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Modulators of the human CCR5 receptor. Part 1: Discovery and initial SAR of 1-(3,3-diphenylpropyl)-piperidinyl amides and ureas

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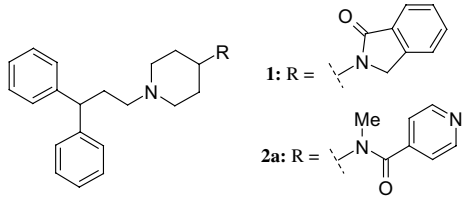
Abstract—Investigation of weak screening hits led to the identification of *N*-alkyl-*N*-[1-(3,3-diphenylpropyl)piperidin-4-yl]-2-phenylacetamides and *N*-alkyl-*N*-[1-(3,3-diphenylpropyl)piperidin-4-yl]-*N*′-benzylureas as potent, selective ligands for the human CCR5 chemokine receptor.

The chemokine receptor CCR5 is expressed on T-lymphocytes, monocytes, macrophages, dendritic cells, microglia and other cell types. These receptors detect and respond to several chemokines, principally 'regulated on activation normal T-cell expressed and secreted' (RANTES) and macrophage inflammatory proteins (MIP) MIP-1α and MIP-1β, resulting in the recruitment of cells of the immune system to sites of disease. CCR5 is also a co-receptor for HIV-1 and other viruses, allowing these viruses to enter cells. Individuals who are homozygous for a 32-base pair deletion in the gene encoding CCR5, whilst otherwise healthy, are strongly protected against HIV-1 infection.[1] Other studies indicate a role for CCR5 and its ligands in disorders such as rheumatoid arthritis,[2] multiple sclerosis,[3] transplant rejection[4] and inflammatory bowel disease.[5] These observations suggest that molecules that modulate the CCR5 receptor would have potential benefit in a wide range of diseases.

CCR5 is a member of the seven trans-membrane G-protein coupled receptor family, many other members of which have proved tractable targets for small molecule drugs. Over the last few years many groups have successfully engaged in the search for small molecules that interfere with the interaction between chemokines and their receptors.[6] In particular CCR5 has generated much interest as a drug target[1] and a recent clinical trial has provided proof of concept for this approach in the treatment of HIV-1 infection.[7]

We began our search for small molecule CCR5 modulators by screening the company compound library for compounds which inhibited the binding of [^{125}I]RANTES to membranes prepared from Chinese hamster ovary (CHO) cells stably expressing recombinant human CCR5.[10] 1-(3,3-Diphenylpropyl)-piperidines **1**[8] and **2a** were identified as weak ligands for CCR5 (binding IC_{50}s in the low micromolar range). We then set out to investigate the effect on potency of varying the piperidine 4-susbstituent.[9]



The advanced amine intermediates **3** (Scheme 1) allowed us to explore the SAR rapidly using multiple parallel techniques. Intermediate **3a** (R^1 = Me) was prepared in a straightforward manner from 4-Boc-amino-piperidine by alkylation followed by reduction of the Boc group to a methyl group. The other intermediates **3** were

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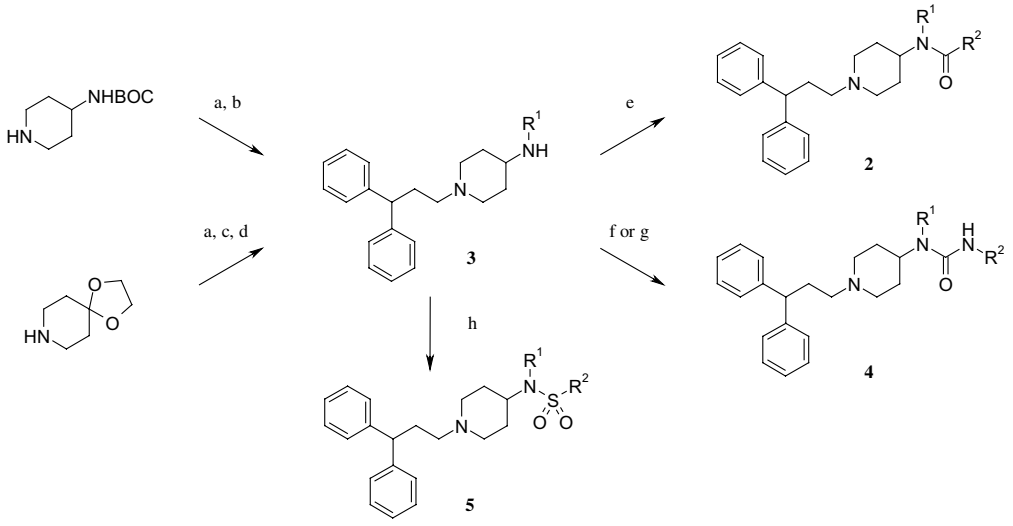


Scheme 1. Reagents and conditions: (a) 3,3-diphenylpropyl bromide, K_2CO_3, $^nBu_4NI$ (0.1 equiv), MeCN, reflux, 5 h; (b) (R^1 = Me) $LiAlH_4$, THF, reflux, 5 h; (c) 6 M HCl, reflux, 3 h; (d) R^1NH_2, $NaBH_3CN$, AcOH, MeOH, rt; (e) R^2CO_2H, DIPCDI, HOBT (0.1 equiv), DMF/DCM, rt; (f) R^2NCO, DCM, rt; (g) R^2CO_2H, DPPA, Et_3N, THF, 0 °C then toluene, 100 °C then **3**, EtOAc, rt; (h) R^2SO_2Cl, (^{i}Pr)$_2$NEt, MeCN/pyridine.

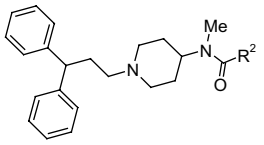
prepared by reductive amination of 1-(3,3-diphenylpropyl)-4-piperidone, which was obtained from 4-piperidone ethylene ketal by alkylation followed by deprotection of the ketone. The intermediates **3** were converted to amides **2** and sulfonamides **5** by standard methods. Ureas **4** were prepared by reaction with the appropriate isocyanate, which was either commercially available or prepared from a carboxylic acid by reaction with diphenylphosphoryl azide with subsequent Curtius rearrangement.

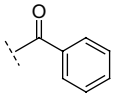
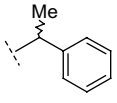
Several hundred analogues were prepared in this way; we describe herein the results for representative examples, which illustrate the main SAR findings.

The CCR5 binding affinity of the two initial screening hits **1** and **2a** is similar (Table 1), suggesting that there is no potency advantage in the amide *N*-substituent being cyclised onto the phenyl ring as in the 2,3-dihydroisoindol-1-one **1**. Therefore we decided to focus on the more synthetically accessible amides **2** and began by varying the amide acyl substituent.

Compounds with substituted phenyl (such as **2b** and **2c**), heteroaryl (**2d**, **2e**), cycloalkyl (**2f**) and alkyl (**2g**, **2h**) groups gave no appreciable improvement in potency. However one particular analogue, the phenylacetyl derivative **2i**, showed sub-micromolar potency and we decided to examine this further. Substitution of the alpha carbon of the phenylacetyl group with carbonyl (**2j**) or small alkyl groups (**2k**) led to a reduction in potency. We then aimed to improve the potency further by exploring the SAR around the phenyl of the phenylacetyl group (Table 2). *ortho* Substitution with halo is accompanied by a reduction in potency, as shown by **2l**, **2p** and **2q**. Halo and methoxy groups in the *meta* and *para* positions generally have a neutral effect on potency. A wide range of substituents was examined in the *para* position; some led to a drop in potency such as phenyl (**2ab**) and benzyloxy (**2aa**), others gave similar potencies to the unsubstituted phenyl such as acetylamino (**2ae**) and methoxycarbonyl (**2aj**). Increases in potency were seen with more strongly electron-withdrawing groups such as trifluoromethyl (**2ac**) and trifluoromethoxy (**2ad**), and particularly with polar electron-withdrawing groups such as nitro (**2al**), sulfonamido (**2ag**), cyano (**2af**, IC_{50} 60 nM) and *N*,*N*-dimethyl-sulfonamido (**2ah**, IC_{50} 46 nM).

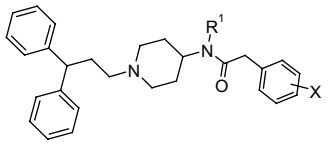
Table 1. Binding data for selected amides



Compound	R^2	IC_{50} (μM)[a]
1	—	4.1
2a	4-Pyridinyl	6.1
2b	4-F-phenyl	7.2
2c	3-NO_2-phenyl	5.1
2d	2-Thienyl	8.7
2e	2-Furanyl	7.9
2f	Cyclobutyl	7.4
2g	Isobutyl	3.4
2h	Neopentyl	5.5
2i	Benzyl	0.81
2j		5.9
2k		6.8

[a] IC_{50}'s were derived from triplicate measurements whose standard errors were normally <5% in a given assay. Assay to assay variability was within ±2-fold based on the results of a standard compound.

Table 2. Binding data for selected phenylacetamides



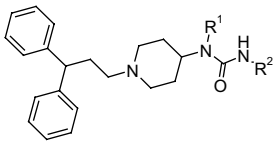
Compound	R^1	X	IC_{50} (μM)[a]
2i	Me	H	0.77
2l	Me	2-Cl	3.6
2m	Me	3-Cl	2.2
2n	Me	4-Cl	0.80
2o	Me	3,4-di-Cl	0.78
2p	Me	2,4-di-Cl	2.6
2q	Me	2-F	1.9
2r	Me	3-F	1.4
2s	Me	4-F	0.66
2t	Me	3,4-di-F	0.69
2u	Me	3-OMe	0.68
2v	Me	4-OMe	0.58
2w	Me	3,4-di-OMe	0.65
2x	Me	3,5-di-OMe	2.7
2y	Me	2,4,5-tri-OMe	1.1
2z	Me	4-Br	0.58
2aa	Me	4-Benzyloxy	3.5
2ab	Me	4-Phenyl	2.3
2ac	Me	4-CF_3	0.37
2ad	Me	4-OCF_3	0.29
2ae	Me	4-NHCOMe	0.68
2af	Me	4-CN	0.060
2ag	Me	4-SO_2NH_2	0.091
2ah	Me	4-$SO_2N(Me)_2$	0.046
2ai	Me	4-SMe	0.56
2aj	Me	4-CO_2Me	0.63
2ak	Me	4-OH	0.47
2al	Me	4-NO_2	0.15
2am	Et	4-OCF_3	0.31
2an	Et	4-CN	0.066
2ao	Et	4-SO_2NH_2	0.038
2ap	Et	4-$SO_2N(Me)_2$	<0.010
2aq	Et	4-SO_2Me	0.076
2ar	Et	4-NO_2	0.11
2as	Cyclopropyl	4-SO_2NH_2	0.033
2at	Cyclopropyl	4-SO_2Me	0.051
2au	Cyclopropyl	4-NO_2	0.31
2av	Allyl	4-OCF_3	0.35
2aw	Allyl	4-SO_2Me	0.037
2ax	Allyl	4-NO_2	0.18

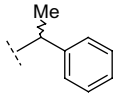
[a] See footnote a of Table 1.

We selected some of these preferred groups in a survey of the substitution on the nitrogen of the amide group (compounds **2am**–**2ax**, Table 2). Replacing the methyl with ethyl, cyclopropyl or allyl had little effect on the binding potency when compounds with the same phenyl substituent were compared (e.g., the 4-nitrophenyl compounds **2al**, **2ar**, **2au** and **2ax** all have IC_{50} values between 0.1 and 0.3 μM).

We also synthesised a large number of compounds in the urea (**4**) and sulfonamide (**5**) series. Binding results for selected compounds are shown in Table 3. In the ureas the preferred substituents on the nitrogen distal to the piperidine were found to be *para*-substituted phenyl

Table 3. Binding data for selected ureas



Compound	R^1	R^2	IC_{50} (μM)[a]
4a	Me	Cyclohexyl	1.8
4b	Me	3-CN–phenyl	4.9
4c	Me	3-Me–phenyl	5.9
4d	Allyl	Phenyl	2.1
4e	Me	3,4-di-Cl–phenyl	0.37
4f	Me	4-F–phenyl	0.19
4g	Et	4-Me–phenyl	0.32
4h	Me	Benzyl	0.10
4i	Et	Benzyl	0.062
4j	Et		5.4
4k	Allyl	3-Me–benzyl	0.24
4l	Allyl	4-OMe–benzyl	0.11
4m	Et	3-Me–benzyl	0.035
4n	Et	4-OMe–benzyl	0.042
4o	Et	4-SO_2Me–benzyl	0.049

[a] See footnote a of Table 1.

and benzyl. The SAR reflected to some extent that found in the phenylacetamide series, for example the alpha methyl benzyl **4j** had reduced potency compared to its benzyl analogue **4i**. However, the substitution pattern on the phenylmethyl group required to achieve binding potency less than 100 nM appeared less restricted than in the phenylacetamide series: groups such as 3-methyl (**4m**) and 4-methoxy (**4n**) were as potent as 4-methanesulfonyl (**4o**). Also in this series the compounds with the proximal nitrogen substituted with allyl tended to show lower potency compared to ethyl and methyl, as illustrated by comparing **4k** with **4m** and **4l** with **4n**. In the sulfonamide series it did not prove possible to achieve potency below 1 μM (data not shown), so further work in this area was abandoned.

The observed differences in structure–activity requirements for the group R^2 between the amides **2**, ureas **4** and sulfonamides **5** is not surprising since the orientation of that group relative to the rest of the molecule will be different due to the differing geometries of the linker groups. However it is interesting to note the higher activity for compounds having R^2 as benzyl in both the amide and the urea series.

Compounds in both the amide and the urea series are highly selective for the human CCR5 receptor over other chemokine receptors. Amides **2ah**, **2ap**, **2aw** and urea **4h** (which all have CCR5 binding IC_{50} values of 100 nM or lower) were screened at 10 μM for binding to human CCR1, CCR2b, CCR3, CXCR1 and CXCR2 using assays analogous to the CCR5-[^{125}I]RANTES binding assay (data not shown). All four compounds showed less than 50% inhibition in all five assays, implying that their selectivity for CCR5 versus these other

chemokine receptors is of the order of 100-fold or better. In order to survey the selectivity profile of the series representative compounds were tested in a panel of assays for binding to a range of GPCRs and ion channels (data not shown).[11] Binding to muscarinic and serotoninergic receptors was seen at 1–10 μM. On the basis of these results we established radioligand binding assays to measure activity at human M_1 and 5-HT_{2A} receptors, compound **2ap** showed IC_{50} values of 0.4 and 0.8 μM, respectively. In addition, compound **2aw** showed micromolar activity at the hERG ion channel. These results indicated to us that further optimisation was required to increase the selectivity with respect to these other activities.

We then studied the effects of a representative compound, **2aw**, in functional systems. Compound **2aw** was tested for its ability to inhibit MIP-1β-stimulated calcium transients in CHO cells stably expressing recombinant human CCR5 and was found to have an IC_{50} of 78 nM. Also **2aw** was shown to have an IC_{50} of 38 nM in an assay measuring the inhibition of the chemotaxis of human AlloT cells in response to MIP-1β. In the same system **2aw** had no effect on the response to MIP-1γ at concentrations up to 10 μM indicating that the inhibition of chemotaxis is mediated through CCR5. The similar potencies seen in these assays, in which MIP-1β is used, to those previously observed in the RANTES binding assay is evidence that the compound is acting as an antagonist at the receptor since it is able to block different CCR5 ligands.

In summary SAR studies around two weak screening hits have led to the identification of a series of potent and selective CCR5 functional antagonists (exemplified by **2aw**), which provide good leads for further optimisation. Our further development of this series to give potent, orally bioavailable CCR5 antagonists will be the subject of future publications.

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References and notes

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11. Work carried out on behalf of AstraZeneca by MDS Pharma Services, Taipei, Taiwan.