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Novel 2-aminooctahydrocyclopentalene-3a-carboxamides as potent CCR2 antagonists

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ABSTRACT

Novel CCR2 antagonists with a novel 2-aminooctahydrocyclopentalene-3a-carboxamide scaffold were designed. SAR studies led to a series of potent compounds. For example, compound **51** had a good PK profile in both dog and monkey, and exhibited excellent efficacy when dosed orally in an inflammation model in hCCR2 KI mice. In addition, an asymmetric synthesis to the core structures was developed.

Chemoattractant cytokines (chemokines) are a family of small heparin-binding proteins and involved in recruiting, chemo-trafficking and activation of leukocytes. There are four subfamilies of chemokines defined by the arrangement of the cysteine (C) residue of the mature proteins: the C, CC, CXC and CX3C chemokines.[1] The monocyte chemoattractant protein (MCP)-1 or CC ligand 2 (CCL2), a member of the CC chemokine subfamily, and its receptor, CC-chemokine receptor 2 (CCR2), have been implicated in both acute and chronic inflammatory and autoimmune diseases associated with infiltration of monocytes, macrophages, lymphocytes, dendritic cells, NK cells, esinophils, basophils, natural killer (NK) cells and memory T-cells.[2] While modified MCP-1 peptides, which were still bound but no longer active CCR2, demonstrated the therapeutically potential of MCP-1 inhibitors in animal models of arthritis,[3] several classes of small molecules with CCR2 antagonism have also been shown to inhibit chemotaxis in response to MCP-1 in-vitro and in animal models.[4]

A majority of these small molecule CCR2 antagonists with low nanomolar potency in a receptor binding assay share a common structural pattern: a basic center flanked either by two aromatic rings or one aromatic and one aliphatic group (Fig. 1).[4] Based on this CCR2 pharmacophore model, we designed a series of fused bicyclic chemotypes to define the linker 2. Here we reported a series of novel 5/5 and 5/6 fused bicycles.

The first generation synthesis of the fused bicycle is illustrated in Scheme 1. Alkylation of commercially available ethyl 2-oxocyclopentanecarboxylate **1a** with 1-chloropropan-2-one, followed by subsequent annulation gave **2a** ($n = 1$). The resulting fused bicycle **2** was hydrogenated to give ketone **3** as racemate. Reduction of ketone **3** by $NaBH_4$ generated the desired alcohol **4** as the major isomer, which was converted with inversion of configuration to amine **5** by mesylation, azidation and reduction. Protection of the amine with a Boc and hydrolysis led to acid **6** as a racemic mixture. The 5/6 fused bicycle was made in the same fashion.

The key intermediate **6** was coupled with a variety of amines to give amides **7**, which were subject to reductive amination with ketone or aldehyde, or coupling with acids, to afford the final compounds **8** Scheme 2.

A brief look at the SAR on the right side revealed that bicyclic amides were superior to mono substituted amides. We decided to use 3-(trifluoromethyl)-5,6,7,8-tetrahydro-1,6-naphthyridine for the SAR study on the left hand side (Table 1). 3-Pentanyl analog **9** showed only micro molar level binding affinity, while potency of its cyclic analog **10** slightly increased. Expansion of the ring from 5 to 7 members led to a trend of increasing potency and arrived at a submolar compound **12**. Insertion of sulfur into the cyclohexane ring **13**, similar in size to **12**, retained potency, but had weak hERG inhibition. Increasing polarity of S to SO_2 reduced hERG binding, but also reduced the potency for CCR2. Replacing cyclohexyl with tetrahydropyranyl afforded the most potent CCR2 compound **15** with IC_{50} of 125 nM. Further modification of **15** by either increasing flexibility or removal of basicity of the nitrogen (**16** and **17**) failed to provide active compounds. Since polarity seems to suppress hERG binding, the methoxy-tetrahydropyranyl group was examined. To our delight, the CCR2 potency was maintained and

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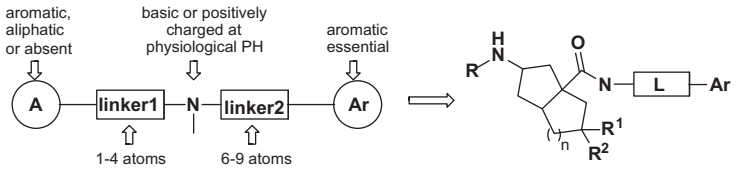
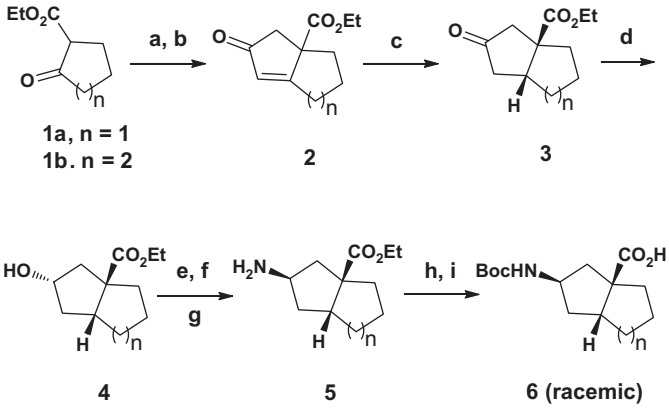


Figure 1. Design of fused ring systems.



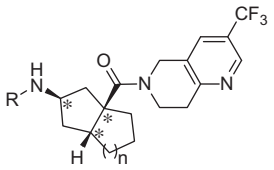
Scheme 1. Regents and conditions: (a) CH_3COCH_2Cl, Na_2CO_3, acetone, rt (n = 1) or $CH{=}C(CH_3)OAc$, $Mn(OAc)_3$, $Cu(OAc)_2$, DCM, 40 °C (n = 2); (b) NaH, toluene, reflux; (c) H_2, Pd/C, EtOH and EtOAc; (d) $NaBH_4$, THF, MeOH, 0 °C to rt; (e) MsCl, TEA, DCM, 0 °C; (f) NaN_3, DMF, 50 °C; (g) H_2, Pd/C, EtOH; (h) $(Boc)_2O$, DCM; (i) LiOH, H_2O, MeOH, rt.

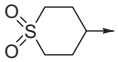
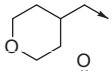
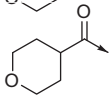
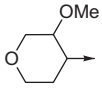
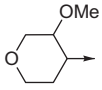
hERG liability was eliminated (**18** vs **15**). To compare the 5/6 fused bicycles to 5/5 analogs, we incorporated the two most potent substituents (tetrahydropyranyl and methoxy-tetrahydropyranyl) on the 5/6 system to generate compounds **19** and **20**. Because **19** and **20** were less potent, we focused our efforts on the 5/5 system in later discussion.[5]

Compound **18** is a mixture of 8 possible diastereoisomers. In order to determine the stereochemical influence of the fused ring on biological activity and provide the handle for further modification in the middle, we developed an asymmetric synthetic route to a key enantiopure intermediate **27**, which turned out to be the active configuration.[6] All SAR from this point on was conducted using the pure active enantiomer for the central bicycle.

As shown in, the optically pure enantiomer **21**[7] was protected with a second Boc group.[8] 1,3-Dipolar addition of bis-Boc protected **22** with silylated allylic acetate gave the desired bicycle **23** as a major isomer.[9] Ozonolysis of olefin **23** gave ketone **24**, which was deoxygenated via tosyl hydrazone to **25**.[10] Removal of both Boc groups, and reprotection of the amine with one Boc group yielded **26**, which was hydrolyzed to give **27**. Acid **27** was subject to coupling with a variety of amines to yield optically pure amide **28**. TFA-mediated deprotection and subsequent reductive amination with previously optimized methoxy-tetrahydropyranyl ketone provided candidate **29** for the right side aromatic ring SAR investigation.

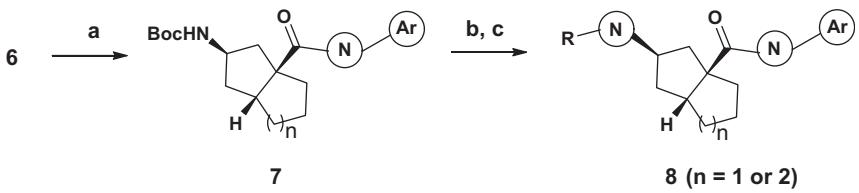
Table 1
Left side SAR using the racemic bicycle core



Compd	n	R	hCCR2 binding IC_{50} (nM)	hERG binding IC_{50} (μM)
9	1		3300	>50.0
10	1		2329	33.5
11	1		1529	33.6
12	1		699	14.7
13	1		874	23.1
14	1		3558	>50.0
15	1		125	25.7
16	1		>25,000	47.0
17	1		>25,000	>50.0
18[a]	1		170	>50.0
19	2		7927	38.9
20[a]	2		1070	40.9

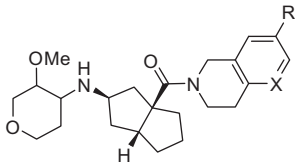
[a] Methoxytetrahydropyrans are mixtures of diastereoisomers.

As illustrated in Table 2, unsubstituted pyridine **30** was inactive. When the pyridine ring was substituted with electron withdrawing groups such as Cl (**31**), Br (**32**), CF_3 (**33**) and NO_2 (**34**) at the 3 posi-



Scheme 2. Regents and conditions: (a) the amine, EDAC, HOBt, TEA, THF; (b) TFA, DCM; (c) ketone/aldehyde, $NaBH(OAc)_3$, TEA, DCM or acid, EDAC, HOBt, TEA, DCM.

Table 2
Right side SAR using the enantiopure bicycle core



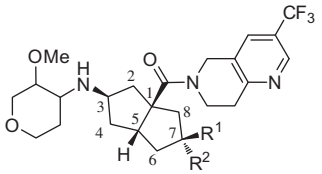
Compd	R	X	hCCR2 binding IC_{50} (nM)	hERG binding IC_{50} (μM)
30	H	N	>25,000	>50.0
31	Cl	N	1100	33.3
32	Br	N	200	48.9
33	CF_3	N	76	>50.0
34	NO_2	N	1500	>50.0
35	OMe	N	>25,000	46.6
36	O^iPr	N	5600	46.9
37	F	CH	4400	23.5
38	Cl	CH	400	42.4
39	Br	CH	290	20.3
40	CF_3	CH	34	27.6
41	CN	CH	1800	33.7
42	OCF_3	CH	16	7.4

∗Single enantiomers for the bicycle core; mixtures of diastereoisomers for methoxy-tetrahydropyranyl moiety.

tion, the potency of these analogs was found to be enhanced, with CF_3 being the most potent compound. Compounds with electron donating groups (**35**, **36**) at the same position were less potent. This indicates that both lipophilicity and electronic effects influence the potency at this position. To investigate the influence of electron density in the right aromatic ring, we synthesized a series of phenyl analogs substituted with both electron withdrawing (F, Cl, Br, CF_3 and CN) and donating (OCF_3) groups. This modification resulted in two low nanomolar potent compounds **40** (34 nM) and **42** (16 nM). However, both **40** and **42** suffered from strong hERG inhibition and failed to provide enough separation between CCR2 and hERG activity.

Having executed both left hand side (R) and right hand side (Ar) modifications in **8**, we directed our attention to the middle bicycles. Taking advantage of the newly developed synthetic method, we were able to conduct our SAR study at the 7-position by functionalization from intermediates **23** and **24** (Scheme 3). It is worth mentioning that mono substitutions on the prochiral 7-position would generate two epimers with the substituent oriented towards (R^1) and away from (R^2) the naphthyridine ring.

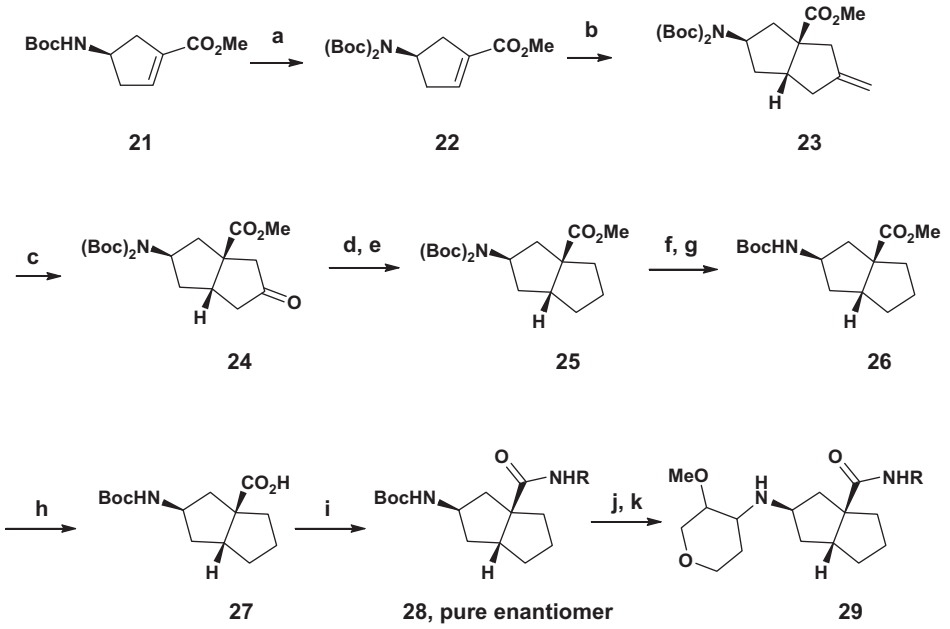
Table 3
Bicycle core SAR[a]



Compd	R^1	R^2	hCCR2 binding IC_{50} (nM)	hERG binding IC_{50} (μM)
33	H	H	76	>50
43	F	F	95	>50
44a	F	H	23	>50
44b	H	F	150	>50
45a	Cl	H	20	>50
45b	H	Cl	45	>50
46a	CF_3	H	1258	>50
46b	H	CF_3	263	>50
47a	CO_2NH_2	H	88	>50
47b	H	CO_2NH_2	130	>50
48a	OH	H	59	>50
48b	H	OH	58	>50
49a	OMe	H	210	>50
49b	H	OMe	200	>50
50a	NMe_2	H	8095	>50
50b	H	NMe_2	247	>50

[a] Single enantiomers for the bicycle core; mixtures of diastereoisomers for methoxy-tetrahydropyranyl moiety.

Many potent compounds were identified by this study. Both lipophilic and polar groups are tolerated (Table 3). The bisfluoro analog **43**, which was designed to block metabolism on the fused ring, displayed equal potency to the parent compound **33**. Mono



Scheme 3. Regents and conditions: (a) $(Boc)_2O$, DMAP, THF, 58 °C; (b) $AcOCH_2C(=CH_2)CH_2TMS$, $Pd(OAc)_2$, P(*i*-PrO)$_3$, toluene, 100 °C; (c) O_3, EtOH, −78 °C; (d) NH_2NHTs, Na_2SO_4, MeOH, 80 °C; (e) catecholborate, $CHCl_3$, 0 °C to rt, then NaOAc, reflux; (f) TFA, DCM, rt; (g) $(Boc)_2O$, DCM; (h) LiOH, H_2O, MeOH, rt; (i) the amine, EDAC, HOBt, TEA, THF; (j) TFA, DCM; (k) 3-methoxydihydro-2*H*-pyran-4(3*H*)-one, $NaBH(OAc)_3$, TEA, DCM.

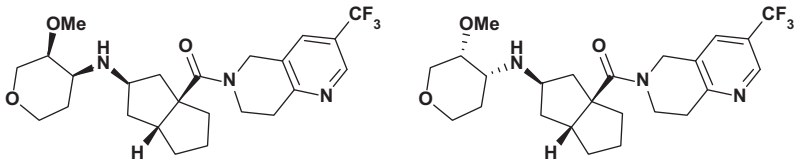


Figure 2. Enantiopure **51** and **52.**

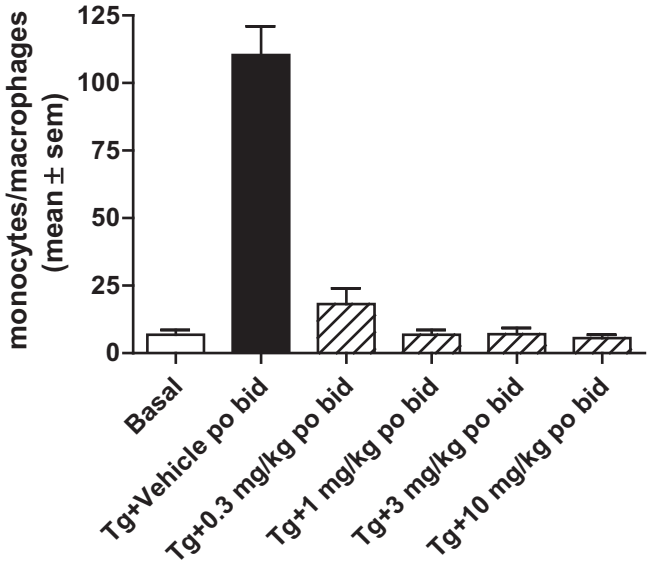


Figure 3. In vivo efficacy in thioglycollate induced peritonitis of **51.**

substitution with small groups such as F, Cl, OH or a flat substituent such as CO_2NH_2 either increased or retained the binding potency in both epimers. Increasing the size of the substitution (OMe, CF_3 and NMe_2) resulted in decrease in potency regardless of the electronic property of the substitution, which indicates the receptor binding pocket is limited in size. In the cases of **46a** and **50a**, the significant loss of activity may be caused by repulsive interaction between the substituent and the naphthyridine ring leading to a disfavored confirmation.

As a result, the SAR on the left, right and the middle led to several compounds with desirable profiles (**33**, **43**, **44a**, **45a**, **45b**, **47a**, **48a** and **48b**). Some of these analogs were further evaluated as potential clinical candidates. For example, compound **33**, which was still a mixture of two diastereomers due to the chirality of the methoxy-tetrahydropyranyl moiety, was separated with chiral Kromasil® AD column (25% isopropanol in ethanol) to give two major diastereoisomers **51** and **52** (Fig. 2).[11] Both **51** and **52** exhibited equal binding affinity (53 nM for **51**, 61 nM for **52**) against the CCR2 receptor. In CCR2 functional assays, they are potent antagonists of MCP-1-induced chemtaxis (22 nM for **51**, 24 nM for **52**) and sub-molar antagonists of MCP-1 induced calcium flux efficacy. Compound **51** has no hERG activity in both the binding assay (>50 μM) and the functional assay (15.6% @ 3 μM, patch clamp). It did not inhibit the major isoforms of human CYP enzymes with an IC_{50} of >10 μM against CYP1A2, 2C19, 2D6, 2C9 and 7.5 μM against CYP 3A4.

When orally administered in an acute inflammation model (thioglycollate induced peritonitis) in mCCR2 knock-out/hCCR2 knock-in mice, compound **51** markedly inhibited the infiltrate of macrophages by 89%, 100%, 100% and 101% at 0.3, 1, 3 and 10 mpk dose, respectively (ED_{50} = 0.1 mpk; EC_{50} = 22 nM in plasma)(Fig. 3).

In addition to its favorable in vivo efficacy, compound **51** displayed excellent pharmacokinetic profiles in higher species (dog and monkey). In dog PK (10 mpk in 0.5% methocel, po and 2 mpk in 20% $HP_\beta CD$, iv), it has moderate systemic clearance (CL: 15.2 mL/min/kg), large volume of distribution (V_{dss}: 2.3 L/kg), reasonable oral half-life ($t_{1/2}$: 3.4 h) and good oral exposure (AUC: 12128 h∗ng/mL). The oral bioavailability (F%) of compound **51** was 100%.

In conclusion, we identified novel 2-aminooctahydrocyclo-pentalene-3a-carboxamides as potent CCR2 antagonists. Modifications in the middle bicycle as well as the left and right hand sides led to the discovery of a series of promising CCR2 antagonists with excellent in-vivo efficacy, and desirable PK and safety profiles. Further modifications on the middle bicyclic ring are in progress and will be reported in due course.

Acknowledgments

We are grateful to Dr. Christopher Teleha for providing large quantities of the intermediate **23** and Dr. Derek Beauchamp for the crystallography analysis of **51**. We also thank the ADME/PK, Secondary Pharmacology and Lead Generation Biology Teams for their contributions to this work.

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5. During our research process, scientists at Abbott Laboratories reported octahydropentalene derivatives as chemokine receptor antagonists in a patent (George, D. M.; Wang, L.; Li, B.; Ericsson, A. M.; Ansell, G. K. PCT Int. Appl. WO 2009042193). Although they did not have the compounds with the same structures as in this paper and neither the specified active stereochemistry, they shared the same 5/5 fused bicycles.
6. For example, compound **33** (IC_{50}, 76 nM), which was made from intermediate **27**, was more potent than its diastereoisomer (IC_{50}, 4070 nM, structure not shown) made from the other enantiomer of **27**.
7. Compound **21** was prepared from (+)-2-azabicyclo[2.2.1]hept-5-ene-3-one according to the procedure described in: Smith, M. E. B.; Derrien, N.; Lloyd, M. C.; Taylor, S. J. C.; Chaplin, D. A.; McCague, R. Tetrahedron Lett. **2001**, *42*, 1347.
8. The second Boc group not only prevents the nucleophilic addition of the nitrogen to the silylated allylic acetate to generate the undesired product, but also plays an important role in controlling the stereochemistry in favor of the desired, enantiomer **23**.
9. Trost, B. M.; Chan, D. M. T. *J. Am. Chem. Soc.* **1979**, *101*, 6429.
10. Corey, E. J.; Desai, M. C.; Engler, T. A. *J. Am. Chem. Soc.* **1985**, *10*, 4339.
11. The stereochemistry of compound **51** was determined by X-ray crystallography of the single crystal of its semi-succinate from acetonitrile.