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Biological Evaluation and Interconversion Studies of Rotamers of SCH 351125, an Orally Bioavailable CCR5 Antagonist

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Abstract—The separation and biological evaluation of rotamers as well as interconversion studies on rotamers of our clinical candidate SCH 351125 are described.

The development of small molecule CCR5 antagonists that inhibit viral entry into host cells has emerged as an attractive new approach for the treatment of HIV-1 infection. Earlier work has established that the HIV-1 virus invades macrophages and primary T-cells by binding to the cell surface protein CD4, as well as to the chemokine receptor CCR5.[1,2] The CCR5 receptor is a member of the seven transmembrane G-protein coupled receptor family, and its natural ligands are the chemokines RANTES, MIP-1α and MIP-1β.[3] Importantly, it was reported from genetic studies that individuals who are homozygous for a 32-base pair deletion in the gene encoding CCR5 are highly resistant to HIV-1 infection. Furthermore, heterozygous individuals, who possess only one intact CCR5 allele advance more slowly to AIDS compared with patients having no deletion.[4,5] These findings suggest that a small molecule that inhibits HIV-1 viral entry into cells could be a potential anti-HIV-1 therapeutic agent with a mechanism of action different from the currently available treatments for HIV-1 infection.

The design and synthesis of small molecule CCR5 antagonists is an active area of research in our labs as well as in many others.[6] We have previously communicated our extensive structure activity studies at the oxime and amide regions of our original lead that gave rise to our clinical candidate SCH 351125 (**1**), as shown in Figure 1.[7] Compound **1** showed excellent oral bioavailability in rats, dogs and monkeys in addition to superior antiviral potency and receptor specificity. More importantly, in our recent clinical studies, compound **1** provided an important proof of concept in humans, by producing a dose-dependent reduction of HIV-1 RNA levels in infected patients.[8]

From a structural perspective, compound **1** possesses an interesting intrinsic molecular property. It exists as an equal mixture of four rotational isomers due to hindered bond rotation at both the amide bond (a) and the bond linking the amide carbonyl to the unsymmetrical nicotinamide-*N*-oxide (b) as shown in Figure 1.[9] As a result, we observe four peaks on a HPLC chromatography using chiral mobile phase, each corresponding to an individual rotational isomer as shown in Figure 2. We describe herein separation and interconversion studies, as well as the biological evaluation of the individual rotamers of **1**.

Two restricted bond rotations create two diastereoisomeric pairs, giving rise to the four rotamers as shown in Figure 3. Rotamers **2** and **3**, as well as **4** and **5**,

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are diastereomeric with respect to each other. Similarly, rotamers **2** and **5**, as well as **3** and **4**, are enantiomeric pairs. Slow rotation 'a', around the amide N–CO bond, is a well known phenomenon for hindered tertiary amides. Rotation 'b', which is a rotation around the CO-aryl bond, is normally rapid. However, steric hindrance caused by the bis-(*ortho*)-methyl substituents on the aromatic ring results in a slow rotation process. We studied compound **6**, a symmetrical amide analogue of compound, **1** to further establish the identity of the slow and fast rotation process. As expected we observed two peaks in chiral HPLC chromatography for compound **6** (Fig. 4). These two peaks correspond to rotamers arising from amide bond rotation 'a'. Since the pyridine ring in **6** is symmetrical, no new isomers result from rotation 'b'. The amide rotamers seen on HPLC can be isolated and equilibrate rapidly under physiological conditions (<2 h at 37 °C).

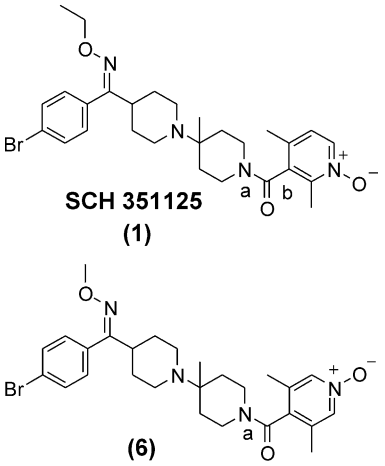

Figure 1. Structure of SCH 351125 (**1**) and symmetrical amide compound **6**.

Next, we investigated the rate of interconversion between rotamers and the final equilibrium ratio in various solvents at 37 °C for compound **1** (Table 1). The rates and equilibrium ratios were measured using HPLC chromatography. The interconversion rates are solvent and temperature dependent. As expected, the interconversions become more rapid as the temperature increases. Also, the interconversions are more rapid in organic solvents relative to aqueous solvents. The interconversion between rotamers **2** and **5**, rotamer **3** and **4** are rapid interconversions (rotation process 'a'). The half-life for this process is approximately 5 h in aqueous solvent. In contrast, the half-life for the slow interconversion process between rotamers **2** and **3**, rotamer **4** and **5** (process 'b') is approximately 25 h in aqueous solvent. The final equilibrium rotamer ratio is approximately 1:1:1:1 in all solvents examined. The equilibrium plasma half-life for interconversion between rotamers **2** and **3** to **4** and **5** appears to be the same under both in vitro and in vivo conditions.[10]

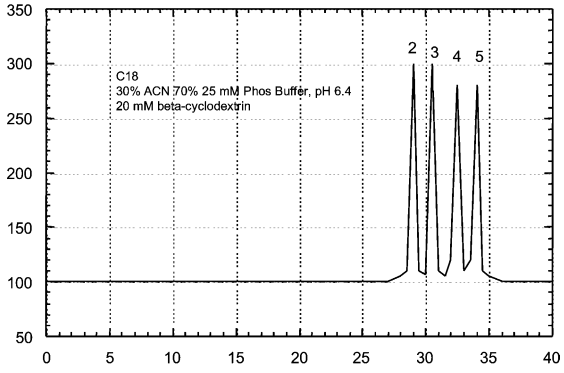

Figure 2. HPLC chromatogram of compound **1**. Chromatographic conditions: column: YMC Pro C18, 5 μm, 25 cm×4.6 mm ID; mobile phase: acetonitrile: 0.025 mM β-cyclodextrin in 0.05 M phosphate buffer, pH 6.4, 30:70, v/v.

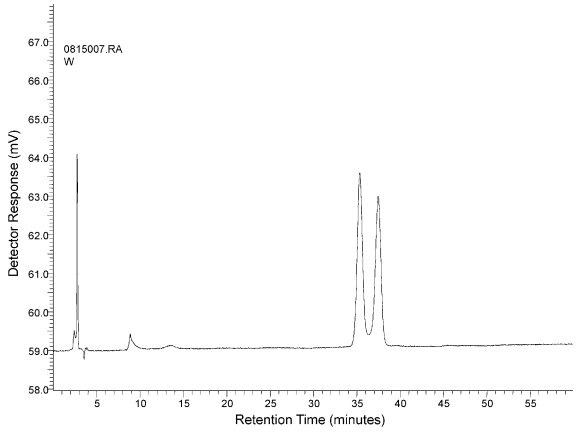

Figure 4. HPLC chromatogram of compound **6**. Chromatographic conditions: column: YMC Pro C18, 5 μm, 25 cm×4.6 mm ID; mobile phase: acetonitrile: 0.025 mM β-cyclodextrin in 0.05 M phosphate buffer, pH 6.4, 25:75, v/v.

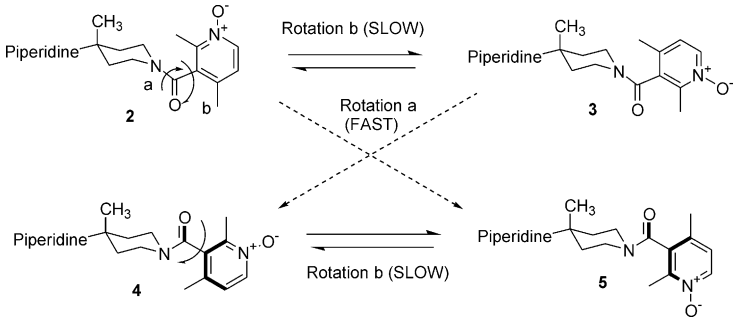

Figure 3. Rotamers of compound **1**.[12]

Table 1. Equilibrium half-life of rotamer interconversions in various solvents at 37 °C

Solvents	$t_{1/2}$ (h) for 'Fast' interconversion[a] (rotamer 2 and 3 to 4 and 5)	$t_{1/2}$ (h) for 'Slow' interconversion[b] (rotamer 3 to 4)
Water	5.5	21
Phos. Buffer (pH 7.0, 50 mM)	5.6	30
Saline (0.9% NaCl)	4.6	23
Glycerin	3.4	7
PEG 400	2.4	5

[a]'Fast' interconversion $t_{1/2}$ is the time required for total concentration of rotamer 2 and 3 to reach a value that is half-way between the initial (100%) and final values (50%), that is, 75% (assuming 100% rotamer 2 present initially).
[b]'Slow' interconversion $t_{1/2}$ is the time required for concentration of rotamer 2 to reach a value that is half-way between the initial (0% and final value (25%), i.e., 12.5%.

Table 2. Biological activity of SCH 351125 rotamers

Fraction	K_i (nM)[a,b]	IC_{50} (nM)[c]
Mixture	7.80	5.0
2	47.63	5.1
3	92.93	10.9
4	97.75	47.6
5	5.10	0.74

[a]Data for the inhibition of RANTES binding and 23 °C for 60 min.
[b]The standard error was 10% and variability was 2–3 fold from assay to assay.
[c]Concentration required to inhibit by 50% the entry of HIV-1 reported 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.

The individual rotamers of **1** were separated using chiral HPLC chromatography. The HPLC fractions containing each rotamer were combined and concentrated at low temperatures (0 °C), and the binding and entry assays were performed immediately under slightly modified conditions to minimize equilibrium between rotamers.[11] The binding and antiviral activity of each rotamer are shown in Table 2. Although all the rotamers show antiviral activity, rotamer **5** was found to be slightly more potent than the equilibrated mixture and the other three rotational isomers.

The presence of rotamers in compound **1** has no significant impact on its antiviral activity but creates some challenges for development. The accompanying paper describes our continued investigation of the design, synthesis and biological evaluation of C2 symmetrical tertiary amide analogues of **1**.

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10. The equilibrium half-life for interconversion between rotamers **2** and **3** (A) to **4** and **5** (B) in rat plasma is 1.5 h. The equilibrium end point is 60% A: 40% B.
11. Modified RANTES binding assay: Assay buffer: 50 mM HEPES pH = 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA; CCR5 membranes: CCR5–CHO cell membranes; Radiolabeled ligand: ^{125}I-RANTES; standard inhibitor: rhMIP-1β. Add components to 96-well format in the order listed above, saving resuspension or dilution of drug samples to the end in order to minimize rotamer equilibration. Incubate plates at room temperature for 1 h, then harvested through glass fiber filters, washed with 10 mM HEPES (pH = 7.4, 150 mM NaCl) at 4 °C, and counted to determine the amount of bound RANTES. The binding affinity constant K_i, was determined from the experimental IC_{50} valued using GraphPad PRISM software analysis. Modified viral entry assay: U-87 astroglioma cells expressing human CD4 and CCR5 were seeded into 96-well plates (7×10^3/well) and pretreated with the individual SCH 351125 rotamers or culture media (DMEM, with

10% FBS) at 4 °C for 1 h. The medium was aspirated and replaced with 20 μL of fresh medium containing compound and then infected with an equal volume of HIV-1 luciferase reporter virus stock for 3 h at 4 °C. The viral inoculum was then removed and the cells washed with PBS. Culture media containing compound was replaced and the cultures were incubated for 3 days at 37 °C. Luciferase activity was measured in cell lysates using the Luciferase Assay System (Promega, Madison, WI, USA). The IC_{50} values represent the concentration of compound required to inhibit luciferase production by 50% compared with control cultures. For a description of all original or typical bio-assays, see ref 7b.

12. The number given for rotamers **2**–**5** in Figure 3 is arbitrary and does not correspond to fractions isolated from HPLC. The structural assignments were not carried out for compounds **2**–**5**.