Electronic Supplementary Information (ESI)

A Novel Two-photon fluorescent probe with Long Stokes Shift and High Signal-to-Background Ratio for human NAD(P)H:Quinone Oxidoreductase-1 (hNQO1) detection and imaging in living cells and tissues

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Calculation equation of TP absorption cross sections(δ)

The two-photon absorption cross sections of samples (δ_s) was calculated as the following equation:[1]

$$\delta_S = \frac{S_S}{S_R} \cdot \left[\frac{\Phi_S \cdot C_R \cdot n_S}{\Phi_R \cdot C_S \cdot n_R}\right] \cdot \delta_S$$

Subscript S and R denote the sample and the reference, respectively. S represents the intensity of TPE fluorescence emission, Φ is the fluorescence quantum yield, C denotes the concentration, and n represents the refractive index of the solvents.

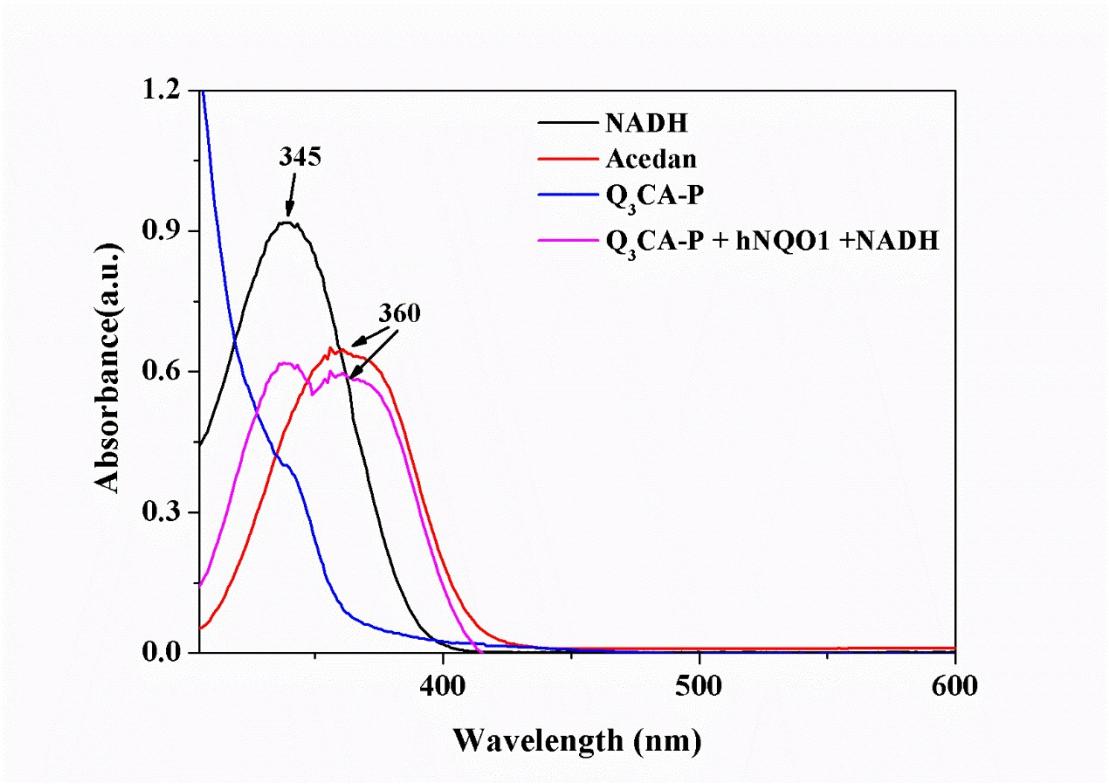


Figure S1. UV-vis absorption spectra of NADH (400 μM, black line), Acedan (50 μM, red line), Q_3CA-P (50 μM) before (blue line) and after (magenta line) reacted with hNQO1 (500 ng/mL) in the presence of NADH (250 μM) at 37 °C for 30 min.

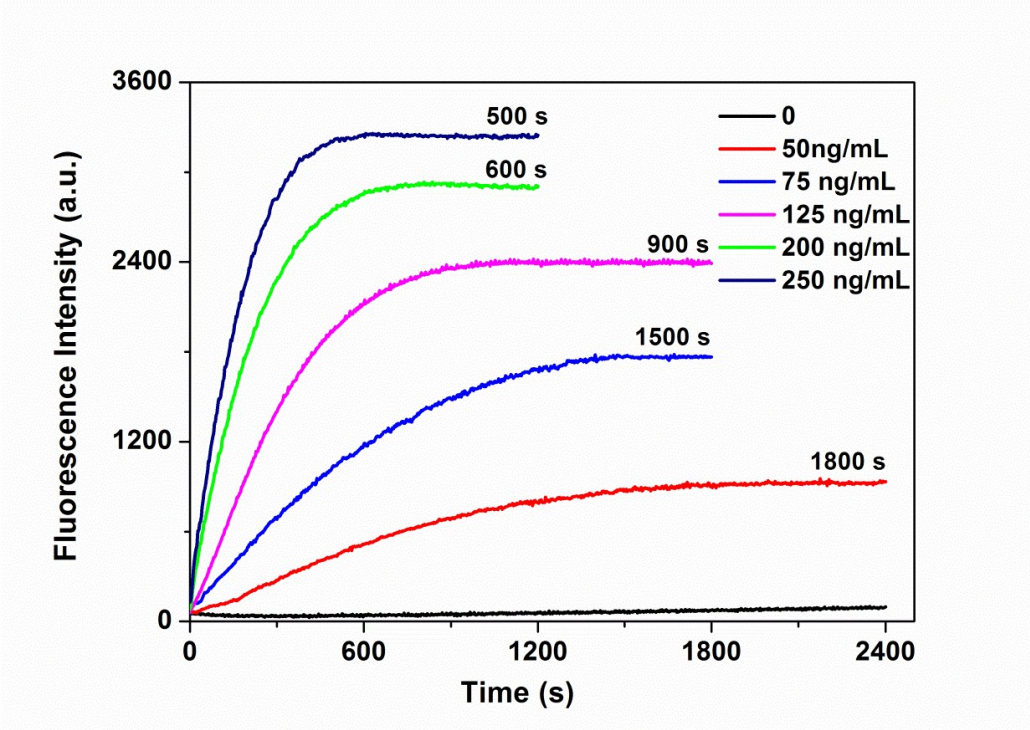


Figure S2. Time dependent fluorescence emission intensity (λex/em = 360/502 nm) of Q_3CA-P (5 μM) reacted with different hNQO1 concentration of 250 ng/mL (navy line), 200 ng/mL (green line), 125 ng/mL (magenta line), 75 ng/mL (blue line), 50 ng/mL (red line), and without hNQO1 (black line).

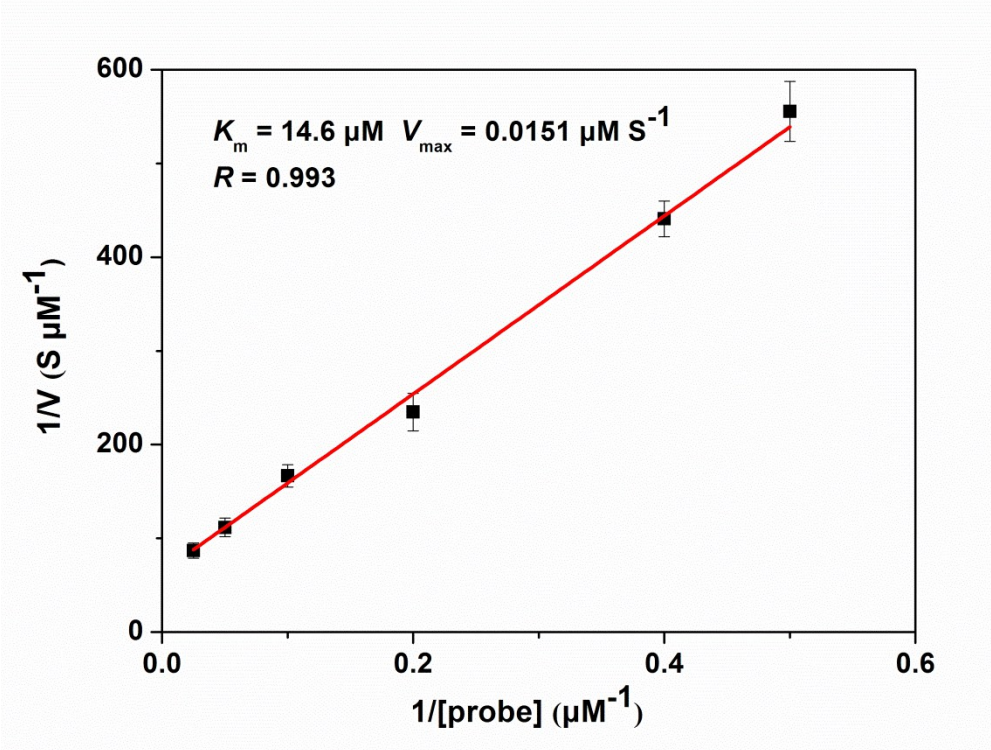


Figure S3. Lineweaver-Burk plot for the enzyme-catalyzed reaction. The Michaelis-Menten equation was described as: $V = V\text{max}\,[\text{probe}] / (K\text{m}+[\text{probe}])$, where V is the reaction rate, [probe] is the Q_3CA-P concentration (substrate), and Km is the Michaelis constant. Conditions: 125 ng/mL hNQO1, 50 μM NADH, 2-40 μM of Q_3CA-P, $\lambda_{ex/em}$ = 360/502 nm.

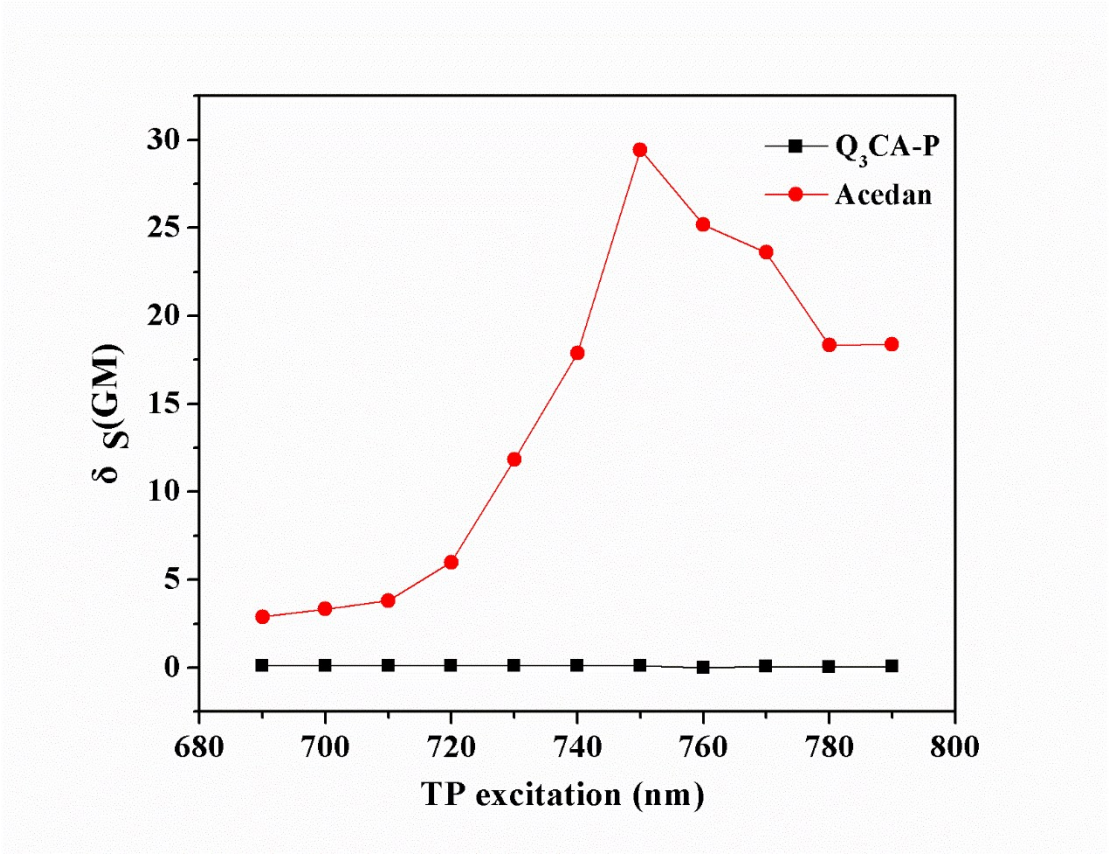


Figure S4. Two-photon action spectra of Q_3CA-P and Acedan in PBS buffer (10 mM, pH 7.4). The estimated uncertainties for the two-photon action cross section values (δ_s) are ± 15 %.

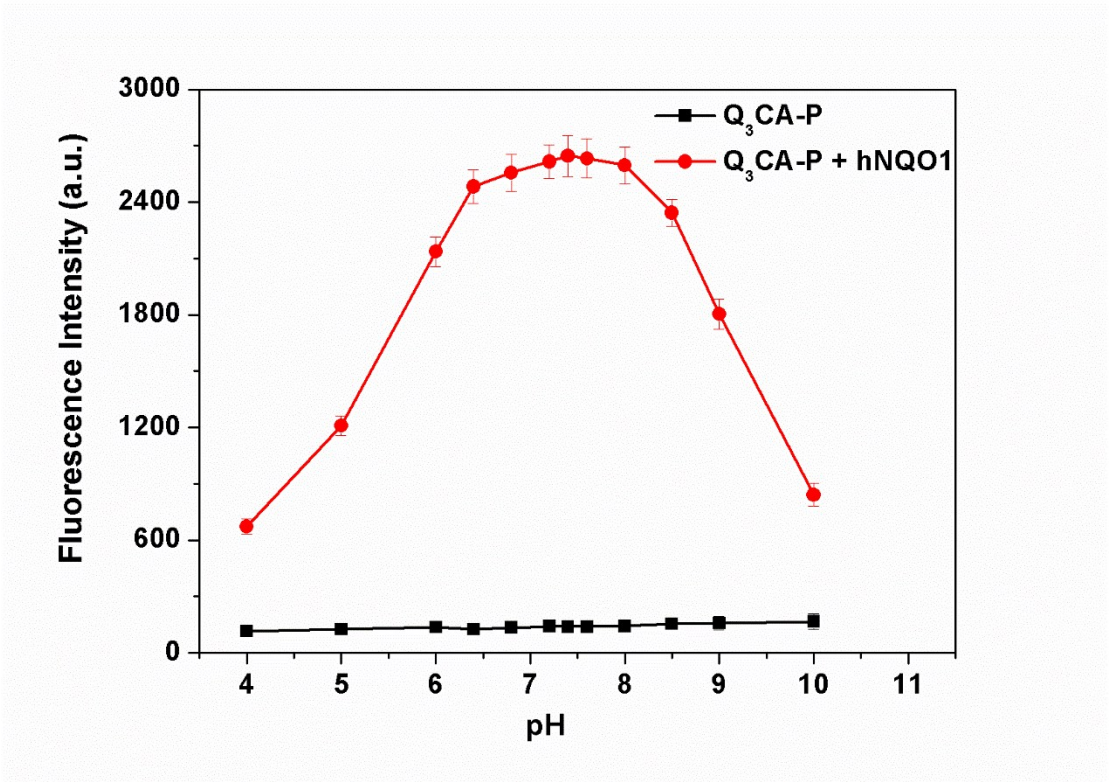


Figure S5. Effect of pH value on the fluorescence intensity of Q_3CA-P (5 μM) reacted with hNQO1 (125 ng/mL) in the presence of NADH (50 μM) at 37 °C. $\lambda_{ex/em}$ = 360/502 nm.

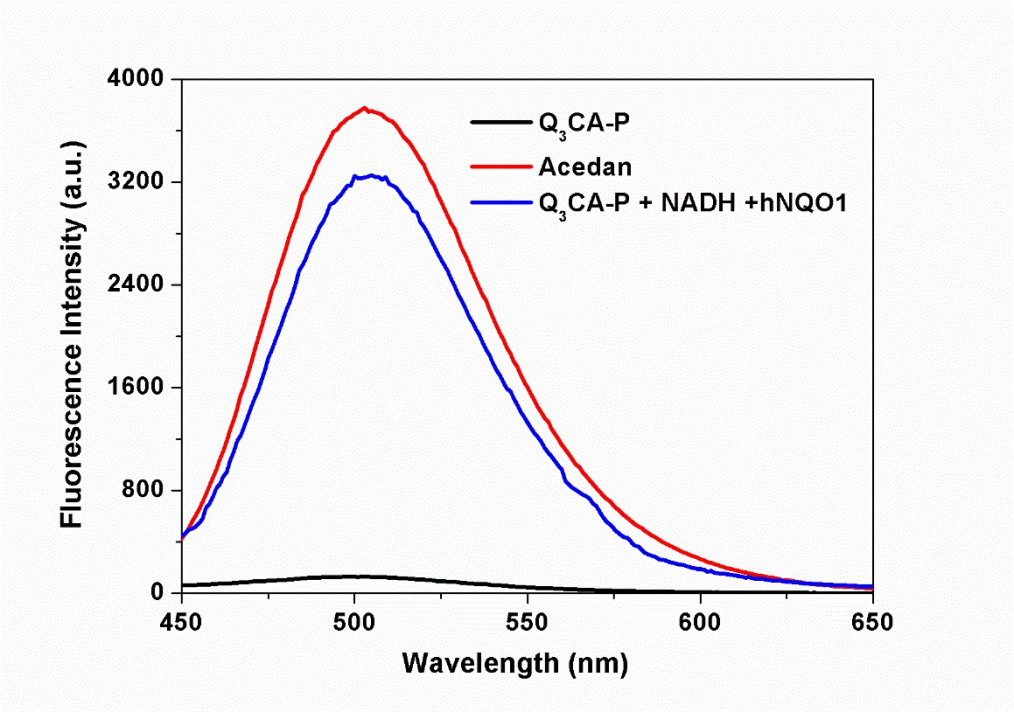


Figure S6. Fluorescence intensity spectra of Acedan (5 μM, red line), Q_3CA-P (5 μM) before (black line) and after (blue line) reacted with hNQO1 (250 ng/mL) in the presence of NADH (50 μM) at 37 °C for 30 min. $\lambda_{ex/em}$ = 360/502 nm.

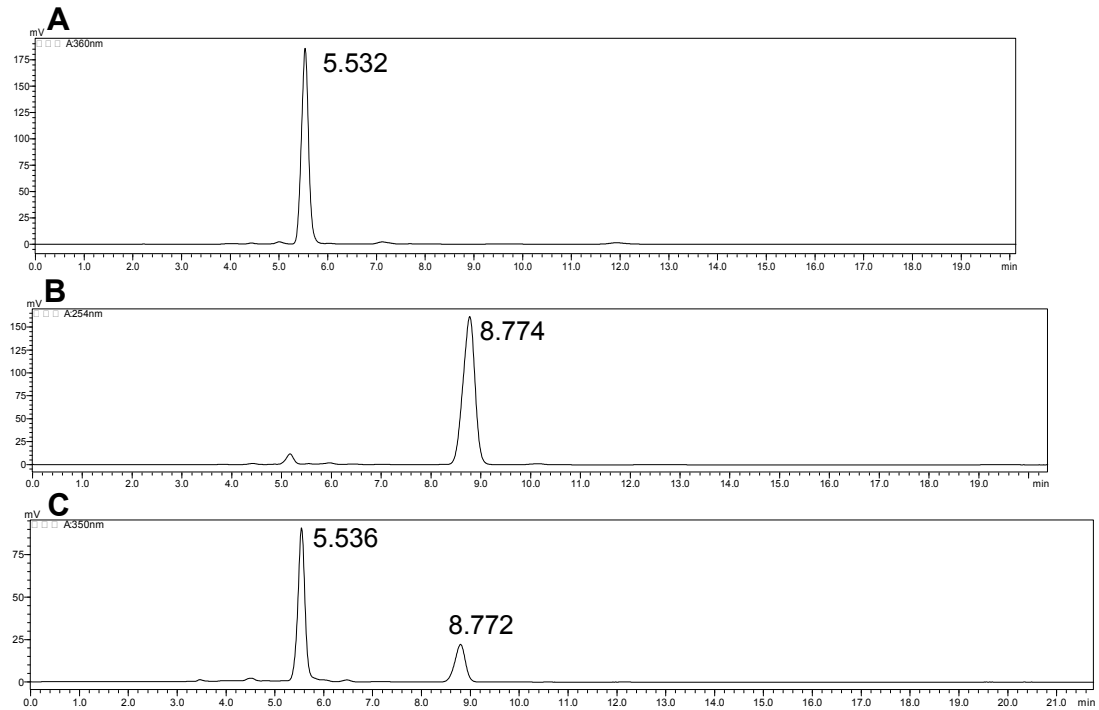


Figure S7. HPLC profiles of (A) 50 μM Acedan, (B) 50 μM Q_3CA-P, (C) 25 μM Q_3CA-P mixed with 625 ng/mL hNQO1 in the presence of 250 μM NADH for 30 min.

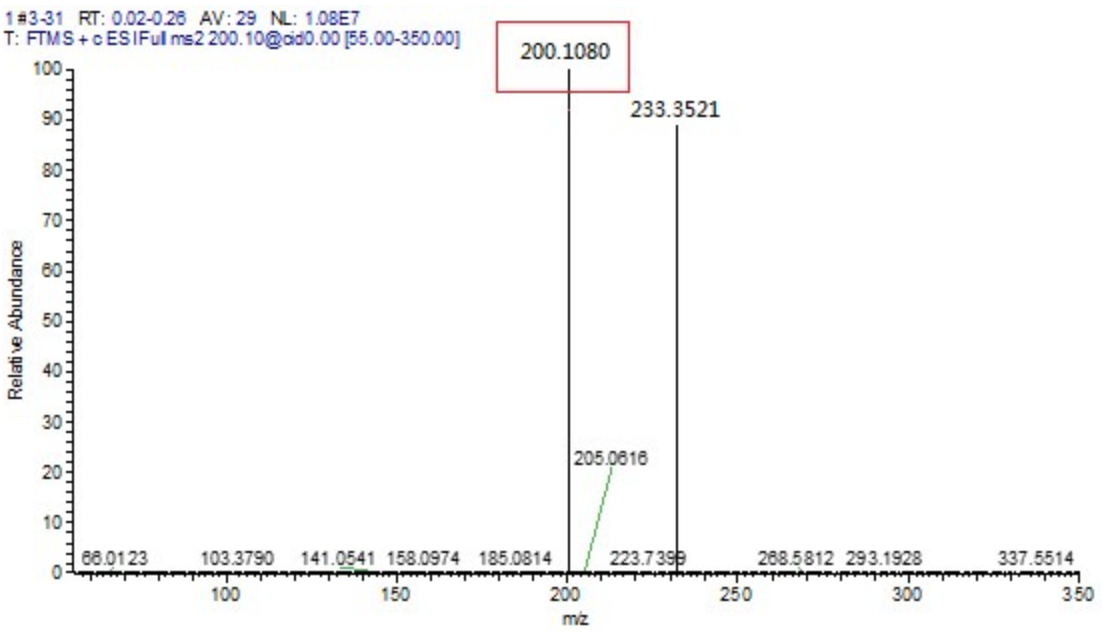


Figure S8. ESI-MS spectrum of reaction product

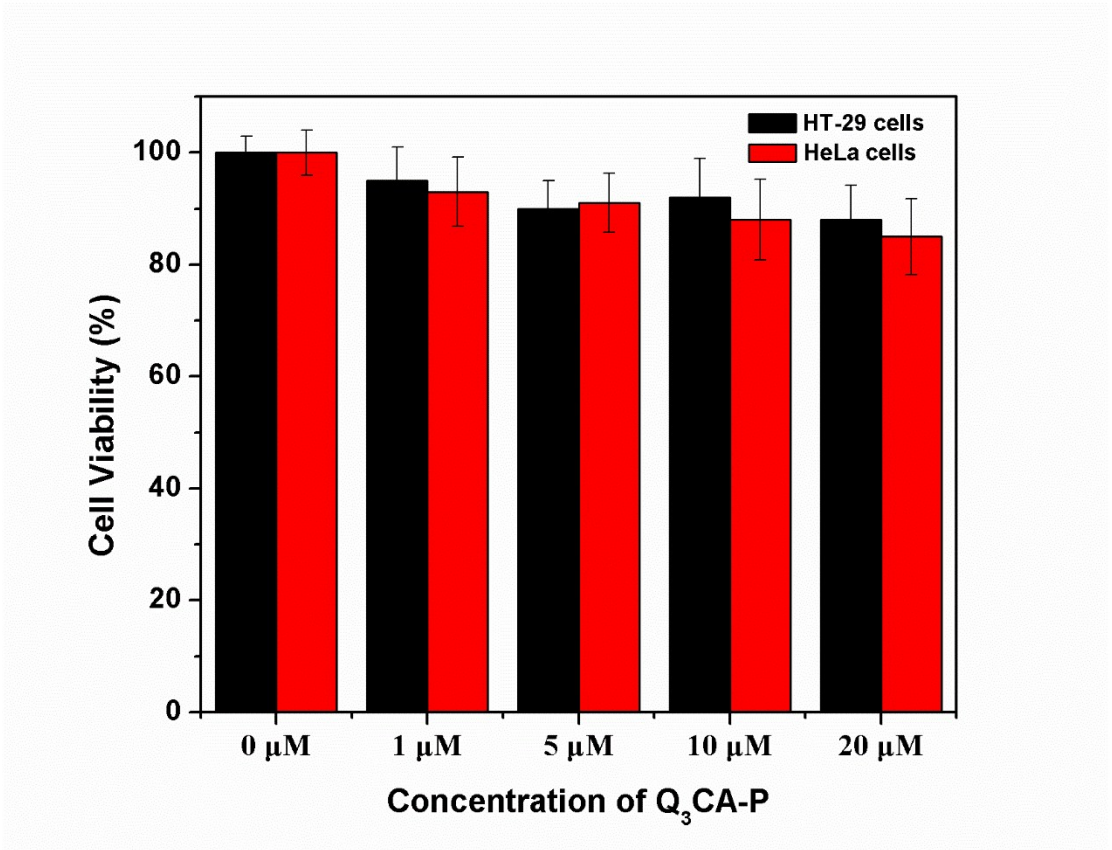


Figure S9. Effects of Q_3CA-P with varied concentrations (1-30 μM) on the viability of HT-29 cells and HeLa cells. The viability of cells without Q_3CA-P is defined as 100%, The results are the means ± SD of three experiments.

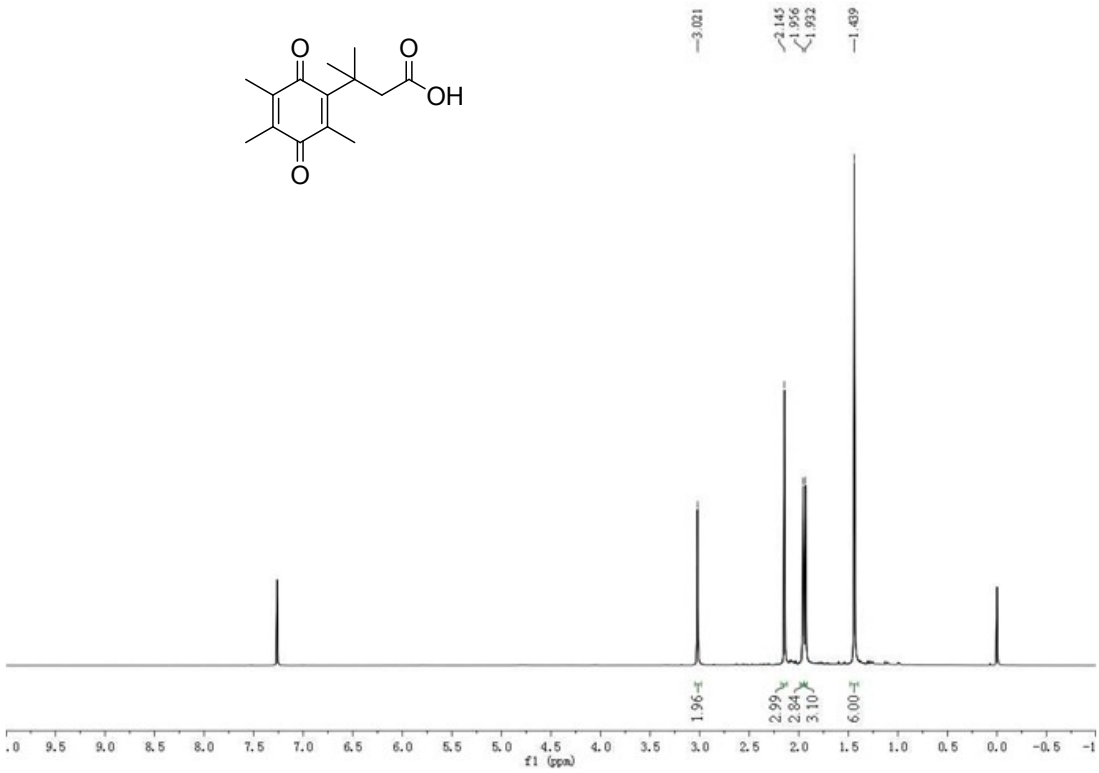


Figure S10. ^{1}H NMR spectrum of compound 2 in $CDCl_3$

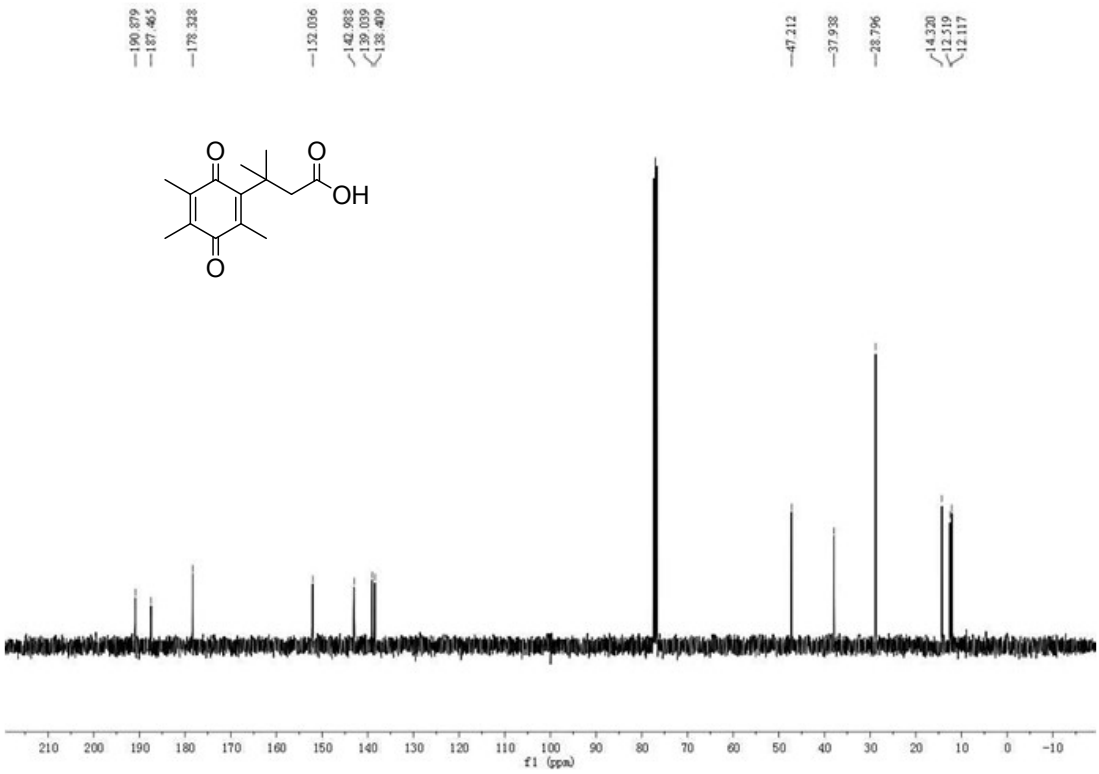


Figure S11. ^{13}C NMR spectrum of compound 2 in $CDCl_3$

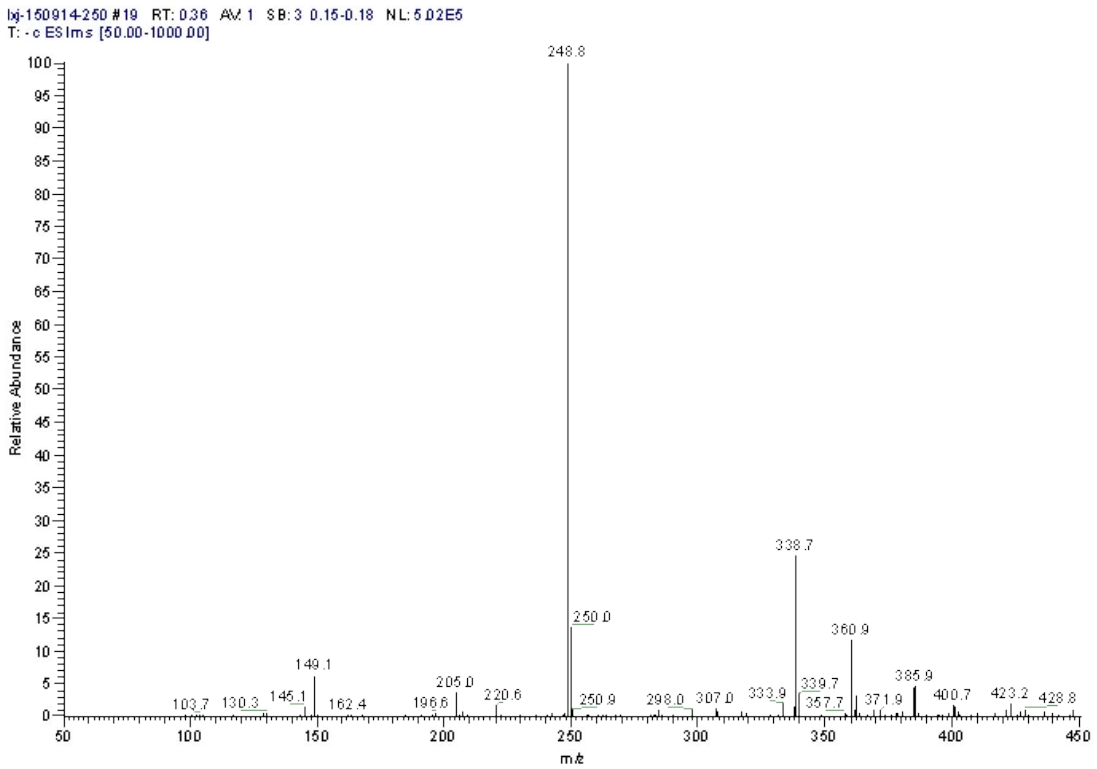


Figure S12. ESI-MS spectrum of compound 2

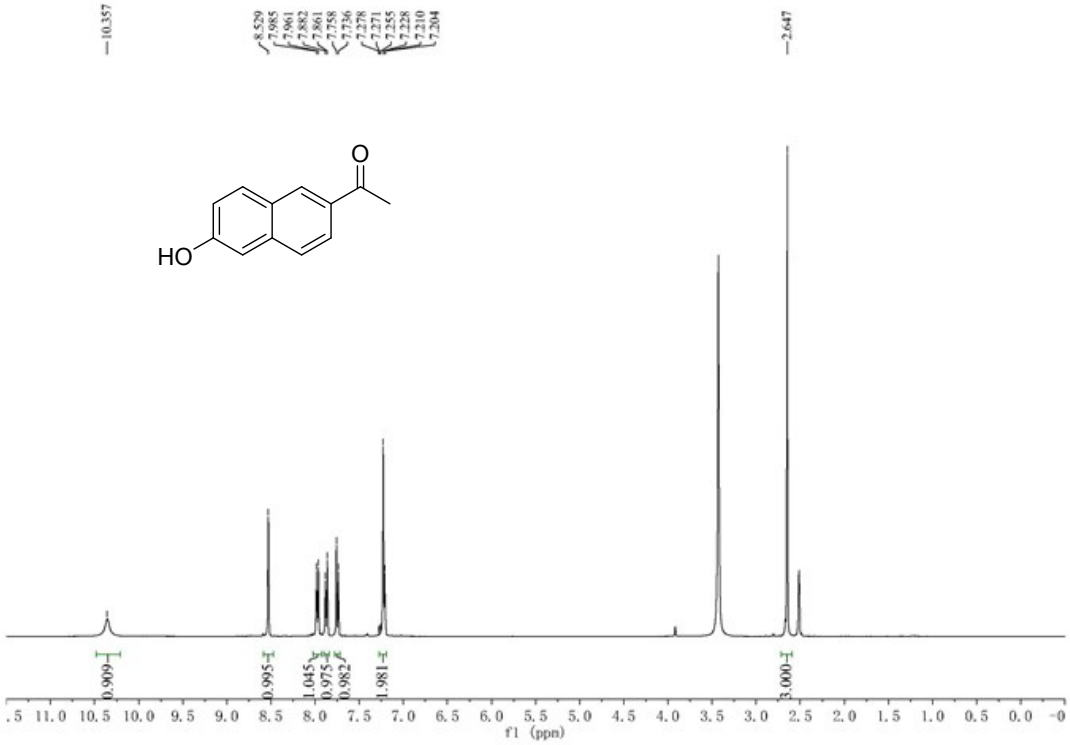


Figure S13. ^{1}H NMR spectrum of compound 3 in DMSO-d_6

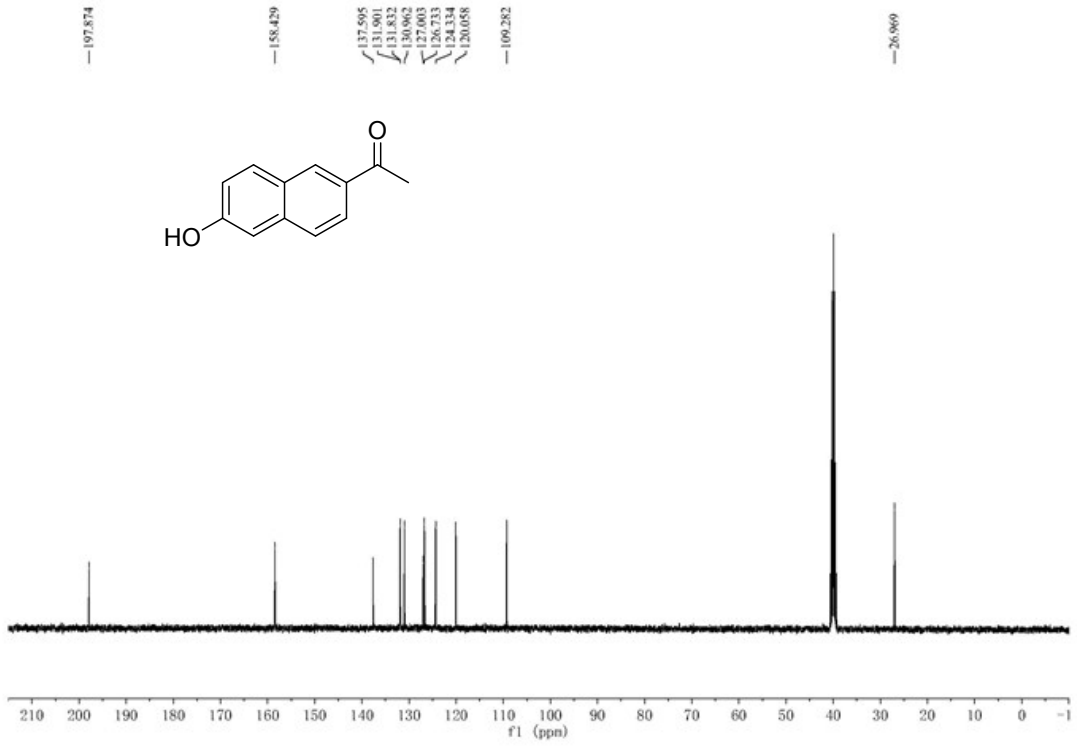


Figure S14. ^{13}C NMR spectrum of compound 3 in DMSO-d_6

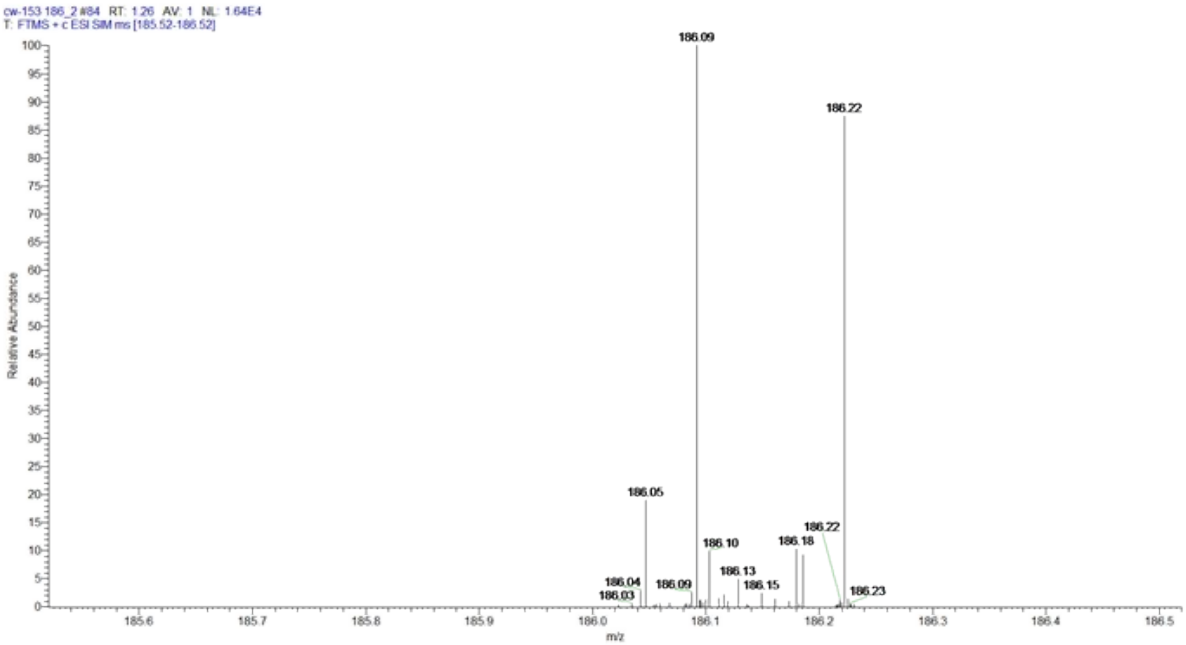


Figure S15. ESI-MS spectrum of compound 3

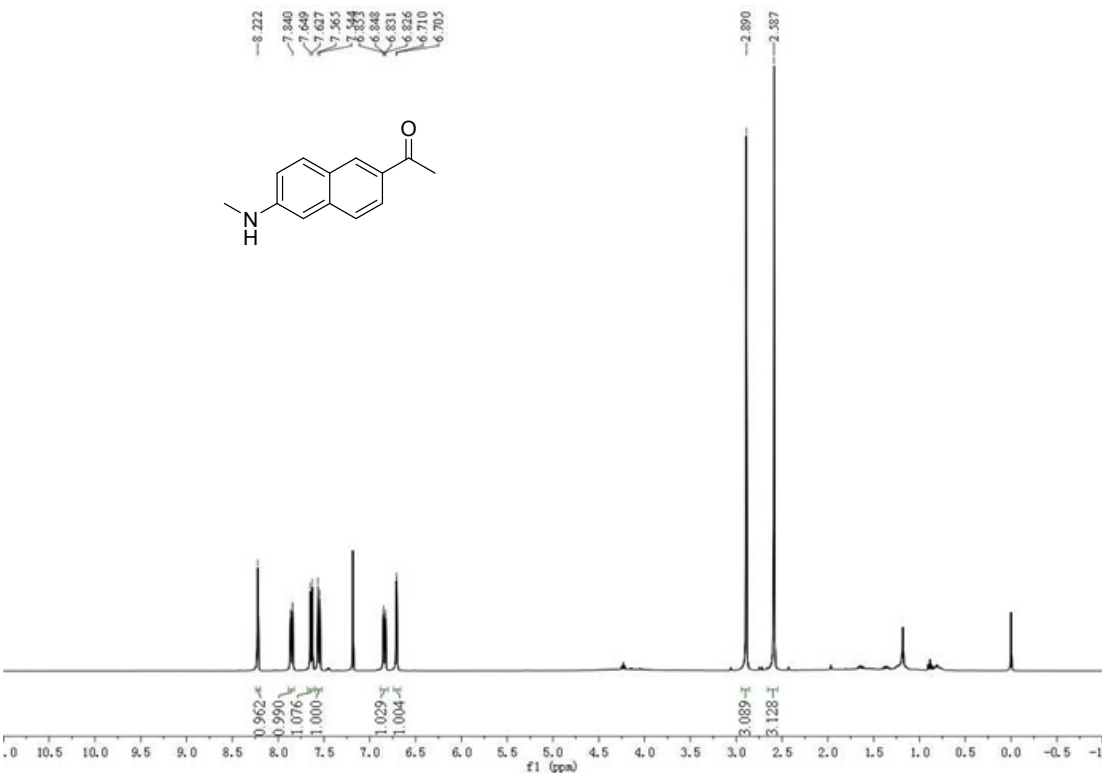


Figure S16. ^{1}H NMR spectrum of Acedan in $CDCl_3$

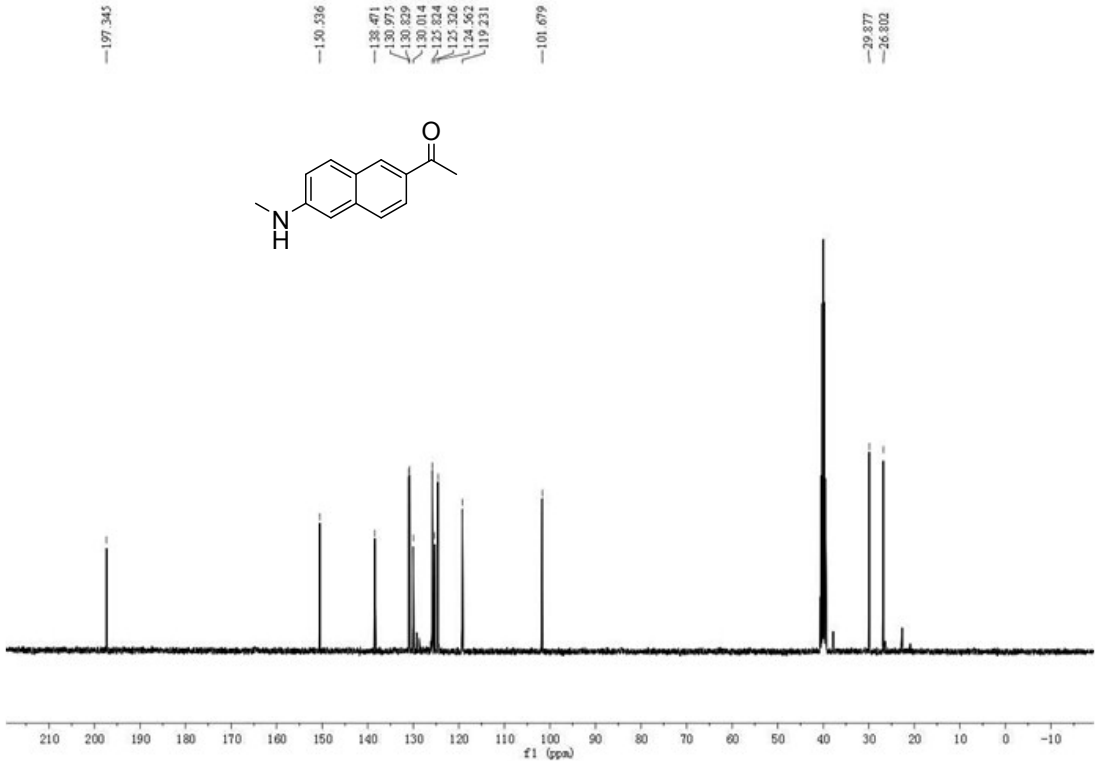
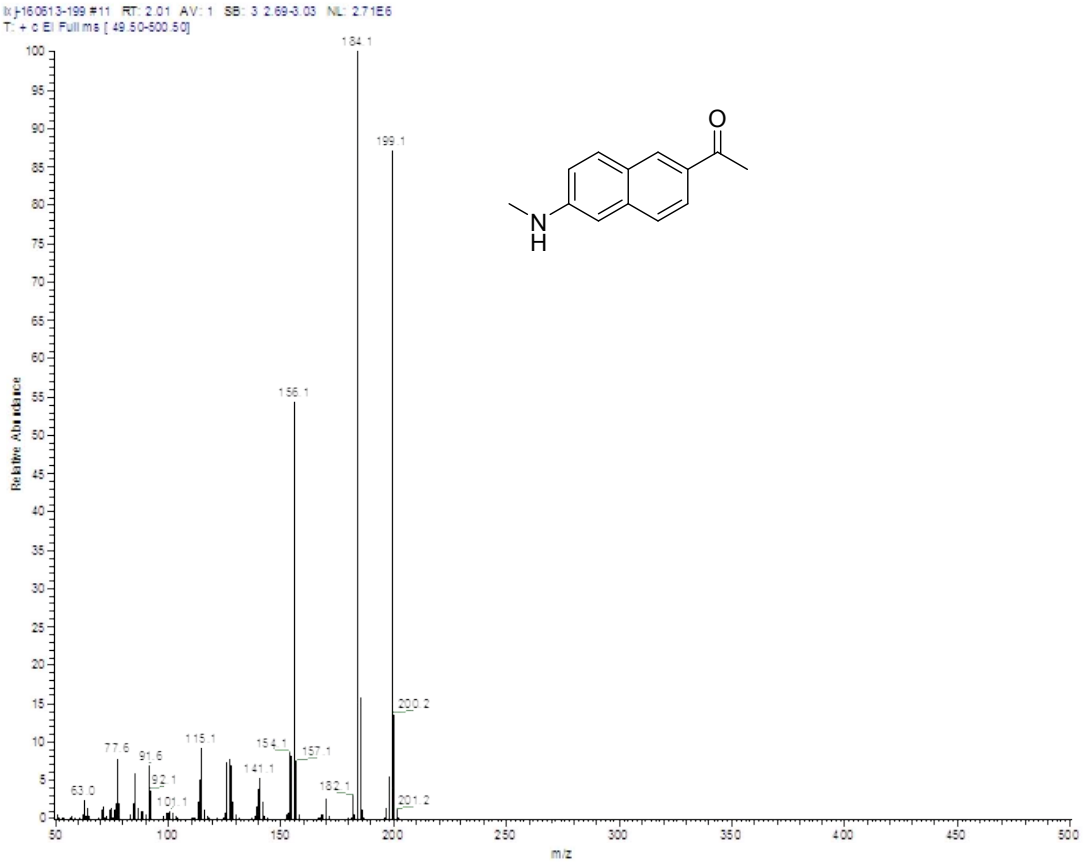


Figure S17. ^{13}C NMR spectrum of Acedan in $CDCl_3$



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Full ms [189.500 - 208.500] - Range: 189.500 - 208.500
Scan No. 1 of 1

Mass	Relative Intensity	Theoretical Mass	Delta [ppm]	Delta [mmu]	Composition
199.0993	100.0	199.0992	0.7	0.1	$C_{13}H_{13}O_1N_1$
		199.1117	-62.5	-12.4	$C_{14}H_{15}O_1$
		199.1356	-182.0	-36.3	$C_{14}H_{17}N_1$
		199.0542	226.4	45.1	$C_{16}H_7$
		199.1481	-245.2	-48.8	$C_{15}H_{19}$

Figure S18. EI-MS spectrum of Acedan

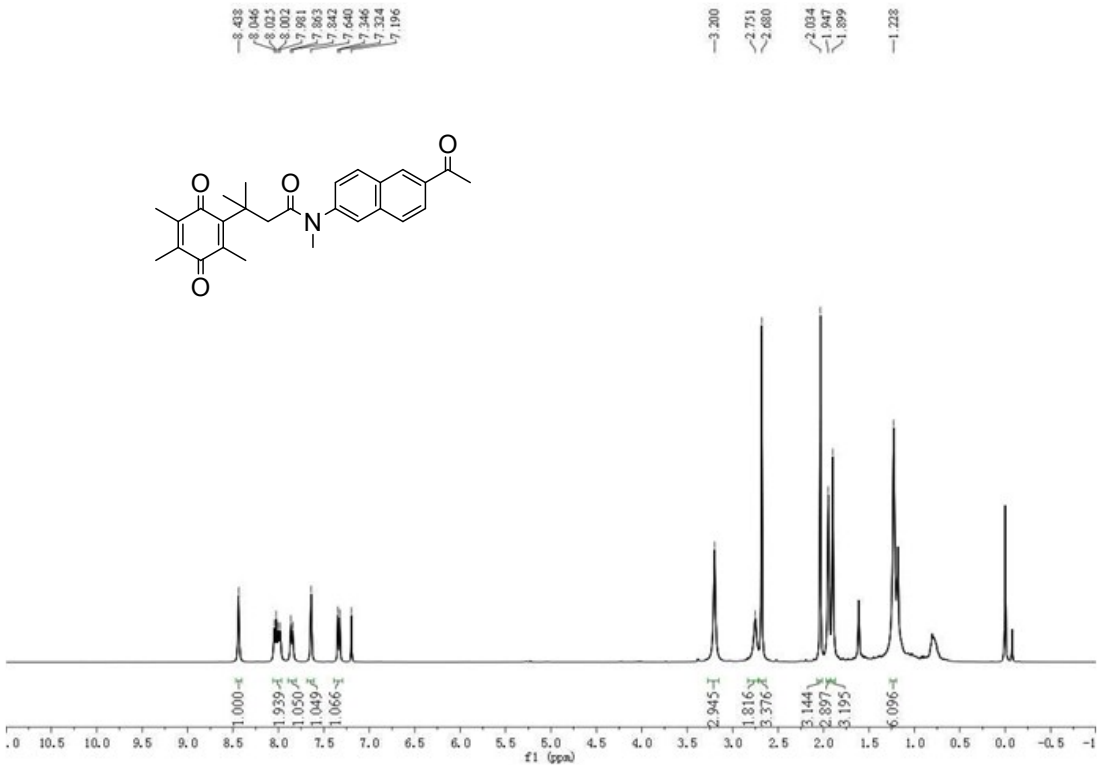


Figure S19. ^{1}H NMR spectrum of Q_3CA-P in $CDCl_3$

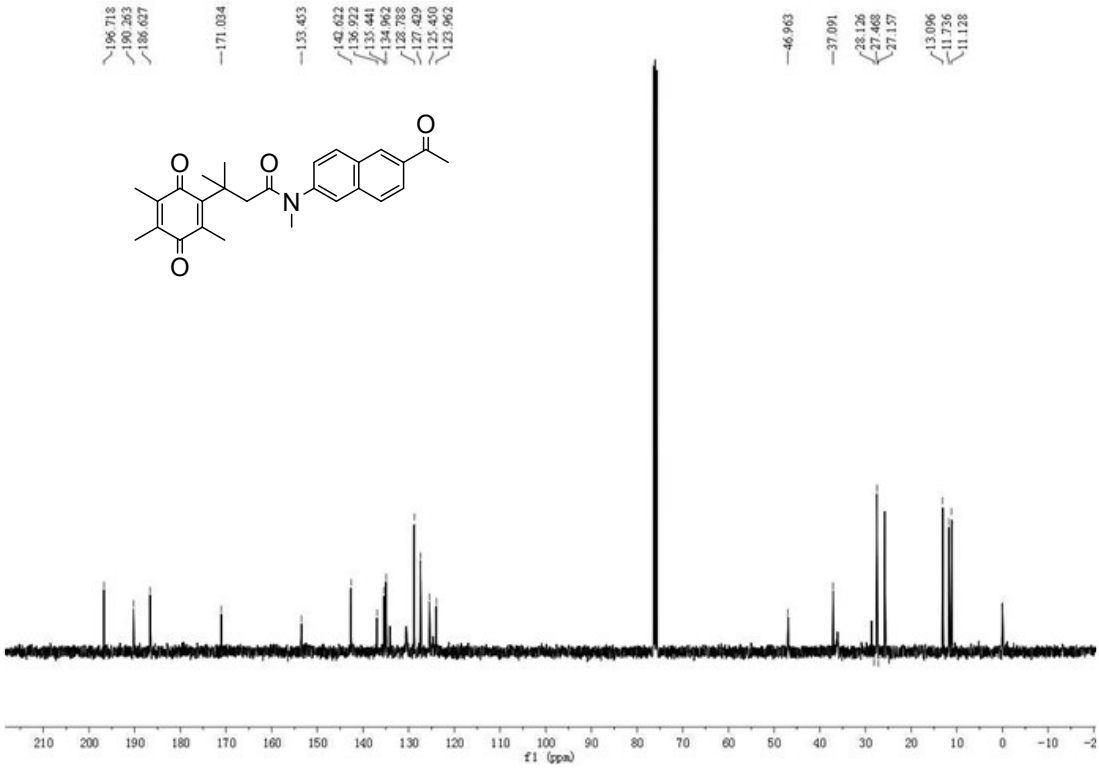
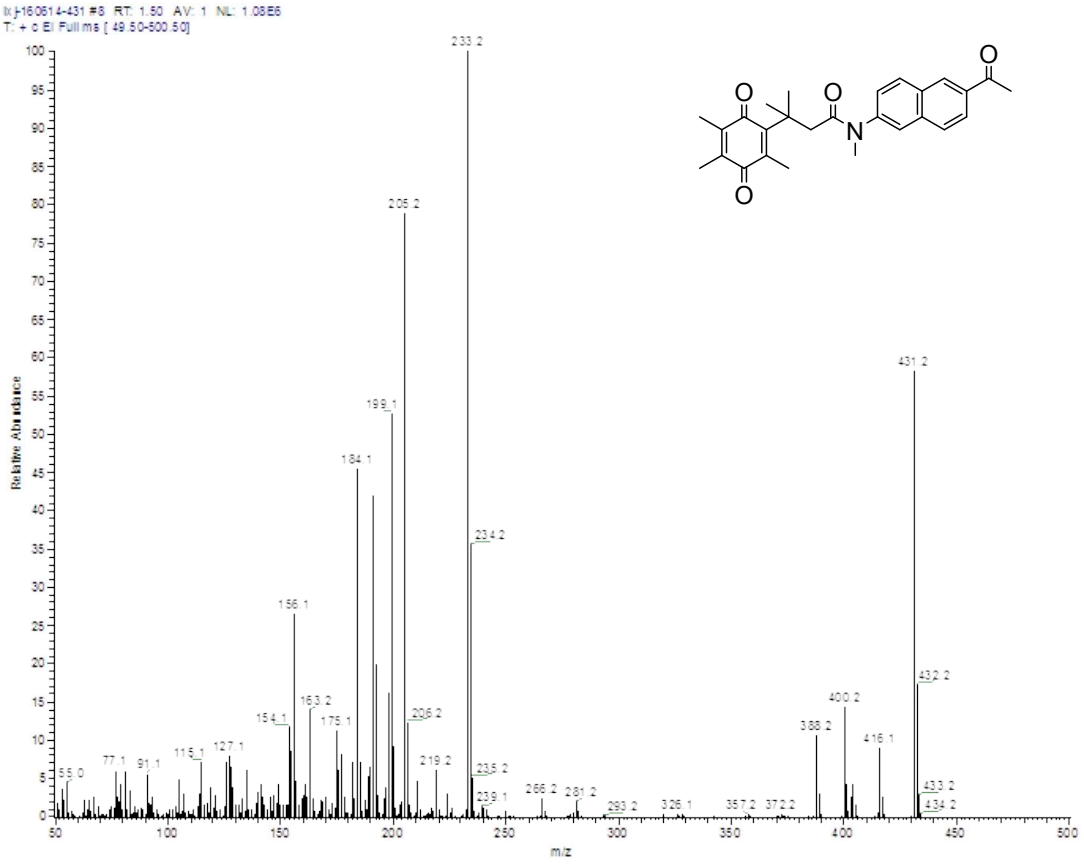


Figure S20. ^{13}C NMR spectrum of Q_3CA-P in $CDCl_3$



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Scan No. 1 of 1

Mass	Relative Intensity	Theoretical Mass	Delta [ppm]	Delta [mmu]	Composition
431.2082	100.0	431.2091	-2.2	-0.9	$C_{27}H_{29}O_4N_1$
		431.2006	17.7	7.6	$C_{31}H_{27}O_2$
		431.2217	-31.3	-13.5	$C_{28}H_{31}O_4$
		431.2244	-37.6	-16.2	$C_{31}H_{29}O_1N_1$
		431.1880	46.8	20.2	$C_{30}H_{25}O_2N_1$

Figure S21. EI-MS spectrum of Q_3CA-P

Table S1. The comparison of Q_3CA-P with other NQO1 probes

Probe	Abs Max (nm)	Em Max (nm)	Stokes shift (nm)	Signal-to-Background Ratio	LOD (ng/ml)
Probe 1 [2]	432	540	108	95:1	ND
Q_3MJSNR [3]	585	624	39	30:1	ND
TPQ [4]	400	520	120	9:1	ND
prodrug 1 [5]	365	550	185	4:1	ND
Prodrug [6]	365	436	71	NK	ND
Q_3CA-P	360	502	142	25:1	5.6

LOD means limit of detection; ND, not determined; NK, not known.

Notes and references

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