# Facilely Synthesized Multifunctional Photosensitizers for Mitochondrial and Bacterial Imaging and Photodynamic Anticancer and Antibacterial Therapy

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# 1. Experimental Procedures

## 1.1 Materials and general instruments

All chemical reagents were obtained from J&K Scientific and were used without further purification. H2DCF-DA (2`,7`-dichlorofluorescein diacetate) Detection Kit and propidium iodide (PI) were purchased from Sigma-Aldrich. Annexin V Alexa Fluor™ 555 conjugate was from Thermo Fisher Scientific. TLC analyses were performed on silica gel GF 254. Column chromatography purification was carried out on silica gel (200–300 mesh). NMR spectra were recorded using a Bruker AMX-600. Chemical shifts were given in ppm relative to the internal reference TMS, $CDCl_3$ or DMSO-$d_6$ as the internal standard. The following abbreviations were used in $^1H$ NMR: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet. High resolution mass spectra were recorded on a Bruker Daltonics Bio TOF mass spectrometer. Fluorescence spectra were obtained using a Horiba Duetta spectrofluorimeter with a 10 mm quartz cuvette. UV-Vis absorption spectra were recorded on a Hitachi PharmaSpec UV-1900 UV-Visible spectrophotometer. Confocal fluorescence images were recorded using a Zeiss LSM 880 confocal laser scanning microscope. The light source was a white LED illuminant from Opple Lighting Co., LTD with the power of 5 W, diameter of 5 cm and intensity of 5 mW/cm$^2$.

## 1.2 ROS detection in solution

The ROS generation was studied using H2DCF-DA as an indicator as the fluorescent intensity at 517 nm increase upon reaction with ROS. H2DCF-DA (5 μM) was mixed with probe in PBS and exposed to white light (power at around 5 mW/cm$^2$) irradiation for 0, 60, 120 and 180 min. The fluorescent intensity at 517 nm was monitored by the fluorescence spectrum with the excitation of 488 nm.

## 1.3 MTT assay for the cell cytotoxicity and photo toxicity

The standard MTT assay was applied to determine the toxicity in HeLa cells. About 7000 cells per well were seeded in 96-well plates in 100 μL of culture medium and cultured overnight for 70–80% cell confluence. After that the medium was replaced with 100 μL of fresh medium containing different concentrations of probes (0–20 μM), and DMSO was used as a negative control. Then, the cells were exposed to white light

(power at around 5 mW/cm$^2$) for 0, 60, 120 and 180 min. After 24 hours of incubation, 10 μL of 12 mM MTT stock solution mixed in 90 μL of PBS was added to each well for additional 4 h incubation. The absorbance at 570 nm was measured using an ELISA plate reader. Cell viability (%) = ($OD_{570}$ sample/$OD_{570}$ control) × 100%.

### 1.4 Cell culture

HeLa cells, MDA-MB-231 cells, B16-F10 cells, HFF cells and HEK-293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies) supplemented with 10% (*v*/*v*) fetal bovine serum (Life Technologies) and 1% antibiotic-antimycotic (Sigma-Aldrich). HepG2 and T47D cells were cultured in RPMI 1640 medium (Life Technologies) supplemented with 10% (*v*/*v*) fetal bovine serum and 1% antibiotics-antimycotic. HUVEC cells were cultured in vascular cell basal medium (ATCC) supplemented with endothelial cell growth kit-BBE (ATCC). All of the cell lines were maintained in the presence of 5% $CO_2$ in a humidified incubator at 37 °C.

### 1.5 Cell imaging

For laser scanning confocal microscopy imaging, the cells ($4 \times 10^3$ per well) were placed on a 35 mm glass bottom cell culture dish and incubated for 24 h. Then, the cells were incubated with 10 μM probe and TPE-Ph-In for 30 min. After washing with PBS three times, the confocal fluorescence images were recorded in the wavelength range of 560–650 nm with excitation at 488 nm for TPE-Ph-In. For **LIQ** probes, please find the detail excitation and emission wavelength in the corresponding Figure caption. In Mitochondria Co-localization experiment, we have selectively collected the images from 5 different viewshed with about 3-8 cells and analysed the overlapped fluorescence from LIQ probe and commercial mitochondrial dye. The final Pearson's correlation coefficient (Pr) was chosen from the one approximated to the average Pr.

### 1.6 Intracellular ROS Detection

The ROS generation within living cells was detected by using H2DCF-DA. HeLa cells were incubated with **LIQ-6** (10 μM) and H2DCF-DA (40 μM) under dark for 30 min. Cells were washed with PBS and irradiated with white light for 30 min (5 mW/cm$^{-2}$ ). For control experiments, the same protocol was performed in the absence of LIQ-6 or

white light irradiation. The images were collected. The excitation wavelength was 488 and the emissions were collected in the range of 500-550 nm.

### 1.7 Propidium iodide co-staining assay for cancer cell photodynamic therapy

HeLa cells were pretreated with **LIQ-6** (10 μM) for 30 min and washed with PBS for three times, while cells were pretreated with PBS as control. Then, cells were stained with 1 μM PI for 5 min and then exposed to white light (about 5 mW/cm$^2$) for 0, 30, 60, 120 and 180 min. After washing cells with PBS three times, the confocal fluorescence images were recorded. The excitation wavelengths for **LIQ-6** and PI were 405 and 561 nm, respectively. And the emissions for **LIQ-6** and PI were collected in the range of 480-580 nm and 600-720 nm, respectively.

### 1.8 Annexin V Alexa Fluor™ 555 conjugate assay for cell apoptosis

HeLa cells were pretreated with **LIQ-6** (10 μM) for 30 min and washed with PBS for three times. Then, cells were exposed to white light (5 mW/cm$^2$) for 0, 30, 60, 120 and 180 min. After washed with PBS three times, cells were stained with Annexin V Alexa Fluor™ 555 conjugate or PI following the standard protocol (Life Technologies). The confocal fluorescence images were recorded. For Annexin V: $\lambda_{ex}$ = 561 nm, $\lambda_{em}$ = 580-650 nm; PI: $\lambda_{ex}$ = 561 nm, $\lambda_{em}$ = 590-720 nm. The control experiments were carried out under white light irradiation for 3h in the absence of LIQ-6 treatment.

### 1.9 Bacterial killing

Two Gram-positive bacteria (*B. subtilis* and *S. aureus*) and two Gram-negative bacteria (*E. coli Nissle* and *P aeruginosa*) are from China General Microbiologocal CultureCollection Center (CGMCC), while *E. coli K12* (Gram-negative) and *S. epidermidis* (Gram-positive) were bought from American Type Culture Collection (ATCC).

A single colony of bacteria on solid culture medium (LB) was transferred to 10 ml of liquid culture medium and grown at 37 °C for 6 h. The antibacterial activity of **LIQ-6** was determined by incubating the bacterial suspensions with certain concentration of **LIQ-6** for 20 min under dark. After centrifuged the bacterial suspensions at 8000 rpm for 5 min, the supernatant was removed and washed with PBS three times. The bacteria were dispersed again in PBS and either exposed to white light (5 mW/cm$^2$) or incubated

under dark for 30 min (for *P. aeruginosa*, 120 min). Then, the bacteria were transferred onto an agar plate and incubated at 37 °C overnight. The bacteria viability was then determined and quantified by the plate count method.

### 1.10 *In vivo* antibacterial test on infected skin wound

All animal procedures were carried out under the guidelines set of the Institutional Animal Care and Use Committee of Sichuan province, and the overall project protocols were approved by the Animal Ethics Committee of Southwest Jiaotong University.

Wistar mice from Chengdu Dashuo Experimental Animal Co. LTD were divided into four groups: (1) control group without bacterial infection and treatment; (2) *S. aureus*-infected wounds with PBS treatment; (3) *S. aureus*-infected wounds with **LIQ-6** treatment; (4) *S. aureus*-infected wounds with **LIQ-6** plus white light irradiation treatment. The mice were anesthetized by intraperitoneal injection of 10% chloral hydrate. Then, about two 8×8 $mm^2$ wounds were cut on the both sides of the spine. 50 μL of *S. aureus* suspension ($10^8$ CFU/mL) was inoculated over each wound and covered with sterile nonwoven fabrics for 1 day. Then, 50 μL of **LIQ-6** (10 μM) or PBS was smeared on the wounds, and the wounds were irradiated by white light with a power density of 20 mW/$cm^2$ for 30 min or under dark. On the Day 1, Day 3, Day 7 and the Day 14 after surgery, the entire wound with adjacent normal skin were obtained. The infectious tissues were separated and homogenized in normal saline. The homogenates were diluted 1000 times with normal saline. 20 μL of the bacteria solution was sprayed onto LB agar plate and subjected to culturing at 37 °C. After 24 h, the bacterial colonies on the plate were counted for analysis. The other tissues were fixed in 4% paraformaldehyde for the histological analysis.

Histological analysis of the wounds was carried out on day 1, 3 and day 14 postoperation. The wound tissues were collected and fixed in 4% formaldehyde solution for 30 min. The pathological sections of wound tissues were analyzed by HE staining. Histological images were taken using an inverted microscope (BA400Digital, Motic China Group Co. Ltd.).

## 2. Photophysical properties

Table S1 Optical properties of all probes in different polarity solvent and in solid state.

| Probe | Solvent | $\lambda_{abs}$ (nm) | $\lambda_{em}$ （nm） | $\Phi_F$ (%) | ε ($M^{-1}.cm^{-1}$) | ΔSS/nm |
|---|---|---|---|---|---|---|
| **LIQ-3** | DCM | 422 | 521 | 40.824 | 14344 | 99 |
| | THF | 413 | 550 | 9.098 | 11240 | 137 |
| | EtOH | 415 | 545 | 14.222 | 13890 | 131 |
| | DMSO | 418 | 563 | 7.032 | 13334 | 145 |
| | PBS | 405 | 556 | 2.374 | 13180 | 151 |
| | Solid | | 518 | 13.44 | | |
| **LIQ-4** | DCM | 416 | 512 | 28.456 | 16050 | 96 |
| | THF | 409 | 536 | 15.023 | 14590 | 127 |
| | EtOH | 411 | 532 | 23.065 | 16060 | 121 |
| | DMSO | 412 | 556 | 13.657 | 15720 | 144 |
| | PBS | 400 | 544 | 5.072 | 15770 | 144 |
| | Solid | | 531 | 3.78 | | |
| **LIQ-5** | DCM | 404 | 500 | 17.211 | 11850 | 96 |
| | THF | 399 | 527 | 27.813 | 9040 | 128 |
| | EtOH | 399 | 516 | 32.522 | 11010 | 117 |
| | DMSO | 402 | 539 | 23.863 | 12740 | 137 |
| | PBS | 389 | 531 | 8.765 | 12070 | 142 |
| | Solid | | 523 | 9.32 | | |
| **LIQ-6** | DCM | 401 | 504 | 9.698 | 15390 | 103 |
| | THF | 399 | 524 | 5.160 | 19397 | 125 |
| | EtOH | 398 | 520 | 6.892 | 15070 | 122 |
| | DMSO | 400 | 535 | 3.683 | 16847 | 135 |
| | PBS | 384 | 533 | 5.156 | 12250 | 150 |
| | Solid | | 540 | 1.59 | | |
| **LIQ-7** | DCM | 454 | 580 | 6.605 | 4040 | 126 |
| | THF | 441 | 563 | 0.338 | 2548 | 122 |
| | EtOH | 441 | 576 | 0.297 | 4589 | 135 |
| | DMSO | 444 | 597 | 1.233 | 3438 | 153 |
| | PBS | 430 | 539 | 0.092 | 5576 | 108 |
| | Solid | | 620 | 3.02 | | |
| **LIQ-8** | DCM | 464 | 598 | 0.269 | 11200 | 134 |
| | THF | 447 | 548 | 0.180 | 7587 | 101 |
| | EtOH | 450 | 537 | 0.052 | 10968 | 87 |
| | DMSO | 453 | 550 | 0.091 | 9208 | 97 |
| | PBS | 441 | 524 | 0.012 | 10624 | 83 |
| | Solid | | 653 | 0 | | |

Fluorescence quantum yields were determined using quinine sulfate in 0.1 M sulphuric acidas as standard (ΦF = 0.55).

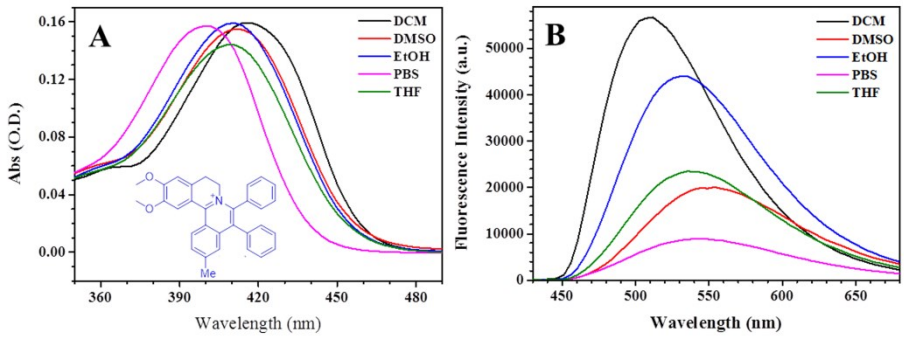



**Fig. S1** (A) UV-vis spectra and (B) fluorescence spectra of **LIQ-4** (10 μM) in DCM, THF, EtOH, DMSO and PBS solution. Excitation wavelength = 416 nm (DCM), 412 nm (DMSO), 411 nm (EtOH), 400 nm (PBS), 409 nm (THF).

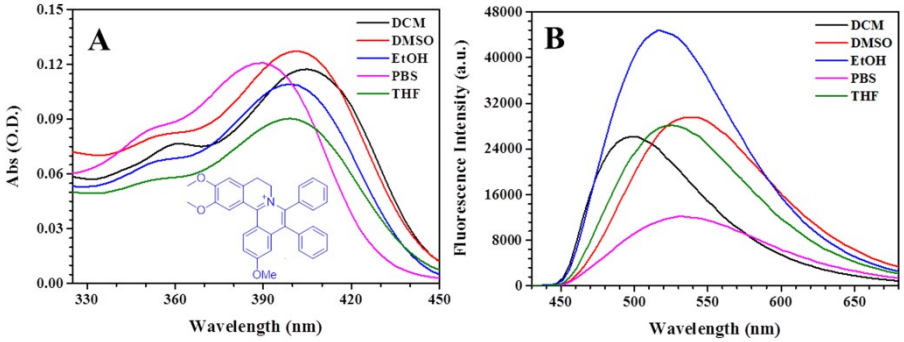



**Fig. S2** (A) UV-vis spectra and (B) fluorescence spectra of **LIQ-5** (10 μM) in DCM, THF, EtOH, DMSO and PBS solution. Excitation wavelength = 404 nm (DCM), 402 nm (DMSO), 399 nm (EtOH), 389 nm (PBS), 399 nm (THF).

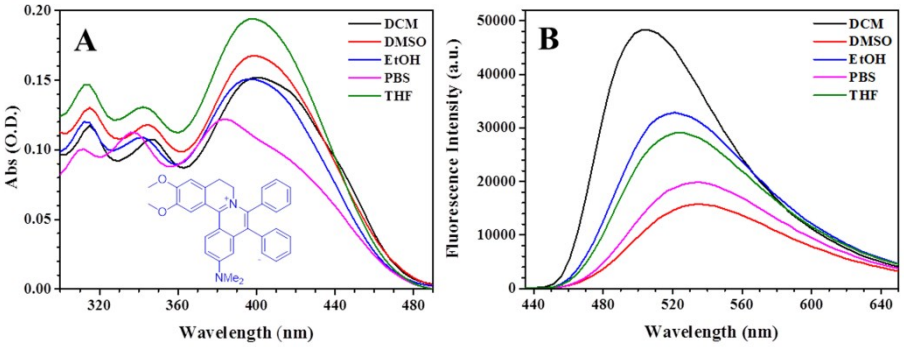



**Fig. S3** (A) UV-vis spectra and (B) fluorescence spectra of **LIQ-6** (10 μM) in DCM, THF, EtOH, DMSO and PBS solution. Excitation wavelength = 401 nm (DCM), 400 nm (DMSO), 398 nm (EtOH), 384 nm (PBS), 399 nm (THF).

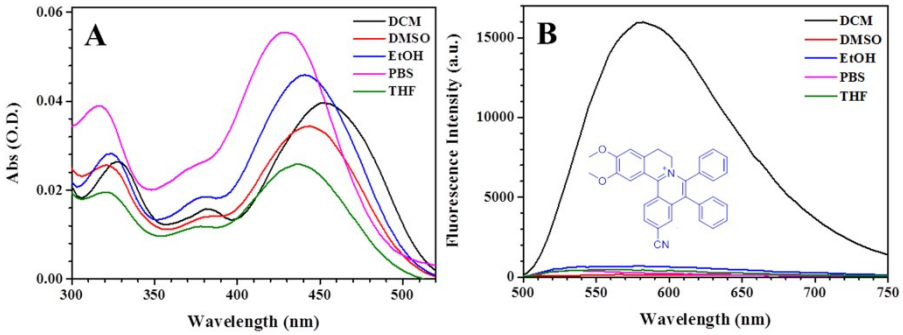



**Fig. S4** (A) UV-vis spectra and (B) fluorescence spectra of **LIQ-7** (10 μM) in DCM, THF, EtOH, DMSO and PBS solution. Excitation wavelength = 454 nm (DCM), 444 nm (DMSO), 441 nm (EtOH), 430 nm (PBS), 441 nm (THF).

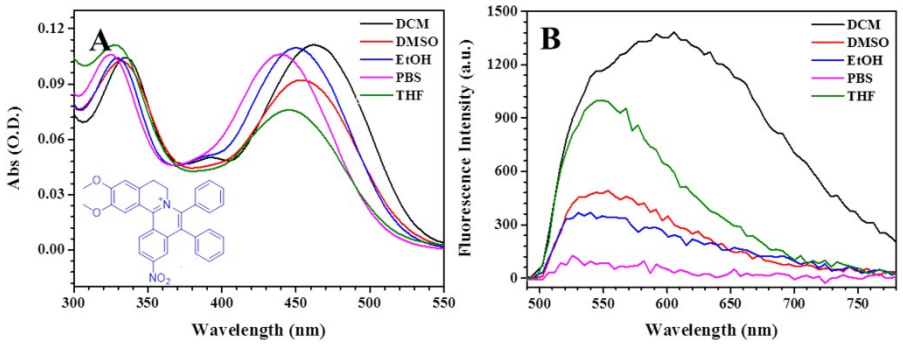



**Fig. S5** (A) UV-vis spectra and (B) fluorescence spectra of **LIQ-8** (10 μM) in DCM, THF, EtOH, DMSO and PBS solution. Excitation wavelength = 464 nm (DCM), 453 nm (DMSO), 450 nm (EtOH), 441 nm (PBS), 447 nm (THF).

## 3. Mitochondria co-localization experiment

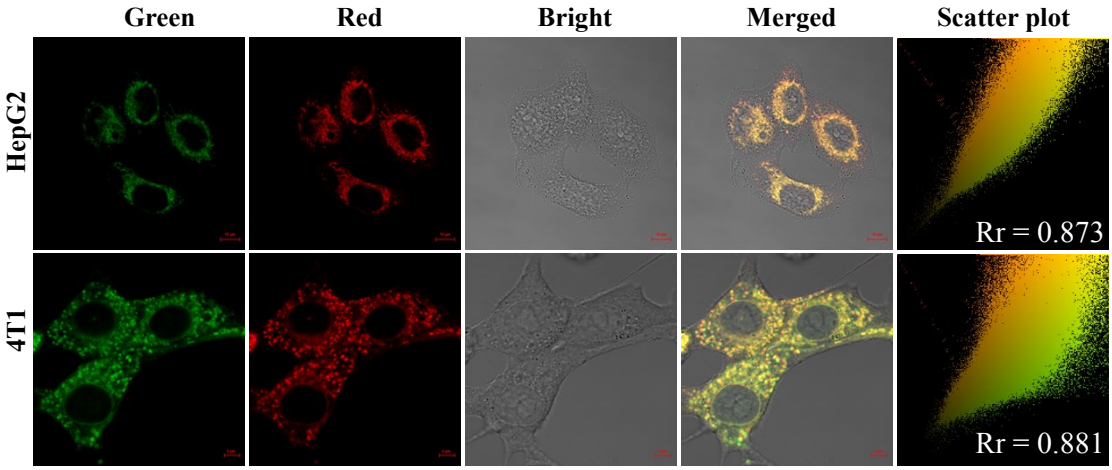



**Fig. S6** Confocal fluorescence imaging of 4T1 and HepG2 cells with staining of **LIQ-6** (10 μM) and TPE-Ph-In (5 μM) for 30 min. Green fluorescence were from **LIQ-6** ($\lambda_{ex}$ = 405 nm, $\lambda_{em}$ = 480-580 nm); Red fluorescence were from TPE-Ph-In ($\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 620-720 nm).

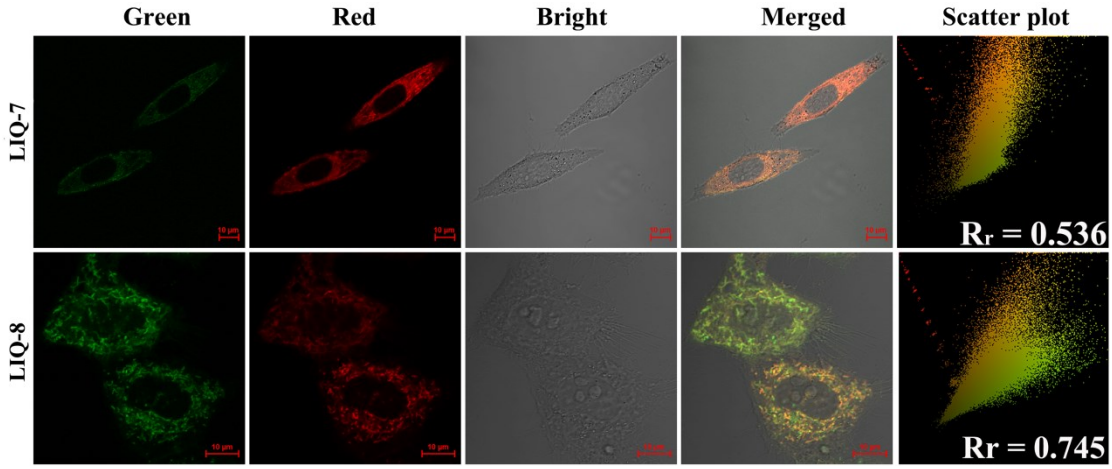



**Fig. S7** Confocal fluorescence imaging of HeLa cells with staining of **LIQ** (10 μM) and TPE-Ph-In (5 μM) for 30 min. Green fluorescence were from **LIQ-7**, **LIQ-8** ($\lambda_{ex}$ = 405 nm, $\lambda_{em}$ = 500-610 nm),; Red fluorescence were from TPE-Ph-In ($\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 620-720 nm).

## 4. Cytotoxicity and phototoxicities

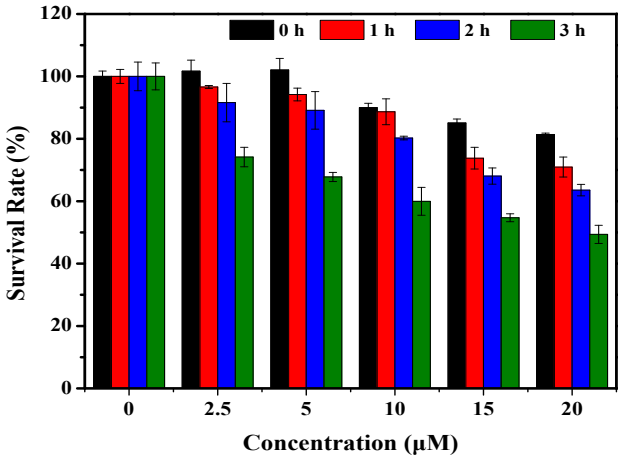



**Fig. S8** HeLa cell survival rates with incubation of different concentrations of **LIQ-3** (2.5, 5, 10, 15, 20 μM) in the presence of 5 mW/cm$^2$ white light irradiation for different time (0 h, 1 h, 2 h or 3 h).

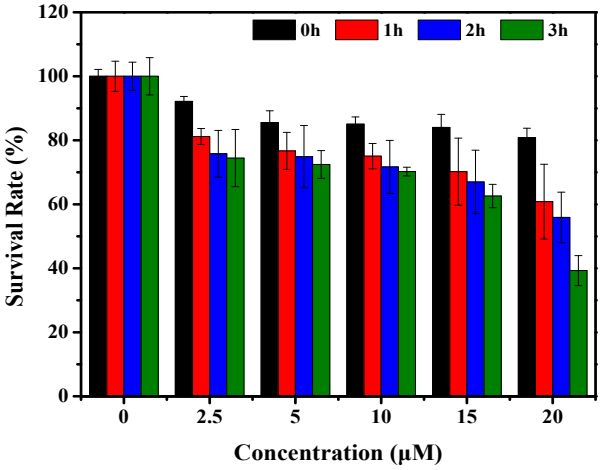



**Fig. S9** HeLa cell survival rates with incubation of different concentrations of **LIQ-4** (2.5, 5, 10, 15, 20 μM) in the presence of 5 mW/cm$^2$ white light irradiation for different time (0 h, 1 h, 2 h or 3 h).

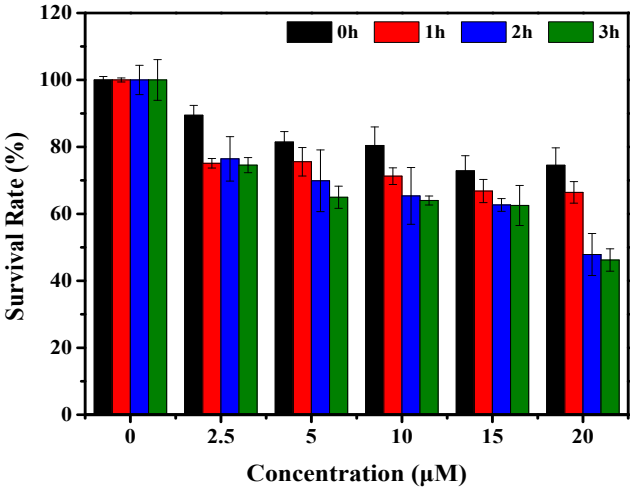



**Fig. S10** HeLa cell survival rates with incubation of different concentrations of **LIQ-5** (2.5, 5, 10, 15, 20 μM) in the presence of 5 mW/cm$^2$ white light irradiation for different time (0 h, 1 h, 2 h or 3 h).

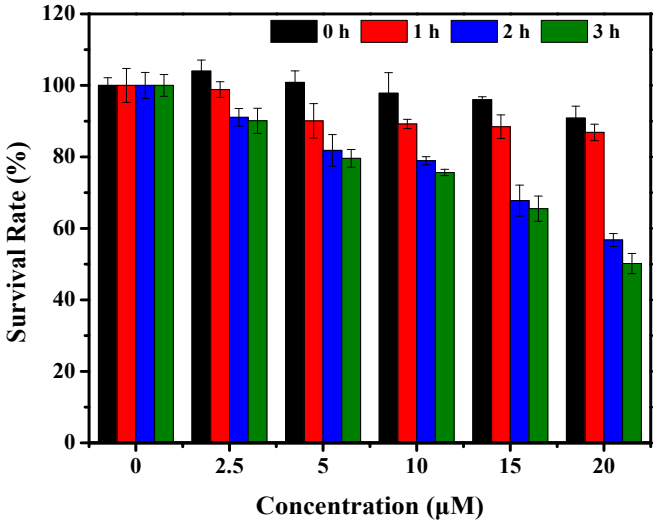



**Fig. S11** HeLa cell survival rates with incubation of different concentrations of **LIQ-7** (2.5, 5, 10, 15, 20 μM) in the presence of 5 mW/cm$^2$ white light irradiation for different time (0 h, 1 h, 2 h or 3 h).

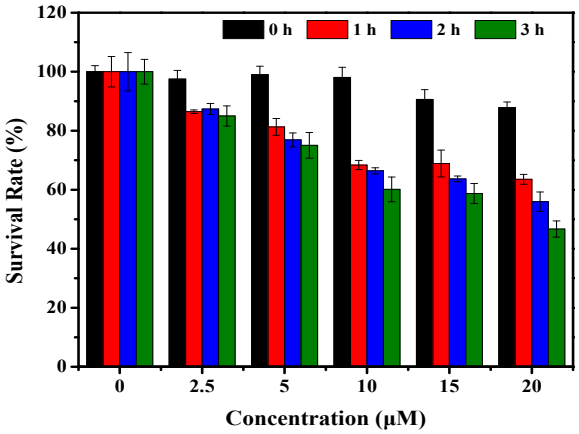



**Fig. S12** HeLa cell survival rates with incubation of different concentrations of **LIQ-8** (2.5, 5, 10, 15, 20 μM) in the presence of 5 mW/cm$^2$ white light irradiation for different time (0 h, 1 h, 2 h or 3 h).

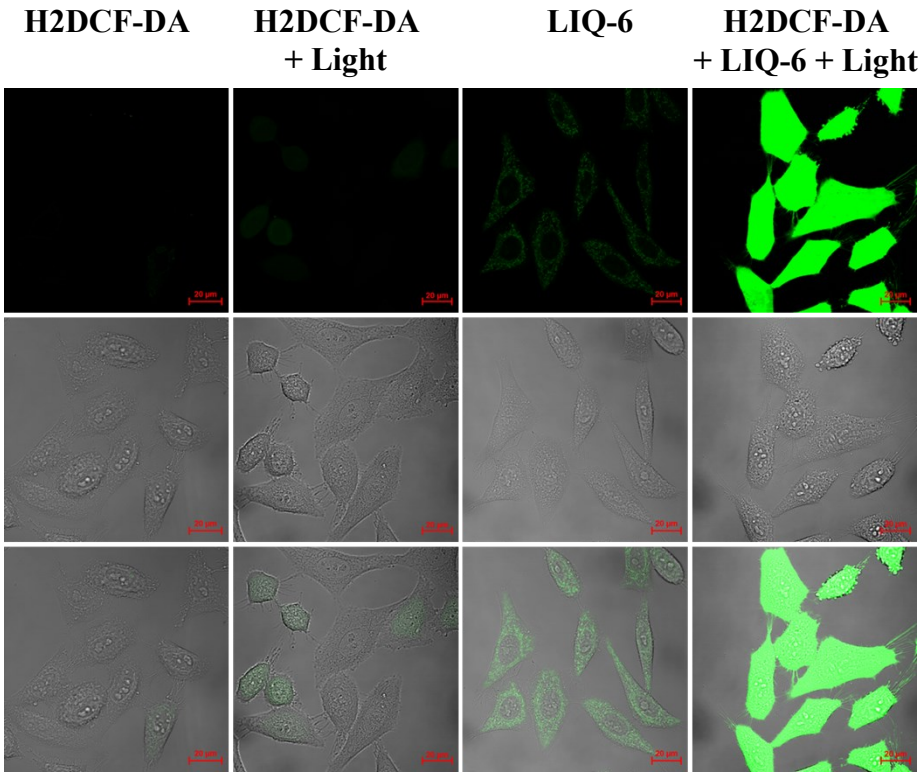



**Fig. S13** Fluorescence images of HeLa cells stained with H2DCF-DA (40 μM) and **LIQ-6** (10 μM) in the absent or present of white light exposure (5 mW/cm$^2$). $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 500-550 nm.

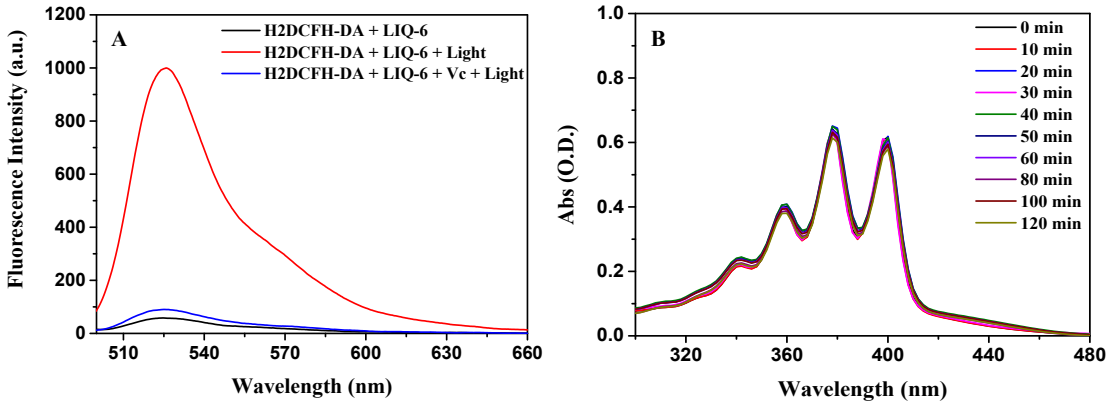



**Fig. S14** ROS generation of **LIQ-6** upon white light irradiation. (A) Fluorescence spectra of H2DCF-DA (40 μM) probe in the absence or presence of **LIQ-6** (10 μM) and Vc (100 μM) under the irradiation for 3 h for free radical ROS detection. (B) UV/Vis absorption spectra of ABDA (50 μM) in the presence of **LIQ-6** (5 μM) under white-light irradiation for 0-120 min for singlet oxygen detection.

## 5. Photo induced cell apoptosis

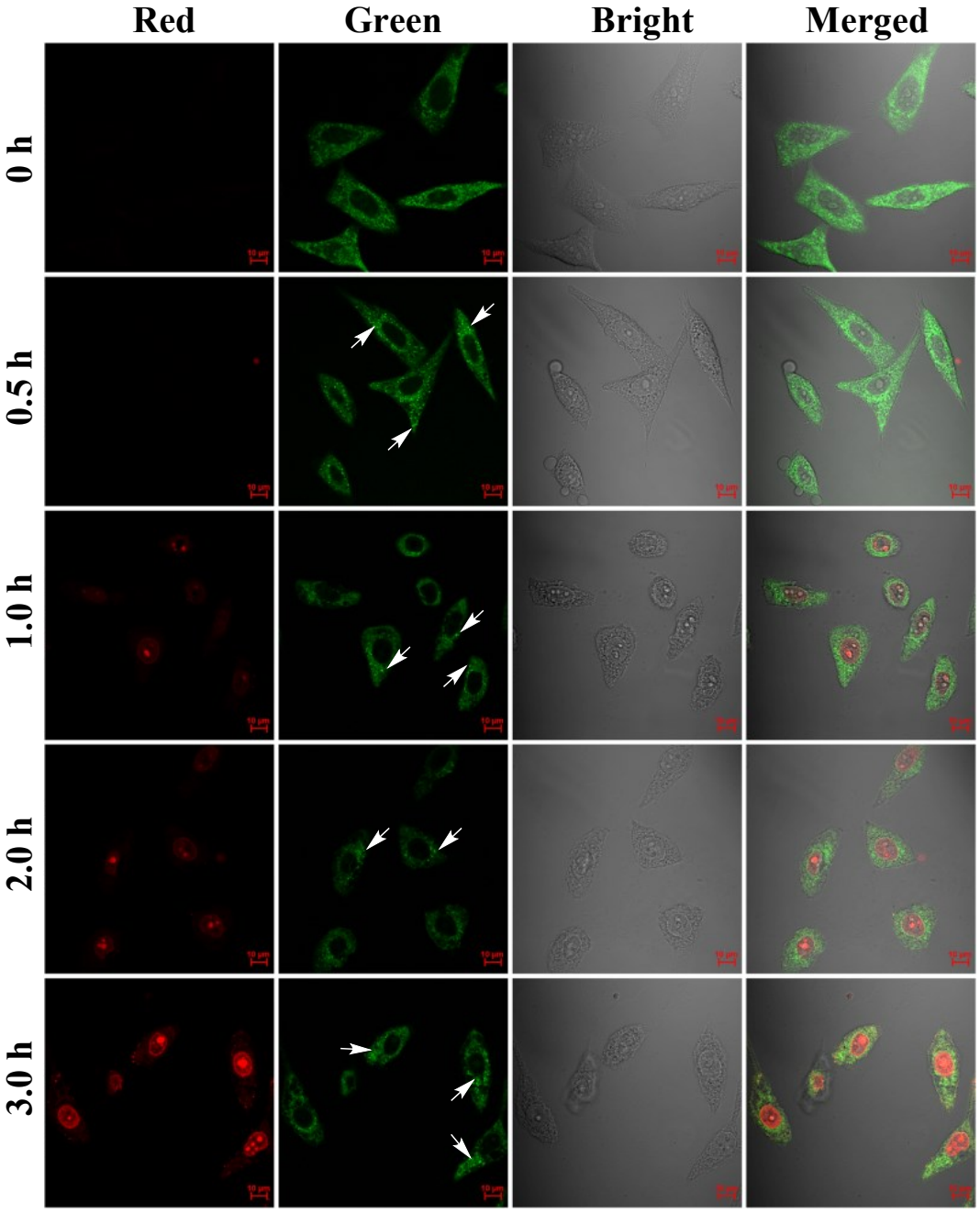



**Fig. S15** Images of HeLa cells stained with PI (1 μM) and **LIQ-6** (10 μM) under white light exposure (5 mW/cm$^2$) for different period of times (0, 0.5, 1, 2, or 3 h). Red channel from PI: $\lambda_{ex}$ = 561 nm, $\lambda_{em}$ = 590-720 nm; Green channel from **LIQ-6**: $\lambda_{ex}$ = 405 nm, $\lambda_{em}$ = 480-580 nm. White arrows indicate bright spherical vesicles or dots from lysosome.

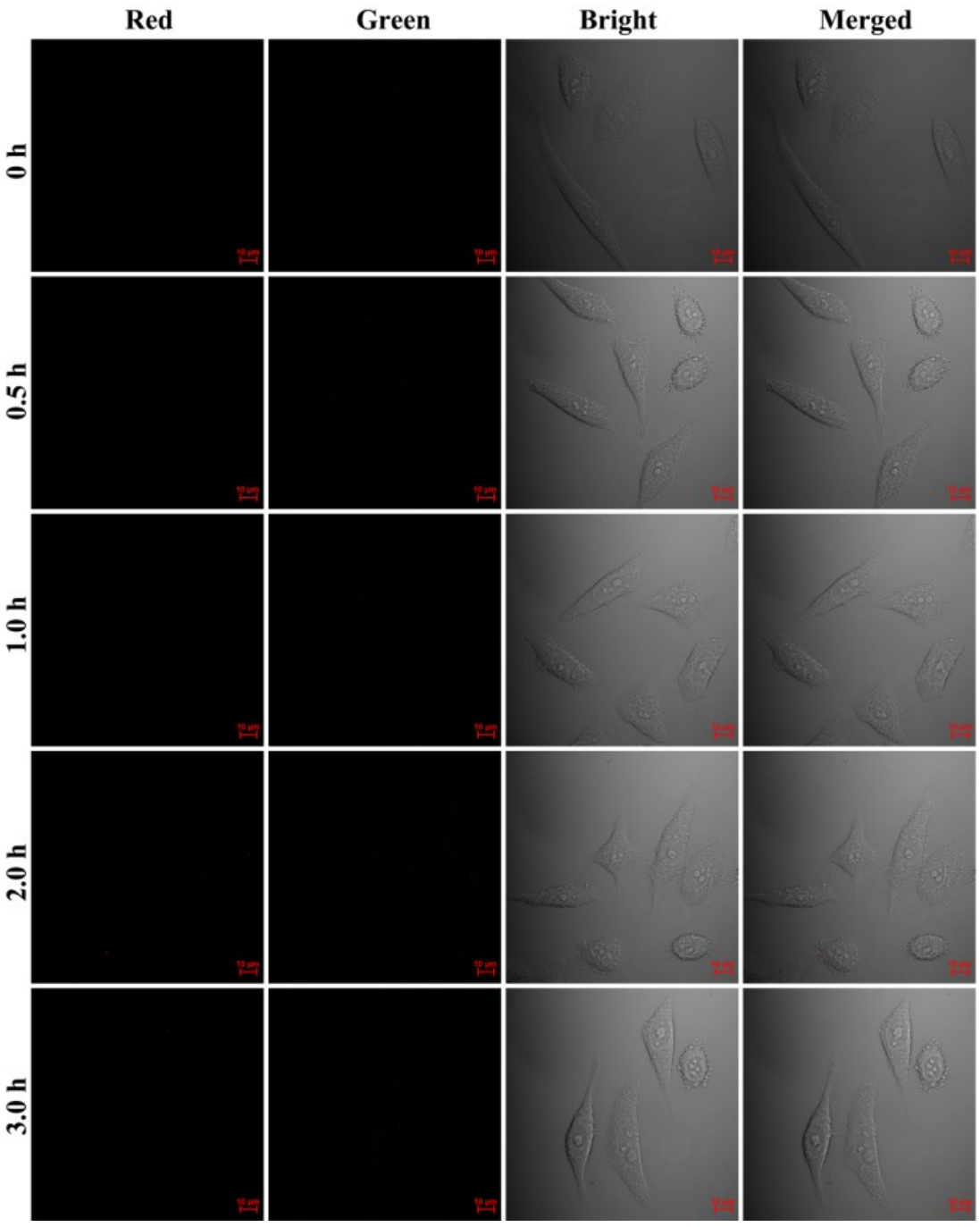



**Fig. S16** Confocal imaging of HeLa cells staining with PI (1 μM) under white light (5 mW/cm$^2$) irradiation for different time (0, 0.5, 1, 2 or 3 h) in the absence of **LIQ-6**. Red channel from PI: $\lambda_{ex}$ = 561 nm, $\lambda_{em}$ = 590-720 nm; Green channel from **LIQ-6**: $\lambda_{ex}$ = 405 nm, $\lambda_{em}$ = 480-580 nm.

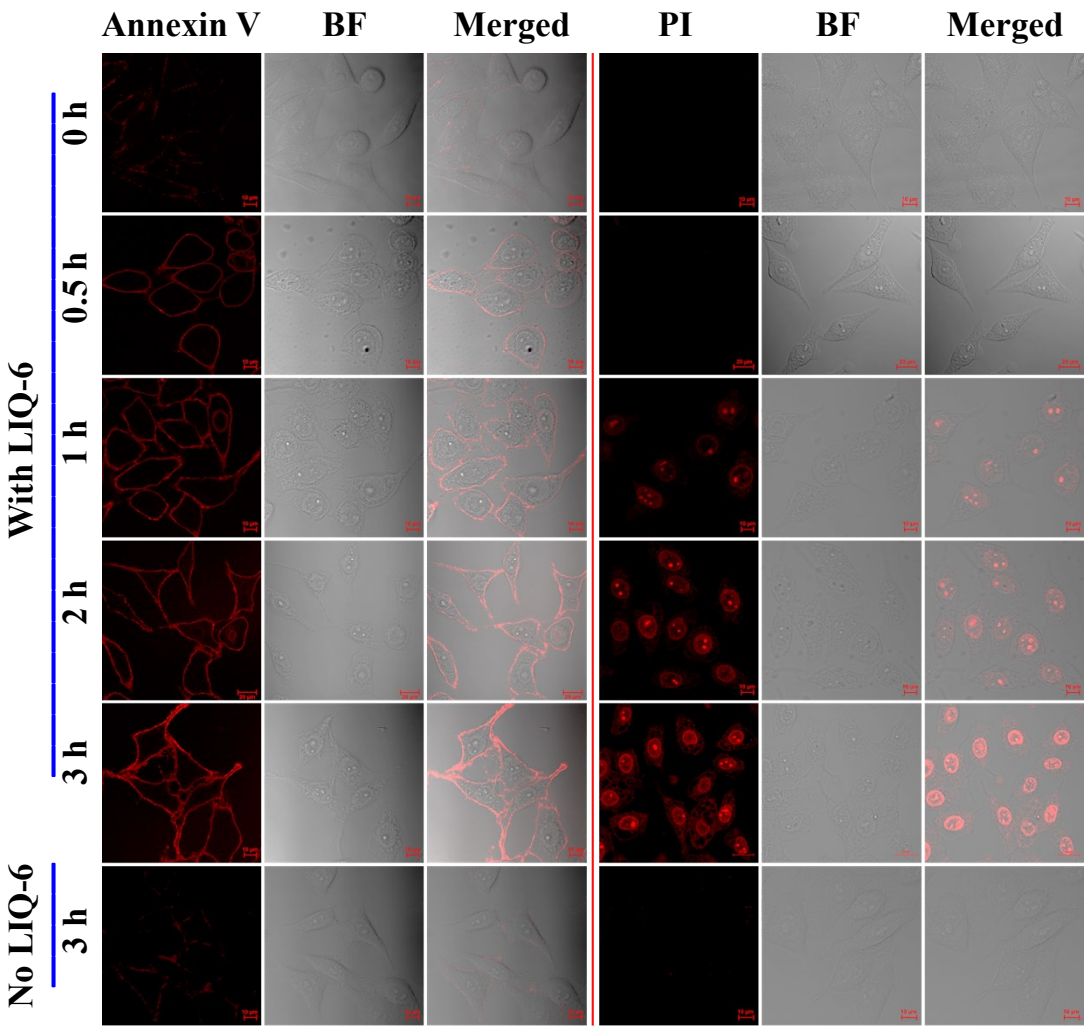



**Fig. S17** Confocal Images of HeLa cells stained with Annexin V, Alexa Fluor™ 555 conjugate or PI with or without **LIQ-6** (10 μM) under white light exposure (5 mW/cm$^2$) for different period of times (0, 0.5, 1, 2, or 3 h). Annexin V: $\lambda_{ex}$ = 561 nm, $\lambda_{em}$ = 580-650 nm; PI: $\lambda_{ex}$ = 561 nm, $\lambda_{em}$ = 590-720 nm.

## 6. Photodynamic antibacterial

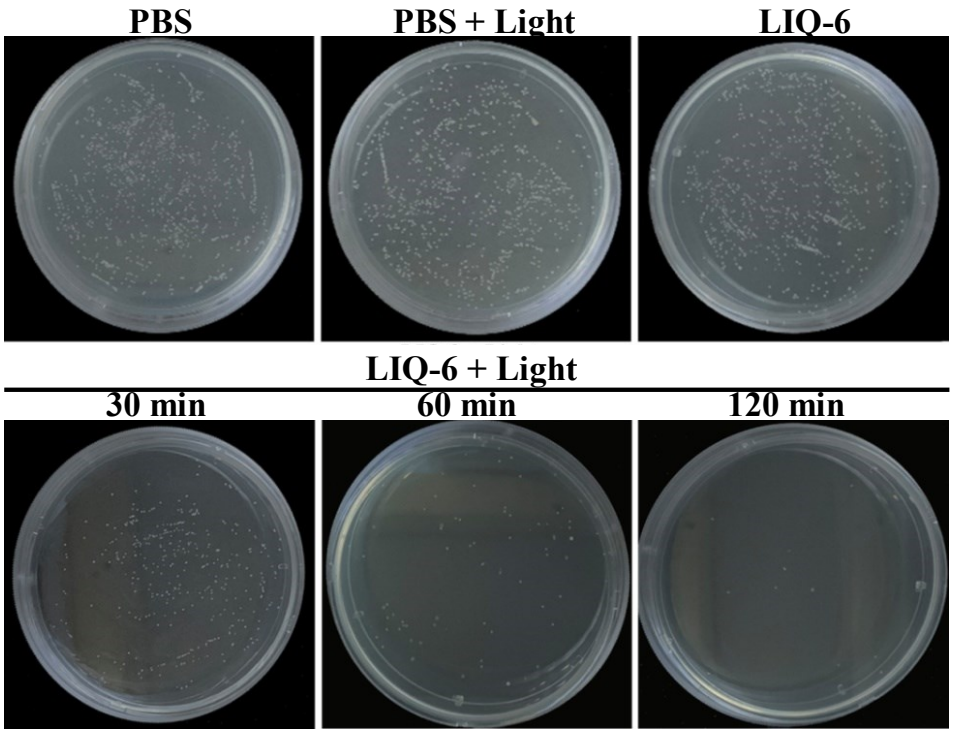
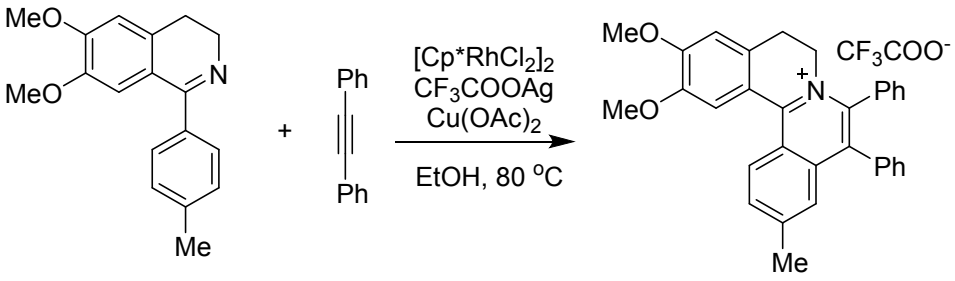


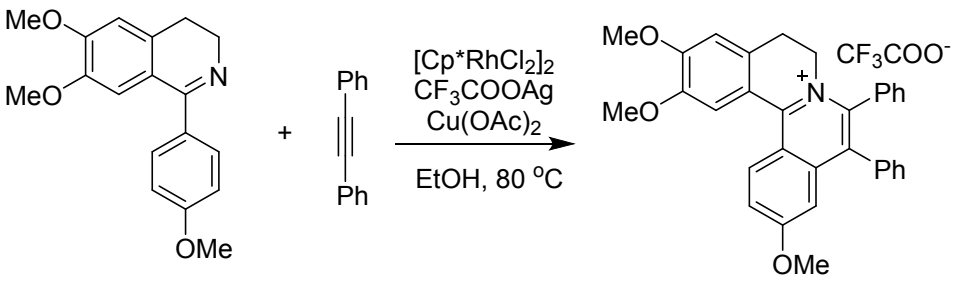

**Fig. S18** Plates of *P aeruginosa* with PBS or **LIQ-6** (10 μM) in the absence and presence or white light irradiation (5 mW/cm$^2$).

## 7. Synthesis and Characterization[1]

To 10 mL of ethyl alcohol were added 0.5 mmol dihydroisoquinoline derivative[2], 0.5 mmol 1,2-diaryl acetylene, 0.005 mmol [($Cp^*RhCl_2)_2$, 0.75 mmol silver trifluoroacetate, and 0.5 mmol copper acetate. After heated to reflux with magnetic stirring for 30 min under $N_2$, the reaction mixture was filtered through diatomite and washed with ethanol. The solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography on silica gel (DCM to DCM/MeOH = 20/1, v/v) to afford the product.

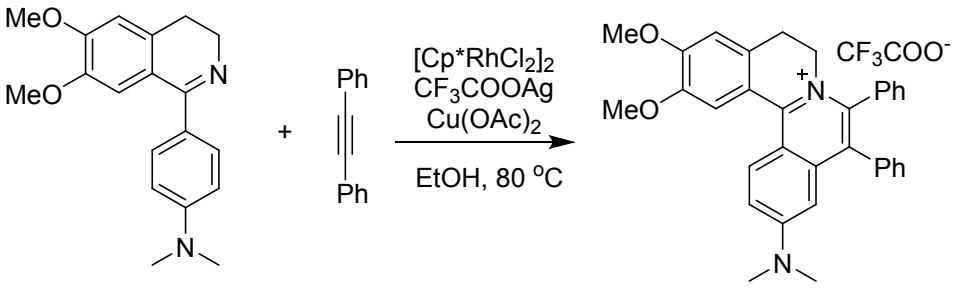



**LIQ-4**: bright yellow solid，99 % yield. $^1$H NMR (600 MHz, DMSO-$d_6$), δ. 8.76 (d, 1H, $J$ = 7.8 Hz), 7.92 (dd, 1H, $J$ = 1.8Hz, $J$ = 13.2 Hz), 7.53 (s, 1H), 7.48-7.46 (m, 2H), 7.41-7.31 (m, 8H), 7.27-7.14 (m, 2H), 4.21 (t, 2H, $J$ = 9.0 Hz), 3.97 (s, 3H), 3.92 (s, 3H), 3.14 (t, 2H, $J$ = 9.0 Hz), 2.53 (s, 3H). $^{13}$C NMR (150 MHz, DMSO-$d_6$), δ. 157.7 (q, $J$ = 30 Hz) (158.0, 157.8, 157.6, 157.4), 153.5, 152.7, 147.6, 147.3, 144.0, 138.2, 137.4, 134.2, 134.0, 133.4, 132.7, 132.2, 130.9, 130.3, 130.2, 129.7, 128.9, 128.6, 128.4, 128.3, 128.2, 125.3, 124.8, 122.7, 118.6, 117.4 (q, $J$ = 300 Hz) (120.4, 118.4, 116.4, 114.4), 116.0, 110.9, 56.2, 56.0, 51.8, 26.0, 22.0. $^{19}$F NMR (DMSO-$d_6$), δ. -73.48. HRMS (ESI): m/z [M - $CF_3COO^-$]$^+$ calcd for $C_{32}H_{28}NO_2$: 458.2115; found 458.2115.

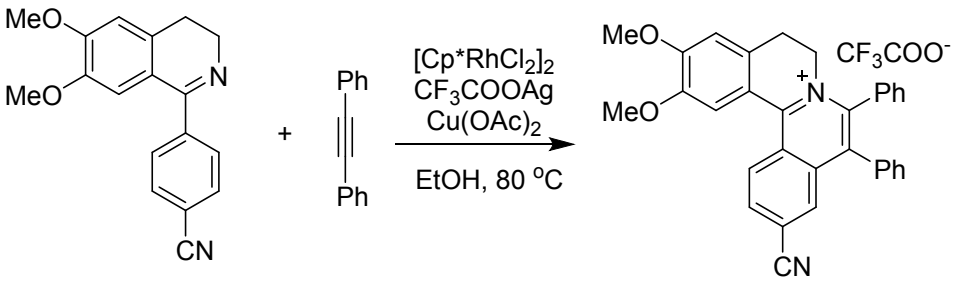



**LIQ-5**: bright yellow solid，96 % yield. $^1$H NMR (600 MHz, DMSO-$d_6$), δ. 8.78 (d, 1H, $J$ = 8.4 Hz), 7.70 (dd, 1H, $J$ = 4.2 Hz, $J$ = 14.4 Hz), 7.50 (s, 1H), 7.48-7.46 (m, 2H), 7.39-7.34 (m, 6H), 7.30 (s, 1H), 7.23 (d, 2H, $J$ = 9.6 Hz), 6.80 (d, 1H, $J$ = 4.2 Hz), 4.16

(t, 2H, $J$ = 9.0 Hz), 3.96 (S, 3H), 3.91 (s, 3H), 3.82 (s, 3H), 3.12 (t, 2H, $J$ = 9.0 Hz). $^{13}$C NMR (150 MHz, DMSO-$d_6$), δ. 164.5, 157.7 (q, $J$ = 30 Hz) (158.0, 157.8, 157.6, 157.4), 153.4, 151.9, 147.6, 144.2, 140.8, 134.2, 133.6, 133.4, 133.1, 132.2, 130.3, 130.2, 129.7, 128.6, 128.5, 128.3, 122.0, 119.5, 118.5, 117.4 (q, $J$ = 300 Hz) (120.4, 118.4, 116.4, 114.4), 115.9, 110.9, 105.3, 56.2, 56.1, 56.1, 51.4, 26.2. $^{19}$F NMR (DMSO-$d_6$), δ. -73.49. HRMS (ESI): m/z [M - $CF_3COO^-$]$^+$ calcd for $C_{32}H_{28}NO_3$: 474.2064; found 474.2055.

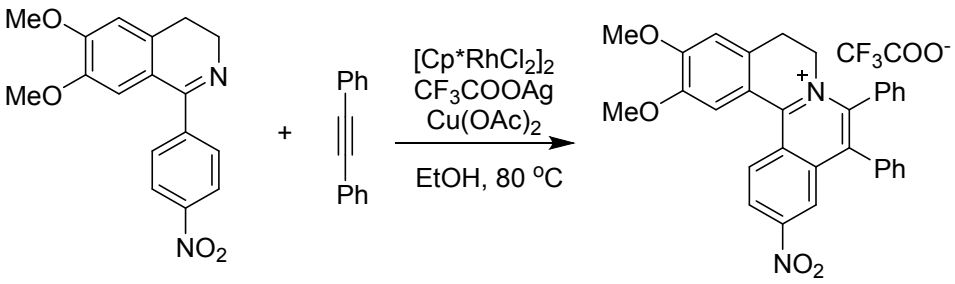



**LIQ-6**: orange solid, 88% yield. $^1$H NMR (600 MHz, $CDCl_3$), δ. 8.43 (d, 1H, $J$ = 9.6 Hz), 7.43-7.41 (m, 2H), 7.36-7.33 (m, 3H), 7.30-7.28 (m, 3H), 7.26-7.25 (m, 2H), 7.14 (d, 2H, $J$ = 6.6 Hz), 7.11 (s, 1H), 6.38 (d, 1H, $J$ = 2.4 Hz), 4.10 (t, 2H, $J$ = = 6.0 Hz), 4.05 (s, 3H), 4.00 (s, 3H), 3.16 (t, 2H, $J$ = 6.0 Hz), 3.11 (s, 6H). $^{13}$C NMR (150 MHz, DMSO-$d_6$), δ. 157.8 (q, $J$ = 31.5) (158.1, 157.9, 157.7, 157.5), 153.6, 152.8, 150.0, 147.4, 143.3, 140.4, 137.4, 133.1, 132.8, 131.0, 130.3, 130.2, 129.4, 128.9, 128.5, 128.4, 128.3, 128.0, 125.4, 118.8, 118.7, 117.3 (q, $J$ = 300 Hz) (120.3, 118.3, 116.3, 114.3), 115.8, 115.7, 111.0, 101.7, 56.1, 56.0, 50.5, 26.6, 21.1. $^{19}$F NMR (DMSO-$d_6$), δ. -73.57. HRMS (ESI): m/z [M - $CF_3COO^-$]$^+$ calcd for $C_{33}H_{31}N_2O_2$: 487.2380; found 487.2379.




**LIQ-7**: orange solid, 86 % yield. $^1$H NMR (600MHz, DMSO-$d_6$), δ. 8.97 (d, 1H, $J$ = 13.2 Hz), 8.31 (dd, 1H, $J$ = 2.4 Hz, $J$ = 13.2 Hz), 7.97 (d, 1H, $J$ = 2.4 Hz), 7.50 (s, 1H), 7.49-7.46 (m, 2H), 7.41-7.34 (m, 6H), 7.26-7.14 (m, 3H), 4.27 (t, 2H, $J$ = 9.6 Hz), 3.98 (s, 3H), 3.91 (s, 3H), 3.16 (t, 2H, $J$ = 9.6 Hz), $^{13}$C NMR (100 MHz, DMSO-$d_6$), δ. 158.0

(q, $J$ = 30 Hz) (158.3, 158.1, 157.9, 157.7), 154.2, 153.9, 147.9, 145.5, 137.4, 137.3, 134.7, 133.9, 133.2, 132.9, 131.6, 131.3, 131.0, 130.4, 130.2, 129.0, 128.7, 128.6, 128.3, 126.1, 125.4, 118.4, 117.5, 117.4, 117.3 (q, $J$ = 300 Hz) (120.3, 118.3, 116.3, 114.3), 110.0, 56.4, 56.2, 52.3, 25.8. $^{19}$F NMR (DMSO-$d_6$), δ. -73.60. HRMS (ESI): m/z [M - $CF_3COO^-$]$^+$ calcd for $C_{32}H_{25}N_2O_2$: 469.1911; found 469.1904.

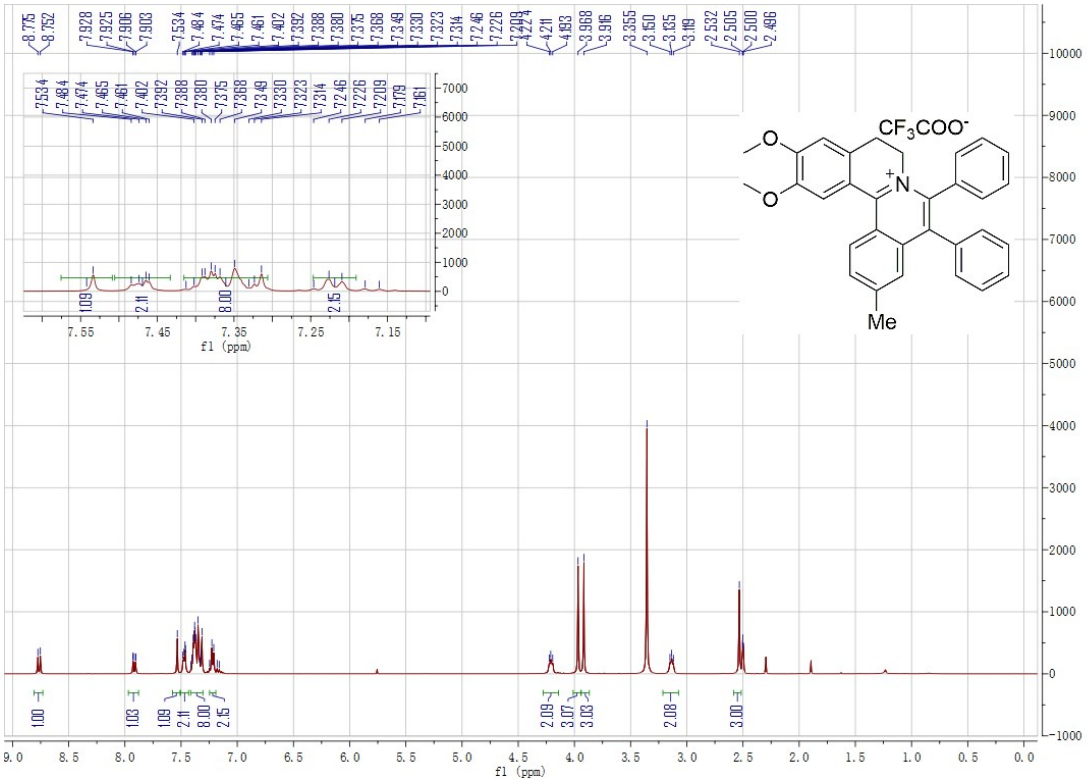



**LIQ-8**: red solid, 83% yield. $^1$H NMR (600 MHz, DMSO-$d_6$), δ. 9.08 (d, 1H, $J$ = 14.4 Hz), 8.65 (dd, 1H, $J$ = 3.6 Hz, $J$ = 14.4 Hz), 8.25 (d, 1H, $J$ = 3.6 Hz), 7.52-7.49 (m, 3H), 7.45-7.37 (m, 6H), 7.36 (s, 1H), 7.29-7.27 (m, 2H), 7.25-7.12 (m, 1H), 4.30 (t, 2H, $J$ = 9.6 Hz), 4.00 (s, 3H), 3.93 (s, 3H), 3.18 (t, 2H, $J$ = 9.6 Hz). $^{13}$C NMR (150 MHz, DMSO-$d_6$), δ. 157.8 (q, $J$ = 30 Hz) (158.1, 157.9, 157.7, 157.5), 154.3, 153.9, 150.6, 150.0, 145.6, 138.1, 137.4, 135.7, 134.3, 134.0, 133.3, 131.6, 130.4, 130.2, 130.1, 129.0, 128.9, 128.8, 128.8, 128.6, 128.3, 127.0, 125.4, 123.4, 121.0, 118.5, 116.0, 111.0, 56.4, 56.1, 52.4, 25.7. $^{19}$F NMR (DMSO-$d_6$), δ. -73.52. HRMS (ESI): m/z [M - $CF_3COO^-$]$^+$ calcd for $C_{31}H_{25}N_2O_4$: 489.1809; found 489.1806.

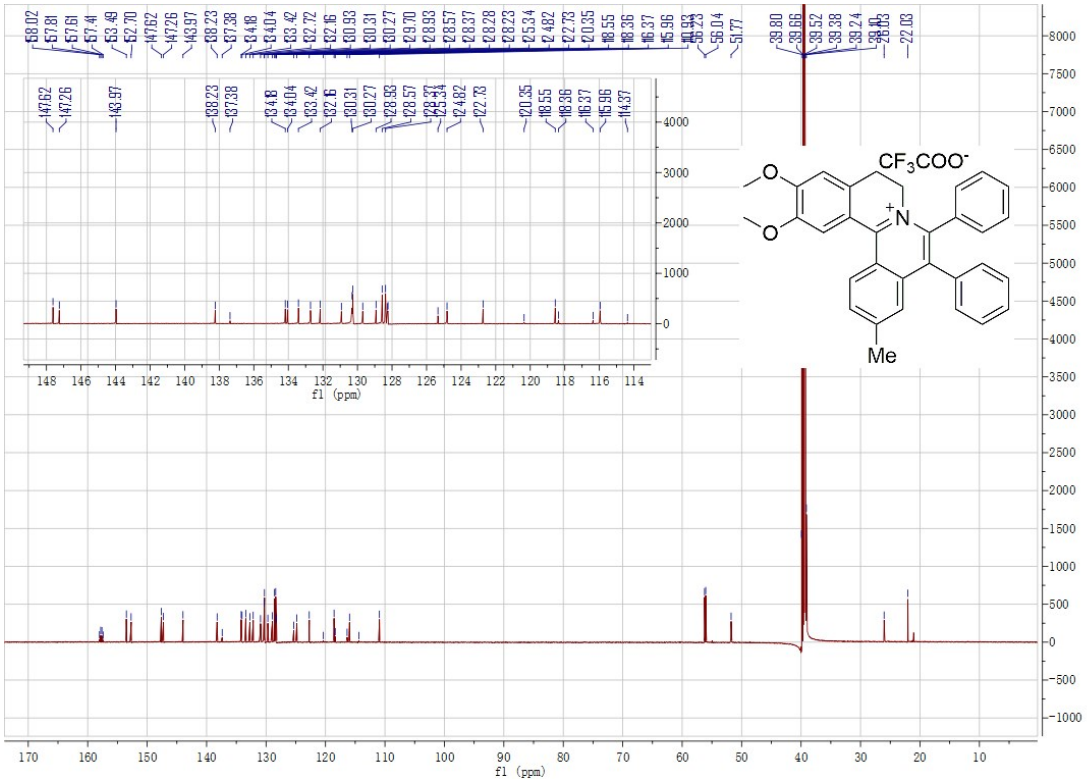
## 8. Reference

[1] L. Liu, Q. Zou, J.-K. Leung, J.-L Wang, C. Kam, S. Chen, S. Feng and M.-Y Wu, Chem. Commun., 55(2019), 14681-14684.
[2] M. Movassaghi, M. D. Hill, Org. Lett., 10(2008), 3485-3488

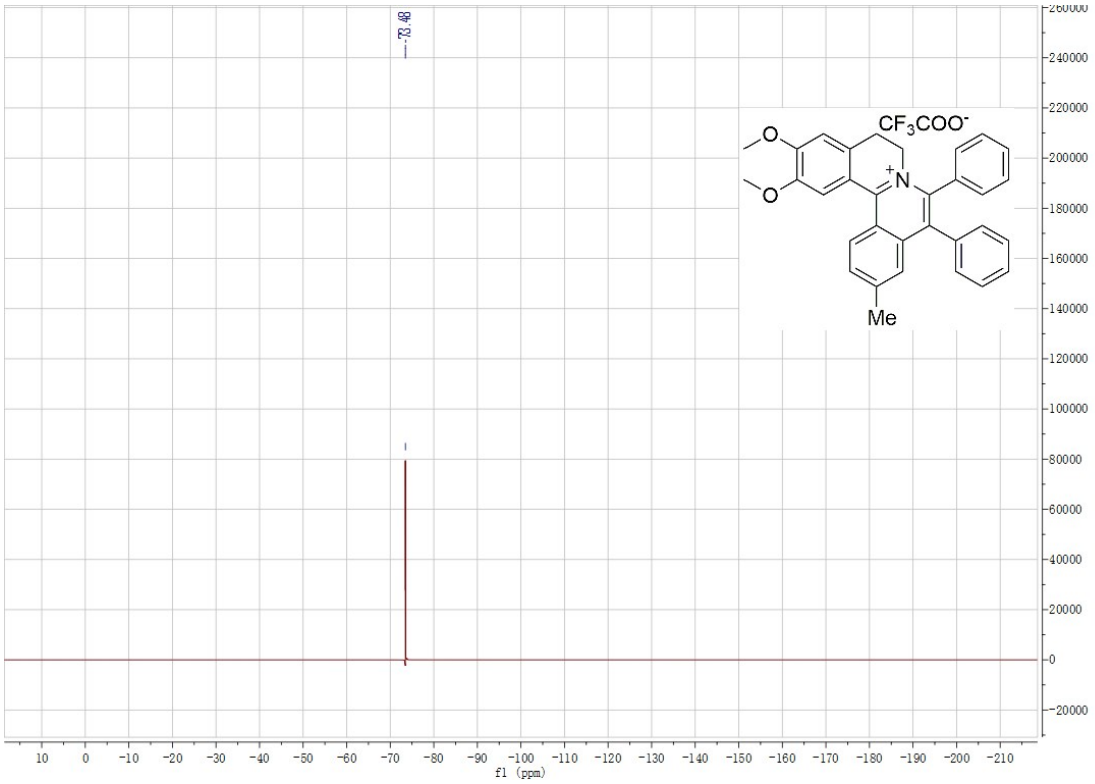
## 9. NMR and HRMS Spectrum



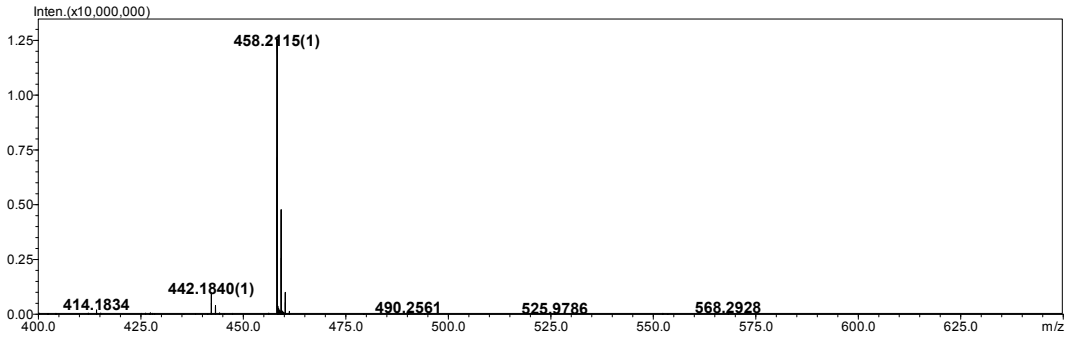

$^{1}$H NMR spectrum of **LIQ-4**.



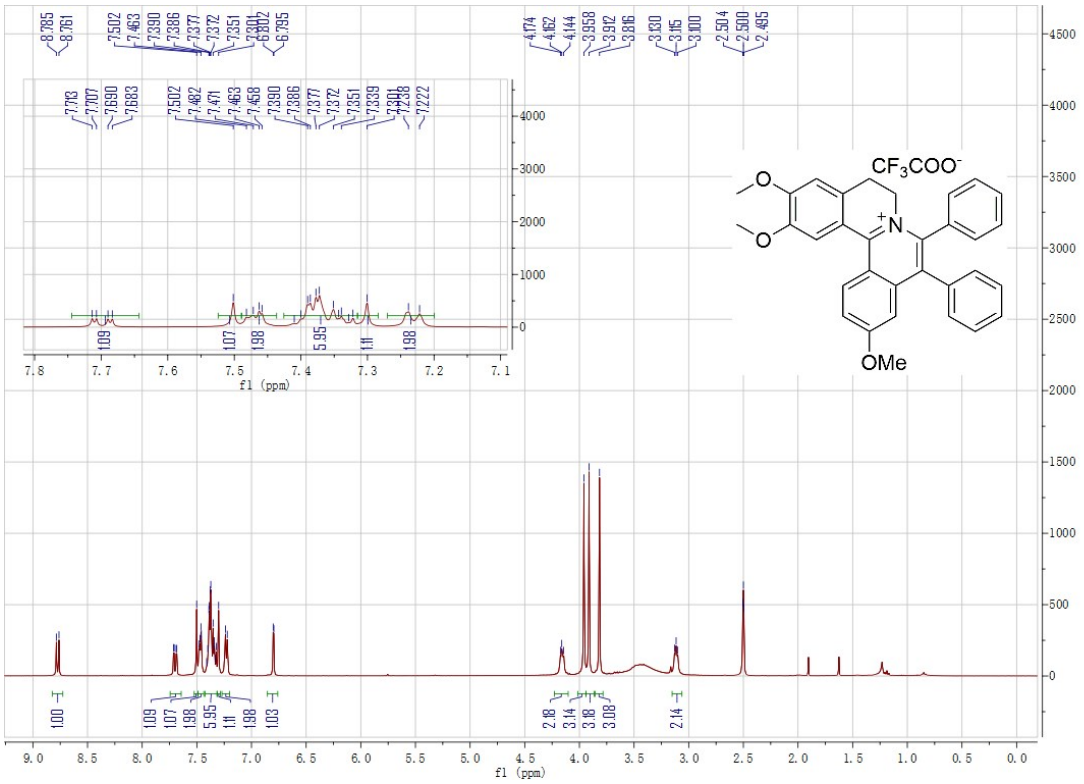
$^{13}$C NMR spectrum of **LIQ-4**.



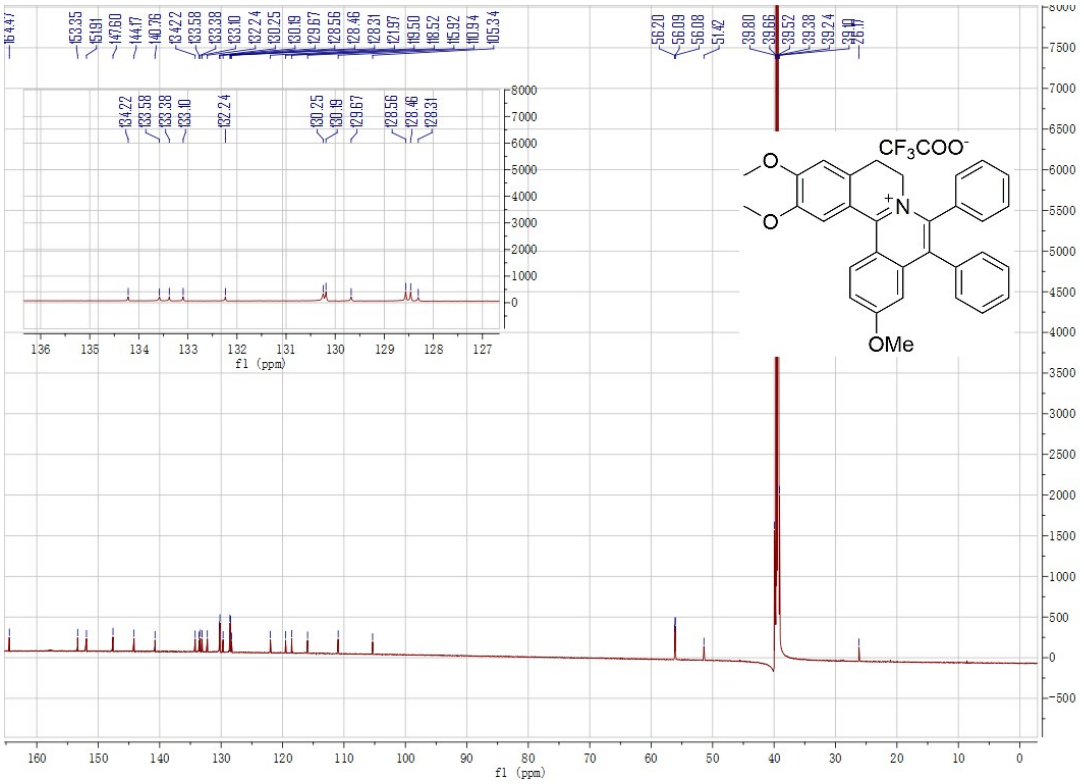

$^{19}$F NMR spectrum of **LIQ-4**.



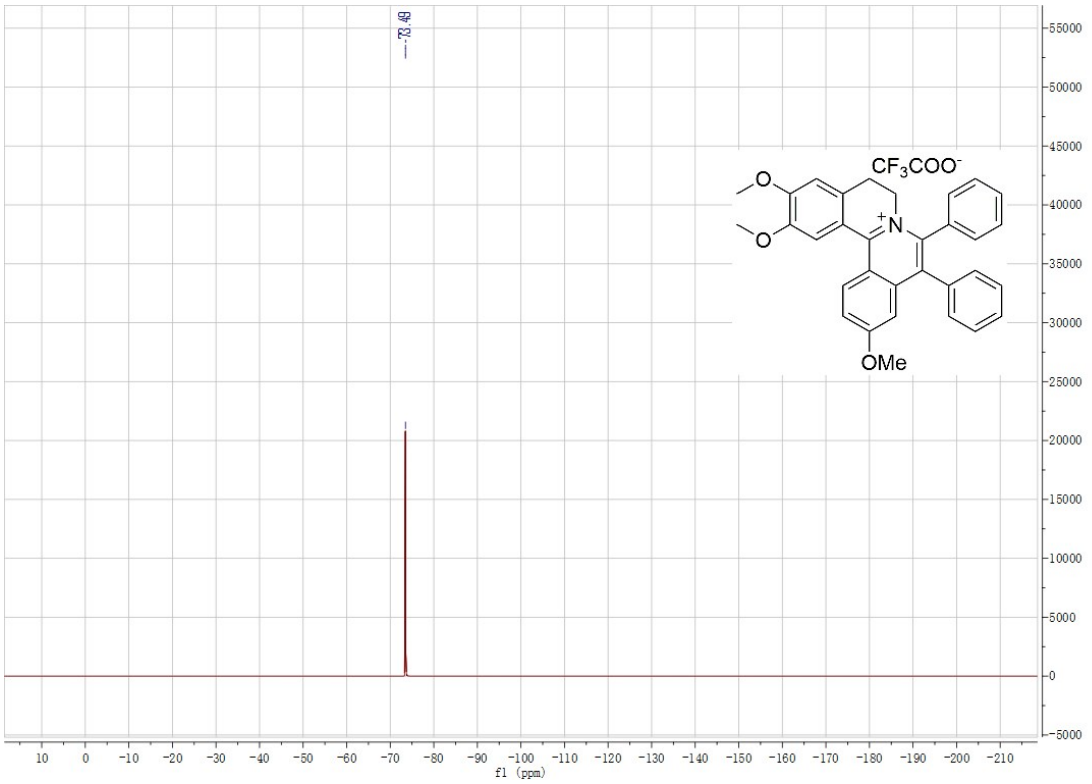

HRMS spectrum of **LIQ-4**.



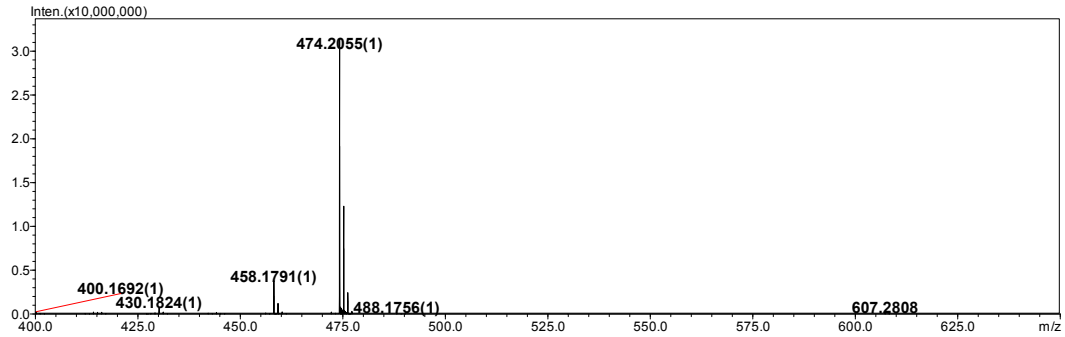

$^{1}$H NMR spectrum of **LIQ-5**.



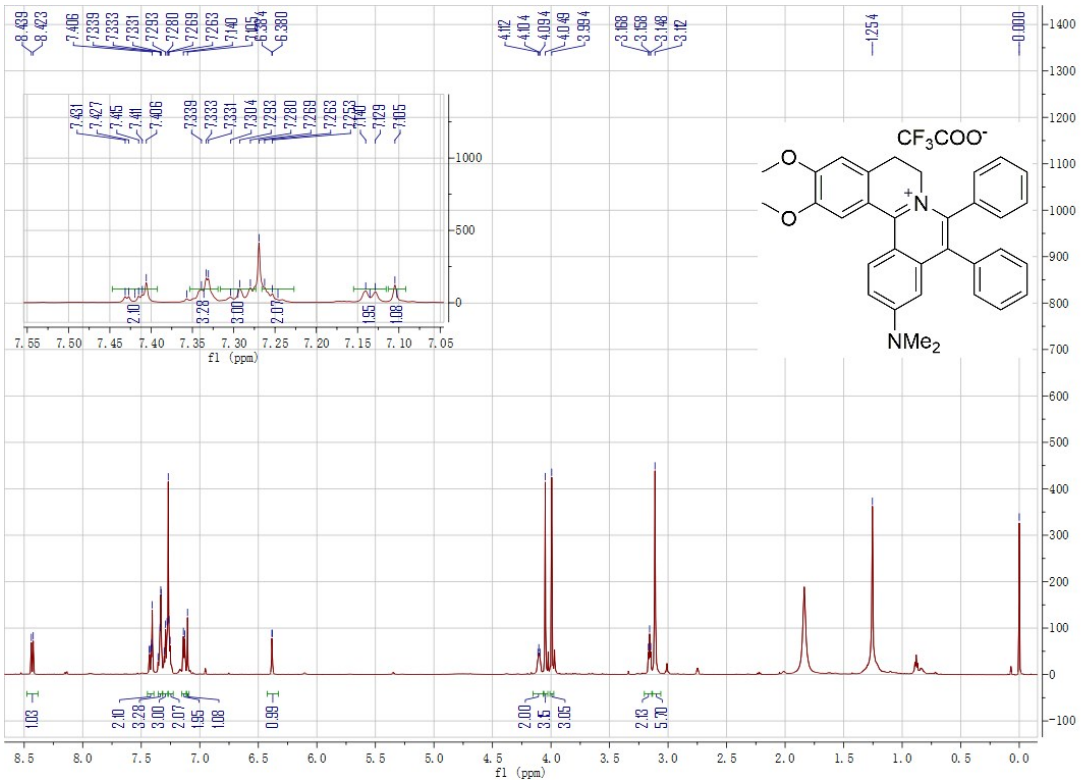

$^{13}$C NMR spectrum of **LIQ-5**.



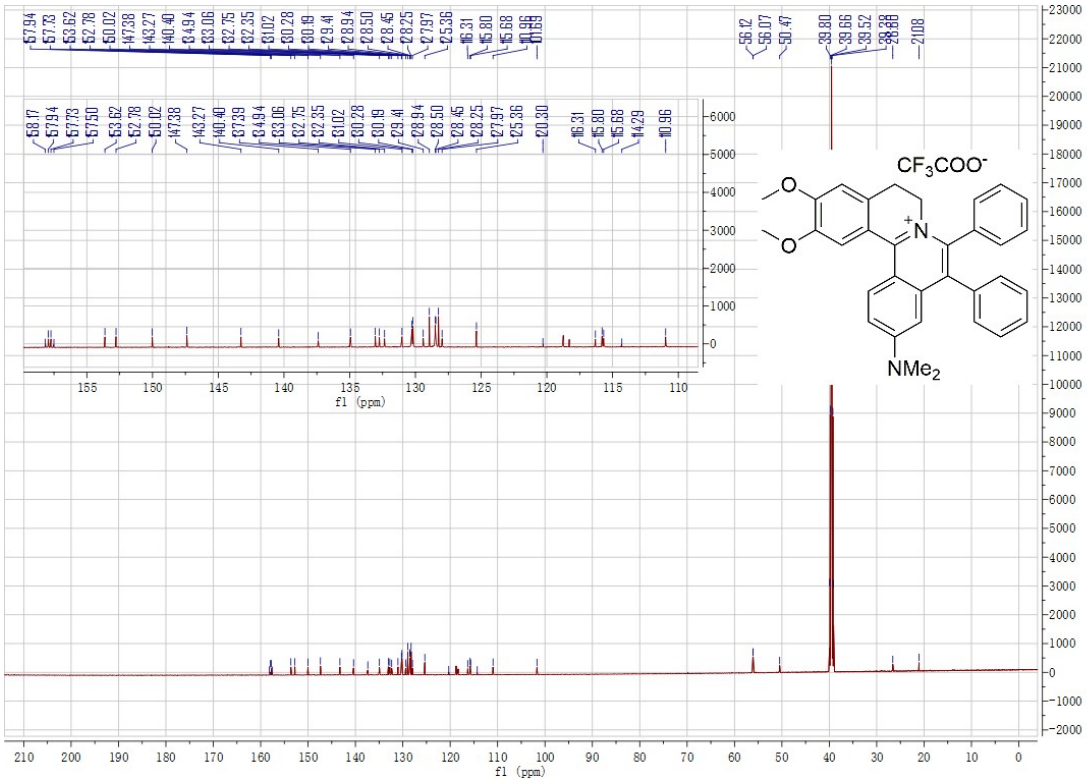

$^{19}$F NMR spectrum of **LIQ-5**.



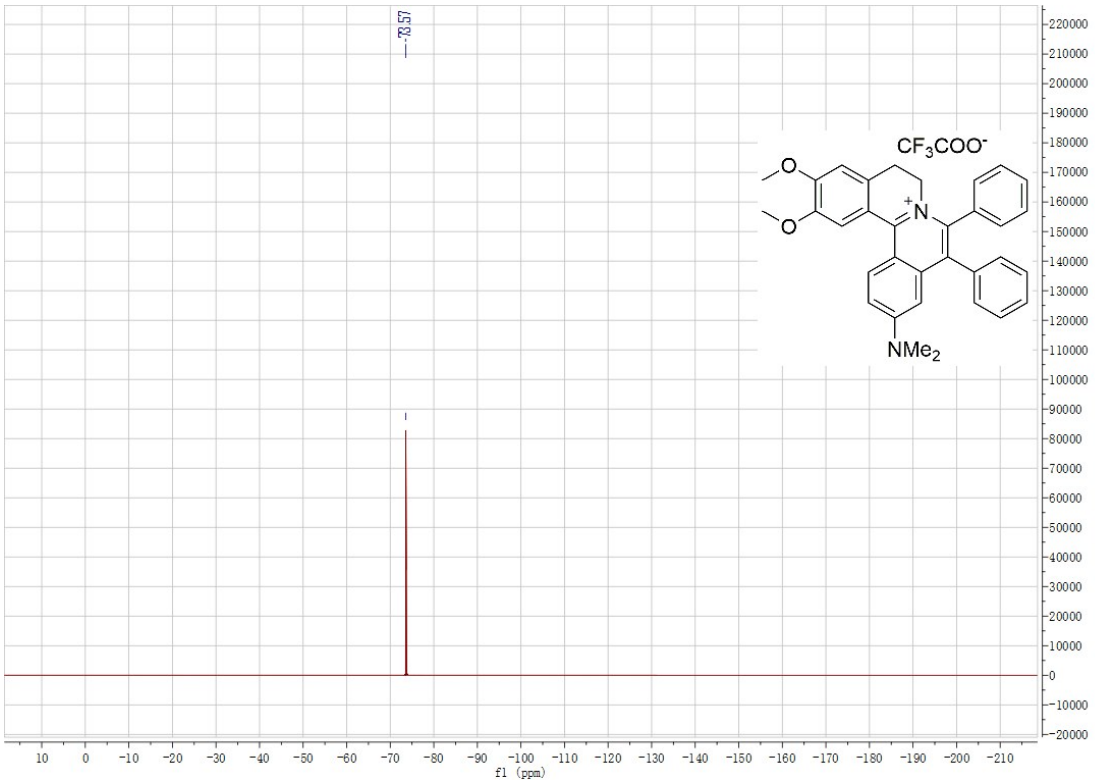

HRMS spectrum of **LIQ-5**.



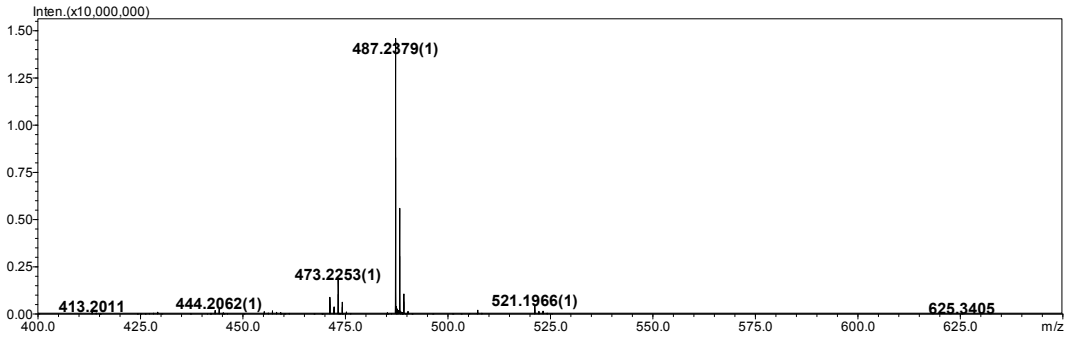

$^{1}$H NMR spectrum of **LIQ-6**.



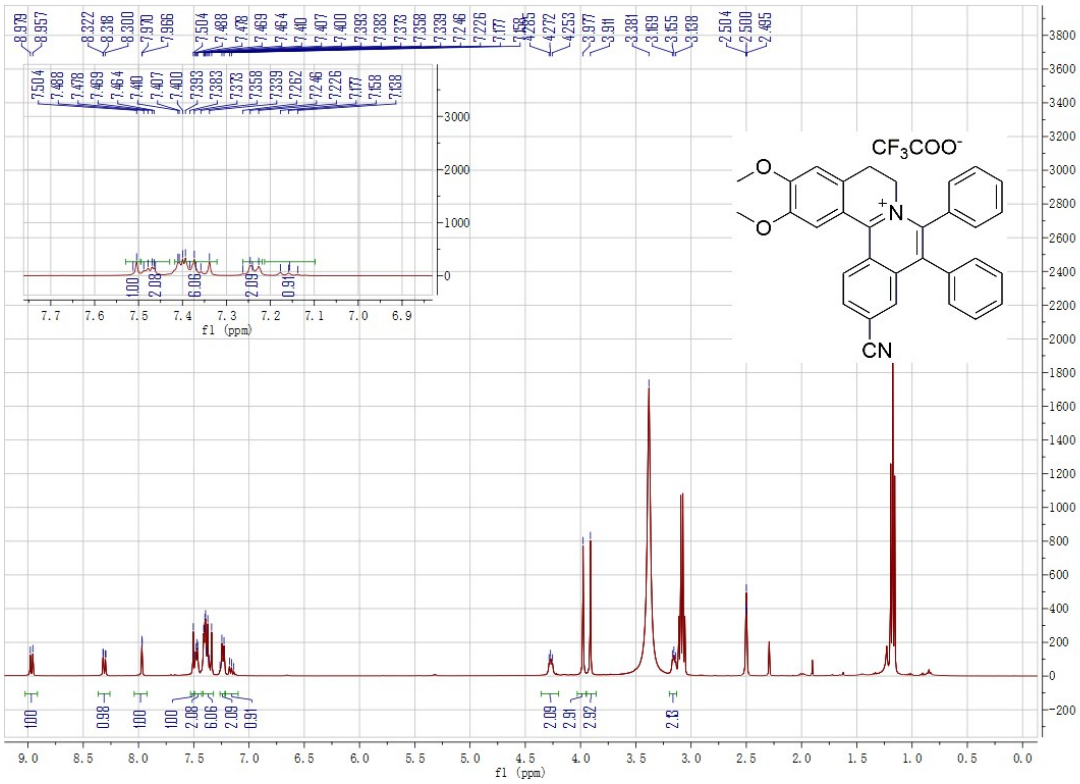

$^{13}$C NMR spectrum of **LIQ-6**.



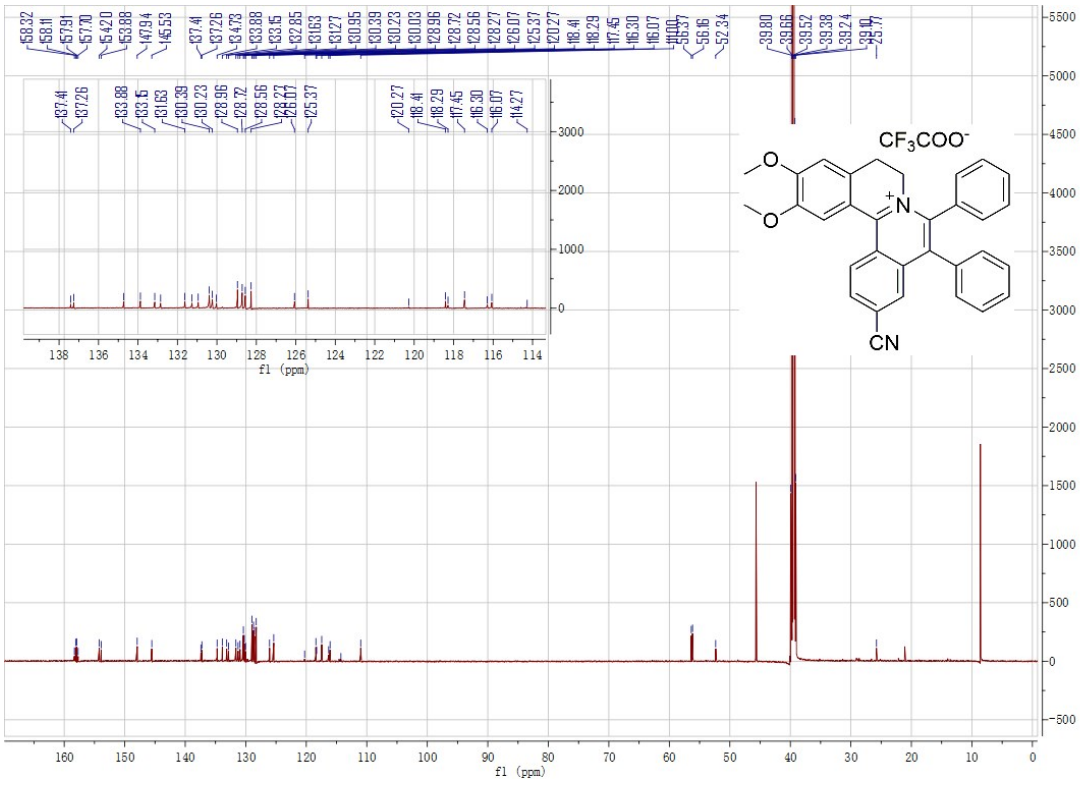

$^{19}$F NMR spectrum of **LIQ-6**.



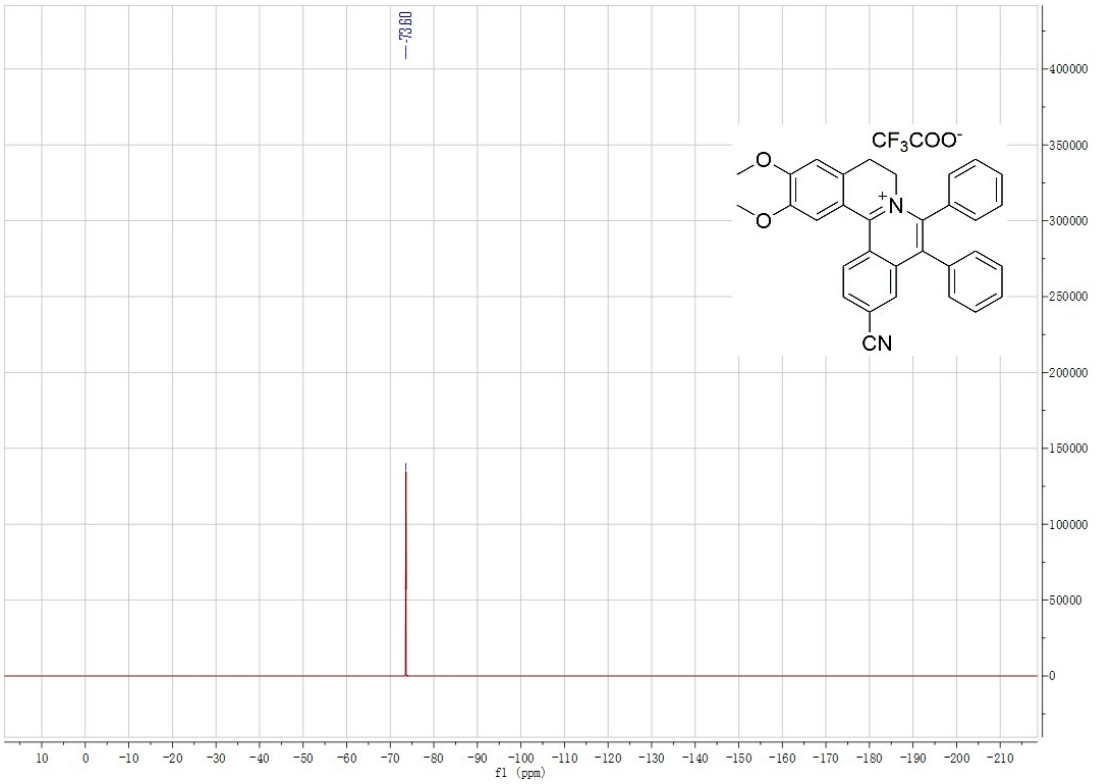

HRMS spectrum of **LIQ-6**.



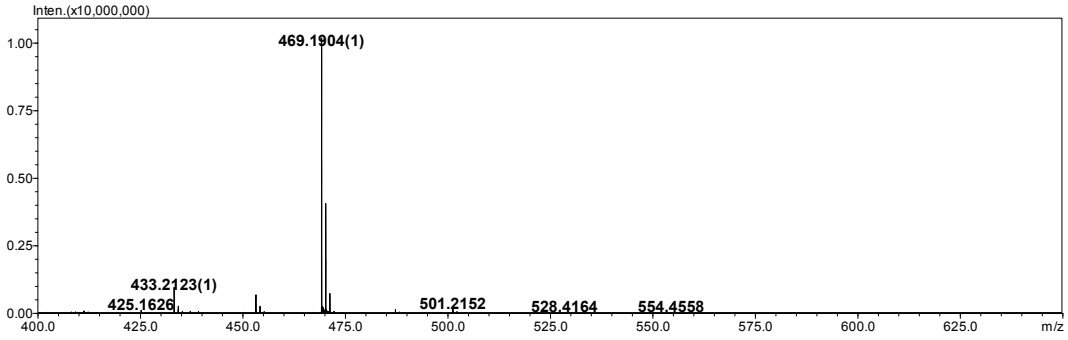

$^{1}$H NMR spectrum of **LIQ-7**.



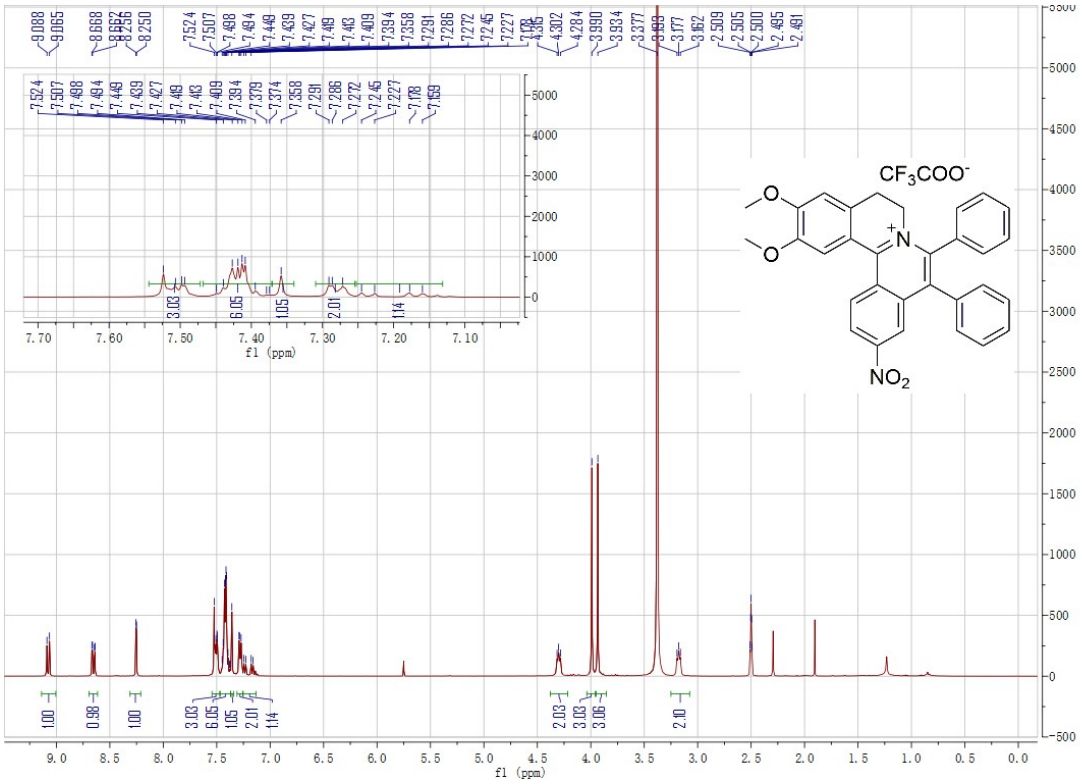

$^{13}$C NMR spectrum of **LIQ-7**.



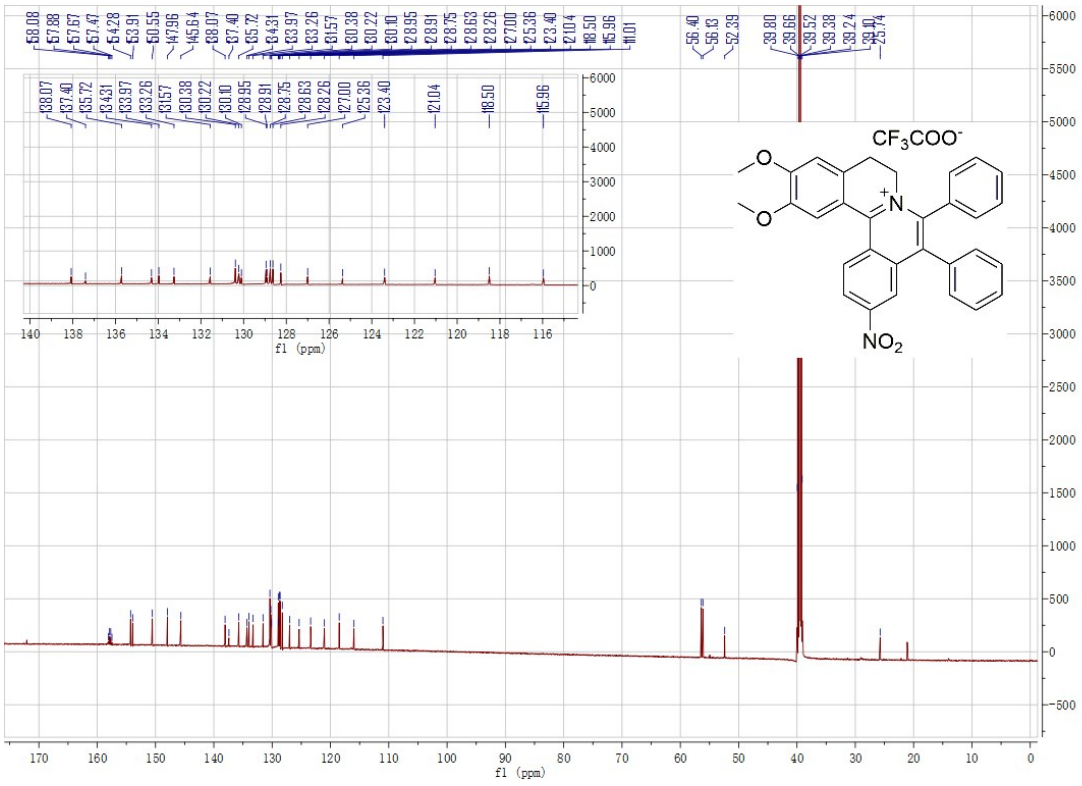

$^{19}$F NMR spectrum of **LIQ-7**.



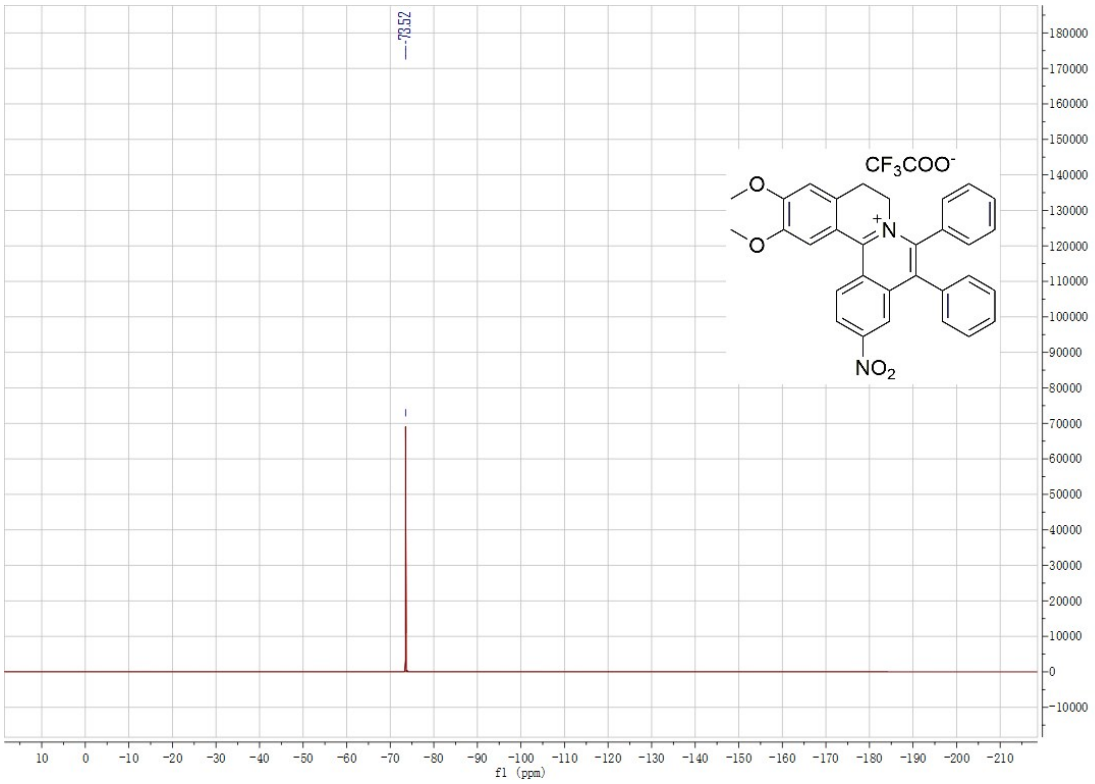

HRMS spectrum of **LIQ-7**.



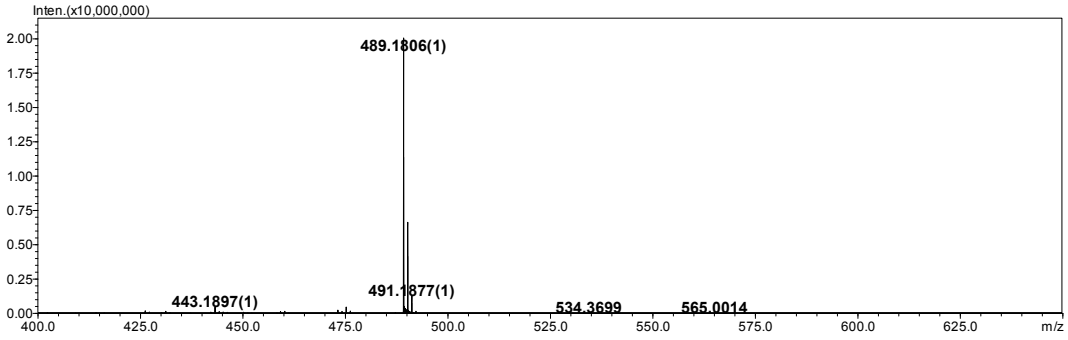

$^{1}$H NMR spectrum of **LIQ-8**.




$^{13}$C NMR spectrum of **LIQ-8**.




$^{19}$F NMR spectrum of **LIQ-8**.




HRMS spectrum of **LIQ-8**.