Brief Articles

Design and Synthesis of Dipeptidyl Glutaminyl Fluoromethyl Ketones as Potent Severe Acute Respiratory Syndrome Coronovirus (SARS-CoV) Inhibitors

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This paper describes the design and synthesis of dipeptidyl *N*,*N*-dimethyl glutaminyl fluoromethyl ketones (fmk) as severe acute respiratory syndrome coronovirus (SARS-CoV) inhibitors. The compounds were tested against SARS-CoV-induced cell death in Vero or CaCo2 cells as a measurement of the inhibiting effects of the compounds on the replication of the virus. *Z*-Leu-Gln(NMe_2)-fmk (**6a**) was found to be a potent inhibitor with low toxicity in cells, protecting cells with an EC_{50} value of 2.5 μM and exhibiting a selectivity index of >40.

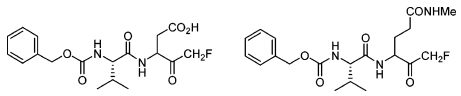
Introduction

Through a collaborative international effort that was initiated during the 2003 severe acute respiratory syndrome (SARS) outbreak, the causative pathogen of SARS has been identified as a novel coronavirus (SARS-CoV).[1] Human coronaviruses (HCoVs) were previously associated with predominantly mild respiratory illnesses, occasionally causing serious infections of the lower respiratory tract in children and adults. In contrast, SARS is marked by an unusually high mortality rate, having infected over 8000 individuals in 29 countries and leaving nearly 800 dead during its 2003 outbreak. The rapid spread of SARS to developed countries within a few months of its origin in Asia highlights the vulnerability of large, diverse, and geographically distant populations to the disease. SARS-CoV is the first coronavirus that is known to regularly cause severe disease in humans.

Coronaviruses, including SARS-CoV, are known to encode several viral proteases for the proteolytic processing of polyproteins to release the functional proteins. Coronavirus proteases have been found to be cysteine proteases, including a chymotrypsin-like main protease, M^{pro}, which is analogous to the main picornavirus protease, $3C^{pro}$, and is essential for viral replication.[2] The conservation of substrate specificities within the M^{pro} protease family of coronaviruses has been reported with the amino acid sequence L-Q-S or L-Q-A as the preferred P_2-P_1-P_1' sequences.[3] M^{pro} exhibits a similarity of cleavage site specificity to that of $3C^{pro}$ of picornaviruses, a family of viruses that also cause respiratory illnesses. The functional significance of M^{pro} in the coronavirus life cycle, as well as its extremely low sequence similarity to other cellular proteinases, makes it an ideal target for the discovery and development of novel drugs against SARS.

The rapid emergence and spread of the SARS-CoV caught public health agencies off guard, and currently there is no effective treatment for SARS. Major efforts to develop SARS vaccines and immune therapies have been initiated and have encountered some problems, including evasion of antibody neutralization due to genetic diversity among SARS viruses.[4] Therefore it is critical to discover and develop novel and effective treatments for SARS in preparation for future outbreaks.

Chart 1



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Several compounds have been reported as inhibitors of SARS-CoV.[5] Through the screening of a library of >10000 compounds to inhibit viral replication, a peptide inhibitor of HIV protease was found to inhibit SARS-CoV M^{pro} with a K_i value of 0.6 μM and showed protective effect in the cell based viral replication assay at a concentration of 10 μM.[6] Another group screened 50240 small molecules in the cell based viral replication assay. One of the active compounds was identified as an inhibitor of SARS-CoV M^{pro} with an IC_{50} value of 2.5 μM in the enzyme assay and an EC_{50} value of 7 μM in the cell protection assay.[7] Through the screening of 50000 compounds in a quenched fluorescence resonance energy transfer assay against SARS-CoV M^{pro}, followed by additional studies, five novel small molecules that show inhibitory activity (IC_{50} = 0.5−7 μM) against SARS-CoV M^{pro} were identified.[8] A series of keto-glutamines with a phthalhydrazido group in the α-position were synthesized based on the enzyme recognizing peptide substrates sequence as inhibitors of SARS-CoV M^{pro}. These tetrapeptide based molecules were found to have IC_{50} values ranging from 0.60 to 70 μM in the enzyme assays.[9] In addition, glycyrrhizin (GL) has been reported to inhibit SARS-CoV replication in vitro with an EC_{50} value of 365 μM, and several more active analogues of GL have also been discovered.[10]

We have been studying the design and synthesis of inhibitors of caspases, which are a class of cysteine proteases that play a critical role in apoptosis. Our dipeptide based caspase inhibitors, such as **7** (MX1013, *Z*-Val-Asp-fmk, Chart 1), are active in

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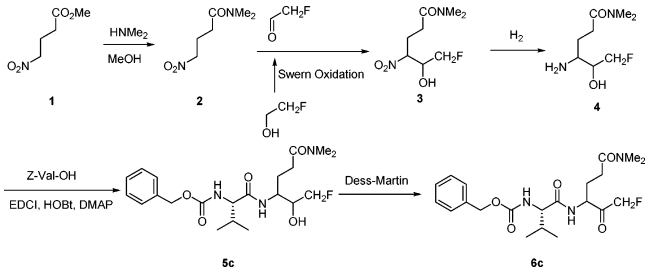
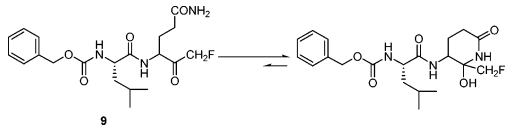
Scheme 1



Scheme 2



inhibiting apoptosis in cell culture models (IC_{50} < 500 nM) and are highly efficacious in animal models, including mouse acute liver failure model and mouse and rat models of brain ischemia/reperfusion injury and acute myocardial infarction.[11] Since SARS-CoV M^{pro} is also a cysteine protease, we sought to apply our knowledge from the studies of caspase inhibitors toward the discovery of potential SARS-CoV inhibitors. Herein we report the design and synthesis of a series of dipeptidyl glutaminyl fluoromethyl ketones as novel inhibitors of SARS-CoV.

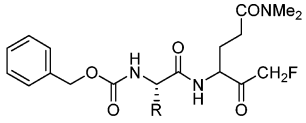
Results and Discussion

Synthesis. It is known from the substrate cleavage site specificity of SARS-CoV M^{pro} that glutamine is essential as the P_1 amino acid. Leucine is the preferred P_2 amino acid, while valine and phenylalanine are also allowed.[12] We therefore designed and synthesized a series of dipeptides, using *N*,*N*-dimethyl-Gln-fmk as the P_1 amino acid and warhead, in combination with different P_2 amino acids. These dipeptide-fmk inhibitors were prepared in five steps as shown in Scheme 1 for Cbz-Val-*N*,*N*-dimethyl-Gln-fmk (**6c**). The intermediate amino-alcohol **4** was prepared by conversion of the ester **1** to the amide **2** by reaction with dimethylamine, followed by reaction of amide **2** with the aldehyde intermediate that is produced by Swern-oxidation of 2-fluoroethanol to produce the nitro-alcohol **3**, which was then reduced by hydrogenation. Coupling of **4** with a *Z*-protected valine produced amide **5c**, which was oxidized by Dess−Martin reagent to produce **6c**. Dipeptides **6a**, **6b**, **6d**, and **6e** were prepared similarly by replacing *Z*-valine with other *Z*-protected amino acids. We also explored the synthesis of dipeptides with *N*-methyl-glutamine and glutamine as the P_1 amino acid. The *N*-methyl-glutamine analogue was prepared by using $MeNH_2$ instead of $NHMe_2$, and the glutamine analogue was prepared by using ammonia instead of $NHMe_2$. As expected, the NH_2 of the glutamine side chain in compound **9** was found to predominantly cyclize with the carbonyl of the fmk group (Scheme 2), as observed by ^1H NMR in solution,[13] similar to what has been reported for a glutamine-aldehyde based inhibitor,[14] whereas the *N*,*N*-dimethyl-glutamine analogues **6a**−**6e** could not form the cyclized structure.[15] The *N*-methyl-glutamine analogue **8** was found to partially form the cyclized structure as observed by ^1H NMR in solution.[16]

Biological Results. The dipeptides were first tested against SARS-CoV infected Vero cells as described previously.[10] The assays measure the protective effects of compounds against SARS-CoV-induced cell death (cytopathic effect (CPE)) as a measurement of the inhibiting effects of compounds on the replication of SARS-CoV. Antiviral activity assessed by CPE inhibition in SARS-CoV infected Vero cultures revealed that compounds **6a**−**6c** were effective inhibitors of both FFM1 and 6109 strains of SARS-CoV replication (Table 1). Compound **6a** has an EC_{50} value of 2.5 μM in the Vero cells infected with strain 6109. In the toxicity assay, incubation of confluent CaCo2 or Vero cell layers with different concentrations of the dipeptides for 3 days did not reveal any toxic effects at concentrations up to 100 μM, indicating that the toxicity CC_{50} values for all compounds are >100 μM. This provides a selectivity index (SI) value of >40 for compound **6a**. Compound **6a** was about 2-fold more potent than compounds **6b** and **6c**. This is consistent with the substrate specificity of SARS-CoV M^{pro} that leucine is the dominant P_2 amino acid (>75%). Valine and phenylalanine also can be the P_2 amino acid,[12] suggesting a preference for a hydrophobic side chain at the P_2 position. Compounds **6d** and **6e** with glycine and alanine as the P_2 amino acid did not inhibit virus replication at concentrations up to 100 μM (maximum concentration tested), confirming that a hydrophobic side chain in the P_2 position such as those in leucine, isoleucine, and valine is important for activity. Compound **7**, a potent caspase inhibitor with an aspartic acid as P_1, did not inhibit virus replication up to 100 μM. This is in agreement with the specificity of SARS-CoV M^{pro} substrate that glutamine is essential in the P_1 position. The high potency of compounds **6a**−**6c** also suggests that the NH_2 group of the glutamine side chain might not be important for hydrogen bonding interactions with SARS-CoV M^{pro}. It has been reported that for hepatitis A virus (HAV) 3C proteinase, a class of protease having similar substrate specificity as that of M^{pro} of coronavirus, the P_1 glutamine can be replaced by *N*,*N*-dimethylglutamine with no impact on substrate peptide recognition or cleavage.[17]

To show that drugs exert the antiviral activity independently of cell type, effects of compounds **6a**−**6e** on CPE were tested in the human colon carcinoma cell line CaCo2 infected with the FFM1 virus strain. The results were similar to those obtained in Vero cells (Table 1). Compound **6a** was 3- to 5-fold more potent than compounds **6b** and **6c**, respectively. Compounds **6d** and **6e** were again not active up to 100 μM.

Table 1. Effect of SARS-CoV Inhibitors on FFM1 or 6109 SARS-CoV Replication in Vero and CaCo2 Cells



		EC_{50} (μM)[a]			CC_{50} (μM)[b]	SI[c]
		Vero		CaCo2	Vero	
Entry	R	FFM1	6109	FFM1		
6a		3.6 ± 1.3	2.5 ± 0.4	2.4 ± 0.56	>100	>40
6b		8.9 ± 2.9	5.3 ± 1.7	8.8 ± 2.5	>100	>18
6c		6.2 ± 1.9	6.6 ± 3.0	12.6 ± 4.1	>100	>15
6d	H	>100	>100	>100	>100	-
6e	Me	>100	>100	>100	>100	-
7		>100	>100	>100	>100	-

[a] Concentration of compound inhibiting cytopathic effect to 50% of untreated cells. Values represent the mean ± SD from three independent experiments. [b] Incubation of confluent CaCo2 or Vero cell layers with different concentrations of all the dipeptides for 3 days did not reveal any toxic effects at concentrations up to 100 μM. [c] Ratio of CC_{50} to EC_{50} in Vero cells with strain 6109.

It is known that picornaviruses, including human rhinoviruses (HRV), also encode a viral 3C protease with specificity for glutamine−glycine cleavage for the processing and releasing of viral proteins that are essential for the replication of the viruses.[18] We therefore sought to test our compounds against rhinoviruses. Interestingly, compounds **6a**−**6c** were found to be inactive against rhinovirus type-2 in a cell based assay,[19] suggesting that compounds **6a**−**6c** might be specific against SARS-CoV and may not inhibit nonspecifically all the viruses that encode a 3C protease. There are several possible reasons. For example, the P_1 *N*,*N*-dimethyl-glutamine might not be preferred by rhinovirus 3C,[20] or a tripeptide structure might be important for inhibition of rhinovirus. Interestingly, rupintrivir (AG7088), the novel HRV 3C protease inhibitor with broad-spectrum antiviral activity against all HRV serotypes,[21] has been reported to be inactive against SARS-CoV (IC_{50} > 100 μM),[22] suggesting that there might be significant differences between the structure of HRV 3C protease and SARS-CoV M^{pro}.

The toxicity of compound **6c** in mice was tested by IV injection (5% dextrose/water solution) of a single dose at 25, 50, and 100 mg/kg, followed by observation for one week. No weight loss or behavioral changes nor any gross pathology change of the major organs was observed at the tested doses. This is in agreement with the low toxicity observed in cell assay for **6c** (CC_{50} > 100 μM). It is expected that related analogues of **6c** should also have low toxicity.

In conclusion, we have designed and synthesized a group of dipeptidyl glutaminyl fluoromethyl ketones as potential inhibitors of SARS-CoV M^{pro}. The antiviral activity of these compounds was assessed by CPE inhibition in SARS-CoV infected Vero and CaCo2 cultures. Compound **6a** was found to have an EC_{50} value of 2.5 μM in the CPE inhibition assay with a SI value of >40, presumably through the inhibition of SARS-CoV M^{pro}. Preliminary SAR studies showed that glutamine is important as the P_1 amino acid and *N*,*N*-dimethyl-glutamine is tolerated at P_1. Leucine is the preferred P_2 amino acid, which can be replaced by isoleucine and valine. Compound **6c** also has been found to have low toxicity in mice, suggesting that these SARS-CoV inhibitors should have a good safety profile for animal efficacy studies.

In addition, picornaviruses, including several important human and veterinary pathogens, such as poliovirus and coxsackievirus (enterovirus), foot-and-mouth disease virus (aphthovirus), encephalomyocarditis virus (cardiovirus), hepatitis A virus (hepatovirus), and human rhinoviruses (rhinovirus), also encode a viral 3C protease with specificity for glutamine−glycine cleavage for the processing and releasing of viral proteins that is essential for the replication of the viruses.[18] Therefore our approach for the design and synthesis of protease inhibitors of SARS-CoV could also be applied to the discovery of inhibitors of picornaviral 3C proteases for the treatment of diseases that are caused by picornaviruses. Additional studies, such as incorporating other amino acids at P_2, replacing the dipeptide structure with a tripeptide, modifying the P_1 glutamine side chain, converting the dipeptide structure into a non-peptide structure to improve oral bioavailability, and animal efficacy studies, will be reported in the future.

Experimental Section

General Methods and Materials. Commercial-grade reagents and solvents were obtained from Aldrich; *Z*-amino acids were obtained from Advanced ChemTech and were used without further purification. The ^{1}H NMR spectra were recorded at 300 MHz. Chemical shifts are reported in parts per million (ppm) downfield from TMS (0.00 ppm), and *J* coupling constants are reported in hertz. Elemental analyses were performed by Numega Resonance Labs, Inc. (San Diego, CA). For cell based antiviral assays, the compounds were dissolved in DMSO at a concentration of 50 mM and stored in aliquots at −20 °C.

***N*,*N*-Dimethyl-4-nitrobutyramide (2).** To a solution of methyl-4-nitrobutyrate (1.0 g, 6.8 mmol) in methanol (10 mL) was added 40% dimethylamine water solution (12.5 mL, 102 mmol). The solution was stirred at room temperature for 48 h, concentrated by vacuum, and diluted with ethyl acetate (20 mL). The organic layer was dried and evaporated. The residue was purified by flash chromatography over silica gel (hexane/EtOAc = 1:1) to give **2** (0.99 g, 91%). ^{1}H NMR ($CDCl_3$): 4.53 (t, J = 6.6, 2H), 3.02−2.94 (m, 6H), 2.48−2.43 (m, 2H), 2.36−2.31 (m, 2H).

6-Fluoro-5-hydroxy-4-nitrohexanoic Acid Dimethylamide (3). To a cooled (−78 °C) solution of oxalyl chloride (0.9 mL, 9.6 mmol) in anhydrous dichloromethane (5 mL) was added anhydrous DMSO (1.4 mL, 19 mmol) dropwise, and the reaction mixture was stirred for 15 min. A solution of 2-fluoroethanol (0.44 mL, 7.5 mmol) in dichloromethane (2 mL) was slowly added into the reaction flask. The reaction mixture was stirred for 15 min; then it was diluted with dichloromethane (60 mL), followed by addition of dry Et_3N (4.4 mL, 31 mmol). The reaction mixture was stirred for 15 min, then warmed to 0 °C and stirred at 0 °C for 2 h. To the reaction mixture was added a solution of *N*,*N*-dimethyl-4-nitrobutyramide (0.99 g, 6.3 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred at 0 °C for 3 h and then stirred at room-temperature overnight. The mixture was concentrated and extracted with ethyl acetate. The organic layer was dried and evaporated. The residue was purified by silica gel (CH_2Cl_2/EtOAc = 1:3) to give 1.2 g (89%) of **3** as a colorless viscous oil. ^{1}H NMR ($CDCl_3$): 5.19 (bs) and 5.02 (bs, 1H), 4.80−4.40 (m, 3H), 4.25 (bs) and 4.20 (bs, 1H), 3.02−2.94 (m, 6H), 2.45−2.20 (m, 4H).

4-Amino-6-fluoro-5-hydroxyhexanoic Acid Dimethylamide (4). To a solution of 6-fluoro-5-hydroxy-4-nitrohexanoic acid dimethylamide (1.2 g, 5.5 mmol) in MeOH (20 mL) was added Ranny Ni (about 500 mg), and the mixture was hydrogenated under 40−45 psi H_2 at room temperature for 6 h. The catalyst was removed. The MeOH solution was evaporated, and the residue oil (0.92 g, yield 87%) was used without further purification. ^{1}H NMR ($CDCl_3$): 4.70−4.40 (m, 2H), 3.80−3.50 (m, 1H), 3.20−2.80 (m, 8H), 2.60−2.40 (m, 2H), 2.00−1.65 (m, 2H).

4-(*Z*-Val-amido)-6-fluoro-5-hydroxyhexanoic Acid Dimethylamide (5c). To a solution of *Z*-valine (1.2 g, 5.0 mmol) in THF (30 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI; 0.95 g, 5.0 mmol), 1-hydroxybenzotriazole (HOBT; 0.67 g, 5.0 mmol), and 4-dimethylaminopyridine (DMAP) (0.30 g, 2.5 mmol). The resulting mixture was stirred for 10 min; then a solution of 4-amino-6-fluoro-5-hydroxyhexanoic acid dimethylamide (0.95 g, 5.0 mmol) in THF (2 mL) was added, and the mixture was stirred at room temperature overnight. The solvent was evaporated, and the residue was diluted with ethyl acetate (100 mL). The solution was washed with brine (10 mL), and the organic layer was dried and evaporated. The residue was purified by chromatography over silica gel (hexane/EtOAc = 1:1 to EtOAc/MeOH = 3:1) to give 1.2 g (58%) of **5c** as a white solid. ^{1}H NMR (acetone-d_6): 7.40−7.30 (m, 5H), 7.10 (m, 1H), 6.40 (m, 1H), 5.05 (s, 2H), 4.60−4.15 (m, 3), 4.05−3.80 (m, 3H), 3.00−2.85 (m, 6H), 2.40 (m, 2H), 2.20−2.09 (m, 1H), 2.00−1.75 (m, 2H), 1.00−0.90 (m, 6H).

4-(*Z*-Val-amido)-6-fluoro-5-oxohexanoic Acid Dimethylamide (6c). To a solution of 4-(*Z*-Val-amido)-6-fluoro-5-hydroxyhexanoic acid dimethylamide (0.90 g, 2.1 mmol) in CH_2Cl_2 (60 mL) at 0 °C was added Dess−Martin periodinane (2.7 g, 6.0 mmol). The resulting mixture was stirred at room temperature for 4 h, the solvent was evaporated, and the residue was diluted with ethyl acetate (80 mL). It was washed with brine (30 mL), and the organic layer was dried and concentrated. The residue was purified by column chromatography (EtOAc/MeOH = 8:1) to give **6c** (0.87 g, 97%) as a solid. ^{1}H NMR (acetone-d_6): 7.95 (bs, 1H), 7.38−7.29 (m, 5H), 6.43 (bs, 1H), 5.30−5.20 (m, 1H), 5.14−5.05 (m, 3H), 4.55−4.51 (m, 1H), 4.09−4.00 (m, 1H), 2.98−2.82 (m, 6H), 2.50−2.46

(m, 2H), 2.17−2.09 (m, 2H), 2.00−1.88 (m, 1H), 0.99−0.92 (m, 6H). Anal. ($C_{21}H_{30}FN_3O_5 \cdot 0.7H_2O$) C, H, N.

The following compounds were prepared from the corresponding *Z* protected amino acid and **4** in two steps by a procedure similar to that described for the preparation of compounds **5c** and **6c**.

4-(*Z*-Leu-amido)-6-fluoro-5-oxohexanoic Acid Dimethylamide (6a). Colorless solid. ^{1}H NMR (acetone-d_6): 8.00−7.94 (m, 1H), 7.38−7.30 (m, 5H), 6.57 (m, 1H), 5.29−5.18 (m, 1H), 5.13−5.09 (m, 3H), 4.50−4.47 (m, 1H), 4.22−4.14 (m, 1H), 2.99−2.80 (m, 6H), 2.49−2.44 (m, 2H), 2.15−2.11 (m, 1H), 1.96−1.89 (m, 1H), 1.79−1.70 (m, 1H), 1.64−1.59 (m, 2H), 0.95−0.91 (m, 6H). Anal. ($C_{22}H_{32}FN_3O_5 \cdot 0.5H_2O$) C, H, N.

4-(*Z*-Ile-amido)-6-fluoro-5-oxohexanoic Acid Dimethylamide (6b). Oil. ^{1}H NMR (acetone-d_6): 7.95−7.93 (m, 1H), 7.38−7.29 (m, 5H), 6.43 (m, 1H), 5.30−5.20 (m, 1H), 5.14−5.04 (m, 3H), 4.54−4.48 (m, 1H), 4.09−4.00 (m, 1H), 2.99−2.80 (m, 6H), 2.50−2.46 (m, 2H), 2.16−2.11 (m, 1H), 1.97−1.88 (m, 2H), 1.61−1.52 (m, 1H), 1.30−1.17 (m, 1H), 0.96−0.86 (m, 6H). Anal. ($C_{22}H_{32}FN_3O_5$) C, H, N.

4-(*Z*-Gly-amido)-6-fluoro-5-oxohexanoic Acid Dimethylamide (6d). Oil. ^{1}H NMR (acetone-d_6): 7.90 (bs, 1H), 7.83−7.25 (m, 5H), 6.70 (bs, 1H), 5.40−5.20 (m, 1H), 5.13−5.04 (m, 3H), 4.55 (bs, 1H), 3.83 (d, J = 6.3, 2H), 2.98−2.85 (m, 6H), 2.45 (m, 2H), 2.16−2.07 (m, 1H), 1.98−1.85 (m, 1H). Anal. ($C_{18}H_{24}FN_3O_5 \cdot 0.8H_2O$) C, H, N.

4-(*Z*-Ala-amido)-6-fluoro-5-oxohexanoic Acid Dimethylamide (6e). Oil. ^{1}H NMR ($CDCl_3$): 7.85−7.78 (m, 1H), 7.30−7.20 (m, 5H), 5.40 (m, 1H), 5.15 (m, 3H), 4.98 (bs, 1H), 4.65 (bs, 1H), 4.25 (m, 1H), 2.98 (s, 3H), 2.95 (s, 3H), 2.60−2.25 (m, 2H), 2.25−2.00 (m, 2H), 1.40 (m, 3H). Anal. ($C_{19}H_{26}FN_3O_5 \cdot H_2O$) C, H, N.

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Supporting Information Available: Antiviral assay, animal studies, and table of elemental analysis data for the targeted compounds **6a**−**6e**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Peiris, J. S.; Guan, Y.; Yuen, K. Y. Severe acute respiratory syndrome. *Nat. Med.* **2004**, *10*, S88−97.

(2) Anand, K.; Ziebuhr, J.; Wadhwani, P.; Mesters, J. R.; Hilgenfeld, R. Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs. *Science* **2003**, *300*, 1763−1767.

(3) Hegyi, A.; Ziebuhr, J. Conservation of substrate specificities among coronavirus main proteases. *J. Gen. Virol.* **2002**, *83*, 595−599.

(4) Yang, Z. Y.; Werner, H. C.; Kong, W. P.; Leung, K.; Traggiai, E.; Lanzavecchia, A.; Nabel, G. J. Evasion of antibody neutralization in emerging severe acute respiratory syndrome coronaviruses. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 797−801.

(5) Shie, J. J.; Fang, J. M.; Kuo, C. J.; Kuo, T. H.; Liang, P. H.; Huang, H. J.; Yang, W. B.; Lin, C. H.; Chen, J. L.; Wu, Y. T.; Wong, C. H. *J. Med. Chem.* **2005**, *48*, 4469−4473.

(6) Wu, C. Y.; Jan, J. T.; Ma, S. H.; Kuo, C. J.; Juan, H. F.; Cheng, Y. S.; Hsu, H. H.; Huang, H. C.; Wu, D.; Brik, A.; Liang, F. S.; Liu, R. S.; Fang, J. M.; Chen, S. T.; Liang, P. H.; Wong, C. H. Small molecules targeting severe acute respiratory syndrome human coronavirus. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 10012−11017.

(7) Kao, R. Y.; Tsui, W. H.; Lee, T. S.; Tanner, J. A.; Watt, R. M.; Huang, J. D.; Hu, L.; Chen, G.; Chen, Z.; Zhang, L.; He, T.; Chan, K. H.; Tse, H.; To, A. P.; Ng, L. W.; Wong, B. C.; Tsoi, H. W.; Yang, D.; Ho, D. D.; Yuen, K. Y. Identification of novel small-molecule inhibitors of severe acute respiratory syndrome-associated coronavirus by chemical genetics. *Chem. Biol.* **2004**, *11*, 1293−1299.

(8) Blanchard, J. E.; Elowe, N. H.; Huitema, C.; Fortin, P. D.; Cechetto, J. D.; Eltis, L. D.; Brown, E. D. High-throughput screening identifies inhibitors of the SARS coronavirus main proteinase. *Chem. Biol.* **2004**, *11*, 1445−1453.

(9) Jain, R. P.; Pettersson, H. I.; Zhang, J.; Aull, K. D.; Fortin, P. D.; Huitema, C.; Eltis, L. D.; Parrish, J. C.; James, M. N.; Wishart, D. S.; Vederas, J. C. Synthesis and evaluation of keto-glutamine analogues as potent inhibitors of severe acute respiratory syndrome 3CLpro. *J. Med. Chem.* **2004**, *47*, 6113−6116.

(10) Hoever, G.; Baltina, L.; Michaelis, M.; Kondratenko, R.; Baltina, L.; Tolstikov, G. A.; Doerr, H. W.; Cinatl, J. Jr. Antiviral Activity of Glycyrrhizic Acid Derivatives against SARS−Coronavirus. *J. Med. Chem.* **2005**, *48*, 1256−1259.

(11) (a) Yang, W.; Guastella, J.; Huang, J.-C.;Wang, Y.; Zhang, L.; Xue, D.; Tran, M.; Woodward, R.; Kasibhatla, S.; Tseng, B.; Drewe, J.; Cai, S. X. MX1013, a dipeptide caspase inhibitor with potent in vivo antiapoptotic activity. *Br. J. Pharmacol.* **2003**, *140*, 402−412. (b) Wang, Y.; Huang, J.-C.; Zhou, Z.-L.; Yang, W.; Guastella, J.; Drewe, J.; Cai, S. X. Dipeptidyl aspartyl fluoromethylketones as potent caspase-3 inhibitors: SAR of the P_2 amino acid. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1269−1272.

(12) Gao, F.; Ou, H. Y.; Chen, L. L.; Zheng, W. X.; Zhang, C. T. Prediction of proteinase cleavage sites in polyproteins of coronaviruses and its applications in analyzing SARS-CoV genomes. *FEBS Lett.* **2003**, *553*, 451−456.

(13) In anti-SARS-CoV screening studies done by the National Institute of Allergy and Infectious Diseases (NIAID) and managed by Southern Research Institute (SRI), compound **9** was found to be >200-fold less active than the corresponding *N*,*N*-dimethyl-glutamine analogue **6a** (personal communication from Dr. Joseph A. Maddry of SRI). The low activity of compound **9** most probably is due to the dominant cyclized form, which is not expected to interact effectively with the SARS-CoV M^{pro} enzyme.

(14) Kong, J. S.; Venkatraman, S.; Furness, K.; Nimkar, S.; Shepherd, T. A.; Wang, Q. M.; Aube, J.; Hanzlik, R. P. Synthesis and evaluation of peptidyl Michael acceptors that inactivate human rhinovirus 3C protease and inhibit virus replication. *J. Med. Chem.* **1998**, *41*, 2579−2587.

(15) Morris, T. S.; Frormann, S.; Shechosky, S.; Lowe, C.; Lall, M. S.; Gauss-Muller, V.; Purcell, R. H.; Emerson, S. U.; Vederas, J. C.; Malcolm, B. A. In vitro and ex vivo inhibition of hepatitis A virus 3C proteinase by a peptidyl monofluoromethyl ketone. *Bioorg. Med. Chem.* **1997**, *5*, 797−807.

(16) In anti-SARS-CoV studies done by Southern Research Institute (SRI), compound **8** was found to have an EC_{50} value of 1.2 μM against the cytopathic effect (personal communication from Dr. Joseph A. Maddry of SRI). Therefore there might be advantage of *N*-methyl-glutamine over *N*,*N*-dimethyl-glutamine due to potential hydrogen bonding of the NH group with the SARS-CoV M^{pro} enzyme.

(17) Malcolm, B, A.; Lowe, C.; Shechosky, S.; McKay, R. T.; Yang, C. C.; Shah, V. J.; Simon, R. J.; Vederas, J. C.; Santi, D. V. Peptide aldehyde inhibitors of hepatitis A virus 3C proteinase. *Biochemistry* **1995**, *34*, 8172−8179.

(18) Matthews, D. A.; Dragovich, P. S.; Webber, S. E.; Fuhrman, S. A.; Patick, A. K.; Zalman, L. S.; Hendrickson, T. F.; Love, R. A.; Prins, T. J.; Marakovits, J. T.; Zhou, R.; Tikhe, J.; Ford, C. E.; Meador, J. W.; Ferre, R. A.; Brown, E. L.; Binford, S. L.; Brothers, M. A.; DeLisle, D. M.; Worland, S. T. Structure-assisted design of mechanism-based irreversible inhibitors of human rhinovirus 3C protease with potent antiviral activity against multiple rhinovirus serotypes. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11000−11007.

(19) In antiviral studies done by Utah State University, compounds **6a**−**6c** were tested against rhinovirus type-2 in a cell based assay and found to be inactive (EC_{50} > 100 μg/mL) (personal communication from Dr. Joseph A. Maddry of SRI).

(20) Dragovich, P. S.; Webber, S. E.; Babine, R. E.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Reich, S. H.; Marakovits, J. T.; Prins, T. J.; Zhou, R.; Tikhe, J.; Littlefield, E. S.; Bleckman, T. M.; Wallace, M. B.; Little, T. L.; Ford, C. E.; Meador, J. W., 3rd; Ferre, R. A.; Brown, E. L.; Binford, S. L.; DeLisle, D. M.; Worland, S. T. Structure-based design, synthesis, and biological evaluation of irreversible human rhinovirus 3C protease inhibitors. 2. Peptide structure−activity studies. *J. Med. Chem.* **1998**, *41*, 2819−2834.

(21) Binford, S. L.; Maldonado, F.; Brothers, M. A.; Weady, P. T.; Zalman, L. S.; Meador, J. W. 3rd; Matthews, D. A.; Patick, A. K. Conservation of amino acids in human rhinovirus 3C protease correlates with broad-spectrum antiviral activity of rupintrivir, a novel human rhinovirus 3C protease inhibitor. *Antimicrob. Agents Chemother.* **2005**, *49*, 619−626.

(22) Shie, J. J.; Fang, J. M.; Kuo, T. H.; Kuo, C. J.; Liang, P. H.; Huang, H. J.; Wu, Y. T.; Jan, J. T.; Cheng, Y. S.; Wong, C. H. Inhibition of the severe acute respiratory syndrome 3CL protease by peptidomimetic alpha,beta-unsaturated esters. *Bioorg. Med. Chem.* **2005**, *13*, 5240−5252.

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