Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 947–952

Antagonists of human CCR5 receptor containing 4-(pyrazolyl)piperidine side chains. Part 3: SAR studies on the benzylpyrazole segment

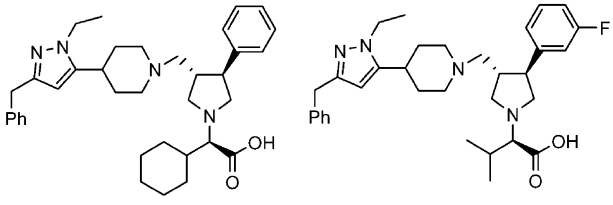
Min Shu,[a,*] Jennifer L. Loebach,[a] Kerry A. Parker,[a] Sander G. Mills,[a] Kevin T. Chapman,[a] Dong-Ming Shen,[a,*] Lorraine Malkowitz,[b] Martin S. Springer,[b] Sandra L. Gould,[b] Julie A. DeMartino,[b] Salvatore J. Siciliano,[b] Jerry Di Salvo,[b] Kathy Lyons,[a] James V. Pivnichny,[a] Gloria Y. Kwei,[c] Anthony Carella,[d] Gwen Carver,[d] Karen Holmes,[d] William A. Schleif,[d] Renee Danzeisen,[d] Daria Hazuda,[d] Joseph Kessler,[d] Janet Lineberger,[d] Michael D. Miller[d] and Emilio A. Emini[d]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[b]*Department of Immunology, Merck Rearch Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[c]*Department of Drug Metabolism, Merck Rearch Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[d]*Department of Antiviral Research, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA*

Received 2 September 2003; revised 24 November 2003; accepted 2 December 2003

Abstract—Extensive SAR studies in our benzylpyrazole series of CCR5 antagonists have shown that both lipophilic and hydrophilic substituents on the phenyl of the benzyl group increase antiviral potency. However, improvements in pharmacokinetic profiles were generally only observed with more lipophilic substitutions. 4-Biphenyl (**51**) performed the best in this regard. Highly lipophilic substituents impart undesirable ion channel activity to these CCR5 antagonists. Alkoxy substituents provide a good balance of antiviral activity, pharmacokinetic parameters, and selectivity. Compounds **42b** and **42d**, containing a 3,4-dimethoxy substituent, are considered the most promising improvements over parent compounds **9**. They demonstrate improved antiviral activity while retaining good pharmacokinetic profile and selectivity.

Since the discovery of the chemokine receptor CCR5 as a co-receptor with CD4 for HIV-1 cell entry, there has been an intense interest to discover small molecule CCR5 antagonists as potential agents for the treatment of HIV-1 infection.[1–12] Our colleagues from these laboratories have reported on 1-amino-2-aryl-4-(piperidin-1-yl)-butanes,[1,2,12] *trans*-1,3,4-trisubstituted pyrrolidines,[2–7] and related compounds[11] as CCR5 antagonists. Excellent in vitro antiviral activities against HIV-1 and good pharmacokinetic profiles in animals have been demonstrated for some of these compounds.



	1	**9a**
CCR5 IC_{50}, nM	1.2	1.5
HeLa (BAL) IC_{90}, nM	0.4	3.7
PBMC (BAL) IC_{95}, nM	< 8	22 ± 16

In the preceding two papers, we reported that replacing a phenylpropyl side chain with a heterocycle such as a benzylpyrazole or a benzylisoxazole provided compounds such as **1** with potent CCR5 binding and in vitro antiviral activities.[13] Although compounds in this

Keywords: CCR5 antagonist; HIV-1; Antiviral; Pyrazole.
* Corresponding authors. Fax: +1-732-594-8080; e-mail: min_shu@merck.com; dongming_shen@merck.com

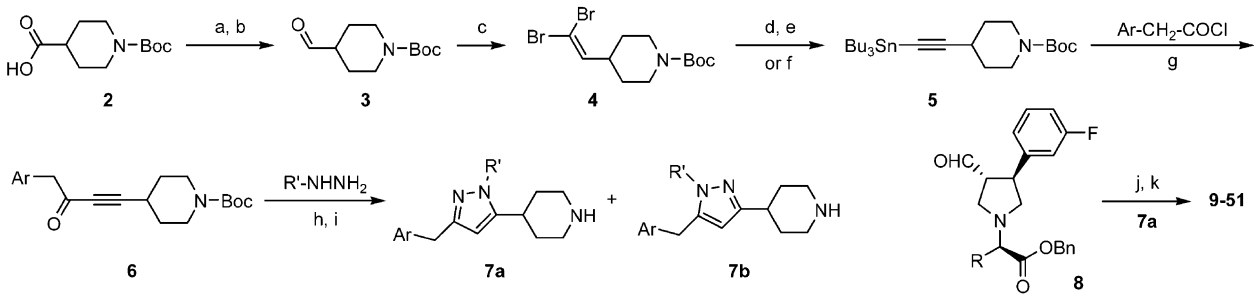
0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.12.006

cyclohexyl series do not have suitable off-target selectivity or pharmacokinetic profiles, further investigation led to the isopropyl compound **9a** having excellent selectivity and enhanced pharmacokinetics.[14] However, **9a** showed considerably less antiviral activity in comparison to **1**. Herein we report the results of our structure–activity relationship studies on the benzylpyrazole region of these compounds. The aim was to discover compounds having the antiviral activity of **1** and the selectivity and pharmacokinetic profile of **9a**. Three isomeric butyl analogues of **9a**[14] were also pursued during this study.

Our initial route for the syntheses of the 4-(pyrazolyl)piperidines gave low selectivity for the desired pyrazole isomer **7a** over **7b**.[13] In order to improve the selectivity and functional group compatibility, a new route to pyrazole **7a** was developed (Scheme 1). The commercially available acid **2** was converted to the dibromo olefin **4** via the aldehyde **3**. Converting **4** to the terminal acetylene anion[15] and quenching with chlorosilane followed by Si–Sn exchange[16] gave the acetylenic tin compound **5**. We also found that the above two steps could be combined using tri-*n*-butyltin chloride during quenching of the lithium acetylide. Pd-catalyzed coupling of **5** with acid chlorides provided acetylenic ketone intermediates **6** in good yields.[17] Cyclization of **6** with hydrazines gave **7a**/**7b** isomeric ratios as high as 20 when the hydrazine was in its free-base form (R′=Me). When ethylhydrazine oxalate and DIEA were used (R′=Et), the ratio was about 8 compared to 2–3 from the diketone route described previously.[13] Both routes were used to prepare **7a** for this study. Reductive amination of **7a** with aldehyde **8** or its *p*-methoxybenzyl analogues[18] followed by deprotection afforded the desired compounds for testing (Table 1). The parent benzyl compounds **9a**–**e**[14] are listed for comparison.

In general, all compounds in Table 1 showed very good CCR5 binding activities with IC_{50}'s in the low nM range or below. Antiviral activities in a cell culture HIV-1 infectivity inhibition assay using HeLa cells (hereafter referred to as HeLa assay), especially in the presence of 50% normal human serum, were considered a more critical measure of activity for these compounds. The antiviral activities of compounds with various R-groups in Table 1 parallel their parent compounds **9a**–**e**. Thus, the isopropyl and (*R*)-*s*-Bu compounds were usually the least active analogues.

Replacing the benzyl in **9** with cyclohexylmethyl (**10**) resulted in about a 10-fold loss of intrinsic antiviral activity. The losses after accounting for serum protein binding are about 3-fold. A series of halogen substitutions (**11**–**17**) showed little impact on antiviral activity compared to the parent compound **9a** in the isopropyl series, but in the cyclohexyl series, there was some improvement in antiviral activity. Adding one trifluoromethyl group (**18**) enhanced antiviral activity slightly. However, the 3,5-bis(trifluoromethyl)phenyl compounds (**19**) were much less active than their 4-trifluoromethylphenyl analogues **18** in the antiviral assay. As was observed previously in the 4-(3-phenylpropyl)-piperidine series,[7] appending electron-withdrawing groups, such as cyano, to the benzyl ring enhanced antiviral activity in these pyrazole compounds (**20** and **21**), especially for **20b** and **20d** after consideration of their serum protein binding. However, this substitution sometimes had a negative effect on the selectivity of these compounds. For example, compound **20d** had a K_i of 0.99 μM for the hERG K^+ channel versus 14.3 μM for **9d**.[20] Inhibition of the hERG K^+ channel has caused several product withdrawals in the industry and is currently a widespread concern in drug discovery.[21,22] Therefore, structural changes that increase the hERG K^+ channel inhibition activity are to be avoided. However, the hERG K^+ channel K_i's for **9c** and **20c** were similar at 29 and >10 μM. The isomeric 3-cyano compounds **21** offered no advantage in activity over **20**. On the other hand, the presence of a polar 4-methylsulfonyl group as in **22** gave compounds which were inactive at the hERG K^+ channel while retaining the CCR5 binding and antiviral activity. Increasing the size of the lipophilic group on the sulfone (**23**) afforded compounds with similar antiviral activity. However, their selectivity was diminished. For example, **23d** had a K_i of 3.2 μM at the hERG K^+ channel compared to >10 μM for **22d**. Increasingly basic functional groups showed larger decreases in intrinsic antiviral activity in **24** and **25**. However, these compounds exhibited substantially less serum protein binding than the parent compounds. Therefore, in the presence of human serum, they generally showed better antiviral activity than the parent compounds **9**.

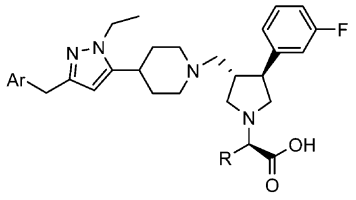


Scheme 1. Reagents: (a) BH_3·THF, THF, 100%; (b) oxalyl chloride, dimethyl sulfoxide, Et_3N, dichloromethane, −78 °C–rt, 86%; (c) CBr_4, Ph_3P, DCM, 80–90%; (d) (1) *n*-BuLi, THF; (2) Me_3SiCl, 89%; (e) Bu_4NF, $(Bu_3Sn)_2O$, 60%; (f) (1) *n*-BuLi, THF; (2) Bu_3SnCl, 80%; (g) 2% $Pd(PPh_3)_4$ or $Pd(PPh_3)_2Cl_2$, 1,2-dichloroethane, reflux, N_2, 50–93%; (h) 60–80 °C, ethanol; (i) HCl in MeOH or TFA, anisole, dichloromethane, rt, 46–85% (**7a**), 4–11% (**7b**); (j) $NaBH(OAc)_3$, DIEA, 1,2-dichloroethane; (k) Pd/C, H_2, MeOH or alternatively in the case of PMB esters, 96% formic acid, rt, 60–80% (two steps).

Surprisingly, adding electron-donating groups such as alkoxyls also improved antiviral activity over the parent compounds **9** in most cases. For example, the 4- and 3-methoxy and 4-ethoxy derivatives (**26**–**28**) were more active than **9**. However, the 3-ethoxy derivatives (**29**) showed no such improvement. As the normal alkyl groups become longer, the 4-*n*-propoxy analogues (**30**) are more active than parent compounds **9** while *n*-butoxy derivatives (**31**) are less active. Branched and cyclic alkoxy compounds (**32**–**35**) also showed better antiviral activity with the *t*-butoxy compounds (**35**) being the most active. 4-Benzyloxy and 4-phenoxy compounds (**36**–**37**) were all less active. Substitution with 4-trifluoromethoxy and 4-(2,2,2-trifluoroethoxy) (**38** and **39**) gave similar antiviral activity as **9** in the presence of human serum. More polar compounds (**40**) showed better antiviral activity, even in the presence of serum. Yet another example of polar substituent improving activity is the phenol (**41**).

Next, four pairs of substituents that enhanced antiviral activity when present individually were combined (**42**–

Table 1. CCR5 binding affinity and antiviral activity of substituted benzylpyrazole compounds[a]



Compd, Ar =	**a** R = *i*-Pr	**b** R = (*S*)-*s*-Bu[b]	**c** R = (*R*)-*s*-Bu[b]	**d** R = *t*-Bu	**e** R = *c*-Hex
9, Phenyl	1.5 (3.7/300)	0.8 (<0.14/100)	0.9 (0.4/300)	0.8 (<0.14/100)	1.6 (0.4/100)
10, *c*-Hexyl	2.3 (33/>300)	1.2 (1.2/300)		1.1 (3.7/300)	
11, 4-Fluorophenyl	1.1 (3.7, 11/>300)				1.1 (33, ND)
12, 3-Fluorophenyl	1.3 (11/300)				1.0 (<0.14/33)
13, 2-Fluorophenyl	2.2 (11/>300)				0.5 (<0.14/33)
14, 3,4-Difluorophenyl	1.1 (3.7, 11/300)				
15, 3,5-Difluorophenyl	2.6 (11/300)				0.9 (<0.14/11)
16, 2,4-Difluorophenyl	5.0 (100/>300)				1.5 (0.4/33)
17, 4-Chlorophenyl	1.3 (3.7/300)				0.9 (0.4/100)
18, 4-Trifluoromethylphenyl	2.0 (3.7/100)	0.5 (<0.14/33)	0.5 (0.4/100)	0.3 (0.4/33)	1.5 (0.4/33)
19, 3,5-Bis(trifluoromethyl)phenyl	3.2 (100/>300)	2.1 (11/>300)	1.6 (33/>300)	2.3 (11/>300)	
20, 4-Cyanophenyl	3.1 (1.2/100)	1.3 (<0.14/3.7)	1.3 (0.4/33)	1.3 (<0.14/3.7)	1.7 (0.4/11)
21, 3-Cyanophenyl	1.7 (3.7/100)	0.9 (<0.14/11)		1.0 (<0.14/11)	
22, 4-Methylsulfonylphenyl	3.5 (3.7/33)			1.6 (<0.14/11)	2.3 (0.4/11)
23, 4-Phenylsulfonylphenyl	1.3 (0.4/33)	0.6 (<0.14/11)	1.1 (0.4/33)	1.1 (<0.14/3.7)	0.8 (0.4/33)
24, 3-Pyridinyl	13.1 (11/300)				3.9 (0.4/33)
25, 3-(Aminomethyl)phenyl		0.5 (1.2/11)	2.7 (3.7/33)		
26, 4-Methoxyphenyl	1.3 (0.4/100)			1.5 (<0.14/33)	1.1 (<0.14/33)
27, 3-Methoxyphenyl		1.2 (<0.14/100)		0.7 (<0.14/33)	
28, 4-Ethoxyphenyl	1.0 (0.4/300)	0.6 (<0.14/33)		0.9 (0.4/33, 100)	1.0 (<0.14/33)
29, 3-Ethoxyphenyl		0.9 (<0.14/100)	0.8 (<0.14/>300)	0.4 (<0.14/100)	
30, 4-*n*-Propoxyphenyl	0.8 (3.7/>300)	1.1 (<0.14/100)	1.2 (1.2/300)	0.8 (0.4/300)	0.7 (1.2/100)
31, 4-*n*-Butoxyphenyl	0.5 (11/300)	1.0 (0.4/11)	0.8 (3.7/100)	0.8 (1.2/33)	0.8 (0.4/33)
32, 4-Cyclopropoxyphenyl	1.0 (1.2/300)	0.3 (<0.14/11)	0.3 (<0.14/33)	0.4 (<0.14/33)	0.2 (<0.14/33)
33, 4-Cyclobutoxyphenyl	0.6 (1.2/100)	1.3 (<0.14/11)	0.4 (<0.14/33)	0.7 (0.4/33)	
34, 4-*i*-Propoxyphenyl		0.8 (<0.14/33)		0.7 (<0.14/11)	
35, 4-*t*-Butoxyphenyl	0.8 (<0.14/100)	1.3 (<0.14/11)	1.6 (<0.14/3.7)	2.0 (11/300)	0.5 (<0.14/11)
36, 4-Benzyloxyphenyl		0.7 (3.7/300)	0.5 (3.7/100)	1.1 (3.7/300)	
37, 4-Phenoxyphenyl	0.6 (11/>300)	0.4 (1.2/100)	0.5 (3.7/300)	0.4 (3.7/300)	0.5 (1.2/100)
38, 4-Trifluoromethoxyphenyl	0.8 (3.7/300)	1.1 (0.4/33)	0.6 (1.2/100)	0.6 (1.2/100)	
39, 4-(2,2,2-Trifluoroethoxy)phenyl		0.6 (0.4/33)	0.5 (3.7/100)	0.6 (1.2/100)	
40, 4-Difluoromethoxyphenyl	0.5 (<0.14/100)	0.3 (<0.14/11)	0.6 (<0.14/11)	0.5 (<0.14/11)	
41, 4-Hydroxyphenyl	0.6 (1.2/33)	1.4 (0.4/11)	0.6 (<0.14/11)	1.1 (0.4/11)	0.5 (11/33)
42, 3,4-Dimethoxyphenyl		1.5 (0.4/33)	2.6 (0.4/33)	1.2 (<0.14/11)	
43, 1,3-Benzodioxol-5-yl		0.5 (<0.14/100)		0.7 (<0.14/33)	
44, 4-Methoxy-3-cyanophenyl	0.8 (1.2/300)	0.5 (<0.14/33)		0.9 (<0.14/33)	
45, 4-Ethoxy-3-fluorophenyl	0.4 (<0.14/100)	0.7 (<0.14/11)	0.4 (<0.14/33)	0.7 (<0.14/11)	
46, 1-Naphthyl		0.4 (<0.14/11)	0.2 (<0.14/33)	0.2 (<0.14/11)	
47, 2-Naphthyl		0.3 (<0.14/300)	0.2 (<0.14/300)	0.2 (<0.14/100)	
48, 4-Tolyl		0.3 (<0.14/11)		0.6 (<0.14/33)	
49, 4-*i*-Propylphenyl		0.3 (<0.14/11)		0.6 (0.4/33)	
50, 4-*t*-Butylphenyl		0.3 (0.4/11)	0.8 (1.2/300)	0.4 (0.4/33)	
51, 4-Biphenyl	0.3 (<0.14/100)	0.3 (0.4/33)	0.5 (1.2/300)	0.6 (1.2/100)	0.3 (0.4/11)

[a] Data shown as CCR5 IC_{50}, nM (HeLa IC_{90}, nM/HeLa IC_{90}, nM in the presence of 50% normal human serum). CCR5 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. CCR5 IC_{50}'s were measured as displacement of [^{125}I]-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes. See ref 3 note 20 for assay protocol. See ref 19 for HeLa assay conditions.

[b] The structures of **9b** and **9c** are shown in ref 23.

45). These compounds did not show any additive improvements in antiviral activity (compare **44** with **21** and **26**, for example).

Replacing the phenyl group in **9** with a 1-naphthyl group (**46**) improved antiviral activity. However, 2-naphthyl (**47**) did not show an improvement in the presence of human serum. Adding alkyl groups to the 4-position of the benzyl group (**48**–**50**) enhanced the antiviral activity. The size of the alkyl groups did not appear to be important since methyl, *i*-propyl and *t*-butyl all gave the same activity. The 4-biphenyl compounds (**51**) showed some enhancement in antiviral activity, especially for **51e**.

The pharmacokinetic (PK) profiles of selected compounds from Table 1 were first evaluated in the rat (Table 2). In a related series of compounds, some compounds were extremely tightly bound to rat plasma (>99.97%) but only moderately bound to human plasma (~97%). In the rat, this correlated with very low volumes of distribution, low clearances and long half-lives. However, due to the discordance in protein binding, it was recognized that good rat PK results could not be considered predictive of the human profiles for those compounds. Thus, compounds having very low volume were often not evaluated further in our series because of difficulties in measuring plasma binding for a large number of compounds. It should be noted, however, **9a** had similar binding to rat and human plasmas.[14]

Addition of 4-fluoro in 3-phenylpropyl series[7] reduced clearance. However, the trend was the opposite for 4-fluoro analogue **11a**. The 3-fluoro compound **12a** had similar or only slightly better exposure than **9a** considering the data were obtained using mixture dosing. Compounds **22a**, **23a**, and **41a** were the most active antivirals in the isopropyl series. The PK properties of **22a** and **23a** were similar and poor in the rat. Compound **41a** was not dosed because **41b**–**e** all showed poor pharmacokinetics. Mixture dosing of **14a**, **17a**, **18a**, **20a**, **26a**, **38a**, and **40a** gave similar pharmacokinetics as **9a**. The only compound showing a clearly better PK profile than **9a** in this series was **51a**, albeit with a very low Vd_{ss}. It appeared difficult to improve the antiviral activity of **9a** in the isopropyl series while preserving the good PK and selectivity.[24]

The PK parameters and selectivity profile of the (*S*)-*s*-Bu series is typical among the related series. The PK of **9b** was characterized by moderate clearance, short half-life, and low oral AUCN. Most of the compounds in this series displayed similar or only marginally improved PK's over **9b**. Compounds demonstrating better PK parameters than the parent **9b** included the alkoxy compounds **28b**, **31b**–**33b**, **35b**, **42b**, fluoroalkoxy analogue **38b**, alkyl derivatives **49b**, **50b**, and biphenyl **51b**. However, their ion channel activities increase with increasing lipophilicity and decreasing polarity of the substituents. The best combination of antiviral activity, pharmacokinetics, and selectivity was found in the 3,4-dimethoxy compound **42b**. The improved selectivity of dialkoxy compounds appears general. Another example was **43b** with a K_i of 27.5 μM at the hERG K^+ channel. One more interesting compound was **38b** which showed a reasonable iv profile in the dog (Table 3), in addition to its good antiviral activity.

The less active (*R*)-*s*-Bu series generally showed better PK profiles. This appears to hold with less lipophilic substituents such as **33c**, **35c**, **40c**, **45c**, and **46c**. For

Table 2. Pharmacokinetic parameters of CCR5 antagonists in the rat and activity at the hERG K^+ ion channel[a]

Compd	Cl_p (mL/min/kg)	$AUCN_{po}$ (μM·h/dose)	Vd_{ss} (L/kg)	$t_{1/2}$ (h)	F (%)	hERG K^+ K_i (μM) (n)
9a	3.2	1.1	0.28	1.4	11	>10
11a	7.6	0.33	0.35	0.91	7.8	ND
12a[b]	3.9	3.3	0.16	0.80	40	ND
22a[b]	11	0.17	0.25	0.38	5.4	ND
51a[b]	0.32	22	0.12	5.7	26	ND
9b	25	0.16	0.64	0.54	13	30 (2)
28b	4.4	0.38	0.15	0.98	5.9	16
31b[b]	4.8	1.2	0.40	1.2	22	ND
32b[b]	1.9	5.7	0.07	1.0	36	ND
33b[b]	2.7	7.5	0.21	1.2	68	5.7 (2)
35b[b]	4.0	5.2	0.31	1.1	76	ND
38b[b]	8.8	1.3	0.73	2.3	41	ND
39b[b]	3.5	2.6	0.21	1.1	36	3.8 (2)
42b[b]	7.4	0.66	0.53	3.3	17	22 (3)
49b[b]	3.3	1.7	0.18	1.18	19	8.1 (1)
50b[b]	1.6	1.7	0.15	1.4	9.2	ND
51b[b]	0.52	20	0.15	4.1	36	3.0 (2)
20c	9.8	0.09	0.33	0.74	3.1	>10 (1)
33c[b]	0.75	9.9	0.10	2.1	26	3.4 (2)
35c[b]	0.97	6.6	0.17	2.7	23	5.6
42c[b]	1.8	1.3	0.05	0.73	8.3	37 (2)
9d[b]	5.7	3.7	0.24	0.79	71	14
20d[b]	2.4	5.2	0.13	1.2	43	1.0 (2)
28d[b]	1.7	12	0.20	1.4	68	ND
33d[b]	0.62	28	0.18	3.5	65	3.5 (2)
34d	0.98	14	0.15	1.4	49	9.6
38d	3.9	2.7	0.27	1.1	39	6.0 (2)
42d	2.1	4.2	0.13	1.1	33	11 (3)
50d[b]	1.1	19	0.22	2.5	77	2.1 (2)
51d[b]	0.23	39	0.14	8.9	33	2.3 (2)
9e	84	0.01	3.9	0.83	3	ND
35e[b]	27	0.18	1.6	1.0	19	ND
51e[b]	5.3	0.88	0.53	1.3	18	0.4 (2)

[a] All compounds were dosed at 0.5 mpk iv and 2.0 mpk orally.
[b] Dosed as a mixture of five compounds. In cases where comparisons were available, such as **9a**, **11a**, and **42d**, mixture dosing increased $AUCN_{po}$ and %F by about 2- to 3-fold while other parameters were similar.

Table 3. Pharmacokinetic parameters of CCR5 antagonists in the dog[a]

Compd	Cl_p (mL/min/kg)	$AUCN_{iv}$, (μM·h/dose)	Vd_{ss} (L/kg)	$t_{1/2}$ (h)	F (%)
38b[b]	3.8	6.8	0.87	3.1	ND
9d	13	2.3	0.50	0.64	44
9d[b]	25	1.2	2.1	0.75	ND
18d[b]	5.5	4.9	0.86	2.8	ND
20d	22	1.3	0.99	2.7	7.9
22d[b]	108	0.24	4.6	0.55	ND
28d[b]	13	2.3	1.8	1.8	ND
42d[b]	14	1.9	1.8	2.1	ND

[a] All compounds were dosed at 0.5 mpk iv and 2.0 mpk orally.
[b] Dosed as a mixture of five compounds at 0.5 mpk each via iv only.

Table 4. Antiviral activity in a PBMC cell culture viral spread assay[25] and selectivity profile of select CCR5 antagonists

Compd	PBMC viral spread inhibition, IC_{95}		IC_{50} (μM) or % inhibition at 10 μM on selected counter-screen targets				
	nM (n)	50% NHS, nM (n)	Human adrenergic NE transporter (%)	Rat L-type Ca^{2+} benzothiazepine (%)	Human dopamine D_3 (%)	Human dopamine transporter (%)	Rat Na^+ channel, site 2 (%)
9a	22±16 (26)	350±320 (14)	21, 27	−3, 13	45, 36	15, 48	32, 18
38b	2.5±1.5 (11)	20±19 (8)	42	46	2.7 μM	3.6 μM	1.2 μM
42b	5.5±4.1 (11)	28±16 (11)	15	36	35	−4	7.2 μM
20d	1.9±1.4 (13)	9.3±8.5 (6)	23	1	4 μM	17	44
22d	6.2±2.1 (6)	8.1±2.7 (6)	13	15	39	36	7
42d	1.6±0.8 (6)	31±35 (6)	3	−8	44	9	3.2 μM

more polar groups, PK parameters are either similar to those of the (*S*)-*s*-Bu series (**20c**, **23c**, and **41c**) or worse as in the case of **42c** (Table 2).

Although the parent compound in the *t*-Bu series, **9d**, had good PK, most compounds having more polar substituents in this series had less favorable pharmacokinetics. Compounds with mostly lipophilic substituents showed better PK profiles than **9d**. In both the alkoxy (**26d**–**35d**) and alkyl/aryl (**48d**–**51d**) subclasses, increasing the size of the substituents is consistently associated with higher oral AUCN, longer half-life, and lower clearance. However, increasing lipophilicity was again associated with higher activity at the hERG K^+ channel. Because the parent compound **9d** did not show a good half-life in the dog, several compounds from this series were screened (Table 3). The results showed that **18d**, **28d**, and **42d** showed improvements over **9d**. Overall, **42d** showed the most promising combination of antiviral activity, PK profiles, and selectivity among the *t*-Bu series.

Most compounds in the cyclohexyl series had similarly inferior rat PK profiles as the parent **9e**. Only the *t*-butoxy compound **35e** and 4-biphenyl **51e** showed a significant improvement in PK parameters (Table 2). The latter displayed an acceptable PK profile. However, its selectivity was inadequate.

A selected group of compounds was further evaluated in an in vitro peripheral blood mononuclear cell (PBMC) HIV-1 spread assay[25] and selectivity in five counter-screens (Table 4). They all showed significantly improved antiviral activity over our lead **9a** in the PMBC assay. However, poor selectivity at the hERG K^+ channel for **20d**, multiple low μM activities in the counter-screens for **38b**, and a poor dog PK profile for **22d** precluded their further study. On balance, the 3,4-dimethoxy derivatives **42b** and **42d** showed the best combination of antiviral activity, PK profile, and selectivity.

Extensive SAR studies in five series of compounds have shown that both lipophilic and hydrophilic substituents on the phenyl of the benzyl group in compounds **9a**–**e** increase their antiviral potency. However, improvements in pharmacokinetic profiles in the rat and/or dog were generally only observed with more lipophilic substitutions. For example, 4-biphenyl compounds **51** are bioavailable, even in the cyclohexyl series (**51e**). Highly lipophilic substituents imparted undesirable ion channel activity. Alkoxy substituents generally provided a good balance of antiviral activity, pharmacokinetic parameters, and selectivity. Among these, the 3,4-dimethoxy group was the most outstanding. For example, compounds **42b** and **42d** represent the most promising improvements over their parent compounds **9**. They showed improved antiviral activity while retaining good pharmacokinetic profile and selectivity.

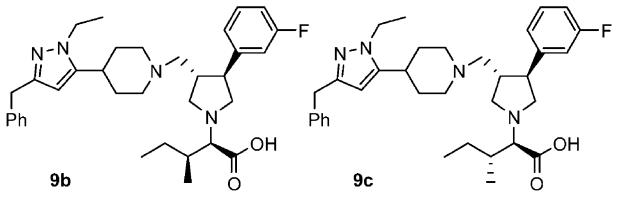
Acknowledgements

The authors would like to thank Dr. G. R. Kieczykowski, Mr. J. F. Leone and Mr. F. Wong for synthetic assistance and Drs. C. G. Caldwell and P. E. Finke for reviewing the manuscript.

References and notes

1. Finke, P. E.; Oates, B.; Mills, S. G.; MacCoss, M.; Malkowitz, L.; Springer, M. S.; Gould, S. L.; DeMartino, J. A.; Carella, A.; Carver, G.; Holmes, K.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M.; Schlief, W. A.; Emini, E. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2475 and references cited therein.
2. Willoughby, C. W.; Berk, S. C.; Rosauer, K. G.; Delgrado, S.; Chapman, K. T.; Gould, S. L.; Springer, M. S.; Malkowitz, L.; Schlief, W. A.; Hazuda, D.; Miller, M.; Kessler, J.; Danzeisen, R.; Holmes, K.; Lineberger, J.; Carella, A.; Carver, G.; Emini, E. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 3137.
3. Hale, J. J.; Budhu, R. J.; Mills, S. G.; MacCoss, M.; Malkowitz, L.; Siciliano, S.; Gould, S. L.; DeMartino, J. A.; Springer, M. S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1437.
4. Hale, J. J.; Budhu, R. J.; Holson, E. B.; Finke, P. E.; Oates, B.; Mills, S. G.; MacCoss, M.; Gould, S. L.; DeMartino, J. A.; Springer, M. S.; Siciliano, S.; Malkowitz, L.; Schlief, W. A.; Hazuda, D.; Miller, M.; Kessler, J.; Danzeisen, R.; Holmes, K.; Lineberger, J.; Carella, A.; Carver, G.; Emini, E. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2741.
5. Lynch, C. L.; Hale, J. J.; Budhu, R. J.; Gentry, A. L.; Mills, S. G.; Chapman, K. T.; MacCoss, M.; Malkowitz, L.; Springer, M. S.; Gould, S. L.; DeMartino, J. A.; Siciliano, S. J.; Cascieri, M. A.; Carella, A.; Carver, G.; Holmes, K.; Schlief, W. A.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M.; Emini, E. A.

Bioorg. Med. Chem. Lett. **2002**, *12*, 3001 and references cited therein.
6. Willoughby, C. W.; Rosauer, K. G.; Hale, J. J.; Budhu, R. J.; Mills, S. G.; Chapman, K. T.; MacCoss, M.; Malkowitz, L.; Springer, M. S.; Gould, S. L.; DeMartino, J. A.; Siciliano, S. J.; Cascieri, M. A.; Carella, A.; Carver, G.; Holmes, K.; Schlief, W. A.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M.; Emini, E. A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 427.
7. Lynch, C. L.; Willoughby, C. W.; Hale, J. J.; Holson, E. J.; Budhu, R. J.; Gentry, A. L.; Rosauer, K. G.; Caldwell, C. G.; Chen, P.; Mills, S. G.; MacCoss, M.; Berk, S.; Chen, L.; Chapman, K. T.; Malkowitz, L.; Springer, M. S.; Gould, S. L.; DeMartino, J. A.; Siciliano, S. J.; Cascieri, M. A.; Carella, A.; Carver, G.; Holmes, K.; Schlief, W. A.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M.; Emini, E. A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 119.
8. Baba, M.; Nishimura, O.; Kanzaki, N.; Okamoto, M.; Sawada, H.; Iizawa, Y.; Shiraishi, M.; Aramaki, Y.; Okonogi, K.; Ogawa, Y.; Meguro, K.; Fujino, M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5698.
9. Tagat, J. R.; Steensma, R. W.; McCombie, S. W.; Nazareno, D. V.; Lin, S.-I.; Neustadt, B. R.; Cox, K.; Xu, S.; Wojcik, L.; Murray, M. G.; Vantuno, N.; Baroudy, B. M.; Strizki, J. M. *J. Med. Chem.* **2001**, *44*, 3343 and references cited therein.
10. For a review, see: Kazmierski, W.; Bifulco, N.; Yang, H.; Boone, L.; DeAnda, F.; Watson, C.; Kenakin, T. *Bioorg. Med. Chem.* **2003**, *11*, 2663.
11. Lynch, C. L.; Gentry, A. L.; Hale, J. J.; Mills, S. G.; MacCoss, M.; Malkowitz, L.; Springer, M. S.; Gould, S. L.; DeMartino, J. A.; Siciliano, S. J.; Cascieri, M. A.; Doss, G.; Carella, A.; Carver, G.; Holmes, K.; Schlief, W. A.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M.; Emini, E. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 677.
12. Kim, D.; Wang, L.; Caldwell, C. G.; Chen, P.; Finke, P. E.; Oates, B.; MacCoss, M.; Mills, S. G.; Malkowitz, L.; Gould, S. L.; DeMartino, J. A.; Springer, M. S.; Hazuda, D.; Miller, M.; Kessler, J.; Danzeisen, R.; Carver, G.; Carella, A.; Holmes, K.; Lineberger, J.; Schlief, W. A.; Emini, E. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 3099 and 3103.
13. Shen, D.-M.; Shu, M.; Mills, S. G.; Chapman, K. T.; Malkowitz, L.; Springer, M. S.; Gould, S. L.; DeMartino, J. A.; Siciliano, S. J.; Kwei, G. Y.; Carella, A.; Carver, G.; Holmes, K.; Schleif, W. A.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M. D.; Emini, E. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, preceding ref 14 in this issue. doi:10.1016/j.bmcl.2003.12.004.
14. Shen, D.-M.; Shu, M.; Willoughby, C. W.; Shah, S.; Lynch, C. L.; Hale, J. J.; Mills, S. G.; Chapman, K. T.; Malkowitz, L.; Springer, M. S.; Gould, S. L.; DeMartino, J. A.; Siciliano, S. J.; Lyons, K.; Pivnichny, J. V.; Kwei, G. Y.; Carella, A.; Carver, G.; Holmes, K.; Schleif, W. A.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M. D.; Emini, E. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, preceding paper in this issue. doi:10.1016/j.bmcl.2003.12.005.
15. Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett.* **1972**, *13*, 3769.
16. Waner, B. P.; Buchwald, S. L. *J. Org. Chem.* **1994**, *5*, 5822.
17. Logue, M. W.; Teng, K. *J. Org. Chem.* **1982**, *47*, 2549.
18. For the preparation of **8** and its *p*-methoxybenzyl analogues, see ref 5 and 14 above.
19. Hazuda, D. J.; Felock, P.; Witmer, M.; Wolfe, A.; Stillmock, K.; Grobler, J. A.; Espeseth, A.; Gabryelski, L.; Schleif, W.; Blau, C.; Miller, M. D. *Science* **2000**, *287*, 646.
20. Inhibition of radio-labeled MK-499 binding using human embryonic kidney (HEK) cells expressing hERG. For procedure, see: Wang, J.; Della Penna, K.; Wang, H.; Karczewski, J.; Connolly, T. M.; Koblan, K. S.; Bennett, P. B.; Salata, J. J. *Am. J. Physiol.-Heart Circ. Physiol.* **2003**, *284*, H256.
21. Crumb, W.; Cavero, I. *Pharm. Sci. Technol. Today* **1999**, *2*, 270.
22. Fermini, B.; Fossa, A. A. *Nat. Rev. Drug Discov.* **2003**, *2*, 439.
23. The structures of **9b** and **9c** are:



24. The hERG K^+ channel activity of **51a** was not measured. It was noted that both **51b** and **51d** had considerable increases in hERG K^+ channel activity compared to **9b** and **9d**, respectively.
25. Saag, M. S.; Emini, E. A.; Laskin, O. L.; Douglas, J.; Lapidus, W. I.; Schleif, W. A.; Whitley, R. J.; Hildebrand, C.; Byrnes, V. W.; Kappes, J. C.; Anderson, K. W.; Massari, F. E.; Shaw, G. M. *New Engl. J. Med.* **1993**, *329*, 1065.