Small peptide and amino acid derivatives in the search for SARS-CoV-2 main protease inhibitors

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Despite the fact that the World Health Organization (WHO) declared the COVID-19 outbreak a pandemic less than a year ago (March 11th, 2020), the knowledge about the causative etiological agent, the coronavirus SARS-Cov-2, its genome and the essential proteins of the viral cycle have advanced enormously [1]. There are also numerous investigations on vaccines, with several already approved, some candidates in clinical phase III [2,3], and massive studies on drug repurposing, with an approved drug, remdesivir (formerly under development for ebola virus) [4], and many others being investigated in advanced clinical phases all over the world [5]. This fast progress in such a short period of time is, in my opinion, mainly due to the previous basic research knowledge generated by academic groups and to the results of applied research by pharmaceutical and biotechnological companies working for years on other more or less related viruses.

In a previous peptide highlight, Susan J. Tzotzos called our attention on the interest of peptides for COVID-19, tabulating those under clinical trials for treating severe manifestations of the disease (hyper-inflammation, coagulopathy, respiratory failure), and visualizing a promising future for peptides aimed at preventing virus cell entry (inhibiting SARS-CoV-2 spike protein-ACE2 receptor interaction) [6]. Here, I will focus on recent discoveries on amino acid and short peptide derivatives as inhibitors of SARS-CoV-2 main protease, which are mostly born under the light of earlier results on SARS- and MERS-CoV protease inhibitors.

SARS-CoV-2 main protease (also named Mpro, or 3CL, or nsp5 protein) is a non-structural protein, considered a key enzyme in the viral replication cycle, and because of that it is probably the most pursuit viral target for antiviral drug discovery programs to fait against COVID-19 [7]. Numerous X-ray structures isolated or in complexes with different kind of inhibitors are already available at the Protein Data Bank. SARS-CoV-2 Mpro has high homology with SARS-CoV protease (95% sequence identity) [8]. Because of this homology, the search of inhibitors of SARS-CoV-2 Mpro started by the evaluation (virtual and experimental) of compounds targeting SARS-CoV Mpro. Most of these inhibitors are small peptide derivatives with a *C*-terminal electrophilic moiety (aldehyde, ketoamide, Michael acceptor) able to covalently react with Cys145 residue of the protein.

Starting from previously described inhibitors of SARS and MERS Mpro [9], researchers at ShanghaiTech University, using computer-aided drug design, enabled the identification of the mechanism-based inhibitor N3 (**1**, Figure 1) and the determination of its crystal structure in complex with SARS-CoV-2 Mpro [10]. In this structure, the Cys145 S atom forms a covalent bond with the vinyl group of the tetrapeptide derivative, the N-benzyl moiety extends into the S1' subsite, the lactam occupies the S1 cavity (H-bond with H163), and Leu, Val and Ala side-chains insert into S2, S3 and S4 pockets. This structure was further used for the high-throughput screening of a big library of diverse molecules, resulting in the identification of non-peptide compounds able to inhibit SARS-CoV-2 Mpro at micromolar concentrations, and one of them exhibiting promising antiviral activity in a Vero cell-based assay [10].

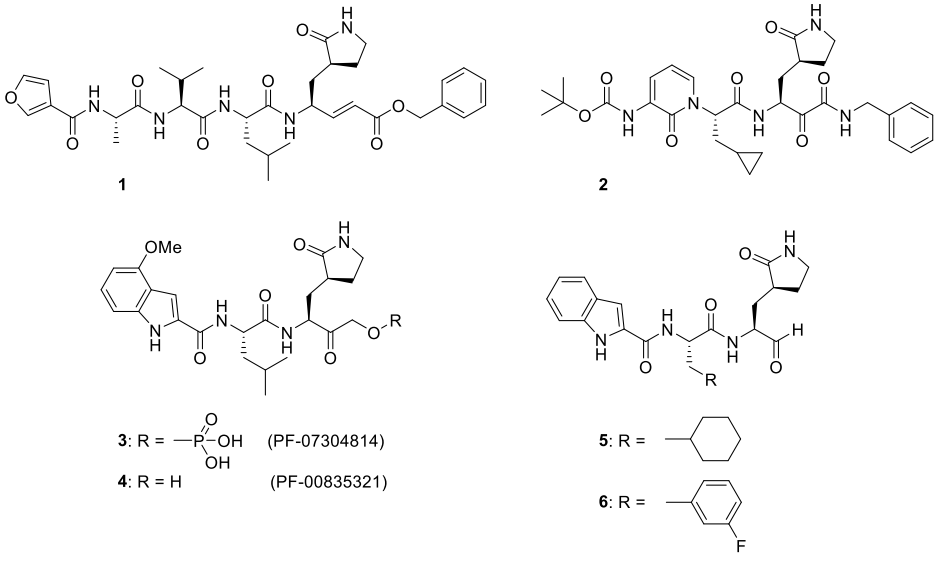

Figure 1. Small peptide derivatives described as SARS-CoV-2 Main protease inhibitors.

Shorter dipeptide derivative **2** (Figure 1) was crystalized in complex with SARS-CoV-2 Mpro, indicating again that the γ–lactam moiety inserts into the S1 subsite, acting as a Gln surrogate [11]. The CO and NH groups of this moiety form H–bonds with the imidazole NH of His166 and the CO of Phe 140, respectively. The cyclopropyl substituent accommodates at S2 subsite and the pyridone ring is situated within the substrate main chain, with the urethane NH forming a hydrogen bond with Glu166 (main chain CO). This compound is able to bind to purified recombinant SARS-CoV-2 Mpro (**2**, IC_{50} = 0.67 μM), shows antiviral activity in the micromolar range in Cau-3 infected cells, and shows a quite good ADME profile. This was a collaborative research among academic German and Chinese research groups. Several modifications in this family of compounds, following structure-guided optimization approaches, led to the discovery of potent, broad-spectrum inhibitors of Mpro of SARS-CoV coronavirus and different enteroviruses [12]. Given the high homology between coronavirus main proteases, it is expected than these compounds could also have activity against SARS-CoV-2.

Pfizer pharmaceutical company, in collaboration with several American academic groups, announced recently the first SARS-CoV-2 Mpro inhibitor, PF-07304814 (**3**, Figure 1), entering clinical trials for the treatment of COVID-19 (NCT04627532) [13]. This compound is a phosphate prodrug, precursor of PF-00835321 (**4**), a potent inhibitor *in vitro* of Mpro's of the coronavirus family. It shows IC_{50} values of 4 and 0.27 nM for SAR-CoV and SARS-CoV-2 Mpro, respectively, and has high selectivity against different human proteases [14]. Compound **4** binds covalently to Mpro of SAR-CoV and SARS-CoV-2 Mpro through the ketone group and the Cys145 sulfur atom, as shown in the co-crystal structures. In addition, an extended network of H-bond interactions, contributes to stabilization of the complex structure, specifically, the primary hydroxyl group of 3 and the catalytic His41, Leu CO and His163 side-chain, indole NH and CO with Glu 166 backbone CO and NH, respectively, and Leu NH with Gln189 side-chain. Hydrophobic Leu side-chain inserts deeply into the S2 subsite and the indole aromatic ring partially occupies the S3 subsite. Compound **4** displays potent in vitro antiviral activity in VeroE6 cells infected with SARS-CoV-2 when added alone, and has an additive activity in combination with remdesivir. The scarce aqueous solubility of **4** fosters its transformation into the corresponding phosphate derivative **3** (PF-00835231), which displays much higher solubility and is metabolized into the active compound **4** by the action of ubiquitously expressed alkaline phosphatases. This prodrug shows good ADME and safety profiles, which supports its progression into phase I clinical trials.

Quite similar indolyl covalent Mpro inhibitors have been described by the group of Wenhao Dai (Shanghai Institute of Materia Medica) and collaborators (Figure 1, compounds **5** and **6**) [15]. The structural features of the SARS-CoV-2 Mpro complexes with these indole derivatives are essentially the same as those previously commented for compound **4**, with the aldehyde group as warhead moiety and the indole group exposed to solvent in this case. In isolated SARSCoV-2 Mpro inhibition assay, compound **5** and **6** show excellent inhibitory potency (IC_{50} = 53 and 40 nM respectively). In good agreement, these compounds inhibit SARS-CoV-2 in vitro at submicromolar concentrations and are not cytotoxic, showing >130 selectivity index. Pharmacokinetic and toxicity studies suggest compound **5** as a possible candidate for clinical studies.

In 2013, the groups of Shaun R. Staffer and Andrew Mesecar (Vanderbilt and Purdue Universities) described non-covalent inhibitors of SARS-CoV Mpro, synthetically accessible through an Ugi multicomponent reaction, and defined important structure-activity relationships [16]. The best compound within this series (**7**, Figure 2), has *R*-configuration, and binds into the active site of Mpro through the pyridine moiety, which inserts in the S1 subsite and establishes a H-bond with His163 side-chain, while the tert-butyphenyl, tert-butylamide and furyl amide groups occupy S2, S3 and S1' subsites, respectively. The structure of one of these compounds (**8**), co-crystalized with SARS-CoV-2 Mpro was recently deposited at the Protein Data Bank (code 6W63), indicating identical mode of interaction as in the case of **7** with SARS-CoV isoform [17]. Lately, the Moonshot consortium disclosed some molecular modeling studies that results in the identification of optimized analogue **9**. This new derivative bears a longer, hydrophobic C-terminal carboxamide substituent, displays submicromolar binding affinity for SARS-CoV-2 Mpro (IC_{50} = 0.1 μM), and significant micromolar antiviral activity in infected cells [18].

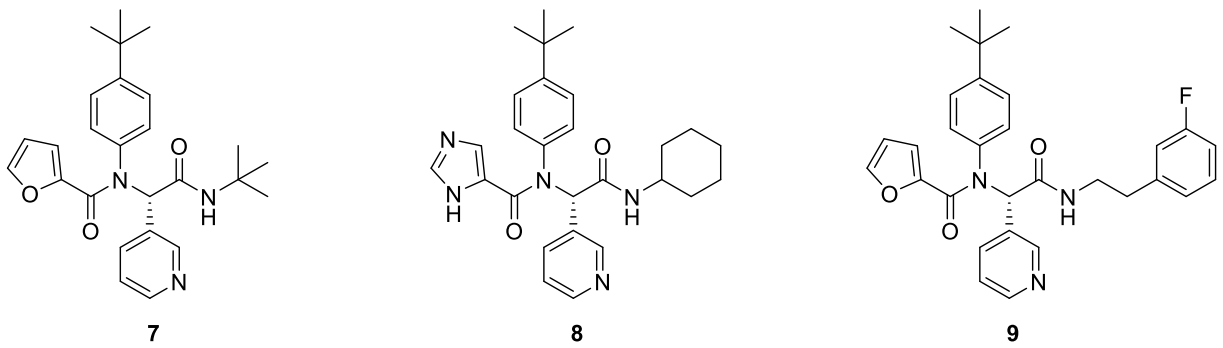

Figure 2. Amino acid derivatives as inhibitors of SARS-CoV-2 Mpro

In conclusion, substrate-derived small peptides having warhead moieties, and quite simple amino acid derivatives, all coming from previous academic campaigns directed to inhibit main protease of SARS-CoV and MERS-CoV, are being the basis for the discovery of SARS-CoV-2 antivirals. Even if this new virus emerged very recently, these efforts, channeled through the potential of a big pharmaceutical company, result in the first Mpro inhibitor reaching clinical trials. Disrupting the dimeric active form of coronaviruses Mpro enzymes just emerged as an alternative approach to antiviral agents [19]. The prospective of amino acid derivatives, peptides and peptidomimetics as covalent and non-covalent competing inhibitors, as well as dimerization disruptors, can easily be envisaged.

A last thought: all these examples are an indication of the added value of basic research in academic centers or non-profit research institutes, too often understood as superfluous expenses, and of the importance of coordination between public-private research partnerships. There is a need for closer interconnection between academia and pharma/biotech companies, and from these latter and hospitals, which provide the adequate framework for the advancement in clinical phase studies. The main lessons learned from COVID-19 pandemic is the recognition by society of the importance of research, and the requirement for collaboration at all levels, rather than competition, that would direct future research policies. Scientist's should continuously be pushing to better understand and to find treatments for unresolved medical needs, and be prepared enough for anticipating and facing emerging diseases.

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