Investigating therapeutic potential of mushroom derivatives for combating COVID-19

Project Thesis

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Department Of Biotechnology and Bioinformatics

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M. TECH IN BIOTECHNOLOGY

Project by

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Under the Supervision of

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DECLARATION

We hereby affirm that the work reported in the M. Tech project report allowed **"Investigating therapeutic potential of mushroom derivatives for combating COVID-19"** submitted at **Jaypee University of Information Technology, Waknaghat, India** is a reliable documentation of our work approved under the administration of **Dr. Raj Kumar**. We have not submitted this work in a different place for any other degree or qualification.

Himadri Hauta

202554

This is to certify that the above statement made by the candidates is truthful to the best of my acquaintance.

Dr. Raj Kumar

Date:

SUPERVISOR CERTIFICATE

This is to endorse that the work named "Investigating therapeutic potential of mushroom derivatives for combating COVID-19." Submitted by Himadri Hauta during the end semester in May 2022 in fulfillment for the decoration of grade of Masters of Technology in Biotechnology of Jaypee University of Information Technology, Solan has been approved out under my command.

Signature of Supervisor

Name of supervisor: Dr. Raj Kumar

Designation: Assistant Professor

Department of Biotechnology and Bioinformatics

Jaypee University of Information Technology

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Thank you.

Abstract

The outbreak of SARS CoV-2 lead to pandemic in the year 2020, the virus is a single stranded viral genome with the genome size of ~32kb that has a positive sense RNA. The main hosts whom the virus targets are mostly mammals. Since the outbreak numerous efforts have been put forward for the discovery of an effective drug against the virus. After a whole year, in Dec 2020 the first drug against the virus was approved by FDA. Drugs available against the virus can be counted on fingertips; therefore the search for new and effective drugs cannot be put to halt as the function of the viral particle can be inhibited by a number of inhibitor molecules. In the following study we tried screening a compound database available on fungal particles to find an effective inhibitor for SARS CoV-2 Nsp16. Virtual screening techniques were used dock the MeFSAT library to the binding site/ binding pocket of Nsp16. 3 compounds were chosen as reference inhibitors for the present study. The binding scores of reference inhibitors were: ref1 (Hydroxychloroquine) -6.9 kcal/mol; ref2 (Remdesivir) -6.3kcal/mol; ref3 (Sinefungin) -8.0kcal/mol. 12 molecules out of 373 showed binding scores > 8.0 kcal/mol. It was revealed that out of these 12 molecules as well, only 6 ligands formed hydrogen bonds with the active site and exhibit important molecular interactions for SARS-CoV-2 Nsp16 inhibition. We propose 6 compounds as potential inhibitors of SARS-CoV-2 Nsp16 and may be considered for further pre-clinical studies.

CHAPTER 1

1.1 Introduction

COVID-19 is an acute respiratory syndrome the scientific name of which is SARS-CoV-2, discovered in December 2019 in Wuhan, China which has a current population of 11 million. The causative agents of this disease were identified as a beta-Corona virus, which is the same as SARS-CoV. The disease was first reported in a wholesale seafood market in Wuhan. The World Health Organization (WHO) has temporarily named this illness as the 2019 novel Corona Virus. Interestingly, the first whole-genome sequencing for a new corona virus was released on January 10, 2020 [1]. This provided researchers with additional sources of information. RT-PCR was used for the detection of virus in the patients. On 21 January, the International Committee on Taxonomy of Viruses (ICTV) Coronaviridae Study Group (CSG) renamed 2019-nCoV SARS-CoV-2, reporting that 2019-novel corona virus is associated with the beta-corona virus group that shares 79.5% and 95% with the SARS-CoV and bat corona virus. However, a Chinese virology group later gave the virus a conventional name and called it HCoV-19 [2]. The WHO classified the 2019-nCoV pandemic a Public Health Emergency of International Concern on January 30. (PHEIC). On January 31, several organizations submitted 51 SARS CoV-2 whole-genome sequences. In Feb, 2020 WHO finally named the virus as 2019- novel corona virus and the pathogen was SARS-CoV-2. The novel infected state was called the corona virus disease 2019 or COVID-19. Unfortunately, SARS-Cov-2 couldn't be contained and swiftly stretched to over 100 countries across the world. Eventually, in February 2020, WHO formally stated COVID-19 as pandemic with a total of 79,331 confirmed virus infected cases, together with 2618 deaths within China along with other 27 countries, prompting worldwide alarm and advocating for pharmaceutical intervention in COVID-19 treatment and prevention. The worldwide death rate was 9,913 on March 19th, 2020, with 2, 42,650 laboratory-confirmed cases. In various nations infected people were dying at different rates. However, in April the worldwide mortality rate was about 3.92%. The WHO also developed a list of priority for the epidemic and pandemic diseases [3

1.3 Corona virus

Corona viruses are a family of RNA viruses that infect birds and mammals, and their structure resembles the sun's corona. They have a 30kb genome and enveloped positive sense ssRNA. It was first found in hens in the year 1930, long before the pandemic, and was known as infectious bronchitis virus at the time. It was finally isolated for the first time in 1937. Although many corona viruses have been discovered in animals, only seven have infected humans. Based on their occurrence after mutation from the initial SARS Cov-2 strain, the variants are divided into four groups. The variants are divided into three groups: Variants of Concern (VOC), which are variants for which there is clear evidence of the risks they represent to public health. Variants of Interest (VOI) are variants for which data is available but the hazards remain unknown. Then there is the Variants Being Monitored (VBM), which are the variants that are being kept an eye on because the risk is low [4]. The main varieties of concern (VOC) are Alpha, Beta, Gamma, & Delta. WHO recommends. The variants of interest (VOI) are Eta, Iota, Lambda, & Kappa. The alpha, beta, gamma, and delta strains of the virus are summarized in Table 1. COVID-19 is known to be the third zoonotic outbreak of the beta-corona virus and the seventh corona virus to infect humans [5].

Table 1: Alpha, Beta, Gamma and Delta variants

Characteristics	Alpha	Beta	Gamma	Delta
Name of the strain	B.1.1.7	B.1.351	P.1	B.1.617.2
Year of detection	December 2020	October 2020	January 2021	December 2020
Place of detection	United Kingdom	South Africa	Brazil	India
Transmissibility (as compared to original)	40%-80% more	50% more	1.7 to 2.4 times more	97% more

7 Human Corona viruses are:

1. The 229E (alpha-corona virus)
2. The NL63 (alpha-corona virus)
3. The OC43 (beta-corona virus)
4. The HKU1 (beta-corona virus)
5. The MERS-CoV (beta-corona virus)
6. The SARS- C0V (beta-corona virus)
7. The SARS-CoV-2 (novel corona virus, COVID-19)

1.2 Dynamics of transmission of novel corona virus

Because of a paucity of evidence, the transmission dynamics of SARS-CoV-2 have remained unknown since the pandemic. Initial research on the virus was either done in a short amount of time or based on its close relatives, such as SARS-CoV and MERS-CoV. It spreads primarily through direct contact between people or by the transmission of respiratory droplets. Whatever the situation may be, the virus should be discharged into the environment for initial transmission. SARS CoV-2 exhibits an 88 percent similarity to the coronavirus-driven strain. Despite the fact that it is genetically distinct from SARS-CoV and MERS-CoV, it has a 79 percent resemblance. While studying SARS CoV, RATG13 and SARS CoV2 it was revealed that SARS CoV2 had ~ 96.2% similar identity of the genome sequence with BAT CoV, RATG13 [6,7].

CHAPTER 2

Structure

SARS-CoV-2 is round or elliptic and is pleomorphic in nature, which makes its research a task to do, it has a diameter of ~60-140nm and is sensitive to UV rays and heat. The complete structure consists of structural and non-structural proteins encoded by ORF1a and ORF1ab. The genome size of the virus is ~30kb which includes 14 open reading frames (ORFs) and 27 proteins, in which 4 are structural proteins and the others are non-structural proteins or accessory proteins. Whole genome analysis shows 96% similarity of SARS-CoV-2 with that of Bat's coronavirus, which makes it possible to believe that bats might be the host of SARS-CoV-2 [7]. On the basis of detection techniques like metagenomics, electron microscopy and molecular biology pangolins also showed 99% similarity with the SARS CoV-2 strain, making pangolins a possible host. The genomic structure of SARS CoV-2 contains:

- The 5'cap
- The ORF1ab
- The Replicase gene
- The Spike ORF
- The Envelop ORF
- The Membrane ORF
- The Nucleocapsid ORF
- The 3' Poly A tail

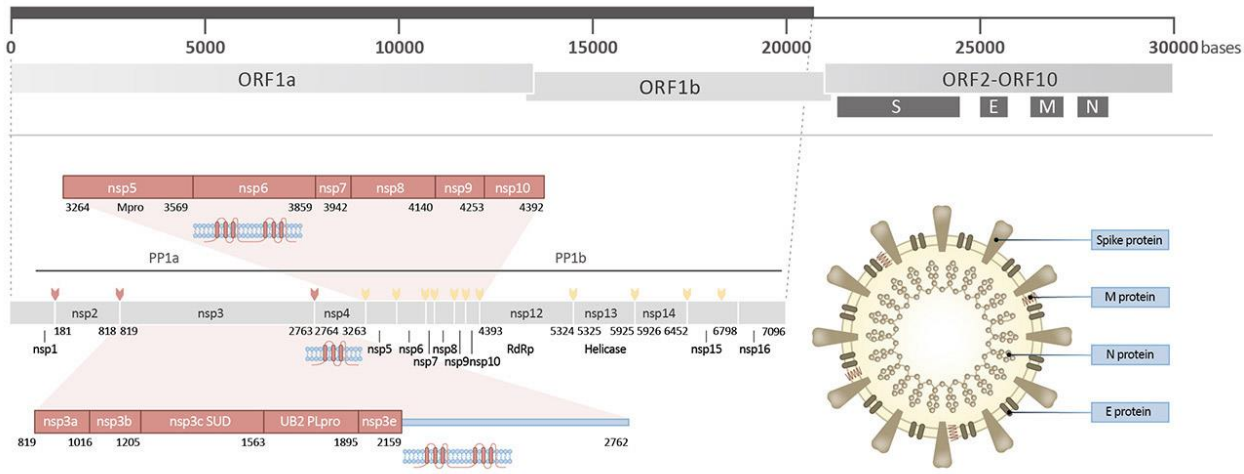


Figure 1: SARS- CoV-2 genome.

2.1Structural proteins

Structural proteins are proteins with certain characteristics, such as a sequence that repeats itself to build a skeleton. These proteins help the virus offer structure, or we can say they contribute to the virus's mechanical attributes. Four main structural proteins are:

1. Nucleocapsid protein (N)
2. Spike glycoprotein (S)
3. Membrane glycoprotein (M), and
4. The Envelope

The main protein that allows the virus to attach to the host surface is the spike protein, it contains of 3 domains which are: N-terminal, which further consists of two subunits, S1 & S2 respectively, followed by the cytoplasmic domain, and then the transmembrane domain. The S1 and S2 subunits of N-terminal are glycosylated and highly conserved. In the host body, ACE2 is found in the lungs, heart, kidneys, and intestines. Spike protein consists of a receptor binding motif that helps the protein in its accurate binding with the ACE2 receptor of the host membrane. The N-terminal domain along with the receptor binding domain are parts of the S1 subunit, while S2 subunit consists of fusion peptides, heptad repeat1 (HR1) and heptad repeat2 (HR2), the transmembrane region, and the intercellular domain. Fusion peptides are proteins that have two domains, each of which is encoded by a distinct gene, but which are fused together so that they can be transcribed and translated as a single unit, resulting in a single polypeptide. Heptad

repeats are structural motifs made up of a seven-amino-acid pattern that repeats. The hydrophobic part of the protein is the transmembrane region [5] [6]. The protein's S1 unit binds to the receptor on the host cell membrane, while the S2 unit facilitates the fusion of the viral particle with the cellular membrane. Membrane protein (M) is also known as the viral assembly's central organizer. It is the maximum found protein on the viral surface, defining the envelope shape and interacting with other structural proteins. Out of all the four major structural proteins that are present on the viral membrane is the envelope protein (E). It is largely localized near the endoplasmic reticulum and the Gogi apparatus, and holds great significance as it is necessary for the assembly of the virus and its release from host cells, as well as during the replication process of the virus [7].

2.2 Non-structural proteins (Nsp)

Nsps are those proteins that are encoded through virus however not present in the viral particle; they are present in the infected cell and perform specific tasks within the host cell. Viruses require NSPs, which are enzymes and transcription factors, to replicate. In the SARS-CoV-2 genome, the ORF1ab polyprotein encodes for 16NSPs. NSPs play a key role in corona virus survival and virulence by assisting in the synthesis and processing of RNA. NSPs go through post-translational changes to control the activity of replicative proteins [8]. The interaction of NSPs leads to the creation of a replication-transcription complex (RTC). Nsp1 reduces the host's gene expression while also inhibiting the infected cells' innate immune response. Nsp3, Nsp4, and Nsp6 keep dsRNA from being degraded by the immune system. Nsp5, also known as 3CLpro, is created automatically at first, after which it cleaves downstream Nsps, allowing nsp4 and 16 to be released. Nsp12 and 8 play a vital part in the RNA polymerase replicative machinery's construction. In order to accomplish successful RNA synthesis, Nsp8 and 12 require the presence of Nsp7 as well [8] [9]. The exonuclease domain at the N-terminus of nsp14 is involved in proofreading. Other Nsps implicated in mRNA capping include 10, 13, 14, and 16. Membrane and envelope proteins transform the host cell into a viral workshop, allowing the host and viral cells to collaborate to create new viral particles. The viral envelope is the outermost coat of the viral cell that is made up of the host cell membrane. It is found beneath the surface proteins. The SARS-CoV-2 envelope is a fatty coating that breaks down and kills the virus when it comes into touch with soap [10]. The virus's genetic material is contained within the capsid, which is located beneath the envelope. The nucleocapsid, also known as the n-protein, is now

found inside this capsid. The nucleocapsid stores all of the virus's genetic information, allowing it to replicate. The virus's ssRNA is linked to N-protein. N-proteins thwart the host cell's defense mechanisms while simultaneously assisting viral RNA replication [11] [12].

Table 2: Nsp1-16 along with the number of structures available for them, PDB IDs, their common names and PubMed IDs

S.No.	*Name*	*No. of structures available*	*PDB IDs*	*Common names*	*PMID*
1.	Nsp1	1	7K3N	Virulent Factor/ Ribosome Inhibitor	33135884, 33151142,34110264,34121824
2.	Nsp2	1	7MSW	Endosome associated protein/ Still unknown	34031651, 34013269
3.	Nsp3	11	7KAG 6WEY 7THH 6W9C 6W9C 7LGO 7T9W 7RQG	Cutting and untagging protein/ Papain-like protease	
4.	Nsp4	2		Double membrane vesicle maker/ Membrane Anchoring	34844509, 34121824
5.	Nsp5		7BQY	3CL proteases/ Main Protease	
6.	Nsp6	1		Double-membrane vesicle	34121824, 32965508

				factory/ Membrane Anchoring	
7.	Nsp7	1	7BV2	Copy assistant/ Processivity Factor for RdRp	
8.	Nsp8	1	7BV2	Primase/ Processivity factor for RdRp	
9.	Nsp9	1	6W9Q	RNA binding protein	33454715,34352100,34844119, 33639316
10.	Nsp10	1	6W4H	Methyltransferase stimulator/ Binds nsp14 and nsp16	32994211,34250275,34684735
11.	Nsp11			Still Unknown	
12.	Nsp12	1	7BV2	RNA-dependent RNA polymerase	
13.	Nsp13	6	6XEZ	Helicase, RNA 5'-Triphosphatase	34117992,34455933,32875166,34996837,34198322, 34381037, 32536457,33052685,32980406,34995115,32817950, 33596235,35012384, 34777769
14.	Nsp14	1	7EGQ	Proofreading exonuclease/ Exonuclease (3'-5"), N7-Methyltransferase	33724070,34942146,34198328,32567979,34303749 32563813,33870746,35194610,32875166,35018374 34845015,33619486,34192965
15.	Nsp15	3	6WLC	Endonuclease	34198324,33886614,32943302,33564093,34778776 35208797,35128681,34639036,33776343,34015930 33963942,33713492,33485847,33824127,32552462 33418408,32835078
16.	Nsp16	1	6W4H	Methyltransferase/	33850334,34766876,34959647,35252541,32835632

2'-o-Methytransferase	33685335,32994211,34250275,33016237,32738360,32266873,32741322,32573355,34099976,34762019

Fungus as a ligand: Fugal habitats are rich sources secondary metabolites. Fungal secondary metabolites are bioactive molecules and can be proved really useful for drug discovery that is natural product based. Bioactive molecules are the molecules produced in response to a microbial activity; they are basically secondary metabolites that can perform therapeutic and immunostimulating activities.

2.3 MeFSAT

Medicinal Fungi Secondary Metabolite and Therapeutics (MeFSAT) is an organized database of 184 medicinal fungi along with the information about their secondary metabolites and their therapeutic uses. The database has information of about 1830 secondary metabolites and almost 149 of their therapeutic uses. The efforts to create this database was made after realizing that there was no such database that gives the information about the secondary metabolites of the fungal kingdom, despite their use in traditional medicines since ages. The data related to secondary metabolites of fungus is dispersed and that is what limits it use for effective drug discovery.

2.4 Molecular Docking

It is a method to perform structure based drug design in –D, it predicts the mode/pose, i.e. the orientation of the ligand when bound to the target molecule at specific binding site. It is a key tool for drug-designing that is computer assisted. There are number of software's available to perform docking, for example: AutoDock Vina, AutoDock, ArgusLab 4.0.1, Open Babel, PyRx, etc. In my research study I learned to operate PyRx Software, which is freely available.

We need to check the binding affinity of the molecules in the database to our target protein, i.e. 6YZ1. After Docking we will compare our binding affinities to that of the binding affinities of certain reference drugs that has already been in use for the treatment of the virus.

CHAPTER 3

Materials and Methods

3.1 Protein selection

The very first step was to explore UniProt for filtering the SRAS-CoV-2 proteins based on the research articles available on them, which eventually led us to know how much research has been done on that particular protein.

After exploring the proteins on UniProt, we decided to shift our focus onto the non-structural proteins (Nsps). To filter out the Nsps I explored their PDBs, to filter the NSPs according to their structures available. The protein structures were procured using the Protein Data Bank database (https://www.rcsb.org). After exploring both, UniProt and PDB, 6YZ1 (PDB ID) is the protein that was finally selected as the target protein. 6YZ1 is Nsp16-Nsp10 conjugate.

3.2 Ligand selection

MeFSAT database was chosen for the ligands. Out of 1830 molecules available in the database only a few would be appropriate as binding energy of our ligand molecule should be more than the reference ligand molecules. The reference ligand molecules were chosen from already approved drugs.

3.3 Molecular Docking

Molecular docking was done for the selected protein and the ligands from the MeFSAT database, along with the reference drugs using the software PyRx, which is a virtual screening tool. The software is automated. The main reason for performing the molecular docking is to understand the mechanism of protein-ligand binding and to see the orientation of the protein-ligand complex.

Steps followed

i. Open PyRx
ii. File > load molecule (pdb file)
iii. File > Import > Chemical table file SDF
iv. Right click on the protein > Go to autodock> Make micromolecules (proteins are automatically converted into pdbqt format)
v. Right click on the ligand > Minimize all (for energy minimization)
vi. Again right click on the ligand > Convert selected to pdbqt (in case of the entire database Convert all in pdbqt)
vii. Press ctrl > select ligand
viii. Press ctrl > select protein
ix. Go to Vina Wizard > select molecules > forward (grid box appears, we want it to cover our entire protein, so by dragging grid box can be adjusted) > forward
x. Visualization using discovery studio.
xi. All the results from PyRx are automatically saved in C drive > users > HP > .mgltools>PyRx.

CHAPTER 4

Results and discussions

4.1 We docked our reference drugs and the ligand database with the protein initially to know the binding energies to compare:

Drug designing on the basis of structure seeks to produce molecules that show high affinities to their specific target proteins. Binding free energy helps us in getting better understanding of how a particular protein finds and binds to its biologically significant ligand which ultimately helps us in designing the dominant drug. Out of 373 molecules that were docked, 61 molecules had binding energies above -6.9 and 12 molecules had binding energy above -8.0. The 12 molecules that had binding energy above -8.0 were selected for studying the molecular interactions, basically the conventional hydrogen bonds.

Table 3: Best binding scores of reference molecules and mushroom ligands.

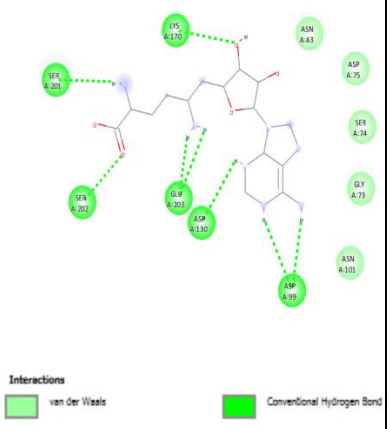
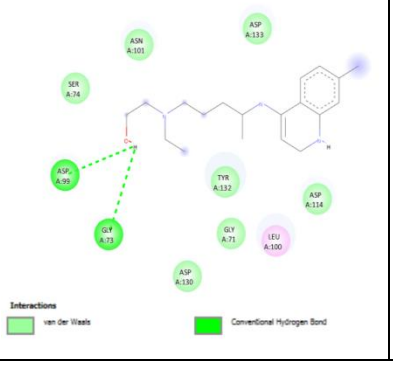
MSID	Binding Energy (kcal/mol)
MSID000059	-8.1
MSID000106	-8.1
MSID000107	-8.4
MSID000108	-8.1
MSID000282	-8.2
MSID000284	-8.3
MSID000285	-8.6
MSID000293	-8.0
MSID000330	-8.6
MSID000331	-8.2
MSID000332	-8.0
MSID000342	-8.0

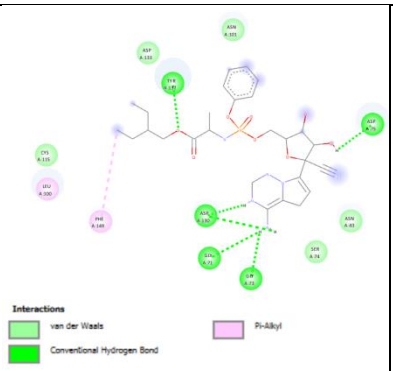
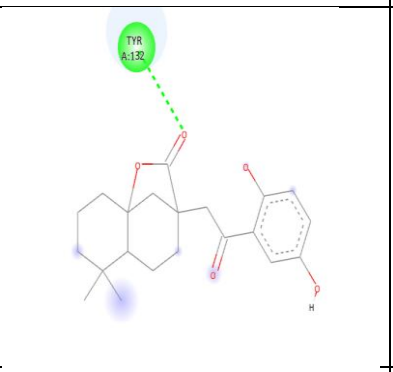
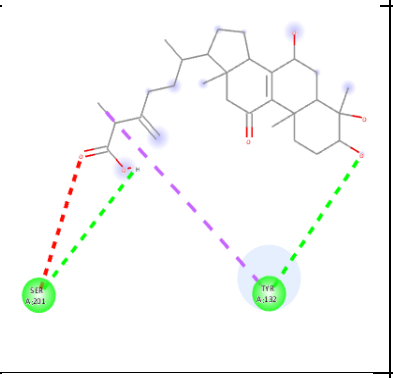
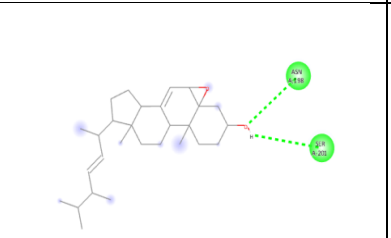
Hydroxychloroquine	-6.9
Remdesivir	-6.3
Sinefungin	-8.0

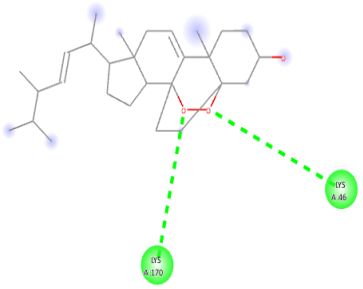
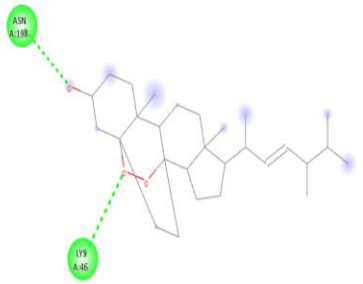
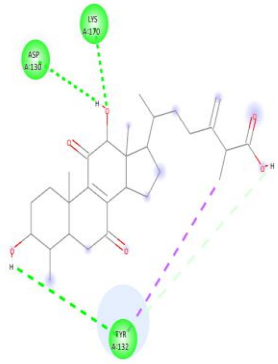
4.2 Reference inhibitors and ligand molecules molecular interactions

Out of the above 12 molecules that had binding scores more than the reference inhibitors only 6 mushroom derivatives showed hydrogen bonds similar to the reference inhibitors. Table 4 shows the results.

Table 4: Hydrogen bond in 2D and hydrophobic interactions of reference molecules and mushroom ligands.

S.No	Molecule	Interactions	2-D diagram	Hydrophobic Interactions
1.	Sinefungin	ASP (A:99) ASP (A:130) LYS (A:170) SER (A:201) SER (A:202) GLU(A:203)		CYS115, LEU100, PHE70, SER98, PHE149, MET131
2.	Remdesivir	GLY (A:73) ASP (A:99)		LEU100, MET131, ASP133, ASN101

3.	Hydroxychloroquine	GLY (A:71) GLY (A:73) ASP (A:75) ASP (A:130) TYR (A:132)		LEU100, MET131, ASN101
4.	MSID000059	TYR (A:132)		CYS115, LEU100, PHE70, SER98, PHE149, MET131
5.	MSID000108	TYR (A:132) SER (A: 201)		LEU100, MET131, ASP133, ASP94
6.	MSID000282	SER (A:201)		LEU100, MET131, ASP133, ASN101

7.	MSID000284	LYS (A:46) LYS (A:170)		CYS115
8.	MSID000285	LYS (A:46) ASN (A:198)		LEU100, MET131, CYS115, PHE149
9.	MSID000342	TYR (A:132)		LEU100, MET131,ASN10 1

CHAPTER 5

Conclusion

Drugs based on natural compounds have revolutionized the drug discovery process as natural compounds have the capability to mimic certain metabolites for example hormones and natural ligand molecules. The study aimed at exploring the potential compounds from medicinal mushrooms with the help of bioinformatics tools. The target protein was selected to be the Nsp16 protein. For ligands selection MeFSAT database was used, which is a library of mushroom derivatives. Molecular docking was performed in order to see the binding energies and conformations of the ligands to the protein molecules. 3 reference inhibitors were used in order to get the similar molecular interactions that are present in the reference molecules. Only 6 molecules out of 372 compounds showed molecular interaction similarity to the reference molecules. The highest binding score was of the ref3, i.e., Sinefungin (-8.0kcal/mol), as for the ref1 (Hydroxychloroquine) and ref2 (Remdesivir) the scores were -6.9kcal/mol and -6.3kcal/mol. The scores for the 6 mushroom derivates were, MSID00059 (-8.1kcal/mol), MSID000108 (-8.1kcal/mol), MSID000282 (-8.2kcal/mol), MSID000284 (-8.3 kcal/mol), MSID000285 (-8.6 kcal/mol), MSID000342 (-8.0 kcal/mol) .These 6 molecules can now be used for further studying their potential as an effective drug against the COVID-19.

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