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Discovery of *N*-propylurea 3-benzylpiperidines as selective CC chemokine receptor-3 (CCR3) antagonists

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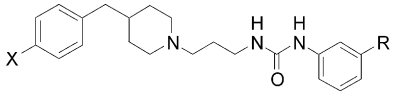
Abstract—The discovery of novel and selective small molecule antagonists of the CC Chemokine Receptor-3 (CCR3) is presented. Simple conversion from a 4- to 3-benzylpiperidine gave improved selectivity for CCR3 over the serotonin $5HT_{2A}$ receptor. Chiral resolution and exploration of mono- and disubstitution of the *N*-propylurea resulted in several 3-benzylpiperidine *N*-propylureas with CCR3 binding IC_{50}s under 5 nM. Data from in vitro calcium mobilization and chemotaxis assays for these compounds ranged from high picomolar to low nanomolar EC_{50}s and correlated well with antagonist binding IC_{50}s.

Asthma is one of the most common chronic diseases in industrialized nations.[1,2] Clinical studies have linked symptom severity in asthma patients to the gross accumulation of eosinophils in the lungs.[3] Eosinophils are recruited and directed to sites in the body via chemoattraction by the chemotactic cytokine (chemokine) eotaxin. Eotaxin binds exclusively to the seven transmembrane G protein-coupled receptor CC chemokine receptor-3 (CCR3), which is predominantly expressed on eosinophils and basophils, suggesting that eotaxin is a specific chemoattractant for these leukocytes.[4] Supported by studies using monoclonal antibodies for the CCR3 receptor[5] and animal eotaxin knockout models,[6] we have targeted antagonism of the CCR3 receptor as a mechanism of inhibiting eosinophil recruitment to the lungs by eotaxin.

We previously reported the design and synthesis of potent antagonists of the CCR3 receptor (Table 1).[7,8] The acetyl- and (1-methyl-tetrazol-5-yl)phenyl ureas (**5**–**6**)[9] were among the most potent antagonists discovered. However, our compounds also demonstrated affinity for several serotonin receptors, particularly $5HT_{2A}$. The potential for adverse side effects stemming from interactions with receptors of the central nervous system required that we improve the selectivity of our antagonists.

We hoped that the known sensitivity of some G protein-coupled receptors to structural changes in the piperidine functionality could be used to improve the selectivity of our antagonists for the CCR3 receptor. To that end we synthesized a series of 4-benzylpiperidine analogues, which are represented by entries **7**–**12** of Table 2. The

Table 1. 4-Benzylpiperidine CCR3 IC_{50} versus serotonin $5HT_{2A}$ binding K_i



Compd	X	R	CCR3 IC_{50}, nM	$5HT_{2A}$ K_i, nM
1	H	MeO	300±100	613
2	H	CN	200±100	770±290
3	F	MeO	30±10	224±64
4	F	CN	20±10	167±31
5	F	Ac	10±4	193±91
6	F	1-Me-tetrazol-5-yl	5±3	—

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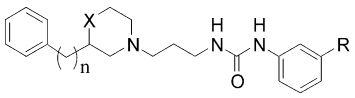
Table 2. Unsubstituted 3-benzylpiperidines and morpholine analogues[a]



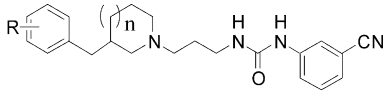
Compd	X	n	R	CCR3% Inh.[b]	CCR3 IC_{50}, nM	$5HT_{2A}$ K_i, nM
7	CH_2	1	MeO		55±9	913±88
8	CH_2	1	CN		41±22	2940±354
9	CH_2	2	MeO	84	787±4	
10	CH_2	2	CN	66		
11	O	1	MeO	14		
12	O	1	CN	36		

[a] All compounds are racemic.
[b] Percent inhibitions determined using 5 μM antagonist.

3-methoxy and 3-cyanophenyl urea 3-benzylpiperidines (**7**–**8**) were approximately 5-fold more potent for the CCR3 receptor than their corresponding 4-benzylpiperidine analogues (**1**–**2**) and were 16- and 70-fold more selective, respectively, for CCR3 than $5HT_{2A}$. In contrast, increasing the distance between the aromatic and piperidine rings of the 3-benzylpiperidine moiety (**9**–**10**) or incorporation of an oxygen atom into the piperidine ring (**11**–**12**) led to decreased binding affinity.

Aromatic substitution of the 3-benzylpiperidine moiety was examined (Table 3) to try and find a substitution pattern that offered improved selectivity and/or potency for the CCR3 receptor. Of particular interest is the *para*-fluorobenzylpiperidine, one of the most potent pharmacophores tested in the 4-benzylpiperidine series. We were encouraged that the 4-fluoro- and 2,4-difluorobenzylpiperidines (**17**–**18**) exhibited slightly greater binding affinities than the nonfluorinated 3-benzylpiperidine parent compound (**8**) and gave modestly better selectivity for CCR3 over $5HT_{2A}$. However, in contrast to the 8- to 10-fold increase in binding observed for the 4-benzylpiperidines, fluorine incorporation at the 4-position in the 3-benzylpiperidines gave only a modest ($\leq$2-fold) boost in potency. A small number of 3-(4-fluoro)benzylpyrrolidines were also prepared to test the effect of ring size. As shown by 3-(4-fluorobenzyl)-pyrrolidine **19**, this resulted in a 10-fold decrease in selectivity for $5HT_{2A}$.

Table 3. 3-Benzylpiperidine aromatic ring substitutions[a]

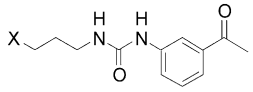


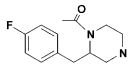
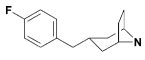
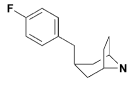
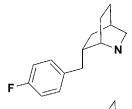
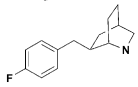
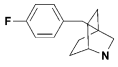
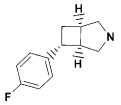
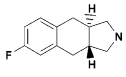
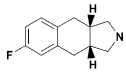
Compd	R	n	CCR3% Inh.[b]	CCR3 IC_{50}, nM	$5HT_{2A}$ K_i, nM
13	4-CF3	1	11		
14	3-MeO	1	31		
15	3-F	1		243±94	
16	2-F	1		38±15	2235±304
17	4-F	1		29±9	2310±354
18	2,4-F	1		19±7	2355±1351
19	4-F	0		31±5	178±30

[a] All compounds are racemic.
[b] Percent inhibitions determined using 1 μM antagonist.

Incorporating the *para*-fluoro moiety, we synthesized a number of structurally diverse and conformationally restricted 3-benzylpiperidine analogues to further elucidate the binding requirements for the CCR3 receptor (Table 4). Heteroatom incorporation resulted in complete (**20**) loss of activity, similar to previous results for the 2-benzylmorpholines (**11**–**12**). Bicyclo[3.2.1]octane **21**, with its benzyl moiety in an equatorial orientation, had a CCR3 IC_{50} of 7.4 nM and exhibited greater than 800-fold selectivity for the CCR3 receptor (6400±424 nM $5HT_{2A}$ K_i). In contrast, the same bicyclo[3.2.1]octane with the benzyl group in an axial orientation (**22**) was approximately 6-fold less potent than **21**. Several bicyclic and tricylcic head pieces (**23**–**28**) were also prepared, three of which (**25**, **27**–**28**) were comparable in potency to the simple benzylpiperidine **17**.

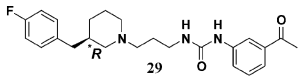
Table 4. Conformationally constrained benzylpiperidine analogues[a,b]



Compd	X	CCR3% Inh.	CCR3 IC_{50}, nM
20		0[c]	—
21			7.4±3.8
22			47±8
23			802±239
24			169±6
25			39±9
26		68[d]	—
27			49±23
28			56±21

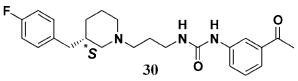
[a] All compounds are racemic.
[b] Structures of configurational isomers were determined by nOe experiments.
[c] Percent inhibition determined using 5 μM antagonist.
[d] Percent inhibition determined using 1 μM antagonist.

To make the appropriate comparison between the 3-cyanophenyl ureas of Table 3 and the 3-acetylphenyl ureas of Table 4, we synthesized enantiomerically pure 3-(4-fluorobenzyl)-piperidine acetyl ureas **29** and **30**.



29

39 ± 2.8 nM CCR3 IC_{50}



30

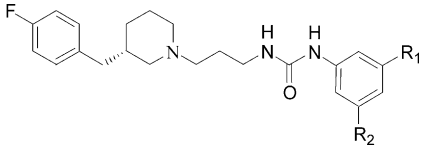
2.5 ± 1.2 nM CCR3 IC_{50}
2.4 nM Ca^{2+}efflux EC_{50}
25 nM Chemotaxis EC_{50}
1350 ± 71 nM $5HT_{2A}$ IC_{50}

The stereochemistry for each benzylpiperidine was assigned based on the crystal structure of the camphor sulfonamide derived from the (+)-enantiomer of 3-(*p*-fluorobenzyl)-piperidine. The (*S*)-enantiomer (**30**) was approximately 16-times more potent than the (*R*)-enantiomer (**29**) and was 4-fold more potent than **5**. Also, **30** had 540-fold selectivity for CCR3 over $5HT_{2A}$. Data for compound **30** in eotaxin-induced calcium mobilization[10] and eotaxin chemotaxis assays were in reasonable correlation with CCR3 binding and proved that **30** is a functional antagonist of the CCR3 receptor.

During the course of optimizing the 3-benzylpiperidine head piece, we also reexamined the 3-substituent of the phenyl urea to improve potency and selectivity (Table 5; **31**–**36**). Several 3-substituted ureas were designed based on the premise that aromatic heterocycles could be used to mimic or improve the binding interactions of the acetyl group of the 3-acetylphenyl urea (**30**). We focused on the 3-position of the phenyl urea because this substitution pattern in the 4-benzylpiperidine series gave the greatest binding affinities.[7] A number of different heterocycles were tested, but only a few compounds exhibited binding affinities comparable to that of **30**. Both oxadiazole **31** and 1-iPr-tetrazol-5-yl **34** were slightly less active than **30**, while the 1-imidazole (**32**) and 5-oxazole (**33**) phenyl ureas were less active by approximately 3-fold. In contrast, the 1-Et- and 1-Me-tetrazol-5-yl phenyl ureas (**35**–**36**) were approximately twice as active as the 3-acetylphenyl urea. Further testing of **36** gave calcium mobilization and chemotaxis EC_{50}'s of 1.3 ± 1.1 and 3.2 ± 1.6 nM, respectively. The close correlation between these three in vitro results was an improvement over data for the acetyl urea (**30**), which had a chemotaxis EC_{50} that was 10-fold less potent than the corresponding CCR3 IC_{50}.

We proposed that a 3,5-substitution pattern might offer an advantage over monosubstitution because disubstitution could increase the likelihood of maintaining binding interactions with the CCR3 receptor even with rotation about the *N*-phenyl bond of the urea. To test this hypothesis, several 3,5-disubstituted phenyl ureas were prepared, the most potent of which are shown in Table 5 (**37**–**45**). The 3,5-disubstituted bis(acetyl)phenyl urea (**37**) gave a CCR3 IC_{50} of 1.2 nM and an EC_{50} for chemotaxis that agreed well with CCR3 binding data. Some improvement in binding was also observed for disubstituted analogues of the 3-(1-Me-tetrazol-5-yl)phenyl urea (**36**) when small polar functionalities (**38**–**39**) were introduced at the 5-position. In contrast, hydrophobic groups (**40**–**42**, **44**) at the 5-position were slightly less potent than the parent 3-(1-Me-tetrazol-5-yl)phenyl urea (**36**). The tetrazole-acetylphenyl urea (**43**) was less active than **36**, while the 3,5-bis(1-Me-tetrazol-5-yl)phenyl urea (**45**) had a CCR3 IC_{50} of 0.7 nM. This was an approximate 2-fold improvement over **36** and was matched in potency only by **38** and **39.** In addition, bistetrazole **45** had an EC_{50} of 0.4 nM in the chemotaxis assay and is the most potent compound reported to date.

Table 5. Structure–activity relationships for phenyl urea substitution[a,b]



Compd	R_1	R_2	CCR3 IC_{50}, nM[a]	Ca^{2+} EC_{50}, nM[a]	Chemotaxis EC_{50}, nM
30	Ac	H	2.5 ± 1.2	2.4	25
31	Oxadiazol-2-yl	H	3.6 ± 1.7	45 ± 34	
32	Imidazol-1-yl	H	7.1 ± 3.7		
33	Oxazol-5-yl	H	7.9 ± 0.3		
34	1-iPr-Tet	H	3.1 ± 1.9		
35	1-Et-Tet	H	1.2 ± 1.6		
36	1-Me-Tet	H	0.6 ± 0.4	1.3 ± 1.1	3.2 ± 1.6
37	Ac	Ac	1.2 ± 0.4		3
38	1-Me-Tet	CH_2OH	0.9 ± 0.3		
39	1-Me-Tet	$CONHCH_3$	0.7 ± 0.4	0.6	< 1
40	1-Me-Tet	Me	1.5 ± 1.0	0.9 ± 0.7	1.0 ± 0.4
41	1-Me-Tet	CF_3	1.2 ± 0.2		
42	1-Me-Tet	Ph	2.4 ± 1.0		
43	1-Me-Tet	Ac	2.6 ± 1.4		
44	1-Me-Tet	Br	3.1 ± 1.0	3.4 ± 1.5	< 1
45	1-Me-Tet	1-Me-Tet	0.7 ± 0.5	0.76 ± 0.03	0.4 ± 0.4

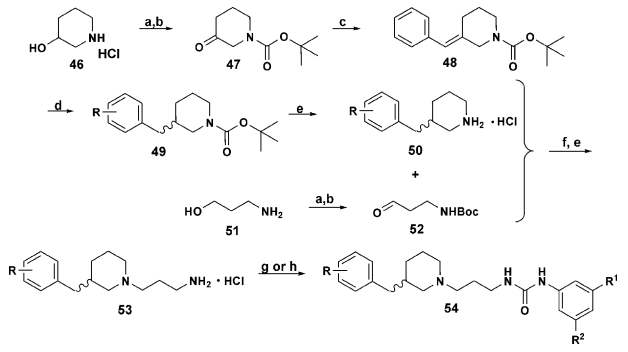
[a] Tet = tetrazol-5-yl.
[b] All compounds were > 100-fold selective versus $5HT_{2A}$.

The 3-benzylpiperidines and their analogues were prepared according to Scheme 1, which depicts the preparation 3-benzylpiperidin-1-yl-*n*-propylureas from commercially available 3-hydroxypiperidine hydrochloride (**46**, Scheme 1). Protection of the piperidine nitrogen with Boc_2O and oxidation with tetrapropylammonium perruthenate (TPAP)[11] afforded the 3-piperidone *tert*-butyl carbamic acid (**47**). A Wittig reaction between **47** and an appropriately substituted benzyltriphenylphosphonium bromide resulted in olefin **48**; reduction then gave racemic Boc-protected benzylpiperidine **49**. Chiral 3-(4-fluorobenzyl)-piperidines were prepared upon chiral resolution of **49**.

Deprotection of **49** under acid conditions and reductive amination with Boc-protected 3-aminopropanal (**52**) and sodium triacetoxyborohydride was followed by acid-mediated cleavage of the resulting Boc-carbamate to give hydrochloride **53**. Urea **54** was obtained by reacting **53** with commercially available 3-substituted phenyl isocyanates in the presence of triethylamine. Where the desired 3-substituted phenyl isocyanates were not commercially available, the corresponding phenolic carbamates were prepared from the appropriately substituted aniline (C_6H_5OCOCl, Et_3N, CH_2Cl_2, 90%) and used instead.

The 3-phenethylpiperidines were prepared by substituting phenethyltriphenylphosphonium bromide[12] for benzyltriphenylphosphonium bromide in Scheme 1. The 2-benzylmorpholines were prepared according to the procedure of Brown et al.[13] Piperazine **20** was prepared from commercially available 2-*tert*-butoxycarbonylamino-3-(4-fluorophenyl)propionic acid using the procedure of Hendrix et al.[14] The strategies for preparing compounds **21**–**28** have been previously reported.[15] The 3-tetrazolephenyl ureas were prepared from the corresponding benzoic acid.[16] Ureas **31** and **33** were prepared from phenolic carbamates derived from commercially available nitro benzenes. Imidazole **32** was prepared from commercially available 1-bromo-3-nitrobenzene.[17]

In conclusion, we have taken potent non-selective 4-benzylpiperidines (**5**–**6**) and prepared selective CCR3 antagonists with binding affinities under 5 nM. This was accomplished by replacing the 4-(4-fluorobenzyl)piperidine of our molecules with a 3-(4-fluorobenzyl)piperidine discovered in an effort to improve selectivity for the CCR3 receptor. Further improvements in binding were found by substituting the 5-position of the 3-(1-Me-tetrazol-5-yl)phenyl urea of these molecules with small polar functional groups. This resulted in a CCR3 antagonist with a binding IC_{50} and in vitro EC_{50} binding data for calcium mobilization and eotaxin chemotaxis assays in the mid to high picomolar range. Having demonstrated that our compounds are functional antagonists of the CCR3 receptor, we have focused on determining the in vivo efficacy of these molecules, the results of which will be reported in a future publication.



Scheme 1. (a) Boc_2O, $NaHCO_3$, THF, 14 h, 90%; (b) TPAP, NMO, CH_2Cl_2/CH_3CN, 4Å MS, 1 h, 60–80%; (c) R-$C_6H_4CH_2P^+Ph_3Br^-$, *n*-BuLi, THF, −78 °C, 6–8 h, 60–70%; (d) H_2 (40 psi), Pd/C, MeOH, 12 h, quant; (e) 4M HCl, diox, 3 h, quant.; (f) **52**, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, 14 h, 80%; (g) 3-R^1-5-R^2-C_6H_4NCO, Et_3N, CH_2Cl_2, 5 min, 60–85%; (h) 3-R^1-5-R^2-$C_6H_4NHC(O)OC_6H_5$, CH_3CN, 50 °C, 1 h, 55–85%.

Acknowledgements

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9. The binding assay was carried out using 150 pM ^{125}I labeled human eotaxin, 5×10^5 CHO cells, and 0.0001–1 μM compound in 150 μL of binding buffer (0.5% bovine serum albumen, 10 mM HEPES buffer and 5 mM magnesium chloride in RPMI 1640 media) in 96-well filtration plates (Millipore) pretreated with 5 μg/mL protamine in phosphate buffered saline, pH 7.2. The assay was incubated at room temperature for 30 min. The plates were vacuum filtered and the remaining cells were washed three times with binding buffer containing 0.5 M NaCl added.

Radiolabel binding was quantified upon filter removal via liquid scintillation counting.
10. The Ca^{+2} mobilization assay was carried out using 10 nM eotaxin, 2×10^5 eosinophils preloaded with Fluo-3AM (Molecular Probes) and 0.0001–1 μM compound in 200 μL of buffer (0.1% bovine serum albumen, 20 mM HEPES buffer and 2.5 mM Probenecid in RPMI 1640 media) in 96-well plates in a fluorescent imaging plate reader. Data was generated as arbitrary fluorescence units and compound dependent inhibition was calculated as a percentage of the response of eotaxin alone.
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