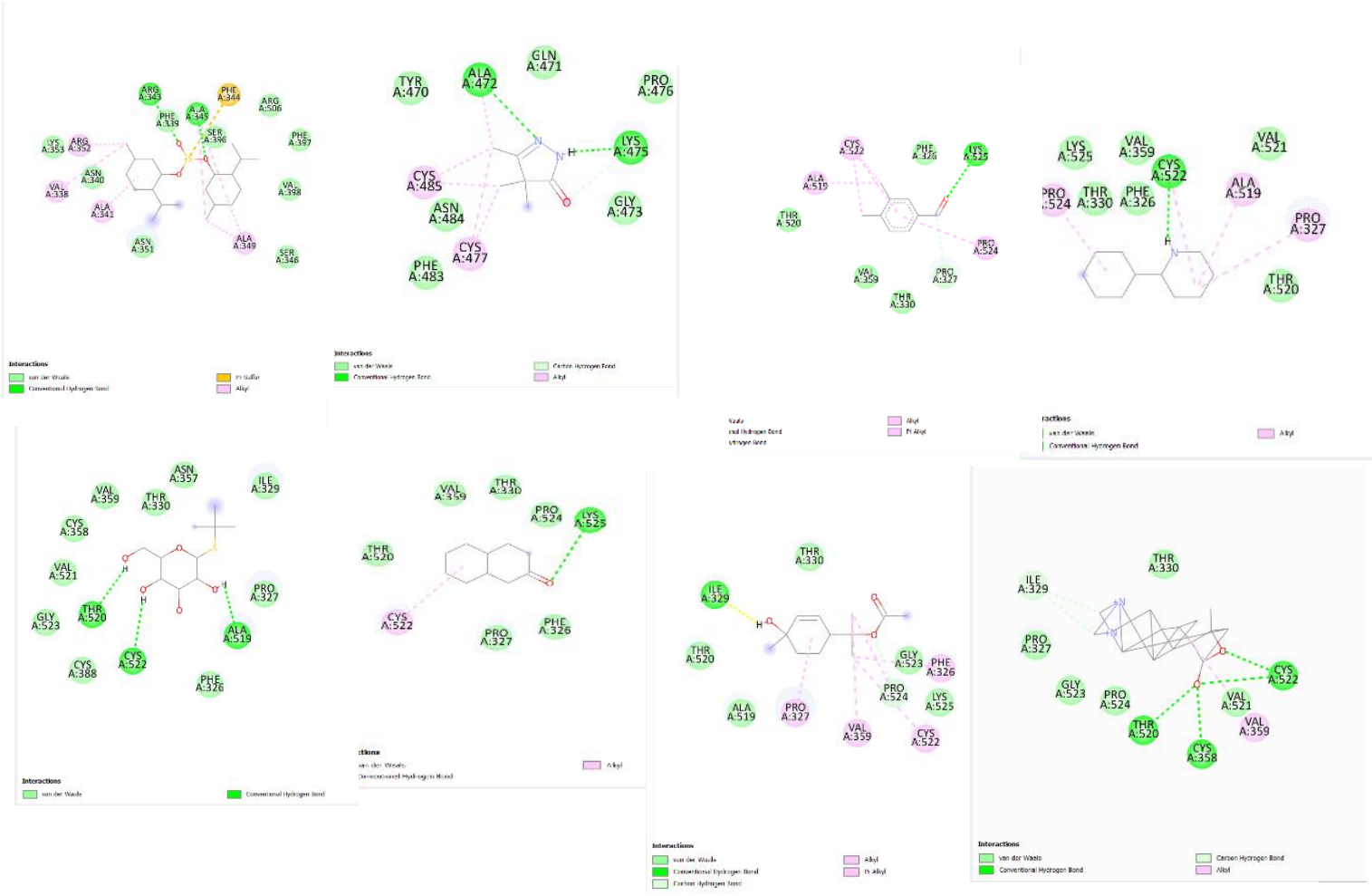

Fig 5.6: From left to right the interaction of 1) *Cyclohexanol, 5-methyl-2-(1-methylethyl)-, sulfite (2:1), [1R-[1.alpha.(1R*,2S*,5R*),2.beta.,5.alpha.]],* **2)** *Benzaldehyde, 3,4-dimethyl-,* **3)** *3H-Pyrazol-3-one, 2,4-dihydro-4,4,5-trimethyl-,* **4)** *2-Cyclohexylpiperidine,* **5)**, *t-Butyl 1-thio-.alpha.-D-glucopyranoside,* **6)** *2(1H)-Naphthalenone, octahydro-, trans-,***7)** *1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol,* **8)** *8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-* **with spike protein 7QO7 recepor binding domain.**

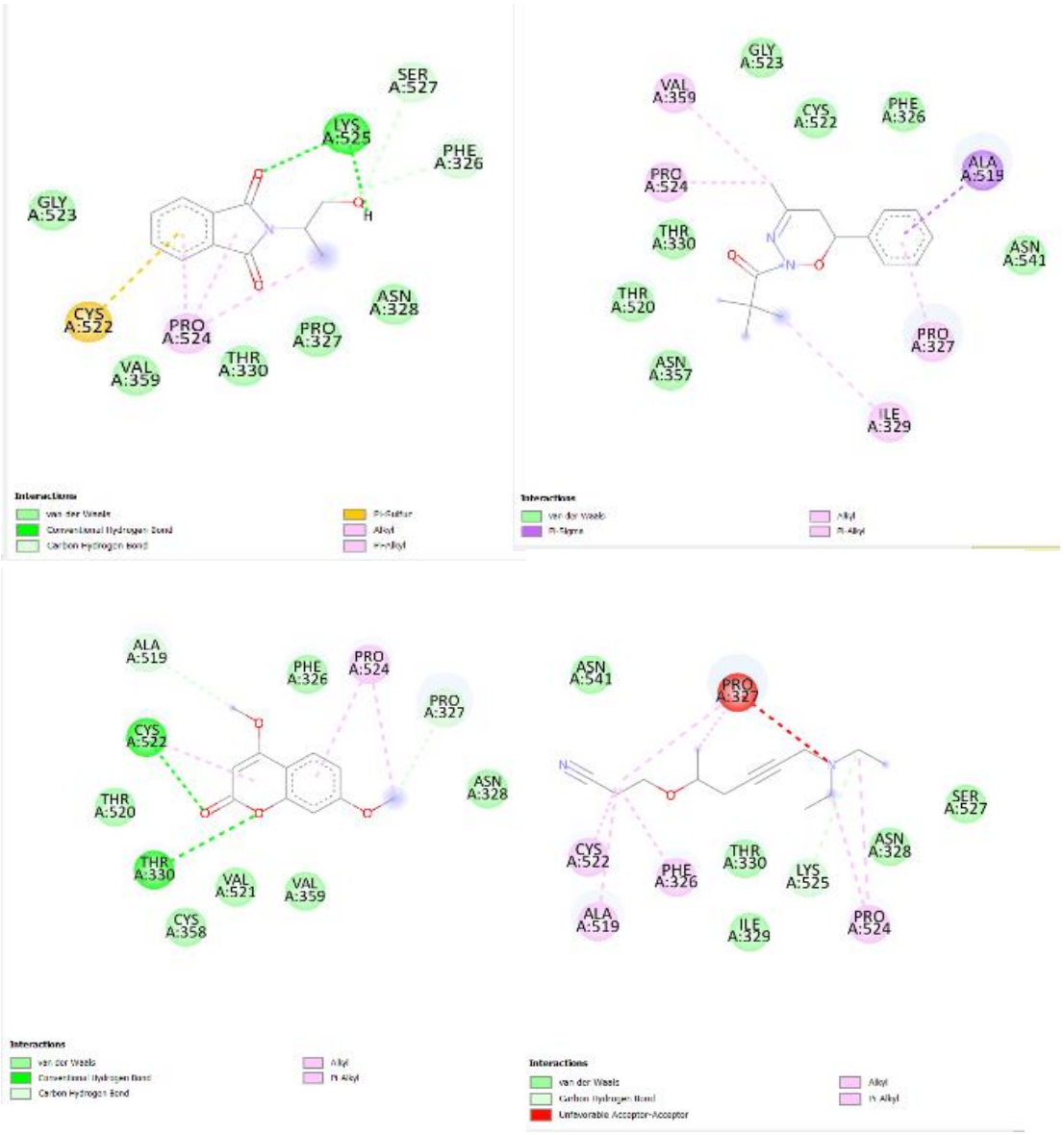

Fig 5.7: From left to right the interaction of 1)*Phthalimide, N-(1-hydroxy-2-propyl)-***, 2)** *Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-***, 3)** *2H-1-Benzopyran-2-one, 4,7-dimethoxy-,* **4)** *Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)* **with spike protein 7QO7 recepor binding domain.**

CHAPTER 6

SUMMARY AND CONCLUSION

The Omicron variant of SARS CoV-2 has higher transmission rate compared to any other variants. The mutations found in the spike protein and its receptor binding domain increases its affinity to the ACE-2 receptor and was thus, used as the target in this study. The phytochemicals were isolated through GC-MS from a polyherbal mixture consisting of 9 siddha herbs, namely, *Coleus amboinicus, Citrus limon, Leucas aspera, Curcuma longa L, Mentha piperita, Ocimum basilicum, Ocimum gratissimum, Vitex negundo* and *Allium sativum*. On docking, the hydrogen bonds and the binding energy, were taken into account. Hydrogen bonds with Distance less than 2.80 are considered as strong bonds. The lowest binding energy displays the strongest intermolecular bond formation between the protein and ligand. The results are briefly described in Table 6.1 below. The least binding energy of the 7QO7 chain A and its receptor binding domain that contains hydrogen bonds is described

Table 6.1: Comparison of the east binding energies of spike protein 7QO7A and its Receptor binding domain.

Name of Compound	7QO7 Chain A	7QO7 RBD
Bicyclo[4.3.0]nonan-4-one, 9-(2-methoxyethoxymethoxy)-1-methyl-	-4.81	-5.80
Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4- [(2-methoxyethoxy)methoxy] - 5, 9 - dimethyl-	-4.26	-6.41
Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy] - 2 - methyl-	-4.38	-6.35
1H-Pyrazole-1-carboximidamide, 3,5-dimethyl-	-4.47	-4.77
6,6-Dimethyl-1,5-diazabicyclo[3.1.0]hexane	N.A.	N.A.
N-(2-Isopropoxyphenyl)-2-thiophenecarboxamide	-4.40	-5.21
7-Diethoxymethylbicyclo[3.2.0]heptan-2-one	-4.27	-5.17
Pyrazolidin-3-one, 2-(4-methylbenzoyl)-1-phenyl-	-5.49	
1,2-Phenylene bis(mesitylsulfonate)	-5.33	-6.30
Cyclohexanone, 3-ethyl-3,5,5-trimethyl-	-5.69	-5.96
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, sulfite (2:1), [1R-[1.alpha.(1R*,2S*,5R*),2.beta.,5.alpha.]]-	-5.26	-7.86
Benzaldehyde, 3,4-dimethyl-	-4.90	-5.09
3H-Pyrazol-3-one, 2,4-dihydro-4,4,5-trimethyl-	-4.49	-4.47
2-Cyclohexylpiperidine	-5.31	-5.81
beta.-(3,4-Dichlorophenyl)ethylamine, N-fluoroacetyl-N-(2-pyrrolidinoethyl)-	-3.44	N.A.
t-Butyl 1-thio-.alpha.-D-glucopyranoside	-3.17	-4.07
2(1H)-Naphthalenone, octahydro-, trans-	-6.14	-6.40
1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol	-4.85	-5.82
8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-	-4.59	-4.88

Phthalimide, N-(1-hydroxy-2-propyl)-	-4.81	-5.80
Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-	-5.54	-6.23
Propiohydrazide, 2,2-dimethyl-N2-(1-methyl-3-oxo-3-phenylpropylideno)-	-5.29	N.A.
2H-1-Benzopyran-2-one, 4,7-dimethoxy-	N.A.	-5.46
Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-	-3.14	-3.00

All the compounds except *2H-1-Benzopyran-2-one, 4,7-dimethoxy-, Propiohydrazide, 2,2-dimethyl-N2-(1-methyl-3-oxo-3-phenylpropylideno)-, beta.-(3,4-Dichlorophenyl)ethylamine, N-fluoroacetyl-N-(2-pyrrolidinoethyl)-, Pyrazolidin-3-one, 2-(4-methylbenzoyl)-1-phenyl-, 6,6-Dimethyl-1,5-diazabicyclo[3.1.0]hexane and beta.-(3,4-Dichlorophenyl)ethylamine, N-fluoroacetyl-N-(2-pyrrolidinoethyl)-* exhibited hydrogen binds for both the targets. *6,6-Dimethyl-1,5-diazabicyclo [3.1.0]hexane* did not bind to both the targets.

The bond between Receptor binding protein (RBD) and the drug candidates were stronger as number of hydrogen bonds formed were higher. The distance of the hydrogen bonds and the binding energy was also lower in the RBD target as compared to chain A. the lowest energy in Chain A is -6.14 while in the RBD it is -7.86. Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4- [(2-methoxyethoxy)methoxy] - 5, 9 - dimethyl- showcased a total of 5 hydrogen bonds with Chain A with binding energy -4.26. 8-Azabicyclo [3.2.1] oct-6-en-3-one, 8-methyl- showed 6 hydrogen bond formations with binding energy as -4.88. The compounds that show minimum energy and more hydrogen bonds could be better candidates. The receptor binding domain (RBD) interacts with ACE-2 receptor in humans and hence, stronger bond formation between the phytochemicals and RBD is important in drug development process.

Among all the compounds, *Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4- [(2-methoxyethoxy)methoxy] - 5, 9 - dimethyl-, Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy] - 2 - methyl-,1,2-Phenylene bis(mesitylsulfonate), Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-, Phthalimide, N-(1-hydroxy-2-propyl)-, 2(1H)-Naphthalenone, octahydro-, trans-, t-Butyl 1-thio-.alpha.-D-glucopyranoside* and *8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-* show higher hydrogen bond formation and minimal binding energy and hence are the best compounds in this study and can be considered potential drug candidates for furthur study in the development of treatment against Omicron variant.

This study shows that the prepared polyherbal mixture can be used as a potential inhibitor of Omicron Variant spike protein from binding to ACE-2 receptor. The study is only a preliminary analysis of the potential of phytochemicals against the SARS Co-2 variant. These selected best phytochemicals should be subjected to further studies in-vivo to evaluate accuracy of the in-silico investigation.

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