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BIOLOGICALLY-VALIDATED HIV INTEGRASE INHIBITORS WITH NUCLEOBASE SCAFFOLDS: STRUCTURE, SYNTHESIS, CHEMICAL BIOLOGY, MOLECULAR MODELING, AND ANTIVIRAL ACTIVITY

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□ *Integrase, an enzyme of the* pol *gene of HIV, is a significant viral target for the discovery of anti-HIV agents. In this presentation, we report on the continuation of our work on the discovery of diketo acids, constructed on nucleobase scaffolds, that are inhibitors of HIV integrase. An example of our synthetic approach to inhibitors with purine nucleobase scaffolds is given. Comparison is made between integrase inhibition data arising from compounds with pyrimidine versus purine nucleobase scaffold. Antiviral results are cited.*

Keywords Integrase; nucleobase scaffold; inhibitors; anti-HIV

INTRODUCTION

HIV-1 integrase is a 32 kDa protein encoded at the 3′-end of the HIV *pol* gene and is responsible for the integration of HIV DNA into host chromosomal DNA.[1–3] Prior to the initiation of integration, there is assembly of viral DNA, previously produced by reverse transcription, on HIV integrase. Following this assembly, endonucleatic cleavage of two nucleotides from each 3′-end of double-stranded viral DNA (3′-processing) produces tailored viral DNA recessed by two nucleotides. In the next step, which occurs in the nucleus and is identified as strand transfer, there is staggered nicking of chromosomal DNA and joining of each 3′-end of the recessed viral DNA to the 5′-ends of the host DNA. The strand transfer step, occurring in the nucleus, is partitioned from 3′-processing and is carried out after transport of the processed, preintegration complex from the cytoplasm into the nucleus. While a number of structurally diverse compounds have been reported to be inhibitors of HIV integrase, only some compounds of one group, the

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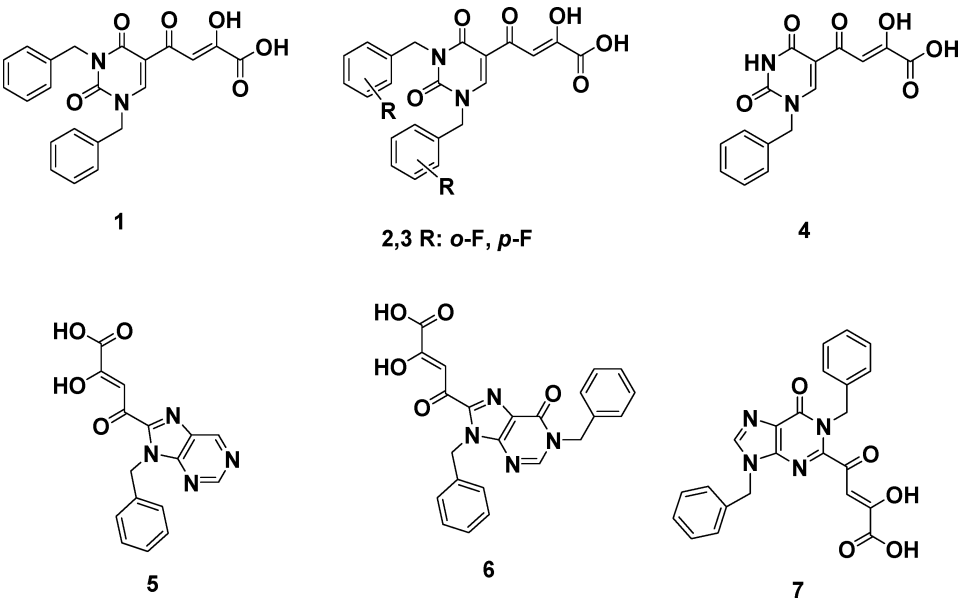
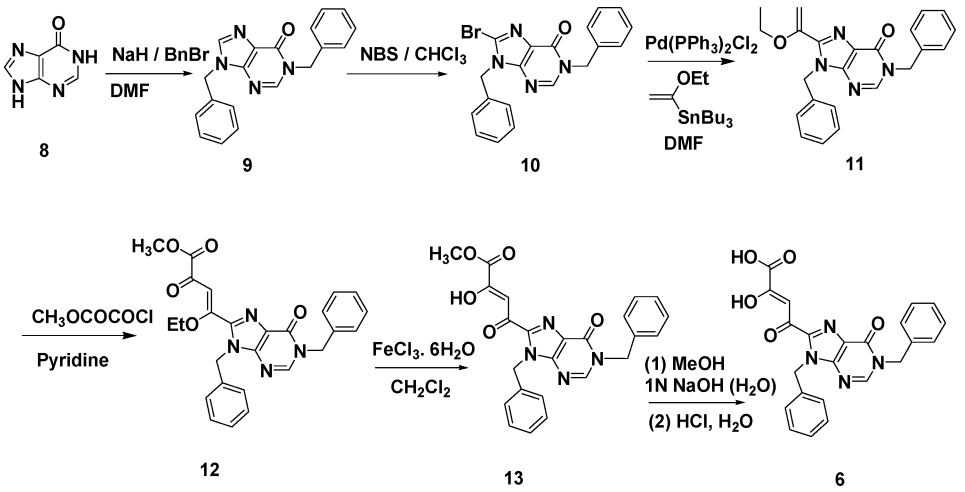


FIGURE 1 Structures of representative diketo acids.

β-diketo acids and their related systems,[4–10] represent the most convincing, biologically-validated inhibitors of this viral enzyme. In this report, we disclose the synthesis and inhibition data of β-diketo acids with purine nucleobase scaffolds (Figure 1) that show selectivity of inhibition of the strand transfer step of HIV-1 integrase. Comparison is made between integrase inhibition data arising from compounds with purine versus pyrimidine nucleobase scaffold (Figure 1). Relevant in vitro antiviral results are cited.

RESULTS AND DISCUSSION

A representative example of the synthesis of a diketo acid with a purine scaffold is illustrated in Scheme 1. Hypoxanthine (**8**), the starting material, was benzylated with NaH/benzyl bromide and then converted to **10** utilizing a free-radical bromination with N-bromosuccinimide (NBS). Palladium-catalyzed cross-coupling with ethoxyvinyl tributylstannane gave **11**. An addition/ elimination reaction of **11** with methyl chlorooxalate and pyridine furnished **12**, which was deprotected with ferric chloride hexahydrate to give ketoester **13**. Final deprotection of the latter in methanol with sodium hydroxide followed by acid work-up resulted in the target molecule **6**. Integrase inhibition studies were conducted with recombinant wild-type HIV-1 integrase and a 21-mer oligonucleotide substrate.[7,9,10] The data are summarized in Table 1. Compounds **6** and **7**, with the β-keto acid functionality at the 8- and 2-positions, respectively, were strong inhibitors of the strand



SCHEME 1 Synthetic methodology for the preparation of a purine diketo acid.

transfer step of HIV-1 integrase. However, both compounds showed much lower inhibition of the 3′-processing step of HIV integrase action. In sharp contrast, β-diketo acids with pyrimidine nucleobase scaffolds (**1–3**) are potent inhibitors of both the 3′-processing and strand transfer steps. The reason for this difference is not entirely clear. However, our molecular modeling data reveal that the regiochemical arrangement and preferred conformation of the β-diketo acids with pyrimidine nucleobase scaffolds allow for more effective overlap of these diketo acids with both the 3′-processing and strand transfer pockets within the catalytic site.

These compounds were evaluated for anti-HIV activity in a PBMC cell-based, microtiter anti-HIV assay against the clinical isolate, HIV-1_{TEKI} (NSI phenotype), and HIV-$1_{NL4\text{-}3}$ (SI phenotype).[7] Compounds **1-3** exhibited potent to highly potent in vitro anti-HIV activity. However, the purine-based compounds, **6** and **7**, while showing strong inhibition of the strand transfer step of HIV integrase, exhibited low anti-HIV activity. Investigations of

TABLE 1 Data summarizing the inhibition of wild type HIV-1 integrase by inhibitors **1–7**

Inhibitors	3′-processing (μM)	Strand transfer (μM)
1	3.7	0.2
2	4.1	<0.6
3	3.9	<0.7
4	10.0	0.5
5	100	10.0
6	31.5	4.1
7	30.0	2.7

other integrase inhibitors related to the anti-HIV active compounds are in progress.

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