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1,3,4 Trisubstituted Pyrrolidine CCR5 Receptor Antagonists Bearing 4-Aminoheterocycle Substituted Piperidine Side Chains

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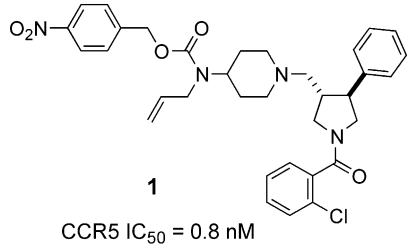
Abstract—A new class of 4-(aminoheterocycle)piperidine derived 1,3,4 trisubstituted pyrrolidine CCR5 antagonists is reported. Compound **4a** is shown to have good binding affinity (1.8 nM) and antiviral activity in PBMC's ($IC_{95} = 50$ nM). Compound **4a** also has improved PK properties relative to **1**.

In 1996, it was shown that HIV-1 infection of macrophages, monocytes and T-cells is mediated by interaction with, in addition to the cell surface molecule CD4, the β-chemokine receptor CCR5.[1] This discovery initiated an intense research effort directed at the development of CCR5 antagonists as potential anti-HIV therapeutic agents.[2] Our own work in this area has led to the discovery of 1,3,4 trisubstituted pyrrolidine derivatives that have been found to be high affinity CCR5 receptor antagonists with excellent anti-HIV activity in vitro.[3]



1

CCR5 IC_{50} = 0.8 nM
HeLa IC_{90} = 1 nM
PBMC IC_{95} = 31 nM

Compound **1** was recently reported as an example of these compounds.[3b] Although **1** has excellent antiviral activity, its poor in vivo properties prompted further optimization. In this paper we wish to report the modification of the carbamate moiety in **1** to an aromatic heterocycle with the aim of improving the pharmacokinetic parameters while maintaining the desired antiviral activity.

The synthesis of these 4-aminoheteroaryl piperidine derivatives is illustrated in Scheme 1. The 1-Boc-4-aminopiperidine was prepared in two steps from commercially available *N*-Boc-piperidin-4-one by reductive amination with benzyl amine followed by hydrogenolysis of the benzyl group. Arylation of the 4-amino group was carried out in one of two ways depending on the reactivity of the aryl halide. For aryl halides known to undergo electrophilic substitution, such as 2-chloropyrimidine derivatives, simple substitution by heating the aryl halide and 4-amino-1-Boc-piperidine in isopropanol afforded products in reasonable yields. Less

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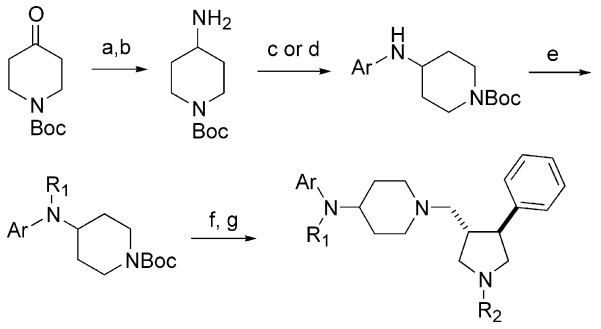
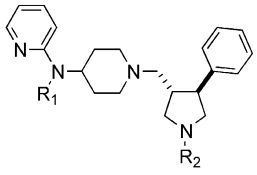


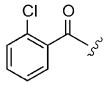
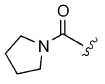
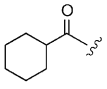
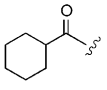
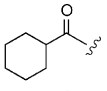
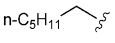
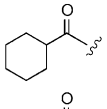
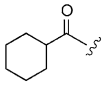
Scheme 1. Reagents and conditions: (a) benzyl amine, $NaHB(OAc)_3$, DCE; (b) Pd/C, H_2, MeOH; (c) ArCl, DIEA, *i*PrOH, 90 °C (Ar = 2-chloropyrimidine derivatives); (d) ArX, $Pd(OAc)_2$, BINAP, NaO*t*Bu, dioxane, 100 °C (Ar = phenyl, pyridyl or unreactive pyrimidine derivatives); (e) $KNTMS_2$, alkyl halide, THF; (f) HCl/MeOH; (g) aldehyde, $NaHB(OAc)_3$, DIEA, DCE.

reactive aryl halides were incorporated by palladium mediated coupling according to method of Buchwald.[4] Alkylation of the nitrogen was then carried out by deprotonation with potassium hexamethyldisilazide and reaction with an appropriate alkyl halide. Removal of the Boc group with HCl and reductive alkylation with the requisite 3-formyl-4-phenyl pyrrolidine derivatives afforded after deprotection, the final pyrrolidine products.

Based on the assumption that the carbamate carbonyl in **1** is acting as an H-bond acceptor our initial studies focused on 2-pyridyl derivatives. Receptor binding and antiviral activities are shown in Table 1. Data are reported as IC_{50}'s for both binding versus MIP-1α,[5] and antiviral activity in a single-cycle infection assay.[6] We were pleased to find that although the activity for this series was weaker than that for **1**, low nano-molar binding affinity and modest antiviral activity were observed for this class (antiviral data is reported as IC_{50}'s since the compounds in this series were of modest activity). For example 2-chlorobenzoyl derivative **2a** was 14 nM in the binding assay and blocked 50% of the viral infection at 300 nM. Simple modifications to the pyrrolidine *N*-group such as with the urea derivative **2b** or the cycloalkyl derivatives **2c** and **2d**, also provided similar activity. When the pyrroldine substituent was a cyclohexylcarbonyl group, changing the R group on the

Table 1. CCR5 binding affinity and antiviral activity of pyrrolidine amides



Compd	R_1	R_2	MIP-1α IC_{50} (nM)[a]	HeLa IC_{50} (nM)[b]
2a			14	300
2b			9	300
2c			10	300
2d			12	100
2e			6	11
2f			9	33
2g			64% inh @ 1000 nM	
2h			78% inh @ 1000 nM	

[a]Values are means of three experiments for displacement of 125-I labeled MIP-1α from CCR5 receptor expressed on cell (CHO) membranes, see ref 5 for assay conditions.
[b]Inhibition over 48 h of the BAL strain of HIV in HeLa Magi cells expressing CCR5 and CXCR4, SD were generally + 20% of average, see ref 6 for assay conditions.

4-amino position of the piperidine to an allyl (**2e**) or a cyclopropylmethyl (**2f**) group improved the antiviral activity ~3- to 10-fold. Larger groups such as *n*-hexyl or benzyl, as in **2g** and **2h**, resulted in loss of activity.

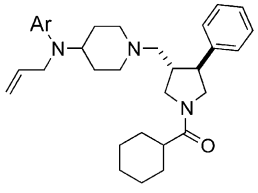
We then investegated a series of *N*-allyl compounds with different aromatic groups substituted at the 4-aminopiperidine position. The data for these compounds are shown in Table 2. Moving the pyridine nitrogen from the 2 position (**2e**) to the 3 position (**2i**) gave a compound with similar antiviral activity but 4-fold weaker receptor binding affinity. The 4-pyridyl derivative **2j** was weaker still, with a binding affinity of 66 nM and no appreciable antiviral activity. Similarly, the phenyl derivative **2l** was also unable to block viral infection and was less potent in the binding assay. Pyrimidine derivative **2k** was equipotent to **2e** in the antiviral assay but displayed a 2-fold weaker binding affinity. Substitution of the 2-pyridine with a trifluoromethyl group resulted in a ~4-fold loss in both binding and antiviral activity.

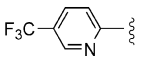
At this point in our studies it was shown that incorporation of a carboxylic acid at the N-1 position of the pyrrolidine resulted in improved antiviral activity as well as better selectivity for the CCR5 receptor versus other targets.[3d] As a result of this observation, we prepared a series of analogues to probe the effect of acidic functionality in the 4-(*N*-propylamino heterocyclic) piperidine series. The data for these compounds are shown in Table 3. We were gratified to find that these compounds had low nano-molar binding affinity and indeed were better inhibitors of viral infection (data reported as IC_{90}'s since compounds in this series were more potent). For example, compound **3a** was 3.9 nM at the receptor and blocked 90% of viral infection at 100 nM. This compares to **2d** which at the same concentration only blocked 50% of the viral infection. Similarly, compounds **3b** and **3c** were potent antivirals both having IC_{90}'s of 33 nM.

We tested compounds **3a**–**3c** for their in-vivo properties by dosing them in rats at 0.5 mpk iv and 2 mpk po. Unfortunately, pyridyl derivatives **3a** and **3b** had high clearances (53 and 106 mL/min/kg, respectively) and poor bioavailabilities (20 and 8%, respectively) resulting in low plasma levels ($C_{max} = 53$ and 19 nM, respectively). Although pyrimidine derivative **3c** had a much lower clearance (17 mg/mL/kg), its bioavailibility was only 8%. During the course of this optimization we found that by incorporating a 3-fluorophenyl rather than phenyl at the 4 position of the pyrrolidne the in vivo profile was substantially improved. Thus the 20% bioavailability of **3d** was 2.5-fold better than that of **3c** and the clearance was similar at 13 mg/mL/kg. This resulted in an increased plasma exposure for **3d** ($C_{max} = 140$ nM vs 49 nM for **3c**).

To further enhance the binding and antiviral activity of this series of compounds we prepared a series of analogues with a N-1 cyclobutylmethyl in place of a N-1 cyclohexyl group off the pyrrolidine nitrogen. In previous work this modification was shown to provide a 10-fold improvement in binding and/or antiviral potency.[3d]

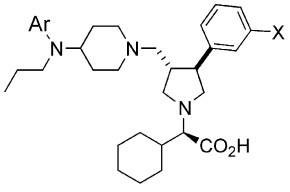
Table 2. Modification of the piperidine 4-aryl group



Compd	Ar	MIP-1α IC_{50} (nM)[a]	HeLa IC_{50} (nM)[b]
2e		6	11
2i		23	12
2j		66	n/a
2k		12	12
2l		42	n/a
2m		21	37

[a]See note a Table 1.
[b]See note b Table 1.

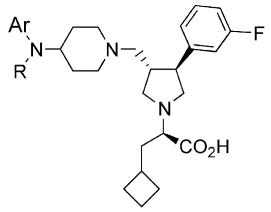
Table 3. Effects of incorporation of a carboxylic acid

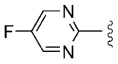
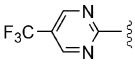
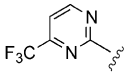
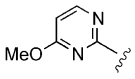


Compd	Ar	X	MIP-1α IC_{50} (nM)[a]	HeLa IC_{50} (nM)[b]
3a		H	3.9	100
3b		H	5	33
3c		H	6.6	33
3d		F	1.6	33

[a]See note a Table 1.
[b]See note b Table 1.

Table 4. Incorporation of cyclobutylmethyl and 3-fluorophenyl substituents



Compd	Ar	R	MIP-1α IC_{50} (nM)[a]	HeLa IC_{50} (nM)[b]
4a		n-C_3H_7–	1.8	11
4b		n-C_2H_5–	1.5	100
4c			1.1	11
4d		n-C_3H_7–	1.7	11
4e		n-C_3H_7–	1.2	33
4f		n-C_3H_7–	0.4	33
4g		n-C_3H_7–	0.5	100
4h		n-C_3H_7–	1.2	450
4i		n-C_3H_7–	0.8	100

[a]See note a Table 1.
[b]See note b Table 1.

The binding and antiviral data for this series of compounds are shown in Table 4 and pharmacokinetic paramaters for some analogues are shown in Table 5.

In this series of compounds the substitution of the cyclobutylmethyl for the cyclohexyl group at the pyrrolidine N-1 position resulted in only a modest (3-fold) increase in antiviral activity, compound **4a** having a binding affinity of 1.8 nM and an IC_{90} in the HeLa assay of 11 nM. Changing the *n*-propyl group to an ethyl group at the piperidine-4-amino position resulted in a 10-fold loss in antiviral activity. In contrast to the earlier studied pyrrolidine N-1 amide series (**2d** vs **2f**) the cyclopropylmethyl derivative **4c** showed no improvement in activity versus **4a**. Isomeric pyrimidine derivatives **4d** and **4e** had similar binding affinity and antiviral activity. Since the 2-aminopyrimidine derivatives showed the best in vivo profiles (see Table 5) we examined ring substitution in this series of compounds. Substitution at the 5 position with either a fluoro (**4f**) or a trifluoromethyl (**4g**) group resulted in ~3-fold increase in binding affinity versus **4a**, however, the antiviral activity was 3-fold (**4f**) and 10-fold (**4g**) less than **4a**. Compounds **4h** and **4i**, having a 4-trifluoromethyl or a 4-methoxy substituent, respectively, were also potent in the binding assay but showed decreased antiviral activity compared to **4a**.

Table 5. Pharmacokinetic paramaters for compounds **4a–e**, **4g**, and **4h** dosed 0.5 mpk iv and 2 mpk orally in rats

Compd	Clp (mL/min/kg)	t1/2 (h)	%F	C_{max} (nM)
4a	28	1.1	29	256
4b	26	1.1	33	210
4c	28	0.7	iv[a]	iv[a]
4d	81	0.3	8	28
4e	90	0.9	iv[a]	iv[a]
4g	9	2.8	72	433
4h	13	1.5	25	177

[a]iv, tested via iv dose only.

We investigated the in vivo properties of some of these compounds by dosing in rats (Table 5). Compound **4a**, the most interesting antiviral compound, had a moderate clearance rate (28 mg/mL/kg) and good bioavailability of 29%. Although the half life was somewhat short (1.1 h), the plasma exposure was good, C_{max} being 256 nM. The ethyl derivative **4b** had a very similar profile. The isomeric pyrimidine derivative **4d** had a much higher clearance and shorter half life. Plasma exposure was poor for this compound which had a C_{max} of 28 nM. In contrast, the 5-trifluoromethyl derivative **4g** had significantly enhanced PK parameters. The low clearance of 9 mg/mL/kg and the increased bioavailability of 72% resulted in good plasma levels, the C_{max} of 433 nM was the best in this series. These enhancements were not observed with the 4-trifluoromethyl derivative **4h**. On balance, compound **4a** had the best profile so we assayed it, along with the cyclohexyl analogue **3d**, for antiviral activity in a more rigorous 7-day assay in primary blood mononuclear cells.[6] Compound **3d** was able to block 95% of the viral infection (IC_{95}) at 375 nM while **4a** was equally effective at 50 nM. The activity of **4a** compares well with that of compound **1** (31 nM) in the PBMC assay.

In summary, we have developed a new class of 4-(heteroarylamino)piperidine derived 1,3,4 trisubstituted pyrrolidine CCR5 antagonists. Many of these compounds are potent antivirals and have good in vivo properties.

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