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# Design and synthesis of novel CCR2 antagonists: Investigation of non-aryl/heteroaryl binding motifs

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ABSTRACT

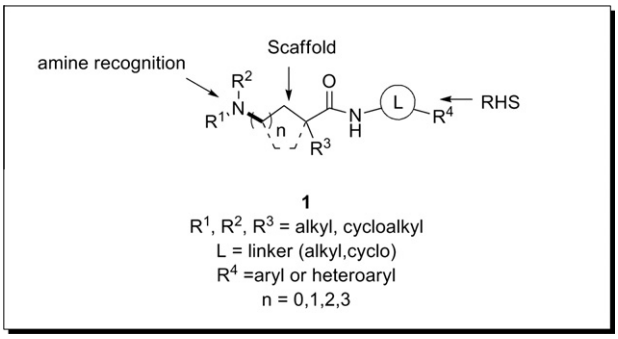
This report describes the design and synthesis of a series of CCR2 antagonists incorporating novel non-aryl/heteroaryl RHS (right hand side) motifs. Previous SAR in the area has suggested an aryl/heteroaryl substituent as a necessary structural feature for binding to the CCR2 receptor. Herein we describe the SAR with regards to potency (binding to hCCR2), dofetilide activity and metabolic stability (in vitro HLM) for this series. The resulting outcome was the identification of compounds with excellent properties for the investigation of the role of CCR2 in disease.



The CC chemokine, Monocyte Chemotactic Protein-1 (MCP-1) serves to recruit monocytes, dendritic cells, natural killer cells and T-lymphocytes.[1] Various chemokines and their respective receptors have been shown to play important roles in many physiological and pathological processes. Evidence exists that MCP-1 is an important biomarker for inflammatory diseases such as rheumatoid arthritis[2] and atherosclerosis.[3] Investigations of CCR2 (ko) and MCP-1 (ko) mice suggest that antagonism of the interaction of MCP-1 with CCR2 may be beneficial in treating inflammatory diseases.[4]

Recent literature has highlighted a range of CCR2 receptor antagonists of varying structural types.[5] In our laboratories, the CCR2 antagonists (Fig. 1, **1**) have been disclosed and served as a basis for further chemical modification.[6] The compounds possess excellent in vitro binding potency and functional activity.

In order to expand the chemical space and by extension the modulation of the biological properties (potency, hERG binding and metabolic stability) a series of compounds were designed and synthesized. Initial SAR suggested that an aryl/heteroaryl RHS (right hand side) motif is required for potency in compounds of the general structural type (see Fig. 1)[5] However, the incorporation of an aryl/heteroaryl motif brings with it some potential deficiencies, for example reduced metabolic stability due to an increase in *c* log *D*, as well as a potential anchor point for binding to




**1**
$R^1$, $R^2$, $R^3$ = alkyl, cycloalkyl
L = linker (alkyl,cyclo)
$R^4$ =aryl or heteroaryl
n = 0,1,2,3

**Figure 1.** General structural motif of selected CCR2 antagonists.

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various ion channels.[7] Therefore, an exploration of non-aryl containing CCR2 antagonists was of interest to avoid these potential pitfalls and expand the chemistry space.

From previous work, compound **2** (see Fig. 2) was identified as a potent CCR2 antagonist (hCCR2 binding $IC_{50}$ = 3.0 nM, human whole blood assay = 3.9 nM, human liver microsome stability $t_{1/2}$ = 93 min), with modest activity on hERG ($IC_{50}$ = 1.7 μM).[8] Initial analog work investigating the removal of the N-linked aryl group and replacement with an isosteric *t*-butoxycarbonyl group disappointingly resulted in a significant loss of binding affinity (see Fig. 3). Further expansion of the SAR led us to the incorporation of the bridged 2.2.1 piperazine ring system for the piperazine ring leading to compound **5**. Compound **5** gratifyingly possessed equivalent activity to **2**, but without an aryl/heteroaryl ring system on the RHS. An overlay of compound **6** onto **4** suggested that perhaps alternate hydrophobic pocket was being accessed (see Fig. 4). Alternatively, the conformational restriction introduced by the methylene bridge may be locking the RHS into the more active conformation. In order to explore the SAR for this compound a series of analogs were prepared wherein the *t*-butoxycarbonyl was replaced with other carbamates, amides and ureas. The syntheses of these compounds are described in Schemes 1 and 2. The activities for these compounds are depicted in Tables 1–3.

The initial investigations of the SAR were done wherein the LHS (left hand side) pyran was unsubstituted. Thus the key intermediate **7** was prepared as previously described.[8] To the acid **7** was coupled the boc-2.2.1 piperazine, followed by removal of the 2,5-dimethylpyrrole protecting group and reduction to give **10**. Reductive amination with 4-pyranone provided **6**, which was then protected with trifluoroacetic anhydride. The Boc group was removed to give the free amine to which a series of carbamates, amides and ureas were prepared. The methoxy pyran and F-pyran series were prepared in a similar manner (see Scheme 2).

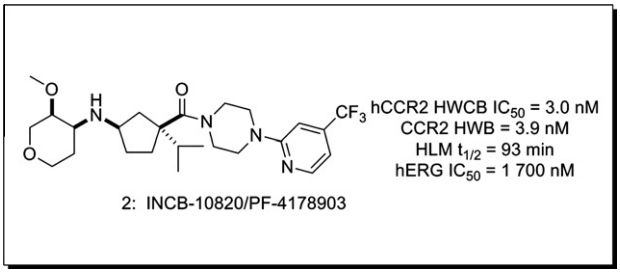



**Figure 2.** Structure of lead compound **2** and biological data.

**Figure 4.** Overlay of compound **4** and **6**.

In order to gauge the steric requirements of the RHS region of the binding site a series of amides were prepared with gradual increases in steric bulk ($CH_3$, $CH_3CH_2$, $^{i}$Pr, $^{t}$Bu, $^{c}$Pr, $^{c}$Bu, $^{c}$Pen, $^{c}$Hex, compounds **14**–**21**). From the activity displayed it was clear that the receptor required a minimal degree of steric bulk, with the *cyclo*-pentyl and *cyclo*-hexyl possessing activity most similar to the parent *N*-Boc compound. The isosteric 3,3-trimethylbutanamide displayed a slight reduction in potency (37 nM vs 5 nM) for the parent. Interestingly, when the *c*-hexyl group **21** was replaced with a pyran to give **22,** a significant decrease in activity was observed suggesting a very lipophilic pocket intolerate of polarity. From previous studies in the aryl series[8], incorporation of a $CF_3$-group enhanced potency, thus a group of $CF_3$ containing amides were synthesized. The activities of these compounds (**23–25**) were equivalent with compound **6**, exhibiting a slight reduction in metabolic stability, due to the increased lipophilicity.

With the SAR of the pyran series showing a clear requirement for a minimal degree of steric bulk for activity, a subset of compounds wherein the pyran was replaced with the methoxy pyran was prepared. Previous work had shown the methoxy pyran to bring activity on the mouse homolog of CCR2[9] allowing target validation in in vivo murine models. Very similar SAR was observed with regards to 3,3-trimethylbutanamide **30** (17 nM) and *t*-butyl amide **31** (40% at 300 nM). Interestingly, in the methoxy pyran

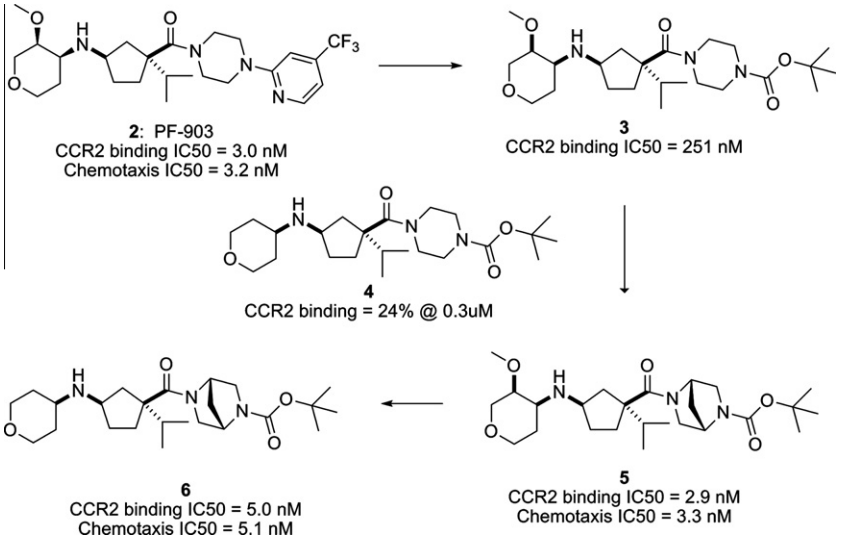
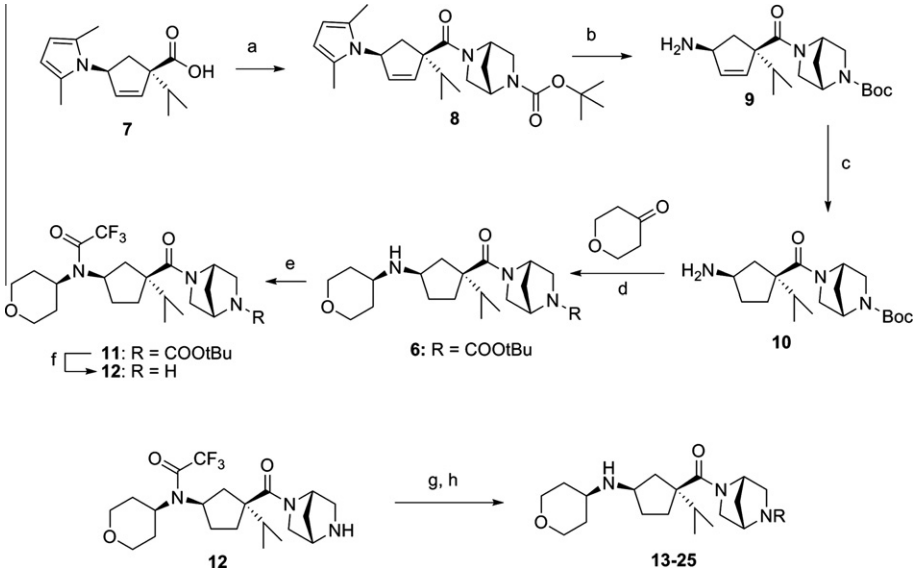
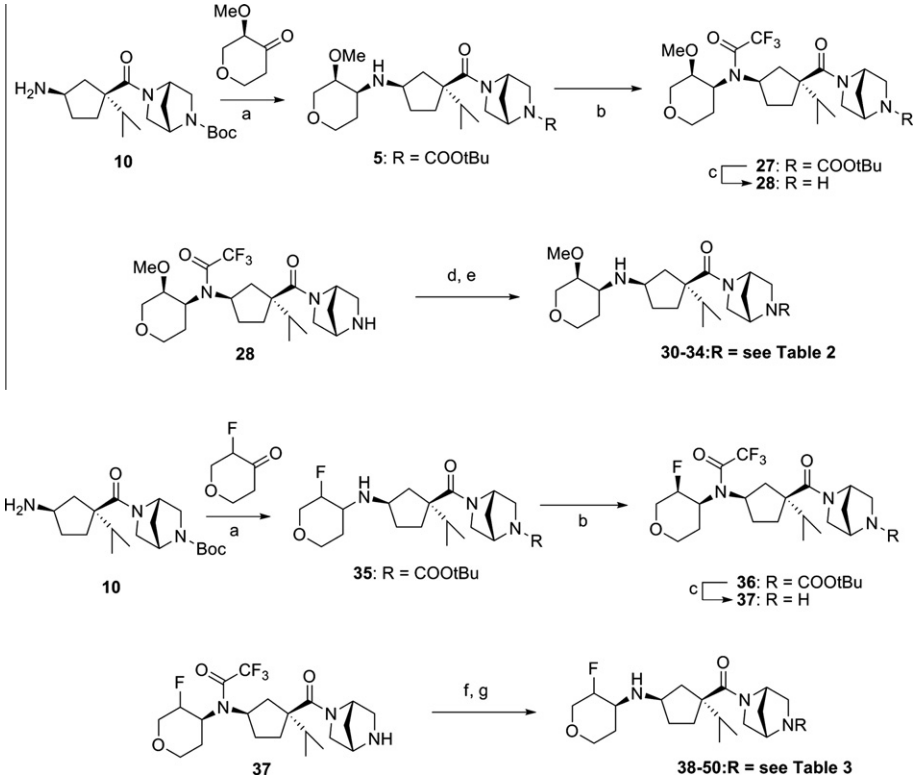



**Figure 3.** Structure of lead compound **2**–**6** and binding data.




**Scheme 1.** Preparation of compounds **6** and **13–25**. Reagents and conditions: (a) (1*S*,4*S*)-*tert*-butyl 2,5-diazabicyclo[2.2.1]heptane-2-carboxylate, TBTU, $Et_3N$, DMF, 0 °C, 95%; (b) $H_2NOH$, THF/$H_2O$, 90%; (c) $H_2$, Pt/C, HOAc, 40 psi; (d) dihydro-2*H*-pyran-4(3*H*)-one, $Et_3N$, $NaBH(OAc)_3$, 95%; (e) TFAA, $Et_3N$, $CH_2Cl_2$, 0 °C, 95%; (f) 30% TFA, $CH_2Cl_2$, rt, 90% yield; (g) (i) RCOCl, $Et_3N$, $CH_2Cl_2$, 0 °C, 90% or (ii) $RCO_2H$, TBTU, $Et_3N$, DMF, 91%; (h) $K_2CO_3$, MeOH, rt, 80–94%.

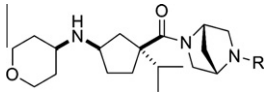


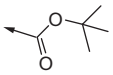
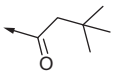
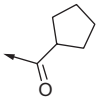
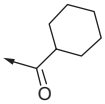
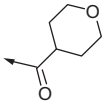
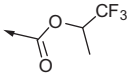
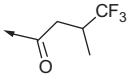
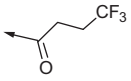

**Scheme 2.** Preparation of compounds **30–34** and **38–50**. Reagents and conditions: (a) pyranone, $Et_3N$, $NaBH(OAc)_3$, 95%; (b) TFAA, $Et_3N$, $CH_2Cl_2$, 0 °C, 95%; (c) 30% TFA, $CH_2Cl_2$, rt, 90% yield; (d) (i) RCOCl, $Et_3N$, $CH_2Cl_2$, 0 °C, 90% or (ii) $RCO_2H$, TBTU, $Et_3N$, DMF, 91% or (iii) RNCO, $Et_3N$, $CH_2Cl_2$, 0 °C; (e) $K_2CO_3$, MeOH, rt, 80–94%.

series a slight enhancement in potency was observed with the *c*-butyl amide (**19** vs **32**, threefold). Finally, analogs **33** (*c*-pentyl) and **34** (benzamide) showed good activity and metabolic stability.

Recent work in the CCR2 field has suggested penetrance into the CNS as a possible requisite for activity in certain disease states (e.g., neuropathic pain).[10] As a strategy to enhance the permeability for the chemical series the pyran was fluorinated beta to the secondary amine resulting in a reduction of basicity (~1.7 p$K_a$ units). Compound **35** exhibited very good potency (4.2 nM), however with a reduction in metabolic stability, likely due to the increased *c* log *D* (~1.1 units). A similar reduction in metabolic stability was observed for the F-pyran series across the various substitutions (amide and carbamate), again likely due to the increased *c* log *D*. On the other hand, compounds **42** and **43** retained their metabolic stability along with good affinity to CCR2 despite the fluorination of the pyran. Further evaluation of these compounds

**Table 1**
Binding affinities, dofetilide and hlm stability of unsubstituted pyran series



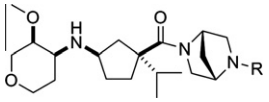
| No. | R | CCR2 HWCB (nM) | Dof (%inh) | HLM ($t_{1/2}$) |
|---|---|---|---|---|
| **6** |  | 5.0[b] | 4.8 | >120 |
| **13** |  | 36.9[b] | 5.7 | >120 |
| **14** |  | 1% at 0.30[a] | 0 | >120 |
| **15** |  | 28% at 0.30[a] | 6 | >120 |
| **16** |  | 40% at 0.30[a] | 3.7 | >120 |
| **17** |  | 23% at 0.30[a] | 2 | >120 |
| **18** |  | 130[a] | 2.3 | >120 |
| **19** |  | 32[b] | 6 | >120 |
| **20** |  | 12.2[b] | 2 | >120 |
| **21** |  | 11.6[a] | 4 | 93.2 |
| **22** |  | 46% at 0.30[a] | 0 | >120 |
| **23** |  | 3.5[a] | 2 | 78 |
| **24** |  | 13[a] | 5 | >120 |
| **25** |  | 14.6[a] | 1 | >120 |

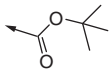
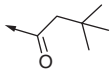
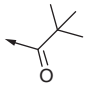
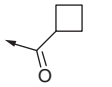
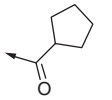
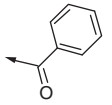
Abbreviations: HWCB = human whole cell binding assay (a = 30 min preincubation, b = 1 h preincubation); Dof = hERG dofetilide assay, %inh at 10 μM; HLM = human liver microsome-half life, 1 μM dose.

in an efflux assay showed low levels of efflux suggesting that they would be reasonable substrates for passage across the blood–brain barrier (BBB), compound **40** (MDCK A/B = $11.8 * 10^{-6}$ cm/s; B/A $10.2 * 10^{-6}$ cm/s; BA/AB = 0.8), compound **41** (MDCK A/B = $8.35 * 10^{-6}$ cm/s; B/A = $5.3 * 10^{-6}$ cm/s; BA/AB = 0.6).

In conclusion, we have identified a series of CCR2 antagonists bearing non-aryl/heteroaryl RHS motifs with excellent potency in human whole cell binding.[11] The compounds displayed good in vitro human metabolic stability and low affinity for the hERG

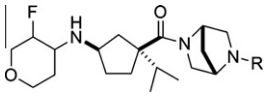
**Table 2**
Binding affinities, dofetilide and hlm stability of methoxy pyran series



| No. | R | CCR2 HWCB (nM) | Dof (%inh) | HLM ($t_{1/2}$) |
|---|---|---|---|---|
| **5** |  | 2.9[b] | 0 | >120 |
| **30** |  | 17.0[b]<br>24.2[a] | 0 | >120 |
| **31** |  | 40% at 0.3[b] | 6 | >120 |
| **32** |  | 11.7[b] | 4 | >120 |
| **33** |  | 8.5[b]<br>11.1[a] | 6.7 | >120 |
| **34** |  | 6.5[b]<br>12.0[a] | 7.8 | 94.2 |

Abbreviations: HWCB = human whole cell binding assay (a = 30 min preincubation, b = 1 h preincubation); Dof = hERG dofetilide assay, %inh at 10 μM; HLM = human liver microsome-half life, 1 μM dose.

**Table 3**
Binding affinities, dofetilide and hlm stability of fluropyran series



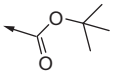
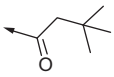
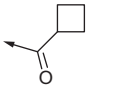
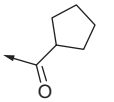
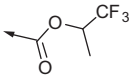
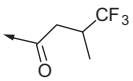
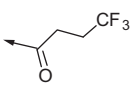
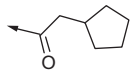
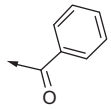
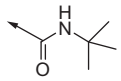
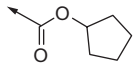
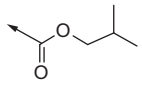
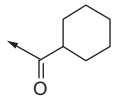
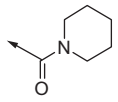
| No. | R | CCR2 HWCB (nM) | Dof (%inh) | HLM ($t_{1/2}$) |
|---|---|---|---|---|
| **35** |  | 4.2[b] | 6 | 37.2 |
| **36** |  | 39.5[a] | 0 | 76 |
| **37** |  | 38.4[a] | 17 | 21 |
| **38** |  | 13.9[a] | 3 | >120 |
| **39** |  | 3.2[a] | 3 | 26 |
| **40** |  | 12.8[a] | 0 | >120 |
| **41** |  | 17.3[a] | 5 | >120 |

**Table 3** (*continued*)

| No. | R | CCR2 HWCB (nM) | Dof (%inh) | HLM ($t_{1/2}$) |
|---|---|---|---|---|
| **42** |  | 14.7[a] | 3 | 48 |
| **43** |  | 17.5[a] | 0 | — |
| **44** |  | 28[a] | 0 | 106 |
| **45** |  | 3.1[a] | 0 | 17 |
| **46** |  | 4.3[a] | 0 | 28 |
| **47** |  | 15.2[a] | 0 | 55 |
| **48** |  | 75.9[a] | 0 | — |

Abbreviations: HWCB = human whole cell binding assay (a = 30 min preincubation, b = 1 h preincubation); Dof = hERG dofetilide assay, %inh at 10 μM; HLM = human liver microsome-half life, 1 μM dose.

ion channel. In addition, compounds **40** and **41** possessed properties amenable to CNS penetrance. Further studies with the compounds disclosed in this publication will be the subject of future communications.

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## References and notes

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