Supplementary Information

Cross-Talk Modulation of Excited State Electron Transfer to Reduce False Negative Background for High Fidelity Imaging *in vivo*

Ao Jiang[a,b], Yuxia Liu[b], Guang Chen[a,b*], Yong Li[a] and Bo Tang[a*]

[a]College of Chemistry, Chemical Engineering and Materials Science, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Collaborative Innovation Center of Functionalized Probes for Chemical Imaging in Universities of Shandong, Institutes of Biomedical Sciences, Shandong Normal University, Jinan 250014, P. R. China.

[b]The Key Laboratory of Life-Organic Analysis; Key Laboratory of Pharmaceutical Intermediates and Analysis of Natural Medicine, College of Chemistry and Chemical Engineering, Qufu Normal University, Qufu 273165, P. R. China

*Phone: (86)531-86180017. Fax: (86)531-86180017

E-mail: tangb@sdnu.edu.cn; chenandguang@163.com

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1. Materials and Reagents

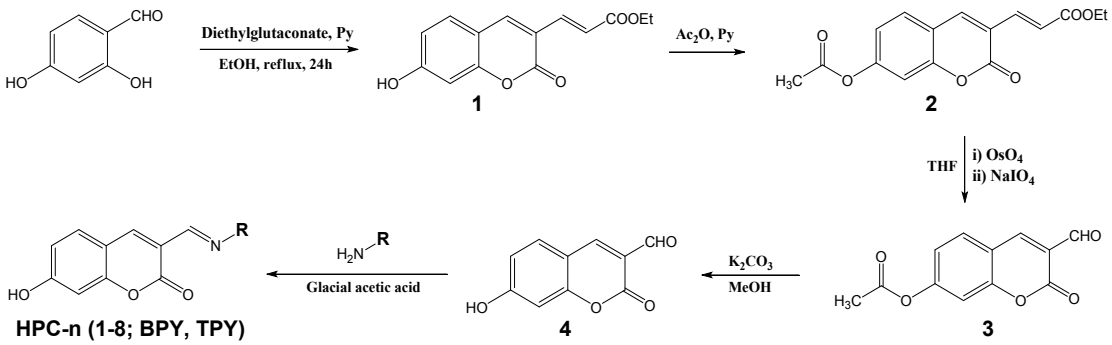
2,4-Dihydroxybenzaldehyde, 2-hydrazinylpyridine, 5-chloro-2-hydrazinylpyridine, 5-bromo-2-hydrazinylpyridine, 2-hydrazinyl-5-phenylpyridine, 6-hydrazinylnicotinic acid, 2-hydrazinyl-5-nitropyridine and 6-hydrazinylnicotinonitrile were purchased from Aladdin Chemistry Co. (Shanghai, China). Diethyl glutaconate was purchased from Sigma-Aldrich Co. (Steinheim, Germany). EGTA (ethylenebis(oxyethylenenitrilo) tetraccetic acid) and HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid) were purchased from J&K Chemical Co. (Beijing, China) . Solutions for metal ions (K^+, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Fe^{2+}, Fe^{3+}, Ag^{2+}, Hg^{2+}, Co^{2+}, Cr^{3+}, Cd^{2+}, Cu^{2+}, Ca^{2+}) were prepared by dissolving the corresponding salts in deionized water. HEPES buffer solution was used to modify the pH. All reagents were analytical grade, and used without further purification. Double-distilled water was used in the experiments.

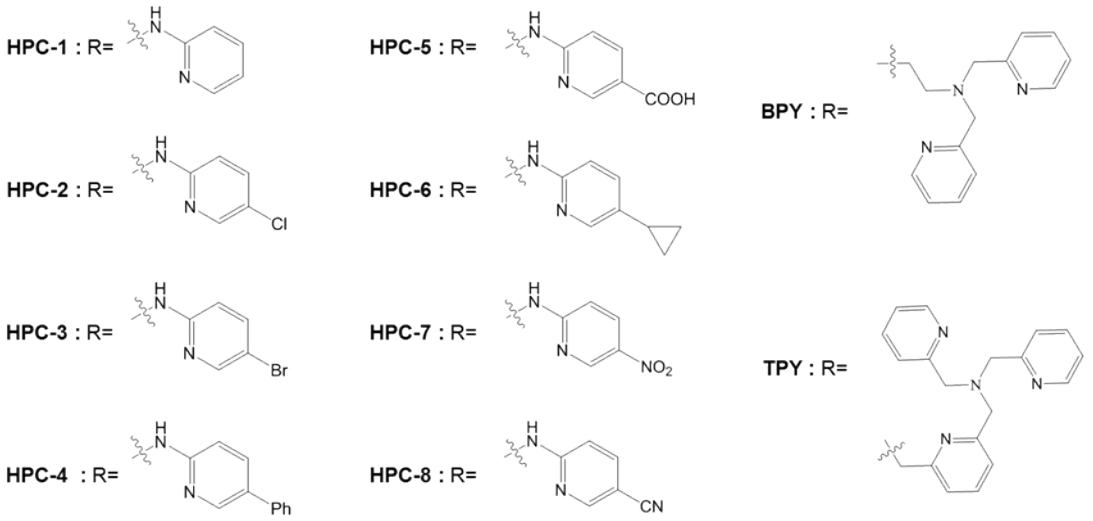
2. Instrumentation

Fluorescence spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer (HITACHI, Japan). UV-visible spectra were collected on a Cary 300 Bio UV-vis spectrophotometer (VARIAN, USA). ^{1}HNMR and ^{13}CNMR spectra were measured in the given solvent at RT on a Bruker ascend 500 (500.1 MHz, ^{1}H; 125.8 MHz, ^{13}C) instrument operated at the denoted spectrometer frequency given in mega Hertz (MHz). Chemical shifts are given in parts per million (ppm) relative to tetramethylsilane (TMS) as an external standard for ^{1}H and ^{13}C-NMR spectra and calibrated against the solvent residual peak. HR-MS date were recorded on a maxis ultra-high resolution-TOF MS system (Bruker Co., Ltd., Germany). LSM 880 confocal laser scanning microscopy were used for the cells, tissus and zebrafish images. (Zeiss Co., Ltd. Germany). Fluorescence imaging of mouse was acquired by Perkin Elmer IVIS Spectrum in Vivo Imaging System. All pH measurements were performed using a pH-3c digital pH-meter with glass-calcium electrode (Shanghai Lei Ci Device Works, Shanghai, China). Thin layer chromatography separation was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 300-400), both of which were purchased from the JiangYou Chemical Company (Yan Tai, China).

3. Synthesis & characterization

3.1 Scheme S1. Synthesis procedures of the probe.





3.2 Synthesis of Compound 1.

2,4-Dihydroxybenzaldehyde (0.74 g, 5.36 mmol) and diethyl glutaconate (1.0 mL, 5.65 mmol) were dissolved with 30 mL EtOH. Then, 3 drops of dry piperidine were added to the solution, and the mixture was refluxed for 24 h. The reaction was then kept below -10 °C for 12 h. After filtering, the resulting solid was washed twice with EtOH and then purified through a silica gel chromatography (200 - 300 mesh) (CH_3CN:CH_2Cl_2 = 1:20 (v/v). A yellow solid was obtained with yield of 76%. Characterizations: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.41(s, 1H), 7.55(d, J=8.6 Hz, 1H), 7.49(d, J=10.0 Hz, 1H), 6.84(dd, J=5, 10.0 Hz, 2H), 6.73(s, 1H), 4.16(q, J=5.1 Hz, 2H), 1.24(t, J=5.1Hz, 3H), ^{13}C NMR (DMSO-d_6, 125 MHz): δ 166.73, 163.35, 159.67, 155.83, 145.97, 139.37, 131.33, 120.19, 116.73, 114.49, 111.92, 102.41, 60.53, 14.63 ppm.

3.3 Synthesis of Compound 2.

Compound **1** (0.2 g, 0.77 mmol) was dissolved in the mixture of 4 mL pyridine and 4 mL acetic anhydride. The solution was stirred at room temperature for 1 h and kept in ice for another 0.5 h. The crude product was filtered and dissolved in DCM. The solution was washed respectivly with water (3×100 mL) and NaCl saturated solution (3×200 mL). After drying over Na_2SO_4, the solvent was removed under reduced pressure to provide crude product as an off-white solid. The solid was purified through silica gel chromatography (200 - 300 mesh) eluted with CH_3CN:CH_2Cl_2 = 1:5 (v/v). A white solid was obtained with a yield of 82%. Characterizations: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.84(s, 1H), 7.53(t, J=10.0Hz, 2H), 7.12(d, J=5.2 Hz, 1H), 7.09-7.05(m, 2H), 4.25(q, J=5.0 Hz, 2H), 2.34(s, 3H), 1.32(t, J=5.1 Hz, 3H), ^{13}C NMR (DMSO-d_6, 125 MHz): δ168.56, 166.87, 158.71, 154.13, 142.80, 137.62, 129.25, 123.83, 121.75, 118.90, 116.76, 110.16, 60.78, 20.14, 14.27 ppm.

3.4 Synthesis of Compound 3.

Compound **2** (2.2 g, 7.28 mmol) was dissolved with 200 mL THF. To the solyution, OsO_4 was added (0.1 g dissolved in 2 mL water). After 0.5 h of stirring, $NaIO_4$ (3.42 g, 16 mmol) was added. The mixture was stirred at room temperature for about 5 days. The resultant product was obtianed after evaporation under vacumn and then extracted with water-CH_2Cl_2 (1:1=v:v). After drying over Na_2SO_4 and the vacumn evaporation, the off-white solid was obtained, which was

purified through a silica gel chromatography (200 - 300 mesh) with gradient elution (CH_3CN : CH_2Cl_2= from 0% : 100% to 16% : 84%; v/v). A white solid was obtained with yield of 43%. Characterizations: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.02(s, 1H), 8.69 (s, 1H), 8.03(d, J=10.1 Hz, 1H), 7.38-7.24(m, 2H), 2.32(s, 3H), ^{13}C NMR (DMSO-d_6, 125 MHz): δ 188.48, 169.06, 159.44, 156.08, 155.79, 146.79, 132.91, 121.48, 119.54, 116.59, 110.77, 21.38 ppm.

3.5 Synthesis of Compound 4.

Compound **3** (0.5 g, 2.15 mmol) and K_2CO_3 (0.595 g, 4.31 mmol) was dissolved in 40 mL of MeOH. The mixture was stirred at room temperature for 1h. The pH of this mixture was adjusted to 3~4 with 1N HCl solution. After filtrating and washing twice with water, the solvent was removed under vacuum, and the solid was dissolved with DCM and dried over Na_2SO_4. The solid residue was purified through a silica gel chromatography (200 - 300 mesh) eluted with CH_3OH:CH_2Cl_2 = 1:10 (v/v). A yellow solid was obtained with a yield of 67%. Characterizations: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.95(s, 1H), 8.56 (s, 1H), 7.80(d, J=10.0 Hz, 1H), 6.88-6.77(m, 2H), ^{13}C NMR (DMSO-d_6, 125 MHz): δ 188.22, 165.41, 160.20, 158.02, 147.68, 133.80, 117.48, 114.99, 111.30, 102.72 ppm.

3.6 Synthesis of BPY.

Compound **4** (0.19 g, 1 mmol) and N',N'-bis(pyridin-2-ylmethyl)ethane-1,2-diamine (0.24 g, 1 mmol) were dissolved with 20 mL MeOH, to which then 3 drops of glacial acetic acid were added. The mixture was stirred at 50℃ for 10 min and the solvent was evaporated under reduced pressure. The obtained solid residue was purified through a silica gel chromatography (200 - 300 mesh) eluted with CH_3OH:CH_2Cl_2 = 1:30 (v/v). A yellow solid was obtained, with a yield of 19%. Characterizations for **BPY**: ^{1}H NMR (CD_3OD-d_4, 500 MHz): δ 8.47-8.44(m, 3H), 7.71-7.68(m, 3H), 7.51(q, J=7.85 Hz, 2H), 7.32-7.24(m, 5H), 3.87(d, J=15.1 Hz, 6H), 3.12 (t, J=5.3 Hz, 2H), ^{13}C NMR (CD_3OD-d_4, 125 MHz): δ 172.09, 161.95, 160.91, 155.44, 154.63, 152.31, 151.02, 147.13, 141.59, 141.21, 130.03, 127.49, 126.35, 125.52, 123.56, 119.74, 113.42, 111.28, 101.83, 98.87, 58.67, 56.98, 56.37, 41.47 ppm. HRMS：calculated for $C_{24}H_{22}N_4O_3$ $[M+H]^+$=415.1765. Found 415.1761.

3.7 Synthesis of TPY.

Compound **4** (0.19 g, 1 mmol) and 1-(6-(aminomethyl)pyridin-2-yl)-N,N-bis(pyridin-2-ylmethyl)methanamine (0.32 g, 1 mmol) were dissolved with 20 mL MeOH, to which 3 drops of glacial acetic acid were added. The mixture was stirred at 50℃ for 10 min and the solvent was evaporated under reduced pressure. The obtained solid residue was purified through a silica gel chromatography (200 - 300 mesh) eluted with CH_3OH:CH_2Cl_2 = 1:30 (v/v). A brown solid was obtained, with a yield of 16%. Characterizations for **TPY**: ^{1}H NMR (CD_3OD-d_4, 500 MHz): δ 8.86(d, J=5.3 Hz, 2H), 8.61-8.58(m, 2H), 8.21(d, J=7.9 Hz, 2H), 8.03-7.94(m, 5H), 7.65-7.61(m, 2H), 7.50 (d, J=10.1 Hz, 1H), 6.82-6.80(m, 2H), 4.56(d, J=5.1 Hz, 6H), 4.20(s, 2H), ^{13}C NMR (CD_3OD-d_4, 125 MHz): δ 188.22, 175.38, 173.61, 172.02, 162.00, 160.96, 155.49, 154.98, 152.50, 151.49, 147.05, 141.52, 141.19, 140.32, 129.96, 127.32, 126.22, 125.03, 122.79, 119.76, 113.35, 111.29, 102.72, 101.76, 98.87, 65.51, 59.09, 56.32, 41.72 ppm. HRMS：calculated for $C_{29}H_{25}N_5O_3$ $[M+H]^+$=492.2030. Found: 492.2236.

3.8 Synthesis of HPC-1.

Compound **4** (0.19 g, 1 mmol) and 2-hydrazinylpyridine (0.11 g, 1 mmol) were dissolved in 20 mL EtOH, to which then 3 drops of glacial acetic acid were added. The mixture was refluxed for 3 h. The solvent was evaporated under reduced pressure. The obtained solid residue was purified through a silica gel chromatography (200 - 300 mesh) eluted with CH_3OH:CH_2Cl_2 = 1:30 (v/v). A yellow solid was obtained with a yield of 41%. Characterizations for HPC-1: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.12(s, 1H), 10.67(s, 1H), 8.39(s, 1H), 8.11(d, J=2.0 Hz, 1H), 8.06(s, 1H), 7.67-7.63(m, 2H), 7.31(d, J=8.5 Hz, 1H), 6.82(dd, J=2.0, 2.0 Hz, 1H), 6.78(dd, J=5.5, 5.5 Hz, 1H), 6.74(d, J=2.0 Hz, 1H), ^{13}C NMR (DMSO-d_6, 125 MHz): δ 161.33, 160.33, 156.73, 154.81, 147.78, 137.88, 135.63, 132.37, 130.31, 117.59, 115.29, 113.70, 111.91, 106.54, 102.03 ppm. HRMS: calculated for $C_{15}H_{11}N_3O_3$ $[M-H]^-$ =280.0717. Found: 280.0696.

3.9 Synthesis of HPC-2.

Compound **4** (0.19 g, 1 mmol) and 5-chloro-2-hydrazinylpyridine (0.14 g, 1 mmol) were dissolved with 20 mL EtOH, to which then 3 drops of glacial acetic acid were added. The mixture was refluxed for 3 h. The solvent was evaporated under reduced pressure. The obtained solid residue was purified through a silica gel chromatography (200 - 300 mesh) eluted with CH_3OH:CH_2Cl_2 = 1:20 (v/v). A yellow solid was obtained with a yield of 45%. Characterizations for **HPC-2**: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.28(s, 1H), 10.69(s, 1H), 8.40(s, 1H), 8.13(d, J=2.5 Hz, 1H), 8.07(s, 1H), 7.72(dd, J=2.0, 2.0 Hz, 1H), 7.66(d, J=8.5 Hz, 1H), 7.31(d, J=9.0 Hz, 1H), 6.82(dd, J=2.0, 2.0Hz, 1H), 6.74(d, J=1.5Hz, 1H), ^{13}C NMR (DMSO-d_6, 125 MHz): δ 161.48, 160.26, 155.37, 154.90, 145.94, 137.63, 136.08, 133.39, 130.39, 120.77, 117.30, 113.73, 111.85, 107.81, 102.04 ppm. HRMS：calculated for $C_{15}H_{10}ClN_3O_3$ $[M-H]^-$ =314.0327. Found: 314.0304.

3.10 Synthesis of HPC-3.

Compound **4** (0.19 g, 1 mmol) and 5-bromo-2-hydrazinylpyridine (0.18 g, 1 mmol) were dissolved in 20 mL EtOH, to which then 3 drops of glacial acetic acid were added. The mixture was refluxed for 3 h and the solvent was evaporated under reduced pressure. The solid residue was purified through a silica gel chromatography (200 - 300 mesh) eluted with CH_3OH:CH_2Cl_2 = 1:25 (v/v). A yellow solid was obtained with a yield of 51%. Characterizations for **HPC-3**: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.29 (s, 1H), 10.69 (s, 1H), 8.40 (s, 1H), 8.20(d, J=2.5 Hz, 1H), 8.08 (s, 1H), 7.82(dd, J=2.0, 2.5 Hz, 1H), 7.66 (d, J=8.5 Hz, 1H), 7.28(d, J=9.0 Hz, 1H), 6.82 (dd, J=2.0, 2.0 Hz, 1H), 6.74 (d, J=2.5 Hz, 1H), ^{13}C NMR (DMSO-d_6, 125 MHz): δ 161.49, 160.25, 155.58, 154.91, 148.12, 140.18, 136.13, 133.51, 130.41, 117.28, 113.74, 111.85, 108.72, 108.46, 102.04 ppm. HRMS：calculated for $C_{15}H_{10}BrN_3O_3$ $[M-H]^-$ =359.9802 and 357.9821. Found: 359.9769 and 357.9792.

3.11 Synthesis of HPC-4.

Compound **4** (0.19 g, 1 mmol) and 2-hydrazinyl-5-phenylpyridine (0.19 g, 1 mmol) were dissolved with 20 mL EtOH, to which then 3 drops of glacial acetic acid were added. The mixture was refluxed for 3 h and the solvent was evaporated under reduced pressure. The obtained solid residue was purified through a silica gel chromatography (200 - 300 mesh) eluted with

CH_3OH:CH_2Cl_2 = 1:30 (v/v). A yellow solid was got, with a yield of 44%. Characterizations for **HPC-4**: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.60 (s, 1H), 10.72 (s, 1H), 8.48 (s, 1H), 8.20(d, J=9.0 Hz, 1H), 8.13(s, 1H), 7.79(d, J=8.0 Hz, 1H), 7.70(d, J=7.5 Hz, 2H), 7.63(d, J=8.0 Hz, 1H), 7.59(t, J=7.5 Hz, 1H), 7.30(t, J=7.5 Hz, 1H), 6.84 (dd, J=2.5, 2.5 Hz, 1H), 6.76(d, J=2.0 Hz, 1H), ^{13}C NMR (DMSO-d_6, 125 MHz): δ 161.48, 160.31, 155.50, 154.92, 147.07, 137.97, 136.13, 133.30, 130.42, 129.78, 127.85, 125.84, 124.30, 122.76, 117.41, 113.76, 111.91, 109.52, 102.06 ppm. HRMS：calculated for $C_{21}H_{15}N_3O_3$ $[M+H]^+$ =332.1029. Found: 332.1105.

3.12 Synthesis of HPC-5.

Compound **4** (0.19 g, 1 mmol) and 6-hydrazinylnicotinic acid (0.15 g, 1 mmol) were dissolved with 20 mL EtOH, to which then 3 drops of glacial acetic acid were added. The mixture was refluxed for 3 h and the solvent was evaporated under reduced pressure. The obtained solid residue was purified through a silica gel chromatography (200 - 300 mesh) eluted with CH_3OH:CH_2Cl_2 = 1:25 (v/v). A yellow solid was obtained, with a yield of 42%. Characterizations for **HPC-5**: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.61(s, 1H), 8.66(d, J=2.0 Hz, 1H), 8.45 (s, 1H), 8.16 (s, 1H), 8.09(dd, J=2.0, 2.0 Hz, 1H), 7.68(d, J=8.5 Hz, 1H), 7.35(d, J=9.0 Hz, 1H), 6.83(dd, J=2.5, 2.0 Hz, 1H), 6.75(d, J=2.0 Hz, 1H),1.05(t, J=7.0 Hz, 2H), ^{13}C NMR (DMSO-d_6, 125 MHz): δ 166.42, 161.74, 160.24, 159.05, 155.09, 150.54, 138.78, 136.81, 135.31, 130.60, 117.69, 117.03, 113.82, 111.81, 105.71, 102.08 ppm. HRMS：calculated for $C_{16}H_{11}N_3O_5$ $[M-H]^-$ =324.0615. Found 324.0601.

3.13 Synthesis of HPC-6.

Compound **4** (0.19 g, 1 mmol) and 5-cyclopropyl-2-hydrazinylpyridine (0.14 g, 1 mmol) were dissolved with 20 mL EtOH, to which then 3 drops of glacial acetic acid were added. The mixture was refluxed for 3 h and the solvent was evaporated under reduced pressure. The obtained solid residue was purified through a silica gel chromatography (200 - 300 mesh) eluted with CH_3OH:CH_2Cl_2 = 1:30 (v/v). A brown solid was obtained, with a yield of 23%. Characterizations for **HPC-6**: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.02(s, 1H), 10.65(s, 1H), 8.35(s, 1H), 8.02(s, 1H), 7.95(d, J=2.0 Hz, 1H), 7.68(d, J=8.5 Hz, 1H), 7.34(dd, J=2.0, 2.5 Hz, 1H), 7.21 (d, J=8.5 Hz, 1H), 6.82(dd, J=2.5, 2.5 Hz, 1H), 6.73(d, J=2.0 Hz, 1H), 1.87-1.84(m, 1H), 0.91-0.89(m, 2H), 0.88(d, J=2.0 Hz, 2H), ^{13}C NMR (DMSO-d_6, 125 MHz): δ 161.21, 160.33, 154.92, 154.71, 145.45, 135.22, 134.90, 131.58, 130.24, 130.03, 117.71, 113.66, 111.94, 106.25, 102.01, 12.07, 8.13 ppm. HRMS：calculated for $C_{18}H_{15}N_3O_3$ $[M-H]^-$ =320.1029. Found: 320.1009.

3.14 Synthesis of HPC-7.

Compound **4** (0.19 g, 1 mmol) and 2-hydrazinyl-5-nitropyridine (0.15 g, 1 mmol) were dissolved with 20 mL EtOH, to which then 3 drops of glacial acetic acid were added. The mixture was refluxed for 3 h and the solvent was evaporated under reduced pressure. The solid product was purified through a silica gel chromatography (200 - 300 mesh) eluted with CH_3OH:CH_2Cl_2 = 1:10 (v/v). A red solid was obtained with a yield of 22%. Characterizations for **HPC-7**: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.12(s, 1H), 9.02(s, 1H), 8.52(s, 1H), 8.40-8.38(m, 1H), 8.25(s, 1H), 7.96(d, J=8.5 Hz, 1H), 7.39(d, J=9.0 Hz, 1H), 6.84 (d, J=8.5 Hz, 1H), 6.75(s, 1H), ^{13}C NMR (DMSO-d_6, 125 MHz): δ 162.18, 160.09, 159.61, 155.35, 146.22, 138.20, 137.91, 136.75, 133.48, 130.84,

116.43, 113.94, 111.66, 105.77, 102.10 ppm. HRMS：calculated for $C_{15}H_{10}N_4O_5$ $[M-H]^-$ =325.0567. Found: 325.0539.

3.15 Synthesis of HPC-8.

Compound **4** (0.19 g, 1 mmol) and 6-hydrazinylnicotinonitrile (0.13 g, 1 mmol) were dissolved with 20 mL EtOH, to which then 3 drops of glacial acetic acid were added. The mixture was refluxed for 3 h and the solvent was evaporated under reduced pressure. The obtained solid residue was purified through a silica gel chromatography (200 - 300 mesh) eluted with CH_3OH:CH_2Cl_2 = 1:30 (v/v). A yellow solid was obtained with a yield of 31%. Characterizations for **HPC-8**: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.76(s, 1H), 10.76(s, 1H), 8.53(s, 1H), 8.47(s, 1H), 8.17(s, 1H), 8.02(d, J=6.5 Hz, 1H), 7.67(d, J=7.0 Hz, 1H), 7.37(d, J=6.5 Hz, 1H), 6.83 (d, J=6.5 Hz, 1H), 6.74(s, 1H), ^{13}C NMR (DMSO-d_6, 125 MHz): δ 161.89, 160.15, 158.27, 155.19, 152.71, 140.73, 137.33, 136.47, 130.67, 118.32, 116.73, 113.84, 111.74, 106.42, 102.07, 98.32 ppm. HRMS：calculated for $C_{16}H_{10}N_4O_3$ $[M-H]^-$ =305.0669. Found: 305.0640.

4 Fluorometric analysis

Fluorescent spectra were recorded at the excitation wavelength of around 410 nm, with the excitation / emission slit width of 10 nm/10 nm. Optical properties were evaluated under the physiological conditions using 1 μM of HPC-n (n: 1-8), BPY and TPY in HEPES buffer solution (pH=7.2, 50 mM HEPES, 100 mM KNO_3) with 1% DMSO. We tested photochemical properties of HPC-n (n: 1-8), BPY and TPY (Table S1).

5 Photostability

To estimate the feasibility for detecting intracellular HPC-n, BPY, TPY and Zn^{2+}, penetration and photostability of these compounds in Hek293t cells and SHSY-5Y cells were investigated. Dynamic fluorescence imaging (Fig. S6, Fig. S7) indicated that these compounds in Hek293t cells or SHSY-5Y cells could exhibit the rapid and strong fluorescence. Then, the fluorescence intensity remained almost unchanged for 50 min, displaying the satisfactory photostability of these compounds.

6 MTT assay

MTT assay was carried out to evaluate the cytotoxicity of HPC-n (n: 1-8), BPY and TPY. The Hek293t cells and SHSY-5Y cells were first incubated with the 10% Fetal Bovine Serum (FBS, Invitrogen) in a humidified incubator containing 5% CO_2 gas at 37℃ for 24 h. Then, the cells were respectivly treated by various concentrations of HPC-n (n: 1-8), BPY and TPY (1 μM, 10 μM, 30 μM, 0.1 mM, 0.5 mM, 1 mM) for 24 h. Next, 25 μL of methylthiazolyl tetrazolium (MTT) (5 mg mL-1) was added to each well and was incubated for 4 h. The cytotoxicity tests were performed using MTT (n=7 replicates). The cell viability was expressed by the mean values ± standard deviation (SD). (Fig. S8)

7 Determination of Apparent Dissociation Constants (Kd)

A series of HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid) buffer solutions (50 mM, pH=7.2, 100 mM KNO_3) containing various amounts of $ZnCl_2$ (0-9 mM) and 10 mM EGTA (ethylenebis(oxyethylenenitrilo) tetraccetic acid) were prepared. The concentration of free Zn^{2+} was calculated with $[Zn^{2+}]_{total}$, $[EGTA]_{total}$ and $K'_{Zn\text{-}EGTA}=3.80\times10^8$ M^{-1}. The fluorescence intensity data were fitted to Eq. 1, and K_d was calculated:

$$F=F_0+(F_{max}-F_0)[Zn^{2+}]_{free}/(K_d+[Zn^{2+}]_{free}) \quad \text{(Eq. 1)}$$

Where F is the fluorescence intensity, F_0 is the fluorescence intensity with no addition of Zn^{2+}, F_{max} is the maximum fluorescence intensity, and $[Zn^{2+}]_{free}$ is the concentration of free Zn^{2+}. (Fig. S5)

8 Optimization of sensing conditions

Before applying HPC-n (n: 1-8) as a probe, sensing conditions were optimized. Time-dependent tests showed that HPC-n (n: 1-8) responded rapidly to Zn^{2+} (Fig. S1), ensuring the instant monitoring in biological systems. As cellular pH values are in dynamic equilibrium, the reversibility for detecting H^+ is a very important parameter for a pH probe. Therefore, pH values were switched between 4.0 and 9.0 using aqueous solutions of hydrochloric acid and sodium hydroxide (Fig. S2). The selectivity of HPC-n (n: 1-8) toward Zn^{2+} against other possible interference was tested. Various biologically relevant metal ions (K^+, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Fe^{2+}, Fe^{3+}, Ag^{2+}, Hg^{2+}, Co^{2+}, Cr^{2+}, Cd^{2+}, Cu^{2+}, Ca^{2+}) were evaluated (Fig. S3).

9 Cell culture and imaging

Hek293t cells and SHSY-5Y cells were seeded into 18 mm glass-bottom dishes with 10% fetal bovine serum, 1% penicillin and 1% streptomycin, maintained at 37°C in 5% CO_2/95% air incubator for 24 hours. Images were captured using 405 nm as excitation and channel (430–650 nm) as emission. Hek293t cells were incubated with HPC-n (n:1, 6, 7, 8) (1 μM), BPY (0.01 μM) and TPY (0.01 μM) for 20 min at 37℃; SHSY-5Y cells were pretreated with TPEN (50 μM) before the incubation with HPC-7 (1 μM) for 20 min. Hek293t cells were incubated with H_2O_2 (200 μM) for 5 h to cause apoptosis, then treated with HPC-8 (1 μM). For further confirmation, Hek293t cells was pretreated with H_2O_2 (200 μM) for 5 h, then treated with TPEN (50 μM) for 0.5 h, and then treated with HPC-8 (1 μM) for 0.5 h. The resultant cells were washed with PBS for imaging analysis.

10 Fluorescence confocal imaging in zebrafish

For background experiments, zebrafish (~5 day) were soaked with TPEN (50 μM) for 60 min, then treated with HPC-8 (1 μM) for 30 min (Fig. 6A, 6B). For imaging of endogenous zinc, zebrafish (~5 day) were incubated with TPEN (50 μM) for 60 min and then treated with HPC-8 (1 μM) for 30 min.

11 Cardiac perfusion for imaging in tissues

Male nude mice (~20 g) were fasted for 12 h to avoid the fluorescence interference from possible food. The mice was injected with 50 mL PBS buffer solution containing 20 μM saline

solution of HPC-8, HPC-5 and HPC-1, through the cardiovascular to ensure the perfusion to liver. The flow rate averages two drops per second (lasted 10 minutes). Ten minutes later, mice were dissected to isolate the livers for confocal imaging. The tissue imaging was excited at 405 nm laser and the emission was collected green channel (430–650 nm).

12 Fluorescence imaging in tumor-bearing mice

To acheive the best conditions, male nude mice (~20 g) were incubated respectively with HPC-n and Zn-HPC-n (n: 1-8) (100 μL 20 μM). To monitor tumor development, male nude mice (~20 g) were subcutaneously inoculated with HepG2 cells. When tumors were visible to naked eye, 100 mL of zinc chloride (0 uM; 10 uM; 30 uM; 60 uM; 100 uM; 150 uM; 200 uM; 300 uM) was injected into each group for 4 weeks. HPC-5 (100 μL; 20 μM) was subcutaneously injected into tumor site and imaging analysis was performed 20 minutes later. A Perkin Elmer In-vivo Imaging System was used for bio-imaging of animal experiments. Mice were anesthetized prior to injection. The excitation wavelength was 465 nm and the emission wavelength was 520 nm.

13 Live subject statement

Nude mice were purchased from Changzhou Cavens Lab Animal Co. Ltd. All experiments were performed in compliance with Chinese national standard Laboratory Animal-Guideline for ethical review of animal welfare (GB/T 35892-2018) and all experiments followed institutional guidelines. All protocols were approved by the Institutional Animal Care and Use Committee in Binzhou Medical University, Yantai, China. Approval number: no. BZ2014-102R.

14 Statistical analysis

All data are expressed as the mean ± S.D. The data under each condition were accumulated from at least three independent experiments. For each experiment, unless otherwise noted, n represents the number of individual biological replicates. For each biological replicate and for all in vitro and ex vivo studies, n ≥10. The statistical analyses were performed using Student's t-test. $P < 0.01$ was considered statistically significant.

15 Supporting Figures

15.1 Time-dependent fluorescence changes of HPC-n toward Zn^{2+}

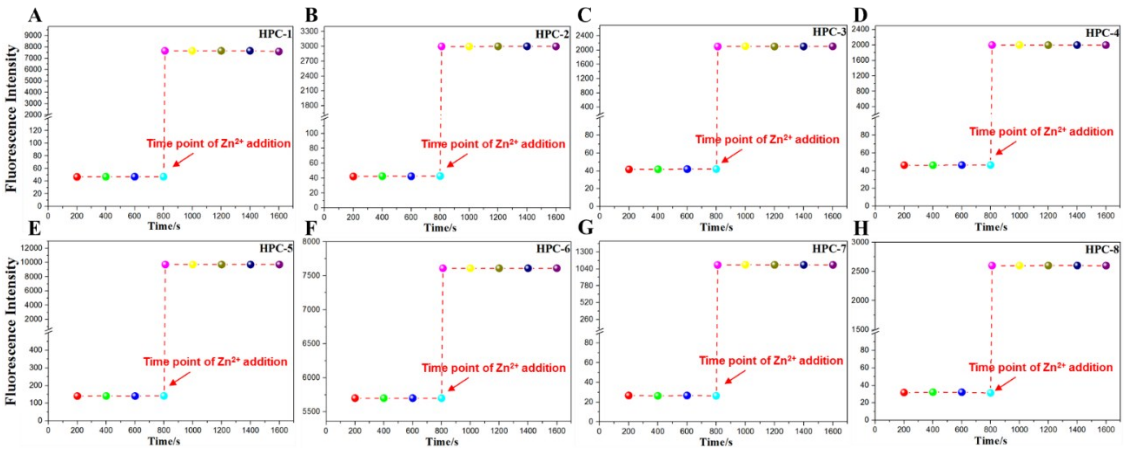


Fig. S1 Fluorescence intensity with the reaction time for HPC-n (n:1-8) (1 μM) with Zn^{2+} (20 μM)

in HEPES buffer solution (50 mM HEPES, 100mM KNO_3, pH=7.2) at 37℃ with 1% DMSO.

15.2 Effects of pH values on HPC-n and Zn-HPC-n

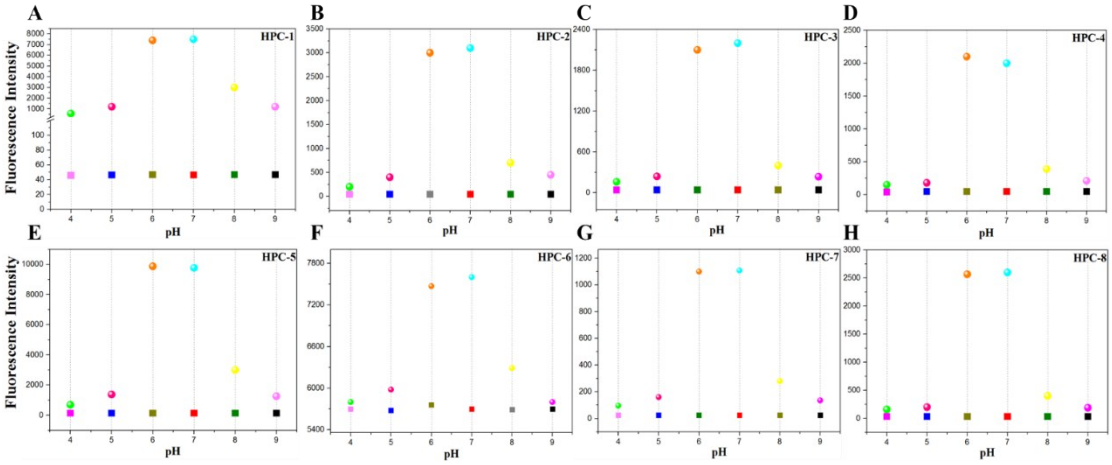


Fig. S2 Fluorescence intensity change of HPC-n (n: 1-8) (1 μM) as the addition Zn^{2+} (20 μM) under different pH values (4.0, 5.0, 6.0, 7.0, 8.0, 9.0) by using HEPES buffer solution at 37℃ with 1% DMSO.

15.3 Selectivity of HPC-n

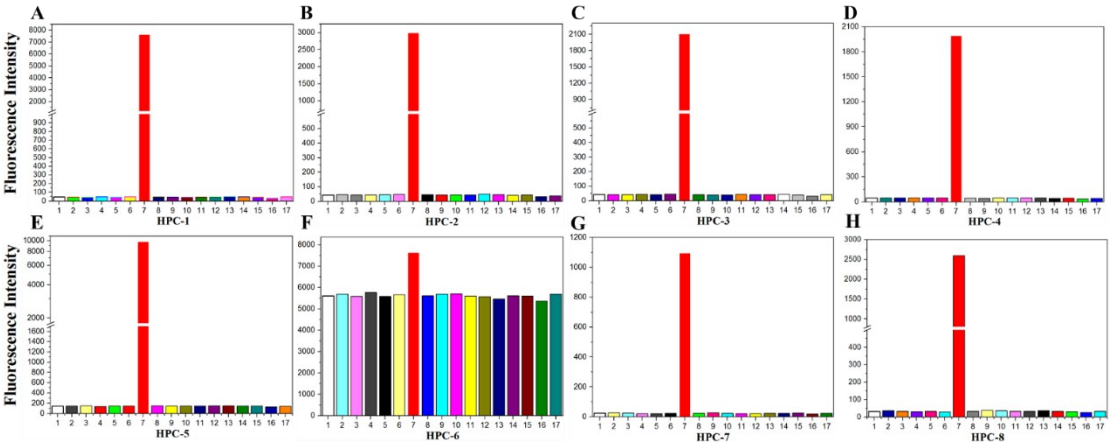


Fig. S3 Fluorescence intensity of **HPC-n** (n: 1-8) (1 μM) in HEPES buffer solution (50 mM HEPES, 100mM KNO_3, pH=7.2) at 37℃ with 1% DMSO containing tested compounds, including 1. Probe only(1 μM); 2. K^+(0.1 mM); 3. Na^+(0.1 mM); 4. Mg^{2+}(0.1 mM); 5. Mn^{2+}(0.1 mM); 6. Ni^{2+}(0.1 mM); 7. Zn^{2+}(20 μM); 8. Pb^{2+}(0.1 mM); 9. Fe^{2+}(0.1 mM); 10. Fe^{3+}(0.1 mM); 11. Ag^{2+}(0.1 mM); 12. Hg^{2+}(0.1 mM); 13. Co^{2+}(0.1 mM); 14. Cr^{2+}(0.1 mM); 15. Cd^{2+}(0.1 mM); 16.Cu^{2+}(0.1 mM); 17. Ca^{2+}(0.1 mM).

15.4 Photochemical Properties of HPC-n, BPY and TPY [a]

Table S1 [a] All data were obtained at pH 7.2 in 50 mM HEPES buffer solution containing 1% DMSO as a cosolvent. [b] The concentration of each compound is 1 μM. [c] The concentration of each compound is 1 μM and Zn^{2+} is 20 μM. [d] Quantum yields of fluorescence were determined by using fluorescein in 0.1 N NaOH as a standard.

Compound	probe only [b]			probe + Zn^{2+} [c]		
	Absorption	Emission	Fluorescence quantum yield [d]	Absorption	Emission	Fluorescence quantum yield [d]
HPC-1	410	480	0.021	415	506	0.62
HPC-2	411	481	0.023	417	510	0.24
HPC-3	410	484	0.022	415	511	0.21
HPC-4	409	486	0.024	416	509	0.22
HPC-5	409	502	0.032	415	501	0.64
HPC-6	410	477	0.179	416	477	0.34
HPC-7	423	477	0.021	427	475	0.19
HPC-8	412	500	0.015	417	530	0.23
BPY	410	475	0.046	415	475	0.22
TPY	410	476	0.052	416	476	0.21

15.5 Fluorescence titration of Zn^{2+} with HPC-n, BPY and TPY

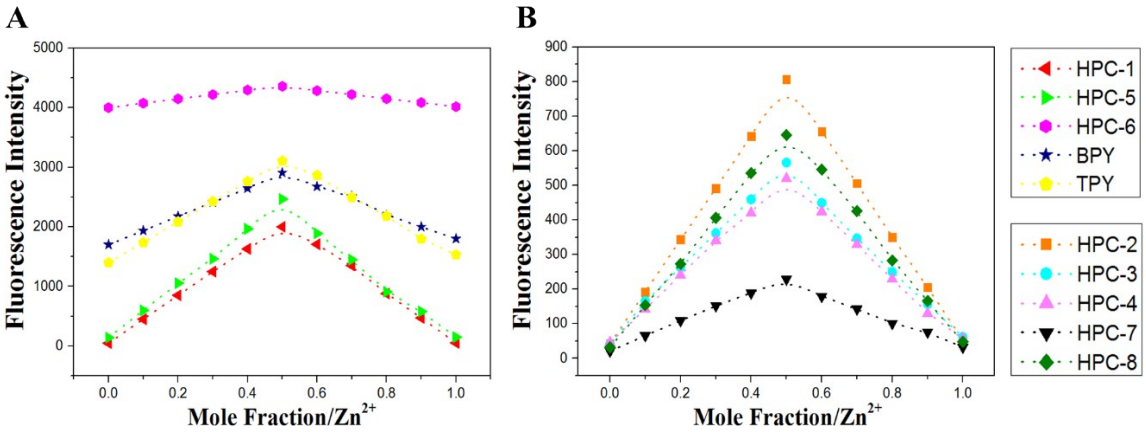
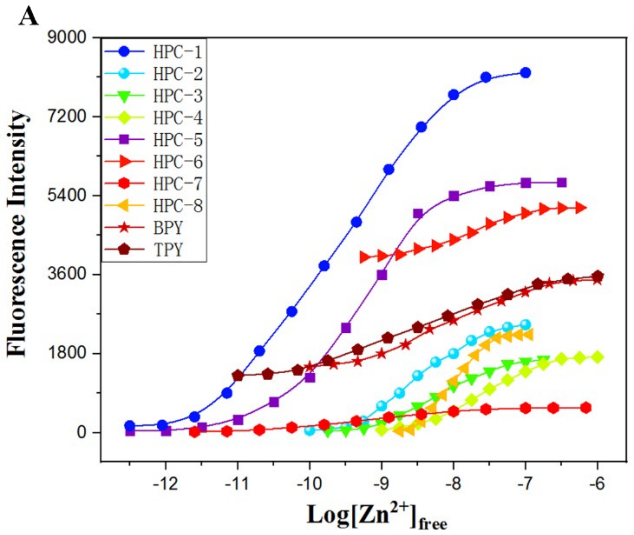


Fig. S4 Job's plot of with Zn^{2+} in HEPES buffer solution (pH=7.2, 50 mM). Maintaining the total concentration to 5 μM, solutions of **HPC-n** (n: 1-8)**, BPY, TPY** and $ZnCl_2$ were prepared in appropriate ratios in different mole fractions. It shows a maximum when the mole fraction is 0.5, indicating the formation of a 1:1 complex.

15.6 Apparent Dissociation Constants (Kd) of HPC-n, BPY and TPY



Compound	K_d (M)
HPC-1	1.3×10^{-9}
HPC-2	9.4×10^{-9}
HPC-3	10.8×10^{-9}
HPC-4	26.2×10^{-9}
HPC-5	2.6×10^{-9}
HPC-6	42.1×10^{-9}
HPC-7	10.7×10^{-9}
HPC-8	16.7×10^{-9}
BPY	8.1×10^{-9}
TPY	7.3×10^{-9}

Fig. S5 (A) Fluorescence intensity of **HPC-n** (n: 1-8), **BPY** and **TPY** (1 μM) in total Zn^{2+} (0-9 mM) with EGTA buffer system (pH=7.2, 50mM HEPES, 100mM KNO_3, 10 mM EGTA) at 0, 0.14, 0.29, 0.66, 1.1, 1.8, 2.6, 4.0, 6.1, 11.0, 24.0 nM, respectively. (B) List of apparent dissociation constants (K_d) of HPC-n, BPY and TPY.

15.7 Dynamic fluorescence imaging for penetration and photostability

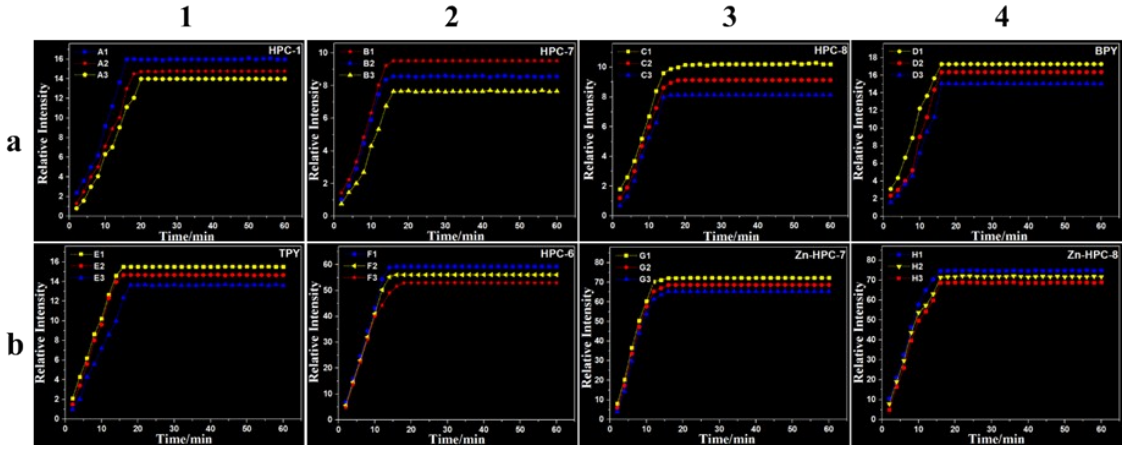


Fig. S6 Dynamic fluorescence imaging of Hek293t cells. Intact cells which were incubated with HPC-1 (1 μM), HPC-7 (1 μM), HPC-8 (1 μM), BPY (0.01 μM), TPY (0.01 μM), HPC-6 (0.01 μM), Zn-HPC-7 (1 μM), Zn-HPC-8 (1 μM) in fresh medium to obtain images that temporal profile of relative fluorescence intensity in regions of interest (An,Bn,…,Hn) (n:1-3). Images were captured using 405 nm for excitation (emission window: 430-650 nm).

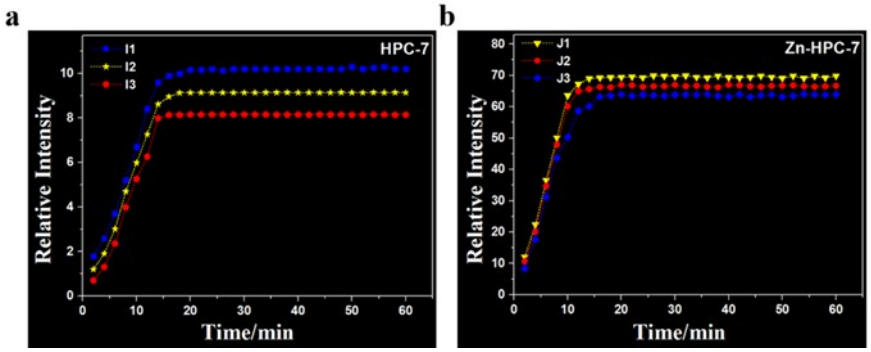


Fig. S7 Dynamic fluorescence imaging of SHSY-5Y cells. Intact cells which were incubated with HPC-7 (1 μM) and Zn-HPC-7 (1 μM) in fresh medium to obtain images that temporal profile of relative fluorescence intensity in regions of interest (I1, I2, I3, J1, J2 and J3). Images were captured using 405 nm for excitation (emission window: 430-650 nm).

15.8 Cell viability assay

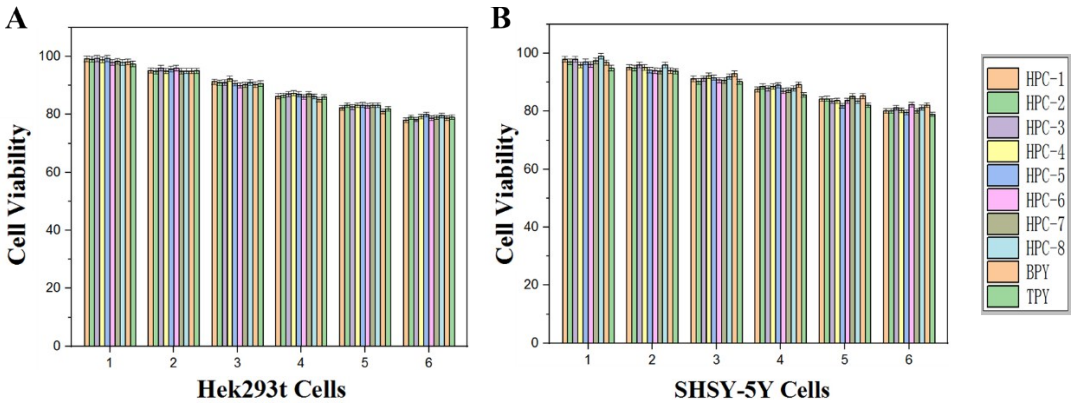
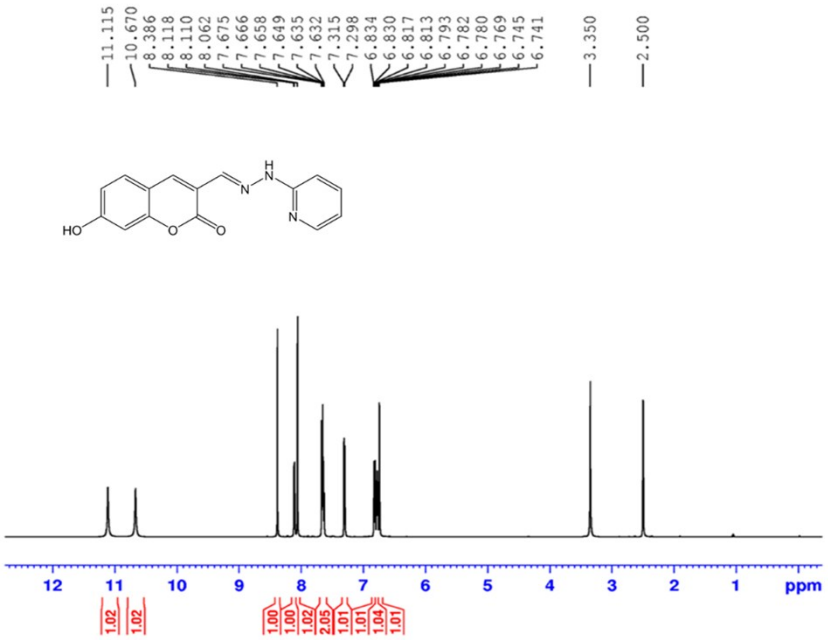


Fig. S8 Cell viability of (A) Hek293t cells and (B) SHSY-5Y cells treated with different concentrations of **HPC-n** (n: 1-8), **BPY** and **TPY** (1 μM, 10 μM, 30 μM, 0.1 mM, 0.5 mM, 1 mM) for 24 hours in fresh medium.

15.9 ^{1}HNMR, ^{13}CNMR and HRMS



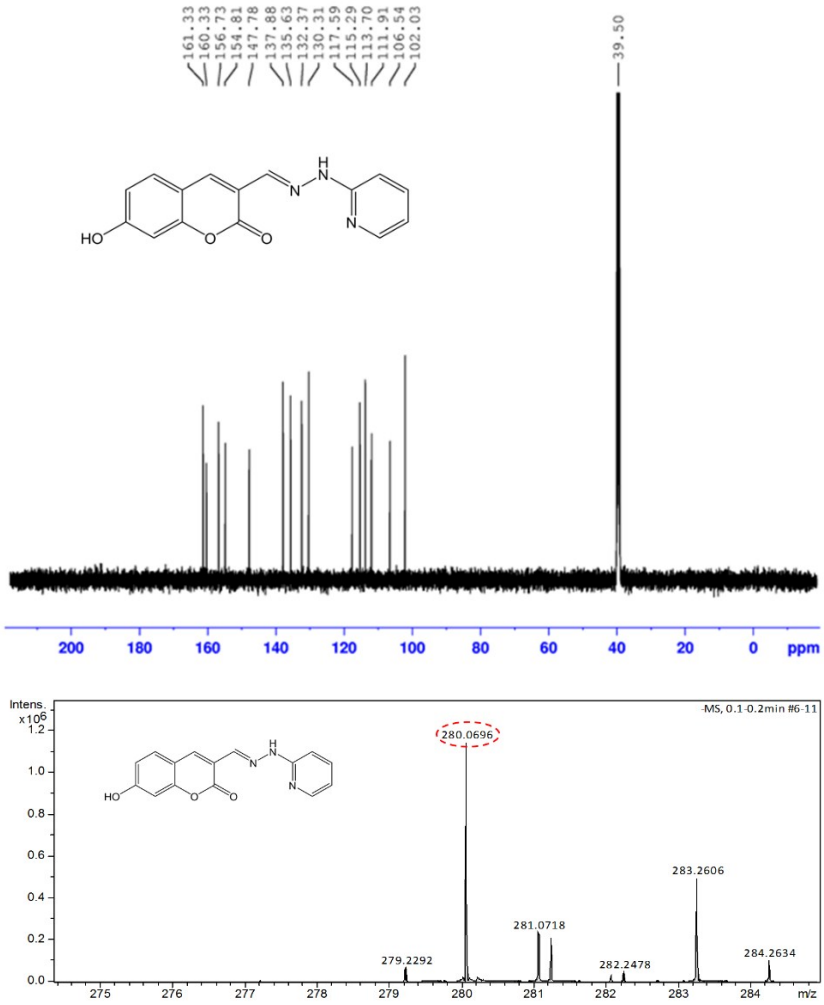
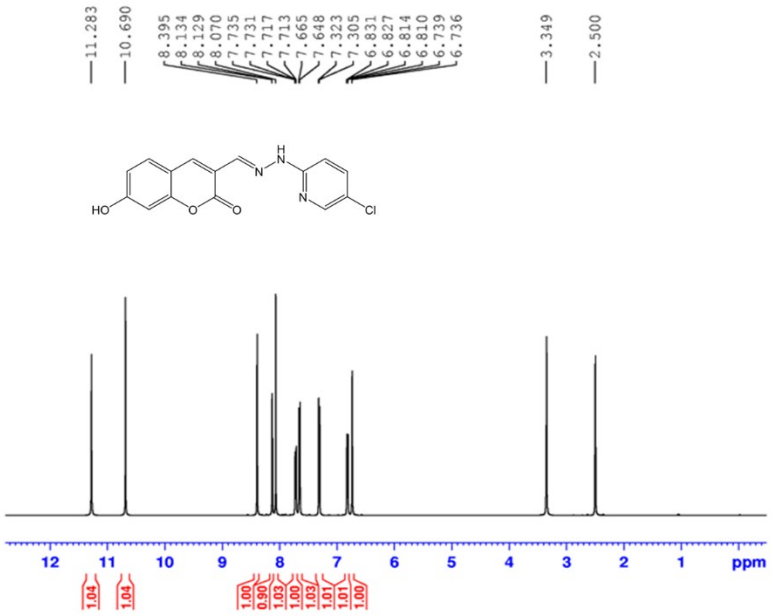


Fig. S9 [1]HNMR, [13]CNMR and HRMS of **HPC-1**.



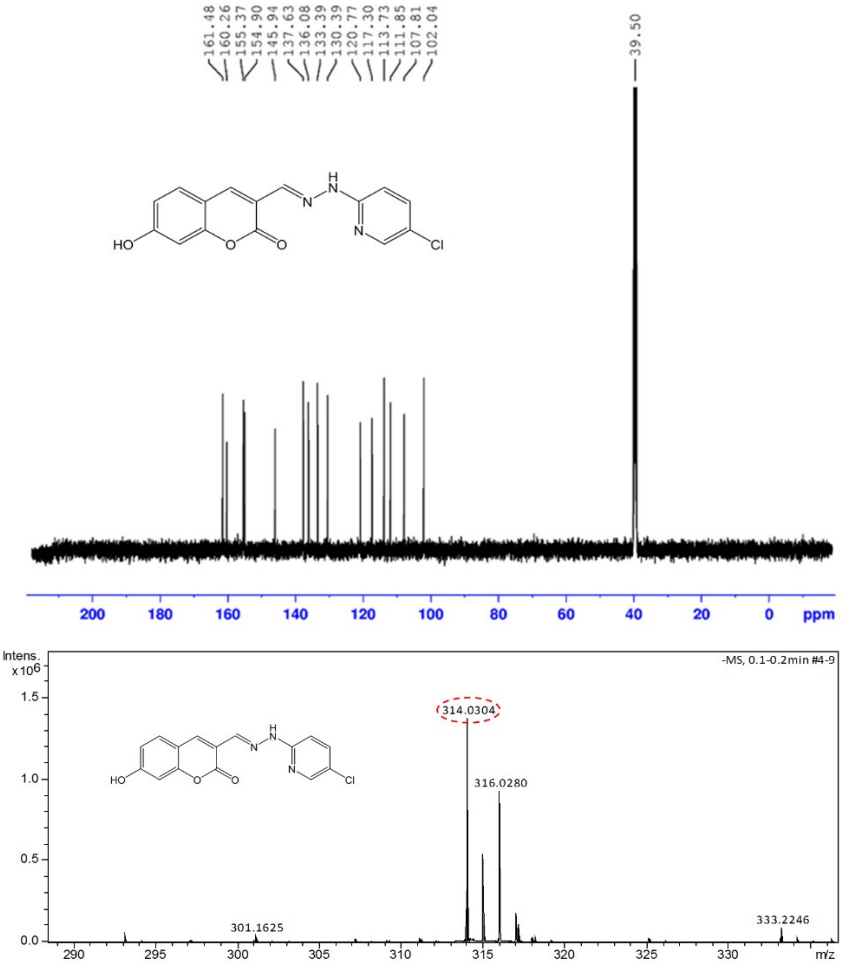
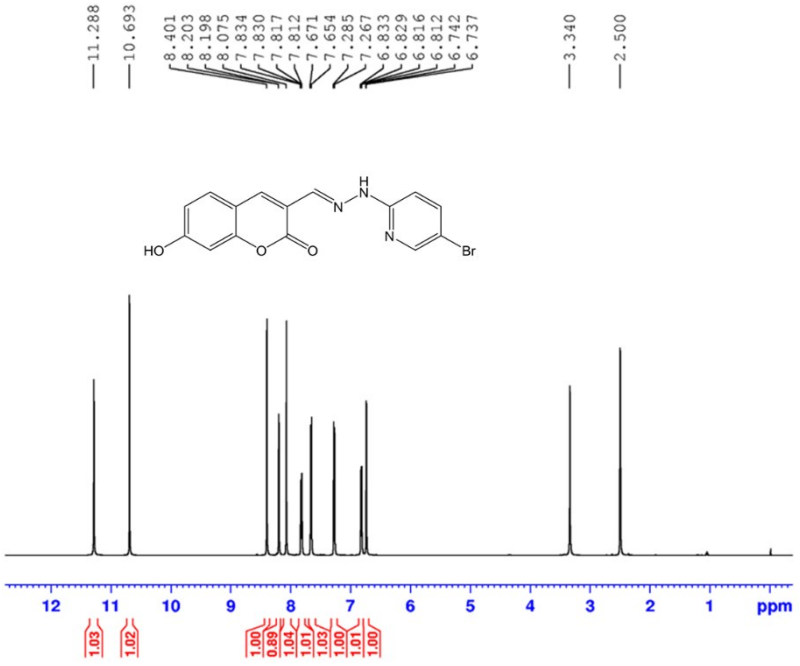


Fig. S10 [1]HNMR, [13]CNMR and HRMS of **HPC-2**.



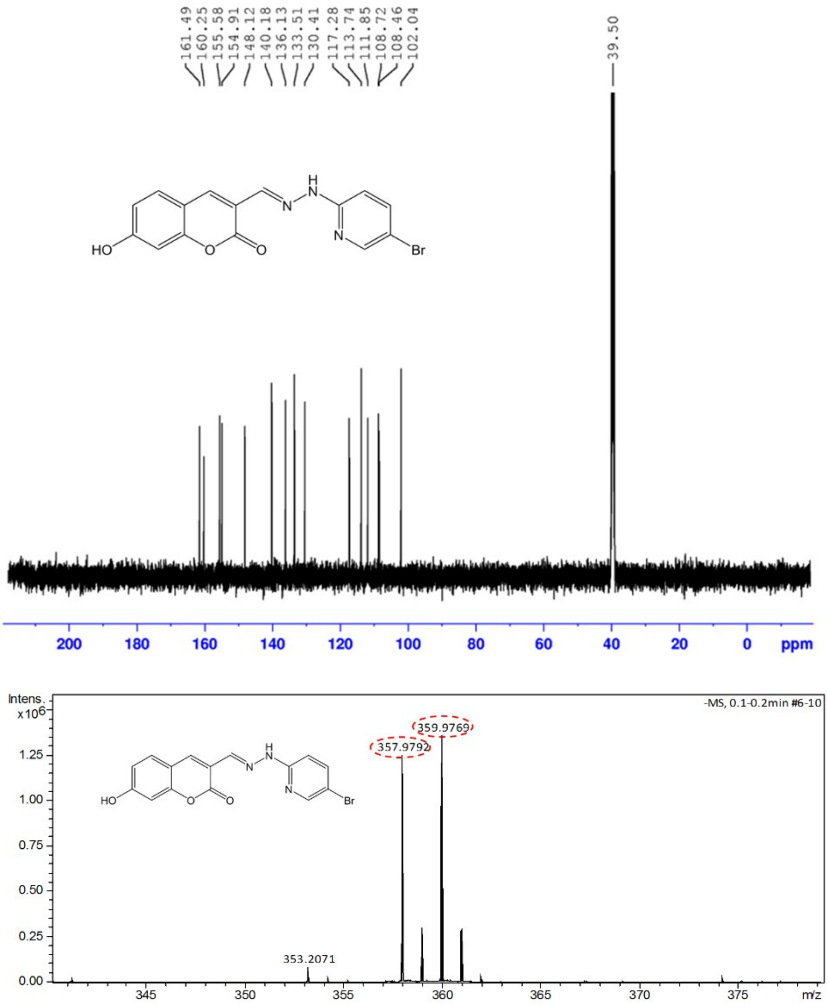
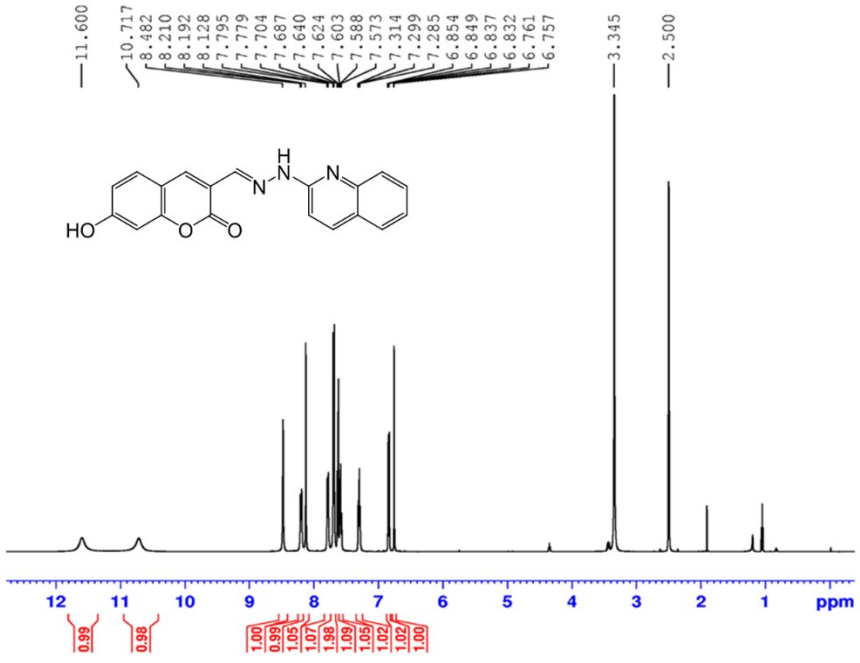


Fig. S11 [1]HNMR, [13]CNMR and HRMS of **HPC-3**.



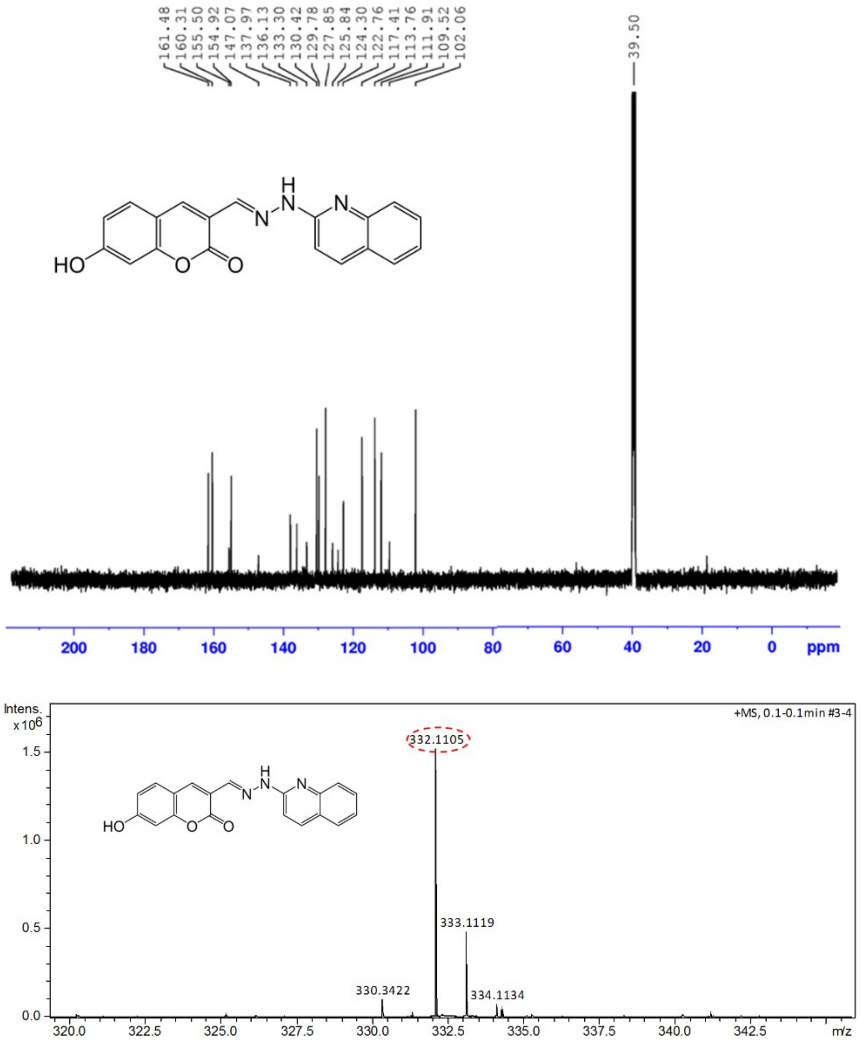
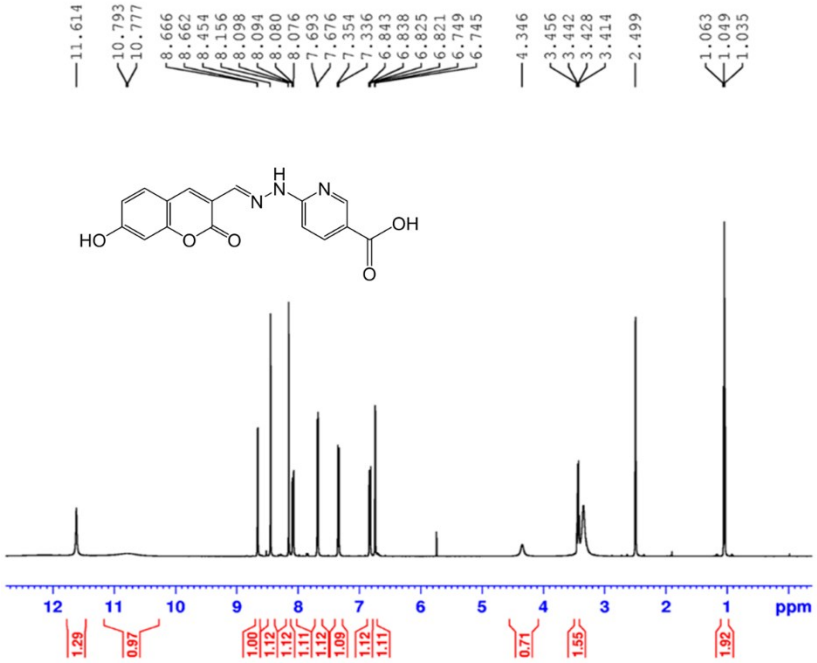


Fig. S12 [1]HNMR, [13]CNMR and HRMS of **HPC-4**.



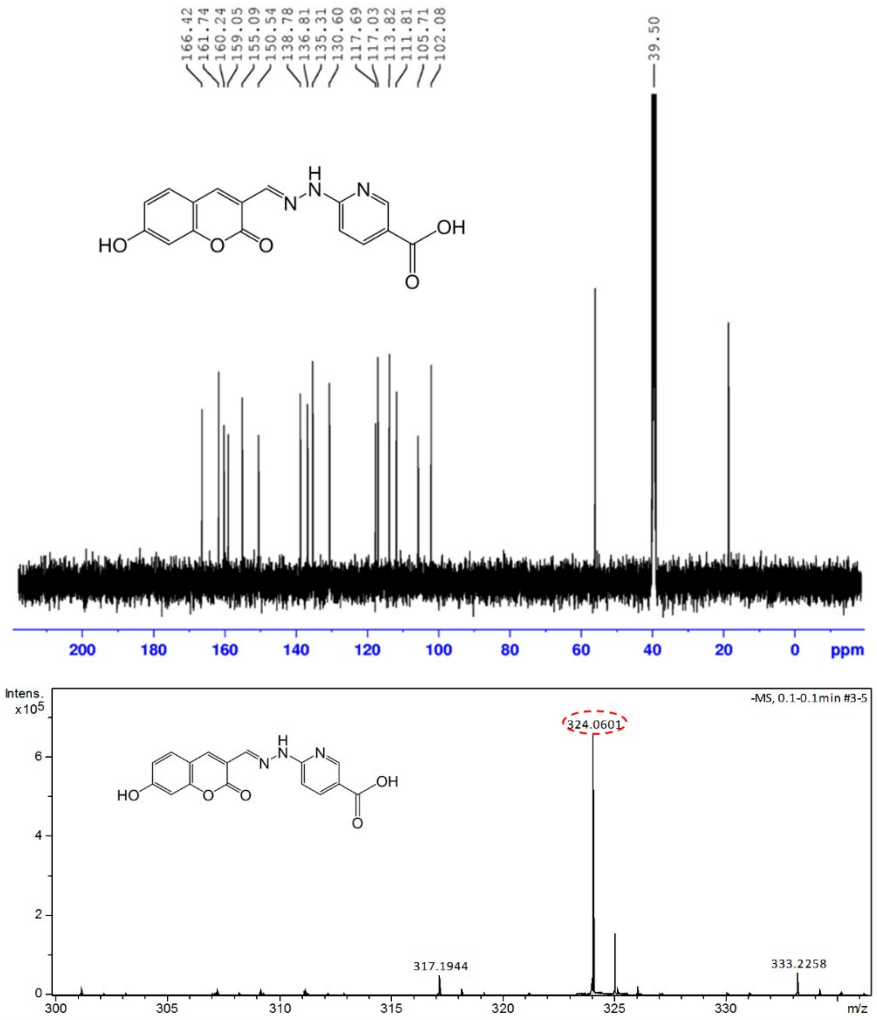
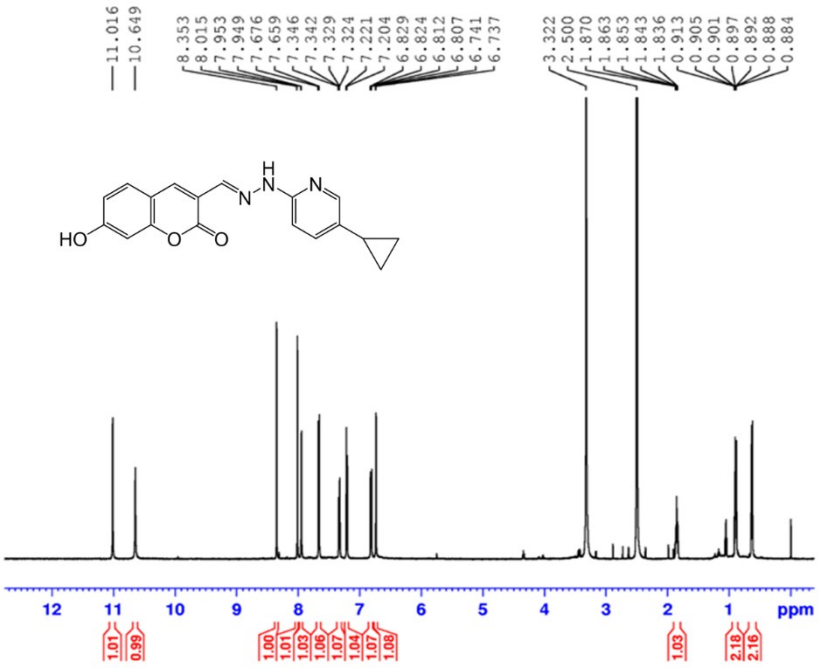


Fig. S13 ^{1}HNMR, ^{13}CNMR and HRMS of **HPC-5**.



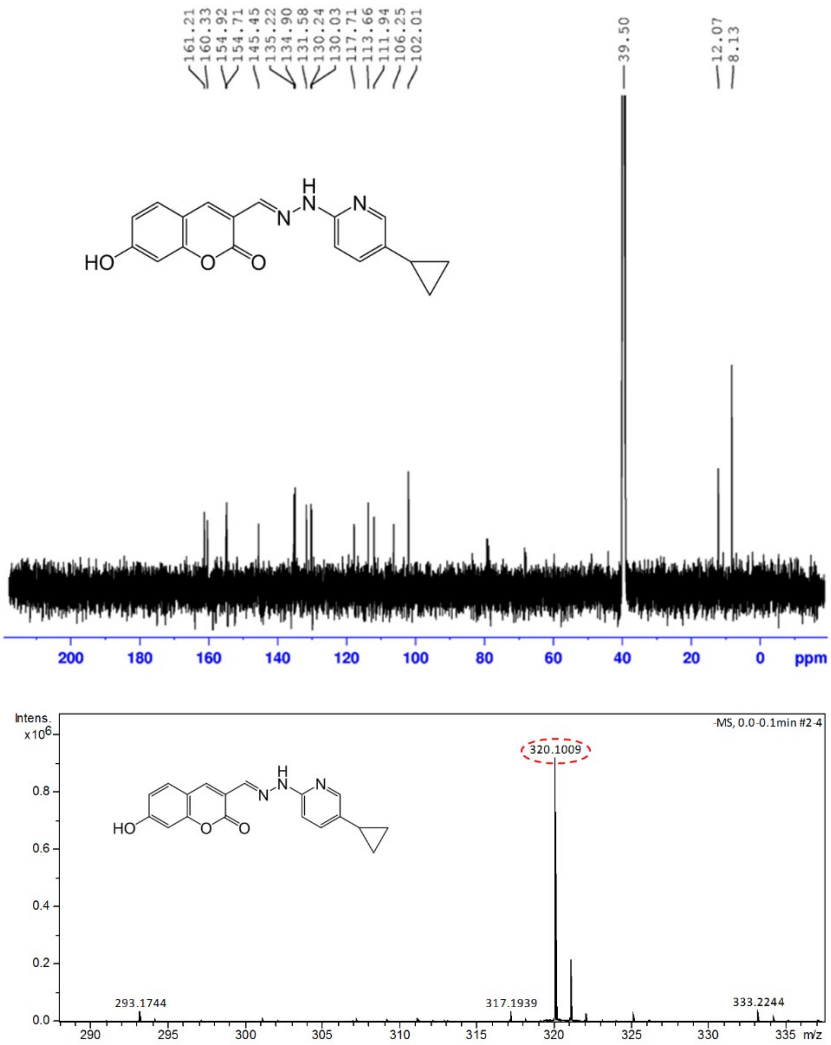
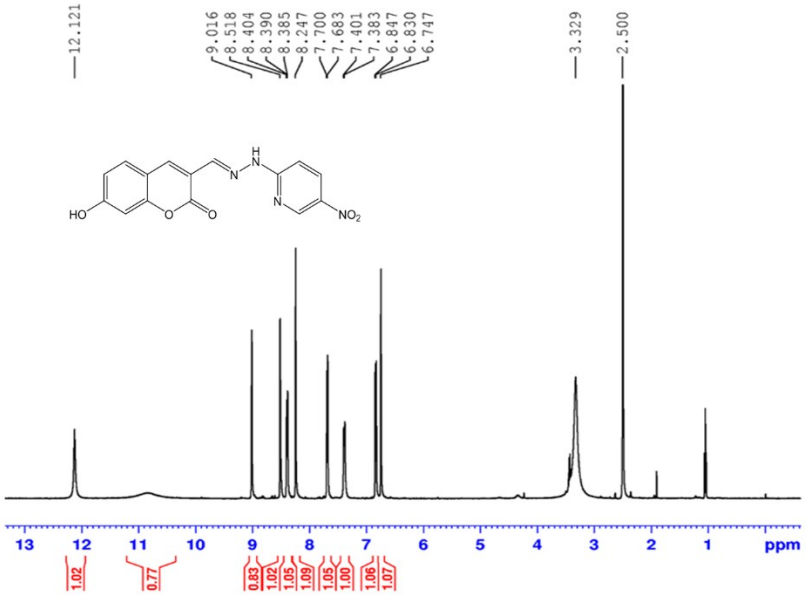


Fig. S14 [1]HNMR, [13]CNMR and HRMS of **HPC-6**.



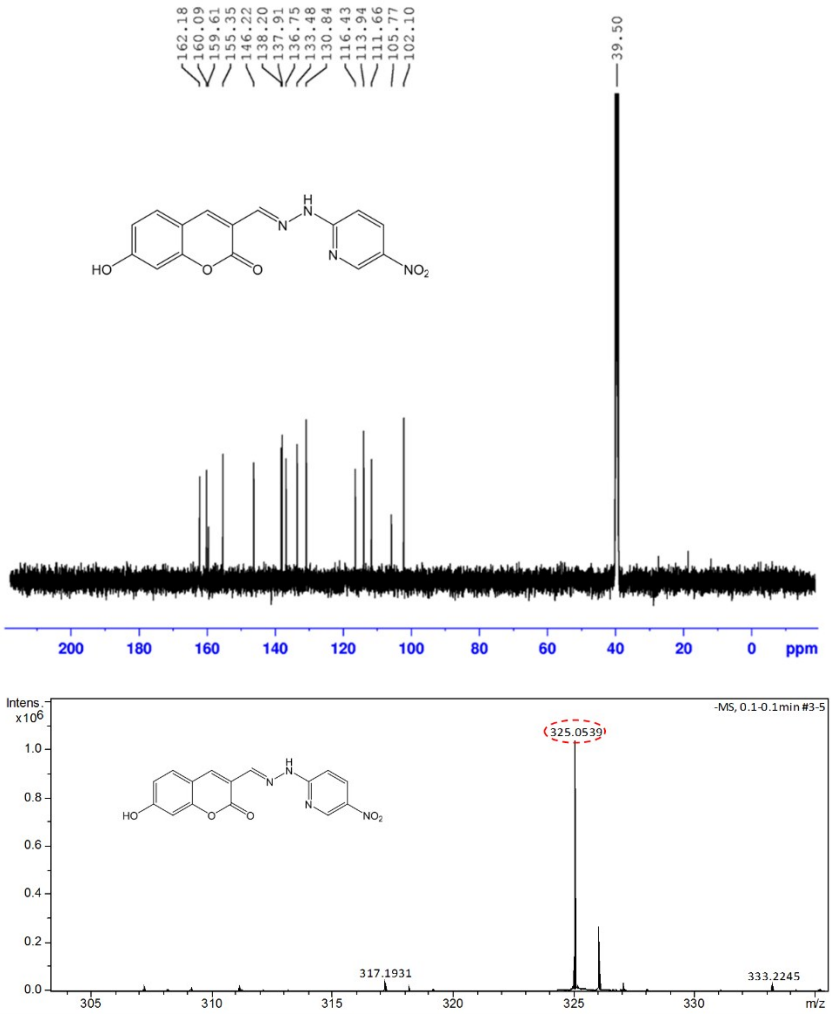
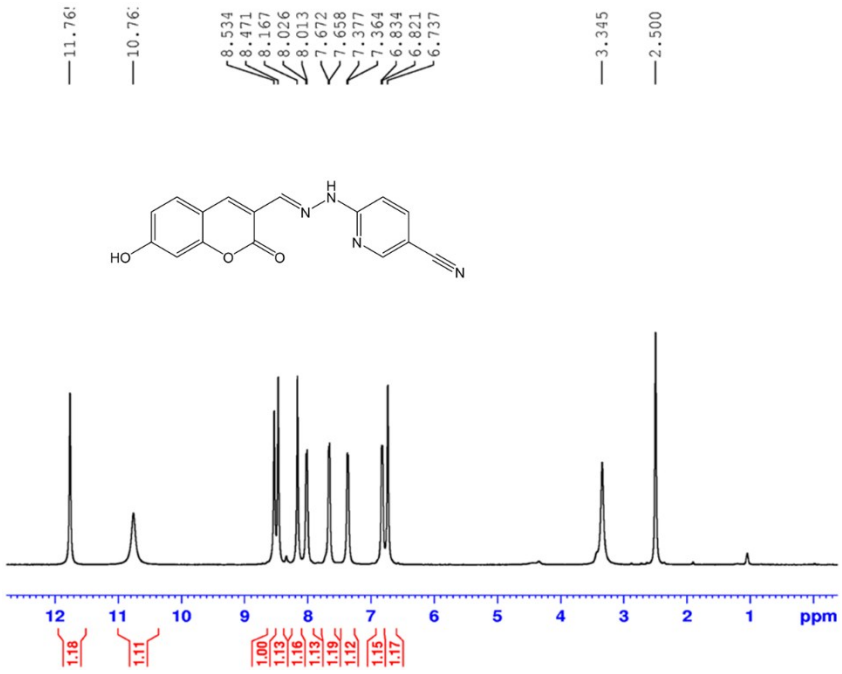


Fig. S15 [1]HNMR, [13]CNMR and HRMS of **HPC-7**.



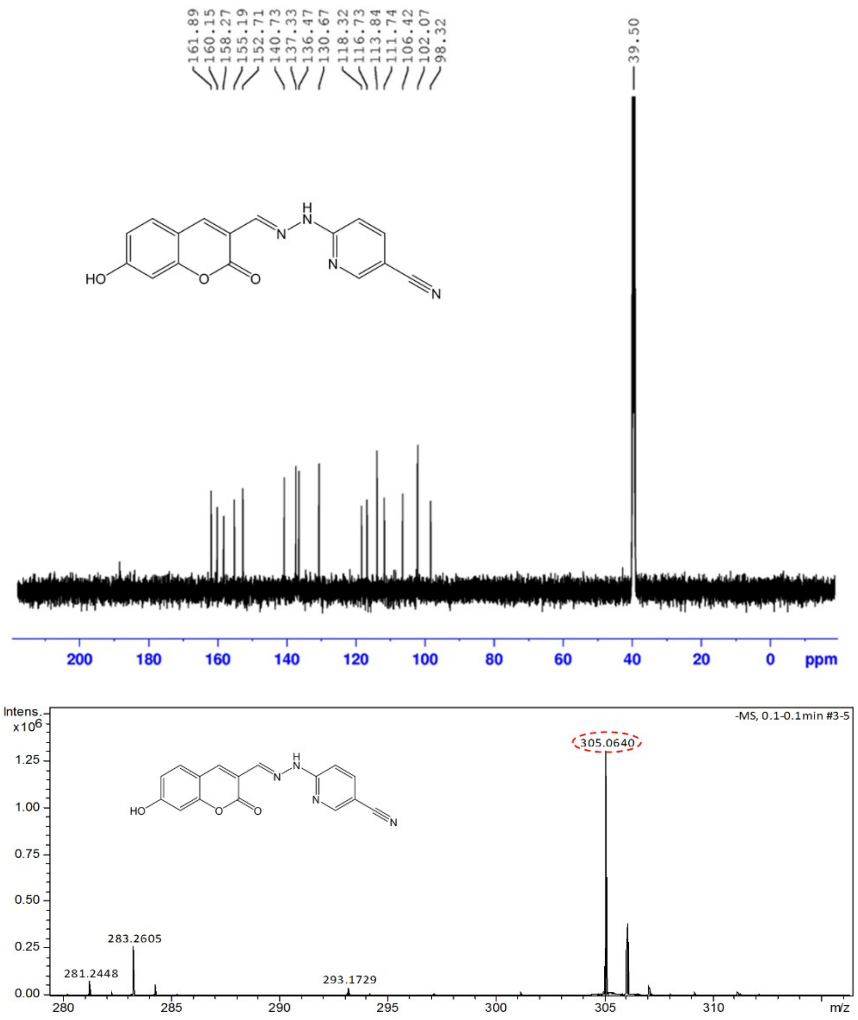
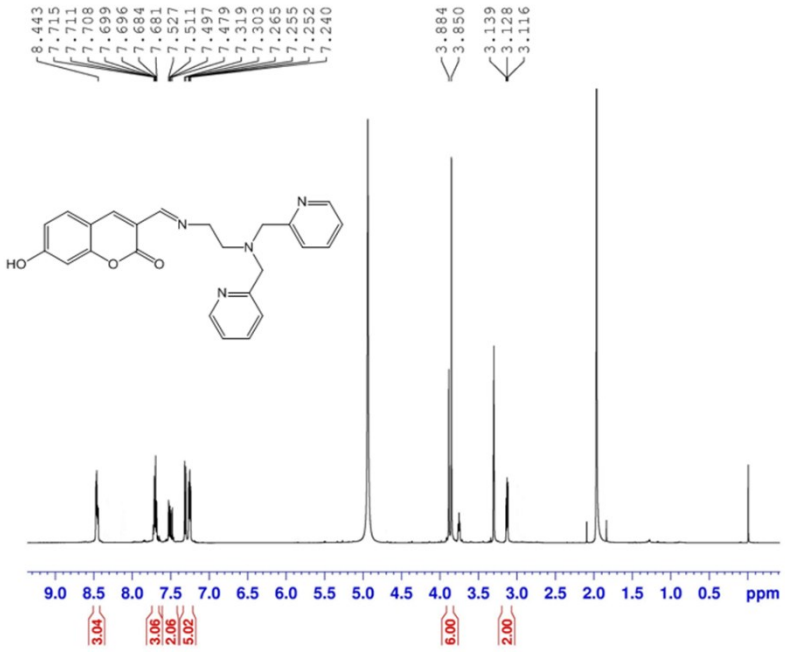


Fig. S16 [1]HNMR, [13]CNMR and HRMS of **HPC-8**.



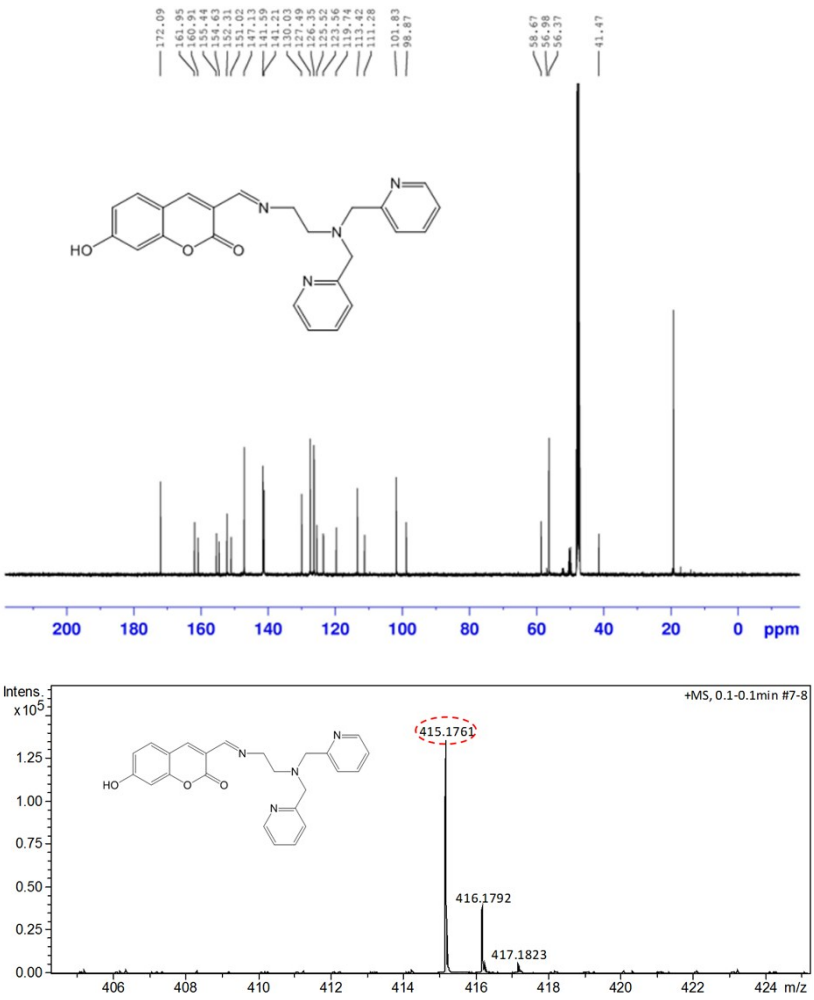
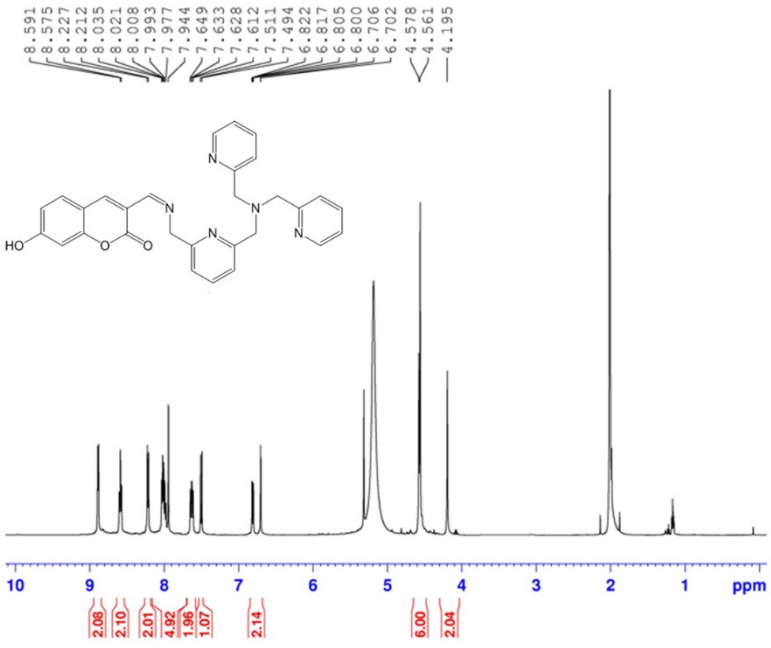


Fig. S17 ^{1}HNMR, ^{13}CNMR and HRMS of **BPY**.



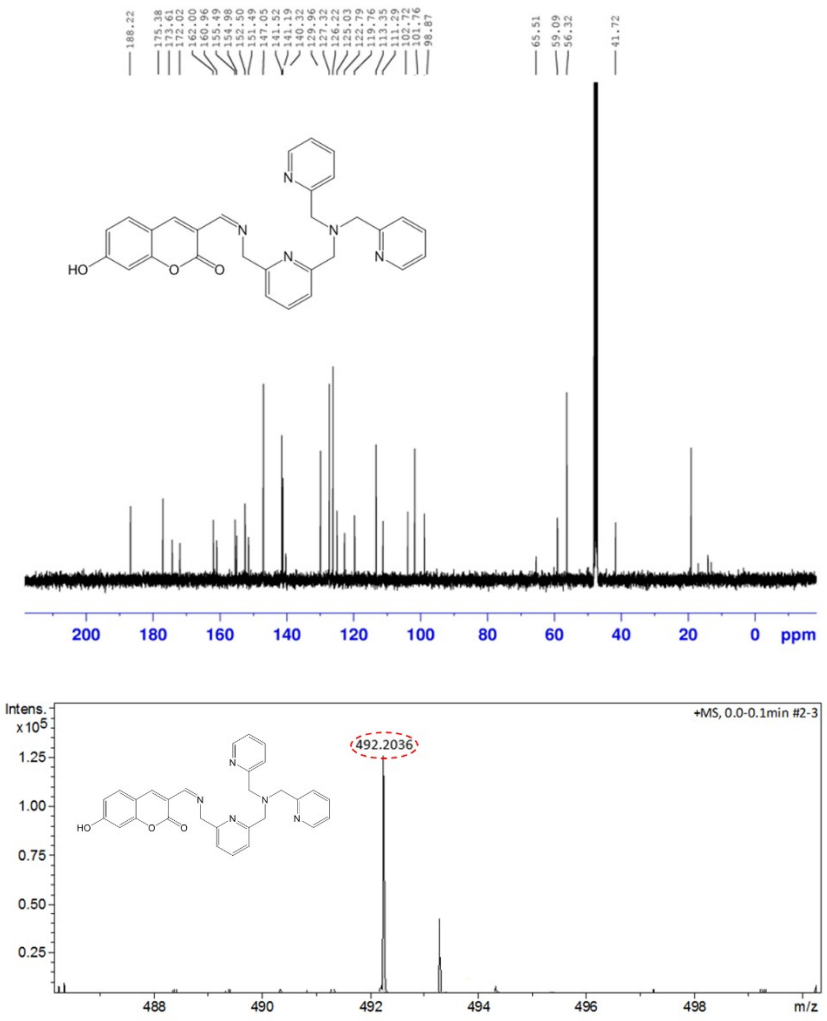


Fig. S18 [1]HNMR, [13]CNMR and HRMS of **TPY**.