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Amine substituted *N*-(1*H*-benzimidazol-2ylmethyl)-5,6,7,8-tetrahydro-8-quinolinamines as CXCR4 antagonists with potent activity against HIV-1

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ABSTRACT

Several novel amine substituted *N*-(1*H*-benzimidazol-2ylmethyl)-5,6,7,8-tetrahydro-8-quinolinamines were synthesized which had potent activity against HIV-1. The synthetic approaches adopted allowed for variation of the substitution pattern and resulting changes in antiviral activity are highlighted. This led to the identification of compounds with low and sub-nanomolar anti-HIV-1 activity.

CXCR4 is a 7-transmembrane chemokine receptor.[1] Unlike most of the other receptors within the chemokine family, CXCR4 has only one known natural ligand, stromal cell-derived factor (SDF-1). CXCR4, and its interaction with SDF-1, has been shown to play a role in a number of physiological processes, including the homing of immune cells to sites of inflammation[2] and maintaining the cellular micro-environment of the bone marrow.[3] The CXCR4/SDF-1 interaction is involved with metastatic spread and in directly regulating the growth and/or survival of several types of cancer.[4] CXCR4 is a co-receptor for some strains of HIV-1 that are associated with late stage disease and progression to AIDS.[5] The CCR5 (R5) utilizing HIV-1 strains are generally associated with the initial infection and asymptomatic phase, however as the disease progresses towards AIDS, variant forms of the virus emerge that can utilize both CCR5 and CXCR4 chemokine receptors (dual tropic viruses) or solely utilize the CXCR4 chemokine receptor (CXCR4 tropic) to gain entry and infect new host cells.[6]

Therefore, CXCR4 antagonists have potential therapeutic uses for HIV infection and several other indications, including mobilization of hematopoietic stem cells from the bone marrow.[7] In addition, by blocking the homing of inflammatory cells to inflamed joints, CXCR4 antagonists may be of value in the treatment of rheumatoid arthritis.[8]

Several CXCR4 antagonists, both peptides and small molecules, have been described. Peptide-based CXCR4 antagonists include ALX-40-4C,[9] CTCE-9908,[10] FC131,[11] and POL3026.[12] Small molecule CXCR4 antagonists, have been developed by Kureha (KRH1636)[13] and AnorMED (acquired by Genzyme in 2006); (AMD3100, Plerixafor, Mozobil,[14] and AMD070[15]). AMD3100 was recently approved by the FDA for stem cell mobilization. We were interested in further studying AMD070 (**1**, Fig. 1).

We were especially interested in investigating whether more potent analogs of AMD070 could be obtained by moving the basic side chain to the benzimidazole portion of the molecule. In addition we wanted to explore the effect of conformationally restraining the side chain. Herein we describe the synthesis and antiviral SAR of a series of *N*-(1*H*-benzimidazol-2yl-methyl)-5,6,7,8-tetrahydro-8-quinolinamine derivatives with basic side chains attached to the N-1 and C-4 position of the benzimidazole.

For the synthesis of benzimidazole derivatives containing basic side chains attached to the benzimidazole N-1 nitrogen the first

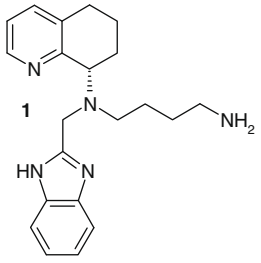

Figure 1. AMD070 (**1**).

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step involved coupling of 2-(aminomethyl)benzimidazole **2** and tetrahydroquinolinone **3**[16] via reductive amination to give **4**. Methylation of **4** with formaldehyde under reductive conditions gave **5**. The basic side chains were subsequently installed using one of two methods outlined in Scheme 1. Treatment of **5** with bromoalkylnitriles **6a** and **6b** under basic conditions afforded nitrile intermediates that were subjected to Raney Nickel reduction to afford primary amine derivatives **9** and **11**, respectively. Alternatively, alkylation of **5** with Boc-protected chloroalkylamines **7a** and **7b**, followed by TFA deprotection, gave compounds **8** and **10**, respectively.

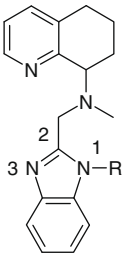
Changing the tether length between the benzimidazole N-1 and the basic nitrogen (Table 1) showed that a 3-carbon propylene linker (as for **9**) was optimal for HIV activity, while the shorter (2 carbon) or longer (4–5 carbon) linkers gave compounds with 5–10-fold less anti-HIV activity. Alkylation of the primary amino group of **9** gave the tertiary amine **12**, which also showed good anti-HIV activity. Similar activity was observed for the guanidine derivative **13**. However reducing the basicity of the amine via trifluoroacetylation gave compound **14**, which showed 10-fold reduction in antiviral activity.

We next investigated the effect of shifting the basic side chain from the N-1 nitrogen to the C-4 position of the benzimidazole. For initial exploration of the optimum distance between the benzimidazole and the basic amine, several linkers tethering the amine to the C-4 position of the benzimidazole via an amide were prepared (Scheme 2). Starting from commercially available methyl 2-amino-3-nitrobenzoate (**15**), hydrogenation gave the phenylenediamine **16**. Coupling of Cbz-protected glycine and **16**, followed by heating in acetic acid to affect imidazole ring closure, gave the methyl 4-benzimidazolecarboxylate **17**. The tetrahydroquinoline was attached by reductive amination with **17**, to give **18**. A second reductive amination with formaldehyde gave the versatile methyl 4-benzimidazolecarboxylate intermediate **19**. Saponification gave **20** and coupling with suitable amines gave desired amides (**21**–**23**), with antiviral activity shown in Table 2.

For these amide-linked analogs, the ethylene diamine derived amine **21** showed the best activity (Table 2), while amides with longer tethers (**22** and **23**) were about 10-fold less potent. This prompted us to synthesize an additional set of compounds (**24**–**26**), with the basic nitrogen incorporated into an imidazole or piperazine heterocycle, and the distance between the benzimidazole and the basic nitrogen approximately the same as for the ethylene diamine **21**. These derivatives (**24–26**) showed similar anti-HIV activity as **21**.

To preclude any possible metabolic instability associated with the amide in analogs **21**–**26**, a series of alkyl analogs was synthesized. The versatile 4-benzimidazolecarboxylate **19**, was used as the starting point. First the methyl ester was reduced to the alcohol, followed by oxidation to the aldehyde **27** (Scheme 3). Aldehyde **27** was used to prepare compounds **28**–**31** by reductive aminations, where the basic side chain is linked to the C-4-position of the benzimidazole via a methylamine linker. While **28** and **29** showed only limited activity, compounds **30** and **31** looked promising. Notable is the >10-fold difference in activity between **29** and **31**, where the more conformationally constrained piperazine derivative **31** shows much more potent activity. To pursue the replacement of the amide linker further, propylamine derivatives were synthesized from the aldehyde **27**. Wittig olefination of **27**, followed by $LiAlH_4$ reduction gave alcohol **32** (Scheme 3). Oxidation of **32** to the aldehyde, followed by reductive amination gave

Table 1
Anti-HIV activity for N-1 substituted benzimidazoles



Compd	R	IC_{50}[a] (μM)	CC_{50}[b] (μM)
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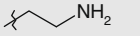
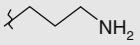
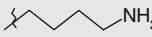
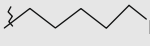
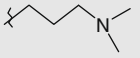
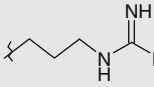
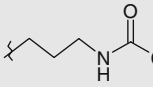
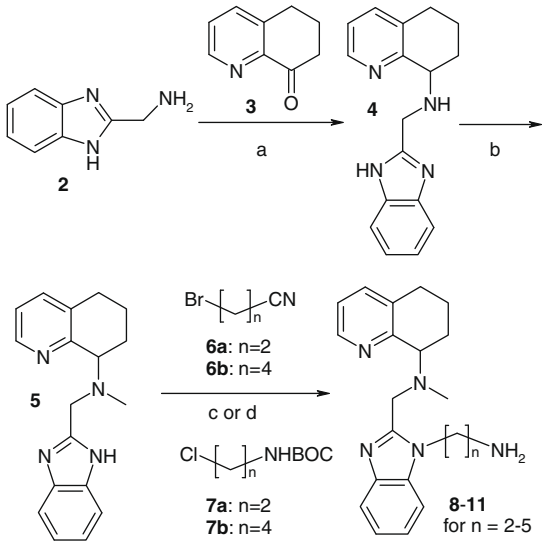
8		0.197	>20
9		0.044	>20
10		0.144	2.9
11		0.228	4.5
12		0.017	16.8
13		0.026	>20
14		0.243	10.4
1[c]	AMD070	0.010	>10

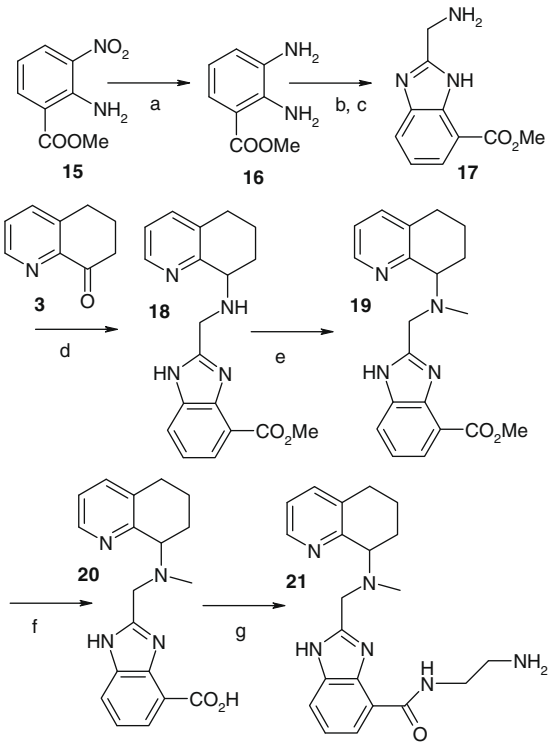
[a] HOS cells (expressing hCXCR4/hCCR5/hCD4/pHIV-LTR-luciferase), HIV-1, CXCR4 strain (IIIB). Compounds were tested for their ability to block infection of the HOS cell line. IC_{50} is the concentration at which 50% efficacy in the antiviral assay is observed Ref. 18.

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

[c] AMD070 included for comparison.



Scheme 1. Reagents and conditions (a) $NaBH_4$, MeOH, 20 °C, 8 h (87%); (b) H_2CO (37% aqueous), $NaBH(OAc)_3$, AcOH, $ClCH_2CH_2Cl$, rt, 18 h, 82%; (c) **6**, K_2CO_3, DMF, 80–100 °C (50–75%); then H_2 (50 psi), Raney Ni, 7 N NH_3 in MeOH, rt, 16 h (70–85%); (d) **7**, K_2CO_3, DMF, 80–100 °C (40–80%); then CF_3COOH, CH_2Cl_2, rt, 8 h (90–100%).



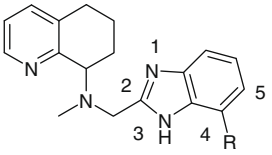
Scheme 2. Reagents and conditions (a) Pd/C (10% w/w), H_2 (1 atm), MeOH, 16 h, rt (99%); (b) BOPCl, carbobenzyloxyglycine (Cbz-glycine), Et(*i*Pr)$_2$N, CH_3CN, 16 h, rt; then AcOH, 70 °C, 150 min (87%); (c) Pd/C (10% w/w), H_2 (1 atm), EtOH, 16 h, rt; (d) **3**, $NaBH(OAc)_3$, AcOH, $ClCH_2CH_2Cl$, rt, 2 h; (e) H_2CO (37% aqueous), $NaBH(OAc)_3$, AcOH, $ClCH_2CH_2Cl$, rt, 18 h (68% from **17**); (f) LiOH (aq), MeOH, THF, 70 °C, 72 h (84%); (g) BOPCl, Et(*i*Pr)$_2$N, CH_3CN, $H_2NCH_2CH_2NHBoc$, 16 h, rt; then MP-TsOH, 7 M NH_3 in MeOH (10%).

C-4 linked propylamine derivatives **33**–**36**, all of which showed very impressive anti-HIV activity. We subsequently became interested in studying whether the basic amine could be incorporated into a heterocycle, such as piperazine, given our previous success with improving activity of flexible amines by incorporation into the conformationally constrained piperazine.

The piperazine derivatives were synthesized from 3-chloro-2-nitroaniline (**37**) as shown in Scheme 4. Condensation of **37** and Boc-protected piperazine, followed by catalytic hydrogenation gave phenylenediamine **38**. BOPCl catalyzed coupling of **38** with *N*-Cbz-glycine followed by acid catalyzed ring closure gave protected methylaminobenzimidazole **39**. Removal of the Cbz-protecting group gave amine **40**. Compound **41** was obtained by coupling ketone **3** with amine **40** by reductive amination. Alkylation of **41** gave **42**. Finally, acidic removal of the Boc group gave piperazine **43** and reductive amination of **43** with formaldehyde gave the desired methylpiperazine **44**. Methylpiperazine derivative **44** showed extremely potent anti-HIV activity at 2 nM, which was about 10-fold more active than AMD070 or other derivatives we had prepared.

The potent anti-HIV activity of the racemate **44** prompted us to prepare this compound in a chirally pure form as outlined in Scheme 5. Thus, the known *S*-4-(methoxyphenylethyl)amine derivative **45** was used as a chiral starting material.[17] Reductive amination with formaldehyde, followed by deprotection with trifluoroacetic acid gave tetrahydroquinoline methylamine **46**. Treatment of **46** with bromobenzylacetate in the presence of base, followed by removal of the benzyl group with Pd on carbon under a hydrogen atmosphere gave glycinate **47**. Amide **49** was formed by BOPCl mediated coupling of **47** and **48**. Finally the desired *S*-ste-

Table 2
Anti-HIV activity and cytotoxicity of substituted *N*-(1*H*-benzimidazol-2ylmethyl)-5,6,7,8-tetrahydro-8-quinolinamines



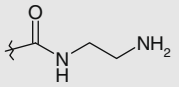
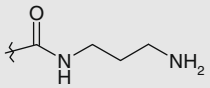
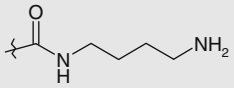
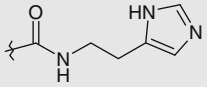
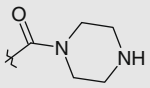
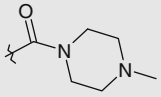
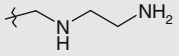
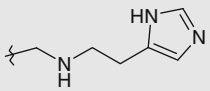
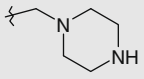
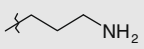
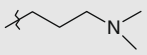
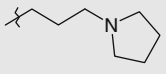
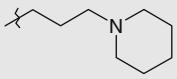
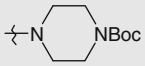
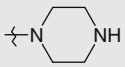
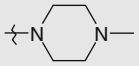
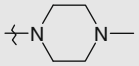
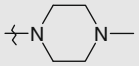
Compd	R	IC_{50}[a] (μM)	CC_{50}[b] (μM)
21		0.027	>10
22		0.134	>20
23		0.396	>20
24		0.019	>10
25		0.046	>20
26		0.033	>10
28		1.3	>20
29		0.54	>20
30		0.041	>20
31		0.013	12
33		0.024	10
34		0.022	7.5
35		0.023	8.2

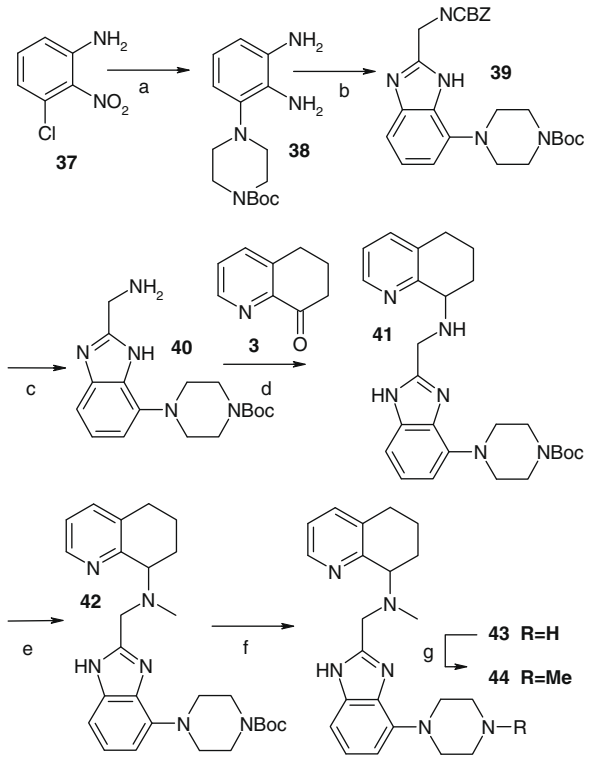
Table 2 (*continued*)

Compd	R	IC_{50}[a] (μM)	CC_{50}[b] (μM)
36		0.025	8.7
42		0.271	>10
43		0.006	>20
44 (rac)		0.002	14
50 (S)		0.0006	12.5
51(R)		0.0069	12.3
1[c]	AMD070	0.010	

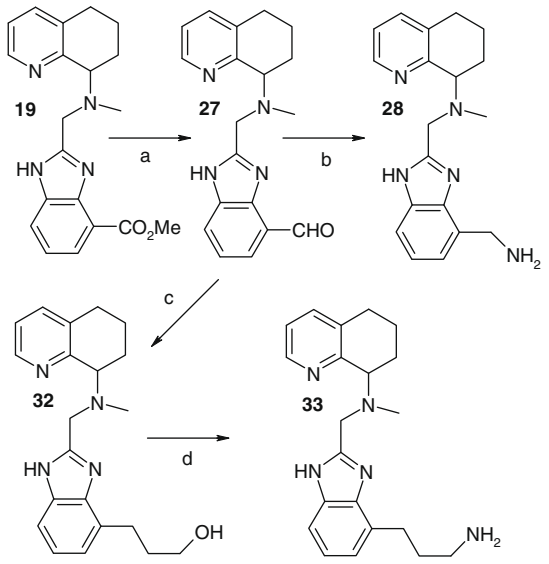
[a] HOS cells (expressing hCXCR4/hCCR5/hCD4/pHIV-LTR-luciferase), HIV-1, CXCR4 strain (IIIB). Compounds were tested for their ability to block infection of the HOS cell line. IC_{50} is the concentration at which 50% efficacy in the antiviral assay is observed Ref. 18.

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

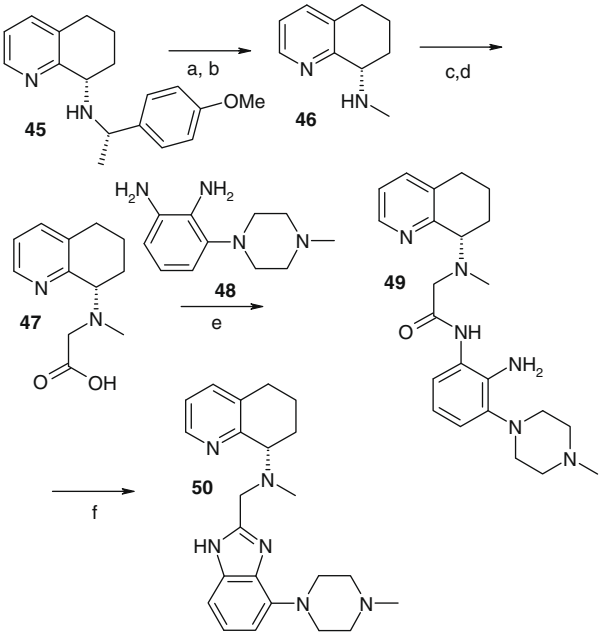
[c] AMD070 is included for comparison.



Scheme 4. Reagents and conditions (a) *t*-butyl 1-piperazinecarboxylate, K_2CO_3, DMF, 130 °C, 72 h (40%); then Pd/C (10% w/w), H_2 (1 atm), EtOH, 3 h, rt (99%); (b) Cbz-Gly, BOPCl, Et(*i*Pr)$_2$N, MeCN, 14 h; then AcOH, 65 °C, 3 h (69% from **40**); (c) Pd/C (10% w/w), H_2 (1 atm), EtOH, 16 h, rt (99%); (d) $NaBH(OAc)_3$, AcOH, $ClCH_2CH_2Cl$, rt, 16 h; (e) CH_2O (37% aq), AcOH, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, rt, 24 h (35% from **41**); (f) HCl (4 N in 1,4-dioxane), MeOH, rt, 12 h (76%); (g) CH_2O (37% aq), AcOH, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, rt, 24 h (60%).



Scheme 3. Reagents and conditions (a) $LiAlH_4$, THF, 0 °C (96%); then DMSO $(COCl)_2$, TEA, CH_2Cl_2 (49%); (b) NH_4OAc, MeOH, $NaCNBH_3$, 50 °C, 16 h (99%); (c) $Ph_3PCHCO_2CH_3$, toluene, 16 h; then $LiAlH_4$, THF; then Pd/C (10% w/w), H_2 (50 psi), MeOH, 48 h, rt (37% from **29**); (d) IBX Resin, CH_2Cl_2 (99%); then NH_4OAc, MeOH, $NaCNBH_3$, 50 °C, 16 h (13%).



Scheme 5. Reagents and conditions (a) CH_2O (37% aq), AcOH, $NaBH(OAc)_3$, $ClCH_2$-CH_2Cl, 12 h, rt; (b) CF_3COOH, CH_2Cl_2, 12 h, rt (91% for a and b); (c) $BrCH_2COOBn$, Et(*i*Pr)$_2$N, CH_2Cl_2, 12 h, rt; (d) Pd/C (10% w/w), H_2 (1 atm), EtOH, 16 h, rt (80% for c and d); (e) **48**, BOPCl, Et(*i*Pr)$_2$N, MeCN, 14 h, rt; (f) AcOH, 70 °C, 1 h (75% from **47**).

reoisomer **50** was formed by treating **49** with acetic acid at elevated temperature.

The *S*-enantiomer **50** showed impressive sub-nM anti-HIV activity, while the *R*-enantiomer (**51**) was over 10-fold less active. Pharmacokinetic studies in rats showed **50** to be orally bioavailable. Screening against a panel of enzymes and receptors (PanLabs) did not show enzyme or receptor inhibition at concentrations close

to those demonstrating anti-HIV activity for **50** (over 500-fold selectivity for all).

Because of the very good anti-HIV potency and good pharmacokinetic properties, **50** was progressed for further studies and served as a template for synthesis of additional analogs.

References and notes

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18. All compounds were also characterized pharmacologically in a cell based functional assay and shown to be non-competitive antagonists of the CXCR4 receptor.