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Azido-Containing Aryl β-Diketo Acid HIV-1 Integrase Inhibitors

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Abstract—Aryl β-diketo acids (ADK) comprise a general class of potent HIV-1 integrase (IN) inhibitors, which can exhibit selective inhibition of strand transfer reactions in extracellular recombinant IN assays and provide potent antiviral effects in HIV-infected cells. Recent studies have shown that polycyclic aryl or aryl rings bearing aryl-containing substituents are components of potent members of this class. Reported herein is the first use of azido functionality as an aryl replacement in β-diketo acid IN inhibitors. The ability of azido-containing inhibitors to exhibit potent inhibition of IN and antiviral protection in HIV-infected cells, renders the azide group of potential value in the further development of ADK-based IN inhibitors.

Along with reverse transcriptase (RT) and protease (PR), HIV integrase (IN) is recognized as an important target for treatment of acquired immunodeficiency syndrome (AIDS). Among these three enzymes, FDA-approved therapies include PR and RT inhibitors, where combination regimes have been effective in reducing the incidence and mortality of AIDS.[1] However, toxicities and resistance to these latter inhibitors, necessitate the examination of complimentary targets for therapeutic development.[2–4] Accordingly, significant effort is being devoted to finding inhibitors against the third viral enzyme, IN.[5–8]

Integrase catalyzes the insertion into the host genome, of proviral DNA derived by reverse transcription of viral RNA. Integration occurs via a multi-step sequence of reactions, which includes cleavage of a dinucleotide pair from the 3′-end (termed 3′-processing or 3′-P) followed by transfer of the resulting shortened strands into the target DNA (termed 'strand transfer' or ST). Although a large number of IN inhibitors have been described, most lack selectivity for the 3′-P versus ST reactions. Additionally, many agents, which exhibit potent inhibition of integrase in extracellular assays, fail to provide anti-viral efficacy in HIV-infected cells.[9] Recently, a promising new class of inhibitors has emerged, which contain aryl β-diketo (ADK) functionality. These are typified by 5CITEP (**1**)[10] and L-708,906 (**2**)[11] (Fig. 1). This general family is broadly characterized by an ability to afford preferential inhibition of ST versus 3′-P reactions.[12,13] Additionally, members of the ADK class not only selectively inhibit ST in extracellular assays using recombinant IN, but also provide anti-viral protection in HIV-infected cells by mechanisms consistent with inhibition of IN.[11,14] Although ADK-based agents are among the most promising IN inhibitors currently known, outside of the patent literature (for a brief overview, see refs [7 and 8]) only a small number of synthetic studies on these agents have been reported.[15,16] Accordingly, described herein is the first examination of azido functionality as a useful motif in ADK-based IN inhibitor design.

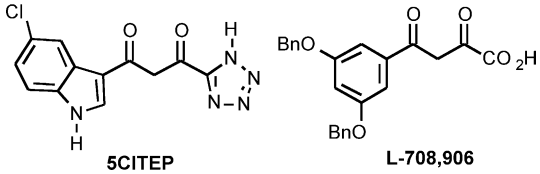

Figure 1. Structure of two representative previously reported ADK-based IN inhibitors.

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In an effort to prepare variants of previously reported[11] L-708,906 (**2**), benzylamine-containing analogues of general structure **3** were designed (Fig. 2).[17] The synthetic route to these agents involved synthesis of azidomethyl-containing intermediates, as shown with compound **4**. The IN inhibitory potency of **4** was evaluated, as well as its anti-viral potency in HIV-1-infected cells. Aryl substituents had previously been shown to be important for effective IN inhibition.[13,15,16] We found that azido-containing **4** (ST IC_{50} ≈2 μM) was as potent as L-708,906 (**2**, ST IC_{50} ≈0.5 μM) in extracellular IN assays. Additionally, antiviral potency was observed in an assay using HIV-1-infected cells (Table 1). It was also noted that **4** exhibited low cytotoxicity.

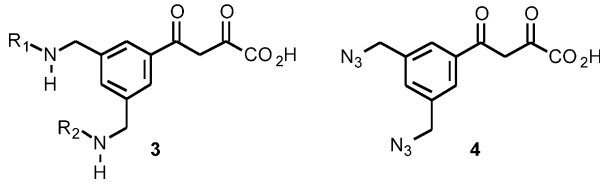
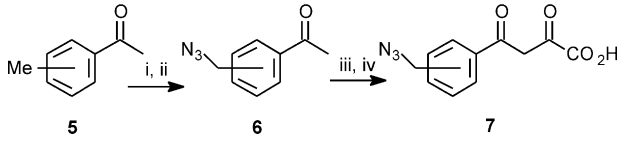


Figure 2. Structures of final target molecules (**3**) and the azide-containing synthetic intermediate (**4**).

The unexpected ability of the azide group to replace aryl functionality prompted us to more fully examine its use. A series of di- and mono-azido-containing ADK inhibitors were prepared. As outlined in Scheme 1,[18] synthesis of azido-containing ADK inhibitors started with benzylic bromination of aryl ketones (**5**), followed by nucleophilic substitution with azide. The resulting benzylic azides (**6**) were coupled with diethyl oxalate to yield the corresponding diketo ethyl esters, which were hydrolyzed to the free acids (**7**) using aqueous sodium hydroxide.



Scheme 1. Synthetic approach to azido-containing ADK inhibitors: (i) NBS, benzoyl peroxide, CCl_4, reflux; (ii) NaN_3, acetone–H_2O (5:1), reflux; (iii) $(CO_2Et)_2$, NaH, toluene, 60° C; (iv) 1 N NaOH, dioxane, 1 h.

Integrase inhibitory potencies of azido-containing ADK-based compounds are provided in Table 1. The isomeric 3,4-di-(azidomethyl) analogue **8** (ST IC_{50} ≈0.2 μM) was approximately 10-fold more potent than the original 3,5-substituted compound **4**. This enhanced potency could potentially be attributable to preferential orientation of the azido functionality induced by *o*-neighboring group crowding. In order to determine conformational effects, constrained analogues **9** and **10** were prepared. The IN inhibitory potencies of these two compounds were maintained relative to compound **8** and were greater than for parent **4**. However, the antiviral potency of cyclopentane-containing **10** was reduced relative to **8**.

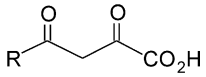
In order to gain an understanding of the individual importance of azidomethyl substituents at the 3- and 4-positions of **8**, the respective mono-substituted analogues **11** and **12** were prepared. The 3-substituted isomer (ST IC_{50} = 1.4 μM) was approximately 5-fold more potent than the 4-substituted analogue (ST IC_{50} ≈6.5 μM). The fact that monoazide-containing **11** was approximately equivalent to diazide-containing **4** in both IN and antiviral assays, suggests that the 3-substitution is sufficient for high potency. However, neither of the monoazide-containing agents **11** or **12** alone achieved the potency of parent **8**. While this indicated that both azidomethyl groups might contribute to the overall inhibitory potency of **8**, it was unclear whether generic substitution at the 4-position could also achieve a similar effect through restriction of rotation of the 3-azidomethyl group. To examine this possibility, 4-methoxy-substituted analogue **13** was prepared. The nearly 40-fold reduction in potency (**13**, ST IC_{50} ≈8.5 μM) incurred by replacement of the 4-azidomethyl group of **8** with a methoxy group, potentially indicated that both azido groups were important for the high inhibitory potency of **8**.

Although the 3,4-di-azido-containing analogues **8**–**10** exhibited from 5- to 10-fold higher IN inhibitory potency than the mono-azide-containing **11**, the antiviral potency of **11** was equivalent to or greater than these di-substituted analogues. The 3-azidomethyl arrangement was therefore examined, since it had been reported previously for 3-benzyl-substituted ADK acid inhibitors, that 6-methoxy and 6-isopropoxy substituents could enhance IN inhibitory potency.[14] Unlike the previously reported 3-benzyl series, the resulting compounds (**14** and **15**, respectively) did not show enhanced IN inhibition relative to **11**.

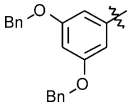
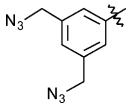
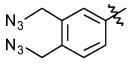
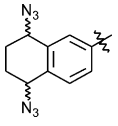
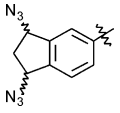
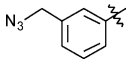
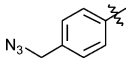
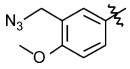
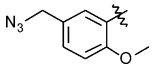
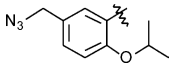
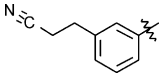
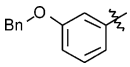
Finally, it was of interest to examine whether steric or electronic properties of the azido functionality were important for IN inhibitory potency. Azides are sterically characterized by their linear geometry. Therefore, nitrile-containing **16** was prepared as a non-azide-containing steric equivalent to **11**. Equal IN inhibitory potency of **16** relative to **11** was supportive of shape as a contributor to the inhibitory potency of **11**. However, the markedly reduced antiviral potency of **16**, potentially indicated that electronic properties of the azide group as well as shape contribute to the antiviral potency of **11**. Therefore, in order to investigate 3-dimensional structural features of **11** and **16**, which might potentially contribute to the difference in their antiviral potency, the subset of molecules **4**, **11**, **16**, and **17** contained in Table 1, was modeled at the *ab initio* level,[19–22] with electrostatic potentials (EP) and electron densities also being determined for each fully optimized structure.[23–25]

Because it was of particular interest to spatially compare the azido-containing side chain of **11** with the nitrilomethano-containing side chain of **16**, local energy-minimized conformations were selected which allowed superposition of these groups (Fig. 3). It is apparent from Figure 3 that the azido group of **11** and the nitrilomethano substituent of **16** are nearly superimposable, with both having less extension than the

Table 1. Structures of inhibitors and associated biological potencies in the indicated assays



General Structure

No.	R	Integrase assay (IC_{50} μM)[a]		Anti-viral potency[a] (IC_{50} μM)	Cytotoxicity[a] (CC_{50} μM)
		3′-P	ST		
2		> 100	0.48 ± 0.08[b]	1.9 ± 0.6	> 50
4		> 100	2.0, 2.8	5 ± 2.5	> 50
8		> 100	0.26, 0.15	14.3 ± 4.7	> 50
9		70	0.32	15.6	ND
10		> 100	0.36	> 25	ND
11		> 100	1.53 ± 0.27	2.1 ± 0.8	> 50
12		> 100	6.1, 7.1	> 25	ND
13		> 100	8.5, 9.0	> 25	ND
14		> 100	24, 18	13.5 ± 2.5	ND
15		> 100	25, 23	5.9 ± 1.1	ND
16		> 100	1.5, 1.8	> 25	ND
17		85, 100	0.35 ± 0.13[b]	0.6[b]	ND

[a]Assays were conducted as previously reported in refs 15 and 16.
[b]Value as previously reported in ref 16.

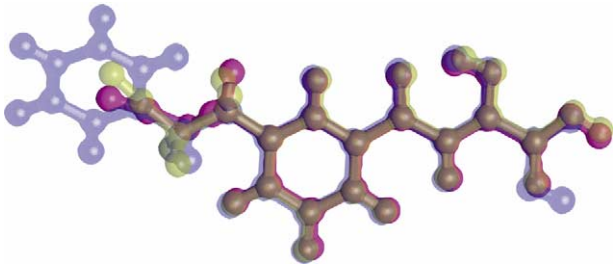


Figure 3. Superposition of local energy minimized conformations of **11** (magenta), **16** (green) and **17** (blue).

benzyloxy group of **17**. Therefore functionality at the 3-position of all three inhibitors could potentially occupy the same binding region in the enzyme.

Since conformational/steric properties of **11** and **16** seemed similar, EP maps, which were calculated after initial *ab initio* minimization, were compared (Fig. 4). These maps show that **4**, **11**, and **16**, which contain linear groups having terminal nitrogens (azido or nitrile), all exhibit a region of negative electrostatic potential at the end of their respective side chain(s), centered on the terminal nitrogen atom(s). This arrangement of negative EP might be capable of interacting with a divalent metal cation, which is situated in the catalytic center. Sequestration of catalytically-bound metal cations is viewed as a potential mechanism by which ADK-type IN inhibitors exert their inhibitory effects.[26]

The IC_{50} values presented in Table 1 were obtained in the presence of manganese using a soluble double mutant (F185K/C280S) HIV-1 integrase.[27] However, in vivo, the wild-type enzyme most probably utilizes magnesium. This might account for discrepancies between in vitro enzyme inhibition and in vivo antiviral potency.[26] Therefore, the potencies of both **11** and **16** in the presence of either manganese or magnesium were compared using wild-type enzyme. Both compounds were found to be inhibitory in the presence of magnesium with potencies similar to those exhibited in the presence of manganese (data to be published elsewhere).

In conclusion, reported herein is the first use of azido functionality in ADK acid-type IN inhibitors. Agents bearing azide groups were found to elicit potent inhibition of IN and to provide antiviral effects in HIV-infected cells at non-cytotoxic concentrations. Although nitrilomethano functionality provided similar inhibitory potency in in vitro IN assays, this moiety failed to provide antiviral potency observed with the azido-containing inhibitors. Since both azido and nitrilomethano groups were found to be sterically and electronically similar and to exhibit similar metal dependencies, the reasons for their in vivo differences are not clear. Azido functionality should be included among the list of useful substitutents for development of ADK-family IN inhibitors.

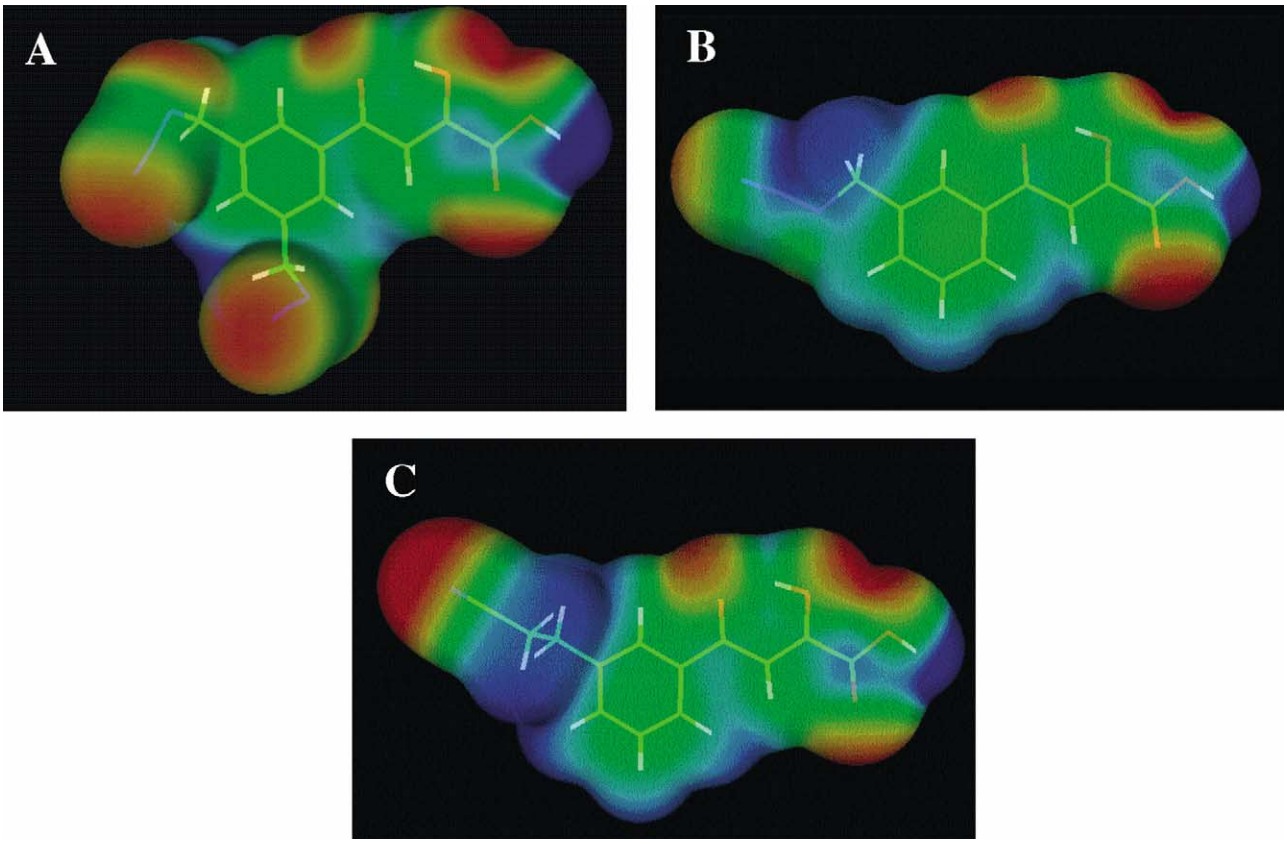


Figure 4. Contour map of the electrostatic potential (EP) mapped onto the electron density for compounds **4** (A), **11** (B), and **16** (C). The ligand structures, shown inside the contour maps as stick figures, are colored by atom type (C, green; N, blue: O, red). Blue EP contours indicate areas with positive electrostatic potential and red contour indicate areas of negative electrostatic potential.

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22. Three-dimensional structures of the compounds were first constructed in Macromodel 7.0, and energy minimized using the Merck Molecular Force Field (MMFF).[19] The geometry of these local energy minima were then used as initial structures for conformational analysis by subjecting to 1000 steps of Monte-Carlo (MC) simulation. The minimum conformation reached by the MC simulations was then fully optimized at the Density Functional Theory (DFT) level of theory, using B3LYP/6-31G* in gas phase, in Gaussian 98 Rev. A11.[20] All the minimum energy structures were confirmed by normal mode analysis, with no negative frequency found.
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