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Antagonists of human CCR5 receptor containing 4-(pyrazolyl)piperidine side chains. Part 1: Discovery and SAR study of 4-pyrazolylpiperidine side chains

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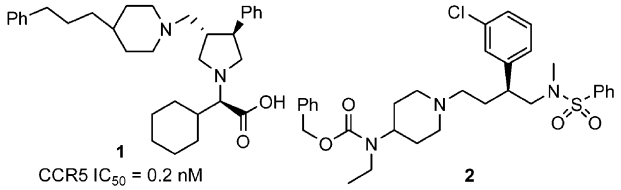
Dedicated to the fond memory of our colleague and friend Dr. Christopher A. Willoughby

Abstract—Replacement of the flexible connecting chains between the piperidine moiety and an aromatic group in previous CCR5 antagonists with heterocycles, such as pyrazole and isoxazole, provided potent CCR5 antagonists with excellent anti-HIV-1 activity in vitro. SAR studies revealed optimal placement of an unsubstituted nitrogen atom in the heterocycle to be *meta* to the bond connected to the 4-position of piperidine. Truncation of a benzyl group to a phenyl group afforded compounds with dramatically improved oral bioavailability, albeit with reduced activity.

CCR5 is a chemokine receptor from the family of seven-transmembrane G-protein coupled receptors. It is the primary co-receptor with CD4 for macrophage tropic HIV-1 virus strains' entry into host cells.[1,2] HIV-1 strains using CCR5 for cell entry, now named R5 variants, are believed to be primarily responsible for the transmission of HIV-1 between individuals. They are usually present throughout the course of HIV-1 infection, both before and after the onset of AIDS. Persons homozygous for a 32-base pair deletion in their CCR5 gene do not express functional CCR5 at the cell surface. These individuals are highly resistant to HIV-1 infection.[3] Heterozygous individuals, while susceptible to HIV-1 infections, showed delayed progression to AIDS compared to infected homozygous persons.[4,5] These observations highlight the central role CCR5 receptor plays in the establishment and perpetuation of HIV-1 infection in vivo. Individuals without cell surface CCR5 receptor appear to be physiologically normal. Thus, CCR5 antagonists have become a potential target for the treatment and prevention of HIV-1 infection.[6–8] Other reports have also suggested a linkage between the CCR5Δ32 deletion and age of onset of multiple sclerosis.[9]



Previous reports from our laboratories disclosed the discovery and SAR studies on several series of CCR5 antagonists including **1**[13] and **2**.[10] They all have piperidines with a flexible side chain at the 4-position

Keywords: CCR5 antagonists; HIV-1; Antiviral; Pyrazole.
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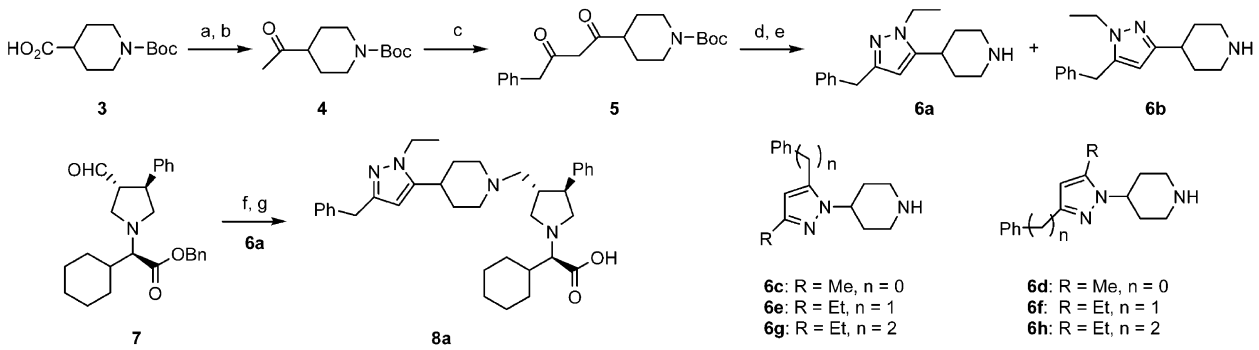


Scheme 1. Reagents: (a) *O*,*N*-dimethylhydroxylamine hydrochloride, HOBt, EDC, DIEA, DMF, 97%; (b) CH_3MgBr (2.25 equiv), ether, 0 °C, 100%; (c) methyl phenylacetate, NaH, THF, rt, 66%; (d) ethylhydrazine oxalate, MeOH, 50 or 60 °C; (e) 10% TFA in DCM, anisole or HCl in MeOH, rt, 58% **6a** and 23% **6b** (two steps); (f) $NaBH(OAc)_3$, DIEA, 1,2-dichloroethane; (g) Pd/C, H_2, MeOH, 68% (two steps).

connected to an aromatic group.[10–13] To improve antiviral activity and in vivo properties, we wished to constrain this side chain by replacing the flexible linking group with an aromatic moiety. Our interest in pyrazole chemistry[14] led us to initiate these studies with this heterocycle.[15,16]

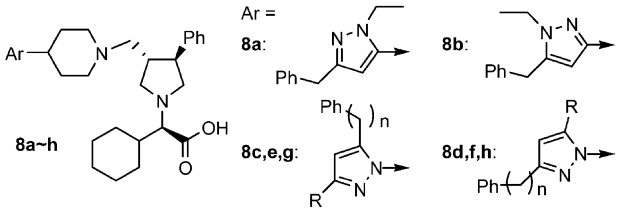
The synthesis of an initial group of pyrazolyl piperidines is illustrated by the preparation of **8a** in Scheme 1. Also shown are other pyrazolylpiperidine intermediates **6b–h** used for the similar preparation of **8b–h**. The Boc precursors to **6a** and **6b** can be separated by RP-HPLC (acetonitrile and water with 0.1% trifluoroacetic acid) or silica gel chromatography (methylene chloride and acetonitrile). The ratio of **6a**/**6b** varies from 2 to 3. Their structures were established by NOE difference or NOESY experiments. Ratios of isomeric mixtures of **8e**/**f** and **8g**/**h** were determined by ^1H NMR. Compounds **6c–h** were prepared from appropriate β-diketones and 1-benzyl-4-hydrazinopiperidine followed by debenzylation using ammonium formate and $Pd(OH)_2$. The diketone precursors themselves were either commercially available (for **6c** and **6d**) or prepared according to a literature method from 2-butanone and an ester.[17] The synthesis of the aldehyde **7** has been reported previously.[13]

After compounds were tested for their activity in displacing [^{125}I]-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes,[19] the more potent compounds were further evaluated as antivirals in a cell culture HIV-1 infectivity assay using HeLa cells (hereafter referred to as HeLa assay).[20] Table 1 shows these results for pyrazoles **8a–h**. Among these compounds, compound **8a** emerged as a new lead structure with potent CCR5 binding and in vitro antiviral activity.

The dramatic difference in the antiviral activities of **8a** and **8f** demonstrated the importance of the correct placement of the nitrogen atoms on the pyrazole ring. These results prompted us to prepare compounds **13** and **14** having the alternative benzyl placement from compound **8a** (Scheme 2). When alkylhydrazines were used in the cyclization of **11** to **12**, the major isomer was **12b** by about 4 to 5-fold over **12a**. We believe that steric factors overrode the usual preference of aldehyde bonding to the unsubstituted nitrogen of alkyl hydrazines. Isomeric pairs of **12** were prepared from separated benzyl precursor and coupled to give **13** and **14**. Among the two isomeric products, **13** and **14**, the more active isomer **13** showed similar activities as **8a** in both CCR5 binding and HeLa assays (Table 2). In this series, phenylethyl is more active than benzyl based on comparison of **13a**/**b** and **13c**/**d**. Among substitutions on the pyrazole nitrogen, the antiviral activity decreases in the order of Et, Me, *n*-Pr, and H. Some isomeric pyrazoles **13** and **14** have similar binding activities (see **13b** and **14b**). However, in the antiviral assay, **13** was much more active than **14**.

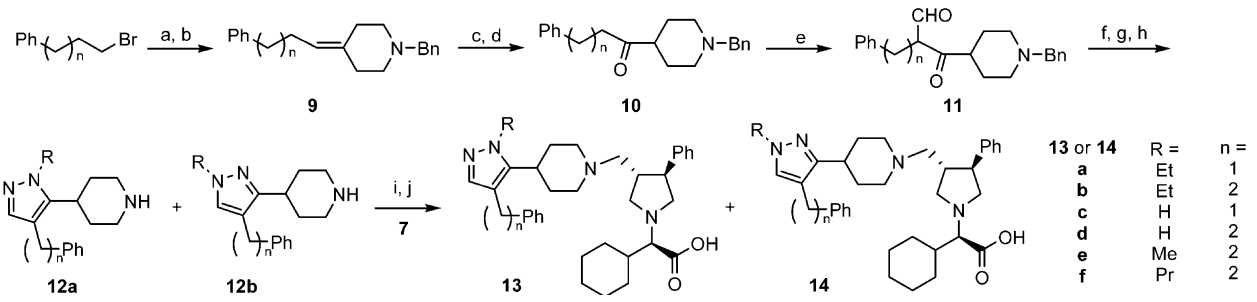
Parallel investigation of the SAR of the alkyl group on the pyrazole ring of compound **8a** showed a similar

Table 1. CCR5 binding affinity and antiviral activity of pyrazole compounds **8a–h**



Compd (ratio)	R =	*n* =	CCR5 IC_{50} (nM)[a,b]	HeLa IC_{90}, (nM)[e]
8a			1.2,[c] 2.1	0.4, 0.4
8b			76	>300
8c	Me	0	65% Inh. @ 2 μM	ND[d]
8d	Me	0	79% Inh. @ 2 μM	ND[d]
8e and **8f** (3:2)				
	Et	1	78	>300
8g	Et	2	82% Inh. @ 2 μM	ND[d]
8g and **8h** (1:1)				
	Et	2	105	>300

[a] IC_{50}'s reported are averages of triplicate measurements whose standard errors were normally <15% in a given assay. Assay to assay variability was within ±2-fold based on a standard compound.
[b] Unless otherwise noted, data from a GP120-membrane-based assay. See ref 19 for assay protocol.
[c] Displacement of [^{125}I]-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes. See ref 12 footnote 20 for assay protocol.
[d] ND = Not determined.
[e] See ref 20 for assay conditions.



Scheme 2. Reagents: (a) triphenylphosphine, toluene, reflux, 70–90%; (b) *N*-benzylpiperidone, $KN(TMS)_2$, toluene, 40–62%; (c) BH_3·THF (3 equiv), THF; (d) CrO_3, H_2SO_4, H_2O, 25% (two steps); (e) methyl formate, KO-*t*-Bu, THF, 60%; (f) $RNHNH_2$, MeOH, 50 °C; (g) semi-preparative HPLC separation; (h) ammonium formate, $Pd(OH)_2$, MeOH, 60 °C, for three steps: 98% (R=H), 22–39% (**12a**), 50–76% (**12b**); (i) $NaBH(OAc)_3$, DIEA, 1,2-dichloroethane; (j) Pd/C, H_2, MeOH, 50–89% (two steps).

trend, except here the parent compound **8i** was equipotent with the ethyl compound **8a** (Table 3). Bulkier and polar groups were not well tolerated at this position.

The marked difference in antiviral activity of the isomeric compounds **8a**/**8b** and **13a**/**14a** might be due to a steric effect of the ethyl group or preferred location of the unsubstituted nitrogen atom in **8a** and **13a**. To distinguish these two possibilities, isoxazole analogues were prepared (Scheme 3). The isomeric isoxazoles **15a** and **15b** were assigned using ^1H NMR.[18] Even though they have similar CCR5 receptor binding activities, compound **16** was about 10-fold more active than isomer **17** in the antiviral assay (Table 4). This result suggests that placement of the unsubstituted nitrogen atom *meta* to the bond connected to the piperidine provides optimal antiviral activity.

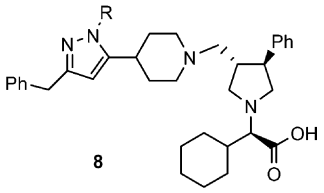
Compounds **8a**, **13b**, and **16** all showed excellent antiviral activity in a more rigorous 7-day in vitro viral infectivity assay using peripheral blood mononuclear cells (PBMC),[21] with IC_{95}'s of <8 nM against the BAL-1 strain of HIV-1. Therefore, they are more potent antivirals than the compounds with flexible connecting groups between phenyl and piperidine rings such as **1**. However, these compounds showed poor pharmacokinetic profiles in the rat (Table 5). Truncation of the benzyl group in **8a** to a phenyl group (**18a**) dramatically improved its pharmacokinetic profile in the rat, but unfortunately at the expense of a considerable loss

Table 2. CCR5 binding affinity and antiviral activity of pyrazole compounds **13** and **14** with alternative benzyl placement

Compd	R =	*n* =	CCR5 IC_{50} (nM)[a]	HeLa IC_{90}, (nM)
13a	Et	1	3.0	3.7, 10
13b	Et	2	0.6	0.4
13c	H	1	4.8	100
13d	H	2	1.2	33
13e	Me	2	1.0[b]	3.7
13f	*n*-Pr	2	0.6[b]	11
14a	Et	1	20	>300
14b	Et	2	2.3	300
14e	Me	2	23[b]	ND
14f	*n*-Pr	2	9.0[b]	ND

[a] See footnote b of Table 1.
[b] See footnote c of Table 1.

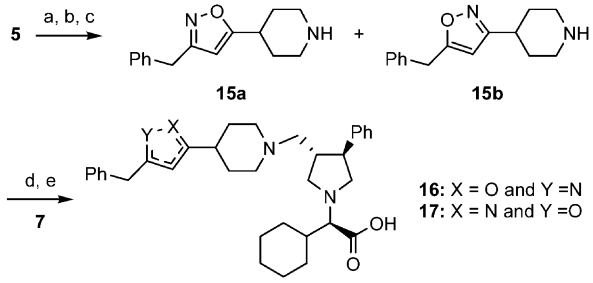
Table 3. CCR5 binding affinity and antiviral activity of pyrazoles



8

Compd	R =	CCR5 IC_{50} (nM)[a]	HeLa IC_{90}, (nM)
8a	Et	2.1	0.4, 0.4
8i	H	2.2	0.4
8j	Me	4.2	3.7
8k	*n*-Pr	4.6	1.2
8l	*n*-Bu	3.0	11
8m	CH_2CF_3	4.8	33
8n	CH_2CO_2Et	9.0	11, 33
8o	CH_2CO_2H	33	ND

[a] See footnote b of Table 1.



Scheme 3. Reagents: (a) hydroxylamine hydrochloride, DIEA, EtOH, 50 °C; (b) TFA, anisole, DCM, rt; (c) RP-HPLC separation, 18% each **15a** and **15b** (three steps); (d) $NaBH(OAc)_3$, DIEA, 1,2-dichloroethane; (e) Pd/C, H_2, MeOH, 67% (**16**) and 92% (**17**).

Table 4. CCR5 binding affinity and antiviral activity versus location of the key nitrogen atom in the heterocycle

Compd	CCR5 IC_{50} (nM)[a]	HeLa IC_{90}, (nM)
16	0.8	0.4
17	1.2	3.7

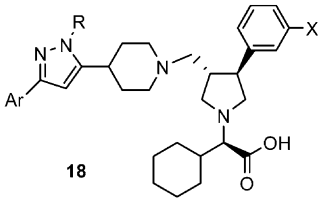
[a] See footnote c of Table 1.

Table 5. Pharmacokinetic parameters of CCR5 antagonists in the rat

Compd	Cl_p, mL/min/kg	$AUCN_{po}$, μM·hr/dose	$t_{1/2}$, h	F,%
8a	64	0.02	0.82	4.1
13b[a]	79	0.01	ND	2.6
16[a]	67	0.03	ND	5.9
18a	3.8	1.7	1.5	21
18j[a]	72	0.01	0.45	3.2

[a] Dosed as a mixture of five compounds. Parameters for **18a** from mixture dosing were similar to those reported for single compound dosing. For **8a**, $AUCN_{po}$ and %F were about 3- and 2-fold higher in mixture dosing, respectively.

Table 6. CCR5 binding affinity and antiviral activity in the truncated and elongated pyrazole series



18

Compd				CCR5 IC_{50} (nM)[a]	HeLa IC_{90} (IC_{50}, nM)
	Ar =	R =	X =		
18a	Ph	Et	H	22	>300 (100)
18b	4-CNPh	Et	H	16	>300 (33)
18c	4-H_2NCH_2Ph	Et	H	112	ND
18d	4-CNPh	H	H	7.5[b]	>300 (100)
18e	1-Naphthyl	H	H	7.5[b]	>300
18f	1-Naphthyl	Et	F	16[b]	ND
18g	2,4-Cl_2Ph	H	F	9[b]	>300
18h	4-ClPh	H	F	4[b]	>300
18i	4-MeOPh	H	F	12[b]	>300
18j	$PhCH_2CH_2$	Et	F	2.2[b]	11

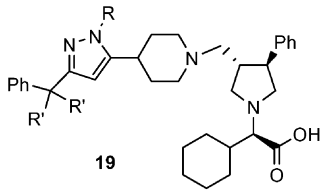
[a] See footnote b of Table 1.
[b] See footnote c of Table 1.

of binding and antiviral activities (Table 6). A survey of several different aryl groups failed to improve the antiviral potency significantly in this series. In addition, elongation of the benzyl group in **8a** to phenethyl in **18j** did not offer any advantage over **8a** in its pharmacokinetics. It also resulted in a considerable loss of antiviral activity in **18j**.

The dramatic difference in pharmacokinetic profiles of **8a** and **18a** prompted us to block the benzylic methylene position of **8a** to prevent metabolism at this site (Table 7). Some of these compounds retained good binding affinity, such as the *gem*-difluoro compound **19a**, *gem*-dimethyl compound **19e**, and cyclopropane compound **19f**. However, their antiviral activities were greatly diminished compared to the parent compound **8a**. In addition, compounds **19a**, **19e**, and **19f** showed no significant improvements in clearance or oral AUC when dosed as mixtures in the rat compared to **8a** (data not shown).

In conclusion, replacement of flexible connecting chains between phenyl and piperidine in previous CCR5 antagonists, such as **1** and **2**, with a heterocycle, such as pyrazole or isoxazole, afforded compounds such as **8a**, **13a**, and **16** which were potent binders to the CCR5 receptor. These compounds also showed improved antiviral activity in vitro against HIV-1. SAR studies indicated the optimal placement of the unsubstituted nitrogen atom in these heterocycles to be *meta* to the bond connected to the piperidine ring for the best antiviral activity. Truncation of the benzyl group to phenyl group led to compound **18a** which had excellent oral bioavailability. Further studies of these and related series leading to compounds with both oral bioavailability and potent antiviral activity are reported in the following paper.

Table 7. CCR5 binding affinity and antiviral activity of metabolically blocked benzyl pyrazoles



19

Compd			CCR5 IC_{50} (nM)[a]	HeLa IC_{90} (nM)
	R =	R′,R′ =		
19a	H	F_2	2.1	300
19b	H	=O	41	ND
19c	Et	=O	23	ND
19d	H	Me_2	11.5	ND
19e	Et	Me_2	6.9	300
19f	H	$–CH_2CH_2–$	4.4	>300
19g	Et	$–CH_2CH_2–$	11	ND

[a] See footnote c of Table 1.

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