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Supplementary data

Thermally Activated Delayed Fluorescence Emitters Thionation Approach toward Next-Generation Photosensitizers

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Abbreviations

DCM = Dichloromethane

DFT = density functional theory

DMSO = Dimethyl Sulfoxide

EA = Ethyl Acetate

ESI = Electrospray Ionization

HOMO = Highest Occupied Molecular Orbitals

HRMS = High Resolution Mass Spectrometry

IPTG = Isopropyl-*β*-D-Thiogalactopyranoside

LUMO = Lowest Unoccupied Molecular Orbital

Min = Minutes

NMR = Nuclear Magnetic Resonance

OD = Optical Density

PE = Petroleum Ether

rpm = Revolutions Per Minute

r.t. = Room Temperature

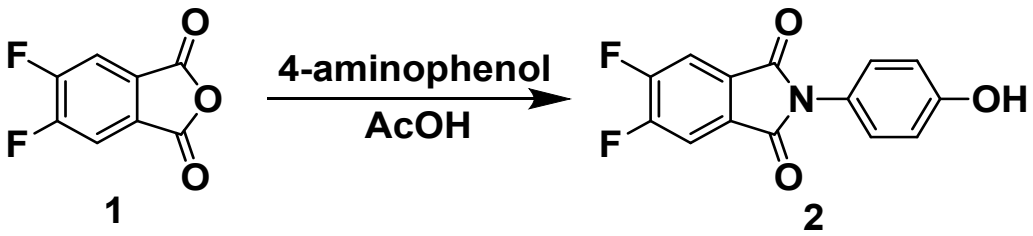
THF = Tetrahydrofuran

TDDFT = time-dependent density functional theory

General methods

All the reagents and solvents were commercially available and used without further purification. All ^{1}H NMR spectra were recorded at 400 MHz and 500 MHz. ^{13}C NMR spectra were recorded at 100 MHz and 125 MHz. HRMS were measured with Thermo LCQ Deca XP Max mass spectrometer for ESI. Six bacterial strains (*Escherichia coli* (*E. coli*) (ATCC 25922), *Klebsiella pneumoniae* (*K. pneumoniae*) (ATCC 700603), *Pseudomonas aeruginosa* (*P. aeruginosa*) (ATCC 27853), *Enterobacter cloacae* (*E. cloacae*) (ATCC 13047), Methicillin resistant *Staphylococcus aureus* (*MRSA*) (ATCC 33592)) were purchased from American Type Culture Collection (ATCC), USA. *Enterococcus faecium* (*E. faecium*) (CICC 10840) and *Acinetobacter baumannii* (*A. baumannii*) (CICC 22933) were purchased from China Center of Industrial Culture Collection, CICC®. Fluorescence emission spectra and full wavelength absorption spectra were performed on 2300 EnSpire multimode plate reader. Fluorescence and phosphorescence spectra were recorded on a Hitachi F-7000 spectrophotometer. The transient photoluminance decay characteristics and temperature dependence experiments were measured using an Edinburgh Instruments FLS1000 spectrometer. Light sources (white light, 20 mW / cm^2) for chemical reactions and bioassays were from LED Light provided by PURI Materials, Shenzhen. OD values were recorded in a 10 mm path quartz cell on a Metash UV-5100B spectrometer. The confocal laser scanning microscopic imaging studies were conducted with ZEISS LSM 800 Confocal Microscope.

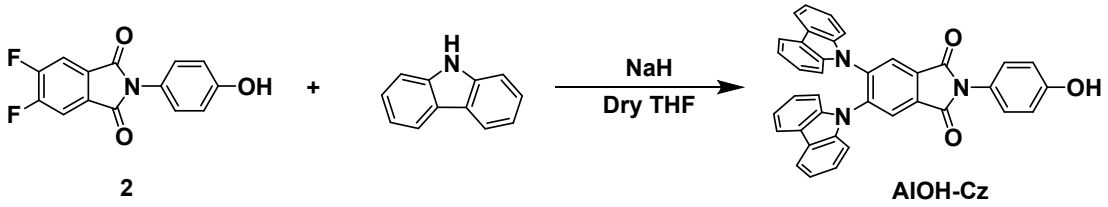
Synthetic procedures and characterized data



Scheme S1. Synthesis of compound **2**.

Compound **2**

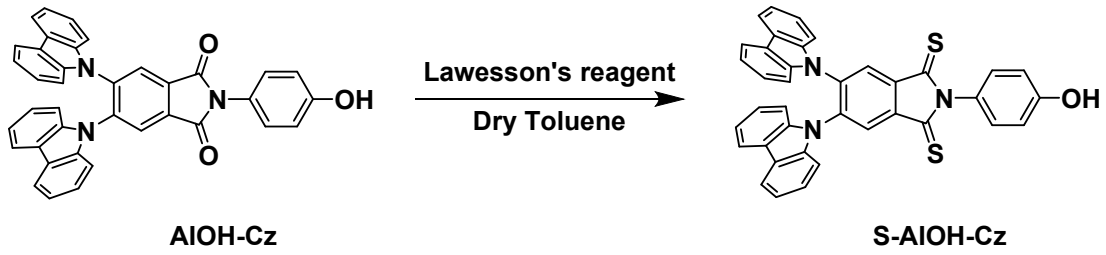
In a 25 mL round bottom flask, compound **1** (920.5 mg, 5 mmol, 1.0 equiv) and 4-aminphenol (545.6 mg, 5 mmol, 1.0 equiv) were reflux for 4 hours in 5 mL glacial acetic acid. The mixture was cooled to r.t., and then kept at 4 °C for 12 hours. The solids were filtered from the solution and washed by water for three times, and compound **2** (1.238 g, 90 %) was obtain as yellow powder without further purification. ^{1}H NMR (400 MHz, DMSO-D_6) δ 9.78 (s, 1H), 8.17 (t, J = 7.8 Hz, 2H), 7.21 (d, J = 8.8 Hz, 2H), 6.89 (d, J = 8.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-D_6) δ 165.59, 157.40, 128.70, 122.63, 117.48, 115.43. HRMS (ESI): Calcd for $C_{14}H_7F_2NO_3$ ([M]$^+$), 275.0389, found, 275.0378.



Scheme S2. Synthesis of **AIOH-Cz**.

AIOH-Cz

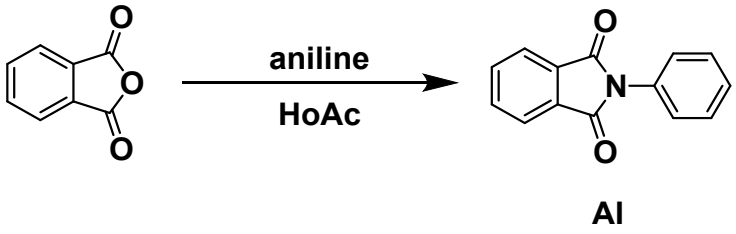
In a 100 mL two-necked round bottom flask, sodium hydride (60% in mineral oil, 144 mg, 3.6 mmol, 1.2 equiv.) was added and a solution of carbazole (501.6 mg, 3 mmol, 1.0 equiv.) in 20 mL of dry THF under argon atmosphere was added portion wise at r.t., and mixture was stirred for 25 minutes. Then, compound **2** (412.8 mg, 1.5 mmol, 0.5 equiv.) was added, and the mixture was stirred for 2 hours. After the reaction was completed, the solution was poured in 20 mL water. The solution was extracted with DCM (3 × 50 mL). The combined organic layer was washed with saturated brine and dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (PE : DCM = 1 : 4) to give yellow solid 376 mg, yield 44 %.^{1}H NMR (500 MHz, $CDCl_3$) δ 8.46 (s, 2H), 7.80 (d, J = 7.5 Hz, 4H), 7.37 (d, J = 8.5 Hz, 2H), 7.15 (d, J = 7.5 Hz, 4H), 7.12-7.05 (m, 8H), 6.99 (d, J = 8.0 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.35, 138.99, 131.15, 128.32, 126.01, 124.18, 121.13, 120.32, 116.31, 109.55. HRMS (ESI): Calcd for $C_{38}H_{23}N_3O_3$ ([M]$^+$), 569.1734, found, 569.1759.



Scheme S3. Synthesis of **S-AIOH-Cz**.

S-AIOH-Cz

In a 100 mL round bottom flask, **AIOH-Cz** (284.8 mg, 0.5 mmol, 1.0 equiv.) and Lawesson's reagent (606.7 mg, 1.5 mmol, 3.0 equiv.) in 10 mL dry toluene was reflux for 12 h under argon atmosphere. Then, the solution was cooled to r.t. and mixture was extracted with water (40 mL) and EA (3 × 50 mL). The combined organic layer was washed with saturated brine and dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (PE : DCM = 1 : 2) to give orange solid 61 mg, yield 20.3 %.^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (s, 2H), 7.80 (d, J = 7.0 Hz, 4H), 7.27-7.24 (m, 3H), 7.17 (d, J = 7.7 Hz, 3H), 7.10-7.06 (m, 8H), 7.04-7.02 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.52, 140.26, 139.18, 130.54, 128.69, 126.58, 125.93, 125.72, 125.09, 124.19, 124.04, 121.20, 120.90, 120.70, 120.24, 116.17, 112.82, 110.00, 109.64. HRMS (ESI): Calcd for $C_{38}H_{24}N_3OS_2$ ($[M+H]^+$), 602.1355, found, 602.1373.

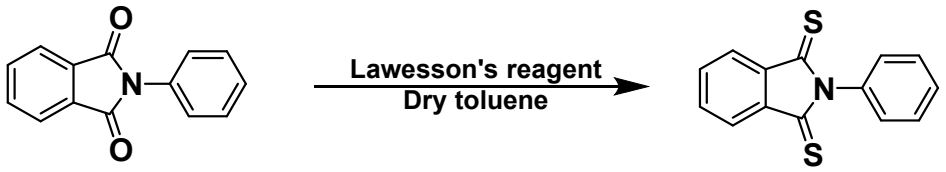


Scheme S4. Synthesis of **AI**

AI

In a 25 mL round bottom flask, o-phthalic anhydride (6 g, 40.5 mmol, 1.0 equiv) and aniline (4.527 g, 48.6 mmol, 1.2 equiv) were reflux for 4 hours in 20 mL glacial acetic acid. The mixture was cooled to r.t., and then kept at 4 °C for 12 hours. The solids were filtered from the solution and washed by water for three times, and **AI** (8.808 g, 97.5%) was obtain as

white powder without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (dd, J = 5.4, 3.1 Hz, 2H), 7.79 (dd, J = 5.5, 3.0 Hz, 2H), 7.54 – 7.49 (m, 2H), 7.47 – 7.43 (m, 2H), 7.43 – 7.38 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.39, 134.52, 131.88, 129.24, 128.22, 126.70, 123.87. HRMS (ESI): Calcd for $C_{14}H_9NO_2$ ([M]$^+$), 223.0628, found, 223.0626.



AI **S-AI**

Scheme S5. Synthesis of **S-AI.**

S-AI

In a 100 mL round bottom flask, **AI** (446.5 mg, 2 mmol, 1.0 equiv.) and Lawesson's reagent (1941 mg, 4.8 mmol, 2.4 equiv.) in 20 mL dry toluene was reflux for 12 h under argon atmosphere. Then the solution was cooled to r.t. and the mixture was extracted with water (40 mL) and EA (3 × 50 mL). The combined organic layer was washed with saturated brine and dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (PE : EA = 5 : 1) to give orange solid 396 mg, yield 77.6 %.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (dd, J = 5.5, 3.1 Hz, 2H), 7.77 (dd, J = 5.6, 3.1 Hz, 2H), 7.59 – 7.49 (m, 3H), 7.33 – 7.28 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.33, 136.32, 135.00, 133.60, 129.53, 129.49, 129.24, 123.74. HRMS (ESI): Calcd for $C_{14}H_9NS_2$ ([M]$^+$),255.0171, found, 255.0190.

DFT Calculations

To ancillary support TADF properties of our probe, the ground state geometry of **AlOH-Cz** is optimized by density functional theory (DFT) using the LCY-PBE functional at DZP (basis set) with the Amsterdam Density Functional (ADF) 2021 program. In order to investigate the energies and the transition characters of the low-lying excited singlet and triplet states, time-dependent density functional theory (TDDFT) with LCY-PBE at DZP (basis set) is used to calculate the vertical excitation energies for S_1, T_1 and T_2 as well as ΔE_{ST}. Due to the steric effect, the torsion angles between the carbazolyl and phthalimide moieties are

large. The HONTO locate mainly on carbazolyl moieties for their strong electron donating property, and LUNTO locate mainly on phthalimide moiety because of intense electron withdrawing ability, showing obvious spatial separation, showing the small spatial overlap between the LUNTO and HONTO. The energy gap between S_1 and T_1 (ΔE_{ST}) estimated by TDDFT with the LCY-PBE functional is these calculations suggest that the ΔE_{ST} is relatively small for **AIOH-Cz** due to the large separation between the LUNTO and HONTO. The small ΔE_{ST} indicate that the **AIOH-Cz** showed TADF property.

The ground state geometry of **S-AIOH-Cz** is optimized by density functional theory (DFT) using the PBE0 functional at DZP (basis set) with the Amsterdam Density Functional (ADF) 2021 program. In order to reveal the mechanism responsible for fluorescence quenching in thiocarbonyl substituted **S-AIOH-Cz**, the DFT and time-dependent density functional theory (TDDFT)with LCY-PBE at DZP (basis set) is used to calculate the vertical excitation energies for S_1, T_1 and T_2 as well as ΔE_{ST}.

Table S1. Calculated spin-orbit coupling (SOC) constants $\langle S_n | H_{SO} | T_m \rangle$ between singlet and triplet states of **S-AIOH-Cz.**

$\langle S_n \| H_{SO} \| T_m \rangle$	T_1	T_2	T_3	T_4
S_1	1.73	73.55	19.66	27.8
S_2	0.87	3.85	1.06	1.33
S_3	21.02	40.96	8.38	16.63
S_4	19.33	145.88	3.13	56.66

UV-Vis absorption and fluorescence emission spectra of AIOH-Cz

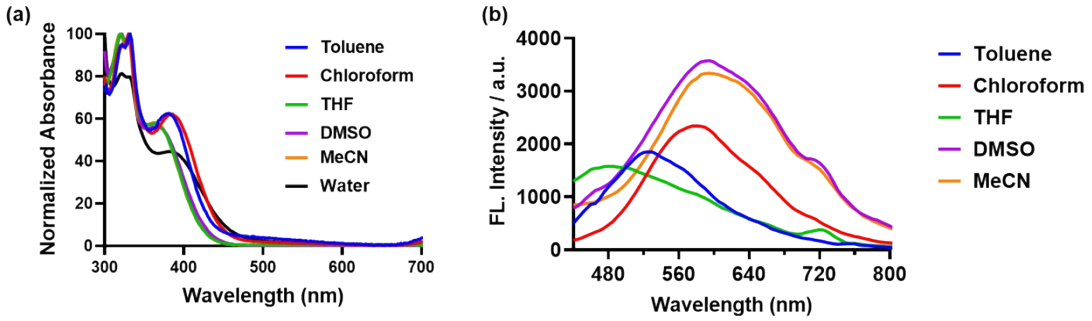


Fig S1. (a) UV-Vis absorption spectra and (b) fluorescence emission spectra of AIOH-Cz (20 μM) in different solvents, λ_{ex} = 390 nm.

The water solubility measurements of the AIOH-Cz

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	9181	Peak 1:	0.000	0.0	0.000
PdI:	1.000	Peak 2:	0.000	0.0	0.000
Intercept:	1.38	Peak 3:	0.000	0.0	0.000
Result quality	Refer to quality report				

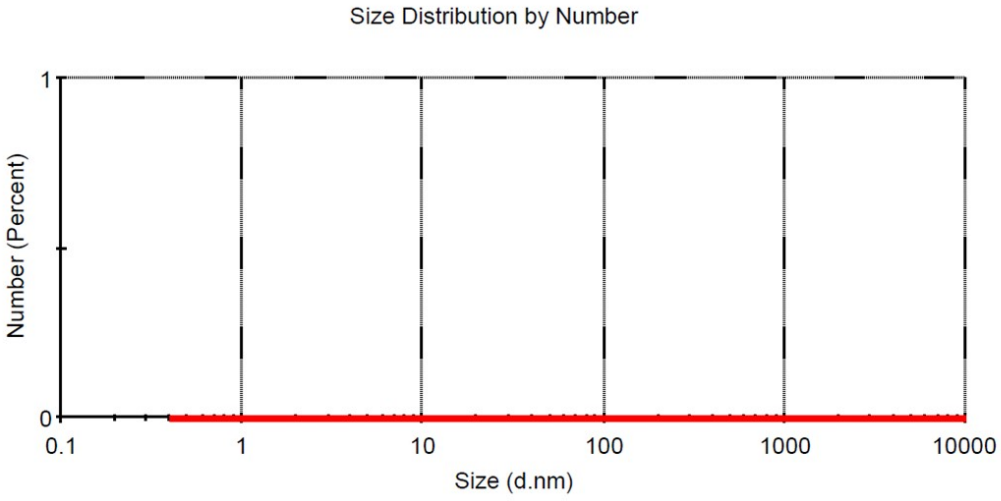


Fig. S2 Representative size distributions of AIOH-Cz measured by DLS (n = 3).

Reactive oxygen species (ROS) measurements of S-AlOH-Cz, AlOH-Cz, and S-Al.

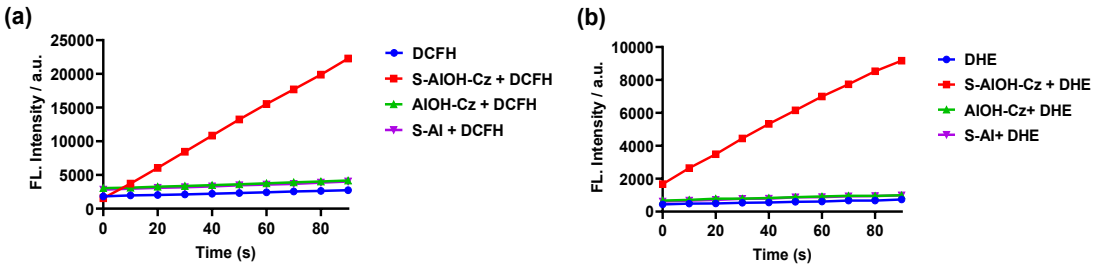


Fig S3. (a) ROS generation of **AlOH-Cz, S-AlOH-Cz, S-Al** and 2 % DMSO as co-solvent in water after irradiation by a white light source (20 mW / cm^2) DCFH-DA as ROS probe (20 μM, λ_{ex} = 480 nm, λ_{em} = 525 nm). (b) $O_2^{\cdot-}$ generation of **AlOH-Cz**, **S-AlOH-Cz**, **S-Al** and 2 % DMSO as co-solvent in water after irradiation by a white light source (20 mW / cm^2) DHE as $O_2^{\cdot-}$ probe (20 μM, λ_{ex} = 480 nm, λ_{em} = 610 nm). The samples were continuously irradiated by white light source (20 mW / cm^2).

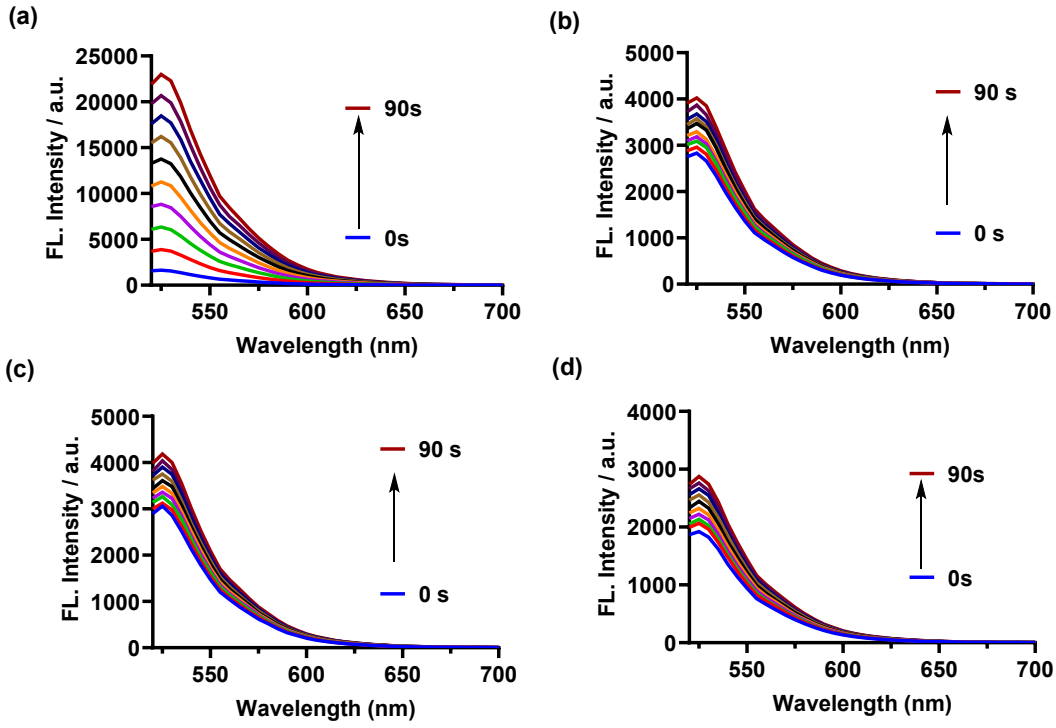


Fig S4. Time-dependent decrease of 20 μM DCFH in the presence of 20 μM (a)**S-AlOH-Cz**, (b) **AlOH-Cz**, (c) **S-Al** and (d) 2 % DMSO as co-solvent in water. The samples were continuously irradiated by white light source (20 mW / cm^2), λ_{ex} = 480 nm.

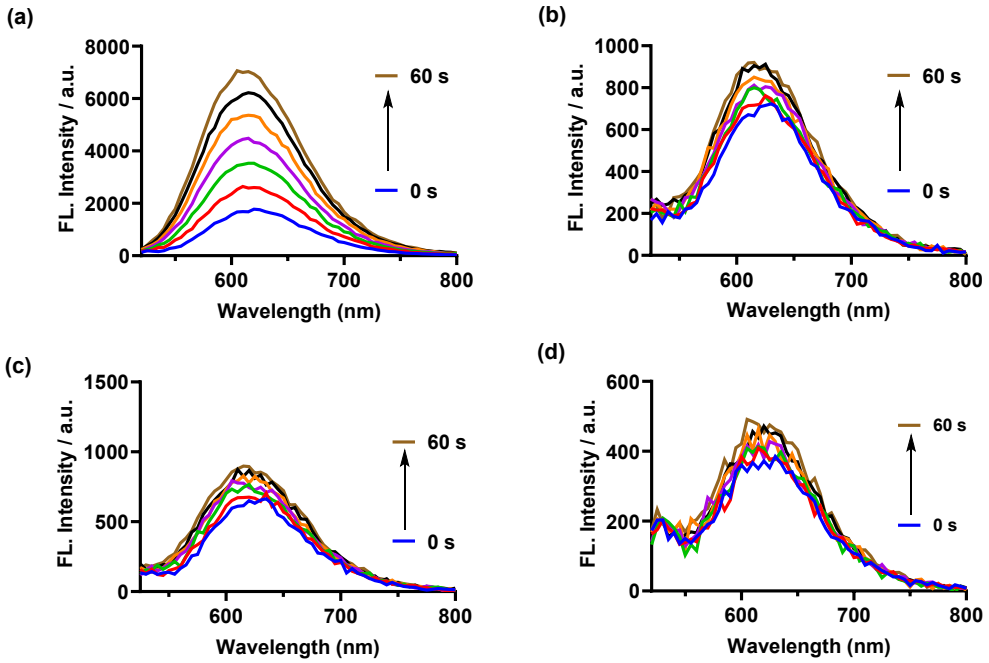


Fig S5. Time-dependent decrease of 20 μM DHE in the presence of 20 μM (a)**S-AIOH-Cz**, (b) **AIOH-Cz**, (c) **S-AI** and (d) 2 % DMSO as co-solvent in water. The samples were continuously irradiated by white light source (20 mW / cm^2), λ_{ex} = 480 nm.

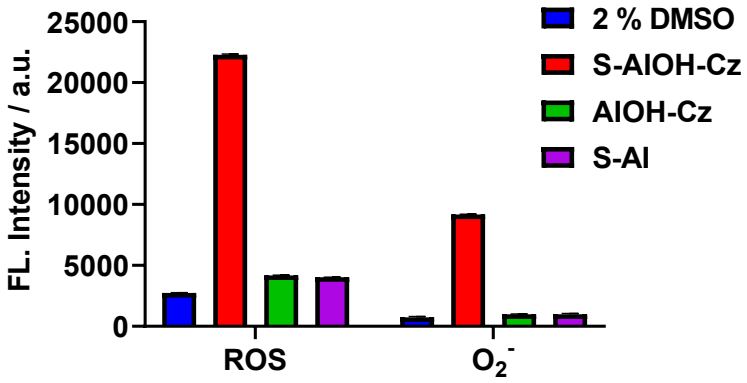


Fig S6. ROS and $O_2^{\cdot-}$ generation of **AIOH-Cz, S-AIOH-Cz, S-AI** and 2 % DMSO as co-solvent in water after 90 s irradiation by a white light source (20 mW / cm^2) DCFH-DA as ROS probe (20 μM, λ_{ex} = 480 nm, λ_{em} = 525 nm). DHE as $O_2^{\cdot-}$ probe (20 μM, λ_{ex} = 480 nm, λ_{em} = 610 nm).

The total ROS signal of the **S-AIOH-Cz** in water was measured using 2,7-dichlorofluorescin diacetate (DCFH-DA) as a probe[1]. The $O_2^{\bullet-}$ production of **S-AIOH-Cz** in water was investigated using dihydroethidium (DHE) as a probe[1,2].

Cell studies

Bacteria cell culture

E. faecium (CICC 10840), *MRSA* (ATCC 33592), *K. pneumoniae* (ATCC 700603), *A. baumannii* (CICC 22933), *P. aeruginosa* (ATCC 27853), *E. cloaca* (ATCC 13047), *E. coli*_GFP and *E. coli* (ATCC 25922) were used in this study. The pET28a_GFP_His plasmid was obtained from Cusabio technology LLC. Transetta (DE3) Chemically Competent Cells (TransGen Biotech, Beijing, China) were transformed with pET28a_GFP_His plasmid. They were selected for kanamycin resistance yielding *E. coli*_GFP. *E. coli*_GFP strain grew in the LB medium (containing 100 μg/mL kanamycin) to an OD_{600} between 0.6 and 0.8, respectively. The cultures were then induced with 1μM IPTG and grown at 20 °C overnight on a rotary shaker shaking at 180 rpm. LB medium was used for culture *E. coli* (ATCC 25922). Tryptone Soya Broth (TSB) medium was used for culture of methicillin sensitive *S. aureus* and *P. aeruginosa*. Nutrient broth (NB) was used for culture of *K. pneumoniae* and *E. cloacae*. Brain Heart Infusion Broth (BHI) was used for culture of *A. baumannii*. M.R.S. Broth (MRS) was used for culture of *E. faecium*. A single colony from the stock agar plate was added to 20 mL of liquid medium, then was grown at 37 °C in a shaker incubator (180 rpm) overnight, and OD_{600} reached approximately 2.5-3.0.

In vitro water disinfection characterization of S-AIOH-Cz

A glycerol stock of *E. coli* (ATCC 25922) and *MRSA* (ATCC 33592) was thawed, and 20 μL were used to inoculate 20 mL LB medium in the 100 mL flasks, respectively. The cultures were incubated with shaking 180 rpm on a rotary shaker at 37 °C overnight. The cultures were harvested and washed twice with ddwater. The washed cells were resuspended in water with an OD_{600} of 0.5. Then the 200 μL aliquots *E. coli* (ATCC 25922) and *MRSA* (ATCC 33592) strains were incubated with or without 20 μM of **S-AIOH-Cz** upon 20 mW / cm^2 white light irradiation for 0, 15, 30, 60 minutes and the cells were treated with 10 μg/mL of PI at 37 °C for 5 min., then measured the fluorescence emission (λ_{ex} = 535 nm, λ_{em} = 575- 750 nm).

Confocal imaging of water disinfection characterization of S-AIOH-Cz

A glycerol stock of *E. coli* (ATCC 25922) and *MRSA* (ATCC 33592) was thawed, and 20 μL were used to inoculate 20 mL LB medium in the 100 mL flasks, respectively. The cultures were incubated with shaking 180 rpm on a rotary shaker at 37 °C overnight. *E. coli*_GFP strain grew in the LB medium (containing 100 μg/mL kanamycin) to an OD_{600} between 0.6 and 0.8, respectively. The cultures were then induced with 1μM IPTG and grown at 20 °C overnight on a rotary shaker shaking at 180 rpm. The cultures were harvested and washed twice with ddwater. The washed cells were resuspended in water with an OD_{600} of 0.5. Then 200 μL aliquots *E. coli*_GFP*, E. coli* (ATCC 25922) and *MRSA* (ATCC 33592) strains were incubated with 20 μM of **S-AIOH-Cz**. After incubation upon 20 mW / cm^2 white light irradiation for 30 min, the cells were treated with 10 μg/mL of PI at 37 °C for 30 min. Then a drop of the suspension was added into an 8-well chamber followed by covering with agarose pads. Fluorescence images were acquired with ZEISS LSM 710 Confocal Microscope (Nikon Eclipse TE2000-E, CFI Plan-Apochromat VC 63 × oil immersedoptics), using a white light laser and argon ion laser for excitation (PI signal: λ_{ex} = 535 ± 20 nm λ_{em} = 615 ± 30nm; GFP signal: λ_{ex} = 488 ± 20 nm λ_{em} = 510 ± 20nm).

The 200 μL aliquots *E. coli* (ATCC 25922) and *MRSA* (ATCC 33592) strains were incubated with 10 μM of DHE at 37 °C for 30 min in dark. Then the strains incubated upon 20 mW / cm^2 white light irradiation for 30 min. Then a drop of the suspension was added into an 8-well chamber followed by covering with agarose pads. Fluorescence images were acquired with ZEISS LSM 710 Confocal Microscope (Nikon Eclipse TE2000-E, CFI Plan-Apochromat VC 63 × oil immersedoptics), using a white light laser and argon ion laser for excitation (DHE signal: λ_{ex} = 480 ± 20 nm λ_{em} = 600 ± 20nm).

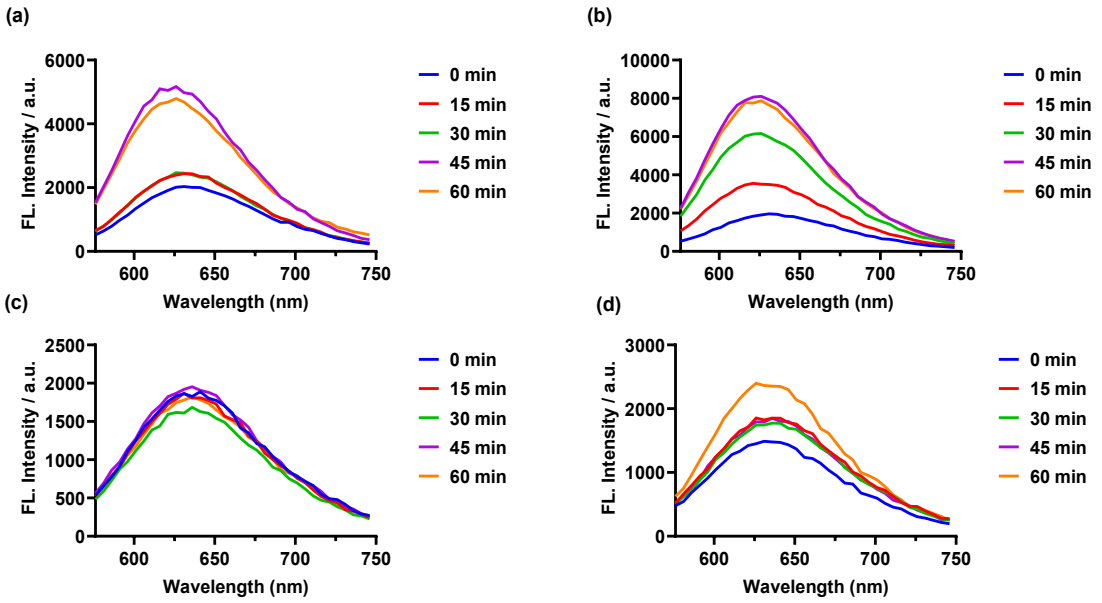


Fig S7. Time-dependent increase of PI in the presence of **S-AlOH-Cz** incubated with (a) *E. coli* (ATCC 25922) and (b) *MRSA* (ATCC 33592) strains and in the absent of **S-AlOH-Cz** incubated with (c) *E. coli* (ATCC 25922) and (d) *MRSA* (ATCC 33592) strains in water. The samples were continuously irradiated by white light source (20 mW / cm^2), PI as death cells marker (1 μg / mL, λ_{ex} = 535 nm, λ_{em} = 575-750 nm).

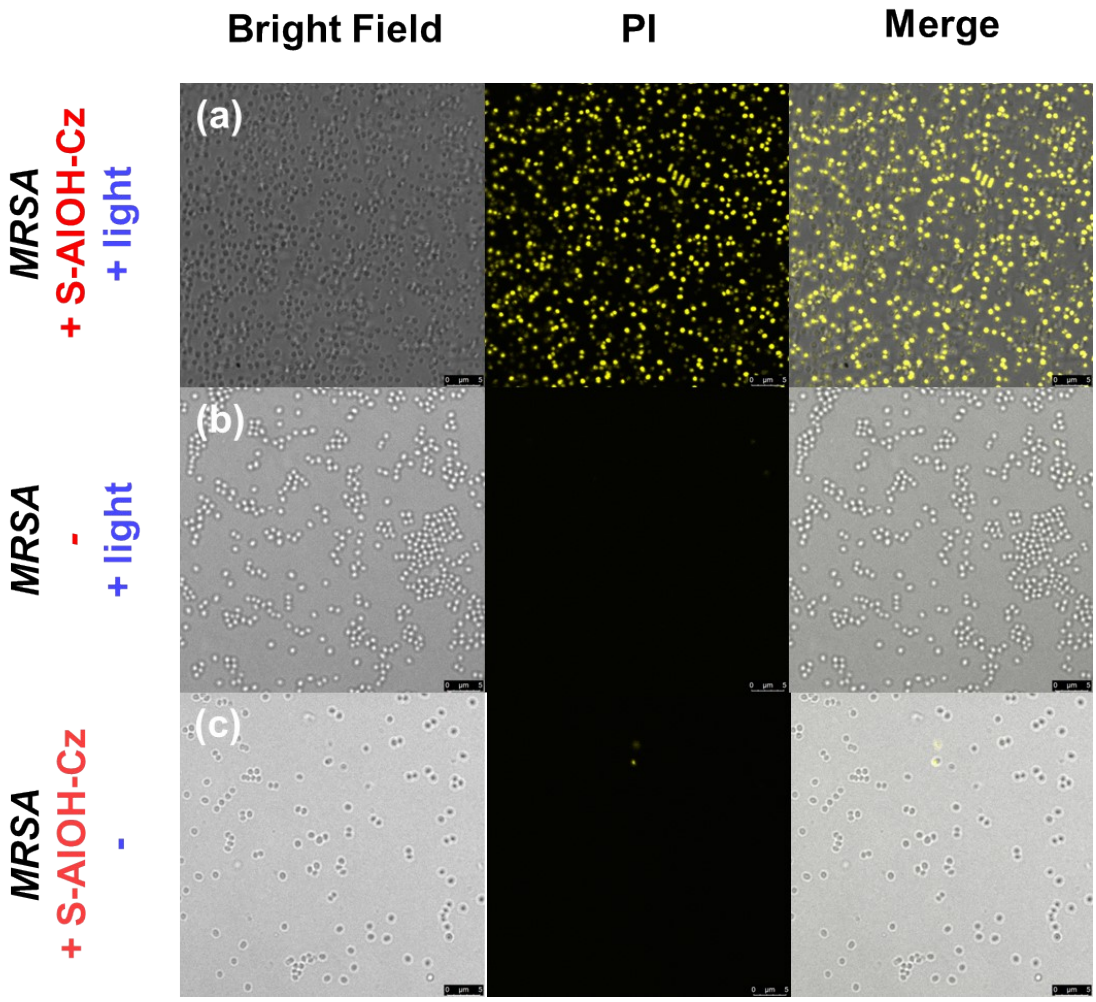


Fig S8. Confocal microscopic images of a) *MRSA* (ATCC 33592) incubated with **S-AIOH-Cz** (20 μM) upon 20 mW / cm^2 white light irradiation for 30 min in water. b) *MRSA* (ATCC 33592) incubated upon 20 mW / cm^2 white light irradiation for 30 min in water. c) *MRSA* (ATCC 33592) incubated with **S-AIOH-Cz** (20 μM) without light irradiation for 30 min in water. Yellow (PI): 585 - 645 nm signals, λ_{ex} = 535 ± 20 nm. Scale bar = 5 μm.

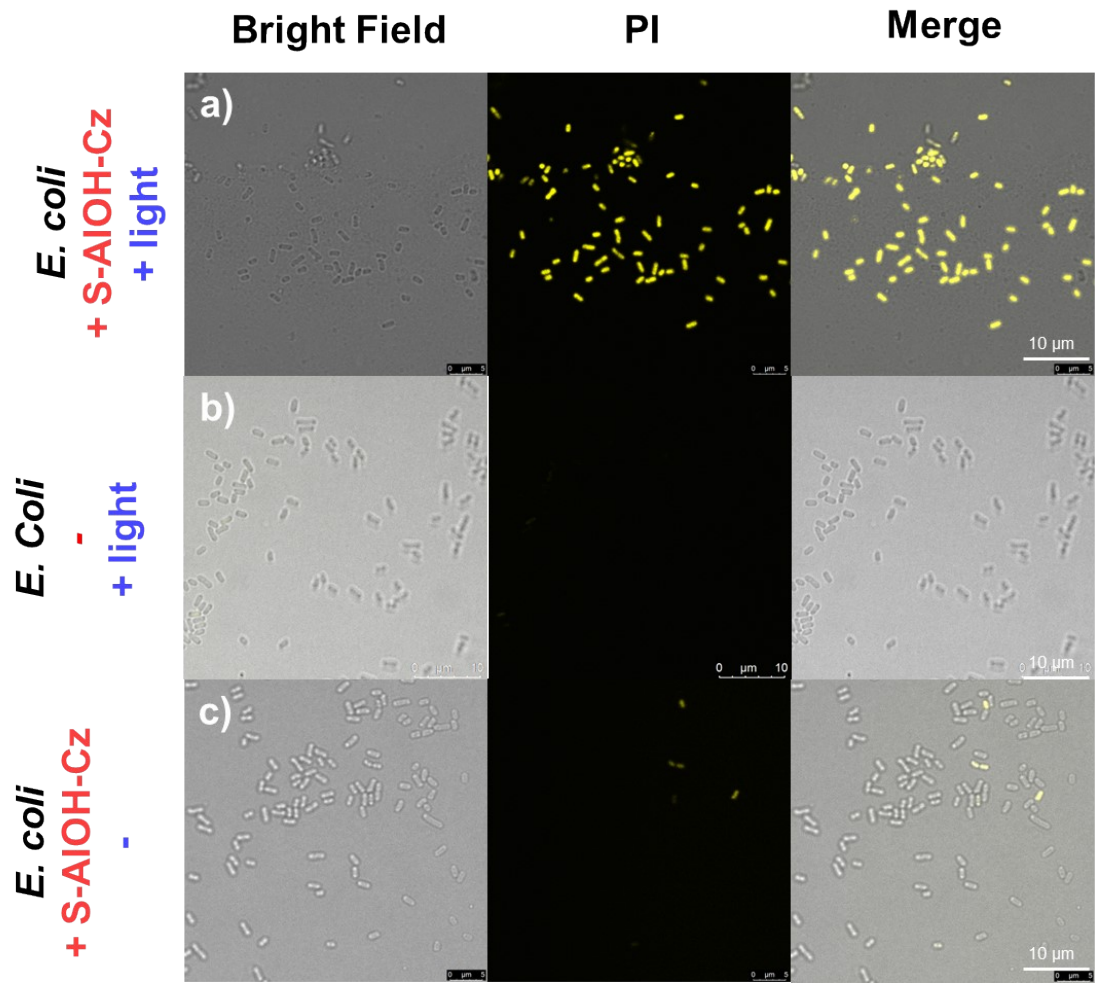


Fig S9. Confocal microscopic images of a) *E. coli* (ATCC 25922) incubated with **S-AIOH-Cz** (20 µM) upon 20 mW / cm^2 white light irradiation for 30 min in water. b) *E. coli* (ATCC 25922) incubated upon 20 mW / cm^2 white light irradiation for 30 min in water. c) *E. coli* (ATCC 25922) incubated with **S-AIOH-Cz** (20 µM) without light irradiation for 30 min in water. Yellow (PI): 585 - 645 nm signals, λ_{ex} = 535 ± 20 nm. Scale bar = 10 µm.

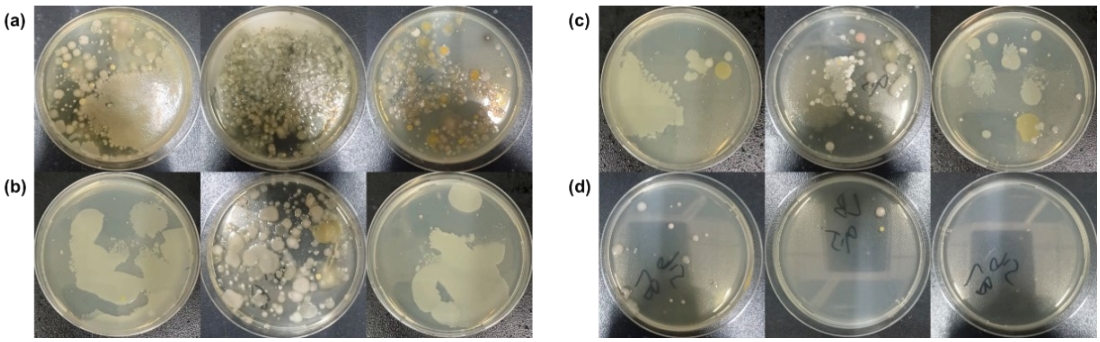
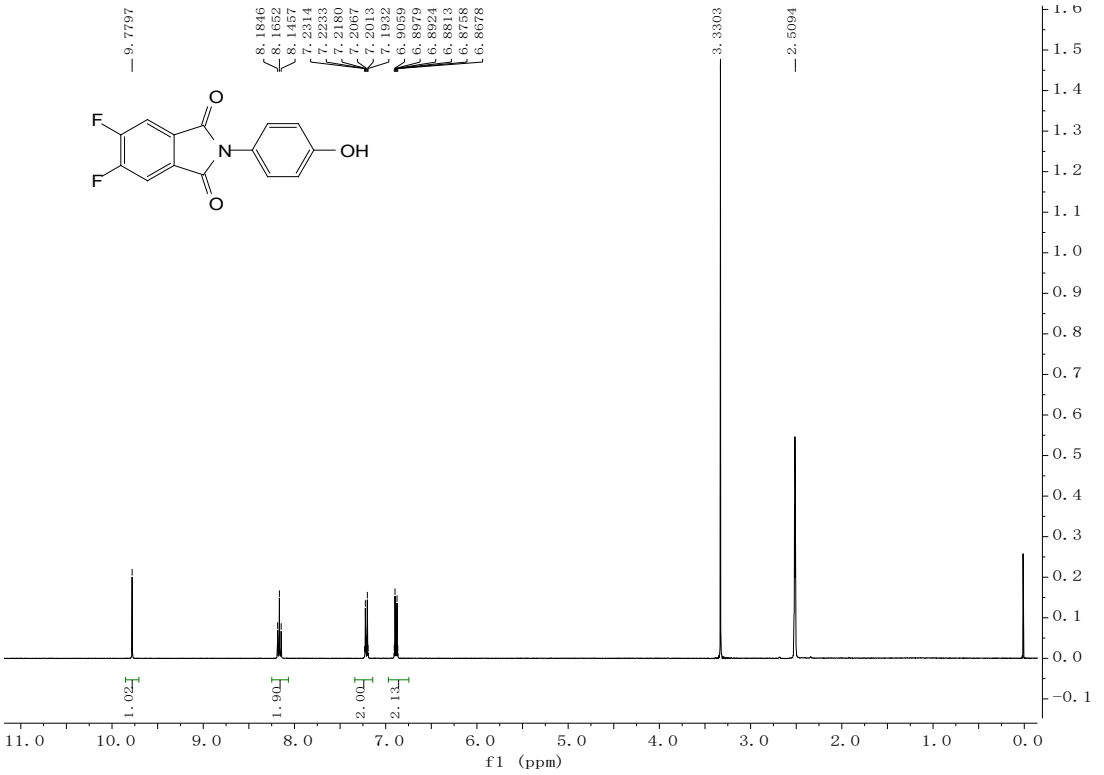
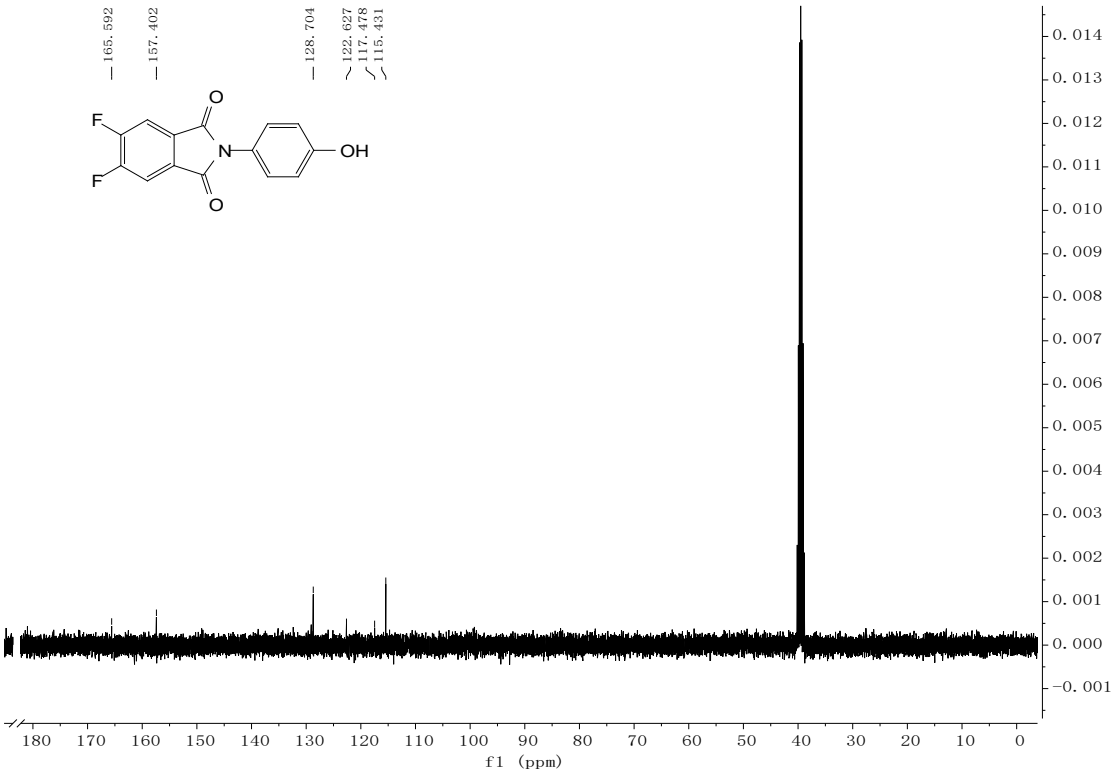


Fig S10. Plate photographs of (a)1 x, (b) 10 x , (c) 100 x, (d) 1000 x diluted water sample from “NianTan” Park in Beijing on LB agar plates supplemented without light irradiation.

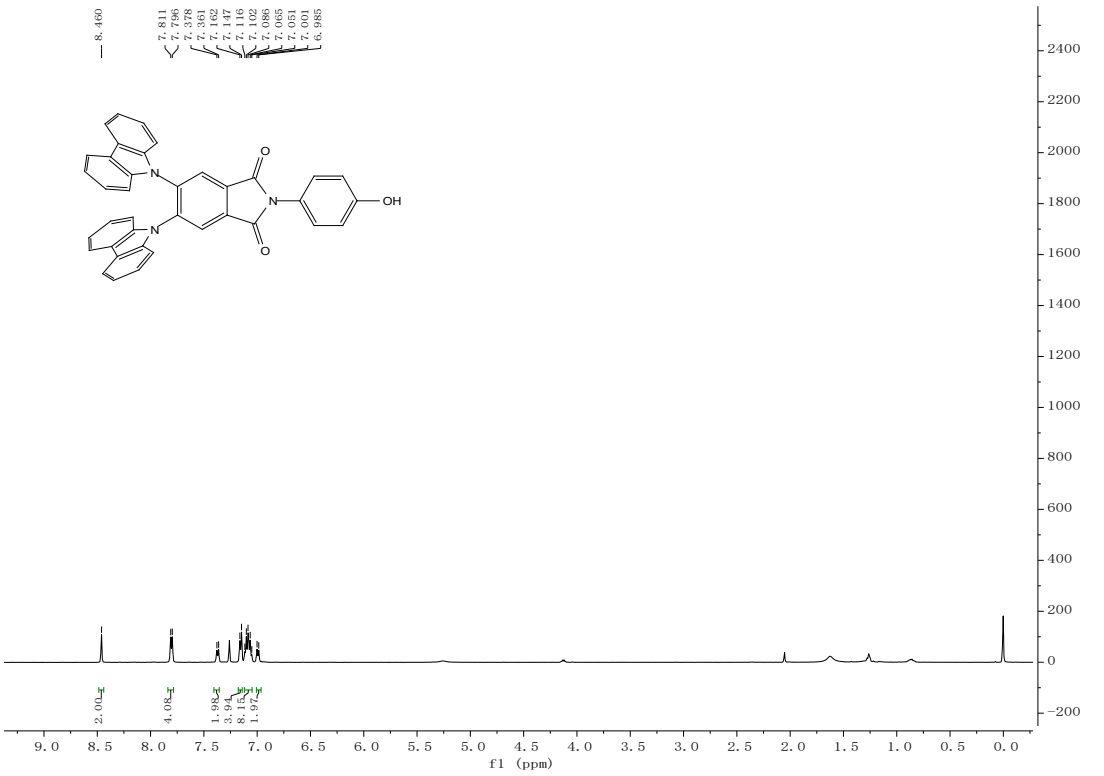
Copies of ^{1}H NMR and ^{13}C NMR spectrum of compounds



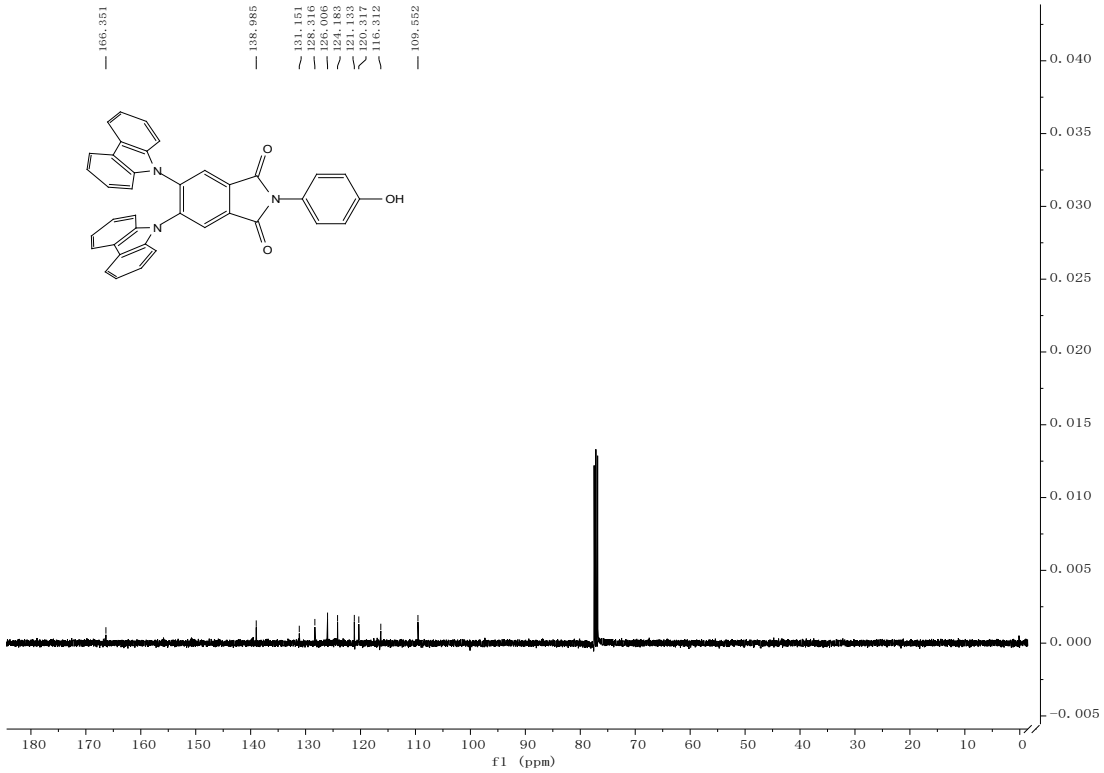
^{1}H NMR spectra of compound 2



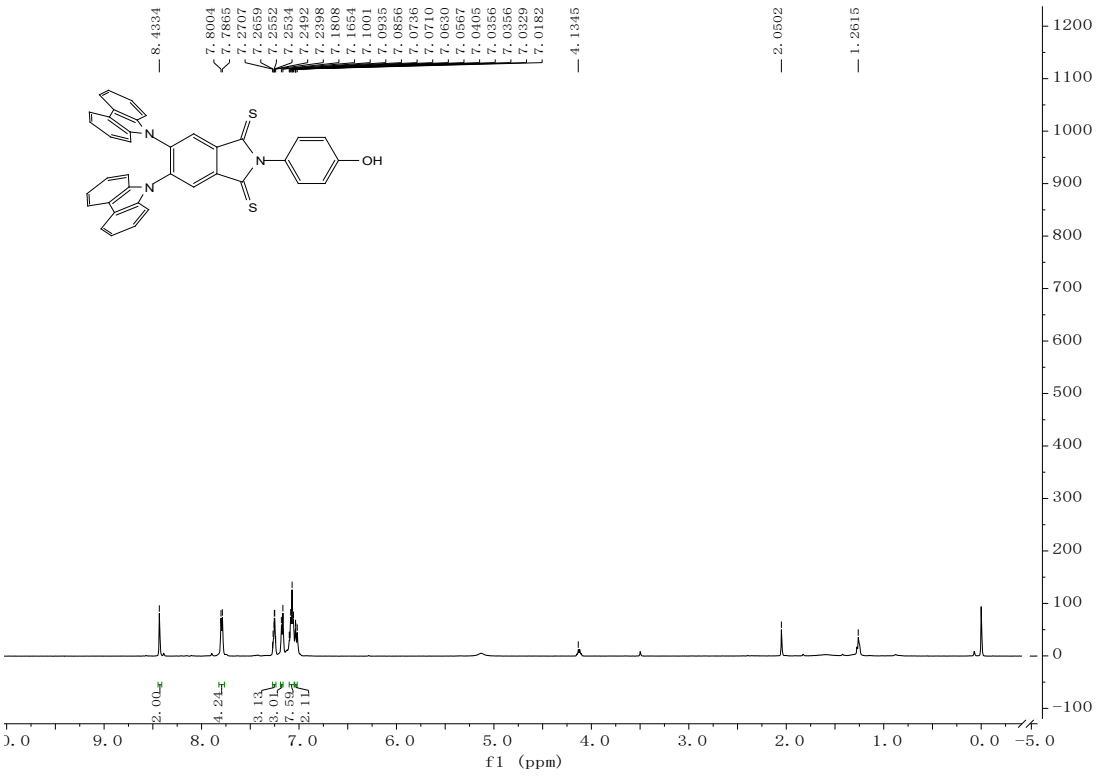
^{13}C NMR spectra of compound 2



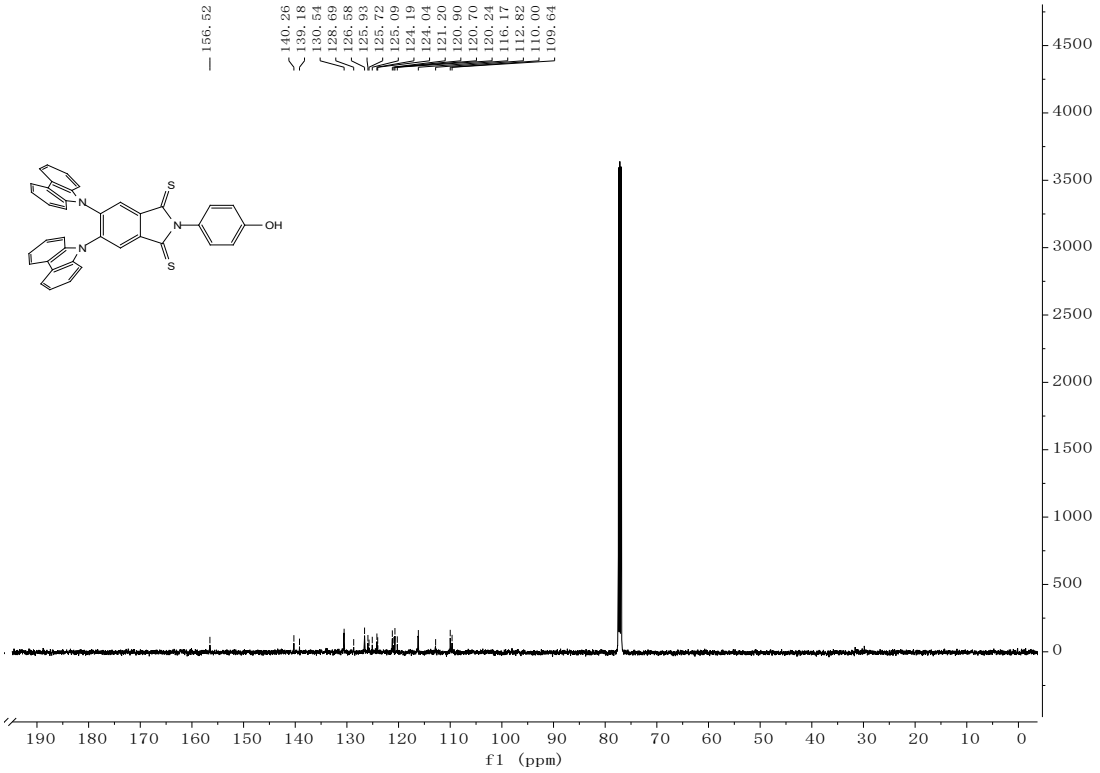
^{1}H NMR spectra of AIOH-Cz



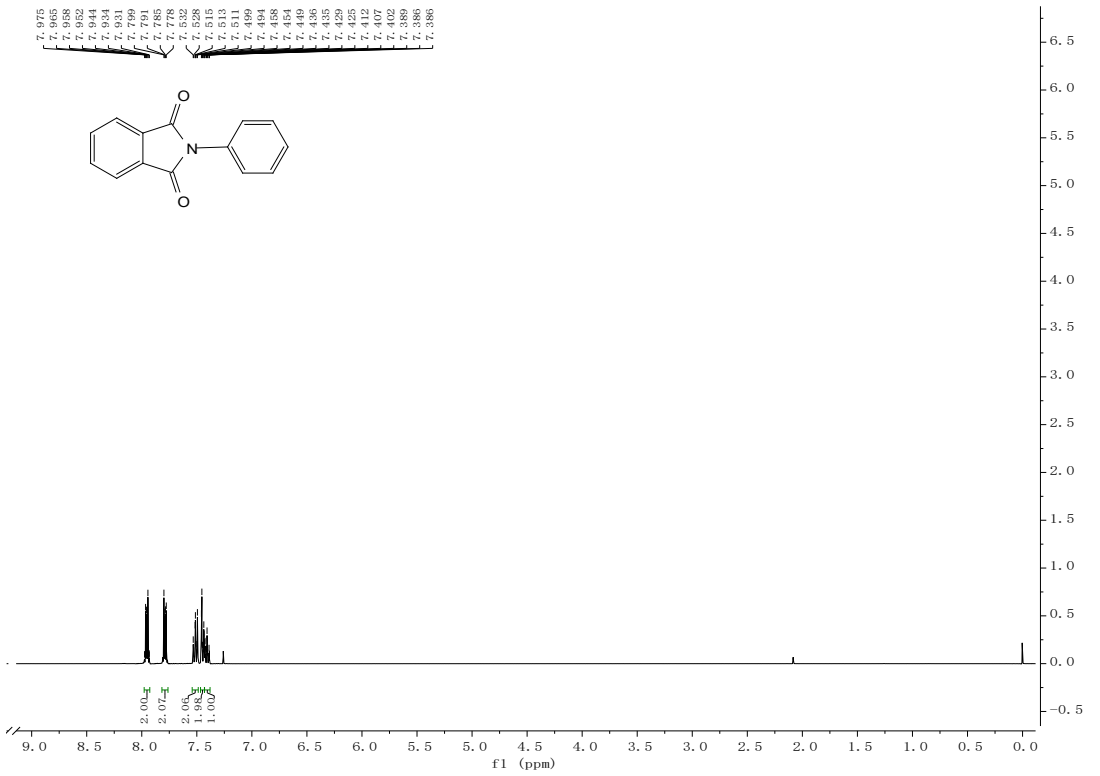
^{13}C NMR spectra of AIOH-Cz



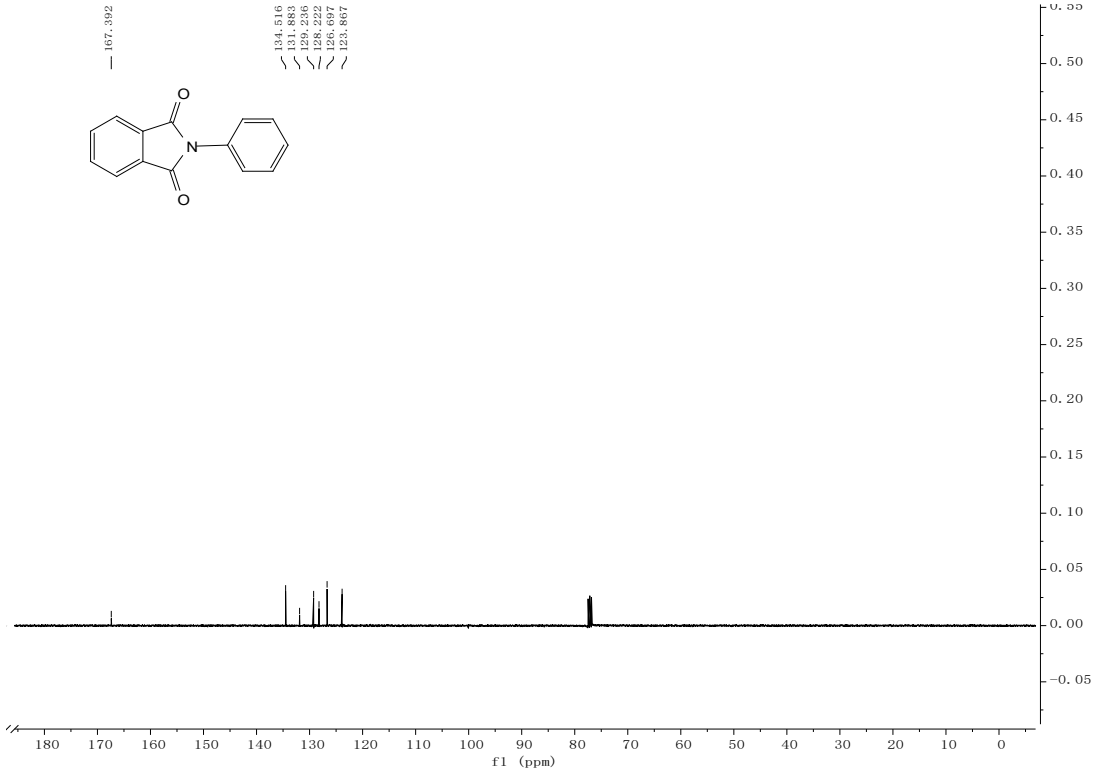
^{1}H NMR spectra of S-AIOH-Cz



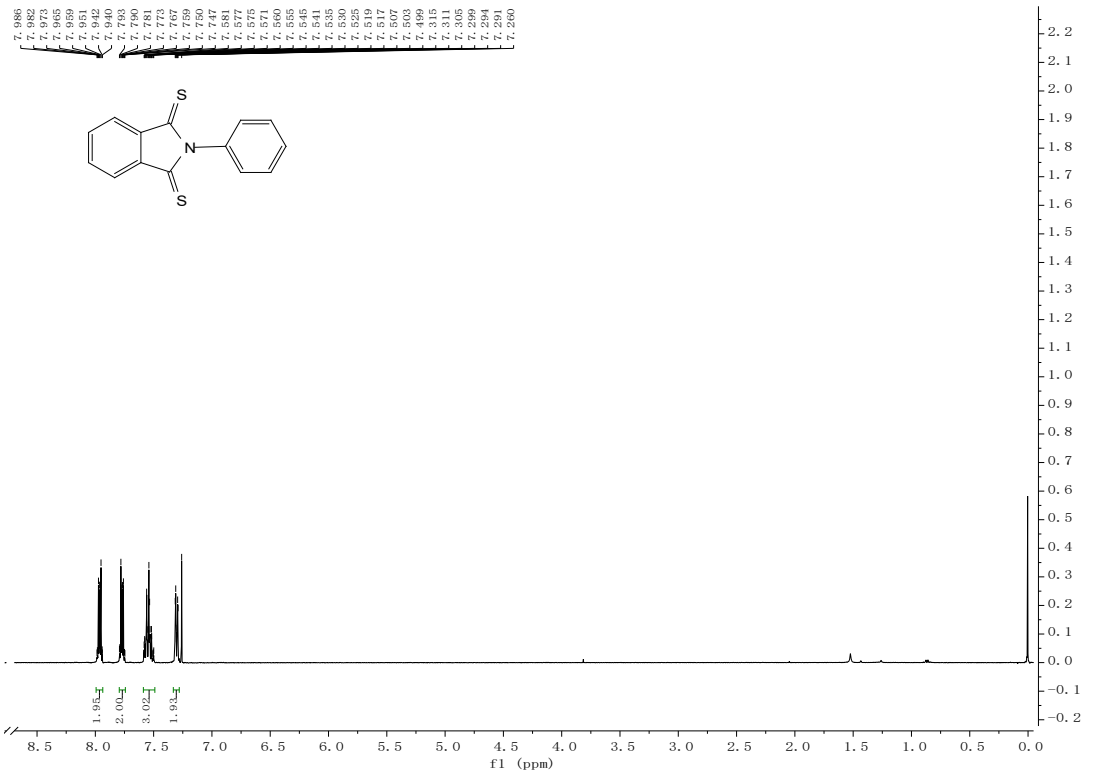
^{13}C NMR spectra of S-AIOH-Cz



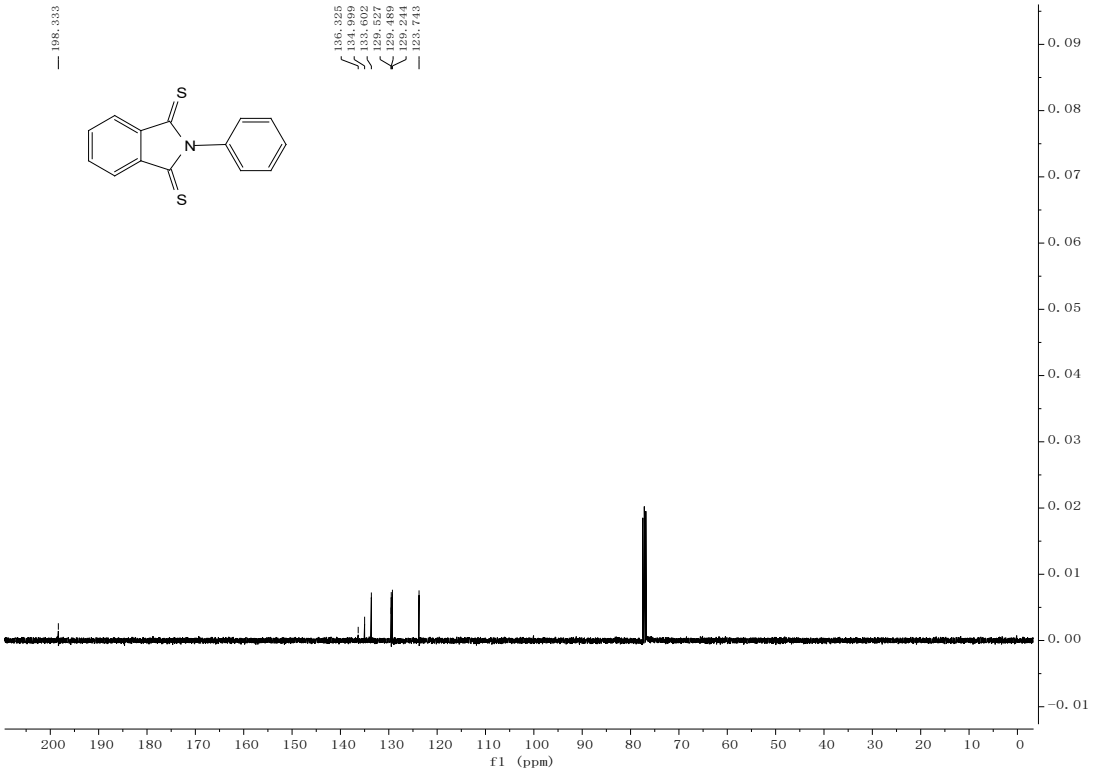
^{1}H NMR spectra of AI



^{13}C NMR spectra of AI



^{1}H NMR spectra of S-Al



^{13}C NMR spectra of S-Al

References

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