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III. Identification of novel CXCR3 chemokine receptor antagonists with a pyrazinyl–piperazinyl–piperidine scaffold

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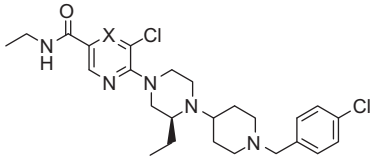
ABSTRACT

The SAR of a novel pyrazinyl–piperazinyl–piperidine scaffold with CXCR3 receptor antagonist activity was explored. Optimization of the DMPK profile and reduction of hERG inhibition is described. Compound **16e** with single-digit CXCR3 affinity, good rat PK and hERG profiles has been identified as a lead for further study.

Chemokines are a family of cytokines that are produced during inflammation and are able to induce chemotaxis of immune cells.[1] CXCR3 is a chemokine receptor that binds CXCL10 (IP-10), CXCL9 (MIG), and CXCL11 (I-TAC) ligands to facilitate the recruitment of Th1 cells to the site of inflammation.[2] CXCR3 has been implicated in inflammatory diseases (psoriasis and inflammatory bowel disease), autoimmune diseases (multiple sclerosis and rheumatoid arthritis), type I diabetes, and acute cardiac allograft rejection. Therefore small molecule antagonists of the CXCR3 receptor have attracted significant interest and may provide therapeutic tools for the treatments of such diseases.[3]

Compounds exemplified by **1** (Fig. 1) have been reported as potent CXCR3 antagonists.[4] However, compounds in this series exhibited only modest rat PK and undesirable hERG K^+ channel activity in a Rb efflux assay. As part of the efforts to identify a new chemotype, the pyridine ring of **1** has been replaced by a pyrazine (**2**). The systematic SAR around the core motif including variations on pyrazine, piperidine, and piperazine rings to generate compounds with good CXCR3 activity as well as improved hERG and PK profiles are described.

The initial efforts were focused on 6-amino-3-chloropyrazine scaffold **8** which was derived from the reported optimization on 2-(*S*)-ethyl piperazinyl–piperidine fragment.[4b] Preparation of the



1 (X = CH)
hCXCR3 IC_{50} = 0.3 nM
rat AUC = 0.4 μM·h

2 (X = N)

Figure 1. Lead compound in pyridine core series.

compounds listed in Tables 1 and 2 was accomplished by the method outlined in Scheme 1. The 2-chlorine of commercially available pyrazine **3** was regioselectively displaced with (*S*)(+)-2-ethylpiperazine in the presence of a Pd-catalyst. Subsequent reductive amination and deprotection gave key tricyclic amine intermediate **5**, which reacted with aldehydes or acid chlorides to afford the benzyl amine and amide derivatives (**6**), respectively. Modification of the pyrazine amide (R^1) was accomplished by conversion of the methyl ester **6** or the acid **7** with amines to afford compounds **8a–r**.

Table 1 summarizes the selected SAR of 5-carboxamide analogs with variations on R^2. Many of the carboxamide derivatives exhibited sub-nanomolar human CXCR3 binding affinity. Primary amides displayed good to excellent activities when R^2 was a benzylic aromatic derivative (**8a–d**) whereas the corresponding amide analog (**8e**) exhibited weaker binding affinity. Fixing the R^2 substituent as a 4-chlorobenzyl, the SAR at R^1 was explored. Alkyl and

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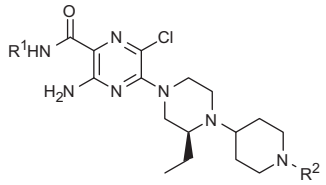
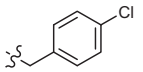
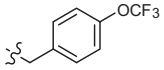
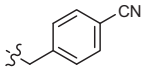
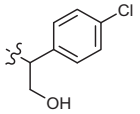
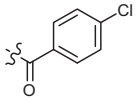
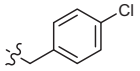
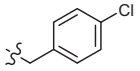
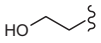
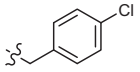
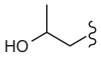
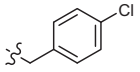
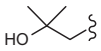
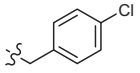
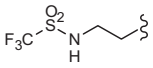
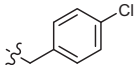
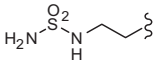
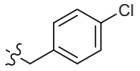
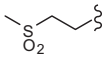
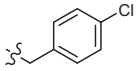
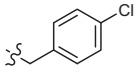
Table 1
Binding affinity of 6-amino-3-chloropyrazine-5-carboxamides



Compd	R^1	R^2	hCXCR3 IC_{50}[a], nM
8a	H		0.8
8b	H		3.7
8c	H		8.5
8d[b]	H		1.9
8e	H		76
8f	Me		0.3
8g	CF_3CH_2		0.8
8h			0.4
8i			0.3
8j			8.4
8k			0.9
8l			1.9
8m			6.4
8n			1.5

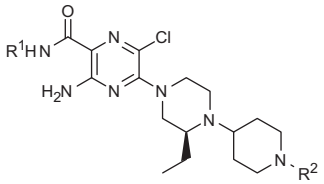
[a] See reference 10.
[b] Single diastereomer.

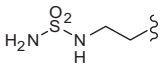
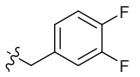
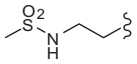
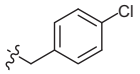
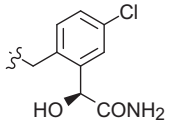
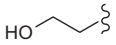
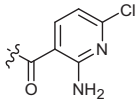
hydroxyalkyl amides (**8f–i**) maintained good CXCR3 affinity whereas the tertiary hydroxyl analog (**8j**) displayed moderate activity. Polar groups such as sulfones (**8k–m**) and lactone (**8n**) maintained moderate to good CXCR3 affinities as well. Although we identified several compounds with sub-nanomolar CXCR3 activity, many compounds including **8a**[5] (rat AUC = 5.1 μM·h) were found to have undesired hERG K^+ channel inhibition (80–90% inhibition measured by Rb efflux assay).[6]

Our strategy to improve the hERG profile was to further explore polar substituents[7] in R^1 and add polar groups to R^2 (Table 2). Sulfonamide substitution at R^1 did suppress hERG inhibition and maintained moderate binding affinity (**8o and 8p**). Compound (**8q**) which contains primary amide on the R^2 aryl moiety also improved the hERG profile and maintained good binding affinity. A similar result was obtained with the 6-amino-2-chloropyridine-5-carboxamide[8] derivative (**8r**). In addition to the hERG improvement, compound **8r** also showed good rat exposure (AUC = 5.9 μM·h) in comparison with the analogs in Table 1. Based upon the profile of **8r** R^2 was fixed as the 6-amino-2-chloropyridine-5-carboxamide while attention was concentrated toward optimization of other areas of the molecule.

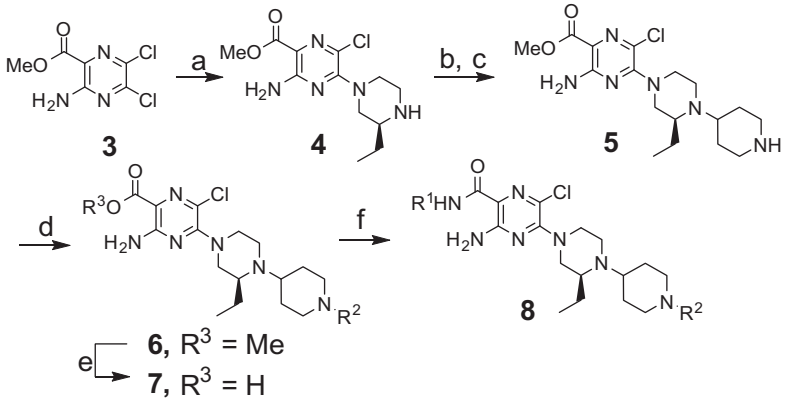
As the previous pyridine series[4] demonstrated good binding to the receptor without the amino motif on pyrazine 6-position, we next investigated the effect of different R^1 groups with elimination

Table 2
Binding affinity and hERG inhibition of 6-amino-3-chloropyrazine-5-carboxamides



Compd	R^1	R^2	hERG[a]%	hCXCR3 IC_{50}[b], nM
8o			4	9
8p			39	4.4
8q	H		14	4.1
8r			17	3.4

[a] % Inhibition measured by Rb efflux assay at 10 μM,
[b] See reference 10.



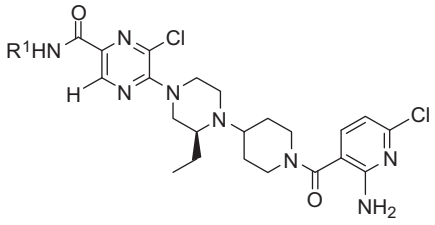
Scheme 1. Reagents and conditions: (a) (*S*)(+)-2-ethylpiperazine, $Pd(OAc)_2$, Cs_2CO_3, DBPD, dioxane, 90 °C; (b) *N*-Boc-piperidin-4-one, $Ti(OiPr)_4$, $NaBH_3CN$, 1,2-dichloroethane, 60 °C; (c) TFA, CH_2Cl_2, RT; (d) ArCHO, $NaBH(OAc)_3$, 1,2-dichloroethane, or ArCOCl, TEA, THF, or $ArCO_2H$, EDCI, HOBt, DMF, RT; (e) LiOH, THF-MeOH, RT; (f) with **6**, R^1NH_2, MeOH, 60–80 °C, with **7**, R^1NH_2, EDCI, HOBt, DMF-THF, RT.

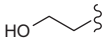
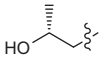
of the pyrazine amino group (Table 3). The synthesis of des-6-aminopyrazine is described in Scheme 2. The diazonium salt of aminopyrazine **4** was converted to the bromide **9** followed by hydrogenolysis to provide the des-amino intermediate **10**. Subsequent structural manipulation as in Scheme 1[9] afforded compounds **11a–f**.

As shown in Table 3, primary and alkyl amides (**11a–f**) exhibited similar CXCR3 affinity and maintained good hERG profile with exception of isopropyl amide (**11d**). While the elimination of 6-amino motif of pyrazine produced several good compounds, no significant improvements on CXCR3 affinity and hERG profile were achieved as illustrated in direct comparison of the compound **11e** to **8r**. These results demonstrated that the 6-amino motif was not necessary for the positive ligand-protein interactions while eliminating it may increase the liability of 3-chlorine of pyrazine.

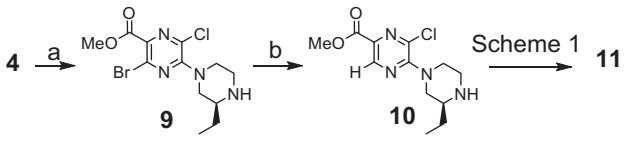
Next we investigated the SAR with variations at R^1 and R^4 to replace the 3-chlorine with a more stable surrogate. The trifluoromethyl compounds were synthesized by the methods described in Scheme 3. The bromide **13** obtained from **12** via NBS bromination was subjected to trifluoromethylation conditions[8] affording **14** ($R^4 = CF_3$). Following the routes described in Scheme 1 afforded compound **15a–c**. The trifluoromethyl analogs (**15a–c**, Table 4) were well tolerated with significantly improved hERG profiles with the exception of cyclopropyl amide (**15c**). In addition to the good affinity and hERG, compounds **15a** and **15b** also displayed good rat PK profiles. The methyl analogs, **15d–f**, were prepared by the methods described in Scheme 3 in which bromopyrazine **13** was converted to the methylated compound **14** (R^4 = Me) via Suzuki coupling with methylboronic acid. Replacement of trifluoromethyl by methyl group was well tolerated. Primary and methyl amides (**15d** and **15e**) had similar CXCR3 affinities and hERG profiles to the corresponding trifluoromethyl analogs, however, the rat PK profiles were significantly improved by the methyl replacement. The cyclopropyl amide (**15f**) demonstrated significant improvements in rat PK and hERG profiles while maintaining the similar potency as compared to **15c**. The maintained 3-methyl substitution unreactive the hERG

Table 3
Binding affinity and hERG inhibition of 6-desamino-3-chloropyrazine-5-carboxamides



Compd	R^1	hERG[a]%	hCXCR3 IC_{50}[b], nM
11a	H	7	2.5
11b		13	1.5
11c		19	1.2
11d		56	0.9
11e		11	2.9
11f		17	2.9

[a] % Inhibition measured by Rb efflux assay at 10 μM.
[b] See reference 10.



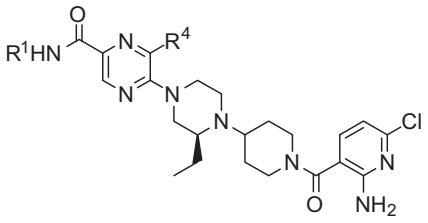
Scheme 2. Reagents and conditions: (a) Br_2, NaONO, 48% HBr, AcOH, 0 °C; (b) H_2 (1 atm), 5% Pd/C, THF, RT.

profile of the 3-chloro analogs and compounds with good overall profile examplified by **15d** have been identified.

After optimization of pyrazine ring, we revisited the investigation of the 4-chlorobenzamide at R^2 to address the potential reactivity of 6-amino-2-chloropyridine motif. Table 5 summarizes the selected 4-chlorobenzamide analogs with variations at R^1. The primary amide **15g** displayed fourfold reduced CXCR3 affinity as compared to **15d** demonstrating the effect of 6-amino-2-chloropyridine motif on the affinity. However, if compared to the compound with same R^2 exemplified by **8e** (Table 1), **15g** showed significantly improved binding affinity while maintaining good hERG profile. Increasing the size of R^1 alkyl improved affinity significantly as illustrated with **15g–j**, but compromised the hERG profile. Particularly the cyclopropyl amide analog **15j** displayed excellent combination of CXCR3 activity and rat PK (AUC = 8.4 vs 2.9 μM·h of **15i**) and was selected for further optimization to improve the hERG profile.

Finally, our efforts were focused on exploration of substitutions of the piperazine ring. Based on our previous findings in pyridine series[4b] small alkyl groups (R^6) were explored with variations of

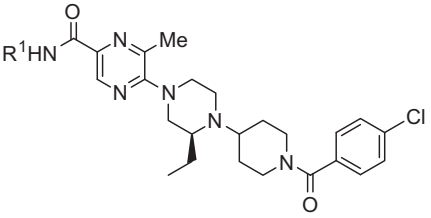
Table 4
Effects of pyrazine C3-substituents of 6-amino-2-chloropyridine amide derivatives



Compd	R^1	R^4	hERG[a]%	hCXCR3 IC_{50}[b], nM	AUC[c] μM·h
15a	H	CF_3	0	1.9	15.3
15b	Me	CF_3	0	1.5	6.4
15c		CF_3	53	1.8	3.6
15d	H	Me	0	4.0	30.2
15e	Me	Me	5	2.4	12.1
15f		Me	16	1.9	22.0

[a] % Inhibition measured by Rb efflux assay at 10 μM.
[b] See reference 10.
[c] Rat PK (10 mpk, po, 6 h, MC).

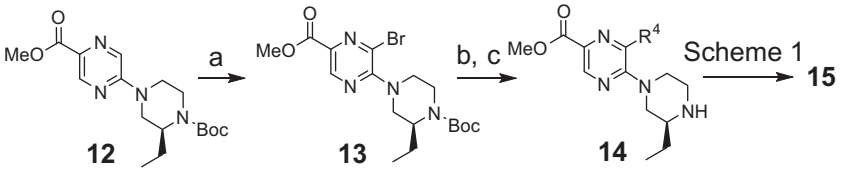
Table 5
Effects of pyrazine C3-substituents of 4-chlorobenzamide derivatives



Compd	R^1	hERG[a] %	hCXCR3 IC_{50}[b], nM
15g	H	6	18
15h	Me	41	3.6
15i	Et	44	2.0
15j		74	1.1

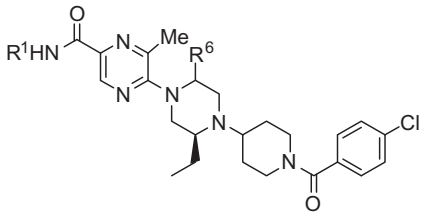
[a] % Inhibition measured by Rb efflux assay at 10 μM
[b] See reference 10.

R^1 to improve hERG profile without compromising binding affinity and PK profiles. First, primary amides of 2-ethyl-5-alkyl-piperazines[11,12] were tested and were tolerated when R^6 was (*R*)-Me as summarized in Table 6 (**16a–c**). Compound **16a** also maintained good hERG profile. Thus, the 2-ethyl-5-(*R*)-methyl-piperazine scaffold was incorporated with other R^1 analogs (**16d** and **16e**) and the cyclopropyl amide **16e** showed significant improvement of hERG profile while maintaining good CXCR3 affinity as compared to **15j**. Furthermore compound **16e** displayed good PK profile as well (rat AUC 9.8 μM·h at 10 mpk, po, 6 h, MC).



Scheme 3. Reagents and conditions: (a) NBS, DMF, RT; (b) for R^4 = CF_3, $ClCF_2CO_2Me$, KF, CuI, DMF, 110 °C, sealed tube, for R^4 = Me, $MeB(OH)_2$, K_2CO_3, $Pd(PPh_3)_2Cl_2$, DMF, 85 °C; (c) TFA, CH_2Cl_2, RT.

Table 6
Effects of 5-alkyl substitutions of piperazine ring



Compd	R^1	R^6	hERG[a] %	hCXCR3 IC_{50}[b], nM
16a	H	(*R*)-Me	9	5.4
16b	H	(*R*)-Et	13	235
16c	H	(*S*)-Me	na	>1000
16d	Me	(*R*)-Me	30	4.2
16e		(*R*)-Me	7	1.3

[a] % Inhibition measured by Rb efflux assay at 10 μM.
[b] See reference 10.

In summary, we have discovered a new chemotype, pyrazinyl–piperazinyl–piperidine, as a potent CXCR3 antagonist. Extensive SAR around the core, R^1, and R^2 fragments of the initial lead **8a** addressed the undesired hERG inhibition and identified compound **16e** which exhibited good human CXCR3 activity and improved hERG profile. Particularly the pyrazine core compound **16e** demonstrated significantly improved rat exposure as compared to pyridine core compound **1** (Fig. 1). Further optimization of these analogs will be reported in due course.

References and notes

1. (a) Liu, L.; Callahan, M. K.; Huang, D.; Ransohoff, R. M. *Curr. Top. Dev. Biol.* **2005**, *68*, 149; (b) Turner, J. E.; Steinmetz, O. M.; Stahl, R. A.; Panzer, U. *Mini-Rev. Med. Chem.* **2007**, *7*, 1089.
2. (a) Loetscher, M. et al *J. Exp. Med.* **1996**, *184*, 963; (b) Cole, K. E. et al *J. Exp. Med.* **1998**, *187*, 2009; (c) Szczuciński, A.; Losy, J. *Acta Neurol. Scand.* **2007**, *115*, 137.
3. For reviews of small molecule CXCR3 antagonists see: (a) Wijtmans, M.; Verzijl, D.; Leurs, R.; de Esch, I. J. P.; Smit, M. J. *Chem. Med. Chem.* **2008**, *3*, 861; (b) Collins, T. L.; Johnson, M. G.; Medina, J. C. *Chem. Biol. Basic Res. Clin. Appl.* **2007**, *2*, 79; (c) Medina, J. C.; Johnson, M. G.; Collins, T. L. *Annu. Rep. Med. Chem.* **2005**, *40*, 215.
4. (a) McGuinness, B. F.; Carroll, C. D.; Zawacki, L. G.; Dong, Z.; Yang, C.; Hobbs, D. W.; Biji, J.-S.; Hall, J. W., III; Jenh, C.-H.; Kozlowski, J. A.; Anilkumar, G. N.; Rosenblum, S. B. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5205; (b) Shao, Y.; Anilkumar, G. N.; Carroll, C. D.; Dong, G.; Hall, J. W., III; Hobbs, D. W.; Jiang, Y.; Jenh, C.-H.; Kim, S. H.; Kozlowski, J. A.; McGuinness, B. F.; Rosenblum, S. B.; Schulman, I.; Shih, N.-Y.; Shu, Y.; Wong, M. K. C.; Yu, W.; Zawacki, L. G.; Zeng, Q. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1527.
5. Compound **8a** has been selected for the proof-of-concept (POC) evaluation in a rat EAE model. Jenh, C.-H., et al., manuscript in preparation.
6. Vaz, R. J.; Li, Y.; Rampe, D. *Prog. Med. Chem.* **2005**, *43*, 1.
7. Blum, C. A.; Zheng, X.; De Lombaert, S. *J. Med. Chem.* **2004**, *47*, 2318.
8. Zeng, Q.; Yang, D.-Y.; Rosenblum, S. B.; Wong, M. K. C.; Anilkumar, G.; Kim, S. H.; Yu, W.; Kozlowski, J. A.; Shih, N.-Y.; McGuinness, B. F.; Zawacki, L. G.; Hobbs, D. W. WO Patent 2006/091428.
9. R^2 was installed under amide coupling condition (EDCI, HOBt, DMF, RT) with sodium 6-amino-2-chloropyridine carboxylate.[8] R^1 variations were performed using the conditions described in Scheme 1.
10. The CXCR3 binding affinity was measured by displacement of ^{125}I-human CXCL10 from recombinant Ba/F3 cells expressing human CXCR3. IC_{50}'s are the mean values (n = 2) with SD of ~13%. For reference see: Jenh, C.-H.; Cox, M. A.; Hipkin, W.; Lu, T.; Pugliese-Sivo, C.; Gonsiorek, W.; Chou, C.-C.; Narula, S. K.; Zavodny, P. J. *Cytokine* **2001**, *15*, 113.
11. 1-Boc-2(*S*)-ethyl-5(*R*)-methylpiperazine and 1-Boc-2(*S*)-ethyl-5(*S*)-methylpiperazine were prepared by the method described in reference 8.
12. 2(*S*)-Ethyl-5(*R*)-ethylpiperazine was purchased from the commercial source.