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Synthetic and computational efforts towards the development of peptidomimetics and small-molecule SARS-CoV 3CLpro inhibitors

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A B S T R A C T

Severe Acute Respiratory Syndrome (SARS) is a severe febrile respiratory disease caused by the beta genus of human coronavirus, known as SARS-CoV. Last year, 2019-nCoV (COVID-19) was a global threat for everyone caused by the outbreak of SARS-CoV-2. 3CLpro, chymotrypsin-like protease, is a major cysteine protease that substantially contributes throughout the viral life cycle of SARS-CoV and SARS-CoV-2. It is a prospective target for the development of SARS-CoV inhibitors by applying a repurposing strategy. This review focuses on a detailed overview of the chemical synthesis and computational chemistry perspectives of peptidomimetic inhibitors (PIs) and small-molecule inhibitors (SMIs) targeting viral proteinase discovered from 2004 to 2020. The PIs and SMIs are one of the primary therapeutic inventions for SARS-CoV. The journey of different analogues towards the evolution of SARS-CoV 3CLpro inhibitors and complete synthetic preparation of nineteen derivatives of PIs and ten derivatives of SMIs and their computational chemistry perspectives were reviewed. From each class of derivatives, we have identified and highlighted the most compelling PIs and SMIs for SARS-CoV 3CLpro. The protein–ligand interaction of 29 inhibitors were also studied that involved with the 3CLpro inhibition, and the frequent amino acid residues of the protease were also analyzed that are responsible for the interactions with the inhibitors. This work will provide an initiative to encourage further research for the development of effective and drug-like 3CLpro inhibitors against coronaviruses in the near future.

1. Introduction

In November 2002, Severe Acute Respiratory Syndrome (SARS) was first identified in Guangdong Province of Southern China and immediately within a few months was considered to be a global threat because of its highly contagious and deadly nature. From November 2002 to 2003, it spread rapidly over 29 countries. Total 8439 cases were reported globally till 3rd July 2003, and among which 812 patients died. The etiological agent solely responsible for this SARS was found to be a novel human coronavirus called SARS-CoV. Some changes in the phylogenetic characters and gene sequence of SARS-CoV distinguish it from other previously discovered SARS-CoV-1,2 SARS is characterized by fever, and after a few days, it is followed by shortness of breath and dry nonproductive cough, headache, dyspnea, lower respiratory tract infection, lymphopenia, and diarrhoea.3

Coronaviruses are enveloped, positive-stranded RNA virus and belong to order Nidovirales, family Coronaviridae, sub-family Coronavirinae genus Coronavirus. Genus is of four types alpha, beta, gamma, and delta Coronavirus. The beta coronavirus with “b” lineage is mainly responsible for SARS-CoV disease4,5. The schematic representation of the taxonomy is described in Figure 1. Coronaviruses are zoonotic viruses, primarily originated from non-humans (animal reservoirs, especially bats and avian species) and then transmitted to humans. The seven coronaviruses known to infect humans include HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV, MERS-CoV, and recently SARS-CoV-2. Amongst these seven SARS-CoV, Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and SARS-CoV-2 are the most infectious and fatal. MERS-CoV was first reported in June 2012 in Saudi Arabia and caused severe respiratory problems. By the end of August 2015, 1511 cases were reported with 574 deaths (fatality

Abbreviations: SARS-CoV, Severe Acute Respiratory Syndrome-coronavirus; 3CLpro, Chymotrypsin-like protease; PIs, Peptidomimetic inhibitors; SMIs, Small-molecule inhibitors; K, Inhibition constant; IC50, half maximal inhibitory concentration; FRET, Fluorescence Resonance Energy Transfer; SAR, Structure-Activity Relationship; uM/μM, Micromolar; SKEA, Sharpless-Katsuki asymmetric epoxidation; SAD, Sharpless asymmetric dihydroxylation.

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The disease caused by SARS-CoV-2, also known as coronavirus disease 19 (COVID-19), as the first case was reported in Wuhan, China, in December 2019. COVID-19 has been declared a worldwide pandemic by World Health Organization (WHO) on March 11, 2020. Till the mid of August 2020, 21.2 million cases were reported, including 76,100 deaths. Even though the death rate is lower than SARS-CoV, the infection rate is quite high, so SARS-CoV-2 is considered a severe medical emergency. In recent times, several pharmaceutical companies successfully developed vaccines to treat COVID-19, but no specific anti-viral drugs have been discovered. Chymotrypsin-like cysteine protease (3CLpro) is the major protease involved in viral replication of SARS-CoV, and it has also been found that the sequence similarity between SARS-CoV and SARS-CoV-2 is 96%—8. By targeting this protease enzyme, many researchers have designed and developed potential inhibitors against coronaviruses. The potential SARS-CoV 3CLpro inhibitors have a particular parent moiety that can be modified structurally to develop a new effective drug. The most advanced pre-clinical therapeutic drugs for SARS-CoV-2 3CLpro are PF-00835231, PF-07304814, GC-376, and PF-07321332 (Figure 2). Based on the identical sequence similarity between SARS-CoV and SARS-CoV-2, the inventors of PF-00835231 have recently developed a new covalent inhibitor for treating COVID-19. Another potent and selective SMI against SARS-CoV-2 3CLpro is ALG-097111 which was evaluated in the COVID-19 infected Syrian Hamster Model for antiviral activity.

To combat the current outbreak of COVID-19, the scientific community is trying to develop effective treatment within a feasible time. Many well-established strategies are taken by the research organizations to eradicate the COVID-19 pandemic. In the field of drug discovery and development, repurposing of existing drugs is one of the organized and economic strategies to fight against novel diseases, like novel coronavirus. The bioinformatics and pharmacoinformatic approaches have played a major role in repurposing the existing drugs. By this strategy, many scientific workers have generated and identified numerous newer inhibitors against target diseases. In the battle of COVID-19, the anti-SARS-CoV compounds are mostly explored and repurposed for screening the best-fit inhibitors. With the help of computational chemistry, recently, Bhowmik and their collaborators identified Nafamostat and VR23 as promising candidates for treating COVID-19 by targeting SARS-CoV-2 3CLpro (Figure 2). The pharmacodynamic study revealed that the Nafamostat and VR23 are promising for drug-likeness but further pre-clinical/clinical investigation studies are needed to be procured for understanding the efficacy and potency of the compounds.

Apart from the pharmacoinformatic approaches, chemical synthesis is an integral part of drug discovery and development; to synthesize the novel molecules for the target diseases. Since now, many organic chemists have explored and discovered the different types of novel inhibitors targeting the SARS-CoV 3CLpro, such as peptidomimetic inhibitors (PIs), small-molecule inhibitors (SMIs), and metal conjugate inhibitors. Researchers can’t evaluate the in vitro or in vivo efficacy and potency of inhibitors without the chemical synthesis of designed inhibitors. So, wet lab chemical synthesis has always a great interest for researchers in the pharmaceutical industry and scientific field to develop novel chemical entities as a drug.

Considering the global pandemic of COVID-19, this review focuses on the chemical synthesis and computational chemistry perspectives of SARS-CoV 3CLpro inhibitors, specially PIs and SMIs, which has been discovered by applying chemical synthesis, in vitro biological assay, and computational chemistry from 2004 to 2020.

The journey of different analogues towards development of SARS-CoV 3CLpro inhibitors is provided in Figure 3. Here, we have classified the SARS-CoV 3CLpro inhibitors into two categories: 1) Peptidomimetic inhibitors (PIs), and 2) Small- molecule inhibitors (SMIs). The PIs consist of different analogues, including: Keto-Glutamine analogues, Anilide analogues, Peptidomimetic α, β-unsaturated esters, Glutamic acid and glutamine peptides, Peptidomimetic (TG-0205221), Phthalhydrazide peptide analogues, Cinanserin analogues, Trifluoromethyl ketone containing peptides, Tri fluoromethyl, benzoazainyl or thiazoyl ketone containing peptidomimetics, Michael type of peptidomimetics, Cysteine protease inhibitors, Potent dipeptide type of peptidomimetics, Novel dipeptide type of peptidomimetics, Nitrile based peptidomimetics, Tripeptide type of peptidomimetics, Peptidomimetics containing cinnaminoyl warhead, Peptidomimetics containing decahydroisoquinolin moiety, Ketone-based covalent inhibitors, and α-Ketoamide derivatives. The SMIs are classified into: isatin derivatives, Chloropyridyl ester derivatives, pyrazoline derivatives, pyrimidine derivatives, macrocyclic inhibitors, 5-sulphonyl isatin derivatives, pyrazoline derivatives, serine derivatives, phenylsulfonyl derivatives, and Octahydrocromene derivatives.

We have also identified the most promising anti-SARS compounds from each class of derivatives and highlighted their structural features and binding information. This work, chemical synthesis, and computational chemistry aspect of SARS-CoV 3CLpro inhibitors will provide an initiative to encourage further research to the organic chemist and medicinal chemist for the design and synthesis of new 3CLpro inhibitors effective against coronaviruses in coming years.

2. SARS-CoV 3CLpro and its structural features:

Coronaviruses are positive polarity family of polyadenylated single-stranded RNA. In several animal species, even human beings, this virus can cause acute and chronic pulmonary and nervous system diseases. Coronavirus virions feature the largest viral genomes ranging from 27 to 31 kb long found to date. The genome is helically encapsidated by nucleocapsid (N) proteins. Virus cores are formed by the matrix proteins that surround the filamentous nucleocapsid. The virus membrane includes various spike-like protruberances called spike (S) proteins, a type of glycoprotein I along with membrane protein (M) that covers the
membrane, and a hydrophobic protein called envelope protein (E) that surrounds the entire virus structure. These proteins make a crown-like appearance, hence the justification of the name Corona, which is derived from a Latin word, while seen in an electron microscope. S proteins act as a protein that binds with the receptor of the host and is a fusion of the viral and cellular membranes. The schematic figure is given in Figure 4.

The SARS-CoV genome consists of two open reading frames bound by a ribosomal frameshift that encodes two overlapping polyproteins, pp1a (approximately 450 kDa) and pp1ab (approximately 750 kDa), that helps to moderate the mechanism of viral replication and transcription. Complex proteolytic processing is involved in the maturation of coronavirus that controls viral gene expression and replication. Some viruses from the same family make use of three proteases in the proteolytic process. In comparison, it is known to encode only two proteases in the case of SARS-CoV. Other than 3CLpro, it also has papain-like cysteine protease (PLpro). 3CLpro, also known as the Main Protease (Mpro), plays a crucial role in the mechanism of viral replication and infection, allowing this macromolecule a prime candidate for antiviral therapy (PDB ID: 1UJ1). Yang et al solved the X-ray crystallographic structure of hexapeptidylchloromethyl ketone (CMK) inhibitor, which is bound to Mpro at various pH values. It was stated that with the two promoters referred to as “A” and “B” are focused almost perpendicular to each other, SARS-CoV 3CLpro acts as a dimer. As shown in the schematic diagram (Figure 5), the SARS-CoV Mpro crystal structure, analogous to other 3CLpro crystals, contains three domains. Domain I has 8–101 residues, and Domain II has 102–184 residues. Domain I and II contain β-sheets

Figure 3. Journey of different analogues in the discovery of SARS-CoV 3CLpro inhibitors.

Figure 4. Structure of SARS-CoV and its proteins (S, M, E, and N).
that form the framework of chymotrypsin, while domain III, having 201–306 residues, consists mainly of alpha-helices. The Cysteine-Histidine (Cys-His) catalytic dyad is present in SARS-CoV Mpro, and between domain I and domain II in a cleft, the binding site for the inhibitor is present. The inhibitor showing in Figure 6, having binding subsite S1 specificity of a coronavirus protease in protomer A bestows complete specificity for the enzyme residue of the P1-Gln substrate. Squeezed between domain II and domain III of the parent monomer and domain II of the other monomer, each N-terminus residue (N-finger) plays an essential part in the dimerization process and development of the Mpro active site. The Mpro SARS-CoV dimer is intensely active, while the monomer is somewhat inactive. The study revealed that the pH-dependent triggering switch is a unique feature of SARS-CoV 3CLpro, resulting in significant, cooperative activities of the Glu-166, Phe-140, Leu-141, and Tyr-118 side chains and the dimer N terminus.

3. Chemical synthesis of SARS-CoV 3CLpro inhibitors:

3.1. Peptidomimetic inhibitors (PIs):

There are various instances of PPI-targeting peptides and peptidomimetics that’s been in clinical trials. For example, the first potent and specific p53 re-activator, an important tumor suppressor protein that binds and acts as an inhibitor of two p53 suppressor proteins, namely MDM2 and MDMX, is Aileron (ALRN-6924). p53 is one of the most favoured targets of antineoplastic drugs activated by its vital role in stopping multiple cancers from initiating and advancing. KAI Pharmaceuticals developed a small peptide named KAI-9803, which directly acts as dPKC inhibitor with its intracellular receptor, RACK. During reperfusion, dPKC activation initiates molecular pathways for cell death, which gradually leads to inflammation and injury during a stroke in the heart or brain. In phase II clinical trial to determine safety and effectiveness, KAI-9803 was evaluated for its efficacy in acute myocardial infarction patients.

The chemical synthesis of PIs under Keto-Glutamine analogues, Anilide analogues, Peptidomimetic α,β-unsaturated esters, Glutamic acid and glutamine peptides, Peptidomimetic (TG-0205221), Phthalhydradize peptide analogues, Cinanserin analogues, Trifluoromethyl ketone containing peptides, Trifluoromethyl, benzo-thiazolyl or thiazolyl ketone containing peptidomimetics, Michael type of peptidomimetics, Cysteine protease inhibitors, Potent dipeptide type of peptidomimetics, Novel dipeptide type of peptidomimetics, Nitrile based peptidomimetics, Tripeptide type of peptidomimetics, Peptidomimetics containing cinnamoyl warhead, Peptidomimetics containing decahydroisoquinolin moiety, Ketone-based covalent inhibitors, and α-Ketoamidine derivatives; are discussed below:

3.1.1. Keto-Glutamine analogues:

In 2004, Jain and co-researchers synthesized a series of keto-glutamine analogues targeting the SARS-CoV 3CLpro. The synthesized compounds have been evaluated against SARS-CoV 3CLpro by

![Figure 5. Schematic diagram demonstrating the essential role of the N-finger in both dimerization and preservation of the enzyme’s active form.](image-url)

![Figure 6. Structure of SARS-CoV 3CLpro inhibitor.](image-url)
using fluorometric assays and found the most potent inhibitors with IC\textsubscript{50} values ranging from 0.60 to 70 \(\mu\text{M}\). The compounds have been designed based on their previously synthesized molecule previously evaluated against human rhinovirus-14 (HRV-14) and hepatitis A virus (HAV) targeting on picornaviral 3C protease enzyme. The reported keto-glutamine analogues 1 and 2 of HAV 3C protease inhibitors with IC\textsubscript{50} values are shown in Figure 7. Jain et al designed the three synthesis scheme (Scheme 1: \(1A, 1B, \text{ and } 1C\)) by modification at P2, P3, and P4 position with amino acids of previously reported compound\textsuperscript{56,60}.

The synthetic route of a tetrapeptide, Scheme 1A initiated according to the previously reported literature\textsuperscript{56,60} to produced 4a-c. After that, it reacts with trifluoroacetic acid for removal of the Boc group. Simultaneously, tripeptide Ac-Val-Thr(Obn)-Leu-OH was added in the reaction medium of DMF to generate tetrapeptide 5a-b. In Scheme 1A, the 5a and 5b compounds were reacted with respective reagents under specific reaction conditions to generate compounds 5c and 5d as shown in Scheme 1B, the 9a and 9b compounds were reacted with appropriate reagents under specific reaction conditions to get another 9c and 9d compounds\textsuperscript{67,68}. Another compound 11 was synthesized without the keto-phthalhydrazide group to analyze the effect of tetrapeptide moiety in the compound 9a-d, as shown in Scheme 1C. In-silico studies of the inhibitor 9b revealed that the pyrrolidine-2-one of S1 site interacted with His163 and nitro group of 5-nitro-2,3-dihydrophthalazine-1,4-dione of S2 site interacted with A3n142 residue of the protease (Figure 8). From all synthesized molecules, 9b has been found the most potent reversible inhibitors against SARS-CoV 3CLpro with IC\textsubscript{50} value 0.6 \(\mu\text{M}\) (Figure 8)\textsuperscript{23}.

### 3.1.2. Anilide analogues:

A multiple series of peptide anilides were developed by Shie and co-workers in 2005 based on anthelmintic drug (Niclosamide)\textsuperscript{59} and evaluated the anti-SARS activity targeting the 3CLpro enzyme\textsuperscript{54}. The anilide analogues were derived from 2-chloro-4-nitroaniline, \(\alpha\)-phenylalanine, and 4-(dimethylamino)benzoic acid. Based on 2-chloro-4-nitroanilide containing dipeptide (Scheme 2, 2A), Shie et al synthesized various anilide analogues, such as tripeptide anilide, tetrapiptide anilide, dimeric anilide, and ketomethylene anilide (Scheme 2B and 2C).

The di-peptide anilides analogues 14a-e were synthesized by following previously reported synthetic strategy\textsuperscript{70}. First, 12 was reacted with Boc-\(\alpha\)-phenylalanine to produced 13 and then, 13 was again treated with substituted carboxylic acid to produce the di-peptides 14a-e via coupling reaction (Scheme 2, 2A). The intermediate compound 13 was taken further to synthesize the tripeptide 15a-x and tetrapiptide analogues 16a-x analogues via coupling with appropriate peptide (Scheme 2, 2B). The synthesis of tripeptide ketomethylene anilide analogues 22a-p was synthesized via multistep organic reaction. First, intermediate 19 was prepared by adding two starting compounds, 17, 18, and then 19 was again reacted with HCl to remove Boc protection and substituted with respective R\textsubscript{3}COI to form compound 20. The compound 20 further on coupling with another 2-chloro-4-nitroaniline derivative 21 in the presence of Palladium-tetrakis(triphenylphosphine) form tetrapiptide anilide analogues 22a-p.

All the synthesized molecules were screened via biological assay with fluorogenic tetrapeptide substrate \textsuperscript{1}, and the molecular docking study with PDB ID (1UK4) showed the binding affinity towards SARS-CoV 3CLpro enzyme. Under anilide peptide analogues, the most potent structure is 14a (shown in Figure 7), which acts as a competitive inhibitor of SARS-CoV 3CLpro with IC\textsubscript{50} value 0.06 \(\mu\text{M}\) and \(K\textsubscript{i}\) value 0.03 \(\mu\text{M}\). Computer-modelling of the inhibitor 14a binding with SARS-CoV 3CLpro found that the benzyl group of S1 site was occupying Cys145, Ser144, His163, and Phe140 or the pocket formed by Thr25, His41, Cys44, Thr45, and Ala46. The 2-chloro-4-nitro benzyl group comprising the S2 site was found to interact with Ala46, while the chlorine atom is within 3 Å of Cys145 and His41. The dimethylamino benzyl of S3 site was found to be fitted into the pockets formed by Gln189-Gln192 and Met165-Pro68 residues (Figure 8)\textsuperscript{24}.

### 3.1.3. Peptidomimetic \(\alpha,\beta\)-unsaturated esters:

In 2005, Shie and co-researchers synthesized a series of peptidomimetics containing \(\alpha,\beta\)-unsaturated esters targeting the 3CLpro for SARS-CoV\textsuperscript{25}. Researchers have synthesized a compound AG7088 and its related tripeptide \(\alpha,\beta\) unsaturated ester, and ketomethylene isosteres (Scheme 3) and evaluated their inhibitory properties against SARS-CoV 3CLpro enzyme. These unsaturated peptidomimetic esters and ketomethylene isosteres showed moderate inhibition against the same enzyme. AG7088, previously reported as a potent PIs active against HRV-3CLpro that contain an \(\alpha,\beta\)-unsaturated ester side chain\textsuperscript{22}. Based on this evidence, it has been thought that AG7088 could be a potent inhibitor of SARS-CoV 3CLpro but, it turned out to have no inhibition even in the concentration of 100 \(\mu\text{M}\) in an enzymatic assay. Although the related \(\alpha,\beta\) unsaturated ester and ketomethylene isosteres were showed moderate inhibitory activity against SARS-CoV 3CLpro enzyme with IC\textsubscript{50} value ranging from 11 to 39 \(\mu\text{M}\textsuperscript{26,27}\).

The synthesis of PIs (AG7088) containing \(\alpha,\beta\)-unsaturated ester warhead and related compounds were synthesized based on previously developed literature\textsuperscript{26,27}. The synthesis strategy was divided into three parts (Scheme 3, 3A), for the synthesis of intermediate 27; Scheme 3, 3B, for the synthesis of Intermediate 30; and Scheme 3, 3C, for the synthesis of PIs (AG7088) via coupling of 27 and 30 intermediates.

Based on molecular modelling study, four different series (1, 2, 3, and 4) of AG7088 were designed and established. The series 1, 2, 3, and 4 were synthesized by changing in the three locations of the AG7088 (Scheme 3, 3C). From that above series, it was found that the series 4 compounds were most promising for SARS-CoV 3CLpro inhibition. The set of Phe-Phe dipeptides containing conjugated amido moieties at the N-terminals were designed with the help of software. The series 4 compounds 38a-e were synthesized by using previously reported methods\textsuperscript{21}. The main starting material for the synthesis of 38a-e is Phe-Phe dipeptidyl \(\alpha,\beta\)-unsaturated ester (Scheme 3, 3C). Amidation of N-Boc-phenylalanine by 4-amino-5-phenyl-2-pentanone and subsequent removal of Boc group with the help of 1,4-dioxane and HCl produces compound Phe-Phe dipeptides \(\alpha,\beta\)-unsaturated ester. Then, with compound Phe-Phe dipeptides \(\alpha,\beta\)-unsaturated ester, several \(\alpha,\beta\)-unsaturated carboxylic acid were reacted to produce a series of analogues (Scheme 3D).
Before isolating the compounds, they have done an enzymatic assay and found that the compound 38c was the most potent inhibitor against SARS-CoV 3CLpro enzyme (Figure 8). Molecular modelling study reveals that, out of four series of compounds, the compounds of series 3 and 4 showed better inhibitory results than the compounds of series 1 and 2 against SARS-CoV 3CLpro enzyme. The in-silico study of compound 38c indicates that the (dimethylamino)cinnamyl group of S1 site was occupied by Glu166, Gln189, and Gln192 residues via hydrogen bonding (Figure 8).

3.1.4. Glutamic acid and glutamine peptides:

It has been established that glutamic acid and glutamine peptides with trifluoromethyl ketone groups show good inhibitory activity against SARS-CoV 3CLpro enzyme (Figure 9). Molecular modelling study reveals that, out of four series of compounds, the compounds of series 3 and 4 showed better inhibitory results than the compounds of series 1 and 2 against SARS-CoV 3CLpro enzyme. The in-silico study of compound 38c indicates that the (dimethylamino)cinnamyl group of S1 site was occupied by Glu166, Gln189, and Gln192 residues via hydrogen bonding (Figure 9).

The Synthesis of CF₃-ketone containing glutamic acid and glutamine peptides can be accomplished by starting with compound 39. The synthesis of target compounds were divided into three parts: Scheme 4, 4A, synthesis of intermediate 45 (trifluoromethyl-β-amino alcohol), Scheme 4, 4B, synthesis of intermediate 48 (glutamic acid and glutamine peptides with a CF₃-ketone unit), and Scheme 4, 4C, Synthesis of the target compound (glutamic acid and glutamine peptides with a CF₃-ketone unit) by coupling reaction. First, compound 39 was treated with paraformaldehyde and para-toluene sulfonic acid in a toluene mixture to produce oxazolidinone acid 40. The formation of oxazolidinone acid 40 was developed by Luesch et al. Then, compound 40 was transferred into 41, and subsequently, compound 41 was also converted into 42 under specific reaction conditions (Scheme 4, 4A). The compound 42 was formed in two forms of stereoisomers, but the diastereomer form of 42 was further reacted with TBAF to produce 43. Then, the compound 42 and 43 were also transferred into compound 44 by reacting with sodium borohydride and methanol. Finally, by hydrogenation using Pd/C, compound 44 was transferred into final product 45 (Scheme 4, 4A). The second part of synthesis is focused on peptide formation. The target compound 48 was synthesized based on published literature.  

3.3C). Before isolating the compounds, they have done an enzymatic assay and found that the compound 38e was the most potent inhibitor against SARS-CoV 3CLpro enzyme (Figure 9). Molecular modelling study reveals that, out of four series of compounds, the compounds of series 3 and 4 showed better inhibitory results than the compounds of series 1 and 2 against SARS-CoV 3CLpro enzyme. The in-silico study of compound 38c indicates that the (dimethylamino)cinnamyl group of S1 site was occupied by Glu166, Gln189, and Gln192 residues via hydrogen bonding (Figure 9).
first, compound 46 was treated with H\(_2\) and Pd/C for the hydrogenation process that produced compound 47. Further, it was coupled with Cbz-L-Ala-OSu under basic medium to produce target peptide 48 (Scheme 4, 4B). In the third part of the synthesis, the starting compound 45 was synthesized as shown in Scheme 4, 4A. The compound 45 was reacted with different synthesized peptides such as: 46, 48, and 49 to get respective product 50, 51, and 52. Then, compounds 50, 51, and 52 reacted with TFA for removal of tert-butyl group to form compound 53, 54, and 55.
In the final step, the last forming compounds 53, 54, and 55 were converted into respective compounds 56, 57, and 58 under suitable reaction conditions (Scheme 4).

3.1.5. Peptidomimetic (TG-0205221):
TG-0205221 is a synthetic compound designed and synthesized by the scientists Syaulan Yang and their research group in 2006. The compound was compared with one natural peptide substrate and it was found that the natural compound showed moderate inhibition against the 3Cpro enzyme but a lower inhibitory activity against 3CLpro of SARS-CoV. Also, from the crystal structure of the drug-receptor complex, it was established that TG-0205221 binds firmly with the 3CLpro enzyme with extensive hydrophobic contact and involves ten hydrophobic bonds and one covalent bond. The compound TG-0205221 (69) was synthesized by a multi-step organic reaction shown in Scheme 5 and evaluated the inhibitory activity against SARS-CoV 3CLpro by using 229E and MRC-5 cells. The cytopathic effect was also performed with infected SARS-CoV Vero E6 cells. TG-0205221, 69 showed exceptional activity against SARS-CoV with $K_i$ value 0.053 µM shown in Figure 8.

The synthesis of TG-0205221 (69) can be accomplished by a multi-step organic reaction. First, the $\mathrm{L}$-Glutamic acid 59 was reacted with trimethylsilyl chloride and methanol as a solvent for 15 h and continued the reaction. After the desired time, the amine group was protected with Boc by adding Di-tert-butyl decarbonate and tri-ethylamine; and formed the compound 60. The compound 60 was again treated with strong base Lithium bis(trimethylsilyl)amide and bromo acetonitrile to get the compound 61. The intermediate compound 62 was formed by the hydrogenation of compound 61 and further reacted with base triethylamine to form cyclization product 63. Next, Boc protection of compound 63 was removed by adding the HCl at room temperature. After that, the addition of Boc-β-cyclohexyl-Ala-OH to the compound 64 produces the compound 65 and further deprotection of Boc produces the compound 66. In the next step, the Cbz-Thr(But)-OH was reacted with compound 66 to form compound 67. In the final step, the ester group of compound 67 was transferred to the alcohol by reacting with lithium hydride and forming compound 68. Further, reaction with triethylamine produced the final compound 69 (Scheme 5).

An in-silico study detected the binding interaction of compound 69 with 3CLpro. The result showed that the pyrrolidine-2-one of the S1 site was interacting with His163, Glu166, Phe140, and Asn142 residue. The amino acid residues, such as His164, Cys145, and Gly143, also interacted with the S2 site of aldehyde moiety. Similarly, in the N of -NHCbz and O of acetamide S3 site, the Glu166 amino acid residue interacted. The S4 site comprising amide residue was found to interact with Gln189 residue and cyclohexane moiety of S5 site interacted with His41. The trimethyl group of S6 site interacted with Met165, Leu167, Pro168, Asp187, Met49, Ala191, Arg188, and Thr190 residue (Figure 8).

3.1.6. Phthalhydrazide peptide analogues:
Zhang et al designed and synthesized a series of tetrapeptide containing phthalhydrazide ketones, pyridinyl esters, and their different analogues. They have designed the molecules based on a previous literature report on potent SARS-CoV 3CLpro inhibitor and screened the three compounds, such as keto-glutamine analogues, AG7088, and aromatic ester; for the development of new potent SARS-CoV 3CLpro inhibitor molecule. They synthesized the designed peptides molecules via Scheme 6: 6A, 6B, and 6C. The pyridinyl esters and their different analogues were also synthesized by two methods (method A and method B), shown in Scheme 6E, Scheme 6F, Scheme 6G, and Scheme 6H. All synthesized molecules were evaluated as SARS-CoV 3CLpro inhibitors.
Scheme 3. Synthesis of peptidomimetics (AG7088) containing α,β-unsaturated ester warhead. Scheme 3, 3A: Synthesis of intermediate 27; Scheme 3, 3B: Synthesis of intermediate 30; Scheme 3, 3C: Synthesis of peptidomimetics (AG7088) via coupling of 27 and 30 intermediates.
by enzyme assay (FRET assay). The assay result revealed that some pyridinyl esters were a potent inhibitor against SARS-CoV 3CLpro with \( IC_{50} \) value ranging from 50 to 65 nM. Among them, the most potent inhibitor is \( 83a \) (\( IC_{50} \) value - 0.05 µM) (Figure 8).

The synthesis of tetrapeptide 73 started via bromination of Boc-L-phenylalanine 70 in the presence of Ethyl chloroformate and triethylamine. The brominated product 71, then reacted with Phthalhydrazide using DMF as the solvent and formed a new substituent product 72. In the last step of the reaction, compound 72 was treated with analogue of peptide molecule Ac-Val-Thr(OBn)-Leu-OH under suitable reaction conditions to produce the final tetrapeptide compound 73 (Scheme 6, 6A). The other two tetrapeptides, 77 and 78 were also synthesized by changing the carboxylic part of compound 74. At first, the Boc-protected cyclic glutamic acid 74 was reacted with N,O-Dimethylhydroxylamine hydrochloride and other reactants, formed compound 75. Then, compound 75 was substituted with 2-Thienylmagnesium bromide under Isopropylmagnesium chloride solution to form compound 76. From this substituted product 76, the two tetrapeptides 77 and 78 were formed by reacting with different peptides (Scheme 6, 6B). Based on Scheme 6, 6B, they have synthesized another tetrapeptide 82, where the starting compound is Boc-protected cyclic glutamic acid ester 79. In the same fashion, the intermediates compounds were synthesized by reacting with peptide molecule, and treatment of lithium hydroxide leads to the compound 81. In the last step, the 5-Chloro-3-pyridinol was treated with compound 81 to produce the tetrapeptide compound 82. To explore the effect of Chloropyridinyl Esters in
tetrapeptides, they have designed two methods (Method A and B) for synthesizing a series of tetrapeptides containing 3-Chloropyridinyl Esters group (Scheme 6, D). The 3-Chloro-5-furan-(2-ylmethoxy)pyridine 85 was formed by reacting compound 84, 3-Chloro pyridinol and triphenylphosphine (Scheme 6, E). The Furan-2-yl Nicotinate 87 was synthesized by the reaction of compound 86, thionyl chloride, and 2-Furanone (Scheme 6, F). Another N-(Pyridin-3-yl)thiophene-2-sulfonamide 89 was also synthesized by treating compound 88 with 3-Amino-pyridine (Scheme 6, G). The synthesis of 2-(Pyridin-3-yl)-1-(thiophen-2-yl)ethanone 92 and 2-(5-Bromopyridin-3-yl)-1-(furan-2-yl)ethanone 95 are shown in Scheme 6 H and 6 I. To explore the effect of aldehyde group, they synthesized the thiazole-4-carbaldehyde 97 and 5-(4-Chlorophenyl)furan-2-carbaldehyde 99 (Scheme 6, J and 6 K).

The result obtained from the in-silico study of compound 83a showed that the pyridine ring in the S1 site of the compound interacted with the Phe140, Leu141, Asn142, Glu166, His163 amino acid residue and that of the keto group of S2 site interacted with the Ser144, Gly143, Cys145 amino acid residue of the protease (Figure 8)²⁸.

3.1.7. Cinanserin analogues:
Cinanserin is a 5-HT receptor antagonist which was discovered in the 1960s and clinically used in the treatment of neurological disorder in 1973. In 2005, Chen et al identified the cinanserin as an inhibitor of SARS-CoV 3CLpro, which was virtually screened from the structural database of existing drugs (more than 8000) and identified the best-fitted molecule to the target (SARS-CoV 3CLpro) through docking study. The virtual screening has identified the top 10 potential molecules that can act as anti-SARS agents and cinanserin is one of them. To confirmed the anti-SARS activity, the cinanserin was further evaluated in HCoV-229E tissue culture assays. It showed that the cinanserin has strong anti-SARS activity (IC₅₀ ranging from 19 to 34 µM) via inhibiting the 3CLpro.⁸⁰

Based on the above research findings, the same research group designed and synthesized a series of cinanserin analogues (Figure 8) for SARS-CoV 3CLpro inhibition by collaborating the work with another research group and published the potential cinanserin analogues in 2008. All synthesized compounds were evaluated against SARS-CoV 3CLpro by FRET assay and showed that some of the compounds are potent and selective inhibitors with IC₅₀ values ranging from 1.06 to 43.7 µM. The most potential cinanserin analogue is shown in Figure 8 (108a, IC₅₀ value- 1.06 µM) and molecular interaction data showed that, the compound 108a binds tightly with 3CLpro enzyme subsite than

![Scheme 5. Synthesis of inhibitor TG-0205221 (69).](image-url)
Scheme 4A:

1. BOC-protected S, HCl, EtOH, 20°C, 48 h, 60%

2. Mesityl sulfide, EtOH, 20°C, 24 h, 94%

3. TFA, 0°C, 1 h

4. HCl, EtOH, 20°C, 24 h, 81%

Scheme 4B:

1. HBr, CH₂Cl₂, 0°C, 1 h

2. NH₂OH·HCl, CH₂Cl₂, 0°C, 2 h

3. TFA, 0°C, 1 h

Scheme 4C:

1. HBr, CH₂Cl₂, 0°C, 1 h

2. NH₂OH·HCl, CH₂Cl₂, 0°C, 2 h

3. TFA, 0°C, 1 h

Scheme 4D:

Method A: K₂CO₃, 5-(chloro-2-pyridinyl) 5%

Method B: K₂CO₃, 5-(chloro-2-pyridinyl), Pyr, CH₂Cl₂

Scheme 4E:

Scheme 4F:

Scheme 4G:

Scheme 4H:

Scheme 4I:

1. LHMDS, THF, 0°C

2. N-fluoro-1,2-diazepane, MeN⁺Cl⁻, 0°C

Scheme 4J:

1. LHMDS

2. DCM, 2,2-dimethoxyethanol

Scheme 4K:

1. HCl, EtOH, 20°C

2. NH₂OH·HCl, CH₂Cl₂, 0°C, 2 h

3. TFA, 0°C, 1 h

(caption on next page)
3.1.8. Trifluoromethyl ketone containing peptides:

The cinanserin analogues were designed and synthesized via three series; series I, series II, and series III. In series I (Scheme 7), the compound 100 was reacted with various alkyl halides in the presence of isopropyl alcohol to produce the respective substituted product 101a-n. Again, these products are treated with substituted acyl chloride in the reaction medium and produce the final substituted product 102a-n (Scheme 7, Series I). Similarly, using another substituted starting material 103a-b, they synthesized 104a-b and 105a-b (Scheme 7, Series II). In series III, they reacted the substituted compounds 107a-b with previous starting material 100 and by simple condensation process, the final compound was formed 108a-b (Scheme 7, Series III).

The hydrophobic interaction atom pairs between the compound 108a and the enzyme were detected by using the LIGPLOT and it was evident that the benzyl group of S1, S4, S5 positions interacts with Thr25, Leu27, Gly143 (S1); Gln189, Met165, Cys145, Glu166 (S2); and His163, Phe140 (S3) respectively. The unsaturated alkyl chain of S2 and Thr25, Leu27, Gly143 (S1); Gln189, Met165, Cys145, Glu166 (S2); and His163, Phe140 (S3) respectively. The unsaturated alkyl chain of S2 and S3 sites was found interacting with His41 and Met49 amino acid residues (Figure 8)²⁹.

3.1.9. Trifluoromethyl, benzothiazolyl or thiazolyl ketone containing peptides:

In 2006, Cai and co-workers reported moderate activity of trifluoromethyl ketone containing peptides can be accomplished in four steps. In the first step, the substituted compound 109a was reacted with sodium nitrate in the presence of DMF and formed the intermediate compound 110a-c. Then, the compound 110a-c reacted with Trifluoroacetaldehyde ethyl hemiacetal under reaction conditions to form compound 111a-e and hydrogenation as well as reaction with N-protected amino acids transform the compound into 112a-g. In the last step, reduction of compound 112a-g leads to final compound 113a-h, as trifluoromethyl ketone containing peptides (Scheme 8).

From the computational studies, the interaction between inhibitor 113e and enzyme was studied. The interaction result showed that the benzyl group of S1 site interacting with Met165, Met49, His41, and Cbz-Ala of S2 site interacted with Glu189 amino acid residue. The cbz group of S3 site and alanine of S4 site interacting with amino acid residues, such as Pro168 (S3) and Gln186 (S4). Similarly, amino acid His163 and Phe40 were associated with leucine of S5 site; and keto group of S6 was also found to interact with amino acid residues Gly143 and Cys145 (Figure 8)³⁰.

3.1.10. Trifluoromethyl, benzothiazolyl or thiazolyl ketone containing peptidomimetic inhibitors:

In 2006, Cai and co-workers reported moderate activity of trifluoromethyl ketone containing peptides against SARS-CoV 3CLpro. It
was observed that the moderate inhibitory activity of the compounds was due to cyclization of the compounds, which decreases its interaction with the active site of the SARS-CoV 3CLpro. To increase the inhibitory activity of trifluoromethyl ketone containing peptides, Regnier T and co-workers in 2009 designed and synthesized a new trifluoromethyl series benzothiazolyl or thiazolyl ketone-containing peptidic compounds. The two approaches utilized to enhance the inhibitory activity were - Firstly, they modified the side chains of amino acid residues at the P1,

Scheme 8. Synthesis of trifluoromethyl ketone containing peptides.

Scheme 9. Synthesis of trifluoromethyl, benzothiazolyl or thiazolyl ketone containing peptidomimetic inhibitors. Scheme 9, 9A: synthesis of CF₃-ketone containing glutamic acid with Cbz protection, Scheme 9, 9B: Synthesis of peptides with thiazolyl and benzothiazolyl groups, Scheme 9, 9C: synthesis of pyrrolidone containing peptides, and Scheme 9, 9D: synthesis of dithiazolyl containing peptide inhibitor 137.
P2, and P3 sites keeping the trifluoromethyl ketone moiety intact. Secondly, they replaced the trifluoromethyl moiety with electron-withdrawing groups such as thiazolyl and benzothiazolyl group. The synthetic route for target compounds are shown in Scheme 9A, 9B, 9C, and 9D. All the synthesized compounds were subjected to in vitro protease inhibition assay to check their 3CLpro inhibitory activity. Compounds 137 showed good inhibitory activity with Kᵢ values 2.20 ± 0.8 (Figure 8).13

The synthetic strategy of target compounds were divided into four parts, first part: synthesis of CF₃-ketone containing glutamic acid with Cbz protection (Scheme 9A), second part: replacing the trifluoromethyl moiety with electron-withdrawing groups such as thiazolyl and benzothiazolyl groups (Scheme 9B), third part: synthesis of pyrrolidone containing peptides (Scheme 9C), and fourth part: synthesis of dithiazolyl containing peptides (Scheme 9D).

The synthesis of target compounds 119a-e were started from N-Cbz-L-glutamine 114 and converted to the compound 115 under specific reaction conditions. Then, compound 115 was reacted with different amines to form the following compounds 116a-d and further converted to the compound 117a-d by using trifluoromethyltrimethylsilane. The compound 118a-d was prepared by a one-pot reaction from 117a-d under appropriate reaction conditions. In the final step, specific peptide fragments20,21 were reacted with previous compound 118a-d and produced the final compounds 119a-e (Scheme 9, 9A).15

The compounds 125a-f were synthesized in four steps. In the first step, the compound 120 was converted to amides 121 and then reacted with thiazole or benzothiazole in the presence of n-Butyllithium to get compounds 122a-b. In the next step, treatment with formic acid compounds 122a-b produces the compounds 123a-b and further converted into 124a-e by using substituted amines under appropriate reaction conditions. In the last step, the final compounds 125a-f were formed by reacting the compounds 124a-e with peptide molecule under appropriate reaction conditions (Scheme 9, 9B).82

The compound 132 was synthesized following the previously published literature via multi-step process69. In the first step, the compound 127 was prepared from previous literature methods68 by using third

Scheme 10. Synthesis of Michael type of peptidomimetic inhibitors. Scheme 10, 10A: synthesis of Michael type inhibitor; Scheme 10, 10B: synthesis of peptide aldehyde 149a-f; Scheme 10, 10C: optimization and synthesis of potent peptide aldehyde inhibitor 156.
staring compound 126. Then, the compound 127 was converted to the 128 by using lithium hydroxide and further converted to 129 under reaction conditions. After that, the compound was treated with thiazole and n-Butyllithium to get compound 130. The reaction of trifluoroacetic acid and compound 130 converts into compound 131 and further converted to the final product 132 by reacting with Cbz-Val-Leu-OH under reaction conditions (Scheme 9, 9C).

Similarly, dithiazolyl compound 137 was prepared from Cbz-Val-Leu-OH 133 and converted to the compound 134 by reacting with n-hydroxy succinimide. Then, compound 134 reacted with glutamic acid to form the compound 135 and converted to the double-wirenbe amide 136. In the final step, the compound 136 reacted with thiazole and n-Butyllithium to get the final compound 137 (Scheme 9, 9D).

The binding interaction of compound 137 with the enzyme as observed in the in silico study showed that the keto group in the S1 site interacted with Gly143, Ser144, Cys145, the thiazole ring in the S2 site interacted with His41, keto group present in the 2nd position of pyrrolidine ring in the S3 site interacted with His163. Further, the Val-Leu and NH group of pyrrolidine comprise the S4 site found to interact with Glu166, Thr190, and further concomitant to the thiol in BF3-Et2O, and after quenching with H2O obtained thioacetate amide 155. The thioacetol on treatment with NBS yields final peptide aldehyde 156.

The binding interaction of compound 149a with the 3Clpro was detected from computational modeling. The result showed the interaction of pyrazole in the S1 site of the compound with His163, Phe140, Leu41, Glu166 amino acid residues of the protease. Leucine in the S2 interacted with Met45, Met65, His41. Further, Valine in S3, Alanine in S4, and aldehyde group in S5 site interacted with Glu166, Thr190, Cys145 amino acid residue, respectively (Figure 8).

3.1.10. Michael type of peptidomimetic inhibitors:

Akaji et al reported the design, synthesis and evaluation of peptide aldehyde as an inhibitor of SARS 3CLpro. The compounds were designed based on an initially reported pentapeptide aldehyde inhibitor (IC50 \(-37 \mu M\)) and the optimum side-chain structure was determined by comparing inhibitory activity with Michael type inhibitors. The synthetic outline and associated reaction conditions are depicted in Scheme 10: 10A, 10B, and 10C, respectively.

Protease inhibition assay was carried out on R188I mutant protease by simultaneous addition of both inhibitor and substrate to determine IC50 value. The result of the experiment suggested that the peptide aldehyde is a competitive inhibitor of SARS 3CLpro because there was no evidence in the formation of a stable covalent bond between inhibitor and protease.

In literature, structural optimization following X-ray crystallographic analysis of inhibitor-protease complex gives a potent tetrapeptide aldehyde with IC50 value 98 nM. The result demonstrated that the peptide aldehyde could be a more effective inhibitor of SARS 3CLpro than Michael-type inhibitors.

The synthesis of Michael type inhibitor (Scheme 10, 10A) was started from Boc-c-Glu-Obn (138). Coupling of the side chain carboxyl group with dimethylamine in BOP presence as a coupling reagent produces side-chain amide, simultaneous reduction with LiAlH4 gave alcohol upon Swern oxidation afforded aldehyde 139. Horner – Wadsworth – Emmons olefination of aldehyde 139 produced \(\text{N}^2\)-protected amino acid ester 140. Cleavage of the protected group by TFA and following coupling with tetrapeptide yield compound 141. For the preparation of 145, aldehyde 139 was converted to 142 by Wittig reaction and following an acid treatment. The Horner – Wadsworth – Emmons olefination and coupling with tetrapeptide converted 142 to 145. To synthesize the analogues 146, 142 was further subjected to Wittig reaction and an acid treatment as above. The following Horner – Wadsworth – Emmons olefination and coupling with the tetrapeptide yielded 146.

Peptide aldehyde 149(a-f) was synthesized by using conventional Weinreb amide resin (Scheme 10, 10B). The conventional Fmoc-based solid-phase synthesis was used to introduce amino acid residues. After peptide chain construction, the resin was reduced with LiAlH4 to obtain desired compounds.

The synthesis of structurally optimized and potent peptide aldehyde is given in Scheme 10, 10C. The synthesis was started with commercially available 9,10-decene-1-ol 150. Which on reaction with mCPBA and following hydrolysis obtained triol linker 151. Under appropriate reaction conditions, 151 was reacted with Fmoc-His (Trt) al to afford corresponding acetal 152. The acetal alcohol on oxidation by Jones reagent yielded carboxylic acid, which in coupling reaction produced amide resin 153. The amide resin on treatment with TFA converted to peptide acetal amide 154. After dissolving in AcOH treated with ethane thiol in BF3-Et2O, and after quenching with H2O obtained thioacetate amide 155. The thioacetol on treatment with NBS yields final peptide aldehyde 156.

The synthesis of tripeptide cysteine protease inhibitors is divided into two parts: synthesis of tripeptide compounds 162a-e with aldehyde side chain (Scheme 11, 11A) and second part involve the synthesis of \(\alpha\)-Ketoamide tripeptide cysteine protease inhibitors (Scheme 11, 11B). In the synthesis of tripeptide compounds 162a-e, the starting material 158 was synthesized based on previously published literature. First, the compound 158 was reacted with N-Boc-\(\text{L}\)-leucine 157 in the presence of EDCl to form compound 159. After that, reaction with different N-Cbz-amino acids 160a-e and previous compound 159 produces the tripeptide compounds 161a-e and further treatment with sodium borohydride and methanol converted to the final compounds 162a-e (Scheme 11, 11A). In the second part of the synthesis, the starting material was 162b, which reacts with different conditions and reagents to transforms the final compounds 163 (Scheme 11, 11B).

3.1.12. Potent dipeptide type of peptidomimetic inhibitors:

To develop a low molecular weight compound with 3CLpro inhibitory activity, Thanagaimalai et al designed a series of dipeptide-type compounds based on a previously reported lead compound C-terminal benzothiazolyl ketone containing dipeptide. Initial docking study involving binding interaction between the lead compound and 3CLpro, encouraged the replacement of P3 N-(3-methoxyphenyl) glycine with various rigid heterocyclic P3 moieties in the lead compound. The synthesis of the target compounds (172a-p) was achieved by assembling two key moieties: peptides, 167 and C-terminal benzothiazole derivative, 171. Synthetic outline along with associated reaction conditions depicted in Scheme 12: 12A and 12B. The inhibition constant value \((K_i)\) of the compounds against 3CLpro were determined by accessing kinetic parameters in a fluorometric protease inhibition assay using constant substrate concentration. The IC50 value of only some potent inhibitor determined based on cleavage activity of R188I SARS-CoV 3CLpro on a synthetic peptide (H-TSAVLQGFRK-NH2). The \(K_i/IC50\) value was reported as a mean of three independent experiments. The SAR study revealed some potent inhibitors having \(K_i/IC50\) value in the sub-micromolar to nanomolar range. The best potent inhibitor is compound.
172a with \( K_i \) value, 0.006 \( \mu \)M (6 nM) and IC\(_{50}\) value, and 0.74 \( \mu \)M (Figure 8). \( K_i \) value of some compounds was also in good agreement with the binding affinity observed in the isothermal titration calorimetry (ITC) study.

The synthesis of dipeptide cysteine protease inhibitors is divided into two parts: the first part involves the synthesis of peptide fragments 167a-r with carboxylic acid side chain (Scheme 12, 12A) and the second part involves the synthesis of dipeptide cysteine protease inhibitor 172a-r (Scheme 12, 12B). The peptide fragments 167a-r were synthesized via starting with compound 164 which reacted with different substituted carboxylic acid 165a-r under reaction conditions and formed the compounds 166a-r. Further, the compounds 166a-r reacted with trifluoroacetic acid to convert into final compounds 167a-r under reaction conditions and formed the compounds 166a-r. Further, the compounds 166a-r reacted with trifluoroacetic acid to convert into final compounds 167a-r (Scheme 12, 12A). In the synthesis of dipeptide cysteine protease inhibitor 172a-r, the main starting compound is 168 and the compound was converted to the 169 by using the method given in published literature. Then, the compound 169 was further reacted with N,O-Dimethylhydroxylamine hydrochloride and formed the Weinreb amide 170. The compound 170 converted to compound 171 by coupling with benzothiazole in the presence of n-Butylamine. Lastly, the reaction of trifluoroacetic acid and previously synthesized compounds 167a-r produces the final dipeptide compounds 172a-r.

The –NH of the indole group along with the carbonyl moiety and the –NH group of the pyrolidin-2-one moiety comprises the S1 site, and this site is found to interact with the Glu166 residue. The –NH group of S2 and S3 interacted with Gln189 and His164 residues, respectively. The carbonyl moiety of S4 position interacted with Ser144 and the ketone group of the pyrolidin-2-one moiety was found to have an interaction with His163 residue of the protease (Figure 8).

### 3.1.13. Novel dipeptide type peptidomimetic inhibitors:

In search of novel low molecular weight SARS-CoV 3CLpro inhibitors Thanigaimalai et al designed some dipeptidic compounds based on their previously reported peptide lead compound Z-Val-Leu-Ala(-pyrrolidone-3-yl)-2-benzothiazole (\( K_i \) = 4.1 nM) by replacing P3 valine unit with varieties of substituents keeping P1 and P1 fixed. Synthesis of the designed dipeptide was achieved through a coupling reaction involving two intermediates, and N-protected amino acid esters 175a-f and a
gamma-lactam benzothiazole 179, those were synthesized in separate steps. The steps of synthesis depicted in Scheme 13A and 13B. The biological activity of the compounds was determined following a fluorometric protease inhibition assay against 3CLpro. Kinetic parameters were accessed at constant substrate concentration and inhibitor concentration varied to report $K_i$ values. $IC_{50}$ values were determined based on cleavage activity of SARS-CoV 3CL mutant protease (R188I) on a synthetic peptide substrate (H-Thr-Ser-Ala-Val-Leu-Gln-Ser-Gly-Phe-Arg-Lys-NH2). SAR study revealed two potential inhibitor 180a ($K_i = 0.39 \mu M, IC_{50} = 10.0 \mu M$) and 181b ($K_i = 0.33 \mu M, IC_{50} = 14.0 \mu M$). Further, a molecular docking study was also performed to understand the binding interaction of HKU1 and infectious bronchitis virus. The study revealed that the inhibitor previously synthesized compounds 175a-f produces the final dipeptide compounds 180a-h, 181a-n, and 182a-d (Scheme 13, 13B).

In silico studies revealed that the –NH group of the methoxy aniline, the ketone group and the –NH of pyrrolidin-2-one moiety was found to interact with the Glu166 residue. The –NH group of S2 and S3 interacted with Gln189 and His164 residues, respectively. The carbonyl moiety of S4 position interacted with Ser144 and Cys145. In contrast, the pyrrolidin-2-one moiety was found to interact with His163 residue of the protease (Figure 8).

3.1.14. Nitrile based peptidomimetic inhibitors

In 2013, Wong and co-workers developed a four type of nitrile based broad-spectrum PIs for SARS-CoV 3CLpro. The four molecules were designed and synthesized with different N-terminal protective groups, such as, Boc (tert-butyloxycarbonyl), Cbz (carboxybenzyl), Mic (5-methylisoxazole-3-carboxyl) and different peptide length (Scheme 14). All four PIs 201, 202, 203, and 204 were evaluated by in vitro enzymatic assay against SARS-CoV 3CLpro and the four inhibitors showed potent inhibitory activity against the SARS-CoV 3CLpro with $IC_{50}$ values ranging from 4.6 to 49.6 $\mu M$ and among the compounds, 204 showed the best inhibitory activity against the SARS-CoV 3CLpro ($IC_{50} = 4.6 \mu M$) (Figure 8). Due to the best inhibitory effects towards SARS-CoV 3CLpro, the PIs 204 was taken for further study for broad-spectrum inhibition against different human coronavirus strains, such as 229E, NL63, OC43, HKU1 and infectious bronchitis virus. The study revealed that the inhibitor 204 has broad-spectrum activity and effective against SARS-CoV 3CLpro, with $IC_{50}$ values ranging from 1.3 to 3.7 $\mu M$.

The synthesis of nitrile based PIs was focused on: synthesis of different small peptide compounds containing Boc (tert-butyloxycarbonyl), Cbz (carboxybenzyl), and Mic (5-methylisoxazole-3-carboxyl) 195, 196, 199, and 200; and joined with peptide 187 to get final PIs 201, 202, 203 and 204. First, the synthesis of 187 starts via
coupling of compound 183 and Gln(Trt)–OH in N,N′-dicyclohexylcarbodiimide N-hydroxysuccinimide; to form the compound 184 and reacts with ammonia, which converts the compound 184 to compound 185. Next, the primary amine transfer to the nitrile group via treatment with trifluoroacetic anhydride and formed the compound 186 and further deprotection of Boc can convert to the final peptide 187 (Scheme 14). Like previous compound 187 synthesis, the rest 195, 196, 199, and 200 intermediate peptides were formed via different reaction conditions shown in Scheme 14. These intermediate peptides were formed via reacting with different starting material, compound 188 and 192. In the last step, compound 187 was reacted with different previously synthesized peptides 195, 196, 199, and 200 to form the target compounds 201, 202, 203, and 204; via coupling the two peptides in mixed anhydride conditions 36.

The cyano group of the S1 site was found to interact with the Glu166, Phe140 residues. The amide group of S2 interacted with Cys145. Leu167 was found to interact with the dimethyl group of S3, the amide groups of S4 forms interaction with Thr190, Gln189 and the S5 site comprising the benzyl group interacted with Pro168 of the protease (Figure 8) 36.

3.1.15. Tripeptide type of peptidomimetic inhibitors:

With an aim to develop novel peptidomimetics as inhibitors of SARS-CoV 3CLpro, Kanno et al designed a series of tripeptide-type compounds containing 4,5-dimethyl thiazole, 5-methyl thiazole, bezothiazole, and a series of 5-arylated thiazole at P1′ position, other substituents at P2 and P4 positions were also rigorously optimized to achieve the best fit for protease inhibition activity. The initial molecular modelling study involving a thiazolyl ketone-containing tripeptidic lead compound (of previous research work on SARS-CoV by the authors) and 3CLpro demonstrated a spacious requirement warhead group rather than a small thiazole in P1′ position, encourage to introduce those large groups. Synthetic outline of designed tripeptide-type compounds and associated reaction conditions depicted in Scheme 15. The protease inhibitory activity of synthesized compounds evaluated using R188I SARS-CoV 3CLpro (SARS-CoV mutant protease) activity on a substrate (H-TSAVLQSGFRK-NH2). A fluorogenic substrate (Dabcyl-KTSAVLQSGFRKME-Edans) was also used for initial rate measurements. IC50 and/or Ki values were reported as the mean of three independent experiments. The study results revealed some compounds with benzothiazolyl moiety exhibit IC50 or Ki value in submicromolar to nanomolar range with two potential compound 214i (Ki = 4.1 nM) and 214p (Ki =...
The docking study of a potential compound, 214o of the series, showed strong binding interaction (Minimization energy: −43.65 kcal/mol) with protease enzyme than previously reported lead compound (Minimization energy: −37.56 kcal/mol). Docking structure was elucidated by X-ray crystallography (PDB ID: 1WOF, K<sub>i</sub> = 10.7 µM) (Figure 8).

The synthesis outline of tripeptide type of PbS divided into three parts; the first part involves the synthesis of dipeptide key fragment; the second part involves the synthesis of tripeptide inhibitor 214a-u, 215a-d, and 216a-i; and the third part involves the synthesis of tripeptide inhibitor-containing imidazole moiety 221a-c.
that has been synthesized with the help of peptide chemistry (Scheme 15, 15A), the second part involves the synthesis of tripeptide inhibitor 214a-u, 215a-d, and 216a-i via synthesizing the key intermediate 213 (Scheme 15, 15B), and third part involve the synthesis of tripeptide inhibitor-containing imidazole moiety 221a-c via synthesizing the key intermediate 220 (Scheme 15, 15C)37.

The dipeptide key fragment 209 has been synthesized via starting with compound Wang-resin which reacted with different substituted Fmoc-amino acids and coupling reagent diisopropyl-cobamideimide under reaction conditions and formed the compound 206. To remove the Fmoc group, the compound 206 has been reacted further with 20% piperidine in DMF and treated with Fmoc-valine to produce compound 207. Next, the compound 207 has been converted to compound 208 by reacting with different acid chlorides or acyl chlorides and 20% piperidine in DMF and further transferred to target fragment 209 by treating with trifluoroacetic acid under specific reaction conditions (Scheme 15, 15A)37.

In the synthesis of tripeptide inhibitors 214a-u, 215a-d, and 216a-i, the main starting compound is 210 and the compound was converted to 211 by following the published literature method46,47. Then, the compound 211 has been further reacted with N,O-Dimethyldihydroxylamine hydrochloride under reaction conditions and formed the Weinreb amide 212. The Weinreb amide 212 has been further converted to the compound 213 by coupling with substituted benzothiazole or 5-arylated thiazole in the presence of n-Butylamine or LDA. Lastly, reaction with trifluoroacetic acid and previously synthesized compound 209 produces the final tripeptide compounds 214a-u, 215a-d, and 216a-i (Scheme 15, 15B)68,69.

The synthesis of tripeptide inhibitor-containing imidazole moiety 221a-c has been started via commercially available compound 217. First, the compound 217 has been reacted with N,O-Dimethyldihydroxylamine hydrochloride under reaction conditions and formed the respective Weinreb amide 218. Then, the Weinreb amide 218 has been treated with thiazole in the presence of n-Butylamine to produce compound 219 and further converted to compound 220 by reacting with tosyl chloride and triethylamine. Lastly, reaction with trifluoroacetic acid and previously synthesized compound 209 produces the final tripeptide inhibitors 221a-c (Scheme 15, 15C)37.

The synthesis strategy was divided into two parts: Scheme 16, 16A, for the synthesis of intermediate 25; Scheme 16, 16B, for the synthesis of target compounds 226a-d. At first, in the synthesis of intermediate compound 25, L-glutamic acid was used as a starting material and similar synthetic protocols were used which describe in Scheme 16, 16A. Further, the intermediate compound 25 was coupled with Cbz-L-Phe under suitable reaction conditions to produce target peptide 222 and under reduction with Dess-martin periodinane and lithium hydride, the compound 222 converted into 223 (Scheme 16). In the final step, the respective compounds 222, 223, and 224 were reacted with substituted cinnamoyl derivatives 225a-d under specific reaction conditions to get desire compounds 226a-d (Scheme 16).

### 3.1.16. Peptidomimetic containing cinnamoyl warhead:

In 2017, Vathan Kumar and co-workers have identified and evaluated six peptidomimetics as SARS-CoV 3CLpro inhibitors68. They developed peptidomimetics with some modifications based on previous literature. The designed inhibitors were synthesized using previously published literature that was used in the synthesis of 3CLpro inhibitors for enterovirus37. All the synthesized compounds were tested in vitro against SARS-CoV 3CLpro as well as MERS-CoV by using previously reported fluorescence assay. The biological evaluation data have reported some potent inhibitors against SARS-CoV 3CLpro, and compound 226d is one of them with an IC₅₀ value of 0.2 ± 0.07 μM (Figure 8).

The synthesis of PIs containing cinnamoyl warhead and the related compound was synthesized based on previously developed literature38. The synthesis strategy was divided into two parts: Scheme 16, 16A, for the synthesis of intermediate 25; Scheme 16, 16B, for the synthesis of target compounds 226a-d. The synthesis of target compounds 226a-d was performed by coupling with Cbz-L-Phe under suitable reaction conditions to produce target peptide 222 and under reduction with Dess-martin periodinane and lithium hydride, the compound 222 converted into 223 (Scheme 16). In the final step, the respective compounds 222, 223, and 224 were reacted with substituted cinnamoyl derivatives 225a-d under specific reaction conditions to get desire compounds 226a-d (Scheme 16).

### 3.1.17. Peptidomimetic containing decahydroisoquinolin moiety:

With an aim to develop SARS-CoV 3CLpro inhibitor, Ohnishi K et al designed some compounds containing decahydroisoquinolin moiety...
with an additional substituent at non-prime site and warheads based on previous C-terminal aldehyde peptide inhibitor. Initially designed compound showed moderate activity, and the analysis of X-ray crystallographic structure of inhibitor in complexation with R188I SARS 3CLpro displayed the absence of interactions covering the P3 to P4 regions of substrate-based inhibitor, which led to the incorporation of a substituent at the non-prime site on the 4-position carbon of decahydroisoquinolin moiety. The synthetic outline and associated reaction condition for the compounds are depicted in Scheme 17. The compound 247 (IC$_{50}$ = 26 µM) exhibited clear but moderate inhibition potency on R188I SARS-CoV 3Cpro (Figure 8). The result of the study suggested increased interaction between protease and inhibitor 247 after incorporation of the substituent at the non-prime site. In addition, it is revealed from the study that the thioacetal warhead in association with decahydroisoquinolin moiety could be an efficient strategy for the design of potent SARS 3Cpro inhibitor.

The synthesis of PIs containing decahydroisoquinolin moiety and the related compound was performed based on the retrosynthetic route. From the retrosynthetic analysis, it was revealed that a diol compound 227 is the starting compound for the synthesis of targeted derivatives. Hence, the synthesis of precursor molecule 233 is started from compound 227. The synthesis of decahydroisoquinolin derivatives has been divided into five parts, the first part has focused on the synthesis of precursor molecule 233 (Scheme 17, 17A); the second part has focused on the synthesis of precursor molecule epi-233 (Scheme 17, 17B); the third part has focused on the synthesis of intermediate compound 237 (Scheme 17, 17C); the fourth part has focused on the synthesis of intermediate compound 242a, 242b, and 243 (Scheme 17, 17D); and the

Scheme 17. Synthesis of peptidomimetics containing Decahydroisoquinolin moiety. Scheme 17, 17A: synthesis of precursor molecule 233; Scheme 17, 17B: synthesis of precursor molecule epi-233; Scheme 17, 17C: synthesis of intermediate compound 237; Scheme 17, 17D: synthesis of intermediate compound 242a, 242b, and 243; and Scheme 17, 17E: synthesis of target compounds including 247.
fifth part has focused on the synthesis of target compounds including 247 (Scheme 17, 17E). Synthetic route of precursor compound 233 for the Pd(II) catalyzed cyclization given in Scheme 17, 17A. Benzylic protection of one alcohol group and oxidation of other alcohol group to the aldehyde of diol 227, and then Wittig reaction deliver compound 228. Protection of a 1,2 diol with TBDMSCl group, obtained via asymmetric dihydroxylation of compound 228, yielded an alcohol 229 as a mixture of epimer. Amine 230 with Boc-protection was obtained via Mitsunobu reaction and catalytic hydrogenation of 229 in the presence of (Boc)₂O. Mitsunobu reaction followed by reduction changed the alcohol group of 230 to amine group, which on acylation by p-bromobenzoic acid gave amide 231. Oxidation of primary alcohol group of 231 to an aldehyde, followed by Wittig reaction gave ether 232. The precursor compound 233 is obtained by reduction of 232 with DIBALH. After that epi 233 with different amine configuration was achieved via synthetic Scheme 17, 17B. The olefin presents at the terminal of 228 is transferred to epi-1,2-diol via asymmetric dihydroxylation by the use of different ligand that was used for 229. Protection of primary alcohol group with TBDMS yield epi-229 as 8:1 diastereomixture. Then the mixture in appropriate reaction conditions gave 234 as a single diastereomer, in addition to the required epi-230 diastereomixture. To yield a single diastereomeric configuration of epi-13, the diol 234 was used. The compound 234 in a sequence of reaction gave compound 236. Then a single diastereomer epi 233 was obtained via the desired sequence of reactions. Diastereoselective cyclization catalyzed by Pd(II) was then investigated (Scheme 17, 17C). Bis-acetonitrile palladium chloride catalyzed cyclization of precursor 233 gave decahydropseudoisoquinolin compound 237. Non-prime site substituents were introduced using compound 237. The preparation of P1 site fragment (242a-b) and nonprime site substituents covering P3 to P4 (243), to be incorporated on the decahydropseudoisoquinolin moiety, depicted in Scheme 17, 17D. For the synthesis of P1 site residue, an easily accessible Fmoc-His(Trt)-OH (240) was transferred to Weinreb amide, and reduction of the amide with DIBALH produced an aldehyde 241. The resulting compound 241 was then transformed to α, β-unsaturated ester or thioacetal, and under appropriate reaction conditions yield 242a or 242b. EDC/DMAP mediated coupling and additional debenzylation by catalytic hydrogenation of H-Gly-OBn and Ac-Thr-OH gave P3 to P4 site substituent were introduced into the cyclic compound 237 (Scheme 17, 17E). Boc group of 237 was detached using HCl, and then coupling of amine with 243 in presence of BOP yielded 244. Oxidation of terminal olefin followed by reductive coupling with 242b, the aldehyde product of 244 gave 245b. Then 245b was converted to 246b via elimination of the Trt group of imidazole in Histidine. To assess the influence of warheads on inhibitory potency, P1 residue 242a and H-His(Trt)-N(OMe)Me were similarly coupled with

**Scheme 18.** Synthesis of Ketone-based covalent inhibitors. Scheme 18, 18A: synthesis halomethylketone (HMK) intermediates 251, 252, 253, and 254; Scheme 18, 18B: Synthesis of chloromethylketone (CMK) intermediate 255; and Scheme 18, 18C: synthesis of target compounds including 274 and 275.
244 to yield 246a and 246c. Treatment 246b with NBS changed the thioacetal to an aldehyde group and after purification of the product via preparative HPLC gave compound 247.15

3.1.18. Ketone-based covalent inhibitors:

In continuation of research efforts to develop novel molecule as a SARS-CoV 3CLpro inhibitor, Robert L. Hoffman et al pursued the design of ketone-based reversible and irreversible inhibitors based on previous Michael acceptor by changing warhead moiety and retaining P1 lactam, while P2 and P3 sites were randomly optimized. The synthesis of the designed compound and associated reaction condition depicted in Scheme 18; 18A, 18B, and 18C. The biological experiment was carried out following SARS-CoV-1 protease FRET assay and analysis, based on the proteolytic activity of 3CLpro on fluorogenic peptide substrate following sequence DABCY-LKTSAVLQ-SGFRKME-EDANS. Viral inhibition assay of the compounds was also carried out on SARS-CoV-1 and hCoV 229E. Two classes of potential inhibitors of SARS-CoV-1 have been developed viz. acylxymethylketones and hydroxethyl ketones. The biological evaluation data have reported some potent inhibitors against SARS-CoV 3CLpro under acylxymethylketone derivatives, and compound 256 is one of them with an IC_{50} value of 0.017 ± 2 μM (Figure 8). Solving crystal structure of the ligand protein complex by X-ray crystallography analysis led to the identification of potent inhibitor PF-00835231 (IC_{50} = 4 ± 0.3 nM) with additional viral inhibition potent. Further, preclinical investigation of PF-00835231 revealed desirable pharmacokinetic property, which warrants the development of PF-00835231 as a drug candidate against COVID-19 patient.16

The halomethylketone (HMK) intermediates were obtained via a two-step synthetic procedure as given in Scheme 18, 18A. Aminoester 248 on saponification yielded amino acid 249. The Reaction of acid 249 with isobutylchloroforomate and trimethylamine produced mixed anhydride, which after reaction with diazomethane provided 250. Diazoketone 250 was reacted with HCl for concurrent deprotection of nitrogen and transformation into the intermediate chloromethylketone (CMK) 253. On the other hand, reaction of 250 with stoichiometric amounts of 48% HBr in dichloromethane gave 254. Chloromethylketone (CMK) intermediate can be prepared differently by the treatment of 248 with too much LDA to produce 251. Conversion of 249 to a Weinreb amide, followed by reaction with Grignard of benzylchloromethyl ether produced oxymethylketone intermediate 252.

Substituted methyl ketone derivatives was achieved via generation of the fully elaborated chloromethylketone (CMK) intermediate 255 (Scheme 18, 18B). Compound 253 in a series of reaction produce 255 in moderate yields without epimerization. Reaction of 255 with numerous carboxylic acids in a medium containing CsF gave acylxymethylketones 256–271. The other derivatives were also obtained by following above synthetic procedure via substitution with appropriate functionalities.

The derivatives containing Hydroxymethylketone (HMK) functionality were obtained via two complementary processes depicted in Scheme 18, 18C. Oxymethylketone intermediate 252 via a series of reactions yielded benzyl ether derivatives 274 in moderate yields. Moreover, by using above synthetic procedures along with the substitution of appropriate functionalities yielded other inhibitors.

The result obtained from the in silico study of compound 256 showed that the pyridine ring in the S1 site of the compound interacted with the Cys345 amino acid residue and that of the carbonyl group of S2 site interacted with the Cys145, Gly143, amino acid residues of the protease (Figure 8).17

3.1.19. α-Ketoamides derivatives:

Zhang et al in their research work, developed broad-spectrum antivirals against enterovirus, alpha coronavirus, and beta coronavirus. Because the protease of these virus shares a common active site architecture and an unique specificity for substrate recognition, they pursued a structure-based design of peptidomimetic α-ketoamides near equipotent against the three virus genera. The proteases targeted in this study specifically cleave peptide following glutamine residue in the P1 position, they have incorporated 5-membered γ-lactam ring (a derivative of glutamine) in P1 position, which causes ten-fold increase in potency of the inhibitors. Synthetic efforts were, therefore, aimed at optimizing the substrates at the P1', P2, and P3 positions of the α-ketoamides. Chemical synthesis of α-ketoamides started with Boc protected l-glutamic acid dimethyl ester, synthetic outline and associated reaction conditions are depicted in Scheme 19; 19A, 19B, and 19C.18 Synthesized compounds were tested against the recombinant viral proteases, viral replicons, and virus-infected cell cultures; most of them were non-toxic to the cell. The SAR study revealed that optimization at P2 substituent is important for achieving near equipotency against alpha coronavirus, beta coronavirus and enterovirus. Among the compounds, the best near equipotent compounds were 289a (P2 = cyclopentylmethyl) and 289r (P2 = cyclohexylmethyl). These compounds achieved the best fit between the different requirements for P2 substituent. However, in terms of viral protease inhibition activity, compound 289a (IC_{50} = 0.24 μM) and 289n (IC_{50} = 0.93 μM) showed the best inhibition activity towards SARS-CoV 3CLpro (Figure 8). This study also demonstrated the fact that as there is a high similarity between the main protease of SARS-CoV and novel beta CoV (SARS-CoV-2/ Wuhan/2019). All results reported here for the inhibition of SARS-CoV will most likely also apply to the new virus as well.19

The synthesis process of α-Ketoamides derivatives has been divided into three parts, the first part involves the synthesis of key lactam fragment 279 (Scheme 19, 19A), the second part involves the synthesis of amino acids intermediates 283a–u (Scheme 19, 19B), and third part involves the addition of two synthesized intermediate compounds 279 and 283a–u to produce the target compounds 289a–u (Scheme 19, 19C).20

The key lactam fragment 279 has been synthesized via three steps which started with the N-Boc glutamic acid dimethyl ester 276 and reacted with bromoacetonitrile under specific reaction conditions to form compound 277. In the next step, the intermediate 277 has undergone a hydrogenation reaction and formed a cyclization product 278. In the last step, the cyclization product 278 has been reacted with trifluoroacetic acid to remove the Boc protection and transferred into final lactam fragment 279 (Scheme 19, 19A).21

Similarly, the second key amino acid intermediates 283a–u has been synthesized in two steps, which started with the substituted acyl chloride 280 and α-amino acid methyl ester 281. These two starting compounds have reacted with trifluoroacetic acid to produce compounds 282a–u and under alkaline hydrolysis, the compounds 282a–u have transferred into target amino acid intermediates 283a–u (Scheme 19, 19B).

The synthesis of α-Ketoamides derivatives has been started via the addition of two synthesized intermediates 279 and 283a–u. Under specific reaction conditions, these intermediates have transferred into compounds 284a–u. In the next step, the compounds 230a–u have undergone a reduction reaction to form compounds 231a–u by reducing agent sodium borohydride and further reaction with Dess – Martin periodinane 285a–u has converted into 286a–u. In the next step, the compounds 286a–u has transferred into 287a–u by nucleophilic addition of isocyanides in acidic medium. The nucleophilic product 287a–u has converted into 288a–u by removing the acetyl group. In the last step, the alcohol group of 287a–u has converted to keto group by oxidation process with Dess – Martin periodinane and formed the α-Ketoamides 289a–u (Scheme 19, 19C).22

In silico studies between inhibitor 289a and 3CLpro revealed the interaction of cyclopropane of S1 site with amino acid residues such as: Gln189, Arg188, Asp187, Met49, and Met165 (Figure 8).23 3.2. Small-molecule inhibitors (SMIs):

The chemical synthesis of SMIs under isatin derivatives 289, and 3CLpro revealed the interaction of cyclopropane of S1 site with amino acid residues such as: Gln189, Arg188, Asp187, Met49, and Met165 (Figure 8).23
Chloropyridyl ester derivatives, pyrazolone derivatives, pyrimidine derivatives, macrocyclic inhibitors, 5-sulphonyl isatin derivatives, serine derivatives, phenylisoserine derivatives, and Octahydroisochromene derivatives; are discussed below:

3.2.1. Isatin derivatives:

N-Substituted isatin derivatives were synthesized and evaluated in vitro against SARS-CoV 3CLpro by Li-Rung Chen and co-researchers. A series of isatin derivatives has been prepared from the reaction of isatin and various bromides (BrR\textsubscript{4}) in the presence of a strong base via a single-step process\textsuperscript{41}. The in vitro SARS-CoV 3CL protease assays have been conducted via FRET analysis for biological evaluation. The assay result showed that some compounds were potent and selective inhibitors with IC\textsubscript{50} values ranging from 0.95 to 17.50 \textmu M against SARS-CoV 3CLpro\textsuperscript{71}. The organic synthesis of isatin derivatives 291a-z is shown in Scheme 20 and among the synthesized compound, the most potent compound is 291a (IC\textsubscript{50} = 0.95 \textmu M), as shown in Figure 9\textsuperscript{41}.

The N-Substituted isatin derivatives 291a-z have been synthesized via a single-step process, which started with substituted isatin 290. Compound 236 reacted with different bromides in the presence of a strong base to obtain target compounds 291a-z (Scheme 20). In compound 291a, the benzothiophene of the S1 site interacted with His164, the carbonyl groups of isatin of S2 site interacted with His41, Cys145, Gly143, and Iodine group of isatin of S3 site interacted with Phe140 amino acid residues of protease as detected in the in silico study (Figure 9)\textsuperscript{41}.

3.2.2. Chloropyridyl ester derivatives:

Chloropyridyl ester is a class of heteroaromatic organic molecule that was used previously in the development of SARS-CoV 3CLpro inhibitors. The first chloropyridyl ester-containing SARS-CoV 3CLpro inhibitor was reported by the Jianmin Zhang and their co-workers in Scheme 19A: synthesis of key lactam fragment 279; Scheme 19, 19B: synthesis of amino acids intermediates 283a-u, and Scheme 19, 19C: synthesis of α-Ketoamides derivatives 289a-u by addition of two intermediates 279 and 283a-u.
Based on the previous history of Chloropyridyl ester as an antiviral potency, in 2008, Ghosh et al. developed a series of SMIs containing Chloropyridyl ester and indole moiety, for SARS-CoV 3CLpro. Similarly, they have used another potent SARS-CoV 3CLpro inhibitor (i.e. Benzotriazole ester derivative) for designing the target molecule, which was developed by the Wong research group in 2006. They synthesized the designed SMIs via Scheme 21. All the synthesized compounds were tested against SARS-CoV 3CLpro by using previously reported FRET enzyme assay as well as SARS-CoV cell-based assay. The assay result revealed that some Chloropyridyl esters were a potent inhibitor against SARS-CoV 3CLpro and among them, the most potent inhibitor is \(294a\) \((IC_{50} = 0.03 \pm 0.01 \mu M, EC_{50} = 6.9 \pm 0.9 \mu M)\) \(\text{(Figure 9)}\).

The Chloropyridyl ester derivatives \(294a-f\) have been synthesized via a single step. Various carboxylic acid derivatives \(292a-f\) were coupled with 5-chloro-3-pyridinol \(293\) in presence of DCC and DIMAP under appropriate reaction conditions and produces Chloropyridyl ester derivatives \(294a-f\) \(\text{(Scheme 21, 21A)}\). After synthesis of \(294a-f\), a few of the indole compounds \(294a-b\) were converted to acetyl products \(295a-b\) via acetylation reaction \(\text{(Scheme 21, 21B)}\). They have also incorporated substituted phenyl sulphonyl group to the indole by direct sulfonamidation reaction but it has been found that the sulphonyl-indole Chloropyridyl products were not active in the anti-viral activity.

The binding interaction of compound \(294a\) obtained by Gold dock with the enzyme as observed in the in silico study showed that the carbonyl group in the S2 site interacted with Gly143, Ser144, Cys145, the nitrogen of the chloropyridinyl in the S1 site is in close proximity with His163 residue of the macromolecule \(\text{(Figure 9)}\).

3.2.3. Pyrazolone Derivatives:

A major class of heterocyclic compounds that exist in many drugs and synthetic products are pyrazolone derivatives. Such molecules has excellent analgesic, antituberculosis, antifungal, antimicrobial, anti-inflammatory, antioxidant and anti-tumor functions. The
pyrazolone structure plays an important role because of its easy preparation and rich biological activity and represents a fascinating template for combinatorial and medicinal chemistry. In 2010, Po-Huang Liang et al have reported a series of pyrazolone compounds having inhibitory activity against SARS-CoV 3CLpro. The compounds have been designed based on reported pyrazole containing drugs (like, Edaravone, a medicine that is effective for myocardial ischemia), synthesized and tested for anti SARS-CoV 3CLpro inhibitory activity by in vitro protease inhibition assay using fluorogenic substrate peptide in which several compounds exhibited potent inhibitions towards 3CLpro (Scheme 22). The most potent inhibitor is 300c with IC50 value 8.4 nM (Figure 9). Incidentally, one of the potent inhibitors also showed better effectiveness against Coxsackievirus B3 3C protease. They have also determined the cytotoxicity of the synthesized compounds using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay and found that, none of them were cytotoxic at 200 nM.

The pyrazolone derivatives 300a-u have been synthesized via two steps. The reaction started with substituted phenylhydrazine hydrochloride 296 and ethyl benzoyleacetate 297. These two starting materials have reacted in the presence of triethylamine to produce intermediate compounds 298 and further reaction with substituted aldehyde under desired reaction conditions, the compounds 298 has converted to the target compounds 300a-u (Scheme 22)48. The computational studies of compound 300c showed that the phenyl ring of the S1 site interacted with Met-49, Arg-188, and Gln-189; the 4-carboxy benzyl of the S2 site interacted with Gln-192 residues of protease as detected in Figure 9.

3.2.4. Pyrimidine Derivatives:

In 2010, Ramajayam and co-workers developed a series of 2-(benzylthio)-6-oxo-4-phenyl-1,6-dihydropyrimidine derivatives as SARS-CoV 3CLpro inhibitors. They have been identified various heterocycles as a novel anti-SARS agent against SARS-CoV 3CLpro from high throughput screening. They have designed the compounds from the data of complete pharmacophore modelling, and synthesized some potential acyclic intermediates through the inhibition of intracellular protein complexes. Several compatible strategies were already developed over the past decade to synthesize and screen the macrocycle libraries for binding to pharmacologically important targets. Due to its important chemical features, such as higher affinity, higher metabolic stability, and selectivity towards proteins, macrocyclic inhibitors have attracted considerable attention to researchers in drug discovery. The first macrocyclic inhibitor (316a) for SARS-CoV 3CLpro has been designed and synthesized by Sivakoteswara and their research group in 2013. The macrocyclic inhibitor (316a) has been evaluated in vitro against different families of virus for anti-viral activity, such as picornaviridae, coronaviridae, and caliciviridae; which showed potent inhibition against 3CLpro of enterovirus (CVB3 Nancy strain), moderate inhibition against 3CLpro of norovirus and weak inhibition against 3CLpro of SARS-CoV with IC50 value 15.5 nM (Figure 9). The macrocyclic inhibitor (316a) has been synthesized via the macrocyclization process (Scheme 24; 24A and 24B)49.

The synthesis process of macrocyclic inhibitors 316a-e has been divided into three parts. The first part involves the synthesis of key acid fragment 309 (Scheme 24, 24A), the second part involves the synthesis of acyclic intermediates 312 (Scheme 24, 24B), and the third part is the addition of two synthesized intermediates compounds 309 and 312 to produce the target compounds 316a-e (Scheme 24, 24C)50.

The key acid fragment 309 has been synthesized in two-steps by using previously published literature51-53, which started with the Boc-propargyl-Gly-OH 307 and reacted with l-Leucine methyl ester hydrochloride under specific reaction conditions to form dipyridyl ester 308. In the final step, the dipyridyl ester 308 has been reacted with dry HCl under reaction conditions to remove the Boc protection (N-terminal) and further reaction with carbobenzoxy chloride transferred to the final cbz-protected acid fragment 309 (Scheme 24, 24A).

Similarly, the second key azide intermediates 312 has been synthesized via two-step as described in previously published literature, which started with the Boc-Glu-ome 310 and NH2(CH2)2N2 (n = 3). These two compounds have been coupled via EDCI mediated reaction and converted to compound 311. Further treatment with HCl in dioxane, the compound 311 has converted to final azide intermediate 312 (Scheme 24, 24B)54.

The synthesis of macrocyclic inhibitors 316a-e has been started via the addition of two synthesized intermediates 309 and 312 and by coupling reaction conditions, the intermediates have transferred into

![Scheme 22. Synthesis of pyrazolone derivatives.](image_url)
acyclic compound 313. In the next step, the compound 313 has undergone a cyclization reaction by applying click chemistry in the presence of Cu(I)Br/DBU and converted to cyclized product 314. The compound 314 further reacted with lithium borohydride in presence of THF to produce corresponding alcohol 315 and by oxidation of compound 315 with Dess–Martin periodinane produced the final macrocyclic compounds 316a-e (Scheme 24, 24C).

3.2.6. 5-sulphonyl isatin derivatives:

The substitution of a carboxamide group with a series of substituted sulfonamide moieties have been explored by Liu and co-workers in 2014 to enhance the inhibitory effect towards SARS-CoV 3CLpro. They have designed the target compounds based on previously reported non-covalent SARS-CoV 3CLpro inhibitors (N-substituted 5-carboxamide-isatin derivatives), which Zhou and co-workers developed in 2006. A series of designed compounds have been synthesized via multistep organic reaction (Scheme 25, 25B) and screened by in vitro protease assay using fluorogenic substrate peptide. From the assay result, it has been found that several compounds have potent inhibitory activity against the SARS-CoV 3CLpro and among all the compounds, 328a...
showed the best inhibitory activity against the SARS-CoV 3CLpro with IC₅₀ = 1.04 ± 0.01 μM (Figure 9). The synthesis of isatin derivatives has been divided into two parts, the first part has focused on the synthesis of two compounds 321 and 322 (Scheme 25, 25A); the second part has focused on the synthesis of series of 5-sulphonyl isatin derivatives 328a-m (Scheme 25, 25B). The target compound 321 and 322 have been synthesized by reacting two corresponding starting material 317 and 318. Under reaction with hydroxylamine hydrochloride and chloral hydrate, the compounds 317 and 318 have converted to corresponding compounds 319 and 320. Lastly, reaction with concentrated sulfuric acid converts the compounds 319 and 320 to target isatin compounds 321 and 322 (Scheme 25, 25A).

In the second part of the synthesis, a series of 5-sulphonyl isatin derivatives 328a-m has been prepared by referring to published literature. First, the isatin molecule 323 has been treated with chlorosulfonic acid that produces two compounds, 324 and 325. In the next step, both the compounds 324 and 325 have been isolated and reacted with a secondary amine under reaction condition and converted to 326. The compound 326 has directly converted to 327a-m by the treatment of secondary amine with high yields. In the last step, the compounds 327a-m have been alkylated with various bromides or iodides in the presence of sodium hydride to produce 5-sulphonyl isatin derivatives 328a-m (Scheme 25, 25B).

The substituted furfural aldehyde fragment 332 has been synthesized by using published literature methods. At first, the diazonium salts 330 have been converted to final substituted furfural aldehyde fragment 332 by reacting with furfural and Copper (II) chloride (Scheme 26, 26A).

Scheme 25. Synthesis of small-molecule inhibitors containing isatin moiety. Scheme 25, 25A: synthesis of isatin compounds 321 and 322; Scheme 25, 25B: synthesis of a series of 5-sulphonyl isatin derivatives 328a-m.
In the second part of the synthesis, a series of pyrazolone derivatives 337a-s was prepared using a multistep organic reaction. To synthesize intermediate compounds 335a-f, the substituted hydrazines 333 and ketoesters 334 has been reacted in the presence of AcOH under reflux condition. Then, the intermediate compounds 335a-f have been converted to pyrazolone derivatives 337a-s by reacting with previously synthesized 336 (Scheme 26, B).

Similarly, another pyrazolone inhibitor 340 has been synthesized by one-step process via reaction of compounds 338 and 339; to explore the effect of aldehyde substitution (Scheme 26, C). In the last part, another pyrazolone inhibitor 344 has been synthesized by a two-step process via the reaction of para-hydroxy benzaldehyde 283 and benzyl bromide under appropriate reaction conditions to produce compound 342. Further, compound 342 has been converted to final compound 344 by reacting with compound 343 under appropriate reaction conditions (Scheme 26, D).

The S1 site composed of the chlorobenzoic acid moiety was observed to interact with Gly143, His163, Ser144, and Cys145 residues and the trimethyl group of S2 fit in the hydrophobic pocket formed by Met49 and Gln189. The carbonyl group of pyrazolone derivative present in the S3 was found to interact with the His41 residue of the 3CLpro (Figure 9).

3.2.8. Serine derivatives:

Konno et al has been aimed to design non-peptidyl SARS-CoV 3CLpro inhibitors based on previous lead compounds, tetrapeptide aldehyde Ac-Thr-Val-Cha-His-H and Bai’s inhibitors of SARS 3CLpro. They have designed a series of functionalized serine derivatives focusing on P1, P2, P4 site interaction of peptide aldehyde inhibitor with 3CLpro. The compounds have been generated through virtual screening on GOLD software with additional docking simulation. The synthetic outline and associated functionalities to prepare serine derivatives are given in table 1, 2 & 3 of their research article. The biological activity of the compounds has been evaluated using cleavage activity of SARS-3CL mutant protease, R188I, on a synthetic decapeptide with the S01 cleavage sequence as the substrate. In parallel with biological study, a cytotoxicity study of serine derivatives has also been performed on Hela cells. The SAR study, in combination with docking simulation, revealed two potential inhibitors ent-290 (SK23) and ent-291 (SK69), with D-configuration on serine template, have high protease selectivity. The potential compounds of this study practically do not have any cytotoxicity. The most potential inhibitor under serine derivatives is 348 (SK23) with IC₅₀ = 30 µM (Figure 9).

A series of serine derivatives 348a-o, 349b-o, and 350a-c has been prepared using a three-step organic reaction. The synthesis has been started via a coupling reaction of Fmoc-Ser(But)-OH 345 and various amines (like cyclohexylamine, piperidine, morpholine, benzylamine, and cyclohexyl methylamine) under reaction conditions and transferred to the intermediate compounds 346a-e. Using different coupling
reaction conditions (A, B, and C) and deprotection of t-butyl group, the compounds 288a-e have been converted to the 347a-o. In the last step, different acylation reaction conditions have been employed to convert the compounds 347a-o to the final serine derivatives 348a-o, 349b-o, and 350a-c (Scheme 27).

Results obtained from in silico study of compound 348 showed that the carbonyl group in the S1 site and amino group in S2 interacted with different acylation reaction conditions have been employed to convert compounds 288a-e have been converted to the final serine derivatives 347a-o, 349b-o, and 350a-c (Scheme 27).

3.2.9. Phenylisoserine derivatives:

Hiroyuki Konno, has designed and synthesized phenylisoserine derivatives from reported molecule tetrapeptide aldehyde (IC50 = 98 nM) and SK23 (IC50 = 30 µM) for SARS-CoV 3CLpro inhibition by the combination of SAR and docking simulation study. The molecular design of phenylisoserine derivatives has been started using three steps: serine to isoserine backbone, induction of the functionality at the P2 position, and SAR study. The inhibitory activity has been determined using a synthetic decapeptide as a substrate against SARS 3CL R188I mutant protease. The inhibitory potency of the related compound was performed based on the retrosynthetic route. Inhibitors and nonpeptide inhibitor having decahydroisoquinolin moiety. However, in the previous work the effect of substituent on decahydroisoquinolin ring was not fully elaborated. In this study the effect of four substituents on 1-position of the decahydroisoquinolin scaffold was rigorously analyzed. A novel hydrophobic core, octahydroisochromene scaffold has been introduced for interaction with the S2 pocket of the protease enzyme and substituents at 1-position was incorporated to form additional interactions.

Octahydroisochromene scaffold was built via Sharpless-Katsuki asymmetric epoxidation (SKAE) and Sharpless asymmetric dihydroxylation (SAD). The P1 site His-al and the substituent at 1-position was incorporated through subsequent reductive amination reactions. The synthetic outline and associated reaction condition for the construction of compounds are depicted in Scheme 29. The inhibitory potency of the compounds (375a-d) with single diastereomeric configuration clearly indicates that a particular stereo-isomer of the octahydroisochromene moiety, (1S, 3S) 375b (IC50 = 95 µM), guides the P1 site imidazole, substituent at the 1-position and the warhead aldehyde of the fused ring to their proper pockets in the 3CL protease (Figure 9).

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### Table 1: Summary of protein-inhibitor interaction for SARS-CoV 3CLpro.

| SL No. | Compound no. | Derivatives | IC_{50} / K_{i} (µM) | Docking Study | Ref. |
|--------|--------------|-------------|----------------------|---------------|-----|
| 1 | 14a | Anilide analogues | 0.06 | 1UK4 | H = Ala46; Pockets involved = Cys145, Ser144, His163, Phe140. Or Thr25, His41, Cys44, Thr45. |
| 2 | 38c | Peptidomimetic α,β unsaturated esters | 1 | 1c145a | H = Gln166, Gln189, Gln192. |
| 3 | 53 | Glutamic acid and glutamine peptides | K_i = 116.1 | N.A. | N.A. |
| 4 | 69 | Peptidomimetic (TG-0205221) | 0.6 | 1ZII | H = Gln189, His163, His164, Gln166, Cys145, Gly143, Phe140. |
| 5 | 83a | Phthalhydrazide peptide analogues | 0.05 | 2ASK | H = His163, Ser144, Gly143, Cys145. |
| 6 | 108a | Cinanserin analogues | 1.06 | 1UJ | H = His163, Ser144, Gly143, His163, His164, Gln166, His164. |
| 7 | 113c | Trifluoromethyl ketone containing peptides | 10 | 1UK4 | C = Cys145. H = Gln166, Gln189, Thr190. |
| 8 | 137 | Trifluoromethyl, benzothiazolyl or thiazolyl ketone containing peptidomimetics | K_i = 2.20 ± 0.8 | 1WOF | C = Cys145. H = His41, Ser144, Gly143, His163, Gln189, Lys166. |
| 9 | 149a | Michael type of peptidomimetics | 5.7 | 3AW0 | H = His163, Gln166, Thr190. |
| 10 | 162b | Cysteine protease inhibitors | 0.23 ± 0.1 | N.A. | N.A. |
| 11 | 172a | Potent dipeptide type of peptidomimetics | 0.74 | 1WOF | H = Gln166, Gln189, His164, Ser144, His163. |
| 12 | 180a | Novel dipeptide type of peptidomimetics | 10 | 1WOF | H = Gln166, Gln189, His164, Ser144, His163. |
| 13 | 204 | Nitrile based peptidomimetics | 4.6 | 3VBS | C = Cys145. H = Gln166, Phe140, Gln189, Thr190. |
| 14 | 214a | Tripeptide type of peptidomimetics | 0.65 | 1WOF | H = His41, Gln166, His164, Ser144, Gly143. |
| 15 | 226d | Peptidomimetic containing Cinnamoyl warhead | 0.2 ± 0.07 | N.A. | N.A. |
| 16 | 247 | Peptidomimetic containing Decahydroisoquinolin moiety | 26 | 4TWV | N.A. |
| 17 | 256 | Ketone-based covalent inhibitors | 0.017 ± 2 | 6XIN | H = Gly143, Cys145. |
| 18 | 289a | α-Ketoamides derivatives | 0.24 ± 0.08 | 2BX4 | H = Gln189, Phe140, Gln166, Gly143, Ser144, His41. |
| 19 | 291a | Iatin derivatives | 0.95 | 1UK4 | H = Gly143, Cys145, His41. |
| 20 | 294a | Chloropyridyl ester derivatives | 0.03 ± 0.01 | 2H0B | H = Cys145, Ser144, Gly143. |
| 21 | 300c | Pyrazolone Derivatives | 8.4 | 1UK4 | H = Gly143, Gln192, Gln166. |
| 22 | 306c | Pyrimidines Derivatives | 6.1 ± 1.1 | 1UK4 | H = Gly143, Gln166, Gly143. |
| 23 | 316a | Macrocyclic inhibitors | 15.5 | 22US | N.A. |
| 24 | 328a | 5-sulphonyl iatin derivatives | 1.04 ± 0.01 | 1UK4 | H = Gly143, Gly163, Cys145. |
| 25 | 337a | Pyrazole derivatives | 5.8 ± 1.5 | 2ALV | H = His41, Ser144, His163, Cys145, Gly143. |
| 26 | 348 | Serine derivatives | 30 | 3AW1 | H = Cys145, Gly189. |
| 27 | 356 | Phenylsissioureline derivatives | 43 | 3AW1 | H = Gln189, His41, Cys145, Gly164. |
| 28 | 357b | Octahydroisoquironene derivatives | 95 | 4TWV | N.A. |

N.A. = Not available.

### Table 2: List of amino acid residues for SARS-CoV 3CLpro.

| Function in SARS-CoV 3CLpro | Amino acid residues |
|-----------------------------|--------------------|
| Catalytic dyad | His41, Cys145 |
| Ligand binding | Cys145, Gly163, Gly189, His163, Gly143, Ser144, Phe140, His41, Met49, His164, Met165, Asn142, Glu192, Pro168, Arg188, Thr190, Ala191, Leu141, Thr25, Ala46, Leu67, Asp187, His172, Leu27. |

From the retrosynthetic analysis it was revealed that a diol compound 364 is the starting compound for the synthesis of targeted derivatives. Hence, the synthesis of precursor molecule 369 is started from compound 364. The key intermediate 369 was synthesized starting with compound 364 following the route shown in Scheme 29, 29A. Protection of one hydroxyl group with TBDPSCI (tert-butyldiphenylsilyl chloride) and oxidation of another alcohol group via Ley-Griffith oxidation reaction using TPAP (tetrakis ammonium phenylacetonitrile) of diol 8, produced the corresponding aldehyde. The aldehyde without purification after reacting with (methoxymethyl)
triphenylphosphonium chloride yielded 365. The reaction between 365 and 10-camphorsulfonic acid produced corresponding aldehyde. The isolated aldehyde after reaction with methyl triphenylphosphonium chloride gave terminal olefin. Removal of TBDPS group in the product upon treatment with TBAF (tetra-n-butylammonium fluoride) produced the intermediate 366. Oxidation of the primary alcohol in 366 via a Ley–Griffith oxidation reaction and homologation of the corresponding aldehyde through Horner–Wadsworth–Emmons (HWE) reaction using ethyl 2-(diethoxyphosphoryl) acetate produced 367.

Reduction of ethyl ester in 367 with DIBAL-H and stereo-specific oxidation of corresponding aliphatic alcohol via a SKAE reaction utilizing TBHP (tert-butyl hydroperoxide) mediated by (S,S)-diethyltartrate ((–)–DET) and titanium tetraisopropoxide (TTIP) gave 368 as a single diastereomer. The terminal olefin in 368 was oxidized via a Sharpless asymmetric dihydroxylation reaction under desired conditions. The production of the anticipated diol was confirmed by 1H NMR spectroscopy with the concurrent formation of a small proportion of the cyclic product 369 produced via the nucleophilic addition of the resultant alcohol. Then, desired cyclized product 369 was obtained via treatment of the mixture with p-toluenesulfonylic acid without further purification.

The key intermediate 369 was transferred into the final product following the synthetic route displayed in Scheme 29, 29B. Oxidative cleavage using sodium periodate followed by reductive amination of the

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Table 3
Summary of cell-based assay for SARS-CoV 3CLpro.

| Sl. No. | Compound no. | Derivatives | Cell-based assay | Ref. |
|-------|-------------|-------------|----------------|------|
| 1     | 38c         | Peptidomimetic α,β unsaturated esters | EC_{50} (µM) | Cell line |
| 2     | 69          | Peptidomimetic (TG-0205221) | 0.6 | Vero |
| 3     | 256         | Keto-based covalent inhibitors | 5 ± 1.4 | Vero |
| 4     | 289s        | α-Ketoamides derivatives | 18.4 ± 6.7 | Vero |
| 5     | 294a        | Chloropyridyl ester derivatives | 6.9 ± 0.9 | Vero |

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Scheme 28A: Synthesis of phenylisoserine derivatives. Scheme 28, 28A: synthesis a series of phenylisoserine derivatives 356 and 357a-e; Scheme 28, 28B: synthesis of phenylisoserine containing inhibitor 360; Scheme 28, 28C: synthesis of phenylisoserine containing inhibitor 363.
corresponding aldehyde with four distinct amines by using NaBH$_3$CN leads to aminated products. Methylation of aminated products by paraformaldehyde gave $370a$-$d$. Oxidation of primary alcohol in the products and followed by reductive condensation of associated aldehyde with H-His-N(OMe)Me leads to Weinreb amides ($371a$–$d$). Reduction of each amide to aldehyde by treatment of DIBAL-H and followed by purification via LC/MS gave the required aldehyde products ($372a$–$d$). The synthetic route for the preparation (1-$S$, 3-$R$) $375a$ is depicted in Scheme 29, 29C (i). The allylic alcohol produced after reducing the ester was oxidized via Sharpless-Katsuki asymmetric epoxidation reaction in presence of ($-$)-DET as the chiral ligand to yield a single diastereoisomer $368$. Oxidation of terminal olefin in $368$ by Sharpless asymmetric dihydroxylation using a chiral ligand, (DHQ)$_2$Pyr gave a primary product $369a$. Oxidative breakdown of the 1,2-diol in $369a$ and followed by reductive amination reaction using $n$-butyl amine in presence of paraformaldehyde were performed as illustrated for compound $370a$. Finally coupling reaction with H-His-N(OMe)Me and following reduction by DIBAL-H was conducted as mentioned for compound $372a$ (Scheme 29, 29C-i) yield $375a$ as the primary product. The diastereoisomer Compound $375b$ with the (1-$S$, 3-$S$) configuration was synthesized by using a combination of (DHQ)$_2$Pyr and ($-$)-DET, those were employed in the SKAE reaction and the Sharpless asymmetric dihydroxylation reaction, respectively. Diastereoisomers, $375c$ and $375d$, were synthesized with the (1-$R$, 3-$R$) and (1-$R$, 3-$S$) configurations, respectively; this was accomplished by using synthetic outline displayed in Scheme 29, 29C-ii. To create the (1-$R$, 3-$R$) configuration in $13c$, a combination of ($-$)-DET and (DHQ)$_2$Pyr was utilized, those were used in the SKAE reaction (producing a single diastereoisomer) and the SAD reaction, respectively. In a similar way the (1-$R$, 3-$S$) configuration in $369d$ build using ($-$)-DET and (DHQ)$_2$Pyr as ligand. Both the product then transferred to $375c$ and $375d$ following the given procedure.

4. Computational chemistry aspects:

Computational chemistry and pharmaceutical chemistry plays a tremendous role in drug discovery and development from the last decade. Nowadays, discovering lead molecules or identifying potential drug candidates for various diseases is one of the challenging scenarios for researchers. To overcome this challenge, the research groups are focusing on using pharmacoinformatic tools to minimize the time, cost, manpower, and errors for developing the potential drugs. By using the above tools, searching thousands of molecules from various databases and simultaneous identification of the best fit molecules for target proteins becomes less tedious. The interactive information derived between proteins (macromolecule) and ligands can help the chemist to design and synthesize the most potent inhibitors against SARS-CoV by analyzing the SAR. Here, we have summarized the computational chemistry aspects of SARS-CoV 3CLpro inhibitors, including the various SARS-CoV 3CL proteases (PDB IDs), types of bond between the ligands and various amino acids of the protease, frequency of amino acid residues involved in the interaction (Table 1).

For virus replication and infection cycles, Mpro enzyme is critical, allowing it a perfect tool for the development of antiviral drugs. The binding site of 3CLpro comprises a catalytic dyad where a cysteine residue (Cys145) behaves as a nucleophile and a histidine residue
(His41) serves either as general acid or base\textsuperscript{4,115}. The list of residues of amino acids that serve a vital part in ligand binding with the SARS-CoV 3CLpro are shown in Table 2. As found from various literature surveys amino acid residues and their percentage frequency are Cys145 (13.2%), Glu166 (11.1%), Gln189 (10.4%), His163 (8.3%), Gly143 (9%), Ser144 (6.3%), Phe140 (5.6%), His41 (5.6%), Met49 (4.9%), His164 (4.2%), Met165 (4.2%), Asn142 (2.8%), Gin192 (2.1%), Pro168 (2.1%), Thr190 (2.1%), Arg188 (1.4%), Ala191 (1.4%), Leu141 (1.4%), Thr25 (0.7%), Ala46 (0.7%), Leu167 (0.7%), Asp187 (0.7%), His172 (0.7%), Leu27 (0.7%) plays a vital role for the various interactions which includes hydrogen-bonding (55.9%), covalent (3.4%), hydrophobic (37.2%) and van der waals interactions (3.4%) in Figure 10, 10A. It was observed that the 11 frequent amino acid residues are mainly responsible for the interactions, which include Cys145, Glu166, Gln189, His163, Gly143, Ser144, Phe140, His41, Met49, His164, Met165 (Figure 10, 10B).

Figure 10. Amino acid residues predominantly involved in protein–ligand interactions. 10A. Amino acid residues occurrence in percentage. 10B. This figure represents the 11 most crucial amino acids responsible for interaction with the ligands present in the SARS-CoV 3CLpro (PDB ID: 1UK4)\textsuperscript{58} with 5-mer peptide of inhibitor (10C).
5. Cell-based assay:

Cell-based therapies can revolutionize contribution to currently unfulfilled healthcare problems, hence making effective manufacturing critical. Before any of this could become a possibility, numerous barriers must be solved, as well as a deeper understanding of the specific requirements for cell-based technologies. Vero E6, Vero 76, and other commonly known cell lines may be utilized for virus replication in vitro with evident cytopathic effects. The cytopathic effect of several viruses is known to be mediated by virus-induced apoptosis or cell necrosis. The ‘Vero’ cells that are used, are extracted from kidney epithelial cells of African green monkey. Vero E6 cells have been widely employed in SARS-CoV research in cell culture-based infection models since 2003, owing to their ability to sustain viral replication to high titers, due to their higher expression of the ACE-2 receptor. Translation cell-based assays for SARS-CoV had given proper insight into the mechanism of action of the potential inhibitors that are may be helpful for the drug discovery process in the near future. Table 3 shows the activities of cell-based assays of various inhibitors that are used in this review.

6. Conclusion & future perspective:

3CLpro is an important drug target for SARS-CoV, as virus-encoded protease involves the processing of the two viral polyproteins and responsible for the viral replication and infection process. This review deals with the chemical synthesis and medicinal chemistry perspectives of PIs and SMIs targeting SARS-CoV 3CLpro, which has been chemically synthesized and biologically screened as newer derivatives from 2004 to 2020 for therapeutic inventions of SARS-CoV. In this overview, we have also identified the most potent compounds from each derivative and highlighted their structural features and binding modes.

In the current scenario, the warhead-based strategy plays a high impact in the medicinal chemistry for the development of SMIs and PIs. Most of the PIs and SMIs for SARS-CoV 3CLpro have been designed by applying a warhead-based strategy, but few inhibitors have shown better inhibition and potency. Most of the inhibitor has been evaluated via in vitro SARS-CoV 3CL protease assays using FRET analysis. The majority of PIs have not been studied further for in-vivo evaluation due to their less optimal physicochemical characteristics and undesirable frameworks.

In the development of potential compounds against SARS-CoV 3CLpro, it has been observed that the first synthetic peptidomimetic compounds are under keto-glutamine analogue. It has been designed and synthesized in 2004 from previously reported anti-viral compounds like hepatitis virus 3C and human rhinovirus 3C inhibitors (Figure 6). Compound 9b is an example of a peptidomimetic inhibitor-containing cyclic keto-glutamine moiety that showed better inhibition against SARS-CoV 3CLpro with IC₅₀ value 0.60 μM. Comparing the molecular docking study, inhibitor 9b is the only compound under SARS-CoV 3CLpro inhibitors studied with human rhinovirus-14 (PDB ID: 1CQQ).

From the keto-glutamine analogues other peptidomimetic inhibitors have been explored during this timeframe for the development of potential drug candidates, such as peptidomimetic containing α,β-unsaturated esters, anilide analogues, glutamic acid–glutamine peptides, and phthalhydrazide peptide; to improve the biological efficacy and potency against SARS-CoV 3CLpro. The chemical synthesis of many SMIs is also discussed in this review. The majority of inhibitors showed micromolar potency against SARS-CoV 3CLpro. The most promising inhibitors under SMIs are 291a, 294a, 300c, 306c, 316a, 328a, 337a, 348, and 356 have been also analyzed with different PDB IDs, except inhibitors 53, 162b, 226d, 247, 316a, and 357b (Table 1).

3CLpro inhibitors have been extensively studied, enabling the production of a percentage frequency graph that displays the most critical amino acid residues detected for interaction with the ligands. From this, it was verified that these compounds interact via hydrogen-bonding (55.9%), covalent (3.4%), hydrophobic (37.2%), and van der waals interactions (3.4%), where the Cys145 residue is the most frequent to exhibit hydrogen bond formation. His41-Cys145 is a part of a catalytic dyad. Met165 residue exhibits the most frequent hydrophobic interacting sites. Cys145 is the amino acid residue for which the covalent interaction is responsible. The 11 frequent amino acid residues responsible for the interactions includes Cys145, Glu166, Gln189, His163, Gly143, Ser114, Phe140, His41, Met49, His164, and Met165 that are discussed earlier in the medicinal chemistry perspective section and will be crucial for drug development process in future.

However, some of the peptidomimetics showed less inhibition activity against the SARS-CoV 3CLpro and poor absorption, distribution, metabolism, and excretion parameters to be a drug-like molecule. Peptidomimetic has few drawbacks over the SMIs, like high molecular weight, poor pharmacokinetic properties and pharmacodynamic property. To develop potential 3CLpro inhibitors against coronavirus, the strategy should be focused on developing low-molecular weight peptidomimetic inhibitors and its lead optimization for improvement of pharmacokinetic and pharmacodynamic properties. This project may soon emerge as a pioneer in the discovery of SARS-CoV 3CLpro inhibitors to succeed in the fight against coronavirus.

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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