Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2024.1001.06**

LOVASTATIN: AN ANTIVIRAL DRUG DESIGN APPROACH FOR CANCER AND COVID-19

Krishna Kumar Das[1], Smaranika Pattnaik[1*], Santosh Kumar Behera[2]

1. Dept. of Biotechnology and Bioinformatics, Laboratory of Medical Microbiology, School of Life Sciences, Sambalpur University, JyotiVihar, Burla,
2. NIPER, Gujrat, India.

ABSTRACT: Lovastatin, also known as the brand name product Mevacor, is a lipid-lowering drug and fungal metabolite derived synthetically from a fermentation product of Aspergillus terreus. Originally named Mevinolin, lovastatin belongs to the statin class of medications, which are used to lower the risk of cardiovascular disease and manage abnormal lipid levels by inhibiting the endogenous production of cholesterol in the liver. Increasing use of the statin class of drugs is largely due to the fact that cardiovascular disease (CVD), which includes heart attack, atherosclerosis, angina, peripheral artery disease, and stroke, has become a leading cause of death in high-income countries and a major cause of morbidity around the world. As the Lovastatin metabolites have the anti-viral pharmacological effect, therefore by treating the drug compound as the precaution and novel drug compound for the Cancer and COVID-19. Being the target gene of interest "HER2, PIK3CA and BRAF" for cancer diseases and "ANPEP, ACE2 and TRIM56" for COVID-19. The molecular docking studies have reported the 6 ligand-protein interactions. Seeking the importance of genetic background of Cancer and COVID-19 patients further studies can be done by mining of non-synonymous SNPs associated with genes for causing both Cancer and COVID-19.

Keywords: Cancer; COVID-19; Lovastatin; molecular docking; cardiovascular disease (CVD)

Article History: Received: Jan 14, 2024; Revised: Jan 20, 2024; Accepted: Jan 25, 2024.

Corresponding Author: Dr. Smaranika Pattnaik Ph.D., D.Sc.
School of Life Sciences, Sambalpur University, JyotiVihar, Burla 768019, Odisha
Email Address: smaranika2010@suniv.ac.in

1. INTRODUCTION

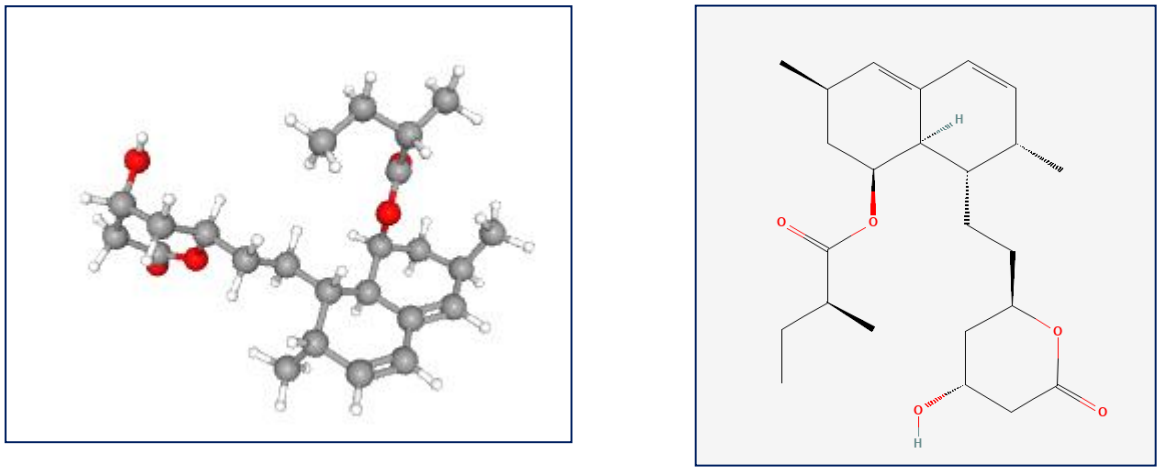
Human epidermal growth factor receptor 2 (HER2) is a membrane tyrosine kinase and oncogene that is over expressed and gene amplified in about 20% of breast cancers. When activated it provides the cell with potent proliferative and anti-apoptosis signals and it is the major driver of tumor development and progression for this subset of breast cancer [1].HER2 over expression has also been seen in other cancers like ovary, endometrium, bladder, lung, colon, and head and neck. The introduction of HER2 directed therapies has dramatically influenced the outcome of patients with HER2 positive breast and gastric/gastro esophageal cancers; however, the results have been proved disappointing in other HER2 over expressing cancers [2].Human epidermal growth factor receptors (HER/erbB) constitute a family of four cell surface receptors involved in transmission of signals controlling normal cell growth and differentiation. A range of growth factors serve as ligands, but none is specific for the HER2 receptor [3]. The year 2007 marks exactly two decades since Human Epidermal Growth Factor Receptor-2 (HER2) was functionally implicated in the pathogenesis of human breast cancer. The subsequent two decades have brought about an explosion of information about the biology of HER2 and the HER family. Here the evidence supporting the oncogenic function of HER2, the mechanisms that are felt to mediate its oncogenic functions, and the evidence that links the experimental evidence with human cancer pathogenesis [4]. Phosphatidylinositol 3-kinases (PI3Ks) are a group of lipidkinases that regulate signaling pathways involved in cell proliferation, adhesion, survival and motility. The PI3K pathway is considered to play an important role in tumorigenesis. Activating mutations of the p110α subunit of PI3K (PIK3CA) have been identified in a broad spectrum of tumors [5]. PIK3CA is mutated in diverse human cancers, but the functional effects of these mutations have not been defined. To evaluate the consequences of PIK3CA alterations, the two most common mutations were inactivated by gene targeting in colorectal cancer (CRC) cells. Biochemical analyses of these cells showed that mutant PIK3CA selectively regulated the phosphorylation of AKT and the fork head transcription factors FKHR and FKHRL1 [6]. The phosphatidylinositol 3-kinase signalling pathway has previously been implicated in tumorigenesis, and evidence over the past year suggests a pivotal role for the phosphatidylinositol 3-kinase catalytic subunit, PIK3CA, in human cancers. PIK3CA mutations in a variety of human malignancies, and discuss their possible implications for diagnosis and therapy [7].The PI3K/Akt/mTOR (phosphoinositol 3-kinase/protein kinase B/mammalian target of rapamycin) pathway is dyes regulated in cervical cancer and is used as a biomarker for therapy. PI3K is a kinase that consists of a regulatory and catalytic domain and has phosphorylation capability. Class I components like the catalytic part (PIK3CA and PIK3CD) and regulatory part (like PIK3R1, PIK3R2, PIK3R3, and PIK3R5) are associated with oncogenesis and growth factors in cervical cancer. This is aimed at discussing the involvement of the PI3K component of the PI3K/Akt/mTOR network in cervical cancer [8].Gene mutations can induce cellular alteration and malignant

transformation. Development of many types of cancer is associated with mutations in the B-raf proto-oncogene (BRAF) gene. The encoded protein is a component of the mitogen-activated protein kinases/extra cellular signal-regulated kinases (MAPK/ERK) signaling pathway, transmitting information from the outside to the cell nucleus [9].The BRAF gene encodes for a serine/ threonine protein kinase that participates in the MAPK/ERK signalling pathway and plays a vital role in cancers and developmental syndromes(RASopathies). The clinical significance of the BRAF gene and other members of RAS/RAF cascade in human cancers and RAS/MAPK syndromes, and focuses the molecular basis and clinical genetics of BRAF to better understand its parallel involvement in both tumourigenesis and RAS/MAPK syndromes—Noonan syndrome, cardio-facio-cutaneous syndrome and LEOPARD syndrome [10]. All mutations are within the kinase domain, with a single substitution (V599E) accounting for 80%. Mutated BRAF proteins have elevated kinase activity and are transforming in NIH3T3 cells. As BRAF is a serine/threonine kinase that is commonly activated by somatic point mutation in human cancer, it may provide new therapeutic opportunities in malignant melanoma [11]. ANPEP/CD13, DPP IV/CD26, and ACE2 are the three protein receptors that are known to be exploited by several human corona viruses. These receptors are moonlighting enzymes involved in several physiological processes such as digestion, metabolism, and blood pressure regulation; moreover, the three proteins are expressed in kidney, intestine, endothelium, and other tissues/cell types [12]. The SARS-CoV-2 beta-corona virus, which caused a pandemic of severe acute respiratory viral infection COVID-19 (from Corona Virus Disease 2019), was first detected. The susceptibility to SARSCoV-2 and the nature of the course of the COVID-19 clinical picture are determined by many factors, including genetic characteristics of both the pathogen and the human. In humans, the genes that are significant for the initial stages of infection include ACE2, ANPEP, DPP4 (encode receptors for corona virus binding); TMPRSS2, FURIN, TMPRSS11D, CTSL, CTSB (encode proteases involved in the entry of the corona virus into the cell); DDX1 (the gene of ATP-dependent RNA helicaseDDX1, which promotes replication of corona viruses); and IFITM1, IFITM2, and IFITM3 (encode interferon-induced transmembrane proteins with an antiviral effect). The expression level of genes that are important for the formation of the susceptibility to SARS-CoV-2 is affected by epigenetic modifications, co-morbidities at the time of infection, taking medications, and bad habits [13]. Different inflammatory genes mined here, including TNFR, IL-8, CXCR1, CXCR2, ADAM10, GPR84, MME,ANPEP, and LAP3, might be druggable targets in efforts to limit SARS-CoV-2 inflammation in severe clinical cases [14].Both SARS-CoV-2 and SARS-CoV enter host cells via the angiotensin-converting enzyme 2 (ACE2) receptor, which is expressed in various human organs. ACE2 catalyzes angiotensin II conversion to angiotensin-(1–7), and the ACE2/angiotensin-(1–7)/MAS axis counteracts the negative effects of the renin-angiotensin system (RAS), which plays important roles in maintaining the physiological and patho-physiological balance of the body. The SARS-CoV-2 spike glycoprotein, which binds to

ACE2, is a potential target for developing specific drugs, antibodies, and vaccines. Restoring the balance between the RAS and ACE2/angiotensin-(1–7)/MAS may help attenuate organ injuries [15]. Angiotensin-converting enzyme (ACE) and its homologue, ACE2, are commonly allied with hypertension, renin–angiotensin–aldosterone system pathway, and other cardiovascular system disorders [16]. ACE2 helps transport amino acids across the membrane. ACE2 sheds from the membrane, producing soluble ACE2 (sACE2). Previous studies have pointed out that sACE2 plays a role in the pathology of the disease, but the underlying mechanism is not yet clear [17]. TRIM56 has been shown to possess deubiquitinase activity and the ability to bind RNA. This adds to the complexity of the regulatory mechanism of TRIM56. TRIM56 was initially found to be able to regulate the innate immune response. TRIM56 in TLR and cGAS-STING pathways of innate immune response, the mechanisms and structural specificity of TRIM56 against different types of viruses, and the dual roles of TRIM56 in tumorigenesis [18]. TRIM56 is a cytoplasmic protein whose expression is stimulated by type I interferon and may function as an antiviral agent. The potential of a TRIM56-based antiviral against COVID-19 from the family Corona viridae, containing single-stranded positive-sense RNA genome [19]. The antiviral activity of TRIM56 depended on its E3 ubiquitin ligase activity as well as the integrity of its C-terminal region but was not attributed to a general augmentation of the interferon antiviral response. Over expression of TRIM56 did not inhibit the replication of vesicular stomatitis virus or hepatitis C virus, a virus closely related to BVDV [20]. Further research is needed to characterize the impact of TRIM proteins on the severity of COVID-19 [21]. Actinomycetes are recognized as a prolific source for bioactive metabolites. Screening adequate numberof strains by appropriate high quality screening protocol determines success of drug discovery programs. Actinomycetes were cultivated in eppendorf tubes (2 ml)using 0.5 ml of yeast extract malt extract agar and modified nutrient glucose agar medium for 10 days and extracted using 1 ml of methanol. The crude extracts were tested against *S. aureus*, *B. subtilis* and *E. coli* by disc-diffusion method. Antagonistic activity, requirements, timeduration and cost effect of all the three methods were compared [22]. Lovastatin is a potent hypercholesterolemic drug used for lowering blood cholesterol. Lovastatin acts by competitively inhibiting the enzyme, 3-hydroxy-3-methylglutaryl coenzyme A reductase involved in the biosynthesis of cholesterol. Commercially Lovastatin is produced by a variety of filamentous fungi including Penicillium species, Monascusruber and *Aspergillus terreus* as a secondary metabolite. Production of Lovastatin by fermentation decreases the production cost compared to costs of chemical synthesis [23].The Enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMG CoA reductase) catalyses conversion of HMG CoA to mevalonate during cholesterol biosynthesis. Lovastatin is used as an anti-cholesterol drug which blocks HMG CoA reductase activity [24].The fungus *Aspergillus terreus* has dominated the biological production of the “crackerjack” drugs known as statins, particularly lovastatin. The aim of this research was the production of lovastatin

which is a known cholesterol-lowering drug, through microbial fermentation using *A. terreus*. The *Aspergillus terreus* NBRC (IFO) 31217 (Strain I) and ATCC 11877 (Strain II) produce lovastatin and they also produce important bioactive compounds of high commercial value like isovaline ($C_5H_{11}NO_2$), 2-oxo-nvaleric acid and silane etc [25].The docking studies prove that lovastatin can effectively help in the treatment of various diseases by effectively binding to various proteins which are responsible for cancer, apoptosis and Alzheimer's [26]

The structure of the Lovastatin drug is shown in the below Figure 1.



3D Structure of Lovastatin **2D Structure of Lovastatin**

Figure 1 2D and 3D structure of Lovastatin.

2. MATERIALS AND METHODS

Preparation of Actinomycetes Cell Free Extract

The Actinomycetes isolates namely, LMA4 (*Microbacterium barkeri*) was subjected to cell free extraction of secondary bio-metabolites. For this purpose, a set of conical flasks containing nutrient broth were taken and the experimental was designed in the following flow chart (Figure 2).

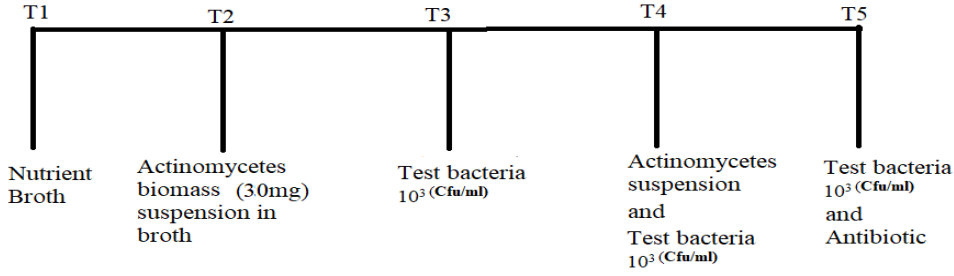


Figure 2 The flow chart showing the experimental design made for preparation of

Prediction of genes from the literature survey and database

The genes associated with the disease are retrieved from the research papers which are already published in the worldwide journals and their details were collected from different databases like

NCBI and UniProt. NCBI has a multi-disciplinary research group composed of computer scientists, molecular biologists, mathematicians, biochemists, research physicians, and structural biologists concentrating on basic and applied research in computational molecular biology. These investigators not only make important contributions to basic science but also serve as a wellspring of new methods for applied research activities. NCBI assumed responsibility for the GenBank DNA sequence database in October 1992. NCBI staffs with advanced training in molecular biology build the database from sequences submitted by individual laboratories and by data exchange with the international nucleotide sequence databases, European Molecular Biology Laboratory (EMBL) and the DNA Database of Japan (DDBJ).

Retrieval of Drugs and Protein compound

The Universal Protein Resource (UniProt) is a comprehensive resource for protein sequence and annotation data. The corresponding protein sequences encoded by these genes were retrieved from UniProtKB database [27-29].The Structure Data Format (SDF) 3D structure of the reported drugs was retrieved from the NCBI PubChem [30-31]database (http://www.ncbi.nlm.nih.gov/ pccompound/) along with its PubChem ID, Molecular weight and Molecular formula. The compounds were converted into pdb format structure using the PyMol [32] (academic version) tool, Discovery Studio v4.1 visulizer tools[33].

3D Structure Validation

The phi and psi torsion angles are less restricted, but due to steric hindrance, there are several preferred combinations of phi, psi values. A scatter plot of phi,psi values for all residues in a protein model is called a Ramachandran plot. This holds even for proline and glycine residues, although their distributions are atypical. Glycine - no side chain, adopts < and =angles in all 4 quadrants of Ramachandran plot. Good models have most of the residues clustered tightly in the most favoured regions with very few outliers. Good, but low-resolution models, may have less pronounced clustering, but still have few outliers. Poor models have no clustering and there are many outliers.

Generation of gene network and its interactions

Gene networks present a graphical view at the level of gene activities and genetic functions and help us to understand complex interactions in a meaningful manner. The GeneMANIA and STRING database (http://string-db.org/) aims to provide such a global perspective for as many organisms as feasible and predict the function of your favorite genes of interest. Known and predicted associations are scored and integrated, resulting in comprehensive protein networks covering >1100 organisms [34-35].

Docking approach

PyRx 0.8 (https://pyrx.sourceforge.io/) was used for docking studies which is widely distributed public domain molecular docking software. The docking analysis was carried out for the reported drugs (can be said as ligands) with their corresponding targets (proteins) using PyRx 0.8 tool. The

interactions of ligand and proteins were studied using Discovery Studio Visulizer. The various bonding interactions of ligand and proteins were explored using the above tools [36]

3. RESULTS AND DISCUSSION

Preparation of Cell Free Extract (CFE)

The flasks (Figure 3 and Figure 4 respectively) containing the biomass of actinomycetes, bacteria and antibiotic were observed for growth of both bacteria and actinomycetes, at the time interval of 0hr, 3hrs, 6 hrs , 9 hrs, 12 hrs and 24 hrs, were found to be turbid except the control flask containing only Nutrient Broth.

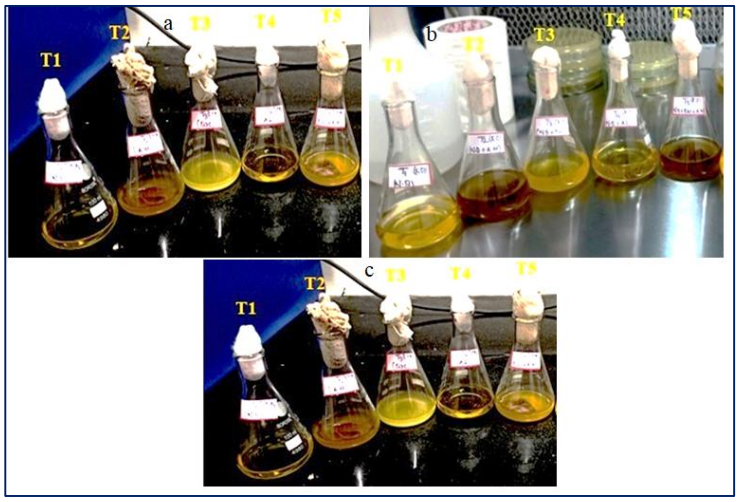


Figure 3 The Flasks containing the Actinomycetes biomass, Test Bacteria (BMS4) and Test Antibiotic (Vancomycin).

Figure 4 The Flasks containing the Actinomycetes biomass, Test Bacteria (BME4) and Test Antibiotic (Azithromycin).

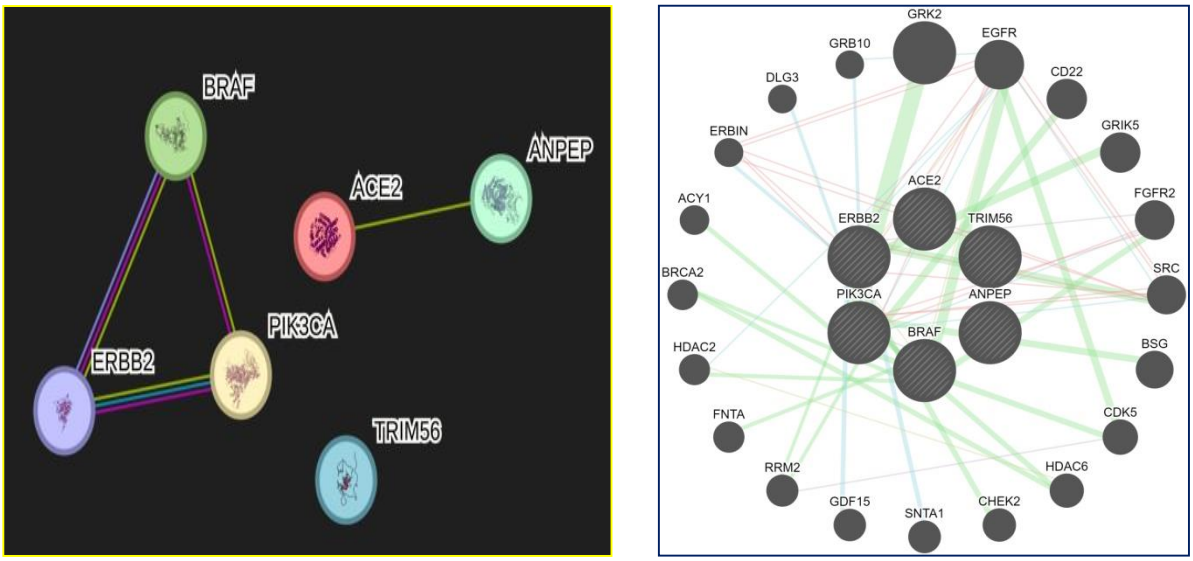
The genome studies of Cancer and COVID-19 diseasewith a total of 3+3 unique genes mapped to discrete genomic locations of human genome from the different literature survey which are given in the Table 1. The genes associated with the cancer diseases are HER2, PIK3CA and BRAF and so on

the gene association with COVID-19 disease are ANPEP, ACE2 and TRIM56.

Table 1 The associated human genes for Cancer and COVID-19

CANCER						
Gene name	**UniProt ID**	**Protein Name**	**Identifier**	**Resolution**	**Chain**	**Position**
HER2 / ERBB2	P04626	Receptor tyrosine-protein kinase erbB-2	1N8Z	2.52 Å	C	23-629
PIK3CA	P42336	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform	2RD0	3.05 Å	A	1-1068
BRAF	P15056	Serine/threonine-protein kinase B-raf	3C4C	2.57 Å	A/B	444-721
COVID-19						
Gene name	**UniProt ID**	**Protein Name**	**Identifier**	**Resolution**	**Chain**	**Position**
ANPEP	P15423	Spike glycoprotein	6ATK	3.50 Å	D/E/F	293-435
ACE2	Q9BYF1	Angiotensin-converting enzyme 2	1R42	2.20 Å	A	1-615
TRIM56	Q9BRZ2	E3 ubiquitin-protein ligase TRIM56	5JW7	2.85 Å	B	1-93

Based on the essential role of 6 genes obtained from significantly literature survey are termed as key genes that were used for network construction of recent trend antiviral diseases named Cancer and COVID-19. The key genes were used for network construction of Cancer and COVID-19 was analyzed through the GeneMANIA and STRING database shown in Figure 5.



Interaction in STRING database **Interaction in GeneMANIA database**

Figure 5 The network construction of the six viral disease genes HER2, PIK3CA, BRAF, ANPEP, ACE2 and TRIM56 analyzed through the GeneMANIA and STRING database

The result of the network analysis in Interaction in GeneMANIA and STRINGis represented in Figure 5. The network of STRING database reported the genes namely HER2/ERBB2, BRAF and PIK3CA genes are mostly interacted which are responsible for Cancer disease but ACE2 and ANPEP interacted with each other with a light single bond at the core region of the network. But in the interaction structure of GeneMANIA it was shown the interaction between the genes namely HER2, PIK3CA, BRAF, ANPEP, ACE2 and TRIM56 are less bonded and their correlated genes and their interactions are so vast. These 6 genes may be said to play a key role in Cancer (HER2, PIK3CA, and BRAF) and COVID-19 (ANPEP, ACE2 and TRIM56) as well as can be differentially expressed in these viral diseases.

Gene Set Enrichment Analysis (GSEA)(https://www.gsea-msigdb.org/gsea/index.jsp) is a computational method that determines whether an a priori defined set of genes shows statisticallysignificant, concordant differences between two biological states(e.g. phenotypes), which is shown in Figure 6.

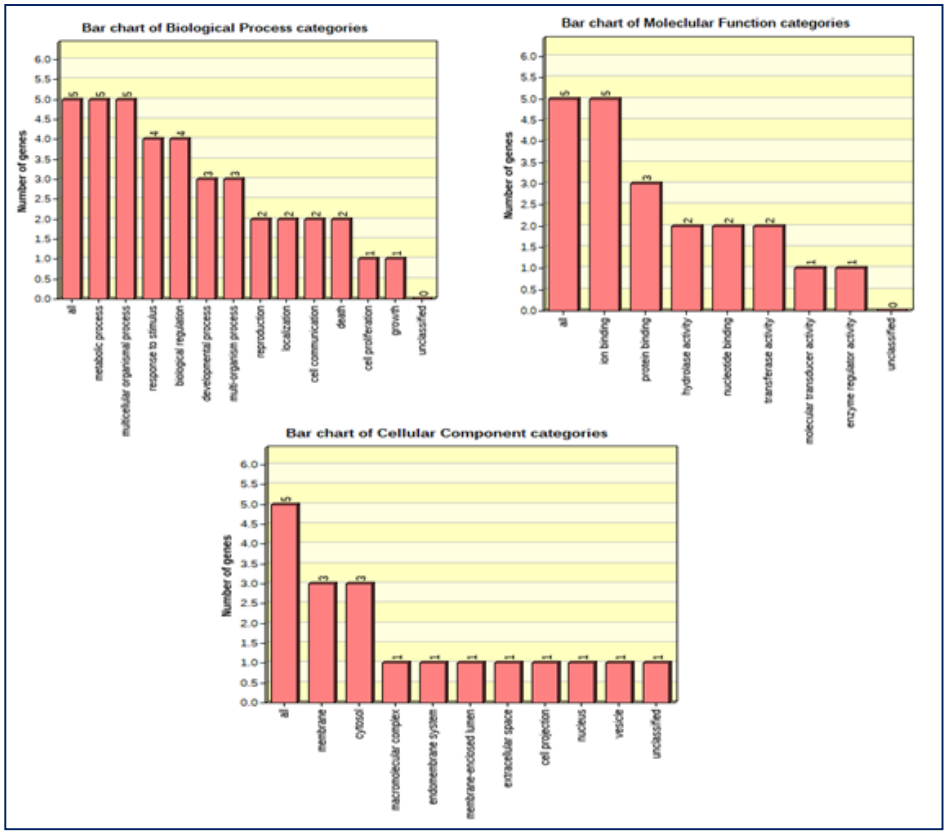
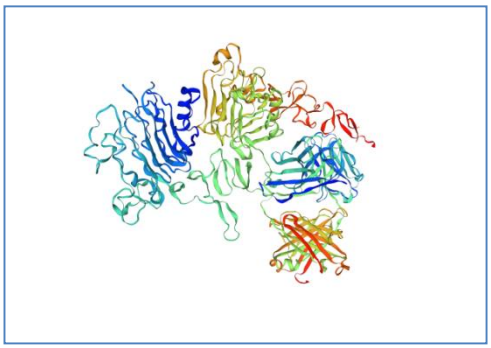


Figure 6 The gene enrichment analysis of the 6 viral disease causing genes by three aspects namely Biological Process (BP), Moleclular Function (MF) and Cellular Component (CC).

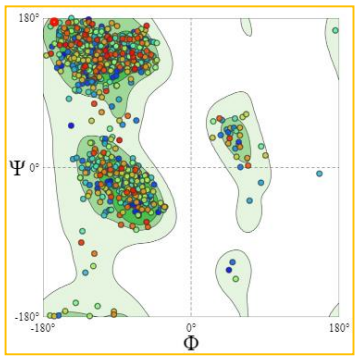
Here the classifications for 6 gene sets are based on three different aspects of biological function in the Human body i.e. Biological Process (BP), Moleclular Function (MF) and CellularComponent (CC). Each of the specific Function of the BP, MF and CC category is represented by a bar and the height of the bar represents the number of user list genes observed in the category.

The amino acid sequences of corresponding proteins encoded by the reported 6 genes (HER2, PIK3CA, BRAF, ANPEP, ACE2 and TRIM56) against Lovastatin drug was retrieved from UniProtKB database. The 3D structures of all the 6 proteins are predicted by homology modelling and threading tool due to unavailability of information at Protein data Bank. Validation of the generated structure using different online server (SWISS-Model) suggests that the quality of the generated structure is good and can be used for protein-ligand studies shown in Figure 7.

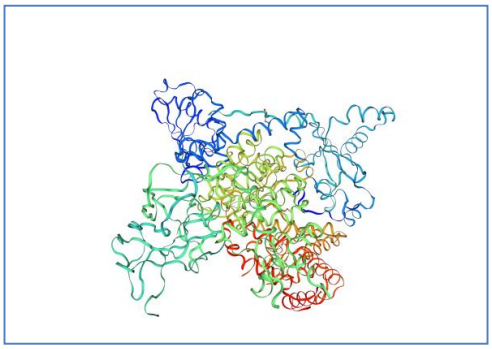
CANCER



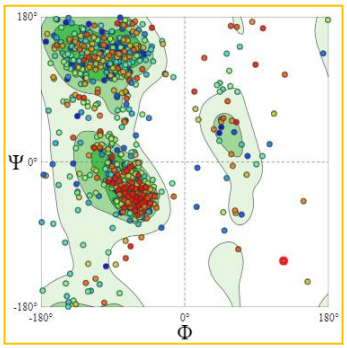
3D Structure of HER2 Protein



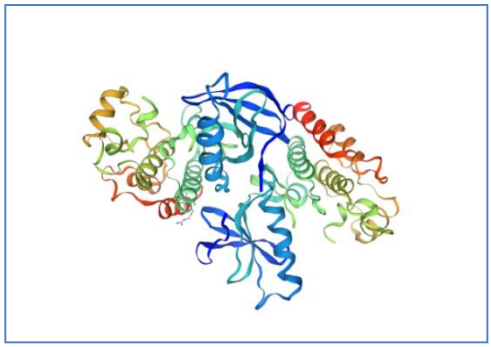
Structural validation of the protein structure HER2



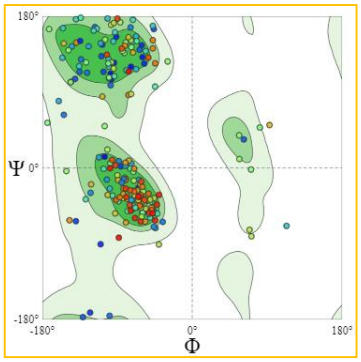
3D Structure of PIK3CA Protein



Structural validation of the protein structure PIK3CA

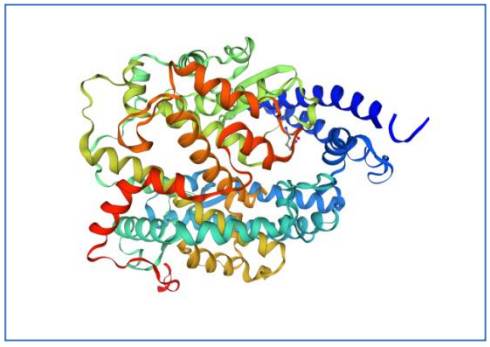


3D Structure of BRAF Protein

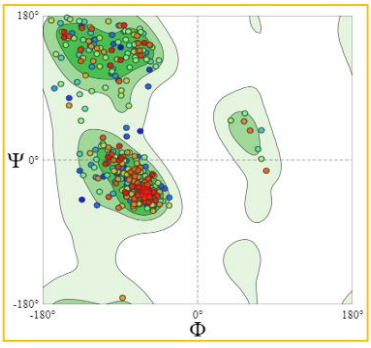


Structural validation of the protein structure BRAF

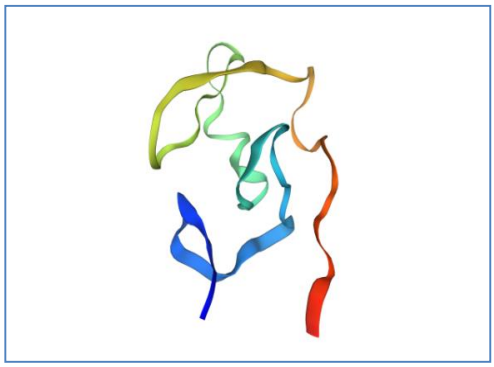
COVID-19



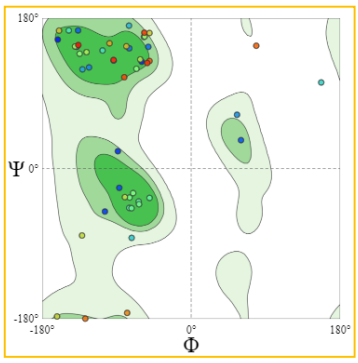
3D Structure of ACE2 Protein



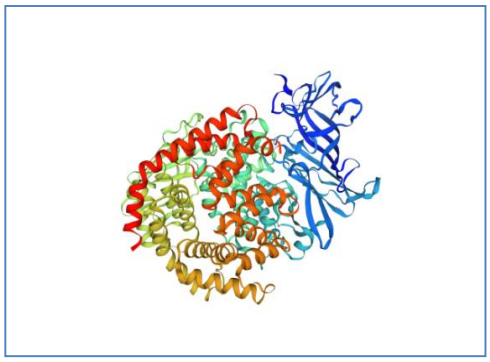
Structural validation of the protein structure ACE2



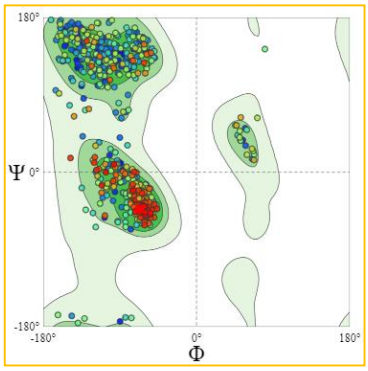
3D Structure of TRIM56 Protein



Structural validation of the protein structure TRIM56



3D Structure of ANPEP Protein



Structural validation of the protein structure ANPEP

Figure 7 Ramachandran plot structure validation of structures of the associated genes of the viral diseases.

From the Output report, Lovastatin drug interacted with 6 genes or its corresponding proteins, docking was performed for the drug Lovastatin and its 6 potential targets. The selected/screened 6 targets are found to be key regulators in Cancer and COVID-19 disease based on the existing records and network analysis in the above. PyRx 0.8 (https://pyrx.sourceforge.io/) was used for docking studies revealed docking score with energy minimization values, Binding energy, Ligand Efficiency, Inhibition Constant and Electrostatic energy for ligand/drug compound-6 potential targets interactions are represented at Table 2.

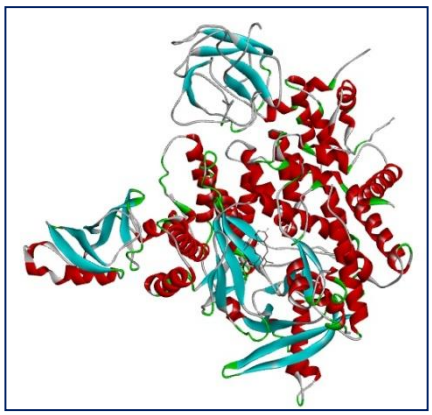
Table 2 Molecular docking analysis of Lovastatin drugs against 6 target proteins using PyRx 0.8 tool.

Sl. no.	Target	Drug	Binding Affinity	rmsd/ub	rmsd/lb
1	HER2	Lovastatin	-7.1	0	0
2	PIK3CA	Lovastatin	-8.4	0	0
3	BRAF	Lovastatin	-7.4	0	0
4	ANPEP	Lovastatin	-8.8	0	0
5	ACE2	Lovastatin	-7.3	0	0
6	TRIM56	Lovastatin	-7.2	0	0

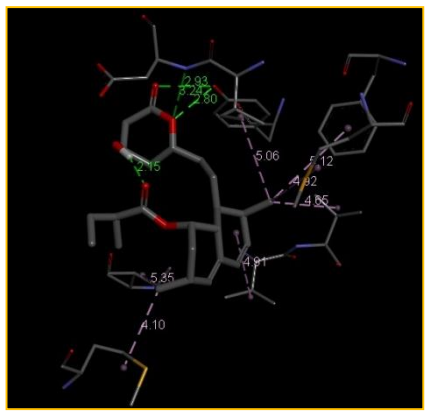
The molecular docking studies have reported the drug-target interactions of ANPEP-Lovastatin was -8.8, PIK3CA-Lovastatin was -8.4, BRAF-Lovastatin was -7.4, ACE2-Lovastatin was-7.3, TRIM56-Lovastatin was -7.2 and HER2-Lovastatin was -7.1 as the highest docking scores with energy minimization out of 6 ligand-protein interactions.

The compound (ligand)-target complex was performed and its interaction studies was visualized in Discovery Studio Visualizer visualize depicted in the Figure 8.

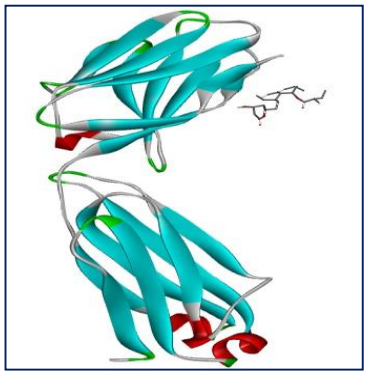
CANCER



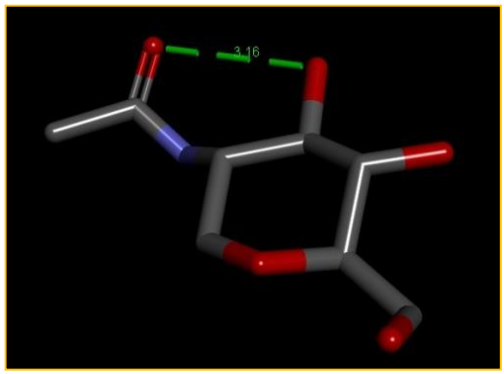
Complex Structure of PIK3CA-Lovastatin



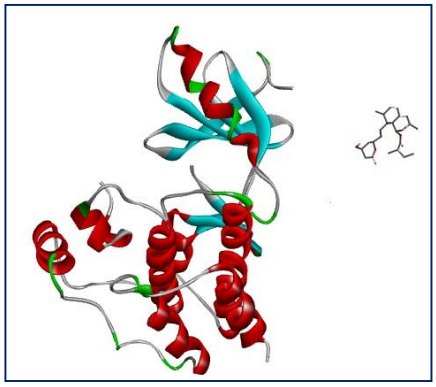
Interaction of Lovastatin with PIK3CA



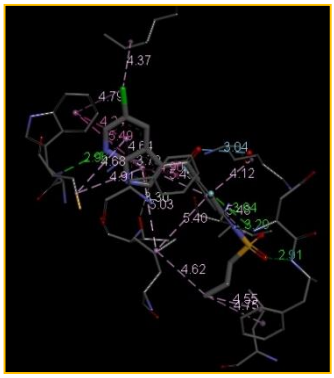
Complex Structure of HER2-Lovastatin



Interaction of Lovastatin with HER2

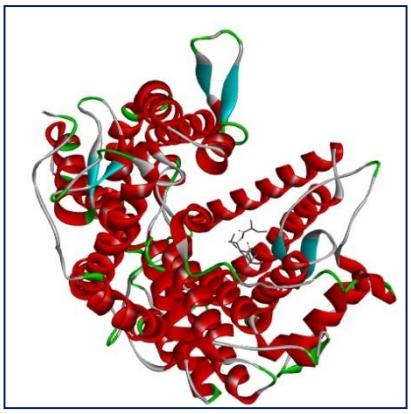


Complex Structure of BRAF-Lovastatin

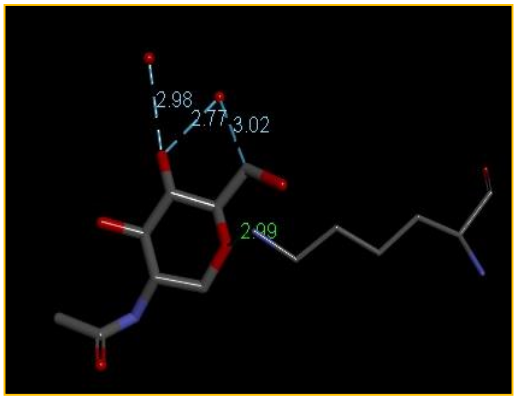


Interaction of Lovastatin with BRAF

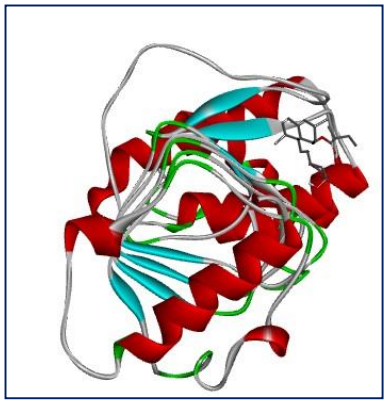
COVID-19



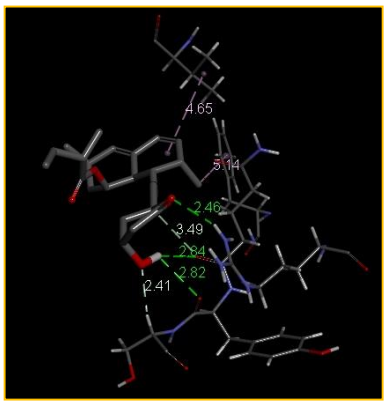
Complex Structure of ACE2-Lovastatin



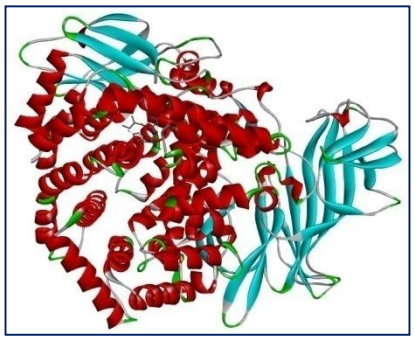
Interaction of Lovastatin with ACE2



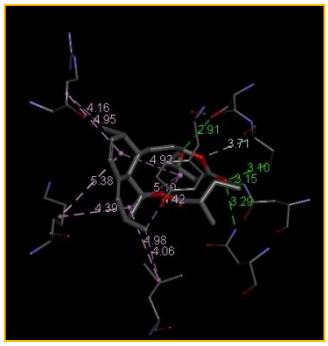
Complex Structure of TRIM56-Lovastatin



Interaction of Lovastatin with TRIM56



Complex Structure of ANPEP-Lovastatin



Interaction of Lovastatin with ANPEP

Figure 8 Compound (ligand)-target complex was performed and its interaction Visualized structures.

From this report it is clear that Cancer and COVID-19 differs from person to person based on their genes and genetic interactions and expressions which recommend the clinicians to go for personalized medicine rather that generalized medicine for the patients with Cancer and COVID-19.

4. CONCLUSION

The present investigation was carried out to explore the genes and their interactions pertaining to Cancer and COVID-19 which is through In-silico and molecular docking analysis. The present strategy of bioinformatics analysis is to exploit the current data available both on gene and genome association study meta-analysis of Cancer and COVID-19 to integrate these at novel levels of understanding of gene network interactions and expression levels.

ACKNOWLEDGEMENT

This research is part of my thesis work registered under Sambalpur University.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

There is no conflict of interest exists.

REFERENCES

1. Gutierrez C, Schiff R. HER2: biology, detection, and clinical implications. Archives of pathology & laboratory medicine. 2011 Jan 1;135(1):55-62.
2. Iqbal N, Iqbal N. Human epidermal growth factor receptor 2 (HER2) in cancers: overexpression and therapeutic implications. Molecular biology international. 2014;2014.

3. Rubin I, Yarden Y. The basic biology of HER2. Annals of oncology. 2001 Jan 1;12:S3-8.
4. Moasser MM. The oncogene HER2: its signaling and transforming functions and its role in human cancer pathogenesis. Oncogene. 2007 Oct;26(45):6469-87.
5. Ligresti G, Militello L, Steelman LS, Cavallaro A, Basile F, Nicoletti F, Stivala F, McCubrey JA, Libra M. PIK3CA mutations in human solid tumors: role in sensitivity to various therapeutic approaches. Cell cycle. 2009 May 1;8(9):1352-8.
6. Samuels Y, Diaz LA, Schmidt-Kittler O, Cummins JM, DeLong L, Cheong I, Rago C, Huso DL, Lengauer C, Kinzler KW, Vogelstein B. Mutant PIK3CA promotes cell growth and invasion of human cancer cells. Cancer cell. 2005 Jun 1;7(6):561-73.
7. Karakas B, Bachman KE, Park BH. Mutation of the PIK3CA oncogene in human cancers. British journal of cancer. 2006 Feb;94(4):455-9.
8. Sun G, Zhang Q, Liu Y, Xie P. Role of phosphatidylinositol 3-kinase and its catalytic unit PIK3CA in cervical cancer: a mini-review. Applied Bionics and Biomechanics. 2022 Aug 21;2022.
9. Śmiech M, Leszczyński P, Kono H, Wardell C, Taniguchi H. Emerging BRAF mutations in cancer progression and their possible effects on transcriptional networks. Genes. 2020 Nov 12;11(11):1342.
10. Hussain MR, Baig M, Mohamoud HS, Ulhaq Z, Hoessli DC, Khogeer GS, Al-Sayed RR, Al-Aama JY. BRAF gene: From human cancers to developmental syndromes. Saudi journal of biological sciences. 2015 Jul 1;22(4):359-73.
11. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N. Mutations of the BRAF gene in human cancer. Nature. 2002 Jun 27;417(6892):949-54.
12. López-Cortés GI, Palacios-Pérez M, Hernández-Aguilar MM, Veledíaz HF, José MV. Human coronavirus cell receptors provide challenging therapeutic targets. Vaccines. 2023 Jan 13;11(1):174.
13. Kucher AN, Babushkina NP, Sleptcov AA, Nazarenko MS. Genetic control of human infection with SARS-CoV-2. Russian Journal of Genetics. 2021 Jun;57(6):627-41.
14. Didangelos, A. COVID-19 hyperinflammation: what about neutrophils?.MSphere,(2020) 5(3), 10-1128.
15. Ni W, Yang X, Yang D, Bao J, Li R, Xiao Y, Hou C, Wang H, Liu J, Yang D, Xu Y. Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. Critical Care. 2020 Dec;24(1):1-0.
16. Shirbhate E, Pandey J, Patel VK, Kamal M, Jawaid T, Gorain B, Kesharwani P, Rajak H. Understanding the role of ACE-2 receptor in pathogenesis of COVID-19 disease: a potential approach for therapeutic intervention. Pharmacological Reports. 2021 Dec 1:1-2.

17. Wang J, Zhao H, An Y. ACE2 Shedding and the Role in COVID-19. Frontiers in Cellular and Infection Microbiology. 2022 Jan 14;11:789180.
18. Fu L, Zhou X, Jiao Q, Chen X. The Functions of TRIM56 in Antiviral Innate Immunity and Tumorigenesis. International Journal of Molecular Sciences. 2023 Mar 6;24(5):5046.
19. Heidary F, Gharebaghi R. Systematic review of the antiviral properties of TRIM56: a potential therapeutic intervention for COVID-19. Expert Review of Clinical Immunology. 2020 Oct 2;16(10):973-84.
20. Wang J, Liu B, Wang N, Lee YM, Liu C, Li K. TRIM56 is a virus-and interferon-inducible E3 ubiquitin ligase that restricts pestivirus infection. Journal of virology. 2011 Apr 15;85(8):3733-45.
21. Tavakoli R, Rahimi P, Hamidi-Fard M, Eybpoosh S, Doroud D, Ahmadi I, Anvari E, Aghasadeghi M, Fateh A. Expression of TRIM56 gene in SARS-CoV-2 variants and its relationship with progression of COVID-19. Future Virology. 2023 Jun;18(9):563-74.
22. Radhakrishnan M, Priya J, Balagurunathan R, Kumar V. Miniaturized fermentation in eppendorf tubes for the detection of antagonistic actinomycetes. International Journal of Bioassays. 2012;1(9):30-5.
23. Seenivasan A, Subhagar S, Aravindan R, Viruthagiri T. Microbial production and biomedical applications of lovastatin. Indian Journal of Pharmaceutical Sciences. 2008 Nov;70(6):701.
24. VK P. Solid State Fermentation: An Effective Method for Lovastatin Production by Fungi–A Mini Review. The Open Tropical Medicine Journal. 2012 Mar 9;5(1).
25. Raj R, Gupta SK, Verma M. Microbial fermentation of lovastatin and other bioactive seconary metabolites using Aspergillus terreus. Research Journal of Life Sciences, Bioinformaticcs, Pharmaceutical and Chemical Sciences. 2019;5(3):34-46.
26. Bhatt RP, Singh U, Uniyal P. Healing mushrooms of Uttarakhand Himalaya, India. Curr Res Environ Appl Mycol. 2018;8(1):1-23.
27. Burley SK, Berman HM, Christie C, Duarte JM, Feng Z, Westbrook J, Young J, Zardecki C. RCSB Protein Data Bank: Sustaining a living digital data resource that enables breakthroughs in scientific research and biomedical education. Protein Science. 2018 Jan;27(1):316-30.
28. Šali A, Fiser A, Sánchez R, Martı-Renom MA, Yerkovich B, Badretdinov A, Melo F, Overington JP, Feyfant E. Departments of Biopharmaceutical Sciences and Pharmaceutical Chemistry, and California Institute for Quantitative Biomedical Research Mission Bay Genentech Hall 600 16th Street, Suite N472D University of California, San Francisco.
29. Sitao Wu Yang Zhang, Nucleic Acids Research, Volume 35, Issue 10, 1 May 2007, Pages 3375–3382,https://doi.org/10.1093/nar/gkm251.Published:03 May 2007. https://academic.oup.com/nar/article/35/10/3375/1100889#82620642.

30. Källberg M, Wang H, Wang S, Peng J, Wang Z, Lu H, Xu J. Template-based protein structure modeling using the RaptorX web server. Nature protocols. 2012 Aug;7(8):1511-22.
31. Wang Z, Zhao F, Peng J, Xu J. Protein 8-class secondary structure prediction using conditional neural fields. In2010 IEEE International conference on bioinformatics and biomedicine (BIBM) 2010 Dec 18 (pp. 109-114). IEEE.
32. Liang J, Edelsbrunner H, Fu P, Sudhakar PV, Subramaniam S. Analytical shape computation of macromolecules: I. Molecular area and volume through alpha shape. Proteins: Structure, Function, and Bioinformatics. 1998 Oct 1;33(1):1-7.
33. Kawabata T. Detection of multiscale pockets on protein surfaces using mathematical morphology. Proteins: Structure, Function, and Bioinformatics. 2010 Apr;78(5):1195-211.
34. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker BA, Wang J. PubChem substance and compound databases. Nucleic acids research. 2016 Jan 4;44(D1):D1202-13.
35. Wang Y, Bryant SH, Cheng T, Wang J, Gindulyte A, Shoemaker BA, Thiessen PA, He S, Zhang J. Pubchem bioassay: 2017 update. Nucleic acids research. 2017 Jan 4;45(D1):D955-63.
36. Hakeem IJ. Molecular docking analysis of acetylcholinesterase inhibitors for Alzheimer's disease management. Bioinformation. 2023;19(5):565-70.