Supporting Information

Shedding Light by Cancer Redox – Human NAD(P)H:quinone Oxidoreductase 1 Activation of a Cloaked Fluorescent Dye

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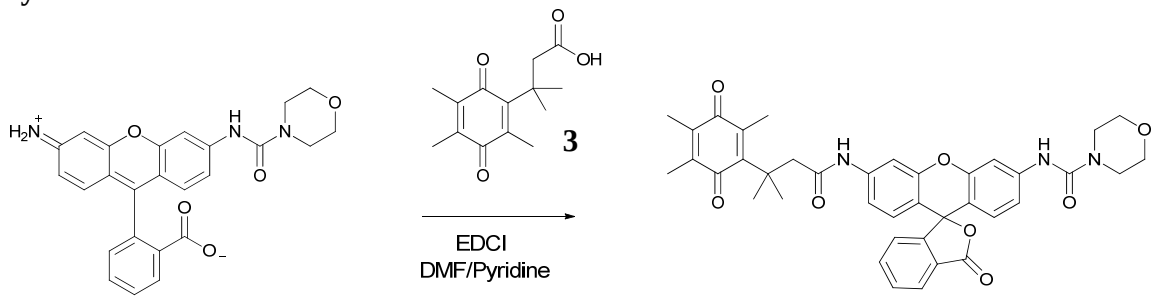
1. Materials and general methods.

Column chromatography was performed on 10 g silica columns using a Flashmaster Personal from Biotage. All chemicals were purchased from Sigma-Aldrich or Fisher Scientific. ^{1}H-NMR spectra were collected in $CDCl_3$ at 25 °C on a Bruker AV-400 spectrometer, and ^{13}C-NMR spectra were collected in $CDCl_3$ on a Varian 700. All chemical shifts are reported in the standard δ notation of parts per million using tetramethylsilane as an internal reference, and the coupling constants J are expressed in Hz. Mass spectral analyses were carried out using an Agilent 6210 ESI-TOF. Fluorescence data were collected using a Perkin Elmer LS55 spectrophotometer. Enzyme assays were performed with a FLUOstar OPTIMA from BMG LABTECH.

2. Synthesis



2 **1**

Scheme S1. Synthetic route for probe **1**.

Synthesis of 1. Synthesis of probe **1** is outlined in **Scheme S1**. Rhodamine-morpholino urea **2**[2] (53.6 mg, 0.121 mmol) was dissolved in anhydrous DMF (0.9 mL) and anhydrous pyridine (0.6 mL) under Ar. To this was added EDCI (43.72 mg, 0.228 mmol) and quinone **3**[1] (74.91 mg, 0.299 mmol). This solution was stirred at room temperature for 24 hours. Solvent was removed under reduced pressure, and the resulting residue was purified using column chromatography (1:1 DCM:ethyl acetate, $R_f = 0.43$) to give probe **1** as a yellow solid (27.8 mg, 34 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.51 (d, $J = 4.16$, 4H), 1.99 (d, $J = 4.16$, 4H), 2.18 (s, 3H), 3.05 (s, 3H), 3.53 (d, $J = 2.48$, 4H), 3.75 (d, $J = 2.36$, 4H), 6.61 (m, 2H), 6.95 (d, $J = 4.8$, 1H), 7.02 (m, 3H), 7.39 (d, $J = 4.88$, 1H), 7.60 (m, 2H), 7.88 (s, 1H), 7.98 (d, $J = 4.16$, 1H). ^{13}C NMR (175 MHz, $CDCl_3$) δ (ppm): 12.2, 12.8, 14.3, 29.0, 38.4, 44.4, 50.2, 66.6, 83.4, 107.8, 107.8, 112.8, 114.0, 115.5, 115.8, 124.2, 125.0, 126.2, 128.2, 128.3, 129.9, 135.4, 138.1, 138.3, 139.9, 141.4, 143.4, 151.6, 151.7, 153.1, 154.9, 170.1, 170.9, 187.6, 191.5. ESI-MS: For $C_{39}H_{37}N_3O_8$: expected $m/z = 676.2659$ $[M+H]^+$; found $m/z = 676.2656$ $[M+H]^+$; 0.4 ppm error.

3. Quantum yield

Quantum yield of dyes 1 and 2 were determined according to a literature method.[3]

$$\phi_F = \phi_S \left(\frac{\int F_{em,F}}{\int F_{em,S}} \right)$$

Where ϕ_F is the quantum yield, $F_{em,F}$ is the integrated emission spectrum for the dye in question, and $F_{em,S}$ is the integrated emission spectrum of the standard. Fluorescein was used as the standard with a quantum yield of 0.95. Quantum yields of compounds **1** and **2** were calculated to be 0.005 and 0.48, respectively.

4. Stability studies of compound 1

All stability studies were performed with a final concentration of probe **1** being 5.0×10^{-6} M, using quartz cuvettes (final volume of 3.0 mL) and a Perkin Elmer LS55 spectrophotometer (λ_{ex} = 490 nm and λ_{em} = 520 nm). Buffer used was 0.1 M PBS (pH = 7.4, 0.007% BSA, 0.1 M KCl). Stability against NADH was performed in a 100×10^{-6} M solution of NADH, with and without the presence of human NQO1 (5×10^{-5} g); the fluorescence intensity was monitored every 40 seconds for 2.2 hours. Stability against biological reductants was tested by adding enough reductant to achieve 1.0 mM for glutathione, ascorbic acid, and dithiothreitol and 0.4 mM sodium dithionite in a 5.0×10^{-6} M solution of compound **1** in buffer. The fluorescence intensity was monitored every 0.5 minutes for 6 hours.

5. NQO1 assay of compound 1

NQO1 assays for the conversion of probe **1** to dye **2** were performed by following fluorescence intensity (excitation at 485 nm and emission at 520 nm) every 1 minute for at least 20 minutes using a FLUOstar OPTIMA instrument and Falcon 96-well plates (black with clear bottoms). Recombinant human NQO1 (Sigma-Aldrich) in 0.1 M PBS, pH 7.4, and supplemented with 0.007% bovine serum albumin (BSA) was used. Stock solutions of compound **1** were prepared in ethanol and diluted in buffer to a final concentration between 2.5×10^{-6} and 60×10^{-6} M. Total volume per well was 200×10^{-6} L with a final NQO1 content of 1×10^{-5} g. Assays were initiated by the instrument-injection of NADH so as to yield a final NADH concentration of 100×10^{-6} M. The concentration of released **2** at any given time was determined from a fluorescence intensity vs. concentration calibration curve for dye **2** in 0.1 M PBS, pH 7.4, and 0.007% BSA. Rate versus [**1**] curves were fitted with a non-linear least-squares algorithm so as to obtain K_M and V_{max} values

6. References

1. L. A. Carpino, S. A. Triolo and R. A. Berglund, *J. Org. Chem.*, 1989, **54**, 3303-3310.
2. L. D. Lavis, T. Y. Chao and R. T. Raines, *ACS Chem. Biol.*, 2006, **1**, 252-260.
3. S. Fery-Forgues and D. Lavabre, *J. Chem. Ed.*, 1999, **76**, 1260-1264.