Supporting Information

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

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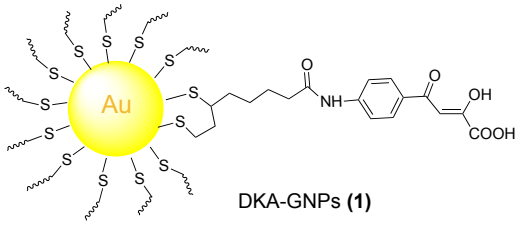
Contents

Experimental Section

Chemistry

All solvents, including anhydrous solvents, and chemicals, were purchased from Aldrich Co., Alfa Aesar, or Carlo Erba, and were used without further purification. All reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere using oven-dried glassware and syringes to transfer solutions. 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 a stationary phase. Melting points (mp) were determined using an Electrothermal melting point or a Köfler apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian XL-200 (200 MHz), or VXR-300 (300 MHz) or 400 MHz Bruker Avance III. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS), used as an internal standard. Splitting patterns are designated as follow: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; brs, broad singlet; dd, double doublet. The assignment of exchangeable protons (OH and NH) was confirmed by the addition of D_2O. Mass spectra were obtained on a Hewlett-Packard 5989 mass engine spectrometer, or a MALDI micro MX (Waters, Micromass) equipped with a reflectron analyzer. 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}). Transmission electron microscopy (TEM) observations were carried out at 100 kV (FEI Tecnai 120 kV). UV-Visible spectra was obtained on the UV-Visible Perkin-Elmer Lambda 35 spectrometer in the wavelength range from 200 to 800 nm. An estimation of the Au/S ratio was performed by using Induced Coupled Plasma - Optical Emission Spectroscopy (ICP-OES) tecnique on a Varian 720-ES instrument. The Au/S ratio estimation was assessed by using an Environmental Scanning Electron Microscope (ESEM) FEI Quanta 200 FEG, coupled with an electron dispersive spectroscope Oxford INCA detector, at the Laboratorio di Microanalisi – University of Florence. Elemental analyses for purity of compounds **4-9** were performed on a Perkin-Elmer Elemental Analyzer 2400-CHN at Laboratory of Microanalysis, Department of Chemistry and Pharmacy, University of Sassari (Italy), and were within ±0.4% of the theoretical values, thus confirming ≥95% purity.

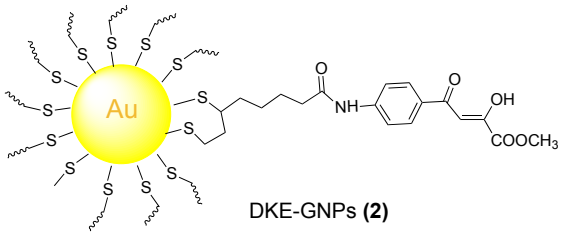
Preparation of DKA coated gold nanoparticles (DKA-GNPs) (1)



DKA-GNPs **(1)**

The hydrolysis of DKE-GNPs **(2)** was performed by the method reported by Sechi et al.[1] A solution of the DKE-GNPs (70 mg) in methanol (15 mL) was treated with 2N NaOH (1.35 mL) and stirred at room temperature for 5 h. After dilution with water, the reaction mixture was acidified with 1N HCl. The precipitate was collected by centrifugation, washed with ethanol, and dried under vacuum, to obtain a black powder (40 mg). Average size of gold nanoparticles DKE-GNP = ~3.7 nm. Elemental analysis calcd (%) for $[Au_{1434}(C_{18}H_{21}NO_5S_2)_{193}]$: Au, 78.72; S, 3.45, found: Au, 78.88; S, 3.77.

Preparation of DKE coated gold nanoparticles (DKE-GNPs) (2)

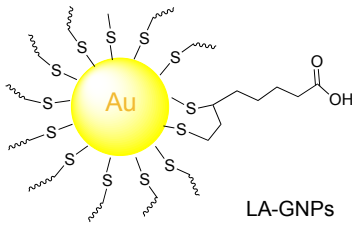


DKE-GNPs **(2)**

The synthesis of DKE-GNPs was carried out by modification of the method reported by Stiti et al.[2] To a solution of 38 mg of $NaBH_4$ and 3.0 mg of **9** in 10 mL of DMSO, a solution of $HAuCl_4 \cdot 4H_2O$ (25 mg) in 10 mL of DMSO was added. The reaction mixture turned deep purple immediately, and was stirred at room temperature for 2 hours. A black precipitate was collected by centrifugation, washed five times with ethanol, dried under vacuum and afforded 23 mg of black solid. Average size of gold nanoparticles DKE-GNP = ~3.7 nm. Elemental analysis calcd (%) for $[Au_{1434}(C_{19}H_{23}NO_5S_2)_{193}]$: Au, 78.13; S, 3.42, found: Au, 78.27; S, 3.38.

Preparation of lipoic acid coated gold nanoparticles (LA-GNPs)

To a solution of 40 mg of $NaBH_4$ and 4.0 mg of lipoic acid in 10 mL of DMSO, a solution of $HAuCl_4 \cdot 4H_2O$ (26 mg) in 10 mL of DMSO. The reaction mixture turned purple immediately, and it was stirred at room temperature for 2 hours. A black precipitate was collected by centrifugation, washed five times with ethanol, dried under vacuum and afforded 22 mg of black solid. Average size of LA-GNPs = ~3.3 nm. Elemental analysis was in agreement with $[Au_{992}(C_8H_{14}O_2S_2)_{124}]$.



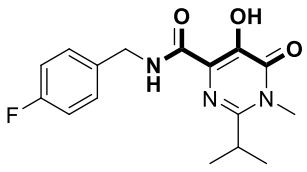
LA-GNPs

Transmission electron microscopy (TEM) analysis

The diameter and homogeneity of the GNPs were characterized using TEM (FEI Tecnai 120 kV) apparatus operating at 100 kV accelerating voltage. TEM measurements were assessed by dissolving GNP suspension in ethanol (30 µL of a diluted 50% nanoparticle solution), and placing a drop onto a carbon Formvar coated copper TEM grid, incubated for 1 min., then side-blotted with Whatman #1 filter paper, followed by evaporation of the remaining solvent. The resulting images were analyzed using Image Pro Analyzer, and at least 100 GNPs were evaluated per sample.

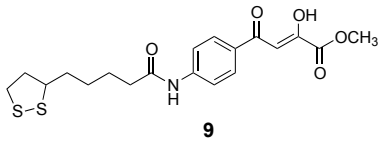
Structure of reference compound HL

For details see in ref. 3.



HL

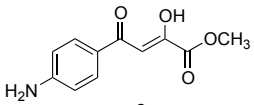
Synthesis of (Z)-methyl 4-(4-(5-(1,2-dithiolan-3-yl)pentanamido)phenyl)-2-hydroxy-4-oxobut-2-enoate (9)



9

A solution of of α-lipoic acid (0.233 g mg 0.00113 mol, 1 eq.) in 3 mL of dimethylacetamide, (0.259 g, 0.00136 mol, 1.2 eq.) of EDCI, and a catalytic amount of DMAP, were stirred at room temperature for 1 h. Next, DKE **6** (0.250 g, 0.00113 mol, 1 eq.) was added, and the mixture was stirred at room temperature for 3 h. Then, the reaction was diluted with water and extracted twice by ethyl acetate. After evaporation *in vacuo*, the residue was purified on silica gel using a mixture ethyl acetate – petroleum ether (1:1) as eluent to afford a yellow oil. Yield: 100%. IR (nujol): v (cm^{-1}) 2923, 2724, 1729, 1691. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.78 (d, 2H, Ar-H), 7.39 (s, 1H, Olefinc-H), 6.65 (d, 2H, Ar-H), 4.22 (brs, 1H, COOH), 3.87 (s, 3H, $COOCH_3$), 3.62 (m, 1H), 3.45 (m, 2H), 3.15 (m, 2H), 2.60-2.38 (m, 2H), 2.03 (t, 2H), 1.85 (m, 2H), 1.31 (m, 2H). MS (EI): *m/z* [M^+ 409], 162 (100%). Anal. Calc. for ($C_{19}H_{23}NO_5S_2$): C, 55.72; H, 5.66; N, 3.42. Found: C, 55.46; H, 5.79; N, 3.55.

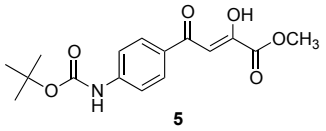
Synthesis of (Z)-methyl 4-(4-aminophenyl)-2-hydroxy-4-oxobut-2-enoate (6)



6

A mixture of Boc-protected DKE (**5)** (0.1 g, 0.0003 mol) and formic acid (2.5 mL) was stirred at room temperature for 1 hour. The mixture was washed by sodium carbonate solution (until no effervescence evolved), and then extracted by CH_2Cl_2 several times, dried over anhydrous $MgSO_4$, followed by evaporation of the organic layer under reduced pressure to give a red powder. The crude product was purified by silica gel column chromatography eluting with petroleum ether-ethyl acetate (1:1) to afford a red powder. Yield: 95%. M.p. 148-149 °C. IR (nujol): v (cm^{-1}) 3444, 2925, 2724, 1745. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 15.64 (brs, 1H, OH), 7.89-7.85 (d, 2H, Aromatic-H), 6.99 (s, 1H, Olefinc-H), 6.71-6.66 (d, 2H, Aromatic-H), 4.30 (brs, 2H, NH_2), 3.93 (s, 3H, $COOCH_3$). ^{13}C-NMR (DMSO-d_6): ppm 189.2, 165.6, 162.6, 155.4, 130.9, 129.6, 120.8, 113.1, 97.3, 52.8. MS (GC-MS): *m/z* [M^+ 221], 162 (100%). Anal. Calc. for ($C_{11}H_{11}NO_4$): C, 59.73; H, 5.01; N, 6.33. Found: C, 59.87; H, 5.22; N, 6.44.

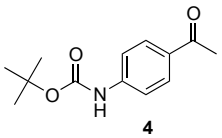
Synthesis of (Z)-methyl 4-(4-((tert-butoxycarbonyl)amino)phenyl)-2-hydroxy-4-oxobut-2-enoate (5)



5

A solution of Boc-protected-aminoacetophenone (**4**, 0.93 g, 0.004 mol) in THF (10 mL) added to a solution of freshly prepared sodium methoxide (sodium metal 0.29 g, 0.0126 mol, 3.15 eq. in 10 mL of dry methanol), further dimethyl oxalate (0.61 g, 0.005 mol, 1.25 eq.) was added to the mixture and refluxed under nitrogen atmosphere for 2 h. The mixture was cooled at room temperature and diluted with cold water. The solution was acidified with 1N HCl. The yellow precipitate formed was filtered off, washed with water and dried. Yield: 82%. M.p. 70-71 °C. IR (nujol): v (cm^{-1}) 3504, 2927, 2724, 1743, 1704, 1639. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 15.65 (brs, 1H, OH), 7.99-7.94 (d, 2H, Aromatic-H), 7.53-7.48 (d, 2H, Aromatic-H), 7.05 (s, 1H, Olefinic-H), 6.79 (brs, 1H, NH), 3.94 (s, 3H, $COOCH_3$), 1.53 (s, 9H, $C(CH_3)_3$). ^{13}C-NMR ($CDCl_3$): ppm 196.3, 190.0, 168.0, 152.0, 143.8, 129.5, 118,1, 117.6, 97.9, 81.5, 53.2, 29.7, 28.2. MS (EI): *m/z* [M^+ 321], 206 (100). Anal. Calc. for ($C_{16}H_{19}NO_6$): C, 59.81; H, 5.96; N, 4.36. Found: C, 59.77; H, 5.89; N, 4.42.

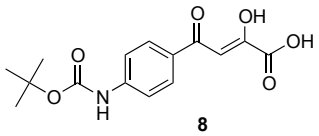
Synthesis of tert-butyl (4-acetylphenyl)carbamate (4)



4

A mixture of *p*-aminoacetophenone (**3**, 1 g, 0.0074 mol) and di-*tert*-butyl dicarbonate (1.78 g, 0.0081 mol, 1.09 eq.) in THF (10 mL) was refluxed for 5 h. After dilution with diethyl ether (25 mL), the mixture was washed sequentially with saturated aqueous citric acid solution, 1N $NaHCO_3$, and brine. The organic layer was dried over anhydrous $MgSO_4$ and evaporated to give white crystals. Yield: 94%. M.p. 114-115 °C. IR (nujol): v (cm^{-1}) 3282, 3056, 2911, 1718, and 1671. ^{1}H-NMR (200MHz, $CDCl_3$): δ 7.94-7.89(d, 2H, Aromatic-H), 7.48-7.45(d, 2H, Aromatic-H), 6.75 (brs, 1H, NH), 2.56(s, 3H, $COCH_3$), 1.53 (s, 9H, $C(CH_3)_3$). ^{13}C-NMR ($CDCl_3$): ppm 196.9, 152.2, 143.0, 131.7, 129.8, 117.3, 81.8, 28.2, 26.3. MS (EI): *m/z* [M^+ 235], 205, 180, 179 (100%). Anal. Calc. for ($C_{13}H_{17}NO_3$): C, 66.36; H, 7.28; N, 5.95. Found: C, 66.52; H, 7.02; N, 6.23.

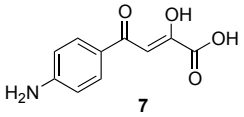
Synthesis of (Z)-4-(4-((tert-butoxycarbonyl)amino)phenyl)-2-hydroxy-4-oxobut-2-enoic acid (8)



8

A solution of the diketoester **5** (0.32 g, 0.001 mol) in methanol (15 mL) was treated with 2N NaOH (0.16 g, 0.004 mol, 4 eq., 2 mL) and stirred at room temperature for 5 h. After dilution with water, the reaction mixture was acidified with 1N HCl. The yellow precipitate that formed was filtered off, and washed with water, and dried to obtain the desired product. Yield: 68%; M.p. 298-299 °C. IR (nujol): v (cm^{-1}) 3517, 2846, 2724, 1733, 1689, 1604. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.99-7.93(d, 2H, Aromatic-H), 7.54-7.49(d, 2H, Aromatic-H), 7.05 (s, 1H, Olefinc-H), 6.85 (s, 1H, NH), 1.54 (s, 9H, $C(CH_3)_3$). ^{13}C-NMR (DMSO-*d_6*, $CDCl_3$): ppm 189.8, 168.17, 163.36, 152.15, 144.49, 128.6, 117.23, 97.1, 79.97, 52.47, 27.72. MS (EI): *m/z* [M^+ 307], 206 (100%). Anal. Calc. for ($C_{15}H_{17}NO_6$): C, 58.6; H, 5.58; N, 4.56. Found: C, 58.45; H, 5.65; N, 4.62.

Synthesis of (Z)-4-(4-aminophenyl)-2-hydroxy-4-oxobut-2-enoic acid (7)



7

A solution of 4M HCl/Dioxane (a new Aldrich sureseal bottle) (13 mL) in a 50 mL round-bottom flask equipped with a magnetic stirrer was cooled by an ice-water bath under nitrogen. The diketoester **6** (0.2 g, 0.0007 mol) was added in one portion with stirring. The ice-bath was removed and the mixture was kept on stirring. After 30 min, TLC indicated that the reaction was complete. The reaction mixture was concentrated under high vacuum at room temperature. The residue was then washed with diethyl ether and collected by filtration to give dark brown powder. Yield: 89%. M.p. 300 °C (dec). IR (nujol): v (cm^{-1}) 3363, 2925, 2724, 1737, 1716. ^{1}H-NMR (DMSO-*d_6*): δ 15.65 (brs, 1H, COOH), 7.83-7.88 (d, 2H, Aromatic-H), 6.93 (s, 1H, Olefinic-H), 6.60-6.65 (d, 2H, Aromatic-H), 4.93 (brs, 2H, NH_2). MS (GC-MS): *m/z* [M^+ 207], 167, 149 (100%). Anal. Calc. for ($C_{10}H_9NO_4$): C, 57.97; H, 4.38; N, 6.76. Found: C, 57.78; H, 4.22; N, 6.81.

HIV-1 IN inhibition assays

Schematic of IN activity in vitro is shown in Table 1: a 21-mer oligonucleotide corresponding to the U5 LTR 5'-end-labeled with 32P is reacted with purified IN. The first step, 3'-processing, involves nucleolytic cleavage of two bases from the 3'-end resulting in a 19-mer oligonucleotide. The 3'-ends are subsequently covalently joined at several sites to another identical oligonucleotide (i.e. strand transfer reaction) that serves as the target DNA.

Biological Materials, Chemicals, and Enzymes. All compounds were dissolved in DMSO and the stock solutions were stored at –20 °C. The $\gamma[^{32}P]$-ATP was purchased from PerkinElmer. The expression system for wild-type IN was a generous gift from Dr. Robert Craigie, Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD.

Preparation of Oligonucleotide Substrates. The oligonucleotides 21top, 5'-GTGTGGAAAATCTCTAGCAGT-3' and 21bot, 5'-ACTGCTAGAGATTTTCCACAC-3' were purchased from Norris Cancer Center Core Facility (University of Southern California) and purified by UV shadowing on polyacrylamide gel. To analyze the extent of 3'-processing and strand transfer using 5'-end labeled substrates, 21top was 5'-end labeled using T_4 polynucleotide kinase (Epicentre, Madison, WI) and $\gamma[^{32}P]$-ATP (Amersham Biosciences or ICN). The kinase was heat-inactivated and 21bot was added in 1.5-molar excess. The mixture was heated at 95 °C, allowed to cool slowly to room temperature, and run through a spin 25 mini-column (USA Scientific) to separate annealed double-stranded oligonucleotide from unincorporated material.

Integrase Assays. To determine the extent of 3'-processing and strand transfer, wild-type IN was preincubated at a final concentration of 200 nM with the inhibitor in reaction buffer (50 mM NaCl, 1 mM HEPES, pH 7.5, 50 μM EDTA, 50 μM dithiothreitol, 10% glycerol (w/v), 7.5 mM $MnCl_2$, 0.1 mg/ml bovine serum albumin, 10 mM 2-mercaptoethanol, 10% DMSO, and 25 mM MOPS, pH 7.2) at 30°C for 30 min. Then, 20 nM of the 5'-end ^{32}P-labeled linear oligonucleotide substrate was added, and incubation was continued for an additional 1h. Reactions were quenched by the addition of an equal volume (16 μL) of loading dye (98% deionized formamide, 10 mM EDTA, 0.025% xylene cyanol and 0.025% bromophenol blue). An aliquot (5 μL) was electrophoresed on a denaturing 20% polyacrylamide gel (0.09 M tris-borate pH 8.3, 2 mM EDTA, 20% acrylamide, 8M urea). Gels were dried, exposed in a PhosphorImager cassette, analyzed using a Typhoon 8610 Variable Mode Imager (Amersham Biosciences) and quantitated using ImageQuant 5.2.

Percent inhibition (% I) was calculated using the following equation:
% I = 100 X [1 - (D - C)/(N - C)], where *C*, *N*, and *D* are the fractions of 21-mer substrate converted to 19-mer (3'-proc product) or ST products for DNA alone, DNA plus IN, and IN plus drug, respectively. The IC_{50} values were determined by plotting the logarithm of sample concentration *versus* percent inhibition to obtain concentration that produced 50% inhibition.

Cytotoxicity (MTT) assays

Cytotoxicity was assessed by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay Briefly, Cytotoxicity of compounds was evaluated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[4] Cells were placed in 96-well plate at 3000–8000 cells/well. After the cells were allowed to attach overnight, compounds were added to the wells at sequential dilutions (30 nM–10 μM). After 72 h of treatment, MTT was added into the media to a final concentration of 300 μg/mL. Cells were incubated for 3 h at 37 °C, and the insoluble formazan converted by viable cells were dissolved in 150 μL of DMSO. After removal of the medium, DMSO was added and the absorbance was read at 570 nm. All assays were done in triplicate. The IC_{50} was then determined for each drug from a plot of log (drug concentration) versus percentage of cells killed. Absorbance at 570 nm was read by microplate reader (Molecular devices, Sunnyvale, CA), and inhibition of cell proliferation was calculated using the following formula: Inhibition of cell proliferation (%) = $(1 - OD_{treatment} / OD_{control})$ Å~ 100%.

References

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