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Alkylsulfone-containing trisubstituted cyclohexanes as potent and bioavailable chemokine receptor 2 (CCR2) antagonists

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Abstract

We describe novel alkylsulfones as potent CCR2 antagonists with reduced hERG channel activity and improved pharmacokinetics over our previously described antagonists. Several of these new alkylsulfones have a profile that includes functional antagonism of CCR2, in vitro microsomal stability, and oral bioavailability. With this improved profile, we demonstrate that two of these antagonists, **2** and **12**, are orally efficacious in an animal model of inflammatory recruitment. CCR2

Keywords

CCR2; antagonist; Chemokine antagonist; GPCR

Monocyte chemoattractant protein-1 (MCP-1 or CCL2) is a CC chemokine overexpressed in many autoimmune and inflammatory conditions.¹ Its native receptor is CC chemokine receptor 2 (CCR2), which is a G protein-coupled receptor.² A primary function of this pair (MCP-1/CCR2) is the activation and migration of inflammatory cells to areas of inflammation. MCP-1 and CCR2 have been implicated in several diseases, including rheumatoid arthritis,³ atherosclerosis,⁴ multiple sclerosis⁵ and insulin resistance.⁶ This has resulted in a large effort focused on the design and synthesis of CCR2 antagonists.⁷ In this communication, we explore structural changes to a series of sulfone-containing CCR2 antagonists with the goal of reducing hERG channel activity and obtaining orally bioavailable compounds.

Recent reports from this laboratory have described the design and synthesis of cyclohexane-based CCR2 antagonists.⁸ The major focus of these early studies was to explore and define the SAR of this novel cyclohexyl template, so as to achieve maximum binding affinity and functional antagonism of CCR2. Unfortunately, the majority of our high affinity CCR2

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antagonists suffered from hERG channel inhibition: a well known liability within the chemokine antagonist field.⁹ In an effort to moderate this hERG liability, we explored structural changes that would lower our overall lipophilicity. As shown in Table 1, when the starting phenyl sulfone **1** was modified to the methyl sulfone **2**, the hERG inhibition was eliminated as observed via a hERG FLIPR assay. However, as is often the case in Log *P* lowering one can sacrifice binding affinity on the target, as was observed here for the methyl sulfone **2**, which lost almost 2-fold in CCR2 binding affinity¹⁰ as compared to **1**. Even though this transformation did eliminate the hERG activity, other channel binding issues were known to exist with our previous antagonists, and therefore, we employed a sodium channel binding assay¹¹ to help monitor this issue. In agreement with the hERG assessment, methylsulfone **2** also showed very little sodium channel binding (3% @ 10 μ M). Continuing with the modifications, we added a nitrogen to the trifluoromethylbenzamide to give **3**, which essentially retained the CCR2 binding affinity and channel profile of **2**. Compounds **4** and **5** had a *tert*-butyl group substituted on the benzamide (instead of a trifluoromethyl), and although they had improved CCR2 binding affinity versus **2** and **3**, respectively, compounds **4** and **5** did display an increase in sodium channel binding (52% and 46% @ 10 μ M, respectively). For other substitutions of the benzamide, both **6** (4-methyl-3-trifluoromethyl) and **7** (3-trifluoromethoxy) had a deleterious effect on CCR2 binding. From here, we turned to the ethylsulfone, but **8** showed an increase in sodium channel activity, and 3-phenylbenzamide **9** revealed more hERG channel activity. The *iso*-propylsulfone **10** increased the CCR2 binding affinity 6-fold as compared to **2** without displaying hERG inhibition. However, the combination of 3-*tert*-butylbenzamide and *iso*-propylsulfone to give **11** increased CCR2 binding but also increased hERG channel binding. *tert*-Butylsulfones also proved to be compatible with CCR2 as **12**, **13** and **14** all showed excellent CCR2 binding affinity with no hERG channel activity and only moderate sodium channel binding. From this data set, an increase in CCR2 binding affinity trended with the alkylsulfone group in this order: Me < Et < *t*-Bu < *i*-Pr.

As shown in Table 2, four alkylsulfones (**2**, **8**, **10**, and **12**) were selected for chemotaxis, in vitro microsomal incubation, Caco-2, hERG patch-clamp¹² and sodium patch-clamp¹³ evaluation. All four alkylsulfones displayed excellent chemotaxis values, hence confirming their ability to operate as potent functional antagonists. Another benefit of the alkylsulfone was observed in the in vitro microsomal stability assay, as all four alkylsulfones were extremely stable as compared to phenylsulfone **1**. In addition, although permeability was universally poor, as measured by Caco-2, the *tert*-butylsulfone **12** did show a measurable value. Three of these compounds (**2**, **8**, and **12**) were also taken into hERG and sodium patch-clamp assays. The patch-clamp values were in-line with the in vitro assessment and indicated a low to moderate liability.

With promising antagonists in hand, we selected two compounds for further evaluation in four species pharmacokinetic (PK) studies. As shown in Table 3, compound **2** displayed some oral exposure across the four species with dog being the best (*F*% = 51). Compound **2** showed a wide disparity in clearance (iv) with high clearance recorded in mouse (*CL* = 70 mL/min/kg) and low clearance recorded in dog (*CL* = 3 mL/min/kg). As shown in Table 4, the *tert*-butylsulfone **12** had more consistent oral bioavailability across the four species than

the methylsulfone **2**. This improved bioavailability for **12** may be a reflection of improved permeability as noted in the Caco-2 value. Compound **12** also had low clearance values across three species with rat being the outlier.

With both **2** and **12** showing oral bioavailability, it was our desire to test these CCR2 antagonists in a mouse model of inflammatory cellular recruitment. The MCP-1/CCR2 pair plays a major role in mediating the egress of inflammatory monocytes (defined as Ly6C⁺F4/80⁺) from bone marrow to blood,¹⁴ and this can be emulated with the thioglycollate (TG)-induced peritonitis model.¹⁵ However, **2** and **12** have poor activity versus mouse CCR2, hence our TG model had to be performed in a human-CCR2 knock-in mouse (we did dose **12** in a TG-induced peritonitis model using wild-type mice, however **12** did not show any activity—data not shown). As shown in Table 5 with human-CCR2 knock-in mice, compounds **2** and **12** were orally dosed in separate experiments one hour before thioglycollate challenge, and both **2** and **12** showed a significant reduction of inflammatory monocytes in blood as compared to vehicle (similar findings were observed with these monocytes in the peritoneal cavity—data not shown). Hence, these results validate the in vivo activity of compounds **2** and **12**.

The synthesis of compound **12**, shown in Scheme 1, is used as a representative example of these alkylsulfone antagonists. The synthesis commenced with mesylation of the homochiral alcohol **15**.¹⁶ The resulting mesylate was used, without purification, in a displacement reaction to give **16**, which was subsequently oxidized to sulfone **17**. The carbamate of **17** was then removed prior to coupling with a methionine derivative to yield **18**. The lactam was formed under our modified Freidinger¹⁷ conditions (MeI and then Cs₂CO₃ in DMF) to give **19**. Final elaboration was performed by way of benzamide installation followed by tertiary amine formation to afford **12**.

In summary, we have demonstrated that trisubstituted cyclohexanes containing alkylsulfones are potent functional antagonists of CCR2 that have an improved hERG channel profile as compared to our previously described antagonists. Two of these alkylsulfone antagonists, **2** and **12**, also displayed in vitro microsomal stability and oral bioavailability. With this improved profile, we established that these CCR2 antagonists are orally efficacious in an animal model of monocyte recruitment, one of the hallmarks of autoimmune disease.

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Scheme 1.

Reagents and conditions: (a) (i) MsCl, TEA, DCM, 0 °C, quant; (ii) NaS-*t*-Bu, DMF, 78%; (b) oxone, IPA, H₂O, 79%; (c) H₂, Pd/C, MeOH; (ii) BOP, NMM, *N*-Cbz-L-Met-OH, DMF, 95% (two steps); (d) (i) MeI; (ii) Cs₂CO₃, DMF, 76%; (e) (i) H₂, Pd/C, MeOH; (ii) BOP, NMM, 3-trifluoromethylbenzoic acid, DMF, 90% (2 steps); (f) (i) TFA, DCM, quant; (ii) acetone, NaBH(OAc)₃, DCM; (iii) 37% HCHO, NaBH(OAc)₃, DCM, 96% (two steps).

Evaluation of alkylsulfone derivatives

Compd #	R	R ¹	R ²	X	CCR2 binding ^a IC ₅₀ (nM)	hERG ^b IC ₅₀ (μM)	Na ⁺ binding ^c %Inh @10 μM
1	Ph	CF ₃	—	C	1.0 ± 0.2 (7)	30	NT
2	Me	CF ₃	—	C	1.8 ± 0.8 (12)	>80	3
3	Me	CF ₃	—	N	2.2 ± 0.1 (2)	>80	0
4	Me	<i>t</i> -Bu	—	C	1.2 (1)	>80	52
5	Me	<i>t</i> -Bu	—	N	0.7 (1)	>80	46
6	Me	3-CF ₃	4-CH ₃	C	5.2 (1)	>80	36
7	Me	OCF ₃	—	C	5.0 (1)	>80	32
8	Et	CF ₃	—	C	1.3 ± 0.02 (2)	>80	26
9	Et	Ph	—	C	1.1 (1)	60	NT
10	<i>i</i> -Pr	CF ₃	—	C	0.3 (1)	>80	NT
11	<i>i</i> -Pr	<i>t</i> -Bu	—	C	0.5 ± 0.2 (2)	77	NT
12	<i>t</i> -Bu	CF ₃	—	C	0.96 ± 0.26 (19)	>80	48
13	<i>t</i> -Bu	OCF ₃	—	C	1.2 (1)	>80	61
14	<i>t</i> -Bu	3-CF ₃	5-F	C	1.3 (1)	>80	48

^aIC₅₀ values (*n*) are displayed as mean ± SD (*n* = 2) and mean ± SEM (*n* > 2).^bhERG FLIPR assay ($n = 1$).^cRat Na⁺ channel binding assay (*n* = 1). NT = not tested.

Table 2

Evaluation of alkylsulfone derivatives

#	R	IC ₅₀ ^a (nM)		Human microsomal stability ^c (% remaining)	hERG ^d IC ₅₀ (μM)	Caco-2 P _{AP-BL} (nm/s)	hERG patch clamp (4 Hz) (% Inh)	Na ⁺ patch clamp (4 Hz) (% Inh @ 10 μM)
		CCR2 binding	Chemotaxis ^b					
1	Ph	1.0 ± 0.2 (7)	0.5	88	30	<15 ^e	NT	NT
2	Me	1.8 ± 0.8 (12)	2.6	100	>80	<15	4% @ 10 μM	22%
8	Et	1.2 ± 0.07 (4)	0.6	100	>80	<15	3.6% @ 3 μM	4.2%
10	<i>i</i> -Pr	0.34 (1)	0.2	100	>80	<15	NT	NT
12	<i>n</i> -Bu	0.96 ± 0.26 (19)	0.2	100	>80	30	4.2% @ 10 μM	19%

^a IC₅₀ values (*n*) are displayed as mean ± SD (*n* = 2) and mean ± SEM (*n* > 2).^b Chemotaxis in human monocytes (*n* = 1) with 0.1 M BSA.^c Percent remaining after 10 min incubation in human hepatic microsomes.^d hERG FLIPR assay (*n* = 1). NT = not tested.^e <15 is limit of detection for this assay.

Table 3Pharmacokinetic data for compound **2**

Species	Dose (mpk) iv/po	<i>F</i> % ^a	CL _{iv} ^a (mL/min/kg)
Mouse	3/54	16	70
Rat	6/72	1	42
Cyno	1/14	9	14
Dog	1/14	51	3

^aValues are an average from two animals.

Table 4Pharmacokinetic data for compound **12**

Species	Dose (mpk) iv/po	F% ^a	CL _{iv} ^a (mL/min/kg)
Mouse	5/100	13	25
Rat	4/43	14	54
Cyno	1/10	26	12
Dog	1/10	74	5

^aValues are an average from two animals.

Table 56-h TG model in human-CCR2 knock-in mice with **2** and **12**

Dose information: **2** dosed at 50 mpk po
12 dosed at 100 mpk po

^a % of Ly6C+F4/80+ cells vs. TG control in peripheral blood.

^a % of Ly6C+F4/80+ cells vs. TG control in peripheral blood.