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

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ABSTRACT

An early lead from the AMD070 program was optimized and a structure–activity relationship was developed for a novel series of heterocyclic containing compounds. Potent CXCR4 antagonists were identified based on anti-HIV-1 activity and Ca^{2+} flux inhibition that displayed good pharmacokinetics in rat and dog.

Although the number of therapeutics available to treat HIV infection has steadily increased over the last two decades there remains a need for new treatment options due to the emergence of drug resistance and poor patient compliance. While HAART treatment[1] has had a dramatic impact on patient outcomes significant research efforts continue in an attempt to identify drugs with novel mechanisms of action. Consequently, the first in class CCR5 chemokine receptor antagonist maraviroc[2] was approved in 2007.

The chemokine receptors CCR5 and CXCR4 belong to the seven-transmembrane G-protein-coupled receptor (GPCR) superfamily and function as co-receptors facilitating fusion of the viral membrane with the host cell. The CCR5 receptor is utilized by the commonly transmitted M tropic (R5) HIV strains to gain entry into T cells whereas CXCR4 is used by the more virulent T tropic (X4) HIV strains. We have been actively involved in the development of CXCR4 antagonists, first with the prototype compound AMD3100,[3] which provided proof of concept that blocking CXCR4 can result in a viral load reduction in T tropic (X4) HIV-1 infected patients.[4] That was followed by the discovery and development of the first orally bioavailable CXCR4 antagonist (AMD070)[5] that was administered to T tropic HIV-1 infected patients and also demonstrated viral suppression.[6] In addition to HIV, CXCR4 and its specific ligand, stromal cell derived factor-1 (SDF-1), play an important role in the homing and retention of progenitor cells in the bone marrow microenvironment.[7] Based on these observations AMD3100 was developed as a stem cell mobilizer that culminated in the US approval of plerixafor (AMD3100) in 2008 for mobilization of hematopoietic stem cells in patients with non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM).[8,9]

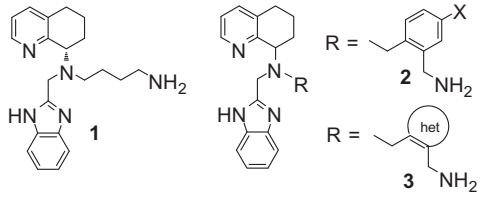

Figure 1. Structure of AMD070 (**1**) and related analogs **2** and **3**.

Recently we reported on the pharmacophore necessary for activity[10] and applied this knowledge to the design and synthesis of oral agents as exemplified by the identification of AMD070 (**1**) which is a potent inhibitor of T tropic (X4) HIV-1 viral replication with an IC_{50} of 2 nM[5] (Fig. 1). During this effort we also discovered a benzylic version of **1** that was 10-fold less active with an IC_{50} of

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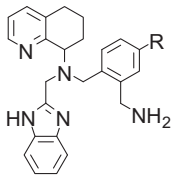
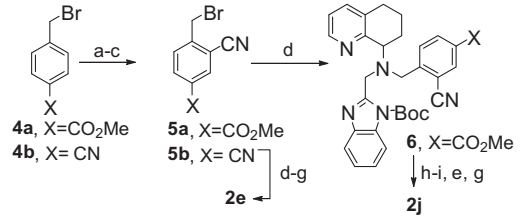
Table 1
Anti-HIV-1 activity, cellular cytotoxicity and calcium signaling (SDF-1) inhibition of compounds **2**[a,b]



Compd	R	HIV-1 IC_{50}[c] (μM)	CC_{50}[c] (μM)	Ca^{2+} flux IC_{50}[d] (μM)
2a	H	0.021	29.4	0.028
2b	F	0.165	45.6	0.219
2c	CF_3	0.104	26.6	0.051
2d	OMe	0.018	36.5	0.005
2e	CH_2NH_2	0.002	122.1	0.010
2f	CH_2OH	0.017	37.5	0.011
2g	CH_2OMe	0.020	201.2	0.021
2h	CO_2H	0.069	137.1	0.126
2i	CO_2Me	0.105	42.2	0.039
2j	$CONH_2$	0.012	120.4	0.015
2k	CHNOMe	0.022	26.8	0.005

[a] Assay conditions reported in Ref. 5.
[b] Assays were performed in duplicate and values represent the mean with standard deviations <30% of the mean.
[c] $CD4^+CXCR4^+$ lymphocytic MT-4 cell line infected with the X4 HIV-1 NL4.3 strain. IC_{50} is the concentration of the compound required to inhibit 50% of the virus-infected cells against viral cytopathicity. CC_{50} is the concentration required to reduce the viability of MT-4 cells by 50%.
[d] CEM-CCRF ($CD4^+CXCR4^+$) T cell line. IC_{50} is the concentration of the compound required to inhibit 50% of the SDF-1-induced Ca^{2+} signaling.



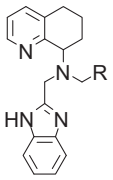
Scheme 1. Reagents and conditions: (a) Zn, TMSCl, $Br(CH_2)_2Br$, THF; (b) TsCN, THF, −78 °C to rt; (c) NBS, AIBN or $(C_6H_5CO)_2O_2$, CCl_4, reflux; (d) *tert*-butyl 2-((5,6,7,8-tetrahydroquinolin-8-ylamino)methyl)-1*H*-benzo[*d*]imidazole-1-carboxylate, KI, DIPEA, CH_3CN, 40–60 °C; (e) H_2, Raney Ni, MeOH, NH_3; (f) $(Boc)_2O$, DIPEA, THF; (g) HBr, AcOH; (h) LiOH, H_2O, THF, 50 °C; (i) $(COCl)_2$, CH_2Cl_2 then NH_3 (g).

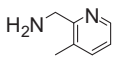
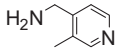
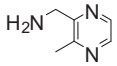
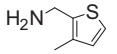
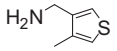
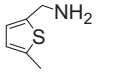
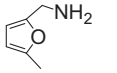
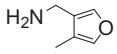
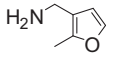
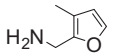
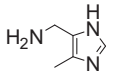
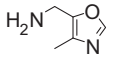
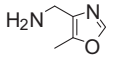
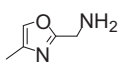
21 nM. In this Letter, we describe the SAR of a series of CXCR4 antagonists where the butyl amine moiety has been replaced by a substituted benzylic aminomethyl group **2** or a heterocyclic aminomethyl moiety **3** (Fig. 1) resulting in the identification of highly potent inhibitors against HIV-1 replication. More recently researchers at Glaxo SmithKline have published several papers detailing an extensive analog program on AMD070.[11]

The synthetic strategy employed to prepare the compounds in Table 1 is exemplified by the synthesis of compounds **2e** and **2j** (Scheme 1). The key step was the selective introduction of an *o*-substituted nitrile which served as the precursor to the aminomethyl moiety. This was accomplished by selective cyanation using tosyl cyanide,[12] for example, conversion of methyl 4-(bromomethyl)benzoate **4a** to the corresponding benzylic zinc bromide followed by treatment with tosyl cyanide and NBS to regenerate the 4-bromomethyl functionality afforded the desired 3-substituted nitrile **5a**.[13] N-Alkylation of **5a** with *tert*-butyl 2-((5,6,7,8-tetrahydroquinolin-8-ylamino)methyl)-1*H*-benzo[*d*]imidazole-1-carboxylate[5] under reflux in the presence of KI and DIPEA afforded the corresponding nitrile ester **6**[14] which was used as a key intermediate for the synthesis of analogs **2f–2k** using standard functional group interconversions. For example, in the synthesis of amide **2j**[15] the methyl ester of **6** was first hydrolyzed to the acid using LiOH and subsequently converted to the primary amide via generation of the acid chloride which was treated with NH_3 (g). The nitrile moiety was reduced in good yield using H_2 in the presence of Raney Ni to afford the aminomethyl group which was treated with HBr in acetic acid resulting in the removal of the BOC group to afford the hydrobromide salt **2j**. Similarly for the synthesis of compound **2e** the nitrile **5b** underwent N-alkylation as above followed by reduction of the bis nitrile, Boc protection to simplify purification and deprotection with HBr in acetic acid to afford the hydrobromide salt **2e**.[†]

The primary data used to drive the SAR were the ability of these compounds to inhibit replication of HIV-1 NL4.3, using exclusively CXCR4 for viral entry into its target cells. In addition, the ability of

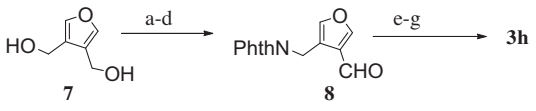
Table 2
Anti-HIV-1 activity, cellular cytotoxicity and calcium signaling (SDF-1) inhibition of compounds **3**[a]



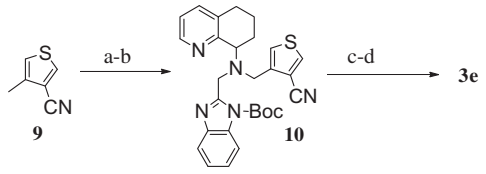
Compd	R	HIV-1 IC_{50} (μM)	CC_{50} (μM)	Ca^{2+} flux IC_{50} (μM)
3a		0.021	150	0.021
3b		0.124	128	0.160
3c		>1.23	6.1	na
3d		0.014	31.5	0.06
3e		0.103	32.9	0.013
3f		2.48	28.5	na
3g		3.57	28.5	na
3h		0.0109	30.2	0.025
3i		0.0004	28.5	0.012
3j		0.008	50.0	0.021
3k		>1.16	5.8	na
3l		2.83	32.9	na
3m		0.0072	64.5	0.0013
3n		20.1	28.5	na

[a] See footnotes in Table 1.

[†] Experimental details and characterization data for compounds **2e–2i** is available in supplementary data.



Scheme 2. Reagents and conditions: (a) NaH, THF, 45 min then TBDMSCl, THF; (b) phthalimide, DEAD, PPh_3, THF, 0 °C; (c) 4 N HCl/THF (2:1); (d) TPAP, NMO, 3 Å mol sieves, 0 °C, 2 h; (e) *tert*-butyl 2-((5,6,7,8-tetrahydroquinolin-8-ylamino)methyl)-1*H*-benzo[*d*]imidazole-1-carboxylate, $NaBH(OAc)_3$, CH_2Cl_2, 60 °C; (f) 1 N HCl/THF (2:1), 2 h; (g) $H_2NNH_2{\cdot}H_2O$, EtOH.



Scheme 3. Reagents and conditions: (a) NBS, AIBN, CCl_4, reflux; (b) *tert*-butyl 2-((5,6,7,8-tetrahydroquinolin-8-ylamino)methyl)-1*H*-benzo[*d*]imidazole-1-carboxylate, KI, DIPEA, CH_3CN, 60 °C; (c) H_2, Raney Ni, MeOH, NH_3; (d) HBr, AcOH.

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

Previously we had shown that the *o*-substituted amine **2a** was eightfold and 27-fold more potent then the corresponding *p*- and *m*-substituted analogs therefore in an attempt to improve the anti-HIV-1 activity of **2a** substituents were introduced at the corresponding *p*-position (Table 1). The simple introduction of an aminomethyl moiety compound **2e**, resulted in a 10-fold enhancement in anti-HIV-1 potency, 2 nM versus 21 nM for **2a**. However, results from the Caco-2 cell permeability assay predicted a low absorption potential for **2e** likely due to a +2 charge at physiological pH compared to **2a** which was predicted to have a moderate absorption potential. This result was corroborated by rat and dog pharmacokinetics which showed low oral bioavailability in both species. In an attempt to reduce the charge of **2e** the *O*-methyloxime **2k** was prepared but this resulted in a 10-fold loss in activity, comparable to **2a**. On the other hand substitution of electron withdrawing groups on the phenyl ring to reduce the basicity of the amine such as F **2b** and CF_3 **2c** did result in compounds with a high predicted absorption potential but unfortunately the potency was reduced five and eightfold, respectively, compared to **2a**. Incorporation of a hydroxymethyl moiety **2f** or the corresponding methyl ether **2g** resulted in comparable potency to **2a** but did not improve the permeability. Interestingly the primary amide **2j** resulted in an eightfold improvement in potency whereas the acid **2h** and methyl ester **2i** were threefold and fivefold less potent. Unfortunately, additional attempts to increase the potency while maintaining permeability were unsuccessful so we shifted our attention to heterocyclic replacements of the phenyl group (Table 2).

Introduction of a 2,3-disubstituted pyridine ring with the aminomethyl moiety at the 2-position **3a**, resulted in a compound with comparable potency to **2a**. Interestingly, a sixfold reduction in potency was observed with the 4,5-disubstituted pyridine ring **3b** when the aminomethyl moiety was at the 4-position. An even greater reduction in potency (>60-fold) was observed upon the incorporation of the 2,3-disubstituted pyrazine ring **3c**. These results did suggest however that the appropriate placement of a heteroatom was tolerated as in the case of **3a**. We next turned our attention to the incorporation of five-membered heterocyclic rings and prepared a series of regioselectively substituted thiophenes **3d–f**. Interestingly the 2,5-disubstituted analog **3f** was substantially less potent with an IC_{50} of 2.48 μM compared to the 2,3- or 3,4-disubstituted analogs, **3d** and **3e** which have an IC_{50} of 0.014 and 0.103 μM. Encouraged by these results we prepared all four regioisomers of the corresponding furan **3g–j**. Similar to the thiophene analog **3f** the 2,5-disubstituted furan **3g** was inactive with an IC_{50} of 3.57 μM and the 3,4-disubstituted analog **3h** had comparable activity to the thiophene **3e** of approx. 0.010 μM. Both regioisomers of the 2,3-disubstituted furan potently inhibited HIV-1 replication but the analog with the aminomethyl group in the 3-position **3i** was 50-fold more potent then **2a** with an IC_{50} of 0.0004 μM whereas the other regioisomer **3j** was three fold more active with an IC_{50} of 0.008 μM. This result prompted us to investigate substitution with an oxazole ring and as a consequence the regiosomers **3l–n** were prepared. As in the other examples the substitution pattern had a dramatic effect on potency. For example,

Table 3
Anti-HIV-1 activity, fold protein shift and SDF-1 binding inhibition of compounds **3**

Compd	HIV-1 MT-4 IC_{50}[a] (nM)	HIV-1 PBMC IC_{50}[a,b] (nM)	Fold IC_{50} protein shift[c]	^{125}I-SDF-1 binding IC_{50}[d] (nM)
(*S*)-**3a**	4.2	9.9	24	19.8
(*S*)-**3e**	0.99	2.0	28	88.9
(*S*)-**3h**	0.31	4.1	17	44.5

[a] Assays were performed in triplicate and values represent the mean with standard deviations <30% of the mean.
[b] Peripheral blood mononuclear cells (PBMC) were isolated from healthy volunteers as reported in Ref. 5.
[c] Fold IC_{50} protein shift is the shift in the X4 HIV-1 PBMC antiviral assay in the presence of 1 mg/mL of α-acid glycoprotein.
[d] Competitive binding studies against CXCR4 were performed in human $CD4^+CXCR4^+$ CEM-CCRF cells as reported in Ref. 5.

Table 4
Pharmacokinetics of (*S*)-**3a**, **3e** and **3h** in rat and dog

Compound	Species	C_{max} (μM)	AUC_{0-inf} (h μM)	CL (mL/min/kg)	*V* (L/kg)	$T_{1/2}$ (h)	*F* (%)
(*S*)-**3a**	Rat	1.2	5.4	70.0	6.6	0.8	22
(*S*)-**3e**		1.4	3.7	27.2	5.2	1.7	6
(*S*)-**3h**		3.0	12.5	23.3	3.6	2.1	17
(*S*)-**3a**	Dog	4.2	13.5	13.3	2.6	5.7	84
(*S*)-**3e**		2.6	3.7	5.0	2.2	3.4	9
(*S*)-**3h**		4.7	16.4	3.7	0.7	2.7	28

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

when the aminomethyl group was adjacent to the oxygen atom of the oxazole **3l** the compound was inactive with an IC_{50} of 2.83 μM whereas substitution adjacent to the nitrogen atom **3m** resulted in a potent compound with an IC_{50} of 0.0072 μM. On the other hand substitution of the aminomethyl group at the 2-position **3n** had a deleterious impact on potency with an IC_{50} of 20 μM. Incorporation of the imidazole **3k** resulted in an inactive compound that was also cytotoxic to MT-4 cells. As was the case with the previous series the effects on SDF-1 induced Ca^{2+} flux tracked with the anti-HIV-1 activity.

The methodology used to prepare the compounds in Table 2 was similar to that utilized in Scheme 1. The key step in many of these examples was the generation of the bromomethyl (**3a–b**, **3d–e**) or aldehyde moiety (**3c**, **3f–n)** of the heterocycle which was then coupled with *tert*-butyl 2-((5,6,7,8-tetrahydroquinolin-8-ylamino)methyl)-1*H*-benzo[*d*]imidazole-1-carboxylate[5] via N-alkylation or reductive amination. In most of the reductive amination examples the amine was masked by use of the phthalimide group. For example, the furan **3h** was synthesized by mono protection of furan-3,4-diyldimethanol **7** as the TBDMS ether followed by Mitsunobu reaction of the alcohol with phthalimide to install the requisite protected amine, acid mediated cleavage of the silyl ether to give the alcohol and finally TPAP oxidation to afford the aldehyde **8** (Scheme 2). Reductive amination with the fully elaborated secondary amine[5] in the presence of $NaBH(OAc)_3$, following an acidic workup to remove any complexed boron species, and deprotection of the phthalimide with hydrazine furnished **3h** as the free-base. In the N-alkylation examples the bromomethyl moiety was readily accessed from commercially available starting materials. For instance, as exemplified by the synthesis of **3e**, 4-methylthiophene-3-carbonitrile **9** was readily converted to the corresponding bromide using NBS in the presence of AIBN which then underwent N- alkylation with the secondary amine to give compound **10**. Reduction of the nitrile to the primary amine using H_2 in the presence of Raney Ni followed by deprotection afforded compound **3e** (Scheme 3).

Based on ADME considerations representative compounds from the pyridine **3a**, thiophene **3e** and furan **3h** series were prepared (Table 3) as the (*S*)-enantiomer using methodology previously reported[16] since AMD070 and related (*S*)-enantiomers[5] had shown a marked increase in potency. This observation was corroborated, for example, the IC_{50} of (*S*)-**3a**, **3e**, and **3h** was 4.2, 1.0, and 0.3 nM, respectively, a 5-, 103-, and 35-fold increase in potency over the corresponding racemates (Tables 2 and 3). The IC_{50} was also evaluated for X4 HIV-1 infection in PBMC and was found to be twofold higher for compounds **3a** and **3e** (IC_{50} of 9.9 and 2.0 nM) and 10-fold higher for compound **3h** (IC_{50} of 4.1 nM) consistent with other compounds when comparing inhibition of infection in the MT-4 $CD4^+$ T cell line and in PBMC. However, in the presence of 1 mg/mL of α-acid glycoprotein (AGP) there was a large 17–28-fold shift in anti-HIV-1 activity. Additional evidence that these compounds are interacting with CXCR4 was provided by the competitive binding with ^{125}I-SDF-1 in $CD4^+CXCR4^+$ T cells with the IC_{50} ranging from 20 to 89 nM (Table 3).

Due to the ease of handling these compounds were prepared as their hydrochloride salts and evaluated in rat and dog pharmacokinetics (Table 4). The oral bioavailability of the thiophene compound (*S*)-**3e** was poor in both species while the furan compound (*S*)-**3h** showed moderate oral bioavailability ($F = 17\%$ in rat and 27% in dog). In contrast, the pyridine compound (*S*)-**3a** had excellent oral bioavailability in dog ($F = 84\%$) and moderate in rat ($F = 22\%$).

In conclusion we identified a novel series of orally bioavailable heterocyclic aminomethyl compounds of the chemokine receptor CXCR4 based on inhibition of SDF-1 induced calcium signaling and ^{125}I-SDF-1 binding that retained potent inhibition of HIV-1 replication. These compounds displayed good pharmacokinetics in rat and dog but because of the large anti-HIV-1 fold shift in potency in the presence of α-AGP these compounds were not further advanced.

Supplementary data

Supplementary data (experimental procedures and characterization data for the synthesis of compounds **2e**–**2i**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.023.

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13. A mixture of 5-cyano-2-methyl-benzoic acid methyl ester (see Ref. 12 for preparation) (894 mg, 5.10 mmol), NBS (1.00 g, 5.62 mmol), and AIBN (125 mg, 0.761 mmol) in CCl_4 (20 mL) was heated at reflux for 3 days then allowed to cool to room temperature. The mixture was filtered, concentrated and the crude purified by column chromatography on silica gel (5% EtOAc/hexanes) to afford methyl 4-(bromomethyl)-3-cyanobenzoate **5a** as a yellow solid (800 mg, 62%). ^{1}H NMR ($CDCl_3$) δ 3.99 (s, 3H), 4.96 (s, 2H), 7.61 (d, 1H, $J = 8.1$ Hz), 7.77 (dd, 1H, $J = 8.1$, 1.8 Hz), 8.27 (d, 1H, $J = 1.8$ Hz).
14. A mixture of *tert*-butyl 2-((5,6,7,8-tetrahydroquinolin-8-ylamino)methyl)-1*H*-benzo[*d*]imidazole-1-carboxylate (253 mg, 0.668 mmol), **5a** (170 mg, 0.669 mmol), KI (6 mg, 0.04 mmol), and DIPEA (0.17 mL, 0.98 mmol) in CH_3CN (6.7 mL) was heated at 60 °C for 18 h. Saturated $NaHCO_3$ (aq) (15 mL) was added, the mixture was extracted with CH_2Cl_2 (3 × 15 mL) and the organic extracts were dried ($MgSO_4$) and concentrated. The crude was purified by column chromatography on silica gel (500:5:1 CH_2Cl_2/MeOH/NH_4OH) to give *tert*-butyl 2-(((2-cyano-4-(methoxycarbonyl)benzyl)(5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-1*H*-benzo[*d*]imidazole-1-carboxylate **6** as a yellow foam (360 mg, 98%). ^{1}H NMR ($CDCl_3$) δ 1.74 (m, 10H), 1.99 (m, 2H), 2.29 (m, 1H), 2.74 (m, 2H), 3.86 (s, 3H), 4.22 (d, 1H, $J = 17$ Hz), 4.33 (m, 1H), 4.36 (d, 1H, $J = 17$ Hz), 4.59 (d, 1H, $J = 14$ Hz), 4.65 (d, 1H, $J = 14$ Hz), 6.98 (dd, 1H, $J = 8.0$, 4.7 Hz), 7.25 (m, 3H), 7.33 (dd, 1H, $J = 8.1$, 1.8 Hz), 7.50 (m, 1H), 7.68 (m, 2H), 8.11 (d, 1H, $J = 8.1$ Hz), 8.41 (m, 1H).

15. (a) To a stirred solution of LiOH (120 mg, 5.0 mmol) in H_2O (5 mL) was added a solution of **6** (273 mg, 0.49 mmol) in THF (5 mL) and the mixture was heated to 50 °C for 17 h. The solution was cooled to room temperature, concentrated and neutralized with 1 N HCl. The aqueous was extracted with $CHCl_3$ (3 × 25 mL) and the combined organic extracts were dried ($MgSO_4$) and concentrated to give the acid as a yellow solid (224 mg). (b) A stirred solution of the acid (220 mg, 0.41 mmol) in CH_2Cl_2 (1.6 mL) was treated with $(COCl)_2$ (0.41 mL, 0.82 mmol) and heated to reflux for 1 h. The red solution was cooled to room temperature and NH_3 (g) was bubbled through the solution for 10 min. The crude mixture was poured into saturated $NaHCO_3$ (aq), extracted with $CHCl_3$ (5 × 10 mL) and the combined organic extracts dried ($MgSO_4$) and concentrated. The crude was purified by column chromatography on silica gel (20:1:1 CH_2Cl_2/MeOH/NH_4OH) to afford the amide as an orange foam (106 mg, 50% over two steps). ^{1}H NMR ($CDCl_3$) δ 1.70–1.93 (m, 1H), 1.99–2.11 (m, 2H), 2.34–2.38 (m, 1H), 2.74–2.88 (m, 2H), 3.88–3.99 (m, 2H), 4.13–4.28 (m, 3H), 5.70 (br s, 1H), 6.05 (br s, 1H), 7.17–7.22 (m, 3H), 7.46 (d, 1H, *J* = 7.5 Hz), 7.50–7.68 (br m, 2H), 7.79–7.95 (m, 3H), 8.64 (d, 1H, *J* = 3.0 Hz). (c) A solution of 3-cyano-4-{[(1*H*-benzimidazole-2-ylmethyl)-(5,6,7,8-tetrahydro quinolin-8-yl)-amino]-methyl}-benzamide (105 mg, 0.24 mmol) in MeOH (10 mL) was treated with Raney Ni (50 mg) and placed under 50 psi H_2 on a Parr shaker for 3.5 h. The slurry was filtered through Celite 521, concentrated and purified by column chromatography on silica gel (20:1:1 CH_2Cl_2/MeOH/NH_4OH) to afford the corresponding aminoamide (35 mg). (d) A saturated solution of HBr in AcOH (2 mL) was added dropwise to a stirred solution of the amide (35 mg, 0.079 mmol) in AcOH (2 mL) and the mixture was stirred for 10 min. Ether (50 mL) was added resulting in a white precipitate, the ether was decanted, the white solid was washed with ether (5 × 50 mL) and dried in vacuo at 50 °C for 17 h to give **2j** as a white solid (49 mg, 25% over two steps). ^{1}H NMR (D_2O) δ 1.84–2.00 (m, 1H), 2.19–2.35 (m, 2H), 2.46–2.54 (m, 1H), 3.00–3.08 (m, 2H), 3.97 (d, 1H, *J* = 15 Hz), 4.21–4.58 (m, 5H), 4.75–4.92 (m, 1H, overlaps with HOD), 7.40 (s, 1H), 7.40 (s, 1H), 7.44–7.55 (m, 6H), 7.93 (t, 1H, *J* = 6.8 Hz), 8.40 (d, 1H, *J* = 8.0 Hz), 8.77 (d, 1H, *J* = 5.5 Hz). ^{13}C NMR (D_2O) δ 20.43, 21.17, 27.94, 40.12, 49.11, 53.67, 63.17, 113.83 (2C), 126.33, 127.19 (2C), 128.29, 129.24, 130.38, 132.10, 132.51, 132.66, 139.96 (2C), 140.12, 141.33, 148.49, 150.15, 150.57, 170.44. ES-MS *m*/*z* 441 (M+H). Anal. Calcd for $C_{26}H_{28}N_6O{\cdot}3.3HBr{\cdot}2.4H_2O{\cdot}0.7NH_4Br$: C, 38.11; H, 4.79; N, 11.45; Br, 39.01. Found: C, 37.73; H, 4.64; N, 11.56; Br, 39.27.

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