Supporting information

One- and two-photon responsive sulfur dioxide donor: A combinatorial drug delivery for improved antibiotic therapy

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1. General Information: All commercially available anhydrous solvents dimethylformamide (DMF), dichloromethane (DCM), petroleum ether (PE) and ethyl acetate (EA) and other chemicals were used without further purification. Acetonitrile (ACN) and dichloromethane (DCM) were distilled from CaH_2 before use. NMR spectra were recorded on a 600 and 400 MHz instrument. ^{1}H NMR chemical shifts were referenced to the tetramethylsilane signal (0 ppm), ^{13}C NMR chemical shifts were referenced to the solvent resonance (77.23 ppm, Chloroform-d ($CDCl_3$)). Chemical shifts (δ) are reported in ppm, and spin−spin coupling constants (J) are given in Hz. The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet. UV/vis absorption spectra were recorded on a Shimadzu UV-2450 UV/vis spectrophotometer and fluorescence spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer. High-resolution mass spectra (HRMS) were recorded on ESI-TOF (electrospray ionization-time-of-flight). Photolysis was carried out using a 125 W medium pressure mercury lamp. RP-HPLC was taken using mobile phase acetonitrile/water (9:1), at a flow rate of 0.5 mL / min (detection: UV 254 nm). Chromatographic purification was done with 60–120 mesh silica gel. For reaction monitoring, precoated silica gel 60 F254 TLC sheets were used.

2. Experimental Procedure and spectroscopic data:

***S*-4,5-dimethoxy-2-nitrobenzyl ethanethioate (2):** Methane sulfonyl chloride (0.214 g, 1.88 mmol) was added to a solution of (4,5-dimethoxy-2-nitrophenyl)methanol (1) (0.2 g, 0.94 mmol) in DCM at 0 °C under nitrogen atmosphere. To this pyridine (0.223 g, 2.82 mmol) was added and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was acidified with 10 mL 2M HCl, and was further diluted with dI water (100 mL) and sat. NaCl (25 mL). The aqueous layer was extracted with DCM (3 × 30 mL). The combined organic layers were dried over Na_2SO_4, and evaporated under reduced pressure. The light yellow solid product was directly taken for the next step without further purification. To this solution of mesylated compound (0.18 g, 0.62 mmol) in ACN (20 mL), was added potassium thioacetate (0.084 g, 0.74 mmol) and the reaction mixture was stirred for 1 h. White solid formed during the reaction was filtered away and the filtrate was concentrated by removing the ACN under reduced temperature. The residue was purified over silica gel column (hexane/EtOAc) to afford compound **3** (0.159 g, 95%) as a yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.09 (s, 1H), 4.44 (s, 2H), 4.01 (s, 3H), 3.96 (s, 3H), 2.35 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 195.9, 153.3, 148.2, 140.1, 128.9,

114.0, 108.24, 56.5, 56.4, 31.8, 30.2. HRMS (ESI^+) calcd for $C_{11}H_{13}NO_5S$ $[M + H]^+$, 272.0587; found: 272.0594.

(*E*)-ethyl 3-(4-(((4,5-dimethoxy-2-nitrobenzyl)sulfonyl)oxy)-3-methoxyphenyl)acrylate (5a):

N-chlorosuccinamide (0.836 g, 7.37 mmol) was added to a 3 mL mixture of 2M HCl:ACN (1:5) and cooled in ice water. The solution of **3** (0.5 g, 1.84 mmol) in 1 mL ACN was added dropwise to the mixture. The resulting solution was stirred for 30 min. The temperature of the ice water bath was maintained below 10 °C. On completion, the reaction mixture was further diluted with 20 mL dI water. ACN was evaporated under reduced pressure while maintaining the water bath at 20 °C. The mixture was extracted with isopropyl ether (2 × 30 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure to yield sulfonyl chloride **4** (0.495 g, 91%). The yellow solid was used in next step without further purification.

A solution of **4** (0.145 g, 0.49 mmol) in DCM (1 mL) was added dropwise to a solution of Ferulic acid ethyl ester (FAEE) (0.108 g, 0.49 mmol), and TEA (0.066 g, 0.66 mmol), in DCM (25 mL) at 0 °C under nitrogen atmosphere. The resulting mixture was stirred for 30 min. Reaction mixture was further diluted with dI water (100 mL) and the aqueous layer was extracted with DCM (3 × 30 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified over silica gel column chromatography, using hexane/EtOAc as the mobile phase. **5** (0.169 g, 72%) was isolated as light yellow solid. ^1H NMR (400 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.62 (d, J = 16.0 Hz, 1H), 7.25 (s, 1H), 7.17 – 7.09 (m, 2H), 7.08 (s, 1H), 6.40 (d, J = 16.0 Hz, 1H), 5.25 (s, 2H), 4.27 (q, J = 7.1 Hz, 2H), 3.98 (d, J = 4.4 Hz, 6H), 3.93 (s, 3H), 1.35 (d, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7, 153.0, 151.8, 149.5, 143.4, 142.2, 139.5, 134.8, 124.6, 121.3, 119.7, 116.6, 114.9, 111.7, 108.8, 60.7, 56.7, 56.5, 56.1, 53.8, 14.3. HRMS (ESI^+) calcd for $C_{21}H_{23}NO_{10}S$ $[M + Na]^+$, 504.0935; found: 504.0923.

Phenyl (4,5-dimethoxy-2-nitrophenyl) methanesulfonate (5b): A solution of **4** (0.150 g, 0.50 mmol) in DCM (1 mL) was added dropwise to a solution of phenol (PhOH) (0.047 g, 0.50 mmol), and TEA (0.076 g, 0.75 mmol), in DCM (25 mL) at 0 °C under nitrogen atmosphere. The resulting mixture was stirred for 30 min. Reaction mixture was further diluted with dI water (100 mL) and the aqueous layer was extracted with DCM (3 × 30 mL).

The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified over silica gel column chromatography, using hexane/EtOAc as the mobile phase. **5** (0.129 g, 72%) was isolated as light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.40 (t, *J* = 7.7 Hz, 2H), 7.31 (t, *J* = 7.2 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.00 (s, 1H), 5.17 (s, 2H), 3.99 (s, 3H), 3.98 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.2, 149.8, 149.2, 142.3, 130.2, 127.6, 122.0, 116.5, 115.0, 109.1, 56.9, 56.7, 53.2. HRMS (ESI$^+$) calcd for $C_{15}H_{15}NO_7S$ $[M + H]^+$, 354.0642; found: 354.0643.

(*E*)-ethyl 3-(4-((((4,5-dimethoxy-2-nitrobenzyl)oxy)carbonyl)oxy)-3-methoxyphenyl)acrylate (6): To a solution of (4,5-dimethoxy-2-nitrophenyl)methanol (1) (0.100 g, 0.47 mmol) and Et_3N (0.071 g, 0.7 mmol) in dry DCM (20 mL) at 0 °C, was added a solution of 4-nitrophenyl chloroformate (0.095 g, 0.47 mmol) in DCM (20 mL) dropwise. After stirring at room temperature for 30 minutes the solvent was evaporated to give the product as yellow crystals (0.186 g, 86%) and in situ reaction with ferulic acid ethyl ester (FAEE) (0.099 g, 0.49 mmol) in the presence of Et_3N in dry DMF at room temperature, and the reaction mixture was stirred for 4 h. After completion of the reaction, it was extracted with EtOAc and washed with saturated NaCl solution and dried over Na_2SO_4. The solvent was removed by rotary evaporation under reduced pressure followed by column chromatography using 30% EtOAc in pet ether to give the product as a light yellow solid (0.163 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.64 (d, J = 16.0 Hz, 1H), 7.20 – 7.13 (m, 3H), 7.13 (s, 1H), 6.40 (d, J = 16.0 Hz, 1H), 5.72 (s, 2H), 4.27 (q, J = 7.1 Hz, 2H), 4.01 (s, 3H), 3.98 (s, 3H), 3.86 (s, 3H), 1.34 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 154.0, 152.7, 151.6, 148.6, 143.7, 141.6, 139.9, 134.1, 126.7, 122.9, 121.4, 119.2, 111.7, 110.0, 108.5, 67.4, 60.8, 56.7, 56.7, 56.3, 14.5. HRMS (ESI$^+$) calcd for $C_{22}H_{23}NO_{10}$ $[M + Na]^+$, 484.1214; found: 462.1242.

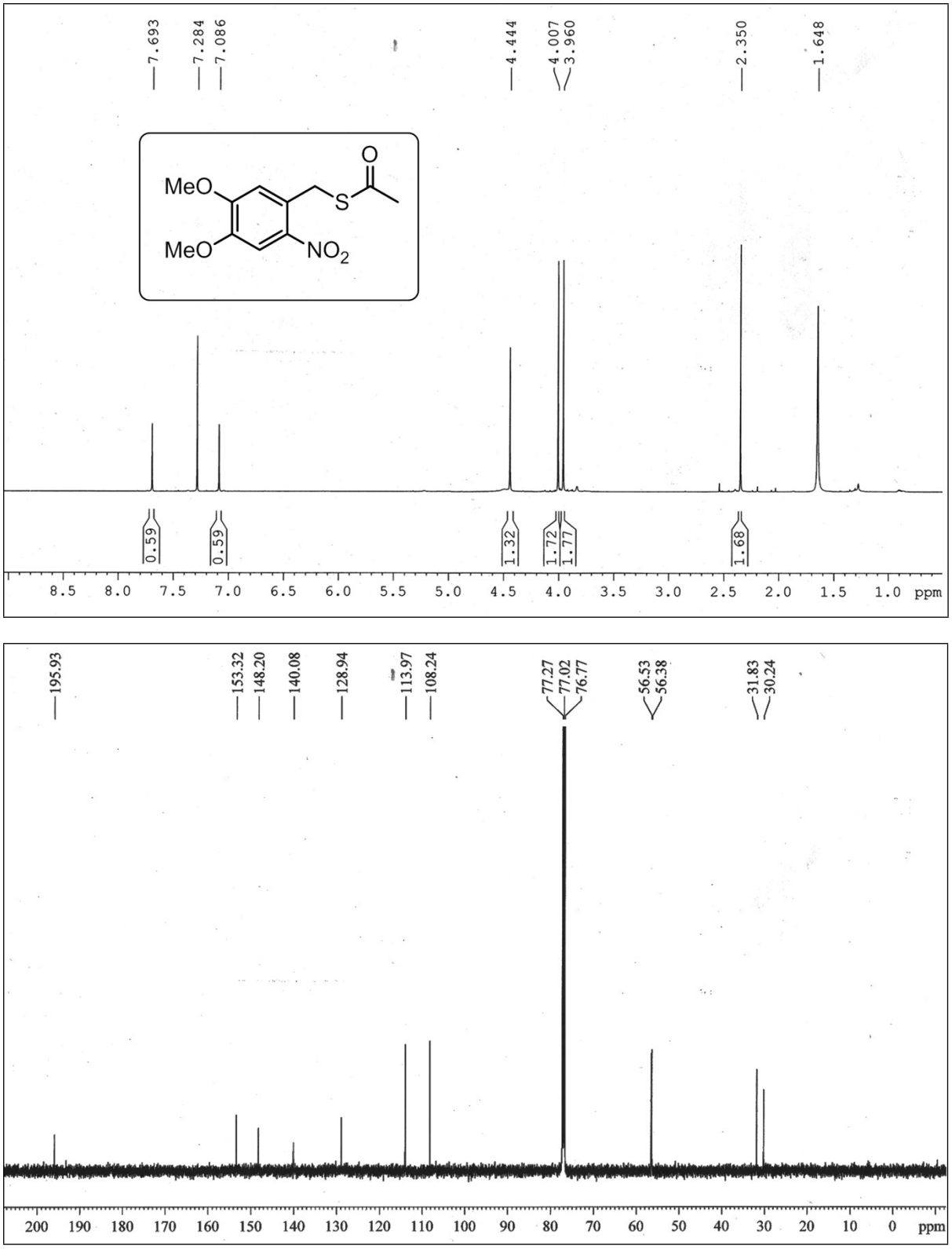
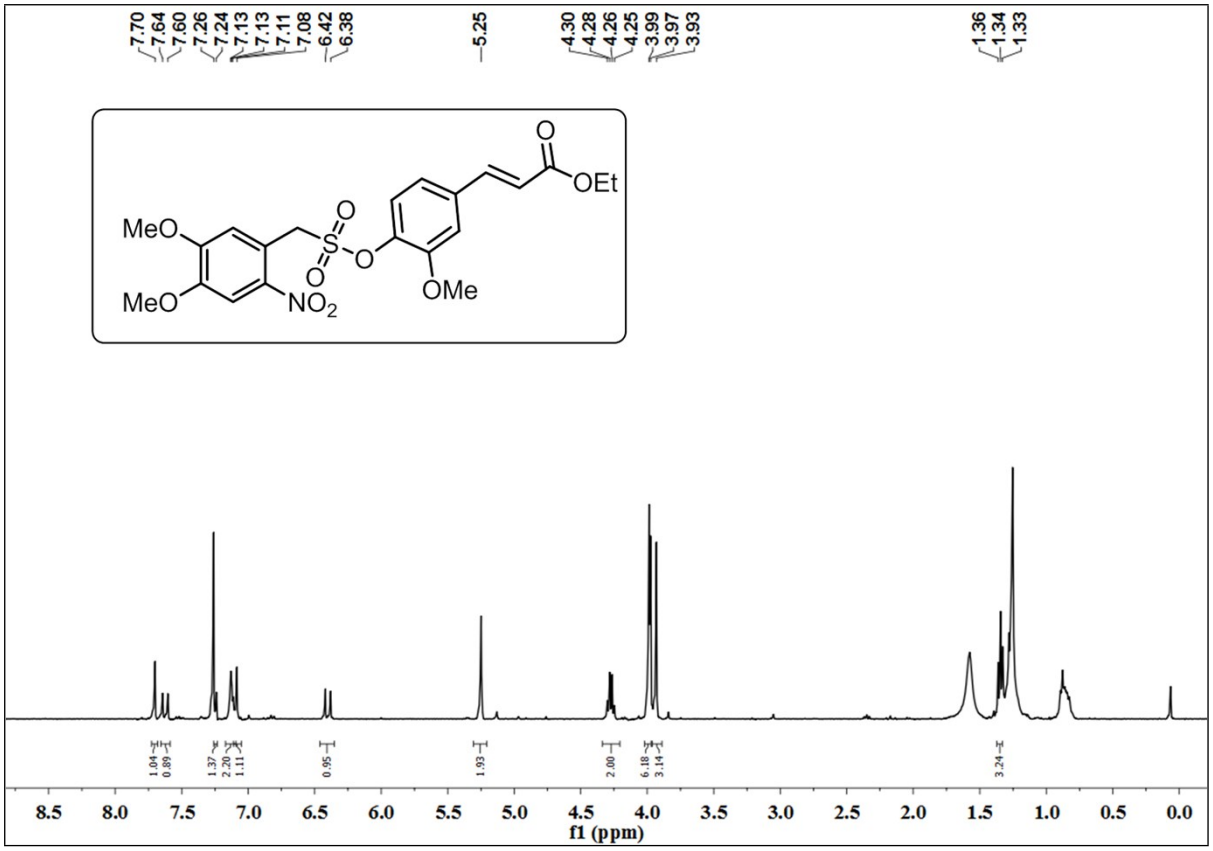


Figure S1. ^{1}H and ^{13}C NMR spectra of **2**



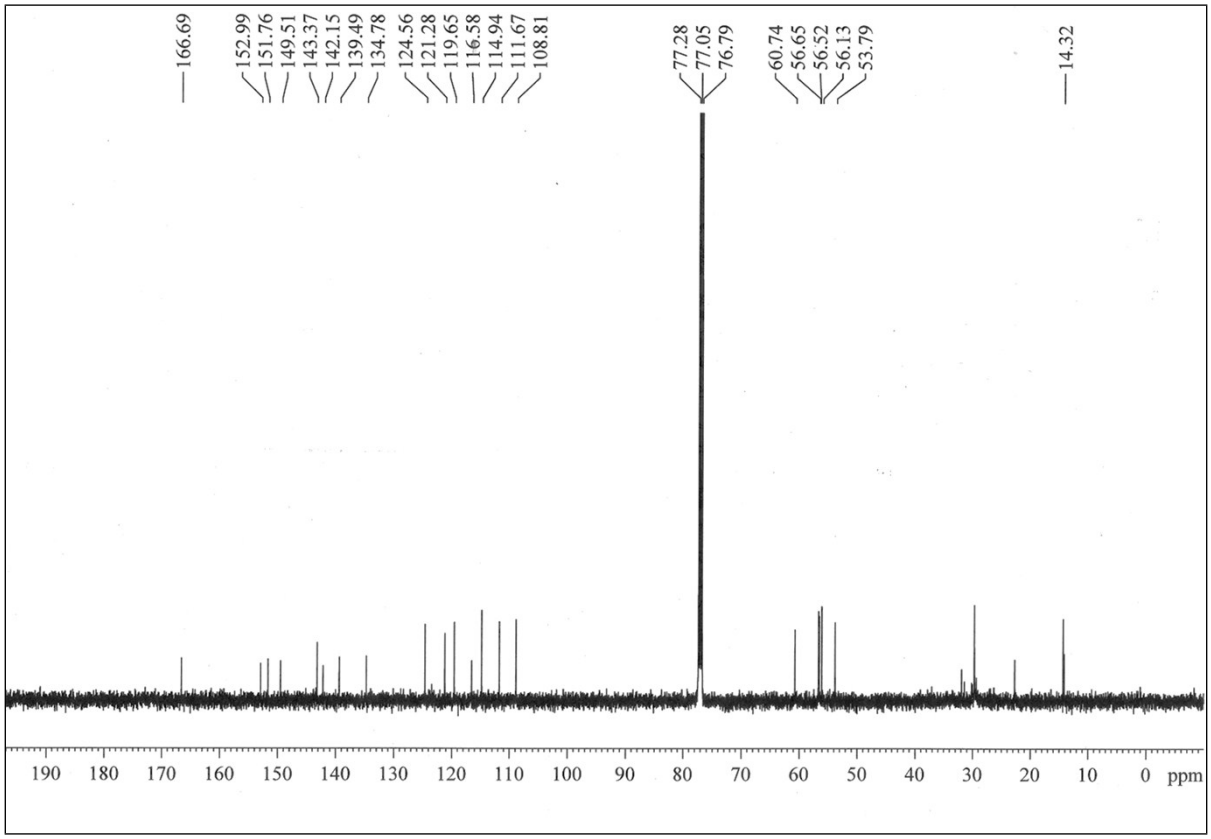


Figure S2. ^{1}H and ^{13}C NMR spectra of **5a**

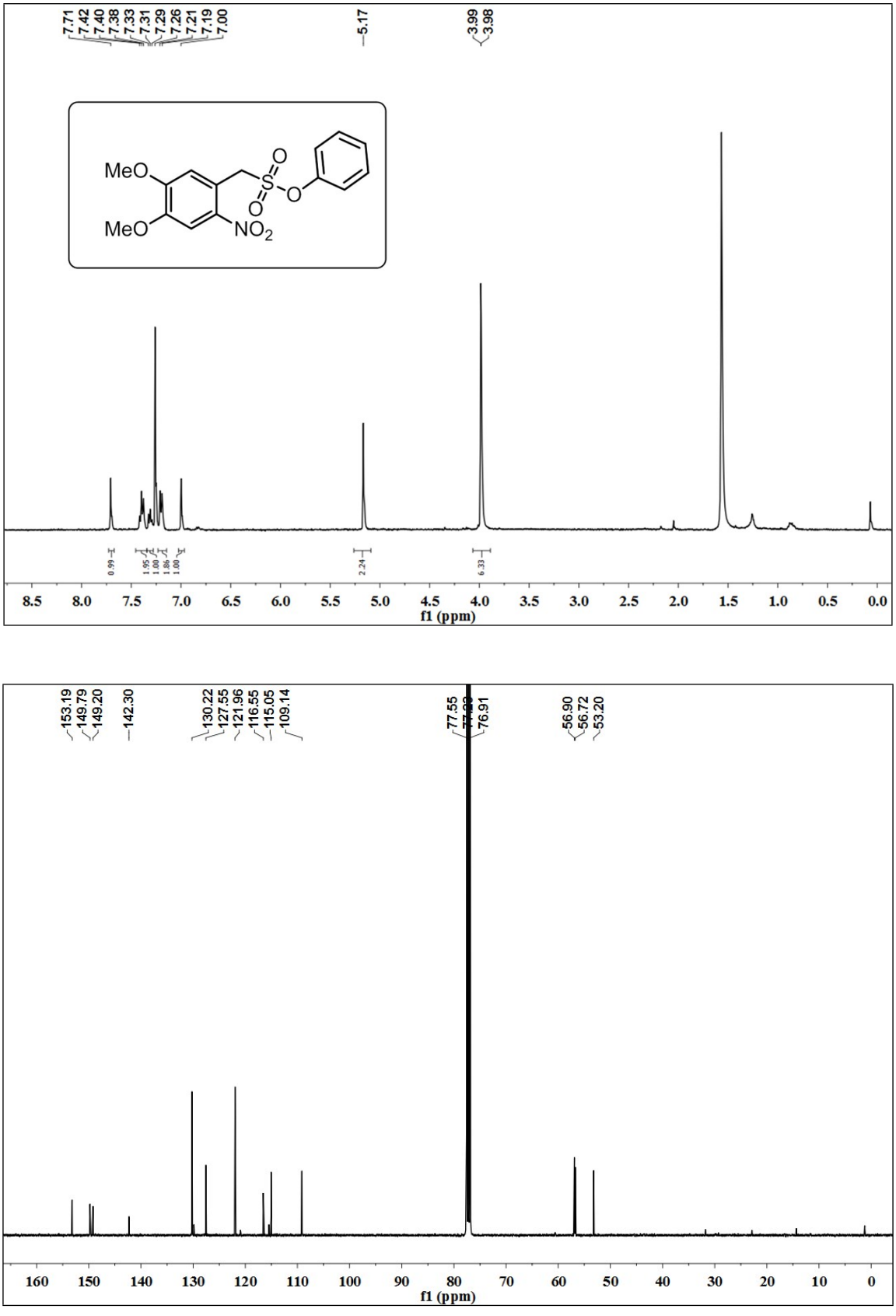


Figure S3. ^{1}H and ^{13}C NMR spectra of **5b**

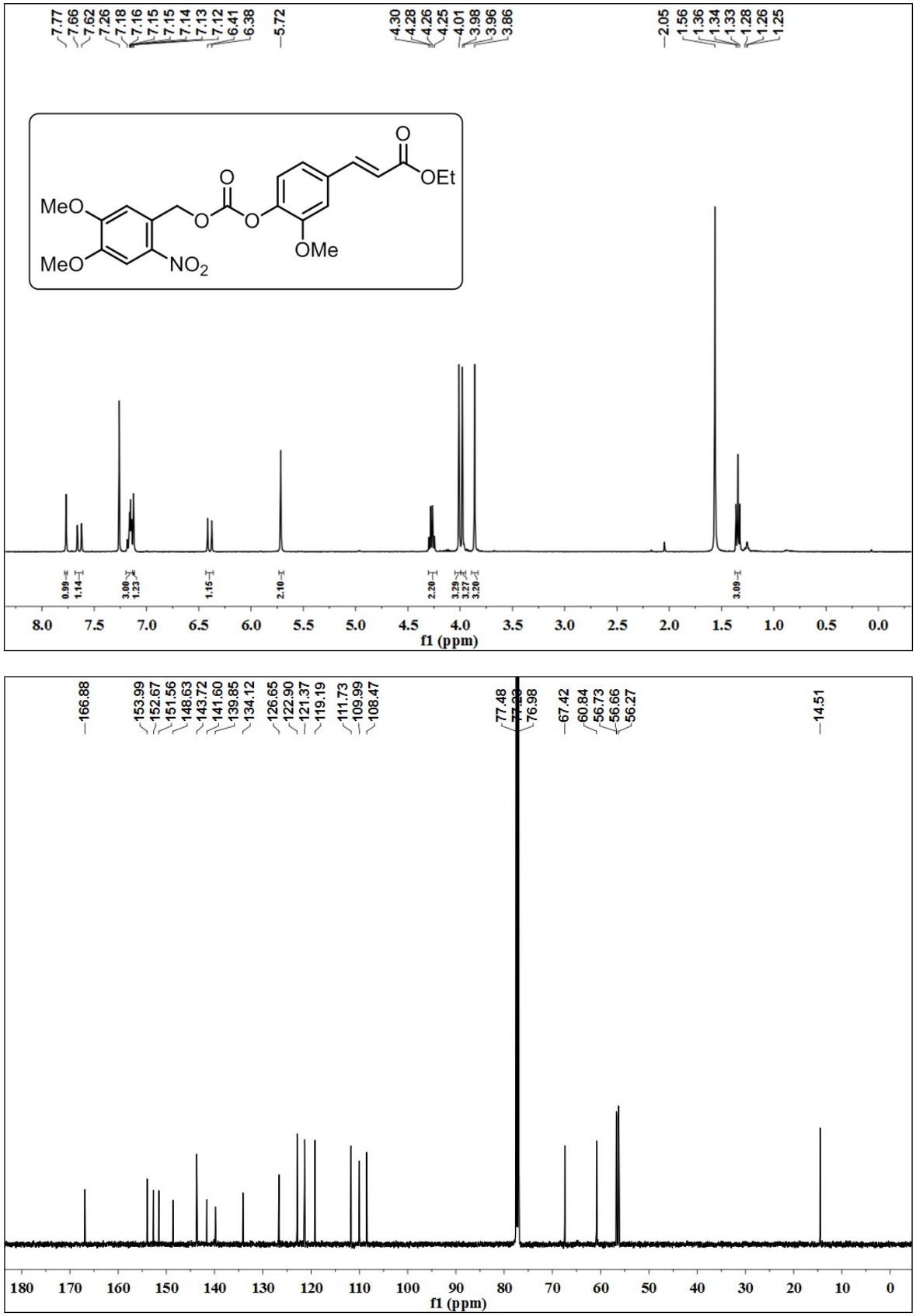


Figure S4. ^{1}H and ^{13}C NMR spectra of **6**

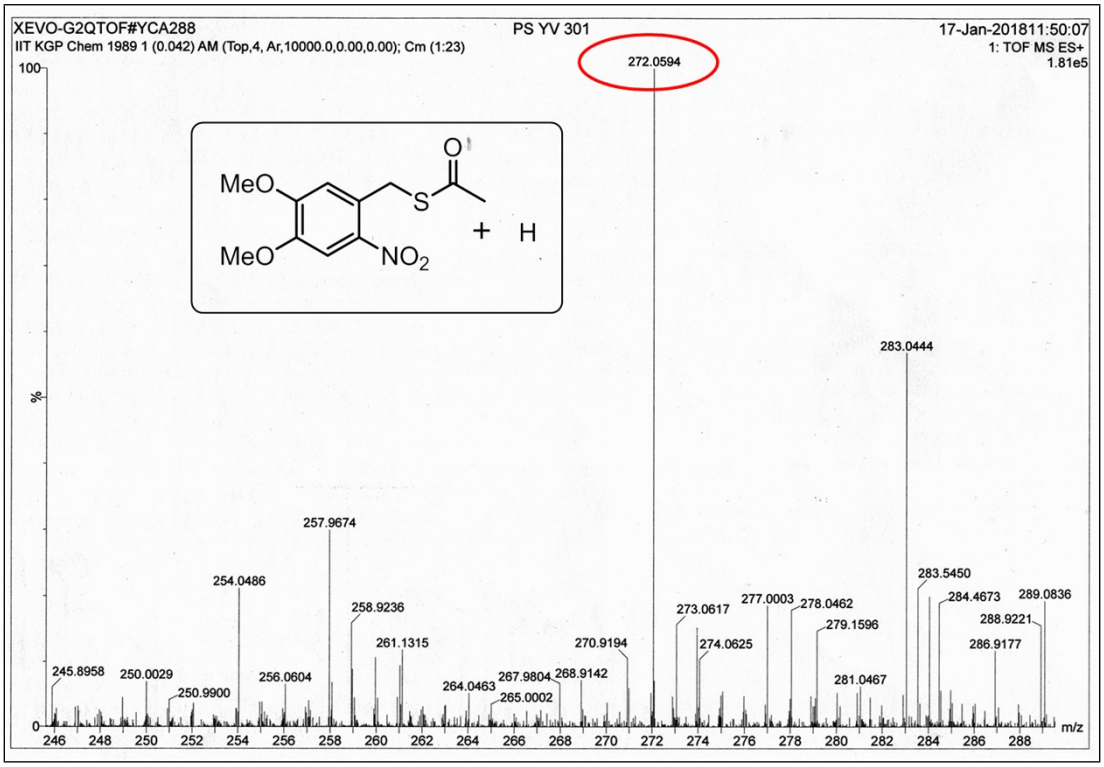


Figure S5. HRMS of **2**

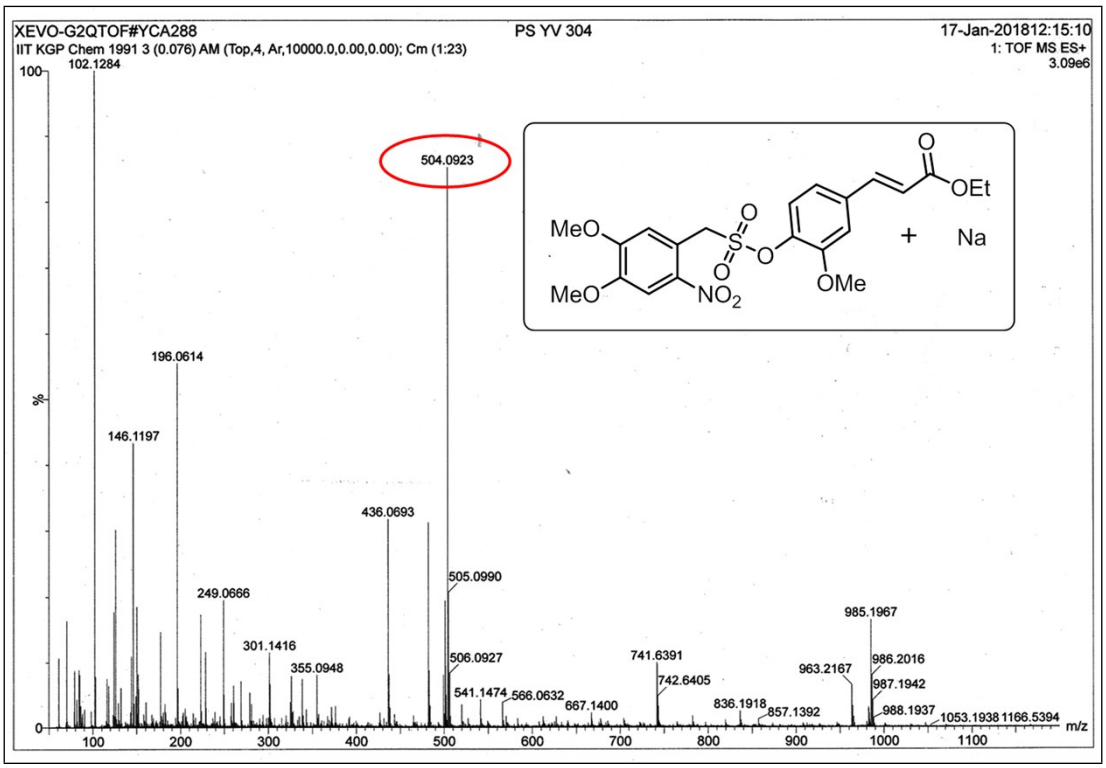


Figure S6. HRMS of **5a**

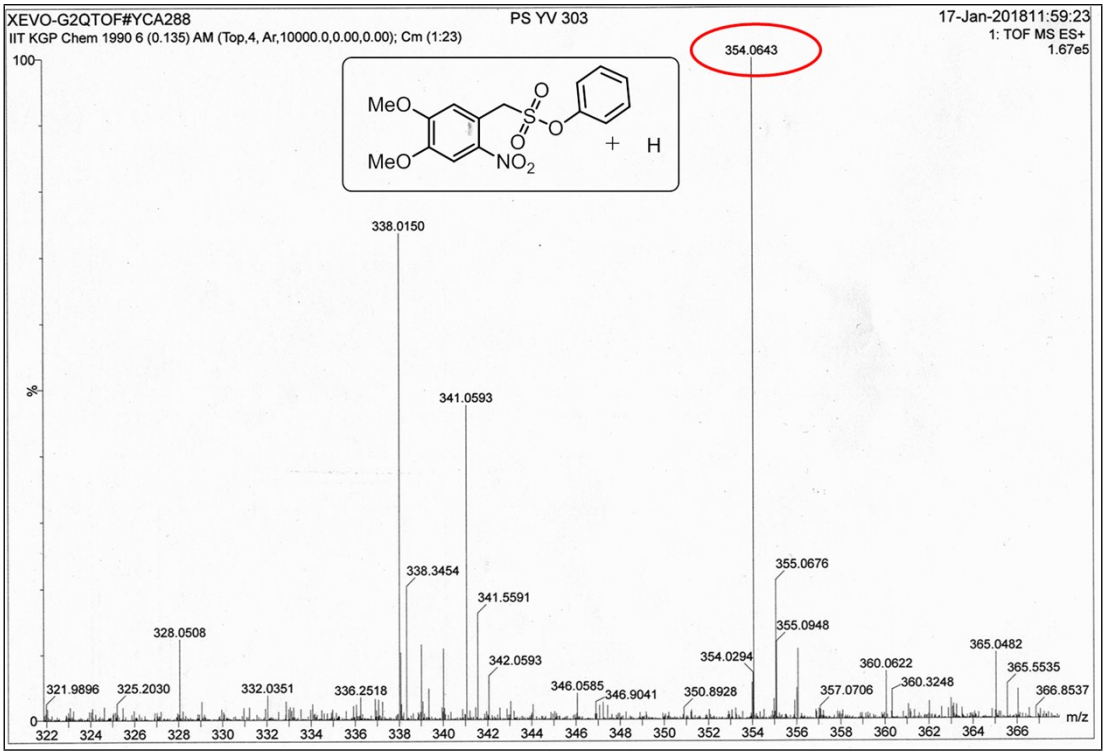


Figure S7. HRMS of **5b**

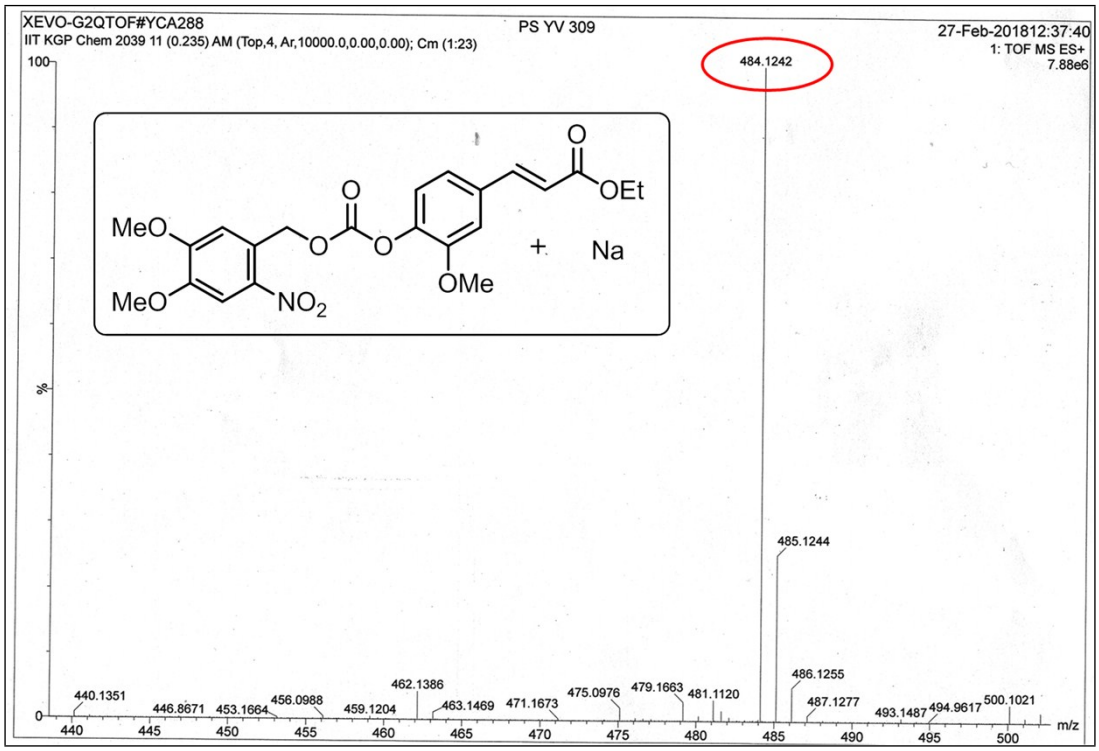


Figure S8. HRMS of **6**

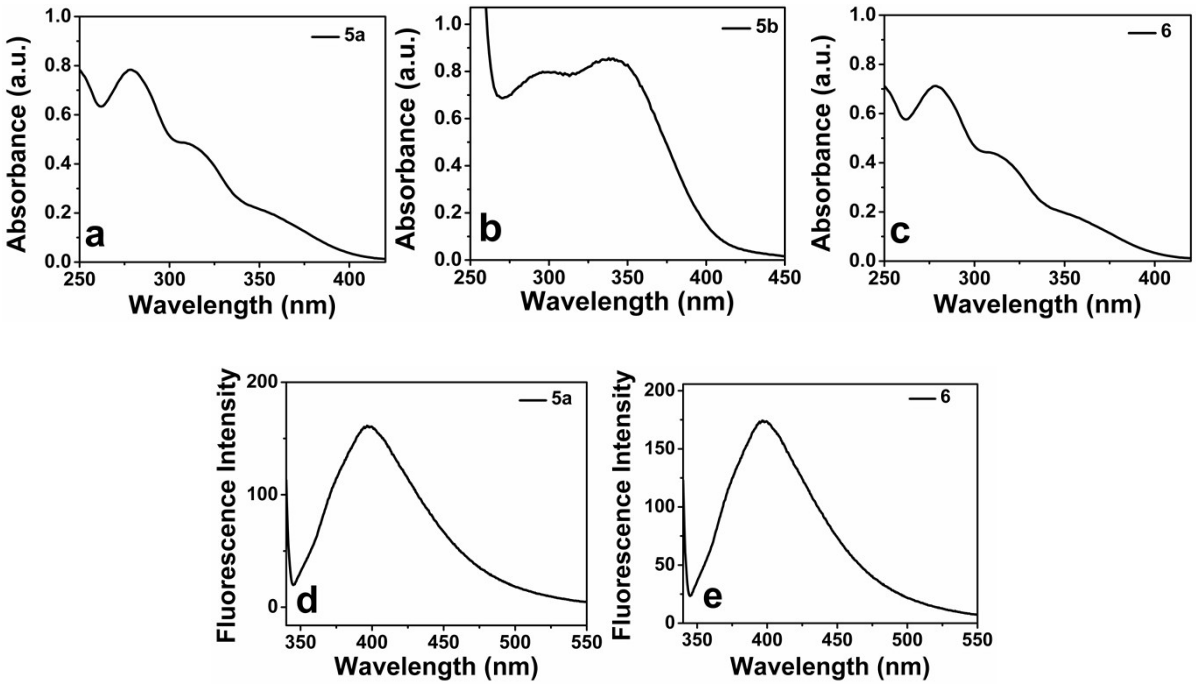


Figure S9. Absorption spectrum of **5a** (a), **5b** (b), and **6** (c). Emission spectrum of **5a** (d) and **6** (e). Excitation wavelength: 330 nm.

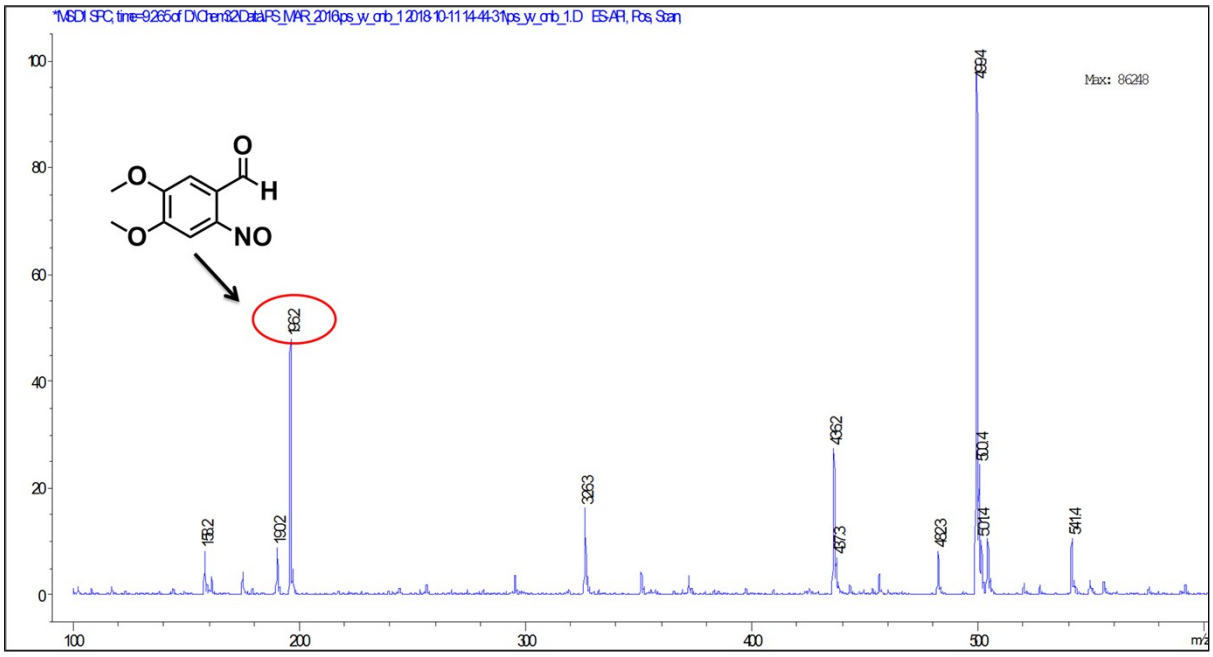


Figure S10. ESI-MS spectrum of **11**

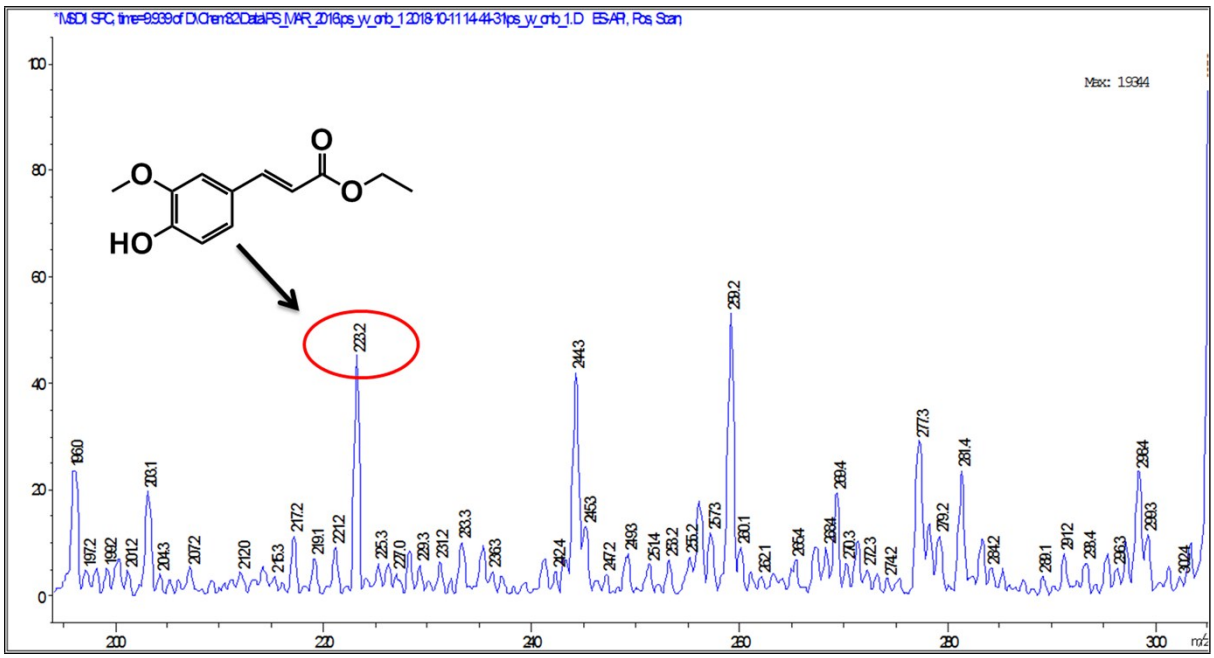


Figure S11. ESI-MS spectrum of **4a**

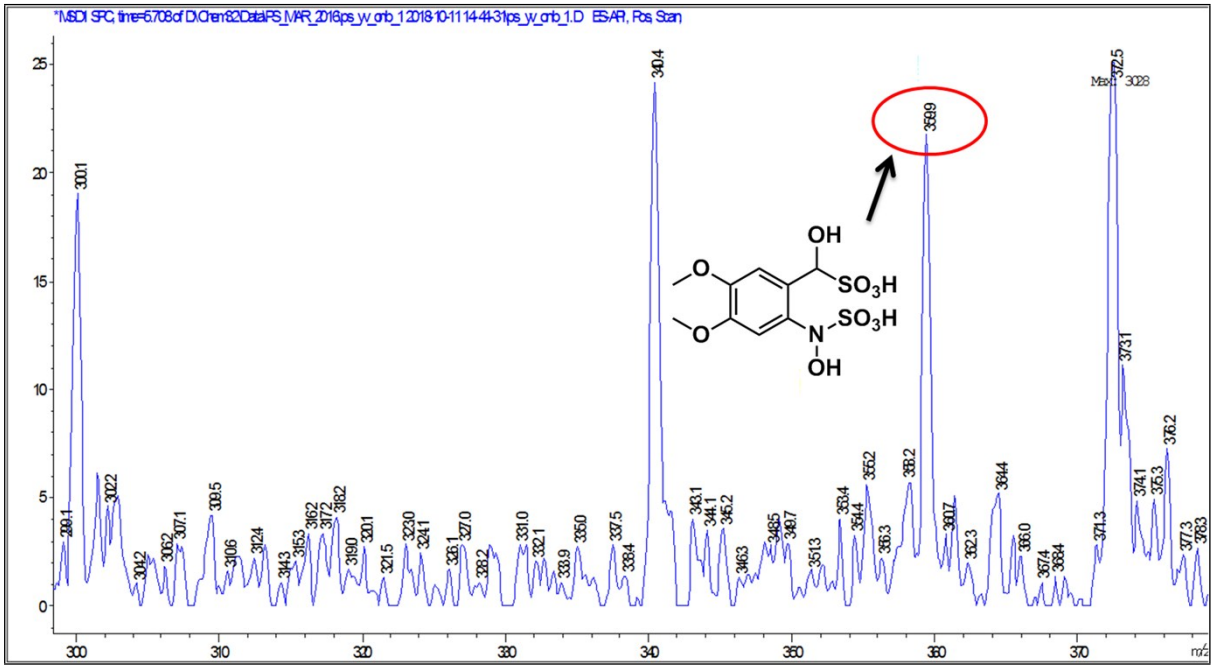


Figure S12. ESI-MS spectrum of adduct

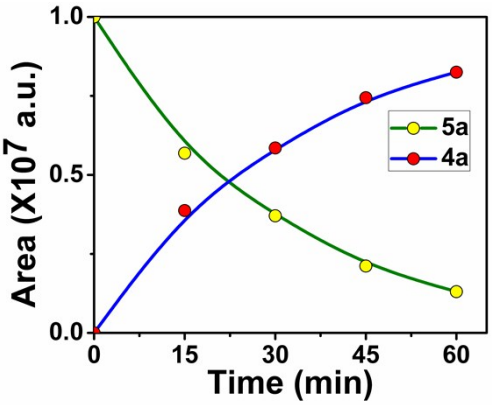


Figure S13. Time course of disappearance of **5a** (green line) and formation of **4a** (FAEE) (blue line) was determined by HPLC analysis at regular interval of time (0-60 min).

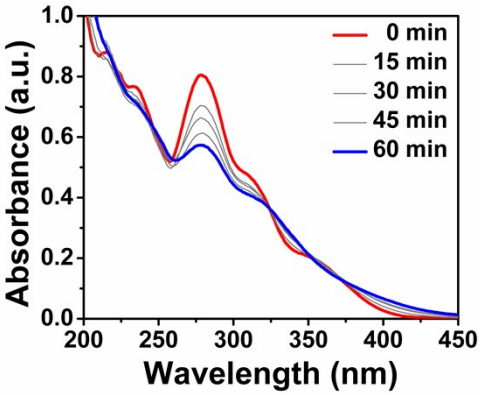


Figure S14. Representative absorption spectra of sulfonate **5a** during photolysis at different time intervals (0-60 min).

Table S1. Photochemical Properties of **5b** and **6**

Donors	**% of release at 365 nm[a]**	**Φ_u[b] at 365 nm**	**δ_u (GM) at 730 nm[c]**
5b	91 (4b)	0.0023	0.016
6	94 (4a)	0.0031	0.022

[a]% of the release (4a and 4b) was determined by reverse-phase HPLC, [b]Photochemical quantum yield after one-photon excitation at $\lambda \geq 365$ nm for 60 min, [c]Two-photon uncaging cross section at 730 nm (1 GM = 10^{-50} cm4 s/photon).

3. Detection of sulfite by SO_2 donors (5a and 5b) with sulfite dye 7 by absorption spectroscopy: 1 mM stock solutions of the sulfonates **5a, 5b** and dye **7** were prepared in ACN independently. In a typical reaction mixture sulfonate **5a** (100 µL, final conc. 100 µM), **7** (10 µL, final conc. 10 µM**)** were added independently to 890 µL PBS (pH 7.4, 10 mM) and was incubated at 37 ºC on Eppendorf thermomixer comfort (800 rpm) for 30 min. The reaction mixture was transferred into a quartz cuvette and subjected to photolysis at $\lambda \geq 365$ nm for 60 min. At a regular interval of time, the aliquots were taken and the absorption spectra were recorded on a Shimadzu UV-2450 UV/vis spectrophotometer.

4. Detection of sulfite from SO_2 donors 5a and 5b with sulfite dye 7 by fluorimetry: 1 mM stock solutions of the sulfonates **5a, 5b** and dye **7** were prepared in ACN independently. In a typical reaction mixture sulfonate **5a** (100 µL, final conc. 100 µM), **7** (10 µL, final conc. 10 µM**)** were added independently to 890 µL PBS (pH 7.4, 10 mM) and was incubated at 37 ºC on Eppendorf thermomixer comfort (800 rpm) for 30 min. The reaction mixture was transferred into a quartz cuvette and subjected to photolysis at $\lambda \geq 365$ nm for 60 min. At a regular interval of time, the aliquots were taken and fluorescence measurements were carried out using a Hitachi F-7000 fluorescence spectrophotometer.

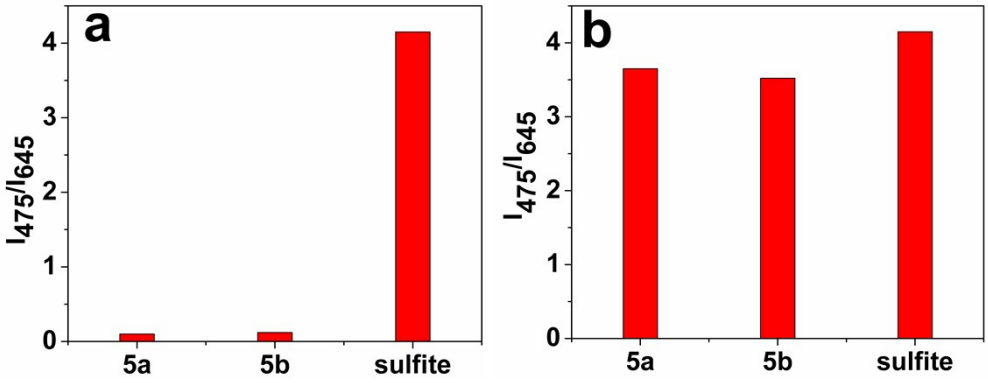


Figure S15. (a) Ratio of fluorescence intensity (I_{475}/I_{645}) when SO_2 donors **5a** and **5b** (100 µM) and dye **7** were incubated in the absence of light. No significant increase in fluorescence intensity ratio was observed and (b) Ratio of fluorescence intensity (I_{475}/I_{645}) when SO_2 donors **5a** and **5b** (100 µM) and dye **7** were incubated in the presence of light irradiation for 60 min at $\lambda \geq 365$ nm. Significant increase in the fluorescence intensity ratio was observed by decrease in the fluorescence at 645 nm of sulfite dye and a corresponding increase in fluorescence at 475 nm of sulfite adduct.

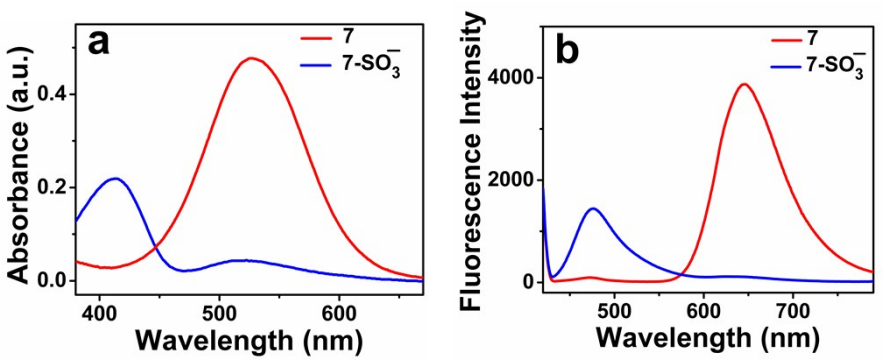


Figure S16. (a) Absorption spectra and (b) Emission spectra of dye 7 (10 μM, 3: 7 ACN/PBS buffer at pH 7.4) were recorded in the presence of $NaHSO_3$ (150 μM). Excitation wavelength: 410 nm.

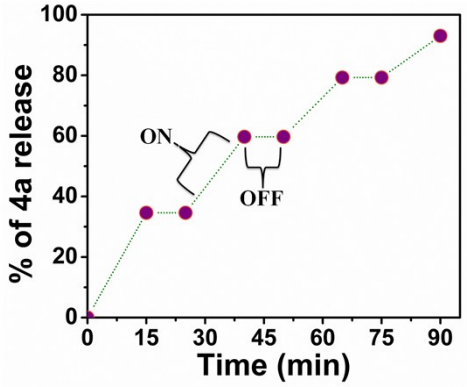


Figure S17. Progress of the release of **4a** (FAEE) from sulfonate **5a** under light and dark conditions (ON indicates the start of light irradiation and OFF indicates the end of light irradiation.

5. Measurement of fluorescence quantum yields:

The fluorescent quantum yields (QY) of sulfonate **5a** and carbonate **6** in acetonitrile/PBS buffer (3:7) were determined by reference point method. 9, 10-Diphenylanthracene in ethanol (literature quantum yield: 95%) was used as the standard sample to calculate the fluorescent QY of **5a** and **6**. The absorbance values corresponding to donors **5a** and **6** at the excitation wavelength were measured on a Shimadzu UV-2450 UV/vis spectrophotometer. Photoluminescence (PL) emission spectra of all the sample solutions were recorded by a Hitachi F-7000 fluorescence spectrophotometer at an excitation wavelength of 350 nm. Further, the fluorescence quantum yields of **5a** and **6** were calculated using equation (1).

$$\frac{\Phi_S}{\Phi_R} = \frac{A_S}{A_R}\frac{(Abs)_R}{(Abs)_S}\frac{\eta_S^2}{\eta_R^2} \qquad (1)$$

Where **Φ** represents quantum yield, **Abs** represents absorbance, **A** represents area of the fluorescence curve, and **η** is refractive index of the medium. The subscripts **S** and **R** denote the corresponding parameters for the sample and reference, respectively.

6. Determination of incident photon flux (I_0) of the UV lamp by potassium ferrioxalate actinometry:

Potassium ferrioxalate actinometry was used for the determination of incident photon flux (I_0) of the UV lamp used for irradiation. Solution of potassium ferrioxalate, 1, 10-phenanthroline and the buffer solution were prepared following the literature procedure. [1]

Solution (0.006 M) of potassium ferrioxalate was irradiated using 125W medium pressure Hg lamp as UV light source (≥ 365 nm) and 1M $CuSO_4$ solution as UV cut-off filter. At regular interval of time (3 min), 1mL of the aliquots was taken out and to it 3 mL of 1,10 phenanthroline solution and 2 mL of the buffer solution were added and the whole solution was kept in dark for 30min. The absorbance of red phenanthroline-ferrous complex formed was then measured spectrophotometrically at 510 nm. The amount of Fe^{2+} ion was determined from the calibration graph. The calibration graph was plotted by measuring the absorbance of phenanthroline-ferrous complex at several known concentration of Fe^{2+} ion in dark. From the slope of the graph the molar absorptivity of the phenanthroline-ferrous complex was calculated to be 1.10×10^4 M^{-1} cm^{-1} at 510 nm which is found to be similar to reported value. Using the known quantum yield (1.283 ± 0.023) for potassium ferrioxalate actinometer at 363.8 nm, [2] the number of Fe^{2+} ion formed during photolysis and the fraction of light absorbed by the actinometer, the incident photon flux (I_0) at 350 nm of the 125W Hg lamp was determined as 1.55×10^{17} photons $s^{-1}cm^{-2}$.

7. Photolysis of SO_2 donors 5a, 5b and carbonate 6 and measurement of photochemical quantum yields:

a) Photolysis of SO_2 donors 5a, 5b and carbonate 6 using UV irradiation (≥ 365 nm):

A solution of 1×10^{-4} M of the SO_2 donors **5a**, **5b** and carbonate **6** were prepared in 3:7 ACN: PBS; pH 7.4. Half of the solution was kept in dark and to the remaining half nitrogen

was passed and irradiated using 125 W medium pressure Hg lamp as UV light source ($\lambda \geq$ 365 nm) and 1M $CuSO_4$ solution as UV cut–off filter for 60 min. At a regular interval of time, 20µl of the aliquots were taken and analyzed by RP-HPLC using mobile phase acetonitrile/water (9:1), at a flow rate of 0.5 mL/min (detection: UV 254 nm). Peak areas were determined by RP-HPLC, which indicated a gradual decrease of **5a**, **5b** and carbonate **6** with time, and the average of three runs. The reaction was followed until the decomposition is more than 90%. Based on HPLC data for decomposition, we plotted normalized [A] (HPLC peak area) versus irradiation time. We observed an exponential correlation for the decomposition of starting material, which suggested a first order reaction. Further, the photochemical quantum yields for the decomposition of starting material was calculated using equation (2).

$$(\Phi_p)_S = (\Phi_p)_{act} \frac{(k_p)_S}{(k_p)_{act}} \frac{(F_{act})}{(F_S)} \qquad (2)$$

Where, the subscript 'S' and 'act' denotes sample (**5a, 5b** and **6**) and actinometer respectively. Potassium ferrioxalate was used as an actinometer. Φ_p is the photolysis quantum yield, k_p is the photolysis rate constant and F is the fraction of light absorbed.

b) Two-photon uncaging cross-section (δ_u) of 5a, 5b and carbonate 6 at 730 nm:

The two-photon uncaging cross-section was measured by comparing the photolysis rates of **5a, 5b and 6** with that of **DMNB-OAc** (4,5-dimethoxy-2-nitrobenzyl acetate) as a reference (δ_u = 0.035 at 730 nm). Aliquots (100 µL) containing **5a, 5b and 6** (1×10^{-4} M in ACN/PBS buffer (3:7), pH 7.4) were irradiated independently with the 730 nm Ti: sapphire laser (pulse width 100 fs, 80 MHz) emitting at an average of 300 mW for 60 min. Each sample was monitored by RP-HPLC using mobile phase acetonitrile/water (9:1), at a flow rate of 0.5 mL / min (detection: UV 254 nm). Similar photolysis experiments were conducted using 100 µL aliquots of **DMNB-OAc** (1×10^{-4} M in ACN/PBS buffer (3:7), pH 7.4). The photolysis solution of **DMNB-OAc** was also analyzed by RP-HPLC. The compounds were eluted with acetonitrile/water (9:1), at a flow rate of 0.5 mL / min and monitored by absorbance at 254 nm and the first-order decay constants for the compounds were analyzed, To calculate the value of δ_u for **5a,** we used the formula $\delta_u\Phi_u(5a) = \delta_u\Phi_u(\text{reference}) \times k_{obs}(5a)/k_{obs}(\text{reference})$, where $\delta_u\Phi_u(\text{reference}) = 0.035$ GM.

8. Experimental procedure for *in vitro* biological application study:

Cellular Imaging: To investigate cellular uptake and the capability of SO_2 generation from **5a**, the dye **7** was used. Thereafter, cell imaging studies were carried out in *E. cloacae* cells by varying the growth time (after growing for 3 h and 18 h). First, the *E. cloacae* cells (MTCC 509) were grown in nutrient broth medium was seeded ($1x10^6$ cells per well) into three different 12 well plates (A, B, and C) and incubated for 3 h at 37 °C. After incubation, medium was removed, and cells were washed thrice with sterile PBS (1X). Then the plate A stained with 1 mL of fresh SO_2 sensitive dye **7** (10 μM) and the cells were incubated for 30 min at 37 °C. After 30 min of incubation, dye was removed and cells were washed thrice with PBS. Then, the imaging was done with DMi8 Leica microscope at different wavelength (410/470 nm) and (530/605 nm). Then the plate B stained with 1 mL of SO_2 sensitive dye **7** (10 μM) and sulfonate **5a** (100 μM) dissolved in PBS and the cells were incubated for 30 min at 37 °C. After 30 min of incubation, the plate B was irradiated (keeping the cell-culture plate 5 cm apart from the light source) under UV light ($\lambda \geq 365$ nm) by a 125 W medium pressure Hg lamp using a suitable filter (1 M $CuSO_4$ solution) for 60 min. After irradiation, the cells were further incubated for another 30 min and the imaging was done by DMi8 Leica microscope at two different wave length (410/470 nm) and (530/605 nm) respectively and analyzed by Las x. The plate C stained with 1 mL of fresh SO_2 sensitive dye **7** (10 μM) and $NaHSO_3$, then the cells were incubated for 30 min at 37 °C. After 30 min of incubation, dye was removed and cells were washed thrice with PBS. Then, the imaging was done with DMi8 Leica microscope at different wavelength (410/470 nm) and (530/605 nm).

Next, the cell imaging studies were carried out in the *E. cloacae* cells after growth period of 18 h. The *E. cloacae* cells (MTCC 509) were grown in nutrient broth medium was seeded (1×10^6 cells per well) into two different 12 well plates (D and E) and incubated for 18 h at 37 °C. After incubation, medium was removed, and cells were washed thrice with sterile PBS (1X). Then the plate D stained with 1 mL of fresh SO_2 sensitive dye **7** (10 μM) and the cells were incubated for 30 min at 37 °C. After 30 min of incubation, dye was removed and cells were washed thrice with PBS. Then, the imaging was done with DMi8 Leica microscope at different wavelength (410/470 nm) and (530/605 nm). Then the plate E stained with 1 mL of SO_2 sensitive dye **7** (10 μM) and sulfonate **5a** (100 μM) and the cells were incubated for 30 min at 37 °C. After 30 min of incubation, the plate B was irradiated (keeping the cell-culture plate 5 cm apart from the light source) under UV light ($\lambda \geq 365$ nm) by a 125 W medium

pressure Hg lamp using a suitable filter (1 M $CuSO_4$ solution) for 60 min. After irradiation, the cells were further incubated for another 30 min and the imaging was done by DMi8 Leica microscope at two different wave length (410/470 nm) and (530/605 nm) respectively and analyzed by Las x.

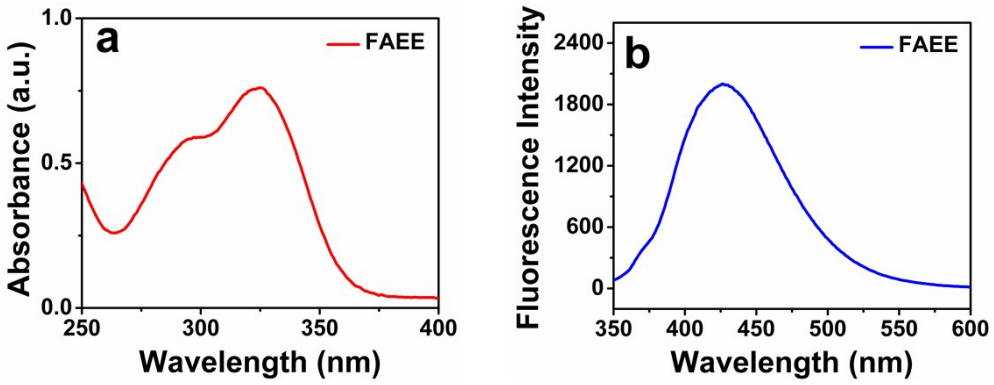


Figure S18. (a) Absorbance spectrum of FAEE, (b) Emission spectrum of FAEE. Excitation wavelength: 330 nm.

9. Fluorescence enhancement study (self-reporting ability) of the designed sulfonate 5a in *in vitro*:

In order to study the self-reporting ability of the designed sulfonate **5a** in *in vitro*, the cell imaging studies were carried out in the *E. cloacae* cells after growth period of 18 h. The *E. cloacae* cells (MTCC 509) were grown in nutrient broth medium was seeded (1×10^6 cells per well) into three different 12 well plates (A and B) and incubated for 18 h at 37 °C.

The plate A was incubated with sulfonate **5a** independently for 3 h followed by imaging using a fluorescence microscope. The emission signal at 410 nm (excitation wavelength 330 nm) was observed before photolysis (at 0 min). Next, the plate B was incubated with sulfonate **5a** and then irradiated (keeping the cell-culture plate 5 cm apart from the light source) under UV light ($\lambda \geq 365$ nm) by a 125 W medium pressure Hg lamp using a suitable filter (1 M $CuSO_4$ solution) for 60 min. After irradiation, the cells were further incubated for another 30 min and the imaging was done by fluorescence microscopy (Ex/Em: 330/410 nm), and we observed an increase in the fluorescence signal at 410 nm. A significant enhancement in fluorescence signal was recorded (**Figure S19**, dark versus light (1P and 2P excitation)).

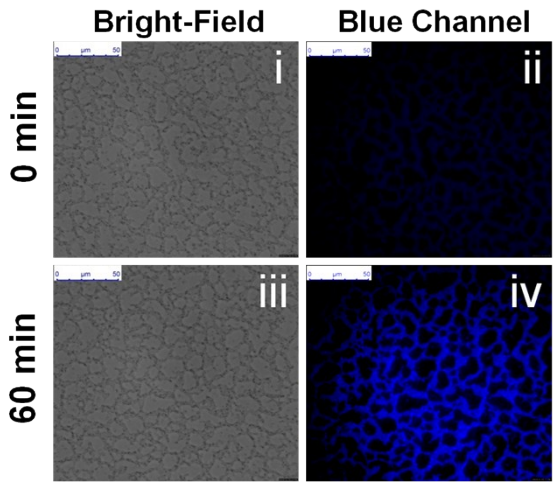


Figure S19. (ii and iv) *E.cloacae* (MTCC 509) cells pre-treated with **5a** (100 μM) and fluorescence microscopy images recorded at 0 min and 60 min of irradiation respectively. Scale bar=50 μm.

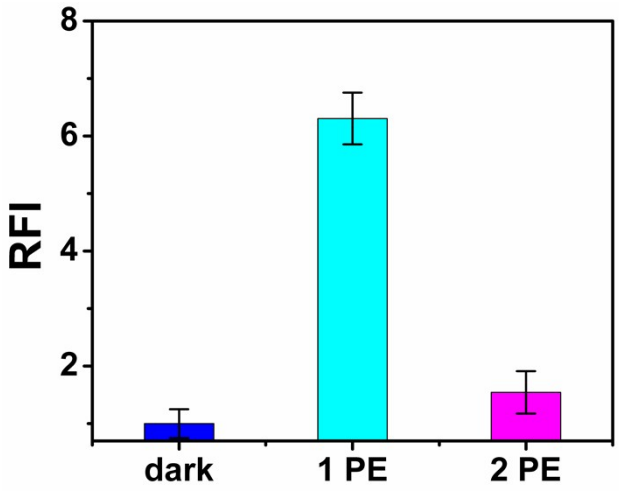


Figure S20. Fluorescence enhancement data for the cellular experiment upon 1 PE and 2 PE. Ex/Em: 330/408 nm.

10. Antibacterial activity assay of sulfonates 5a, 5b and carbonate 6 against *E.cloacae* (MTCC 509):

The antibacterial activity of sulfonates **5a**, **5b** and carbonate **6** were evaluated against *E.cloacae* (MTCC 509) cells, using a broth dilution method[3]. The mid-logarithmic phase bacteria was obtained by growing bacteria in a nutrient broth and then diluted in the same medium to give approximately 10^6 CFU (colony forming units) mL^{-1}.

Antibacterial activity assay of 5a, 5b and 6 before photolysis:

Aliquots (150 µL) of a suspension containing bacteria in a culture medium were added to 150 µL of DMSO and PBS buffer (1:99 v/v) containing **5a, 5b** and **6**, and it was serially diluted from100 µM/mL to 0.195 µM/mL. The inhibition of growth was determined by measuring the absorbance at 600 nm with a microplate reader after an incubation of 18 h at 37 °C.

Antibacterial activity assay of 5a, 5b and 6 after photolysis:

Aliquots (150 µL) of a suspension containing bacteria in a culture medium were added to 150 µL of DMSO and PBS buffer (1:99 v/v) containing **5a, 5b** and **6**, and it was serially diluted from 100 µM/mL to 0.195 µM/mL. Plates were kept at 37 °C for 4 h and then irradiated (keeping the cell-culture plate 5 cm apart from the light source) under UV light ($\lambda \geq 365$ nm) by a 125 W medium pressure Hg lamp using a suitable filter (1 M $CuSO_4$ solution) for 60 min. The plate was again incubated at 37 °C for 14 h. The inhibition of growth was determined by measuring the absorbance at 600 nm with a microplate reader.

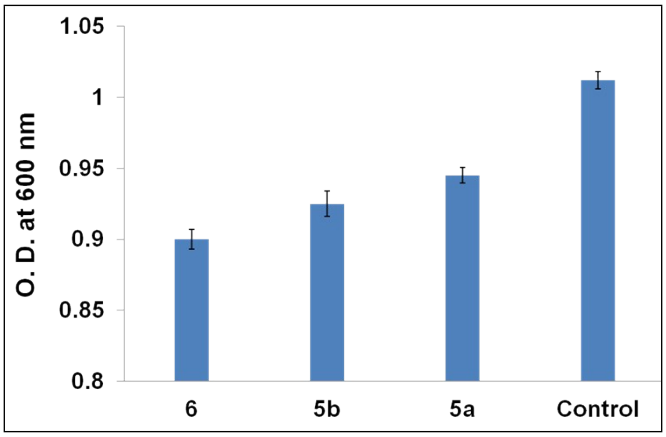


Figure S21. Antibacterial activity of **5a**, **5b** and **6** (100 µM) before photolysis. The culture medium was supplemented with measured at 600 nm after 18 h at 37 ± 2 °C. Data are the mean of triplicate experiments ± S.E.

Agar well Diffusion Test:

Principle:

The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Method:

Petriplate containing 20 mL of nutrient broth medium was seeded with overnight culture of *E.cloacae* (MTCC 509) cells. Wells were cut and 15 μM of test compounds (**5a, 5b, 6, and FAEE**) were added. Petriplate was kept at 37 °C for 4 h and then irradiated (keeping the cell-culture plate 5 cm apart from the light source) under UV light ($\lambda \geq 365$ nm) by a 125 W medium pressure Hg lamp using a suitable filter (1 M $CuSO_4$ solution) for 60 min. The plates were then incubated at 37 °C for 12 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

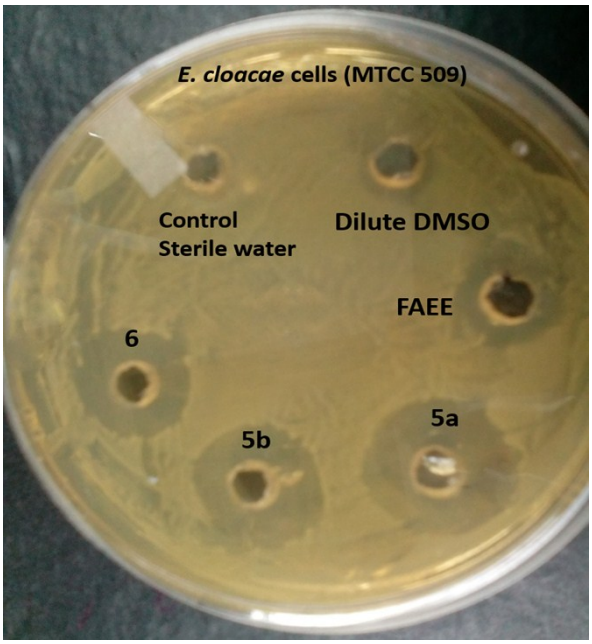


Figure S22. Antibacterial screening of **5a, 5b, 6,** and **FAEE** (15 μM) after photolysis for 60 min by agar well diffusion method against *E.cloacae* (MTCC 509) cells.

11. References:

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2. Demas, J. N.; Bowman, W. D.; Zalewski, E. F.; Velapoldi, R. A. *J. Phys. Chem*. **1981**, *85*, 2766.

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