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Tricyclic HIV integrase inhibitors V. SAR studies on the benzyl moiety

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ABSTRACT

SAR studies on the *para*-fluorobenzyl moiety of tricyclic HIV integrase inhibitors are discussed and lead compounds with potency and PK properties comparable to raltegravir were identified.

The integrase (IN) of Human immunodeficiency virus (HIV), the causative pathogen of AIDS, is an essential enzyme encoded in the HIV pol gene, together with reverse transcriptase (RT) and protease (PR). The recent progress in researches directed towards identifying clinically useful IN inhibitors has lead to the market approval of raltegravir (MK-0518, **1**),[1] a powerful addition to HIV therapeutics aimed at overcoming drug resistance problems and improving the life of AIDS patients (Fig. 1).

In our previous publications we described the design and SAR studies of a class of novel and highly organized tricyclic HIV IN inhibitors.[2] The lead compound from the series, compound **2**, exhibited not only excellent potency against both IN strand transfer activity in the enzymatic assay and HIV replication in the cell culture, but also good pharmacokinetics in both rat and dog.[2d]

In the systematic efforts to optimize both potency and pharmacokinetic profile of the tricyclic integrase inhibitors, we started examining the roles of hydrophobic *p*-fluorobenzyl moiety highlighted in **2**, in addition to other portions of the scaffold that have been evaluated previously.[2] A modeling system developed in-house[3] indicated that besides the *p*-fluorine, many other hydrophobic/hydrophilic substituents could be accommodated at site 1 (Fig 2). We noticed that SAR studies on the hydrophobic *p*-fluorobenzyl moiety on a raltegravir-based pyrimidinone scaffold were recently published by Merck scientists.[4] In this manuscript, we report the results of our investigations on the roles of substitutions on the phenyl portion of *p*-fluorobenzyl moiety and a preferred substitution pattern emerged from these studies.

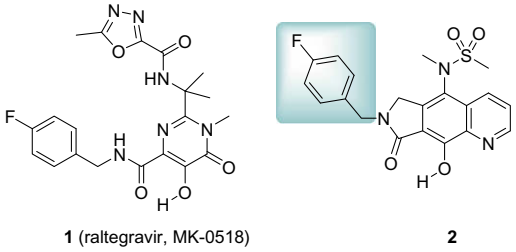

Figure 1. The structures of **1** and **2**.

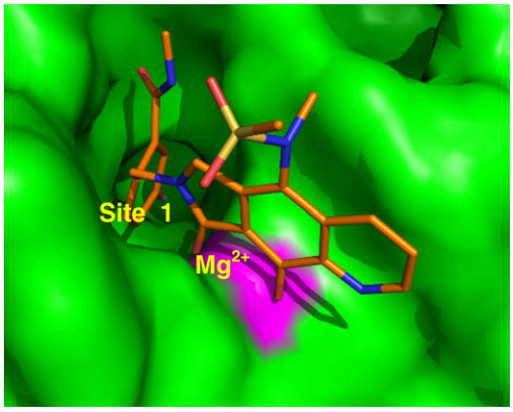

Figure 2. Modeling indicates that the hydrophobic pocket (site 1) may accommodate additional substituents on the *p*-F-benzyl moiety.

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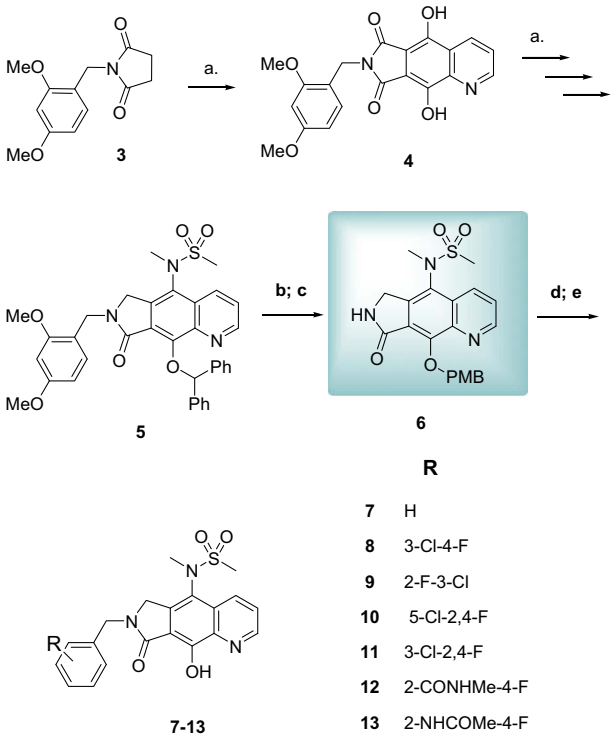

Scheme 1. PMB: *p*-methoxybenzyl. Reagents and conditions: (a) For conditions see: Ref. 2a and 2d; (b) TFA, triethylsilane, rt 1 h then 78 °C, 2 h, 78%; (c) Cs_2CO_3, *p*-methoxybenzyl chloride, tetrabutylammonium iodide, 65°C, 2 h, 80%; (d) substituted benzyl bromides, sodium hydride, DMF, 0 °C (see Ref. 6 for for the preparation of the substituted benzyl bromides); (e) TFA, triethylsilane, rt, 50–93%.

To access a variety of substituted benzyl moieties in **2**, we envisaged a convergent synthetic plan utilizing key intermediate **6** shown in Scheme 1. Compound **3** was prepared from 2,4-dimethoxybenzylamine and succinic anhydride under heating condition. Dieckmann condensation between **3** and dimethyl 2,3-pyridinedicarboxylate yielded **4** which was converted to C5 aza intermediate **5** according to the procedures described in our previous publications.[2a,d] Upon treating **5** with acid in the presence of triethylsilane, free phenol was generated which was re-protected as *p*-methoxybenzyl ether **6**. Alkylation of **6** with benzyl bromide or 3-Cl-4-F-benzyl bromide yielded **7** and **8**. Other substituted benzylating agents were prepared with the standard procedures and used to convert **6** to **9**–**12**.[6]

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

All the analogs tested showed enzymatic activity in the IN strand transfer assay at submicromolar concentrations. An analysis of the potency revealed a unique substitution pattern that is preferred in the series. First, the role of the fluorine for the potency is essential since removal of the fluorine (**7**) led to a 20-fold loss in potency by comparing **7** to **2**. All the multi-halogenated analogs (**8**–**11**) were highly potent in anti-HIV assay in cell culture. Further more, the cell-based potency of these compounds was shifted by human serum proteins (HSP) to small degrees (2.8–3.6 folds of shifting) and they were equipotent to raltegravir (**1**) when tested in parallel. A noteworthy feature is that these halogens can be incorporated in different combinations of substituted positions to maintain good activities.

On the other hand, installing relatively polar functional groups such as carboxamide at the ortho position (**12** and **13**) compromised the cell culture activity in anti-HIV assay. These observations suggest that the preferred substituents on the benzyl moiety of tricyclic scaffold were hydrophobic functional groups. This trend is in contrast to the raltegravir based scaffold, which tolerated carboxamides in anti-HIV assay.[4] One of the possible explanations is the reduced capability of these compounds to penetrate the cell membrane since their enzymatic activity is comparable to the other analogs.

Table 1
Integration strand transfer inhibition and anti-HIV proliferation assay results for compounds **2**, **7**–**13**[a]

Compd	IC_{50}[b]	EC_{50} (10%FBS)[c]	EC_{50} (HSP)[d]	Shift fold (HSP/FBS)
2	28	1.7	11.4	6.7
7	120	35	nd	nd
8	52	3.5	12	3.4
9	61	6	18	3
10	39	6	17	2.8
11	135	5	18	3.6
12	74	32	90	2.8
13	5	52	nd	nd
1[e] (raltegravir)	40	4	20	5

[a] Values are means of at least two experiments, given in nM, nd: not determined.
[b] Ref. 5a.
[c] Ref. 5b.
[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. 5c for details.
[e] In-house data.

Table 2
Pharmacokinetics of **2**, **8** and **10** 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)
2	15	1.1	0.28	45	7	0.4
8	7	1.36	0.23	41	7.13	0.15
10	9	1.38	0.29	79	16	0.02
1 (raltegravir)[b]	37	2	2.34	45	11 (β $T_{1/2}$)	0.36

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 ivdosing 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 p.o.); 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.
[b] Data adapted from Ref. 1.

The results of pharmacokinetic studies performed on both **8** and **10** in rat and dog are summarized in Table 2. Similar to **2**,[2d] both **8** and **10** were found to be orally bioavailable in these two species. A remarkable feature of **8** and **10** is their excellent PK profiles in dog. Both compounds exhibited low clearance, long half-life time ($T_{1/2}$) and high oral bioavailability. The major PK parameters compare very favourably to those of raltegravir (**1**) reported by the Merck group.[1] The noticeably low systemic clearance of both **8** and **10** in dog PK studies can be a combination of a few factors including their intrinsic metabolic stabilities and plasma protein binding in dog.

In conclusion, analogs of the C5 aza tricyclic quinoline derived by modifying the *p*-fluorobenzyl moiety were prepared. Examination of SAR revealed a preferred substitution pattern that favors the incorporation of two or three halogens with flexible substituted positions. More notably, addition of halogens greatly improved dog PK properties. These lead compounds were selected for the clinical investigation and the results will be reported in due course.

Acknowledgments

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lation group for supporting the PK studies; Gregg Jones for carrying out biological assays.

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5. (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 horse radish peroxidase detection was used.; (b) For antiviral assay, 50 μl of 2× test concentration of fivefold serially diluted drug in culture medium were 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 microlitres of infected cell suspension in culture medium ($\sim 1.5 \times 10^4$ cells) were then added to each well containing the drug dilutions. The plates are incubated at 37 °C for 5 days. One hundred microlitres of CellTiter-Glo™ Reagent (catalog # G7571, Promega Biosciences, Inc., Madison, WI) were 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.
6. For 3-Cl-4-F-benzyl bromide (**8**): DeGraw, J. I.; Cory, M.; Skinner, W. A.; Theisen, M. C.; Mitoma, C. *J. Med Chem.* **1967**, *10*, 64; For 5-Cl-2,4-F-benzyl bromide (**9** and **10**), 2-CONHMe-4-F benzyl analog () and 2-NHAc-4-F-benzyl analog (**13**): US2007/72831; For 3-Cl-2,4-F-benzyl bromide (**11**): WO2007/61670 example 68.