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The synthesis of substituted bipiperidine amide compounds as CCR3 antagonists

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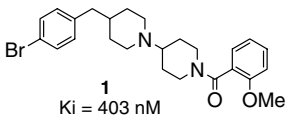
Abstract—Bipiperidine amide **1** has been identified as a CC chemokine receptor 3 (CCR3) antagonist. Optimization of its structure–activity relationship has resulted in the identification of *cis* (*R*,*R*)-4-[(3,4-dichlorophenyl)methyl]-3-hydroxymethyl-1′(6-quinolinyl-carbonyl)-1,4′-bipiperidine **14n**, which exhibits potent receptor affinity and inhibition of both calcium flux and eosinophil chemotaxis.

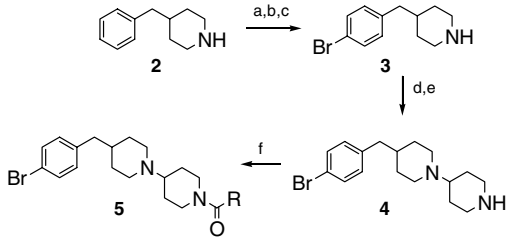
Despite advances in the treatment of asthma, including long acting β-agonists and inhaled corticosteroids, the incidence of morbidity and mortality due to this disease has increased. Therefore, additional therapeutic approaches are needed. A significant characteristic of airway diseases such as asthma, is the migration of eosinophils into the lung tissue. The CC chemokine receptor 3 (CCR3) is a member of the seven transmembrane G-protein coupled receptor family, and is the predominant receptor on eosinophils responsible for eosinophil chemotaxis.[1] It is activated by several chemokines including eotaxins, RANTES, and macrophage chemoattractant protein-4.[2] An antibody to eotaxin has been shown to decrease eosinophil migration to the lung and airway hyperreactivity after allergen challenge in mice.[1] The importance of CCR3 antagonists in allergic inflammation is still being determined.



1
Ki = 403 nM

High-throughput screening of our internal corporate compound database using a CCR3 membrane binding assay has led to the identification of several lead structures as CCR3 antagonists. In particular, compound **1** exhibits reasonable affinity for the CCR3 receptor and is amenable to a structure–activity relationship study.

The synthesis of compound **1** is depicted in Scheme 1. Since the amide moiety was introduced at the last step, a library of various amides and sulfonamides was rapidly surveyed, and we discovered that the



2 **3** **5** **4**

Scheme 1. Reagents and conditions: (a) TFAA, CH_2Cl_2, 88%; (b) CH_3SO_3H, 1,3-dibromo-5,5-dimethylhydantoin, CH_2Cl_2, 45%; (c) K_2CO_3, MeOH, H_2O, 99%; (d) N-*t*BOC-4-piperidone, $NaB(OAc)_3H$, AcOH, $ClCH_2CH_2Cl$, 58%; (e) 4 N HCl in dioxane, MeOH, CH_2Cl_2, Δ, 100%; (f) acyl chlorides or sulfonyl chlorides, TEA, CH_2Cl_2; carboxylic acids, DEC, HOBT, CH_2Cl_2, DMF.

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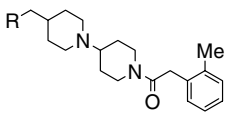
Table 1. In vitro CCR3 membrane binding activity of bipiperidines **5a** and **8a–n**



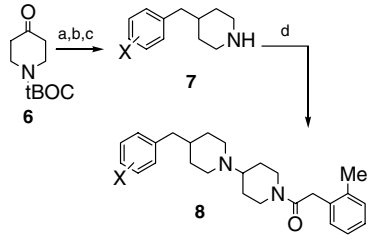
Compd	R	K_i (nM)[a]
5a	4-Br–Ph	66 ± 5
8a	4-Cl–Ph	142 ± 37
8b	4-F–Ph	111 ± 5
8c	4-MeO–Ph	792 ± 95
8d	3-Cl–Ph	128 ± 53
8e	2-Cl–Ph	20%[b]
8f	3,5-diF–Ph	2255 ± 135
8g	3,5-diCl–Ph	938 ± 195
8h	3,4-diCl–Ph	40 ± 4
8i	2,3-diCl–Ph	103 ± 22
8j	2,4-diCl–Ph	56 ± 21
8k	2,5-diCl–Ph	251 ± 33
8l	2-F–4-Cl–Ph	145 ± 26
8m	3-F–4-Cl–Ph	191 ± 48
8n	3-F–5-Cl–Ph	504 ± 84

[a] The assay protocol is described in Refs. 4 and 5.
[b] % Inhibition @ 1 μM ($n = 2$).

2-toluoylacetyl-1,4-bipiperidine **5a** showed improved CCR3 affinity (see Table 1).

We next decided to optimize the substitution pattern on the phenyl ring, and these analogues were synthesized according to the route outlined in Scheme 2. The key step involved a hydroboration and subsequent palladium coupling reaction (Suzuki protocol) to incorporate the appropriate phenyl ring.[3]

Membrane binding data for selected phenyl analogues are shown in Table 1. Replacement of the 4-bromo substituent in **5a** with the electron withdrawing chloro **8a** or fluoro **8b** resulted in a smaller decrease in CCR3 potency when contrasted with the electron donating methoxy group in **8c**. Comparison of the *ortho* (**8e**), *meta* (**8d**), and *para* (**8a**) chloro analogues indicated that substitution was better tolerated at the *meta* and *para* positions. A number of disubstituted analogues **8f–n** were examined, and only the 3,4-dichlorophenyl-bipiperidine **8h** exhibited a slight improvement in CCR3 affinity.

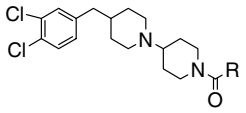


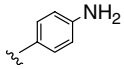
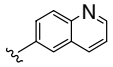
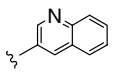
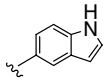
Scheme 2. Reagents and conditions: (a) Ph_3PMeBr, *n*BuLi, PhMe, −78 °C to rt, 79%; (b) 9-BBN, X-PhBr, $Pd(dppf)Cl_2$, K_2CO_3, THF, DMF, Δ 21–77%; (c) 4 N HCl in dioxane, CH_2Cl_2, 100%; (d) *N*-2′-toluoylacetamido-4-piperidone, $NaB(OAc)_3H$, AcOH, $ClCH_2CH_2Cl$, 19–57%.

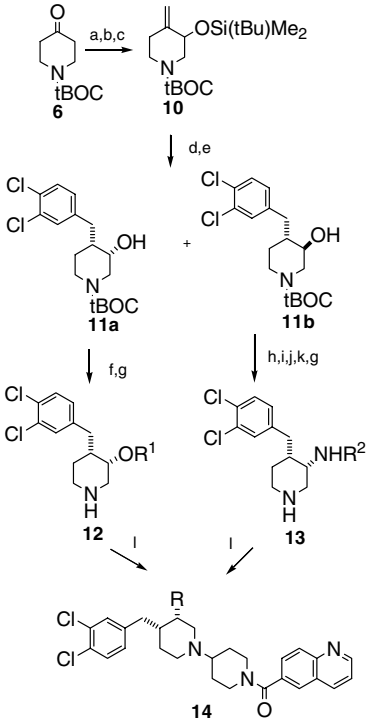
With the 3,4-dichlorobenzyl moiety in hand, we returned to optimization of the amide moiety. As summarized in Table 2, the 2-tolylacetamide could be replaced with the cinnolinylamide **9d** or the quinolinylamides **9e–g** and retain CCR3 affinity. However, the isomeric quinolinylamides **9h–i**, the indolinylamides **9j–k**, and the anilines **9a–c** (**9c** could be considered the acyclic open analogue of **9e**) showed reduced CCR3 affinity.

An incremental improvement in the biological activity was observed with modification of the bipiperidine core. In particular, substitution was well tolerated at the 3-position of the second piperidine ring. Synthesis of this subset of analogues is illustrated in Scheme 3. Wittig

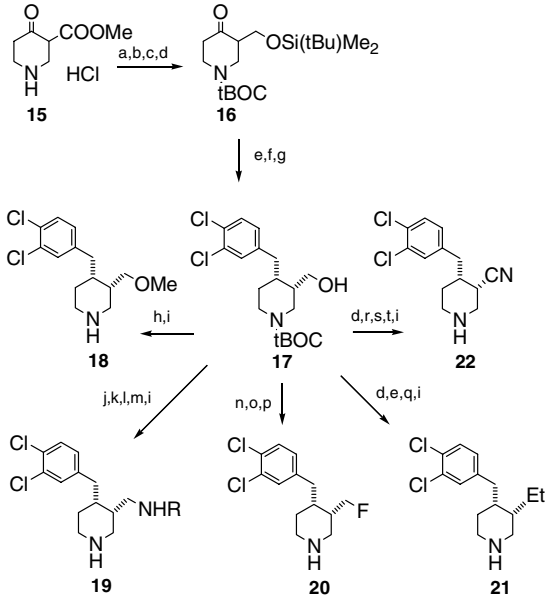
Table 2. In vitro CCR3 membrane binding activity of bipiperidines **8h** and **9a–k**



Compd	R	K_i (nM)
8h		40 ± 4
9a		100 ± 11
9b		122 ± 10
9c		136 ± 16
9d		46 ± 5
9e		30 ± 2
9f		38 ± 6
9g		68 ± 8
9h		140 ± 21
9i		458 ± 4
9j		111 ± 10
9k		119 ± 13



Scheme 3. Reagents and conditions: (a) Ph_3PMeBr, *n*BuLi, PhMe, −78 °C to rt, 79%; (b) SeO_2, *t*BuOOH, CH_2Cl_2, 49%; (c) Me_2(*t*Bu)SiCl, Et_3N, DMAP, CH_2Cl_2, 92%; (d) 9-BBN, 1,2-dichloro-4-iodobenzene, Pd(dppf)Cl_2, K_2CO_3, THF, DMF, Δ 36% of **11a** and 38% of **11b**; (e) nBu_4NF, THF, 95%; (f) KN(TMS)$_2$, MeI or EtI, DMF; (g) 4 N HCl in dioxane, CH_2Cl_2, 100%; (h) MsCl, Et_3N, CH_2Cl_2, 100%; (i) NaN_3, DMF, Δ 56%; (j) Ph_3P, THF, H_2O, Δ 73%; (k) AcCl or MsCl, Et_3N, CH_2Cl_2; (l) *N*-6-quinolinylcarbonyl-4-piperidone, NaB(OAc)$_3$H, AcOH, $ClCH_2CH_2Cl$.



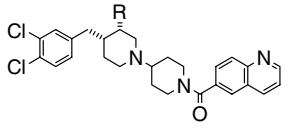
Scheme 4. Reagents and conditions: (a) *t*(BOC)$_2$O, Et_3N, MeOH, CH_2Cl_2, 100%; (b) $NaBH_4$, THF, MeOH, 92%; (c) Me_2(*t*Bu)SiCl, Et_3N, DMAP, CH_2Cl_2, 83%; (d) oxalyl chloride, DMSO, Et_3N, CH_2Cl_2, 97%; (e) Ph_3PMeBr, *n*BuLi, PhMe, −78 °C to rt, 79%; (f) 9-BBN, 1,2-dichloro-4-iodobenzene, Pd(dppf)Cl_2, K_2CO_3, THF, DMF, Δ 69%; (g) nBu_4NF, THF, 63%; (h) NaH, MeI, DMF, 100%; (i) 4 N HCl in dioxane, CH_2Cl_2, 100%; (j) MsCl, Et_3N, CH_2Cl_2, 100%; (k) NaN_3, DMF, Δ; (l) Ph_3P, THF, H_2O, Δ; (m) AcCl or MsCl, Et_3N, CH_2Cl_2; (n) trichloroethyl chloroformate, Et_3N, CH_2Cl_2, 68%; (o) bis-(2-methoxyethyl)amino-sulfur trifluoride, CH_2Cl_2, 50%; (p) Zn, AcOH, Δ 81%; (q) H_2, $(Ph_3P)_3RhCl$, EtOH, 55%; (r) $KMnO_4$, acetone, H_2O, 88%; (s) DEC, HOBT, NH_3, CH_2Cl_2; (t) Burgess reagent, CH_2Cl_2.

reaction of ketone **6**, allylic oxidation, and alcohol protection provided intermediate **10**.[3] Hydroboration, palladium coupling, and removal of the silyl protecting group produced the *cis*/*trans* mixture of **11a/11b**, which were separable by flash chromatography. The hydroxy group was elaborated or converted into an amino group through the corresponding azide intermediate. Reductive amination with *N*-6-quinolinylcarbonyl-4-piperidone gave the target compounds **14**.

Additional 3-substituted bipiperidines **14** were constructed by the synthetic route pictured in Scheme 4. Methyl 4-oxo-3-piperidinecarboxylate **15** was protected as the BOC derivative, reduced to the diol with sodium borohydride,[6] silylated to protect the primary hydroxyl group, and subjected to Swern oxidation[7] to yield ketone **16**. In this case, Wittig reaction, hydroboration, and palladium coupling, and removal of the silyl protecting group produced only the *cis* isomer of alcohol **17**. The hydroxyl group in **17** was further functionalized into a variety of different groups including ether **18**, amine **19**, fluoride **20**, alkane **21** or nitrile **22**.

The biological activity of the 3-substituted bipiperidines **14** is tabulated in Table 3. The *cis* isomer displayed significantly more activity than the *trans* isomer (e.g., the *trans* isomer of compound **14a** has $K_i = 263 \pm 26$ nM).

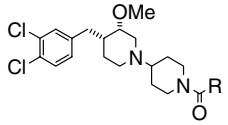
Table 3. In vitro CCR3 membrane binding activity of 3-substituted bipiperidines **9f** and **14a–r**

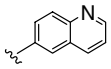
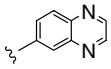
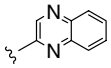
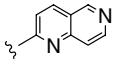
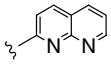


Compd	R	K_i (nM)
9e	H	30 ± 2
14a	Me	14 ± 2
14b	Et	29 ± 7
14c	CH=CH_2	23 ± 1
14d	CH_2F	46 ± 2
14e	CN	15%[a]
14f	$CONH_2$	29%[a]
14g	OMe (enant A)	326 ± 33
14h	OMe (enant B)	7.7 ± 2
14i	OEt (enant A)	32%[a]
14j	OEt (enant B)	33 ± 1
14k	$NHSO_2Me$	1002 ± 162
14l	NHCOMe	164 ± 28
14m	CH_2OH (enant A)	50 ± 14
14n	**CH_2OH (enant B)**	**3.5 ± 1**
14o	CH_2OMe (enant A)	394 ± 99
14p	CH_2OMe (enant B)	41 ± 2
14q	CH_2NHSO_2Me	331 ± 90
14r	$CH_2NHCOMe$	205 ± 30

[a] % Inhibition @ 1 μM ($n = 2$).

Table 4. In vitro CCR3 membrane binding and calcium flux activity of bipiperidines **15a–i**.



Compd	R	K_i (nM)	Calcium flux IC_{50} (nM)[a]
15a		86 ± 41	95[b]
15b		11 ± 2	25 ± 11
15c		26 ± 7	80 ± 13
15d		18 ± 5	34 ± 16
15e		20 ± 10	32 ± 2
15f		48 ± 10	169 ± 34
15g		34 ± 8	82 ± 7
15h		35 ± 1	66 ± 27
15i		15 ± 3	24 ± 5

[a] The assay protocol is described in Refs. 4 and 11.
[b] $n = 1$.

Enantiomers of highly potent compounds were separated by HPLC on a Chiralcel AD column (eluant was 10–30% IPA in hexane with 0.5% diethylamine).[8] The preferred absolute stereochemistry of *R,R* was determined by a chiral synthesis.[4] Based on these results, the CCR3 receptor is quite sensitive to the conformation of the bipiperidine core. A sterically small substituent is preferred at the 3-position, and the methyl **14a**, methoxy **14h**, and hydroxymethyl **14n** compounds exhibited the most potent CCR3 affinity (K_i = 3–14 nM). The ethyl **14b**, vinyl **14c**, fluoromethyl **14d**, ethoxy **14j**, and methoxymethyl **14p** showed slightly lower affinity (K_i = 23–46 nM). In contrast, the more electron withdrawing nitrile **14e**, carboxamide **14f**, sulfonamide **14k** and **14q**, and acetamide **14l** and **14r** were less active. Final combination of the 4-[(3,4-dichlorophenyl)methyl]-3-methoxy-1,4′-bipiperidine core with various quinolinyl amides are represented in Table 4. Unexpectedly, the cinnolinylamide **15a** did not display the potent CCR3 affinity of the 3-unsubstituted bipiperidine **9d**. However, the quinolinylamides **15b–e** all showed good CCR3 affinity and activity in the calcium flux assay. Heterocyclic amides with two nitrogen atoms such as **15f–i** also exhibited reasonable biological profiles.

In conclusion, compound **14n** was identified as a very potent CCR3 antagonist[9] and exhibited minimal activity at the CCR4 (11% at 1 μM) or CCR8 receptor (8% at 1 μM). It was active in the calcium flux assay with IC_{50} = 9.2 ± 3.2 nM and in the human eosinophil chemotaxis assay with IC_{50} = 160 ± 129 nM. For our intact cell binding assays (n = 2), compound **14n** also possessed good affinity for the human (K_i = 3.3 nM) and monkey (K_i = 7.2 nM) CCR3 receptors, but lower affinity for the rat (K_i = 2981 nM) and mouse (K_i = 606 nM) CCR3 receptors. Although bipiperidine **14n** displayed a reasonable AUC of 1341 ng h/mL at 10 mpk po in a rat pharmacokinetic study, the lower affinity for the rat receptor resulted in significant challenges with in vivo rat profiling. In addition, **14n** produced an undesired 85% inhibition at 1 μM in the hERG voltage clamp assay.[10] Therefore, due to the liabilities with this series of compounds, further advancement was precluded.

References and notes

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8. Chiralcel HPLC columns obtained from Chiral Technologies, 730 Springdale Dr., Exton, PA 19341.
9. For compound **14n**, K_i = 3.1 ± 0.1 nM when MCP-4 is the substrate in the membrane binding assay.
10. The hERG voltage clamp assay was conducted at Chan-Test Inc., 14656 Neo Parkway, Cleveland, OH 44128.
11. Calcium flux assay protocol: This assay used CREM3 cells at 37 °C, in which the human CCR3 receptor was expressed in the rat Y3 cell line. 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.