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Design, synthesis, and biological evaluation of novel dipeptide-type SARS-CoV 3CL protease inhibitors: Structure–activity relationship study

Pillaiyar Thanigaimalai [a], Sho Konno [a], Takehito Yamamoto [a], Yuji Koiwai [a], Akihiro Taguchi [a], Kentaro Takayama [a], Fumika Yakushiji [a], Kenichi Akaji [b], Yoshiaki Kiso [b], Yuko Kawasaki [c], Shen-En Chen [c], Aurash Naser-Tavakolian [c], Arne Schön [c], Ernesto Freire [c], Yoshio Hayashi [a,*]

[a] Department of Medicinal Chemistry, Tokyo University of Pharmacy and Life Sciences, Tokyo 192-0392, Japan
[b] Department of Medicinal Chemistry, Kyoto Pharmaceutical University, Kyoto 607-8412, Japan
[c] Department of Biology, Johns Hopkins University, Baltimore, MD, USA

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ABSTRACT

This work describes the design, synthesis, and evaluation of low-molecular weight peptidic SARS-CoV 3CL protease inhibitors. The inhibitors were designed based on the potent tripeptidic Z-Val-Leu-Ala(-pyrrolidone-3-yl)-2-benzothiazole (**8**; K_i = 4.1 nM), in which the P3 valine unit was substituted with a variety of distinct moieties. The resulting series of dipeptide-type inhibitors displayed moderate to good inhibitory activities against $3CL^{pro}$. In particular, compounds **26m** and **26n** exhibited good inhibitory activities with K_i values of 0.39 and 0.33 μM, respectively. These low-molecular weight compounds are attractive leads for the further development of potent peptidomimetic inhibitors with pharmaceutical profiles. Docking studies were performed to model the binding interaction of the compound **26m** with the SARS-CoV 3CL protease. The preliminary SAR study of the peptidomimetic compounds with potent inhibitory activities revealed several structural features that boosted the inhibitory activity: (i) a benzothiazole warhead at the S1′ position, (ii) a γ-lactam unit at the S1-position, (iii) an appropriately hydrophobic leucine moiety at the S2-position, and (iv) a hydrogen bond between the *N*-arylglycine unit and a backbone hydrogen bond donor at the S3-position.

1. Introduction

Since its first appearance in Southern China in late 2002, severe acute respiratory syndrome (SARS) has been recognized as a global threat [1,2]. Its rapid and unexpected spread to 32 countries has affected more than 8000 individuals and caused nearly 800 (~10%) fatalities worldwide within a few months [1–3]. The causative SARS pathogen is a novel coronavirus, SARS-CoV [4,5]. SARS-CoV is a positive-strand RNA virus with a genome sequence that is only moderately homologous to other known coronaviruses [6,7]. SARS-CoV encodes a chymotrypsin-like protease ($3CL^{pro}$), also referred to as the main protease (M^{pro}), which plays a pivotal role in processing viral polyproteins and controlling replicase complex activity [8]. This enzyme is indispensable for viral replication and infection processes, making it an ideal target for the design of antiviral therapies. The $3CL^{pro}$ active site contains a catalytic dyad in which a cysteine residue (Cys145) acts as a nucleophile and a histidine (His41) residue acts as a general acid or base [9,10]. The SARS epidemic was successfully controlled in 2003; however, the potential reemergence of pandemic SARS-CoV continues to pose a risk, and new strains of SARS could potentially be more virulent than the strains that contributed to the 2003 outbreak. Since 2003, two additional human coronaviruses, NL63 and HKU1, have been identified in patients around the world [11,12]. Recently, a new SARS-like virus, HCoV-EMC, was identified in at least two individuals, one of whom died [13]. Very recently, the first case of a fatal respiratory illness similar to the deadly SARS was confirmed in Britain [14]. The World Health Organization (WHO) has announced that it is closely monitoring the situation and is working to “ensure a high degree of preparedness,

* Corresponding author. Tel./fax: +81 42 676 3275.
E-mail address: yhayashi@toyaku.ac.jp (Y. Hayashi).

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should the new virus be found to be sufficiently transmissible to cause a community outbreak". There is a significant need to develop anti-SARS agents that are capable of treating this potentially fatal respiratory illness. Several reports of crystalline forms of the SARS-CoV 3CL^{pro} protein bound to hexapeptidyl chloromethyl ketone inhibitors have been reported [6,9], and numerous peptidic structures have been reported in the context of targeted antiviral drug design [15–22]. The reported protease inhibitors are generally peptidic in nature, often five to three residues in length, and bear a reactive warhead group at the *C*-terminus which forms an interaction with the protease catalytic Cys145 (Fig. 1, **1**–**9**). Two of these compounds (**8** and **9**), recently described in a separate report from our group [22], exhibited excellent potent inhibitory activities with K_i values of 4.1 and 3.1 nM, respectively. These peptidic inhibitors provided valuable insight into the design constraints for this system and quickly led to the development of non-peptidic small molecule inhibitors (Fig. 1, **10**–**17**) [23–30]. These small molecular inhibitors generally showed moderate to good activities.

Recently, we performed a structure–activity relationship study based on the lead compound, Z-Val-Leu-Ala(pyrrolidone-3-yl)-2-thiazole (**7**) [21]. This study led to the discovery of the potent compounds **8** and **9**, with K_i values in the low nanomolar range [22].

Extending our studies toward the development of new anti-SARS agents, we now report the design, synthesis, and evaluation of a series of low-molecular weight dipeptide-type compounds in which the P3 valine unit is removed from the previous lead Z-Val-Leu-Ala(pyrrolidone-3-yl)-2-benzothiazole compound (**8**, Fig. 1). A preliminary SAR study led to the identification of inhibitors with moderate to good inhibitory activities. In particular, compounds **26m** and **26n** exhibited potent inhibitory activities with K_i values of 0.39 and 0.33 μM, respectively. The binding interactions of **26m** were predicted using molecular modeling studies. We describe the results of these extensive studies in detail, including the design, synthesis, molecular modeling, and biological evaluation of a series of SARS-CoV 3CL^{pro} inhibitors.

2. Results and discussion

2.1. Synthesis

The synthesis of the title inhibitors was achieved through a coupling reaction involving two key fragments, as shown in Scheme 1. One of the key fragment intermediates (**19**) was synthesized from the amino acid esters **18** with either corresponding carboxylic acids *via* 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride–1-hydroxybenzotriazole (EDC·HCl–HOBt)

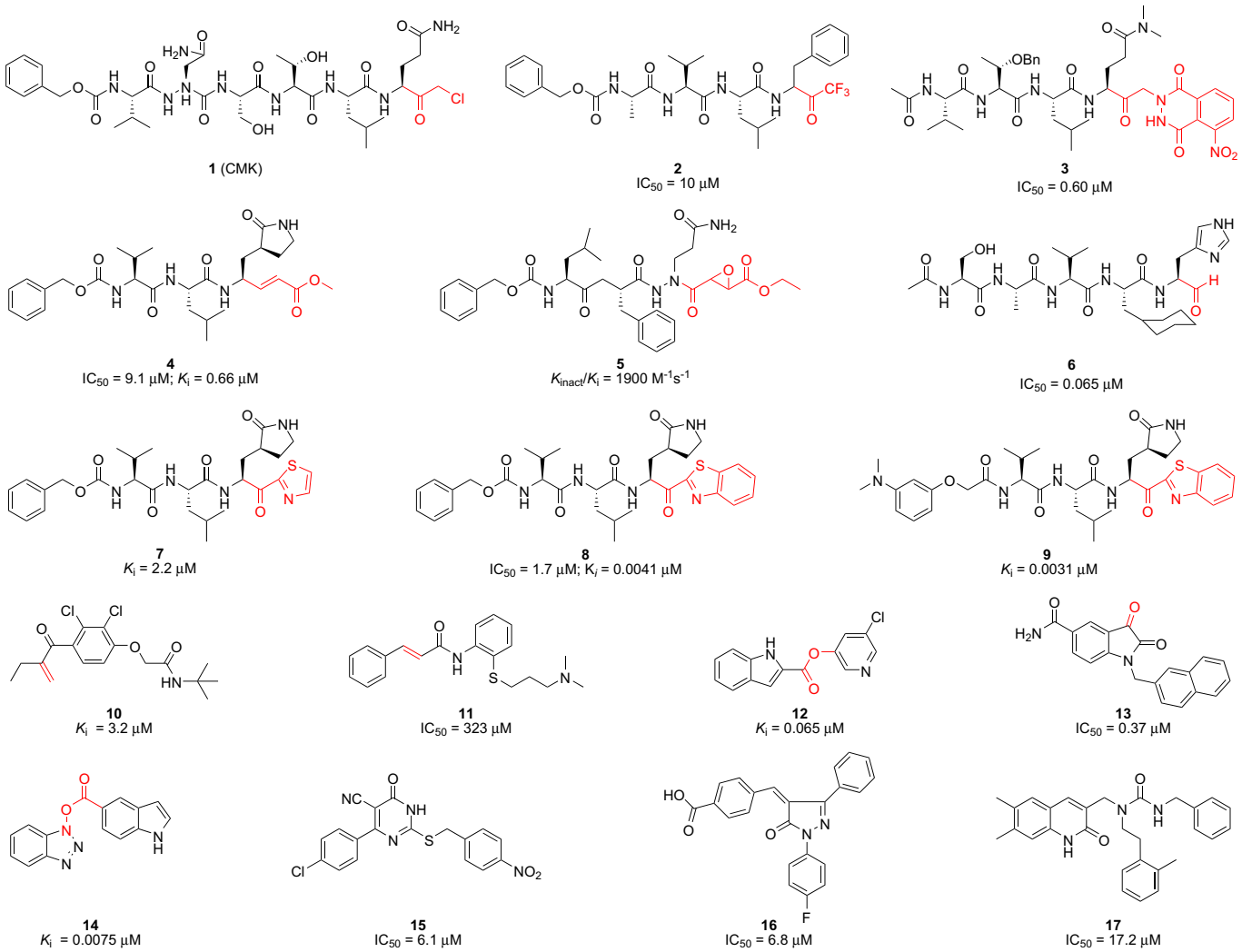

Fig. 1. Representative peptidomimetics (**1**–**9**) and small molecular (**10**–**17**) 3CL^{pro} inhibitors highlighting reactive warhead groups (red).