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# Design, synthesis, and SAR studies of novel and highly active tri-cyclic HIV integrase inhibitors

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**Abstract**—A novel class of tri-cyclic HIV integrase inhibitors were designed based on conformational analysis of 1,6-naphthyridine carboxamide compound L-870810 and docking the designed inhibitor into the active site of our integrase enzyme model. The efficient syntheses of pyrroloquinoline tri-cyclic analogs are described. The SAR studies resulted in the identification of a lead compound that is more potent and more soluble than L-870810.


Many marketed anti-HIV therapeutic agents target either of the two viral enzymes, HIV reverse transcriptase or HIV protease. However, because of continuously emerging HIV mutants that are resistant to most of these drugs, there is the need for HIV inhibitors with a novel mechanism of action.[1] One of the key steps in the life cycle of HIV is the integration of a pro-viral DNA into the genome of the host cell in the nucleus. This crucial event is catalyzed by a third virally encoded and packaged enzyme, HIV integrase. The integration process involves multiple steps and factors from both the virus and the host.[2] The recent clinical trials of an HIV integrase inhibitor L-870810 (**1**) by Merck showed encouraging results of HIV viral load reduction in AIDS patients,[3] thus validating integrase as an attractive HIV therapeutic target.

As depicted in Figure 1, there are two rotational isomers associated with the amide bond in **1**. While conformer B has been postulated as the active form for binding,[3b] conformer A is the lower energy species in the free form based on our calculations.[4] Although there is a difference of only 1.7 kcal/mol between A and B, conversion from A to B will cost approximately 5 kcal/mol to overcome the rotational barrier.

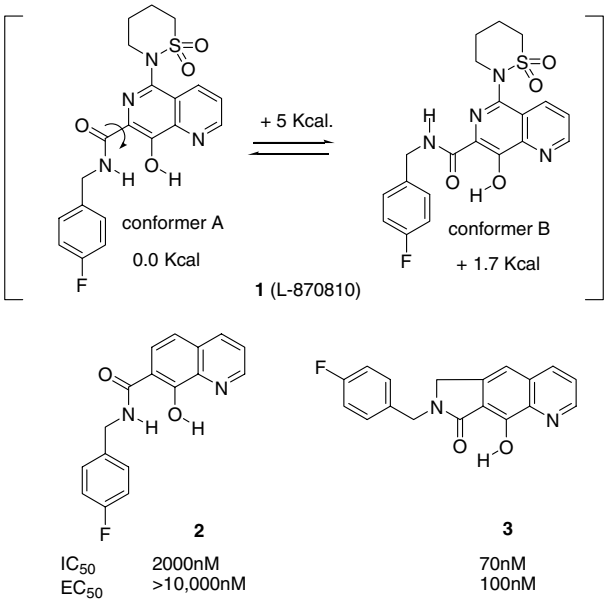


**Figure 1.** The structures of L-870810 (**1**), quinoline carboxamide (**2**), and tri-cyclic pyrroloquinoline (**3**). $IC_{50}$: the concentration required to inhibit 50% of integrase strand transfer activity; $EC_{50}$: anti-HIV activity in MT-4 cells; for details, see Ref. 9a) and 9b).

Hence, it was hypothesized that a pre-organized scaffold such as 3 would provide an energetic benefit for binding in the active site. To directly evaluate the effects of ring

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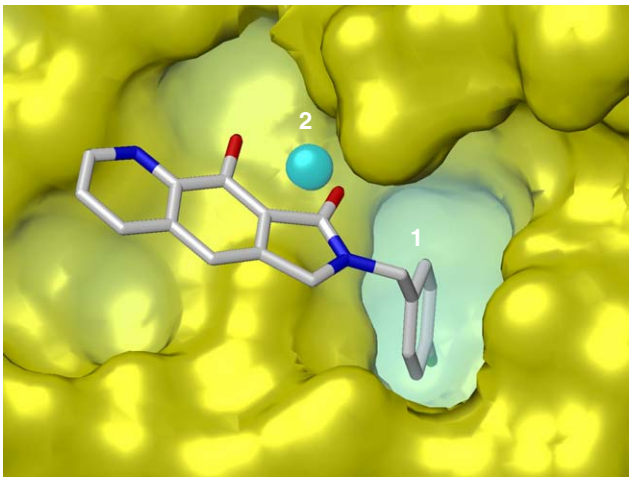



**Figure 2.** Top view of compound **3** docked into the active site of an integrase/DNA complex model.

closure on the activity, we examined a pair of prototype compounds **2** and **3** shown in Figure 1.[5]

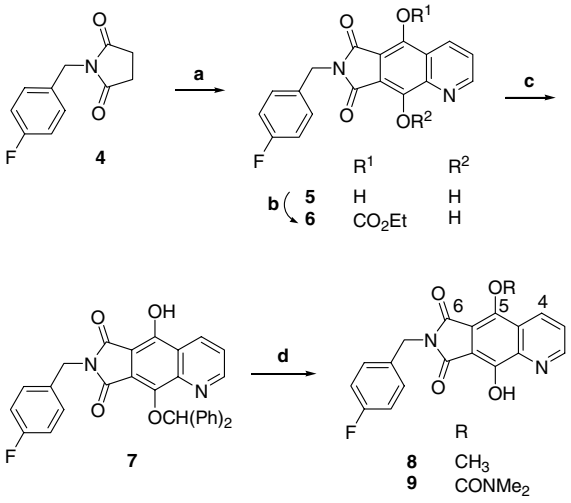
As expected, compound **3** is significantly more potent than compound **2**. The results are consistent with our modeling which docked compound **3** into the active site (Fig. 2) of an integrase/DNA complex model.[6] In the model, a hydrophobic pocket (labeled 1) exists and is well utilized by the *p*-fluorobenzyl moiety that is held tightly in the optimal direction by the lactam ring. This discovery prompted us to initiate a program to investigate several series of HIV integrase inhibitors designed on the pre-organized tri-cyclic core.[7,8] Our active site model also depicts specific polar interactions between inhibitors and enzyme[6] in addition to the critical interaction between the magnesium and the inhibitor (labeled 2).

Our synthetic strategies were planned based upon (a) quick assembly of a tri-cyclic scaffold and (b) the ability to create opportunities for optimizing both the potency and other pharmacological properties of analogs. The synthesis of the tri-cyclic core **5** and two immediate analogs is depicted in Scheme 1 starting with the Dieckmann condensation between **4**, prepared easily from succinimide, and the commercially available pyridine-2,3-dicarboxylic acid dimethyl ester. The differentiation

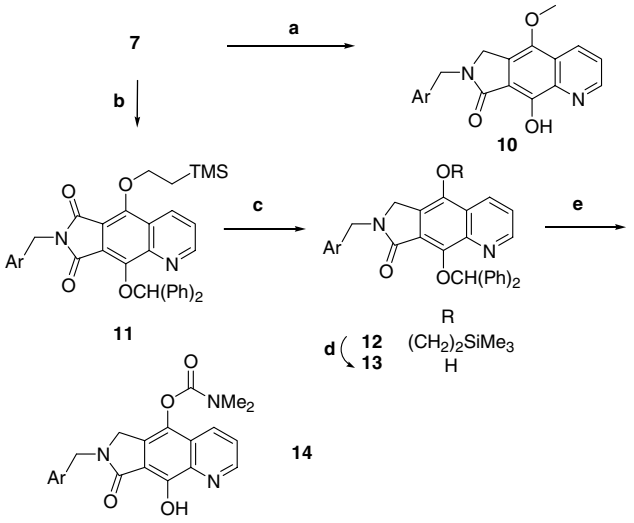
**Table 1.** Integration strand transfer inhibition, anti-HIV proliferation and cytotoxicity assay results for compounds **8**–**10**, **14**, and **16**–**19**[a]

| Compound | $IC_{50}$[b] | $EC_{50}$[c] (MT-4 cell) | $CC_{50}$[d] (MT-4 cell) | Solubility[e] |
|---|---|---|---|---|
| **8** | 0.08 | 7.5 | 19 | <1.4 |
| **9** | 2.4 | 1.18 | 22 | 2.5 |
| **10** | 0.05 | 0.089 | 5.1 | 3 |
| **14** | 0.76 | 0.003 | 1.5 | nd |
| **16** | 1.2 | 0.78 | 1 | nd |
| **17** | 2.1 | 0.34 | 1 | nd |
| **18** | 0.35 | 0.07 | 0.4 | nd |
| **19** | 0.335 | 0.011 | 0.3 | nd |

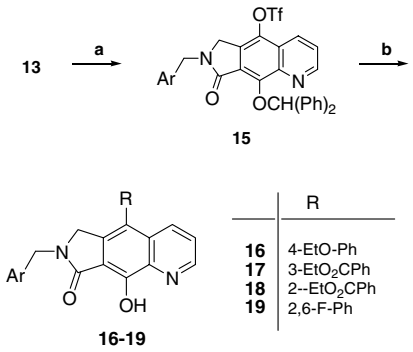
[a] Values are means of at least two experiments, given in μM, nd: not determined.
[b] Ref. 9a.
[c] Ref. 9b.
[d] Ref. 9c.
[e] Measured by dissolving the solid of testing compound in 20% of acetonitrile or 20% of ethanol in phosphate buffer (pH 7.3) and determining the concentration of supernatants by HPLC after incubation at rt overnight.



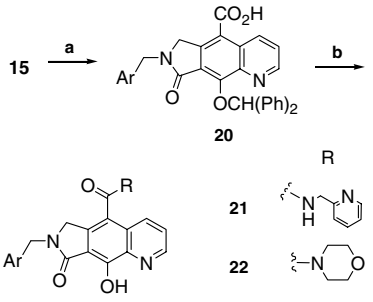

**Scheme 1.** Reagents and conditions: (a) NaOMe, THF, pyridine-2,3-dicarboxylic acid dimethyl ester, reflux, 24 h, 66%; (b) ethyl chloroformate, NaOH, 1,4-dioxane/water, rt, 1 h, 63%; (c) 1—$(Ph)_2CN_2$, 1,2-dichloroethane, 70 °C, 3 h, 80%; 2—$K_2CO_3$, THF/water, cat. 4-dimethylaminopyridine, rt, 12 h, 100%; (d) 1—for **8**: iodomethane, $K_2CO_3$, DMF, 40 °C, 1 h, 85%, for **9**: $ClCONMe_2$, TEA, DMAP, dichloromethane, rt, 12 h, 50%; 2—trifluoroacetic acid, triethylsilane, rt, 10–30 min, 64–68%.




**Scheme 2.** Reagents and conditions: Ar: *p*-fluorophenyl; (a) 1—MeI, $K_2CO_3$, DMF, 40 °C, 1 h; 2—$NaBH_4$, THF/MeOH, rt, 15 h, 3—triethylsilane, TFA, 80% for three steps; (b) 2-trimethylsilylethanol, DEAD, $Ph_3P$, dichloromethane, rt, 3 h, 82%; (c) 1—$LiBH_4$, THF/water, 0 °C to rt, 1 h; 2—$Ac_2O$, DMAP, DCM, rt, 12 h, 3—triethylsilane, TMSOTf, 2,6-lutidine, rt, 12 h, 47% for three steps; (d) TBAF, THF, rt, 1 hr, 100%; (e) 1—dichloromethane, $ClCONMe_2$, DMAP, rt, 4 h, 47%; 2—trifluoroacetic acid, triethylsilane, rt, 30 min, 93%.




**Scheme 3.** Reagents and conditions: Ar: *p*-fluorophenyl; (a) $Cs_2CO_3$, *N*-phenyltrifluoromethane sulfonamide, dichloromethane, reflux, 1 h, 43%; (b) (**16–19**) 1—4-ethoxyphenolboronic acid (for **16**); 3-ethoxycarbonylphenylboronic acid (for **17**); 2-ethoxycarbonylphenylboronic acid (for **18**) or 2,6-difluorophenylboronic acid (for **19**), tetrakis-(triphenylphosphine)-palladium(0), $K_2CO_3$, toluene, ethanol, water, 120 °C: 3 h, 17–48%, 2—triethylsilane, TFA, dichloromethane, rt, 1 h, 30–68%.




**Scheme 4.** Reagents and conditions: Ar: *p*-fluorophenyl; (a) 1—$Pd(OAc)_2$, dppp, TEA, DMF/water, CO (1 atm), 70 °C, 2.5 h; 2—$Cs_2CO_3$, iodomethane, rt, 45 min, 70%, two steps; 3—LiOH, THF/water, rt, 15 h, 98%; (b) 1—2-aminomethylpyridine (for **21**) or morpholine (for **22**), diisopropylethylamine, HATU, DMF, rt, 15 h, 2—triethylsilane, TFA, dichloromethane, rt, 1 h, 30–53% for two steps.

of the two phenolic hydroxyl groups was accomplished using the Schotten–Baumann protocol to form monoethyl carbonate **6**. The free hydroxyl was then protected as a diphenylmethyl (DPM) ether, which possesses both stability to basic conditions and desirable steric hindrance to facilitate the control of regioselectivity in the subsequent modification of the succinimide moiety. Upon standard hydrolysis, compound **7** was converted to **8** and **9**. Their activities are reported in Table 1.

Succinimide analog **8** lacked significant anti-HIV potency in the cell-based assay, while showing good integrase inhibitory activity. Similar structures were reported by researchers from the Tibotec.[10] Encouraged by the activity of **8** in the strand transfer assay, we undertook further modification by removing the C6 carbonyl to improve the potency in the cell-based assay. As shown in Scheme 2, compound **7** was converted to the corresponding methyl ether **10** and carbamate **14**.

Compared to **8** and **9**, compounds **10** and **14** exhibited substantially improved potency in the cell-based assay (Table 1). The improved potency of **10** over **8** could be due to the increased solubility and permeation through the cell membrane. The enhanced activity of C5 carbamate **14** in the cell-based assay as compared to C5 methyl ether **10** was also noticeable. Therefore, we explored other functional groups at C5 in addition to the carbamate. Particularly, compounds containing the C5 substituents linked with a C–C bond were investigated because of their stability. Schemes 3 and 4 depict the syntheses of selected bi-aryl and carboxamide analogs.

Compound **18** is the most active among the three simple mono-substituted bi-aryl analogs **16–18** (Table 1). The potency in the HIV cell-based assay was further enhanced when a 2,6-difluorophenyl group was introduced in compound **19**. It is unclear what factors contributed to the potency improvement in this series of C5 bi-aryl analogs.

Both **21** and **22**[11] displayed $IC_{50}$ in sub-micromolar and $EC_{50}$ in low nanomolar ranges (Table 2). We also tested these compounds for the protein-shifted anti-HIV activity. When taking into account the binding effects by both human serum albumin and α-1-acid glycoprotein, these compounds showed potency similar to or better than **1**. A plausible explanation for the reduced effect of these proteins on the potency of **22** as compared to **1** is the higher polarity of **22**, as shown by the calculated log *P*. We also measured the solubility of compounds **1**, **21**, and **22** at pH 7.3 and found that both **21** and **22** are more soluble than **1** in water.[12] These data point to the potential of tri-cyclic analogs as promising candidates for further pharmacological evaluation.

**Table 2.** Activities of carboxamide analogs and selected data of protein binding effect on potency, clog P and solubility[a]

| Compound | $IC_{50}$[b] | $EC_{50}$[c] (MT-2 cell) | $CC_{50}$[d] (MT-4 cell) | Protein-shifted $EC_{50}$[e] (MT-2 cell) | Calcd log *P*[f] | Solubility[g] |
|---|---|---|---|---|---|---|
| **21** | 0.19 | 0.0098 | 1.6 | 0.094 | 2.12 | 36 |
| **22** | 0.036 | 0.0034 | 4 | 0.054 | 0.97 | 97 |
| **1** | 0.05 | 0.0038 | 1.5 | 0.088 | 2.24 | 7.2 |

[a] All values are given in μM except for calcd log *P*.
[b] Ref. 9a.
[c] Ref. 9b.
[d] Ref. 9b.
[e] Ref. 9c.
[f] The program used for calculations: Pallas.
[g] Measured by dissolving the solid of testing compound in 20% of acetonitrile or 20% of ethanol in phosphate buffer (pH 7.3) and determining the concentration of supernatants by HPLC after incubation at rt overnight.

The synthetic schemes described above have allowed us to examine a variety of structures based on a pre-organized pyrolloquinoline tri-cyclic core and evaluate their inhibitory activities against HIV integration in both the strand transfer and cytopathic anti-HIV assays. We have discovered novel, highly organized and potent HIV integrase inhibitors from this class of compounds. Furthermore, our lead compound **22** is more soluble and more potent than the clinically efficacious compound **1** when tested under the physiologically relevant conditions. The identification of a compound demonstrating in vivo pharmacological properties for further development as a clinically useful HIV inhibitor is the subject of our ongoing research program and results will be published in due course.

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## References and notes

1. Mugavero, M. J.; Hicks, C. B. *Drug Discovery Today: Therapeutic Strategies* **2004**, *1*, 529.
2. Esposito, D.; Craigie, R. *Adv. Virus Res.* **1999**, *52*, 319.
3. (a) Little, S.; Drusano, D.; Schooley, R. Haas, D.; Kumar, P.; Hammer, S.; McMahon, D.; Squires, K.; Asfour, R.; Richman, D.; Chen, J.; Saah, A.; Leavitt, R.; Hazuda, D. J.; Nguyen, B. Y. Protocol 004 Study Team, Abstracts of Papers, # 161, 12th Conference on Retroviruses and Opportunistic Infections, Boston, MA, February 22–25, 2005; Foundation of Retrovirology and Human Health, Alexandria, VA, 2005; (b) Hazuda, D. J.; Anthony, N. J.; Gomez, R. P.; Jolly, S. M.; Wai, J. S.; Zhuang, L.; Fisher, T. E.; Embry, M.; Guare, J. P., Jr.; Egbertson, M. S.; Vacca, J. P.; Huff, J. R.; Felock, P. J.; Witmer, M. V.; Stillmock, K. A.; Danovich, R.; Grobler, J.; Miller, M. D.; Espeseth, A. S.; Jin, L.; Chen, I.-W.; Lin, J. H.; Kassahun, K.; Ellis, J. D.; Wong, B. K.; Xu, W.; Pearson, P. G.; Schleif, W. A.; Cortese, R.; Emini, E.; Summa, V.; Holoway, M. K.; Young, S. D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11233.
4. Since molecular mechanics force-field methods do differ (based on the parameterization of each specific method) and may produce different results for specific molecular systems, we decided to utilize several methods (Ref.: 1, Sybyl v.6.9, Tripos, Molecular Modeling Software, St. Louis MI; and 2, MacroModel, Schrodinger Inc., Molecular Modeling Software) in order to evaluate consistency in the results. The lowest energy structures for conformer A and B was computed using six different molecular mechanics methods. The energy difference ($\Delta E$) between A and B was computed from each method. All six methods dictate that conformer A may be more favorable and the average $\Delta E$ is 1.7 kcal/mole for conformer A.
5. See Refs. 9a for $IC_{50}$ and 9b (MT-4 cell) for $EC_{50}$. Compound **2** was prepared from commercially available 8-hydroxyl-quinoline-7-carboxylic acid and 4-fluorobenzylamine by standard amide-forming conditions similar to reaction b in Scheme 4. Compound **3** was obtained from triflate **15** in Scheme 3 according to condition b.
6. Chen, X, Tsiang, M. and coworkers, manuscript in preparation.
7. Chen, J. M.; Chen, X.; Fardis, M.; Jin, H.; Kim, C. U. and Schacherer, L. N. PCT. Int. Patent WO2004035577; *Chem. Abstr.* **2004**, *140*, 375156.
8. (a) For two other series of tricyclic HIV integrase inhibitors: (a) Fardis, M. et al., *Bioorg. Med. Chem. Lett*. **2006**, accepted for publication; (b) Metobo, S. et al., *Bioorg. Med. Chem. Lett*. **2006**, accepted for publication.
9. (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 2X test concentration of 5-fold serially diluted drug in culture medium was added to each well of a 96-well plate (nine 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) 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 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 $\alpha_1$-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.
10. (a) Verschueren, W. G.; Dierynck, I.; Surleraux, D. L. N. G.; Daeyaert, F. F. D. PCT. Int. Patent WO2004096807; *Chem. Abstr.* **2004**, *141*, 410963; (b) Verschueren, W. G.; Dierynck, I.; Amssoms, K. I. E.; Hu, L.; Boonants, P. M. J. G.; Pille, G. M. E.; Daeyaert, F. F. D.; Hertogs, K.; Surleraux, D. L. N. G.; Wigerinck, P. B. T. P. *J. Med. Chem.* **2005**, *48*, 1930.
11. The characterization of compound **22**: $^1$H NMR ($CDCl_3$) 9.08 (dd, 1H), 8.15 (s, 1H), 8.06 (dd, 1H), 7.76 (dd, 4H), 7.55 (dd, 1H), 7.30 (m, 8H), 7.07 (dd, 2H), 4.95 (d, $J = 15$ Hz, 1H), 4.70 (d, $J = 15$ Hz, 1H), 4.42 (d, $J = 15$ Hz, 1H), 4.14 (d, $J = 15$ Hz, 1H), 3.94–3.79 (m, 4H), 3.41 (m, 2H), 2.99 (m, 2H.) MS: 588 (M+1).
12. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3.