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# Individual and common inhibitors of coronavirus and picornavirus main proteases

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ABSTRACT

**Picornaviruses (PV) and coronaviruses (CoV) are positive-stranded RNA viruses which infect millions of people worldwide each year, resulting in a wide range of clinical outcomes. As reported in this study, using high throughput screening against ~6800 small molecules, we have identified several novel inhibitors of SARS-CoV $3CL^{pro}$ with $IC_{50}$ of low μM. Interestingly, one of them equally inhibited both $3C^{pro}$ and $3CL^{pro}$ from PV and CoV, respectively. Using computer modeling, the structural features of these compounds as individual and common protease inhibitors were elucidated to enhance our knowledge for developing anti-viral agents against PV and CoV.**


## 1. Introduction

Picornaviruses (PV) are small nonenveloped RNA viruses with a single strand of genomic RNA of 7500–8000 nucleotides [1]. The members of PV include rhinoviruses (RV), enteroviruses (EV), coxsackieviruses (CV), polioviruses, echoviruses, encephalomyocarditis viruses, meningitis virus, foot and mouth viruses, hepatitis A virus, and so on. Among them, RV is the major cause of the common cold, whereas EV and CV infection can cause hand, foot, and mouth diseases in human and animals. In severe cases, EV can damage the central nervous systems leading to viral meningitis, encephalitis, and severe myocarditis, as well as fatal pulmonary edema [2–5]. CV strain B is a major human pathogen that causes meningitis and mycocarditis leading to heart failure in young adults and congestive heart failure [6]. In these PV, a chymotrypsin-like protease (named $3C^{pro}$) is required to process polyproteins into mature proteins for viral replication, which represents a promising anti-viral drug target [7].

On the other hand, coronaviruses (CoV) are the positive-stranded RNA viruses with larger genome of 27–32 kb, which typically cause respiratory and enteric diseases, pneumonia, exacerbation of asthma, neurological symptoms, and myocarditis in humans and domestic animals. An outbreak of severe acute respiratory syndrome (SARS), caused by a novel human CoV, was spread from China to 29 countries in 2003, infecting a total of ~8000 people and killing ~800 patients [8]. SARS-CoV contains a 3C-like protease ($3CL^{pro}$) analogous to the $3C^{pro}$ of PV, responsible for processing two overlapping polyproteins, pp1a (486 kDa) and pp1ab (790 kDa). Other members of human CoV including CoV-229E, CoV-OC43, CoV-HKU1, and CoV-NL63 also require a $3CL^{pro}$ in the maturation of viral proteins.

Several inhibitors have been developed to inhibit the $3C^{pro}$ of RV and EV [9–12] and $3CL^{pro}$ of SARS-CoV [13–15]. However, their inhibitors can not be mutually used without modification. For example, AG7088, a potent inhibitor of RV and other picornaviral $3C^{pro}$ [16], failed to inhibit SARS-CoV $3CL^{pro}$ [17]. Unlike the $3CL^{pro}$, which is dimeric and in which each subunit is composed of three domains, the $3C^{pro}$ is a monomer with only the two catalytic domains. The structure-based sequence alignment (Fig. 1) shows some sequence differences, which may alter inhibitor specificity. In this study, we performed high throughput screening using a library of ~6800 compounds to find five novel inhibitors of the

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SARS-CoV 3CL$^{pro}$, 4 of which also inhibited another human CoV-229E 3CL$^{pro}$, but did not inhibit the 3C$^{pro}$ from RV14, CVB3, and EV71. But, one compound was found to almost equally inhibit these 3CL$^{pro}$ and 3C$^{pro}$. From computer modeling, we rationalized the binding discrepancy of the inhibitors against these proteases. The information is useful to further develop more potent individual or common inhibitors of 3C$^{pro}$ and 3CL$^{pro}$ of PV and CoV for antiviral drug discovery.

## 2. Methods

### 2.1. Expression and purification of the proteases

Two types of proteases including 3CL$^{pro}$ from SARS-CoV and CoV-229E and 3C$^{pro}$ from CVB3, EV71, and RV14 were used to assay the inhibitors in this study. The SARS-CoV 3CL$^{pro}$ and EV71 3C$^{pro}$ were prepared as reported previously [12,18]. For expressing CVB3, RV14, and CoV-229E proteases, the genes were cloned from viral cDNAs by using polymerase chain reaction (PCR) as reported elsewhere.

### 2.2. Primary screening

For screening, 0.05 μM SARS 3CL$^{pro}$, 6 μM fluorogenic substrate Dabcyl-KTSAVLQSGFRKME-Edans, and 50 μM of approximately 6800 compounds provided by Korea Chemical Bank (Daejeon, Korea) were used. Enhanced fluorescence of the reactions in the buffer of 20 mM Bis-Tris at pH 7.0 was monitored at 538 nm with excitation at 355 nm using a fluorescence plate reader. The compounds which inhibited more than 50% of the protease activity at 50 μM were selected for the next assay run at 10 μM.

### 2.3. $IC_{50}$ determination

The five hits that inhibited SARS-CoV 3CL$^{pro}$ at 10 μM were also evaluated against CoV-229E 3CL$^{pro}$, EV71 3C$^{pro}$, CVB3 3C$^{pro}$, and RV14 3C$^{pro}$. In the assay solution, the activities of these proteases (0.5 μM) with 10 μM fluorogenic substrate in the buffers of 10 mM MES at pH 6.5 and 6.0 (the optimal pH for EV71 and RV14 proteases, respectively) and 10 mM HEPES at pH 7.5 (for CoV-229E and CVB3 proteases) were measured in the presence

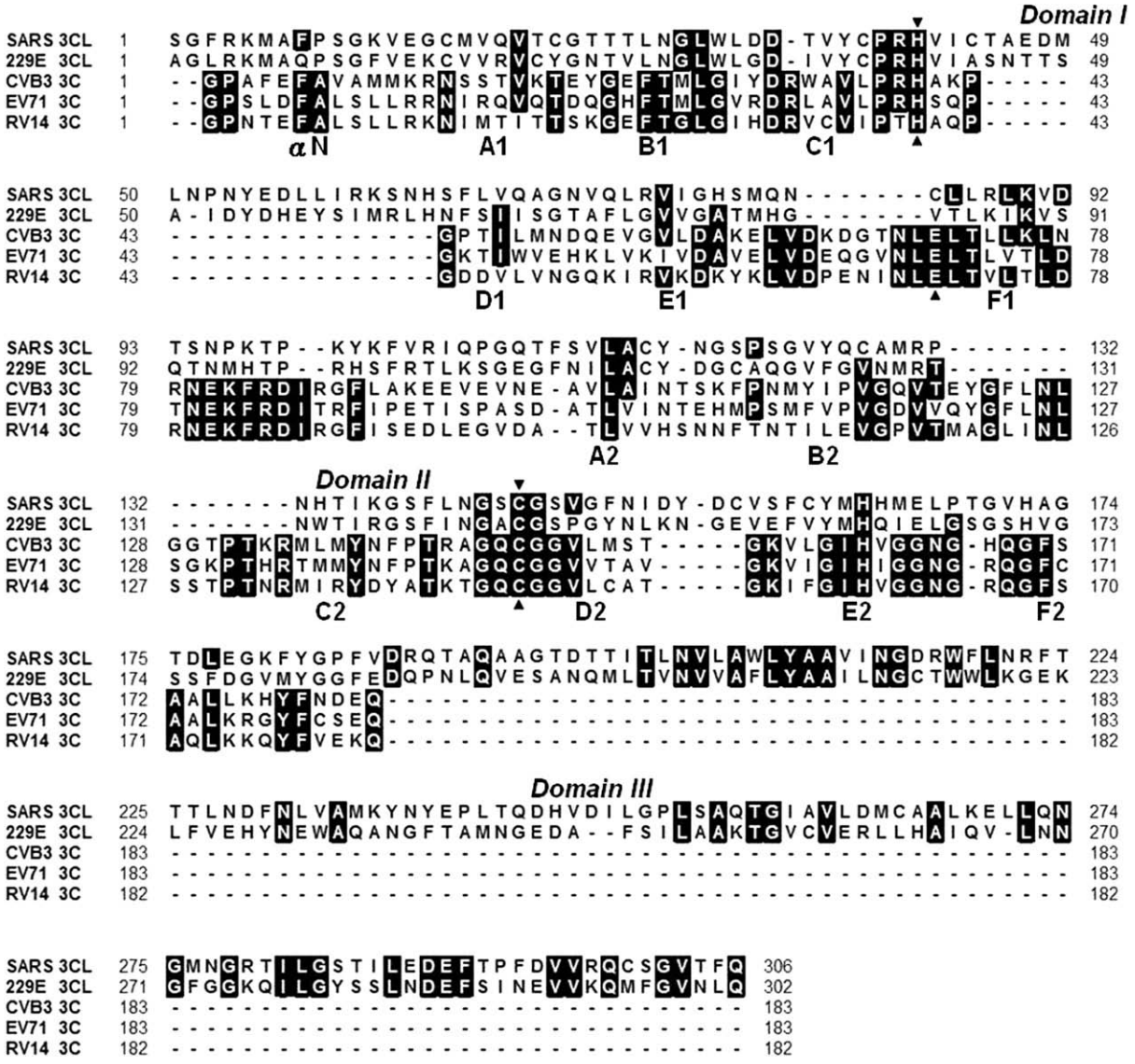


**Fig. 1.** A structure-based sequence alignment of SARS-CoV 3CL$^{pro}$, CoV-229E 3CL$^{pro}$, CVB3 3C$^{pro}$, EV71 3C$^{pro}$, and RV14 3C$^{pro}$. The domains according to 3CL$^{pro}$ are shown above the sequence and the secondary elements according to the 3C$^{pro}$ structure are shown below it. Arrows indicate the essential catalytic amino acids His and Cys for 3CL$^{pro}$ and 3C$^{pro}$, and Glu (only for 3C$^{pro}$).