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Design and synthesis of photoactivatable aryl diketo acid-containing HIV-1 integrase inhibitors as potential affinity probes

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Abstract—Aryl diketo acids (ADKs) represent an important new class of HIV-1 integrase (IN) inhibitors. In order to facilitate examination of the structural basis underlying IN·ADK interaction, biphenyl ketone and phenyl azide photophores were incorporated into ADK structures. Of particular note is the novel dual utilization of azide and phenyketone moieties for both enzyme recognition and for crosslinking. The resulting analogues maintained low micromolar inhibitory potency against IN in recombinant in vitro assays. These potential HIV-1 integrase photoaffinity labels may provide useful tools for studying enzyme interactions of the ADK inhibitor class.
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1. Introduction

Along with reverse transcriptase (RT) and protease (PR), integrase (IN) is an enzyme critically required for replication of the human immunodeficiency virus type-1 (HIV-1). Although all three enzymes represent targets for anti-AIDS therapeutic development,[1] unlike the first two enzymes for which drugs are currently marketed, IN has yet to yield an FDA-approved inhibitor. Recently a promising new class of aryl diketo (ADK) inhibitors has emerged,[2,3] however a lack of structural information regarding binding of these inhibitors to IN has frustrated the structure-based design of next-generation agents.[4,5] Wide precedence exists for the application of photoaffinity labeling to elucidate ligand enzyme interactions.[6] Among photophores, biphenyl ketones and aryl azides have shown particular use as photo-activatable handles in variety of biologically relevant systems.[7] The high level of interest in ADK family inhibitors, coupled with the paucity of information regarding the structural basis of their interaction with IN, makes them particularly attractive candidates for similar derivation as photoaffinity labels. We have previously reported biphenyl ketone-containing derivatives of coumarin compounds (**1a** and **b**, Fig. 1) as HIV-1 IN photoaffinity labels.[8] ADK family IN inhibitors, which are typified by compounds **2a** and **b**, exhibit high HIV-1 binding affinity that is dependent on both the nature and location of substituents on the ADK phenyl ring. For the unsubstitued analogue **2a**, inhibition of HIV-1 IN catalyzed strand transfer (ST)[9] occurs with an IC_{50} value of 25 μM.[10] Introduction of substituents at the 3-position can enhance inhibitory potency, as exemplified by **2b** ($IC_{50} = < 0.10$ μM)[11] and **3** ($IC_{50} = 0.35$ μM).[10] Accordingly, the phenyl 3-position was chosen as the site to incorporate biphenyl ketone and azide photo-activatable functionality. This resulted

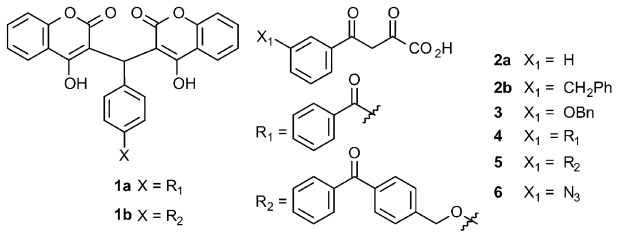

Figure 1. Structures of HIV-1 IN inhibitors and photoaffinity labels.

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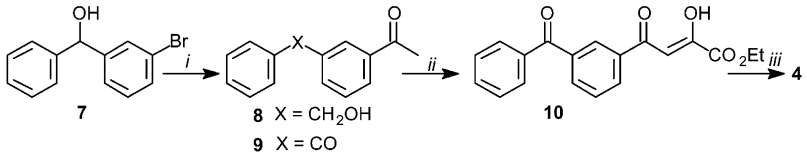
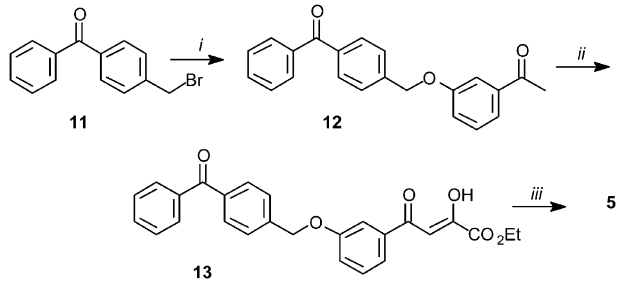


Scheme 1. Reagents and conditions: (i) *N*-methoxy-*N*-methylacetamide, *n*-BuLi, THF, −78° C (75% yield); (ii) diethyl oxalate, NaH, toluene, 60° C (quantitative); (iii) 1 N NaOH, dioxane:H_2O, rt (53% yield).

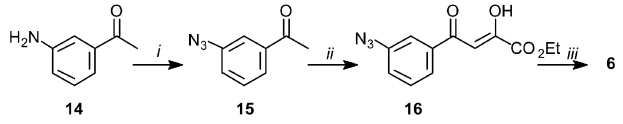
in selection of diketo acids **4–6** as target compounds (Fig. 1). Of note, compounds **4** and **6** both utilize phenyl rings of the parent DKA itself as integral components of their respective biphenyl ketone and aryl azide photophores, while compound **5** appends biphenyl ketone functionality as a separate entity onto the parent DKA structure. This latter substitution may be viewed as replacing the benzylic phenyl group of ADK inhibitor **3** with the photophore.

2. Synthesis

Preparation of final ADKs **4–6**,[12] relied on condensation of appropriate phenyl methyl ketones **9**, **12** and **15** with diethyl oxalate to yield ethyl esters **10**, **13** and **16**, respectively (Schemes 1–3). Base-catalyzed hydrolysis provided the title free acids **3–5**. Incorporation of biphenyl ketone moieties onto **9** and **12** was achieved either by elaborating the phenyl ADK phenyl ring as one half of the biphenyl ketone (Scheme 1), or by appending a preformed diphenyl ketone via the ether linkage using ((4-bromomethyl)phenyl) phenyl ketone (**11**, Scheme 2). Synthesis of 3-(azidophenyl) methyl ketone (**15**) was accomplished from the corresponding 3-aminophenyl methyl ketone precursor (**14**, Scheme 3).



Scheme 2. Reagents and conditions: (i) K_2CO_3, 1-(3-hydroxyphenyl)-ethane-1-one, DMF, 70 °C (56% yield); (ii) diethyl oxalate, NaH, toluene, 60 °C (quantitative); (iii) 1 N NaOH, dioxane:H_2O, rt (66% yield).



Scheme 3. Reagents and conditions: (i) (a) $NaNO_2$, HCl, H_2O, 0 °C; (b) NaN_3, 0 °C (83% yield); (ii) diethyl oxalate, NaH, toluene, 60 °C (quantitative); (iii) 1 N NaOH, dioxane:H_2O, rt (70% yield).

Table 1. Inhibition of HIV-1 integrase as measured in an in vitro assay

Compd	IC_{50} (μM)	
	3′-P	ST
4	>100	2.7, 2.1
5	>100	19.0, 29.0
6	>100	5.3, 5.2

3. Biological evaluation

Integrase functions in a two step manner: (1) Initial removal of a dinucleotide unit from the 3′-ends of the viral DNA (termed '3′-processing' or 3′-P) and (2) Integration of the 3′-processed strands into the host DNA (termed 'strand transfer' or ST). Using viral long terminal repeat oligonucleotide sequences as substrate, an in vitro recombinant HIV-1 integrase assay[13] was used to evaluate the ability of final products **4–6** to inhibit HIV-1 integrase. The assay provides IC_{50} values against both 3′-P and ST processes. This capability is important since as a class, ADK family inhibitors are distinguished by their selective inhibition of ST versus 3′-P catalysis.[10,14] Results indicate that all three compounds inhibit ST processes with IC_{50} values in the low micromolar range. The more potent biphenyl ketone-based inhibitor **4**, exhibits approximately 10-fold higher affinity than its counterpart **5**, while aryl azide-containing **6** has an IC_{50} value between these two. Consistent with other members of the AKD family, selective inhibition against ST is observed relative to 3′-P (Table 1).

4. Conclusions

Reported herein is the design and synthesis of novel ADK family HIV-1 integrase inhibitors that contain functionality intended to allow photo-activated cross coupling. Of particular note is the novel dual utilization of azide and phenyketone moieties for both enzyme recognition and for crosslinking. These agents may potentially prove useful as photoaffinity labels in studies designed to gain greater understanding of the manner in which ADK family inhibitors interact with HIV-1 IN. Such information may aid the structure-based design of next generation agents.

Acknowledgements

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12. Analytical data for final products: *2-hydroxy-4-oxo-4-(3-(phenylcarbonyl)phenyl)but-2-enoic acid* (**4**) (obtained as a white solid following HPLC purification) mp 93°C (dec), ^{1}H NMR (DMSO-d_6) δ 8.35 (1H, d, J=8.5 Hz), 8.29 (1H, s), 8.01 (1H, dd, J=6 Hz, 1.5 Hz), 7.78–7.57 (6H, m), 7.08 (1H, s), FAB-MS ($^-$VE) m/z: 295 (M−H); *2-hydroxy-4-oxo-4-(3-((4-(phenylcarbonyl)phenyl)methoxy)phenyl)but-2-enoic acid* (**5**) mp 107–109°C, ^{1}H NMR (DMSO-d_6) δ 7.74–7.68 (4H, m), 7.65–7.59 (5H, m), 7.53–7.50 (2H, t, J=8 Hz), 7.46 (1H, t, J=8 Hz), 7.32 (1H, d, J=8 Hz), 7.05 (1H, s), 5.30 (2H, s), FAB-MS ($^-$VE) m/z: 401(M−H); *4-(3-azidophenyl)-2-hydroxy-4-oxobut-2-enoic acid* (**6**) mp 118–120 °C, ^{1}H NMR (DMSO-d_6) δ 7.87 (1H, d, J=8 Hz), 7.68 (1H, t, J=2 Hz), 7.59 (1H, t, J=7.8 Hz), 7.43 (1H, m), 7.09 (1H, s). FAB-MS ($^-$VE) m/z: 232 (M−H).
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