

Identifying potential compounds from *Bacopa monnieri* (brahmi) against Coxsackievirus A16 RdRp targeting HFM disease (tomato flu)

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Abstract

The most promising drug target to treat hand, foot, and mouth disease (HFMD) caused by coxsackievirus (CVA16) is RNA-dependent RNA polymerase (RdRp). This study offered an in-silico pipeline to identify possible RdRp inhibitors against CVA16 RdRp. Here, 91 natural compounds derived from *Bacopa monnieri* (*brahmi*) were virtually screened against the RdRp of CVA16. Bacobitacin D, a hit screened in this study, exhibited eight hydrogen bonds with RdRp targeting catalytic site residues (Asp²³⁸ and Asp³²⁹). Later, the molecular dynamics (MD) simulation and MM/GBSA binding free energy calculations were performed with the top three hits selected on the exhaustive docking score (≤ -9.55 kcal/mole). Bacobitacin D showed the minimum fluctuation ($\langle \text{RMSD} \rangle = 0.75$ nm, standard deviation = 0.02) over the 100 ns MD simulation time. However, the ΔG_{TOTAL} binding free energy of Bacopaside IV was the lowest with -23.70 kcal/mole while Bacobitacin D showed comparable ΔG_{TOTAL} with -19.14 kcal/mole. Later, the middle structures of the most populated cluster obtained from MD simulation were studied for the interaction. Bacobitacin D showed direct interaction with the catalytic residues (Asp²³⁸ and Ser²⁸⁹). This study proposed Bacobitacin D as active inhibitor against RdRp of CVA16 that need to be validated in an experiment to ascertain its inhibitory action.

Introduction

The new disease called the 'tomato flu' is also widely known as hand, foot, and mouth disease (HFMD) [1]. Recently, 82 cases have been reported in children less than 5 years old in Kerala, India [2]. This disease is not only limited to children, but it can also affect adults to a comparable extent. The symptoms include red blisters all over the body, particularly the hands, feet, and oral cavity, while other common symptoms include fever, fatigue, nausea, vomiting, diarrhoea, dehydration, joint swelling, and body aches. The name 'tomato flu' came to be known in India due to the red blister's resemblance to tomatoes. HFMD was first reported in New Zealand, Toronto in 1957 [3], and since the 1970s there have been many outbreaks reported [4–7]. The HFMD can be transmitted through contacts with the infected person, such as nose and throat secretions, saliva, drool, or nasal mucus, fluid from blisters or scabs, and faeces [8]. Although India has not yet reported any fatal cases, the illness has recently been reported to have spread to other states.

HFMD is caused by Coxsackievirus A16 (CVA16), and Enterovirus 71 (EV71), but the most common causative virus is CVA16. Both, EV71 and CVA16 belong to the *Enterovirus* genus of the *Picornaviridae* family and have RNA single-stranded positive sense genomes [9]. The membrane-associated replication complex responsible for CVA16's genome replication that uses RNA-dependent RNA polymerase (RdRp) as its primary catalytic enzyme [10]. Except for *Retroviridae*, all members of the RNA virus superfamilies share the RNA-dependent RNA polymerase (RdRp), a highly conserved enzyme [11]. A sequence and structure-based comparison of CVA16 RdRp was performed with its close homologs (sequences and structures sourced from Protein Data Bank and UniProt database where Ids are shown in Supplementary Table S1). The phylogenetic tree and the heat map of the identity matrix has been provided in the

Supplementary Fig. S1 and S2 which showed that the CVA16 RdRp had > 45% similarity with Human rhinovirus 16, Human enterovirus D68, Poliovirus type 1, Coxsackievirus B3, and Human enterovirus 71. The heat map had only a small green patch that showed the high sequence similarity of CVA16 RdRp with limited RdRp from different viruses. Most of the sequences showed lower sequence similarity with the reference protein. Human enterovirus 71. Though their sequence similarity was found to be very low in phylogenetic studies, but the core structural features of RdRps were found to be conserved in structure based phylogenetic studies. Supplementary Fig. S3, showed the structural similarity bars for all 32 proteins compared to CVA16 RdRp. Here, the representation of RMSDs below 2 Å, 4 Å, 6 Å and above 6 Å were depicted with green, yellow, orange and red colors, respectively. Apart from the *Reovirus*, all other viruses showed high structural similarity with the CVA16 RdRp (< 2 Å) for different sequence patches as observed in Supplementary Fig. S3.

Moreover, the enzyme alone is responsible for the fast mutation of RNA viruses caused by the high rate of transcription error [12]. During the virus replication cycle, the enzyme is essential for the transcription process as well as the development of genomic RNA [13]. Interestingly, this protein does not have any close homologs among the human protein [12]. The essentiality and uniqueness of RdRp proposed it a desirable drug target for a therapeutic molecule to inhibit the growth of RNA viruses. The crystal structure was solved for the RNA-dependent RNA polymerase protein from CVA16 and its functional site was structurally determined [10]. There have been studies to design therapeutic peptides and small molecules against CVA16 and EVA71 [14–16]. Here, the Molnupiravir drug and its active form were used against EVA71 in one study [15], while activities of Rupintrivir analogs were studied against CVA16 and EVA71 targeting cysteine protease (C_{pro}) enzyme in another study [14]. Similarly, Yongqi Yang *et.al.*, studied the action of doxepin hydrochloride against group B coxsackievirus [17]. Additionally, a study conducted on EV-A71 and CVA16 designed a peptide against the structure of EV-A71 enteroviral protein 2C [16]. Encouraging results from these studies suggested that growth of CVA16 can be inhibited by small compounds targeting their essential proteins.

Herbal medicine contains a comprehensive list of chemical compounds that have evolved over time to control pathogen infections. The medicinal properties of herbs with their inherent phyto-compound combinations and independent actions in medicine are extensively studied. *Bacopa monniera* (L.), most known as Brahmi, has shown therapeutic activity and it is largely accepted as an ayurvedic medicine for neuronal disorders and loss of memory. In addition, Bacopa is a key component of ayurvedic medicine and is used to treat cognitive impairment. There have been studies reported on the cognitive and memory performance enhancement of elderly people with the use of *B. monniera* extract [18, 19]. Bacosides-enriched standardized extract of *Bacopa* were found to functionally activate the synaptic proteins [20]. Neuronal disorders such as Alzheimer's Dementia, anterograde/retrograde amnesia, memory impairments caused by Okadaic acid, a selective inhibitor of protein phosphatase, aluminium-chloride which causes oxidative damage, autistic symptoms induced by sodium valproate, a weak blocker of sodium ion channels, and inhibitor of GABA transaminase have shown improvement by Bacopa treatment [18–20]. The extract from *B. monniera* was identified with 90 compounds in a study conducted in 2016 through

literature curation [21] and with 52 compounds in a study conducted in 2020 using literature curation and data mining with set of filter criteria [22]. Out of these, the duplicates were removed that resulted in 91 unique compounds. Interestingly, it was found that the compound Monnierasides II was the same compound as Bacopaside B, therefore only one was considered for the further study that resulted into screening of total 90 compounds. However, 71 bioactive compounds were found in PubChem, which could be suitable to use directly in the virtual screening. The rest 19 compounds were drawn separately and used in this study, as shown in Supplementary Fig. S4.

The HFMD infection has been developing as an outbreak in recent times, yet no drug has been identified against this disease. High-performance computational approaches can be used to cut down the cost and the effort required for the drug research and development process. Elucidating the compound's binding mechanisms could be beneficial in the design and development of novel anti-HFMD drugs. This study attempted to screen compounds that originated from *B. monniera* that could be used against RdRp of CVA16 using in-silico methodology. In this study, virtual screening, molecular docking, molecular dynamic (MD) simulation, and MM/GBSA binding energy estimation were performed to determine the binding efficiency of screened compounds of *B. monniera* against RdRp of CVA16 to inhibit the growth of the virus and control the HFMD infection.

Materials and Methods

Structural Collection and Preparation

The crystal structure of RdRp of coxsackievirus A16 (CVA16) was taken from polymerase elongation complex that was retrieved from the Protein Data Bank [23] (PDB ID: 5Y6Z [10]). The structure has two polypeptide chains solved at the resolution of 2.50 Å, chain A was extracted separately and used in this study after preparing in the AutoDock Vina suite [24, 25]. This structure was co-complexed with the synthetic construct of template and product RNA. The water molecules were removed from the protein structure. Later, hydrogen atoms were added, and Gasteiger charges were assigned to each atom of protein. The prepared structure was further used for virtual screening and re-docking protocol. Similarly, the compounds of *B. monniera* was prepared. The *B. monniera* compounds were collected using literature curation and searched in the PubChem database [26]. Here, 91 compounds were collected from the literatures, and it was found that the Monnierasides II was the same compound as Bacopaside B. Thus only one was considered in the virtual screening that resulted in molecular docking of 90 compounds against the target of interest. Out of these 90 compounds, the Canonical SMILES of only 71 compounds were found in the PubChem database. The 2D structure of the missing 19 compounds were drawn using "Name To Structure" tool of DataWarrior server [27] followed by their translation into SMILES using ChemSpider [28]. The Canonical SMILES were further collected and stored for each compound. They were then converted to MOL2 files and eventually prepared in PDBQT format using OpenBabel tool [29].

Virtual Screening of *B. monniera* Compounds

The binding pocket of the CVA16 RdRp was predicted with the help of CASTp web server [30]. The CASTp web server is a geometric and topological properties determining tool that predicts the binding cavity of the protein structure. In the CASTp server, the PDB ID: 5Y6Z (chain: A) was used, and the top ranked binding pocket was selected. The binding pocket residues were retrieved from the results and were stored to create the grid for virtual screening process. The 90 compounds of *B. monniera* were docked with the target protein crystal structure of the coxsackievirus A16 RNA-dependent RNA polymerase. The grid box dimensions used for virtual screening were 58 Å × 56 Å × 56 Å in x, y, and z axis, with residues covering the binding sites centered at -92.48 Å, 2.76 Å and 19.62 Å. The AutoDock Vina programme was used to screen these compounds using physical molecular docking. The parameters for the docking protocols in virtual screening were binding modes 20, exhaustiveness 100, and maximum energy difference 4 (kcal/mol). Eventually, all 90 compounds were docked in the binding pocket of the protein CVA16 RdRp and the top ten hits were then used for further analysis.

Molecular Re-docking

The top ten hits obtained from the virtual screening were re-docked at the same binding pocket of the protein CVA16 RdRp to confirm their binding. The re-docking was performed with the AutoDock Vina programme with different set of parameters compared to virtual screening. Exhaustiveness parameter allows to control the consistency of the docking experiment, this suggest similar docked poses will be obtained by increasing the exhaustiveness parameter. The second parameter, binding modes, controls the number of conformations generated for the protein-ligand complex. Here, the exhaustiveness was increased to 500 while the binding modes were at 20 and the maximum energy difference was 4 (kcal/mol). All the top ten hits were docked within the grid box, dimension of 58 Å × 56 Å × 56 Å that centered at -92.48 Å, 2.76 Å and 19.62 Å for x, y and z respectively. PoseView in the ProteinPlus server was used for predicting the interactions of the protein-ligand complexes [31–33]. After docking, the best-ranked docked pose complex for the top three ligands was chosen for a 100-ns MD simulation.

Molecular Dynamics (MD) Simulation

The molecular dynamic simulations of the protein-ligand complexes were performed with the Gromacs2021 package [34–36]. The top three complexes were used post re-docking for the 100 ns MD simulation. CHARMM force field parameters [37] were used to define topology parameters, which were then assigned to both ligands and protein. The distance electrostatic force was calculated using the Ewald Particle Mesh technique [38]. Na⁺ and Cl⁻ ions were added to create neutral system, and the TIP3P water cube model was used to solvate the system. Steepest descent (SD) method of 50,000 minimization steps were applied to the system to remove steric clashes. Further, the entire system was heated to 310 K with a timestep of 2 fs for 100 ps of simulation time (NVT). Later, the system was further equilibrated at 1 atm pressure for 1 ns of simulation time (NPT). All hydrogen bonds were limited using the SHAKE method [39], and finally the coordinate of the structure after every 10 ps were recorded over the complete 100 ns (100,000 ps) production run. The results of the MD simulation were analysed using the RMSD (root mean square deviation), or RMSF (root mean square fluctuation) tool in Gromacs to determine the

conformational stability and variation. The velocity-rescaling scheme was used as a temperature coupling scheme by GROMACS to simulate at constant temperature [40]. Temperature control was combined with pressure coupling that used Parrinello-Rahman pressure coupling giving the NPT ensemble [41]. Later, the top three hits were clustered to use the middle structure for interaction analysis. Here, g_cluster packages of GROMACS was used for clustering with a threshold RMS of 0.3 nm applying the gromos cluster method. The middle structure from the most populated cluster was selected for the catalytic residue interaction analysis.

Binding Free Energy

The gmx MMPBSA programme was used to determine the binding free energy of the protein-ligand complex using the Molecular Mechanics Generalized Born Surface Area (MM/GBSA) technique [42, 43]. The final 20 ns of the MD simulation trajectory were used to compute the ΔG_{TOTAL} binding free energy for the top three hits after re-docking of the top ten complexes. The solvation parameter (igb) was set to 5, and the system's salt concentration was set to 0.154 M. Other parameters, such as the internal and exterior dielectric constants, were set to their default values, which are 1.0 and 80.0, respectively. The MM/GBSA calculation approach is demonstrated in Eq. 1.

$$\Delta G = G_{\text{complex}} - [G_{\text{receptor}} + G_{\text{ligand}}]$$

1

Here, < > sign stands for the average of free energy for the complex, receptor, and ligand over the last 20 ns simulation trajectory frames. Energetic components used in the ΔG calculation are determined using the equations (2–6).

$$\Delta G_{\text{binding}} = \Delta H - T\Delta S$$

2

$$\Delta H = \Delta G_{\text{GAS}} + \Delta G_{\text{SOLV}}$$

3

$$\Delta G_{\text{GAS}} = \Delta E_{\text{EL}} + \Delta E_{\text{VDWAALS}}$$

4

$$\Delta G_{\text{SOLV}} = \Delta E_{\text{GB}} + \Delta E_{\text{SURF}}$$

5

$\Delta E_{\text{SURF}} = \gamma \cdot \text{SASA}$ (6) Additionally, ΔH is the enthalpy change that is made up of solvation free energy and gas-phase energy (ΔG_{GAS}) (ΔG_{SOLV}). Here, $T\Delta S$ is the free binding energy's entropy contribution. van der Waals and electrostatic terms form ΔG_{GAS} (ΔE_{EL} and $\Delta E_{\text{VDWAALS}}$, respectively). The polar solvation energy (ΔE_{GB}) and the nonpolar solvation energy (ΔE_{SURF}) form the total solvation energy

(ΔG_{SOLV}). Here, ΔE_{SURF} was computed from the product of solvent-accessible surface (SASA) area and solvent surface tension parameter (γ).

Results and Discussions

Binding pocket

The binding pocket of the coxsackievirus A16 RdRp with PDB Id: 5Y6Z was predicted using the CASTp web server and the residues were listed to determine the binding grid box for virtual screening. The largest binding pocket had an area SA (solvent-accessible surface area) of 7764.046 Å² and a volume SA (solvent-accessible surface volume) of 16672.565 Å³, which was selected for virtual screening. The binding site residues predicted for CVA16 were used to build the grid box for the virtual screening procedure. The Fig. 1(a) showed the binding pocket of the RdRp CVA16 predicted by CASTp server.

As shown in the Fig. 1(a), the binding site estimated from the CASTp server had a significantly larger area that covers the major binding pockets of the protein. This indicates that target docking explored the possible geometrical pockets of the protein and made the site-specific docking equivalent to the blind docking. RdRps are a distinct class of nucleic acid polymerases that are encoded by RNA viruses. The 3D structure of the CVA16 RdRp showed interactions with the RNA chain co-crystallized with the protein structure (5Y6Z). LigPlot + was used to visualize the interaction of RNA with the RdRp protein as illustrated in the Fig. 1(b) [44]. There were multiple H-bonds observed with the residues Ser⁴⁰¹, Lys⁴⁰⁶, His¹¹³, Asp⁴¹³, Tyr³²⁷, Ser²⁹⁵ and Asp³²⁹ of the RdRp.

Virtual screening

The PubChem database, "Name To Structure" tool of DataWarrior server and ChemSpider were collectively used to generate the 3D structures of 90 compounds found in *B. monniera* plants. These chemical compounds processed in virtual screening using the monomer of CVA16 RdRp protein. The compound names and the corresponding best binding scores calculated for the best pose are shown in Table 1. The top ten hits had the highest docked binding scores of ≤ -9.9 kcal/mole were selected for additional exhaustive re-docking. The 2D structure of the top ten hits are shown in the Fig. 2. The top ten hits selected after screening had a Bacobitacin D molecule with a maximum binding affinity score of -11 kcal/mol. Here, the compounds Bacoside A4 and Bacoside A6 had the same binding energy of -10.2 kcal/mol. However, the binding energies for the compounds Bacopasaponin A, Bacopaside IV and Bacopaside X were -10.1 kcal/mol. Moreover, the binding energies of the compounds Bacopasaponin C, Bacopasaponin G, Cucurbitacin B, and Bacoside A2 were -9.9 kcal/mol, which were selected for further analysis. These ten compounds were then re-analysed by exhaustive re-docking in the same binding site of the protein CVA16 RdRp with increased exhaustiveness parameter.

Table 1

Best binding energy of 90 compounds of *B. monnieri* predicted from the virtual screening in RdRp along with the reference compound Favipiravir.

Compounds of <i>B. monnieri</i>	Binding Energy (Kcal/mol)	Compounds of <i>B. monnieri</i>	Binding Energy (Kcal/mol)
Bacobitacin D	-11	Ursolic acid	-9
Bacoside A4	-10.2	Bacopasaponin H	-8.9
Bacoside A6	-10.2	Bacopasaponin B	-8.9
Bacopasaponin A	-10.1	Bacopaside C	-8.9
Bacopaside IV	-10.1	Lupeol	-8.8
Bacopaside X	-10.1	Bacopaside XII	-8.7
Bacopasaponin C	-9.9	Bacoside B	-8.7
Bacopasaponin G	-9.9	20 hydroxy lupeol	-8.6
Cucurbitacin B	-9.9	B daucosterin / Sitogluside	-8.6
Bacoside A2	-9.9	Bacosine	-8.5
Bacopasaponin F	-9.8	Bacoside A	-8.4
Bacopaside II	-9.8	Bacopasaponin D	-8.4
Luteolin-7-O- β -glucopyranoside	-9.7	Cucurbitacin E	-8.4
Bacopaside III	-9.7	Rosavin	-8.3
Bacopaside VIII	-9.7	Quercetin	-8.3
Asiaticoside	-9.7	Luteolin	-8
Bacopaside N1	-9.7	β -sitosterol/ Stigmast-5-en-3 β -ol	-7.9
Bacopaside XI	-9.7	Pectic acid	-7.9
Bacoside A3	-9.7	28 hydroxy lupeol	-7.7
Bacopaside IX	-9.6	Bacogenin A1	-7.7
Cucurbitacin C	-9.6	Bacogenin A2	-7.7
Bacopaside VI	-9.6	Plantainoside B	-7.7
Bacopaside VIII	-9.6	Apigenin	-7.6
Bacobitacin C	-9.6	Stigmastanol	-7.5
*Compounds are selected based on value of binding energy in their best poseRe-docking Analysis			

Compounds of <i>B. monnieri</i>	Binding Energy (Kcal/mol)	Compounds of <i>B. monnieri</i>	Binding Energy (Kcal/mol)
Bacosaponin C	-9.5	Monnierasides II/Bacopaside B	-7.5
Bacosaponin A	-9.5	Stigmasterol	-7.4
Saponin	-9.5	Bacogenin A4	-7.3
Bacosaponin D	-9.5	Monnierasides III	-7.3
Bacoside A5	-9.4	Wogonin	-7.2
Bacoside C	-9.4	Monnierasides I	-7.2
Bacopaside I	-9.4	Bacosterol	-6.6
Bacosaponin B	-9.4	Bacopaside A	-6.4
Bacobitacin A	-9.3	Loliolide	-5.9
Bacopasaponin E	-9.3	3,4Dimethoxycinnamic acid	-5.7
Cucurbitacin A	-9.3	Ascorbic acid	-5.5
Ebelin lactone	-9.3	D-Mannitol	-5.1
Bacoside A1	-9.3	Stearic acid	-5
Bacobitacin B	-9.2	Hydroxyl benzoic acid	-4.8
Bacopaside V	-9.2	Nicotine	-4.8
Betulinic acid	-9.2	Heptacosane	-4.6
Cucurbitacin D	-9.2	Dotriacontane	-4.5
Asiatic acid	-9.1	Hentriacontane	-4.4
Brahmic acid	-9.1	Octacosane	-4.2
Bacopaside N2	-9.1	Nonacosane	-4.1
Oroxindin	-9	Triacontane	-4.1
Reference (Favipiravir)	-7.6		
<i>*Compounds are selected based on value of binding energy in their best pose</i> Re-docking Analysis			

Post virtual screening, the top ten hits were re-docked to confirm their binding with the protein at increased exhaustiveness parameter. These ten compounds were docked into the same binding pocket of the protein using AutoDock Vina. Variation from the virtual screening was seen in the average binding affinity after re-docking of the same compounds. Here, the algebraic average of binding energy was

calculated for all poses generated for given compounds with their Standard Deviation (SD) and Standard Error of Mean (SEM) as listed in the Table 2. The binding affinity was best for Bacobitacin D with average binding energy of -10.73 kcal/mol, which was similar to the results obtained in the virtual screening. The average binding energy for Bacopaside IV was -9.57 kcal/mol. Bacoside A2 had an average binding energy of -9.55 kcal/mol, while Bacopaside X and Bacoside A6 had the same average binding energy of -9.47 kcal/mol. Bacopasaponin G and Bacopasaponin C had average binding energies of -9.41 kcal/mol and -9.37 kcal/mol, respectively. The average binding energy after re-docking of Bacopasaponin A was -8.91 kcal/mol, while that for Bacoside A2 and Cucurbitacin B was -8.78 kcal/mol and -8.69 kcal/mol, respectively. Here, it was observed that the top three hits had similar binding energies with higher binding affinity compared to the other seven hit compounds. Furthermore, Table 2 showed that the top three hits had similar SD (0.13–0.17) and SEM (0.03–0.04) values. However, the rest of the hit compounds showed SD of 0.2 to 0.56 while their SEM were 0.04 and 0.12. This indicates the higher consistency in binding energies for top three compounds (Bacopaside IV, Bacobitacin D, and Bacoside A2) that further confirmed their superior interaction with the target protein compared to the other hit compounds.

Additionally, a reference ligand (Favipiravir) was docked with the protein using same parameters for comparative analysis. Supplementary Table S2 listed the docking energies of 19 poses for Favipiravir when bound with the target protein. It was observed that Favipiravir had an average binding energy of -7.2 kcal/mol, which was higher than the binding energies shown by top ten hits. Comparison study showed that the top ten hits had better binding affinity with the protein CVA16 RdRp than the reference ligand (Favipiravir) which validated their selection.

Table 2

Average binding energy score of the top 10 hits calculated in re-docking with CVA16 RdRp with higher exhaustiveness.

Compounds	Average binding energy kcal/mole	Standard Deviation (SD)	Standard Error of Mean (SEM)
Bacobitacin D	-10.73	0.15	0.03
Bacopaside IV	-9.57	0.17	0.04
Bacoside A2	-9.55	0.13	0.03
Bacopaside X	-9.47	0.28	0.06
Bacoside A6	-9.47	0.56	0.12
Bacopasaponin G	-9.41	0.23	0.05
Bacopasaponin C	-9.37	0.21	0.04
Bacopasaponin A	-8.91	0.37	0.08
Bacoside A4	-8.78	0.40	0.09
Cucurbitacin B	-8.69	0.50	0.11

Protein-Ligand interaction and Physio-chemical analysis

The interactions of the top ten hits with binding site residues were predicted with the help of the ProteinPlus server using PoseView [31–33]. The protein-ligand interaction was illustrated in the 2D and 3D structural representation in Fig. 3 and Supplementary Fig. S5. The best docking score was for Bacobitacin D with – 10.73 kcal/mol, had multiple hydrogen bonds with Lys⁶¹, Tyr²³⁷, Asp³²⁹, Ser¹⁸⁴, Ser¹⁷⁹, Thr¹¹⁴, Lys¹⁵⁹, Asp²³⁸ and had a single hydrophobic contact with Ile¹⁷⁶ of chain A of CVA16 RdRp, as shown in Fig. 3(a). Bacopaside IV had multiple hydrophobic contacts and H-bond interactions with Tyr¹⁵⁷, Ile¹⁷⁶, Thr¹¹⁴, and Gly²⁹³, Ser²⁹², Ser²⁹⁵, respectively, as shown in Fig. 3(b). Bacoside A2 had minimum interactions of only three hydrogen bonds with the residues His¹⁹⁹, Glu¹⁰⁸, and Tyr¹⁰⁶ with the chain A of the RdRp protein as illustrated in Fig. 3(c). Bacopaside X had multiple interactions with the residues Ser¹¹⁵, Asp¹¹¹, Lys¹²⁷, Ser¹⁸⁴, and Gly²⁹⁰ forming hydrogen bonds and Thr¹¹⁴, Tyr¹⁵⁷, and Ser¹¹⁵ were involved in hydrophobic contacts as shown in Fig. 3(d). Here, Bacoside A6 had multiple hydrogen bonds with residues Lys⁶¹, Leu¹⁵⁷, Gly²⁹⁰, Ser¹⁷⁹, Ser¹⁸⁴, Asn²⁹⁸, Asp²³⁸, Thr²⁹⁴, Ala²³⁹ and no hydrophobic contacts as depicted in the Fig. 3(e). Figure 3(f) showed that the compound, Bacopasaponin G had multiple hydrogen bonds with residues Ser¹⁸⁴, Lys¹²⁷, Arg¹⁸⁸, Ser²⁹², Gly²⁹³ and a single hydrophobic contact with Thr²⁹⁴. It was observed in the Fig. 3(g) that Bacopasaponin C had only three

hydrogen bonds with residues Glu¹⁰⁸, Cys²¹² and Ser¹⁸⁴. As illustrated in Fig. 3(h), Bacopasaponin A had multiple hydrogen bonds with residues Ala¹⁰⁹, Thr²⁹⁴, Asn²⁹⁸, Asp²³⁸ and Arg¹⁸⁸, and three hydrophobic contacts with His¹⁹⁹, Thr²⁹⁴ and Gly²⁹⁰ residues. Bacoside A4 had multiple hydrogen bonds with the residues Gly²⁹⁰, Thr¹¹⁴, Ser¹⁷⁹, Ser¹⁸⁴ and His¹⁹⁹ as shown in Fig. 3(i). It was observed in Fig. 3(j) that Cucurbitacin B had only two hydrogen bonds with residues Ser¹⁸⁴ and Lys¹²⁷.

Noticeably, the protein residues (Ser²⁹⁵ and Asp³²⁹) that formed hydrogen bonds with the natural substrate RNA as shown in Fig. 1(b), were also detected in protein-ligand interactions for Bacopaside IV and Bacobitacin D. It was also observed that these two ligands, Bacopaside IV and Bacobitacin D, were part of the top three hits after the re-docking analysis.

The 3D conformations of the top ranked ten compounds in the binding pocket of RdRp are shown in Supplementary Fig. S5. It showed the top scoring pose computed after the re-docking of the compounds to the binding site of the CVA16 RdRp. Here, in the Supplementary Fig. S5, it is shown that these ten compounds were bound in the same binding site at different cavity region. Here, the top three hits ((1) Bacopaside IV, (2) Bacobitacin D, and (3) Bacoside A2) based on re-docking analysis were selected for further MD simulation and MM/GBSA analysis.

Furthermore, the ADMET analysis of the top three hit compounds was performed using the SwissADME server [45] and the ProTox-II - Prediction Of Toxicity Of Chemicals server [46]. The Supplementary Table S3 show the ADME properties and the Toxicity class report. The compounds Bacobitacin D and Bacoside A2 showed moderately soluble characteristic while Bacopaside IV showed poor solubility. Here, the GI Absorption was lower while they bind with P-glycoprotein that confirms the high distribution and uptake of these compounds. These compounds predicted as CYP1A2 inhibitors that allows its high plasma concentration. These three hits also showed 0 alerts in PAINS (pan assay interference compounds) that suggest their promising outcomes in in-vitro and in-vivo studies. The Toxicity analysis of the top three showed that the compounds belong to toxicity class 4 and 3 which is acceptable according to Hazard Communication Standard (HCS). This toxicity class is based on GHS (Globally Harmonized System of Classification and Labeling of Chemicals). These top three hits showed decent ADMET properties to be selected for further experimental studies.

Protein-Ligand Dynamics

The molecular dynamics (MD) simulation was used to estimate the strength of the interaction between the protein and ligand molecule. The root mean square deviation (RMSD) and root mean square fluctuation (RMSF) for the MD trajectory for protein-ligand complexes provide important information on the system's flexibility. The top three compounds: (1) Bacopaside IV, (2) Bacobitacin D, and (3) Bacoside A2 had the highest binding scores in re-docking and were therefore selected for the MD simulation. The binding free energies of these top three hit complexes were calculated to evaluate the binding affinities of the ligands to the protein molecule in dynamics mode.

Root Mean Square Deviation (RMSD)

The stability of the top three hits upon binding to the protein were examined by evaluating the root mean square deviation (RMSD). RMSD measures the conformational change that proteins and ligands undergo during and after binding. Figure 4(a) shows the RMSD of the protein Cα atoms compared to the initial equilibrated structure. The protein conformation upon binding with the top hits - Bacopaside IV, Bacobitacin D, and Bacoside A2 were highly stable and showed the RMSD ranging from 0.1 nm to 0.25 nm, during the 100 ns MD simulation. The protein did not show significant deviation from its equilibrated state and exhibited a stagnant RMSD throughout the simulation. Here, the CVA16 RdRp-Bacopaside IV complex showed RMSD of 0.14 nm during the first 10 ns of the simulation and increased to 0.2 for the next 10 ns simulation. However, later, till 80 ns of the MD simulation, the RMSD of the protein upon binding to Bacopaside IV showed the RMSD value of 0.15 nm. This showed stable and consistent conformation of the protein upon binding to the ligand. The RMSD of the protein upon binding to the compound Bacobitacin D showed most fluctuation where it jumped from 0.13 nm to 0.2 nm during the first 25 ns simulation, while it again jumped to 0.27 nm during the last 20 ns simulation. In contrast, RMSD of the protein fluctuated between 0.15 nm to 0.17 nm throughout the simulation for the CVA16 RdRp- Bacoside A2 complex. Overall RMSD of the protein for all the top three hits were stable with magnitude lower than 0.3 nm.

Figure 4(b) shows the RMSD for the top three compounds bound to the protein. Here, the structures were fitted and aligned using the protein as a reference molecule, and the RMSD of the ligand compounds was calculated. The RMSD of the ligand Bacopaside IV showed the most stable conformation, with a value of 0.3 nm for the first 45 ns simulation and 0.5 nm for the last 55 ns time frame. The overall RMSD of the ligand Bacopaside IV showed consistency throughout the simulation. The ligand Bacobitacin D, after binding to the protein CVA16 RdRp, showed an increase in RMSD from 0.3 nm to 0.75 nm for the first 25 ns of and settled consistently for the rest of the 75 ns of MD simulation. It was observed that the Bacoside A2 showed the least stable conformation, where it fluctuated to a large extent compared to the other two hits. Bacoside A2 showed an increase of RMSD from 0.3 nm to 1.25 nm for the first 25 ns of the MD simulation. The overall RMSD pattern of the ligand showed that it fluctuated between the range of 0.9 nm and 1.1 nm for the rest of the simulation. Both the compounds Bacopaside IV and Bacobitacin D showed stable and consistent RMSDs of 0.5 nm and 0.75 nm for the last 20 ns of the MD simulation, validating their binding to the protein CVA16 RdRp. The overall RMSD suggested that top three hits were strongly bound to CVA16 RdRp.

Additionally, the RMSD of the reference ligand (Favipiravir) was calculated over the 100 ns MD simulation for comparative study. The Supplementary Fig. S6 showed the RMSD of the proteins and the reference ligand (Favipiravir). The protein upon binding to Favipiravir showed stable RMSD of < 0.25 nm consistently during the 100 ns MD simulation. However, the ligand RMSD showed a different trend with high fluctuations in the first 50 ns of simulation time and then stabilized in the last 50 ns of the simulation. The RMSD spiked from 0.5 nm to 2.75 nm in the first 50 ns. Later, it dropped down to 1.5 nm and remained in this range for the rest of the 50 ns simulation. The hit compounds Bacobitacin D and

Bacopaside IV had shown the higher stability in their binding poses. These compounds reached their corresponding stable state after 30 ns and 50 ns, respectively. Moreover, the magnitude of RMSD for these compounds in the stable state (RMSD: 0.5 nm, 0.75 nm) were also lower than the reference compound.

Root Mean Square Fluctuation (RMSF)

RMSF values for the protein and ligand molecules were computed to ascertain the unique variations of each residue/molecule in the bound state. Figure 5(a) shows the RMSF of the CVA16 RdRp protein structure following binding of the top three hit compounds. In the complex state of Bacopaside IV, the RMSF for the protein had two residues with RMSF > 0.3 nm. However, in the Bacobitacin D complex, there were three residues with RMSF > 0.3 nm RMSF. In contrast to these two hits, Bacoside A2 had six residues with RMSF > 0.3 nm. Overall, only few peaks were observed with RMSF > 0.3 nm during the 100 ns MD simulation for the top three hits with a similar trend of variations.

Figure 5(b) shows the RMSF calculated for the top three ligands (averaged for all atoms) for the 100 ns MD simulation. Here, the compounds Bacobitacin D and Bacoside A2 showed similar trends, where the RMSF was 0.17 nm and 0.18 nm, respectively. However, compared to the other two hit compounds, Bacopaside IV showed a lower RMSF of 0.10 nm. It was observed that Bacopaside IV displayed the minimum fluctuations in this study, which suggested it as a potential compound for stable binding with the CVA16 RdRp.

Hydrogen Bonds

Based on intramolecular hydrogen bonding, it is possible to predict the stability of the complex and the binding of the ligand to the protein. The top three hits, as shown in Fig. 6, had a total of 1 to 13 hydrogen bonds over the 100 ns MD simulation. In 78.7% of the frames, the Bacopaside IV showed 1–3 hydrogen bonds with the least fluctuations and 5–6 hydrogen bonds with high fluctuations in 0.24% of the frames (Fig. 6(a)). However, the compound Bacobitacin D showed the highest number of hydrogen bonds compared to the other top two hits. It was observed that the compound Bacobitacin D showed 3–7 hydrogen bonds with minimum fluctuation in 78.7% of the frames and 8–10 hydrogen bonds with maximum fluctuations in 12.9% of the frames (Fig. 6(b)). Here, Bacoside A2 showed 4–6 hydrogen bonds with high fluctuations in 0.93% of the frames, while it displayed 1–3 hydrogen bonds with the least fluctuations in 74.4% of the frames (Fig. 6(c)). As shown in Fig. 6, the number of hydrogen bonds in the protein-ligand complex for the Bacobitacin D compound was higher in comparison, indicating the possibility of the compound stronger bonding to the protein CVA16 RdRp.

MM/GBSA Analysis

The binding free energy for the top three hits was determined using the gmx MMPBSA test tool [42, 43]. The gmx_MMPBSA_ana visualisation tools were used to plot the graphs and analyse the energy components of the MM/GBSA. The bar plots for the energy values for the individual (ΔE_{EL} , $\Delta E_{VDWAALS}$) and summed components (ΔG_{GAS} , ΔG_{SOLV} , and ΔG_{TOTAL}) are shown in Fig. 7. Figure 7 also shows the ΔG_{GAS} and ΔG_{SOLV} for each of the three complexes, which is consisted of ΔE_{EL} , $\Delta E_{VDWAALS}$, ΔE_{GB} , and ΔE_{SURF} . It was observed that the compound, Bacopaside IV showed the lowest ΔG_{TOTAL} binding free energy with a value of -23.70 kcal/mol (Fig. 7(a)). However, the compounds Bacobitacin D and Bacoside A2 showed -19.14 kcal/mol and -14.37 kcal/mol ΔG_{TOTAL} binding free energy (Fig. 7(b, c)). ΔG_{TOTAL} is an essential metric for assessing the ligands binding characteristics to proteins as it computes the total binding energy changes as a result of complex formation. The ΔG_{TOTAL} value in this case demonstrated preferential binding by Bacopaside IV compared to compounds Bacobitacin D and Bacoside A2. Furthermore, the binding affinity of reference ligand (Favipiravir) was evaluated using MM/GBSA analysis as shown in the Supplementary Fig. S7. Here, the binding free energy was calculated for the last 20 ns and average score is shown in Supplementary Fig. S7. The ΔG_{TOTAL} binding free energy of Favipiravir showed -16.57 kcal/mol average score over last 20 ns. Moreover, compared to Favipiravir, the two hit compounds (Bacopaside IV and Bacobitacin D) showed better ΔG_{TOTAL} binding free energy while Bacoside A2 has highly comparative binding free energy with the reference.

Additionally, the variation of total binding free energy (ΔG_{TOTAL}) with a running average (red line) was plotted in Supplementary Fig. S8. The 200 frames taken from the last 20 ns of the MD simulation and showed with the ΔG_{TOTAL} in Supplementary Fig. S8, where the dashed red line shows the running average of the ΔG_{TOTAL} . It was observed that the ΔG_{TOTAL} for the compound Bacopaside IV sloped down from 75 to 125 frames in the range of -22 kcal/mol to -24 kcal/mol and then remained stable for the rest of the frames (Supplementary Fig. S8(a)). The line graph for the compound Bacobitacin D showed a dip for the 50 to 125 frames from -17 kcal/mol to -21 kcal/mol, while it again increased to -19 kcal/mol for the 175 to 200 frames (Supplementary Fig. S8(b)). There was a minor dip in the ΔG_{TOTAL} for the compound Bacoside A2 where the line graph reached -18 kcal/mol but later increased to -11 kcal/mol in the 140 frames before slopping down again (Supplementary Fig. S8(c)). Overall, the top three ligands demonstrated high binding free energy (ΔG_{TOTAL}) trends in the last 20 ns of the simulation.

Catalytic Residues

Earlier studies detected that the binding site of the protein CVA16 RdRp contains catalytic residues. The residues Asp²³⁸ and Ser²⁸⁹ initiate the conformational switch during NTP binding and catalysis. Here, both residues form a defined hydrogen bonding network involving side chain rotamer changes of these conserved residues. Asp²³⁸ is a NTP ribose hydroxyls, a conserved motif A while Ser²⁸⁹ is a conserved motif B serine [10]. Previous studies documented that the other conserved aspartic acid, Asp²³³ and a conserved motif C aspartic acid, Asp³²⁹ move toward the NTP substrate after the rearrangement around the ribose hydroxyls [47]. These two residues are universally conserved for the nucleic acid polymerases,

and both coordinate with the catalytic divalent metal ions (naturally being magnesium ions). These four catalytic residues (Asp²³⁸, Ser²⁸⁹, Asp²³³, and Asp³²⁹) found in the binding pocket of CVA16 RdRp as shown in Fig. 8(a). Interestingly, one of the top three hit compounds, Bacobitacin D, showed hydrogen bonding with the catalytic residues Asp²³⁸ and Asp³²⁹ after the re-docking analysis, as shown in Fig. 3(a). Bacobitacin D was found to have significant binding with CVA16 RdRp in this study.

Reference Ligand Interactions

Additionally, for validation, the binding of the top three hits was compared with the known ligands of the homologous proteins. The protein CVA16 RdRp (PDB ID: 5Y6Z) was searched for homologous proteins with co-crystallized inhibitors that had 90% sequence similarity. Here, five complexes (PDB IDs: 6KWR, 5F8I, 7W9S, 3N6N and 3N6M) were found similar to CVA16 RdRp. These protein structures were selected with their ligands as references to compare the top three hits' binding. The 3D structural representation of the reference ligands in the binding pocket of the CVA16 RdRp is shown in Fig. 8(b). It was observed that all the reference ligands were bound at the same binding location of the binding pocket.

The interaction between the protein and ligand molecule was analysed using these homologous PDB structures, as shown in Table 3. The reference ligands formed multiple hydrogen bonds and no hydrophobic contacts. The homologous proteins of CVA16 RdRp found in the PDB database belonged to the enterovirus (EV71) RNA-dependent RNA polymerase (RdRp) and genome polyprotein bound to DCT (2',3'-Dideoxycytidine 5'-Triphosphate), CTP (2',3'-Dideoxycytidine 5'-Triphosphate), GTP (Guanosine-5'-triphosphate), or BUP (5-bromouridine 5'-(tetrahydrogen) triphosphate), as mentioned in Table 3. The compound DCT showed two hydrogen bonds with Arg¹⁷⁴, Gly²³⁶ and the CTP molecule formed hydrogen bonds with the residues Arg¹⁶³, Tyr²³⁷, Asp²³⁸, Ser²⁸⁹, Asn²⁹⁸, Asp³²⁹, respectively. Here, CTP molecule in another complex (PDB ID:7W9S) formed hydrogen bonds with Arg¹⁷⁴, Gly²³⁶, Tyr²³⁷, Asp²³⁸, Ser²⁸⁹, Asn²⁹⁸, Lys³⁶⁰ and BUO compound formed hydrogen bonds with residues Arg¹⁶³, Ser²³⁵, Gly²³⁶, Asp²³⁸ while, from PDB entry 3N6M, GPT formed hydrogen bonds with Arg¹⁷⁴, Leu¹⁷⁵, Arg¹⁶³, Ser²³⁵, Gly²³⁶, Asp²³⁸. Interestingly, four complexes with PDB entries 5F8I, 7W9S, 3N6N and 3N6M showed hydrogen bond formation with the catalytic residue Asp²³⁸. Additionally, 5F8I and 7W9S showed hydrogen bond formation with catalytic residue Ser²⁸⁹ while 7W9S also had a hydrogen bond with Asp³²⁹ that is the critical catalytic site.

Hits Interaction

The top three hits were selected for further interaction analysis where clustering was performed the most populated conformation generated during the MD simulation. Here, the middle structure was selected from the most populated cluster for the interaction detailing. The 2D and 3D forms of protein-ligand interaction were shown in Fig. 9. Single hydrogen bond interaction with the residue Glu¹⁰⁸ and two hydrophobic contacts with residues Ile¹⁷⁶ and Tyr¹⁵⁷ were detected for the protein-ligand complex of Bacopaside IV (Fig. 9(b)). It was observed that the compound Bacobitacin D showed multiple hydrogen

bonds with the residues Tyr³²⁷, Asp²³⁸, Ser²⁸⁹ and Asp¹¹¹ (Fig. 9(d)). However, the minimum number of interactions was seen in the protein-ligand complex of the compound Bacoside A2 with one hydrogen bond with the residue Arg³⁷⁷ (Fig. 9(f)). It was noticed that Bacobitacin D formed hydrogen bond interaction with two of the catalytic residues Asp²³⁸ and Ser²⁸⁹. The Fig. 9(c) showed that the compound Bacobitacin D was bound at the same binding pocket compared to the reference ligands, as shown in the Fig. 8(b).

The Supplementary Fig. S9 shows the 2D and 3D interaction plot of the reference ligand (Favipiravir) with the protein during the 0 ns, 60 ns and 80 ns MD simulation. Here, two poses of the most stable state (60 ns and 80 ns) were evaluated for interaction analysis. Later, the initial pose (0 ns) was also considered for evaluating the docked conformation. The 2D interaction plot showed that Favipiravir had multiple hydrogen bond interactions with residues Gly²⁹³, Ser²⁹², Arg¹⁸⁸, Ser¹⁸⁴, Ser¹⁷⁹, Lys¹²⁷, and Asp¹¹¹ at the 0 ns simulation (Supplementary Fig. S9(b)). It was observed that Favipiravir showed hydrogen bonds with Ala¹¹⁶, Ser¹¹⁵, Val¹²⁵ and Lys²⁴ at 60 ns simulation (Supplementary Fig. S9(d)), however, at the 80 ns simulation except Lys²⁴, the rest of the hydrogen bonds were same (Supplementary Fig. S9(f)). The 3D interaction showed that (Favipiravir) changed its conformation from the initial state (0 ns simulation) to the 60 ns and 80 ns simulation as shown in the Supplementary Fig. S9(a, c, e). Comparing the top three hits with the reference ligand (Favipiravir) interaction showed that only the compound Bacobitacin D had the same binding residue Asp¹¹¹ formed hydrogen bond with the protein. Moreover, this residue was only shown in the initial binding complex of reference ligand (0 ns). Later, in the most stable phase there were no overlapping of hydrogen bond forming residues between reference compound and top hit compounds.

Minimum Distance Analysis

Furthermore, the top three hits were analysed for the minimum distance from the catalytic residues (Asp²³³, Asp²³⁸, Ser²⁸⁹, and Asp³²⁹) using mindist programme of GROMACS packages for the last 20 ns simulation. The minimum distance from the catalytic sites was plotted in the graph, as shown in Fig. 10. The minimum distance of Bacoside A2 was highest for all the four catalytic residues with a distance ranging from 0.3 nm to 1.7 nm during the last 20 ns simulation (Fig. 10(a, b, c, d)). It was observed that Bacoside A2 showed major fluctuation in distance from the catalytic residues. Here, the compound Bacopaside IV showed high minimum distance for two catalytic residues Asp²³³ and Asp²³⁸ with distance of 1.5 nm and 0.9 nm, respectively (Fig. 10(a, b)). However, the distance was 0.3 nm for both the catalytic residues Ser²⁸⁹, and Asp³²⁹ (Fig. 10(c, d)) in the Bacopaside IV-protein complex. The minimum distance for Bacobitacin D showed acceptable interacting distance ranging from 0.2 nm to 0.3 nm for three of the catalytic residues Asp²³⁸, Ser²⁸⁹, and Asp³²⁹ (Fig. 10(b, c and d)). Further it also showed minimum distance of 0.5 nm to 0.9 nm with the catalytic residue Asp²³³ (Fig. 10(a)). The compounds Bacobitacin D showed stable and consistent minimum distance with the catalytic residues indicating probable strong inhibitory effect.

Additionally, for comparative study, the reference ligand (Favipiravir) was also used to calculate the minimum distance from the catalytic residues (Asp²³³, Asp²³⁸, Ser²⁸⁹, and Asp³²⁹). The Supplementary Fig. S10 shows the minimum distance plot of Favipiravir with the catalytic residues over the 100 ns simulation. It was observed that the minimum distance was relatively higher (0.8–2.7 nm) for Favipiravir at 0 ns, 60 ns and 80 ns simulation, however it only showed a comparative minimum distance of 0.5 nm with residue Ser²⁸⁹ for initial state (0 ns simulation).

Conclusion

The hand, foot, and mouth disease (HFMD) also known as ‘tomato flu’ is caused by RNA single-stranded virus, Coxsackievirus A16 (CVA16). The most essential protein involved in the replication of RNA viruses is the RNA-dependent RNA polymerase (RdRp). All members of the RNA virus superfamilies, with the exception of *Retroviridae*, shared highly conserved enzyme RdRp. It is the main catalytic enzyme of the membrane-associated replication complex that replicates the genome of CVA16. RdRp of CVA16 is a potential drug target for the HFMD natural inhibiting compound. It consists of four major catalytic residues Asp²³³, Asp²³⁸, Ser²⁸⁹, and Asp³²⁹ at the binding site of the RdRp of CVA16. In this in silico study, 90 compounds from *B. monniera* with therapeutic properties were virtually screened against RdRp of CVA16. The top ten hits based on binding energy score ≤ -9.9 kcal/mol, (a) Bacobitacin D, (b) Bacoside A4, (c) Bacoside A6, (d) Bacopasaponin A, (e) Bacopaside IV, (f) Bacopaside X, (g) Bacopasaponin C, (h) Bacopasaponin G, (i) Cucurbitacin B, (j) Bacoside A2 were selected for exhaustive re-docking. Interaction analysis showed that the compound Bacobitacin D had the maximum hydrogen bonds with RdRp of CVA16. Interestingly, Bacobitacin D also showed hydrogen bonding with the catalytic residues Asp²³⁸ and Asp³²⁹ designating it as top hit candidate against RdRp. Later, top three hits among these compounds were selected for MD simulation and MM/GBSA analysis. Other studies indicated the acceptable activities for molnupiravir, rupintrinvir, doxepin hydrochloride against coxsackievirus and human enterovirus targeting RdRp protein. These compounds showed minimal structural similarity with the top three hit compounds (explained in Supplementary Fig. S11 and Table S4). This indicated the identification of novel chemical scaffold against CVA16 RdRp. The top three hit compounds, Bacopaside IV, Bacobitacin D, Bacoside A2 showed stable protein RMSD during the 100 ns MD simulation. Among these top three hits, Bacobitacin D showed the most consistent and stable RMSD while Bacopaside IV showed lowest RMSD during the last 50 ns simulation. Further, the MM/GBSA results showed Bacopaside IV had the lowest ΔG_{TOTAL} binding free energy while the other two compounds (Bacobitacin D and Bacoside A2) showed acceptable ΔG_{TOTAL} energies indicating strong affinity. Later, the middle structure from the most populated cluster was analysed for protein-ligand interaction, where it was observed that the compound Bacobitacin D showed hydrogen bonds with the catalytic site residues Asp²³⁸ and Ser²⁸⁹ of RdRp of CVA16. Comparative study with the reference ligand, Favipiravir showed that only the compound Bacobitacin D had the same binding residue Asp¹¹¹ having hydrogen bond, which also had interaction of Favipiravir with the protein. This computational study suggested Bacobitacin D as the most potential inhibitor for treatment of HFMD caused by targeting RdRp of

coxsackievirus A16. The inhibitory action of these hit compounds must be further established through additional experimental methods.

Declarations

Competing Interests: The authors declare that there is no competing interest either financially or non-financially.

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Authors Contribution: Avinash Mishra designed the study and supervised the complete execution and writing process. Parveen Punia performed the initial screening of the compounds. Arun Prajapati has performed the simulations, Priyasha Maitra prepared the figures, tables and assisted in the analysis of the simulation results. All author contributed in writing and reviewing the manuscript.

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Table

Table 3 is available in the Supplementary Files section.

Figures

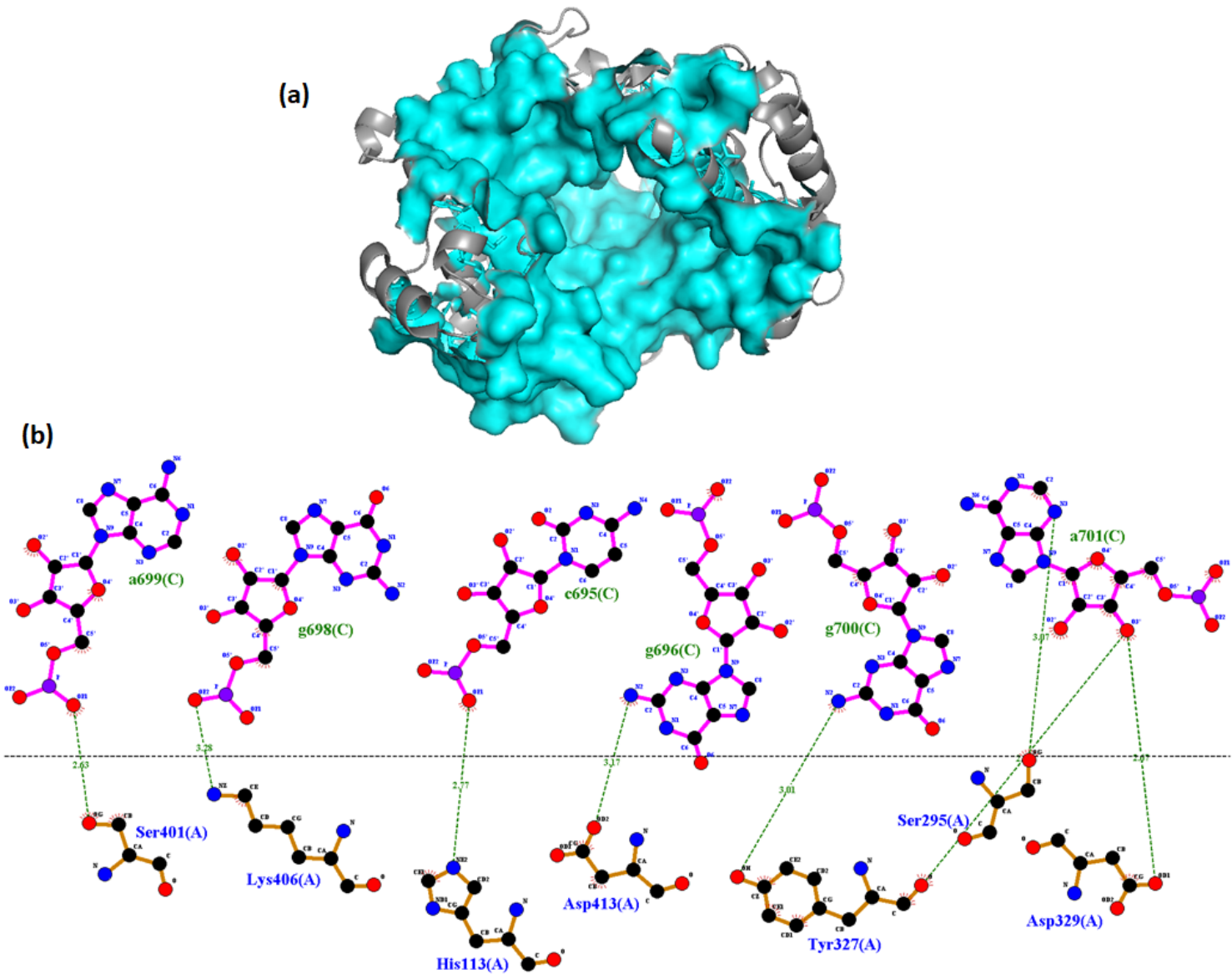


Figure 1

(a) Top rank binding pocket of 5Y6Z (CVA16 RdRp) predicted by CASTp web server, (b) The H-bond interactions between the RNA molecule and the residues of CVA16 RdRp protein.

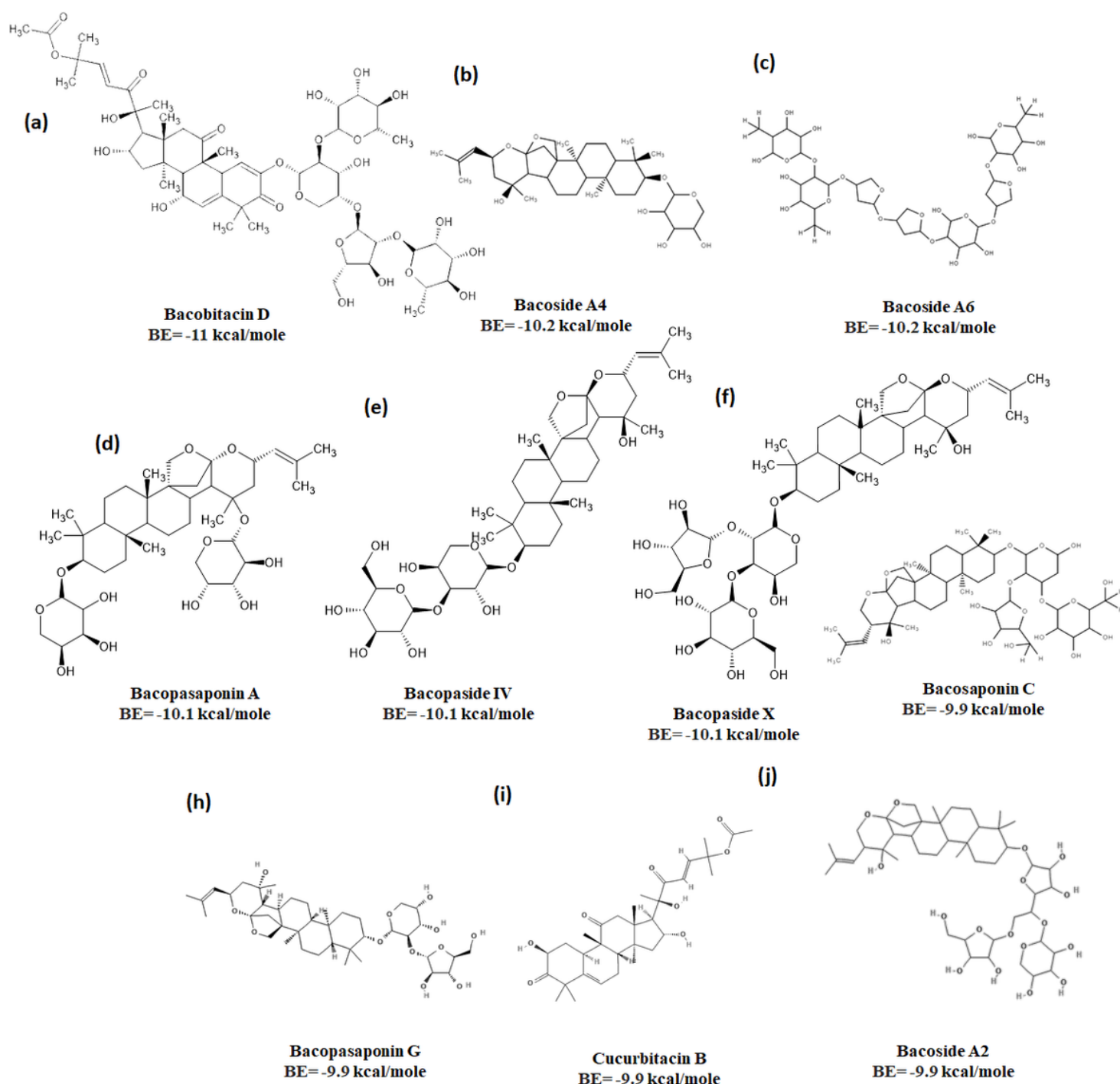


Figure 2

2D Structure of the top ten hits of *B. monnieri*, (a) Bacobitacin D, (b) Bacoside A4, (c) Bacoside A6, (d) Bacopasaponin A, (e) Bacopaside IV, (f) Bacopaside X, (g) Bacopasaponin C, (h) Bacopasaponin G, (i) Cucurbitacin B, (j) Bacoside A2

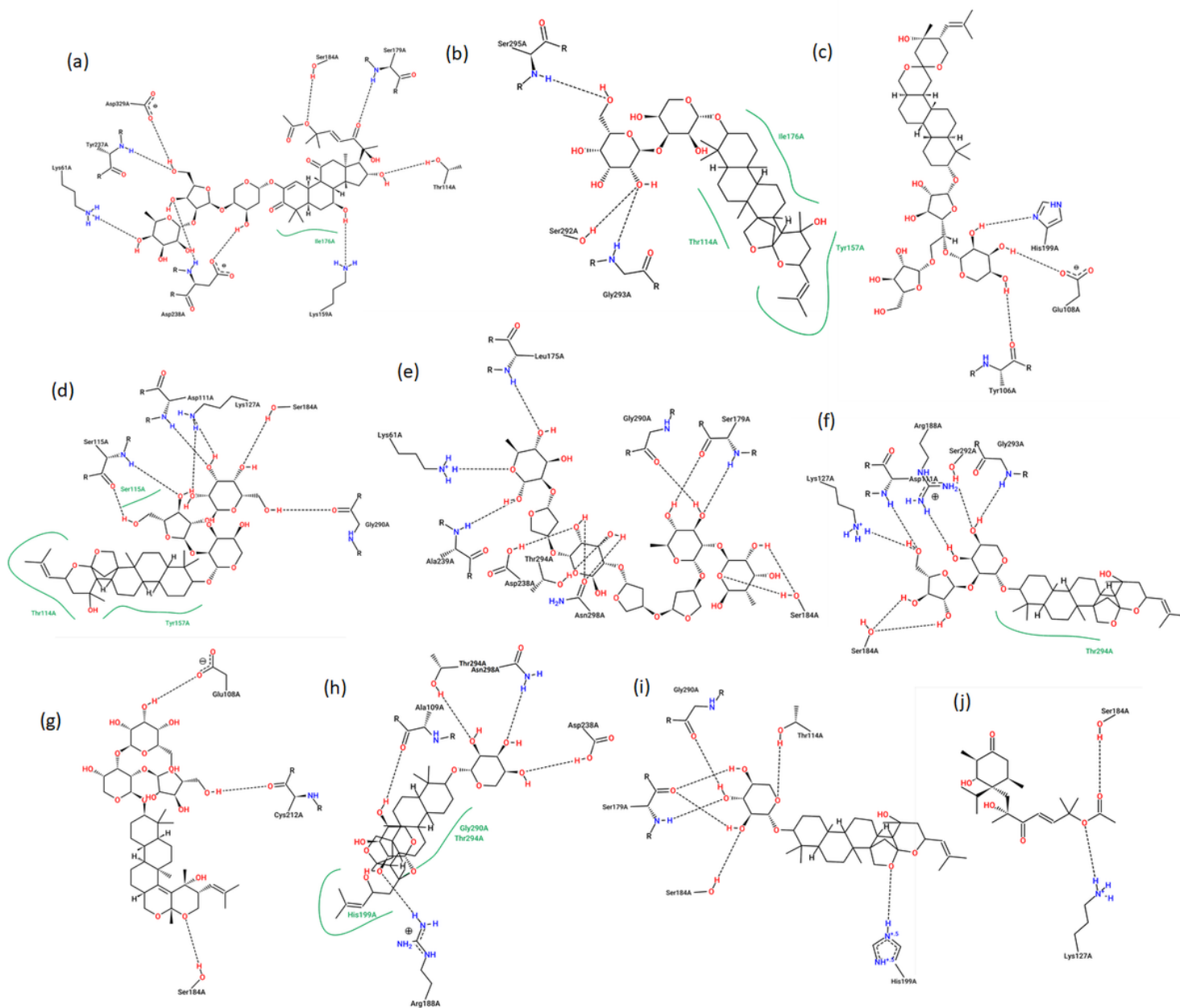


Figure 3

2D Interactions plot of the protein-ligand complexes for (a) Bacobitacin D, (b) Bacopaside IV, (c) Bacoside A2, (d) Bacopaside X, (e) Bacoside A6, (f) Bacopasaponin G, (g) Bacopasaponin C, (h) Bacopasaponin A, (i) Bacoside A4, and (j) Cucurbitacin B.

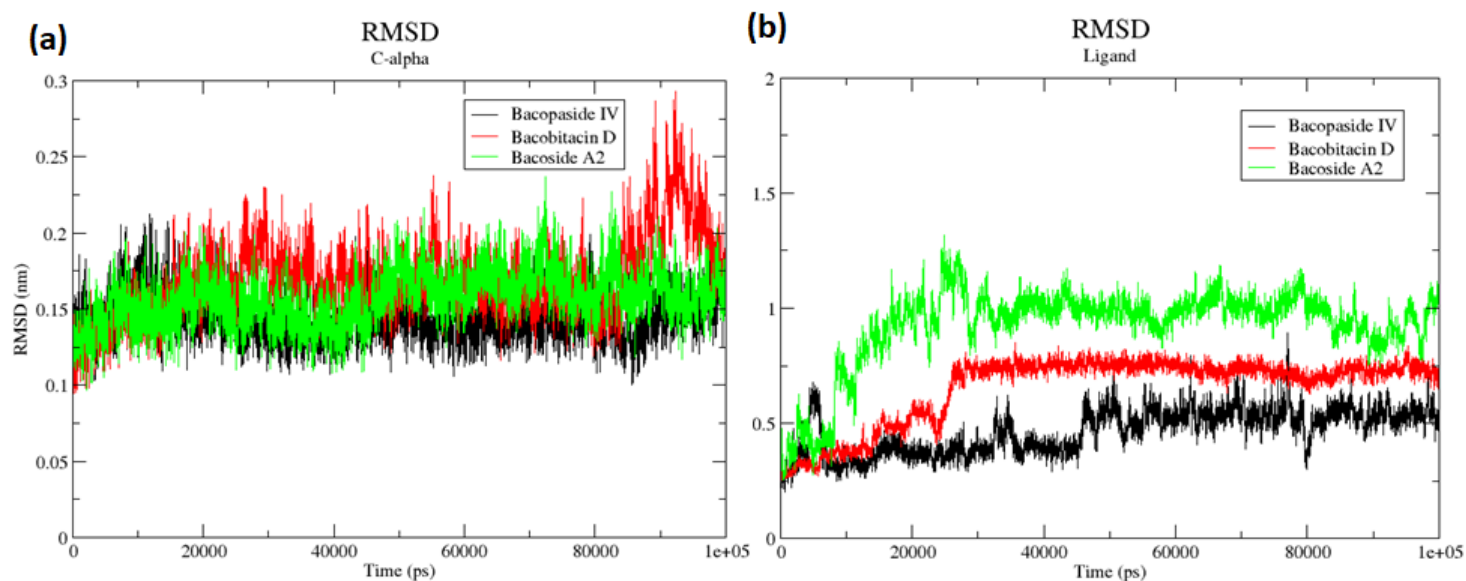


Figure 4

(a) The RMSD of protein Cα atoms of CVA16 RdRp for the top three hit ligands, Bacopaside IV, Bacobitacin D, and Bacoside A2 calculated over 100 ns MD simulation trajectories. (b). The RMSD of hit compounds after binding with the CVA16 RdRp, for the top three hits Bacopaside IV, Bacobitacin D, and Bacoside A2 calculated over 100 ns MD simulation trajectories using protein as aligning molecule.

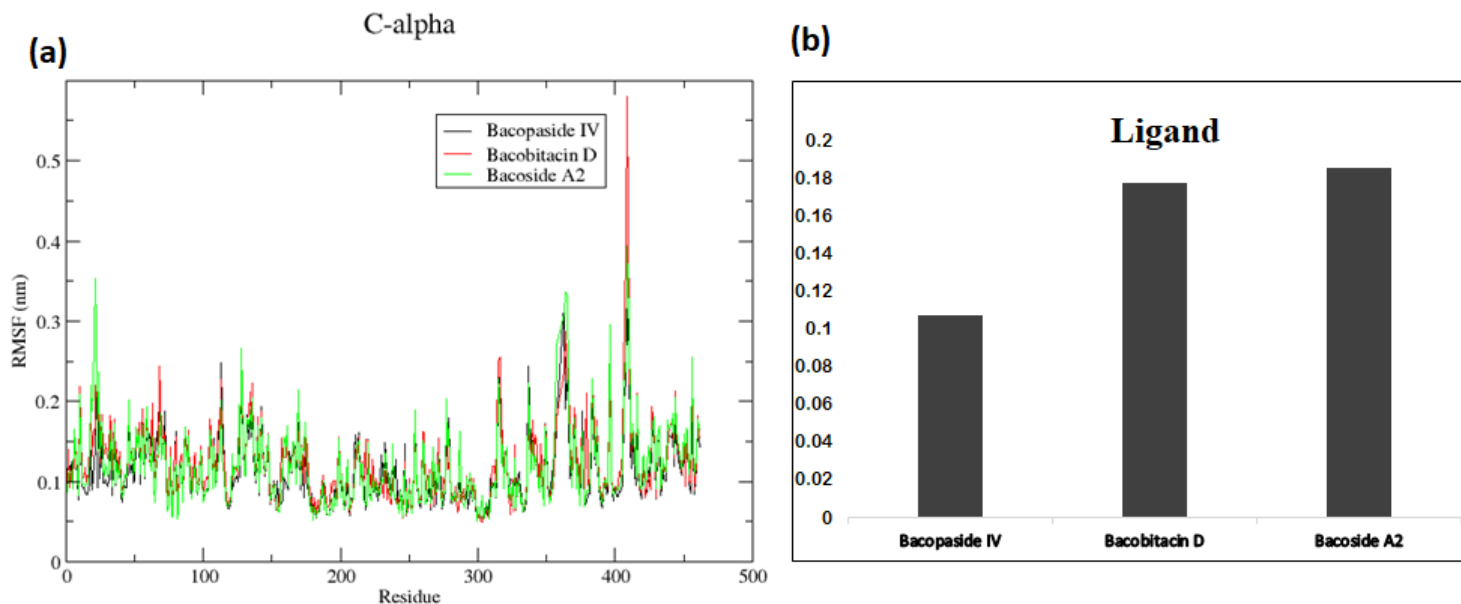


Figure 5

(a) The RMSF of CVA16 RdRp Cα atoms bound with the ligands, Bacopaside IV, Bacobitacin D, and Bacoside A2 calculated over 100 ns MD simulation trajectories. (b) The RMSF of hit molecules after binding with the CVA16 RdRp calculated over 100 ns MD simulation time frame for Bacopaside IV, Bacobitacin D, and Bacoside A2.

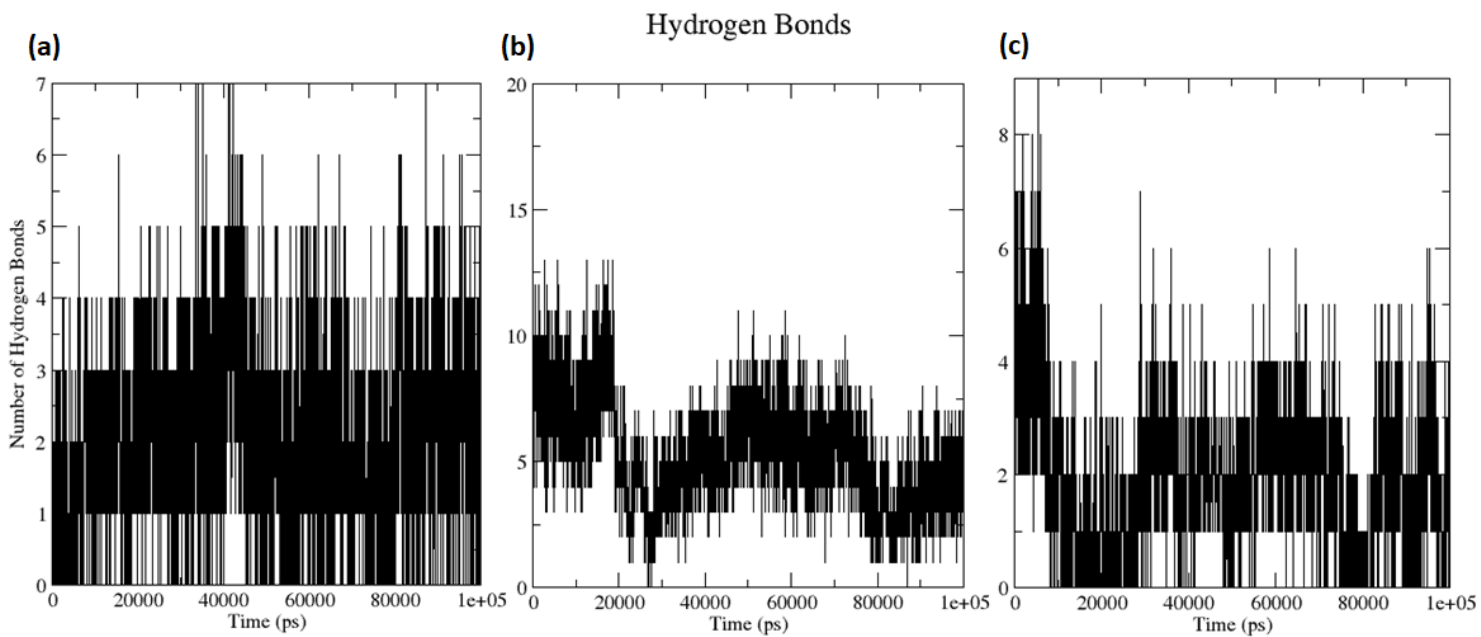


Figure 6

Hydrogen bond counts over the 100 ns MD simulation trajectories for (a) Bacopaside IV, (b) Bacobitacin D, (c) Bacoside A2

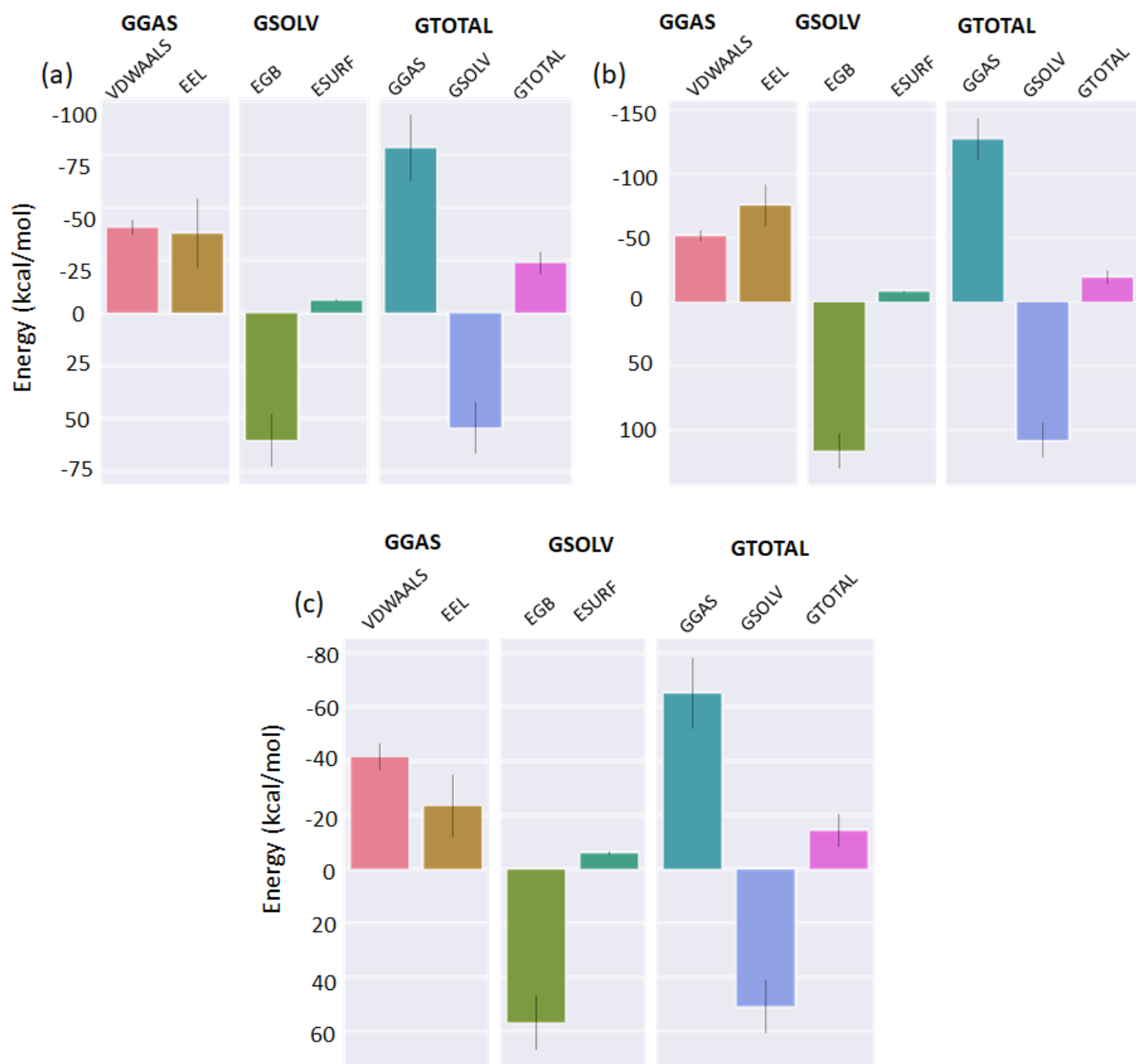


Figure 7

Binding free energy components computed based on MM/GBSA analysis for the top three hits (a) Bacopaside IV, (b) Bacobitacin D, (c) Bacoside A2

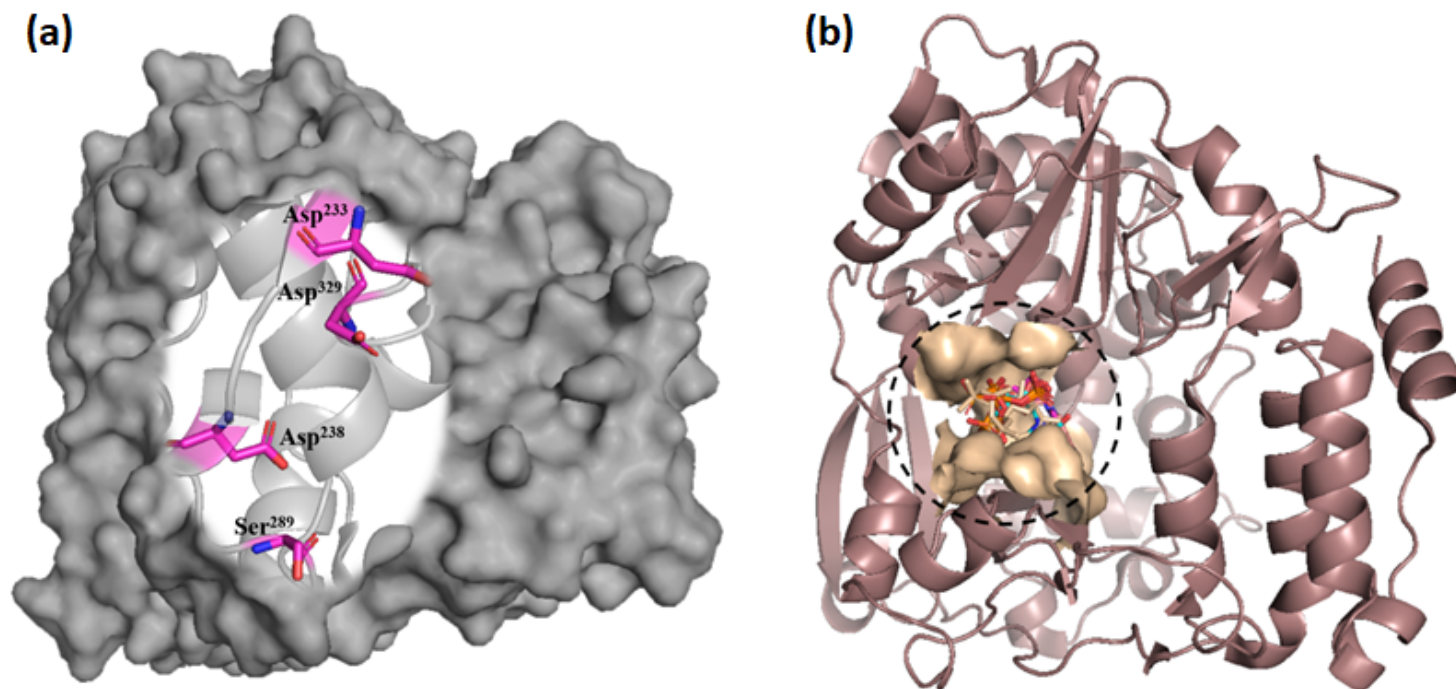


Figure 8

(a) The Catalytic residues Asp²³³, Asp²³⁸, Ser²⁸⁹ and Asp³²⁹ of CVA16 RdRp. (b). The 3D Structure of the binding of reference ligands in the binding pocket of the protein, CVA16 RdRp.

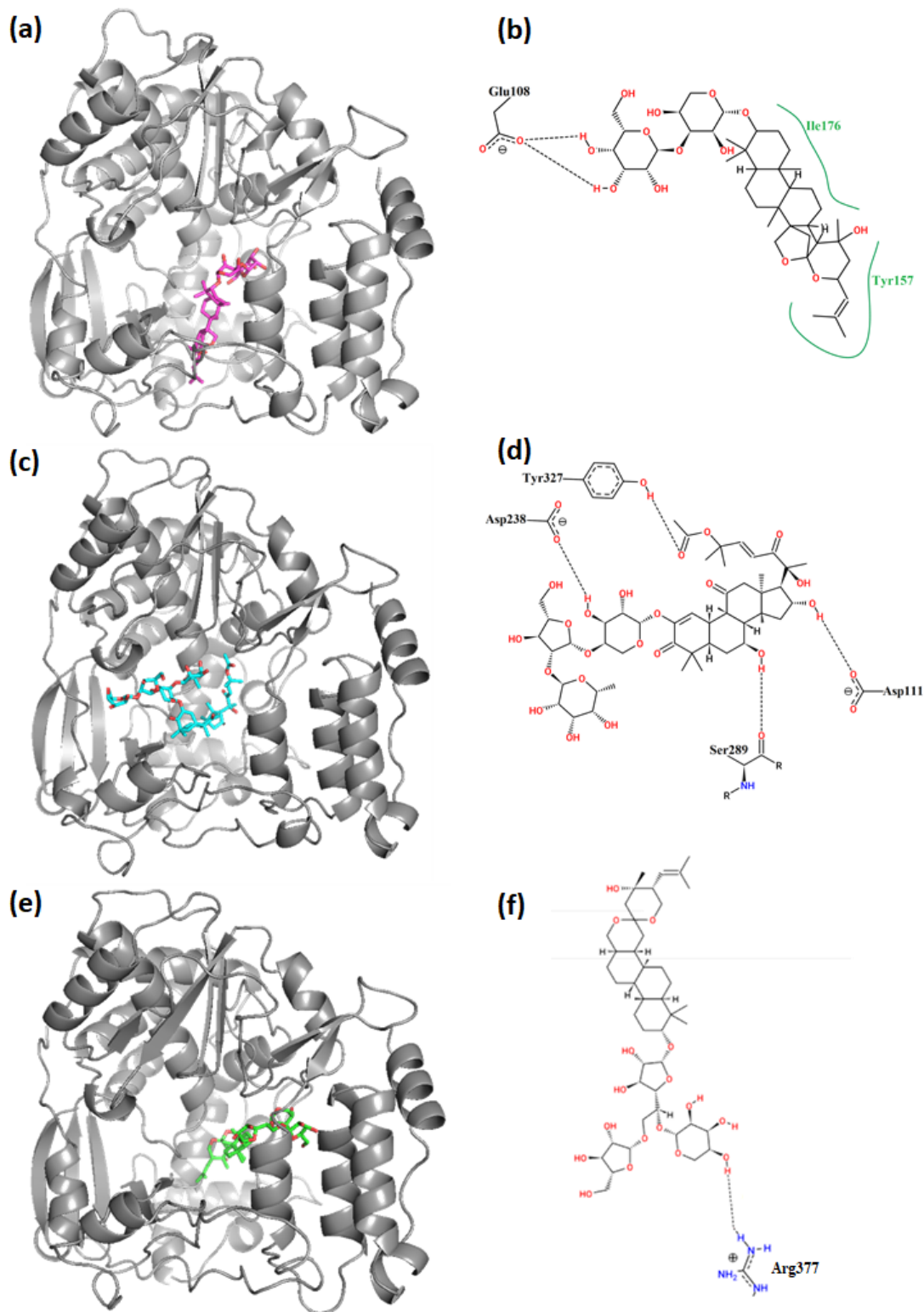


Figure 9

2D and 3D interaction plots for the protein-ligand complexes of the middle structure of the most populated clusters in the MD simulation for the top three hits (a, b) Bacopaside IV, (c, d) Bacobitacin D, (e, f) Bacoside A2

Minimum Distance

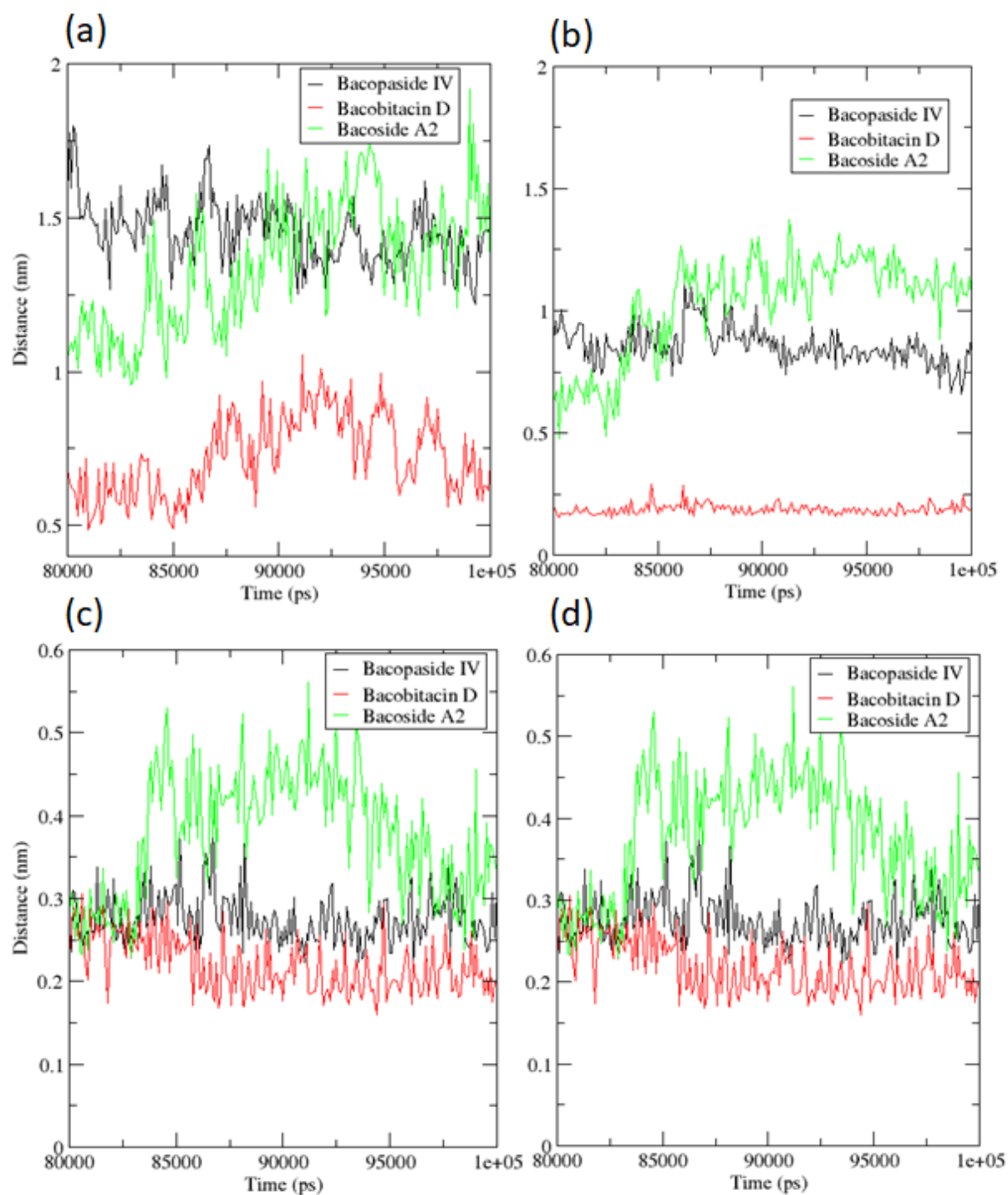


Figure 10

The minimum distance plots of the catalytic residues of the protein-ligand complexes with ligands Bacopaside IV, Bacobitacin D, Bacoside A2, (a) Asp²³³, (b) Asp²³⁸, (c) Ser²⁸⁹, and (d) Asp³²⁹.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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