An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

SCREENING AND ASSESSMENT OF SELECTED ALKALOIDS AS POTENTIAL INHIBITORS OF COVID-19 PROTEASE ENZYME

Shailendra Sanjay Suryawanshi [1*], Rajkumar Sanjay Patil[1], Pooja Bhavakana Jayannache[1], M. S. Palled[2], S. G. Alegaon[2], Zaranappa[3]

[*1,2] Department of Pharmaceutical Chemistry, [1]Department of Pharmacology, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Nehru Nagar, Belagavi, Karnataka, India.
[3]Department of Pharmaceutical Chemistry, Government College of Pharmacy, Bengaluru, Rajiv Gandhi University of Health Sciences, Bengaluru, Karnataka, India.
*Corresponding author E -mail: shailendrasss80@gmail.com

ARTICLE INFO

Key Words
COVID-19, Berberine, Molecular Docking, Alkaloids, Discovery Studio 2019.

Access this article online
Website: https://www.jgtps.com/
Quick Response Code:

ABSTRACT

The aim of the present research work is to screen and assess the selected plant alkaloids as potential COVID-19 main protease enzyme (Mpro) inhibitors, using molecular docking study. We have selected several plant alkaloids which are having antiviral activity which includes berberine, columbamine, leurocristine, lycoricidine, lycorine, narciclasine, palmitine, periformaline, perivine and vincaleucoblastine used as ligands to study the interaction with selected target of COVID-2019. Nelfinavir and lopinavir were used as standard antiviral agents for comparison. COVID-19 Mpro was docked with selected compounds using PyRx 0.8 and docking was analysed by PyRx 0.8 and Biovia Discovery Studio 2019. The binding energies obtained from the docking of 6LU7 with lopinavir, nelfinavir, berberine, columbamine, leurocristine, lycoricidine, lycorine, narciclasine, palmitine, periformyline, perivine and vincaleucoblastine were -8, -8.3, -6.8, -7, -6.5, -7.8, -7, -8.1, -7.3, -7.2, -7.3 and -7.3 kcal/mol, respectively. From the binding energy calculations we can conclude that nelfinavir and lopinavir may represent potential treatment options and berberine, columbamine, leurocristine, lycoricidine, lycorine, narciclasine, palmitine, periformyline, perivine and vincaleucoblastine found to possess the best inhibitors of COVID-19 Mpro.

INTRODUCTION

The 2019-novel coronavirus (nCoV) is a major source of disaster in the 21th century and the finding of specific drugs treatment to prevent the attack of Coronaviruses is a major need [1] Coronaviruses (CoVs) have a single stranded RNA genome structure with a size ranging from 26.2 to 31.7 kb and covered by an enveloped structure [2]. We have total seven strains of human CoVs namely NL63, 229E, HKU1, OC43, Middle East respiratory syndrome (MERS)-CoV, Severe Acute Respiratory Syndrome (SARS)-CoV, and 2019-novel coronavirus (nCoV), which are responsible to cause infections of both lower and upper respiratory tract which includes pneumonia, common cold, bronchiolitis, rhinitis, pharyngitis, sinusitis etc. [3,4]. Strain SARS-CoV, MERS-CoV, and 2019-nCoV are proved for their high pathogenecity which caused endemic of severe CoV disease [4]. New coronavirus (CoV) strain was identified in Wuhan, China, in the year 2019 [5]. The Emergency Committee of the World Health

Organization declared an outbreak in China on 30 January 2020, which was considered as Public Health Emergencies of International Concern (PHEIC) [6]. Officially, WHO named this disease as COVID-19 (coronavirus disease 2019) on 11 February 2020 [7]. Currently, no specific therapies for COVID-19 are available and investigations regarding the treatment of COVID-19 are lacking. Potential combinations of protease inhibitor lopinavir/ritonavir, which is commonly used to treat human immunodeficiency virus, for the treatment of COVID-19-infected patients have been investigated and reported [8]. An investigation carried out by Xu et al. (2020) showed that among 4 tested drugs namely nelfinavir, pitavastatin, perampanel, and praziquantel, nelfinavir was identified potent inhibitor against COVID-19 Mpro, based on binding free energy calculations. Liu *et al*. (2020) have successfully crystallized the main protease (Mpro) from COVID-19, which has been structured and repositioned in the Protein Data Bank (PDB) and is accessible by the public. This protease represents a potential target for the inhibition of CoV replication [9].

The main proteases in CoVs PDB ID 6LU7 is acts as potential target proteins for COVID-19 treatment. 6LU7 is the Mpro in COVID-19 that has been structured and repositioned in PDB and has been accessible by the public since early February 2020 [10]. The discovery of the Mpro protease structure in COVID-19 provides a great opportunity to identify potential drug candidates for treatment [11]. Plant secondary metabolites play an important role in the treatment, prevention, mitigation and cure of various diseases and disorders in mankind. Plant alkaloids are one of the largest groups of natural products found to possess diverse group of chemical entities and also found to possess various biological activities [12, 13]. Several researchers have studied the effect of alkaloids on viral reproduction. The studies revealed that about 40 alkaloids possess antiviral properties. Berberin, columbamine, leurocristine, lycoricidine, lycorine, narciclasine, palmitine, Periformyline, perivine and vincaleurocblastine are the alkaloids which are reported for various antiviral activities. [14, 15, 16, 17, 18]

Literature search revealed that, selected alkaloids have potent antiviral effect against different viruses and may be effective against COVID-19. Hence there is need of screening and assessing the selected alkaloids against molecular targets of COVID-19 using molecular docking techniques. No studies have been reported on molecular docking studies of selected alkaloids against selected target of COVID-19. This promoted us to carry out present research work.

MATERIALS AND METHODS

Software's: PyRx 0.8, Biovia Discovery Studio 2019, Molsoft, marvinsketch.

Alkaloids: berberine, columbamine, leurocristine, lycoricidine, lycorine, narciclasine, palmitine, periformyline, perivine, vincaleucoblastine used as ligands.

Standard drugs: Nelfinavir and lopinavir were used as standard for comparison.

Determination of Drug Likeness Properties of Selected Ligands:

In our study we have selected alkaloids as Ligands. Almost all the selected alkaloids are available in market in various pharmaceutical and ayurvedic dosage form and they are safe also consumed by large number of population. In order to find out drug-like properties of each ligands we have followed the Lipinski's rule of five. With the adherence of Lipinski's rule of data about drug likeness was compiled. The Canonical simplified molecular line-entry systems (SMILES) were retrieved from PubChem and used in Molsoft software to obtain data.

Preparation of Macromolecules: Structure of COVID-19 3clpro/Mpro (PDB ID: 6LU7) macromolecule was retrieved from PDB (https://www.rcsb.org/), website in pdb format. The retrieved protein is associated with water molecules and hetero-atoms. All hetero atoms, water molecules and native ligand were removed by using Discovery studio 2019 to avoid docking interference and saved in the PDB format. The 6LU7 protein contains two chains, A and C. Chain A contains SARS-CoV-2 main protease enzyme hence Chain A was used for macromolecule preparation.

Preparation of ligand: All the 3D structures of the ligand molecules were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in structural data format (SDF) and converted to protein data bank (PDB) format using Discovery studio 2019. In the present study

lopinavir, nelfinavir, berberine, columbamine, leurocristine, lycoricidine, lycorine, narciclasine, palmitine, periformyline, perivine and vincaleucoblastine were used as ligands.

Determination of Active Sites: The amino acids in the active site of a protein were determined by using the Biovia Discovery Studio 2019. The determination of the amino acids in the active site was used to analyse docking evaluation results [19]

Molecular Docking: PyRx 0.8 was used for molecular docking. After the completion of docking, autodock preferences were obtained for both ligand and target in PDBQT format. The docking analysis was performed by Biovia Discovery Studio 2019. The pose for minimum binding energy was selected as best interaction.

RESULTS

Drug likeness properties of selected ligands were calculated. Ligands and drug candidate compounds have been previously selected, based on adherence to Lipinski's rule of five. The drug scanning results were calculated and data were presented in Table 1.

The 6LU7 is the main protease (Mpro) found in COVID-19, which been structured and repositioned in PDB and can be accessed by the public, as of early February 2020. The PDB ID, structure of macromolecule, native ligand and amino acids found in the active site pockets of 6LU7 are presented in Table 2.

Molecular docking analysis of selected alkaloids, selected drugs and its 2D interaction with different amino acids on targets were presented in Table 3.

Molecular docking analysis results for several compounds against 6LU7 and its binding energy/Gibbs Energy were presented in Table 4. Graph showed molecular docking results between 6LU7 and several drug candidate compounds (the binding energy value ΔG is shown in minus kcal/mol) and presented in (Figure 1).

DISCUSSION

The present study focused on the main proteases in CoVs PDB ID 6LU7 as potential target proteins for COVID-19 treatment. 6LU7 is the Mpro in COVID-19 that has been structured and repositioned in PDB and has been accessible by the public since early February 2020 [21]. The discovery of the Mpro protease structure in COVID-19 provides a great opportunity to identify potential drug candidates for treatment [22]. In many viruses, proteases play essential roles in viral replication; therefore, proteases are often used as protein targets during the development of antiviral therapeutics [23]. In the present study, we have used nelfinavir and lopinavir as standards for comparison. Several alkaloids from medicinal plants have been reported to show antiviral bioactivities [12–18].

We investigated Berberin, Columbamine, Leurocristine, Lycoricidine, Lycorin, Narciclacin, Palmitine, Periformyline, Perivine and Vincaleurocblastine as potential inhibitors of the COVID-19 Mpro. The binding energies obtained from docking 6LU7 with Berberin, Columbamine, Leurocristine, Lycoricidine, Lycorin, Narciclacin, Palmitine, Periformyline, Perivine and Vincaleurocblastine were -8, -8.3, -6.8, -7, -6.5, -7.8, -7, -8.1, -7.3, -7.2, -7.3, -7.3 kcal/mol, respectively.

The docking analysis in the present study showed the interaction of several compounds with COVID-19 Mpro, ranked by affinity (ΔG); Nelfinavir >narciclastine> lopinavir > Lycoricidine >Palmitine, Perivine and Vincaleuroblastine>Periformyline> Columbamine and Lycorine >Berberin>Leucocristine were the most recommended alkaloids found in medicinal plants as potential inhibitors of COVID-19 Mpro, which should be explored in future research.

CONCLUSION

Currently available drugs for COVID-19 treatment primarily act on the main protease (Mpro). The aim of this study was to screen and assess the selected alkaloids from plant that may inhibit the COVID-19 protease enzyme. The binding affinity of narciclastine is higher as compared to others whereas leurocristine found to show low affinity amongst all the compounds. Nelfinavir and lopinavir may represent potential treatment options, and berberin, columbamine, leurocristine, lycoricidine, lycorin, narciclacin, palmitine, periformyline, perivine and vincaleurocblastine may be recommended as most potential inhibitors of COVID-19 Mpro.

Table 1: Drug likeness properties of selected drugs and ligands

Sr. No.	Name of the Ligands	Mol Formula	Mol Weight	Log p	HBD	HBA	Drug Likeness Score
1	Nelfinavir	$C_{32}H_{45}N_3O_4S$	567.31	5.13	4	6	1.14
2	Lopinavir	$C_{37}H_{48}N_4O_5$	628.36	5.71	4	5	1.10
3	Leurocristine	$C_{46}H_{56}N_4O_{10}$	824.4	2.96	3	12	1.36
4	Periformyline	$C_{21}H_{22}N_2O_4$	366.16	2.24	1	4	0.05
5	Perivine	$C_{20}H_{22}N_2O_3$	338.16	2.23	2	4	0.05
6	Vincaleucoblastine	$C_{46}H_{56}N_4O_9$	808.4	4.27	2	11	1.53
7	Berberine	$C_{20}H_{18}NO_4$	336.12	4.39	0	4	0.77
8	Columbamine	$C_{20}H_{20}NO_4$	338.14	3.7	1	4	0.84
9	Palmitine	$C_{21}H_{22}NO_4$	352.15	3.96	0	4	0.69
10	Lycorine	$C_{16}H_{17}NO_4$	287.12	1.16	2	5	-0.26
11	Lycoricidine	$C_{14}H_{13}NO_6$	291.07	0.89	4	6	4
12	Narciclasine	$C_{14}H_{13}NO_7$	257.11	2.68	1	3	0.29

Table 2: PDB ID, target, native ligand and active sites

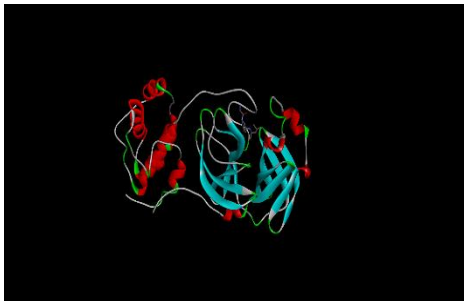
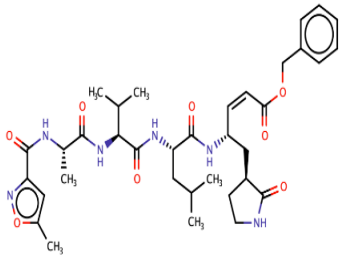
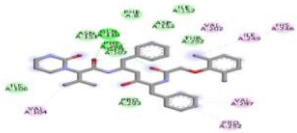
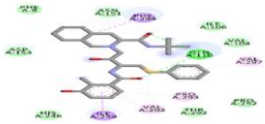
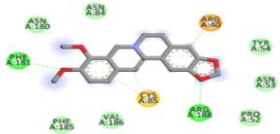
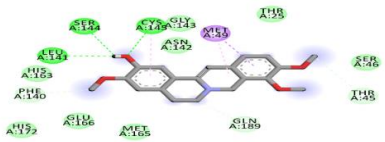
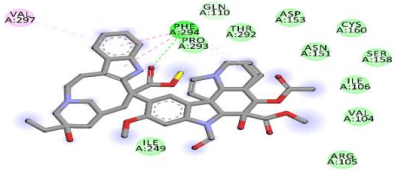
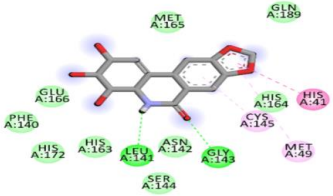
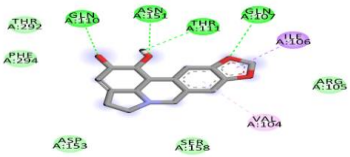
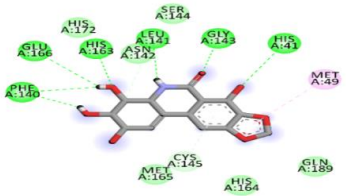
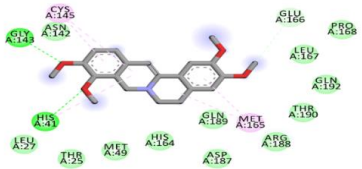
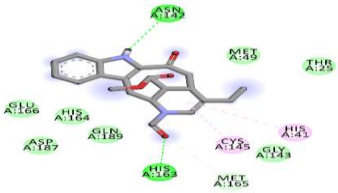
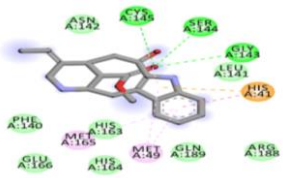
PDB ID	Macromolecule	Native Ligand	Active Sites
6LU7			THR24, THR26, PHE140, ASN142, GLY143, CYS145, HIS163, HIS164, GLU166, HIS172

Table 3: Molecular docking analysis data of ligands and target

Sr. No.	Ligands Name	Molecular structure and Interaction with 6LU7	Amino acids involved in the interaction
1	Lopinavir		HIS 41, PHE 140, ASN 142, GLU 166, PRO 168, ASP 187GLN 189
2	Nelfinavir		GLN 110, VAL 202ILE 249, PHE 294VAL 297
3	Berberin		ARG 40, CYS 85, PHE 181, ARG 188

4	Columbamine		MET 49, LEU 141, SER 144, CYS 145
5	Leurocristine		PRO 290, PHE 294, VAL 297
6	Lycoricidine		HIS 41, MET 49, LEU 141, GLY 143, CYS 145, HIS 164
7	Lycorine		VAL 104, ILE 106, GLN 107, GLN 110, THR 111, ASN 151
8	Narciclasine		HIS 41, MET 49, PHE 140, LEU 141, GLY 143,
9	Palmitine		HIS 41, GLY 143, CYS 145, MET 165, GLU 166
10	Periformyline		HIS 41, ASN 142, CYS 145, HIS 163
11	Perivine		HIS 41, MET 49, GLY 143, SER 144, CYS 145, MET 165

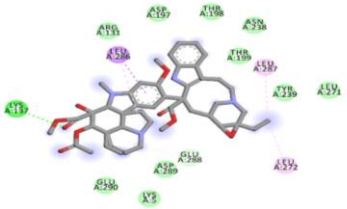
12	Vincaleurocblastine		LYS 137, LEU 272, LEU 286, LEU 287

Table 4: Pub Chem ID of ligand and binding energy

Protein	Ligands	Pub Chem ID	Binding energy (kcal/mol)
6LU7	Lopinavir	97727	-8
	Nelfinavir	64143	-8.3
	Berberine	2353	-6.8
	Columbamine	72310	-7
	Leurocristine	5388993	-6.5
	Lycoricidine	73065	-7.8
	Lycorine	72378	-7
	Narciclasine	72376	-8.1
	Palmitine	19009	-7.3
	Periformyline	11969538	-7.2
	Perivine	6473766	-7.3
	Vincaleucoblastine	11969554	-7.3

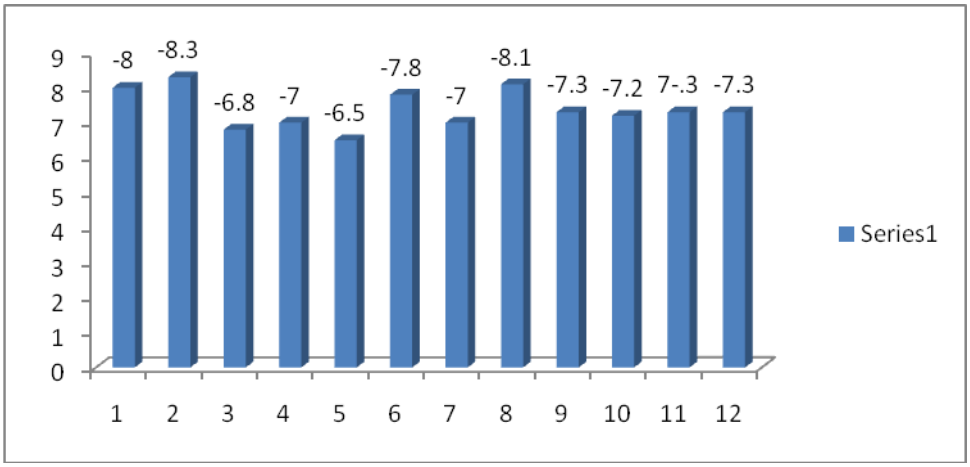


Figure 1: Graph showing the binding energy value ΔG is shown in minus kcal/mol on y-axis *vs* compounds 1 to 12 on x-axis. (1-Lopinavir, 2-Nelfinavir, 3- Berberine, 4- Columbamine, 5-Leurocristine, 6- Lycoricidine, 7- Lycorine, 8- Narciclasine, 9- Palmitine, 10- Periformaline, 11-Perivine, 12- Vincaleucoblastine)

Figure 2 (a to k) showed the binding between 6LU7 and native ligand, Lopinavir, Nelfinavir, Berberine, Columbamine, Leurocristine, Lycoricidine, Lycorine, Narciclasine, Palmitine, Periformyline, Perivine, Vincaleucoblastine as potential inhibitor of COVID-19 Mpro.

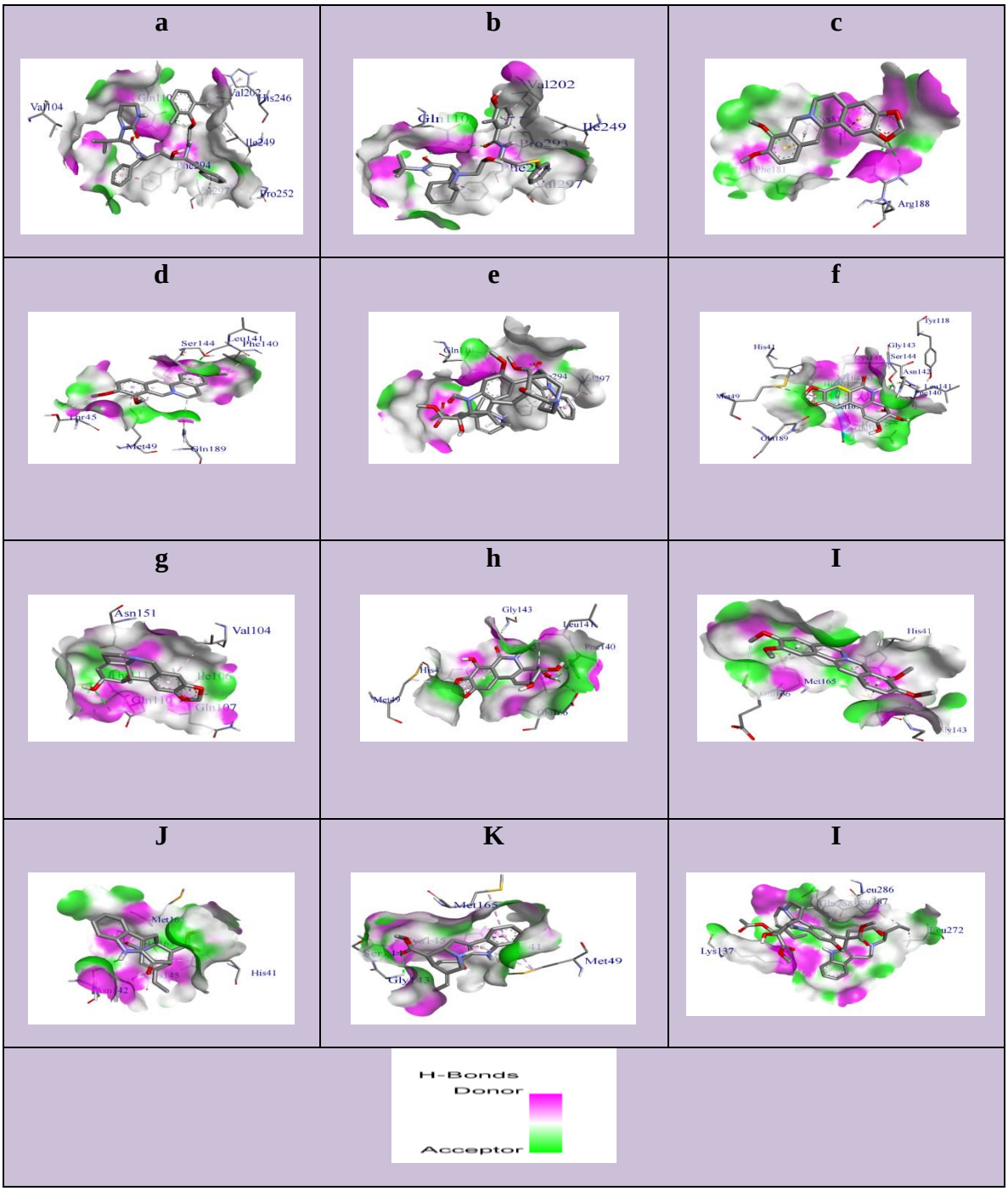


Figure 2: Docking analysis visualization of 6LU7 binding with a) lopinavir b) nelfinavir c) berberin d) columbamine e) leurocristine f) lycoricidine g) lycorin h) narciclastine) palmitine j) periformyline k) perivine l) vincaleublastine

Acknowledgement: Authors acknowledge Miss. Rohini S Kavalapure, Assistant Professor, Department of Pharmaceutical Chemistry, KLE College of Pharmacy, Belagavi for her guidance and help during docking analysis and determination of drug likeness profile in research work.

REFERENCES

1. Prajapa M, Sarma P, Shekhar N, Avti P, Sinha S, Kaur H, Kumar S, Bhattacharyya A,Kumar H, Bansal S, Medhi B. Drug targets for corona virus: A systematic review. Indian Journal of Pharmacology 2020; 52(1): 56-65.

2. Yang H, Bartlam M, Rao Z. Drug design targeting the main protease, the Achilles' heel of coronaviruses. Curr Pharm Des 2006; 12: 4573-90.
3. Chang CK, Lo SC, Wang YS, Hou MH. Recent insights into the development of therapeutics against coronavirus diseases by targeting N protein. Drug Discov Today 2016; 21: 562-72.
4. Paules CI, Marston HD, Fauci AS. Coronavirus infections-Morethan just the common cold. JAMA 2020; 323: 707.
5. Malik YS, Sircar S, Bhat S, Sharun K, Dhama K, Dadar M, Tiwari R, Chaicumpa W. Emerging novel Corona virus (2019- nCoV) - Current scenario, evolutionary perspective based on genome analysis and recent developments. The Veterinary Quarterly 2020; 40(1): 68-78.
6. Morales AJ, MacGregor K, Kanagarajah S, Patel D, Schlagenhauf P. Going global – Travel and the 2019 novel corona virus. Travel Med Infect Dis 2020; 33: 101578.
7. Zhang D, Wu K, Zhang X , Deng S , Peng B. In silico screening of Chinese herbal medicines with the potential to directly inhibit 2019 novel corona virus. Journal of Integrative Medicine 2020; 18(2): 152-158.
8. [5] H. Lu. Drug treatment options for the 2019-new corona virus (2019-nCoV). Biosci Trends 2020; 14(1): 69-71.
9. [10] Xu Z, Peng C, Shi Y, Zhu Z, Mu K, Wang X. Nelfinavir was predicted to be a potential inhibitor of 2019-nCov main protease by an integrative approach combining homology modelling, molecular docking and binding free energy calculation. Biorxiv. https://doi.org/10.1101/2020.01.27.921627.
10. Liu X and Wang XJ. Potential inhibitors against 2019-nCoV corona virus M protease from clinically approved medicines. J. Genet. Genomics 2020. doi: https://doi.org/10.1101/2020.01.29.924100.
11. Chang KO, Kim Y, Lovell S, Rathnayake AD, and Groutas WC. Antiviral drug discovery: Norovirus proteases and development of inhibitors. Viruses 2019; 11(2):1-14.
12. Bribi N. Pharmacological activity of Alkaloids: A Review. Asian Journal of Botany 2018; 1: 1-6.
13. Feng Zuo, Norio Nakamura, Teruaki Akao, et al. Pharmacokinetics of berberine and its main metabolites in conventional and pseudo germ-free rats determined by liquid chromatography/ion trap mass spectrometry. Drug Metab Dispos 2006; (34): 2064–2072.
14. Patil, P.J.; Ghosh, J.S. Antimicrobial activity of Catharanthus roseus—A detailed study. Br. J. Pharmcol. Toxicol 2010; 1:40–44.
15. Song, B.; Yang, S.; Jin, L.H.; Bhadury, P.S. Environment-Friendly Antiviral Agents for Plants; Springer Science & Business Media: Guizhou, China, 2011.
16. Manske, R.H.F.; Holmes, H.L. The Alkaloids: Chemistry and Physiology; Elsevier: New York, NY, USA, 2014.
17. Ududua, U.O.; Monanu, M.O.; Chuku, L.C. Proximate Analysis and Phytochemical profile of Brachystegia eurycoma Leaves. Asian J Res Biochem 2019; 4: 1–11.
18. Petrov, N.M. Antiviral Activity of Plant Extract from Tanacetum Vulgare Against Cucumber
    Mosaic Virus and Potato Virus Y. J. Biomed Biotechnol 2016; 5: 189–194.
19. Xu J, Zhao S, Teng T, Abdalla AE, and Zhu W. Systematic Comparison of Two Animal- to-Human Transmitted Human Corona viruses : SARS-CoV-2 and SARS-CoV. Viruses 2020; 12(2): 244.
20. Liu X and Wang XJ. Potential inhibitors against 2019-nCoV corona virus M protease from clinically approved medicines. J. Genet. Genomics 2020. doi: https://doi.org/10.1101/2020.01.29.924100.
21. Chang KO, Kim Y, Lovell S, Rathnayake AD, and Groutas WC.Antiviral drug discovery: Norovirus proteases and development of inhibitors. Viruses 2019; 11(2): 1-14.
22. Li JY et al., The epidemic of 2019-novel-coronavirus. Microbes Infect 2020; 22(2): 80-85.
23. Patick AK, Potts KE. Protease inhibitors as antiviral agents. Clinical microbiology reviews. 1998 Oct 1;11(4):614-27