Letters

Piperazine-Based CCR5 Antagonists as HIV-1 Inhibitors. IV. Discovery of 1-[(4,6-Dimethyl-5-pyrimidinyl)carbonyl]-4-[4-{2-methoxy-1(*R*)-4-(trifluoromethyl)-phenyl}ethyl-3(*S*)-methyl-1-piperazinyl]-4-methylpiperidine (Sch-417690/Sch-D), a Potent, Highly Selective, and Orally Bioavailable CCR5 Antagonist

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Abstract: The nature and the size of the benzylic substituent are shown to be the key to controlling receptor selectivity (CCR5 vs M1, M2) and potency in the title compounds. Optimization of the lead benzylic methyl compound **3** led to the methoxymethyl analogue **30**, which had excellent receptor selectivity and oral bioavailability in rats and monkeys. Compound **30** (Sch-417690/Sch-D), a potent inhibitor of HIV-1 entry into target cells, is currently in clinical trials.

Despite the advances in our knowledge of the pathogenesis of the human immunodeficiency virus type 1 (HIV-1) and in therapeutic intervention, the AIDS pandemic continues to be the globe's leading public health issue. Although the inhibitors of reverse transcriptase and viral protease enzymes have contained the disease progression in HIV patients, the development of resistance to these drugs highlights the need for new, mechanistically novel therapies.[1] About seven years ago, the chemokine receptors CCR5 and CXCR4 were identified as essential coreceptors for the attachment of HIV-1 to the surface of $CD4^+$ macrophages and T-cells.[2] The coreceptor engagement leads to a conformational change in the gp 41 glycoprotein on the viral envelope, revealing a peptide which fuses the HIV envelope to the cell membrane, thus aiding viral entry into the cell. The chemokine receptors CCR5 and CXCR4 which control this key event in the HIV entry process belong to the extensively studied super family of G-protein-coupled receptors (GPCRs), making them attractive targets for drug design.[3] Studies of HIV tropism indicated that the R5 viral strains that utilize CCR5 are responsible for the initial establishment and development of the disease, which prompted several research groups to design small molecule ligands to block the HIV−CCR5 interaction.[4] Individuals lacking functional CCR5 receptors (Δ32-homozygotes) are resistant to HIV infection while their immune systems are apparently not compromised, supporting the blockade of CCR5 as a mechanism for inhibiting HIV.[5]

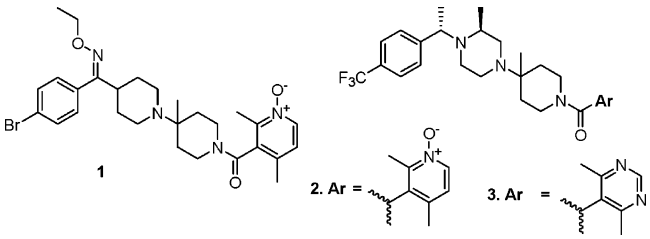

Figure 1. Piperidine and piperazine-based CCR5 Antagonists.

We have previously reported on the development of two related series of compounds as CCR5 antagonists (Figure 1). From the piperidino-piperidine series emerged compound **1** (Sch-351125/Sch-C), which is currently in clinical trials as the first orally active agent to block HIV entry.[6] The corresponding compound (**2**) in the piperazino-piperidine series[7] satisfied many of the initial criteria but showed acute CV and CNS side effects in ancillary pharmacology. This compound possessed modest affinity for the muscarinic receptors. In addition to brain, M1 and M3 muscarinic receptors are also prevalent in the GI tract, and M2 receptors are expressed in heart and lung tissues. We surmised that the high oral blood levels that we deemed necessary for an anti-infective agent such as **2** were not compatible with even modest affinities for other receptors. Additionally, compound **2** exhibited four rotational isomers (rotamers) arising from hindered rotations about the two bonds forming the unsymmetrical tertiary piperidino-amide moiety. One pair of these rotamers can be rendered degenerate by making the amide symmetrical, with the 4,6-dimethylpyrimidine carboxamide (**3**) being optimal.[8] Although the CV profile of the pyrimidine carboxamide **3** was much better than that of the *N*-oxide **2** in rats, some GI effects were still noted at higher doses of **3**. Hence, we decided to further refine the template represented by **3** with the goal of minimizing or eliminating its affinity for the muscarinic receptors and perhaps improving its CCR5 affinity. The following is an account of the key aspects of our back-up program which resulted in the discovery of the titled compound **30**, our second generation orally active CCR5 antagonist, which is currently in clinical trials as an HIV entry inhibitor.[9]

The piperidino-piperidine **1** and the piperazino-piperidine **2** and **3** have similar scaffolds and the SAR is very similar for the two ends of the molecules. However, there are distinguishing features to the two structures in the middle which contributes to the differences in their activity. Hence we focused our attention on the central portion of the piperazino-piperidine structure with the view of modulating receptor selectivity. Replacing the 2(*S*)-methyl group on the piperazine ring with an ethyl or propyl group resulted in substantial loss of binding at CCR5, and so we turned our attention to the

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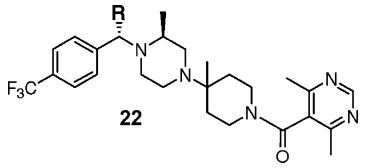
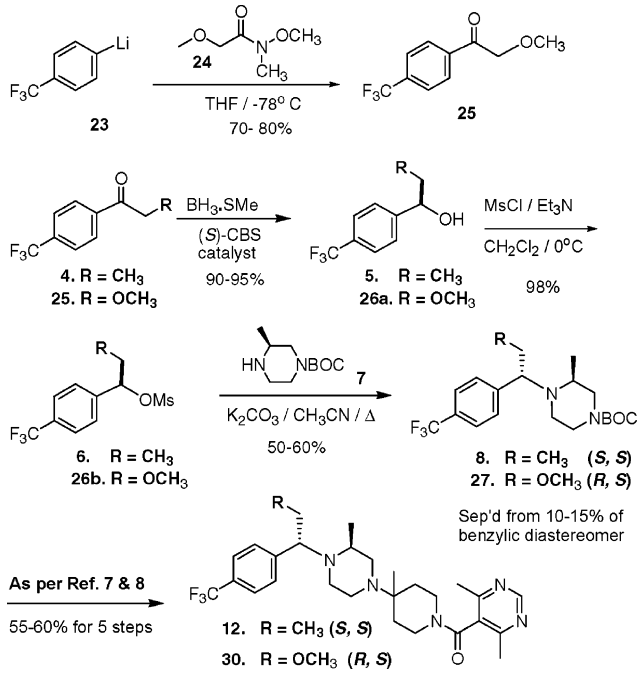
Table 1. Larger Benzylic Substituents Improve Receptor Selectivity[a]



	R in **22**	CCR5 K_i (nM)	muscarinic K_i (nM) M1	M2	M3	HIV entry (nM)[b]	rat PK (po) AUC_{0-6h}[c]
3	CH_3	2.8	575	456	716	0.39	6210
12	CH_3CH_2	5.2	3900	4750	9089	0.18	7310
20	$CH_3CH_2CH_2$	1.1	6075	4760	>5300	0.35	4600
21	$PhCH_2$	1.4	>3900	>5700	>5300	0.13	2030

[a] For descriptions of antiviral, muscarinic and rat PK assays, please see refs 7 and 8. [b] IC_{50} values for inhibiting the entry of replication defective HIV-1 (YU-2) in to U-80 cells. [c] Oral blood levels of compound in rats (10 mg/kg, n=2). Area under curve = ng/mL × h.

Scheme 1. The S_N2 Displacement Route



Scheme 2. The Cyanoamine Alkylation Route

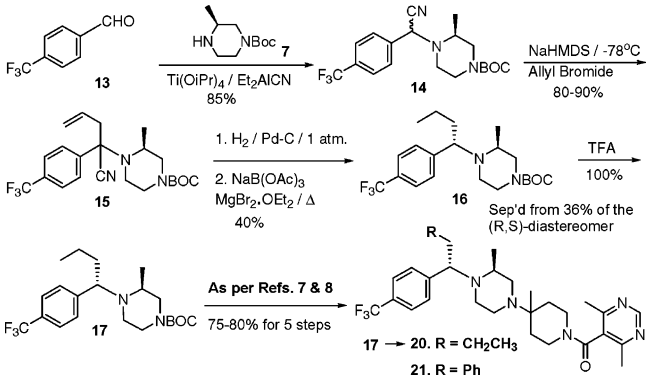


Table 2. Larger Benzylic Substituents Enhance Potency vs HIV-Isolates[a]

	R in **22**	IC_{90} (nM) values for the inhibition of HIV-1 clinical isolates in PBMC JrFL	ADA-M	301657	JV1083	RU 570
1	oxime-piperidine	19	25	43	10	400
3	CH_3	40	19	13	31	>1000
12	CH_3CH_2	19	21	16	18	1000
20	$CH_3CH_2CH_2$	0.01	0.03	0.09	0.27	0.43
21	$PhCH_2$	0.19	0.04	0.6	0.25	4
30	CH_3OCH_2	3.3	2.8	1.8	4.9	10

[a] For a description of antiviral assays, please see refs 6 and 7.

benzylic substituent, leaving the 2(*S*)-methyl piperazinopiperidine intact. Two different scalable synthetic routes were developed to facilitate the systematic variation of the benzylic substituent.

Initially, we approached the synthesis of the benzylic ethyl analogue by adapting our published S_N2 displacement route (Scheme 1).[7] Thus, the commercially available propiophenone **4** was reduced by the CBS protocol[10] to furnish benzylic alcohol **5** enriched in the *R*-enantiomer. Activation to the mesylate **6** and reaction with the chiral piperazine **7** led to the desired (*S*,*S*) diastereomeric product **8**, which was processed to the final target **12**.[8] Extension of the S_N2 displacement route to larger benzylic substituents was inefficient.

Our second synthetic strategy (Scheme 2) exploited the alkylation of a cyanoamine, followed by decyanation to introduce larger benzylic substituents.[11] Commercial aryl aldehydes were condensed with the chiral piperazine **7** under modified Strecker conditions[6,7] to obtain the cyanoamines **14**. Treatment of **14** with NaHMDS under scrupulously anaerobic conditions and quenching with allyl bromide provided the α-allyl cyanoamine **15** in good yield. Saturation of the allyl group (H_2/Pd−C) and reductive removal of the nitrile [$MgBr_2$·OEt_2/$NaB(OAc)_3H$] resulted in the formation of the benzylic propyl derivative **16**, as a 1:1 mixture of diastereomers. Flash chromatography served to isolate the desired (*S*,*S*) diastereomer which was parlayed to the target **20**. The benzyl group was similarly introduced by treating the cyanoamine anion with benzyl bromide, decyanation, and further elaboration to **21**. Propyl and larger iodides failed to alkylate the hindered cyanoamine anion.

Targets bearing ether side chains at the benzylic position were obtained by the S_N2 displacement route, as the alkoxy alkyl halides were not reactive enough to alkylate the cyanoamine. For example (Scheme 1), α-methoxyacetophenone **25** was made efficiently by the addition of aryllithium **23** to the Weinreb amide **24**.[12] CBS reduction of the α-alkoxyacetophenone, activation to a mesylate, and reaction with monoprotected chiral piperazine **7** proceeded in high overall yields. Completion of the sequence as before secured the target **30**.

Even with a one carbon homologation to the ethyl derivative **12**, we observed (Table 1) an order of magnitude improvement in receptor selectivity (CCR5 vs M1-M3) that was further improved with larger groups

Table 3. Analogues of the *n*-Propyl Compound

	R in **22**	CCR5 K_i (nM)	muscarinic K_i (nM) M1	muscarinic K_i (nM) M2	entry[b] IC_{50} (nM)	HIV-PBMC[a] IC_{90} (nM)	rat PK[c] AUC_{0-6h}
20	$CH_3CH_2CH_2$	1.1	6075	4760	0.35	0.03−0.45	4600
30	CH_3OCH_2	2.5	>10000	>10000	0.46	1−10	6900
31	Cyc-Pr-CH_2	1.6	1894	3512	0.8	0.01−4	5900
32	$CF_3CH_2CH_2$	ND	>3700	>6700	1.44	0.4−20	4600

[a] HIV isolates included: JrCSF, ASM57, Bal, JV1083, and RU570. [b,c] Please refer to footnotes *b*, *c* in Table 1 for details.

such as propyl (**20**) and benzyl (**21**). However, we had to balance the increase in receptor selectivity against the observed oral blood levels of these benzylic analogues in our high throughput pharmacokinetic (PK) screen in rats.[7] Compounds bearing lipophilic side chains were metabolized readily by liver enzymes, resulting in lower blood levels after oral administration. Thus, the serum level for the propyl compound **20**, though acceptable**,** was much less than that of the ethyl analogue **12**, and the benzyl derivative **21** had the lowest oral blood level among this group.

Another significant benefit that accrued from the presence of larger benzylic substituents was enhanced potency of inhibition of HIV-1 strains in peripheral blood mononuclear cells (PBMCs). The IC_{90} (nM) values for inhibiting genetically diverse HIV-1 isolates for the new piperazino-piperidines were compared with the first clinical candidate **1** (Table 2). In terms of their potency, the methyl and ethyl derivatives were about equal to **1**, but were not as broad spectrum in eliciting their response. The effect of further carbon homologation from ethyl to propyl was very dramatic, and the *n*-propyl compound **20** was the most potent compound in this panel. The benzyl analogue **21** was almost as potent as **20**, but it had the lowest oral blood levels in rat among this group (Table 1).

Complete profiling of the very potent propyl compound **20** showed that this compound was orally well absorbed in rat and monkey (Table 4). It did not inhibit or induce liver enzymes, and was tolerated without significant GI and CV/CNS side effects up to a dose of 10 mg/kg in rats. On the negative side, compound **20** was >99% protein bound in human plasma (compound **1** is only 84% protein bound), and during a chronic dosing study in rats, lack of body weight gain relative to control was observed at 10 and 30 mg/kg doses. Further study showed that the propyl side chain was oxidized to carboxylic acid which forms an acyl glucuronide (observed in rat bile), a potentially toxic metabolite.[13]

To block the oxidation of the terminal methyl group in the propyl side chain, we prepared three related derivatives. The methoxymethyl (**30**), cyclopropylmethyl (**31**), and the 3,3,3-trifluoropropyl (**32**) analogues were prepared via the S_N2 displacement route. Comparison of the data for these three compounds (Table 3) showed that **30** had the best overall profile: Introduction of an oxygen atom in the propyl side chain, in the form of the methoxymethyl (MOM) derivative (**30**), reduced its lipophilicity and essentially eliminated affinity for the muscarinic receptors. The MOM compound **30** was more potent than the first generation piperidino-piperidine **1**, but less potent than the *n*-propyl derivative **20** (Table 2). However, the modest drop in potency in going from the *n*-propyl (**20**) to the MOM compound (**30**) was compensated by improvements in the oral blood level (Table 3) and protein binding (84%) for the latter. The cyclopropylmethyl analogue **31** had the best potency in this triad vs a small panel of isolates, but its receptor selectivity (CCR5 vs M1 and M2) was diminished and it was >99% protein bound. In a head-to-head chronic dosing study in rats, there were no significant adverse side effects with **30** whereas some lack of weight gain and mild enzyme induction were noted with **31.** Compared to **30**, the trifluoropropyl compound **32** showed lower oral blood levels, lacked the broad spectrum potency against diverse HIV isolates, and was 99% protein bound.

Table 4. Pharmacokinetic Profiles of Compounds **20** and **30**

	species (dose, mg/kg)	iv admin AUC_{0-24h}[a]	iv admin $T_{1/2}$ (h)	oral admin C_{max}[b]	oral admin AUC_{0-24h}[a]	BA (%)[c]
20	rat (2)	4550	15.5	311	3270	72
	monkey (2)	2670	6.3	240	1760	66
30	rat (10)	21000	8	2300	21000	100
	monkey (2)	2240	3.5	670	1990	89

[a] AUC (area under curve of concn vs time plot) = ng/mL × h. [b] C_{max} = ng/mL. [c] BA (bioavailability) = $[AUC]_{po}/[AUC]_{iv}$.

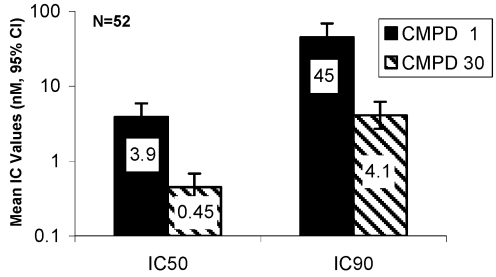


Figure 2. Potencies of compounds **1** and **30** vs HIV isolates.

Direct comparison (Figure 2) of compound **30** with our first generation CCR5 antagonist **1** against a geographically diverse panel of HIV isolates ($n = 52$) showed that **30** is an order of magnitude more potent than **1**, with a mean $IC_{50} = 0.45$ nM and $IC_{90} = 4$ nM.

Further work confirmed that the methoxymethyl compound **30** had high oral bioavailability in rodents and primates (Table 4), in addition to very good potency and receptor selectivity. It has good CNS penetration (brain/plasma ratio = 0.86 for **30** and 0.17 for **1**). The major route of metabolism (rats) for **30** is through the liver, where O-demethylation of the side chain followed by glucuronidation was observed.

While we were developing compound **30** in the back-up program, QT prolongation was observed with **1** (doses ≥ 400 mg/day) in the electrocardiogram of healthy human volunteers. This is thought to arise from the interaction of the putative drug with the hERG potassium ion channel in heart tissue.[14] In the voltage clamp assay,[15] the piperazino-piperidine **30** had weaker

hERG activity (IC_{50} = 5.8 μM) than **1** (IC_{50} = 1.1 μM). In an ancillary study in cyanomolgus monkeys dosed with compound **30** and monitored with cardiac telemetry, no cardiovascular effects (including QT prolongation) were observed up to a dose of 40 mg/kg. Upon chronic dosing of **30** in rats, there was neither inhibition nor induction of liver enzymes. Additionally, no acute CNS or GI effects were noted in an ancillary study with **30** (10 mg/kg oral dose in rats). Thus our premise of designing piperazino-piperidine leads with high receptor selectivity (CCR5 vs M1, M2) as the basis for improved side effect profile had fulfilled its promise.

In summary, during our back up program, we discovered that the size of the benzylic substituent is the key to enhanced potency and receptor selectivity in the piperazino-piperidine series. These efforts initially led to the *n*-propyl analogue **20** with excellent broad spectrum potency. However, the formation of an acylglucuronide metabolite from this compound in vivo, and undesirable side effects during chronic dosing, prompted us to introduce a heteroatom (O) in the propyl side chain to alter its metabolic fate. The resulting methoxymethyl derivative **30** had the best overall profile among all the compounds in the second phase of our program: It was extremely selective for the CCR5 receptor with almost no affinity for muscarinic receptors or other GPCRs. It inhibited a broad spectrum of HIV isolates with a mean IC_{90} of 4 nM, an order of magnitude more potent than the first generation compound (**1**). The MOM compound (**30**) had excellent oral bioavailability in rats and monkeys, reduced affinity for the hERG K^+ channel, and no significant CNS or GI side effects at an oral dose of 10 mg/kg in rats. Compound **30** is currently in clinical trials.

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Supporting Information Available: Experimental procedures and spectral data for the preparation and characterization of compounds **12**, **20**, **30**−**32** are available free of charge at http://pubs.acs.org.

References

(1) Pomerantz, R. J. Primary HIV-1 resistance. *J. Am. Med. Assoc.* **1999**, *282* (12), 1177−1178.

(2) Berger, E. A.; Murphy, P. M.; Farber, J. M. Chemokine receptors as HIV-1 co-receptors: roles in viral entry, tropism and disease. *Annu. Rev. Immunol.* **1999**, *17*, 657−700.

(3) Hunt, S. W. III; LaRosa, G. J. Chemokine receptors as HIV co-receptors: targets for therapeutic intervention in AIDS. *Annu. Rep. Med. Chem.* **1998**, *33*, 263−272.

(4) Kazmierski, W.; Bifulco, N.; Yang, H.; Boone, L.; DeAnda, F.; Watson, C.; Kenakin, T. Recent progress in discovery of small-molecule CCR5 chemokine receptor ligands as HIV-1 inhibitors. *Bioorg. Med. Chem.* **2003**, *11*, 2663−2676.

(5) Liu, R.; Paxton, W.; Choe, S.; Ceradini, D.; Martin, S.; Horuk, R.; MacDonald, M.; Stuhlmann, H.; Koup, R.; Landau, N. R. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply exposed individuals to HIV-1 infection. *Cell* **1996**, *86*, 367−377.

(6) Palani, A.; Shapiro, S.; Clader, J.; Greenlee, W.; Cox, K.; Strizki, J.; Endres, M.; Baroudy, B. Discovery of 4-[(*Z*)−(4-Bromophenyl-(ethoxyimino)methyl]-1′-[(2,4-dimethyl-3-pyridinyl)carbonyl]-4′-methyl-1,4′-bipiperidine *N*-Oxide (SCH 351125): An Orally Bioavailable Human CCR5 Antagonist for the Treatment of HIV Infection. *J. Med. Chem.* **2001**, *44*, 3339−3342.

(7) Tagat, J. R.; Steensma, R.; McCombie, S.; Nazareno, D.; Lin, S.-I.; Neustadt, B.; Cox, K.; Xu, S.; Wojcik, L.; Murray, M.; Vantuno, N.; Baroudy, B.; Strizki, J. Piperazine-Based CCR5 Antagonsits as HIV-1 Inhibitors. II: Discovery of 1-[(2,4-Dimethyl-3-pyridinyl)carbonyl]-4-methyl-4-[3(*S*)-4-methyl-4-[1(*S*)-[4-(trifluoromethyl)phenyl}ethyl}-1-piperazinyl}piperidine *N*1-Oxide (Sch-350634), an Orally Bioavailable, Potent CCR5 Antagonist. *J. Med. Chem.* **2001**, *44*, 3343−3346.

(8) McCombie, S.; Tagat, J. R.; Vice, S.; Lin, S.-I.; Steensma, R.; Palani, A.; Neustadt, B.; Baroudy, B.; Strizki, J.; Endres, M.; Cox, K.; Dan, N.; Chou, C.-C. Piperazine-Based CCR5 Antagonists as HIV-1 Inhibitors. III: Synthesis, Antiviral and Pharmacokinetic Profiles of Symmetrical Heteroaryl Carboxamides. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 567−571.

(9) Tagat, J.; McCombie, S.; Nazareno, D.; Steensma, R.; Labroli, M.; Baroudy, B.; Strizki, J.; Cox, K. Discovery of potent, selective and orally active CCR5 antagonists for the potential treatment of HIV infection. *Abstracts of Papers*; 225th ACS National Meeting, New Orleans, LA, March 23−27, 2003; American Chemical Society: Washington, D. C., 2003; MEDI 15.

(10) Corey, E. J.; Bakshi, R. K.; Shibata, S. Highly enantioselective borane reductions of ketones catalyzed by chiral oxaborolidines. *J. Am. Chem. Soc.* **1987**, *109*, 5551−5553.

(11) (a) Ahlbrecht, H.; Raab, W.; Vonderheid, C. Dehydrocyanation of α-aminonitriles: A versatile and convenient enamine and dienamine synthesis. *Synthesis* **1979**, 127−129. (b) Bunnelle, W. H.; Shevlin, C. G. On the reduction of α-aminonitriles with sodium. *Tetrahedron Lett.* **1989**, 30, 4203−4206.

(12) Nahm, S.; Weinreb, S. W. *N*-methoxy-*N*-methyl amides as effective acylating agents. *Tetrahedron Lett.* **1981**, *22*, 3815−3818.

(13) Shipkova, M.; Armstrong, V. W. Oellerich, M.; Wieland, E. Acyl glucuronide drug metabolites: toxicological and analytical implications. *Therap. Drug Monit.* **2003**, *25*, 1−16.

(14) Pearlstein, R.; Vaz, R.; Rampe, D. Understanding the structure−activity relationship of the Human Ether-a-go-go-Related Gene cardiac K^+ channel. A model for bad behavior. *J. Med. Chem.* **2003**, *46*, 1−6.

(15) Fermini, B.; Fossa, A. The impact of drug-induced QT interval prolongation on drug discovery and development. *Nat. Rev. Drug Discovery* **2003**, *2*, 439−447.

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