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Potent small molecule CCR1 antagonists

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Abstract—The present manuscript details structure–activity relationship studies of lead structure **1**, which led to the discovery of CCR1 antagonists >100-fold more potent than **1**.

The preceding manuscript in this series highlighted fundamental SAR work in our CCR1 (CC-chemokine receptor-1) antagonist program aimed at finding potent small molecule drugs for the treatment of autoimmune diseases and organ transplant rejection.[1] This effort began with the identification of the structurally unique screening hit, compound **1** (Fig. 1). This compound was shown to be a micromolar inhibitor of both CCL3 binding and CCL3 induced chemotaxis of THP-1 cells.[2,3] Initial medicinal chemistry efforts demonstrated that the absolute stereochemistry present in compound **1** (2*S*,4*S*,5*R*) was required for optimal potency. Additional work provided compound **2**, the 5-benzyl variant of **1** whose increased activity in both CCL3 binding and chemotaxis assays validated it as a suitable lead for further optimization.

Aiming to improve upon the potency of **2**, we set out to investigate SAR involving the N-terminal amide functionality. In doing so, our primary aim was to systematically investigate the heteroaromatic moiety. A series of amide derivatives (Table 1) was prepared in two steps from lactone **3**.[4,5] The loss in activity observed with the naphthalene-2-carboxamide analog **4** highlighted the importance of the quinoline nitrogen present in **2**. Furthermore, it was also discovered that the positioning of

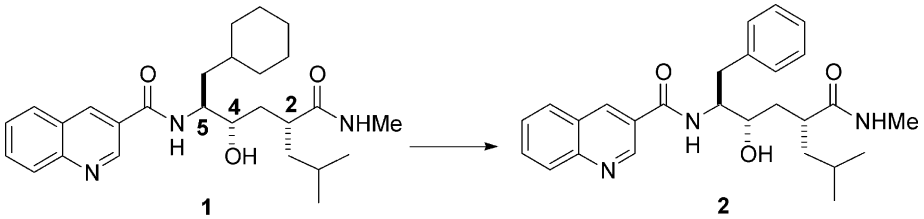

Figure 1.

Keywords: CCR1; Chemokine.
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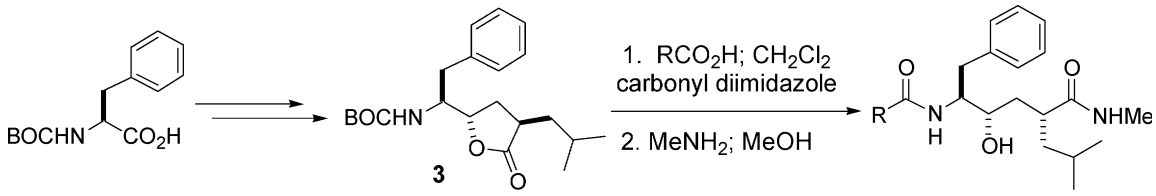
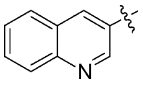
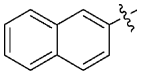
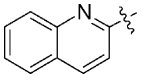
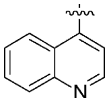
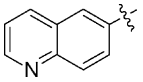
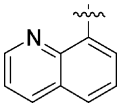
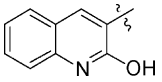
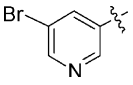
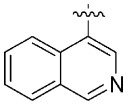
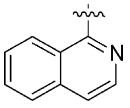
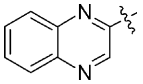
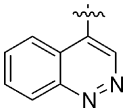
Table 1.

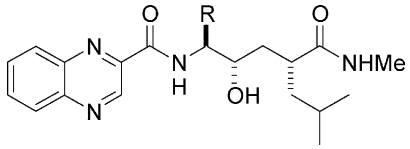


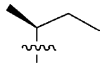
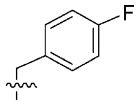
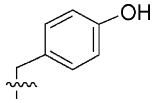
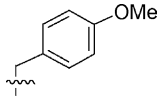
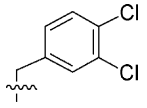
Compound	R	CCL3 binding IC_{50} (μM)	CCL3 chemotaxis IC_{50} (μM)
2		0.65	0.46
4		10.3	>25
5		0.13	0.89
6		18.5	4.3
7		2.49	3.30
8		>32	>25
9		16.7	>25
10		>32	>25
11		0.51	NA
12		5.15	6.60
13		0.58	2.40
14		0.064	0.064
15		2.57	1.10
16		>32	>25

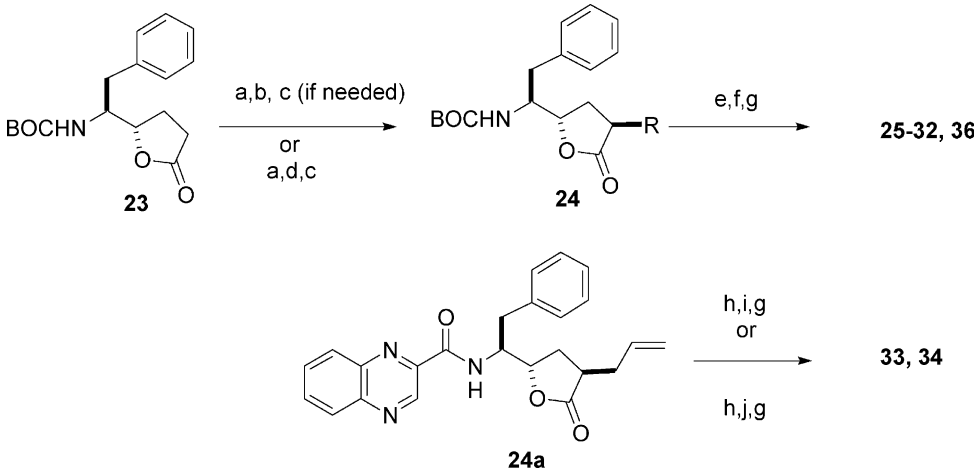
the carboxamide functionality on the quinoline ring played an important role in determining the potency of analogs. The quinoline-2-carboxamide **5** was notable in that it provided improved inhibition of CCL3 binding, but in contrast, the corresponding quinoline-4-carboxamide, -6-carboxamide, and -8-carboxamide derivatives (**6**–**8**, respectively) showed decreased activity both in CCL3 binding and chemotaxis assays. In addition, the 2-hydroxyquinoline-3-carboxamide analog **9** showed a considerable loss in activity relative to the parent quinoline-3-carboxamide. Removing the fused phenyl ring from the quinoline also proved detrimental, as the nicotinamide **10** was completely inactive. However, the corresponding 5-bromo-nicotinamide **11** provided activity comparable to quinoline **2**, possibly due to an increase in lipophilicity and/or a decrease in the Lewis basicity of the pyridine nitrogen. Converting the quinoline-carboxamide to an isoquinoline-carboxamide provided mixed results with the isoquinoline-4-carboxamide **12** losing a log in activity and the isoquinoline-1-carboxamide **13** nearly equipotent to **2** in the CCL3 binding assay. Building on the finding that optimal activity was achieved when the amide functionality was one or two carbon atoms removed from the heteroaromatic nitrogen and the implication that lipophilicity and/or decreased Lewis basicity led to increased potency, we prepared the quinoxaline-2-carboxamide **14**. This arrangement of the heteroaromatic nitrogens relative to the carboxamide functionality and each other proved quite favorable and provided a log increase in potency relative to **2**. In comparison, the related cinnoline-3-carboxamide derivative **15** was slightly less active than the parent quinoline-carboxamide. In addition to a favorable relative placement of the heteroaromatic nitrogens, lipophilicity also played an important role in the activity of **14**. This was evident in that the pyrazine-2-carboxamide derivative **16** was completely inactive.

Having discovered a more optimal N-terminal amide, we turned our attention to modifications of the C-5 benzyl group. The presence of the quinoxaline-2-carboxamide did not alter the C-5 SAR trend reported previously[1] as the benzyl substituent in **14** provided the best potency, followed by the cyclohexylmethyl (**17**) and 2-methyl-propyl (**18**) groups (Table 2). These results focused our attention squarely on the 5-benzyl derivative, which served as the starting point for an investigation into the effect of substitution of the phenyl ring.

Table 2.

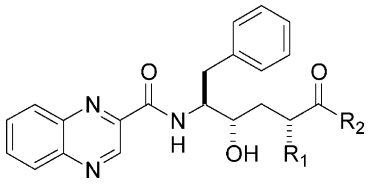


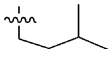
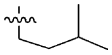
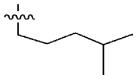
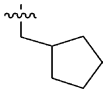
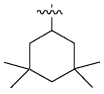
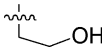
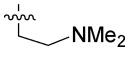
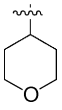
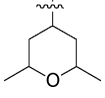
Compound	R	CCL3 binding IC_{50} (μM)	CCL3 chemotaxis IC_{50} (μM)
14		0.064	0.064
17		0.67	0.56
18		4.2	NA
19		2.6	3.0
20		0.61	1.3
21		2.9	2.0
22		0.16	0.15



Scheme 1. Reagents and conditions: (a) LHMDS or LDA, THF, −78 °C; (b) ROTf or RBr; (c) 10% Pd/C, 35 psi H_2, THF/MeOH; (d) ketone, Burgess reagent, benzene, reflux; (e) TFA, CH_2Cl_2; (f) quinoxalyl chloride, TEA, CH_2Cl_2; (g) amine, MeOH, rt; (h) O_3, MeOH, SMe_2; (i) $NaBH_4$, MeOH; (j) $NaBH(OAc)_3$, $HN(CH_3)_2$, dichloroethane.

Table 3.



Compound	R1	R2	CCL3 binding IC_{50} (μM)	CCL3 chemotaxis IC_{50} (μM)
14		NHMe	0.065	0.064
25		NHMe	0.022	0.008
26		NH_2	0.028	0.002
27		NH_2	0.42	0.069
28		NH_2	0.048	0.012
29		NH_2	0.026	0.028
30		NH_2	0.008	0.005
31		NH_2	44.8	4.6
32		NH_2	0.28	0.12
33		NH_2	4.6	>25
34		NH_2	55.1	>25
35		NH_2	3.5	12.5
36		NH_2	0.015	0.005

To this end, 4 differentially substituted benzyl derivatives (**19**–**22**) were prepared beginning with the appropriately substituted Boc-amino acid. With the exception of the 3,4-dichlorobenzyl derivative **22**, the remaining three analogs were ⩾10–fold less active than their unsubstituted counterpart.

Concurrent with work around the C-5 group was an effort investigating alternative functionality at the C-2 position. Rather than the multi-step process involved to prepare analogs with alternative C-5 substituents, the synthesis of C-2 alkyl derivatives was much more straightforward, beginning with the common lactone intermediate **23**[4,5] (see Scheme 1). The differentiating step was the addition of an alkylating agent (allyl/benzyl bromide, alkyl triflate, or ketone) to the lithium enolate of **23**. The reaction proceeded with a high degree of stereoselectivity with allyl/benzyl bromides or alkyl triflates to provide **24**. Condensation reactions of the lithium enolate of **23** with cyclic ketones were less stereoselective producing separable mixtures of diastereomers, which, following a dehydration/hydrogenation

sequence (Burgess reagent; Pd/C, 35 psi H_2), provided **24**. Subsequent Boc deprotection, N-acylation with quinoxaline-2-carbonyl chloride and lactone opening with the appropriate amine ($MeNH_2$ or NH_3) provided the final compounds. For the preparation of **33** and **34**, the allyl intermediate **24a** was converted to the corresponding aldehyde via ozonolysis. Reduction with $NaBH_4$ provided the alcohol functionality of **33**; reductive amination provided the amine functionality of **34**.

A variety of lipophilic substituents were well tolerated at C-2. Homologation of the isobutyl C-2 substituent of **14** led to a modest improvement in potency (**25**) (Table 3). In an attempt to reduce lipophilicity, the primary amide **26** was prepared and found to be equipotent to the methyl amide **25**. Homologation of **26** led to diminished potency (**27**) suggesting that the C-2 substituent binds in a lipophilic pocket of defined dimensions. A variety of cycloalkyl substituents were well tolerated at C-2 (**28**–**30**). However, as alluded to above, large groups were not well tolerated (compare **30** and **31**). Benzyl substitution at C-2 provided moderate potency (**32**), however, incorporation of polar functionality at C-2 imparted greatly reduced potency (**33**–**35**). In some cases, masking polar functionality led to improved potency (compare **35** and **36**).

Potent analogs were evaluated in a number of secondary assays. For example, in addition to inhibiting CCL3 induced chemotaxis of THP-1 cells, compound **26** also effectively blocked chemotaxis induced by CCL5 (RANTES), the other primary ligand for CCR1. This represents a >100-fold increase in activity relative to the lead compound **1**. Activation of the CCR1 receptor leads to upregulation of the integrin CD11b on monocytes. Expression of this integrin plays an important role in monocyte adhesion and extravasation thereby leading to the delivery of these cells to inflammatory sites. Lead compound **1** demonstrated that CCR1 antagonism effectively inhibits CCL3 induced CD11b upregulation on monocytes in human whole blood ($IC_{50} \sim 5\,\mu M$). Compound **26** was also evaluated in this assay and found to be 10–20×more potent than lead compound **1**.[6]

In summary, optimization of the N-terminal heterocycle and C-2 alkyl group of lead compound **1** led to a number of CCR1 antagonists with enhanced potency.

References and notes

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