Bioorganic & Medicinal Chemistry Letters 19 (2009) 1187–1190

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Tricyclic HIV integrase inhibitors: VI. SAR studies of 'benzyl flipped' C3-substituted pyrroloquinolines

Sammy Metobo, Michael Mish *, Haolun Jin, Salman Jabri, Rachael Lansdown, Xiaowu Chen, Manuel Tsiang, Matthew Wright, Choung U. Kim

Gilead Sciences, Medicinal Chemistry, Inc. 333 Lakeside Drive, Foster City, CA 94404, USA

ARTICLE INFO

Article history:
Received 20 November 2008
Accepted 18 December 2008
Available online 25 December 2008

Keywords:
AIDS
HIV
Integase inhibitors
Tricyclic pyrroloquinoline
C3 Benzyl SAR

ABSTRACT

A series of C3 halobenzyl-substituted tricyclic HIV integrase inhibitors was prepared. Improvement in cell-based inhibitor potency was observed in comparison to previously disclosed tricyclic pyrroloquinolines carrying the 'halobenzyl tail' at the lactam nitrogen. Animal PK for several of the C3-substituted inhibitors was examined, with a dihaloaryl analog achieving good balance in protein-shifted EC_{50} and $t_{1/2}$ in animal PK studies.

Introduction: The causative pathogen of AIDS, human immunodeficiency virus (HIV), requires three essential enzymes encoded in the HIV pol gene for replication. While there are many licensed therapeutics that target protease (PR) and reverse transcriptase (RT), by comparison it has been only recently that promising integrase (IN) inhibitors have been advanced through clinical development.[1]

Previous reports from our group have examined the SAR of IN inhibitors based on a pyrroloquinoline-containing tricyclic scaffold such as **1** (Fig. 1).[2] Here, we report the discovery of a next-generation class of analogs in the pyrroloquinoline series, shown generally as **2**, where the benzyl group is appended to the pyridine C3 position. These new inhibitors display improved potency and in vivo PK when compared to many of the previously disclosed analogs from our research program.

The rationale to pursue this SAR was provided by a few key considerations. Based on examination of our integrase/DNA active site model, initially developed for earlier leads in this program,[3] we hypothesized that these 'benzyl flipped' analogs would possess excellent inhibitor potency. Figure 2 presents an overlay of the previously reported analog **1**, with a prototypical 'flipped tail' inhibitor **9**. It can be seen that the integrase active site can accommodate the new inhibitor **9** especially well if the scaffold binds in such a way as to orient the benzyl tail into the 'lipophilic pocket' of the enzyme that our model has postulated previously.

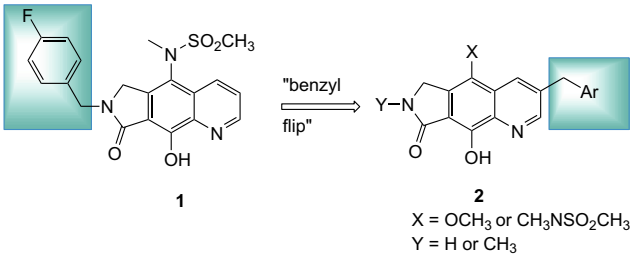

Figure 1. Tricyclic pyrroloquinoline inhibitor **1**, and general 'benzyl flipped' inhibitor structure **2**.

Additionally, protein-shifted inhibitor potency and metabolic stability were recognized as essential considerations in lead identification. Evaluation of these new 'benzyl flipped' analogs was undertaken as part of our ongoing effort to develop a new series of potent IN inhibitors with excellent PK.

Synthesis, results and discussion: Our synthesis plan for the series hinged on the use of a C3-substituted pyridine dielectrophile[4] as the reacting partner in a Dieckmann condensation to quickly build up the tricyclic core of the scaffold. The synthesis of analog **8** is shown in Scheme 1, where condensation between *N*-methyl succinimide and the pyridine dicarboxylate **3** is carried out to give bis-phenol **4**. Treatment with excess triisopropylsilyl (TIPS) chloride in the presence of TEA gives the intermediate bis-ether, which then furnishes the mono-TIPS ether **6** upon reaction with an equivalent of water added to the reaction mixture. Reduction of the imide to the lactam with $LiBH_4$, followed by alkylation of the free

* Corresponding author. Tel.: +1 650 522 5316; fax: +1 650 522 5169.
E-mail address: mmish@gilead.com (M. Mish).

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doi:10.1016/j.bmcl.2008.12.079

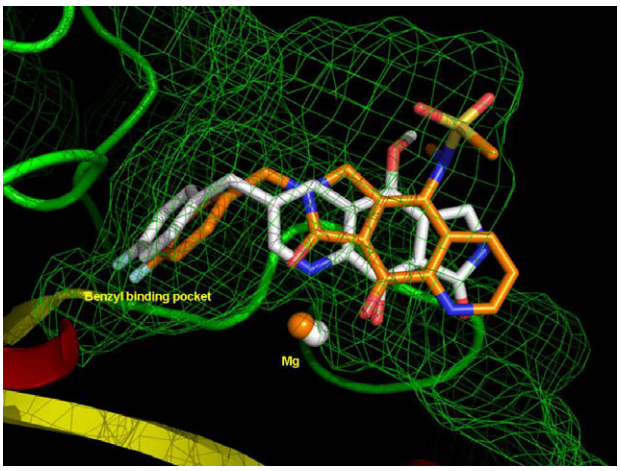


Figure 2. Overlay of 'flipped-tail' compound **9** (white scaffold) onto **1** (orange scaffold) docked into integrase/DNA complex model.

phenol with MeI gave the final compound **8** after TIPS hydrolysis employing TFA/TES in dichloromethane (DCM).

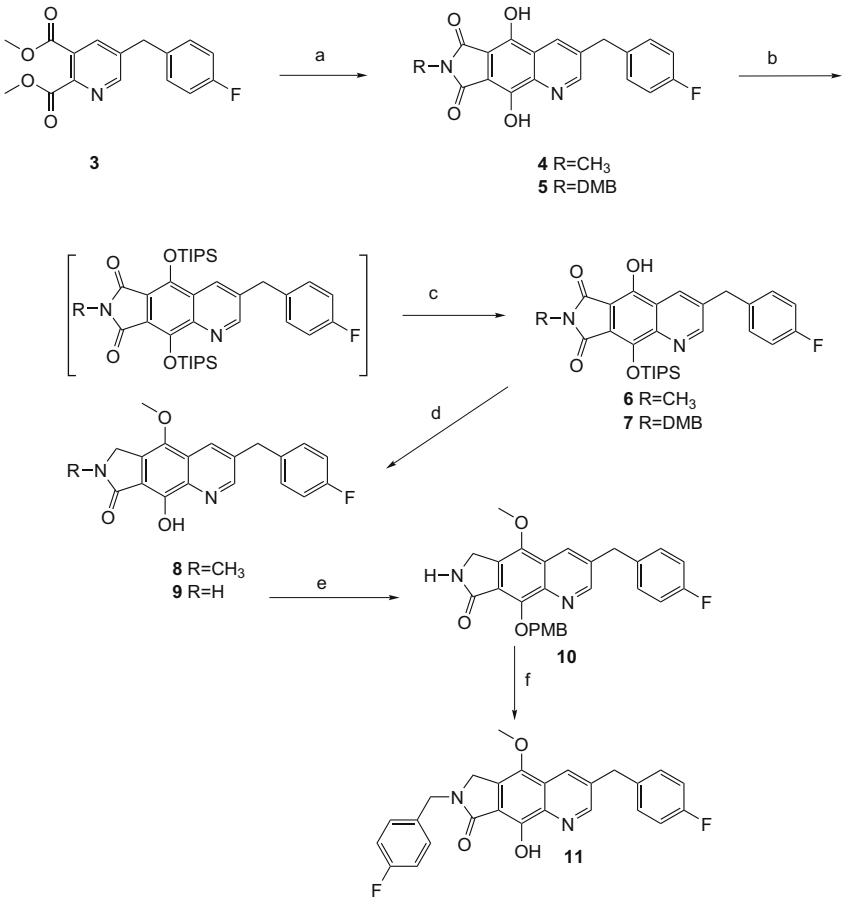
For the related analogs **9** and **11**, the *N*-3,5-dimethoxybenzyl (DMB) protected imide was utilized in the Dieckmann condensation. The synthetic sequence is analogous to the one previously described, until unmasking of the DMB-lactam via heating in TFA/THF (to furnish analog **9**) is followed by recapping the C8-phenol to yield intermediate **10**. Further elaboration via lactam *N*-alkylation with *p*-F benzyl bromide gave, after PMB removal, the bis-*p*-F benzyl analog **11**.

Synthesis of these first analogs in the 'flipped-tail' series was followed by efforts that focused on two additional elements of SAR. Primarily, we examined leads featuring a C5-*N*-methyl, *N*-mesyl group (exemplified by previously reported compounds in the program such as **1**). The impact of a dihalogenated aryl 'tail' was also of interest based on results from our original inhibitor series. In the context of our earlier work, both lines of SAR were expected to result in meaningful improvements in potency and PK behavior.

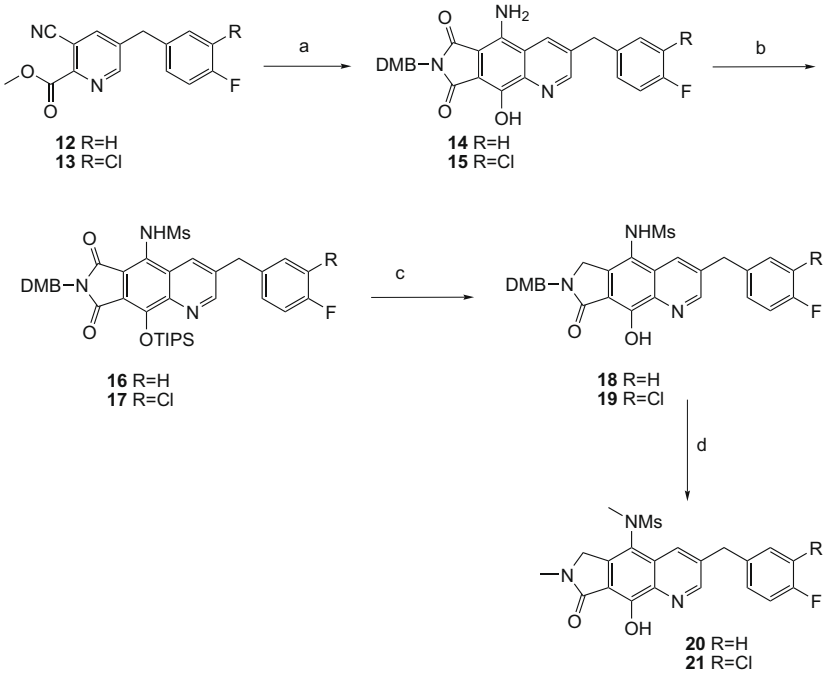
The synthesis of these latter analogs is detailed in Scheme 2. Here, Dieckmann condensation of *N*-DMB-imide with a nitrile-ester **12** led to aniline **14**. Mesylation followed by reduction provided lactam **18**. Subsequent DMB removal, PMB recapping of the C8-phenol, lactam *N*-methylation, and final deprotection gave analog **20**. A similar synthetic sequence involving dihaloaryl 'tail'-bearing-pyridine **13** ultimately furnished analog **21**.

Comparison of strand transfer IC_{50} and antiviral EC_{50} values (Table 1) for C5-methoxy analogs **8**, **9** and **11** showed a trend where a decrease in potency is seen upon increasing the size of the lactam *N*-substituent.

Within this set, the most potent analog in the series is the free-lactam compound **9**. Despite the presence of two aryl 'tails' in compound **11**, a moderate level of both enzymatic and cell-based antiviral activity was maintained. This observation suggests that the presence of two extended lipophilic moieties on the pyrroloquinoline core results in a comparatively poor inhibitor fit relative



Scheme 1. Reagents and conditions: (a) *N*-methyl succinimide, NaOMe, THF, reflux, 24 h, 66%; (b) TIPSCl (2 equiv), DMF, DIPEA; (c) i—H_2O, 16 h, 84% across 2 steps; ii—$LiBH_4$, THF/MeOH, rt–60 °C, 76–85%; (d) i—MeI, NaH, 80–85%; ii—TFA/THF/H_2O, 82–88%; (e) PMBBr, CS_2CO_3,DMF, 44%; (f) i—NaH, *p*-F benzylbromide, DMF, 65%; ii—TFA/TES/DCM, 82%.



Scheme 2. Reagents and conditions: (a) LiHMDS (2.5 equiv), THF, 0 °C–rt, 24 h, 50–86%; (b) i—TIPSCl (1.2 equiv), DMF, TEA, 65–75%; ii—MsCl, TEA (excess, then KOtBu), 60–65%; (c) $LiBH_4$, THF/MeOH, rt–80 °C; (d) i—MeI, Cs_2CO_3, 74–82% across 2 steps; ii–TFA/TES/DCM, rt to 70 °C, 90–94%; iii—PMBBr, Cs_2CO_3, DMF, 40–52%, (iv) MeI, NaH, DMF, 70–78% (v) TFA/TES/DCM, 80%.

Table 1
In vitro data for C3-substituted tricyclic inhibitors[a]

Compound	IC_{50}[b] (nM)	EC_{50}, (10% FBS)[c]	EC_{50} (HSP)[d]
8	100	20	nd
9	14	16	27
11	600	105	nd
20	80	2	7
21	265	1.1	35

[a] Values are means of at least two experiments, given in nM, nd, not determined.
[b] Ref. 6a.
[c] Ref. 6b.
[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. 6c for details.

Table 2
Comparison of PK results for new analogs **20** and **21** in rat and dog, presented alongside PK data for clinically approved IN inhibitor raltegravir (RAL data is adapted from Ref. 1)

Compound	Rat			Dog		
	F (%)	$T_{½}$ (h)	CL (L/h/kg)	*F* (%)	$T_{½}$ (h)	CL (L/h/kg)
Raltegravir	45	2	2.4	69	11 (β $T_{1/2}$)	0.36
20	12	0.4	0.49	47	4.4	0.13
21	11	1.7	0.22	60	8.8	0.12

F (%): fraction available to the general circulation upon oral dosing of test compounds as compared to i.v. dosing, calculated based on AUC from i.v. and p.o. groups, expressed as %; CL: total body clearance obtained from i.v. dosing groups. 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 $T_{½}$ was generated from the i.v. dosing group. The data represent the mean value obtained from three animals in each study. Data adapted from Ref. 1.

to inhibitors carrying only one benzyl 'tail'.[5] On the other hand, evaluation of the C5-aza substituted, 'flipped-tail' analogs showed, most notably in the case of analog **20**, significant improvement in the EC_{50} and protein shifted EC_{50} values.

Of the compounds thus obtained, we chose to advance several that showed compelling potency in the protein shifted assay to evaluation in rat and dog PK studies (summarized in Table 2). The resulting rat and dog PK for analogs **20** and **21** are presented alongside the animal PK data reported for the clinically approved IN inhibitor raltegravir (MK-0518).[1] While the bioavailability of this new tricyclic series is comparable to that of the reference compound, the clearance of **20** was seen to be significantly lower than that of raltegravir in both species. Further gains in the DMPK properties of the 'benzyl flipped' pyrroloquinoline leads were seen with the dihaloaryl-tail compound **21**. Specifically, the in vivo clearance was further driven down in rat as compared to the 4-fluorobenzyl analog **20**. Overall, **21** showed improvement in terms of $t_{1/2}$ in both rat and dog, while at the same time showing protein-shifted potency that was only moderately attenuated when compared to the related compound **20**.

Conclusions: The synthetic work presented above enabled the examination of a new series of pyrroloquinoline HIV integrase inhibitors that moved the 'benzyl tail' portion of the inhibitor to the C3-position of the inhibitor scaffold. This change to the tricyclic scaffold was found to be well-tolerated with respect to the resulting potency of the compounds as measured by the integrase strand transfer and tissue culture anti-HIV assays. PK evaluation of a subset of these leads showed that fine-tuning of both the C5-substituent and the aryl portion of the 'benzyl tail' proved beneficial in terms of identifying diahaloaryl 'flipped tail' analog **21**. This lead showed good oral bioavailability (F), low in vivo clearance, and high antiviral activity. These results support possible further clinical evaluation of this new series.

Acknowledgments

The authors are grateful to Xubin Zheng and Bing Lu for analyzing samples in PK studies; Vahid Zia and co-workers in the formulation group for supporting the PK studies; Gregg Jones and Fang Yu for carrying out biological assays.

References and notes

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4. The preparation of pyridine dielectrophile **3** has been reported in Johns, B.; Boros, E.; Kawasuji, T.; Koble, C. S.; Kurose, N.; Murai, H.; Sherrill, R.; Weatherhead, J. W.O. Patent 2005/077050(A2), 2005, while the preparation of pyridines **12** and **13** is based on chemistry reported in Cai, Z.; Jabri, S.; Jin, H.; Lansdown, R.; Metobo, S.; Mish, M.; Pastor, R. W.O. Patent 2007/136714(A2), 2007.
5. Consideration of the ST IC_{50} of compound **11** alongside either **9**, or a related inhibitor originally reported in Ref. 2a wherein the bulky C3-*p*-fluorobenzyl substituent of **11** is replaced with a proton, illustrates a large decrease in the potency of **11** compared to either of these nearly identical analogs that carry only a single benzyl tail group.
6. (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 5-fold 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 microliters 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 microliters 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 3-fold 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.