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Potent heteroarylpiperidine and carboxyphenylpiperidine 1-alkyl-cyclopentane carboxamide CCR2 antagonists

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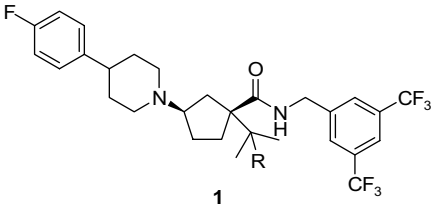
Abstract—This report describes replacement of the 4-(4-fluorophenyl)piperidine moiety in our CCR2 antagonists with 4-heteroaryl piperidine and 4-(carboxyphenyl)-piperidine subunits. Some of the resulting analogs retained potency in our CCR2 binding assay and had improved selectivity versus the I_{Kr} channel; poor selectivity against I_{Kr} had been a liability of earlier analogs in this series.

Chemokines are secreted by proinflammatory cells, leukocytes, and endothelial cells in response to a stimulus to the immune system. Monocyte chemoattractant protein-1 (MCP-1, CCL2), included within the CC class of chemokines,[1] mediates chemotaxis of monocytes to inflammatory sites primarily through interactions with its receptor, CCR2.[2] CCR2 is a member of the G-protein-coupled seven-transmembrane receptor superfamily and is most abundantly expressed on monocytes. Numerous recent studies have linked MCP-1 and CCR2 to various inflammatory diseases[3] including rheumatoid arthritis[4] and atherosclerosis.[5] Consequently, the therapeutic potential of CCR2 antagonists in treating inflammatory diseases has stimulated considerable interest.[6] Our group has recently disclosed the discovery and properties of a new class of potent CCR2 antagonists based upon a 3-[4-(aryl)piperidinyl]-1-alkyl-cyclopentane carboxamide core typified by **1**.[7] Counterscreening of a number of compounds within this family revealed a high affinity at the outward delayed rectifier potassium channel (I_{Kr}, human ether-a-go-go-related gene, hERG), which has been associated with QTc prolongation in vivo.[8] Prolongation of the QTc interval, in turn, has been linked to cardiac arrhythmias for a broad range of drugs.[8] With this in mind we set out to modify our CCR2 antagonists so as to tune out the I_{Kr} inhibition, while retaining potency in our primary screen. This letter describes modification of the 4-(4-fluorophenyl)piperidine moiety in lead type **1** by introduction of more polar aryl and heteroaryl groups in the piperidine 4-position. Some of the resulting analogs had significantly diminished I_{Kr} inhibition and good potency in the CCR2 binding assay. A 4-(5-pyrimidyl)piperidine containing analog with improved selectivity and good oral bioavailability in rats will be highlighted.



1

R = H, CCR2 IC_{50} = 3 nM
IKr IC_{50} = 16 nM

R = OH, CCR2 IC_{50} = 1.3 nM
IKr IC_{50} = 54 nM

In an attempt to minimize the potent I_{Kr} binding affinity characteristic of **1**, we decided to explore analogs in which the 4-fluorophenyl group was replaced with more polar aryl and heteroaryl groups. From SAR in a precursor acyclic CCR2 antagonist series[9] we knew that various functional groups could be tolerated in the phenyl 3-position without a major loss in potency (while only small groups were tolerated in the 2- and 4-positions). Similarly, of the three possible 4-pyridylpiperidine isomers, the 4-(3-pyridyl)piperidine subunit was

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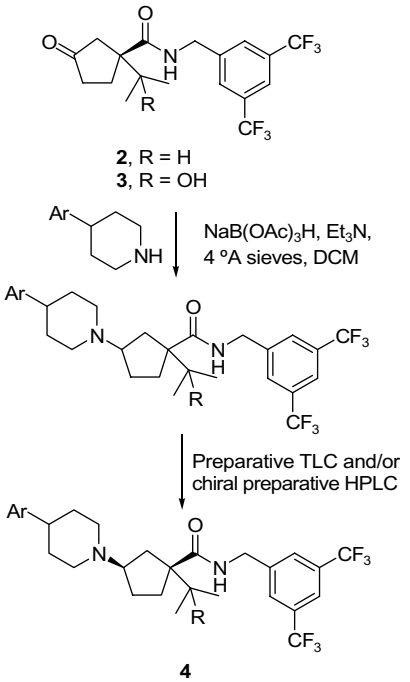
shown to be the best. This information guided us in the selection of our initial aryl and heteroaryl piperidine containing analogs. The requisite aryl and heteroaryl piperidines were prepared according to the procedure of Wustrow and Wise by palladium-mediated coupling of aryl and heteroarylboronic acids with *tert*-butyl 4-{[(trifluoromethyl)sulfonyl]oxy}-3,6-dihydropyridine-1(2*H*)-carboxylate, followed by hydrogenation and Boc removal.[10] The resulting piperidines were reductively alkylated with previously described[7b] intermediates **2** and **3** by treatment with sodium triacetoxyborohydride (Scheme 1) over 2–3 days. The cis and trans-isomers could usually be separated by preparative TLC (with the cis-isomers normally being obtained as the major isomers). Slow reductive amination times were tolerated because of good yields and favorable cis-selectivities; alternative methods were not evaluated. The two cis-diastereomers could then be separated by preparative chiral HPLC (using either a Chiralcel OD or a Chiralpak AD column, obtained from Chiral Technologies, Inc.) to afford the homochiral analogs. In some cases functional groups on the phenyl ring were modified to give the final analogs (i.e., esters hydrolyzed).

CCR2 Binding affinities were determined using a radioligand competition binding assay measuring inhibition of 125I-MCP-1 binding to the endogenous CCR-2 receptor on human monocyte whole cells.[7] I_{Kr} binding data were obtained by measuring displacement of 33S-MK499 from HEK cells stably expressing hERG.[11] Functional blockade of I_{Kr} was not evaluated for these analogs. An initial series of analogs with corresponding CCR2 and I_{Kr} binding data is presented in Table 1. Introduction of methoxy and hydroxy groups in the phenyl 3-position provided only modest improvement in selectivity as measured by the ratio of I_{Kr} binding to CCR2 binding when compared to the 4-fluoro (**1a**) and 3-fluoro (**4a**) direct analogs. Similarly, introduction of a 3-pyridyl (**4f**) in place of the phenyl (**1a**) gave only a slight improvement in selectivity, although potency remained high at CCR2 indicating a tolerance for heteroaryl groups at this position. The largest decrease in I_{Kr} affinity was observed upon introduction of a carboxylic acid in the 3-position (**4d**), however this was accompanied by a simultaneous decrease in CCR2 binding affinity. The effect was more pronounced in the hydroxypropyl side chain series (**4e**) where I_{Kr} binding dropped to 20 μM, while CCR2 binding, though diminished, remained at a respectable 45 nM.

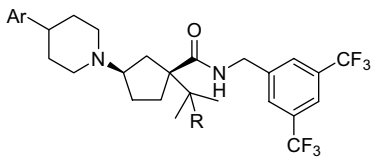


Scheme 1. Synthesis of target analogs **4**.

The modest selectivity enhancement and the CCR2 potency retention observed with 3-pyridyl analog **4f** prompted us to prepare more polar heteroarylpiperidine containing analogs. In particular, we were interested in preparing all possible two nitrogen containing 6-membered heteroaryl isomers, as these would be more polar than the pyridylpiperidine but of similar steric dimensions. At the time of this work we could find no literature reference to the target pyrimidyl, pyridazyl, and pyrazyl piperidines. Since the heteroarylboronic acids were not available, we decided to devise a synthesis that could make use of commercially available haloheteroaryl precursors. Recently Billotte, in a letter focusing on aryl azetidine synthesis from an azetidinyl zincate reagent, briefly described the application of the piperidinyl zincate **6** to the synthesis of two aryl piperidines via palladium mediated coupling to the corresponding aryl halides.[12] As shown in Scheme 2 and Table 2, we successfully applied Billotte's methodology to the synthesis of all of our target isomeric heteroarylpiperidines, leading to the first disclosure of a general preparation of these interesting and potentially useful new compounds. 4-Substituted piperidines prepared according to Scheme 2 were reductively alkylated with **2** and **3** as described in Scheme 1 to give, after separation of the isomers, target analogs for evaluation in our assays. Some of the heteroarylpiperidines were prepared with di or trihalo heteroaryl precursors. The resulting haloheteroarylpiperidines could be hydrogenated prior to Boc removal (Scheme 2, step d and **8e**) or they could be carried on to target CCR2 antagonists according to Scheme 1 and then hydrogenated so that both the haloheteroaryl and reduced heteroarylpiperidines could be evaluated in our assays.

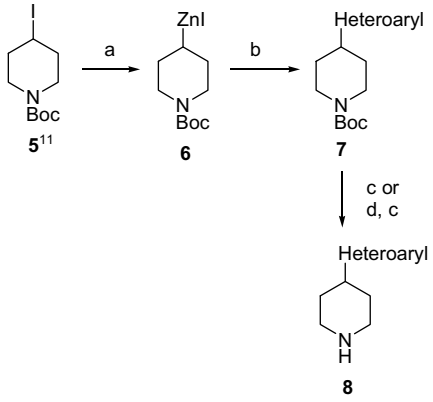
Table 3 lists the target heteroarylpiperidine containing analogs prepared, along with corresponding binding data. Only analogs deemed of sufficient potency in our CCR2 binding assay were evaluated in the I_{Kr} assay. Most analogs were assayed as a mixture of two *cis*-diastereomers, although we know from previously described work that only the 1*S*,3*R*-isomer (as shown) contributes to CCR2 binding affinity.[7] All heteroarylpiperidine containing analogs except **4h**, **4i**, and **4j** were significantly less potent than the original lead **1** as well as the pyridylpiperidine analog **4f**. Compounds **4h**, **4i**, and **4j**, all containing the same 5-(pyrimidyl)piperidine subunit, were of similar potency to lead **1** and pyridylpiperidine **4f**. Compounds **4i** and **4j** are single 1*S*, 3*R*-enantiomers obtained by preparative chiral HPLC

Table 1. Initial aryl and heteroarylpiperidine analogs



Compound	R	Ar	CCR2 IC_{50} ± SD[d] (nM)	I_{Kr} IC_{50}[e] (nM)	I_{Kr} IC_{50}/CCR2 IC_{50}
1a	H	4-F–Ph	3 ± 1	16	5
1b	OH	4-F–Ph	1.3 ± 0.49	54	42
4a[a]	H	3-F–Ph	4 ± 4	45	11
4b[a]	H	3-(OMe)–Ph	6 ± 4	217	36
4c[a]	H	3-(OH)–Ph[b]	6 ± 2	67	11
4d[a]	H	3-(CO_2H)–Ph[c]	62 ± 4.9	2786	45
4e[a]	OH	3-(CO_2H)–Ph[c]	45 ± 6.4	19,720	438
4f[a]	H	3-Pyridyl	6 ± 0.7	224	37

[a] Assayed as a mixture of two cis-enantiomers.
[b] Obtained from **4b** by treatment with BBr_3 in DCM.
[c] Obtained from the corresponding ethyl ester intermediates by hydrolysis with LiOH in ethanol/water.
[d] CCR2 IC_{50} is an average of 2–5 determinations.
[e] I_{Kr} IC_{50} is an average of two determinations; all SD values are within 30% of the value of the average.



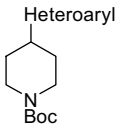
Scheme 2. Reagents and conditions: (a) Zn powder, THF, 1,2-dibromoethane; TMSCl; **5**; (b) heteroarylhalide, $Pd_2(dba)_3$, P(2-furyl)$_3$, 80 °C, 3–5 h; (c) 4 N HCl/dioxane, rt, 1 h; (d) H_2, cat. Pd/C (10%), $NaHCO_3$ (2 equiv), EtOAc.

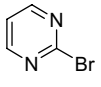
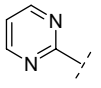
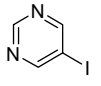
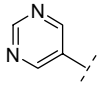
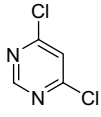
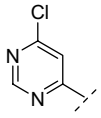
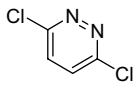
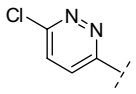
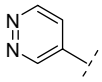
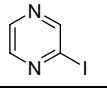
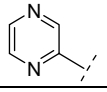
separation of the cis-mixtures (like **4h**). Of the analogs evaluated in the I_{Kr} binding assay, only those containing this optimal 5-(pyrimidyl)piperidine subunit showed improvement in selectivity as measured by the ratio of I_{Kr} binding affinity to CCR2 binding affinity. Moreover, the selectivity of analogs **4i** and **4j** is further improved over that of the pyridylpiperidine analog **4f**. These two analogs show a greater than 100-fold selectivity window. The dramatic synergistic improvement in selectivity that was observed upon changing the side chain from isopropyl to hydroxy-isopropyl in the carboxyphenyl series (**4d** to **4e**) was not observed in the pyrimidyl series (**4i** to **4j**).

The oral bioavailability of analog **4h** was evaluated in rats (3 mpk po) and found to be good (F = 33%, Cl = 7 mL/min/kg, $t_{1/2}$ = 3.8 h, AUCN = 1.53 μM h).

Analogs **4i** and **4j** were evaluated in a functional assay measuring inhibition of MCP-1-mediated chemotaxis of monocytes[7] and were both found to be potent CCR2 antagonists (IC_{50} of 2 nM and 2 nM, respectively).

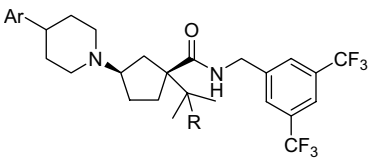
Table 2. *N*-Boc-heteroarylpiperidines prepared according to Scheme 2

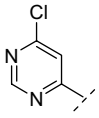
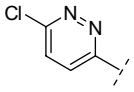
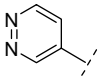


Compound	Halide	Ar-	Yield (%)
8a			70
8b			24
8c			15
8d			45
8e			14[a]
8f			56

[a] Hydrogenation followed Pd coupling as per Scheme 2. Yield given is for two steps (15%, 92%). Starting halide for **8e** was prepared in one step from commercially available 4,5-dibromopyridazine-3-one by heating with $POCl_3$.

Table 3. Heteroarylpiperidine containing analogs



Compound	R	Ar	CCR2 IC_{50} ± SD[c] (nM)	I_{Kr} IC_{50}[d] (nM)	I_{Kr}/CCR2
4g[a]	H		144 ± 16.3	—	—
4h[a]	H		12 ± 3.0	1413	118
4i[b]	H		5 ± 0.5	1800	360
4j[b]	OH		6 ± 0.7	844	141
4k[a]	H		24 ± 1.4	186	8
4l[a]	H		44 ± 1.4	130	3
4m[a]	H		165 ± 24.7	—	—
4n[a]	H		50 ± 4.9	—	—
4o[a]	H		64 ± 2.8	—	—
4p[a]	H		41 ± 5.7	600	15

[a] Assayed as a mixture of two cis-enantiomers.
[b] Resolved by preparative chiral HPLC; data shown for single (1*S*,3*R*)-enantiomer.
[c] CCR2 IC_{50} is an average of 2–5 determinations.
[d] I_{Kr} IC_{50} is an average of two determinations; all SD values are within 30% of the value of the average.

In addition, analogs **4i** and **4j** were inactive when assayed against CCR5 (3% at 1 μM and 3% at 1 μM, respectively), while our original lead **1** retained the CCR5 activity (R = H, CCR5 IC_{50} = 69% at 1 μM, R = OH, CCR5 IC_{50} = 69% at 1 μM) common to many known CCR2 antagonists.[6]

In summary, we have explored the effect on CCR2 potency, and selectivity versus the I_{Kr} ion channel, of replacement of the 4-(4-fluorophenyl)piperidine moiety in our lead series **1** with substituted phenylpiperidines and heteroarylpiperidines. Analogs containing the 3-carboxyphenylpiperidine and 5-(pyrimidyl)piperidine subunit were found to have notably improved selectivity. Analogs having the 5-(pyrimidyl)piperidine were of particular interest in that they retained most or all of the potency of lead **1**, they had greater than 100-fold selectivity against the I_{Kr} channel, and analog **4h** was found to have good oral bioavailability in rats. Moreover, 5-(pyrimidyl)piperidine analogs **4i** and **4j** were shown to be highly potent antagonists of CCR2 in a functional assay measuring inhibition of MCP-1-mediated chemotaxis of monocytes. Finally, analogs having the 5-(pyrimidyl)piperidine subunit were found to be selective against CCR5, contrary to our initial lead as well as other published CCR2 antagonists.

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