Automated Parallel Solid-Phase Synthesis of Non-Peptide CCR1 Receptor Antagonists

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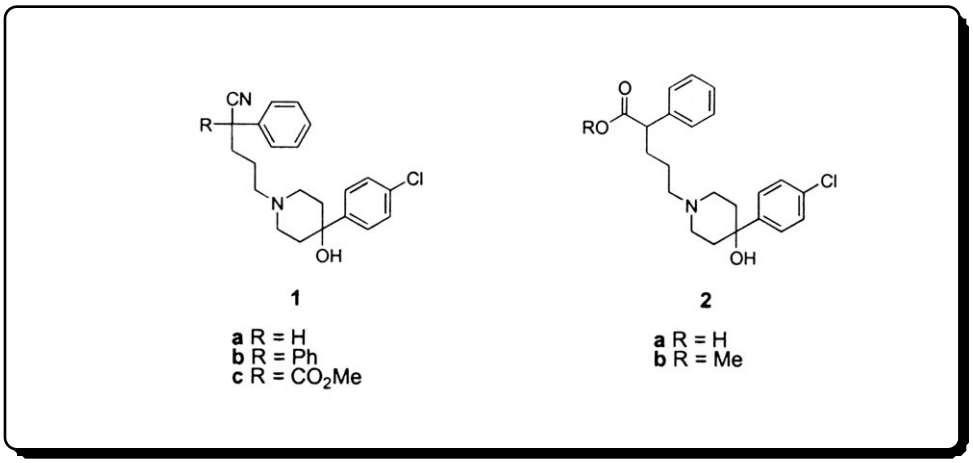
Abstract—An automated, parallel, solid-phase synthesis and screening strategy using commercially available aryl acetic acids as starting materials has discovered novel, non-peptide CCR1 antagonists (K_i < 100 nM).

The CCR1 receptor is a member of the chemokine receptor family [1]. The role of chemokines in the immune response is to engage the immune cells against pathogenic organisms by direct recruitment and activation. Recent studies provide strong evidence for the important role of the chemokines RANTES and MIP-1α in chronic inflammatory diseases, such as multiple sclerosis and rheumatoid arthritis [2]. Since RANTES and MIP-1α are ligands for CCR1, antagonists for this receptor are prime targets for the discovery of new therapies.



The discovery in our laboratories of hydroxypiperidines **1** as potent CCR1 receptor antagonists has been described (Table 1) [3]. Des-cyano analogs **2** show slightly weaker activity; within this series, the methyl carboxylate **2b** has approximately 3-fold greater activity than the carboxylic acid **2a**. Since **2a** contains a carboxylic acid handle for attachment to a solid-phase polystyrene resin, we envisioned a synthesis using a diverse selection of commercially available phenyl- and heteroaryl acetic acids as starting materials.

Automated, parallel, solid-phase synthesis of small non-peptide molecules for lead refinement has recently emerged as a focus of drug discovery research [4]. In this case, our strategy was to rapidly synthesize single compounds to elucidate the effect of varying the aryl acetic acid moiety of **2**. Furthermore, we chose to screen unpurified reaction products and only re-synthesize or purify interesting, active molecules. Compounds found in this manner could be esterified to give the more potent esters (*i.e.* **2a** compared to **2b**, Table 1). In this paper, we describe the automated synthesis, SAR, screening strategy and follow-up re-synthesis of novel, non-peptide CCR1 receptor antagonists **2**, **7a-q**, and **8-10**.

Our synthetic strategy was to optimize the previously reported solution-phase synthesis of compounds **1** [3] for use on an automated solid-phase synthesizer [5]. The first step required coupling of the diverse aryl acetic acid to the resin. Once this unique step was completed, the subsequent steps to attach the non-variant propylpiperidinol moiety would be both identical and parallel for all compounds. This strategy ensures that the synthesis be very amenable to automation.

The synthesis is described in Scheme 1 [6]. Each aryl acetic acid **3** was coupled to Wang resin using DIC and DMAP in CH_2Cl_2 to afford resin-bound inter-mediate **4**. Treatment with KHMDS in THF followed by 1-chloro-3-iodopropane afforded chloro intermediate **5**. Addition of 4-*para*-chlorophenyl-piperidin-4-ol with DMAP in DMF afforded completed molecule **6** which was now ready to be

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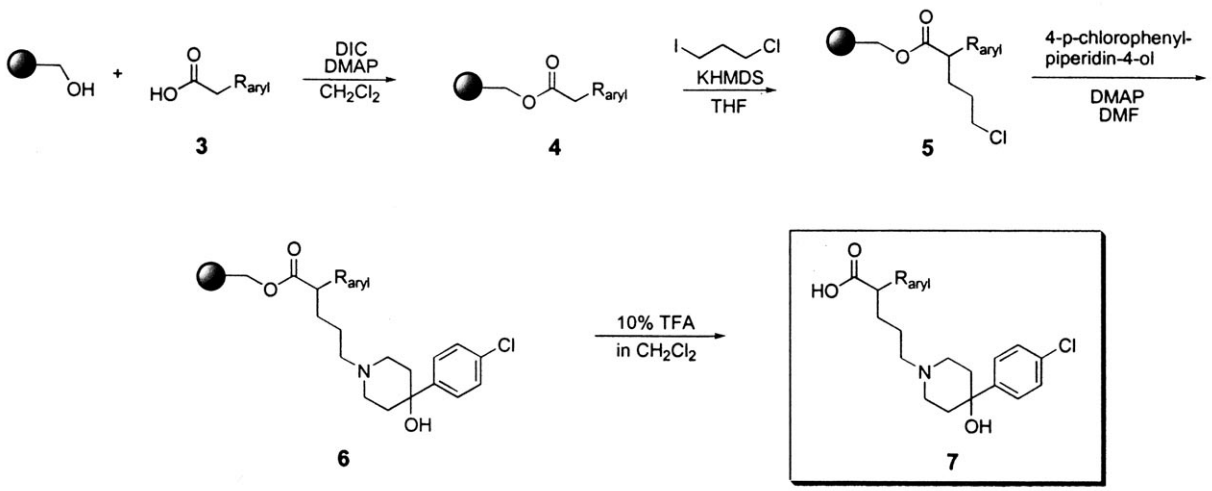
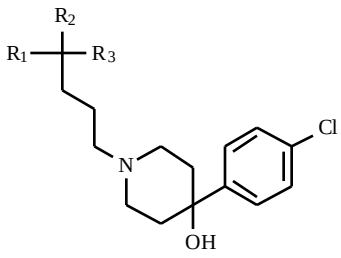


Scheme 1.

cleaved from the resin. Cleavage was accomplished with 10% TFA in CH_2Cl_2 to afford final product **7**. In this manner, over 100 analogs were synthesized.

Table 1. Inhibition Data for Pure CCR1 Antagonists 1, 2, 8-11



cmpd	R_1	R_2	R_3	K_i (nM)[a]
1a[b]	H	CN	Ph	54
1b[b]	Ph	CN	Ph	52
1c[b]	CO_2Me	CN	Ph	53
2a[c]	CO_2H	H	Ph	500
2b[c]	CO_2Me	H	Ph	145
8[c]	CO_2Me	H	2-CF_3-Ph	74
9[c]	CO_2Me	H	3-Cl-Ph	187
10[c]	CO_2Me	CN	2-CF_3-Ph	3730
11[b]	H	CN	2-CF_3-Ph	25% @ 10 μM

[a]K_i values are derived from competitive binding on CCR1 with ^{125}I MIP-1α. Values represent the mean of n ≥ 2. Standard deviations are <30% of the mean. See ref. 3b. [b]ref. 3a. [c]ref. 7].

Table 2. Inhibition Data for Unpurified Library Synthesis Products 7

cmpd	R aryl	K_i (nM)[a]
7a	**2-CF_3-Ph**	**213**
7b	3-(5-chlorobenzothiophene)	221
7c	2-pyridyl	228
7d	3,5-F_2-Ph	229
7e	2-F-Ph	334
7f	3-F-Ph	575
7g	2-naphthyl	852
7h	3-Cl-Ph	870
7i	Ph	880[b]
7j	2,4-Cl_2-Ph	896
7k	2-thienyl	916
7l	3-MeO-Ph	1510
7m	2,5-MeO_2-Ph	1610
7n	2,4-F_2-Ph	1730
7o	3,4-F_2-Ph	1865
7p	2-Me-Ph	2635
7q	3,4-Cl_2-Ph	2940

[a]K_i values are derived from competitive binding on CCR1 with ^{125}I MIP-1α. Values represent the mean of n ≥ 2. Standard deviations are <30% of the mean. See ref. 3b [b]Average of assay results from four unpurified synthetic preparations. Std dev = 363.

Sampled library members were analyzed by HPLC, ^{1}H NMR and MS. The isolated crude yields were in the range of 0-90%. The variety of acids used as starting materials demonstrates that the synthesis tolerated a wide range of substituents (Table 2). Both electron-rich aryl rings (**7l**, **7m**, **7p**), electron poor (**7a**, **7d**, **7j, 7n, 7o, 7q**) aryl rings, and heteroaryl rings (**7b**, **7c**, **7k**) were tolerated.

The isolated, unpurified reaction products were screened for their activity to inhibit binding of radio-labeled human MIP-1α in HEK 293 cells expressing the human CCR1 receptor in a dose range, and these are reported as K_i values [3b]. The data for compounds that have a K_i < 3 μM are summarized in Table 2. The K_i data for the unpurified parent phenyl compound **7i** agrees with that of the purified **2a** (Table 1). The lower activity of **7i** is due to the fact that the screening concentration for **7i** is determined from the theoretical yield. The most potent compound discovered is 2-CF_3-phenyl-substituted **7a**. Other aryl groups with *ortho* (**7c**, **7e**, **7k**) or *meta* substituents (**7d**, **7f**, **7h**) and the 2-pyridyl analog **7c** show moderate activity.

To assess the activity of compounds generated from the automated, parallel library synthesis, the parent phenyl compound (**7i**) was synthesized in each library as an internal control. By comparing the activity of the parallel-synthesized **7i** to the independently synthesized, purified **2a**, compounds **7** could be ranked relative to each other and to the internal standard **7i**. This strategy allows rapid assessment of compounds for re-synthesis.

Our knowledge of the 3-fold increase in potency of the methyl carboxylate **2b** over the carboxylic acid **2a** encouraged us to synthesize methyl carboxylates **8** and **9** derived from carboxylic acids **7a** and **7h**. Gratifyingly, both methyl carboxylates **8** (K_i = 74 nM) and **9** (K_i = 187 nM) are approximately 3 to 5-fold more potent than the carboxylic acids analogs **7a** and **7h**. Intriguingly, 2-trifluoromethylphenyl-substituted **7a** is nearly 2-fold more potent than the parent unsubstituted phenyl compound **2b**, and is nearly equipotent with the cyano-containing series **1a-c**. Expecting a similar increase in potency, we chose to synthesize the 2-trifluoromethylphenyl analogs of cyano-containing **1b** and **1a** (e.g. **10** and **11**, respectively). Unfortunately, both **10** and **11** displayed a surprising lack of potency, demonstrating that the SAR of the two series **1** and **2** may not be paralleled. The discrepancy of the activities of **1b** and **1a** versus **10** and **11** probably indicates that this portion of the template interacts fairly closely with the CCR1 receptor [3a].

In conclusion, we have shown that an automated, parallel, solid-phase synthesis and screening strategy of over 100 compounds **7**, using commercially available aryl acetic acids as starting materials, has discovered novel, non-peptide CCR1 antagonists **8** and **9** (K_i = 74 and 187 nM, respectively).

REFERENCES AND NOTES

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[4] a) Krchnak, V.; Padera, V. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3261-3264 (b) Brennan, T.; Biddison, G.; Frauendorf, A.; Schwarcz, L.; Keen, B.; Ecker, D. J.; Davis, P. W.; Tindler, R.; Swayze, E. E. *Biotechnol. Bioeng*. **1998**, *61*, 33-45.

[5] Argonaut Nautilusä, Argonaut Technologies, San Carlos, CA.

[6] *Synthesis Procedure*: To Wang resin (0.15 g, 1.2 mmol/g, 0.18 mmol) and a diverse aryl acetic acid (0.27 mmol, 1.5 equiv.) was added 3 mL of a solution of DIC (1.5 equiv.) and DMAP (1.5 equiv.) in methylene chloride. The resin was agitated for 8 h followed by extensive washing with THF. THF (1 mL) was added followed by KHMDS in toluene (0.8 mL, 0.5 M, 2.2 equiv.) and then THF (0.8 mL). The resin was agitated for 0.5 h followed by addition of 1-chloro-3-iodopropane (0.8 mL, 4.1 equiv.) and THF (0.8 mL). The resin was heated to 60°C with agitation for 45 min, cooled and quenched with THF-water. The resin was washed extensively with THF. To the resin was added 3 mL of a solution of *para*-4-chlorophenylpiperidin-1-ol (5 equiv.) and DMAP in DMF (0.3 equiv.) The resin was then heated to 70°C with agitation for 6 h. The resin was washed extensively with THF and the product was cleaved from the resin with 10 % TFA in methylene chloride for 20 min. The product **7** was collected with 2 methylene chloride rinses (1 mL each).

[7] All new compounds have MS, ^{1}H NMR and elemental analysis consistent with their structure.