Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Bioorganic & Medicinal Chemistry Letters 23 (2013) 3709–3712

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Macrocyclic inhibitors of 3C and 3C-like proteases of picornavirus, norovirus, and coronavirus

Sivakoteswara Rao Mandadapu [a,†], Pathum M. Weerawarna [a,†], Allan M. Prior [b], Roxanne Adeline Z. Uy [a], Sridhar Aravapalli [a], Kevin R. Alliston [a], Gerald H. Lushington [c], Yunjeong Kim [d], Duy H. Hua [b], Kyeong-Ok Chang [d], William C. Groutas [a,*]

[a] Department of Chemistry, Wichita State University, Wichita, KS 67260, USA
[b] Department of Chemistry, Kansas State University, Manhattan, KS 66506, USA
[c] LiS Consulting, Lawrence, KS 66046, USA
[d] Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, USA

ARTICLE INFO

Article history:
Received 2 April 2013
Revised 29 April 2013
Accepted 7 May 2013
Available online 16 May 2013

Keywords:
Macrocyclic inhibitors
3C and 3CL proteases
Picornavirus-like supercluster pathogens

ABSTRACT

The design, synthesis, and in vitro evaluation of the first macrocyclic inhibitor of 3C and 3C-like proteases of picornavirus, norovirus, and coronavirus are reported. The in vitro inhibitory activity (50% effective concentration) of the macrocyclic inhibitor toward enterovirus 3C protease (CVB3 Nancy strain), and coronavirus (SARS-CoV) and norovirus 3C-like proteases, was determined to be 1.8, 15.5 and 5.1 μM, respectively.

The picornavirus-like protease supercluster includes viruses in the *Picornaviridae*, *Coronaviridae*, and *Caliciviridae* families. Many human pathogens of major medical and economic importance belong to these virus families. For instance, the family *Picornaviridae* includes enterovirus (enterovirus, EV; coxsackievirus, CV; poliovirus, PV), human rhinovirus (HRV), and hepatitis A virus (HAV).[1,2] Non-polio enteroviruses are responsible for 10–15 million symptomatic infections in the US each year,[3] while HRV is the major causative agent of upper respiratory tract infections.[4] In the *Coronaviridae* family, severe acute respiratory syndrome (SARS) caused by SARS-coronavirus (SARS-CoA) is a recognized global threat to public health.[5] Noroviruses belong to the *Norovirus* genus of the *Caliciviridae* family and are highly contagious human pathogens that are the most common cause of food borne and water borne acute viral gastroenteritis.[6] Thus, norovirus infection constitutes an important public health problem. There are currently no vaccines (except for poliovirus) or specific antiviral agents for combating infections caused by the aforementioned viruses; thus, there is an urgent and unmet need for the discovery and development of broad spectrum small-molecule therapeutics and prophylactics for these important pathogens.[7–10]

The picornaviral genome consists of a positive sense, single-stranded RNA of ~7.5 kb in length that encodes a large precursor polyprotein that requires proteolytic processing to generate mature viral proteins.[1,2] Processing of the polyprotein is primarily mediated by the viral 3C protease (3Cpro). Likewise, the ~30 kb genome of SARS-CoV comprises both nonstructural and structural regions. Two polyproteins (designated as pp1a and pp1ab) encoded by the viral genome undergo proteolytic processing by two proteases: a chymotrypsin-like cysteine protease (3C-like protease, 3CLpro) and a papain-like protease (PLpro), to generate functionally active proteins. Finally, the 7–8 kb RNA genome of noroviruses encodes a polyprotein that is processed by a 3C-like protease (3CLpro) to generate mature proteins.[11] Although there is high genetic diversity among these viruses, 3Cpro and 3CLpro are highly conserved, as well as essential for virus replication.

Inspection of the crystal structures of picornavirus 3Cpro[12–15] and norovirus 3CLpro,[16–19] reveals that the proteases share in common a chymotrypsin-like fold, a Cys-His-Glu/Asp catalytic triad (EV and CV 3Cpro, and NV 3CLpro) or Cys-His dyad (SARS-CoV 3CLpro),[20] an extended binding site, and a preference for cleaving at Gln-Gly ($P_1 - P'_1$) junctions in protein and synthetic peptidyl substrates (vide infra). The confluence of structural similarities in the active sites, mechanism of action, and substrate specificity preferences of EV and CV 3Cpro,[12,13] SARS-CoV 3CLpro,[20,21] and NV 3CLpro[11,17,22] (Table 1) suggests that a drug-like entity can be fashioned that displays inhibitory activity against all three

* Corresponding author. Tel.: +1 (316) 978 7374; fax: +1 (316) 978 3431.
E-mail address: bill.groutas@wichita.edu (W.C. Groutas).
† These authors contributed equally to this work.

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.05.021

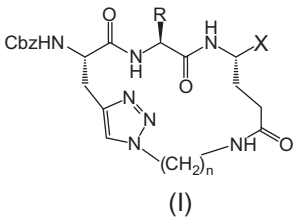
Table 1
Substrate specificity of the 3C and 3C-like proteases of viruses in the picornavirus-like protease supercluster

Viral 3Cpro or 3CLpro	P_5	P_4	P_3	P_2	P_1	P'_1	P'_2
EV71	E	A	V/L/T	L/F	Q	G	P
CVA16	E	A	L	F	Q	G	P
SARS-CoV	S	A	V/T/K	L	Q	A/S	G
NV	D/E	F/Y	H/Q/E	L	Q	G	P

proteases, making them appealing targets for the discovery of broad spectrum antiviral agents.[16,23]

Picornavirus 3Cpro,[2] SARS-CoV 3CLpro[23] and NV 3CLpro[24] have been the subject of intense investigations. We report herein the design, synthesis, and in vitro evaluation of a representative member of a new class of macrocyclic transition state inhibitors (**I**) (Fig. 1) that is effective against all three proteases. To our knowledge, this is the first report describing the inhibition of 3Cpro and 3CLpro of pathogens belonging to the picornavirus-like protease supercluster, by a macrocyclic inhibitor.

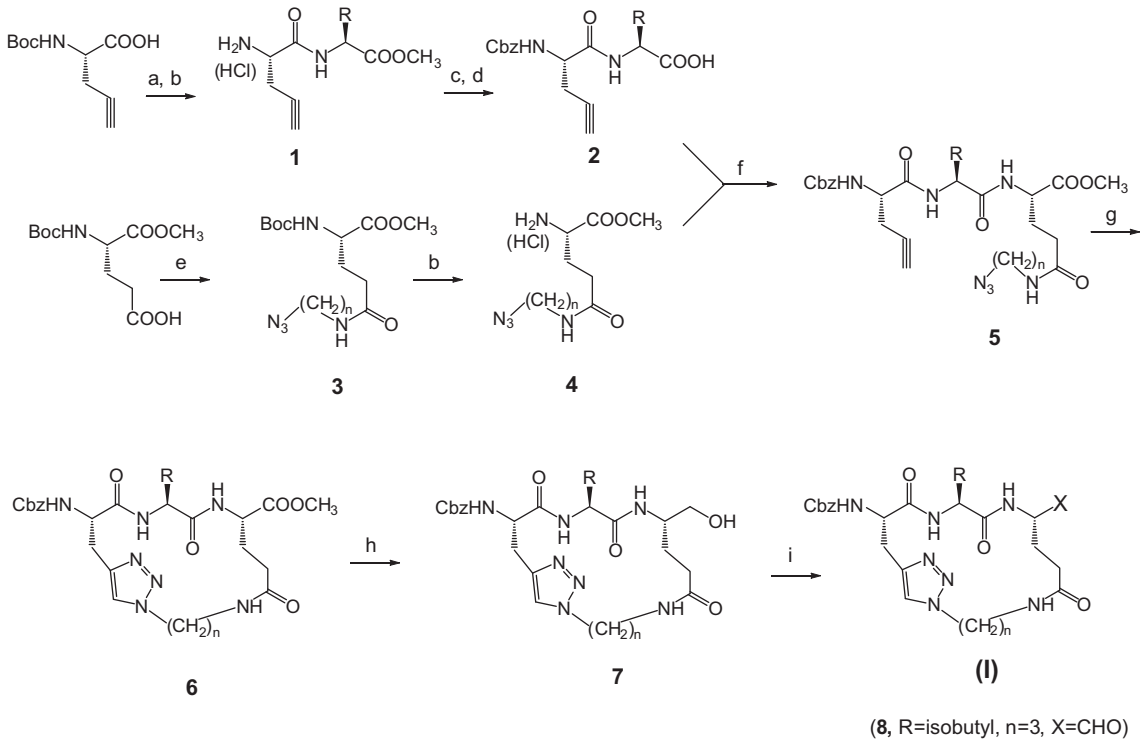
The design of macrocyclic inhibitor (**I**) rested on the following considerations: (a) proteases are known to recognize their ligands in the β-strand conformation;[25] (b) macrocyclization is an effective way of pre-organizing a peptidyl transition state mimic in a β-strand conformation suitable for binding to the active site of a protease;[26–28] (c) in general, macrocyclization increases affinity by reducing the loss of entropy upon inhibitor binding, as well as cellular permeability, and proteolytic stability;[29] (d) macrocyclization improves drug-like characteristics;[30,31] (e) the plasticity of the S_3 subsite in the 3C and 3CL proteases was exploited in the design of macrocyclic inhibitor (**I**) by tethering the P_1 Gln side chain to the P_3 residue side chain; and, (e) computational and modeling studies suggested that a ring size corresponding to $n = 3$ would produce good receptor binding and minimal intra-ligand strain.



(I)

Figure 1. General structure of macrocyclic inhibitor (**I**).

Based on the aforementioned considerations, inhibitor (**I**) was assembled in a convergent fashion by first constructing fragments **2** and **4**, followed by subsequent coupling of the two fragments to generate acyclic precursor **5** (Scheme 1). Cyclization was subsequently accomplished using click chemistry.[32–35] Thus, fragment **2** was synthesized by coupling (L) Boc-protected propargyl glycine with (L) leucine methyl ester using EDCI/HOBt/DIEA/DMF to yield the dipeptidyl ester which was subsequently treated with dry HCl in dioxane to remove the N-terminal Boc protecting group. Reaction with benzylchloroformate yielded the Cbz-protected ester which was hydrolyzed with LiOH in aqueous THF to yield the corresponding acid **2**. EDCI-mediated coupling of commercially available (L) Boc-Glu-OCH_3 with $NH_2(CH_2)_nN_3$ ($n = 3$), followed by removal of the Boc group, yielded fragment **4**.[36] The amino alkyl azide was conveniently synthesized by converting $BocNH(CH_2)_nOH$ to the mesylate via treatment with methanesulfonyl chloride in the presence of triethylamine, followed by reaction with sodium azide in DMF and removal of the protective group. Coupling of fragment **2** with **4** using standard coupling conditions yielded acyclic precursor **5** which was treated with Cu(I)Br/DBU in dichloromethane to furnish compound **6** in 45% yield. Compound **6** was treated with lithium borohydride to yield alcohol **7** (84% yield) which, upon Dess–Martin periodinane oxidation,[37] and subsequent purification gave macrocyclic aldehyde **8** (Scheme 1, structure (**I**), $n = 3$,



Scheme 1. Reagents and conditions: (a) EDCI/HOBt/DIEA/DMF then (L) $NH_2CHRCOOCH_3$; (b) HCl/dioxane; (c) benzylchloroformate/TEA/DCM; (d) LiOH/aq THF; (e) EDCI/HOBt/DIEA/DMF then $NH_2(CH_2)_nN_3$; (f) EDCI/HOBt/DIEA/DMF; (g) Cu(I)Br/DBU/DCM; (h) $LiBH_4$/THF; (i) Dess–Martin periodinane.

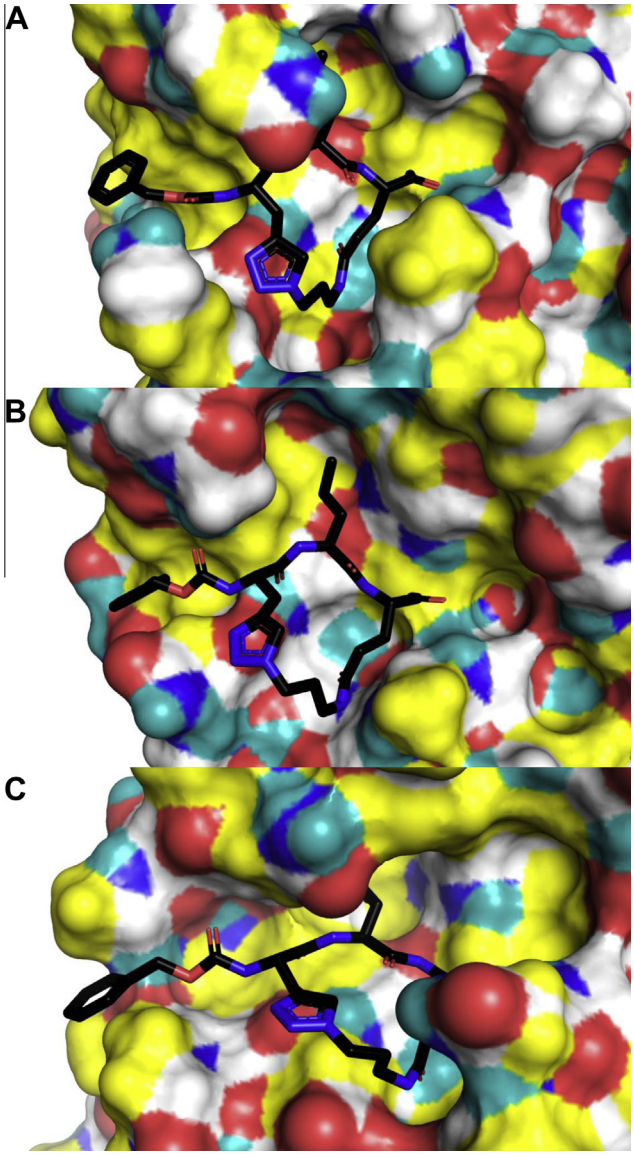


Figure 2. Computationally predicted conformers for inhibitor **8** bound to (A) norovirus 3CLpro, (B) coxsackie virus 3Cpro, and (C) SARS-CoV 3CLpro. Inhibitor is rendered as CPK-colored sticks with black carbon atoms. Protein receptors are shown as Connolly surfaces colored as follows: yellow = nonpolar aryl, alkyl and thioalkyl; white = weakly polar aryl and alkyl; cyan = polar H; blue = polar N; red = polar O.

R = isobutyl, X = CHO), as a white solid.[38] The inhibitory activity of aldehyde **8** was evaluated in vitro as previously described.[16,39–42] Compound **8** displayed inhibitory activity against NV 3CLpro (IC_{50} 5.1 μM), enterovirus (CVB3 Nancy strain) 3Cpro (1.8 μM), and SARS-CoV 3CLpro (IC_{50} 15.5 μM).

In order to gain insight and understanding into the binding of inhibitor **8** to the active site of each protease, computer modeling was used (Fig. 2). Thus, the receptor structures were prepared from the following protein data bank (PDB) crystal structures: (A) NV 3CLpro from 2IPH,[17] (B) CV 3C pro from 3ZZB,[43] and (C) SARS-CoV 3CLpro from 2ZU5.[44] These three receptor models were chosen by virtue of having cocrystallized ligands that each displayed the following three features consistent with the likely binding mode of inhibitor **8**: (i) a covalent attachment to the catalytically active cysteine (analogous to the terminal aldehyde in inhibitor **8**), (ii) branched alkyl, as per isobutyl group in **8**, and (iii) aryl (phenylalanine or Cbz), as per Cbz in **8**. This permitted the intelligent prepositioning of inhibitor **8** into each of the three protease receptors, which was accomplished in PYMOL[45] via manual docking. PYMOL was then used to produce a computational framework for refining the docked conformation as follows: a ligand–receptor complex was generated by protonating the preliminary receptor–ligand complex (according to physiological pH with anionic aspartate and glutamate residues, and cationic lysine and arginine residues), then retaining only the ligand plus all complete residues with at least one atom located within no more than 6.0 Å from any ligand atom. The resulting complex models were then permitted to undergo 1000 molecular mechanics optimization steps in Avogadro[46] using the MMFF94 force field and electrostatic charge model.[47] The resulting complexes were then rendered in PYMOL. The computational studies indicate that inhibitor **8** is capable of nestling snugly in the active site of the 3C and 3CL proteases.

In summary, we report herein for the first time the inhibition of the 3Cpro and 3CL pro of viral pathogens belonging to the picornavirus-like protease supercluster by a macrocyclic inhibitor. A full account describing the exploration of R, linker, n (ring size), and the nature of warhead X, will be reported in due course.

Acknowledgment

The generous financial support of this work by the National Institutes of Health (AI081891) is gratefully acknowledged.

References and notes

1. Racaniello, V. R. In *Picornaviridae: The Viruses and their Replication in Fields Virology*; Knipe, D. M., Howley, P. M., Eds.; Lippincott, Williams & Wilkins: Philadelphia, 2007; Vol. 1, pp 795–838.
2. *The Picornaviruses*; Ehrenfeld, E., Domingo, E., Roos, R. P., Eds.; ASM Press: Washington, DC, 2010.
3. (a) Solomon, T.; Lewtwaite, P.; Perera, D.; Cardosa, M. J.; McMinn, P.; Ooi, M. H. *Lancet Infect. Dis.* **2010**, *10*, 778; (b) McMinn, P. C. *Curr. Opin. Virol.* **2012**, *2*, 199.
4. (a) Turner, R. B.; Couch, R. B. In *Rhinoviruses in Fields Virology*; Knipe, D. M., Howley, P. M., Eds.; Lippincott, Williams & Wilkins: Philadelphia, 2007; Vol. 1, pp 895–909; (b) Winther, B. *Proc. Am. Thorac. Soc.* **2011**, *8*, 79; (c) Ren, L.; Xiang, Z.; Wang, J. *Curr. Infect. Dis. Rep.* **2012**, *14*, 284.
5. (a) Perlman, S.; Netland, J. *Nat. Rev. Microbiol.* **2009**, *7*, 439; (b) Khan, G. *Virol. J.* **2013**, *10*, 66.
6. (a) Atmar, R. L. *Food Environ. Virol.* **2010**, *2*, 117; (b) Patel, M. M.; Hall, A. J.; Vinje, J.; Parashar, U. D. *J. Clin. Virol.* **2009**, *44*, 1.
7. (a) Ramajayam, R.; Tan, K. P.; Liang, P. H. *Biochem. Soc. Trans.* **2011**, *39*, 1371; (b) Tong, T. R. *Infect. Disord. Drug Targets* **2009**, *9*, 223.
8. Thibaut, H. J.; De Palma, A. M.; Neyts, J. *Biochem. Pharmacol.* **2012**, *83*, 185.
9. Steuber, H.; Hilgenfeld, R. *Curr. Top. Med. Chem.* **2010**, *10*, 323.
10. Eckardt, A. J.; Baumgart, D. C. *Recent Pat. Antiinfect. Drug Disc.* **2011**, *6*, 54.
11. Blakeney, S. J.; Cahill, A.; Reilly, P. A. *Virology* **2003**, *308*, 216.
12. (a) Cui, S.; Wang, J.; Fan, T.; Qin, B.; Guo, L.; Lei, X.; Wang, J.; Wang, M.; Jin, Q. *J .Mol. Biol.* **2011**, *408*, 449; (b) Wang, J.; Fan, T.; Yao, X.; Guo, L.; Lei, X.; Wang, J.; Jin, Q.; Cui, S. *J. Virol.* **2011**, *85*, 10021.
13. (a) Lu, G.; Qi, J.; Chen, Z.; Xu, X.; Gao, F.; Lin, D.; Qian, W.; Liu, H.; Jiang, H.; Yan, J.; Gao, G. F. *J. Virol.* **2011**, *85*, 10319; (b) 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.; Shi, S.-R.; Liang, P.-H. *Bioorg. Med. Chem.* **2008**, *16*, 7388.
14. Allaire, M.; Chernaia, M. M.; Malcolm, B. A.; James, M. N. *Nature* **1994**, *369*, 72.
15. Seipelt, J.; Guarne, A.; Bergmann, E.; James, M.; Sommergruber, W.; Fita, I.; Skern, T. *Virus Res.* **1999**, *62*, 159.
16. Kim, Y.; Lovell, S.; Tiew, K. C.; Gunnam, G. R.; Alliston, K. R.; Battaille, K. P.; Groutas, W. C.; Chang, K. O. *J. Virol.* **2012**, *86*, 11754.
17. 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.
18. Zeitler, C. E.; Estes, M. K.; Venkataraman, P. B. V. *J. Virol.* **2006**, *80*, 5050.
19. Nakamura, K.; Someya, Y.; Kumasaka, T.; Ueno, G.; Yamamoto, M.; Sata, T.; Takeda, N.; Miyamura, T.; Tanaka, N. *J. Virol.* **2005**, *79*, 13685.
20. Akaji, K.; Konno, H.; Mitsui, H.; Teruya, K.; Shimamoto, Y.; Hattori, Y.; Ozaki, T.; Kusunoki, M.; Sanjoh, A. *J. Med. Chem.* **2011**, *54*, 7962. and references cited therein.
21. (a) Goetz, D. H.; Choe, Y.; Hansell, E.; Chen, Y. T.; McDowell, M.; Jonsson, C. B.; Roush, W. R.; McKerrow, J.; Craik, C. S. *Biochemistry* **2007**, *46*, 8744; (b) Chuck, C. P.; Chong, L. T.; Chen, C.; Chow, H. F.; Wan, D. C. C.; Wong, K. B. *PLoS One* **2010**, *5*, e13197; (c) Zhu, L.; George, S.; Schmidt, M. F.; Al-Gharabli, S. I.; Rademann, J.; Hilgenfeld, R. *Antiviral Res.* **2011**, *92*, 204; (d) Chuck, C. P.; Chow, H. F.; Wan, D. C.; Wong, K. B. *PLoS One* **2011**, *6*, e27228.

22. (a) Hardy, M. E.; Crone, T. J.; Brower, J. E.; Ettayebi, K. *Virus Res.* **2002**, *89*, 29; (b) Someya, Y.; Takeda, N.; Miyamura, T. *Antiviral Res.* **2005**, *110*, 91.
23. Barnard, D. L.; Kumaki, Y. *Future Virol.* **2011**, *6*, 615.
24. (a) 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; (b) 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; (c) Dou, D.; Mandadapu, S. R.; Alliston, K. R.; Kim, Y.; Chang, K.-O.; Groutas, W. C. *Bioorg. Med. Chem.* **2011**, *19*, 5749; (d) Dou, D.; Mandadapu, S. R.; Alliston, K. R.; Kim, Y.; Chang, K.-O.; Groutas, W. C. *Eur. J. Med. Chem.* **2012**, *47*, 59; (e) Dou, D.; He, G.; Mandadapu, S. R.; Aravapalli, S.; Kim, Y.; Chang, K.-O.; Groutas, W. C. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 377; (f) 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; (g) Mandadapu, S. R.; Gunnam, M. R.; Tiew, K. C.; Uy, R. A. Z.; Prior, A. M.; Alliston, K. R.; Hua, D. H.; Kim, Y.; Chang, K. O.; Groutas, W. C. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 62; (h) Mandadapu, S. R.; Weerawarna, P. M.; Gunnam, M. R.; Alliston, K. R.; Lushington, G. H.; Kim, Y.; Chang, K. O.; Groutas, W. C. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4820; (i) Pokheil, L.; Kim, Y.; Thi, D.; Nguyen, T.; Prior, A. M.; Lu, J.; Chang, K. O.; Hua, D. H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3480.
25. (a) Tyndall, J. D.; Nall, T.; Fairlie, D. P. *Chem. Rev.* **2005**, *105*, 973; (b) Madala, P. K.; Tyndall, J. D.; Nall, T.; Fairlie, D. P. *Chem. Rev.* **2010**, *110*, 3299.
26. Tyndall, J. D.; Fairlie, D. P. *Curr. Med. Chem.* **2001**, *8*, 893.
27. Gilon, C.; Halle, D.; Chorev, M.; Selinger, Z.; Byk, G. *Biopolymers* **1991**, *31*, 745.
28. Glenn, M. P.; Pattenden, L. K.; Reid, R. C.; Tyssen, D. P.; Tyndall, J. D.; Birch, C. J.; Fairlie, D. P. *J. Med. Chem.* **2002**, *45*, 371.
29. Marsault, E.; Peterson, M. L. *J. Med. Chem.* **1961**, *2011*, 54.
30. McCreary, R. P.; Fairlie, D. P. *Curr. Opin. Drug Disc. Dev.* **1998**, *1*, 208.
31. (a) Meanwell, N. *Chem. Res. Toxicol.* **2011**, *24*, 1420; (b) Lipinski, C. A. *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235; (c) Veber, D. F. *J. Med. Chem.* **2002**, *45*, 2615; (d) Ritchie, T. J.; Ertl, P.; Lewis, R. *Drug Discovery Today* **2011**, *16*, 65; (e) Gleeson, M. P. *J. Med. Chem.* **2008**, *51*, 817; (f) Adessi, C.; Soto, C. *Curr. Med. Chem.* **2002**, *9*, 963; (g) Edwards, M. P.; Price, D. A. *Annu. Rep. Med. Chem.* **2010**, *45*, 381.
32. Roper, S.; Kolb, H. C. *Methods Princ. Med. Chem.* **2006**, *34*, 313.
33. Kelly, A. R.; Wei, J.; Kesavan, S.; Marie, J. C.; Windmon, N.; Young, D. W.; Marcaurelle, L. A. *Org. Lett.* **2009**, *11*, 2257.
34. Pehere, A. D.; Abell, A. D. *Org. Lett.* **2012**, *14*, 1330.
35. Zhang, J.; Kemmink, J.; Rijkers, D. T. S.; Liskamp, R. M. J. *Org. Lett.* **2011**, *13*, 3438.
36. All compounds were characterized by ^{1}H NMR and HRMS, and had a >95% purity.
37. 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. *Bioorg. Med. Chem.* **1854**, *2010*, 18.
38. *Compound* ***8***: ^{1}H NMR (DMSO-d_6): δ 9.49 (s, 1H), 7.83 (s, 1H), 7.30 (m, 5H), 5.10 (m, 2H), 4.50 (m, 1H), 4.40 (m, 2H), 3.80 (m, 2H), 3.11 (m, 2H), 2.88 (m, 2H), 2.98–2.24 (m, 5H), 1.49–1.80 (m, 5H), 0.81–0.99 (m, 6H). HRMS. Calculated M+Na 578.2703. Found mass: 578.2702.
39. Chang, K. O.; Sosnovtsev, S. V.; Belliot, G.; King, A. D.; Green, K. Y. *Virology* **2006**, *2*, 463.
40. Chang, K. O.; George, D. W. *J. Virol.* **2007**, *22*, 12111.
41. Chang, K. O. *J. Virol.* **2009**, *83*, 8587.
42. Kim, Y.; Thapa, M.; Hua, D. H.; Chang, K.-O. *Antiviral Res.* **2011**, *89*, 165.
43. Tan, J.; George, S.; Kusov, Y.; Perbandt, M.; Anemuller, S.; Mesters, J. R.; Norder, H.; Coutard, B.; Lacroix, C.; Leyssen, P.; Neyts, J.; Hilgenfeld, R. J. *Virol.* **2013**, *87*, 4339. Protein data bank entry 3ZZB.
44. Lee, C. C.; Kuo, C. J.; Ko, T. P.; Hsu, M. F.; Tsui, Y. C.; Chang, S. C.; Yang, S.; Chen, S. J.; Chen, H. C.; Hsu, M. C.; Shih, S. R.; Liang, P. H.; Wang, A. H.-J. *J. Biol. Chem.* **2009**, *284*, 7646.
45. The PYMOL Molecular Graphics System, Version 1.5, Schrödinger, LLC; 2012.
46. Hanwell, M. D.; Curtis, D. E.; Lonie, D. C.; Vandermeersch, T.; Zurek, E.; Hutchison, G. R. *J. Cheminf.* **2012**, *4*, 17.
47. Halgren, T. A. *J. Comput. Chem.* **1998**, *5–6*, 490.