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Novel N-substituted benzimidazole CXCR4 antagonists as potential anti-HIV agents

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ABSTRACT

The lead optimization of a series of N-substituted benzimidazole CXCR4 antagonists is described. Side chain modifications and stereochemical optimization led to substantial improvements in potency and protein shift to afford compounds with low nanomolar anti-HIV activity.

In spite of nearly three decades of intensive research toward the treatment and eradication of HIV/AIDS, the disease remains a global health crisis.[1] The advent of highly active antiretroviral therapy (HAART) in the mid 1990s, which employs various combinations of reverse transcriptase and protease inhibitors, led to dramatic improvements in clinical outcomes.[2] More recently we have seen the approval of the first examples of two newer classes of small molecule antiretrovirals including the CCR5 antagonist Maraviroc[3] and the integrase inhibitor Raltegravir,[4] both in 2007. While the diversity of drugs now available to the clinician has dramatically improved HIV treatment, significant challenges remain. Of particular concern are long term treatment side effects[5] and the emergence of drug resistant viral strains.[6]

One area that has received a great deal of attention in recent years is the concept of blocking viral entry by targeting the chemokine receptors CCR5 and CXCR4 which function as co-receptors, along with CD4, to facilitate fusion of the viral membrane with the host cell.[7] CXCR4 is a G-protein coupled seven-transmembrane receptor utilized by T-tropic HIV strains to gain entry into T-cells.[8] The appearance of CXCR4 utilizing strains of HIV is associated with a decrease in the number of T-cells and accelerated disease progression.[9] In vitro studies have shown that the addition of the natural CXCR4 ligand, SDF-1, or small molecule antagonists (e.g., AMD3100, AMD070) can block HIV infection.[10] In addition, AMD3100[11] and AMD070[12] showed antiviral efficacy in recent human clinical studies. Therefore the CXCR4 receptor represents a promising HIV therapeutic target.

In a recent report our research group described SAR studies based on AMD070 leading to the N-substituted benzimidazole **1** with potent anti-HIV activity.[13] These studies clearly showed that the 3-carbon tether provided the optimal distance between the benzimidazole ring nitrogen and the distal amino group. This result prompted us to ask if further improvements in activity could be achieved by conformationally constraining the basic amino group by incorporating the 3-carbon chain into various ring systems (general structure **A**, Fig. 1). This report describes the synthesis and SAR of a series of novel CXCR4 antagonists containing various nitrogen heterocycles attached to the *N*-1 position of the benzimidazole.

The first set of compounds that we prepared contained four, five and six-membered rings attached to the benzimidazole nitrogen

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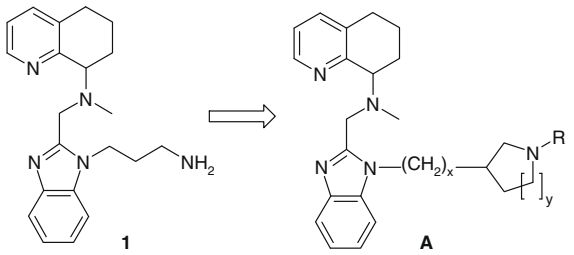
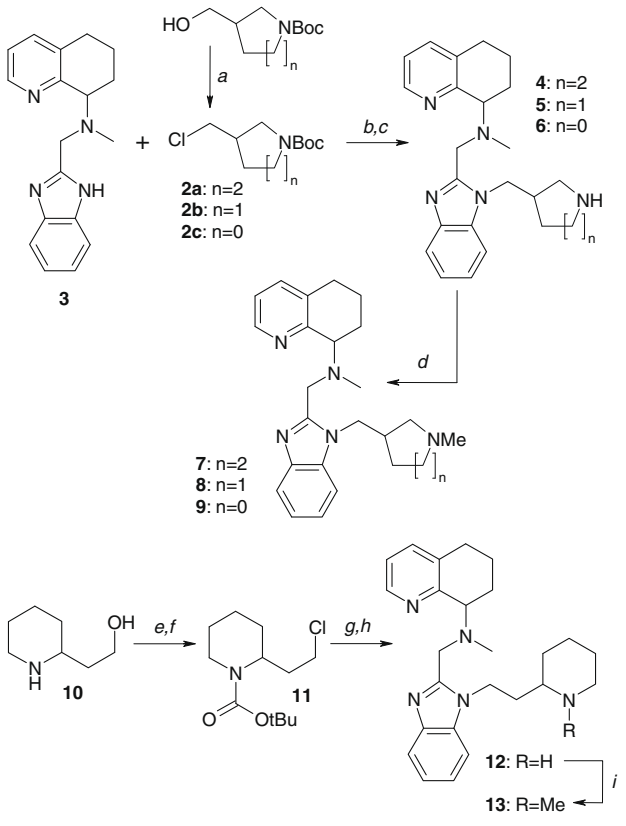


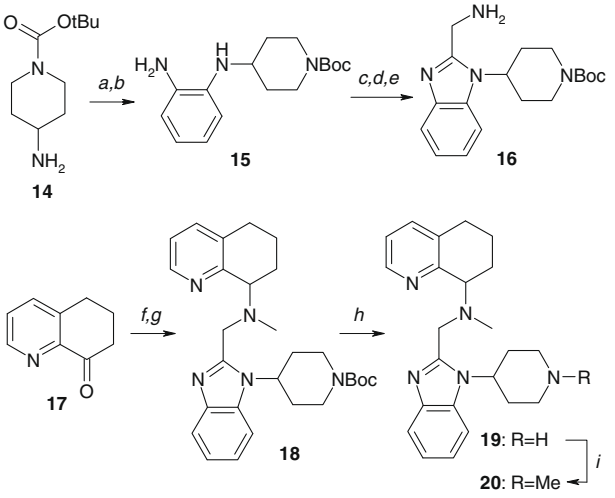
Figure 1.

through a methylene linker (Scheme 1). The syntheses were based on alkylation of the previously reported racemic intermediate **3**[13] with chlorides **2a–c** which were themselves obtained by PS-PPh_3 mediated chlorination of the corresponding alcohols. Potassium carbonate promoted alkylation of **3** with **2a–c** followed by acidic deprotection gave compounds **4**, **5** and **6**. The secondary amines were then methylated by reductive alkylation with formaldehyde to afford methyl derivatives **7**, **8** and **9**. The second side chain motif that we were interested in contained a piperidine ring attached to the benzimidazole nitrogen through an ethylene linker (compounds **12** and **13**). The synthesis was directly analogous to that described above and is also illustrated in Scheme 1.

Finally, we wanted to explore the effect of incorporating the distal nitrogen into a piperidine ring attached directly to the benzimidazole nitrogen (Scheme 2). This route begins with the commercially available Boc-protected 4-aminopiperidine **14**. This



Scheme 1. Reagents and conditions: (a) PS-PPh_3, CCl_4, CH_2Cl_2 (93% for **2a**); (b) K_2CO_3, KI, DMF, 90–120 °C (79% for $n = 2$); (c) 4 N HCl in dioxane, MeOH, (94% for **4**); (d) 37% aqueous formaldehyde, $NaBH(OAc)_3$, 1,2-dichloroethane (80% for **7**); (e) $(Boc)_2O$, CH_2Cl_2 (91%); (f) PS-PPh_3, CCl_4, CH_2Cl_2 (90%); (g) compound **3**, K_2CO_3, DMF, 90 °C (50%); (h) 4 N HCl in dioxane, MeOH, (87%); (i) 37% aqueous formaldehyde, $NaBH(OAc)_3$, 1,2-dichloroethane (83%).



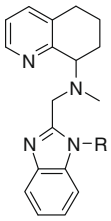
Scheme 2. Reagents and conditions: (a) 2-nitrochlorobenzene, K_2CO_3, DMF, 120 °C (33%); (b) H_2 (40 psi), 10% Pd(C), MeOH (97%); (c) CBz-glycine, BOP-chloride, $(iPr)_2EtN$, MeCN (86%); (d) glacial AcOH, 60 °C (87%); (e) H_2 (40 psi), 10% Pd(C), MeOH (92%); (f) compound **16**, $NaBH(OAc)_3$, AcOH, 1,2-dichloroethane (76%); (g) 37% aqueous formaldehyde, $NaBH(OAc)_3$, 1,2-dichloroethane (78%); (h) TFA, CH_2Cl_2 (74%); (i) 37% aqueous formaldehyde, $NaBH(OAc)_3$, 1,2-dichloroethane (88%).

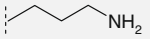
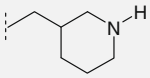
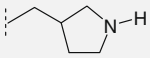
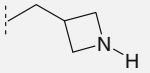
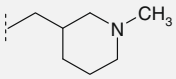
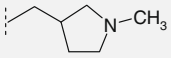
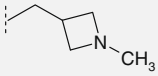
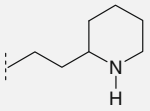
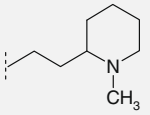
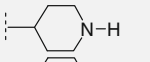
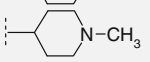
compound was reacted with 2-nitrochlorobenzene under basic conditions at elevated temperature. The resulting nitroaniline derivative was subjected to catalytic hydrogenation to afford diamine **15**. Coupling of **15** with CBz-glycine followed by thermal cyclization in AcOH and then hydrogenolysis of the CBz group gave primary amine **16**. Compound **16** was coupled with ketone **17**[14] by reductive amination. This was followed by reductive methylation of the central nitrogen to afford **18**. The Boc group was cleaved by treatment with TFA to give secondary amine **19** which was then converted to the methyl derivative **20** by reductive methylation with formaldehyde.

Table 1 shows antiviral and cytotoxicity data for 10 cyclic side chain analogs along with the open chain parent compound **1** for comparison. In general, cyclization of the amine side chain leads to a relatively modest effect on antiviral activity ranging from twofold less to twofold more active than **1**. A comparison of the methylene tethered *N*–H derivatives **4**, **5** and **6** shows that the five-membered ring is preferred providing a twofold increase in activity relative to **1**. Interestingly, methylation leads to an increase in activity for the four and six-membered ring compounds **6** and **4** and a twofold loss in potency for the five-membered ring analog **5** suggesting that hydrophobicity alone is not a significant determinant in the SAR. The ethylene linked piperidine analog **12** showed a modest loss in activity relative to **1** with methylation conferring a twofold potency increase. The equipotent *N*-piperidinyl analogs **19** and **20** were the least active in the entire set, probably due to the higher degree of conformational rigidity associated with the side chain. The six-membered ring *N*-methyl analog **7** emerged as the most promising compound from this exercise with a 19 nM antiviral activity and a 160-fold cytotoxicity window.

Having determined that the optimal side chain structure was the six-membered ring associated with compound **7**, we next turned our attention to stereochemical considerations. Compound **7** contains two stereocenters and was initially prepared as a mixture of four stereoisomers. We sought to deconvolute any potential stereochemical SAR by individually preparing all four discreet stereoisomers. The synthesis of the *S,S* stereoisomer is illustrated in Scheme 3. Reaction of the commercial, enantiopure, Boc-protected diamine **21** with 2-nitrofluorobenzene followed by catalytic reduction afforded diamine **22**. Cyclization by treatment of **22** with

Table 1
Anti-HIV IC_{50}s ± standard deviation (n) and CC_{50}s for N-substituted benzimidazoles containing cyclic amine sidechains



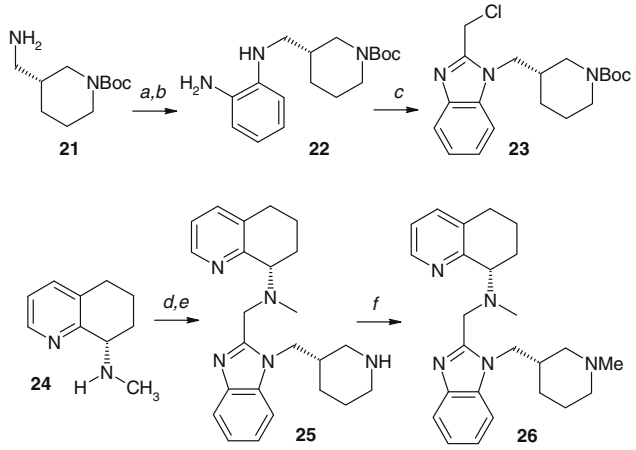
Compound	R	IC_{50}[a] (nM)	CC_{50}[b] (nM)
1		44 ± 3 (2)	9600
4		51 ± 2 (2)	15,000
5		24 ± 1 (2)	7700
6		84 ± 7 (2)	15,000
7		19 ± 2 (2)	3000
8		57 ± 2 (2)	15,000
9		66 ± 5 (2)	14,000
12		64 ± 4 (2)	8300
13		33 ± 1 (2)	4900
19		99 ± 6 (2)	13,000
20		95 ± 8 (2)	16,000

[a] HOS cells expressing hCXCR4/hCCR5/hCD4/pHIV-LTR-luciferase, HIV-1, CXCR4 strain (IIIB). Compounds were tested for their ability to block infection of the HOS cell line. IC_{50} is the concentration at which 50% efficacy in the antiviral assay was observed.[15]

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

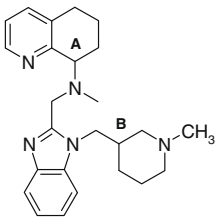
chlorotrimethylorthoacetate under acid catalysis gave benzimidazole derivative **23**. Alkylation of the previously reported, enantiopure, tetrahydroquinoline **24**[13] with **23** under basic conditions followed by TFA mediated Boc deprotection afforded secondary amine **25**. Reductive alkylation with formaldehyde gave methyl derivative **26** as the *S,S* stereoisomer. The remaining three stereoisomers were prepared in a directly analogous manner using the appropriate enantiopure starting materials.

The SAR data associated with the four individual stereoisomers and the isomer mixture **7** is illustrated in Table 2. The absolute configuration at the tetrahydroquinoline attachment point (position A) has the largest effect on potency showing a 20–40-fold activity preference for the *S* configuration. This is consistent with our previous observations of stereochemical SAR with the tetrahydroquinoline ring system.[13,16] The effect of the absolute configuration of the side chain (position B) is more subtle but shows a consistent 2–4-fold preference for the *S* configuration. The *S,S* isomer **26** showed a greater than sevenfold increase in activity over the original racemic, open chain parent compound **1**.



Scheme 3. Reagents and conditions: (a) 2-nitrofluorobenzene, K_2CO_3, MeCN, reflux (98%); (b) H_2 (50 psi), 10% Pd(C), MeOH (99%); (c) $(MeO)_3CCH_2Cl$, PTSA, CH_2Cl_2 (85%); (d) compound **23**, $(iPr)_2EtN$, KI, MeCN, 65 °C (86%); (e) TFA, CH_2Cl_2 (97%); (f) 37% aqueous formaldehyde, $NaBH(OAc)_3$, 1,2-dichloroethane (94%).

Table 2
Anti-HIV IC_{50}s ± standard deviation (n) and CC_{50}s for stereochemically defined N-substituted benzimidazoles



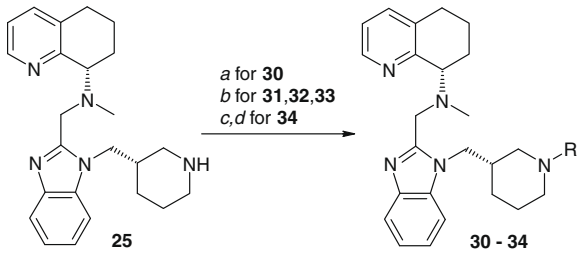
Compound	A config	B config	IC_{50}[a] (nM)	CC_{50}[b] (nM)
7	*R/S*	*R/S*	19 ± 2 (2)	3000
26	*S*	*S*	6.0 ± 1 (2)	3600
27	*S*	*R*	21 ± 1 (2)	3500
28	*R*	*S*	210 (1)	3600
29	*R*	*R*	500 (1)	2800

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

While compound **26** showed an impressive 6 nM antiviral activity, it suffered from a 10-fold reduction in activity when assayed in the presence of serum proteins (human serum albumin and α-acid glycoprotein). Therefore, our final SAR exercise was focused on reducing the protein shift by replacing the side chain methyl group with several alternate substituents. This was easily accomplished via reductive alkylations of secondary amine **25** with aldehydes and ketones (Scheme 4).

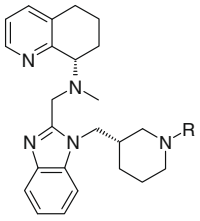
Antiviral and protein shift data for our final set of analogs is shown in Table 3. Replacement of the side chain methyl group with a hydrogen atom (compound **25**) leads to a nearly complete loss of protein shift with retention of antiviral activity. Substitution with an isopropyl group (compound **30**) reduced the protein shift down to twofold and provided a threefold activity increase. The larger branched alkyl analogs **31** and **32** gave protein shifts in the 5–6-fold range accompanied by modest reductions in potency. Interestingly, the polar side chains associated with compounds **33** and **34** provided minimal improvements in protein shift relative to **26**. From this study, compound **30** was identified as the most promising analog showing a very impressive 2 nM antiviral activity and a twofold protein shift.

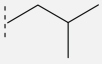
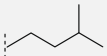
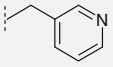
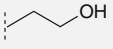
In order to further assess its viability as a potential drug candidate, compound **30** was subjected to pharmacokinetic analysis in



Scheme 4. Reagents and conditions: (a) acetone, $NaBH(OAc)_3$, 1,2-dichloroethane (80%); (b) RCHO, $NaBH(OAc)_3$, 1,2-dichloroethane (89% for **31**); (c) $TBSOCH_2CHO$, $NaBH(OAc)_3$, 1,2-dichloroethane; (d) TBAF, THF (56% for two steps).

Table 3
Anti-HIV IC_{50}s ± standard deviation (n), CC_{50}s and protein shifts for distal N-substituent optimization



Compound	R	IC_{50}[a] (nM)	CC_{50}[b] (nM)	Fold IC_{50} protein shift[c]
26	Me	6.0 ± 1 (2)	3600	10
25	H	8.0 ± 2 (2)	3500	1.2
30		2.0 ± 1 (4)	2100	2.0
31		8.1 ± 2 (2)	>2000	6.2
32		15 ± 2 (2)	2300	5.1
33		33 ± 3 (2)	2500	8.7
34		12 ± 3 (2)	2500	9.1

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

[c] Protein shift is the shift in concentration at which 50% efficacy in the antiviral assay is observed in the presence of human serum albumin (45 mg/mL) and α-acid glycoprotein (1 mg/mL).

both rats and dogs. Following a 1 mg/kg IV dose in rats, **30** showed a 5.8 h half life and an 8.5 mL/min/kg clearance value. Oral dosing in rats at 3 mg/kg produced an 11% oral bioavailability. After IV dosing at 1 mg/kg in dogs, an 11 h half life and a 9.4 mL/min/kg clearance value was observed. Dog oral dosing at 1 mg/kg yielded a 22% oral bioavailability. In addition, screening of compound **30** against a panel of enzymes and receptors showed little risk of undesirable off-target activity including hERG.

Using the open chain analog **1** as a starting point for SAR studies, we have demonstrated that by employing the appropriate conformational modifications, in combination with stereochemical and N-substituent optimization, it was possible to substantially improve the antiviral activity (22-fold relative to **1**). From these studies, compound **30** emerged as a promising candidate showing a 2 nM antiviral activity, a 1000-fold cytotoxicity window, and an attractive twofold protein shift. In addition, compound **30** showed acceptable pharmacokinetic properties in both rats and dogs. The results described herein led us to pursue several additional structure–activity directions in the tetrahydroquinoline series. These studies will be discussed in future reports.

Acknowledgements

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