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Combinatorial Synthesis of CCR5 Antagonists

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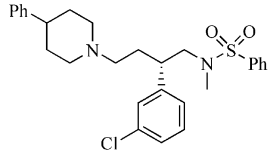
Abstract—Herein we report the preparation of a combinatorial library of compounds with potent CCR5 binding affinity. The library design was aided by SAR generated in a traditional medicinal chemistry effort. Compounds with novel combinations of subunits were discovered that have high binding affinity for the CCR5 receptor. A potent CCR5 antagonist from the library, compound **11** was found to have moderate anti-HIV-1 activity.

Over the past several years, combinatorial chemistry has emerged as a novel tool for the discovery and optimization of new leads in the pharmaceutical industry. We have previously described the preparation of libraries leading to the discovery of potent and selective receptor antagonists,[1] as well as various protease inhibitors.[2] While this work has been largely successful, we were interested in determining the impact that combinatorial techniques could have on an already established medicinal chemistry effort. In this paper, we report our findings on the combinatorial preparation of CCR5 antagonists.

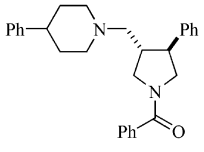
The CCR5 chemokine receptor is a member of the superfamily of seven-transmembrane spanning G-protein coupled receptors.[3] It has recently been discovered that the CCR5 receptor acts as primary co-receptor, together with the cell surface molecule CD4, for fusion then cell entry of certain HIV-1 viral strains.[4] Compelling evidence for the role of CCR5 in HIV-1 infection comes from a study of individuals who, due to a 32 base-pair deletion in the gene for CCR5, lack functional receptor. Individuals who are homozygous for this defect are highly resistant to HIV-1 infection[5] while heterozygous individuals show significantly delayed progression to AIDS.[6] As a result of these discoveries, many efforts to develop CCR5 antagonists have been undertaken.[7]

Compounds represented by structures **A** and **B** (Fig. 1) were being investigated by our colleagues in the medicinal chemistry group.[8] Extensive SAR was being developed around both the acyclic (structure **A**) and cyclic scaffolds (structure **B**). Our strategy was to use this SAR to design a library of compounds with the hope of discovering novel pharmacophore elements or novel combinations of known elements.

Our synthesis of a CCR5 antagonist library is shown in Scheme 1. We chose to employ the Kenner sulfonamide linker[9] recently popularized by Ellman.[10] Anchoring of the requisite scaffolds, **C** (Scheme 1), as the Boc



A



B

CCR5 IC_{50} = 30 nM vs MIP-1α CCR5 IC_{50} = 67 nM vs MIP-1α

Figure 1. CCR5 antagonists under investigation by medicinal chemistry.

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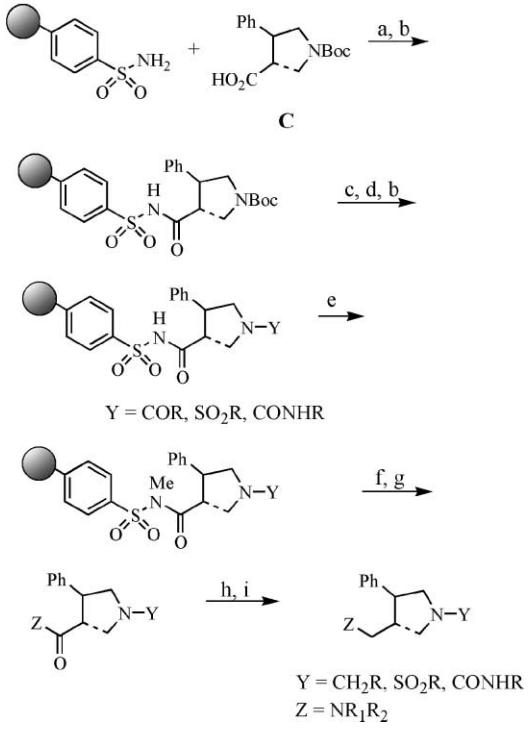
protected amino acid was accomplished using DIC in THF/DCM to form the symmetrical anhydride. Addition of the polystyrene bound arylsulfonamide[11] to the preformed mixed anyhydride in the presence of DMAP resulted in efficient coupling. Three separate scaffolds (used as racemic mixtures) were coupled and a portion of each resin was archived. The remaining resin was mixed and split into 39 equal pools. The Boc group was removed by treatment with TFA in methylene chloride and then an acylating agent was added to each pool. We chose 39 different 'Y subunits' as acylating agents from a variety of acid chlorides, sulfonyl chlorides and isocyanates. The selection of subunits was based on the SAR generated by our colleagues in the medicinal chemistry group.[8] Again a portion of resin from each pool was archived and the remaining resin was mixed and split into 100 equal portions. Alkylation of the sulfonamide with trimethylsilyl diazomethane followed by displacement with 2 equivalents of the amine 'Z subunits' afforded 100 pools of 117 compounds/pool. Again the selection of subunits was guided by the SAR generated by the med-chem effort.[8] The excess amine was removed by scavenging with a isothiocyanate resin.[12]

The 100 pools were then treated with borane–methyl sulfide complex in dioxane to reduce the amide bonds. Heating the products in HCl/MeOH followed by removal of the solvent[13] afforded the desired product pools. To evaluate the composition of the pools LC/MS analysis was performed and the results were compared with calculated spectra using a method previously described.[14]



Scheme 1. Solid-phase synthesis of CCR5 antagonist library: (a) DIC, THF/DCM then add resin and DMAP; (b) archive, mix and split resin; (c) TFA, DCM; (d) RCOCl or RSO_2Cl or RNCO (39 different 'Y' Subunits); (e) $TMSCHN_2$, THF; (f) R_1R_2NH, 50 °C (100 different 'Z' Subunits); (g) Polystyryl isothiocyanate resin; (h) BMS, dioxane, 50 °C; (i) HCl/MeOH, 50 °C then azeotrope 3×.

Evaluation of Biological Activity

The 100 pools of compounds were assayed for CCR5 affinity by measuring the ability of the mixtures to inhibit binding of ^{125}I-MIP-1α or ^{125}I-GP-120 (the HIV-1 envelope glycoprotein) to the CCR5 receptor in Chinese hamster ovary (CHO) cell membranes.[15] Assay results for a select set of Z pools is shown in Table 1.

The **Z1** pool containing 4-phenylpiperidine showed a 63% inhibition of MIP-1α binding and a 23% inhibition of GP-120 binding, both at a concentration of 1 micromolar. The 4-phenylpiperidine pool represents a standard for comparison since much of the early medicinal chemistry effort was focused on this subunit.[8a–d] Three pools, **Z8**, **Z9** and **Z10**, stood out from the others. These pools exhibited good inhibition (>70% @ 1 μM) of both GP-120 and MIP-1α binding while most of the other pools had much weaker affinity. Although the 4-(carbobenzyloxyethylamino)piperidine (amine in the **Z10** pool) was being investigated by the medicinal chemistry group the 4-(3-phenylpropyl) piperidine derivatives had not been extensively explored in previous work. Thus we chose to deconvolute the **Z8** and **Z9** pools.

The required Y pools were prepared from the archived resin using the chemistry described above and screened

Table 1. CCR5 binding of representative **Z** pools (117 compounds/pool)

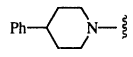
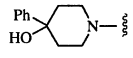
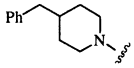
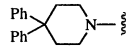
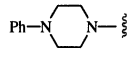
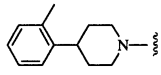
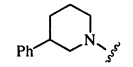
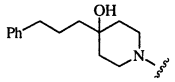
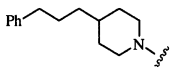
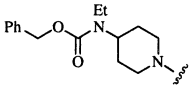
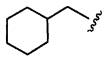
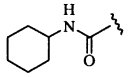
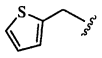
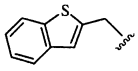
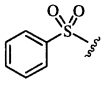
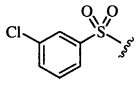
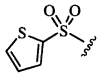
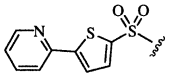
Pool no.	Amine	MIP-1α %Inhib@1 μM	GP-120 %Inhib@1 μM
Z1		63	23
Z2		49	19
Z3		61	13
Z4		5	0
Z5		11	0
Z6		22	11
Z7		16	0
Z8		75	84
Z9		79	88
Z10		76	72

Table 2. CCR5 binding of representative Y pools (three compounds/pool)

Pool no.	Subunit	MIP-1α %Inhib@100 nM		GP-120 %Inhib@160 nM	
		Amine **Z8**	Amine **Z9**	Amine **Z8**	Amine **Z9**
Y1		74	74	86	83
Y2		39	53	53	73
Y3		66	67	65	65
Y4		11	5	28	0
Y5		56	57	68	80
Y6		31	16	58	36
Y7		71	67	77	95
Y8		89	74	79	95

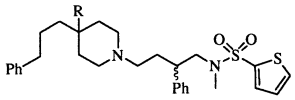
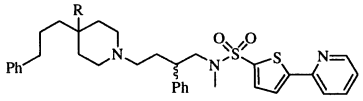
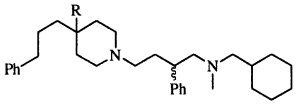
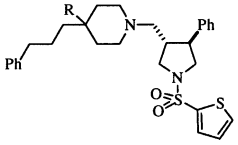
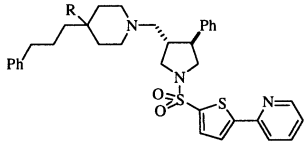
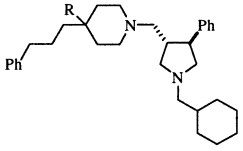
in the CCR5 binding assays. Table 2 shows the binding affinity for select Y pools for both amines **Z8** and **Z9**. Pools **Y5** contained the phenyl sulfonyl subunit used in the initial SAR work and serves as a basis for comparison. As can be seen three pools, **Y1**, **Y7** and **Y8** showed better inhibition against both MIP-1α and GP-120 than pool **Y5** for both amines **Z8** and **Z9**. Also pools **Y1** and **Y8** contained novel structural motifs, **Y1** having a methylcyclohexyl group and **Y8** having a 5-(2-pyridyl)thiophenyl-2-sulfonyl group. Based on these considerations of novelty and activity profiles pools **Y1**, **Y7** and **Y8** were selected for follow up.

From the data shown in Tables 1 and 2, a set of 12 single compounds were resynthesized and screened for binding affinity and antiviral activity. These results are shown in Table 3. Some interesting features are evident from the CCR5 binding data. When the 4-(3-phenylpropyl)piperidine derivatives were combined with the acyclic scaffold and with the sulfonamides as 'Y' subunits as in compounds **1**, **2**, **3**, and **4**, potent binding was observed. The IC_{50}'s of these compounds ranged from 2 to 7 nM. However, combination of the 4-(3-phenylpropyl)piperidine, the acyclic scaffold and the methyl cyclohexyl group as in compounds **5** and **6** resulted compounds with weak affinity for the receptor. Interestingly the situation was reversed for the cyclic pyrrolidine scaffold. Combination of the 4-(3-phenyl propyl)piperidine, the pyrrolidine scaffold and sulfonamide 'Y' subunits gave moderately potent compounds (**7**, **8**, **9**, and **10**) with IC_{50}'s in the 20 to 60 nM range. However, with the methylcyclohexyl substitution potent binding was observed. For example, compound **12** had a 6.5 nM binding affinity and the most potent compound, compound **11** had a 1 nM binding affinity.

Some of most potent compounds were then assayed for antiviral activity. In the acyclic series it was found that compound **2** had no activity, however compounds **3** and **4** blocked 95% of viral replication at 6.2 and 3.1 μM, respectively. Although these compounds all had similar binding affinity incorporation of the pyridiyl substituent is critical for antiviral activity in this series. The reason for this apparent discrepancy is unclear.[16] Compound **11**, based on the pyrrolidine scaffold was the most potent compound in the library having an IC_{95} of 580 nM in the antiviral assay and a binding affinity of 1 nM. Thus, the aliphatic cyclohexyl substituent, the aryl propyl piperidine side chain and the rigid pyrrolidine scaffold in combination give rise to a compound with potent binding and antiviral activity.

In summary, we have developed a solid phase synthesis of CCR5 antagonists and constructed a library based on SAR generated from a classical medicinal chemistry study. From this library, a novel combination of pharmacophore elements resulted in compound **11** which has potent receptor binding and moderate anti-viral activity. This discovery has opened up a new direction for the design of CCR5 antagonists based on a pyrrolidine scaffold with arylpropylpiperidine and aliphatic side chains. Further elaboration of this class of molecules will be reported in due course.

Table 3. Activity of single compounds

Compd	Structure	CCR5 Binding[a] IC_{50} (nM)	Antiviral[b] IC_{95} (μM)
1, R = OH		6.5	
2, R = H		3	NA[c]
3, R = OH		4	6.2
4, R = H		2	3.1
5, R = OH		NA[c]	
6, R = H		NA[c]	
7, R = OH		20	
8, R = H		25	
9, R = OH		41	
10, R = H		60	
11, R = OH		1	0.58
12, R = H		6.5	

[a]Binding against MIP-1α, average of three titrations.
[b]Inhibition of viral growth over 7 days vs YU-2 strain of HIV-1.
[c]No significant activity at 10 micromolar.

References and Notes

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