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Bioorganic & Medicinal Chemistry Letters 14 (2004) 2175–2179
Bioorganic & Medicinal Chemistry Letters

Novel CCR1 antagonists with improved metabolic stability

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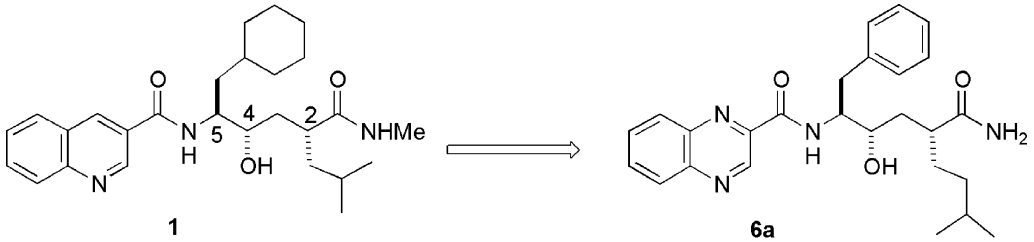
Received 8 January 2004; revised 4 February 2004; accepted 5 February 2004

Abstract—The synthesis, biological activity, and pharmacokinetic profile of novel CCR1 antagonists are described.

Chemokines represent a growing family of proteins that have been shown to play an important role in leukocyte activation and migration.[1–3] The chemokines CCL3 (MIP-1α, macrophage inflammatory protein-1α) and CCL5 (RANTES, regulated on activation normal T-cell expressed) bind to their shared receptor, CCR1, which is expressed on a number of cell types including monocytes, immature dendritic cells, and T cells. Blocking the action of CCR1 signaling may be useful for the treatment of diseases in which these cells play a role including autoimmune diseases and organ transplant rejection.[4,5]

Recently we describe a novel series of CCR1 antagonists derived from compound **1**.[6,7] Through optimization of the N-terminal, C-5 and C-2 substituents, a variety of analogues with much improved potency in both a ^{125}I-CCL3 binding assay[8] as well as a CCL3 induced chemotaxis assay[9] were identified (e.g. **6a**). While efforts were ongoing to optimize potency in this series, parallel efforts were ongoing to optimize pharmacokinetic parameters. For example, in vitro oxidative metabolic stability was evaluated by measuring the rate of drug consumption in human liver microsomes (HLM) thus providing intrinsic clearance values (Cl_{int}). These values provide the medicinal chemist with a powerful tool for addressing metabolic issues. [10] Early on in the program, the majority of analogues in this series were rapidly metabolized by HLM, suggesting the potential for



Keywords: CCR1; Chemokine; Microsome.
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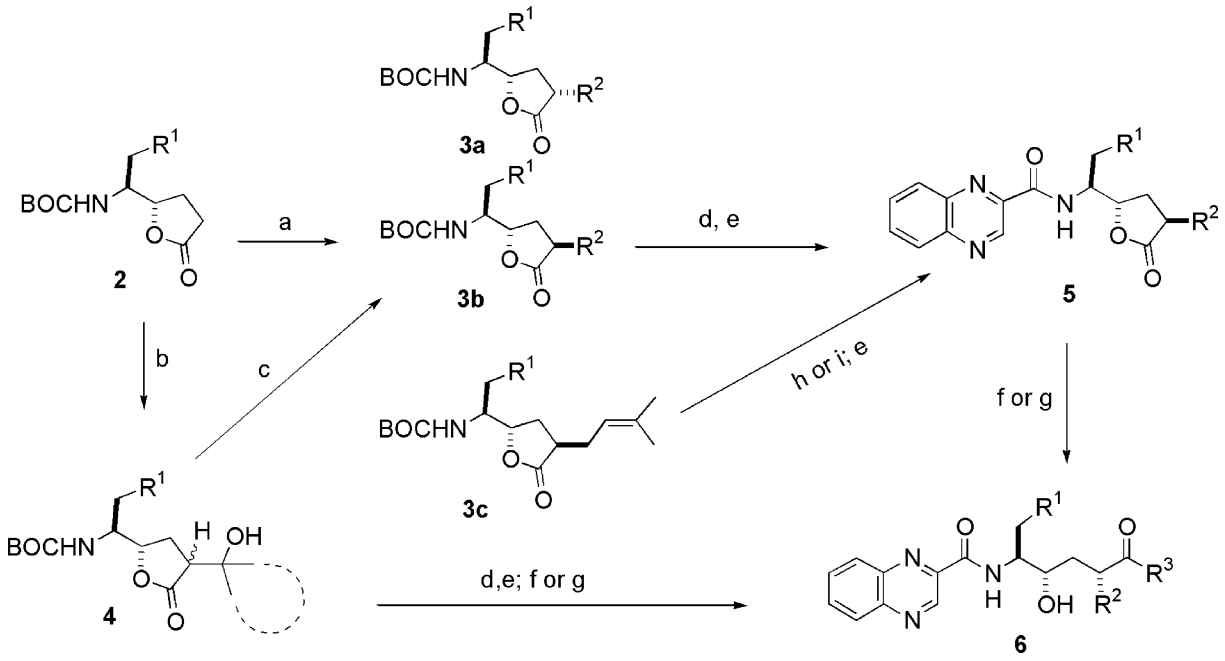
doi:10.1016/j.bmcl.2004.02.022



Scheme 1. Reagents and conditions: (a) LHMDS, THF, R^2OTf or R^2Br, −78 °C; (b) LDA, THF, ketone, −78 °C; (c) Burgess reagent, benzene, reflux; H_2 35 psi, 10% Pd/C, THF/MeOH; (d) TFA, CH_2Cl_2; (e) quinoxalyl chloride, TEA, CH_2Cl_2; (f) amine, MeOH, rt; (g) amine, HOAc, dioxane, reflux; (h) HF·pyridine, benzene; (i) TFA, neat.

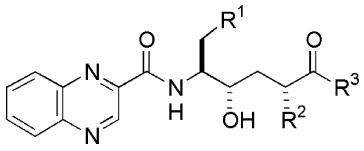
unacceptably high hepatic clearance in humans. Herein we further discuss the SAR of this series in the context of simultaneously improving HLM stability.

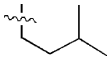
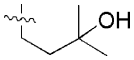
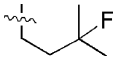
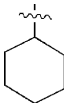
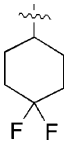
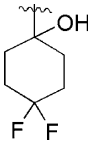
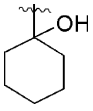
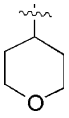
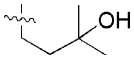
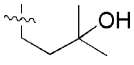
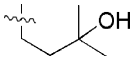
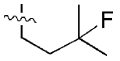
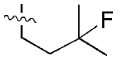
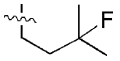
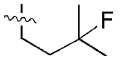
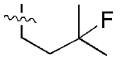
Compounds were prepared as depicted in Scheme 1. Alkylation of lactone **2**[6,7] was accomplished by enolate formation (LHMDS or LDA, THF) followed by addition of an alkyl bromide or triflate providing a separable mixture of diastereomers **3a/b** wherein **3b** was the major component. Removal of the Boc protecting group of **3b** followed by reacting the resulting amine with quinoxalyl chloride provided lactone **5**, which was then treated with the required primary or secondary amine to give the target analogue **6** with (2*R*,4*S*,5*S*) stereochemistry previously reported to provide the greatest potency.[6] Alternatively, reacting the enolate of lactone **2** with a cyclic ketone provided the hydroxy intermediate **4** as a mixture of diastereomers, which was readily converted to a separable mixture of diastereomers **3a/b** (Burgess reagent; 35 psi H_2, Pd/C) then converted to target analogues **6** as described above. For analogues containing cycloalkanol C-2 substituents (i.e., **6f**,**g**), intermediate **4** was directly converted to the final compounds by removal of the Boc protecting group, coupling the resultant amine with quinoxalyl chloride followed by lactone ring-opening (NH_3,MeOH). The fluorinated analogues **6c**,**l**–**q** were prepared by reacting the olefin **3c** with HF·pyridine to install both the methine fluorine as well as remove the Boc protecting group. Reacting the resulting amine with quinoxalyl chloride providing **5**. Tertiary hydroxyl analogues **6i**–**k** were prepared by treating **3c** with neat TFA, which converted the olefin to a trifluoroacetate and also removed the Boc protecting group. As before, reacting with quinoxalyl chloride gave the desired intermediate **5**, which when treated with the required amine simultaneously opened the lactone ring and deprotected the trifluoroacetate to give the final products **6**.

As described previously, low nanomolar potency in this series was achieved with a variety of analogues containing lipophilic C-2 substituents.[7] However, the majority of these compounds were found to undergo rapid metabolism when incubated with HLM. Experiments conducted to elucidate the site(s) of metabolism suggested that the C-2 alkyl substituent was particularly prone to oxidative metabolism. For example, incubation of compound **6a** in HLM produced a hydroxylated compound as the major metabolite.[11] In order to produce the metabolite in quantities sufficient for structure determination and potency evaluation, microbial bioconversion of **6a** was explored. Incubation of **6a** with *Streptomyces griseolus* (strain ATCC 11796) for 5 days provided milligram quantities of the metabolite whose structure was determined to be **6b**, demonstrating that oxidative metabolism of the C-2 sidechain had indeed occurred.[12] Not surprisingly, **6b** was found to be considerably less potent than the parent compound **6a** due to the reduced lipophilicity of the C-2 sidechain. However, we were encouraged by the significant improvement in HLM stability of **6b**, suggesting that the incorporation of C-2 substituents designed to block metabolism and/or provide reduced lipophilicity could lead to drug candidates with improved pharmacokinetic profiles. Indeed, replacement of the hydroxyl group in **6b** with a blocking fluorine provided an analogue with a lipophilic C-2 substituent that displayed excellent potency and metabolic stability (**6c**).

Now that we had demonstrated the ability to produce a potent, microsome stable compound, we attempted to apply this 'metabolic blocking' strategy to other potent

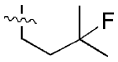
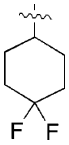
Table 1.



Compound	R^1	R^2	R^3	CCL3 binding IC_{50} (μM)	CCL3 chemotaxis IC_{50} (μM)	Cl_{int} (mL/min/kg)	ClogP
6a	Phenyl		$-NH_2$	0.028	0.002	202	4.1
6b	Phenyl		$-NH_2$	0.58	1.18	4.9	1.9
6c	Phenyl		$-NH_2$	0.009	0.013	8.0	3.4
6d	Phenyl		$-NH_2$	0.008	0.005	242	4.2
6e	Phenyl		$-NH_2$	0.020	0.022	35	3.6
6f	Phenyl		$-NH_2$	0.056	0.068	6.4	2.5
6g	Phenyl		$-NH_2$	0.054	0.12	47.7	3.1
6h	Phenyl		$-NH_2$	3.46	12.50	NT	1.8
6i	2-Fluorophenyl		$-NH_2$	0.052	0.68	16.5	2.0
6j	3-Fluorophenyl		$-NH_2$	0.046	0.065	6.0	2.0
6k	4-Fluorophenyl		$-NH_2$	>25	>25	NT	2.0
6l	Phenyl		–NHEt	0.075	0.13	NT	4.1
6m	Phenyl		–NHBn	0.85	0.44	299	5.5
6n	Phenyl		–Pyrrolidino	2.32	0.74	836	4.2
6o	Phenyl		4-*N*-Methyl piperazino	5.60	7.05	647	4.6
6p	Phenyl		–NHOH	0.027	0.031	3.6	3.0

(*continued on next page*)

Table 1. (*continued*)

Compound	R^1	R^2	R^3	CCL3 binding IC_{50} (μM)	CCL3 chemotaxis IC_{50} (μM)	Cl_{int} (mL/min/kg)	ClogP
6q	Phenyl		$-NHNH_2$	0.007	0.001	32.7	3.0
6r	Phenyl		–NHOH	0.007	0.008	6.1	3.2

analogues lacking microsome stability. For example, compound **6d**, which contains a C-2 cyclohexyl group, was one of the more potent analogues in the program. In this case, we did not perform experiments to determine the metabolic site(s), but thought it reasonable to assume that the 4-position of the cyclohexyl ring could be the liability. We then prepared a number of analogues to probe the feasibility of blocking potential metabolism at this site. The 4,4-difluoro analogue **6e** maintained good potency, but showed only a modest improvement in microsome stability. In contrast, the analogous compound containing a tertiary alcohol did show a significant improvement in microsomal stability (**6f**). The reason for the enhanced stability of **6f** versus **6e** was not entirely clear, however it may have been due to the reduced lipophilicity of **6f** relative to **6e** (ClogP: 2.5 vs 3.6).[13] Removal of the blocking fluorine atoms in **6f** to give **6g** resulted in diminished microsome stability, which could be explained by the greater polarity of **6f** versus **6g** (ClogP 2.5 vs 3.1) or perhaps the difluoro substitution is indeed blocking metabolism in this case. Incorporation of heteroatoms at the 4-position of the cyclohexyl ring provided analogues with greatly reduced potency (**6h**), in line with SAR suggesting the requirement for lipophilic substituents at this position.

Another area of focus involved follow-up to the hydroxy metabolite **6b**. While this analogue was significantly less potent than the des-hydroxy analogue **6a**, we felt it worthwhile to reexamine the SAR around this compound due to its promising metabolic stability. Exploration of the C-5 position revealed that a number of halobenzyl C-5 substituents imparted a significant improvement in potency. Most notably, the 3-fluorobenzyl analogue **6j** was shown to be >10-fold more potent than the desfluoro analogue **6b** and also retained excellent HLM stability. Interestingly, the SAR in this region of the molecule was quite sensitive to minor structural changes. For example, while the 3-fluorobenzyl analogue **6j** showed good potency, the closely related 4-fluorobenzyl analogue **6k** was inactive.

One final area of focus involved exploration of the C-terminal SAR using compound **6c** as the starting point. Substituted amides (**6l–o**) were generally less potent or offered no pharmacokinetic advantage when compared to the primary amide **6c**. However, the hydroxamic acid **6p** and hydrazide **6q** maintained potency and **6p** also displayed acceptable microsomal stability. Based on this finding, a number of additional hydroxamic acids were prepared, some of which had promising profiles (e.g. **6r**) (Table 1).

With a number of potent, HLM stable compounds in hand, additional information was necessary for further advancement toward a potential development candidate. Pharmacokinetic studies conducted in dogs and monkeys indicated that these compounds generally had moderate volumes of distribution (V_{dss}) and plasma clearances (Cl_p), resulting in elimination half-lives in vivo in the range of 0.9–1.7 h (Table 2). The hydroxamic acid analogues (compounds **6p** and **6r**) were found to undergo glucuronidation, resulting in their higher clearance rates.[14] For the series, oral bioavailability was often low (<10%), which may reflect on generally poor aqueous solubility. Indeed, the compound with the highest aqueous solubility (**6j**) also provided the highest oral bioavailability for this set of analogues. The HLM stable compounds in Table 2 were also evaluated for their ability to inhibit the upregulation of CD11b on monocytes induced by CCL3 in human whole blood.[15] Compounds in this series retained good activity in this assay as all the analogues in Table 2 provided IC_{50} values less than 0.5 μM.

Table 2.

Compound	Species	(%) F	Cl_p (mL/min/kg)	V_{dss} (L/kg)	$T_{1/2}$ (h)	Aqueous solubility (μg/mL)
6c	Dog	1.6	12	1.2	1.0	ND
	Monkey	2.9	18	1.2	1.2	
6j	Dog	49	13	1.0	1.4	157
	Monkey	9.5	18	1.3	0.92	
6p	Dog	3.4	28	1.2	1.7	21
	Monkey	6.5	29	1.5	1.1	
6r	Dog	NT	38	1.3	0.5	12
	Monkey	<1.1	33	1.8	1.1	

In summary, a number of potent CCR1 antagonists with improved HLM stablility were described. Analogues were designed to inhibit metabolism either by reducing lipophilicity or by addition of moieties to block metabolism. Additional studies with compound **6j** have revealed a high degree of selectivity for CCR1 versus a broad panel of receptors (including other chemokine receptors), as well as a high degree of selectivity for the human receptor.[16]

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11. As determined by mass spectral analysis.
12. **6b** was prepared by bioconversion of **6a** using *Streptomyces griseolus* strain ATCC 11796 following the procedures outlined in: Smith, R. V.; Rosazza, J. P. *Microbial Transformations of Bioactive Compounds*; Rosazza, J. P., Ed.; CRC: Boca Raton, FL, 1982; Vol. 2, pp 1–42. The metabolite was extracted from the broth with ethyl acetate following pH adjustment to 3.5 and partially purified using a Sep-Pak® C-18 cartridge. Final purification was achieved by HPLC on an Inertsil C8 column (250×200 mm) with a 65:35 H_20/CH_3CN mobile phase. ^1H NMR and mass spectral data was used to assign the structure.
13. Calculated with ClogP version 4.0 from BioByte.
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