Supporting Information for

Cell Membrane-Anchored Fluorescent Probe for Monitoring Carbon Monoxide Released out of Living Cells

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EXPERIMENTAL SECTION

Reagents and Apparatus. Unless otherwise stated, all chemical materials are purchased from commercial suppliers without further purification. Cell membrane tracker (Dio) and LPS was purchased from Beyotime Biotechnology. Zinc protoporphyrin (ZnPP), Hemin and CORM-2 were purchased from Sigma-Aldrich. Ultrapure water was obtained from a Milli-Q system. Photoluminescent spectra were measured with a HITACHI F4600 fluorescence spectrophotometer at room temperature. UV-vis-NIR absorption spectra were recorded on a Shimadzu UV-3600 plus. Continuous wave X-band ESR spectra were recorded on a JES-FA200 spectrometer. The pH measurements were performed on a Mettler-Toledo Delta 320 pH meter. The used Silica gel (200-300 mesh) for column chromatography was obtained from Qingdao Ocean Chemicals (Qingdao, China). ^{1}H and ^{13}C NMR were performed on a Bruker

DRX-400 spectrometer (Bruker) system, using TMS as an internal standard. Mass spectra were recorded using an LCQ Advantage ion trap mass spectrometer (ThermoFinnigan). One- and two-photon fluorescence imaging experiments were obtained using an Olympus FV1000-MPE multiphoton laser scanning confocal microscope (Japan). **ANRP** was synthesized as shown in **Scheme S1.** The newly synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and ESI.

Spectrophotometric Experiments. Spectrophotometric experiments were carried out in buffered aqueous DMSO solution (DPBS/DMSO = 19:1, v/v). For the probe respond to CO, a volume of 2 μL of **ANRP** stock solution (0.5 mM), CORM-2 solution, and DPBS were added into a tube to make the final volume 200 μL. After the samples incubated at 37 °C for 30 min, the fluorescence spectra were measured with both excitation and emission slits set at 10 nm. The samples were excited by 580 nm and the emission wavelength was collected from 600 nm to 750 nm. The solutions of various potential interfering species were prepared with ultrapure water.

Cytotoxicity Study. A standard MTS assay was used to study the cytotoxicity of **ANRP**. Hela cells were seeded at 1×10^{5} cells per well in 96-well plates and cultured for 24 h. After that, Hela cells were treated with different concentrations (0-20 μM) of **ANRP** for 24 h. The culture media was removed and the wells were washed with DPBS (200 μL) for three times. MTS solution was added to each well and incubated at 37 °C for 40 min. Then the cell viability was determined by a multimode microplate.

Cell Culture and Imaging. HeLa, HepG2 and HL7702 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS, GIBCO) and 1% penicillin-streptomycin at 37 °C in humidified atmosphere containing 20% O_2 and 5% CO_2. Fluorescence imaging of cells was performed on an Olympus FV1000 MPE multiphoton laser scanning microscope (Japan) with a 60× oil immersion objective lens. The fluorescence signal of **ANRP** was collected from 600 to 700 nm with excitation at 543 nm.

Flow cytometric analysis. HeLa cells, HepG2 cells and HL-7702 cells were cultured in 6-well plate for 24 h, respectively. The cells were incubated with **ANRP** for 30 min, then treated with EDTA. After that, the cells were subjected to flow cytometry analysis using Cyan-LX (DakoCytomation). The cells without any treatment were used as control. The mean

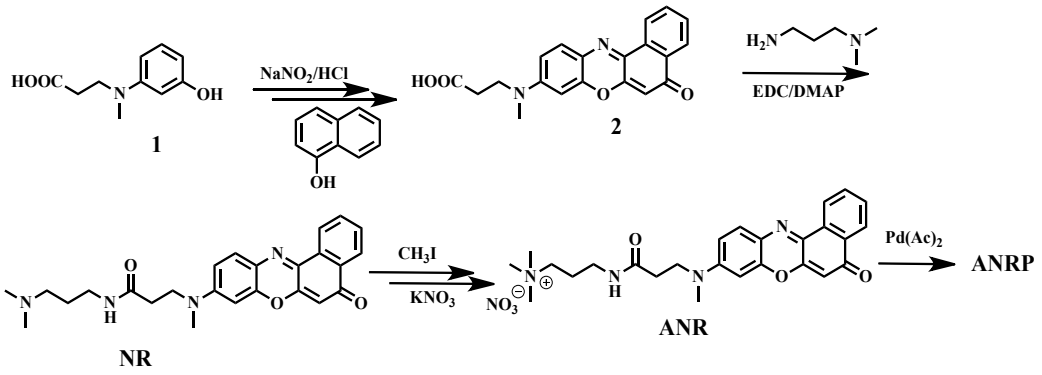
fluorescence was determined by counting 10,000 eventson the BD FACSVerse™ flow cytometer

Animals. We obtained ~8 weeks old Male Balb/c mice from Hunan SJA Laboratory Animal Co., Ltd. and used under protocols approved by Hunan University Laboratory Animal Center.

Organs imaging. The mice were euthanized and the organs including heart, liver, spleen, lung and kidney were isolated. These organs were cultured with DPBS or 10 μM **ANRP** for 30 min, respectively, and imaged using a Caliper VIS Lumina XR small animal optical in vivo imaging system. Excitation scan was chosen as imaging mode for all experiments, and Input/Em was selected as 543 nm for excitation with DsRed filter for emission channel.

Two-Photon Fluorescence Microscopy Images of Endogenous CO in Liver Tissue Slices. Liver tissue slices were obtained from the **ANRP** treated liver tissue mentioned above and imaged with a mode-locked titanium-sapphire laser source set at a wavelength of 780 nm and the emission wavelength was collected at 605-680 nm.

In Vivo Imaging. Before *in vivo* imaging, Balb/c mice were anesthetized by inhalation of 5% isoflurane in 100% oxygen. The mice were intraperitoneally injected with 50 μL of 30 μM **ANRP**, followed by an injection of 100 μL of 100 μM CORM-2. Time-dependent fluorescence images of CO in the living mice were performed using a Caliper VIS Lumina XR small animal optical in vivo imaging system. Excitation scan was chosen as imaging mode for all experiments, and Input/Em was selected as 543 nm for excitation with DsRed filter for emission channel.



Scheme S1. Synthesis Route for **ANRP**

Synthesis of Compound 2. Compound **1** was synthesized as previous reported[1]**.** Sodium nitrite (1.52 g, 22 mmol) in 10 mL water was slowly added to the solution of compound **1** (3.3 g, 16.9 mmol) in 20 mL concentrated HCl at 0 °C. The mixture was stirred for 3 h at this

temperature. After that, the solvent was evaporated under reduced pressure and this residue was used in the next step without further purification.

To the solution of the residue in 50 mL DMF was added 1-hydroxynaphthalene (3.45 g, 24.0 mmol) and the reaction mixture was heated to 130 °C for 4 h. After cooling the reaction to room temperature, the solvent was evaporated and the residual material was purified by chromatography on silica gel with CH_2Cl_2/ EtOH (10:1) to afford compound **2** as a dark solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (d, J = 7.8 Hz, 1H), 8.11 (d, J = 7.7 Hz, 1H), 7.79 (m, 1H), 7.71 (m, 1H), 7.57 (d, J = 9.0 Hz, 1H), 6.79 (dd, J = 9.0, 2.3 Hz, 1H), 6.62 (d, J = 2.3 Hz, 1H), 6.25 (s, 1H), 3.71 (t, J = 7.0 Hz, 2H), 3.04 (s, 3H), 2.57 (t, J = 7.0 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.48, 173.40, 152.17, 146.40, 139.45, 132.05, 131.93, 131.52, 131.09, 130.56, 125.46, 124.89, 123.88, 110.86, 105.19, 97.12, 48.35, 38.82, 32.07. ESI-MS calculated for [M] = 348.1, found 346.9

Synthesis of NR. A mixture of compound **2** (0.69 g, 2 mmol), EDCI (0.58 g, 3 mmol), DMAP (0.25 g, 2 mmol) and HOBt (0.32 g, 2.4 mmol) in 50 mL DCM was stirred for 30 min at room temperature. 3-dimethylaminopropylamine (0.62 g, 6 mmol) was then added to the mixture and the reaction was stirred at room temperature overnight. After removal of solvent, the residual was purified over silica gel using CH_2Cl_2/EtOH (5:1) as the eluent to yield **NR** as a dark solid. ^{1}H NMR (400 MHz, MeOD) δ 8.36 (d, *J* = 7.7 Hz, 1H), 8.03 (d, *J* = 7.2 Hz, 2H), 7.61 (m, 2H), 7.56 (m, 2H), 7.28 (d, *J* = 9.0 Hz, 1H), 6.54 (d, *J* = 8.7 Hz, 1H), 6.28 (s, 1H), 6.03 (s, 1H), 3.60 (t, *J* = 5.8 Hz, 2H), 3.14 (t, *J* = 6.0 Hz, 2H), 2.89 (s, 3H), 2.43 (t, *J* = 5.7 Hz, 2H), 2.29 (m, 2H), 2.19 (s, 6H), 1.65 -1.58 (m, 2H). ^{13}C NMR (100 MHz, MeOD) δ 183.79, 172.13, 152.12, 151.96, 145.99, 138.49, 131.75, 131.21, 130.94, 130.59, 129.48, 125.14, 124.79, 123.44, 110.41, 104.15, 96.19, 56.59, 48.71, 43.91, 37.56, 37.30, 29.36, 26.56. ESI-MS calculated for [M] = 432.2, found 433.3

Synthesis of ANR. CH_3I (0.14 g, 1 mmol) was added to the solution of **NR** (0.22 g, 0.5 mmol) in CH_3CN (20 mL) and the mixture was refluxed overnight. After cooling the reaction to room temperature, the solvent was removed and the residual material was purified by chromatography on silica gel with CH_2Cl_2/EtOH (3:1) to provide a dark solid. The dark solid was dissolved in methanol (5 mL) and a saturated KNO_3 solution was then added. After stirring for 30 min, methanol was evaporated and the precipitation was collected as a dark

solid. ^{1}H NMR (400 MHz, MeOD) δ 8.45 (d, *J* = 4.0 Hz 1H), 8.09 (d, *J* = 4.0 Hz, 1H), 7.69 (t, *J* = 8.0 Hz 1H), 7.62 (t, *J* = 8.0 Hz 1H), 7.39 (d, *J* = 4 Hz, 1H), 6.65 (d, *J* = 4 Hz, 1H), 6.42 (s, 1H), 6.13 (s, 1H), 3.66 (t, *J* = 4 Hz, 2H), 3.34 (m, 2H), 3.27 (t, *J* = 4 Hz, 2H), 3.10 (s, 3H), 2.96 (s, 9H), 2.51 (t, *J* = 8 Hz, 2H), 2.0-1.92 (m, 2H). ^{13}C NMR (100 MHz, MeOD) δ 183.90, 172.60, 152.27, 152.13, 146.12, 131.83, 131.32, 131.03, 130.67, 129.61, 125.19, 124.84, 123.48, 110.51, 104.24, 96.32, 64.29, 52.25, 48.60, 37.43, 36.00, 33.34, 22.94. ESI-MS calculated for [M] = 447.2, found 447.2.

Synthesis of ANRP. A mixture of **ANR** (50 mg, 0.1 mmol) and $Pd(Ac)_2$ (30 mg) in 15 mL acetic acid was heated to 60 °C for 6 h then at room temperature for 12 hours under nitrogen protection. After that, the solution was filtered and the solvent was removed under vacuum. Then, the crude product was redissolved in methanol and filtered using syringe filter. The solvent was removed under vacuum and the crude product was purified by recrystallization to give a dark solid. ^{1}H NMR (400 MHz, DMSO) δ 8.43 (d, *J* = 4.0 Hz 1H), 8.08 (d, *J* = 7.2 Hz, 1H), 7.61 (d, *J* = 7.5 Hz, 1H), 7.29 (m, 1H), 6.78 (d, *J* = 4 Hz, 1H), 6.68 (s, 1H), 6.27 (s, 1H), 3.75 (t, *J* = 4 Hz, 2H), 3.32-3.29 (m, 4H), 3.07 (s, 12H), 2.45 (m, 2H), 1.83 (m, 2H). ESI-MS (m/z) found 611.2, calculated 611.1; HRMS (m/z) found 611.1488, calculated 611.1480. Anal. calcd for $C_{60}H_{72}N_8O_{14}Pd_2^{2+}$ C, 53.70; H, 5.41; N, 8.35 found: C, 54.03; H, 5.11; N,8.63.

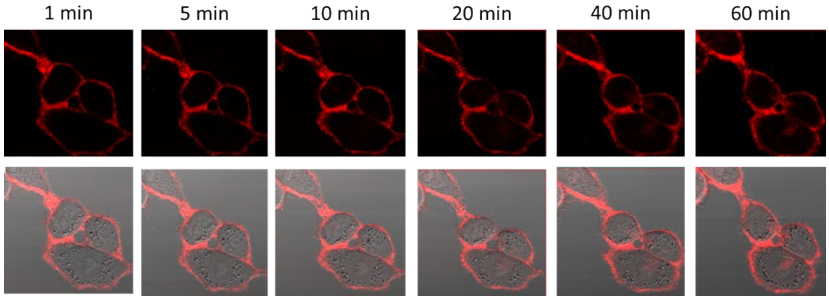


Fig. S1 The feasibility of **ANR** staining cell membrane. λ_{ex} = 543 nm, λ_{em} = 600-700 nm.

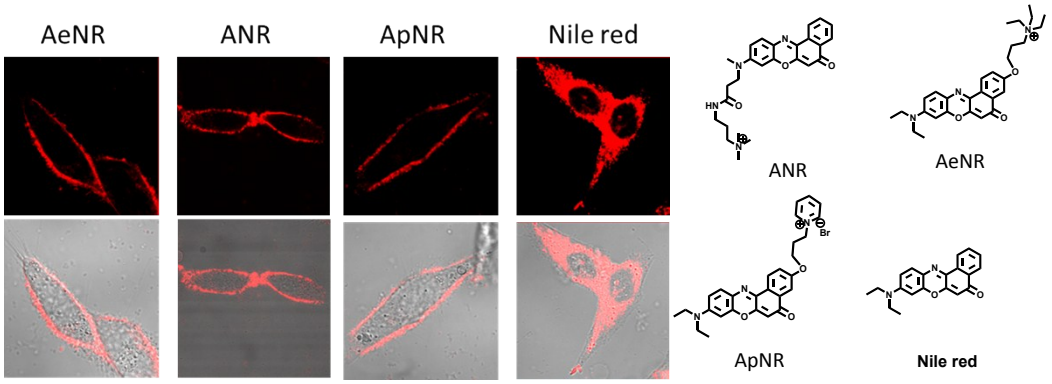


Fig. S2 The ability of **ANR**, **AeNR**, **ApNR** and **Nile red** to stain cell membrane. λ_{ex} = 543 nm, λ_{em} = 600-700 nm.

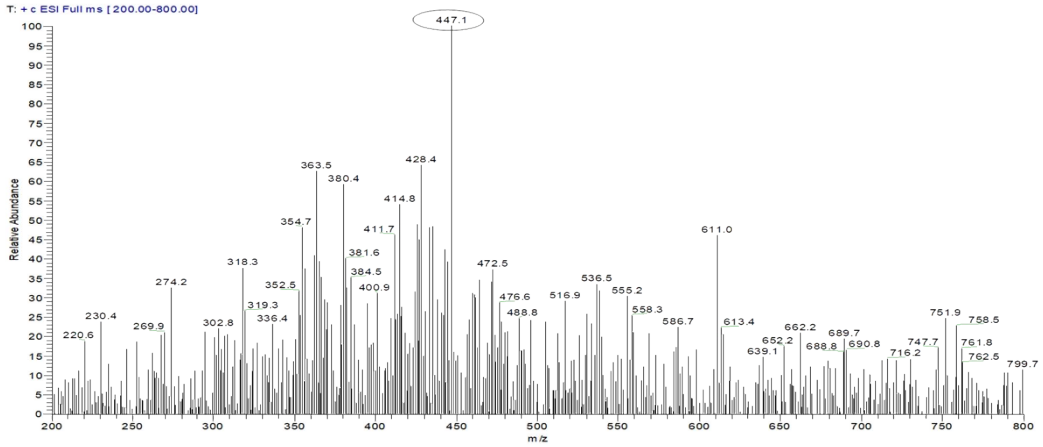


Fig. S3 ESI spectrum of **ANRP** (5 μM) treated with excessive CORM-2.

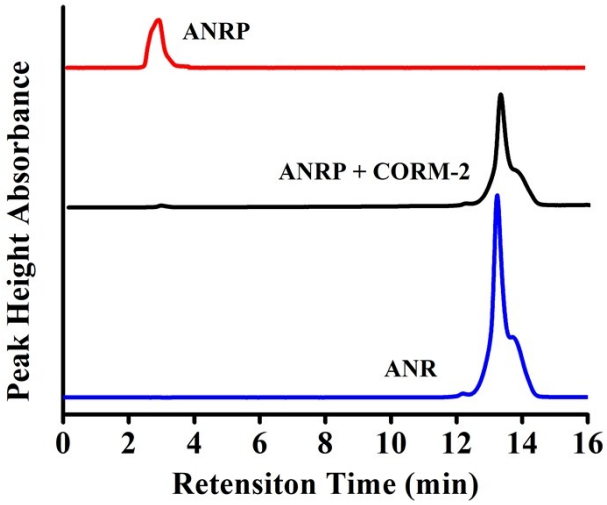


Fig. S4 HPLC traces of different systems.

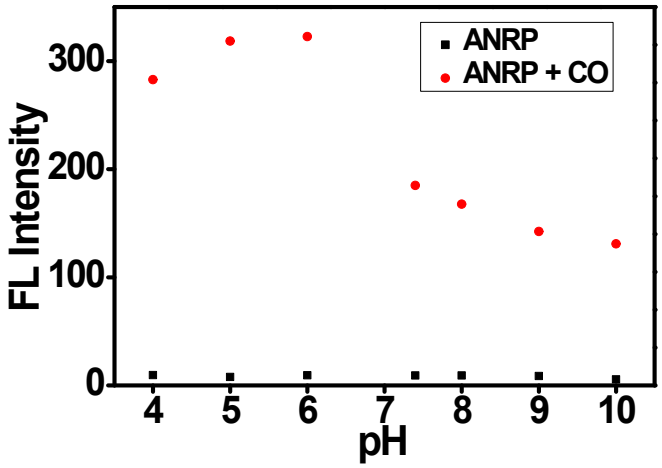


Fig. S5 The effects of pH on the reaction of **ANRP** to CO.

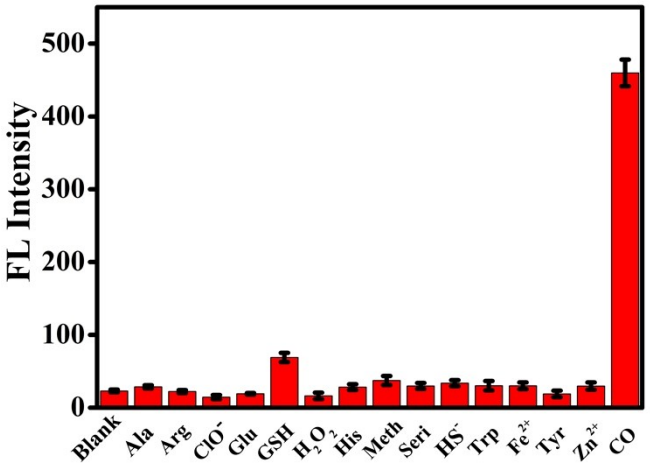


Fig. S6 Selectivity of **ANRP** (5 μM) towards 100 μM CO, 5 mM GSH, 200 μM other species.

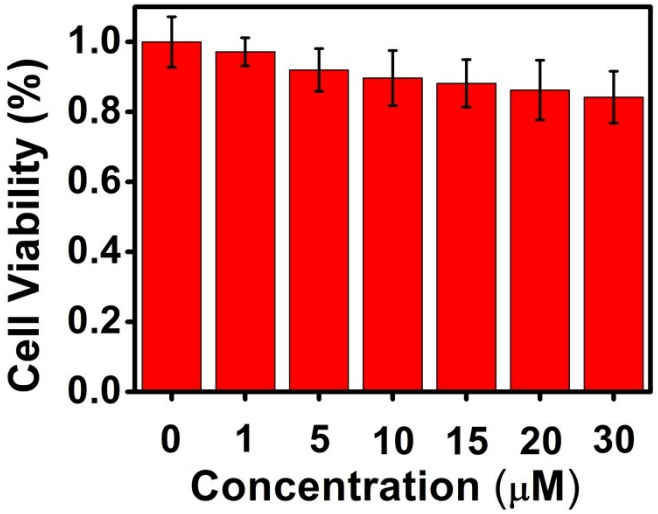


Fig. S7 Cell viability of HeLa cells treated with different concentrations of **ANRP** for 24 h

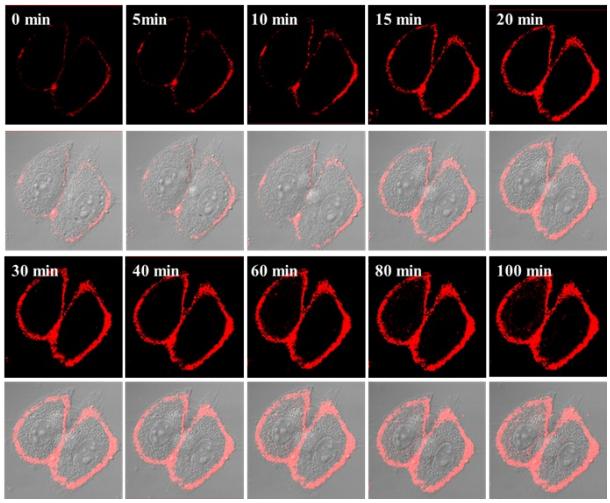


Fig. S8 Real-time imaging of HepG2 cells loaded with 5 μM **ANRP** upon treatment with 40 μM CORM-2. λ_{ex} = 543 nm, λ_{em} = 600-700 nm.

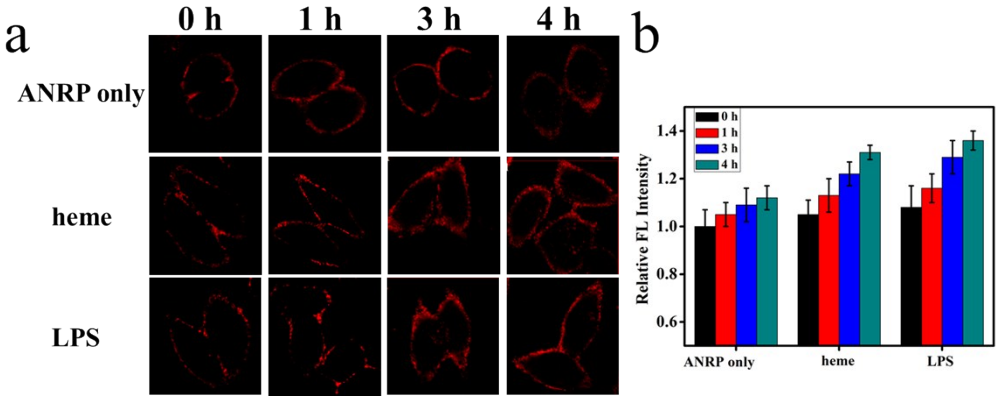


Fig. S9 Fluorescence images of **ANRP** (5 μM) in HepG2 cells under different conditions. Cells pretreated with **ANRP** (5 μM) for 30 min, then washed and treated with 20 μg/mL LPS, 30 μM heme for different time. (b) the relative fluorescence intensities on HepG2 cells membrane in panel (a). λ_{ex} = 543 nm, λ_{em} = 600-700 nm

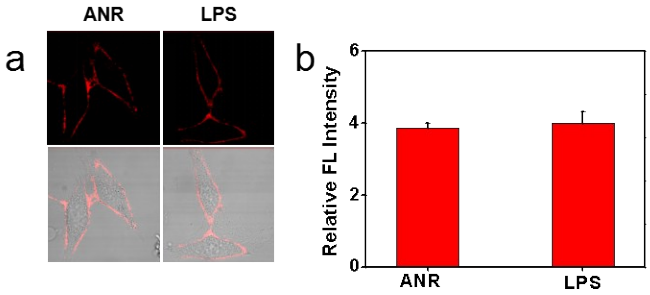


Fig. S10 Fluorescence images of HepG2 cells loaded with 5 μM **ANR**. Cells cultured with **ANR** only, or pretreated with LPS (1 μg/mL) for 24 h, then **ANR** at 37 °C for 30 min. λ_{ex} = 543 nm, λ_{em} = 600-700 nm

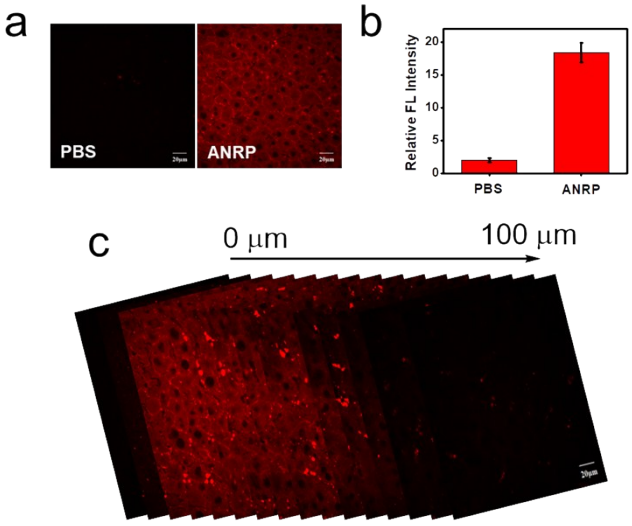


Fig. S11 Two-photon microscopy images of liver tissue at different depths after treatment with PBS or **ANRP** (50 μM) for 1 h.

References

1. J. Jose and K. Burgess, *J.Org. Chem.*, 2006, **71**, 7835-7839.

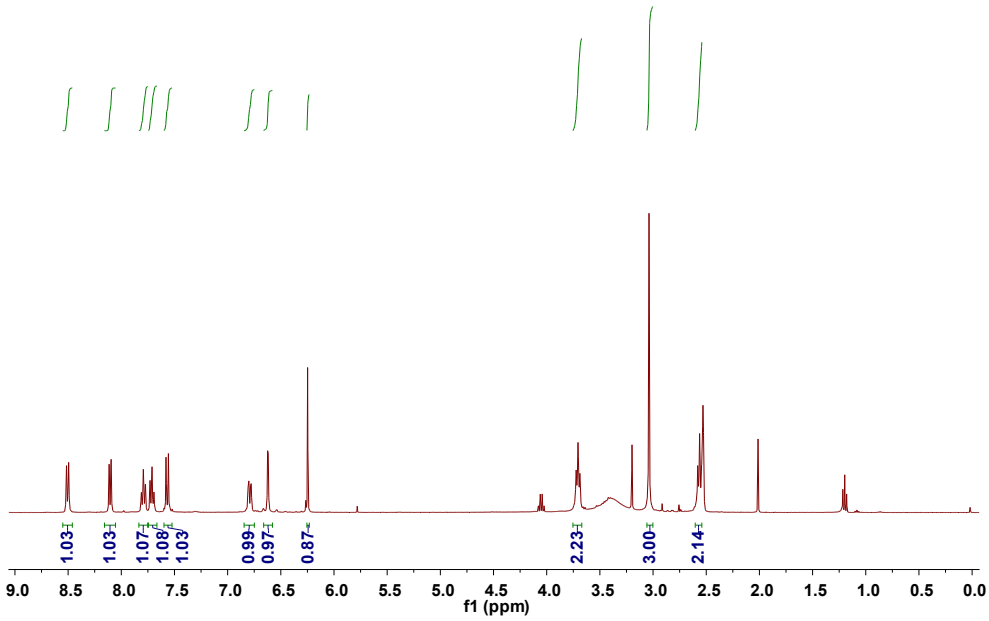


Fig. S12 ^{1}H NMR spectrum of compound **2**

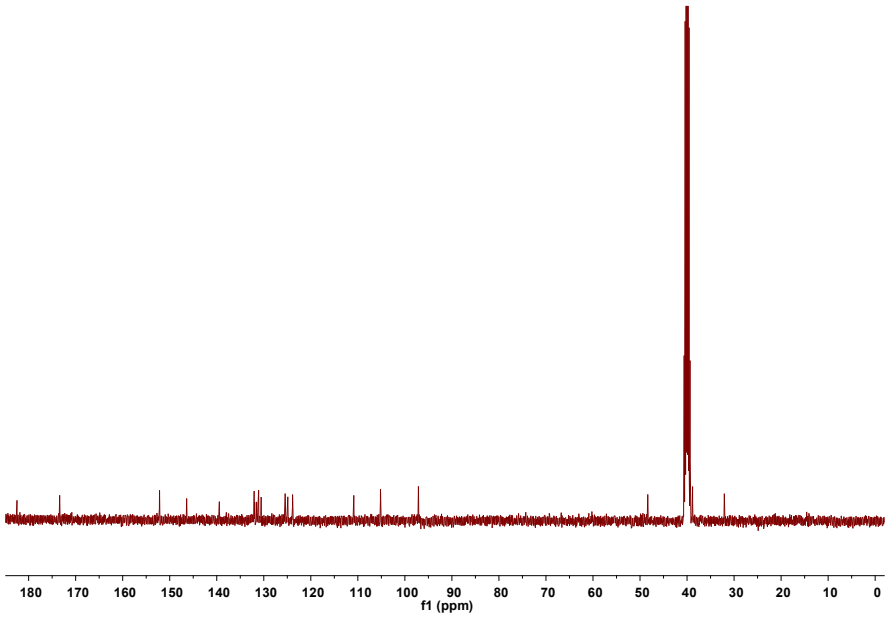


Fig. S13 ^{13}C NMR spectrum of compound **2**

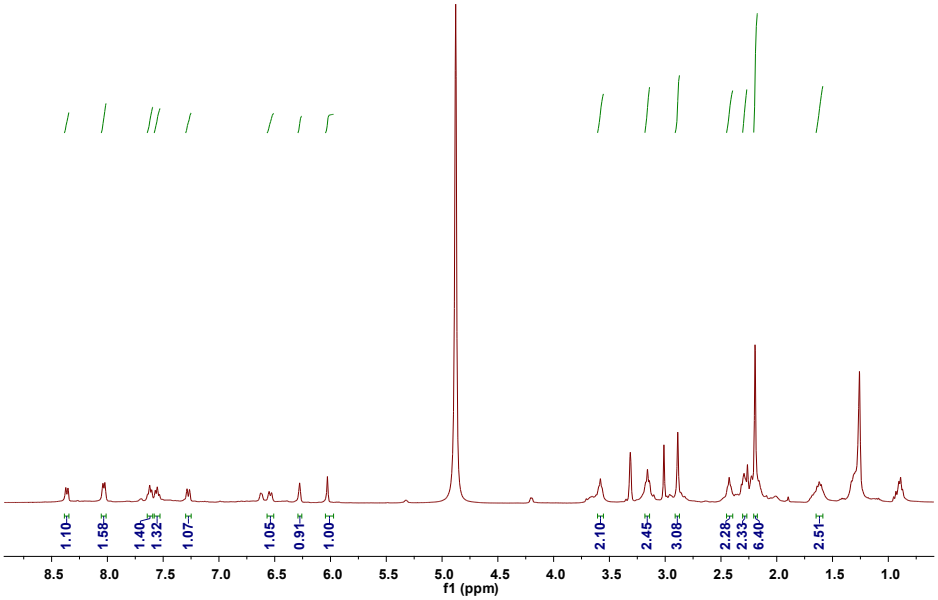


Fig. S14 ^{1}H NMR spectrum of compound **NR**

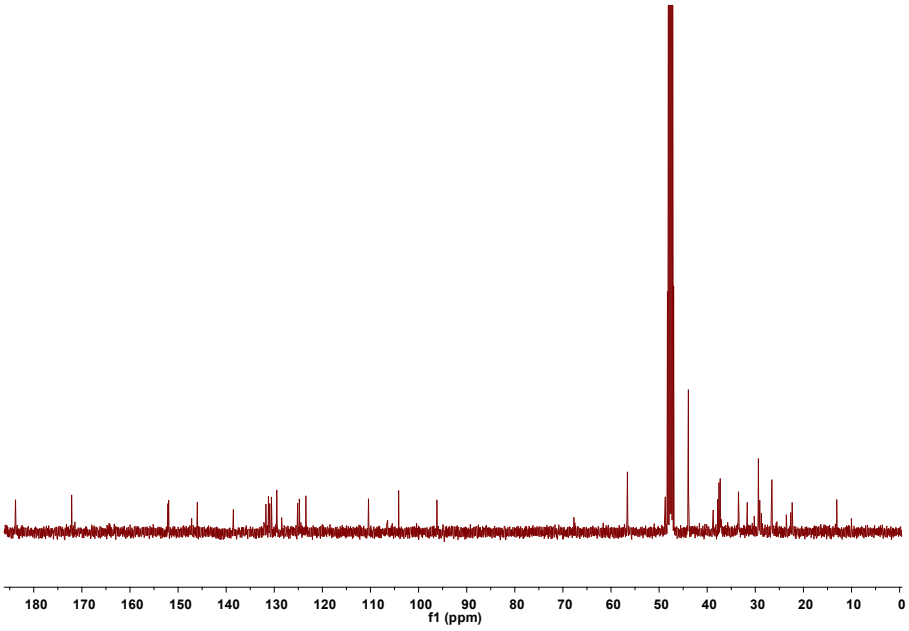


Fig. S15 ^{13}C NMR spectrum of compound **NR**

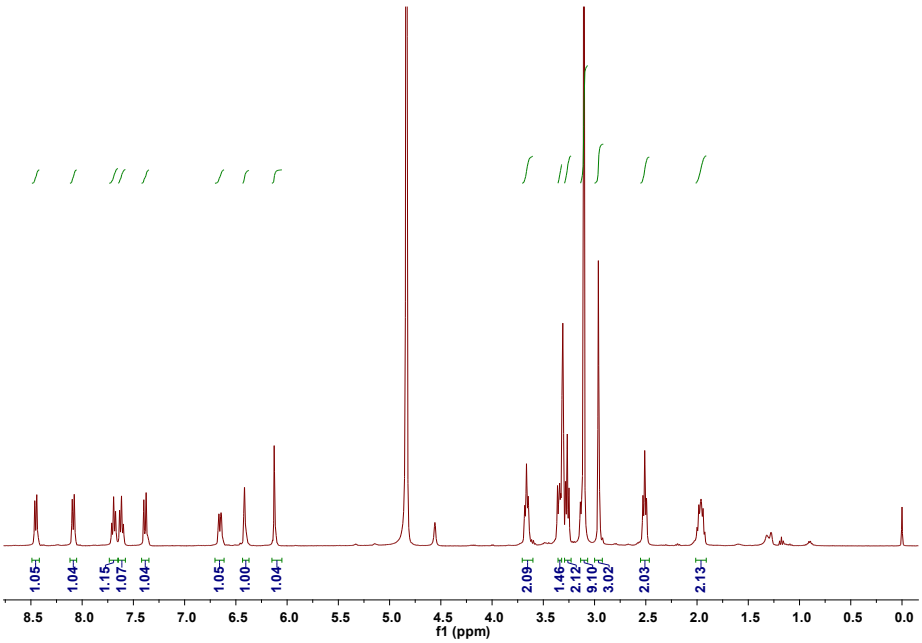


Fig. S16 ^{1}H NMR spectrum of compound **ANR**

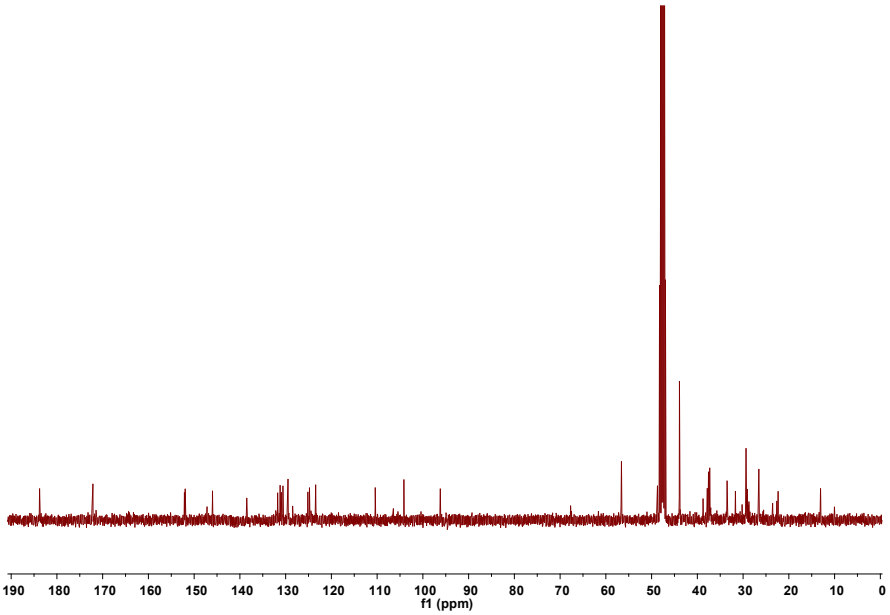


Fig. S17 ^{13}C NMR spectrum of compound **ANR**

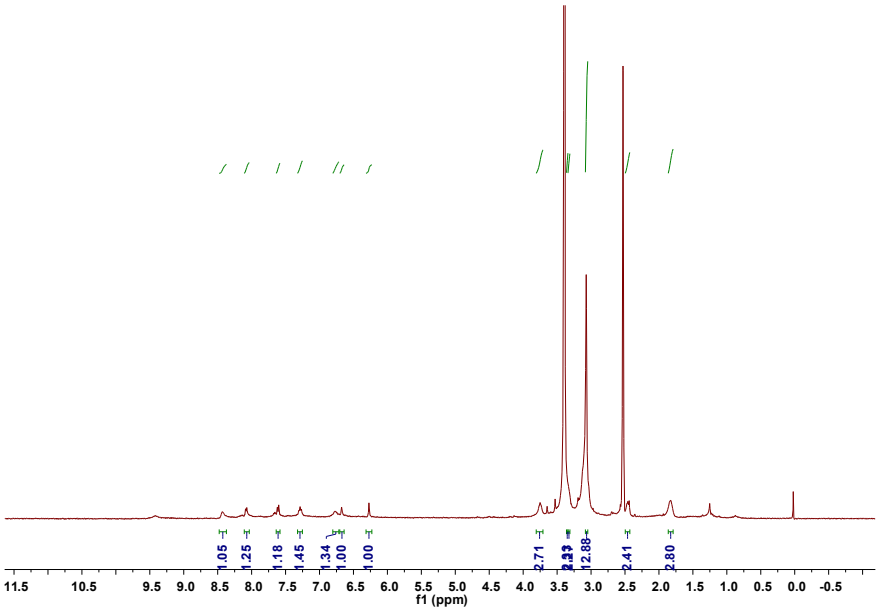


Fig. S18 ^{1}H NMR spectrum of compound **ANRP.**

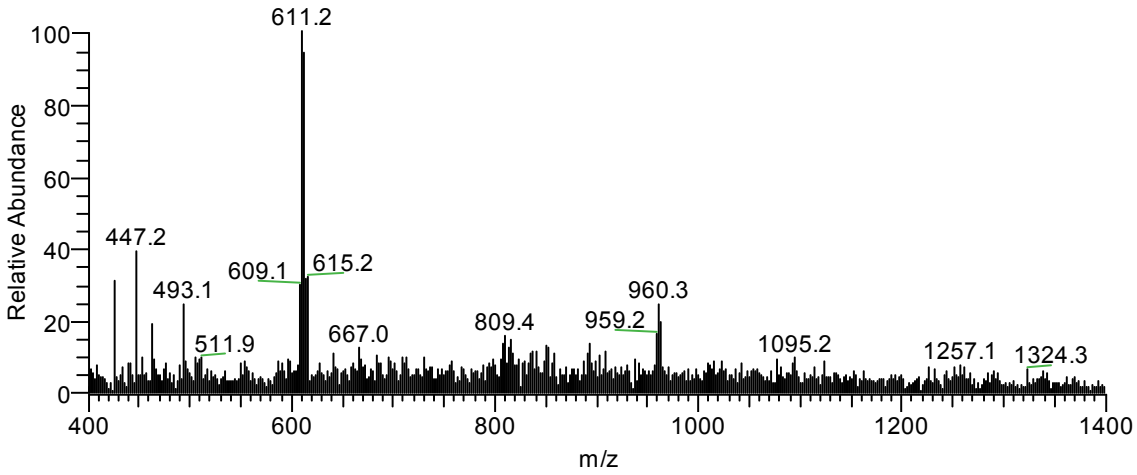


Fig. S19 ESI-MS spectrometric analysis of **ANRP.**

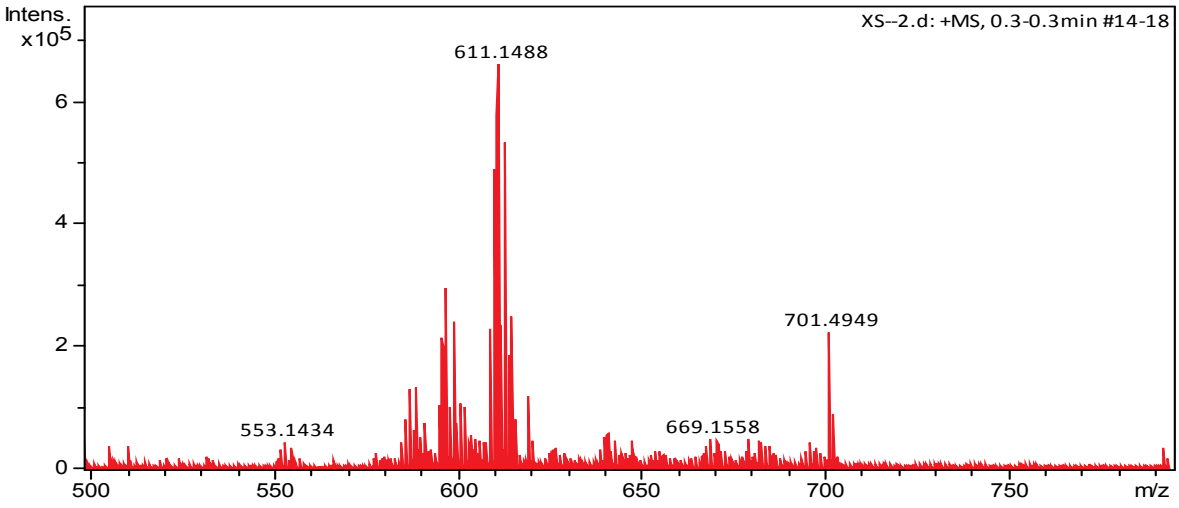


Fig. S20 HRMS data of **ANRP.**

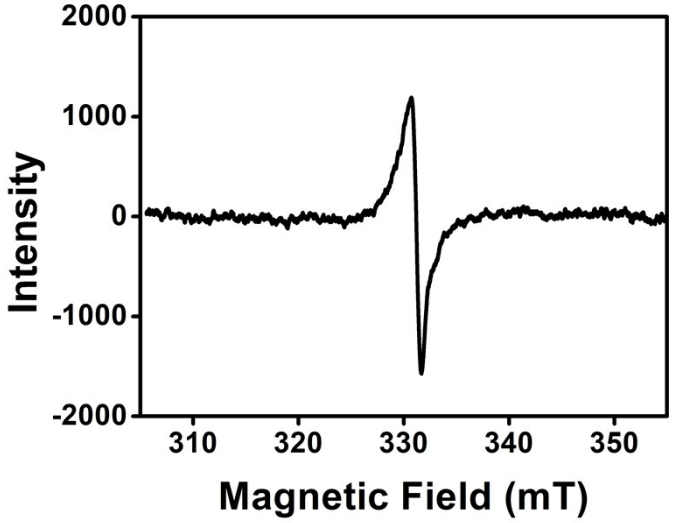


Fig. S21 ESR spectra of **ANRP**.

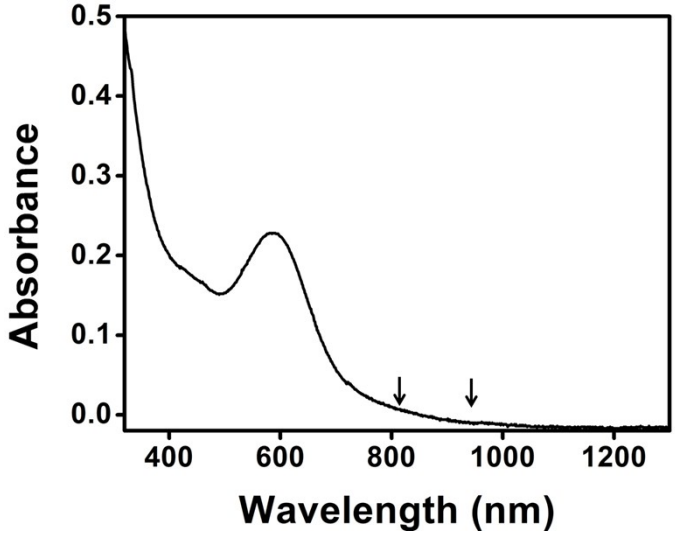


Fig. S22 UV-vis-NIR of **ANRP**.