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Discovery and optimization of a series of quinazolinone-derived antagonists of CXCR3

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Abstract—A series of quinazolinone-derived inhibitors of the CXCR3 receptor have been synthesized and their affinity for the receptor evaluated. Compounds were evaluated in a ^{125}I-IP10 displacement assay and in in vitro cell migration assays to IP10, ITAC, and MIG using human peripheral blood mononuclear cells.

CXCR3 is a chemokine receptor primarily expressed on activated CD4+ and CD8+ T cells with a Th_1 phenotype;[1] it is also expressed on B cells,[2] natural killer (NK) cells,[3] malignant T cells[4], and astrocytes.[5] The ligands for CXCR3 are Mig (CXCL9), IP-10 (CXCL10), and ITAC (CXCL11). These ligands are induced primarily by IFN-γ and are produced by macrophages as well as other cell types in inflamed tissue.[6] CXCR3 and its ligands have been implicated in a number of inflammatory diseases including rheumatoid arthritis,[7] multiple sclerosis,[8] inflammatory bowel diseases,[9] psoriasis,[10] and transplant rejection.[11] Therefore, it has been postulated that blockade of CXCR3 may play a beneficial role in the treatment of these diseases.[12]

High-throughput screening led to the discovery of structure (**1**) as a moderate CXCR3 antagonist.[13] Compound **1** inhibited binding of ^{125}I-IP10 to CXCR3 receptors expressed on activated peripheral blood lymphocytes with an IC_{50} of 0.25 μM (Table 1). In addition, **1** also inhibited calcium mobilization induced by MIG with IC_{50} of 0.50 μM. Pharmacokinetic studies with **1** in rats revealed high clearance (2.8 L/kg/h) after a 0.5 mg/kg iv dosing. The bioavailability after oral gavage dosing at 2 mg/kg is very low (1.5%). In this report, we describe our effort in improving the potency and pharmacokinetic properties of compound **1** that led to the identification of AMG 487 (**47**), which was selected for evaluation in clinical studies.

A series of quinazolinone derivatives were prepared as described in Scheme 1.[14] The critical intermediates **2** were easily synthesized by treatment of 2-aminobenzoic acid and the appropriate BOC-protected amino acid with triphenyl phosphite and pyridine, followed by reaction with anilines in one pot.[15] Deprotection of **2** using TFA provided the primary amines, which were subsequently converted to secondary amine **3** by reductive amination reactions with aldehydes. In the case where R^2 is dimethylamino methyl, the corresponding aldehyde was prepared by heating dimethylamino acetaldehyde dimethyl acetal in concentrated HCl at 100 °C for 1 h and neutralizing with 10 N NaOH at 0 °C (The prepared aldehyde in aqueous solution was used in the next step without purification.). Final functionalization

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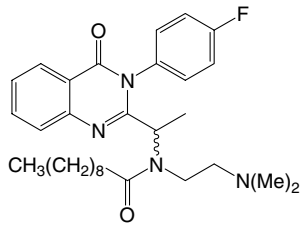
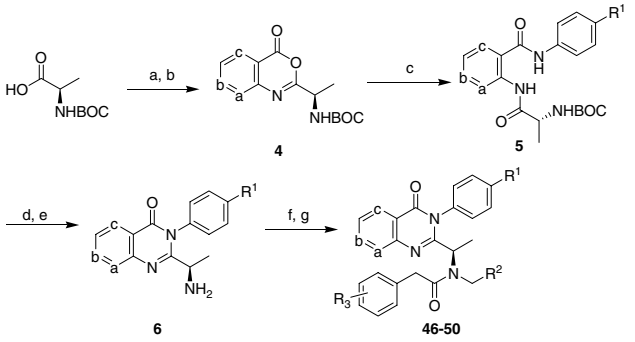
Table 1. Exploration of the stereocenter



1

Compound	^{125}I -IP10 IC_{50}[a,b] (μM)
1	0.25
1(R-enantiomer)	0.146
1(S-enantiomer)	>10

[a] Values are means of three experiments, standard deviation is ±30%.
[b] Displacement of ^{125}I-labeled IP10 from the CXCR3 receptor expressed on PBMC. See Ref. 16 for assay protocol.



Scheme 2. Reagents and conditions: (a) isobutylchloro- formate, NMM, DCM, −25 °C, 1 h; (b) 2-amino aza-benzene carboxylic acid, −25 °C, 12 h; (c) anilines, DCM, −25 °C, 2 h; (d) isobutylchloroformate, DCM, −25 to −15 °C, 6 h, 30% four steps; (e) TFA, DCM, rt, 2 h, >90%; (f) aldehydes, $Na(OAc)_3BH$, $ClCH_2CH_2Cl$, rt, 2 h, ~80%; (g) acetic acids, EDC, HOBt, DMF, rt, 1 h, 50–90%.

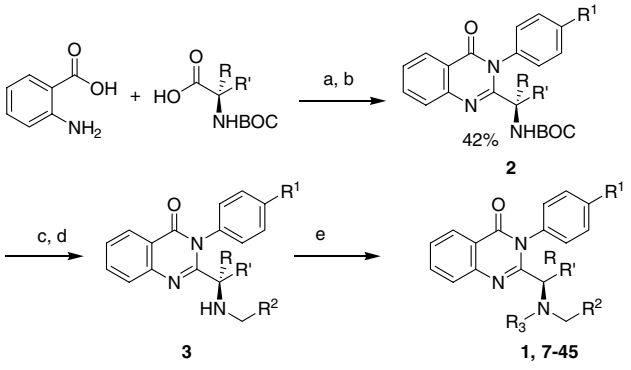
with the appropriate carboxylic acids afforded compounds **1**, **7**–**9**, **14**–**45**. Tertiary amines **10** and **11** were obtained by a second reductive amination of **3** with the respective aldehydes. Formation of sulfonamides **12** and **13** was achieved by treatment of **3** with the sulfonyl chlorides.

In addition, a series of aza-quinazolinones were synthesized according to Scheme 2. The cyclization method from Scheme 1 resulted in significant racemization in the case of 2-amino aza-benzene carboxylic acid. Thus, the oxazinones **4** were obtained by addition of the appropriate 2-amino aza-benzene carboxylic acid to a solution of the amino acid pretreated with isobutylchloroformate. Ring opening by anilines provided bisamides **5**, which upon treatment with isobutylchloroformate followed by deprotection with TFA afforded the amine **6**. Amine **6** was converted to compounds **46**–**50** using transformations described in Scheme 1.

The lead optimization was primarily guided by a ^{125}I-IP10 ligand replacement assay.[16] Table 1 shows the significant difference in activty between the enantiomers of **1**. Enantioselective synthesis established that the R stereoisomer is the more active of the two enantiomers.

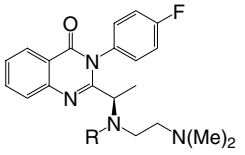
Our initial effort focused on identifying replacements for the long alkyl chain and the dimethylamine groups of **1** which were identified as the major metabolic sites in stability studies with microsomes. These groups were presumed to be responsible in part for the high clearance of the compound in vivo.

When evaluating replacements to the long alkyl-amide moiety, it was found that the amide carbonyl was important for activity (Table 2). Replacement of the amide by an amine **10**–**11** or a sulfonamide **12**–**13** resulted in significant loss of activity. However, it was discovered that the long alkyl amide group could be effectively replaced



Scheme 1. Reagents and conditions: (a) $P(OPh)_3$, pyridine, 70 °C, 3 h; (b) anilines, 55 °C, 1 h, ~50% for two steps; (c) TFA, DCM, rt, 2 h, >90%; (d) R^2CHO, $Na(OAc)_3BH$, $ClCH_2CH_2Cl$, rt, 2 h, 50–80%; (e) for amides: acetic acid, EDC, HOBt, DMF, rt, 1 h, 50–90%; for sulfonamides: sulfonyl chloride, pyridine, rt, 3 h, 60%; for amines: aldehydes, $Na(OAc)_3BH$, $ClCH_2CH_2Cl$, rt, 2 h, ~80%.

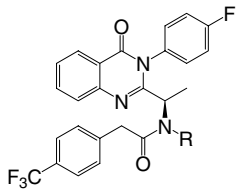
Table 2. Optimization of the amide moiety



Compound	R	^{125}I -IP10 IC_{50}[a,b] (μM)
1	$-CO(CH_2)_8CH_3$	0.146
7	$-CO(CH_2)_{10}CH_3$	0.154
8	$-CO(CH_2)_6CH_3$	0.375
9	$-CO(CH_2)_4CH_3$	>10
10	$-(CH_2)_8CH_3$	0.710
11	$-(CH_2)_7CH_3$	0.79
12	$-S(O)_2(CH_2)_9CH_3$	0.587
13	$-S(O)_2(CH_2)_7CH_3$	1.4
14	$-COCH_2$Ph-4-Ph	0.075
15	–COPh-4-Ph	>10
16	$-COCH_2$Ph	>10
17	$-COCH_2$Ph-4-CH_3	>10
18	$-COCH_2$Ph-4-CF_3	0.088
19	$-COCH_2$Ph-4-OCF_3	0.156

[a] Values are means of three experiments, standard deviation is ±30%.
[b] Displacement of ^{125}I-labeled IP10 from the CXCR3 receptor expressed on PBMC. See Ref. 16 for assay protocol.

Table 3. Exploration of *N*-substituents



Compound	R	^{125}I –IP10 IC_{50}[a,b] (μM)
18	$-(CH_2)_2N(CH_3)_2$	0.088
20	$-(CH_2)_2OMe$	0.30
21	$-(CH_2)_2OEt$	0.04
22	$-(CH_2)_2CH_3$	>10
23	$-CH_2$-2-thiazolyl	0.10
24	$-CH_2$-2-imidazoyl	0.23
25	$-CH_2$-4-imidazoyl	0.65
26	$-CH_2$-4-(1-methyl-imidazoyl)	0.24
27	$-CH_2$-2-pyridyl	0.073
28	$-CH_2$-3-pyridyl	0.013

[a] Values are means of three experiments, standard deviation is ±30%.
[b] Displacement of ^{125}I-labeled IP10 from the CXCR3 receptor expressed on PBMC. See Ref. 16 for assay protocol.

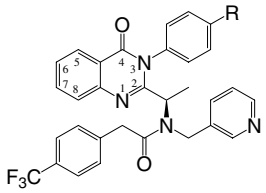
by a biphenyl-acetyl moiety (**14**). It was also discovered that the orientation of the biphenyl group played an important role in maintaining CXCR3 antagonistic activity. Removal of the methylene group from the biphenyl-acetamide moiety resulted in complete loss of activity (**15**). In addition, the importance of the terminal phenyl group was established with phenyl acetamide **16**, which exhibited significantly lower activity. The series of para-substituted phenyl acetamides **17**–**19** established that CF_3 and OCF_3 groups, but not CH_3 (**17**), could serve as a replacement for the terminal phenyl ring.

Evaluation of the dimethylamine moiety demonstrated that while this group is important for activity, it could be substituted by other polar groups (Table 3). Substitution of the dimethylamine group by a methyl group, as in **22**, resulted in significant loss of activity. However, the dimethylamine moiety could be effectively replaced by alkoxy (**20**–**21**) or a heterocycle (**23**–**28**).

Substitutions on the 3-*N*-phenyl were also explored (Table 4). Alkoxy and propargyl groups (**33**–**35**) were preferred for activity, however, the propargyl analogs exhibited significant CYP 3A4 inhibition. Fluoro, chloro, and cyano were also well tolerated (**30**, **31**, **37**), while inclusion of groups such as carboxylic acid, sulfone or amide resulted in dramatic reduction in potency (**38**–**40**).

Investigation of the 2-alkylamino group is illustrated in Table 5 with the *N*-ethoxyethyl-biphenylacetyl scaffold. Efforts to remove the stereo center by replacing the methyl with hydrogen (**41**) or adding an additional methyl group to the stereocenter (**43**) resulted in significant loss of CXCR3 activity (Table 5). The methyl group attached to the stereocenter can be replaced by an ethyl group (**44**) while maintaining potency. However, replacement of the methyl group by phenyl (**45**) resulted in decreased activity.

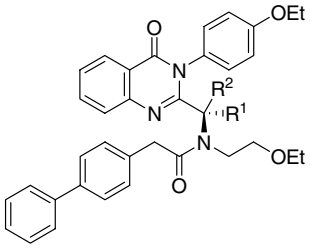
Table 4. Exploration of 4-phenyl substitutions



Compound	R	^{125}I -IP10 IC_{50}[a,b] (μM)
29	–H	0.299
30	–F	0.012
31	–Cl	0.022
32	$-CH_3$	0.025
33	$-OCH_3$	0.014
34	$-OCH_2CH_3$	0.006
35	$-C{\equiv}CCH_3$	0.004
36	$-NO_2$	0.007
37	–CN	0.011
38	$-SO_2Me$	10
39	–COOH	1.37
40	–NHCOMe	25

[a] Values are means of three experiments, standard deviation is ±30%.
[b] Displacement of ^{125}I-labeled IP10 from the CXCR3 receptor expressed on PBMC. See Ref. 16 for assay protocol.

Table 5. Exploration of the stereocenter



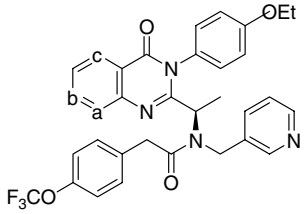
Compound	R^1	R^2	^{125}I -IP10 IC_{50}[a,b] (μM)
41	H	H	2.3
42	H	Me	0.075
43	Me	Me	>10
44	H	Et	0.009
45	H	Ph	4

[a] Values are means of three experiments, standard deviation is ±30%.
[b] Displacement of ^{125}I-labeled IP10 from the CXCR3 receptor expressed on PBMC. See Ref. 16 for assay protocol.

In order to increase polarity we explored the effect that introducing nitrogen atoms in the central quinazolinone core have in potency (Table 6). It was discovered that quinazolinone **46** and 8-azaquinazolinone (**47**, AMG 487) derivatives were similar in activity, while the 5-, 7-azaquinazolinone and 5,8-diazaquinazolinone derivatives (**48**–**50**) were significantly less potent.

Further evaluation of AMG 487 (**47**) demonstrated that, in addition of inhibiting ^{125}I-IP-10 binding, AMG 487 also inhibits binding of ^{125}I-ITAC to CXCR3 with an IC_{50} value of 8.2 nM. Evaluation of AMG 487 in in vitro functional assays demonstrated that it inhibits CXCR3-mediated cell migration by the three CXCR3 chemokines (IP-10 IC_{50} = 8 nM, ITAC IC_{50} = 15 nM, and MIG IC_{50} = 36 nM). Furthermore, AMG 487

Table 6. Replacement of the quinazolinone core



Compound	*a*	*b*	*c*	^{125}I -IP10 IC_{50}[a,b] (μM)
46	C	C	C	0.006
47	N	C	C	0.008
48	C	N	C	0.144
49	C	C	N	1.40
50	N	C	N	0.480

[a] Values are means of three experiments, standard deviation is ±30%.
[b] Displacement of ^{125}I-labeled IP10 from the CXCR3 receptor expressed on PBMC. See Ref. 16 for assay protocol.

Table 7. Pharmacokinetic profile of AMG 487 (**47**)

Parameter	Rat	Dog	Cyno
CL (L/h/kg)[a]	1.6	1.1	0.12
$t_{1/2}$ (h)[a]	9.3	0.4	7.6
F_{po} (%)[b]	12–57	85	19

[a] Following iv dosing in rat at 0.5 mg/kg and dog and cyno at 1 mg/kg.
[b] Following po dosing in rat at 2 mg/kg, dog at 2.5 and cyno at 5 mg/kg.

inhibits calcium mobilization in response to ITAC (IC_{50} = 5 nM).

The pharmacokinetic parameters of AMG 487 were markedly improved relative to (**1**). AMG 487 showed moderate to low clearance after iv dosing in rat, dog or cyno and was well absorbed after oral administration in rat and dog (Table 7).

In order to evaluate the ability of AMG 487 to inhibit cell migration in vivo we used a mouse model of bleomycin-induced cellular recruitment into the lung. In this model bleomycin is introduced in the lungs of mice via intra-tracheal instillation after tracheostomy.[17] Six days after bleomycin challenge a bronchoalveolar lavage (BAL) is performed and the number of cells collected in the BAL are counted using a hemocytometer. All but the lowest dose treatment group exhibited significant reduction in cellular infiltration into the lungs ($p < 0.005$ as determined by Student's *t*-test). A dose of 3 mg/kg given subcutaneously twice daily gave similar migration inhibition results as the CXCR3 deficient mice (n = 8–12 mice per group) (Fig. 1).

Based in part on the evaluation of CXCR3 activity and pharmacokinetic studies, we selected AMG 487 (**47**) as a candidate compound for evaluation in clinical studies.

In summary, we have discovered and optimized a novel series of potent CXCR3 antagonists. Several observations, including identification of the R-enantiomer as the active molecule, discovery of fluorinated-phenyl acyl groups and 3-pyridylmethyl as replacements of the alkyl

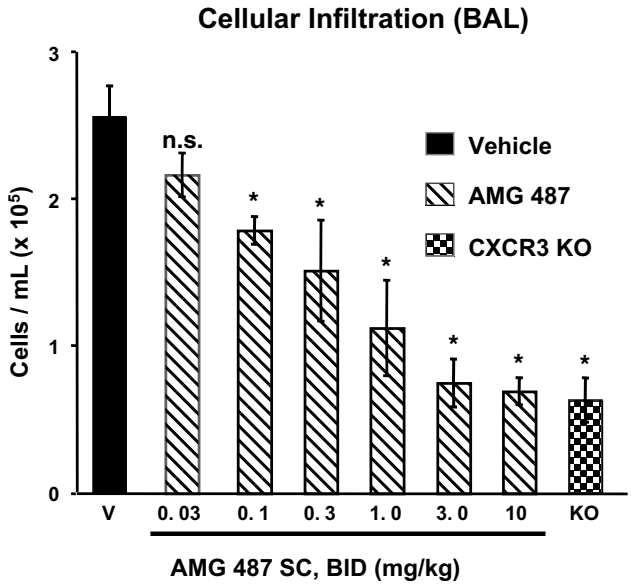


Figure 1. Evaluation of AMG 487 in a mouse bleomycin model of cellular recruitment. $^{*}p < 0.005$ as determined by Student's *t* tests.

moiety and the dimethylaminoethyl moieties of the lead compound, led to the discovery of **47** (AMG 487) which exhibited good potency in binding and functional assays and good in vivo pharmacokinetic properties across species. This compound was shown to inhibit in vitro cell migration mediated by IP-10, Mig, and ITAC. Furthermore, this compound is a potent inhibitor of cellular recruitment in vivo using a bleomycine-mouse model.

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