

In Silico Discovery and Evaluation of Inhibitors of the SARS–CoV–2 Spike Protein–HSPA8 Complex Towards Developing COVID-19 Therapeutic Drugs





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Query	Liver Toxicity		Metabolism							Membrane Transporters			Others			
	DILI	Cyto-toxicity	HLM	Cyp Inhibitors for					BBB	P-gp Inhibitor	P-gp Substrate	hERG Blocker	MMP	AMES	MRTD (mg/day)	
				1A2	3A4	2D6	2C9	2C19								
	Yes	No	Yes	No	No	No	No	No	No	Yes	No	No	Yes	No	196	
	Yes	No	No	No	No	No	No	No	No	Yes	No	No	Yes	Yes	209	
	No	No	Yes	No	Yes	No	No	No	No	No	No	No	No	Yes	2092	
	No	No	Yes	No	No	No	No	No	Yes	Yes	No	No	Yes	No	65	

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⚠ - Outside applicability domain - no high-confidence prediction available




Figure S1. Results from the ADMET analysis of the four small molecules NSC44175, NSC11926, NSC36398, and NSC281245 [40]. The meaning of the colour coding is as follows: Green: desirable or optimal properties; Red: undesirable or poor properties; Red stripes: may pose risks or fail to meet desirable criteria; Green stripes: favourable properties.

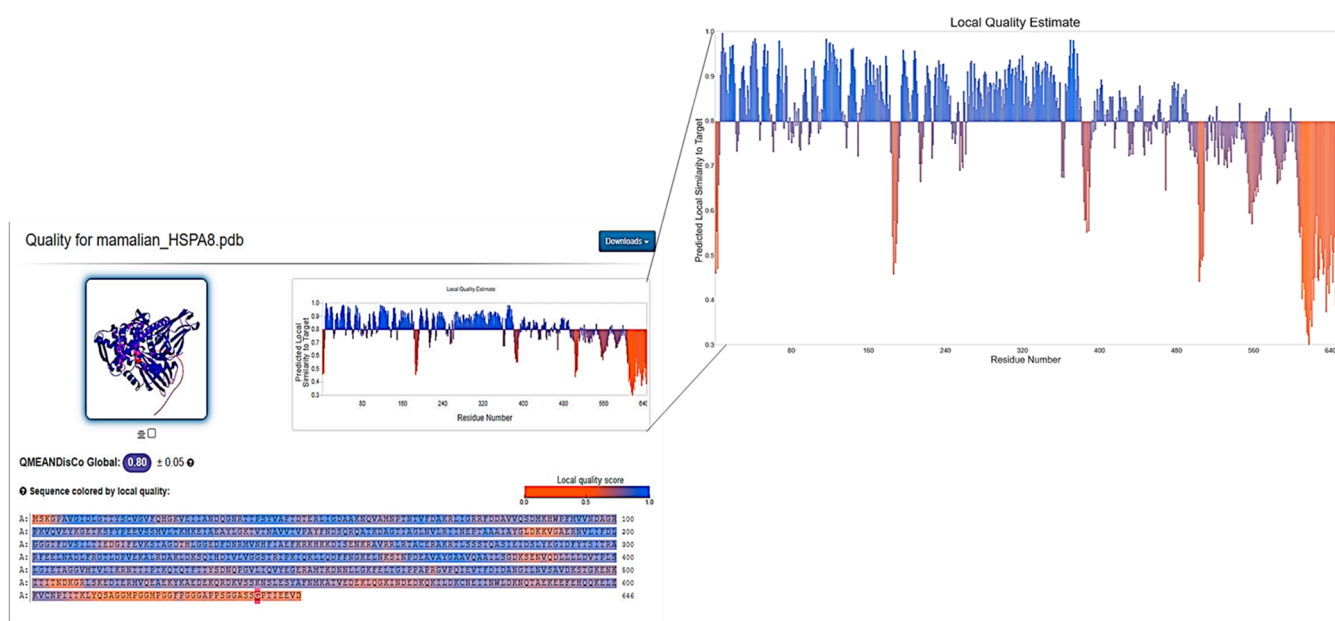


Figure S2. Qmean validation results of the modelled human HSPA8 [27].



Figure S3. Qmean validation results of the modelled SARS-CoV-2 spike protein [27].

Table S1: Protein-protein docking best pose from Bioluminate4.6 (Maestro, Schrödinger suite) [31].

Title	PIPER Cluster Size	PIPER pose energy (kcal/mol)	PIPER pose score	PIPER Model Number
prot-prot-docking_2_pose_1	65	-748.301	-101.189	11
prot-prot-docking_2_pose_2	64	-736.403	-125.859	17
prot-prot-docking_2_pose_3	50	-622.428	-156.643	736
prot-prot-docking_2_pose_4	24	-616.269	-32.505	847
prot-prot-docking_2_pose_5	22	-694.401	-39.79	86
prot-prot-docking_2_pose_6	22	-618.513	-112.32	809
prot-prot-docking_2_pose_7	19	-635.099	-33.974	519
prot-prot-docking_2_pose_8	16	-644.73	-98.929	392
prot-prot-docking_2_pose_9	16	-867.403	337.213	2
prot-prot-docking_2_pose_10	15	-643.026	-63.452	414
prot-prot-docking_2_pose_11	15	-820.341	105.533	3
prot-prot-docking_2_pose_12	15	-660.966	300.027	233
prot-prot-docking_2_pose_13	13	-617.408	-141.853	826
prot-prot-docking_2_pose_14	12	-878.788	283.618	1
prot-prot-docking_2_pose_15	12	-796.446	73.679	5
prot-prot-docking_2_pose_16	12	-652.552	-117.022	313
prot-prot-docking_2_pose_17	12	-624.942	-91.458	682
prot-prot-docking_2_pose_18	11	-907.046	288.66	0
prot-prot-docking_2_pose_19	11	-699.018	310.646	75
prot-prot-docking_2_pose_20	11	-697.958	287.631	76
prot-prot-docking_2_pose_21	11	-641.266	387.979	434
prot-prot-docking_2_pose_22	10	-716.187	440.715	47
prot-prot-docking_2_pose_23	10	-702.358	179.046	67
prot-prot-docking_2_pose_24	10	-680.55	248.557	127
prot-prot-docking_2_pose_25	10	-659.919	-109.081	246
prot-prot-docking_2_pose_26	10	-654.341	294.355	297
prot-prot-docking_2_pose_27	10	-637.711	98.584	489
prot-prot-docking_2_pose_28	9	-687.039	-175.113	103
prot-prot-docking_2_pose_29	7	-661.378	-49.428	228
prot-prot-docking_2_pose_30	5	-649.847	62.952	341

Table S2: Overall protein-protein interactions from the SARS-CoV-2 spike protein-HSPA8 complex [31].

HSPA8 residues	Spike protein residues	Distance(A)	Specific Interactions	#HB
H:THR 411	A:THR 478	1.6	1x hb to A:Thr 478	1
H:PRO 468	B:ASN 370	1.6	1x clash to B:Asn 370	0
H:VAL 471	B:SER 371	1.8		0
H:LYS 493	B:LEU 335	1.8		0
H:THR 405	A:THR 478	1.9		0
H:TYR 431	A:PHE 486	1.9		0
H:GLN 426	A:SER 477	1.9	1x hb to A:Ser 477	1
H:TYR 431	A:TYR 489	1.9		0
H:ALA 406	A:GLY 485	1.9		0
H:ARG 469	A:GLU 484	2.0	1x hb, 1x salt bridge to A: Glu 484	1
H:PHE 428	A:ASN 487	2.0	1x clash to A:Asn 487	0
H:THR 429	A:PHE 486	2.1	1x clash to A:Phe 486	0

H:ARG 469	A:GLN 493	2.1	1x hb to A:Gln 493	1
H:GLY 407	A:VAL 483	2.2		0
H:THR 429	A:TYR 489	2.2		0
H:ALA 406	A:CYS 488	2.2		0
H:THR 429	A:ALA 475	2.2		0
H:GLN 473	B:ASP 364	2.2	1x hb to B:Asp 364	1
H:ARG 469	A:PHE 490	2.2		0
H:GLY 470	A:PHE 456	2.2		0
H:ALA 406	A:PHE 486	2.2		0
H:ALA 467	B:SER 371	2.2		0
H:GLN 473	B:VAL 367	2.2		0
H:VAL 438	A:PHE 486	2.3		0
H:THR 405	A:PHE 486	2.3		0
H:GLN 473	B:LEU 335	2.3		0
H:TYR 431	A:GLY 485	2.3		0
H:GLY 470	A:TYR 489	2.3		0
H:ILE 403	A:PHE 486	2.3		0
H:PRO 468	B:SER 371	2.3		0
H:GLU 404	A:PHE 486	2.4		0
H:THR 430	A:TYR 489	2.4		0
H:VAL 471	B:VAL 367	2.4		0
H:VAL 409	A:ASN 481	2.4		0
H:ASP 433	A:GLN 493	2.4	1x hb to A:Gln 493	1
H:LYS 493	B:THR 333	2.5	1x hb to B:Thr 333	1
H:VAL 409	A:PRO 479	2.5		0
H:THR 411	A:SER 477	2.5		0
H:THR 429	A:ASN 487	2.5		0
H:ARG 469	A:PHE 456	2.5		0
H:THR 405	A:ASN 487	2.6		0
H:VAL 471	B:ASN 370	2.6		0
H:VAL 409	A:THR 478	2.6		0
H:ILE 440	A:PHE 486	2.6		0
H:GLU 475	B:THR 333	2.7		0
H:SER 494	B:GLY 339	2.7		0
H:LYS 493	B:ASN 334	2.8		0
H:MET 410	A:THR 478	2.8		0
H:PHE 428	A:THR 478	2.8		0
H:ALA 406	A:VAL 483	2.9		0
H:ALA 406	A:ASN 487	2.9		0
H:SER 494	B:PRO 337	2.9		0
H:THR 427	A:GLY 476	2.9		0
H:PHE 428	A:PHE 486	3.0		0
H:ALA 406	A:GLU 484	3.0		0
H:THR 411	A:PRO 479	3.0		0
H:ARG 469	A:LEU 492	3.0		0
H:THR 430	A:PHE 486	3.1		0
H:THR 425	B:THR 333	3.1		0
H:PRO 465	B:ASN 343	3.1		0
H:ARG 469	A:LEU 455	3.2		0
H:ARG 469	A:TYR 489	3.2		0
H:SER 494	B:CYS 336	3.2		0

H:MET 410	A:PRO 479	3.2	0
H:VAL 409	A:CYS 480	3.3	0
H:GLY 407	A:GLY 485	3.3	0
H:SER 494	B:PHE 338	3.3	0
H:PRO 466	B:ASN 343	3.4	0
H:SER 494	B:GLU 340	3.4	0
H:VAL 476	A:PHE 486	3.4	0
H:GLN 426	A:GLY 476	3.4	0
H:PRO 468	A:GLN 493	3.5	0
H:GLN 426	A:THR 478	3.5	0
H:LYS 493	B:PRO 337	3.6	0
H:PHE 428	A:GLY 476	3.6	0
H:PRO 468	A:LEU 455	3.6	0
H:VAL 471	A:PHE 456	3.6	0
H:THR 427	A:ALA 475	3.6	0
H:VAL 412	A:SER 477	3.7	0
H:THR 495	B:GLU 340	3.7	0
H:LYS 493	B:CYS 336	3.7	0
H:ALA 406	A:CYS 480	3.7	0
H:THR 427	A:ASN 487	3.7	0
H:ARG 469	B:ASN 370	3.8	0
H:ILE 474	A:PHE 486	3.8	0
H:PRO 465	B:GLY 339	3.8	0
H:THR 411	A:GLY 476	3.8	0
H:LYS 493	B:VAL 362	3.9	0

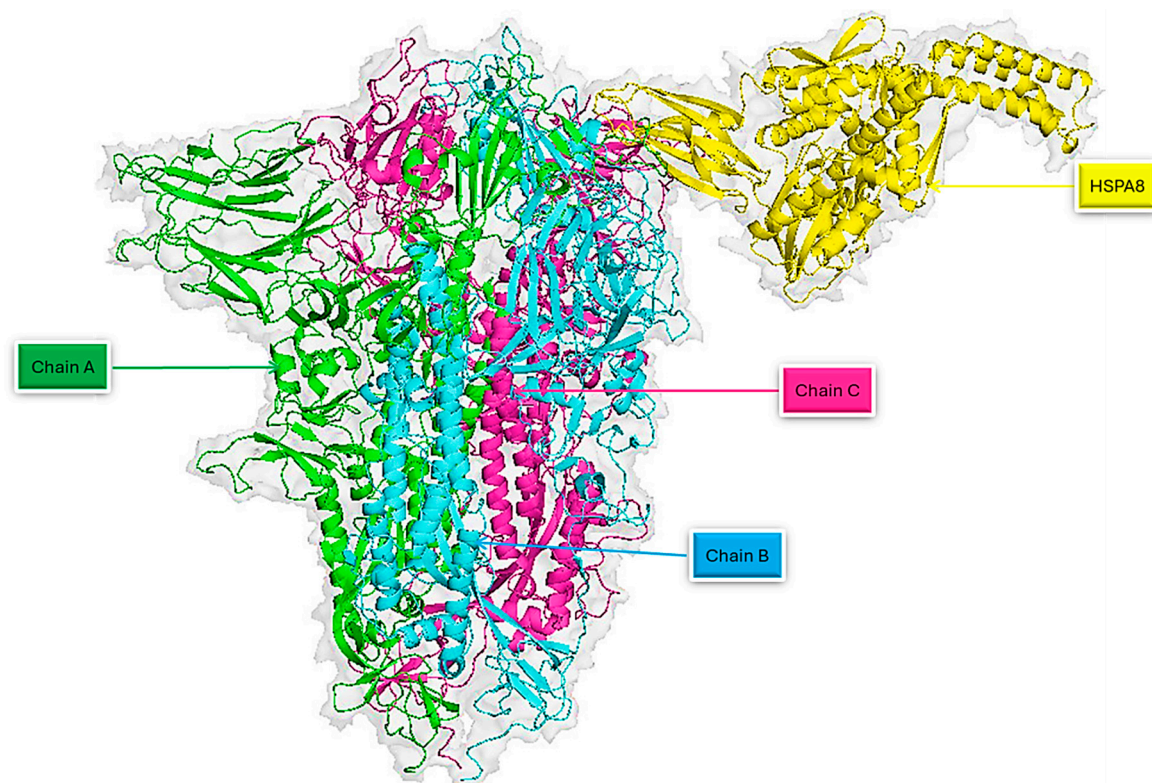


Figure S4: Structural insight into the HSPA8:spike protein complex: A BioLuminate-derived protein-protein docking study revealing intriguing interactions [31,44].

Table S3: Sitescore, dscore, volume, exposure, enclosure, contact, phobic, philic and balance of human HSPA8 and SARS-CoV-2 spike protein from the identified active sites obtained from SiteMap [33–35].

Protein	Site	Sitescore	Size	Dscore	Volume	Exposure	Enclosure	Contact	Phobic	Philic	Balance
HSPA8	1	1.114	230	0.935	341.2854	0.307	0.868	1.128	0.301	1.614	0.186
	2	1.031	165	0.922	407.827	0.531	0.744	0.971	0.181	1.423	0.127
	3	1.005	100	0.991	288.806	0.543	0.706	0.965	0.318	1.142	0.278
	4	0.927	87	0.940	166.698	0.571	0.655	0.859	0.542	1.012	0.536
	5	0.835	64	0.745	161.896	0.579	0.671	0.892	0.136	1.257	0.108
Spike protein	4	1.105	877	1.148	3367.574	0.507	0.791	0.936	0.990	0.817	1.212
	3	1.057	916	1.034	2404.773	0.448	0.783	0.992	0.434	1.151	0.377
	5	1.054	715	1.034	2190.398	0.516	0.779	1.017	0.570	1.148	0.497
	2	1.033	966	1.031	2715.188	0.535	0.748	0.929	0.419	1.097	0.382
	1	1.031	1344	1.052	4515.595	0.582	0.734	0.897	0.439	1.006	0.437
HSPA8:spike complex	4	1.096	875	1.135	3361.057	0.516	0.786	0.925	0.902	0.849	1.063
	5	1.049	682	1.03	2181.48	0.553	0.771	0.993	0.648	1.144	0.566
	1	1.042	1174	1.044	3309.95	0.508	0.761	0.933	0.541	1.082	0.5
	2	1.037	1126	1.03	3186.813	0.514	0.753	0.93	0.438	1.109	0.395
	3	1.035	1086	1.036	3155.6	0.521	0.751	0.928	0.443	1.086	0.408

Table S4: Amino acid residues located on the most preferred binding sites of human HSPA8, SARS-CoV-2 spike protein, and HSPA8:spike protein complex [34,45].

Amino acid residues on the most preferred binding site of human HSPA8.	Amino acid residues on the most preferred binding site of the SARS-CoV-2 spike protein.	Amino acid residues on the most preferred binding site of the HSPA8:spike protein complex
ASP10, LEU11, GLY12, THR13, THR14, TYR15, SER16, CYS17, THR37, PRO39, SER40, TYR41, LYS56, LYS71, THR145, PRO147, ALA148, GLU175, PRO176, ASH199, LEU200, GLY201, GLY202, GLY203, THR204, PHE205, ASP206, GLY230, GLU231, ASP234, ARG261, ARG264, THR265, GLU268, LYS271, ARG272, SER275, VAL337, GLY338, GLY339, SER340, ARG342, ILE343, PRO365, ASP366, GLU367, ALA368, VAL369, ALA370, TYR371	GLU725, ILE726, LEU727, PRO728, MET731, THR732, GLN762, LEU763, ARG765, ALA766, LEU767, GLY769, ILE770, VAL772, GLU773, GLN774, LYS776, GLU780, VAL781, GLN784, GLY946, LYS947, LEU948, ASP950, VAL951, GLN954, ASN955, GLN957, ALA958, LEU959, THR961, LEU962, GLN965, SER1003, THR1006, TYR1007, GLN1010, GLN1011, LEU1012, ILE1013, ARG1014, ALA1015, ALA1016, GLU1017, ILE1018, ARG1019, ALA1020, SER1021, ALA1022, ASN1023, LEU1024, ALA1026, THR1027, LYS1028, SER1030, GLU1031, LEU1034, ARG1039, VAL1040, ASP1041,	THR723, GLU725, ILE726, LEU727, PRO728, MET731, THR732, PHE759, GLN762, LEU763, ARG765, ALA766, LEU767, GLY769, ILE770, VAL772, GLU773, GLN774, LYS776, GLU780, GLN784, SER946, LYS947, LEU948, ASP950, VAL951, LEU952, LEU953, GLN954, ASN955, GLN957, ALA958, THR961, LEU962, GLN965, SER1003, GLN1005, THR1006, TYR1007, VAL1008, GLN1010, GLN1011, LEU1012, ILE1013, ARG1014, ALA1015, ALA1016, GLU1017, ILE1018, ARG1019, ALA1020, SER1021, ALA1022, ASN1023, LEU1024, ALA1026, THR1027, LYS1028, SER1030, GLU1031, LEU1034, ARG1039, VAL1040, ASP1041, PHE1042, CYX1043, GLY1044, LYS1045, HIE1064

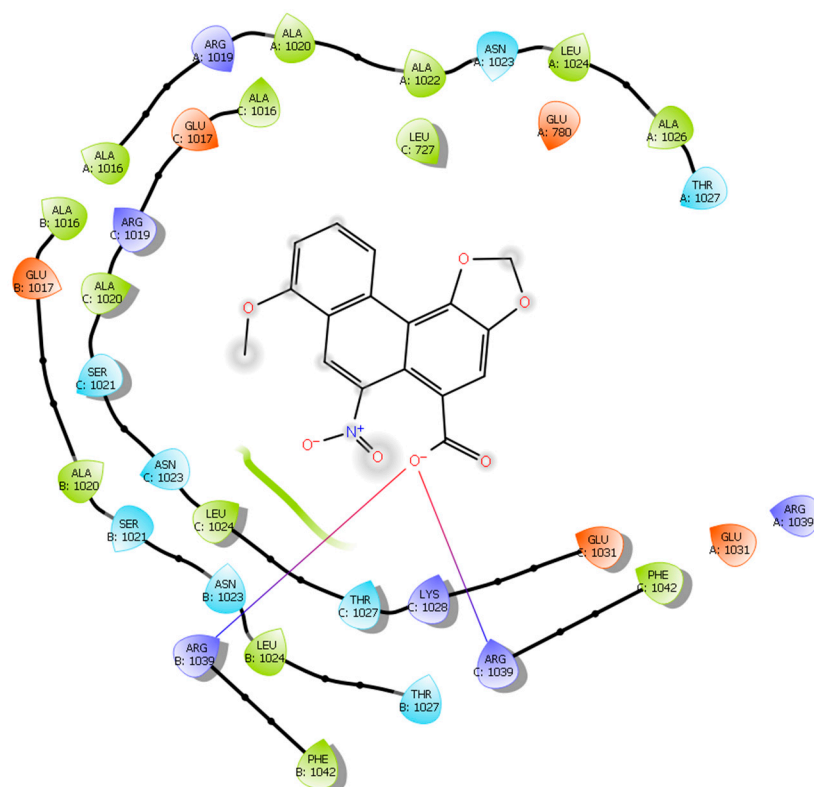
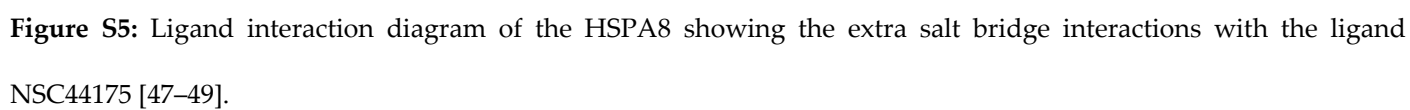


Figure S6: Ligand interaction diagram of the protein complex, HSPA8:spike protein, showing the additional salt bridge interactions with NSC11926 [38,48,49].