Piperazine-Based CCR5 Antagonists as HIV-1 Inhibitors. II. Discovery of 1-[(2,4-Dimethyl-3-pyridinyl)carbonyl]-4-methyl-4-[3(*S*)-methyl-4-[1(*S*)-[4-(trifluoromethyl)phenyl]ethyl]-1-piperazinyl]-piperidine *N*1-Oxide (Sch-350634), an Orally Bioavailable, Potent CCR5 Antagonist

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Abstract: Truncation of the original piperidino-2(*S*)-methyl piperazine lead structure **2**, from a family of muscarinic antagonists, gave compound **8** which has improved selectivity for the HIV-1 co-receptor CCR5 over muscarinic receptors. Further optimization for pharmacokinetic properties afforded Sch-350634 (**1**), a prototypical piperazine-based CCR5 antagonist, which is a potent inhibitor of HIV-1 entry and replication in PBMCs. The title compound (**1**) has excellent oral bioavailability in rat, dog, and monkey.

Introduction. Human immunodeficiency virus type 1 (HIV-1) infection, which eventually leads to the acquired immunodeficiency syndrome (AIDS), continues unabated as an epidemic of major proportions in sub-Saharan Africa and Asia.[1] In the Western Hemisphere, the emergence of viral strains resistant to combinations of antiretroviral drugs still makes HIV-1 a primary healthcare issue.[2] The recent discovery of CCR5, a co-receptor essential for HIV recognition and entry into CD4+ macrophages and T-cells, was therefore received with excitement in the scientific community as a potential new target for antiviral therapy.[3] The expectation was that suitable chemical agents which bind to CCR5 would prevent HIV from entering the CD4+ target cells.[3a] Further impetus to this approach came from studies in which individuals homozygous for a 32 base pair deletion in the gene encoding CCR5 and thus lacking functional receptors were shown to be resistant to HIV-1 infection; heterozygous individuals, who lack one of the two alleles needed for CCR5 expression, show delayed disease progression following HIV infection.[3a]

CCR5 is a chemokine receptor belonging to the super family of G-protein coupled receptors (GPCRs). The design and synthesis of small molecule ligands for GPCRs has been an active area of research over the past decade, and the importance of chemokines and their receptors in viral disease processes is now well-recognized.[4] Consequently, the goal of discovering an orally active, potent, and selective small molecule CCR5 antagonist has stimulated vigorous research programs at several pharmaceutical companies.[5] We have recently disclosed studies in which the initial hits from our database of privileged structures that also have muscarinic M2 antagonist activity were optimized with regard to CCR5 binding activity to afford piperazine- and piperidine-based CCR5 antagonists.[6] In this paper, we describe the design, synthesis, and biological activity of Sch-350634 (**1**), an orally bioavailable CCR5 antagonist that inhibits the replication of HIV-1 via blockade of its entry into cells.

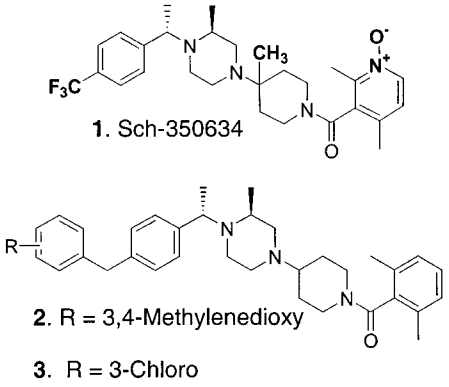

Figure 1.

Our preliminary studies showed that 2(*S*)-methyl piperazine and the 2,6-dimethyl benzamide moiety in structure **2** (K_i: CCR5 = 18 nM; M2 = 760 nM) are essential to enhance affinity for CCR5 over the muscarinic receptors (particularly M2).[7] Although compound **2** satisfied preliminary in vitro criteria, we were aware that the presence of a methylenedioxyphenyl ring in this structure could be a metabolic liability (Figure 1). In our earlier work, CCR5 and M2 receptor binding data for compound **3** (K_i: CCR5 = 45 nM; M2 = 1400 nM) indicated that substitution on the left side of the molecule was tolerated. We then set out to define the minimum structural elements that were needed for a CCR5 antagonist of this structural class, with selectivity over binding at muscarinic M2 receptor,[8] good potency in anti-viral assays,[9] and good oral absorption in rat[10] as our preliminary overall criteria.

Chemistry. As shown in Scheme 1, elaboration of commercially available α-(*S*)-methyl benzylamine (**4**) via halogenation and S_N2 displacement of an activated (*R*)-methyl lactate gave the diketo piperazine **6**, which was processed to the target **8**. Compound **8** exhibits strong affinity for CCR5 with a significant decrease in the binding at the M2 muscarinic receptor (CCR5: $K_i = 20$ nM; M2: 32% inhibition at 1 μM). This result suggested that truncation on the left side to simplify the structure and reduce the molecular weight can provide piperidino-piperazines, which are more selective ligands for CCR5. Replacing the benzylic carbon in **8** with groups such as

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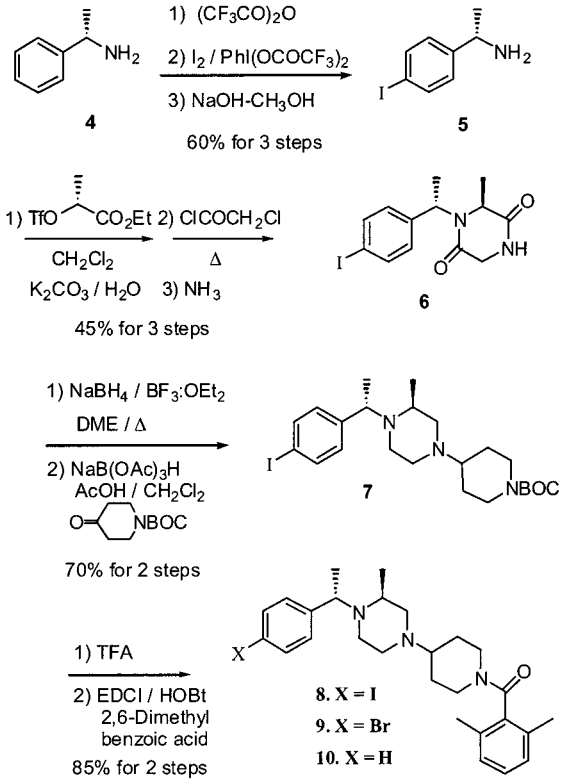
Scheme 1. Diketopiperazine Route



amides, carbamates, or sulfonamides resulted in loss of binding at CCR5, as did attempts to replace the amide group on the right side with alkyl groups, sulfonamides, or carbamates. Thus, the structure **8** became a starting point for further refinement of structure−activity−pharmacokinetic relationships.

The iodine substituent in **7** served as a functional handle to introduce less labile groups at the para-position via standard methodology.[11] Alternatively, targets such as **16** and **17** containing electron-withdrawing para-substituents that are not readily accessible from the intermediate **7** were prepared by a route which is enantio- and diastereo-selective (Scheme 2).[12] In this route, it is noteworthy that a modified Strecker reaction (**14** → **15**) served to introduce an alkyl or aryl group at the 4-position of the piperidine.[13] Standard reductive amination introduces a hydrogen atom.[14]

Results and Discussion. In the truncated structure **8**, as in compounds **2** and **3**, the (*S*)-methyl group in the piperazine ring is essential for effective binding at CCR5 (Table 1). Introduction of a methyl group at the 4-position of the piperidine (compounds **8b**, **8d**, and **8e**) results in a 3−7-fold improvement in the binding potency (K_i), with a proportional increase in the antiviral potency (IC_{50}) in the functional assay. The methyl group was found to be optimal at the ring junction (R^3: CH_3 > H > Et, *i*-Pr).

In a related study, we prepared the other three diastereomers arising from the two chiral centers. The (*S*,*S*)-diastereomer depicted (**8**) was about 8 times more potent in binding to CCR5 than the (*R*,*S*)-diastereomer (K_i = 125 nM), whereas the (*S*,*R*) and (*R*,*R*) diastereomers were inactive (K_i > 1 μM), again underscoring the importance of the 2(*S*)-methyl piperazine in the pharmacophore.

Scheme 2. S_N2 Displacement Route

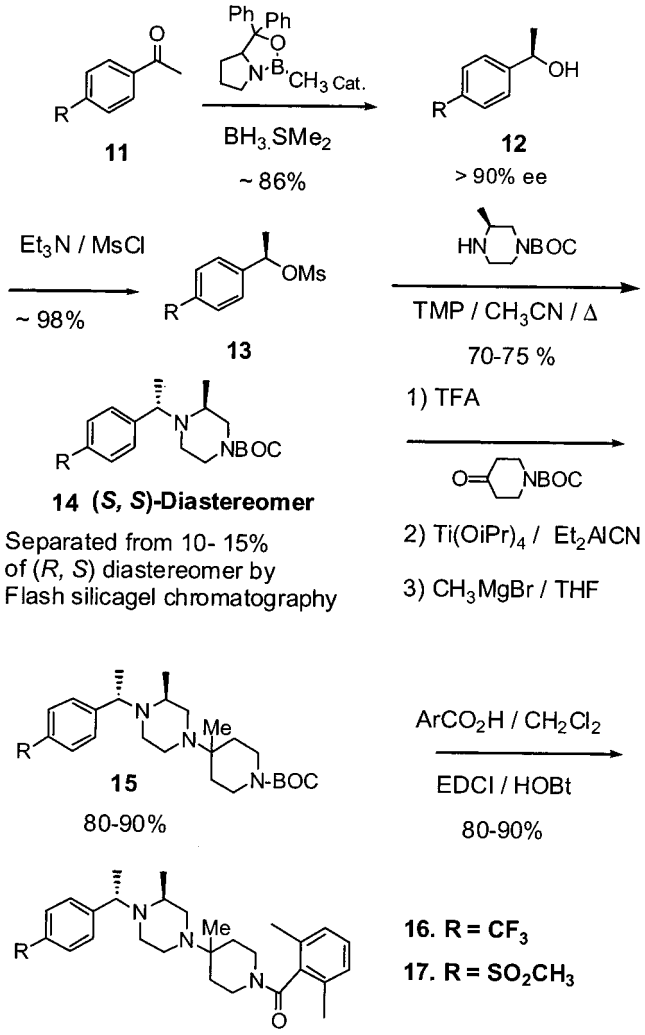
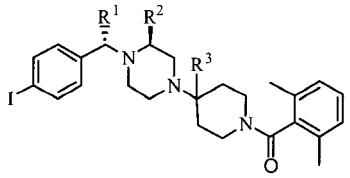


Table 1. Methylation Patterns Determine CCR5 Affinity



compd	R^1	R^2	R^3	CCR5 binding K_i (nM)[a]	HIV-1 entry IC_{50} (nM)[b]
8a	H	H	H	440	ND[c]
8b	H	H	CH_3	62	ND[c]
8c	H	CH_3	H	30	ND[c]
8	CH_3	CH_3	H	20	7
8d	H	CH_3	CH_3	8	2.7
8e	CH_3	CH_3	CH_3	5	1

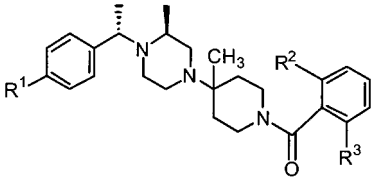
[a] See Supporting Information for details. Standard error was 10%, and assay-to-assay variability was 2−3-fold. [b] Concentration required to inhibit by 50% the entry of HIV-1 reporter virus (JrFl) into U-87 cells. For IC_{50} values, the 95% confidence limit was within 1 log and the intra-assay variation was less than 0.5 log. [c] ND = not determined.

The bromo-derivative **9** (K_i = 23 nM; IC_{50} = 6 nM), which was prepared in a manner analagous to **8**, was equipotent to the latter. However, the des-halo compound **10** (K_i = 275 nM) was significantly less active, and the ortho- and meta-substituted derivatives were 5−6 times less active than **8** and **9**, indicating the

requirement of a para-substituent of medium size in the pharmacophore.

With the in vitro structure−activity relationships in the new, truncated piperidino-piperazine series well-established, we turned our attention to pharmacokinetic (PK) characterization of our CCR5 antagonists. Following oral administration of compounds (10 mg/kg) to rats, their plasma concentrations over a 6 h period were determined.[10] The benzamides exhibited only modest blood levels in the rat model following oral administration due to oxidative metabolism in the benzamide portion. Consequently, the two methyl groups were replaced with heteroatoms (Cl, OH, NH_2). The in vitro and rat PK data for representative new amides are presented in Table 2.

Table 2. Effect of Varying the Amide on Oral Blood Levels in Rat (10 mg/kg)



				CCR5 data		rat PK (0−6 h)
compd	R^1	R^2	R^3	*Ki* (nM)[a]	IC_{50} (nM)[b]	AUC (ng/mL h)
8e	I	CH_3	CH_3	5	1	822
8f	I	Cl	NH_2	12	2	1984
8g	I	CH_3	OH	7	1	2806
16	CF_3	CH_3	CH_3	1	0.4	922
16a	CF_3	Cl	NH_2	5	1.2	1872
16b	CF_3	CH_3	OH	5	3.2	2543
17	SO_2CH_3	CH_3	CH_3	3	0.5	ND[c]
17a	SO_2CH_3	Cl	NH_2	5	0.8	447
17b	SO_2CH_3	CH_3	OH	15	4	384

[a] Standard error was 10%, and assay-to-assay variability was 2−3-fold. [b] Concentration required to inhibit by 50% the entry of HIV-1 reporter virus (ADA) into U-87 cells. For IC_{50} values, the 95% confidence limit was within 1 log and the intra-assay variation was less than 0.5 log. [c] ND = not determined.

Replacing the lipophilic benzamide with the polar anthranilamides and salicylamides significantly improved oral blood levels, expressed as the area under the curve (AUC) of concentration vs time plots. While several para-substituents satisfied the CCR5 binding criteria, the trifluoromethyl group was optimal based on PK parameters.

The concept of introducing heteroatoms in the amide portion was extrapolated by replacing the 2,6-dimethyl benzamide with the 2,4-dimethyl nicotinamide (Figure 2). The combination of a para-trifluoromethyl substituent on the left and 2,4-dimethyl nicotinamide on the right side of the piperidino-piperazine core afforded a compound (**18**) that satisfied all our preliminary criteria in terms of its anti-viral properties and good oral blood levels in rat. Additionally, **18** has reduced affinity for the M2 muscarinic receptor (K_i = 2500 nM) and does not inhibit the liver enzymes 3A4 and 2D6. Compound **18** also has excellent potency in anti-viral assays: It inhibits HIV entry in a single cycle infectivity assay with an IC_{50} = 0.5 nM (HIV-1/ADA) and the replication of several primary HIV-1 isolates in peripheral blood mononuclear cells (PBMC), with IC_{50} values in the 0.2−2.0 nM range.[15]

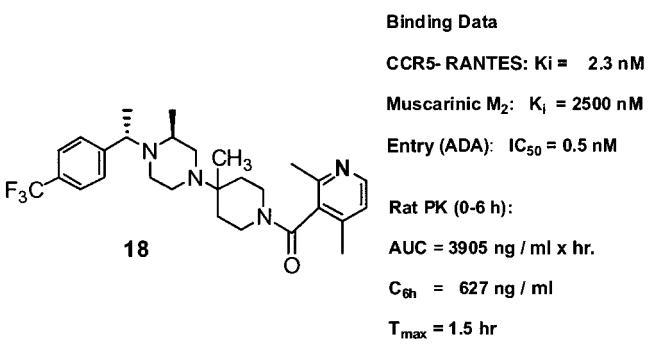


Figure 2.

Pharmacokinetic data for **18** (administered as the amorphous hydrochloride salt in 0.4% methyl cellulose) in beagle dogs and in cynomolgus monkeys are summarized in Table 3.

Table 3. Pharmacokinetic Profile of Compound **18**

	iv administration[a]		oral administration[b]		
species	C_o[c]	AUC_{0-24h}[d]	C_{max}[c]	AUC_{0-24h}[d]	BA (%)[e]
dog[e]	346	1609	226	1583	33[e]
monkey[e]	390	996	281	2742	27.5[e]

[a] Intravenous (iv) dose = 1 mg/kg. [b] Oral dose = 3 mg/kg (dog) or 10 mg/kg (monkey). [c] C_o and C_{max} are in ng/mL. [d] AUC is in ng/mL h. [e] Bioavailability (BA) is dose-corrected.

Following oral administration of **18** to rats, analysis of plasma samples showed that a single (M+16) metabolite is rapidly produced in amounts that could exceed the remaining parent compound. This reflects a high level of absorption, followed by first-pass oxidation. A similar situation was later found in both dogs and monkeys.

Surmising that the rapid metabolism of **18** may be due to oxidation at the pyridyl nitrogen, we prepared the corresponding nicotinamide-*N*-oxide (**1**). Hindered rotation about the two bonds that constitute the unsymmetrical tertiary amide on the right side results in the observation of four rotational isomers (rotamers) under achiral conditions for compound **1**.[16]

Compound **1**, which has good affinity for CCR5 binding and good potency in the entry assay, showed almost a 3-fold improvement in oral blood level in rats, relative to **18** (Figure 3).

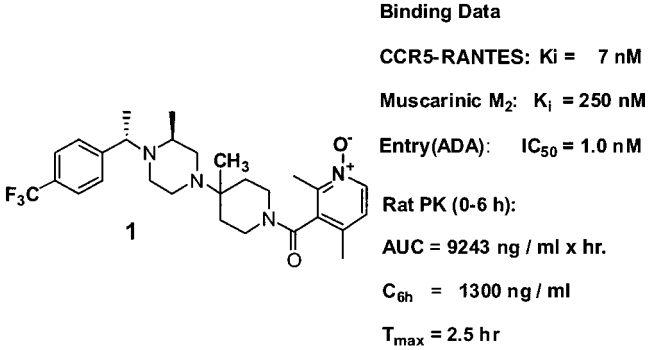


Figure 3.

Detailed pharmacokinetic evaluation in beagle dogs and cynomolgus monkeys (Table 4) clearly demonstrated that compound **1** (amorphous HCl salt) is capable of achieving and sustaining high blood levels

Table 4. Pharmacokinetic Profile of the Title Compound (**1**)

	iv administration[a]		oral administration[b]		
species	AUC_{0-24h}[d]	$T_{1/2}$ (h)	C_{max}[c]	AUC_{0-24h}[d]	BA (%)[e]
dog[e]	3240	6	690	6290	65[e]
monkey[e]	2610	4	1420	15400	59[e]

[a] Intravenous (iv) dose = 1 mg/kg. [b] Oral dose = 3 mg/kg (dog) or 10 mg/kg (monkey). [c] C_{max} is in ng/mL. [d] AUC is in ng/mL h. [e] Bioavailability (BA) is dose-corrected.

following oral administration. The major route of excretion is through the urine in rats and through the bile in dogs and in monkeys. The major metabolite arises via oxidative cleavage of the CH_3CHCH_2 region of the chiral piperazine. The reduction of the *N*-oxide (**1**) back to the nicotinamide **18** was not observed.

Compound **1** shows 30−50-fold selectivity for CCR5 over the M_1 (K_i = 350 nM) and the M_2 (K_i = 250 nM) muscarinic receptors. It has no appreciable affinity for other related receptors of current interest.[17] There is neither inhibition nor induction of the liver enzymes with this compound. In the PBMC based assay, compound **1** inhibited the replication of HIV-1 isolates with IC_{50} values in the 2−20 nM range.[15] Importantly, compounds **1** and **18** also bind to primate CCR5, suggesting the possibility of evaluation in primate-based models of the disease.

In conclusion, truncation of the original piperidino-2(*S*)-methyl piperazine lead structure **2** gave compound **8** with reduced molecular weight and improved selectivity for the HIV co-receptor CCR5 over the muscarinic M2 receptor. Optimization of this pharmacophore for pharmacokinetic properties afforded the title compound (**1**), a prototypical piperazine-based CCR5 antagonist, which is a potent inhibitor of HIV-1 entry and replication and has excellent oral bioavailability in rat, dog, and monkey. Starting with potent muscarinic antagonists with weak affinity for CCR5,[7] we have designed structure **1**, which is a potent, orally absorbed CCR5 antagonist with modest affinity for the muscarinic M2 receptor. The further improvement of such structures toward greater selectivity for CCR5 will be reported in due course.

Acknowledgment. We thank Dr. Jean Lachowicz (CNS Pharmacology) for the muscarinic antagonist data and the physical and analytical chemistry department for spectral and analytical data of our compounds. Drs. Anandan Palani, John Clader, and William Greenlee are thanked for many helpful discussions. We are grateful to Drs. Ashit Ganguly, John Piwinski, and Gregory Reyes for their strong support of this project.

Supporting Information Available: Description of antiviral assays and experimental procedures and spectral data for the preparation and characterization of compounds **1**, **8**, and **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) The conversion of **7** to targets bearing diverse para-substituents will be published elsewhere.

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(15) Primary HIV-1 isolates included: US-1, 92US715, BAL, ASM57, JRFL, JrCSF, QZ4589, 302056, SF162, DJ258, 92RW026, 94ZW103, CM235, and BZ162. The cytotoxicity (CC_{50}) of **1** and **18** is >40 μM in PBMC cultures using MTS cell titer 96 protocol.

(16) Studies of the physicochemical phenomenon of rotamers will be reported in due course.

(17) Compounds **1** and **18** (1−5 μM) show 0−20% binding at CCR1−3, CCR7, NK1−3, CB1−2, H1, H3, NPY, D1−2, A2a, nociceptin, opioid, and thrombin receptors.

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