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Triketoacid inhibitors of HIV-integrase: A new chemotype useful for probing the integrase pharmacophore

Michael A. Walker,[a,*] Timothy Johnson,[a] Zhuping Ma,[a] Jacques Banville,[c] Roger Remillard,[c] Oak Kim,[a] Yunhui Zhang,[a] Andrew Staab,[a] Henry Wong,[a] Albert Torri,[b] Himadri Samanta,[b] Zeyu Lin,[b] Carol Deminie,[b] Brian Terry,[b] Mark Krystal[b] and Nicholas Meanwell[a]

[a]*Department of Medicinal Chemistry, Pharmaceutical Research Institute, Bristol-Myers Squibb, The Richard L Gelb Center for Pharmaceutical Research and Development, 5 Research Parkway Wallingford, CT 06492, USA*
[b]*Department of Virology, Pharmaceutical Research Institute, Bristol-Myers Squibb, The Richard L Gelb Center for Pharmaceutical Research and Development, 5 Research Parkway Wallingford, CT 06492, USA*
[c]*Department of Medicinal Chemistry, Pharmaceutical Research Institute, 100 de l'Industrie Boulevard, Que., Canada J5R1J1*

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Abstract—Integrase is one of three enzymes expressed by HIV and represents a validated target for therapy. This study reports on the discovery of a new triketoacid-based chemotype that selectively inhibits the strand transfer reaction of HIV-integrase. SAR studies showed that the template binds to integrase in a manner similar to the diketoacid-based inhibitors. Moreover, comparison of the new chemotype to two different diketoacid templates led us to propose two aryl-binding domains in the inhibitor binding site. This information was used to design a new diketoacid template with improved activity against the enzyme.
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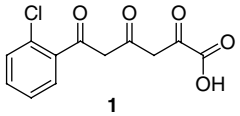
HIV-integrase is the viral enzyme responsible for inserting a DNA-copy of the virus into the host chromosome. Without this occurring, the virus cannot replicate, making integrase an attractive target for the development of novel anti-HIV agents.[1] However, the discovery of suitable drug candidates targeting HIV-integrase has proven to be a formidable task. A number of drug discovery campaigns have been launched over the last 15 years, and it is only recently that compounds suitable for development have been described.[2]

HIV-integrase catalyzes two reactions. In the first, referred to as 3′-processing, the enzyme cleaves two base pairs from the 3′-ends of the transcribed viral DNA (vDNA), leaving 3′-CA dinucleotides at each end. The second reaction occurs in the nucleus, wherein the 3′-ends of the vDNA are inserted into the host DNA. This step is referred to as strand transfer and is currently the preferred target for the development of inhibitors. It is believed that this reaction occurs via a Mg^{2+}-mediated phosphate ester cleavage involving the 3′-hydroxyl group of the vDNA. According to this mechanism, the 3′-hydroxyl group of the incoming vDNA attacks the phosphate ester of the host DNA-strand to form a pentavalent intermediate.[3] Two Mg^{+2} ions facilitate the flow of negative charge from the deprotonated 3′-hydroxyl of the viral strand to that of the leaving 3′-hydroxyl of the host strand. This mechanism is similar to that invoked for structurally related enzymes such as RNAse H, *Escherichia coli* exonuclease, MuA-transposase, Tn5 transposase, and the RuvC-Holliday junction resolvase.[4] An analogous pentacovalent phosphorus intermediate has been observed in the X-ray structure of β-phosphoglucomutase.[5]



1
IC_{50} = 1 μM
EC_{50} = 120 μM

Recent efforts from our labs have uncovered an authentic lead exemplified by compound **1** bearing a triketoacid structure.[6] Compound **1** is a moderately potent inhibitor

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* Corresponding author. Tel.: +1 203 677 6686; e-mail: walkerma@bms.com

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of the isolated enzyme (1 μM)[7] and of HIV-1 in cell culture. Additionally, the compound is able to inhibit integrase in the context of the pre-integration complex (PIC) (IC_{50} = 0.6 μM) isolated from infected cells. In counter screens, it did not inhibit other viral or DNA-processing enzymes (HIV-RT, HIV-protease, RNase H, BVDV-polymerase, T3-polymerase, and Klenow fragment) and was devoid of activity against other viruses (BVDV, RSV, Flu, and Rhinovirus). Time-of-addition experiments showed that antiviral activity coincided with the viral integration step.

In order to confirm that integrase is the target, multiple populations of the virus were maintained in the presence of **1**. Serial passage of virus in the presence of increasing amounts of inhibitor yielded two isolates with reduced susceptibility, R1 and R2, after 47 passages. As shown in Table 1, the two resistant isolates exhibited a >3-fold decrease in susceptibility to compound. Sequence analysis indicated two unique sets of mutations for each resistant strain (R1 = T66I and L172F, R2 = V151A and V165I), located in the integrase enzyme. These residues are found in the core domain of the enzyme and are close to the active site catalytic DDE triad (Asp64, Asp116, and Glu152). In subsequent experiments, the T66I mutation was introduced into the HIV-NL_{4-3} (HIV-1) strain which resulted in resistance to **1** in cell culture. Similar results have been reported for the diketoacid-based inhibitor, L-731,988, where the T66I, S153Y, and M154L amino acid changes were observed to confer resistance.[8] Recombinant bacterially expressed integrase containing the T66I and M154I substitutions also exhibited decreased sensitivity to **1** (not shown). Thus, the results clearly show that the triketoacid inhibitor **1** targets integrase.

Table 1. Activity of **1** against wild-type (HIV-1) and resistant virus

strain	EC_{50} (μM)
HIV-NL_{3-4}	55
HIV-R1	>200
HIV-R2	>200
HIV-NL_{3-4} (T66I)	>200

Published studies have concluded that the diketoacid inhibitors bind to the two catalytic Mg^{2+} atoms located in the active site, as depicted in Figure 1.[9] In contrast to L-731,988, compound **1** offers the potential for two different modes of binding to Mg^{2+}, illustrated by model A and model B in Figure 1. A brief SAR investigation was conducted in an effort to resolve this ambiguity. L-731,988 is very sensitive to modifications to the Mg^{2+}-binding elements, as shown for compound **2**, where the C-terminal carboxylic acid has been replaced by a tetrazole. Even though the tetrazole moiety is well established as a carboxylic acid mimic, in this setting activity is severely compromised. The triketoacid **1** behaves in a similar fashion, since compound **3** is essentially inactive. However, the activity of **1** is only moderately affected by removal of the δ-carbonyl, since compound **4** is only an order of magnitude weaker. Therefore, since the SAR for the Mg^{2+}-binding domain of **1** matches that of L-731988, it is likely that the triketoacid binds to integrase in the manner depicted by model B.

Next, the SAR related to the phenyl ring was evaluated and the results of that survey are shown in Figure 2. Using the unsubstituted parent **5** as a reference point, it is clear that the introduction of an electron-withdrawing group at the *ortho*- (e.g., **6**–**9**) or *meta*- (e.g., **10**) position enhances in vitro activity. The phenyl ring can be replaced by pyridine, but its activity is sensitive to regiochemistry with the more exposed 3-isomer **12** weaker than the 2-isomer **11**. The improved activity observed for **1** and **6**–**9**, compared to **11** and **12**, suggests that a hydrophobic binding site exists in the region between the *ortho*- and *meta*-sites. Compound **13**, wherein this region is occupied by a fused phenyl ring, was designed to test this concept. In accordance with the hypothesis, **13** showed a noticeable improvement in inhibitory activity. In contrast, compounds **14** and **15**, which also have

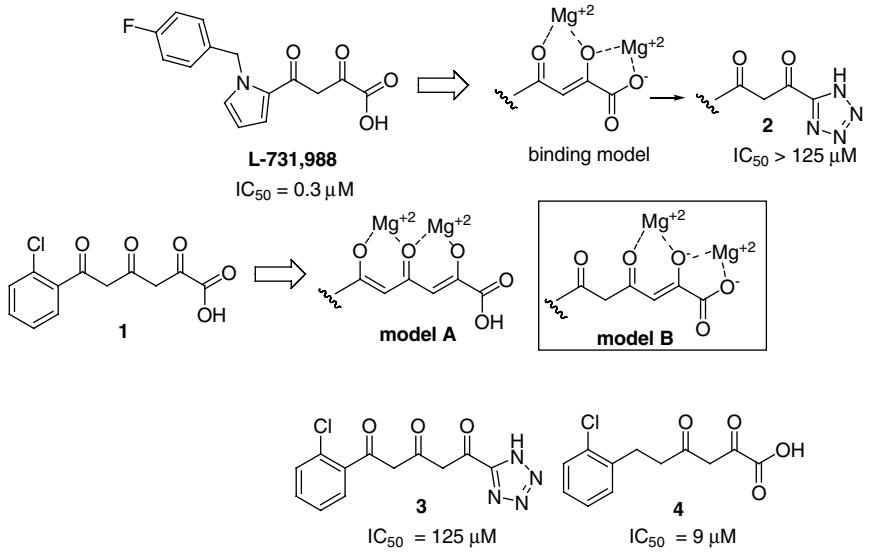
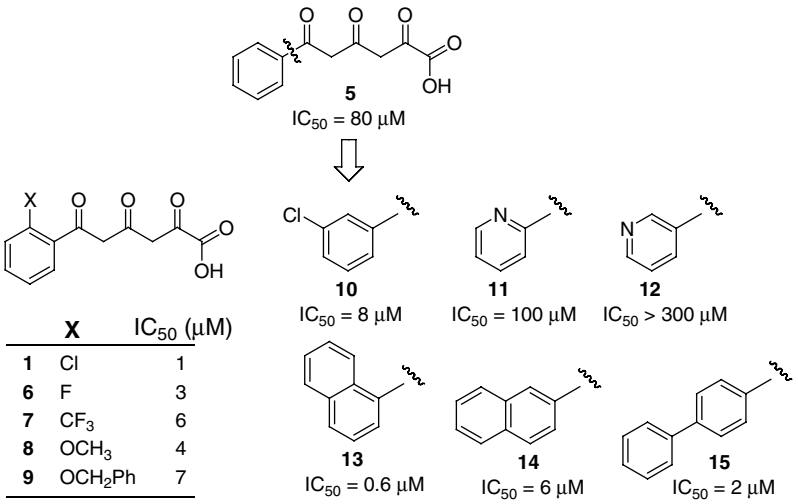


Figure 1. Binding models for L-731,988 and compound **1**. In vitro inhibitory activities (reference 7) for each compound are indicated.



	X	IC_{50} (μM)
1	Cl	1
6	F	3
7	CF_3	6
8	OCH_3	4
9	OCH_2Ph	7

Figure 2. SAR around the aryl group of the triketoacid chemotype.

an additional phenyl ring, do not demonstrate increased potency, indicating that the improved activity seen with **13** is not simply due to non-specific, hydrophobic binding. This suggests a specific aryl-binding domain exists within the integrase enzyme that is capable of distinguishing subtle differences in structure.

The SAR survey was further expanded in order to map the binding site occupied by the aryl portion of the triketoacid template. It was assumed that the fully enolized form of the triketoacid was more consistent with the SAR than the keto form. As such, there are two possible configurations for the enol bond, *Z* and *E*. In order to help delineate this issue, diketoacids L-731,988 and **16** were used as model templates. Since L-731,988 was specifically designed to take full advantage of the interaction of the aryl group with integrase, it represents a compelling template for the purpose of comparison.[10] Compound **16** is also very potent and, as such, should be useful to compare to the triketoacid template Figure 3.

Despite the fact that L-731,988 and **16** are equipotent, they differ in the positioning of their aryl groups, with respect to the diketoacid template. This is illustrated in Figure 4, where it is observed that an overlay of the

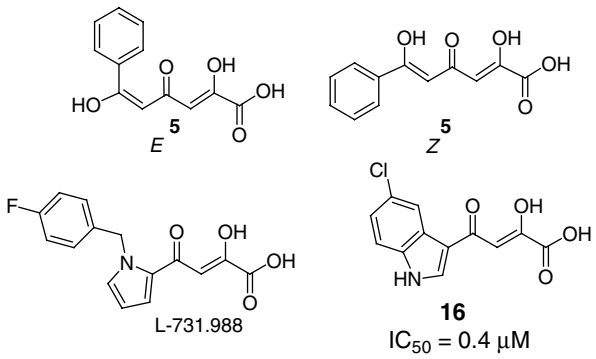


Figure 3. Possible enol configurations for **5**.

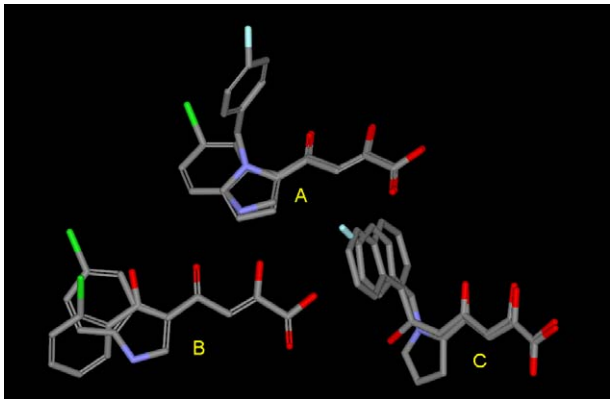
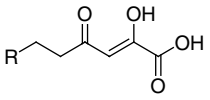
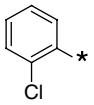
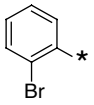
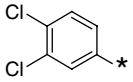


Figure 4. Overlay of L-731,988 and **16** (A). Overlay of compound *Z*-**5**l and **16** (B). Overlay of *E*-**13** and L-731,988 (C). Halogen atoms are not shown.

two yields a misalignment of the aryl groups.[11] This result can be interpreted as that there are two aryl-binding domains available in the active site of integrase. When the triketoacid template is compared to compound **16**, the *Z*-enol configuration was found to overlay better than the *E*. As seen in Figure 4, although the alignment of the aryl groups of **16** and **1** is not optimal, the *ortho* chloro group of the phenyl ring can project toward the fused phenyl ring of the indole, allowing it to occupy this region. This provides an explanation for the activity enhancing effect of the *ortho*-substituent. In contrast, when L-731,988 is used as a model, the *E*-enol provides a better overlay. Figure 4 shows an overlay of **13**, the most active triketoacid, and L-731,998, and here the aryl groups exhibit very good alignment. In addition, it is expected that the *ortho* or *meta* substituents of **1** and **6**–**10** would occupy this region. Although model compounds **16** and L-731,988 predict different binding geometries for the enol bond of the triketoacid, both allow reasonable interpretation of the SAR presented in Figure 2. More significantly, assuming two different aryl-binding domains exist in the enzyme active site, it is apparent that the triketoacid is able to access either one with a simple change of enol configuration.

Table 2. In vitro activity of diketoacid analogues



Compound	R	IC_{50} (μM)
17		30
4		26
18		5
19		0.01[a]

[a] Assay protocol different to that utilized for 4, 17, and 18 – see reference 12.

The final phase of the survey was to re-examine the effect of removing the enol group connecting the phenyl ring to the triketoacid moiety. Since the saturated derivative **4** is an effective inhibitor of integrase, compounds **17**–**19**, were synthesized and evaluated in vitro, as summarized in Table 2. As anticipated, this modification was well tolerated and aryl ring substitution resulted in increased potency. While the SAR trends are similar to that seen with the enol-containing series, there are some subtle differences. For example, an *ortho* Cl substituent is less effective than an *ortho* Br. The 3,4-dichloro analogue **19** is highly active, but it should be noted that the data reported for this compound were determined using a different assay than that used to assess the potency of compounds **4**, **17**, and **18**, preventing a direct comparison.[12] However, under the modified assay conditions, compounds **1**, L-731,988, and **16** had IC_{50}s = 0.2, 0.02, and 0.08 μM, respectively, indicating that **19** is indeed potent when compared to other diketoacid-based inhibitors.

In summary, we disclose the basic SAR associated with a new class of triketoacid-based inhibitors of HIV-integrase. Preliminary SAR studies indicate that this chemotype binds to integrase in a manner similar to the diketoacid-based inhibitors. Preferred substitution on the phenyl group was also studied and it was discovered that an appropriately positioned naphthyl group yielded optimal activity. Comparison of the triketoacids with two different diketoacid-based inhibitors provided some insight into the SAR and helped to establish a model for the binding conformation. The data are consistent with dual aryl-binding domains within the integrase protein. Using this information, we were able to modify the diketoacid template by removing the carbonyl group adjacent to the aryl group, which led to the discovery of **19** as a potent inhibitor of integrase.

Diketoacid-based inhibitors have proven to be very useful as a starting point for the design of clinically verified agents, thus the discovery of **1** represents a useful lead toward the goal of developing compounds for the treatment of HIV infection. The objective of the current investigation was to gain a better understanding of the tri/diketoacid inhibitor pharmacophore, in order to develop a more 'drug like' template. Toward that end, the current study provides information regarding the binding of the aryl domain of the diketoacid-based

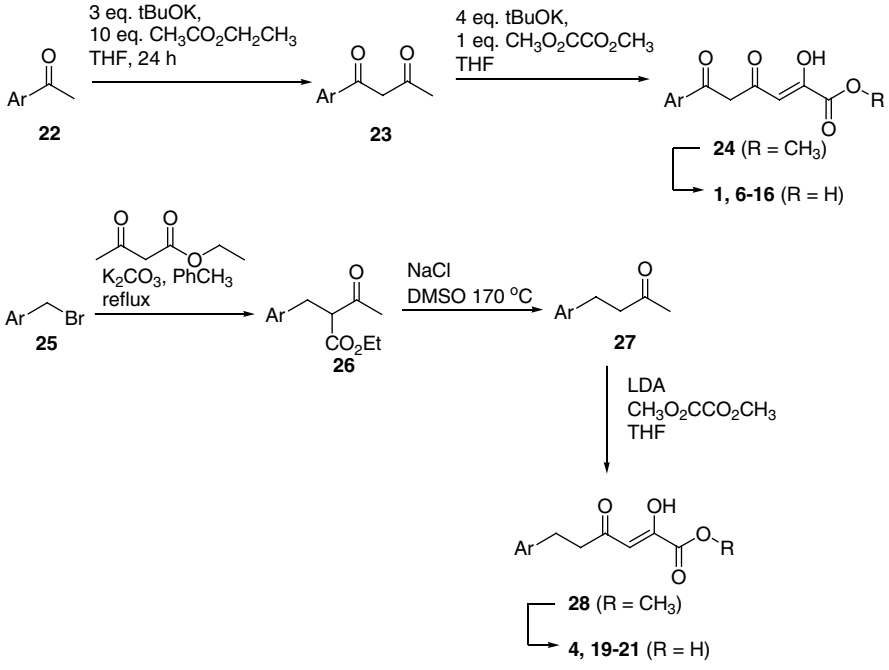


Figure 5. Synthesis of tri- and diketoacids.

inhibitors. Future publications will demonstrate how this information can be used to introduce structural modifications to this portion of the template.

The syntheses of the compounds described in this letter are illustrated in Figure 5. The triketoacids were prepared using a modified version of the method reported by Stiles, which is simply a series of two Claisen condensation reactions starting from the methyl-arylketone, ethylacetate, and dimethyl oxalate.[13] Compounds **4**, and **17**–**19**, were also synthesized using Claisen methodology as demonstrated.

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7. In vitro activity was measured in the following manner which is similar to previously disclosed methods. Purified recombinant HIV-1 integrase was incubated with immobilized precleaved substrate DNA in a 96-well plate for 20 min at 37 °C. After the integration complex was formed, compounds at desired concentrations were added to the wells followed by a 10 min incubation at 37 °C. Biotinylated target DNA was then added and the reaction was carried out for an additional 1 h at 37 °C. Wells were washed thoroughly to remove any free DNA and integration activity was measured by using a commercial kit to quantitate the amount of biotinylated target DNA integrated into the substrate.
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11. The overlaid structures were generated in the following manner: the equilibrium conformations of compounds were determined in Spartan (Wavefunction Inc.) using molecular mechanics (MMFF94 forcefield). The structures were then manually overlaid using the alignment feature in DSViewerPro (Accelrys Inc.).
12. To evaluate in vitro activity against HIV-integrase, 5 pmol of biotin labeled substrate DNA was bound to 100 μg of Streptavidin coated PVT SPA beads (Amersham Pharmacia Biotech). Recombinant integrase (0.26 ng) was incubated with the beads for 90 min at 37 °C. Unbound enzyme was removed by washing the complex followed by addition of inhibitors and 0.1 fmol of P33 labeled target DNA. The reaction was stopped by adding EDTA to a final concentration of 40 mM. Samples were counted in TopCountNXT (Packard) and the CPM was used as a measure of integration. The reaction conditions were as described in A. Engelman and R. Craigie, J. Virol. **1995**, *69*, 5908–5911. The sequences of substrate and target DNA were described in Nucleic Acid Research **1994**, *22*, 1121–1122.
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