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Discovery and SAR of 5-aminooctahydrocyclopentapyrrole-3a-carboxamides as potent CCR2 antagonists

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ABSTRACT

SAR study of 5-aminooctahydrocyclopentapyrrole-3a-carboxamide scaffold led to identification of several CCR2 antagonists with potent activity in both binding and functional assays. Their cardiovascular safety and pharmacokinetic properties were also evaluated.

Chemokines (chemotactic cytokines) are low molecular weight proteins (8–10 kDa), and involved in recruiting, chemo-trafficking and activation of leukocytes such as monocytes, macrophages, lymphocytes, activated T cells, dendritic cells, eosinophils, basophiles, natural killer (NK) cells, by acting as a chemoattractant to guide the migration of cells. Based on the position of the cysteine residues, the chemokine family can be classified into four families: the C, CC, CXC and CX3C chemokines.[1] The Monocyte Chemoattractant Protein-1 (MCP-1), also known as CC chemokine ligand-2 (CCL2), is a member of the CC chemokine which specifically acts on monocytes and some T cells in inflammatory reactions.[2] The biological effects of MCP-1 are reflected by binding to its primary receptor, the CC chemokine receptor-2 (CCR2). This MCP-1 driven activation of CCR2 initiates a cascade of intracellular signaling events which might be associated with a variety of diseases such as rheumatoid arthritis (RA),[3] atherosclerosis,[4] multiple sclerosis,[5] inflammatory airway diseases,[6] neuropathic pain[7] and diabetic nephropathy.[8] Growing evidence suggests that interrupting the MCP-1-CCR2 axis by mutant MCP-1,[9] anti-MCP-1 antibodies[10] or peptide CCR2 antagonists[11] inhibits the progression of those CCR2-dependent diseases. Therefore, blocking CCR2 could provide a potential approach for development of novel therapy.

In the last decade, enormous efforts have been invested by many research laboratories including ours to find small molecules acting as mediators of biological phenomenon such as inflammation. To date, significant progress has been made and several different chemotypes of CCR2 agonists have already advanced into clinical trials.[12] In our previous manuscript,[13] we disclosed a series of potent 2-aminooctahydrocyclopentalene-3a-carboxamides **A** as CCR2 antagonists. To eliminate one chiral center and provide a more chemically accessible handle, we replaced the carbon at the 7 position with a nitrogen and discovered a novel series of 5-amino-octahydrocyclopenta-pyrrole-3a-carboxamides **1** (Fig. 1). Here in, we report the synthesis and biological evaluation of this new scaffold.

The synthesis of the unsubstituted pyrrolidinone **11** was illustrated in Scheme 1. Commercially available **2** was converted to ester **3** according to the reported procedure.[14] A 1,3-dipolar cycloaddition of **3** gave optically pure diastereoisomer **4** as the major isomer. Removal of the benzyl group by hydrogenation and subsequent protection with a Cbz group generated **6**. A hydrolysis / coupling sequence afforded compound **8**. Deprotection of the Boc group, followed by a reductive amination gave compound **10**. Finally, removal of the Cbz group by hydrogenation led to the target

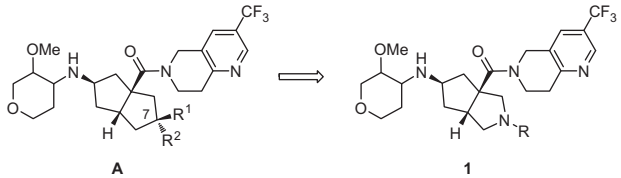

Figure 1.

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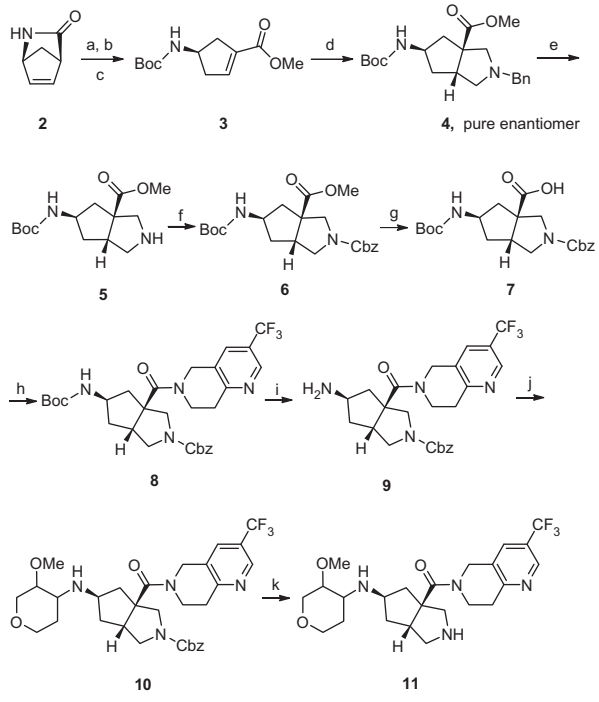
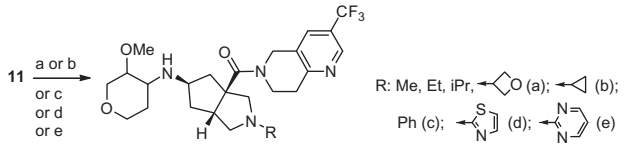


Scheme 1. Regents and conditions: (a) HCl, MeOH, reflux; (b) $(Boc)_2O$, TEA, DCM, 0 °C to rt; (c) DBU, DCM; (d) $TMSCH_2N(CH_2OMe)Bn$, 5% TFA in DCM; (e) H_2, $Pd(OH)_2$, MeOH, 25 psi, rt; (f) CbzCl, TEA, DCM, 0 °C to rt; (g) 6*N* KOH, THF; (h) the amine, EDAC, HOBt, TEA, THF; (i) TFA, DCM; (j) 3-methoxydihydro-2*H*-pyran-4(3*H*)-one, $NaBH(OAc)_3$, TEA, DCM; (k) H_2, Pd/C, MeOH.

compound **11**. Evan though the central bicyclic core is optically pure, **11** and all compounds discussed in this paper are a mixture of 4 possible diastereoisomers due to the two chiral centers on the methoxytetrahydropyanyl moiety.

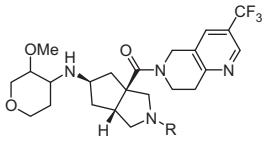
Compound **11** was subjected to substitution on the endocyclic nitrogen atom to provide a variety of analogs. For example, alkylation on the nitrogen was fulfilled by reductive amination of **11** with corresponding aldehyde (**12**, **13**), ketone (**14**, **16**), and ketone derivative (**15**) (Scheme 2). Aromatic substitution was achieved by cross-coupling of **11** with phenyl boronic acid (**17**) or aryl bromides (**18**, **19**).

Initial SAR of the R group was evaluated and summarized in Table 1. As a starting point, compound **11** possessed an IC_{50} of 780 nM in binding affinity. Methylation of **11** afforded the more potent compound **12** (IC_{50} = 300 nM). Increasing size of substitution (R) from Me (**11**), Et (**13**) to *i*-Pr (**14**) showed decreasing potency. On the other hand, the potency of analogs with less flexible substitution such as aliphatic rings (**15**, **16**) or aromatic groups (**17**, **18**), increased slightly. The improved potency of cyclic substitution over acyclic ones may come from their entropic contribution (**14** vs **15**). In the case of **19**, loss of activity may result from the enhanced basicity from the pyrimidinyl group. This early SAR indicated that CCR2 activity was mostly influenced by both the



Scheme 2. Regents and conditions: (a) aldehyde/ketone, $NaBH(OAc)_3$, DCM (for **12**, **13**, **14**, **16**); (b) (1-ethoxycyclopropoxy)-trimethylsilane, $NaBH_3CN$, AcOH, MeOH, reflux (for **15**); (c) $PhB(OH)_2$, $Cu(OAc)_2$, 2,6-lutidine, myristic acid, toluene, rt (for **17**); (d) 2-bromothiazole, KF, TEA, EtOH, 90 °C (for **18**); (e) 2-bromopyrimidine, TEA, EtOH, 90 °C (for **19**).

Table 1
SAR of aliphatic and aromatic analogs



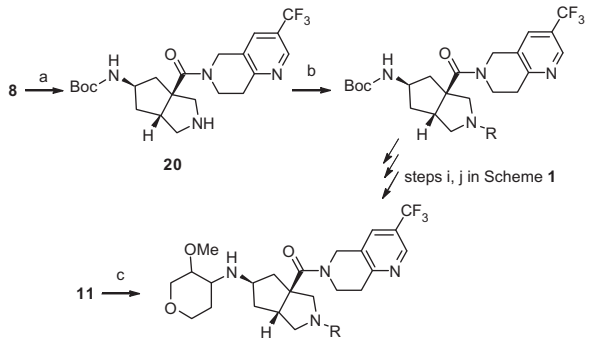
Compd	R	hCCR2 IC_{50} (nM)		hERG IC_{50} (μM)
		Binding	CTX[b]	Binding
11	H	780	770	>50
12	Me	300	320	>50
13	Et	629	nt	42.6
14	iPr	1215	nt	>50
15		180	nt	42.1
16		126	nt	>50
17	Ph	62	90	22.9
18		152	nt	>50
19		1300	nt	>50

[a]Single enantiomers for the central bicycle core; mixtures of diastereoisomers for methoxytetrahydropyranyl moiety.
[b] MCP-1 induced chemotaxis in THP-1 cells (Ref. 15).

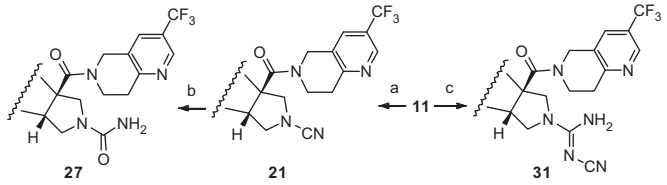
size of the substitution and the basicity on the nitrogen atom. This prompted us to search for appropriate substitution which could reduce the basicity of the nitrogen atom while also filling the binding pocket.

The first set of non-basic analogs, the amide and urea derivatives, were prepared either directly from unsubstituted **11** or from amine **20** (Scheme 3). Thus, hydrogenation of **8** gave amine **20**. Carbonylation of **20** or **11** with an acid or an acid chloride gave the amide. Condensation with isocyanate gave the urea except for unsubstituted urea **27**, which was prepared by hydrolysis of cyanoamide **21** (Scheme 4). Cyanoguanidine **31** was obtained from condensation of **11** and sodium dicyanamide.

As shown in Table 2, reduction of the basicity at the nitrogen by the acetylation of **11** led to a moderately potent compound **22** (Table 2). Unsaturated amides (**23**, **24**, **25**) exhibited double digit nanomolar activity both in binding and functional assays. The most potent compound **23** suffered from potential cardiovascular liability in hERG screening. Saturation of the double bond in **23** provided **26**, which suppressed the hERG inhibition, but failed to return the potency. To our delight, non-basic urea moieties were also tolerated. Unsubstituted urea **27** and its bioisostere **31** exhibited low nanomolar binding affinities and potently inhibited MCP-1-induced chemotaxis, without hERG activity (IC_{50}>50 μM). Both methylated urea **28** and isopropylated urea **29** barely retained activity. Aryl substituted urea (**30**) resulted in loss of activity. eADME pro-

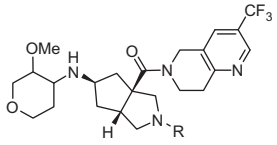


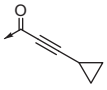
Scheme 3. Regents and conditions: (a) H_2, $Pd(OH)_2$, MeOH, 25 psi, rt; (b) acid chloride, TEA, DCM, 0 °C (for **22**, **23**, **26**); or isocyanate, THF, rt (for **28**, **29**, **30**); (c) the acid, EDAC, HOBt, TEA, DCM, rt (for **24**, **25**).



Scheme 4. Regents and conditions: (a) BrCN, K_2CO_3, ACN, rt; (b) TEA, DCM, rt; (c) $NaN(CN)_2$, 5% H_2O in *i*-PrOH, 120 °C.

Table 2
SAR of amide, urea and cyanoguanidine



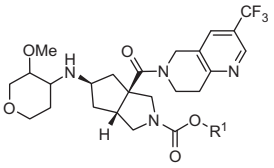
Compd	R	hCCR2 IC_{50} (nM)		hERG IC_{50} (μM)
		Binding	CTX	Binding
22		395	230	>50
23		30	33	24.1
24		68	88	>50
25		87	39	49.6
26		110	87	>50
27		64	27	>50
28		77	71	>50
29		410	81	>50
30		57	220	>50
31		37	29	>50

*Single enantiomers for the central bicycle core; mixtures of diastereoisomers for methoxytetrahydropyranyl moiety.

filing of selected potent compounds (**27**, **28** and **31**) showed low oral exposure in mouse PK due to their poor lipophilicity. For example, the CLogD (at pH 7)[16] of urea **27** and cyanoguanidine **31** are -3.1 and -2.1, respectively. To decrease the polarity of target molecules, we turned our attention to carbamates.

Synthesis of the carbamate is illustrated in Scheme 5. The carbamoylation of **20** either with available chloroformate (**33** to **37**), or alcohol and *N*, *N*′-disuccinimidyl carbonates (DSC) (**38** to **43**) gave intermediate **32**. Deprotection and alkylation on the left part of **32** led to carbamates **33** to **43**.

Table 3
SAR of carbamate



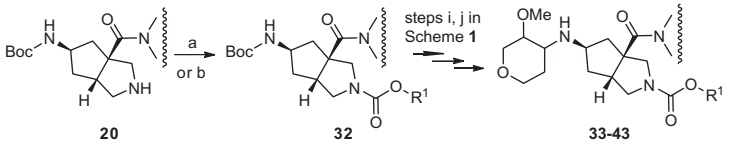
Compd	R^1	hCCR2 IC_{50} (nM)		hERG IC_{50} (μM)
		Binding	CTX	Binding
33	Me	410	69	>50
34	Ph	560	950	>50
35		118	190	>50
36		59	99	>50
37		35	21	>50
38		82	41	>50
39		32	65	>50
40		31	32	>50
41		45	18	>50
42		33	28	>50
43		41	97	>50

*Single enantiomers for the central bicycle core; mixtures of diastereoisomers for methoxytetrahydropyranyl moiety.

In-vitro screening result of the carbamates is summarized in Table 3. Methyl carbamate **33** possessed moderate binding affinity, but good activity in the chemotaxis (CTX) functional assay. Replacement of methyl by phenyl (**34**) resulted in a greater than 10-fold loss of hCCR2 activity in CTX. Increasing flexibility (**35**) brought some degree of activity back. Further replacement of phenyl with an aliphatic group (**36**, **37**) showed a trend of increasing activity. It was also observed that activity was retained with oxygen or nitrogen-containing groups on the ester side (**38** to **43**). This finding indicates that there may be binding to a polar or H-bonding residue in the pocket (Table 3).

Selected potent carbamates were further evaluated in PK study. For example, in rat PK (oral dose at 10 mpk in 0.5% methocel), compound **40** showed better maximum plasma concentration (*C*max: 145 ng/mL) and oral exposure (AUC: 427 h ng/ml) than urea **27**(Cmax: 70.6 ng/mL, AUC: 74 h ng/ml). It also exhibited significantly greater CCR2 selectivity over hERG inhibition in binding and function assay (PatchXpress: 7.3% at 3 μM), and was ideally suitable for further characterization.

In summary, we identified a series of novel CCR2 antagonists feathering 5-amino-octahydrocyclopentapyrrole-3a-carboxamides as the core structure and systemically evaluated the SAR for the substitution on its endocyclic nitrogen atom. The early SAR exploration afforded compound **27**, which possessed good CCR2 binding affinity and functional activity, and showed no safety liability. Further optimization led to compound **40** which retained good CCR2 activity, and also displayed low cardiovascular liability and good



Scheme 5. Regents and conditions: (a) $ClCOR^1$, TEA, DCM, 0 °C to rt (for **33**, **34**, **35**, **36, 37**); (b) R^1OH, DSC, DMAP, ACN, DCM, DMF, rt (for **38**, **39**, **40**, **41**, **42, 43**).

pharmacokinetic properties. These findings merit our further investigation of this series of analogous compounds to identify a development candidate.

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15. MCP-1 induced chemotaxis in THP-1 cells is to examine whether the compounds are antagonists of CCR2 by evaluation of the compounds in a cell migration assay (chemotaxis assay) in human monocytic cell line, THP-1 cells, in the presence of MCP-1. 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% CO2. An aliquot of the cells which had migrated to the bottom chamber taken and counted then compared to vehicle.
16. CLogD value was calculated from Third Demension owned by JNJ.