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Evaluation of amide replacements in CCR5 antagonists as a means to increase intrinsic permeability. Part 2: SAR optimization and pharmacokinetic profile of a homologous azacyle series

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ABSTRACT

Replacement of a secondary amide with a piperidine or azetidine moiety in a series of CCR5 antagonists led to the discovery of compounds with increased intrinsic permeability. This effort led to the identification of a potent CCR5 antagonist which exhibited an improved in vivo pharmacokinetic profile.

CCR5 and its endogenous ligands RANTES, MIP1-α, and MIP1-β are implicated in organ transplant rejection as well as in the pathophysiology of inflammatory diseases such as rheumatoid arthritis (RA). Additionally, in 1996 it was discovered that CCR5 acts as the co-receptor for cellular entry of R5-tropic HIV-1 viruses, which predominate during the early stages of viral infection.[1] Since individuals who are homozygous for the CCR5 delta 32 mutation (which results in non-functional CCR5) are resistant to HIV infection and experience less severe symptoms of RA,[2] CCR5 presents an exciting target for the development of small molecule antagonists for the treatment of HIV, inflammatory diseases and/or to control transplant rejection.[3]

We previously introduced a new series of CCR5 antagonists exemplified by compound **1** (Fig. 1).[4]

Compound **1** contains the four pharmacophores found in many of the series reported in the literature: a tertiary basic amine, two hydrophobes in the western part of the molecule (the tail), with hydrophobe 1 tolerating a limited level of polarity, and a (hetero)aryl group in the eastern part of the molecule (the head).[5]

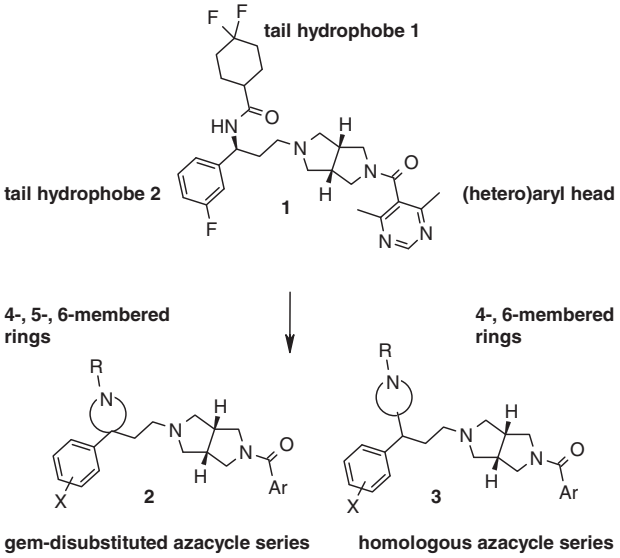

Figure 1. Compound **1**, a representative example of our CCR5 antagonist series, the gem-disubstituted azacycle series (**2**), and the homologous piperidine and azetidine azacycle series (**3**).

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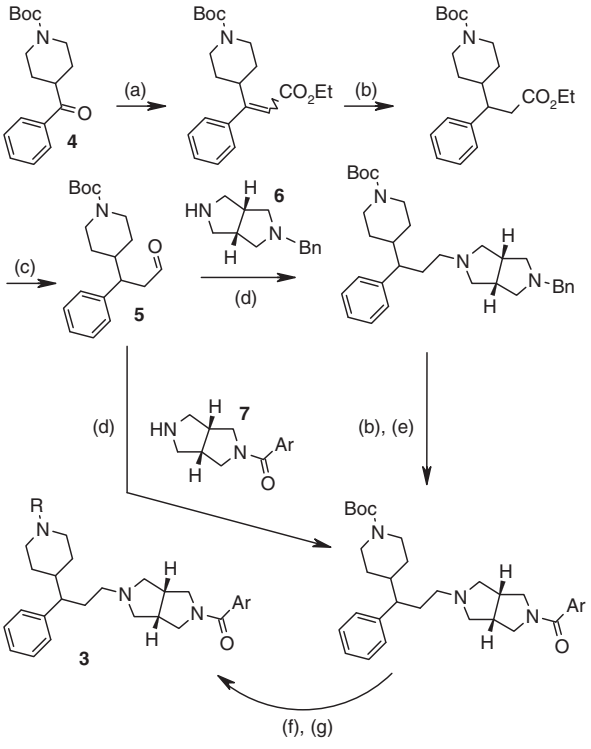
Not unexpectedly, based on the physicochemical properties of the series (molecular weight above 500, a number of hydrogen-bonding functionalities, and a basic amine), most compounds in this series showed borderline membrane permeability[6] (intrinsic permeability[7] in our assay ranging from 2 to 5 × 10^{-6} cm/s) and were subject to transport by the efflux pump P-glycoprotein (P-gp). In some cases, the low permeability translated into poor pharmacokinetic profiles (low oral bioavailability, high clearance). A similar challenging preclinical pharmacokinetic profile was reported for maraviroc (Selzentry®), the first FDA-approved small molecule HIV entry inhibitor.[8] We reasoned that by (re)moving the tail primary amide we might be able to improve the intrinsic permeability by reducing the desolvation energy cost (i.e., leading to a decrease in the membrane/solvent partition coefficient) and/or by reducing the affinity for P-gp.[9]

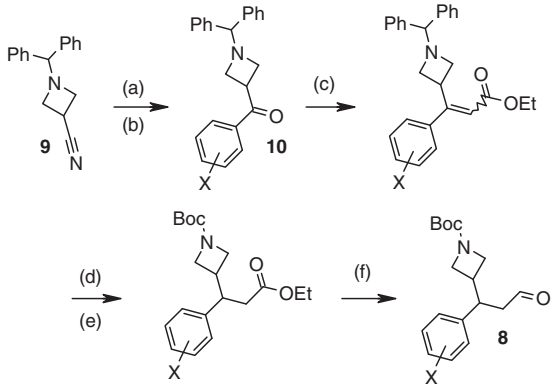
In an earlier publication we presented a first set of compounds in which the tail amide of our initial series had been replaced by gem-disubstituted azacycles (Fig. 1, **2**).[10] These compounds, as hypothesized, showed improved intrinsic permeability in vitro; unfortunately, due to the lack of metabolic stability, no in vivo effect of the improvement could be demonstrated.

In this paper, we describe the optimization of a parallel series in which the tail amide was replaced with homologous piperidine and azetidine moieties (Fig. 1, **3**). These replacements were attractive not only because they allowed us to test our hypothesis, but also because similar groups were described previously in the CCR5 literature.[11]

As shown in Scheme 1, the synthesis of the piperidine-containing compounds began with elaboration of the known 4-benzoyl-piperidine-1-carboxylic acid *tert*-butyl ester **4** via a Horner–Wadsworth–Emmons reaction. Hydrogenation of the double bond followed by selective reduction of the racemic ester yielded the key racemic aldehyde intermediate **5**. We were able to separate both enantiomers of the ester and in some cases to use the enantiomerically pure fractions in the subsequent chemical steps. The final compounds were prepared according to two general procedures. In the first procedure, aldehyde **5** was treated under reductive amination conditions with the benzyl-protected bispyrrolidine core **6**.[12] Removal of the benzyl group was followed by acylation of the free amine. In the second procedure, the fully elaborated system **7** was employed in the reductive amination reaction. In both cases, the completion of the synthesis involved deprotection of the tail amine and treatment with acyl chlorides, sulfonyl chlorides, isocyanates, chloroformates or alkyl triflates.



Scheme 1. General synthetic methods for the preparation of compounds in the piperidine sub-series. Reagents and conditions: (a) (diethoxy-phosphoryl)-acetic acid ethyl ester, NaH, THF, 0–60 °C, overnight, 90–95%; (b) H_2 (1 atm), 20% $Pd(OH)_2$, EtOH, rt, 24 h, >85%; (c) DIBAL-H, CH_2Cl_2, −78 °C to rt, overnight; Tempo, bleach, CH_2Cl_2/H_2O, 70–80% over two steps; (d) $NaBH(OAc)_3$, HOAc, CH_2Cl_2, rt, 2 h overnight, 47–73%; (e) ArCOOH, EDCI, HOBt, Et_3N (or DIPEA), CH_2Cl_2, rt, overnight, 30–80%; (f) HCl 4 M in dioxane, CH_2Cl_2, RT, 2 h; (g) RCOOH, EDCI, HOBt, Et_3N, CH_2Cl_2, rt, overnight, or RCOCl (or RSO_2Cl, $TfOCH_2CF_3$, RCNO, or RCOCl) Et_3N, CH_2Cl_2, 0 °C to rt, overnight, 50%.



Scheme 2. Synthesis of compounds in the azetidine series. Reagents and conditions: (a) PhMgBr, 3-F-PhMgBr or 3,5-diF-PhMgBr, benzene, 0 °C to rt, overnight; (b) aqueous NaOH, EtOH, 60 °C, 50–75% over two steps; (c) (diethoxy-phosphoryl)-acetic acid ethyl ester, NaH, THF, 0–60 °C, overnight, 90–95%; (d) H_2 (1 atm), 20% $Pd(OH)_2$, EtOH, RT, 24 h, >95%; (e) Boc_2O, NaOH, THF/H_2O, rt, overnight, 80–90%.

The azetidine-containing compounds were synthesized using the same general procedure, but using aldehyde **8** instead of aldehyde **5**. Aldehyde **8** was prepared from commercially available 1-benzhydryl-azetidine-3-carbonitrile **9** according to the route outlined in Scheme 2.

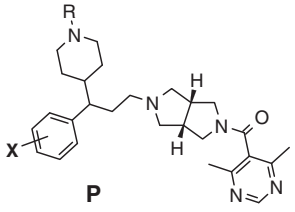
Grignard addition with either phenylmagnesium bromide, 3-fluorophenylmagnesium bromide or 3,5-difluorophenylmagnesium bromide, and hydrolysis of the resulting imine gave the ketone intermediates **10**, which were then employed in a Horner–Wadsworth–Emmons reaction. Hydrogenation of the double bond and concomitant removal of the benzhydryl group gave the free amine, which was Boc protected. Selective reduction of the ethyl esters yielded the racemic aldehydes **8**. Reductive amination and tail piece introduction were carried out following the steps described in Scheme 1.

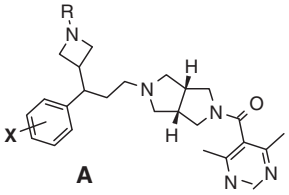
The fluoro analogs in the piperidine subset were synthesized starting from known commercially available Boc-protected 4-cyano-piperidine in a sequence analogous to the one described in Scheme 2 for the azetidine subset.

To explore the largest chemical space possible, we prepared an array of ureas, carbamates, sulfonamides, amides, and fluoroalkylamines. Unless specified, compounds in both subsets were prepared as racemic mixtures. In this initial array the head heteroaryl was kept constant (4,6-dimethyl-pyrimidine-5-yl). The compounds were tested as inhibitors of RANTES binding, of cell fusion, and of viral entry (Table 1).

The most potent compounds of this array contained small fluorinated alkyl chains (**12**–**15**) or sulfonamides (**17**) in the piperidine subset and sulfonamides in the azetidine subset (**24**–**27**). Urea, carbamate and amide substituents were not tolerated and resulted in loss of cell–cell fusion inhibition (**11**, **16**, **18**, **22** and **23**), even if the

Table 1
RANTES binding inhibition,[14] cell–cell fusion inhibition,[15] and antiviral activity[16] of representative piperidine- and azetidine-tail compounds





Compd	Tail	X	R	Binding IC_{50}[a] (nM)	Cell–cell fusion inhibition IC_{50}[a] (nM)	Antiviral activity IC_{50}[a] (nM)
11	P	H		>500	>5000	—[b]
12	P	H		17	34	64
13	P	H		27	137	55
14	P	3-F		35	11	14
15	P	3,5-di-F		25	19	20
16	P	H		102	744	—[b]
17	P	H		47	99	48
18	P	H		32	>5000	—[b]
19	P	H		72	>5000	—[b]
20	P	H		68	2819	—[b]
21	A	H		>500	>5000	—[b]
22	A	H		207	2832	—[b]
23	A	H		>500	>5000	—[b]
24	A	H		23	93	100
25	A	H		40	313	103
26	A	3-F		39	30	156
27	A	3,5-di-F		35	63	90

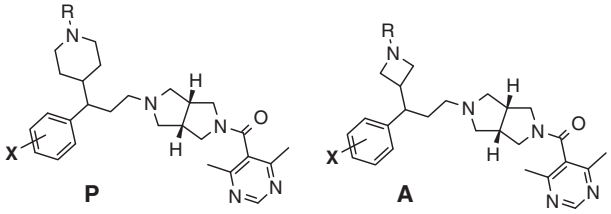
[a] Values are means of at least two experiments.
[b] Not determined.

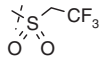
compounds inhibited RANTES binding. As has been observed by others,[13] in some cases RANTES binding inhibition did not translate into functional activity (cell fusion, antiviral) due to different conformational change requirements for binding inhibition and functional activity. In the piperidine subset, for example, carbamate **16** and amide **18** showed RANTES binding inhibition (102 nM and 32 nM, respectively), but essentially no cell–cell fusion inhibition was observed. In general, we used the RANTES binding inhibition assay and the cell fusion assay as first activity screens, and used the functional antiviral assay to compare compounds. It is worth noting that we never encountered a compound inactive in the binding assay and active in the functional assays.

Earlier SAR suggested that antiviral activity could be improved by the introduction of halogen atoms in the aromatic ring of tail hydrophobe 2. In the case of the homologous piperidine and azetidine sub-series, we did not observe any significant activity effect (compounds **13** and **14** vs **12** and compounds **26** and **27** vs **25**).

With the encouraging potencies observed for the sulfonamide analogs in either subset (**17**, **24**–**27**), we focused on the optimization

Table 2
RANTES binding inhibition,[14] antiviral activity[16] and metabolic stability of piperidine- and azetidine-sulfonamides



Compd	Tail	X	R	Binding inhibition IC_{50}[a] (nM)	Antiviral activity IC_{50}[a] (nM)	HLM[b]
25	A	H		40	103	40
28	A	H		52	>625	—[c]
29	A	H		22	31	294
30	P	H		48	207	—[c]
31	P	H		27	71	—[c]
32	P	H		25	39	90
33	P	H		23	59	—[c]
34	P	H		34	43	41
35	P	3-F		44	16	67
36	P	H		38	6	—[c]

[a] Values are means of at least two experiments.
[b] Human liver microsomal intrinsic clearance (μl/min/mg protein); in our assay, values ⩾35 μl/min/mg protein were considered to be high clearance values.
[c] Not determined.

of antiviral potency by incorporating different sulfonamide tail pieces. Representative compounds are shown in Table 2.

In the azetidine subset, larger sulfonamide tail pieces led to compounds with higher potencies, as seen in analog **29**, which showed an antiviral IC_{50} of 31 nM. However, we were not able to increase antiviral activity further by modifying the azetidine sulfonamide substituent. In the piperidine subset, with the exception of the trifluoro ethane group, compound **30**, various sulfonamide groups (**31**–**36**) were well tolerated, but did not significantly improve antiviral activity. The best substituent in this sub-series was the phenyl sulfonamide (**36**). Unfortunately, the phenyl substituent introduced cytochrome P450 inhibition liabilities. Various modifications such as incorporation of heteroatoms and substituents did not result in compounds with better profiles (data not shown). In this array, **34** was one of the most active and metabolically stable compounds and was used as starting point for the preparation of a set of compounds in which the head piece was varied (Table 3).

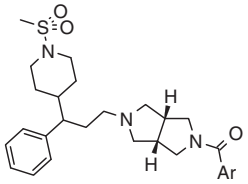
The incorporation of sulfonamides in the head region was not tolerated, as seen with compounds **37** and **38**. We then introduced two of the best heads in our original series. While the 4,6-dimethyl-pyran-2-one (**39**) did not improve antiviral potency, the incorporation of the 4,6-dimethyl-pyridine-2-carbo-nitrile[17] head piece increased the antiviral potency approximately 10-fold (compound **40**, 4 nM, compared to compound **34**, 43 nM). However, compound **40** lacked metabolic stability in our in vitro human liver microsome assay and was not further profiled.

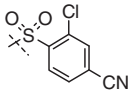
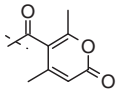
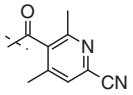
We prepared both enantiomers of compounds **34** and **35** using enantiomerically pure aldehyde **5**. It was found that the activity resided fully in one enantiomer as shown in Table 4. We did not determine the absolute stereochemistry of the active enantiomers **41** and **43**.

In order to evaluate our hypothesis that the intrinsic permeability could be improved by removing the tail primary amide portion, we tested representative compounds in both subsets in a 21-day Caco-2 permeability assay in the presence and absence of the P-gp inhibitor elacridar.

Most of the compounds synthesized possessed at least twofold higher intrinsic permeability[7] values than the representative primary-amide-tail compound **1**, as shown in Table 5. This trend was shown to be general, and no correlation between *c* log *P*, log *D* or polar surface area and the obtained AB fluxes (with or without

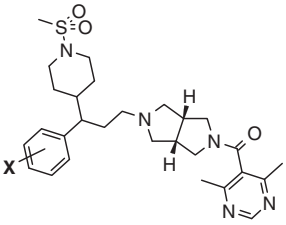
Table 3
RANTES binding inhibition,[14] cell–cell fusion inhibition,[15] antiviral activity[16] and metabolic stability of piperidine methylsulfonamides



Compd	R	Binding inhibition IC_{50}[a] (nM)	Cell–cell fusion inhibition IC_{50}[a] (nM)	Antiviral activity IC_{50}[a] (nM)	HLM[b]
34		34	57	43	41
37		104	2173	>625	—[c]
38		215	4123	—[c]	—[c]
39		27	8	37	63
40		31	1.2	4	162

[a] Values are means of at least two experiments.
[b] Human liver microsomal intrinsic clearance (μl/min/mg protein).
[c] Not determined.

Table 4
RANTES binding inhibition[14] and antiviral activity[16] of both enantiomers of **34** and **35**



Compound	X	Binding inhibition IC_{50}[a] (nM)	Antiviral activity IC_{50}[a] (nM)
Enantiomer 1 (**41**)	H	25	42
Enantiomer 2 (**42**)	H	>500	>5000
Enantiomer 1 (**43**)	F	32	17
Enantiomer 2 (**44**)	F	>500	—[b]

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

elacridar) could be established. It is also noteworthy to point out that the AB apparent fluxes (without elacridar) also improved at least twofold, with flux values in some cases moving from the low permeability category in our assay to the medium category. The efflux ratios, however, remained relatively high in the in-vitro assay, indicating that under the assay conditions P-gp might transport the compounds quite efficiently despite the increase in intrinsic permeability.

Table 5
Apparent fluxes in 21-day Caco-2 assays[a] with or without elacridar and apparent efflux ratio

Compd	Papp: AB[b] 21-day Caco-2 without elacridar[c]	Papp: AB 21-day Caco-2 with 2 μM elacridar	Efflux ratio BA[d]/AB 21-day Caco-2 without elacridar
1	0.22	5.41	55.38
29	1.4	12.40	11.30
34	0.87	16.50[e]	24.10
40	0.85	18.10	30.00
41	0.5	10.20	42.90
43	0.5	12.60	41.40

[a] Mass recovery was within set target values in all cases.
[b] Apparent apical to basolateral fluxes (10^{-6} cm/s).
[c] Permeability category for Caco-2 assay (0–1, low; 1–10, medium; >10, high).
[d] Apparent basolateral to apical fluxes (10^{-6} cm/s).
[e] Papp: AB 21-Caco-2 in the presence of 1 μM elacridar.

Table 6
Key properties of **43**

Antiviral activity IC_{50}[a] (nM)	hERG[b] IC_{20} (μM)	Intrinsic clearance[a] (μL/min/mg protein)			Intrinsic permeability[c]
		RLM	DLM	HLM	
16	15.4	40	18	60	12.6

[a] Values are means of at least two experiments.
[b] Measured at 37 °C.
[c] Unit: 10E-6 cm/s; see also Ref. 7.

Table 7
Pharmacokinetic parameters of **43** in rat[a] and dog[a]

Rat	Dog
Dose	Dose
Oral: 10 mg/kg	Oral: 5 mg/kg
IV: 1 mg/kg	IV: 1 mg/kg
F = 6.6%	F = 96%
Cl = 40 mL/min/mg	Cl = 31 mL/min/kg
IV $T_{1/2}$ = 0.37 h	IV $T_{1/2}$ = 4 h
C_{max} = 110 ng/mL	C_{max} = 1300 ng/mL

[a] Values are means of two experiments.

Compound **43** was selected for further evaluation. A summary of key properties is shown in Table 6. Compound **43** had good antiviral potency, was not an inhibitor of the hERG[18] potassium ion channel and the cytochrome P450 CYP enzymes,[19] and showed a medium microsomal stability.[20] The improved intrinsic permeability of **43** together with its moderate in vitro clearance translated into an appealing pharmacokinetic profile in rat and dog, with an oral bioavailability of 7% and 94%, respectively (Table 7). Additionally, an in-house comparison showed that **43** maintained higher exposures compared to maraviroc in both species (Fig. 2).

In summary, we described the synthesis and optimization of azacyclic CCR5 antagonists. Careful tuning of the head and tail substituents resulted in the identification of potent compounds. Additionally, compounds of this azacyclic series showed increased intrinsic permeability compared to the original amide tail series. The improved intrinsic permeability of **43** together with its moderate in vitro clearance translated into an appealing pharmacokinetic profile.

Acknowledgements

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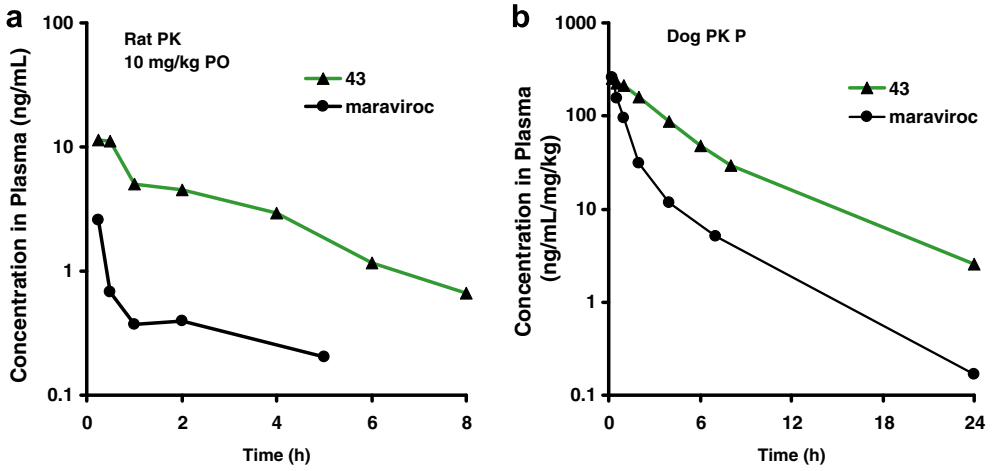

Figure 2. Dose-normalized exposures; (a) rat PO PK comparison of **43** (5 mg/kg) and maraviroc (5 mg/kg); (b) dog PO PK comparison of **43** (5 mg/kg) and maraviroc (10 mg/kg). Values are means of two experiments.

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