

In Silico* Screening of Some Naturally Occurring Bioactive Compounds Predicts Potential Inhibitors against SARS-COV-2 (COVID-19) Protease

Ashok Kumar Mishra¹ and Satya Prakash Tewari

*Department of Physics, Dr.Shakuntala Misra National Rehabilitation University, Lucknow,
Uttar Pradesh, India-226017*

¹ Corresponding Author: akmishra@dsmnru.ac.in; akmishra2k5@gmail.com (A.K. Mishra)

- This research is dedicated to the peoples who have lost the lives, been struggling for the lives, been working hard to save the lives as well as been frightened for the lives while fighting against the pandemic COVID-19 i.e. the all CORONA WARRIORS.

Abstract

SARS-COV-2 identified as COVID-19 in Wuhan city of China in the month of December, 2019 has now been declared as pandemic by World Health Organization whose transmission chain and cure both have emerged as a tough problem for the medical fraternity. The reports pertaining to the treatment of this pandemic are still lacking. We firmly believe that Nature itself provides a simple solution for any complicated problem created in it which motivated us to carry out *In Silico* investigations on some bioactive natural compounds reportedly found in the fruits and leaves of *Anthocephalus Cadamba* which is a miraculous plant found on the earth aiming to predict the potential inhibitors against aforesaid virus. Having modeled the ground state ligand structure of the such nine natural compounds applying density functional theory at B3LYP/631+G (d, p) level we have performed their molecular docking with SARS-COV-2 protease to calculate the binding affinity as well as to screen the binding at S-protein site during ligand-protein interactions. Out of these nine studied naturally occurring compounds; Oleanic Acid has been appeared to be potential inhibitor for COVID-19 followed by Ursolic Acid, IsoVallesiachotamine, Vallesiachotamine, Cadambine, Vincosamide-N-Oxide, Isodihydroaminocadambine, Pentyle Ester of Chlorogenic Acid and D-Myo-Inositol. Hence these bioactive natural compounds or their structural analogs may be explored as anti-COVID19 drug agent which will be possessing the peculiar feature of cost-less synthesis and less or no side effect due to their natural occurrence. The solubility and solvent-effect related to the phytochemicals may be the point of concern. The *In-vivo* investigations on these proposed natural compounds or on their structural analogs are invited for designing and developing the potential medicine/vaccine for the treatment of COVID-19 pandemic.

Keywords: COVID-19 inhibitors; anti- SARS-COV-2 bioactive natural compounds; Molecular-Docking; DFT; Phytochemicals; Molecular -modeling

Introduction

The novel corona virus disease (COVID-19) identified in Wuhan City of China [1] spread across the earth as pandemic putting the whole world on high alert [2–5] led to 823626 total cases and 40598 deaths all over the world till 01 April 2020 [6]. Available evidence indicates that this virus is transmitted through the respiratory droplets (such as coughing) and by fomites that can propagate through air at distances of 1 meter [7–9] and no evidence is available about its airborne transmission in the current study [10] which establishes the principle of maintaining the distance of more than 1 meter termed as ‘social distancing’/‘physical distancing’ along with hand-hygiene for the prevention of COVID-19. A recent study based on the mathematical modeling conducted by Indian Council of Medical Research (ICMR) has suggested the proper quarantine and isolation as the preventive measures for stopping the outbreak of COVID-19 through community transmission [11]. It appears that in view of this, country- wide lock-down has been declared in India since the midnight of the 23 rd March, 2020 in continuation to pre-lock down action of evacuating the possible places expected for people’s gathering as well as the social awareness drive already started from the very beginning of February, 2020 which we welcome.

Appreciable contribution in the development of diagnostics, therapeutics and vaccines for this novel corona virus has been indicated [11] and based on some clinical investigations, an anti-malarial drug namely chloroquine phosphate has been reported to be having a certain curative effect on the COVID-19 [12]. ICMR has also recommended an anti-malarial drug namely Hydroxy-chloroquine for those individuals which are asymptomatic healthcare workers involved in the care of suspected or confirmed cases of COVID-19 and asymptomatic household contacts of laboratory confirmed cases [13]. The possibility of high risk-factor associated with these suggested drugs has not been ruled out which projected it to be a trial measure. No specific therapy and medicine for the treatment of COVID-19 has ever been reported to the best of our knowledge which inspired us to think on the natural products relying on the concept of particle-antiparticle theory of Nature implying that if there is a novel corona virus then there must be its anti-virus material in the Nature itself and we have obtained motivating results in the present research carried out with the help of available computational facility at our home during lock-down period.

We have selected total eleven bioactive natural compounds embedded spontaneously in *Anthocephalus Cadamba* which has been reported to be a miraculous tree having crucial significance in Hindu Mythology containing the largest number of phytochemicals and secondary metabolites having pharmacological and biological properties, however, the solubility and solvent-effect to be the point of concern [14]. The four bio molecules contained in the leaves of the said tree namely 7-hydroxy-6-methoxy coumarian ($C_{10}H_8O_4$), Methyl ester of chlorogenic acid ($C_{17}H_{20}O_9$), Pentyle Ester of Chlorogenic Acid ($C_{21}H_{28}O_9$), D-Myo-Inositol ($C_7H_{14}O_6$); 07 biomolecules contained in fruits of the said tree namely Oleanic Acid ($C_{30}H_{48}O_3$), Ursolic Acid

(C₃₀H₄₈O₃), Vallesiachotamine (C₂₁H₂₂N₂O₃), Iso-Vallesiachotamine (C₂₁H₂₂N₂O₃), Cadambine (C₂₇H₃₂N₂O₁₀), Vincosamide-N-Oxide (C₂₆H₃₁N₂O₉), Isodihydroamino-cadambine (C₂₆H₃₃N₃O₇) already reported to be isolated and characterized in Central Drug Research Institute, Lucknow, India [15-16] and the electronic properties of the few of them have already been studied by us computationally [17-21]; were screened as ligands to interact with the targeted SARS-COV-2 protease. The first two of the aforesaid compounds responded negatively but rest nine compounds exhibited the positive results which have been presented in this paper. We expect that the present study will offer a new dimension in developing the drug/vaccine for the COVID-19.

Methodology

The *In silico* optimized ligand-structure of the aforementioned eleven bioactive natural compounds were obtained as per reported approach of density functional theory at B3LYP/631+G (d, p) level [22-23] implemented through Gaussian 09 program-package [24]. We have used main protease of SARS-COV-2 retrieved from the RCSB protein data bank having PDB ID: 6Y84 [25] and PDB ID: 6LU7 [26] as potential target protein for the binding of these ligands obtained using molecular docking approach implemented through Autodock 4.2 program package [27-29]. The binding of these natural bioactive ligands with the PDB ID: 6CRV which is SARS Spike Glycoprotein for corona virus SARS-COV emerged in 2002 as a highly transmissible [30] has also been obtained with the help of same docking approach in order to investigate the consistency of the performance of these compounds with the class of viral protein. The binding of these compounds with the PDB ID: 6VXX which is the SARS-COV-2 spike glycoprotein for COVID-19 [31] has also been examined to illustrate the potential of these compounds to deactivate this novel corona virus at the receptor end.

Results and Discussion

The *In silico* optimized molecular structure of 09 bioactive natural compounds modeled using DFT-B3LY/6-31+G (d,p) level of theory which exhibited the property of inhibitor against main protease of COVID-19, have been displayed in figure 1. The binding affinity for all displayed molecules as ligand with the target protease of the SARS-Cov-2 virus (PDB ID: 6Y84 and 6LU7) as well as with the SARS Spike Glycoprotein for corona virus (PDB ID: 6CRV) have been depicted in table 1 to table 9. We observe a complete cycle for the binding of all these compounds with the said protease in maximum ten cluster run and the final free energy of binding is significant for all the ligand-protein interactions. The insignificant result for the binding of molecule no.8 (pentyl ester of chlorogenic acid) with 6LU7 and 6CRV receptors have been obtained where the same molecule exhibits a significant final free energy of binding with 6Y84 protease of COVID-19 as obvious from the table 8. The significant binding of these natural compounds with the said protease results in the conformational changes in the main protease of SARS-COV-2 as displayed in figure 2 to figure 10. It is, therefore, predicted that the naturally occurring bio molecules displayed in figure 1 may be explored as potential inhibitors for the COVID-

19 protease. The docking results of these molecules are presented in decreasing order of the binding affinity i.e. the binding affinity with the target protein is largest for molecule 1 followed by molecule 2,3,4,5,6,7,8 and 9.

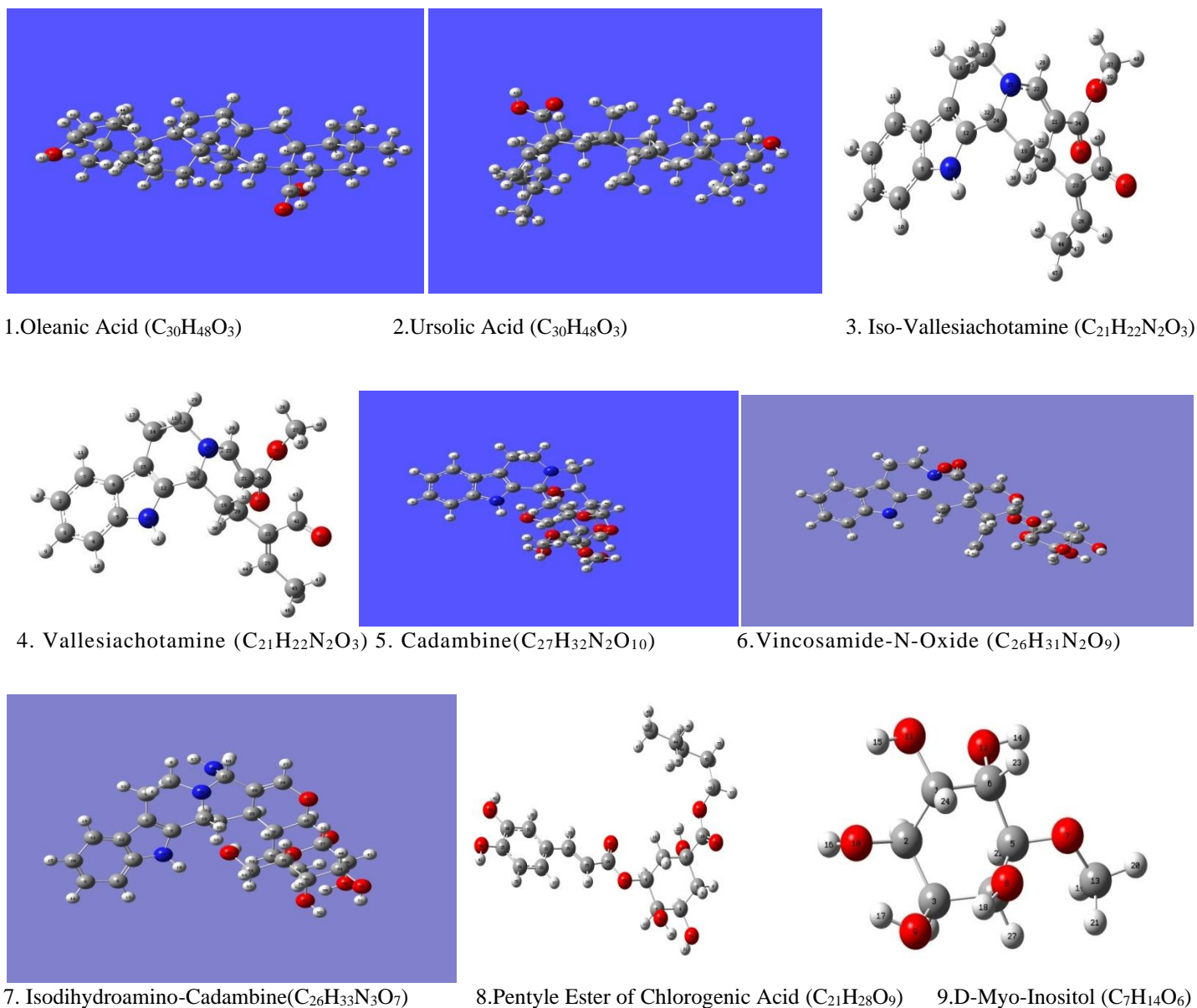


Figure 1: The optimized molecular structure of Nine Bioactive Natural Compounds *reported to be found in fruits (*molecule no 1 to 7*) and leaves (*molecule no 8 to 9*) of *Anthocephalus cadamba* [* 14-16]. Blue balls: N-atoms, black balls: C-atoms, white balls: H-atoms

The significantly negative value of the final free energy of binding obtained through the molecular docking of these compounds with the SARS-COV-2 protease reveals that these molecules may be explored as potential inhibitor against COVID-19. Since these compounds are naturally occurring, hence they do not cost for their synthesis and bears less or nil side-effect which enables them to be explored as user-friendly drug. The solubility and the effect of the solvent which are usually associated with the phytochemicals may be the point of

attention while using these compounds as drug candidate. The other chemical structure may also be derived from these bioactive natural compounds to further design potential drug agent for COVID-19.

Table 1: Binding affinity of Oleanic Acid ($C_{30}H_{48}O_3$) with the target proteins

Cluster Rank	SARS-COV-2 protease (PDB ID: 6Y84)		SARS-COV-2 protease (PDB ID: 6LU7)		SARS-COV Spike Glycoprotein (PDB ID: 6CRV)	
	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant
1	-11.28 kcal/mol	5.38nM	-6.11 kcal/mol	33.45 μ M	-5.90 kcal/mol	47.30 μ M
2	-9.78 kcal/mol	67.90nM	-5.83 kcal/mol	52.86 μ M	-5.73 kcal/mol	63.60 μ M
3	-9.60 kcal/mol	91.21nM	-5.70 kcal/mol	66.07 μ M	-5.41 kcal/mol	107.78 μ M
4	-9.10 kcal/mol	213.24nM	-5.59 kcal/mol	79.53 μ M	-4.91 kcal/mol	249.99 μ M
5	-----	-----	-5.57 kcal/mol	82.47 μ M	-4.39 kcal/mol	607.04 μ M
6	-----	-----	-5.44 kcal/mol	102.54 μ M	-4.28 kcal/mol	723.95 μ M
7	-----	-----	-5.08 kcal/mol	189.25 μ M	-4.25 kcal/mol	768.82 μ M
8	-----	-----	-4.89 kcal/mol	258.48 μ M	-----	-----
9	-----	-----	-4.77 kcal/mol	318.64 μ M	-----	-----
10	-----	-----	-----	-----	-----	-----

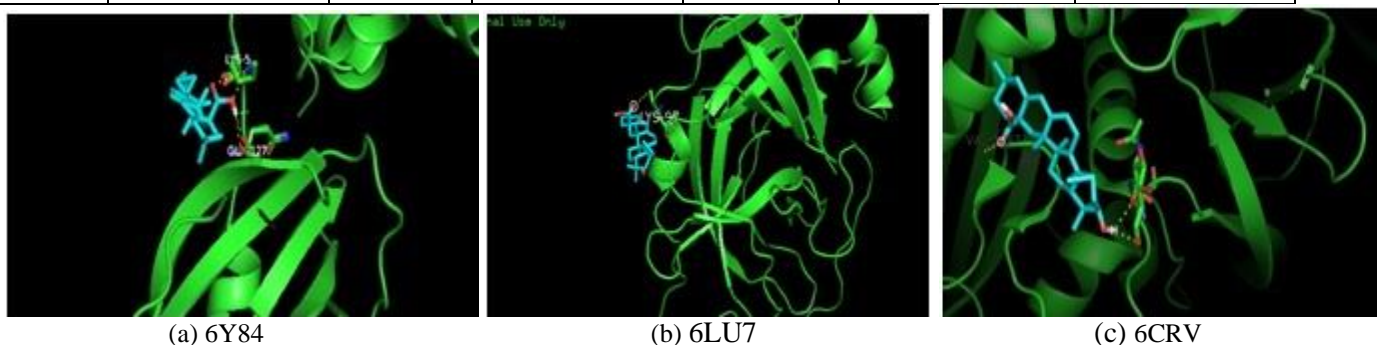
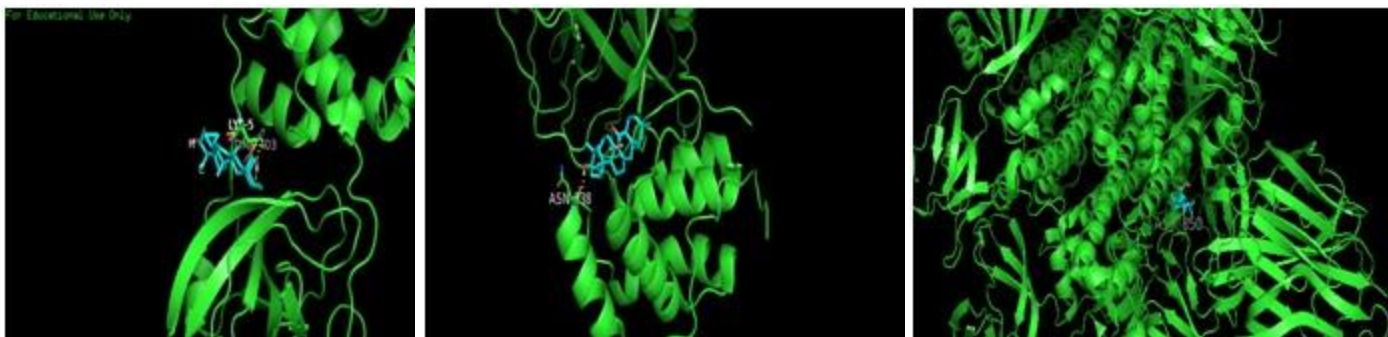


Figure 2: Conformational changes observed due the binding of ligand-1 (Oleanic Acid) with the COVID-19 protease and S-protein receptor

Table 2: Binding affinity of Ursolic Acid ($C_{30}H_{48}O_3$) with the target proteins

Cluster Rank	SARS-COV-2 protease (PDB ID: 6Y84)		SARS-COV-2 protease (PDB ID: 6LU7)		SARS-COV Spike Glycoprotein (PDB ID: 6CRV)	
	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant
1	-10.94 kcal/mol	9.63nM	-6.40 kcal/mol	20.38 μ M	-6.96 kcal/mol	7.88 μ M
2	-10.30 kcal/mol	28.35nM	-6.34 kcal/mol	22.70 μ M	-6.81 kcal/mol	10.26 μ M
3	-9.74 kcal/mol	72.73nM	-6.00 kcal/mol	40.32 μ M	-6.76 kcal/mol	11.17 μ M
4	-9.72 kcal/mol	74.54nM	-5.89 kcal/mol	47.97 μ M	-6.61 kcal/mol	14.24 μ M
5	-9.36 kcal/mol	137.19nM	-5.34 kcal/mol	122.36 μ M	-6.30 kcal/mol	24.24 μ M
6	-8.90 kcal/mol	299.83nM	-4.96 kcal/mol	232.96 μ M	-6.19 kcal/mol	29.17 μ M
7	-----	-----	-----	-----	-6.14 kcal/mol	31.59 μ M
8	-----	-----	-----	-----	-6.08 kcal/mol	34.90 μ M
9	-----	-----	-----	-----	-5.91 kcal/mol	46.80 μ M
10	-----	-----	-----	-----	-5.86 kcal/mol	50.93 μ M



(a)6Y84

(b) 6LU7

(c) 6CRV

Figure 3: Conformational changes observed due the binding of ligand-2 (Ursolic Acid) with the COVID-19 protease and S-protein receptor

Table 3: Binding affinity of Iso-Vallesiachotamine (C₂₁H₂₂N₂O₃) with the target proteins

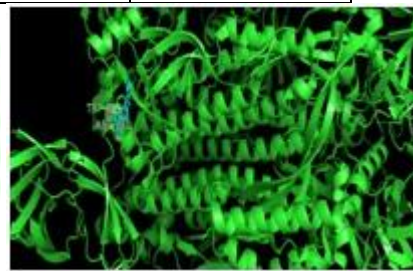
Cluster Rank	SARS-COV-2 protease (PDB ID: 6Y84)		SARS-COV-2 protease (PDB ID: 6LU7)		SARS-COV Spike Glycoprotein (PDB ID: 6CRV)	
	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant
1	-9.55 kcal/mol	99.42nM	-6.00 kcal/mol	39.82μM	-5.16 kcal/mol	165.89μM
2	-7.93 kcal/mol	1.53μM	-5.71 kcal/mol	65.58μM	-5.00 kcal/mol	214.52μM
3	-7.63 kcal/mol	2.54μM	-5.70 kcal/mol	66.53μM	-4.94 kcal/mol	237.57μM
4	-7.61 kcal/mol	2.63μM	-5.35 kcal/mol	20.56μM	-4.81 kcal/mol	296.22μM
5	-7.45 kcal/mol	3.43μM	-5.11 kcal/mol	178.30μM	-4.75 kcal/mol	332.16μM
6	-6.51 kcal/mol	16.94μM	-4.86 kcal/mol	275.30μM	-4.70 kcal/mol	357.55μM
7	-----	-----	-----	-----	-4.68 kcal/mol	370.48μM
8	-----	-----	-----	-----	-4.45 kcal/mol	548.28μM
9	-----	-----	-----	-----	-4.36 kcal/mol	640.89μM
10	-----	-----	-----	-----	-4.03 kcal/mol	1.1 mM



(a) 6Y84



(b) 6LU7



(c) 6CRV

Figure 4: Conformational changes observed due the binding of ligand-3 (Iso-Vallesiachotamine) with the COVID-19 protease and S-protein receptor

Table 4: Binding affinity of Vallesiachotamine(C₂₁H₂₂N₂O₃) with the target proteins

Cluster Rank	SARS-COV-2 protease (PDB ID: 6Y84)		SARS-COV-2 protease (PDB ID: 6LU7)		SARS-COV Spike Glycoprotein (PDB ID: 6CRV)	
	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant
1	-9.50 kcal/mol	109.66nM	-4.98 kcal/mol	224.33μM	-6.35 kcal/mol	22.14μM
2	-9.32 kcal/mol	148.51nM	-4.80 kcal/mol	302.86μM	-6.04 kcal/mol	37.63μM
3	-8.77 kcal/mol	371.00nM	-4.43 kcal/mol	564.68μM	-5.52 kcal/mol	89.42μM
4	-8.33 kcal/mol	784.52nM	-4.31 kcal/mol	693.59μM	-5.05 kcal/mol	197.72μM
5	-6.91 kcal/mol	8.64μM	-4.30 kcal/mol	699.66μM	-4.88 kcal/mol	262.69μM

6	-6.87 kcal/mol	9.28 μ M	-4.28 kcal/mol	723.92 μ M	-4.77 kcal/mol	320.94 μ M
7	-6.62 kcal/mol	13.94 μ M	-3.94 kcal/mol	1.30mM	-4.36 kcal/mol	633.00 μ M
8	-----	-----	-3.73 kcal/mol	1.84mM	-4.25 kcal/mol	769.77 μ M
9	-----	-----	-2.84 kcal/mol	8.22mM	-4.02 kcal/mol	1.12mM
10	-----	-----	-----	-----	-3.90 kcal/mol	1.39mM

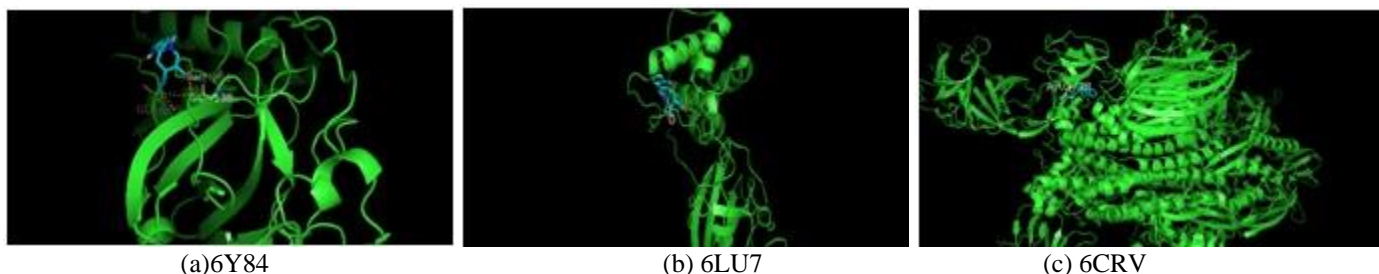


Figure 5: Conformational changes observed due the binding of ligand-4 (Vallesiachotamine) with the COVID-19 protease and S-protein receptor

Table 5: Binding affinity of Cadambine(C₂₇H₃₂N₂O₁₀) with the target proteins

Cluster Rank	SARS-COV-2 protease (PDB ID: 6Y84)		SARS-COV-2 protease (PDB ID: 6LU7)		SARS-COV Spike Glycoprotein (PDB ID: 6CRV)	
	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant
1	-8.85 kcal/mol	323.97nM	-6.93 kcal/mol	13.86 μ M	-5.35 kcal/mol	118.85 μ M
2	-7.57 kcal/mol	2.81 μ M	-4.80 kcal/mol	301.24 μ M	-4.83 kcal/mol	287.35 μ M
3	-7.46 kcal/mol	3.39 μ M	-4.44 kcal/mol	553.34 μ M	-4.65 kcal/mol	391.37 μ M
4	-7.18 kcal/mol	5.45 μ M	-4.26 kcal/mol	756.51 μ M	-4.56 kcal/mol	451.55 μ M
5	-6.79 kcal/mol	10.57 μ M	-4.24 kcal/mol	779.20 μ M	-4.33 kcal/mol	673.01 μ M
6	-6.22 kcal/mol	27.54 μ M	-4.05 kcal/mol	1.07mM	-4.24 kcal/mol	783.98 μ M
7	-6.15 kcal/mol	30.85 μ M	-4.03 kcal/mol	1.11mM	-3.78 kcal/mol	1.71mM
8	-5.85 kcal/mol	51.89 μ M	-3.72 kcal/mol	1.88mM	-3.50 kcal/mol	2.70mM
9	-5.72 kcal/mol	64.44 μ M	-3.33 kcal/mol	3.60mM	-3.06 kcal/mol	5.75mM
10	-5.45 kcal/mol	100.34 μ M	-3.31 kcal/mol	3.77mM	-2.88 kcal/mol	7.78mM

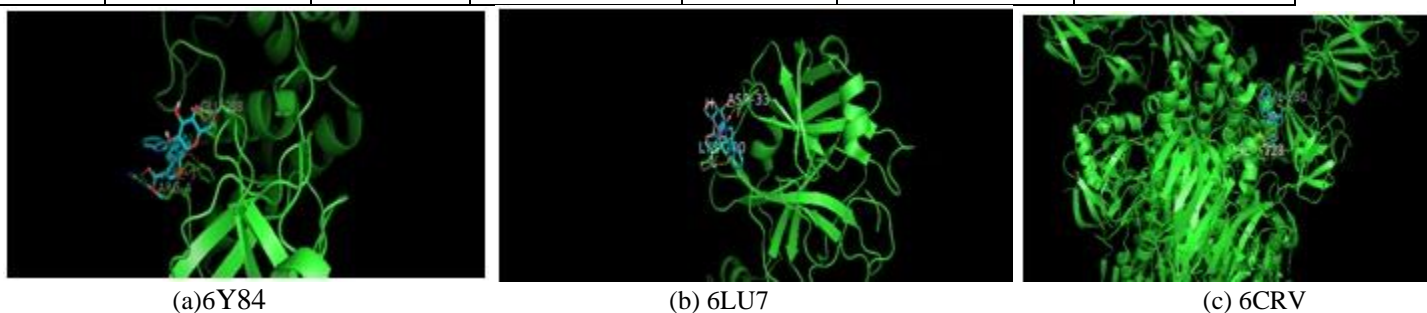


Figure 6: Conformational changes observed due the binding of ligand-5 (Cadambine) with the COVID-19 protease and S-protein receptor

Table 6: Binding affinity of Vincosamide-N-Oxide (C₂₆H₃₁N₂O₉) with the target proteins

Cluster Rank	SARS-COV-2 protease (PDB ID: 6Y84)		SARS-COV-2 protease (PDB ID: 6LU7)		SARS-COV Spike Glycoprotein (PDB ID: 6CRV)	
	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant
1	-8.81 kcal/mol	348.65nM	-5.81 kcal/mol	55.52 μ M	-4.59 kcal/mol	434.05 μ M
2	-8.77 kcal/mol	370.30nM	-4.78 kcal/mol	312.98 μ M	-4.47 kcal/mol	532.26 μ M
3	-8.41 kcal/mol	686.48nM	-4.49 kcal/mol	511.38 μ M	-4.28 kcal/mol	730.11 μ M

4	-8.08 kcal/mol	1.19 μ M	-3.93 kcal/mol	1.31mM	-4.05 kcal/mol	1.07mM
5	-7.92 kcal/mol	1.58 μ M	-3.89 kcal/mol	1.40mM	-4.00 kcal/mol	1.17mM
6	-7.70 kcal/mol	2.26 μ M	-3.75 kcal/mol	1.79mM	-3.49 kcal/mol	2.78M
7	-7.22 kcal/mol	5.08 μ M	-3.48 kcal/mol	2.83mM	-3.44 kcal/mol	3.02mM
8	-6.26 kcal/mol	25.76 μ M	-3.41 kcal/mol	3.15mM	-3.38 kcal/mol	3.31mM
9	-5.08 kcal/mol	187.55 μ M	-3.33 kcal/mol	3.64mM	-3.29 kcal/mol	3.91mM
10	-----	-----	-3.03 kcal/mol	5.96mM	-3.04 kcal/mol	5.93mM

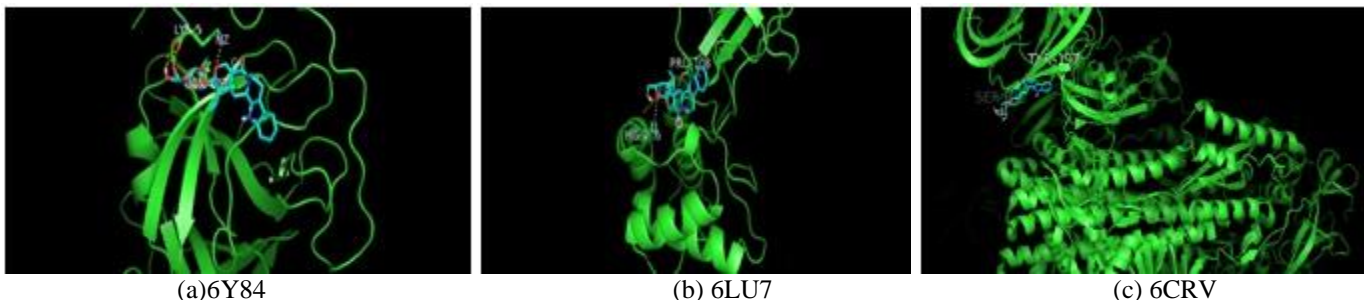


Figure 7: Conformational changes observed due the binding of ligand-6 (Vincosamide-N-Oxide) with the COVID-19 protease and S-protein receptor

Table 7: Binding affinity of Isodihydroamino-cadambine($C_{26}H_{33}N_3O_7$)with the target proteins

Cluster Rank	SARS-COV-2 protease (PDB ID: 6Y84)		SARS-COV-2 protease (PDB ID: 6LU7)		SARS-COV Spike Glycoprotein (PDB ID: 6CRV)	
	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant
1	-8.57 kcal/mol	520.33nM	-4.85 kcal/mol	277.88 μ M	-4.10 kcal/mol	922.19 μ M
2	-7.88 kcal/mol	1.67 μ M	-4.10 kcal/mol	990.69 μ M	-3.90 kcal/mol	1.39mM
3	-6.49 kcal/mol	17.43 μ M	-4.08 kcal/mol	1.02mM	-3.53 kcal/mol	2.60mM
4	-5.27 kcal/mol	137.23 μ M	-3.09 kcal/mol	5.42mM	-3.44 kcal/mol	2.99mM
5	-5.11 kcal/mol	181.03 μ M	-2.92 kcal/mol	7.26mM	-3.31 kcal/mol	3.73mM
6	-4.76 kcal/mol	324.79 μ M	-2.58 kcal/mol	12.88mM	-3.00 kcal/mol	6.28mM
7	-4.21 kcal/mol	816.29 μ M	-2.53 kcal/mol	14.04mM	-2.97 kcal/mol	6.63mM
8	-3.90 kcal/mol	1.39mM	-2.51 kcal/mol	14.54mM	-2.88 kcal/mol	7.71mM
9	-----	-----	-1.04 kcal/mol	172.81 mM	-2.84 kcal/mol	8.27mM
10	-----	-----	-----	-----	-2.69 kcal/mol	10.75mM

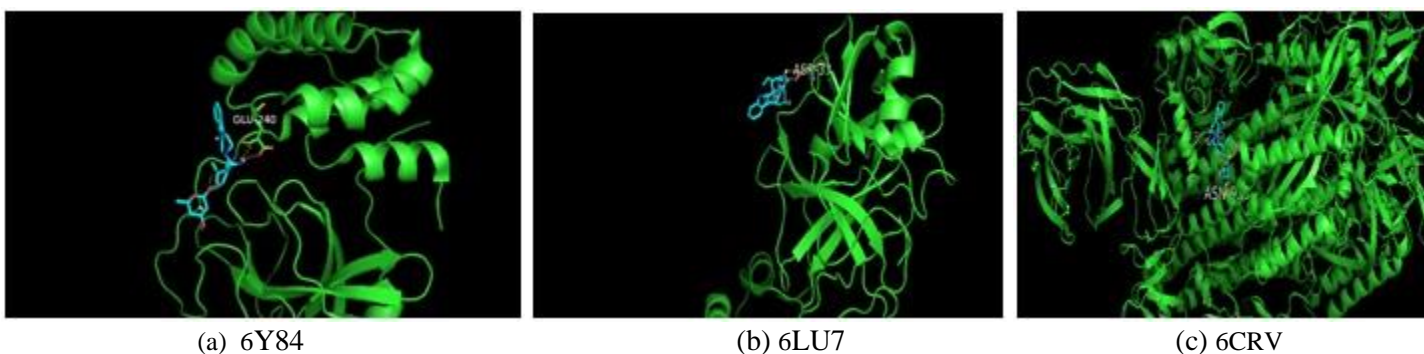


Figure 8: Conformational changes observed due the binding of ligand-7 (Isodihydroamino-cadambine) with the COVID-19 protease and S-protein receptor

Table 8: Binding affinity of Pentyle ester of chlorogenic acid ($C_{21}H_{28}O_9$) with the target proteins

Cluster Rank	SARS-COV-2 protease (PDB ID: 6Y84)		SARS-COV-2 protease (PDB ID: 6LU7)		SARS-COV Spike Glycoprotein (PDB ID: 6CRV)	
	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant
1	-5.61 kcal/mol	77.71 μ M	-1.95 kcal/mol	37.57mM	-2.35 kcal/mol	19.10mM
2	-5.10 kcal/mol	182.54 μ M	-1.52 kcal/mol	76.24mM	-1.77 kcal/mol	50.49mM
3	-5.06 kcal/mol	194.58 μ M	-1.46 kcal/mol	85.21mM	-1.66 kcal/mol	60.43mM
4	-5.05 kcal/mol	198.85 μ M	-1.03 kcal/mol	176.88mM	-1.64 kcal/mol	62.51mM
5	-4.18 kcal/mol	869.07 μ M	-0.86 kcal/mol	233.66mM	-1.37 kcal/mol	99.72mM
6	-3.40 kcal/mol	3.21mM	-0.61 kcal/mol	356.69mM	-1.23 kcal/mol	126.32mM
7	-3.30 kcal/mol	3.80mM	-0.42 kcal/mol	490.95mM	-1.03 kcal/mol	176.85mM
8	-3.15 kcal/mol	4.87mM	-0.37 kcal/mol	531.76mM	-0.20 kcal/mol	713.11mM
9	-3.03 kcal/mol	6.04mM	-0.06 kcal/mol	903.01mM	-0.10 kcal/mol	840.36mM
10	-2.86 kcal/mol	8.01mM	+0.05 kcal/mol	-----	-0.00 kcal/mol	991.66mM

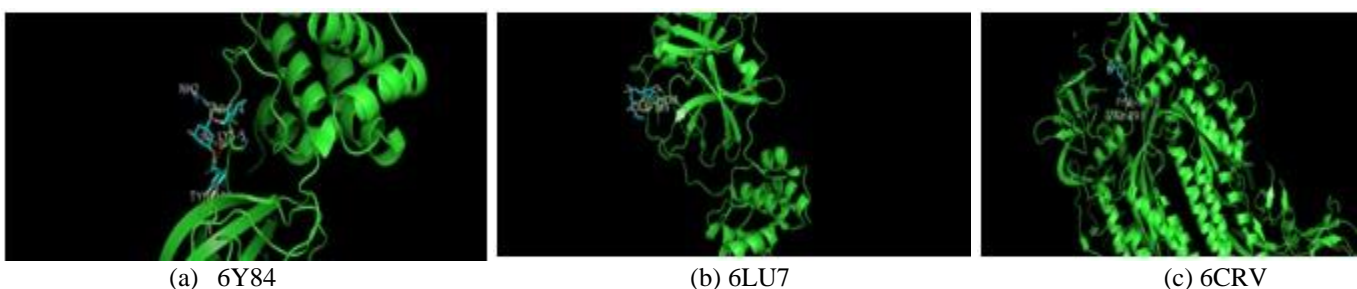
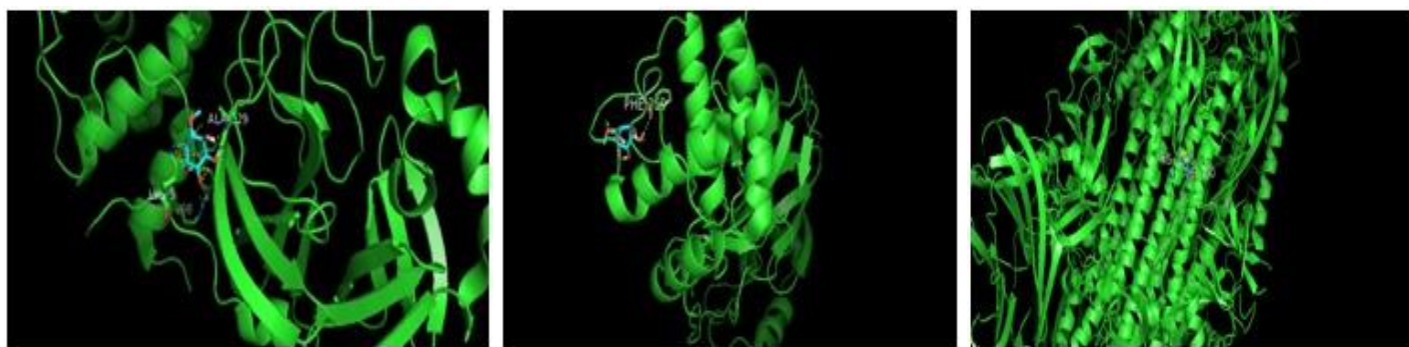


Figure 9: Conformational changes observed due the binding of ligand-8 (Pentyle Ester of Chlorogenic Acid) with the COVID-19 protease and S-protein receptor

Table 9: Binding affinity of D-Myo-Inositol ($C_7H_{14}O_6$) with the target proteins

Cluster Rank	SARS-COV-2 protease (PDB ID: 6Y84)		SARS-COV-2 protease (PDB ID: 6LU7)		SARS-COV Spike Glycoprotein (PDB ID: 6CRV)	
	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant
1	-5.51 kcal/mol	90.67 μ M	-2.22 kcal/mol	23.63mM	-3.11 kcal/mol	5.22mM
2	-5.06 kcal/mol	195.69 μ M	-2.05 kcal/mol	31.17mM	-2.77 kcal/mol	9.34mM
3	-4.30 kcal/mol	702.05 μ M	-1.86 kcal/mol	43.37mM	-2.64 kcal/mol	11.57mM
4	-3.56 kcal/mol	2.47 μ M	-1.74 kcal/mol	53.02mM	-2.53 kcal/mol	13.88mM
5	-3.04 kcal/mol	5.86mM	-1.72 kcal/mol	54.66mM	-2.32 kcal/mol	20.09mM
6	-3.00 kcal/mol	6.30mM	-1.53 kcal/mol	75.47mM	-2.30 kcal/mol	20.46mM
7	-2.93 kcal/mol	7.09mM	-1.38 kcal/mol	98.07mM	-2.11 kcal/mol	28.50mM
8	-----	-----	-1.10 kcal/mol	154.91mM	-1.98 kcal/mol	35.50mM
9	-----	-----	-1.07 kcal/mol	164.03mM	-1.94 kcal/mol	37.54mM
10	-----	-----	-----	-----	-1.56 kcal/mol	71.41mM



(a) 6Y84

(b) 6LU7

(c) 6CRV

Figure 10: Conformational changes observed due the binding of ligand-9 (D-Myo-Inositol) with the COVID-19 protease and S-protein receptor. The significantly negative value of free energy of binding of these molecules with PDB ID: 6VXX (SARS-COV-2 spike glycoprotein for COVID-19) in ligand-protein interaction as depicted in table 10 and 11 reveals that these molecules or their structural-derivatives may stop the replication of the virus at the receiver end in the human body. The results regarding binding affinity of the some of these compounds and corresponding conformational changes occurred in the target protein are presented in figure 11 and 12.

Table 10: Binding affinity in SARS-COV-2 spike glycoprotein interaction

Oleanic Acid (C₃₀H₄₈O₃)-6VXX interaction			Ursolic Acid(C₃₀H₄₈O₃) -6VXX interaction	
Cluster Rank	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant
1	-6.76 kcal/mol	11.05μM	-7.15 kcal/mol	5.73μM
2	-6.54 kcal/mol	16.17μM	-7.07 kcal/mol	6.53μM
3	-6.41 kcal/mol	19.85μM	-6.74 kcal/mol	11.56μM
4	-6.41 kcal/mol	19.90μM	-6.72 kcal/mol	11.78μM
5	-6.12 kcal/mol	32.71μM	-6.48 kcal/mol	17.64μM
6	-6.04 kcal/mol	37.45μM	-6.39 kcal/mol	20.63μM
7	-5.80 kcal/mol	56.45μM	-6.29 kcal/mol	24.67μM
8	-5.70 kcal/mol	66.58μM	-6.22 kcal/mol	27.76μM
9	-5.45 kcal/mol	101.12μM	-6.20 kcal/mol	28.40 μM
10			-5.81 kcal/mol	55.41μM

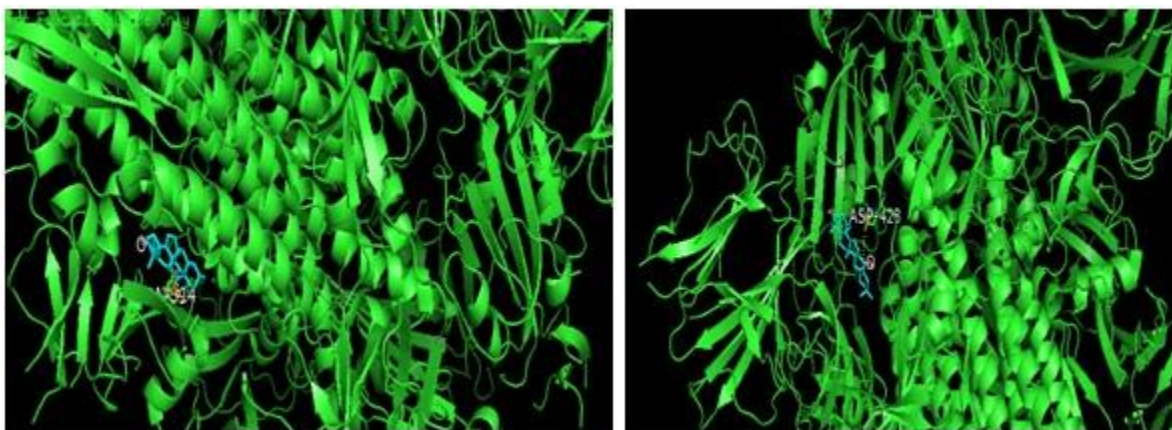


Figure 11 (a) Ligand [Oleanic Acid] - Protein [6VXX] interaction

(b) Ligand [Ursolic Acid] - Protein [6VXX] interaction

Table 11: Binding affinity in SARS-COV-2 spike glycoprotein interaction

Iso-Vallesiachotamine (C ₂₁ H ₂₂ N ₂ O ₃)-6VXX interaction			Isodihydroamino-cadambine (C ₂₆ H ₃₃ N ₃ O ₇) -6VXX interaction	
Cluster Rank	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant
1	-5.18 kcal/mol	160.30μM	-5.36 kcal/mol	118.39μM
2	-5.12 kcal/mol	177.97μM	-3.88 kcal/mol	1.44mM
3	-5.01 kcal/mol	211.92μM	-3.56 kcal/mol	2.46mM
4	-4.76 kcal/mol	322.60μM	-3.48 kcal/mol	2.80mM
5	-4.72 kcal/mol	344.73μM	-3.35 kcal/mol	3.49mM
6	-4.55 kcal/mol	462.95μM	-3.22 kcal/mol	4.39mM
7	-4.32 kcal/mol	676.74μM	-2.88 kcal/mol	7.78mM
8	-4.25 kcal/mol	762.33μM	-2.65 kcal/mol	11.39mM
9	-3.58 kcal/mol	2.37mM	-2.62 kcal/mol	12.08mM
10	-3.40 kcal/mol	3.20mM	-2.53 kcal/mol	14.00mM

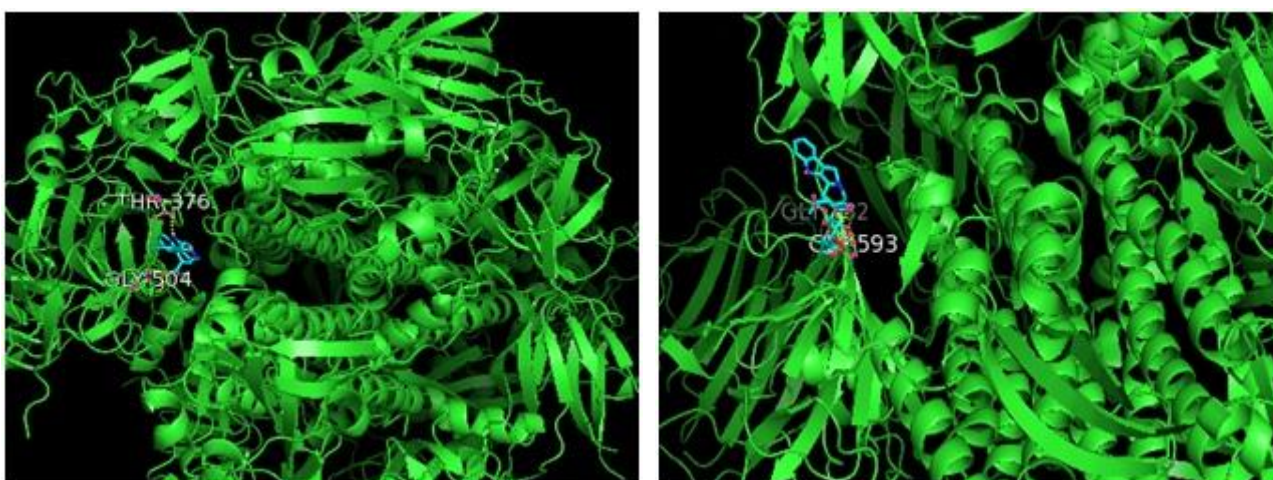


Figure 12 (a) Ligand [Iso-Vallesiachotamine] - Protein [6VXX] interaction (b) Ligand [Isodihydroamino-cadambine] - Protein [6VXX] interaction

Similar types of significant binding affinity have been obtained in all the SARS-COV-2 spike glycoprotein – ligand (studied compounds) interactions whose results are available on demand but not depicted due to space limitations.

Concluding Remarks

We have carried out a schematic *In Silico* investigation applying density functional theory and molecular docking approach on the nine naturally occurring bioactive compounds listed in figure 1 spontaneously found in the leaves and fruits of *Anthocephalus cadamba*. On the basis of the binding affinity, we have found these compounds as potential inhibitors against the COVID-19 having final free energy of binding in the order of molecule 1>2>3>4>5>6>7>8>9. These molecules have also emerged as potential inhibitors against the SARS-

COV-2 spike glycoprotein for COVID-19 in our investigation. These compounds may be explored as drug candidate and their molecular structure may be exploited to develop a vaccine for COVID-19. In-vivo study is invited on these compounds for developing user friendly drug for COVID-19. The further study on derived structures of these compounds is going on in our laboratory.

Acknowledgements

Authors are thankful to Professor Neeraj Misra, Department of Physics University of Lucknow, India for permitting us to use his computational facility. Thanks are due to Dr. D.P. Mishra, former Research fellow, Central Drug Research Institute, Lucknow, India for immense help regarding the information about bioactive natural products.

References

- [1] C. Huang, Y. Wang, X. Li, et al., *Lancet* 395 (2020) 497–506
- [2] J. F. W. Chan, K. H. Kok, Z. Zhu, H. Chu, K. K. W. To, S. Yuan, K. Y. Yuen, *Emerging Microbes & Infections* 9 (2020) 221–236.
- [3] J. F. W. Chan, S. Yuan, K. H. Kok, K. K. W. To, et al., *Lancet* 395 (2020) 514–523.
- [4] N. Chen, M. Zhou, X. Dong, J. Qu, F. Gong, Y. Han, Y. Qiu, J. Wang, Y. Liu, Y. Wei, J. Xia, T. Yu, X. Zhang, L. Zhang, *Lancet* 395 (2020) 507–513
- [5] L. Roujian, X. Zhao, J. Li, P. Niu, et al., *Lancet* 395 (2020) 565–574
- [6]https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200317-sitrep-57-covid-19.pdf?sfvrsn=a26922f2_2 (accessed on 2 April, 2020)
- [7] J. Liu, X. Liao, S. Qian et al. Community transmission of severe acute respiratory syndrome coronavirus 2, Shenzhen, China, 2020. *Emerg Infect Dis* (2020) doi.org/10.3201/eid2606.200239
- [8] J. Chan, S. Yuan, K. Kok et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* (2020) doi: 10.1016/S0140-6736(20)30154-9
- [9] Q. Li, X. Guan, P. Wu et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med* (2020); doi:10.1056/NEJMoA2001316.
- [10] N van Doremalen, D. Morris, T. Bushmaker et al. Aerosol and Surface Stability of SARS-CoV-2 as compared with SARS-CoV-1. *New Engl J Med* 2020 doi: 10.1056/NEJMc2004973
- [11] S. Mandal, T. Bhatnagar, N. Arinaminpathy et al. Prudent public health intervention strategies to control the coronavirus disease 2019 transmission in India: A mathematical model-based approach, *Indian J Med Res*, Epub ahead of print (2020), DOI: 10.4103/ijmr.IJMR_504_20.
- [11] G. Li and E. De Clercq, *Nat. Rev. Drug Discovery* 19 (2020) 149-150

- [12] J. Gao, Z. Tian, X. Yang, *BioScience Trends* (2020) doi:10.5582/bst.2020.01047
- [13] <https://icmr.nic.in/content/covid-19> accessed on 25 March, 2020
- [14] A. Dwevedi, K. Sharma, and Y. K. Sharma, *Cadamba: A miraculous tree having enormous pharmacological implications*, *Pharmacognosy Review*, 9(18) (2015) 107-113
- [15] D P Mishra , R Maurya, *Isolation and Characterization of Bioactive Natural Products from Indian Medicinal Plants*, PhD Thesis, Central Drug Research Institute, Lucknow, India, (2014)
- [16] D P Mishra, M A Khan, D K Yadav, A K Rawat, R K Singh, T Ahamad, M K Hussain, M Saquib and M F Khan, *Chemistry Select*, 3(2018) 8468-8472
- [17] A.K. Mishra A and S.P. Tewari, 7-Hydroxy-6-Methoxy-Coumarin to be a Multifunctional Bioactive Natural molecule: A Theoretical Study. *Materials Today: Proceedings* (2019) doi.org/10.1016/j.matpr.2019.04.100
- [18] A.K. Mishra and S.P. Tewari, Spectroscopic and Bioactivity Analysis of Naturally occurring Methyl Ester of Chlorogenic Acid Using Density Functional Theory Based Quantum Chemical Computation: *Sensor Letters*, 17 (2019) 822-825
- [19] A. K. Mishra and S.P. Tewari, Structure, spectra and bioactivity of pentyl ester of chlorogenic acid: DFT study. *Emerging Materials Research* 8(4) (2019) <https://doi.org/10.1680/jemmr.18.00129>
- [20] A.K. Mishra and S.P. Tewari, DFT based modeling of a natural molecule D-Myo-Inositol explores it to be a multifunctional biologically active, *AIP Conference Proceedings* (2019) <https://doi.org/10.1063/1.5098621>
- [21] A. K. Mishra and S. P. Tewari, Density functional theory towards bioactivity analysis of Isovallesiachotamine natural bio molecule, *International Journal of Scientific Research in Physics and Applied Sciences* (2019) <https://doi.org/10.26438/ijsrpas/v7i.118131>
- [22] A.D Becke, Density-functional thermochemistry: III. The role of exact exchange. *Journal of Chemical Physics* 98 (1993) 5648–5652.
- [23] C. Lee, W. Yang and R.G. Parr, Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density, *Physical Review B* 37(2) (1988) 785–789.
- [24] M.J. Frisch, G.W. Trucks, H.B. Schlegel et al. (2010) Gaussian 09, Revision B.01. Gaussian Inc., Wallingford, CT, USA.
- [25] <http://www.rcsb.org/structure/6Y84> (accessed on 27 March, 2020).
- [26] <http://www.rcsb.org/structure/6LU7> (accessed on 27 March, 2020).

- [27] G.M. Morris, R. Huey, W. Lindstrom et al. Autodock4 and AutoDock-Tools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, 30(10) (2009) 2785–2791.
- [28] S. Cosconati, S. Forli, A.L. Perryman et al. Virtual screening with AutoDock: Theory and practice. *Expert Opin. Drug Discovery*, 5(6) (2010) 597–607.
- [29] S. Forli and A.J. Olson. A force field with discrete displaceable waters and desolvations entropy for hydrated ligand Docking, *Journal of Medicinal Chemistry* 55(2), (2012) 623–638.
- [30] <http://www.rcsb.org/structure/6CRV> (accessed on 26 March, 2020).
- [31] <http://www.rcsb.org/structure/6VXX> (accessed on 01 April, 2020).