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Synthesis and HIV-integrase strand transfer inhibition activity of 7-hydroxy[1,3]thiazolo[5,4-*b*]pyridin-5(4*H*)-ones

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Abstract—An efficient synthesis of methyl 7-hydroxy[1,3]thiazolo[5,4-*b*]pyridin-5(4*H*)-one-6-carboxylates (**8**–**10** and **16**) and 6-carboxamides (**17**–**20**) is described. Sub-micromolar enzyme inhibition of HIV integrase was achieved with several carboxamide analogs which were superior to their carboxylic ester congeners.

Acquired immunodeficiency syndrome (AIDS) in humans results from infection by the human immunodeficiency virus type 1 (HIV-1). Highly active antiretroviral therapy (HAART) for HIV currently targets two of the three virally encoded enzymes; namely, reverse transcriptase and protease. This approach to therapy usually involves treating the patient with an HIV-protease inhibitor in combination with both a nucleoside reverse transcriptase inhibitor and a non-nucleoside reverse transcriptase inhibitor. Although HAART therapy is highly successful in warding off the development of AIDS, incidences of drug resistance and toxic side effects emphasize the need for new targets in the treatment of HIV.[1]

Significantly, the third and final HIV-encoded enzyme, HIV-integrase (IN), has yet to yield to drug discovery efforts.[2] Integrase catalyzes the integration of reverse transcribed viral DNA into host cell DNA through a two-step, metal-dependent process. Step one, known as 3′ processing,[3] effects cleavage of two nucleotides from the two 3′ ends of double stranded viral DNA. The second step, strand transfer,[4] integrates the viral DNA into the host cell DNA through a series of phosphodiester transesterification reactions. A final non-integrase-dependent step, involving cellular DNA repair enzymes, fills in the remaining gaps. Integrase is an especially attractive target for HIV therapy because the enzyme is needed for viral infectivity and there are no known host cell counterparts.

The search for a clinically viable HIV-integrase inhibitor for HIV therapy has spanned more than a decade and recently led to clinical evaluations of heteroaryl diketone S-1360 (**1**)[5] and naphthyridine carboxamide L-870,810 (**2**).[6] These molecules inhibit the strand transfer step of HIV-1 integrase and possess potent antiviral activities. More recently, other heteroaromatic scaffolds with HIV-integrase inhibition activity have emerged, including the naphthyridinone carboxamides **3**.[7] All of these compounds (**1**–**3**) contain a diketoacid-like motif implicated in binding of metal ions within the catalytic core domain of the integrase enzyme.[8] The substituted benzyl group in **1**–**3** is also necessary for potent antiviral activity. In this report, we describe an expedient synthesis of 7-hydroxythiazolopyridinones **8**–**10** and **16**–**20**, and their HIV-integrase strand-transfer inhibition activity.

Synthesis of the title compounds was achieved by the chemistry depicted in Schemes 1, 2 and Eqs. 1–3. Structures of the title compound esters (**8**–**10** and **16**) and carboxamides (**17**–**20**) are shown in Charts 1 and 2, respectively. Enzyme inhibition data are provided in Tables 1 and 2. The requisite intermediate 5-aminothiazoles **6a**–**i** were synthesized from commercially available methyl isocyanoacetate **4** and the appropriate isothiocyanate **5a**–**i** (Scheme 1).[9] Treatment of **6a**–**i** with methyl malonyl chloride in 1,2-dichloroethane (DCE) at reflux temperature afforded malonyl amides **7a**–**i** which were cyclized under basic conditions to thiazolopyridinones **8a**, **8c**–**e**, **9a**–**b**, and **10a**–**c** (see Chart 1).

Keywords: HIV-integrase; Strand-transfer inhibition.
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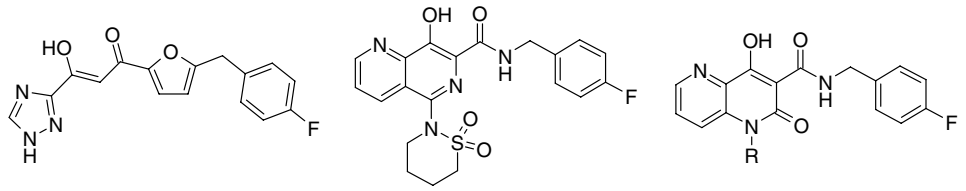

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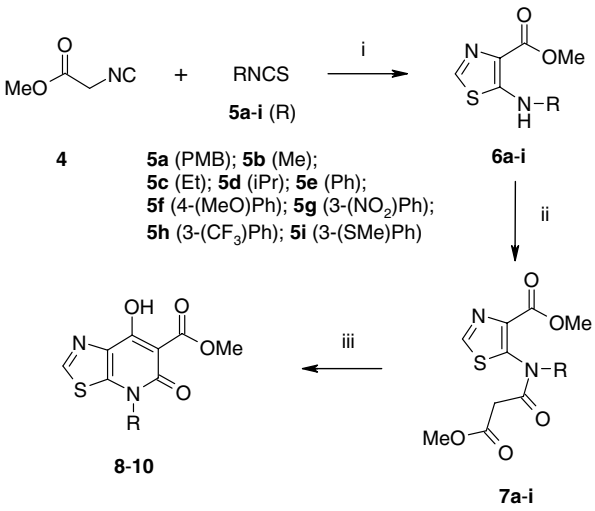
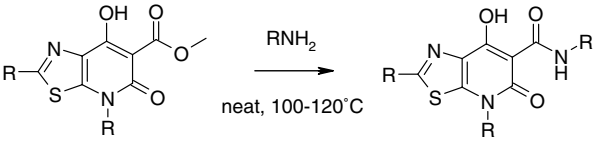


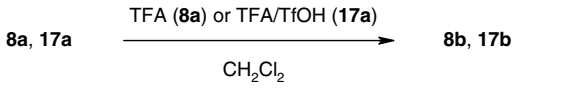
Scheme 1. Synthetic route to compounds **8a**, **8c**–**e**, **9a**,**b**, and **10a**–**c**. Reagents and conditions: (i) *t*-BuOK, THF; (ii) methyl malonyl chloride, DCE, reflux; (iii) NaOMe, MeOH.

Treatment of aminothiazoles **6b** and **6e** with NBS in acetonitrile provided 2-bromothiazoles **11a**,**b**, respectively (Scheme 2). Amino group protection of **11a**,**b** with $(Boc)_2O$/DMAP produced N-Boc derivatives **12a**,**b**. Bromine–magnesium exchange[10] of **12a**,**b** with isopropyl magnesium bromide followed by reaction with 4-fluorobenzaldehyde afforded intermediate alcohols **13a**,**b** which were N-deprotected and reduced to 5-aminothiazoles **14a**,**b** in one step with TFA/triethylsilane.[11] Reaction of **14a**,**b** with malonyl chloride and treatment of the resulting malonyl amides **15a**,**b** with NaOMe/MeOH afforded the thiazolopyridinones **16a**,**b**.

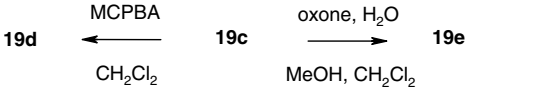
Equations 1-3. Synthesis of Compounds **17-20**



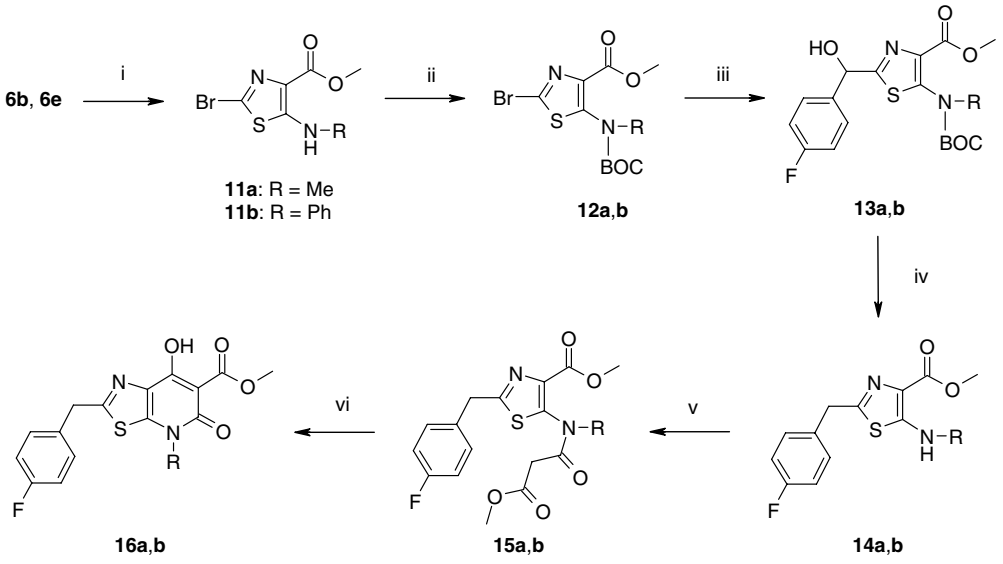
(1)



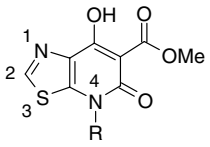
(2)



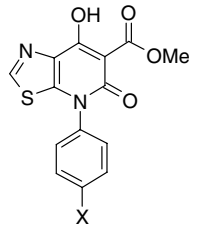
(3)



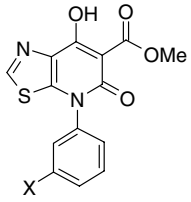
Scheme 2. Synthetic route to compounds **16a**,**b**. Reagents and conditions: (i) NBS, CH_3CN; (ii) $(Boc)_2O$, DMAP, THF, reflux; (iii) *i*-PrMgBr, 4-fluorobenzaldehyde, THF; (iv) Et_3SiH, TFA, CH_2Cl_2; (v) methyl malonyl chloride, DCE, reflux; (vi) NaOMe, MeOH.



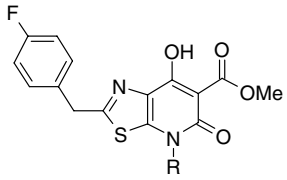
8a: R = PMB
8b: R = H
8c: R = Me
8d: R = Et
8e: R = i-Pr



9a: X = H
9b: X = OMe



10a: X = NO_2; R = H
10b: X = CF_3; R = H
10c: X = SMe; R = H



16a: R = Me
16b: R = Ph

Chart 1.

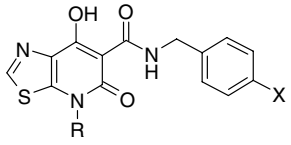
Methyl esters **8**–**10** and **16** were converted to carboxamides **17**–**20** by reaction with the appropriate amines under neat thermal conditions (Eq. 1). Reaction of the *p*-methoxybenzyl (PMB) analogs **8a** and **17a** with TFA or TFA/triflic acid afforded compounds **8b** and **17b**, respectively (Eq. 2). Oxidation of *m*-thioanisole analog **19c** with MCPBA or oxone gave the corresponding sulfone (**19d**) and sulfoxide (**19e**) products, respectively (Eq. 3).

Methyl esters **8**–**10** and **16** were significantly less active in the strand transfer assay[12] (Table 1) compared with S-1360 (**1**) (IC_{50} = 0.16 μM); esters containing substituted (**8a**), linear (**8d**) or branched (**8e**) N-4 alkyl groups showed weak inhibition activity (IC_{50} = 4–11 μM); N-4 aryl derivatives (**9a**,**b** and **10a**–**c**) were inactive. Interestingly, incorporation of a *p*-fluorobenzyl moiety at C-2 in the ester series (**16a**,**b**) enhanced inhibition activity compared to **8c** and **9a**.

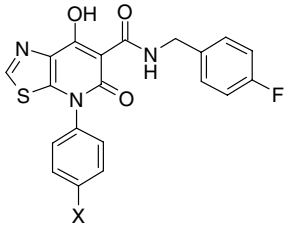
Conversion of carboxylic esters **8**–**10** and **16** to carboxamides **17**–**20** improved inhibition activity with the exception of **8a** and **16a**. In these latter cases, conversion to the corresponding *p*-fluorobenzylcarboxamides produced compounds of similar (**17a**) or lesser (**20a**) potency. Carboxamides having N-4 ethyl (**17d**) and N-4 isopropyl (**17e**) substitution were the most potent analogs (IC_{50} = 0.03–0.19 μM). Replacing the fluorine atom of **17c** with chlorine (**17f**) produced a 3-fold drop in strand transfer inhibition activity.

In the N-4 aryl series (**18**–**19**), *meta*-phenyl substitution (**19a**–**e**) reduced strand transfer inhibition activity compared to the unsubstituted phenyl compound **18a**. Oxidation of thioanisole derivative **19c** to the corresponding sulfone (**19d**) and sulfoxide (**19e**) improved potency by less than an order of magnitude. Interestingly, none of the title compounds showed antiviral activity separate from cellular toxicity; this observation is unusual considering that many potent strand transfer inhibitors have submicromolar cellular potency values.[13]

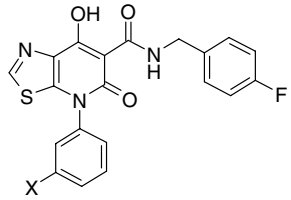
In summary, an efficient synthesis of thiazolo[5,4-*b*]pyridin-5(4*H*)-one HIV integrase strand transfer inhibitors was presented. The methodology described herein



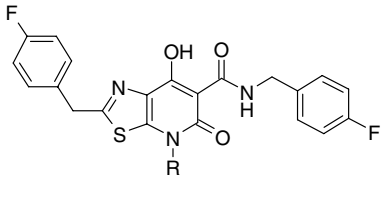
17a: R = PMB; X = F
17b: R = H; X = F
17c: R = Me; X = F
17d: R = Et; X = F
17e: R = i-Pr; X = F
17f: R = Me; X = Cl



18a: X = H
18b: X = OMe



19a: X = NO_2
19b: X = CF_3
19c: X = SMe
19d: X = SO_2Me
19e: X = S(O)Me



20a: R = Me
20b: R = Ph

Chart 2.

Table 1. HIV integrase strand transfer inhibition activity of methyl 7-hydroxy[1,3]thiazolo[5,4-*b*]pyridin-5(4*H*)-one-6-carboxylates **8–10**, and **16**[a]

Compound	N[b]	IC_{50}[c,d,e] (μM)
8a	2	4.2 ± 3.6
8b	1	>500
8c	1	>500
8d	1	1.23
8e	1	11
9a	1	>500
9b	1	>500
10a	1	>500
10b	1	>500
10c	1	>500
16a	1	35
16b	1	282

[a] See Chart 1 for molecular formulas.
[b] N = number of experiments.
[c] Data are expressed as means ± SE ($N > 1$).
[d] IC_{50} data for S-1360 (**1**) = 0.16 ± 0.04 μM ($N = 33$).
[e] See Ref. 12 for assay conditions.

Table 2. HIV integrase strand transfer inhibition activity of 7-hydroxy[1,3]thiazolo[5,4-*b*]pyridin-5(4*H*)-one-6-carboxamides **17–20**[a]

Compound	N[b]	IC_{50}[c,d,e] (μM)
17a	2	4 ± 2
17b	2	1.5 ± 0.1
17c	2	0.7 ± 0.3
17d	1	0.03
17e	2	0.19 ± 0.03
17f	2	2.1 ± 0.1
18a	2	0.4 ± 0.1
18b	1	1
19a	2	17 ± 3
19b	1	7
19c	2	7 ± 3
19d	2	0.93 ± 0.04
19e	2	2.0 ± 0.2
20a	1	151
20b	2	4 ± 2

[a] See Chart 2 for molecular formulas.
[b] N = number of experiments.
[c] Data are expressed as means ± SE ($N > 1$).
[d] IC_{50} data for S-1360 (**1**) = 0.16 ± 0.04 μM ($N = 33$).
[e] See Ref. 12 for assay conditions.

affords rapid access to diverse analogs in this series and provides a general perspective on their structure–activity relationships. Potent enzyme inhibition of HIV integrase was achieved with several C-6 carboxamide-containing derivatives which were generally more active than their ester counterparts. Within the carboxamide series, simple N-4 alkyl substituents showed greater potency with IC_{50} values as low as 0.03 μM. These findings contribute to the growing understanding of SAR in the field of 2-metal-binding HIV integrase inhibitors.

Acknowledgment

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References and notes

1. Richman, D. D. *Nature* **2001**, *410*, 995.
2. Pommier, Y.; Johnson, A. A.; Marchand, C. *Nat. Rev. Drug Disc.* **2005**, *4*, 236.
3. Benard, C.; Zouhiri, F.; Normand-Bayle, M.; Danet, M.; Desmaele, D.; Leh, H.; Mouscadet, J.-F.; Mbemba, G.; Thomas, C.-M.; Bonnenfant, S.; Bret, M. L.; d'Angelo, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2473.
4. Guochen, C.; Neamati, N.; Nair, V. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4815.
5. Fujishita, T.; Yoshinaga, T.; Sato, A. WO00/39086 A1, 2000; *Chem Abstr.* **2000**, *133*, 89529.
6. Hazuda, D. J.; Anthony, N. J.; Gomez, R. P.; Jolly, S. M.; Wai, J. S.; Zhuang, L.; Fisher, T. E.; Embrey, 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.; Holloway, M. K.; Young, S. D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11233.
7. Egbertson, M.; Melamed, J. Y.; Langford, H. M.; Young, S. D. WO03/062204 A1 2003; *Chem. Abstr.* **2003**, *139*, 133554.
8. Kiyama, R.; Kawasuji, T. WO01/95905 A1 2001; *Chem. Abstr.* **2001**, *136*, 53570.
9. Suzuki, M.; Moriya, T.; Matsumoto, K.; Miyoshi, M. *Synthesis* **1982**, 874.
10. Abarbri, M.; Thibonnet, J.; Berillon, L.; Dehmel, F.; Rottlander, M.; Knochel, P. *J. Org. Chem.* **2000**, *65*, 4618.
11. Carey, F. A.; Tremper, H. S. *J. Am. Chem. Soc.* **1969**, *91*, 2967.
12. Compounds were tested as inhibitors of recombinant HIV integrase in the following in vitro strand transfer assay. A complex of integrase and biotinylated donor DNA–streptavidin-coated SPA beads was formed by incubating 2 μM recombinant integrase with 0.66 μM biotinylated donor DNA–4 mg/ml streptavidin-coated SPA beads in 25 mM sodium MOPS, pH 7.2, 23 mM NaCl, 10 mM $MgCl_2$, and 10 mM dithiothreitol, and 10% DMSO for 5 min at 37 °C. Beads were pelleted by centrifugation, supernatant removed, and then beads resuspended in 25 mM sodium MOPS, pH 7.2, 23 mM NaCl, and 10 mM $MgCl_2$. Beads were again spun down, supernatant removed, and then beads resuspended in a volume of 25 mM sodium MOPS, pH 7.2, 23 mM NaCl, 10 mM $MgCl_2$ that would give 570 nM integrase (assuming all integrase bound the DNA-beads). Test compounds dissolved and diluted in DMSO were added to the integrase–DNA complex to give 6.7% DMSO (typically 1 μl of compound added to 14 μl of integrase complex), and preincubated for 60 min at 37 °C. Then [^{3}H] target DNA substrate was added to give a final concentration of 7 nM substrate, and the strand transfer reaction mixture was incubated at 37 °C typically for 25–45 min which allowed for a linear increase in covalent attachment of the donor DNA to the radiolabeled target DNA. A 20 μl reaction was quenched by adding 60 μl of the following: 50 mM sodium EDTA pH 8, 25 mM sodium MOPS, pH 7.2, 0.1 mg/ml salmon testes DNA, and 500 mM NaCl. Streptavidin-coated SPA were from GE Healthcare, oligos to make the donor DNA were from Oligos Etc, and [^{3}H] target DNA was a custom synthesis from Perkin Elmer. Sequences of donor and target DNA were previously described (Hazuda, D. J.; Hastings, J. C.; Wolfe, A. L.; Emini, E. A. *Nucleic Acid Res.* **1994**, *22*, 1121) with the addition of seven terminal A's on each end of the target DNA that allowed for the

incorporation of 14 tritiated T's (specific activity of target DNA approximately 1300 Ci/mmol). The K_m of substrate target DNA and IC_{50} of standard inhibitors were checked with this percentage of DMSO (6.7%) vs no DMSO and no difference was observed (with potencies in very good agreement with literature values if applicable).

13. Young, S. D. *Annu. Rep. Med. Chem.* **2003**, *38*, 173.