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1,3,4-Trisubstituted Pyrrolidine CCR5 Receptor Antagonists. Part 2: Lead Optimization Affording Selective, Orally Bioavailable Compounds with Potent Anti-HIV Activity

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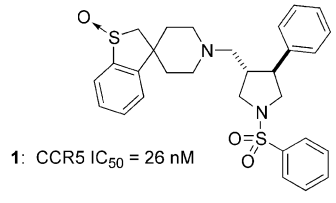
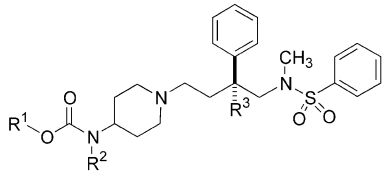
Abstract—Investigations of the structure–activity relationships of 1,3,4-trisubstituted pyrrolidine human CCR5 receptor antagonists afforded orally bioavailable compounds with the ability to inhibit HIV replication in vitro.

The inhibition of viral entry into host cells has recently emerged as an attractive new target for human immunodeficiency virus (HIV) therapy. Agents that interfere with the interaction of the HIV envelope glycoprotein gp120 and the cellular receptor CD4 as well as agents that inhibit the function of the HIV fusion peptide gp41 have been described.[1] A Phase II clinical trial with the entry inhibitor T-20 has provided an important proof-of-concept experiment in humans as it was demonstrated that this compound could successfully block HIV entry in vivo.[2] The β-chemokine receptor CCR5 has been identified as the major co-receptor required for the entry of HIV-1 into monocytes, macrophages and primary T-cells,[3,4] and reports from Takeda[5] and Schering–Plough[6] describing small molecule human CCR5 receptor antagonists that inhibit HIV replication have recently appeared.

In a previous communication,[7] we described the discovery of 1,3,4-trisubstituted pyrrolidines (exemplified by **1**) as a novel lead class of CCR5 receptor antagonists. Investigations of acyclic 2-aryl-4-(piperidin-1-yl)butanamine CCR5 antagonists has led to the identification of several analogues (such as **2**–**4**) with good affinity for the CCR5 receptor and potent anti-HIV activity.[8–11] Our efforts to incorporate some of the favorable pharmacophoric elements from the acyclic compounds into the pyrrolidines has resulted in the identification of a series of selective, orally bioavailable pyrrolidine analogues possessing the ability to inhibit HIV replication in vitro.



1: CCR5 IC_{50} = 26 nM



2: R^1 = CH_3-, R^2 = c-$C_5H_{11}CH_2$-
R^3 = H (racemate)
CCR5 IC_{50} = 22 nM

3: R^1 = $PhCH_2$-, R^2 = CH_3CH_2-
R^3 = H (racemate)
CCR5 IC_{50} = 7 nM

4: R^1 = (4-NO_2)$PhCH_2$-
R^2 = CH_2=$CHCH_2$-, R^3 = CH_3-
CCR5 IC_{50} = 0.1 nM
PBMC CIC_{95} (YU-2) ≤ 8 nM
PBMC CIC_{95} (BAL) = 13 nM

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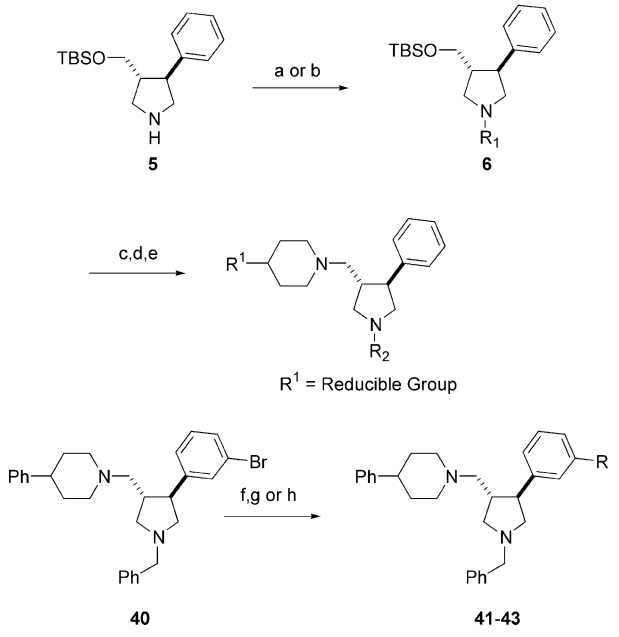
The synthetic chemistry used to prepare the majority of the pyrrolidine analogues in this work has been previously described.[7] Analogues with structural elements that were incompatible with the catalytic hydrogenation employed in the earlier synthetic route were prepared as shown in Scheme 1. Three analogues (**41**–**43**) that were used to probe the SAR of the C3 phenyl ring of the pyrrolidines were prepared by adding a palladium-catalyzed coupling step to the previously described route. The syntheses of the 4-(*N*-alkyl-*N*-carbamoyl) aminopiperidine subunits used to prepare analogues **46**–**63** have been reported.[10]

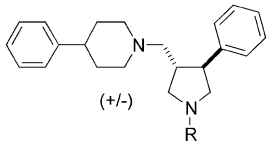
The discovery that several distinct piperidine subunits could impart significant CCR5 binding affinity to the acyclic series of compounds[8–11] prompted the incorporation of these same subunits into the pyrrolidine scaffold. Racemic pyrrolidine analogues containing the structurally simple 4-phenylpiperidine subunit[9] were among the first targeted and the phenylsulfonamide **7** was prepared (Table 1). Compound **7** was found to be of comparable potency to the initial pyrrolidine lead compounds in an assay to measure the ability of test compounds to displace ligand [^{125}I]-MIP-1α from the CCR5 receptor expressed on Chinese Hamster Ovary (CHO) cell membranes,[7] but a result obtained with one of the intermediates used in its preparation provided a point of divergence from the acyclic antagonists. While the *N*-phenylsulfonyl substituent in the acyclic compounds (such as **2**–**4**) was absolutely required for CCR5 activity,[8] it quickly became apparent that this was not so with the pyrrolidines as *N*-benzyl analogue **8** was only 2-fold less potent than phenylsulfonamide **7**. Following up on this observation, chain extension (**9**–**11**) was found to offer little apparent advantage while truncation (**12**–**13**) afforded less potent compounds. Other alterations of the functionality linking the pendant phenyl ring to the pyrrolidine nitrogen (**14**–**16**) led to the most potent analogue of the series, benzamide **15**. The phenyl sulfonamide substituent of the acyclic antagonists was determined to be metabolically labile when incubated with rat liver microsomes;[11] while similar experiments were not carried out with these initial analogues, they did provide a flexible starting point for further investigations.



Scheme 1. Reagents: (a) RCOCl, DIEA or TEA, CH_2Cl_2; (b) RCO_2H, EDC or BOP-Cl, DIEA or TEA, CH_2Cl_2; (c) TBAF, THF, 0 °C to rt; (d) $(COCl)_2$, DMSO, DIEA, CH_2Cl_2, −78 to 0 °C; (e) 4-substituted piperidine, $NaB(OAc)_3H$, TEA, CH_2Cl_2; (f) $PhB(OH)_2$, Na_2CO_3, $(Ph_3P)_4Pd$, EtOH/toluene, reflux (R = –Ph, **41**, 44%); (g) $(CH_3)_4Sn$, $(Ph_3P)_3PdCl_2$, DMF, 100 °C (R = –Me, **42**, 61%); (h) CO, TEA, $(Ph_3P)_4Pd$, MeOH, reflux (R = $-CO_2CH_3$, **43**, 38%).

A series of substituted benzamide derivatives based on **15** was prepared and the CCR5 binding affinities of

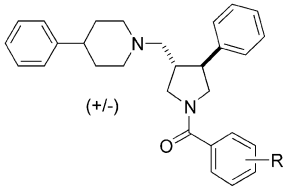
Table 1. Inhibition of [^{125}I]-MIP-1α binding[a] by various N1 analogues



Compound	R	CCR5 IC_{50} (nM)
7	$PhSO_2$–	152 ± 16
8	$PhCH_2$–	255 ± 40
9	$PhCH_2CH_2$–	200 ± 17
10	$PhCH_2CH_2CH_2$–	> 1000
11[b]	$Ph(CH_3)CH$–	480 ± 120
12	Ph–	> 1000
13	H–	980 ± 580
14	PhNHCO–	610 ± 170
15	PhCO–	67 ± 7
16	$PhCH_2CO$–	155 ± 10

[a]Displacement of ^{125}I-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes. Data are reported as mean ± SD for $n = 3$ determinations. See ref 7 for assay protocol.
[b]1:1 Mixture of racemic diastereomers.

Table 2. Inhibition of [^{125}I]-MIP-1α binding by N1 benzamide analogues[a]



Compound	R	CCR5 IC_{50} (nM)
15	H	67 ± 7
17	2-Cl	52 ± 3
18	3-Cl	94 ± 14
19	4-Cl	750 ± 100
20	2-MeO	140 ± 11
21	3-MeO	140 ± 11
22	4-MeO	> 1000
23	$2\text{-}CF_3$	97 ± 17
24	2-F	96 ± 5
25	2-Ph	89 ± 8
26	2-Br	48 ± 5
27	$2\text{-}CO_2CH_3$	32 ± 3

[a]Displacement of ^{125}I-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes. Data are reported as mean ± SD for $n = 3$ determinations. See ref 7 for assay protocol.

these compounds are shown in Table 2. Substitution at the 2-position of the benzamide was found to be tolerated and electron-withdrawing groups seemed to be favored over electron-donating groups (**17**–**22**). Exchanging other functional groups for the 2-chloro substituent of **17** was generally deleterious with the methyl ester **27** being the only new compound with greater CCR5 affinity than **15**. All of the six possible dichlorobenzamide analogues of **15** were prepared and all were less potent than the 2-chloro analogue **17** (data not shown).

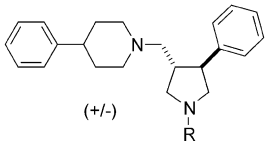
Since substitution of the benzamide phenyl ring of **15** did not generally afford compounds with enhanced CCR5 affinity, the nature of the amide substituent itself was examined (Table 3). The 1-naphthoyl analogue **28** was about 3-fold more potent than benzamide **15**; interestingly, the corresponding 2-naphthoyl analogue was at least 50-fold less active (data not shown). The introduction of a basic pyridine ring (**29**) gave a less active compound, but other neutral heterocycles, such as furan and thiophene, were found to be suitable phenyl substitutes (**30**–**32**). An aromatic group was not required for activity (**33**–**35**), while cyclic substituents seemed to be favored over an acyclic one (cf. **34** and **36**).

A series of N1-benzyl pyrrolidine compounds was prepared in order to the examine the SAR of the C3 aromatic ring (Table 4). Substitution with a variety of functional groups at the 3-position of the phenyl ring was tolerated, but was sensitive to the steric requirements of the substituent as evidenced by the loss in potency seen with any group larger than methyl or fluoro. 3-Thienyl (**45**) was also a suitable phenyl substitute; these observations paralleled those seen for the central aromatic ring of the acyclic 2-aryl-4-(piperidin-1-yl)butanamine antagonists.[9] 4-(*N*-Alkyl-*N*-carbamoyl)-amino piperidine pharmacophores had been demonstrated to impart appreciable CCR5 affinity to acyclic antagonists such as **2**–**4**; the ability to significantly inhibit HIV replication in vitro was observed of **4**.[11] Three of these piperidine subunits were incorporated into pyrrolidine compounds containing various N1 and C3 pharmacophores identified from the previously examined (4-phenyl)piperidine analogues (Table 5). In addition to determining the [^{125}I]-MIP-1α binding affinity for this set of compounds, selected analogues were also examined for their ability to inhibit a single HIV infection cycle in a CCR5-expressing HeLa Magi cell line.[12] The potency trends observed in the [^{125}I]-MIP-1α binding assay with the less active (4-phenyl)piperidine analogues (**8**, **15**, **17**, **28**, **33**, and **34**) approximately carried over to analogues **46**–**57**, while the more potent analogues **58**–**63** appeared to diverge from the expected result. Gratifyingly, the antiinfectivity activity of these compounds as determined by their CIC_{90} values in the HeLa Magi cells followed the predicted trends with (2-chloro)-benzoyl analogue **60** being the most active.[13]

The pharmacokinetics and the ability of analogues **54** and **60** to inhibit HIV replication in normal human donor PMBCs[14] were examined (Table 6). While being a relatively weak antiviral compound, **54** did exhibit the cross-species oral bioavailability that was lacking in acyclic analogue **4**. Analogue **60** demonstrated that potent anti-HIV activity could be obtained with the 1,3,4-trisubstituted pyrrolidine compounds. Both **54** and **60** were found to bind in a highly selective manner to CCR5 as compared to other chemokine receptors in competition assays similar to that used to examine the inhibiton of [^{125}I]-MIP-1-α binding to CCR5. Both **54** and **60** were determined to have IC_{50} values greater than 1 μM for the CCR1, CCR2, CCR3, CCR4, CXCR3, and CXCR4 receptors. Additionally, **60** was found to be a full antagonist of CCR5.[15]

Investigation of the SAR requirements of 1,3,4-trisubstituted pyrrolidine CCR5 receptor antagonists led to

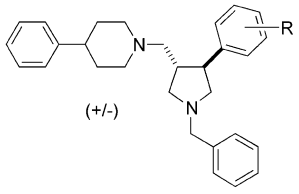
Table 3. Inhibition of [^{125}I]-MIP-1α binding by N1 amide analogues[a]



Compound	R	CCR5 IC_{50} (nM)
15	PhCO–	67±7
28	1-Naphthoyl	19±2
29	Nicotinoyl	260±34
30	2-Furanoyl	55±5
31	3-Furanoyl	33±3
32	2-Thienoyl	46±4
33	Cyclohexanoyl	34±3
34	Cyclopentanoyl	19±2
35	Cyclobutanoyl	48±5
36	2-(Ethyl)butanoyl	57±6

[a]Displacement of ^{125}I-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes. Data are reported as mean±SD for $n=3$ determinations. See ref 7 for assay protocol.

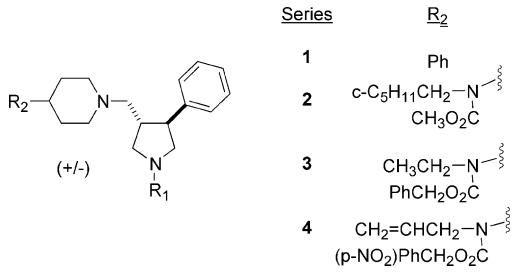
Table 4. Inhibition of [^{125}I]-MIP-1α binding by C3 aryl analogues[a]



Compound	R	CCR5 IC_{50} (nM)
8	H	255±40
37	2-Cl	>1000
38	3-Cl	810±350
39	4-Cl	>1000
40	3-Br	>1000
41	3-Ph	>1000
42	3-CH_3	390±40
43	3-CO_2CH_3	>1000
44	3-F	150±15
45		220±35

[a]Displacement of ^{125}I-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes. Data are reported as mean±SD for $n=3$ determinations. See ref 7 for assay protocol.

Table 5. Inhibition of [^{125}I]-MIP-1α binding by analogues with various piperidine pharmacophores[a]



R_1	Series			
	1	2	3	4
	8	**46**	**52**	**58**
$PhCH_2$–	255±40	46±3	9±1	0.1±0.04[b] (100)[c]
	15	**47**	**53**	**59**
PhCO–	67±7	31±5	5±1	0.2±0.02[b] (11)[c]
	17	**48**	**54**	**60**
(2-Cl)PhCO–	52±3	12±2	2±0.2[b] (50)[c]	0.8±0.5[b] (1)[c]
	28	**49**	**55**	**61**
1-Naphthoyl	19±2	22±3	4±0.4	0.8±0.2[b] (4)[c]
	33	**50**	**56**	**62**
Cyclohexanoyl	34±3	17±2	4±0.4	0.8±0.2[b] (4)[c]
	34	**51**	**57**	**63**
Cyclopentanoyl	19± 2	15± 1	2±0.2	0.2±0.02[b] (4)[c]

[a]Displacement of ^{125}I-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes. Data are reported as mean±SD for $n=3$ determinations. See ref 7 for assay protocol.
[b]Data for 3-(*S*),4-(*S*) enantiomer.
[c]Inhibition (CIC_{90}) over a 48 h period of a single HIV (BAL) infection cycle in HeLa Magi cells expressing both CXCR4 and CCR5. See ref 12 for assay protocol.

Table 6. Anti-HIV activity and pharmacokinetics

	Compound		
	4	**54**	**60**
Anti-HIV activity[a]			
PBMC CIC_{95} (nM)	13 (BAL)	1500 (YU-2)	31 (BAL)
Rat pharmacokinetics[b]			
Cl_p (mL/min/kg)	14.2	12.2	41
V_{dss} (L/kg)	2.2	0.8	1.7
$t_{1/2}$ (h)	2.0	1.0	1.2
%F	29	18	39
Dog pharmacokinetics[c]			
Cl_p (mL/min/kg)	24	20.2	Nd[d]
V_{dss} (L/kg)	14.1	1.0	Nd[d]
$t_{1/2}$ (h)	10.2	1.0	Nd[d]
%F	<1	34	Nd[d]

[a]Inhibition of HIV expansion in uninfected normal donor PBMCs over a 7 day period. CIC_{95} values were determined by comparing mean viral p24 antigen levels in test cultures with the levels expressed in control cultures. HIV strain used in the assay is indicated in parentheses. See ref 14 for assay protocol.
[b]Average data generated after 5 mg/kg po and 1 mg/kg iv doses in $n=3$ animals/dose.
[c]Average data generated after 2 mg/kg po and 0.5 mg/kg iv doses in $n=3$ animals/dose.

the identification of novel elements in these compounds, which are diverse from those found in acyclic antagonists such as **4**. Potent anti-HIV activity was achieved in several analogues; compound **60** was determined to be of comparable potency to **4** and to the key compounds featured in reports from Takeda[5] and Schering–Plough.[6] Cross-species oral bioavailability was demonstrated in analogue **54**. The incorporation of additional diverse structural elements into the pyrrolidine class of CCR5 receptor antagonists is in progress and reports of the further optimization of these compounds will be forthcoming.

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