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Piperazine-Based CCR5 Antagonists as HIV-1 Inhibitors. III: Synthesis, Antiviral and Pharmacokinetic Profiles of Symmetrical Heteroaryl Carboxamides†

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Abstract—The unsymmetrical nicotinamide-*N*-oxide moiety in compound **1** was replaced with symmetrical isonicotinamides as well as 4,6-dimethyl pyrimidine-5-carboxamides. Compound **16** from the latter set reduced the number of rotamers, improved potency of inhibiting UIV entry, slightly diminished the affinity for the muscarine receptors and showed very good oral absorption.

The discovery of the chemokine receptor CCR5 as an essential co-receptor for the type 1Human Immunodeficiency Virus (HIV-1) to dock and gain entry into $CD4^+$ macrophages and T-cells has stimulated wide-spread programs to discover small molecule antagonists to block the HIV-1-CCR5 interaction.[2] We have recently disclosed our initial efforts in this regard leading to the discovery of a prototypical piperazine-based CCR5 antagonist **1**.[3] Sch-350634 (**1**) had excellent oral bioavailability in rodents and in primates, as well as good antiviral activity (CCR5 $K_i = 7$ nM; $IC_{50} = 2$–20 nM vs HIV isolates in cell-based assays). However, the moderate affinity of **1** for the muscarinic receptors (M1 = 350 nM; M2 = 250 nM) was a concern.

The nicotinamide derivative **2** had better receptor selectivity (K_i: CCR5 = 2 nM; M2 = 2500 nM) and potency ($IC_{50} = 0.2$–2.0 nM vs HIV isolates) than **1**. Since the nicotinamide **2** was rapidly oxidized to its *N*-oxide **1** in vivo, **1** was selected for ancillary studies (Fig. 1).

The prevalence of M1 receptors in the intestine and M2 receptors in the heart and the forebrain coupled with the very high oral blood levels exhibited by **1** led to the observation of cardiovascular effects (at 10 mg/kg, po) and CNS and GI effects (at 30 mg/kg, po) in rats.

Another interesting feature of **1** was the observation of four rotational isomers (rotamers) for this compound under chiral conditions.[4] The hindered rotations about the N–CO and the CO–pyridyl bonds in **1** result in four diastereomeric rotamers for this structure, which due to their relatively slow equilibration are observable on HPLC (Fig. 2) using a chiral mobile phase.[5]

The lower energy N–CO bond rotation due to the tertiary amide is quite common and leads to a rapidly equilibrating pair of rotamers. The hindered rotation

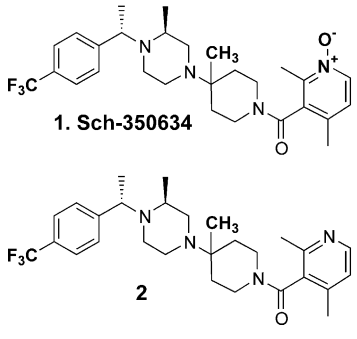

Figure 1. Unsymmetrical heteroaryl carboxamides.

†For a preliminary account of this work, see ref 1.

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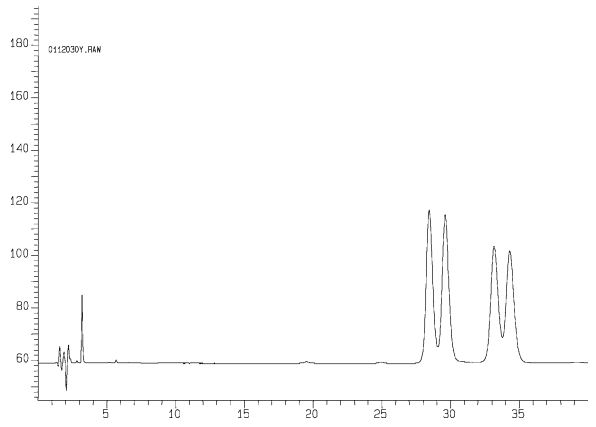


Figure 2. HPLC chromatogram of compound **1**.

about the CO–pyridyl bond requires more energy and leads to a slowly equilibrating pair of rotamers due to the unsymmetrical nature of the pyridine moiety (Fig. 3). Experimentally, the higher energy rotamers equilibrated to a steady state mixture of ~2:1 in 5 h at 37 °C, 18 h at 25 °C, and 4 days at 10 °C.

Tertiary amides bearing an aryl/heteroaryl ring with a C_2 axis of symmetry would lead to a degenerate pair of rotamers for the CO–Aryl bond rotation. In practice, this should result in the observation of a single pair of rapidly equilibrating rotamers for such compounds.

We decided to explore the effect of replacing the unsymmetrical nicotinamide-*N*-oxide in **1** with symmetrical hetroaryl amides in order to minimize the number of rotamers and possibly affect the receptor selectivity (CCR5 vs M2) in the process. As polar amides are needed to obtain high blood levels orally, we focused our attention on two classes of symmetrical amides: isonicotinamides and pyrimidine carboxamides.

Deletion of one or both the methyl groups from the nicotinamide-*N*-oxide moiety in **1** resulted in loss of binding at CCR5 ($K_i > 30$ nM). Based on our published SAR, we decided to keep the two substituents flanking the carbonyl group as methyl groups or chlorine atoms.[6] The requisite isonicotinic acids **4** and **6** and their corresponding *N*-oxides **5** and **7** were prepared as described in Scheme 1.[7] Coupling of the acids **4**–**7** with the piperazino-piperidine **12** (R = CF_3; see Scheme 2) under standard conditions (EDCI/HOBt/CH_2Cl_2/Et_3N; yield = 80–90%) gave the isonicotinamides and their *N*-oxides listed in Table 1.

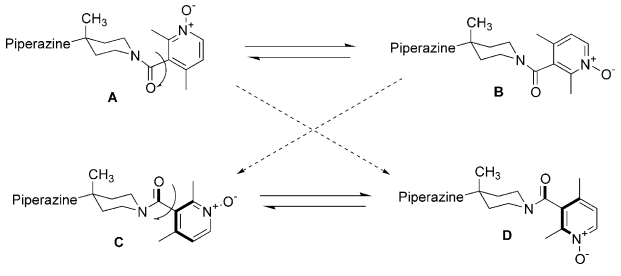
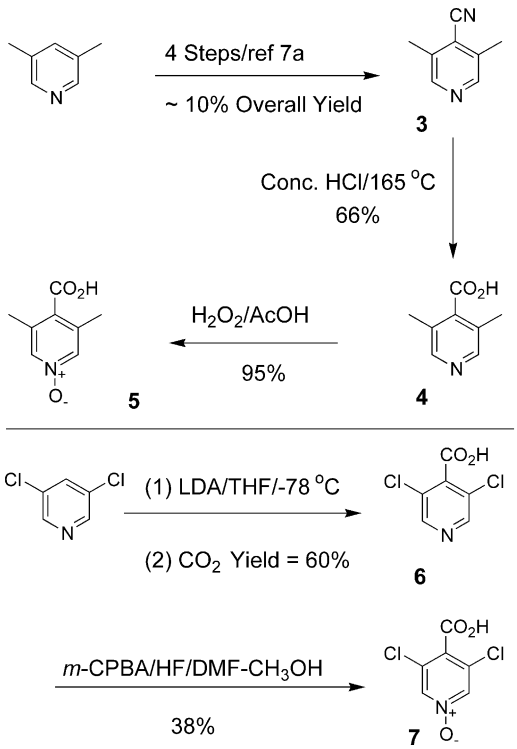


Figure 3. Rotamers of compound **1**.



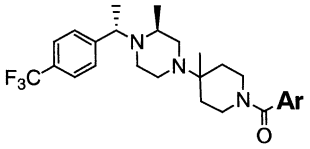
Scheme 1. Synthesis of isonicotinic acids and their *N*-oxides.

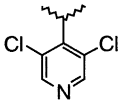
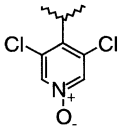
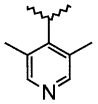
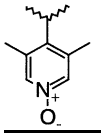
As Table 1 indicates,[9] desirable levels of potency, receptor selectivity (CCR5/M2) and oral blood levels[10] in the rat were not observed together for any single compound in this set and so the isonicotinamides were not pursued further.

It is well known that the direct condensation of the diketo-ester **8** with nucleophiles such as the amidines results in deacylation whereas using the *O*-alkylated derivative **9** furnishes the desired heterocycle.[11] Thus, the reaction of **8** with methyl triflate in the presence of Cs_2CO_3 in acetonitrile gave exclusively the *O*-methylated product **9**. Proton NMR analysis revealed no evidence of C-alkylation but ~20% unreacted diketo-ester **8** remained even when excess methyl triflate was used. Condensation of **9** with amidines under standard conditions formed the pyrimidine esters **10**. We have found that the hydrolysis of the *tert*-butyl ester **10b** avoided the loss of material due to water solubility that occurred with the saponification of the ethyl ester **10a**, and generally gave higher yields of the pyrimidine carboxylic acids **11**. The piperidino-piperazine cores **12**, prepared as described previously,[3] were then coupled to the newly made pyrimidine carboxylic acids under standard conditions (Scheme 2).

Several core amines **12** were capped with the parent 4,6-dimethyl pyrimidine-5-carboxamide (e.g., **13**–**16**). While many *para*-substituents (*R*) were tolerated, the *p*-CF_3 derivative (**16**) had optimal binding at CCR5 ($K_i = 3$ nM), slightly reduced affinity for the muscarinic receptors (K_i: M1 = 575; M2 = 456 nM) compared to **1** and very good oral absorption as well (Table 2). Here

Table 1. Data for isonicotinamides and their *N*-oxides[8]



Ar	Ki (nM)[a]		HIV Entry[b]	Rat PK[c]
	CCR5	M2	IC_{50} (nM)	AUC_{0-6h} (ng/ml×h)
	0.7	2031	0.3	450
	2.7	348	0.45	1810
	5.23	1678	ND	ND
	18	255	0.90	6760

[a]K_i (nM).[6]
[b]IC_{50} (nM) for inhibiting HIV (ADA) entry into cells.[9]
[c]AUC_{0-6h} (ng h/mL; oral dose = 10 mg/kg).[10]

Table 2. Data for parent pyrimidine carboxamides[8]

Compd	R	K_i (nM)[a]		Entry[b] (nM)	Rat PK[c]
		CCR5	M2		AUC_{0-6h}
13	I	11	169	ND	ND
14	CN	20	164	1.8	6740
15	CF_3O	11	68	1.2	8100
16	**CF_3**	**3**	**456**	**0.5**	**6210**

[a]K_i (nM).[6]
[b]IC_{50} (nM) for inhibiting HIV (ADA) entry into cells.[9]
[c]AUC (ng h/mL; oral dose = 10 mg/kg).[10]

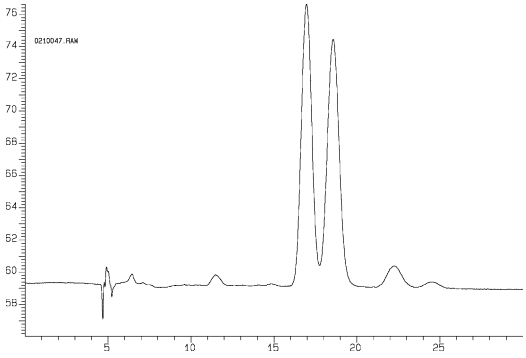
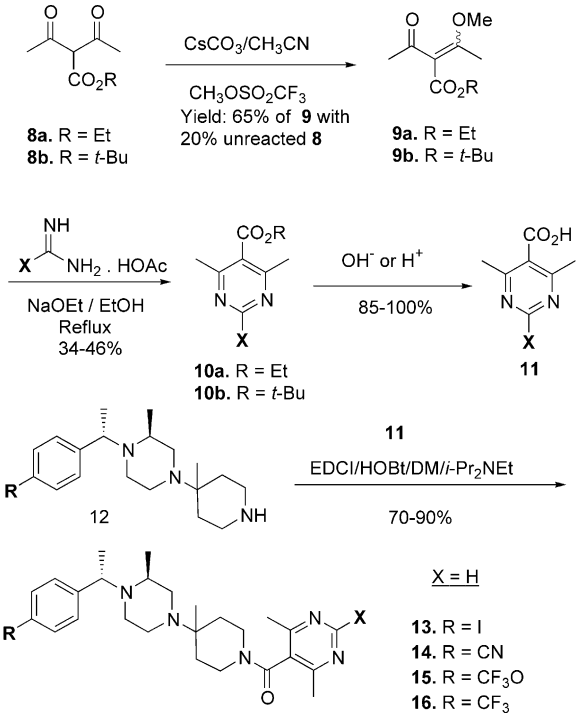


Figure 4. HPLC chromatogram of compound **16**.

again, deletion of the methyl group(s) on the pyrimidine ring diminished activity as did *N*-oxidation (*N*-oxide of **16** is not a significant metabolite, unlike **1** is of **2**).

As expected, compound **16** exhibits only the two rotamers characteristic of a tertiary amide due to hindered rotation about the N–CO bond. The atropisomers arising from hindered rotation about the CO–pyrimidine ring are now degenerate and are not observable on chiral HPLC (Fig. 4). The amide rotamers seen on HPLC[12] can be isolated and equilibrate rapidly under physiological conditions (< 2 h at 37 °C).

In keeping with the design of C_2 symmetric amides, we then turned our attention to the synthesis of 2-substituted-4,6-dimethyl pyrimidine-5-carboxamides. The requisite acids **11** (X ≠ H) were prepared by employing the appropriate amidines as shown in Scheme 2 and coupled to several piperazino-piperidines in the usual manner. Table 3 summarizes data for selected pyrimidine carboxamides of general structure **17**.

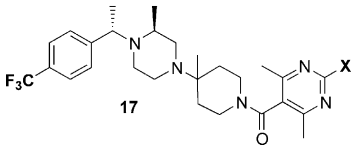


Scheme 2. Synthesis of pyrimidine carboxamides.

Table 3. Data for 2-substituted pyrimidine amides (**17**)[8]

Compd	X	K_i (nM)[a]		Entry[b] (nM)	Rat PK[c]
		CCR5	M2		AUC_{0-6h}
16	**H**	**3**	**456**	**0.5**	**6210**
18	CH_3	10	985	1.45	5520
19	CF_3	10	613	**0.1**	1970
20	Ph	12	1225	1.8	1290
21	CH_3O	11	202	ND	1970
22	NH_2	38	1100	ND	ND

[a]K_i (nM).[6]
[b]IC_{50} (nM) for inhibiting HIV (ADA) entry into cells.[9]
[c]AUC (ng h/mL; oral dose = 10 mg/kg).[10]



17

The 2-substituent (X) appeared to affect binding at both CCR5 and M2 receptors (e.g., **18**, **20**, and **22**) initially. However, this effect turned out to be very inconsistent.

As can be seen from Table 3, none of the 2-substituted-4,6-dimethyl pyrimidine-5-carboxamides improved the overall profile of the parent compound **16**.[13] Hence, **16** was selected for complete antiviral, metabolic and pharmacokinetic characterization (Tables 4 and 5).[3,8]

In summary, the symmetrical 4,5-dimethyl-5-pyrimidine carboxamide **16** eliminated the pair of rotamers observed due to very slow rotation about the CO-heteroaryl ring in compounds such as **1**. The only two rotamers seen for **16** are characteristic of all tertiary amides and equilibrate to a steady state (1:1) fairly rapidly. Compound **16** satisfied our criteria for strong binding to CCR5 and inhibition of HIV entry as well as very good oral absorption in rodents and primates. It also inhibited a small panel of HIV isolates of U.S origin, although it was less effective against clades from other geographical areas. There was neither inhibition nor induction of liver enzymes with this compound. The compound was shown to be a true antagonist of the CCR5 receptor in RANTES induced calcium flux assay.[9] Compounds **16**, **2** and **1** inhibited the MIP-1β (0.1 nM) induced migration of recombinant mouse pro-B cell line Ba/F3 that expresses human CCR5 with IC_{50} values of 0.01, 0.03 and 0.1 nM, respectively.[9] Compound **16** is 92% protein bound (**1** is 84% protein bound) and crosses the blood brain barrier ($AUC_{brain}/AUC_{plasma} = 1.7$). The lower blood levels after oral administration in rats and monkeys shown by the pyrimidine carboxamide **16** compared to the nicotinamide *N*-oxide **1**,[3] coupled with the modest improvement in the CCR5/M2 ratio (152 vs 35) helped **16** to show statistically significant improvement over **1** in its ancillary pharmacological profile. The mean BP, heart rate and ECG in rats were clean after oral administration of **16** up to a dose of 30 mg/kg and there were no negative effects on CNS related behavior at the lowest tested dose of 3 mg/kg. However, some effects at higher doses were noted in the gastrointestinal emptying and transit experiments, implicating the effect of **16** on the muscarinic receptors. Overall, although the new pyrimidine carboxamide **16** was a significant improvement over the nicotinamide-N-oxide **1**, its ancillary pharmacology profile was not as favorable as the leading compound Sch-351125 (Sch-C) from the related but distinct piperidino-piperidine series.[14] The oximino-piperidinio-piperidine Sch-351125 cleared the hurdle of toxicology and was advanced to the clinic as a possible treatment for HIV infection. Our studies to further improve the CCR5/Muscarinic receptor selectivity as well as the broad-spectrum potency of structures like **16** while retaining their desirable pharmacokinetics will be reported in due course.

Table 4. Pharmacokinetic profile of compound **16**

Species	iv administration[a]		Oral administration[b]		
	AUC[d]	$T_{1/2}$ (h)	C_{max}[c]	AUC	F (%)[e]
Rat	22,700	22	1490	22,000	97
Monkey	4710	8	340	2370	50

[a]iv dose = 1 mg/kg.
[b]Oral dose = 10 mg/kg (rat) and 2 mg/kg (monkey).
[c]C_{max} = ng/mL.
[d]$AUC_{0\text{-}24\,h}$ (ng/mL h).
[e]Bioavailability (%F). See ref 3 for details.

Table 5. Inhibition of HIV isolates by **16** in PBMC[a]

Virus	Clade/Origin	IC_{50} (nM)	IC_{90} (nM)
US-1	B/USA	0.7	4
ASM 108 (wt)	B/USA	7	17
ASM 108 (hz)	B/USA	0.2	3
301657 (wt)	B/USA	3.2	24
301657 (hz)	B/USA	26.8	>1000
Bal (hz)	B/USA	6.4	60
YU-2 (wt)	B/USA	2.3	8
YU-2 (hz)	B/USA	1.3	100
JV 1083	G/Nigeria	166	>300
BCF01	O/Cameroon	200	>1000

[a]Peripheral blood mononuclear cells. See ref 9.

Acknowledgements

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5. HPLC conditions for comp. **1**: Column: YMC pro, C18, 3 μm, 150 × 4.6 mm ID. Mobile phase: 30% ACN: 70% 25 mM potassium phosphate buffer, pH 6.4 + 20 mM β-cyclodextrin. Flow rate: 1.0 mL/min. Detection: 220 nm. Injection vol.: 10 uL. Sample concn: ~0.3 mg/mL in water. Column temperature: 20 °C.

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8. For the CCR5-RANTES binding assay, the standard error was 10% and variability was 2- to 3-fold from assay to assay. For details of the muscarinic receptor binding assay, see: Lachowicz, J. E.; Lowe, D.; Duffy, R. A.; Ruperto, V.; Taylor, L. A.; Guzik, H.; Brown, J.; Berger, J. G.; Tice, M.; McQuade, R.; Kozlowski, J.; Clader, J.; Strader, C. D.; Murgolo, N. *Life Sci.* **1999**, *64*, 535. The viral Entry assay refers to a single-cycle HIV replication assay adapted from: Connor, R. I.; Chen, B. K.; Choe, S.; Landau, N. R. *Virology* **1995**, *206*, 935 Typically, the 95% confidence limit for the IC_{50} values for inhibiting HIV entry was within 1 log and the R^2 variance values between 0.9 and 1.0. Intra-experimental IC_{50} values varied less than 0.5 log. The differences between the observed IC_{50} values in the Entry assay and the K_i values in the binding assay may reflect the difference between the ligand (RANTES versus HIV) and the target (cell membrane versus live U-87 cells).

9. For details of all the assays used in this study, see: Strizki, J. M.; Xu, S.; Wagner, N. E.; Wojcik, L.; Liu, J.; Endres, M.; Palani, A.; Shapiro, S.; Clader, J. W.; Greenlee, W. J.; Tagat, J. R.; McCombie, S.; Cox, K.; Fawzi, A. B.; Chou, C.-C.; Pugliese-Sivo, C.; Davies, L.; Moreno, M. E.; Ho, D. D.; Trkola, A.; Stoddart, C. A.; Moore, J. P.; Reyes, G. R.; Baroudy, B. M. *Proc. Nat. Acad. Sci. U.S.A.* **2001**, *98*, 12718.

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12. HPLC Conditions for compound **16**: Column: Chiralcel OD-R, 25 cm × 4.6 mm ID. Mobile phase: 40:10:50, ACN: IPA: 50 mM potassium phosphate buffer, pH 7.8. Flow rate: 0.6 mL/min. Detection: 220 nm. Injection vol.: 10 uL. Column temp: 10 °C.

13. All new compounds reported herein were fully characterized by NMR, MS and HRMS. Spectral data for the key compound **16**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 (s, 3H), 1.14 (d, J = 6 Hz, 3H), 1.16–1.27 (m, 1H), 1.28 (d, J = 6 Hz, 3H), 1.4 (m, 1H), 1.8 (br-t, J = 9 Hz, 1H), 2.0 (br-t, J = 9 Hz, 1H), 2.3–2.44 (m, 5H), 2.45 (s, 3H), 2.48 (s, 3H), 2.5–2.65 (m, 1H), 3.0 (m, 2H), 3.4 (dd, J = 14, 7 Hz, 2H), 4.0 (br-s, 1H), 4.22 (br-d, J = 9 Hz, 1H), 7.53 (dd, J = 7, 3 Hz, 4H), 8.94 (s, 1H). FABMS (MH^+): 504 (100%). HRMS calcd for $C_{27}H_{37}N_5OF_3$ (MH^+): 504.2937; Found 504.2950.

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