Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supplementary Information

An ICT-based fluorescent ratiometric probe for monitoring mitochondrial peroxynitrite in living cells

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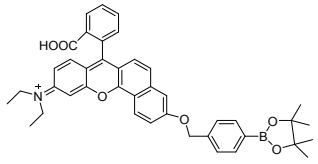
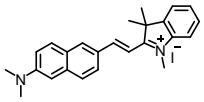
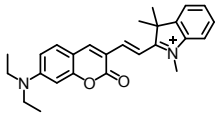
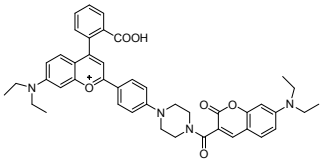
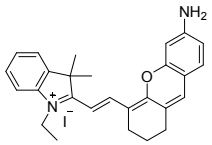
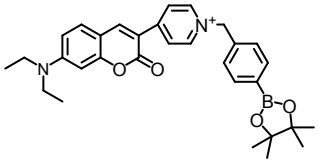
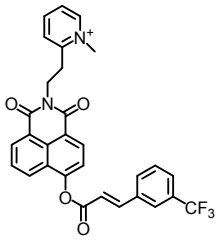
Table S1 A comparison of the ratiometric fluorescent probes for the detection of $ONOO^-$.

Probe	Response mechanism	λ_{em1} / λ_{em2} (nm)	Emission shift (nm)	Mitochon dria-targeting	Ref.
	ICT	560/630	70	No	50
	Oxidization, Cleavage	535/718	183	Yes	33
	Oxidization, Cleavage	515/635	120	Yes	32
	FRET	473/651	173	Yes	31
	Oxidization, Cleavage	460/708	248	Yes	34
	Oxidization, Cleavage	500/565	65	No	51
	Oxidization, Cleavage	454/558	104	Yes	52

Synthesis of compound 2

To a solution of 40% dimethylamine solution (5 mL, 44 mmol) was added compound 1 (2.0 g, 9 mmol) and $Na_2S_2O_5$ (3.57 g, 19 mmol) in H_2O (30 mL). The mixture was stirred at 140◦ C for 48 h. After cooling to room temperature, the mixture was filtered and wash with water. Next, it was extracted with dichloromethane and water. Purification on silicagel afforded compound 2 as solid (1.1 g, 49%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.84 (d, *J* = 1.6 Hz, 1H), 7.62 (d, *J* = 9.2 Hz, 1H), 7.53 (d, *J* = 8.8 Hz, 1H), 7.44 (dd, *J* = 8.8, 2.0 Hz, 1 H), 7.18 (dd, *J* = 9.2, 2.8 Hz, 1 H), 6.88 (d, *J* = 1.6 Hz, 1H), 3.02 (s, 6H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 148.72, 133.47, 129.39, 127.88, 127.85, 127.80, 117.09, 115.08, 106.14, 40.76.

Synthesis of compound 3

Under N_2 atmosphere, n-BuLi (1.056 mL, 2.5 M in hexane, 2.9 mmol) was added to compound 2 (689 mg, 2.8 mmol) in dry THF and cooled to -78 ℃. The mixture was stirred at refluxed for 45 min. Then, dimethylformamide (DMF) (0.44 mL, 5.5 mmol) was added and continue reacted 2 h. After cooling to room temperature, the reaction was quenched with a saturated ammonium chloride solution and extracted with ethyl acetate. the solvent was evaporated under reduced pressure. The residue was purified by the silica gel chromatography using Petroleum ether\ethyl acetate as eluent to afford compound 3 as a atrovirens solid (480 mg, 85% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.00 (s, 1H), 8.13 (s, 1H), 7.82 (m, 2H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.17 (dd, *J* = 9.2, 2.4 Hz, 1 H), 6.88 (s, 1H), 3.11 (s, 6H). ^{13}C-NMR (100 MHz, DMSO-d6) δ 191.81, 150.57, 138.64, 134.78, 130.77, 130.69, 126.85, 125.14,

123.54, 116.26, 105.59, 40.43.

Synthesis of Compound DMANI

Under N_2 atmosphere, compound 3 (80 mg, 0.4 mmol) and 1,2,3,3-tetramethyl-3H-indol-1-ium (121 mg, 0.7 mmol) were dissolved in 8 mL ethyl alcohol. Then, 0.5 mL of piperidine was added to the flask. The reaction mixture was refluxed overnight. After being cooled to room temperature, the mixture was filtered and wash with diethyl ether to obtain an atrovirens solid. The crude product was then purified by flash silica gel chromatography column to yield atrovirens solid **DMANI** (55 mg, 39% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.53 (d, *J* = 9.2 Hz, 1H), 8.48 (s, 1H), 8.19 (d, *J* = 8.8 Hz, 1H), 7.85 (dd, *J* = 7.2, 3.6 Hz, 3H), 7.77 (d, *J* = 8.8 Hz, 1H), 7.59 (t, 3H), 7.30 (dd, *J* = 9.2, 2.0 Hz, 1H), 7.03 (d, *J* = 2.0 Hz, 1H), 4.11 (s, 3H), 3.13 (s, 6H), 1.81 (s, 6H). ^{13}C-NMR (100 MHz, DMSO-d6) δ 181.26, 154.40, 151.29, 143.67, 142.43, 138.20, 136.12, 131.45, 129.33, 129.08, 128.30, 127.28, 125.47, 125.06, 123.27, 125.47, 125.08, 123.27, 116.99, 115.01, 110.08, 105.84, 56.48, 52.08, 34.37, 26.18, 19.04. HRMS (ESI) Calcd. for $C_{25}H_{27}N_2$ $(M+H)^+$: 355.2158,Found355.2169

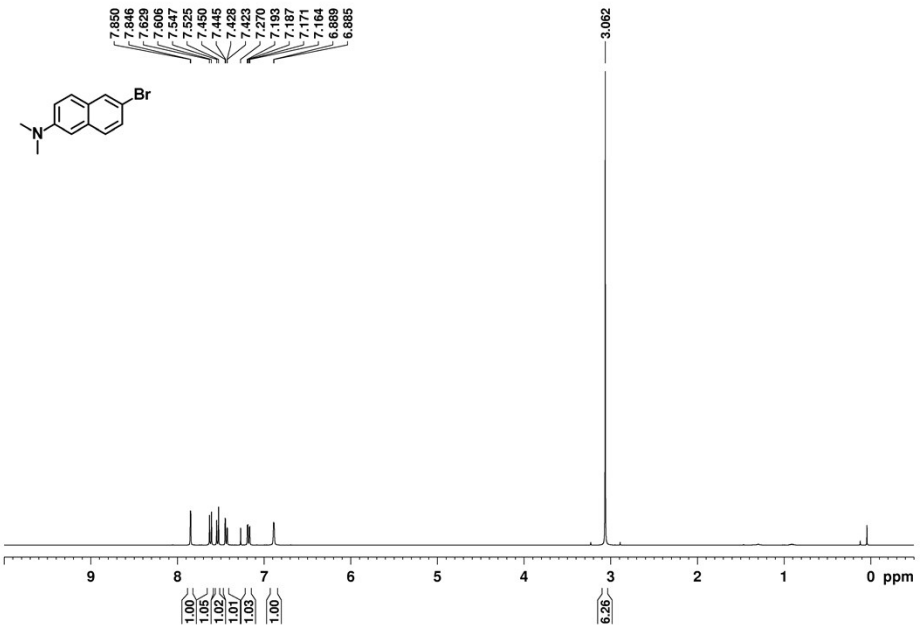


Figure S1 ^{1}H NMR spectra of compound 2 in $CDCl_3$.

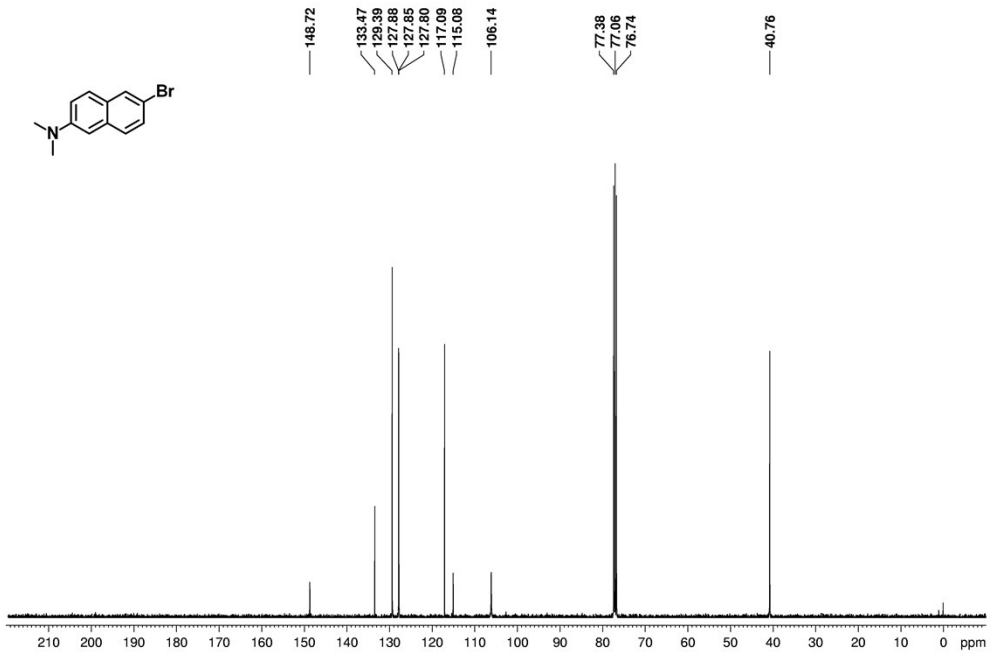


Figure S2 ^{13}C NMR spectra of compound 2 in $CDCl_3$.

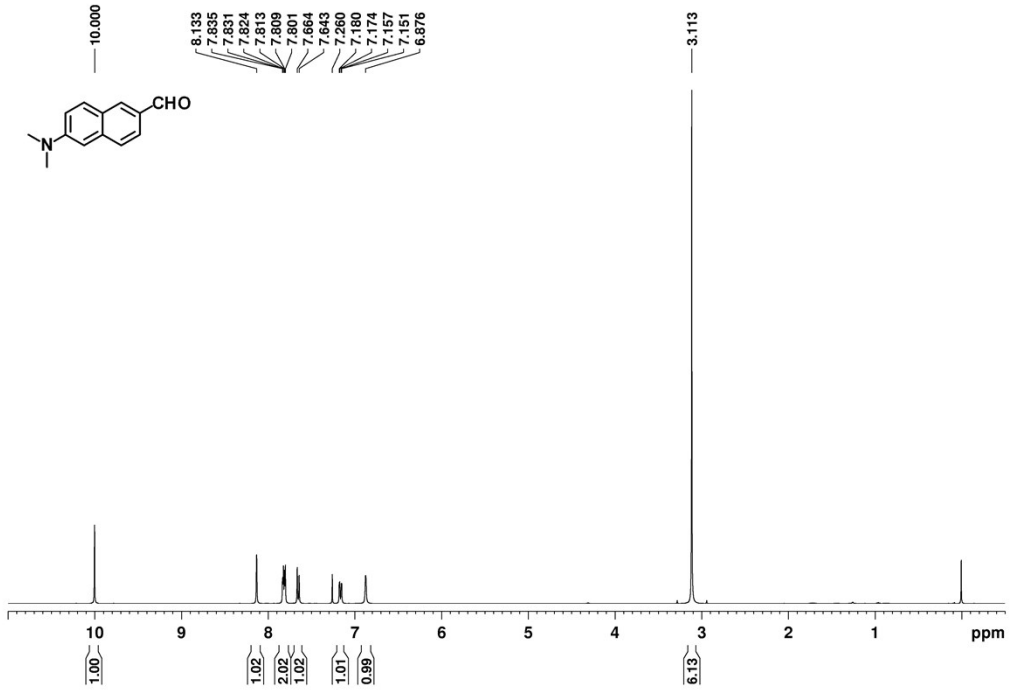


Figure S3 ^{1}H NMR spectra of compound 3 in $CDCl_3$.

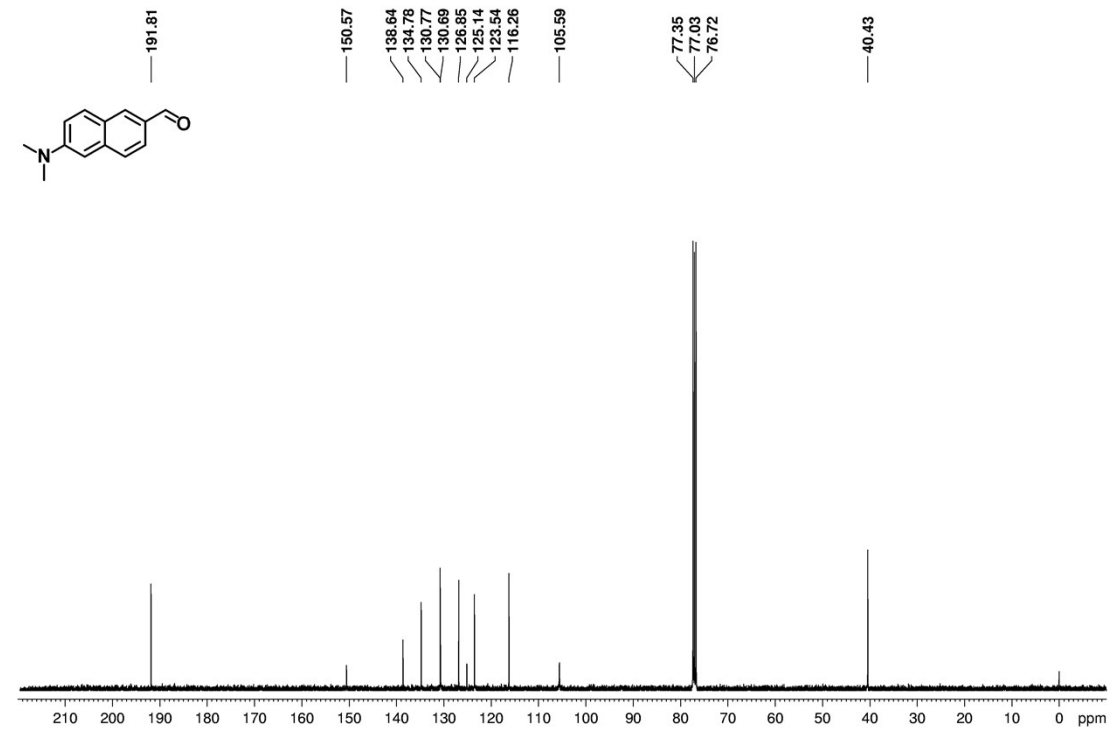


Figure S4 ^{13}C NMR spectra of compound 3 in $CDCl_3$.

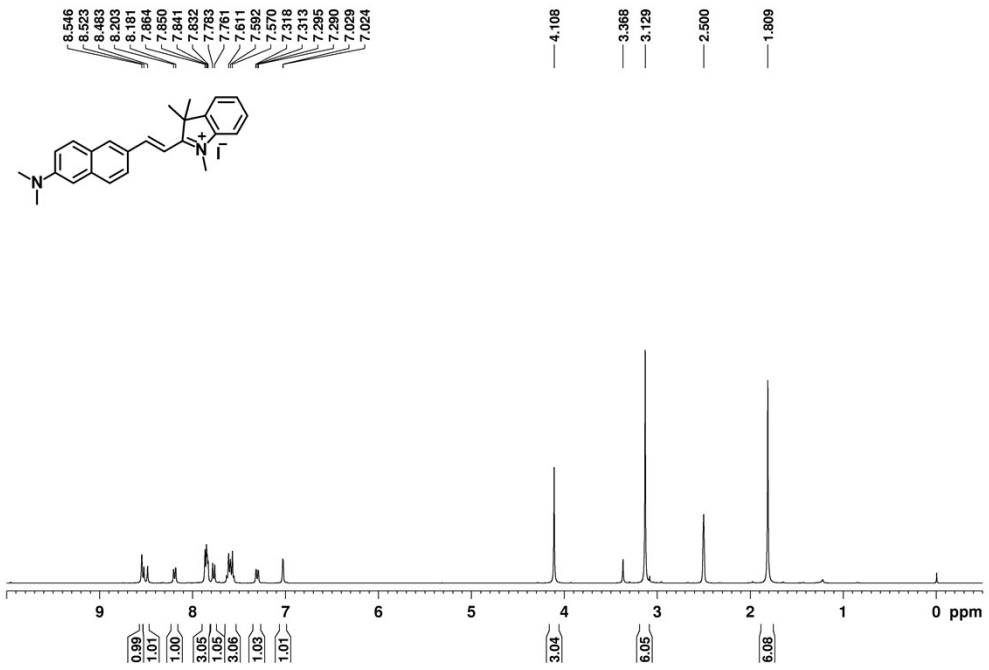


Figure S5 ^{1}H NMR spectra of **DMANI** in DMSO-d6.

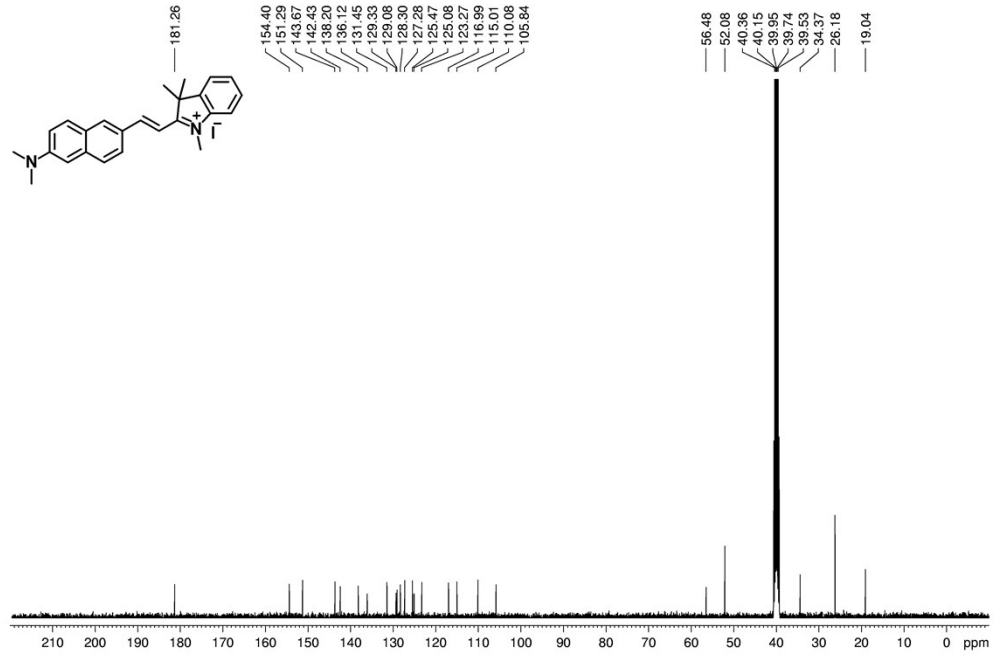


Figure S6 ^{13}C NMR spectra of **DMANI** in DMSO-d6.

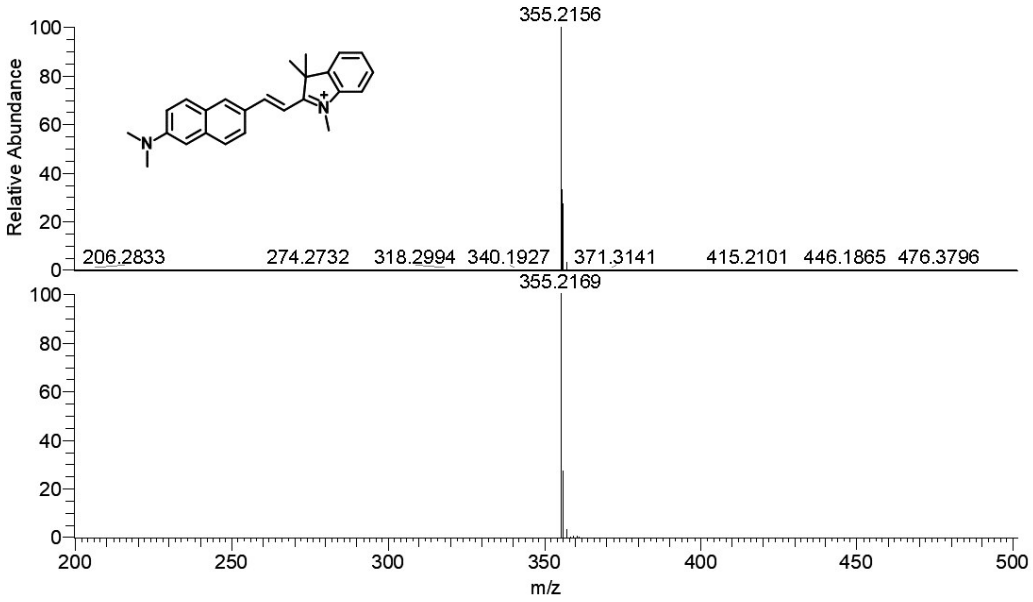


Figure S7 Mass spectra of **DMANI**.

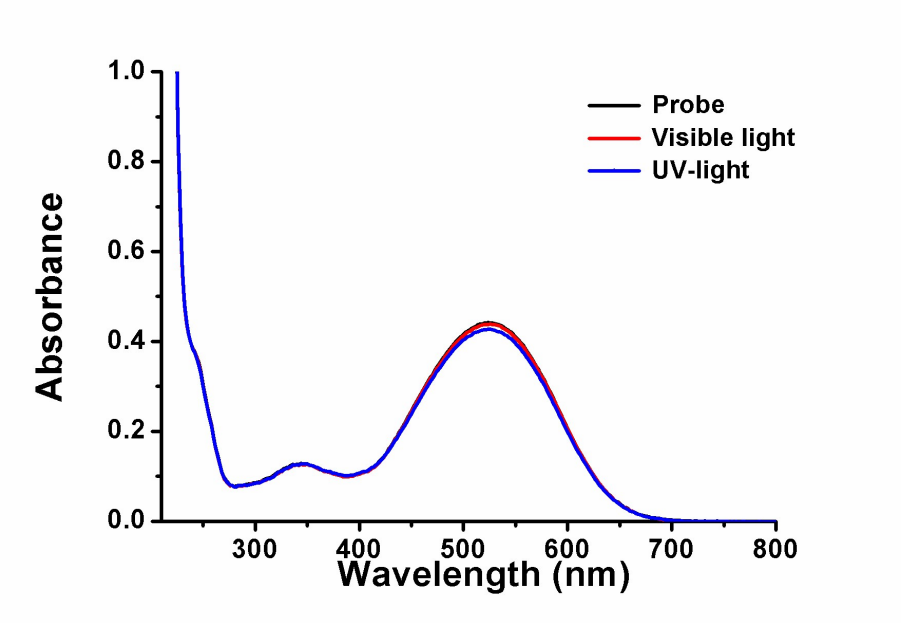


Figure S8 Photostability of the probe **DMANI**.

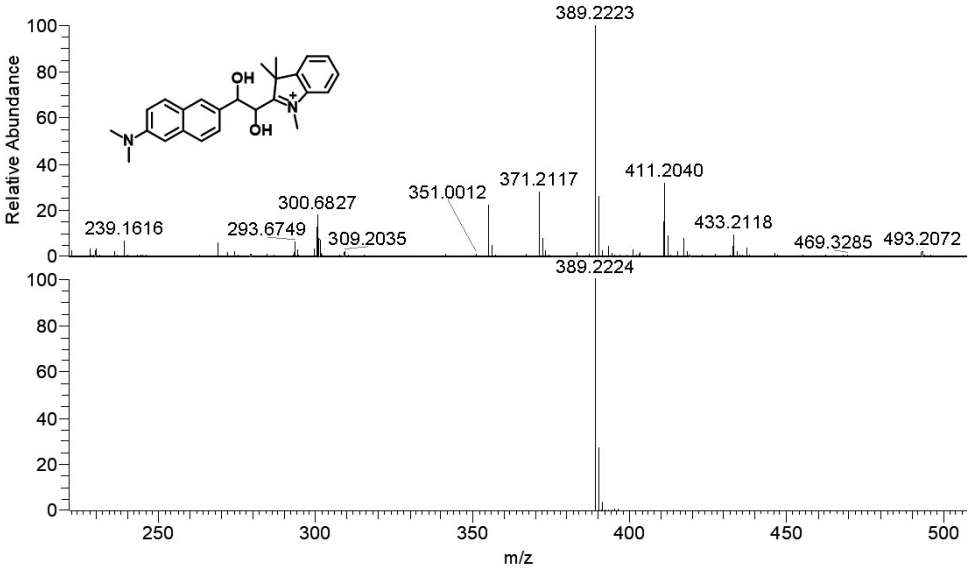


Figure S9 HRMS spectrum recorded after mixing **DMANI** (5 μM) and $ONOO^-$ (30 μM).

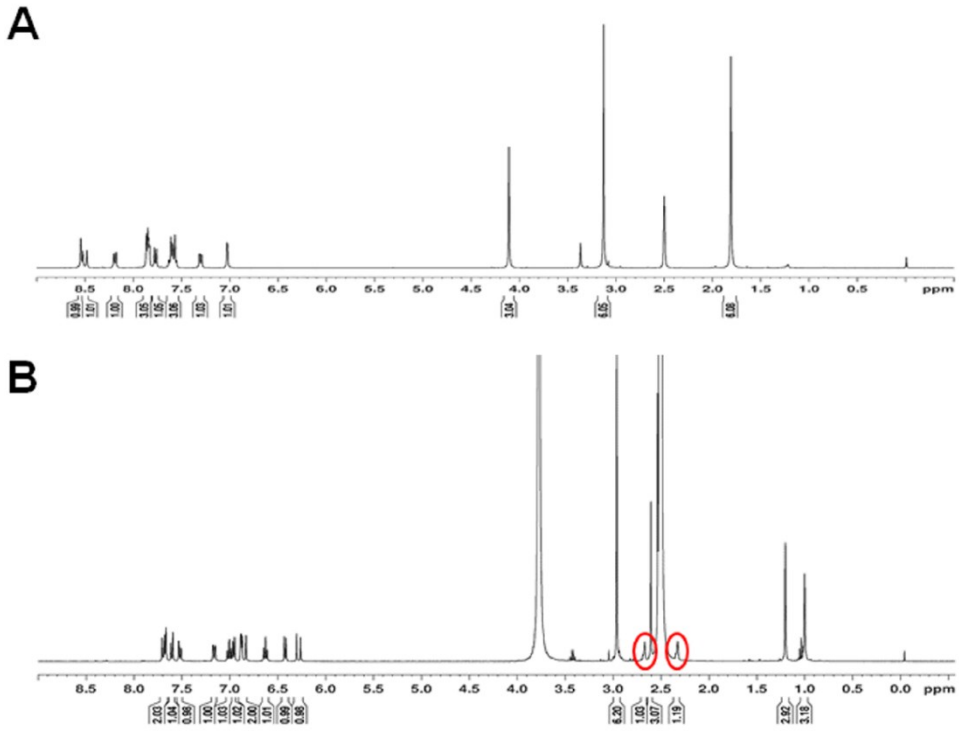


Figure S10 ^1H NMR spectrum recorded **DMANI** (A) or after mixing **DMANI** (5 μM) and $ONOO^-$ (30 μM).

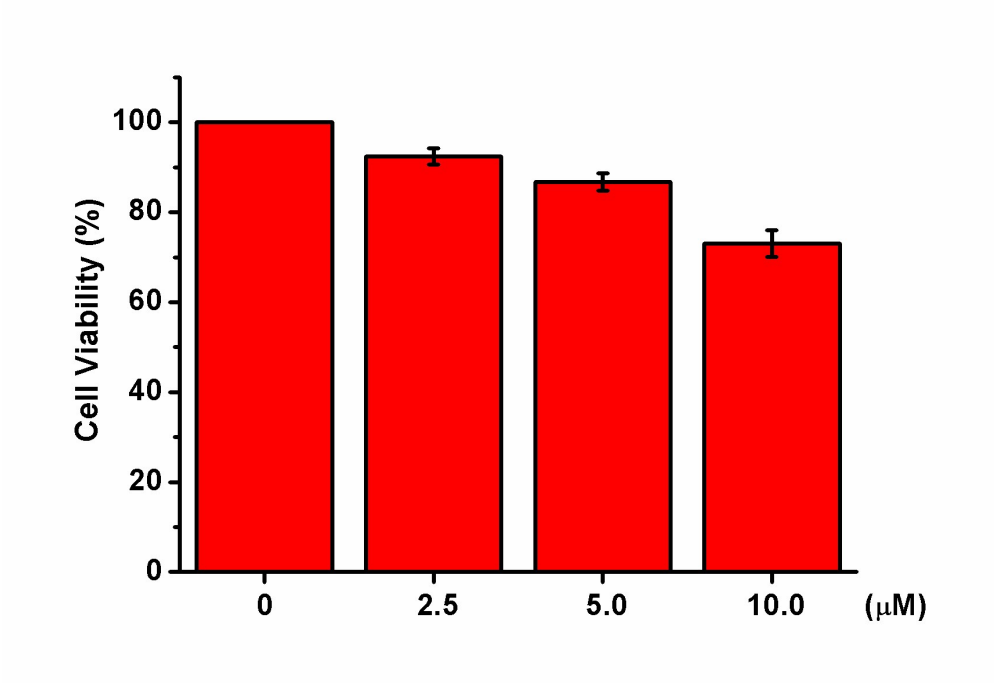


Figure S11 Cell viability of HepG2 cells treated with different concentrations of **DMANI** for 24 h.