Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Crystallography based investigation of weak interaction for drug designing against COVID-19

Nayim Sepay,*[a,b] Pranab Saha,[a] Zarrin Shahzadi,[b] Aratrika Chakraborty[b] and Umesh Chandra Halder*[a]

[a]Department of Chemistry, Jadavpur University, Kolkata 700 032, India

[b]Department of Chemistry, Lady Brabourne College, Kolkata 700 017, India.

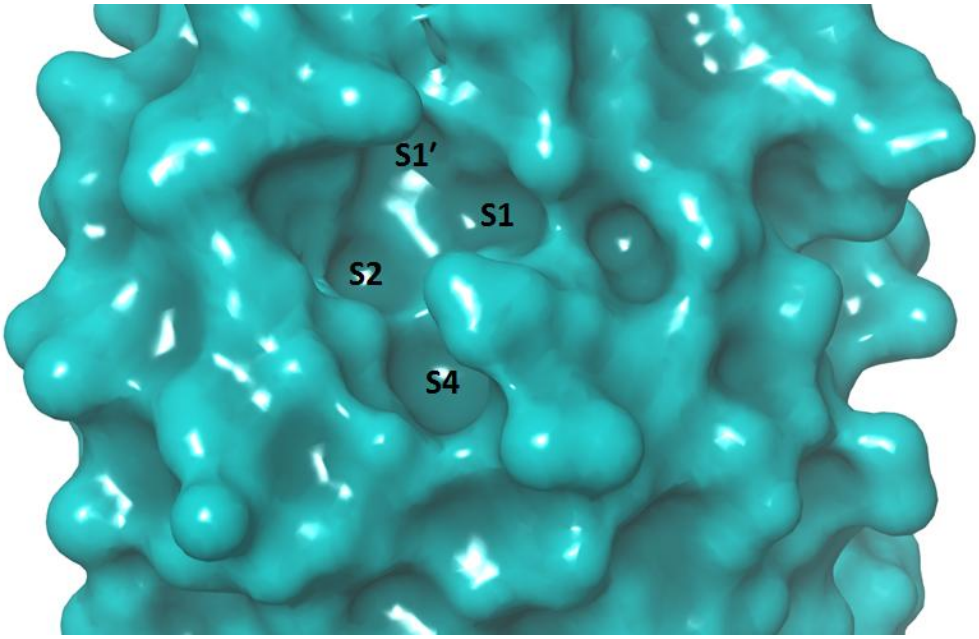


Figure S1: The major domains inside the active site of SARS-CoV-2 M^{pro} protein

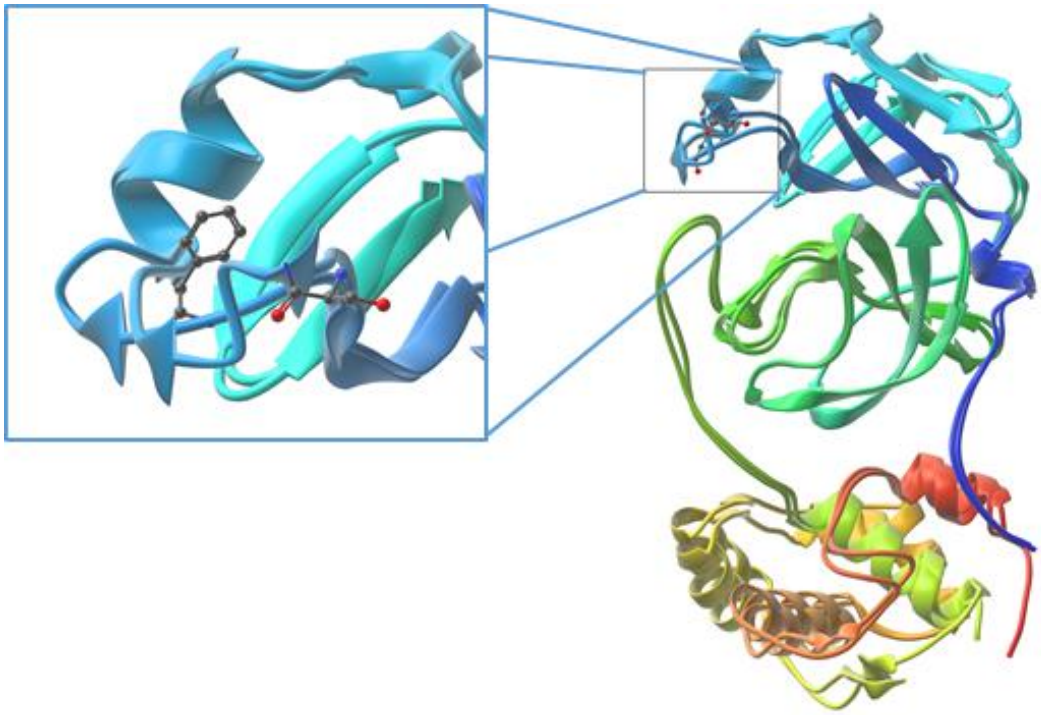


Figure S2: The mutation of amino acid 46 of SARS-CoV M^{pro} with Ser residue was found at the active site of SARS-CoV-2 M^{pro} (PDB ids of SARS-CoV M^{pro} and SARS-CoV-2 M^{pro} is 1P9S and 6LU7).

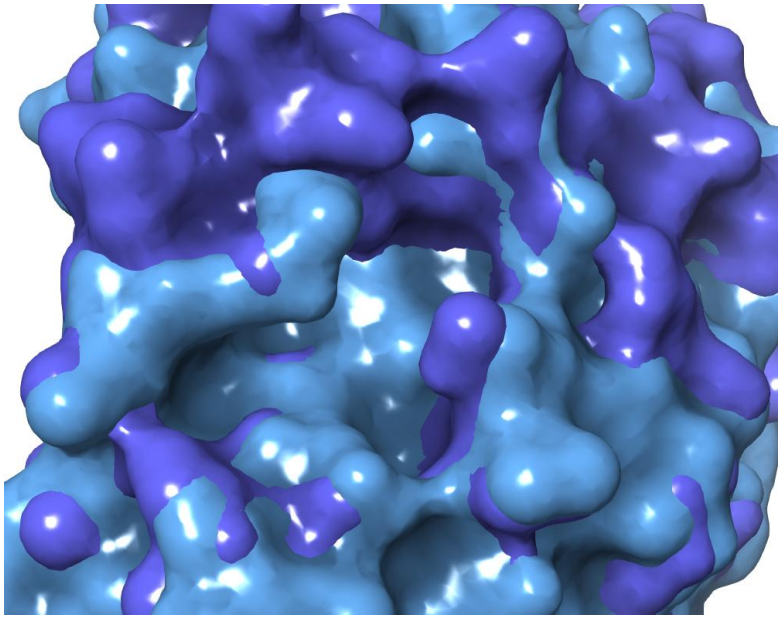


Figure S3: The change of depth and size of the active site of SARS-CoV-2 M^{pro} for the mutation (PDB ids of SARS-CoV M^{pro} and SARS-CoV-2 M^{pro} is 1P9S and 6LU7).

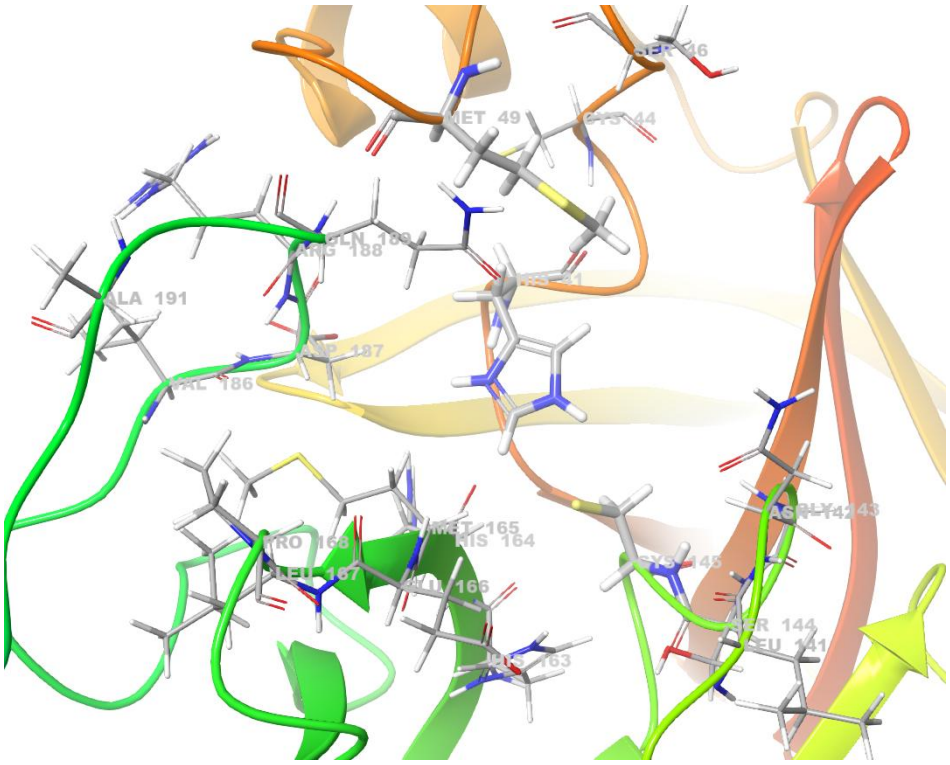
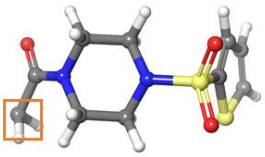


Figure S4: The constituent amino acid residues of SARS-CoV-2 M^{pro}.

Covalently attached small molecules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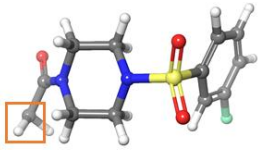
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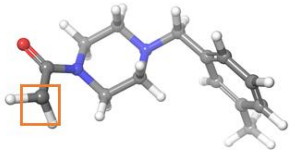
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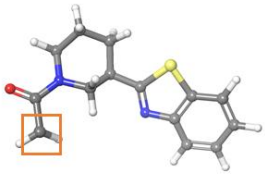
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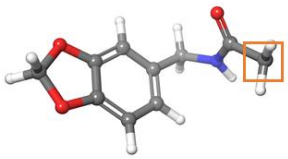
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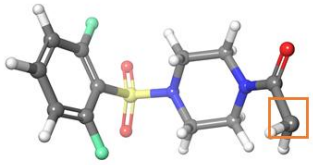
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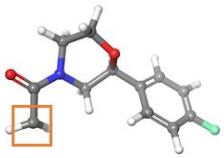
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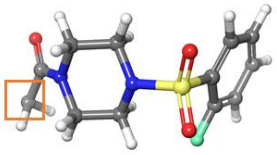
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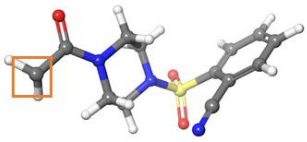
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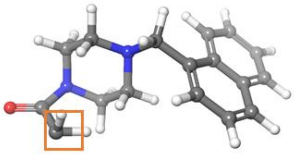
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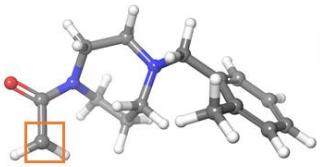
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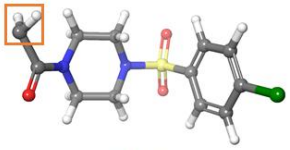
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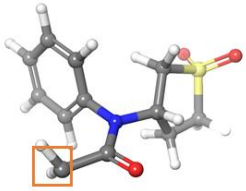
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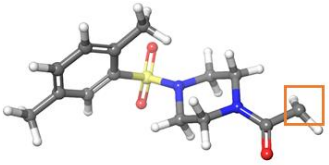
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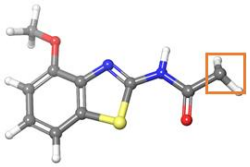
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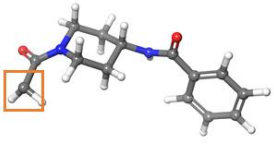
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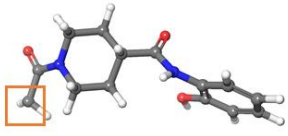
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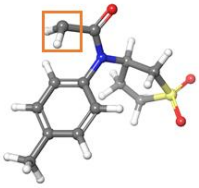
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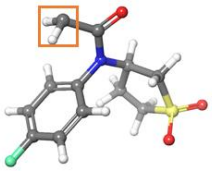
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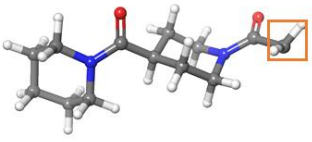
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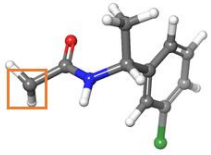
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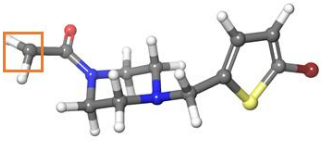
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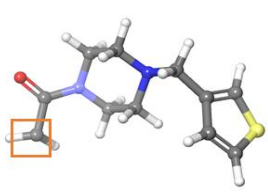
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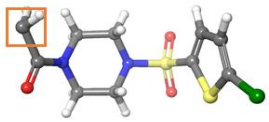
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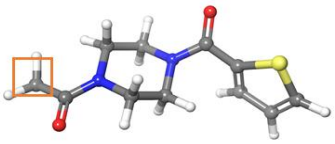
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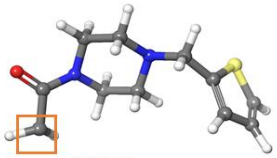
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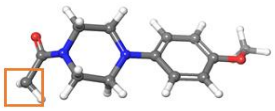
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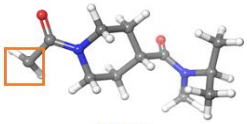
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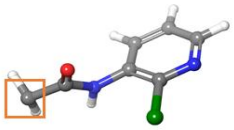
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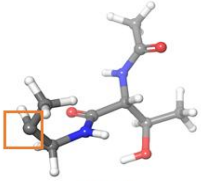
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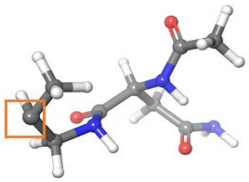
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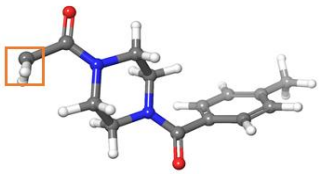
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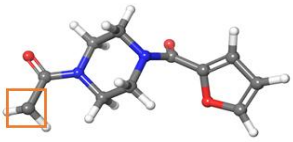
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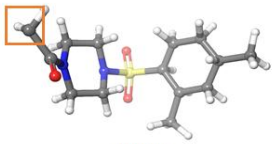
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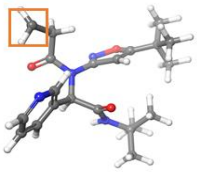
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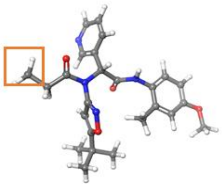
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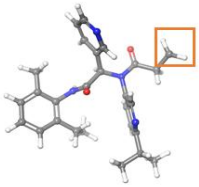
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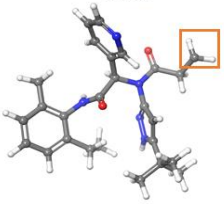
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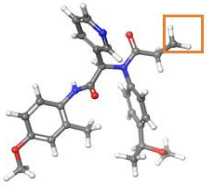
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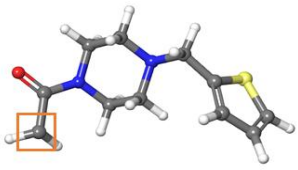
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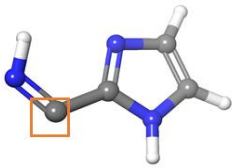
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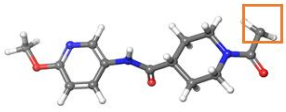
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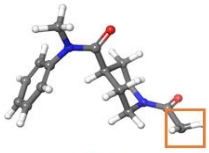
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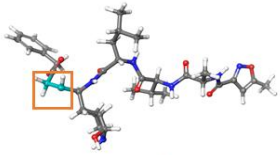
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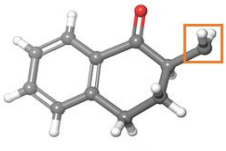
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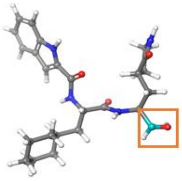
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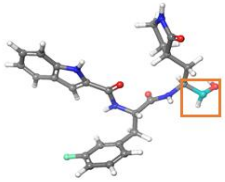
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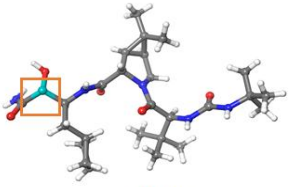
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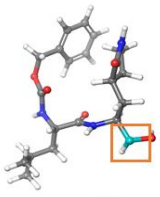
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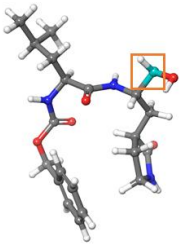

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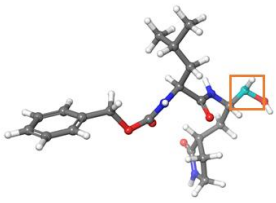

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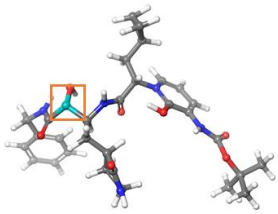

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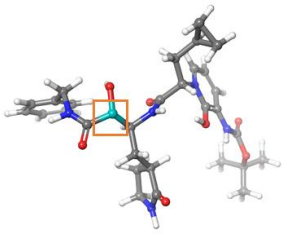

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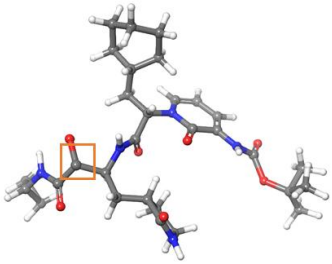

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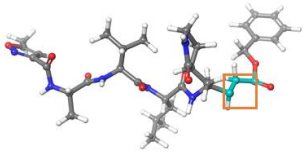

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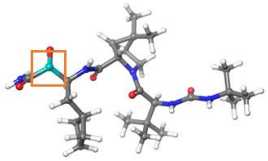

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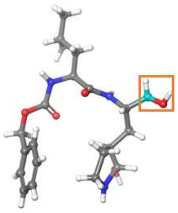

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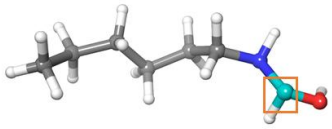

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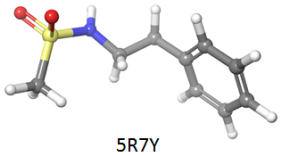
Non-covalently attached small molecules



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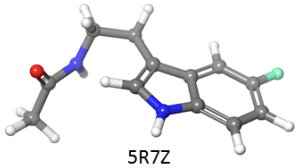

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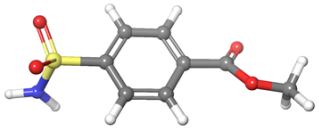

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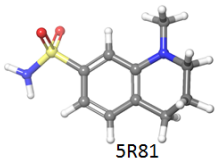

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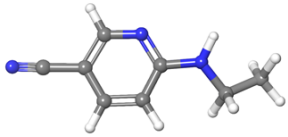

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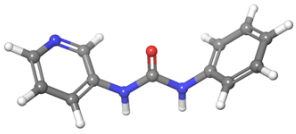

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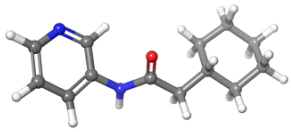

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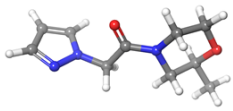

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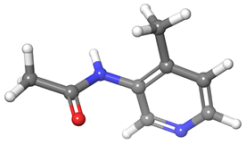

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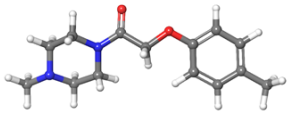

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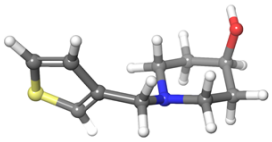

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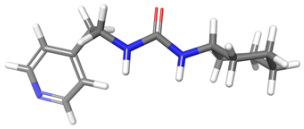

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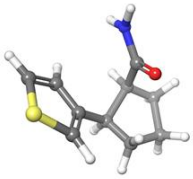

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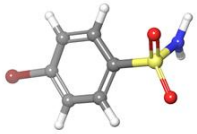

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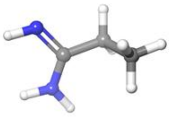

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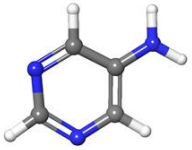

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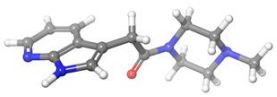

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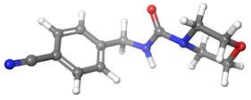

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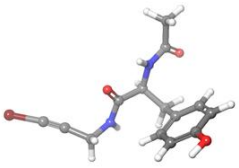

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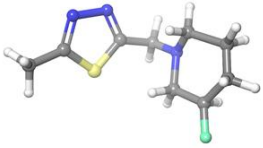

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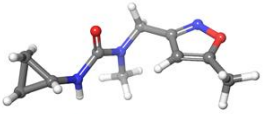

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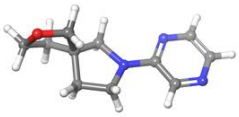

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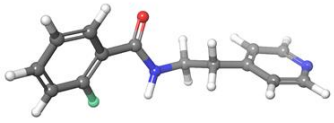

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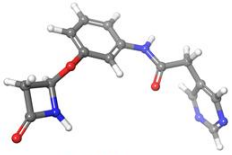

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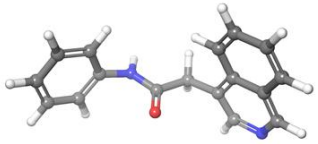

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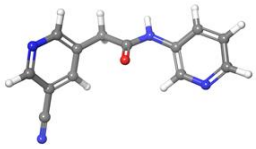

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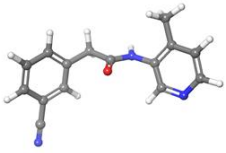

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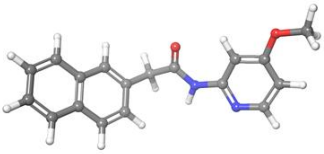

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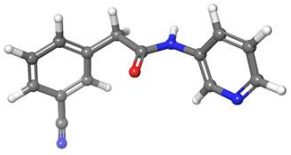

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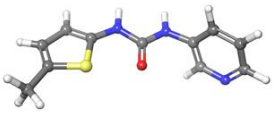

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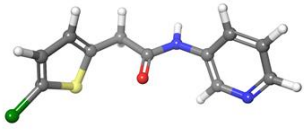

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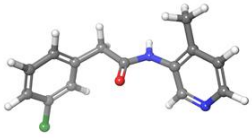

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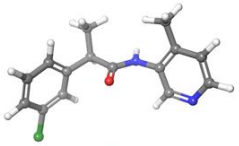

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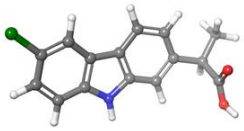

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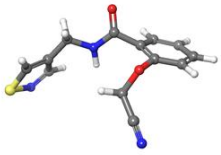

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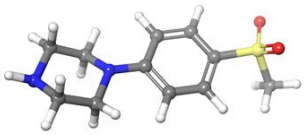

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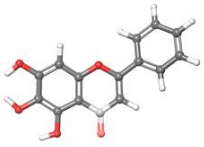

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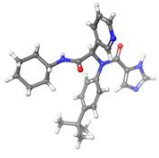


6W63

Small molecules attached non-covalently at outside of the active side

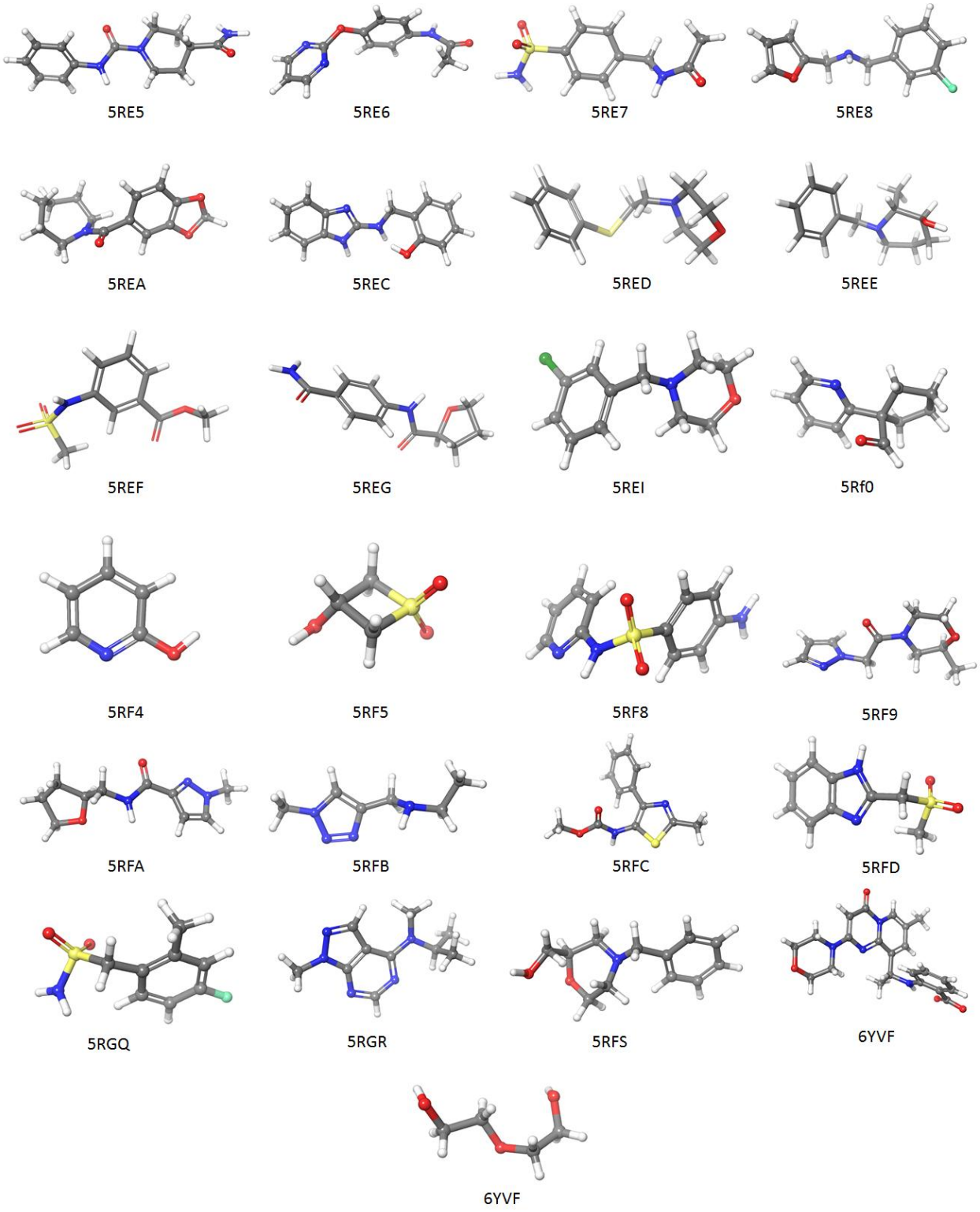


Figure S5: Small molecules found in the SARS CoV-2 M^{pro} protein crystal as a bound ligand through different binding modes.

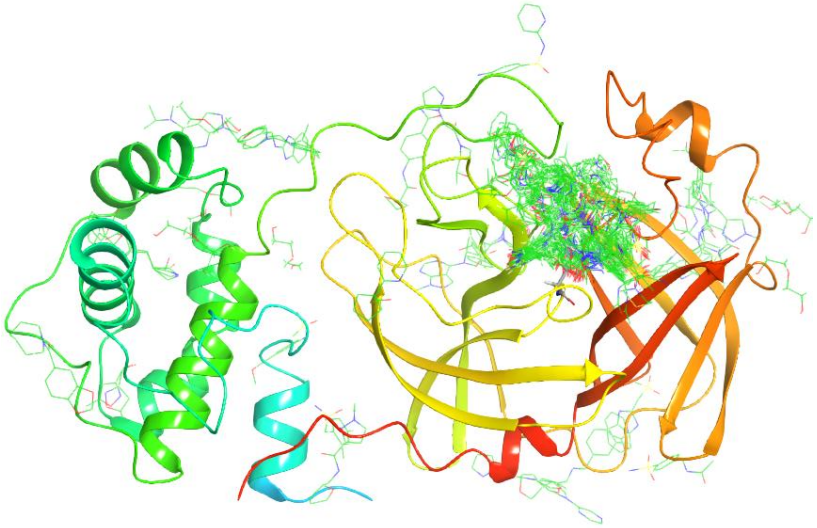
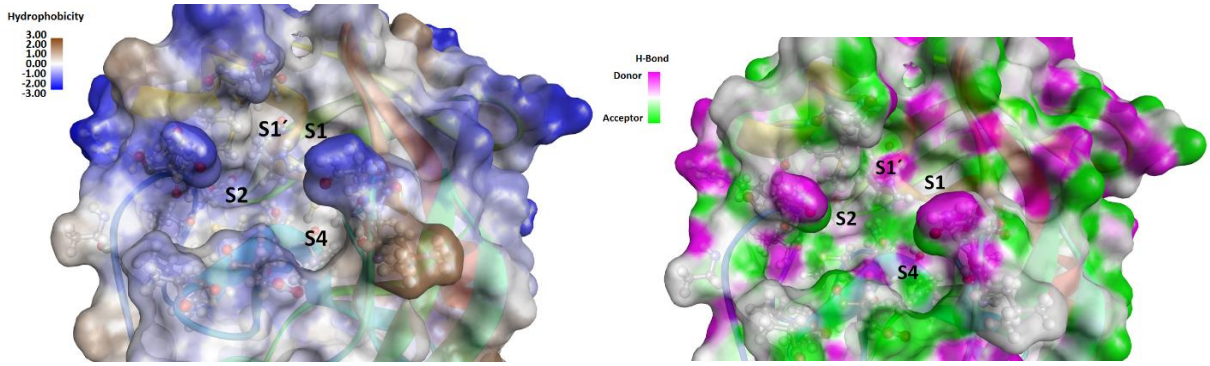


Figure S6: Distribution of small molecule ligand around the SARS-CoV-2 M^{pro} enzyme.



(a) (b)

Figure S7: The map of (a) hydrophobicity-hydrophilicity and (b) hydrogen bond donor-acceptor regions in the active site of the SARS-CoV-2 M^{pro} protein.

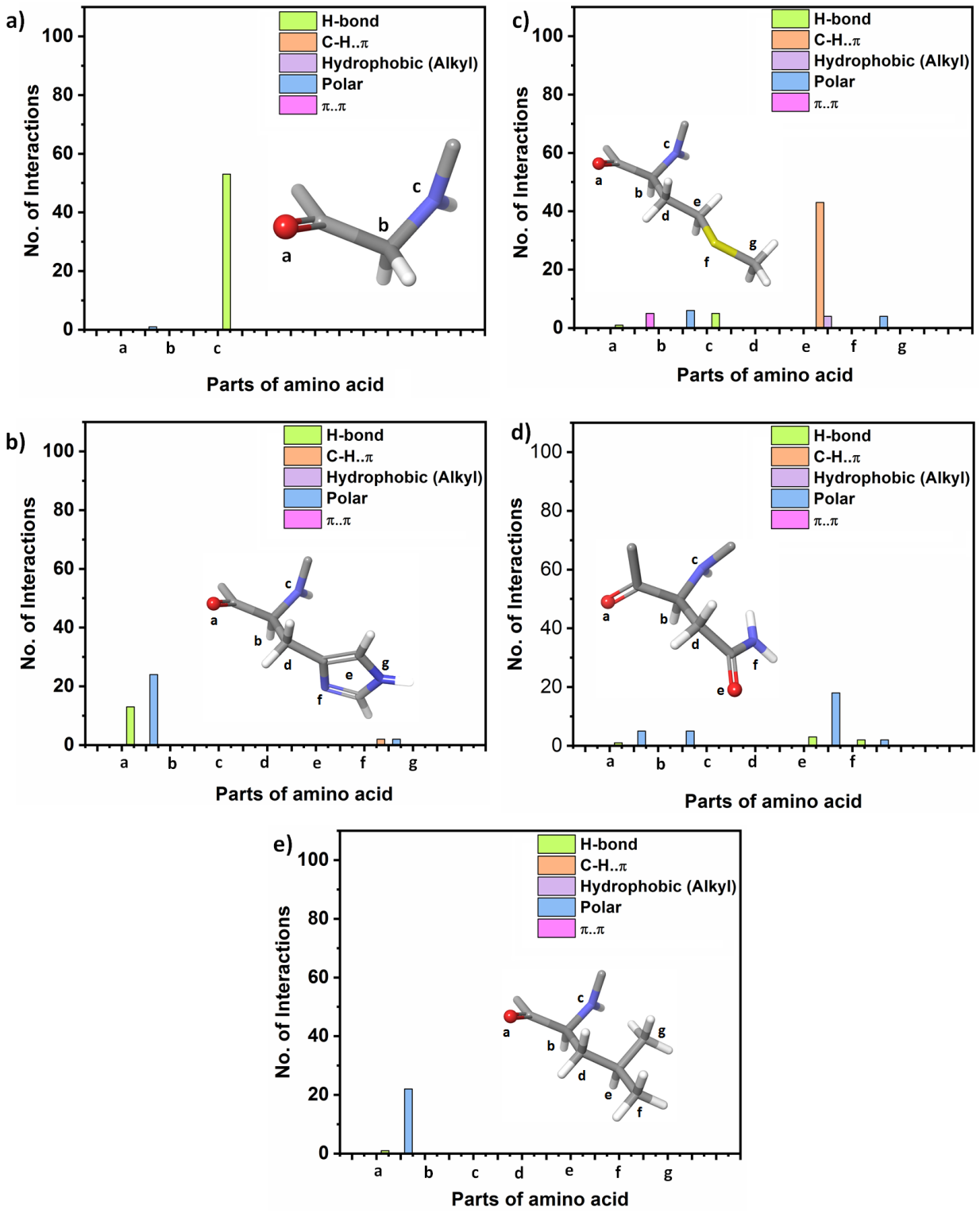


Figure S8: In the active site of the SARS-CoV-2 M^{pro} enzyme, the population of H-bond, C-H…π, hydrophobic, polar and π…π interactions at different parts of (a) G143; (b) G143, (c) M165, (d) N142 and (e) L141 amino acids.

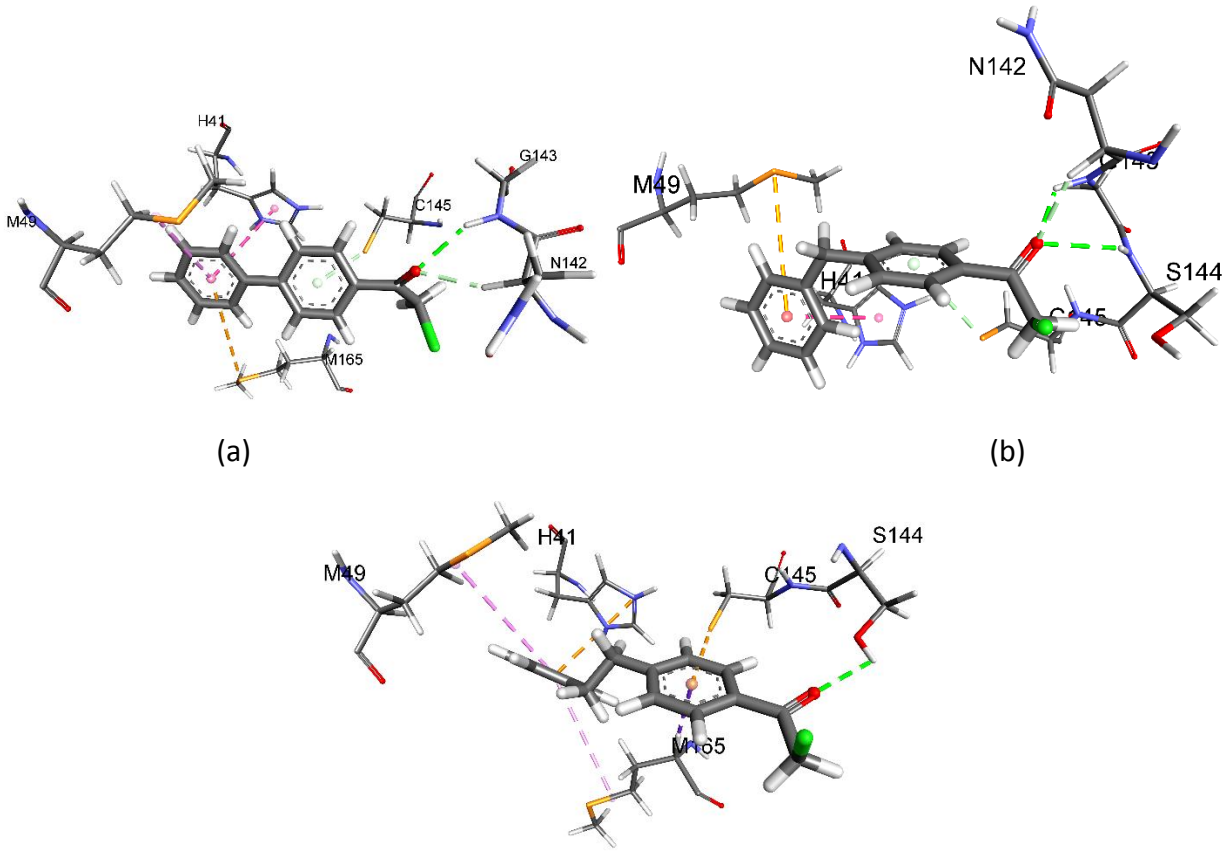
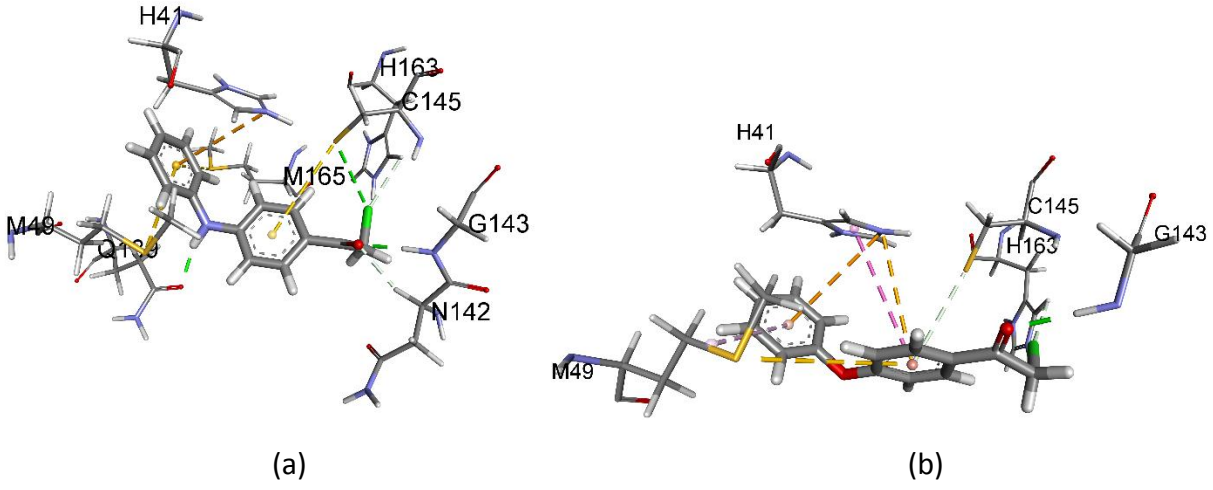
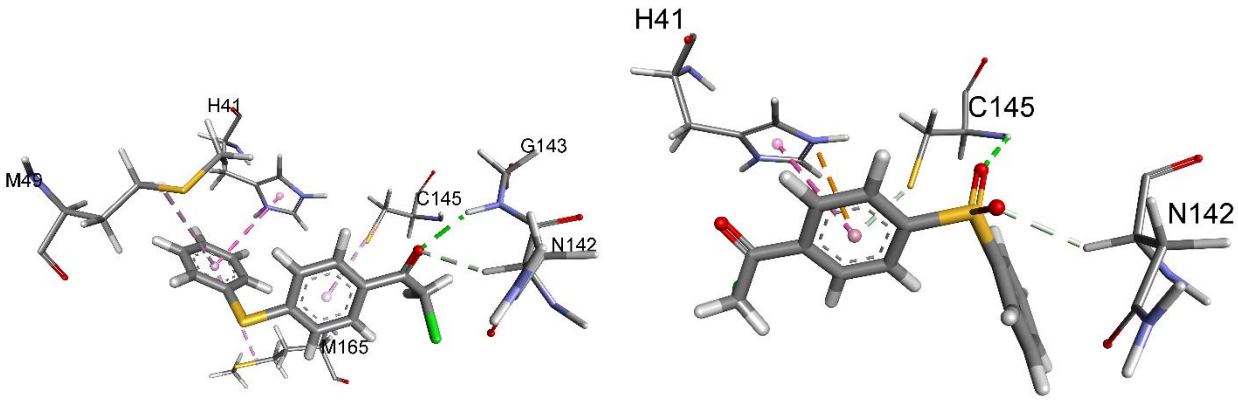


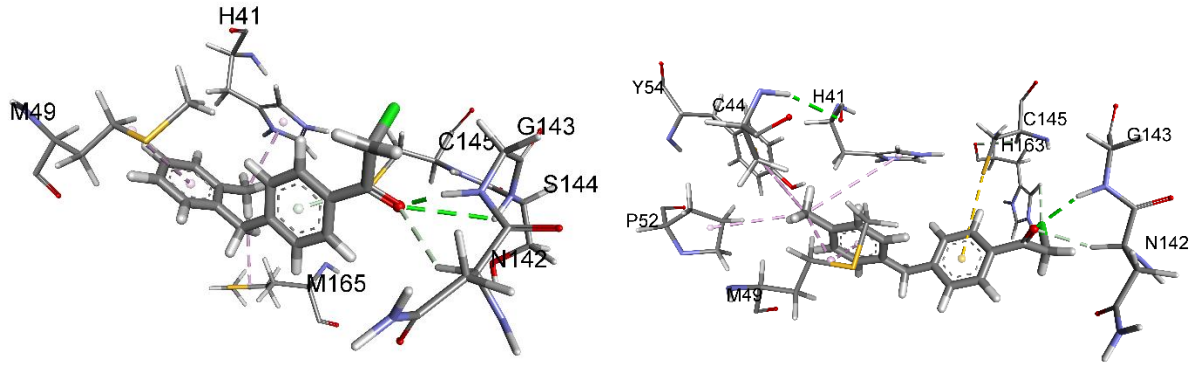
Figure 9: Non-covalent interactions of (a) compound **1**, (b) compound **2** and (c) compound **3** inside the active site of SARS-CoV-2 M^{pro} enzyme.





(c) (d)

Fig. S10: Non-covalent interactions of (a) compound **4**, (b) compound **5**, (c) compound **6** and (d) compound **7** inside the active site of SARS-CoV-2 M^{pro} enzyme.



(a) (b)

Fig. S11: Non-covalent interactions of (a) compound **8** and (b) compound **10** inside the active site of SARS-CoV-2 M^{pro} enzyme.

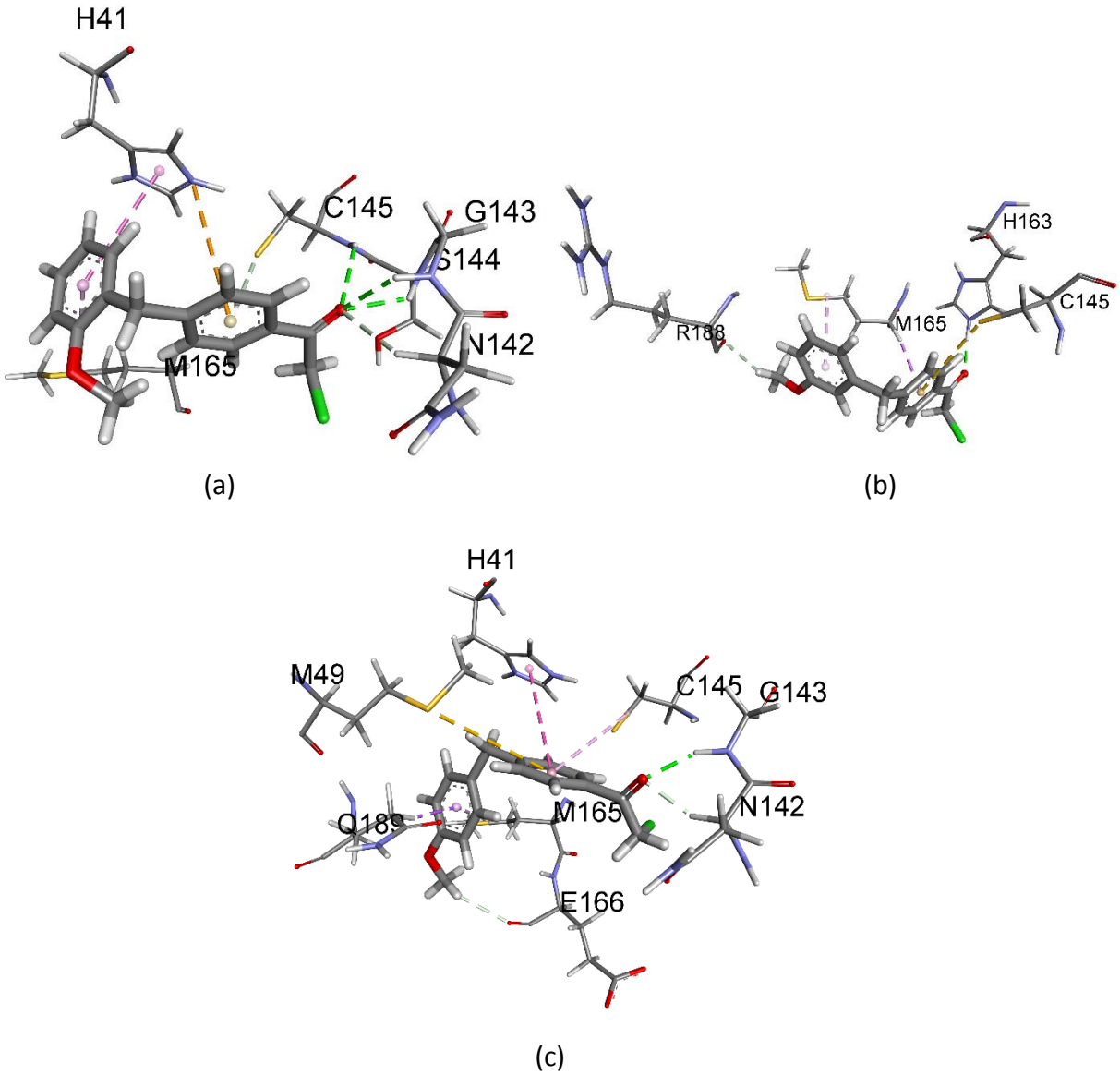


Fig. S12: Non-covalent interactions of (a) compound **11**, (b) compound 1**2** and (c) compound 1**3** inside the active site of SARS-CoV-2 M^{pro} enzyme.

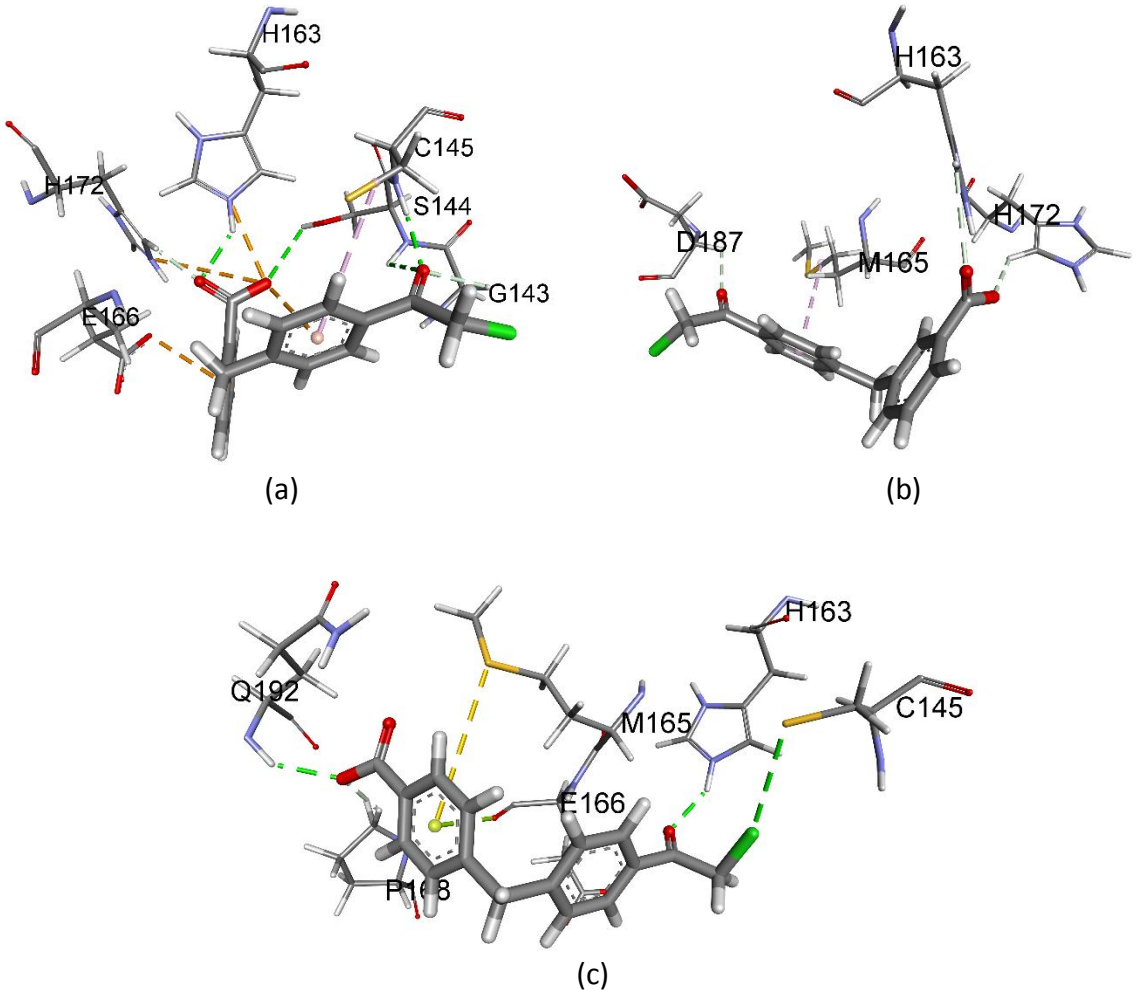


Fig. S13: Non-covalent interactions of (a) compound **14**, (b) compound **15** and (c) compound **16** inside the active site of SARS-CoV-2 M^{pro} enzyme.

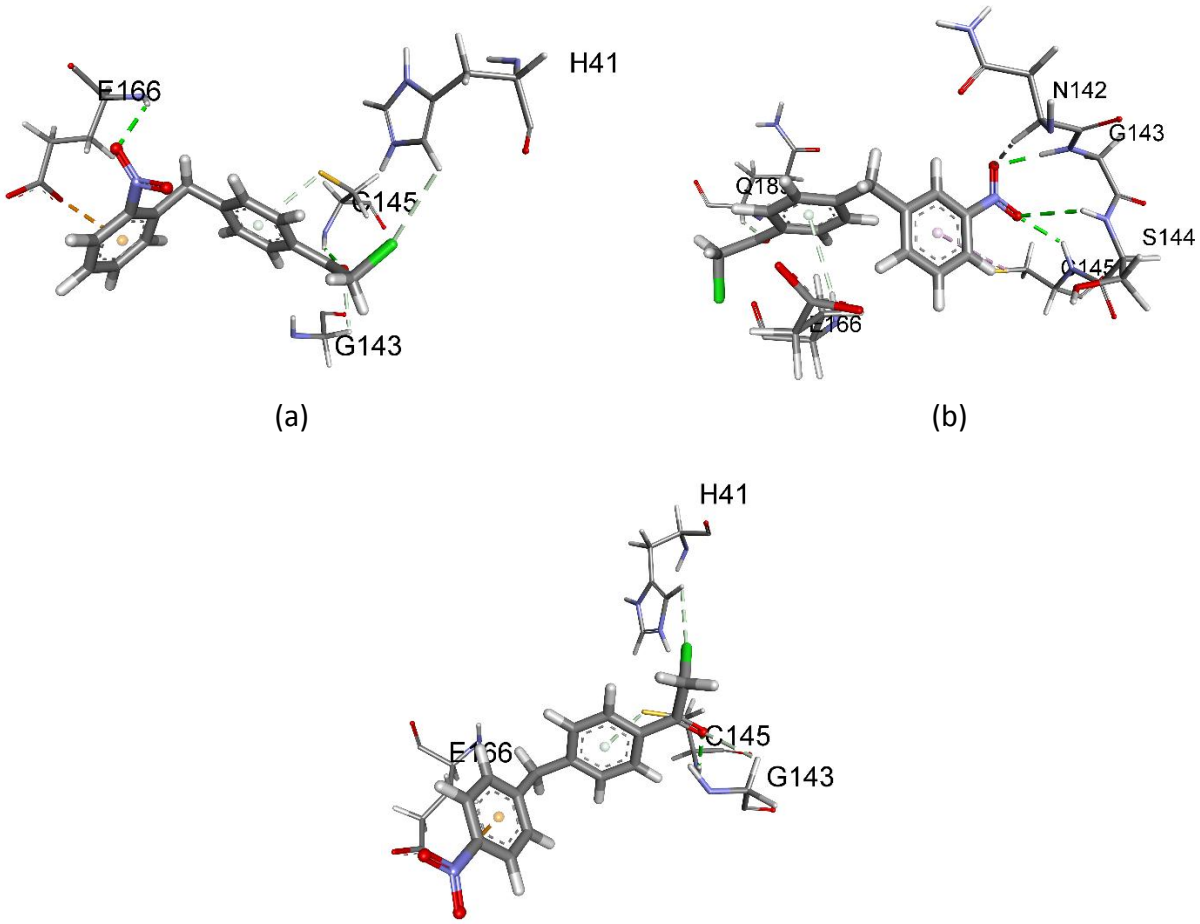


Fig. S14: Non-covalent interactions of (a) compound **17**, (b) compound **18** and (c) compound **19** inside the active site of SARS-CoV-2 M^{pro} enzyme.

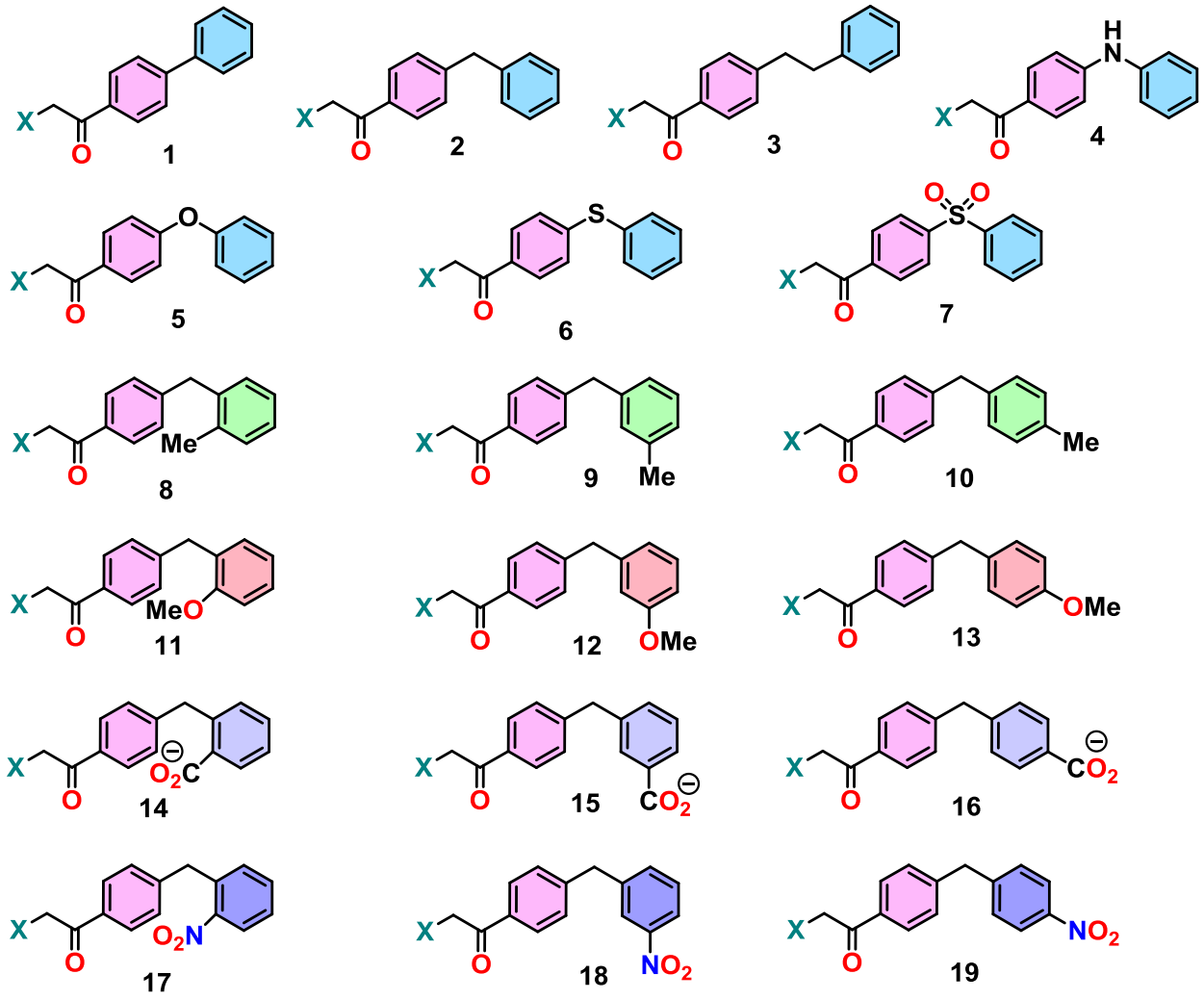
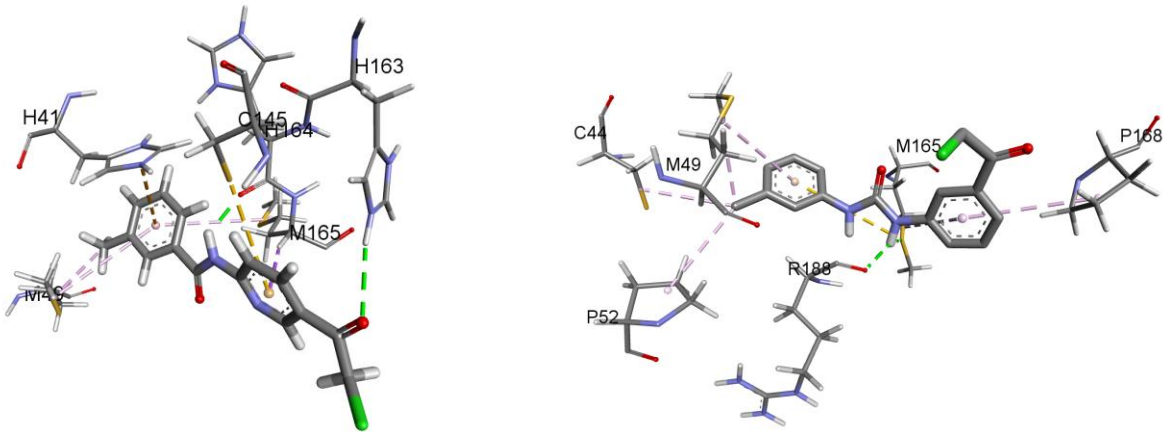
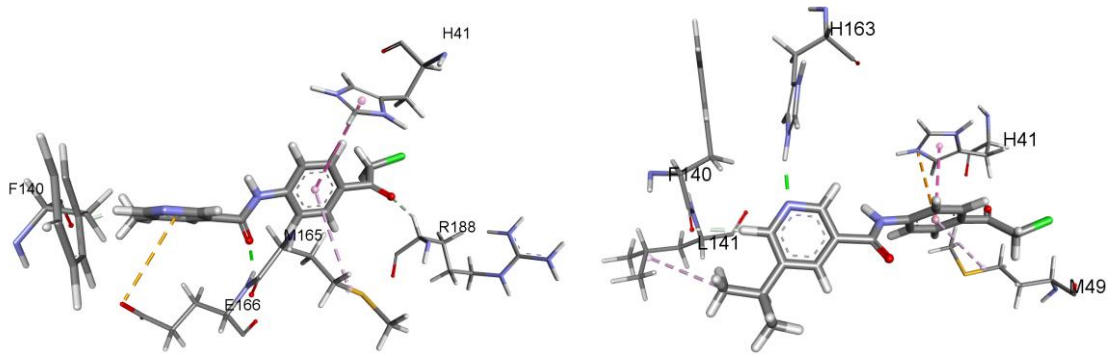


Fig. S15: 2D chemical structure of the designed compound.



(a) (b)



(c) **(d)**

Fig. S16: Non-covalent interactions of (a) compound **21**, (b) compound **22**, (c) compound **23** and (d) compound **24** inside the active site of SARS-CoV-2 M^{pro} enzyme.

Table S1. PDB IDs of SARS-CoV-2 M^{pro} enzyme

Position of Small molecules		PDB ID
No Small molecules (apo)		5R8T, 6M2Q, 6M03, 6WQF, 6WTM, 6Y2E, 6Y84, 6Y87, 7BRO.
Outside of the active site		5RE5, 5RE6, 5RE7, 5RE8, 5REA, 5REC, 5RED, 5REE, 5REF, 5REG, 5RF1, 5RF4, 5RF5, 5RF8, 5RF9, 5RFA, 5RFB, 5RFC, 5RFD, 5RGQ, 5RGR, 5RGS, 6VYF.
Inside of the active site	Covalently attached	5REJ, 5REK, 5REL, 5REM, 5REN, 5REO, 5REP, 5RER, 5RES, 5RET, 5REU, 5REV, 5REW, 5REX, 5REY, 5RFF, 5RFG, 5RFH, 5RFI, 5RFJ, 5RFK, 5RFL, 5RFM, 5RFN, 5RFO, 5RFP, 5RFQ, 5RFR, 5RFS, 5RET, 5REU, 5REV, 5RFW, 5RFX, 5RFY, 5RFZ, 5RG0, 5RG2, 5RG3, 5RGL, 5RGM, 5RGN, 5RGO, 5RGP, 5RGT, 5RH5, 5RH6, 5RH7, 5RH9, 5RHA, 5RHB, 5RHC, 5RHE, 5RHF, 6LU7, 6QMQ, 6LXE, 6MOK, 6WNP, 6WTJ, 6WTK, 6WTM, 6Y2F, 6Y2G, 6Y7M, 7BQY, 7BRP, 7BRR, 7BUY.
	Non-covalently attached	5R7Y, 5R7Z, 5R80, 5R81, 5R82, 5R83, 5R84, 5R89, 5RE4, 5RE9, 5REB, 5REH, 5REZ, 5RF1, 5RF2, 5RF3, 5RF6, 5RF7, 5REE, 5RG1, 5RG2, 5RG3, 5RG4, 5RG8, 5RHD, 6M2N, 6W63.

Table S2. The p-values for testing the difference between each of amino acids

	H41	C145	M49	G143	E166	M165	H164	N142	L141	S144	C44	P168	Q189	H163	R188	A 191	S46	L167	V 186	D187
H41																				
C145	0.063																			
M49	0.00049	0.0375																		
G143	1.9E-09	5E-06	0.0034																	
E166	1.1E-09	3E-06	0.0026	0.4614																
M165	4E-14	6E-10	5E-06	0.0374	0.0459															
H164	0	2E-12	5E-08	0.003	0.004	0.1626														
N142	0	3E-14	1E-08	0.0013	0.0018	0.1056	0.3947													
L141	0	5E-14	8E-10	0.0002	0.0003	0.0354	0.2027	0.286												
S144	0	0	1E-14	5E-08	8E-08	9E-05	0.0022	0.0047	0.02											
C44	0	0	4E-15	2E-08	3E-08	4E-05	0.0012	0.0026	0.012	0.4137										
P168	0	0	4E-15	2E-08	3E-08	4E-05	0.0012	0.0026	0.012	0.4137	0.5									
Q189	0	0	4E-15	2E-08	3E-08	4E-05	0.0012	0.0026	0.012	0.4137	0.5	0.5								
H163	0	0	0	3E-10	5E-10	1E-06	5E-05	0.0001	8E-04	0.1126	0.1587	0.1587	0.1587							
R188	0	0	0	3E-11	4E-11	1E-07	7E-06	2E-05	1E-04	0.0354	0.0544	0.0544	0.0544	0.2637						
A 191	0	0	0	7E-12	1E-11	4E-08	2E-06	6E-06	4E-05	0.0163	0.0261	0.0261	0.0261	0.1587	0.3527					
S46	0	0	0	2E-12	3E-12	1E-08	6E-07	2E-06	1E-05	0.0063	0.0105	0.0105	0.0105	0.0787	0.2073	0.3274				
L167	0	0	0	2E-12	3E-12	1E-08	6E-07	2E-06	1E-05	0.0063	0.0105	0.0105	0.0105	0.0787	0.2073	0.3274	0.5			
V 186	0	0	0	2E-12	3E-12	1E-08	6E-07	2E-06	1E-05	0.0063	0.0105	0.0105	0.0105	0.0787	0.2073	0.3274	0.5	0.5		
D187	0	0	0	2E-12	3E-12	1E-08	6E-07	2E-06	1E-05	0.0063	0.0105	0.0105	0.0105	0.0787	0.2073	0.3274	0.5	0.5	0.5	

Method of calculations

Group	**1**	**2**
Sample Size	N_1	N_2
Number of Events	X_1	X_2
Event Rate	λ_1	λ_2
Distribution of X	Poisson(λ_1)	Poisson(λ_2)

Mathews (2010) proposed two test statistics that can be used to test statistical hypotheses about the rate difference. The first is based is the *large-sample z-test* of the hypotheses H0: $\lambda_1 = \lambda_2$ versus Ha: $\lambda_1 \neq \lambda_2$.

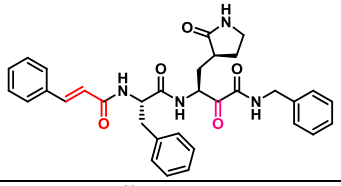
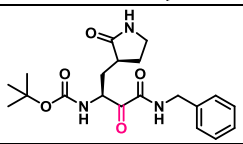
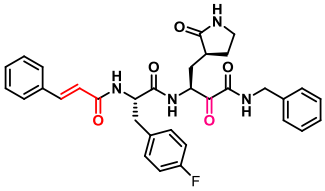
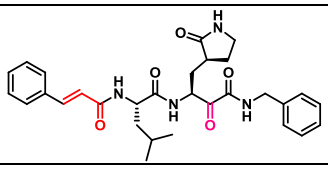
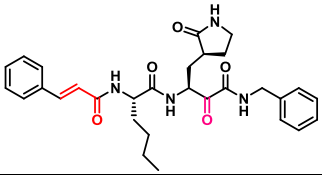
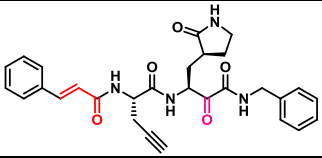
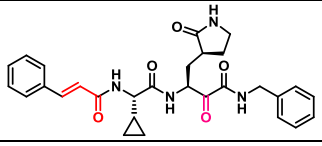
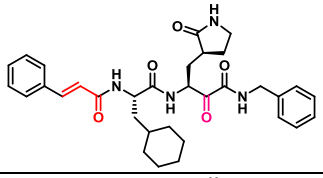
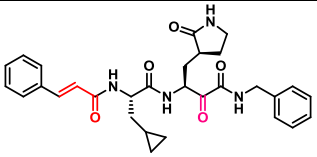
$$Z_{LS} = \frac{\lambda_2 - \lambda_1}{\sqrt{\frac{\lambda_1}{N_1} + \frac{\lambda_2}{N_2}}}$$

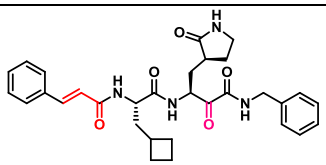
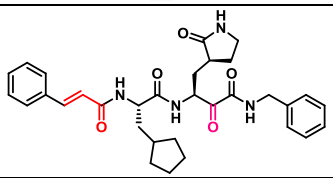
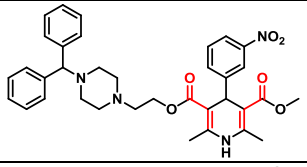
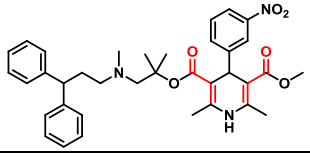
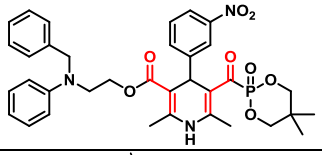
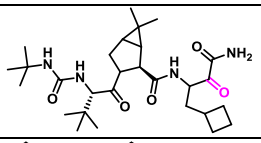
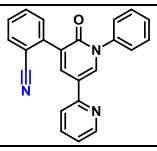
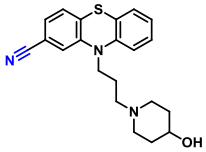
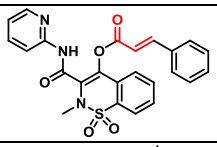
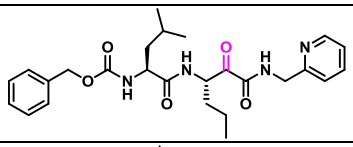
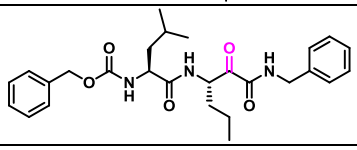
$p = P(Z > Z_{LS})$ Where Z follows N (0, 1)

Smith, P.G. and Morrow, R.H. 1996. Field Trials of Health Intervention in Developing Countries: A Toolbox. Macmillan Education. Oxford, England.

Campbell, M.J. and Walters, S.J. 2014. How to Design, Analzse and Report Cluster Randomised Trials in Medicine and Health Related Research. John Wiley. New York.

Table S3: Few examples of crystallographically non-characterized SARS-CoV-2 M^{pro} enzyme inhibitor small molecules

Entry	Structure of molecules	Reference
1		*J. Med. Chem.* **2020**, *63*, 4562−4578
2		*J. Med. Chem.* **2020**, *63*, 4562−4578
3		*J. Med. Chem.* **2020**, *63*, 4562−4578
4		*J. Med. Chem.* **2020**, *63*, 4562−4578
5		*J. Med. Chem.* **2020**, *63*, 4562−4578
6		*J. Med. Chem.* **2020**, *63*, 4562−4578
7		*J. Med. Chem.* **2020**, *63*, 4562−4578
8		*J. Med. Chem.* **2020**, *63*, 4562−4578
9		*J. Med. Chem.* **2020**, *63*, 4562−4578

10		*J. Med. Chem.* **2020**, *63*, 4562−4578
11		*J. Med. Chem.* **2020**, *63*, 4562−4578
12		*ACS Med. Chem. Lett.* **2020**, *11*, 2526−2533
13		*ACS Med. Chem. Lett.* **2020**, *11*, 2526−2533
14		*ACS Med. Chem. Lett.* **2020**, *11*, 2526−2533
15		*ACS Med. Chem. Lett.* **2020**, *11*, 2526−2533
16		*ACS Med. Chem. Lett.* **2020**, *11*, 2526−2533
17		*ACS Med. Chem. Lett.* **2020**, *11*, 2526−2533
18		*ACS Med. Chem. Lett.* **2020**, *11*, 2526−2533
19		*Sci. Adv*. **2020**, *6*, eabe0751
20		*Sci. Adv*. **2020**, *6*, eabe0751

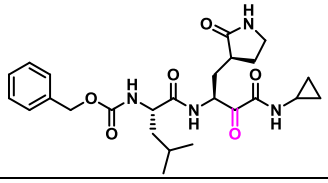
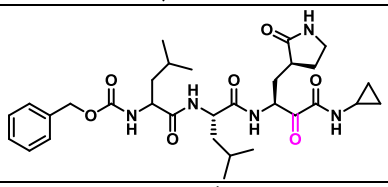
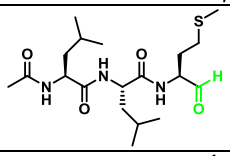
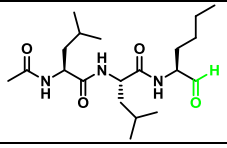
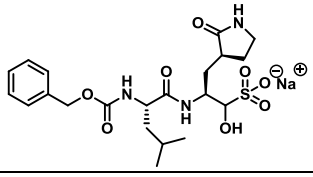
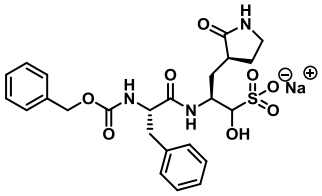
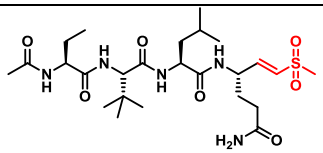
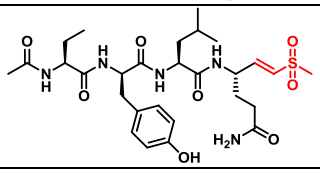
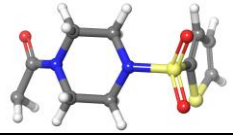
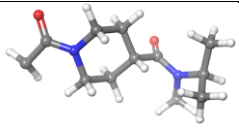
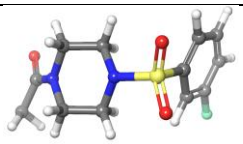
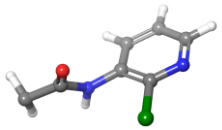
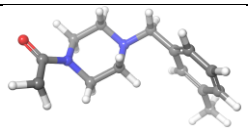
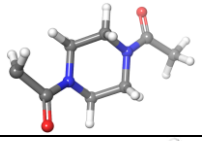
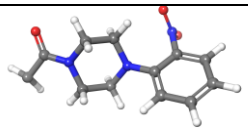
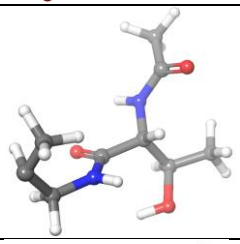
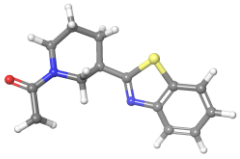
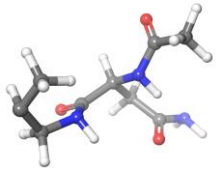
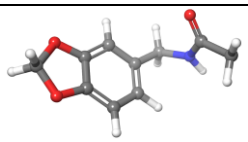
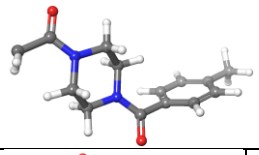
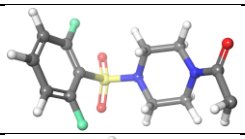
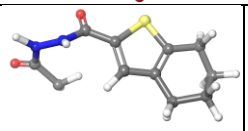
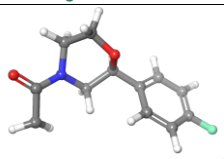
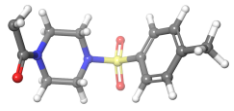
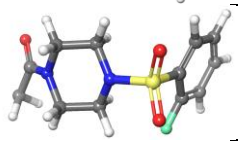
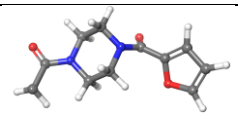
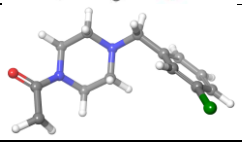
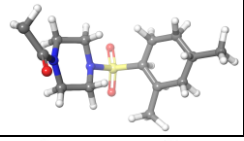
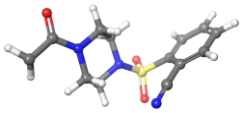
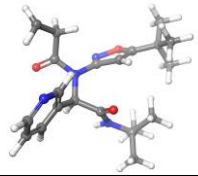
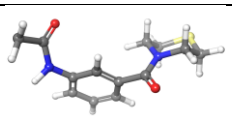
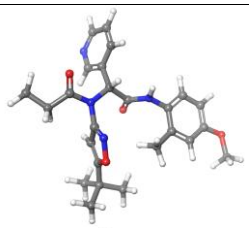
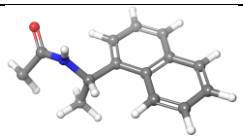
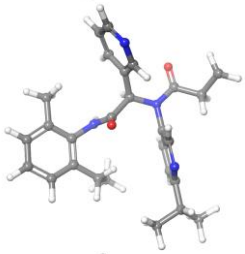
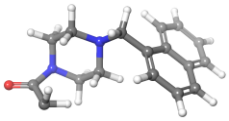
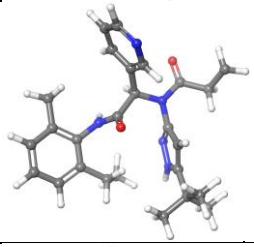
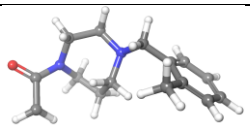
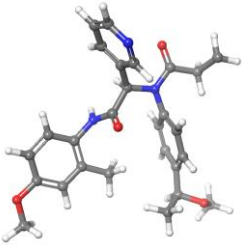
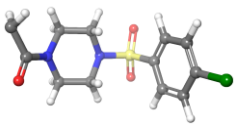
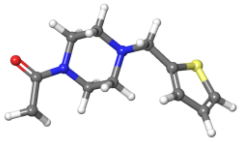
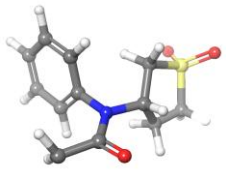
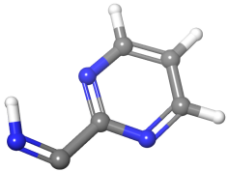
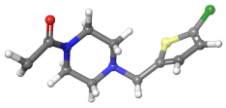
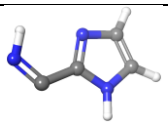
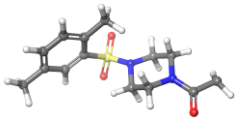
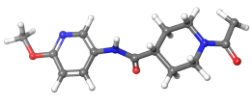
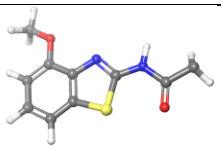
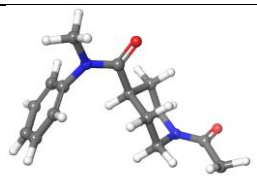
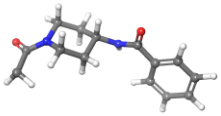
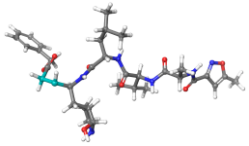
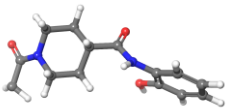
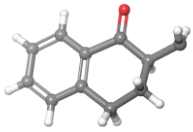
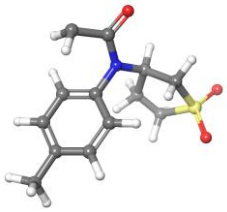
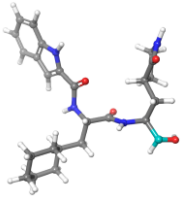
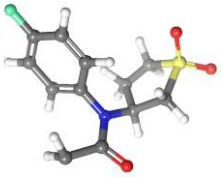
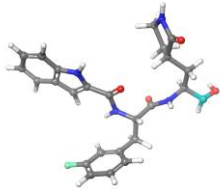
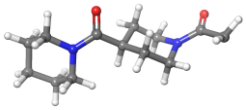
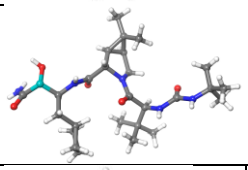
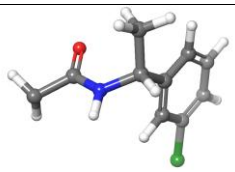
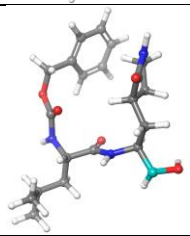
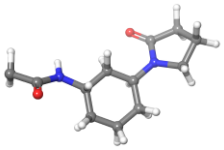
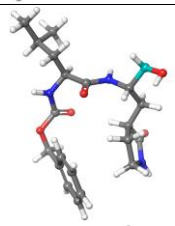
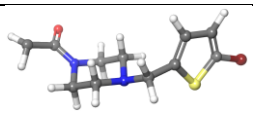
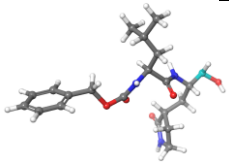
21		*Sci. Adv*. **2020**, *6*, eabe0751
22		*Sci. Adv*. **2020**, *6*, eabe0751
23		*Sci. Adv*. **2020**, *6*, eabe0751
24		*Sci. Adv*. **2020**, *6*, eabe0751
25		*Sci. Adv*. **2020**, *6*, eabe0751
26		*Sci. Adv*. **2020**, *6*, eabe0751
27		*Nat Chem Biol*, **2020**, https://doi.org/10.1038/s41589-020-00689-z
28		*Nat Chem Biol*, **2020**, https://doi.org/10.1038/s41589-020-00689-z

Table S4: Binding energy (ΔG) of ligands found in active site of SARS-CoV-2 M^{pro} enzyme through covalent bond.

Entry	PDB id	Ligand	ΔG (KJ/mol)	Entry	PDB id	Ligand	ΔG (KJ/mol)
1.	5REJ		-24.94	**35**	5RFY		-19.28

2.	5REK		-26.92	**36**	5RFZ		-15.92
3.	5REL		-25.24	**37**	5RG0		-14.66
4.	5REM		-21.71	**38**	5RG2		-12.55
5.	5REN		-23.39	**39**	5RG3		-10.25
6.	5REO		-21.50	**40**	5RGL		-27.26
7.	5REP		-24.74	**41**	5RGM		-23.05
8.	5RER		-24.36	**42**	5RGN		-28.10
9.	5RES		-24.25	**43**	5RGO		-25.99
10	5RET		-21.38	**44**	5RGP		-17.59
11	5REU		-21.21	**45**	5RGT		-30.61

12	5REV		-28.06	**46**	5RH5		-31.21
13	5REW		-25.28	**47**	5RH6		-34.82
14	5REX		-29.82	**48**	5RH7		-34.39
15	5REY		-28.09	**49**	5RH9		-33.35
16	5RFF		-25.99	**50**	5RHA		-24.95
17	5RFG		-23.89	**51**	5RHB		-12.34
18	5RFH		-23.14	**52**	5RHC		-11.97
19	5RFI		-24.95	**53**	5RHE		-22.89

20	5RFJ		-17.26	**54**	5RHF		-23.86
21	5RFK		-20.45	**55**	6LU7		-8.86
22	5RFL		-20.66	**56**	6YNQ		-13.06
23	5RFM		-23.98	**57**	6LZE		-29.61
24	5RFN		-24.31	**58**	6M0K		-29.86
25	5RFO		-17.34	**59**	6WNP		-8.61
26	5RFP		-18.94	**60**	6WTJ		-12.39
27	5RFQ		-19.70	**61**	6WTK		-12.10
28	5RFR		-26.25	**62**	6WTM		-12.51

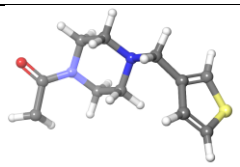
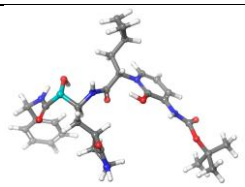
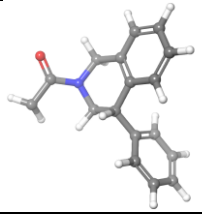
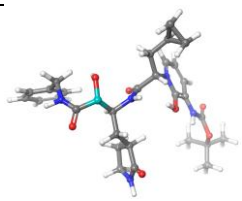
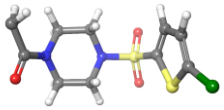
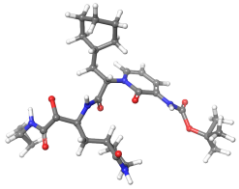
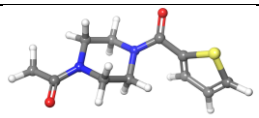
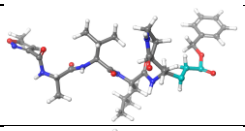
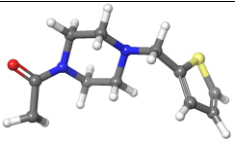
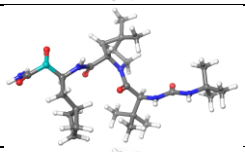
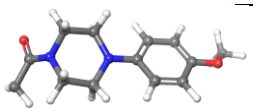
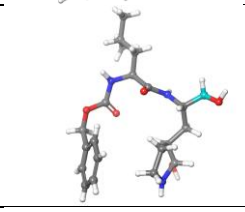
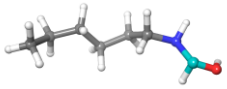
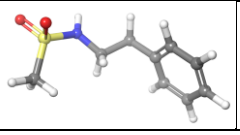
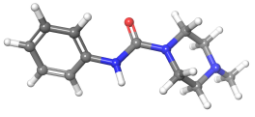
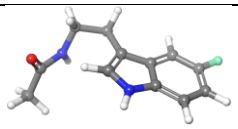
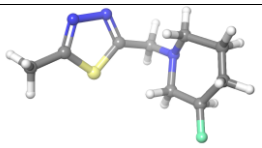
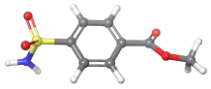
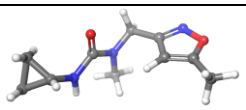
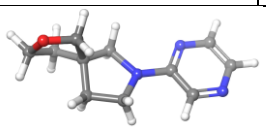
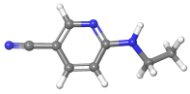
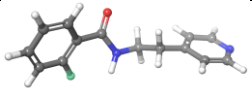
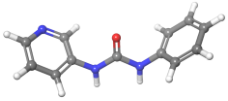
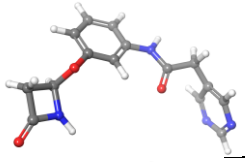
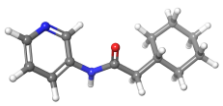
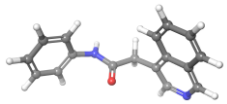
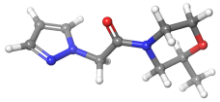
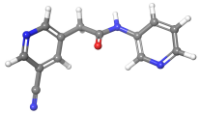
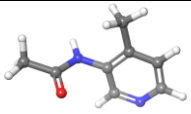
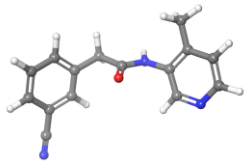
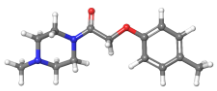
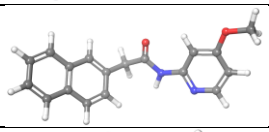
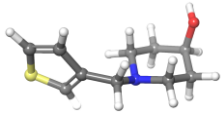
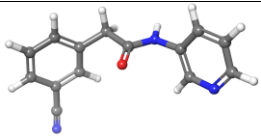
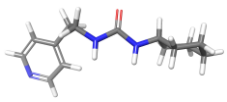
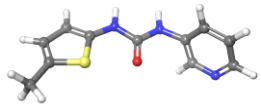
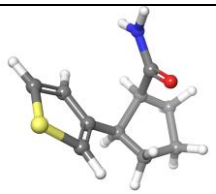
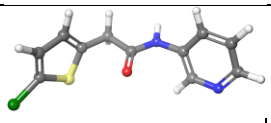
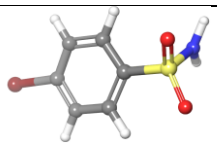
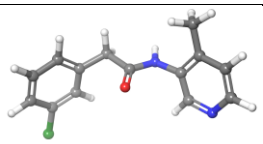
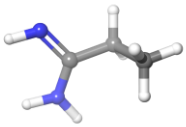
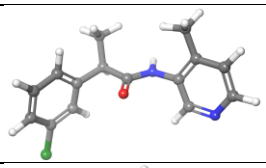
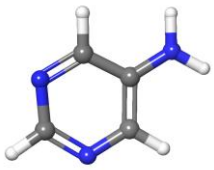
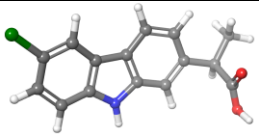
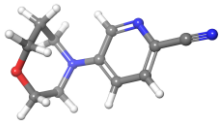
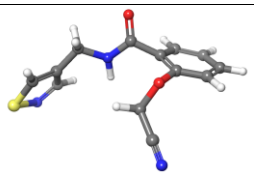
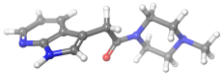
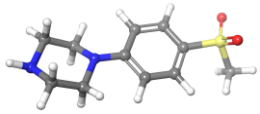
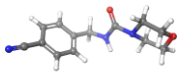
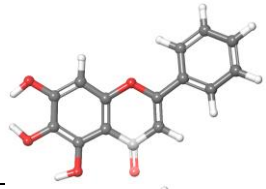
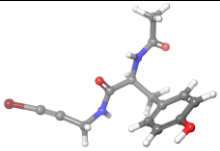
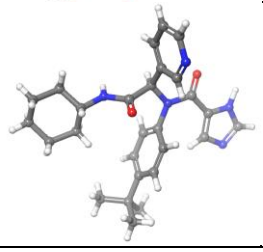
29	5RFS		-26.96	**63**	6Y2F		-11.80
30	5RET		-28.14	**64**	6Y2G		-1138
31	5REU		-22.76	**65**	6Y7M		-16.59
32	5REV		-21.50	**66**	7BQY		-15.58
33	5RFW		-21.29	**67**	7BRP		-12.22
34	5RFX		-23.48	**68**	7BRR		-16.00
				69	7BUY		-7.60

Table S5: Binding energy (ΔG) of ligands found in active site of SARS-CoV-2 M^{pro} enzyme through non-covalent bond.

Entry	**PDB Id**	**Ligand**	**ΔG (KJ/mol)**	**Entry**	**PDB Id**	**Ligand**	**ΔG (KJ/mol)**
1	5R7Y		-18.94	**21**	5RGG		-23.73

2	5R7Z		-20.83	**22**	5RGH		-21.42
3	5R80		-18.10	**23**	5RGI		-23.44
4	5R81		-22.26	**24**	5RGJ		-23.05
5	5R82		-22.09	**25**	5RGK		-24.31
6	5R83		-24.94	**26**	5RGU		-20.12
7	5R84		-21.92	**27**	5RGV		-28.52
8	5R89		-19.53	**28**	5RGW		-25.99
9	5RE4		-18.44	**29**	5RGX		-28.48
10	5RE9		-26.84	**30**	5RGY		-25.91
11	5REB		-24.69	**31**	5RGZ		-27.84
12	5REH		-25.07	**32**	5RH0		-25.75
13	5REZ		-23.98	**33**	5RH1		-26.25

14	5RF1		-21.42	**34**	5RH2		-26.75
15	5RF2		-4.62	**35**	5RH3		-26.96
16	5RF3		-10.96	**36**	5RH4		-27.89
17	5RF6		-23.44	**37**	5RH8		-25.49
18	5RF7		-28.06	**38**	5RHD		-24.02
19	5REE		-24.28	**39**	6M2N		-26.50
20	5RG1		-20.08	**40**	6W63		-29.06