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Benzyl amide-ketoacid inhibitors of HIV-integrase

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Abstract—Integrase is one of three enzymes expressed by HIV and represents a validated target for therapy. Previous reports have demonstrated that the diketoacid-based chemotype is a useful starting point for the design of inhibitors of this enzyme. In this study, one of the ketone groups is replaced by a benzylamide resulting in a new potent chemotype. A preliminary SAR study is carried out to investigate the substitution requirements on the phenyl ring and methylene group of the benzylamide.

The ability of the viral DNA of HIV to become incorporated into the genome of the host is a hallmark of retroviral infection. This event is necessary[1] and it ensures the indefinite survival of the virus in the host as long as the infected pool of cells remains alive. Integrase, which is coded for by the virus, is responsible for this event in the viral lifecycle. In the cytosol, intergrase trims the 3′-ends of both strands of the viral DNA (vDNA) immediately downstream of a conserved CA dinucleotide, leaving a 2-base overhang at each 5′-end. The enzyme and processed vDNA are transported to the nucleus where the two 3′-ends of the DNA are inserted into the host DNA, an event designated as 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 for two major reasons. First, distinguishing false leads from authentic leads was difficult under the early assay conditions. Second, the integrase-inhibitor pharmacophore appears to be under-represented in most compound collections. Recently, optimized assay conditions have been developed which are more selective for finding *bona fide* leads. This has led to the discovery of the diketoacid derived inhibitors.[2] These compounds selectively inhibit the strand transfer reaction of the enzyme. The mechanism of inhibition appears to involve direct binding of the compounds to the divalent metal (Mg^{2+}) co-factor located at the enzyme active site. Kinetic studies indicate that the inhibitors intercept the enzyme-vDNA complex formed after the 3′-processing reaction and prevent the binding of this complex to host DNA.

Evolution of this chemotype has yielded a number of compounds which have been examined in the clinic (see Fig. 1), including S-1360 (Shionogi, GlaxoSmithKline),[3] L-870810, and MK-0518 (Merck).[4,5] Figure 2 shows a generic diketoacid and the minimal pharmacophore-elements needed to bind to integrase. The diketoacid portion of the template is believed to bind to the Mg^{2+} co-factor located in the active site, while the aryl group participates in a specific interaction with an adjacent hydrophobic pocket or surface. SAR studies on the aryl group indicate that the structural elements of the enzyme which form this hydrophobic

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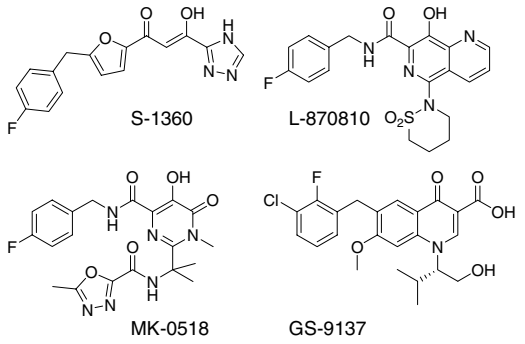


Figure 1. Clinically studied integrase inhibitors.

domain are well defined. This being the case, the linker group which couples the aryl group to the ketoacid is a critical feature in controlling activity by altering the position of the aryl group with respect to the Mg^{2+}-binding portion of the template.[6] Very recently another apparently new chemotype has emerged represented by the clinical compound GS-9137 (Fig. 1, Japan Tobacco and Gilead).[7]

The diketoacid based inhibitors have been under investigation in our laboratories, in particular the SAR associated with the linker group.[8] Our efforts in this area began with the characterization of diketoacid **1** and triketoacid **2**. Using these two compounds, we have previously shown that there are probably two different aryl binding sites located adjacent to the Mg^{2+} binding Asp, Asp, Glu (DDE)-triad. Access to these binding sites is controlled by the structure of the linker group, in the present case the pyrrole ring of **1** and the enol moiety of **2**. Of interest, deannulation of **1** to provide **3** leads to a complete loss in activity, but the anilide ketoacid derivative **4** displays very good inhibitory activity.[9] Based on this result it is tempting to speculate that an H-bond donating group is required for activity; however, our results suggest that the primary contribution of the amide bond is to serve as a conformational lock.

Since it is our contention that **1** and **2** possess different integrase-binding configurations it was of interest to see what effect the analogous modification would have on **2**. In contrast to the results obtained on going from **1** to **3**, the diketoacid derivative **5** retained activity, in the absence of the enol. Since it is logical to assume that the 2-carbon spacer group of **5** is associated with even a larger entropic penalty for binding compared to the single carbon linker of **3**, the difference in activity between these homologues suggests that the longer linker allows the phenyl group to reach a hydrophobic domain on the enzyme that is not accessible by the 1 carbon linker. Taking advantage of the improved activity associated with introducing a conformational lock into the ring via an amide bond the same approach was applied to **5**. The 1000-fold increase in activity for compound **6** relative to **5** provides support for this hypothesis.[10]

With this new chemotype in hand, a survey of the phenyl ring SAR was carried out to probe the nature of the hydrophobic pocket believed to be spatially distinct from that of the anilide series. While a report by Daeyaert et al.[11] reported the use of the benzylamide ketoacid template to develop a QSAR model for integrase, based on molecular properties such as sterics, polarizability, charge, H-bond acceptors, and donors, we were more interested in examining the SAR with a higher degree of precision, optimizing activity, and to relate this to the diketoacid chemotype represented by compounds **1** and **2**. Substituents were chosen in order to examine the effects of electron donation/withdrawal and sterics. It should be noted that substitution of the phenyl ring also has the potential of changing the electronics of the adjacent amide bond. This in turn will alter the ability of amide-carbonyl to bind to Mg^{2+} in the active site. As shown in Table 1, ortho-substitution reduces activity relative to the unsubstituted compound **6**. The SAR of the *meta*-position appears to be driven primarily by electron induction, in that inductive electron withdrawal is unfavorable. In contrast, electron withdrawal at the *para*-position leads to improved activity (cf. **9c**), but this effect is offset somewhat by the size of the group.

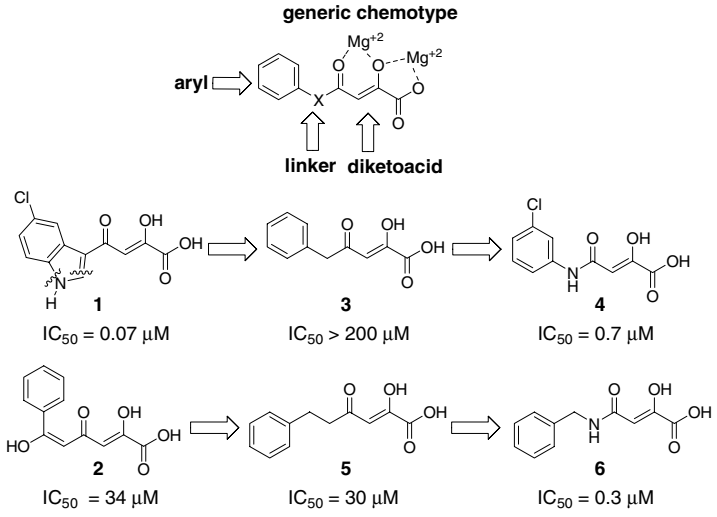
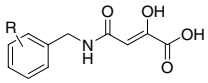


Figure 2. Diketoacid pharmacophore and the corresponding activity of related diketoacid and amide-ketoacid inhibitors.

Table 1. In vitro SAR of phenyl ring substitution[12]



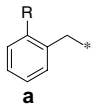
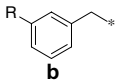
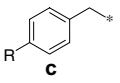
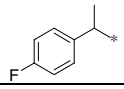
Compound	R	IC_{50} (μM)		
		 a	 b	 c
6	–H	0.3		
7a,b,c	–Me	1.3	0.3	1.4
8a,b,c	–OMe	0.5	2.4	4.2
9a,b,c	–F	1.8	1.2	0.2
10a,b,c	–Cl	1.7	0.6	1.1
11	$–NO_2$	—	—	2.1
12	$–CF_3$	—	—	9
13	–*t*-Bu	—	—	180

Table 2. In vitro SAR of benzylic methylene

Compound	R	IC_{50} (μM)
14		>300
15		24
16		3

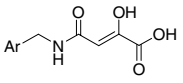
Apparently, the depth of the pocket which surrounds the benzyl group appears to be limited, therefore only small groups can be accommodated. Altogether, these results suggest a well-organized hydrophobic pocket which is sensitive to the electronic nature of the π-surface and size of the phenyl group.

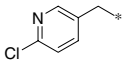
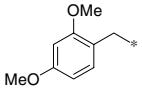
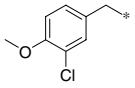
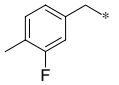
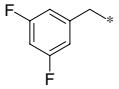
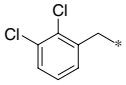
Apart from the phenyl group, substitution of the benzylic methylene was examined. As illustrated by compounds **14**, **15**, and **16** methylation results in attenuation of activity indicating that there is restricted tolerance for substitution at this site. Interestingly, this site is sensitive to the enantiomeric configuration of the chiral carbon atom. The *R*-isomer has much lower activity than the *S*-isomer.

As shown in Table 3, the SAR of multiply functionalized aryl groups is not straightforward in that the substituent effects are not additive. When combined, the groups appear to be acting synergistically to reduce or increase activity beyond what is expected based on the SAR shown in Table 1.

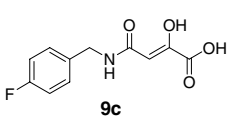
In order to evaluate the antiviral activity of the amide-ketoacid chemotype the compounds were tested in cell culture against a luciferase containing non-infectious HIV-reporter virus.[13] As might be expected based on the number of H-bond donor and acceptor functionality

Table 3. SAR of multiple substituents

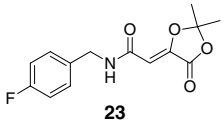


Compound	Ar	IC_{50} (μM)
17		0.7
18		7.5
19		0.3
20		0.2
21		1.7
22		0.2

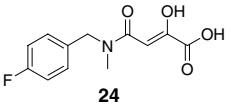
present on the molecules as well as the presence of a highly acidic carboxylate the antiviral activity was modest. As shown in Figure 3, compound **9c**, which possesses potent in vitro activity, exhibited an EC_{50} = 5.9 μM. Assuming that the H-bond donating groups were adversely affecting the penetration of the compound into the cell these groups were blocked in a systematic fashion. There was only a slight improvement in activity when **9c** was delivered as the corresponding dioxolane pro-drug derivative **23** probably due to premature hydrolysis of the acetonide-ester prior to entering the cell. On the other hand, methylation of the amide to yield **24** provides a 10-fold increase in cell culture activity while having only a marginal effect on in vitro activity, apparently due to increased cellular uptake. It is interesting to point out the necessity of the methyl group to achieve improved activity for this chemotype compared to the aza-naphthyridine and pyrimidine chemotypes (cf. L-870810 and MK-0518) where the opposite effect is seen.



9c
EC_{50} = 5.9 μM



23
EC_{50} = 1.2 μM



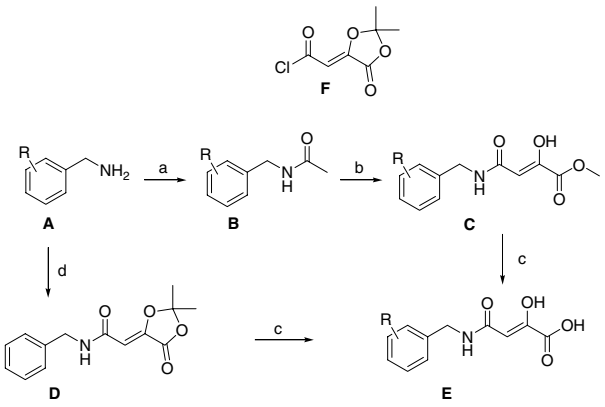
24
IC_{50} = 0.11 μM
EC_{50} = 0.6 μM

Figure 3. Cell culture activity of amide-ketoacid derivatives of **9c**.

The synthesis of the benzylic amide-ketoacid compounds is readily accomplished using either of the methods shown in Scheme 1.[14] Acylation of benzylamine **A** provides amide **B** which can be readily condensed with dimethyloxylate forming the amide-ketoester **C**. Likewise, the benzylamide can be amidated with **F**[15] to yield **D**. The desired benzyl amide-ketoacid is easily generated following saponification of **C** and **D**. The simplicity of these methods is readily apparent and affords a convenient platform for the parallel synthesis of small libraries.

In summary, the results reported above demonstrate that benzylic amide-ketoacids are potent inhibitors of HIV-integrase. Moreover, the amide-based chemotype yields a dramatic improvement in activity over the corresponding diketoacid based template represented by compound **5**. This is believed to be the result of the reduced rotational freedom of an amide C–N bond compared to an acyclic C–C bond. Thus, as demonstrated in previous publications, the relative position of the aryl group with respect to the Mg^{2+}-binding portion of the molecule is important for activity.

In general, the unsubstituted benzylic group of compound **6** appears to be fairly well optimized for binding to integrase. The SAR associated with the benzylic element appears to be straightforward when substituted by a single group, as illustrated in Tables 1 and 2. However, the SAR of multiple substituents attached to the phenyl ring is more complicated. Nonetheless, the SAR of the benzylic group points to a well-defined hydrophobic pocket on integrase that can discriminate groups based on their size and electronic properties.



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

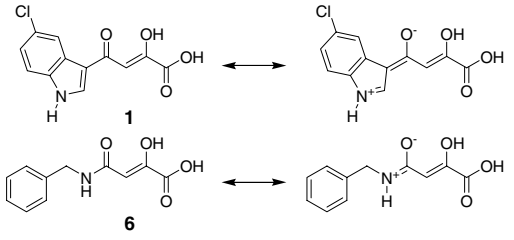


Figure 4. Resonance structures of compounds **1** and **6**.

Returning briefly to Figure 2, one can see where the diketoacid inhibitor **1** derives its activity. As illustrated in Figure 4, in analogy to the amide group of **6**, the pyrrole-ring locks the attached phenyl group into what is likely the preferred conformation via resonance and also pushes electron density back toward the diketoacid through conjugation, increasing its affinity toward Mg^{2+}. This is similar to the proposed function of the amide group of the amide-ketoacid inhibitors. However, the amide group is less structurally complicated and therefore a more efficient functional group.

The amide-ketoacid chemotype provides a very versatile platform for investigating the pharmacophore requirements for the design of integrase inhibitors. The relatively straightforward synthesis provides rapid assembly of compounds for SAR studies. As mentioned above, both the ester and acid forms[16] of the amide-ketoacid chemotype have been exploited by others, for example, Daeyaert et al. for the development of a QSAR model. While this model proved useful for predicting the binding of amide-ketoacid esters and acids to integrase it did not provide information regarding the binding configuration to the enzyme.

A feature worth mentioning is the high 'binding efficiency' of this chemotype where $\geqslant$2/3's of the atoms of the chemotype are directly interacting with the enzyme. Another interesting feature of this chemotype is the large response in cell culture activity achieved by methylation of the benzylamide nitrogen. This provides an additional vector for compound modification and leaves open the possibility for improving activity even further by attaching additional functional groups. More complex structural variations have been studied in our laboratories and will reported in due course.

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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 10 mM. Samples were counted in TopCountNXT (Packard) and the CPM was used as a measure of integration. The reaction condition was as described in Engelman, A.; Craigie, R. *J. Virol.* **1995**, *69*, 5908–5911. The sequences of substrate and target DNA were described in Nucleic Acid Research **1994** *22*, 1121–1122.
13. A recombinant NL-Rluc virus was constructed in which a section of the nef gene from NL4-3 was replaced with the *Renilla* Luciferase gene. The NL-RLuc virus was prepared by co-transfection of two plasmids, pNLRLuc and pVSVenv. The pNLRLuc contains the NL-Rluc DNA cloned into pUC18 at the PvuII site, while the pVSVenv contains the gene for VSV G protein linked to an LTR promoter. Transfections were performed at a 1:3 ratio of pNLRLuc to pVSVenv on 293T cells using the LipofectAMINE PLUS kit from Invitrogen (Carlsbad, CA) according to manufacturer's instruction, and the pseudotype virus generated was titered in MT-2 cells. Susceptibility of viruses to compounds was determined by incubation in the presence of serial dilutions of the compound. The 50% effective concentration (EC_{50}) was calculated by using the exponential form of the median effect equation where (Fa) = 1/[1 + (ED_{50}/drug concn)m] (Johnson, V. A,; Byington, R.T. Infectivity Assay. In Techniques in HIV Research, Aldovini, A., Walker, B. D., Eds.; Stockton Press: New York, 1990, pp 71–76).
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16. In our own studies, we have found that the acid form is preferred for binding to integrase. For example, the ethyl ester of compound **6** had IC_{50} = 10.5 μM.