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Design and Synthesis of Novel CCR3 Antagonists

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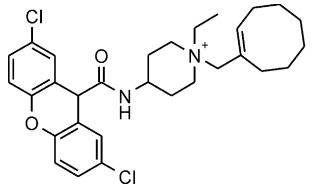
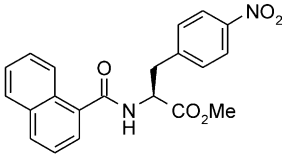
Abstract—As part of our investigation into the development of potent CCR3 antagonists, a series of piperidine analogues was designed and prepared. Exploration of the piperidine core examined both the basicity and the location of a nitrogen, as well as conformational variants. The bicyclo-piperidine **24c** was found to be the most potent inhibitor of CCR3 with an IC_{50} of 0.0082 μM in the binding assay and 0.0024 μM in the chemotaxis assay.

Asthma is a chronic inflammatory disease of the airways in which bronchial hyperresponsiveness appears to correlate with increased numbers of tissue inflammatory cells such as eosinophils, Th2-type T lymphocytes, basophils and mast cells.[1] These cells, and their mediators or granules, play a critical role in induction and maintenance of the inflammatory process in asthma and allergic diseases. Targeting cell influx and activation may therefore be of therapeutic benefit.

Chemokine ligands and their receptors have an important role in recruiting leukocytes such as eosinophils and Th2-type lymphocytes into areas of inflammation in diseases such as asthma and allergy.[2] CCR3 is a β-chemokine receptor present on these cells,[3–5] and its antagonism should block their recruitment and mast cell activation.[6] Recent examples of CCR3 antagonists include the xanthene carboxamide (**A**) which antagonizes both CCR1 (IC_{50} = 0.9 nM) and CCR3 (IC_{50} = 0.58 nM),[7] the non-basic phenylalanine derived CCR3 antagonist (**B**) (IC_{50} = 5 nM),[8] and the 2-(benzothiazolethio)acetamide (**C**) with an IC_{50} of 2.3 nM against CCR3.[9]



A



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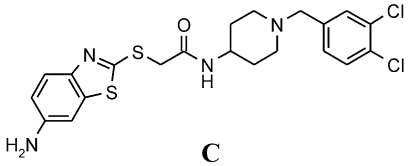
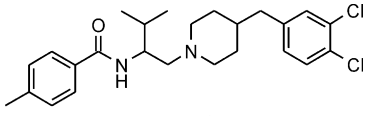


C

Herein, we describe an SAR evaluation of an early piperidine lead **1**. Our investigation focused on the nature of the piperidine core. We sought to investigate the importance of the basic nitrogen, the position of the nitrogen atom around the ring, and the effect of ring constraint to determine a preferred binding conformation.



1

The preparation of a cyclohexane analogue is described in Scheme 1.

Compound **2** was prepared from commercially available 1,4-dioxa-spiro[4,5]decan-8-one, which was reacted with 3,4-dichlorobenzyl triphenylphosphonium bromide in a Wittig reaction. Subsequent hydrogenation and acid hydrolysis afforded **3**, which was subjected to

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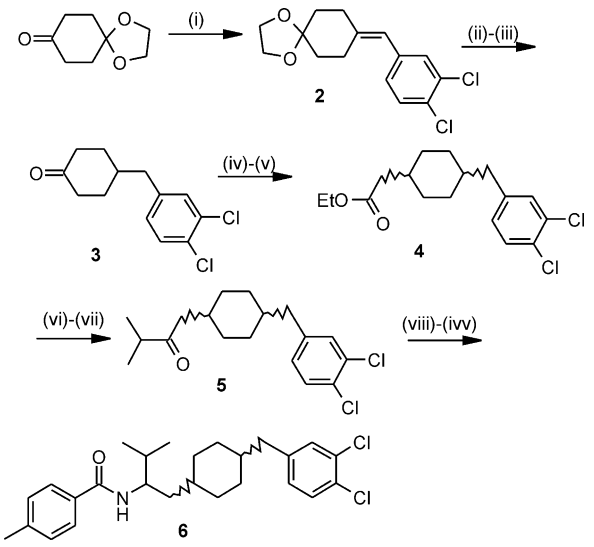
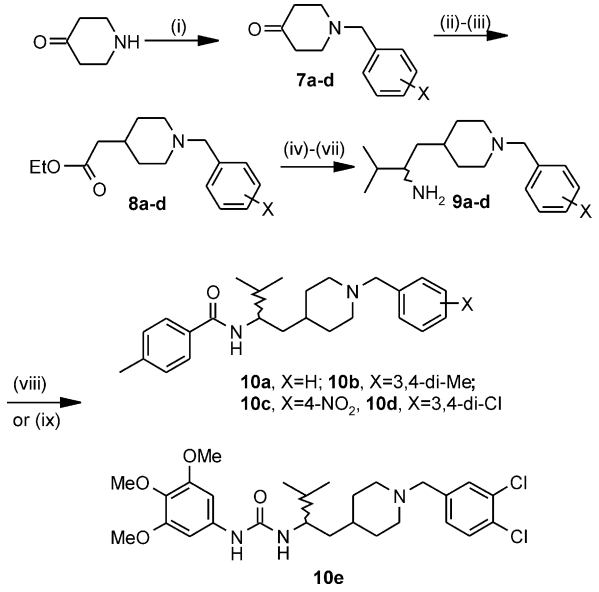


Scheme 1. (i) 3,4-di-ClPhCH$_2$P$^+$Ph$_3$Br$^-$/*n*BuLi, 0 °C, THF, 50%; (ii) H$_2$, PtO$_2$, EtOAc, 95%; (iii) HCl (aq), reflux in EtOH, 98%; (iv) (EtO)$_2$P(O)CH$_2$CO$_2$Et, NaH, THF, 97%; (v) H$_2$, PtO$_2$, EtOAc, 98%; (vi) (a) *i*Bu$_2$AlH, toluene, −60 °C; (b) *i*PrMgBr, THF, 0 °C, 95%; (vii) PDC, CH$_2$Cl$_2$, 54% (viii) ammonium formate/NaCNBH$_3$, 95%; (ix) *p*-MePhCOCl/Et$_3$N, CH$_2$Cl$_2$, 65%.

a Horner–Emmons reaction and then hydrogenation to give **4**. Upon reaction with isopropyl magnesium bromide and oxidation by PDC, **5** was obtained. A point worth mentioning is that the propensity of dechloronation of the aromatic ring during hydrogenation with Pd/C can be avoided with the use of PtO$_2$.

The *N*-transposed analogues of **1** can be prepared according to Scheme 2, starting from 4-piperidone and substituted benzyl bromides to give **7a–d**. A similar



Scheme 2.* (i) X-PhCH$_2$Br/Et$_3$N, CH$_2$Cl$_2$, 83%; (ii) (EtO)$_2$P(O)CH$_2$CO$_2$Et, NaH in THF, 97%; (iii) H$_2$, PtO$_2$, EtOAc, 99%; (iv) *i*Bu$_2$AlH, toluene, −60 °C, 46%; (v) *i*PrMgBr, THF, 0 °C, 84%; (vi) PDC, CH$_2$Cl$_2$, 81%; (vii) ammonium formate/NaCNBH$_3$, 96%; (viii) *p*-MePhCOCl/Et$_3$N, CH$_2$Cl$_2$ to give **10a–d**, 90%; (ix) 3,4,5-tri-MeOPhNCO, CH$_2$Cl$_2$ to give **10e**, 50%. *Yields are given for X = 3,4-di-Cl.

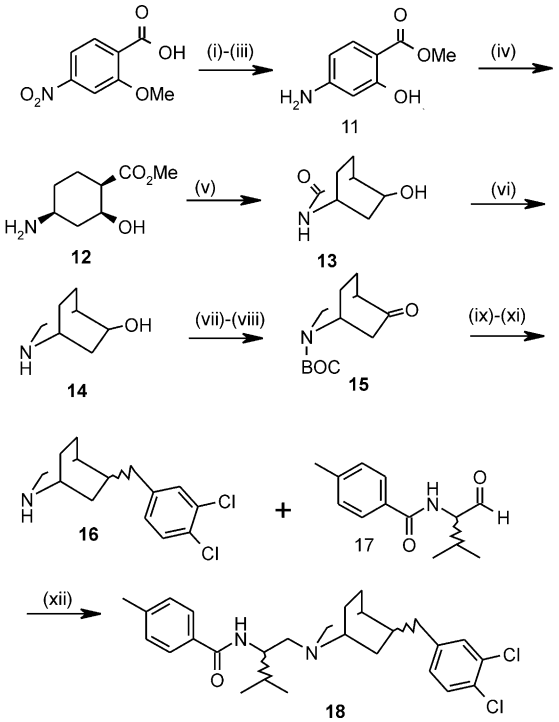
reaction sequence as described in Scheme 1 gave **9a–d**, which upon reacting with 4-toluoyl chloride gave **10a–d.** Reaction of **9d** with 3,4,5-trimethoxyphenyl isocyanate gave **10e**.

The preparation of a 2,5-constrained piperidine analogue is shown in Scheme 3.

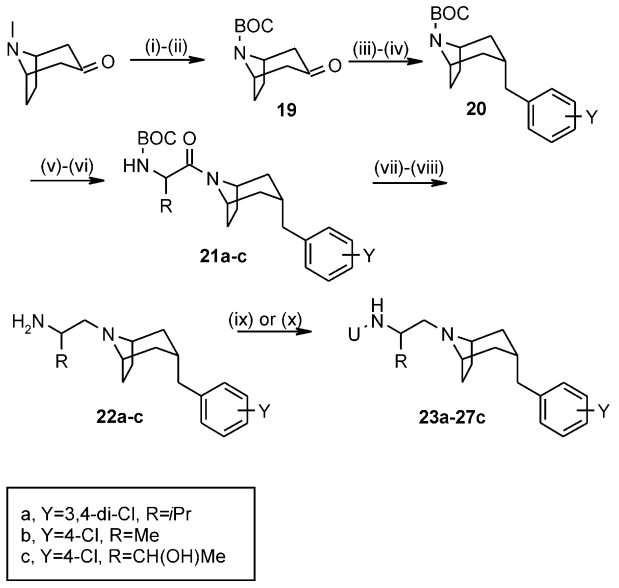
Demethylation of commercially available 2-methoxy-4-nitrobenzoic acid via BBr$_3$, followed by esterification and reduction gave **11**. Reduction of the aromatic ring was accomplished by using 5% Rh/Al in warm acetic acid. Subsequent ring closure was effected by refluxing **12** in mesitylene at 165 °C. The resulting lactam **13** was reduced to **14** by LAH, which was then protected using a BOC group. PDC oxidation gave ketone **15**. The 3,4-dichlorobenzyl group was incorporated via a Wittig reaction and subsequent hydrogenation to afford **16**. The stereochemistry of **16** was determined by ^{1}H NMR to be a 1:1 mixture of *endo*/*exo* isomers relative to the NH containing bridge. It was coupled with intermediate **17** via reductive amination to give compound **18**. The intermediate **17** was made by acylation of valinol with *p*-toluoyl chloride, followed by PDC oxidation.

An alternative constraint of the piperidine core was through a bridging ethylene at the 2/6 positions. The preparation of these analogues is described in Scheme 4.

The commercially available *N*-methyltropinone was reacted with 1-chloroethyl chloroformate in methanol to give the corresponding demethylated product, which



Scheme 3. (i) BBr$_3$, CH$_2$Cl$_2$, 97%; (ii) SOCl$_2$, MeOH, 95%; (iii) H$_2$, 10% Pd/C, EtOAc, 98%; (iv) H$_2$ (53 psi), 5% Rh on Al, acetic acid (70 h), 58 °C, 58%; (v) mesitylene, 165 °C, 80%; (vi) LAH, THF, reflux, 98%; (vii) (BOC)$_2$O, 86%; (viii) PDC, CH$_2$Cl$_2$, 96%; (ix) 3,4-di-ClPhCH$_2$P(O)(OEt)$_2$, 0 °C, THF, 61%; (x) H$_2$, PtO$_2$, EtOAc, 88%; (xi) TFA, 99%; (xii) Me$_4$NB(OAc)$_3$H, CH$_2$Cl$_2$, 36%.



Scheme 4.* (i) 1-Cl-ethyl chloroformate, MeOH, 88%; (ii) $(BOC)_2O$, 98%; (iii) Y-$PhCH_2P(O)(OMe)_2$, potassium *t*-amylate, toluene, reflux, 65%; (iv) H_2, PtO_2, EtOAc, 65%; (v) TFA, CH_2Cl_2, 71%; (vi) BOCNHCH(R)COOH, EDCI. CH_2Cl_2, TFA; (vii) BH_3, THF, reflux; (viii) HCl (aq), 70 °C, 53–55% from vi; (ix) 4-toluoyl chloride, Et_3N, CH_2Cl_2; (x) substituted phenyl isocynate, 55–65%. *Yields are given for Y = 3,4-di-Cl, R = *i*Pr.

was subsequently protected with a BOC group to give **19**. A similar sequence of synthetic manipulations to that described in Scheme 3 gave the final intermediates **22a–c**, which were converted to the final amide or ureas (**23a–27c**). The stereochemistry of **20** was determined by ^1H NMR to be 2:1 *exo*/*endo* relative to N containing bridge, resulting from preferential H_2 attack from the less sterically hindered side (N vs CH_2CH_2). This is also supported by literature evidence.[10] The 4-chlorobenzyl tail was used in this series as it was less lipophilic than dichlorobenzyl, but still maintained potency against CCR3. It was later found that the monochloro compounds tend to bind to plasma protein to a lesser degree than their dichloro counterparts.

The compounds described above were screened in a binding assay using ^{125}I-labelled eotaxin.[11] The results are shown in Table 1.

In this series of compounds, the presence of a basic nitrogen appears to be crucial for binding to the CCR3 receptor (compare **1** vs **6**). It has been previously found that the glutamic acid in TM7, conserved in most chemokine receptors, is an important recognition element for some small molecule antagonists.[12] This series takes advantage of this interaction.

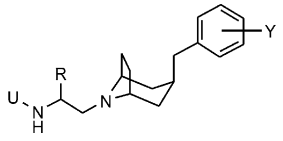
When the basic nitrogen of the piperidine ring is positioned near the benzylic group (**10d**), the binding potency is only 3-fold less as compared to **1**. This marginal loss of activity suggests that the side chain of the glutamic acid residue in TM7 of CCR3 may be flexible and can adopt a productive binding interaction with the nitrogen of both inhibitors. It might be argued that the slightly diminished activity could be attributed to the difference in basicity of that nitrogen, influenced by the benzyl group and its substituents. However, compounds **10a**, **10b** and **10c**, with hydrogen, 3,4-dimethyl or 4-nitro as substituents, indicate this is not the case. The fact that the 3,4-dichlorobenzyl moiety is most favored illustrates that this group provides the best binding interaction in this lipophilic region of the receptor. Compound **10e** was prepared based upon the SAR investigation around **1**, where a urea linker to a trimethoxyphenyl group was shown to substantially improve potency against CCR3.[13]

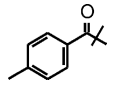
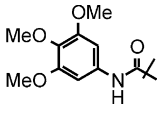
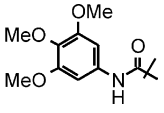
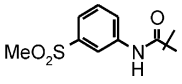
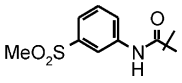
A piperidine ring can adopt a number of conformations, and we tried to constrain its conformation to discover the most preferred presentation of the nitrogen. Compound **18** is constrained as a boat conformation while

Table 1. Binding affinity of compound **1**, **6**, **10a–e** and **18** to CCR3 receptor ($n \geq 2$)

Compd	IC_{50} (μM)
1	0.5
6	>200
10a	>200
10b	7.8
10c	8.5
10d	1.7
10e	0.5
18	5.3

Table 2. IC_{50} values of compound **23a–27c** in binding and chemotaxis[14] assays ($n \geq 2$)



No.	R	U	IC_{50} binding (μM)	IC_{50} chemotaxis (μM)
23a	(R/S)		1.8	N.D.
24c			0.0082	0.0024
25b			0.011	0.0047
26b			0.065	0.012
27c			0.012	0.0044

23a–**27c** are constrained in the chair conformation. As seen in Table 1, compound **18**, the boat conformation is less active ($IC_{50}=5.3$ μM) than the original lead compound **1** ($IC_{50}=0.5$ μM). The chair conformation of **23a** also did not look promising for this series ($IC_{50}=1.8$ μM, Table 2). Possible explanations are unfavorable steric interactions due to the ethylene bridge or that the central scaffold is now overly rigid. However, this chair scaffold does result in more active compounds when paired with the urea linker as in **24c**, **25b**, **26b**, and **27c**. The combination of side chain modification [MeCH(OH) vs Me] and urea head substitution (trimethoxy vs methylsufonyl) ultimately gives the most potent CCR3 antagonist, **24c** ($IC_{50}=0.0082$ μM). A similar trend is also observed in the chemotaxis assay where **24c** exhibited an IC_{50} of 0.0024 μM.

The pharmacokinetic profiles of several compounds in Table 2 were also evaluated. The iv data in rats of compound **23a** demonstrated high clearance (7.06 l/h/kg) coupled with a high volume of distribution (30.1 l/kg) and $t_{1/2}$ of 3.5 h. This is characteristic of lipophilic compounds with basic nitrogen and the PK profile of **23a** is similar throughout this series. A subsequent po study in rats (30 mpk) for more potent compounds **24c**, **25b** and **27c** gave exposure values [AUC (0–24 h, μg/mL h)] of 0.19, 0.28, and 0.71, respectively. The low po exposure levels of these compounds are indicative of a high volume of distribution, which was quantified in the iv study with **23a**.

We designed and synthesized a novel class of CCR3 antagonists by modifying the lead compound **1**. Functional antagonism is demonstrated by their ability to inhibit a chemotactic response. The potencies of these compounds appear to be dependent on the presence of a basic nitrogen, which presumably interacts with the glutamic acid in TM7 of CCR3. Optimization of these di-substituted piperidines, based on the aryl urea **24c**, will be the subject of future communications.

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