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Potent inhibition of norovirus 3CL protease by peptidyl α-ketoamides and α-ketoheterocycles

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ABSTRACT

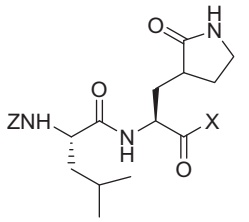
A series of structurally-diverse α-ketoamides and α-ketoheterocycles was synthesized and subsequently investigated for inhibitory activity against norovirus 3CL protease in vitro, as well as anti-norovirus activity in a cell-based replicon system. The synthesized compounds were found to inhibit norovirus 3CL protease in vitro and to also exhibit potent anti-norovirus activity in a cell-based replicon system.

Noroviruses belong to the *Norovirus* genus of the Caliciviridae family.[1] They are highly contagious human pathogens that have attracted considerable attention because they are the most common cause of foodborne and waterborne acute gastroenteritis.[2–5] Outbreaks of acute gastroenteritis are common, particularly in crowded settings, such as schools, nursing homes, and cruise ships. There are currently no vaccines or specific antiviral agents for combating norovirus infection;[6] thus, there is an urgent and unmet need for the discovery and development of small-molecule anti-norovirus therapeutics.

Noroviruses are small, enveloped viruses with a single-stranded, positive sense 7.7-kb RNA genome, which encodes a polyprotein precursor which is co- and post-translationally processed by a virus-encoded cysteine protease to generate mature non-structural proteins.[7] Processing of the polyprotein by norovirus 3CL protease (3CLpro) is essential for virus replication; consequently, norovirus 3CLpro has emerged as an attractive target for the discovery of therapeutics for norovirus infection. [8]

Norovirus 3CLpro is a cysteine endoprotease with a Cys-His-Glu catalytic triad and a substrate specificity for a -D/E-F-X-L-Q-G-P- sequence, where X is H, Q, E or D, corresponding to the subsites S_5-S_4-S_3–S_2–S_1–S_1'–S_2'–. Cleavage is at the P_1–P_1' (Q–G) scissile bond. X-ray crystal structures of norovirus 3CLpro alone[9,10] or covalently-bound to an inhibitor, such as a peptidyl Michael acceptor[11] or a peptidyl aldehyde,[12] have been reported.

We have recently described the cell-based inhibition of noroviruses by an array of structurally-diverse series of compounds[13–17] and have, furthermore, disclosed the results of preliminary studies related to the design, synthesis, and evaluation of peptidyl aldehydes as transition state inhibitors of norovirus 3CLpro.[8] In an attempt to identify suitably-functionalized dipeptidyl inhibitors that possess pharmacological activity and molecular properties that are important for oral bioavailability and favorable ADMET characteristics,[18–24] we describe herein the synthesis and utilization of a series of peptidyl α-ketoamides and α-ketoheterocycles (Fig. 1, structures **I–II**) in the in vitro inhibition of norovirus 3CLpro, as



X = (C=O) NHR (I)
= heterocycle (II)

Figure 1. General structures of inhibitors (**I–II**).

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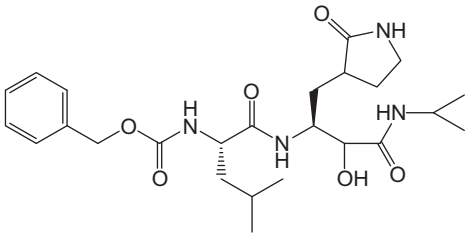
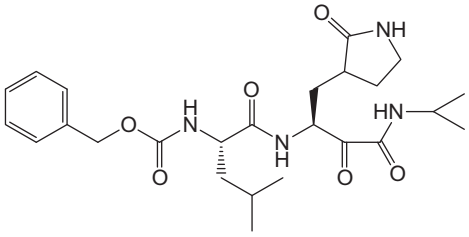
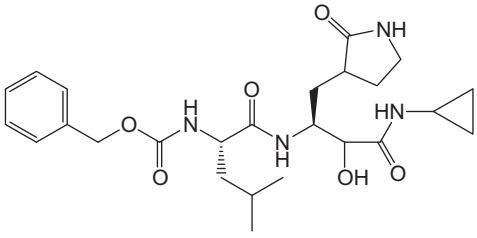
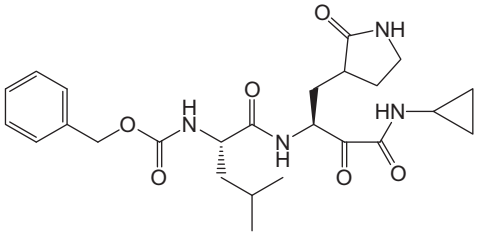
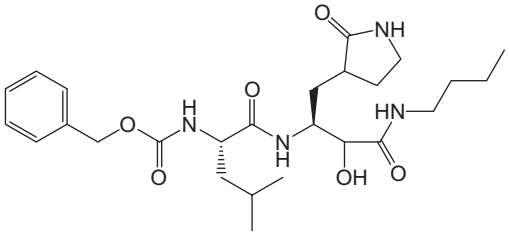
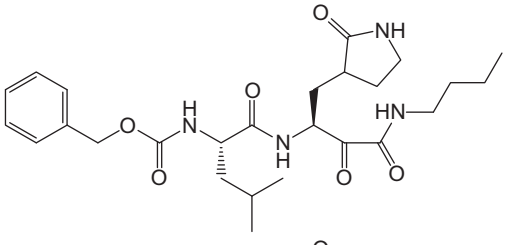
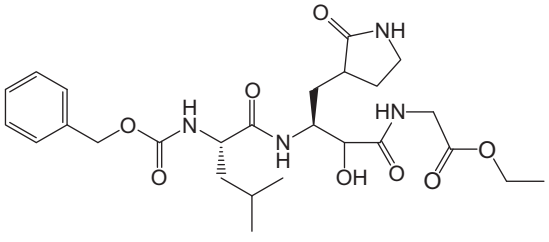
Table 1

Compound	Structure	IC_{50} (μm)	ED_{50} (μm)
5a		45.3	4.1
6a[42]		5.3	2.8
5b		>50	>10
6b		3.4	1.1
5c		>50	>20
6c		4.1	1.3
5d		>50	>10

(*continued on next page*)

Table 1 (*continued*)

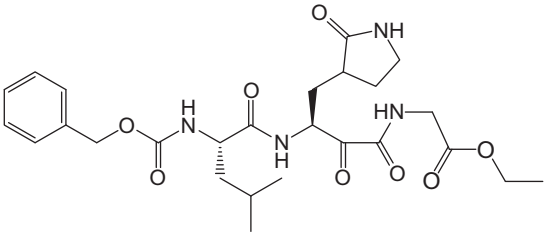
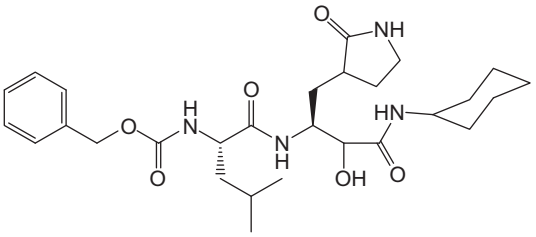
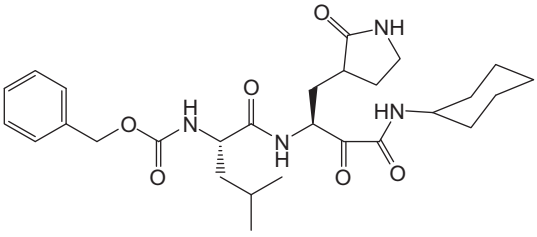
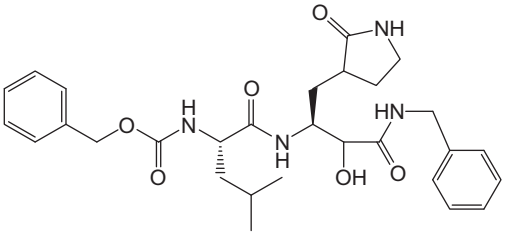
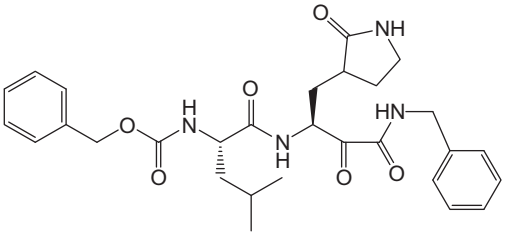
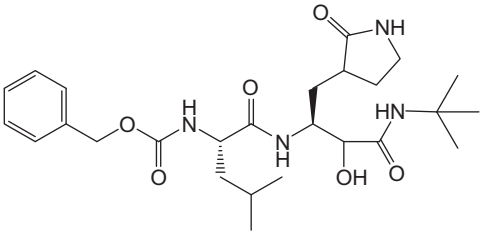
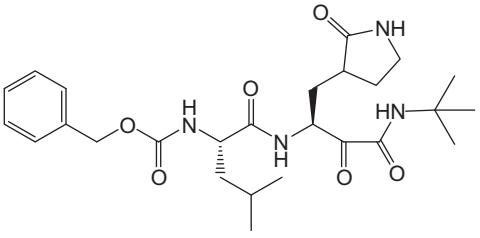
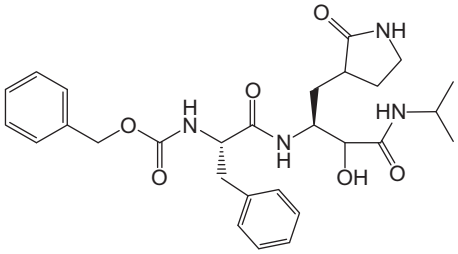
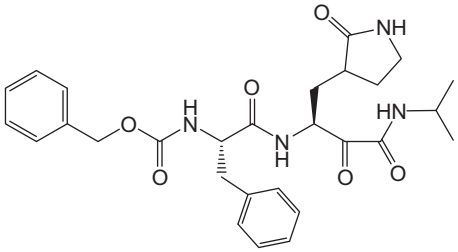
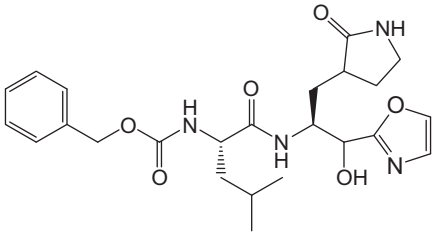
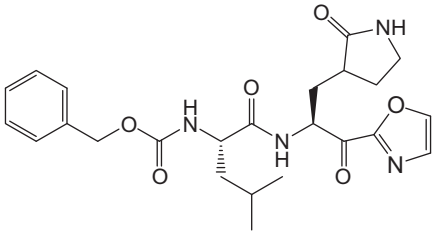
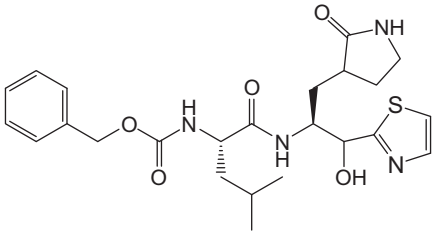
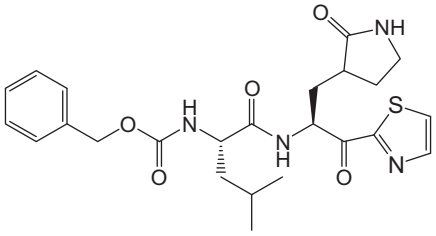
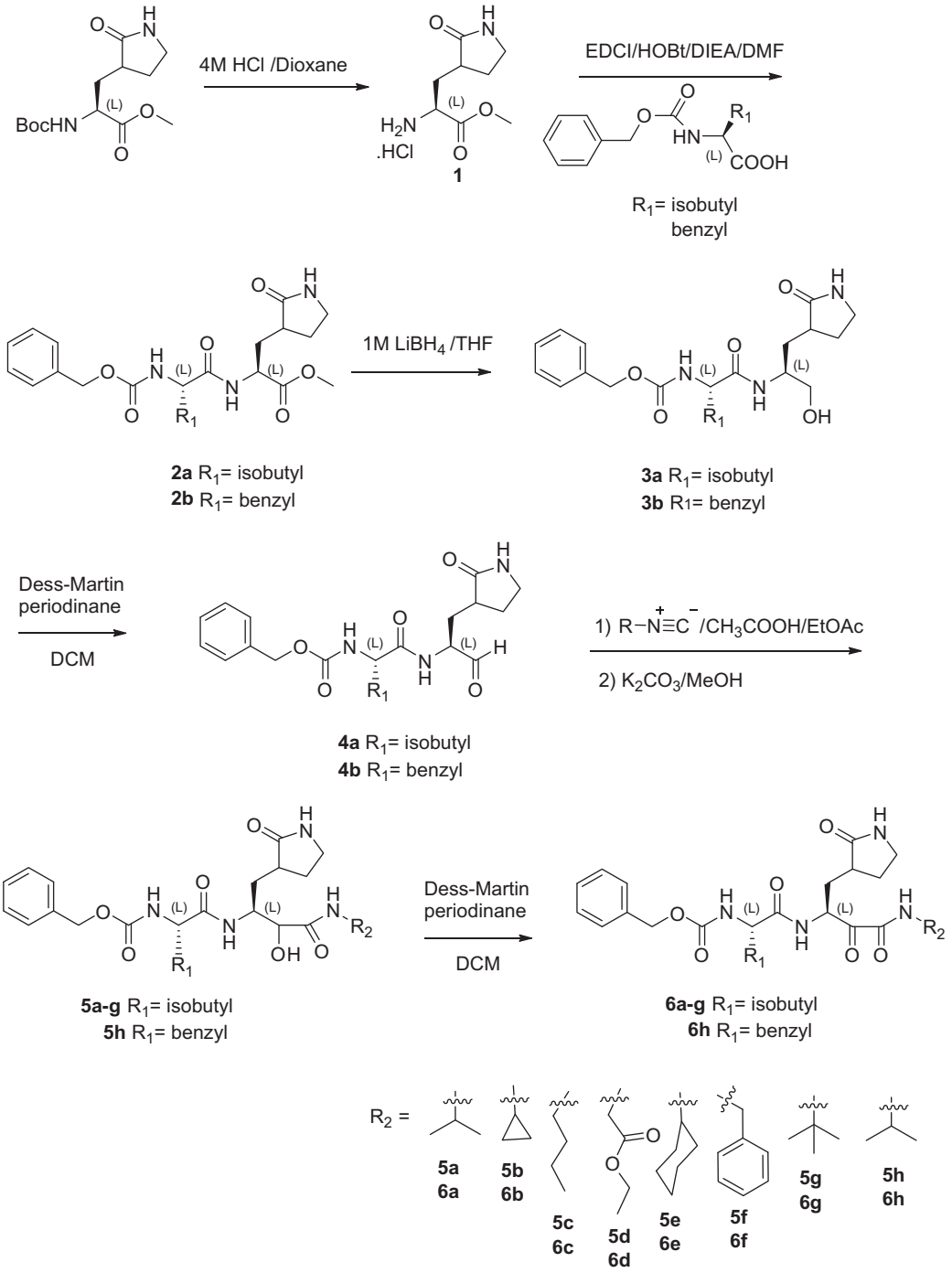
Compound	Structure	IC_{50} (μm)	ED_{50} (μm)
6d		2.1	0.8
5e		>50	>20
6e		3.5	1.2
5f		>50	>20
6f		7.1	1.8
5g		>50	>20
6g		2.8	1.1

Table 1 (*continued*)

Compound	Structure	IC_{50} (μm)	ED_{50} (μm)
5h		23.1	6.3
6h		21.5	4.2
7a		>50	>20
8a		2.3	0.9
7b		12.3	4.3
8b		8.8	4.2

well as the inhibition of norovirus using a cell-based replicon system. The synthesized compounds were also used to probe the S′ subsites of the enzyme.

The syntheses of α-ketoamides ***6a–h*** and α-ketoheterocycles ***8a–b*** (Table 1) were carried out as illustrated in Schemes 1 and 2, respectively.[25] A glutamine surrogate, previously shown to be highly effective in the design of rhinovirus 3C[26] and enterovirus 3C[27] proteases, was utilized as the primary specificity (P_1) residue. The Boc-protected surrogate was synthesized using literature procedures[28] and was subsequently deprotected to yield compound ***1*** (Scheme 1). EDCI-mediated coupling with Z-(L)-Leu-OH or Z-(L)-Phe-OH yielded compounds ***2a, b*** which were reduced to the corresponding alcohols using lithium borohydride. Dess–Martin oxidation furnished aldehydes ***4a–b*** which were reacted with an array of structurally-diverse isonitriles to generate a series of precursor alcohols ***5a–g*** and ***5h*** which, upon oxidation, yielded

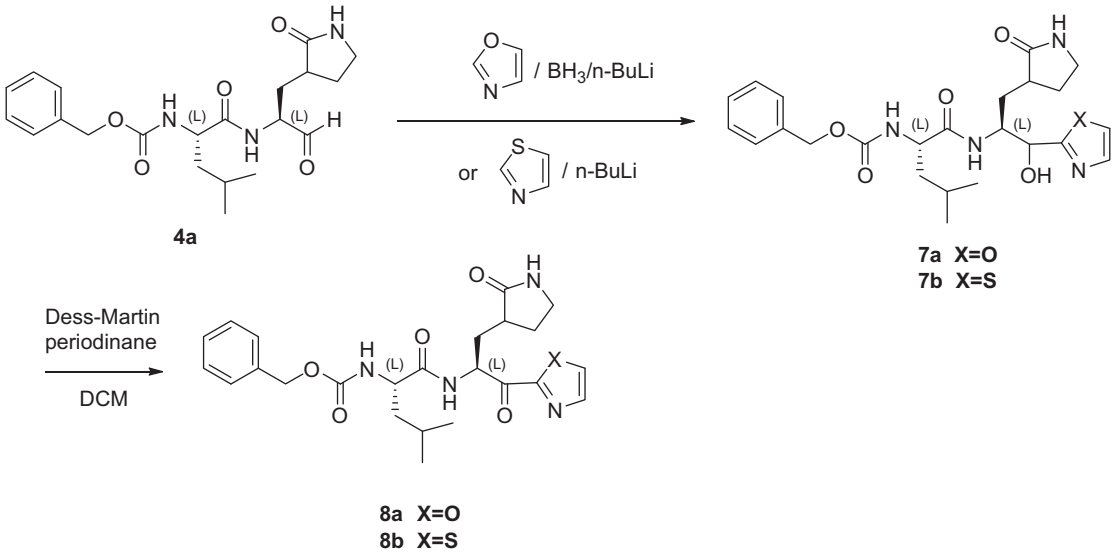


Scheme 1. Synthesis of inhibitors ***6a–h***.

the desired α-ketoamides ***6a–h***.[29] α-Ketoheterocycle ***8a*** was synthesized by sequentially treating a solution of oxazole in THF with borane and *n*-butyl lithium,[30] followed by reaction with aldehyde ***4a***, to yield precursor alcohol ***7a*** which was subsequently oxidized to form α-ketoheterocycle ***8a***. Reaction of compound ***4a*** with the anion generated by reacting thiazole with *n*-butyl lithium,[31] followed by Dess–Martin oxidation of the isolated precursor alcohol, yielded α-ketoheterocycle ***2*** (Scheme 2). The interaction of the generated precursors and final compounds with norovirus 3CLpro was investigated in vitro as previously described.[8] The activity of the generated compounds against norovirus was also investigated in a cell-based system[32–35] and the combined results are summarized in Table 1.

We have recently demonstrated that peptidyl aldehydes function as transition state inhibitors of NV 3CLpro.[8] In this Letter we demonstrate that α-ketoamides and α-ketoheterocycles inhibit norovirus 3CLpro in vitro, and also exhibit potent anti-norovirus activity in a cell-based system. S′–P′ interactions have been previously utilized to increase the affinity and selectivity of inhibitors, including transition state analogs;[30,36,37] consequently, the S′ subsites of norovirus 3CLpro were probed using a series of structurally-diverse α-ketoamides.

It is evident from the results summarized in Table 1 that peptidyl α-ketoamides (compounds ***6a–g***) potently inhibit norovirus 3CLpro in vitro. Most importantly, the compounds exhibit potent anti-norovirus activity in a cell-based replicon system. In order



Scheme 2. Synthesis of inhibitors ***8a–b***.

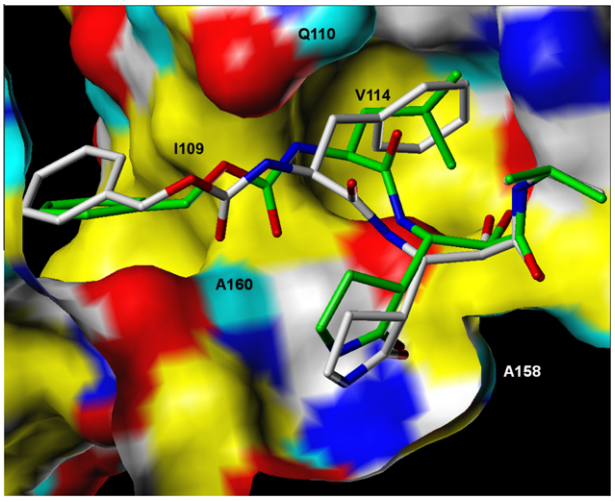


Figure 2. Predicted binding modes for norovirus 3CLpro inhibitors ***6a*** and ***6h***. The norovirus 3CLpro receptor is rendered as a Connolly surface, and is colored as follows: red = polar O, blue = polar N, cyan = polar H, white = polarized alkyl or aryl (C, H), and yellow = hydrophobic. The ligands are represented as CPK-colored sticks, with carbon atoms colored as follows: green = compound ***6a*** and white = compound ***6h***. A selection of receptor residues with key ligand interactions are labeled.

to enhance further the pharmacological activity of the compounds by exploiting favorable binding interactions between the R group in (I) (assumed to be projecting toward the S′ subsites) and the enzyme, the nature of the R group was varied. Although the precise orientation of the R group will have to await the determination of the X-ray crystal structure of a ligand–enzyme complex (in progress), the results indicate that a wide range of R groups can be tolerated. As anticipated, the corresponding precursor alcohols (compounds ***5a–h***, Table 1) were substantially less active (vide infra). Furthermore, replacement of P2 Leu with Phe decreased potency fourfold (compare compounds ***6a*** and ***6h***, Table 1), a finding that is consistent with the strong preference of the enzyme for a Leu residue at P_2. Intriguingly, precursor alcohols ***5a, 5h*** and ***7b*** exhibited noteworthy activity in the cell-based replicon system despite their weak in vitro inhibitory activity against norovirus 3CLpro.

In order to computationally predict binding modes for compounds ***6a*** and ***6h***, a receptor structure for norovirus 3CLpro was prepared using the reported crystal structure[11] by extracting the co-crystallized covalently-bound peptidyl ligand and all resolved water.[38] The two inhibitors are capable of adopting similar low-energy conformations (Fig. 2) and engage in multiple favorable binding interactions with the enzyme, including lipophilic interactions involving the –(CH_2CH_2)– segment of the glutamine surrogate with the corresponding –(CH_2CH_2)– segment of Pro136 (above the viewing plane in Fig. 2), the Leu side chain in each inhibitor with His30 (also above plane), Ile109 and Val114, and interactions of the phenyl ring in the Cbz cap—partially occupying the S4 pocket—with Ile109. A network of hydrogen bonds involving Ala158 (backbone carbonyl), Gln110 (side chain amide) and Ala160 (backbone amide hydrogen) are also evident. Comparison of the binding modes of ***6a*** and ***6h*** suggests that the decline in potency in the latter may arise from the substitution of a more bulky group (benzyl) into the relatively small hydrophobic pocket (defined by Val114 in Fig. 2), which tends to shift the ***6h*** binding mode outwards, disrupting the ligand H-bond with Gln110.

α-Ketoheterocycles ***8a–b*** were also found to inhibit norovirus 3CLpro in vitro, with the oxazole derivative being about fourfold more potent than the corresponding thiazole compound (Table 1). Both compounds were found to inhibit norovirus in a cell-based replicon system, with isoxazole ***8a*** being the most effective (ED_{50} 900 nM).

In summary, a series of structurally-diverse α-ketoamides and α-ketoheterocycles has been synthesized and shown to potently inhibit norovirus 3CLpro in vitro, as well as norovirus in a cell-based replicon system.

Acknowledgments

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References and notes

1. Green, Y. K. In *Caliciviridae: The Noroviruses in Fields Virology*; Knipe, D. M., Howley, P. M., Eds.; Lippincott, Williams & Wilkins: Philadelphia, 2007; Vol. 1, pp 949–979.
2. Patel, M. M.; Hall, A. J.; Vinje, J.; Parashar, U. D. *J. Clin. Virol.* **2009**, *44*, 1.
3. Eckardt, A. J.; Baumgart, D. C. *Rec. Patents Anti-Infect. Drug Discov.* **2011**, *6*, 54.
4. Atmar, R. L. *Food Environ. Virol.* **2010**, *2*, 117.

5. Khan, M. A.; Bass, D. M. *Curr. Opin. Gastroenterol.* **2010**, *26*, 26.
6. (a) Glass, R. I.; Parashar, U. D.; Estes, M. K. *N. Eng. J. Med.* **2009**, *361*, 1776; (b) Tan, M.; Jiang, X. *Curr. Opin. Invest. Drugs* **2008**, *9*, 146.
7. Blakeney, S. J.; Cahill, A.; Reilly, P. A. *Virology* **2003**, *308*, 216.
8. Tiew, K.-C.; He, G.; Aravapalli, S.; Mandadapu, S. R.; Gunnam, M. R.; Alliston, K. R.; Lushington, G. H.; Kim, Y.; Chang, K.-O.; Groutas, W. C. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5315.
9. Zeitler, C. E.; Estes, M. K.; Prasad Venkatarman, B. V. *J. Virol.* **2006**, *80*, 5050.
10. Nakamura, K.; Someya, Y.; Kumasaka, T.; Ueno, G.; Yamamoto, M.; Sato, T.; Takeda, N.; Miyamura, T.; Tanaka, N. *J. Virol.* **2005**, *79*, 13685.
11. Hussey, R. J.; Coates, L.; Gill, R. S.; Erskine, P. T.; Coker, S.-F.; Mitchell, E.; Cooper, J. B.; Wood, S.; Broadbridge, R.; Clarke, I. N.; Lambden, P. R.; Shoolingin-Jordan, P. M. *Biochemistry* **2011**, *50*, 240.
12. Kim, Y.; Lovell, S.; Tiew, K-C.; Mandadapu, S. R.; Alliston, K. R.; Battaille, K. P.; Groutas, W. C.; Chang, K.-O., submitted for publication.
13. Dou, D.; Tiew, K.-C.; He, G.; Mandadapu, S. R.; Aravapalli, S.; Alliston, K. R.; Kim, Y.; Chang, K.-O.; Groutas, W. C. *Bioorg. Med. Chem.* **2011**, *19*, 5975.
14. Dou, D.; Mandadapu, S. R.; Alliston, K. R.; Kim, Y.; Chang, K.-O.; Groutas, W. C. *Bioorg. Med. Chem.* **2011**, *19*, 5749.
15. Dou, D.; Mandadapu, S. R.; Alliston, K. R.; Kim, Y.; Chang, K.-O.; Groutas, W. C. *Eur. J. Med. Chem.* **2012**, *47*, 59.
16. Dou, D.; He, G.; Mandadapu, S. R.; Aravapalli, S.; Kim, Y.; Chang, K.-O.; Groutas, W. C. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 377.
17. Dou, D.; Tiew, K.-C.; Mandadapu, S. R.; Gunnam, M. R.; Alliston, K. R.; Kim, Y.; Chang, K.-O.; Groutas, W. C. *Bioorg. Med. Chem.* **2012**, *20*, 2111.
18. Lipinski, C. A. *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235.
19. Veber, D. F. *J. Med. Chem.* **2002**, *45*, 2615.
20. Ritchie, T. J.; Ertl, P.; Lewis, R. *Drug Discovery Today* **2011**, *16*, 65.
21. Gleeson, M. P. *J. Med. Chem.* **2008**, *51*, 817.
22. Johnson, T. W.; Dress, K. R.; Edwards, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5560.
23. Perola, E. *J. Med. Chem.* **2010**, *53*, 2986.
24. Edwards, M. P.; Price, D. A. *Ann. Rep. Med. Chem.* **2010**, *45*, 381.
25. All compounds were characterized by ^{1}H NMR and HRMS and had a >95% purity.
26. (a) Webber, S. E.; Okano, K.; Little, T. L.; Reich, S. H.; Xin, Y.; Fuhrman, S. A.; Matthews, D. A.; Love, R. A.; Hendrickson, T. F.; Patick, A. K.; Meador, J. W.; Ferre, R. A.; Brown, E. L.; Ford, C. E.; Binford, S. L.; Worland, S. T. *J. Med. Chem.* **1998**, *41*, 2786; (b) Dragovich, P. S.; Prins, T. J.; Zhou, R.; Webber, S. E.; Marakovits, J. T.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Lee, C. A.; Ford, C. E.; Burke, B. J.; Rejto, P. A.; Hendrickson, T. F.; Tuntland, T.; Brown, E. L.; Meador, J. W.; Ferre, R. A.; Harr, J. E.; Kosa, M. B.; Worland, S. T. *J. Med. Chem.* **1999**, *42*, 1213.
27. Kuo, C.-J.; Shie, J.-J.; Fang, J.-M.; Yen, G.-R.; Hsu, J. T.-A.; Liu, H.-G.; Tseng, S.-N.; Chang, S.-C.; Lee, C.-Y.; Shih, S.-R.; Liang, P.-H. *Bioorg. Med. Chem.* **2008**, *16*, 7388.
28. Mou, K.; Xu, B.; Ma, C.; Yang, X.; Zou, X.; Lu, Y.; Xu, P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2198.
29. Bogen, S. L.; Arasappan, A.; Velazquez, F.; Blackman, M.; Huelgas, R.; Pan, W.; Siegel, E.; Nair, L. G.; Venkatraman, S.; Guo, Z.; Dolle, R.; Shi, N.-Y.; Njoroge, F. G. *Bioorg. Med. Chem.* **1854**, *2010*, 18.
30. Ezzili, C.; Mileni, M.; McGlinchey, N.; Long, J. Z.; Kinsey, S. G.; Hochstatter, D. G.; Stevens, R. C.; Lichtman, A. H.; Cravatt, B. F.; Bilsky, E. J.; Boger, D. L. *J. Med. Chem.* **2011**, *54*, 2805.
31. Edwards, P. D.; Wolanin, D. J.; Andisik, D. W.; David, M. W. *J. Med. Chem.* **1995**, *38*, 76.
32. Chang, K. O.; Sosnovtsev, S. V.; Belliot, G.; King, A. D.; Green, K. Y. *Virology* **2006**, *2*, 463.
33. Chang, K. O.; George, D. W. *J. Virol.* **2007**, *22*, 12111.
34. Chang, K. O. *J. Virol.* **2009**, *83*, 8587.
35. Kim, Y.; Thapa, M.; Hua, D. H.; Chang, K.-O. *Antiviral Res.* **2011**, *89*, 165.
36. Imperiali, B.; Abeles, R. H. *Biochemistry* **1987**, *26*, 4474.
37. Dai, Y.; Hedstrom, L.; Abeles, R. H. *Biochemistry* **2000**, *39*, 6498.
38. The receptor structure was prepared by extracting the co-crystallized covalently-bound peptidyl ligand in 2IP7[11] and all resolved water. Protons were added to the remaining structure using SYBYL[39] and the covalently-bound ligands (compounds ***6a*** and ***6h***) were assembled sequentially as follows: beginning with the bare sulfur on Cys 139 left from deletion of the co-crystallized ligand, SYBYL was used to add the nearest ligand heavy atom, protons were added to the resulting complex, and then the ligand position was optimized using molecular mechanics (Tripos Molecular Force Field[40]), Gasteiger–Marsili electrostatics[41] and default convergence criteria. All protein atomic coordinates were held fixed except for those of Cys139. From the resulting optimized structure, terminal protons on the ligand fragment were replaced with those heavy atoms corresponding to next-nearest neighbors to the Cys139 sulfur attachment point, protons were added to the resulting complex, and the structure re-optimized. Next–next nearest neighbors were then added in a similar manner and continued incrementally until the ligand was fully constituted.
39. SYBYL 8.1, Tripos International, St. Louis, MO 63144, USA.
40. Clark, M.; Cramer, R. D., III; van Opdenhosch, N. *J. Comput. Chem.* **1989**, *10*, 982.
41. Gasteiger, J.; Marsili, M. *Tetrahedron Lett.* **1978**, *34*, 3181.
42. Previously reported[8] to be inactive due to compound decomposition during handling