Supplementary Information

Stress response decay with aging visualized using a dual-channel logic-based fluorescent probe

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1. Materials and general methods

Unless specifically stated, all chemical reagents were used of the highest available quality and were used directly without further purification or distillation. XL413 was purchased from MedChemExpress (USA). All solvents and inorganic salts were analytical grade unless otherwise noted. Distilled water was used for all experiments. Analytical thin-layer chromatography (TLC) used commercial silica gel plates (GF254). Flash column chromatography was performed using Merck silica gel (200-300 mesh). The composition of mixed solvents is given by the volume ratio (v/v). The $^1$H NMR and $^{13}$C NMR spectra were acquired on a Varian Unity INOVA-400 spectrometer, Bruker 400, Bruker 600 instruments and JEOL spectrometer operating at 400 MHz. High-resolution mass spectra (HRMS) were performed with a Bruker micro-TOF-QII mass spectrometer (Bruker Daltonics Corp, Germany) in electrospray ionization (ESI) mode. The single crystal structures were determined by X-ray crystallography with a Bruker SMART APEX-CCD system and a Bruker VEBTURE system. The pictures of solid powders were taken on Nikon SMZ18 solid microscope. Absolute fluorescence quantum yields were measured using the Absolute PL quantum yield spectrometer C9920-02G (Hamamatsu). The pH measurements were carried out on a Mettler Toledo pH meter. The refractive indices were measured using the abbe refractometer (INESA WYA, China). The fluorescence spectra were measured with a Hitachi F-2700 fluorescence spectrophotometer (Tokyo, Japan) with a 10 mm quartz cuvette. The UV-visible absorption spectra were determined by a MAPADA UV-1800 spectrophotometer with a 10 mm quartz cuvette. Fluorescence imaging studies of cells were observed using a confocal laser scanning microscope (Nikon A1plus, Japan) with excitation wavelength at 488 nm and 561 nm. Fluorescence imaging studies of C. elegans were recorded using a confocal laser scanning microscope (Leica TCS SP8) with excitation wavelength at 488 nm and 561 nm.

X-ray crystallography details

The single crystal of ROB was obtained by diffusion method and the crystal was purple and transparent. The single crystals of ROCL, ROCZ and ROE were grown from a mixture of ethanol and dichloromethane by volatilization method and the crystals were colourless and transparent. The single crystals of ROKS and ROZ were obtained by diffusion method and the crystals were yellow and transparent. Single-crystal X-ray experiments were performed using a Bruker Smart Apex CCD diffractometer equipped with a graphite-monochromatized Mo Kα radiation ($\lambda = 0.71073$ Å) using $\omega$ and $\varphi$ scan mode at room temperature and a Bruker VENTURE system with a Ga-target liquid METALJET D2 PLUS X-ray Source ($\lambda = 1.34139$ Å) at 174K. The data integration and reduction were processed with SAINT software. The structures were solved by the direct method using SHELXS and refined by a full-matrix least-
squares method on F2 with the SHELXL-2014 program.\textsuperscript{1,2} Hydrogen atoms were placed in geometrically calculated positions. A summary of the crystallographic data and the structure refinement parameters of ROB, ROCL, ROCZ, ROE, ROKS and ROZ are given in Table S2-S3, respectively. The crystallographic data for ROB, ROCL, ROCZ, ROE, ROKS and ROZ can be found in the CCDC with the numbers 2018837, 2018838, 2018841, 2018842, 2018843 and 2018844.

\textbf{Preparation of UV-Vis and fluorescence spectral measurements}

ROKS, ROCL, ROB, ROE, ROCZ and ROZ were dissolved in analytical grade DMF to obtain stock solutions (1 mM), respectively. The stock solution of thiophenol (10 mM) were prepared in analytical grade DMF. The stock solution of various ROS including HOCl, O$_2^\cdot$, NO$^\cdot$, TBHP, H$_2$O$_2$, ‘OH, $^1$O$_2$, TBO’ and ONOO$^\cdot$ were prepared according to previous reports.\textsuperscript{3,4} The stock solutions of various ions (1 mM) including Fe$^{3+}$, Al$^{3+}$, Zn$^{2+}$, Mg$^{2+}$, HSO$_3^-$, HSO$_4^-$, S$_2$O$_3^{2-}$, SCN$^-$, NO$_2^-$, NO$_3^-$, HCO$_3^-$, CO$_3^{2-}$, F$^-$, Cl$^-$, Br$^-$ and I$^-$ were prepared by direct dissolution of corresponding salt in distilled water. Small biological molecules (Cys, Hcy and GSH) were dissolved in distilled water to obtain stock solutions (1 mM).

\textbf{Measurement of relative fluorescence quantum yields}

The relative fluorescence quantum yield of compound ROB was determined in PBS buffer solution (10 mM, pH = 7.4, containing 10\% DMF) using Rhodamine B as a reference with a known $\Phi$ value of 0.89 in ethylene glycol excited at 535 nm.\textsuperscript{5} The relative fluorescence quantum yields of compounds ROCL, ROCZ and ROE were determined in PBS buffer solution (10 mM, pH = 7.4, containing 10\% DMF) using quinine sulfate as a reference with a known $\Phi$ value of 0.55 in 0.1 M H$_2$SO$_4$ excited at 365 nm.\textsuperscript{6} The relative fluorescence quantum yields of compounds were calculated following the equation (1):

\begin{equation}
\frac{\Phi_S}{\Phi_R} = \frac{(F_S/F_R)}{(A_R/A_S)} \times \left(\frac{\eta_S^2}{\eta_R^2}\right)
\end{equation}

Where S and R are the sample and the reference, respectively. $\Phi$ is the fluorescence quantum yield. $F$ is the relative integrated fluorescence emission area. $A$ is the absorbance at the excitation wavelength. $\eta$ is the refractive index of the solvent. The refractive indices of ethylene glycol, H$_2$SO$_4$ (0.1 M) and PBS buffer solution (10 mM, pH = 7.4, containing 10\% DMF) were measured respectively to be 1.4274, 1.3335 and 1.3463 by abbe refractometer (INESA WYA, China).

\textbf{Measurement of absolute fluorescence quantum yields}

The absolute fluorescence quantum yields of compounds in solid form were determined by the Absolute PL quantum yield spectrometer C9920-02G.
**Determination of detection limit**

The limit of detection (LOD) was determined from the fluorescence titration data. According to the results of the titrating experiment, a good linear relationship between the fluorescence intensity and the determinand concentration was obtained. The detection limit was calculated by means of equation (2):

$$\text{LOD} = \frac{3\sigma}{K}$$  \hspace{1cm} (2)

Where $\sigma$ is the standard deviation of the blank measurement and K is the slope of the calibration curve.

**Density functional theory calculations**

Gaussian 09 program at $\omega$B97X-D/6-31G(d,p) level of theory was employed to optimize the ground state (GS) of 1,3-dinitrobenzene and the triplet state of ROCL, and then the orbital energies were calculated. To mimic the d-PeT process, 1,3-dinitrobenzene was calculated using the GS where the LUMO orbital was occupied by an electron pair. However, ROCL was calculated using the triplet state in which each HOMO and LUMO were occupied by a single electron, respectively.

**Cell culture**

The HepG2 cells line were obtained from Procell Life Science & Technology Co., Ltd (Wuhan, China). The HepG2 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS). The HepG2 cells were seeded at a density of $1 \times 10^5$ cells per dish and cultured in a humidified atmosphere of 5% CO$_2$ and 95% air at 37 °C. For senescence induction, HepG2 cells were supplemented with media including XL413 (5 μM) for different times (3 days and 5 days). For cell fixation, HepG2 cells were added with 4% paraformaldehyde and incubated for 10 min.

**Cytotoxicity assay**

The cytotoxicity of ROKS to HepG2, MRC-5, HL-7702, Hela and A549 cells and ROCL to HepG2 cells was assessed by performing standard Cell Counting Kit-8 (CCK-8) assay. Cells ($5 \times 10^3$ cells/well) were seeded into 96-well plates in DMEM medium containing 10% FBS and cultured at 37 °C under 5% CO$_2$ for 24 h. Different concentrations of ROKS and ROCL (0, 10, 20, 30, 40 and 50 μM) were added to the wells. After incubation for 24 h, 100 μL CCK-8 solution (10% in serum free culture medium) was added to each well, and the plate was incubated for another 2 h. The absorbance of each well at 450 nm were measured by a microplate reader, and then the viability of cells was calculated using equation (3):

$$\text{cell viability} = \frac{(\text{OD}_{\text{positive}} - \text{OD}_{\text{control}})}{(\text{OD}_{\text{negative}} - \text{OD}_{\text{control}})}$$  \hspace{1cm} (3)
Western blot analysis
HepG2 cells were incubated with XL413 (5 μM) under 5% CO₂ in air and humidified atmosphere at 37 °C for 3 days. Then the XL413-incubated cells were washed with PBS three times, removed from plates and dipped in RIPA buffer that contain protease inhibitor. The concentration of proteins was determined using a BCA proteins assay kit. Protein samples were mixed with SDS-PAGE loading buffer and were heated for 10 min at 100 °C to fully denature the proteins. A 10% SDS-PAGE gel was used to separate the proteins, followed by transfer onto a PVDF membrane at 300 mA for 2 h to measure the expression level of the targeted proteins. 5 % skim milk in 0.1% tris buffered/tween 20 (TBST) were utilized to block membrane for one hour at room temperature and the membrane were incubated along with antibodies (γ-H2AX: Abcam, ab81299, 1/10000 dilution; GAPDH: Abclonal, ac033, 1/2000 dilution) at 4 °C for 12 h. Finally, the membrane was washed with TBST and further incubated with secondary antibodies (Anti-rabbit IgG antibody, CST, 7074P2; Goat anti-Mouse IgG antibody, Arigo, ARG65350) for 1 h and then chemiluminescence enhancement method was used to check protein’s signal. Tanon-4600SF image-capturing system was utilized to image the gels.

X-gal staining assay
X-gal staining was performed according to the X-gal staining kit protocols (Beyotime Biotechnology). The HepG2 cells pre-incubated with XL413 (5 μM) for 5 days were cultured at 37 °C and then fixed with 4% paraformaldehyde for 10 min. Subsequently, the HepG2 cells were treated with X-gal solution (1 mg/mL) and cultured at 37 °C overnight. After that, the HepG2 cells were washed by PBS buffer solution for three times and the change of blue color was observed under an inverted microscope.

C. elegans culture and imaging
C. elegans were grown and maintained on nematode growth medium (NGM) agar plates at 20 °C. They were fed Escherichia coli strain OP50 (Escherichia coli) and cultured under standard conditions until the worms reached the young adult, then picked the worms which were cultured for 6 days and 12 days to the next experiment. The C. elegans were incubated with ROKS (30 μM) in centrifuge tubes for 60min and treated with PhSH (3 μM) for different times (0, 15, 30, 45 and 60 min), then picked for imaging.
2. Spectroscopic properties

**Figure S1.** (A-B) Absorption (A) and fluorescence spectra (B) of ROKS, ROCL and ROB in PBS buffer solution (10 mM, pH = 7.4, containing 10% DMF). (C-D) Absorption (C) and fluorescence spectra (D) of ROZ, ROE and ROCZ in PBS buffer solution (10 mM, pH = 7.4, containing 10% DMF). $\lambda_{ex} = 350$ nm for ROKS, ROCL, ROZ, ROE and ROCZ; $\lambda_{ex} = 550$ nm for ROB, slit widths: 2.5 nm/5.0 nm.

**Figure S2.** (A) Fluorescence spectra of ROCL (10 µM) in a mixture of DMF and water with diverse water fractions ($f_w$). (B) Fluorescence spectra of ROCZ (10 µM) in a mixture of DMF and water with diverse water fractions ($f_w$). $\lambda_{ex} = 350$ nm, slit widths: 2.5 nm/5.0 nm.
Figure S3. Photostability profiles of ROKS (10 µM) (A-B), ROKS (10 µM) upon addition of PhSH (200 µM, 11 min) (C) and ROKS (10 µM) upon addition of PhSH (200 µM, 11 min) followed by HOCl (100 µM, 30 s) (D) in PBS buffer solution (10 mM, pH = 7.4, containing 10% DMF). Slit widths: 2.5 nm/5.0 nm.
Figure S4. Frontier orbital energy diagram. Illustration of the thermodynamic simulation of the fluorescence OFF/ON switch by the d-Pet process.

Table S1. The calculated HOMO-LUMO orbital energies of the 1,3-dinitrobenzene and ROCL.

| Compound                  | HOMO (eV)<sup>a</sup> | LUMO (eV)<sup>a</sup> |
|---------------------------|-----------------------|------------------------|
| 1.3-dinitrobenzene (GS)<sup>b</sup> | (-10.51)             | (-1.22)                |
| ROCL(trp)<sup>c</sup>     | (-5.10)               | (1.10)                 |

<sup>a</sup> Orbital energies (in eV) were calculated at the B3LYP/6-31G level of theory.

<sup>b</sup> “GS” denotes the compound calculated in ground state.

<sup>c</sup> “triplet” denotes the compound calculated in triplet state.
Figure S5. (A) Normalized emission spectra of ROCL and the reaction system of ROKS with PhSH in PBS buffer solution (10 mM, pH = 7.4, containing 10% DMF). (B) Normalized emission spectra of ROB and the reaction system of ROCL with HOCl in PBS buffer solution (10 mM, pH = 7.4, containing 10% DMF). λ_ex = 350 nm, slit widths: 2.5 nm/5.0 nm. (C) Normalized absorbance spectra of ROCL and the reaction system of ROKS with PhSH in PBS buffer solution (10 mM, pH = 7.4, containing 10% DMF). (D) Normalized absorbance spectra of ROB and the reaction system of ROCL with HOCl in PBS buffer solution (10 mM, pH = 7.4, containing 10% DMF).
Figure S6. (A) HRMS spectra of ROKS in methanol. (B) HRMS spectra of reaction product of ROKS with PhSH in methanol. (C) HRMS spectra of ROCL in methanol. (D) HRMS spectra of reaction product of ROCL with HOCl in methanol.
Figure S7. (A) Absorption spectra of ROKS (10 µM) upon addition of PhSH (0-200 µM) in PBS buffer solution (10 mM, pH = 7.4, containing 10% DMF). (B) Absorption spectra of ROCL (10 µM) upon addition of HOCl (0-100 µM) in PBS buffer solution (10 mM, pH = 7.4, containing 10% DMF).

Figure S8. (A) Time-course fluorescence spectra of ROKS (10 µM) in the presence of PhSH (200 µM) in PBS buffer solution (10 mM, pH = 7.4, containing 10% DMF). (B) Time-course fluorescence spectra of ROCL (10 µM) in the presence of HOCl (40 µM) in PBS buffer solution (10 mM, pH = 7.4, containing 10% DMF). λ_{ex} = 350 nm, slit widths: 2.5 nm/5.0 nm.

Figure S9. (A) Fluorescence spectra of ROKS (10 µM) in the absence (black) and presence (red) of PhSH (50 µM) at various pH values in PBS buffer solutions (10 mM, containing 10%
Figure S10. (A) Selectivity and competition response of ROKS (10 µM) to PhSH (100 µM) against GSH (1 mM) or other analytes (100 µM) in PBS buffer solution (10 mM, pH = 7.4, containing 10% DMF). λ_ex = 350 nm, slit widths: 2.5 nm/5.0 nm. (Blank, 2. Fe³⁺, 3. Al³⁺, 4. Zn²⁺, 5. Mg²⁺, 6. HSO₃⁻, 7. HSO₄⁻, 8. S₂O₃²⁻, 9. SCN⁻, 10. NO₂⁻, 11. NO₃⁻, 12. HCO₃⁻, 13. CO₃²⁻, 14. F⁻, 15. Cl⁻, 16. Br⁻, 17. I⁻, 18. Cys, 19. Hcy, 20. GSH, 21. H₂O₂, 22. •OH, 23. O₂⁻, 24. O₃, 25. NO', 26. TBO', 27. TBHP, 28. ONOO⁻, 29. HOCl, 30. PhSH).
3. Cytotoxicity studies

![Figure S11](image_url)

**Figure S11.** (A-E) Survival rate of HepG2 cells (A), MRC-5 cells (B), HL-7702 cells (C), Hela cells (D) and A549 cells (E) treated with different concentrations of ROKS for 24 h. (F) Survival rate of HepG2 cells treated with different concentrations of ROCL for 24 h. Cell survival rate was assayed by the CCK-8 method (values: mean ± standard deviation). Error bars are represented as the standard deviation (± S.D.) with n = 5.
4. Images of compounds

![Images of ROCZ, ROZ and ROE](image)

**Figure S12.** Images of ROCZ, ROZ and ROE in solid state under natural light and UV light of 365 nm, respectively.

5. Confocal imaging

![Confocal imaging](image)

**Figure S13.** (A) Representative confocal fluorescence images from HepG2 cells treated with ROCL (30 μM, 30 min) and then imaged at different times (0, 15, 30, 45, 60 and 90 min). (B) Average fluorescence intensity in the red channel from parallel images including (A). Scale bar: 25 μm. Red channel for HOCl: λ<sub>ex</sub> = 561 nm, λ<sub>em</sub> = 570–620 nm. Error bars are represented as the standard deviation (± S.D.) with n = 3. The number of dots represents that of samples. Significant differences (n.s., not significant) are analyzed with two-sided Student’s t-test.
Figure S14. (A) Representative confocal fluorescence images from HepG2 cells treated with ROCL (30 µM, 30 min) and then stimulated with zymosan (10 µg/mL) for different times (0, 5, 15, 30, 45 and 60 min). (B) Average fluorescence intensity in the red channel from parallel images including (A). Scale bar: 20 µm. Red channel for HOCl: λ_{ex} = 561 nm, λ_{em} = 570-620 nm. Error bars are represented as the standard deviation (± S.D.) with n = 3. The number of dots represents that of samples. Significant differences (*P < 0.05, **P < 0.01) are analyzed with two-sided Student’s t-test.

Figure S15. (A) Representative confocal fluorescence images from HepG2 cells treated with ROCZ (30 µM, 30 min) and then stimulated with LPS (10 µg/mL) for different times (0, 15, 30, 45, 60 and 120 min). (B) Average fluorescence intensity in the red channel from parallel images including (A). Scale bar: 25 µm. Red channel for HOCl: λ_{ex} = 561 nm, λ_{em} = 570–620 nm. Error bars are represented as the standard deviation (± S.D.) with n = 3. The number of dots represents that of samples. Significant differences (n.s., not significant) are analyzed with two-sided Student’s t-test.
Figure S16. (A) Representative confocal fluorescence images from fixed HepG2 cells stained with ROKS (30 µM, 10 min) (a) and incubated with PhSH (100 µM, 10 min) (b), and then treated with HOCl (100 µM, 10 min) (c). (B) Average fluorescence intensity in the green channel and the red channel from parallel images including (A). (C) Average fluorescence ratio \( \frac{I_{\text{Red}}}{I_{\text{Green}}} \) from parallel images including (A). Scale bar: 25 µm. Green channel for PhSH: \( \lambda_{\text{ex}} = 488 \text{ nm}, \lambda_{\text{em}} = 500-550 \text{ nm} \); red channel for HOCl: \( \lambda_{\text{ex}} = 561 \text{ nm}, \lambda_{\text{em}} = 570-620 \text{ nm} \). Error bars are represented as the standard deviation (± S.D.) with \( n = 3 \). The number of dots represents that of samples. Significant differences (n.s., not significant, **\( P < 0.01 \), ***\( P < 0.001 \)) are analyzed with two-sided Student’s t-test.

6. Synthetic methods

Synthesis of Compound 1

\[
\text{H}_2\text{N} \quad \text{OH} \quad + \quad \text{Cl} \quad \text{Br} \quad \overset{\text{Na}_2\text{CO}_3}{\text{DMF, 80°C}} \quad \overset{1}{\text{OH}}
\]

A mixture of \( m \)-aminophenol (2.18 g, 20 mmol), 1-Bromo-3-chloropropane (10 mL, 100 mmol) and anhydrous sodium carbonate (4.24 g, 40 mmol) was dissolved in \( N,N \)-dimethylformamide (15 mL). Under the protection of argon gas, the reaction mixture was
stirred at 80 °C for 15 h. After cooling, about 100 mL water was added and the resulting solution was extract with dichloromethane (100 mL × 3). The organic phase was dried with anhydrous MgSO₄ and evaporated under reduced pressure affording the crude product, which was further purified by chromatography using EA/PE (1:15, v/v) as eluent to afford compound 1 as a gray solid (2.62 g, 69%). Mp = 138-140 °C. ¹H NMR (400 MHz, CDCl₃) δ (ppm): 6.67 (d, J = 8.0 Hz, 1H), 6.06 (d, J = 8.0 Hz, 1H), 4.54 (s, 1H), 3.10 (q, J = 5.2 Hz, 4H), 2.72-2.65 (m, 4H), 2.03-1.93 (m, 4H); HRMS (ESI) m/z calcd for C₁₂H₁₆NO [M+H]^+: 190.1226, found: 190.1222.

**Synthesis of Compound 2**

Compound 1 (2.84 g, 15 mmol) and o-phthalic anhydride (2.96 g, 20 mmol) in toluene (30 mL) was refluxed for 24 h. The solvent was evaporated under reduced pressure, and the residue was purified by chromatography using MeOH/DCM (1:40, v/v), and obtained compound 2 as a light green solid (2.33 g, 46%). Mp = 188-190 °C. ¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 13.01 (s, 1H), 12.94 (s, 1H), 7.96-7.93 (m, 1H), 7.69-7.64 (m, 1H), 7.59 (q, J = 7.4 Hz, 1H), 7.35-7.32 (m, 1H), 6.40 (d, J = 5.3 Hz, 1H), 3.25-3.21 (m, 4H), 2.58 (t, J = 6.1 Hz, 2H), 2.40 (t, J = 6.1 Hz, 2H), 1.87-1.82 (m, 2H), 1.78-1.73 (m, 2H); HRMS (ESI) m/z calcd for C₂₀H₂₀N₄O₄ [M+H]^+: 338.1387, found: 338.1403.

**Synthesis of Compound 3**

A mixture of 2,4-dihydroxybenzaldehyde (1.38 g, 10 mmol), o-amino thiophenol (1.07 mL, 10 mmol) and sodium metabisulfite (1.90 g, 10 mmol) was dissolved in N,N-dimethylformamide (30 mL) and the reaction solution was heated under refluxed for 2 h with vigorous stirring. The reaction mixture was cooled to room temperature and then was added dropwise into water (100 mL). The precipitate was filtered, washed with water (10 mL × 3), and then dried to give compound 3 as a yellow solid (1.83 g, 61%). Mp = 205-206 °C. ¹H NMR (600 MHz, DMSO-d₆) δ (ppm): 11.68 (s, 1H), 10.19 (s, 1H), 8.07 (d, J = 7.8 Hz, 1H),
7.96 (d, J = 8.0 Hz, 1H), 7.92 (d, J = 9.1 Hz, 1H), 7.49 (t, J = 7.5 Hz, 1H), 7.38 (t, J = 7.4 Hz, 1H), 6.46 (s, 2H); HRMS (ESI) m/z calcd for C_{13}H_{10}NO_2S [M+H]^+: 244.0435, found: 244.0439.

Synthesis of Compound ROB

A mixture of 2 (0.34 g, 1 mmol) and 3 (0.37 g, 1.5 mmol) in methanesulfonic acid (5 mL) was heated to 90 °C for 24 h under argon protection. After cooling, the reaction solution was poured into ice water (100 mL). The precipitate was filtered, and then was washed with brine (10 mL × 3) and ethyl ether (10 mL × 3), and purified by chromatography using MeOH/DCM (1:30, v/v) to afford ROB as a purple solid (0.30 g, 56%). Mp > 250 °C. ¹H NMR (400 MHz, DMSO-d₆ + drops of CF₃COOD) δ (ppm): 8.30 (d, J = 7.7 Hz, 1H), 8.06 (d, J = 7.4 Hz, 1H), 7.95-7.90 (m, 2H), 7.87-7.81 (m, 2H), 7.61 (d, J = 7.3 Hz, 1H), 7.44-7.36 (m, 2H), 7.28 (s, 1H), 6.46 (s, 1H), 3.65-3.56 (m, 4H), 2.95 (s, 2H), 2.48 (s, 2H), 2.10-1.98 (m, 2H), 1.79-1.73 (m, 2H); HRMS (ESI) m/z calcd for C_{33}H_{25}N_2O_4S [M+H]^+: 545.1530, found: 545.1552; FT-IR (KBr, cm⁻¹): 3415.78, 2929.75, 2850.67, 1755.15, 1616.28, 1448.48, 1276.82, 1197.74, 1099.38, 854.43, 761.58, 713.63.

Synthesis of Compound ROCL

A solution of ROB (0.54 g, 1 mmol) in ethanol (30 mL) was added slowly hydrazine monohydrate (20 mL, 80%) and then the mixture was refluxed. After reaction for 5 h, the solvent was removed under reduced pressure. To the resulting residue was purified by chromatography using MeOH/DCM (1:200, v/v) to give ROCL as a gray solid (0.24 g, 42%). Mp > 250 °C. ¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 11.77 (s, 1H), 8.04 (d, J = 7.8 Hz, 1H), 7.93 (d, J = 8.1 Hz, 1H), 7.86-7.84 (m, 1H), 7.53-7.51 (m, 3H), 7.44 (t, J = 7.4 Hz, 1H), 7.36 (d, J = 7.5 Hz, 1H), 7.07-7.06 (m, 1H), 6.89 (s, 1H), 5.95 (s, 1H), 4.50 (s, 2H), 3.12-3.07
(m, 4H), 2.86 (t, \(J = 6.0\) Hz, 2H), 2.47-2.37 (m, 2H), 1.95 (s, 2H), 1.77 (s, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\): 168.41, 166.48, 159.15, 155.79, 151.68, 151.29, 148.29, 144.05, 133.03, 132.33, 129.71, 128.70, 127.85, 126.77, 125.57, 123.91, 123.81, 123.41, 122.02, 121.55, 118.37, 114.10, 111.62, 108.14, 104.92, 104.23, 65.83, 49.99, 49.53, 27.38, 21.90, 21.33, 21.25; HRMS (ESI) m/z calcd for C\(_{33}\)H\(_{27}\)N\(_{4}\)O\(_3\)S \([\text{M+H}]^+\): 559.1798, found: 559.1742; FT-IR (KBr, cm\(^{-1}\)): 3425.43, 2927.82, 2842.95, 1697.28, 1610.49, 1498.63, 1431.12, 1370.68, 1168.81, 935.44, 711.70.

**Synthesis of Compound ROKS**

![Reaction Scheme](attachment:image.png)

ROCL (0.56 g, 1 mmol), K\(_2\)CO\(_3\) (0.14 g, 1 mmol) and 1-fluoro-2, 4-dinitrobenzene (0.28 g, 1.5 mmol) were added to DMF (10 mL) and the mixture was stirred for 2 h at room temperature. After the reaction was complete, the resulting reaction mixture was poured into ice water. The precipitate was collected by filtration, and then purified by chromatography using MeOH/DCM (1:80, v/v) to give ROKS as a pale yellow solid (0.41 g, 57%). Mp > 250 °C. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) (ppm): 8.88 (d, \(J = 2.8\) Hz, 1H), 8.26 (dd, \(J = 2.6, 9.2\) Hz, 1H), 7.98-7.96 (m, 1H), 7.86 (d, \(J = 8.4\) Hz, 2H), 7.72 (d, \(J = 8.0\) Hz, 1H), 7.49-7.47 (m, 2H), 7.36 (t, \(J = 7.3\) Hz, 1H), 7.28-7.24 (m, 1H), 7.15-7.11 (m, 3H), 6.08 (s, 1H), 3.16-3.10 (m, 4H), 2.89 (t, \(J = 6.5\) Hz, 2H), 2.54-2.49 (m, 2H), 2.01-1.98 (m, 2H), 1.88-1.84 (m, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\): 167.08, 160.02, 155.07, 155.03, 152.55, 151.47, 151.14, 147.76, 143.91, 142.39, 139.96, 135.45, 135.37, 133.27, 130.75, 129.28, 129.19, 129.00, 126.56, 125.57, 123.79, 123.70, 123.41, 122.35, 122.00, 121.42, 119.43, 119.17, 119.13, 118.90, 110.09, 65.97, 65.57, 50.04, 49.54, 27.39, 21.76, 21.24, 15.40; HRMS (ESI) m/z calcd for C\(_{39}\)H\(_{28}\)Na\(_2\)N\(_6\)O\(_7\)S \([\text{M+Na}]^+\): 747.1632, found: 747.1635; FT-IR (KBr, cm\(^{-1}\)): 3423.50, 3107.19, 3045.47, 2933.60, 2839.09, 2356.91, 1701.14, 1608.56, 1533.34, 1454.26, 1348.18, 1307.68, 1263.32, 1161.10, 916.15, 730.99.

**Synthesis of Compound ROE**
Under an argon atmosphere, ROCL (0.28 g, 0.5 mmol), K₂CO₃ (0.07 g, 0.5 mmol) and Iodoethane (44 μL, 0.55 mmol) were mixed in acetonitrile (10 mL) and the solution was refluxed for 16 h. The reaction solvent was evaporated off under reduced pressure to afford pale yellow, which was purified by chromatography using MeOH/DCM (1:100, v/v) to obtain ROE as a white solid (0.29 g, 75%). Mp > 250 °C. ¹H NMR (400 MHz, CDCl₃) δ (ppm): 8.00 (d, J = 8.2 Hz, 1H), 7.96 (d, J = 8.1 Hz, 1H), 7.91 (s, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.47-7.39 (m, 3H), 7.30 (t, J = 8.1 Hz, 1H), 7.08 (d, J = 6.3 Hz, 1H), 6.87 (s, 1H), 6.06 (s, 1H), 4.33 (q, J = 7.2 Hz, 2H), 3.85 (s, 2H), 3.16 (t, J = 5.5 Hz, 2H), 3.11 (t, J = 5.5 Hz, 2H), 2.96 (t, J = 6.6 Hz, 2H), 2.59-2.45 (m, 2H), 2.07-2.01 (m, 2H), 1.90-1.84 (m, 2H), 1.66 (t, J = 6.9 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ: 167.01, 162.02, 157.38, 155.03, 151.97, 151.72, 148.07, 143.92, 135.85, 132.94, 129.39, 128.93, 128.50, 125.87, 124.43, 123.70, 123.66, 123.48, 122.77, 121.06, 118.49, 118.31, 111.87, 107.92, 104.77, 100.57, 65.86, 65.40, 49.95, 49.45, 27.35, 21.89, 21.36, 21.33, 14.88; HRMS (ESI) m/z calcd for C₃₁H₂₁N₄O₃S [M+H]⁺: 587.2111, found: 587.2097; FT-IR (KBr, cm⁻¹): 3440.80, 2937.40, 1687.61, 1610.46, 1421.45, 1307.65, 1184.22, 761.83.  

Synthesis of Compound ROCZ

ROCL (1.28 g, 2.3 mmol) were refluxed in acetone (15 mL) for 5 h. The reaction solvent was removed under vacuum, and the residue was then purified by chromatography using MeOH/DCM (1:100, v/v) to give ROCZ as a white solid (1.10 g, 80%). Mp > 250 °C. ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.98-7.96 (m, 1H), 7.89 (d, J = 4.8 Hz, 1H), 7.76 (d, J = 7.9 Hz, 1H), 7.53-7.49 (m, 2H), 7.46-7.42 (m, 1H), 7.33 (t, J = 7.6 Hz, 1H), 7.14 (d, J = 6.6 Hz, 1H), 7.04 (s, 1H), 6.87 (s, 1H), 6.16 (s, 1H), 3.17-3.10 (m, 4H), 2.93 (t, J = 6.7 Hz, 2H), 2.57-2.47 (m, 2H), 2.05-2.02 (m, 2H), 1.98 (s, 3H), 1.88 (s, 5H); ¹³C NMR (100 MHz, CDCl₃) δ: 174.30, 168.65, 160.92, 158.78, 155.71, 151.75, 151.48, 148.00, 143.73, 132.62,
132.25, 130.30, 128.59, 128.24, 126.45, 124.45, 123.74, 123.36, 121.95, 121.47, 117.86, 113.63, 112.98, 107.84, 105.95, 104.57, 66.03, 49.97, 49.51, 27.36, 25.64, 21.97, 21.82, 21.41, 21.21; HRMS (ESI) m/z calcd for C_{36}H_{31}N_{4}O_{3}S [M+H]^+: 599.2111, found: 599.2101; FT-IR (KBr, cm^{-1}): 3436.94, 2929.69, 1703.04, 1623.96, 1494.74, 1444.59, 1309.58, 1178.43, 756.05.

**Synthesis of Compound ROZ**

ROZ (0.39 g, 0.65 mmol), K_{2}CO_{3} (0.14 g, 1 mmol) and 2,4-dinitrofluorobenzene (84 µL, 0.72 mmol) were added to DMF (10 mL) and the mixture was stirred for 2 h at room temperature. After the reaction was complete, the resulting reaction mixture was poured into ice water. The precipitate was collected by filtration and then purified by chromatography using MeOH/DCM (1:100, v/v) to give ROZ as a pale yellow solid (0.27 g, 47%). Mp > 250 °C. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) (ppm): 8.92 (s, 1H), 8.30 (d, \(J = 6.4\) Hz, 1H), 7.99 (d, \(J = 8.6\) Hz, 1H), 7.87 (d, \(J = 11.1\) Hz, 2H), 7.74 (d, \(J = 7.8\) Hz, 1H), 7.54-7.51 (m, 2H), 7.40 (t, \(J = 7.7\) Hz, 1H), 7.29 (t, \(J = 7.1\) Hz, 1H), 7.16 (d, \(J = 5.4\) Hz, 1H), 7.09 (t, \(J = 8.6\) Hz, 2H), 6.21 (s, 1H), 3.17-3.12 (m, 4H), 2.89 (t, \(J = 6.4\) Hz, 2H), 2.6-2.48 (m, 2H), 2.02 (s, 5H), 1.96 (s, 3H), 1.90-1.85 (m, 2H); HRMS (ESI) m/z calcd for C_{42}H_{33}N_{6}O_{7}S [M+H]^+: 765.2126, found: 765.2101; FT-IR (KBr, cm^{-1}): 3448.51, 2923.90, 1701.11, 1614.32, 1535.24, 1446.52, 1305.72, 1168.79, 730.97.

7. Characterization of compounds

**Table S2. Crystal data and structure refinement summary for ROKS, ROCL and ROB.**

|         | ROKS          | ROCL          | ROB           |
|---------|---------------|---------------|---------------|
| Empirical formula | C_{36}H_{31}N_{4}O_{3}S | C_{33}H_{26}N_{4}O_{3}S | C_{33}H_{24}N_{2}O_{2}S |
| Formula weight | 724.75 | 558.64 | 544.60 |
| Temperature/K | 296 | 296 | 296 |
| Crystal system | monoclinic | triclinic | triclinic |
| Space group | P 1 21/c 1 | P-1 | P-1 |
| \(a/\text{Å}\) | 17.669(5) | 10.077(2) | 7.8981(18) |
|        | ROE          | ROCZ         | ROZ          |
|--------|--------------|--------------|--------------|
| **b/Å** | 9.336(3)     | 10.691(2)    | 10.981(2)    |
| **c/Å** | 23.887(7)    | 13.476(3)    | 15.616(4)    |
| **α/°** | 90           | 101.301(3)   | 72.196(4)    |
| **β/°** | 102.697(6)   | 96.718(3)    | 88.661(4)    |
| **γ/°** | 90           | 105.176(3)   | 80.402(4)    |
| Volume/Å³ | 3844.3(19)   | 1352.1(4)    | 1270.9(5)    |
| **Z**  | 4            | 2            | 2            |
| ρcalc g/cm³ | 1.380        | 1.372        | 1.423        |
| µ/mm⁻¹ | 0.149        | 0.163        | 0.172        |
| F(000) | 1672.0       | 584          | 568          |
| hmin,max | -20, 21      | -12, 9       | -9, 9        |
| kmin,max | -11, 11      | -12, 11      | -12, 13      |
|  lmin,max | -18, 29      | -16, 16      | -19, 17      |
| No. of Reflns. | 20245/7633 | 7038/4942    | 6981/4981    |
| R₁ [I > 2σ(I)] | 0.0740      | 0.0567       | 0.0529       |
| wR₂ (all data) | 0.2298      | 0.1683       | 0.1375       |
| CCDC   | 2018843      | 2018838      | 2018837      |

**Table S3.** Crystal data and structure refinement summary for ROE, ROCZ and ROZ.
|        |       |       |       |
|--------|-------|-------|-------|
| \(\alpha^\circ\) | 90    | 90    | 90    |
| \(\beta^\circ\)   | 117.634(9)° | 94.57(2)° | 101.185(9)° |
| \(\gamma^\circ\)  | 90    | 90    | 90    |
| Volume/Å³       | 6482(4) | 6508(10) | 3693.0(13) |
| \(Z\)           | 8     | 8     | 4     |
| \(\rho_{\text{calc}}\) g/cm³ | 1.235 | 1.309 | 1.376 |
| \(\mu/\text{mm}^{-1}\) | 0.143 | 0.224 | 0.837 |
| \(F(000)\)      | 2528.0 | 2680.0 | 1592.0 |
| \(h_{\text{min-max}}\)  | -27, 27 | -21, 38 | -19, 21 |
| \(k_{\text{min-max}}\)  | -21, 23 | -14, 14 | -11, 11 |
| \(l_{\text{min-max}}\)  | -19, 16 | -19, 17 | -28, 28 |
| No. of Reflns.  | 15991/5714 | 14140/5725 | 31566/6969 |
| R_{\text{int}} | 0.1629 | 0.1635 | 0.0547 |
| GOF on \(F^2\)  | 0.903  | 0.957  | 1.049  |
| R_{\text{I}}^{[\text{a}]}[I > 2\sigma(I)] | 0.0975 | 0.0994 | 0.0759 |
| wR_{\text{2}}^{[\text{b}]} (all data) | 0.3382 | 0.2962 | 0.2599 |
| CCDC           | 2018842 | 2018841 | 2018844 |
Figure S17. $^1$H NMR spectrum of compound 1 in CDCl$_3$.

Figure S18. HRMS spectrum of compound 1.
Figure S19. $^1$H NMR spectrum of compound 2 in DMSO-$d_6$.

Figure S20. HRMS spectrum of compound 2.
Figure S21. $^1$H NMR spectrum of compound 3 in DMSO-$d_6$.

Figure S22. HRMS spectrum of compound 3.
Figure S23. $^1$H NMR spectrum of compound ROB in DMSO-$d_6$ + drops of CF$_3$COOD.

Figure S24. HRMS spectrum of compound ROB.
Figure S25. $^1$H NMR spectrum of compound ROCL in DMSO-$_d_6$.

Figure S26. $^{13}$C NMR spectrum of compound ROCL in CDCl$_3$. 
Figure S27. HRMS spectrum of compound ROCL.

Figure S28. ¹H NMR spectrum of compound ROKS in CDCl₃.
Figure S29. $^{13}$C NMR spectrum of compound ROKS in CDCl$_3$.

Figure S30. HRMS spectrum of compound ROKS.
Figure S31. $^1\text{H}$ NMR spectrum of compound ROE in CDCl$_3$.

Figure S32. $^{13}\text{C}$ NMR spectrum of compound ROE in CDCl$_3$.  

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Figure S33. HRMS spectrum of compound ROE.

Figure S34. $^1$H NMR spectrum of compound ROCZ in CDCl$_3$. 
Figure S35. $^{13}$C NMR spectrum of compound ROCZ in CDCl$_3$.

Figure S36. HRMS spectrum of compound ROCZ.
Figure S37. $^1$H NMR spectrum of compound ROZ in CDCl$_3$.

Figure S38. HRMS spectrum of compound ROZ.

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