Letter

Inhibition of Human Immunodeficiency Virus-1 Integrase by #-Diketo Acid Coated Gold Nanoparticles

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Inhibition of Human Immunodeficiency Virus-1 Integrase by β-Diketo Acid Coated Gold Nanoparticles

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KEYWORDS: HIV-1 Integrase Inhibitors; β-Diketo Acids; Gold Nanoparticles; Multivalent Ligands.

ABSTRACT: Gold nanoparticles (GNPs) have been proposed as carriers for drugs to improve their intrinsic therapeutic activities and to overcome pharmacokinetic problems. In this study, novel nanosystems constituted by a model β-diketo acid (DKA) grafted to the surface of GNPs were designed and synthesized following the "multivalent high-affinity" binding strategy. These first nanoscale DKA prototypes showed improved inhibition of HIV-1 integrase (HIV-1 IN) catalytic activities as compared with free DKA ligands.

HIV-1 integrase (HIV-1 IN) has been validated as a target for developing novel antiretroviral agents.[1-3] It catalyzes the insertion of retro-trascribed viral cDNA into the host genome (Chart 1, A) to form a stable provirus through two coordinated reactions, 3′-processing (3'-proc) and strand transfer (ST).[4-6] 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 has improved the outcome of therapy by potential synergism, without exacerbating toxicity.[1,2,7]

Among a plethora of compounds with diverse structural features reported as IN inhibitors,[7] a class of compounds bearing a β-diketo acid (DKA, Chart 1, B) functionality has emerged and validated as the most effective chemical space in anti-IN drug discovery, and some compounds belonging to this family have had clinical success.[8-13] Several DKA congeners selectively inhibit the strand transfer reaction, and in cell-based assays they inhibit integration without affecting earlier phases of the HIV-1 replication cycle.[8] Mechanistically, DKA pharmacophoric motif is involved in a functional sequestration of divalent metal ions (Chart 1, C), which are critical cofactors at the enzyme catalytic site.[7,12-15] This would subsequently block the transition state of the IN-DNA complex.[2,7,13,14] Despite the outstanding advances in the HIV-IN drug discovery, it was of paramount importance to explore further possibilities to optimize the action of DKA-based compounds as IN inhibitors by the investigation of newer chemical entities.[16,17]

Starting from the complexity of biological systems to exploit widespread advantage of multivalency, i.e. the combination of several entities involved in individual interactions or binding events, this characteristic has been followed as suitable strategy in the generation of high-affinity ligands, which were able to produce simultaneous and reversible molecular recognition interactions.[18-20]

In the past decade, several prototypes as multimeric ligands targeting tumor/viral receptors have been exploited, and multivalency can be envisaged as a way to improve the binding performance of small molecule–drug construct, thus expecting to reach a similar efficiency of the antibody–drug conjugates.[18,21-23]

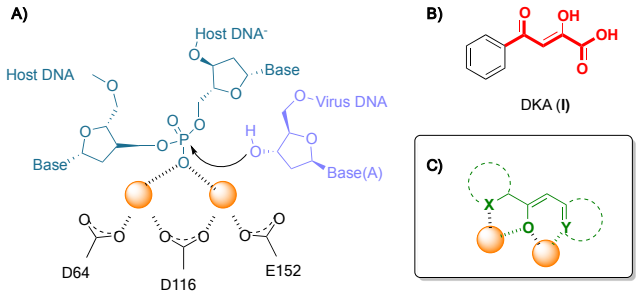


Chart 1. A) HIV-1 IN catalytic mechanism; B) Representative DKA and pharmacophoric motif (in red); C) DKA-based chemotype chelates metal ions (orange spheres) cofactors on the active site.

On the other hand, the application of nanotechnology in drug design is having an impact on diagnostics and drug delivery and, more recently, in drug discovery.[24-26] Therefore, the concept of multivalency, by nanoparticle (NP)-based platforms with multiple ligands coated on their surface, has emerged as suitable strategy to enhance the binding affinity of

simple monovalent ligands. Nanoparticles (NPs) can be synthetically engineered to present multiple high-affinity molecules on their surface to tune binding affinity over several orders of magnitude. Moreover, such nanosystems may be effective in disrupting key protein/protein interactions involved in several pathogenic events. NP-based prototypes possessing multivalent ligands are also expected to produce a high local concentration of binding molecules which, consequently, can statistically promote formation of more ligand-receptor interactions, thus enhancing affinity and selectivity at the biological target.

In this context, gold nanoparticles (GNPs) are extensively investigated for various biomedical applications due to their large surface area to volume ratio and thermal stability, as well as their less toxicity, synthetic accessibility and amenability of functionalization.[27-29] Some gold nanosystems have been proposed as carriers for drugs, to overcome pharmacokinetic problems and to improve their intrinsic therapeutic activities.[30]

The demonstration that GNPs convert a weak interaction of active small ligands into multivalent more effective and biologically active therapeutics have been offered by several seminal reports, which include, for example, inhibition of HIV fusion and antiretroviral delivery,[31,32] activation/inhibition of carbonic anhydrase,[33,34] and tumor targeting.[35,36]

In this scenario, we sought to explore the development of novel GNPs that could effectively inhibit viral enzymes such as HIV-1 IN (Figure 1). Therefore, a nanoparticulate carrier was designed simultaneously with a model DKA inhibitor.

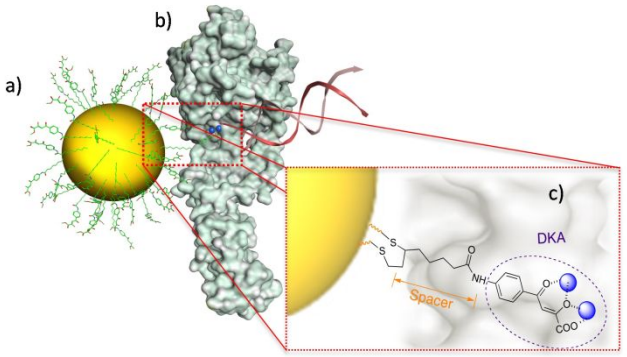


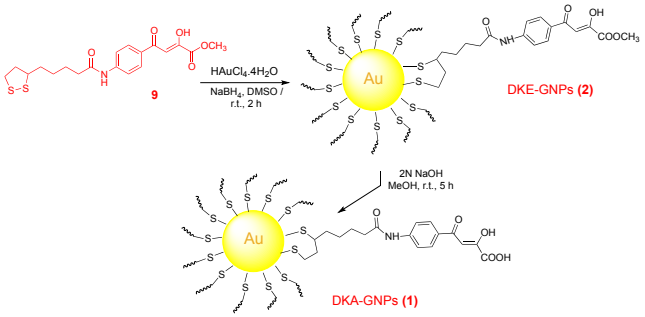
Figure 1. a) Graphical representation of a GNP coated with DKA ligands on its surface; b) virtual interaction of GNPs with the HIV-1 IN enzyme; c) interaction of a conjugated DKA ligand with the metal ions on the catalytic site.

In this work, we developed ~3.5 nm diameter DKA-coated GNPs as a nanoscale platform to construct novel HIV-1 IN multivalent therapeutics. These particles were designed starting from a model derivative of the simple 2-hydroxy-4-oxo-4-phenylbut-2-enoic acid (**I**, Chart 1); it was coupled with lipoic acid to have both a) a flexible linker between the ligand and the gold surface, and b) the appropriate thiolic terminal groups needed for anchoring such ligands to the NP surface (Figure 1).

The obtained gold nanosystems resulted in precise and monodisperse nanoscale constructs, which were tested for anti-HIV-1 IN activities.

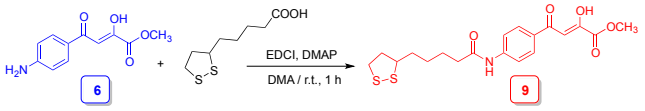
The preparation of DKA-coated GNPs (DKA-GNPs, **1**) was achieved in a two-step reaction by reduction of chloroaurate with $NaBH_4$ in the presence of the lipoic acid-tailed diketoester (DKE-GNPs, **2**), followed by an alkaline hydrolysis (Scheme 1).

Scheme 1. Preparation of DKE- and DKA-GNPs prototypes 1 and 2



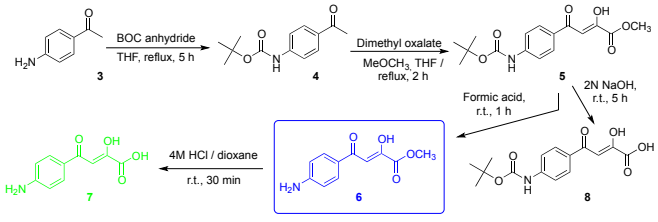
The key synthone **9** was synthesized by coupling lipoic acid with the 4-amino-DKE **6** in the presence of EDCI/DMAP, as outlined in Scheme 2.

Scheme 2. Preparation of the synthon 9



Synthetic approaches for the preparation of the key intermediate **6**, and for the other free ligands **7** and **8** and their precursors are depicted in Scheme 3.

Scheme 3. Synthetic routes for the preparation of free ligands and intermediates



Briefly, **6** was obtained by following the Boc protection of the commercially available 4-amino-acetophenone **3** to give the 4-Boc-amino-acetophenone **4**, which was converted to the diketoester derivative **5** by Claisen condensation with dimethyl oxalate and sodium methoxide, using a procedure previoulsy reported by us, with slight modification.[11] Subsequent Boc deprotection of **5** by formic acid in mild conditions gave **6** in good yield. The acids **7** and **8** were obtained upon acid or alkaline hydrolysis of diketoesters **6** and **5**, respectively (Scheme 3), for comparison purpose.

All small molecules have been characterized by means of NMR, IR, mass spectrometry (ESI and FAB) and elemental analysis.

GNPs **1** and **2** were characterized by transmission electron microscopy (TEM) (Figure 2). These particles are monodispersed, roughly spherical in shape, having an average particle size of ~3.7 nm; the number of gold atoms is calculated as ~1430 (for DKEs-GNPs **1** and DKAs-GNPs **2**).[27] Elemental analysis and ICP-OES measurements indicated that the number of ligands grafted on the NP' surface resulted 193 for both systems (considering the Au/S w/w ratio).[27,33,34,37]

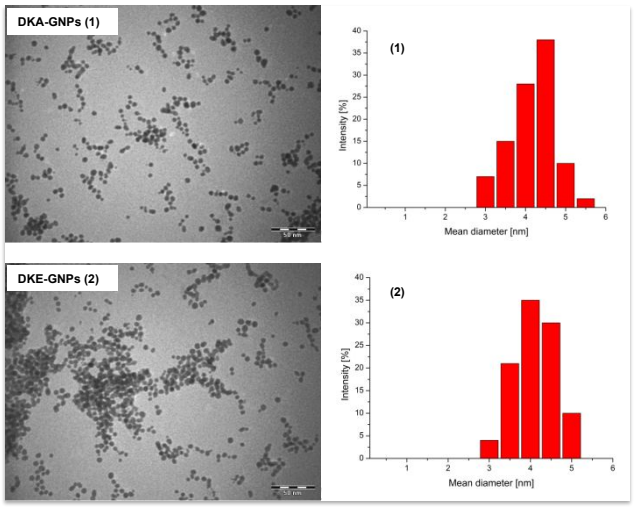


Figure 2. Left: TEM images of the GNPs **1** and **2** (scale bar = 50 nm); right, the corresponding nanoparticle size distribution.

Therefore, the obtained GNPs were consistent with a proposed empirical formula of $[Au_{1434}(C_{18}H_{21}O_5NS_2)_{193}]$ and $[Au_{1434}(C_{19}H_{23}O_5NS_2)_{193}]$, for **1** and **2**, respectively.

IR spectrum of GNPs-DKE **2** is given in Figure 3A. The C=O stretching bands of the $COOCH_3$/COOH functionalities were found at approximately 1700 cm^{-1} in the spectrum of lipoic acid-tailed DKE **9**, used for comparison.

Moreover, Figure 3B shows the UV–Vis spectrum of DKE- and DKA-capped GNPs **1** and **2**. A strong absorption at about 540 nm, a resonance corresponding to excitation of surface plasmon vibrations in the GNPs,[27] was observed for both gold nanosystems.

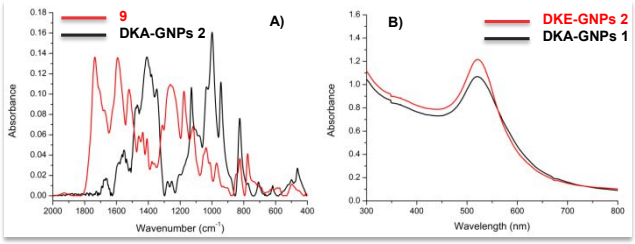
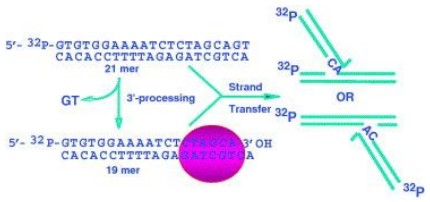


Figure 3. A) FT-IR spectra of DKEs-GNPs **2** and DKEs free ligand **9.** B) UV-Vis spectra of DKE-GNPs **2** and DKA-GNPs **1**.

GNPs **1** and **2**, and ligands **5-8** were tested for their ability to inhibit IN catalytic activity in *in vitro* assays employing purified enzyme, using the raltegravir-based derivative (HL^2)[38] as reference compound, and LA-GNPs, used as a control (Supporting information). Inhibition of IN catalytic activities, 3'-proc and ST, were evaluated using oligonucleotide-based assays and the results are reported in Table 1 (see below for schematic of IN activity, and in Supporting information for experimental details).

With the exception of the amino-DKEs **6** and **9**, all tested compounds showed anti-IN activity in (low) concentration ranges (IC_{50s} from 0.96 ± 0.34 to 44 ± 6 μM), with more potency exhibited towards the catalyzed ST process for free ligands **5** and **7** versus 3'-proc, as evidenced by their selectivity indexes. The ligands differ in activities as previously reported, thus confirming that the nature of the substituents on the aromatic ring significantly influence the potency.

Table 1. *In vitro* HIV-1 integrase inhibition and MTT assay



Cpds	3'-Processing [a]IC_{50} (μM)	Strand Transfer [a]IC_{50} (μM)	[b]SI	[c]CC_{50}
5	>100	44 ± 6	>2.3	-
8	15 ± 1	14 ± 1	~1	-
6	>100	>100	-	-
7	74 ± 6	14 ± 3	5.3	-
9	>50	>50	-	-
DKAs-GNPs (1)	0.96 ± 0.34	1.2 ± 0.85	0.8	>10
DKEs-GNPs (2)	2.5 ± 0.12	2.4 ± 0.1	~1	>10
[d]**LA-GNPs**	>5	>5	-	-
[e]**HL^2**	10 ± 8	0.14 ± 0.03	71.4	-

[a]IC_{50}: inhibitory concentration 50% (average data for inhibition of 3'-processing and strand transfer of purified IN), data is presented as mean ± SD from three independent experiments. [b]SI: Selectivity Index. [c]CC_{50}: Cytotoxic concentration 50% in MT-4 cells, determined by MMT assays. [d]Lipoic acid-coated GNPs. [e]Data from ref. 38.

Surprisingly, with respect to that showed from compounds **5** and **7**, as well as from the reference compound, the Boc-protected DKA **8** shared a similar inhibition profile for both catalytic activities (IC_{50s} = 15 ± 1 and 14 ± 1 μM for 3'proc and ST, respectively).

This behavior was translated to the GNPs, which exhibited inhibition profiles against both catalytic processes. Specifically, GNP-DKA **1** was the most potent compound

(IC_{50} values of the 1.2 ± 0.85 μM and 0.96 ± 0.34 μM for ST and 3'-proc, respectively). While the lipoic acid-coated GNPs (LA-GNPs) were not active (at >5 μM) as expected, GNPs-DKE **2** was approximately 2-fold less potent than the corresponding diketoacid **1**, thus demonstrating that the acid- and ester- terminal functionalities of the nanosystems similarly inhibited HIV-IN functions. Interestingly, these GNPs (i.e. **1** and **2**) did not display any selectivity for IN reactions, and did not show cytotoxicity in human MT-4 cells at 10 μM (Table 1).

In this study, we proposed the first application of small molecule (β-DKE/DKA)-coated GNPs as effective HIV-1 IN inhibitors. These nanoscale anti-IN prototypes demonstrated an improved IN inhibition potency against purified enzyme with respect to the free compounds. Analysis of preliminary results seems to support the hypothesis that these nanoprotopypes could act by interfering with both catalytic processes, thus competing with IN and DNA. Both the volume of the nanogold and the presence of multiple conjugated ligands in proximity of the enzyme active site can play a role in chelating the metal ions and in interfering with protein/DNA complex. Work is in progress to clarify the mechanism of action of these gold nanosystems, which can be useful for development of powerful nanogold-based antiretrovirals.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Experimental methods. Synthesis of DKA/DKE-GNPs (**1** and **2**) and LA-GNPs, TEM analyses, synthesis and characterization details of compounds **5-8**, HIV-1 IN inhibition and cytotoxicity (MTT) assays. (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors dedicate this work to the memory of the Prof. Maurizio Botta. The authors declare no competing financial interest.

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ABBREVIATIONS

HIV-1, Human Immunodeficiency Virus-1; IN, Integrase; DKAs, β-diketo acids; NP, nanoparticles; GNPs, gold nanoparticles; cDNA; dexoxyribonuleic acid; TEM, transmission electron microscopy; EDCI, *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide; DMAP, 4-(Dimethylamino)pyridine.

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SYNOPSIS TOC

Graphical representation of a GNP decorated with DKA ligands on its surface. DKA-coated GNPs as a nanoscale platform to construct novel HIV-1 IN multivalent therapeutics have been developed. These anti-IN prototypes demonstrated an improved IN inhibition potency against purified enzyme as compared with free DKA ligands.

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