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1,3,4-Trisubstituted Pyrrolidine CCR5 Receptor Antagonists. Part 4: Synthesis of N-1 Acidic Functionality Affording Analogues with Enhanced Antiviral Activity Against HIV

Christopher L. Lynch,[a,*] Jeffrey J. Hale,[a] Richard J. Budhu,[a] Amy L. Gentry,[a] Sander G. Mills,[a] Kevin T. Chapman,[a] Malcolm MacCoss,[a] Lorraine Malkowitz,[b] Martin S. Springer,[b] Sandra L. Gould,[b] Julie A. DeMartino,[b] Salvatore J. Siciliano,[b] Margaret A. Cascieri,[b] Anthony Carella,[c] Gwen Carver,[c] Karen Holmes,[c] William A. Schleif,[c] Renee Danzeisen,[c] Daria Hazuda,[c] Joseph Kessler,[c] Janet Lineberger,[c] Michael Miller[c] and Emilio A. Emini[c]

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

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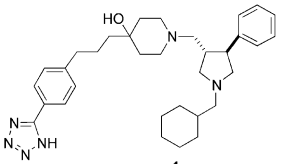
Abstract—A series of α-(pyrrolidin-1-yl)acetic acids is presented as selective and potent antivirals against HIV. Several of the pyrrolidine zwitterions demonstrated reasonable in vitro properties, enhanced antiviral activities and improved pharmacokinetic profiles over pyrrolidine **1**.

The discovery that the β-chemokine receptor CCR5 is a co-receptor utilized by the human immunodeficiency virus (HIV) to gain entry into host cells has led to the pursuit of small molecule antagonists as novel antiviral agents.[1–3] Recent reports from these laboratories have described 1,3,4-trisubstituted pyrrolidines as potent inhibitors of viral entry in the HeLa cell anti-infectivity assay.[4] In the preceding paper, the introduction of acidic functionality into a combinatorial chemistry lead[5] increased both the in vitro selectivity and antiviral activity within this class of CCR5 antagonists, as exemplified by tetrazole **1**.[6] Although **1** was found to possess desirable in vitro properties (e.g., L-Type Ca^{+2} channel $IC_{50} > 10$ μM), its rat pharmacokinetic (PK) profile was not acceptable. In an effort to further explore zwitterionic pyrrolidines, this paper will present the synthesis and SAR of α-(pyrroldin-1-yl)acetic acids as selective and potent antivirals with improved pharmacokinetics.



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CCR5 (MIP 1α) IC_{50} = 0.2 nM
HeLa (BAL) IC_{90} = 4 nM
L-Type Ca^{+2} Channel IC_{50} > 10 μM

Rat Pharmacokinetics:
Cl_p = 61 mL/min/kg
$t_{1/2}$ = 0.3 h
%F = 0

The synthesis of the α-(pyrrolidin-1-yl)acids was executed through an alkylation of a 3,4-disubstituted pyrrolidine intermediate[7] with triflates derived from enantiopure α-hydroxy esters which occurs with clean S_N2 inversion.[8] The required esters (see Scheme 1) were obtained from commercially available chiral α-hydroxy acids (A)[9] or were synthesized through a variety of routes, including enolate oxidation (B),[10] Grignard addition to an oxalate (C),[11] diazotization of cyclopropylalanine (D)[12] and an asymmetric reduction of an α-ketoamide (E).[13] In addition, the *para*-methoxybenzyl (PMB) ester was prepared in specific examples to accommodate reducible functionality under catalytic hydrogenation. In the examples (B and C) employing

*Corresponding author. Fax: +1-908-594-5966; e-mail: chris_lynch@merck.com

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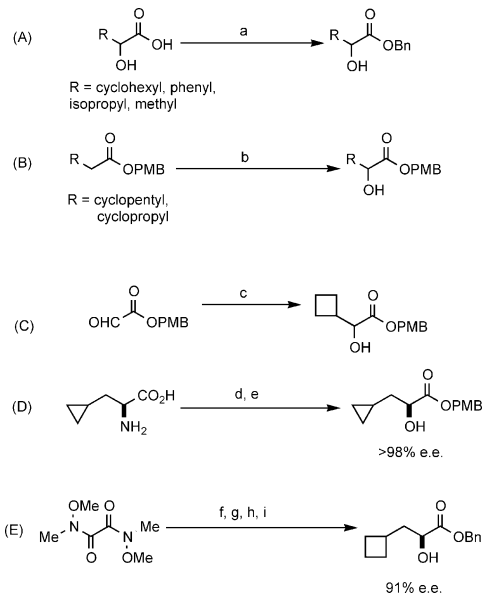
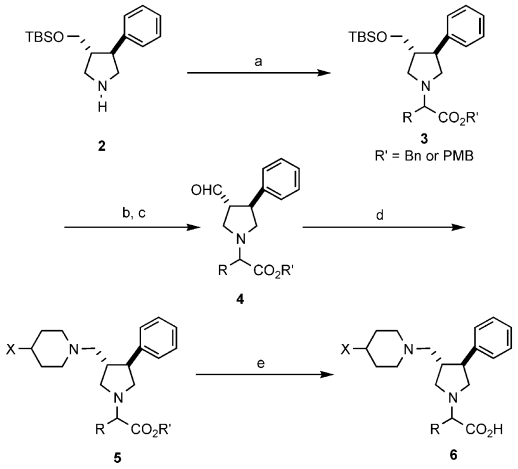


Scheme 1. Reagents and conditions: (a) 0.5 N KOH, Bu_4NI, Bn–Br, $CHCl_3$; (b) NaHMDS, THF, −78 °C; 2-(Phenylsulfonyl)-3-phenyloxaziridine; (c) Cylcobutylmagnesium bromide, Et_2O, −78 °C; (d) 2 N H_2SO_4, $NaNO_2$; (e) PMB–Cl, TEA, DMF; (f) Mg–K, (Bromomethyl)cyclobutane, THF; (g) (*R*)-Alpine Borane, THF; (h) KO*t*Bu, THF, H_2O; (i) Bn–Br, TEA, DMF.

racemic α-hydroxy esters, the diastereomers were separated by HPLC after cleavage of the TBS ether (see Scheme 2). Upon separation of the diastereomers, the assignment of relative stereochemistry was based on the correlation of ^1H NMR data with the products derived from the enantiomerically pure starting materials.

The general route to the α-(pyrrolidin-1-yl)acids is presented in Scheme 2. With routes to the hydroxy esters established, alkylation of **2** with the intermediate triflates was straight forward to give **3**. The cleavage of the silyl ether with tetrabutylammonium fluoride, followed by a Swern oxidation, gave aldehyde **4**. Reductive amination with several 4-substituted piperidines[5] under the conditions of Abdel-Magid and co-workers[14] provided

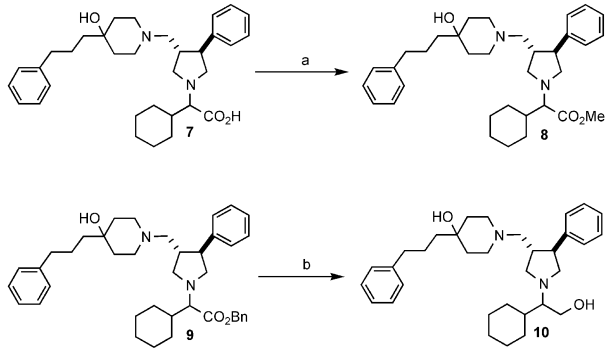


Scheme 2. (a) Triflate, DIEA, CH_2Cl_2, −78 to 0 °C; (b) TBAF, THF; (c) $(COCl)_2$, DMSO, TEA, CH_2Cl_2; (d) Piperidine analogue, $NaB(OAc)_3H$, CH_2Cl_2; (e) H_2, Pd–C, MeOH (or HCO_2H, 50 °C for PMB esters).

esters **5**. Final cleavage of the benzyl group under catalytic hydrogenation or with warm formic acid for the PMB-esters provided the desired target compounds.

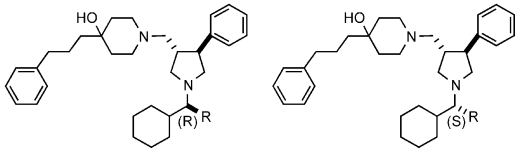
Derivatives other than carboxylates were also explored (Scheme 3). Standard chemistry allowed the preparation of the methyl esters **8** and primary alcohols **10** from intermediates generated along the synthetic route in Scheme 2.

The CCR5 receptor affinity and antiviral data for the compounds are presented in Tables 1, 2, and 4. Initially, analogues were screened for their ability to displace [^{125}I]-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes.[15] The most potent compounds were further evaluated as antivirals in a HeLa cell antiinfectivity assay versus the BAL strain of HIV.[16] Based on the functionality within **1**, the α-(pyrrolidin-1-yl)cyclohexylacetic acid and its derivatives were prepared to study the initial SAR (Table 1). Several trends became apparent with the modifications of this scaffold. This class of pyrrolidines favors the (*R*)-stereochemistry at the pyrrolidine center (comparing **11**–**14** with **15**–**18**). In every case, the (*S*)-stereochemistry led to a loss in both CCR5 binding and antiviral activity. Although both the free acid and primary alcohol are tolerated as determined



Scheme 3. (a) $TMSCHN_2$, MeOH, THF; (b) $LiAlH_4$, THF, 0 °C.

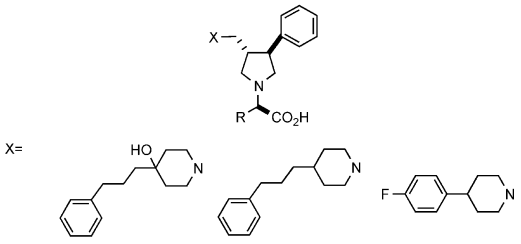
Table 1. CCR5 receptor affinity and antiviral activity of α-(pyrrolidin-1-yl)cyclohexylacetic acid derivatives



No.	R	MIP 1α[a] (HeLa)[b]	No.	R	MIP 1α[a] (HeLa)[b]
11	CH_2OH	1 ± 0.2 (300)	**15**	CH_2OH	1 ± 0.4 (NT[c])
12	CO_2Bn	3.6 ± 1.0 (> 300)	**16**	CO_2Bn	24 ± 10 (> 300)
13	CO_2Me	1 ± 0.3 (NT[c])	**17**	CO_2Me	4 ± 0.7 (NT[c])
14	CO_2H	0.5 ± 0.05 (11)	**18**	CO_2H	5 ± 0.6 (> 300)

[a]Displacement of [^{125}I]-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes (IC_{50}, nM). Data are reported as mean ± SD for $n = 3$ determinations. See ref 15 for assay protocol.
[b]IC_{90} (nM) values obtained in the HeLa cell antiinfectivity assay versus BAL. See ref 16 for assay protocol.
[c]NT = Not tested.

Table 2. CCR5 receptor affinity and antiviral activity of α-(pyrrolidin-1-yl)acetic acid analogues[a]



R			
c-Hex	**14** 0.5±0.05 (11)	**23** 0.1±0.03 (11)	**25** 4±0.4 (100)
Ph	**19** 0.8±0.1 (33)		
i-Pr	**20** 2±0.2 (333)	**24** 0.7±0.03 (111)	**26** 36±4 (>100)
CH_3	**21** 3±0.5 (333)		
H	**22** 6±1 (3000)		

[a]Displacement of [^{125}I]-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes (IC_{50}, nM). Data are reported as mean±SD for $n=3$ determinations. See ref 15 for assay protocol. Numbers in parentheses are the IC_{90} (nM) values obtained in the HeLa cell antiinfectivity assay versus BAL. See ref 16 for assay protocol.

by the receptor binding assay (**11** and **14**), the zwitterion **14** displays a 30-fold increase in antiviral activity.

Further studies (Table 2) demonstrated that decreasing the size of the side chain of the α-(pyrrolidin-1-yl)acetic acids (**14** and **19**–**22**) resulted in a loss of antiviral activity. Although substituting an isopropyl group for the cyclohexane (**14** and **20**) maintained the affinity for the CCR5 receptor (0.5 vs 2 nM), a drop in the HeLa assay suggested a large hydrophobic moiety was required for antiviral activity. Introduction of a simple aromatic side chain as in **19** led to a 3-fold loss in antiviral activity. Removal of the side chain to provide the acetic acid **22** eliminated all antiviral activity. In general, the drop in CCR5 binding with smaller side chains was not substantial, however the antiviral activity was highly dependent on the size of the substituent. This result suggests that measurements of MIP-1α binding do not rigorously predict antiviral activity.

Although the (*R*)-α-(pyrrolidin-1-yl)cyclohexylacetic acid **14** possessed comparable antiviral activity to pyrrolidine **1**, the rat pharmacokinetics of **14** were poor (Table 3). Based on this data, our focus shifted to strategies for improving the PK parameters. Synthesis of more compact molecules, and thus minimizing the functionality of the piperidine, appeared a logical step for improving the oral bioavailability. Initially, the 4-hydroxy of **14** was removed to provide the 4-(3-phenpropyl)piperidine analogues **23** and **24**. Immediately, it was observed that neither the MIP 1α nor the HeLa data was compromised within **23**, however the antiviral activity decreased with **24**. More importantly, the PK profile of **23** improved with a decrease in clearance and an increase in bioavailability in three species (see Table 3). In order to determine the minimum requirement for the piperidine side chain and further enhance the PK, a 4-(4-fluorophenyl)piperidine was introduced (**25** and **26**). Although the oral bioavailability was maintained or improved slightly with **26**, the viral activity dropped with this truncated side chain.

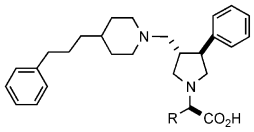
Since it was clear from the above data that a cyclohexyl side chain with the 4-(3-phenpropyl)piperidine was optimal for both antiviral and PK properties (e.g., **23**), additional cyclohexyl replacements were then investigated (Table 4). The cyclopropane **27** did not show adequate antiviral activity, which was comparable to the isopropyl analogue **24**. However, analogues **28**, **29**, and **31** maintained the activity of **23**. An enhancement in antiviral activity came with introduction of the cyclobutyl methylene moiety (**30**). Although the receptor binding data of **30** was similar to **23** and **28**, the antiviral activity displayed a 10-fold increase in potency. The pharmacokinetics and Ca^{+2} channel activity of **23** were maintained with this improvement in antiviral activity of **30** (Table 3).

In summary, the goal of our studies was to improve the pharmacokinetics of **1** while maintaining selectivity and antiviral activity. Although (*R*)-α-(pyrrolidin-1-yl)cyclohexylacetic acid **14** maintained both potency against the BAL strain in the PBMC assay and selectivity

Table 3. PBMC and Ca^{+2} channel activities[17] with the pharmacokinetic profiles of **14**, **23**, **26**, and **30**

	14	**23**	**26**	**30**
PBMC (BAL) IC_{95} nM (n) (ref 16)	187 (3)	77 (2)	>3000 (1)	<8 (2)
L-Type Ca^{+2} Channel IC_{50} μM (n)	>10	5.3	>10	2.7
Rat				
Clp (mL/min/kg)	128	17.6	68.3	26.5
Vd_{ss} (L/kg)	15	3.3	8.4	5.9
$t_{1/2}$ (h)	1.8	2.1	1.9	3.0
%F	0	26	50	29
Dog				
Clp (mL/min/kg)		10.9	13.1	
Vd_{ss} (L/kg)		3.1	8.6	
$t_{1/2}$ (h)		9.2	6.4	
%F		43	34	
Rhesus				
Clp (mL/min/kg)		17.1	14.6	
Vd_{ss} (L/kg)		6.2	4.2	
$t_{1/2}$ (h)		6.3	7.8	
%F		10	84	

Table 4. CCR5 receptor affinity and antiviral activity of α-(pyrrolidin-1-yl)acetic acid analogues[a]



R	
c-Pr	**27** 3±0.5 (300)
c-Bu	**28** 0.1±0.03 (11)
(c-Pr)CH_2	**29** 0.1±0.01 (11)
(c-Bu)CH_2	**30** 0.1±0.02 (1.2)
c-Pent	**31** 0.2±0.03 (11)

[a]Displacement of [^{125}I]-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes (IC_{50}, nM). Data are reported as mean±SD for $n=3$ determinations. See ref 15 for assay protocol. Numbers in parentheses are the IC_{90} (nM) values obtained in the HeLa cell antiinfectivity assay versus BAL. See ref 16 for assay protocol.

against the L-type Ca^{+2} channel (IC_{50} 10 μM; Table 3), compound **14** lacked oral bioavailability. However, further efforts uncovered several improved analogues (**23**, **26**, and **30**). Both **23** and **30** possessed potent antiviral activity in the PBMC assay and improved rat PK properties over **14**. In addition, **23** and **26** demonstrated that this class of pyrrolidine zwitterions could display reasonable PK profiles in three separate species and antiviral activity comparable to tetrazole **1**.

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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.
2. Panali, A.; Shapiro, S.; Clader, J. W.; Greenlee, W. J.; Cox, K.; Strizki, J.; Endres, M.; Baroudy, B. M. *J. Med. Chem.* **2001**, *44*, 3339.
3. 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 USA* **1999**, *96*, 5698.
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. Willoughby, C. A.; Berk, S. C.; Rosauer, K. G.; Degrado, S.; Chapman, K. T.; Gould, S. L.; Springer, M. S.; Malkowitz, L.; Schlief, W. A.; Hazuda, D.; Miller, M.; Kessler, J.; Danzisen, R.; Holmes, K.; Lineberger, J.; Carella, A.; Carver, G.; Emini, E. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 3137.
6. Hale, J. J.; Budhu, R. J.; 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.* **2002**, *12*, 2997.
7. 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.
8. Feenstra, R. W.; Stokkingreef, E. H. M.; Nivard, R. J. F.; Ottenheijm, H. C. J. *Tetrahedron Lett.* **1987**, *28*, 1215.
9. Lhommet, G.; Richaud, M.-G.; Maitte, P. *C. R. Hebd. Seances Acad. Sci.* **1980**, *290*, 445.
10. Davis, F. A.; Haque, M. S.; Ulatowski, T. G.; Towson, J. C. *J. Org. Chem.* **1986**, *51*, 2402.
11. Whitesell, J. K.; Buchanan, C. M. *J. Org. Chem.* **1986**, *51*, 5443.
12. For a related diazotization see: Kolasa, T.; Miller, M. J. *J. Org. Chem.* **1987**, *52*, 4978.
13. For a related reduction of ketoesters see: Brown, H. C.; Pai, G. G. *J. Org. Chem.* **1985**, *50*, 1384.
14. Abdel-Magic, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849.
15. Siciliano, S. J.; Kuhmann, S. E.; Weng, Y.; Madani, N.; Springer, M. S.; Lineberger, J. E.; Danzeisen, R.; Miller, M. D.; Kavanaugh, M. P.; DeMartino, J. A.; Kabat, D. *J. Biol. Chem.* **1999**, *274*, 1905.
16. 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.
17. Reynolds, I. J.; Snowman, A. M.; Snyder, S. H. *J. Pharmacol. Exp. Ther.* **1986**, *237*, 731.