Identification of Compounds from *Curcuma longa* with *In Silico* Binding Potential against SARS-CoV-2 and Human Host Proteins Involve in Virus Entry and Pathogenesis

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Kumar et al.: Curcuma longa Phytochemicals Possess SARS-Cov-2 Viral Entry and Virulence

Severe acute respiratory syndrome coronavirus 2 and associated coronavirus disease 2019 is a newly identified human coronavirus has imposed a serious threat to global health. The rapid transmission of severe acute respiratory syndrome coronavirus 2 and its ability to spread in humans have prompted the development of new approaches for its treatment. Severe acute respiratory syndrome coronavirus 2 requires RNA-dependent RNA polymerases for life cycle propagation and Spike (S)-protein for attachment to the host cell surface receptors. The virus enters the human body with the assistance of a key functional host receptor dipeptidyl peptidase-4 primed by transmembrane serine protease 2 which are putative targets for drug development. We performed screening of 267 compounds from Curcuma longa L. (Zingiberaceae family) against the viral S-protein and RNA-dependent RNA polymerases and host receptor proteins dipeptidyl peptidase-4 and transmembrane serine protease 2 using *in silico* molecular docking. Compounds C1, ((4Z,6E)-1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-4,6-dien-3-one) and C6 ((4Z,6E)-1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-4,6dien-3-one) exhibited tight binding to the S1 domain of the Spike protein than VE607 and with RNAdependent RNA polymerase protein more effectively than ribavirin and remdesivir. These compounds also interacted with the human host proteins dipeptidyl peptidase-4 and transmembrane serine protease 2 with higher efficiency than standard inhibitors sitagliptin and camostat mesylate. The lead compounds showed favorable free binding energy for all the studied protein-ligand complexes in Molecular mechanics/ Generalized born model and solvent accessibility analysis. Besides, other Curcuma longa compounds C14 and C23 exhibited almost similar potential against these target proteins. The structure based optimization and molecular docking studies have provided information on some lead Curcuma longa compounds with probability for advancement in preclinical research.

Key words: Severe acute respiratory syndrome coronavirus 2, *Curcuma longa*, transmembrane serine protease 2, dipeptidyl peptidase-4, spike glycoprotein, RNA dependent RNA polymerase

Coronavirus Disease 2019 (COVID-19) is an acute respiratory illness caused by a novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV -2)^[1]. COVID-19 was first identified in December 2019, spreading from its likely origin in Wuhan, China and throughout the virus has affected millions of people worldwide. On March 11th, 2020, World Health Organization (WHO) announced the SARS-CoV-2 outbreak as pandemic with a state of public health emergency. As of May 7th, 2020, more than 3.77 million cases have been reported across 187 countries and territories, resulting in more than 265 000 deaths (https://www.worldometers.info/coronavirus/). According to current reports, SARS-CoV-2 transmission initiated from bats to humans, followed by human to human transmission occurring through small droplets produced while coughing, sneezing and talking or during close contact^[2]. The rapidly increasing number

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of patients worldwide has prompted researchers to find treatment(s) and cure against this viral infection. Currently there is lack of therapy against SARS-CoV-2 infection, though several compounds have been investigated against some druggable targets for this disease.

The single-stranded SARS-CoV-2 virus enter cells via endocytosis through surface spike (S) proteins and bind to the Angiotensin-Converting Enzyme 2 (ACE-2) and Dipeptidyl Peptidase-4 (DPP4/CD26) receptors. Binding of S-protein to ACE-2 triggers a conformational change in the S-protein facilitating proteolytic digestion by Transmembrane Serine Protease 2 (TMPRSS2) and allows fusion of virus to the cell membrane^[3]. Upon entering the host cell, the viral particle is decoded and assembled for translation utilizing the Open Reading Frames 1a (ORF1a) and Open Reading Frames 1ab (ORF1ab) generating Polyproteins (pp1a and pp1ab). These polyproteins undergo cleavage to form structural proteins for the Ribonucleic acid (RNA) replicasetranscriptase complex responsible for the replication and transcription of viral RNA^[4]. The disruption of the replication processes could lead to identification of potential molecular target(s) to develop effective treatment strategies.

It has been known that many viruses lack preventive vaccines and/or potential antiviral therapy because of the high rate of genomic mutation in the virus allowing them to rapidly evolve and adapt to the host environment^[5]. Under these circumstances traditional herbal medicines and phytochemicals are excellent source for antiviral drug discovery. Some studies demonstrated that natural phytochemicals have different kinds of activities against microorganism and germs including viruses^[6-8]. Natural compounds such as scutellarein, silvestrol, tryptanthrin, amentoflavone, quercetin, myricetin and lectins have shown promise in the suppression of viral attachment and inhibitors of viral enzymes such as proteases, helicases and polymerases^[9,10]. Griffithsin, a liptin derived from red algae inhibits the binding of SARS-CoV-2 spike protein with the human ACE-2 receptor (viral attachment) by virtue of its Receptor-Binding Domain (RBD) (glycosylation site in S1 subunit of spike protein) binding potential^[11]. Previously, Keyaerts et al. showed the SARS-CoV spike protein mannose binding potential of Hippeastrum Hybrid Agglutinin (HAA) lectin *in vitro*^[12]. In another study, Cheng *et al.* reported that saikosaponin B2 natural compound has anticoronaviral activity at micromolar concentrations. The study suggested that saikosaponin B2 possess viral attachment and penetration inhibition potential^[13]. Curcuma longa L. (C. longa) (Zingiberaceae family) is a well-known medicinal plant in Ayurveda and other traditional medicinal systems^[14]. It has been used as a dietary spice and herbal supplement. Phytochemicals present in C. longa possess various pharmacological properties including antiviral activity against dengue and hepatitis C virus^[15,16] and have demonstrated suppression of infection against Zika and Chikungunya virus through inhibition of binding at the cell surface^[17]. Curcumin and related compounds present in C. longa inhibit Human Immunodeficiency Virus (HIV) replication by targeting HIV-1 integrase and HIV-2 proteases^[13]. Curcumin, demethoxycurcumin and bisdemethoxycurcumin inhibit attachment of influenza virus by targeting neuraminidase protein^[18]. Recently our research group reported that C. longa phytochemicals have potential to inhibit SARS-CoV-2 viral virulence by inhibiting Main Protease (Mpro) enzvme^[19].

In the present study, we screened a small library of compounds from *C. longa* against SARS-CoV-2 proteins *viz.* spike glycoprotein (S-protein) and RNA dependent RNA polymerase (RdRp) and human host proteins including CD26 and TMPRSS2 as potential targets and drug designing candidates against SARS-CoV-2.

MATERIALS AND METHODS

C. longa compound retrieval and preparation:

A total of 267 compounds present in C. longa plant were obtained from different literature and search engine platforms such as PubMed, Google Scholar, Web of Science, Science Direct, Scopus, Semantic Scholar, Medline and PubMed Central^[20]. The structures of compounds present in C. longa were prepared by using Marvin Sketch software^[21]. The Two Dimensional (2D) or Three Dimensional (3D) structure of standard compounds against targeted proteins was retrieved from the National Center for Biotechnology Information (NCBI) PubChem in Spatial Data File (.sdf) format^[22]. Open Babel molecule format converter was used to perform conversion of 2D to 3D conformation and their conversion from .sdf to Molecular (.mol) data file^[23]. Ligand energy was minimized by applying Merck Molecular Force Field (MMFF94) and conjugate gradients optimization algorithm using PyRx-Python prescription 0.8 for 200 steps^[24].

Receptor retrieval:

3D structure of SARS-CoV-2 S-protein (Protein Data Bank (PDB) ID: 6VSB) and human CD26 (PDB ID: 4PNZ) receptor were obtained from PDB (https://www.rcsb.org/)^[25]. The resolutions of the retrieved structures were between 1.9 Å to 3.46 Å.

Homology modeling of RdRp and TMPRSS2 protein:

The crystal structure of SARS-CoV-2 RdRp and human TMPRSS2 protein has not been elucidated. Thus, the 3D structure of these two proteins was modeled using the Swiss-model structural bioinformatics server^[26]. Amino Acid (AA) sequences of SARS-CoV-2 RdRp (NCBI reference sequence: YP 009725307.1) and TMPRSS2 (NCBI reference sequence: NP 001128571.1) were retrieved from the NCBI database. We used SARS coronavirus Non-Structural Protein 12 (NSP12) (PDB ID-6NUR) and serine protease hepsin (PDB ID-5CE1) as respective templates for modeling of SARS-CoV-2 RdRp and TMPRSS2. For the alignment of TMPRSS2 and RdRp target with their respective template sequences the T-Coffee server was used^[27]. Representation of the alignment was made using ESPript 3.0 server^[28]. Modelled structures were refined using ModRefiner sever^[29].

Receptor preparation:

3D structure of SARS-CoV-2 S-protein and human CD26 protein was loaded onto the University of California, San Francisco (UCSF) Chimera for molecular docking preparation^[30]. Protein models were cleaned and optimized by removing ligands and other heteroatoms including water. After this step, the energy minimization of protein structures was performed by steepest descent method having 100 steps (step size 0.02 Å) and a conjugate gradient method with 10 steps (step size 0.02 Å) using UCSF Chimera.

Active site prediction of RdRp and TMPRSS2 modelled protein:

It is anticipated that an effective drug ligand to dock either within the protein pocket or the functional active region. The possible binding sites were searched for RdRp and TMPRSS2 modeled proteins using Computer Atlas of Surface Topography of Proteins (CASTp) 22 tool. The CASTp22 tool predicts potential pockets of target protein and confirms whether the highest frequency binding sites of the heat map were located within the protein pocket^[31].

Molecular docking studies:

Auto Dock Tools 1.5.6 (ADT) was used to dock the test ligands on targeted protein^[32]. Gasteiger partial charges assigned to the ligands and docking calculations were performed. Polar hydrogen atoms, Kollman charges and solvation parameters were applied using appropriate Auto Dock tool. Lamarckian Genetic Algorithm (LGA) was used to explore the active binding region in this study. The grid box included the entire binding site of the protein providing enough space for the ligands translational and rotational walk. For each of the 30 independent runs, a maximum number of 27 000 Genetic Algorithm (GA) operations were generated on a single population of 150 individuals. Operator weights for the rate of crossover, gene mutation and elitism were set as 0.80, 0.02 and 1 respectively. LigPlot⁺ (v.1.4.5) and UCSF chimera (v.1.10.2) online tools were used for protein-ligand interaction visualization^[33].

Molecular Mechanics/Generalized Born Model and Solvent Accessibility (MM-GBSA) analysis:

Prime MM-GBSA analysis was used to evaluate the receptor/protein and receptor/protein-ligand binding energies, which includes the VSGB solvent model, Optimized Potentials for Liquid Simulations (OPLS)-2005 force field and rotamer search algorithms. The Prime MM-GBSA simulation was performed by using the Glide pose viewer file to compute the total free energy of binding. The MM-GBSA calculations were attained to evaluate the relative binding affinity of test molecules and their respective complexes with lead ligands (reported in kcal/mol). As the MM-GBSA binding energies are approximate free energies of binding, a more negative value indicates stronger binding.

RESULTS AND DISCUSSION

In the present study, a total of 267 *C. longa* compounds were searched from the literature and docked against two SARS-CoV-2 proteins including S-protein and RdRp and two human host proteins CD26 and TMPRSS2. Binding score of lead compounds against SARS-CoV-2 S-protein and RdRp modelled, CD26 and TMPRSS2 modelled proteins are shown in fig. 1A -fig. 1D with a cut-off score of \leq -7, \leq -4, \leq -6 and \leq -5 kcal/mole. The structure of lead compounds with their name and targets are represented in Table 1^[34-47]. CASTp server was utilized to predict the binding pockets in RdRp and TMPRSS2 modeled protein. The predicted solvent-accessible surface area



Fig. 1: Docking score of *C. longa* compounds against SARS-CoV-2 virus and human host proteins, (A) Docking score of lead compounds (\leq -6 kcal/mol) against human CD26 protein; (B) Docking score of lead compounds (\leq -5 kcal/mole) against modeled human TMPRSS2 protein; (C) Docking score of lead compounds (\leq -7 kcal/mole) against SARS-CoV-2 spike glycoprotein; (D) Docking score of lead compounds (\leq -4 kcal/mole) against SARS-CoV-2 RdRp; (E) Structure of lead *C. longa* compound [(4Z,6E)-1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-4,6-dien-3-one] against human CD26 and SARS-CoV-2 spike glycoprotein; (F) Structure of lead *C. longa* compound [(4Z,6E)-1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-4,6-dien-3-one] against human TMPRSS2 and RdRp modeled protein; (a) Sitagliptin; (b) Vildagliptin; (c) Linagliptin; (d) Saxagliptin; (e) Alogliptin; (f) Teneligliptin; (a') Ribavirin; (b') Remdesivir; (c') Galidesivir; (d') Tenofovir; (e') Sofosbuvir; CM-Camostat mesylate

and volume for the RdRp protein was 2796.437 Å2 and 5657.063 Å3 respectively. Similarly, the predicted solvent-accessible surface area and volume for the TMPRSS2 protein was 338.267 Å2 and 264.585 Å3 respectively (fig. 2A and fig. 2B).

The *in silico* models SARS-CoV-2 RdRp and human TMPRSS2 proteins were generated by homology modeling employing the Swiss Model web server and CASTp22 prediction tool^[26,31]. The homology model of the TMPRSS2 was adopted from our recently published article^[47]. Alignment analysis of the RdRp and TMPRSS2 with their respective template sequences utilized for the homology modelling is depicted in fig. 3. The generated modelled proteins were respectable at different modelling parameters such as MolProbity score, Clash score, local similarity to the target, normalized QMEAN score, Ramachandran favored, Ramachandran outliners, Bad angles and Bad

bonds (fig. 4 and Table 2)^[48]. AA residue of targeted proteins (PDB 4PNZ, PDB 6VSB; RdRp modeled and TMPRSS2 modeled), type of interaction, binding energy of protein-ligand complex and binding affinity of some lead compounds against respective protein has been summarized in Table 3. MM-GBSA analysis of the lead C. longa phytochemicals and standard inhibitors with their respective protein/receptor complexes and unbound proteins was performed and the results are shown in Table 4. Results showed favorable free energy of protein/receptor-ligand complexes in comparison to unbound proteins in MM-GBSA analysis. Various standard inhibitors from published studies were engaged to compare the target protein binding potential of C. longa compounds^[49-53] (fig. 1A-fig. 1D. Binding pattern of the compounds against viral and human host proteins are shown in fig. 5 and fig. 6. Docking score of standard compound against SARS-CoV-2 S-protein,

TABLE 1: LIST OF LEAD *C. longa* PHYTOCHEMICALS AGAINST SARS-COV-2 AND HUMAN HOST PROTEINS RELATED TO VIRAL INFECTION

S. No	Compound name	Structure	Reference	Targeted protein
1	(4Z,6E)-1,5-dihydroxy-1,7-bis(4-hydroxy-3-	HO - 0H	[28]	Spike [#] , CD26,
	methoxyphenyl)hepta-4,6-dien-3-one	"Daard"		TMPRSS2*, RdRp*
		оно он		
2	(1E)-1,7-bis(4-hydroxy-3-methoxyphenyl)	0 Q	[29,30]	Spike, CD26, TMPRSS2
	hept-1-ene-3,5-dione	gystige		
З	Tetrabydroxycurcumin	ног 🗸 🗸 он	[31]	Spike CD26 TMPRSS2
5		HO O O HO		RdRp
		но он он он		·
4	(1E)-7-(4-hydroxy-3-methoxyphenyl)-1-(4-	0 0	[29,30]	CD26
	hydroxyphenyl)hept-1-ene-3,5-dione	HOLOCOL		
5	(1E)-1,7-bis(4-hydroxyphenyl)hept-1-	0 0	[29,30]	CD26
	ene-3,5-dione	min		
		но	1001	
6	(42,6E)-1,5-dihydroxy-1-(4-hydroxy-3-		[28]	Spike, CD26, TMPRSS2,
	hepta-4 6-dien-3-one	но		какр
7	2-methyl-5-(6-methylhept-5-en-2-yl)		[32]	CD26
	cyclohex-2-ene-1,4-diol	LA OH		
		HOLY		
8	(4Z,6E)-1,5-dihydroxy-7-(4-hydroxy-3-	HO OH	[28]	Spike, CD26
	methoxyphenyl)-1-(4-hydroxyphenyl)	appling		• •
9	hepta-4,6-dien-3-one (6E)-3-bydroxy-1 7-bis(4-bydroxyphenyl)	но	[28]	Spike CD26 TMPRSS2
,	hept-6-ene-1,5-dione	C C C C C C C C C C C C C C C C C C C		RdRp
10	Cyclocurcumin	но	[33]	CD26
10	cycocalcalini	S.		0020
		\$~~~{		
11	(4Z,6E)-1,5-dihydroxy-1,7-bis(4-	OH O OH	[28]	Spike, CD26, TMPRSS2,
	hydroxyphenyl)hepta-4,6-dien-3-one	ностолон		RdRp
12	Santalol A	<i>(</i>],	[34]	CD26
		N.		
		HO		
13	Bisacurone	0	[28]	CD26, RdRp
		OH		
14	5-hvdroxy-6-(3-hvdroxy-4-methylphenyl)-2-	Ton	[35]	CD26, RdRp
	methylhept-2-en-4-one	LIL_OH		02_0,p
		но		
15	Bisacurone B	0	[36]	CD26. RdRn
		LULOH		
		COH		

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16	6-(5-hydroxy-2-methoxy-4- methylcyclohex-3-en-1-yl)-2-methylhept-2-	АЛАН	[37]	CD26		
17	en-4-one 2-hydroxy-4-(6-methyl-4-oxohept-5-en-2- yl)benzaldehyde	ОН	[37]	CD26		
18	(1E,6E)-1-(3,4-dihydroxyphenyl)-7-(4- hydroxyphenyl)hepta-1,6-diene-3,5-dione	СССССССС	[37]	TMPRSS2, RdRp		
19	(1E,4E)-1,5-bis(4-hydroxy-3- methoxyphenyl)penta-1,4-dien-3-one	HO CON	[32]	TMPRSS2		
20	(1E,4E,6E)-1,7-bis(4-hydroxyphenyl) hepta-1,4,6-trien-3-one		[38]	TMPRSS2		
21	1-(5-hydroxy-3,6-dimethyl-2,3,3a,4,5,7a- hexahydro-1-benzofuran-2-yl)-3- methylbut-2-en-1-one		[32]	TMPRSS2		
22	(1E)-1-(4-hydroxy-3-methoxyphenyl)-7-(4- hydroxyphenyl)hept-1-ene-3,5-dione	<u> </u>	[29,30]	TMPRSS2		
23	Calebin A	HO CONTRACTOR	[30]	TMPRSS2, RdRp		
24	Curcumin	HO CON	[39]	Spike		
25	1,6-Heptadiene-3,5-dione, 1,7-bis(4- hydroxy-3-methoxyphenyl)	AND LONG	[40]	Spike		
26	Curculonone D	СОН	[32]	RdRp		
27	Vanillin	OT OH	[41]	RdRp		



Fig. 2: The surface of the ligand binding pocket calculation of (A) RdRp and (B) TMPRSS2 modeled protein using CASTp 3.0. server. The binding pocket is shown in red color

A	1 10	20	30	40	50	60	70	80	90
6NUR-Template	MGSADASTFLNR	VCGVSAARLTPC	GTGTSTDVV	YRAFDIYNEK	VAGFARFLETN	CCRFQEKDE	EGNLLDSYFV	VKRHTMSNYQH	EETIYN
RDRP-Target									
consensus>70									
	100	110	120	130	140	150	160	170	180
SNUR-Template	LVKDCPAVAVHD	FFRFRVDGDRVP	AISROLLTA	YTMADLVIAL	RHFDEGNCDTL	KEILVTINC:	CODDYFNERD	WYDFVENPDIL WYDFVENPDIL	BUYANI
consensus>70			QRLTK	YTMADLVYAL	RHFDEGNCDTL	KEILVTYNC	CDDDYFNEED	WYDFVENPDIL	RVYANL
	190	200	210	220	230	240	250	260	270
6NUR-Template	GERVROSLLKTV	OFCDAME AGIV	GVLTLDNQD	LNGNWYDFGD	FVQVAPGCGVP	IVDSYYSLL	PILTLTRAL	ARSENDADIA	SPI ISS
RDRP-Target	GERVECALLETV	OFCDAME AGIV	GVLTLDNOD	LNGNWYDFGD	TIQTTPGSGVP	VDSYYSLL	MPILTLTRAL	TAESEVOTOLT	30° 1030
consensus> /0	GERVEQ. LLETT	QFCDARREAGIV	GVLTLDNQD	LNGNWIDFGD	FIQ PG. GVP	VDSIISLL	AFILTLTRAL	AESH.D.DL.	AP.IAN
(MIII)- Ferry lake	DITIONNER	2 9 V	500		329	330		359	100
RDRP-Target	DLLKYDFTEERL	LFDRYFKYWDO	TYHPNCVNC	LDDRCILHCA	NFNVLFSTVFP	PISFGPLVR	LIFVDGVPFV	VSTGYRFRELG	VVENOD
consensus>70	DLLKYDFTEERL	LFDRYFKYWDQ	TYHPNCINC	LDDRCILHCA	NFNVLFSTVFP	PISFGPLVR	KIFVDGVPFV	VSTGYHFRELG	VVENQD
	370	380	390	400	410	420	430	440	450
6NUR-Template	VNLRSSRLSFKE	LLVYAADPANHA	ASGNILLDK	RTTCFSVAAL	TNNVAFQTVKP	GNFNKDFYD	AVSKOPPKE	GSSVELKEFFF	AQDGNA
consensus>70	VNLHSSRLSFKE:	LLVYAADPAMHA	ASGNLLLDK	RTTCFSVAAL	TNNVAFOTVEP	GNFNKDFYD	FAVSKGFFKE	GSSVELKHFFF	AQDGNA
	460	470	480	490	500	510	520	530	540
6NUR-Template	AISDYDYYRYNL	PTHCDIRQLLFV	VEVVDEYFD	CYDGGCINAN	QVIVNNLDKSA	GFPFNKWGK	RLYYDSMSY	EDQDALFAYTE	RNVIPT
RDRP-Target	AISDYDYYRYNL	PIMCDIRQLLFV	VEVVDEYFD	CYDGGCINAN	QV IVNNLDKSA	GFPFNKWGK.	RLYYDSMSY	EDQDALFAYTE	RNVIPT
consensus>70	AISDIDITRINL	PTHCDIRQLLFV	VEVVDKIFD	CIDGGCINAN	QVIVNNLDKSA	GFPFNKWGK	RLIIDSMSI	EDQUALFATTE	RNVIPT
Chillion Barrellacka	550	260	570	580	590	600	610	620	630
RDRP-Target	ITOMNLEYAISA	ENRARIVAGUSI	CSTMTNROF	HOKLLKSIAA	TRGATVVIGTS	EFYGGWHNM	LETVYSDVEN	PRLNGWDYPKC	DRAMPN
consensus>70	ITQMNLKYAISA	KNRARTVAGVSI	CSTMINRQF	HQKLLKSIAA	TRGATVVIGTS	KFYGGWHNM	LKTVYSDVE .	PHLMGWDYPKC	DRAMPN
	640	650	660	670	680	690	700	710	720
6NUR-Template	MERIMASLVEAR	KENTCCHLSERF	TRLANECAQ	VLSENVMCGG	SLYVEPOGTES	GDATTAYAN	VFNICQAVT	ANVNALLSTDG	NKIADK
RDRP-Target	MLRIMASLVLAR	KR <mark>TTCC</mark> SLSRRF	TRLANECAO	VLSENVMCGG	SLYVEPOGTSS	GDATTAYAN	EVFNICQAVT	ANVNALLSTDG	NKIADK
consensus>70	ALKINASLVLAK	AR. TCC. LSHKP	TREANECAU	V LSERVACGG	SLIVAPGGISS	GDATTAIAN	TOO	ANVNALLSTDG	ALADA
OWID-Ferrylate	THE REAL PROPERTY OF				Nove States and a				
RDRP-Target	TVRNLOHRLYECI	TRNRDVDTOFV	NEFTATLER	RESMMILSDD	AVVOTNSTYAS	OGLVASIEN	KSVLTTONN	VFMSEAKCHTE	TDLTKG
consensus>70	YVRNLQHRLYECI	LYRNRDVD FV	# EFYAYLRK	HFSMMILSDD	AVVCNNS.YA.	QGLVASIKNI	K. VLYYQNN	VFMSEAKCWTE	TDLTKG
	820	830	840	850	860	870	880	890	900
6NUR-Template	PREFCEQUENTIALV	830 KQGDDYVYLPYP	DPSRILGAG	850 CFVDDIVKTD	860 GTLMIERFVSL	870 AIDAYPLTK	S S O	890 HAYLQYIRKLE	900 DELTGR
6NUR-Template RDRP-Target	820 PHEFCSQHTMLVI PHEFCSQHTMLVI	830 KOGDDYVYLPYP KOGDDYVYLPYP	840 DPSRILGAG DPSRILGAG	850 CFVDDIVKTD CFVDDIVKTD	860 GTLMIERFVSL GTLMIERFVSL	870 AIDAYPLTK AIDAYPLTK	PNQEYADVF	890 HLYLQYIRKLE ELYLQYIRKLE	900 DELTGE DELTGE
6NUR-Template RDRP-Target consensus>70	820 PHEFCSQHTMLVI PHEFCSQHTMLVI PHEFCSQHTMLVI	830 CODDIVILOIO CODDIVILOIO KQGDDIVILOIO	840 DPSRILGAG DPSRILGAG DPSRILGAG	S 5 0 CFVDDIVKTD CFVDDIVKTD CFVDDIVKTD	860 TIMIERFVSL GTIMIERFVSL GTIMIERFVSL	870 AIDAYPLTK AIDAYPLTK AIDAYPLTK	SBO PNOLYADVI PNOLYADVI IPNOLYADVI	890 LYLQYIRLE BLYLQYIRLE BLYLQYIRKLE	900 Deltgr Deltgr Deltgr
6NUR-Template RDRP-Target consensus>70	820 PHEFCSQRTMLVI PREFCSQRTMLVI PHEFCSQRTMLVI 910	830 CCDDTVILPTP CCDDTVILPTP KQCDDTVILPTP 920	840 DPSRILGAG DPSRILGAG 930	SSO CFVDDIVKTD CFVDDIVKTD CFVDDIVKTD 940	860 Timierfysi Timierfysi Gtimierfysi 950	870 AIDAYPLTK AIDAYPLTK AIDAYPLTKI	SBO PROTADVE PROTADVE IPROEYADVE	890 HLYLQYIRKLE HLYLQYIRKLE	900 DELTGE DELTGE DELTGE
GNUR-Template RDRP-Target consensus>70 GNUR-Template RDRP-Target	820 PHEFCSQHTMLVF PHEFCSQHTMLVF 9HEFCSQHTMLVF 910 MLONTSVHLTNO	830 QGDDTYYLPYP QGDDTYYLPYP RQGDDYYYLPYP 920 TSPYTEPE FYE	840 DPSRILGAG DPSRILGAG DPSRILGAG 930 AMYTPHTVL	SSO CFVDDIVKTD CFVDDIVKTD CFVDDIVKTD 940 LVPRGSGHHHI	860 TLMIERFVSL TLMIERFVSL GTLMIERFVSL 950 HHHAWSHPQFE	870 AIDAYPLTK AIDAYPLTK AIDAYPLTK K	SSO IPNOEYADVF IPNOEYADVF IPNOEYADVF	890 HIYLQYIRKLE HIYLQYIRKLE HIYLQYIRKLE	900 Deltgr Deltgr Deltgr
6NUR-Template RDRP-Target consensus>70 6NUR-Template RDRP-Target consensus>70	820 PHEFCSQHTMLVI PHEFCSQHTMLVI PHEFCSQHTMLVI 910 MLDNYSVMLTNDI MLDNYSVMLTNDI	830 QGDDYYLDYP KQGDDYYLDYP 920 TSBYLDP TSBYLDP NTSRYWEPE	940 DPSRILGAG DPSRILGAG 930 AMYTPHTVL	850 CFVDDIVKTO CFVDDIVKTO CFVDDIVKTO 940 LVPRGSGHHH	SGO TLMIERFVSL TLMIERFVSL GTLMIERFVSL 950 HHRAWSHPQFE	870 AIDAYPLTK AIDAYPLTK AIDAYPLTK AIDAYPLTK K	880 IPNCETADVF IPNCETADVF IPNCETADVF	890 ELYLQYIRKLE RLYLQYIRKLE	900 DELTGR DELTGR DELTGR
GNUR-Template RDRP-Target consensus>70 GNUR-Template RDRP-Target consensus>70	820 PHEFCSQHTMLV PHEFCSQHTMLV 910 HOMYSVNLTND MLDMYSVMLTND	830 QGDDYYLPYP KQGDDYYLPYP 920 TSRYMPP NTSRYMEPE	940 DPSRILGAG DPSRILGAG 930 AMYTPHTVL	850 CIVDDIVKTO CIVDDIVKTO CIVDDIVKTO 940 LVPRGSGHHH	960 TLMIERFVSL STLMIERFVSL 950 HHHAWSHPQFE	970 AIDAYPLTK AIDAYPLTK AIDAYPLTK K	880 IPNOLTADVI IPNOLTADVI IPNOLTADVI	890 HLYLQYIRKLE HLYLQYIRKLE HLYLQYIRKLE	900 DELTGR DELTGR DELTGR
GNUR-Template RDRP-Target consensus>70 GNUR-Template RDRP-Target consensus>70 R	920 PHEFCSQHTMLV PHEFCSQHTMLV 910 100 YSVLTND MDMSVLTND MDMSVMLTND	830 QGDJYYIAPYP QGDDYYIAPYP XQGDDYYYLPYP 920 TSRYAPIFYE TSRYAPIFYE	840 DPSRILGAG DPSRILGAG DPSRILGAG 930 AMYTPHTVL	250 CIVDDLVKTD CIVDDLVKTD CIVDDLVKTD 940 LVPRGSGHHH	960 TLMIERFVSL STLMIERFVSL 950 HHRAWSHPQFE	870 AIDAYPLTKI AIDAYPLTKI AIDAYPLTKI K	BEQ IDNQETADVF IDNQETADVF	890 HLYLQYIRKLE HLYLQYIRKLE HLYLQYIRKLE	900 Deltgr Deltgr Deltgr
GNUR-Template RDRP-Target consensus>70 GNUR-Template RDRP-Target consensus>70 B	820 820 820 820 820 820 810 810 810 80 80 80 80 80 80 80 80 80 8	830 000019101090 0000191091090 0000191090 0000190 000100 000100 000100 000100 000100 000100 000100 0000 00000 0000	840 DPSRILGAG DPSRILGAG 930 AMYTPHTVL	850 CPVDDVKTD CPVDDVKTD CPVDDIVKTD 940 LVPRGSGHHH	860 TLM ERFVSE GTLMTERFVSE 950 HHHAWSHPQFE 50	870 AIDAYPLTKI AIDAYPLTKI K 60	SEQ IDNQETADVF IDNQETADVF IDNQETADVF	890 80	900 DELTGR DELTGR DELTGR 90
GNUR-Template RDRP-Target consensus>70 GNUR-Template RDRP-Target consensus>70 B SCE1 1-Template SCE1 1-Template	620 DIACSOLTMLY DIACSOLTMLY PREFCSQRTMLY 510 MONEY PREFCSQRTMLY 100 100 100 100 100 100 100 10	830 (CODDYVLEYP (CODDYVLEYP 920 920 1097 1	840 DPSRILGAG DPSRILGAG 930 AMYTPHTVL: 30 TEGTWRLLCS	250 CPVDDIVKTD CPVDDIVKTD 940 LVPRGSGHHH LVPRGSGHHH	860 STAN STANSA STAN STANSA GTAN ERFVSA 950 HHHAWSHPQFE SCEEMGFLRAL	879 HDAYPLTK HDAYPLTK AIDAYPLTK K - - - - - - - - - - - - - - - - - -	70 AGANGTSGFF	B 9 0 LYLOY DELT HYLOY DELT HLYLOY DELT RLYLOY DELT CVDEGRLPHTQI	900 DELTGR DELTGR DELTGR 90 RLLEVI
GNUR-Template RDRP-Target consensus>70 GNUR-Template RDRP-Target consensus>70 B 5CE1_1-Template TMPR52-Target consensus>70	820 011 C 50 H 14 011 C 50 H 14 011 C 50 H 14 010 010 C 50 H 14 010 010 010 010 010 010 010 0	630 CODIVILEYP CODIVILEYP 200 920 10011100 1001100 1001100 1001100 1001100 1001100 1001100 1001100 100100	840 DPSRLGAG DPSRLGAG 930 AMYTPHTVL 30 TEGTWRLLCS	40 SSRSNARVAGL	860 CIANA STAVEN CIANA STAVEN CIANA STAVEN CIANA STUMIERFVSL SSO SCEEMGFLRAL	870 HDAYDETK AIDAYDETK K GQ THSELDVRT/	70 AGANGTSGFF	890 11410410411 HLYLQYIRKIH 60 CVDEGRIPHTQ	900 DELTGR DELTGR DELTGR 90 RLLEVI
GNUR-Template RDRP-Target consensus>70 GNUR-Template RDRP-Target consensus>70 B SCE1 1-Template TMPRSS2-Target consensus>70	820 910 C 50 C 1 M LV 910 C 50 C 1 M LV 910 M S V 1 A 10 10 D S D 2 F L Y P V 1 10 D S D 2 F L Y 1 10 D S D 2	030 CCDDYVILDYD CCDDYVILDYD 920 10871102 1087110 1097110000000000	B40 DPSRIGAG DPSRIGAG 930 AMYTPHTVL 30 TEGTWRLLCS	40 SSRSNARVAGL	860 DIANE DIAVSE DIANE DIAVSE CILMIERPASE 950 HHHAWSHPQFE 50 SCEEMGFLRAL	60 THSELDVRT	80 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	B90 EVECTORE HULCYTORE RLYLQYIRKER CVDEGREPHTQ	900 DELTGR DELTGR DELTGR 90 RLLEVI
GNUR-Template RDRD-Target consensus>70 GNUR-Template RDRD-Target consensus>70 B SCE1 1-Template TMPDES2-Target consensus>70 SCE1 1-Template	B20 DIFCEQUENTAL PREFCQUENTLY PREFCQUENTLY PREFCQUENTLY 1 10 SDQEPLYPVQVI 1 10 SDQEPLYPVQVI 100 SVCDCBEFLI	20 20 20 20 20 20 20 20 20 20	840 DPSR146AG DPSR146AG 930 AMYTPRTVL: 30 TEGTWRLLCS 120 PUDTVF	850 EV/0 D V V 50 EV/0 D V 50 EV/0 D V 50 940 LVPRGSGHHHI LVPRGSGHHHI 40 SSRSNARVAGL	860 2514 8.0 14 20 2524 8.0 14 20 2524 8.0 14 20 2550 2550 8.0 14 14 14 20 2550 8.0 14 14 14 14 14 14 14 14 14 14 14 14 14	60 THORPETK AIDAYPETK AIDAYPETK K 	POQUADUF	BOO DVLQVIRKI NLVLQVIRKI RLVLQVIRKI CVDEGRLPHTQI	900 DELTGR DELTGR DELTGR 90 RLLEVI
GNUR-Template RDRP-Target consensus>70 GNUR-Template RDRP-Target consensus>70 B SCE1_1-Template TMPRSS2-Target SCE1_1-Template		830 600 600 600 600 600 600 800 80	840 DDSR16AG DDSR16AG S30 AMYTPHTVL: 30 TEGTWRLLCS 120 PVDR1VC. RCV_LYCPHE	850 CAVDDIVA.CO CAVDDIVA.CO CAVDDIVA.CO CAVDDIVA.CO SPUDIVA.CO SPUDIVA.CO SPUDIVA.CO SSRSNARVAGL	BEO DIA BOANDA STLMIERFUL 950 IRHANSHPOPE 50 SCEEMOFIRAL SNEPVCQDDMN	670 10A 79 LTK 10A 79 LTK A 10A 79 LTK K 60 THSELDVRT. ENYDRAACS.	POLYADY POLYADY POLYADY POLYADY IPNQEYADY 70 AGANGTSGFF	BOO BOO BULLOUIDER BLULOUIDER CUDECELPHIQ SQGIUDDSOST	900 DELTGR DELTGR DELTGR 90 RLLEVI SFMKLN
GNUR-Template RDRP-Target RDRP-Target GNUR-Template RDRP-Target Consensus 70 B SCEL 1-Template TMPRSS2-Target SCEL 1-Template TMPRS2-Target SCEL 1-Template	820 820 840 840 840 840 840 840 840 84	830 830 800 800 800 800 800 800	B 4 0 DDSRILGAG DDSRILGAG S30 AMYTPHTVL 30 TEGTNRLLCS 120 PVD VC. RCVLYCPNI R. GVPI	eso evod divato evod divato cfvd divato 940 Lvprgschnhi ssrsnarvaci pilqvyssqrk	BEO DEL LE SEVER DEL LE SEVER DEL LE REVEL STLMIERFYSL 950 INNANSNPOFE SCEEMGFLRAL SWRPVCQDDWN	60 THSELDVRT	70 AGANGTSGFF	BO BO BO CVDECRLPHTQ SQGIVDDSCST	900 DELTGH DELTGH 90 RLLEVI SFMKLN
GNUR-Template RDRP-Target consensus>70 GNUR-Template RDRP-Target consensus>70 B SCEL 1-Template TMPRSS2-Target consensus>70 SCEL 1-Template	B20 DIFCEQUENTAL PREFCQUENTLY PREFCQUENTLY PREFCQUENTLY DIFC	20 CODYVIL P CODYVIL P RQCDYVIL P P P P P P P P P P P P P P	B 4 0 DPSRIEAC DPSRIEAC 930 AMYTPHTVL: 30 TEGTWRLLCS 120 PUDTVC. RCVLYCPNI RCVLYCPNI	eso evod Divato evod Divato crvd Divato 240 Lvprgschnei 55rsnarvagl Filqvyssqrr	SOUTH A SOUTH AND	60 THORPETA A IDAYPETA A IDAYPETA K 	70 AGANGTSGPF	80 80 80 CVDEGRLPHTQI SQGIVDDSGST 50 16	900 900 900 RLLEVI 500 800 800 800 800 800 800 800 800 800
GNUR-Template BDRD-Target Connersus>70 GNUR-Target Consensus>70 B SCEL1-Template SCEL1-Template SCEL1-Template Consensus>70 SCEL1-Template Consensus>70 SCEL1-Template	B20 B1CCSQTMLV PHEPCSQTMLV PHEPCSQTMLV PARTCSQTMLV PARTCSQTMLV SLO SDQEPLYPVQV 100 SVCDCPRGRPL	S30 S30 S00 S00 S00 S00 S00 S00	840 DFSRIGAG DFSRIGAG 930 ANYTPHTVL: 30 16 120 PUDIVG R. GUILOS	650 6700 514 4 50 6700 514 4 50 6700 514 4 50 940 1998 50 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	SWHPVCQDDWN	60 THORN PLTK AIDAYPLTK K C THSELDVRT ENYCRAACR G G C C C C C C C C C C C C C C C C C	70 AGANGTSGFF	B90 B90 B90 B90 B90 B90 CVDECRLPHTQI CVDECRLPHTQI SQCIVDDSGST 50 16	900 DELTGE DELTGE 90 RLLEVI SFMKLN 9 FPERNE
GNUR-Template RDRP-Target connensus>70 GNUR-Template RDPP-Target connensus>70 B SCE1 1-Template TMPRS2-Target connensus>70 SCE1 1-Template TMPRS2-Target connensus>70 SCE1 1-Template TMPRS2-Target connensus>70	820 820 840 840 840 840 840 840 840 84	830 830 830 830 830 830 830 830	B 40 DPSRIEAC DPSRIEAC 930 AMYTPHTVL: 120 PVDEIVE. RCVEYEPH VSIRCIACO	e 50 EVO DIVATO EVO DIVATO CFV DDIVATO 940 UVPRGSCHNHI SSRSNARVAGL FILQVYSSQRR	BEO DEL HER AVEN DEL HER AVEN DEL HER AVEN STIMTERFYSI 950 SCEEMFPOPE SCEEMFPLRAL SHEPVCQDDMN 13 VGCESALPCA	60 60 10 A 10 A	70 AGANGTSGFF CMGYKNNFYS 10 10 10 10 10 10 10 10 10 10	80 60 CVDECRLPHTQ SQGIVDDSGST 50 14 60 CVDECRLPHTQ 14 70 70 70 70 70 70 70 70 70 70	900 95565 95576 90 RLLEVI SFMKLN 9 FFERNA V. EK
GNUR-Template RDRP-Target consensus 70 GNUR-Target consensus 70 GNUR-Target consensus 70 SCE1_1-Template TMPRSS2-Target consensus 70 SCE1_1-Template TMPRSS2-Target consensus 70 SCE1_1-Template TMPRSS2-Target consensus 70	820 820 820 820 820 820 820 820	830 830 800 800 800 800 800 800 800 800	B 40 DPSRLGAG DPSRLGAG S30 AMYTPRTVL 30 TEGTWRLLCS 120 PVDCTVC R.CVLTCPRS VSLRCIACCO	eso evod Siva eo evod Siva eo e	BEO BEAL HE AND AN DA CILL HE AND AND AN CILL HE RANDA SO SO SO SO SO SO SO SO SO SO SO SO SO	670 10 N P L T K 10 N P L T K 10 N P L T K 10 N P L T K 60 T H S E L D V R T 	70 AGANGTSGPF AGANGTSGFFF AGANGTSGFFF AGANGTSGFFF AGANGTSGFFF AGANGTSGFFF AGANGTSGFFF AGANGTSGFFF AGANGTSGFFF AGANGTSGFFF AGANGTSGFFF AGANGTSGFFF AGANGTSGFFF AGANGTSGFFF AGANGTSGFFFF AGANGTSGFFFF AGANGTSGFFFF AGANGTSGFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF	80 80 80 80 CVDEGRLPHTQI 80 CVDEGRLPHTQI 80 80 80 80 80 80 80 80 80 80	900 015 - 50 015 - 50 0
GNUR-Template DDRD-Target DDRD-Target consensus>70 GNUR-Template DDRD-Target consensus>70 B SCEL 1-Template TMPASS2-Target consensus>70 SCEL 1-Template TMPASS2-Target consensus>70 SCEL 1-Template TMPASS2-Target SCEL 1-Template		830 830 830 830 830 830 830 830	840 DISALGAG DISALGAG DISALGAG 930 AMYTPHTVL: 30 120 PVDIVE RCVIVE RCVIVE RCVIVE RCVIVE 190 190 190 190 190 190		8 6 0 1 1 1 8 2 4 5 1 1 2 1 4 8 2 4 5 1 1 2 1 4 8 2 4 5 1 5 5 0 5 0 5 0 5 0 5 0 5 0 5 0 5	60 60 10 A 10 L TX A 10 A YP L TX 60 THSELDVRT 70 THSELDVRT 70	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	80 80 80 80 CVDECRLPHTQI 80 CVDECRLPHTQI 80 CVDECRLPHTQI 80 16 80 16 16 16 16 16 16 16 16 16 16	900 915 CCI 925 CCI 925 CCI 90 RLLEVI SPMKLN 90 FPERNR 90 FPERNR 90 FPERNR 90 FPERNR 90 FPERNR
GNUR-Template RDRP-Target Consensus>70 GNUR-Template ERP2-Target Consensus>70 B SCEL 1-Template TMPR52-Target consensus>70 SCEL 1-Template TMPR52-Target consensus>70 SCEL 1-Template TMPR52-Target Consensus>70 SCEL 1-Template TMPR52-Target Consensus>70 SCEL 1-Template	820 14 CEQUENIX 910 910 910 910 910 910 910 910	830 830 830 830 830 830 830 830	840 DPSR14GAG DPSR14GAG 930 AMTTPHTVL: 120 PVDC1VC. RCV61VC. RCV61VC. RCV61VC. RCV61VC. RCV61VC. 190 LOF61VC. 190 LOF61VC.		860 511 4 5 3 4 5 4 521 4 5 3 4 5 4 521 4 5 3 4 5 4 950 50 50 50 50 50 50 50 50 50	60 60 10A PD LTX A IDA VP LTX A IDA VP LTX A IDA VP LTX A IDA VP LTX COMPANY	70 70 70 70 70 70 70 70 70 70	800 140041400 14004140000000000	900 015 40 015 40 010000000000000000000000000000000000
GNUR-Template BDRD-Target Connersus>70 GNUR-Template EDRD-Target consensus>70 BSCIII-Template TMP2552-Target Consensus>70 SCIIII-Template TMP2552-Target consensus>70 SCIIII-Template TMP2552-Target consensus>70 SCIIII-Template	B20 B20 B20 B20 B20 B20 B20 B20	830 830 800 800 800 800 800 800 800 800	B 40 DISALGAG DISALGA		50 50 50 50 50 50 50 50 50 50 50 50 50 5	670 10 A 10 A	TO TO TO TO TO TO TO TO TO TO	890 1140041441 1140041441 1140041441 1140041441 1140041441 800 CVDEGRLPHTQI 800 CVDEGRLPHTQI 800 110005057 1100005057 1100005057 1100005057 1100005057 1100005057 11000050	900 SLYCH SLYCH DELTGR 90 RLLEVI SPMKLN 9 PPERNR V. EX F
GNUR-Template RDRR-Target CORPERANDO GNUR-Template RDRR-Target consensus 70 B SCE1 1-Template TMPRSS2-Target consensus 70 SCE1 1-Template TMPRSS2-Target CONSENSUS 7 SCE1 1-Template TMPRSS2-Target CONSENSUS 7 SCE1 1-Template TMPRSS2-Target CONSENSUS 7 SCE1 1-Template TMPRSS2-Target CONSENSUS 7 SCE1 1-Template SCE1 1-Templa	820 11 C C C C H LV 91 C C C H LV 10 M C V LV LV 10 M C V LV LV 10 M C V LV LV 10 C C P C R C F L 10 C C P C R F L 10 C C P C C P C R F L 10 C C P C P C C P C C P C C P C C P C C P C C P C C P C C P C C P C C P C C P C C P C C P C C P	300 300 300 300 300 300 300 300	840 DPSRL4GAC DPSRL4GAC 930 AMYTPHTVL: 30 120 PVD IVE RCV IVE VSLRCIACOV 190 200 200 200 200 200 200 200 2		860 541 H 8 2 W 54 541 H 8 2 W 54 550 550 550 50 50 50 50 50 50	60 60 10 A UP LTX A IDA YP LTX A IDA YP LTX K 	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	800 14404144 14404144 14404144 14404144 14404144 14404144 14404144 14404144 14404144 14404144 1440414 1440414 1440414 14404144 14404144 14404144 14404144 14404144 1440414 1440414	900 015 cG 015 cG 0
GNUR-Template RDRP-Target consensus>70 GNUR-Template ERP2-Target consensus>70 B SCEL 1-Template TMPR552-Target consensus>70 SCEL 1-Template TMPR552-Target consensus>70 SCEL 1-Template TMPR552-Target consensus>70 SCEL 1-Template TMPR552-Target consensus>70 SCEL 1-Template SCEL 1-Template SCEL 1-Template SCEL 1-Template	820 14 CEQUENTAL 910 910 910 910 910 910 910 910		B 40 DPSRLEAG DPSRLEAG P30 AMTTPHTVL: 120 PVD 147 RCV 147 RCV 147 PUD 147 SHOCIACGY 190 LONG 190 LONG		860 141 H 3 A 4 5 A 4 5 A 141 H 4 3 A 4 5 A 51 H 1 8 A 7 5 A 950 50 50 50 50 50 50 50 50 50	60 60 10A PD LTX A IDA VP LTX A IDA VP LTX K 60 THSELD VRT 60 THSELD VRT 9 10 10 10 10 10 10 10 10 10 10	70 A CANGTSCFF C C C C C C C C C C C C C C C C C C C	800 14 004 14 0 14 004 14 0	900 DILIGN DILIGN DILIGN DILIGN DILIGN DILIGN SPMKLN SPMKLN SPMKLN SPMKLN SPMKLN SPMKLN SPMKLN SPMKLN
GNUR-Template DDRD-Target DDRD-Target GNUR-Template DDRD-Target consensus>70 SCE1_1-Template DDRDS2-Target consensus>70 SCE1_1-Template TMPRS52-Target consensus>70 SCE1_1-Template TMPRS52-Target consensus>70 SCE1_1-Template TMPRS52-Target consensus>70 SCE1_1-Template TMPRS52-Target consensus>70 SCE1_1-Template TMPRS52-Target Consensus>70 SCE1_1-Template TMPRS52-Target Consensus>70 SCE1_1-Template TMPRS52-Target SCE1_1-Template TMPRS52-Target SCE1_1-Template	B20 B20 B10 C C C B MLV 910 910 910 910 910 910 910 910	3.0 CODAWAR CODAWAR CODAWAR CODAWAR CODAWAR SCODAW	840 DISALGAG DISALGAG DISALGAG 930 ANYTPHTVL: 30 120 PUDITC: 80 120 120 120 120 120 120 120 12		50 50 50 50 50 50 50 50 50 50 50 50 50 5	60 10 A 10 A YP L TKI K 	T O C V A VY T	800 1140041441 11400414441 11400414444 11400414444 11400414444 1140041444 1140041444 1140041444 114004144 11400444	900 DILTCH DILTCH DELTCH SPMKLN PPERNE V.EX SPMKLN V.EX SPMKLN SPMKLN SPMKLN SPMKLN SPMKLN SPMKLN SPMKLN SPMKLN SPMKLN SPMKLN SPMKLN SPMKLN SPMKLN
GNUR-Template RDRP-Target DRP-Target consensus>70 GNUR-Template RDRP-Target SCEL 1-Template TMPRSS2-Target consensus>70 SCEL 1-Template TMPRSS2-Target consensus>70 SCEL 1-Template TMPRSS2-Target consensus>70 SCEL 1-Template TMPRSS2-Target consensus>70 SCEL 1-Template TMPRSS2-Target consensus>70 SCEL 1-Template SCEL 1-Template	820 11 C C C C H LV 910 C C C H LV 100 C C C C C C C C C C C C C C C C C C		840 DPSRLGAG DPSRLGAG 930 AMYTPHTVL: 120 PVDETV: RCVFLCACO 120 PVDETV: RCVFLCACO 120 PVDETV: RCVFLCACO 190 SHOVEGADD SHOVEGADD SRVFGSRV 		860 141 H 3 A V 54 141 H 4 3 A V 54 141 H 4 3 A V 54 55 50 50 50 50 50 50 50 50 50	60 10AVPLTX AIDAVPLTX AIDAVPLTX AIDAVPLTX AIDAVPLTX AIDAVPLTX AIDAVPLTX AIDAVPLTX AIDAVPLTX AIDAVPLTX COMPTON	70 AGANGTSGFF AGANGTSGF AGANGTSGFF AGANGTSGF	800 800 CVDECRLPHTQI SQGIVDDSOST 50 144 144 144 144 144 144 144 14	900 SLEVE DELTGE 90 RLLEVI SPMKLN 9 PERNM V. EK V. EK C. I.
GNUR-Template RDRP-Target Consensus 70 GNUR-Target consensus 70 GNUR-Target consensus 70 B SCE1 1-Template TMPDSS2-Target consensus 70 SCE1 1-Template TMPDSS2-Target consensus 70 SCE1 1-Template TMPDSS2-Target consensus 70 SCE1 1-Template TMPSS2-Target consensus 70 SCE1 1-Template TMPSS2-Target consensus 70 SCE1 1-Template TMPSS2-Target SCE1 1-Template TMPSS2-Target SCE1 1-Template TMPSS2-Target SCE1 1-Template SCE1 1-Template SCE		830 830 830 830 830 830 830 830	B 40 D S FLGAC D S FLGAC D S FLGAC D S FLGAC S S C S S S S S S S S S S S S S S S S	950 950 940 109 RATED 940 109 RGS GHHHI 40 55 RS NA RVA GL 91 LQVY SSQRR 11 LQVY SSQRR 12 LQVY SSQRR	8 6 0 1 4 4 8 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	60 10 A UP L TX 10 A UP L TX	70 AGANGTSGPF AGANGTSGPF AGANGTSGF AGANGTSGF AGANGTSGF AGANGTSGFF AGANGTSGF AGANGTS	BOO SOCIUDISCST SOCIUDISCST SOCIUDISCST SOCIUDISCST SOCIUDISCST SOCIUDISCST SOCIUDISCST SOCIUDISCST SOCIUTION S	900 SILEVI SILEVI SIMKLN PPRNK V. EX SIMKLN COLLAND SIMKLN COLLAND SIMKLN
GNUR-Template DDRD-Target DDRD-Target Consensus>70 GNUR-Template DDRD-Target consensus>70 SCEL 1-Template DDRDS2-Target consensus>70 SCEL 1-Template TMPASS2-Target consensus>70 SCEL 1-Template		30 30 30 30 30 30 30 30 30 30	840 DPSRL4CAC DPSRL4CAC DPSRL4CAC 930 ANYTPHTVL: 30 120 120 120 120 120 120 120 12		50 50 50 50 50 50 50 50 50 50 50 50 50 5	60 10 A 10 A YP L TX 10 A YP L TX 10 A YP L TX 60 THSELDVRT: 	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	800 14404 44 14404 44 14404 44 1	900 DELTGE DELTGE DELTGE 90 RLLEVI SPMKLN 9 9 9 9 9 9 9 9 9 9 9 9 8 8 9 0 8 9 9 8 9 9 9 8 9 9 8 9 9 9 8 9 8

Fig. 3: Alignment of the target protein with the template protein sequence, (A) Alignment of RdRp with the template and (B) Alignment of TMPRSS2 with the template



Fig. 4: Modelling parameters of SARS-CoV-2 RdRp modeled protein, (A) Ramachandran plot; (B) Local quality estimate and (C) Z-score of the modeled protein

TABLE 2: PROTEIN MODELLING PARAMETERS AND THEIR VALUES

Davameters	TMPRSS2	RNA polymerase
Parameters	protein	protein
MolProbity score	1.78	1.03
Clash score	5.45	0.85
Ramachandran favored	92.15 %	96.05 %
Ramachandran outliers	0.87 %	0.56 %
Bad bonds	0/2785	0/7370
Bad angles	31/3791	47/9999

RdRp, CD26 and TMPRSS2 proteins are shown in fig. 1A-fig. 1D. Result showed that compound C1 (-7.88 kcal/mole) and C6 (-6.62 kcal/mole) showed minimum binding score against CD26 and TMPRSS2 proteins. Compound C1 ((4Z,6E)-1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-4,6-dien-3-one) showed Hydrogen Bonding (HB) and hydrophobic interaction pattern with the CD26 protein (Table 3). Compound C6 ((4Z,6E)-1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-4,6-

TABLE 3: SARS-COV-2 AND HUMAN HOST PROTEIN AA RESIDUES INVOLVED IN THE INTERACTION WITH C. longa COMPOUNDS

	CN	AA residue	AA residue involve		
Protein		involve in HB	in HI/DSI/Pi-Pi interactions		
		Asp739, Asp545,	Glu205. Asn709. Trp201. Arg125. Trp124. Lys122. His740. Gly741. Ala743. Tyr547.		
	C1	Asn710, Lys554	Val546. Trp627. Glv628. Tvr752. Trp629. Ser630		
		,, .e, _ ,	Asp739, ASH709, Asp545, Lvs554, Asn710, Lvs122, Trp124, Arg125, His740, Glv741,		
CD26	C2	NF	Glu205. Trp201. Tvr752. Val546. Tvr547. Trp627. Glv628. Trp629. Ser630. Glv632		
	C 2	Asn710, ASH709,	Glu205, Trp201, His740, Gly741, Lys122, Trp124, Arg125, Trp629, Gln553, Ser552,		
	C3	Asp739, Lys554	Tyr547		
	~ /		Leu188, Gly190, Phe193, Val283, Ala280, Ile279, Arg277, Ile489, Thr324, Pro325,		
	C6	NF	Cyx278*, Cyx281*, Tyr227, Phe231		
	62	T 007 4 077	Phe231, Phe193, Gly190, Tyr189, Leu188, Ile489, Val283, Ala280, Ile279, Pro325,		
	C2	Tyr227, Arg277	Thr324, Pro400, Lys399, Phe394, Cyx281*, Cyx278*		
	C11	Leu188	Phe193, Gly190, Thr227, Arg277, Ile279, Ala280, Pro335, Thr324, Cyx278*, Phe231		
			Ile489, Arg277, Ala280, Ile279, Val283, Leu188, Tyr189, Gly190, Phe193, Phe394,		
	C3	Tyr227	Pro400, Pro325, Thr324, Phe231, Cvx278*, Cvx281*		
	-	Tyr227, Arg277,	Leu188, Gly190, Phe193, Pro325, Thr324, Pro400, Ala280, Ile279, Cyx281*, Cyx278*,		
	C18	Ala280	Phe394		
		Gln493 Tvr351	1vs417 11e418 Asn422 1eu492 Gln493 Ser494 Tvr351 Tvr495 Val350 Ser349		
	C1	Val350 Val401	Dhe_347 e/0.2 Acr/4.2 Acr/4.3 Acr		
			$r_1r_2r_1$, r_2r_2 , r_2r_2 , $r_1r_2r_2$, $r_1r_2r_2$, $r_1r_2r_2$		
	C8	Val401, Val350,	Tvr495 Ser494 Gln493 Leu492 Lvs417 lle418 Tvr471 Asn472 Ser349 Phe347		
		Tyr351, Pro491,	Acndd? Phed97		
		Gln493,			
Spike	C 2	Phe347, Val350,	Asp442, Ala348, Ser349, Tyr351, Lys417, Ile418, Tyr421, Asn422, Pro491, Leu492,		
	CΖ	Tyr495	Gln493, Ser494, Phe497, Val401,Arg509, Tyr495		
	C 0	Tyr351, Gln493,	Pro491, Leu492, Ser494, Phe497, Arg509, Val401, Phe347, Ala348, Ser349, Val350,		
	69	Tyr495, Asp442	Asn422, Lys417, Ile418		
		Gln493, Pro491,			
	C6	Tyr351, Val350,	Ile402, Ser349, Asn422, Leu492, Ser494, Tyr495, Asp442, Ile418, Lys417, Phe497		
		Val401			
		Asp623, Asp618,	Arg624, Cys622, Lys621, Pro620, Tyr619, Trp617, Gly616, Arg553, Lys551, Lys798,		
	C6	Asp761	Cvs799, Trp800, Asp760, Ala762, Glu811, Phe812		
		Asp623, Asp760,			
	C1	Asp761, Ser814,	Asn691, Thr680, Cys622, Arg553, Arg555, Tyr619, Asp618, Trp617, Gly616, Cys799,		
polymerase		Lvs798	Trp800, Glu811, Phe812, Cys813, Ser759		
		Ser814, Glu811,	Cys813, Phe812, Cys799, Lys798, Gly616, Trp617, Asp618, Ala762, Asp760, Ser759,		
	C14	Asp761, Trp800	Leu758		
		-F -) - F - 3 -	Val762 Ala762 Aca761 Aca760 Ara624 Aca622 Ara552 Aca452 Ara555 Val557		
	C23	Thr556, Lys798	valius, Alaruz, Asprol, Asprou, Algoz4, Aspoz5, Algoz5, Asp452, Algoz5, Valo57,		
			Lys343, Glyoto, Trpotz, Aspoto, Prieotz, Gluott, Trpoud, Cys799		

Note: HB-Hydrogen bond; HI-Hydrophobic interaction; *-indicates residues with Pi-Pi interaction; DSI-Digital sequence information

TABLE 4: MM-GBSA ANALYSIS OF THE SARS-COV-2 TARGETED PROTEIN AND PROTEIN-STANDARD INHIBITOR/LEAD MOLECULE COMPLEX

Decenter name	Receptor/Protein	Receptor/Protein-standard ligand	Receptor/Protein-lead ligand complex
Receptor name	energy	complex energy	energy
CD26	-63281.79373	-63286.47008	-63381.1174
Spike	-128584.6622	-128598.4453	-128681.7845
RNA pol	-33846.13982	-33825.36073	-33957.28006
TMPRSS2	-13641.03537	-13788.34184	-13772.66361

Note: All the numerical values are shown in the table are given in kcal/mole. Sitagliptin, VE607, Ribavirin and CM were used as standard ligand for CD26, Spike, RdRp and TMPRSS2 proteins respectively. *C. longa* compound [(4Z,6E)-1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-4,6-dien-3-one] was the lead ligand for CD26 and SARS-CoV-2 spike glycoprotein. *C. longa* compound [(4Z,6E)-1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-4,6-dien-3-one] was the lead ligand for TMPRSS2 and RdRp



Fig. 5: Interaction of *C. longa* compounds with CD26 and TMPRSS2 human host proteins, (A) Surface structure and type of interaction involved in the compound C1 ((4Z,6E)-1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-4,6-dien-3-one) and CD26 protein interaction. Protein and ligand are shown in red and yellow color; (B) Surface structure and type of interaction involved with compound C6 ((4Z,6E)-1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-4,6-dien-3-one) and TMPRSS2 modeled protein. Protein and ligand are shown in cyan and purple color



Fig. 6: Interaction of *C. longa* compounds with SARS-CoV-2 and RdRp proteins, (A) Surface structure and type of interaction involved with compound C1 ((4Z,6E)-1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-4,6-dien-3-one) and SARS-COV-2 spike glycoprotein. Protein and ligand are shown in yellow and red color; (B) Surface structure and type of interaction involved with the compound C6 ((4Z,6E)-1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-4,6-dien-3-one) and RdRp modeled protein. Protein and ligand are shown in green and red color

dien-3-one) showed disulfide/HB, hydrophobic and pi-pi interaction pattern with the modeled TMPRSS2 protein (Table 3).

Camostat mesylate (CM) is a known, *in vitro* validated human TMPRSS2 protein inhibitor^[53,54]. We predicted the active site (ligand binding site) of the TMPRSS2 modeled protein. Docking of CM and C1 (4Z,6E)-1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4hydroxyphenyl)hepta-4,6-dien-3-one was performed against the site to predict the comparative binding of both compounds. The result showed that *C. longa* compound binds more tightly (-6.62 kcal/mole) to the TMPRSS2 predicted active site in comparison to known CM inhibitor (-5.14 kcal/mole) (fig. 7A and fig. 7B). Moreover, the lead ligand bound structures of the CD26 and modeled TMPRSS2 protein showed slight changes in Root-Mean-Square Deviation (RMSD) values (0.304Å and 0.371Å) in comparison to unbound structure (fig. 8A-fig. 8E).

Compound C1 ((4Z,6E)-1,5-dihydroxy-1,7-bis (4-hydroxy-3-methoxyphenyl)hepta-4,6-dien-3-one) exhibited potential binding (-8.51 kcal/mole) at SARS-



Fig. 7: Binding of lead and standard compounds at predicted binding site at TMPRSS2 protein compared with the unbound protein inhibitors at allosteric and predicted active site of the protein, (A) Binding of (4Z,6E)-1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-4,6-dien-3-one phytochemical (red color) at TMPRSS2 predicted active site with binding score; (B) Binding of *in vitro* validated TMPRSS2 inhibitor CM (red color) at predicted active site and its binding score. Ligand bound (yellow color) and unbound (cyan color) protein structures were superimposed to show the change in protein conformation. Change in ligand bound and unbound protein conformation is provided in the form of RMSD value and binding score of ligand at the predicted active site is given in kcal/mole



Fig. 8: *C. longa* compounds bound and un-bound 3D superimposed structure of SARS-CoV-2 and human host protein. Yellow color indicates unbound protein structure and ligand bound protein structure is shown in cyan color, (A) Superimposed ligand ((4Z,6E)-1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-4,6-dien-3-one) bound and unbound CD26 protein structures; (B) Superimposed ligand ((4Z,6E)-1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-7-(4-hydroxyphenyl)hepta-4,6-dien-3-one) bound and unbound TMPRSS2 modeled protein structures; (C) Superimposed ligand ((4Z,6E)-1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-4,6-dien-3-one) bound and unbound ((4Z,6E)-1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-4,6-dien-3-one) bound and unbound SARS-CoV-2 spike glycoprotein structures; (D) Superimposed ligand ((4Z,6E)-1,5-dihydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-4,6-dien-3-one) bound and unbound RdRp modeled protein structures; (E) Difference in RMSD values of ligand bound and unbound proteins

CoV-2 S1 domain and ACE-2 protein binding interface. Result showed HB and hydrophobic interactions between compound C1 and S1 domain (Table 2 and fig. 6A). Besides S-protein, we also targeted SARS-CoV-2 RdRp protein. Results showed that *C. longa* compounds (C1, C6, C14 and C23) have potential to bind SARS-CoV-2 RdRp approximately with similar potential ranging from -5.57 to -5.10 kcal/mole. Compound C1 interacts with RdRp protein through HB and hydrophobic interaction with a minimum binding score among all screened compounds (Table 3 and fig. 6B).

In the next experiment, the comparative docking pose of respective standard inhibitors against target proteins were studied (fig. 9A-fig. 9E). AA residues and type of interactions involved with the standard inhibitortarget protein binding is summarized in Table 3. Result showed that docking pose of standard inhibitor and lead compounds against CD26 protein were different (fig. 9A and Table 3). Compound C6 and CM interacted with the same AA residues *viz*. Arg277, Tyr227, Ile279, Thr324, Pro325, Phe231, Ala280, Phe193, Cys278, Gly190 and Leu188 of TMPRSS2 protein; although the type of interaction was different (fig. 9B and Table 3). Additional interactions noted with compound C6 were with Leu188, Gly190, Phe193, Val283, Ala280, Pro325, Cys281 and Phe231 AA residues. Compound C1 and VE607 showed similar pattern of binding with AA www.ijpsonline.com



Fig. 9: Superimposed *C. longa* compounds and standard inhibitors at active site of SARS-CoV-2 and human host target proteins, (A) Binding of compound C1 (green) and sitagliptin (red) at human host CD26 active site; (B) Binding of compound C6 (yellow) and CM (warm pink) at human host TMPRSS2 active site; (C) Binding of compound C1 (green) and VE607 (blue) at SARS-CoV-2 spike glycoprotein active site; (D) Binding of compound C6 (yellow) and ribavirin (cyan) at SARS-CoV-2 RNA-polymerase active site; (E) Binding of compound C6 (yellow), remdesivir prodrug (blue) and remdesvir active drug (nucleoside triphosphate) (red) at SARS-CoV-2 RNA-polymerase active site

resides viz. Gln493, Tyr495, Ser494, Asn422, Leu492, Tyr351, Val350, Asp442 and Phe497 against SARS-CoV-2 S-protein (Table 2). Moreover, compound C1 showed additional binding with Lys417, Ile418, Tyr351, Val350, Ser349, Phe347, Ile402, Arg509 and Val401 residues (Table 3). Ribavirin, remdesivir (prodrug) and nucleoside triphosphate (active metabolite of remdesivir) were engaged as SARS RdRp standard inhibitors for docking pose comparison with C. longa compounds (Table 3). Similar to ribavirin, compound C6 showed interactions with Ala762, Asp761, Glu811, Lys798, Trp617, Asp760, Trp800 and Cys799 residue of SARS-CoV-2 RdRp enzyme (Table 3). Compound C6 showed binding with some additional AA residues including Asp623, Asp618, Arg624, Cys622, Lys621, Pro620, Tyr619, Gly616, Arg553, Lys551 and Phe812, respectively (Table 3). It should be noted that lead compound C6 and recently proposed SARS-CoV-2 RdRp inhibitor remdesivir prodrug and its active metabolite nucleoside triphosphate exhibited similar binding pattern on protein active site with binding energy -5.81 and -6.85 kJ/mole (fig. 9E). Compound C6 and remdesivir active drug (nucleoside triphosphate) interacted with almost similar AA residues including Asp618, Asp761, Lys621, Pro620, Trp617, Gly616, Lys551, Lys798, Trp800 and Glu811 (Table 3).

Virus entry in the human cells is initiated through the interaction of viral protein with the human receptor(s)

followed by conformational change in the viral protein which induces the internalization process^[55]. Thus, the agents which can prevent the viral entry and their attachment to the host cells are important candidates for anti-viral drug discovery. Several natural products show their anti-viral potential by inhibiting the viral attachment. Several natural compounds show their antiviral potential by inhibiting the viral attachment to the host cell. For example, natural tannin compounds viz. chebulagic acid and punicalagin inhibit the attachment and fusion of different viruses with the host receptor by deactivating the free viral particles^[56]. It has been reported that some natural compounds including epigallocatechin-3-gallate a major constituent of green tea, has potential to inhibit the viral attachment, its replication, assembly or release of their progeny virions. The antiviral mechanism inhibits cell-to-cell spread of the virus and lowers the infectivity^[57]. In the present study, we identified the binding potential of C. longa phytochemicals with the SARS-CoV-2 and human proteins involve in the viral-host protein interaction, internalization and replication process.

CD26, also known as DPP4, is a 110 kDa human cellsurface glycoprotein that exerts varying functions in cell type and physiological conditions in context-based manner. Lu *et al.* (2013) demonstrated that Middle East Respiratory Syndrome Coronavirus (MERS-CoV) spike protein bound with CD26 mediates attachment www.ijpsonline.com



Fig. 10: C. longa compounds targeting SARS-CoV-2 and human host proteins involved in viral entry and virulence

and fusion to host cells thereby initiating infection^[54]. Human CD26 is an important immuno-regulator used by viruses for immune hijacking and virulence. TMPRSS2 is a serine protease that proteolytically cleaves and activates viral spike protein and facilitates virus-cell membrane fusion. It has been reported that TMPRSS2 plays a critical role in the proteolytic activation of SARS-CoV and MERS-CoV^[58]. Therefore, targeting human CD26 and TMPRSS2 proteins is an efficient step to inhibit the cellular entry and virulence of SARS-CoV-2.

Our study corroborates with previous publications demonstrating binding of CD26 inhibitors at Tyr125, Glu205, Lys122, Trp124 and Ap739 amino residue of CD26^[59,60]. In the present study, *C. longa* compounds binds to the predicted ligand binding (fig. 2B) of TMPRSS2 protein. Our study also corroborates with the recently published data on remdesivir which binds at the similar binding site of TMPRSS2 protein was relatively involved in the SARS-CoV-2 S-protein and TMPRSS2 protein interaction interface. Comparison of ligand and CM bound TMPRSS2 modeled protein showed that the binding of lead compound at the predicted site of the protein binds more tightly than the binding of CM

(fig. 7A and fig. 7B). Thus, the comparative results indicate the TMPRSS2 binding potential of *C. longa* compounds at the predicted site. Minor change in RMSD values of ligand bound and unbound CD26 and TMPRSS2 structures indicate stable docking pose of *C. longa* compounds at respective binding sites of the protein.

SARS-CoV-2 spike glycoprotein is composed of S1 and S2 subunits^[61]. The S1 and S2 domains are involved in host cell receptor binding and membrane fusion. The S1 subunit comprise of a signal peptide, N-terminal domain and RBD. The S1 domain interacts with human ACE-2 receptor and forms a fusion core that passages viral and human cellular membrane to initiate fusion and infection. Beside ACE-2 receptor, the S1 domain (RBD) of the spike protein interacts with several other host proteins such as CD26 and TMPRSS2. S2 subunit of SARS-CoV-2 S-protein consists of conserved fusion peptide mediating membrane fusion process^[62]. Recent studies indicated that similar to SARS-CoV and MERS-CoV, the S1 domain of SARS-CoV-2 possesses conserved sequences which interact with human proteins underlying cell adhesion and virulence^[63].

Tai *et al.* characterized the receptor binding domain of SARS-CoV-2 and reported the variable amino residues

(331aa-524aa) among SARS-CoV-2 and SARS-CoV^[64]. Wrapp et al. reported that S1 subunit of SARS-CoV-2 has 10-20 fold higher binding affinity for ACE-2 receptor than SARS-CoV which may contribute to its higher infectivity and transmission potential in comparison to SARS-CoV^[65]. Tight binding of compound C1 at SARS-CoV-2 S1 domain and ACE-2 protein binding interface indicates the disruption of protein-protein interaction between these two molecules (fig. 5A). Our study corroborates with the recent findings showing the binding of hesperidin, a flavanone glycoside found in citrus fruits, at the SARS-CoV-2 S1 domain and ACE-2 protein binding interface^[60]. It should be noted that the compound C1 binds with some key AA residues (Gln493, Ile402, Lys417, Gln493 and Ser494) which are unique to the SARS-CoV-2 S1 domain. This result indicates the selective SARS-CoV-2S1 domain targeting potential with C. longa compounds might disrupt the viral adherence on human host cells. Since polymerases play a central role in viral genome synthesis, replication and transcription, thus, targeting SARS-CoV-2 RdRp by C1 could emerge as a potential antiviral drug. Tight binding of other C. longa compounds C1, C6, C14 and C23 at SARS-CoV-2 RdRp active site indicates that the compounds might have inhibitory potential against viral replication.

In our study, similar binding pattern of compound C6 was noted as compared to standard inhibitor CM interaction with TMPRSS2 protein (fig. 7A, fig. 7B and fig. 8B). Binding with an additional AA residue might be the reason behind lower binding energy of compound C6 against TMPRSS2 protein than standard inhibitor (fig. 1). It has been reported that VE607 compound binds with SARS-CoV S-protein pseudotype HIV-1 virus and thereby inhibit the viral entry in ACE-2 expressing 2393T cells^[66]. Binding of compound C1 with additional residues of spike protein S1 domain might be responsible for its lower binding energy than VE607 compound (Table 3). Similar to SARS-CoV-2 RdRp inhibitors, compound C6 interacted with similar AAs. Altogether, C. longa compound show additional binding with the protein which might be responsible for its lower binding energy. The overall results indicate significant SARS-CoV-2 RdRp inhibition potential by C. longa compounds. Potent binding of the lead C. longa phytochemicals to the targeted SARS-CoV-2 and human host proteins are depicted in fig. 10.

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The authors declared no conflict of interest.

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