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Supporting Information

Design, synthesis and application in biological imaging of a novel red fluorescent dye based on rhodanine derivative

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1. Experimental section

General information

All solvents and materials were used as received from commercial sources without further purification. ^{1}H NMR spectra was measured on a BRUKER AVANCE AV-500 spectrometer. Mass spectra (MS) was determined by Bruker autoflex matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF). Elemental analysis of carbon, hydrogen and nitrogen were performed on a Vario EL III microanalyzer. UV–Vis absorption spectra was recorded in film situation on a Shimadzu UV-2500 spectrophotometer. Photoluminescence (PL) spectra of the target compound was performed by a Hitachi F-4600 fluorescence spectrophotometer. The the time-resolved fluorescence and phosphorescence spectra at 77 K were tested through Hitachi F-4600 with 0.1 s delay. The transient lifetime in neat film were measured by the Edinburgh FLS-980 Instruments. The fluorescence quantum yield was measured with an integrating sphere.

Thermo analysis

Thermogravimetric analysis (TGA) was carried out using a NETZSCH STA 449C instrument. The thermal stability of the sample under the nitrogen atmosphere was measured by detecting their weight loss with the heating rate of 20 °C min^{-1} from 25 to 600 °C.

Electrochemical analysis

Cyclic voltammetry were performed on CHI voltammetric analyzer in the nitrogen purged dichloromethane (oxidation process) at a scan rate of 100 mV s^{-1} with a platinum plate as the working electrode, a silver wire as the pseudo-reference electrode, and a platinum wire as the auxiliary electrode. The supporting electrolyte was tetrabutylammonium hexafluorophosphate ($TBAPF_6$) (0.1 M) and ferrocenium-ferrocene (Fc^+/ Fc) was selected as the internal standard. The half-wave potential ($E_{1/2}$) value for Fc^+/Fc are calculated from the average of cyclic voltammetric anodic and cathodic peaks. According to the formula: [4.8 eV + (E_{onset} - $E_{1/2}$(Fc/Fc) $^+$)], the HOMO energy levels were calculated from the oxidation curves. And LUMO energy level was deduced from the energy band gap (E_g) from absorbance and HOMO level.

Theory calculations

All calculations were performed utilizing the Gaussian 09 program package. Geometry optimizations of 2RDNTPA was conducted in the framework of the density functional theory (DFT) at the B3LYP(Becke three parameters hybrid functional with Lee-Yang-Perdew correlation functional) level. The 6-31G(d,p) basis set was used for all the elements. The molecular orbitals were visualized using Gaussview.

Synthesis

Synthesis of 2-triphenylaminebenzene-1,3-dialdehyde (2AIDTPA)

A mixture of 2-bromobenzene-1,3-dialdehyde (0.11, 0.5 mmol), N,N-Diphenyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (0.17 g, 0.55 mmol), tetrakis(triphenylphosphine)palladium (0) (0.029 g, 0.025 mmol) and K_2CO_3 (0.34 g, 2.5 mmol) were dissolved in the solvent mixture of toluene (5 mL), methanol (1 mL) and water (2 mL) under nitrogen atmosphere. The mixture was refluxed at 110 °C for 24 h. After cooling to room temperature, the mixture was poured into water,

extracted with dichloromethane, and then purified by column chromatography over silica gel with CH_2Cl_2/petroleum ether (1:4) as the eluent to afford a yellow solid yielding 173 mg, 92%. ^{1}H NMR (400 MHz, $CDCl_3$): 9.95 (t, *J* = 1.4 Hz, 2H), 8.23 (dd, *J* = 7.7, 2.7 Hz, 2H), 7.62 (t, *J* = 7.7 Hz, 1H), 7.37-7.27 (m, 4H), 7.26-7.22 (m, 1H), 7.20-7.16 (m, 5H), 7.15-7.05 (m, 4H).

Synthesis of 2-triphenylamine-1,3-dia[(2-(3-ethyl-4-oxo-thiazolidin-2-ylidene) -malononitrile] (2RDNTPA)

A mixture of 2AIDTPA (0.19 g, 0.5 mmol), and 2-(3-ethyl-4-oxo-thiazolidin-2-ylidene)-malononitrile (0.22 g, 1.1 mmol), were dissolved in the solvent of 1.4-dioxane (5 mL) and triethylamine (1 mL) under nitrogen atmosphere. The mixture was refluxed at 110 °C for 8 h. After cooling to room temperature, the mixture was poured into water, extracted with dichloromethane, and then purified by column chromatography over silica gel with CH_2Cl_2/petroleum ether (4:1) as the eluent to afford a orange-red solid yielding 290 mg, 80%. ^{1}H NMR (400 MHz, $CDCl_3$): 7.77-7.63 （m, 5H）, 7.30 (d, *J* = 4.0 Hz, 2H), 7.21 (d, *J* = 3.3 Hz, 4H), 7.15-7.06 (m, 3H), 6.89 (d, *J* = 14.0 Hz, 2H), 4.29 (q, *J* = 7.1 Hz, 4H), 1.41 (t, *J* = 7.2 Hz, 6H). MS (MALDI-TOF) [m/z]: Calcd for $C_{42}H_{29}N_7O_2S_2$, 727.86; Found: 726.705. EA calcd for $C_{42}H_{29}N_7O_2S_2$: C 69.31, H 4.02, N 13.47, S 8.81%; found: C 69.26, H 3.88, N 13.48, S 8.70%.

Cytotoxicity assay

The cytotoxicity of 2RDNTPA to HepG2 liver cancer cells were determined via MTT assay (MTT, [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The HepG2 cells were cultured in 96-well plates in RPMI-1640 medium (containing 10% heat-inactivated fetal bovine serum) in 5% CO_2 atmosphere at 37°C. The minimum of just prepared dimethyl sulfoxide (DMSO) stock solutions of 2RDNTPA was added into the culture media in the designated wells. The final DMSO content was lower than 0.5%, and the final concentration of 2RDNTPA was 0~100 μM. Then the cells were incubated for 24 h at 37℃. A short 5 min 420 nm light irradiation was administrated after 1 hour of dark incubation. Then 20 μL MTT (5 mg mL–1 in PBS buffer) was added to each well and incubated for another 4 h. The medium was then carefully removed and DMSO (150 μL per well) was added. Subsequently, the dark blue crystals were solubilized in DMSO, and the absorbance of the solution was measured at 490 nm using a multifunction micro-plate reader (Molecular Devices, Flex Station 3). All the tests were carried out in triplicate. The cells without photoirradiation were also determined.

Confocal fluorescence imaging

Confocal imaging was carried out by laser scanning confocal microscope (FV1000, Olympus) using a 60 × oil-immersion objective. HepG2 cells were cultured in RPMI-1640 medium (containing 10% fetal bovine serum, FBS) in an atmosphere of 5% CO_2 and 95% air at 37°C for 24 h. Then the cells were seeded in glass-bottomed cell culture dishes at 40% confluence. The culture media containing 2RDNTPA were

prepared by adding the minimum of just prepared DMSO stock solution of 2RDNTPA into the RPMI-1640 medium, and the final 2RDNTPA concentration was 10 μM, and DMSO content was lower than 0.5%.

2. Figures

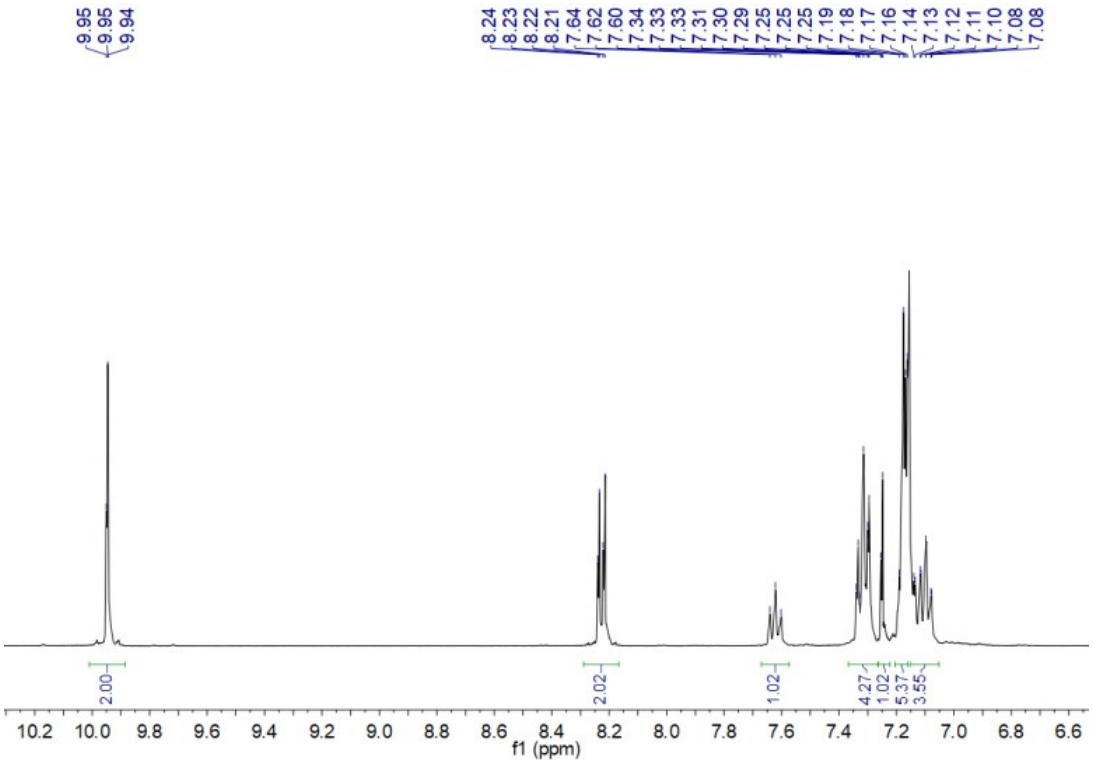

Fig. S1 ^{1}H NMR spectrum of 2AIDTPA

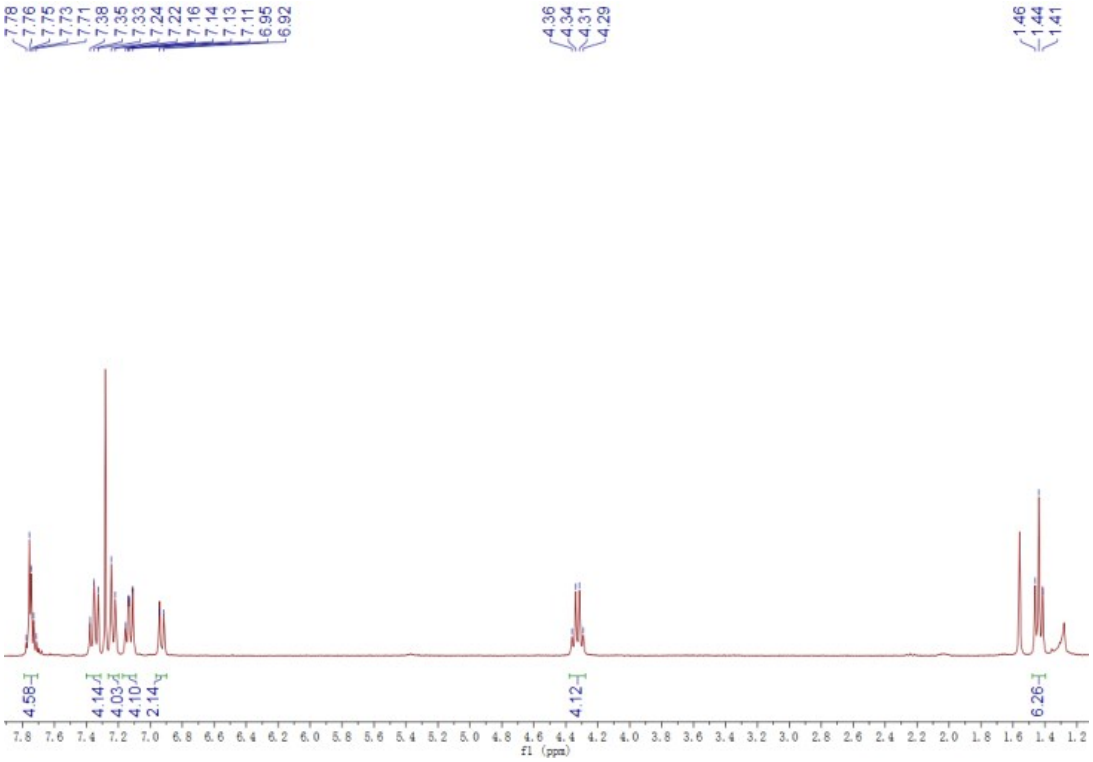

Fig. S2 ^{1}H NMR spectrum of 2RDNTPA

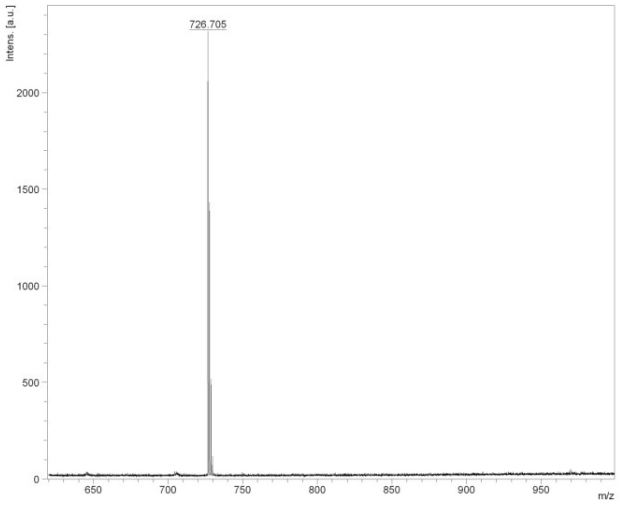

Fig. S3 MS spectrum of 2RDNTPA

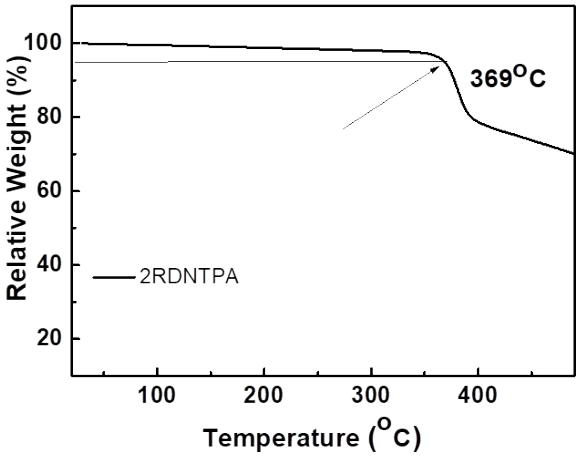

Fig. S4 TGA curves of 2RDNTPA.

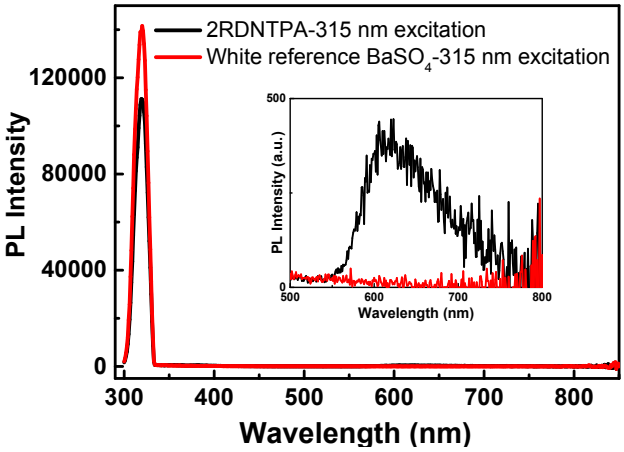

Fig. S5 Fluorescence quantum yield determination of 2RDNTPA.

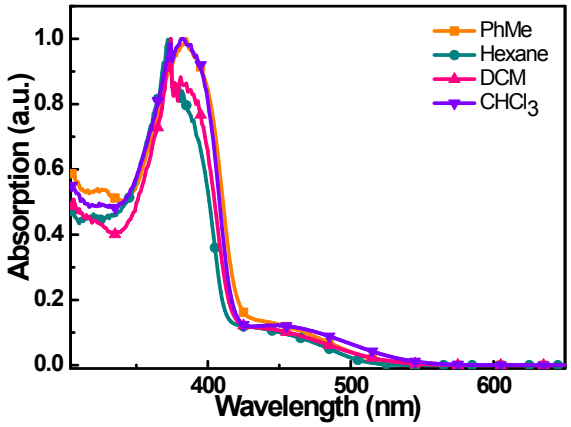

Fig. S6 UV-Vis spectra for 2RDNTPA in various solutions (10^{-5} M) with different solvent polarity.

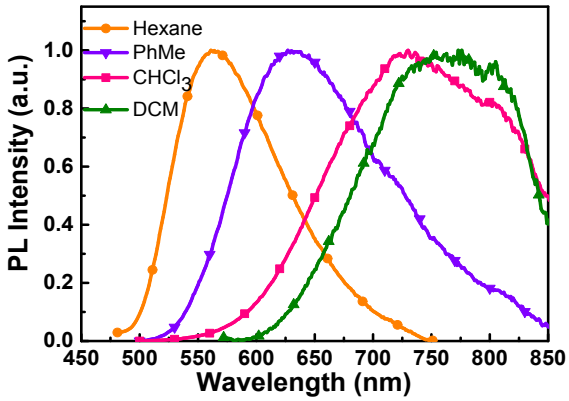

Fig. S7 PL spectra for 2RDNTPA in various solutions with different solvent polarity (excitation of 450 nm).

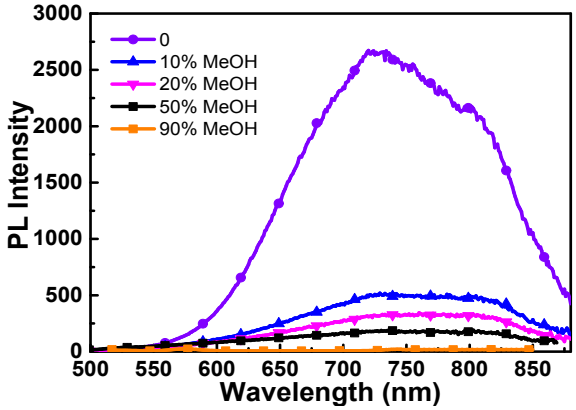

Fig. S8 PL spectra of 2RDNTPA in chloroform/methanol mixtures with different methanol fractions (f_w).

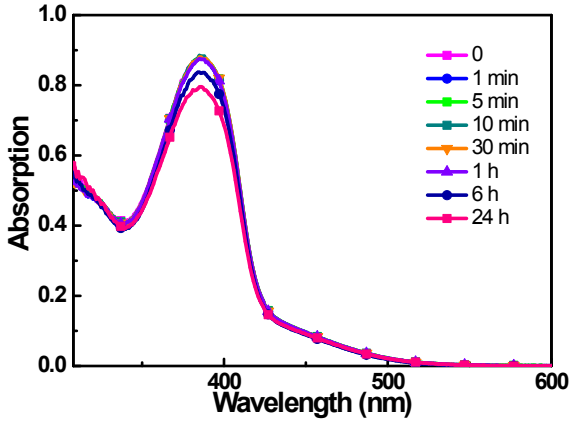

Fig. S9 UV–Vis absorption of 2RDNTPA in dimethyl sulfoxide (DMSO) (10^{-5} M) at room temperature after different times of exposure under 365 nm UV lamp.

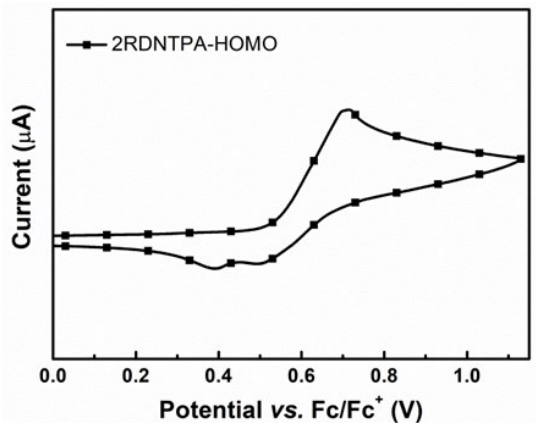

Fig. S10 Oxidation behavior of 2RDNTPA.

Table S1 Spectral properties of 4CzPTANMe in different solvents (10^{-5} M).

Solvent	λ_{abs} (nm)	λ_{em} (nm)
Hexane	381, 451	562
PhMe	382, 447	627
$CHCl_3$	382, 458	725
DCM	378, 446	759