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The discovery of structurally novel CCR1 antagonists derived from a hydroxyethylene peptide isostere template

John C. Kath,* Amy P. DiRico, Ronald P. Gladue, William H. Martin, Eric B. McElroy, Ingrid A. Stock, Laurie A. Tylaska and Deye Zheng

Pfizer Global Research and Development, Eastern Point Road, Groton, CT 06340, USA

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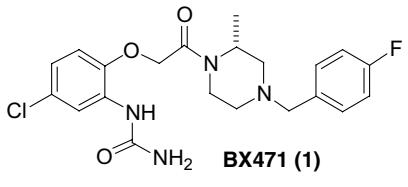
Abstract—The present manuscript details the discovery and early fundamental structure–activity relationship studies involving compound **3**, a novel hydroxyethylene peptide isostere derived molecule that provides micromolar inhibition of CCL3 binding to its receptor CCR1. Initial studies established this screening hit as a legitimate lead for further medicinal chemistry optimization.

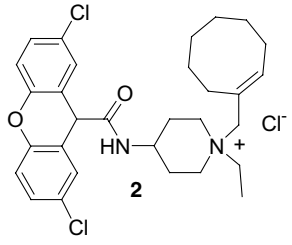
Chemokines are low molecular weight proteins (8–10 kDa) that are best known for their potent chemotactic activity.[1–3] They exert their functions by interacting with 7-transmembrane (7TM) G-protein coupled receptors that are differentially expressed on subsets of leukocytes. Chemokines are divided into four subclasses (CC, CXC, CX_3C, and C) depending on the spacing between their N-terminal cysteine residues. The majority of chemokines are 'inducible proteins', that are upregulated in response to inflammatory stimuli suggesting that they play a pivotal role in the progression of autoimmune diseases,[4–7] allograft rejection,[8,9] asthma,[10] atherosclerosis,[11] AIDS,[12,13] and cancer.[14]

The CC-chemokine receptor-1 (CCR1) is expressed on monocytes, T cells, immature dendritic cells, eosinophils, and in some cases neutrophils. CCR1, as well as its primary ligands, CCL3 (MIP-1α), and CCL5 (RANTES) have been shown to be expressed at inflammatory sites in several diseases. Furthermore, neutralizing antibodies and/or receptor deficient animals have provided evidence that blockade of CCR1 may be beneficial for of the treatment of multiple sclerosis, rheumatoid arthritis, and allograft rejection.[15] Recently, small molecule antagonists of CCR1 have been reported by Berlex (**1**)[16] and Banyu (**2**).[17] The most well characterized of these, BX-471 (**1**) has shown efficacy in animal models of multiple sclerosis and organ transplant rejection[18,19] and is reported to be in clinical trials.[20]

All of the known CCR1 antagonists, in addition to the majority of other small molecule chemokine receptor antagonists, are structurally related in that most possess a positively charged nitrogen under physiological conditions.[21] This common structural element is typical not only for small molecule chemokine receptor antagonists



BX471 (1)



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* Corresponding author. Tel.: +1-860-441-3852; fax: +1-860-441-4111; e-mail: john_c_kath@groton.pfizer.com

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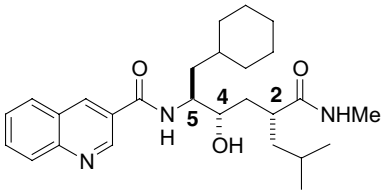
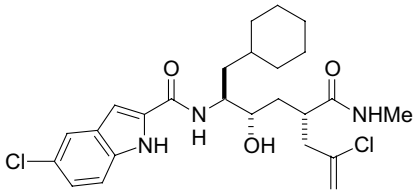


3

CCL3 binding: IC_{50} 2.3 μM
CCL3 chemotaxis: IC_{50} 0.84 μM
CCL5 chemotaxis: IC_{50} 0.63 μM
Renin: IC_{50} >10 μM



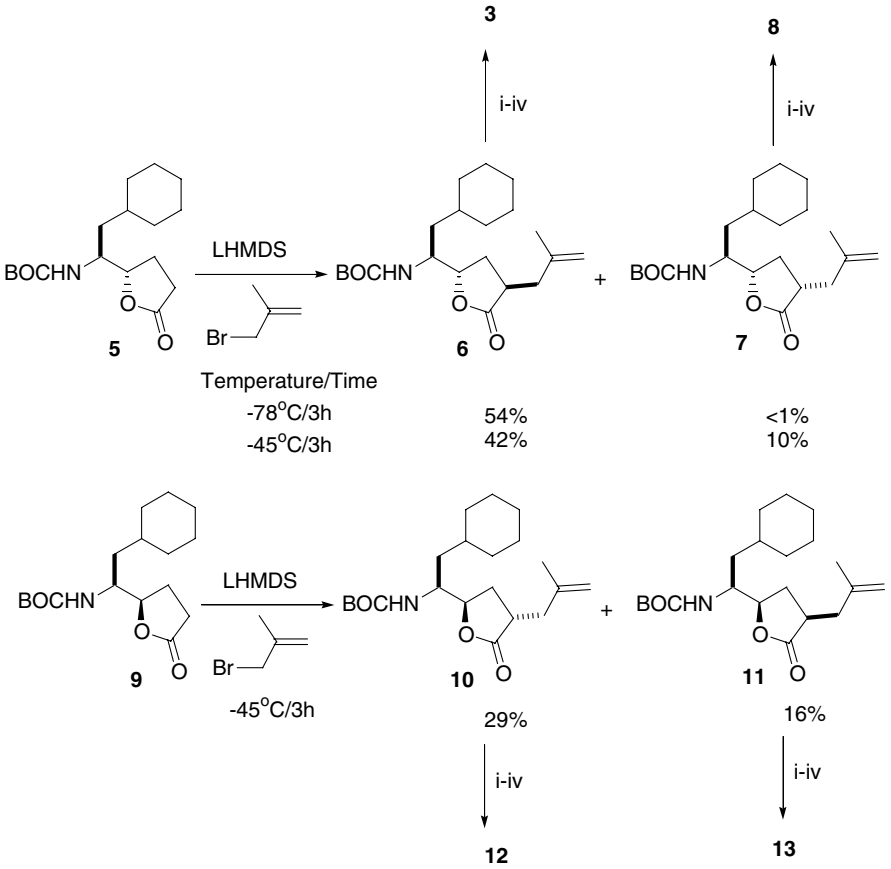
4

Renin: IC_{50} 0.041 μM
CCL3 binding: IC_{50} >32 μM
CCL5 chemotaxis: IC_{50} >25 μM

Figure 1.

but, in a larger sense, to small molecule G-protein coupled receptor antagonists. We disclose here the discovery and preliminary structure–activity relationships of a structurally novel class of chemokine receptor antagonists.

In the course of screening our compound file for inhibitors of ^{125}I–CCL3 binding to CCR1, compound **3** was identified as a potential lead compound (Fig. 1). Compound **3**, which contains a hydroxyethylene peptide isostere more commonly found in aspartyl protease inhibitors, is a 2.3 μM inhibitor of CCL3 binding and blocks both CCL3 and CCL5 induced chemotaxis of THP-1 cells at submicromolar levels. Compound **3** was originally prepared for a renin inhibitor program, where structurally similar 5-chloro-indole-2-carboxamide derivatives such as **4** provided optimal activity. In contrast, the quinoline-3-carboxamide found in **3** rendered it inactive against renin. Within the subset of compounds prepared for the renin program, the CCR1 activity of **3** was unique. Compound **4** and other structurally similar renin inhibitors did not inhibit CCL3 binding nor CCL3 and CCL5 induced chemotaxis of THP-1 cells. Because (a) it was felt that **3** represented an atypical chemotype for a 7TM antagonist and (b) the set of hydroxyethylene peptide isostere-derived renin



Scheme 1. Reagents and conditions: (i) H_2; Pd/C; EtOH; rt, (ii) TFA, CH_2Cl_2; 0 °C, (iii) quinoline-3-carboxylic acid; EDCI, CH_2Cl_2; rt, (iv) $MeNH_2$, MeOH; rt.

inhibitors in our file was limited to the 2*R*,4*S*,5*S* stereochemistry; our initial exploration of SAR involved the synthesis of the remaining seven possible stereoisomers of **3**.

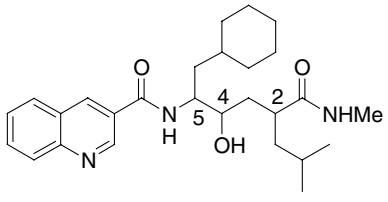
Compound **3** was synthesized starting with known lactone **5**, which can be prepared by a variety of methods.[22,23] Low temperature alkylation of **5** with methallyl bromide provided lactone **6** nearly exclusively (see Scheme 1). Lactone **6** was converted to compound **3** in 83% overall yield by hydrogenation of the alkene, removal of the Boc protecting group, formation of the N-terminal quinoline-3-carboxamide, and ring opening of the lactone with methylamine to form the C-terminal methyl amide. Preparation of the 2*S* diastereomer of compound **3** required lactone **7**, which was not obtained in any appreciable amount in the aforementioned −78 °C alkylation. However, carrying out the alkylation at a higher temperature (−45 °C) reduced the diastereoselectivity thereby allowing adequate quantities of lactone **7** to be obtained after separation from **6** by silica gel chromatography. Following the identical four steps required for the synthesis of **3**, diastereomer **8** was obtained in similar yields.

The precursor for the preparation of the two remaining analogs possessing 4*R*,5*S* stereochemistry was lactone **9**, the minor diastereomer formed in the synthesis of **5**. The nonselective alkylation of the lithium enolate of **9** with methallyl bromide at −45 °C provided lactone diastereomers **10** and **11** in a 2:1 ratio. The two diastereomers were separated by silica gel chromatography and their relative stereochemistry confirmed by an X-ray crystal structure of **10**. Both **10** and **11** were transformed to the 2*S*,4*R*,5*S* and 2*R*,4*R*,5*S* stereoisomers **12** and **13** employing chemistry used to obtain **3** and **8**. Identical procedures starting with the enantiomers of **5** and **9** (*ent*-**5** and *ent*-**9**) provided *ent*-**3**, *ent*-**8**, *ent*-**12**, and *ent*-**13**.

With this set of eight stereoisomers in hand, comparative testing for both inhibition of CCL3 binding to CCR1 and inhibition of CCL3 induced chemotaxis of THP-1 cells was undertaken. The results from these assays (perhaps surprisingly) confirmed the superiority of the original 2*R*,4*S*,5*S* stereochemistry present in the initial lead **3** (Table 1). Furthermore, the apparent stereospecificity of these interactions provided initial evidence that compound **3** was a viable lead.

Having answered the questions regarding relative and absolute stereochemistry, attention was turned toward other fundamental components of **3**. Beginning at the N-terminal amide, preparation of the corresponding quinoline-3-*sulfonamide* **14** spoke to the importance of N-terminal *amide* functionality contributing to CCR1 activity (Table 2). At the opposite end, the C-terminal *N*-methyl amide was replaced with a *N*,*N*-dimethyl amide **15** and a primary amide **16**. While removal of a hydrogen bond donor in the C-terminal amide completely abolished activity, the removal of the *N*-methyl to provide a primary amide only resulted in a moderate loss of potency. Also evident early on was that C-2 alkyl functionality was a critical component contributing to the activity of these molecules as removal of the C-2 isobutyl group (**17**) abolished activity. At the C-5 position, replacement of the cyclohexylmethyl group with the considerably smaller and less lipophilic isobutyl group (**18**) resulted in a 5-fold loss of potency while the differentially branched 2-butyl isomer **19** was inactive. These results suggested that the C-5 position might be a fruitful area for additional SAR exploration. Indeed, continuing efforts at the C-5 position next focused on replacing the original cyclohexylmethyl group with a benzyl group (**20**), which provided improvements in both CCL3 binding as well as CCL3 induced chemotaxis relative to **3**. That compound **20** was even more potent when tested for inhibition of CCL5 induced chemotaxis (IC_{50}: 50 nM) only strengthened our conviction that this novel, nonbasic series of chemokine receptor antagonists had a great deal of potential. As a result, the 5(*S*)-benzyl-4(*S*)-hydroxy-2(*R*)-alkyl-5-amino-pentanoic acid template found in **20** was used as a platform for lead

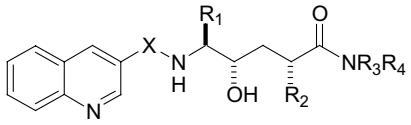
Table 1.

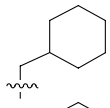
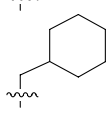
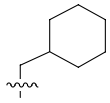
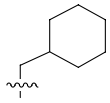
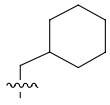
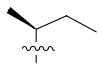
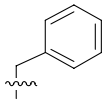


Compound	C2 stereochem.	C4 stereochem.	C5 stereochem.	CCL3 binding[24] IC_{50} (μM)[a]	CCL3 chemotaxis[25] IC_{50} (μM)[a]
3	*R*	*S*	*S*	2.3	0.77
8	*S*	*S*	*S*	30	12.0
12	*S*	*R*	*S*	Inactive	7.70
13	*R*	*R*	*S*	Inactive	Inactive
ent-**3**	*S*	*R*	*R*	15	Inactive
ent-**8**	*R*	*R*	*R*	Inactive	Inactive
ent-**12**	*R*	*S*	*R*	Inactive	Inactive
ent-**13**	*S*	*S*	*R*	Inactive	Inactive

[a] Compounds deemed inactive provided <50% inhibition at the highest concentration tested (32 μM in the binding assay and 25 μM in the chemotaxis assay).

Table 2.



Compound	X	R_1	R_2	R_3	R_4	CCL3 binding IC_{50} (μM)	CCL3 chemotaxis IC_{50} (μM)
3	CO			H	Me	2.3	0.84
14	SO_2			H	Me	Inactive	Inactive
15	CO			Me	Me	Inactive	Inactive
16	CO			H	H	8.0	6.3
17	CO		H	H	Me	Inactive	Not tested
18	CO			H	Me	11.8	Not tested
19	CO			H	Me	Inactive	Inactive
20	CO			H	Me	0.65	0.46

development efforts aimed at finding CCR1 antagonists with improved potency.

Acknowledgements

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