Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 16 (2006) 5695–5699
Bioorganic & Medicinal Chemistry Letters

2,4-Disubstituted piperidines as selective CC chemokine receptor 3 (CCR3) antagonists: Synthesis and selectivity

Paul S. Watson,*,† Bin Jiang, Kim Harrison, Nao Asakawa, Patricia K. Welch, Maryanne Covington, Nicole C. Stowell, Eric A. Wadman, Paul Davies, Kimberly A. Solomon, Robert C. Newton, George L. Trainor, Steven M. Friedman, Carl P. Decicco and Soo S. Ko

Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 5400, Princeton, NJ 08542-5400, USA

Received 9 June 2006; revised 31 July 2006; accepted 1 August 2006
Available online 23 August 2006

Abstract—Linear unselective CCR3 antagonist leads with IC_{50} values in the 200 nM range were converted into low nM binding compounds selective at CCR3 by moving the piperidine nitrogen substituent to the carbon at the 2-position of the ring. Substitution of the piperidine nitrogen with simple alkyl and acyl groups was found to improve the selectivity of this new compound class. In particular, *N*-{3-[(2*S*, 4*R*)-1-(propyl)-4-(4-fluorobenzyl)piperidinyl]propyl}-*N*′-(3-acetylphenyl)urea exhibited single digit nanomolar IC_{50} values for CCR3 with >100-fold selectivity against an extensive counter screen panel.

Infiltration of eosinophils into the airways is a common feature of allergic asthma and, in animal models, contributes to airway hyperresponsiveness and remodeling. CC chemokine receptor-3 (CCR3) is a principal mediator of eosinophil migration, and its ligands, such as eotaxin, are prominently expressed in asthmatic airways. Moreover, mice in which the CCR3 or eotaxin genes are disrupted exhibit reduced airway eosinophilia following allergen challenge. Thus, small molecule antagonists of CCR3 may provide a novel therapy in asthma by inhibiting eosinophilic inflammation.

Previously disclosed efforts identified compound **1** as a lead structure for further optimization (see Fig. 1).[1] Compound **1** is moderately potent at CCR3 (binding IC_{50} = 200 nM),[2] but it exhibited little selectivity against several other protein targets surveyed (for example, 5-HT_{2a}). In efforts to improve potency and selectivity, two chemical series that altered the spacial arrangements of two of the three key binding motifs (benzyl group and phenyl urea) were chosen for further exploration. The results for path A have been described previously.[3]

The following letter describes the SAR observed by moving the urea alkyl chain from the nitrogen to the

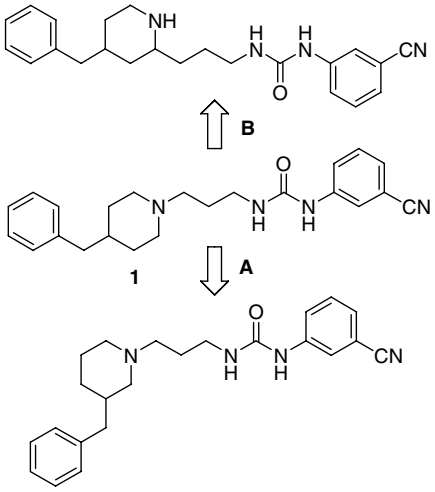


Figure 1. Structural modifications explored to address selectivity issues of compound **1**.

Keywords: Eotaxin; CCR3; Antagonist; Piperidine; Eosinophil.
* Corresponding author. Tel.: +1 919 287 1268; fax: +1 919 941 9177; e-mail: pwatson@inspirepharm.com
† Present address: Inspire Pharmaceuticals Inc., 4222 Emperor Boulevard, Suite 200, Durham, NC 27703, USA.

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.08.012

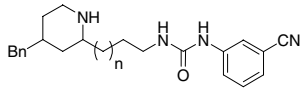
2-carbon (path B). 2,4-Disubstituted piperidines were considered interesting targets due to the introduction of stereochemical diversity (both relative and absolute) and the availability of the free nitrogen atom as an additional site of derivation.

The CCR3 binding affinities of the *cis* and *trans*-2,4-disubstituted urea diastereomers are shown in Table 1. The propyl *cis*-diastereomer (**2**) showed twofold less binding affinity to CCR3 with regard to linear achiral **1**. However, the propyl *trans*-diastereomer (**3**) demonstrated a 10-fold improvement in binding affinity to CCR3. Separation of the enantiomers of the propyl *trans*-racemic mixture by HPLC provided both antipodes. The binding results for **4** and **5** established that the affinity for CCR3 resides in the (+)-*trans*-diastereomer. Evaluation of the ''linker'' chain length (at the level of the racemate) found the ethyl and butyl *trans*-diastereomers (compounds **6** and **7**, respectively) to be 10-fold less active than the propyl *trans*-diastereomer (**3**). The optimal length of the linker chain in this new series was in complete agreement with our previous work on other related series.[1,3]

Concurrent with the discovery that the propyl *trans*-diastereomer was a potent CCR3 antagonist was the observation that this unsubstituted piperidine structural motif also demonstrated potent inhibitory activity for norepinephrine (NET), dopamine (DAT), and serotonin reuptake transporters (5-HTT). Table 2 illustrates that both enantiomers of the propyl *trans*-diastereomer (**4** and **5**) are very potent inhibitors of all three transporters. Substitution of the 3-cyano group with a 3-acetyl functionality (compare compounds **4** and **8**) provided no improvement in selectivity. Previous observation that installation of a *p*-fluoro substituent on the piperidine benzyl ring was found to improve CCR3 affinity by 10-fold[1] led to the preparation of compounds **9** and **10**. Unlike the previous chemotypes, the *p*-fluoro substituent only provided for a twofold improvement in potency (compare **8** to **9**). The *p*-fluoro substituent on the piperidine benzyl ring improved the selectivity for DAT and NET (compare **8** to **9**), but eroded the selectivity for 5-HTT. As in our other series of CCR3 antagonists,[3] symmetrical 3,5-disubstitution on the urea phenyl group improved CCR3 binding affinity by threefold (compare **9** to **10**). Symmetrical 3,5-disubstitution on the urea phenyl group was not beneficial to any transporter selectivity.

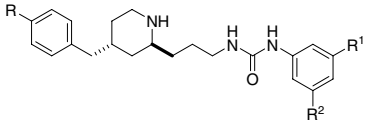
In an attempt to address the transporter selectivity issue, we examined substitution on the piperidine nitrogen. The 3- and 3,5-diacetyl phenyl urea substituents are used to illustrate these general SAR trends (Table 3). As observed previously in this and other series,[3] disubstitution on the urea phenyl group was again found to be more potent than monosubstitution (cf. **12**–**16**, **14**–**17**, and **15**–**18**, respectively). Substitution at the piperidine nitrogen was found to be well tolerated and did not significantly alter binding affinity to CCR3 in most derivatives. However, substitution with a 2-trifluoroethyl substituent (compound **25**) eliminated all activity for CCR3. Fortunately, simple alkyl substitution of the piperidine nitrogen provided selectivity over the CNS transporters. All nitrogen substituents tested were found to provide reasonable (at most occurrences >100-fold) selectivity over NET and DAT. In regard to 5-HTT selectivity, simple alkyl groups (e.g., compounds **11**–**14** and **16**–**19**) provided 40- to 300-fold selectivity. The presence of an oxygen atom γ to the piperidine nitrogen (compounds **15** and **18**) was found to significantly reduce the 5-HTT selectivity. Notably, acylation of the nitrogen (compounds **26** and **27**) yielded selective compounds across all three CNS transporters with only about 5-fold loss of the CCR3-binding affinity (compare to compounds **10** and **16**). The maintenance of the CCR3 binding affinity in these derivatives could be speculated to derive from the enhancement of a hydrogen bond or the rigidifying effect of the nitrogen substituent (pseudo $A_{1,3}$ strain) on the overall conformation of the system. This result is quite interesting, given that compound **25** is poorly active at CCR3. Finally, urea **12**

Table 1. CCR3 binding affinities of 2,4-disubstituted ureas



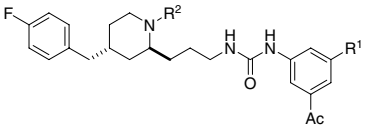
Compound	Optical purity	Isomer	CCR3 IC_{50} (nM)	*n*
2	(±)	*cis*	386	1
3	(±)	*trans*	22	1
4	(+)	*trans*	13	1
5	(−)	*trans*	644	1
6	(±)	*trans*	185	0
7	(±)	*trans*	142	2

Table 2. Transporter selectivity issues of 2,4-disubstituted piperidine ureas



Compound		R	R^1	R^2	CCR3 IC_{50} (nM)	5-HTT K_i (nM)	DAT K_i (nM)	NET K_i (nM)
4	(+)	H	CN	H	13.0	35	34	37
5	(−)	H	CN	H	644	54	10	46
8	(±)	H	Ac	H	6.0	42	32	39
9	(+)	F	Ac	H	1.4	18	219	233
10	(+)	F	Ac	Ac	0.5	3.5	119	95

Table 3. Binding affinities and transporter selectivity profiles for selected N-substituted piperidine ureas



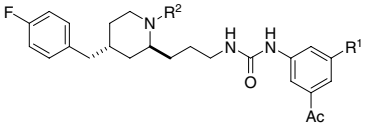
Compound	R^1	R^2	CCR3 IC_{50} (nM)	5-HTT, K_i (nM)	DAT, K_i (nM)	NET, K_i (nM)
9	H	H	1.4	18	219	233
11	H	Me	1.7	572	96	257
12	H	Pr	2.0	296	756	4168
13	H	Cyclopropyl methyl	2.5	117	251	3449
14	H	Allyl	4.1	217	855	1230
15	H	$(CH_2)_2OH$	7.0	50	2240	1820
10	Ac	H	0.5	3.5	119	95
16	Ac	Pr	0.7	166	157	1480
17	Ac	Allyl	1.0	69	297	876
18	Ac	$(CH_2)_2OH$	0.9	40	451	1390
19	Ac	$(CH_2)_3OH$	1.3	212	609	497
20	Ac	$CH_2C(O)Me$	0.9	11	754	381
21	Ac	$CH_2C(O)NH_2$	3.6	710	2659	5007
22	Ac	CH_2CH_2F	2.3	49	651	317
23	Ac	Propargyl	1.9	305	621	524
24	Ac	CH_2CHF_2	12.4	328	931	1052
25	Ac	CH_2CF_3	1380	NT	NT	NT
26	Ac	$C(O)CH_3$	3.9	5465	1224	1379
27	Ac	$C(O)CH_2CH_3$	5.8	224	2082	9261
28	Ac	$C(=NH)NH_2$	4.3	19	31	388

(**IS811**) was found to be 170-fold selective over 5-HT_{2a}, a potential liability previously mentioned for this series of compounds.[3]

A representative set of compounds was further characterized in a secondary calcium mobilization assay and in their ability to block the eotaxin-induced human eosinophil chemotaxis assay (Table 4).[4] All 2,4-disubstituted piperidine urea analogs proved to be antagonists of eotaxin-induced calcium mobilization. Consistent with previous data, 3,5-disubstitution proved to be optimal in both functional assays (compare compounds **12** to **16**). Notably, compound **26**, which lacks a basic nitrogen and exhibits an IC_{50} of 3.9 nM in the CCR3 binding assay, proved much less potent in the secondary functional assays. Compounds **12** and **16** proved to be potent inhibitors of chemotaxis and the potency correlated well to the binding assay results.

Compound **12** (**IS811**) was further evaluated in two animal PK models (Table 5) and found to have suitable properties to warrant further preclinical in vivo studies. As shown in Figure 2, **IS811** dose-dependently inhibited eotaxin-induced eosinophil influx to the lung.[5,6]

Table 4. Calcium mobilization and chemotaxis values for selected analogs



Compound	R^1	R^2	CCR3 IC_{50} (nM)	Ca^+ IC_{50}(nM)	Chemotaxis EC_{50} (nM)
12	H	Pr	2.0	26	19
16	Ac	Pr	0.7	1.2	4.7
26	Ac	Ac	3.9	133	>100

The synthesis of the required urea analogs (**2**–**5**) was initially performed according to the sequence described in Scheme 1. Starting from *N*-Boc-4-benzyl piperidine (**29**), metalation with *s*-BuLi and alkylation of the resulting

Table 5. Pharmacokinetic properties of compound **12** (**IS811**) in mouse and Cynolgus monkey

Species and route of administration	Dose (mg/kg)	Cl (L/h/kg)	VD (L/kg)	$T_{1/2}$ (h)	C_{max} (nM)	%*F*
Mouse (IV)	2	10.6	8		—	—
Mouse (PO)	10	—	—	1.4	274	36
Cyno (IV)	2	0.4	3.1		—	—
Cyno (PO)	5	—		9	989	18

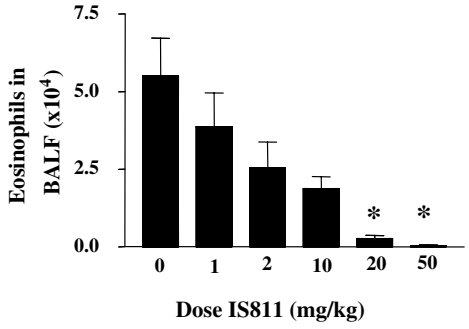
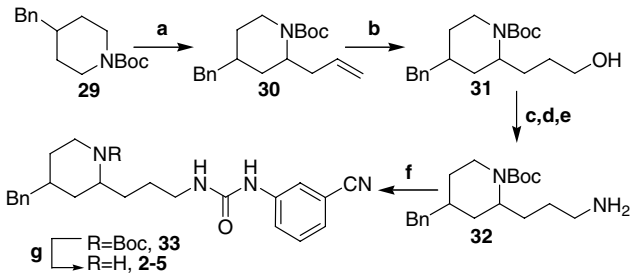
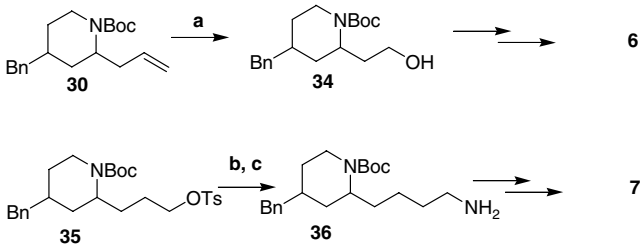


Figure 2. Inhibition of eotaxin-induced eosinophil influx into the lung by compound **12** (**IS811**).[6]



Scheme 1. Reagents and conditions: (a) s-BuLi, THF, 0 °C then allyl iodide; (b) 9-BBN, THF then 30% H_2O_2; (c) TsCl, pyridine, 0 °C; (d) NaN_3, DMSO; (e) H_2, 10% Pd/C, MeOH; (f) m-CN–Ph–NCO, THF; (g) TFA, CH_2Cl_2.



Scheme 2. Reagents and conditions: (a) O_3, CH_2Cl_2, −78 °C then DMS, 50%; (b) NaCN, DMSO, 50 °C, 80%; (c) H_2, 10% Pd/C, MeOH, 90%.

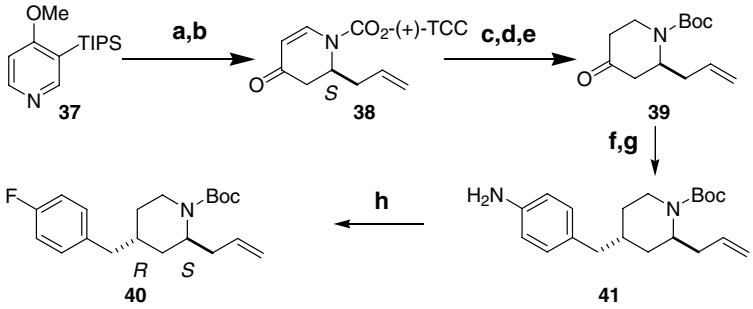
anion with allyl iodide provided olefin **30**. Hydroboration of **30** with 9-BBN upon oxidative workup yielded alcohols **31**. Three-step conversion of alcohols **31** (tosylation, azidation, and reduction) led to amines **32**. Addition of amines **32** to 3-cyanophenyl isocyanate provided a 2:1 mixture of protected ureas **33**. Separation of the *cis* and *trans*-diastereomers was accomplished by HPLC. Subsequent deprotection (TFA) led to racemic mixtures of each urea **2** and **3**. In a similar manner, other derivatives differing by the extent of substitution on the urea phenyl ring were prepared using the corresponding isocyanates.

Amine **30** could also be treated with ozone upon reductive workup to provide alcohol **34** (Scheme 2). Conversion of **34** to *trans*-urea **6** was performed in a similar manner to **2**. Finally, the tosylate **35** derived from alcohol **31** could be displaced with sodium cyanide and reduced to give amine **36**. Subsequent treatment with the isocyanate, acidic cleavage, and separation by HPLC provided *trans*-urea **7**.

During the evolution of this work, a growing body of evidence suggested that an asymmetric synthesis of this structural moiety that could incorporate a *p*-fluoro substituent on the piperidine benzyl group would be beneficial. Therefore, incorporation of these two features became part of the primary focus of an improved synthesis. A racemic version of the following sequence has been described in a different publication.[7] Toward this end, olefin **40** (Scheme 3) was targeted as a versatile intermediate. Addition of allyl magnesium chloride to the chiral acyliminium ion derived from pyridine **37** provided enone **38** after acid catalyzed removal of the TIPS group. The absolute stereochemistry of the 2-position was assigned as *S* using previously reported models.[8] Base catalyzed removal of the chiral auxiliary, protection of the vinylogous amide, and conjugate reduction of the enone yielded ketone **39**. Wittig olefination and dissolving metal reduction provided aniline **41** in good yield.

Previous studies indicated that hydrogenation of the Wittig adduct was not selective for the *trans*-diastereomer.[7] In addition, conditions that did provide high levels of selectivity (dissolving metal reduction) were also found to remove the desired *p*-fluoro substituent. Hence, a modified procedure that utilizes an aniline as a masked fluorine substituent was developed. To this end, conversion of the aniline to the fluoride via the in situ generation of the diazonium salt required the reprotection of the piperidine nitrogen after crude work-up. This procedure provided olefin **41** in gram quantities. Olefin **41** was utilized according to procedures outlined in Scheme 1. Subsequent synthetic derivatives could then be deprotected (TFA) and substituted on the piperidine nitrogen by a number of sequences (reductive amination, acylation, and acylation/reduction).

In conclusion, we have demonstrated that moderately potent nonselective 1,4-disubstituted benzyl piperidines can be converted into potent selective CCR3 antagonists by moving the *N*-substituent to the 2-position and substituting the free nitrogen position with small alkyl



Scheme 3. Reagents and conditions: (a) (+)-TCC–C(O)Cl, toluene/THF, −30 °C then CH_2=$CHCH_2MgBr$, −78; 10% HCl, rt, 68%; (b) TFA, CH_2Cl_2, 100%; (c) K_2CO_3, MeOH, reflux; (d) Boc_2O, DMAP, CH_3CN, 92% for two steps; (e) Zn, HOAc, 50 °C, 90%; (f) 4-(H(Z)N)-$PhCH_2P(Ph)_3Cl$, *t*-BuOK, THF, 90%; (g) Li, NH_3, THF, −78 to −30 °C, 90%; (h) 70% HF/pyridine, −78 to −30 °C then $NaNO_2$, 10 °C then urea, 0 °C; Boc_2O, NaOH, 1,4-dioxane, 20 °C, 80%.

and acyl groups. We have discovered that the potency of these derivatives lies in the 2*S*, 4*R-trans* diastereomer. We have also found that low binding affinities to CCR3 can be maintained without the presence of a key basic nitrogen atom. These derivatives were found to possess very good in vivo PK characteristics in both mouse and monkey models.

Acknowledgments

We gratefully acknowledge the help of A. J. Mical, K. A. Rathgeb, D. R. Wu, and N. C. Caputo of the Separations Group; T. H. Scholz and G. A. Nemeth of the Spectroscopy Group; and Percy Carter for his critical review and helpful suggestions during the preparation of this manuscript.

References and notes

1. Wacker, D. A.; Santella, J. B., III; Gardner, D. S.; Varnes, J. G.; Estrella, M.; DeLucca, G. V.; Ko, S. S.; Tanabe, K.; Watson, P. S.; Welch, P. K.; Covington, M.; Stowell, N. C.; Wadman, E. A.; Davies, P.; Solomon, K. A.; Newton, R. C.; Trainor, G. L.; Friedman, S. M.; Decicco, C. P.; Duncia, J. V. *Bioorg. Med. Chem. Lett.* **2002**, *12*(13), 1785.
2. For binding assay details, see footnote 16 in reference 1.
3. Varnes, J. G.; Gardner, D. S.; Santella, J. B., III; Duncia, J. V.; Estrella, M.; Watson, P. S.; Clark, C. M.; Ko, S. S.; Welch, P. K.; Covington, M.; Stowell, N. C.; Wadman, E. A.; Davies, P.; Solomon, K. A.; Newton, R. C.; Trainor, G. L.; Decicco, C. P.; Wacker, D. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*(7), 1645.
4. For calcium mobilization assay conditions, see footnote 19 in reference 1. Readers are referred to US Patent 6,331,544 (Ko, S. S.; DeLucca, G. V.; Duncia, J. V.; Santella, J. B., III; Wacker, D.A.) for details regarding chemotaxis assay conditions.
5. De Lucca, G. V.; Kim, U. T.; Vargo, B. J.; Duncia, J. V.; Santella, J. B., III; Gardner, D. S.; Zheng, C.; Liauw, A.; Wang, Z.; Emmett, G.; Wacker, D. A.; Welch, P. K.; Covington, M.; Stowell, N. C.; Wadman, E. A.; Das, A. M.; Davies, P.; Yeleswaram, S.; Graden, D. M.; Solomon, K. A.; Newton, R. C.; Trainor, G. L.; Decicco, C. P.; Ko, S. S. *J. Med. Chem.* **2005**, *48*(6), 2194.
6. Das, A. M.; Vaddi, K. G.; Solomon, K. A.; Krauthauser, C.; Jiang, X.; Mcintyre, K.; Wadman, EA.; Welch, P. K.; Covington, M.; Graden, D. M.; Yeleswaram, S.; Trzaskos, J. M.; Newton, R. C.; Mandlekar, S.; Ko, S. S.; Carter, P. H.; Davies, P. *J. Pharmacol. Exp. Ther.* **2006**, *318*, 411.
7. Watson, P. S.; Jiang, B.; Scott, B. *Org. Lett.* **2000**, *2*(23), 3679.
8. Comins, D. L.; Goehring, R. R.; Joseph, S. P.; O'Connor, S. *J. Org. Chem.* **1990**, *55*, 2574.