

Supplementary Information

Aurasperone A inhibits SARS CoV-2 in-vitro: an integrated in-vitro and in-silico study

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1. Characterization of compound 6

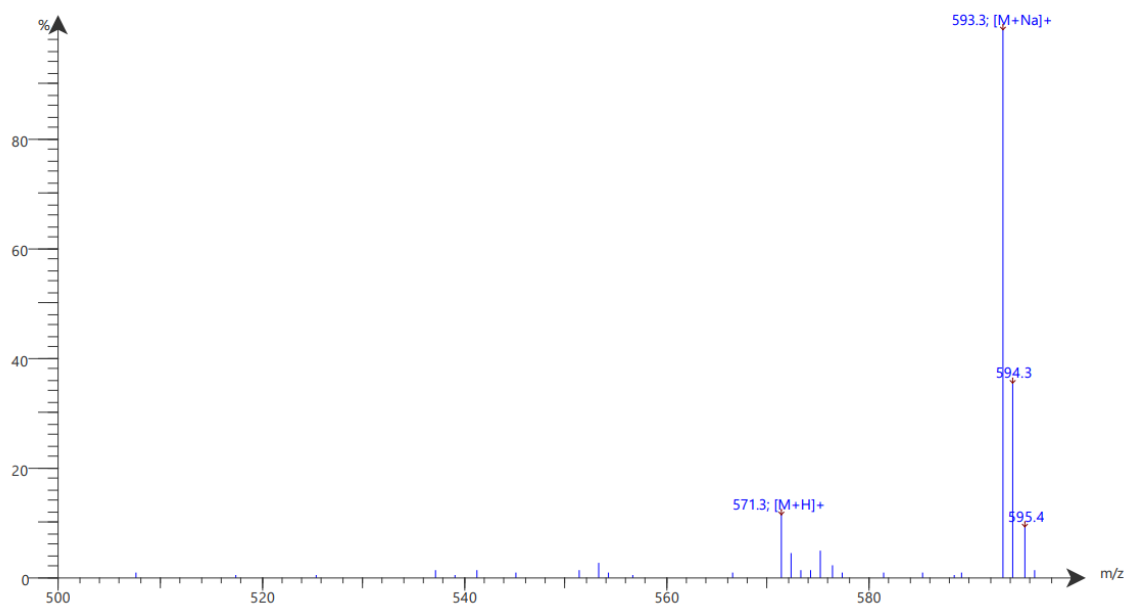


Figure S1: ESI⁺ mass spectrum of compound 6 (Rubasperone B)

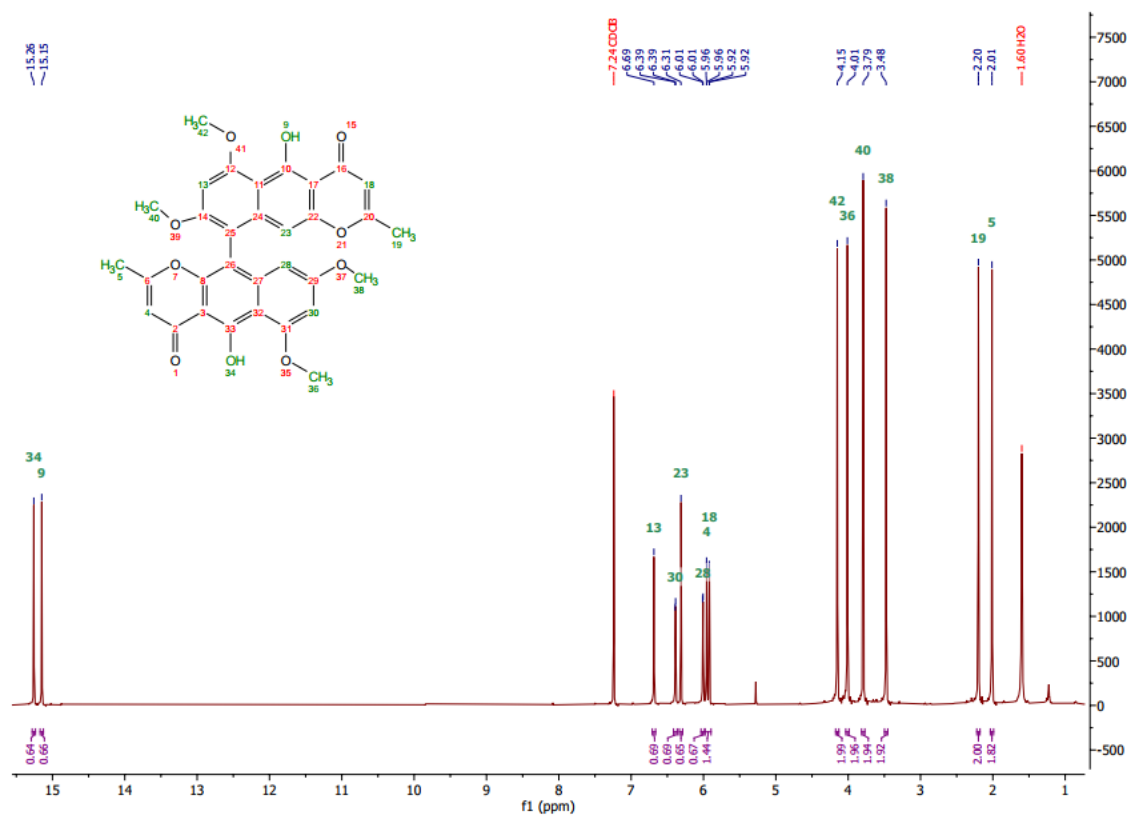


Figure S2: ¹H-NMR spectrum of compound 6 (Rubasperone B)

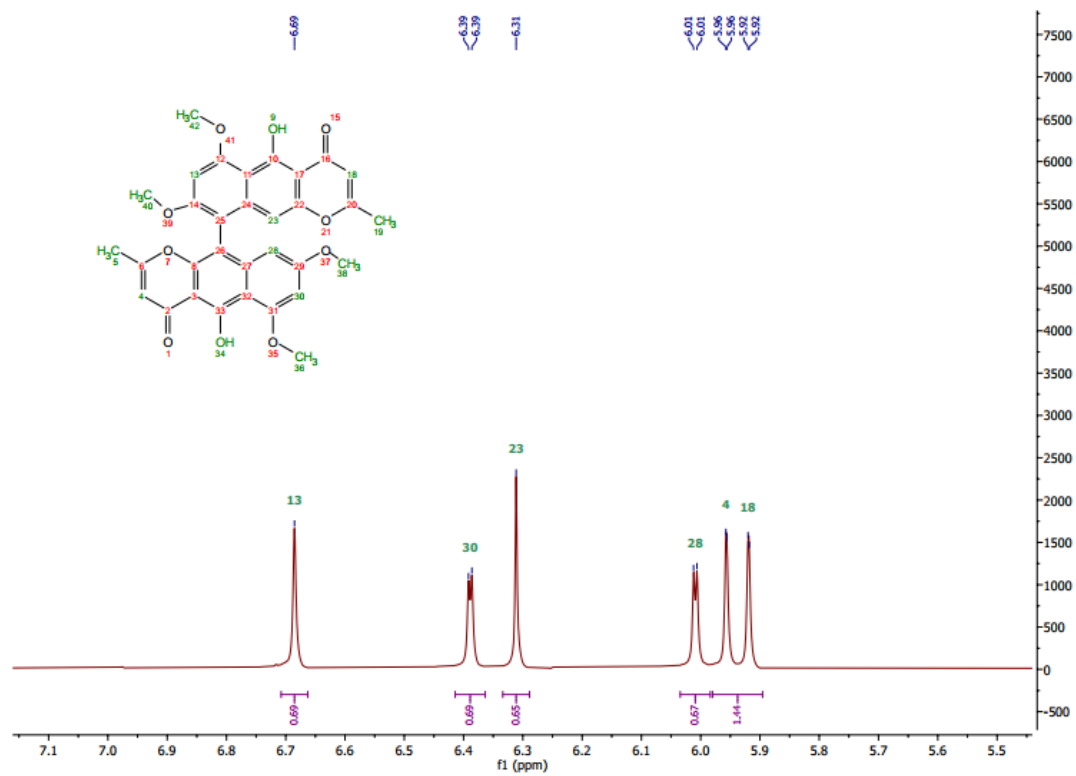


Figure S3: Expanded ¹H-NMR spectrum of compound **6** (Rubasperone B)

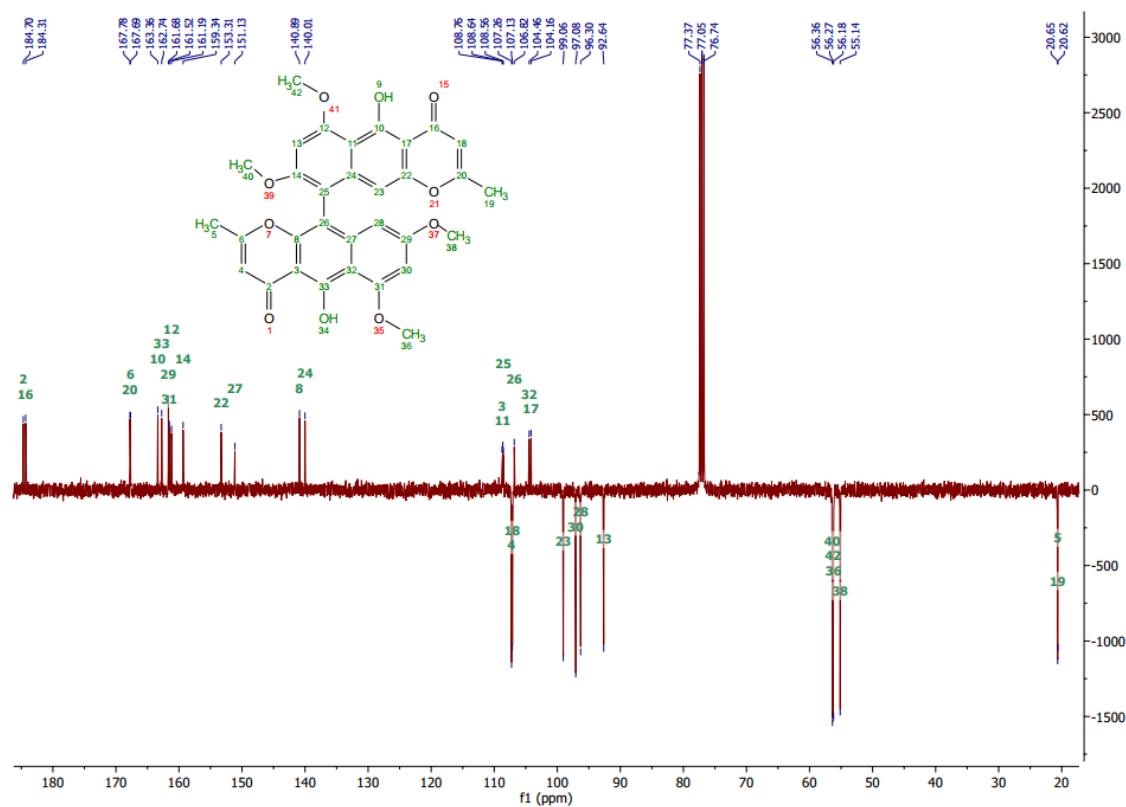


Figure S4: APT spectrum of compound **6** (Rubasperone B)

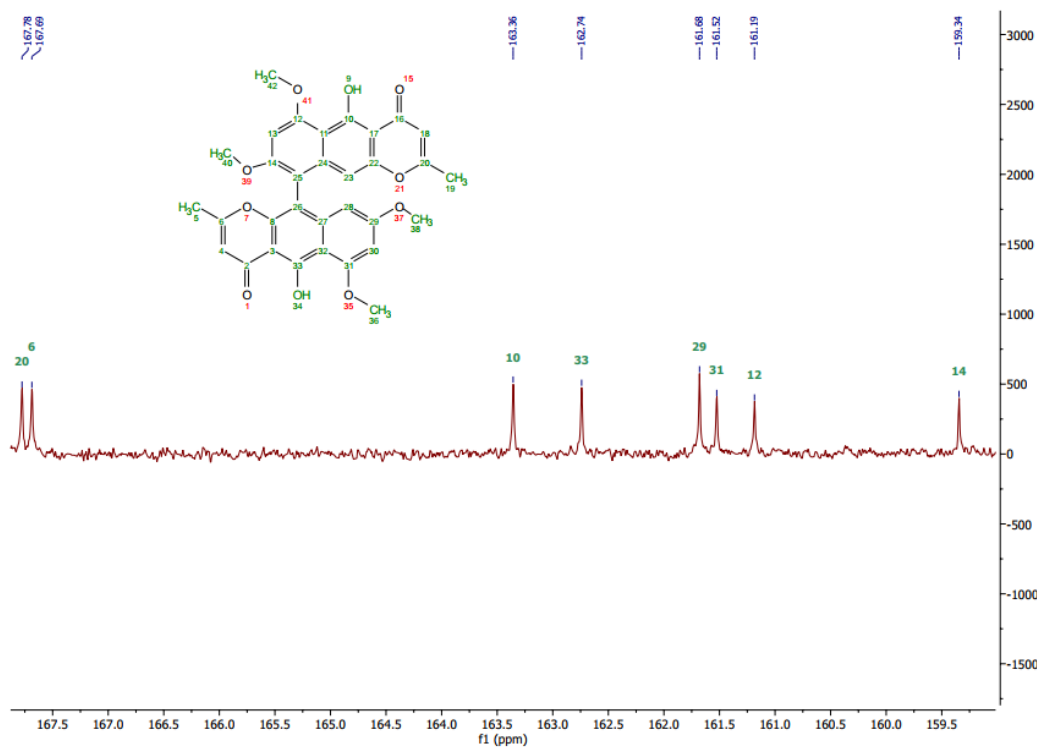


Figure S5: Expanded APT spectrum of compound **6** (Rubasperone B)

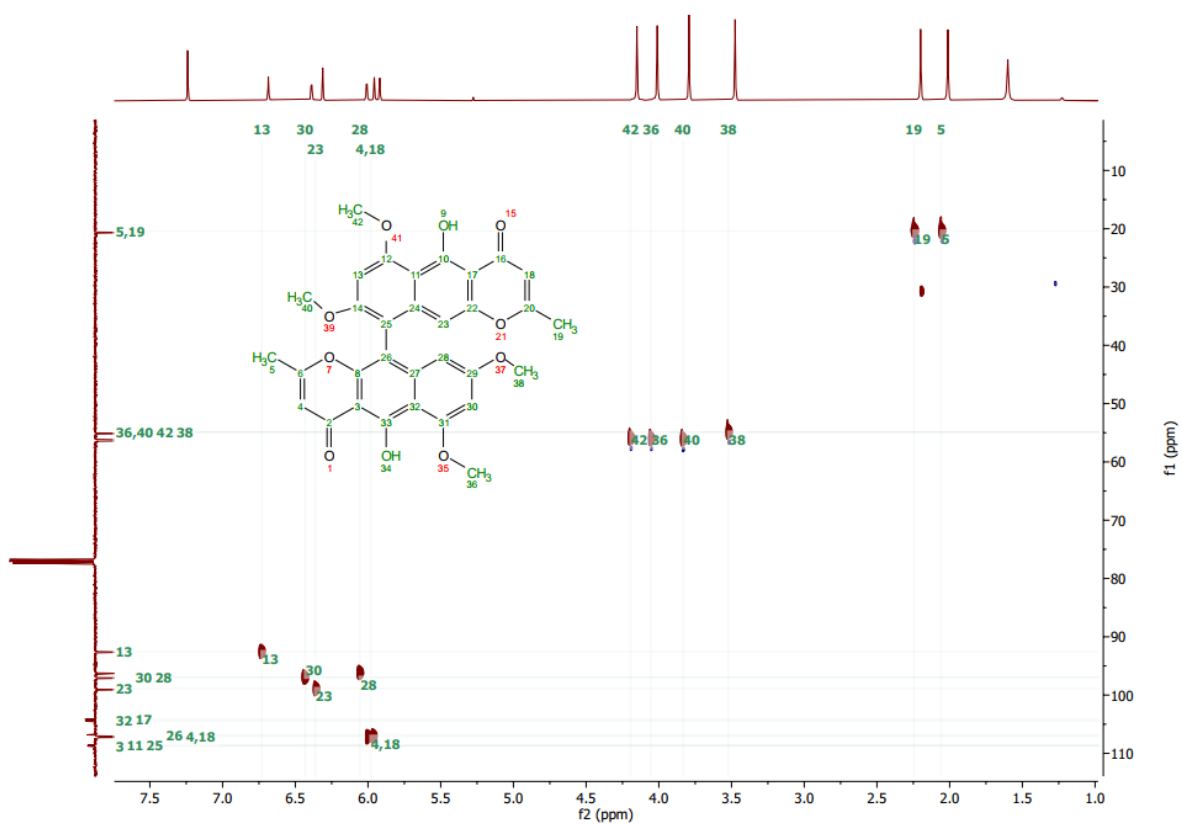


Figure S6: HSQC spectrum of compound **6** (Rubasperone B)

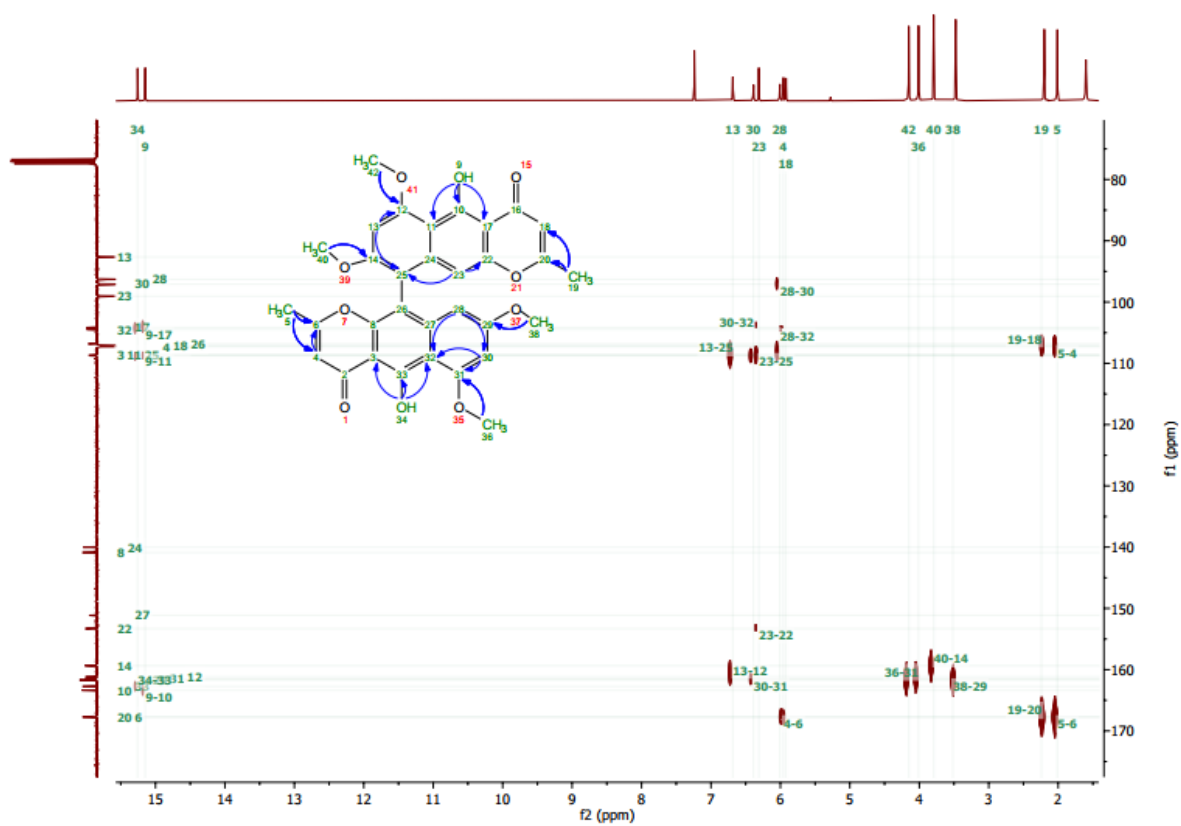


Figure S7: HMBC spectrum of compound **6** (Rubasperone B)

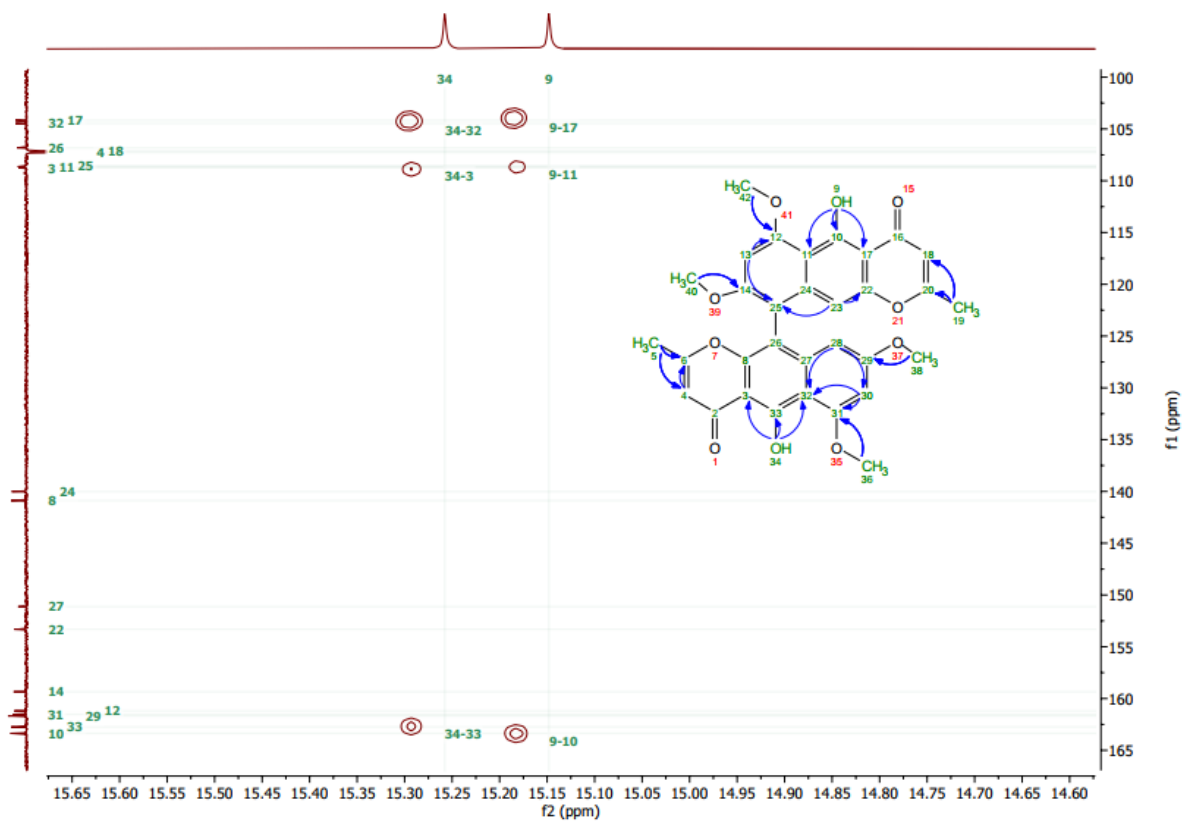


Figure S8: Expanded HMBC spectrum of compound **6** (Rubasperone B)

2. Docking study

Table S1. Docking scores of different modes of the tested *Aspergillus niger* secondary metabolites against the SARS-CoV-2 main therapeutic targets using AutoDock Vina.

Compound	M^{pro}				PL^{pro}			
Flavasperone (1)	mode	affinity (kcal/mol)	dist from best mode		mode	affinity (kcal/mol)	dist from best mode	
			rmsd l.b.	rmsd u.b.			rmsd l.b.	rmsd u.b.
	1	-7.1	0.000	0.000	1	-6.4	0.000	0.000
	2	-6.6	2.231	5.387	2	-5.9	17.761	20.243
	3	-6.6	1.294	3.710	3	-5.9	2.113	5.352
	4	-6.5	1.689	3.875	4	-5.8	1.838	2.760
	5	-6.5	4.044	5.294	5	-5.8	17.779	20.268
	6	-6.4	1.485	2.482	6	-5.7	19.124	21.735
	7	-6.3	2.651	4.350	7	-5.4	1.898	5.806
	8	-6.0	2.970	5.295	8	-5.4	2.376	5.709
	9	-6.0	2.650	7.324	9	-5.4	12.201	14.197
	10	-5.9	2.412	5.818	10	-5.3	19.105	21.772
Rubrofusarin B (2)	mode	affinity (kcal/mol)	dist from best mode		mode	affinity (kcal/mol)	dist from best mode	
			rmsd l.b.	rmsd u.b.			rmsd l.b.	rmsd u.b.
	1	-6.9	0.000	0.000	1	-5.9	0.000	0.000
	2	-6.6	2.281	4.247	2	-5.7	14.572	16.105
	3	-6.3	2.247	4.276	3	-5.7	0.988	5.964
	4	-6.3	2.116	5.631	4	-5.7	1.403	6.169
	5	-6.1	3.099	4.797	5	-5.6	13.230	16.328
	6	-6.1	2.646	4.732	6	-5.6	12.611	15.417
	7	-6.1	3.581	6.627	7	-5.6	12.195	14.620
	8	-6.0	2.617	3.078	8	-5.5	2.238	4.799
	9	-5.9	2.248	4.527	9	-5.5	12.691	14.529
	10	-5.9	15.316	17.291	10	-5.4	11.768	13.897

Aurasperone A (3)	mode	affinity	dist from best mode		mode	affinity	dist from best mode	
		(kcal/mol)	rmsd l.b.	rmsd u.b.		(kcal/mol)	rmsd l.b.	rmsd u.b.
	-----+-----+-----+-----							
	1	-8.1	0.000	0.000	1	-7.4	0.000	0.000
	2	-8.1	2.111	8.838	2	-7.3	3.505	5.716
	3	-7.8	2.067	8.569	3	-6.9	0.828	4.202
	4	-7.5	3.261	7.107	4	-6.7	2.991	5.455
	5	-7.4	3.168	5.677	5	-6.6	3.230	8.285
	6	-7.4	3.340	7.589	6	-6.6	2.942	6.901
	7	-7.1	2.942	5.224	7	-6.4	4.049	6.192
	8	-7.1	2.792	7.130	8	-6.2	9.927	15.348
Fonsecinone A (4)	9	-7.0	16.102	19.379	9	-6.2	3.119	6.988
	10	-6.6	4.166	8.987	10	-6.2	3.294	4.205
	-----+-----+-----+-----							
	mode	affinity	dist from best mode		mode	affinity	dist from best mode	
		(kcal/mol)	rmsd l.b.	rmsd u.b.		(kcal/mol)	rmsd l.b.	rmsd u.b.
	-----+-----+-----+-----							
	1	-8.5	0.000	0.000	1	-7.1	0.000	0.000
	2	-8.0	1.054	4.216	2	-6.9	2.595	6.628
	3	-7.6	3.221	6.325	3	-6.8	2.742	5.963
	4	-7.5	2.024	8.383	4	-6.7	3.228	8.051
	5	-7.1	5.070	9.583	5	-6.7	1.092	4.198
	6	-7.1	2.638	6.338	6	-6.6	3.595	6.780
	7	-7.0	3.835	7.213	7	-6.5	3.826	6.164
	8	-6.7	2.333	8.417	8	-6.3	2.138	4.246
	9	-6.6	4.033	7.799	9	-6.1	2.437	7.614
	10	-6.4	2.972	6.215	10	-6.1	2.536	5.854

Aspernigrin A (5)	mode	affinity	dist from best mode		mode	affinity	dist from best mode	
		(kcal/mol)	rmsd l.b.	rmsd u.b.		(kcal/mol)	rmsd l.b.	rmsd u.b.
	-----+-----+-----+-----							
	1	-6.2	0.000	0.000	1	-6.3	0.000	0.000
	2	-6.1	3.331	6.314	2	-6.1	2.842	3.914
	3	-6.1	2.051	2.719	3	-6.0	3.520	5.996
	4	-6.1	1.648	2.154	4	-5.8	4.555	6.053
	5	-6.0	4.186	5.386	5	-5.8	2.954	6.139
	6	-5.8	1.724	2.235	6	-5.7	2.629	6.604
	7	-5.8	4.008	6.227	7	-5.6	2.858	6.858
	8	-5.7	3.899	5.245	8	-5.4	2.769	6.340
Rubasperone B (6)	9	-5.6	3.273	4.566	9	-5.1	3.870	8.526
	10	-5.5	16.135	17.886	10	-5.1	12.465	14.959
	-----+-----+-----+-----							
	mode	affinity	dist from best mode		mode	affinity	dist from best mode	
		(kcal/mol)	rmsd l.b.	rmsd u.b.		(kcal/mol)	rmsd l.b.	rmsd u.b.
	-----+-----+-----+-----							
	1	-8.5	0.000	0.000	1	-6.8	0.000	0.000
	2	-8.2	1.534	7.601	2	-6.4	10.760	14.566
	3	-8.0	0.917	4.222	3	-6.4	10.255	14.248
	4	-7.2	2.318	5.398	4	-6.3	11.438	16.454
	5	-7.2	2.507	5.546	5	-6.3	11.599	15.282
	6	-7.1	2.525	6.626	6	-6.2	9.957	15.077
	7	-7.1	1.692	7.627	7	-6.1	10.335	14.933
	8	-7.1	2.344	5.022	8	-6.1	10.955	14.434
	9	-7.0	2.427	7.098	9	-6.1	11.210	16.876
	10	-6.8	2.280	7.784	10	-6.0	8.301	12.911

	mode	affinity	dist from best mode		mode	affinity	dist from best mode	
		(kcal/mol)	rmsd l.b.	rmsd u.b.		(kcal/mol)	rmsd l.b.	rmsd u.b.
Reference inhibitor *	1	-7.5	0.000	0.000	1	-6.7	0.000	0.000
	2	-7.3	1.508	2.119	2	-6.1	2.898	4.545
	3	-7.2	2.609	7.639	3	-6.1	2.107	2.846
	4	-7.2	3.889	7.746	4	-5.8	2.513	3.542
	5	-7.2	3.101	7.602	5	-5.7	2.924	7.823
	6	-7.2	2.219	7.211	6	-5.7	2.479	7.621
	7	-7.2	2.412	6.872	7	-5.7	2.730	5.295
	8	-7.0	2.028	7.396	8	-5.7	2.743	4.245
	9	-6.8	2.813	5.776	9	-5.7	2.693	5.104
	10	-6.8	2.805	8.289	10	-5.6	19.595	23.575

Compound	Helicase				RdRp			
Flavasperone (1)	mode	affinity	dist from best mode		mode	affinity	dist from best mode	
		(kcal/mol)	rmsd l.b.	rmsd u.b.		(kcal/mol)	rmsd l.b.	rmsd u.b.
	-----+-----+-----+-----							
	1	-7.6	0.000	0.000	1	-6.9	0.000	0.000
	2	-7.1	2.138	6.313	2	-6.3	21.429	26.697
	3	-6.6	1.902	5.095	3	-6.0	11.156	14.531
	4	-6.5	2.826	5.242	4	-6.0	23.537	26.194
	5	-6.5	1.641	5.606	5	-6.0	22.754	26.133
	6	-6.5	3.340	4.408	6	-6.0	22.161	25.421
	7	-6.4	3.124	5.393	7	-6.0	22.153	25.967
	8	-6.4	1.716	3.717	8	-5.9	24.513	27.463
	9	-6.3	3.519	5.993	9	-5.8	2.616	4.822
	10	-6.2	2.880	5.209	10	-5.8	22.732	25.849
Rubrofusarin B (2)	mode	affinity	dist from best mode		mode	affinity	dist from best mode	
		(kcal/mol)	rmsd l.b.	rmsd u.b.		(kcal/mol)	rmsd l.b.	rmsd u.b.
	-----+-----+-----+-----							
	1	-7.0	0.000	0.000	1	-6.8	0.000	0.000
	2	-6.8	1.665	3.684	2	-6.3	24.457	28.576
	3	-6.7	3.312	6.020	3	-6.2	26.828	29.390
	4	-6.7	1.696	3.528	4	-6.2	2.057	4.741
	5	-6.6	2.496	3.868	5	-6.0	26.933	28.414
	6	-6.6	1.916	6.692	6	-6.0	25.898	28.686
	7	-6.5	2.065	3.810	7	-5.8	23.027	26.744
	8	-6.3	8.228	12.363	8	-5.8	23.261	27.651
	9	-6.3	3.926	5.780	9	-5.7	24.368	27.564
	10	-6.2	2.336	6.455	10	-5.7	4.992	8.055

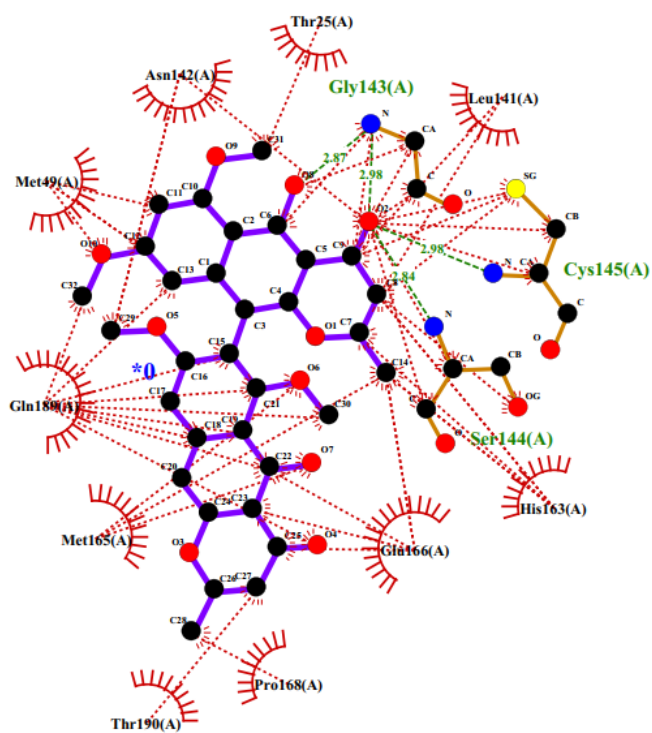
Aurasperone A (3)	mode	affinity	dist from best mode		mode	affinity	dist from best mode	
		(kcal/mol)	rmsd l.b.	rmsd u.b.		(kcal/mol)	rmsd l.b.	rmsd u.b.
	-----+-----+-----+-----							
	1	-8.0	0.000	0.000	1	-7.8	0.000	0.000
	2	-7.7	0.798	4.214	2	-7.7	2.589	8.664
	3	-7.4	2.632	6.858	3	-7.5	2.449	8.013
	4	-7.3	2.345	8.520	4	-7.5	2.826	6.008
	5	-7.0	3.283	4.882	5	-7.4	2.769	9.781
	6	-7.0	2.426	8.861	6	-7.4	2.554	3.889
	7	-6.9	2.509	6.477	7	-7.4	11.542	15.763
	8	-6.8	2.498	8.021	8	-7.3	3.475	9.212
Fonsecinone A (4)	9	-6.7	2.951	5.834	9	-7.2	4.225	8.543
	10	-6.7	2.550	6.257	10	-7.1	3.053	5.036
	-----+-----+-----+-----							
	mode	affinity	dist from best mode		mode	affinity	dist from best mode	
		(kcal/mol)	rmsd l.b.	rmsd u.b.		(kcal/mol)	rmsd l.b.	rmsd u.b.
	-----+-----+-----+-----							
	1	-8.1	0.000	0.000	1	-8.2	0.000	0.000
	2	-7.8	3.163	8.579	2	-8.0	1.329	4.415
	3	-7.6	10.553	14.851	3	-7.8	9.301	13.699
	4	-7.6	13.958	18.707	4	-7.7	25.228	29.473
	5	-7.5	12.482	16.428	5	-7.7	22.936	28.551
	6	-7.3	13.809	18.932	6	-7.7	23.984	29.211
	7	-7.2	12.804	17.450	7	-7.6	25.633	28.818
	8	-7.1	14.581	18.306	8	-7.5	25.924	29.895
	9	-7.1	2.520	5.600	9	-7.4	22.320	28.017
	10	-7.0	13.017	17.499	10	-7.3	7.853	12.962

Aspernigrin A (5)	mode	affinity	dist from best mode		mode	affinity	dist from best mode	
		(kcal/mol)	rmsd l.b.	rmsd u.b.		(kcal/mol)	rmsd l.b.	rmsd u.b.
	-----+-----+-----+-----							
	1	-6.7	0.000	0.000	1	-6.5	0.000	0.000
	2	-6.7	3.257	4.122	2	-6.4	10.658	11.724
	3	-6.6	1.382	2.256	3	-6.3	10.763	12.893
	4	-6.6	3.857	6.217	4	-6.1	13.179	14.281
	5	-6.5	4.619	6.679	5	-6.1	17.227	18.794
	6	-6.3	3.821	5.068	6	-6.1	15.115	16.205
	7	-6.2	4.260	6.464	7	-5.9	12.133	13.260
	8	-5.9	3.716	6.381	8	-5.8	12.810	13.810
	9	-5.9	4.299	6.361	9	-5.8	15.240	16.736
	10	-5.8	8.103	10.154	10	-5.8	10.881	11.855
Rubasperone B (6)	mode	affinity	dist from best mode		mode	affinity	dist from best mode	
		(kcal/mol)	rmsd l.b.	rmsd u.b.		(kcal/mol)	rmsd l.b.	rmsd u.b.
	-----+-----+-----+-----							
	1	-8.0	0.000	0.000	1	-7.9	0.000	0.000
	2	-8.0	1.399	7.722	2	-7.9	20.483	24.573
	3	-7.4	22.483	27.715	3	-7.7	7.246	11.382
	4	-7.4	1.284	7.571	4	-7.6	7.063	11.223
	5	-7.4	7.213	10.523	5	-7.6	19.189	23.222
	6	-7.2	20.221	25.155	6	-7.6	1.199	7.396
	7	-7.2	1.780	4.884	7	-7.6	20.569	24.294
	8	-7.1	17.225	22.425	8	-7.5	16.469	21.113
	9	-6.9	21.880	26.641	9	-7.5	15.575	20.480
	10	-6.9	17.043	21.971	10	-7.4	1.015	4.313

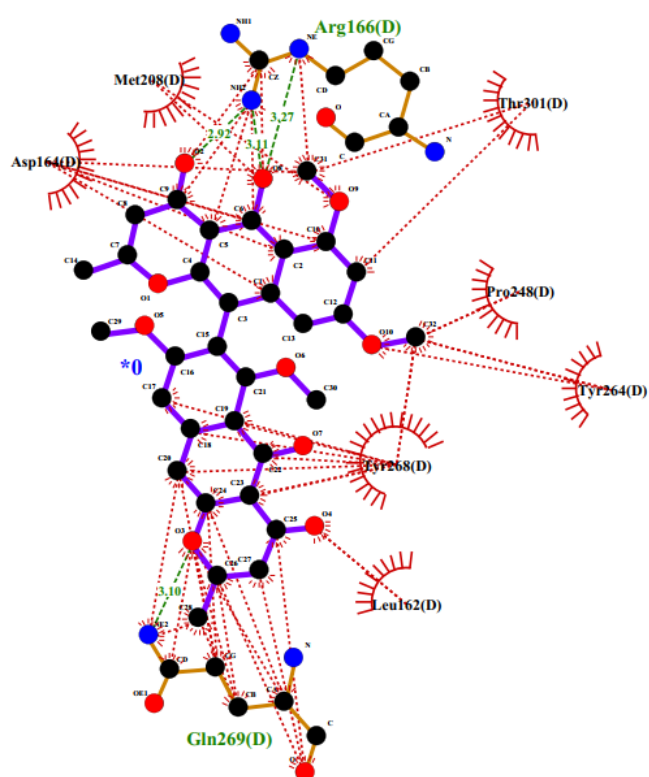
	mode	affinity	dist from best mode		mode	affinity	dist from best mode	
		(kcal/mol)	rmsd l.b.	rmsd u.b.		(kcal/mol)	rmsd l.b.	rmsd u.b.
	-----+-----+-----+-----				-----+-----+-----+-----			
Reference inhibitor *	1	-5.6	0.000	0.000	1	-6.6	0.000	0.000
	2	-5.6	1.327	1.875	2	-6.3	5.397	8.392
	3	-5.5	1.734	2.240	3	-6.2	22.787	24.573
	4	-5.3	5.191	6.949	4	-6.1	4.926	7.054
	5	-5.2	4.914	6.909	5	-5.8	5.815	8.191
	6	-5.2	2.439	3.066	6	-5.7	1.970	2.516
	7	-5.2	2.345	2.985	7	-5.7	1.767	2.708
	8	-5.0	11.659	12.890	8	-5.6	4.653	7.073
	9	-5.0	2.139	2.632	9	-5.6	19.973	22.022
	10	-4.8	3.730	4.446	10	-5.5	4.669	7.025

Spike protein											
Flavasperone (1)				Rubrofusarin B (2)				Aurasperone A (3)			
mode	affinity	dist from best mode		mode	affinity	dist from best mode		mode	affinity	dist from best mode	
	(kcal/mol)	rmsd l.b.	rmsd u.b.		(kcal/mol)	rmsd l.b.	rmsd u.b.		(kcal/mol)	rmsd l.b.	rmsd u.b.
1	-5.8	0.000	0.000	1	-5.8	0.000	0.000	1	-7.0	0.000	0.000
2	-5.6	3.567	6.478	2	-5.6	18.613	21.431	2	-6.8	1.306	2.776
3	-5.5	14.620	16.535	3	-5.6	18.397	21.710	3	-6.6	2.733	7.252
4	-5.5	7.673	12.049	4	-5.6	17.680	20.384	4	-6.3	11.151	16.172
5	-5.4	17.906	20.161	5	-5.6	16.972	19.089	5	-6.3	3.589	5.662
6	-5.4	3.608	6.019	6	-5.5	2.528	6.742	6	-6.3	2.025	5.708
7	-5.4	13.999	16.229	7	-5.4	2.925	7.388	7	-6.2	4.087	6.164
8	-5.4	17.583	19.559	8	-5.3	18.754	21.794	8	-6.1	1.792	5.097
9	-5.4	16.691	19.355	9	-5.3	17.672	21.625	9	-5.9	16.705	20.521
10	-5.2	15.770	18.059	10	-5.3	18.496	22.115	10	-5.9	6.938	14.178
Fonsecinone A (4)				Aspernigrin A (5)				Rubasperone B (6)			
mode	affinity	dist from best mode		mode	affinity	dist from best mode		mode	affinity	dist from best mode	
	(kcal/mol)	rmsd l.b.	rmsd u.b.		(kcal/mol)	rmsd l.b.	rmsd u.b.		(kcal/mol)	rmsd l.b.	rmsd u.b.
1	-7.1	0.000	0.000	1	-5.9	0.000	0.000	1	-7.0	0.000	0.000
2	-6.8	2.697	8.201	2	-5.8	16.990	18.120	2	-6.8	1.450	7.461
3	-6.8	3.821	9.148	3	-5.8	22.004	23.557	3	-6.7	20.708	25.292
4	-6.3	0.924	4.349	4	-5.8	1.075	1.996	4	-6.7	2.118	7.788
5	-6.3	3.822	9.597	5	-5.6	22.261	23.780	5	-6.5	2.491	5.756
6	-6.3	3.825	10.873	6	-5.5	16.056	17.331	6	-6.3	1.621	7.422
7	-6.3	4.851	9.724	7	-5.3	22.120	23.529	7	-6.2	2.818	5.650
8	-6.3	21.666	26.291	8	-5.2	12.286	14.708	8	-6.1	21.286	25.551
9	-6.2	4.801	8.786	9	-5.1	17.421	19.098	9	-6.1	2.561	6.912
10	-6.2	3.765	6.623	10	-5.1	22.748	24.661	10	-6.1	2.627	5.206

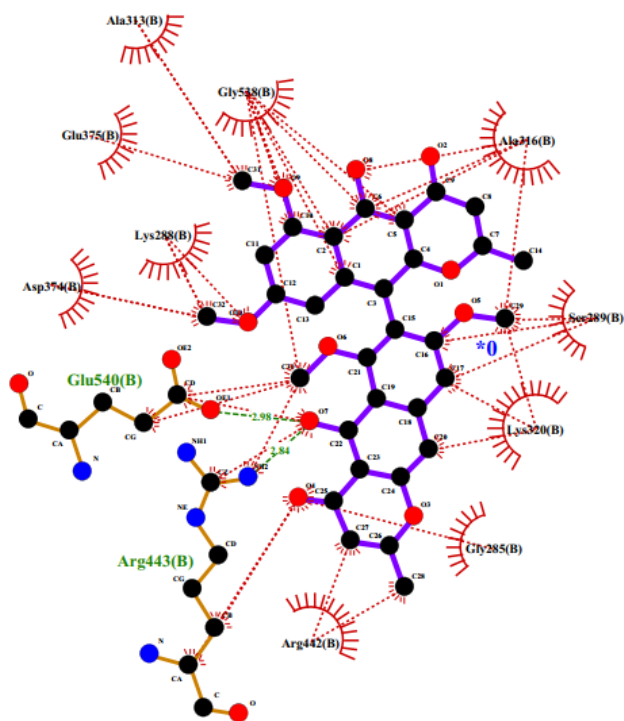
A.



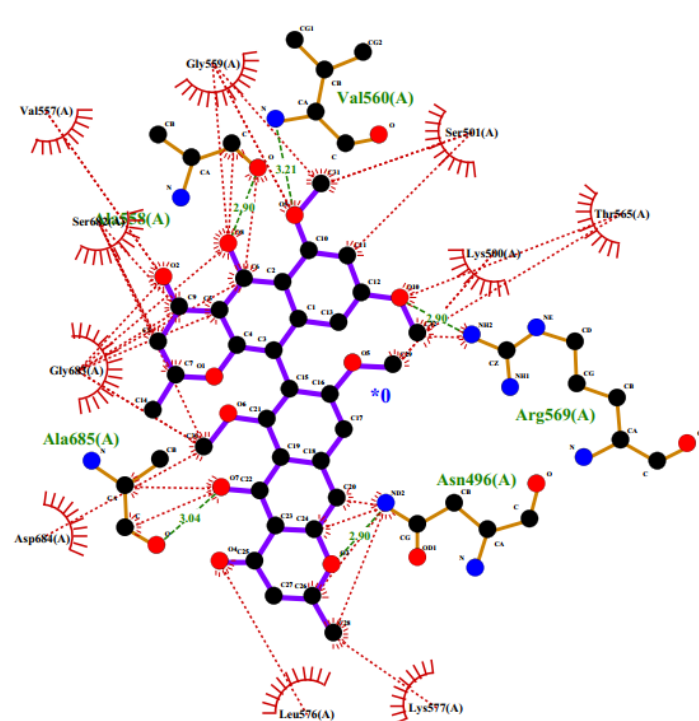
B.



C.



D.



E.

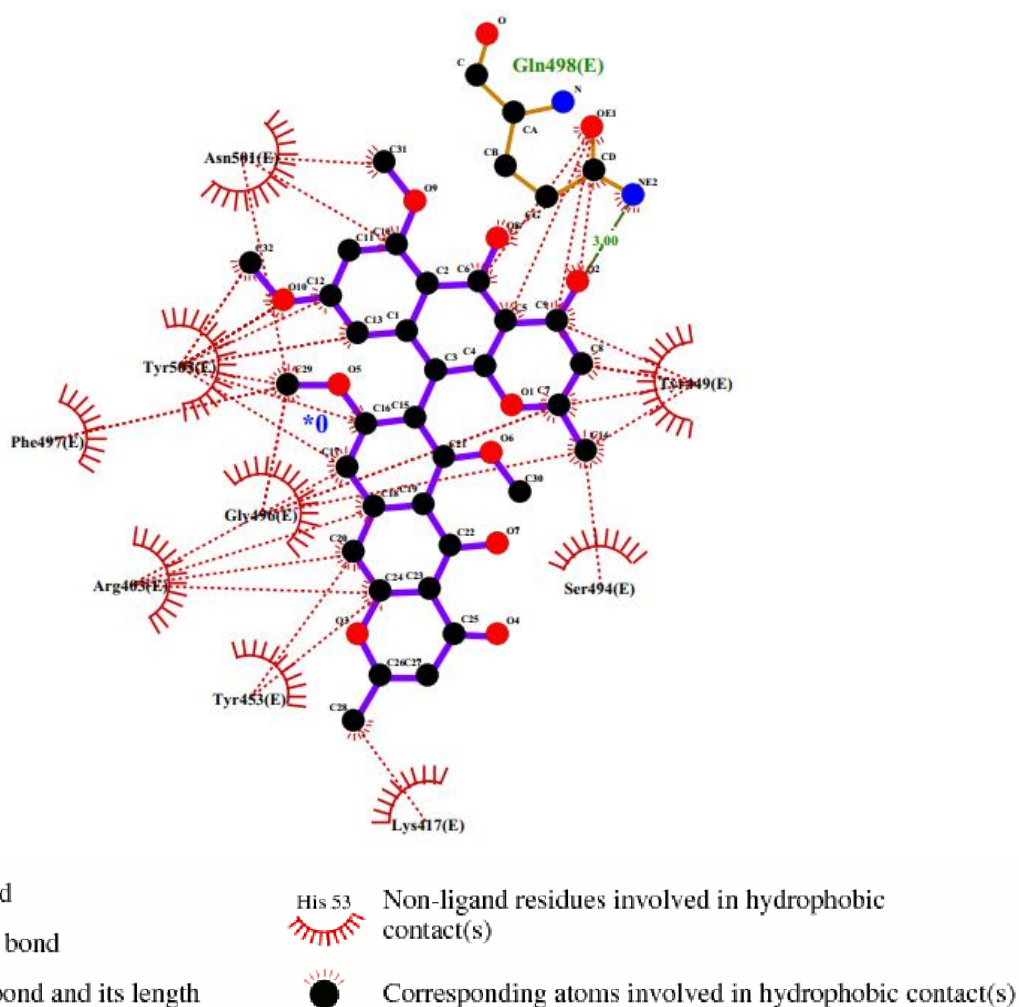


Figure S9. 2D plots of the most active compound (Aurasperone A (**3**)) binding within the active site of: A. M^{pro}; B. PL^{pro}; C. RNA helicase; D. RdRp; E. viral spike protein, showing the amino acid residues involved in the interaction.

3. Molecular Dynamics Simulation

Desmond v. 2.2 software was used for performing MDS experiments [1–3]. This software applies the OPLS-2005 force field. Protein systems were built using the System Builder option, where the protein structure was checked for any missing hydrogens, the protonation states of the amino acid residues were set (pH = 7.4), and the co-crystallized water molecules were removed. Thereafter, the whole structure was embedded in an orthorhombic box of TIP3P water together with 0.15 M Na⁺ and Cl[−] ions in 20 Å solvent

buffer. Afterward, the prepared systems were energy minimized and equilibrated for 10 ns. For protein-ligand complexes, the top-scoring poses were used as a starting points for simulation. Desmond software automatically parameterizes inputted ligands during the system building step according to the OPLS force field. For simulations performed by NAMD [4], the protein structures were built and optimized by using the QwikMD toolkit of the VMD software. The parameters and topologies of the compounds were calculated using the Charmm27 force field with the online software Ligand Reader and Modeler (<http://www.charmm-gui.org/?doc=input/ligandrm>, accessed on 16 April 2021) [5]. Afterward, the generated parameters and topology files were loaded to VMD to readily read the protein–ligand complexes without errors and then conduct the simulation step.

4. Binding Free Energy Calculations

Binding free energy calculations (ΔG) were performed using the free energy perturbation (FEP) method [4]. This method was described in detail in the recent article by Kim and coworkers [4]. Briefly, this method calculates the binding free energy $\Delta G_{\text{binding}}$ according to the following equation: $\Delta G_{\text{binding}} = \Delta G_{\text{Complex}} - \Delta G_{\text{Ligand}}$. The value of each ΔG is estimated from a separate simulation using NAMD software. Interestingly, all input files required for simulation by NAMD can be papered by using the online website CharmmGUI (<https://charmm-gui.org/?doc=input/afes.abinding>). Subsequently, we can use these files in NAMD to produce the required simulations using the FEP calculation function in NAMD. The equilibration was achieved in the NPT ensemble at 300 K and 1 atm (1.01325 bar) with Langevin piston pressure (for “Complex” and “Ligand”) in the presence of the TIP3P water model. Then, 10 ns FEP simulations were performed for each compound, and the last 5 ns of the free energy values was measured for the final free energy values [4]. Finally, the generated trajectories were visualized and analyzed using VMD software. It worth noting that Ngo and co-workers in their recent benchmarking study found that the FEP method of determination of ΔG was the most accurate method in terms of predicting M^{Pro} inhibitors [5].

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