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CCR2B Receptor Antagonists: Conversion of a Weak HTS Hit to a Potent Lead Compound

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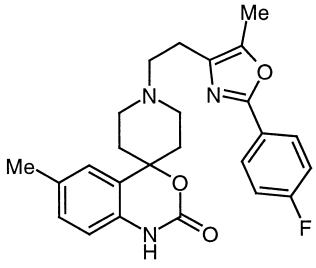
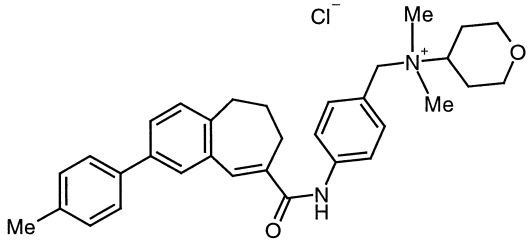
Abstract—A weak HTS hit at the CCR2B receptor has been converted into a potent antagonist by array SAR studies. Selectivity over the closely related CCR5 receptor is also achieved.

Over recent years, there has been a rapid growth in the number of isolated low molecular weight proteins called chemokines (*chemo*tactic cyto*kines*).[1] These proteins are involved in a variety of inflammatory responses via interaction with chemokine receptors located on the cell surface of leukocytes followed by chemotaxis and infiltration into the adjacent tissue. The chemokine proteins can be divided into four families dependent on the arrangement of conserved cysteine residues near the N-terminus.[1] Monocyte chemotactic protein-1 (MCP-1) is a member of the CC class of chemokines, and has been strongly implicated in various inflammatory diseases.[2] The effects of MCP-1 are mediated primarily via the CCR2B receptor,[3] and it has been widely recognised that antagonists of this receptor are potential therapeutic agents for various pathological conditions, e.g., atherosclerosis[4] and rheumatoid arthritis.[5] This hypothesis has been recently validated by studies using MCP-1[6] and CCR2[7] knockout mice. Two series of small molecule CCR2B antagonists have recently been reported, exemplified by the Roche compound **1**[8] and the Takeda compound **2**.[9] Both these compounds have major disadvantages, namely compound **1** possesses poor functional activity, and compound **2** possesses mixed CCR5/CCR2B receptor affinities. We now report the identification of a potent functional antagonist at the CCR2B receptor with additional selectivity over CCR5.



1 IC_{50} CCR2B 32 nM

IC_{50} chemotaxis 1 μM



2 IC_{50} CCR2B 27 nM

IC_{50} CCR5 1.4 nM

High-throughput screening of the SmithKline Beecham compound collection against the cloned human CCR2B receptor identified the indole derivative **3** as a weak ligand (K_i 5.3 μM).[12] Herein we describe the SAR around **3** and its conversion into a potent antagonist at the CCR2B receptor.

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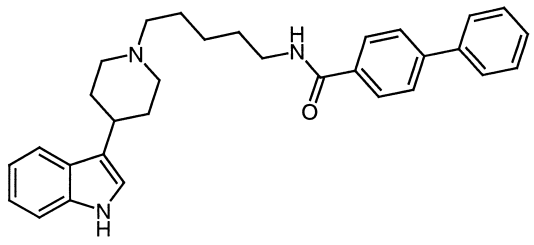


3 K_i 5.3 μM[12]

Our initial chemical programme was divided into three discrete areas: modification to the biphenyl amide moiety, variation of the chain length between the two side-chain nitrogen atoms, and substitution on the indole ring.

Chemistry[10]

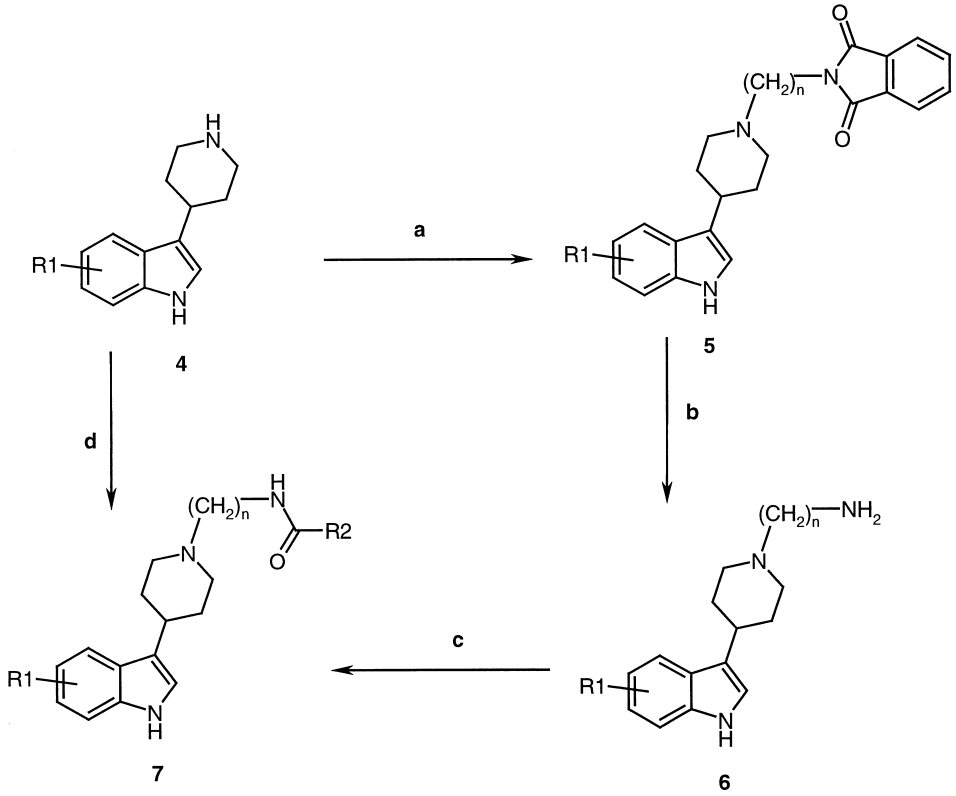
Amide linked compounds were synthesised by either of the routes shown in Scheme 1. Alkylation of the readily available indolopiperidines **4**[11] with a bromoalkylphthalimide gave the chain extended protected amine derivatives **5**, which were deprotected to afford primary amines **6**. Coupling with the appropriate carboxylic acid provided the desired target structures **7**. Alternatively, the products **7** could be obtained via a direct alkylation of **4** with the appropriate bromoalkyl amides. Urea and sulfonamide analogues were prepared by a similar process.

Structure–activity relationships

Initially, we focused on modifications to the aromatic amide group in **3** (see Table 1). Unfortunately, it was found that the majority of substituted benzamides investigated exhibited reduced potency at the CCR2B receptor (data not shown), with the exception of halogen substituted analogues, e.g., **8** and **9**. Replacement of the amide carbonyl in **9** by sulfonyl also resulted in reduced potency. Introduction of a one- or two-carbon linker as in **11** or **12** offered no advantage, however replacement of the methylene group in **11** with NH to form the urea **13** provided a threefold potency increase. More interestingly, replacement of the benz-amide functionality in **9** with 3,4-dichlorocinnamide to give **14** conferred a 10-fold increase in affinity.

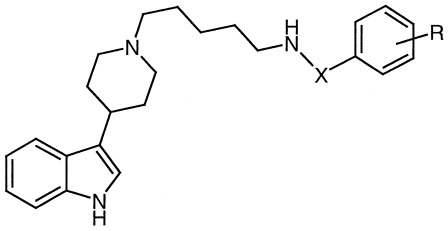
Having identified the cinnamide linker in **14** as optimal, we explored the influence of substitution on the aromatic ring. As illustrated in Table 2, highest affinity was observed with a small lipophilic substituent at positions 3 and/or 4 on the phenyl ring, **14** and **19** representing preferred compounds. Larger substituents as in **25**, or polar groups as in **27**, were disfavoured.

We then studied the effect of varying the carbon chain length between the basic nitrogen and the amide nitrogen. As can be seen from Table 3, a C_5 linkage is preferred, closely followed by C_4 and C_3, with C_2 and C_6 being essentially inactive.



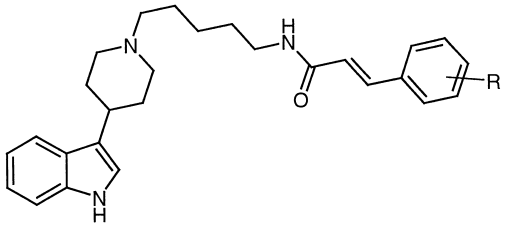
Scheme 1. Synthetic route to amide derivatives. Reagents: (a) $Br(CH_2)_nN$phthalimide, $NaHCO_3$, DMF, 80°C (75–85%); (b) hydrazine, ethanol, reflux (65–90%); (c) $R2CO_2H$, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, HOBT, CH_2Cl_2, room temp. (50–90%); (d) $Br(CH_2)_nNHCOR2$, $NaHCO_3$, DMF, 80°C (60–90%).

Table 1. Amide variations



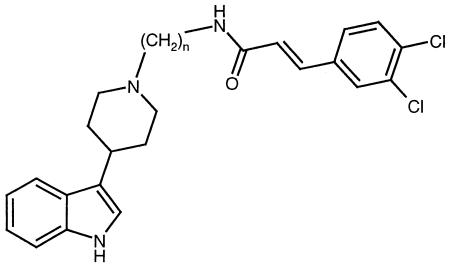
#	X	R	K_i (nM)[12]
3	C=O	4-Ph	5400
8	C=O	4-Br	7100
9	C=O	3,4-diCl	5100
10	SO_2	3,4-diCl	>10,000
11	$C(=O)CH_2$	3,4-diCl	4400
12	$C(=O)CH_2CH_2$	3,4-diCl	5900
13	C(=O)NH	3,4-diCl	1600
14	*trans* C(=O)CH=CH	3,4-diCl	420

Table 2. Cinnamide substitution



#	R	K_i (nM)[12]	#	R	K_i (nM)[12]
14	3,4-diCl	420	**22**	4-Me	550
15	H	870	**23**	3-Me	790
16	4-Cl	520	**24**	4-Ph	1450
17	3-Cl	560	**25**	4-Bu^t	1700
18	2-Cl	4400	**26**	4-NMe_2	1200
19	4-Br	350	**27**	4-OH	6300
20	4-I	460	**28**	4-NHAc	12,900
21	3-CF_3	790	**29**	4-CN	2800

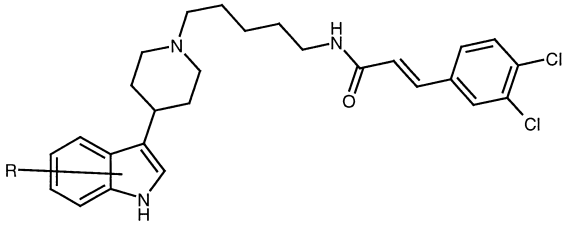
Table 3. Chain length variation



#	*n*	K_i (nM)[12]
30	2	>10,000
31	3	2500
32	4	660
14	5	420
33	6[a]	>10,000

[a]In original 4-phenylbenzamide series.

Table 4. Indole ring substitution



#	R	K_i (nM)[12]
14	H	420
34	5-OH	50
35	5-$MeSO_2NH$	79
36	5-MeO	590
37	4-OH	360
38	6-OH	360
39	5-OH-1-Me	4200
40	5-OH-2-Me	234

Finally, we undertook an SAR study on indole ring substitution, with key findings summarised in Table 4. Significantly, it was found that an H-bond donor at C-5 was beneficial with the 5-OH indole **34** exhibiting a 10-fold increase in affinity. This effect was also mirrored by the 5-$MeSO_2NH$ analogue **35**, which suggests a specific H-bonding interaction, as the corresponding 5-MeO, 4-OH and 6-OH derivatives did not show this increase. The indole NH appears to be crucial for high affinity, since the indole N-Me analogue **39** was ca. 80-fold less active. This hypothesis is also supported by the lack of activity for the corresponding benzofuran and benzothiophene analogues.[13] C-2 substitution, as in **40**, conferred a slight reduction in affinity which can be rationalised by an adverse conformational effect on the active indole-piperidine orientation.

Having identified **34** as a potent ligand at the CCR2B receptor, we then examined its effects in a functional system, and also its effects at the closely related CCR5 receptor. Initially, **34** was tested for its ability to inhibit MCP-1-stimulated calcium transients using fura-2-loaded human monocytes, and was found to have a mean K_b of 26 nM ($n=2$). Similarly, in a functional model based on inhibition of MCP-1 stimulated chemotaxis of human monocytes, it was shown that **34** is a potent antagonist with a mean K_b of 25 nM ($n=2$). Finally, the effects of antagonist **34** at the CCR2B receptor (K_i 50 nM) were demonstrated to be specific, with CCR5 receptor affinity considerably lower (K_i 4260 nM).

In summary we have identifed **34** as a potent CCR2B receptor antagonist, which has comparable activity in binding and functional studies, and which is selective over the CCR5 receptor, its closest chemokine receptor homologue. Unfortunately, **34** was not progressed due to unwanted 5-HT receptor affinities. Modifications to structure **34**, which reduce its propensity to interact with 5-HT receptors, will be the subject of a future publication.

References and Notes

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10. All novel compounds gave satisfactory analytical data in full agreement with their proposed structures.
11. For example see: Freter, K.; Fuchs, V.; Barsumian, E.; Oliver, J. T. *Arzn. Forsch.* **1985**, *35*, 272.
12. Antagonists were tested using a radioligand binding displacement assay. Compounds were dissolved in DMSO and a concentration range (1% final concentration in assay) and were incubated in the presence of 0.14 nM ^{125}I-MCP-1 and membranes (100,000 cell equivalents) from CHO cells stably transfected with the MCP-1 receptor CCR2B. The incubation took place at RT for 2 h in a total volume of 110 μL 50 mM HEPES, 1 mM $CaCl_2$, 5 mM $MgCl_2$, 0.5% bovine serum albumin (fatty acid free) pH 7.4 in 96 well plate format. Membranes were harvested on GF/C filters and filters were washed with 4×1 mL of 50 mM HEPES, 0.5 M NaCl pH 7.0. Filter bound radioactivity was determined by liquid scintillation counting. For IC_{50}'s and K_i's multiple curves were analysed by four parameter fitting using in-house developed Excel software entitled Inflexion 2. All K_i values represent the mean of at least two determinations.
13. SmithKline Beecham, unpublished results.