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Identification of a sulfonamide series of CCR2 antagonists

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ABSTRACT

A series of sulfonamide CCR2 antagonists was identified by high-throughput screening. Management of molecular weight and physical properties, in particular moderation of lipophilicity and study of pK_a, yielded highly potent CCR2 antagonists exhibiting good pharmacokinetic properties and improved potency in the presence of human plasma.

Chemokines are a family of endogenous pro-inflammatory proteins which act primarily via the activation and recruitment of leukocytes yet the inappropriate over-expression of such proteins is implicated in a variety of disease conditions.[1] CCL2, commonly referred to as monocyte chemoattractant protein 1 (MCP-1), activates several molecular targets including the G-protein coupled seven-transmembrane receptor CCR2. This ligand/receptor pair is overexpressed in numerous inflammatory conditions wherein excessive monocyte recruitment is observed, such as atherosclerosis[2] and rheumatoid arthritis.[3]

It is worth noting that while the anti-CCR2 monoclonal antibody ML-1202 was effective in phase II trials for multiple sclerosis it exhibited no benefit in patients with rheumatoid arthritis.[4] A host of patents and peer-reviewed publications have appeared which describe the development of small-molecule CCR2 antagonists.[4] Quaternary ammonium CCR5 antagonist TAK-779[5] (**1**, Fig. 1) interacts with CCR2 and several series containing a non-quaternary basic center have been reported, for example, **2**[6] and **3**[7] (Merck) and **4**[8] (BMS). Acidic CCR2 antagonists such as **5**, described by researchers at AstraZeneca, have also appeared.[9] Herein we describe the discovery of an alternative class of compounds that does not require a strongly ionizable group for activity.

A series of biaryl sulfonamides, exemplified by compound **6**, emerged from a high-throughput screen of the GSK collection. The preliminary SAR observed by early arrays are described in Figure 2. While potency strongly correlated with lipophilicity,

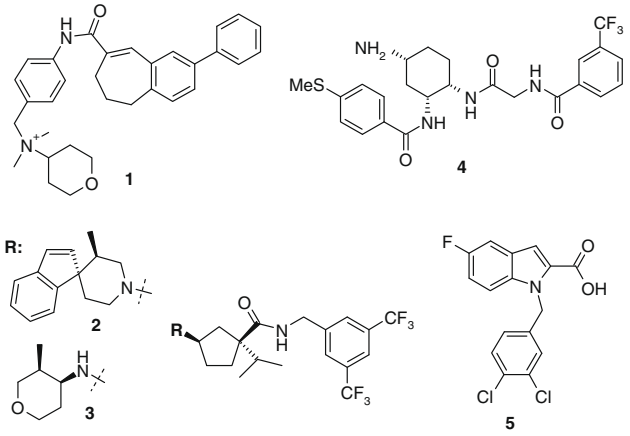

Figure 1. Selected literature CCR2 antagonists.

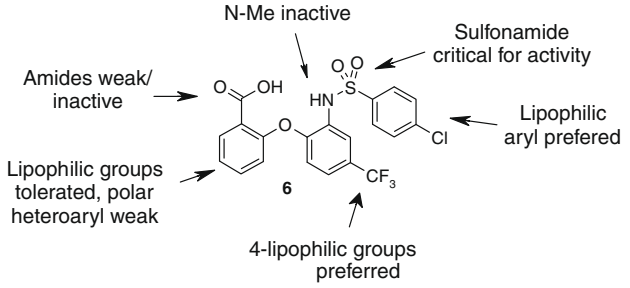

Figure 2. SAR from preliminary lead identification activities.

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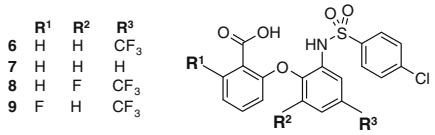
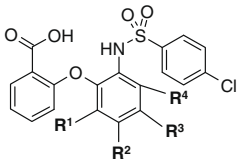
Table 1
Preliminary selectivity and chemotaxis data. CCR2, CCR1 and murine CCR2 conducted in recombinant GTPgS ($n \geqslant 6$). Compounds **6** and **7** also reported below the lower limit of the CCR1 assay (data not shown)



Compound	CCR2 fpK_i	CTX fpK_i		CCR1 fpK_i	Mouse CCR2 fpK_i	Spec. pK_a	
		1% FBS	1% HS			CO_2H	$NHSO_2$
6	6.1	—	—	6.3	5.8	3.2	7.9
7	6.6	6.4	6.4	6.5	5.7	3.3	8.7
8	7.5	7.2	4.9	6.9	6.6	—	—
9	6.9	6.8	5.0	6.9	6.0	2.4	7.7

Table 2
Representative central ring SAR



Compound	R^1	R^2	R^3	R^4	CCR2 fpK_i	CCR1 fpK_i	CTX 10 μM antagonist %I at 1% HS
7					6.6	6.5	—
14			SO_2Me		<5.5	—	—
15			CN		7.2	6.0	—
16			F		7.4	6.6	52
17			Cl		7.7	6.8	65
18	Me				<5.5	<5.5	—
19	Cl				6.4	6.3	—
20		Me			6.2	6.2	—
21				F	<5.5	<5.5	—

examination of four closely related analogs (Table 1) provided encouragement that activity could be modulated independently of log *P*. Inclusion of fluorine adjacent either to the carboxylic acid or biaryl ether increased potency, possibly as a consequence of pK_a modulation. Compounds **8** and **9** demonstrated encouraging potency in monocyte chemotaxis (CTX)[10]; however, replacement of fetal bovine serum (FBS) with human serum (HS) resulted in a dramatic reduction in potency. Conversely, the undecorated analog **7** maintained activity, suggesting that protein binding could be managed. The general selectivity for this series was acceptable although most analogs also interacted with CCR1.

The risk that the observed in vitro activity resulted from non-specific binding to proteins (CCR2 or CCL2) was discharged by addition of detergent to the primary assay[11] and assessment of promiscuity using β-lactamase.[12]

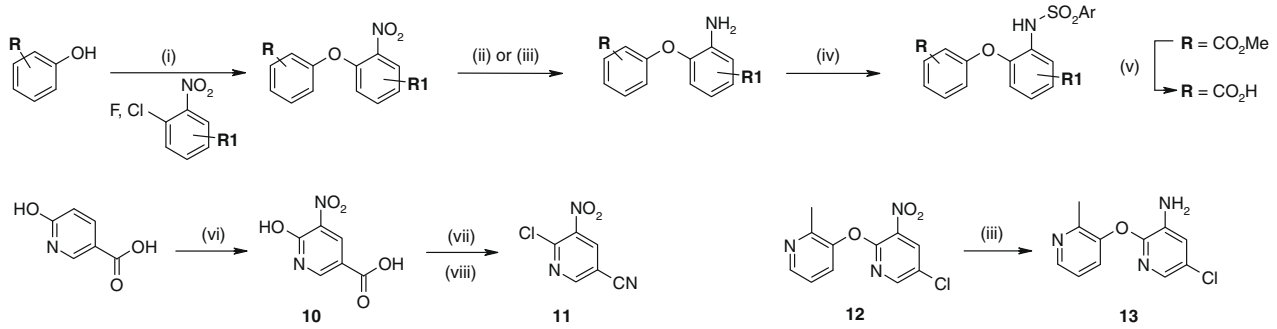
The compounds described herein were prepared according to the general Scheme 1.[13] Synthesis of the previously unreported nitro-pyridine **11** was achieved via dehydration of the primary amide derived from **10**, prepared by nitration of 2-hydroxy 5-carboxy pyridine. Core 2-halo nitro arenes and aromatic alcohols were coupled under basic conditions. Reduction of the nitro group was achieved using standard hydrogenation protocols or tin chloride. We discovered that transfer hydrogenation with ammonium formate over platinum on carbon provided a reliable, facile method for nitro group reduction without disturbing vulnerable chlorines, nitriles and pyridines. For example, reduction of **12**–**13** was achieved in 83% yield with simple filtration providing the product in ⩾90% purity. Standard DMAP/pyridine conditions proved to be optimal for coupling the resulting amines with sulfonyl chlorides.

The medicinal chemistry effort focused on increasing potency while balancing lipophilicity and limiting molecular weight. Table 2 summarizes some of the modifications realized in the central region of the molecule. Substitution at R^3 with fluorine, chlorine or nitrile conferred a 10-fold increase in potency and a modest increase in selectivity over CCR1. Inclusion of a methyl group at R^1 was not tolerated whereas inclusion of a chlorine substituent was. Conversely addition of a weakly donating methyl group at R^2 proved inconsequential. Replacement of hydrogen with fluorine at R^4 (**21**) resulted in a significant loss of activity.

Selected modifications of the sulfonamide group are presented in Table 3. Combination of the 3 chloro and 4-chorlo derivatives provided the optimal 3,4-dichloro benzene sulfonamide **24**. 5-Chloro thienyl sulfonamide **25** was also very active although it is worth nothing that the sulfonamide pK_a was reduced (compare **25** and **17**). Methane sulfonamide **26** was weakly active and larger alkyl sulfonamides were only weakly active. Inhibition of chemotaxis by 1 μM **24** in the presence of 0.1 and 1% human serum was 54% and 21%, respectively. This confirmed earlier observations of a significant serum-protein related effect. A strategy of reducing lipophilicity and managing pK_a was adopted to modulate protein binding.

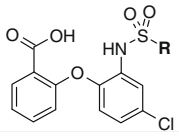
Polar core motifs such as pyrazine and pyrimidine (**27** and **28**, Table 4) were 10-fold weaker than the related pyridines **29** and **30**. Installation of chlorine *para* to the biaryl ether resulted in identification of compound **31**. Compounds **30** and **31** possessed similar chemotaxis activity; however, the shift for compound **31** appears to be greater, possibly a consequence of reduced sulfonamide pK_a.

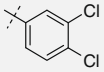
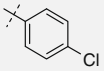
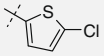
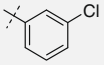
Investigations of the biaryl ether are summarized in Table 5. Compounds **31**–**34** revealed that the position of the acid was not



Scheme 1. Typical reagents and conditions. (i) DMF, K_2CO_3, 70 °C; (ii) $SnCl_2$ or H_2, Pd/C or H_2, Pt/C; (iii) Pt/C, $NH_4{\cdot}HCO_2$, DCM, reflux (83% for **13**); (iv) $ArSO_2Cl$, DMAP, pyridine 95 °C; (v) LiOH, MeOH/H_2O 1:1, 30 °C; (vi) H_2SO_4, HNO_3 (79%); (vii) CDI, NH_3, 60 °C; (viii) $POCl_3$, reflux (59%, two steps).

Table 3
Selected sulfonamide SAR



Compound	R	CCR2 fpK_i	pK_a NHSO_2	Compound	R	CCR2 fpK_i	pK_a NHSO_2
22		6.8	–	**24**		8.5	7.89
17		7.7	8.09	**25**		8.3	7.42
23		8.1	–	**26**		6.4	–

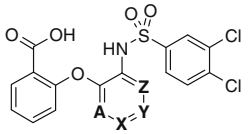
relevant for potency furthermore polar amides such as **35** were tolerated. The 3-pyridyl group introduced a weakly basic moiety thus maintaining solubility in the absence of the carboxylic acid and the 2-methyl derivative **37** possessed the best anti-chemotaxis activity in the presence of increasing human serum. The retention of activity in the presence of plasma proteins[14] may be related to the pK_a of the sulfonamide rather than lipophilicity (e.g., compare **36** and **37**).

The increase in sulfonamide pK_a resulting from the installation of the 2-methyl group was confirmed by repeated testing and may be due to conformational change in the biaryl ether Inclusion of cyano in the central ring (**42**) led to a reduction in chemotaxis activity, possibly due to reduced sulfonamide pK_a.

Table 6 summarizes the pharmacokinetic and selectivity data on a selection of analogs. The acidic compounds **24** and **31** both displayed excellent adsorption and half-life typical of protein-bound carboxylic acids.

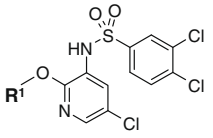
Amide **35** displayed modest bioavailability as a consequence of increased clearance whereas the 2-methyl pyridyl analog **37** exhibited a very promising profile upon oral dosing. The series proved to be generally selective in hERG and p450 assays although the non-acid analogs interfered moderately with 2C9. In general the series also interacts with the CCR1 receptor although dual activity was

Table 4
Aza 6-membered ring derivatives. CH unless otherwise indicated

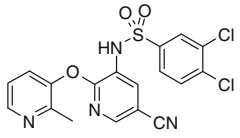


Compound	A	X	Y	Z	CCR2 fpK_i	pK_a NHSO_2	CTX 1 μM antagonist %*I* at 0.1, 1% HS
27	N			N	6.1	–	–
28	N		N		6.4	–	–
29		N			7.3	7.54	15, 0
30	N				7.5	7.32	60, 41
31	N		C–Cl		8.9	6.58	67, 37

Table 5
Activity (CCR2 and chemotaxis) and physical properties of selected biaryl ether modifications



31-41



42

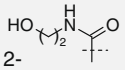
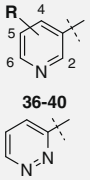
R^1	Compound	R	CCR2 fpK_i	CTX 1 μM % *I* at 0.1, 1% HS	log *D*	pK_a NHSO_2
 **31-35**	**31**	2-CO_2H	8.9	67, 36	0.8	6.58
	32	3-F, 2-CO_2H	8.7	–	–	–
	33	3-CO_2H	8.8	42, 33	0.44	–
	34	4-CO_2H	8.5	–	0.4	–
	35	 2-	7.6	31, 0	2.1	5.6
 **36-40**	**36**	H	7.7	36, 5.4	2.32	5.72
	37	2-Me	8.0	72, 50	2.7	7.64
	38	5-CO_2H	8.0	17, 0	–0.67	6.04
	39	5-Cl	7.9	55, 0	3.1	–
	40	6-CN	7.8	–	2.12	–
	41	–	6.7	–	–	–
–	**42**	–	7.3	43, 12	1.5	5.23

Table 6
Selected DMPK, developability and chemokine selectivity data

Compound	Rat PK summary				In vitro developability profile						Chemokine selectivity			
	F (%)	*T* 1/2 iv, po (h)	C_l (mL/min/kg)	V_d (L/kg)	hERG[a] (pIC_{50})	p450 profile (pIC_{50})					CCR2 (pK_i)	CCR1 (pK_i)	CCR4 (pK_i)	CCR5 (pIC_{50})
						1A2	2C19	2C9	2D6	3A4				
24	70	6.5, 5.9	0.6	0.2	5.1	4.4	4.96	5.38	4.27	5.0	8.5	7.3	6.4	6.2
31	97	4.5, 4.5	0.71	0.12	<4.5	4.4	4.4	4.67	4.0	4.0	8.9	7.7	6.1	6.6
35	37	1.1, —	13	0.47	<4.2	<4.0	5.06	5.3	<4.0	4.69	7.6	6.7	5.8	<10%[b]
37	75	0.8, 2.3	4.3	0.34	<4.5	<4.0	4.65	5.87	<4.0	4.71	8.0	7.0	5.9	40%[b]
39	58	1.2, —	2.4	0.2	—	<4.0	5.18	5.97	<4.0	6.36	7.9	6.8	—	—

[a] hERG fluorescence-polarization binding assay.
[b] % inhibition at 10 μM antagonist.

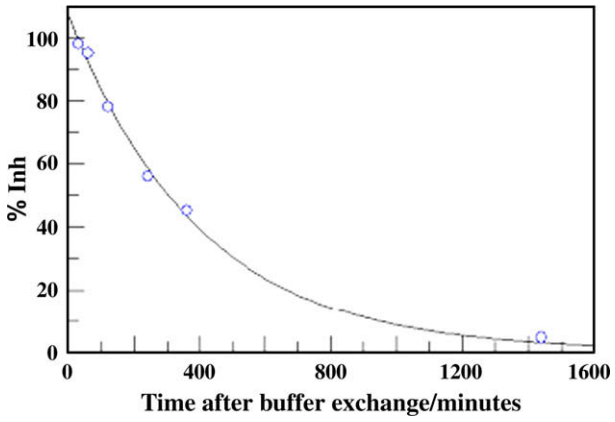


Figure 3. Reversibility of CCR2 blockade at 10 μM **31** in 1% FBS following buffer exchange.

considered to be of benefit given that signaling redundancy is mooted as one of the likely reasons for the poor clinical efficacy observed to date with selective chemokine antagonists.[15]

The time-dependent behavior of compound **31** in chemotaxis was investigated. THP-1 cells were incubated with 10 μM **31** for 30 min and the assay buffer exchanged. Periodic stimulation of chemotaxis provided the reversibility curve illustrated in Figure 3. The half-life for this reversibility was measured as approximately 280 min, suggesting a slow dissociation rate of the compound from the receptor.

HTS hit **6** (CCR2 pK_i 6.1, M_W 471, *c* log *P*[16] 5.1) was optimized through careful attention to molecular size and physical properties to provide compound **37** (CCR2 pK_i 8.0, M_W 443, *c* log *P* 3.4). The apparent influence of plasma proteins on activity was moderated via removal of the carboxylic acid and through reduction of lipophilicity. Measured sulfonamide pK_as were apparently related to the activity shift observed in chemotaxis, as exemplified by the closely related analogs **36**, **37** and **42** whereby the most lipophilic, but least acidic compound proved to be the most effect anti-chemotactic agent.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.142.

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