Supplementary Experiment Procedures

Protein production and crystallization

The genomic region that codes for New SARS-Like Coronavirus 3C-like Protease was chemical synthesized following the sequence of Human betacoronavirus 2c EMC/2012 (HCoV-EMC). *BamH*I and *Xho*I restriction sites were attached to the 5' and 3' ends separately by PCR, and the PCR product was inserted into the pET-28b-SUMO vector. The recombinant plasmids were transformed into *Escherichia coli* strain BL21 (DE3) (TransGen Biotech). kanamycin-resistant colonies were grown in Luria–Bertani medium at 37 °C until the OD_{600} reached 0.8. Isopropyl β-D-1-thiogalactopyranoside was added to a final concentration of 0.1 mM, and the cultures were grown for an additional 20 h at 16 °C. Cells were harvested by centrifugation, resuspended, and homogenized in lysis buffer containing 20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 4 mM $MgCl_2$ using a low-temperature ultra-high pressure cell disrupter (JNBIO, Guangzhou, China). The lysate was centrifuged at 20 000 *g* for 30 min to remove cell debris. The supernatant was loaded onto a Ni^{2+}-NTA agarose column (Qiagen). After washed with 20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 4 mM $MgCl_2$ and 20 mM imidazole, the SUMO moiety was cleaved by His-tagged SUMO protease(Ulp) at 16°C overnight. The elution was concentrated, and then changed into a buffer containing 20 mM Tris-HCl (pH 8.0), 40 mM NaCl, and 4mM $MgCl_2$. The sample was further purified using a Hitrap Q column (GE Healthcare) with a linear gradient from 40mM to 150 mM NaCl with 20 mM Tris-HCl (pH 8.0), and 4mM

$MgCl_2$. The target proteins were concentrated to 10 mg/mL in a buffer with 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, and 4mM $MgCl_2$ for storage.

The N3 inhibitor was synthesized by solid-phase peptide synthesis, and then purified to >98% by HPLC. The purified HCoV-EMC 3CL^{pro} and N3 inhibitor were mixed at 1:2 molar ratio and pretreated at 16 °C for 2 h before crystallization. Crystallization of the complex was performed at 16 °C using the hanging-drop vapor-diffusion method. Crystals appeared and reached their final size within 1 day in a well solution containing 200 mM Trimethylamine N-oxide dihydrate, 0.02 M cadmium chloride hydrate, 100 mM Tris-HCl (pH 8.5), and 16% w/v polyethylene glycol monomethyl ether 2000. Crystals were transferred to a 4.0 M sodium formate solution for 30 s prior to flash freezing, and then stored in liquid nitrogen for data collection.

X-ray diffraction data collection, processing, and structure determination

The diffraction data of the HCoV-EMC 3CL^{pro}-N3 complex were collected to 2.27 Å resolution at 100 K using an ADSC Q270 CCD detector on the beamline BL17A of Photon Factory (PF, Japan) with a wavelength of 1.0000 Å. Data were processed and scaled using the HKL2000 package [1]. The crystal belonged to the space group $P2_12_12_1$, with one HCoV-EMC 3CL^{pro} dimer per asymmetric unit, corresponding to a solvent content of 47% [2]. The complex structure was determined using PHASER [3] with the crystal structure of SARS-CoV 3CL^{pro} (PDB code: 2AMQ) as the initial searching model. The presence of bound N3 molecules was initially revealed by the difference in the density map. Manual model construction and refinement were performed with COOT [4] and PHENIX

[5] following rigid body refinement, energy minimization, and individual B-factor refinement. The quality of the final refined model was verified using the program PROCHECK [6]. The final refinement statistics are summarized in Table S1. Structural figures were drawn using the program PyMOL [7].

In vitro inhibitory assay

The activity of HCoV-EMC 3CL^{pro} was measured by continuous kinetic assays, using an identical fluorogenic substrate MCA-AVLQSGFR-Lys(Dnp)-Lys-NH2 (GL Biochem, China). The fluorescence intensity was monitored with a Fluoroskan Ascent instrument (ThermoLabsystems, Finland) using wavelengths of 320 and 405 nm for excitation and emission, respectively. The experiments were performed with a buffer consisting of 50 mM Tris-HCl (pH 7.3) with 1 mM EDTA. The reaction was initiated by adding protease (final concentration of 1 μM) to a solution containing the substrate. Fluorescence was monitored at 1 point per 2 s. The curves were fitted and IC_{50} was calculated in GraphPad Prism software.

Supplementary Figures

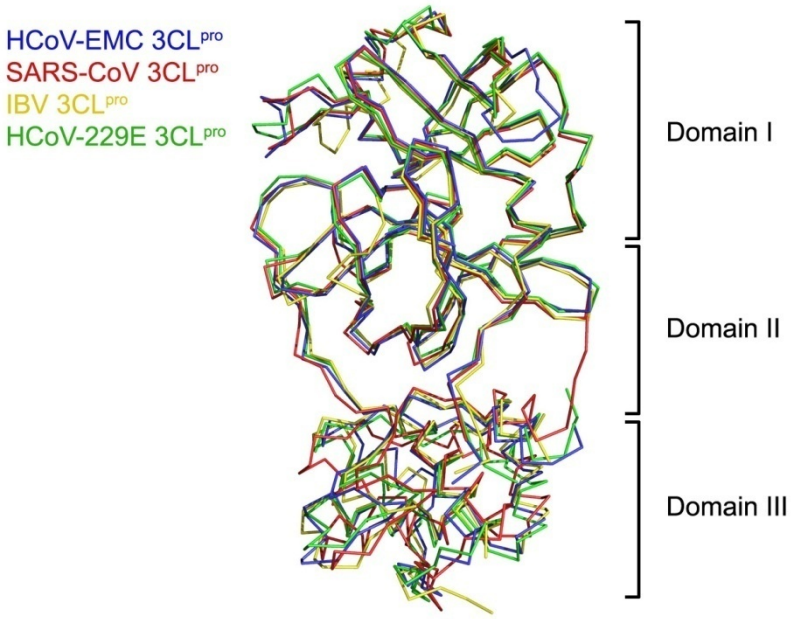

Figure S1. Structural comparison of CoV 3CL^{pro} from different groups. The crystal structures 3CL^{pro} from HCoV-EMC, SARS-CoV (group II), IBV (group III) and HCoV-229E (group I) are aligned and shown as blue, red, yellow and green ribbons. Domain I, II and III for a conserved 3CL^{pro} architecture are labeled in the left panel.

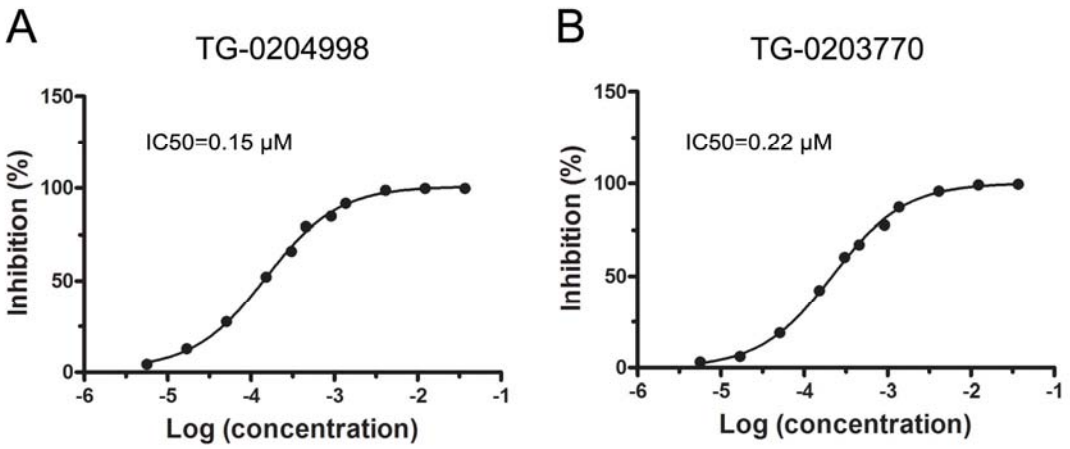

Figure S2 Anti-3CL^{pro} activities of two reported peptidomimetic TG-0204998 (A) and TG-0203770 (B).

Supplementary Tables

Table S1. Data collection and refinement statistics.

Parameters	HCoV-EMC 3CL^{pro}-N3 complex
Data collection statistics	
Cell parameters	
a (Å)	79.6
b (Å)	93.2
c (Å)	103.1
α, β, γ (°)	90.0, 90.0, 90.0
Space group	$P2_12_12_1$
Wavelength used (Å)	1.0000
Resolution (Å)	50.00–2.27 (2.39-2.27) [c]
No. of all reflections	240,588 (33,884)
No. of unique reflections	37,548 (5,134)
Completeness (%)	100.0 (100.0)
Average I/σ(I)	13.5 (6.0)
R_{merge} [a] (%)	10.2 (53.4)
Refinement statistics	
No. of reflections used (σ(F) > 0)	33,516
R_{work} [b] (%)	22.1
R_{free} [b] (%)	27.8
r.m.s.d. bond distance (Å)	0.008
r.m.s.d. bond angle (º)	1.309
Average B-value (Å^2)	45.4
No. of protein atoms	4,586
No. of ligand atoms	98
No. of solvent atoms	257
Ramachandran plot	
Res. in allowed regions (%)	95.0
Res. in generously allowed regions (%)	5.0
Res. in disallowed regions (%)	0.0

[a] R_{merge} = $\Sigma_h\Sigma_i$ | I_{ih}–<I_h> |/$\Sigma_h\Sigma_i$ <I_h>, where <I_h> is the mean of the observations I_{ih} of reflection h.

[b] R_{work} = Σ(||F_p(obs)|–|F_p(calc)||)/ Σ|F_p(obs)|; R_{free} is an R factor for a pre-selected subset (5%) of reflections that was not included in refinement.

[c] Numbers in parentheses are corresponding values for the highest resolution shell.

Supplementary References

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