Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Review article

Protease targeted COVID-19 drug discovery: What we have learned from the past SARS-CoV inhibitors?

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ABSTRACT

The fascinating similarity between the SARS-CoV and SARS-CoV-2, inspires scientific community to investigate deeper into the SARS-CoV proteases such as main protease (Mpro) and papain-like protease (PLpro) and their inhibitors for the discovery of SARS-CoV-2 protease inhibitors. Because of the similarity in the proteases of these two corona viruses, there is a greater chance for the previous SARS-CoV Mpro and PLpro inhibitors to provide effective results against SARS-CoV-2. In this context, the molecular fragments from the SARS-CoV protease inhibitors through the fragment-based drug design and discovery technique can be useful guidance for COVID-19 drug discovery. Here, we have focused on the structure-activity relationship studies of previous SARS-CoV protease inhibitors and discussed about crucial fragments generated from previous SARS-CoV protease inhibitors important for the lead optimization of SARS-CoV-2 protease inhibitors. This study surely offers different strategic options of lead optimization to the medicinal chemists to discover effective anti-viral agent against the devastating disease, COVID-19.

Contents

1. Introduction

The new viral infection COVID-19 was first appeared in the Wuhan province of China at the very end of 2019 and dragged the entire human civilization into a global pandemic by affecting millions of people across the globe [1–5]. This COVID-19 is caused by the novel severe acute respiratory syndrome (SARS) coronavirus-2

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(SARS-CoV-2) which belongs to the *Betacoronavirus* genus and is responsible for lower respiratory tract infection similar to SARS-CoV and MERS-CoV [1,6]. The SARS-CoV-2 is, a 30,000 base pair single-stranded positive-sense RNA virus with an envelope containing spike (S) proteins on the surface, providing a crown-like mien [1,7]. It bears 79% genetic similarity to SARS-CoV and is most similar to bat coronavirus RaTG13 [1]. The incubation period of the COVID-19 infection is 2–14 days and can be up to 24 days [6]. These longer incubation periods, for their transmissibility and asymptomatic nature, are responsible for a large number of infections [6]. To date, billions confirmed COVID-19 cases have been reported with million deaths worldwide [5]. The increasing numbers of COVID-19 cases depict the severity of the current situation and demand an effective solution.

In this situation, no effective drugs have been discovered to combat SARS-CoV-2 infections except a handful of repurposed drugs like chloroquine, hydroxychloroquine, remdesivir, etc. which are being used for the treatment of COVID-19 [2,6]. In case of vaccine development against COVID-19, the safety and efficacy are of major concerns. The majority of the vaccines developed against previous SARS-CoV and MERS-CoV are either inactivated or live-attenuated vaccine in nature [6]. Therefore, systematic rational drug discovery against different targets of COVID-19 is getting increasing attention of different researchers throughout the world.

Researches related to COVID-19 were able to elucidate several druggable targets of SARS-CoV-2 including spike (S) protein, 3-chymotrypsin-like protease/main protease (3CLpro/Mpro), papain-like protease (PLpro), RNA dependent RNA polymerase, etc [3,7]. Out of these, viral proteases (PLpro and Mpro) are considered important targets for drug development (Fig. 1). In corona viruses, viral proteases are responsible for non-structural proteins (nsps) production by processing viral RNA translated polyproteins [3,4,7]. Hence, the Mpro and PLpro acknowledged great attention for their significant role in the enzymatic activity leading to their post-translational processing of replicase polyproteins those are crucial in the corona virus lifecycle [8–16].

The fascinating similarity between the SARS-CoV and SARS-CoV-2 [2–4], inspires us to investigate deeper into the SARS-CoV proteases and their inhibitors for the discovery of SARS-CoV-2 protease inhibitors [17–20]. Because of the similarity in the proteases of these two corona viruses, there is a greater chance for the previous SARS-CoV inhibitors [21–68] to provide effective results against SARS-CoV-2. In this context, the molecular fragments from the SARS-CoV protease inhibitors through the fragment-based drug design and discovery technique can be useful guidance for COVID-19 drug discovery. Thus, in this review, we have focused on the important molecular fragments of SARS-CoV inhibitors to catalyze the drug discovery process for COVID-19.

2. A short trip to human corona virus

SARS-CoV-2 is not the only human corona virus outbreak reported in the 21st century [69,70]. In November 2002, clusters of pneumonia of unknown cause were, disclosed in Guangdong province of China, which was later known as the SARS-CoV outbreak. It infected at least 8422 people globally in 32 countries and causing 916 deaths (fatality rate of ~10%) [71]. Later in 2012, Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV), epidemic surfaced in Middle Eastern countries, has infected more than 1700 people (fatality rate of ~36%) [72,73].

It was more than sixty years past when the first identification of HCoV was proclaimed for respiratory tract infections [74,75]. As of seven species of HCoVs were reported for their association with respiratory tract infections, these strains are - (a) HCoV-229E, (b) HCoV-OC43, (c) HCoV- Hong Kong University 1 (HCoV-HKU1), (d) HCoV-NL63, (e) SARS-CoV, (f) MERS-CoV and (g) 2019-nCoV (now officially renamed as SARS-CoV-2). The seventh strain of HCoV, SARS-CoV-2, is taxonomically belongs to the *Betacoronavirus* genre [7].

HCoV contains a single-stranded positive sense RNA genome [76–80]. The spike glycoprotein of HCoV is the critical conciliator of entry into the host cells [81]. In consequence of virion entry into the host cells, two polyproteins namely pp1a and pp1ab are promptly translated and subsequently, split by two viral proteases such as 3C-like protease (3CLpro) and papain-like protease (PLpro) (Fig. 1). The proteolytic cleavage of these two viral polyproteins fabricated sixteen non-structural proteins (nsp1 to nsp16). The 3CLpro manages the proteolytic cleaving of all junctions downstream of nsp4 while PLpro cleaves nsp1, 2 and 3 [80,82]. Hence, both are climacteric for CoV replication, these two can be considered as druggable targets [82–88].

3. SARS-CoV proteases: a structural overview

SARS-CoV M-pro is a homodimeric protein [7,89]. Its each subunit is also termed as protomer. A number of 306 amino acid residue is found in each protomer and is constructed by three domains [7]. The domain I is 8–100 residues long followed by domain II (101–184 residues) and domain III (from 199 to 306 residues) [83] (Fig. 2).

Domains I and II allocated the same fold *i.e.*, an antiparallel six stranded β-barrel structure. The substrate-binding site or catalytic

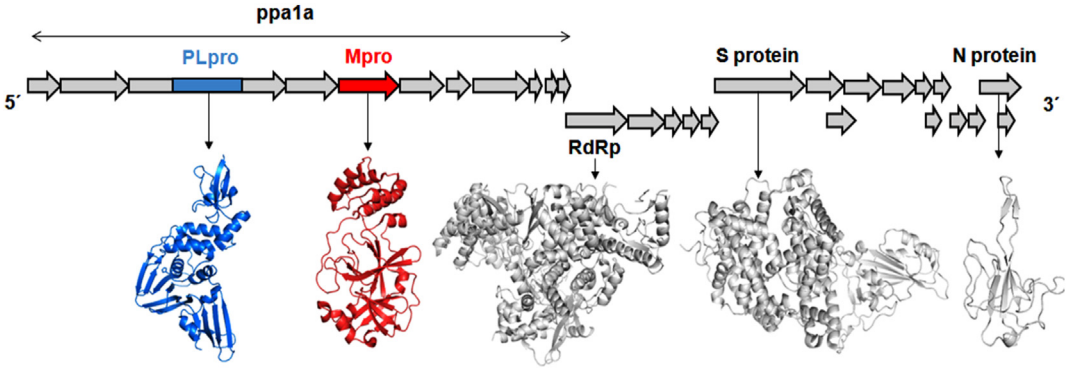

Fig. 1. Schematic plot of the SARS-CoV-2 genome and proteomes encoding the large replicase polyprotein 1a (pp1a) and pp1ab. Two proteases namely papain-like cysteine protease (PLpro) and 3-chymotrypsin-like protease/main protease (3CLpro/Mpro) are responsible for cleaving these polyproteins to produce important enzymes like RNA-dependent RNA polymerase (RdRp) and helicase, necessary in the transcription and replication of the virus.

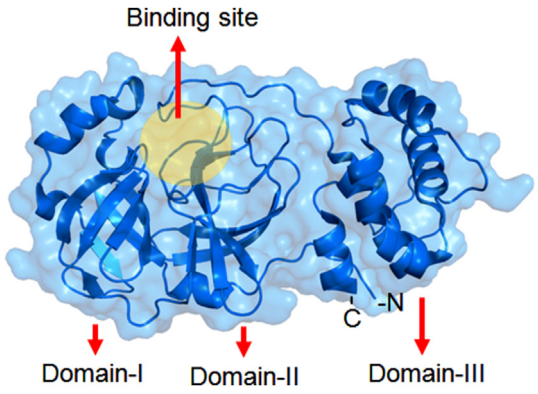

Fig. 2. Structure of SARS-CoV Mpro.

site of SARS-CoV-2 Mpro is located at a cleft between domains I and II. Notably, the domain III helps in the regulation of Mpro dimerization. N-terminal amino acid residue of a protomer interacts with another to form the S1 subsite of the substrate-binding pocket [78]. Apart from S1 amino acid residue (from a protomer), F140, L141, N142, H163, E166 (from the other protomer) are involved in the formation of S1 site. Moreover, the S2 site is formed by M49, Y54, H164, D187 and R188. S4 site is formed by M165, L167, Q189, T190 and Q192. Notably, the S1′ is formed by H41, G143, S144 and C145. Meanwhile, H41 and C145 formed a catalytic dyad which modulates the catalysis [87].

The X-ray structure of SARS-CoV PLpro of the nsp3 polyprotein domains was elucidated [33]. There are three domains in the structure: the thumb, the palm (contains catalytic triad) and the fingers (contains Zn finger motif). The substrate binding site is situated between the thumb and the palm domains. The highly conserved N-terminal region of the nsp3 contains an ubiquitin-like (UBL) domain also (Fig. 3). The PLpro enzyme can recognize and cleave ubiquitin, ISG15 (interferon-induced gene 15) proteins. The preference for cleavage of these substrates is after the LXGG motif. The fold of different PLpro enzymes in CoVs are very similar [20]. Therefore, it is considered as an important target for the development of broad spectrum inhibitors against different CoVs.

Moreover, PLpro not only reveals proteolytic property but also it shows deubiquitinating (DUB) and deISGylating activities [90]. It deubiquitinate or deISGylate host cell proteins and inactivate the NF-κB pathway leading to host cells immune suppression [91,92]. Therefore, inhibiting the PLpro enzyme would potentially block viral replication as well as PLpro-derived host cell immune suppression [82].

The structures of these proteases are also important for the drug development against COVID-19. SARS-CoV-2 proteases and SARS-CoV proteases exhibit high sequence similarities. We have already done a detail structural biology of SARS-CoV-2 proteases in our early communication [69,76]. Thus, in order to minimize the time, cost and uncertainty in drug discovery knowledge from the past SARS-CoV proteases and its inhibitors should be efficiently used into the present drug development/lead optimization against SARS-CoV-2 proteases.

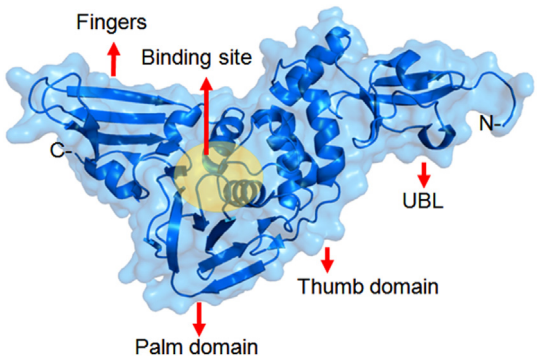

Fig. 3. Structure of SARS-CoV PLpro.

4. An insight into the SARS-CoV protease inhibitors

Since HIV and HCV proteases are fascinating targets for the successful development of antiviral drugs, it is proving the potential of HCoV protease inhibitors to hamper viral replication. In this connection, an enormous medicinal chemistry effort was dedicated to pinpoint effective peptidomimetics and/or small molecules as inhibitors of SARS-CoV Mpro [21–30] and PLpro [31–42]. However, most of the developed compounds displayed SARS-CoV protease inhibitory activities in micromolar (μM) level, only few of them exhibited activity in nanomolar (nM) ranges. Notably, the promising inhibitors with nM affinity towards the protease targets remained in the preclinical or early clinical stage. Nevertheless, these inhibitors are pretended to be an attentive starting point for lead optimization. Hence, the structure-activity relationships (SARs) studies may give rationale behind the effective CoV protease targeting drug design. Besides, we would consider SARs studies the first priority to include a detailed discussion and thus this study offers more insight than the reported studies [17–20]. In addition, we also showed how the fragment-based drug design is preferable towards protease-based drug discovery.

4.1. Understanding chemico-biological interactions of SARS-CoV Mpro inhibitors

With a target to improve SARS-CoV Mpro inhibitory potency of anilide derivatives Shie and co-workers reported a group of anilides as potent Mpro inhibitors [23]. Although these 2-chloro-4-nitroanilides exhibited a decent Mpro inhibition, the keto-methylene isosters displayed comparatively lower potency in compare to the other derivatives. Compound **A001** (Fig. 4) containing a *N*,*N*-dimethylaminophenyl and 2-chloro-4-nitrophenyl features at the R and R_1 position, respectively exhibited promising SARS-CoV Mpro inhibition (IC_{50} = 0.06 μM) as well as excellent binding affinity (*Ki* = 0.03 μM).

The docking study of **A001** inside the SARS-CoV Mpro active site demonstrated the binding pattern of **A001**. The

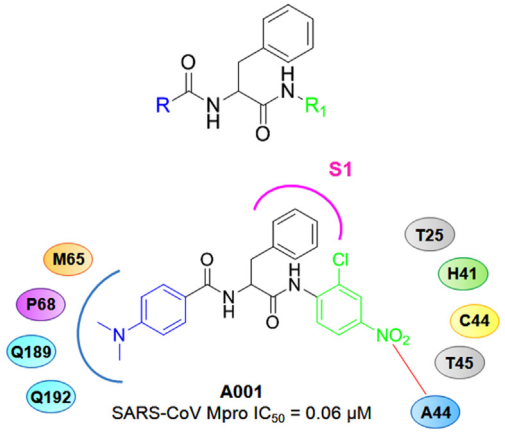

Fig. 4. Structures of **A001** along with the parent molecule.

dimethylaminophenyl moiety fitted inside the P68, M165, Q189 and Q192 created cleft while the phenyl ring of the benzyl moiety entered the S1 pocket of the SARS-CoV Mpro. In addition, the 2-chloro-4-nitorphenyl function fitted inside the pocket formed by T25, H41, C44, A46 and T45 where the NO_2 group interacted with amino acid residue A46 [23].

Inspired by the human rhinovirus (HRV) 3Cpro inhibitor, **A002**, further convening their study on SARS-CoV Mpro inhibitors. The same group developed a set of α,β-unsaturated peptidomimetic molecules as potent inhibitors of coronavirus main protease [22]. From the inhibition assay of these compounds, it has been observed that presence of lactam moiety at P1 (Fig. 5) was the detrimental for Mpro inhibition. Replacement of the lactam moiety with the phenyl ring improved the activity of these molecules (**A002** *vs* **A003**, **A004**).

Among the phenyl containing molecules both the keto-methylene isosteric compounds and tetrapeptidomimetic esters exhibited comparatively similar potency. Form the docking study of **A002** with SARS-CoV Mpro, it would be observed the insertion of fluorophenyl moiety of **A002** into the S2 site. However, the improper fitting of the lactam moiety inside the S1 pocket and disposition of the ester function into the S1′ site led to the prevention of suitable bond formation. In contrast, compound **A005** (Fig. 5), an analog of **A003**, fitted well into the Mpro active site and interacted with H163, G143, E166, Q189 leading to its improved Mpro inhibitory activity than **A002**-**A004**. Notably, **A005** was also unable to form a covalent bond with C145. As a consequence, the authors again design and reported a group of dipeptide inhibitors possessing a Michael acceptor at both ends of these molecules. Among these, **A006** (Fig. 5) showed the highest SARS-CoV Mpro inhibitory potency (IC_{50} = 1 μM) as well as binding affinity (Ki = 0.52 μM). Furthermore, the molecular docking study **A006** with SARS-CoV Mpro displayed that the phenyl rings of the molecules entered into the S2 and S3 pockets whilst the cinnamoyl moiety formed interactions with E166, Q189 and E192 [22].

In a structure-based drug design approach to identify potent non-peptide mimetic novel SARS-CoV Mpro inhibitors, Lu et al. [21] screened a wide array of 58,855 compounds and identified two potential hits. Further, the core structures of these two hits were employed to analogue search which gave rise to 21 analogs having promising Mpro inhibitory activities. Among these, compounds of **A007** and **A008** containing scaffold A and **A009** bearing scaffold B possessed potent inhibition against SARS-CoV Mpro (Fig. 6).

A007 occupied S3 and S5 pockets of Mpro as suggested by the structural analysis. The 2,4-dichloromethyl phenyl ring of **A007** entered deep inside the hydrophobic pocket formed by P39, C145, H41, H163, H164, F182, F185 and Y182. Moreover, the phenyl ring of **A007** and amino acid residue H41 formed a π-π interaction. Furthermore, 2,4-dichloro-5-methyl phenyl moiety of resides between the H41 and C145 allowed by shifting of H41 from C145 causing blocking of catalytic dyad, thus inhibiting SARS-CoV Mpro activity. Interestingly, the triazole functions of **A009** (Fig. 6) entered into the S2 pocket. Moreover, the trifluoromethyl function of triazole moiety made close contact with the catalytic dyad. The furan ring interacted with E166 while the phenyl associated with the furan ring reached the S4 and S5 pockets to form hydrophobic interaction with Q192, P168, Q189, M165 and E166. Besides, carbothioate linker oxygen of compound **A009** was found to interact with E166 and P140 [21].

Wu et al. [24] reported a set of benzotriazole ester-based molecules as potent SARS-CoV Mpro inhibitors. These authors found that among those molecules, benzotriazole esters derived from electron donating group containing benzoic acids are more stable compared to the electron withdrawing group containing analogs which were more susceptible to hydrolysis. Upon analyzing the nature of inhibited enzyme, these authors proposed that the 4-dimethylamino benzoyl analogue, **A010** interacted with the thiol group of C145 to acylate it and thus, irreversibly deactivating the enzyme (Fig. 7) [24].

Tsai and collaborators utilized docking based virtual screening technique and screened 93 compounds on the basis of binding interaction patterns and affinity towards SARS-CoV Mpro (PDB: 1UK4) [25]. Further, twentyone compounds were selected with inhibition against SARS-CoV 3CLpro ($IC_{50} \leq 30$ μM). A common *N*-phenyl-2-(2-pyrimidinylthio)acetamide core structure was pinpointed which was further utilities for analogue search and resulted twentyeight analogs having IC_{50} values in the range of 3−1000 μM. Meanwhile, the 3D-QSAR study on these twenty eight identified analogs and pharmacophore mapping suggested the important sites where steric and electrostatic interactions along with hydrogen-bond acceptor (HBA), hydrogen-bond donor (HBD) and hydrophobic (HY) features that could significantly contribute to SARS-CoV Mpro inhibition (Fig. 8) [25].

In 2008, Mukherjee and colleagues identified two hits, **A013** and

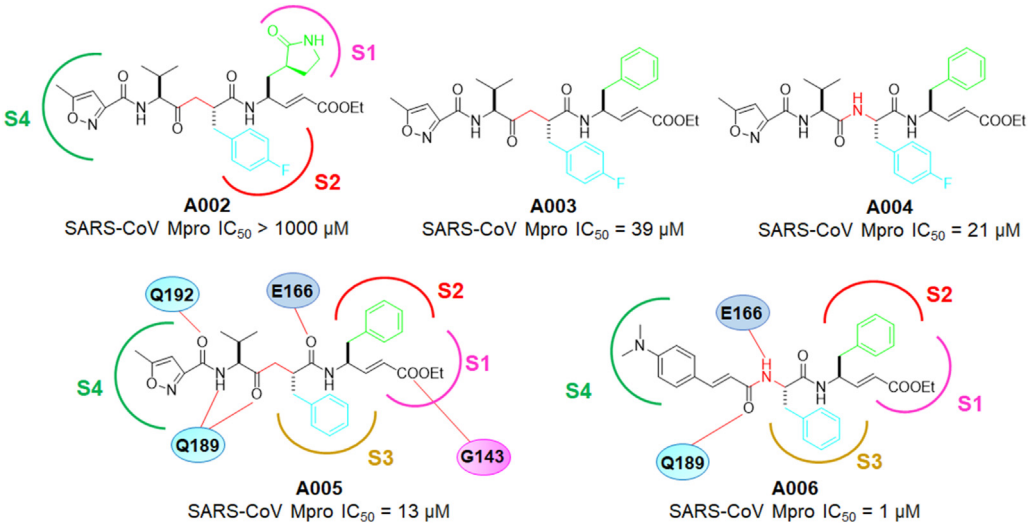

Fig. 5. Structures of **A002-A006**.

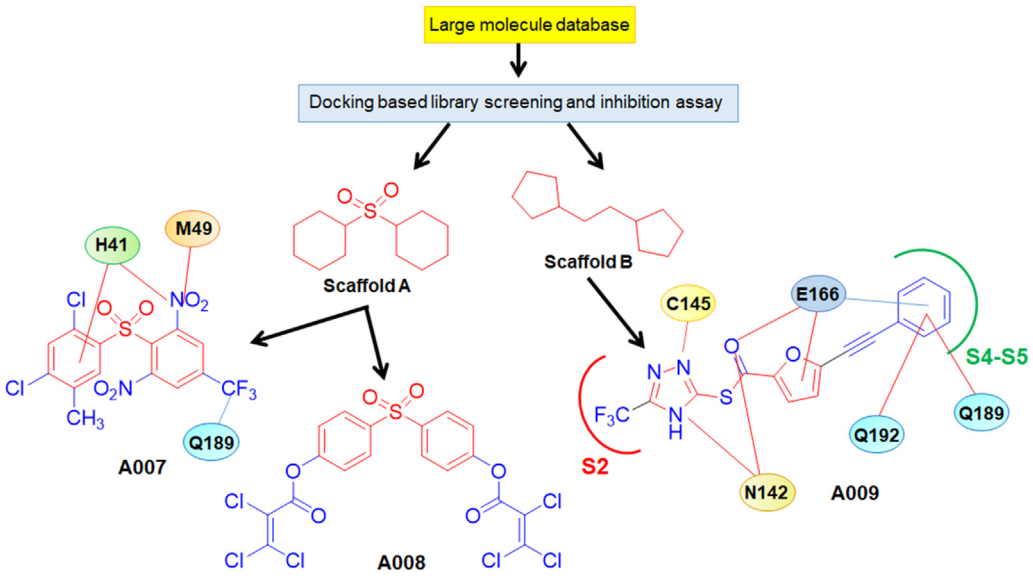

Fig. 6. Development of **A007-A009**.

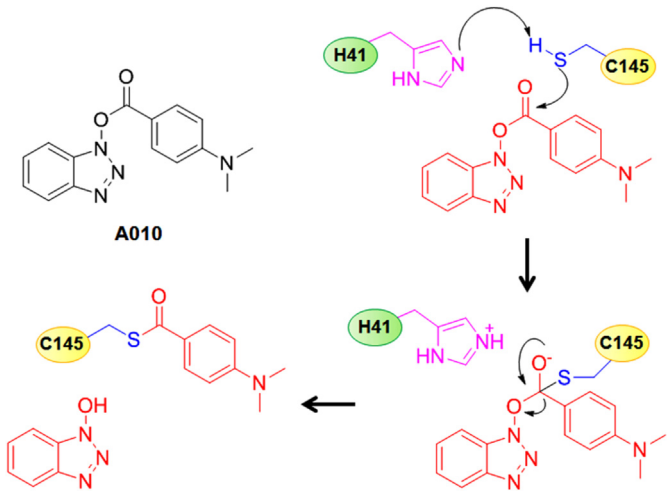

Fig. 7. Structure of **A010** along with its SARS-CoV Mpro inhibitory mechanism.

A014 after extensive virtual screening (VS) study (Fig. 9) [26]. The binding poses of **A013** and **A014** revealed that both of these inhibitors interacted with binding site residues and form hydrophobic as well as hydrogen bond interactions to maintain their Mpro inhibition.

In spite of occupancy of S1, S1′, and S2 pockets by these inhibitors, the S4 pocket remains unoccupied (Fig. 9). Notably, the thiomethylene carboxamido linker of compound **A013** (marked red in Fig. 9) was observed in the **A011** as identified by Tsai et al. [25].

Jacobs and colleagues reported the discovery of several acetamide derivatives using high throughput screening (HTS) and structure-based optimization techniques [28]. In this HTS study, from a large library of molecules, 44 active molecules with Mpro IC_{50} < 10 μM were identified whereas subsequent counter-screening of these compounds against PLpro activity enabled the identification of hit compound **A015** (Fig. 10).

In order to optimize the **A015** for more potent Mpro inhibition, these authors utilizing the HTS data and designed a library of 80 molecules bearing a similar scaffold. From this library of molecules, compound **A016** (Fig. 10) was identified as a potent Mpro inhibitor. Interestingly, the *R*-enantiomer of **A016** was highly active against Mpro whereas the *S*-enantiomer of the molecule exhibited its inactive nature. Also, the *R*-enantiomer displayed its Mpro selective nature while being inactive against PLpro.

From the compound **A016** bound crystal structure of SARS-CoV Mpro (PDB: 3V3M), the molecule was found to occupy the S1—S3 pockets of the enzyme active site (Fig. 10). The 3-pyridyl group entered into the S1 pocket whereas the *tert*-butylamido group occupied the S3 pocket. Also, the *tert*-butyl anilido moiety (P2) of the molecule occupied the S2 pocket of the enzyme (Fig. 10). Moreover, the oxygen atom from the furan ring and its adjacent carbonyl function were found to interact with G143 amino acid via hydrogen bond interaction.

Although further optimization of **A016** was also attempted to improve the Mpro inhibitory potency, the optimized compounds were unable to deliver higher Mpro inhibition than **A016**. The overall structure-activity relationship of these compounds suggested that the 4-*tert*-butyl phenyl group substitution at the R_2 position (P2 substituent) of the scaffold (Fig. 11) is beneficial for Mpro inhibition whereas 4-*iso*-propyl phenyl group at that position reduces the activity. Also, the presence of *cyclo*-propyl or 4-fluoro phenyl groups at R_2 diminished the activity. Regarding the R_3 substitution, the 3-pyridyl group was found favorable for Mpro inhibition whereas 3-thienyl group as R_3 substituent was deleterious for the activity.

Additionally, replacement of the R_3 (P1 substituent) 3-pyridyl group of **A016** with pyrazine or pyridazine groups exhibited well tolerability but were unable to improve the Mpro inhibition. On the other hand, substituting the 3-pyridyl group of **A016** with diazole and triazole groups as well as 2-pyridyl and 4-pyridyl groups exhibited detrimental effects on the activity. Concerning the five-membered furan ring present at the R_1 position (P1' substituent) of **A016**, substituting that furan ring with 2-chloro furan or imidazole groups was well tolerated (Fig. 11). Moreover, substituents like branched alkyl group, 2-pyridyl and 3-pyridyl groups as well as substituted phenyl rings at R_1 position showed detrimental effects on the activity [28].

In 2017, a molecular docking-based VS study of 3,08,307 chemical compounds was adopted by Nguyen et al. [29] in an urge to identify SARS-CoV Mpro inhibitors. Finally, seven compounds were selected with their SARS-CoV Mpro IC_{50} ranged from 38.57 to

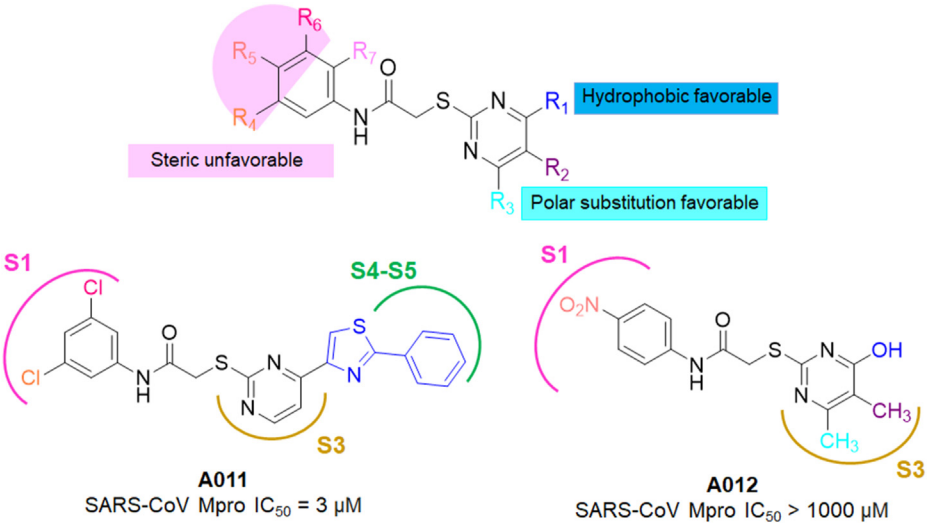

Fig. 8. SARs of *N*-phenyl-2-(2-pyrimidinylthio)acetamides along with the structures of **A011**-**A012**.

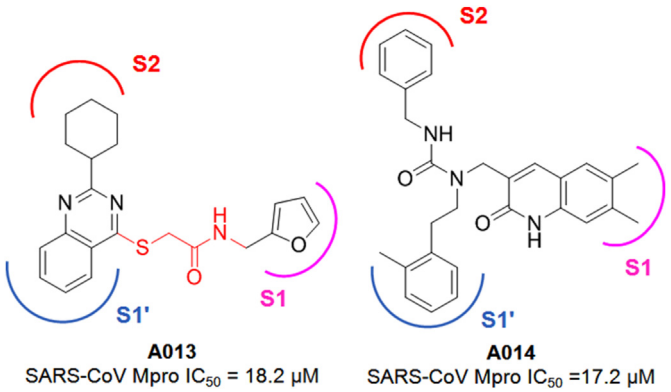

Fig. 9. Structures of **A013**-**A014**.

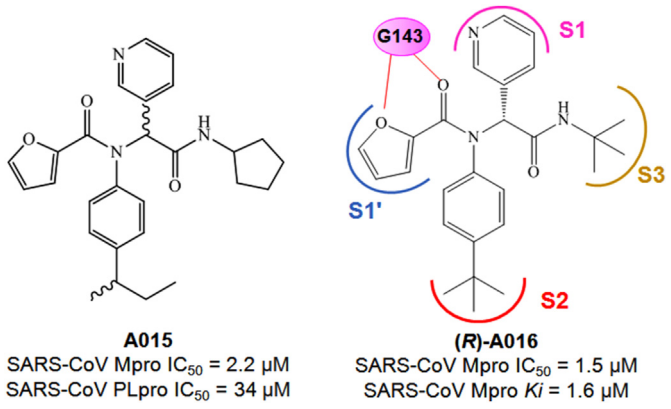

Fig. 10. Structures of **A015**-**A016**.

101.38 μM. The detailed molecular docking interaction of potent compounds **A017** and **A018** (Fig. 12) referred that the inhibitors formed several hydrogen bonds with catalytic residues and also established hydrophobic contacts with hotspot amino acid residues of SARS-CoV Mpro. Moreover, inhibitors, **A017** and **A018**, competitively inhibit Mpro with *Ki* values of 9.11 and 9.93 μM, respectively [29].

Kumar et al. [30] reported a group of neuramide inhibitors by adopting the screening and synthetic approaches on basis of their previous inhibitors. The phenyl function (ring A) connected to a furan ring (ring B) of these new neuramide inhibitors was found to pivotal while, the presence of carboxylic acid groups at both $R_1$and R_4 positions possessed detrimental effect as far as the SARS-CoV Mpro inhibitory activity was concerned (Fig. 13). The SAR study suggested that *iso*-butyl or *tert*-butyl methyl groups at the R_4 position delivered higher SARS-CoV Mpo inhibition compared to their fluorine, carboxylic acid, cyano and methoxy substituted analogs. Additionally, bulky hydrophobic groups such as phenyl ring substitution at the pyrazolone (ring C) R_3 position was found to be beneficial compared to the corresponding trifluoromethyl substituted analogs. Besides, pyrazolone carbonyl function was suggested as an important feature to interact with H41, thus inactivating the catalytic dyad. Moreover, the chlorine substitution at the R_2 position was found to be important for the activity. The molecular docking study of the most active compound of this series, **A019** (Fig. 13), showed that carboxylate function inserted at the S1 sub-site and forming interactions with S144, E143, C145 and H163 whereas, the furan ring was found to interact with L27 side chain via hydrophobic contact [30].

In another study, the same group identified a set of potent SARS-CoV and MERS-CoV Mpro inhibitors by screening the inhibitors of enterovirus 71 (EV71) Mpro [31]. Among these, aldehyde derivatives in presence of fused heterocyclic ring were found to detrimental whilst the substituted phenyl analogs exhibited potent SARS-CoV Mpro inhibition. Also, the compound **A020** containing a 2-fluoro-4-chloro-phenyl moiety showed a 0.2 μM IC_{50} value against SARS-CoV Mpro along with a MERS-CoV Mpro IC_{50} value of 4.7 μM (Fig. 13) [31].

Apart from the above mentioned SARS-CoV Mpro inhibitors, some important peptidomimetic as well as small molecular covalent and non-covalent CoV Mpro inhibitors [27,43–68] are summarised in Tables 4 and 5, respectively (Figs. 14–16).

The close associateship of SARS-CoV-2 Mpro to homologues SARS-CoV Mpro exhibits a high sequence identity (~96.1%). It confers the chance of efficiency of SARS-CoV Mpro inhibitors towards SARS-CoV-2.

4.2. Understanding chemico-biological interactions of SARS-CoV PLpro inhibitors

PLpro reveals proteolytic activity. Besides, it shows

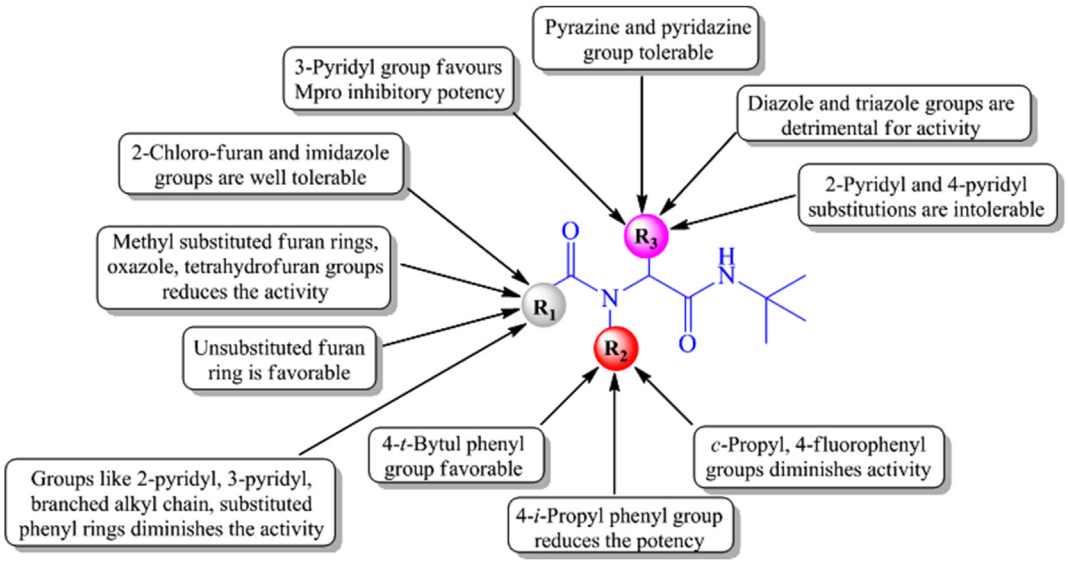

Fig. 11. Structure-activity relationship of acetamide derivatives.

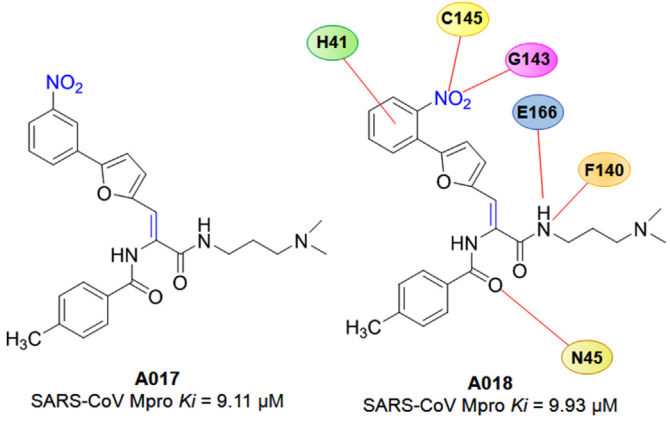

Fig. 12. Structures of **A017**-**A018**.

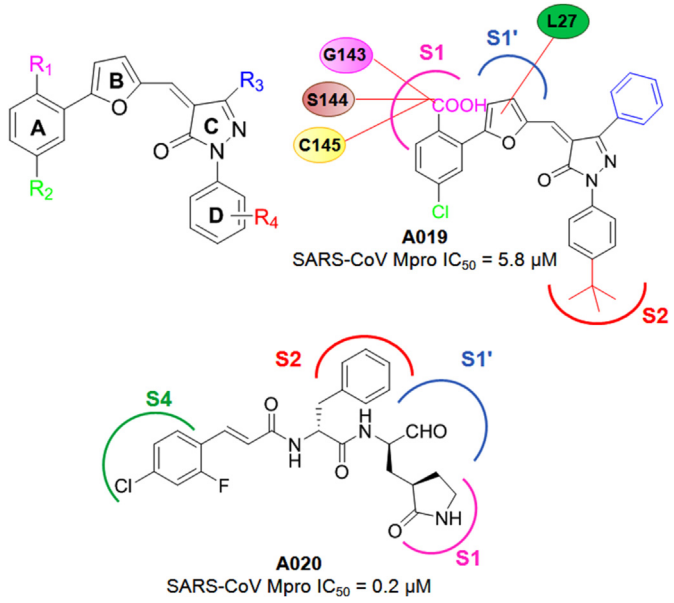

Fig. 13. Structures of **A019**-**A020**.

deubiquitinating (DUB) and deISGylating activities [82]. Ratia et al. [33] reported naphthyl derivatives were as non-covalent competitive inhibitors of SARS-CoV PLpro. Interestingly, these investigated naphthyl derivatives possessing acanonical pharmacophoric features such as head, linker and tail (Fig. 17).

The head region is mostly conserved in between 1 and 2-naphthyl features [33–36]. SAR studies revealed that the 2-naphthyl head disfavors PLpro inhibition over 1-naphthyl analog (compound **B001** *vs* **B002**–**B006**). The structure-based studies revealed that naphthyl derivatives bind within the S4–S3 sub-sites of PLpro which likely to induce a loop closure and subsequently, undergone conformational change and thereby, led to PLpro inactivation.

Besides, the stereochemical pattern of the methyl substituent served as a critical factor to modulate PLpro bining affinity. Báez-Santos et al. [34] reported that increasing steric properties at that position potentially hampered the PLpro inhibitory activity. The potential entropic gain may displace the water molecules resulted in a larger enthalpic penalty of breaking hydrogen bonds. Moreover, the stereo-chemistry of the methyl substituent at R_3 influences the PLpro binding affinity. The (*R*)-methyl enantiomer extends into an interior of the PLpro enzyme between Y265 and T302 [34].

The amide NH (as linker) was found to be a key regulator of PLpro inhibition. Methylation at the aminde nitrogen atom led to loss in SARS-CoV PLpro inhibitory activity (the *N*-methyl derivative **B003**, $IC_{50} = 22.6$ μM *vs* compound **B002**, $IC_{50} = 2.3$ μM). Piperidine function has been pleasurable towards biological activity against PLpro enzyme. It exhibited positive effects on SARS-CoV PLpro inhibitory activity. In addition, the SAR study suggested that bulky R_4 substituents had little influences towards PLpro inhibition (compound **B005** *vs* **B006** in Fig. 17) [34]. At the tail portion, substitutions at the 3rd and/or 4th position of the phenyl ring were suitable (compound **B007**–**B010** *vs* **B006**) to achieve potency in nano-molar ranges (Fig. 18).

Notably, dioxolane derivatives **B011**–**B012** and 4-ethyl prototype **B013** exhibited excellent PLpro inhibitory activities. The interaction between the dioxolane group and catalytic site amino acid Q270 has contributed positively to the PLpro inhibition.

Table 4
Peptidomimetic and small molecular covalent SARS-CoV Mpro inhibitors.

Entry	Comp	PDB	Activity against Mpro	Year	Reference
1	**A021**	–	IC_{50} = 50 nM	2004	[27]
2	**A022**	2A5K	k_{inact}/Ki = 1900 (±400) $M^{-1}s^{-1}$	2005	[44]
3	**A023**	2GX4	*Ki* = 53 nM	2006	[45]
4	**A024**	2QIQ	IC_{50} = 80 μM	2007	[46]
5	**A025**	–	IC_{50} = 50 nM; antiviral activity EC_{50} = 6.9 μM	2008	[47]
6	**A026**	–	IC_{50} = 2.20 μM	2009	[48]
7	**A027**	–	*Ki* = 0.003 μM	2013	[49]
8	**A028**	–	*Ki* = 0.33 μM	2013	[50]
9	**A029**	–	*Ki* = 0.065 μM	2013	[51]
10	**A030**	5N19	IC_{50} = 1.95 μM	2020	[3]
11	**A031**	–	IC_{50} = 0.71 μM	2020	[3]

Table 5
Peptidomimetic and small molecular non-covalent SARS-CoV Mpro inhibitors.

Entry	Comp	PDB	Activity against Mpro	Year	Reference
1	**A032**	–	IC_{50} = 0.60 μM	2004	[52]
2	**A033**	–	*Ki* = 0.6 μM	2004	[53]
3	**A034**	–	IC_{50} = 2.5 μM	2004	[54]
4	**A035**	–	IC_{50} = 4.3 μM	2004	[27]
5	**A036**	–	IC_{50} = 0.37 μM	2006	[56]
6	**A037**	–	IC_{50} = 0.04 μM	2006	[56]
7	**A038**	–	*Ki* = 0.073 μM	2007	[57]
8	**A039**	–	*Ki* = 8.2 μM	2007	[58]
9	**A040**	–	IC_{50} = 5.5 μM	2010	[62]
10	**A041**	–	IC_{50} = 2.6 μM	2010	[62]
11	**A042**	–	IC_{50} = 65 nM	2011	[63]
12	**A043**	3ATW	IC_{50} = 98 nM	2011	[63]
13	**A044**	–	IC_{50} = 3.8 μM	2013	[64]
14	**A045**	4MDS	IC_{50} = 8.2 μM	2013	[64]
15	**A046**	–	IC_{50} = 4.1 μM	2013	[64]
16	**A047**	–	IC_{50} = 0.051 μM	2013	[64]
17	**A048**	–	IC_{50} = 1.04 μM	2014	[65]

Although there should be another influences which likely to contribute largely to the substituted phenyls. Unlike fluorine substitution at the phenyl ring induced conspicuous polarization effects in the π-system of associated phenyl which may elevate the binding affinity [34]. However, the methoxy group at the terminal phenyl ring displayed the negative influence towards SARS-CoV PLpro inhibition.

In spite of promising SARS-CoV PLpro inhibition, naphthyl derivatives exhibited poor MERS-CoV PLpro inhibitory activity. Lee and co-workers very nicely explain the reason behind this poor activity [42]. These authors also reported compound **B014** (Fig. 19) as promising dual PLpro inhibitors of SARS-CoV (IC_{50} = 10.9 μM) and MERS-CoV (IC_{50} = 6.2 μM) after screening 25,000 compounds [42].

In 2012, a group of Scientists from Republic of Korea reported SARS-CoV Mpro and PLpro inhibitory properties of seven isolated tanshinones (**B015**–**B021**) from *Salvia miltiorrhiza* (Laminaceae) [37]. Significantly, all of these isolated compounds exhibited inhibitory activities against both cysteine proteases. In particular, these compounds (**B015**–**B021**) displayed significant SARS-CoV PLpro inhibition ranging from 0.8 to 30.0 μM (Fig. 20) [37].

Diarylheptanoids, isolated from *Alnus japonica*, exhibited promising SARS-CoV PLpro inhibitory activities. Among all these compounds, hirsutenone (**B022**) possessed the most active PLpro inhibitory activity (IC_{50} = 4.1 μM) comparatively better than curcumin (IC_{50} = 5.7 μM) [40].

Later, Cho and collaborators reported geranylated flavonoids from the fruits of *Paulownia tomentosa* to exert SARS-CoV PLpro inhibitory activities [41]. Of the isolated dihydro-2H-pyran moiety, tomentin E (**B023**, Fig. 19) showed the most potent activity against

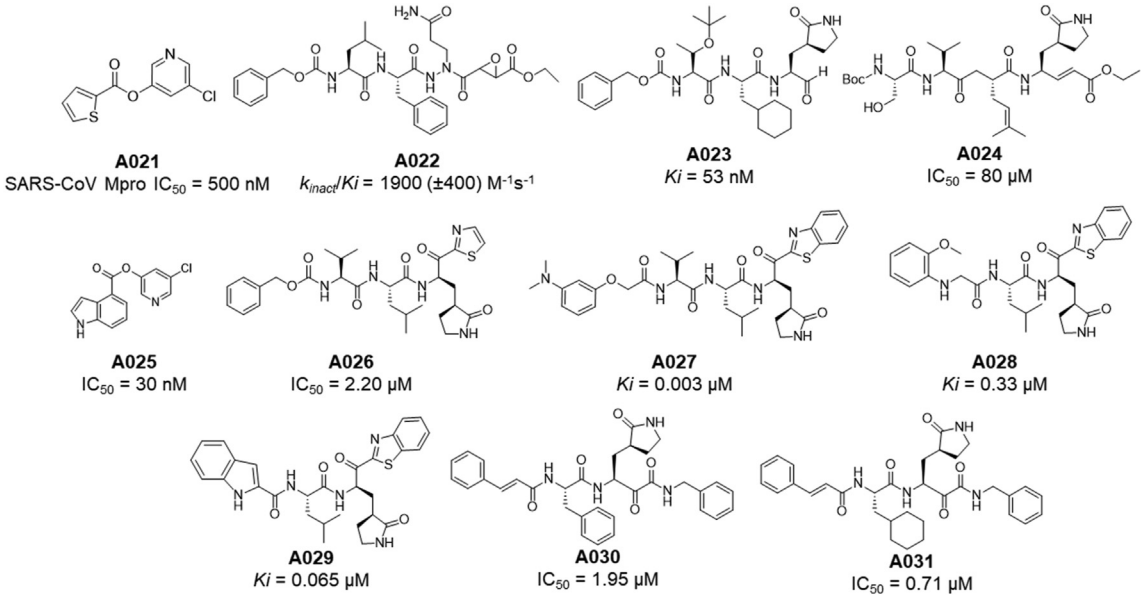

Fig. 14. Structures of **A021**-**A031**.

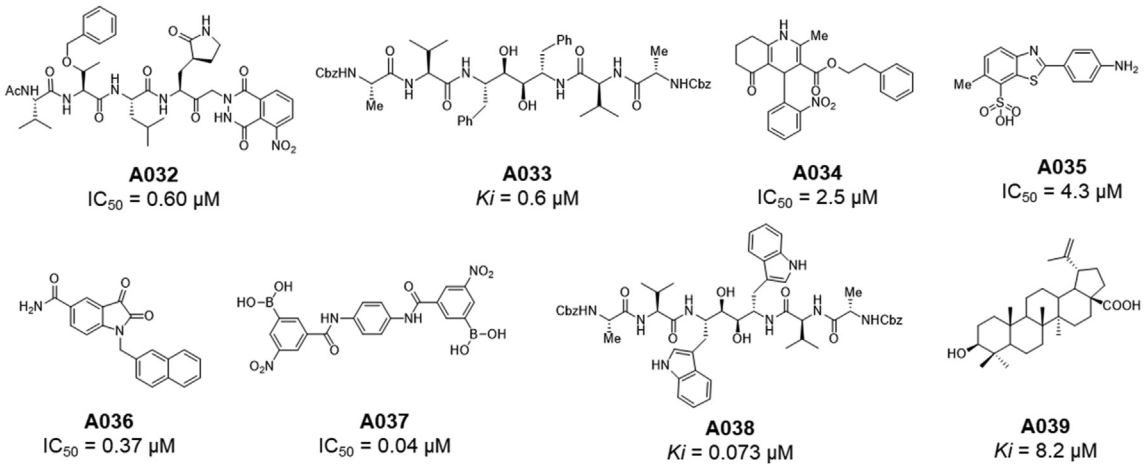

Fig. 15. Structures of **A032**-**A039**.

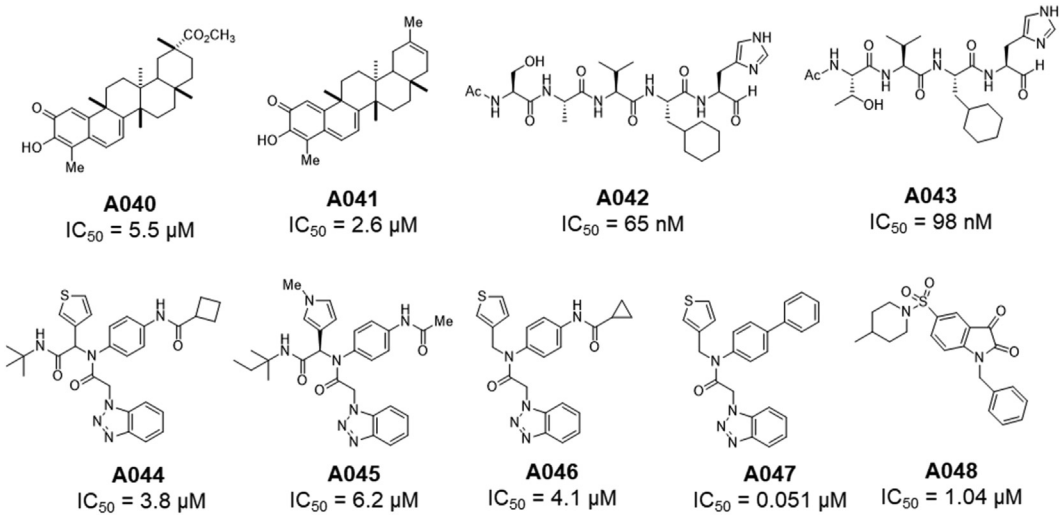

Fig. 16. Structures of **A040**-**A048**.

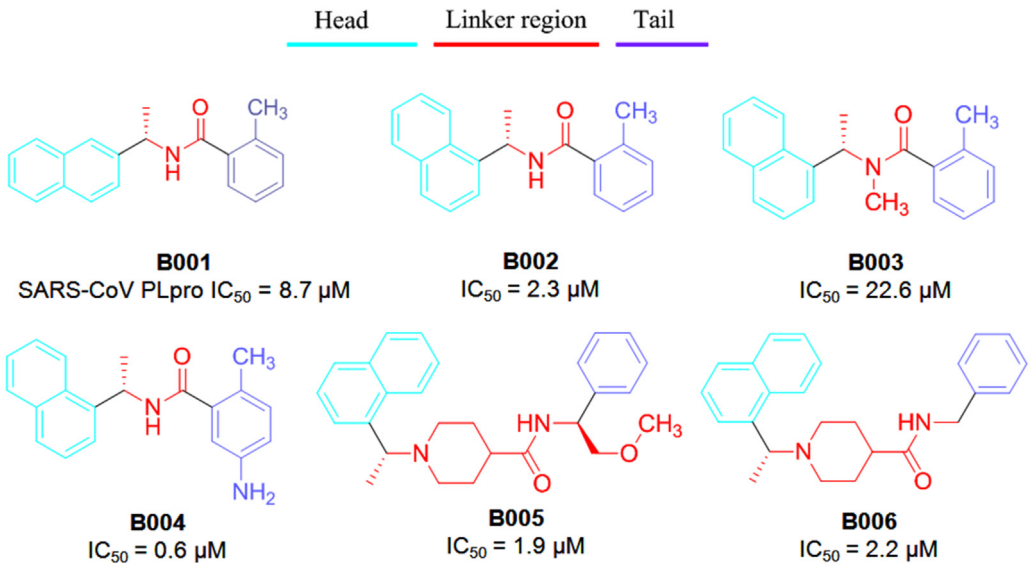

Fig. 17. Structure of SARS-CoV PLpro inhibitors (**B001**–**B006**).

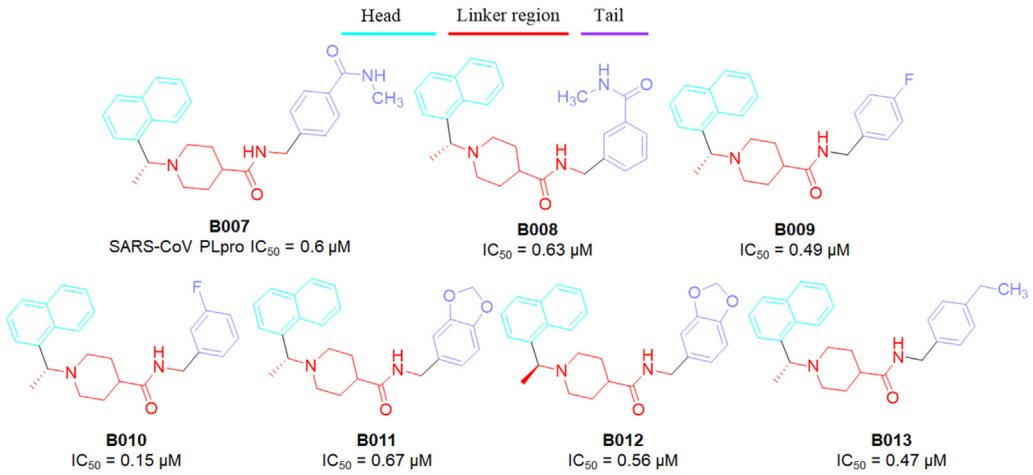

Fig. 18. Structure of SARS-CoV PLpro inhibitors (**B007**–**B013**).

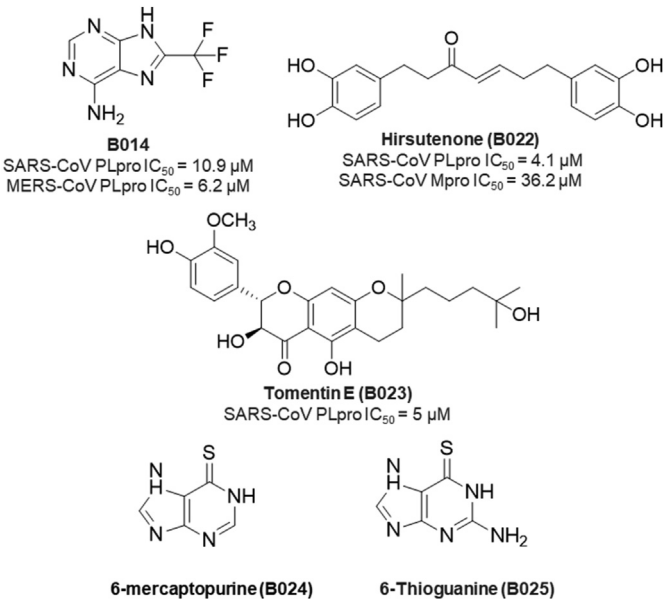

Fig. 19. Structure of some SARS-CoV PLpro inhibitors.

SARS-CoV PLpro (IC_{50} = 5 μM).

Purine analogs such as 6-mercaptopurine (6 MP, **B024**) and 6-thioguanine (6 TG, **B025**) as shown in Fig. 19 were found to inhibit SARS-CoV PLpro. These were categories as reversible and slow-binding inhibitors of SARS-CoV PLpro [39].

5. How the fragment-based drug design is preferable towards protease-based drug discovery?

In the field of drug development, the utilization of different computer-aided drug design techniques such as structure-based and ligand-based drug design approaches, virtual screening of molecule libraries have become efficient means for hit identification and lead optimization [7,93–101]. Fragment-based drug design (FBDD) approaches for lead optimization has also gained quite a reputation in both industrial and academic levels becoming a successful tool for modern drug discovery [99]. FBDD approaches have several advantages such as a) this method can provide lead molecules with better physicochemical properties, b) fragments have chances to produce good interactions, c) fragments with lesser complex structures can give higher rates of hit molecules, d) fragment-derived leads can be less hydrophobic and smaller and e) able to deliver high-quality lead molecules.

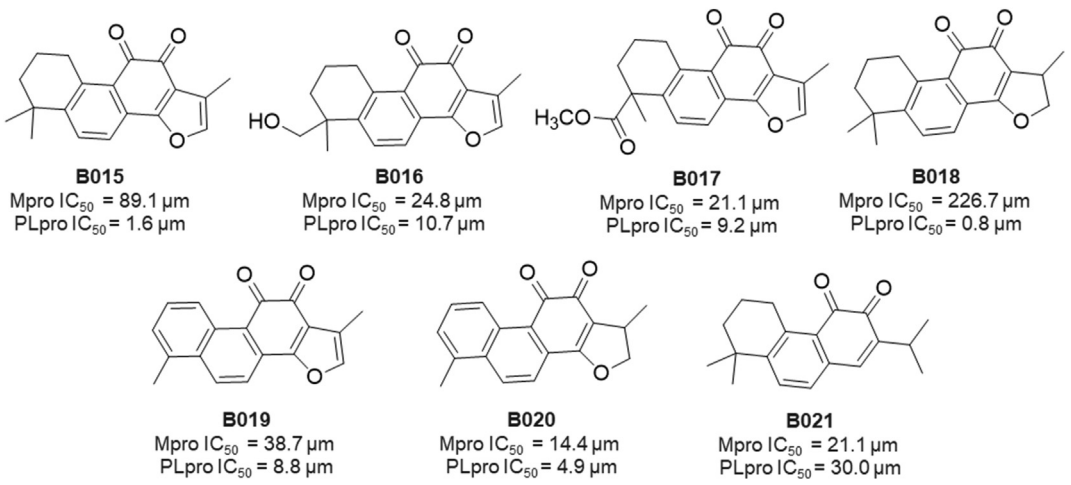

Fig. 20. Structure of SARS-CoV PLpro inhibitors (**B015**–**B021**).

Choudhury from the PGIMER, Chandigarh, India screened a huge library of 191678 fragments against the SARS-CoV-2 Mpro binding cavity to pick fragments with high affinity [8]. Depending on the higher binding affinity, new molecules were designed. Interestingly, fifteen designed molecules formed stable complexes with SARS-CoV-2 Mpro as suggested by the molecular docking and Molecular Mechanics-Generalized Born Surface Area (MM/GBSA) based binding free energy.

In a recent study, Douangamath et al. [9] reported 74 high-value fragment hits after screening 1250 unique fragments against Mpro of SARS-CoV-2. Out of these 74 fragments, 23 non-covalent and 48 covalent hits in the active site and rest hits were distinguished at the vital dimerization interface.

Depending on our previous reported molecular modelling studies [69,76,102], we have identified fragments and marked in Figs. 21 and 22. A compound with furan and/or pyridine exerts effective SARS-CoV Mpro inhibitory activity (Fig. 21). Hence, it may be anticipated that furan and/or pyridine obviously put some more interaction benefit towards biological activity.

For instance, the furan oxygen atom of compound **A016** interacts with the backbone NH of G143 by forming hydrogen bonding interaction (PDB: 3V3M). Likewise, the fingerprint CC(C)NC(=O) CNC(=O) in compounds found to interact with several active site amino acid residues of SARS-CoV Mpro (PDB: 3ATW) as illustrated in Fig. 23.

These distinguishing key molecular fragments revealed influences towards the SARS-CoV Mpro inhibitory activities. The studied fragments may leave considerable space to follow-up anti-SARS-CoV-2 drug design. Similarly, the 1-naphthalene (at the head), piperidine (at the linker) and benzamide (at the tail) moieties are the fragment of interest for the SARS-CoV PLpro inhibitory activities. The naphthalene ring forms interactions with amino acids P248, P249 and Y269 at the solvent exposed site of SARS-CoV PLpro (PDB: 3MJ5) as shown in Fig. 24A and B. Very recently, 1-naphthalene based derivatives were reported to display SARS-CoV-2 PLpro inhibition [103].

The piperidine ring is also found to be pivotal for SARS-CoV PLpro inhibition since it engages in π-sigma interaction with the Y265 of the enzyme (PDB: 3MJ5), as depicted in Fig. 24B. Furthermore, the amide group of benzamide moiety of a compound interacts with the side chain of D165 and the backbone nitrogen atom of Q270 (PDB: 3E9S) by forming hydrogen bonding interaction (Fig. 24A).

In an endeavour, our research team explored the crucial structural fingerprints modulating SARS-CoV PLpro inhibitory activities by the aid of 2D-QSAR, SPCI analysis as well as Monte Carlo optimization based QSAR [69]. Our research group also performed a molecular docking study of some *in-house* isoglutamine derivatives against putative target SARS-CoV-2 PLpro (Fig. 25). These derivatives may serve as a seed which sustain significant hope against corona virsus.

Hence, these fragments identified by the previous studies may be an effective approach to accelerate drug design against SARS-CoV-2. Notably, the identified fragments could be implemented in the medicinal chemistry endeavors of COVID-19 and other CoV drug discovery also. Our modeling approach will give several strategic options to the medicinal chemists for the lead optimization of these protease inhibitors. With the incorporation of these fragments, potent inhibitors may be designed and synthesized. Some fragments are matching for SARS-CoV-2 PLpro, 3CLpro inhibitors and other fragments may give encouraging results in future. Since the protease based COVID-19 drug discovery is in its very early stage, the transferability of patterns may be judged with patience.

6. Conclusion

It is well known fact that many of these viruses encode one or more proteases employed in processes like maturation, viral poly protein processing, etc [104]. As a boon of such involvements, viral proteases have been lucrative targets to design potent viral protease inhibitors. Historically, in diseases like human immune deficiency virus (HIV) and hepatitis C virus (HCV) infections, targeting the viral proteases were able to provide several anti-viral agents which were effective against those infections [99,100]. Moreover, the HIV protease inhibitors lopinavir and ritonavir were effective against SARS-CoV and MERS-CoV and were also tested clinically against COVID-19 [4,6,101]. Hence, the proteases of SARS-CoV-2 are druggable targets to develop anti-viral agents and have higher odds to achieve the desirable anti-viral agent for COVID-19 treatment.

Meanwhile, desirable pharmacokinetic characteristics are very important for drug discovery and development. Reports already supported that macromolecular and/or peptidomimetic compounds are advantageous over the low molecular weight compounds in terms of Mpro and PLpro inhibitory potency as well as selectivity [104]. However, these types of compound may exhibit ADME issues. An analysis of the reported SARS-CoV Mpro and PLpro inhibitors for their drug likeliness properties has revealed that compounds **A002-A003**, **A005-A006**, **A008**, **A019**, **A022-A023**, **A024**, **A026**, **A027**, **A032-A033**, **A037-A038**, **A042-A043** failed to pass *Lipinski rule of five* principle [105] due to combinations of different factors, e.g. AlogP: 5, molecular weight: 500, number of hydrogen bond acceptors: 10, number of hydrogen bond donor: 5 [106]. In this scenario, small molecule based lead optimization

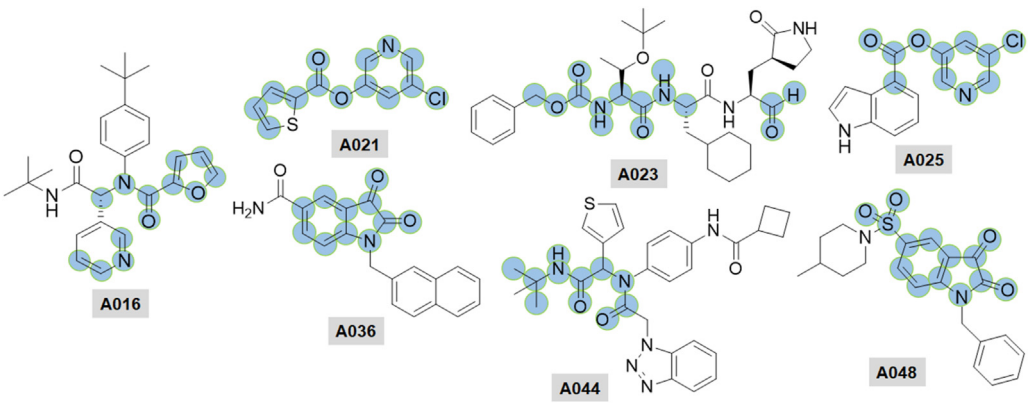

Fig. 21. Important fingerprints are highlighted in the SARS-CoV Mpro inhibitors.

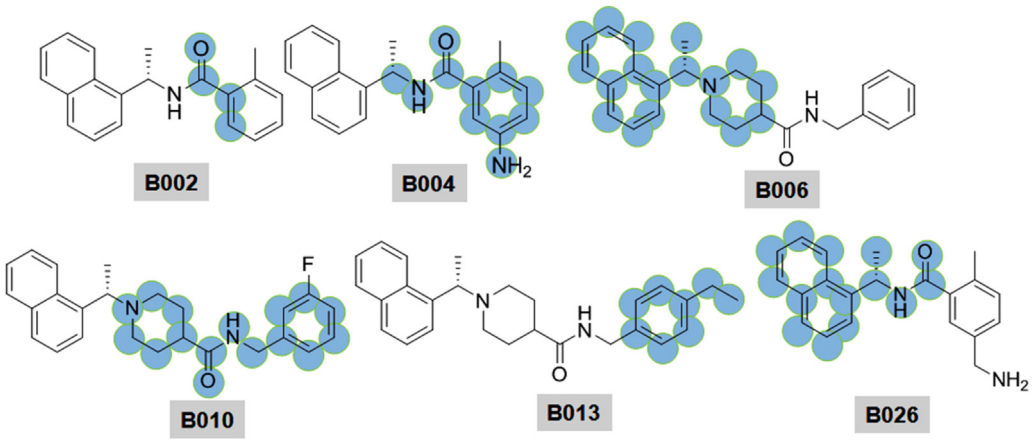

Fig. 22. Important fingerprints are highlighted in the SARS-CoV PLpro inhibitors.

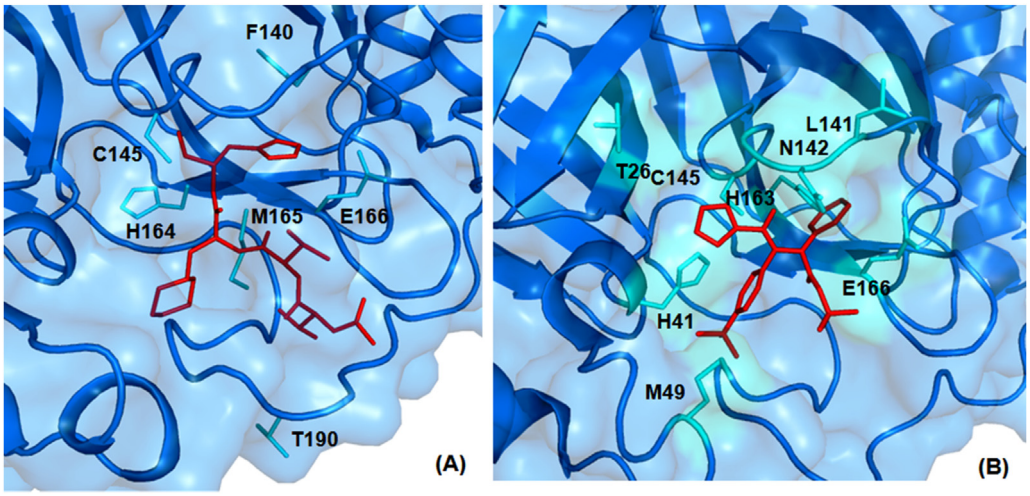

Fig. 23. 3D interaction plot of prototype compounds with SARS-CoV 3CLpro active site amino acid residues (A) PDB: 3ATW and (B) PDB: 3V3M.

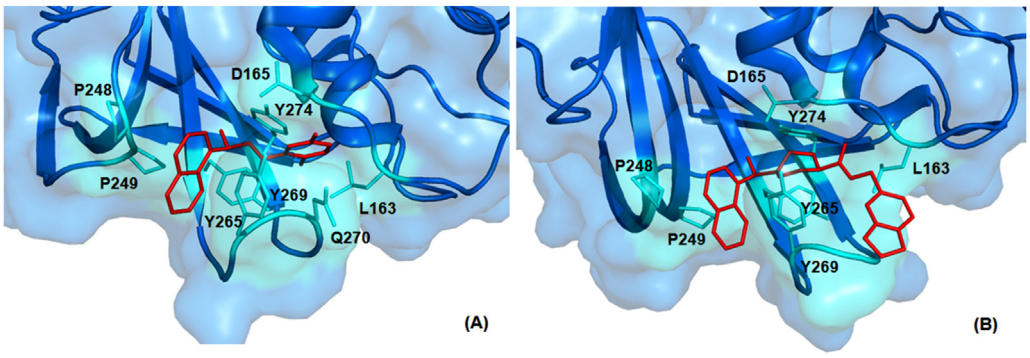

Fig. 24. 3D interaction plots of naphthyl based PLpro inhibitors with SARS-CoV PLpro active site amino acid residues (A) PDB: 3E9S and (B) PDB: 3MJ5.

strategies may be beneficial. In our previous study [107], we have shown that baicalein (MW 270.24 Da) based lead optimization by taking into account different good fragments can generate potential inhibitors and may effectively block the SARS-CoV-2 Mpro target. The design of protease inhibitors should also take into account the PAINS (Pan-assay interference compounds) alert as these compounds can bind nonspecifically with different target. An analysis of the SARS-CoV Mpro and PLpro inhibitors by SwissADME [108] has revealed that compounds **A006**, **B015**–**B021** have possible PAINS alert to be avoided in the lead optimization of protease inhibitors.

In addition to this, there is a high possibility of nonsense mutation to be introduced in the viral genome of SARS-CoV and SARS-CoV-2 due to the higher replication rate as well as mutation frequency of the virus. In this case, rational drug design of protease inhibitors targeting the conserved catalytic residues in the active

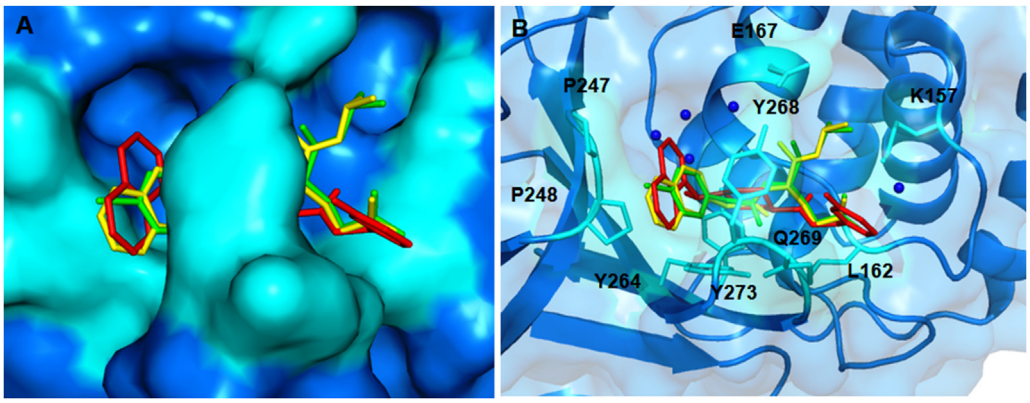

Fig. 25. Binding interaction of a prototype *in-house* naphthyl derivative with SARS-CoV-2 PLpro.

site will be a preferred strategy [109]. Among the two proteases, drug discovery against PLpro target is challenging due to interference of host-cell deubiquitinases [110]. However, Mpro can be an ideal target of reduced off-target effects [111,112] as no human host-cell proteases are known with similar substrate specificity.

Declaration of competing interest

The authors have no conflict of interests.

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Abbreviations used

3CLpro	3C-like protease or main protease
CoV	coronavirus
COVID-19	coronavirus disease 2019
E protein	envelope protein
EBOV	Ebola virus
Mpro	main protease
M protein	membrane protein
MERS-CoV	Middle East respiratory syndrome coronavirus
N protein	nucleocapsid protein
Nsp	non-structural proteins
NTD	N-terminal domain
ORF	open reading frame
PLpro	papain-like protease
QSAR	Quantitative structure-activity relationship
RdRp	RNA-dependent RNA polymerase
S protein	spike protein
SAR	Structure-activity relationship
SARS-CoV	severe acute respiratory syndrome coronavirus
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SPCI	Structural and physico-chemical interpretation
WHO	World Health Organization

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