

## Supporting Information

### **Novel Self-assembled Organic Nanoprobe for Molecular Imaging and Treatment of Gram-positive Bacterial Infection**

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#### **1. Synthesis**

**2-hydroxy-1,2-diphenylethanone (compound 1):** In a 50 mL round bottom flask, 1.75 g vitamin VB1, 3.5 mL water and 15 mL ethanol were added and the flask was allowed to cool in an ice bath. At the same time, 5mL of 10% sodium hydroxide solution in a test tube was also placed in the ice bath to cool. Then with cooling in ice bath, the sodium hydroxide solution was added to the reaction solution, and continued to shake. During the process, pH of the solution was adjusted to 9-10 until the solution was yellow, then the ice bath was removed. This solution was next added with the newly steamed benzaldehyde and installed with reflux condenser in a water bath with the temperature of 65-75 °C for 1.5 h, without heating the mixture to boiling the whole time. The pH of the solution was maintained in the range of 8 to 9 during the reaction. At the end, the reaction mixture became an orange uniform solution. The reaction mixture was cooled

to room temperature before precipitating pale yellow crystals 1.80 g (85% yields).  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  4.56 (s, 1H), 5.94 (s, 1H), 7.27–7.44 (m, 7H), 7.50 (t, 1H), 7.94 (d, 2H).

**Benzil (compound 2):** Compound 1 (1.0 g, 0.094 mol) and 10 mL conc.  $\text{HNO}_3$  was taken in a 50 mL round bottom flask and heated on a boiling water bath with occasional shaking until the evolution of oxides of nitrogen seized. The reaction mixture was then poured onto crushed ice, and stirred well till the yellow solid separated out. It was then filtered and recrystallized in ethanol to yield benzil 892 mg (90% yields). The obtained substance was identical with a commercial one.

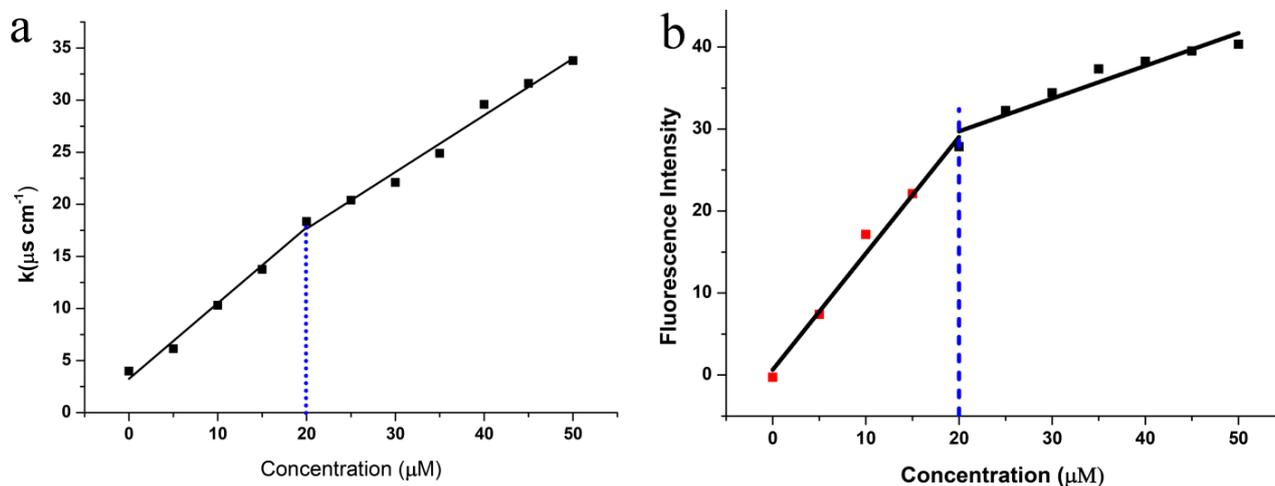
**Compound 3:** Aniline (0.91 mL, 10.00 mmol) and 4-pyridine carboxaldehyde (1.06 g, 10.00 mmol) were dissolved in acetic acid (100 mL) and stirred for 1 h at room temperature. Benzil (2.10 g, 10.00 mmol) and ammonium acetate (3.55 g, 46.00 mmol) were added subsequently. The mixture was heated at 120 °C overnight. After quenching the reaction, the dark solution was poured into copious amounts of water. After neutralization, the mixture was filtered and washed with water. Reprecipitation in methanol from dichloromethane solution afforded a white powder (1.57 g, yield = 42%).  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 8.48 – 8.47 (d,  $J = 2\text{H}$ ), 7.58 - 7.60 (d,  $J = 2\text{H}$ ), 7.31-7.35 (m, 4H), 7.22-7.29 (m, 7H), 7.13-7.14 (d, 2H), 7.09-7.10 (d,  $J = 6.71\text{ Hz}$  2H).  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 149.71, 143.84, 139.20, 137.78, 136.65, 133.96, 132.46, 131.05, 130.00, 129.48, 129.01, 128.47, 128.34, 128.30, 128.27, 127.32, 126.97, 122.32. HRMS (ESI)  $m/z$ : calcd for  $\text{C}_{26}\text{H}_{19}\text{N}_3$ , 373.1579 [M]; found 374.1651 [M + H] $^+$ .

**Compound 4:** Compound 3 (0.19 g, 0.50 mmol) was reflux with 1,12-dibromododecane (0.17 g, 0.52 mmol) in 15 mL acetonitrile for 10 h. After the mixture cooled to room temperature, it was evaporated under reduced pressure to remove the solvent. The residue was purified by a silica gel column chromatography using a DCM and methanol mixture (120:1 v/v) as the elution solvent to give compound 4 as a green powder (157 mg, yield = 45%).  $^1\text{H NMR}$  (400 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ ): 8.90- 8.91 (d, 2H), 7.77 - 7.79 (d, 2H), 7.49 -7.53 (m, 7H), 7.27 -7.37 (m, 8H), 4.45 - 4.49 (t, 2 H), 3.50-3.54 (t, 2 H), 1.87-1.93 (m, 2H), 1.74-1.81 (m, 2H), 1.24-1.36 (m,

16H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ ): 142.84, 141.93, 138.44, 138.14, 134.31, 133.83, 131.41, 129.24, 128.50, 128.28, 127.59, 127.17, 127.01, 126.85, 126.79, 125.86, 124.87, 121.92, 58.36, 53.26, 30.55, 28.66, 27.20, 27.18, 27.16, 27.06, 26.66, 26.42, 25.83, 23.70.

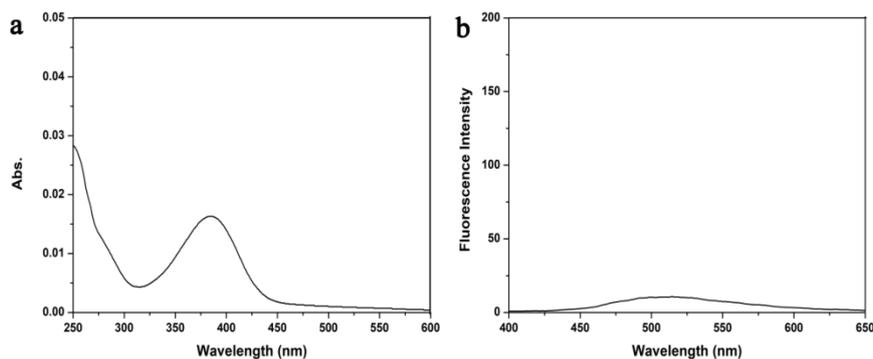
**Compound TPIP:** Compound 4 (0.14 g, 0.020 mol) was reflux with 10 mL pyridine for 8 h. After the mixture cooled to room temperature, it was evaporated under reduced pressure to remove the pyridine. The residue was purified by a silica gel column chromatography using a DCM and methanol mixture (15:1 v/v) as the elution solvent to give compound TPIP as a green powder (66.1 mg, yield = 42%).  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ ): 9.18-9.19 (d, 2H), 8.99 - 9.00 (d, 2H), 8.62-8.66 (t, 1H), 8.18-8.21 (t, 2H) 7.80-7.82 (d, 2H), 7.51-7.55 (m, 7H), 7.29-7.39 (m, 8H), 4.63-4.67 (t, 2H), 4.50-4.54 (t, 2H), 1.88-1.95 (m, 4H), 1.25-1.36 (m, 16H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ ): 145.94, 145.29, 145.07, 144.03, 140.58, 140.24, 136.42, 133.55, 131.38, 130.63, 130.41, 129.70, 129.32, 129.13, 128.98, 128.90, 128.53, 127.96, 126.99, 123.99, 61.08, 60.34, 52.58, 45.71, 31.23, 30.88, 30.30, 29.32, 29.24, 28.86, 28.84, 25.86.

## 2. Determination of the CAC



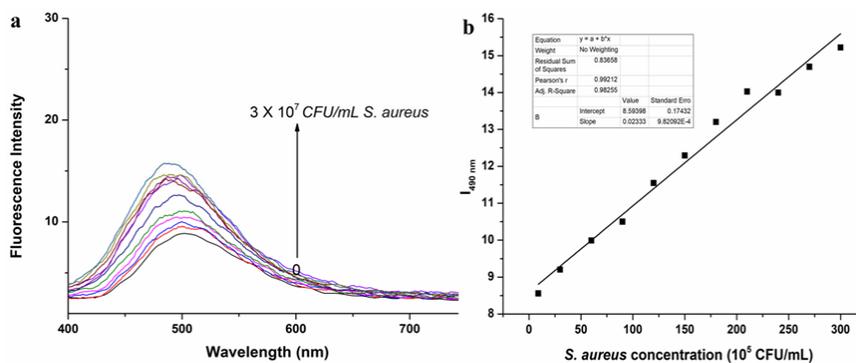
**Figure S1.** (a) Plot of conductivity versus the concentration of TPIP. (b) Plot of fluorescence intensity at 500 nm versus the corresponding TPIP concentration.

### 3. Spectroscopy characterization of TPIP-FONs



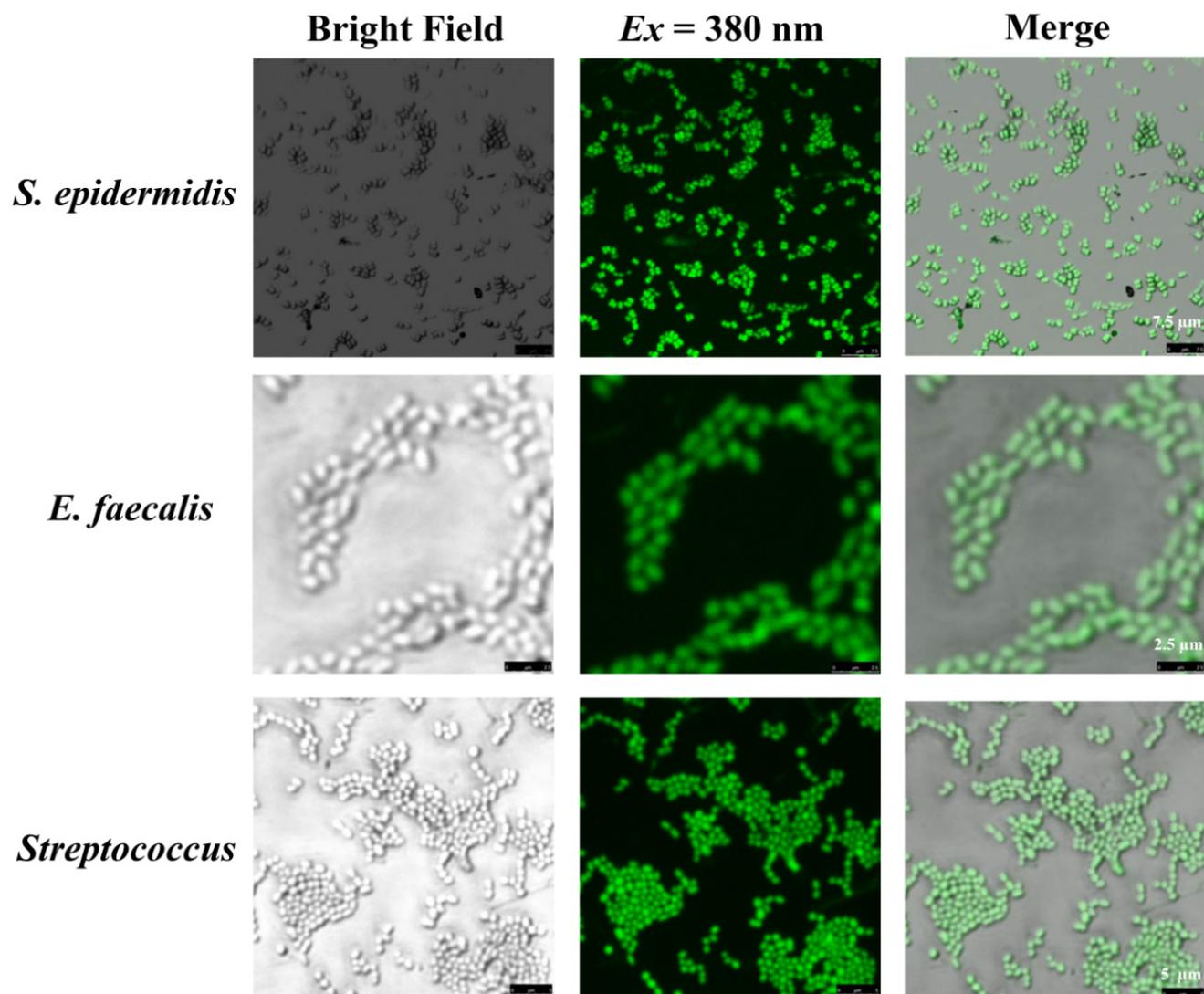
**Figure S2.** (a) UV-vis absorption spectrum of TPIP-FONs (5 μM). (b) PL spectra of TPIP-FONs (5 μM) in water aqueous.

### 4. Bacteria detection



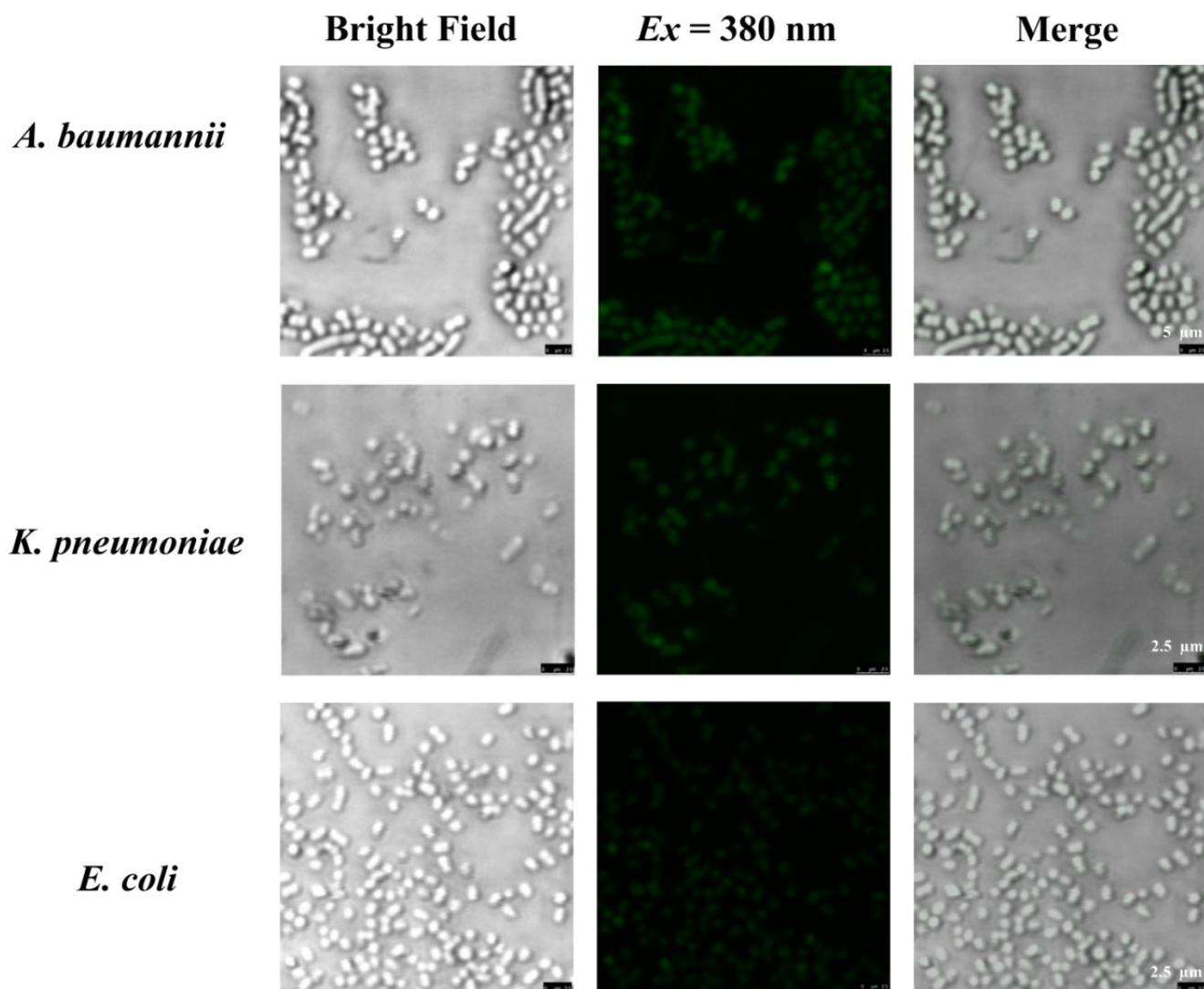
**Figure S3.** (a) Fluorescence intensity of TPIP-FONs aqueous solution upon addition of varying amount of *S. aureus* cells. (b) Fluorescence intensity at 490 nm against concentrations of *S. aureus* cells.

### 5. Fluorescent imaging of Gram-positive bacteria



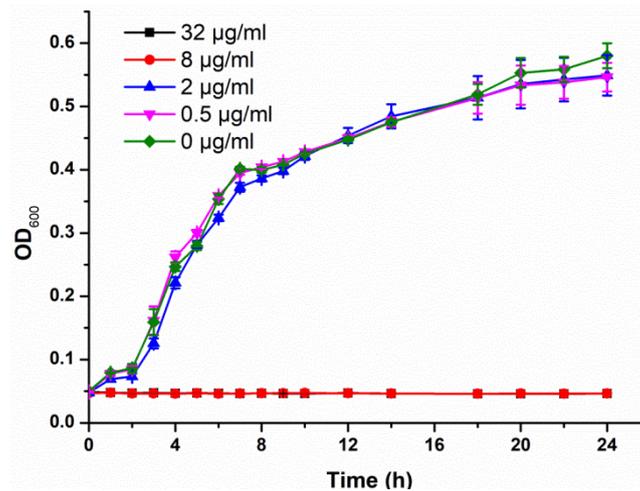
**Figure S4.** Confocal fluorescence images of three Gram-positive bacteria treated with 5  $\mu$ M TPIP-FONs.

## 6. Fluorescent imaging of Gram-negative bacteria



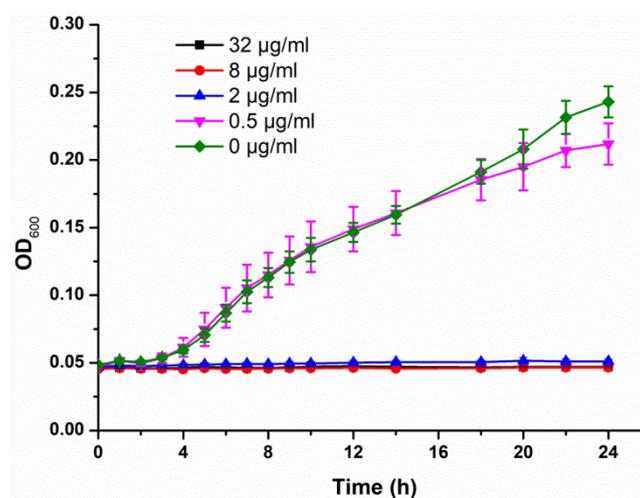
**Figure S5** Confocal fluorescence images of three Gram-negative bacteria treated with 5  $\mu\text{M}$  TPIP-FONs.

## 7. Concentration-dependent antimicrobial of TPIP-FONs for *E. coils*



**Figure S6.** Inhibition effect of different concentrations of TPIP-FONs on the growth of *E. coli* as a function of incubation time.

### 8. Concentration-dependent antimicrobial of TPIP-FONs for *C. albicans*



**Figure S7.** Inhibition effect of different concentrations of TPIP-FONs on the growth of *C. albicans* as a function of incubation time.

### 9. Determination of minimum inhibitory concentration

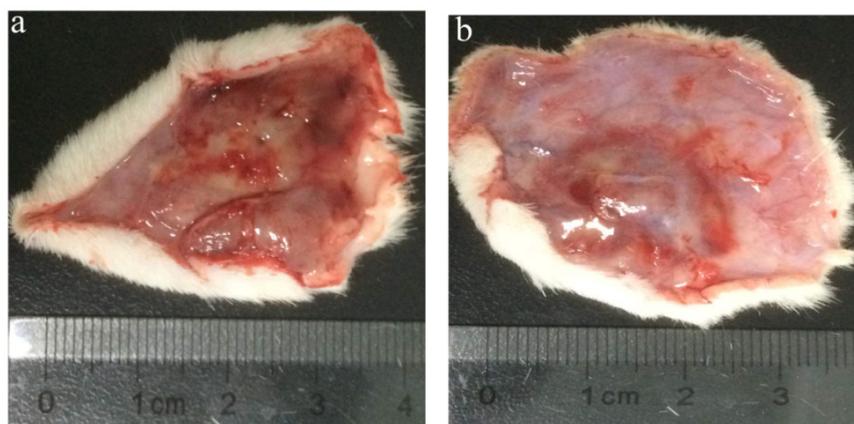
TPIP-FONs were dispersed in sterile phosphate-buffered saline and diluted from 0.5 to 32 µg/mL. Bacterial strains (1.0 mL, aliquots) cultured in respective solution were collected and centrifuged. Then the bacterial suspension was diluted 1000-fold to obtain the final concentration of  $10^5$  CFU mL<sup>-1</sup>. The diluted bacterial solutions were placed into a 96-well plate,

and then added into various concentrations of **TPIP-FONs**. The cultures were incubated at 37 °C for 24 h. After incubation, the bacterial viability was determined by measuring the OD<sub>600</sub> values. We defined the MIC values as the lowest sample concentration at which more than 90% bacteria was inhibited. The bacteria along was used as a negative control and the whole experiment was conducted for three times.

**Table S1.** Representative minimal inhibitory concentrations (MIC) for various bacterial strains.

Species	Type	MIC ( $\mu\text{g/ml}$ )
<i>S. aureus</i>	Gram-positive bacteria	2
<i>P. aeruginosa</i>	Gram-negative bacteria	> 32
<i>E.coli</i>	Gram-negative bacteria	8
<i>C.albicans</i>	Fungus	2

## 10. Photos of infected skin tissue

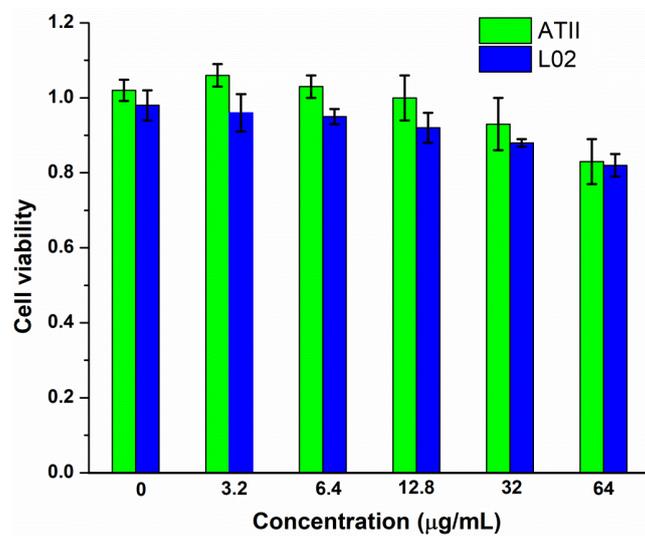


**FigureS8.** (a) Photographs of infected rat skin from the control group. (b) Photographs of infected rat skin from the **TPIP-FONs** treated group.

## 11. Cytotoxicity assay

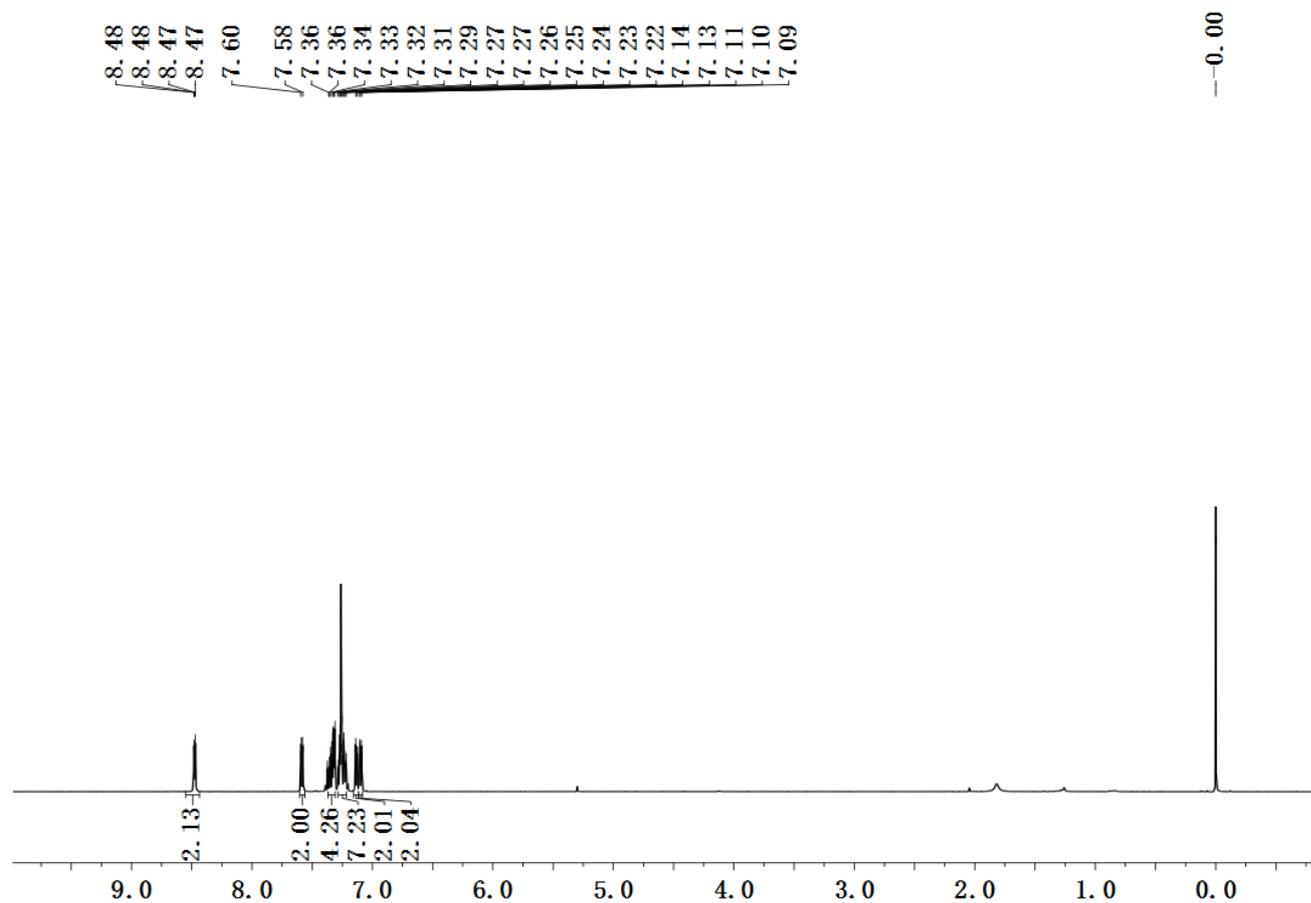
AT II cells (normal lung cells) and L02 (normal liver cells) were used to evaluate the cytotoxicity of **TPIP-FONs**. In this study the cells were cultured in cell media DMEM supplemented with 10% fetal bovine serum, 100 U of penicillin and  $100 \mu\text{g mL}^{-1}$  streptomycin in a humidified incubator at  $37 \text{ }^\circ\text{C}$  and 5%  $\text{CO}_2$ . The cells ( $5 \times 10^4$  cells per well) were seeded in a 96-well plate in cell media overnight and then incubated with different concentrations of **TPIP-FONs** (0, 3.2, 6.4, 12.8, 32, and  $64 \mu\text{g mL}^{-1}$ ) for 24 h. Then  $10 \mu\text{L}$  5% MTT was added

into each well and the cells were incubated for another 4 h. The culture medium containing MTT was removed and 150  $\mu$ L DMSO was added to each well to dissolve the formazan crystals with low-speed shaking 10 min. The absorbance at 490 nm was measured by a microplate reader.

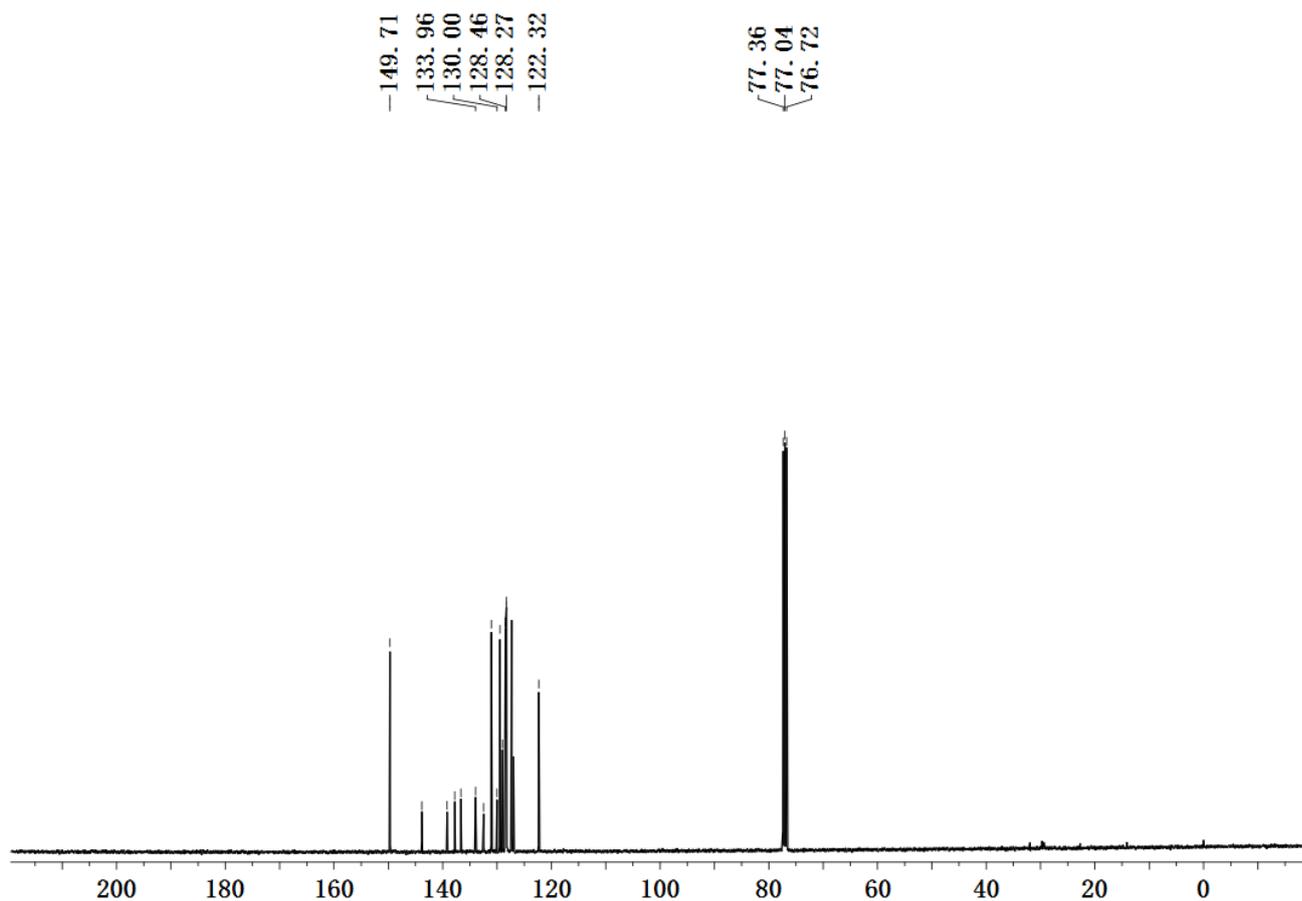


**FigureS9.** Cell viabilities of AT II and L02 cells treated with different concentrations of **TPIP-FONs**. Results shown were normalized to the control.

## 12. Characterization of compounds

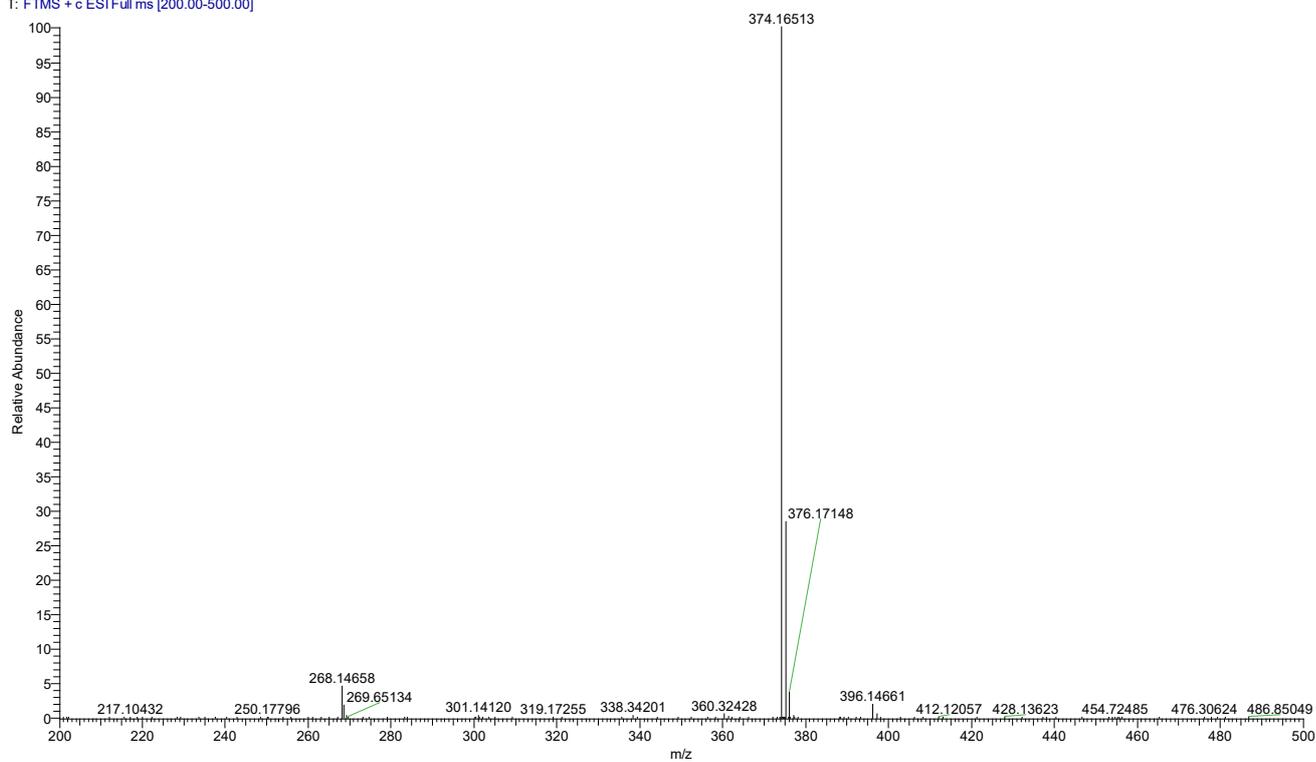


**Figure S10.**  $^1\text{H}$  NMR spectrum of compound **3** ( $\text{CDCl}_3$ ).

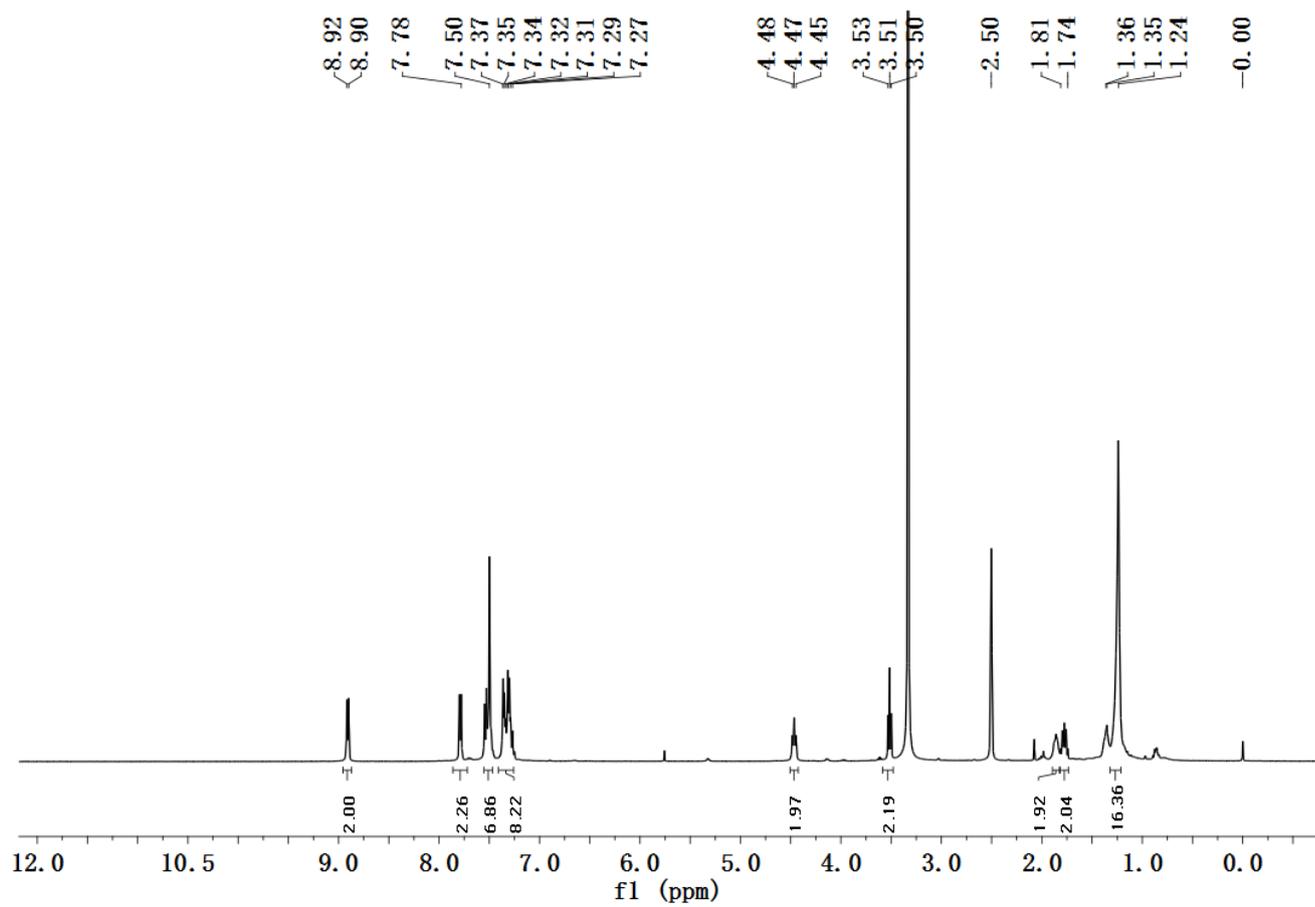


**Figure S11.**  $^{13}\text{C}$  NMR spectrum of compound **3** ( $\text{CDCl}_3$ ).

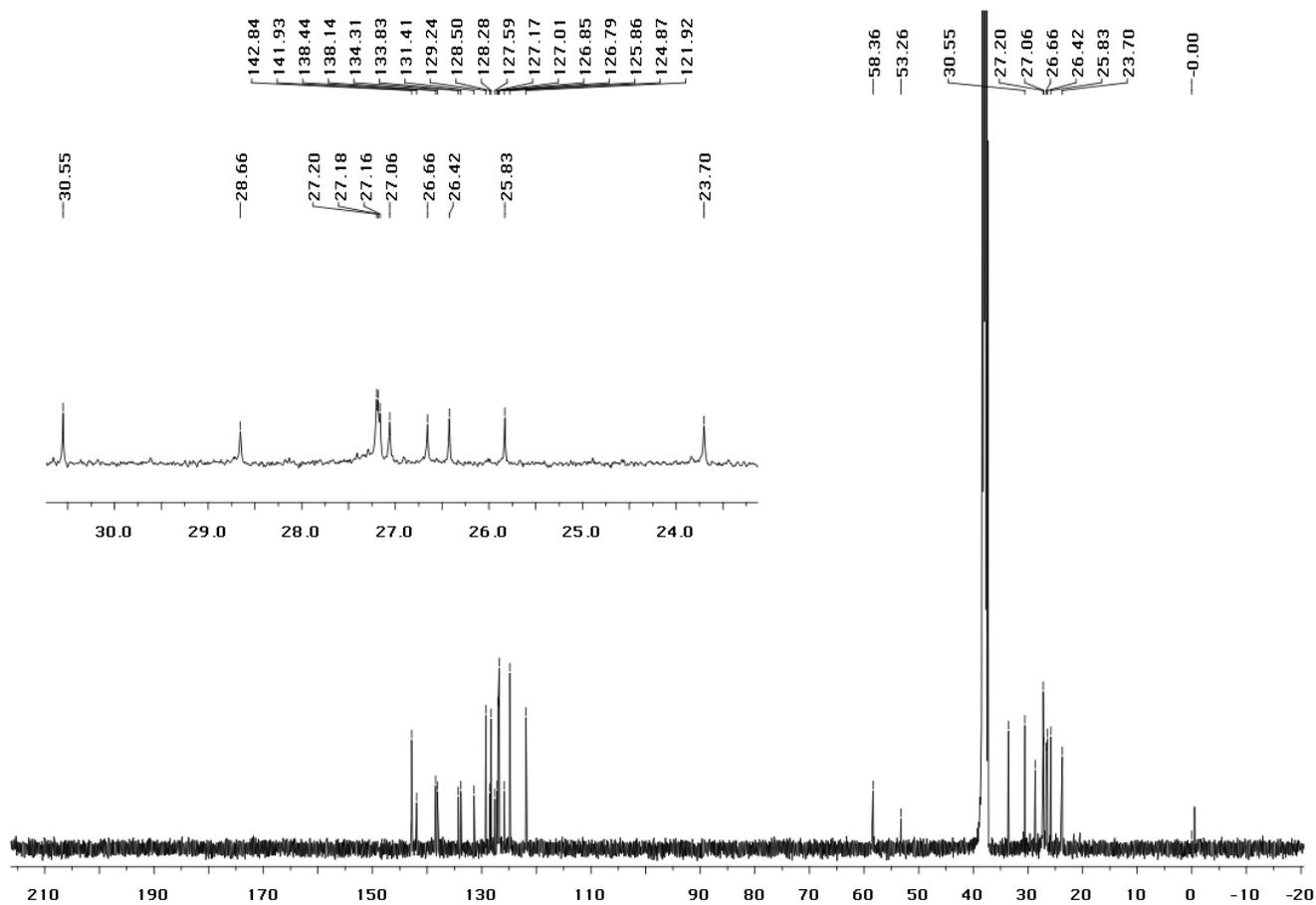
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T: FTMS + c ESI Full ms [200.00-500.00]



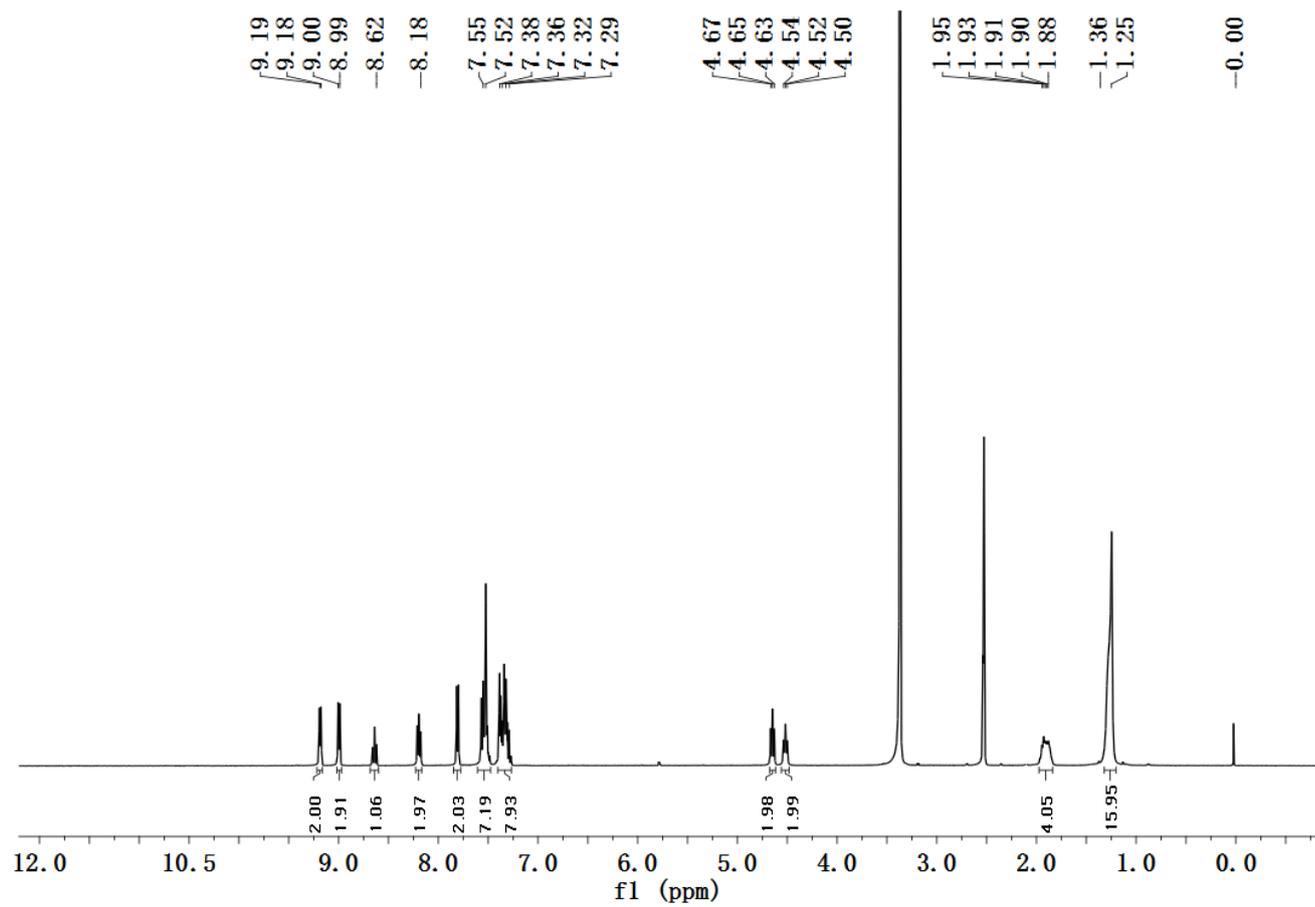
**Figure S12.** HRMS spectrum of compound **3**.



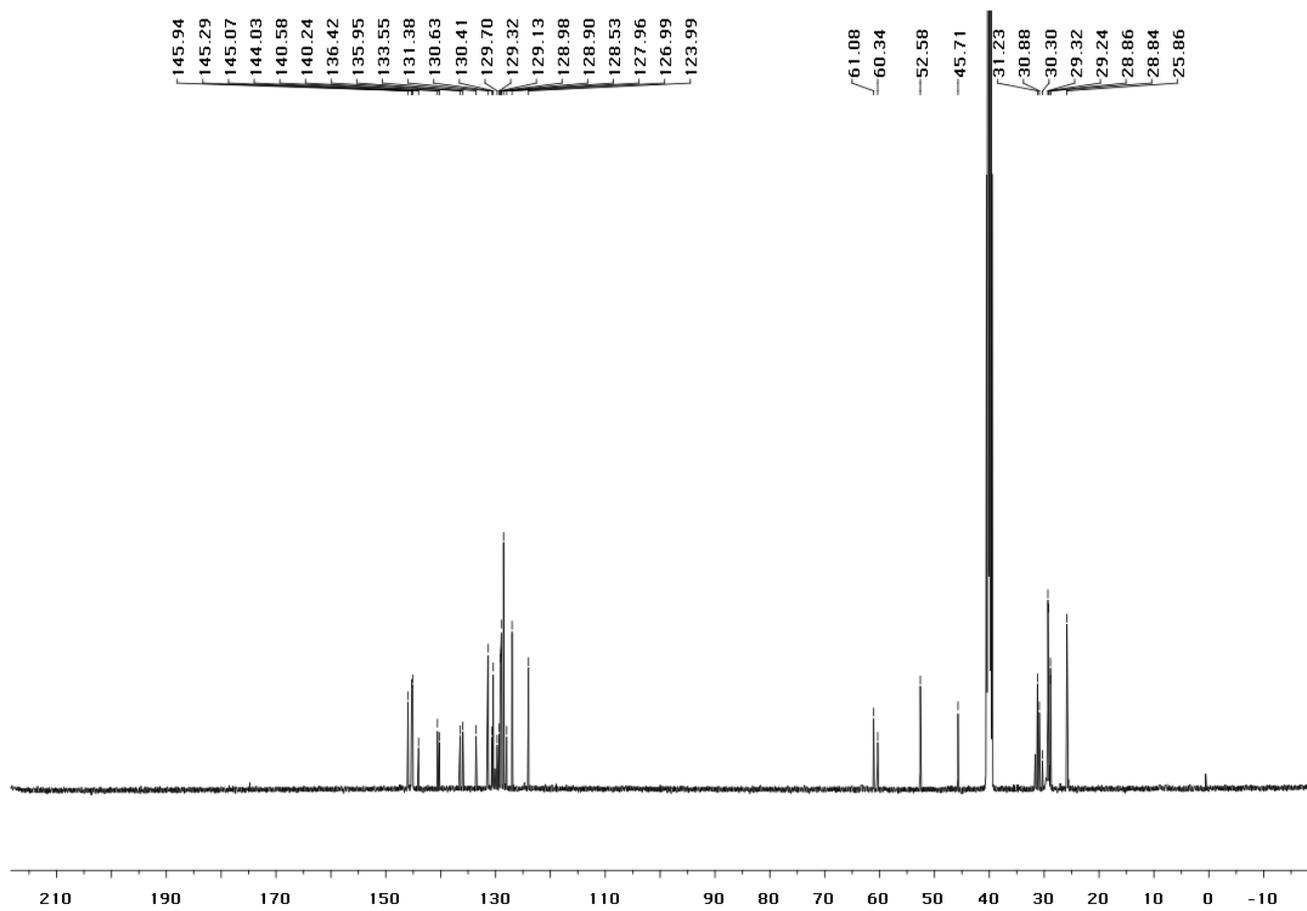
**Figure S13.**  $^1\text{H}$  NMR spectrum of compound **4** (DMSO- $d_6$ ).



**Figure S14.**  $^{13}\text{C}$  NMR spectrum of compound 4 ( $\text{DMSO-d}_6$ ).



**Figure S15.**  $^1\text{H}$  NMR spectrum of compound **TPIP** ( $\text{DMSO-d}_6$ ).



**Figure S16.**  $^{13}\text{C}$  NMR spectrum of compound TPIP ( $\text{DMSO-d}_6$ ).