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A novel series of *N*-(azetidin-3-yl)-2-(heteroarylamino)acetamide CCR2 antagonists

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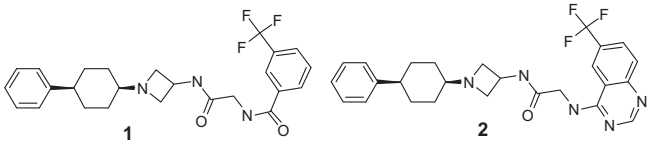
ABSTRACT

The inflammatory response associated with the activation of C–C chemokine receptor CCR2 via it's interaction with the monocyte chemoattractant protein-1 (MCP-1, CCL2) has been implicated in many disease states, including rheumatoid arthritis, multiple sclerosis, atherosclerosis, asthma and neuropathic pain. Small molecule antagonists of CCR2 have been efficacious in animal models of inflammatory disease, and have been advanced into clinical development. The necessity to attenuate hERG binding appears to be a common theme for many of the CCR2 antagonist scaffolds appearing in the literature, presumably due the basic hydrophobic motif present in all of these molecules. Following the discovery of a novel cyclohexyl azetidinylamide CCR2 antagonist scaffold, replacement of the amide bond with heterocyclic rings was explored as a strategy for reducing hERG binding and improving pharmacokinetic properties.

Activation of the C–C chemokine receptor CCR2 via its interaction with the monocyte chemoattractant protein-1 (MCP-1, CCL2) leads to monocyte/macrophage recruitment and the resulting inflammatory response. Studies using rodent models have implicated the inflammatory response associated with MCP-1/CCR2 interaction to many disease states, including rheumatoid arthritis, multiple sclerosis, atherosclerosis, asthma and neuropathic pain.[1–10] Mouse models have also demonstrated that adipose tissue macrophage accumulation via MCP-1 interaction with CCR2 leads to the development of insulin resistance related with obesity. Small-molecule CCR2 antagonist strategies to ameliorate inflammation-mediated disease has been extensively pursued over the last decade and several compounds have been advanced into clinical development.[11] Our CCR2 antagonist program has explored several chemotypes over the last few years. Following our discovery of the (1-cyclohexylazetidin-3-yl)-*N*-acylglycineamide CCR2 antagonist scaffold **1**,[12] an ongoing effort has been to identify heterocyclic replacements for the *N*-acylglycine moiety with the intention of improving the in vivo pharmacokinetic profile. Continuing this amide bond replacement strategy, compound **2** was identified as a potent CCR2 antagonist, but with significant affinity at the hERG channel. Here we describe some of our SAR studies geared towards reducing hERG binding while attempting to maintain a favorable pharmacokinetic profile.



1 **2**

Initially, the cyclohexyl portion of compound **2** was modified while maintaining the rest of the molecule constant. These compounds (Table 1) were synthesized according to Scheme 1. The **commercially** available amine **19** was coupled to Cbz-glycine and subjected to hydrogenolysis to give the amine **20**. 6-(Trifluoromethyl)-quinazolin-4-ol[13] was converted to chloroquinazoline **21**, which was coupled to amine **20** to give compound **27**.

The BOC-group was removed and the resulting amines, **28** were subjected to reductive amination conditions in the presence of various ketones,[12] **29** to give compounds **2**–**18**. The cis and trans isomers were separated by silica column chromatography (8 N NH_3/

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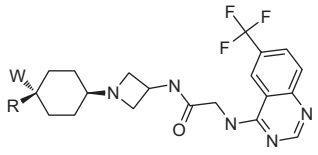
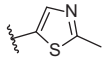
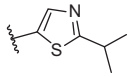
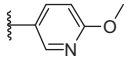
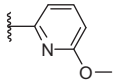
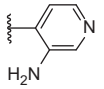
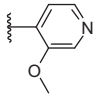
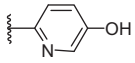
Table 1
Arylcyclohexyl modifications



Compound	R	W	CCR2 binding IC_{50}[a] (nM)	CTX IC_{50}[a,b] (nM)	*hERG* binding IC_{50}[c] (μM)
2		H	15	4	5.2
3		OH	15	18	16.9
4		OH	10	8	>50
5		OH	22	24	28.4
6		OH	40	18	>50
7		OH	27	11	>50
8		OH	22	6	37.5
9		OH	19	4	28.5
10		OH	56	65	13.4
11		H	36	NT[d]	46
12		H	7	2	20.2
13		H	5	7	16.0
14		H	7	6	12.9
15		H	17	5	8.3
16		H	5	2	7.5
17		H	7	2	6.6
18		H	21	43	2.1

[a] IC_{50} values are reported as the average of at least two separate determinations if IC_{50} <100 nM with a typical variation of less than ±25%. MCP-1 receptor binding assay in THP-1 cells (Ref. 14).
[b] MCP-1 induced chemotaxis in THP-1 cells (Ref. 15).
[c] hERG ^{3}H-astemizole binding activity on HEK-293 cell (Ref. 16).
[d] NT = not tested.

MeOH in EtOAc). The trans isomers have marginal potency towards CCR2.

Various heterocyclic replacements for the quinazoline ring (Table 2) were also synthesized. Compounds **31**, **32**, **37** and **38** were synthesized according to Scheme 1, using heteroaryl chlorides, **22**–**25**.[17]

The cyanoquinoline analog **34** was also prepared according to Scheme 1; however instead of displacing a chloride to form the glycine to quinoline bond, the hydroxyl residue on quinoline **26** was activated with bromo-tris-pyrrolidinophosphonium-hexafluoro-phosphate (PyBrOP) to form a phosphonium leaving group.[18] Chloro quinazolines **22** and **23** were prepared by treating the corresponding quinazolinone with oxalyl chloride and catalytic DMF. Phthalazine, **24** was synthesized according to Scheme 2. Lactone **39** was nitrated and reduced to give the aniline **40**, which was diazotized in the presence of KI to give the iodide **41**. Copper catalyzed trifluoromethylation of **41** gave compound **42**. Compound **42** was brominated and heated in the presence of hydrazine to give the phthalazine **44**, which was converted to the chloride **24**.

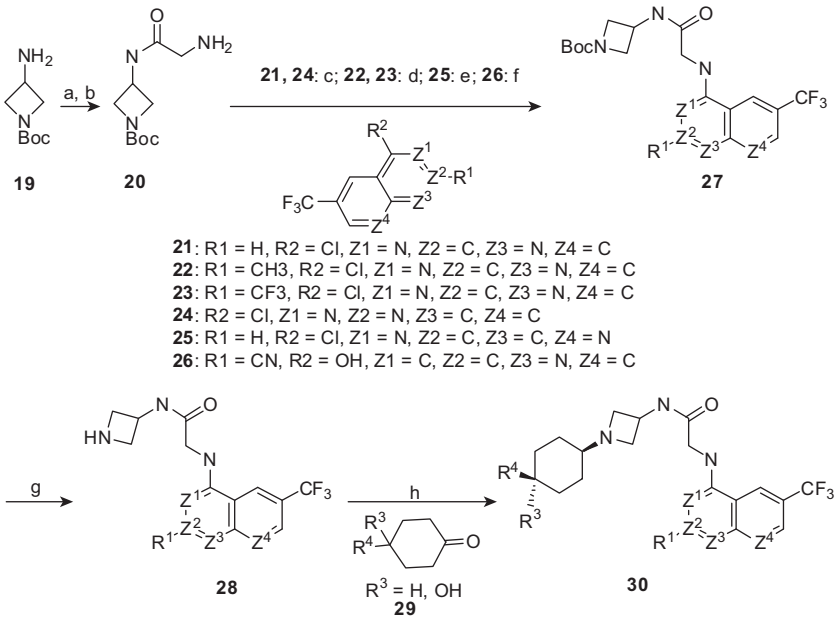
Dihydronaphthyridine **45** (Scheme 3) was oxidized to the lactam, **46**. DDQ oxidation gave 1,6-naphthyridinone **48**, which was converted to the chloro naphthyridine **25**. Cyanoquinoline **26** was prepared according to Scheme 4. Commercially available 4-hydroxyl quinoline ester **49** was protected as the *p*-methoxybenzyl ether and converted to the amide **51**.

Dehydration of **51** with trifluoroacetic anhydride provided the nitrile **52**, which was deprotected to give 4-hydroxy cyanoquinoline **26**. Compound **33** was prepared according to Scheme 5. Reductive amination of ketone **53** with Boc-aminoazetidine, followed by deprotection gave amine **55**, which was coupled to Boc-gly to give compound **56**. Compound **56** was deprotected and coupled to 4-hydroxyquinoline, **58** to give compound **33**.

Compound **35** was synthesized according to Scheme 6. 4-Aminoquinoline **59** was CBz-protected and the carbamide was alkylated with bromoacetic acid to give compound **61**. The ester was hydrolyzed and the acid was coupled to 1-Boc-amino azetidine to give the amide **62**. Both protecting groups were removed and the resulting azetidine amine was coupled to the ketone **53** give compound **35**. Chloroisoquinoline[20] **64** (Scheme 7) was converted to the iodide **56** by halogen metal exchange followed by treatment with iodine. Copper catalyzed trifluoromethylation gave compound **66**, which was converted to the chloroisoquinoline **67**. Initially an unsuccessful attempt was made to couple chloroisoquinoline **67** to amine **20** in a manner similar to that in Scheme 1, which led us to the rather lengthy route to compound **36** described here.

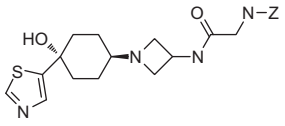
Chloroisoquinoline **67** was converted to the phenoxy derivative by heating in molten phenol in the presence of KOH. The phenoxide **68** was heated in molten ammonium acetate to give the aminoisoquinoline **69**. The amine was protected as its Cbz derivative and alkylated with bromoacetic acid methyl ester. Methyl ester was hydrolyzed and the acid was coupled to amine **55**. Deprotection via catalytic hydrogenation gave compound **36**.

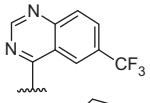
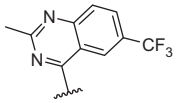
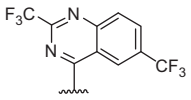
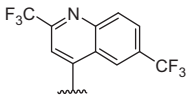
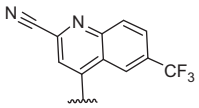
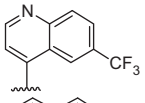
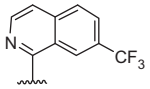
Our previous work[12] had suggested that introducing a hydroxyl residue on the cyclohexane ring as in compound **3** attenuated hERG binding in cyclohexylazetidinyl CCR2 inhibitors. While compound **3** did have reduced hERG binding, increasing hydrophilicity by replacing the phenyl ring with various thiazoles (**4**, **6**, **7**) and isothiazole **5** resulted in a much more significant reduction in hERG binding while maintaining good CCR2 potency. Increasing the hydrophobicity of the thiazole ring (**8**), results in an increase in hERG binding. However, comparing compounds **6**, **7** and **8** lipophilic substitutions at the 2-possition of the thiazole ring does seem to improve CCR2 potency. While 2-methoxypyridin-5-yl substitution (**9**) also reduced hERG binding significantly, 2-methoxypyridin-6-yl substitution (**10**) was comparable to phenyl substitution. Although thiazole carbinol analogs (**4**, **6**, **7**) provided the necessary reduction in hERG binding while maintaining good potency their pharmacokinetic properties were not optimal. Based on our observation that des-hydroxy analogs in this series had



Scheme 1. Reagents and conditions: (a) CbzGly-OH, DIEA, EDCI, HOBT; (b) Pd/C H_2; (c) TEA, iPrOH, 90 °C, 1 h; (d) TEA, THF, reflux 18 h; (e) TEA, diglyme, 120 °C 18 h; (f) PyBrOP, TEA; (g) 50% TFA, DCM; (h) $Na(OAc)_3BH$, DCM.

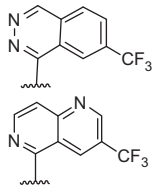
Table 2
Heterocyclic amide bond replacements



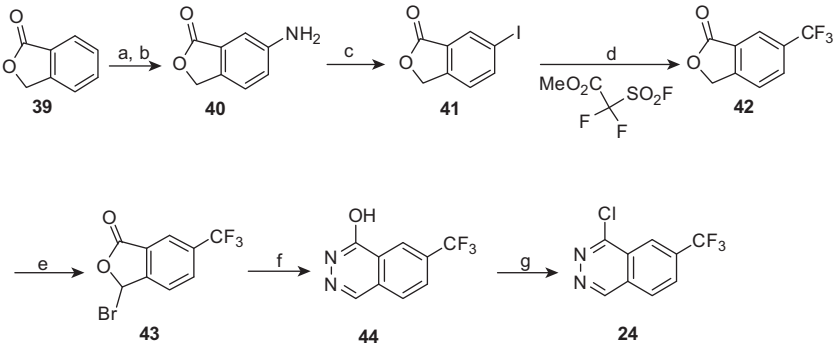
Compound	Z	CCR2 binding IC_{50}[a] (nM)	CTX IC_{50}[a,c] (nM)	*hERG* binding IC_{50}[d] (μM)	*hERG* patch[b] % inh @ 3 μM (sol con.)
6		40	18	>50	10.8 ± 2.1 (6.5 ± 3.9)
31		8	7	25.7	NT[e]
32		39	17	32.7	NT
33		50	64	36.1	25(17)
34		23	25	35.1	9.6 ± 1.9 (6.7 ± 3.9)
35		72	59	11.2	NT
36		20	8	32.9	NT
37		19	NT	NT	12.4 ± 2.4(7.5 ± 3.7)

(*continued on next page*)

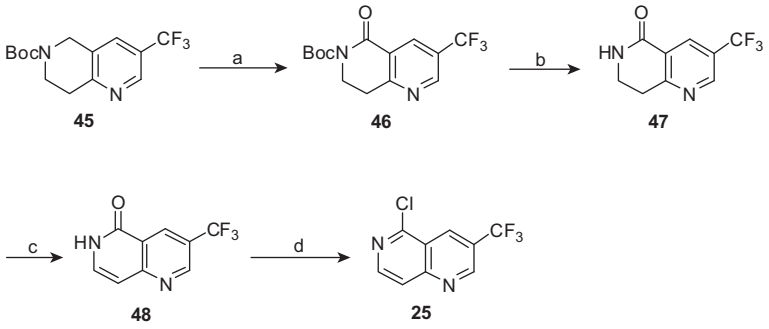
Table 2 (*continued*)

Compound	Z	CCR2 binding IC_{50}[a] (nM)	CTX IC_{50}[a,c] (nM)	*hERG* binding IC_{50}[d] (μM)	*hERG* patch[b] % inh @ 3 μM (sol con.)
38		38	NT	>50	8.5 ± 1.7 (7.5 ± 3.7)

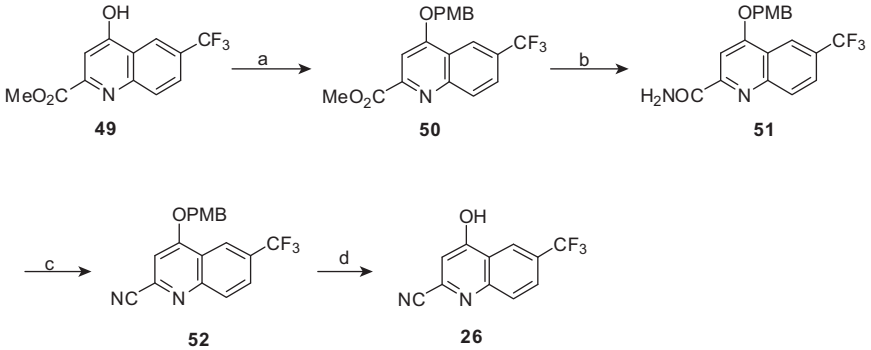
[a] IC_{50} values are reported as the average of at least two separate determinations if IC_{50} <100 nM with a typical variation of less than ± 25%.
[b] The membrane K+ current IKr in HERG-transfected HEK293 cells (solvent control), see Ref. 19.
[c] MCP-1 induced chemotaxis in THP-1 cells (Ref. 15).
[d] hERG ^{3}H-astemizole binding activity on HEK-293 cell (Ref. 16).
[e] NT = not tested.



Scheme 2. Reagents and conditions: (a) H_2SO_4/KNO_3; (b) Fe, AcOH; (c) $NaNO_2$/HCl, KI; (d) CuI, DMF, 100 °C, 18 h; (e) NBS, AIBN, CCl_4, 65 °C, 3 h; (f) NH_2NH_2, EtOH, 60 °C, 18 h; (g) $POCl_3$, 100 °C, 2 h.



Scheme 3. Reagents and conditions: (a) CCl_4/CH_3CN (10:1), $NaIO_4$ aq, $RuCl_3.H_2O$, 12 h; (b) TFA, DCM; (c) DDQ, 100 °C, 6 h; (d) $POCl_3$, CH_3CN, 90 °C, 2 h.



Scheme 4. Reagents and conditions: (a) Cs_2CO_3, *p*-MeO-$PhCH_2Cl$; (b) NH_3; (c) TFAA, TEA; (d) TFA, DCM.

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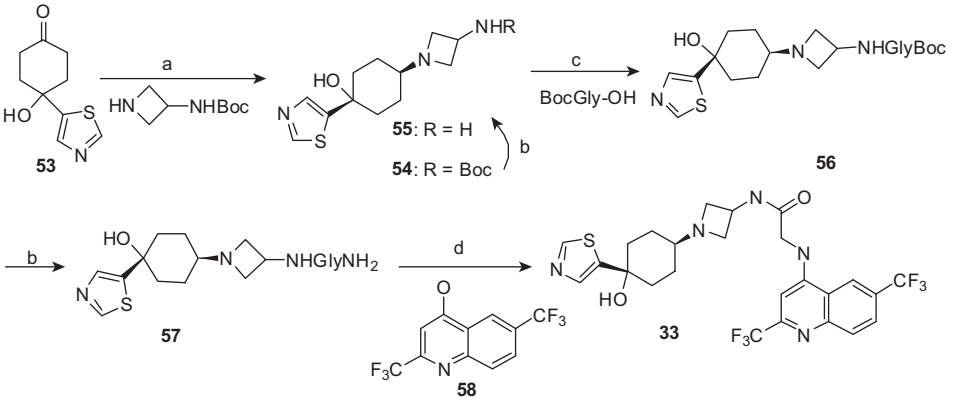
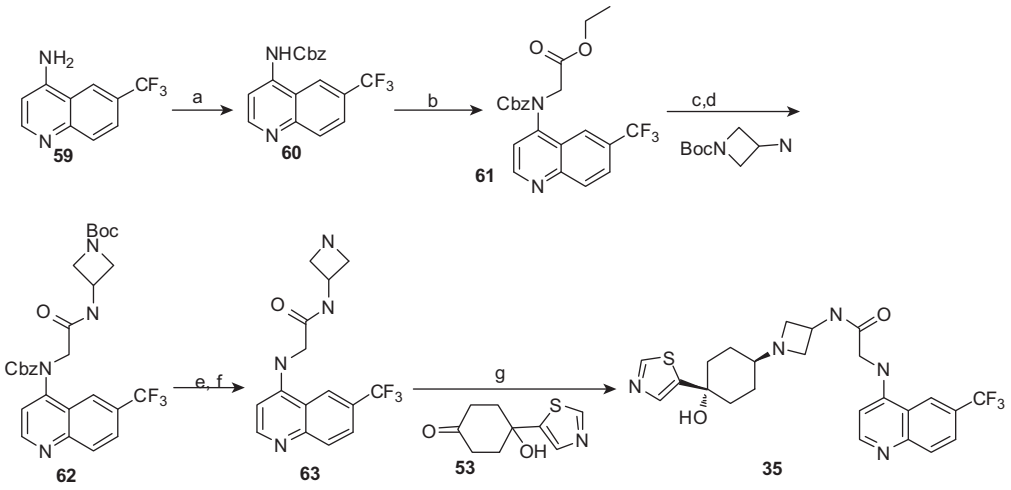
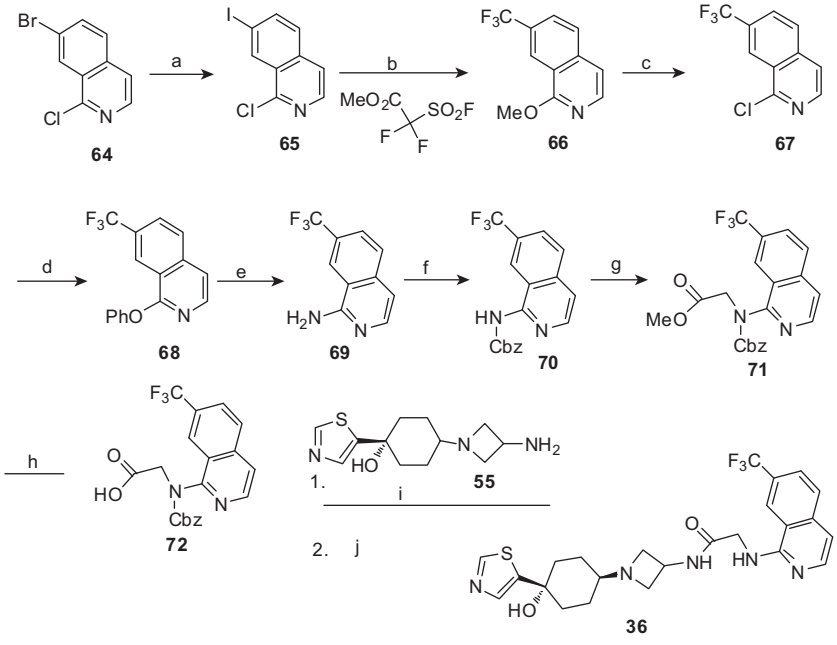


Scheme 5. Reagents and conditions: (a) $Na(OAc)_3BH$, DCM; (b) TFA, DCM; (c) EDCI, HOBT, TEA, DCM; (d) PyBrOP, DIEA, dioxane, rt, 3 days.



Scheme 6. Reagents and conditions: (a) CbzCl, $NaHCO_3$; (b) Methyl bromoacetate, Cs_2CO_3, DMF, rt, 18 h; (c) LiOH, THF/MeOH, 3 h; (d) EDCI, HOBT, TEA, DCM, 18 h; (e) Pd/C H_2; (f) TFA, DCM; (g) $Na(OAc)_3BH$, NMM, DCM.



Scheme 7. Reagents and conditions: (a) nBuLi, I_2; (b) CuI, DMF, 100 °C, 18 h; (c) $POCl_3$, 150 °C, 18 h; (d) PhOH, KOH, 100 °C; (e) NH_4OAc, 160 °C; (f) CbzCl, NMM; (g) Methyl bromoacetate, NaH; (h) NaOH; (i) EDC, HOBT, TEA; (j) Pd/C H_2.

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better PK properties than their hydroxyl counterparts we examined the possibility of identifying des-hydroxy analogs with reduced hERG binding by replacing the phenyl ring in compound **2** with more hydrophilic aryl residues. Out of the several pyridyl, amino and hydroxyl-pyridyl analogs synthesized (**11**–**16**), only the 3-aminopyridyl analog **11** showed significant reduction in hERG binding. Hydrophilic phenyl substitutions such as 2-hydroxy, (**17**) or 2-cyano (**18**) did not produce analogs with significantly reduced hERG binding.

Reduced hERG activity for compound **6** was confirmed using a hERG patch clamp study. Minimal Inhibition of hERG-mediated K+ current in HEK293 cells was observed at 3 μM (Table 2). Reducing hydrophilicity and basicity were considered as two potential approaches to improve the poor oral bioavailability of compound **6**. However, we were aware that increasing lipophilicity within this series could result in greater hERG affinity.

Using compound **6** as a template with significantly reduced hERG binding and good CCR2 potency we continued to explore various heterocyclic replacements for the quinazoline ring (Table 2). These compounds had good CCR2 binding and functional activity. Increased lipophilicity due to the 2-methyl substitution on the quinazoline ring (**31**), while improving CCR2 potency, increased hERG binding as well. Introducing an electron withdrawing trifluoromethyl group that is also lipophillic instead of the 2-methyl residue provides reduced hERG binding, possibly due to reduced basicity of the quinazoline ring.[21] Replacing the quinazoline ring with a similarly substituted quinoline ring provides comparable hERG binding. In comparison with the qinazoline analog **6**, compound **33** has significantly increased inhibition in the hERG patch assay. Replacing the trifluoromethyl residue with a cyano group provides a compound (**34**) with good potency and similar hERG binding to compound **33**, but with significantly reduced inhibition in the patch clamp assay.

Table 3
In vivo rat pharmacokinetic parameters for compounds **6** and **34**

Compound	$t_{1/2}$	AUC_{last} (h ng/mL)	Vss (L/kg)	Cl (mL/min/kg)	*F* (%)
6	1.8	10.6	8.5	89	0.3
34	1.8	6.0	13.0	148	0.5

IV: 2 mg/kg in 20% HPβCD; PO: 10 mg/kg in 0.5% Methocel.

Quinoline analog **35** has increased hERG binding compared to the quinoline analogs **33**–**34**, and quinazoline **6**. Here again increased basicity[21] of the unsubstituted quinoline might be a contributing factor. In contrast the less basic isoquinoline **36**, has significantly reduced hERG binding. Compared to quinazoline **6**, the more basic quinoline **35** has significantly reduced CCR2 binding and functional activity, where as the less basic isoquinoline **36** has improved binding and functional CCR2 potency.

While reducing the basicity of the quinoline ring by introducing a trfluoromethyl residue (**33**) has little effect on CCR2 potency, 2-cyano substitution (**34**) significantly improves both CCR2 binding and functional potency. The more hydrophilic and less basic phthalazine **37** and naphthyridine **38**, with comparable CCR2 activity to quinazoline **6**, are clean in the hERG patch assay.

Compound **34** was evaluated for efficacy in a thioglycollate-induced peritoneal leukocyte infiltration model in mouse CCR2 knock-out/human CCR2 knock-in mice[22] (Fig 1). When compound **34** was dosed orally at 3, 10 and 30 mpk, a dose dependent inhibition of peritonitis was observed. At the 30 mpk dose >50% inhibition of T-lymphocyte, Monocyte/macrophage and total leukocyte infiltration was observed.

In summary, we have evaluated a series of heterocyclic replacements for the aryl amide potion of (1-cyclohexylazetidin-3-yl)-*N*-acylglycineamide, CCR2 antagonist. While compounds devoid of

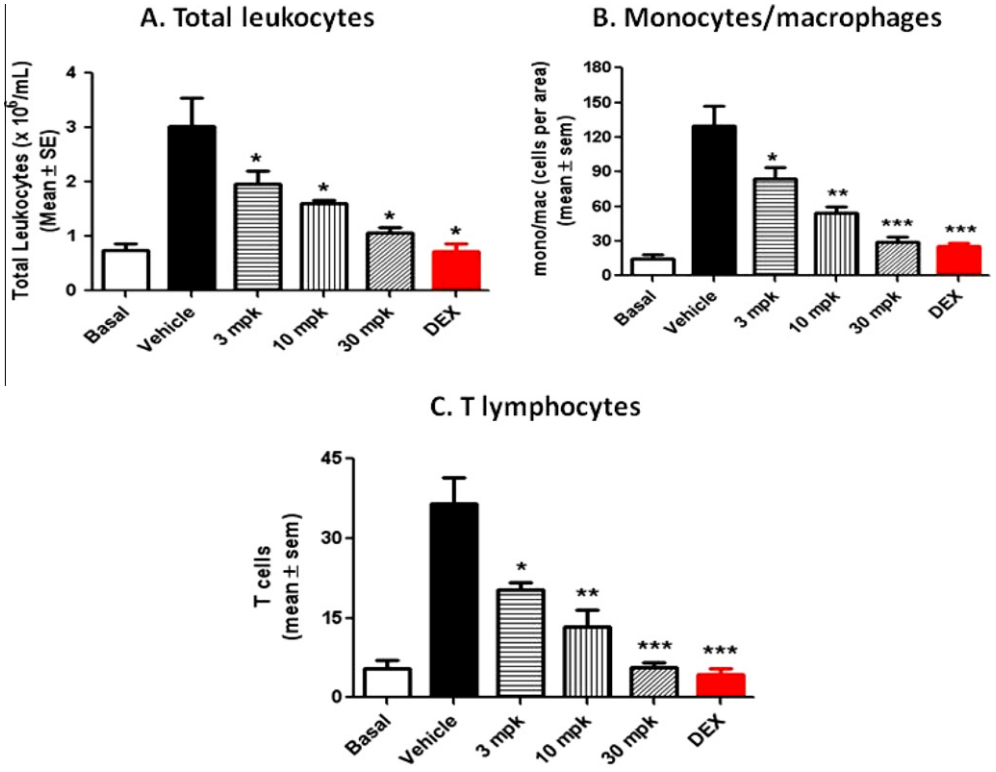

Figure 1. Effect of compound **34** on thioglycollate-induced peritoneal leukocyte infiltration in hCCR2KI mice (Ref. 22). Compound **34** dosed po, BID. Positive control dexamethasone (DEX) dosed 5 mg/kg, po, QD. Statistical significance was determined using unpaired *t*-test. In all analyses, p <0.05 was taken to indicate statistical significance. $^{*}p$ <0.05, $^{**}p$ <0.01 and $^{***}p$ <0.001 versus control.

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hERG activity and with good CCR2 binding and functional activity were identified (**6**, **34**, **37**, **38**), none of these compounds possessed a suitable pharmacokinetic profile (Table 3) for further advancement.

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14. MCP-1 receptor binding assay in THP-1 cells: THP-1 cells were obtained from American Type Culture Collection (Manassas, VA, USA). The THP-1 cells were grown in RPMI-1640 supplemented with 10% fetal bovine serum in a humidified 5% CO_2 atmosphere at 37 °C. The cell density was maintained at 0.5 × 106 cells/mL. THP-1 cells were incubated with 0.5 nM 125I labeled MCP-1 (Perkin–Elmer Life Sciences Inc., Boston, MA) in the presence of varying concentrations of either unlabeled MCP-1 (R&D Systems, Minneapolis, MN) or test compound for 2 h at 30 °C in a 96 well plates. Cells were then harvested onto a filter plate, dried, and 20 μL of Microscint 20 was added to each well. Plates were counted in a TopCount NXT, Microplate Scintillation & Luminescence Counter (Perkin–Elmer Life Sciences Inc., Boston, MA, USA). Blank values (buffer only) were subtracted from all values and drug treated values were compared to vehicle treated values. 1 μM cold MCP-1 was used for nonspecific binding.
15. MCP-1 induced chemotaxis in THP-1 cells: MCP-1 induced chemotaxis was run in a 24-well chemotaxis chamber. MCP-1 (0.01 μg/mL) was added to the lower chamber and 100 μL of THP-1 cells (1 × 107 cell/mL) was added to the top chamber. Varying concentrations of test compound were added to the top and bottom chambers. Cells were allowed to chemotax for 3 h at 37 °C and 5% CO_2. An aliquot of the cells which had migrated to the bottom chamber was taken and counted then compared to vehicle.
16. hERG [3H]-astemizole binding experiment: this assay is a 384well in-plate vacuum filtration binding assay. Assay reagents are added into a prepared/blocked 384 well assay plate in the following order: (1) hERG membrane diluted in assay buffer; (2) test compound; and (3) 3H astemizole diluted in assay buffer. Assay reagents are incubated in the filter plate for 1 h and then washed six times with ice-cold wash buffer. Plates are allowed to dry overnight at room temperature. The following morning, plates are sealed and scintillant is added to each well. Following a 2 h incubation with scintillant, plates are placed on the TopCount and counted 1 min per well. Data is calculated using raw CPM. Where applicable, IC_{50} values are calculated using raw CPM values. Curves are fitted individually from singlet 11 point dosing curves +1% DMSO control.
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19. Patch express experiment: experiments were performed using HEK293 cells stably expressing the HERG potassium channel. Cells were grown at 37 °C and 5% CO_2 in culture flasks in MEM medium supplemented with 10% heat inactivated fetal bovine serum, 1% L-glutamine–penicillin–streptomycin solution, 1% nonessential amino acids (100x), 1% sodium pyruvate (100 mM) and 0.8% geneticin (50 mg/ml). Before use the cells were subcultured in MEM medium in the absence of 5 ml L-glutamine–penicillin–streptomycin. For use in the automated patch-clamp system PatchXpress 7000A (Axon Instruments) cells were harvested to obtain cell suspension of single cells. Extracellular solution contained (mM): 150 NaCl, 4 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 HEPES, five glucose (pH 7.4 with NaOH). Pipette solution contained (mM): 120 KCl, 10 HEPES, 5 EGTA, 4 ATP–Mg_2, 2 $MgCl_2$, 0.5 $CaCl_2$ (pH 7.2 with KOH). Patch-clamp experiments were performed in the voltage-clamp mode and whole-cell currents were recorded with an automated patch-clamp assay utilizing the PatchXpress 7000A system (Axon Instruments). Current signals were amplified and digitized by a Multiclamp amplifier, stored and analyzed by using the PatchXpress, DataXpress software and Igor 5.0 (Wavemetrics). The holding potential was −80 mV. The HERG current (K+-selective outward current) was determined as the maximal tail current at −40 mV after a 2 s depolarization to +60 mV. Pulse cycling rate was 15 s. Before each test pulse a short pulse (0.5 s) from the holding potential to −60 mV was given to determine (linear) leak current. After establishing whole-cell configuration and a stability period, the vehicle was applied for 5 min followed by the test substance by increasing concentrations of 3×10^{-6} M, 10^{-5} M and 3×10^{-5} M. Each concentration of the test substance was applied twice. The effect of each concentration was determined after 5 min as an average current of three sequential voltage pulses. To determine the extent of block the residual current was compared with vehicle pre-treatment. Data are presented as mean values ± standard error of the mean (SEM).
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21. Calculated (measured) Pka of heterocycles: quinazolin-4-amine = 4.84 (5.73); 2-methylquinazolin-4-amine = 5.76; 2-(trifluoromethyl)quinazolin-4-amine = 3.06; isoquinolin-1-amine = 4.22 (7.62, Guenter Grethe, Isoquinolines, Vol. 1, John Wiley & Sons, Feb 20, 1981); quinolin-4-amine = 9.07 (9.08); 2-(trifluoromethyl)quinolin-4-amine = 6.35; 4-aminoquinoline-2-carbonitrile = 5.34.
22. Thiolycollate-induced peritonitis in mice: animals were intraperiponeally injected with sterile thioglycollate (25 mL/kg, ip, Sigma) for induction of peritonitis. Animals were orally dosed with vehicle or CCR2 antagonists at 3, 10 or 30 mg/kg twice daily. At the 72-hour time point, peritoneal cavities were lavaged with 10 mL of sterile saline. Total cell counts in the peritoneal lavage fluid were performed using a microscope and cell differentiation was performed using cytospinanalysis after Giemsa staining (Hema Tek 2000). Percent inhibition of the thioglycollate induced peritonitis was calculated by comparing the change in number of leukocytes of CCR2 antagonist treated mice to the vehicle-treated mice.