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Bioorganic & Medicinal Chemistry Letters 18 (2008) 688–693
Bioorganic & Medicinal Chemistry Letters

Optimization of the heterocyclic core of the quinazolinone-derived CXCR3 antagonists

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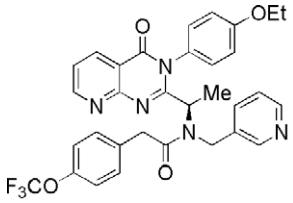
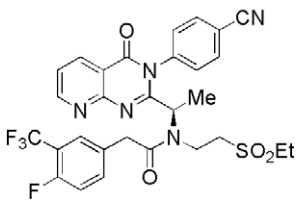
Received 18 October 2007; revised 14 November 2007; accepted 15 November 2007
Available online 21 November 2007

Abstract—A series of six–six and six–five fused heterocyclic CXCR3 antagonists has been synthesized and their activities evaluated in an [^{125}I]-IP-10 displacement assay and an ITAC mediated in vitro cell migration assay. The pharmacokinetic properties of several top compounds have also been studied. This effort led to the discovery of compounds with increased potency and improved pharmacokinetic properties that could serve as useful tools to study the role of the CXCR3 receptor in vivo.

The recent development of various biologic agents such as Raptiva® (efalizumab)[1,2] and Tysabri® (natalizumab)[3,4] demonstrates the therapeutic value of blocking T cell recruitment into inflamed tissue. These agents are efficacious in the treatment of autoimmune diseases such as psoriasis, multiple sclerosis (MS), and inflammatory bowel disease (IBD), and thus there is great interest in novel mechanisms that inhibit T cell recruitment. Chemokine receptors and their ligands play an important role in mediating leukocyte trafficking. CXCR3 is a chemokine receptor primarily expressed on activated $CD4^+$ and $CD8^+$ T cells with the Th1 phenotype.[5,6] The ligands for CXCR3, MIG (CXCL9), IP-10 (CXCL10), and ITAC (CXCL11), mediate migration of CXCR3-expressing cells and are found at increased concentrations in inflamed tissue from patients suffering from IBD, MS, rheumatoid arthritis, and transplant rejection.[7–18] It has been hypothesized that blockade of CXCR3 will prevent inflammatory cells from reaching sites of inflammation, thereby alleviating a variety of inflammatory and autoimmune diseases.



AMG 487



1

Several research groups, including ours, have been involved in the discovery of small molecule antagonists of CXCR3.[5,19–22] We previously reported that a series of 2,3-disubstituted-quinazolinone and 2,3-disubstituted-8-aza-quinazolinone derivatives, typified by AMG 487, are potent and selective CXCR3 antagonists.[19] AMG 487 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 in vitro cell migration by IP-10 (IC_{50} = 8 nM), ITAC (IC_{50} = 15 nM), and MIG (IC_{50} = 36 nM). In this article, we report the replacement of the 8-aza-quinazolinone core of AMG 487 with alternative bicyclic-ring systems to evaluate the role that the core has on the affinity of these antagonists for the CXCR3 receptor. Since the 8-aza-quinazolinone-derivative **1** exhibits potencies similar to those of AMG 487 in the ligand displacement assays ([^{125}I]-IP-10 IC_{50} in buffer = 11 nM;

Keywords: Chemokine; CXCR3; Mig; ITAC; IP10; CXCL9; CXCL10; CXCL11.
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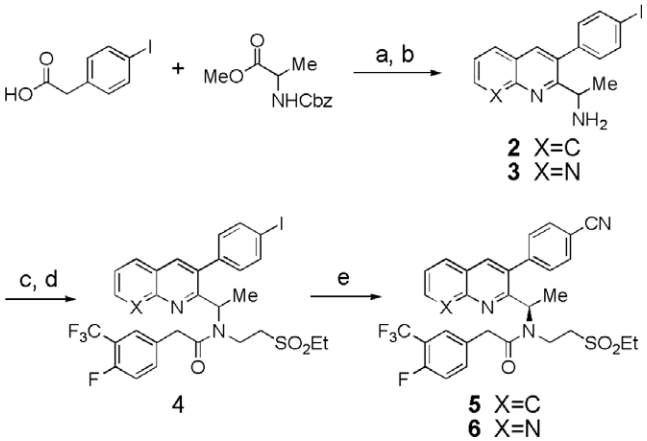
doi:10.1016/j.bmcl.2007.11.060

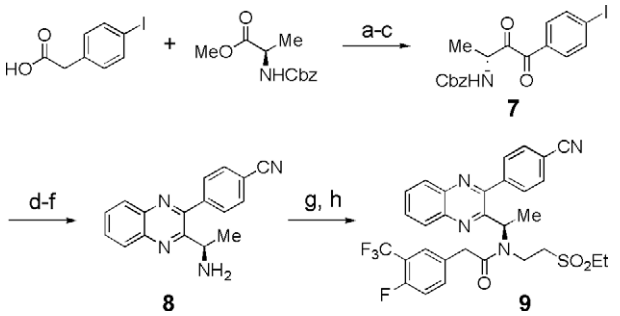
IC_{50} in plasma = 25 nM) and the in vitro cell migration assay (ITAC IC_{50} = 115 nM), but is devoid of the time-dependent CYP 3A4 inactivation that results from *O*-deethylation of AMG 487, we decided to explore the structure activity relationship of the core with reference to **1**. Compounds were evaluated in a [^{125}I]-IP-10 ligand replacement assay run both in the presence and in the absence of plasma.[23] A selected group of compounds was also evaluated in an ITAC-induced in vitro migration assay using activated human peripheral blood mononuclear cells (PBMC) in the presence of plasma.[23] Additionally, the pharmacokinetic profile in the rat was determined for several compounds.

We demonstrated previously that the majority of the CXCR3 activity in this series resides with the (*R*)-stereoisomers.[19] Here we assayed enantiomerically enriched compounds (>95% ee) obtained either by synthesis from optically pure starting materials or by separation of the enantiomers from racemic product mixtures *via* chiral phase HPLC. When enantiomers were separated by HPLC the (*R*)-stereochemistry was putatively assigned to the stereoisomer with higher binding affinity in the in vitro [^{125}I]-IP-10 displacement assay.

Quinoline **5** and 1,8-naphthyridine **6** were obtained as shown in Scheme 1. Treatment of 2-(4-iodophenyl) acetic acid with Cbz-protected alanine methyl ester in the presence of lithium hexamethyldisilazide led to the formation of the Cbz-protected 3-amino-1-(4-iodophenyl)butan-2-one,[24] which cyclized with 2-aminonicotinaldehyde or 2-aminobenzaldehyde to afford com-pound **2** or **3**. Michael addition of **2** or **3** to ethylsulfonylethene, followed by phenylacylation with 4-fluoro-3-(trifluoromethyl)phenylacetic acid and palladium-mediated transformation of the iodophenyl group into a cyanophenyl group, afforded the racemic products **5** and **6**. Optically pure **5** and **6** were isolated by chiral phase HPLC.



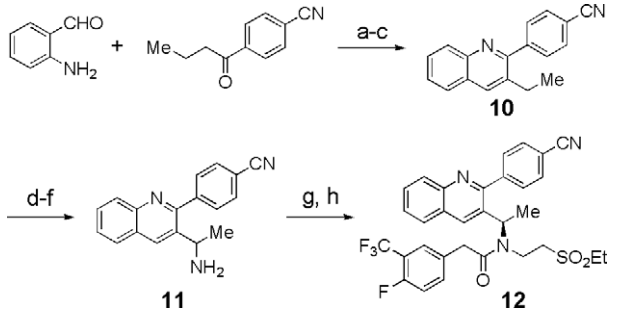
Scheme 1. Reagents and conditions: (a) LHMDS, THF, −78 °C to rt, 20%; (b) 2-aminonicotinaldehyde or 2-aminobenzaldehyde, KOH, EtOH, reflux, 47%. (c) ethylsulfonylethene, TEA, MeOH, reflux; (d) 4-fluoro-3-trifluoromethylphenylacetic acid, EDC, HOBt, NMM, DMF, rt, 80–90% two steps. (e) $Pd(PPh_3)_4$, CuI, NaCN, MeCN, microwave, 120 °C, 80–90%. Enantiomerically pure products were isolated by chiral phase HPLC.



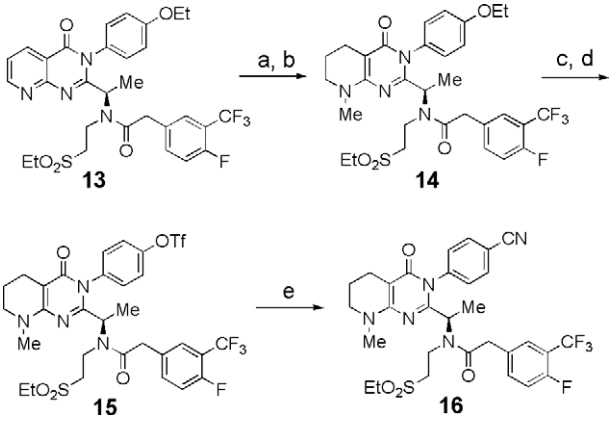
Scheme 2. Reagents and conditions: (a) LHMDS, THF, −78 °C to rt, 20–30% over three steps; (b) *tert*-butoxy-*N,N,N′,N′*-tetramethylmethanediamine, THF, 60 °C; (c) O_3, DCM, −78 °C; (d) benzene-1,2-diamine, EtOH, AcOH, reflux; (e) $Pd(PPh_3)_4$, CuI, NaCN, MeCN, microwave, 120 °C, 80–90% (f) $PdCl_2$, Et_3SiH, TEA, DCM, rt, 85%; (g) ethylsulfonylethene, TEA, MeOH; (h) 4-fluoro-3-trifluoromethylphenylacetic acid, EDC, HOBt, NMM, DMF, rt, 80–90% two steps.

In the case of quinoxaline **9** (Scheme 2), the intermediate 3-Cbz-amino-1-(4-iodophenyl)butan-2-one obtained in Scheme 1 was reacted with *tert*-butoxy-*N,N,N′,N′*-tetramethylmethanediamine and then oxidized with ozone to generate intermediate dione **7**, which cyclized with 1,2-diaminobenzene to furnish Cbz-protected (*R*)-1-(3-(4-iodophenyl)quinoxalin-2-yl)ethanamine. Subsequent conversion of the iodide to the nitrile and removal of the Cbz group provided intermediate **8.** Compound **9** was synthesized by Michael addition to ethylsulfonylethene and acylation with 4-fluoro-3-(trifluoromethyl)phenylacetic acid as described in Scheme 1.

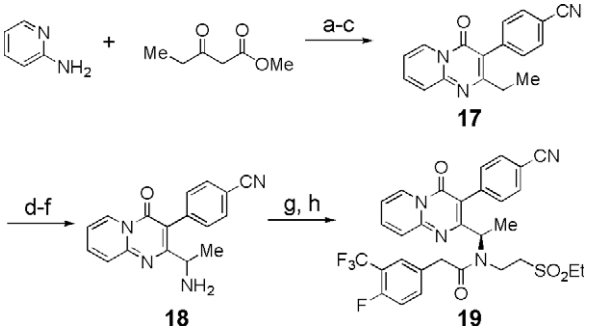
Quinoline **12** was synthesized from 2-aminobenzaldehyde and 4-butyrylbenzonitrile in eight steps according to Scheme 3. Cyclization to form the quinoline ring system **10** was carried out as in Scheme 1. During the cyclization the nitrile was hydrolyzed to the acid and a two-step amide formation from the acid followed by dehydration to the desired nitrile was required to afford 4-(3-ethylquinolin-2-yl)benzonitrile. Installation of the amino group was achieved by radical bromination followed by azide substitution of the bromide and reduction of the azide to the primary amine **11**, which



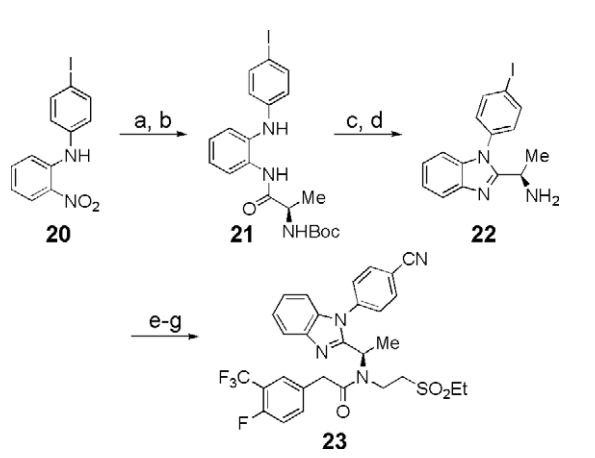
Scheme 3. Reagents and conditions: (a) KOH, EtOH, 80 °C, 90%; (b) oxalyl chloride, DCM, then NH_4OH,; (c) Tf_2O, TEA, DCM, two step 36%; (d) NBS, CCl_4, benzoylperoxide, reflux, 97%; (e) NaN_3, DMF, 85 °C (f) PPh_3, DMF/H_2O, 40 °C, 85% over two steps; (g) ethylsulfonylethene, TEA, MeOH, 99%; (h) 4-fluoro-3-trifluoromethylphenylacetic acid, EDC, HOBt, NMM, DMF, rt, 61% over two steps. Enantiomerically pure products were isolated by chiral phase HPLC.



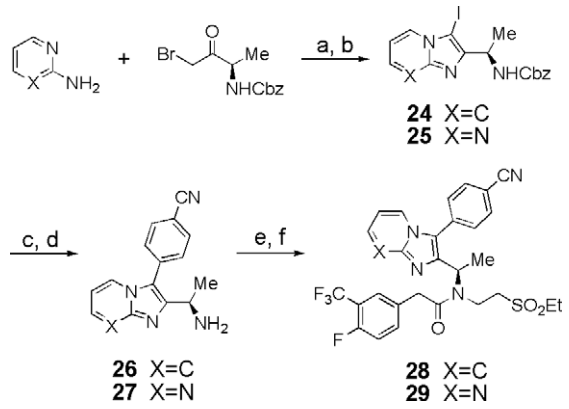
Scheme 4. Reagents and conditions: (a) H_2, 10% Pd/C, 90%; (b) paraformaldehyde, $Na(OAc)_3BH$; $ClCH_2CH_2Cl$, rt, 60%; (c) BBr_3, DCM, rt, 71%; (d) Tf_2O, 2,6-dimethylpyridine, DCM, rt, 83%; (e) $Pd(PPh_3)_4$, CuI, NaCN, MeCN, microwave, 120 °C, 80–90%.



Scheme 5. Reagents and conditions: (a) EtOH, reflux; (b) NBS, MeCN, rt, 50% over two steps; (c) 4-cyanophenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, 80 °C, DME, 95%; (d) NBS, CCl_4, benzoylperoxide, reflux; (e) K_2CO_3, phthalimide; (f) NH_2NH_2, 91% over two steps; (g) ethylsulfonylethene, TEA, MeOH; (h) 4-fluoro-3-trifluoromethylphenylacetic acid, EDC, HOBt, NMM, DMF, rt, 80–90% two steps. Enantiomerically pure products were isolated by chiral phase HPLC.



Scheme 6. Reagents and conditions: (a) $SnCl_2$, EtOAc, reflux, 95%; (b) (R)-2-(tert-butoxycarbonyl)propanoic acid, EDC, HOBt, NMM, rt, 85%; (c) AcOH, 80 °C, 81%; (d) TFA, DCM, rt, 75%; (e) ethylsulfonylethene, TEA, MeOH; (f) 4-fluoro-3-trifluoromethylphenylacetic acid, EDC, HOBt, NMM, DMF, rt, 80–90% two steps. (g) $Pd(PPh_3)_4$, CuI, NaCN, MeCN, microwave, 120 °C, 92%.

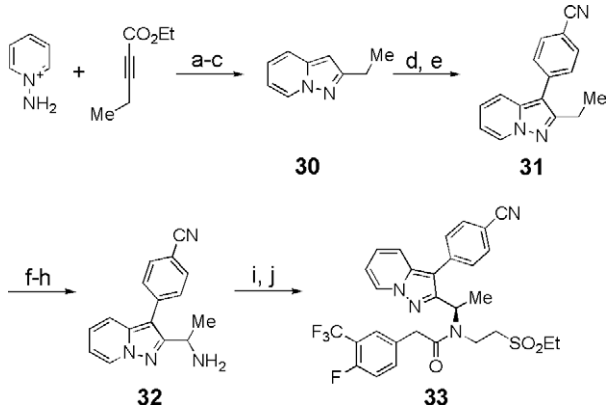


Scheme 7. Reagents and conditions: (a) EtOH, reflux; (b) NIS, CH_3CN, rt, 80–90%; (c) 4-cyanophenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, 80 °C, DME, 90%; (d) BBr_3, DCM, −10 °C to rt, 92%; (e) ethylsulfonylethene, TEA, MeOH; (f) 4-fluoro-3-trifluoromethylphenylacetic acid, EDC, HOBt, NMM, DMF, rt, 80–90% two steps.

was converted to racemic **12** in two steps as previously described. Enantiopure **12** was isolated by chiral HPLC.

Tetrahydropyridopyrimidinone **16** was readily obtained starting from the 8-aza-quinazolinone **13** as shown in Scheme 4. Reduction of **13** followed by reductive amination with paraformaldehyde provided compound **14**, which was deethylated and transformed to the aryl triflate **15** before conversion to racemic final product by palladium catalyzed cyanation.

Pyridopyrimidine **19** was prepared as described in Scheme 5. Treatment of 2-amino-pyridine with methyl 3-oxopentanoate followed by bromination and then Suzuki coupling to introduce the 3-(4′-cyanophenyl) group generated intermediate **17**. The amino group in **18** was installed in three steps via bromination of **17**, nucleophilic displacement of the bromide with phthalimide,



Scheme 8. Reagents and conditions: (a) K_2CO_3, air, DMF; (b) 3N NaOH, MeOH; (c) H_2SO_4/H_2O, 70 °C, 48% over three steps; (d) NIS, AcOH, rt; (e) 4-cyanophenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, 80 °C, 37% over two steps; (f) NBS, CCl_4, benzoylperoxide, reflux, 97%; (g) NaN_3, DMF, 85 °C; (h) 10% Pd/C, MeOH, rt, 80% over three steps; (i) ethylsulfonylethene, TEA, MeOH, reflux; (j) 4-fluoro-3-trifluoromethylphenylacetic acid, EDC, HOBt, NMM, DMF, rt, 80–90% two steps. Enantiomerically pure products were isolated by chiral phase HPLC.

and hydrolysis of the phthalimide with hydrazine. The transformation of **18** to racemic **19** was achieved using the same methods as described in Scheme 1. Enantiopure **19** was isolated by chiral HPLC.

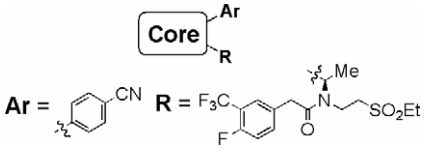
The synthesis of benzimidazole **23** is outlined in Scheme 6. Reduction of nitroaryl **20** followed by coupling with Cbz-protected D-alanine yielded **21**, which was cyclized in acetic acid and deprotected to afford **22**. Compound **23** was obtained by employing the two-step Michael addition and phenylacylation sequence described in Scheme 1, followed by palladium-catalyzed cyanation.

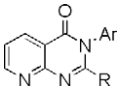
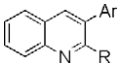
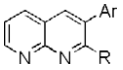
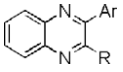
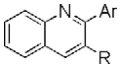
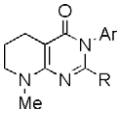
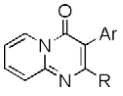
The intermediates **26** and **27** to H-imidazo[1–24] pyridine **28** and imidazo[1,2-*a*]pyrimidine **29** were obtained in four steps starting from aminopyridine or aminopyrimidine as described in Scheme 7. The cyclization with (*R*)-3-amino-1-bromobutan-2-one followed by iodination yielded **24** and **25**. Compounds **26** and **27** were obtained *via* Suzuki coupling followed by removal of the Cbz group. Michael addition and phenylacylation described in Scheme 1 afforded **28** and **29**.

The synthesis of compound **33** is described in Scheme 8. Treatment of 1-aminopyridinium iodide with ethyl pent-2-ynoate followed by decarboxylation afforded 2-ethylpyrazolo[1,5-*a*]pyridine **30**. Subsequent iodination and Suzuki coupling led to formation of compound **31**. Radical bromination, followed by azide displacement of the bromide and reduction of the azide, afforded the primary amine **32**. Racemic final product **33** was obtained after Michael addition with ethylsulfonylethene, followed by acylation with 4-fluoro-3-(trifluoromethyl)phenylacetic acid as previously described in Scheme 1. Enantiomerically enriched **33** was obtained by chiral HPLC.

Initially, we designed a series of six–six fused heterobicyclic derivatives such as quinoline **5**, 1,8-naphthyridine **6**, and quinoxaline **9** to determine the influence of the carbonyl and the 3-position nitrogen atom of the 8-aza-qui-

Table 1. In vitro activities of six–six fused heterocyclic ring systems[a]



ID	Core (Ar, R)	Binding IC_{50}[b] (nM)		Lymphocyte migration IC_{50}[e] (nM)
		Buffer[c]	Plasma[d]	
1		11	25	115
5		0.80	21	72
6		2.0	27	48
9		9.0	121	n.d.
12		5.0	115	n.d.
16		4.0	60	88
19		2.0	17	41

[a] Values are means of three experiments, standard deviation is ±30% (see Ref. 23).
[b] Displacement of [^{125}I]-IP-10 from the CXCR3 receptor expressed on activated PBMC.
[c] RPMI-1640 buffer supplemented with 0.5% BSA.
[d] EDTA-anti-coagulated human plasma to 50%.
[e] ITAC mediated migration of PBMC in the presence of 100% human plasma.

nazolinone lead **1** on binding and functional activities. These compounds were evaluated in [^{125}I]-IP-10 displacement and ITAC-mediated lymphocyte migration assays (Table 1). Relative to **1**, compounds **5**, **6**, and **9** exhibited similar or improved affinities for the CXCR3 receptor as evident from their IC_{50}'s in the [^{125}I]-IP-10 displacement assay run in buffer.[23] Clearly the carbonyl and the 3-position nitrogen atom of the parental 8-aza-quinazolinone lead **1** are not essential for binding of these compounds to the receptor. It was also found that quinoline **5** showed a greater than 10-fold increase in affinity for the CXCR3 receptor in buffer compared to **1**. However, the increase in potency was negligible when taking into consideration protein binding as demonstrated by the [^{125}I]-IP-10 ligand displacement and lymphocyte migration assays run in the presence of human plasma. Compound **16** was designed to evaluate the necessity of a fully aromatic bicyclic ring system for high binding affinity. This compound had similar potency to the lead compound **1** across all assays, confirming that an extensive aromatic bicyclic ring system is not essential for potent binding or functional activity in the in vitro assays.

We also investigated six–five fused heterocycles in place of the 8-aza-quinazolinone core (Table 2). Benzoimidazole **22**, imidazopyridine **28**, imidazopyrimidine **29**, and pyrozolopyridine **33** afforded good affinity for the CXCR3 receptor as evident from the [^{125}I]-IP-10 ligand displacement assay in buffer. The imidazopyridine-derived compound **28** afforded the highest affinity for the receptor ([^{125}I]-IP-10 IC_{50} in buffer = 3 nM; IC_{50} in plasma = 21 nM), while the pyrazolopyridine-derivative **33** had the weakest binding affinity ([^{125}I]-IP-10 IC_{50} in buffer = 18 nM; IC_{50} in plasma = 523 nM). Compound **28** also had improved CXCR3 activity in the migration assay (ITAC IC_{50} = 72 nM) relative to the lead **1** (ITAC IC_{50} = 115 nM). While the benzoimidazole **22** and the imidazopyrimidine **29** exhibited strong affinity for the receptor in the [^{125}I]-IP-10 ligand displacement assay in buffer, these compounds afforded only modest activity in the binding and migration assays run in the presence of human plasma.

The pharmacokinetic properties of selected compounds were determined in the rat (Table 3). In general compounds evaluated showed good oral absorption in the rat. In addition, compound **19** showed lower clearance after i.v. administration compared to the 8-azaquinazolinone lead **1**, while compounds **5** and **6** had comparable pharmacokinetic profiles to **1**.

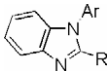
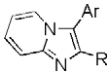
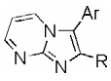
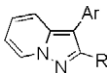
In summary, we have discovered that a variety of six–six fused heterocycles, such as quinoline, 1,8-naphthyridine, and quinoxaline, and six–five fused heterocycles, such as benzoimidazole, imidazopyridine, and imidazopyrimidine, yield viable replacements of the 8-azaquinazolinone core featured in AMG 487 and **1**. We have demonstrated that the carbonyl and the 3-position nitrogen atom of the parental 8-aza-quinazolinone lead are not essential for the binding of these compounds to

Table 3. Pharmacokinetic profiles of select analogs in rat

ID	Cls(L/h/Kg)[a]	MRT(h)[a]	F(%)[b]
1	2.38	0.53	47
5	2.53	1.5	17
6	2.38	0.37	31
16	3.78	0.35	n.d.
19	0.24	1.4	24
28	2.18	0.26	24

[a] Following i.v. dosing in rat at 0.5 mg/kg.
[b] Following p.o. dosing in rat at 2 mg/kg.

Table 2. In vitro activities of six–five fused heterocyclic ring systems[a]

ID	Core	Binding IC_{50}[b] (nM)		Lymphocyte migration IC_{50}[e] (nM)
		Buffer[c]	Plasma[d]	
22		6.0	35	521
28		3.0	21	72
29		10	79	643
33		18	523	n.d.

[a] Values are means of three experiments, standard deviation is ±30% (see Ref. 23).
[b] Displacement of [^{125}I]-IP-10 from the CXCR3 receptor expressed on activated PBMC.
[c] RPMI-1640 buffer supplemented with 0.5% BSA.
[d] EDTA-anti-coagulated human plasma to 50%.

the receptor. While it is clear that the heterocyclic core contributes to the affinity of these molecules to the receptor, the broad variety of structural changes tolerated suggests that the main role of the heterocyclic core is to arrange the substituents in the correct orientation. Of the six–six bicycles, pyridopyrimidine **19** exhibited good potency in the binding and functional assays and good in vivo pharmacokinetic properties in the rat making this compound a useful tool to evaluate the role that the CXCR3 receptor plays in vivo. Quinoline **5** and naphthyridine **6** are also promising with regard to their increased in vitro activity and comparable pharmacokinetic profile relative to **1**.

References and notes

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23. [^{125}I]-IP-10 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 antagonists and recombinant human [^{125}I]-IP-10 (50 pM) for 2 h at rt. 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]-IP-10 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 ("plasma") was added to the assay buffer, to 50% final assay concentration. ITAC in vitro cell migration assay: 100 ng/ml of human ITAC resuspended in 100% plasma was added to the lower chamber of a migration plate. Human PBMC (prepared as above) were resuspended in 100% plasma and placed in the upper chamber of the migration plate. Compounds were measured for their ability to inhibit CXCR3 mediated migration of cells into the lower chamber, in response to ITAC.
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