Bioorganic & Medicinal Chemistry Letters 20 (2010) 3026–3030

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and SAR of novel isoquinoline CXCR4 antagonists with potent anti-HIV activity

John F. Miller [a,*], Kristjan S. Gudmundsson [a], Leah D'Aurora Richardson [a], Stephen Jenkinson [d], Andrew Spaltenstein [a], Michael Thomson [b], Pat Wheelan [c]

[a] Department of Medicinal Chemistry, Infectious Diseases Center for Excellence in Drug Discovery, GlaxoSmithKline Research and Development, Five Moore Drive, Research Triangle Park, NC 27709-3398, USA
[b] Department of Virology, Infectious Diseases Center for Excellence in Drug Discovery, GlaxoSmithKline Research and Development, Five Moore Drive, Research Triangle Park, NC 27709-3398, USA
[c] Department of DMPK, Infectious Diseases Center for Excellence in Drug Discovery, GlaxoSmithKline Research and Development, Five Moore Drive, Research Triangle Park, NC 27709-3398, USA
[d] Department of Biochemical and Analytical Pharmacology, Infectious Diseases Center for Excellence in Drug Discovery, GlaxoSmithKline Research and Development, Five Moore Drive, Research Triangle Park, NC 27709-3398, USA

ARTICLE INFO

Article history:
Received 10 March 2010
Revised 30 March 2010
Accepted 31 March 2010
Available online 3 April 2010

Keywords:
CXCR4
Isoquinoline
Tetrahydroquinoline
AMD070
HIV
Antiviral

ABSTRACT

Using AMD070 as a starting point for structural modification, a novel series of isoquinoline CXCR4 antagonists was developed. A structure–activity scan of alternate lower heterocycles led to the 3-isoquinolinyl moiety as an attractive replacement for benzimidazole. Side chain optimization in the isoquinoline series led to a number of compounds with low nanomolar anti-HIV activities and promising rat PK properties.

In recent years the medicinal chemist's focus in the battle against HIV/AIDS has broadened to include molecular targets beyond the HIV reverse transcriptase and protease enzymes, which are the cornerstones of the highly active antiretroviral therapy (HAART).[1] One area that has received a great deal of attention is the concept of blocking viral entry by targeting the chemokine receptors CCR5 and CXCR4, which function as co-receptors, along with CD4, to facilitate fusion of the viral membrane with the host cell.[2] CXCR4 is a G-protein coupled 7-transmembrane receptor utilized by T-tropic HIV strains to gain entry into T-cells. The appearance of CXCR4 utilizing strains of HIV is associated with a decrease in the number of T-cells and accelerated disease progression.[3] In vitro studies have shown that addition of the natural CXCR4 ligand, SDF-1, or small molecule antagonists, can block HIV infection.[2e] SDF-1 is a highly basic protein with about 20% of its 68 amino acids (for SDF-1α) being arginine, lysine or histidine. This observation, along with the fact that the extracellular binding regions of the CXCR4 receptor are particularly rich in aspartic acid and glutamic acid residues, points to a receptor–ligand binding model in which charge-charge interactions play a prominent role.[4] Not surprisingly, the currently known small molecule CXCR4 antagonists are also highly basic in nature. A particularly notable example is the tetrahydroquinoline derivative AMD070 (Fig. 1) which was recently shown to possess significant anti-HIV efficacy in human clinical studies.[5] Key features of the AMD070 pharmacophore include a triad of basic nitrogen atoms and a distal amino group attached to the central nitrogen by a 4-carbon tether.

We recently reported a structure–activity study toward improving the antiviral potency and/or ADME properties of AMD070 through iterative structural modifications. Specifically, we showed that it was possible to shift the distal amine side chain from the central nitrogen to either ring of the benzimidazole with retention of potent antiviral activity (Fig. 1, compounds **1** and **2**).[6] In a separate report we described related efforts in which we replaced the benzimidazole with an imidazopyridine in combination with the side chain shift.[7] The results of these studies prompted us to explore the possibility of replacing the benzimidazole with other heteroaromatic ring systems capable of maintaining the required basic nitrogen triad.

* Corresponding author. Tel.: +1 919 483 2750; fax: +1 919 315 6923.
E-mail address: john.6.miller@gsk.com (J.F. Miller).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.03.118

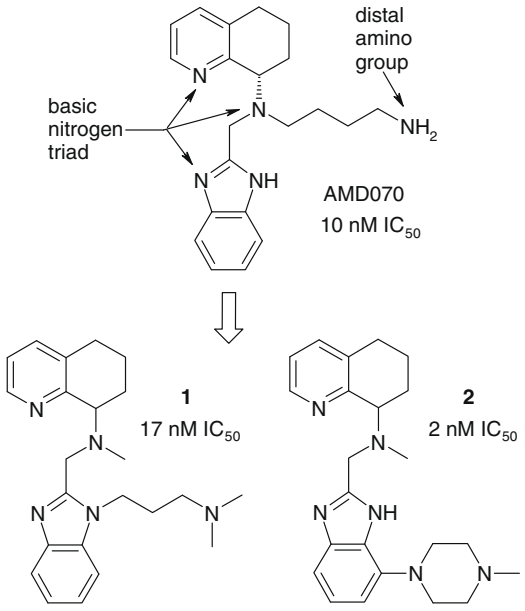
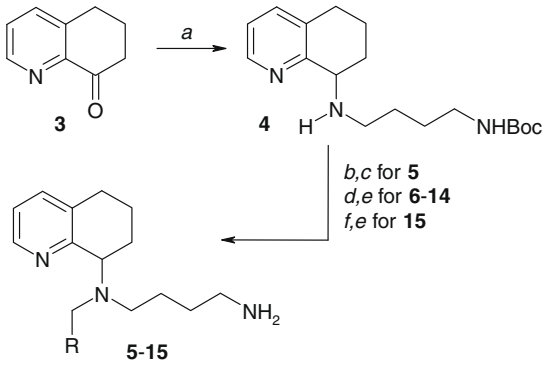


Figure 1. Tetrahydroquinolines with potent anti-HIV activity.

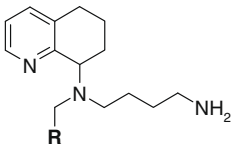
In order to identify viable alternative ring systems, we conducted a scan of various heteroaryl fragments with the distal amine side chain attached to the central nitrogen analogous to AMD070, with the exception that this study was done in the racemic series. Our synthetic approach is illustrated in Scheme 1. The key secondary amine intermediate **4** was synthesized by reductive amination of ketone **3**[8] with *N*-Boc-1,4-diaminobutane. The benzimidazole derivative **5** was prepared via alkylation with *N*-Boc-2-(chloromethyl)benzimidazole followed by TFA deprotection. Compounds **6**–**14** were prepared by reductive alkylations with the appropriate heteroaryl aldehydes followed by HCl mediated cleavage of the Boc protecting group. The benzothiazole analog **15** was synthesized by alkylation with 2-(bromomethyl)-1,3-benzothiazole followed by acidic deprotection.

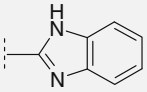
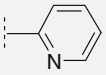
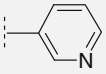
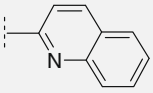
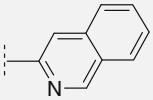
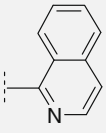
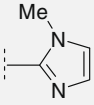
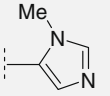
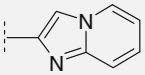
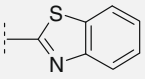
Antiviral and cytotoxicity data for the alternate lower heterocycle derivatives is shown in Table 1. Most of the ring systems studied retain appreciable antiviral activity with several in the same potency range as the benzimidazole derivative **5** (racemic version of AMD070). Exceptions include the 3-pyridyl and imidazole derivatives **7** and **12**, which due to the position of the basic ring nitrogens, are incapable of maintaining the required nitrogen triad



Scheme 1. Reagents and conditions: (a) *N*-Boc-1,4-diaminobutane, $NaBH(OAc)_3$, AcOH, 1,2-dichloroethane (73%); (b) *N*-Boc-2-(chloromethyl)benzimidazole, KI, $(iPr)_2EtN$, MeCN, rt (83%); (c) TFA, CH_2Cl_2 (71%); (d) RCHO, $NaBH(OAc)_3$, AcOH, 1,2-dichloroethane (55% for **6**); (e) 4 N HCl/dioxane, MeOH (62% for **6**); (f) 2-(bromomethyl)-1,3-benzothiazole, KI, $(iPr)_2EtN$, MeCN, 80 °C (59%).

Table 1
Anti-HIV IC_{50}s ± standard deviation (n) and CC_{50}s for alternate lower heterocycle derivatives



Compound	R	IC_{50}[a] (nM)	CC_{50}[b] (nM)
5[c]		23 ± 2 (4)	13,000
6[d]		70 ± 8 (4)	11,000
7		>20,000 (1)	>20,000
8[d]		34 ± 7 (2)	12,000
9		12 ± 1 (2)	13,000
10		11 ± 1 (2)	8200
11[e]		120 ± 3 (2)	>20,000
12		>20000 (2)	>20,000
13		42 ± 8 (2)	>20,000
14[d]		340 ± 29 (2)	9700
15[d]		200 ± 12 (2)	13,000

[a] HOS cells expressing hCXCR4/hCCR5/hCD4/pHIV-LTR-luciferase, HIV-1, CXCR4 strain (IIIB). Compounds were tested for their ability to block infection of the HOS cell line. IC_{50} is the concentration at which 50% efficacy in the antiviral assay was observed.[9]

[b] CC_{50} is the concentration at which 50% cytotoxicity is observed in the HOS cell line.

[c] Compound previously reported in Ref. 10.

[d] Compound previously reported in Ref. 11.

[e] Compound previously reported in Ref. 12.

geometry. The isoquinoline derivatives **8**–**10** were particularly interesting with antiviral activities in the 10–30 nM range. A comparison of these compounds with the pyridyl derivative **6**, shows that benzo ring fusion confers a consistent improvement in activity. Compounds **14** and **15** show reduced activities relative to the corresponding nitrogen analogs (i.e., **15** compared to **5**), perhaps due to the reduced basicity of the thiazole and benzothiazole ring nitrogens.

Having identified several suitable replacements for the benzimidazole ring system, we turned our attention to shifting the distal amine side chain from the central nitrogen to the lower heterocycle. Based on a combination of attractive potency and synthetic tractability, we chose the isoquinoline system **9** for further study. We explored two different modes of attaching the amino group to the 4-position of the isoquinoline ring system: 1- and 3-carbon tethers (Fig. 2, general structure **A**).

Scheme 2 illustrates the synthesis of the 1-carbon primary amine **23**. In order to assess the pharmacodynamic effect of the distal amine side chains, we also prepared the unsubstituted parent compound **19** for comparison. The bromomethyl intermediate **17** was prepared by NBS bromination of commercially available 3-methylisoquinoline **16**. Reaction of **17** with the enantiopure tetrahydroquinoline derivative **18**[6] gave **19** in high yield. The *S* absolute configuration of **18** was chosen based on the clear activity preference observed in our previous studies.[6,7] Substitution at the 4-position of the isoquinoline was achieved by NIS iodination to give intermediate **20**, which was then subjected to Pd catalyzed cyanation followed by NBS bromination. Benzylic bromide **21** was reacted with **18** to afford nitrile **22** which was subjected to Raney nickel reduction to give primary amine **23**. Scheme 3 details the subsequent side chain elaboration of **23** to give target compounds **24**–**29**.

Our synthetic route to the 3-carbon series is shown in Scheme 4. Heck reaction of iodide **20** with acrylonitrile followed by NBS bromination gave **30** as an *E*/*Z* isomer mixture. Base promoted S_N2 substitution of bromide **30** with **18** gave **31** in high yield. Sodium borohydride reduction of **31** afforded nitrile **32,** which upon Raney nickel reduction gave **33**. Primary amine **33** was then further elaborated as described in Scheme 4 to afford compounds **34**–**37**.

Table 2 shows SAR data for the 4-substituted isoquinoline series. Interestingly, the unsubstituted analog **19** shows significant activity even in the absence of a basic, distal amine side chain. Attaching the 1-carbon primary amine moiety (compound **23**) affords a sixfold increase in activity relative to **19**. Dimethylation of the amino group leads to a twofold loss in activity. A comparison of **23** and **24** with the longer chain amino derivatives **33** and **34,** shows that the 3-carbon chain is favored, a result consistent with our previously reported chain length SAR in the benzimidazole series.[6] The equipotent guanidine derivatives **25** and **35** showed impressive activities and the best cytotoxicity windows in the entire series. The equivalent activities of these 1- and 3-carbon analogs contrasts with the modest but consistent potency preference for the 3-carbon chain length that is generally observed. Perhaps this is due to the ability of the guanidine moiety to present two basic nitrogens in a 1,3-relationship thereby allowing the proximal nitrogen of **35** to mimic the distal nitrogen of **25**. A surprising aspect of the SAR in this series is the unexpectedly potent activity of the non-basic,

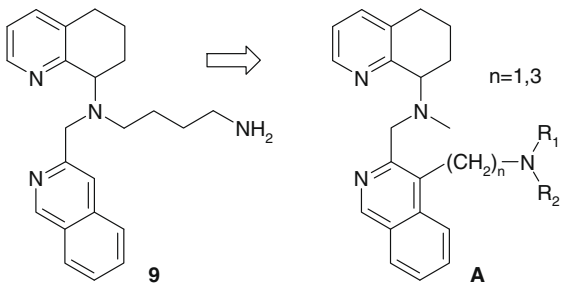
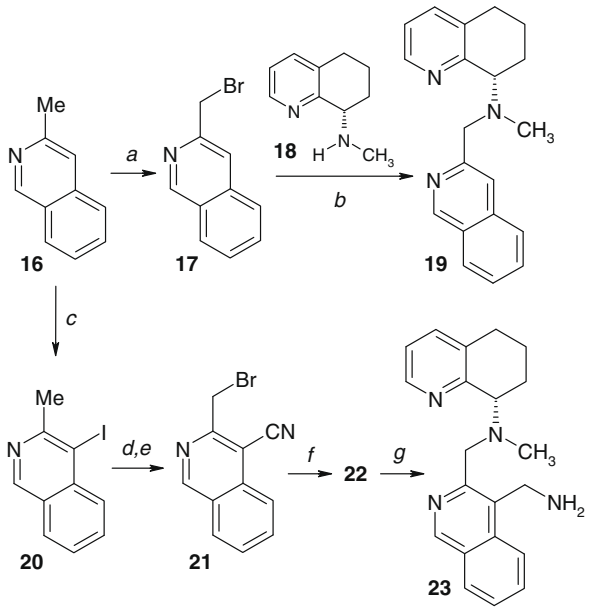
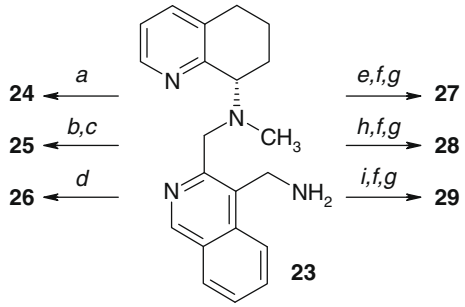


Figure 2.



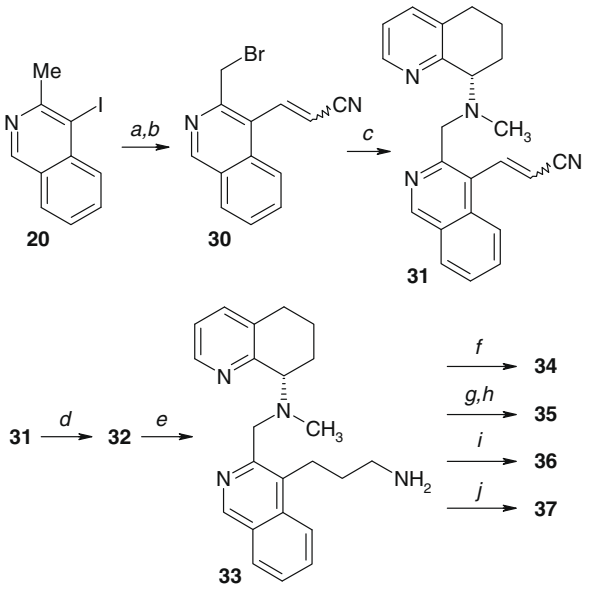
Scheme 2. Reagents and conditions: (a) NBS, AIBN, CCl_4, reflux (66%); (b) compound **18**, $(i\text{Pr})_2\text{EtN}$, DMF, rt (98%); (c) NIS, glacial AcOH, 80 °C (56%); (d) $Zn(CN)_2$, $Pd(Ph_3P)_4$, DMF, 120 °C (96%); (e) NBS, AIBN, CCl_4, reflux (62%); (f) compound **18**, $(i\text{Pr})_2\text{EtN}$, DMF, rt (92%); (g) H_2 (50 psi), Raney Ni, 2 M NH_3/MeOH (53%).



Scheme 3. Reagents and conditions: (a) 37% aqueous formaldehyde, $NaBH(OAc)_3$, AcOH, 1,2-dichloroethane (77%); (b) *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine, 1:1 THF/CH_2Cl_2 (68%); (c) TFA, CH_2Cl_2 (87%); (d) $MeSO_2Cl$, $(i\text{Pr})_2\text{EtN}$, CH_2Cl_2 (70%); (e) Boc-glycine, HATU, $(i\text{Pr})_2\text{EtN}$, MeCN (72%); (f) TFA, CH_2Cl_2 (89% for **30**); (g) 37% aqueous formaldehyde, $NaBH(OAc)_3$, AcOH, 1,2-dichloroethane (84% for **30**); (h) Boc-L-proline, HATU, $(i\text{Pr})_2\text{EtN}$, MeCN (63%); (i) Boc-D-proline, HATU, $(i\text{Pr})_2\text{EtN}$, MeCN (71%).

capped amine derivatives **26**, **36** and **37**. In fact, the 3-carbon sulfonamide **37** is nearly as active as the corresponding primary amine **33**. Similarly interesting is the activity of nitrile derivatives **22** and **32**. This contrasts sharply with our previous observations in the benzimidazole series, where non-basic, acylated amine side chains showed substantial reductions in activity. Within the set of lower heterocycles that we have explored, there is clearly something unique to the isoquinoline system that apparently facilitates side chain-receptor interactions through non-charged forces (i.e., hydrogen bond and/or dipole-dipole interactions).

The amide derivatives **27**–**29** allowed us to examine 4-atom tethers between the basic nitrogen and the ring system, albeit in a more conformationally rigid manner. The dimethylaminoglycine analog **27** was quite potent with a 12 nM IC_{50}. Proline derivatives **28** and **29** show that conformationally constraining the basic amine into a five-membered ring leads to fourfold



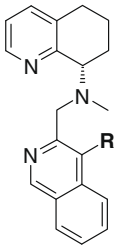
Scheme 4. Reagents and conditions: (a) acrylonitrile, $Pd(OAc)_2$, Ph_3P, Et_3N, DMF, 100 °C (77%); (b) NBS, AIBN, CCl_4, reflux (57%); (c) compound **18**, $(iPr)_2EtN$, DMF, rt (98%); (d) $NaBH_4$, *i*PrOH, rt, 5 days (71%); (e) H_2 (1 atm), Raney Ni, 2 M NH_3/MeOH (70%); (f) 37% aqueous formaldehyde, $NaBH(OAc)_3$, 1,2-dichloroethane (81%); (g) *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine, 1:1 THF/CH_2Cl_2 (71%); (h) TFA, CH_2Cl_2 (91%); (i) *N*,*N*-dimethylcarbamyl chloride, $(iPr)_2EtN$, CH_2Cl_2 (75%); (j) $MeSO_2Cl$, $(iPr)_2EtN$, CH_2Cl_2 (83%).

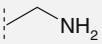
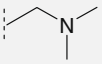
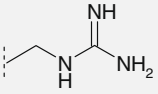
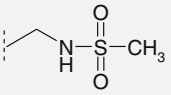
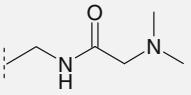
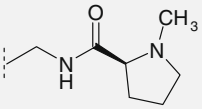
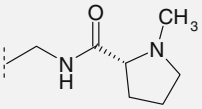
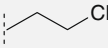
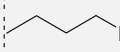
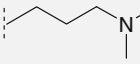
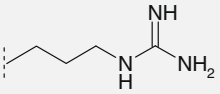
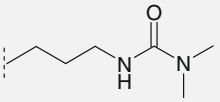
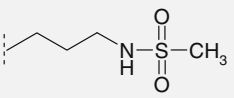
reduction in activity for the *S*-enantiomer **28** with the *R*-enantiomer **29** remaining unchanged relative to the open chain analog. The 3-carbon amine derivative **34** is the most active analog in the entire series with a 5 nM IC_{50} and a 130-fold cytotoxicity window. In comparing the overall SAR of the isoquinoline series with our previously reported benzimidazole[6] and imidazopyridine series,[7] we were able to achieve similar antiviral activities, however, the isoquinolines show somewhat diminished cytotoxicity windows.

To further explore the therapeutic potential of the isoquinoline series, a representative set of compounds was chosen for pharmacokinetic analysis in rats. Table 3 illustrates the PK parameters associated with five selected analogs. All of the compounds showed acceptable half life and clearance values and with the exception of the guanidine derivative **25**, they also showed at least some level of systemic exposure. The dimethyl urea derivative **36** showed the best exposure with a 20% oral bioavailability, thus demonstrating the potential of this structural class in regards to lower species PK.

Using AMD070 as a starting point for structural modification, we identified several viable heterocyclic replacements for the benzimidazole ring system. The 3-isoquinolinyl moiety was chosen for further structural manipulation, specifically by using our previously successful approach of shifting the distal amine side chain to the lower heterocyclic ring system. This led to a number of compounds with low nanomolar antiviral activities, and to the surprising observation of potent activity with non-basic side chain derivatives. In addition, several analogs were shown to have acceptable rat PK properties. While we were able to achieve potency and pharmacokinetic profiles similar to our benzimidazole and imidazopyridine series, the isoquinolines suffered from generally less favorable cytotoxicity windows. However, this exercise effectively validates the concept of combining heterocyclic replacements with side chain shifts to generate a pharmacologically viable and structurally novel chemotype in the tetrahydroquinoline series.

Table 2
Anti-HIV IC_{50}s ± standard deviation (*n*) and CC_{50}s for 4-substituted isoquinoline derivatives



Compound	R	IC_{50}[a] (nM)	CC_{50}[b] (nM)
19	H	160 ± 19 (2)	650
22	CN	72 ± 9 (2)	1300
23		27 ± 11 (2)	3100
24		48 ± 7 (5)	5900
25		20 ± 5 (4)	9000
26		41 ± 9 (4)	2000
27		12 ± 4 (5)	480
28		44 ± 9 (2)	540
29		12 ± 5 (2)	690
32		21 ± 2 (2)	950
33		12 ± 2 (2)	1000
34		5.0 ± 2.0 (5)	650
35		17 ± 3 (2)	6500
36		38 ± 12 (4)	750
37		16 ± 2 (4)	690

[a,b] See footnotes a and b from Table 1.

Table 3
Pharmacokinetic parameters of representative isoquinoline CXCR4 antagonists in rats

Compound	$t_{1/2}$ (h)	Cl (mL/min/kg)	V_d (L/kg)	%*F*
25	12	16	9.0	0
27	1.5	11	1.5	10
34	2.9	6.1	2.9	6
36	3.1	16	1.7	20
37	5.8	9.5	1.4	4

Half life ($t_{1/2}$), clearance (Cl) and volume of distribution (V_d) calculated following 1 mg/kg IV doses. Percent oral bioavailability (%*F*) calculated following solution doses of 3 mg/kg.

Acknowledgments

We thank Richard Hazen, Wendell Lawrence, David McCoy for providing HIV-1 antiviral data.

References and notes

1. Agrawal, L.; Lu, X.; Jin, Q.; Alkhatib, G. *Curr. Pharm. Des.* **2006**, *12*, 2031.
2. (a) Qian, K.; Morris-Natschke, S. L.; Lee, K. *Med. Res. Rev.* **2009**, *29*, 369; (b) Kuritzkes, D. R. *Curr. Opin. HIV AIDS* **2009**, *4*, 82; (c) Kazmierski, W. M.; Gudmundsson, K. S.; Piscitelli, S. C. *Ann. Rep. Med. Chem.* **2007**, *42*, 301; (d) Mosley, C. A.; Wilson, L. J.; Wiseman, J. M.; Skudlarek, J. W.; Liotta, D. C. *Expert Opin. Ther. Patents* **2009**, *19*, 23; (e) Grande, F.; Garofalo, A.; Neamati, N. *Curr. Pharm. Des.* **2008**, *14*, 385.
3. Schuitemaker, H.; Koot, M.; Kootstra, N. A.; Dercksen, M. W.; de Goede, R. E.; van Steenwijk, R. P.; Lange, J. M.; Schattenkerk, J. K.; Miedema, F.; Tersmette, M. *J. Virol.* **1992**, *66*, 1354.
4. (a) Dealwis, C.; Fernandez, E. J.; Thompson, D. A.; Simon, R. J.; Siani, M. A.; Lolis, E. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6941; (b) Hatse, S.; Princen, K.; Gerlach, L.; Bridger, G.; Henson, G.; De Clercq, E.; Schwartz, T. W.; Schols, D. *Mol. Pharm.* **2001**, *60*, 164.
5. Moyle, G.; DeJesus, E.; Boffito, M.; Wong, R. S.; Gibney, C.; Badel, K.; MacFarland, R.; Calandra, G.; Bridger, G.; Becker, S. *Clin. Infect. Dis.* **2009**, *48*, 798.
6. Gudmundsson, K. S.; Sebahar, P. R.; Richardson, L. D.; Miller, J. F.; Turner, E. M.; Catalano, J. G.; Spaltenstein, A.; Lawrence, W.; Thomson, M.; Jenkinson, S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5048.
7. Gudmundsson, K. S.; Boggs, S. D.; Catalano, J. G.; Svolto, A.; Spaltenstein, A.; Thomson, M.; Wheelan, P.; Jenkinson, S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6399.
8. Kelly, T. R.; Lebedev, R. L. *J. Org. Chem.* **2002**, *67*, 2197.
9. All compounds were also characterized in a cell fusion CXCR4 receptor binding assay. The resulting SAR tracked closely with the HOS antiviral data. For example, compounds **5**, **27** and **34** showed IC_{50}s in the receptor binding assay of 36, 16, and 3.4 nM, respectively. In addition, representative compounds were characterized in a cell based functional assay and were shown to be non-competitive antagonists of the CXCR4 receptor. For assay protocols see: Gudmundsson, K.; Miller, J. F.; Turner, E. M. International Patent WO 2006/020415, 2006.
10. Bridger, G. J.; Skerlj, R. T.; Kaller, A.; Harwig, C.; Bogucki, D.; Wilson, T.; Crawford, J.; McEachern, E. J.; Atsma, B.; Nan, S.; Zhou, Y.; Schols, D.; Smith, C. D.; Di Fluri, R. M. International Patent WO 2003/055876, 2003.
11. Bridger, G.; McEachern, E. J.; Skerlj, R.; Schols, D. U.S. Patent US 2004/020992, 2004.
12. Bridger, G.; Kaller, A.; Harwig, C.; Skerlj, R.; Bogucki, D.; Wilson, T. R.; Crawford, J.; McEachern, E. J.; Atsma, B.; Nan, S.; Zhou, Y.; Schols, D.; Smith, C. D.; Di Fluri, M. R. U.S. Patent US 2004/0019058, 2004.