Supplementary information

Photosensitized DNA cleavage promoted by amino acids

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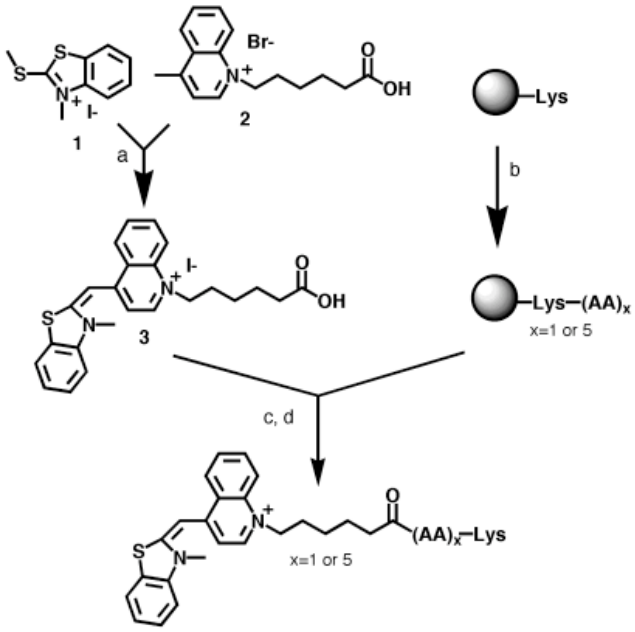
Experimental procedures

A. Synthesis of TO-peptide conjugates



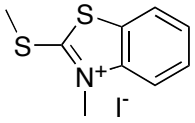
(a) TEA/ EtOH/ 50°C/ 3h/ $(CH_3)_2CO$/ Et_2O/ 2h. (b) 20% piperidine:DMF/ Fmoc-AA/ HBTU/ DIPEA/ DMF/ 23°C/ 3h. (c) **3**/ HBTU/ DIPEA/ DMF/ 23°C/ 3h. (d) TFA:DCM (1:1)/rt/30 min. See supporting information for synthesis details.

General Procedures

Solvents were purchased from Fisher and reagents were purchased from Aldrich Chemical Co. or Acros Organics. Amino acids were purchased from Advanced ChemTech. Both solvents and reagents were used without further purification. Reverse phase HPLC was performed on a Varian 250 x 4.6 mm stainless steel column packed with Microsorb-MV 300 C18 (5 μm). A flow rate of 1.0 mL/min. was used with an

aqueous solution buffered with 50 mM ammonium acetate and a linear gradient from 20 to 100% acetonitrile over 80 min. ^{1}H NMR spectra were recorded on a Varian 400 MHz spectrometer. Proton chemical shifts are reported in ppm (δ) relative to the solvent reference relative to tetramethylsilane (TMS) (d_6-DMSO, δ 2.50). Data are reported as follows: chemical shift (multiplicity [singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m)], coupling constants [Hz], integration). Carbon NMR spectra were recorded on a Varian 500 (125 MHz) spectrometer with complete proton decoupling. Carbon chemical shifts were reported in ppm (δ) relative to TMS with the respective solvent resonance as the internal standard (DMSO, 39.5). Mass spectral analysis was performed by the Boston College Mass Spectrometry Facility. Samples were analyzed by accurate mass electrospray mass spectrometry (ES-MS) operating in positive mode on a Micromass LCT mass spectrometer. Absorbance spectroscopy was performed on a Hewlett Packard 8452A Diode Array Spectrophotometer.

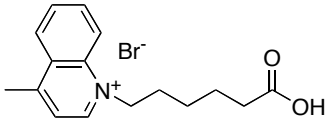
Methods



Preparation of compound 1

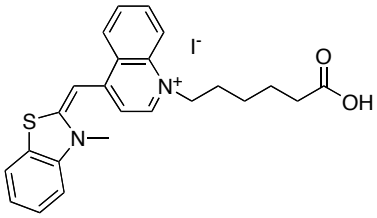
3-methylbenzothiazole-2-thione (2.00 g, 11.0 mmol, 1.00 equiv.) and methyl iodide (3.00 g, 21.1 mmol, 1.92 equiv.) were combined in a 50 mL round bottom flask sealed with a rubber septa and heated to 45 ^{O}C for 5 hours. The reaction mixture became a white solid. After cooling to 25 ^{O}C, the solid was resuspended in 100 mL methanol. Diethyl ether (75 ml) was added to ensure that the product had fully precipitated. The precipitate was collected by vacuum filtration and dried under reduced pressure to yield 2.74 g (77%) of a white solid. **UV-Vis**: λ_{max} (H_2O): 226 nm (ε= 24,700 $M^{-1}cm^{-1}$), 306 nm (ε= 17,200 $M^{-1}cm^{-1}$); **^{1}H NMR** (400 MHz, DMSO) δ 3.13 (s, 3H, SCH_3), 4.12 (s, 3H, NCH_3), 7.73 (m, 1H, Ar), 7.85 (m, 1H, Ar), 8.20 (d, J= 8.42 Hz, 1H, Ar), 8.40 (d, J= 8.42 Hz, 1H, Ar); **Exact mass** calculated for [$C_9H_{10}NS_2^+$] requires *m/z* 192.0255. Found 192.0256 (ESI+).

Preparation of compound 2



Lepidine (2.21 g, 15.5 mmol, 1.00 equiv.) and 6-bromohexanoic acid (3.29 g, 16.9 mmol, 1.09 equiv.) were combined in a 100 mL round bottom flask sealed with a rubber septa and heated at 125 ^{O}C for 5 hours. The resulting brown residue was dissolved in 28 mL of methylene chloride and the product was precipitated upon addition of 16 mL of acetone. The precipitate was collected by vacuum filtration and dried under reduced pressure to yield 2.87 g (55%) of a tan solid. **UV-Vis**: λ_{max} (H_2O) 234 nm (ε= 33,200 $M^{-1}cm^{-1}$), 314 nm (ε= 8,300 $M^{-1}cm^{-1}$); **^{1}H NMR** (400 MHz, DMSO) δ 1.40 (m, 2H, CH_2), 1.56 (m, 2H, CH_2), 1.96 (m, 2H, CH_2), 2.23 (t, J= 7.2 Hz, 2H, CH_2), 3.01 (s, 3H, CH_3), 5.01 (t, J= 7.5 Hz, 2H, NCH_2), 8.07 (m, 2H, Ar), 8.27 (m, 1H, Ar), 8.58 (m, 2H, Ar), 9.42 (d, J= 5.85 Hz, 1H, Ar); **Exact mass** calculated for [$C_{16}H_{20}NO_2^+$] requires *m/z* 258.1494. Found 258.1498 (ESI+).

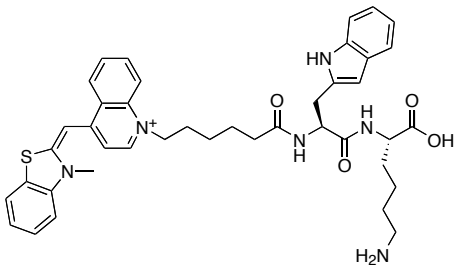
Preparation of compound 3



Compound **1** (1.21 g, 3.58 mmol, 1.00 equiv.) and compound **2** (1.16 g, 3.58 mmol, 1.00 equiv.) were suspended in 36 mL of ethanol and heated to 70 ^{O}C for 1 hour to dissolve. Triethylamine (1.10 mL, 7.82 mmol, 2.18 equiv.) was then added and the reaction was cooled to 50 ^{O}C. After 3 hours, the slurry was allowed to cool to 25 ^{O}C, and diethyl ether (107 mL) was added to fully precipitate a red solid. The crude solid was suspended in acetone (70 mL)/ ether (100 mL) for 1 hour, collected by vacuum filtration and dried under reduced pressure to yield 1.06 g (56%) of a red solid. **UV-Vis**: λ_{max} (H_2O) 502 nm (ε= 66400 $M^{-1}cm^{-1}$); **^{1}H NMR** (400 MHz, DMSO) δ 1.38 (m, 2H, CH_2), 1.56 (m, 2H, CH_2), 1.86 (m, 2H, CH_2), 2.21 (t, J= 7.2 Hz, 2H, CH_2), 4.03 (s, 3H, NCH_3), 4.61 (t, J= 7.2 Hz, 2H, NCH_2), 6.94 (s, 1H, CH), 7.40 (m, 2H, Ar), 7.62 (m, 1H, Ar), 7.77 (m, 2H, Ar), 8.00 (m, 1H, Ar), 8.07 (d, J=

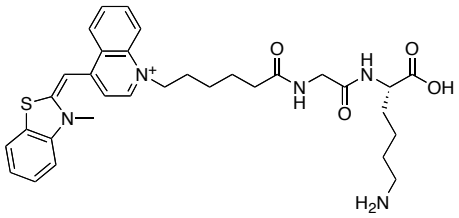
7.69 Hz, 1H, Ar), 8.15 (d, J= 9.16 Hz, 1H, Ar), 8.65 (d, J= 6.96 Hz, 1H, Ar), 8.81 (d, J= 8.62 Hz, 1H, Ar); **^{13}C NMR** (125 MHz, DMSO): □ 175.6, 161.2, 149.7, 145.6, 141.7, 138.2, 134.5, 129.4, 128.0, 127.0, 126.0, 125.4, 125.1, 124.1, 119.3, 114.2, 109.0, 89.3, 55.2, 35.1, 34.9, 29.8, 26.7, 25.3; **Exact mass** calculated for [$C_{24}H_{25}N_2O_2S^+$] requires *m/z* 405.1637. Found 405.1641 (ESI+); **HPLC retention time**: 19.7 min.; **mp**: 249-251 ^{O}C.

General protocol for the synthesis of peptides and TO-peptide conjugates. TO-peptide conjugates were synthesized on solid support using commercially available Wang-Fmoc-Lys(Boc) (0.7 mmol/g, Advanced ChemTech). Couplings were performed using 4 equivalents of Fmoc protected amino acid, 4 equivalents of HBTU and 8 equivalents of diisopropylethylamine in DMF for 3 hours. Deprotection of the Fmoc group was achieved using 20% piperidine in DMF for 30 minutes (after the coupling of the first amino acid to the resin, Fmoc deprotection was achieved using 50% piperidine in DMF for 5 minutes in order to minimize diketopiperazine formation). The TO-dipeptide conjugates (TO-GK, TO-WK, and TO-YK), KWK and WK were simultaneously deprotected and cleaved from the resin with 50% TFA in methylene chloride for 30 minutes. The solution was concentrated under reduced pressure in the presence of toluene in order to remove any residual TFA. TO-dipeptide conjugates were dissolved in a minimal amount of methanol, which was then removed under vacuum to yield a red solid. The identity of individual conjugates was confirmed by high-resolution mass spectrometry.



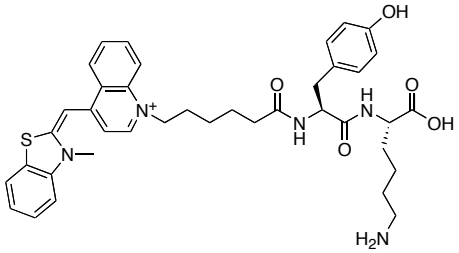
TO-WK

Exact mass calculated for [$C_{41}H_{47}N_6O_4S^+$] requires m/z 719.3380. Found 719.3389 (ESI+); **HPLC retention time**: 21.59 min.



TO-GK

Exact mass calculated for [$C_{32}H_{40}N_5O_4S^+$] requires m/z 590.2801. Found 590.2808 (ESI+); **HPLC retention time**: 15.37 min.



TO-YK

Exact mass calculated for [$C_{39}H_{46}N_5O_5S^+$] requires m/z 696.3220. Found 696.3206 (ESI+); **HPLC retention time**: 17.51 min.

B. Procedures for TO-peptide conjugate characterization

Measurement of fluorescence quantum yields

Fluorescence spectra were collected at 25˚C with λ_{ex}= 470 nm and λ_{em} of 490-650 nm on a Jobin-Yvon FluoroMax spectrometer. Samples contained 1.5 μM TO or TO-conjugate, 50 mM sodium phosphate/10 mM NaCl (pH 7), and 45 μM bp calf thymus CT DNA, and quantum yield values were calculated relative to that reported for DNA-bound TO by Kubista and coworkers in reference 4.

Measurement of DNA binding affinities

Binding constants were measured by monitoring changes in fluorescence intensity upon addition of calf thymus DNA (CT DNA) in dilute solutions of TO or TO conjugate containing 50 mM sodium phosphate (pH 7) and 100 mM NaCl. The changes in intensity observed in the presence of DNA were fit using the Scatchard equation to obtain K_d values.

DNA photocleavage experiments

20 μM TO or TO-peptide conjugate was added to 75 μM (bp) pUC18 in 25 mM sodium phosphate (pH 7) in the dark ([underivatized peptides] = 200 μM to ensure complexation with DNA). Irradiation was performed with a 365 nm transilluminating lamp. Cleavage efficiencies in aliquots removed from reactions were visualized after 1% agarose gel electrophoresis by ethidium staining. Minimal cleavage was observed when identical samples were incubated in the dark or when DNA samples were irradiated alone. In Figure 2B and 3A, percent cleaved values represent averages of 5-10 replicates, and a correction factor of 1.22 was used to adjust for the decreased stainability of supercoiled DNA. The data shown in Figure 3A was obtained with 10 mM NaN_3 and 200 μM trolox. These scavenger concentrations produced minimal fluorescence quenching for DNA-bound TO-WK.

Modified FOX assay

The procedure for the modified FOX assay was based on that developed by Gebicki and coworkers and described in ref. 15. 50 μM TO or TO-WK was added to a solution of 70% glycerol and 30% H_2O or D_2O in the dark. Samples were irradiated for 0, 15, 35, 45, or 60 minutes with a 365 nm transilluminating lamp. After irradiation, one volume of glacial acetic acid was added, followed by 700 μM xylenol orange and 280 μM ammonium iron(II) sulfate hexahydrate. 50 μM H_2O_2 was added to a solution of

70% glycerol to provide a standard. Samples were thoroughly mixed after adding FOX reagents, and then incubated for 30 minutes in the dark. Samples were diluted with one volume of water, mixed, and the absorbance at 595 nm was measured using a Molecular Devices Thermomax microplate reader at 595 nm. The absorbance values for TO-WK were corrected for the small signals obtained with TO, and the activity of TO-WK was reported relative to that obtained with the H_2O_2 standard.