Bioorganic & Medicinal Chemistry Letters 22 (2012) 357–362

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of potent and specific CXCR3 antagonists

Xiaoqi Chen *, Jeff Mihalic, Jeff Deignan, Darin J. Gustin, Jason Duquette, Xiaohui Du, Johann Chan, Zice Fu, Michael Johnson, An-Rong Li, Kirk Henne, Tim Sullivan, Bryan Lemon, Ji Ma, Shichang Miao, George Tonn, Tassie Collins, Julio C. Medina

Amgen Inc., 1120 Veterans Boulevard, ASF2-3, South San Francisco, CA 94080, USA

ARTICLE INFO

Article history:
Received 3 October 2011
Revised 28 October 2011
Accepted 31 October 2011
Available online 9 November 2011

Keywords:
CXCR3
Antagonist
Inflammation

ABSTRACT

The optimization of a series of 8-aza-quinazolinone analogs for antagonist activity against the CXCR3 receptor is reported. Compounds were optimized to avoid the formation of active metabolites and time-dependent-inhibitors of CYP3A4. In addition, antagonists showed potent against CXCR3 activity in whole blood and optimized to avoid activity in the chromosomal aberration assay. Compound **25** was identified as having the optimal balance of CXCR3 activity and pharmacokinetic properties across multiple pre-clinical species, which are reported herein.

Leukocyte migration is a critical step in the pathophysiology of autoimmune and inflammatory conditions. Recent clinical data with protein therapeutics such as Raptiva and Tysabri, and with small molecules such as Gilenya indicates that blockade of T cell migration into inflammed tissue provides an effective way of treating inflammatory diseases. CXCR3 is the predominant chemokine receptor expressed on activated T-cells of Type-1 (Th1) and mediates recruitment and activation of Th1 cells. In addition to Th1 cells, CXCR3 is also expressed on NK cells, B cells and on a fraction of circulating $CD4^+$ and $CD8^+$ T cells. CXCR3 and its ligands, MIG (CXCL9), IP-10 (CXCL10), and I-TAC (CXCL11), are upregulated in several inflammatory diseases including multiple sclerosis,[1] inflammatory bowel disease,[2] rheumatoid arthritis,[3] and solid organ transplant rejection.[4] The established clinical utility of interdicting lymphocyte recruitment coupled with strong human disease association data and compelling pre-clinical data provide the rationale for proposing blockade of CXCR3-mediated T-cell recruitment as a therapeutic target with opportunity for development in several immunologic inflammatory diseases. Therefore, we and others have been interested in discovering CXCR3 antagonists as potential therapeutics.[5]

Our earlier efforts led to the discovery of a potent and selective CXCR3 antagonist, AMG 487 (Fig. 1), which was evaluated in phase I clinical trials.[6] AMG 487 is a potent and selective inhibitor of CXCR3. It inhibits binding of [^{125}I]-IP-10 and [^{125}I]-ITAC to CXCR3 with IC_{50} values of 8.0 and 8.2 nM, respectively, and inhibits CXCR3-mediated migration in response to IP-10 (IC_{50} = 8 nM), ITAC (IC_{50} = 15 nM), and MIG (IC_{50} = 36 nM) in vitro. However, daily doses above 100 mg of AMG 487 resulted in drug accumulation in humans receiving multiple doses that was not observed in preclinical species. Evaluation of AMG 487 using in vitro and in vivo preclinical models led to the discovery of a major metabolite, M1, and a minor metabolite M2 (Fig. 1). M1 was found to be a circulating active metabolite in human, while M2 was found to be a time-dependent inhibitor (TDI) of CYP3A4.[7] M2 is believed to be responsible for the accumulation seen in humans with AMG 487.

We have previously reported that replacement of the pyridyl methyl group with an ethyl sulfone group, as shown in compound **3**, increases CXCR3 affinity.[8] Thus, we decided to focus on analogs bearing this moiety since they would also avoid the formation of the pyridine *N*-oxide metabolite.

In order to address the potential formation of a phenol metabolite similar to M2 new cores were explored. It was hypothesized that a inhibitor wherein the 4-ethoxy-phenyl moiety is attached to the core through a carbon atom would be more stable to oxidation than through a nitrogen atom. This was considered a viable strategy given that earlier optimization efforts demonstrated that a broad variety of structural changes at the 8-aza-quinazolinone core were tolerated.[9] Unfortunately, it was found that while the *O*-ethyl substituted phenol analogs, like compounds **1** and **4**, were not time-dependent inhibitors of CYP3A4, the phenol congeners **2** and **5** were time-dependent inhibitors of CYP3A4 (Fig. 2). This finding suggested that the 4-ethoxy-phenyl functional group would have to be replaced. Toward this end a cyano group was tested as a potential replacement for the ethoxy moiety.

The synthesis of cyano-substituted analogs is outlined in Scheme 1. In the first step of the synthesis, 4-iodoaniline **7** was

* Corresponding author.
E-mail address: xiaoqic@amgen.com (X. Chen).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.10.120

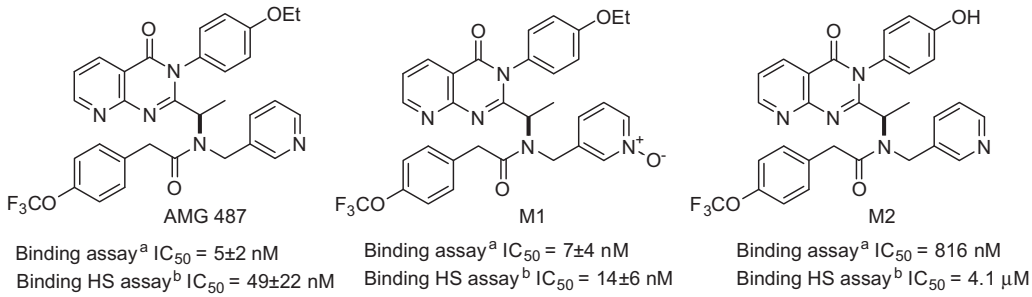


Figure 1. AMG 487 and metabolites M1 and M2. [a]Binding assay: [^{125}I]-IP-10 in buffer; [b]binding HS (human serum) assay: [^{125}I]-IP-10 in buffer in the presence of 100% human serum.

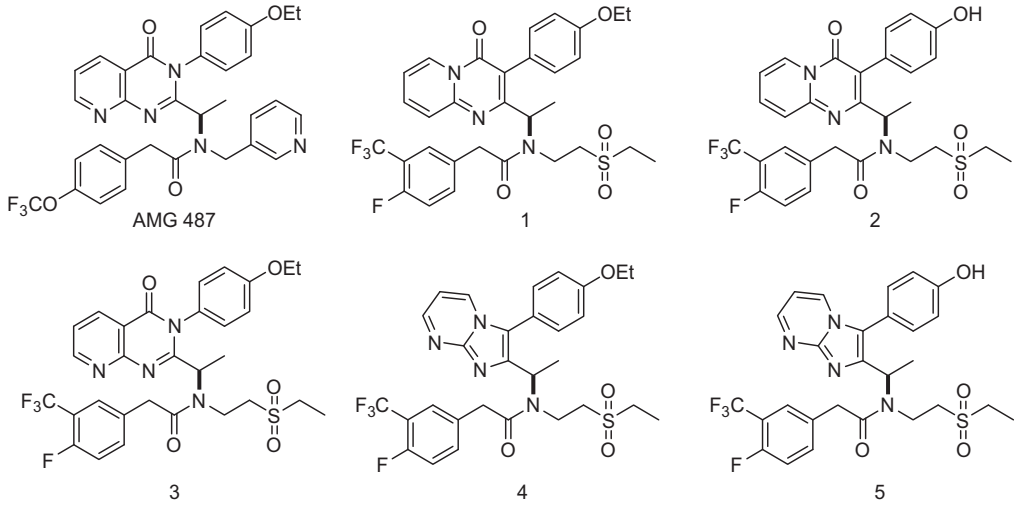
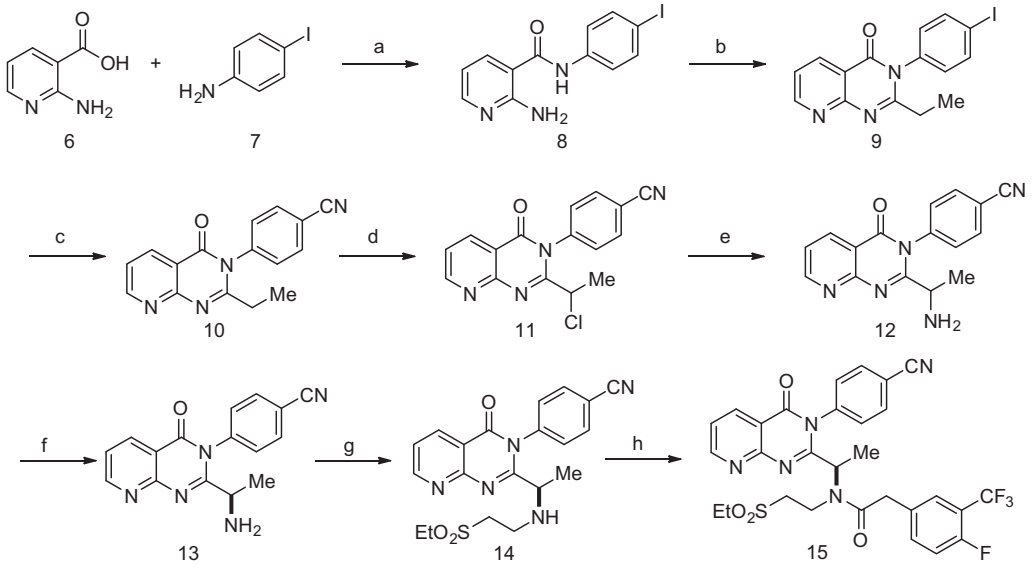


Figure 2. Ethoxide substituted CXCR3 analogs. AMG 487, compound **1**, **3**, **4** were not CYP3A4 time dependent inhibitors. Compound **2**, **5** were CYP3A4 time dependent inhibitors.



Scheme 1. Synthesis of analog **15**. Reagents: (a) EDC, HOBT, Et_3N in CH_2Cl_2, 75%; (b) HOAc, triethyl orthopropionate, 60 °C, 78%; (c) $Pd_2(dba)_3$, dppf, $Zn(CN)_2$, DMF, 115 °C, 96%; (d) NCS, HOAc, 40 °C, 99%; (e) NaN_3, THF/H_2O, then PMe_3, 0 °C, 60% (two steps); (f) L-malic acid, resolution, 36%, 99% ee; (g) ethylvinyl sulfone, 65 °C; (h) 4-fluoro-3-trifluoromethyl-phenyl acetic acid, EDC, HOBT, Et_3N, 73% (two steps).

coupled with 2-aminonicotinic acid **6** using a standard peptide coupling. The resulting amide **8** was subsequently cyclized with triethyl orthopropionate to form the 8-aza-quinazolinone **9**.[10] Iodide **9** was converted to the cyano group by palladium-catalyzed coupling with $Zn(CN)_2$. This sequence of reactions was required since 4-aminobenzonitrile was unreactive towards the formation

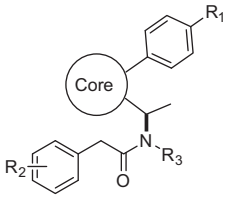
Table 1
Pharmacokinetic profile for analog **15**[a]

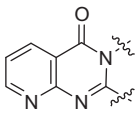
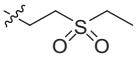
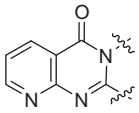
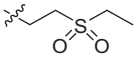
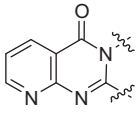
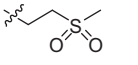
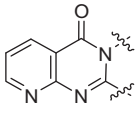
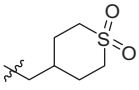
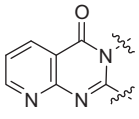
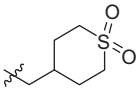
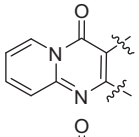
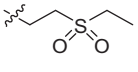
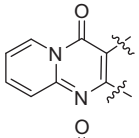
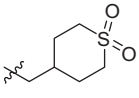
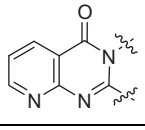
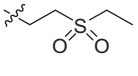
	Mouse	Rat	Beagle dog	Cynomolgus monkey
CL[b] (L/h/kg)	0.35	2.4	1.0	0.74
MRT[b] (h)	0.70	0.53	1.2	1.7
%*F*[c]	17	47	69	13

[a] Amorphous solid.
[b] iv Dose 1 mg/kg in mouse and 0.5 mg/kg in rat, dog and monkey in 10% *N*,*N*-dimethylacetamide, 10% ethanol, 30% propylene glycol, and 50% water.
[c] p.o. Dose 5 mg/kg in mouse and 2 mg/kg in rat, beagle dog, and cynomolgus monkey as a suspension in 1% Tween 80 in 1% methylcellulose.

of the pyridopyrimidin-4-one intermediate used in our original synthesis. The benzylic position of **10** was chlorinated with NCS to provide racemic chloride **11**. Treatment of **11** with sodium azide in wet THF followed by the addition of trimethylphosphine gave primary amine **12**. Compound **12** could be resolved by chiral chromatography or through selective recrystallization of the diastereomeric salt complex with L-malic acid to obtain enantiomerically pure 8-aza-quinazolinone **13**. The amine of **13** was then alkylated with ethylvinyl sulfone and followed by a standard amide coupling with 4-fluoro-3-(trifluoromethyl)phenyl acetic acid to generate the final product **15**. Compound **15** was a highly potent CXCR3 antagonist. The binding IC_{50} of [^{125}I]-IP-10 in buffer and [^{125}I]-IP-10 in the present of human serum were 10 and 18 nM, respectively. Compound **15** effectively inhibited CXCR3-mediated in vitro cell migration in response to ITAC (IC_{50} = 62 nM). The compound was further evaluated in animals for its PK profile. It was found that **15** demonstrated moderate Cl in rat and beagle dog, with rates of 2.4 and 1.0 L/h/kg, respectively. However, the clearance rates were

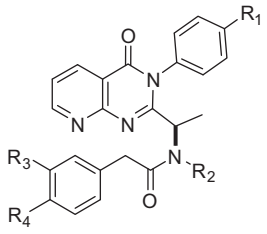
Table 2
Analogs evaluated in the chromosomal aberrations assay

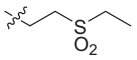
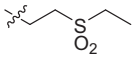
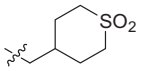
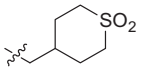
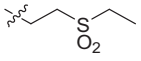
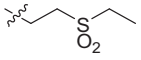
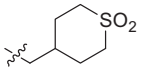
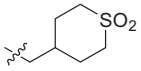


Compd.	Core	R^1	R^2	R^3	[^{125}I]-IP-10 binding		ITAC migration IC_{50} (nM)	Chromosomal aberrations assay[a]
					Buffer IC_{50} (nM)	With HS IC_{50} (nM)		
15		CN	3-CF_3, 4-F		11	46	–	+
16		CN	4-CF_3, 3-F		10	23	47	+
17		CN	3-CF_3, 4-F		13	29	–	+
18		CN	4-CF_3, 3-F		7	15	37	+
19		CN	3-CF_3, 4-F		8	12	39	–
20		CN	3-CF_3, 4-F		2	17	–	+
21		CN	4-OCF_3		6	22	60	+
22		F	4-CF_3, 3-F		47	58	292	–

Binding assay: [^{125}I]-IP-10 in buffer; binding HS (human serum) assay: [^{125}I]-IP-10 in buffer in the presence of 100% human serum; migration: ITAC mediated migration in the presence of 100% human serum.
[a] Chromosomal aberrations in cultured human peripheral blood lymphocytes at concentration of 7.81–1000 μg/mL (+S9 and −S9) at Covance Inc.

Table 3
Analogs which tested negative in the chromosomal aberrations assay



Compd.	R^1	R^2	R^3	R^4	[^{125}I]-IP-10 binding assay		ITAC migration IC_{50} (nM)
					Buffer IC_{50} (nM)	With HS IC_{50} (nM)	
23	Cl		CF_3	F	6	22	102
24	Cl		F	CF_3	7	21	107
25	Cl		CF_3	F	6	12	45
26	Cl		F	CF_3	7	13	55
22	F		CF_3	F	42	50	254
27	F		F	CF_3	47	58	292
28	F		CF_3	F	7	17	72
29	F		F	CF_3	7	22	90

Binding assay: [^{125}I]-IP-10 in buffer; binding HS (human serum) assay: [^{125}I]-IP-10 in buffer in the presence of 100% human serum; migration: ITAC mediated migration in the presence of 100% human serum.

Table 4
Pharmacokinetic profile of analogs **25**[a] and **28**[a] in multiple species

	Mouse		Rat		Beagle dog		Cynomolgus monkey	
	25	**28**	**25**	**28**	**25**	**28**	**25**	**28**
CL[b] (L/h/kg)	0.13	0.34	0.49	2.1	1.5	0.89	0.12	0.34
MRT[b] (h)	3.9	2.1	3.5	2.4	1.1	1.5	8.7	6.4
V_{dss}[b] (L/kg)	0.48	0.73	1.9	5.0	1.4	1.3	1.2	2.2
%F[c]	45	59	69	>100	>100	>100	44	51

[a] Amorphous solid.
[b] iv Dose 1 mg/kg in mouse and 0.5 mg/kg in rat, beagle dog and cynomolgus monkey in 10% *N*,*N*-dimethylacetamide, 10% ethanol, 30% propylene glycol, and 50% water.
[c] p.o. Dose 5 mg/kg in mouse and 2 mg/kg in rat, cynomolgus monkey and beagle dog as a suspension in 1% Tween 80 in 1% methylcellulose.

low in mice and cynomolgus monkeys of 0.35 and 0.74 L/h/kg, respectively (Table 1).

The cyano replacement of the ethoxy substituent in **3** afforded a compound (**15**) that did not produce any metabolites with CYP3A4 time-dependent inhibitory (TDI) activity. However, while compound **15** was negative in the Ames test for mutagenic potential, it tested positive in the in vitro chromosomal aberration assay. To this end, a series of related analogs, **16**–**22** were prepared and submitted for the chromosomal aberration assay for a better understanding of this issue (Table 2). With the exception of compounds **19** and **22**, the compounds featuring the nitrile moiety were positive in the chromosomal aberration assay. It was found that replacement of the ethyl sulfone group with a cyclic sulfone to generate compounds **18** and **19** afforded CXCR3 antagonists with increased potency. The IC_{50} of ITAC mediated migration assay of **18** and **19** in the presence of 100% human serum was 37 and 39 nM. Switching the position of the fluoro and trifluoromethyl group in the acetamide of **15** did not affect the CXCR3 inhibitory activity. The list of compounds which were tested in the chromosomal aberration assay is tabulated in Table 2. With the exception of compound **19**, the remaining compounds that feature the CN moiety were positive in the chromosomal aberration assay. Evaluation of the pharmacokinetic properties of **19** showed that the compound had a low rate of clearance in mice, beagle dogs and cynomolgus monkeys, the clearance was 0.49, 0.5, and 1.3 L/h/kg after iv dosing of 1.0, 0.5, and 0.5 mg/kg, respectively. However, the oral bioavailability of this molecule was rather poor. The oral bioavailability was 17% in mouse and only 1.6% and 1% in beagle dog and cynomolgus monkey following a p.o. dosing of 5, 2, and 2 mg/kg, respectively. It was possible that the poor oral bioavailability of compound **19** was the result of low permeability. The PAMPA permeability[11] of **19** measured 0–0.1 $\times$ 10^{-6} cm/s perme-

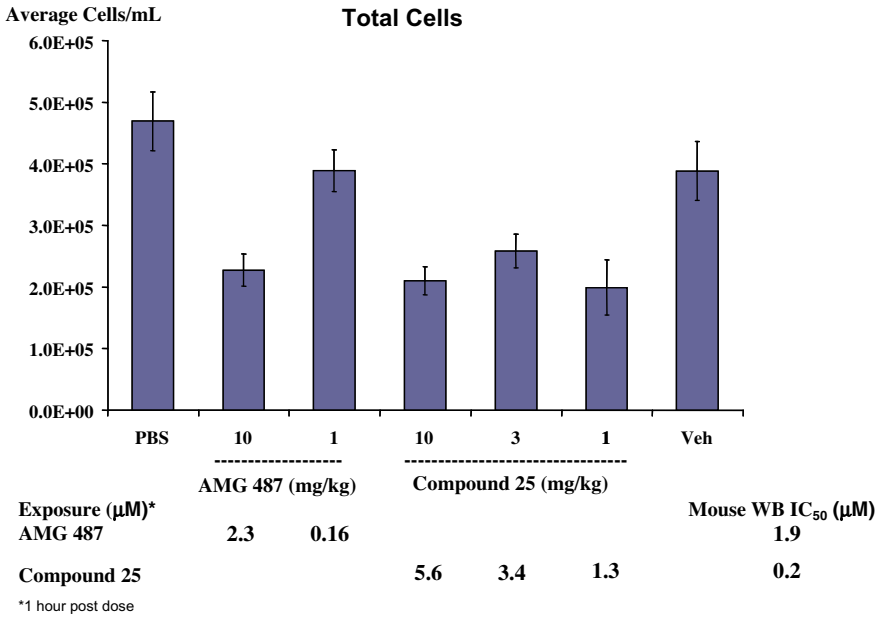


Figure 3. Mouse Bleomycin efficacy model results of AMG 487 and compound **25** against lung inflammation.

ability between pH 5 and 7.4. It was speculated that the favorable chromosomal aberration results might also be attributed to the intrinsically low permeability of the compound.

Replacement of the cyano group with a fluorine resulted in compound **22**, which tested negative in the chromosomal aberration assay. This SAR trend indicated that the cyano group could be the cause for the positive results in the chromosomal aberration assay. In addition, compound **22** had a better PAMPA permeability of 0.8–1.4 $\times$ 10^{-6} cm/s at pH of 5–7.4 than compound **19**. However, its potency as a CXCR3 antagonist was diminished (Table 2). The potency was in agreement with the previous SAR that showed the fluoride-substituted analogs had lower activity in the binding and migration assays when compared to the ethoxy or cyano substituted phenyl analogs. However, it was interesting to note that **22** showed relatively good potency in the CXCR3 binding in the presence of human serum, with an IC_{50} of 58 nM. Based on this evidence and previous SAR, a logical choice was to further investigate the chloro-substituted analogs. A number of chloro-substituted ethyl sulfone analogs were synthesized and tested in the CXCR3 assay (Table 3). Compounds **23** and **24** displayed good potency in the binding and migration assays (Table 3). Cyclic sulfone analogs **25** and **26** demonstrated increased potency when compared to the ethyl sulfone analogs **23** and **24**, especially in the CXCR3-mediated in vitro cell migration assay mediated by ITAC. Compounds **25** and **26** were profiled in the CYP inhibition assay. It was found that both **25** and **26** showed IC_{50} values of greater than 10 μM against CYP 3A4 and 2D6. In addition, compounds **25** and **26** tested negative for inducing chromosomal aberrations, with and without metabolic activation, confirming the hypothesis that the cyano-substitution was likely responsible for the positive chromosomal aberration results.

Based on their potency in the CXCR3 in vitro assays compounds **25** and **28** were selected for further evaluation. The in vivo pharmacokinetic properties of compounds **25** and **28** were profiled in multiple species including, mice, rats, beagle dogs, and cynomolgus monkeys and the results are summarized in Table 4. In general, compound **25** demonstrated lower clearance and longer half lives across the species tested with the exception of dogs. The plasma clearance (CL) of **25** was low in mice, rats, and monkeys (3–15% hepatic blood flow), but higher in dogs (82% hepatic blood flow). The volume of distribution (V_{dss}) varied across species, ranging from 0.48 L/kg in mouse to 1.9 L/kg in rats. This variance followed the same rank order as the extent of plasma protein binding across species. The unbound fractions were 0.7%, 6.1%, 1.5%, and 3.7% in mice, rats, dogs, and monkeys, respectively. The half-life ($T_{1/2}$) of **25** after intravenous dosing was approximately 4 h in mice and rats, 1 h in dogs, and 9 h in monkeys.

Following oral gavage administration, **25** exhibited moderate to high oral bioavailability (%*F*). Oral bioavailabilities of **25** ranged from 44% to 69% in mice, rats, and cynomolgus monkeys. In beagle dogs, the apparent bioavailability exceeded 100% due to nonlinear pharmacokinetic behavior.

Compound **25** was further evaluated in a CXCR3 receptor occupancy assay in whole blood. The CXCR3 occupancy assay was performed via FACS analysis by using a fluorescently-tagged anti-CXCR3 antibody. Addition of the CXCR3 ligand, ITAC, results in the loss of fluorescent signal of antibody. The CXCR3-antagonists blocks ITAC binding against receptor, but this series of compounds does not block binding of the anti-CXCR3 antibody to the CXCR3 receptor. An ITAC concentration of 500 ng/mL was used in the assay. In this assay, compound **25** demonstrated IC_{50} values of 200 and 9.8 nM using mouse and human whole blood, respectively.

It has been shown that CXCR3 plays an important role in a mouse model of lung injury induced by bleomycin administered by the intra-tracheal route.[12] Changes in bronchoalveolar lavage (BAL) fluid attributed to bleomycin treatment include increased total cell count followed by a sustained increase in lymphocyte count. In this efficacy experiment, C57BL/6 mice were treated by a single intra-tracheal instillation 1 mg/kg of bleomycin. The previous clinical candidate, AMG 487, was used as a positive control in this experiment. AMG 487 and compound **25** were dosed subcutaneously with the use of an Alzet osmotic mini-pump on the same day of bleomycin challenge for 6 days.[13] A BAL was performed on day six and the number of cells was counted. Plasma samples were collected at the end of the experiment to determine drug level.

Compound **25** was effective in blocking leukocyte migration into the lungs of mice following bleomycin challenge with an effective dose of 1 mg/kg (Fig. 3). The level of blocking of cell penetration was similar to the CXCR3 knockout animals. This dose results in blood levels of 1.3 μM 1 h post dose, which is six-fold the mouse whole blood receptor occupancy assay IC_{50}.

In conclusion, through detailed compound optimization efforts, several CXCR3 antagonists with improved in vitro potency over AMG 487 were discovered. These compounds lack the ethoxy group responsible for the circulating metabolite M2, which causes the CYP3A4 TDI in vivo with AMG 487. Furthermore, these compounds avoid the formation of the pyridine *N*-oxide active metabolite, M1 also observed with AMG 487. Compound **25** showed good oral bioavailability in multiple preclinical species and demonstrated efficacy in a mouse model of bleomycin-induced leukocytes trafficking into the lung. Moreover, **25** showed potent inhibitory activity in a CXCR3 receptor occupancy assay using human and mouse whole blood.

References and notes

1. (a) Mahad, D. J.; Howell, S. J. L.; 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.
2. (a) Uguccioni, M.; Gionchetti, P.; Robbiani, D. F.; Rizzello, F.; Peruzzo, S.; Campieri, M.; Baggiolini, M. *Am. J. Pathol.* **1999**, *155*, 331; (b) Yuan, Y. H.; Hove, T.; The, F. O.; Slors, J. F.; van Deventer, S. J.; Velde, A. A. *Inflamm. Bowel Dis.* **2001**, *7*, 281.
3. (a) Patel, D. D.; Zachariah, J. P.; Whichard, L. P. *Clin. Immunol.* **2001**, *98*, 39; Qin, S.; Rottman, J. B.; Myers, P.; Kassam, N.; Weinblatt, M.; Loetscher, M.; Koch, A. E.; Moser, B.; Mackay, C. R. *J . Clin. Invest.* **1998**, *101*, 746.
4. (a) 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; (b) Melter, M.; Exeni, A.; Reinders, M. E.; Fang, J. C.; McMahon, G.; Ganz, P.; Hancock, W. W.; Briscoe, D. M. *Circulation* **2001**, *104*, 2558; (c) Goddard, S.; Williams, A.; Morland, C.; Qin, S.; Gladuem, R.; Hubscher, S. G.; Adams, D. H. *Transplantation* **2000**, *72*, 1957; (d) Hancock, W. W.; Wang, L.; Ye, Q.; Han, R.; Lee, I. *Curr. Opin. Immunol.* **2003**, *15*, 479; (e) 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.
5. (a) Crosignani, S.; Missotten, M.; Cleva, C.; Dondi, R.; Ratinaud, Y.; Humbert, Y.; Mandal, A. B.; Bombrun, A.; Power, C.; Chollet, A.; Proudfoot, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3614; (b) McGuinness, B. F.; Carroll, C. D.; Zawacki, L. G.; Dong, G.; Yang, C.; Hobbs, D. W.; Jacob-Samuel, B.; Hall, J. W.; Jenh, C. H.; Kozlowski, J. A.; Anilkumar, G. N.; Rosenblum, S. B. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5205; (c) Bongartz, J. P.; Buntinx, M.; Coesemans, E.; Hermans, B.; Van Lommen, G.; Van Wauwe, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5819; (d) Watson, R. J.; Allen, D. R.; Birch, H. L.; Chapman, G. A.; Galvin, F. C.; Jopling, L. A.; Knight, R. L.; Meier, D.; Oliver, K.; Meissner, J. W. G.; Owen, D. A.; Thomas, E. J.; Tremayne, N.; Williams, S. C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 147; (e) Knight, R. L.; Allen, D. R.; Birch, H. L.; Chapman, G. A.; Galvin, F. C.; Jopling, L. A.; Lock, C. J.; Meissner, J. W. G.; Owen, D. A.; Raphy, G.; Watson, R. J.; Williams, S. C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 629; (f) Watson, R. J.; Allen, D. R.; Birch, H. L.; Chapman, G. A.; Hannah, D. R.; Knight, R. L.; Meissner, J. W. G.; Owen, D. A.; Thomas, E. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6806; (g) Storelli, S.; Verzijl, D.; Al-Badie, J.; Elders, N.; Bosch, L.; Timmerman, H.; Smit, M. J.; De Esch, I. J. P.; Leurs, R. *Archiv der Pharmazie (Weinheim, Germany)* **2007**, *340*, 281; (h) 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; (i) 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; (j) Heise, C. E.; Pahuja, A.; Hudson, S. C.; Mistry, M. S.; Putnam, A. L.; Gross, M. M.; Gottlieb, P. A.; Wade, W. S.; Kiankarimi, M.; Schwarz, D.; Crowe, P.; Zlotnik, A.; Alleva, D. G. *J.Pharm. Exp. Therap.* **2005**, *313*, 1263; (k) Ondeyka, J. G.; Herath, K. B.; Jayasuriya, H.; Polishook, J. D.; Bills, G. F.; Dombrowski, A. W.; Mojena, M.; Koch, G.; DiSalvo, J.; DeMartino, J.; Guan, Z.; Nanakorn, W.; Morenberg, C. M.; Balick, M. J.; Stevenson, D. W.; Slattery, M.; Borris, R. P.; Singh, S. B. *Mol. Divers.* **2005**, *9*, 123; (l) 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.
6. Johnson, M.; Li, A. R.; 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. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3339.
7. Tonn, G. R.; Wong, S. G.; Wong, S. C.; Johnson, M. G.; Ma, J.; Cho, R.; Floren, L. C.; Kersey, K.; Berry, K.; Marcus, A. P.; Wang, X.; Van Lengerich, B.; Medina, J. C.; Pearson, P. G.; Wong, B. K. *Drug Metab. Dispos.* **2009**, *37*, 502.
8. Liu, J.; Fu, Z.; Li, A. R.; Johnson, M.; Zhu, L.; Marcus, A.; Danao, J.; Sullivan, T.; Tonn, G.; Collins, T.; Medina, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5114.
9. Du, X.; Gustin, D. J.; Chen, X.; Duquette, J.; McGee, L. R.; Wang, Z.; Ebsworth, K.; Henne, K.; Lemon, B.; Ma, J.; Miao, S.; Sabalan, E.; Sullivan, T. J.; Tonn, G.; Collins, T. L.; Medina, J. C. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5200.
10. Chan, J.; Gustin, D. J.; Divirgilio, E.; Guram, A.; Faul, M. M. *Synthesis* **2007**, *23*, 3678.
11. Balimane, P.; Han, Y. H.; Saeho; Chong, S. *AAPS J.* **2008**, *8*, E1.
12. Jiang, D.; Liang, J.; Hodge, J.; Lu, B.; Zhu, Z.; Yu, S.; Fan, J.; Gao, Y.; Yin, Z.; Homer, R.; Gerard, C.; Noble, P. W. *J. Clin. Invest.* **2004**, *114*, 291–299.
13. Alzet osmotic mini-pump with a volume of 0.2 mL was implanted subcutaneously. The infusion rate was 1 μL/h. For more information, please visit http://www.alzet.com/downloads/TIM.pdf.