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Effect of substitution on novel tricyclic HIV-1 integrase inhibitors

Maria Fardis,[a,*] Haolun Jin,[a] Salman Jabri,[a] Ruby Z. Cai,[a] Michael Mish,[a] Manuel Tsiang[b] and Choung U. Kim[a]

[a]*Department of Medicinal Chemistry, Gilead Sciences, 333 Lakeside Dr., Foster City, CA 94404, USA*
[b]*Department of Biology, Gilead Sciences, 333 Lakeside Dr., Foster City, CA 94404, USA*

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Abstract—A series of novel tricyclic inhibitors of HIV-1 integrase enzyme was prepared. The effect of substitution at C-6 of the 9-hydroxy-6,7-dihydropyrrolo[3,4-*g*]quinolin-8-one compounds was studied *in vitro*. Inhibitors with small side chains at C-6 were generally well tolerated by the enzyme, and the physicochemical properties of the inhibitors were improved by substitution of a small alkyl group at this position. A second series of analogs bearing a sulfamate at the C-5 position with various C-6 substituents were prepared to explore the interplay between the two groups. The SAR of the two classes are not parallel; modification at C-5 impacts the effect of substitutions at C-6.

Acquired immunodeficiency syndrome (AIDS) has become a serious epidemic since first reported in 1981. Currently, more than 38 million people are infected worldwide and the disease still remains a significant problem.[1] Human immunodeficiency virus (HIV), which causes AIDS, contains three essential enzymes: reverse transcriptase (RT), protease (PR), and integrase (IN). Inhibitors of RT and PR are known, and various combinations of them are used in highly active antiretroviral therapy (HAART). The effectiveness of HAART is limited by poor adherence to the demanding dosing regimen, cost, and the development of viral resistance. Therefore, new drugs, particularly those with a novel mode of action, are highly sought after.[2]

Recently, attention has been focused on the development of inhibitors targeted at HIV IN. Lack of a close analog of this enzyme in mammals provides further incentive toward development of a nontoxic antiviral therapeutic. A large number of inhibitors of IN have been identified *in vitro*, but compounds that have proven effective *in vivo* remain scarce.[3] We and others have previously identified tricyclic compounds such as **1** that demonstrate good potency against the enzyme (Fig. 1).[4,5] An extensive series of analogs prepared

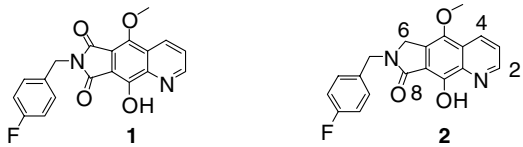

Figure 1. Activity of pyrroloquinoline-6,8-dione **1** versus pyrroloquinoline-8-one **2**.

in-house indicated that the pyridyl nitrogen, the C-9 phenol, and the C-8 carbonyl form the pharmacophore of the compounds in binding to the enzyme. However, the cellular antiviral activity was limited. In an effort to improve the potency and pharmacokinetic profile of our inhibitors, we noted that the cell-based antiviral activity of the pyrroloquinoline-8-one **2** was substantially improved compared with the pyrroloquinoline-6,8-dione **1**.[6] We hypothesized that the presence of the C-6 carbonyl in **1** yields a planar molecule with poor solubility due to π-stacking, and that the better cellular activity of **2** might be due to improved solubility. We therefore decided to further elaborate that position.

Our initial goal was to investigate the effect of substitution at the C-6 position of 5-methoxy-pyrrolo-

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* Corresponding author. Tel.: +1 650 522 5194; fax: +1 650 522 5899; e-mail: mfardis@gilead.com

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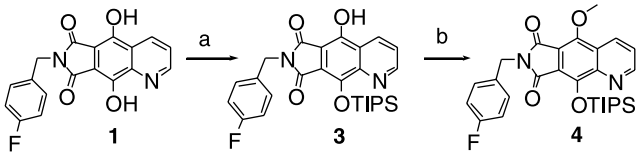
quinolin-8-ones on enzymatic and cell-based antiviral activity. In addition, since C-6 substituents have the potential for additional interactions with the enzyme, we wanted to explore the relationship between substitutions at the C-5 and the C-6 positions. To address the above questions, an initial series of C-6 analogs carrying 5-methoxy was prepared. Subsequently, we also explored a series of sulfamates at C-5.

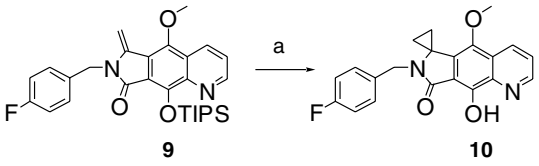
The common intermediate **3**, which was utilized in the preparation of all these analogs, was synthesized as shown in Scheme 1. The pyrroloquinoline-6,8-dione **1** was prepared as described in the literature.[7] The C-9 phenol can be preferentially protected under thermodynamic conditions by heating the reaction. The effect of various protecting groups on the chemoselectivity of subsequent reactions for the C-6 position was explored. TIPS proved to be optimal, presumably for steric reasons.

A series of small alkyl groups at C-6 were prepared by selective nucleophilic addition of alkyl anions to the carbonyl group (Scheme 2). Specifically, addition of methyl Grignard to the appropriately protected compound **4** led to the rapid and clean production of **5**.[8] Quenching the reaction with 1 N aqueous HCl conveniently gave **8** in one step. Reduction of **5** using triethylsilane with $BF_3{\cdot}OEt_2$ in dichloromethane yielded **6** in near quantitative yield. Removal of the TIPS group was achieved using TFA in dichloromethane to provide **7**. Repeating this reaction sequence, it was found that extended exposure of compound **5** to $BF_3{\cdot}OEt_2$ results in the cleavage of the protecting group, providing **7** in one step.

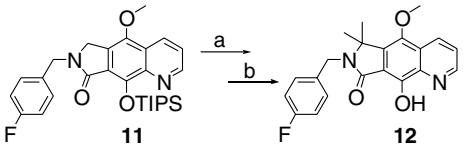
Preparation of the spirocyclopropyl **10** was accomplished from compound **9** using the (iodomethyl)zinc species activated with TFA (Scheme 3).[9]



Scheme 1. Reagents: (a) TIPSCl, Im, DMF; (b) MeI, K_2CO_3, ACN (72% over two steps).



Scheme 3. Reagents and conditions: (a) i—Et_2Zn, CH_2Cl_2, 0 °C; ii—TFA, 0 °C, 15 min; iii—CH_2I_2, CH_2Cl_2; iv—Compound **9**, CH_2Cl_2, 0 °C to rt, overnight.

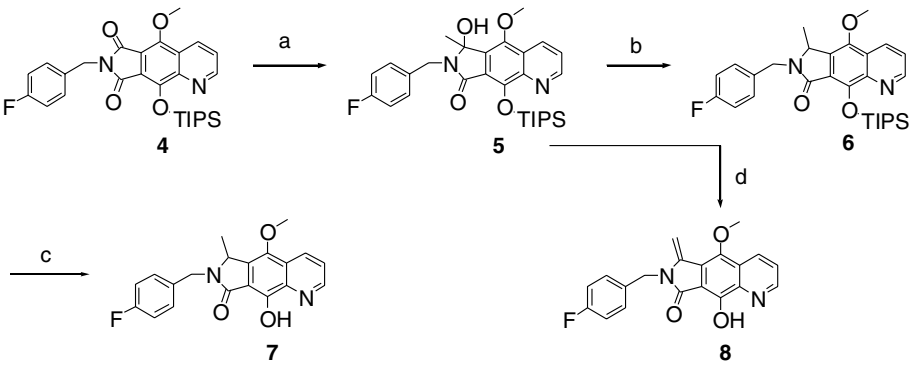


Scheme 4. Reagents and condition: (a) LiHMDS, THF, 0 °C, MeI (58%); (b) TFA, CH_2Cl_2.

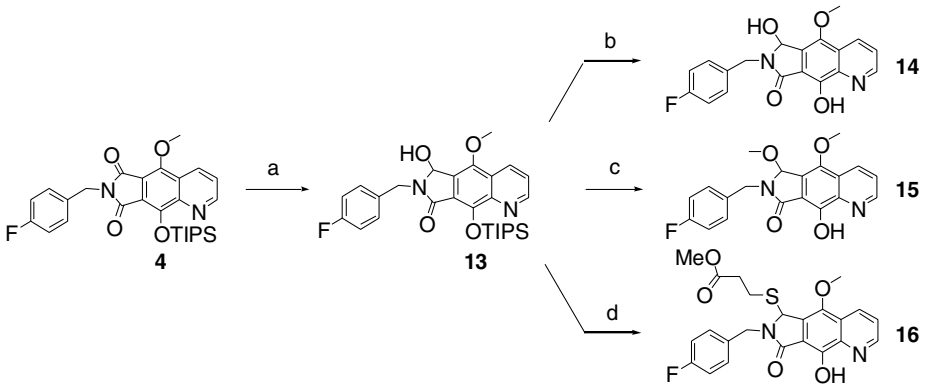
To complete the SAR of this series of compounds, we also prepared the C-6 *gem*-dimethyl compound **12**. This analog was prepared by direct alkylation of the protected analog **11** under strongly basic conditions at low temperature (Scheme 4).

The tolerance of the enzyme for solubility-enhancing substituents at C-6 was explored next. We chose to attach different substituents onto the C-6 position via nucleophilic addition to *N*-acyliminium ions prepared from the protected 6-hydroxy-pyrroloquinolin-8-one **13** (Scheme 5). Reduction of the imide **4** was accomplished using an excess of $NaBH_4$ in MeOH[10] to provide **13** as the only regioisomer. Deprotection provided compound **14**. Exposure of **13** to methanol in TFA provided methyl ether **15**. We concluded that the reactive acyliminium ion can be generated under mild conditions from the 6-OH compound **13**. The formation of the thioether **16** required forcing conditions leading us to believe that stability of **16** is improved compared with that of **15**.

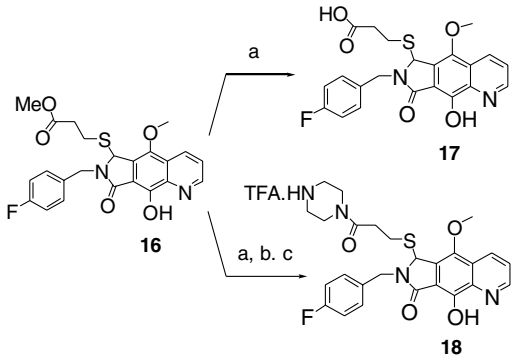
We were particularly interested in the antiviral activity of the charged analogs. We utilized compound **16** to rapidly provide access to both carboxylic acid **17** and amine **18** (Scheme 6).



Scheme 2. Reagents: (a) i—MeMgBr, THF, rt; ii—satd aq NH_4Cl, EtOAc (99%); (b) $BF_3{\cdot}OEt_2$, TES, CH_2Cl_2, (99%); (c) and (d) TFA, CH_2Cl_2.



Scheme 5. Reagents and conditions: (a) $NaBH_4$, MeOH, CH_2Cl_2, overnight; (b) TBAF, THF, (100%); (c) TFA, MeOH, 1 h (100%); (d) $HS(CH_2)_2CO_2Me$, TFA, CH_2Cl_2, 1 day.

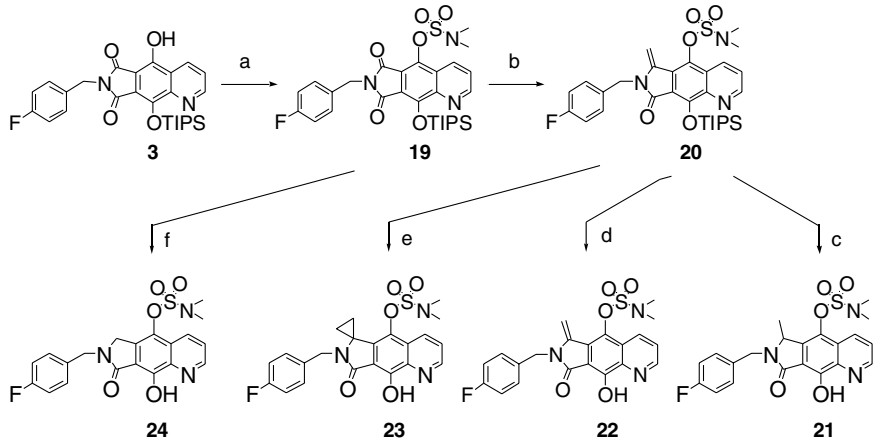


Scheme 6. Reagents and conditions: (a) LiOH, THF, MeOH, H_2O (58% over three-steps—see Scheme 5); (b) 1-BOC-piperazine, EDC, CH_2Cl_2, TEA; (c) TFA, CH_2Cl_2,, 1 h.

Of various C-5 substitutions, dimethyl sulfamate (**24**) gave the best cell-based antiviral activity (**2**: EC_{50} = 89 nM, **24**: EC_{50} = 7 nM). Therefore, a series of compounds carrying this substituent at C-5 with small alkyl groups at C-6 was prepared (Scheme 7). The monoprotected compound **3** was transformed to sulfamate **19** using dimethyl sulfamoyl chloride and DMAP in good yield. Removal of the C-6 carbonyl was performed using a two-step procedure: reduction using $NaBH_4$ to form the aminal, followed by a second reductive elimination of the aminal using triethylsilane and TFA to provide the dihydrosulfamate **24**. Preparation of compounds **21**–**23** was accomplished following the procedures described for **7**, **8**, and **10**.

All compounds were evaluated in the IN strand transfer assay, as well as a cell-based antiviral assay using the MT4 cell line.[11] The data are summarized in Table 1.

Comparison of compound **1**, our initial lead, with the rest of the analogs demonstrates the benefit of modification at C-6 on antiviral activity. Compound **8** which maintained the planar conformation showed weaker activity among **2**–**10**. Compounds **2** and **7** had very similar enzymatic activities, demonstrating the tolerance of the enzyme toward substitution with small alkyl groups. The spirocyclopropyl variant **10** had excellent activity against the enzyme. The solubility of the spirocyclic analog **10** similar to **2** and **7** was improved over our initial lead **1**. Further substitution at C-6 was not tolerated by the enzyme as the activity of the *gem*-dimethyl compound **12** dropped by over an order of magnitude



Scheme 7. Reagents and conditions: (a) $ClSO_2N(Me)_2$, CH_2Cl_2, DMAP, TEA (75%); (b) i—MeMgBr, THF, rt; ii—aq 1 N HCl, CH_2Cl_2 (87%); (c) $BF_3{\cdot}OEt_2$, TES, CH_2Cl_2 (75%); (d) TFA, CH_2Cl_2 (75%); (e) i—Et_2Zn, CH_2Cl_2, 0 °C; ii—TFA, 0 °C, 15 min; iii—CH_2I_2, CH_2Cl_2; iv—Compound **20**, CH_2Cl_2, 0 °C to rt, overnight; (f) i—$NaBH_4$, MeOH, THF (85%); ii—TES, TFA, CH_2Cl_2 (95%).

Table 1. Strand transfer inhibition, antiviral activity, cytotoxicity, and selected physicochemical properties of C-6 pyrrolloquinoline-8-ones

Compound	IC_{50} (nM)	EC_{50} (nM)	CC_{50} (nM)	Solubility (μM) pH: 7.3/2.2
1	80	7500	19,000	<1.4/<1.4
2	60	89	5100	3/285
7	88	77	1600	3/193
8	150	375	6100	
10	7	124	3300	26/49
12	210	1110	427	
14	36	256	9740	
15	64	415	11,400	
16	310	430	2800	
17	295	3400	24,000	
18	59	685	5250	
21	815	8	755	2/11
22	420	52	1650	<1/<1
23	357	443	14,200	2.1/11
24	255	7	1400	<2.3/7

compared to the spirocyclopropyl analog **10**. Therefore, subsequent analogs were prepared using a linear side chain to avoid a steric clash with the enzyme.

In an attempt to install polar groups at the C-6 position, compounds **14**, **17**, and **18** were prepared. Chemical stability studies indicated that compound **16** was stable under the assay conditions. Thus, while **16** lacked the necessary antiviral activity, we used it as a precursor for preparation of acidic analog **17** and basic compound **18**. The compounds with polar substitutions had poor potency in the antiviral assay and were not further pursued.

The enzymatic activity of compounds bearing a sulfamate at C-5 was generally poorer compared with analogs with a methyl group at this position. However, the methyl sulfamate **21** as well as the dihydrosulfamate **24** both had impressive antiviral activities. This class of compounds is less soluble than the C-5 methyl series and did not offer an improvement in the physicochemical profile of the compounds.

In general, the SAR of C-5 sulfamates does not parallel that of C-5 methyl series. This may be due to the steric or electronic factors. Sterically, the presence of substituents at C-6 may alter the preferred conformation of C-5 side chain compared with the dihydro C-6 analogs. Electronically, the presence of an electron-withdrawing group at C-5 might affect the pK_a of the C-9 phenol which in turn changes the binding properties of the pharmacophore region. Further studies are necessary to clarify the importance of each factor affecting the enzymatic activity.

In summary, a new class of inhibitors of IN with favorable *in vitro* potency is reported herein. The pharmacophore region of the molecule, the 1-nitrogen, the 8-carbonyl, and the 9-phenol, was left intact. A number of analogs with various substitutions at C-6 were evaluated in order to understand the relationship between C-6 and C-5 groups. In general, the antiviral activity of the pyrroloquinoline-6,8-diones was improved by removing the C-6 carbonyl group. Among the substitutions placed at the C-6, small alkyl groups such as the methyl analog **7** and the spirocyclopropyl compound **10** had the most attractive *in vitro* profiles. Additionally, the solubility of C-6 substituted analogs was improved compared to the parent imide **1**. This class of compounds has favorable drug-like properties and is being further pursued.

Acknowledgments

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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.2006.05.008.

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11. (a) Strand transfer assay was modified from a previous report (Hazuda, et al. *Nucleic. Acids 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 2X test concentration of 5-fold serially diluted drug in culture medium was 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 were 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 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.