Protease targeted COVID-19 drug discovery and its challenges: Insight into viral main protease (Mpro) and papain-like protease (PLpro) inhibitors

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) brutally perils physical and mental health worldwide. Unavailability of effective anti-viral drug rendering global threat of COVID-19 caused by SARS-CoV-2. In this scenario, viral protease enzymes are crucial targets for drug discovery. This extensive study meticulously focused on two viral proteases such as main protease (Mpro) and papain-like protease (PLpro), those are essential for viral replication. This review provides a detail overview of the targets (Mpro and PLpro) from a structural and medicinal chemistry point of view, together with recently reported protease inhibitors. An insight into the challenges in the development of effective as well as drug like protease inhibitors is discussed. Peptidomimetic and/or covalent coronavirus protease inhibitors possessed potent and selective active site inhibition but compromised in pharmacokinetic parameters to be a drug/drug like molecule. Lead optimization of non-peptidomimetic and/or low molecular weight compounds may be a better option for oral delivery. A masterly combination of adequate pharmacokinetic properties with coronavirus protease activity as well as selectivity will provide potential drug candidates in future. This study is a part of our endeavors which surely dictates medicinal chemistry efforts to discover effective anti-viral agent for this devastating disease.

1. Introduction

In late December 2019, the newly emerged highly contagious novel coronavirus disease 2019 (COVID-19) was identified in Humans.[1,2] The outburst of virus containing a single positive-stranded RNA first found to occur in Wuhan, China and was named as severe acute respiratory syndrome (SARS)-CoV-2 (SARS-CoV-2).[3–10] Worldwide more than millions of cases have been registered.[11,12] According to World Health Organization (WHO), the highly transmissible disease COVID-19 has so far, more than millions confirmed cases and deaths have been reported from 216 countries around the globe.[12]

Currently, this virus is far more contagious and more catastrophic compared to other flu-viruses with several symptoms like fever, cough, pneumonia, nausea, and fatigue.[13] Hence, the World Health Organization was forced to declare a state of global health emergency to organized scientific and medical efforts to quickly develop a cure for patients.[14] Presently, there is no specific targeted therapy against this novel virus. Thus, the scientific community is making great efforts to explore diverse mechanisms to restrict the virus replication. As a result, diverse antiviral drugs with similar viral infections were tested on patients. Several drugs like: Remdesivir (designed for the Ebola virus),[14] Lopinavir/Ritonavir (designed for the HIV),[15] chloroquine and hydroxychloroquine (designed for anti-malarial action)[14] and Tocilizumab (designed for rheumatoid arthritis)[16] were found to be effective against this deadly virus, but their efficacy still remains controversial.[17]

The current impact of COVID-19 outbreak and the possibility of forthcoming CoV epidemics prove that there is a need for rapid discovery of anti-COVID-19 drugs. Recent studies revealed that SARS-CoV-

2 has a comparable genomic pattern to other corona viruses.[18] These viruses mainly comprises of a 5′-untranslated region (UTR), a replicase complex for encoding non-structural proteins (nsps), spike protein (S) gene, envelope protein (E), membrane protein (M) gene, nucleocapsid protein (N) gene, 3′-UTR, and numerous unknown non-structural parts which provide them support against the environmental factors.[19–22] Usually, these viruses harvest several polypeptides which promote proteolytic breakdown to produce 20 additional proteins during their lifecycle. Among them two crucial proteases such as main protease (Mpro) and papain-like protease (PLpro) are vital for virus replication.[22–24] Meanwhile, a tremendous effort has been spent on studying these proteases in order to discover specific inhibitors against this noxious COVID-19.[25–30]

Among these the two proteases, the coronavirus Main protease (Mpro) also recognized as 3C like protease (3CLpro) acknowledged great attention for its significant role in enzymatic activity leading to its post-translational processing of replicase polyproteins.[26,27] The Mpro consist of 306 amino acid long and has high structural and sequence resemblance to that of SARS-CoV Mpro.[25] SARS-CoV-2 Mpro monomer comprises of three domains (*i.e.*, *N*-terminal domain-I, *N*-terminal domain-II, and C-terminal domain-III).[25] The Mpro active site consists of two catalytic dyad C145 and H41 (Fig. 1A–B).

On the other hand, papain-like protease (PLpro) active site consists of catalytic triad (Fig. 1C–D). PLpro functions by cleaving ISG15, a two-domain Ub-like protein, and Lys48-linked polyUb chains. Hence, their main function lies in the processing of the viral polypeptide into functional proteins, which further deubiquitinize and dampen host anti-viral reactions by hijacking the ubiquitin (Ub) an enzyme playing the pivotal role in host defense mechanism.[31] Therefore, the two proteases are equally important for viral lifecycle and are supported by several studies which reveal that most of the *coronaviridae* genome encrypts two polyproteins, pp1a and pp1ab during their translation stage through ribosomal frame shifting mechanism.[32] These polyproteins were further processed into mature non-structural proteins (nsps) by Mpro and PLpro which plays a vital role in the transcription/replication.[33] Targeting these may hence institute a valid tactic for antiviral drug design and discovery.

In the 21st century, drug repurposing, screening of databases and designing different inhibitors are the only fastest possibility in terms drug discovery to prevent the catastrophe caused by COVID-19 outbreak. Diverse approaches have also been made in order to get

insights into the mechanism of these proteases and to inhibit their functions but still there has been a lot of groundwork to be done for drug discovery and development against these targets. This study, as a part of rational drug design and discovery,[9,10,34–37] aims to sketch out the current status of SARS-CoV-2 protease inhibitors based drug discovery. We also try to provide a new insight into coronavirus protease structural biology and discuss the challenges in the development of effective as well as drug like protease inhibitors. The study will offer an initiative to stimulate further research by providing useful guidance to the medicinal chemists for designing of new protease inhibitors effective against COVID-19 in near future.

2. Structural biology of SARS-CoV-2 proteases

CoV is a single-stranded positive sense RNA virus where genome is encapsulated within a membrane envelope.[38–43] The spike glycoprotein of CoV regulates its entry into the host cells.[43–45] Two polyproteins (pp1a and pp1ab) are translated after virion entry into the host cells, which are promptly split by two viral proteases including Mpro and PLpro.[46] Further proteolytic cleavage of these two viral polyproteins resulted in sixteen non-structural proteins (nsp1 to nsp16). The PLpro manages the proteolytic cleaving of nsp 1–3, whereas all junctions downstream of nsp4 are cleaved by Mpro.

The Mpro cleaves at no fewer than 11 sites on the large polyprotein 1ab with the recognition sequence of L-Q↓ (S, A, G) (↓ refers the cleavage site).[25] The Mpro of SARS-CoV-2 is a 67.6 kDa homodimeric cysteine protease having huge sequence identity with SARS-CoV Mpro (Fig. 1). Mpro of CoV forms a dimer where each monomer consists of *N*-terminal catalytic region and the C-terminal region. Moreover, the *N*-terminal residues form a typical chymotrypsin fold while the C-terminal residues form an extra domain. In addition, each protomer containing three domains such as domains I (residues 8–101), II (residues 102–184) and domain III (residues 201–303).[22] Domains I and II espouses a double β-barrel fold and the active site is located in a shallow cleft between two antiparallel β-barrels (Fig. 1). Notably, C terminal helical-bundle domain, Domain III, might involve in stabilization of their active homodimer forms. The active site can be further divided into several (sub)sites. Notably, the catalytic dyad formed by H41-C145 is observed at the S1 site (Fig. 2). The hydrophobic side chains are found mostly at the S2 and S4 sites. The list of amino acid residues play key role in SARS-CoV-2 Mpro is highlighted in Table 1.
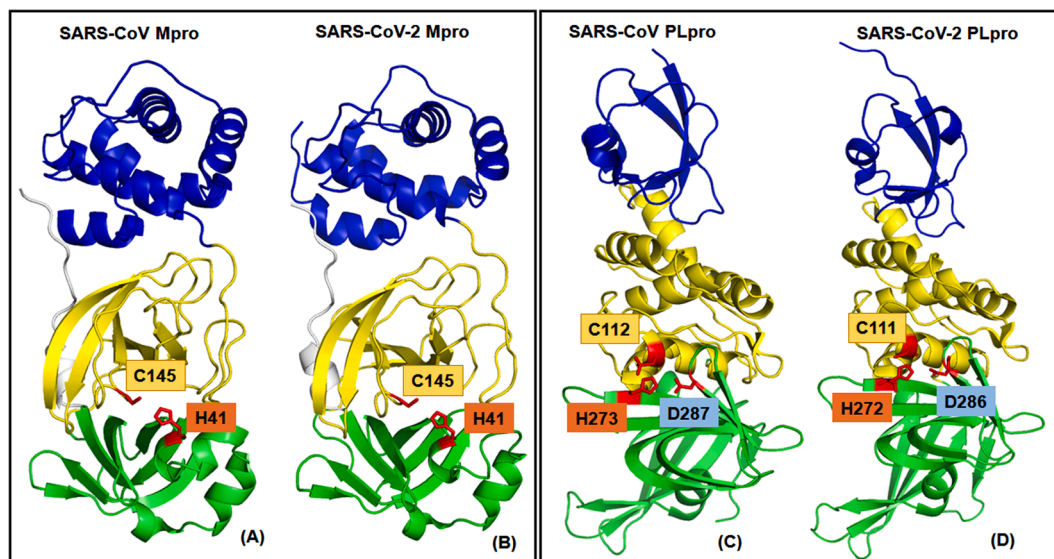

Fig. 1. Three-dimensional structures of (A) SARS-CoV Mpro (PDB: 3ATW), (B) SARS-CoV-2 Mpro (PDB: 6LU7), (C) SARS-CoV PLpro (PDB: 4OW0), (D) SARS-CoV-2 PLpro (PDB: 6WUU). The amino acids formed catalytic dyad for Mpro and catalytic triad for PLpro are also highlighted.
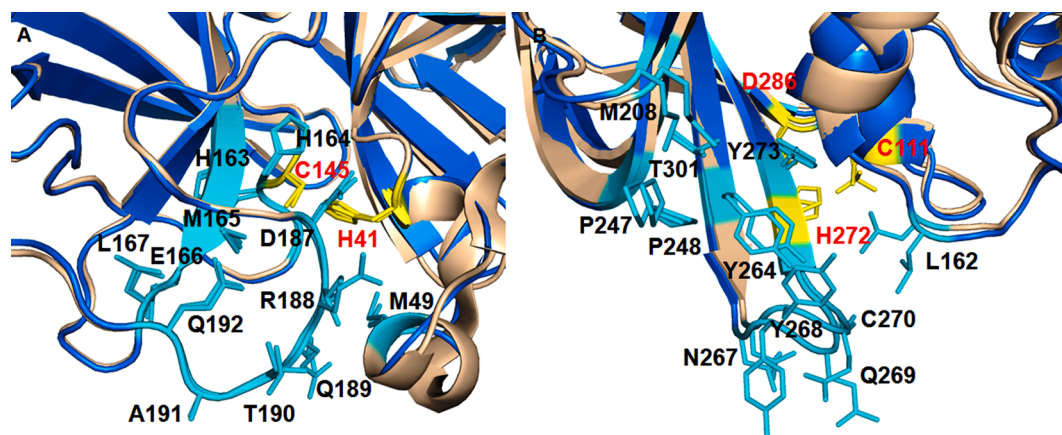
Fig. 2. SARS-CoV-2 (A) Mpro and (B) PLpro binding sites.

Table 1
List of amino acid residues play important role in SARS-CoV-2 Mpro.

Role in SARS-CoV-2 Mpro	Residue
Catalytic dyad	H41, C145
Substrate binding	H41, M49, G143, S144, H163, H164, M165, E166, L167, D187, R188, Q189, T190, A191, Q192
Dimerization	R4, S10, G11, E14, N28, S139, F140, S147, E290, R298

Since the sequences of SARS-CoV-2 and SARS-CoV Mpro share 96% of identity and the minimum differences between both enzymes resides at the surface of the proteins. Therefore, inhibitors against SARS-CoV Mpro are expected to inhibit SARS-CoV-2 Mpro.

The ligand-bound X-ray structure of SARS-CoV-2 PLpro was elucidated few days ago.[7] A close comparisons of apo SARS-CoV-2 PLpro (PDB: 6W9C) to inhibitor-bound SARS-CoV-2 PLpro (PDB: 6WUU and 6WX4) possessed similar overall structures with little exception of the β14- β15 loop proximal to the catalytic site.[7] Catalytic C111 of SARS-CoV-2 PLpro engages in Michael addition to the warhead of inhibitors and rendering the formation of a covalent thio-ether linkage.

The list of amino acid residues play key role in SARS-CoV-2 PLpro is highlighted in Table 2.

Notably, SARS-CoV-2 PLpro and SARS-CoV PLpro only differ by 54 residues.[24] The residues of active site are almost identical. Moreover, the PLpro of SARS-CoV-2 and SARS-CoV demonstrated an almost comparable architecture of S4-S2 site suggesting comparable substrate preferences at positions *P*4-*P*2. Interestingly, SARS-CoV-2 PLpro harbors deISGylating activities similar to SARS-CoV PLpro but its potential to hydrolyze K48-linked Ub chains is declined.[7] In contrast, SARS-CoV-2 PLpro fails to possess potent interferon-antagonizing and deubiquitinase activities.[23]

3. Search of protease inhibitors against COVID-19

3.1. Molecular modeling and in silico virtual screening against SARS-CoV-2

Novel coronavirus pandemic caused by SARS-CoV-2 severely

Table 2
List of amino acid residues play key role in SARS-CoV-2 PLpro.

Role in SARS-CoV-2 PLpro	Residue
Catalytic triad	C111, H272, D286
Substrate binding	Y268, M208, P247, P248, T301, P248, Y264, N267, Q269, L162, C270, G271 and Y273

threatens public health globally. In its infancy, little knowledge about the exact molecular mechanisms of the disease is obstructing the attempts to develop promising anti-viral drugs.[9] Hence, bioinformatics and molecular modeling approaches are the only handy strategy until precise molecular and structural biology is known.

FDA-approved drugs surely claim safe alternatives if it exhibits at least modest activity against SARS-CoV-2. Currently, scientific community are largely focused in the screening of – (i) FDA-approved drug databases, (ii) clinical trials molecules and/or (iii) previously reported coronavirus inhibitors.[9] *In silico* virtual screening (VS) techniques are proficient to explore CoV protease inhibitors.[47–86] Yu and co-workers[40] reported the computational screening and findings with regard to potential binding luteolin and other natural compounds against Mpro. Notably, luteolin has also been found to bind effectively with other targets (PLpro, Spike protein, and RdRp) of SARS-CoV-2.[40,87,88]

Vast amount of *in silico* VS studies against SARS-CoV-2 Mpro has been reported over past months.[26–30,48–86] As the detailed description on the molecular modeling studies is out of Scope for this current communication, readers interested in learning more about recent molecular modeling studies to identify probable CoV protease inhibitors are directed to mentioned references[48–90] and others.

Since the SARS-CoV-2 Mpro shares about 96% sequence similarity with SARS-CoV Mpro, previously reported SARS-CoV Mpro inhibitors may have huge prospect to show their efficacy against SARS-CoV-2 Mpro also. By May this year, we have endorsed our rational anti-viral drug design efforts through data mining and molecular docking studies.[10] 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. Further, QSAR derived virtual screening of some *in-house* molecules were done which rendered some important hits.

3.2. What efforts are taken to identify COVID-19 protease inhibitors?

In February 2020, the first crystal structure of SARS-CoV-2 virus Mpro (PDB: 6LU7) with covalent inhibitor **N3** (Fig. 3) was reported by Jin and co-workers.[3]

The isobutyl function of **N3** embed itself in the hydrophobic S2 site formed by H41, M49, and M169 (Fig. 4A–B). This study forms the basis of rapid target-based discovery of lead molecules against 2019-nCoV Mpro.

After that lot of target-based virtual screening were performed to find out promising protease inhibitors against COVID-19. Jin *et al* screened more than 10,000 molecules underwent SARS-CoV-2 Mpro structure-based virtual and high-throughput screening, which finally identified six small-molecule inhibitors including two FDA-approved drugs (Disulfiram and Carmofur) and clinical trials compounds
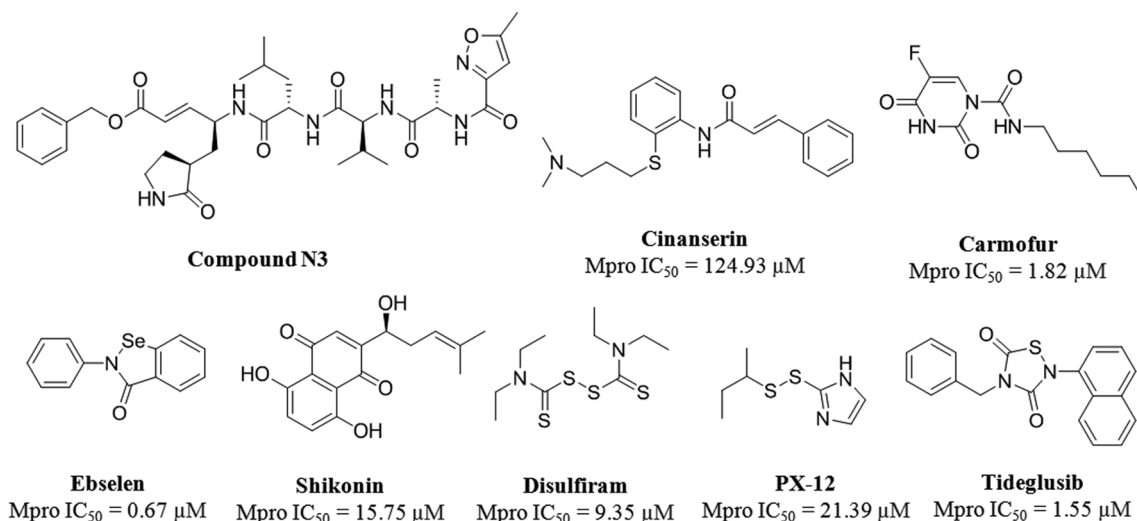
Fig. 3. Structure of SARS-CoV-2 Mpro inhibitors.

Compound N3

Cinanserin
Mpro IC$_{50}$ = 124.93 μM

Carmofur
Mpro IC$_{50}$ = 1.82 μM

Ebselen
Mpro IC$_{50}$ = 0.67 μM

Shikonin
Mpro IC$_{50}$ = 15.75 μM

Disulfiram
Mpro IC$_{50}$ = 9.35 μM

PX-12
Mpro IC$_{50}$ = 21.39 μM

Tideglusib
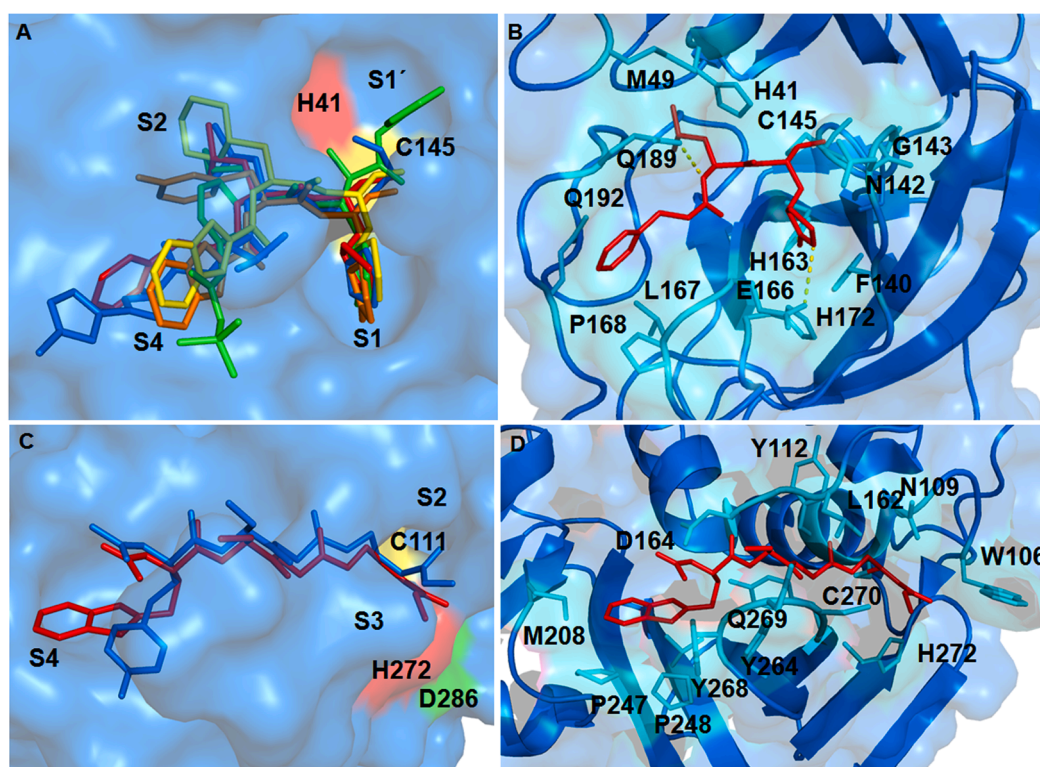Mpro IC$_{50}$ = 1.55 μM

Fig. 4. (A) Binding modes of SARS-CoV-2 Mpro inhibitors (N3, GC-376, 13b, 11a and 11b) at the active site, (B) Interaction of GC-376 with the amino acid residues, (C) Binding modes of SARS-CoV-2 PLpro inhibitors (**VIR250** and **VIR251**) at the active site, (D) Interaction of **VIR250** with the amino acid residues at active site of SARS-CoV-2 PLpro.

(Ebselen, Tideglusib, Shikonin and PX-12) with a IC$_{50}$ range of 0.67–21.4 μM (Fig. 3). Notably, Ebselen (SARS-CoV-2 Mpro IC$_{50}$ = 0.67 μM) showed promising anti-viral activity in cell-based assays (EC$_{50}$ = 4.67 μM).[3]

Su and co-workers elucidated the SARS-CoV-2 Mpro crystal structure in complex with a non-covalent, non-peptidomimetic inhibitor baicalein (Fig. 5) at a resolution of 2.2 Å (PDB: 6M2N).[8] Interestingly, baicalein is snugly fitted in the core of the substrate-binding site by interacting catalytic oxyanion loop (residues 138–145), E166, those are critical not only for recognition of substrates but also peptidomimetic inhibitors. Nevertheless, baicalein revealed a unique binding mode in comparison

with other covalent or peptidomimetic inhibitors.

A comparison analysis of the inhibitor-bound CoV Mpro crystal structures suggested that a peptidomimetic inhibitor portrayed like a 'sword', while baicalein set a 'shield' near the two catalytic dyads to restrict the binding of the substrate. In addition, baicalein was also found promising in an enzymatic assay against SARS-CoV-2 Mpro (IC$_{50}$ = 0.94 μM). It also exhibited a dose-dependent inhibition on the replication of SARS-CoV-2 with a half-maximal effective concentrations (EC$_{50}$ = 1.69 μM).[8] The unique binding mode and promising ligand-binding efficiency of baicalein will inspire Researchers for further lead optimization.
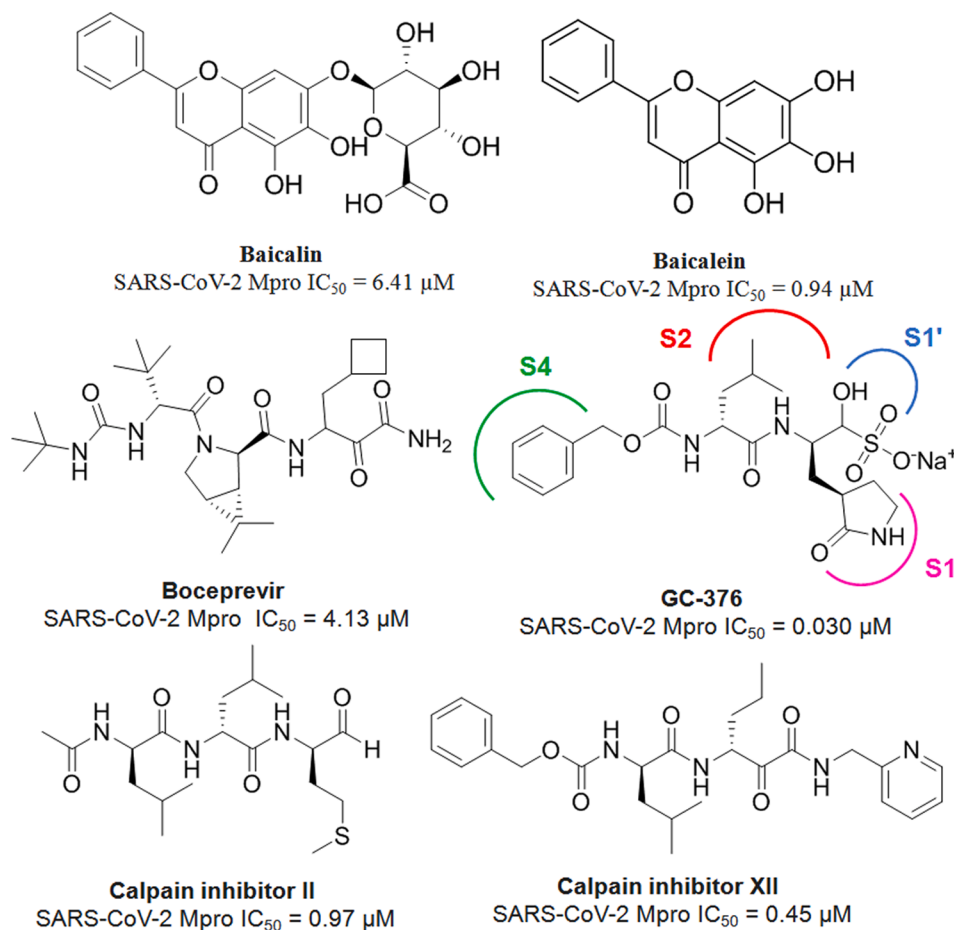
Fig. 5. Structure of SARS-CoV-2 Mpro inhibitors namely Baicalin, Baicalein, Boceprevir, GC-376, calpain inhibitors II and XII.

Ma *et al* employed a fluorescence resonance energy transfer (FRET)-based SARS-CoV-2 Mpro enzymatic assay to explore a library of known protease inhibitors.[17] Among these, four inhibitors such as boceprevir (HCV NS3/4A protease inhibitor, SARS-CoV-2 Mpro IC$_{50}$ = 4.13 μM), GC-376 (calpain protease inhibitor, SARS-CoV-2 Mpro IC$_{50}$ = 0.030 μM), calpain inhibitors II (SARS-CoV-2 Mpro IC$_{50}$ = 0.97 μM) and XII (SARS-CoV-2 Mpro IC$_{50}$ = 0.45 μM) exhibited promising SARS-CoV-2 Mpro inhibitory activities (Fig. 5).

Structure-based design, synthesis and activity assessment by Zhang *et al* facilitated the development of peptidomimetic α-ketoamides as broad-spectrum inhibitors of beta coronavirus and alpha coronavirus Mpro.[4] The most potent compound **11r** of this series displayed EC$_{50}$ of 400 pM against MERS-CoV in virus-infected Huh7 cells (Fig. 6). Notably, **11r** exhibited broad-spectrum anti-viral activity due to its *P*2 cyclohexyl moiety which intended to fit the pocket in the enterovirus Mpro.

In another study, the same group modified the chemical structure of **11r** by replacing the hydrophobic cinnamoyl moiety by comparatively less hydrophobic Boc group and concealing the *P*3-*P*2 amide bond within a six member pyridone ring.[25] This led to the development of **13a** (Fig. 6) with improved solubility in plasma and also reduced binding to plasma proteins, however, the SARS-CoV-2 Mpro inhibition was compromised (**13a**: SARS-CoV-2 Mpro IC$_{50}$ = 2.39 μM *vs* **11r**: SARS-CoV-2 Mpro IC$_{50}$ = 0.18 μM). Further replacement *P*2 cyclohexyl moiety of **13a** with cyclopropyl resulted an increase in the anti-viral property against SARS-CoV-2 Mpro (**13b**: IC$_{50}$ = 0.67 μM, Fig. 6). This molecule **13b** also showed potency against Mpro of SARS-CoV (IC$_{50}$ = 0.90 μM) and MERS-CoV (IC$_{50}$ = 0.58 μM). Furthermore, the X-ray crystal structure of **13b**-bound SARS-CoV-2 Mpro conferred that the carbonyl oxygen of pyridone in the *P*3-*P*2 position formed hydrogen bond with the main-chain amide of E166. Despite the protecting Boc

group on *P*3 unable to occupy the canonical S4 site of Mpro the protease, it was found at close enough to P168 and subsequently, directed outward by more than 2 Å relative to apo-Mpro structure of SARS-CoV-2. Further removal of Boc group in compound **14b** (Fig. 6) led to fall in the inhibitory action, suggesting that hydrophobicity and bulkiness of Boc group would be important to cross the cellular membrane.[25]

Dai *et al* elucidated two crystal structures of SARS-CoV-2 Mpro in complex with two indole based covalent inhibitors (Fig. 6) at a high resolution.[21] The indole ring of **11a** at *P*3 occupied the solvent exposed S4 site to form a 2.6-Å hydrogen bond with E166 along with hydrophobic interactions with side chains of residues P168 and Q189. Since the S2 site of CoV Mpro withholds bulky *P*2 fragment, the cyclohexyl moiety of **11a** is buried snugly into the S2 pocket of SARS-CoV-2 Mpro and stacking with the imidazole ring of H41. It also interacts with the side chains of M49, Y54, M165, D187 and R188. In contrast, fluophenyl function of **11b** at *P*2 undergoes a significant downward rotation and form additional hydrogen bond Q189 that is likely to enhance Mpro inhibitory activity. Notably, the aldehyde functions of both **11a** and **11b** act as a warhead in *P*1 to form a covalent bond with cysteine residue. Moreover, the (*S*)-γ-lactam ring immerses into the S1 site of CoV Mpro to form several hydrogen bonds with H163, F140 and E166. Both of these inhibitors displayed excellent Mpro inhibitory activities (**11a**: SARS-CoV-2 Mpro IC$_{50}$ = 0.053 μM; **11b**: SARS-CoV-2 Mpro IC$_{50}$ = 0.040 μM) along with good PK properties *in vivo*.[21] Hence, from the above studies[3,17,21,25] it may be observed that S2 site in SARS-CoV-2 Mpro can board a broad range of hydrophobic substitutions. The isobutyl, cyclopropyl, cyclohexyl and 3-FPh moieties of inhibitors embed themselves in the hydrophobic S2 site formed by H41, M49, and M169.[17]

Despite huge research efforts on SARS-CoV-2 Mpro inhibitors, proteomic and structural biology works on SARS-CoV-2 PLpro and its
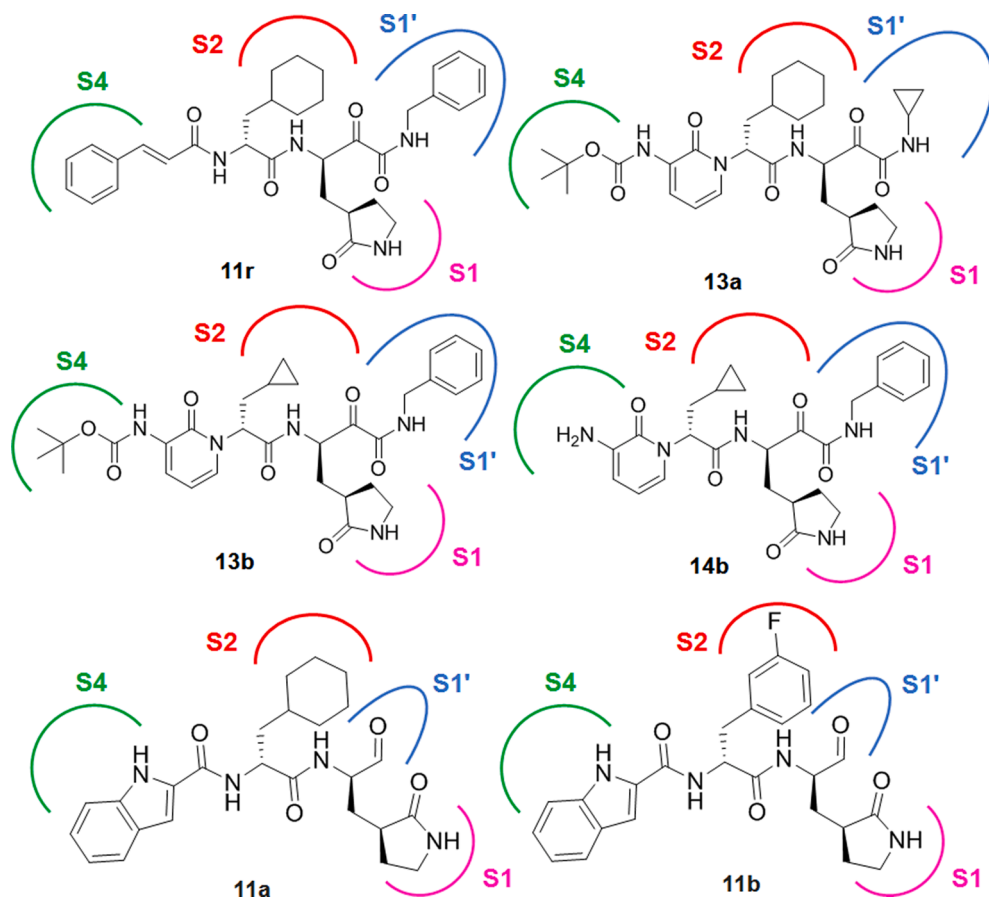
Fig. 6. Structure of SARS-CoV-2 Mpro inhibitors (**11r**, **13a**, **13b**, **14b**, **11a** and **11b**).
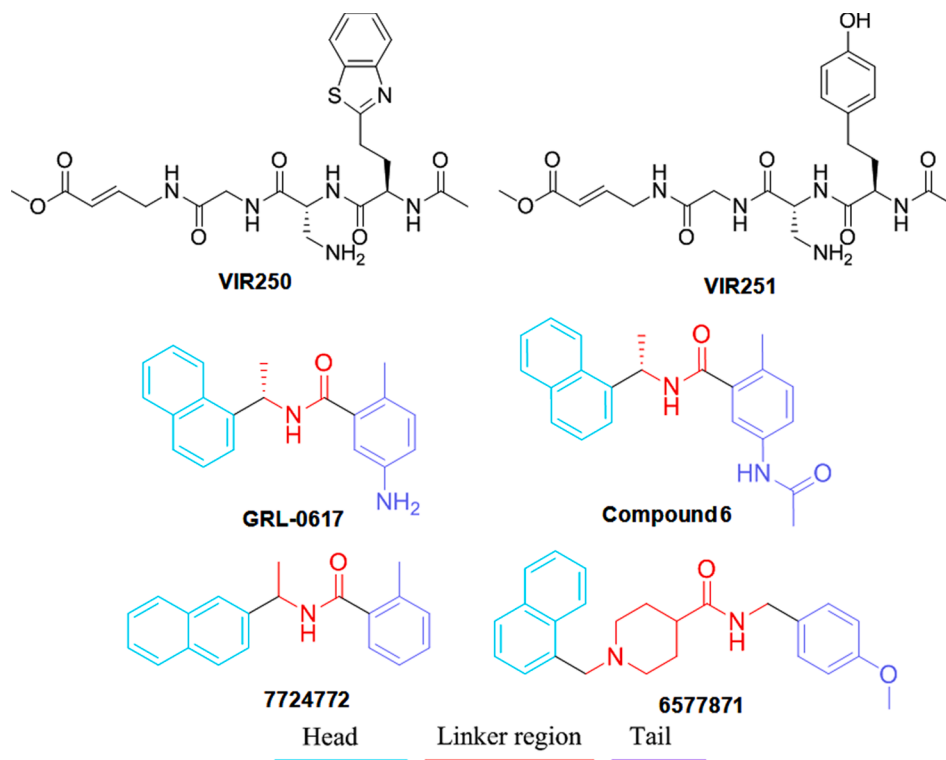

Fig. 7. Structure of SARS-CoV-2 PLpro inhibitors (VIR250 and VIR251) and naphthyl based SARS-CoV-2 PLpro inhibitors.

inhibitor have been very few. Nevertheless, Rut and co-workers utilised HyCoSuL (Hybrid Combinatorial Substrate Library) to scrutinize substrate specificity of SARS-CoV-2 PLpro enzymes.[7] Two irreversible inhibitors namely **VIR250** and **VIR251** having high degree of PLpro selectivity over other proteases were identified (Fig. 7). The same study first time reported the inhibitor-bound crystal structures of SARS-CoV-2 PLpro. Altogether, these crystal structures in complex with **VIR250** (PDB: 6WUU) and **VIR251** (PDB: 6WX4) provide a basic for rapid rational drug design against SARS-CoV-2 PLpro (Fig. 4C–D).

We have reported the SARs and QSAR of naphthalene based SARS-CoV PLpro inhibitors.[10] Very recently, Freitas and co-workers reported these naphthalene based derivatives against SARS-CoV-2 PLpro and SARS-CoV-2 replication.[24] Since the residues in the BL2 loop of PLpro are almost identical between SARS-CoV-2 and SARS-CoV-1, this probably facilitated the binding of naphthalene based derivatives. **GRL-0617** (IC_{50} of 2.4 μM and 600 nM against SARS-CoV-2 PLpro and SARS-CoV-1 PLpro, respectively), compound **6** (IC_{50} of 5 μM and 2.6 μM against SARS-CoV-2 PLpro and SARS-CoV-1 PLpro, respectively) exhibited excellent potency against SARS-CoV-2 replication (Fig 7). In addition, the lead compounds, **77247723** and **6577871** exhibited IC_{50} values of 23.5 μM and 100.7 μM, respectively against SARS-CoV-2 PLpro. Previously they (**77247723** and **6577871**) displayed 20 μM and 59 μM SARS-CoV PLpro IC_{50} values, respectively.

Therefore, it may be postulated that previously designed naphthalene based SARS-CoV PLpro inhibitors follows the similar trend of structure–activity relationship against SARS-CoV-2 PLpro also.[24]

3.3. Challenges in drug discovery efforts for SARS-CoV-2 protease inhibitors

The corona virus protease inhibitors discovery effort targeting Mpro and PLpro have presenting a substantial challenge owing to poor pharmacokinetic properties of peptidomimetic/macromolecular compounds and low inhibitory potency of non-peptidomimetic and/or compounds having low molecular weight.[91,92] To be an effective drug/drug candidate, a molecule must have ability not only to reach its desire target in the body in sufficient concentration but also to possess expected biological responses. Drug discovery and development markedly depends on assessment of absorption, distribution, metabolism and excretion (ADME) characteristics. Notably, the macromolecule approach of developing SARS-CoV-2 Mpro as well as PLpro inhibitors has been advantageous over the low molecular weight compounds in terms of inhibitory potency and selectivity. In fact the former can occupy the different parts of the active sites of the Mpro and PLpro enzyme to manifest potential inhibitory property. We have collected recently published different SARS-CoV-2 Mpro inhibitors and analyzed for their drug likeness and other physicochemical properties. From the analysis we have seen that most of these compounds fail to pass the drug-likeness
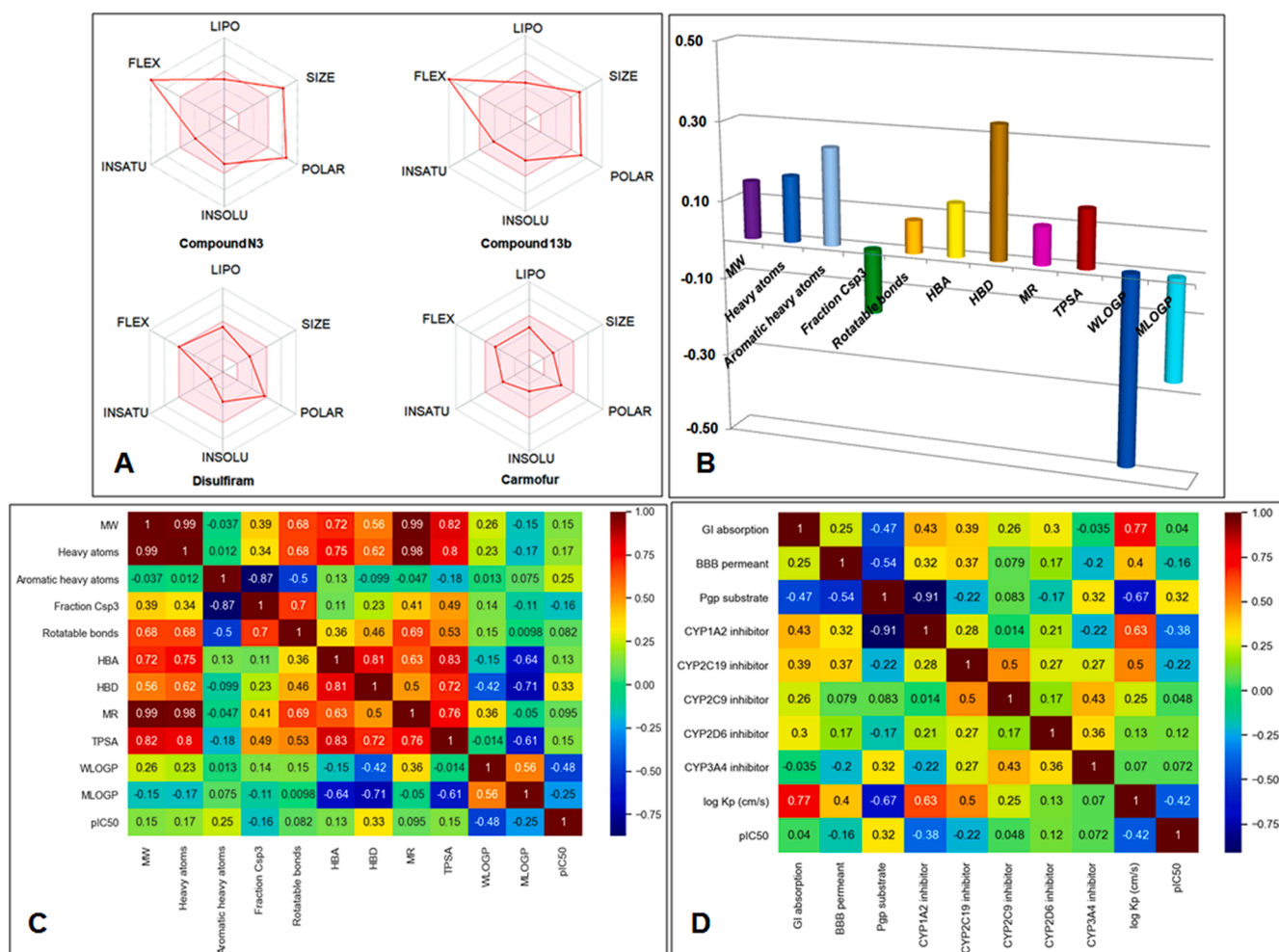
Fig. 8. (A) Radar plot of four prototype compounds N3, 13b, Disulfiram, Carmofur after calculating ADME data (http://www.swissadme.ch/) suggesting the drug-likeness. Pink area represents the optimal range of each property. LIPO = Lipophilicity, SIZE = Molecular weight, POLAR = Polarity, INSOLU = Solubility, INSATU = Saturation, FLEX = Flexibility; (B) Correlation of SARS-CoV-2 Mpro inhibitory activity with eleven molecular descriptors ($N = 25$); (C) The correlation matrix of the physic-chemical and structural properties along with SARS-CoV-2 Mpro pIC_{50} ($N = 25$) at significant p-statics; (D) The correlation matrix of the ADME properties along with SARS-CoV-2 Mpro pIC_{50} ($N = 25$) at significant p-statics.

criteria (Fig. 8A–D).

Molecules including Baicalein, Disulfiram, Carmofur (Fig. 8A), Ebselen, Tideglusib, Shikonin and PX-12 coherently passed the drug likeness to be suitable drug but adding challenge to achieve potency and selectivity against coronavirus protease. A similar trend is seen with the SARS-CoV-2 PLpro inhibitors also. Hence, this should be addressed in Mpro and PLpro protease-based drug discovery which could help harness the therapeutic potential against COVID-19.

Meanwhile, a pool of 2D and fingerprint descriptors for these twenty five SARS-CoV-2 Mpro inhibitors (those having exact biological endpoint from Table 3) was calculated to frisk the linear relationships. However, the similar analysis has not been possible for the SARS-CoV-2 PLpro inhibitors due to the insufficient number of reported compounds. The correlation of SARS-CoV-2 Mpro inhibitory activity with eleven molecular descriptors ($N = 25$) at significant p-statics is graphically represented in Fig. 8B.

A positive coefficient of the eight descriptors referred to the favourable effect on the SARS-CoV-2 Mpro inhibitory potential of the studied compounds, while the negative coefficient of three descriptors, *i. e.*, *Fraction Csp3*, *WLOGP*, *MLOGP*, exhibited detrimental effect on the inhibitory properties. The correlation matrix of the physico-chemical and structural properties along with SARS-CoV-2 Mpro pIC_{50} ($N = 25$) at significant p-statics is depicted in Fig. 8C.

Commonly, among these 25 derivatives, several molecules carrying hydrogen bond donor (*HBD*) > 3 and total polar surface area (*TPSA*) > 150 were poorly active. Besides, molecules with *TPSA* < 90 and *HBD* < 3 frequently possessed lower Mpro inhibitory potency. Regarding the influence of lipophilicity in Mpro activity, highly hydrophobic (*WLOGP* > 2.9 and *MLOGP* > 1.6) derivatives were ill-active against Mpro. Interestingly, in this dataset, the least active compound namely cinanserin possessed a lower molecular weight (*MW* = 340.48), lesser hydrogen bond donor (*HBD*) and acceptor (*HBA*) groups (*HBD* = 1 and *HBA* = 2), lower total polar surface area (*TPSA* = 57.64) as well as high hydrophobic nature (*WLOGP* = 4.08 and *MLOGP* = 3.76) compared to the most potent Mpro inhibitor (GC-376: *MW* = 507.53, *HBD* = 4, *HBA* = 8, *TPSA* = 182.34, *WLOGP* = 0.75 and *MLOGP* = −0.08).

Moreover, from the properties such as, blood–brain-barrier permeability (*BBB permiant*), inhibition capability of CYP1A2 (*CYP1A2 inhibitor*), CYP2C19 (*CYP2C19 inhibitor*) as well as permeability coefficient (log *Kp* (cm/s)) of these molecules can be negatively correlated with their Mpro inhibitory potency (Fig. 8D). Noticeably, most of this dataset molecule with a log *Kp* (cm/s) value $\geq$ −6.0 exhibited lesser to-poorly active nature against Mpro. Hence, this should be addressed in Mpro and PLpro protease-based drug discovery which could help harness the therapeutic potential against COVID-19.

4. Conclusion and future perspective

COVID-19 disease is few months old. Until precise molecular and structural biology underlying SARS-CoV-2 replication are available, bioinformatics and multi-target molecular modeling driven *in vitro* antiviral study as well as repurposing of previous SARS-CoV protease inhibitors are the handy strategies.

From the SARs, it may be postulated that peptidomimetic and/or covalent coronavirus protease inhibitors possessed potent and selective active-site inhibition. However, these inhibitors exhibited poor absorption, distribution, metabolism, and excretion as well as toxicology parameters to be a drug/drug like molecule.[92] Consequently, repurposing/new protease inhibitors discovery efforts of peptidomimetic compounds have presenting a substantial challenge owing to poor pharmacokinetic properties. On the other hand, non-peptidomimetic and/or compounds having low molecular weight coherently passed the drug likeness to be suitable drug but adding challenge to achieve potency and selectivity against coronavirus protease. Hence, lead optimization of non-peptidomimetic and/or low molecular weight compounds should be focused. In this scenario, fragment based drug design (FBDD)

Table 3

Reported SARS-CoV-2 Mpro inhibitors and biological properties.

Entry	Name	SARS-CoV-2 Mpro IC$_{50}$ (μM)	pIC_{50}
1	N3	–	–
2	Ebselen	0.67	6.174
3	Disulfiram	9.35	5.029
4	Tideglusib	1.55	5.810
5	Carmofur	1.82	5.740
6	Shikonin	15.75	4.803
7	PX-12	21.39	4.670
8	Cinanserin	124.93	3.903
9	11a	0.053	7.276
10	11b	0.04	7.398
11	11r	0.18	6.745
12	13a	2.39	5.622
13	13b	0.67	6.174
14	14b	–	–
15	Simeprevir	13.74	4.862
16	Boceprevir	4.13	5.384
17	Narlaprevir	5.73	5.242
18	MG-132	3.9	5.409
19	Calpeptin	10.69	4.971
20	Calpain inhibitor III	>20	–
21	Calpain inhibitor VI	>20	–
22	Calpain inhibitor I (ALLN)	8.6	5.066
23	MG-115	3.14	5.503
24	Calpain inhibitor II (ALLM)	0.97	6.013
25	Calpain inhibitor XII	0.45	6.347
26	PSI	10.38	4.984
27	GC-376	0.03	7.523
28	Rupintrivir	>20	–
29	Camostat	>20	–
30	Lopinavir	>20	–
31	Ritonavir	>20	–
32	Baicalin	6.41	5.193
33	Baicalein	0.94	6.027

approaches can play a significant role in designing and developing potential protease inhibitors. The effective strategy for drug discovery of potential protease inhibitors may consist of following steps: step 1: identification of low molecular weight compounds as protease inhibitors; step 2: identification of good fragments from peptidomimetic compounds by different experimental and computational methods; step 3: incorporating of good fragments during the lead optimization of the low molecular weight compounds; step 4: final optimization of these hybrid molecules for satisfactory pharmacokinetic and pharmacodynamics properties. A masterly combination of adequate pharmacokinetic properties with coronavirus protease activity as well as selectivity will provide strong drug candidates in future.

Based on recent mechanistic and structural data on other viral proteases including HIV, we can anticipate or rather suggest to target the allosteric sites of coronavirus proteases as strategies-based drug discovery tool.[92] This effort may soon emerge as frontiers in SARS-CoV-2 Mpro and PLpro drug discovery to triumph the battle against COVID-19.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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