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Discovery of Novel Small Molecule Orally Bioavailable C−X−C Chemokine Receptor 4 Antagonists That Are Potent Inhibitors of T-Tropic (X4) HIV-1 Replication

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The redesign of azamacrocyclic CXCR4 chemokine receptor antagonists resulted in the discovery of novel, small molecule, orally bioavailable compounds that retained T-tropic (CXCR4 using, X4) anti-HIV-1 activity. A structure−activity relationship (SAR) was determined on the basis of the inhibition of replication of X4 HIV-1 NL4.3 in MT-4 cells. As a result of lead optimization, we identified (*S*)-*N*′-((1*H*-benzo[*d*]imidazol-2-yl)methyl)-*N*′-(5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (AMD070) **2** as a potent and selective antagonist of CXCR4 with an IC_{50} value of 13 nM in a CXCR4 ^{125}I-SDF inhibition binding assay. Compound **2** inhibited the replication of T-tropic HIV-1 (NL4.3 strain) in MT-4 cells and PBMCs with an IC_{50} of 2 and 26 nM, respectively, while remaining noncytotoxic to cells at concentrations exceeding 23 μM. The pharmacokinetics of **2** was evaluated in rat and dog, and good oral bioavailability was observed in both species. This compound represents the first small molecule orally bioavailable CXCR4 antagonist that was developed for the treatment of HIV-1 infection.

Introduction

The standard of care in treating patients infected with HIV-1[a] is to begin a HAART regimen following a significant decline in CD4+ T-cells, resulting in suppression of virus replication to low or undetectable circulating HIV-1 RNA levels.[1] Initiation of HAART regimens immediately following infection may have additional benefits such as slowing the decline to AIDS and reducing the transmission of HIV.[2] Typical HAART regimens consist of a combination of three or more HIV-1 protease and reverse transcriptase inhibitors. However, poor compliance due to a high pill burden and significant long-term toxicities have resulted in the emergence of drug resistant virus in many patients and hence the need for new treatment options.

Entry blockade has been validated as a viable target since the approval of enfuvirtide, an injectable anti-HIV drug that prevents HIV entry by blocking *env* gp41-mediated fusion.[3] Since the discovery of the chemokine receptors CXCR4 and CCR5 as HIV coreceptors,[4] a clearer understanding of the mechanistic details of virus cell entry has emerged and a tremendous amount of effort has been expended on discovering novel antagonists of these receptors as potential anti-HIV agents.[5,6] CXCR4 and CCR5 belong to the seven-transmembrane G-protein-coupled receptor (GPCR) superfamily that upon the allosteric binding of a ligand to the receptor results in a ligand-induced conformational change that is unable to support interaction with viral *env* gp120.[5] Given that the most commonly transmitted HIV-1 strains (M-tropic, CCR5 using or R5) utilize the CCR5 coreceptor, most of the research effort has focused on identifying a CCR5 antagonist,[7−9] culminating in the U.S. approval of maraviroc in 2007.[10,11] Maraviroc was indicated for combination antiretroviral treatment of adults infected with exclusively CCR5-tropic HIV-1 whose viral loads remain detectable despite existing antiretroviral treatment or who have multiple drug resistant virus. However, the emergence of T-tropic, CXCR4-using, or X4 HIV-1 variants in a minority of HIV-1 infected persons has been observed following treatment with maraviroc.[12] This development strongly suggests that a combination of CCR5 and CXCR4 antagonists for treatment of dual/mixed tropic HIV-1 infection will be required for complete viral suppression.

We have been actively involved in the development of CXCR4 antagonists beginning with the prototype compound AMD3100 (**1**, Figure 1), which was originally developed as an anti-HIV agent[13−15] and evaluated clinically in HIV-1 infected patients.[16−18] Interestingly, in these initial clinical trials leukocytosis was observed which upon further investigation showed that administration of **1** resulted in consistent increases in the number of peripheral blood $CD34^+$ stem cells.[19] This led to the clinical development of **1** in hematopoietic stem cell mobilization and the subsequent U.S. approval of plerixafor (**1**) in 2008 for use in combination with granulocyte colony-stimulating factor to mobilize hematopoietic stem cells to the peripheral blood for collection and subsequent autologous transplantation in patients with non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM).[20−22] In parallel we also discovered and developed a small-molecule, orally bioavailable

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[a] Abbreviations: HIV, human immunodeficiency virus; HAART, highly active antiretroviral therapy; AIDS, acquired immunodeficiency syndrome; CXCR4, C−X−C chemokine receptor 4; CCR5, C−C−R chemokine receptor 5.

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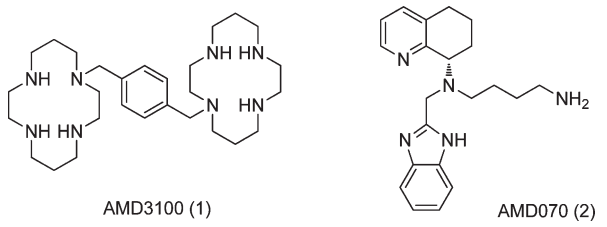


Figure 1. Structures of AMD3100 and AMD070.

CXCR4 antagonist (AMD070) (**2**)[23−25] (Figure 1) that was shown to be a potent inhibitor of T-tropic (X4) HIV-1 replication.[26] In a dose range finding study in healthy volunteers it was demonstrated that at doses ranging from 50 to 400 mg **2** is safe, well tolerated, and orally bioavailable.[24] Administration of **1** and **2** to HIV-1 infected patients, whose virus was confirmed to use CXCR4 for viral entry, suppressed the replication of X4 and dual/mixed strains of viruses.[17,24] The following discussion will focus on our medicinal chemistry efforts leading to the discovery of **2**.

Redesign Criteria

The importance of maintaining a basic amine moiety as part of the redesign criteria is highlighted by several studies that have shown that binding of **1** and related analogues is highly dependent upon the amino acids aspartic acid (Asp) 171 and Asp 262, located in the transmembrane regions TM-IV and TM-VI.[30−34] In these studies it was concluded, on the basis of receptor mutagenesis, that **1** acts on the CXCR4 receptor by binding to the aspartic acid moieties D171 in TM-IV and D262 in TM-VI and that these residues are essential for the ability of **1** to block the binding of the chemokine ligand stromal-cell-derived factor (SDF) 1α as well as the binding of the CXCR4-specific antibody clone 12G5. More importantly it was also found that in U87 cells stably transfected with CD4 and the mutant CXCR4 receptors CXCR4[D171N] and CXCR4[D262N] the ability of **1** to inhibit HIV infection was diminished confirming that **1** binds in a region of the receptor that is critical for X4 HIV-1 coreceptor function.[31] Recently we reported the structure−activity relationship of a series of azamacrocyclic CXCR4 chemokine receptor antagonists and identified the pharmacophore necessary for potent anti-HIV activity.[27] Our original pharmacophore hypothesis was that (1) the minimum macrocyclic requirements for potent activity are the protonated secondary amine groups in a 14-membered ring and (2) the activity is improved by H-bond acceptors which presumably lock the ring in a favorable conformation for antiviral activity.[27] In terms of identifying a small molecule antagonist of CXCR4, these compounds represented a substantial advance over the original bicyclams,[28,29] since a 2-aminomethylpyridine group was identified as a replacement for one of the macrocyclic rings (Figure 2). Although the prototype compound AMD3465 exhibited potent inhibition against X4 HIV-1 replication,[27] the oral bioavailability was still poor, since the overall charge at physiological pH was +2 to +3. As a consequence, a research effort was initiated to find a nonmacrocyclic analogue with an overall reduced charge at physiological pH that retained potent anti-HIV activity and was orally bioavailable. The SAR that we had earlier established was important in our redesign efforts because we had previously shown that one of the nitrogen atoms could be replaced by carbon or a phenyl group as exemplified by compound **A** (Figure 2) while still maintaining

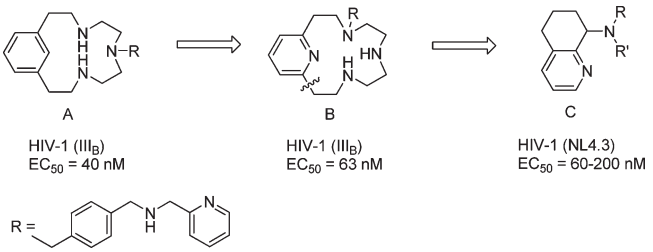


Figure 2. Redesign evolution leading to nonmacrocyclic compounds.

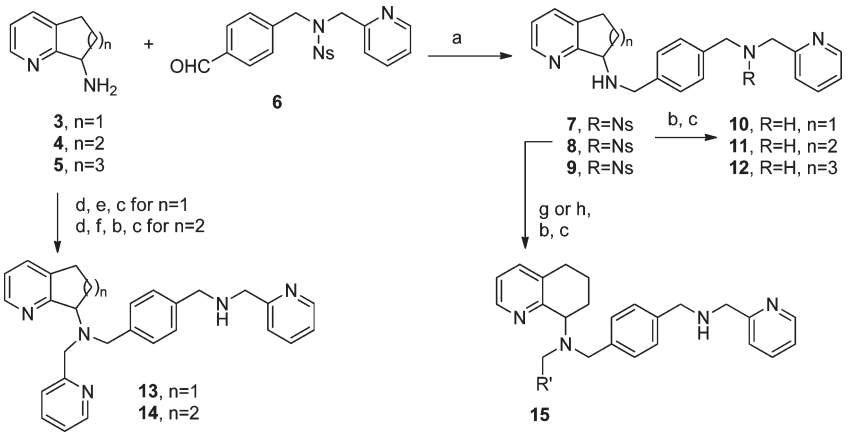
a moderate anti-HIV-1 activity with an EC_{50} of 40 nM against the HIV-1 (III_B) strain. In addition, we had shown that substitution of one of the nitrogen atoms with a pyridine ring where the side chain was now linked to one of the proximal nitrogen atoms as exemplified by compound **B** (Figure 2) resulted in an anti-HIV-1 activity comparable to that of compound **A**, EC_{50} of 63 nM vs 40 nM.[27] Hence, compound **B** represented the ideal starting point in our redesign efforts. In the following discussion we will explain the rationale that resulted in the discovery of nonmacrocyclic analogues represented by structure **C**. These initial compounds had anti-HIV-1 activity equivalent to that of macrocycle **B**, thus supporting our initial redesign criteria.

Chemistry

The five- to seven-membered bicyclic amines **3**−**5** were synthesized by catalytic hydrogenation of the corresponding acetamido substituted quinolines followed by acetamide hydrolysis, as previously reported for the synthesis of 8-amino-5,6,7,8-tetrahydroquinoline[35] **4**. The bicyclic amines **3**−**5** underwent reductive amination with *N*-(4-formylbenzyl)-2-nitro-*N*-(pyridinyl-2-ylmethyl)benzenesulfonamide **6**, which was synthesized by oxidation of the corresponding alcohol,[27] followed by nosyl group deprotection to afford the corresponding secondary amines **10**−**12** (Scheme 1). The functionalization of the intermediate scaffold **8** was performed by N-alkylation with the appropriate alkyl halide or mesylate using either K_2CO_3 or DIPEA in hot DMF or alternatively using K_2CO_3 in CH_3CN. Deprotection of the nosyl protecting group was accomplished using thiophenol, and the resultant amine **15** was isolated as the HBr salt (Scheme 1). Alternatively the bicyclic amines **3** and **4** could be elaborated by first undergoing reductive amination with pyridine-2-carboxaldehyde (imine formation followed by hydrogenation using 10% Pd/C in MeOH) to afford the corresponding secondary amine. N-Alkylation with *N*-[1-methylene-4-chloromethyl-enephenylene]-*N*-(2-nitrobenzenesulfonyl)-2-(aminomethyl)-pyridine[27] or the same moiety with the corresponding diethylphosphoryl protecting group[27] followed by subsequent deprotection and salting gave the corresponding HBr salts **14** and **13**, respectively (Scheme 1).

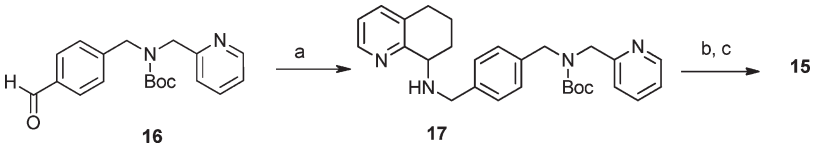
The functionalization of the scaffolds **8** and **17** was performed by one of the two following methods (Schemes 1 and 2). In the first method, the scaffold was N-alkylated with the appropriate alkyl halide or mesylate and base in hot DMF or CH_3CN. Alternatively, the amine was alkylated by reductive amination with the appropriate aldehyde using either $NaBH_3CN$ or $NaBH(OAc)_3$. For intermediates where R = nosyl, the protecting group was removed with thiophenol as described above and the resultant amine isolated as its HBr salt. The Boc-protected derivatives were simply treated

Scheme 1[a]



[a] Reagents: (a) $NaBH_3CN$, MeOH; (b) PhSH, K_2CO_3, CH_3CN; (c) HBr, AcOH; (d) pyridine-2-carboxaldehyde, MeOH, Pd/C, H_2; (e) *N*-[1-methylene-4-chloromethylenephenylene]-*N*-(diethylphosphoryl)-2-(aminomethyl)pyridine, K_2CO_3, CH_3CN; (f) *N*-[1-methylene-4-chloromethylene-phenylene]-*N*-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine, K_2CO_3, CH_3CN; (g) R′X, DIPEA or K_2CO_3, DMF; (h) R′X, K_2CO_3, CH_3CN.

Scheme 2[a]



[a] Reagents: (a) **4**, $NaBH(OAc)_3$, MeOH; (b) R′CHO, $NaBH_3CN$, MeOH; (c) HBr, AcOH.

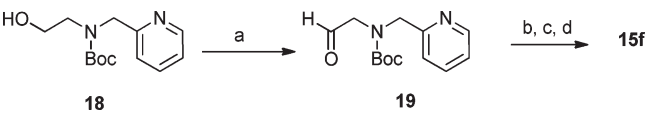
with HBr/AcOH to afford the salts directly. For instance, in the cases of compounds **15c** and **15e** a *tert*-butoxycarbonyl protecting group was utilized (Scheme 2). Reductive amination of the Boc-protected aldehyde **16** with 8-amino-5,6,7,8-tetrahydroquinoline **4** using $NaBH(OAc)_3$ in MeOH gave the Boc-protected scaffold **17**. Subsequent reductive amination with 2-imidazolecarboxaldehyde and *N*-(*tert*-butyloxycarbonyl)-*N*-benzylaminoacetaldehyde followed by concomitant deprotection and salting afforded the HBr salts **15c** and **15e**, respectively (Scheme 2).

Similarly, compound **15f** was synthesized by oxidation of *tert*-butyl 2-oxoethyl(pyridine-2-ylmethylcarbamate **18** to the corresponding aldehyde **19** with Dess−Martin periodinane. Reductive amination with the secondary amine **8** using $NaBH(OAc)_3$ in MeOH followed by sequential nosyl group and Boc deprotection afforded the HBr salt **15f** (Scheme 3).

The chain-extended benzimidazole analogues **22** and **23** were prepared as described in Scheme 4. For example, condensation of phenylenediamine with 4-chlorobutyric acid in refluxing HCl afforded the alcohol (1*H*-benzimidazol-2-yl)-propan-1-ol, which was then protected with Boc_2O and oxidized to the aldehyde **21**. Standard reductive amination of **17** followed by salting gave the propyl linker **23**. On the other hand, condensation of phenylenediamine with 4-chloropropionic acid in refluxing HCl afforded the chloride 2-(2-chloroethyl)-1*H*-benzimidazole **20** which was protected with Boc_2O and used for N-alkylation with **17** followed by salting to give the ethyl linker **22** (Scheme 4).

The amide analogues **26** and **27** were prepared in four steps as outlined in Scheme 5. The coupling of 2-picolinic acid with the appropriate aminobenzyl alcohol using EDC/HOBt to afford the amide followed by mesylation and N-alkylation with 8-amino-5,6,7,8-tetrahydroquinoline **4** gave the desired

Scheme 3[a]



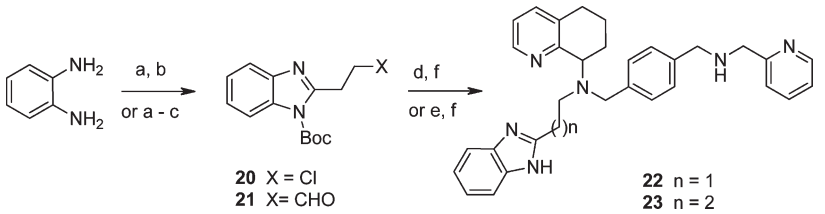
[a] Reagents: (a) Dess−Martin periodinane, CH_2Cl_2; (b) **8**, $NaBH(OAc)_3$, MeOH; (c) PhSH, K_2CO_3, CH_3CN; (d) HBr, AcOH.

amines **24** and **25**, which were then coupled with 1*H*-benzimidazole-2-carbaldehyde by reductive amination to afford the freebases **26** and **27**, respectively (Scheme 5). Similarly the reverse amides **30** and **31** were prepared by first coupling 4-chloromethylbenzoyl chloride with the appropriate aminopyridine and then N-alkylating the resultant benzyl chlorides followed by concomitant Boc deprotection and salting to afford the amides **30** and **31** (Scheme 6).

The benzylic amines **32** and **33** were synthesized by reductive amination of 8-amino-5,6,7,8-tetrahydroquinoline **4** with the regioisomeric cyanobenzaldehydes using $NaBH(OAc)_3$ in CH_2Cl_2 followed by N-alkylation with *N*-Boc-2-chloromethylbenzimidazole[36] and subsequent reduction of the cyano group to the primary amine using H_2 in the presence of Raney Ni (Scheme 7).

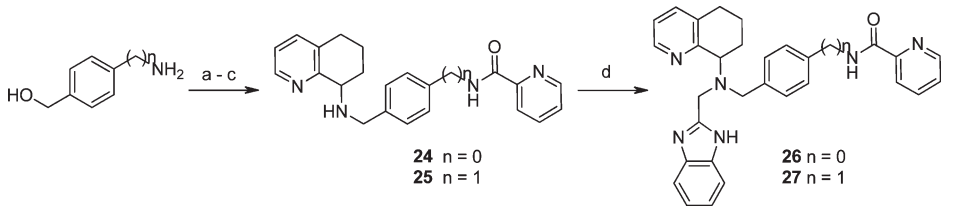
Compounds **36**−**42**, **46**, and **47** were synthesized from the key secondary amine intermediate **35** which was obtained by N-alkylation of **4** with *N*-*tert*-butoxycarbonyl-2-chloromethylbenzimidazole.[36,37] The desired side chain could be installed by either N-alkylation or reductive amination of the secondary amine followed by functional group manipulation to afford the desired target compounds (Scheme 8). For example, the (*Z*)-alkene **37** was readily prepared by N-alkylation of **35** with (*Z*)-*tert*-butyl 4-chlorobut-2-enylcarbamate and subsequent deprotection with TFA to afford the corresponding freebase **37**. Similarly the (*E*)-alkene **38** was prepared

Scheme 4[a]



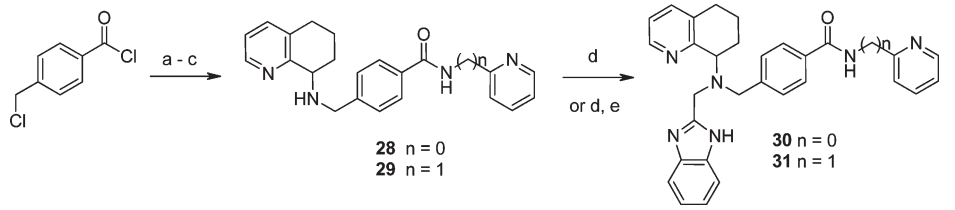
[a] Reagents: (a) $ClCH_2(CH_2)_nCO_2H$, 4 N HCl, reflux; (b) Boc_2O, DIPEA, DMF; (c) Dess−Martin periodinane CH_2Cl_2; (d) **17**, DIPEA, NaI, CH_3CN, 80 °C; (e) **17**, $NaBH(OAc)_3$, AcOH, CH_2Cl_2; (f) HBr, AcOH.

Scheme 5[a]



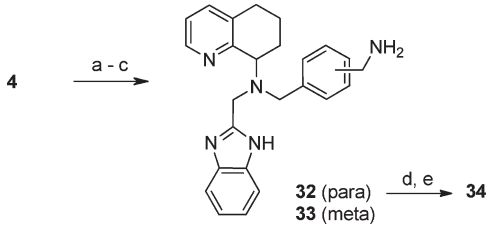
[a] Reagents: (a) 2-$PyCO_2H$, EDC, HOBt, NMM, DMF; (b) MsCl, Et_3N, CH_2Cl_2; (c) **4**, DIPEA, CH_3CN, 80 °C; (d) 1*H*-benzimidazole-2-carbaldehyde, $NaBH(OAc)_3$, THF, 60 °C.

Scheme 6[a]



[a] Reagents: (a) 2-$Py(CH_2)_nNH_2$, EtN, THF, 0 °C; (b) Ns-**4**, K_2CO_3, CH_3CN, 80 °C; (c) PhSH, K_2CO_3, CH_3CN; (d) *N*-Boc-2-chloromethylbenzimidazole, K_2CO_3, CH_3CN, 80 °C; (e) HBr, AcOH.

Scheme 7[a]



[a] Reagents: (a) cyanobenzaldehyde, $NaBH(OAc)_3$, CH_2Cl_2; (b) *N*-Boc-2-chloromethylbenzimidazole, DIPEA, KI, CH_3CN, 60 °C; (c) H_2, Raney Ni, MeOH, NH_3; (d) benzaldehyde, $NaBH_4$, MeOH; (e) HBr, AcOH.

by N-alkylation of **35** with (*E*)-*N*-(4-bromo-2-butenyl)phthalimide followed by phthalimide deprotection with hydrazine to afford the freebase **38**. Compound **42** was prepared by N-alkylation of **35** with 4-bromovaleronitrile followed by Raney Ni catalyzed hydrogenation to afford the primary amine which was converted to the HBr salt **42**. On the other hand the aliphatic amines **40** and **41** were prepared by reductive amination with *N*-(*tert*-butoxycarbonyl)-2-aminoacetaldehyde and *tert*-butyl 3-oxopropylcarbamate, respectively, followed by deprotection and salting to afford the HBr salts.

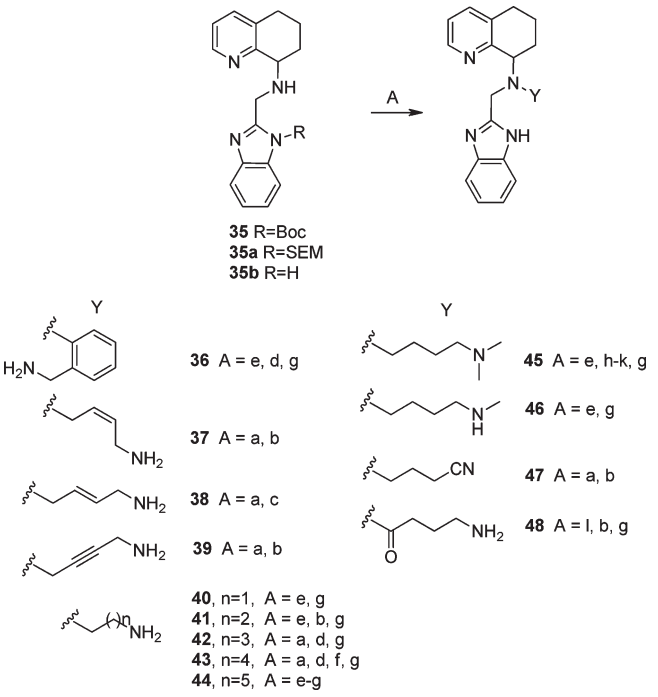
The SEM[38] protected intermediate **35a** was used to prepare compounds **43** and **44**. Compound **35a** was prepared by N-protection of 2-hydroxymethylbenzimidazole with SEM-Cl in the presence of DIPEA followed by oxidation with MnO_2 in CH_2Cl_2 to afford the corresponding SEM protected aldehyde which underwent reductive amination with 8-amino-5,6,7,8-tetrahydroquinoline **4** in the presence of $NaBH(OAc)_3$. N-Alkylation of **35a** with 5-bromovaleronitrile followed by Raney Ni hydrogenation, SEM deprotection with 4 N HCl at 50 °C for 6 h, and salting afforded the HBr salt **43**. In contrast compound **44** was prepared by reductive amination of **35a** with *tert*-butyl (6-oxohexyl)carbamate followed by concomitant deprotection of the Boc and SEM groups using 4 N HCl at 50 °C for 3 h to afford the freebase which was converted to the HBr salt **44** using standard conditions.

The unprotected intermediate **35b**, prepared by the reaction of 2-(aminomethyl)benzimidazole with 6,7-dihydro-5*H*-quinolin-8-one[39] in dry MeOH to form the imine followed by $NaBH_4$ reduction, was used to prepare compounds **45** and **48**. Compound **45** was prepared by a series of reactions starting with the reductive amination of **35b** with 4-(*tert*-butyldimethylsilyloxy)butanal, TBDMS deprotection using HF-pyridine, *N*-Boc protection, Swern oxidation of the alcohol to the aldehyde, reductive amination with *N*,*N*-dimethylamine, and finally Boc deprotection and salting using HBr in acetic acid to afford the HBr salt **45**. In addition, **35b** also underwent EDC coupling with 4-(*tert*-butoxycarbonylamino)butanoic acid to afford the corresponding amide which after standard deprotection and salting afforded the HBr salt **48**.

Results and Discussion

The strategy that we employed in redesigning compound **B** was to fix the side chain as the 2-aminomethylpyridine group

Scheme 8[a]

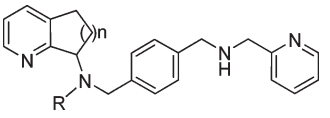


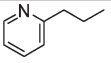
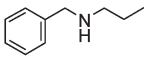
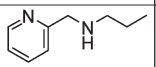
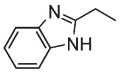
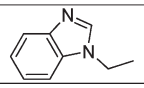
[a] Reagents: (a) RX, DIPEA, KI, CH_3CN, 60 °C; (b) TFA; (c) $H_2NNH_2 \cdot H_2O$, EtOH, reflux; (d) H_2, Raney Ni, MeOH, NH_3; (e) RCHO, $NaBH(OAc)_3$, CH_2Cl_2; (f) 4 N HCl; (g) HBr, AcOH; (h) HF-pyridine, THF; (i) $(Boc)_2O$, DIPEA, THF; (j) $(COCl)_2$, DMSO, CH_2Cl_2; (k) NMe_2, $NaBH(OAc)_3$, CH_2Cl_2; (l) HOBT, EDC, NMM, DMF.

and disconnect the pyridine C−C bond, thus opening up the macrocyclic ring (Figure 2). However, we also recognized that in order to maintain some degree of rigidity, modification of the pyridine ring into a synthetically accessible bicyclic ring system such as the amino substituted cycloalkylpyridines would be necessary. These ring systems, e.g., 8-aminotetrahydroquinoline, satisfied our pharmacophore criteria (H-bond acceptor capability and rigidity) and as a consequence were deemed an appropriate replacement for the macrocylic ring. A logical first step was to investigate the effect of varying the cycloalkylpyridines ring size from 5 to 7. Although the amino group is attached to a stereocenter, we made no attempt to resolve the stereocenter at this early stage of the research program.

The primary data used to drive the SAR were the ability of these compounds to inhibit replication of HIV-1 NL4.3 in MT-4 cells, a strain of HIV that uses exclusively CXCR4 for fusion and viral entry into target cells. The ability of these compounds to inhibit replication of HIV-1 NL4.3 in MT-4 cells showed that the six- and seven-membered rings (**11** and **12**, Table 1) had an IC_{50} of 2.7 and 2.5 μM whereas the five-membered ring **10** was less potent with an IC_{50} of 14 μM. A close examination of molecular models revealed that the "bite angle" of the primary amine increases with decreasing ring size, suggesting that a bite angle that exceeds the six-membered ring is detrimental to anti-HIV-1 activity. To assess whether this compound class would have good absorption, results from the Caco-2 cell permeability assay for **11** predicted a high absorption potential in human and did not show efflux transport potential (ratio P_{app} (B−A/A−B) < 3.0), suggesting that **11** is a not a P-glycoprotein substrate. On the basis of these encouraging preliminary results and ease of synthesis, we prepared a series of simple analogues of **11** to further test our pharmacophore hypothesis (Table 1).

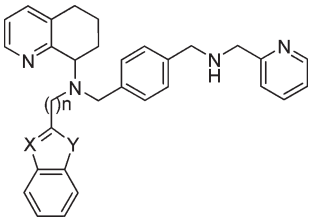
Table 1. Anti-HIV-1 Activity of Small Molecule Antagonists of CXCR4



Compound	n	R	HIV-1 (NL4.3/MT-4) IC_{50} (μM)	MT-4 cells CC_{50} (μM)
10	1	H	14.1	na
11	2	H	2.7	na
12	3	H	2.5	na
13	1		4.9	124
14	2		0.27	44
15a	2		2.1	>118
15b	2		>4.3	21
15c	2		0.20	105
15d	2		>25	126
15e	2		0.92	23
15f	2		0.06	21
15g	2		0.06	23
15h	2		18.8	21

We were gratified to find that the simple addition of a methylpyridine moiety to the secondary amine to afford **14** increased the antiviral potency by an order of magnitude to an IC_{50} of 0.27 μM (Table 1). The importance of the H-bond acceptor was confirmed by comparing the antiviral activity of the phenyl derivative **15b** (IC_{50} > 4.3) versus the pyridine derivative **14**. In addition the length of the linker was important; the two-carbon linker **15a** was 10-fold less active than the one-carbon linker **14**, suggesting that the optimal spacer between the tertiary nitrogen atom and adjoining N was two carbons. Substitution of the pyridine moiety with an alternative H-bond acceptor, an imidazole moiety **15c**, resulted in comparable activity: IC_{50} of 0.20 μM vs 0.27 μM. However, incorporation of an H-bond donor such as the basic amino moiety **15d** rendered the compound inactive (IC_{50} > 25 μM). Interestingly some activity was restored upon substitution of the amine with a lipophilic benzylic moiety as in **15e**, resulting in an IC_{50} of 0.92 μM which could be attributed to a combination of increased lipophilicity and decreased pK_a of the secondary benzylic amine (1 log reduction). A further 15-fold enhancement in antiviral activity was accomplished by replacement of the phenyl group with an H-bond acceptor moiety such as pyridine **15f** which had an IC_{50} of 0.06 μM. A similar antiviral activity (0.06 μM) was obtained with **15g** containing a more rigid and lipophilic benzimidazole moiety.

Table 2. Anti-HIV-1 Activity of Benzimidazole Analogues of **15g**



compd	*n*	X	Y	HIV-1 (NL4.3/MT-4) IC_{50} (μM)	MT-4 cells CC_{50} (μM)
15g	1	N	NH	0.06	23
15i	1	CH	NH	1.6	8.0
15j	1	N	O	9.1	204
15k	1	N	S	4.6	22.3
22	2	N	NH	1.1	23.1
23	3	N	NH	1.2	101

Interestingly linking the benzimidazole side chain directly through the N atom as in **15h** rendered the compound inactive (IC_{50} of 18.8 uM).

With the discovery of several potential lead compounds, these were further evaluated in a Caco-2 cell permeability assay to predict for absorption and P-glycoprotein substrate specificity. Although the antiviral activity of **15f** was good, the compound was predicted to have medium absorption and be a substrate for P-glycoprotein (ratio P_{app} (B−A/A−B) > 3.0). In a pharmacokinetic study to determine oral bioavailability it was observed that **15f** was acutely toxic when given by the iv route with an MTD of 1 mg/kg, suggesting that toxicity is C_{max} related. On the other hand compound **15g** in the Caco-2-cell permeability assay was predicted to have high absorption and not be a substrate for P-glycoprotein (ratio P_{app} (B−A/A−B) < 3.0). The high absorption was confirmed by the in vivo evaluation of the (*S*)-enantiomer of **15g** in rat and dog, demonstrating an excellent oral bioavailability of 47% and 55%, respectively (Table 8). As a result of the good PK data and anti-HIV-1 activity, this compound was selected as a lead for further optimization.

Our strategy to optimize the antiviral activity of **15g** was to modify the tetrahydroquinoline, benzimidazole, and side chain quadrants of the molecule as depicted in Figure 3. Modification of the tetrahydroquinoline moiety was investigated by synthesizing several analogues according to previously published methodology to determine the following: the optimum point of attachment of the amino moiety,[35] the effect of saturation,[40] the effect of replacing pyridine with other heterocycles (bicyclic amines containing fused pyridine, pyrazine, furan, and tetrahydropyran heterocycles), and heteroatom incorporation.[41] The compounds were ranked, relative to **11**, according to the ability to inhibit replication of HIV-1 NL4.3 in MT-4 cells. However, none of the compounds synthesized exhibited an antiviral activity superior to **11**. These results are consistent with our original pharmacophore hypothesis, the requirement for an H-bond acceptor and a protonated amine that are proximal to each other as demonstrated by the 8-aminotetrahydroquinoline moiety. In addition the effect of substitution of the tetrahydroquinoline moiety was also investigated. It was found that substitution had an adverse effect on antiviral activity. The effect was independent of the electronic nature of the substituents, suggesting that steric factors had a detrimental impact on antiviral activity. We concluded, on the basis of these results,

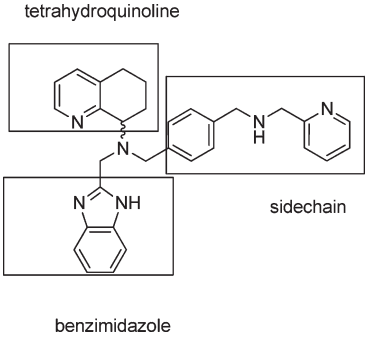


Figure 3. Structure of compound **15g**.

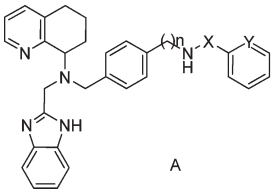
that the ideal moiety on the top left-hand side of the molecule was an unsubstituted tetrahydroquinoline containing a tertiary amine at C8.

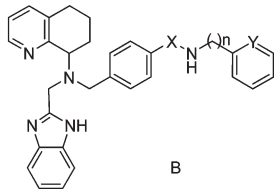
Substitution of the benzimidazole moiety of **15g** was also investigated to determine the impact of electronic and/or steric parameters. However, a clear structure−activity relationship was not evident, and since no enhancement in antiviral activity was observed, we determined that the optimum moiety for the southern quadrant was the unsubstituted benzimidazole. In addition the effect of heteroatom replacement on the benzimidazole moiety was investigated to further understand the impact of basicity and charge on anti-HIV-1 activity and to test our pharmacophore hypothesis (Table 2). It appears that the activity is directly related to the basicity of the heteroaromatic moiety. As the basicity increases through the series O to S to N (**15j**, **15k**, **15g**) the anti-HIV-1 activity (IC_{50}) in MT-4 cells also increases from 9.1 to 4.6 to 0.06 μM, respectively, supporting our original pharmacophore hypothesis of the necessity of a basic moiety linked to the tertiary nitrogen. Linker length was also investigated (Table 2), and it was found that the optimum linker length was one carbon, since the addition of a two-carbon linker **22** or three-carbon linker **23** reduced the anti-HIV-1 activity by approximately 20-fold, supporting the earlier hypothesis (Table 1) that a two-carbon spacer between the tertiary N and adjoining N atoms was the pharmacophore necessary for potency.

The final quadrant explored was the nature of the side chain with the intent to determine the effect of varying the basicity and length as shown by structures **A** and **B** (Table 3). For instance, was a basic amino moiety a requirement for anti-HIV-1 activity? To address this question, an amide was introduced adjacent to the phenyl ring (structure **B**, compound **31**), resulting in a 25-fold loss in anti-HIV-1 activity (IC_{50} of 1.44 μM). Similarly, the incorporation of an amide adjacent to the pyridine group (structure **A**, compound **27**) resulted in an 18-fold loss in anti-HIV-1 activity (IC_{50} of 0.88 μM). On the other hand, truncation of the side chain amides **26** and **30** resulted in comparable activity to the full length side chains **27** and **31** (approximately 1.3-fold difference), indicating that the length of the side chain was not critical for anti-HIV-1 activity. On the basis of these results, it is apparent that a basic amino moiety is required for potent antiviral activity because the introduction of an amide, a neutral H-bond donor, renders the compounds considerably less active.

More importantly, the necessity of the pyridine nitrogen was probed by substitution with a phenyl group, **34**, resulting in a 1.6-fold reduction in anti-HIV-1 activity, IC_{50} of 0.095 μM vs 0.059 μM, suggesting that the pyridine nitrogen was not crucial for anti-HIV-1 activity (Table 3) and the structure could be truncated while still retaining

Table 3. Anti-HIV-1 Activity of Side Chain Modification of **15g**



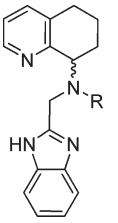


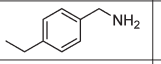
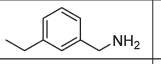
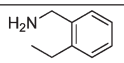
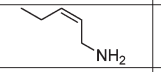
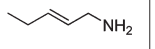
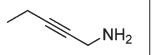
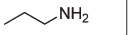
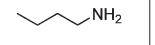
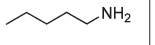
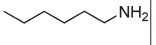
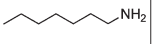
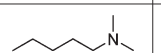
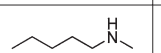
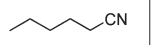
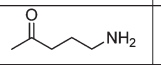
compd	structure	*n*	X	Y	HIV-1 (NL4.3/MT-4) IC_{50} (μM)	MT-4 cells CC_{50} (μM)
26	A	0	CO	N	0.66	37.5
27	A	1	CO	N	0.88	36.8
15g	A	1	CH_2	N	0.059	23.0
34	A	1	CH_2	C	0.095	26.4
30	B	0	CO	N	0.98	34.3
31	B	1	CO	N	1.44	126.8

potency. As a result, the truncated benzylic primary amine **32** was synthesized and found to have comparable anti-HIV-1 activity to **34** with an IC_{50} of 0.167 μM (1.7-fold difference), supporting the notion that an unsubstituted basic amine is sufficient for anti-HIV-1 activity (Table 4). Interestingly, when the meta **33** and ortho **36** substituted benzylic amines were evaluated, **36** was found to be approximately 3-fold more active then **15g**, IC_{50} of 0.021 μM vs 0.059 μM. This result led to the conclusion that the phenyl group may not be necessary for anti-HIV-1 activity, and as a consequence, a series of closely related aliphatic analogues were prepared. We were pleased to find that the direct analogue of the ortho benzylic amine **36**, the (*Z*)-alkene **37**, had an anti-HIV-1 activity of 0.015 μM vs 0.021 μM whereas the anti-HIV-1 activity of the (*E*)-isomer **38** was within 2-fold of that for **37**. Evaluation of the comparable saturated compound containing a butylamine chain **42** resulted in an anti-HIV-1 activity of 0.0048 μM, a 3-fold increase compared to **37**, which was the most potent compound identified within this series. The chain length was investigated to determine the optimal length required for anti-HIV-1 activity. The ethylamine analogue **40** was 275-fold less potent, whereas the propyl analogue **41** was 16-fold less potent then the butylamine **42**. Similarly the pentyl and hexyl analogues **43** and **44** were 15-fold and 20-fold less potent than **42**, indicating that the optimum chain length was the butyl analogue as originally hypothesized. Substitution of the primary amine as the *N*,*N*-dimethyl **45** or *N*-methyl **46** analogues led to a 10-fold and 2-fold loss in potency. The importance of the primary amine was further corroborated by substitution with the nitrile **47** which resulted in a 155-fold reduction in potency. In addition the importance of the tertiary amine was substantiated by the amide **48** which was 550-fold less active then **42**, supporting our initial pharmacophore hypothesis.

All the compounds synthesized at this stage of the research program were racemic mixtures. As a consequence, we set out to separate the enantiomers in order to determine which enantiomer provided more potent antiviral activity. We were able to separate the two enantiomers of **15g** using preparative chiral HPLC to obtain (*S*)-**15g** and (*R*)-**15g**. It was gratifying to find that there was a dramatic difference in anti-HIV-1 activity in MT-4 cells (Table 5); the (*R*)-enantiomer had an IC_{50} of 1.08 μM (18-fold higher than the racemate), whereas the (*S*)-enantiomer had an IC_{50} of 0.03 μM (2-fold more active than the racemate). We were able to assign the absolute configuration of (*S*)-**15g** by analogy to the key intermediate, (*S*)-8-aminotetrahydroquinoline, where we had unequivocally

Table 4. Anti-HIV-1 Activity of Side Chain Modification

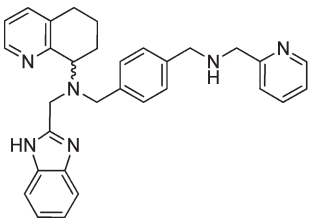


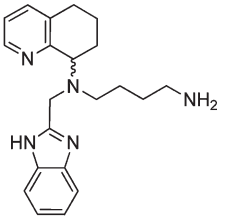
Compound	R	HIV-1 (NL4.3/MT-4) IC_{50} (μM)	Ca-Flux CEM-CCRF IC_{50} (μM)	MT-4 cells CC_{50} (μM)
32		0.167	0.119	238
33		0.575	1.402	45.9
36		0.021	0.050	29.6
37		0.015	0.077	51.9
38		0.028	0.160	50.8
39		0.170	0.973	10.8
40		1.369	1.369	6.86
41		0.077	0.068	32.9
42		0.0048	0.011	160
43		0.074	0.514	30.2
44		0.101	0.075	30.2
45		0.011	0.087	29.5
46		0.051	0.19	28.2
47		0.774	0.442	55.3
48		2.64	3.20	152

established the absolute configuration based on a single X-ray crystal structure of a rhenium complex and reported on the synthesis of (*S*)-8-aminotetrahydroquinoline using a lipase based enzymatic resolution of the racemate.[41] This methodology was used to synthesize the single enantiomers of **15g** and **42**. As expected, a comparable difference in anti-HIV-1 activity was observed for the two enantiomers of **42**; the (*R*)-enantiomer was 30-fold less potent (0.144 μM), whereas the (*S*)-enantiomer was 2-fold more potent (0.002 μM).

The effects of compounds **15g** and **42** and the enantiomers regarding the specific interaction with CXCR4 were studied in more detail. The effects on SDF-1 induced Ca^{2+} flux in $CD4^+CXCR4^+$ T cells (CEM-CCRF) cells were found to correlate with the anti-HIV-1 activity in $CD4^+$ $CXCR4^+$ T cells (Table 5). Compounds **15g** and (*S*)-**15g** both inhibited Ca^{2+} flux with a comparable IC_{50} of 0.28 and 0.38 μM, respectively, whereas the (*R*)-enantiomer was about 10-fold less active with an IC_{50} of 2.47 μM. Similarly, compounds **42** and **2** both inhibited Ca^{2+} flux with a comparable IC_{50} of 0.017 and 0.012 μM, respectively, whereas the (*R*)-enantiomer

Table 5. Correlation of Anti-HIV-1 Activity and CXCR4 Specific Interactions of **2**, **15g**, and **42**





		IC_{50} (μM)			
compd	stereochemistry	HIV-1 (NL4.3/ MT-4)	Ca-Flux CEM-CCRF	12G5 mAb inhibition	^{125}I-SDF-1 binding
15g	*R*/*S*	0.06	0.28	0.25	na
(*S*)-**15g**	*S*	0.03	0.38	0.12	na
(*R*)-**15g**	*R*	1.08	2.47	29.2	na
42	*R*/*S*	0.005	0.017	na	0.022
2	*S*	0.002	0.012	na	0.013
(*R*)-**42**	*R*	0.144	0.108	na	0.206

Table 6. Chemokine Receptor Selectivity Profiles of **2** and **15g**

	IC_{50} (μM)					
compd	CCR1/MIP-1α	CCR2b/MCP-1	CCR4/TARC	CCR5/MIP-1β	CXCR1/interleukin-8	CXCR2/interleukin-8
(*S*)-**15g**	> 10	> 10	> 10	> 10	> 10	> 10
2	> 10	> 10	> 10	> 10	> 10	> 10

Table 7. Anti-HIV-1 Activity of **2** and **15g** against Other HIV-1 Strains, in PBMCs and in the Presence of 10% Human Serum

	HIV-1 NL4.3		HIV-1 IIIb		PBMC NL4.3		10% human serum IC_{50} (μM)	
compd	IC_{50} (μM)	IC_{90} (μM)	IC_{50} (μM)	IC_{90} (μM)	IC_{50} (μM)	IC_{90} (μM)	HIV-1 NL4.3	HIV-1 IIIb
15g	0.051	0.137	0.060	0.108	0.277	0.559	0.069	0.081
2	0.001	0.003	na	na	0.009	0.026	0.016	na

was about 5-fold less active with an IC_{50} of 0.108 μM, demonstrating a good correlation with the anti-HIV-1 activity. To provide further evidence that these compounds were acting through antagonism of the CXCR4 receptor, the ability to inhibit binding of a CXCR4-specific mAb (12G5) to CXCR4 on SUP-T1 cells and competitive binding with ^{125}I-SDF-1 in $CD4^+CXCR4^+$ T cells (CEM-CCRF) cells was also investigated (Table 5). A good correlation between inhibition of HIV-1 replication and inhibition of 12G5 binding to CXCR4 and competitive binding with ^{125}I-SDF-1 was observed. For example, compounds **15g** and (*S*)-**15g** exhibited IC_{50} values of 0.25 and 0.12 μM for 12G5 mAb binding to CXCR4 and IC_{50} values of 0.06 and 0.03 μM for anti-HIV-1 activity. Similarly, compounds **42** and **2** exhibited exhibited IC_{50} values of 0.022 and 0.013 μM for ^{125}I-SDF-1 binding and IC_{50} values of 0.005 and 0.002 μM for anti-HIV-1 activity.

The selectivity of **15g** and **2** against a series of other closely related G-protein-coupled chemokine receptors (GPCRs) (CCR1, CCR2b, CCR4, CCR5, CXCR1, and CXCR2) was evaluated in radiolabeled chemokine-receptor binding assays, and the IC_{50} binding affinities were found to be consistently > 10 μM, indicating that the compounds are selective for CXCR4 (Table 6).

The antiviral activity of **15g** and **2** was confirmed against another X4 strain of HIV-1, HIV-1 IIIb in MT-4 cells. The repeat studies were conducted in duplicate and both IC_{50} and IC_{90} calculated (Table 7). The activity of **15g** was comparable with both strains of HIV-1. For example, the IC_{50} in HIV-1 NL4.3 was 0.051 μM vs 0.060 μM in HIV-1 IIIb. The presence of 10% human serum had little effect on the activity of **15g** against either virus strain. However, in the presence of 10% human serum, **2** was 16-fold less potent (0.016 μM vs 0.001 μM) against the NL4.3 strain. The IC_{50} and IC_{90} were also evaluated for X4 HIV-1 infection in PBMCs. The IC_{50} values for **15g** were 5-fold higher (0.277 μM vs 0.051 μM) and the IC_{90} values were 4-fold higher (0.559 μM vs 0.137 μM) in PBMCs compared to MT-4 cells. Similarly, the IC_{50} values for **2** were 9-fold higher (0.009 μM vs 0.001 μM) and 8.7-fold higher (0.003 μM vs 0.026 μM) in PBMCs compared to MT-4 cells. This difference is comparable to that seen with other compounds when comparing inhibition of infection in the MT-4 $CD4^+$ T cell tumor line and in PBMCs.

Because of ease of handling, the hydrochloride salts of **15g** and **2** were synthesized and evaluated in rat and dog pharmacokinetics (Table 8). The oral bioavailability of **15g** was good in both rat (F = 48%) and dog (F = 55%), resulting in the selection of this compound for lead optimization as discussed previously. Compound **2** showed promising oral bioavailability in rat and dog. The rate of clearance was species dependent with compound **2** having lower clearance in dog compared to rat (1.3 vs 3.7 (mL/min)/kg), and this was reflected in a longer half-life (9.9 h) and excellent oral bioavailability (80%). On the basis of the pharmacokinetic data and ADME data, **2** was selected for safety studies in rat and dog.

In conclusion we successfully redesigned a class of azamacrocyclic compounds that are antagonists of the chemokine

Table 8. Pharmacokinetics of **2** and **15g** in Rat and Dog[a]

compd	C_{max} (μg/mL)	AUC_{0-inf} (mg·h/mL)	Cl ((mL/min)/kg)	V (L/kg)	$T_{1/2}$ (h)	F (%)
			PK in Rat			
(*S*)-**15g**	4.5	8.7	2.2	5.5	2.9	48
2	1.2	5.8	3.7	14.3	3.5	22
			PK in Dog			
(*S*)-**15g**	0.8	4.4	4.4	17.5	5.7	55
2	1.7	3.4	1.3	19.1	9.9	80

[a] Clearance (Cl), volume of distribution (Vdss) and half life ($T_{1/2}$) calculated following a 10 mg/kg iv dose in rat and 5 mg/kg iv dose in dog for **15g** and 5 mg/kg iv dose in rat and 2.5 mg/kg iv dose in dog for **2**. Oral bioavailability (F) calculated following solution doses of 40 mg/kg in rat and 25 mg/kg in dog for **15g** and 100 mg/kg in rat and 6 mg/kg in dog for **2**.

receptor CXCR4 and potent inhibitors of (T-tropic, CXCR4 using, or X4) HIV-1 replication. The azamacrocyclic compounds are not orally bioavailable because at physiological pH they contain a charge of +2 to +3. Through our redesign efforts we synthesized a novel series of small molecule orally bioavailable antagonists of the chemokine receptor CXCR4, based on inhibition of SDF-1 induced chemotaxis and calcium flux and 12G5 binding data, that retained potent inhibition of X4 HIV-1 replication. Recently others have also reported on the discovery of oral CXCR4 agents.[42] In addition these compounds were determined to be selective for CXCR4 and displayed favorable pharmacokinetic properties. The lead compound **2** was selected for preclinical safety evaluation and ultimately entered the clinic for assessment as an antiviral agent in HIV infected patients.[23,24]

Experimental Section

Unless otherwise noted, most of the reagents and solvents were purchased at the highest commercial quality and used without further purification. All reactions involving air- or moisture-sensitive reagents were performed under a nitrogen or argon atmosphere. Silica gel chromatography was performed using glass columns packed with silica gel 60 (40−63 μM, VWR International). ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, on a Bruker Avance 300 spectrometer with shifts referenced to the residual proton shift of the internal deuterated solvent. Electrospray mass spectra were recorded on a Bruker-HP Esquire-LC ion trap mass spectrometer. Microanalyses for C, H, N, and halogen were performed by Atlantic Microlab, Inc. (Norcross, GA) and were within 0.4% of theoretical values. Purity was determined by reversed phase HPLC and was ≥95% for all compounds tested.

General Procedure A: Direct Reductive Amination with $NaBH_3CN$. To a stirred solution of the amine (∼1−2 equiv) in anhydrous MeOH (concentration ∼0.1 M), at room temperature, was added the carbonyl compound (∼1−2 equiv) in one portion. Once the carbonyl compound had dissolved (∼5 min), $NaBH_3CN$ (∼2−4 equiv) was added in one portion and the resultant solution was stirred at room temperature. The solvent was removed under reduced pressure and CH_2Cl_2 (20 mL/mmol of amine) and brine or 1.0 M aqueous NaOH (10 mL/mmol amine) were added to the residue. The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL/mmol amine). The combined organic phases were dried (Na_2SO_4) and concentrated. The crude material was purified by chromatography on silica gel.

General Procedure B: Deprotection of the 2-Nitrobenzenesulfonyl Group (Nosyl). To a stirred solution of the nosyl-protected amine (1 equiv) in anhydrous CH_3CN (or DMF) (∼0.05 M), at room temperature was added thiophenol (4−8 equiv) followed by powdered K_2CO_3 (8−12 equiv). The resulting bright-yellow solution was stirred at room temperature (or 50 °C) for 1−24 h. The solvent was removed under reduced pressure, and CH_2Cl_2 (10 mL/mmol amine) and water (2 mL/mmol amine) were added to the residue. The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phases were dried (Na_2SO_4) and concentrated. Purification of the crude material by chromatography provided the free base. An alternative workup is as follows: The reaction mixture was filtered and concentrated to provide a yellow oil which was purified by chromatography on basic alumina (eluant CH_2Cl_2, then 20:1 CH_2Cl_2/CH_3OH) to provide the free base as a colorless oil.

General Procedure C: Salt Formation Using Saturated HBr (g) in Acetic Acid. To a solution of the free base in glacial acetic acid (or dioxane) (2 mL) was added a saturated solution of HBr (g) in acetic acid (or dioxane) (2 mL). A large volume of ether (25 mL) was then added to precipitate a solid, which was allowed to settle to the bottom of the flask, and the supernatant solution was decanted. The solid was washed by decantation with ether (3 × 25 mL), and the remaining traces of solvent were removed under vacuum. For additional purification (where necessary), the solid can be dissolved in methanol and reprecipitated with a large volume of ether. Washing the solid with ether by decantation, followed by drying of the solid in vacuo (0.1 Torr) gave the desired compound.

General Procedure D: N-Alkylation of Amines with Mesylates or Alkyl Halides. To a solution of the amine (1−1.4 equiv), *N*,*N*,-diisopropylethylamine (or K_2CO_3) (1.5−2 equiv), and KI (0.05−0.16 equiv) in CH_3CN (∼0.1−0.2 M) was added the mesylate or alkyl halide (such as 1-*N*-*tert*-butoxycarbonyl-2-chloromethylbenzimidazole) (1−1.4 equiv). The mixture was stirred at 50−70 °C for 3−25 h, as monitored by analytical thin layer chromatography. The reaction mixture was cooled, diluted with CH_2Cl_2 (10 mL/mmol amine), and poured into either saturated aqueous $NaHCO_3$ or brine (10 mL/mmol alcohol). The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL/mmol amine). The combined organic phases were dried (Na_2SO_4 or $MgSO_4$) and concentrated under reduced pressure. The crude material was purified by chromatography to afford the desired N-alkylated product.

General Procedure E: Direct Reductive Amination with $NaBH(OAc)_3$. To a stirred solution of the amine (1 equiv) in CH_2Cl_2 (∼0.2 M), at room temperature, were added the carbonyl compound (∼1−2 equiv), glacial acetic acid (0−2 equiv), and $NaBH(OAc)_3$ (∼1.5−3 equiv). The resultant solution was stirred at room temperature. The reaction mixture was poured into either saturated aqueous $NaHCO_3$ or 1.0 M aqueous NaOH (10 mL/mmol amine). The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL /mmol amine). The combined organic phases were dried (Na_2SO_4) and concentrated. The crude material was purified by chromatography on silica gel.

General Procedure F: EDC Coupling. To a stirred solution of the amine (1 equiv), acid (1.1 equiv), 1-hydroxybenzotriazole (1.1 equiv), 4-methylmorpholine (1.5 equiv) in anhydrous DMF (∼0.3 M), at room temperature under nitrogen atmosphere, was added *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) (1.1 equiv). The resultant solution was stirred at room temperature. DMF was removed under vacuum. The mixture was diluted with CH_2Cl_2 (100 mL/mmol), washed with $NaHCO_3$, dried (Na_2SO_4), concentrated, and purified by chromatography on silica gel.

General Procedure G: Removal of Boc Group with TFA. A solution of the Boc-protected amine in CH_2Cl_2 (∼0.05 M) and TFA (∼ 1−2 mL/mmol) was stirred at room temperature for ∼1−3 h. The reaction mixture was diluted with CH_2Cl_2 and then concentrated in vacuo. The residue was diluted with CH_2Cl_2 and 1 N NaOH to pH > 11. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo to afford the desired amine,

which was either used without purification in the next step or purified by chromatography on silica gel.

***N*-(2-Nitrobenzenesulfonyl)-*N*-(2-pyridinylmethyl)-*N'*-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine (8).** By use of general procedure A, reaction of **4** (21.2 g, 51 mmol) with **6** (7.61 g, 51 mmol) followed by purification using silica gel chromatography (20:1 CH_2Cl_2/CH_3OH) gave **8** (11 g, 40%) as an orange oil. ^{1}H NMR ($CDCl_3$) δ 1.74−1.84 (m, 2H), 1.99−2.05 (m, 1H), 2.02−2.05 (m, 1H), 2.72−2.86 (m, 2H), 3.13 (br s, 1H), 3.79−3.94 (m, 3H), 4.57 (s, 2H), 4.60 (s, 2H), 7.07−7.11 (m, 4H), 7.20−7.24 (m, 3H), 7.37 (d, 1,H, J = 7.4 Hz), 7.53, (t, 2H, J = 8.4 Hz) 7.64 (br s, 2H), 7.94 (d, 1H, J = 7.8 Hz), 8.40 (t, 2H, J = 5.9 Hz); ^{13}C NMR ($CDCl_3$) δ 19.70, 28.61, 28.85, 51.43, 51.54, 52.37, 57.56, 122.26, 122.78, 122.82, 124.55, 128.91 (2), 129.12 (2), 131.39, 131.98, 132.87, 133.65, 133.98, 134.60, 136.98, 137.28, 140.87, 147.20, 148.30, 149.60, 156.34, 157.77. ES-MS m/z 544 (M + H). Anal. ($C_{29}H_{29}N_5O_4S\cdot 0.1CH_2Cl_2$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-*N*-(2-pyridinylmethyl)-*N'*-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine (17).** By use of general procedure E, reaction of **4** (4.16 g, 28.1 mmol) with **16** (9.15 g, 28.1 mmol) followed by purification on silica gel (EtOAc) gave **17** (9.65 g, 75%) as a yellow oil. ^{1}H NMR ($CDCl_3$) (mixture of rotational isomers) δ 1.41 (br s) and 1.48 (br s) (total 9H), 1.76−1.83 (m, 2H), 2.02−2.06 (m, 1H), 2.15−2.18 (m, 1H), 2.75−2.83 (m, 2H), 3.81−3.85 (m, 1H), 3.86 (d, 1H, J = 12 Hz), 3.97 (d, 1H, J = 12 Hz), 4.44 (br s, 2H), 4.53 (br s, 2H), 7.04 (dd, 1H, J = 7.8, 4.8 Hz), 7.12−7.25 (m, 4H), 7.33−7.37 (m, 3H), 7.62 (td, 1H, J = 7.5, 1.8 Hz), 8.38 (dd, 1H, J = 4.8, 1.2 Hz), 8.52 (dd, 1H, J = 5.7, 1.8 Hz); ^{13}C NMR ($CDCl_3$) (mixture of rotational isomers) δ 19.37, 28.09, 28.34, 28.56, 49.83, 50.08, 51.22, 51.45, 57.21, 79.71, 79.83, 120.47, 121.49, 121.66, 121.73, 127.36, 128.12, 132.10, 136.18, 136.31, 136.52, 139.50, 146.49, 148.89, 155.62, 157.17, 157.91, 158.27. ES-MS m/z 459 (M + H).

***N*-(2-Pyridinylmethyl)-*N'*-(1*H*-benzimidazol-2-ylmethyl)-*N'*-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine Tetrahydrobromide Dihydrate (15g).** By use of general procedure D, reaction of **8** (425 mg, 0.78 mmol) with 2-chloromethylbenzimidazole (129 mg, 0.77 mmol) followed by purification by radial chromatography on silica gel (2 mm plate, 20:1 CH_2Cl_2/CH_3OH containing 1% NH_4OH) gave the protected amine (169 mg, 31%) as a yellow solid. By use of general procedure B, reaction of the protected amine (161 mg, 0.239 mmol) followed by purification using radial chromatography on silica gel (2 mm plate, 50:1:1 $CH_2Cl_2/CH_3OH/NH_4OH$) gave the free base (61 mg, 52%) as a yellow oil. By use of general procedure C, conversion of the free base (61 mg, 0.125 mmol) to the HBr salt gave **15g** (79 mg, 74%) as a white solid. ^{1}H NMR (D_2O) δ 1.93−1.98 (m, 1H), 2.19−2.31 (m, 2H), 2.41−2.46 (m, 1H), 3.20 (br s, 2H), 3.77−3.88 (m, 4H), 4.16 (s, 2H), 4,44 (d, 1H, J = 16.5 Hz), 4.63 (d, 1H, J = 16.5 Hz), 4.73−4.79 (m, 1H, overlaps with HOD), 7.04 (d, 2H, J = 8.1 Hz), 7.23 (d, 2H, J = 7.8 Hz), 7.37 (dd, 2H, J = 3.0, 6.3 Hz), 7.54 (dd, 2H, J = 3.0, 6.3 Hz), 7.67 (d, 1H, J = 7.8 Hz), 7.72 (dd, 1H, J = 6.3, 6.9 Hz), 7.91 (dd, 1H, J = 6.0, 7.8 Hz), 8.20 (t, 1H, J = 7.8 Hz), 8.39 (d, 1H, J = 8.1 Hz), 8.67 (d, 1H, J = 5.1 Hz), 8.75 (d, 1H, J = 5.7 Hz); ^{13}C NMR (D_2O) δ 20.46, 20.97, 27.87, 48.88, 50.22, 50.44, 56.71, 63.26, 113.92, 126.15, 126.43, 126.52, 126.65, 130.04, 130.22, 130.47, 130.92, 138.23, 139.70, 141.05, 142.99, 147.15, 147.95, 148.32, 150.80, 151.79. ES-MS m/z 489 (M + H). Anal. ($C_{31}H_{32}N_6\cdot 4.0HBr\cdot 2.0H_2O$) C, H, N, Br.

***N*-(2-Pyridinylmethyl)-*N'*-(indol-2-ylmethyl)-*N'*-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine (15i).** By use of general procedure E, reaction of **8** (320 mg, 0.58 mmol) and 3*H*-indole-2-carboxaldehyde (85 mg, 0.58 mmol) gave the protected amine (300 mg, 77%) as a yellow oil. By use of general procedure B, reaction of the protected amine (150 mg, 0.22 mmol) gave the free base **15i** (55 mg, 51%) as a pale-yellow solid. ^{1}H NMR ($CDCl_3$) δ 1.63−1.72 (m, 1H), 1.85−1.95 (m, 1H), 1.97−2.09 (m, 1H), 2.12−2.22 (m, 1H), 2.68−2.72 (m, 1H), 2.77−2.90 (m, 1H), 3.65 (d, 1H, J = 13.5 Hz), 3.78 (s, 2H), 3.80 (d, 1H, J = 14.1 Hz), 3.85 (s, 2H), 3.90 (s, 2H), 4.05 (dd, 1H, J = 9.0, 5.7 Hz), 6.24 (s, 1H), 7.02 (t, 1H, J = 7.5 Hz), 7.10−7.15 (m, 3H), 7.24−7.31 (m, 2H), 7.36−7.51 (m, 6H), 7.61 (dt, 1H, J = 7.5, 1.8 Hz), 8.55 (d, 1H, J = 7.2), 8.63 (d, 1H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 21.09, 23.69, 29.15, 47.41, 53.25, 53.82, 54.48, 59.15, 99.14, 110.98, 118.85, 119.65, 120.57, 121.87, 121.89, 122.35, 128.15, 128.68, 128.85, 134.44, 135.89, 136.40, 136.86, 138.75, 138.77, 139.11, 147.01, 149.25, 158.04, 159.78. ES-MS m/z 488 (M + H). Anal. ($C_{32}H_{33}N_5\cdot 0.6H_2O$) C, H, N.

Pyridine-2-carboxylic Acid 4-{[(1*H*-Benzimidazol-2-ylmethyl)-(5,6,7,8-tetrahydroquinolin-8-yl)amino]methyl}benzylamide (27). By use of the procedure described for compound **26**, (4-aminomethylphenyl)methanol (200 mg, 1.46 mmol) and picolinic acid (195 mg, 1.61 mmol) gave the corresponding amido alcohol which was converted directly to 4-(picolinamidomethyl)benzyl methanesulfonate (214 mg, 60%, 2 steps). By use of general procedure D, reaction of **4** (148 mg, 1.00 mmol) and 4-(picolinamidomethyl)benzyl methanesulfonate (214 mg, 0.7 mmol) followed by purification on silica gel ($CH_2Cl_2/CH_3OH/NH_4OH$ 97:1.5:1.5) gave **25** (81 mg, 33%) as a yellow oil. By use of general procedure E, reaction of *N*-(4-((5,6,7,8-tetrahydroquinolin-8-ylamino)methyl)benzyl)picolinamide (**25**) (40 mg, 0.11 mmol) and 1*H*-benzimidazole-2-carbaldehyde (16 mg, 0.11 mmol) followed by purification on silica gel ($CH_2Cl_2/CH_3OH/NH_4OH$ 198:1:1) gave **27** (28 mg, 52%) as a white foam. ^{1}H NMR (CD_3OD) δ 1.60−1.63 (m, 1H), 1.95−2.06 (m, 2H), 2.20−2.22 (m, 1H), 2.66−2.83 (m, 2H), 3.51 (d, 1H, J = 13.2 Hz), 3.58 (d, 1H, J = 13.2 Hz), 3.94 (d, 1H, J = 15.3 Hz), 4.04−4.09 (m, 3H), 4.42 (br s, 2H), 7.09−7.15 (m, 4H), 7.20 (dd, 1H, J = 7.8, 4.8 Hz), 7.20 (d, 1H, J = 8.1 Hz), 7.28 (d, 2H, J = 8,1 Hz), 7.41−7.47 (m, 2H), 7.48−7.52 (m, 2H), 7.91 (ddd, 1H, J = 7.5, 7.5, 1.8 Hz), 8.06 (d, 1H, J = 7.8 Hz), 8.55 (d, 1H, J = 4.5 Hz), 8.60 (d, 1H, J = 4.5 Hz); ^{13}C NMR (CD_3OD) δ 22.84, 24.33, 30.62, 44.13, 51.41, 56.15, 63.13, 123.55, 124.03, 128.13, 128.61, 130.80, 137.39, 138.95, 139.14, 139.51, 139.67, 148.35, 150.17, 151.40, 156.31, 158.31, 166.94. ES-MS m/z 503 (M + H). Anal. ($C_{31}H_{30}N_6O\cdot 0.8H_2O\cdot 0.3CH_2Cl_2$) C, H, N.

4-{[(1*H*-Benzo[*d*]imidazol-2-ylmethyl)(5,6,7,8-tetrahydroquinolin-8-yl)amino]methyl}-*N*-(pyridin-2-yl)benzamide (30). To a solution of 2-aminopyridine (304 mg, 3.22 mmol) and Et_3N (0.8 mL, 5.70 mmol) in anhydrous THF (5 mL) at 0 °C was added a solution of 4-(chloromethyl)benzoyl chloride (282 mg, 1.40 mmol) in THF (5 mL), and the mixture was stirred at 0 °C for 3 h. The mixture was diluted with EtOAc (75 mL) and saturated aqueous NH_4Cl (50 mL). The organic layer was washed with brine (1 × 50 mL), dried (Na_2SO_4), concentrated, and purified on silica gel (hexanes/EtOAc, 9:1) to give 4-(chloromethyl)-*N*-(pyridinyl-2-yl)benzamide (170 mg, 49%) as a white solid. By use of general procedure D, reaction of **8** (230 mg, 0.69 mmol) with 4-(chloromethyl)-*N*-(pyridinyl-2-yl)benzamide (170 mg, 0.69 mmol) followed by purification on silica gel (15:1 CH_2Cl_2/EtOAc) gave the protected amine (344 mg, 92%) as a white foam. By use of general procedure B, reaction of the protected amine (340 mg, 0.62 mmol) followed by purification using radial chromatography on silica gel (2 mm plate, 3:3:94 MeOH/NH_4OH/CH_2Cl_2) afforded **28** (210 mg, 93%) as a pale-yellow oil. By use of general procedure D, reaction of *N*-(pyridin-2-yl)-4-[(5,6,7,8-tetrahydroquinolin-8-ylamino)methyl]benzamide (**28**) (155 mg, 0.43 mmol) with *N*-Boc-2-chloromethylbenzimidazole[36] (115 mg, 0.43 mmol) followed by purification using radial chromatography on silica gel (2 mm plate, 3:3:94 MeOH/NH_4OH/CH_2Cl_2) afforded **30** (100 mg, 47%) as a white foam. ^{1}H NMR ($CDCl_3$) δ 1.68−1.75 (m, 1H), 1.97−2.08 (m, 2H), 2.26−2.32 (m, 1H), 2.71−2.84 (m, 1H), 2.86−2.91 (m, 1H), 3.84 (s, 2H), 3.95 (d, 1H, J = 16.5 Hz), 4.08−4.14 (m, 1H), 4.22 (d, 1H, J = 16.0 Hz), 7.03−7.07 (m, 1H), 7.16−7.22 (m, 3H), 7.45 (d, 1H, J = 6.9 Hz), 7.52−7.61 (m, 3H), 7.65 (d, 1H, J = 7.5 Hz), 7.73 (ddd, 1H, J = 1.8, 7.2, 8.7 Hz), 7.80 (d, 2H, J = 8.1 Hz), 8.27−8.29 (m, 1H), 8.33 (d 1H, J = 8.4 Hz), 8.44 (br s, 1H), 8.72−8.80 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 21.72, 23.30, 29.56,

49.29, 54.16, 60.83, 111.39, 114.52, 120.17, 122.29, 122.81, 127.72, 129.28, 133.58, 135.17, 137.76, 138.77, 144.61, 147.38, 148.21, 152.00, 156.13, 157.54, 165.99. ES-MS m/z 489.2 (M + H). Anal. ($C_{30}H_{28}N_6O \cdot 0.6H_2O \cdot 0.7CHCl_3$) C, H, N.

***N*-[(1*H*-Benzo[*d*]imidazol-2-yl)methyl]-*N*-(4-(aminomethyl)-benzyl)-5,6,7,8-tetrahydroquinolin-8-amine (32).** By use of general procedure E, reaction of **4** (24.3 g, 164 mmol) with 4-cyanobenzaldehyde (21.5 g, 164 mmol) followed by purification on silica gel (5% CH_3OH/CH_2Cl_2) afforded 4-[(5,6,7,8-tetrahydroquinolin-8-ylamino)methyl]benzonitrile (30.9 g, 72%) as a pale-yellow solid. By use of general procedure D, reaction of 4-[(5,6,7,8-tetrahydroquinolin-8-ylamino)methyl]benzonitrile (30.1 g, 114 mmol) with *N*-(*tert*-butoxycarbonyl)-2-chloromethylbenzimidazole (30.5 g, 114 mmol) followed by purification on silica gel (3% CH_3OH/CH_2Cl_2) gave the coupled product (38.0 g, 67%) as a pale-orange foam. This material (35 g, 70.9 mmol) was subsequently dissolved in NH_3 saturated methanol, treated with Raney nickel, and placed under 50 psi of H_2 on a Parr shaker for 16 h. The mixture was filtered through Celite 521, concentrated, and purified on silica gel (5% CH_3OH/ CH_2Cl_2) to give **32** (25.1 g, 90%) as a yellow powder. ^{1}H NMR ($CDCl_3$) δ 1.63−1.74 (m, 1H), 1.98−2.06 (m, 2H), 2.24−2.30 (m, 1H), 2.67−2.91 (m, 2H), 3.69 (s, 2H), 3.75 (s, 2H), 3.91−4.17 (m, 3H), 7.14−7.19 (m, 5H), 7.33 (d, 2H, J = 7.5 Hz), 7.43 (d, 1H, J = 7.5 Hz), 7.54−7.58 (m, 2H), 8.69 (d, 1H, J = 4.2 Hz); ^{13}C NMR ($CDCl_3$) δ 21.66, 23.57, 29.53, 44.85, 49.00, 54.06, 60.93, 115.15, 115.26, 122.09(2), 122.66, 128.46(2), 129.36(2), 135.21, 137.71, 137.78, 138.75, 138.95, 147.12, 155.90, 157.35. ES-MS m/z 398 (M + H). Anal. ($C_{25}H_{27}N_5 \cdot 0.6H_2O \cdot 0.4CH_2Cl_2$) C, H, N.

***N*^{1}-(1*H*-Benzo[*d*]imidazol-2-ylmethyl)-*N*^{1}-(5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine Trihydrobromide Dihydrate (42).** By use of general procedure D, reaction of **35** (169 mg, 0.451 mmol) and 4-bromovaleronitrile (0.10 mL, 1.01 mmol) followed by purification on silica gel (30:1:1 $CH_2Cl_2/CH_3OH/NH_4OH$) provided the tertiary amine (108 mg, 54%) as a yellow foam. The amine (108 mg, 0.24 mmol) was dissolved in NH_3 saturated MeOH (4 mL), treated with Raney nickel (100 mg), and placed under 50 psi of H_2 on a Parr shaker for 24 h. Purification by radial chromatography on silica gel (1 mm plate, 20:1:1 $CH_2Cl_2/CH_3OH/NH_4OH$) provided the free base (33 mg, 39%) as a white foam. By use of general procedure C, conversion of the free base (33 mg, 0.094 mmol) to the HBr salt gave **42** (40 mg, 68%) as a white solid. ^{1}H NMR (D_2O) δ 1.52 (br s, 4H), 1.74−1.88 (m, 1H), 1.95−2.08 (m, 1H), 2.15−2.21 (m, 1H), 2.34−2.39 (m, 1H), 2.50−2.61 (m, 1H), 2.79−2.86 (m, 3H), 2.99−3.02 (m, 2H), 4.38 (d, 1H, J = 16.8 Hz), 4.47−4.56 (m, 2H), 7.58−7.63 (m, 2H), 7.76−7.88 (m, 3H), 8.34 (d, 1H, J = 7.8 Hz), 8.62 (d, 1H, J = 5.7 Hz); ^{13}C NMR (D_2O) δ 20.42 (2 C), 25.03, 25.42, 27.64, 39.50, 48.20, 51.71, 60.64, 114.26, 125.93, 126.93, 131.05, 139.32, 140.62, 148.09, 150.31, 151.82; ES-MS m/z 350 (M + H). Anal. ($C_{21}H_{27}N_5 \cdot 2.9HBr \cdot 2.2H_2O$) C, H, N.

Anti-HIV Activity Assays. The $CD4^+CXCR4^+$ lymphocytic MT-4 cell line was obtained from the American Type Culture Collection (Rockville, MD). PBMCs from healthy donors were isolated by density gradient centrifugation and stimulated with PHA at 1 μg/mL (Sigma Chemical Co., Bornem, Belgium) for 3 days at 37 °C. The activated cells (PHA-stimulated blasts) were washed three times with PBS, and viral infections were done as described by Schols et al.[15] HIV-infected or mock-infected PHA-stimulated blasts were cultured in the presence of 25 U/mL of IL-2 and varying concentrations of compounds. Supernatant was collected at day 10, and HIV-1 core antigen in the culture supernatant was analyzed by the p24 viral Ag ELISA kit (Perkin-Elmer, Boston, MA). Inhibition of HIV-1 replication in MT-4 cells was performed as previously described.[14] Anti-HIV-1 activity and cytotoxicity measurements were carried out in parallel. They were based on the viability of MT-4 cells that had been infected with HIV-1 in the presence of various concentrations of the test compounds. The IC_{50} was defined as the concentration required to inhibit 50% of the virus-infected cells against viral cytopathicity. The 50% cytotoxic concentration (CC_{50}) was defined as the compound concentration required to reduce the viability of mock-infected PBMCs or MT-4 cells by 50%. The "greater than" symbol (>) is used to indicate the highest concentrations at which the compounds were tested and still found to be noncytotoxic. Average IC_{50} and CC_{50} values for several separate experiments are presented as defined above.

^{125}I-Chemokine Competition Binding Assay. Competition binding studies against CXCR4 were performed in human $CD4^+CXCR4^+$ CEM-CCRF cells. CXCR4 inhibitors in a concentration range were incubated for 3 h at 4 °C in binding buffer (PBS containing 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.25% BSA, pH 7.4) with 5×10^5 cells and 100 pM ^{125}I-SDF-1α (Perkin-Elmer, 2200 Ci/mmol) in Millipore Durapore filter plates. Unbound ^{125}I-SDF-1α was removed by washing with 50 mM HEPES, 0.5 M NaCl, pH 7, at 4 °C. The bound radioactivity was counted using a 1450 MicroBeta liquid scintillation counter (Wallac). The data were analyzed by nonlinear regression using PRISM© 4.0 (GraphPAD Software, San Diego, CA).

Radioligand binding studies for CCR1, CCR2b, CCR4, CCR5, CXCR1, and CXCR2 were performed at MDS Laboratories, Bothell, WA.

Anti-CXCR4 mAb (Clone 12G5) Binding Assay. To study the effect of the compounds on anti-CXCR4 mAb (clone 12G5) binding, SUP-T1 T cells were first preincubated with the compounds (with **1** as a control) for 30 min on ice, washed with PBS with 2% FCS, and incubated with PE-conjugated anti-CXCR4 mAb (R&D Systems Europe, Oxon, U.K.) for 30 min on ice. After being washed with PBS, the cell samples were fixed with 1% paraformaldehyde in PBS and analyzed on a FACS Calibur flow cytometery. The dose-dependent inhibitory effects of the compounds on mAb binding were determined using the mean fluorescence intensity values, as described previously.[14]

SDF-1 Calcium Flux Induced Signaling Assay. For further characterization of the CXCR4 inhibitors, CEM-CCRF cells were resuspended (10×10^6 cells/mL) in serum-reduced media (RPMI 1640 containing 2% fetal calf serum) and loaded with the calcium indicator, Fluo-4/AM (1 μM) (Molecular Probes, Inc., Eugene, OR), for 30 min at 37 °C. The dye-loaded cells were washed twice with Hanks balanced salt solution in 20 mM HEPES, 0.2% BSA, 2.5 mM probenecid, pH 7.4. The cells were resuspended in the same buffer (7×10^6 cells/mL) followed by a 20 min incubation at room temperature in the dark. The cells were then incubated in the dark at 37 °C for 15 min with CXCR4 inhibitors. Changes in intracellular calcium concentration upon addition of SDF-1 (15 nM), the specific ligand for CXCR4, were monitored using the FLEXstation (Molecular Devices, Sunnyvale, CA) at 525 nm (excitation λ = 485 nm). The fluorescence data were analyzed using the program Softmax PRO 4.0 (Molecular Devices) and IC_{50} values calculated using GraphPad Prism 4.0 software (San Diego, CA).

Caco-2 Assay. Caco-2 monolayers were grown to confluency on collagen-coated microporous polycarbonate membranes in 12-well Costar Transwell plates. The permeability assay was run in Hank's balanced salt solution (HBSS) containing 10 mM HEPES and 15 mM glucose at a pH of 7.0 ± 0.2. Dosing solution concentrations were 10−100 μM in assay buffer. Cells were dosed on the apical side (A-to-B) or basolateral side (B-to-A) and incubated at 37 °C with 5% CO_2 and 90% relative humidity. After 2 h a 200 μL sample was taken from the receiver chamber. Each determination was performed in duplicate. All samples were assayed by LC/MS using electrospray ionization. The dosing solution was diluted 100-fold and analyzed as an analytical standard. The assay was performed by Absorption Systems (Exton, PA).

Supporting Information Available: Experimental procedures and characterization data for compounds **10**−**14**, **15a**−**f**, **15j**, **15k**, **16**, **18**, **20**−**23**, **26**, **31**, **33**−**35**, **35a**, **35b**, **36**−**41**, and **43**−**48**. This material is available free of charge via the Internet at http://pubs.acs.org.

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