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N-Arylalkylpiperidine urea derivatives as CC chemokine receptor-3 (CCR3) antagonists

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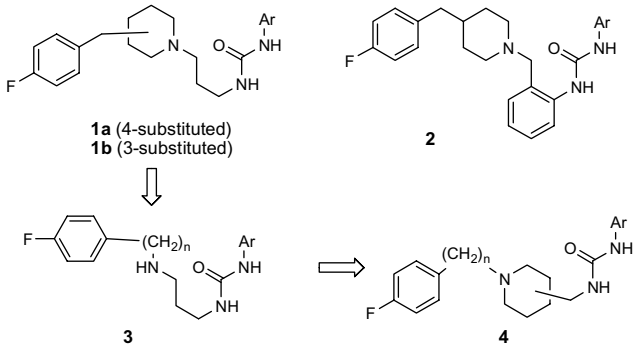
Abstract—The synthesis and structure–activity relationships of *N*-arylalkylpiperidylmethyl ureas as antagonists of the CC chemokine receptor-3 (CCR3) are presented. These compounds displayed potent binding to the receptor as well as functional antagonism of eotaxin-elicited effects on eosinophils.

Many chemokines (chemotactic cytokines) are major mediators of inflammatory responses, and antagonism of their effects has attracted significant attention as a novel approach to anti-inflammatory therapy.[1,2] Eotaxin (CC11), a CC or type 2 chemokine, appears to be a primary chemoattractant responsible for local eosinophilia in some inflammatory conditions, including asthma, allergic rhinitis, contact dermatitis and parasitic infections.[3] The receptor for eotaxin, CCR3, which is a member of the 7-transmembrane G-protein coupled receptor family, is expressed by eosinophils, mast cells and Th2 lymphocytes.[1,4,5] A growing body of evidence supports the importance of eotaxin and CCR3 in the inflammatory component of asthma and other diseases characterized by eosinophilia.[4–8] Since asthma is widespread, debilitating and often life-threatening,[9] the pharmaceutical research community has taken a great interest in discovering small molecule antagonists of CCR3 as a novel approach to an anti-inflammatory therapy for this disease.[1,2]

An earlier report from our group[10] disclosed 4-benzylpiperidin-1-ylpropyureas (**1a**) as potent antagonists for CCR3. Subsequent studies demonstrated that greater potency and selectivity resulted from replacing the 4-benzylpiperidine moiety with *S*-3-benzylpiperidine (**1b**), as well as by the appropriate choice of substituents on the urea aromatic ring.[11] Further work showed that conformational restriction of the propyl chain by fusion with a benzene ring, providing **2**, also gave compounds with good potency.[12]

An alternative rigidification of the central propylene chain of **1** would involve breaking the piperidine ring, to **3**, and forming a new piperidine ring onto this chain, leading to the general structure **4**. We explored the effects such a change would have on CCR3 potency. (Examples of somewhat related CCR3 antagonists containing *N*-benzyl piperazines and morpholines, some linked to ureas, have been reported.)[13]



A series of 1-(4-fluorophenylalkyl)piperidines attached through a methylene linker to an arylurea moiety served

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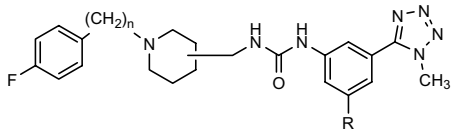
Table 1. Effect of chain length, urea position and absolute configuration on binding and eotaxin-induced Ca^{2+} release



	n	Isomer	R	CCR3 IC_{50} (nM)[a]	Ca^{2+} IC_{50} (nM)[b]
5	1	4(*RS*)	H	54	1200
6	2	4(*RS*)	H	350	ca. 10,000[c]
7	3	4(*RS*)	H	230	2500
8	1	3(*RS*)	H	3500	>10,000[d]
9	2	3(*RS*)	H	6	180
10	3	3(*RS*)	H	36	1500
11	4	3(*RS*)	H	29	
12	2	3(*R*)	H	13	870
13	2	3(*S*)	H	5	260
14	3	3(*R*)	H	15	1500
15	3	3(*S*)	H	100[e]	ca. 10,000[f]
16	2	3(*R*)	Et	1.6	
17	2	3(*S*)	Et	0.7	27
18	3	3(*R*)	Et	2.2	
19	3	3(*S*)	Et	1.9	290

[a] See Ref. 14.
[b] See Ref. 15.
[c] 57% at 10,000 nM.
[d] 0% at 10,000 nM.
[e] 45% at 100 nM.
[f] 67% at 10,000 nM.

to evaluate the optimal chain length and substitution position, as shown by compounds **5** through **11** in Table 1. The 4-fluorophenyl and 3-(1-methyltetrazol-5-yl)-phenyl substituents were chosen for their known potency-enhancing effects in **1a** and **1b**.[10,11]

Substitution at the 3-position of the piperidine ring enhanced potency significantly relative to the 4-substituted analogues. This change, allowing a more pronounced bend in the overall shape of the molecule, is consistent with the enhanced potency displayed by the *ortho*-disubstituted phenyl series **3** over the corresponding *para*-isomers.[12] The effect of chain length on potency differed dramatically between the 3- and 4-substituted series. Maximal binding potency in the 4-substituted series was provided by the *N*-benzyl analogue (**5**), yet this compound was by far the least potent of the 3-substituted compounds examined. In the 4-substituted series, ethylene and propylene linkers were very similar (**6** and **7**), while in the 3-substituted series the ethylene analogue **9** was significantly more potent than either propylene (**10**) or butylene (**11**). In general, inhibition of eotaxin-induced Ca^{2+} mobilization in eosinophils demonstrated that these compounds were receptor antagonists, with the rank-order potency of this effect similar to that of receptor binding.

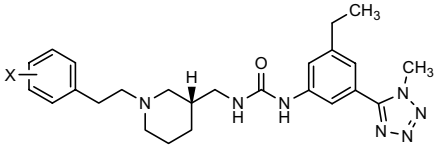
Since shifting the piperidine substituent from the 4- to the 3-position introduced chirality into the molecules, the individual enantiomers were prepared and evaluated. Although both isomers were active, the (*S*) enantiomer in the ethylene series was 3-fold more potent than the optical antipode (**12** vs **13**), yet the (*R*) isomer was more potent with a propylene chain (**14** vs **15**). This may reflect the degree of flexibility inherent in these molecules, allowing the critical binding moieties (presumably the basic nitrogen, the fluorophenyl and the urea) to reach similar conformations in all cases. This suggests that the piperidine ring does not interact significantly with the receptor, serving only as a spacer.

The addition of a second *meta*-substituent on the phenyl ring of the urea (examples **16** through **19** in Table 1) increased potency still further, in agreement with prior results.[11] In this case, the differences in binding potency between (*R*) and (*S*), and between chain lengths of 2 or 3 carbons, were minimal. However, the Ca^{2+} mobilization assay still favoured the ethylene analogue by 10-fold in the (*S*) series (**17** vs **19**).

The substitution on the phenethyl ring was varied in the (*S*) series, as shown in Table 2. In agreement with previous reports for **1a** and **1b**,[10,11] halogens (**17** and **20** through **26**) were preferable to alkyl (**28**), or to larger or more polar substituents (**29** through **31**). The unsubstituted case (**27**) was nearly as potent. Interestingly, in the fluorine-substituted compounds, *para*-substitution was somewhat favoured (**17** vs **20**), while a *meta*-chloro substituent seemed preferable to the *para*-isomer (**25** vs **24**), *ortho*-substitution was slightly disfavoured (**21** and **22** vs **17**, **26** vs **24** and **25**).

The ethylene series possesses five rotatable bonds between the phenyl ring and the urea moiety, which can dramatically effect the overall shape of the molecules. This flexibility may contribute to the similar potency of compounds having different absolute stereochemistry and chain length (e.g., **12** through **14**). Seeking to limit this flexibility, we prepared three compounds (Table 3, **32** through **34**) lacking the methylene spacer between

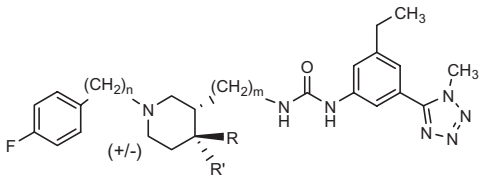
Table 2. Phenyl substitution effects on CCR3 binding



	X	CCR3, IC_{50} (nM)[a]
17	4-F	0.7
20	3-F	12
21	2-F	5.5
22	2,4-F_2	1.8
23	3,4-F_2	3.2
24	4-Cl	3.6
25[b]	3-Cl	0.6
26	2,4-Cl_2	5.5
27	H	3.7
28	Me	9.7
29	CF_3	82
30	OMe	120
31	NMe_2	380

[a] See Ref. 14.
[b] Ca^{2+} IC_{50} 15 nM; see Ref. 15.

Table 3. Effect of attempted conformational restraint on binding and Ca^{2+} release



	n	*m*	R	R′	CCR3 IC_{50} (nM)[a]	Ca^{2+} IC_{50} (nM)[b]
32	2	0	H	H	14	
33	3	0	H	H	1.2	
34	4	0	H	H	7.9	
35[c]	2	1	H	H	3.4	46
36	2	1	Me	H	1.3	17
37	2	1	Et	H	2.2	
38	2	1	*i*-Pr	H	1.6	10
39	2	1	Me	Me	0.63	27
40	2	1	CF_3	H	3.0	
41	2	1	Ph	H	3.0	

[a] See Ref. 14.
[b] See Ref. 15.
[c] This compound is the racemate of **17**.

the piperidine ring and the urea. Comparing compounds with the same atom count between the two aromatic rings (**33** vs **9** and **34** vs **10**), potency was increased about 5-fold by this change. The change was much more dramatic for the shortest analogues (**32** vs **8**), where a 250-fold improvement resulted.

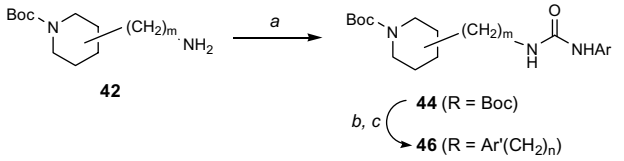
Incorporation of a 4-substituent on the piperidine ring offered another possibility for altering the conformational preference of the urea-containing chain via a buttressing effect. However, several analogues with such a substituent *trans* to the ureidomethyl group (**36** through **41**) showed marginal to no improvement in binding potency relative to **35**. Some slight improvements in antagonism of Ca^{2+} mobilization by the three analogues examined were probably not significant relative to that shown by **35**.

Four of the compounds described were shown to inhibit chemotaxis of human eosinophils in response to eotaxin (Table 4). However, these were clearly weaker than closely related 3-benzylpiperidine analogues of structure **1b**, which had chemotaxis IC_{50} values below 5 nM.[11] Similarly, the calcium mobilization potencies were significantly reduced relative to the binding potencies,

Table 4. Antagonism of eotaxin-induced chemotaxis compared to binding and Ca^{2+} release

	CCR3, IC_{50} (nM)[a]	Ca^{2+}, EC_{50} (nM)[b]	Chemotaxis, % inhib. @ 30 nM[c]
17	0.7	27	51
25	0.6	15	24
35	3.4	46	51
36	1.3	17	42

[a] See Ref. 14.
[b] See Ref. 15.
[c] See Ref. 16.



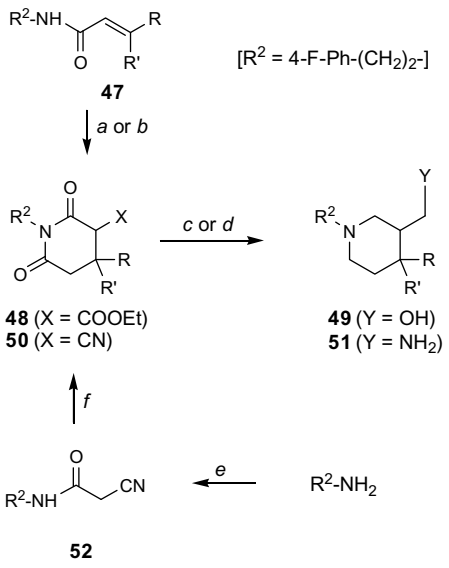
Scheme 1. Reagents and conditions: (a) ArNHCOOPh (**43**), DMF, Et_3N, rt, 70–80%; (b) HCl(g), EtOAc, rt, quant.; (c) $Ar'(CH_2)_nOTs$ (**45**), K_2CO_3, acetone or acetonitrile, reflux, 50–70%.

unlike the observations for previously-reported compounds. The explanation for this might lie in differences in the kinetics of receptor binding between the two series. Perhaps the greater flexibility of the phenethylamine moiety of the present series relative to the benzypiperidine moiety of **1b** results in faster dissociation from the receptor. This could translate to reduced functional effects for the same degree of receptor occupancy.

The compounds described were prepared from readily-available building blocks. As shown in Scheme 1, 1-Boc-protected 4-aminomethyl-,[17] 3-aminomethyl-[18] or 3-amino-piperidine[19] **42** was acylated with a substituted phenylcarbamate **43** (prepared according to published procedures).[20] The enantiomeric 3-aminomethyl-piperidine derivatives **42** ($m = 1$) were prepared from the corresponding alcohols, separated by enzymatic kinetic resolution.[18,21] (The *S* isomers had 97% ee, and the *R* isomers had 92% ee.) Deprotection of the ring nitrogen of **44** was followed by alkylation with the appropriate ω-tosyloxyalkylbenzene derivative **45** under standard conditions in good yields. (The alternative of reductive alkyl- ations with the corresponding aldehydes produced variable yields of products, and the purities of the crude products were generally worse.) Those alcohol precursors to the tosylates not commercially available were prepared by borane reduction of the corresponding acids, which were either purchased or readily prepared by Horner–Emmons reactions from the substituted benzaldehydes.

The 3,4-disubstituted aminomethylpiperidine derivative precursors to compounds **36** through **41** were obtained as shown in Scheme 2. Substituted acrylamides **47** were obtained in high recrystallized yields (70–98%) from the corresponding substituted acryloyl chloride or acrylic acid and 4-fluorophenethylamine. Base-catalyzed addition of diethyl malonate provided the piperidine-2,6-diones **48**. (Only the *trans* isomers were isolated when one of R and R′ was H.) Reduction with $LiAlH_4$ provided moderate yields of the hydroxymethyl compounds **49**, which were converted to the aminomethyl compounds using reported methods.[18] Alternatively, addition of ethyl cyanoacetate[22] to **47** followed by reduction of the cyanoimides **50** provided the aminomethyl derivatives directly, although the yields of the reduction step were modest (30–60%). Acylation with phenyl carbamates **43** as in Scheme 1 provided the desired products **36**–**38**, **40** and **41** in 60–80% yields.

In the case of the 4,4-dimethyl analogue **39**, no reaction of either malonate or cyanoacetate esters with the



Scheme 2. Reagents and conditions: (a) diethyl malonate, NaOEt, toluene, reflux, 75%; (b) ethyl cyanoacetate, NaH, THF, reflux (45–75%); or KOBut, *t*-BuOH, THF, reflux (75–96%); (c) $LiAlH_4$, THF, reflux (40%); (d) BH_3, THF, reflux, then HOAc (30–60%); (e) ethyl cyanoacetate, neat, 100 °C, 52%; (f) ethyl β-methylpropenoate, KOBut, *t*-BuOH, THF, reflux, 85%.

dimethyl acrylamide **47** (R = R′ = Me) was observed under a variety of conditions. In order to take advantage of the increased electrophilicity of esters over amides, the order of assembly was reversed. Thus, the cyanoacetamide **52** was prepared from ethyl cyanoacetate and 4-fluorophenethylamine, followed by base-catalyzed addition to ethyl β-methylpropenoate, to provide the desired imide **50** (R = R′ = Me) in excellent yield. Borane reduction provided **51** (R = R′ = Me).

In conclusion, compounds of general structure **4**, conceptually derived from **1** and **2**, bound to CCR3 with good potency and also displayed functional antagonism of the eotaxin-induced effects on human eosinophils. Broad features of the SAR were similar to the previously-reported series. The greater conformational freedom of **4** relative to **1** and **2** seemed to be reflected in a relatively high tolerance for changes in chain length and absolute stereochemistry, and may also be the cause of the relatively weaker inhibition of eotaxin-induced chemotaxis than was reported for the earlier compounds. Further work addressing these issues will be reported in due course.

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