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Supplementary Materials

Discovery of Chalcone-Based Hybrid Structures as High Affinity and Site-Specific Inhibitors against SARS-CoV-2: A Comprehensive Structural Analysis Based on Various Host-Based and Viral Targets

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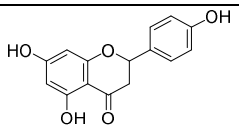
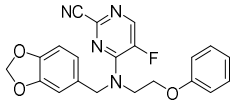
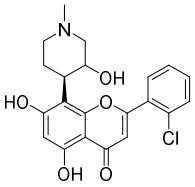
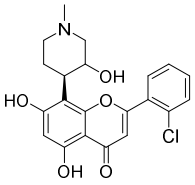
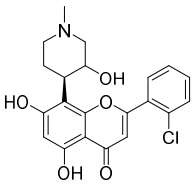
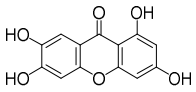
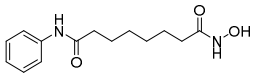
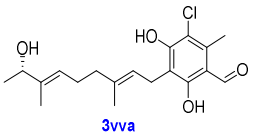
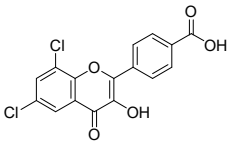
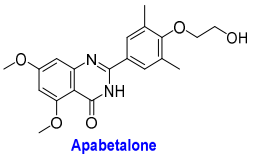
Table S1. The most important host and virus-based targets selected to evaluate the antiviral activity of the 757 chalcone-based compound library, along with PDB codes, chemical structure of their co-crystal ligands, and the authoritative studies proposing these targets as promising strategies for the treatment of viral diseases such as COVID-19.

N.	HBATs	PDB Code	Co-crystallized ligand	HBATs-References
1	p38 MAPK	4EH3	 Naringenin	[1-8]
2	Cathepsin L	5MQY	 PubChem CID: 137653100	[9-16]
3	CDK1	6GU2	 Flavopiridol	[17-22]
4	CDK2/CyclinA	6GUB	 Flavopiridol	[23-25]
5	CDK9/cyclinT1	3BLR	 Flavopiridol	[26-29]
6	ERK2	3SA0	 Norathyriol	[30-34]
7	HDAC2	4LXZ	 Vorinostat	[35-39]
8	DHODH	5ZF7	 3vva	[40-46]
9	CK2 alpha'	5M4U	 PubChem CID: 38997316	[47-52]
10	BRD2	4J1P	 Apabetalone	[53-55]

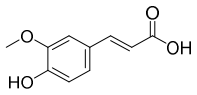
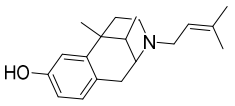
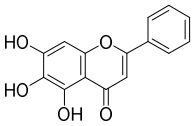
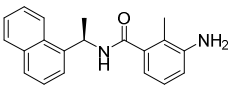
11	BRD4	6HOV	 Ferulic acid	[53, 55-57]
12	Sigma-1 receptor	6DK1	 Pentazocine	[58-64]
13	3CLpro	6M2N	 Baicalein	[65-68]
14	PLpro	7JN2	 PubChem CID: 153835436	[69-72]

Table S2. Some important druglikeness, ADME, and toxicity parameters of the best identified **CHA-12**, **CHA-37**, **CHA-378**, **CHA-384**, and standards quercetin and resveratrol predicted by admetSAR, preADME, and swissADME online servers.

Parameters	**CHA-12**	**CHA-37**	**CHA-378**	**CHA-384**	**Quercetin**	**Resveratrol**
Formula	$C_{26}H_{19}N_5O_3$	$C_{21}H_{15}N_3O$	$C_{22}H_{19}NO_5S$	$C_{23}H_{17}ClN_2O_4$	$C_{15}H_{10}O_7$	$C_{14}H_{12}O_3$
Molecular weight (g/mol)	449.46	325.36	409.45	420.85	302.24	228.24
Lipinski rule of Five	Suitable	Suitable	Suitable	Suitable	Suitable	Suitable
Rule of Five Violations a	0	0	0	0	0	0
BBB permeant	No	Yes	No	No	No	Yes
GI absorption	High	High	High	High	High	High
P-glycoprotein Inhibitor	No	Inhibitor	No	No	No	No
Acute Oral Toxicity	III	III	III	III	II	III
Carcinogens	No	No	No	No	No	No

a: Violations of Lipinski rule of 5 (logP < 5, MW < 500, nHBA < 10, and nHBD < 5).

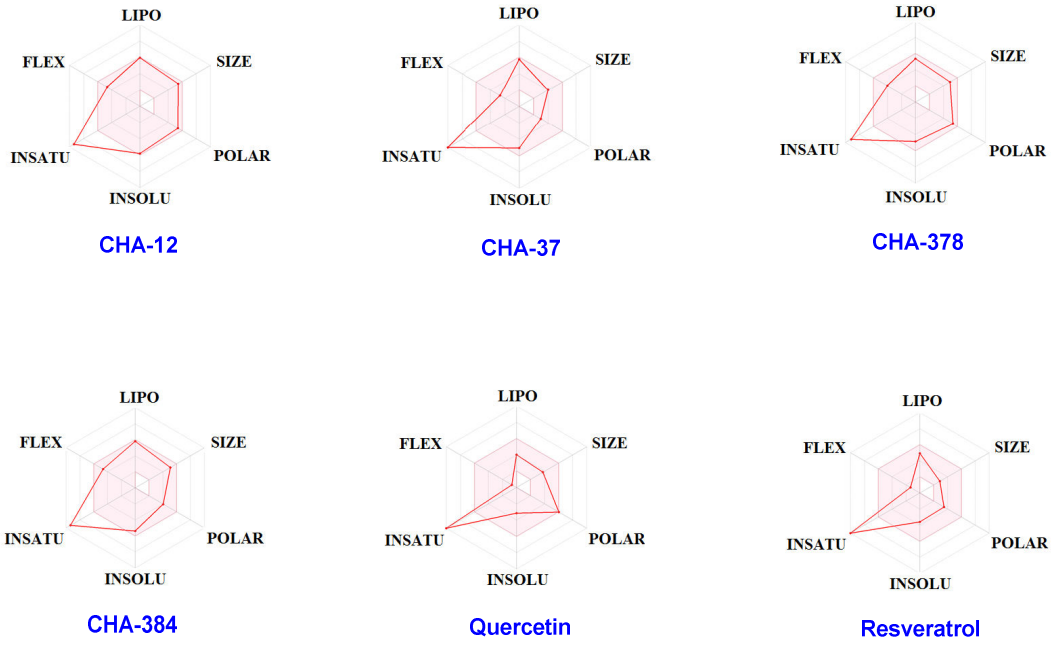


Figure S1. Radar map of the physicochemical properties for oral bioavailability of **CHA-12**, **CHA-37**, **CHA-378**, **CHA-384**, and standards quercetin and resveratrol predicted by SwissADME online server (colored zone is the suitable physicochemical space for oral bioavailability). LIPO (Lipophilicity): -0.7 < XLOGP3 < +5.0**;** SIZE: 150 g/mol < mw < 500 g/mol**;** POLAR (polarity): 20 Å^2 < TPSA < 130 Å^2**;** INSOLU (insolubility): 0 < Log S (ESOL) < 6**;** INSATU (insaturation): 0.25 < Fraction Csp3 < 1**;** FLEX (Flexibility): 0 < Numb. rotatable bonds < 9.

Table S3. Predicted inhibitory effects of **CHA-12**, **CHA-37**, **CHA-378**, **CHA-384**, resveratrol, and quercetin on cytochrome P450 enzymes (predicted by preADME online server).

Compounds	CYP2C19 inhibition	CYP2C9 inhibition	CYP2D6 inhibition	CYP2D6 Substrate	CYP3A4 inhibition	CYP3A4 Substrate
CHA-12	Non	Inhibitor	Non	Non	Non	Weakly
CHA-37	Non	Inhibitor	Non	Non	Non	Weakly
CHA-378	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non
CHA-384	Non	Inhibitor	Non	Non	Non	Non
Resveratrol	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non
Quercetin	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non

Table S4. Important interactions of most active **CHA-12**, **CHA-378**, **CHA-384,** and baicalein as standard in the SARS-CoV-2 3CLpro active site.

Interactions	CHA-12		CHA-378		CHA-384		Baicalein	
	Residues	Interacting groups	Residues	Interacting groups	Residues	Interacting groups	Residues	Interacting groups
Conventional H-bond	Glu166	NH-Tetrazole	Asn142	3-OH (B-Phenyl ring)	Arg188	1-NH (Urea-moiety)	Gly143	6-OH (A-Ring)
	Thr190	OH (A-Phenyl ring)			Arg188	2-NH (Urea-moiety)	Ser144	6-OH (A-Ring)
	Gln192	Carbonyl group						
	His41	O (Etheric linker)						
	His41	N-Quinoline						
Pi-donor	Gln189	B-Phenyl ring	Asn142	B-Phenyl ring				
Pi-Alkyl	Met49	B-Phenyl ring	Met49	Phenyl ring (Sulf-moiety)	Met49	A-Phenyl ring	Met49	B-Ring
	Cys145	Quinoline (Benzene ring)	Met165	A-Phenyl ring	Met165	A-Phenyl ring	Cys44	B-Ring
			Cys145	B-Phenyl ring	Pro168	Phenyl ring (Urea-	Cys145	C-Ring

						moiety)		
			His41	Methyl (Sulf-moiety)				
Pi-Sulfur	Met49	Quinoline (Pyridine ring)					Cys145	A-Ring
Pi-Sigma	Thr25	Quinoline (Pyridine ring)	Gln189	A-Phenyl ring	Thr25	B-Phenyl ring		
			Gln189	A-Phenyl ring				
Acceptor-Acceptor			Asn142	4-OH (B-Phenyl ring)				
			Ser144	4-OH (B-Phenyl ring)				
Donor-Donor							Gly143	5-OH (A-Ring)
Pi-Cation			His41	Phenyl ring (Sulf-moiety)				
Pi-Pi Stacked			His41	Phenyl ring (Sulf-moiety)	His41	A-Phenyl ring	His41	B-Ring
Alkyl					Pro168	Cl (Urea-moiety)		
					Ala191	Cl (Urea-moiety)		
Carbon							Met165	Carbonyl (C-Ring)

Table S5. Important interactions of the most active **CHA-12**, **CHA-37**, **CHA-378** and standard compound GRL0617 in the active site of SARS-CoV-2 PLpro.

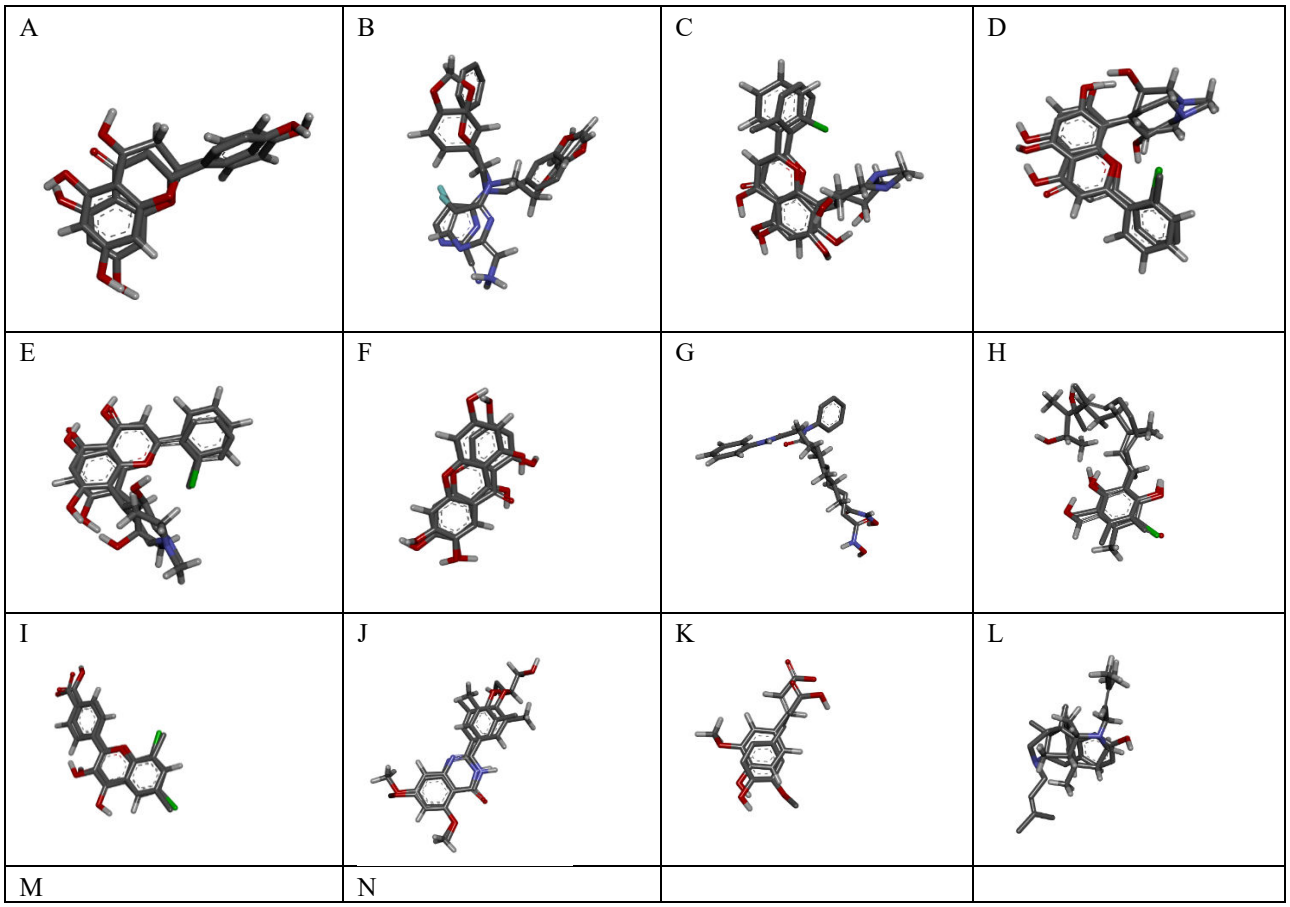
Interactions	**CHA-12**		**CHA-37**		**CHA-384**		**GRL0617**	
	Residues	Interacting groups	Residues	Interacting groups	Residues	Interacting groups	Residues	Interacting groups
Conventional H-bond	Lys157	N1 Tetrazole			Tyr264	Carbonyl group	Gln269	Carbonyl group
	Gln269	N4 Tetrazole			Glu167	NH (Sulf-moiety)	Asp164	NH (Amide)
	Tyr264	OH (A-Phenyl ring)					Gly163	NH2 (A-Phenyl ring)
Pi-donor	Gln269	Tetrazole ring						
Pi-Alkyl	Pro248	B-Phenyl ring	Pro248	B-Phenyl ring	Pro248	B-Phenyl ring	Tyr268	CH_3 (A-Phenyl ring)
			Pro248	Triazole (Benzotriazole)	Leu162	A-Phenyl ring	Pro247	Naphthyl (Closer ring)
			Pro248	Benzene (Benzotriazole)			Pro248	Naphthyl (Closer ring)
			Pro247	Benzene (Benzotriazole)			Pro248	Naphthyl (Farther ring)
Amide-Pi Stacked	Gly163	A-Phenyl ring	Gly163	A-Phenyl ring	Gly163	A-Phenyl ring		
			Asn267	Triazole (Benzotriazole)				
Pi-Sigma					Gln269	A-Phenyl ring	Asp164	A-Phenyl ring
Acceptor-Acceptor	Tyr273	Carbonyl group						
Pi-Cation			Lys157	A-Phenyl ring				
Pi-Anion	Glu167	Tetrazole ring			Asp164	B-Phenyl ring	Asp164	A-Phenyl ring
					Glu167	Phenyl ring (Sulf-moiety)		

Pi-Pi Stacked	Tyr268	Quinoline (Pyridine ring)	Tyr264	B-Phenyl ring					
	Tyr268	Quinoline (Benzene ring)							
Pi-Pi T-Shaped	Tyr268	B-Phenyl ring	Tyr268	Benzene (Benzotriazole)	Tyr264	B-Phenyl ring	Tyr268	Naphthyl ring)	(Closer
							Tyr268	Naphthyl ring)	(Farther
Carbon					Pro248	4-OH (B-Phenyl ring)			

Table S6. The list of host-based and virus-based targets along with their associated PDB codes as well as the essential parameters for the docking protocol used in this study.

Entry	Target	PDB Code	X	Y	Z	Grid box	Docking score[a]
1	p38 MAPK	4EH3	-2.668	0.007	-20836	20,20,20	- 8.40
2	Cathepsin L	5MQY	54.336	48.381	17.63	20,20,20	-6.80
3	CDK1	6GU2	329.218	212.541	192.325	20,20,20	-9.60
4	CDK2/CyclinA	6GUB	-8.411	-21.607	22.226	20,20,20	-10.10
5	CDK9/cyclinT1	3BLR	52.813	-16.066	-12.998	20,20,20	-10.30
6	ERK2	3SA0	-12.826	10.831	40.627	26,22,20	-7.40
7	HDAC2	4LXZ	25.00	-17.005	-0.024	20,20,20	-7.50
8	DHODH	5ZF7	-33.443	14.428	-21.466	20,22.22	-9.60
9	CK2 alpha'	5M4U	10.536	-19.326	-8.976	20,20,20	-11.40
10	RBD2	4J1P	10.105	20.989	-6.705	20,20,26	-7.30
11	RBD4	6HOV	10.887	5.487	-0.524	20,20,20	-6.50
12	Sigma-1 receptor	6DK1	12.009	37.935	-33.62	20,20,22	-9.0
13	SARS-CoV-2 3CLpro	6M2N	-32.488	-63.85	40.962	22,20,20	-7.80
14	SARS-CoV-2 PLpro	7JN2	51.608	30.592	0.79	20,20,20	-8.80

a: Docking score of the re-dock co-crystal ligand for the method validation.



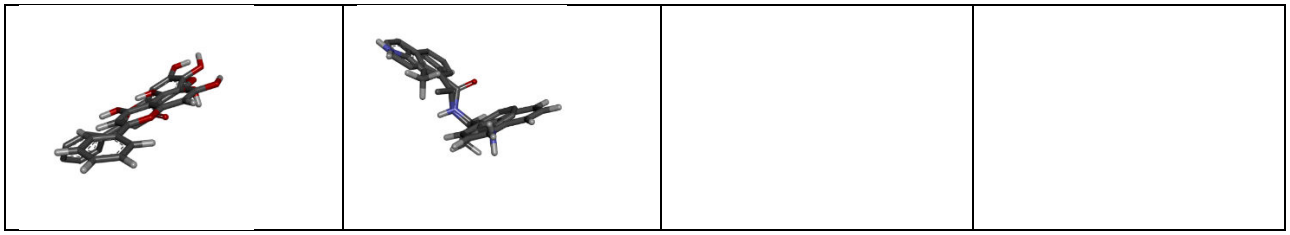


Figure S2. Coordinates of the co-crystal ligand and the re-docked one in the validation of the docking methods with (**A**) p38 MAPK, (**B**) Cathepsin L., (**C**) cyclin-dependent Kinase-1, (**D**) CDK2/CyclinA, (**E**) CDK9/cyclinT1, (**F**) ERK2, (**G**) HDAC2, (**H**) DHODH, (**I**) CK2 alpha, (**J**) RBD2, (**K**) RBD4, (**L**) sigma-1 receptor, (**M**) 3CLpro and (**N**) PLpro.

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