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Novel CXCR3 antagonists with a piperazinyl-piperidine core

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ABSTRACT

High-throughput screening of an encoded combinatorial aryl piperazine library led to the identification of a novel series of potent piperazinyl-piperidine based CXCR3 antagonists. Analogs of the initial hit were synthesized via solid and solution phase methods to probe the influence of structure on the CXCR3 binding of these molecules. Various functional groups were found to contribute to the overall potency and essential molecular features were identified.

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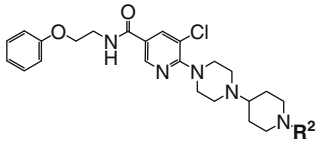
The chemokines IP-10 (CXCL10), MIG (CXCL9), and I-TAC (CXCL11) interact with the CXCR3 receptor, facilitating the recruitment of Th1 cells during a variety of inflammatory responses.[1] Hence, a CXCR3 antagonist offers promise as an anti-inflammatory agent for conditions, such as multiple sclerosis,[2] psoriasis,[3] and transplant rejection,[4] where upregulation of CXCR3+ T lymphocytes is implicated. A variety of chemotypes have been disclosed recently as small molecule CXCR3 antagonists[5] including 2,3-disubstituted quinazolinones and 8-azaquinazolinones,[6] 2-iminobenzimidazoles,[7] aryl-[1,4]diazepane ureas,[8] aminopiperidine and aminotropane homotropene amides,[9] and benzetimide derivatives.[10] High-throughput screening of a portion of an encoded combinatorial library collection[11] revealed the piperazinyl-piperidine compound **1a** as a moderately potent binder (110 nM) of the CXCR3 receptor (Table 1). Characterization in a chemotaxis assay[12] confirmed **1a** as an antagonist and led to further follow-up efforts based on this hit.

Since **1a** was identified from an encoded combinatorial library, a solid-phase pathway to construct analogs probing the right and left hand sides of the lead was in place (Scheme 1). Nucleophilic loading of a variety of amines to Tentagel resin harboring a photocleavable bromomethyl-*ortho*-nitrophenyl linker[15] afforded secondary amine resins **2**. Coupling of carboxylic acid **3** to resin-bound amine **2** via HATU resulted in Boc-protected **4** and deprotection generated intermediate **5.** Reductive amination with *N*-Boc-piperidinone and subsequent removal of the newly added Boc protecting group led to secondary amine resin **6**. This key intermediate was treated with carboxylic acids (amide formation) or alde-

Table 1
Human CXCR3 receptor antagonist hit **1a** and right side analogs



Compds	R^2	hCXCR3 binding K_i (nM)[14]
1a	4-Cyanobenzyl	110 ± 10
1b	Benzyl	1900 ± 50
1c	3-Cyanobenzyl	4100 ± 650
1d	2-Cyanobenzyl	8900 ± 3100
1e	4-Cyanobenzoyl	320 ± 50
1f	4-Chlorobenzyl	70 ± 20
1g	4-Bromobenzyl	85 ± 10
1h	4-Fluorobenzyl	160 ± 40
1i	4-Trifluoromethylbenzyl	510 ± 100
1j	4-Methoxybenzyl	120 ± 10
1k	4-Dimethylamino	2900 ± 50

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† In 2008, Pharmacopeia, Inc. was acquired by Ligand Pharmaceuticals.

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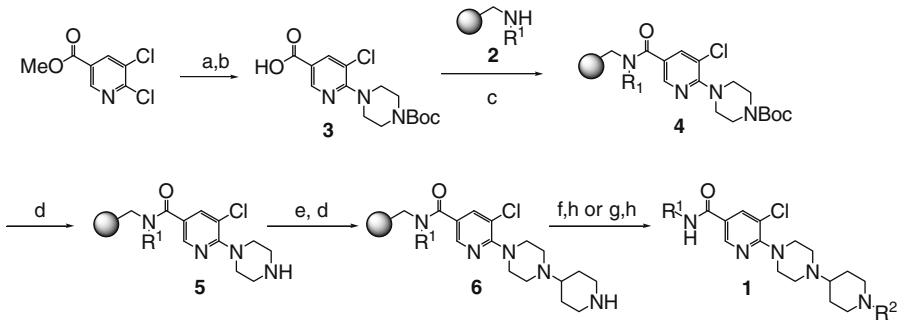
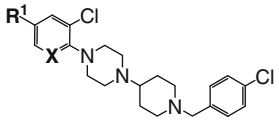
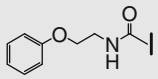
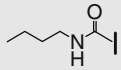
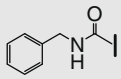
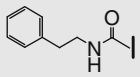
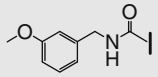
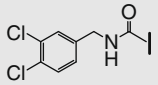
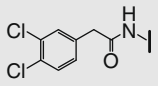
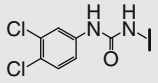
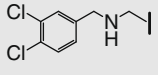
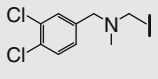
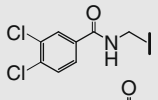
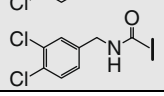


Scheme 1. Synthesis of analogs **1a**–**p**. Reagents and conditions: (a) Boc-piperazine, K_2CO_3, DMF, 90 °C; (b) LiOH, MeOH, H_2O, rt; (c) HATU, DIEA, DMF, rt; (d) TFA, DCM, rt; (e) Boc-piperidinone, $Na(OAc)_3BH$, DCE, rt; (f) substituted benzaldehyde, $Na(OAc)_3BH$, DCE, rt; (g) substituted benzoic acid, HATU, DIEA, DMF, rt; (h) *hν* (365 nm), 3%TFA/MeOH (v/v), 50 °C.

Table 2
Human CXCR3 receptor antagonist binding of left side analogs



Compds	R^1	X	hCXCR3 binding K_i (nM)
1f		N	70 ± 20
1l		N	560 ± 40
1m		N	370 ± 160
1n		N	140 ± 10
1o		N	470 ± 120
1p		N	410 ± 40
1q		N	35 ± 10
12a		N	1900 ± 400
12b		N	>50,000
12c		N	870 ± 10
12d		N	3700 ± 700
12e		N	3800 ± 600
21		CH	330 ± 50

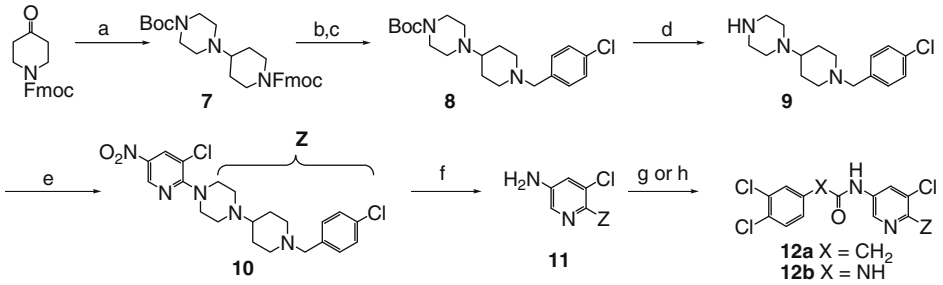
hydes (reductive alkylation) to generate upon resin cleavage, analogs **1a–q**.[16]

Testing of the initial set of analogs synthesized by this methodology (Table 1) indicated a clear preference for a *para*-substituted benzyl functionality on the piperidine as indicated by the dramatic loss of potency exhibited by unsubstituted benzyl analog **1b**. Both the 3-cyano (**1c**), and 2-cyano (**1d**) benzyl derivatives were also less active than **1a**. Replacement of the benzyl linkage with an amide (**1e**) was tolerated, albeit with a threefold drop in potency. Finally, the 4-chlorobenzyl moiety (**1f**) was identified as preferable to benzyl groups bearing either more electron withdrawing (**1h**, **1i**) or electron donating (**1j**, **1k**) *para*-substituents. The SAR of this region of the molecule appears to be similar to the trend exhibited by the benzyl-substituted piperidine moiety in the recently published benzetimide series.[10] It is intriguing to speculate that both inhibitor classes may share a similar binding mode with the CXCR3 receptor.[17]

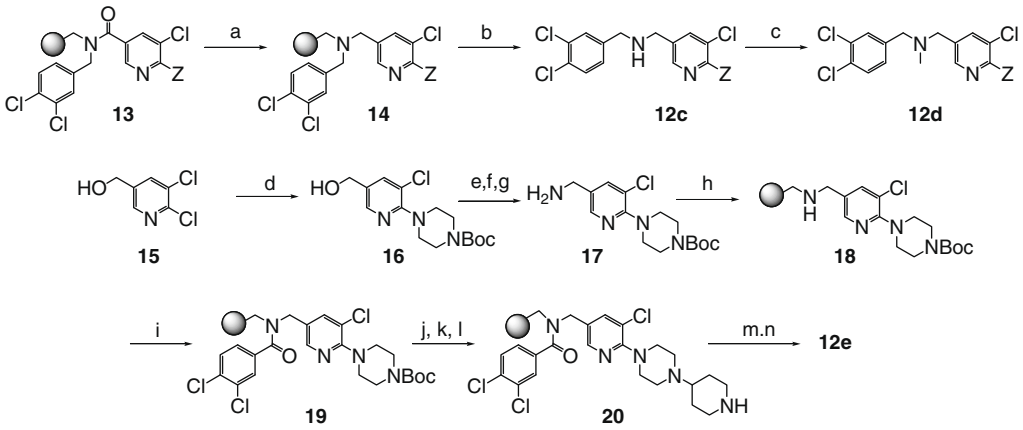
Attention was next drawn to the left hand side nicotinyl amide. Attempts to lower the molecular weight of **1a** by complete removal of the amide moiety led to a loss of activity (data not shown). Various amide substituents, however, were tolerated (**1f, 1l–1q**, Table 2) with a preference for electron-deficient benzyl moieties. Moreover, molecular weight reduction by truncation of the benzylamide to a methylamide (**1l**) resulted in a 15-fold decrease in potency. Therefore, the most potent analog **1q** was chosen as the lead structure moving forward.

The combinatorial library's original solid-phase attachment point generated a residual secondary amide in the analogs discussed so far. Hybrid approaches of solution-phase synthesis (Scheme 2) and alternative solid-phase syntheses (Scheme 3) were employed to prepare analogs which explored the importance of this secondary amide (Table 2). Inversion of the amide to an acylated 3-aminopyridine resulted in a loss of activity (**12a**). The urea analog of this 3-amino pyridine (**12b**) did not rescue receptor affinity. Binding affinity was also lost by reduction of the secondary amide **1q** to the secondary amine **12c** or tertiary amine **12d**. Relocation of the amide in these reduced analogs could not recover the lost activity (**12e**). Hence, the nicotinyl amide linkage was identified as an essential part of the pharmacophore. The combinatorial library from which **1a** was decoded contained multiple un-decoded (inactive) aryl piperazine cores, indicating the 5-substituted nicotinamide was essential for CXCR3 receptor binding. Indeed, replacement of the pyridine ring with a benzene caused a 10-fold drop in affinity (**21**).[18] Remarkably, as indicated by the comparison of analogs **22** and **23** (Table 3), removal of the 5-chloro moiety caused a 100-fold drop in potency.[19]

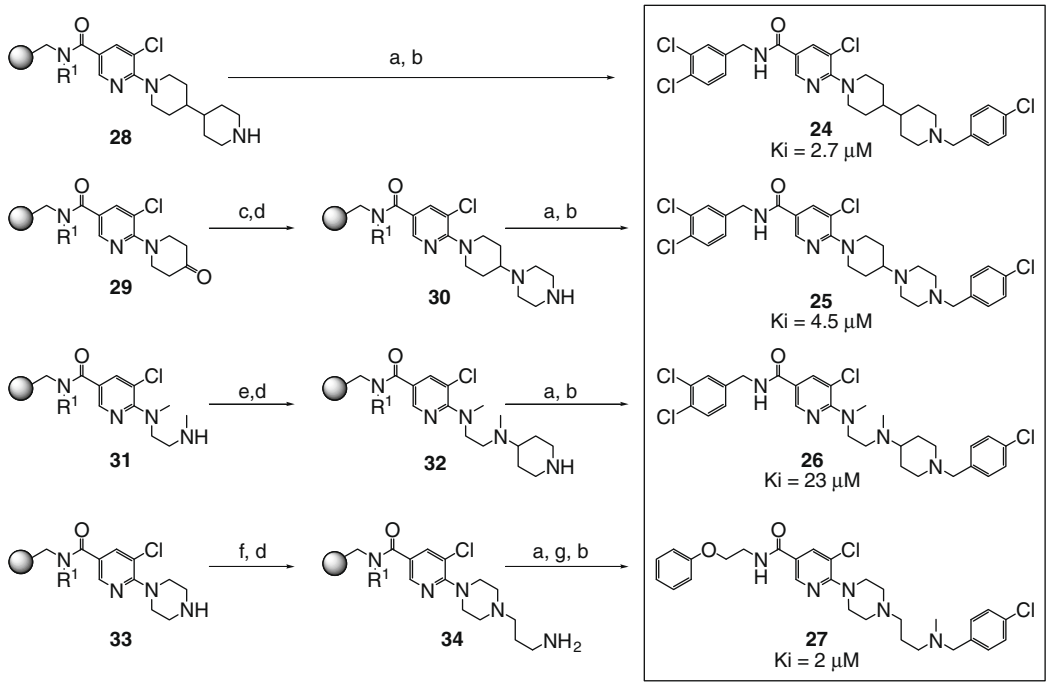
The role of the piperazinyl-piperidine was next explored. The required analogs were synthesized via slight variations on the original solid-phase route as outlined in Scheme 4. Removal of a basic



Scheme 2. Solution synthesis of aminopyridine analogs **12a,b**. Reagents and conditions: (a) Boc-piperazine, $Na(OAc)_3BH$, AcOH, DCE, rt; (b) 20% piperidine in THF, rt; (c) 4-chlorobenzaldehyde, $Na(OAc)_3BH$, AcOH, DCE, rt; (d) HCl, Dioxane, rt; (e) 2,3-dichloro-5-nitropyridine, K_2CO_3, DMF, 90 °C; (f) $Na_2S_2O_4$, EtOH, H_2O; rt; (g) 3,4-dichlorophenylacetyl chloride, DCM; rt; (h) 3,4-dichlorophenyl isocyanate, DCM, rt.

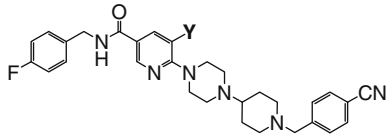


Scheme 3. Synthesis of aminomethylpyridine analogs. Resin bound **13** was synthesized via the method outlined in Scheme 1. Reagents and conditions: (a) BH_3–THF, 65 °C; (b) *hv* (365 nm), 0.2 M methylamine in MeOH, 50 °C; (c) formaldehyde (37 wt % in H_2O), $Na(OAc)_3BH$, AcOH, DCE, rt; (d) Boc-piperazine, $Pd_2(dba)_3$, dppf, K_2CO_3, DMF, 95 °C; (e) methanesulfonyl chloride, Et_3N, DCM, rt; (f) NaN_3, DMSO, 65 °C; (g) Ph_3P, THF; rt; (h) bromomethyl-*ortho*-nitrophenyl linked Tentagel resin, DMF; (i) 3,4-dichlorobenzoyl chloride, 2,6-lutidine, DCM, rt; (j) TFA, DCM, rt; (k) Boc-piperidinone, $Na(OAc)_3BH$, DCE, rt; (l) TFA, DCM, rt; (m) *p*-chlorobenzaldehyde, $Na(OAc)_3BH$, AcOH, DCE, rt; (n) *hv* (365 nm), 3%TFA/MeOH (v/v), 50 °C.



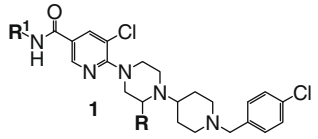
Scheme 4. Synthesis of piperazinyl-piperidine analogs. Resin bound starting compounds were synthesized via the method outlined in Scheme 1. Reagents and conditions: (a) *p*-chlorobenzaldehyde, $Na(OAc)_3BH$, AcOH, DCE, rt; (b) *hv* (365 nm), 3%TFA/MeOH (v/v), 50 °C; (c) Boc-piperazine, $Na(OAc)_3BH$, DCE, rt; (d) TFA, DCM, rt; (e) Boc-piperidinone, $Na(OAc)_3BH$, AcOH, DCE, rt; (f) 3-(Boc-amino)-1-propanal, $Na(OAc)_3BH$, DCE, rt; (g) formaldehyde, $Na(OAc)_3BH$, DCE, rt.

Table 3
Effect of Y substituent on CXCR3 binding affinity



Compds	Y	hCXCR3 binding K_i (nM)
22	Cl	90 ± 30
23	H	7200 ± 550

Table 4
Effect of 2-methyl substitution of the piperidine ring



Compds	R^1	R	hCXCR3 binding K_i (nM)
1l	Methyl	H	560 ± 40
1q	3,4-Dichlorobenzyl	H	35 ± 10
35	3,4-Dichlorobenzyl	R–Me	86 ± 20
36	3,4-Dichlorobenzyl	S–Me	16 ± 2
37	Methyl	S–Me	32 ± 6

nitrogen by replacement of the core piperazine with a piperidine (**24**) was not tolerated. In fact, relocation of the basic nitrogen by the synthesis of the inverted piperidinyl-piperazine analog (**25**) depleted binding affinity. Opening either ring to an acyclic analog (**26**, **27**) also substantially lowered binding activity. Clearly, the pyridinyl piperazinyl-piperidine core was essential for CXCR3 binding activity.

The goal of lowering molecular weight was only achieved after a significant increase in potency was obtained via methyl substitution on the piperazine ring (Table 4).[20] While the 2′-(*R*)-methyl piperazine analog **35** was slightly weaker than **1q**, the 2′-(*S*)-methyl piperazine derivative (**36**) boosted receptor affinity two-fold. More importantly, 2′-(*S*)-methyl piperazine analogs maintained their affinity (32 nM) after truncation of the benzylamide group to a simplified methyl amide (**37**).

In conclusion, high-throughput screening of an encoded combinatorial library uncovered a novel CXCR3 antagonist hit **1a**, with moderate CXCR3 affinity. Initial studies revealed a tight SAR around this lead structure. Ultimately, 2′-(*S*)-methyl piperazine substitution led to **37**, a lead CXCR3 inhibitor with improved affinity and reduced molecular weight. Future communications will further develop the SAR of these CXCR3 antagonists as well as examine their ADME profile and in vivo disease model activity.

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12. In an in vitro chemotaxis assay using a recombinant Ba/F3 cell line expressing hCXCR3 receptor,[13] **1a** exhibited similar IC_{50} values for chemotaxis induced by three ligands: 45 nM (vs 5 nM hIP-10), 20 nM (vs 50 nM hMIG), and 85 nM (vs 5 nM hI-TAC).
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14. A scintillation proximity binding assay was used to measure the affinity of the compound to CXCR3. Ba/F3-CXCR3 membranes (0.5–2 μg per assay) were preincubated for 0.5–20 h with wheat germ agglutinin-coated scintillation proximity assay beads (300 μg per assay). The beads and membranes were incubated in the absence and presence of various concentrations of compound and 25 pM ^{125}I-IP10 in 50 mM Hepes (pH 7.3), 1 mM $CaCl_2$, 5 mM $MgCl_2$, 125 mM NaCl, 0.2% BSA and 1% DMSO. After 1–4 h at room temperature, the membrane bound ^{125}I-IP10 was measured in a Wallac Microbeta. The values are reported as the mean of at least two determinations ± SD.
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18. Analog **21** was synthesized via the route outlined in Scheme 1 starting with 3-chloro-4-fluorobenzoic acid.
19. The des-5-chloropyridyl analog **23** was synthesized via the solid-phase route described in Scheme 1 starting with 6-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)nicotinic acid which was synthesized by palladium-catalyzed amination ($Pd(OAc)_2$, dppf, Cs_2CO_3, 1,4-dioxane, 100 °C) of methyl 6-chloronicotinate with Boc-piperazine.
20. Compounds in Table 4 were synthesized via the route outlined in Scheme 1 starting with Boc-protected *R* and *S* 2-methylpiperazine.