Supporting Information

NO photoreleaser-deoxyadenosine and -bile acid derivatives bioconjugates as novel potential photo-chemotherapeutics

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Content:

General

The reactions for the synthesis of **photocage-CDC** and **photocage-UDC** were monitored by TLC on pre-coatedSilica Gel plates (thickness 0.25 mm, Merck), and phosphomolybdicacid solution was used as the spray reagent to visualize the steroids. The reactions for the synthesis of intermediates **2**, **4**, **6**, **7, 8** and of **photocage-SdAdo** were monitored by HPC-MS.

Flash column chromatography was performed on silica gel 60 (2300 (23mesh) or with a combiflash apparatus.

The microwave (MW) irradiation was performed using a Biotage Initiator apparatus. Optimization experiments were performed in the 'single-run' mode, i.e., by manual filling of reaction vials and by specifyingthe irradiation time and maximum temperature. Melting pointswere determined using a capillary apparatus.

HPLC-MS analyses were performed on a Agilent 1260 with a diode array detector using a Zorbax C8 column (4.6 × 150 mm, 5 μm) with a linear gradient water/acenitrile at a 0.5 mL/min flow rate, detection at λ = 260 nm and an Esquire 3000 Plus Bruker mass spectrometer.

ESI-HRMS were acquired on an Agilent Dual ESI Q TOF 6520 using methanol.

NMR spectra were recorded with a Varian Mercury 400 MHz instrument.

UV/vis absorption and fluorescence spectra were recorded with a Jasco V 650 spectrophotometer. Irradiation experiments were performed in a quartz cell (1 cm path length, 3 mL capacity) with RPR lamps with emission centered at λ = 420 nm in a Rayonet photochemical reactor.

NO release was measured with a World Precision Instrument, ISO-NO meter, equipped with a data acquisition system, and based on direct amperometric detection of NO with short response time (< 5 s) and sensitivity range 1 nM–20 μM. The analog signal was digitalized with a four-channel recording system and transferred to a computer. The sensor was accurately calibrated by mixing standard solutions of $NaNO_2$ with 0.1 M H_2SO_4 and 0.1 M KI according to the reaction:

$$4H^+ + 2I^- + 2NO_2^- \rightarrow 2H_2O + 2NO + I_2$$

Irradiation was in a quartz cell (1 cm path length, 3 mL capacity) NO measurements were carried out under stirring with the electrode positioned outside the light path, to avoid NO signal artefacts due to photoelectric interference on the ISO-NO electrode.

Synthetic procedures and characterizations

The intermediates **2**, **4**, **6**, **7** and **8** were characterized by LC- MS, ^{1}H NMR and ^{13}C NMR. In the case of bioconjugates **photocage-SdAdo**, **photocage-CDC** and **photocage-UDC** also HRMS was performed.

Photocage-SdAdo. 5-(4-Nitro-trifluoromethyl)phenylamino-pentane-1-thiol **2** (3 mmol, 920 mg) and triethylamine (1.5 mL, 10 mmol) were added to a 1.6 mM suspension of commercial 8-bromo-2'-deoxyadenosine **1** (330 mg, 1.0 mmol) in water. The resulting solution was heated at 100° C for 2 h. The warm reaction mixture was extracted with ethylacetate (2 x 100 mL) and the solvent evaporated under reduced pressure. The target compound was obtained in 20 % yield.

^{1}H NMR (CD_3OD) δ 1.59 (2H, m), 1.69 (2H, m), 1.84 (2H, m), 2.21 (1H, ddd, J1 = 1.6 Hz, J2 = 5.6 Hz, J3 = 13.0 Hz, collapsing to dd J = 5.6 Hz, J = 13 Hz upon irradiation at δ 4.60; collapsing to dd J = 1.6 Hz, J = 13.0 Hz upon irradiation at δ 6.38;), 2.99 (1H, ddd, J1 = 5.6 Hz, J2 = 9.0 Hz, J3 = 13.0 Hz, collapsing to dd J = 5.6 Hz, J = 13 Hz upon irradiation at δ 4.60; collapsing to dd J = 9.0 Hz, J = 13.0 Hz upon irradiation at δ 6.38;), 3.20 (2H, t, J = 6.8), 3.36 (2H, t, J = 8.4), 3.80 (2H, ABX system, J_{AB} = 13 Hz, J_{AX} = 3 Hz, collapsing to AB system upon irradiation at δ 4.08), 4.08 (1H, m, collapsing to t J = 3.0 Hz, upon irradiation at δ 4.60), 4.60 (1H, m, collapsing to dd, J1 = 1.6, J2 = 6.0 Hz, upon irradiation to δ 4.08), 6.38 (1H, dd, J1 = J2 = 5.6), 6.70 (1H, dd, J1 = 3 Hz, J2 = 9 HZ), 6.94 (1H, d, J1 = 3 Hz), 7.96 (1H, d, J1 = 9 Hz), 8.05 (1H, s); ^{13}C (CD_3OD) δ 26 (CH_2), 28 (CH_2), 29 (CH_2), 32 (CH_2), 39 (CH_2), 42 (CH_2), 63 (CH_2), 72 (CH), 86 (CH), 89 (CH), 129 (CH), 151 (CH), 120 (q), 121 (q), 150 (q), 151 (CH), 153 (q), 154 (q). HRMS of [M + H]+ ions: calculated for $C_{22}H_{26}F_3N_7O_5S$ 558,1668, found 558,1667.

5-(4-Nitro-trifluoromethyl-phenylamino)pentan-1-olo (6). 5-Amino-pentan-1-olo (2 mmol, 206 mg) and K_2CO_3 (150 mg) were added to a solution of 4-chloro-1-nitro-2-trifluoromethyl benzene **5** (1 mmol, 225 mg, 0.15 mL) in DMSO dry (10 mL) in a sealed tube. The mixture was stirred at 85 °C for 24 h. The reaction mixture was extracted with ethyl acetate (2 x 15 mL) and washed with water/brine 1:1 solution (5 x 25 mL). The organic phase was dried over sodium sulfate and evaporated to dryness under reduced pressure. The residue was purified on silica gel by gradient elution from petroleum ether 100% to petroleum ether/ethyl acetate 40/60 to obtain the target compound (0.6 mmol, 175 mg, 60% yield). ^{1}H NMR ($CDCl_3$) δ 1.50 (2H, m), 1.62 (2H, m), 1.70 (2H, m), 3.22 (2H, t, J = 4.5), 3.67 (2H, J = 4.0), 6.64 (1H, dd, J1 = 1.5, J2 = 5.5), 6.87 (1H, d, J = 1.5), 7.98 (1H, d, J = 5.5); ^{13}C ($CDCl_3$) (selected data): δ 23 (CH_2), 29 (CH_2), 32 (CH_2), 44 (CH_2), 63 (CH_2), 112 (CH), 113 (CH), 121 (q), 124 (q), 129 (CH), 152 (q). ESI-MS (m/z) (ES +) 293 (M+1), 315 (M+23), 331 (M+39).

(5-Bromo-pentyl)-(4-nitro-trifluoromethyl-phenyl) amine (7). Compound **6** (1 mmol, 290 mg) was dissolved in fluorobenzene (10 mL) and PBr_3 1 M solution in dichloromethane was added dropwise (3 eq, 3 mL). The reaction mixture was left under stirring overnight and then evaporated to dryness under reduced pressure. The residue was suspended in water and NaOH 0.2 M was added up to pH = 12. The water solution was extracted with ethyl acetate (2 x 15 mL) and the organic layer washed with water/brine 1:1 solution (2 x 10 mL). The organic phase was dried over sodium sulfate and evaporated to dryness. The residue was purified on silica gel by gradient elution from petroleum ether 100% to petroleum ether/ethyl acetate 80/20 to obtain the target compound (0.5 mmol, 180 mg, 50% yield). ^{1}H NMR ($CDCl_3$) δ 1.60 (2H, m), 1.71 (2H, m), 1.92 (2H, m), 3.25 (2H, t, J = 7.0), 3.43 (2H, J = 6.5), 6.68 (1H, dd, J1 = 2.5, J2 = 8.8), 6.87 (1H, d, J = 2.5), 7.98 (1H, d, J = 9.2); ^{13}C ($CDCl_3$) δ 26 (CH_2), 28 (CH_2), 32 (CH_2), 33 (CH_2), 44 (CH_2), 112 (CH), 113 (CH), 121 (q), 124 (q), 129 (CH), 152 (q). ESI-MS (m/z) (ES +) 355, 357 (M+1).

S-[5-(4-nitro-trifluoromethyl-phenylamine) penthyl] ester (**8).** Compound **7** (1 mmol, 355 mg) was dissolved in DMF dry (10 mL) in a sealed tube. Potassium tioacetate (1.3 mmol, 150 mg) was added and the mixture was stirred at 50 °C for 4 h. The reaction mixture was extracted with ethyl acetate (2 x 15 mL) and washed with water/brine 1:1 solution (5 x 25 mL). The organic phase was dried over sodium sulfate and evaporated to dryness under reduced pressure. The residue was purified on silica gel by gradient elution from petroleum ether 100% to petroleum ether/ethyl acetate 85/15 to obtain the target compound (0.8 mmol, 280 mg, 80% yield). ^{1}H NMR ($CDCl_3$) δ 1.48 (2H, m), 1.71 (2H, m), 1.66 (4H, m), 2.34 (3H, s), 2.89 (2H, t, J = 7.2), 3.21 (2H, t, J = 6.8), 6.65 (1H, dd, J1 = 2.8, J2 = 9.0), 6.88 (1H, d, J = 2.8), 7.98 (1H, d, J = 9.0); ^{13}C ($CDCl_3$) δ 26 (CH_2), 28 (CH_2), 29 (CH_2), 30 (CH_2), 31 (CH_2), 44 (CH_3), 111 (CH), 113 (CH), 121 (q), 124 (q), 129 (CH), 152 (q), 196 (CO). ESI-MS (m/z) (ES +) 351(M+1), 373 (M+23).

5-(4-nitro-trifluoromethyl-phenylamine)-pentane-1-thiol (2). Compound **8** (1 mmol, 350 mg) was dissolved in CH_3OH (50 mL) and cooled down to -78 °C. CH_3COBr (20 mmol, 2 mL) was added dropwise. The reaction mixture was warmed up at room temperature and left under stirring for 4 h. The solvent was evaporated to dryness under reduced pressure and the residue was solved in ethyl acetate and washed with water in order to reach pH = 7. The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was used without further purification (0.95 mmol, 330 mg, 95% yield). ^{1}H NMR ($CDCl_3$) δ 1.35 (1H, t, J = 5.0 Hz; disappeared upon D_2O shake. SH signal), 1.53 (2H, m), 1.68 (4H, m), 2.56 (2H, q, J1 = 4.5 Hz, J2 = 5.0 Hz), 3.23 (2H, J = 4.2), 6.65 (1H, dd, J1 = 1.8, J2 = 5.5), 6.88 (1H, d, J = 1.8), 8.00 (1H, d, J = 5.5); ^{13}C ($CDCl_3$) δ 25 (CH_2), 26 (CH_2), 29 (CH_2), 33 (CH_2), 44 (CH_2), 111 (CH), 113 (CH), 121 (q), 124 (q), 129 (CH), 152 (q). ESI-MS (m/z) (ES +) 309 (M+1).

(4-nitro-trifluoromethyl-phenyl)-pent-4-inyl-amine (4). 4-Pentyn-1-amine (2 mmol, 206 mg) and K_2CO_3 (150 mg) were added to a solution of 4-chloro-1-nitro-2-trifluoromethyl benzene **5** (1 mmol, 225 mg, 0.15 mL) in DMSO dry (10 mL) in a sealed tube. The mixture was stirred at 85 °C for 24 h. The reaction mixture was extracted with ethyl acetate (2 x 15 mL) and washed with water/brine 1:1 solution (5 x 25 mL). The organic phase was dried over sodium sulfate and evaporated to dryness under reduced pressure. The residue was purified on silica gel by gradient elution from petroleum ether 100% to petroleum ether/ethyl acetate 80/20 to obtain the target compound (0.65 mmol, 340 mg, 65% yield). ^{1}H NMR ($CDCl_3$) δ 1.48 (2H, m), 1.71 (2H, m), 1.86 (2H, m), 2.05 (1H, t, J = 2.8), 2.35 (2H, dt, J_d = 2.8, J_t = 6.8; collapsing to d J = 2.8 upon irradiation at δ = 1.87), 3.40 (2H, t, J = 6.8; collapsing to s upon irradiation at δ = 1.87), 6.68 (1H, dd, J1 = 2.8, J2 = 9.2), 6.90 (1H, d, J = 2.8), 8.00 (1H, d, J = 9.2); ^{13}C ($CDCl_3$) δ 16 (CH_2), 27 (CH_2), 43 (CH_2), 70 (CH), 83 (q), 113 (CH), 121 (q), 124 (q), 127 (q), 129 (CH), 152 (q). ESI-MS (m/z) (ES +) 273 (M+1).

General procedure for the click reaction

To a solution of the appropriate alkyne (0.03 mmol) in 1.4 ml of a 1:1:1.5 mixture of H_2O/*t*-BuOH/THF (v/v), sodium ascorbate (0.06 mmol), $CuSO_4 \cdot 5H_2O$ (0.012 mmol), and the proper azide **3a,b** (0.04 mmol) was added. The resulting mixture was premixed for 30 s, then heated in a sealed glass tube in a Biotage Initiator microwave apparatus at 80 °C for 30 min. After cooling at room temperature, solvents were removed in vacuo and the crude material was purified by flash chromatography on silica gel with cyclohexane/EtOAc 2:1 and AcOH 0.1%, as an eluent.

Photocage-UDC. Yellow amorphous solid, yield 66%. ^{1}H NMR (DMSO-d_6): δ = 11.50 (1H, bs), 8.05 (1H, d, J = 9.5), 7.99 (1H, s, 5-H triazole), 7.63 (1H, t, J = 5), 7.03 (1H, bs), 6.79 (1H, dd, J = 9.5, 2.0), 4.43-4.32 (1H, m), 4.90 (1H, bs, D_2O ex), 3.40-3.27 (2H m), 3.23 (2H, dt, J = 7.5, 5.0), 2.69 (2H, t, J = 7.5), 2.35-2.15 (1H, m), 2.13-2.01 (2H, m), 1.93-0.95 (24H, m), 0.92 (3H, s), 0.86 (3H, d, J = 6.5), 0,60 (3H, s). ^{13}C NMR (DMSO-d_6): δ = 174.9, 153.2, 145.8, 133.4, 129.8, 126.6, 124.9 (q, CF3), 123.9, 121.1, 119.9, 69.1, 59.5, 55.4, 54.7, 43.0, 42.5, 41.8, 39.5, 38.4, 37.3, 35.0, 34.8, 34.1, 33.8, 30.73, 30.72, 28.1, 27.8, 27.7, 26.6, 23.1, 22.6, 20.9, 18.3, 12.0. HRMS of $[M - H]^-$ ions: calculated for C36H50F3N5O5 688,3764, found 688,3762.

Photocage-CDC. Yellow amorphous solid, yield 68%. ^{1}H NMR (CD_3OD):δ 8.01 (1H, d, J = 9.1 Hz), 7.82 (1H, s), 6.96 (1H, d, J = 2.5 Hz), 6.72 (1H, d, J = 9.2 Hz), 4.41-4.29 (1H, m), 3.80 (1H, d, J = 2.5 Hz), 3.25 (2H, t, J = 6.9 Hz), 2.84-2-72 (3H, m), 2.39-2.27(1H, m), 2.24-2.14 (1H, m), 2.06-1.05 (32H, m), 1.00 (3H, s), 0.95 (3H, d, J = 6.5 Hz), 0.69 (3H, s). ^{13}C NMR (CD_3OD) δ = 178.08, 154,32, 147.77, 136.13, 127.10 (q, CF3) 126.13, 125.20, 122.20, 121.10, 112.67, 112.17, 68.87, 62.67, 57.34, 51.54, 43.57, 43.41, 43.17, 40.91, 40.68, 38.16, 36.85, 36.76, 36.33, 35.60, 34.09,

32.35, 32.07, 29.40, 29.24, 24.55, 23.72, 23.28, 21.78, 18.79, 12.19. HRMS of $[M - H]^-$ ions: calculated for C36H50F3N5O5 688,3764, found 688,3767.

Biological assay

Cell growth inhibition assays were carried out using the leukemia cell line K562 and colon carcinoma HCT116. Cell lines were obtained from ATCC (Manassas, VA) and maintained in RPMI 1640, supplemented with 10% fetal bovine serum (FBS), penicillin (100 Units mL-1), streptomycin (100 μg mL-1) and glutamine (2mM) (complete medium); the pH of the medium was 7.2 and the incubation was performed at 37 °C in a 5% CO_2 atmosphere. Adherent cells were routinely used at 70% of confluence and passaged every 3 days by treatment with 0.05% Trypsin-EDTA (Lonza). K562 cells were routinely fed every 3 days. The antiproliferative activity of the compounds was tested with 3-(4,5-dimethylthiozol-2-yl)2,5-diphenyltetrazolium bromide solution (MTT) assay. K562 and HCT116 were seeded in triplicate in 96-well trays respectively at the density of $5 \cdot 10^3$ and 10^4 in 50 μL of complete medium. Stock solutions (50 mM) of each compound were made in DMSO and diluted in complete medium to give final concentrations of 50, 25 and 10 μM. Untreated cells were placed in every plate as a negative control. The cells were exposed to the compounds, in 100 μL total volume, for 72 hours.

The photocytotoxicity experiments were carried out by irradiating cells, incubated with photoactive components, with the irradiation source described above for 40 min. In this case, cell viability was measured after 72 h. After each incubation time 25 μL of a 12 mM solution of MTT were added. After two hours of incubation, 100 μL of lysing buffer (50% DMF + 20% sodium dodecyl sulfate (SDS), pH 4.7) were added to convert the MTT solution into a violet colored formazane. After additional 18 hours the solution absorbance, proportional to the number of live cells, was measured by spectrophotometer at 570 nm and converted into % of growth inhibition.