Supporting Information

Fluorescent dyes with multiple quaternary ammonium centers for specific image discrimination and gram-positive antibacterial activity

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Synthesis and Characterization

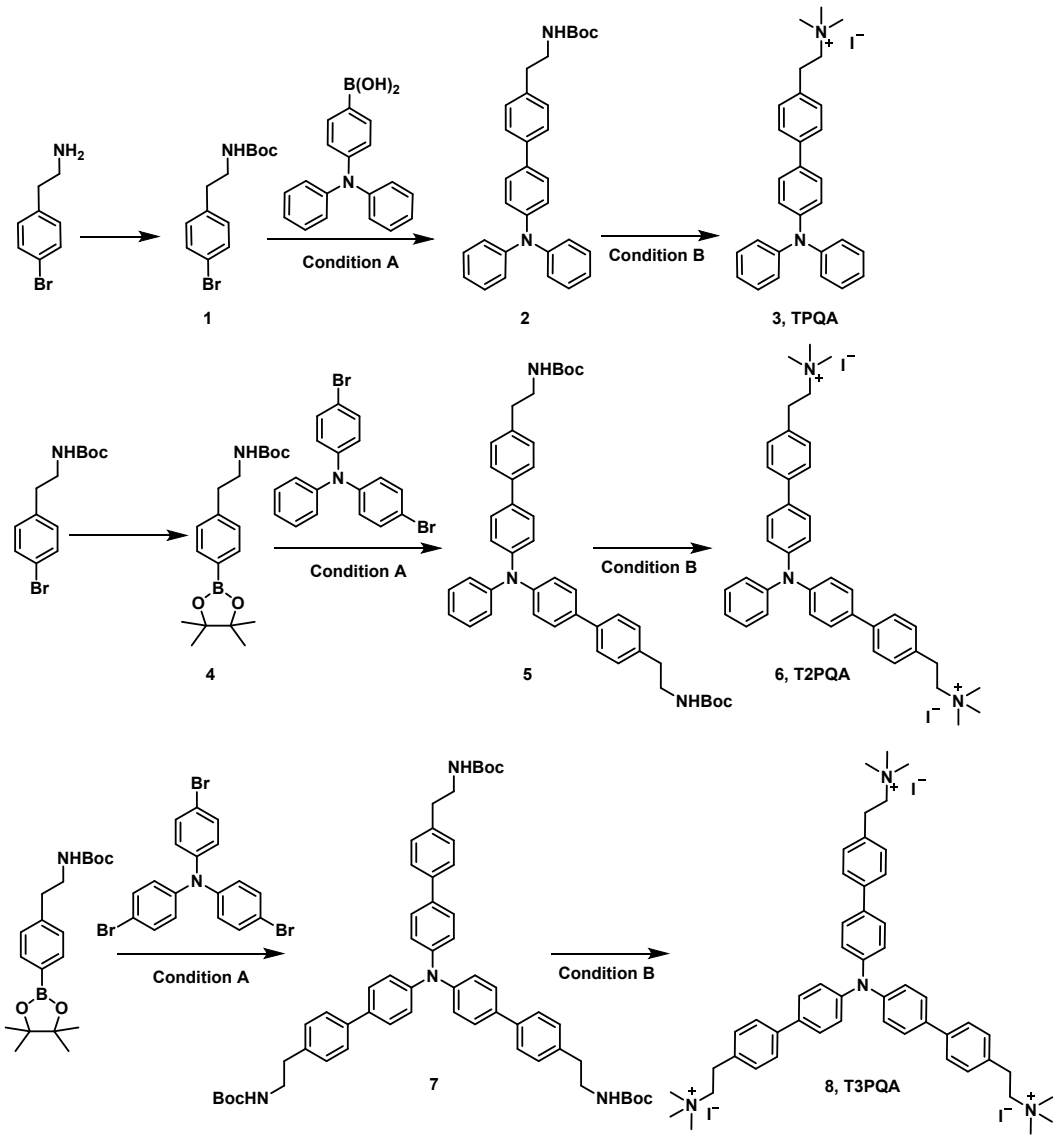


Figure S1. The synthesis routes of **TPQA**, **T2PQA** and **T3PQA**.
Condition A: Pd $(PPh_3)_4$, K_2CO_3, THF: H_2O (3:1), 85 °C, overnight. Condition B: 1): TFA, DCM, r.t., 3 h. 2): K_2CO_3, CH_3I, EA: CH_3OH (1:1), r.t., 18 h.

General Methods for Chemistry: All reagents and solvents were obtained from commercial suppliers and used without further purification. Flash column chromatography was performed over 200−300 mesh silica gel in petroleum ether (bp. 60-90 °C). Thin layer chromatography (TLC) employed glass 0.25 mm silica gel plates. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE NEO 400MHZ Digital NMR Spectrometer. All chemical shifts were reported relative to

tetramethylsilane (0 ppm for ^{1}H), $CDCl_3$ (7.26 ppm for ^{1}H, 77.16 ppm for ^{13}C) and DMSO (2.50 ppm for ^{1}H, 39.52ppm for ^{13}C), respectively. Mass spectra were recorded on AmaZon X LC-MS for electrospray ionization (HRMS).

Intermediate compounds 1 and 4 were synthesized according to the literature method.[1]

***Synthesis of compound 1*:** ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.42 (d, *J* = 8.4 Hz, 2H), 7.06 (d, *J* = 8.3 Hz, 2H), 3.41 – 3.29 (m, 2H), 2.75 (t, *J* = 7.0 Hz, 2H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 155.76, 137.94, 131.56, 130.51, 120.18, 79.28, 41.54, 35.59, 28.33. HRMS (ESI) *m/z* calcd for $C_{13}H_{19}BrNO_2^+$ $[M+H]^+$ 300.05937, found 300.05942.

***Synthesis of compound 4*:** ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.75 (d, *J* = 7.7 Hz, 2H), 7.20 (d, *J* = 7.6 Hz, 2H), 3.37 (t, *J* = 7.2 Hz, 2H), 2.81 (t, *J* = 7.0 Hz, 2H), 1.43 (s, 9H), 1.34 (s, 12H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 155.79, 142.29, 135.01, 128.19, 83.66, 83.42, 41.59，36.29，28.33, 24.95, 24.79. HRMS (ESI) m/z calcd for $C_{19}H_{31}BNO_4^+$ $[M+H]^+$ 348.23407, found 348.23289.

***Synthesis of compound 2*:** A mixture of 4-(Diphenylamino) phenylboronic acid (1.45 g, 5.0 mmol), *tert*-butyl(4-bromophenethyl) carbamate (1.8 g, 5.5 mmol), potassium carbonate (1.2 g, 8.7 mmol), 45 mL of THF, 15 mL of water, and Pd $(PPh_3)_4$ (200 mg) was carefully degassed and charged with argon. Then the reaction mixture was stirred at 85 °C for 10 h (monitored by TLC). When the reaction finished and cooled to ambient temperature, the reaction mixture was stopped by the addition of water, extracted with dichloromethane (10 mL x 3), and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and purified by column chromatography to afford compound **2** (2.23g, yield: 96%) as a yellow foamy solid (petroleum ether: ethyl acetate = 5:1). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.56 – 7.49 (m, 2H), 7.49 – 7.43 (m, 2H), 7.29 – 7.24 (m, 6H), 7.17 – 7.10 (m, 6H), 7.07 – 7.00 (m, 2H), 4.57 (s, 1H), 3.41 (t, *J* = 7.0 Hz, 2H), 2.83 (t, *J* = 7.0 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 155.92, 147.69, 147.09, 138.84, 137.57, 134.83, 129.28, 129.23, 127.61, 126.81, 124.40, 123.94, 122.91, 41.81, 35.88, 29.72, 28.44. HRMS (ESI) *m/z* calcd for $C_{31}H_{33}N_2O_2^+$ $[M+H^+]$ 465.2537, found 465.2545.

***Synthesis of compound TPQA*:** To a stirred solution of compound **2** (232 mg; 0.5 mmol) in dichloromethane (10 mL) was added trifluoroacetic acid (TFA) (2 mL). The solution was stirred at ambient temperature for 3 h. The volatile components were removed under reduced pressure, and the residue was treated with MeOH, then the solvent was evaporated under reduced pressure. The residence was washed with EA:PE=1:10 (55 mL×2), which was employed directly without further purification. Then the residence was dissolved in ethyl acetate (20 mL). Iodomethane (100 μL) and potassium carbonate (445 mg, 2.5 mmol anhydrous powder) were added and the mixture was stirred vigorously for 18 hours. After evaporation, the crude residue was dissolved in washed with EA:PE=1:10 (55 mL×2), then 50 mL dichloromethane (DCM) was added to the residue. Filtration and collects the organic phase to afforded target compound **TPQA** (254 mg, yield: 95%) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.67 – 7.54 (m, 4H), 7.40 (d, J = 7.9 Hz, 2H), 7.33 (t, J = 7.7 Hz, 4H), 7.16 – 6.90 (m, 8H), 3.63 – 3.54 (m, 2H), 3.17 (s, 9H), 3.12 – 3.06 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.51, 147.21, 138.75, 135.44, 134.09, 130.10, 130.03, 128.02, 126.90, 124.59, 123.83, 123.74, 66.23, 52.86, 52.82, 52.79, 28.56. HRMS (ESI) *m/z* calcd for $C_{29}H_{31}N_2O_2^+$ $[M]^+$ 407.2482, found 407.2472.

***Synthesis of compound 5*:** Intermediate **4** (2g，5.6mmol, 2.3eq), 4,4'-dibromotriphenylamin (967mg，2.4mmol，1.0eq), potassium carbonate (1.15g, 8.4mmol, 3.5eq) and $Pd(PPh_3)_4$ (200 mg) were mixed in THF (36 mL) and water (12 mL) in a flask purged with nitrogen. Then the reaction mixture was stirred at 85 °C for 10 h (monitored by TLC). When the reaction finished and cooled to ambient temperature, the reaction mixture was stopped by the addition of water, extracted with dichloromethane (10 mL x 3), and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and purified by column chromatography to afford compound **5** (1.15g, yield: 70%) as a light green solid (petroleum ether: ethyl acetate = 3:1). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.48-7.36 (m, 8H), 7.24 – 7.16 (m, 6H), 7.10-7.05 (m, 6H), 7.05-6.95 (m, 1H), 4.50 (s, 1H), 3.39 – 3.28 (m, 4H), 2.76 (t, J = 7.0 Hz, 4H), 1.37 (s, 18H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 155.87, 147.49, 146.86, 138.75, 137.60, 135.02, 129.31, 129.21, 127.64, 126.79, 124.58, 124.14, 123.10,

79.22, 41.72, 35.78, 28.39. HRMS (ESI) m/z calcd for $C_{44}H_{50}N_3O_4^+$ [M+H]$^+$ 684.37958, found 684.37759.

***Synthesis of compound T2PQA*:** To a stirred solution of compound **5** (342mg，0.5mmol) in dichloromethane (10 mL) was added trifluoroacetic acid(TFA) (4 mL). The solution was stirred at ambient temperature for 3 h. The volatile components were removed under reduced pressure, and the residue was treated with MeOH, then the solvent was evaporated under reduced pressure. The residence was washed with EA:PE=1:10 (55 mL×2), which was employed directly without further purification. Then the residence (24 mg，0.05 mmol) was dissolved in EA: MeOH=1:1 (2 mL). Iodomethane (60 μL) and potassium carbonate (89 mg) were added and the mixture was stirred vigorously for 18 hours. After evaporation, the crude residue was washed with EA:PE=1:10 (5 mL×2), then dissolved in 25 mL water. The residue extracted with dichloromethane (50 mL), and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to afford target compound **T2PQA** (33mg, yield: 80%) as yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.66-7.61 (m, 8H), 7.40-7.34 (m, 5H), 7.14-7.07 (m, 8H), 3.60 – 3.53 (m, 4H), 3.15 (s, 18H), 3.12-3.06 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.91, 146.57, 138.30, 135.12, 134.05, 129.81, 129.64, 129.28, 127.71, 126.54, 124.59, 123.85, 65.81, 52.45, 52.42, 52.38, 28.16. HRMS (ESI) m/z calcd for $C_{40}H_{47}N_3^{2+}$ [M/2]$^+$ 284.68795, found 284.68837.

***Synthesis of compound 7*:** Intermediate **4** (2g，5.6mmol, 3.5eq), tris(4-bromophenyl)amine (770mg，1.6mmol，1.0eq), potassium carbonate (1.15g, 8.4mmol, 3.5eq) and $Pd(PPh_3)_4$ (200 mg) were mixed in THF (36 mL) and water (12 mL) in a flask purged with nitrogen. Then the reaction mixture was stirred at 85 °C for 10 h (monitored by TLC). When the reaction finished and cooled to ambient temperature, the reaction mixture was stopped by the addition of water, extracted with dichloromethane (10 mL x 3), and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and purified by column chromatography to afford compound **7** (578mg, yield: 40%) as a light green solid (petroleum ether: ethyl acetate = 2:1). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.47-7.44 (m, 6H), 7.43-7.19 (m, 6H), 7.19-7.18

(m, 3H), 7.18-7.16 (m, 3H), 7.16-7.13 (m, 6H), 4.51 (s, 3H), 3.37-3.32 (m, 6H), 2.77 (t, J = 7.0 Hz, 6H), 1.37 (s, 27H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 155.87, 146.69, 138.72, 137.66, 135.25, 129.23, 127.71, 126.81, 124.38, 79.24, 41.73, 35.80, 28.39. HRMS (ESI) m/z calcd for $C_{57}H_{67}N_4O_6^+$ [M+H] $^+$ 903.50551, found 903.50290.

***Synthesis of compound T3PQA*:** To a stirred solution of compound **7** (450mg，0.5mmol) in dichloromethane (10 mL) was added trifluoroacetic acid(TFA) (6 mL). The solution was stirred at ambient temperature for 3 h. The volatile components were removed under reduced pressure, and the residue was treated with MeOH, then the solvent was evaporated under reduced pressure. The residence was washed with EA:PE=1:10 (55 mL×2), which was employed directly without further purification. Then the residence (30 mg，0.05 mmol) was dissolved in EA: MeOH=1:1 (2 mL). Iodomethane (100 μL) and potassium carbonate (135 mg) were added and the mixture was stirred vigorously for 18 hours. After evaporation, the crude residue was washed with EA:PE=1:10 (5 mL×2), then dissolved in 25 mL water. The residue extracted with dichloromethane (50 mL), and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to afford target compound **T3PQA** (27mg, yield: 50%) as yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.70-7.65 (m, 12H), 7.42-7.38 (m, 6H), 7.19-7.14 (m, 6H), 3.60-3.54 (m, 6H), 3.16 (s, 27H), 3.11-3.07 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.36, 138.26, 135.20, 134.40, 129.67, 127.80, 126.59, 124.27, 65.79, 52.46, 52.42, 52.38, 28.17. HRMS (ESI) m/z calcd for $C_{51}H_{63}N_4^{2+}$ [M/3] $^+$ 243.83454, found 243.83483.

NMR spectra of Compounds

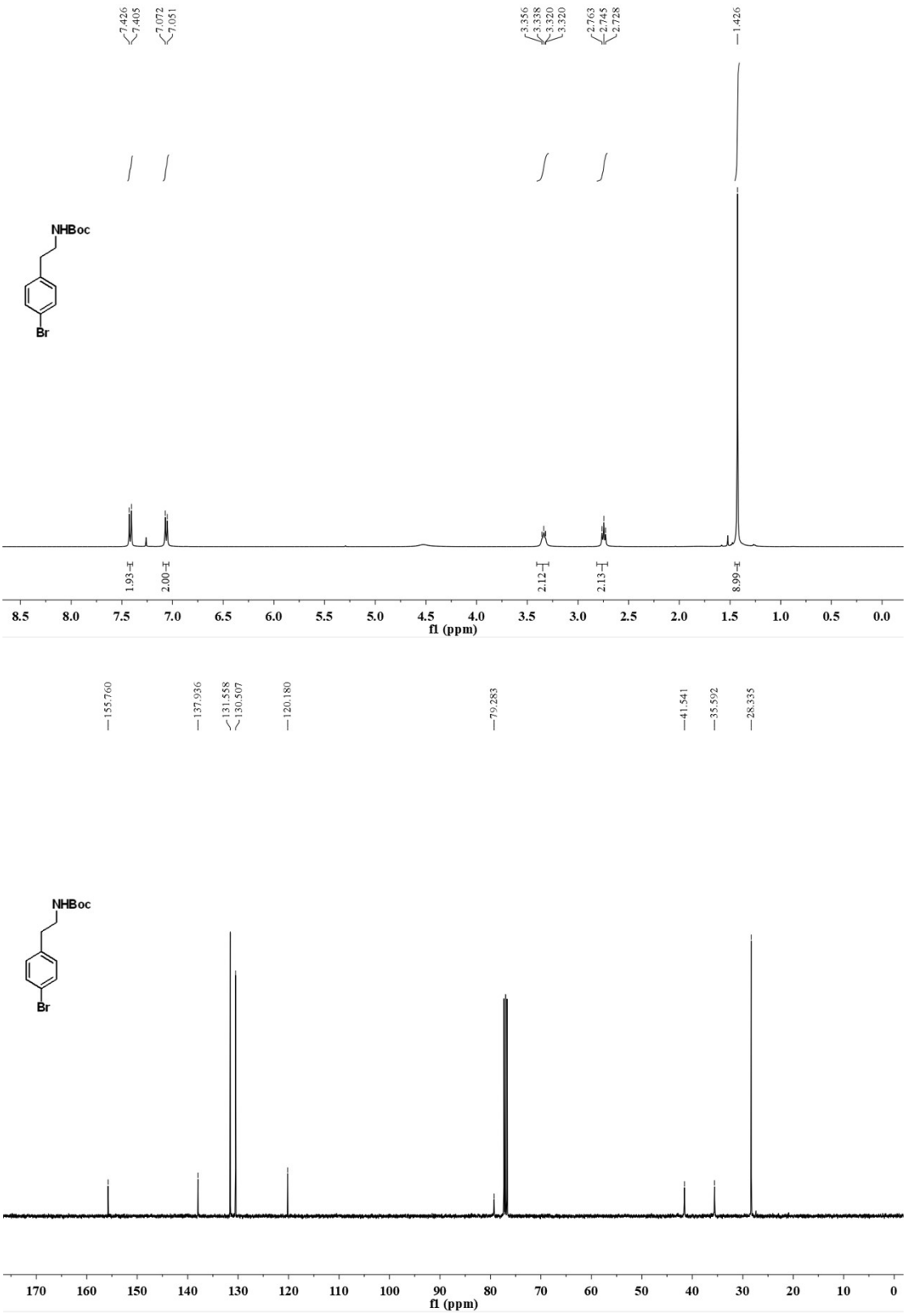


Figure S2. ^{1}H and ^{13}C NMR spectra of compound **1**.

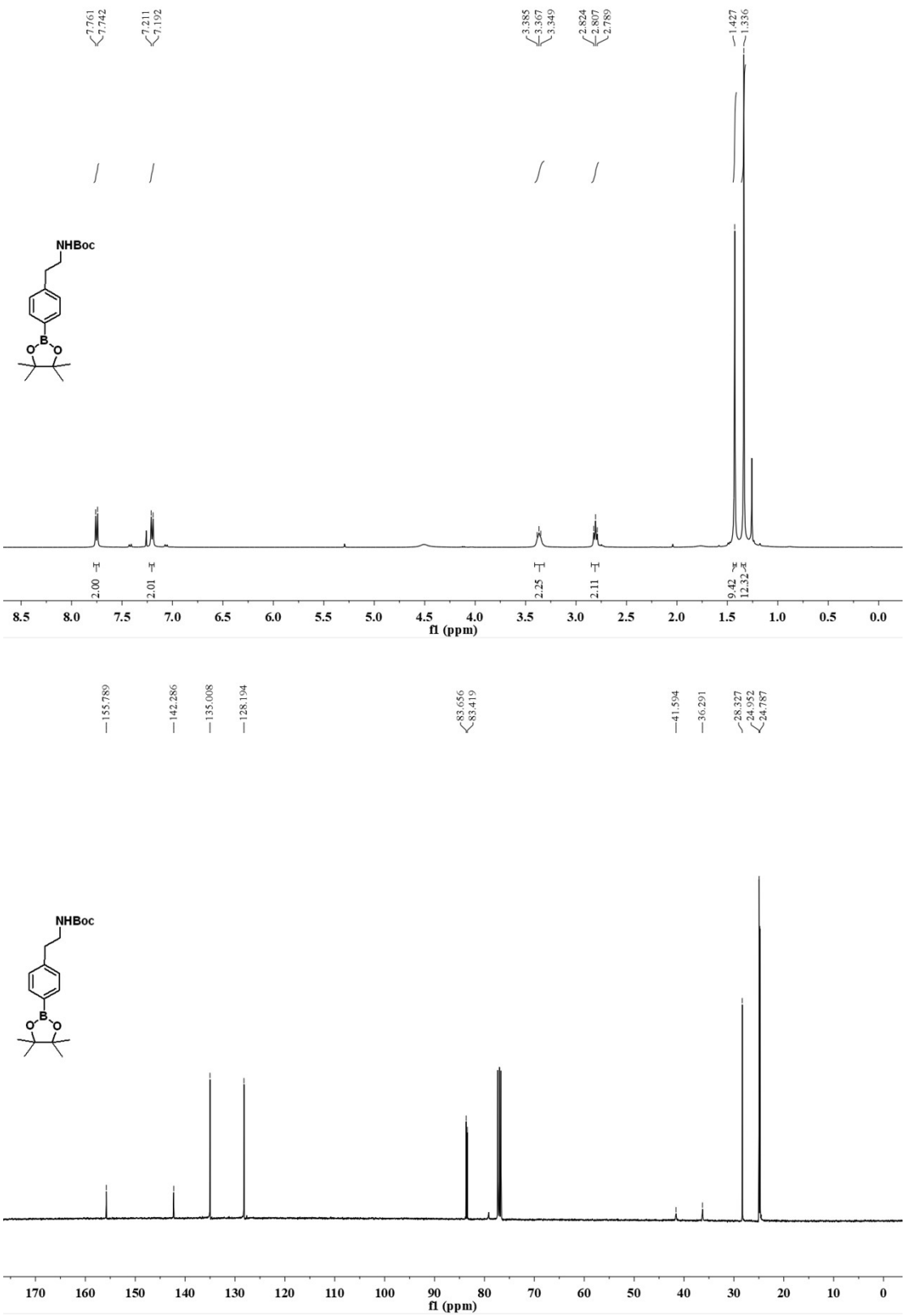


Figure S3. ^{1}H and ^{13}C NMR spectra of compound **4.**

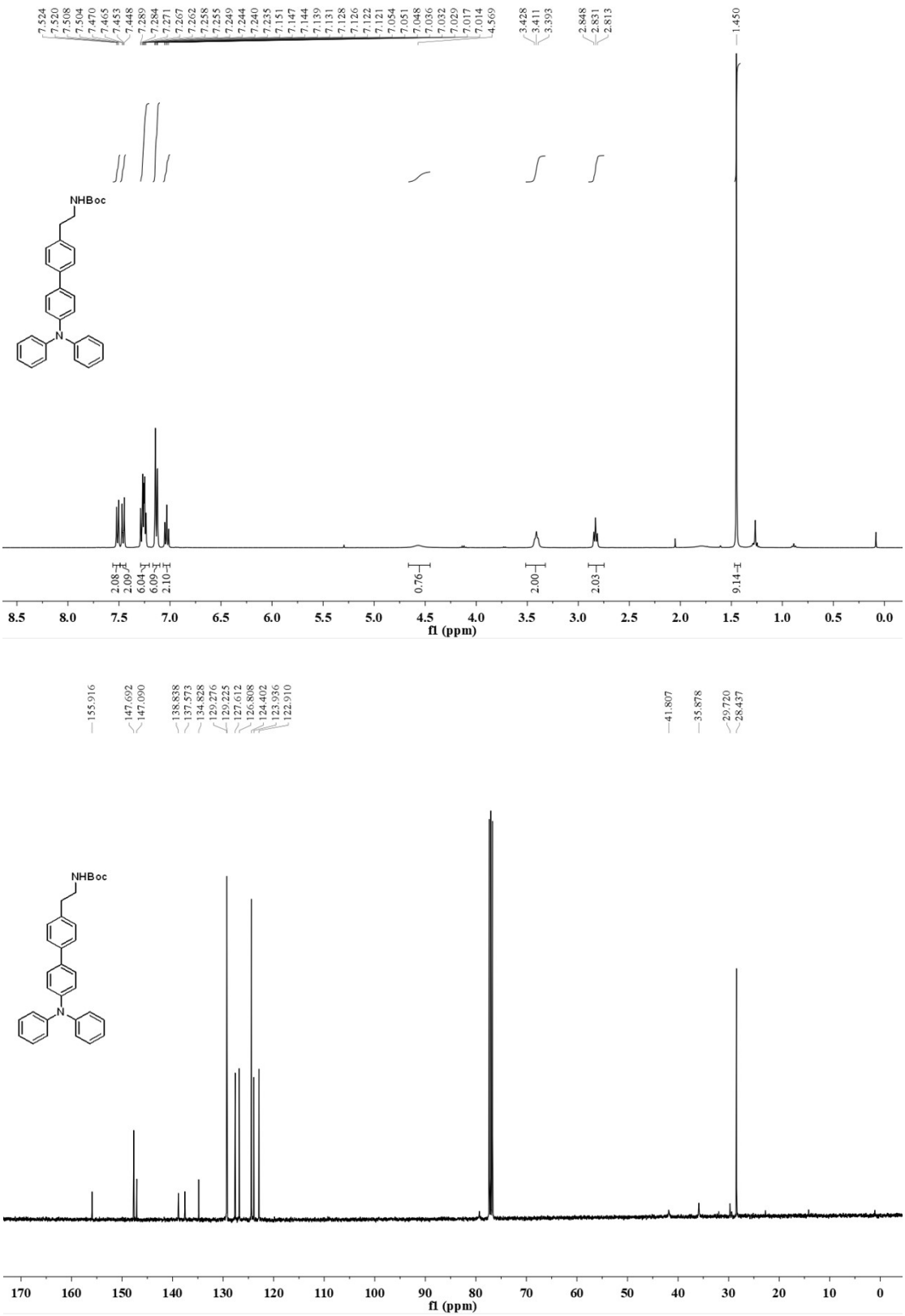


Figure S4. ^{1}H and ^{13}C NMR spectra of compound **2.**

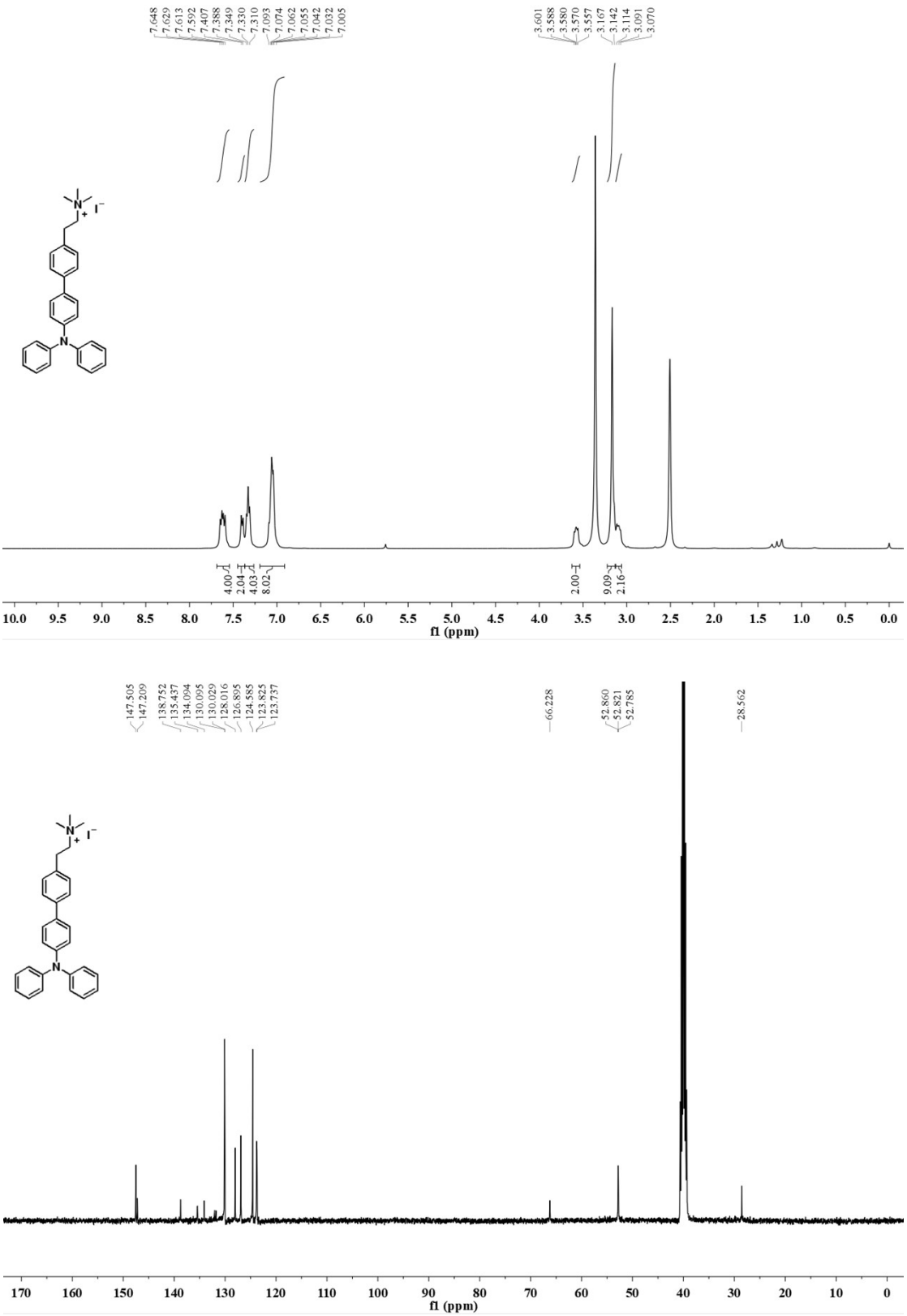


Figure S5. ^{1}H and ^{13}C NMR spectra of **TPQA.**

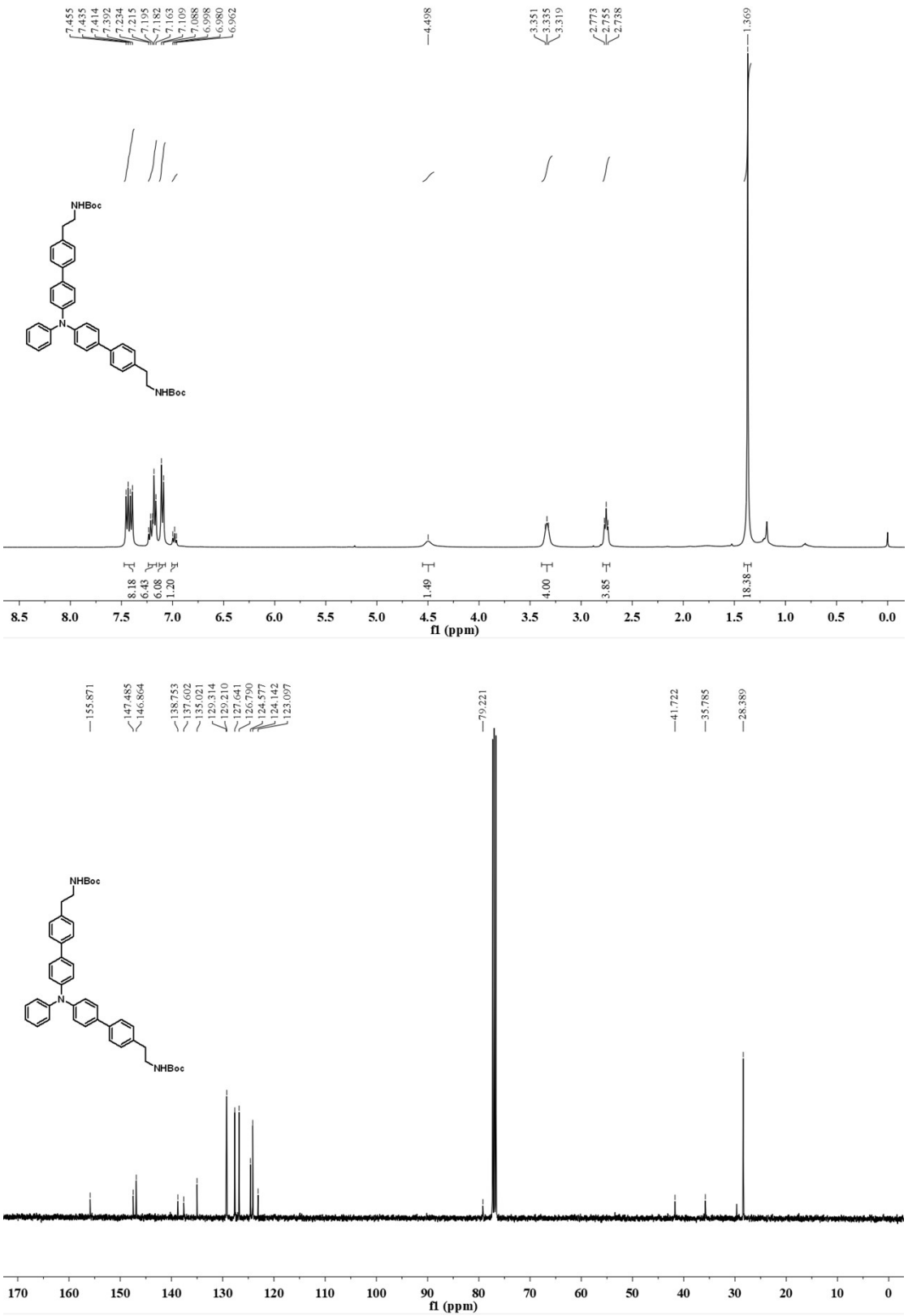


Figure S6. ^{1}H and ^{13}C NMR spectra of compound **5.**

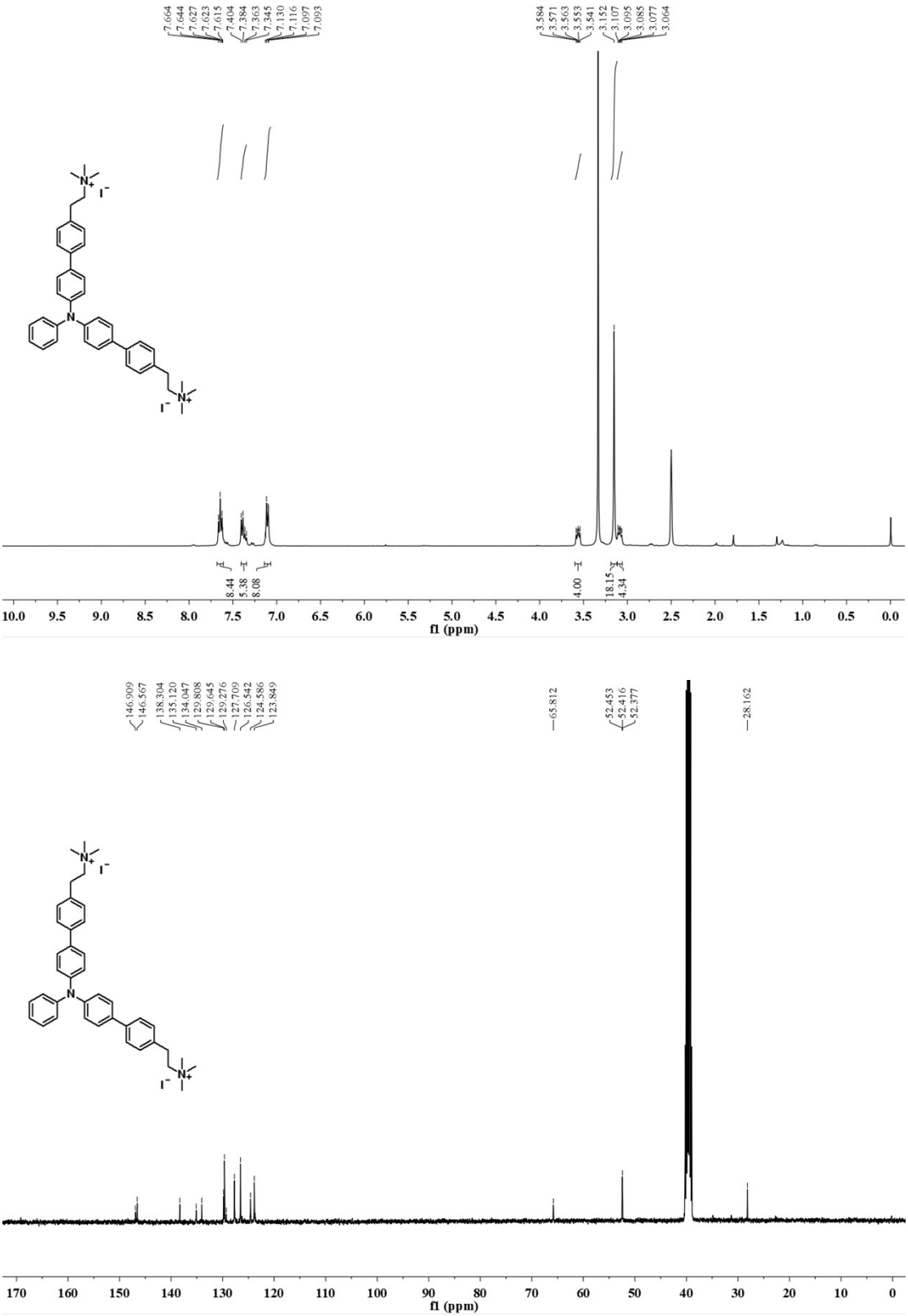


Figure S7. ^{1}H and ^{13}C NMR spectra of **T2PQA.**

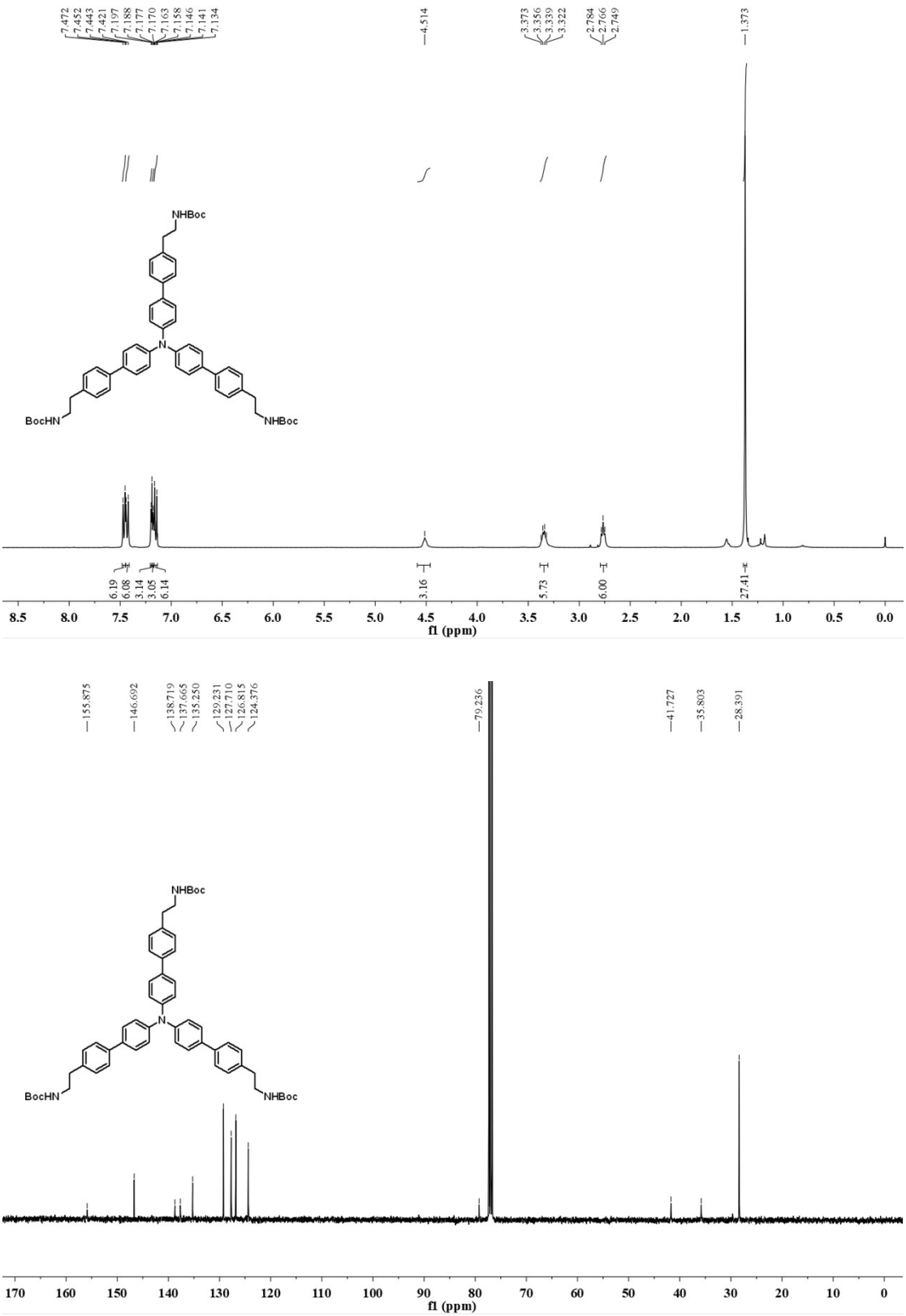


Figure S8. ^{1}H and ^{13}C NMR spectra of compound **7.**

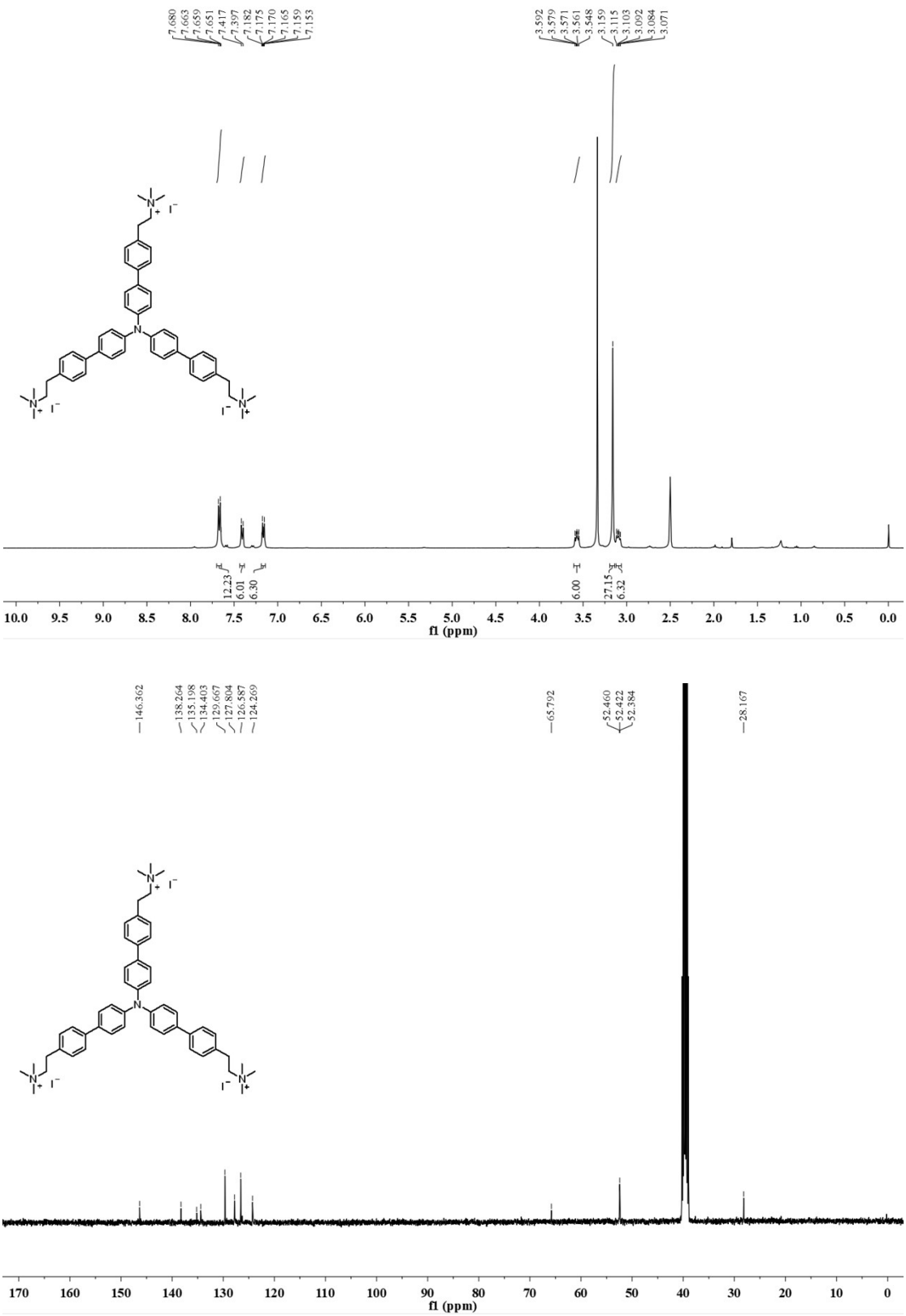


Figure S9. ^{1}H and ^{13}C NMR spectra of **T3PQA.**

According to the CIR effects of triphenylamine (TPA) derivatives discovered in Tang group,[2] TPQA increases the conjugated structure compared with TPA, inducing the rigidity of the whole molecule and the red shift of its absorbance spectrum in DMSO. However, TPQA cannot be simply considered as an absolute planar structure, its stable state in solution concludes a twisted molecular state closed to a 3D structure and a rigid molecular state closed to a planarization structure. The stability of the two states is similar, so that they show similar proportions in the apparent molecular configuration distribution. Therefore, the absorption spectrum is the result of coexistence of the two states, which is reflected in the absorption spectrum as a wider absorption spectrum. When the conjugate effect is further increased, the plane rigidity is further enhanced and the distribution ratio of structures closer to plane rigidity is gradually increased. So, the absorption spectrum of T2PQA is gradually redshift and tend to be symmetrical. Inferably, the absorption spectrum of T3PQA mainly reflects the UV-vis absorbance of the planar rigid structure, corresponding to a narrow absorbance spectrum. In conclusion, the broadening of the absorption spectrum is the manifestation of the coexistence of two states, which is a phenomenon of insufficient red shift.

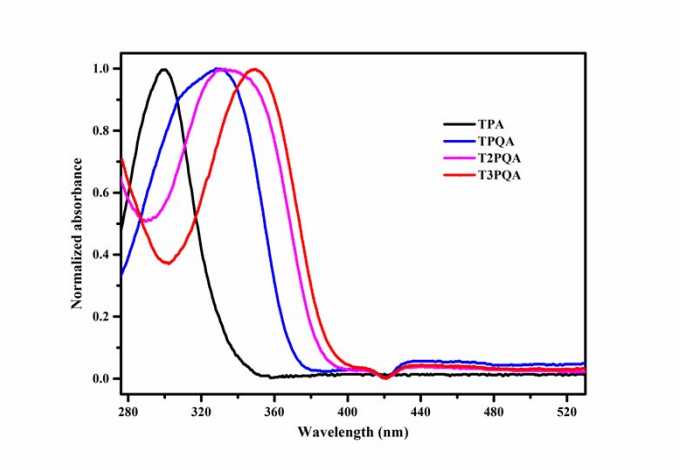


Figure S10. The Normalized absorption spectra of TPA, TPQA, T2PQA and T3PQA dissolved in DMSO.

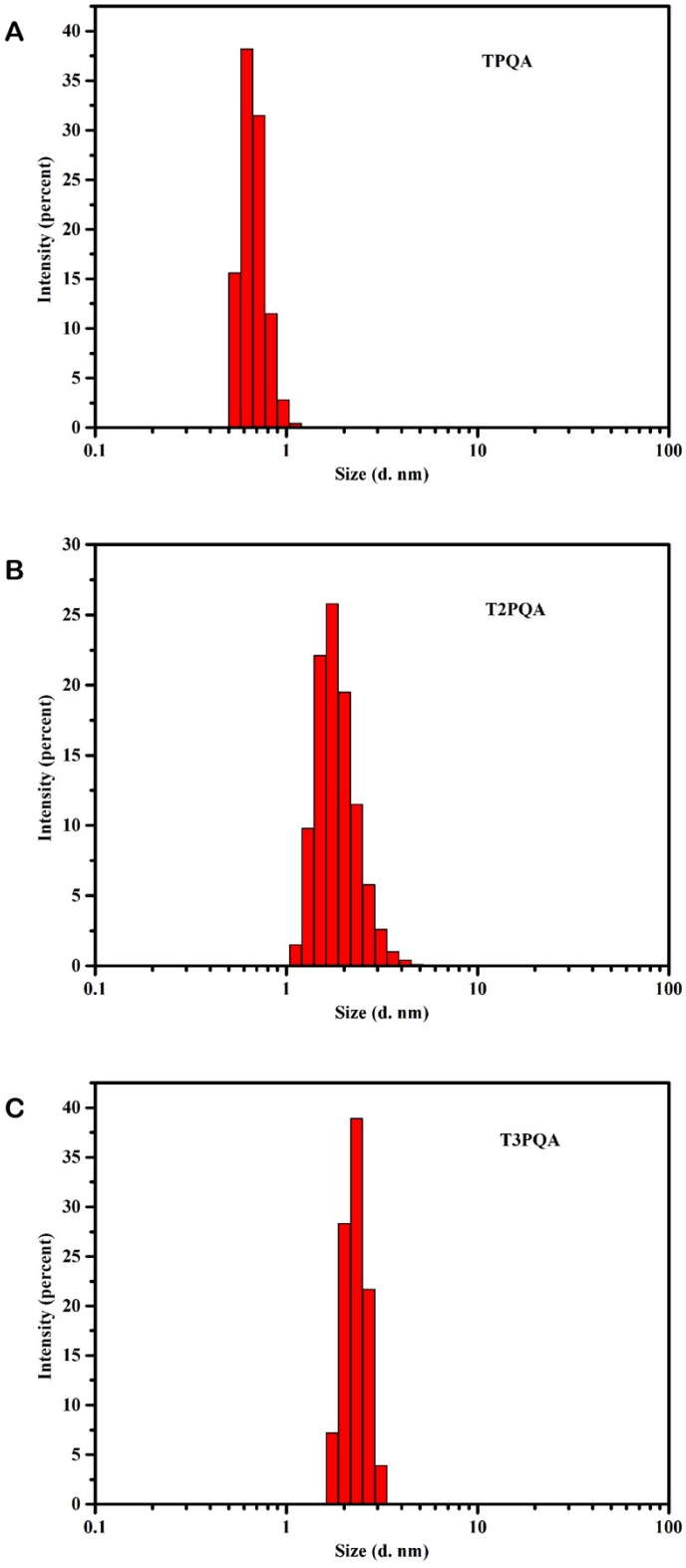


Figure S11. The characterization of hydration particle size of TPQA (A), T2PQA (B) and T3PQA (C) in DMSO-water system with 99% water content at the concentration of 1×10^{-5} M.

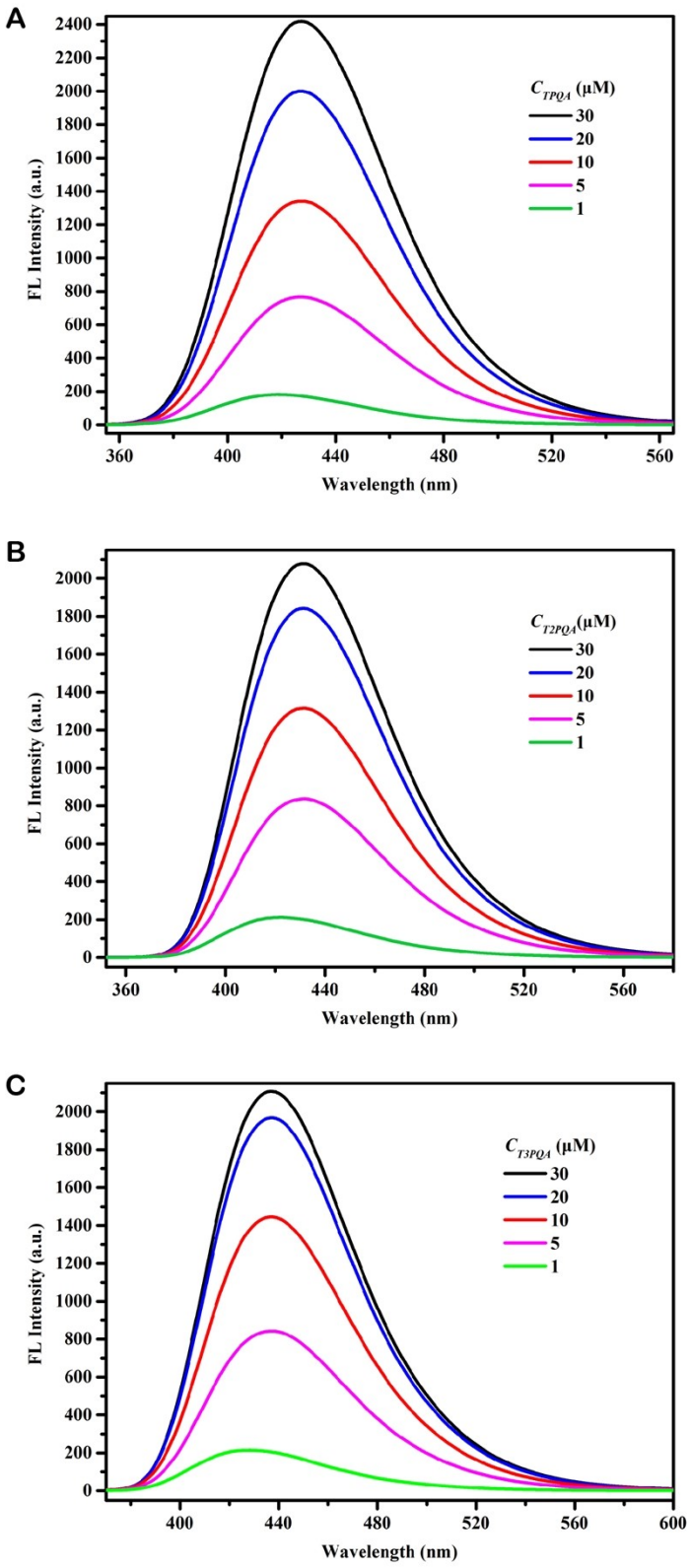


Figure S12. Photoluminescence spectra of TPQA (A), T2PQA (B) and T3PQA (C) in DMSO at different concentrations (1×10^{-6} M, 5×10^{-6} M, 10×10^{-6} M, 20×10^{-6} M and 30×10^{-6} M).

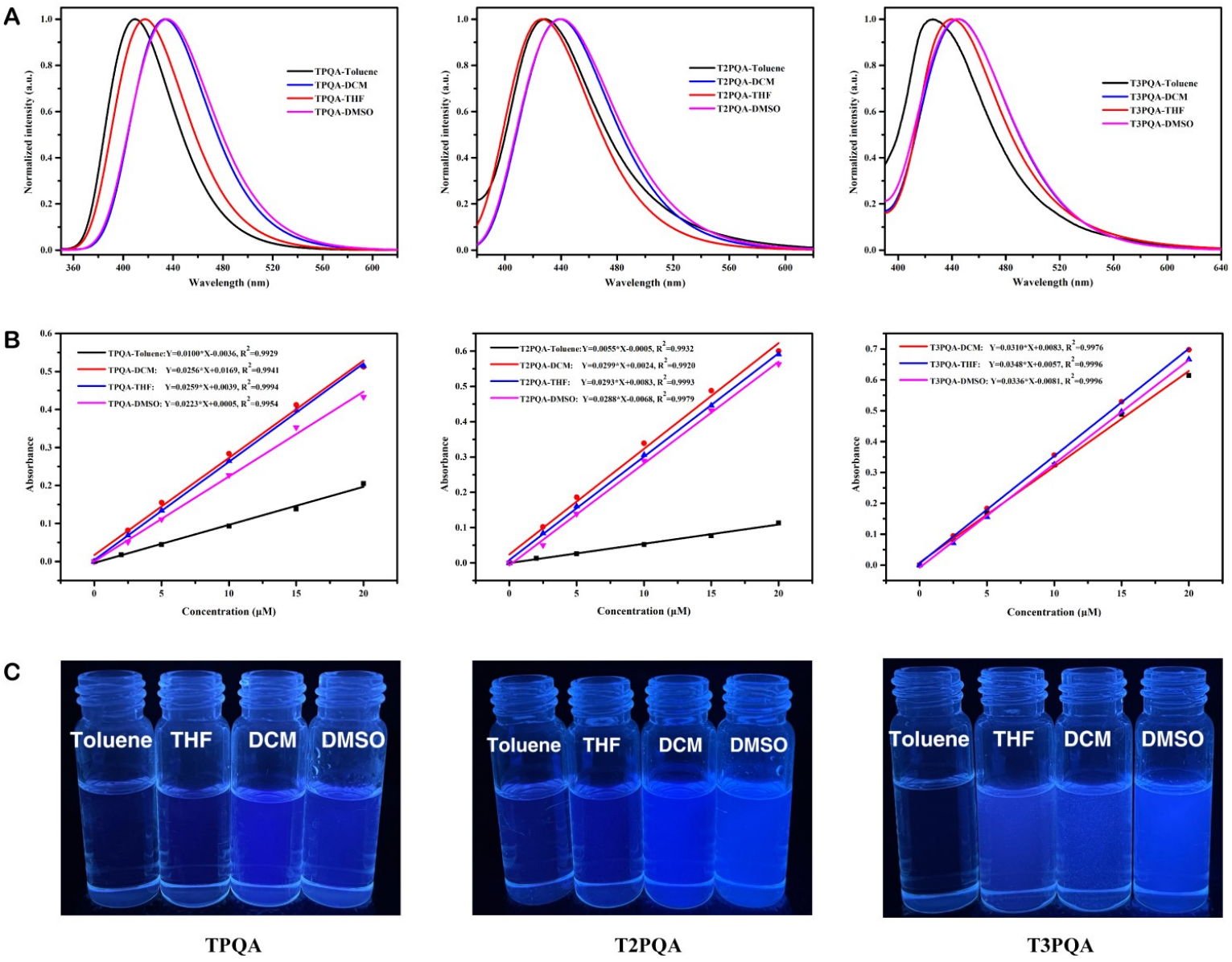


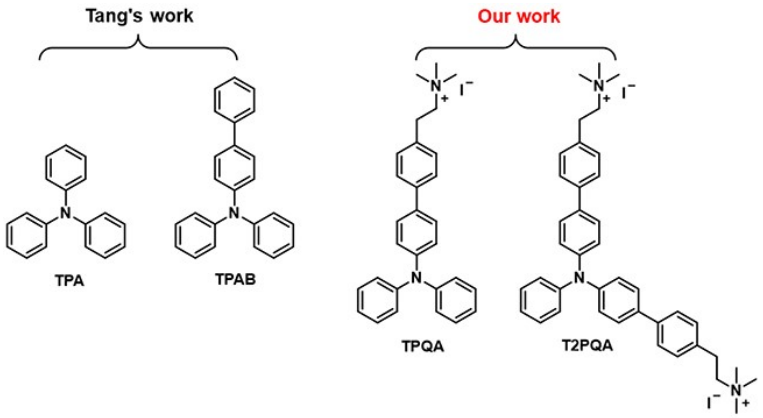
Figure S13. A) Normalized emission spectra of TPQA, T2PQA and T3PQA in different solvents (1×10^{-5} M). B) The plots of the absorbance of TPQA, T2PQA and T3PQA in different solvents curve with concentration. C) Photographs taken under 365nm UV illumination in solution (from left to right: toluene, THF, DCM and DMSO with 1% DMSO content).

Table S1. Properties of TPQA, T2PQA and T3PQA

	Solvent	λ_{abs} (nm)[a)]	λ_{em} (nm)[b)]	Φ_F (%) [c)]	$\Delta\lambda$[d)]	ε (L·cm^{-1}·mol^{-1})[e)]
TPQA	Toluene	321	409	26	61	10000
	DCM	335	435	73	86	25600
	THF	328	419	45	74	25900
	DMSO	331	436	77	89	22300
T2PQA	Toluene	353	428	32	62	5500
	DCM	342	441	59	78	29900
	THF	345	429	31	71	29300
	DMSO	332	441	63	81	28800
T3PQA	Toluene	-	426	10	57	-
	DCM	355	447	42	80	31000
	THF	360	443	9	74	34800
	DMSO	349	446	55	76	33600

a) Absorption maximum; b) Emission maximum; c) Fluorescence quantum yield; d) Stokes' shift; e) the molar absorptivity coefficient.

The three molecules in our study have similar structures to those reported by Tang group. [2] Compared with reported structures of molecules with CIR properties, our molecules only introduce a quaternary amine group that does not participate in conjugation and is relatively small, which will not substantially change their optical properties. Based on the analysis of the structures of three designed CIR molecules, it can be indicated that TPQA has a more conjugated structure compared with TPA which induces its planarization character, corresponding to the result observed by the UV-vis spectrum that TPQA has a red shift of its absorbance spectrum in solution and an increase of its fluorescence quantum yield from 26% to 77%. Identically, T2PQA has a further red shift absorbance spectrum due to its more conjugated structure, while the apparently more rigid structure leads to a stronger Π-stacking effect causing the exciton degradation, resulting in the fluorescence quantum yield decline. All these results stated above are compliance with the CIR phenomenon in twisting molecules reported by Tang et al.



Compound	Φ_f [%]				
	Toluene	THF	DCM	DMF	DMSO
TPA	-	13.0	-	-	-
TPAB	29.9	35.2	39.7	49.1	-
TPQA	26	45	73	-	77
T2PQA	32	31	59	-	63

Figure S14. The chemical structures and fluorescence quantum yields (Φ_f) of TPA, TPAB [2], TPQA and T2PQA. ("-" indicates that no value is given)

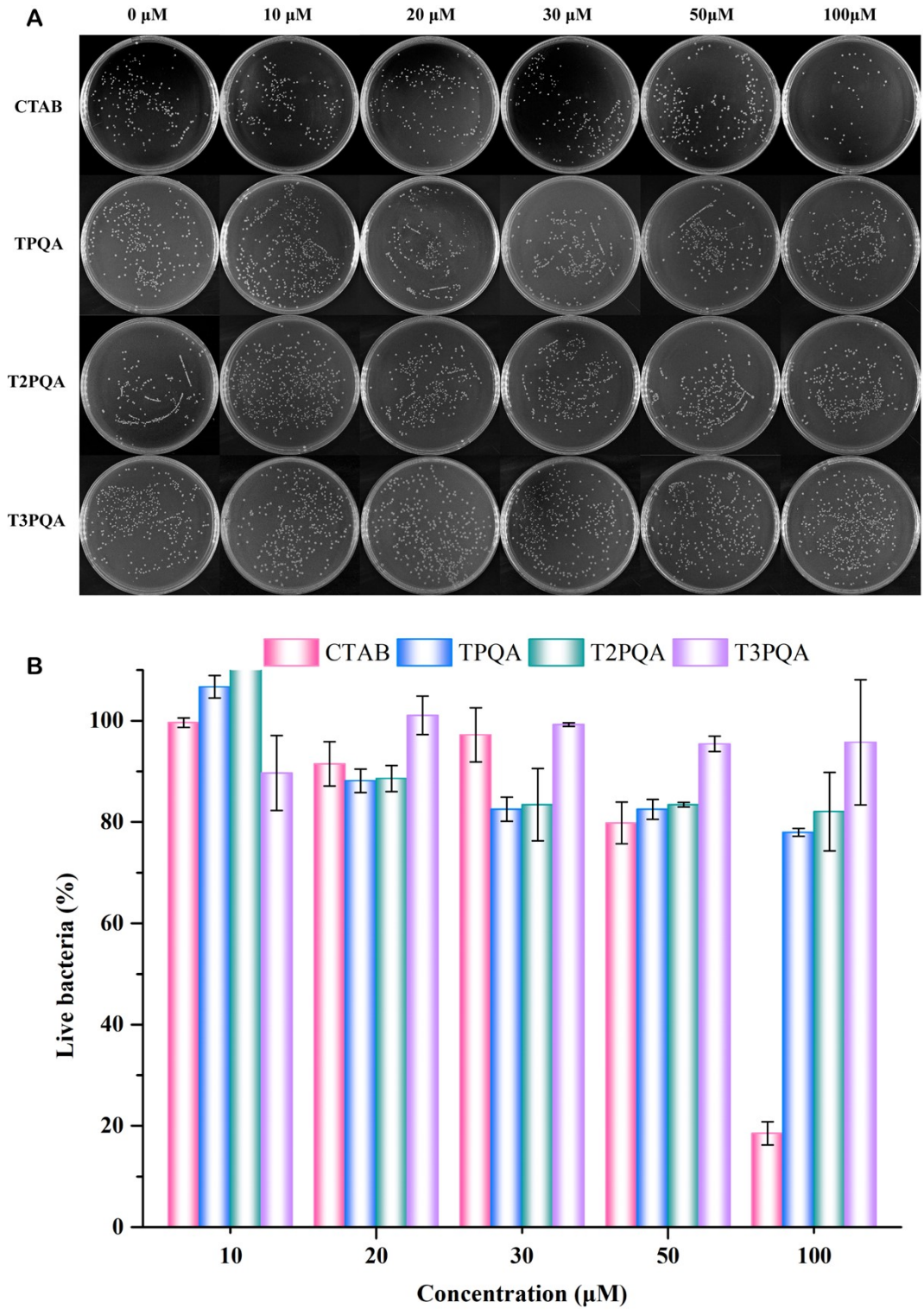


Figure S15. Antibacterial activity (A) and Bacterial survival rate (B) of Gram-negative bacteria *E. coli* incubated with CTAB, TPQA, T2PQA and T3PQA for 30 min under different concentration.

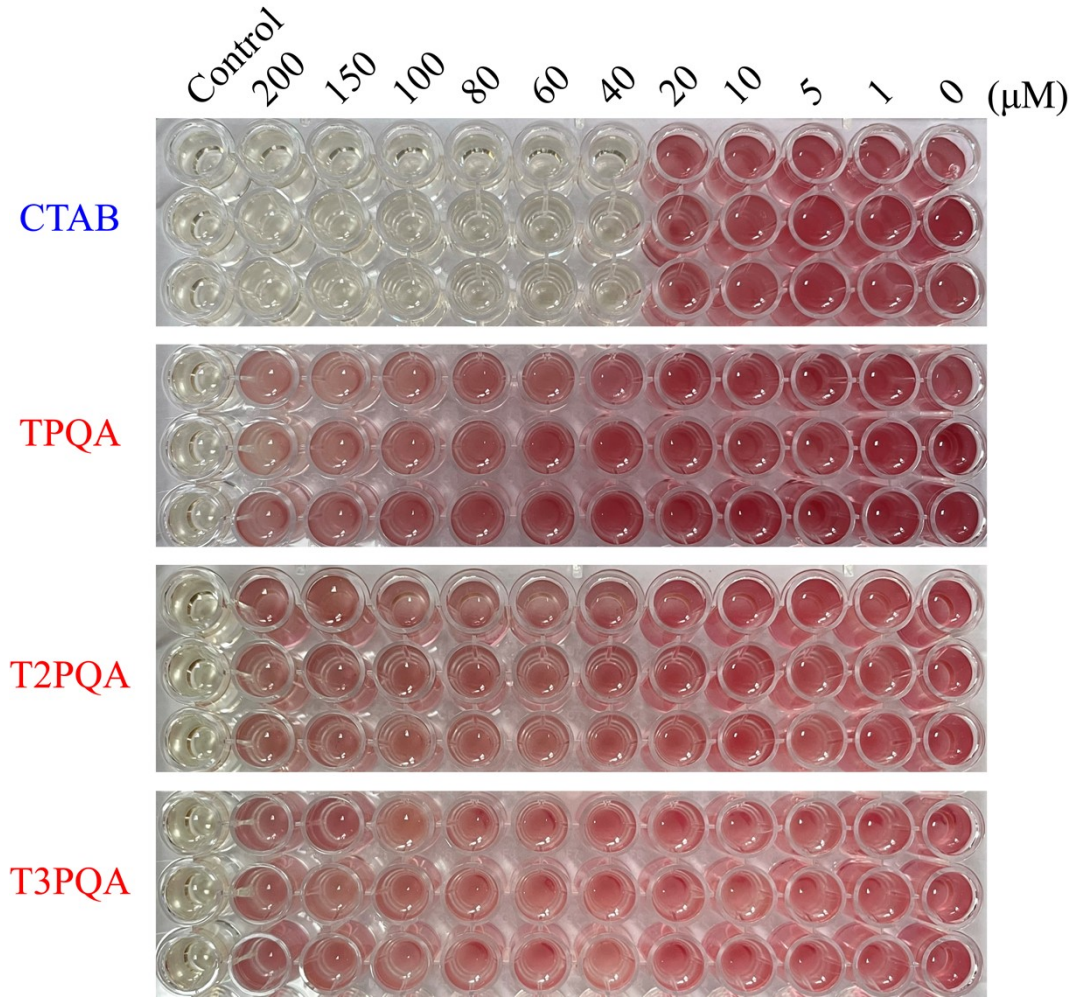


Figure S16. Photographs of the determination of MIC values for CTAB, TPQA, T2PQA and T3PQA against *E. coli*.

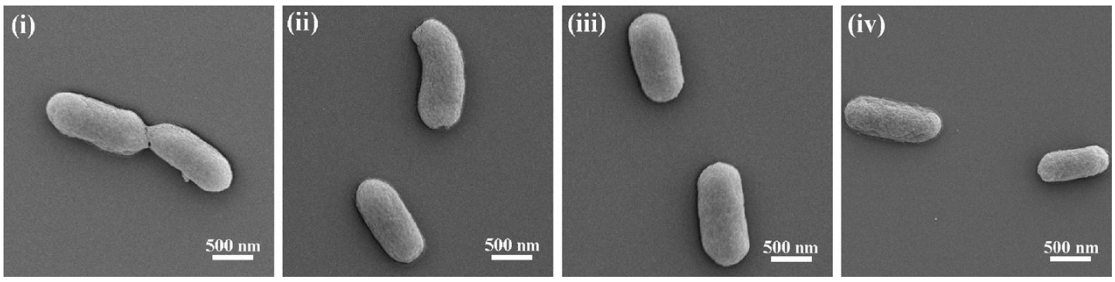


Figure S17. SEM images of *E. coli* incubated with 10 × 10^{-6} M of TPQA (ii), T2PQA (iii) and T3PQA (iv). The control (i) was *E. coli* without TPQA, T2PQA and T3PQA. Scale bar: 500 nm.

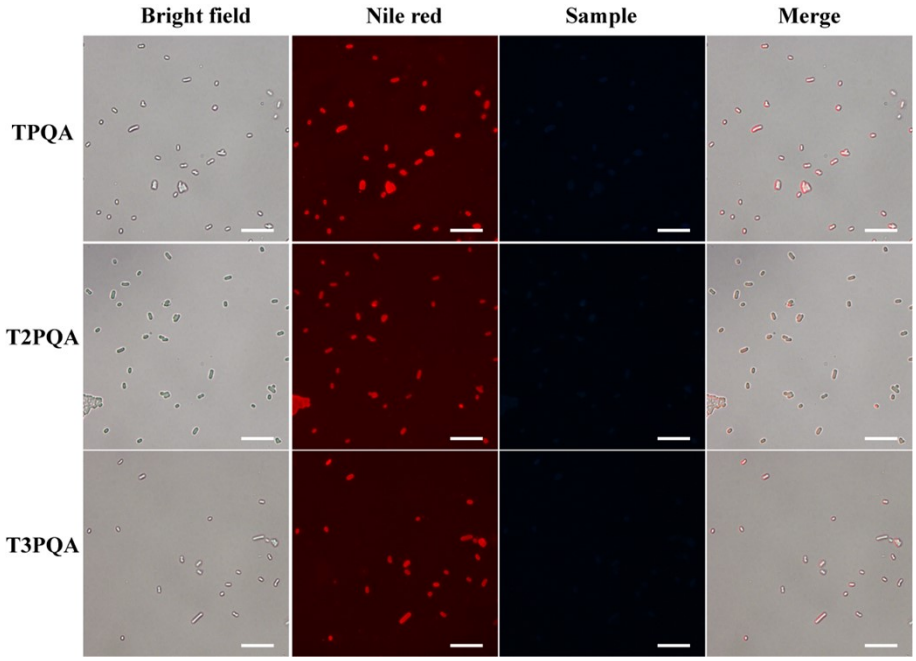


Figure S18. The fluorescence microscope images of Gram-negative bacteria *E. coli* after coincubation with TPQA, T2PQA and T3PQA (10×10^{-6} M) for 30 min. Scale bar: 5μm. (red fluorescence: Nile red).

References

1. Y. Liao, L. Xu, S. Ou, H. Edwards, D. Luedtke, Y. Ge and Z. Qin, *ACS Med Chem Lett*, 2018, **9**, 635-640.
2. G. Chen, W.B. Li, T.R. Zhou, Q. Peng, D. Zhai, H.X. Li, W. Z. Yuan, Y.M. Zhang and B. Z. Tang. *Adv Mater*, 2015, **27**, 4496–4501.