

Supplementary Material

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

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Table S1. The PDB Id and UniProt Id of CVA16 RdRp with its close homologs

VIRUSES	PDB ID	UNIPROT ID
HUMAN_RHINOVIRUS_16	1xr7	Q82122
HUMAN_ENTEROVIRUS_D68	5xe0	F1T146
POLIOVIRUS_TYPE_1	1ra6	P03300
COXSACKIEVIRUS_B3	4zpc	Q5UEA2
COXSACKIEVIRUS_A16	5y6z	L7WS61
HUMAN_ENTEROVIRUS_71	5f8n	E5RPG2
MENGO_ENCEPHALOMYOCARDITIS_VIRUS	4nyz	P12296
FOOT_AND_MOUTH_DISEASE_VIRUS_C	1u09	Q9QCE4
HEPATITIS_C_VIRUS_GENOTYPE	1nb4	O92972
BOMBYX_MORI_CYTOPLASMIC_POLYHEDROSIS_VIRUS	5h0r	A0A0S1LIW6
BOVINE_VIRAL_DIARRHEA_VIRUS	2cjq	Q96662
INFLUENZA_C_VIRUS	5d98	Q9IMP4
INFLUENZA_B_VIRUS	4wrt	Q5V8Y6
INFLUENZA_A_VIRUS	5m3h	H6QM91
REOVIRUS_TYPE_3	1n35	P0CK31
PSEUDOMONAS_PHAGE_PHI12	4gzk	Q94M06
PSEUDOMONAS_PHAGE_PHI6	1hhs	P11124
THOSEA_ASIGNA_VIRUS	4xhi	Q6A562
ROTAVIRUS_A	2r7r	O37061
INFECTIOUS_PANCREATIC_NECROSIS_VIRUS	2yi9	P22173
AVIAN_INFECTIOUS_BURSAL_DISEASE_VIRUS	2pus	Q9Q6Q5
NORWALK-LIKE_VIRUS	4nrt	A0ZNP5
MURINE_NOROVIRUS	3uqs	Q80J95
RABBIT_HEMORRHAGIC_DISEASE_VIRUS	1khw	P27410
SAPPORO_VIRUS	2uut	Q69014
BUNYAVIRUS_LA_CROSSE	5amq	A5HC98
HUMAN_PICOBIRNAVIRUS	5i61	Q50LE4
DENGUE_VIRUS_TYPE_3	4hhj	Q6YMS4
ZIKA_VIRUS	5wz3	A0A109PRQ3
JAPANESE_ENCEPHALITIS_VIRUS	4k6m	P27395
KUNJIN_VIRUS (WEST NILE VIRUS (WNV))	2hcn	P14335
VESICULAR_STOMATITIS_INDIANA_VIRUS	5a22	P03523
ESCHERICHIA_VIRUS_QBETA (BACTERIOPHAGE QB)	3mmp	P14647

Table S2. The binding energies of the reference ligand (Favipiravir) after docking with the protein.

Mode	Affinity (kcal/mol)
1	-7.6
2	-7.6
3	-7.5
4	-7.4
5	-7.4
6	-7.4
7	-7.3
8	-7.2
9	-7.2
10	-7.2
11	-7.1
12	-7.1
13	-7.1
14	-7.1
15	-7
16	-7
17	-7
18	-7
19	-6.9
Average	-7.2

Table S3. The ADME properties and the toxicity prediction of the top three hits.

ADME Properties	Bacobitacin D	Bacopaside IV	Bacoside A2
GI Absorption	Low	Low	Low
Solubility	Moderately soluble	Poorly soluble	Moderately soluble
Lipophilicity (Log Po/w (iLOGP))	3.38	4.6	4.61
CYP1A2 inhibitor	No	No	No
P-gp substrate	Yes	Yes	Yes
PAINS	0 alert	0 alert	0 alert
Toxicity Class	4	4	3

Table S4. Tanimoto similarity coefficient of the top three compounds with molnupiravir, rupintrinvir, doxepin hydrochloride

Compounds Set 1	Compounds Set 1	Tanimoto
Bacoside_A2	Molnupiravir	0.04
Bacoside_A2	Rupintrinvir	0.06
Bacoside_A2	Doxepin_hydrochloride	0.01
Bacobitacin_D	Molnupiravir	0.05
Bacobitacin_D	Rupintrinvir	0.11
Bacobitacin_D	Doxepin_hydrochloride	0.02
Bacopaside_IV	Molnupiravir	0.05
Bacopaside_IV	Rupintrinvir	0.07
Bacopaside_IV	Doxepin_hydrochloride	0.01

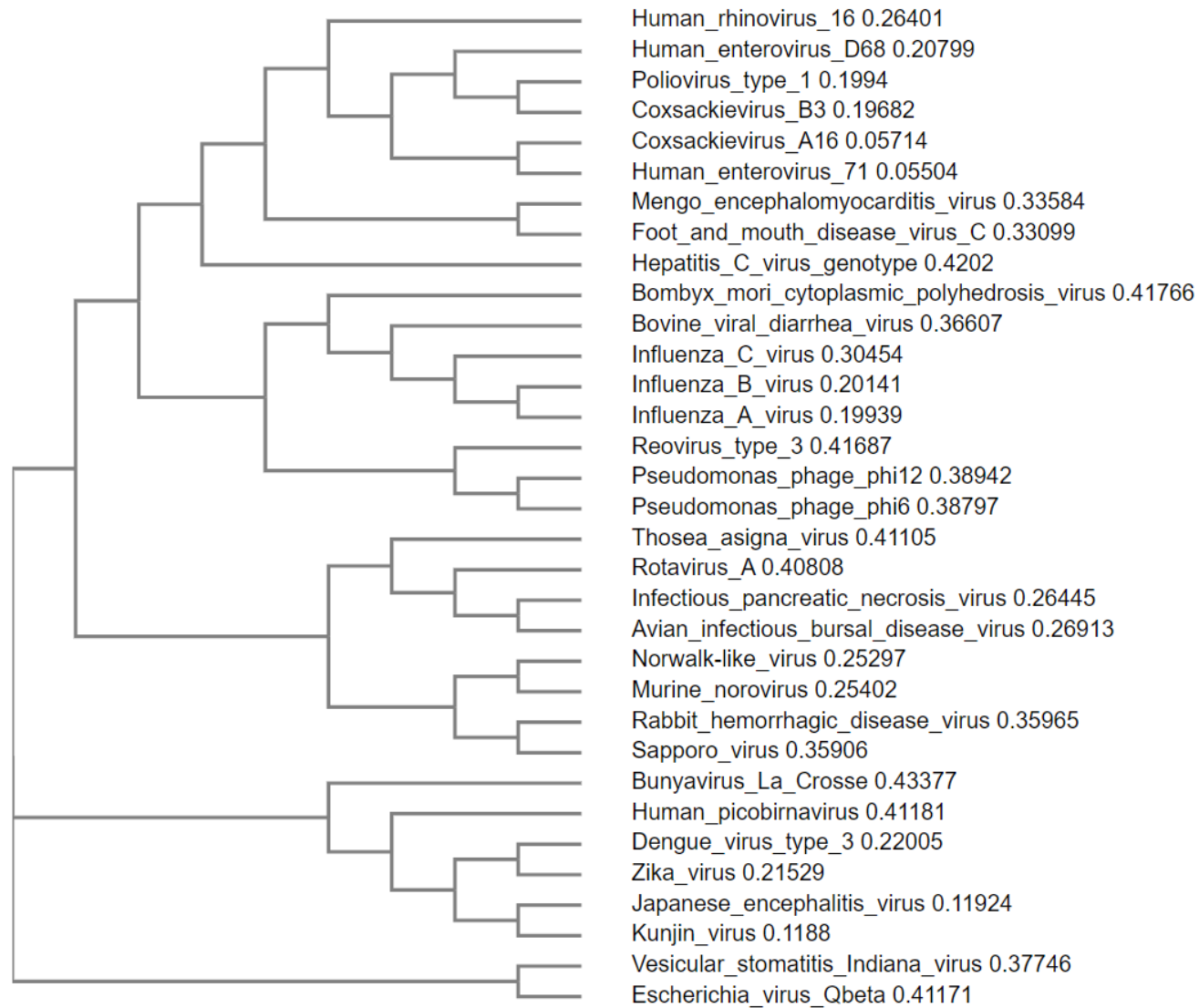


Figure S1. Sequence-based phylogeny of viral RdRps is presented

	Human_rhino_virus_16	Human_eterovirus_D68	Poliovirus_type_1	Coxsackievirus_A16	Human_eterovirus_71	Coxsackievirus_B3	Mengoencephalomyocarditis_virus	Foot_and_mouth_disease_virus_C	Hepatitis_C_virus_genotype	Bombyx_mori_cytoplasmic_polyhedrosis_virus	Reovirus_type_3	Pseudomonas_phage_phi12	Pseudomonas_phage_phi6	Bovine_viral_diarrrhea_virus	Influenza_A_C_virus	Influenza_A_B_virus	Influenza_A_A_virus	Thosea_asigna_virus	Rotavirus_A	Infectio_pancreatic_necrosis_virus	Avian_infectious_bursae_disease_virus	Norwalk-like_virus	Murine_norovirus	Rabbit_hemorrhagic_disease_virus	Sapporo_virus	Bunyavirus_La_Crosse	Human_picobirnavirus	Dengue_virus_type_3	Zika_virus	Japanese_encephalitis_virus	Kunjin_virus	Vesicular_stomatitis_Indiana_virus	Escherichia_virus_Qbeta
Human_rhinovirus_16	100	48.45	48.44	46.89	46.14	49.32	25.73	25.1	13.12	9.7	9.07	11.34	6.87	15.57	10.15	9.14	10.07	9.63	9.07	12.39	8.16	9.84	9.38	9.19	9.54	9.76	4.35	11.92	12.01	12.5	13	9.89	7.92
Human_enterovirus_D68	48.45	100	57.57	55	54.81	59.38	25.35	25.44	14.54	10.7	10.77	10.24	7.42	15.66	10.37	8.87	10.54	8.24	7.01	9.58	7.56	10.76	8.95	8.35	11.89	10.42	7.33	12.72	12.47	13.27	13.36	9.9	8.07
Poliovirus_type_1	48.44	57.57	100	54.96	54.64	60.38	26.71	25.15	15.66	10.1	10.32	10.96	6.18	15.29	9.61	10.32	10.51	8.19	6.28	13.24	6.82	9.78	9.14	7.75	11.06	10.53	9.09	12.76	11.94	12.26	12.5	10.1	6.74
Coxsackievirus_A16	46.89	55	54.96	100	88.78	56.79	26.78	26.49	15.71	10.44	9.72	11.26	5.87	16.13	11.08	9.34	10.02	11.15	7.53	11.34	8.65	11.04	9.22	8.75	10.21	9.52	6.38	11.87	12.28	11.94	12.02	9.67	7.07
Human_enterovirus_71	46.14	54.81	54.64	88.78	100	56.93	27.23	26.6	15.28	10.01	10.32	12.29	5.87	16.31	11.33	9.58	10.02	10.38	7.99	12.84	9.51	11.54	9.57	9.09	11.56	10.09	6.91	12.19	12.36	11.86	11.86	9.77	6.71
Coxsackievirus_B3	49.32	59.38	60.38	56.79	56.93	100	26.05	25.12	15.7	10.76	11.18	11.95	7.62	15.54	10.1	11.06	11	9.3	6.88	9.39	8.16	10.22	8.2	8.28	11.1	10.15	8.56	11.85	11.69	12.66	12.99	10.32	6.67
Mengoencephalomyocarditis_virus	25.73	25.35	26.71	26.78	27.23	26.05	100	33.32	14.52	9.89	8.33	9.75	6.41	14.33	9.71	10.68	11.35	7.04	7.36	9.88	8.24	9.31	10.2	9.43	9.13	9.28	6.05	12.04	11.66	13.03	12.41	11.77	8.97
Foot_and_mouth_disease_virus_C	25.1	25.44	25.15	26.49	26.6	25.12	33.32	100	14.85	9.18	9.75	8.16	7.97	15.02	7.73	9.7	8.66	8.1	9.07	8.82	9.25	11.77	11.71	9.68	9.01	9.45	10.22	13.14	11.95	11.76	11.53	9.62	12.08
Hepatitis_C_virus_genotype	13.12	14.54	15.66	15.71	15.28	15.7	14.52	14.85	100	13.18	13.14	9.95	10.59	21.6	10.91	10.37	7.89	11.73	7.75	7.66	9.07	10.15	11.05	10.04	10.39	9.78	10.48	14.99	15.25	15.26	15.94	12.3	8.81
Bombyx_mori_cytoplasmic_polyhedrosis_virus	9.7	10.7	10.1	10.44	10.01	10.76	9.89	9.18	13.18	100	14.52	13.95	11.03	21.88	11.92	13.13	11.49	8.88	8.39	7.98	6.99	11.21	8.98	8.8	10.02	9.09	9.29	9.49	10.23	10.75	11.09	11.49	8.82
Reovirus_type_3	9.07	10.77	10.32	9.72	10.32	11.18	8.33	9.75	13.14	14.52	100	14.86	14.94	21.17	8.8	8.88	11.21	6.31	7.92	7.2	9.88	9.34	10.12	9.94	9.3	7.11	9.02	10.6	11.02	10.86	10.63	9.19	9.93
Pseudomonas_phage_phi12	11.34	10.24	10.96	11.26	12.29	11.95	9.75	8.16	9.95	13.95	14.86	100	22.26	17.61	7.38	8.16	9.4	9.95	3.49	6.9	6.25	6.67	4.44	6.36	6.02	6.86	4.76	8.87	6.59	6.27	6.27	8.19	7.69
Pseudomonas_phage_phi6	6.87	7.42	6.18	5.87	5.87	7.62	6.41	7.97	10.59	11.03	14.94	22.26	100	18.08	6.62	8.72	7.28	10.58	6.59	3.45	1.56	8.7	4.35	7.46	5.88	7.08	14.29	10.44	9.21	7.54	6.89	8.46	3.08
Bovine_viral_diarrrhea_virus	15.57	15.66	15.29	16.13	16.31	15.54	14.33	15.02	21.6	21.88	21.17	17.61	18.08	100	20.45	19.69	18.03	11.04	8.15	10.45	11.54	13.03	13.1	12.01	10.98	10.78	9.85	16.56	16.39	16.56	17.17	12.69	7.97
Influenza_C_virus	10.15	10.37	9.61	11.08	11.33	10.1	9.71	7.73	10.91	11.92	8.8	7.38	6.62	20.45	100	40.86	38.72	5.1	4.07	5.13	5.15	5.79	7.63	6.3	7.23	6.86	2.04	8.07	9.73	8.98	8.74	9	8.2
Influenza_B_virus	9.14	8.87	10.32	9.34	9.58	11.06	10.68	9.7	10.37	13.13	8.88	8.16	8.72	19.69	40.86	100	59.92	7.84	5.04	10.53	3.16	6.61	5.08	8.82	9.64	7.3	5.21	5.42	7.84	6.6	6.11	7.02	7.38
Influenza_A_virus	10.07	10.54	10.51	10.02	10.02	11	11.35	8.66	7.89	11.49	11.21	9.4	7.28	18.03	38.72	59.92	100	6.45	4.96	9.21	6.32	8.26	8.47	8.4	10.44	7.55	5.21	5.61	7.28	6.78	5.33	8.7	9.02
Thosea_asigna_virus	9.63	8.24	8.19	11.15	10.38	9.3	7.04	8.1	11.73	8.88	6.31	9.95	10.58	11.04	5.1	7.84	6.45	100	15.62	18.76	17.56	11.62	11.63	12.63	13.38	10.73	5.98	15.5	16.87	15.26	13.14	5.95	
Rotavirus_A	9.07	7.01	6.28	7.53	7.99	6.88	7.36	9.07	7.75	8.39	7.92	3.49	6.59	8.15	4.07	5.04	4.96	15.62	100	17.97	19.6	11.13	11.87	11.51	11.76	11.05	11.89	12.54	12.88	13.47	14.93	12.6	9.87
Infectious_pancreatic_necrosis_virus	12.39	9.58	13.24	11.34	12.84	9.39	9.88	8.82	7.66	7.98	7.2	6.9	3.45	10.45	5.13	10.53	9.21	18.76	17.97	100	46.64	10.16	11.35	11.79	8.75	11.6	6.6	11.7	12.86	11.05	11.96	12.47	7.61
Avian_infectious_bursal_disease_virus	8.16	7.56	6.82	8.65	9.51	8.16	8.24	9.25	9.07	6.99	9.88	6.25	1.56	11.54	5.15	3.16	6.32	17.56	19.6	46.64	100	10.31	12.62	11.51	8.81	10	8.42	11.64	12.39	11.38	12.22	13.32	7.56
Norwalk-like_virus	9.84	10.76	9.78	11.04	11.54	10.22	9.31	11.77	10.15	11.21	9.34	6.67	8.7	13.03	5.79	6.61	8.26	11.82	11.13	10.16	10.31	100	49.3	23	25.15	12.45	7.37	13.98	14.19	13.83	13.33	12.92	7.59
Murine_norovirus	9.38	8.95	9.14	9.22	9.57	8.2	10.2	11.71	11.05	8.98	10.12	4.44	4.35	13.1	7.63	5.08	8.47	11.63	11.87	11.35	12.62	49.3	100	22.23	24.29	12.57	10.21	13.73	14.88	14.68	14.17	12.5	7.83
Rabbit_hemorrhagic_disease_virus	9.19	8.35	7.75	8.75	9.09	8.28	9.43	9.68	10.04	8.8	9.94	6.36	7.46	12.01	6.3	8.82	8.4	12.63	11.51	11.79	11.51	23	22.23	100	28.13	10.23	7.14	15.4	14.78	14.96	14.16	13.05	7.57
Sapporo_virus	9.54	11.89	11.06	10.21	11.56	11.1	9.13	9.01	10.39	10.02	9.3	6.02	5.88	10.98	7.23	9.64	10.44	13.38	11.76	8.75	8.81	25.15	24.29	28.13	100	10.18	8.58	13.88	14.48	13.48	13.24	13.45	7.51
Bunyavirus_La_Crosse	9.76	10.42	10.53	9.52	10.09	10.15	9.28	9.45	9.78	9.09	7.11	6.86	7.08	10.78	6.86	7.3	7.55	10.73	11.05	11.6	10	12.45	12.57	10.23	10.18	100	10.99	16.5	16.62	17.38	17.86	13.53	9.77
Human_picobirnavirus	4.35	7.33	9.09	6.38	6.91	8.56	6.05	10.22	10.48	9.29	9.02	4.76	14.29	9.85	2.04	5.21	5.21	5.98	11.89	6.6	8.42	7.37	10.21	7.14	8.58	10.99	100	21.38	22.32	22.15	21.73	10.84	8.51
Dengue_virus_type_3	11.92	12.72	12.76	11.87	12.19	11.85	12.04	13.14	14.99	9.49	10.6	8.87	10.44	16.56	8.07	5.42	5.61	15.5	12.54	11.7	11.64	13.98	13.73	15.4	13.88	16.5	21.38	100	56.47	51.43	51.82	17.49	11.66
Zika_virus	12.01	12.47	11.94	12.28	12.36	11.69	11.66	11.95	15.25	10.23	11.02	6.59	9.21	16.39	9.73	7.84	7.28	16.87	12.88	12.86	12.39	14.19	14.88	14.78	14.48	16.62	22.32	56.47	100	56.06	57.06	18.04	12.82
Japanese_encephalitis_virus	12.5	13.27	12.26	11.94	11.86	12.66	13.03	11.76	15.26	10.75	10.86	6.27	7.54	16.56	8.98	6.6	6.78	15.26	13.47	11.05	11.38	13.83	14.68	14.96	13.48	17.38	22.15	51.43	56.06	100	76.2	18.49	12.76
Kunjin_virus	13	13.36	12.5	12.02	11.86	12.99	12.41	11.53	15.94	11.09	10.63	6.27	6.89	17.17	8.74	6.11	5.33	15.26	14.93	11.96	12.22	13.33	14.17	14.16	13.24	17.86	21.73	51.82	57.06	76.2	100	18.3	12.76
Vesicular_stomatitis_Indiana_virus	9.89	9.9	10.1	9.67	9.77	10.32	11.77	9.62	12.3	11.49	9.19	8.19	8.46	12.69	9	7.02	8.7	13.14	12.6	12.47	13.32	12.92	12.5	13.05	13.45	13.53	10.84	17.49	18.04	18.49	18.3	100	21.08
Escherichia_virus_Qbeta	7.92	8.07	6.74	7.07	6.71	6.67	8.97	12.08	8.81	8.82	9.93	7.69	3.08	7.97	8.2	7.38	9.02	5.95	7.61	9.87	7.56	7.59	7.83	7.57	7.51	9.77	8.51	11.66	12.82	12.76	12.76	21.08	100

Figure S2. Heat map of Percent Identity Matrix of viral RdRps from sequence-based phylogeny (CVA16 RdRp with its close homologs)

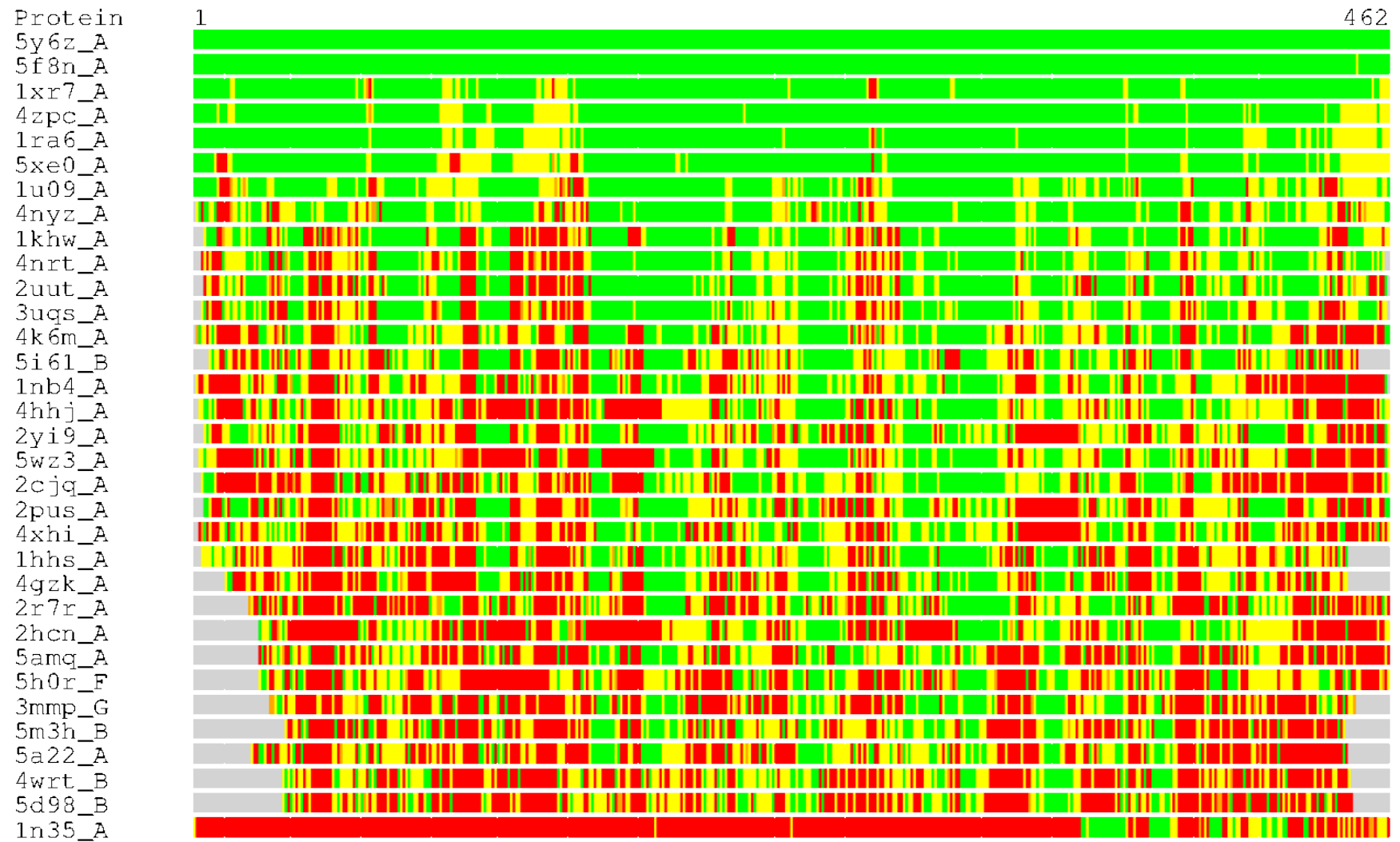


Figure S3. Structural alignment of CVA16 RdRp with its close homologs

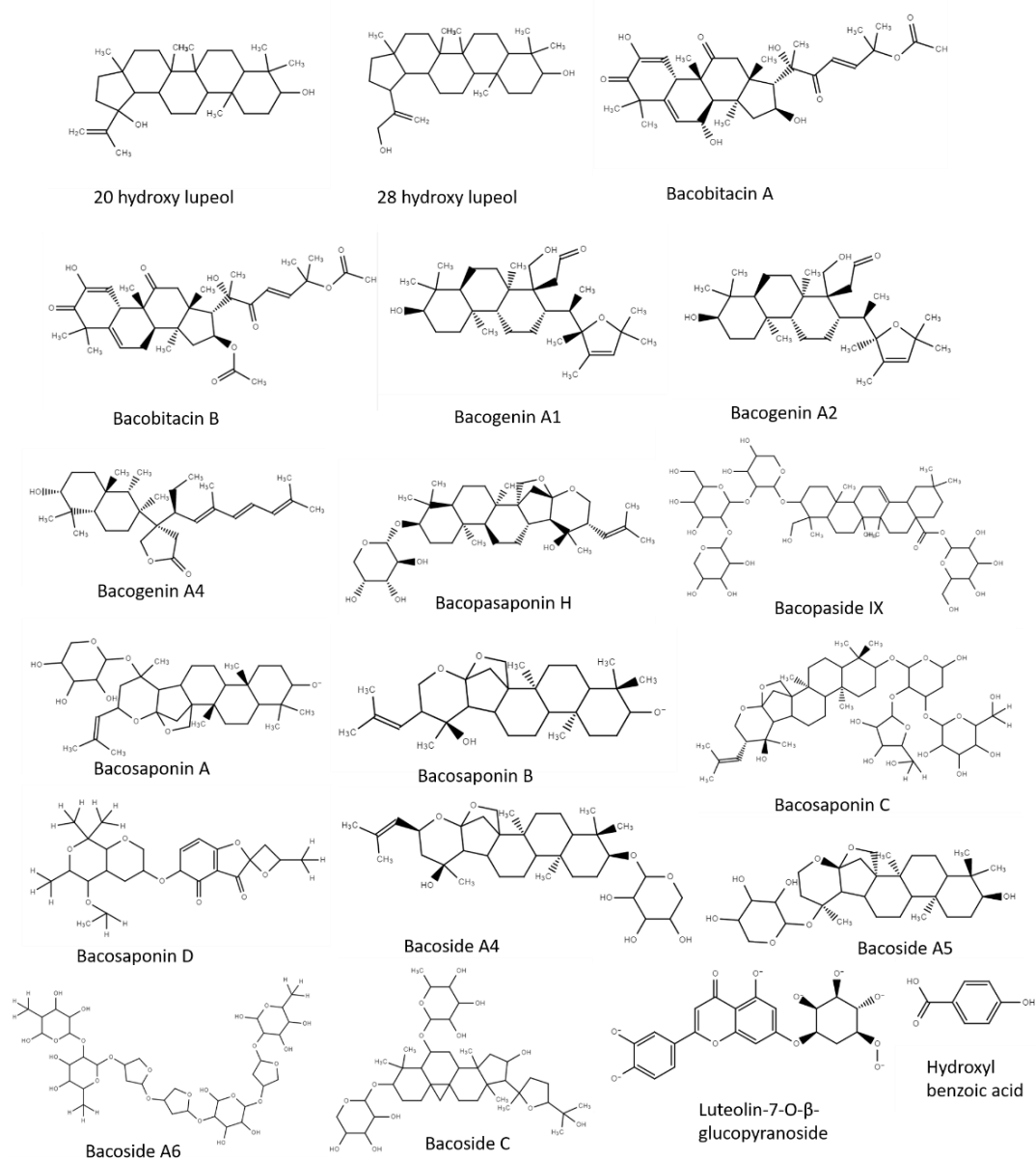


Figure S4. 2D Structural representation of the other 19 compounds predicted using the Name To Structure tool of DataWarrior server and ChemSpider tool.

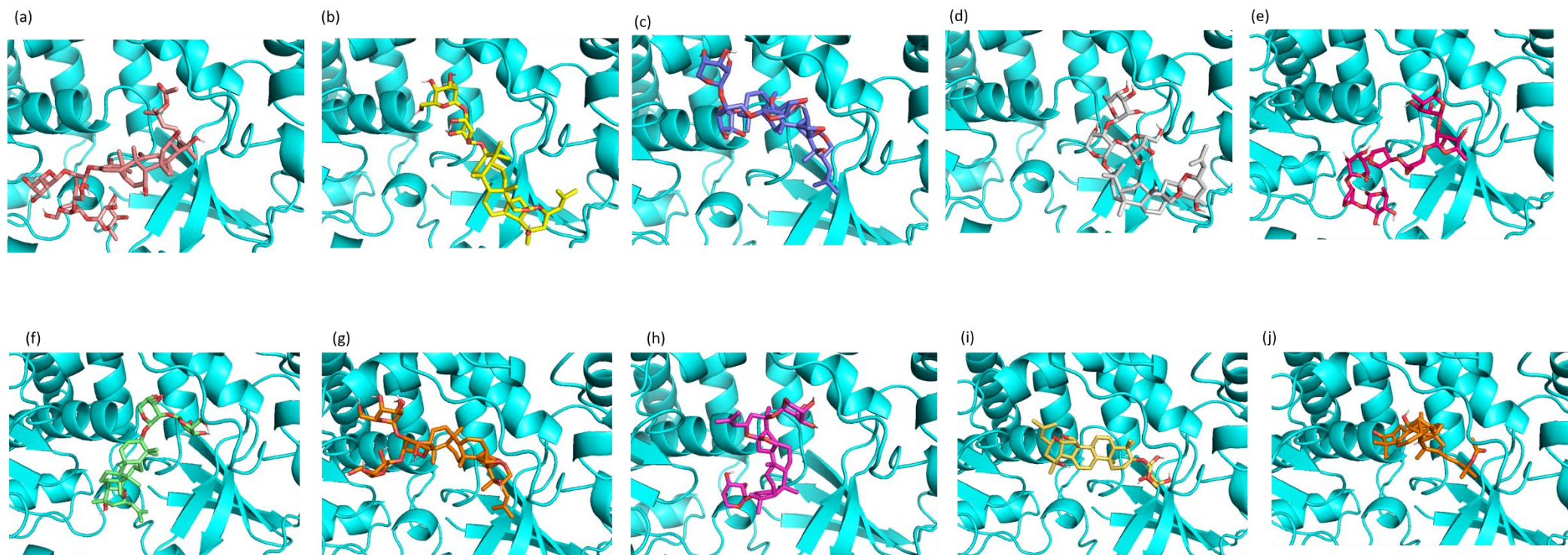


Figure S5. 3D Structure of the binding of protein-ligand complexes for: (a) Bacobitacin D, (b) Bacopaside IV, (c) Bacoside A2, (d) Bacopaside X, (e) Bacoside A6, (f) Bacopasaponin G, (g) Bacopasaponin C, (h) Bacopasaponin A, (i) Bacoside A4, and (j) Cucurbitacin B.

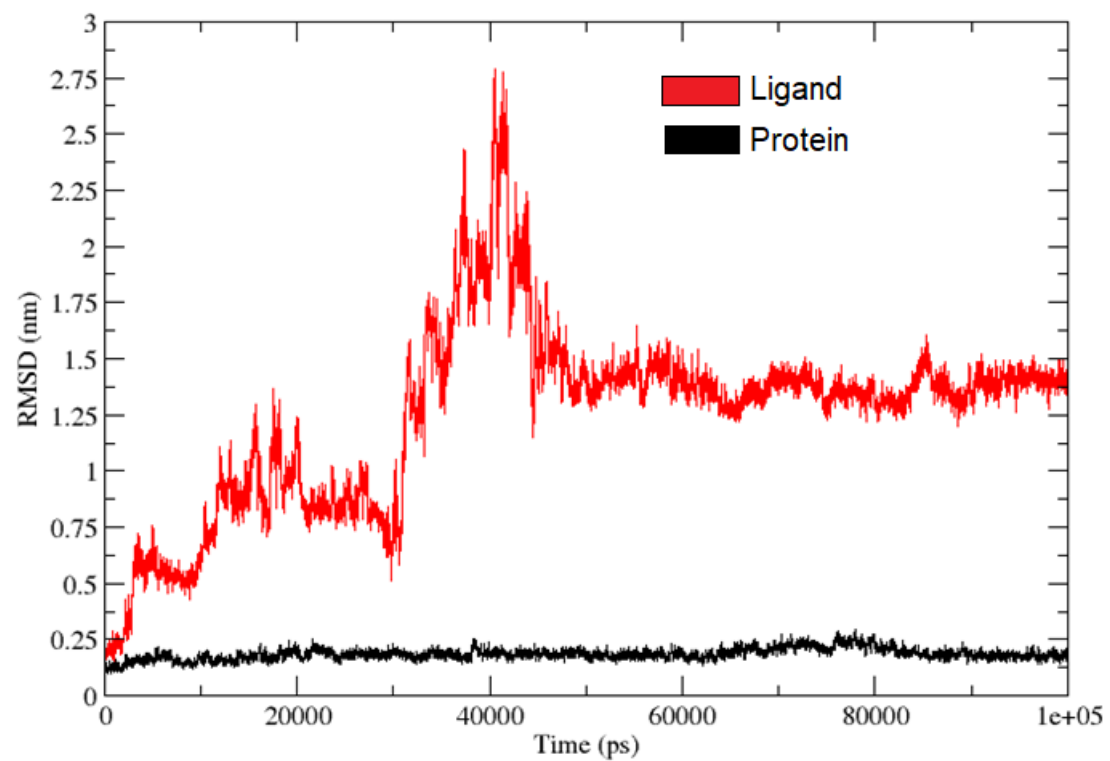


Figure S6. (a) The RMSD of protein C α atoms of CVA16 RdRp and the reference ligand (Favipiravir) calculated over 100 ns MD simulation trajectories.

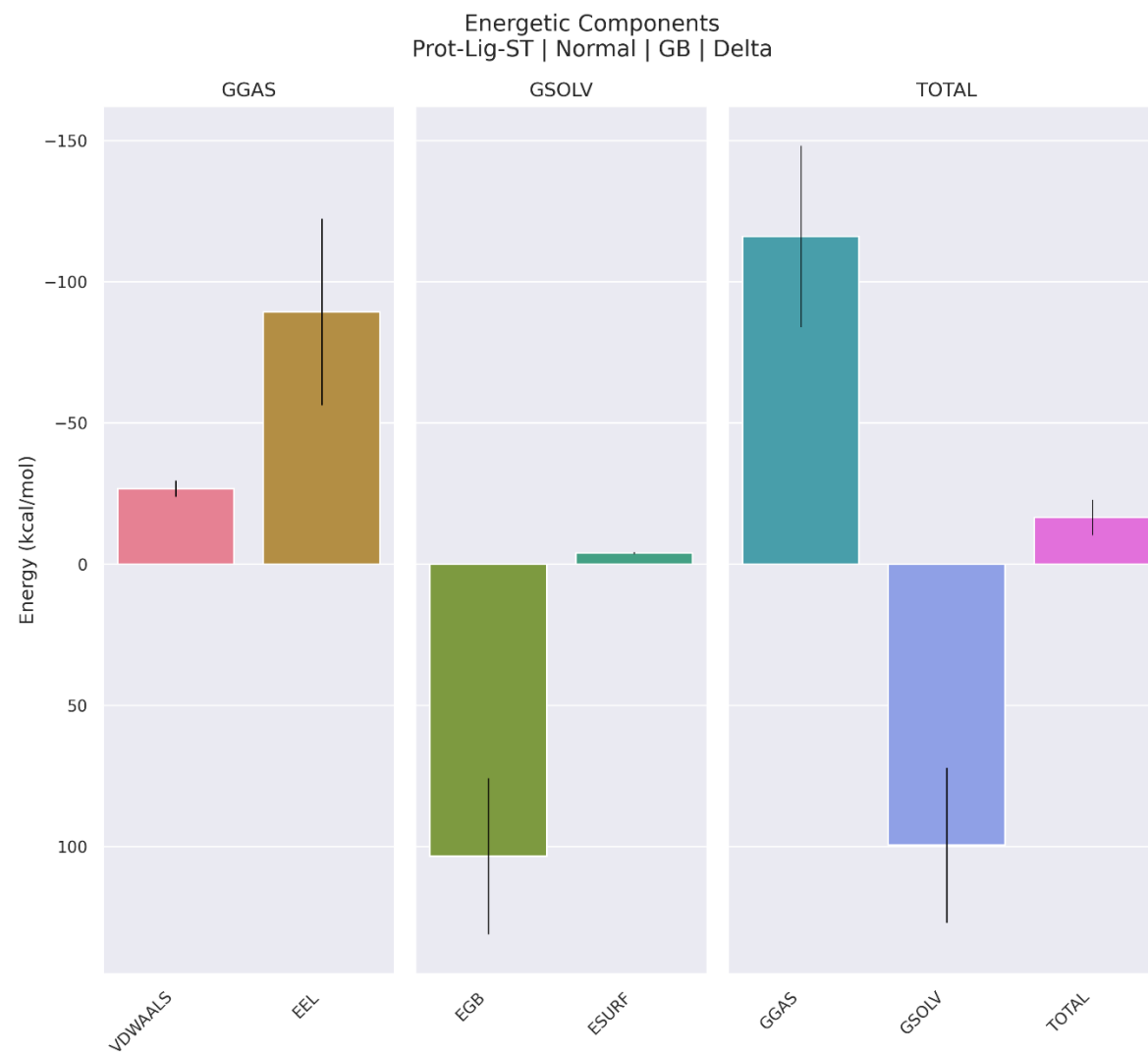


Figure S7. Binding free energy components computed based on MM/GBSA analysis for the reference ligand (Favipiravir) averaged over last 20 ns.

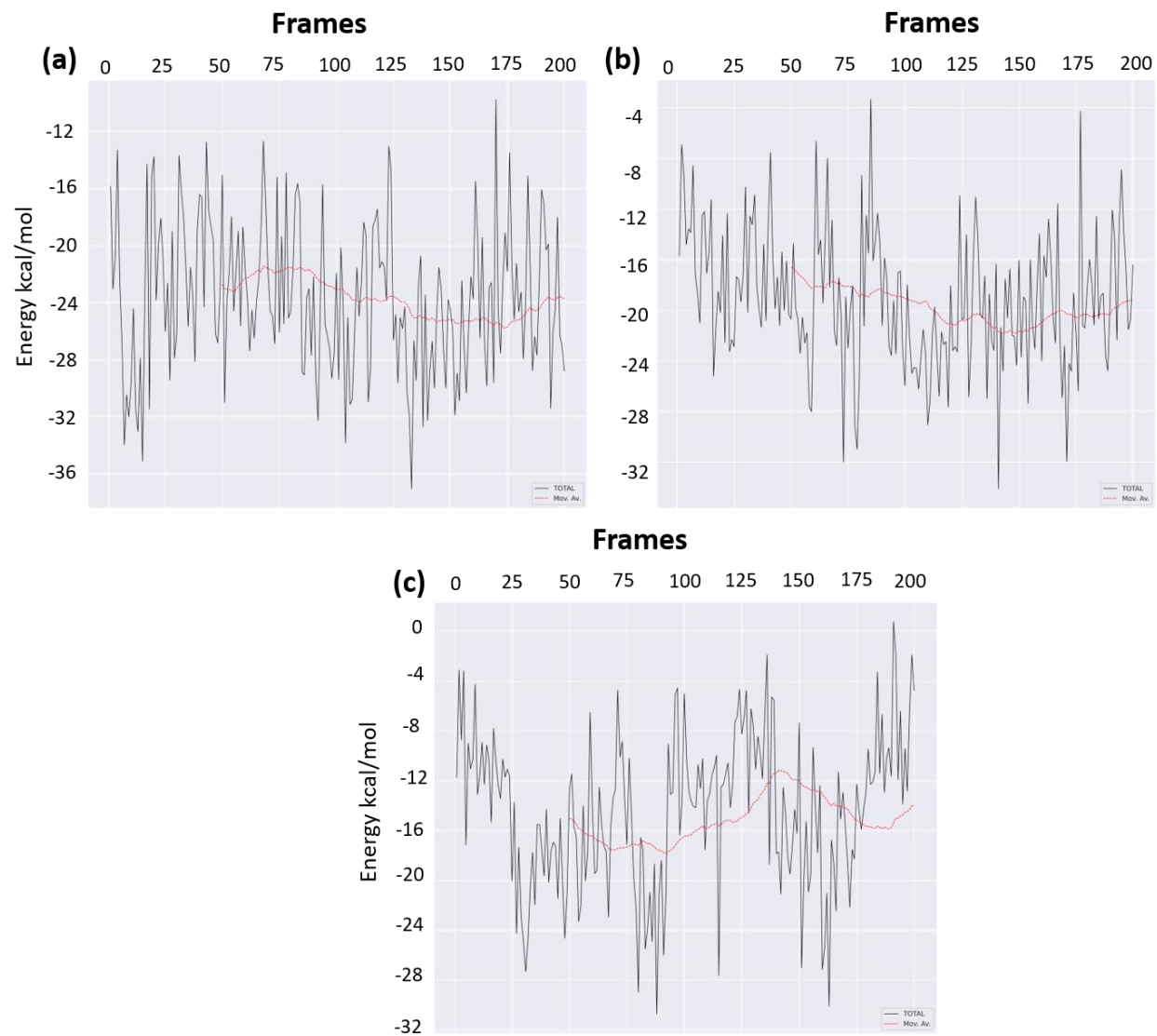


Figure S8. The total binding free energy (ΔG_{TOTAL}) plots from gmx_MMPBSA analysis of the protein-ligand complexes for, (a) Bacopaside IV, (b) Bacobitacin D, (c) Bacoside A2

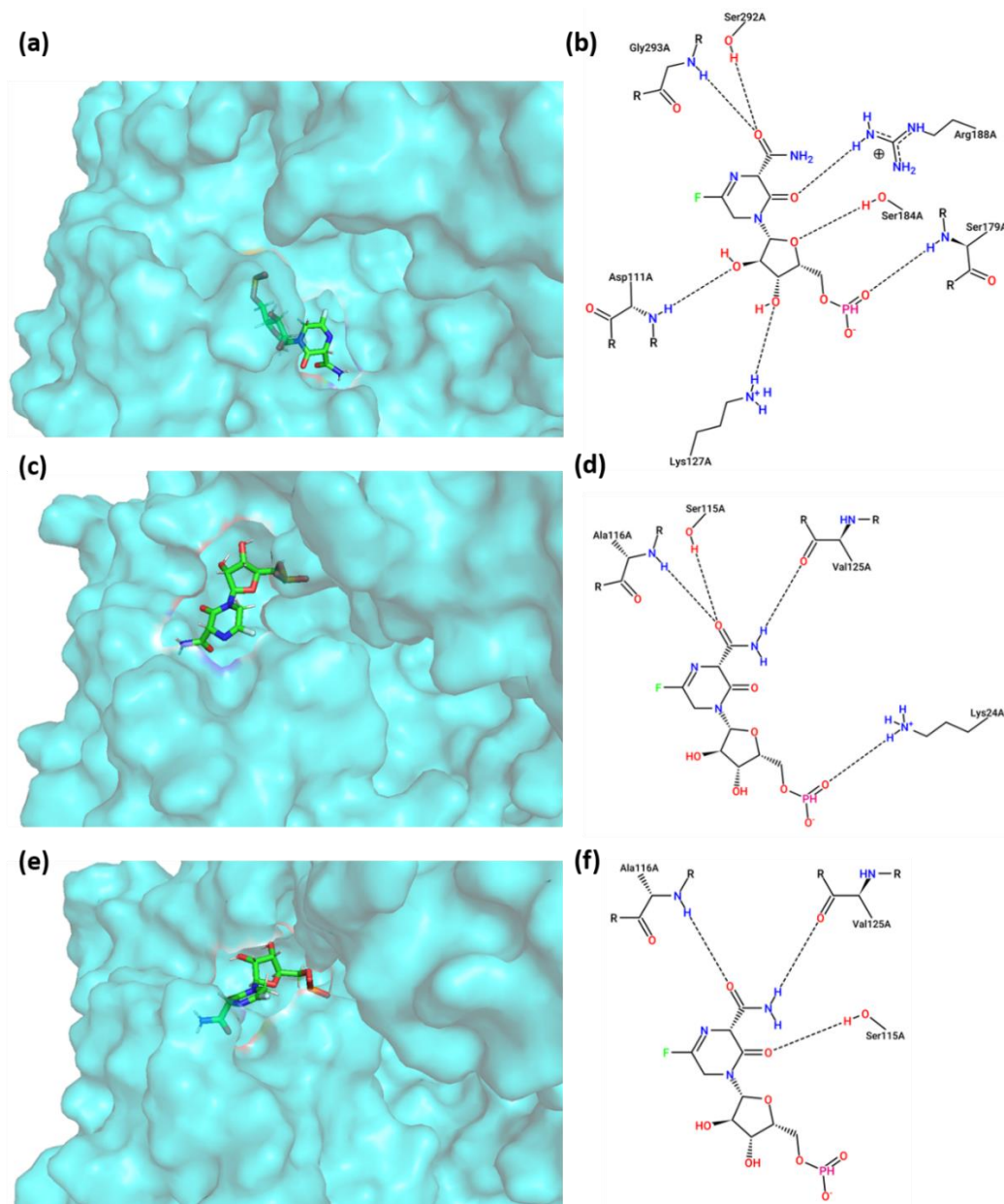


Figure S9. 2D and 3D interaction plots for the protein-ligand complexes from the MD simulation of the reference ligand (Favipiravir) at, (a, b) 0 ns, (c, d) 60 ns, (e, f) 80 ns

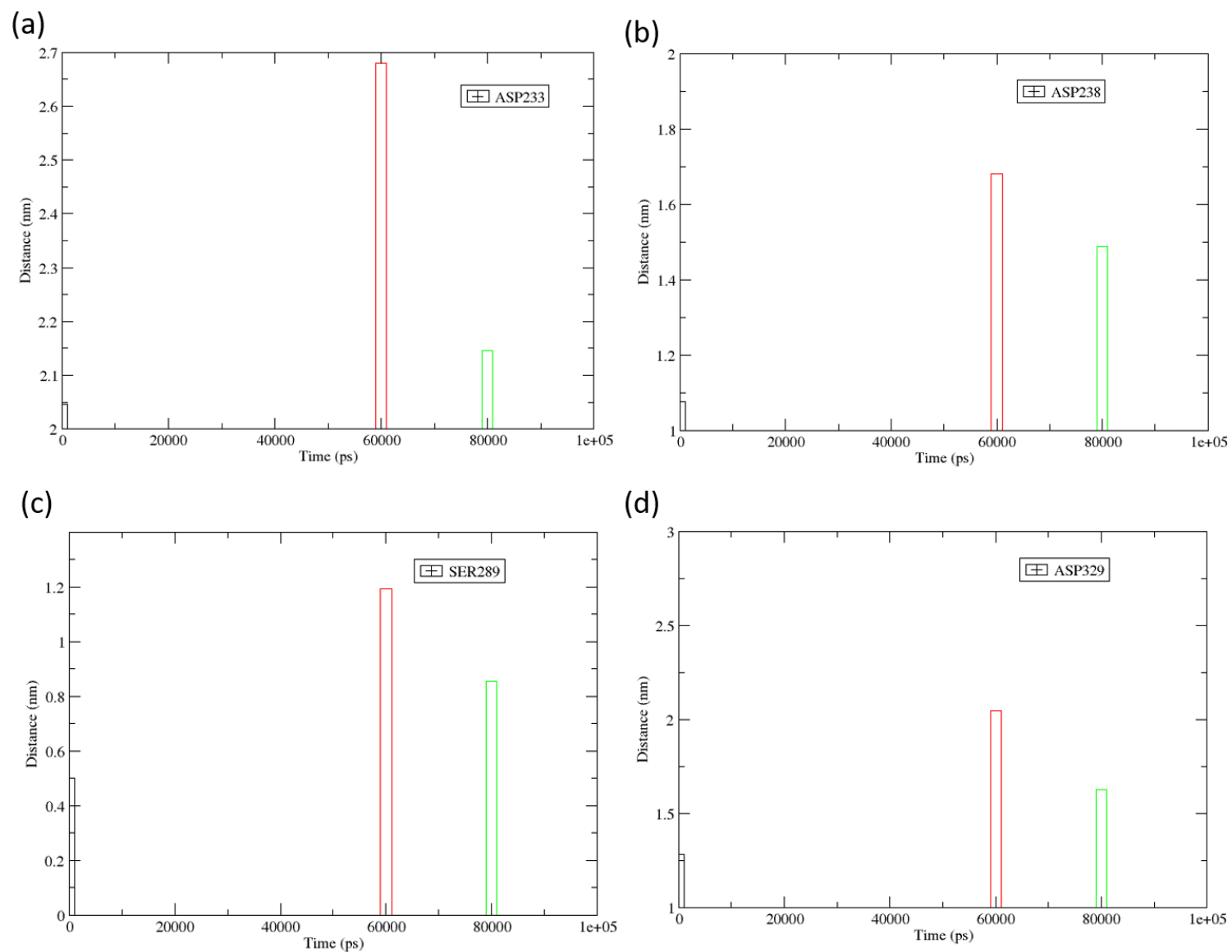


Figure S10. The minimum distance plots of the catalytic residues of the protein-ligand complex with reference ligand (Favipiravir), (a) Asp²³³, (b) Asp²³⁸, (c) Ser²⁸⁹, and (d) Asp³²⁹

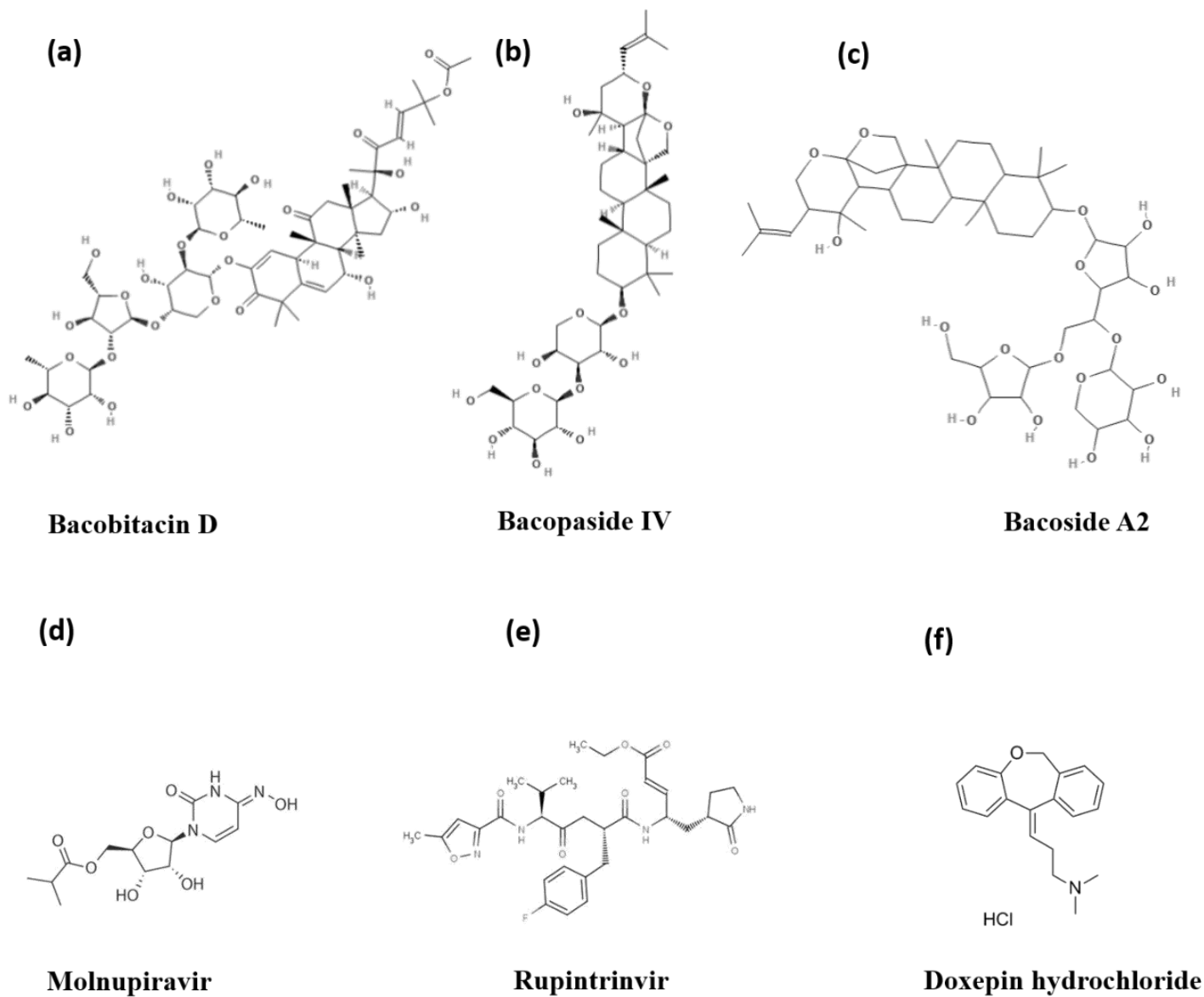


Figure S11. 2D structures of the top three compounds with molnupiravir, rupintrinvir, doxepin hydrochloride