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Design, synthesis and biological evaluation of novel tricyclic HIV-1 integrase inhibitors by modification of its pyridine ring

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Abstract—This communication details both the syntheses and biological evaluation of a novel class of HIV-1 integrase inhibitors. When the quinoline moiety is replaced with the quinoxoline moiety, the antiviral activity is significantly compromised. Similarly, introduction of imidazole to replace the pyridine ring is deleterious to the potency of the compound against the enzyme. Substitution at the 3-position of the pyridine has been investigated. The presence of the pyridine ring in the tricyclic core is preferred for antiviral activity against HIV integrase.

The pol gene of the human immunodeficiency virus (HIV) encodes three essential enzymes; protease, reverse transcriptase and integrase (IN).[1] To date, only HIV protease and reverse transcriptase inhibitors are routinely prescribed in highly active antiretroviral therapy (HAART).[2] However, the continued emergence of multidrug resistance in AIDS patients warrants additional therapeutic approaches based on novel mechanisms of action.[3]

As part of an overall study focused on the structure–activity relationships of novel tricyclic IN inhibitors, compound **1** emerged as an initial lead (Fig. 1).[4] The hydroxyl group plays a key role in binding to IN. The phenol and the 7-carboxamide carbonyl group via a bidentate coordination, chelate to the available Mg^{2+} ion in the catalytic core of IN. This is analogous to the binding interactions seen in the α-enol moiety present in earlier diketoacid (DKA) inhibitors. At the time these DKA inhibitors were emerging, a new generation of IN inhibitors were reported that retained this 1,3-diketo acid motif but replaced the carboxylic acid with heterocyclic bioisosteres such as those present in 5CITEP **2** and S-1360 **3** (Fig. 1).[5] Both of these DKA derivatives contain a nitrogen atom in the heterocycles acting as a third binding point. These heterocycles are isosteric to the pyridine ring in **1**.[6,7]

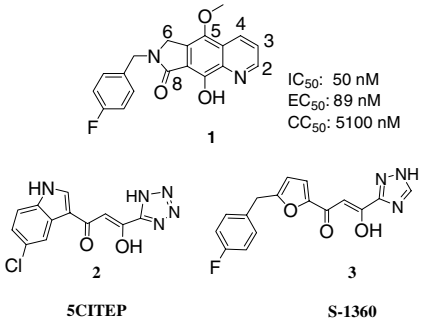

Figure 1. Structure and activity of 5-methoxy pyrrolloquinoline-8-one **1** and structures of 5CITEP **2** and S-1360 **3**.

We sought to study the role of the pyridine moiety played in the context of our highly organized and rigidified tricyclic scaffold to both enzyme- and cell-based anti-HIV activity. We have investigated a number of analogs by modifying the pyridyl ring of our tricyclic lactam core to other heterocyclic systems and studied preliminary substitution effects at C3 to examine the impact on the antiviral potency.

Our general synthetic route was applied to the new inhibitors such as lactams **6** starting from the commercially available diacid **4** (Scheme 1).[4] We noted that imide **5** could be regioselectively reduced to the corresponding aminal by use of sodium borohydride and then to lactam **6** using trifluoroacetic acid and triethylsilane. The fully reduced piperazine **7** was also

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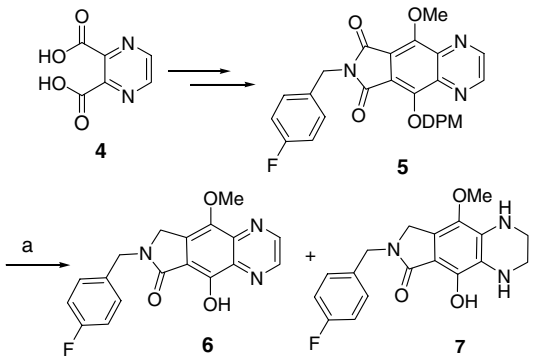


Scheme 1. Syntheses of compounds **6** and **7**. Reagents: (a) i—$NaBH_4$, $MeOH/CH_2Cl_2$; ii—TFA, Et_3SiH, CH_2Cl_2 (21% and 34% yield for **6** and **7**, respectively).

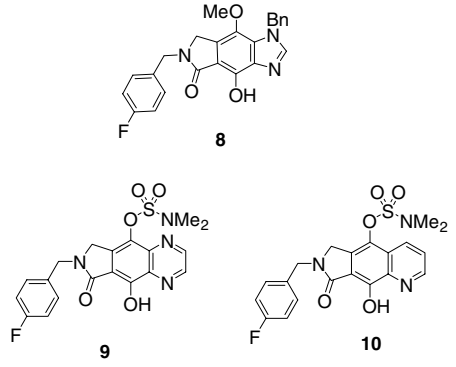


Figure 2. Structures of **8**, **9,** and **10**.

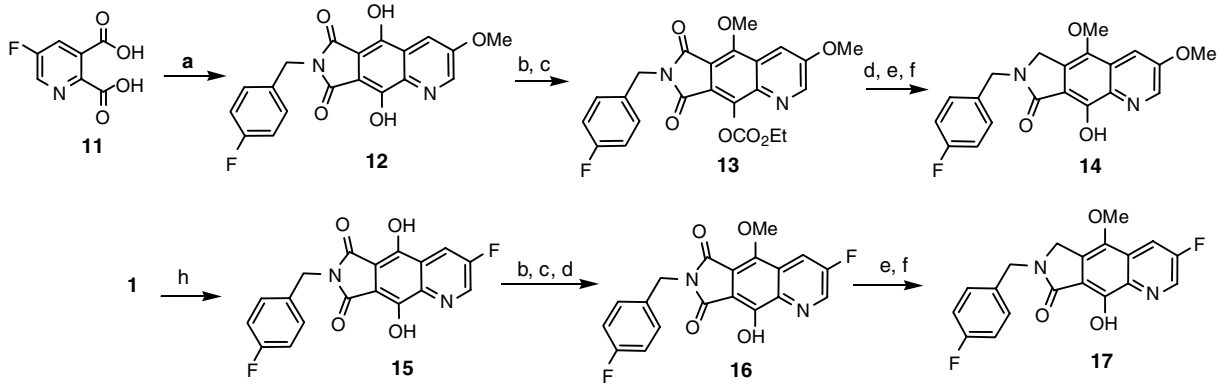
obtained during preparation of **6** under these acidic conditions presumably via ionic reduction of the pyrazine.

Commercially available 1*H*-imidazole-4,5-dicarboxylic acid was converted to compound **8** (Fig. 2) in the manner similar to Scheme 1. The synthesis of compound **9**, (Fig. 2) the *N*,*N*-dimethylsulfamate analog begins with diacid **4**. Its synthesis is analogous to that of **10**, described by Fardis et al.[8]

The synthesis of **11** starting with 3-aminoquinoline has been reported elsewhere (Scheme 2).[9] Esterification of **11** was carried out under standard conditions to furnish the corresponding 2,3 pyridine diester before the ensuing Dieckmann condensation using 1-(4-fluoro-benzyl)-pyrrolidine-2,5-dione was performed. However, only 3-methoxy containing bisphenol **12** was obtained. Apparently, the fluorine at C3 was displaced by methoxide to form **12** exclusively. Bisphenol **12** was converted to the corresponding biscarbonate using excess ethyl chloroformate. In the presence of stoichiometric amounts of DMAP, the biscarbonate was converted to the monocarbonate with complete regioselectivity to expose the phenol at C5. This phenol was converted to its methyl ether **13** under standard conditions. The carbonate was then hydrolyzed and the resulting C8 phenol protected as the diphenylmethyl ether. Reduction of the less sterically encumbered carbonyl of the imide moiety was carried out with sodium borohydride to the aminal before further reduction with trifluoroacetic acid and triethylsilane to form lactam **14**.[13] The preparation of compound **15** proved more challenging than **12**. A large number of bases and various solvents were screened. NaHMDS in THF at low temperature proved to be the optimal conditions to provide the halogen-containing **15** exclusively. The rest of the sequence leading to lactam **17** was uneventful.[13]

The compounds were tested for viral inhibition of HIV-1 IN catalyzed strand transfer and replication in the cell (Table 1).[10] Compounds **1** and **10** were used as standards for comparison.[4,8]

The importance of the lone pair of electrons present in the pyridine nitrogen of the pharmacophore has recently been noted by our in-house data and previously published literature.[11] Our SAR studies clearly confirm that the location and geometry of this pair of electrons are critical determinants of the activity of the inhibitors toward the enzyme. Addition of a second nitrogen lone pair of electrons at the C4 position has apparently a negative effect in the biological activity as exemplified with pyrazine **6** which shows a clear reduction in antiviral activity.



Scheme 2. Syntheses of compounds **14** and **17**. Reagents and conditions: (a) i—MeOH, H_2SO_4, 80 °C, 81%; ii—1-(4-fluoro-benzyl)-pyrrolidine-2,5-dione (see Ref. 4), NaH, THF, MeOH, 0 °C to rt, 49%; (b) i—ECF, pyridine, DMF, 90%; ii—DMAP, H_2O, THF 92%; (c) K_2CO_3, MeI, THF; (d) K_2CO_3, DMAP, THF, H_2O; (e) Ph_2CN_2, 1,2-DCE, 80 °C, 66% over two steps; (f) i—$NaBH_4$, MeOH; ii—TFA, TES, CH_2Cl_2, 73% over two steps; (h) i—MeOH, H_2SO_4; ii—1-(4-fluoro-benzyl)-pyrrolidine-2,5-dione, NaHMDS, THF, −78 °C, 87% over two steps.

Table 1. Integration strand transfer inhibition, anti-HIV proliferation and cytotoxicity assay results for compounds **1**, **6**, **8**, **9**, **10**, **14**, and **17**[10]

Compound	Strand transfer	Anti-HIV in MT4	Cytotoxicity in MT4
	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
1	0.05	0.089	5.1
6	0.21	3.55	87
8	24	>10	25
9	0.033	0.54	2.7
10	0.255	0.007	1.4
14	0.093	0.07	2.1
17	0.0072	1.54	33.3

The aromatic nitrogen lone pair is a Lewis base in the pyridine ring. Thus, when non-aromatic heterocycles are present as in the piperazinyl-based **7**, poor antiviral activity is noted (EC_{50} > 10 μM and IC_{50} 0.85 μM).

Inspired by use of five-membered heterocycles as carboxylate isosteres in **2** and **3**, we synthesized and tested the imidazole analog **8**. This analog completely lost inhibition activity toward the enzyme along with its antiviral potency. However, it is unclear whether the benzyl group on the benzimidazole in **8** was detrimental.

We believe that both the shape of heterocycles and electronic properties of nitrogen in the ring play an important role in formation of an optimal tricyclic integrase inhibitor. Introduction of small five-membered heterocycles proved to be deleterious to both intrinsic potency and the cell-based EC_{50}.

Since the pyridine was confirmed as the optimal moiety for our tricyclic scaffold, and in light of recently disclosed observations,[12] we decided to further evaluate the effect of substitution. Our attention initially was focused to the 3-quinoline position (C3) position of the pyridine moiety of **1**. The preliminary examination of the tolerance to C3 substitution of the pyridine revealed intriguing results. Methoxy substitution at C3 in **14** largely preserved the enzymatic activity while improving anti-HIV potency in the cell assay when compared to **1**. Furthermore, replacement of hydrogen by fluorine at C3 in **17** resulted in significant enhancement of enzymatic activity but at the same time, significant loss of anti-HIV activity in the cell assay. Correlation between activity at the enzyme and activity in the cell culture has not been particularly strong. Generally, this trend was true for most series. This disconnect may be due to inherently poor solubility of the inhibitors. This may contribute to poor permeation through cell membranes. Additionally, the pK_as of the pyridine nitrogen and phenol which comprise the binding motif is altered in analogs **14** and **17** and this may also play a role in the observed changes of activity.

Nevertheless, the discovery that an electron-donating group such as methoxy at C3 is well tolerated implies the opportunity for further expansion and optimization at this position. It has been shown that inhibitors can bind to IN in a 'forward' or 'reverse' direction if overlaid together.[12] Indeed, this C3 position could be used as a point through which these 'reverse' inhibitors may be constructed. The implication of this new series remains to be seen but could have effects ranging from intrinsic potency to resistance mutations.

In summary, based on the compounds we have studied, we have shown that the pyridine ring is optimal in our novel tricyclic-based scaffold. Indeed, observations made from our SAR indicate that an aromatic nitrogen is important for biological activity. Also, substitutions at the 3-position of the quinoline ring are well tolerated. This position is an attractive site for future structural–activity studies to modulate both chemical and pharmacokinetic properties of the tricyclic analogs. Additionally, further work is also underway to explore 2- and 4-substitutions of the quinoline in this scaffold.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.05.018.

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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 or MT-4 cells were infected with HIV-1 IIIB at an moi 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 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.

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13. Selected analytical data. Compound **6**: ^{1}H NMR (300 MHz) $CDCl_3$ δ: 8.95 (d, J = 13. 8 Hz, 2H), 7.23–7.27 (m, 2H), 6.96–7.05 (s, 2H), 4.79 (2H), 4.55 (s, 2H), 4.14 (s, 3H). ^{19}F NMR (300 MHz) $CDCl_3$ δ: 62.80 MS: 340.1 (M+1). Compound **8**: ^{1}H NMR (300 MHz) $CDCl_3$ δ: 9.11 (br s 1H), 7.86 (s, 1H), 7.33–7.23 (m, 5H), 7.01–7.07 (s, 4H), 5.57 (s, 2H), 4.71 (s, 2H), 4.37 (s, 2H), 3.57 (s, 3H). ^{19}F NMR (300 MHz) $CDCl_3$ δ: 62.25. MS: 418.2 (M+1). Compound **14**: ^{1}H NMR (300 MHz) $CDCl_3$ δ: 8.72 (s, 1H), 7.68 (s, 1H), 7.36 (d, 2H), 7.04 (d, 2H), 4.77 (2H), 4.50 (s, 2H), 4.02 (s, 3H), 3.98 (s, 3H). ^{19}F NMR (300 MHz) $CDCl_3$ δ: −114.49 MS: 369.20 (M+1). Compound **17**: ^{1}H NMR (300 MHz) $CDCl_3$ δ: 8.84 (s, 1H), 8.09 (d, 1H), 7.33 (d, 2H), 7.09 (d, 2H), 4.78 (2H), 4.58 (s, 2H), 3.98 (s, 3H). ^{19}F NMR (300 MHz) $CDCl_3$ δ: −114.49, −125.02. MS: 379.23 (M+23).