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Design, synthesis and structure activity relationships of spirocyclic compounds as potent CCR1 antagonists

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ABSTRACT

A series of CCR1 antagonists based upon spirocyclic compounds **1b** and **2b** were synthesised in which substituted aniline moiety was replaced with substituted benzamides. In vitro data revealed that CCR1 potency could be retained in such compounds.

Inflammatory disorders are characterised by the excessive recruitment of leukocytes to the site of inflammation. Leukocytes trafficking in a controlled manner is an important feature of the immune response to infections, while loss of such control results in inflammatory diseases. Chemokines belong to a super family of cytokines and play an important role in various inflammatory diseases by mediating leukocyte recruitment.[1–4] Various diseases such as rheumatoid arthritis,[5,6] multiple sclerosis[7,8] and asthma are characterised by deregulated leukocyte recruitment. Chemokines bind to cell surface chemokine receptors and thus, transmit intracellular signals to their target cells by activating G-proteins coupled to the receptors. Chemokine receptors are attractive therapeutic targets for drug developments due to their central role in regulating leukocyte trafficking. The C–C (cystine–cystine) chemokine receptor-1 (CCR1) and its' major endogenous ligands MIP-1α (CCL3) and RANTES (CCL5) play an important role in chronic inflammatory diseases such as rheumatoid arthritis and multiple sclerosis and inhibition of CCR1 is expected to be beneficial for patients who suffer from such inflammatory disorders. Thus, low molecular weight synthetic CCR1 antagonists could be useful as therapeutic agents. The search for specific and highly potent chemokine receptor antagonists has recently been a popular theme in the literature.[9–14] Here, we report the discovery of a novel class of spirocyclic compounds as potent CCR1 antagonists.

Previously, we have reported a series of conformationally constrained spirocyclic compounds as highly potent CCR1 antagonists[15] (Fig. 1). However, this class of compound contains an N-acetylated aniline moiety which may be prone to deacetylation[16b] by specific human enzymes to generate aniline derivative, thus potentially leading to reactive metabolites formation, resulting in possible genotoxicity or other unwanted side effects. Thus, when such a fragment is present in a drug candidate, a careful analysis is required to make sure that the molecule as a whole and the corresponding aniline fragments are free from genotoxicity[16a] before progression to clinical development. Therefore, it may be desirable to identify possible alternatives to such an aniline moiety in a series of candidate drug molecules. Thus, we focused our attention towards finding a replacement for the *N*-acetyl group in **1b** and **2b** (Fig. 1) without compromising CCR1 potency. Hence, we investigated whether a benzamide derivative of the *N*-acetyl aniline moiety would be feasible. Thus, we designed a series of compounds with the *N*-acetyl moiety replaced by a CONRR group.

Synthesis of the carboxamide derivatives is outlined in Schemes 1–5. The starting material **5** was prepared from **4** according to a published procedure.[17] Reaction of **5** with (2*S*)-oxiran-2-ylmethyl-3-nitrobenzene sulphonate in the presence of Cs_2CO_3 gave epoxide **6** in high yield. Epoxide **6** was opened by spirocyclic amine **1** to yield **7** which was hydrolysed by aqueous NaOH to afford **8** in quantitative yield. Standard amide coupling reaction of **8** with

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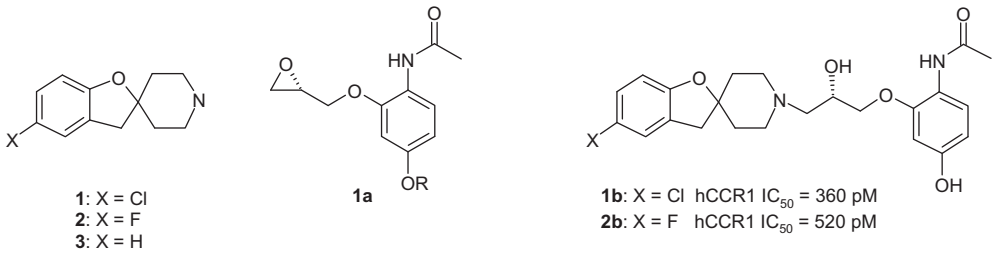
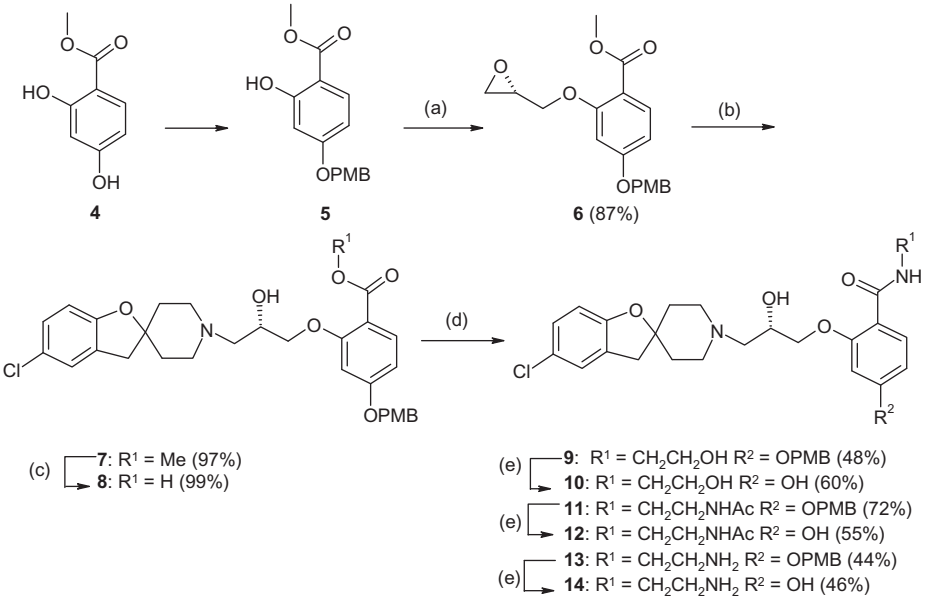
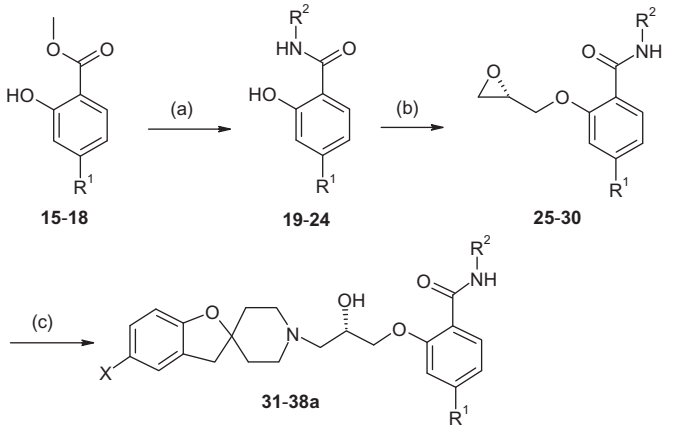


Figure 1. Previously reported CCR1 antagonists.



Scheme 1. Reagents and conditions: (a) (2*S*)-Oxiran-2-yl-methyl-3-nitrobenzene sulphonate, Cs_2CO_3, DMF, rt, 20 h (87%); (b) spirocycle 1, EtOH, reflux 20 h (97%); (c) aq NaOH, EtOH, rt, 3 h (99%); (d) $NH_2CH_2CH_2OH$ or $NH_2CH_2CH_2NHAc$ or $NH_2CH_2CH_2NH_2$, CDI, DMF, rt, 20 h (44–72%); (e) TFA, CH_2Cl_2, rt, 1 h (46–60%).



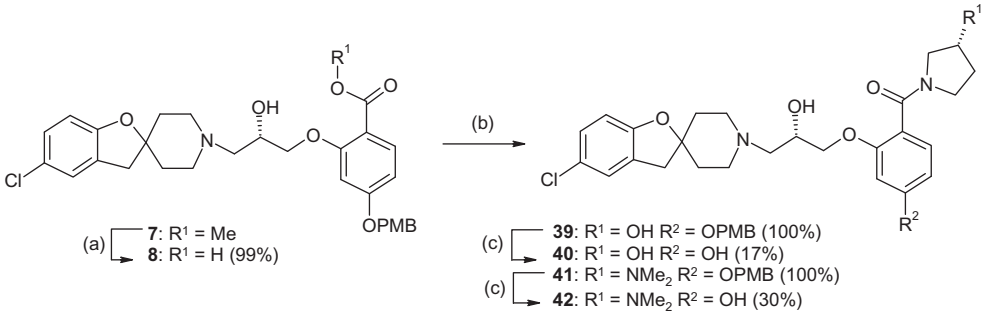
Scheme 2. Reagents and conditions: (a) Cyclopropyl amine, 70–100 °C, 20 h or rt, 20–170 h (31%-quant.); (b) (2*S*)-oxiran-2-yl-methyl-3-nitrobenzene sulphonate, Cs_2CO_3, DMF, rt, 20 h (63–99%); (c) spirocycles 1, 2, EtOH, reflux 20 h (38–69%) (Table 1).

different amines in the presence of CDI afforded **9**, **11** and **13** in moderate yields. Finally, the *para*-methoxybenzyl (PMB) protecting group in **9**, **11** and **13** was removed by TFA treatment to afford final compounds **10** (60%), **12** (55%) and **14** (46%) (Scheme 1).

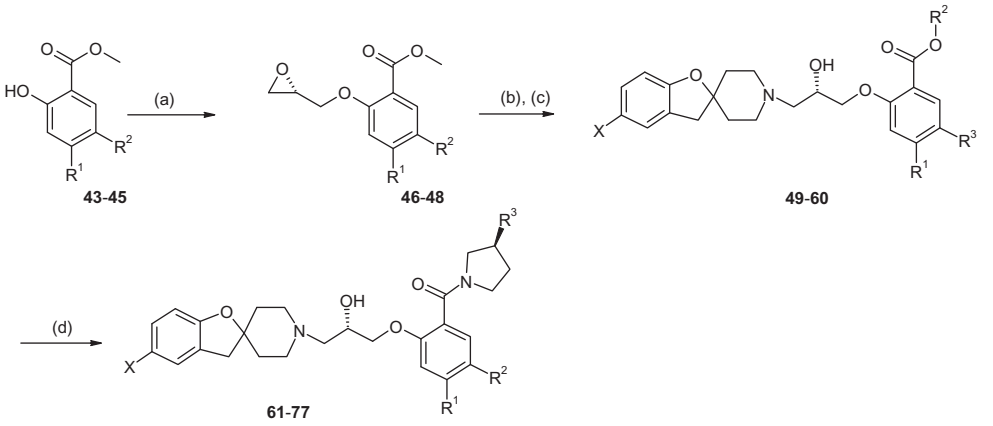
Intermediate **18** was prepared from **4** by treatment with triphenylmethyl (Tr) chloride in DMF in the presence of Et_3N and DMAP at room temperature in 43% yield. Compounds **5**, and **15–18** were treated separately with cyclopropyl amine to give **19–22**, and **24** in 31–84% isolated yield, whilst treatment of **5** with methyl amine gave **23** in quantitative yield. Compounds **19–24**, were treated separately with (2*S*)-oxiran-2-yl-methyl-3-nitrobenzene sulphonate in the presence of Cs_2CO_3 in DMF to give corresponding epoxides **25–30** in good yields. Epoxides **25–30** were opened by spirocyclic amines **1** or **2** in refluxing ethanol to give **31–34**, **36** and **38** in moderate yields. Finally, the *para*-methoxybenzyl protecting group in **34**, **36** and trityl group in **38** were removed by TFA treatment to give **35**, **37** and **38a** in moderate yield (Scheme 2, Table 1).

The acid derivative **8** was converted to amide **39** and **41** in quantitative yield as crude materials, by coupling with the respective amines under standard conditions. The *para*-methoxybenzyl protecting group in **39** and **41** was removed by TFA treatment to afford **40** and **42**, respectively, in low yield (Scheme 3).

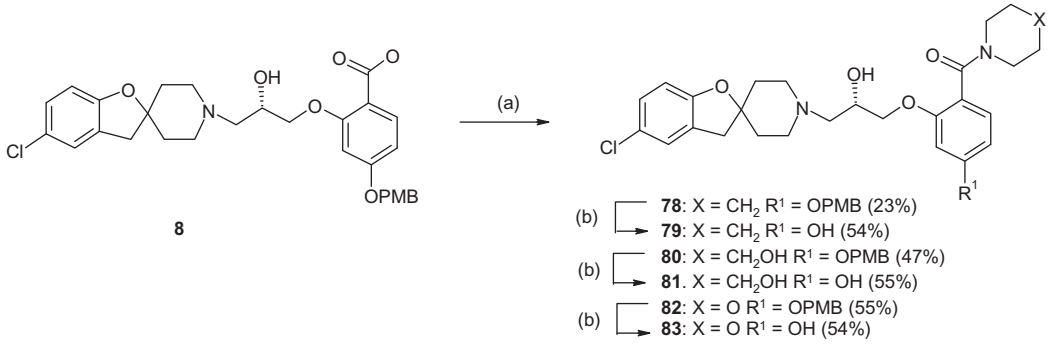
Compound **44** was prepared following a literature procedure.[18] Reaction of **44** with *para*-methoxybenzyl chloride in the presence of K_2CO_3 in acetone to give **45** in good yield. Compounds **43, 16**, and **45** were treated separately with (2*S*)-oxiran-2-yl-methyl-3-nitrobenzene sulphonate and Cs_2CO_3 in DMF to give epoxides **46–48** in high yields. Epoxides **46–48** were opened by spirocyclic amines **1–3** and the corresponding products were hydrolysed to give **50**, **52**, **54**, **56**, **58** and **60** in high yields. Subsequently, these free acids were converted to amides **61–66**, **68**, **70**, **72**, **74** and **76**, in acceptable yields, by coupling with the respective amines



Scheme 3. Reagents and conditions: (a) aq NaOH, MeOH, rt, 3 h (99%); (b) (3*R*)-pyrrolidin-3-ol, CDI, DMF, rt, 20 h (100%) or (3*R*)-*N*,*N*-dimethylpyrrolidin-3-ol, PS-carbodiimide, NMP, CH_2Cl_2, rt, 20 h (100%); (c) TFA, CH_2Cl_2, rt, 1 h (17–30%).



Scheme 4. Reagents and conditions: (a) (2*S*)-Oxiran-2-yl-methyl-3-nitrobenzene sulphonate, Cs_2CO_3, DMF, rt, 20 h (74–96%); (b) spirocycles 1, 2, 3, EtOH, reflux 20 h (42–91%); (c) aq NaOH, EtOH, rt, 3 h (36–100%); (d) amine, CDI, DMF, rt, 20 h (28–100%) (Table 2).



Scheme 5. Reagents and conditions: (a) Amine, CDI, DMF, rt, 20 h (23–55%); (b) TFA, CH_2Cl_2, rt, 1 h (54–55%).

under standard conditions. The protecting groups from **66**, **68**, **70**, **72**, **74** and **76** were removed by TFA treatment to give **67**, **69**, **71**, **73**, **75** and **77** in moderate yields (Scheme 4, Table 2).

Similarly, the acid derivative **8** was converted to the corresponding amides **78**, **80** and **82** via amide coupling reaction in the presence of CDI in DMF and subsequently the protecting group was removed by TFA treatment to give **79** (54%), **81** (55%) and **83** (54%) (Scheme 5).

As a first attempt NHAc in **1b** and **2b** (Fig. 1) was replaced with -CONHMe and thus **35** and **37** (Scheme 2) were designed. Biological evaluations[15] revealed that CCR1 potency of these compounds was dropped to some extend compared to *N*-acetyl analogues **1b** and **2b** but they were nevertheless very potent CCR1 antagonists (Table 3). In addition **35** was found to be stable in human hepatocytes but less stable in rat hepatocytes. On the other hand **37** was less stable both in rat and human hepatocytes. Both of these compounds had good permeability in a caco-2 assay. Moreover, **35** and **37** were potent rodent CCR1 antagonists. Next the tolerability with regard to CCR1 potency of extended chain with polar groups instead of the *N*-methyl group was explored and **10**, **12** and **14** were designed. However, these compounds were found to be 6- to 200-fold less active compared to **35**. In order to investigate whether this was due to the lipophilic effect, a cyclopropyl group was introduced instead of the hydrophilic groups such as hydroxyl, primary amine and one model example **38a** was synthesised. Indeed, **38a** was found to be a potent CCR1 antagonist. To explore further the *meta* hydroxyl group of the substituted phenol was replaced by a hydrogen, *O*-methyl or fluorine and **31**, **32**, **33** were synthesised. Gratify-

Table 1

Compound	R^1	R^2	X	Yield (%)
15	H			
16	F			
17	OMe			
18	OTr			43
19	H	Cyclopropyl		53
20	OMe	Cyclopropyl		31
21	F	Cyclopropyl		84
22	OPMB	Cyclopropyl		71
23	OPMB	Me		100
24	OTr	Cyclopropyl		55
25	H	Cyclopropyl		99
26	OMe	Cyclopropyl		99
27	F	Cyclopropyl		63
28	OPMB	Cyclopropyl		70
29	OPMB	Me		75
30	OTr	Cyclopropyl		73
31	H	Cyclopropyl	Cl	38
32	OMe	Cyclopropyl	Cl	67
33	F	Cyclopropyl	Cl	56
34	OPMB	Me	Cl	69
35	OH	Me	Cl	52
36	OPMB	Me	F	68
37	OH	Me	F	44
38	OTr	Cyclopropyl	Cl	
38a	OH	Cyclopropyl	Cl	52

Table 2

Compound	R^1	R^2	R^3	X	Yield (%)
43	Me	H			
44	OH	Cl			
45	OPMB	Cl			60
46	Me	H			75
47	F	H			96
48	OPMB	Cl			74
49	Me	Me	H	Cl	91
50	Me	H	H	Cl	79
51	Me	Me	H	F	77
52	Me	H	H	F	36
53	F	Me	H	Cl	64
54	F	H	H	Cl	84
55	F	Me	H	F	77
56	F	H	H	F	72
57	F	Me	H	H	42
58	F	H	H	H	70
59	OPMB	Me	Cl	Cl	67
60	OPMB	H	Cl	Cl	quant.
61	Me	H	OH	F	73
62	Me	H	OH	Cl	56
63	F	H	OH	Cl	28
64	F	H	OH	H	40
65	F	H	OH	F	40
66	OPMB	Cl	OH	Cl	37
67	OH	Cl	OH	Cl	59
68	OPMB	H	OH	Cl	52
69	OH	H	OH	Cl	62
70	OPMB	H	NMe_2	Cl	100
71	OH	H	NMe_2	Cl	32
72	OPMB	H	OMe	Cl	34
73	OH	H	OMe	Cl	80
74	OPMB	H	NHAc	Cl	59
75	OH	H	NHAc	Cl	27
76	OPMB	H	H	Cl	37
77	OH	H	H	Cl	72

ingly, these compounds were found to be potent CCR1 antagonists with good permeability. They were also found to be stable in human hepatocytes except **32**. In addition, **32** was found to be very potent rat CCR1 antagonist (Table 3). Next, the possibility of replacing the flexible chain such as in **10** with more constrained five membered ring was explored, thus **40** and the corresponding isomer **69** were designed. In vitro data revealed that constrained compounds **40** was equipotent to **10** whilst **69** was sixfold more potent than the analogue with flexible chain **10**. However, they were less permeable as well. The hydroxyl group in the pyrrolidine ring in **40** and **69** was replaced with a NMe_2 moiety. Thus, **42** and **71** were designed. Compound **42** was equipotent to **69** whilst **71** was almost 100-fold less potent than **69**. Next, **69** was modified either by replacing the *meta* hydroxyl group of the substituted phenol with methyl or fluorine or by replacing the hydroxyl group on the pyrrolidine ring with methoxy, *N*-acetyl or hydrogen. Thus, **62**, **63**, **73**, **75** and **77** were designed and synthesised. These compounds were all found to be potent CCR1 antagonists with good permeability except **75** whilst **63** was also found to be metabolically stable both in human and rat hepatocytes. However, **63** was active in hERG channel (Table 3). To investigate the substitution effect on the phenyl ring of the spirocycle, **63** was compared with **64** and **65**. Compound **65** was found to be fivefold less potent CCR1 antagonist than **63** whereas **64** was more than 60-fold less potent compared to **63**. The drop of potency observed with **64** and **65** was presumed to be due to reduced lipophilicity. Similarly comparison of **69** with **67**, showed that introduction of chlorine in the *para* position of the substituted phenol improved CCR1 potency, albeit to a small extent, whilst good stability in human and rat hepatocytes was retained. However, the permeability of **67** was not improved, relative to **69**, despite increased lipophilicity. The reason for low permeability of **69** is at present unclear. In addition, this compound was less active in hERG channel. Finally, expansion of the five membered ring to a six membered ring was explored, thus **79**, **81** and **83** were designed. Evaluation of these compounds revealed **81** to be potent CCR1 antagonist whilst **79** and **83** were less potent.

Table 3
In vitro data, CCR1 antagonists[19]

Compound	hCCR1 IC_{50} (μM)	hheps (μl/min/10^6 cells)	rheps (μl/min/mg)	Caco2 (cm/s × 10^6)	hERG[20] IC_{50} (μM)	rCCR1 IC_{50} (μM)
10	0.012	n.a.	n.a.	1.4	n.a.	
12	0.071	n.a.	n.a.	n.a	1.2	
14	0.4	n.a.	n.a.	n.a	n.a.	
31	0.0028	5.2	45	24	n.a.	
32	0.0025	15	n.a.	12	n.a.	0.079
33	0.0014	4.6	20	16	0.038	
35	0.0018	<4.6	33	14	n.a.	0.16
37	0.0032	26	22	8.1	n.a.	0.89
38a	0.0014	10.6	28	12	0.3	0.114
40	0.0079	n.a.	n.a.	0.1	n.a.	14
42	0.0013	n.a.	n.a.	0.4	>17.5	
61	0.0089	10	n.a.	5.3	n.a.	
62	0.005	11	n.a.	6.5	n.a.	
63	0.0025	6.8	<4	14	0.7	2.5
64	0.13	n.a.	n.a.	3	n.a.	
65	0.012	n.a.	n.a.	8.1	n.a.	
67	0.00092	<3	8.1	0.6	8.3	0.88
69	0.0019	<3	6.7	0.8	22.7	14
71	0.11	n.a.	n.a.	n.a	n.a.	
73	0.0079	n.a.	n.a.	3.9	n.a.	
75	0.089	n.a.	n.a.	0.2	>10	
77	0.0018	9.6	23	13	n.a.	
79	0.0071	14	n.a.	18	n.a.	1.1
81	0.0011	n.a.	n.a.	0.2	1.6	
83	0.032	n.a.	n.a.	2.6	n.a.	

Data represent the average values of at least two experiments.
n.a.: Not available.
hheps: Human hepatocytes.
rheps: Rat hepatocytes.
Caco: Human colonic adenocarcinoma.
hERG: Human ether-a-go-go-related-gene.

However, whilst **79** was found to have good permeability, this was not the case for **81** and **83**.

In conclusion, we have shown that the *N*-acetyl group (a fragment of pro aniline), of the potent CCR1 antagonists **1b** and **2b**, could be replaced by -CONRR (substituted benzamide) moiety with retention of CCR1 potency and good in vitro pharmacokinetics properties. Often, species cross-over is a challenge in the evaluation of chemokine receptor modulators, making it difficult to evaluate the pharmacodynamic properties of the compounds and the potential clinical relevance of the mechanism. However, this investigation showed that, although compounds in this series are generally much less potent against rat than human CCR1 receptors, some of these compounds may in fact be expected to show moderate efficacy in rodent models of inflammatory diseases. However, it was also observed that these compounds are prone to inhibit hERG channel and this activity could only be minimized at the expense of permeability. Thus, further studies will be required to optimize such parameters before progression of any of these compounds to clinical evaluation.

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