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N-Benzylindole-2-carboxylic acids: potent functional antagonists of the CCR2b chemokine receptor

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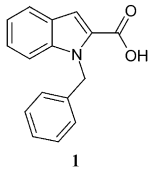
Abstract—Screening of the corporate database led to the discovery of a novel series of *N*-benzylindole-2-carboxylic acid CCR2b chemokine receptor antagonists. These compounds demonstrate high affinity and functional inhibition of the CCR2b receptor. A discussion of the structure–activity relationships is presented, together with evidence for a highly selective receptor binding profile.

1. Introduction

The recruitment and activation of select populations of leukocytes is a key feature of a variety of inflammatory conditions. Whilst this response is crucial for host defence during inflammation, the secretory products of white blood cells may increase injury by damaging surrounding healthy tissue. Monocyte chemoattractant protein-1 (MCP-1) is a member of the chemokine family of pro-inflammatory cytokines that mediate leukocyte chemotaxis and activation.[1–4] These effects are mediated principally through activation of intracellular signalling pathways following binding of MCP-1 to the chemokine receptor CCR2b. MCP-1 is a potent chemotactic and activating factor for monocytes and memory T-cells and has been shown to regulate adhesion molecule expression and cytokine production,[5] and to induce superoxide anion and lysosomal enzyme release from human monocytes.[6] MCP-1 has been implicated in the pathophysiology of a wide range of both acute and chronic inflammatory conditions such as rheumatoid arthritis,[7–9] atherosclerosis,[10–13] asthma,[14–16] psoriasis[17,18] and transplant rejection.[19–22] Evidence for this involvement comes from studies showing elevated MCP-1 expression correlates with leukocyte infiltration in vivo,[23–25] the use of neutralizing antibodies,[26,27] and through both animal receptor[28] and ligand[29] knockout studies.

An MCP-1 receptor antagonist thus represents an attractive target for drug discovery, and this has prompted an intense period of pharmaceutical research. Several companies have reported the discovery of potent small molecule antagonists of the CCR2b receptor, showing varying degrees of selectivity over closely homologous receptors.[30–35] It appears that these agents, discovered independently, share a common pharmacophore, namely a basic moiety (predominantly cyclic) connected by spacing groups to a lipophilic aromatic end group. Indeed, two groups have demonstrated the importance of the interaction of this base with glutamate 291 on helix 7 of the transmembrane domain, through a combination of receptor mutations, and homology modelling.[33,36] It was similarly demonstrated that this residue is important in binding of the natural ligand. Screening of our corporate compound collection for compounds which bound to the CCR2b receptor[37] led to discovery of the *N*-benzylindole-2-carboxylic acid **1**, which had an IC_{50} value of 1.7 μM. This structure is noteworthy as it deviates from the emerging pharmacophore for antagonism described above, suggesting a possible alternative binding site for small molecule intervention.



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A synthetic chemistry program was initiated to explore the structure–activity relationship around this lead

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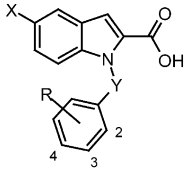
compound. SAR studies initially focused on modification of the benzyl group, with both indole and 5-chloroindole derivatives examined (Table 1). Small, lipophilic substituents were well tolerated (compounds **2**–**4**) in all positions. 3,4-Disubstitution was particularly favoured, resulting in an order of magnitude increase in binding affinity (**5**–**7**). In general, substitution in the 2- and 6-positions of the benzyl group were less well tolerated, likely due to the pronounced effect this pattern can have on the conformation of the benzyl group. Substitution at the 4-position with bulky groups such as phenyl (**8**) or benzyloxy (**9**) led to significantly less activity. It was observed that for monosubstituted benzyl groups, substitution at the 3-position was generally better tolerated than at 4 (compare compounds **10** and **13**), although strong electron donating groups in either position offered no advantage over the unsubstituted compound. Strong electron withdrawing groups were acceptable at the 3-position (**11**, **12**), but the placing of polar groups such as the acid **14** led to much weaker activity. Taken together, these results strongly indicate that the benzyl group occupies a relatively small, hydrophobic pocket within the CCR2b receptor, and is critical for activity (replacement of the benzyl for a number of aliphatic groups, or complete removal, led to inactive compounds—data not shown).

The importance of the conformation of the benzyl group is further highlighted by sulfonyl analogue (**15**), which is less active than the benzyl analogue (**5**), and by the benzoyl derivative (**16**), which is inactive up to 50 μM, although for these analogues, an adverse electronic influence cannot be ruled out.

We next turned our attention to the carboxylic acid. For these studies, the 3,4-dichlorobenzyl analogue (**5**) was selected as a potent lead for further exploration of the SAR. It was observed that this anionic function is a key requirement to provide optimal binding to the CCR2b receptor (Table 2), with a reasonable relationship between decreasing pK_a and increasing affinity. Certain acid mimics were acceptable, though only relatively acidic ones, such as the trifluoromethyl acylsulfonamide (**17**) and tetrazole (**18**), conferred activity close to that of the starting compound. In the case of **17**, the dramatic increase in acidity over the parent acid does not translate into improved binding, indicating that only a threshold of acidity is required for optimal binding, or possibly that other factors are influencing potency for this analogue. It is noteworthy that **20**, the methyl analogue of **17** is greater than an order of magnitude less potent. Whilst relatively bulky acid mimics were tolerated (see **19**), compounds that lacked the ability to generate the negative charge were generally of much weaker activity (**21**).

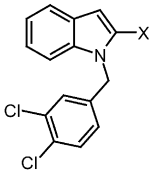
Finally, the effect of substitution on the indole ring in compound **5** was investigated (Table 3). Strong electron withdrawing groups such as nitro (**22**–**25**) and trifluoromethyl (**26**–**29**) were generally poorly tolerated except when substituted in the 5-position (**23**, **27**). The methane sulfonyl group was particularly disfavoured, here even at C-5 (**30**, **31**); however, strong electron-withdrawing groups were better tolerated at the indole 3-position (**52**, **53**). As with the benzyl group, halogens appeared to be optimal for activity with fluorine (**32**–**35**) favoured over chlorine (**36**–**40**). With the electron donating substituents methoxy and amino, substitution in the 4- and 5-positions are acceptable (**42**, **43** and **46**, **47**), but leads to substantial loss in affinity at the other positions examined (**41**, **44**, **45** and **48**). The effect of steric bulk on the indole ring was examined using the phenyl derivatives (**49**–**51**). Here a strong preference for substitution at the 4-position was observed, with a dramatic reduction in affinity on moving to the 5- and 6-positions. In general, it can be seen that the 4- and 5-positions are more tolerant of steric bulk than the 6- and 7-positions. The most sterically demanding group synthesized at the 7-position was the nitro compound (**25**), which showed activity greater than 10 μM, again indicating the importance of the benzyl group conformation for receptor interaction.

Table 1. Binding studies on benzyl analogues of compound **1**



Compd	R	X	Y	CCR2b IC_{50} (μM)
1	H	H	CH_2	1.7
2	2-Cl	Cl	CH_2	1.48
3	3-Cl	H	CH_2	1.13
4	4-Cl	H	CH_2	1.36
5	3,4-diCl	H	CH_2	0.23
6	3,4-diCH_3	Cl	CH_2	0.22
7	3-Cl,4-CH_3	Cl	CH_2	0.12
8	4-Ph	H	CH_2	>50
9	4-OCH_2Ph	Cl	CH_2	>50
10	3-OCH_3	Cl	CH_2	3.01
11	3-CN	Cl	CH_2	2.0
12	3-NO_2	Cl	CH_2	0.65
13	4-OCH_3	Cl	CH_2	5.77
14	3-COOH	Cl	CH_2	27
15	3,4-diCl	H	SO_2	1.02
16	3,4-diCl	H	CO	>50

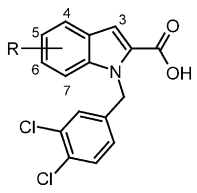
Table 2. Modifications to the acid group of compound **5**



Compd	X	CCR2b IC_{50} (μM)	pK_a
5	COOH	0.23	3.3
17[a]	$CONHSO_2CF_3$	0.37	−3.01[b]
18	Tetrazole	1.03	3.52
19	$CONHSO_2Ph$	4.78	3.87
20	$CONHSO_2CH_3$	6.09	3.9
21	$CONH_2$	12.7	16.73[b]

[a] 5-Fluoroindole derivative.
[b] Estimated from ACD pK_a suite of software.

Table 3. Effect of substitution on the indole ring of compound **5**

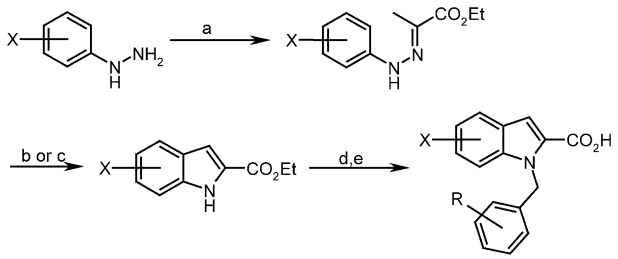


Compd	R	CCR2b IC_{50} (μM)
5	H	0.23
22	4-NO_2	1.31
23	5-NO_2	0.39
24	6-NO_2	1.64
25	7-NO_2	10.84
26	4-CF_3	1.19
27	5-CF_3	0.17
28	6-CF_3	1.05
29	7-CF_3	1.27
30	4-SO_2CH_3	3.88
31	5-SO_2CH_3	2.2
32	4-F	0.099
33	5-F	0.056
34	6-F	0.09
35	7-F	0.125
36	3-Cl	0.26
37	4-Cl	0.15
38	5-Cl	0.11
39	6-Cl	0.34
40	7-Cl	0.34
41	3-OCH_3	0.77
42	4-OCH_3	0.28
43	5-OCH_3	0.41
44	6-OCH_3	2.3
45	7-OCH_3	1.21
46	4-NH_2	0.23
47	5-NH_2	0.5
48	6-NH_2	12.6
49	4-Ph	0.64
50	5-Ph	8.6
51	6-Ph	12.3
52	3-CN	0.30
53	3-COOH	0.096

The optimal indole substituent found in this study was the 5-fluoro group, which had an IC_{50} of 56 nM (compound **33**). The ability of this compound to inhibit chemotaxis of cells expressing CCR2b receptor in response to an MCP-1 stimulus was examined, and an IC_{50} of 312 nM observed for inhibition of this response.[37] In addition, representative compounds were further profiled against a panel of over 40 other seven-transmembrane G-protein coupled receptor binding assays. These included α and β adrenoceptors, 5-HT and muscarinic receptors, and ion channels. All compounds had IC_{50} values greater than 10 μM. Thus, the *N*-benzylindole-2-carboxylic acids represent a novel class of potent, selective and functionally active inhibitors of the CCR2b chemokine receptor.

2. Chemistry

The compounds described herein were synthesized according to the generic route shown in Scheme 1. Indole-2-carboxylic esters were either purchased from commercial sources, or isolated following Fischer indolization of the ethyl pyruvate hydrazones shown.



Scheme 1. Reagents and conditions: (a) CH_3COCO_2Et, EtOH, AcOH; (b) polyphosphoric acid, 90 °C; (c) $ZnCl_2$, AcOH, reflux; (d) NaH, DMF, R-$PhCH_2Cl$; (e) 3 M NaOH, THF, EtOH.

Regioisomers were separated at this stage via column chromatography or crystallization. The indoles were then alkylated with benzyl halides following deprotonation with sodium hydride, and the target compounds obtained upon saponification. 3-Substituted indoles were generally prepared by derivation of indole derivatives prior to benzylation using standard procedures.[38]

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