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Discovery of a 4-Azetidinyl-1-thiazoyl-cyclohexane CCR2 Antagonist as a Development Candidate

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KEYWORDS azetidine, CCR2, hERG, cardiovascular (CV) safety

ABSTRACT: We have discovered a novel series of 4-azetidinyl-1-aryl-cyclohexanes as CCR2 Antagonists. A divergent SAR studies on hCCR2 and hERG activities led to the discovery of compound **8d** , which displayed good hCCR2 binding affinity (IC_{50}, 37 nM) and potent functional antagonism (chemotaxis IC_{50}, 30 nM). It presented an IC_{50} of > 50 μM in inhibition of the hERG channel and had no effect on QTc interval up to 10 mg/kg (i.v.) in anesthetized guinea pig and dog CV studies. It also displayed high selectivity over other chemokine receptors and GPCRs, and amendable oral bioavailability in dogs and primates. In a thioglycollate-induced inflammation model in hCCR2KI mice, it had ED_{50} of 3 mg/kg on inhibition of the influx of leukocytes, monocytes/macrophages and T-lymphocytes.

Monocyte chemotactic protein-1 (MCP-1) is one of the primary molecules controlling the influx of mononuclear leukocytes into sites of inflammation.[1-2] MCP-1 is a potent chemotactic factor for monocytes and memory T lymphocytes, and stimulates the movement of those cells along a chemotactic gradient following binding to its cell-surface receptor, CC chemokine receptor-2 (CCR2). This ligand/receptor pair is overexpressed in numerous inflammatory conditions wherein excessive monocyte recruitment is observed. Indeed, CCR2- and MCP-1-deficient mice and CCR2 or MCP-1 antibody-treated rodents show decreased recruitment of monocytes and produce markedly attenuated inflammatory responses in animal models of rheumatoid arthritis (RAs),[3] multiple sclerosis,[4] asthma,[5] atherosclerosis,[6] diabetes,[7] allograft rejection,[8] and neuropathic pain.[9] Clearly, these observations confirm the role of CCR2 in the pathogenesis of several immune-based inflammatory diseases and identify this chemokine receptor as a potentially valuable therapeutic target. Thus, an antagonist of the binding of MCP-1 to its receptor may be an effective treatment for any inflammatory disease in which monocytes, mast cells, or basophils play major roles.

While a leading CCR2 antagonist MK-0812 failed to show significant improvement on RAs in clinical trials, there has been continuously intensive research interest in many distinct chemical series as CCR2 antagonists for other indications.[10-17] We have recently reported a novel series of 4-azetidinyl-1-aryl-cyclohexanes as CCR2 antagonists.[16] This scaffold is characterized by a general hERG liable structure consisting of a central basic amine flanked by two hydrophobes at the ends. Divergent SARs on hCCR2 and hERG activities enabled us to dial out hERG affinity and generated highly selective hCCR2 antagonists in the scaffold. The lead compound **1** (Figure 1) possessed good hCCR2 binding affinity (IC_{50}, 15 nM), potent functional activity (chemotaxis, IC_{50}, 22 nM) and amendable separation over hERG activities. Disappointingly, i.v. treatment of anesthetized guinea pigs with **1** resulted in a significant dose dependent prolongation of the QTc interval (ΔQTc). At a dose of 10 mg/kg, a +20% ΔQTc was observed, with drug plasma level of 12 μM being attained. Thus, **1** was abandoned from further development due to its unacceptable CV safety profile. Since QTc prolongation has been linked to preferential blockade of the voltage-gated potassium (K^+) channel encoded by the hERG, optimization of a structural series for lack of *in vitro* hERG affinity appears to be generally predictive of decreased potential to cause QTc prolongation *in vivo*. Herein, we report our continuous SAR studies on identifying potent and selective hCCR2 antagonists devoid of this ancillary cardiac activity. Owing to the correlation between the ΔQTc observed with **1** in guinea pigs and the potency of **1** in the hERG binding assay, we defined hERG IC_{50}'s >25 μM must be achieved to address QTc issue. As a critical follow-up, a hERG patch clamp assay was used as a second front-line screening for eliminating compounds that displayed significant blockade of the potassium current, which is another *in vitro* indicator for causing QTc prolongation.

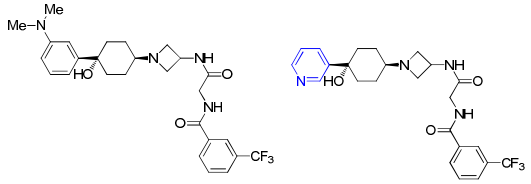
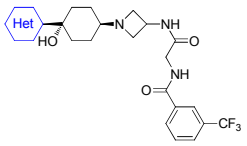
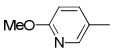
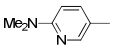
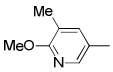
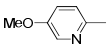
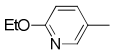
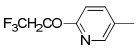
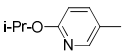
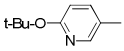
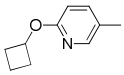


Figure 1. Early leads

Table 1. SAR of nitrogen containing six membered heterocyclic analogues



ID	Het	hCCR2 binding IC_{50} (nM)[a]	CTX IC_{50} (nM)[b]	hERG IC_{50} (μM)[c]	Patch Clamp %@3 μM (control)[d]
2a		190	nt[e]	35.0	28±7
2b		650	nt	43.8	19±7
2c		190	nt	10.0	54±4
2d		170	nt	12.5	61±6
2e		45	74	35.0	12±6
2f		44	56	37.0	29±3
2g		310	nt	22.5	61±10
2h		220	nt	21.6	55±5
2i		71	nt	17.5	43±3
2j		250	nt	25.0	30±3
2k		18	30	14.5	68±2
2l		19	33	7.0	nt
2m		50	44	11.2	49±9
2n		49	17	5.5	nt
2o		27	43	9.8	nt

[a] MCP-1 Receptor Binding Assay in THP-1 Cells, for IC_{50}>100 nM (n=1), for IC_{50}<100 nM (n>2, average values, SEM<±25%); [b] MCP-1 Induced Chemotaxis in THP-1 Cells; [c] hERG ^{3}H-astemizole binding activity on HEK-293 cell ; [d] The membrane K^+ current IKr in hERG-transfected HEK293 cells (n=3); [e] nt: not tested

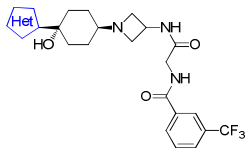
In the discovery of lead compound **1**, it was established that the cyclohexyl azetidine core as well as the right-end bis-amide functionality were critical for hCCR2 activity and might be too sensitive for modifications.[16] The left-end phenyl ring was somehow tolerated for moderate to good hCCR2 activity. For this reason, we focused further optimization on the left-end aromatic group. Replacement of the phenyl group at the 1-position on the cyclohexyl ring with the 3-pyridinyl group resulted in a significant loss of hCCR2 activity but also further attenuated hERG binding affinity (**2a**: hCCR2 IC_{50}, 190 nM; hERG $IC_{50,}$ 35 μM).[15-17] These data prompted us to take nitrogen containing six membered aromatic rings as the starting

point for subsequent optimization (Table 1). Compound **2b** bearing a more polar pyrimidinyl group displayed even weaker hCCR2 binding affinity ($IC_{50,}$ 650 nM) compared with **2a**. We then examined the substituent effect on both hCCR2 and hERG inhibition among the pyridinyl derivatives. Electron withdrawing groups such as fluoro or cyano did not improve hCCR2 binding as indicated with **2c** and **2d** with IC_{50} of 190 nM and 170 nM respectively. In contrast, electron donating group, exemplified by methoxy and methyl groups, had beneficial effect on hCCR2 activity as evidenced by an approximate 3-5 fold increase of binding affinity (**2e** and **2f** vs. **2a**). The improved potency of **2e** and **2f** was also translated into good levels of activity in the chemotaxis assay with IC_{50} of 74 nM and 56 nM, respectively. To our delight, installation of both substituents maintained good selectivity between hCCR2 and hERG binding affinities (hERG binding IC_{50} of 35 and 37 μM). The weak hERG affinities of both compounds were further confirmed with only 12% and 29% of inhibition at 3 μM in the patch clamp assay. The dimethylamino analogue **2g** was ~ 8-fold weaker for hCCR2 binding than **2f**. Addition of a methyl group *ortho* to the methoxy group also resulted in significant loss of hCCR2 affinity as shown by **2h** (IC_{50}, 220 nM). The two methoxy-pyridinyl regioisomers **2i** and **2j** displayed weaker hCCR2 binding affinities (IC_{50}, 71 and 250 nM) compared with **2f** indicating electronic density of the pyridinyl ring had great impact on hCCR2 activity. As expected, installation of more lipophilic 4-alkoxy groups maintained or improved both hCCR2 binding affinity and chemotaxis activity as illustrated by **2k** to **2o**. Steric bulkiness on the alkoxy group was well tolerated for good hCCR2 activity (**2m**, **2n**, **2o**). Unfortunately, the unwanted hERG signals among these derivatives reappeared to be problematic for further evaluations.

Given the good *in vitro* selectivity between hCCR2 and hERG activities, **2e** and **2f** were selected for evaluation in an anesthetized guinea pig CV (GPCV) study. Disappointingly, both **2e** and **2f** still displayed electro-cardiographic effects consisting of dose dependent increase in ΔQTc. With a dosage of 10 mg/kg (i.v.), **2e** and **2f** exhibited +13% and +6% of the ΔQTc respectively. These results led us to re-define our criteria on *in vitro* hERG profile for advancing compounds into GPCV study. Our efforts then focused on identifying compounds with decreased hERG affinity to our detection limit (IC_{50}, 50 μM) while maintaining or improving hCCR2 activities. However, attempt on determining the direct correlation between hERG binding and QTc prolongation could be challenging due to the impact on QTc from other factors, for an example, serum protein binding. Therefore, the final selection on the compounds for further development should be solely determined by comprehensively *in vivo* CV safety evaluation.

Reviewing of recent literatures led us to postulate that the weak basic pyridinyl group of **2e** and **2f** may participate in a cation-π interaction with the Tyr-652 residue of the hERG channel.[18] Thus, elimination of the basic feature of the pyridinyl group would disrupt this interaction and further attenuate hERG binding. For chemistry effort, we decided to examine the left-end aromatic ring with a series of five membered heterocyclic analogues. As illustrated in Table 2, substitution by five membered heterocycle had great impact on hCCR2 affinity. While incorporation of an oxazole (**3**), imidazole (**4**), pyrrozole (**5**) or thiadiazole (**6**) either efficiently attenuated or

Table 2. SAR of five membered heterocyclic analogues



ID	Het	hCCR2 Binding IC_{50} (nM)	hERG Binding IC_{50} (μM)
3		2,820	> 50
4		5,300	15.0
5		2,700	> 50
6		790	> 50
7		100	27.0
8a		62	32.0
9		36	24.0

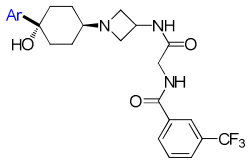
abolished hERG affinity, it also significantly reduced hCCR2 activity. To our satisfaction, installation of a 2-thiophene (**7**), 2-thiazole (**8a**) or 2-isothiazole (**9**) at the 1-postion of the cyclohexyl ring was tolerated for good hCCR2 binding affinity (IC_{50}, 100, 62, 36 nM). Among them, **8a** displayed relatively weak hERG binding affinity with IC_{50} of 32 μM and promising separation between hCCR2 and hERG affinities, which promoted us to investigate this substitution pattern in detail.

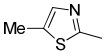
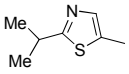
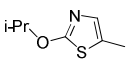
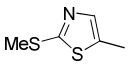
Table 3 highlighted the SAR within a series of thiazole substituted analogues. Installation of a thiazole moiety at the 1-position of cyclohexyl ring was generally well tolerated for good hCCR2 activity. However, modification on the thiazole group had great impact on *in vitro* hERG profile and hence dominated the QTc effect in GPCV study. There was a trend that more lipophilic thiazoles led to stronger hERG *in vitro* activity. Compared to **8a**, simple methylation at the 3-position of the thiazole caused the blockade of hERG potassium current from 35% to 49% at 3 μM (**8b**). It is therefore not surprising that intravenous infusion of **8b** in anesthetized guinea pigs triggered a dose dependent QTc prolongation (ΔQTc, +15% at 10 mg/kg). Compound **8c** bearing a hydrophobic benzothiazole moiety possessed enhanced hERG *in vitro* activity. Installation of a 5-thaizole group onto the 1-position of the cyclohexyl ring (**8d**) remarkably attenuated the hERG binding affinity to greater than 50 μM. Consistent with its weak hERG affinity, **8d** also displayed low inhibition on the potassium current in the patch clamp study (21% inhibition at 3 μM). To our delight, intravenous infusion of **8d** in anesthetized guinea

pigs had no effect on QTc up to 10 mg/kg. Plasma concentration reached at 26 μM. Having identified 5-thiazole group as the preferred left-end moiety, we extended our SAR studies to several substituted 5-thiazole analogues. Compound **8e** and **8f** with small alkyl substitutions displayed good hCCR2 activities and desired *in vitro* hERG profile. Furthermore, **8f** demonstrated no effect on QTc up to 10 mg/kg (i.v.) in GPCV study (plasma level, 10 μM). It is noteworthy that the 5-thiazole is not considered as a general pharmacophore for suppressing hERG *in vitro* activities and eliminating QTc prolongation in GPCV study. As illustrated by **8g** and **8h**, while maintaining good hCCR2 activities, they both possessed enhanced inhibition on the potassium current (49% and 77% inhibition at 3 μM). While compound **8g** displayed no effect on QTc up to 10 mg/kg (i.v.) in GPCV study, the plasma concentration was only ~ 3 μM which did not provide enough safety margin for further development. Here, an extremely narrow line appeared to determine the CV safety profile of this series.

Given its superior *in vitro* hERG and GPCV safety profiles, **8d** was selected to be further evaluated in an anesthetized dog CV safety study. Strikingly, it did not induce dose-dependent or notable effects on most cardiohemodynamic, functional respiratory and electrophysiological parameters up to 10 mg/kg (i.v.) with plasma level at 70 μM. It also did not induce changes in ECG morphology. Overall, **8d** represented the first compound with good CV safety profile as a development candidate.

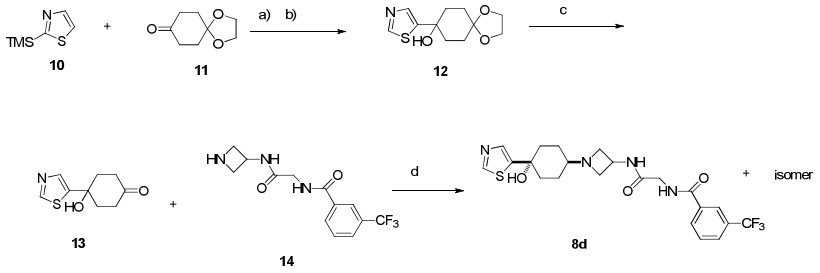
Table 3. SAR of thiazole substituted analogues



ID	Ar	hCCR2 binding IC_{50} (nM)	CTX IC_{50} (nM)	hERG Binding IC_{50} (μM)	Patch Clamp % @ 3 μM (control)	GPCV ΔQTc@10 mg/kg (+%)[a]
8a		62	13	32	30±4	nt
8b		18	60	28	49±3	+15
8c		16	28	6.2	80±9	nt
8d		37	30	> 50	21±1	no effect
8e		78	65	> 50	21±5	nt
8f		57	70	> 50	35±3	no effect
8g		5	4	28	49±6	no effect
8h		41	68	6.0	77±5	nt

[a] Cumulative doses (0.1, 0.3, 1, 3 and 10 mg/kg) were administered incrementally as five-minute i.v. infusions at 0, 20, 40, 60 and 80 minutes. QTc intervals (ΔQTc) were measured and recorded at each time point as a percent change from baseline for each animal. Values are mean ± SEM (n=3)

Scheme 1. Synthesis of **8d**



(a) n-BuLi, -78°C. (b) TBAF, 2 steps, 55%. (C) HCl, acetone, room temperature, 90%. (d) $NaBH(OAc)_3$, TEA, DCM, room temperature, 35% along with its isomer, 40%.

Compound **8d** was a selective hCCR2 inhibitor, showing no significant inhibitory activity at concentration of 10 μM in Cerep and Invitrogen protein kinase panel screens. It did not significantly inhibit the binding of any of the relevant chemokines tested including CCR1, CCR3, CCR4, CCR5, CXCR1, CXCR2, CXCR4, and CX3CR1 at concentration of 25 μM.

Compound **8d** also exhibited amendable DMPK profiles for further development. It was highly orally bioavailable and exhibited oral bioavailability of 70.2% in dogs when dosed at 6.7 mg/kg using 0.5% methocel as a vehicle (C_{max} = 1617 ng/mL, AUC_{last} = 5887 h*ng/mL). In addition, it showed 25.4% oral bioavailability in non-human primates (7.2 mg/kg p.o., C_{max} = 740 ng/mL, AUC_{last} = 3061 h*ng/mL), but only 19% of oral bioavailability in mice (10 mg/kg p.o., C_{max} = 74 ng/mL, AUC_{last} = 204 h*ng/mL), and 15.3% oral bioavailability in rats (10 mg/kg p.o., C_{max} = 100 ng/mL, AUC_{last} = 416 h*ng/mL).

Compound **8d** only displayed a K_i of 9.6 μM for mCCR2 binding. Human CCR2 knock-in/murine CCR2 knock-out mice (hCCR2KI) have been adopted to overcome the species issue and validate in vivo models. Compound **8d** was assessed in several pharmacological models using hCCR2KI mice. In a thioglycollate-induced peritonitis (TG) model, it dose-dependently inhibited the influx of leukocytes, monocytes/macrophages and T-lymphocytes into the peritoneal cavity with an ED_{50} of 3 mg/kg p.o. bid. In an OVA-induced asthma model, **8d** inhibited airway eosinophil infiltration by 89% at a dose of 10 mg/kg p.o. bid. The efficacy of **8d** in mouse model suggests that it may have potential as a single agent in asthma to improve signs and symptoms and reduce airway resistance. The detailed pharmacological profile of **8d** will be reported later.

The synthesis of this series is exemplified by compound **8d** as illustrated in Scheme 1. Thiazole **10** was treated with n-BuLi in THF at –78°C and the resulting anion was reacted with commercially available ketone **11** followed by desilylation with TBAF to obtain ketal **12** in 55% yield. De-protection of the ketal group in 1 N HCl acetone at room temperature gave the corresponding ketone **13** in 90% yield. Reductive amination of **13** with **14**[16] using $NaBH(OAc)_3$ gave a pair of *cis-trans* adducts as mixtures, which were separated by silica gel column chromatography to afford *cis* isomer (in terms of thiazole and azetidine moiety) **8d** in 35% yield along with its *trans* isomer (structure not shown) in 40% yield.

In conclusion, we have discovered a novel series of azetidinyl cyclohexanes as potent and selective hCCR2 antagonists. Through divergent SARs of hCCR2 and hERG on the left-end heterocyclic ring, **8d** was identified as the lead compound. It exhibits potent hCCR2 activity, high selectivity, weak hERG affinity and good oral bioavailability. Evaluated in guinea pigs and dogs, **8d** possesses a clean CV safety profile and provides good safety margin as a development candidate.

ASSOCIATED CONTENT

Supporting Information. Experimental procedures for the synthesis of **8d** and characterization data for **2a**-**2o**, **3**-**7**, **8a**-**h**, **9** as well as in vitro and in vivo biological protocols are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest..

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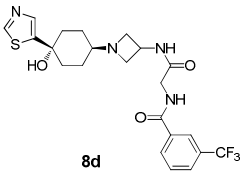

8d

hCCR2 binding: 37 nM
hERG binding: > 50 μM
GPCV: no effect
TG: ED_{50} = 3 mg/kg