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Design, synthesis, molecular modeling, and anti-HIV-1 integrase activity of a series of photoactivatable diketo acid-containing inhibitors as affinity probes

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ABSTRACT

The diketo acid (DKA) class of HIV-1 integrase (IN) inhibitors is thought to function by chelating divalent metal ions on the enzyme catalytic site. However, differences in mutations conferring resistance to various DKA inhibitors suggest that multiple binding orientations may exist. In order to facilitate identification of DKA binding sites, a series of photoactivable analogues of two potent DKAs was prepared as novel photoaffinity probes. In cross-linking assays designed to measure disruption of substrate DNA binding, the photoprobes behaved similarly to a reference DKA inhibitor. Molecular modeling studies suggest that such photoprobes interact within the IN active site in a manner similar to that of the parent DKAs. Analogues **Ia-c** are novel photoaffinity ligands useful in clarifying the HIV-1 binding interactions of DKA inhibitors.

1. Introduction

HIV-1 integrase (IN) is an attractive and validated target for developing novel antiretroviral agents (Neamati, 2001; Anthony, 2004; Pommier et al., 2005). Because of its vital role in the viral replication cycle, with no human counterpart of the enzyme known, the addition of an IN inhibitor to existing components of antiretroviral therapy (Barbaro et al., 2005) is expected to improve the outcome of therapy by potential synergism (De Clercq, 2002, 2005), without exacerbating toxicity (Cohen, 2002; Little et al., 2002).

In the past several years, a plethora of compounds with diverse structural features has been reported as IN inhibitors (Cotelle, 2006; Neamati, 2002). Several of them inhibit both the viral enzyme and viral replication in cell-based assays, as well as in animal models (Pommier et al., 2005). Among all the reported inhibitors, the β-diketo acid (DKA) class of compounds has shown the most promising results (Hazuda et al., 2000; Pais and Burke, 2002; Pommier et al., 2005). After about 15 years of study, the DKA-based derivative, Raltegravir (MK-0518, Plate 1), has been approved by the US Food and Drug Administration (Rowley, 2008; Wang et al., 2007). It is believed that the DKA pharmacophoric motif could be involved in a functional sequestration of one or both divalent metal ions that are critical cofactors at the enzyme catalytic site (Grobler et al., 2002; Pommier et al., 2005; Sechi et al., 2009). This would subsequently block the transition state of the IN-DNA complex (Espeseth et al., 2000). In this scenario it is of paramount importance to acquire information about the mode of action of DKAs that could then be useful in the design of new IN inhibitors.

Photoaffinity-labelling (PL) technology is emerging as a very useful tool for the identification and localization of proteins and their active sites in drug-discovery studies (Dormán and Prestwich, 2000; Fedan et al., 1984; Hatanaka and Sadakane, 2002; Kotzyba-Hibert et al., 1995). This method is particularly useful for the identification of ligand-binding sites of target proteins and for the investigation of ligand–receptor interactions. The use of affinity-labeled inhibitors to covalently modify the site of interaction and subsequent analysis of the protein has been very effective in providing useful information about inhibitor binding for a multitude of therapeutic target proteins. The PL technology enables the direct probing of a target protein through a covalent bond that is photochemically introduced between a ligand and its specific receptor (Fig. 1). Thus, PL could be applied in two levels of drug discovery and development processes. At macro-level, the method is useful for the screening of early leads from the evaluation of affinity by cross-linking, to know which ligand preferentially binds to which protein. If the binding site analysis of a target protein is important for defining a particular pharmacophore, the PL will give the structural information of receptor binding domain at the micro-level.

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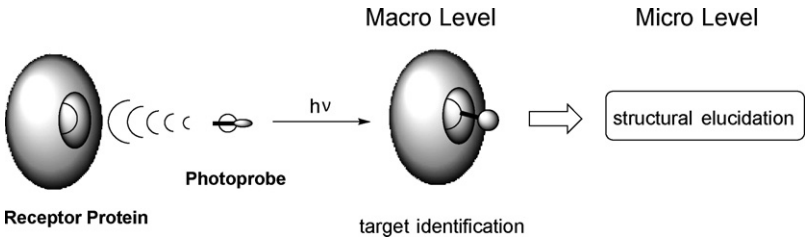

Fig. 1. The photoaffinity-labelling approach.

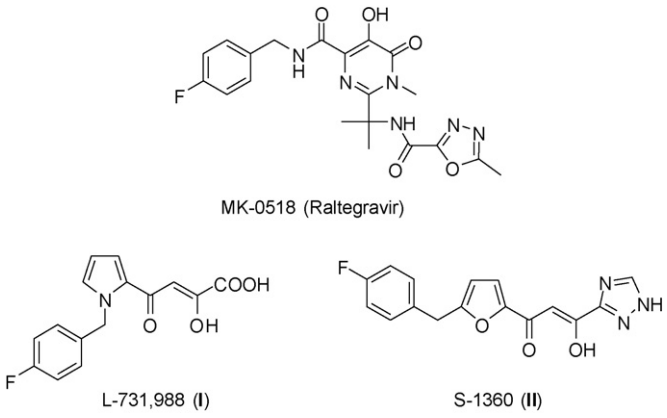

Plate 1. Structure of the first HIV-1 IN inhibitor in therapy (MK-0518), and representative diketo acid-based compounds, L-731,988 (**I**), S-1360 (**II**), used in this study.

Such an approach can be used to obtain structural information detailing the association between the enzyme IN and inhibitors under development. In fact, application of PL to IN has elucidated a small number of inhibitor-binding sites at atomic resolution (Al-Mawsawi et al., 2006).

To facilitate identification of DKA binding sites, a series of photoactivable compounds, related to two potent DKA-based inhibitors, L-731,988 (**I**) (Hazuda et al., 2000) and S-1360 (**II**, Plate 1) (Yoshinaga et al., 2002; Billich, 2003), was prepared as photoaffinity probes (Fig. 2). The latter were designed by replacing the para-fluoro-phenyl moieties of **I** and **II** with the three kinds of photophores, such as 4-azido-phenyl-, 4-azido-tetrafluorophenyl- and benzophenone, as photoreactive groups (Chehade and Spielmann, 2000; Dormán and Prestwich, 1994; Fleming, 1995). These photoprobes are expected to be useful tools for elucidation of the DKA binding mode and subsequent structure-based studies to design novel and selective IN inhibitors.

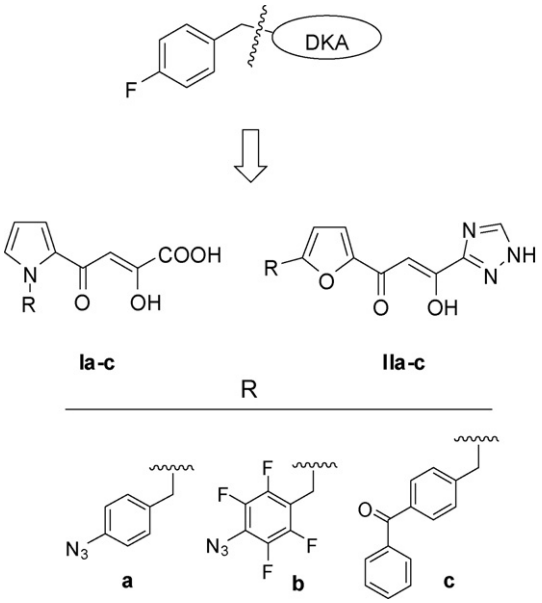

Fig. 2. Design of title compounds.

2. Materials and methods

2.1. Experimental chemistry

Anhydrous solvents and all reagents were purchased from Sigma–Aldrich, Merck or Carlo Erba. Anhydrous diethyl ether was obtained by distillation from Na/benzophenone under a nitrogen atmosphere. All reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere using oven-dried glassware and syringes to transfer solutions. Melting points (mp) were determined using an electrothermal melting point or a Köfler apparatus and are uncorrected. Infrared (IR) spectra were recorded as thin films or nujol mulls on NaCl plates with a Perkin-Elmer 781 IR spectrophotometer and are expressed in ν (cm^{-1}). Nuclear magnetic resonance (^1H NMR, ^{13}C NMR, NOE difference and NOESY) spectra were determined in $CDCl_3$, DMSO-d_6 or $CDCl_3$/DMSO-d_6 (in 3/1 ratio) and were recorded at 200 MHz on a Varian XL-200. Chemical shifts (δ scale) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) used as an internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; brs, broad singlet; dd, double doublet. The assignment of exchangeable protons (O*H* and N*H*) was confirmed by the addition of D_2O. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F-254 plates. Flash chromatography purifications were performed on Merck Silica gel 60 (230–400 mesh ASTM) as the stationary phase. Elemental analyses were performed on a Perkin-Elmer 2400 spectrometer at Laboratorio di Microanalisi, Dipartimento di Chimica, Università di Sassari (Italy), and were within ±0.4% of the theoretical values.

2.1.1. General procedure for the preparation of photoprobes ***[Ia-c]***

A solution of the appropriate β-diketo ester **1a-c** (1 mmol) in MeOH (10 mL) was treated with 2N NaOH (4.0 eq) and was stirred at r.t. for 5 h. Then, the reaction mixture was diluted with water and acidified with 1N HCl. The yellow precipitate formed was filtered off, washed with water and recrystallized from H_2O/EtOH.

*2.1.1.1. 4-[1-(4-Azidobenzyl)-1H-pyrrol-2-yl]-2-hydroxy-4-oxobut-2-enoic acid **[Ia]***. Yield: 50%; mp 171–173 °C dec. IR (nujol): ν (cm^{-1}) 2113 (N_3 azide), 1719 (C=O acid), 1624 (C=O ketone). ^1H NMR (200 MHz, $CDCl_3$ + DMSO-d_6): δ 7.21–6.89 (m, 6H, Ar–H), 6.86 (s, 1H, CH=C), 6.35–6.22 (m, 1H, Ar–H, pyrrole), 5.61 (s, 2H, CH_2). MS: *m*/*z* 312 (M^+). Anal. Calc. for ($C_{15}H_{12}N_4O_4$): C, 57.69; H, 3.87; N, 17.94. Found: C, 57.55; H, 3.79; N, 18.03.