REVIEW ARTICLE
doi: 10.2306/scienceasia1513-1874.2023.047

ScienceAsia 49 (2023): **477–484**

Targeting the main protease for COVID-19 treatment: A comprehensive review of computational screening and experimental studies

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Received 2 Mar 2023, Accepted 23 May 2023
Available online 11 Jun 2023

ABSTRACT: SARS-CoV-2 was the pathogen responsible for triggering the global COVID-19 outbreak in 2020. Encouraging advancements have been made in the research and development of vaccines and antiviral drugs. Noticeably, the coronavirus reproduction process relies heavily on the SARS-CoV-2 Main protease (Mpro), which is essential for viral replication. Therefore, this review presents computational drug discovery and screening methods aiming at identifying repurposed medications and potent new compounds from existing databases to effectively combat COVID-19 by targeting Mpro. This review can aid in understanding Mpro inhibitors and their potential usefulness.

KEYWORDS: COVID-19, SARS-CoV-2, main protease (Mpro), drug discovery, antiviral drugs

INTRODUCTION

The ongoing COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, also known as 2019-nCoV), has resulted in a significant global outbreak and poses an important public health challenge [1–4]. The occurrence of previous outbreaks of SARS-CoV-1 and Middle East respiratory syndrome (MERS) coronavirus suggests that coronavirus outbreaks could recur in the near future [5]. Thus, the development of effective antiviral drugs is critical to supplement vaccine development.

The SARS-CoV-2 replicase gene (Orf1) encodes for polyproteins 1a and 1b (pp1a and pp1b), which are responsible for all necessary actions required for the viral life cycle. The auto-cleavage of pp1a and pp1ab releases the 3-chymotrypsin-like main protease (Mpro), which is crucial for viral replication. This cleavage leads to the release of a functional protein nsp4 through nsp16 [6]. Given the vital role of Mpro in the viral life cycle, it is an attractive target for the discovery of novel anti-coronaviral drugs [7]. The Mpro shares a high degree of structural and sequence similarity with SARS-CoV Mpro [8], thus making drugs that target Mpro potentially effective against future coronavirus outbreaks. The enzyme's active site comprises 2 catalytic dyad residues, C145 and H41 (Fig. 1), while the substrate-binding residues include H41, M49, G143, S144, H163, H164, M165, E166, L167, D187, R188, Q189, T190, A191, and Q192 [9].

Fig. 2A provides a list of representative inhibitors of the SARS-CoV-2 main protease (Mpro), including several peptidomimetics. The substrate-mimicking covalent inhibitor N3 forms a covalent bond with the catalytic residue C145 via Michael addition (Fig. 2B); however, it has low membrane penetration due to its high polarity [10]. GC376, a dipeptide-based inhibitor originally developed to treat feline infectious peritonitis, has potent anti-coronavirus activity [11]. GRL-2420, a tripeptide-based inhibitor discovered during SARS-CoV research, has also been reported [12]. Nirmatrelvir (PF-07321332) and lufotrelvir (PF-07304814) were developed by Pfizer. PF-07321332 is an optimized form of PF-07304814 with a nitrile group added as a covalent warhead to react with the catalytic residue C145 (Fig. 2B) [10, 13–16]. Additionally, ensitrelvir (S-217622), marketed as Xocova, is an oral antiviral drug invented by Shionogi in collaboration with Hokkaido University which acts as a Mpro inhibitor and has demonstrated effectiveness against the Omicron variant [17, 18].

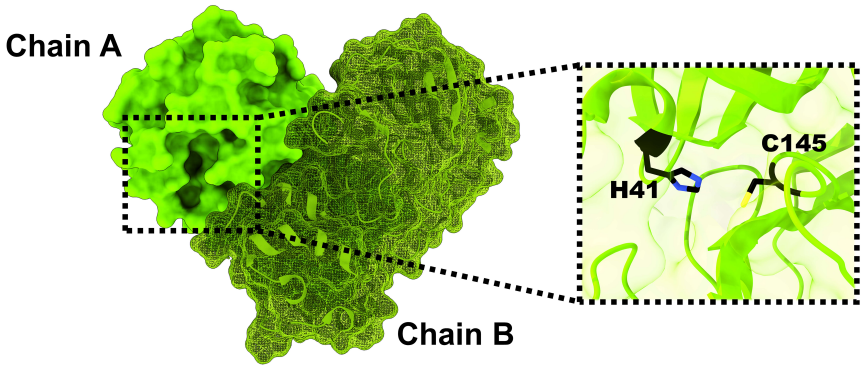

Fig. 1 SARS-CoV-2 main protease (Mpro) as a viral target for the development of therapeutic approaches against COVID-19. Additionally, the catalytic dyad H41 and C145 is depicted, highlighting its significance in the enzymatic activity of Mpro.

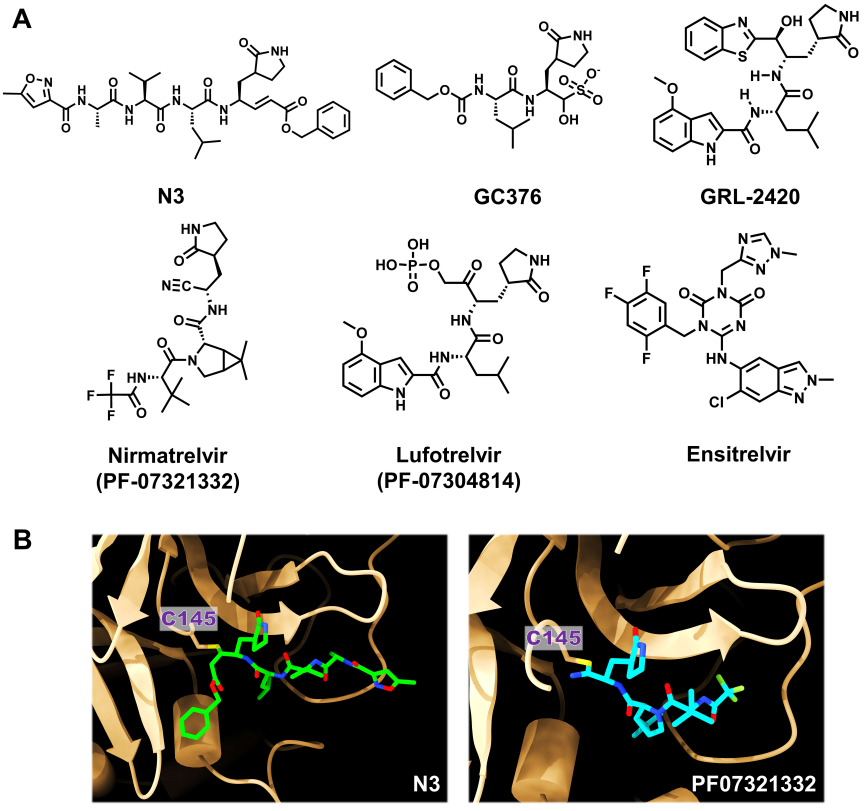

Fig. 2 (A) 2D structure of reported SARS-CoV-2 Mpro inhibitiors. (B) 3D structure of N3 (6LU7.pdb) and PF-07321332 (nirmatrelvir, 7VH8.pdb) binding at the Mpro active site.

Among the 24 residues in the active site that were highly conserved among SARS-CoV-2, G143, H163, D187, and Q192 were found to be extremely prone to mutations, while M49, N142, E166, and Q189 were highly tolerant [19]. These tolerant locations have less potential to produce or evolve drug resistance, as N142, E166, and Q189 mutations are essential for Mpro function. However, the Mpro inhibitor PF-07321332 may be susceptible to 3 mutations: Q189E, E166A, and E166Q (Fig. 3). Other mutations in Mpro such as S144L, S144K, M165I, R188K, T190I, and A191V have also been reported [20].

In the fight against COVID-19, computational drug discovery and screening have been crucial in identi-

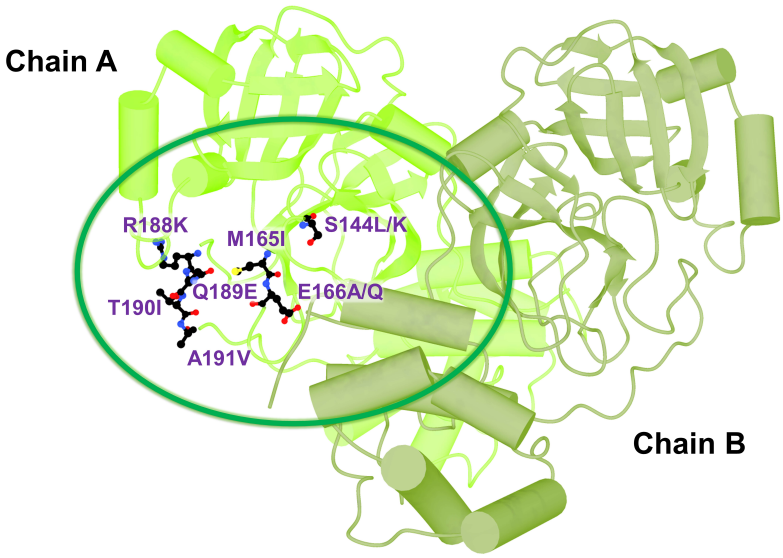

Fig. 3 The represented mutations of the SARS-CoV-2 Mpro.

fying repurposed medications and novel potent compounds from existing databases. Computational methods have been extensively utilized to better understand the actions and mechanisms of SARS-CoV-2. This review provides an overview of the current status of anti-SARS-CoV-2 drug discovery, mainly targeting the SARS-CoV-2 Main protease (Mpro) based on *in silico* studies.

COMPUTATIONAL PREDICTION OF INHIBITORS FOR COVID-19 TREATMENT

The *in silico* studies conducted on potential inhibitors of SARS-CoV-2 Mpro are summarized in Table 1. The review covers Mpro inhibitors from different categories, including (i) repurposed drugs, (ii) natural compounds (or natural products), and (iii) synthetic or household compounds.

(i) Repurposing drugs

In January 2020 [10], the crystal structure of Mpro with covalent inhibitor N3 was first reported, providing a platform for rapid target-based lead drug development against SARS-CoV-2 Mpro. Combining structure-based pharmacophore modeling with molecular docking, COVID-19 Mpro inhibitors were discovered from FDA-approved antiviral drugs such as remdesivir (viral RdRp inhibitor), saquinavir (HIV protease inhibitor), and raltegravir (HIV protease inhibitor) [21]. Jin et al [10] screened over 10,000 clinical trials and pharmacologically active compounds using high-throughput and structure-based virtual screening. Clinical trial drugs such as ebselen for Meniere's Disease, tideglusib for Alzheimer's disease, and PX-12 for Metastatic cancer showed strong SARS-CoV-2 Mpro inhibition with an IC_{50} range of 0.67–21.39 μM. Moreover, by using the active site conformations of SARS-CoV-2 Mpro through pharmacophore clustering, the anti-HCV drugs boceprevir and telaprevir as well as the anti-HIV drug nelfinavir were found to show significant Mpro inhibition and antiviral efficacy in the micromolar range, obtained from a set of 2,122 FDA-approved drugs [22].

A consensus virtual screening has been used to target Mpro using 4 molecular docking techniques including Glide SP, AutoDock Vina, and two protocols with AutoDock 4.2, on a library of 2000 approved oral drugs [23]. From the predicted structures of their complexes with Mpro, 42 drugs were identified as top candidates, and 17 drugs were tested in a kinetic assay for Mpro inhibition. Five compounds showed IC_{50} values below 40 μM, including manidipine (hypertension drug), boceprevir (HCV NS3/4A protease inhibitor), lercanidipine (hypertension drug), bedaquiline (tuberculosis medication), and efonidipine (atherosclerosis treatment). According to the Glide docking, the binding mode predicted for boceprevir suggests that the dimethylcyclopropyl group is positioned at P1, while the side chain containing the cyclobutyl and terminal ketoamide groups occupies P1′. The proximal tert-butyl group is located at P2, and the distal tert-butyl group is situated in the hydrophobic pocket at P4/P5. Narayanan et al [24] discovered 6 compounds with anti-SARS-CoV-2 Mpro action from a library of 64 repurposed drugs. These compounds were then modeled at protease active sites using *in silico* docking. The inhibition of SARS-CoV-2 Mpro by MG-101, lycorine HCl, and nelfinavir mesylate (mesylate salt form of nelfinavir) was found to be interesting, and a covalent

link was formed between the inhibitor and the active site, as evidenced by the crystal structure of Mpro in complex with MG-101. The catalytic residue C145 indicated a substrate-binding blockade in the active site, suggesting that this inhibition is effective.

Li et al [25] proposed *in silico* screening method using accelerated free energy perturbation-based absolute binding free energy (FEP-ABFE) predictions to identify potential SARS-CoV-2 Mpro inhibitors. Their approach utilized a restraint energy distribution (RED) function to enable accurate FEP-ABFE predictions, allowing for the screening of over 2,500 small compounds, including all FDA-approved drugs. From this, they identified 15 drugs predicted to be effective SARS-CoV-2 Mpro inhibitors, with dipyridamole, an anti-aggregating action drug (K_i = 0.04 μM) being the most effective, which was later shown to have positive therapeutic outcomes in clinical investigations for COVID-19 treatment. The antimalarial drugs, hydroxychloroquine (K_i = 0.36 μM) and chloroquine (K_i = 0.56 μM), were also potent inhibitors. In addition, FDA-approved drugs with Mpro inhibitory potential were identified using a combination of virtual screening based on molecular docking with crystal structures of peptidomimetic inhibitors (N3, 13b, and 11a) and experimental verification [9]. Lapatinib for lung cancer treatment demonstrated high levels of Mpro inhibition (IC_{50} = 35 μM and K_i = 23 μM) with molecular dynamics (MD) simulations revealing that the 5 subsites (S1′, S1, S2, S3, and S4) of the Mpro could be suitable binding sites for this drug. Moreover, effective peptidomimetic inhibitors shared crucial hydrogen bond donor (HBD) and acceptor (HBA) features with lapatinib's main chemical pharmacophore.

To discover new covalent non-peptidomimetic inhibitors of Mpro, Xiong et al [26] utilized *in silico* screening-based discovery approach on the ChemDiv database. The enzymatic activity experiment conducted on Mpro identified 3 hit compounds (compounds 2, 3, and 8) that potentially bind covalently. The crystal structure revealed that the most effective hit compound 8 (IC_{50} value of 8.50 μM) interacts with the S1′ and S2 subsites of the ligand binding pocket and also confirmed the covalent binding of the predicted warhead with the catalytic residue C145.

(ii) Natural compounds (or natural products)

Besides repurposing drugs, natural compounds are potential candidates as SARS-CoV-2 Mpro inhibitors. Deetanya et al [27] investigated the fluorescent probe 8-anilinonaphthalene-1-sulfonate (ANS) as a potential alternative assay for identifying inhibitors. When ANS bound to Mpro, fluorescence was enhanced, and this association was antagonistic to a peptide substrate. Baicalein (IC_{50} and K_i of 42 ± 2 and 15.2 ± 0.7 μM, respectively) and rutin (IC_{50} of 31 ± 1 μM and K_i of 11.3 ± 0.4 μM), 2 naturally occurring flavonoids, were used to illustrate the value of an ANS-based competitive binding assay for identifying Mpro inhibitors. With the aid of molecular modeling, the molecular basis of ANS and rutin association with Mpro was investigated. As a consequence of our findings, it was possible to identify new SARS-CoV-2 antiviral compounds by using ANS in a competitive binding assay in addition to the traditional peptide substrate-based activity assay. Additionally, Liu et al [28] reported natural products, baicalin and baicalein, derived from the root of *Scutellaria baicalensis*, which are Traditional Chinese medicine (TCM) herbs as novel inhibitors of the Mpro. In Vero E6 cells, baicalin and baicalein showed potent antiviral activities with respective IC_{50} values of 83.4 and 0.39 μM, indicating a better performance of baicalein over baicalin. Theaflavin 3-gallate, a naturally occurring bioactive compound produced from theaflavin and present in large quantities in black tea leaves, has shown better docking scores than repurposed drugs in previous preliminary molecular docking investigations (Atazanavir, Darunavir, and Lopinavir) [29, 30]. Theaflavin 3-gallate interacts more strongly with the active site residues of Mpro than it does with a standard molecule, GC373 (a known inhibitor of Mpro), according to assessments of MD simulations. Theaflavin 3-gallate had an IC_{50} of 18.48 ± 1.29 μM against SARS-CoV-2 Mpro inhibition. By measuring viral transcripts in Vero cells and treating SARS-CoV-2 (Indian/a3i clade/2020 isolate) with 200 μM of theaflavin 3-gallate *in vitro*, it was discovered that the viral count was reduced by 75%.

Yamamoto et al [31] employed a combination of structure-based virtual screening and *in vitro* experiments to identify hit compounds from the Enamine library. Among the 180 compounds examined at 20 μM, 9 compounds demonstrated inhibition rates greater than 5% in the Mpro. In subsequent dose-response tests, 6 compounds (Z391132396, Z166626994, Z819866548, Z2094146478, Z1159100304, and Z324552662) were found to have IC_{50} values on the order of 100 μM. In a previous study by Jin et al [32], VS10 and VS12 were identified as active compounds using docking-based virtual screening and an enzyme-based assay from the Specs database (http://www.specs.net). The IC_{50} values for VS10 and VS12 were 0.20 μM and 1.89 μM, respectively. VS10 was found to form 4 strong hydrogen bonds with G143, S144, and C45, while VS12 was stabilized within the Mpro pocket through a hydrogen bond formed with E166 and Q189 residues.

Hengphasatporn et al [33] utilized parallel cascade selection molecular dynamics-based ligand binding path sampling (LB-PaCS-MD) and fragment molecular orbital (FMO) calculations to determine the ligand path from an aqueous solution to the SARS-CoV 2 Mpro active site and to create a suitable ligand binding pocket for delivering potent inhibitors from natural

Table 1 Inhibitors targeting SARS-CoV-2 Mpro derived from *in silico* screening and subsequent experimental evaluation.

Type of inhibitor	Name	*In silico* method for screening	Experiment evaluation	IC_{50} (μM)	Ref.
FDA approved drug	Lapatinib	Molecular docking (FlexX) and MD simulations	Enzyme-based assay (FRET-based assay)	35, 23 (K_i)	[9]
Clinical trial compound	ebselen	Molecular docking (Glide)	Enzyme-based assay	0.67	[10]
	tideglusib			1.55	
	shikonin			15.75	
	PX-12			21.39	
FDA approved drug	boceprevir	Combined protease pharmacophore clustering and molecular docking (iGEMDOCK)	Enzyme-based assay	1.63	[22]
			Antiviral activity (Vero cell infected SARS CoV-2)	49.89 (EC_{50})	
	telaprevir		Enzyme-based assay	11.47	
			Cytotoxicity (Vero E6 cell)	35.80 (CC_{50})	
FDA approved oral drug	manidipine	Molecular docking (Glide SP, AutoDock Vina, and two protocols with AutoDock 4.2)	Enzyme-based assay (FRET-based assay)	4.8	[23]
	boceprevir			5.4	
	lercanidipine			16.2	
	bedaquiline			18.7	
	efonidipine			38.5	
FDA approved drug	MG-101	Molecular docking (Glide SP)	Enzyme-based assay (FRET-based assay)	2.89	[24]
			Cytotoxicity (Vero E6 cell)	0.0038	
	lycorine HCl		Cytotoxicity (Vero E6 cell)	0.01	
	nelfinavir mesylate			0.07	
FDA approved drug	dipyridamole	Free energy perturbation-based absolute binding free energy (FEP-ABFE) based screening	Enzyme-based assay (FRET-based assay)	0.06, 0.04 (K_i)	[25]
	hydroxychloroquine			2.9, 0.36 (K_i)	
	chloroquine			3.9, 0.56 (K_i)	
	candesartan cilexetil			2.8, 0.18 (K_i)	
	disulfiram			4.7, 0.31 (K_i)	
ChemDiv database	compound 2	Molecular docking (Glide) and FAF-Drug4 Serve	Enzyme-based assay (FRET-based assay)	19.09	[26]
	compound 3			36.07	
	compound 8			8.50	
Traditional Chinese medicine	baicalin	Molecular docking (Glide)	Enzyme-based assay	0.39	[28]
	baicalein			83.40	
–	theaflavin	Molecular docking (CDOCKER) and MD simulations	Enzyme-based assay (FRET-based assay)	22.22	[29]
	theaflavin 3-gallate			18.48	
	GC376			0.24	
Enamine library	Z391132396	Molecular docking (Molegro Virtual Docker)	Enzyme-based assay (FRET-based assay)	154	[31]
	Z166626994			222	
	Z819866548			189	
	Z2094146478			281	
	Z1159100304			273	
	Z324552662			291	
Specs database	VS10	Molecular docking (GOLD)	Enzyme-based assay (FRET-based assay)	0.20	[32]
	VS12			1.89	
Xanthone	rubraxanthone	Parallel cascade selection molecular dynamics-based ligand binding-path sampling (LB-PaCS-MD)	Enzyme-based assay (FRET-based assay)	74.6 (K_i)	[33]
				9.82 ($K_{i'}$)	
			Antiviral activity (Vero cell infected SARS CoV-2)	4.00 (EC_{50})	
			Cytotoxicity (Vero E6 cell)	26.61 (CC_{50})	
			Cytotoxicity (Calu-3 cell)	> 50 (CC_{50})	
Flavonoid	brominated baicalein (TH024)	Molecular docking (AutoDock VinaXB), FMO-based virtual screening and MD simulations	Enzyme-based assay (FRET-based assay)	56, 33 (K_i)	[34]
N-aryl amide piperine analog	2,5-dimethoxy-substituted phenyl piperamide 5	Molecular docking (AutoDock Vina) and MD simulations	Enzyme-based assay (FRET-based assay)	106.9	[35]
12-dithiocarbamate-14-deoxyandrographolide analog	3l	Molecular docking (AutoDock Vina) and MD simulation	Enzyme-based assay (FRET-based assay)	10, 6.8 (K_i)	[36]
	3m			12, 8.3 (K_i)	
	3t			7, 5 (K_i)	

Table 1 Continue ...

Type of inhibitor	Name	*In silico* method for screening	Experiment evaluation	IC_{50} (μM)	Ref.
α-mangostin and N-containing α-mangostin analog	α-mangostin Analog 2 Analog 3 Analog 4	Molecular docking (AutoDock Vina) and subsequent FMO calculation	Enzyme-based assay (FRET-based assay)	61.6 24.6 325.1 63.8	[37]
N-substituted isatin compound	Compound 26 Compound 27 Compound 23	Molecular docking (Glide)	Enzyme-based assay (FRET-based assay)	0.045 0.047 0.053	[38]
2-(furan-2-ylmethylene)hydrazine-1-carbothioamide derivative	F8-B6	Molecular docking (LigPrep module in Schrodinger software)	Enzyme-based assay (FRET-based assay) Vero cell MDCK cell	1.57 > 100 (CC_{50}) > 100 (CC_{50})	[39]
	F8-B22		Enzyme-based assay (FRET-based assay)	1.55	
Scutellarein and its methylated derivative	4′-*O*-methylscutellarein	Molecular docking (AutoDock)	Enzyme-based assay (FRET-based assay)	0.40	[40]

compound extraction. Among the tested compounds, rubraxanthone from *Garcinia cylindrocarpa* stems and *G. tetrandra* stem bark showed high cellular inhibition (EC_{50} of 4.00 ± 1.75 μM) and mixed inhibition (K_i of 74.6 ± 24.1 μM and $K_{i'}$ of 9.82 ± 3.17 μM) antiviral activity against SARS-CoV-2 Mpro. Rubraxanthone was found to interact with either the active site or allosteric site with a revealed interaction profile, key binding residues, and significant interaction. Similarly, halogenated baicalein (TH024) was identified as a potent inhibitor of SARS-CoV 2 Mpro through molecular dynamics simulation and quantum mechanical techniques [34]. The oxyanion holes, containing G143, S144, and C145, of Mpro were found to act as HBDs, stabilizing the hydroxyl group of brominated baicalein. The compound TH024 was further confirmed as a powerful inhibitor of SARSCoV-2 Mpro through experiments and showed no significant toxicity in both *in vivo* and *in vitro* research. In addition, various other natural compounds from the CH_2Cl_2-soluble fractions of *Garcinia cylindrocarpa* stems and *G. tetrandra* stem bark, including garcinone D, cratoxylone, tetrandraxanthone A, 9-hydroxycalabaxanthone, and γ-mangostin, were computationally and experimentally examined [33].

(iii) Synthetic or household compounds

Studies on the discovery of SARS-CoV-2 Mpro inhibitors from synthetic or household compounds have been conducted. N-aryl amide piperine analogs were synthesized using semi-synthesis [35]. Black pepper, or piperine, is an alkaloid found in the dried seeds of the *Piper nigrum* plant. Compound 5, a 2,5-dimethoxy-substituted phenyl piperamide, demonstrated potent anti-SARS-CoV-2 activity with no cytotoxicity against Vero and Vero E6 mammalian cell lines. In fact, compound 5 was more effective than rutin in inhibiting Mpro activity with an IC_{50} of 106.9 μM. Docking and MD modeling revealed that this compound could bind to the Mpro with increased binding interaction and stability. Nutho et al [36] investigated 21 12-dithiocarbamate-14-deoxyandrographolide analogs as SARS-CoV-2 Mpro inhibitors using *in vitro* and *in silico* experiments. Note that andrographolide is derived from the *Andrographis paniculata* plant. Compounds 3l, 3m, and 3t exhibited promising inhibitory efficacy against Mpro with IC_{50} values of 10 μM, 12 μM, and 7 μM, respectively. The MD results showed that most compounds could bind to the Mpro active site, especially at the S1, S2, and S4 subsites. Essential residues for ligand binding were T25, H41, C44, S46, M49, C145, H163, M165, E166, L167, D187, R188, Q189, and T190. New N-containing xanthone analogs of α-mangostin were also synthesized through a one-pot Smiles rearrangement [37]. The α-mangostin is a xanthone isolated from the pericarp of mangosteen (*Garcinia mangostana*). The biological activities of analogs 2-4 were evaluated, including their anti-lung cancer, antitrypanosomal, and anti-SARS-CoV-2 Mpro properties. It was found that analog 2, which contained an additional ether moiety, inhibited Mpro activity about three-fold better than α-mangostin, with an IC_{50} value of 24.6 ± 1.1 μM. The fragment molecular orbital technique (FMO-RIMP2/PCM) revealed that the improved binding interaction of compound 2 in the Mpro active site was due to the additional ether moiety. A separate study identified potent Mpro inhibitors among N-substituted isatin compounds [38]. The top 3 compounds, 26 (1-(naphthalen-2-ylmethyl)-2,3-dioxoindoline-5-carboxamide), 27 (1-((6-Bromonaphthalen-2-yl)methyl)-2,3-dioxoindoline-5-carboxamide), and 23 (1-(benzo[b]thiophen-2-ylmethyl)-2,3-dioxoindoline-5-carboxamide), demonstrated IC_{50} values of 45 nM, 47 nM, and 53 nM, respectively. Compound 26's oxygens at C-2 and C-3 formed hydrogen bonds with the main-chain amino group of catalytic residue C145,

while its carboxyamide at C-5 formed hydrogen bonds with the side-chain carboxyl groups of N142 and E166.

A new class of non-peptidomimetic inhibitors of SARS-CoV-2 Mpro, 2-(furan-2-ylmethylene)hydrazine-1-carbothioamide derivatives, was identified through in-house library structure-based screening and biological evaluation [39]. F8-B6 and F8-B22 were among the compounds discovered as potent inhibitors of Mpro with IC_{50} values of 1.57 μM and 1.55 μM, respectively. Mass spectrometry and enzyme kinetic experiments demonstrated that F8-B6 was a reversible covalent inhibitor of Mpro. Moreover, F8-B6 showed low cytotoxicity in Vero and MDCK cells with a 50% cytotoxicity concentration (CC_{50}) above 100 μM. These novel non-peptidomimetic SARS-CoV-2 Mpro inhibitors provide a valuable starting point for further structural optimization. Meanwhile, Wu et al [40] evaluated the inhibitory efficacy of scutellarein and its methylated derivatives against the Mpro of the SARS-CoV-2 virus using the fluorescence resonance energy transfer (FRET). In the plant *Scutellaria lateriflora*, there is a flavone known as scutellarein. 4′-*O*-methylscutellarein showed the most promising enzyme inhibitory activity *in vitro* with an IC_{50} value of 0.40 ± 0.03 μM among all the tested methylated derivatives. Docking results indicated that 4′-*O*-methylscutellarein was well-positioned in the substrate-binding pocket and formed hydrogen bonding with L141's carbonyl group.

CONCLUDING REMARKS

Nowadays, there is widespread development of a cure for COVID-19 pandemic. This review highlights the potential of combining computational and experimental techniques to develop new treatments for COVID-19. The review includes reports of *in silico* investigations that have identified promising targets for anti-SARS-CoV-2 Mpro compounds. Although several Mpro inhibitors have been discovered, there is still an opportunity to uncover even more potent and selective compounds for the treatment of COVID-19 through ongoing drug discovery endeavors. Further research on Mpro inhibitors could potentially lead to the development of more effective drugs with better pharmacological properties such as improved oral bioavailability, lower toxicity, and greater specificity for Mpro. By discovering and producing efficient and targeted Mpro inhibitors, researchers may be able to develop a prophylactic therapy that could be used to prevent the spread of future coronavirus outbreaks.

Overall, the prospects for targeting Mpro for COVID-19 treatment are promising, and continued research in this area could lead to the development of effective therapies for COVID-19 and other coronaviruses. Further research in this area is necessary to harness the potential of Mpro inhibitors for COVID-19 treatment fully.

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