Bioorganic & Medicinal Chemistry Letters 21 (2011) 7291–7294

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

CCR2 receptor antagonists: Optimization of biaryl sulfonamides to increase activity in whole blood

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ARTICLE INFO

Article history:
Received 2 September 2011
Revised 7 October 2011
Accepted 11 October 2011
Available online 19 October 2011

Keywords:
CCR2 antagonists
MCP-1
Biaryl sulfonamides
HSA
CCR2 knock-in mouse
Thioglycolate induced peritonitis

ABSTRACT

A series of biarylsulfonamides was identified as hCCR2 receptor antagonist but suffered from high plasma protein binding resulting in a >100 fold shift in activity in a functional GTPγS assay run in tandem in the presence and absence of human serum albumin. Introduction of an aryl amide with ethylenediamine linker led to compounds with reduced shifts and improved activity in whole blood.

The CCR2 chemokine receptor and its primary ligand, monocyte chemoattractant protein-1 (MCP-1), play key roles in attracting monocytes to sites of inflammation. At the site of an atherosclerotic lesion MCP-1 is up- regulated and, upon binding of MCP-1 to the CCR2 receptor, a signaling transduction cascade occurs and monocytes are recruited to the site of the lesion. The aggregation of these immune cells eventually leads to the formation of foam cells and the build-up of plaque on the arterial wall.[1] Deletion of CCR2 in apoE deficient mice, a murine model of human atherosclerosis, resulted in significant protection from both macrophage accumulation and atherosclerotic lesion formation in response to a high-fat diet.[2] Similar studies also showed that CCR2−/− mice fed a regular chow diet were more resistant to the development of atherosclerosis than wild-type mice.[3] Therefore, an antagonist for the CCR2 receptor was targeted as a potential treatment for atherosclerosis.[4] Additional therapeutic indications have also become very attractive including rheumatoid arthritis[5] and multiple sclerosis.[6] A wealth of patents and peer-reviewed journal articles have published in the past decade which describe the development of small molecule CCR2 antagonists.[7]

In a previous report,[8] a series of biarylsulfonamides CCR2 antagonists as exemplified by compound **1** were described (Table 1). Although compound **1** displays promising activity in our primary CCR2 GTPγS assay, there was a shift observed in the chemotaxis (CTX) assay when human serum was increased from 0.1% to 1.0% as shown in Table 1. Due to this loss in activity, our objective was focused on improving the activity of the sulfonamide series in the presence of human serum. To expedite compound evaluation, a high throughput GTPγS assay in the presence of 5% HSA was developed as a surrogate for human serum and run in parallel with the primary GTPγS assay in the absence of HSA. This allowed for rapid identification of compounds with reduced shifts in activity between the two assays. This approach was validated using compound **1** as a standard and when tested in the CCR2 GTPγS assay in the presence of 5% HSA[9,10] the activity of **1** dropped by ~120 fold.

Our chemistry strategy was centered on modulating the polarity and/or basicity of the biaryl sulfonamides to reduce the observed shift in activity. Ultimately, the introduction of polar groups to the left-hand side of the molecule led to a subseries of ethylenediamine linked amides which had significantly reduced shifts in the presence of HSA. Although ortho, meta, and para-substitution all showed improvement, para-substituted amides had the highest activity in the primary assay with and without HSA (Table 2).

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doi:10.1016/j.bmcl.2011.10.038

Table 1
Biarylsulfonamide series identified as CCR2 antagonist

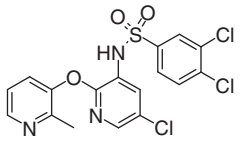
Compound	Structure	CCR2 fK_i (0% HSA) nM	CCR2 fK_i (5% HSA) nM	Fold shift	CTX %I @ 1 μM(0.1% HS)	CTX %I @1 μM(1% HS)	Rat PK (2 mg/kg, po)			
							$T_{1/2}$ (h)	Cl (mL/min/kg)	V_{dss} (L/kg)	F (%)
1		10	1259	126	72	50	2.3	4.3	0.34	75

Table 2
Shift was reduced in the presence of HSA with incorporation of amide

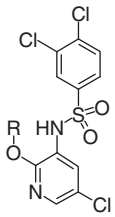
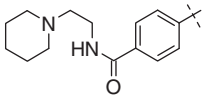
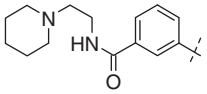
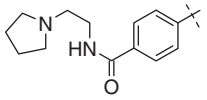
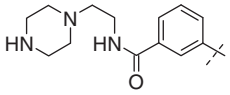
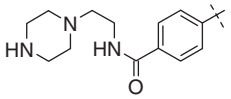
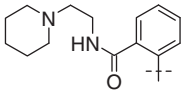
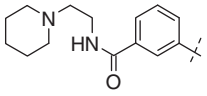
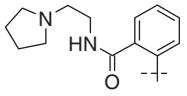
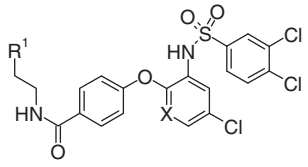
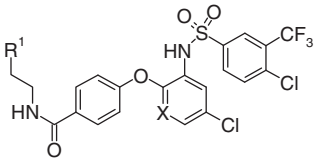
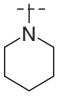
	#	R	CCR2 fK_i 0% HSA	CCR2 fK_i 5% HSA	Fold shift	#	R	CCR2 fK_i 0% HSA	CCR2 fK_i 5% HSA	Fold shift
	**2**		13	20	1.5	**6**		100	1000	10
	3		13	63	5	**7**		200	2511	13
	4		40	251	6	**8**		794	1585	2
	5		79	1259	16	**9**		631	2512	4

Table 3
CF_3 improves activity in both GTPγS assay and human whole blood shape change assay





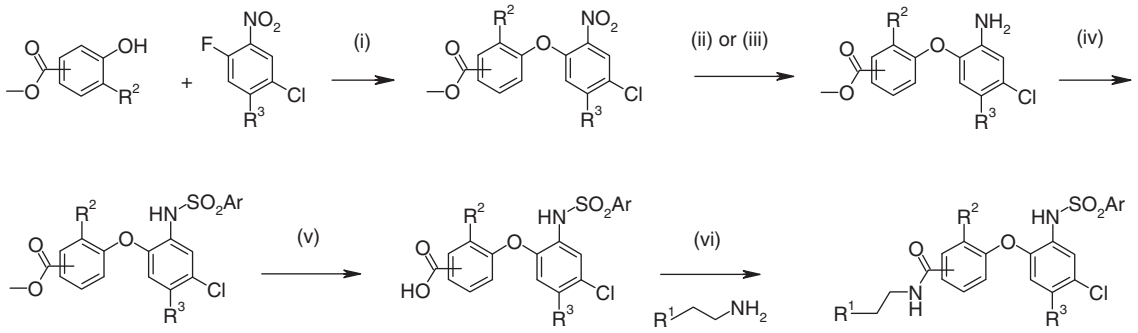
R^1	X	Compound	CCR2 fK_i (0% HSA)	WB shape change pA2	Compound	CCR2 fK_i (0% HSA) (nM)	WB shape change pA2
	C	**10**	63	5.5	**11**	10	6.2
	N	**2**	13	5.8	**12**	3	6.4

Optimization of the substitution on the benzene sulfonamide was also explored in parallel. It was determined that the 4-chloro, 3-trifluoromethyl substitution was the optimal benzene sulfonamide. This group gave consistent boost in activity in both the primary assay in the absence of HSA as well as in our low throughput human whole blood shape change assay[11] as demonstrated by compounds **11** and **12** (Table 3). Unfortunately, when compound **12** was progressed to rat PK studies, a significant loss in oral absorption was observed (Table 4) when compared to the original lead **1** (Table 1).

Due to the minimal activity difference observed in whole blood between the pyridyl and phenyl central cores (Table 3), we chose to move forward with the phenyl core based on a more efficient synthetic route to these compounds (Scheme 1).[12] The core 2-F, 5-Cl nitro arenes and aromatic alcohols were coupled under basic conditions. Reduction of the nitro group was then achieved using either nickel chloride and sodium borohydride or transfer hydrogenation with ammonium formate. Standard DMAP/pyridine

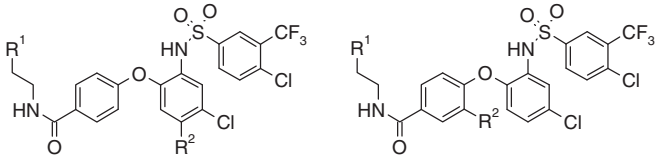
Table 4
Pharmacokinetic[13] profile of compounds **12** and **13**

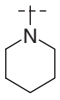
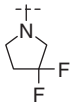
	12 Rat (1 mg/kg iv/2 mg/kg po)	13 Rat (1 mg/kg iv/1.5 mg/kg po)
C_{max} (ng/mL)	624	652
$T_{1/2}$ (h)	0.9	3.9
Cl (mL/min/kg)	39	17
V_{dss} (L/kg)	1.9	2.7
F (%)	10	37



Scheme 1. Typical reagents and conditions. (i) DMF, K_2CO_3, 70 °C; (ii) $NiCl_2$, $NaBH_4$, MeOH; (iii) $HCOONH_4$, Pd/C, DCM, 50 °C; (iv) $ArSO_2Cl$, DMAP, Pyridine 95 °C; (v) LiOH, MeOH/H_2O (1:1), 30 °C; (vi) Amine, BOP, TEA, DCM.

Table 5
Optimization of 4-Cl, 3-CF_3 benzenesulfonamide



R^1	R^2	Compound	CCR2 fK_i (0% HSA) (nM)	WB shape change pA2	Compound	CCR2 fK_i (0% HSA) (nM)	WB shape change pA2
	H	**11**	10	6.2	**11**	10	6.2
	Me	**14**	16	5.9	**20**	25	5.6
	MeO	**15**	10	6.4	**21**	25	6.0
	H	**16**	2	6.3	**16**	2	6.3
	Me	**17**	8	6.4	**22**	8	6.3
	MeO	**18**	4	6.4	**13**	6	6.6
	H	**19**	63	5.4			

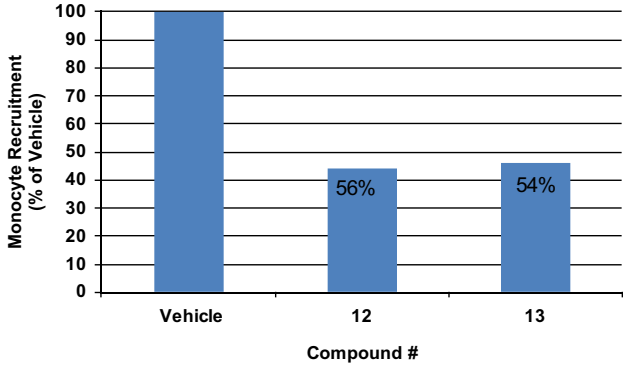


Figure 1. Effect on monocyte recruitment (40 mg/kg oral dose).

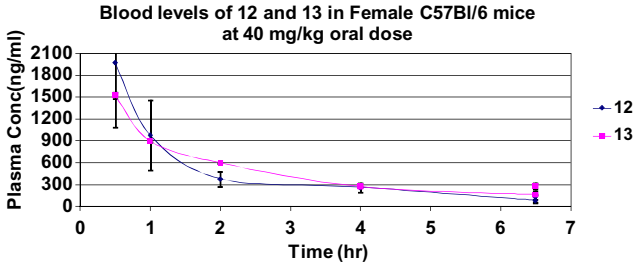


Figure 2. Blood levels of compounds **12** and **13** in satellite mice of in vivo study shown in Figure 1.

conditions proved to be optimal for coupling the resulting anilines with sulfonyl chlorides. Hydrolysis using LiOH afforded carboxylic acids which were then coupled with various amines under standard conditions to yield the desired amides.

With a convergent synthetic scheme in place, we were easily able to investigate substitution around the central and left-hand side phenyl rings to see what effect it may have on the activity and PK of the series. We believed that substitution of either of the phenyl rings may block a metabolic soft spot and/or change the orientation of the biaryl ether which could impact solubility and bioavailability. This led to the identification of compound **13** which has improved oral absorption and lower clearance with a longer half-life in comparison to compound **12** (Table 4). In addition, compound **13** is the most active biaryl sulfonamide tested in the whole blood assay (Table 5). Finally, the incorporation of the gem di-F into the pyrrolidine **19**, resulted in both a loss of activity in the primary assay as well as in human whole blood.

Two of the more promising compounds identified, **12**[14] and **13**,[15] were advanced into a thioglycolate induced peritonitis model in the humanized CCR2 knock-in mouse.[16] PK/PD predictions based on rat PK suggested a dose of 40 mg/kg was needed to achieve an ~80% reduction in monocyte recruitment for **12** and **13**. Surprisingly, the exposure observed in the C57Bl/6 mouse did not correlate well with that predicted from the rat PK. Assuming linearity, the expected

C_{max}'s for **12** and **13** were 12,000 and 17,000 ng/ml at 40 mg/kg oral dose. However, the observed exposure was ~10-fold less than expected (1520 and 1970 ng/ml—Fig. 2). Despite the reduced exposure, compounds **12** and **13** did exhibit significant reductions in monocyte recruitment (Fig. 1).

In conclusion, utilization of a parallel screening approach in which we ran the primary GTPγS assay in tandem with the GTPγS assay plus 5% HSA allowed for the rapid identification of compounds with improved activities in the presence of HSA. Additional optimization led to the identification of the 4-chloro, 3-trifluoromethylphenyl sulfonamides and the tethered amides which display significant improvements in activity in the presence of HSA and more importantly the whole blood assay. Significant reductions in monocyte recruitment were observed in vivo, despite decreased exposure in the mouse. In due course we will disclose alternative series' which were progressed in parallel with improved activities in vivo.

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10. 0.2% w/v HSA was used. That is equal to 5% of physiological concentration or 4% in whole blood.
11. (a) This human whole blood shape change assay was developed to further evaluate compounds in a more physiologically relevant media and was used to confirm the reduced shift observed in the screening assays; (b) Human blood from healthy donors was incubated for 15 minutes with CCR2 antagonist prior to stimulation with various concentrations of MCP-1 for 5 minutes. After stimulation, samples were rapidly fixed and analyzed by flow cytometry. Increases in monocyte Side-Scatter were analyzed and plotted against MCP-1 concentration, and pA2 values calculated by comparison to a dose response from vehicle treated blood.
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13. All studies using animals were conducted after review by the GSK Institutional Animal Care and Use Committee and in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.
14. Log*D*–3.2, tPSA–97, hERG pIC_{50} 5.3, CCR1 pIC_{50} <4.6, CCR4 pIC_{50} <5.0.
15. Log*D*–2.3, tPSA–101, hERG pIC_{50} 4.6, CCR1 pIC_{50} <4.6, CCR4 pIC_{50} <5.0.
16. (a) Prosser, H. M.; Cooper, D. G.; Forbes, I. T.; Geppert, M.; Gribble, A. D.; Grau, E. V.; Groot, P. H.; Harper, A. J.; Moores, K. E.; Pickering, S. J.; Piercy, V. *Drug Dev. Res.* **2002**, *55*, 197; (b) Henderson, R. B.; Hobbs, J. A. R.; Mathies, M.; Hogg, N. *Blood* **2003**, *102*, 328.