Supporting Information

Aggregation-Induced Emission Probe for Light-Up and In Situ Detection of Calcium Ions at High Concentration

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Materials and chemicals

Diethyl iminodiacetate was purchased from Meryer; Hydrazine monohydrate was purchased from Acros; Sodium hydroxide was purchased from Tianjin FuChen; THF was distilled from sodium under dry nitrogen prior to use. All other chemicals and reagents were purchased from commercial sources and used as received without further purification. Ultra pure water was supplied by Milli-Q Plus System (Millipore Corporation, United States). Dulbecco's Modified Essential Medium (DMEM) was purchased from Gibco (Life Technologies). Phosphate buffered saline (PBS), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Thermo Fisher Scientific. Cell Counting Kit-8 (CCK8) was purchased from Dojindo, Japan. The normal human serum was obtained from Department of Laboratory Medicine, Nanfang Hospital.

Equipment and methods

UV-Vis absorption spectra were measured on a Shimadzu UV-2600 spectrophotometer, medium scanning rate, and quartz cuvettes of 1 cm path length. Photoluminescence spectra were recorded on a Fluorescence Spectrophotometer (Hitachi F-7000). The PL changes upon treatment with metal ions and biomolecules was measured on a microplate reader (TECAN, infiniteM200PRO). ^{1}H and ^{13}C NMR spectra were measured on a Bruker AV 500 NMR spectrometer. High resolution mass spectra (HRMS) were recorded on a GCT Premier CAB 048 mass spectrometer operated in MALDI-TOF model. The isothermal titration calorimetry experiments were conducted on VP-ITC (MICROCAL Instruments, USA). The absolute fluorescence quantum yield was measured using a Hamamatsu quantum yield spectrometer C11347 Quantaurus_QY. The fluorescence lifetime was measured using a Hamamatsu Compact Fluorescence Lifetime Spectrometer C11367. Confocal lasing scanning microscopic (CLSM) images were obtained on the confocal microscope (Leica TCS SP8 and Zeiss 710). The Alizarin Red S staining was conducted according to the literature method[1] and the image was obtained by inverted fluorescence microscope (Carl ZESS Microscopy GmbH). The SEM and TEM experiments were respectively conducted by using a scanning electron microscope (FEI Q25) and a transmission electron microscope (JEM-1400 plus).

Fluorescent detection of calcium ions in solution

The probe SA-4CO_2Na (10 μL, 10 mM) was added into the 90 μL PBS buffer solutions of Ca^{2+} at determined concentrations. After standing at room temperature for 0.5 h, the PL spectra was measured on the microplate reader (TECAN).

For detection of Ca^{2+} in human serum, the normal and hypercalcemic samples (1.4, 2.0, 2.5, 3.0 mM) were prepared by addition of Ca^{2+} ions into the normal human serum and the free Ca^{2+} concentrations were determined by a commercial blood gas analyzer (ABL800 FLEX blood gas analyzer). The probe of SA-4CO_2Na (10 μL, 10 mM) was then added into the samples and the PL spectra were then measured on the microplate reader (TECAN).

Isothermal titration

The aqueous solutions of SA-4CO_2Na (0.35 mM) and $CaCl_2$ (3.5 mM) were first degassed before use. The $CaCl_2$ solution was then added into SA-4CO_2Na solution with a series of injections (26 aliquots of 10 μL) at 200 s intervals with a constant stirring rate at 300 rpm under 25 ^{o}C. After completion of the experiment, the results were analyzed with the origin software (MICROCAL).

Cell culture

Mouse bone-derived mesenchymal stem cells (mouse BMSCs) were purchased from ATCC (CRL-12424) and cultured in DMEM with 1% penicillin-streptomycin and 10% FBS at 37 ℃ in a humidified incubator with 5% CO_2.

Cytotoxicity of SA-4CO_2Na

The cell viability was evaluated by Cell Counting Kit-8 (CCK-8) assay according to the manufacturer's method. Mouse BMSCs were seeded into 96-well plates at a density of 5×10^3 cells per well and incubated for 24 h. The cells were then rinsed with PBS for twice and further treated with DMEM solution containing SA-4CO_2Na at different concentrations (0, 100, 200, 300, 400, 500 μM) for 24 h. After rinsing with PBS twice, each well was added with 100 μL of CCK-8 working solution. The plate was incubated at 37 ℃ for 2 h and then the absorption was measured at 450 nm by using a microplate reader.

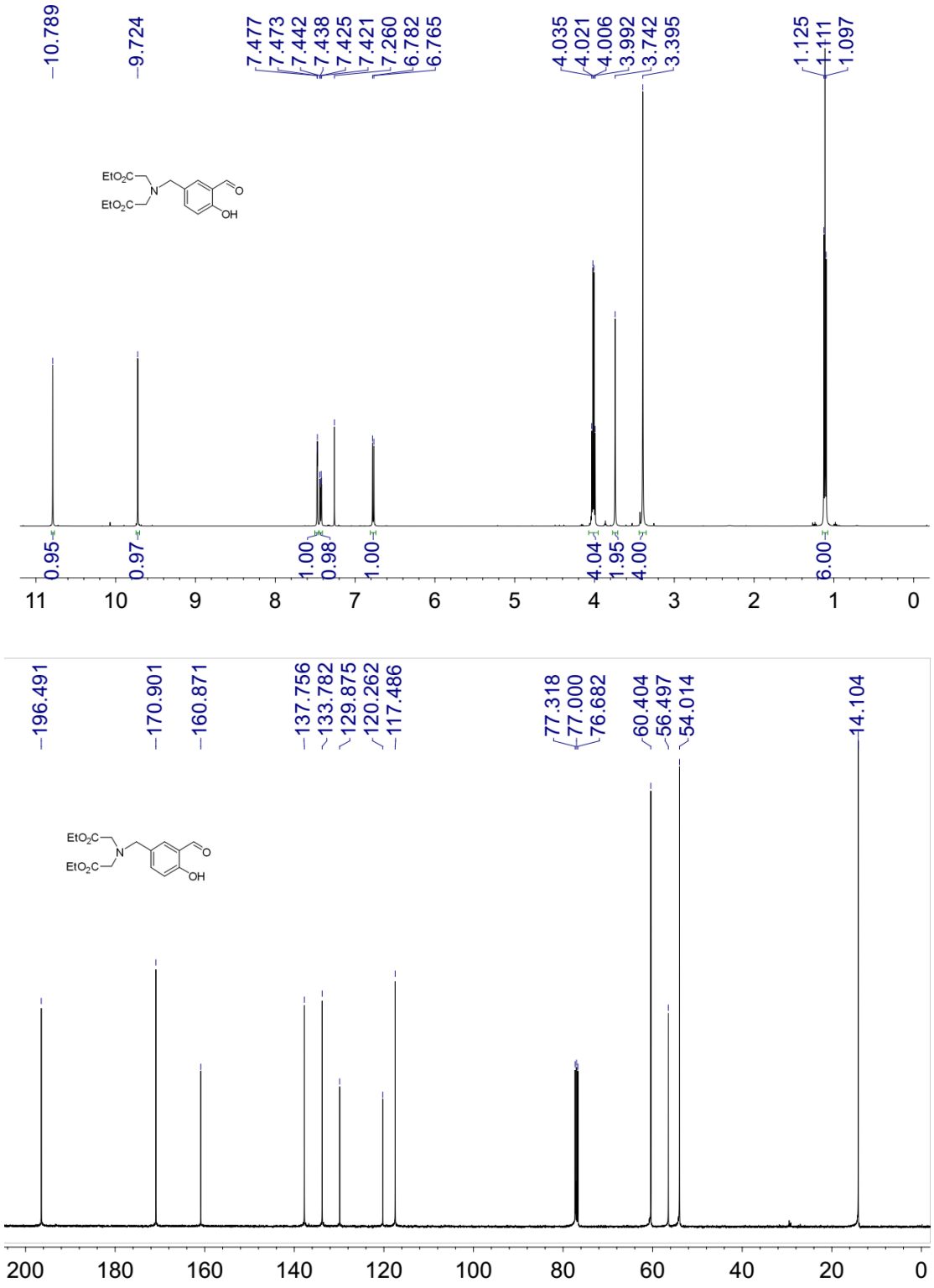

Figure S1. ^{1}H and ^{13}C NMR spectra of compound **3** in $CDCl_3$.

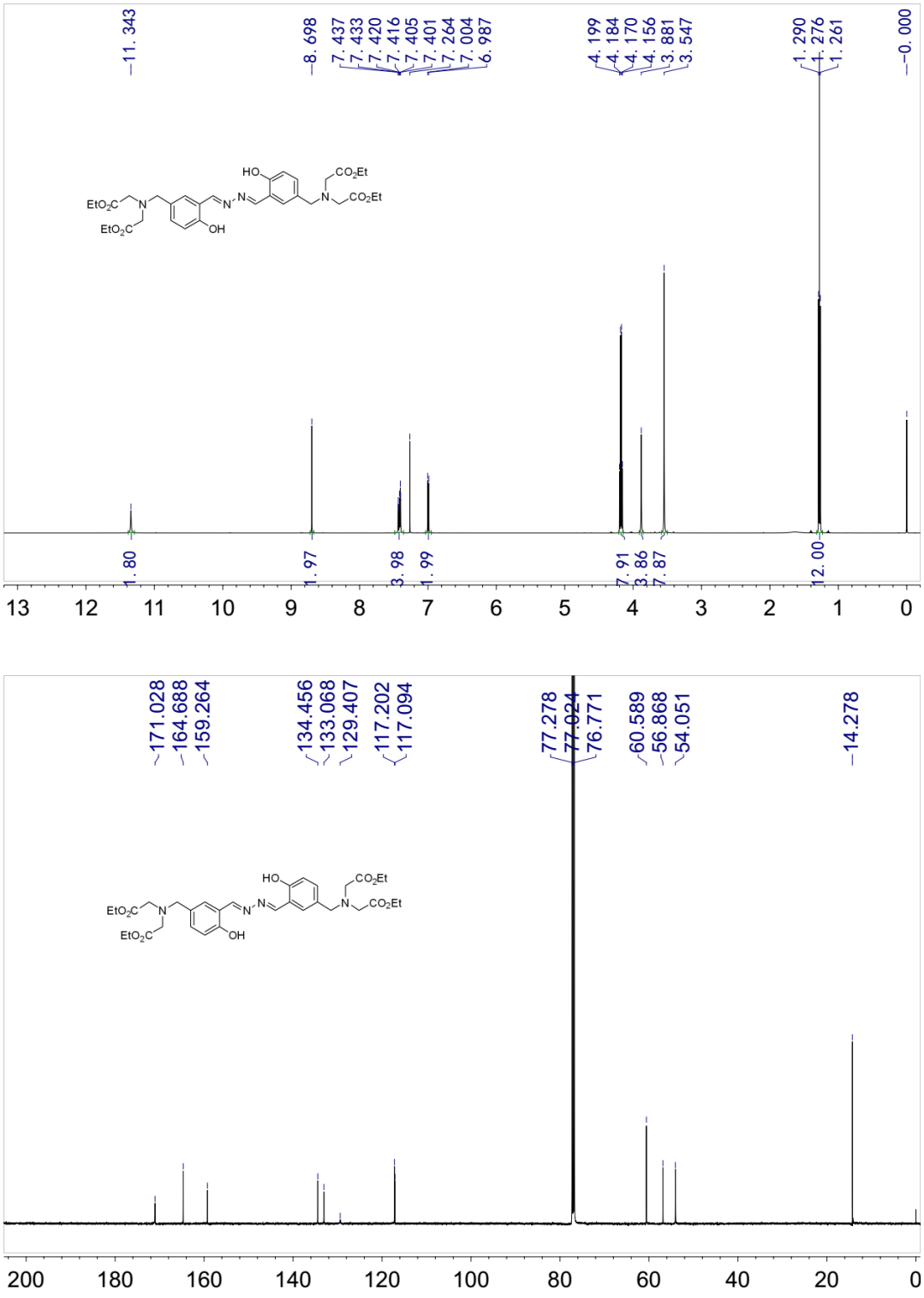

Figure S2. ^{1}H and ^{13}C NMR spectra of SA-4CO_2Et in $CDCl_3$.

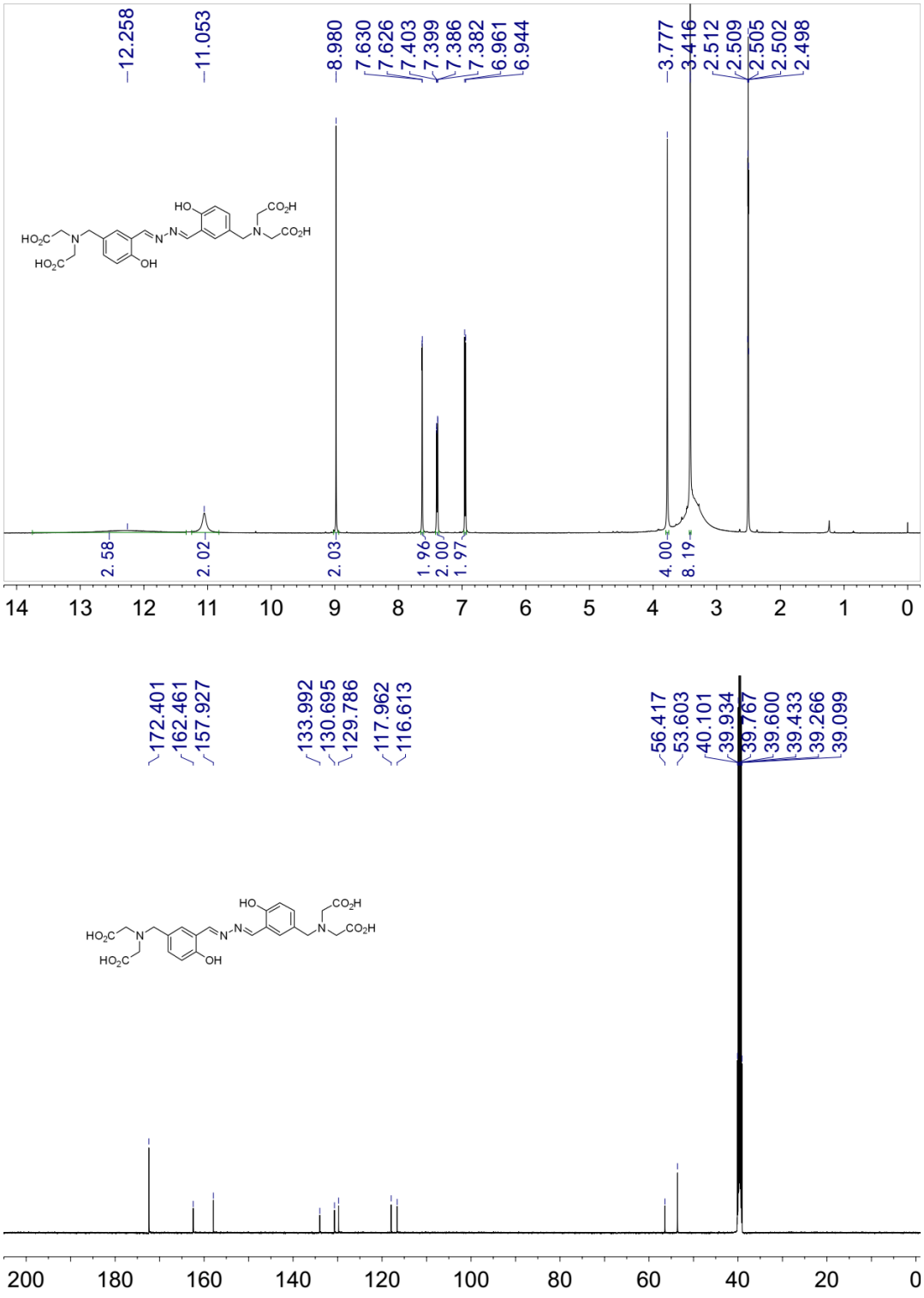

Figure S3. ^{1}H and ^{13}C NMR spectra of SA-4CO_2H in DMSO-d_6.

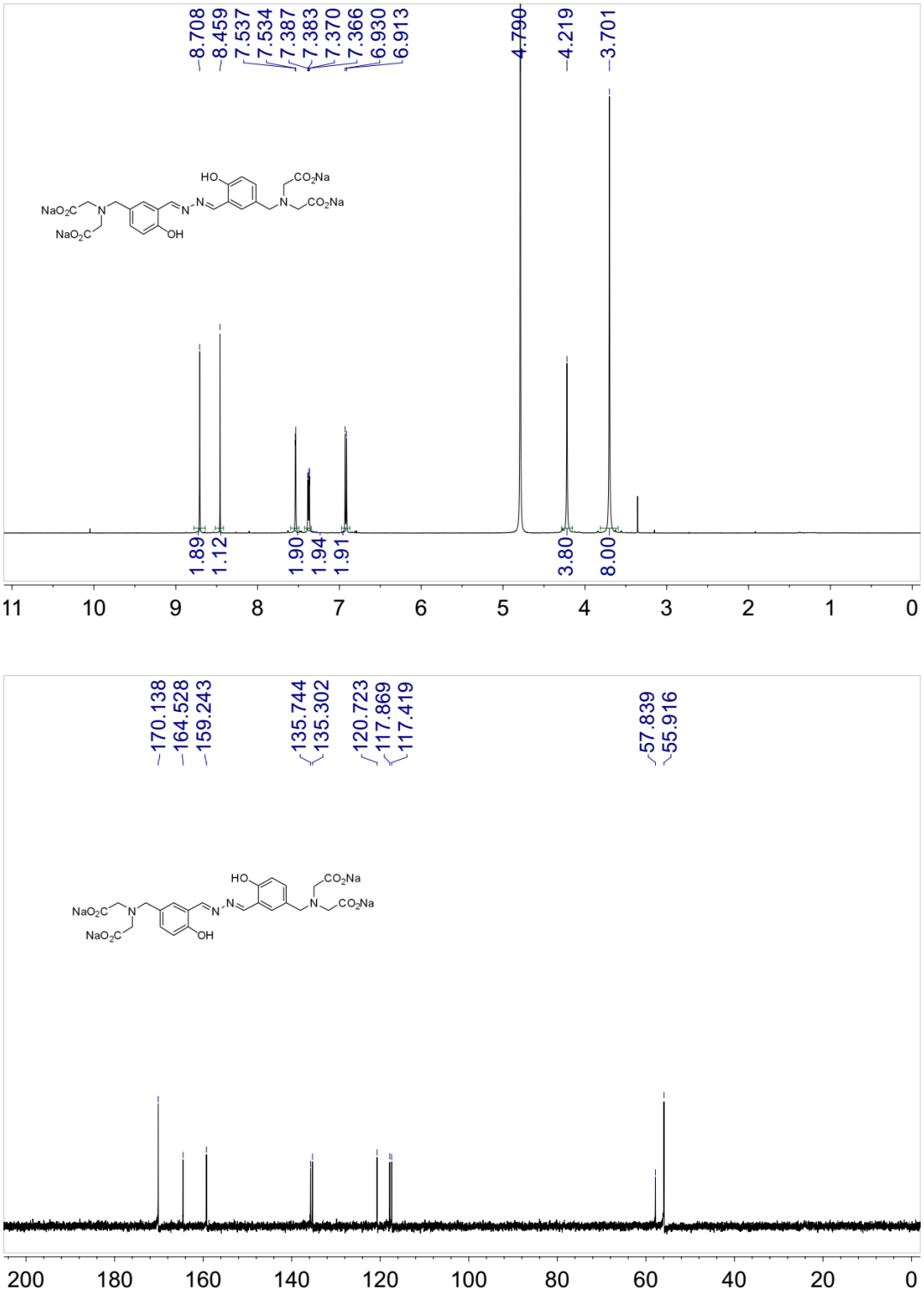

Figure S4. ^{1}H and ^{13}C NMR spectra of SA-4CO_2Na in D_2O.

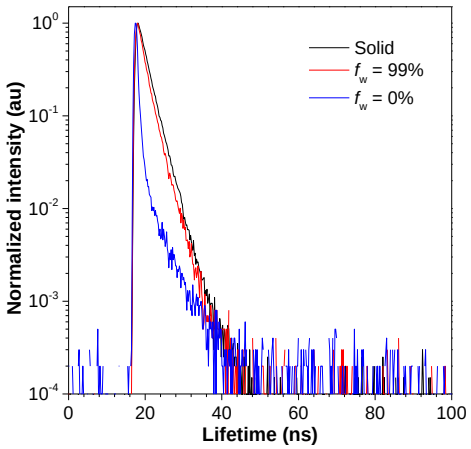

Figure S5. Fluorescence decay spectra of SA-4CO_2Na in solid state and in THF/water mixtures with 0 and 99% water fractions (f_w), respectively.

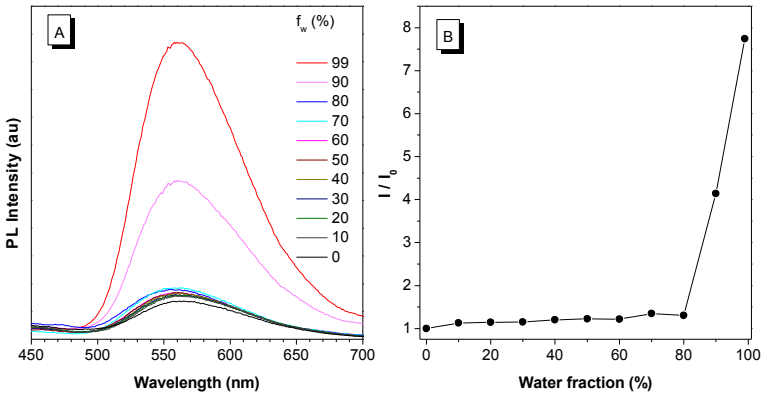

Figure S6. (A) PL spectra of SA-4CO_2Et in THF and THF/water mixtures with different water fractions (f_w); [SA-4CO_2Et] = 10 μM; λ_{ex} = 351 nm. (B) Plot of relative PL intensity (I/I_0) of SA-4CO_2Et at 560 nm versus the solvent composition of THF/water mixture.

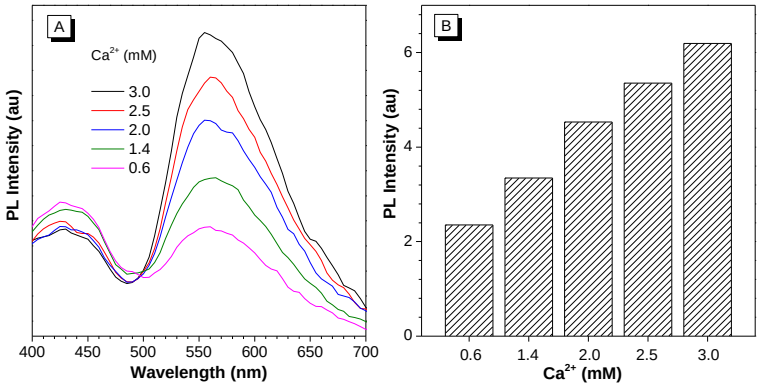

Figure S7. (A) The PL spectra of SA-4CO_2Na in human serum samples with different Ca^{2+} ion concentrations. (B) The relative fluorescence intensity changes of SA-4CO_2Na at 560 nm with different concentrations of Ca^{2+} ions. [SA-4CO_2Na] = 1.0 mM; λ_{ex} = 351 nm.

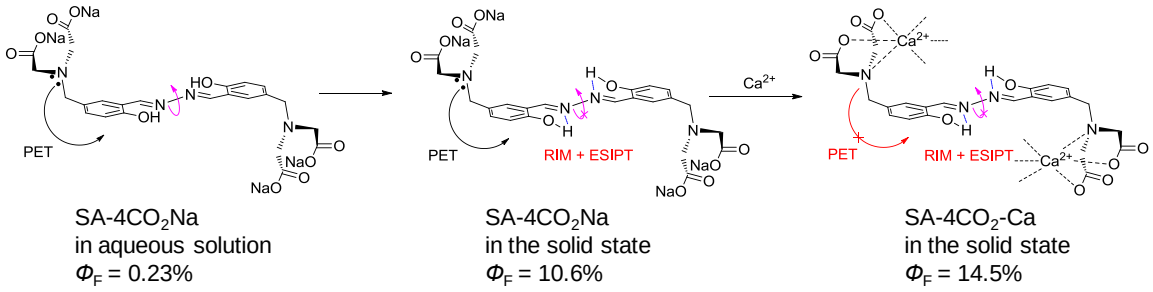

Figure S8. The light-up detection of Ca^{2+} based on the synergistic mechanisms of RIM, ESIPT, and PET.

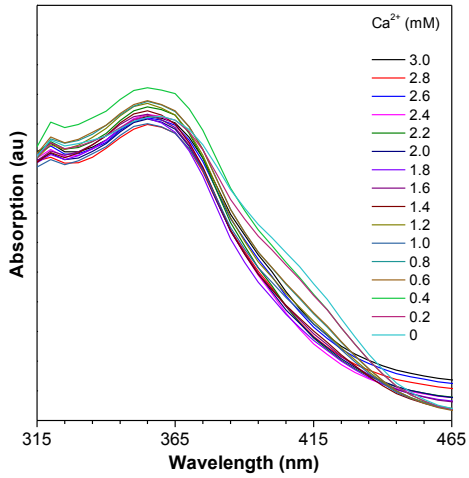

Figure S9. The absorption spectra of SA-4CO_2Na treated with $CaCl_2$ at different concentrations in PBS buffer solution.

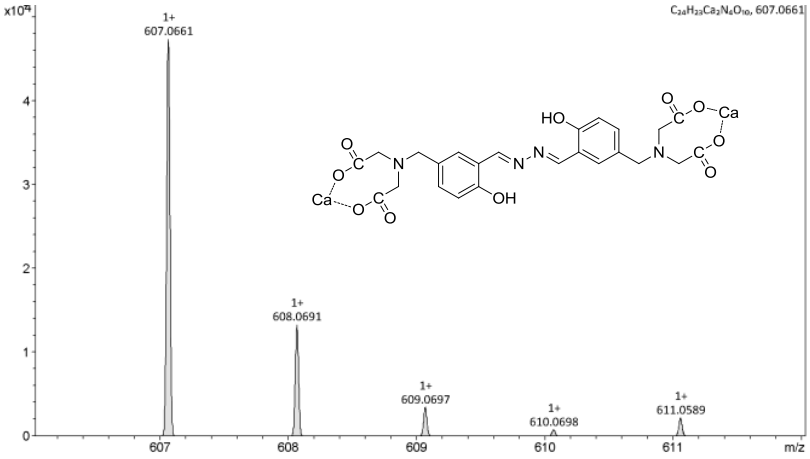

Figure S10. The HRMS spectra of SA-4CO_2-2Ca.

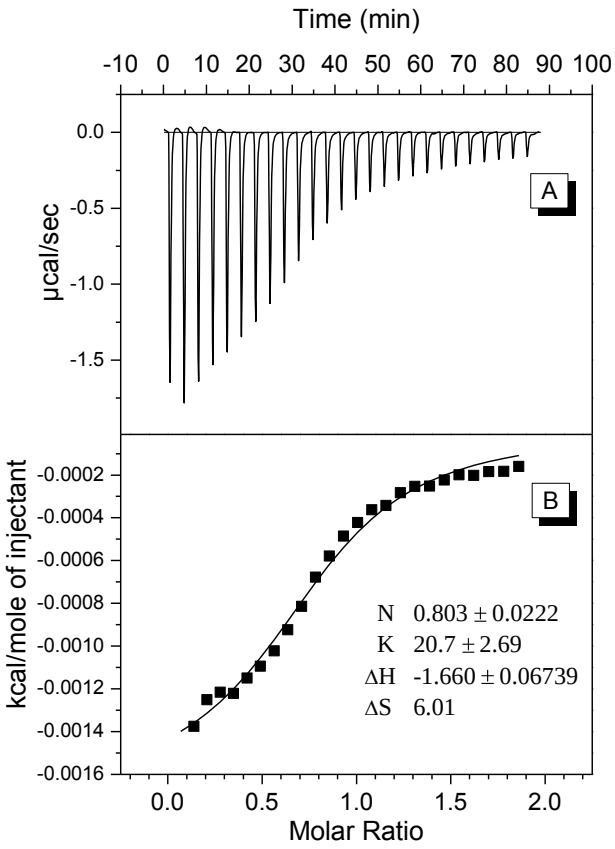

Figure S11. (A) The ITC titration curve of SA-4CO_2Na treated with Ca^{2+}; (B) the processed data. [SA-4CO_2Na] = 0.35 mM, [Ca^{2+}] = 3.5 mM, 25 ^{o}C, all injections of Ca^{2+} with 10 µL and 200 s spacing.

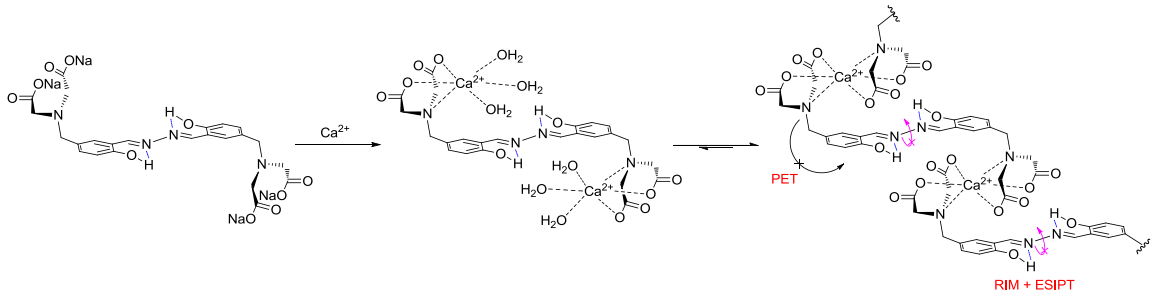

Figure S12. The proposed binding mode of SA-4CO_2Na with Ca^{2+}.

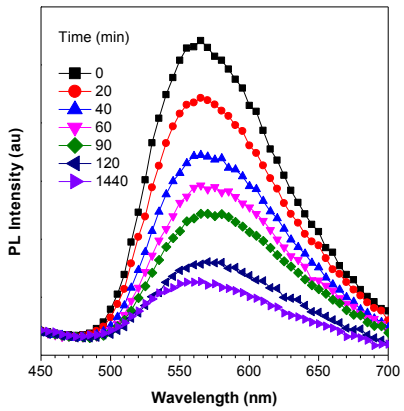

Figure S13. Fluorescence spectra changes of SA-4CO_2Na (1.0 mM)/$CaCl_2$ (3.0 mM) in PBS buffer solution treated with EDTA (3.0 mM); λ_{ex} = 351 nm.

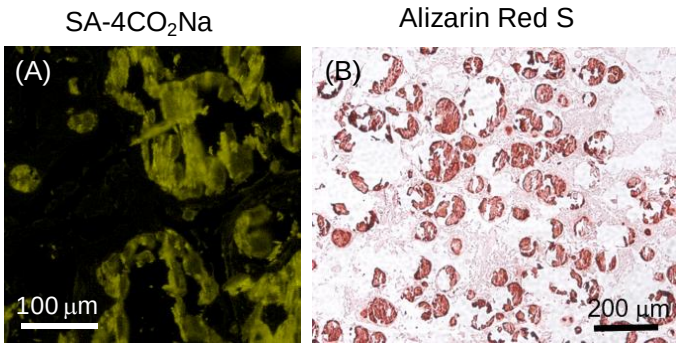

Figure S14. The psammomatous meningioma slice stained with (A) SA-4CO_2Na and (B) Alizarin Red S.

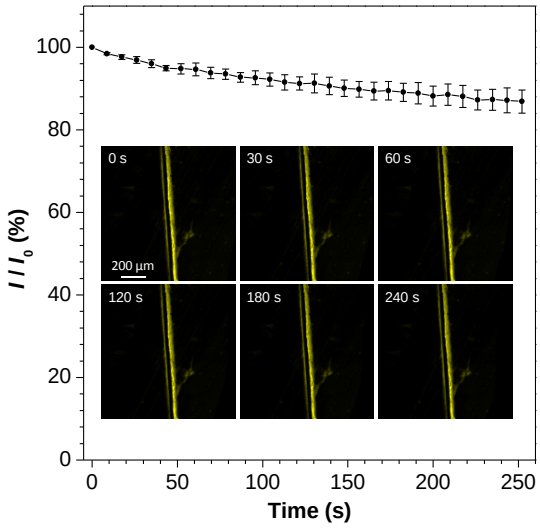

Figure S15. I/I_0 (%) of fluorescence intensity of bone microcracks stained with SA-4CO_2Na (0.5 mM) with increasing time of irradiation at 405 nm with 90% laser power. Inset: Fluorescence images of bone microcracks with increasing time of irradiation.

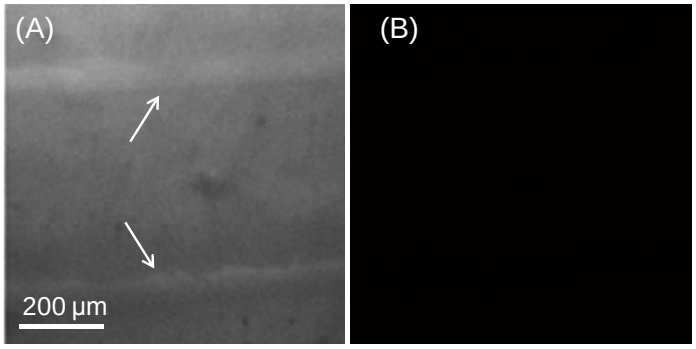

Figure S16. (A) Bright field and (B) fluorescence image of bone microcracks (indicated by white arrows) stained with SA-4CO_2Et (0.5 mM) for 4 h. λ_{ex} = 405 nm; λ_{em} = 460–750 nm.

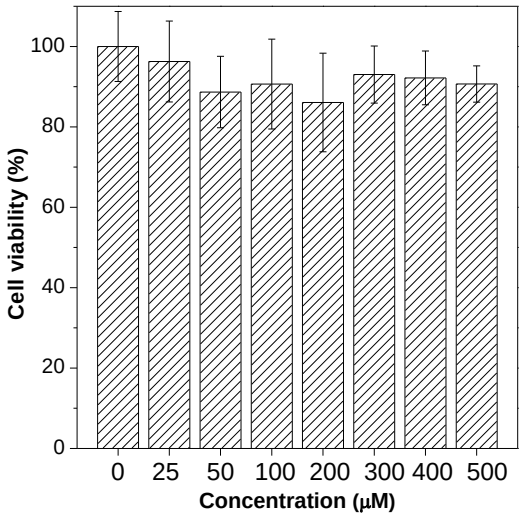

Figure S17. Cell viabilities of mBMSCs after incubation with different concentrations of SA-4CO_2Na (0, 25, 50, 100, 200, 300, 400 and 500 μM) for 24 h.

References

(1) Mori, F.; Tanji, K.; Wakabayashi, K. Widespread Calcium Deposits, As Detected Using The Alizarin Red S Technique, in The Nervous System of Rats Treated with Dimethyl Mercury. *Neuropathology* **2000**, *20*, 210-215.