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Design and optimization of imidazole derivatives as potent CXCR3 antagonists

Xiaohui Du, Xiaoqi Chen, Jeffrey T. Mihalic, Jeffrey Deignan, Jason Duquette, An-Rong Li, Bryan Lemon, Ji Ma, Shichang Miao, Karen Ebsworth, Timothy J. Sullivan, George Tonn, Tassie L. Collins and Julio C. Medina*

Amgen Inc. 1120 Veterans Boulevard, South San Francisco, CA 94080, USA

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Abstract—A series of imidazole derivatives have been designed and optimized for CXCR3 antagonism, pharmacokinetic properties, and reduced formation of glutathione conjugates. Our efforts led to the discovery of potent CXCR3 antagonists with good pharmacokinetic properties. These compounds are useful tools for in vivo studies of CXCR3 function.

The CXCR3 chemokine receptor and its ligands, Mig (CXCL9), IP10 (CXCL10), and ITAC (CXCL11), are believed to play major roles in the recruitment of Type-1 T helper (Th1) cells from the peripheral blood into inflamed tissues, thus aggravating the inflammatory response.[1] This hypothesis is supported by the increased numbers of infiltrating cells that express CXCR3 and the increased levels of Mig, IP10, and ITAC observed in disease tissue from patients suffering from inflammatory bowel disease,[2] multiple sclerosis,[3] psoriasis,[4] rheumatoid arthritis,[5] and solid organ transplant rejection.[6] Further support for the key role that CXCR3 plays in mediating immune disorders comes from evaluation of CXCR3 deficient mice in cardiac allograft experiments where the CXCR3 knock-out mice show increased allograft tolerance when compared to wild-type mice.[7] Likewise, transplant experiments with antibodies to the CXCR3 ligands, Mig and IP10, enhance allograft survival.[7] Furthermore, cardiac allografts from mice lacking IP10 show prolonged allograft survival time when transplanted into a wild-type mice.[8] Additional reports of in vivo transplant models of lung, small bowel, and islet further validate the key role that CXCR3 plays in cellular recruitment and allograft survival.[9] Therefore, there is great interest in discovering small molecule CXCR3 antagonists[1a,10a–d] for the treatment of autoimmune and inflammatory diseases.

We have previously demonstrated[11] that 2,3-substituted-quinazolin-4-one derivatives such as compound **1** are potent and selective CXCR3 antagonists. We also reported that the quinazolin-4-one core could be replaced by several bicyclic–heterocyclic ring systems.[12]

Our previous work demonstrated that significant modifications of the quinazolinone core moiety in **1** are toler-

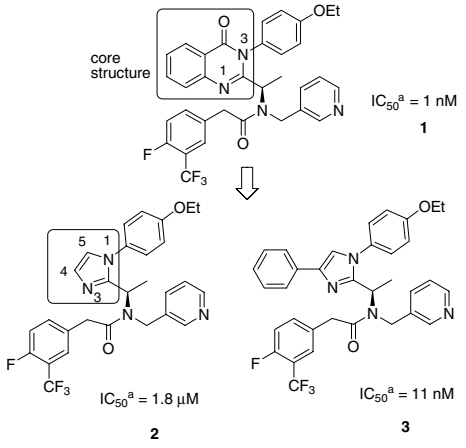

Figure 1. Imidazole derivatives afford potent CXCR3 antagonists. [a]IC_{50} for ^{125}I-IP10 ligand displacement in buffer.

Keywords: CXCR3; IP10; ITAC; Mig; CXCL9; CXCL10; CXCL11; Imidazole; Chemokine.
* Corresponding author. Tel.: +1 6502442425; fax: +1 6502442015; e-mail: medinaj@amgen.com

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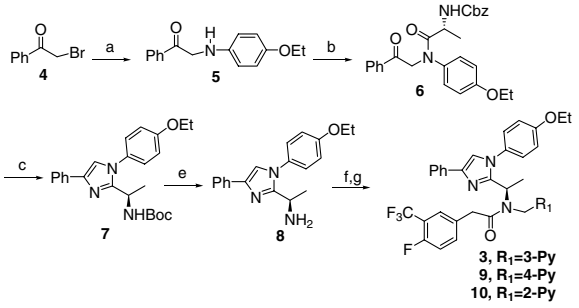
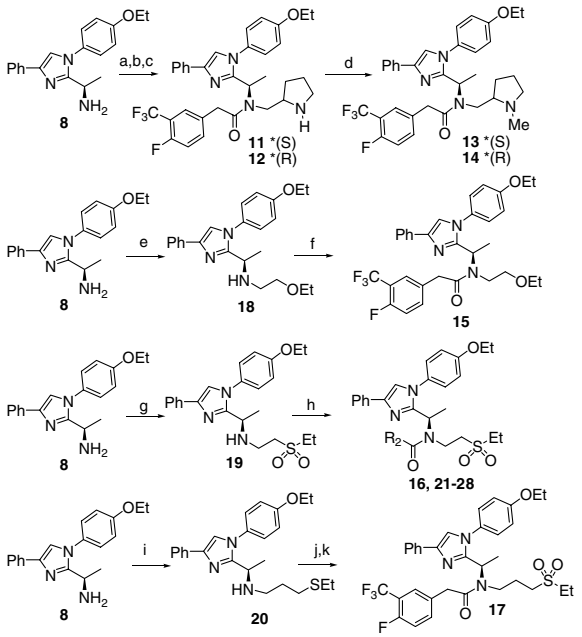
ated and we postulated that the main role of this moiety is to hold the peripheral groups in the correct orientation. We hypothesized that an imidazole ring system would be a good replacement for the quinazolinone core since the imidazole ring system contains nitrogens in a 1,3-relationship similar to the quinazolinone core structure. The resulting compound **2**, however, displayed significant loss of CXCR3 affinity compared to quinazolinone **1**. It was postulated that adding a lipophilic moiety resembling the rest of the phenyl moiety on the quinazolinone core was necessary. Indeed, addition of a phenyl group at the 4-position of the imidazole ring (**3**) significantly increased the compound affinity for the CXCR3 receptor, indicating that a lipophilic moiety connected to the imidazole core structure was preferred for increased potency (Fig. 1). In this article, we report the systematic optimization of the imidazole-derived CXCR3 antagonists that led to compounds[13] with significantly improved potency and good pharmacokinetic properties. The affinity of these compounds for the CXCR3 receptor was evaluated using an ^{125}I-IP10 ligand displacement assay to IL-2 activated human peripheral blood mononuclear cells (PBMC) in the presence or absence of human plasma.[14] Furthermore, the antagonistic activity of these compounds was studied using an ITAC-induced cellular migration assay of PBMC.[14]

Previous work performed on the related quinazolinone series suggested that optimization of the peripheral groups surrounding the heterocyclic core could further increase affinity for the receptor. Therefore, a series of imidazole derivatives with *N*-alkyl modifications was prepared as single enantiomers (Schemes 1 and 2). Alkylation of 4-ethoxyaniline with 2-bromo-1-phenylethanone **4** generated 2-(4-ethoxyphenylamino)-1-phenylethanone **5**. Subsequent coupling with the mixed anhydride of Boc-protected D-alanine and cyclization of compound **6** with ammonium acetate furnished imidazole **7**. Deprotection of the Boc group led to amine **8**. Compound **8** was a common intermediate that led to various CXCR3 antagonists in Tables 1 and 2. Compounds **3**, **9**, and **10** were prepared through reductive amination of **8** and coupling with 4-fluoro-3-trifluor-



Scheme 1. Reagents and conditions: (a) 4-ethoxyaniline, Et_2O, 16 h, 67%; (b) BOC-D-alanine, isobutylchloroformate, NMM, DCM, −20 °C to rt, then **5**, 92%; (c) NH_4OAc, HOAc, 90 °C, 2 h, 96%; (d) TFA, DCM, 100%; (f) various pyridyl aldehyde, $NaBH_3CN$, HOAc, DCE; (g) 4-fluoro-3-trifluoromethylphenylacetic acid, EDC, HOBt, NMM, DMF. Yields for steps f and g combined: **3**: 48%; **9**: 47%; **10**: 9%.

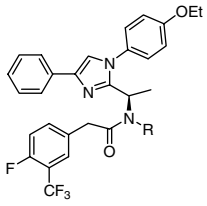


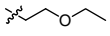
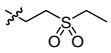
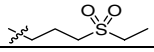
Scheme 2. Reagents and conditions: (a) *tert*-butyl 2-formylpyrrolidine-1-carboxylate, $NaBH_3CN$, HOAc, DCE; (b) 4-fluoro-3-trifluoromethylphenylacetic acid, EDC, HOBt, NMM, DMF; (c) TFA, DCM, **11**: 27% for 3 steps; **12**: 62% for 3 steps; (d) formaldehyde, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, **13**: 100%; **14**: 82%; (e) $BrCH_2CH_2OEt$, DMF, K_2CO_3, NaI, 80 °C, 42%; (f) trifluoromethoxyphenylacetic acid, EDC, HOBt, NMM, DMF, 83%; (g) ethyl vinyl sulfone, Et_3N, MeOH, 50 °C, 18 h, 52%; (h) for **16**: 4-trifluoromethoxyphenylacetic acid, EDC, HOBt, NMM, DMF, 96%; for **21**–**28**: various phenylacetic acid R_2CH_2COOH, BOPCl, Et_3N, THF, 29–94%; (i) 3-(ethylthio)propanal, $NaBH_3CN$, HOAc, DCM, 48%; (j) 4-trifluoromethoxyphenylacetic acid, EDC, HOBt, NMM, DMF, 81%; (k) MCPBA, DCM, 72%.

ophenylacetic acid. The syntheses of compounds **11**–**14** from compound **8** involved additional manipulations of the pyrrolidine moiety. Deprotection of the Boc group gave compounds **11** and **12**, while introduction of a methyl group via reduction amination gave **13** and **14**. Alkylation of compound **8** with 1-bromo-2-ethoxyethane led to amine **18**. Standard amide coupling of **18** generated antagonist **15**. Michael addition of compound **8** with ethyl vinyl sulfone followed by coupling with phenylacetic acid furnished compound **16**. With a different coupling reagent, BOPCl, compounds **21**–**28** can be prepared from the same intermediate **19**. A propyl sulfone side chain could be installed through reductive amination of compound **8** with 3-(ethylthio)propanal, amide coupling, and oxidation of the thio ether to sulfone **17**. With minor modifications, this route was efficient and reproducible in preparing compounds **29**–**34** with substitutions at the 4-position of the imidazole ring. The enantiomeric purity of the final compounds was assessed by chiral HPLC.[15]

In addition, a series of cyclopropyl-substituted imidazole derivatives were prepared as described in Scheme 3. Iodination at the 5-position of the imidazole ring of antagonist **32** resulted in iodide **35**, which was converted to compound **36** via Stille coupling. Saturation of the double bond in **36** led to compound **37**. On the other hand, ozonolysis of the alkene moiety in **36** and reduc-

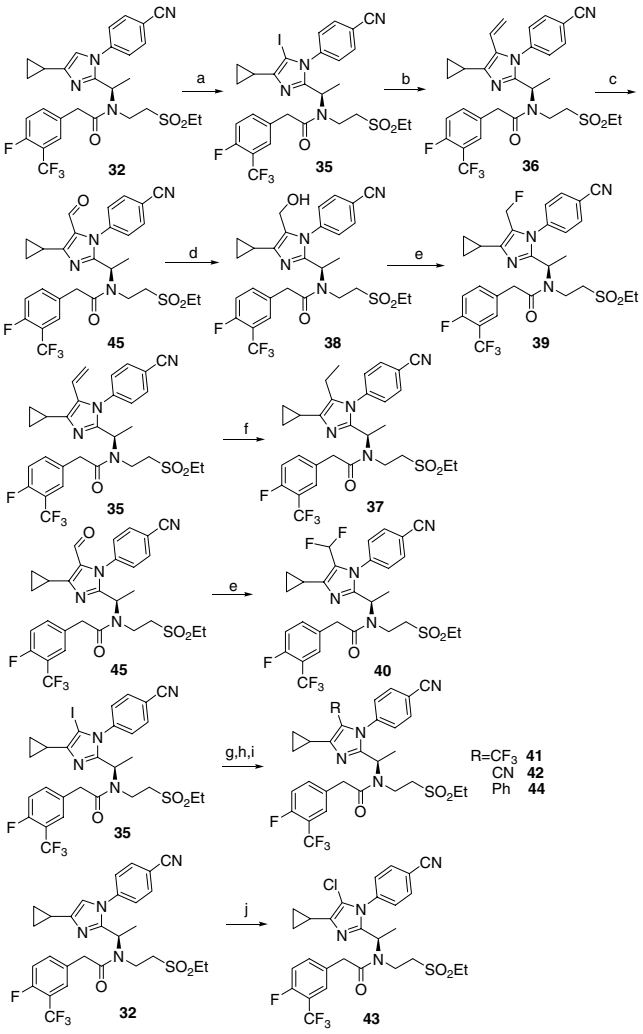
Table 1. SAR of amide N-substituents



Compound	R Group	^{125}I-IP10 binding	
		Buffer[a,b] (nM)	Plasma[a,b] (nM)
3		11	91
9		35	129
10		80	1179
11		46	36
12		11	20
13		14	81
14		6.4	31
15		11	202
16		0.4	4.5
17		2.4	18

[a] Values are means of three experiments. Standard deviations are ±30%.

[b] Displacement of ^{125}I-labeled IP10 from the CXCR3 receptor expressed on PBMC. See Ref. 14 for assay protocol.



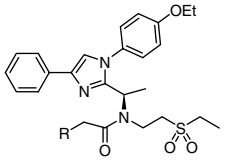
Scheme 3. Synthesis of 4-substituted imidazole analogs. Reagents and conditions: (a) NIS, CH_2Cl_2, 95%; (b) *n*-$Bu_3SnCHCH_2$, $Pd(PPh_3)_4$, toluene, 79%; (c) O_3, CH_2Cl_2–MeOH, −78 °C, then Me_2S, 31%; (d) $NaBH_4$, MeOH, 60%; (e) Deoxo-Fluor, CH_2Cl_2, 81% for **39** and 40% for **40**; (f) H_2, 10% Pd/C, 90%; (g) **41**: FSO_2CF_2COOMe, CuBr, $PdCl_2$, 150 °C, μW, 55%; (h) **42**: CuCN, DMF, 200 °C, μW, 60%; (i) **44**: Bu_3SnPh, $PdCl_2(PPh_3)_2$, 180 °C, μW, 78%; (j) SO_2Cl_2, $ClCH_2CH_2Cl$, 35%.

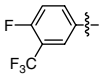
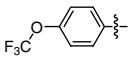
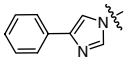
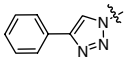
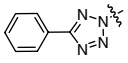
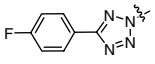
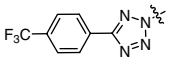
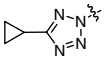
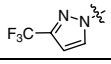
tion of the resulting aldehyde **45** generated alcohol **38**. Fluorination of **38** and **45** generated compounds **39** and **40**. A few additional antagonists could be prepared from iodide **35**, such as trifluoromethyl-substituted compound **41**, compound **42** through cyanation, and compound **44** through Stille coupling. Chlorination of **32** afforded compound **43**.

The compounds in Table 1 demonstrated that a variety of substitutions at the amide-*N*-alkyl moiety were well tolerated. It was interesting to observe that, while a 3-pyridyl moiety was preferred for CXCR3 activity over the 2- or 4-substituted pyridine derivatives, the fact that all regioisomers had significant activity suggested that the position of the nitrogen atom is not critical for activity (**3**, **9**, and **10**). Furthermore, it was observed that amines (**11**–**14**) and alkoxy groups (**15**) also had good potency which suggested that this site tolerated a variety of polar groups. It was also noted that, the potency of some of the antagonists did not shift much in the presence of plasma in the ^{125}I-IP10 binding assay such as compounds **11** and **12**, while the potency of the ethoxyethyl-substituted compound **15** decreased about 20-fold in the presence of plasma. In addition, sulfone groups (**16** and **17**) were identified as potent replacements of the 3-pyridyl moiety. Subsequent studies were carried out by using the ethyl sulfone of **16** as the amide N-substituents.

In an attempt to identify more polar molecules, the phenylacetic acid side chain was replaced with a variety of bicyclic side chains, such as imidazole-phenyl, triazole-phenyl, and tetrazole-phenyl (Table 2). Unfortunately, these were uniformly less potent than the parent **16**, although most only by an order of magnitude (**22**–**26**). The bicyclic replacements with smaller lipo-

Table 2. Replacing amide side chain with polar groups led to potent CXCR3 antagonists



Compound	R Group	^{125}I-IP10 binding	
		Buffer[a,b] (nM)	Plasma[a,b] (nM)
16		0.4	4.5
21		7.1	45
22		4.8	24
23		5.6	49
24		9.4	69
25		5	47
26		2.1	19
27		44	1659
28		40	1124

[a] Values are means of three experiments. Standard deviations are ±30%.

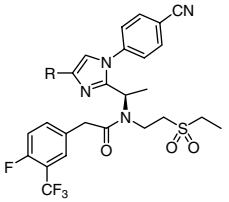
[b] Displacement of ^{125}I-labeled IP10 from the CXCR3 receptor expressed on PBMC. See Ref. 14 for assay protocol.

philic groups such as cyclopropyl **27** or trifluoromethyl **28** all resulted in loss of potency.

Metabolism studies of the 4-ethoxy-phenyl-substituted analogs with human liver microsomes demonstrated the formation of phenolic metabolites that are time-dependent inhibitors of CYP3A4. Therefore, replacement of the ethoxy group was desired. Based on previous SAR studies in the quinazolinone series,[11] the cyano group was found to be a suitable replacement of the ethoxy group. Compound **29** had an IC_{50} of 0.7 nM in the ^{125}I-IP10 binding assay in buffer compared to 0.4 nM of **16**. Therefore, the cyano group was used in the design of subsequent compounds.

Exploration of substitutions at the 4-position of the imidazole ring demonstrated that replacement of the phenyl moiety by pyridyl (**30**–**31**) or a small alkyl group (**32**–**34**) resulted in reduced activity (Table 3). However, evaluation of the pharmacokinetic properties of **29** and **32** (Table 4) demonstrated that **29** was cleared faster than **32** following intravenous administration in rats. Furthermore, **32** had significantly improved solubility and permeability compared to **29**. Therefore, it was decided to study further analogs of **32** bearing a cyclopropyl substituent at the 4-position of imidazole.

Table 3. Effect of substitutions at the 4-position of the imidazole ring



Compound	R Group	^{125}I-IP10 binding		ITAC plasma migration[a,c] (nM)
		Buffer[a,b] (nM)	Plasma[a,b] (nM)	
29		0.7	14	10
30		2.3	29	91
31		4.0	43	33
32		6.6	37	53
33		2.6	19	54
34		2.5	21	100

[a] Values are means of three experiments. Standard deviations are ±30%.

[b] Displacement of ^{125}I-labeled IP10 from the CXCR3 receptor expressed on PBMC. See Ref. 14 for assay protocol.

[c] One hundred nanograms per milliliter of ITAC was used in the presence of 100% human plasma. See Ref. 14 for assay protocol.

Table 4. Pharmacokinetic profiles in rat and physical properties of the CN-substituted antagonists **29** and **32**

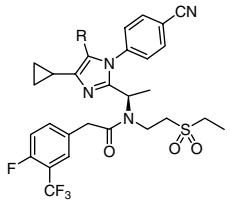
	Compound	**29**	**32**
IV[a]	CL (L/h/kg)	5.5	3.3
	AUC (μg h/L)	92	149
	MRT (h)	1.0	0.3
PO[a]	AUC (μg h/L)	11	95
	MRT (h)	0.9	2.4
	F (%)	3	16
Solubility[b] (μg/mL)		0.5	32
Permeability[c] (10^{-6} cm/s)		0.9	5.1

[a] IV dose: 0.5 mg/kg; PO dose: 2.0 mg/kg. In all cases: $n = 2$.

[b] The solubility assay measures a compound's concentration in the supernatant after a 10 mM DMSO stock solution is added into the assay buffer pH 7.4 with the final DMSO concentration ~1%.

[c] Permeability assay is based on the PAMPA technology (parallel artificial membrane permeability assay) using a phospholipid lipid bilayer. It measures the passive diffusion of the compound across the lipid bilayer.

Table 5. The effect of substitutions at the 5-position of the imidazole ring



Compound	R	^{125}I-IP10 binding		ITAC plasma migration[a,c] (nM)	GSH adduct percentage (%)
		Buffer[a,b] (nM)	Plasma[a,b] (nM)		
32	–H	6.6	37	53	44
36	–$CHCH_2$	3.8	22	45	8
37	–Et	3.2	16	29	14
38	–CH_2OH	10	33	113	1
39	–CH_2F	8	28	119	N.D.[d]
40	–CHF_2	12	18	20	0
41	–CF_3	19	45	268	0
42	–CN	72	276	N.D.[d]	0
43	–Cl	7.8	24	80	2
44	–Ph	752	10,000	N.D.[d]	N.D.[d]

[a] Values are means of three experiments.
[b] Displacement of ^{125}I-labeled IP10 from the CXCR3 receptor expressed on PBMC. See Ref. 14 for assay protocol.
[c] One hundred nanograms per milliliter of ITAC was used in the presence of 100% human plasma. See Ref. 14 for assay protocol.
[d] N.D. = not determined.

Metabolic studies with **32** using human liver microsome indicated a significant amount of glutathione (GSH) conjugates formed primarily at the imidazole ring. Therefore, a number of analogs of **32** with additional substitution at the 5-position of the imidazole ring were synthesized and studied with the aim of reducing metabolic activation and improving metabolic stability.

Small alkyl (**36**, **37**, and **40**) and chloro (**43**) substituents at the 5-position of the imidazole core maintained affinity for the CXCR3 receptor (Table 5). However, some loss in potency was observed when a strong electron-withdrawing group such as a trifluoromethyl group (**41**) and a cyano group (**42**) or a bulkier phenyl group (**44**) was added to the this position. It was also noted that substitutions at the 5-position of the imidazole ring significantly decreased the formation of GSH conjugates. Electron-withdrawing groups such as difluoromethyl (**40**), trifluoromethyl (**41**), cyano (**42**), chloro (**43**) group substitution completely prevented the formation of such adducts.

Compounds **40** with a difluoromethyl substituent and **43** with a chloro substituent not only maintained potency against the CXCR3 receptor and displayed decreased formation of GSH conjugates, but also had improved pharmacokinetic properties when compared to the parental compound **32** (Table 6).

In conclusion, we have discovered that the quinazolinone ring in our first-generation series can be effectively replaced by substituted imidazole derivatives to give a new class of potent CXCR3 antagonists. An efficient synthetic route to this class of compounds was developed. It was discovered that disubstitution of the imidazole core with lipophilic groups improved both potency and pharmacokinetic properties, while reducing the potential for metabolic bioactivation. Our optimization efforts have led to the discovery of compounds **40** and **43** as potent CXCR3 antagonists with oral bioavailability and acceptable pharmacokinetics in rodent, making them useful tool compounds for in vivo studies of CXCR3 function.

Table 6. Pharmacokinetic profiles of **39** and **42** in rat

	Compound	**40**	**43**
IV[a]	CL (L/h/kg)	2.2	2.3
	AUC (μg h/L)	237	219
	MRT (h)	0.9	1.3
PO[a]	AUC (μg h/L)	456	225
	MRT (h)	2.4	5.4
	F (%)	48	26

[a] IV dose: 0.5 mg/kg; PO dose: 2.0 mg/kg. In all cases: $n = 2$.

References and notes

1. (a) Medina, J.; Johnson, M. G.; Collins, T. L. *Ann. Rep. Med. Chem.* **2005**, *40*, 215; (b) Baggiolini, M. *Nature* **1998**, *392*, 565.
2. (a) Yuan, Y. H.; ten Hove, T.; The, F. O.; Slors, J. F.; van Deventer, S. J.; te Velde, A. A. *Inflamm. Bowel Dis.* **2001**, *7*, 281; (b) Uguccioni, M.; Gionchetti, P.; Robbiani, D. F.; Rizzello, F.; Peruzzo, S.; Campieri, M.; Baggiolini, M. *Am. J. Pathol.* **1999**, *155*, 331.
3. (a) Mahad, D. J.; Howell, S. J.; Woodroofe, M. N. *J. Neurol. Neurosurg. Psychiatry* **2002**, *72*, 498; (b) Mahad, D. J.; Lawry, J.; Howell, S. J.; Woodroofe, M. N. *Mult. Scler.* **2003**, *9*, 189; (c) Sorensen, T. L.; Tani, M.; Jensen, J.; Pierce, V.; Lucchinetti, C.; Folcik, V. A.; Qin, S.; Rottman, J.; Sellebjerg, F.; Strieter, R. M.; Frederiksen, J. L.; Ransohoff, R. M. *J. Clin. Invest.* **1999**, *103*, 807; (d)

Sorensen, T. L.; Trebst, C.; Kivisakk, P.; Klaege, K. L.; Majmudar, A.; Ravid, R.; Lassmann, H.; Olsen, D. B.; Strieter, R. M.; Ransohoff, R. M.; Sellebjerg, F. *J. Neuroimmunol.* **2002**, *127*, 59.

4. (a) Gottlieb, A. B.; Luster, A. D.; Posnett, D. N.; Carter, D. M. *J. Exp. Med.* **1988**, *168*, 941; (b) Gottlieb, A. B.; Krueger, J. G.; Wittkowski, K.; Dedrick, R.; Walicke, P. A.; Garovoy, M. *Arch. Dermatol.* **2002**, *138*, 591; (c) Rottman, J. B.; Smith, T. L.; Ganley, K. G.; Kikuchi, T.; Krueger, J. G. *Lab. Invest.* **2001**, *81*, 335.
5. Patel, D. D.; Zachariah, J. P.; Whichard, L. P. *Clin. Immunol.* **2001**, *98*, 39.
6. (a) Melter, M.; Exeni, A.; Reinders, M. E.; Fang, J. C.; McMahon, G.; Ganz, P.; Hancock, W. W.; Briscoe, D. M. *Circulation* **2001**, *104*, 2558; (b) Segerer, S.; Cui, Y.; Eitner, F.; Goodpaster, T.; Hudkins, K. L.; Mack, M.; Cartron, J. P.; Colin, Y.; Schlondorff, D.; Alpers, C. E. *Am. J. Kidney Dis.* **2001**, *37*, 518; (c) Agostini, C.; Calabrese, F.; Rea, F.; Facco, M.; Tosoni, A.; Loy, M.; Binotto, G.; Valente, M.; Trentin, L.; Semenzato, G. *Am. J. Pathol.* **2001**, *158*, 1703.
7. (a) Hancock, W. W.; Lu, B.; Gao, W.; Csizmadia, V.; Faia, K.; King, J. A.; Smiley, S. T.; Ling, M.; Gerard, N. P.; Gerard, C. *J. Exp. Med.* **2000**, *192*, 1515; (b) Hancock, W. W.; Wang, L.; Ye, Q.; Han, R.; Lee, I. *Curr. Opin. Immunol.* **2003**, *15*, 479.
8. Hancock, W. W.; Gao, W.; Csizmadia, V.; Faia, K. L.; Shemmeri, N.; Luster, A. D. *J. Exp. Med.* **2001**, *193*, 975.
9. (a) Hancock, W. W.; Lu, B.; Gao, W.; Csizmadia, V.; Faia, K.; King, J. A.; Smiley, S. T.; Ling, M.; Gerard, N. P.; Gerard, C. *J. Exp. Med.* **2000**, *192*, 1515; (b) Belperio, J. A.; Keane, M. P.; Burdick, M. D.; Lynch, J. P., 3rd; Zisman, D. A.; Xue, Y. Y.; Li, K.; Ardehali, A.; Ross, D. J.; Strieter, R. M. *J. Immunol.* **2003**, *171*, 4844; (c) Zhang, Z.; Kaptanoglu, L.; Tang, Y.; Ivancic, D.; Rao, S. M.; Luster, A.; Barrett, T. A.; Fryer, J. *Gastroenterology* **2004**, *126*, 809; (d) Baker, M. S.; Chen, X.; Rotramel, A. R.; Nelson, J. J.; Lu, B.; Gerard, C.; Kanwar, Y.; Kaufman, D. B. *Surgery* **2003**, *134*, 126.
10. (a) Collins, T. L.; Johnson, M. G.; Medina, J. C. Antagonists of CXCR3: a Review of Current Progress. In *Chemokine Biology-Basic Research and Clinical Application*, Neote, K.; Letts, G. L.; Moser, B., Eds.; Birkhauser Verlag Publishers, **2007**; Vol. II, *2*, pp 79; (b) Allen, D. R.; Bolt, A.; Chapman, G. A.; Knight, R. L.; Meissner, J. W. G.; Owen, D. A.; Watson, R. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 697; (c) Storelli, S.; Verzijl, D.; Al-Badie, J.; Elders, N.; Bosch, L.; Timmerman, H.; Smit, M. J.; De Esch, I. J. P.; Leurs, R. *Arch. Pharm.* **2007**, *340*, 281; (d) Cole, A. G.; Stroke, I. L.; Brescia, M.-R.; Simhadri, S.; Zhang, J. J.; Hussain, Z.; Snider, M.; Haskell, C.; Ribeiro, S.; Appell, K. C.; Henderson, I.; Webb, M. L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 200.
11. Johnson, M.; Li, A.; Liu, J.; Fu, Z.; Zhu, L.; Miao, S.; Wang, X.; Xu, Q.; Huang, A.; Marcus, A.; Xu, F.; Ebsworth, K.; Sablan, E.; Danao, J.; Kumer, J.; Dairaghi, D.; Lawrence, C.; Sullivan, T.; Tonn, G.; Schall, T.; Collins, T.; Medina, J. *Biol. Org. Med. Chem. Lett.* **2007**, *17*, 3339.
12. Li, A.; Johnson, M. G.; Liu, J.; Chen, X.; Du, X.; Mihalic, J. T.; Deignan, J.; Gustin, D. J.; Duquette, J.; Fu, Z.; Zhu, L.; Marcus, A. P.; Bergeron, P.; McGee, L. R.; Danao, J.; Bryan, L.; Carabeo, T.; Sullivan, T.; Ma, J.; Tang, J.; Tonn, G.; Collins, T.; Medina, J. C. *Biol. Org. Med. Chem. Lett.* **2008**, *18*, 688.
13. All compounds were characterized by ^{1}H NMR and LC/MS and their purity was determined to be >95% by reverse phase HPLC.
14. ^{125}I-IP10 binding assay in buffer: human peripheral blood mononuclear cells (PBMC) were activated with anti-CD3 monoclonal antibody and recombinant human IL-2 for 14 days. Cells were co-incubated with CXCR3 antagonist and recombinant human ^{125}I-IP10 for 2 h at room temperature. The assay buffer used was RPMI-1640 (without phenol red), supplemented with 0.5% BSA. Cells were harvested onto 96 well filter plates and radioactivity was counted on a scintillation counter. Assay values were means of three experiments. ^{125}I-IP10 binding assay in plasma: conditions were the same as the ^{125}I-IP10 binding assay in buffer with the exception that EDTA-anti-coagulated human plasma (from frozen stocks) was used instead of the RPMI buffer. ITAC in vitro cell migration assay: 100 ng/ml of ITAC was used in the presence of 100% human plasma. Compounds were measured by their ability to inhibit CXCR3 mediated cell migration in response to ITAC.
15. Chiral purity was analyzed with ChiralTech AD column with hexanes and isopropanol as solvents. The enantiomeric purity was higher than 95% ee with this synthetic route.