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Exploration of the diketoacid integrase inhibitor chemotype leading to the discovery of the anilide-ketoacids chemotype

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Abstract—Integrase is one of three enzymes expressed by HIV and represents a validated target for therapy. A previous study of the diketoacid-based chemotype suggested that there are two aryl-binding domains on integrase. In this study, modifications to the indole-based diketoacid chemotype are explored. It is demonstrated that the indole group can be replaced with secondary but not tertiary (e.g., *N*-methyl) aniline-based amides without sacrificing in vitro inhibitory activity. The difference in activity between the secondary and tertiary amides is most likely due to the opposite conformational preferences of the amide bonds, *s-trans* for the secondary-amide and *s-cis* for the tertiary-amide. However, it was found that the conformational preference of the tertiary amide can be reversed by incorporating the amide nitrogen atom into an indoline heterocycle, resulting in very potent integrase inhibitors.

HIV-integrase is one of only three enzymes expressed by HIV and is responsible for inserting the viral DNA (vDNA) into the host genome. Without this occurring the virus cannot replicate.[1] There are a number of steps involved in this process. In the cytosol, intergrase trims the 3′-ends of both strands of the vDNA immediately downstream of a conserved CA dinucleotide, leaving a 2-base overhang at each 5′-end. The enzyme and processed vDNA are transported in to the nucleus where the two 3′-ends of the DNA are inserted into the host DNA, an event designated strand transfer. The subsequent steps are not well defined but include, pruning the 5′-overhangs on the vDNA and repairing the gaps created by the insertion reaction, processes thought to be completed by host cell enzymes. HIV genome integration yields a provirus that can go on to produce new virus or lie dormant in a latent state.

The discovery of clinically relevant inhibitors of HIV integrase for antiviral therapy has proven to be a challenging task despite the large amount of effort that has gone into it. After 15 years of research, only a small number of compounds have been reported to have been studied in clinical trials: S-1360 (Shionogi, GlaxoSmithKline)[2] and L-870810 and MK-0518 (Merck)[3,4] and GS-9137 (Japan Tobacco and Gilead).[5] Despite the elusiveness of the target, we and others have discovered that certain diketoacid-containing compounds, such as **1**, **2**, and **3**, are selective inhibitors of the strand transfer reaction mediated by HIV-integrase and are antiviral in cell culture.[6] In previous reports of these compounds it was found that the structural requirements around the diketoacid portion of the molecule were very strict, leading to the suggestion that this motif binds to the two active site Mg^{2+} ions.[7] The positioning of the aryl group with respect to the diketoacid was found to be sensitive to

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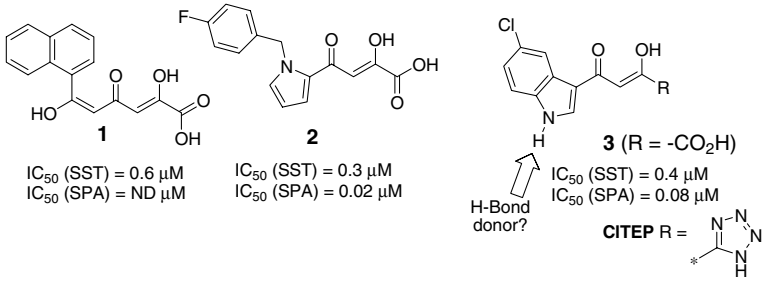


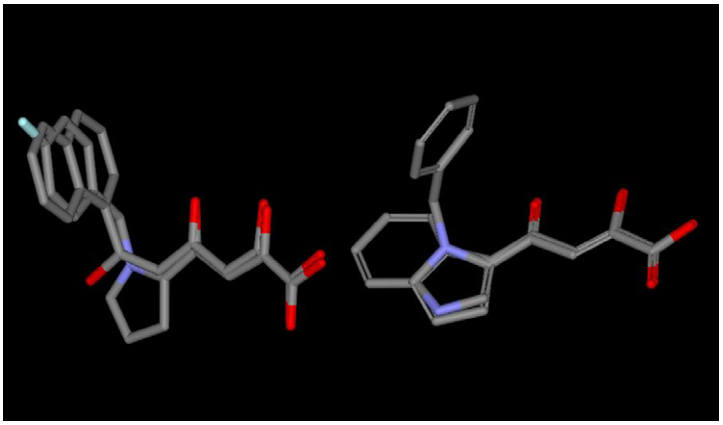


Figure 1. Diketoacid-based inhibitors of HIV-integrase. Overlay of **1** and **2** (left). Overlay of **2** and **3** (right).

modification, indicating a discrete interaction with the enzyme.[8] As shown in Figure 1, superposition of **1** and **2** yields a very good overlay of the naphthyl group with the 4-fluoro-phenyl moiety, suggesting that these compounds can bind to the same aryl-binding domain.[9] In contrast, **2** and **3** are nearly equipotent, despite the fact that they differ in the positioning of their aryl groups, leading to the proposal of two distinct aryl-binding domains. The topology of the triketoacid chemotype **1** appears to better mimic the template represented by **2** rather than that embodied by **3**. Consequently we became interested in designing a template which would better mimic the indole-based template. This would potentially provide an inhibitor with a different resistance profile or allow the design of a compound which could simultaneously interact with both sites.

Despite their structural differences, all three compounds can be described by the generic chemotype shown in Figure 2. Viewed in this way, the main difference between the compounds is the identity of the linker moiety designated X which controls the position of the aryl element with respect to the diketoacid group. As the first step in deconstructing **3**, the pyrrole heterocycle was excised producing compound **4**. Surprisingly, this structural modification resulted in a dramatic reduction in inhibitory potency. In contrast, the anilide derivative, **5**, was found to be a moderately potent inhibitor of integrase-mediated strand transfer. This result is of interest since both **4** and **5** have the same topology as **3** with respect to the relationship between the aryl ring and diketoacid moiety but only **5** retains activity. This suggests that an additional factor in addition to the aryl group and Mg^{2+}-binding moiety is involved in recognition of the diketoacid template by integrase. Of particular interest was a report of a single crystal X-ray structure of the indole-based integrase inhibitor, 5ClTEP, bound to the catalytic core of the enzyme and engaged in a H-bonding interaction between the indole-NH and the enzyme.[10]

In an effort to determine if the –NH group of **3** contributed to binding through the formation of a hydrogen bond to the enzyme, the SAR survey summarized in Figure 3 was conducted. In vitro evaluation of compounds **6** and **7** appeared to confirm that a H-bond donor such as the –NH presented by the anilide contributed to enzyme recognition, since removing it either by methylation (**6**) or incorporation into a ring system (**7**) resulted in a significant loss in activity. Despite these observations, the cyclic amide **8** displays very good in vitro inhibitory activity, a particularly interesting result since it contradicts the suggestion that a H-bond donor is an essential component of the pharmacophore.

This led to a reconsideration of the role of the amide group and attention was focused on conformational aspects of this structural element. Summarized in Figure 3, it is hypothesized that the non-methylated anilide-ketoacid prefers the lower energy, *s-trans* amide conformation (A in Fig. 3) when bound to the enzyme which is consistent with the preferred conformation of **3**. Molecular mechanics calculations[11] were performed which showed that for compound **6** conformation A is slightly higher in energy than the corresponding *s-cis* conformation, depicted as B, presumably because the *N*-methyl group suffers from an unfavorable 1,5-steric interaction with the enol C–H. Thus, for the *N*-methyl anilide derivative conformation B is favored, which directs the

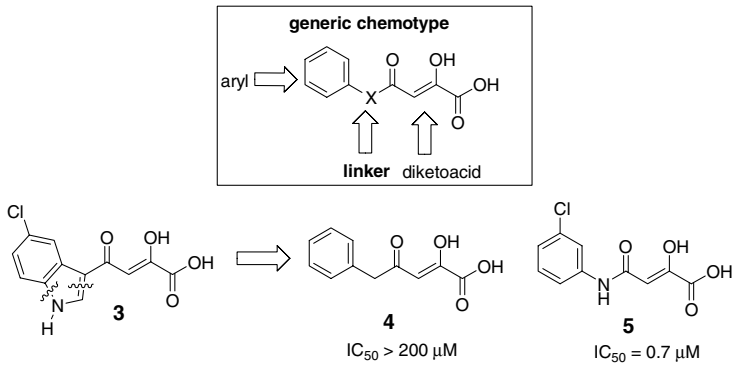


Figure 2. Initial discovery of the anilide-ketoacid chemotype.

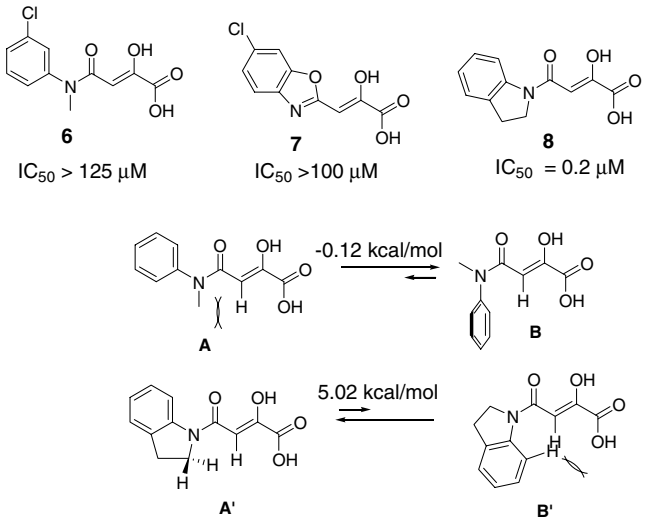
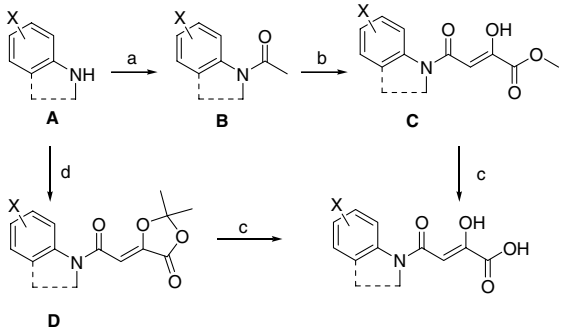


Figure 3. Initial SAR study around anilide chemotype.

phenyl group away from the aryl-binding domain of the enzyme. With respect to the indoline **8**, cyclization pulls the *N*-methylene moiety toward the phenyl ring and directs the methylene protons out of the plane of the enol C–H, reducing the unfavorable 1,3-steric interaction. At the same time, a severely unfavorable interaction is introduced into conformation B′, since the additional ring locks the phenyl group into a planar alignment with the enol moiety. Thus, conformation A′ is favored, providing an explanation for the observed inhibitory activity.

The synthesis of the amide-ketoacid template is straightforward and can be accomplished by either of two routes, as illustrated in Scheme 1. In the first sequence, aniline or indoline is acetylated under standard conditions to afford intermediate **B** which can be readily condensed with dimethyloxoalate using LiHMDS as the base. The resulting ester **C** is hydrolyzed with aqueous base to afford the corresponding acid. An alternate synthetic pathway is demonstrated in which the diketo acid moiety is introduced directly in a protected form to afford **D**.[12] The acetonide group of **D** is readily removed under aqueous alkaline conditions.

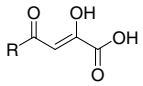


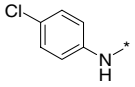
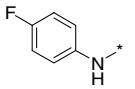
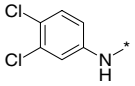
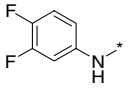
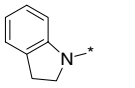
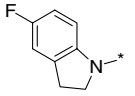
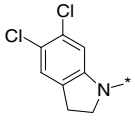
Scheme 1. Reagents: (a) CH_3COCl, *i*-Pr_2NEt, CH_2Cl_2; (b) dimethyloxalate, LiHMDS, THF (c) NaOH (d) C, *i*-Pr_2NEt, CH_2Cl_2.

The synthetic versatility associated with a simple amide moiety allowed a rapid examination of the SAR around this chemotype as illustrated in Table 1. The regiochemistry of substitution of the anilide ring appears to be a critical determinant of activity since the 4-substituted compounds **10** and **11** are an order of magnitude less active than the 3-chloro analogue **5**. Perhaps surprisingly, the dichlorinated derivative **12** is more active than **5**, both in vitro and in cell culture. However, this boost in activity shows some dependence on halogen identity since the difluoro analogue **13** is less active than **5** although introduction of the second fluorine atom (**11**) increases potency 5-fold. The conformational constraints imposed by cyclization to the indoline derivatives are associated with enhanced potency compared to the corresponding aniline. Of particular interest is compound **15**, which is equipotent to the highly active diketoacids **2** and **3**.

In conclusion, we have shown that the aniline- and indoline-derived amide-ketoacids can mimic the diketoacid-based inhibitors of HIV-integrase. The amide-ketoacid template is amenable to rapid exploration of the aryl-portion of the template and SAR studies have provided a more detailed understanding of the recognition requirements at this site of the pharmacophore. The results suggest that H-bond donation to the enzyme by the amide NH does not contribute to binding. In addition, the binding conformation of the aryl group can be rationalized to be in the *trans*-configura-

Table 1.



Compound	R	IC_{50} (μM)[a]
10		5.6
11		13.4
12		0.16
13		2.4
8		0.20
14		0.23
15		0.02

[a] In vitro inhibition of integrase catalyzed strand transfer.

tion and co-planar with the Mg^{2+} binding amide-keto-acid side chain.

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11. The equilibrium conformations of compounds were determined in Spartan (Wavefunction Inc.) using molecular mechanics (MMFF94 forcefield). The dihedral angle of the amide bond (O–C–N–C) was defined as either 180° or 0° in order to compare the *s-trans* and *s-cis* configurations.
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