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Design and synthesis of novel small molecule CCR2 antagonists: Evaluation of 4-aminopiperidine derivatives

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ABSTRACT

A novel *N*-(2-oxo-2-(piperidin-4-ylamino)ethyl)-3-(trifluoromethyl)benzamide series of human CCR2 chemokine receptor antagonists was identified. With a pharmacophore model based on known CCR2 antagonists a new core scaffold was designed, analogues of it synthesized and structure–affinity relationship studies derived yielding a new high affinity CCR2 antagonist *N*-(2-((1-(4-(3-methoxyphenyl)cyclohexyl)piperidin-4-yl)amino)-2-oxoethyl)-3-(trifluoromethyl)benzamide.

The CC chemokine CCL2, through its interaction with the CCR2 G protein-coupled receptor, plays an important role in the recruitment of monocytes, natural killer cells, dendritic cells and T-lymphocytes.[1] Research on CCL2 knockout (KO) and CCR2 KO mice suggests that inhibition of the CCL2/CCR2 axis may be beneficial in the treatment of inflammatory diseases.[2] The pair is thought to be involved in atherosclerosis,[3] insulin resistance,[4] multiple sclerosis,[5] neuropathic pain[6] and asthma.[7] Different in vitro and in vivo models have shown the usefulness of small molecule CCR2 antagonists to inhibit the chemotactic response of CCL2.[8–10] Consequently, the pharmaceutical industry has devoted considerable efforts to the development of CCR2 antagonists to combat these diseases. A vast number of different scaffolds used in the design of CCR2 antagonists has been described.[11,12]

However, the bulk of these antagonists share the same structural motifs: a basic nitrogen atom in the center, flanked by two aromatic rings of which one is connected to the nitrogen atom with an amide containing linker and the other with an aliphatic linker (Fig. 1). In some cases the latter aromatic ring is missing and only the aliphatic group is left on one side.[12] Usually, the central

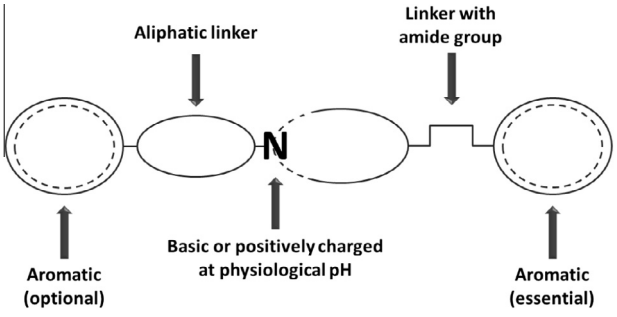

Figure 1. Pharmacophore of CCR2 antagonists.

Abbreviations: AcOH, acetic acid; Boc, *tert*-butyloxycarbonyl; CCL2, chemokine ligand 2; CCR2, chemokine receptor 2; DCE, dichloroethane; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; DMAP, *N*,*N*-dimethylaminopyridine; DMSO, dimethylsulfoxide; INCB3344, *N*-(2-(((3*S*,4*S*)-1-(4-(benzo[*d*][1,3]dioxol-5-yl)-4-hydroxycyclohexyl)-4-ethoxypyrrolidin-3-yl)amino)-2-oxoethyl)-3-(trifluoromethyl)benzamide; JNJ Lead, *N*-(2-((1-((1*R*,4*R*)-4-(3-(dimethylamino)phenyl)-4-hydroxycyclohexyl)azetidin-3-yl)amino)-2-oxoethyl)-3-(trifluoromethyl)benzamide; KO, knockout; KOPh, potassium phenolate; LDA, lithium diisopropylamide; Lit-BuO, lithium *tert*-butoxide; MeOH, methanol; MW, microwave; MS, molecular sieves; $Pd_2(dba)_3$, tris(dibenzylideneacetone)dipalladium(0); $Pd(dppf)Cl_2$, [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane; $Pd(OAc)_2$, palladium acetate; $Pd(PPh_3)_4$, tetrakis(triphenylphosphine)palladium(0); PEMB, 5-ethyl-2-methyl-pyridine borane; PPh_3, triphenylphosphine; PyBroP, bromo-tris-pyrrolidino phosphoniumhexafluorophosphate; SAR, structure–affinity relationships; TFA, trifluoroacetic acid; THF, tetrahydrofuran; U2OS, human bone osteosarcoma cells; XPhos, 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl.

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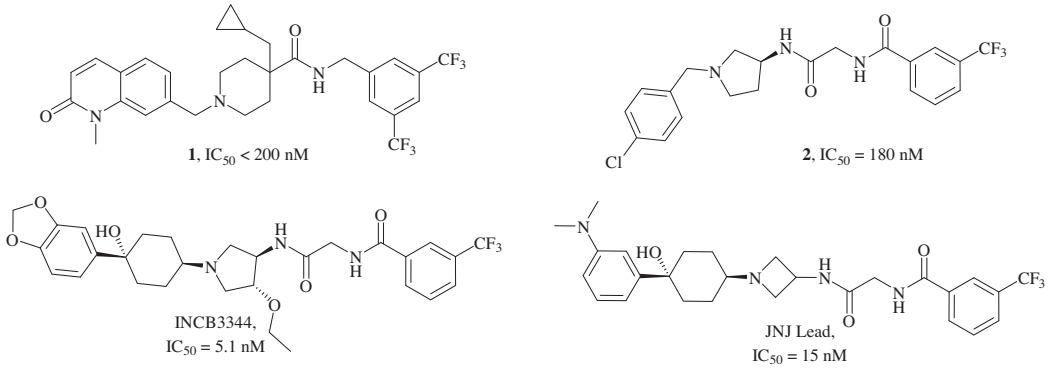
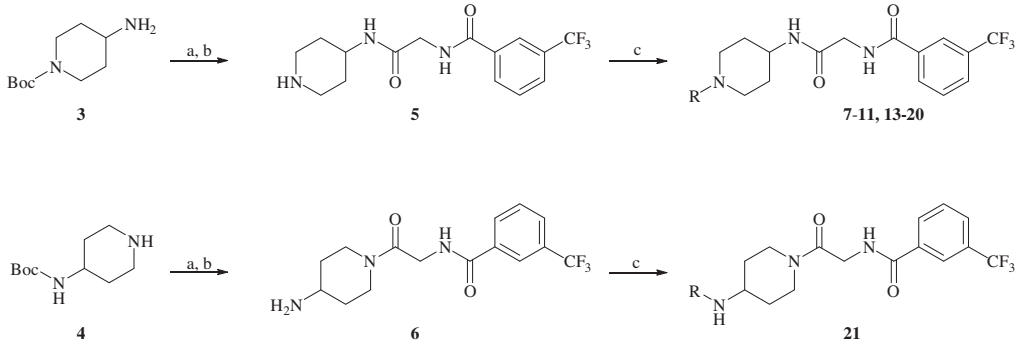
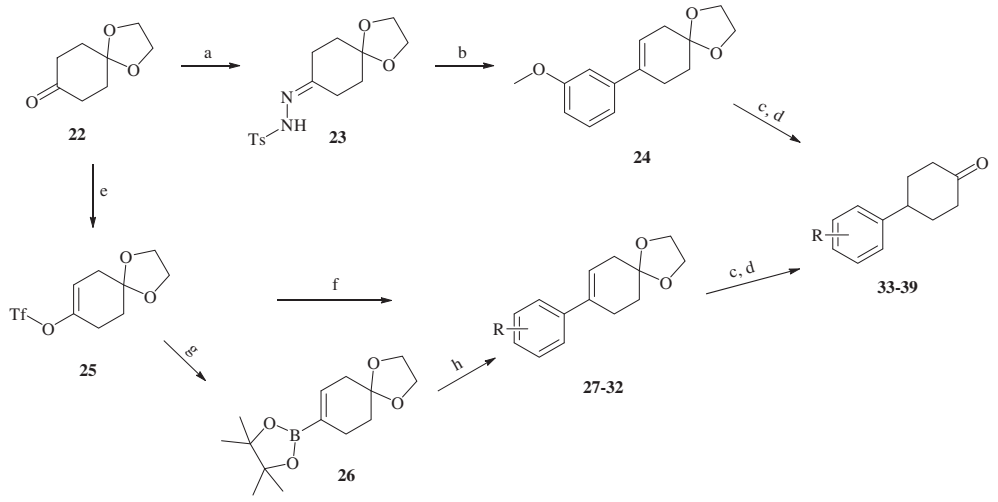


Figure 2. Known CCR2 antagonists.



Scheme 1. Reagents and conditions: (a) PyBroP, DIPEA, DMAP, *N*-(3-(trifluoromethyl)benzoyl)glycine, DCM, MS 4 Å, room temperature; (b) dry 3 M HCl in MeOH, room temperature, yield in two steps: 57–89%; (c) corresponding aldehyde or ketone, $Na(AcO)_3BH$, AcOH, DCE, room temperature, (2–64%).

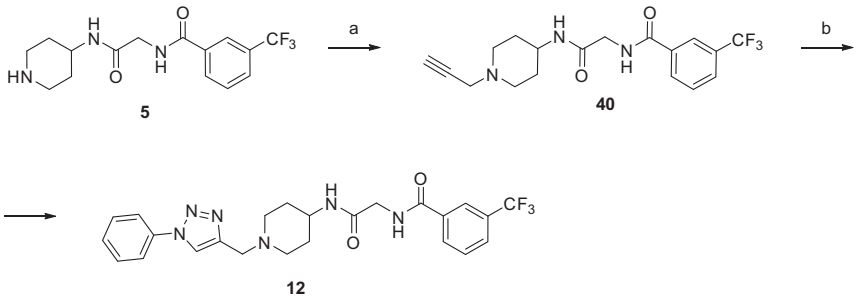


Scheme 2. Reagents and conditions: (a) *p*-toluenesulfonhydrazide, dioxane, 130 °C, 45 min, MW, (65%); b) $Pd_2(dba)_3$, XPhos, Li*t*-BuO 1.0 M in hexanes, dioxane, 110 °C, 2 h, MW, (24%); (c) Pd/C 10% wt, $Pd(OAc)_2$, MeOH, H_2, room temperature, 4–12 h, (85–99%); (d) $FeCl_3{\cdot}6H_2O$, acetone, DCM, 5–12 h, room temperature, (42–99%); (e) (i)1.3 equiv LDA, THF, under N_2, −78 °C → −25 °C, 2 h, cool down to −78 °C; (ii) *N*-phenyl-bis(trifluoromethanesulfonimide), −78 °C → room temperature, 24 h, (80%); (f) 3,4-(methylenedioxy)phenylboronic acid, KF, $Pd(dppf)Cl_2$, room temperature overnight, (58%); (g) $PdCl_2$, PPh_3, bis(pinacolato)diboron, KOPh, toluene, under N_2, 4 h at 50 °C, 24 h at room temperature, (59%); (h) $Pd(PPh_3)_4$, corresponding arylhalogen, Na_2CO_3 2 M in H_2O, dioxane, under N_2, 80 °C, 5 h, MW, (80–99%).

nitrogen is part of an aliphatic heterocycle (e.g., piperidine, **1**[13] pyrrolidine, **2**[14] and INCB3344[15] or azetidine, JNJ Lead[16]) (Fig. 2).

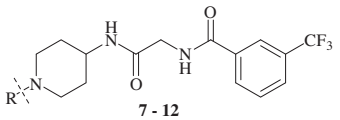
In this Letter, we describe our efforts towards the identification of a new class of CCR2 receptor antagonists. Using the structural knowledge of CCR2 antagonists as in Figure 2 we generated hybrid scaffolds based on a piperidine ring. We explored different linkers between the basic nitrogen and aromatic groups as well as different substituents on the aromatic group. All compounds were evaluated in a ^{125}I-CCL2 displacement assay on a human bone osteosarcoma (U2OS) cell membrane preparation expressing CCR2 as described previously by our group.[17]

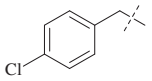
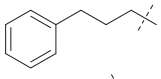
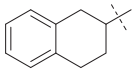
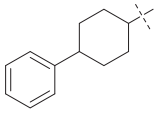
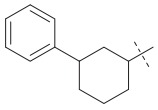
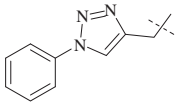
The synthetic methods to arrive at these compounds are depicted in Schemes 1–3. The commercially available *N*-Boc-protected piperidineamines **3** and **4** were used in a peptide-coupling reaction with *N*-(3-(trifluoromethyl)benzoyl)glycine under bromo-tris-pyrrolidino phosphoniumhexafluorophosphate



Scheme 3. Reagents and conditions: (a) propargyl bromide, K_2CO_3, acetone, reflux overnight, (99%); (b) iodobenzene, proline, Na_2CO_3, NaN_3, ascorbic acid, $CuSO_4{\cdot}5H_2O$, DMSO/H_2O 3:1, 80 °C, 48 h, (4%).

Table 1
CCR2 affinities of compounds **7–12**



R	Nr.	K_i, (nM) ± SEM (n = 3)[a]
	**7**	0%
	**8**	6%
	**9**	24%
	**10**	74 ± 9
	**11**	10%
	**12**	12%

[a] Human CCR2 binding affinity in [^{125}I]CCL2 assay or % displ. at 1 μM of [^{125}I]CCL2 binding.

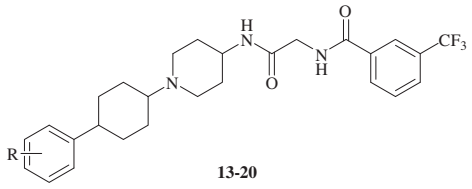
(PyBroP) conditions.[18] Subsequent removal of the Boc-protecting group with dry HCl in methanol produced the free amines **5** and **6**. These amines were used in reductive amination reactions with different aldehydes and ketones to yield the desired products **7–11**, **13–21** (Scheme 1).

For the synthesis of the desired ketones we first used a synthetic route via hydrazone intermediates (Scheme 2) under conditions described by Barluenga et al.[19] Commercially available ketone **22** was reacted with tosylhydrazide to generate hydrazone **23**, which was used subsequently in a Pd-catalyzed cross-coupling reaction to generate **24** with moderate yield. However, attempts to use this method with other substituents on the phenyl ring resulted in very poor yields or no product at all. Another synthetic route was therefore chosen to yield the desired ketones. The acetal protected cyclohexanone **22** was deprotonated with lithium diisopropylamide (LDA) and reacted with *N*-phenyl-bis(trifluoromethanesulfonimide) to generate triflate **25**. This compound was used directly in a Suzuki-coupling with 3,4-methylenedioxy***phenylboronic acid, however, to introduce other substituents we decided to transform the triflate into boronic ester **26** with bis(pinacolato)diboron. This allowed us to use a wider range of arylhalogens as coupling partners in the Suzuki-coupling to eventually generate the desired intermediates with good overall yields. Subsequently, reduction of the double bond and removal of the acetal protecting group yielded the desired ketones **33–39**, which were used in reductive amination reactions to yield the final compounds.

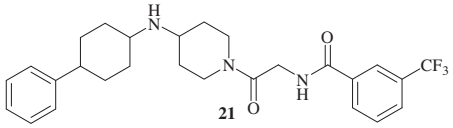
To explore the influence of a methylenetriazole group as a linker between the piperidine and phenyl moieties we used click chemistry. First, we alkylated the piperidine of compound **5** with propargyl bromide to generate compound **40**, which was used in a further reaction with sodium azide and iodobenzene in the presence of proline, ascorbic acid and $CuSO_4$ as described by Feldman et al.[20]

As mentioned before we combined the different scaffolds from two known CCR2 antagonists (compound **1** of Epix Delaware;[13] compound **2** from Tejin[14]) to generate a hybrid scaffold by transfecting the *N*-(3-(trifluoromethyl)benzoyl)glycine part onto the piperidine ring. We argued that the expansion of the central ring to piperidine (compared to INCB3344 and JNJ Lead) might have a minor effect only on the configuration of the molecule. However, the 4-chlorobenzyl group (compound **7**) which had yielded good affinity in combination with the pyrrolidine scaffold[14] (compound **2**), provided no affinity in the case of piperidine (Table 1). Extending the linker to propyl (compound **8**) had a minor effect on the affinity and the rigidification of the linker into tetrahydronaphthalene (compound **9**) yielded negligible improvement (displacement at 1 μM concentration of 6% and 24%, respectively). However, separation of the rings into a 4-phenylcyclohexyl group (compound **10**) resulted in a boost of affinity (K_i = 74 nM). To explore the correct location of the phenyl ring we moved it to the 3 position on the cyclohexane ring (compound **11**), which resulted in a complete loss of affinity. In addition, the cyclohexane's exchange to methylenetriazole as a linker (compound **12**) did not yield any affinity either. Apparently, the distance, 3D orientation and lipophilicity provided by the cyclohexane moiety is just right for the binding of these molecules to the CCR2 receptor and any deviation from it results in complete loss of affinity. This could also be the reason why the 4-aryl-cyclohexane motif is used in so many pyrrolidine[21,22] and azetidine[16,23] derivatives (e.g., INCB3344, JNJ Lead, see Fig. 2). We continued the SAR studies with different substituents on the phenyl ring of the 4-phenyl-cyclohexyl group. Introduction of a methyl group on different positions indicated that substitution on the 2 and 4 positions (compounds **13** and **15**) decreased the affinity (Table 2). The 3 position can tolerate substitution, albeit with a slight decrease in affinity (**14**, K_i = 270 nM). Changing the methyl to methoxy resulted in a regain of the affinity (compound **16**, K_i = 66 nM) pointing to a possible H-bond formation in the receptor binding pocket. However, insertion of two

Table 2
CCR2 affinities of compounds **13-21**



13-20



21

Nr.	R	K_i, (nM) ± SEM (n = 3)[a]
13	2-Me	26%
14	3-Me	270 ± 20
15	4-Me	38%
16	3-OMe	66 ± 12
17	3,5-Di-OMe	41%
18	2,6-Di-OMe	42%
19	4-OH	139 ± 35
20	3,4-OCH_2O–	90 ± 18
21	—	31%

[a] Human CCR2 binding affinity in [^{125}I]CCL2 assay or % displ. at 1 μM of [^{125}I]CCL2 binding.

methoxy groups on either the 3,5 or 2,6 positions (compounds **17** and **18**) yielded a decrease in affinity (displacement of 41% and 42%, respectively). Inserting a hydroxyl group on the 4 position (compound **19**) was tolerated with a twofold affinity decrease compared to **10**. Combining substituents of **16** and **19** into a 3,4-methylendioxy group retained the affinity (compound **20**, K_i = 90 - nM) which is in accordance with observations from the pyrrolidine[21] and azetidine[16] series. Finally, we wanted to explore the possibility of reversing the piperidine ring (compound **21**) in the same fashion as it was described for pyrrolidines[24] where it had only minimal effect on the binding affinity. However, in our case of the piperidine moiety such reversal substantially decreased the affinity for the CCR2 receptor.

In conclusion, we have synthesized a novel series of *N*-(2-oxo-2-(piperidin-4-ylamino)ethyl)-3-(trifluoromethyl)benzamide derivatives and compounds substituted with 4-arylcyclohexanes were identified as good hits for CCR2 antagonism and might be considered for further optimization.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures for the synthesis of the compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.10.060.

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