

# Supplementary Information

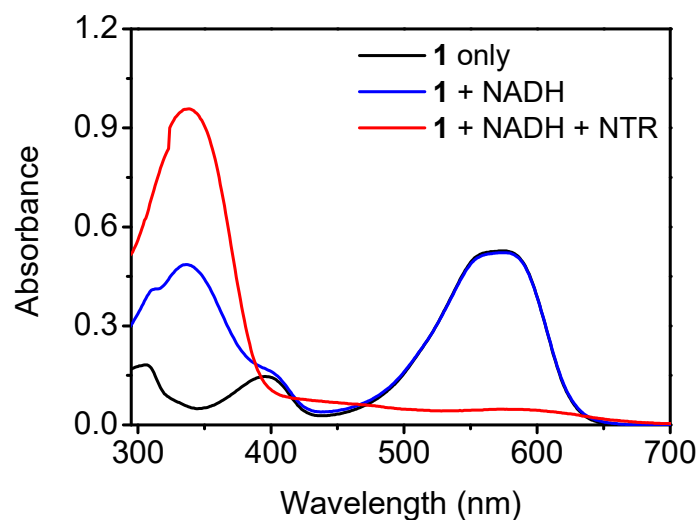
## **Ratiometric Fluorescence Assay for Nitroreductase Activity: Locked-Flavylium Fluorophore as a NTR-sensitive Molecular Probe**

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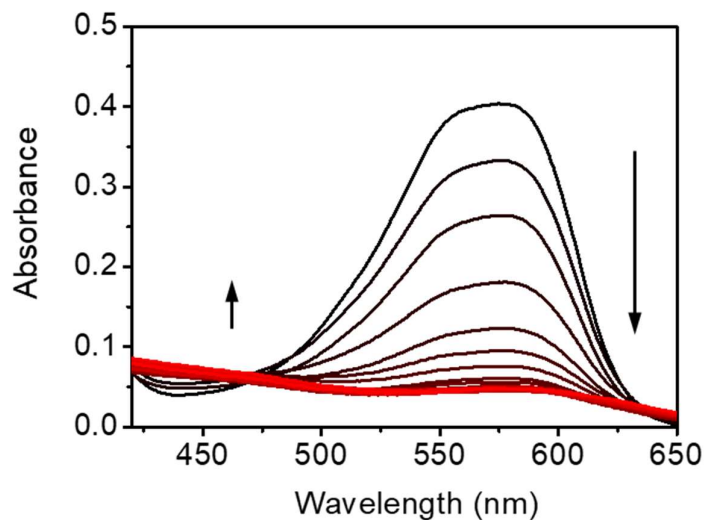
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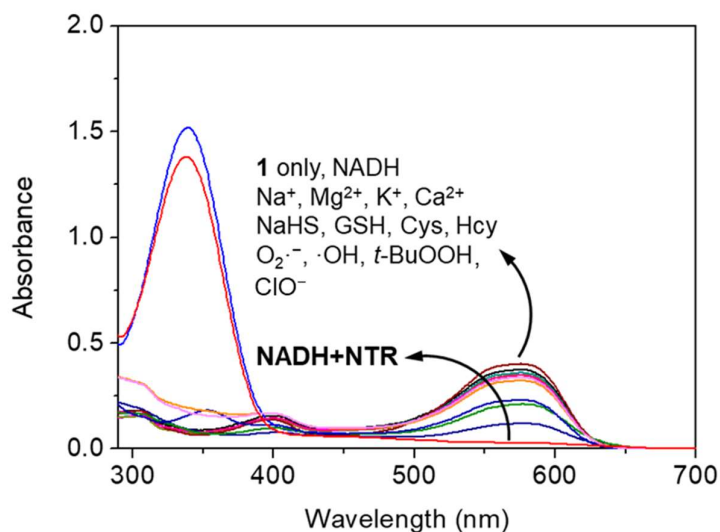
<sup>‡</sup>These authors are equally contributed to this work



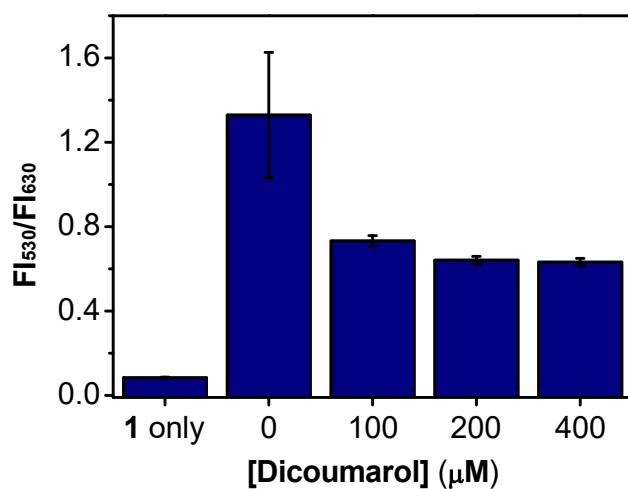
**Figure S1.** UV/Vis absorption spectra of probe **1** with or without NTR and NADH. All data were obtained in PBS buffer (10 mM, pH 7.4)/DMSO (99:1, v/v) with an incubation of 90 min at 37 °C.  $\lambda_{\text{ex}} = 470$  nm.



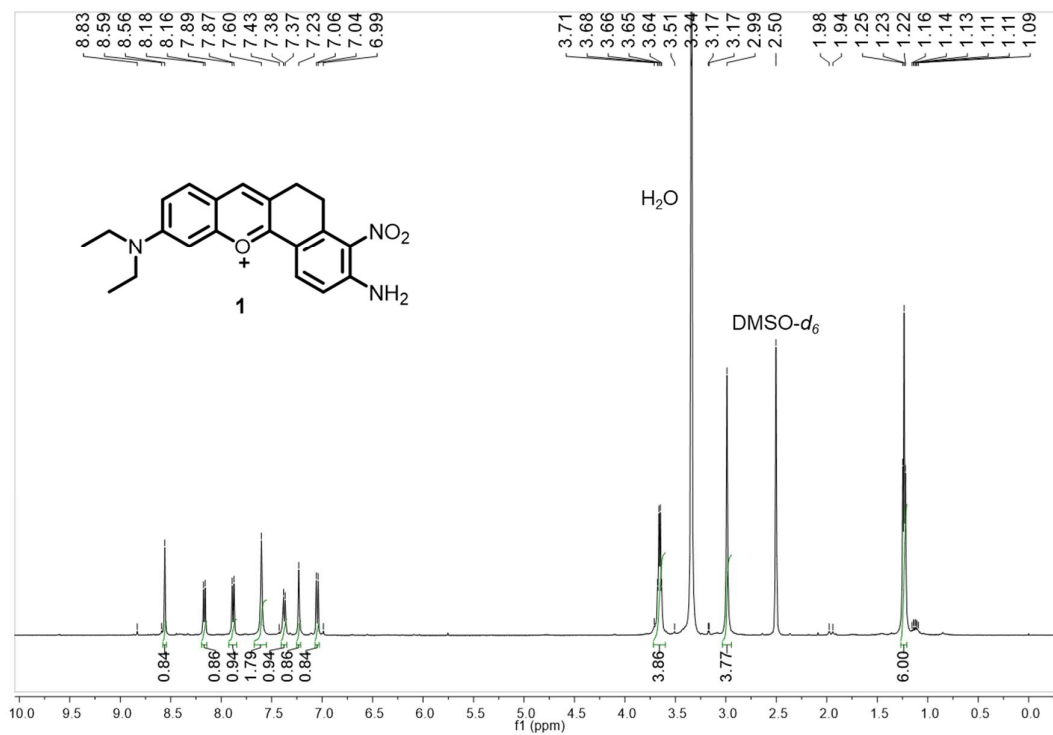
**Figure S2.** Time-dependent absorption changes of probe **1** (10  $\mu\text{M}$ ) in the presence of NTR (2.0  $\mu\text{g/mL}$ ) and NADH (300  $\mu\text{M}$ ). All data were obtained in PBS buffer (10 mM, pH 7.4)/DMSO (99:1, v/v) with an incubation of 90 min at 37 °C.  $\lambda_{\text{ex}} = 470$  nm.



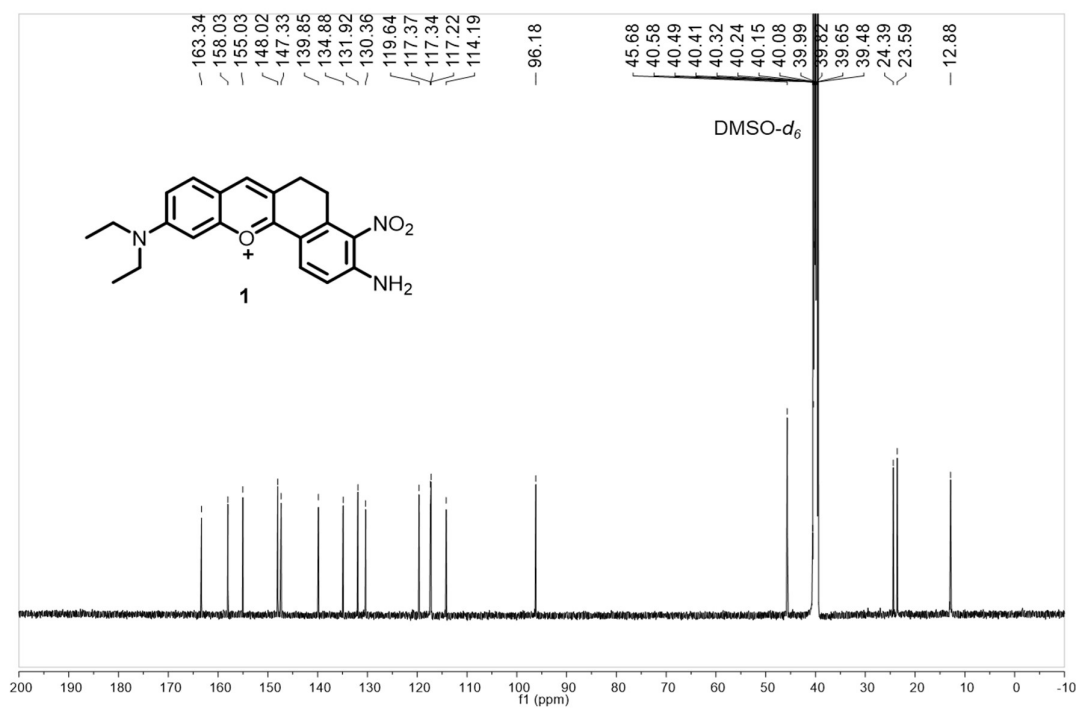
**Figure S3.** UV/Vis absorption spectra of probe **1** (10  $\mu\text{M}$ ) toward NADH (300  $\mu\text{M}$ ), metal ions ( $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$ ; 1 mM, respectively), thiols (NaHS, GSH, Cys, and Hcy; 1 mM, respectively), ROS ( $\text{O}_2^{\cdot-}$ ,  $\cdot\text{OH}$ , *t*-BuOOH, and  $\text{ClO}^-$ ; 100  $\mu\text{M}$ , respectively), and NADH (300  $\mu\text{M}$ )+NTR (2  $\mu\text{g}/\text{mL}$ ). All data were obtained in PBS buffer (10 mM, pH 7.4)/DMSO (99:1, v/v) with an incubation of 90 min at 37  $^\circ\text{C}$ .  $\lambda_{\text{ex}} = 470$  nm.



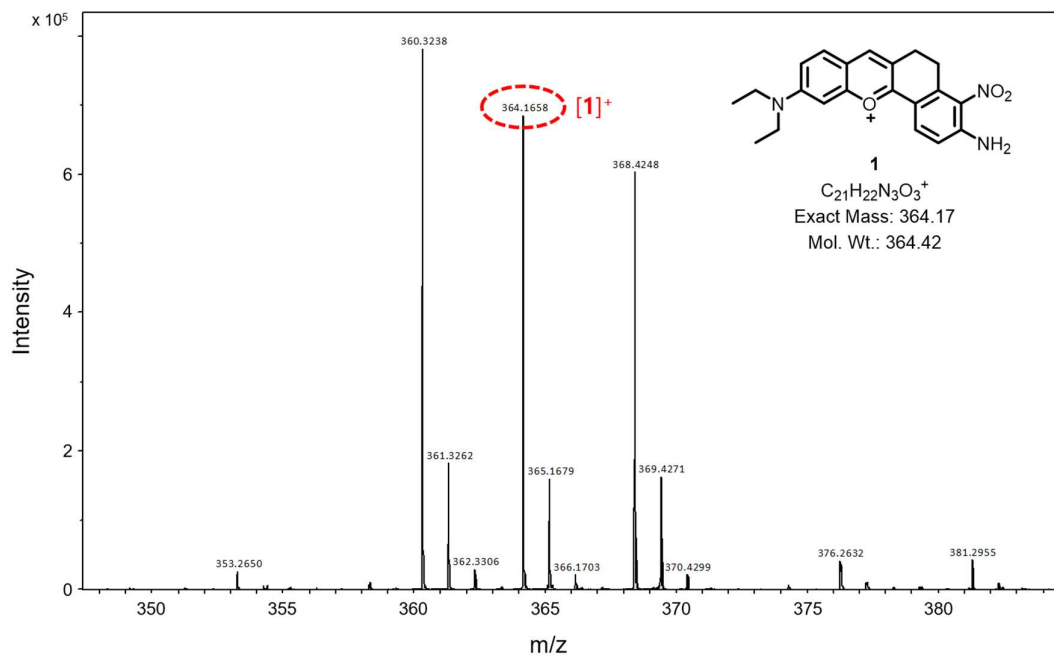
**Figure S4.** Inhibition assay of NTR activity using various concentrations of dicoumarol based on fluorescence ratio ( $\text{FI}_{530}/\text{FI}_{630}$ ) of probe **1** (10  $\mu\text{M}$ ). The fluorescence intensity ratio from probe **1** was measured to NTR activity (2  $\mu\text{g}/\text{mL}$  of NTR and 300  $\mu\text{M}$  of NADH) at different concentrations of dicoumarol (0, 100, 200 and 400  $\mu\text{M}$ ). All data were obtained in PBS buffer (10 mM, pH 7.4)/DMSO (9:1, v/v) with 90 min incubation at 37  $^\circ\text{C}$ .  $\lambda_{\text{ex}} = 470$  nm. Error bars displayed mean  $\pm$  standard deviation (s.d.,  $n = 3$ ).



**Figure S5.** <sup>1</sup>H NMR spectrum of **1** in DMSO-*d*<sub>6</sub>.



**Figure S6.** <sup>13</sup>C NMR spectrum of **1** in DMSO-*d*<sub>6</sub>.



**Figure S7.** HR-ESI-MS spectrum of **1**.