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Supporting Information

2-Styrylquinoline-based two-photon AIEgens for dual monitoring of pH and viscosity in living cells

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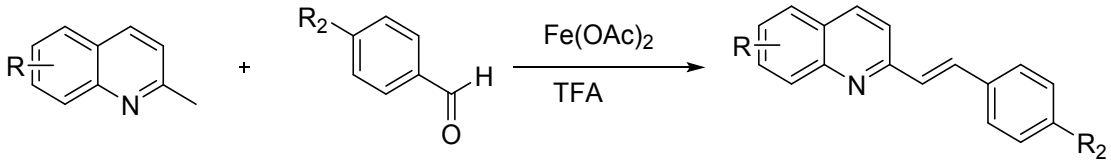
Contents:

1. Experimental Section

General information

All reactions were performed at ambient condition. All chemicals were obtained from commercial sources and used directly without further purification. Solvents used in the experiment have been priorly treated following standard procedure. The reaction process was monitored by TLC. The NMR was recorded in 500 MHz apparatus using $CDCl_3$ or DMSO as solvent, and the frequencies for measuring ^1H, ^{13}C NMR were 126 MHz. Chemical shifts were recorded in ppm by employing TMS (for ^1H NMR) or the solvent peak of $CDCl_3$ (77.0 ppm, for ^{13}C NMR) as internal standard. HRMS data were obtained under ESI model.

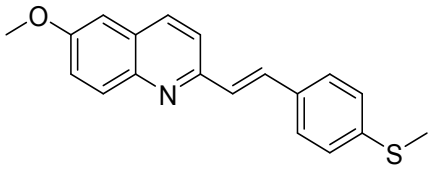
Synthetic procedures



General procedure of product:

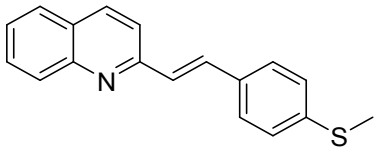
(*E*)-6-methoxy-2-(4-(methylthio)styryl)quinoline

$Fe(OAc)_2$ (3.5 mg, 0.02 mmol), 6-methoxy-2-methylquinoline (58 mg, 0.4 mmol), 4-(methylthio)benzaldehyde (51 mg, 0.48 mmol), and TFA (3 mL, 0.04 mmol) were dissolved in dry toluene (1.0 mL) under N_2 and the mixture was stirred at 100 ℃ for 24 h. The mixture was then cooled to room temperature and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica gel) to give as a white solid.



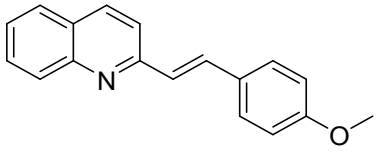
(*E*)-2-(4-(methylthio)styryl)quinoline

$Fe(OAc)_2$ (3.5 mg, 0.02 mmol), 2-methylquinoline (58 mg, 0.4 mmol), 4-(methylthio)benzaldehyde (51 mg, 0.48 mmol), and TFA (3 mL, 0.04 mmol) were dissolved in dry toluene (1.0 mL) under N_2 and the mixture was stirred at 100 ℃ for 24 h. The mixture was then cooled to room temperature and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica gel) to give as a white solid.



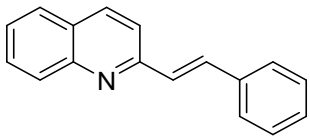
(*E*)-2-(4-methoxystyryl)quinoline

$Fe(OAc)_2$ (3.5 mg, 0.02 mmol), 6-methoxy-2-methylquinoline (58 mg, 0.4 mmol), 4-methoxybenzaldehyde (51 mg, 0.48 mmol), and TFA (3 mL, 0.04 mmol) were dissolved in dry toluene (1.0 mL) under N_2 and the mixture was stirred at 100 ℃ for 24 h. The mixture was then cooled to room temperature and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica gel) to give as a white solid.



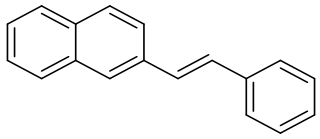
(*E*)-2-styrylquinoline

$Fe(OAc)_2$ (3.5 mg, 0.02 mmol), 6-methoxy-2-methylquinoline (58 mg, 0.4 mmol), benzaldehyde (51 mg, 0.48 mmol), and TFA (3 mL, 0.04 mmol) were dissolved in dry toluene (1.0 mL) under N_2 and the mixture was stirred at 100 ℃ for 24 h. The mixture was then cooled to room temperature and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica gel) to give as a white solid.



(*E*)-2-styrylnaphthalene

$Pd(PPh_3)_2Cl_2$ (0.02 mmol), 2-bromonaphthalene (0.4 mmol), styrene (0.48 mmol), and TEA (3 mL, 0.04 mmol) were dissolved in MeCN (1.0 mL) under N_2 and the mixture was stirred at 100 ℃ for 24 h. The mixture was then cooled to room temperature and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica gel) to give as a white solid.



Cell culture

Adenocarcinoma HeLa cells were cultured in DMEM high glucose media supplemented with 10% fetal bovine serum. The cells were grown overnight at 37 °C incubator with 5% CO_2. A549 cells were seeded at a density of $0.3x10^6$ cells in 35 mm dish and kept overnight prior to cell imaging studies.

Cell viability assay

MTT assay was performed to assess the viability of HeLa cells. The cells were seeded at a density of 4000 cells per well and grown overnight at 37 °C incubator with 5% CO_2. The cells were then exposed to different concentrations of probes for 48 h. After the stipulated time of probe exposure, 10 μL of MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] was added at a concentration of 5 mg/mL and solubilized using 100 μL of MTT solubilizing agent (DMSO) after 2 h. The readings were taken at 590 nm with a reference filter of 620 nm using Synergy HT Multi-Mode Microplate reader (Biotek). The same procedure was followed to assess the viability of the probe-treated A549 cells.

Imaging of pH in HeLa cells

Cells were grown at a density of 0.3 x 10^6 cells in 35 mm dish in DMEM media containing 10% fetal bovine serum. Subsequently, the cells were incubated with the probe **HAPH-1** for 0.5–3 h. After washed twice with PBS buffer, the cells were incubated with different pH buffer. After 15 min incubation, fluorescence imaging was performed using two-photon excitation microscopy (Olympus FVMPE -RS). Ex = 780 nm, Em = 520 nm-580 nm. Blue channel: Ex = 640 nm, Em=420-490 nm, Yellow channel: Ex = 820 nm, 540-620 nm. Scale bar: 20 μm.

Imaging of viscosity in HeLa cells

Cells were grown at a density of 0.3 x 10^6 cells in four dishes in DMEM media containing 10% fetal bovine serum. Before the imaging experiments, the cells were washed with PBS (pH=7.4) buffer three times. HeLa cells were firstly incubated with probe **HAPH-1** (10 μM) (containing 0.1 % DMSO as a cosolvent) for 30 min at 37 ˚C. Cells were washed twice with 1 mL PBS at room temperature, and then 1 mL PBS was added and the cells were observed under a confocal microscopy. Secondly, HeLa cells were co-incubated with nystatin (10.0 μM) for 30 min at 37 ˚C and then washed with PBS twice. The cells were incubated with **HAPH-1** (10 μM) for 20 min at 37 ˚C, and then washed with PBS three times. The other three groups were treated at 4 ˚C, 25 ˚C, and 37 ˚C. The fluorescence images were acquired using two-photon excitation microscopy (Olympus FVMPE-RS). Ex = 780 nm, Em = 520 nm-580 nm. Blue channel: Ex = 640 nm, Em=420-490 nm, Yellow channel: Ex = 820 nm, 540-620 nm.

Fluorescence quantum yields measurement

Fluorescence quantum yield of the probes and the probes after click reaction was determined using the reference of fluorescein (Φ = 0.98) in 0.1 M aqueous NaOH. The quantum yield of probes and the probes after click reaction, were calculated according to following equation.

$\Phi_x=\Phi_s(A_sS_x)/(A_xS_s)$

Φs is the fluorescence quantum yield of fluorescein, $\mathbf{A_x}$ is the absorbance of probes and after click reactions. As is the absorbance of the standard $\mathbf{S_x}$ is integrated fluorescence emission of new dyes while the **Ss** is integrated fluorescence emission of the standard.

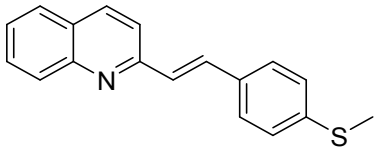
Two-photon absorption cross section measurement.

$$\delta = \frac{S_s \Phi_r \phi_r c_r}{S_r \Phi_s \phi_s c_s} \delta_r$$

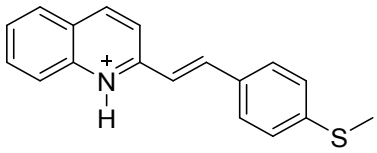
S and r respectively represent the sample and reference molecules, by the photomultiplier detector (PMT, photomultiplier tube) acquisition of signal strength as s, Φ for quantum yield, ϕ experimental instrument to collect fluorescence efficiency, number of molecules in the solution density is expressed as c, the delta two-photon absorption cross section of the sample under test, the delta r is a reference molecules of two-photon absorption cross section.

2. Characterization Data of the Compounds

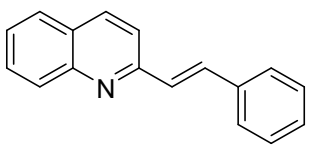
(*E*)-2-(4-(methylthio)styryl)quinoline: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (d, *J* = 8.6 Hz, 1H), 8.07 (t, *J* = 7.2 Hz, 1H), 7.79 – 7.76 (m, 2H), 7.71 (m, *J* = 8.3, 5.7, 1.3 Hz, 1H), 7.66 (d, *J* = 8.4 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.49 (dd, *J* = 11.3, 4.3 Hz, 1H), 7.41 – 7.35 (m, 1H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.29 – 7.27 (m, 1H), 2.54 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.01, 147.91, 139.44, 136.36, 133.90, 132.99, 129.97, 129.76, 129.07, 128.17, 127.55 (d, J = 17.7 Hz), 126.46, 125.23, 122.17, 119.22, 14.68. HRMS (ESI$^+$): Calculated for $C_{18}H_{15}NS$: $[M+H]^+$ 278.3850 , found 278.3853.



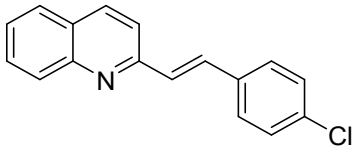
(*E*)-2-(4-(methylthio)styryl)quinolin-1-ium: ^{1}H NMR (500 MHz, DMSO) δ 16.13 (m, 1H), 9.03 (d, *J* = 8.9 Hz, 1H), 8.50 (t, *J* = 8.9 Hz, 2H), 8.42 (d, *J* = 16.4 Hz, 1H), 8.26 (d, *J* = 7.8 Hz, 1H), 8.11 – 8.05 (m, 1H), 7.92 – 7.82 (m, 2H), 7.68 (d, J = 8.4 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H), 2.53 (s, 3H).



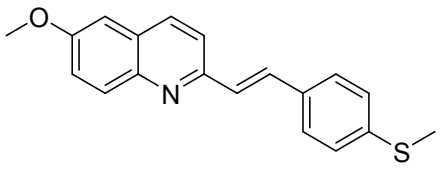
(*E*)-2-styrylquinoline:^{1}H NMR (500 MHz, DMSO) δ 8.36 (d, *J* = 8.6 Hz, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.95 (d, *J* = 7.4 Hz, 1H), 7.87 (dd, *J* = 16.9, 12.5 Hz,2H), 7.75 (d, *J* = 7.2 Hz, 3H), 7.59 – 7.54 (m, 1H), 7.50 (d, *J* = 16.4 Hz, 1H), 7.45 (t, *J* = 7.6 Hz, 2H), 7.36 (t, *J* = 7.3 Hz, 1H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.95, 148.23, 136.50, 136.28, 134.40, 129.69, 129.17, 128.99, 128.76, 128.59, 127.54 – 127.12 (m), 126.12, 119.22. HRMS (ESI$^+$): Calculated for $C_{17}H_{13}N$: $[M+H]^+$ 232.2980, found 232.2983.



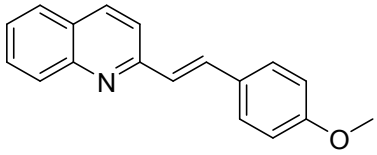
(*E*)-2-(4-chlorostyryl)quinoline: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 (d, *J* = 8.6 Hz, 1H), 7.99 (d, *J* = 8.4 Hz, 1H), 7.94 (d, *J* = 8.0 Hz, 1H), 7.88 – 7.84 (m, 1H), 7.79 – 7.74 (m, 3H), 7.56 (m, *J* = 8.0, 7.0, 1.1 Hz, 1H), 7.48 (dd, *J* = 6.7, 1.7 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.32, 147.61, 136.51 (d, *J* = 13.9 Hz), 135.21, 133.02, 132.67, 129.68 (d, *J* = 38.0 Hz), 128.89, 128.75–128.11 (m), 127.77, 127.05, 126.26, 119.97. HRMS (ESI$^+$): Calculated for $C_{17}H_{12}ClN$: $[M+H]^+$ 266.7400，found 266.7395.



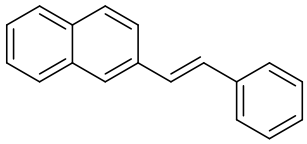
(*E*)-6-methoxy-2-(4-(methylthio)styryl)quinoline: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, *J* = 8.6 Hz, 1H), 7.98 (d, *J* = 9.3 Hz, 1H), 7.65 – 7.59 (m, 2H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.39 – 7.35 (m, 2H), 7.29 (s, 2H), 7.08 (d, *J* = 2.8 Hz, 1H), 3.95 (s, 3H), 2.53 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.69, 153.78, 144.34 , 139.06 , 135.11 , 133.65, 132.67, 130.63, 128.38, 127.52, 126.60, 122.31, 119.59, 105.37, 55.58, 29.71. HRMS (ESI$^+$): Calculated for $C_{19}H_{17}NOS$: $[M+H]^+$ 308.4110，found 308.4116.



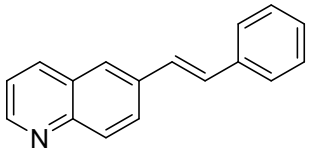
(*E*)-2-(4-methoxystyryl)quinoline: ^{1}H NMR (500 MHz, $CDCl_3$): 8.05-8.09 (m, 2H), 7.76 (dd, *J1* = 8.0 Hz, *J2* = 1.0 Hz, 1H), 7.56-7.70 (m, 5H), 7.45-7.48 (m, 1H), 7.25-7.29 (m, 1H), δ6.91-6.92 (m, 1H,), 4.06 (q, *J* = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 159.5, 156.4, 148.3, 136.2, 134.1, 129.6, 129.1, 128.6, 127.4, 127.2, 126.7, 125.9, 119.1, 114.7, 63.5,. HRMS (ESI$^+$): Calculated for $C_{18}H_{15}NO$: [M+H]+ 262.3240，found 262.3245.



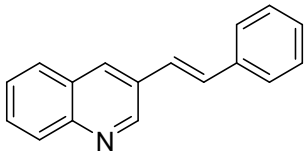
(*E*)-2-styrylnaphthalene: ^{1}H NMR (500 MHz, DMSO) δ 8.03 (s, 1H), 7.95 – 7.87 (m, 4H), 7.69 – 7.64 (m, 2H), 7.55 – 7.48 (m, 2H), 7.42 (dd, J = 12.1, 4.6 Hz, 4H), 7.33 – 7.26 (m, 1H); ^{13}C NMR (126 MHz, DMSO) δ 137.05, 134.65, 133.28, 132.57, 128.97, 128.75, 128.42, 128.20, 127.96 – 127.51 (m), 126.64 – 126.27 (m), 123.63. HRMS (ESI^{+}): Calculated for $C_{18}H_{14}$: $[M+H]^{+}$ 231.3100，found 231.3103.



(*E*)-6-Styrylquinoline: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 (dd, *J* = 4.6, 1.3 Hz, 1H), 8.56 (dd, *J* = 8.6, 1.4 Hz, 1H), 8.07 (d, *J* = 8.4 Hz, 1H), 7.84 – 7.69 (m, 3H), 7.61 (d, *J* = 7.6 Hz, 2H), 7.47 – 7.40 (m, 3H), 7.33 (t, *J* = 7.4 Hz, 1H), 7.18 (d, *J* = 16.0 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.46, 148.70, 137.32, 135.52, 133.00, 132.30, 129.41, 129.39, 128.96, 128.27, 126.89, 126.60, 124.36, 124.03, 121.08. HRMS (ESI^{+}): Calculated for: $C_{17}H_{13}N$ $[M+H]^{+}$: 232.1048; found: 232.1048



(*E*)-3-Styrylquinoline: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.12 (d, *J* = 2.2, 1H), 8.17 (d, *J* = 2.0, 1 H), 8.12–8.10 (m, 1 H), 7.83–7.81 (m, 1H), 7.70–7.66 (m, 1H), 7.59–7.53 (m, 3H), 7.42–7.39 (m, 2H), 7.34–7.29 (m, 1H), 7.33 (d, *J* = 16.5, 1H), 7.24 (d, *J* = 16.5, 1H). ^{13}C NMR (126 MHz, DMSO) δ 149.54, 136.73, 132.06, 130.69, 130.22, 129.25, 128.76 (d, *J* = 9.1 Hz), 128.10, 127.74, 127.03, 126.67, 125.22. HRMS (ESI^{+}): Calculated for: $C_{17}H_{13}N$ $[M+H]^{+}$: 232.1048; found: 232.1048



3. Supporting Figures and Tables

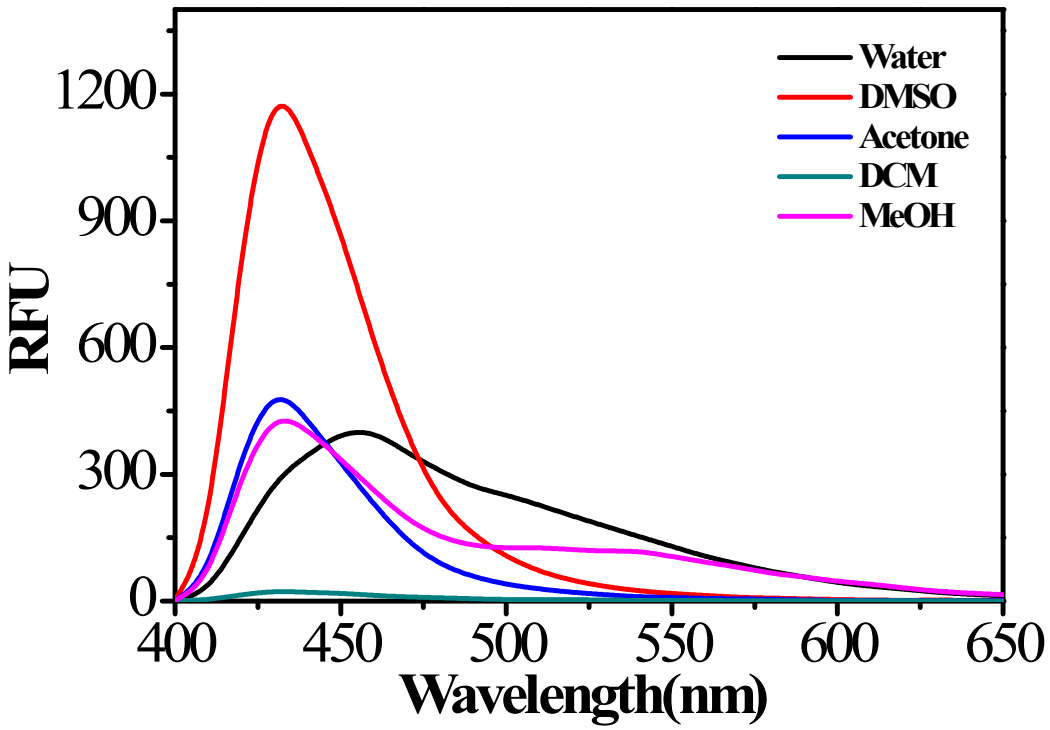


Fig S1. Emission spectra of **AQ-3S** (10 μM) in different solvents.

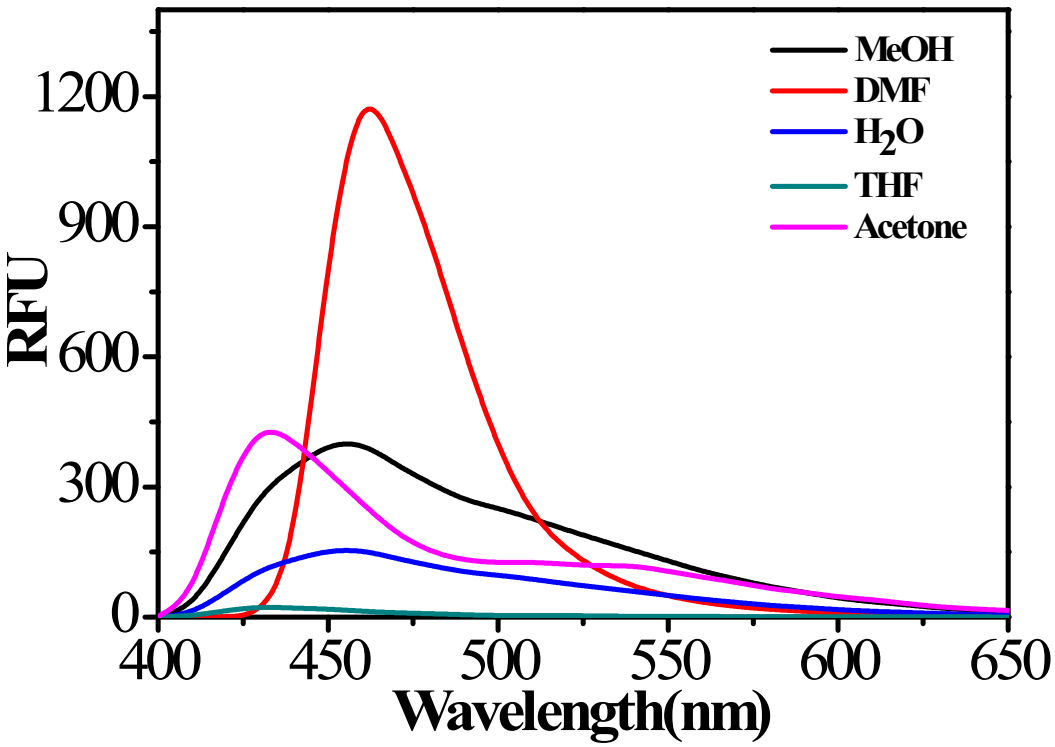


Fig S2. Emission spectra of **AQ-6S** (10 μM) in different solvents.

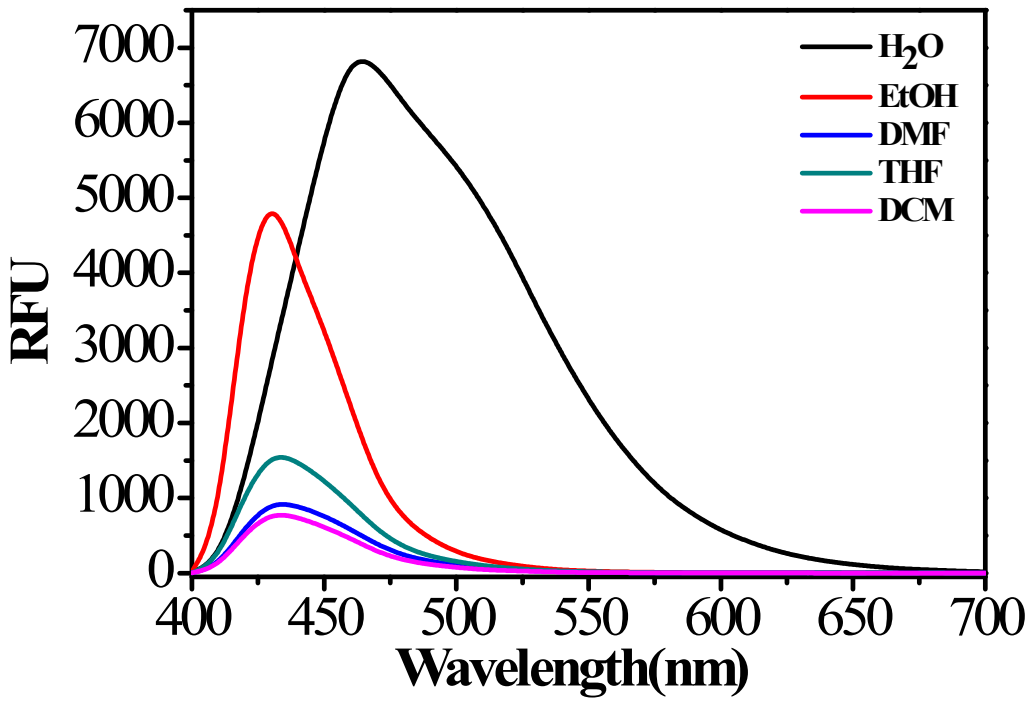


Fig S3. Emission spectra of **HAPH** (10 μM) in different solvents.

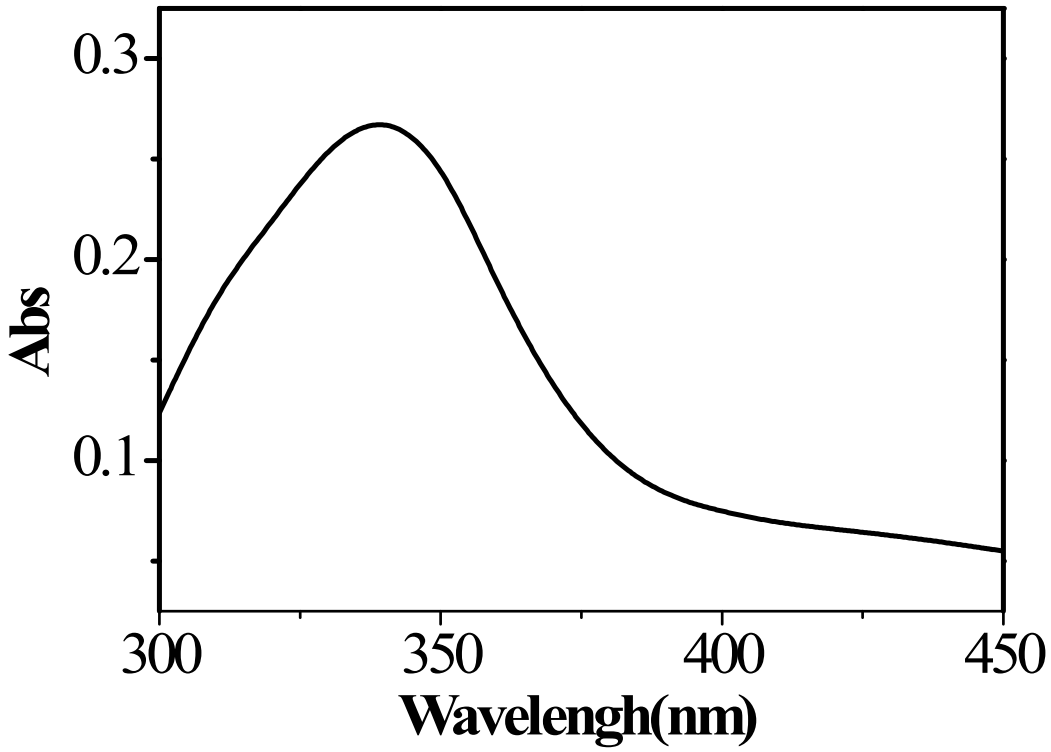


Fig S4. Excitation spectrum of **HAPH-1** （10 uM）in PBS.

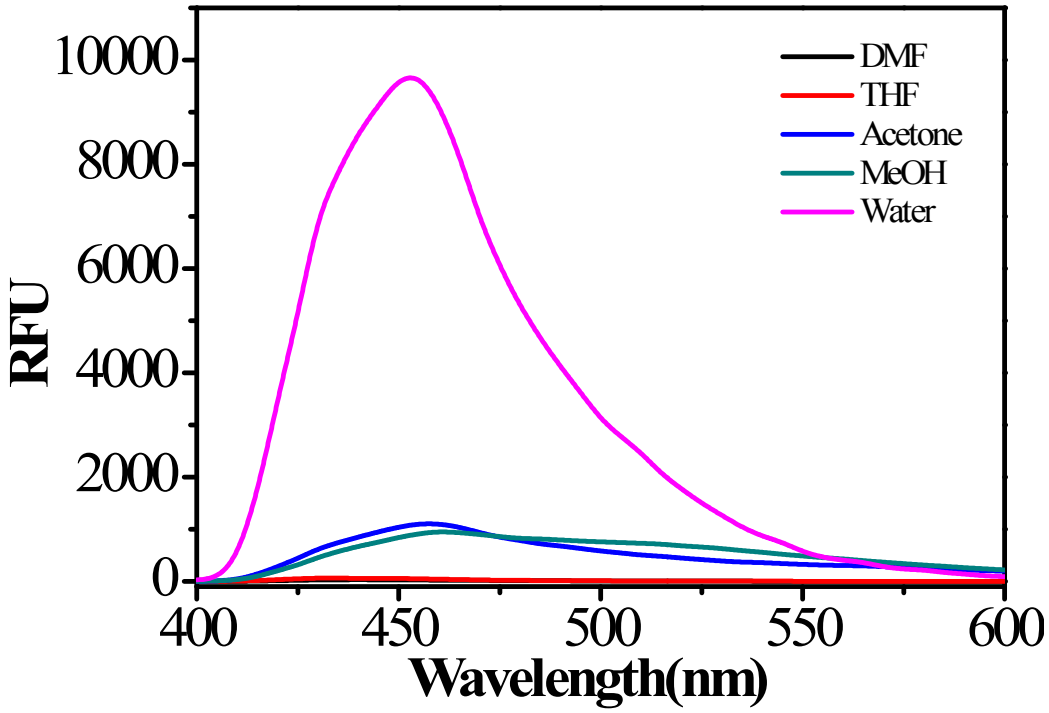


Fig S5. Emission spectra of **HAPH-1**(10 μM) in different solvents.

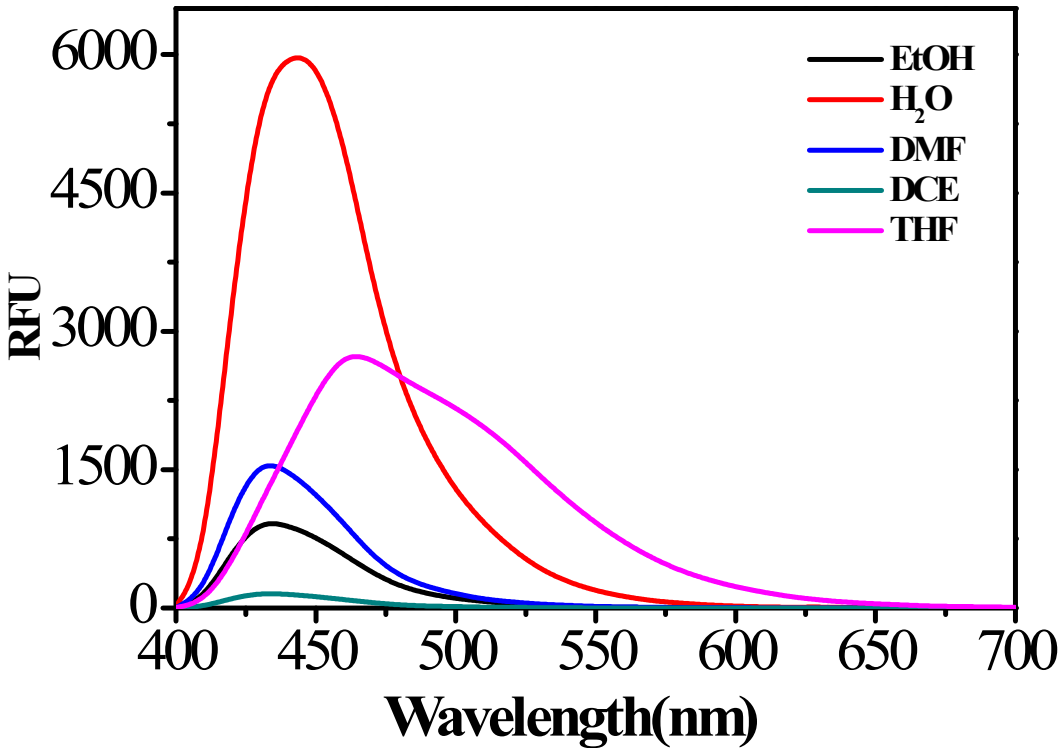


Fig S6. Emission spectra of **HAPH-2** (10 μM) in different solvents.

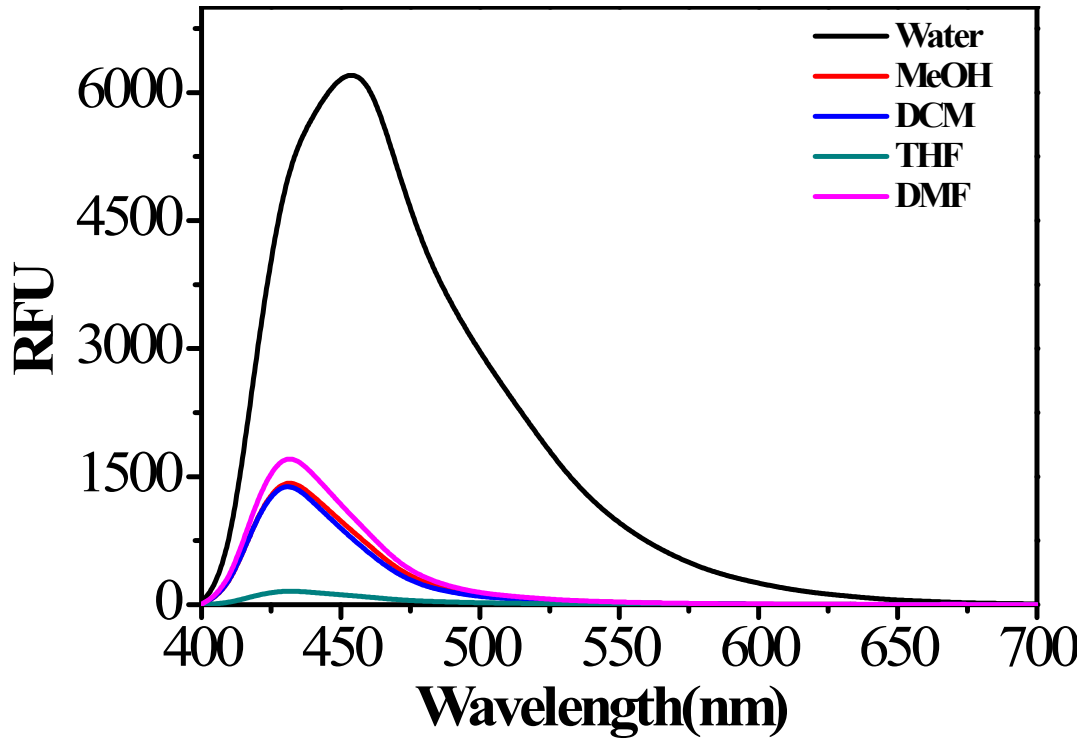


Fig S7. Emission spectra of **HAPH-3** (10 μM) in different solvents.

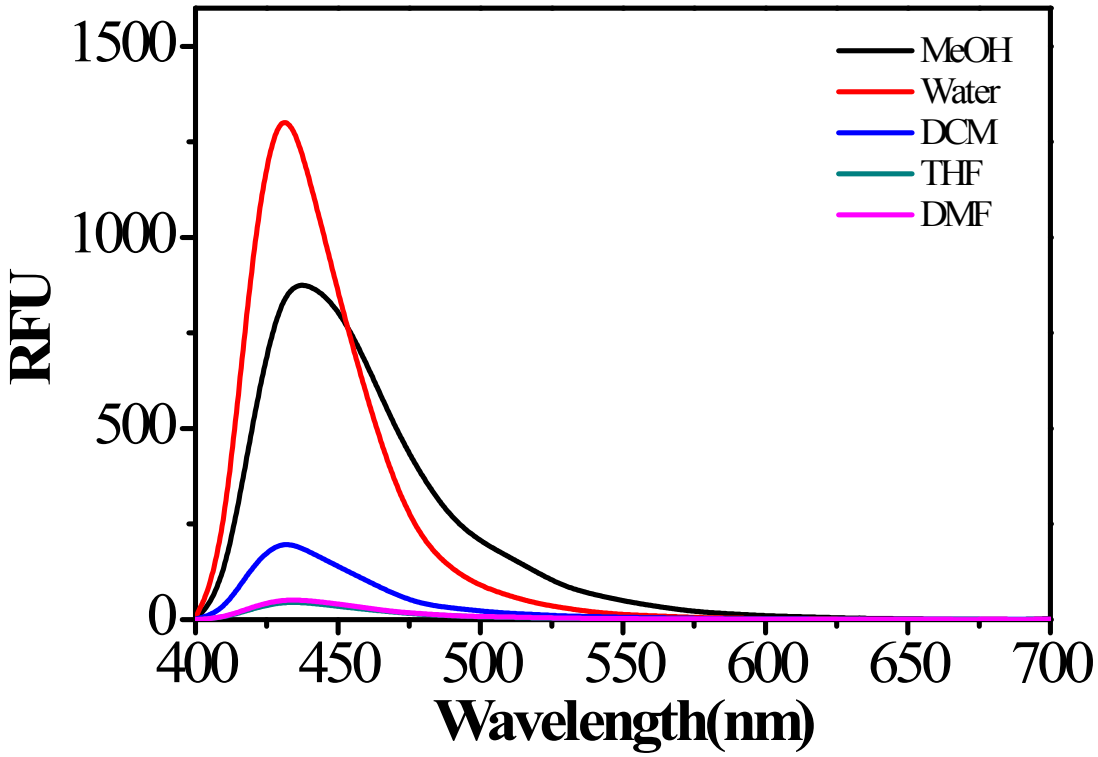


Fig S8. Emission spectra of **HAPH-4** (10 μM) in different solvents.

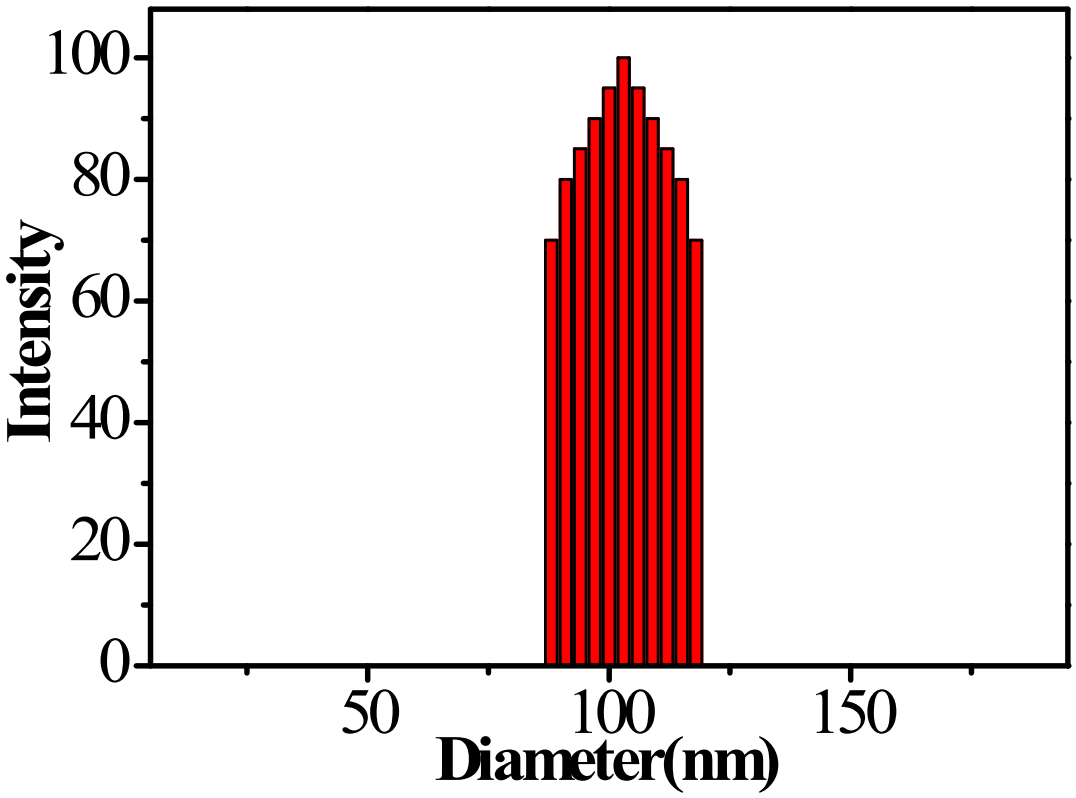


Fig 9. Hydrodynamic diameters of **HAPH-1** in DMSO/H_2O (v/v= 1:99) measured by LLS

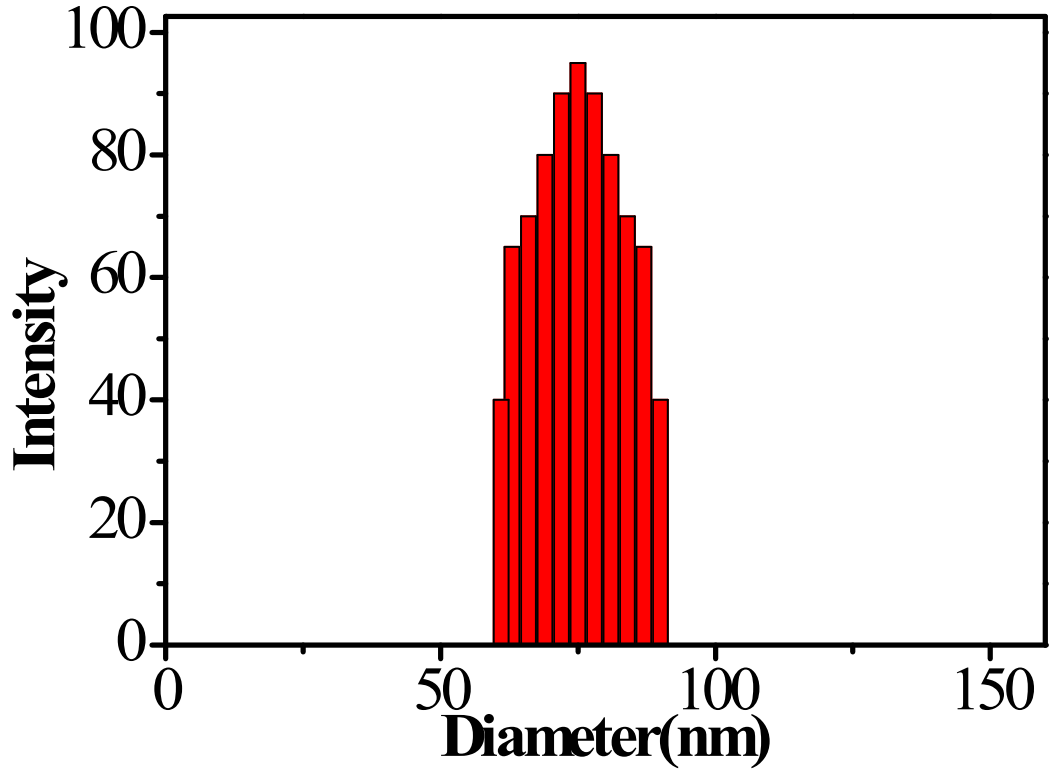


Fig S10. Hydrodynamic diameters of **HAPH-3** in DMSO/H_2O (v/v= 1:99) measured by LLS.

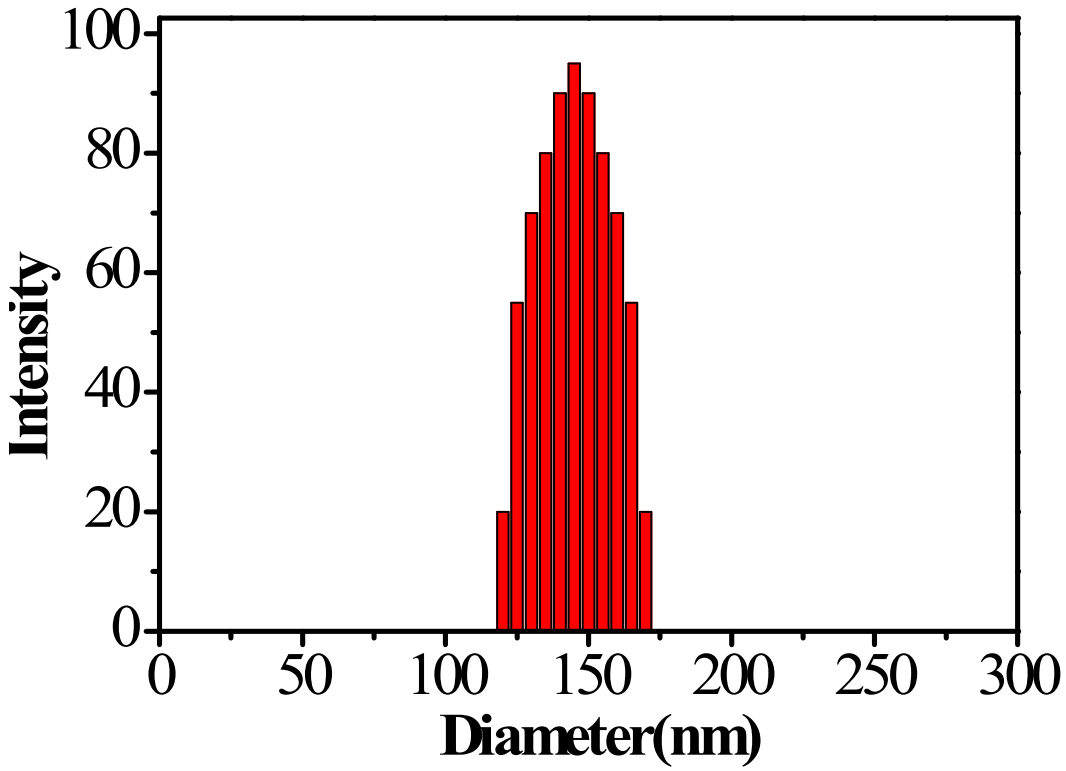


Fig S11. Hydrodynamic diameters of **HAPH-4** in DMSO/H_2O (v/v= 1:99) measured by LLS.

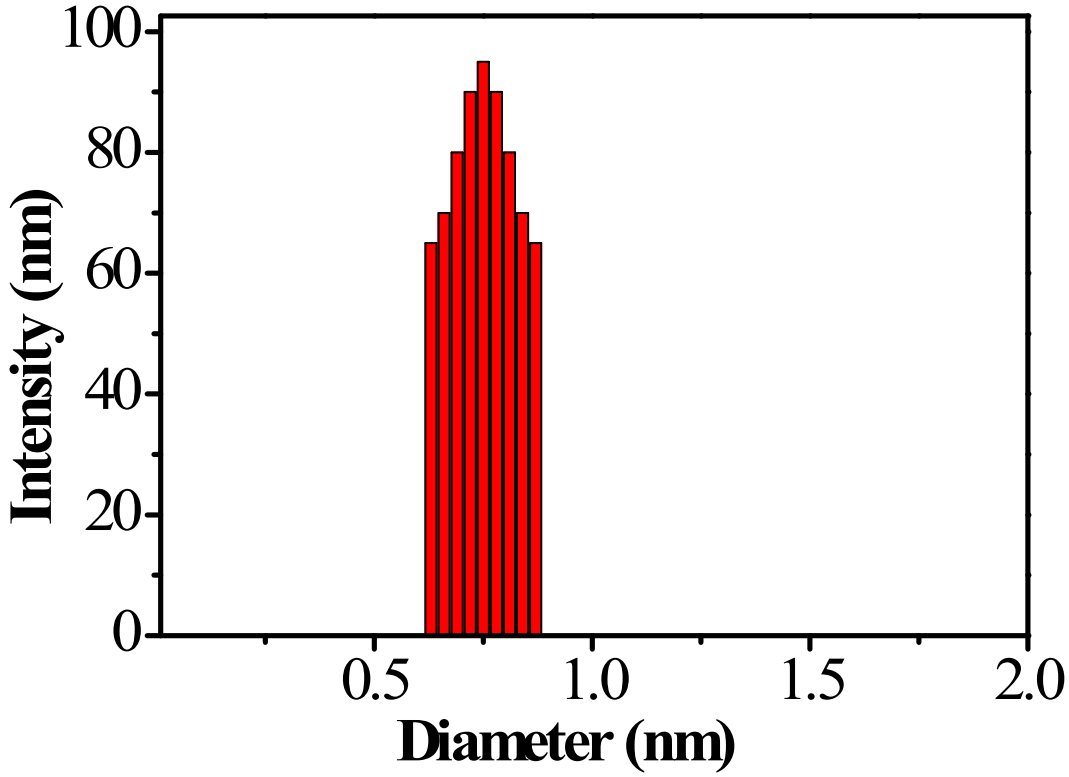


Fig S12. Hydrodynamic diameters of **HAPH-6** in DMSO/H_2O (v/v= 1:99) measured by LLS.

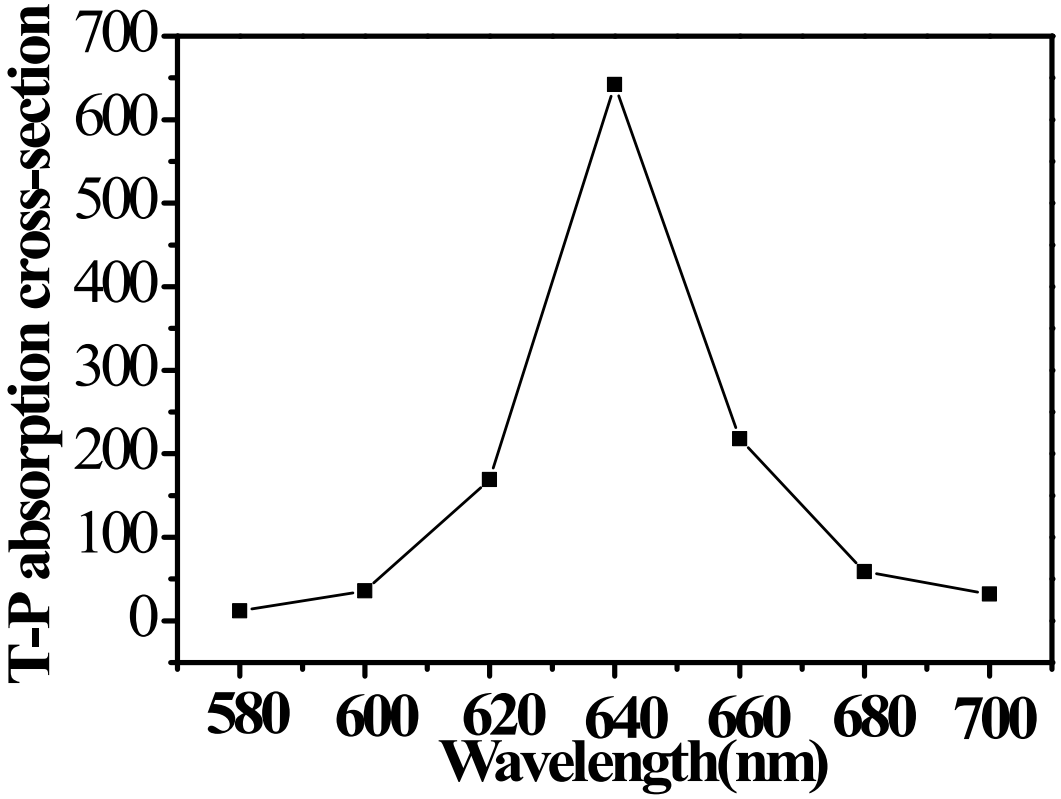


Fig S13. Two-photon absorption cross-section value of **HAPH-1**

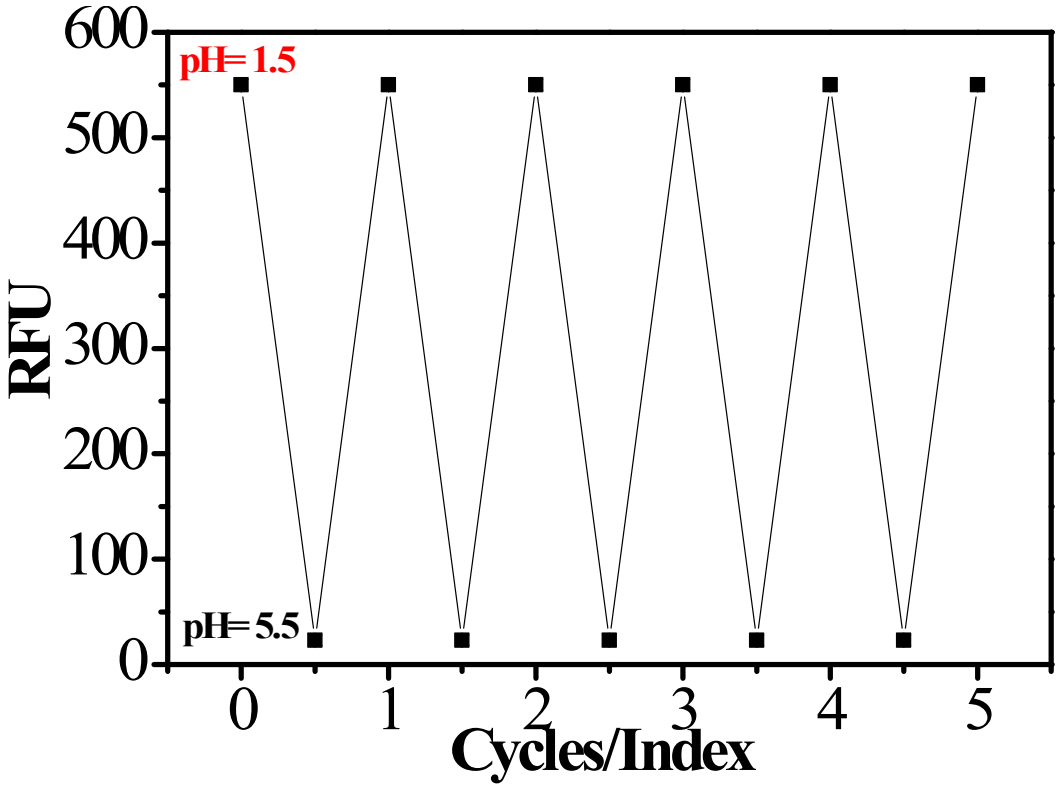


Fig S14. pH reversibility study of **HAPH-1**(2.5 μM) between pH=1.5 and pH=5.5.

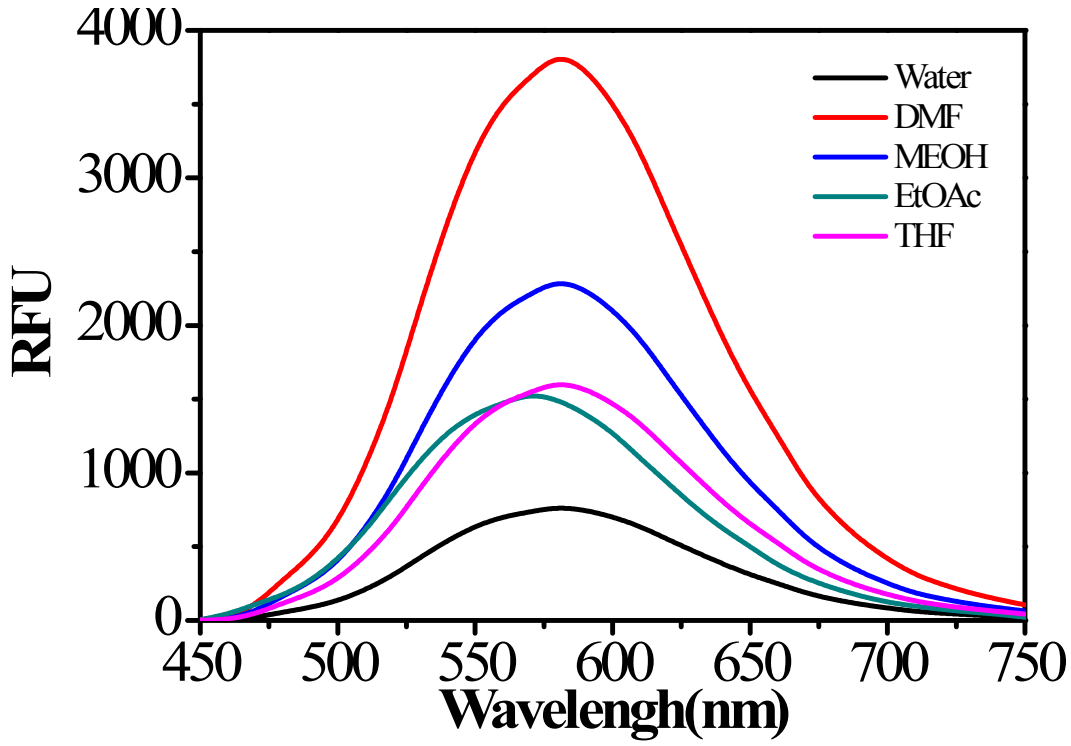


Fig S15. Emission spectra of **HAPH-1** (10 μM) in different solvents at pH=1.0.

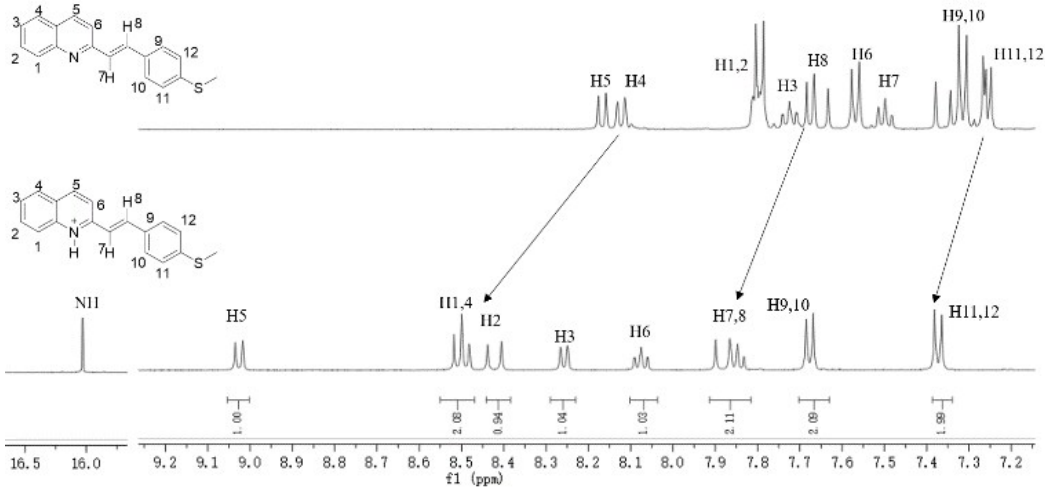


Figure S16. Partial ^{1}H NMR spectra of **HAPH-1** and **HAPH-1-HCl** in d-DMSO.

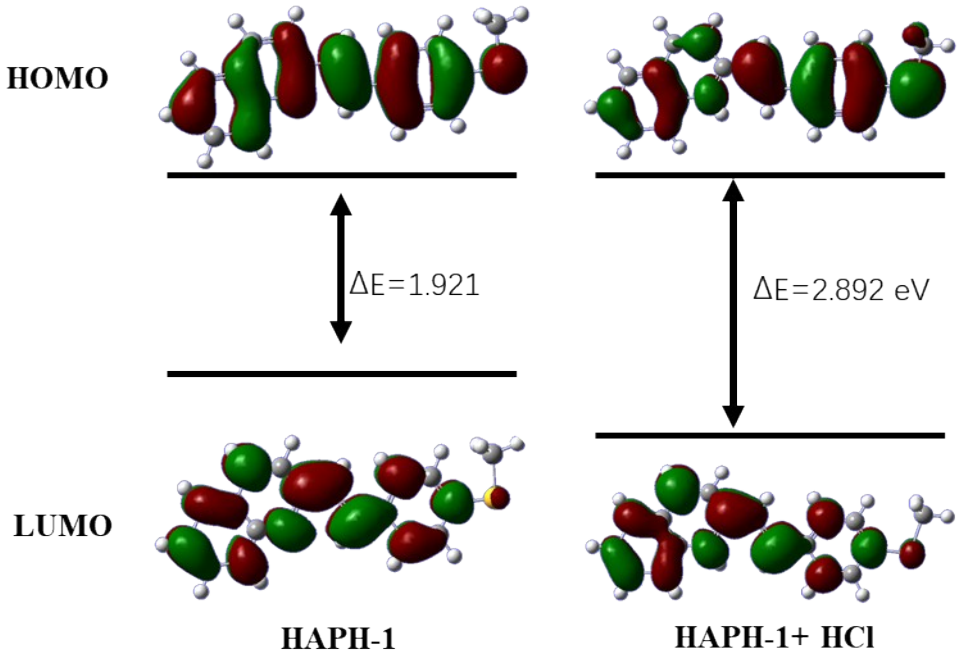


Fig S17. Molecular orbital distribution vertical transition energy by DFT calculation (CAM-B3LYP/6-31G*) of the corresponding first excited-state optimized structures.

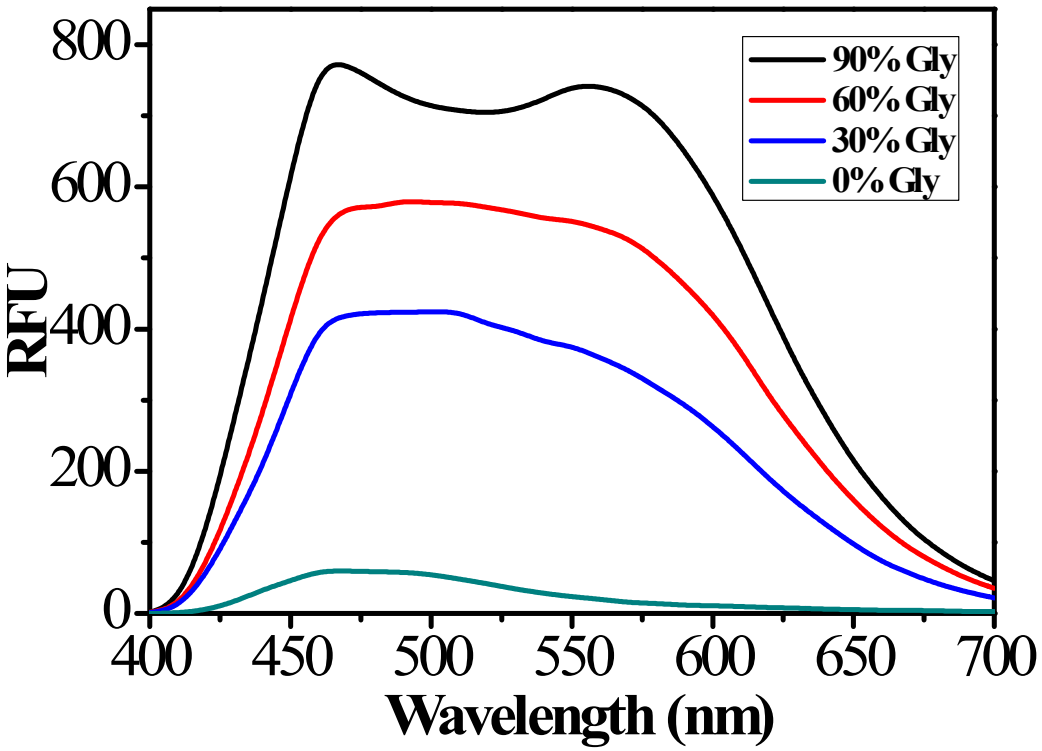


Fig S18. Emission spectra of **HAPH-1** (10 μM) in various glycerol–DMF mixture.

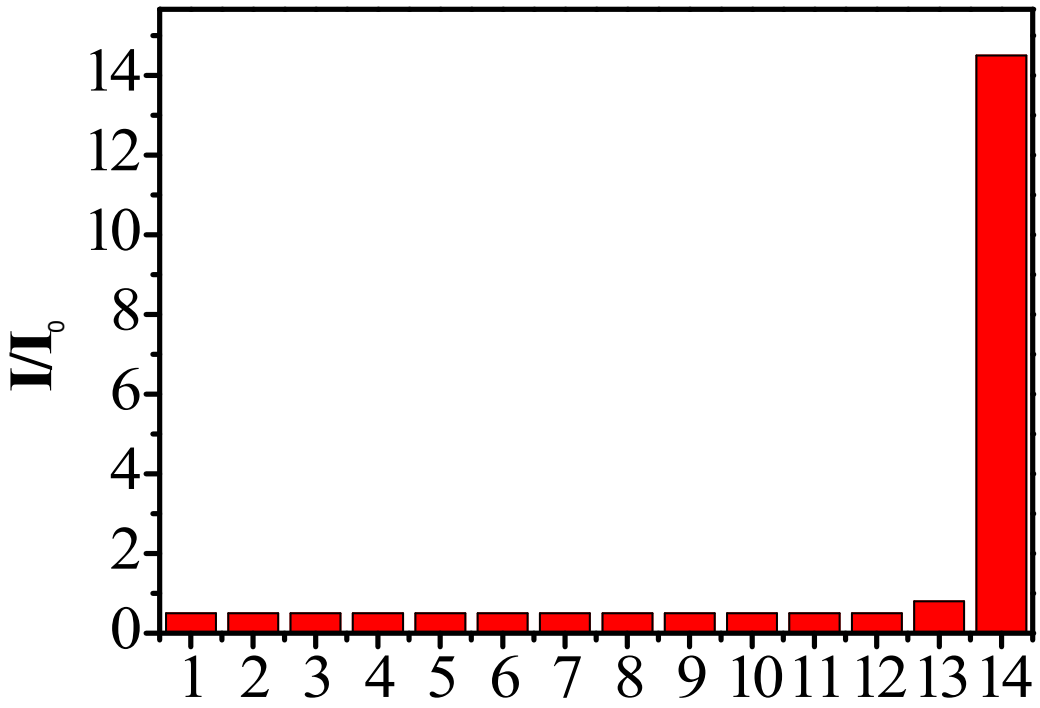


Fig S19. Fluorescence intensity of probe **HAPH-1** (2.5 μM) in the presence of various analytes (50 uM, $ONOO^-$, NO^{2-}, H_2O_2, Co^{2+}, Fe^{2+}, Hg^{2+}, Ag^+, Ni^{2+}, TBHP, GSH, Hcy, Cys) and viscosity (EtOH/Glycerol) solution.

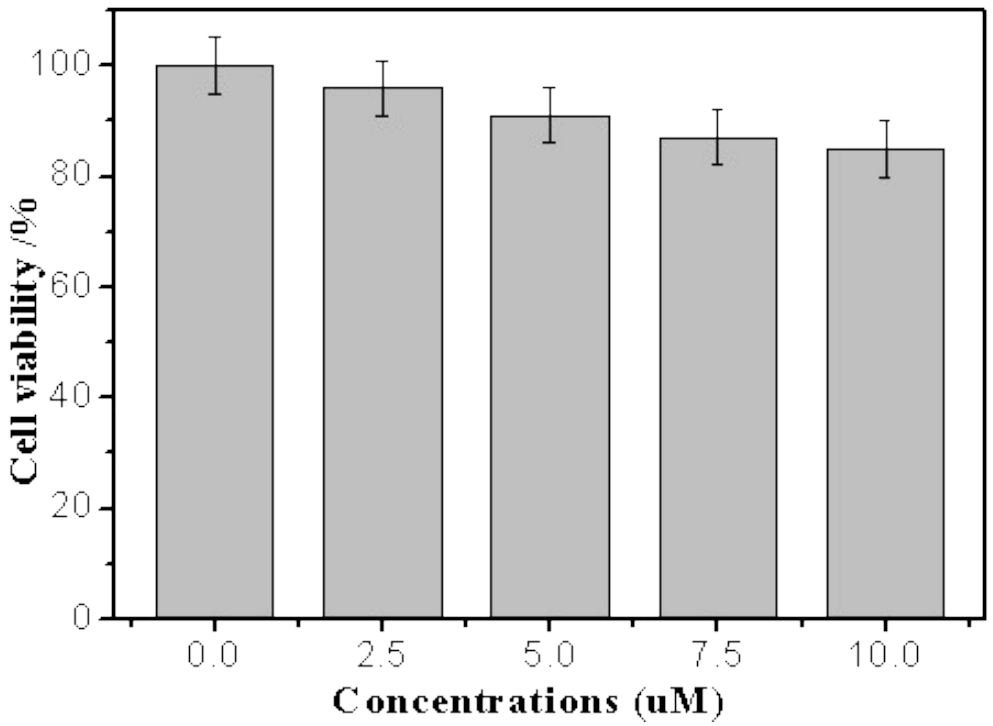
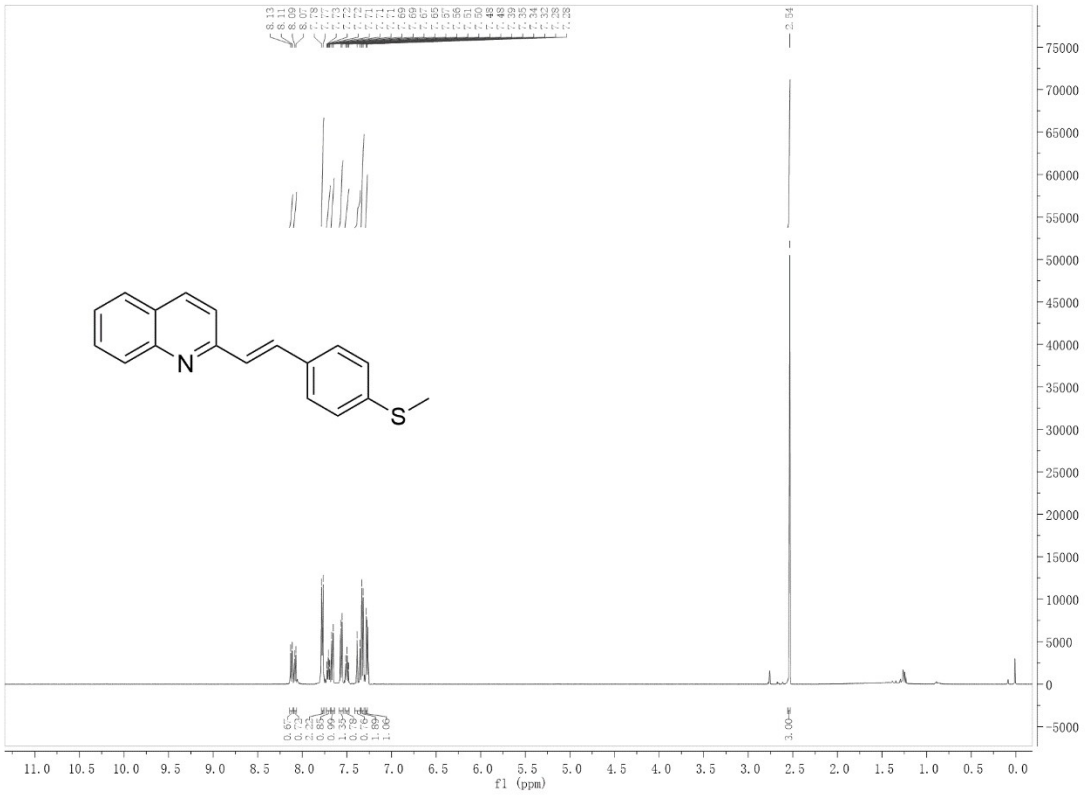


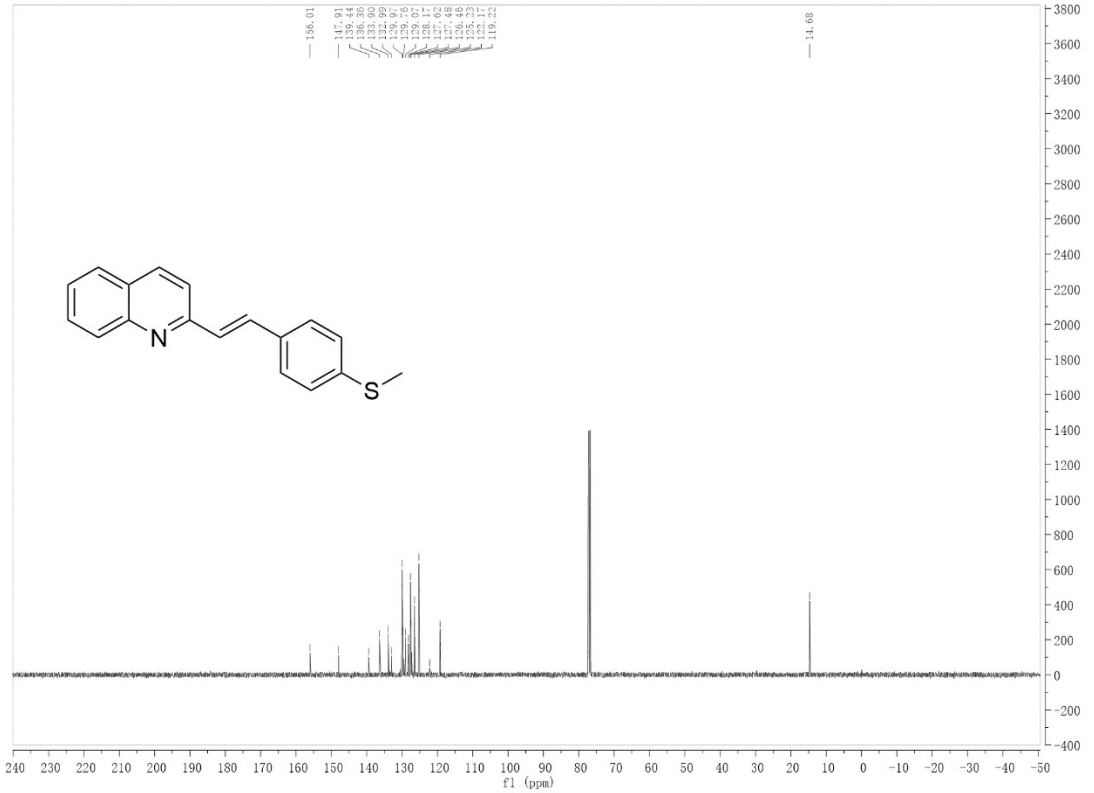
Figure S20. Cell viability of **HAPH-1** by a standard MTT assay (HeLa). The experiments were repeated five times and the data are shown as mean (±S.D.).

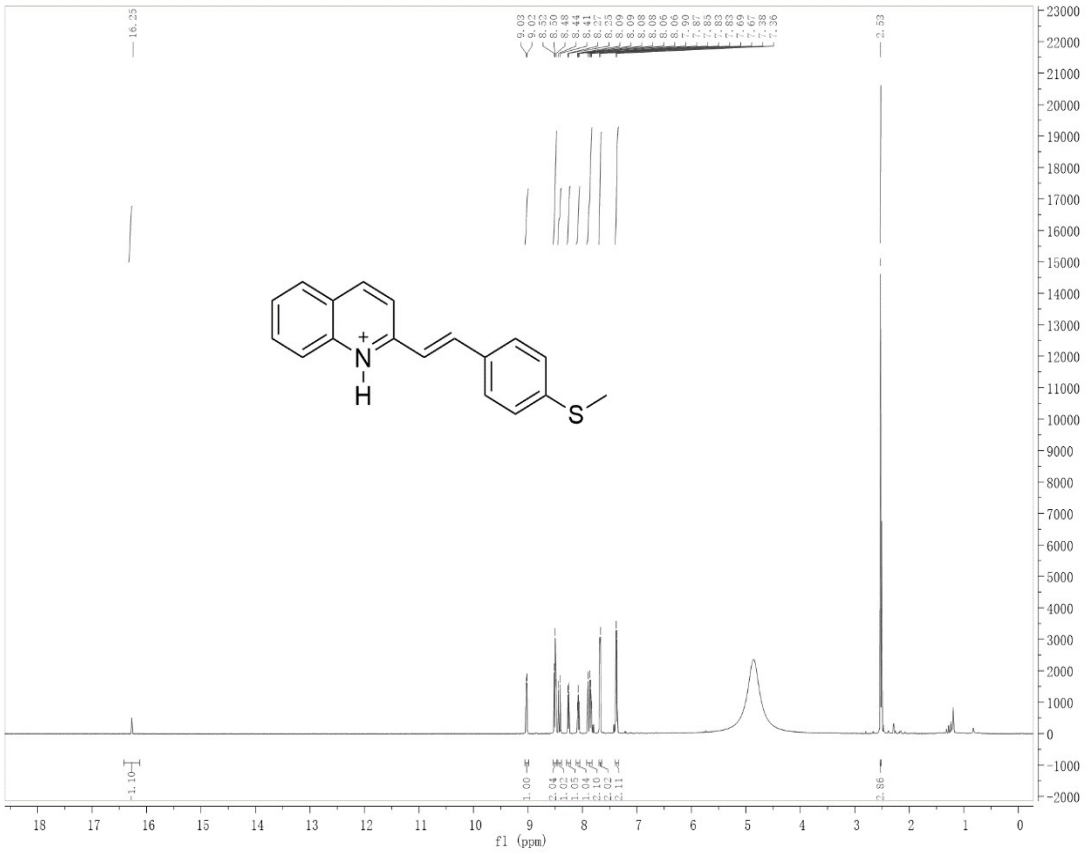
Table S1 Photophysical properties of the fluorophores

Dye	E_X/nm	E_X^{TP}/nm	Em/nm	Quantum yield	TP absorption cross-section
HAPH	340	680	470	0.68	456
HAPH-1	320	640	470	0.81	624
HAPH-1+HCl	410	820	580	0.21	221
HAPH-2	360	720	450	0.65	602
HAPH-3	350	700	460	0.64	365
HAPH-4	360	720	440	0.35	343
HAPH-5	310	--	420	0.45	--

4. NMR Spectra



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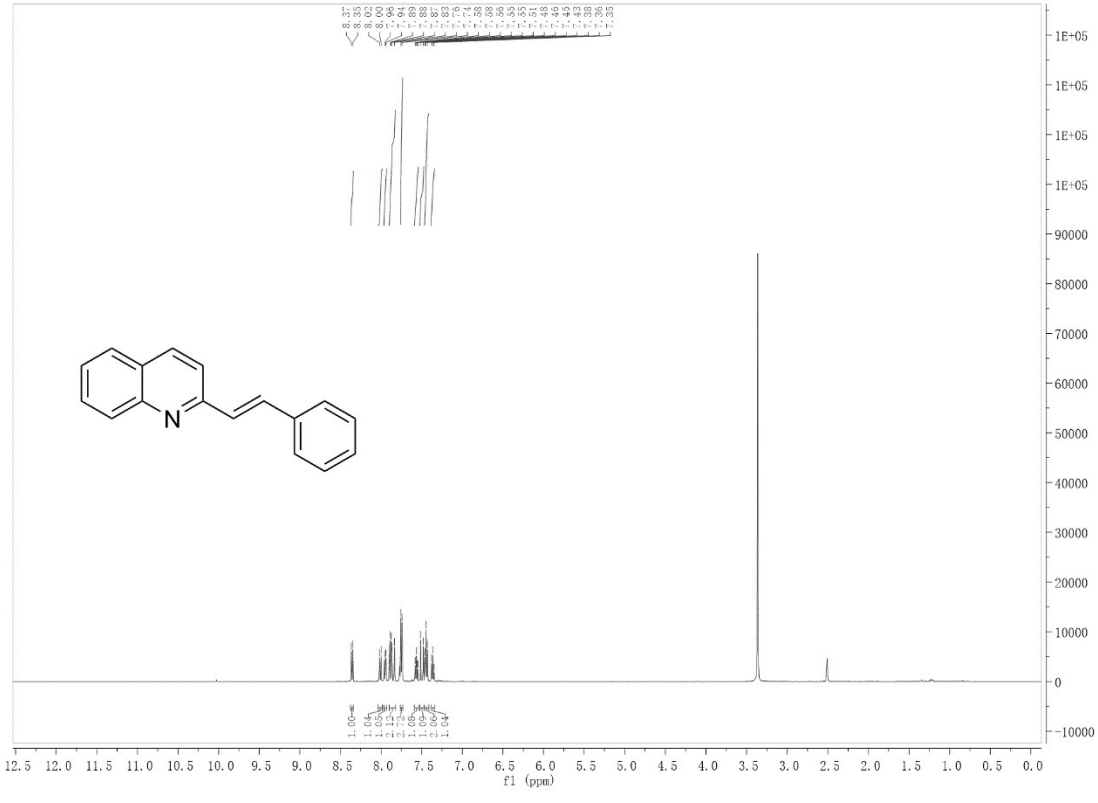
9.02
8.52
8.50
8.48
8.44
8.41
8.37
8.25
8.09
8.09
8.08
8.08
8.06
8.06
8.00
7.87
7.85
7.83
7.83
7.69
7.67
7.38
7.36
2.53
1.10
1.00
2.04
2.02
1.11
1.04
2.10
1.02
2.11
2.86
N
H
S
f1 (ppm)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8.02
8.00
7.96
7.94
7.89
7.88
7.87
7.83
7.75
7.74
7.58
7.56
7.55
7.51
7.48
7.46
7.45
7.43
7.38
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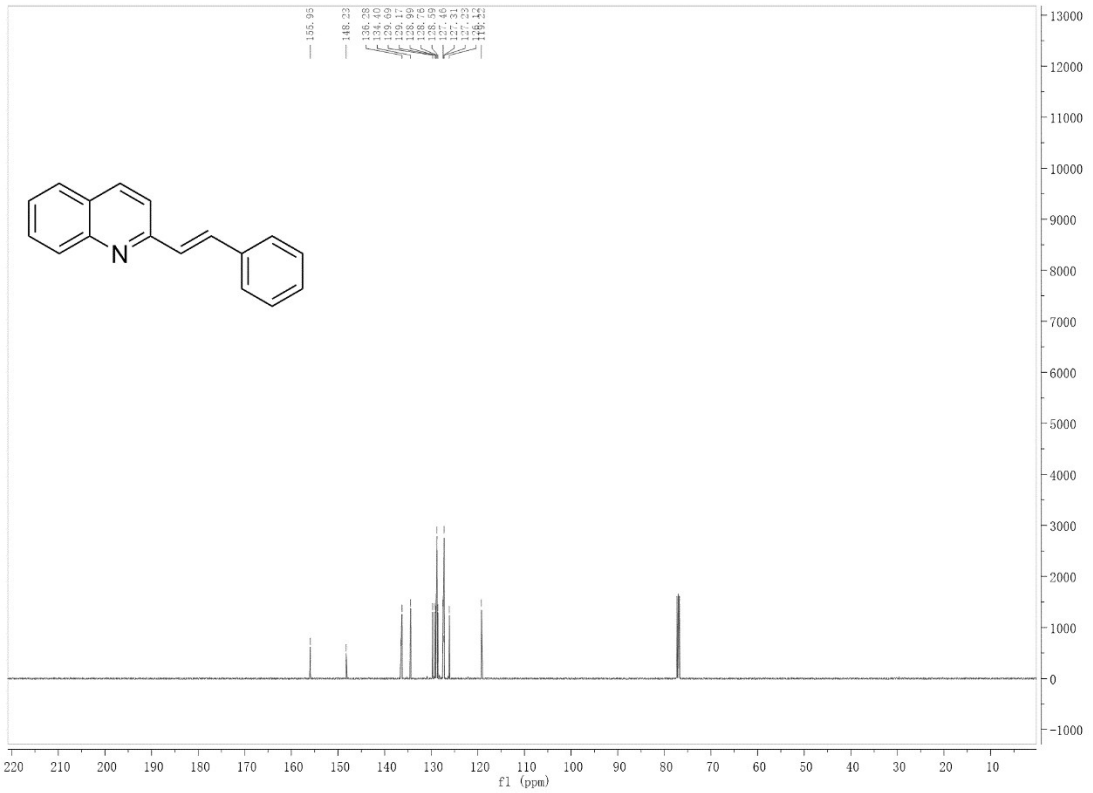
2.24
1.08
1.00
1.04
N
f1 (ppm)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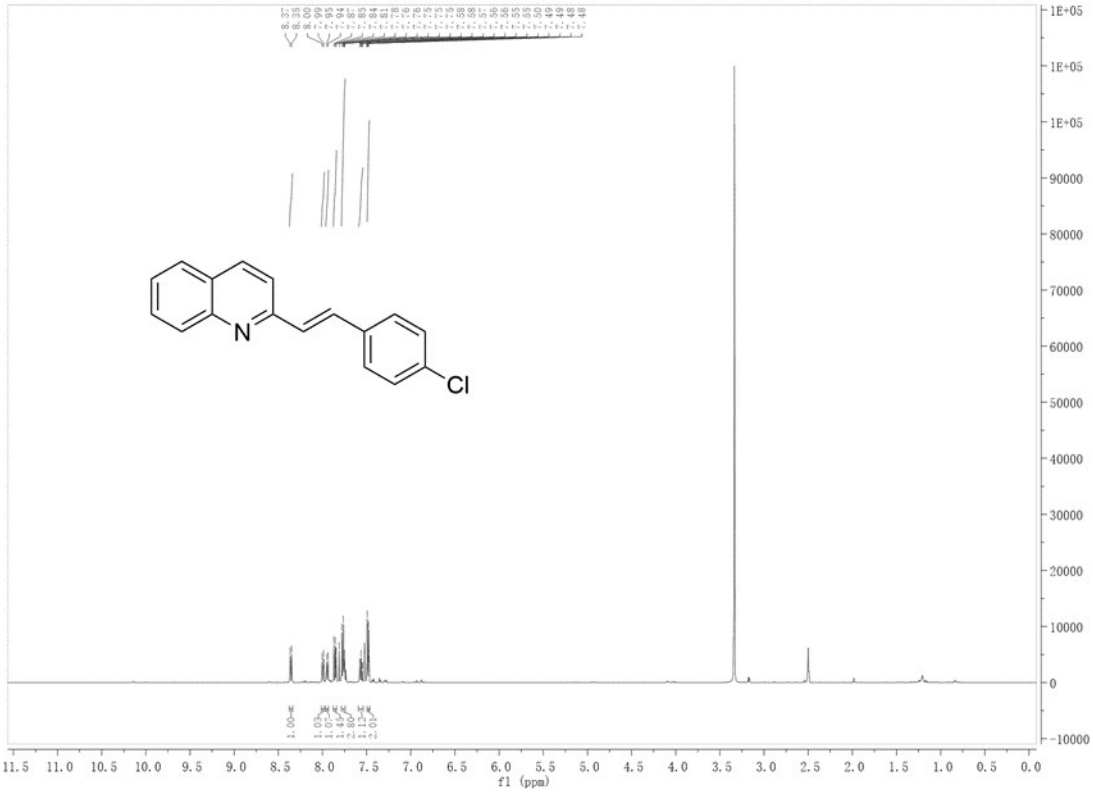
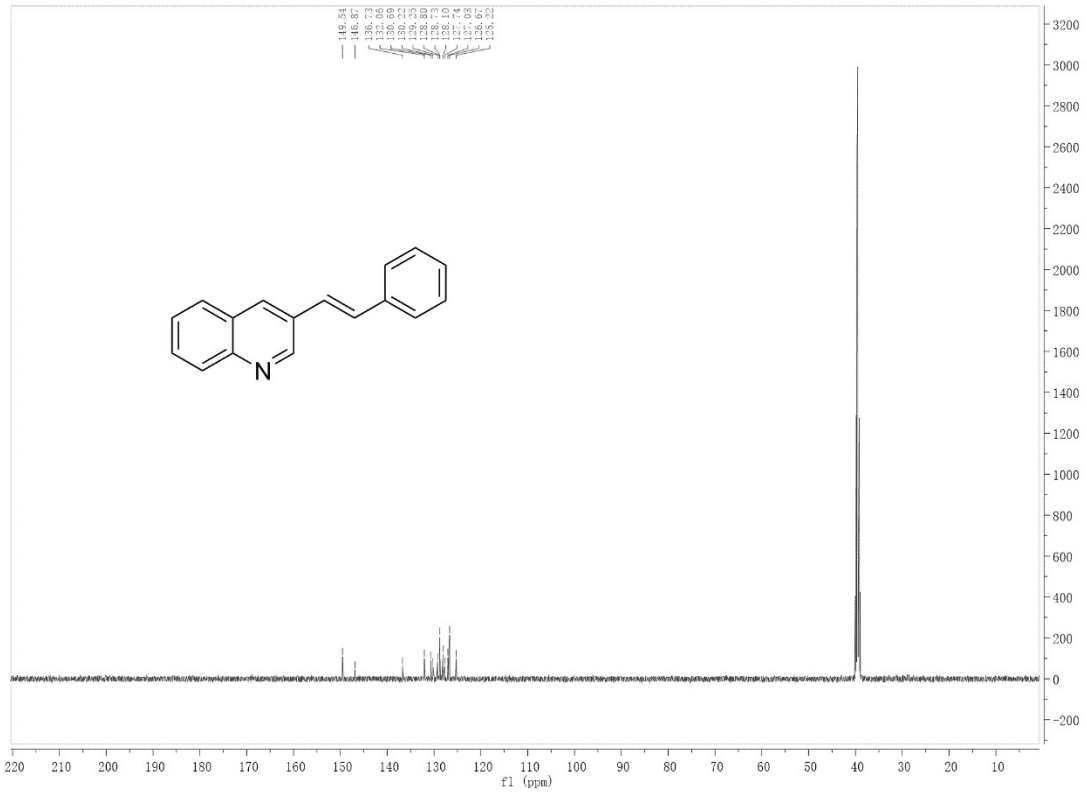
148.23
136.28
134.40
129.69
129.17
128.89
128.76
128.59
127.46
127.31
127.25
126.42
126.22
N
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)


N
Cl
1E+05
1E+05
1E+05
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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132.06
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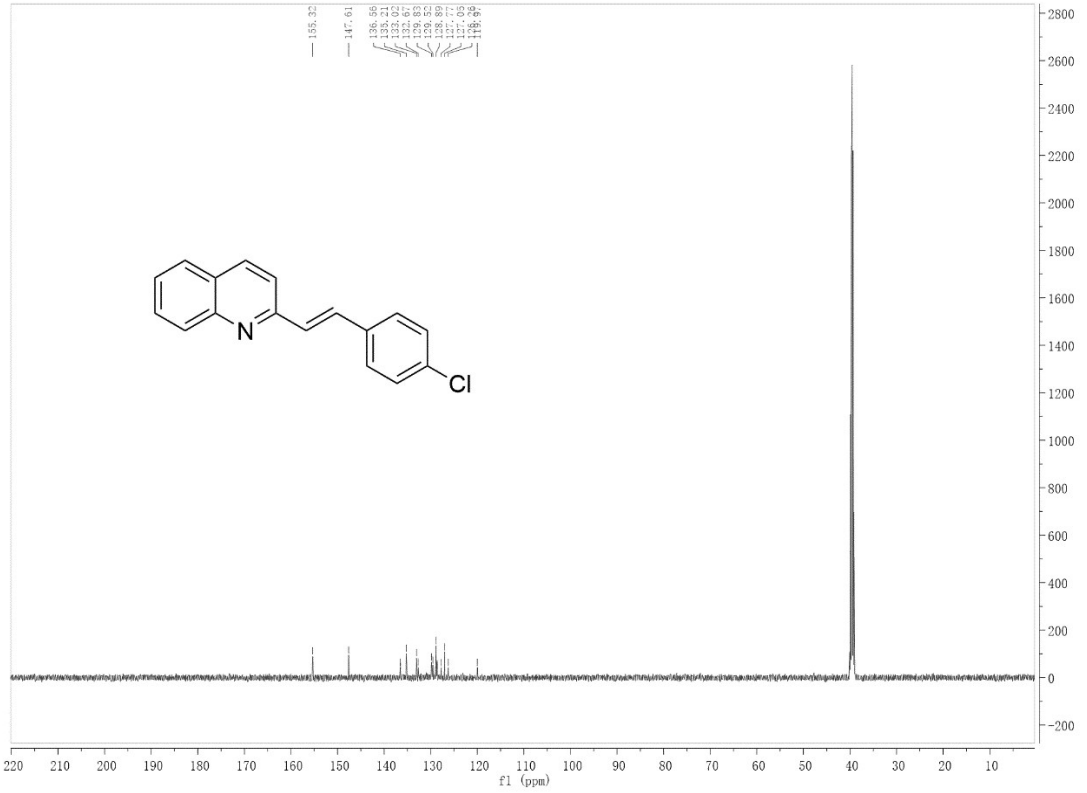
128.80
128.73
128.10
127.74
127.03
126.67
125.22
N
f1 (ppm)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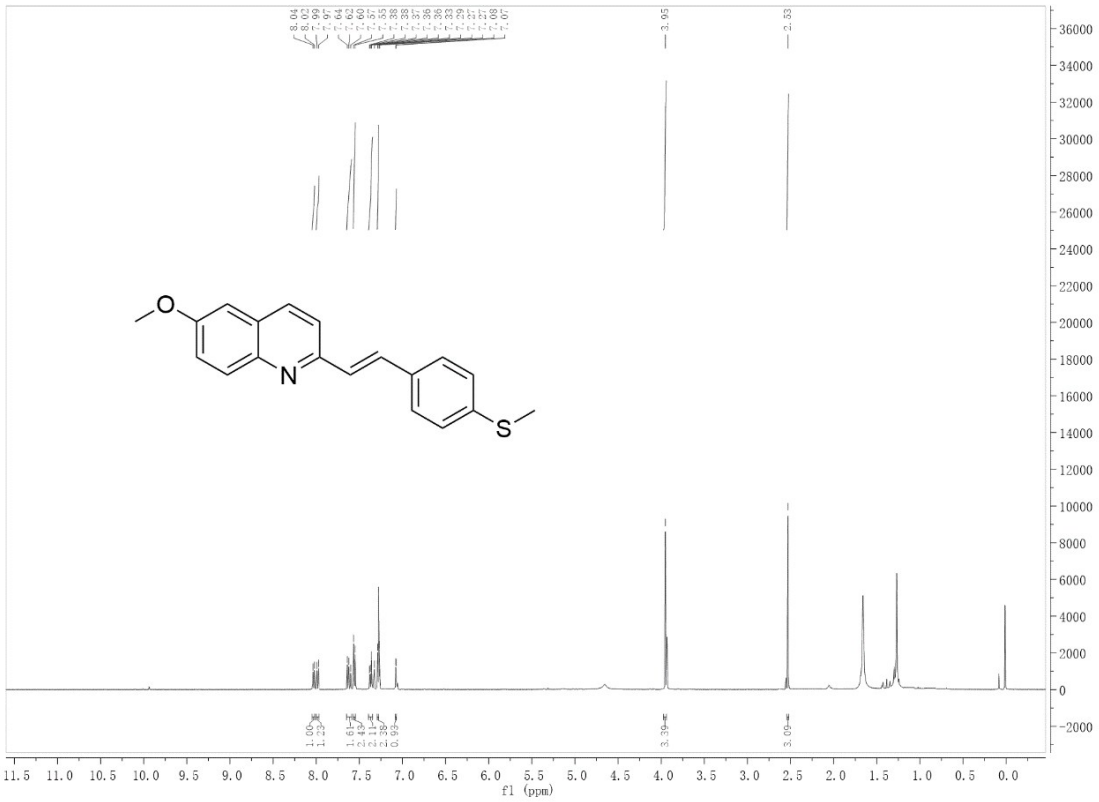
N
Cl
f1 (ppm)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7.38
7.38
7.37
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7.27
7.27
7.08
7.07
3.95
2.53
O
N
S
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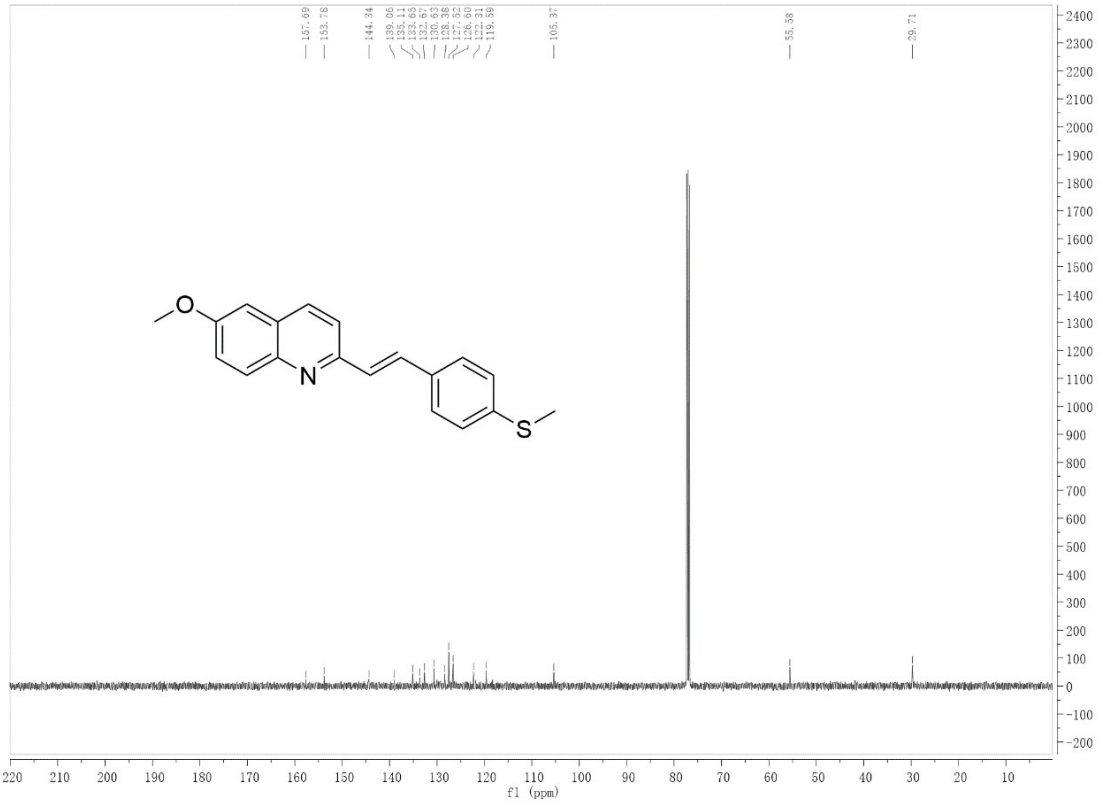
2.43
2.11
2.38
0.99
3.39
3.09
f1 (ppm)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139.06
135.11
132.15
132.07
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127.62
126.60
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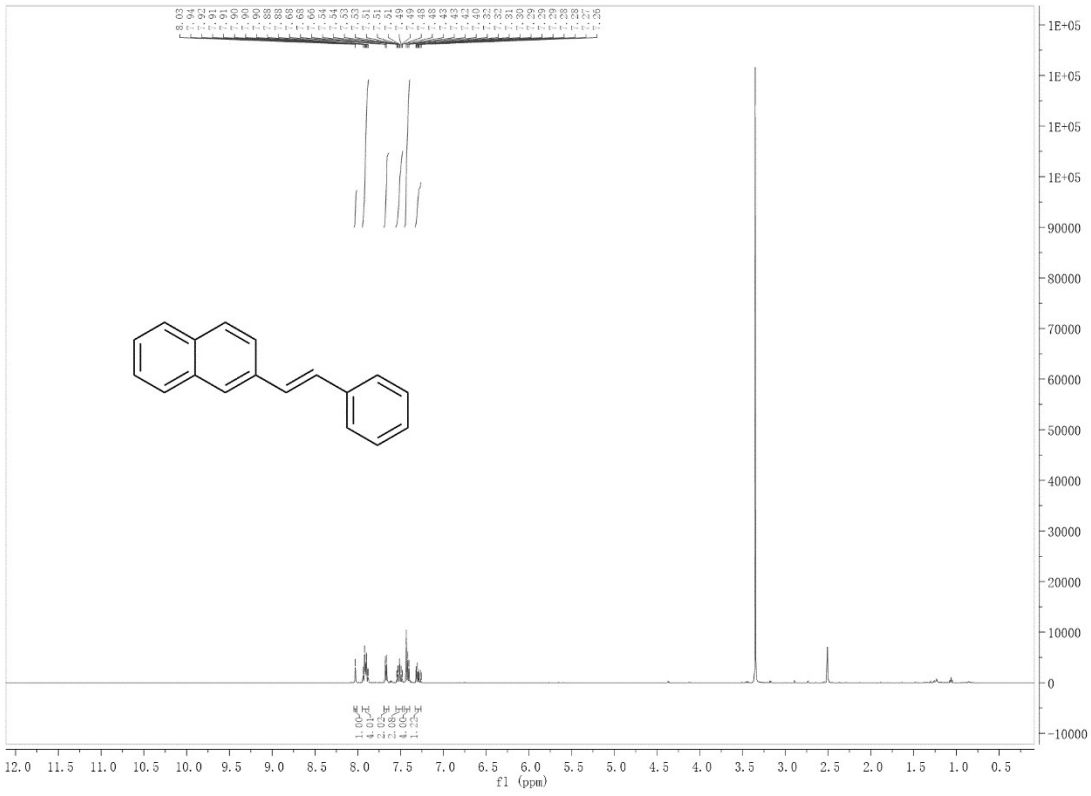
55.58
29.71
O
N
S
f1 (ppm)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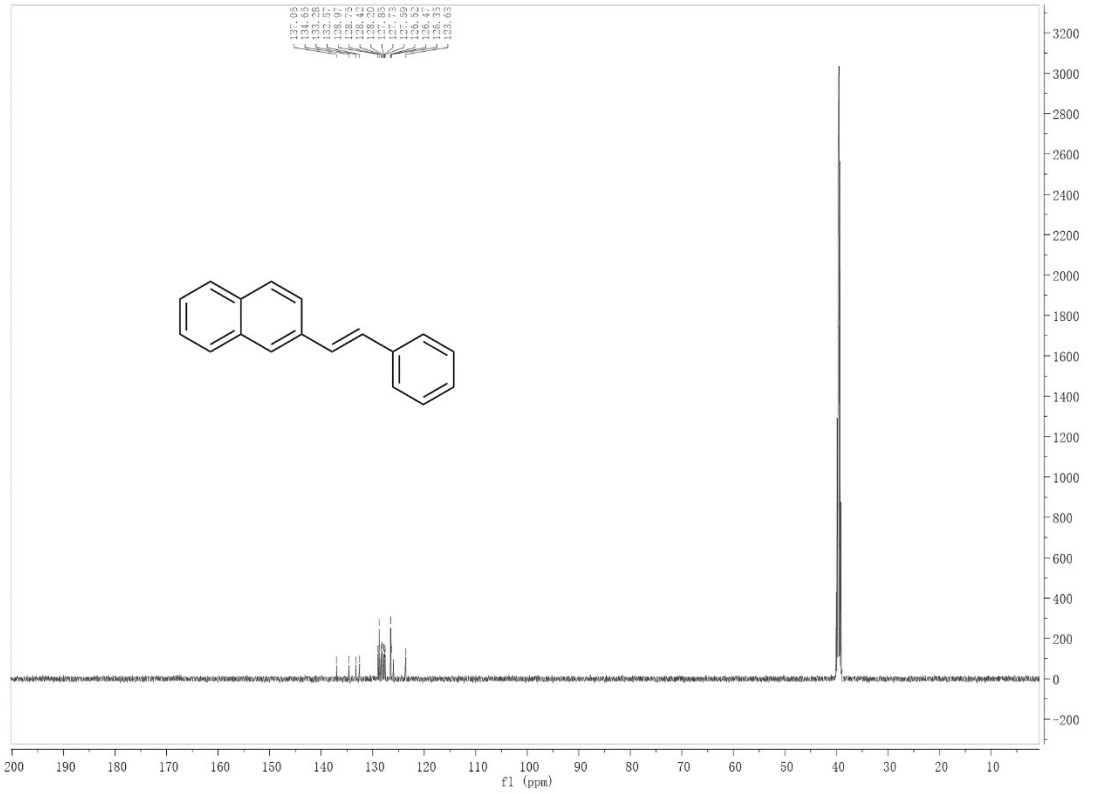
f1 (ppm)