Design of novel CXCR4 antagonists that are potent inhibitors of T-tropic (X4) HIV-1 replication

Renato Skerlj [a,*], Gary Bridger [b], Ernest McEachern [b], Curtis Harwig [b], Chris Smith [b], Alan Kaller [b], Duane Veale [b], Helen Yee [b], Krystyna Skupinska [b], Rossana Wauthy [b], Letian Wang [b], Ian Baird [b], Yongbao Zhu [b], Kate Burrage [b], Wen Yang [b], Michael Sartori [b], Dana Huskens [c], Erik De Clercq [c], Dominique Schols [c]

[a] Genzyme Corp., 153 Second Avenue, Waltham, MA 02451, USA
[b] AnorMED, Inc., 200-20353 64th Avenue, Langley, British Columbia, Canada V2Y 1N5
[c] Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

ARTICLE INFO

ABSTRACT

A novel series of CXCR4 antagonists were identified based on the substantial redesign of AMD070. These compounds possessed potent anti-HIV-1 activity and showed excellent pharmacokinetics in rat and dog.

© 2011 Elsevier Ltd. All rights reserved.

Recent recommendations of the International AIDS Society Panel-USA on antiretroviral therapy (ART) for HIV-infected adults state, that frequent monitoring of patient HIV-1 RNA is necessary to identify and manage treatment failure, with the goal being suppression of HIV-1 RNA below quantification limits.[1] Frequent monitoring is necessary to identify virologic failure due to the emergence of resistance variants. Recently approved drugs with novel mechanisms of action such as raltegravir[2] and maraviroc[3] that target HIV integrase and the chemokine receptor CCR5 add to the armory of drugs available to treat HIV and provide additional treatment options for managing resistance.

The discovery of the chemokine receptors CXCR4 and CCR5 in 1996 as co-receptors for HIV for cell entry by HIV-1[4] prompted a concerted research effort to discover novel antagonists of these receptors. Our longstanding research interest in CXCR4 led to the first clinical proof of concept that blocking CXCR4 with a small molecule antagonist, AMD3100, results in viral load reduction in T-tropic (X4) HIV-1 infected patients.[5] This was followed by the discovery and development of the first orally bioavailable CXCR4 antagonist AMD070[6] that also demonstrated viral load reduction

in T-tropic (X4) HIV-1 infected patients.[7] In addition to HIV, CXCR4 and its specific ligand, CXCL12 [stromal cell derived factor-1 (SDF-1)], have been implicated to play a role in tumor progression, angiogenesis, metastasis and survival.[8] Moreover, the CXCR4/CXCL12 axis also plays an important role in the homing and retention of progenitor cells in the bone marrow microenvironment.[9] Consequently, based on compelling clinical data, plerixafor (AMD3100) was approved in the US in 2008 for mobilization of hematopoietic stem cells in patients with non-Hodgkins's lymphoma (NHL)[10] and multiple myeloma (MM).[11]

Recently we reported on our medicinal chemistry efforts that led to the design and synthesis of oral agents such as AMD070[6] as well as novel heterocyclic analogs of AMD070.[12] In addition researchers from GlaxoSmithKline have reported on an extensive analog program of AMD070.[13] In this Letter, we disclose our efforts on modifying AMD070 (**1**) by sequentially replacing the benzimidazole ring with a substituted pyridine ring (**2**), opening up the tetrahydroquinoline ring (**3**), and combining both of these modifications to afford the open chain analogs (**4**) while still maintaining potent HIV-1 inhibition (Fig. 1).

The primary data used to drive the SAR were the ability of these compounds to inhibit replication of HIV-1 NL4.3, using exclusively CXCR4 for viral entry into its target cells. In addition, the ability of these compounds to specifically interact with CXCR4 were also
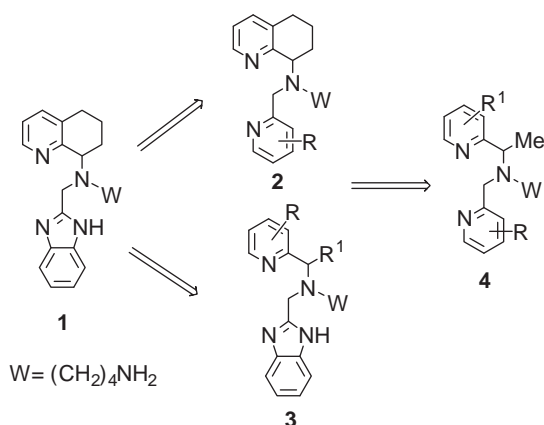
Figure 1. Structure of racemic AMD070 (**1**) and related analogs **2**, **3** and **4**.

measured by the effects on SDF-1 induced Ca^{2+} flux in $CD4^+CXCR4^+T$ cells (CEM-CCRF) cells and were found to correlate with the anti-HIV-1 activity (Tables 1 and 3). Although the molecules contain a stereocenter the SAR was based on the racemic mixture.

Based on our early pharmacophore model,[14] where it was determined that the optimal spacer between the tertiary nitrogen atom and the adjoining nitrogen was a two carbon linker, we chose to replace the benzimidazole ring with a substituted pyridine ring specifically attached at the 2-position (Table 1). In contemplating redesigns to compound **1** we were cognizant of the importance of maintaining a basic amine moiety based on receptor mutagenesis studies showing the dependence on aspartic acid residues Asp 171 and Asp 262 as well as other amino acid residues on the binding affinity for compounds AMD3100[15] and AMD070.[16] The importance of aspartic acid residues was recently corroborated by the first X-ray crystal structure of ligands bound to CXCR4.[17] Consequently a series of 2-substituted pyridines were synthesized and

the effect on antiviral potency examined (Table 1). Gratifyingly the unsubstituted pyridine analog **2a** was threefold less potent when compared to **1**; IC_{50} of 15.5 versus 4.8 nM.[6] Walking a methyl group around the pyridine ring demonstrated the following trend regarding potency: 6-Me (**2e**) > 5-Me (**2d**) > 3-Me (**2b**) > 4-Me (**2c**), for example, compound **2e** was twofold more potent than **1** with an IC_{50} of 2.9 nM whereas **2d** had comparable potency (IC_{50} of 5.3 nM). Interestingly the 3,5-dimethyl analog **2g** resulted in an eightfold enhancement in potency compared to the 5-methyl analog **2d** (IC_{50} of 0.63 vs 5.3 nM). Although the 6-methyl analog **2e** was the most potent of the methyl series, substitution at the 6-position with either an H-bond donor (amino **2h**) or H-bond acceptor (methoxymethyl **2i**) moiety resulted in a 10- and 191-fold drop in potency relative to **2e**. On the other hand, substitution at the 3-position appears to be well tolerated (**2b**, **2j–n**). Apart from the hydroxyl group **2j** other substituents were either equipotent (amino **2k**) or enhanced potency; the bulky *i*Pr group **2l** is approximately threefold more potent than **2b** and the hydroxymethyl **2m** and chloro **2n** were 11- and 78-fold more potent than **2b**.

The synthetic strategy used to prepare compounds **2** is similar to methodology previously reported.[12] The key step in many of these examples was the generation of the appropriately functionalized bromomethyl (**2j–k**) or aldehyde moiety (**2a–i**, **2l–n**) of the pyridine ring which was then coupled with 2-(4-((5,6,7,8-tetrahydroquinolin-8-yl)amino)butyl)isoindoline-1,3-dione[18] **5** via N-alkylation or reductive amination (Scheme 1). The key intermediate **5** was synthesized by phthalamide protection of commercially available 4-aminobutan-1-ol followed by TPAP oxidation to the aldehyde and subsequent reductive amination with 8-amino-5,6,7,8-tetrahydroquinoline.[19] The phthalamide group was easily deprotected using an excess of hydrazine in ethanol at room temperature followed by salting with HBr in acetic acid to afford the final compounds as hydrobromide salts.

Having determined that a substituted pyridine ring in the place of the benzimidazole ring resulted in compounds with enhanced anti-HIV-1 activity (Table 1) we shifted our attention to gauge

Table 1
Anti-HIV-1 activity, cytotoxicity and calcium signaling (SDF-1) inhibition of compounds **2**[a,b]

| Compound	R	HIV-1 IC_{50}[c] (nM)	CC_{50}[d] (nM)	Ca^{2+} Flux IC_{50}[e] (nM)
2a	H	15.3 (*n* = 1)	164,900	74 (*n* = 3)
2b	3-Me	23.3 (*n* = 2)	32,400	18 (*n* = 4)
2c	4-Me	47.8 (*n* = 1)	36,900	91 (*n* = 2)
2d	5-Me	5.3 (*n* = 1)	35,300	35 (*n* = 2)
2e	6-Me	2.9 (*n* = 3)	298,700	53 (*n* = 5)
2f	4,6-diMe	17 (*n* = 2)	149,400	38 (*n* = 3)
2g	3,5-diMe	0.63 (*n* = 3)	32,600	19 (*n* = 4)
2h	6-NH_2	30 (*n* = 2)	157,900	49 (*n* = 1)
2i	6-CH_2OMe	553 (*n* = 1)	>153,500	1621 (*n* = 1)
2j	3-OH	113 (*n* = 2)	148,800	129 (*n* = 3)
2k	3-NH_2	19.7 (*n* = 3)	168,800	27 (*n* = 4)
2l	3-*i*Pr	8.4 (*n* = 1)	33,800	63 (*n* = 4)
2m	3-CH_2OH	2.1 (*n* = 2)	154,800	79 (*n* = 2)
2n	3-Cl	0.4 (*n* = 1)	190,900	44 (*n* = 3)

[a] Assay conditions reported in Ref. 6.
[b] Assays were performed in duplicate and values represent the mean with standard deviations <30% of the mean. Bracketed values represent the number of experiments.
[c] $CD4^+CXCR4^+$ lymphocytic MT-4 cell line were infected with the X4 HIV-1 NL4.3 strain. IC_{50} is the concentration of the compound required to protect 50% of the cells against viral cytopathicity.
[d] CC_{50} is the concentration required to reduce the viability of MT-4 cells by 50%.
[e] CEM-CCRF ($CD4^+CXCR4^+$) T cell line. IC_{50} is the concentration of the compound required to inhibit 50% of the SDF-1-induced Ca^{2+} signaling.
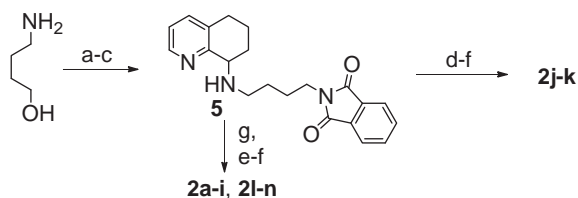
Scheme 1. Reagents: (a) Phthalic anhydride, CHCl$_3$, 70 °C; (b) TPAP, NMO, CH$_2$Cl$_2$; (c) 8-amino-5,6,7,8-tetrahydroquinoline, NaBH(OAc)$_3$, CH$_2$Cl$_2$; (d) RX, KI, DIPEA, CH$_3$CN, reflux; (e) N$_2$H$_4$, EtOH; (f) HBr, AcOH; (g) RCHO, NaBH(OAc)$_3$, CH$_2$Cl$_2$.

the effects of opening up the tetrahydroquinoline ring of **1** (Table 2). Surprisingly a dramatic effect on anti-HIV-1 potency was observed as the steric bulk at the benzylic position (R^1) was increased. For instance, the closest analog to **1**, compound **3a** where R^1 is a methyl group was approximately fourfold less active compared to **1** with an IC$_{50}$ of 18 nM. However, the ethyl analog **3b** was 182-fold less potent and the more sterically demanding isopropyl analog **3c** was 4000-fold less potent. A similar loss in potency was also observed for the phenyl analog **3d** and the gem dimethyl analog **3e** indicating the sensitivity to steric bulk at this position perhaps due to the binding orientation to CXCR4. Interestingly, when R^1 was a hydrogen atom **3f** moderate potency was observed, IC$_{50}$ of 410 nM. To further probe the SAR the addition of an H-bond donor hydroxyl group at the 3-position **3g** of the pyridine ring resulted in the complete abolition of activity. However, if the isopropyl group was substituted at the 3-position **3h** rather than the benzylic position comparable potency to the unsubstituted analog **3f** was observed suggesting that sterically demanding groups were tolerated at the 3-position. The pyridine ring was also found to be sensitive to substitution since the walking of a methyl group (**3i–l**) around the pyridine ring of **3a** resulted in a 24- to 230-fold loss in activity other than the 5-Me analog **3k** which was approximately fivefold more potent than **3a**, for example, IC$_{50}$ of 3.8 versus 18 nM (Table 2).

Table 2
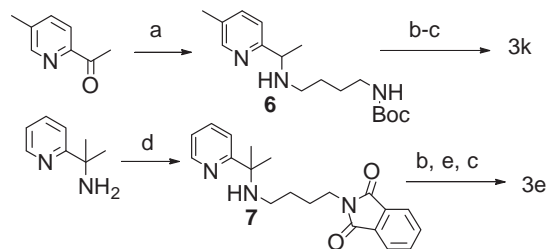Anti-HIV-1 activity and cytotoxicity of compounds **3**[a]
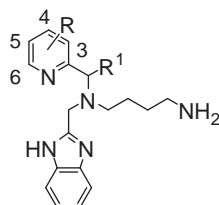
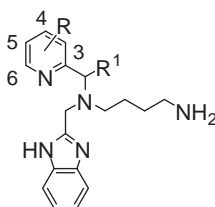| Compound	R	R^1	HIV-1 IC$_{50}$ (nM)	CC$_{50}$ (nM)
3a	H	Me	18 (n = 2)	>160,900
3b	H	Et	874 (n = 1)	>161,800
3c	H	iPr	16300 (n = 1)	>161,900
3d	H	Ph	13300 (n = 1)	>148,300
3e	H	Me$_2$	2500 (n = 1)	>159,300
3f	H	H	41 (n = 1)	151,100
3g	3-OH	H	>149200 (n = 1)	>149,200
3h	3-iPr	H	34 (n = 2)	145,100
3i	3-Me	Me	505 (n = 3)	>146,100
3j	4-Me	Me	438 (n = 2)	>141,600
3k	5-Me	Me	3.8 (n = 1)	>146,200
3l	6-Me	Me	4100 (n = 1)	>167,200
3m	6-NH$_2$	Me	2290 (n = 1)	>149,400

[a] See footnotes in Table 1.

Compounds **3** were prepared either starting from an appropriately functionalized aldehyde (**3a–b**, **3d**, **3f–l**) or primary amine (**3c**, **3e**, **3m**) as exemplified by the preparation of **3k** and **3e** (Scheme 2). For example, in the synthesis of **3k** commercially available 1-(5-methylpyridin-2-yl)ethanone was coupled with tert-butyl (4-aminobutyl)carbamate (prepared by the selective Boc protection of butane 1,4-diamine) in the presence of NaBH(OAc)$_3$ to afford the secondary amine **6**. N-alkylation with N-Boc-chloromethylbenzimidazole[6] in the presence of Hunig's base and KI followed by concomitant Boc deprotection and salting with HBr in acetic acid afforded **3k** as the hydrobromide salt. In the case of **3e** commercially available 2-(pyridin-2-yl)propane-2-amine underwent reductive amination with 4-(1,3-dioxoisoindolin-2-yl)butanal (prepared by phthalamide protection of the amino alcohol followed by oxidation to the aldehyde) to afford the secondary amine **7** which then underwent N-alkylation with N-Boc-chloromethylbenzimidazole[6] as described above. Deprotection of the phthalamide group with hydrazine in ethanol followed by treatment with HBr in acetic acid afforded **3e** as the hydrobromide salt (Scheme 2). Compounds **4** were synthesized using similar methodology.

By demonstrating that compound **1** could be substantially redesigned by first replacing the benzimidazole ring with a substituted pyridine ring and secondly opening up the tetrahydroquinoline ring to a pyridinyl ethanamine while still maintaining potent anti-HIV-1 activity, a series of analogs **4** were synthesized where both of these changes were incorporated (Table 3). We were pleased to find that these compounds had comparable potency to the precursors **2** and **3** verifying our original pharmacophore hypothesis.[14] The SAR in general tracked what was observed for compounds **2**. For example, the IC$_{50}$ of the 3,5-dimethyl analog **2g** was 0.63 versus 1.8 nM for the 3,5-dimethyl analog **4g**. The IC$_{50}$ of the 6-Me analog **2e** was 2.9 versus 15.1 nM for the 6-Me analog **4f**. However, the 3-Me analog **4a** was 21-fold more potent than the corresponding analog **2b**, IC$_{50}$ of 23.3 versus 1.1 nM. Interestingly, the analog **4h** with a 4-Me group on the northern pyridine ring had comparable activity to **4a**, 0.3 versus 1.1 nM.

Several of the more potent compounds with acceptable ADME properties were chosen for further evaluation as anti-viral agents (Table 4). Since we had previously shown that the (S)-enantiomer of AMD070[6] and related compounds had a marked increase in anti-HIV-1 potency relative to the racemates these compounds were synthesized as the hydrochloride salts of the (S)-enantiomer.[20] In general this observation was corroborated although the potency enhancement was not as pronounced as previously observed, for example, the IC$_{50}$ of (S)-**2e**, **2k**, **3a** and **4a** were 2.4, 7.7, 1.6, 1.4 and 1.1 nM, respectively, a 1-, 12-, 13- and 6-fold increase (Tables 1–4). However, we were pleased to find that the IC$_{50}$ for X4 HIV-1 infection in PBMC's was comparable to that of the MT-4 CD4$^+$T cell line for all compounds with a range of 2.0–15.8 nM. In addition, evidence that these compounds are antagonists of CXCR4 was provided by the competitive binding with ^{125}I-SDF-1 in CD4$^+$CXCR4$^+$ T

Scheme 2. Reagents: (a) NH$_2$(CH$_2$)$_4$NHBoc, NaBH(OAc)$_3$, CH$_2$Cl$_2$; (b) N-Boc-2-chloromethylbenzimidazole, KI, DIPEA, CH$_3$CN, reflux; (c) HBr, AcOH; (d) 4-(1,3-dioxoisoindolin-2-yl)butanal, NaBH(OAc)$_3$, CH$_2$Cl$_2$; (e) N$_2$H$_4$, EtOH.

Table 3
Anti-HIV-1 activity, cytotoxicity and calcium signaling (SDF-1) inhibition of compounds **4**[a]

Compound	R	R^1	HIV-1 IC_{50} (nM)	CC_{50} (nM)	Ca^{2+} flux IC_{50} (nM)
4a	3-Me	H	1.1 ($n = 2$)	45,400	23 ($n = 6$)
4b	3-NH_2	H	18.4 ($n = 1$)	167,200	72 ($n = 2$)
4c	3-CF_3	H	39.1 ($n = 2$)	>155,400	111 ($n = 1$)
4d	3-iBu	H	1.6 ($n = 2$)	157,500	18 ($n = 2$)
4e	5-Me	H	48.1 ($n = 1$)	158,300	66 ($n = 1$)
4f	6-Me	H	15.0 ($n = 1$)	>145,400	183 ($n = 2$)
4g	3,5-diMe	H	1.8 ($n = 2$)	163,100	20 ($n = 2$)
4h	3-Me	4-Me	0.3 ($n = 2$)	138,236	8 ($n = 2$)

[a] See footnotes in Table 1.

Table 4
Anti-HIV-1 activity, fold protein shift and SDF-1 binding inhibition of compounds (S)-**2e**, **2g**, **2k**, **3a** and **4a**[a]

Compound	HIV-1 MT-4 IC_{50}[a] (nM)	HIV-1 PBMC IC_{50}[a,b] (nM)	Fold IC_{50} protein shift[c]	^{125}I-SDF-1 binding IC_{50}[d] (nM)
(S)-**2e**	2.4 ($n = 4$)	15.8	0.6	96.1
(S)-**2g**	7.7 ($n = 3$)	3.6	2.8	62.9
(S)-**2k**	1.6 ($n = 3$)	2.1	4.3	10.9
(S)-**3a**	1.4 ($n = 4$)	2.0	5.4	16.6
(S)-**4a**	1.1 ($n = 1$)	2.1	14.2	35.8

[a] Assays were performed in triplicate and values represent the mean with standard deviations <30% of the mean. Bracketed values represent the number of experiments.
[b] Peripheral blood mononuclear cells (PBMC) were isolated from healthy volunteers and infected by X4 HIV-1 as reported in Ref. 6.
[c] Fold IC_{50} protein shift is the shift in the X4 HIV-1 PBMC antiviral assay in the presence of 1 mg/ml of α-acid glycoprotein.
[d] Competitive binding studies against CXCR4 were performed in human $CD4^+CXCR4^+$ CEM-CCRF cells as reported in Ref. 6.

Table 5
Pharmacokinetics of (S)-**2e**, **2g**, **2k**, **3a** and **4a** in rat and dog[a]

Compound	Species	C_{max} (μM)	AUC_{0-inf} (h μM)	CL (ml/min/kg)	V (L/kg)	$T_{1/2}$ (h)	F (%)
(S)-**2e**	Rat	1.46	8.75	163.3	39.8	2.8	87
(S)-**2g**		2.70	20.7	31.7	28.5	10.5	42
(S)-**2k**		1.66	8.73	83.3	24.1	3.4	44
(S)-**3a**		0.18	0.33	113.3	16.6	1.7	2
(S)-**4a**		1.58	11.19	98.3	36.9	4.4	67
(S)-**2e**	Dog	2.06	7.75	11.3	2.1	2.1	45
(S)-**2g**		7.16	22.06	3.3	8.7	39.6	51
(S)-**2k**		7.74	21.20	5.0	9.6	19	84
(S)-**3a**		1.33	1.7	25	8.5	3.1	20
(S)-**4a**		1.38	7.49	11.7	13.8	13	56

[a] Clearance (CL), volume of distribution (V_{dss}) and half life ($T_{1/2}$) calculated following a 10 μmol/kg iv dose in rat and 5 μmol/kg iv dose in dog. Oral bioavailability (F) calculated following solution doses of 100 μmol/kg in rat and 12.5 μmol/kg in dog.

cells with the IC_{50} ranging from 11 to 96 nM (Table 4). Finally, the anti-viral activity of these compounds was assessed in the presence of 1 mg/mL of α-acid glycoprotein (AGP) and we were pleased to observe that the shift in activity was moderate ranging from 0.6- to 5.4-fold (Table 4).

Based on the moderate fold protein shift and the excellent potency these compounds were profiled in rat and dog pharmacokinetics (Table 5). In general these compounds showed moderate to excellent exposure and oral bioavailability other than (S)-**3a** which was rapidly cleared in both rat and dog resulting in low oral bioavailability. For example, the 3,5-dimethyl analog (S)-**2g** showed low clearance and long half life with a corresponding durable exposure and good oral bioavailability in both rat and dog. This was similarly observed for (S)-**2e**, **2k** and **4a**.

In summary we identified a novel series of small molecule antagonists based on the redesign of AMD070. These molecules are antagonists of the chemokine receptor CXCR4 based on inhibition of SDF-1 induced calcium signaling and ^{125}I-SDF-1 binding and are potent inhibitors of HIV-1 replication. Based on encouraging ADME data and pharmacokinetic data these compounds were further advanced to rodent toxicology studies.

Supplementary data

Supplementary data (characterization data for compounds **2a**–**b**, **2d**–**e**, **2g**–**h**, **2k**–**n**, **3a**, **3e**, **3j**–**k**, **4a**, **4d**, **4g**–**h**, **6**, **7**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.021.

References and notes

1. Thompson, M. A.; Aberg, J. A.; Cahn, P.; Montaner, J. S. G.; Rizzardini, G.; Telenti, A.; Gatell, J. M.; Gunthard, H. F.; Hammer, S. M.; Hirsch, M. S.; Jacobsen, D. M.; Reiss, P.; Richman, D. D.; Volberding, P. A.; Yeni, P.; Schooley, R. T. *JAMA* **2010**, *304*, 321.
2. Steigbeigeel, R. T.; Cooper, D. A.; Kumar, P. N.; Eron, J. E.; Schechter, M.; Markowitz, M.; Loufty, M. R.; Lennox, J. L.; Gatell, J. M.; Rockstroh, J. K.; Katlama, C.; Yeni, P.; Lazzarin, A.; Clotet, B.; Zhao, J.; Chen, J.; Ryan, D. M.; Rhodes, R.; Killar, J. A.; Gilde, L. R.; Strohmaier, K. M.; Meibohm, A. R.; Miller, M. D.; Hazuda, D. J.; Nessly, M. L.; DiNubile, M. J.; Isaccs, R. D.; Nguyen, B.-Y.; Teppler, H. *N. Eng. J. Med.* **2008**, *359*, 339.
3. Gulick, R. M.; Lalezari, J.; Goodrich, J.; Clumeck, N.; DeJesus, E.; Horban, A.; Nadler, J.; Clotet, B.; Karlsson, A.; Wohlfeiler, M.; Montana, J. B.; McHale, M.; Sullivan, J.; Ridgway, C.; Felstead, S.; Dunne, M. W.; van der Ryst, E.; Mayer, H. *N. Eng. J. Med.* **2008**, *359*, 1429.
4. (a) Feng, Y.; Broder, C. C.; Kennedy, P. E.; Berger, E. A. *Science* **1996**, *272*, 872; (b) Trkola, A.; Dragic, T.; Arthos, J.; Binley, J. M.; Olson, W. C.; Allaway, G. P.; Cheng-Mayer, C.; Robinson, J.; Maddon, P. J.; Moore, J. P. *Nature* **1996**, *384*, 184.
5. (a) Hendrix, C. W.; Collier, A. C.; Lederman, M. M.; Schols, D.; Pollard, R. B.; Brown, S.; Jackson, J. B.; Coombs, R. W.; Glesby, M. J.; Flexner, C. W.; Bridger, G. J.; Badel, K.; MacFarland, R. T.; Henson, G. W.; Calandra, G. J. *Acquir. Immune Defic. Syndr.* **2004**, *37*, 1253; (b) Franzen, S.; Bridger, G.; Whitcomb, J. M.; Toma, L.; Stawiski, E.; Parkin, N.; Petropoulos, C. J.; Huang, W. *Antimicrob. Agents Chemother.* **2008**, *52*, 2608.
6. Skerlj, R. T.; Bridger, G. J.; Kaller, A.; McEachern, E. J.; Crawford, J.; Zhou, Y.; Atsma, B.; Langille, J.; Nan, S.; Veale, D.; Wilson, T.; Harwig, C.; Hatse, S.; Princen, K.; De Clercq, E.; Schols, D. *J. Med. Chem.* **2010**, *53*, 3376.
7. 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.
8. Teicher, B. A.; Fricker, S. P. *Clin. Cancer Res.* **2010**, *16*, 2927.
9. Ma, Q.; Jones, D.; Springer, T. A. *Immunity* **1999**, *10*, 463.
10. DiPersio, J. F.; Micallef, I. N.; Stiff, P. J.; Bolwell, B. J.; Maziarz, R. T.; Jacobsen, E.; Nademanee, A.; McCarty, J.; Bridger, G.; Calandra, G. *J. Clin. Oncol.* **2009**, *27*, 4767.
11. Dipersio, J. F.; Stadtmauer, E. A.; Nademanee, A.; Micallef, I. N.; Stiff, P. J.; Kaufman, J. L.; Maziarz, R. T.; Hosing, C.; Fruehauf, S.; Horwitz, M.; Cooper, D.; Bridger, G.; Calandra, G. *Blood* **2009**, *113*, 5720.
12. Skerlj, R.; Bridger, G.; McEachern, E.; Harwig, C.; Smith, C.; Wilson, T.; Veale, D.; Yee, H.; Crawford, J.; Skupinska, K.; Wauthy, R.; Yang, W.; Zhu, Y.; Bogucki, D.; Di Fluri, M.; Langille, J.; Huskens, D.; De Clercq, E.; Schols, D. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 262.
13. (a) 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; (b) 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; (c) Miller, J. F.; Turner, E. M.; Gudmundsson, K. S.; Jenkinson, S.; Splatenstein, A.; Thomson, M.; Wheelan, P. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2125; (d) Catalano, J. G.; Gudmundsson, K. S.; Svolto, A.; Boggs, S. D.; Miller, J. F.; Spaltenstein, A.; Thomson, M.; Wheelan, P.; Minick, D. J.; Phelps, D. E.; Jenkinson, S. *Bioorg. Chem. Lett.* **2010**, *20*, 2186; (e) Miller, J. F.; Gudmundsson, K. S.; Richardson, L. D.; Jenkinson, S.; Spaltenstein, A.; Thomson, M.; Wheelan, P. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3026.
14. Bridger, G. J.; Skerlj, R. T.; Hernandez-Abad, P. E.; Bogucki, D. E.; Wang, Z.; Zhou, Y.; Nan, S.; Boehringer, E. M.; Wilson, T.; Crawford, J.; Metz, M.; Hatse, S.; Princen, K.; De Clercq, E.; Schols, D. *J. Med. Chem.* **2010**, *53*, 1250.
15. Gerlach, L. O.; Skerlj, R. T.; Bridger, G. J.; Schwartz, T. W. *J. Biol. Chem.* **2001**, *276*, 14153.
16. Wong, R. S.; Bodart, V.; Metz, M.; Labrecque, J.; Bridger, G.; Fricker, S. P. *Mol. Pharmacol.* **2008**, *74*, 1485.
17. Wu, B.; Chien, E. Y. T.; Mol, C. D.; Fenalti, G.; Liu, W.; Katritch, V.; Abagyan, R.; Brooun, A.; Wells, P.; Bi, F. C.; Hamel, D. J.; Kuhn, P.; Handel, T. A.; Cherezov, V.; Stevens, R. C. *Science* **2010**, *330*, 1066.
18. Crawford, J. B.; Chen, G.; Gauthier, D.; Skerlj, R.; Baird, I. R.; Wilson, T. R. U.S. Patent Application 11/077,896, 2005.
19. Skupinska, K. A.; McEachern, E. J.; Skerlj, R. T.; Bridger, G. J. *J. Org. Chem.* **2002**, *67*, 7890.
20. Skupinska, K. A.; McEachern, E. J.; Baird, I. R.; Skerlj, R. T.; Bridger, G. J. *J. Org. Chem.* **2003**, *68*, 3546.