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Evaluation of secondary amide replacements in a series of CCR5 antagonists as a means to increase intrinsic membrane permeability. Part 1: Optimization of gem-disubstituted azacycles

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ABSTRACT

Replacement of a secondary amide with an *N*-acyl or *N*-sulfonyl gem-disubstituted azacyle in a series of CCR5 antagonists led to the identification of compounds with excellent in vitro HIV antiviral activity and increased intrinsic membrane permeability.

CCR5 is one of the many chemokine receptors involved in the mediation of the inflammation process by activating and inducing migration of several effector T cell subsets, such as antigen-presenting leukocytes and Th1-polarized T cells. It is now recognized that CCR5 is among the major regulators of Th1-cell recruitment in autoimmune diseases.[1] In addition to its role as a receptor on inflammatory leucocytes, CCR5 is also the primary co-receptor for macrophage-tropic HIV-1 virus. The Δ32 mutation in the CCR5 allele causes the expression of a truncated, nonfunctional CCR5 protein. Individuals homozygous for the CCR5 Δ32 mutation are resistant to HIV-1 infection while heterozygosity prolongs seroconversion time. While epidemiologic studies still have to demonstrate more clearly whether or not CCR5 deficiency is associated with overt or covert biologic adverse effects, CCR5 has proven to be an extraordinary target for the pharmaceutical industry, with therapeutic applications in HIV-1/AIDS treatment[2] as well as potentials in various chronic or acute inflammatory diseases.

We recently disclosed a series of CCR5 antagonists (Fig. 1).[3] This series contains the four pharmacophores found in most of the series reported in the literature (i.e., a tertiary basic amine, two hydrophobes in the Western part of the molecule which will be referred to as the tail, and an aryl or heteroaryl in the Eastern part, which will be referred to as the head).

Herein we describe part of our SAR focusing on the tail region. Specifically, we describe the evaluation of alternative ways to present hydrophobe **1** that would not involve the use of a secondary amide. We hypothesized that by (re)moving the amide hydrogen bond donor we would increase the intrinsic membrane permeability of the series by reducing the desolvation energy cost (i.e., leading to a decrease in the membrane/solvent partition coefficient) and/or by reducing interaction with the Pgp efflux pump.[4] We

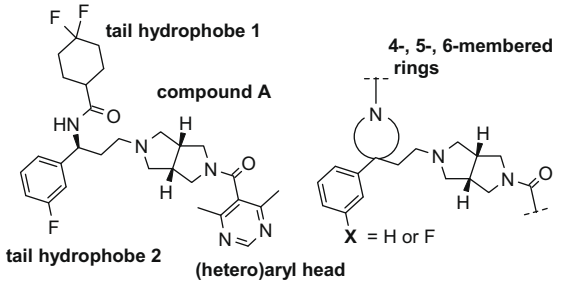

Figure 1. A representative example of our series of CCR5 antagonists, **A**, showing the four required pharmacophores and the proposed azacylic replacements of the tail secondary amide.

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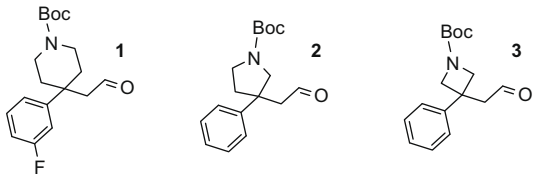
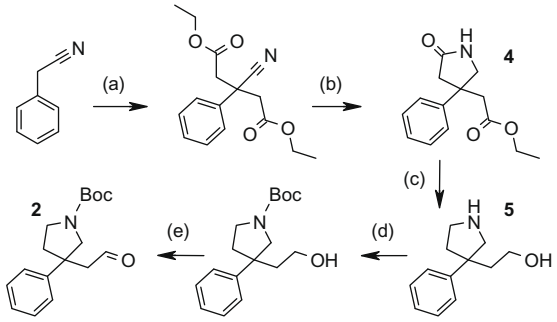


Figure 2. Structure of the three aldehydes required for the synthesis of compounds in the piperidine-tail, pyrrolidine-tail, and azetidine-tail sub-series, respectively.



Scheme 1. Synthesis of aldehyde **2**. Reagents and conditions: (a) LHMDS, ethyl bromoacetate, THF, −78 °C to rt, 24 h, 69%; (b) Raney-nickel, H_2 (50 psi), MeOH, 48 h, 92%; (d) LAH, THF, 70 °C, overnight, 78%; (d) Boc_2O, $NaHCO_3$, THF/H_2O, rt, overnight, 80%; (e) Dess–Martin periodinane, $CH_2Cl_2/^tBuOH$, rt, overnight, used crude.

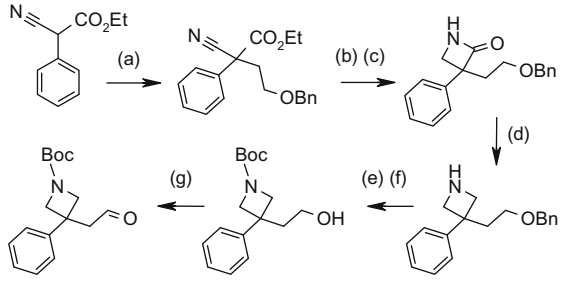
mostly focused on saturated gem-disubstituted 4-, 5-, and 6-azacycles (Fig. 1).

The synthesis of compounds in the piperidine-, pyrrolidine-, and azetidine-tail sub-series required the synthesis of aldehydes **1**, **2**, and **3** (Fig. 2).

Aldehyde **1** was prepared according to literature procedures.[5] Racemic aldehyde **2** was prepared according to the procedure depicted in Scheme 1.

Phenylacetonitrile was bis-alkylated with ethyl bromoacetate. Reduction of the nitrile was carried out with hydrogen and Raney-nickel and the corresponding amine cyclized in situ with one of the appended acetic esters to form lactam **4**. Both the lactam and the remaining ester were reduced with lithium aluminum hydride to give aminoalcohol **5**. The pyrrolidine nitrogen was protected with a Boc group and the alcohol was oxidized to the aldehydes using Dess–Martin periodinane.

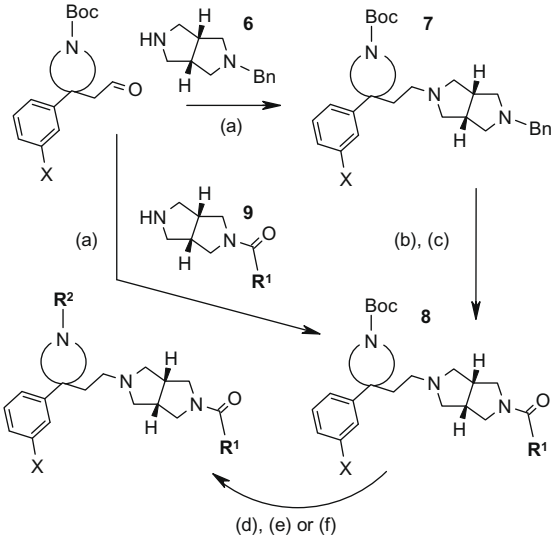
The preparation of aldehyde **3** was carried out by a similar sequence (Scheme 2). Ethyl phenylcyanoacetate was alkylated with benzyl 2-bromoethyl ether. Selective reduction of the nitrile to the corresponding primary amine was performed with sodium



Scheme 2. Synthesis of aldehyde **3**. Reagents and conditions: (a) NaH, benzyl 2-bromoethyl ether, DMF, 0–60 °C, overnight, 60%; (b) $NaBH_4$, $CoCl_2{\cdot}6H_2O$, MeOH, rt, 16 h, 40%; (c) MeMgI, Et_2O/THF, 0 °C to rt, 3 h, 40%; (d) LAH, THF, 60 °C, 6 h; (e) Boc_2O, $NaHCO_3$, THF/H_2O, rt, 3 days, 49% (over two steps); (f) H_2 (1 atm), 20% $Pd(OH)_2$, EtOH, rt, 24 h, >95%; (g) TEMPO, NaOCl, $NaHCO_3$, CH_2Cl_2/H_2O, 0 °C to rt, 4 h, used crude.

borohydride in the presence of cobalt(II) chloride. In this case, the amine did not cyclize spontaneously onto the ester but required the use of methylmagnesium iodide as a base. The azetidinone was reduced with lithium aluminum hydride and the azetidine nitrogen was protected with a Boc group. The benzyl ether was then hydrogenolyzed and the primary alcohol was oxidized to aldehyde **3** with TEMPO/sodium hypochlorite.

The CCR5 antagonists with varying head and tail substituents (**R**^{1}, **R**^{2}, respectively) were prepared according to two general procedures (Scheme 3).



Scheme 3. General synthetic approach of analogs in the three sub-series. Reagents and conditions: (a) $NaBH(OAc)_3$, CH_2Cl_2, rt, 2 h; (b) H_2 (1 atm), 20% $Pd(OH)_2$, EtOH, rt, 24 h, >95%; (c) **R**^{1}COOH, EDCI, HOBt, Et_3N (or DIPEA), CH_2Cl_2, rt, overnight, 40–80%; (d) HCl 4 M in dioxane, CH_2Cl_2, rt, 2 h; (e) **R**^{2}COOH, EDCI, HOBt, Et_3N (or DIPEA), CH_2Cl_2, rt, overnight or **R**^{2}COCl, pyridine (or Et_3N), CH_2Cl_2, 0 °C to rt, overnight, or **R**$^2SO_2Cl$, Et_3N, CH_2Cl_2, 0 °C to rt, overnight, 50–80%.

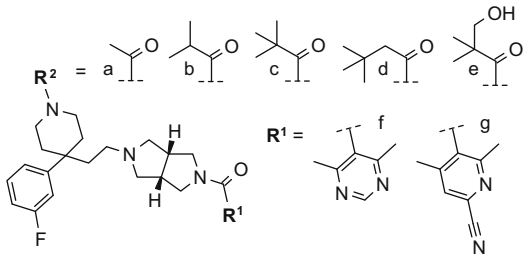


Figure 3. Representative compounds **10**–**16** prepared in the piperidine-tail sub-series.

Table 1
RANTES binding inhibition,[6] cell fusion inhibition,[7] and antiviral activity[8] of piperidine-tail compounds **10**–**16**

Compounds			Binding inhibition	Cell fusion inhibition	Antiviral activity
	R^2	R^1	IC_{50}[a] (nM)	IC_{50}[a] (nM)	IC_{50}[a] (nM)
10	a	f	>500	>2500	nd[b]
11	b	f	102	160	>400
13	c	f	59	129	67
14	d	f	nd[b]	339	447
15	e	f	167	1000	nd[b]
16	c	g	15	37	18

[a] Values are means of at least two experiments.
[b] Not determined.

Reductive N-alkylation of the aldehydes with template **6**[3] formed **7** as an orthogonally protected bis secondary amine intermediate. The pyrrolidine *N*-benzyl group was hydrogenolyzed to give an amine which was acylated using standard procedures to give **8**. Alternatively, the aldehydes were reacted with template **9**[3] in which one of the pyrrolidine amines was already bearing the head acyl group $\mathbf{R^1}$. The tail Boc group was then hydrolyzed and the tail amine was acylated or sulfonylated.

From the first parallel synthesis array that was prepared (Fig. 3), it became apparent that only hydrophobic bulky acyl groups $\mathbf{R^2}$ were tolerated (Table 1).

Introduction of some polarity such as in compound **15** abolished the antiviral activity. The best compound prepared in the array was **13** which showed an antiviral activity of 67 nM. During our SAR studies (data not shown), we discovered that substituted heteroaryl $\mathbf{R^1}$ heads usually increased antiviral activity. We thus replaced the standard $\mathbf{R^1}$ = 5-(4,6-dimethylpyrimidine) head in compound **13** with $\mathbf{R^1}$ = 5-(4,6-dimethyl-2-cyanopyridine)[9] and noticed a close to fourfold improvement in antiviral activity (compound **16**) (see Table 1).

We prepared a set of compounds varying the size of the pyrrolidine nitrogen substituent $\mathbf{R^2}$ (Fig. 4) and observed that in this subseries, the type of groups tolerated was even more limited than in the piperidine-tail sub-series, with cyclopentyl being the best. In this sub-series as well, $\mathbf{R^1}$ = 5-(4,6-dimethyl-2-cyanopyridine) increased activity up to 15-fold compared to $\mathbf{R^1}$ = 5-(4,6-dimethylpyrimidine) (compounds **17** and **18**). We did not separate the enantiomers of **17**–**20**, the activity reported in Table 2 are for the racemic mixtures (see Table 2).

Having learned from the piperidine- and pyrrolidine-tail subseries that CCR5 did not offer much space in the hydrophobe 1 binding region, a limitation that led to a mostly flat SAR, we anticipated that as we reduced the size of the tail azacycle to a four-membered ring we would be able to attach more diverse types of substituents than in the previous sub-series (Fig. 5). This turned out to be the case (see Table 3).

Unlike the piperidine- and pyrrolidine-tail sub-series, the azetidine-tail sub-series accepted both amide and sulfonamide groups (compounds **25** and **30**). Also, while slightly polar groups were tolerated (compounds **27** and **29**), better antiviral activity was seen with hydrophobic substituents. In general, the more hydrophobic the substituent, the more active the compound (**25**, **26**, **28**, **31**, **32**), with 4,4-difluorocyclohexyl being the best. The activity of the series was further improved by introducing $\mathbf{R^1}$ = 5-(4,6-dimethyl-2-cyanopyridine) as the head substituent (compounds **31** and **38**). When the azetidine nitrogen $\mathbf{R^2}$ acyl substituent was 3,3-difluorocyclobutyl, electron-withdrawing groups such as trifluoromethyl at C_2 of the pyrimidine improved potency close to twofold (compounds **35** compared to **32**). When the pyridine head was used instead of the pyrimidine head, C_2-substitution improved potency close to 10-fold (compounds **39** and **41** compared to **37**). The boost in potency was the same for both substituents (cyano or trifluoromethyl). Interestingly, when the azetidine nitrogen $\mathbf{R^1}$ acyl substituent was the optimal 4,4-difluorocyclohexyl, the combined effect of a better tail hydrophobe 1 and an optimal substituted pyridine head seemed to be more than additive. Indeed, compound **40** (substituent = trifluoromethyl) was greater than 10-fold more potent (activity below detection level) than the unsubstituted equivalent, **36**. Compound **38** (substituent = cyano) on the other hand was only sixfold more potent than **36**, which indicated to us that the trifluoromethyl substituent was better than the cyano substituent at increasing the activity. In other words, the right combination of tail and head substituents led to

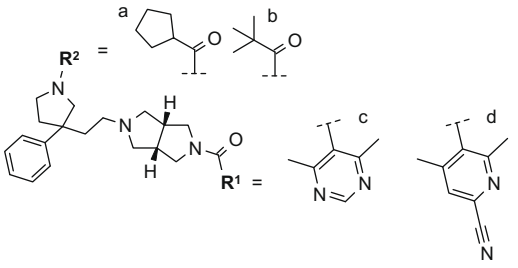


Figure 4. Representative compounds **17**–**20** prepared in the pyrrolidine-tail subseries.

Table 2
RANTES binding inhibition,[6] cell fusion inhibition,[7] and antiviral activity[8] of pyrrolidine-tail compounds **17**-**20**

Compounds			Binding inhibition	Cell fusion inhibition	Antiviral activity
	R^2	R^1	IC_{50}[a] (nM)	IC_{50}[a] (nM)	IC_{50}[a] (nM)
17	a	c	116	361	351
18	a	d	46	85	24
19	b	c	242	1943	nd[b]
20	b	d	69	360	nd

[a] Values are means of at least two experiments.
[b] Not determined.

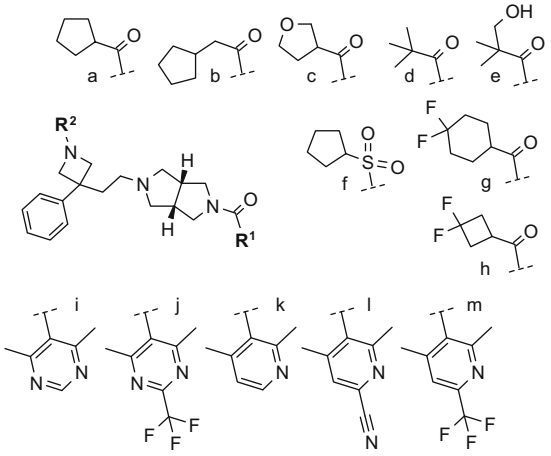
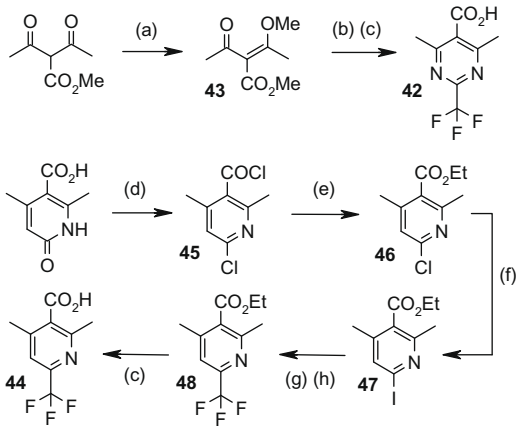


Figure 5. Representative compounds **25**–**41** prepared in the azetidine-tail series.

Table 3
RANTES binding inhibition,[6] cell fusion inhibition,[7] and antiviral activity[8] of the azetidine-tail compounds **25**–**41**

Compounds			Binding inhibition	Cell fusion inhibition	Antiviral activity
	R^2	R^1	IC_{50}[a] (nM)	IC_{50}[a] (nM)	IC_{50}[a] (nM)
25	a	i	67	65	60
26	b	i	28	136	53
27	c	i	55	559	204
28	d	i	27	186	77
29	e	i	56	310	130
30	f	i	27	458	40
31	g	i	28	10	21
32	h	i	30	93	39
33	c	j	38	92	59
34	g	j	30	5	4
35	h	j	66	168	23
36	g	k	38	15	12
37	h	k	60	53	55
38	g	l	17	<0.3	2
39	h	l	24	1	4
40	g	m	21	2	⩽1.2
41	h	m	22	3	4

[a] Values are means of at least two experiments.



Scheme 4. Syntheses of acids **42** and **44**. Reagents and conditions: (a) Cs_2CO_3, MeOTf, (b) trifluoroacetamide, acetone/MeOH, 0 °C to rt overnight, 44%; (c) KOH, EtOH/H_2O, 40 °C, overnight, acidic work-up, 66% for acid **42**, 75% for acid **44**; (d) $POCl_3$, 80 °C; (e) EtOH, 0 °C then rt, 30 min (60% over two steps); (f) NaI, TMSCl, 75 °C, 4 h,%; (g) CuI, KF, $TMSCF_3$, NMP, 70 °C, overnight; (h) H_2 (40 psi), 10% Pd/C, AcOH, MeOH, rt, 48 h, 45% over three steps.

the identification of compounds with substantially better than expected antiviral potencies. The mechanistic explanation of such a cooperative effect is still not clear, but we hypothesized that it could be related to some dynamic effect of such combination leading to a more efficient allosteric rearrangement[10] of the CCR5 receptor which is required for antiviral activity after binding of the antagonist.

The preparation of compounds **33**–**35** and **40**, **41** required the introduction of heteroaryl heads derived from acid **42** (**R**^{1}COOH where **R**^{1} = **j**) and acid **44** (**R**^{1}COOH where **R**^{1} = **m**). They were synthesized according to the sequences depicted in Scheme 4. Acid **42** was synthesized in three steps from the commercially available 3-methyl-pentane-2,4-dione using a modified literature procedure.[11] The enolate of 2-acetyl-3-oxo-butyric acid methyl ester was trapped with methyl triflate to form intermediate **43** which was then treated with trifluoroacetamidine under neutral conditions at room temperature to give the ethyl ester of **42** in 44% yield.[12] The ester was then hydrolyzed to the acid with potassium hydroxide. Evaporation of the reaction mixture and acidification of an aqueous solution of the recovered residue led to the crystallization of the acid **42** which was obtained in 66% yield (see Scheme 4).

The synthesis of acid **44** was carried out in six steps from the commercially available 2,4-dimethyl-6-oxo-1,6-dihydro-pyridine-3-carboxylic acid. The starting material was treated with phosphorus oxychloride to give acyl chloride **45** which was then treated with ethanol to give ester **46**. The chloride was exchanged with an iodide and **47** was then treated with trifluoromethyl carbene generated in situ from trimethylsilyltrifluoromethane and potassium fluoride. Subsequent hydrogenolysis allowed the purification of the trifluoropyridine **48** from the unsubstituted pyridine generated by reduction of chloride **46** carried over from the iodination reaction or reduction of the unreacted iodide **47**. Saponification of the ester led to the isolation of the acid **44** in 75% yield.

Representative compounds from the three sub-series were tested in a 21-day Caco-2 permeability assay.[13] In order to address the potential influence of Pgp in the apparent permeability, the assay (in both directions) was run in the presence or absence of the Pgp inhibitor elacridar.[14] We believe that the results of the assay in the presence of elacridar gave us a good estimate of the intrinsic permeability versus the apparent permeability observed in the assay without elacridar, by eliminating the influence of Pgp on potential substrates. Also, in order to alleviate the possibility of over- or under-estimating the flux values, mass recovery in both compartments were performed and were shown to be within the set target values in all cases. Finally, the good solubility in the assay buffer of the compounds tested did not lead us to believe that the observed results could be biased either way. We describe here the results of only a small set of compounds (see Table 4), but the trend was shown to be general.

Table 4
Apparent fluxes in 21-day Caco-2 assays with or without elacridar and apparent efflux ratio

Compounds	Papp: AB[a] 21-day Caco-2 without elacridar	Papp: AB 21-day Caco-2 with 2 μM elacridar	Efflux ratio BA[b]/AB 21-day Caco-2 without elacridar
A	0.22	5.41	55.38
13	2.98	14.9	6.39
16	2.73	17.5	6.78
18	2.55	14.3	8.25
38	0.53	10.2	32.26
39	0.44	10.7	40
40	1.72	14.6	10.35
41	1.59	13.2	10.69

[a] Apparent apical to basolateral fluxes (cm/s × 10^{-6}).
[b] Apparent basolateral to apical fluxes (cm/s × 10^{-6}), data not shown.

The apical to basolateral (AB) apparent fluxes (with elacridar) of compounds **13**, **16**, **18**, **38**, **39**, **40**, **41** were all at least twofold greater than that of the representative primary-amide-tail compound **A** (Fig. 1). Since no correlation could be made between the AB apparent fluxes (without elacridar) and *c*log *P*, log *D*, or polar surface area, we concluded that this was due to an overall increase in intrinsic permeability of the gem-disubstituted azacyclic series versus our original series. The increase was even noticeable in the AB apparent fluxes (without elacridar) of compounds **13**, **16**, **18**, **40**, and **41** in which the possible efflux influence of Pgp seemed to be reduced and/or compensated. Interestingly, despite the increase in intrinsic permeability, the AB apparent fluxes (without elacridar) of compounds **38** and **39** were fairly low compared to other compounds. To us, this indicated that Pgp might transport those compounds more efficiently and that the efflux might no longer be compensated by the increase in permeability.

While we were able to improve the intrinsic permeability of our lead series in vitro, high metabolic clearance precluded us from observing an effect in vivo (data not shown). However, it will be addressed on a parallel series in part 2.

In this Letter, we described the synthesis and evaluation of three new sub-series of saturated gem-disubstituted azacylic CCR5 antagonists. Careful tuning of head and tail substituents allowed the identification of derivatives with excellent in vitro HIV-1 antiviral activity. Moreover, compounds within the three sub-series showed increased intrinsic permeability compared to that of the original primary amide tail series, as measured in the Caco-2 permeability assay.

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