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The synthesis of substituted bipiperidine amide compounds as CCR3 ligands: Antagonists versus agonists

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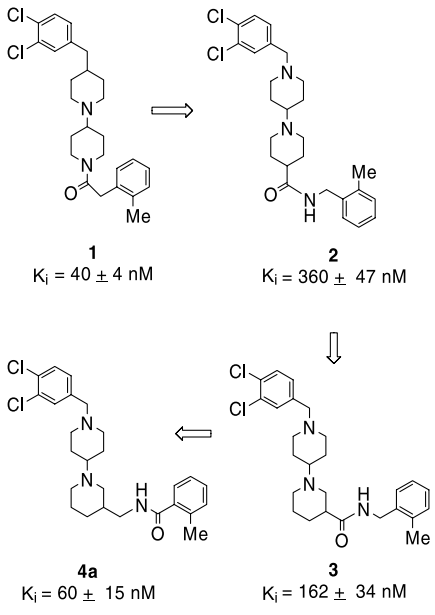
Abstract—Structure–activity relationship study of bipiperidine amide **1** has identified the reverse bipiperidine amide **4a** as a CC chemokine-3 (CCR3) receptor antagonist. Optimization of the structure–activity relationship of compound **4a** has resulted in the identification of a CCR3 antagonist **4i** as well as a CCR3 agonist **13**.

Asthma is a chronic inflammatory disease of the lungs which is characterized by the recruitment of eosinophils, T lymphocytes, basophils, and mast cells into the lung tissue and results in reduced airflow and bronchial hyperresponsiveness. Eosinophil chemotaxis is controlled by the CC chemokine-3 (CCR3) receptor which is a seven transmembrane G-protein coupled receptor activated by several chemokines including eotaxins, RANTES, and macrophage chemoattractant protein-4.[1–3] An antibody to eotaxin has been shown to decrease eosinophil migration to the lung and airway hyperreactivity after allergen challenge in mice.[4] Therefore, a small molecule CCR3 antagonist may inhibit eosinophil chemotaxis and be effective in the treatment of asthma. A number of research organizations are investigating this approach.[5–11]

We have previously reported the identification of compound **1** as a CCR3 antagonist.[12] In our structure–activity relationship (SAR) study of compound **1**, we discovered that the bipiperidine core can be reversed as in compound **2** and retain reasonable CCR3 affinity. The CCR3 affinity can be optimized first, by moving the amide moiety to the 3-position of the reversed bipiperidine core as in compound **3** and second, by repositioning the carbonyl moiety as in compound **4a**. This paper will describe the results of our SAR investigation of compound **4a**.



The construction of compounds **2**–**4** followed the synthetic route depicted in Scheme 1. Alkylation of 4-piperidone (**5**) produced ketone **6** which undergoes a reductive amination reaction with methyl isonipecotate

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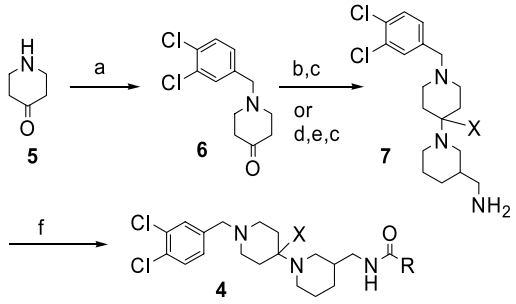


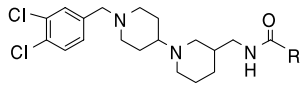
Scheme 1. Reagents and conditions: (a) 3,4-dichlorobenzyl chloride, Et_3N, $ClCH_2CH_2Cl$, Δ, 65%; (b) 3-(*t*BOC-aminomethyl)piperidine, CH_2Cl_2, AcOH, $NaB(OAc)_3H$, 64% for X = H; (c) TFA, CH_2Cl_2, 100%; (d) 3-(*t*BOC-aminomethyl)-piperidine, $Ti(OiPr)_4$, CH_2Cl_2, Et_2AlCN, 96% for X = CN; (e) MeMgBr, THF, 0 °C, 88% for X = Me; (f) RCOOH, Et_3N, EDCI, HOBT, DMF.

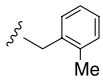
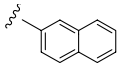
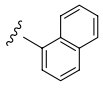
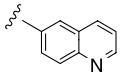
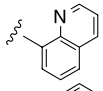
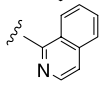
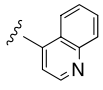
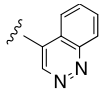
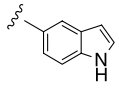
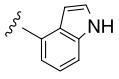
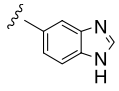
for the formation of compound **2**, ethyl nipecotate for the formation of compound **3**, or 3-(*t*BOC-aminomethyl)-piperidine for the formation of compound **4**. Since the amide moiety was introduced at the last step, a variety of amides were surveyed and selected results are summarized in Table 1. All analogues were studied as a mixture of stereoisomers. Each compound has been assayed for in vitro CCR3 membrane binding activity[13] as well as agonist activity in a [^{35}S]GTPγS assay.[14–17] All of these analogues were found to be antagonists. The spatial shape of the phenyl ring appears to be important for activity since the acetamide **4b** is less potent while the cyclohexyl amide **4c** retains activity. In addition, the methyl group on the phenyl ring can be replaced by a methoxy group as in **4d**, but the electron withdrawing chloro substituent in **4e** reduces CCR3 activity. Insertion of a methylene linker in the toluyl amide **4f** is also tolerated. In particular, the phenyl ring can be replaced with a number of bicyclic rings including naphthalene, quinoline, and indole and retain biological activity. The 6-quinolinyl amide **4i** is the most potent CCR3 antagonist in this subseries.

Next, we decided to examine the SAR of the 3,4-dichlorobenzyl moiety. These analogues were synthesized according to the route outlined in Scheme 2. 1-BOC-3-aminomethylpiperidine (**8**) was converted into the 6-quinolinyl amide **9**. Reductive amination introduced the second piperidine ring of intermediate **10** and variation of the 3,4-dichlorobenzyl subunit is accomplished at the last synthetic step.

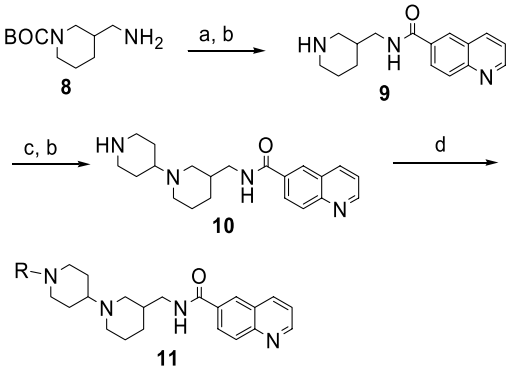
Selected dichlorobenzyl analogues are tabulated in Table 2. The 3,4-dichloro substitution pattern is critical and exhibits the optimal binding interaction in this lipophilic region of the CCR3 receptor. This key moiety has also been established in the published SAR studies of CCR3 antagonists from Gong et al.[7] and Naya et al.[8] Interestingly, the 3,5-dichloro analogue **11a** and the 2,5-dichloro analogue **11b** are less active as well as exhibit a degree of agonist activity in the [^{35}S]GTPγS assay.[15] In terms of characterization of agonist activity, since the E_{max}% of the numerous natural chemokine ligands of human CCR3 ranged from approximately 40 to 115% in our [^{35}S]GTPγS assay, compounds with stimulatory activity in this assay of >40% inhibition are considered to have significant agonist activity. The monosubstituted 2-chloro analogue **11c** is inactive while the 4-chloro analogue **11d** shows reasonable CCR3 affinity. The saturated cyclohexylmethyl analogue **11e** is completely inactive. Methyl substitution at the

Table 1. In vitro CCR3 membrane binding activity of amide bipiperidine analogues **4a–q**



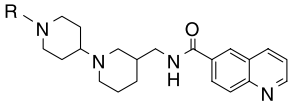
Compd	R	K_i (nM)
4a		60 ± 15
4b	Me	533 ± 83
4c		107 ± 25
4d		92 ± 13
4e		27%[a]
4f		86 ± 23
4g		48 ± 9
4h		58 ± 14
4i		23 ± 1
4j		30 ± 2
4k		34 ± 6
4l		80 ± 12
4m		99 ± 11
4n		29 ± 1
4o		36 ± 6
4p		47 ± 9

[a] % inhibition at 1 μM (n = 2).



Scheme 2. Reagents and conditions: (a) 6-quinolinecarboxylic acid, EDCI, HOBT, CH_2Cl_2, 96%; (b) TFA, CH_2Cl_2, 100%; (c) *N*-BOC-4-piperidone, AcOH, $NaB(OAc)_3H$, CH_2Cl_2, 70%; (d) RCHO, AcOH, $NaB(OAc)_3H$, CH_2Cl_2 or RCOCl, Et_3N, CH_2Cl_2.

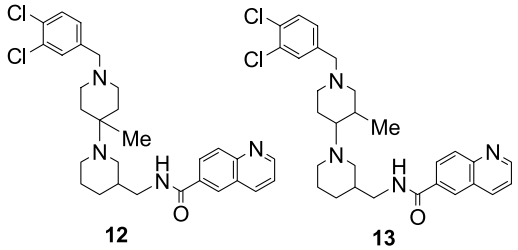
Table 2. In vitro CCR3 membrane binding and agonist (GTPγS) activity of benzyl bipiperidine analogues **4i** and **11a**–**j**



Compd	R	K_i (nM)	E_{max}% GTPγS[a]
4i	3,4-DiCl-PhCH$_2$	23 ± 1	−7
11a	3,5-DiCl-PhCH$_2$	398 ± 59	45
11b	2,5-DiCl-PhCH$_2$	391 ± 44	45
11c	2-Cl-PhCH$_2$	36%[a]	NT
11d	4-Cl-PhCH$_2$	95 ± 7	−8
11e	CyclohexylCH$_2$	17%[b]	NT
11f	3,4-DiCl-PhCHMe	74 ± 3	−12
11g	3,4-DiCl-PhCH$_2$CH$_2$	180 ± 32	2
11h	3,4-DiCl-PhCO	20%[b]	NT
11i	3,4-DiCl-PhCH$_2$CO	767 ± 16	NT
11j	3,4-DiCl-PhNHCONH	6%[b]	NT

NT = not tested.
[a] E_{max}% at 10 μM (n = 2).
[b] % inhibition at 1 μM (n = 2).

benzylic position as in **11f** or extension to the 3,4-dichlorophenethyl as in **11g** also decrease affinity. Replacement of the 3,4-dichlorobenzyl moiety with the corresponding amide moieties as in compounds **11h** and **11i** or the urea moiety as in compound **11j** also produces inactive analogues.



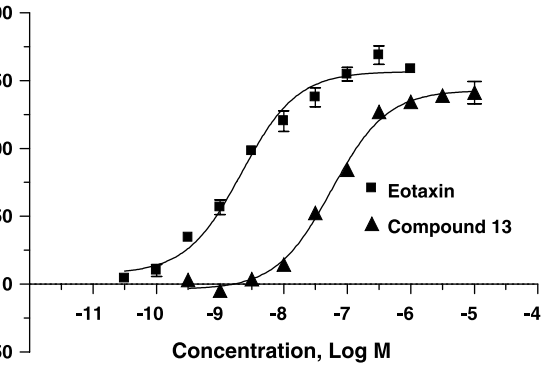
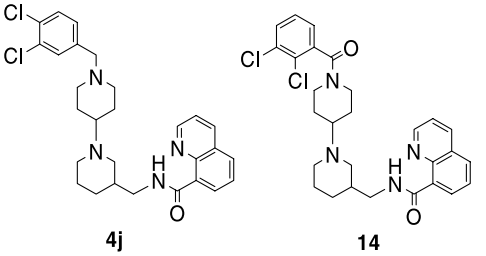


Figure 1. CCR3 ^{35}S-GTPγS binding for eotaxin and compound **13**.

Modification of the reverse bipiperidine core by addition of a methyl group afforded an interesting biological result. A methyl group is tolerated at the 4-position as in compound **12**, but at the 3-position produced a very potent agonist, compound **13**. The synthesis of compound **12** is included in Scheme 1 (X = Me) while the synthesis of compound **13** follows a similar pathway beginning with the commercially available *N*-benzyl-3-methyl-4-piperidone. The dose-response study of agonist **13** in the [^{35}S]GTPγS assay is depicted in Figure 1. This experience of unexpectedly discovering an agonist profile from a structural modification of an antagonist has precedence in the CCR3 research of De Lucca et al.[6] and Anderskewitz et al.[10] De Lucca and co-workers found that quaternary piperidinium salts were potent functional agonists while their corresponding parent piperidine derivatives were antagonists. Anderskewitz and co-workers reported that their lead cyclic amidine structure was a CCR3 antagonist, but addition of an imine moiety to the cyclic amidine in order to modulate the basicity of the compound produced a potent agonist. A second example of a structural modification inducing an agonist profile in our series is illustrated below. The 8-quinolinyl amide **4j** is a potent CCR3 antagonist, and replacement of the 3,4-dichlorobenzyl moiety with the 2,3-dichlorobenzoyl moiety generates the potent agonist **14**.



In conclusion, compound **4i** was identified as a potent CCR3 antagonist from this structural series and exhibited minimal activity at the CCR1 (17% at 1 μM), CCR4 (37% at 1 μM), and CCR8 (−8% at 1 μM) receptors. Compound **4i** was also active in the calcium flux

assay[18] with IC_{50} = 215 ± 84 nM and in the recombinant CCR3 (BaF3 cells) chemotaxis assay[19] with IC_{50} = 136 ± 38 nM. Unfortunately, in a rat pharmacokinetic study, compound **4i** exhibited a very low AUC of 264 ng h/mL at 3 mpk (iv) which precluded advancing into in vivo studies.

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13. Membrane binding assay protocol: This assay was done using ^{125}I eotaxin and membranes from CREM3 cells (a human CCR3 transfected rat Y3 cell line) on PVT-WGA-SPA beads in binding buffer (0.5% bovine serum albumin, 25 mM HEPES buffer, 75 mM NaCl, 1 mM $CaCl_2$, 5 mM $MgCl_2$, adjusted to final pH 7.6). Compounds were tested from 100 μM to 10 pM final concentrations in 96-well plates. The plates were shaken briefly and incubated at room temperature for 5 h before counting on a scintillation counter.
14. Activation of G-protein-coupled receptors with agonists stimulates the exchange of GTP for GDP on the active site of the Gα protein. This activation can be measured by the binding of [^{35}S]GTPγS, a non-hydrolyzable GTP analogue, to receptor-membrane preparations in the presence of excess GDP. E_{max}% is the percent increase over basal binding of [^{35}S]GTPγS achieved for each compound at 10 μM and is expressed as a percent of maximal eotaxin response at 100 nM.
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18. Calcium flux assay protocol: This assay used CREM3 cells at 37 °C. Compounds were dissolved in DMSO and diluted with buffer (pH 7.4 HBSS containing HEPES, BSA, and probenecid). Intracellular calcium levels were measured with a fluorometric imaging plate reader (FLIPR from Molecular Devices, Sunnyvale, CA) at 1 s intervals for 60 s then at 2 s intervals for 60 s.
19. Eotaxin-mediated chemotaxis assay protocol: This assay used BAF3 cells which expressed recombinant human CCR3. The Neuroprobe ChemoTx microplate system with a 5 μm pore size filter was used according to the manufacturer's specifications. Cell chemotaxis in response to 1 nM human eotaxin in the presence and absence of the compound was measured after 3 h incubation at 37 °C. Cells which had migrated in response to eotaxin were quantitated using the LDH assay (Promega).