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Letters

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

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Abstract: Structure−activity studies on piperidino-piperidine **3** led to the discovery of SCH 351125 (**1**), a selective CCR5 antagonist with potent activity against RANTES binding (K_i = 2 nM), which possesses subnanomolar activity in blocking viral entry and has excellent antiviral potency versus a panel of primary HIV-1 viral isolates. Compound **1**, which has good oral bioavailability in rats, dogs, and monkeys, is proposed as a potential therapeutic agent for the treatment of HIV-1 and has entered human clinical trials.

Introduction. Human immunodeficiency virus (HIV) infection, with its clinical progression to AIDS, has become one of the leading causes of death in the world and the number one cause in Africa.[1] Although the use of combination antiretroviral therapy with HIV protease and reverse transcriptase inhibitors is highly successful in suppressing HIV infection and reducing morbidity and mortality, the emergence of drug resistance, long-term toxicity and adverse drug−drug interactions are major health concerns.[2] Recent reports describing the role of chemokines[3] and chemokine receptors in HIV infection revealed new targets for antiretroviral therapy, with implications for developing mechanistically novel classes of anti HIV-1 drugs.[4] CCR5, a cell surface receptor for the β-chemokines MIP-1α, MIP-1β, and RANTES, has been identified as a co-receptor with CD4 for attachment and fusion of macrophage-tropic (M-tropic) HIV-1 isolates.[5] Evidence for the importance of CCR5 in HIV transmission came from a study describing individuals who were homozygous for a 32-base pair deletion in the gene encoding CCR5. Cells from these individuals do not express functional CCR5 and are resistant to infection with M-tropic HIV strains.[6] Furthermore, heterozygous individuals, who express only one CCR5 allele, were found to have slower progression to AIDS compared with patients having no deletion.[7] The above observations provided compelling evidence that functional inhibition of the CCR5 receptor might be highly protective against HIV-1 infection. Therefore, it is hoped that the blockade of viral entry with small molecules targeting the CCR5 co-receptor could represent a new class of anti HIV-1 agent.

Scientists at Takeda Chemical Industries have disclosed their discovery of quaternary ammonium anilide as a small molecule CCR5 antagonist (TAK-779).[8a] Groups from Merck have also disclosed their work in this area.[8b] We initiated our drug discovery program with the similar goal of identifying a potent and orally active CCR5 antagonist. Because the CCR5 receptor belongs to the super family of 7-transmembrane G-protein coupled receptors, we were particularly interested in identifying compounds that were selective for the CCR5 receptor vs other G-protein coupled receptors. Screening of the Schering-Plough compound file led to the identification of compound **2** which was a CCR5 receptor antagonist (CCR5 $K_i = 1.0\ \mu$M). However, this compound also antagonized the muscarinic M_2 receptor (M_2 $K_i = 1.3$ nM). Subsequent SAR investigations using the knowledge gained from concurrent efforts in a related piperazino-piperidine amide series yielded the low molecular weight early lead compound **3** (CCR5 K_i = 66 nM; M2 $K_i = 1323$ nM).[9] The investigation in this series not only improved the CCR5 binding, but also the

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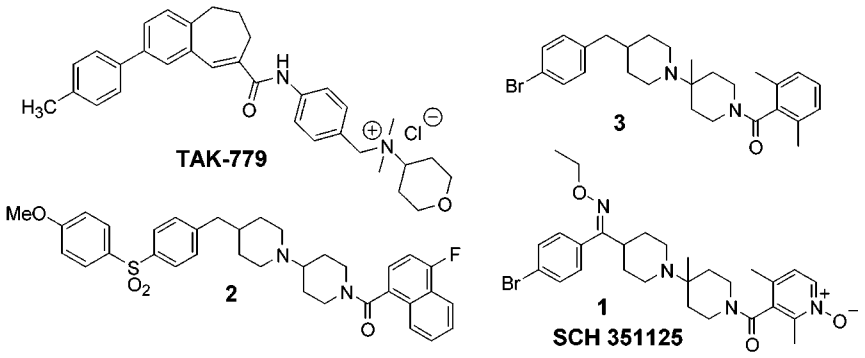
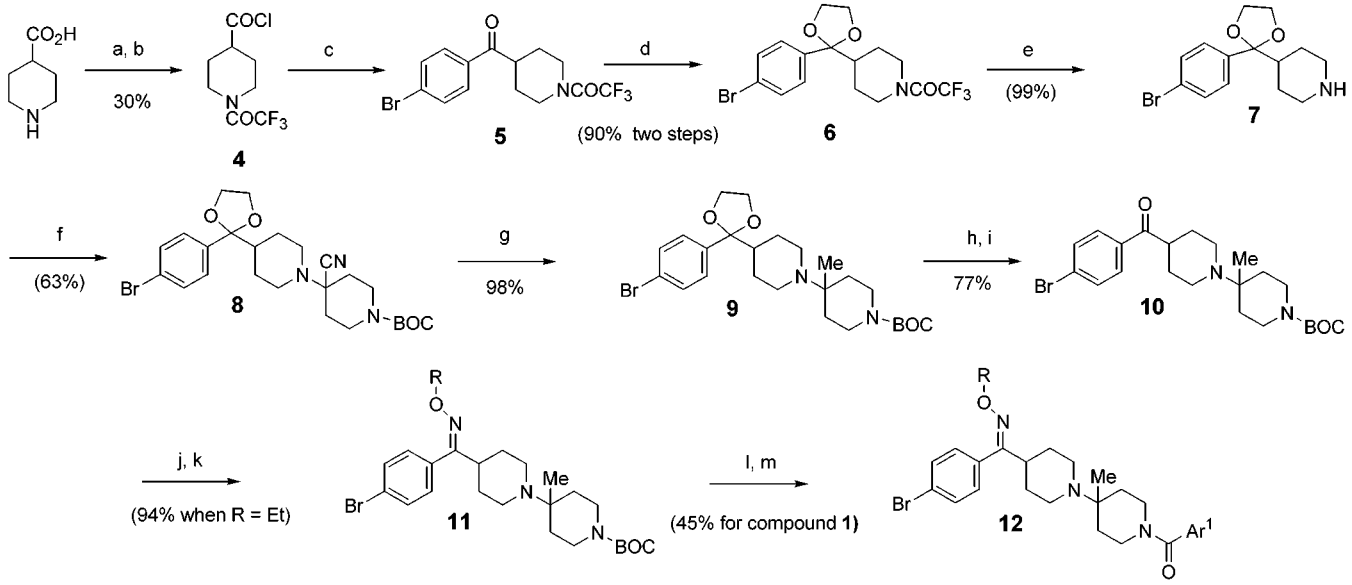


Figure 1. CCR5 antagonists.

Scheme 1. Synthesis of Oximino-piperidino-piperidine Amides[a]



[a] Reagents and conditions: (a) TFAA (excess), reflux, 4 h; (b) $SOCl_2$ (excess), 12 h; (c) $AlCl_3$, bromobenzene (excess), reflux; (d) ethylene glycol, *p*-TSA, toluene, reflux (azeotropically remove water); (e) MeOH, H_2O, K_2CO_3, rt, 12 h; (f) *N*-Boc-piperidine-4-one, $Ti(Oi\text{-}Pr)_4$, 1,2-dichloroethane, rt, 12 h then Et_2AlCN, rt, 3 h; (g) MeMgBr, THF, rt, 2 h; (h) 6N HCl, EtOAc, rt, 12 h; (i) BOC_2O, 10% NaOH, ether, rt, 12 h; (j) $NH_2OR{\cdot}HCl$, NaOAc, MeOH, rt, 24 h; (k) 1 M HCl in ether (3 equiv), DCM, rt, 48 h followed by chromatographic separation; (l) TFA, DCM, rt, 2 h; (m) Ar^1CO_2H, EDCI, DIPEA, HOBT, DCM, rt, 12 h.

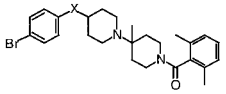
selectivity with respect to the M2 muscarinic receptor. In this Letter, we disclose the design, synthesis, and SAR development of oximino-piperidino-piperidine amides derived from our early lead compound **3**, which led to the discovery of our clinical candidate SCH 351125 (**1**), a potent and orally bioavailable CCR5 antagonist (Figure 1). Preliminary reports on the antiviral activity of this compound have been presented previously using the designation SCH-C.[10]

Chemistry. Oximino-piperidino-piperidine analogues were prepared as shown in Scheme 1. Protection of commercially available isonipecotic acid as the trifluoroacetamide, followed by treatment with thionyl chloride, afforded *N*-trifluoroacetylisonipecotyl chloride (**4**). Friedel−Crafts condensation of acid chloride **4** and bromobenzene afforded ketone **5**, which was then converted to compound **7**. The amine **7** was allowed to react with *N*-Boc-piperidine-4-one in the presence of titanium isopropoxide. Subsequent treatment of the resulting intermediate with diethylaluminum cyanide gave aminonitrile **8**. This aminonitrile was treated with an excess of methylmagnesium bromide to afford the alkylated product **9**, which was then processed to ketone **10** in two steps. The ketone was then converted to the desired oximino-piperidino-piperidine analogues **11** by treatment with either methyl or ethyl hydroxylamine hydrochloride in the presence of sodium acetate. BOC removal and coupling of the resulting free amine with the desired aromatic acids proceeded under standard conditions. The oxime formation step afforded a mixture of *E* and *Z* oxime isomers in a ratio of 1.5:1, which was readily separated by silica gel chromatography. For subsequent large-scale preparation, the *E*/*Z* mixture was first equilibrated with 1 M hydrochloric acid in ether to provide a 1:2.5 mixture favoring the *Z* isomer, which was then separated as above following reprotection of the secondary amine with Boc anhydride.

The in vitro activity of these CCR5 receptor antagonists was determined using a membrane binding assay and HIV-1 viral entry and replication assays.[11] Rat blood plasma levels after oral administration were evaluated using a rapid rat pharmacokinetic screen.[12]

Results and Discussion. With our initial early lead **3** in hand, we began our discovery process with the goal of improving its potency, selectivity versus M2 receptor, and oral bioavailability. Our initial studies had established that the para aromatic substituent was essential for CCR5 potency, with bromine being optimal. Ad-

Table 1. SAR and Pharmacokinetic Properties of Compounds with Benzylic Modifications of **3**



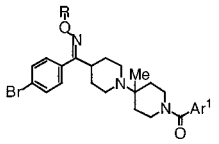
Compound	X	Ki (nM)[a,b]	IC_{50} (nM)[c]	Rat PK (10 mg/kg, po)[d] $AUC_{0\text{-}6h}$ (hr. μg/ml)
3	CH_2	66	10	0.04
13	NH	7.0	5.5	0.15
14	O	29	ND[e]	ND
15	CO	54	13	ND
16	C=N-OMe (E/Z)	11	1.5	2.1
17	C=N-OMe (E)	25	2.8	1.2
18a	C=N-OMe (Z)	2.0	1.2	1.4
19	C=N-OEt (E)	48	9.2	ND
19a	C=N-OEt (Z)	1.8	1.3	1.0

[a] The standard error was 10% and variability was 2−3-fold from assay to assay. See Supporting Information for procedure. [b] Data for inhibition of RANTES binding. [c] Concentration required to inhibit by 50% the entry of HIV-1 reporter virus (ADA) into U-87 cells. For IC_{50} values, 95% confidence limit was within 1 log and intraassay variation less than 0.5 log. [d] See ref 11 for procedure. [e] ND, not determined.

ditionally, both the 2,6-disubstituted benzamide and the methyl group at the ring junction were required for potent CCR5 inhibition in vitro. Although compound **3** showed promising CCR5 activity, oral bioavailability was poor, possibly due to metabolism at the benzylic site. Thus, subsequent SAR efforts were first focused at the benzylic site by introducing substituents that would either block or reduce metabolism (Table 1). Replacement of the methylene linker with an -NH- linker as in **13** provided a 10-fold improvement in binding. Replacements such as oxygen (**14**) or carbonyl (**15**) did not improve the CCR5 potency. The introduction of the oxime linker in **16** not only improved the CCR5 binding affinity 6-fold but also substantially increased oral absorption and drug blood levels in the rat. Separation of the oxime geometric isomers led to the discovery that the *Z*-isomer **18a** was 10-fold more potent than the *E*-isomer **17**. To determine the stability of compound **18a**, the compound was treated with 0.1 N HCl (pH = 1) at 37 °C for 12 h. No evidence of hydrolysis or oxime equilibration was observed. Further SAR optimization of the oxime substitution established that the methyl and ethyl oximes were more potent than other alkyl substitutions.

Having optimized the oxime geometry and alkyl substitution, we turned our attention to address the key metabolic issues associated with compound **18a** in this series. We observed extensive oxidative metabolism (monohydroxylation) in the rat at the 2,6-dimethyl benzamide moiety, along with minor amounts of dihydroxylation and a carboxylic acid metabolite resulting from oxidation of one of the methyl groups. To address these issues, our subsequent efforts were focused on the 2,6-dimethyl moiety to find a suitable replacement in order to block their metabolism and lead to compounds

Table 2. Inhibitory and Pharmacokinetic Properties of Oximino-piperidino-piperidine Amides



Compound	R	Ar^1	Ki (nM)[a,b]	IC_{50} (nM)[c]	Rat PK (10 mg/kg,po)[d] $AUC_{0\text{-}6h}$ (hr. μg/ml)
18b	Me		3.4	0.6	0.61
18c	Me		4.5	0.5	1.3
18d	Me		18	4.0	3.0
18e	Me		1.1	0.5	1.9
18f	Me		18	5.6	2.4
19b	Et		1.1	0.2	2.1
1	Et		2.1	0.6	6.5

[a] The standard error was 10% and variability was 2−3-fold from assay to assay. See Supporting Information for procedure. [b] Data for inhibition of RANTES binding. [c] Concentration required to inhibit by 50% the entry of HIV-1 reporter virus (ADA) into U-87 cells. For IC_{50} values, 95% confidence limit was within 1 log and intraassay variation less than 0.5 log. [d] See ref 11 for procedure.

with improved pharmacokinetic profiles (Table 2). Since the 2,6-disubstitution is necessary for high binding potency, our initial approach was to replace the methyl groups with heteroatoms. This resulted in compounds **18b**, **18c**, and **18d**, which all showed either reduced CCR5 binding potency or decreased oral drug exposure in the rat. However, changing the benzamide to a nicotinamide, while leaving the 2-methyl groups intact, provided potent CCR5 antagonists with significantly improved rat plasma levels after oral administration (**18e** and **19b**). Metabolite identification studies on these nicotinamides now indicated less metabolism at the methyl and aryl groups and the formation of a major (M+16) metabolite. Suspecting that this may have been due to oxidation of the pyridine nitrogen, the corresponding nicotinamide *N*-oxides were prepared.

In the methoxime series, this modification resulted in 10-fold loss of potency with no improvement in rat PK (**18f**). However, in the ethoxime series, we discovered compound **1** with significantly improved rat plasma levels after oral administration and nearly equivalent potency to **19b**. Interestingly, compound **1** exists as a equal mixture of four rotamers due to the presence of restricted bond rotations caused by the unsymmetrical 2,6-dimethyl nicotinic *N*-oxide tertiary amide.[13] Having achieved the desired potency and oral plasma levels in rat PK screen, advanced PK studies were then performed on compound **1**.

Table 3 summarizes details of the in vivo pharmacokinetic studies of **1** in rats, dogs, and cynomolgus

Table 3. Pharmacokinetic Profile of **1**

species	dose (iv/po) (mg/kg)	A (%)	iv administration $AUC_{(0-24h)}$ (μg h/mL)	iv administration $t_{1/2}$ (h)	oral administration C_{max} (μg/mL)	oral administration $AUC_{(0-24h)}$ (μg h/mL)	oral administration BA (%)
rat	10/10	57	13	5	1.4	8.2	63
monkey	2/2	80	7.4	6	0.42	3.9	52
dog	1/3	98	2.8	10	0.58	7.7	92[a]

[a] Dose normalized, A = absorption, BA = bioavailability.

monkeys. All the studies were conducted using the amorphous tartrate salt of **1**. The vehicle used for oral dosing was 0.4% methylcellulose and for intravenous dosing was 20% hydroxypropyl-β-cyclodextrin. As indicated, compound **1** showed excellent oral bioavailability and absorption in rats, dogs, and monkeys. The major route of excretion was determined to be through excretion in the urine of rats and dogs and through the bile of monkeys. The main metabolite of **1**, which was seen in both urine and bile, proved to be the deethylated oxime. There was no evidence for loss of the oxime stereochemistry in vivo.

The anti-HIV-1 activity of **1** was evaluated in both a single cycle infection assay and in replication assays using peripheral blood mononuclear cells (PBMC) infected by primary M-tropic HIV-1 isolates. Compound **1** showed excellent antiviral activity with an $IC_{50} = 0.6$ nM against the HIV-1 reporter virus (ADA) in the entry assay and with a mean $IC_{50} = 2$ nM against a wide range of HIV-1 isolates in the replication assays. In a counter screen assay, compound **1** at a concentration of 2−20 μM showed less than 15% inhibition of other closely related chemokine receptors including CCR1, CCR2, CCR3, and CCR7, as well as muscarinic receptors. The cytotoxicity (CC_{50}) of compound **1** is 92 μM in PBMC cultures using the MTS assay (Promega Corporation, Madison, WI).

In summary, we have identified a series of oximino-piperidino-piperidine amides that potently inhibit HIV-1 entry utilizing the CCR5 co-receptor. Subsequent optimization for pharmacokinetics resulted in the discovery of **1**, a potent inhibitor of HIV-1 viral entry and replication with excellent oral bioavailability in rat, dogs, and monkeys. Compound **1** has completed 1 month preclinical safety and toxicology studies in rats and monkeys and is currently in phase I clinical trial.

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Supporting Information Available: Experimental details and analytical data for the preparation of compounds **17**, **18a**−**f**, and **19a**,**b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Compound **1** is separable into four distinct peaks by analytical chiral HPLC or two peaks by achiral HPLC. Details of the physical−chemical phenomenon of the rotamers will be published in due course.

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