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The design and synthesis of novel, potent and orally bioavailable *N*-aryl piperazine-1-carboxamide CCR2 antagonists with very high hERG selectivity

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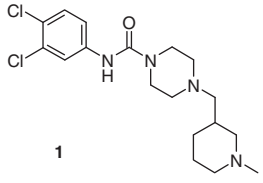
ABSTRACT

A novel *N*-aryl piperazine-1-carboxamide series of human CCR2 chemokine receptor antagonists was discovered. Early analogues were potent at CCR2 but also inhibited the hERG cardiac ion channel. Structural modifications which decreased lipophilicity and basicity resulted in the identification of a sub-series with an improved margin over hERG. The pharmacological and pharmacokinetic properties of the lead compound from this series, *N*-(3,4-dichlorophenyl)-4-[(2*R*)-4-isopropylpiperazine-2-carbonyl]piperazine-1-carboxamide, are described.

CCL2, commonly known as MCP-1 (monocyte chemoattractant protein-1), is a member of the CC chemokine family and plays a key role in the recruitment of monocytes and other cell types in various physiological and pathological processes.[1] CCR2 is the only known high affinity receptor for MCP-1 and is expressed on monocytes, activated T-cells, dendritic cells, basophils, NK cells and microglia cells.[2] Early evidence of a key role for the MCP-1-CCR2 system in inflammatory diseases such as rheumatoid arthritis, multiple sclerosis and atherosclerosis led to a widespread effort to discover and develop small molecule CCR2 antagonists.[3–5,6a] Ongoing studies using CCR2 and MCP-1 knockout mice and CCR2 antagonists have provided evidence of a role for this system in metabolic disease,[6] fibrosis, pain,[7] COPD[8] and even cancer,[3a] adding further stimulus to the search for clinical candidates.

Our CCR2 antagonist programme began with the identification of compound **1** during the screening of a large library designed around a number of general GPCR ligand motifs. **1** was found to displace ^{125}I-labelled MCP-1 from HEK cell membranes expressing human CCR2 with a K_i of 0.40 μM, and showed <50% binding at 10 μM to any of the other 45 GPCRs in the screening panel. This high degree of selectivity together with favourable physicochemical properties and scope for structural modification made this an attractive starting point for drug discovery. Our exploration of the SAR started with varying the terminal piperidine substituent. Compounds[9] were tested for their ability to inhibit binding of MCP-1 to CCR2 expressed in THP-1 cell membranes using SPA and to antagonise an MCP-1-induced calcium flux mediated by CCR2 natively expressed in THP-1 cells using FLIPR technology.[10] Since compound **1** was found to inhibit the hERG cardiac ion channel this activity was also evaluated.[11] The results are shown in Table 1.



1

Removal of the terminal piperidine methyl substituent reduced CCR2 activity (racemic **2a**) whereas increasing the size of the substituent to ethyl (*rac*-2b) gave a small increase in activity. Testing of the two enantiomers of **2b** revealed that the (*R*) absolute configuration is favoured (**2b** is 30-fold more active than *ent*-2b). Subsequent compounds were therefore prepared with the (*R*) absolute configuration. Introduction of a phenyl ring onto the alkyl group increased potency (**2c**, **2e**, **2f**) with the optimal 'spacer' being two

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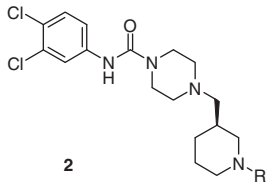
Table 1
CCR2 binding, Ca^{2+} flux and hERG binding data for selected compounds



2

Compd	R	CCR2 binding IC_{50} (nM)[a]	CCR2 Ca^{2+} flux IC_{50}[a] (nM)	hERG binding IC_{50}[a] (μM)
1	Me	170	965	1.6
rac-**2a**	H	780	950	1.6
rac-**2b**	Et	123	627	4.3
2b	Et	37	174	2.1
ent-**2b**	Et	1184	nt	nt
2c	CH_2Ph	99	244	0.56
2d	CH_2c-Pr	52	161	0.93
2e	CH_2CH_2Ph	5	42	<0.3
2f	$(CH_2)_3Ph$	64	280	0.40
2g	*c*-Pr	10	64	2.0
2h	*i*-Pr	16	58	1.3

[a] IC_{50}s were derived from triplicate measurements whose standard errors were normally <5% in a given assay. Assay to assay variability was within twofold based on the results of a standard compound. nt = not tested.

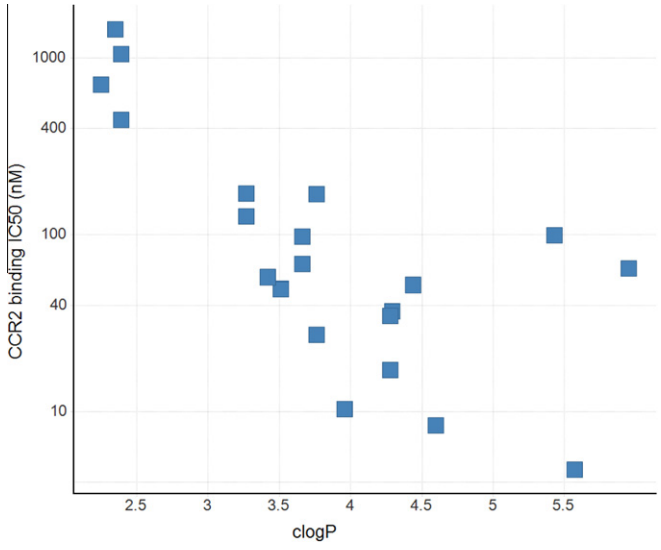


Figure 1. Plot of CCR2 binding against calculated log*P* for **2** and **3**.

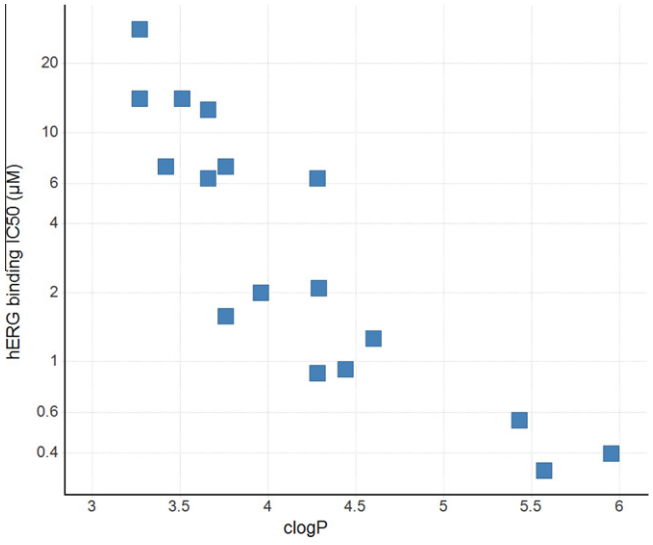


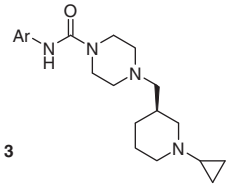
Figure 2. Plot of hERG binding against calculated log*P* for **2** and **3**.

methylenes (**2e**). Replacing this phenyl with cyclopropyl also gave good activity (**2d**) but the *N*-cyclopropyl (**2g**) and *N*-isopropyl (**2h**) analogues were almost as potent as the best phenyl containing compound **2e**. Both CCR2 and hERG binding activity increased with increasing calculated log*P* (see Figs. 1 and 2). The two outliers in Fig. 1 with the poorest ligand lipophilicity efficiency (LLE) are **2c** and **2f**. The cyclopropyl analogue **2g** had the highest CCR2 ligand lipophilicity efficiency (LLE) and therefore gave the best separation between hERG and CCR2b activity. The cyclopropyl substituent was then fixed while the aryl substitution at the far end of the molecule was explored with a view to improving potency and/or LLE. The results are shown in Table 2.

Changes such as one chloro to fluoro (**3a**), methyl (**3d**) or trifluoromethyl (**3b**, **3c**) retained most of the activity, while removing one of the two chlorine atoms (**3e**, **3f**) and replacing the remaining one with trifluoromethyl (**3i**, **3j**) or bromo (**3g**) gave compounds with reduced potency. Unsubstituted phenyl (**3o**) or polar substituents such as methoxy (**3h**), cyano (**3k**, **3l**) or methylsulfonyl (**3n**) reduced or abolished activity. Ortho substitution appeared to be deleterious since the 2-chloro analogue **3m** was inactive. The addition of a methyl group on the urea NH also abolished activity (results not shown). Both CCR2 and hERG activity broadly correlated with lipophilicity for **3** as well as **2** (Figs. 1 and 2). None of the compounds achieved a tenfold increase in the separation between CCR2 and hERG affinities relative to the original 3,4-dichlorophenyl analogue **2g**.

2g was profiled for ADME properties. It had good aqueous solubility (290 μM in pH7.4 buffer), moderate plasma protein binding (5% free in human and rat), and moderate metabolic stability in vitro (intrinsic clearance in rat hepatocytes: 11 μL/min/10^6 cells,

Table 2
CCR2 binding, Ca^{2+} flux and hERG binding data for selected compounds



3

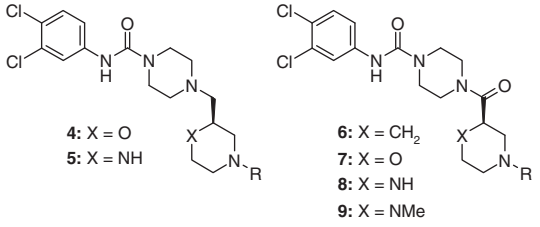
Compd	Ar	CCR2 binding IC_{50}[a] (nM)	CCR2 Ca^{2+} flux IC_{50}[a] (nM)	hERG binding IC_{50}[a] (μM)
3a	3-Cl-4-F-Ph	50	75	14
3b	3-Cl-4-CF_3-Ph	17	31	0.89
3c	3-CF_3-4-Cl-Ph	35	421	6.3
3d	3-Cl-4-Me-Ph	27	184	7.1
3e	4-Cl-Ph	127	220	14
3f	3-Cl-Ph	171	202	28
3g	4-Br-Ph	58	162	7.1
3h	4-OMe-Ph	1458	nt	nt
3i	3-CF_3-Ph	98	578	13
3j	4-CF_3-Ph	68	593	6.3
3k	3-CN-Ph	1055	5870	nt
3l	4-CN-Ph	449	4360	nt
3m	2-Cl-Ph	>10000	nt	nt
3n	3-SO_2Me-Ph	>10000	nt	nt
3o	Ph	710	>3000	nt

[a] IC_{50}s were derived from triplicate measurements whose standard errors were normally <5% in a given assay. Assay to assay variability was within twofold based on the results of a standard compound. nt = not tested.

and in human microsomes: 5 μL/min/mg protein). Rat pharmacokinetic (PK) data was acceptable: iv clearance was 24 mL/min/kg, with a half-life of 5 h and volume of distribution of 8 L/kg, and oral bioavailability was 11%. Compound **2g** inhibited CYPs 3A4 and 2D6 with IC_{50}s of 1.6 and 3.1 μM, respectively, which did not meet our desired profile of >10 μM.

Unwanted activity at the hERG ion channel is a frequent problem in the optimization of chemokine receptor antagonists including CCR2.[3,5] The observed correlation of both CCR2 and hERG with lipophilicity suggested that further changes in the substituents on this scaffold would be unlikely to increase the separation of these activities. Potential approaches to reduce hERG activity include

Table 3
CCR2 binding, Ca^{2+} flux and hERG binding data for selected compounds



4: X = O
5: X = NH

6: X = CH_2
7: X = O
8: X = NH
9: X = NMe

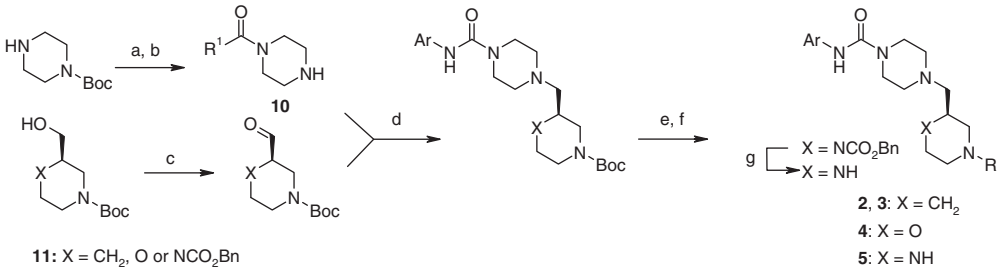
Compd	R	CCR2 binding IC_{50}[a] (nM)	Ca^{2+} flux IC_{50}[a] (nM)	hERG binding IC_{50}[a] (μM)
4a	Et	64	277	7.9
4b	*c*-Pr	267	262	16
4c	*i*-Pr	17	42	6.3
rac-**5a**	Et	797	Nt	8.9
5b	*i*-Pr	26	32	5.6
6a	Et	856	588	5.0
rac-**7a**	Et	2570	>1000	5.0
8a	Et	21	55	10
8b	*c*-Pr	32	38	19
8c	*i*-Pr	3.5	5.8	17
8d	Me	69	362	20
rac-**9a**	Me	5500	Nt	13

[a] IC_{50}s were derived from triplicate measurements whose standard errors were normally <5% in a given assay. Assay to assay variability was within twofold based on the results of a standard compound. nt = not tested.

Table 4
In vitro and in vivo PK data for **8c**

Species	Cl_{int}[a] (μL/min/10^6 cells)	Cl[b] (mL/min/kg)	Vss[b] (L/kg)	$t½$[b] (h)	F[c] %
Rat	<2	30	4.0	3.0	11
Dog	<2	17	8.5	9.1	83
Human	<2	–	–	–	–

[a] For experimental procedures see Ref. 14.
[b] Compounds dosed 4 μmol/kg iv.
[c] Compounds dosed 11 (rat) and 4 (dog) μmol/kg po.



Scheme 1. Reagents and conditions: (a) ArNCO, $CHCl_3$, rt; (b) TFA, CH_2Cl_2, rt; (c) Dess–Martin periodinane; CH_2Cl_2, rt (quant.); (d) $Na(OAc)_3BH$, CH_2Cl_2, rt (89% over three steps); (e) TFA, CH_2Cl_2, rt or 4 M HCl, dioxane, rt; (f) RBr, K_2CO_3, acetone, reflux (48–77% over two steps); (g) 4 M HCl, dioxane; $ZnBr_2$, 10% Pd/C, H_2, NMP, rt (60%).

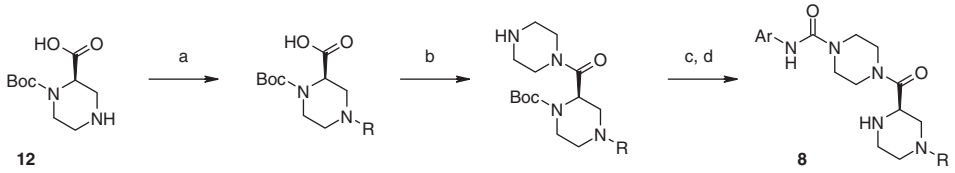
reducing lipophilicity by incorporating polar atoms and attenuating the pK_a of the basic amine group.[12] With this in mind we investigated replacing the piperidine with morpholine or piperazine and changing the methylene linker to a carbonyl. The data for these compounds is shown in Table 3.

Replacing the piperidine in **2** with morpholine (**4**) or piperazine (**5**) mostly retained the activity at CCR2 while reducing hERG activity somewhat. Not all changes were beneficial: replacement of the methylene linker in **2** with carbonyl (**6**) led to a drop in activity which was also seen going from methylene to carbonyl in the morpholine sub-series (**4** to **7**). However, gratifyingly, the combination of the carbonyl linker and the piperazine terminal ring (**8**) gave potent compounds together with a 10-fold reduction in hERG activity (**8b**, **8c**). The proximal nitrogen of the piperazine compounds did not tolerate substitution—racemic **9a** was nearly 100-fold less potent than **8d**. Rationalising the effects of these changes on hERG activity in terms of shifts in log$D_{7.4}$ is not straightforward since the structural changes affect ionization as well as lipophilicity. For example, going from piperidine **2** to morpholine **4** reduces log$P$ by ~0.6 units but increases log$D_{7.4}$ by ~0.8 units due to the reduction in basicity (pK_a from ~10 to ~8) of the most basic terminal nitrogen arising from the inductive effect of the oxygen.[13] Similarly, the change to a piperazine with a carbonyl linker (**2** to **8**) which gave around a 10-fold improvement in hERG while slightly improving the CCR2 activity is accompanied by a reduction in log$P$ but an increase in log$D_{7.4}$ (e.g., matched pair **2h** and **8c** have measured log$D_{7.4}$ values of 1.62 and 2.21, respectively and measured basic pK_a values of 10.0 and 7.8, respectively, giving log$P$ values of 4.2 and 2.7, respectively). The SAR of the terminal alkyl substituent did not recapitulate what had been seen in the original piperidines **2**. Surprisingly the isopropyl group which previously had similar activity to the cyclopropyl and only twofold better than the ethyl (**2h** vs **2g** and **2b**, Table 1) now gave significantly more activity (**4c** vs **4a** and **4b**, **8c** vs **8a** and **8b**). The most potent compound **8c** was selected for further profiling.

Compound **8c** had a clean CYP inhibition profile (IC_{50} >10 μM vs 5 isoforms), excellent physical properties (aqueous solubility >3100 μM), high metabolic stability in hepatocytes across species, acceptable in vivo PK in rat and excellent PK in dog (Table 4).

In the hERG ion current electrophysiology assay **8c** had a mean IC_{50} of 60 μM (std. dev. 19 μM, N = 6), giving a 10,000-fold selectivity for CCR2 over hERG. **8c** inhibited MCP-1-induced chemotaxis in CCR2-expressing THP-1 cells with a mean IC_{50} of 5.1 nM and inhibited MCP-1-induced L-selectin shedding in human peripheral whole blood with a pA_2 of 8.46. In common with other CCR2 antagonists[3a,5g] **8c** displayed a drop-off in activity in non-human species: IC_{50} values in mouse, rat and dog versions of the calcium flux FLIPR assay were 1.2, 0.2 and 1.6 μM, respectively (cf. 5.8 nM in human). In a secondary pharmacology panel consisting of 72 receptor and enzyme assays **8c** showed <33% inhibition at 3 μM in all tests. Compound **8c** had no activity at other human chemokine receptors (CCR1, CCR8, CXCR4, CXCR5) with the exception of CCR5 (FLIPR IC_{50} = 22 nM). Low selectivity over CCR5 is also commonly observed for CCR2 antagonists.[3a]

A general route to the synthesis of compounds **2**, **3**, **4** and **5** is shown in Scheme 1. The left hand side aryl urea was installed by reaction of mono-protected piperazine with the appropriately substituted phenyl isocyanate to give intermediate **10**. The right hand side methyl piperidine, morpholine or piperazine moiety was commercially available as a homochiral Boc-protected



Scheme 2. Reagents and conditions: (a) RI, Na_2CO_3, EtOH, reflux (63%); (b) DMTMM, *N*-methyl morpholine, piperazine, CH_2Cl_2, rt (50%); (c) ArNCO, $CHCl_3$, rt or $ArHNCO_2Ph$, NEt_3, THF, 60 °C; (d) TFA, CH_2Cl_2, rt (56–89% over two steps).

primary alcohol **11**. Dess–Martin oxidation of **11** gave an aldehyde which was coupled with **10** in a reductive amination. Removal of the Boc group with trifluoroacetic acid paved the way for alkylation of the terminal nitrogen to give compounds **2**, **3** and **4**. For compounds **5** having piperazine as the terminal ring, the nitrogen proximal to the methylene was first protected as the benzyl carbamate (Cbz) (X = NCO_2Bn in **11**). Removal of the Boc in step (e) required a change from TFA to HCl to avoid unwanted cleavage of the Cbz. The Cbz was removed in the final step using palladium catalysed hydrogenation of the hydrochloride salt of the starting material in the presence of zinc bromide which avoided hydrogenolysis of the aryl halogen atoms on the left hand side Ar group.[15] The sequence of steps could also be reversed so that the final step was the reaction of a phenyl isocyanate with an advanced intermediate which already contained the *N*-alkyl substituent on the terminal piperidine, morpholine or piperazine ring.[9]

The synthesis of compounds **6–9** containing the carbonyl linker followed a similar strategy and is illustrated for compounds **8** in Scheme 2. Starting from the mono-Boc-protected homochiral piperazine carboxylic acid **12**, N-alkylation was followed by amide coupling to piperazine using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride (DMTMM)[16] and *N*-methyl morpholine avoiding unwanted cleavage of the Boc protecting group. The aryl urea was installed by reaction with either a phenyl isocyanate or an *N*-aryl phenyl carbamate. Finally, removal of the Boc group resulted in compounds **8**.

In summary, testing of a 'GPCR ligand motif' library resulted in screening hit **1**. Exploration of SAR led to the identification of a novel, potent and selective series of *N*-aryl piperazine-1-carboxamide CCR2 antagonists with good DMPK properties but unacceptable hERG margin. Changes to the series scaffold increased ligand lipophilicity efficiency and serendipitously led to a compound, **8c**, which had low nanomolar activity at CCR2 and a 10,000-fold margin to hERG ion channel inhibition. Further studies on this series of CCR2 antagonists will be communicated in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.04.118. These data include MOL files and InChiKeys of the most important compounds described in this article.

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