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Tricyclic HIV integrase inhibitors: Potent and orally bioavailable C5-aza analogs

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Abstract—A series of C5-aza tricyclic HIV integrase inhibitors was prepared. A highly potent and orally bioavailable compound (compound **9**) was identified and selected for development.

Human immunodeficiency virus (HIV), the causative pathogen of AIDS, replicates utilizing three essential enzymes encoded in the HIV pol gene, reverse transcriptase (RT), protease (PR), and integrase (IN). While many licensed anti-HIV agents target either RT or PR, the recent progress in extensive researches by the pharmaceutical industry has led to the emergence of promising IN inhibitors.[1] The most advanced lead, MK-0518 (Raltegravir, **1**) (Fig. 1), has been approved by the FDA as the first HIV integrase inhibitor to be marketed in the US.

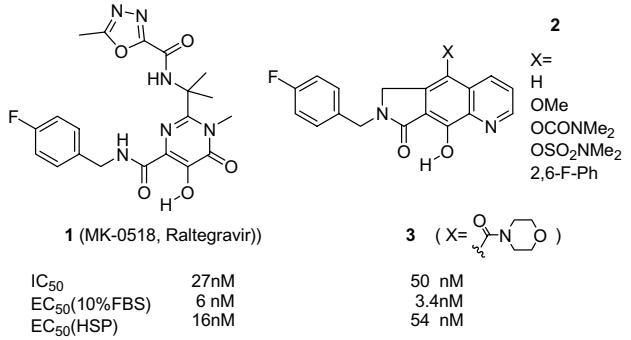

Figure 1. The structures of **1** (K-0518, Raltegravir), **2** and **3**.

Our previous publications described the design and SAR studies of a few series of novel and highly organized tricyclic HIV IN inhibitors shown as **2** (Fig. 1).[2] Those analogs possessed potent activity against both the HIV integrase-catalyzed strand transfer activity in the enzymatic assay as well as HIV replication in cell culture assays. A representative compound (**3**), for example, exhibited the good activity in the presence of either 10% FBS or human serum proteins (HSP) (Fig. 1).[2a]

An important objective for our HIV integrase inhibitor research program is to identify an orally bioavailable compound for clinical development. Its pharmacokinetic properties will allow for the simple dosing regimens, particularly in the combination therapy, a routine clinical practice in the AIDS medication. It was observed that compound **3** showed exposure upon oral dosing in both rat and dog, but its overall pharmacokinetic profiles were sub-optimal.[3] In order to improve the key pharmacokinetic parameters such as half-life ($t_{1/2}$) and the oral absorption of these potent IN inhibitors, we extended the SAR studies beyond those analogs reported previously. For the tricyclic scaffold, a variety of C5 substituents were relatively well tolerated in preserving acceptable potency. These substituents included ether, carbamate, sulfamate, aryl, and carboxamide functionalities. Therefore, we envisaged that further exploitation of effects by other C5 substituents on both potency and pharmacokinetics was possible because of the direct electronic and steric influences of these substituents on the tricyclic pharmacophore. In this communication, we report the

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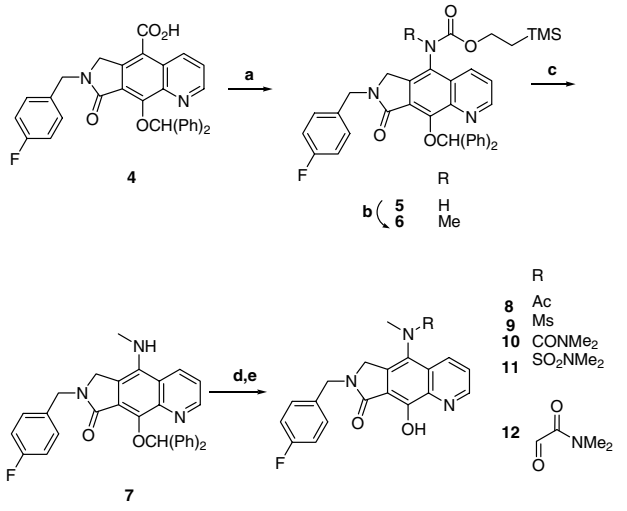
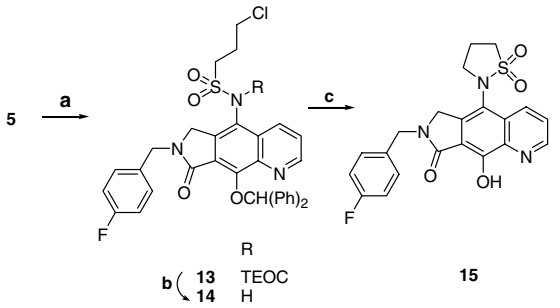
preparation of a series of C5-amino derivatives as potent inhibitors of both HIV integrase-catalyzed strand transfer activity and HIV infection in cell cultures. The pharmacokinetic evaluation of a subset of this SAR series is also described and has led to the identification of a compound for development.

The preparation of these analogs is illustrated in Schemes 1 and 2. Compound **4**, described in our previous publication,[2a] was converted to TEOC- protected C5 amine **5** via the Curtius rearrangement protocol coupled with trapping isocyanate with TMS ethanol. Upon methylation and removal of TEOC, analogs **8**–**12** were obtained by treating C5 methylamine **7** with appropriate reagents and deprotecting diphenylmethyl ether (DPM) of the phenol.



Scheme 1. Ac, acetyl; Ms, methansulfonyl. Reagents and conditions: (a) phosphorazidic acid diphenyl ester, TEA, rt, 5 h, then TMS ethanol, 60 °C, 26 h, 70%; (b) NaH, MeI, DMF, 0 °C–rt, 1 h, 80%; (c) TBAF, rt, overnight, 84%; (d) for **8**: NaH, DMF, acetyl chloride, 0 °C, 2 h, 86%; for **9**: pyridine, methansulfonyl chloride, rt, overnight, 85%; for **10**: $ClCONMe_2$, TEA, DMAP, dichloromethane, rt, 12 h, 50%; for **11**: 3-dimethylsulfamoyl-1-methyl-3H-imidazol-1-ium triflate, 120 °C, microwave, 90 min, 80%; for **11**: 1—TEA, *N*,*N*-dimethylsulfamoyl chloride, for **12**: 1—TEA, methyl chlorooxoacetate, DCM, rt, 30 min; 2—dimethylamine, rt, 10 min, 76%; (e) TFA, triethylsilane, rt, 50–93%.



Scheme 2. Reagents and conditions: (a) NaH, DMF, 3-chloropropansulfonyl chloride, 0 °C–rt, 0.5 h, 60%; (b) TBAF, THF, rt, 90%; (c) 1—NaH, DMF; 2—triethylsilane, TFA, rt, 15 min, 50%.

To examine the cyclization effect of the C5-aza group on the tricyclic pharmacophore, the preparation of compound **15** was undertaken and its synthesis is shown in Scheme 2. Intermediate **5** was reacted with 3-chloropropansulfonyl chloride, followed by removal of the TEOC protecting group. Upon treating with base to form the 5-membered sultam and removal of DPM, **15** was obtained in good overall yield.

All analogs prepared were tested for their activity in both HIV integrase strand transfer assay as well as anti-HIV infection in a cell culture assay. The data are summarized in Table 1.

In general all the compounds showed good to excellent potency in both the enzyme and the cell culture-based assays. C5 Sulfonamide **9**, sulfonylurea **11**, and sultam **15** exhibited improved activity in the cell assay over **3**. Further, the superior potency of **9** and **11** was maintained when human serum proteins, serum albumin and α-1 acidic glycoproteins, were added to reach physiologic concentrations in the cell culture based assay. Interestingly, the simple amide **8**, urea **10**, and oxalylamide **12** were found to be less potent although **10** was the most soluble among those tested. The potency of C5 sultam analog **15** was more affected by the serum proteins than either **9** or **11**. The results that we observed in this work are consistent with those reported by our laboratory previously. Both C5 carbamate and sulfamate groups on the same tricyclic pharmacophore were more potent than other C5 substituents on the tricyclic scaffold.[2a,b] However, C5 amine derived analogs are more attractive due to their greater stability toward hydrolysis as compared to the analogous carbamates or sulfamates.

The two most potent compounds, **9** and **11**, along with compound **15**, were chosen for pharmacokinetic evaluations in both rat and dog and the major pharmacokinetic parameters are shown in Table 2.

Both **9** and **11** were found to be orally bioavailable in these two species. Notably higher oral bioavailability

Table 1. Integration strand transfer inhibition and anti-HIV proliferation assay results for compounds **8**–**12**, **15**[a]

Compound	IC_{50}[b]	EC_{50} (10% FBS)[c]	EC_{50} (HSP)[d]	Solubility[e] (μM)
8	100	47	nd	nd
9	28	1.7	11.4	11
10	71	10	nd	844
11	62	3.1	8.4	4.5
12	265	31	nd	nd
15	13	1.4	49	2.8

[a] Values are means of at least two experiments, given in nM. nd, not determined.
[b] Ref. [4a].
[c] Ref. [4b].
[d] HSP, human serum proteins adjusted EC_{50}, obtained by assaying compounds in the presence of physiological concentrations of human serum albumin and AAG; see Ref. [4c] for details.
[e] Measured by dissolving the solid of testing compound in pH 7.3 aqueous media and determining the concentration by HPLC.

Table 2. Pharmacokinetics of **9**, **11**, and **15** in rat and dog[a]

Species	Rat			Dog		
	F (%)	$T_{1/2}$ (h)	CL (L/h/kg)	*F* (%)	$T_{1/2}$ (h)	CL (L/h/kg)
9	15	1.1	0.28	45	4.9	0.4
11	13	0.9	0.23	16	4.5	0.34
15	4	0.6	1.53	8.3	0.8	0.3

F (%), fraction absorbed upon oral dosing testing compounds as compared to iv dosing, calculated based on AUC from iv and po groups, expressed as %; CL, total body clearance obtained from iv dosing groups.

[a] All compounds were dosed as free parent in a solution form (EtOH, PG, PEG400; and citric acid; pH 3.3 for iv and pH 2.2 for po); and $t_{1/2}$ was generated from the iv dosing group. The values were means of data obtained from samples of three animals in each study.

(45%) and longer half-life (4.9 h) than **11** and **15** in dog were observed for compound **9**. It is interesting to note that although compound **15** exhibited similar clearance in dog as both **9** and **11**, its half-life was much shorter.

We were intrigued by the fact that compound **9** is more potent than MK-0518 in the presence of serum proteins. The X-ray crystal structure of **9** was determined and is shown in Figure 2.[5] It is interesting to note that N of the C5 methanesulfonamido group is an sp^2 hybridized center. *N*-methyl and methansulfonyl moieties are oriented perpendicular to the tri-cyclic core plane. The hydrophobic *p*-F-phenyl moiety is also in a similar orientation versus the plane of the tri-cyclic core. The observed fixed conformation is the same as the one shown in our initial molecular modeling studies[2a] and could be a preferred conformation at the active site during the strand transfer of HIV integration process.

In conclusion, by building upon our previously published SAR studies and by exploring a variety of C5-amine derivatives, we have successfully identified even more highly active HIV integrase inhibitors with good oral bioavailability in pre-clinical PK studies. These results further demonstrated that a design strategy that rigidifies the pharmacophore of the IN inhibitor is critical to the observed activity against both IN strand transfer and HIV replication in cell culture assays. Because of its favorable potency and PK properties, compound **9** was selected for further work in pre-clinical and clinical development.[6]

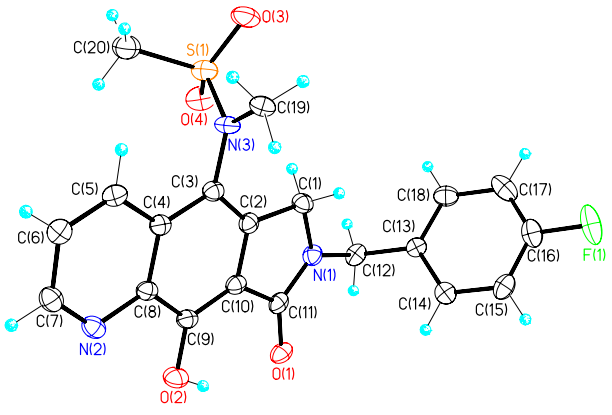


Figure 2. The ORTEP representation of X-ray 3D crystal structure of **9**. Nitrogen atoms are in purple, oxygen atoms in red, the sulfur in yellow, and the fluorine in green.

Acknowledgments

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3. Eisenberg, G. unpublished communication.
4. (a) Strand transfer assay modified from a previous report (Hazuda et al., *Nucleic Acid Res.* **1994**, *22*, 1121). Biotinylated donor DNA was bound to Reacti-Bind High Binding Capacity Streptavidin coated white plates. DIG-tagged target DNA with anti-DIG antibody-conjugated horseradish peroxidase detection was used.; (b) For antiviral assay, 50 μl of 2× test concentration of fivefold serially diluted drug in culture medium was added to each well of a 96-well plate (9 concentrations) in triplicate. MT-2 cells were infected with HIV-1 IIIB at an m.o.i. of 0.01 for 3 h. Fifty microliters of infected cell suspension in culture medium ($\sim 1.5 \times 10^4$ cells) was then added to each well containing the drug dilutions. The plates are incubated at 37 °C for 5 days. One hundred microliters of CellTiter-Glo™ Reagent (catalog # G7571, Promega Biosciences, Inc., Madison, WI) was then added to each well. Cell lysis

was allowed to complete by incubating at room temperature for 10 min. Chemiluminescence was then read. For the cytotoxicity assay, the protocol is identical to that of the antiviral assay, except that uninfected cells and a threefold serial dilution of drugs were used.; (c) The effect of compounds binding to serum protein components was evaluated by determining the antiviral EC_{50} in MT-2 cells in 10% FBS in the presence or absence of serum concentrations of HSA (35 mg/ml) or α_1-AGP (1.5 mg/ml). From the EC_{50} data in the presence of each individual protein, the EC_{50} resulting from the combined effect of both proteins (as in serum) can be calculated. The derivation of the appropriate equation for this calculation can be made through competitive binding assumptions.

5. Crystallographic data for **9** were collected at the University of California at San Diego and have been deposited in the Cambridge Crystallographic Data Centre as supplementary publication number 662530.
6. Other physicochemical characterizations of compound **9**: ^{1}HNMR (300 MHz) (DMSO-d_6) δ: 10.91 (br s, 1H), 8.95 (s, 1H), 8.43 (s, 1H), 7.78 (s, 1H), 7.39–7.33 (m, 2H), 7.21–7.13 (m, 2H), 4.71 (s, 2H), 4.53 (s, 2H), 3.25 (s, 3H), 3.18 (s, 3H); ^{19}F NMR δ: −115.87; MS: 416.1 (M+H).