Supporting Information for

Effect of different substituents on the fluorescence properties of precursors of synthetic GFP analogs and its polarity-sensitive lipid droplets probe with AIE properties for imaging cells and zebrafish

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1. Materials and instruments

High-resolution electrospray mass spectra (HRMS) were gained from Bruker APEX IV-FTMS 7.0T mass spectrometer; NMR spectra were examined from AVANCE II 400 MHz Digital NMR Spectrometer with TMS as an internal standard; Electronic absorption spectra were recorded on a LabTech UV Power spectrometer; Fluorescence spectra were obtained with a HITACHI F4600 fluorescence spectrophotometer; The particle size was obtained by direct testing of Zetasizer Nano ZS90 (Malvern Instruments Ltd., Worcestershire, UK); The fluorescent images of the cells and zebrafish were obtained with Leica SP8 inverted fluorescence confocal microscope. The pH measurements were implemented on a Mettler-Toledo Delta 320 pH meter; analysis was exhibited on silica gel plates and column chromatography was carried out over silica gel (mesh 200-300). Compound melting point data obtained by direct measurement with the micro melting point meter SGW® X-4A. Both TLC and silica gel were purchased from the Qingdao Ocean Chemicals.

2. Yield of dyes 2a-h and its characterisation

Compound **2a**. The purified product was yellow solid. Yield 77%. mp 237.5-239.3 °C (dec.). ^{1}H NMR (400 MHz, DMSO) δ 8.56 (d, J = 8.5 Hz, 2H), 8.36 (d, J = 8.6 Hz, 2H), 8.19 (d, J = 7.7 Hz, 2H), 7.85 – 7.61 (m, 3H), 7.48 (s, 1H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 166.34, 164.77, 147.72, 139.51, 136.18, 134.20, 132.83, 129.33, 128.31, 126.87, 124.73, 123.82. HRMS (ESI) Found: 295.0708 $[M+H]^+$; Molecular formula $C_{16}H_{10}N_2O_4$ requires $[M+H]^+$ 295.0713 (Figure S12-S14).

Compound **2b**. The purified product was light yellow powder. Yield 69%. mp 202.2-

230.7 °C (dec.). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.26 (d, *J* = 8.1 Hz, 2H), 8.15 (s, 2H), 7.76 (d, *J* = 8.3 Hz, 3H), 7.67 (d, *J* = 6.6 Hz, 2H), 7.37 (s, 1H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 166.62, 163.32, 133.83, 133.76, 133.61, 132.49, 131.97, 129.27, 129.10, 127.99, 124.94, 124.86. HRMS (ESI) Found: 327.9962 and 329.9940 $[M+H]^+$; Molecular formula $C_{16}H_{10}BrNO_2$ requires $[M+H]^+$ 327.9968 and 329.9947 (Figure S15-S17).

Compound **2c**. The purified product was white solid. Yield 82%. mp 161.3-164.0 °C (dec.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, *J* = 2.1 Hz, 1H), 8.19 (d, *J* = 2.0 Hz, 2H), 8.17 (d, *J* = 1.6 Hz, 1H), 7.64 – 7.59 (m, 1H), 7.55 – 7.51 (m, 2H), 7.50 – 7.44 (m, 3H), 7.25 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.65, 163.56, 133.52, 133.36, 133.27, 132.47, 131.79, 131.21, 128.94, 128.91, 128.38, 125.60. HRMS (ESI) Found: 250.0858 $[M+H]^+$; Molecular formula $C_{16}H_{11}NO_2$ requires $[M+H]^+$ 250.0863 (Figure S18-S20).

Compound **2d**. The purified product was light yellow powder. Yield 88%. mp 138.4-139.4 °C (dec.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, *J* = 7.6 Hz, 2H), 8.13 – 8.08 (m, 2H), 7.63 – 7.58 (m, 1H), 7.56 – 7.50 (m, 2H), 7.34 – 7.27 (m, 2H), 7.23 (s, 1H), 2.42 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.83, 163.04, 142.12, 133.18, 132.55, 132.45, 132.08, 130.91, 129.75, 128.92, 128.29, 125.75, 21.82. HRMS (ESI) Found: 264.1015 $[M+H]^+$; Molecular formula $C_{17}H_{13}NO_2$ requires $[M+H]^+$ 264.1019 (Figure S21-S23).

Compound **2e**. The purified product was red powder. Yield 83%. mp 215.3-217.7 °C (dec.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 – 7.96 (m, 4H), 7.51 (dt, *J* = 14.7, 7.9 Hz, 3H), 7.18 (s, 1H), 6.75 (d, *J* = 7.8 Hz, 2H), 3.08 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.49, 160.66, 152.04, 134.83, 133.23, 132.31, 128.78, 128.47, 127.78, 126.34,

122.08, 111.99, 40.18. HRMS (ESI) Found: 293.1279 $[M+H]^+$; Molecular formula $C_{18}H_{16}N_2O_2$ requires $[M+H]^+$ 293.1285 (Figure S24-S26).

Compound **2f**. The purified product was dark red solid powder. Yield 85%. mp 132.7-133.6 °C (dec.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 – 8.03 (m, 4H), 7.55 – 7.44 (m, 3H), 7.17 (s, 1H), 6.70 (d, *J* = 8.7 Hz, 2H), 3.43 (q, *J* = 7.1 Hz, 4H), 1.22 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.59, 160.22, 150.08, 135.19, 133.36, 132.16, 128.74, 127.68, 127.06, 126.41, 121.15, 111.43, 44.72, 12.61. HRMS (ESI) Found: 321.1590 $[M+H]^+$; Molecular formula $C_{20}H_{20}N_2O_2$ requires $[M+H]^+$ 321.1598 (Figure S27-S29).

Compound **2g**. The purified product was brownish yellow solid. Yield 78%. mp 210.9-213.6 °C (dec.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 – 8.11 (m, 4H), 7.57 (t, *J* = 7.3 Hz, 1H), 7.51 (t, *J* = 7.4 Hz, 2H), 7.18 (s, 1H), 6.94 (d, *J* = 9.0 Hz, 2H), 3.90 – 3.85 (m, 4H), 3.37 – 3.32 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.19, 161.63, 152.62, 134.51, 132.69, 132.29, 130.01, 128.84, 127.97, 126.06, 124.59, 114.18, 66.49, 47.52. HRMS (ESI) Found: 335.1384 $[M+H]^+$; Molecular formula $C_{20}H_{18}N_2O_3$ requires $[M+H]^+$ 335.1390 (Figure S30-S32).

Compound **2h** (probe **T-LD**). The purified product was red solid powder. Yield 81%. mp 175.5-178.1 °C (dec.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, *J* = 8.1 Hz, 2H), 8.07 (d, *J* = 8.8 Hz, 2H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.49 (t, *J* = 7.6 Hz, 2H), 7.33 (t, *J* = 7.8 Hz, 4H), 7.19 (d, *J* = 3.7 Hz, 3H), 7.16 (d, *J* = 9.9 Hz, 4H), 7.05 (d, *J* = 8.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.12, 161.87, 150.70, 146.34, 134.00, 132.75, 131.91, 130.46, 129.62, 128.84, 128.01, 126.26, 126.07, 125.98, 124.76, 120.35. HRMS (ESI) Found: 417.1591 $[M+H]^+$; Molecular formula $C_{28}H_{20}N_2O_2$ requires $[M+H]^+$ 417.1598

(Figure S33-S35).

3. General information for spectroscopic studies

Oxazolone dyes with different substituents were dissolved separately in dimethyl sulfoxide (DMSO) to obtain a stock solution (1 mM). 10 μM of fluorescence was used for all spectroscopic experiments, and 20 μL of the Oxazolone dyes stock solution was added to the cuvette after dilution to 2 mL with different solvents. Solutions of various interfering substances (10 mM) were prepared in ultrapure water. PBS buffer solutions of different pH values from 1-12 were measured and prepared with a pH meter. The probe **T-LD** excitation wavelength was 470 nm; Vol. 600 v; both of the excitation and emission slit widths are 5 nm.

3. Cell culture and cytotoxicity assays

Hela cells were provided by Jiangsu Kaiji Biotechnology Co., Ltd. The living HeLa cells were cultured in the Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine serum (10% FBS) under the atmosphere containing 5% CO_2 and 95% air at 37 °C. The cytotoxic effects of the probe **T-LD** were tested by the MTT assay. The living cells line were treated in DMEM (Dulbecco's Modified Eagle Medium) supplied with fetal bovine serum (10%, FBS), penicillin (100 U/mL) and streptomycin (100 μg/mL) under the atmosphere of CO_2 (5%) and air (95%) at 37 °C. The HeLa cells were then seeded into 96-well plates, and 0, 2, 5, 10, 20, 30 μM (final concentration) of the probe **T-LD** (99.9% DMEM and 0.1% DMSO) were added respectively. Subsequently, the cells were cultured at 37 °C in an atmosphere of CO_2 (5%) and air (95%) for 24 hours. Then the HeLa cells were washed with PBS buffer,

and DMEM medium (500 μL) was added. Next, MTT (50 μL, 5 mg/mL) was injected to every well and incubated for 4 h. Violet formazan was treated with sodium dodecyl sulfate solution (500 μL) in the H_2O-DMF mixture. Absorbance of the solution was measured at 570 nm by the way of a microplate reader. The cell viability was determined by assuming 100% cell viability for cells without **T-LD**.

4. General procedures for probe T-LD biological experiments

The concentration of the probe in the cell imaging experiments was 10 μM, and the cells were incubated at a temperature of 37°C with 5% CO_2 for 30 min. To remove the residual probe, the cells were rinsed three times with PBS buffer solution before imaging. Starvation cell group: obtained by feeding with fetal bovine serum (10%, FBS) only without any added high sugar for 5h under the same culture conditions; Oleic acid cell group: obtained by adding oleic acid at a concentration of 10 μM for 1h under normal culture conditions; Co-localization experiment: Probe 10 μM and commercial lipid droplet dye Nile Red 5 μM were co-incubated with cells for 30min. Finally, the cells were imaged with a Leica SP8 inverted fluorescence confocal microscope with excitation wavelength of 470 nm and emission wavelengths of 500-580 nm (green channel) and excitation wavelength of 570 nm and emission wavelengths of 610-660 nm (red channel), respectively.

5. Zebrafish imaging experiments

Live zebrafish under different conditions were incubated with 10 μM **T-LD** in PBS buffer for 30 min and then transferred to another imaging plate containing a trace of water and placed under a confocal microscope for imaging with an excitation

wavelength of 470 nm and emission wavelengths of 500-580 nm.

Table S1. Photophysical properties of compound 2a in different solvents.

	λ_{abs} (nm)	λ_{em} (nm)	Stokes shift (nm)	Φ_F	Logε_{max}	FWHM
Tol	**381**	**433**	**52**	**14.5 %**	**4.36**	**65.72**
Dioxane	**378**	**426**	**48**	**0.5 %**	**4.45**	**59.70**
EtOAc	**376**	**467**	**100**	**0.3 %**	**4.35**	**88.21**
DCM	**379**	**436**	**57**	**1.1 %**	**4.26**	**66.75**
Acetone	**374**	**460**	**86**	**0.6 %**	**4.33**	**103.68**
MeCN	**376**	**480**	**104**	**1.4 %**	**4.21**	**131.95**
DMF	**381**	**484**	**103**	**2.5 %**	**4.35**	**144.21**
DMSO	**383**	**505**	**122**	**2.9 %**	**4.29**	**134.30**
EtOH	**375**	**413**	**38**	**2.8 %**	**4.23**	**44.72**
MeOH	**373**	**535**	**162**	**3.9 %**	**4.36**	**133.83**

Table S2. Photophysical properties of compound 2b in different solvents.

	λ_{abs} (nm)	λ_{em} (nm)	Stokes shift (nm)	Φ_F	Logε_{max}	FWHM
Tol	**371**	**433**	**65**	**18.0 %**	**4.42**	**76.42**
Dioxane	**368**	**424**	**56**	**2.5 %**	**4.46**	**68.04**
EtOAc	**367**	**421**	**54**	**1.9 %**	**4.48**	**69.11**
DCM	**370**	**430**	**60**	**1.4 %**	**4.23**	**65.72**
Acetone	**366**	**421**	**55**	**1.8 %**	**4.42**	**69.19**
MeCN	**366**	**429**	**63**	**1.2 %**	**4.46**	**69.26**
DMF	**370**	**424**	**54**	**2.6 %**	**4.47**	**66.60**
DMSO	**372**	**505**	**133**	**4.0 %**	**4.39**	**68.05**
EtOH	**367**	**419**	**52**	**3.9 %**	**4.38**	**66.57**
MeOH	**365**	**421**	**56**	**3.0 %**	**4.51**	**69.84**

Table S3. Photophysical properties of compound 2c in different solvents.

	λ_{abs} (nm)	λ_{em} (nm)	Stokes shift (nm)	Φ_F	(Logε_{max})	FWHM
Tol	**365**	**434**	**69**	**50.8 %**	**4.42**	**69.44**
Dioxane	**381**	**416**	**35**	**1.9 %**	**4.32**	**58.54**
EtOAc	**360**	**415**	**55**	**1.3 %**	**4.41**	**75.91**
DCM	**363**	**415**	**52**	**2.1 %**	**4.15**	**68.12**
Acetone	**360**	**416**	**56**	**1.1 %**	**4.39**	**76.89**

MeCN	**360**	**414**	**54**	**0.7 %**	**4.38**	**70.63**
DMF	**364**	**417**	**53**	**1.7 %**	**4.43**	**64.99**
DMSO	**382**	**421**	**39**	**3.1 %**	**4.26**	**73.15**
EtOH	**380**	**413**	**33**	**2.2 %**	**4.26**	**77.36**
MeOH	**360**	**412**	**52**	**2.7 %**	**4.40**	**80.46**

Table S4. Photophysical properties of compound 2d in different solvents.

	λ_{abs} (nm)	λ_{em} (nm)	Stokes shift (nm)	Φ_F	Logε_{max}	FWHM
Tol	**373**	**434**	**61**	**16.6 %**	**4.45**	**68.67**
Dioxane	**370**	**426**	**56**	**1.1 %**	**4.46**	**69.11**
EtOAc	**368**	**421**	**53**	**0.9 %**	**4.39**	**70.34**
DCM	**372**	**435**	**63**	**1.5 %**	**4.28**	**66.76**
Acetone	**368**	**421**	**53**	**1.5 %**	**4.14**	**71.64**
MeCN	**368**	**415**	**47**	**0.6 %**	**4.41**	**72.41**
DMF	**370**	**413**	**43**	**1.3 %**	**4.37**	**62.27**
DMSO	**374**	**436**	**62**	**2.1 %**	**4.35**	**71.24**
EtOH	**369**	**413**	**44**	**2.1 %**	**4.41**	**61.30**
MeOH	**369**	**420**	**51**	**1.8 %**	**4.34**	**71.43**

Table S5. Photophysical properties of compound 2e in different solvents.

	λ_{abs} (nm)	λ_{em} (nm)	Stokes shift (nm)	Φ_F	Logε_{max}	FWHM
Tol	**467**	**509**	**42**	**1.2 %**	**4.52**	**57.16**
Dioxane	**461**	**515**	**46**	**0.9 %**	**4.51**	**56.69**
EtOAc	**462**	**524**	**62**	**1.3 %**	**4.47**	**54.90**
DCM	**469**	**525**	**56**	**0.7 %**	**4.24**	**51.55**
Acetone	**466**	**535**	**69**	**1.9 %**	**4.39**	**55.01**
MeCN	**465**	**540**	**75**	**1.6 %**	**4.38**	**54.83**
DMF	**473**	**546**	**73**	**2.6 %**	**4.49**	**55.70**
DMSO	**478**	**552**	**74**	**5.8%**	**4.32**	**56.04**
EtOH	**467**	**529**	**62**	**1.2 %**	**4.43**	**57.09**
MeOH	**466**	**534**	**68**	**1.8 %**	**4.49**	**56.43**

Table S6. Photophysical properties of compound 2f in different solvents.

	λ_{abs} (nm)	λ_{em} (nm)	Stokes shift (nm)	Φ_F	Logε_{max}	FWHM
Tol	**476**	**510**	**34**	**1.6 %**	**4.46**	**61.19**

Dioxane	**471**	**517**	**46**	**0.9 %**	**4.57**	**56.85**
EtOAc	**469**	**526**	**57**	**1.3 %**	**4.50**	**52.49**
DCM	**480**	**530**	**50**	**0.7 %**	**4.36**	**50.35**
Acetone	**474**	**537**	**63**	**2.3 %**	**4.40**	**50.45**
MeCN	**473**	**541**	**68**	**1.6 %**	**4.48**	**50.74**
DMF	**479**	**545**	**66**	**2.5 %**	**4.48**	**53.35**
DMSO	**488**	**554**	**66**	**4.6 %**	**4.47**	**52.43**
EtOH	**476**	**532**	**56**	**1.5 %**	**4.43**	**55.64**
MeOH	**474**	**537**	**63**	**3.5 %**	**4.30**	**53.78**

Table S7. Photophysical properties of compound 2g in different solvents.

	λ_{abs} (nm)	λ_{em} (nm)	Stokes shift (nm)	Φ_F	Logε_{max}	FWHM
Tol	**373**	**434**	**61**	**16.6 %**	**4.45**	**68.67**
Dioxane	**370**	**426**	**56**	**1.1 %**	**4.46**	**69.11**
EtOAc	**368**	**421**	**53**	**0.9 %**	**4.39**	**70.34**
DCM	**372**	**435**	**63**	**1.5 %**	**4.28**	**66.76**
Acetone	**368**	**421**	**53**	**1.5 %**	**4.14**	**71.64**
MeCN	**368**	**415**	**47**	**0.6 %**	**4.41**	**72.41**
DMF	**370**	**413**	**43**	**1.3 %**	**4.37**	**62.27**
DMSO	**374**	**436**	**62**	**2.1 %**	**4.35**	**71.24**
EtOH	**369**	**413**	**44**	**2.1 %**	**4.41**	**61.30**
MeOH	**369**	**420**	**51**	**1.8 %**	**4.34**	**71.43**

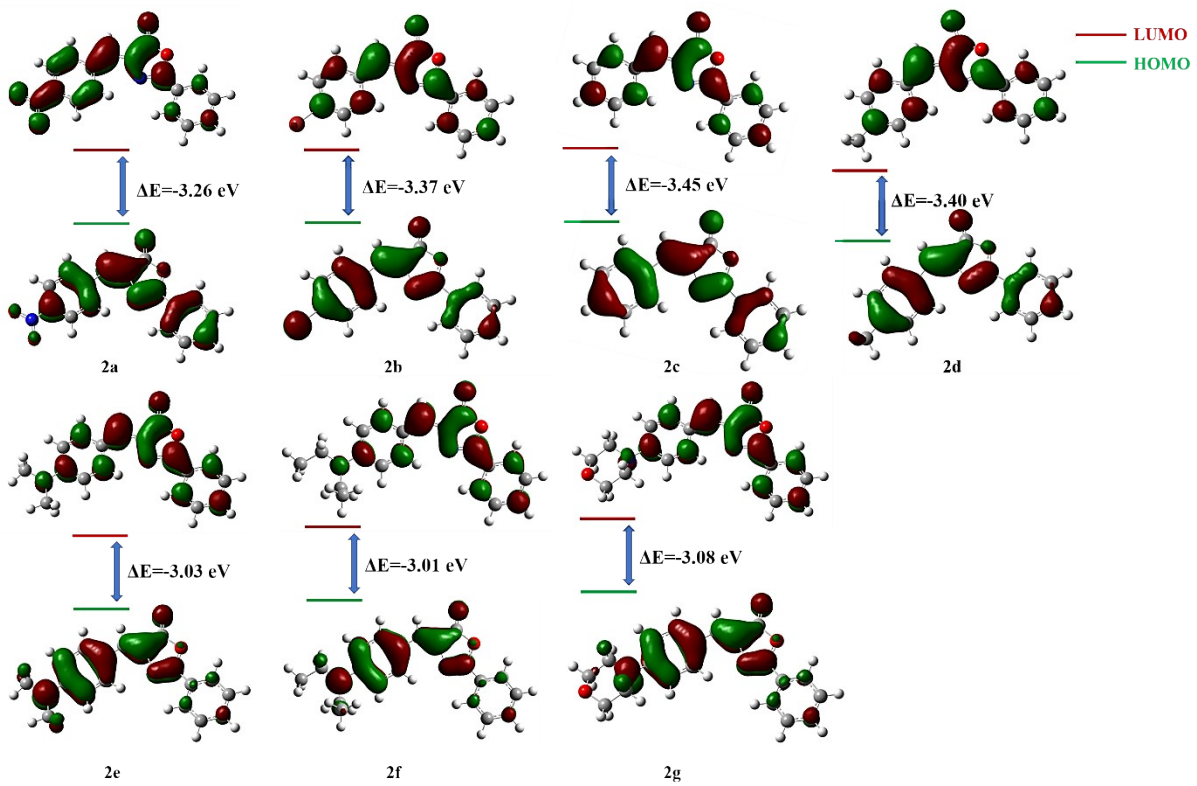

Figure S1. Calculation of the frontier molecular orbitals of the compound (2a-g) in different solvent models, calculated with Gaussian'09 at B3LYP/6-31+G(d, p) level.

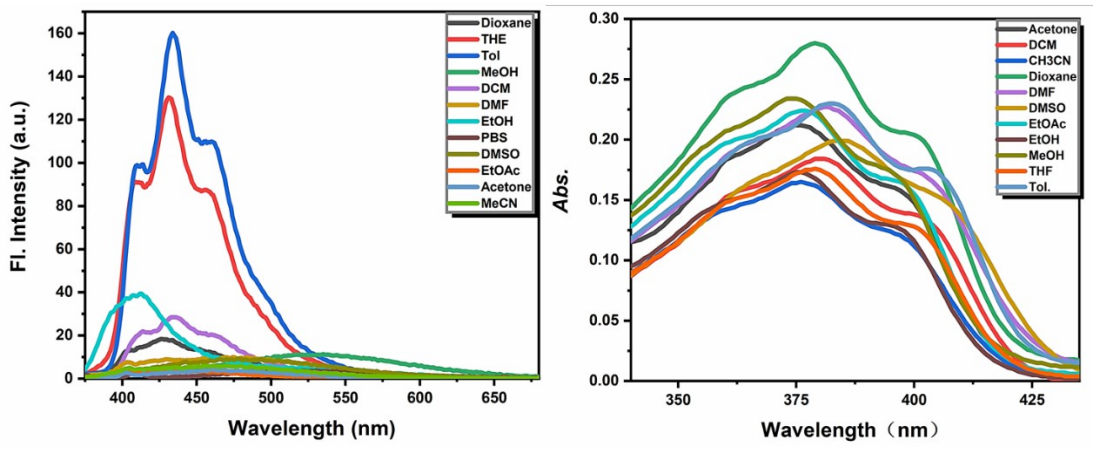

Figure S2. Emission and absorption spectra of compound 2a (10 μM) in different solvents.

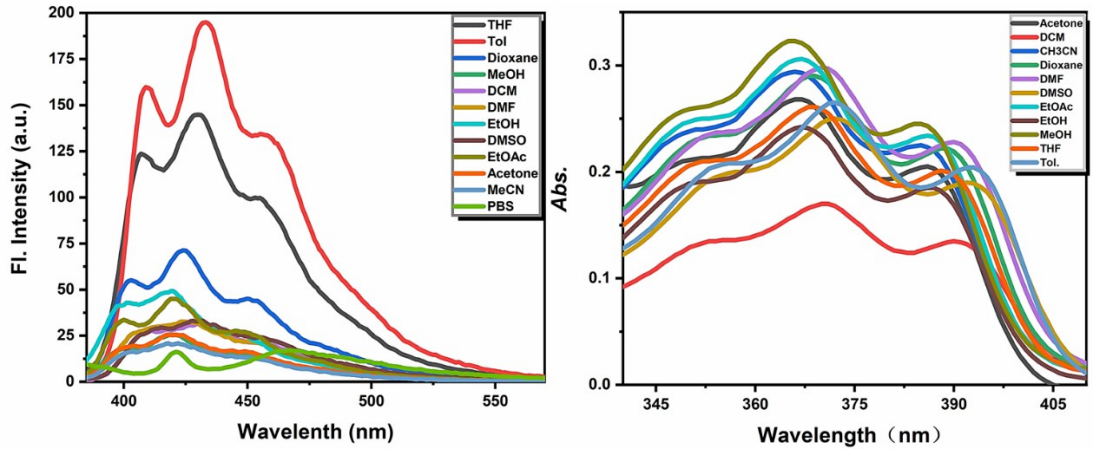

Figure S3. Emission and absorption spectra of compound 2b (10 μM) in different solvents.

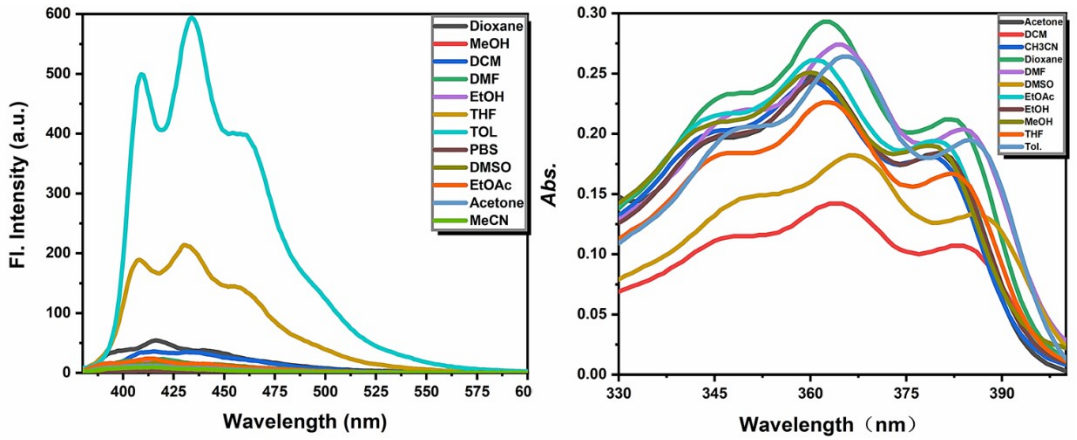

Figure S4. Emission and absorption spectra of compound 2c (10 μM) in different solvents.

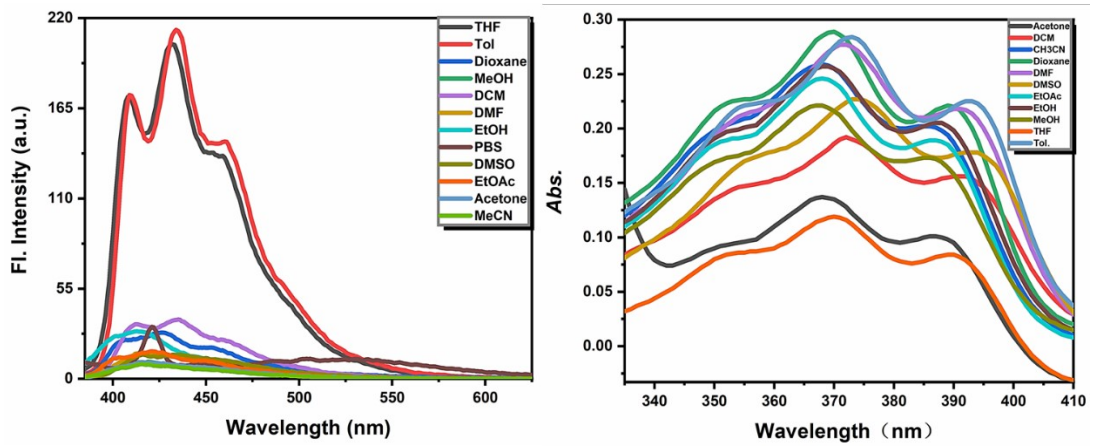

Figure S5. Emission and absorption spectra of compound 2d (10 μM) in different solvents.

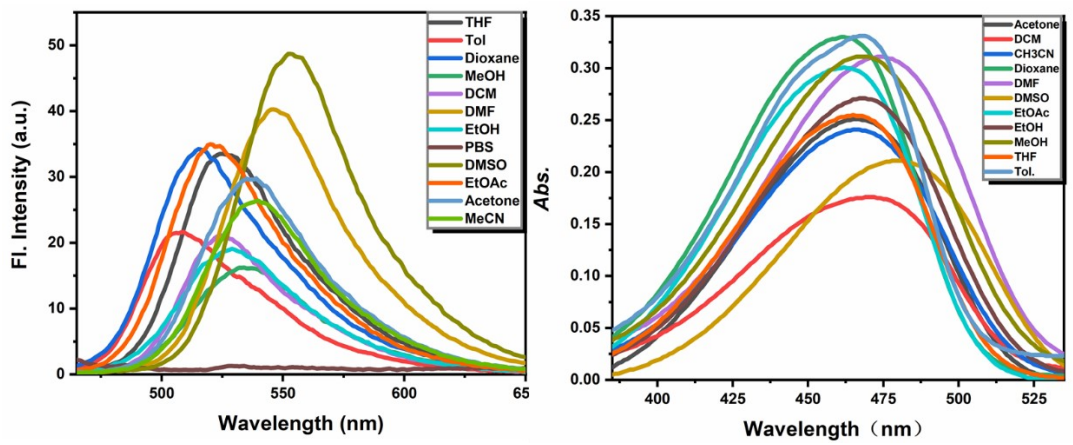

Figure S6. Emission and absorption spectra of compound 2e (10 μM) in different solvents.

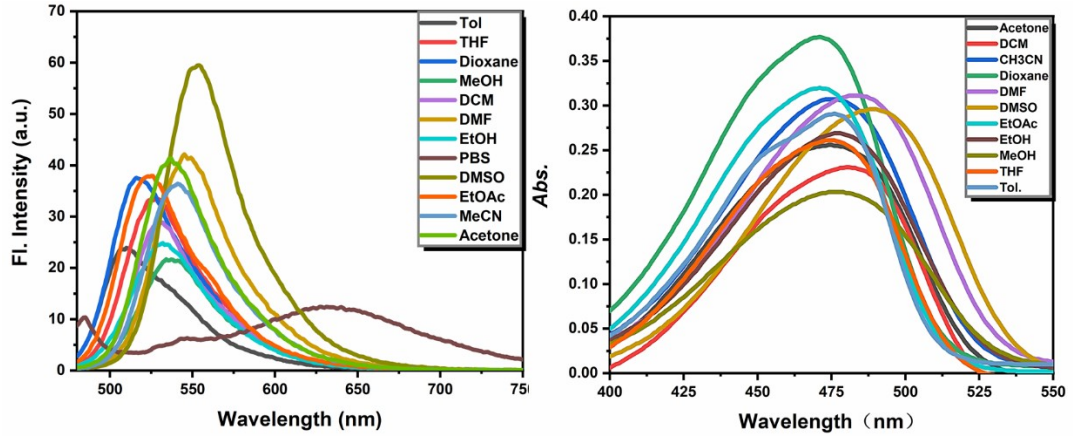

Figure S7. Emission and absorption spectra of compound 2f (10 μM) in different solvents.

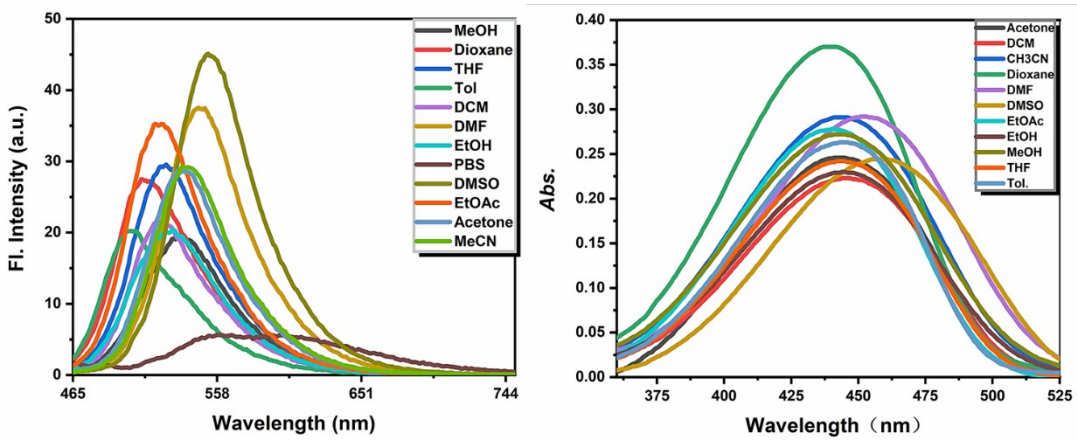

Figure S8. Emission and absorption spectra of compound 2g(10 μM) in different solvents.

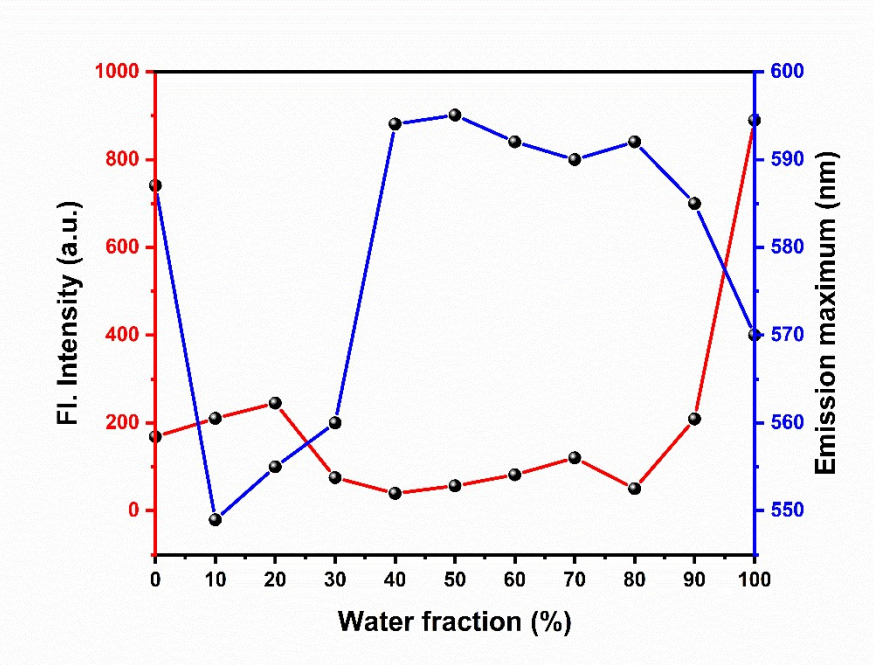

Figure S9. Tendency for maximum fluorescence emission intensity and Stokes shift of the probe **T-LD** (10 μM) in different ratios of THF/H_2O.

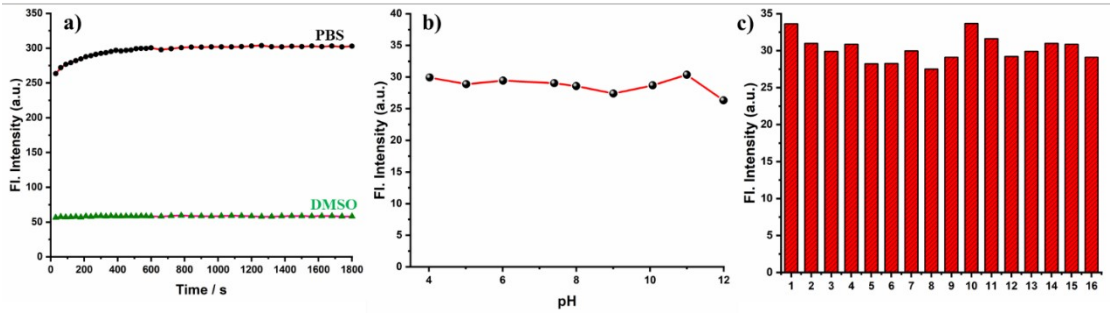

Figure S10 a) Detection of kinetic stability fluorescence spectra of the probe **T-LD** (10 μM) in PBS buffers and DMSO. **b)** Fluorescence emission intensity of the probe **T-LD** (10 μM) in different pH dioxane/PBS buffers (v/v = 1/1). c) Fluorescence intensity of the probe **T-LD** (10 μM) in the presence of different analytes (10 μM) in dioxane/PBS buffers (v/v = 1/1). Where 1-16 represent Cu^{2+}, Ca^{2+}, Mg^{2+}, Al^{3+}, Fe^{2+}, ClO^-, HS^-, Fe^{3+}, Na^+, HCO_3^-, Arg, TBHP, SO_4^{2-}, Cl^-, NO_2^- ,CO_3^{2-} respectively.

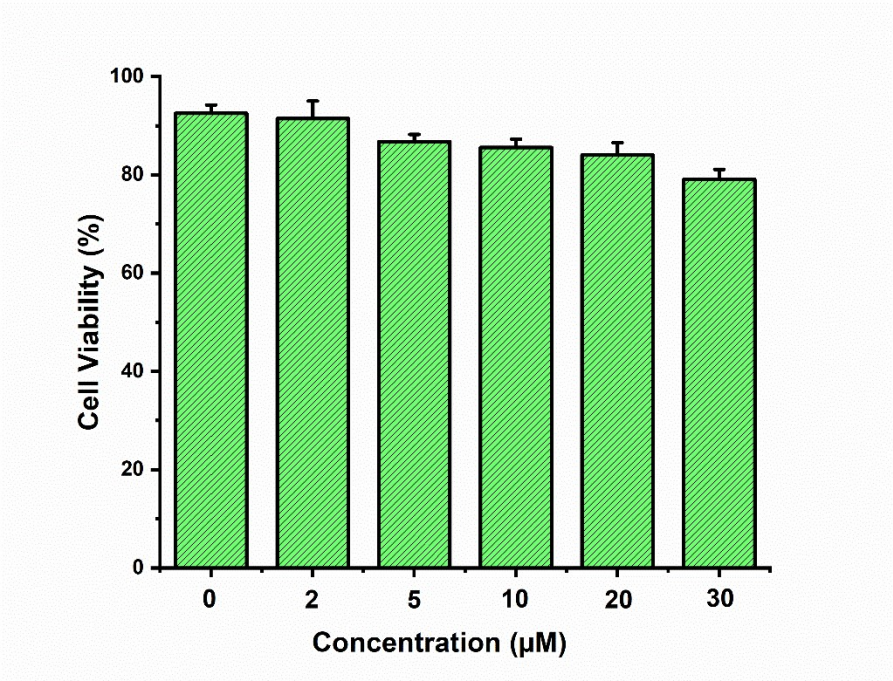

Figure S11. Cytotoxicity assays of probe **T-LD** at different concentrations (0 μM; 2 μM; 5 μM; 10 μM; 20μM; 30 μM) for HeLa cells.

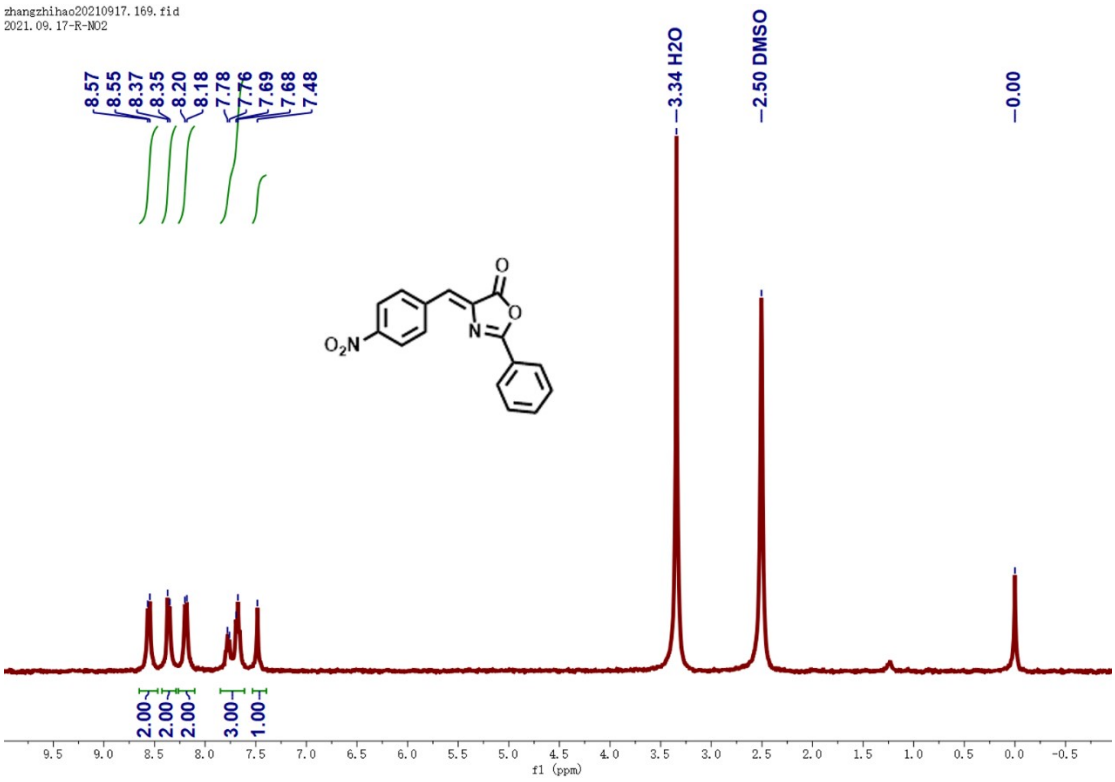

Figure S12. ^{1}H NMR spectrum of compound **2a** in DMSO-*d6*.

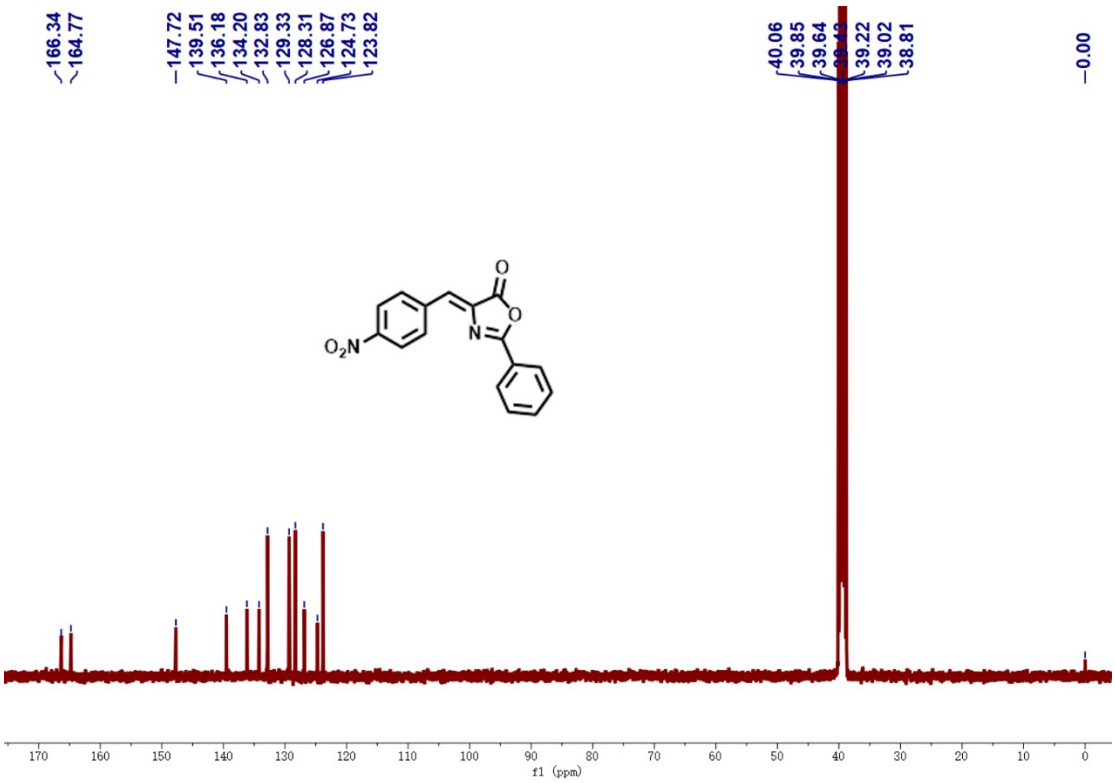

Figure S13. ^{13}C NMR spectrum of compound **2a** in DMSO-*d6*.

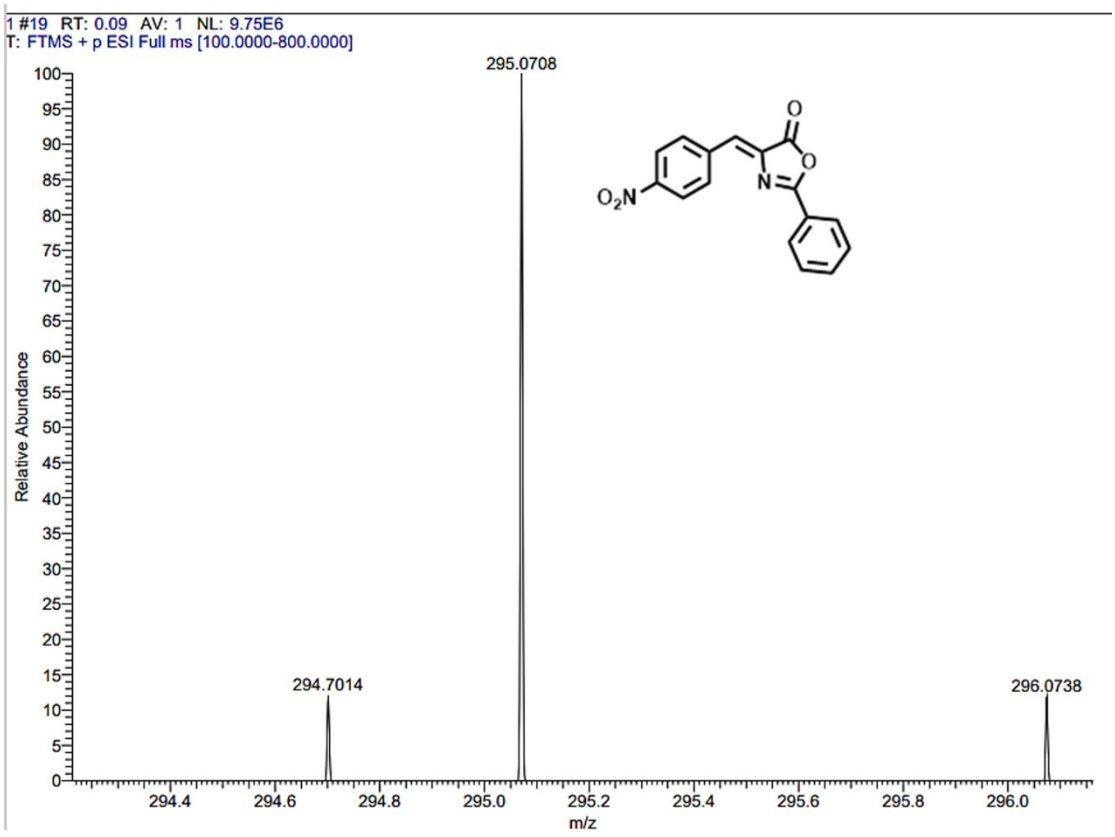

Figure S14. HR-MS spectrum of compound **2a** in CH_3OH.

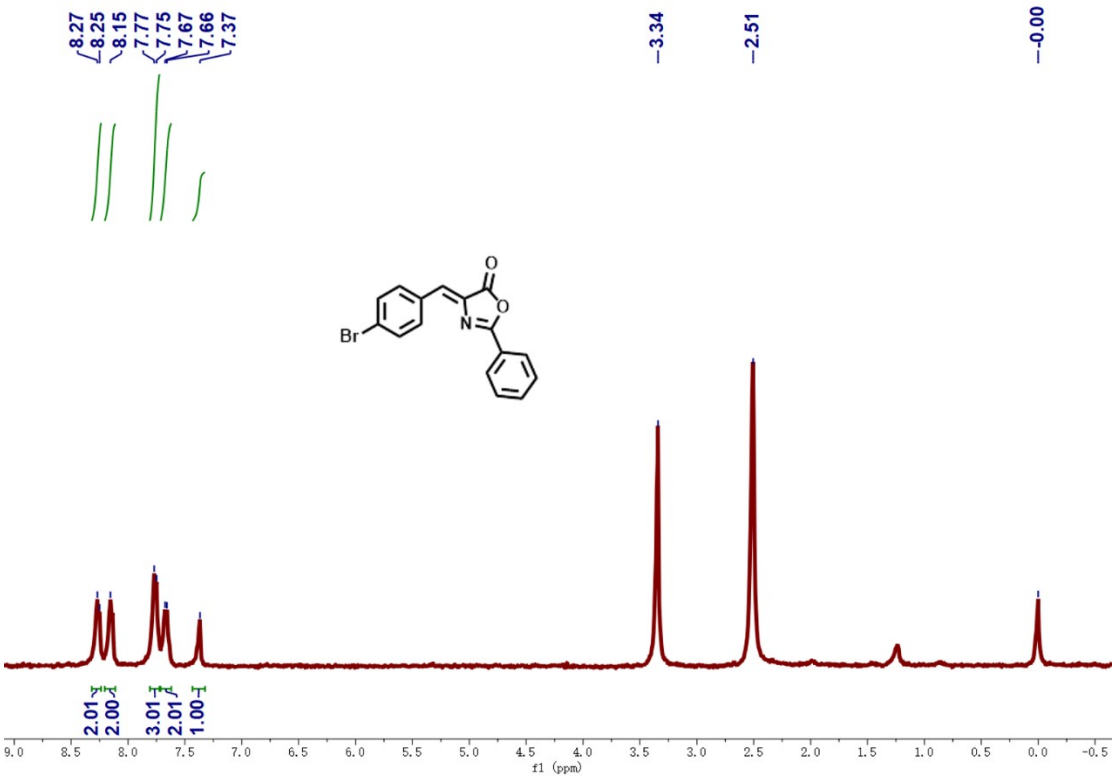

Figure S15. [1]H NMR spectrum of compound **2b** in DMSO-*d6*

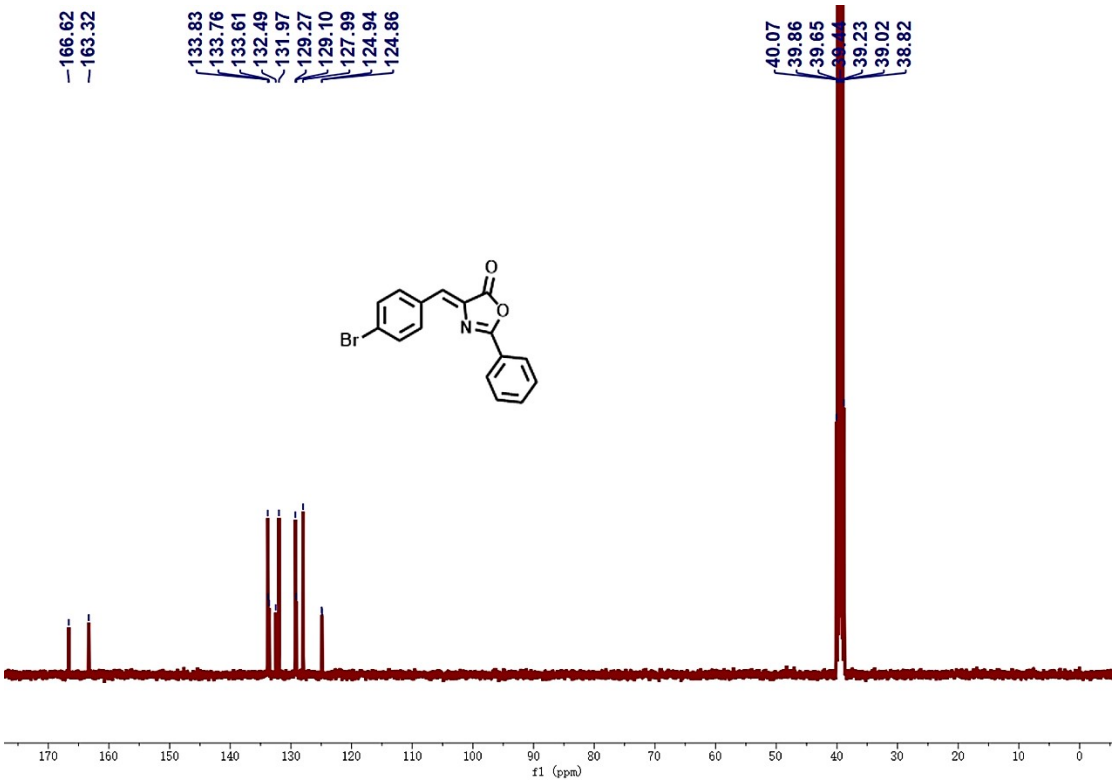

Figure S16. [13]C NMR spectrum of compound **2b** in DMSO-*d6*

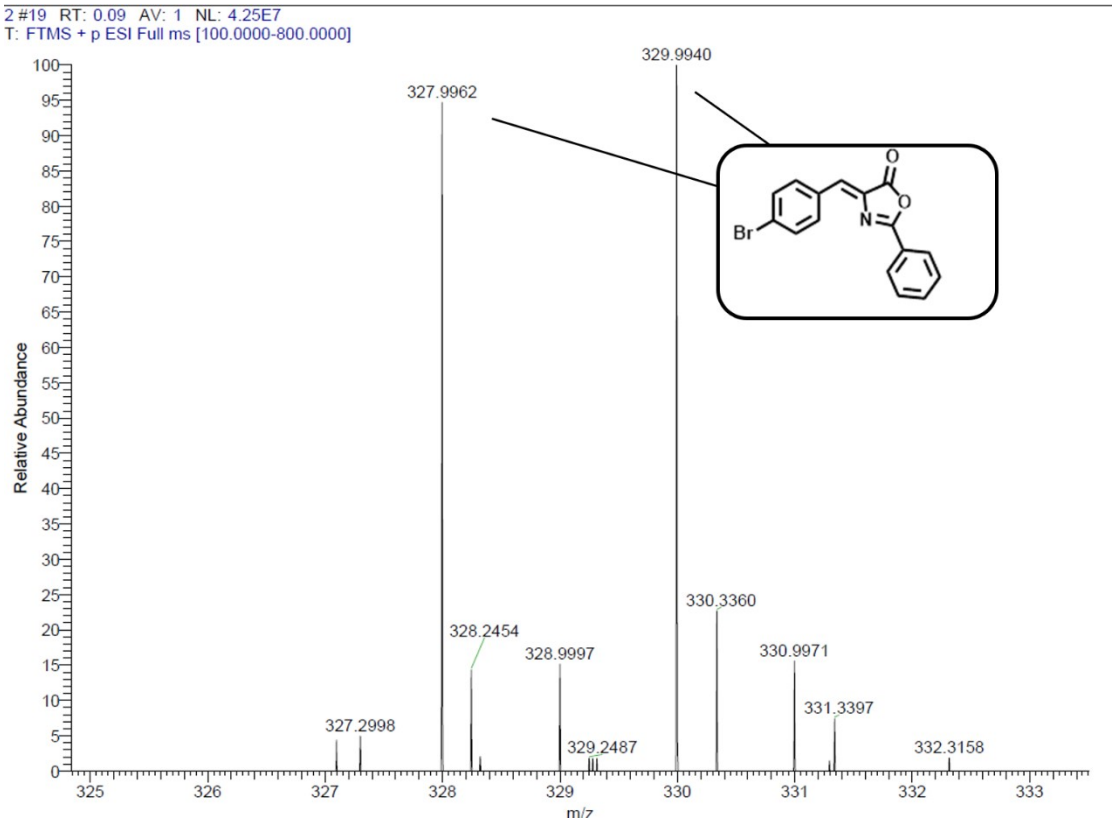

Figure S17. HR-MS spectrum of compound **2b** in CH_3OH.

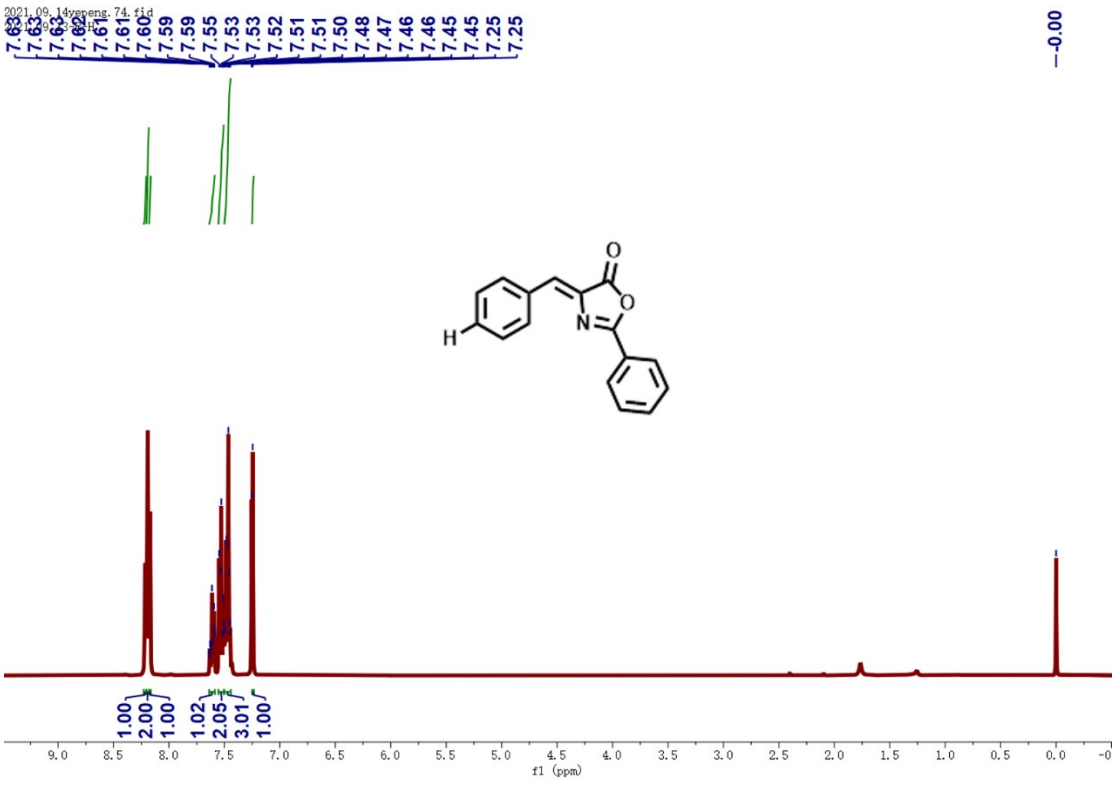

Figure S18. ^{1}H NMR spectrum of compound **2c** in $CDCl_3$.

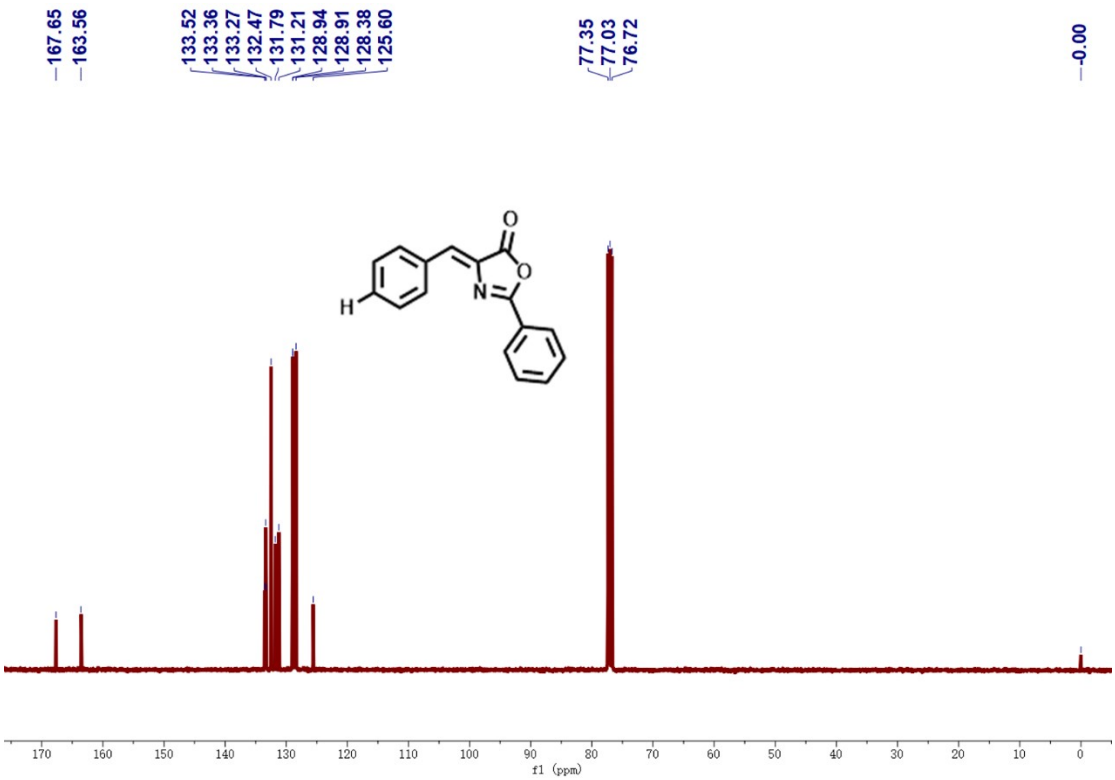

Figure S19. ^{13}C NMR spectrum of compound **2c** in $CDCl_3$.

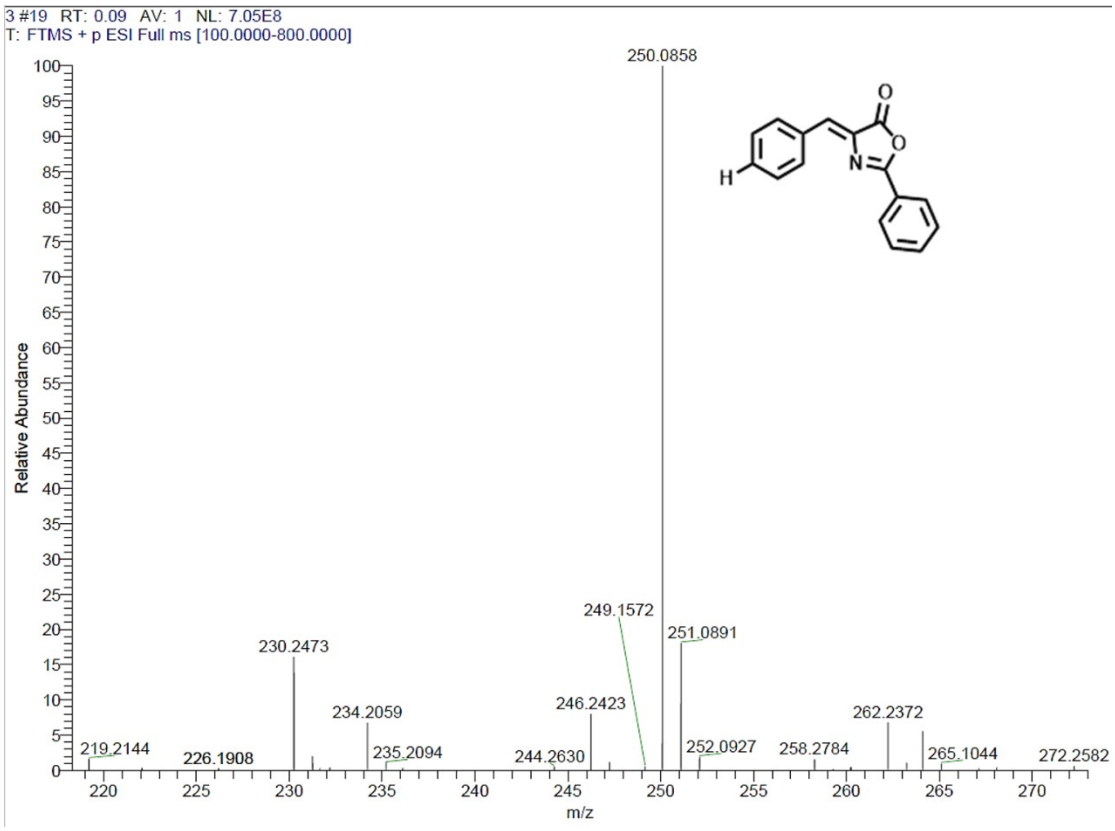

Figure S20. HR-MS spectrum of compound **2c** in CH_3OH.

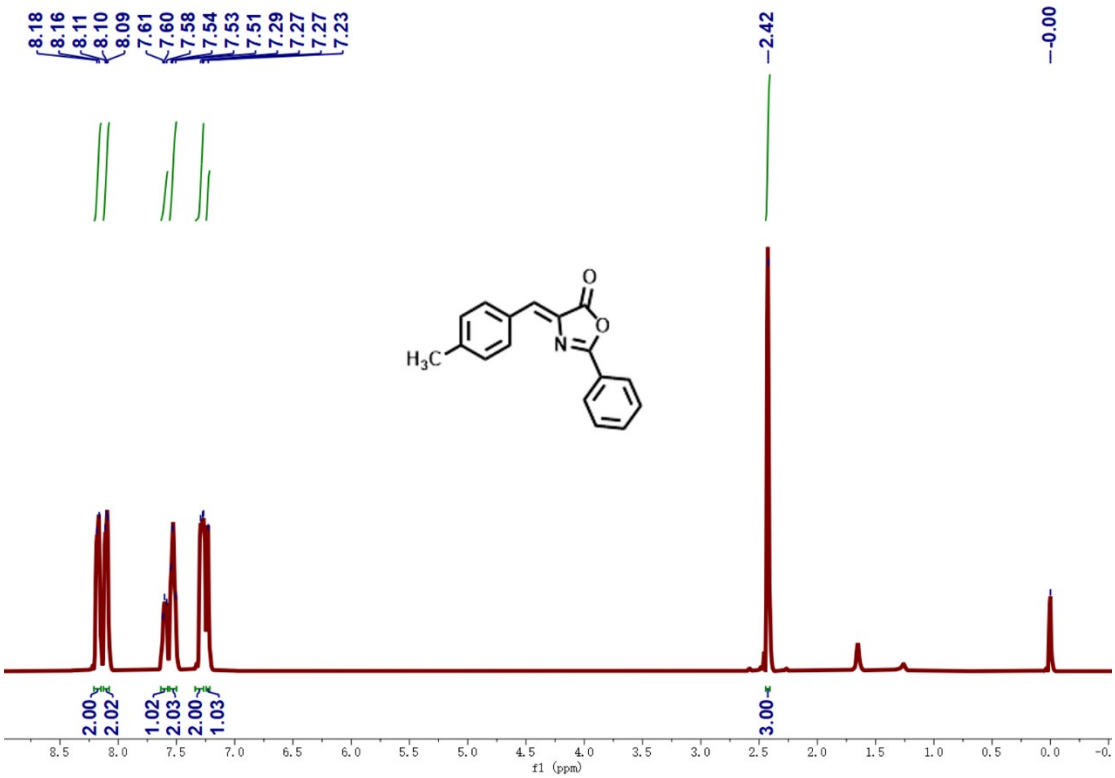

Figure S21. ^{1}H NMR spectrum of compound **2d** in $CDCl_3$.

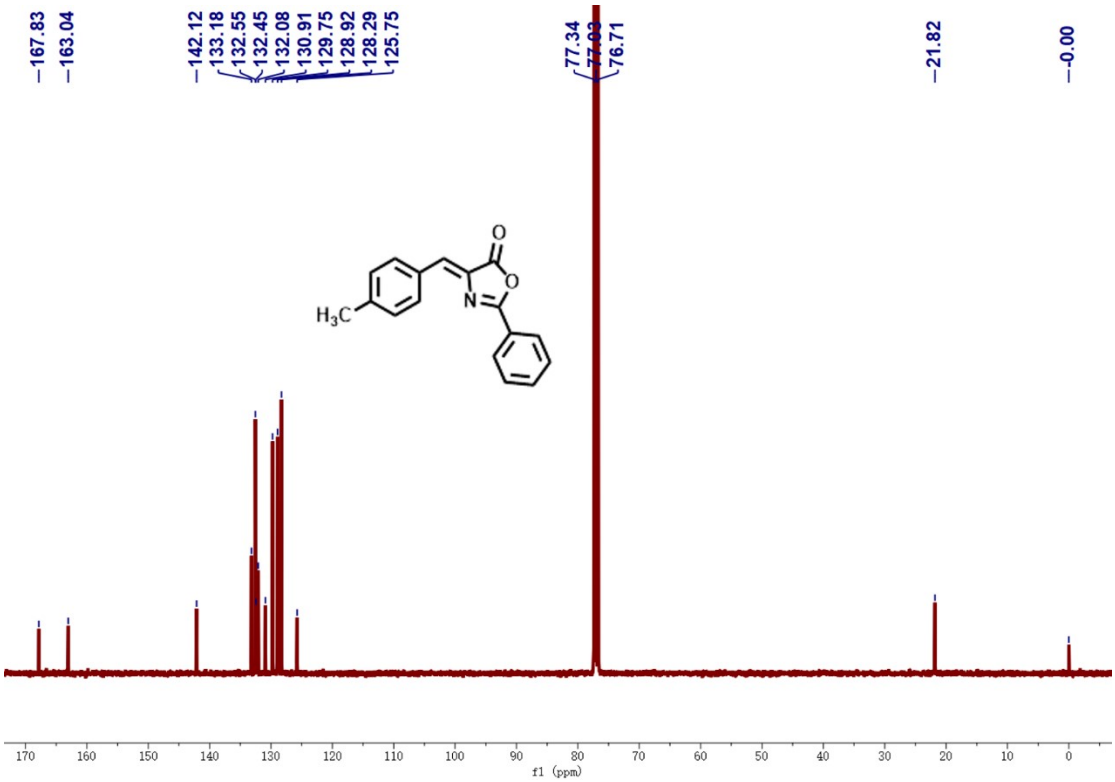

Figure S22. ^{13}C NMR spectrum of compound **2d** in $CDCl_3$.

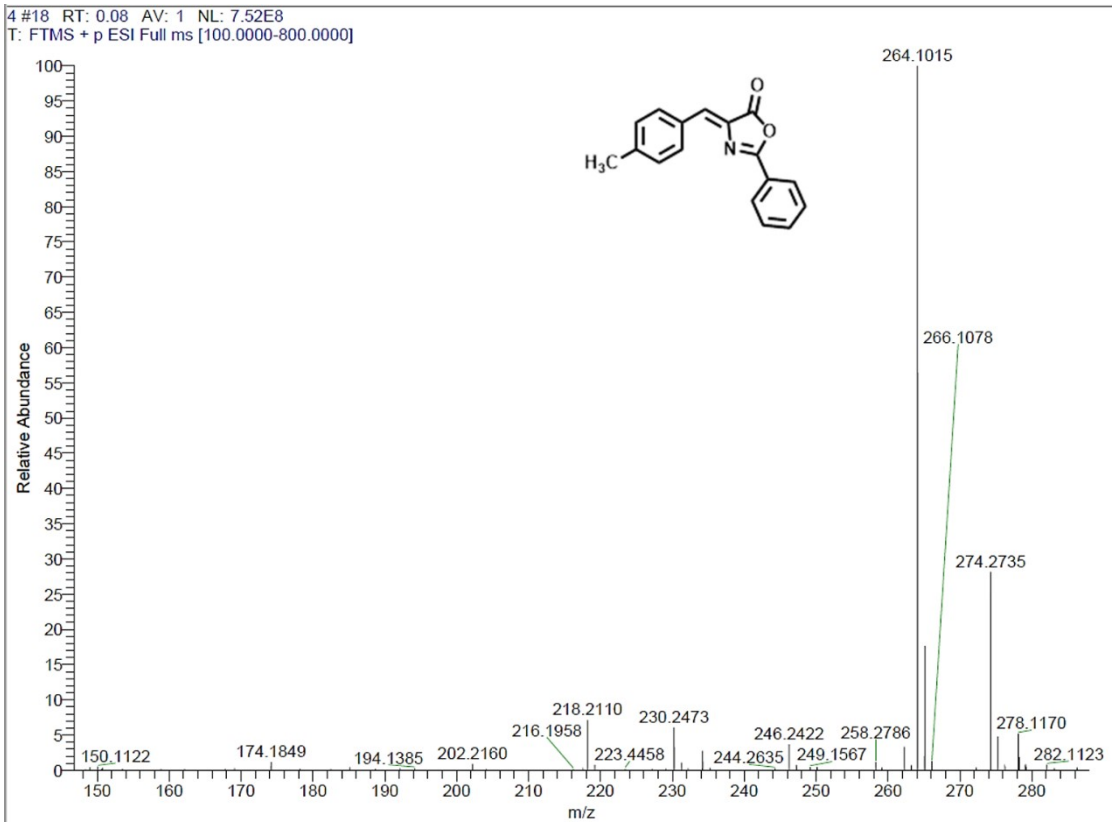

Figure S23. HR-MS spectrum of compound **2d** in CH_3OH.

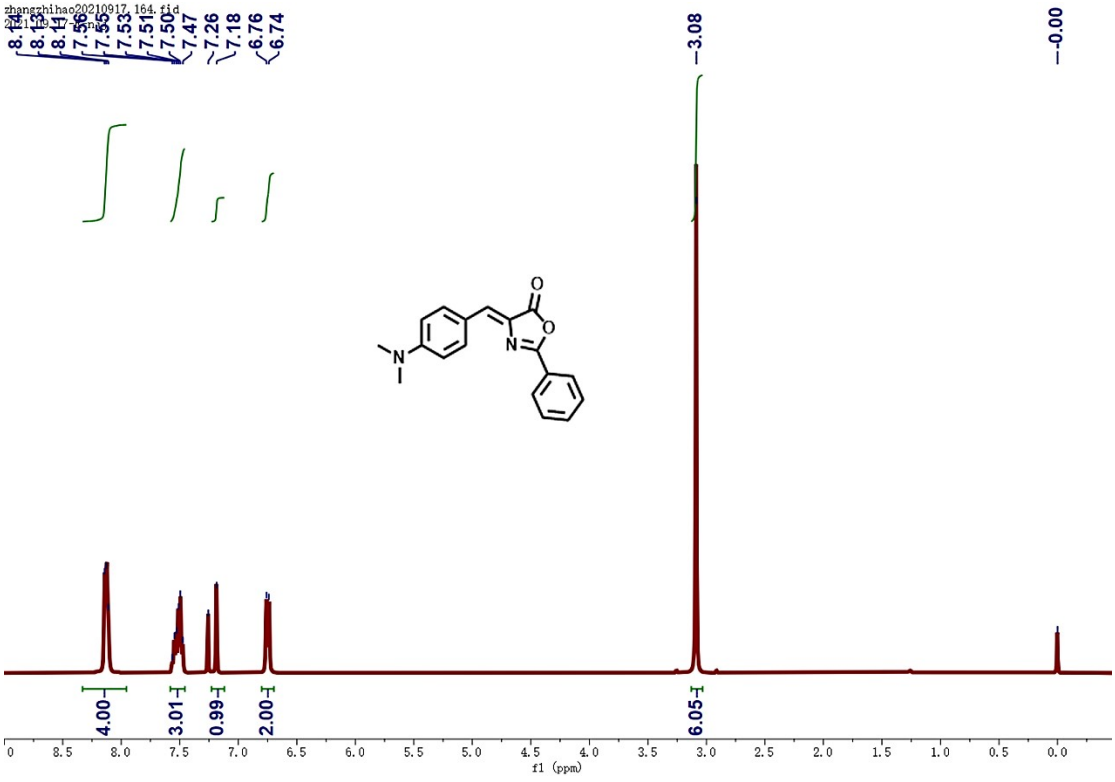

Figure S24. ^{1}H NMR spectrum of compound **2e** in $CDCl_3$.

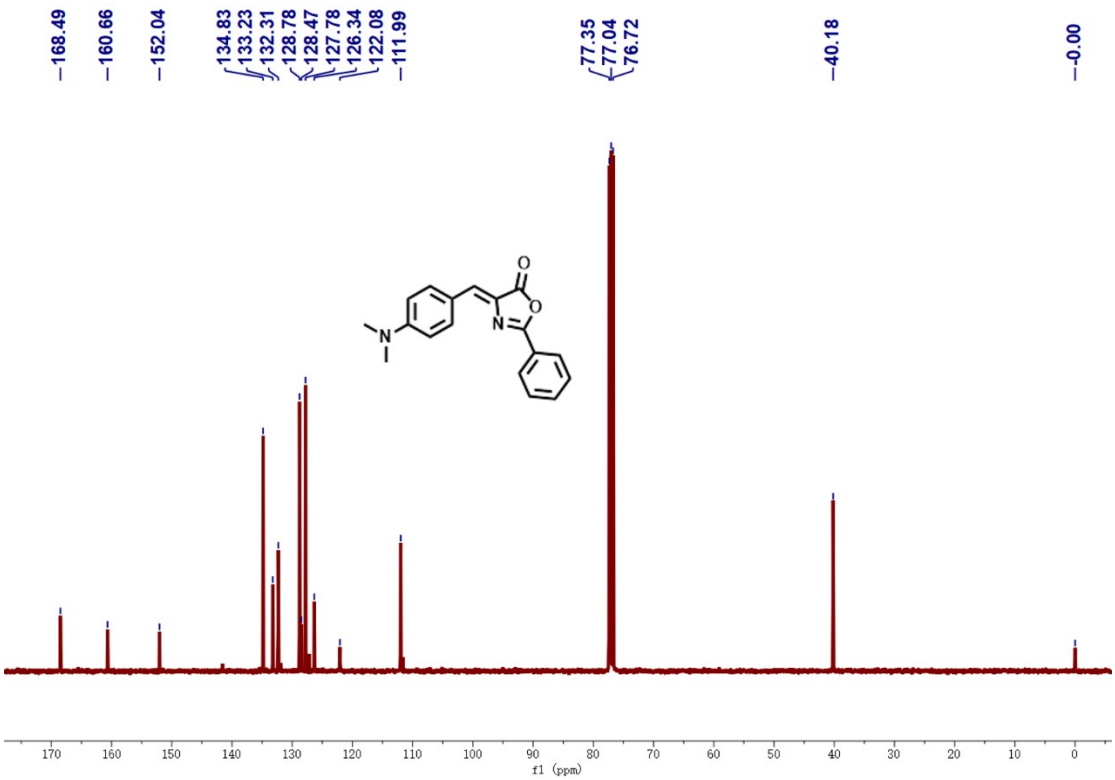

Figure S25. ^{13}C NMR spectrum of compound **2e** in $CDCl_3$.

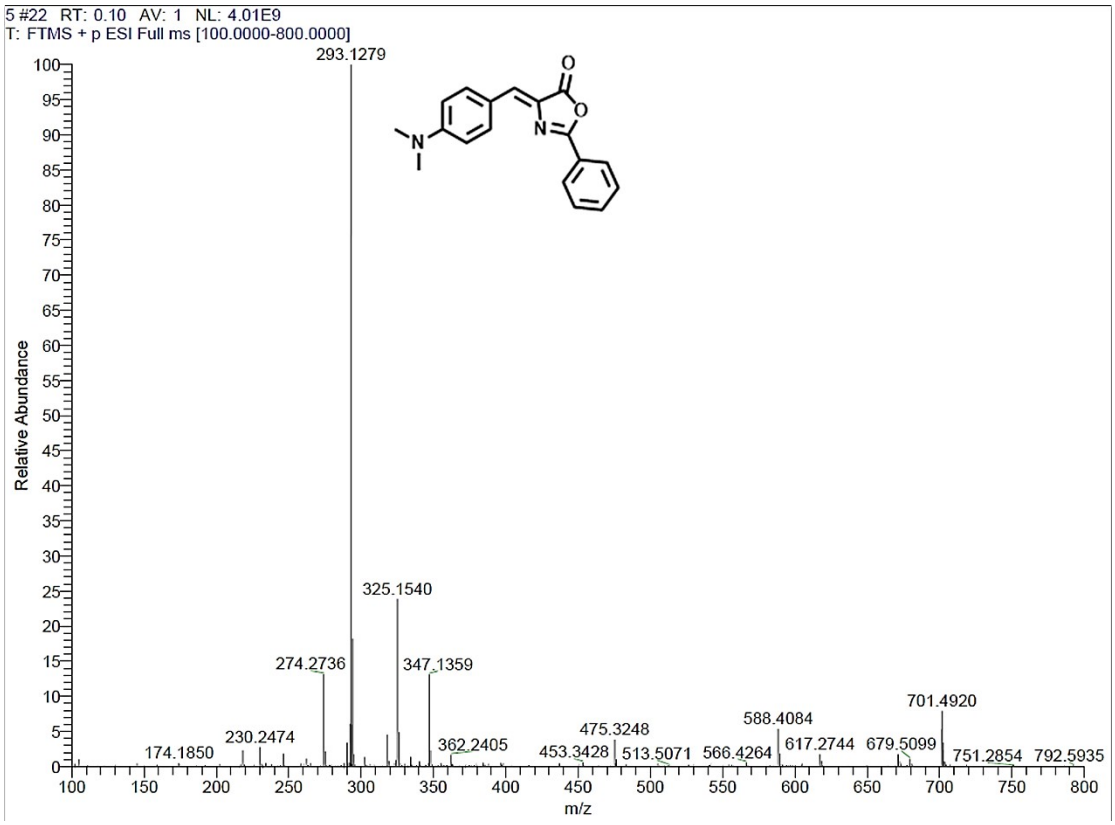

Figure S26. HR-MS spectrum of compound **2e** in CH_3OH.

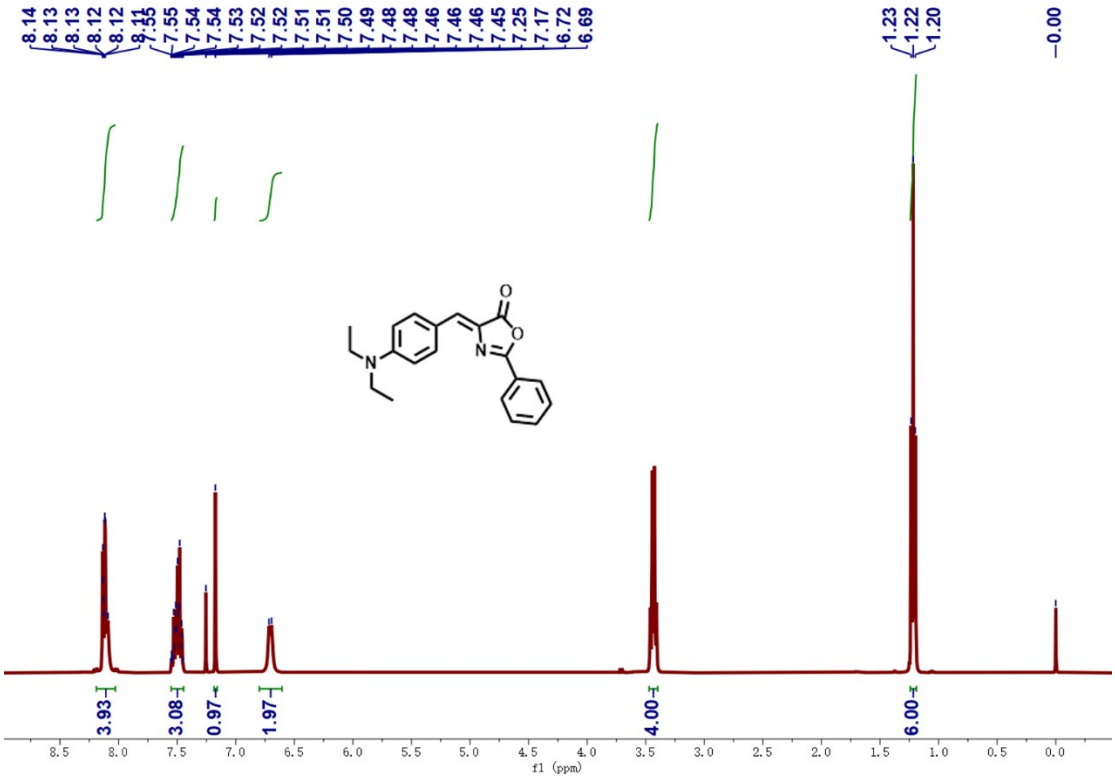

Figure S27. ^{1}H NMR spectrum of compound **2f** in $CDCl_3$.

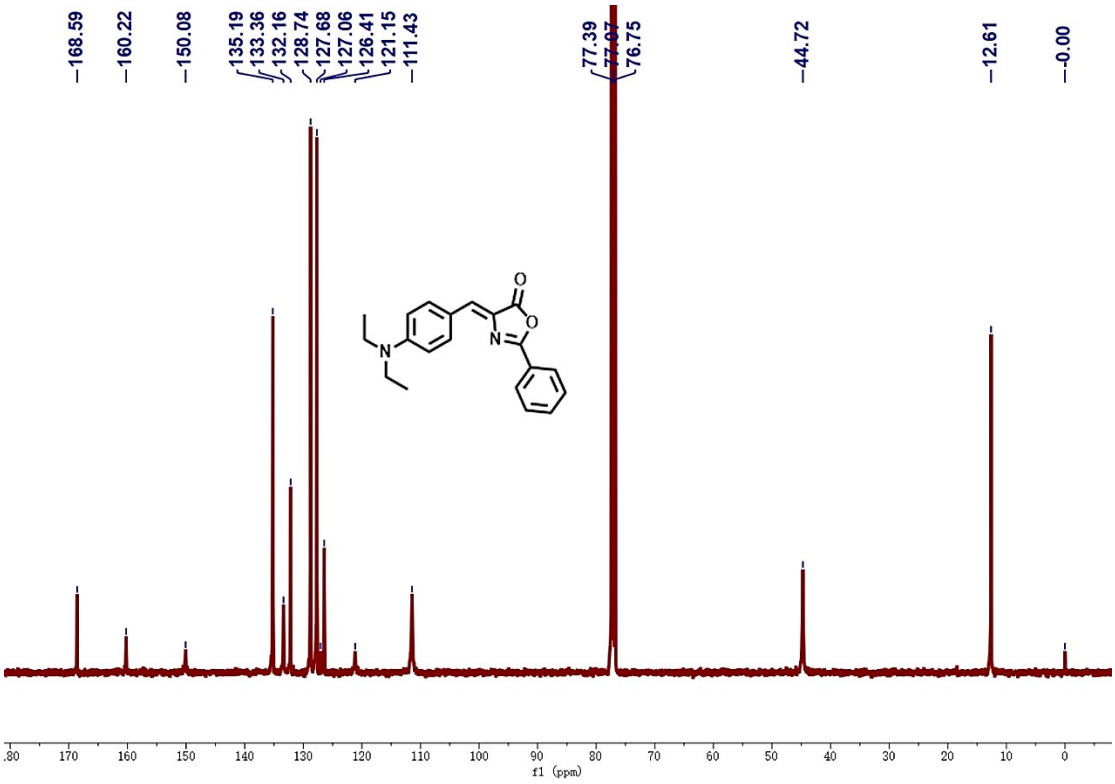

Figure S28. ^{13}C NMR spectrum of compound **2f** in $CDCl_3$.

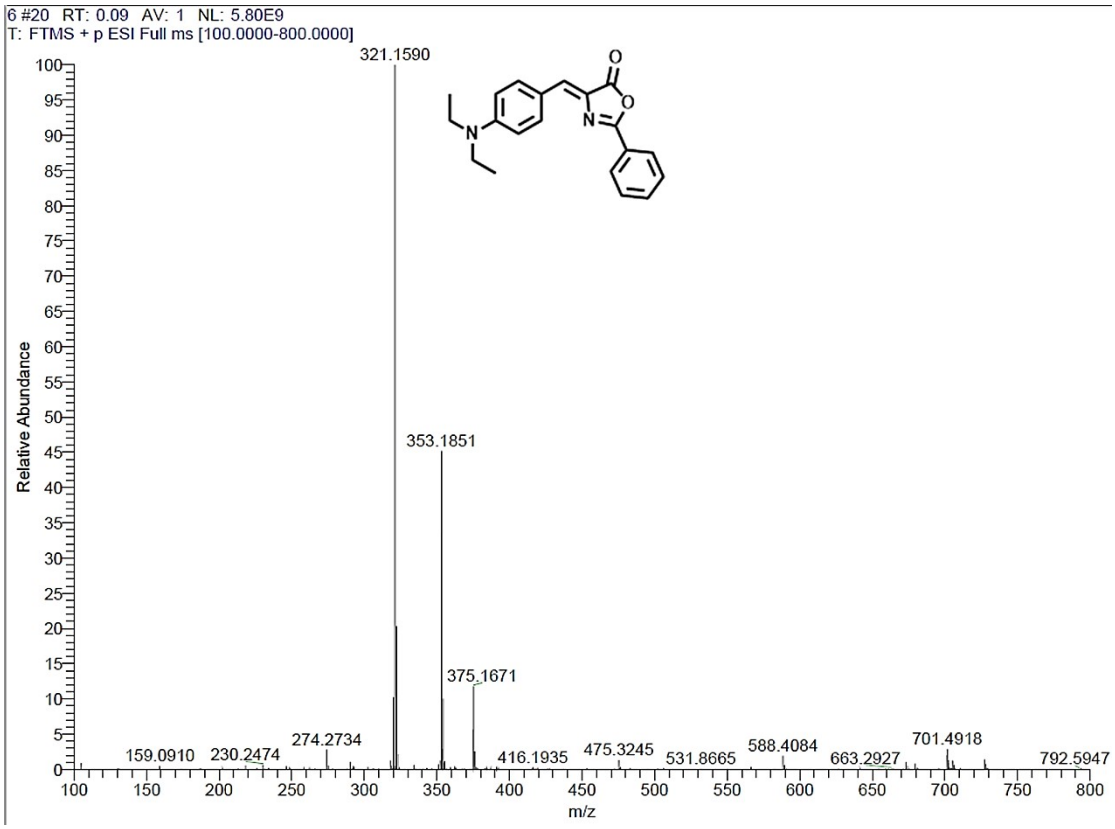

Figure S29. HR-MS spectrum of compound **2f** in CH_3OH.

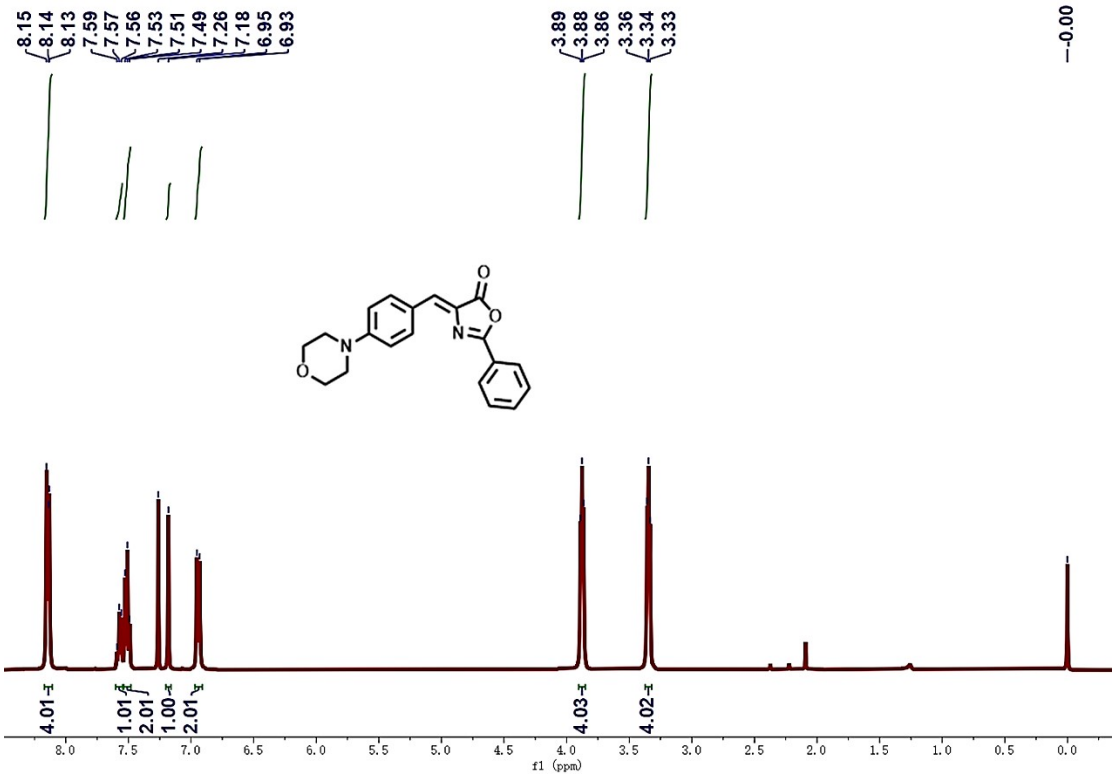

Figure S30. ^{1}H NMR spectrum of compound **2g** in $CDCl_3$.

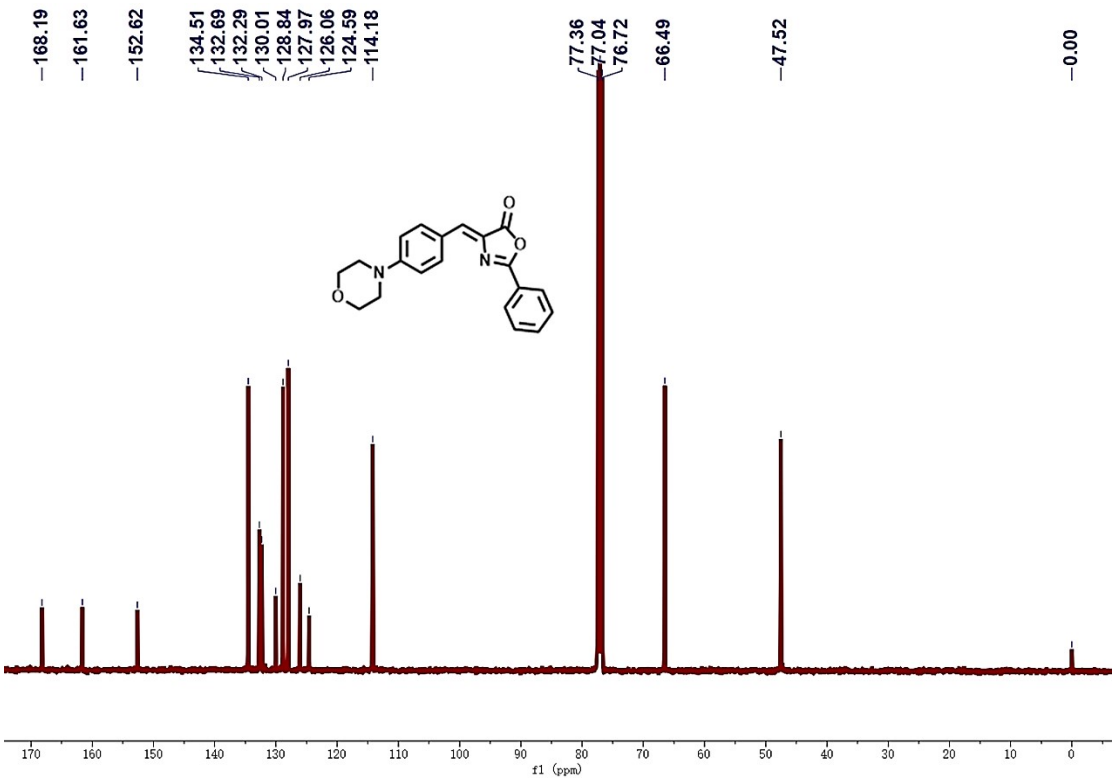

Figure S31. ^{13}C NMR spectrum of compound **2g** in $CDCl_3$.

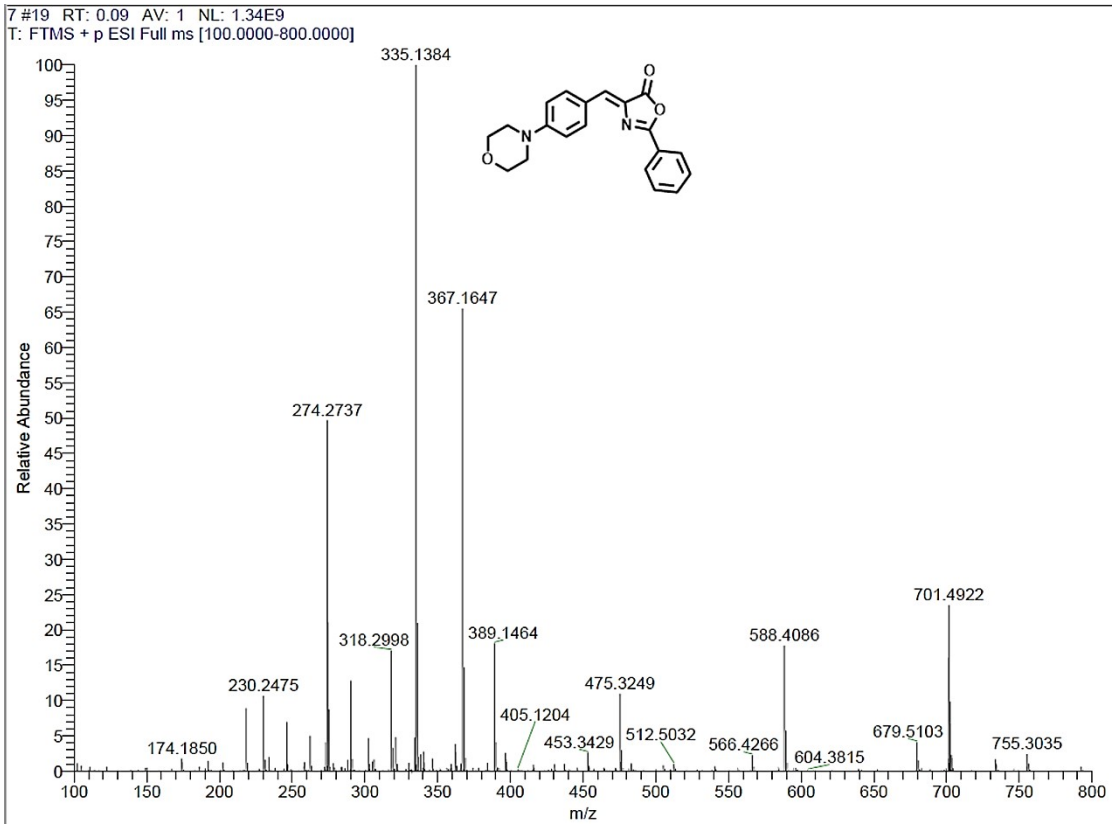

Figure S32. HR-MS spectrum of compound **2g** in CH_3OH.

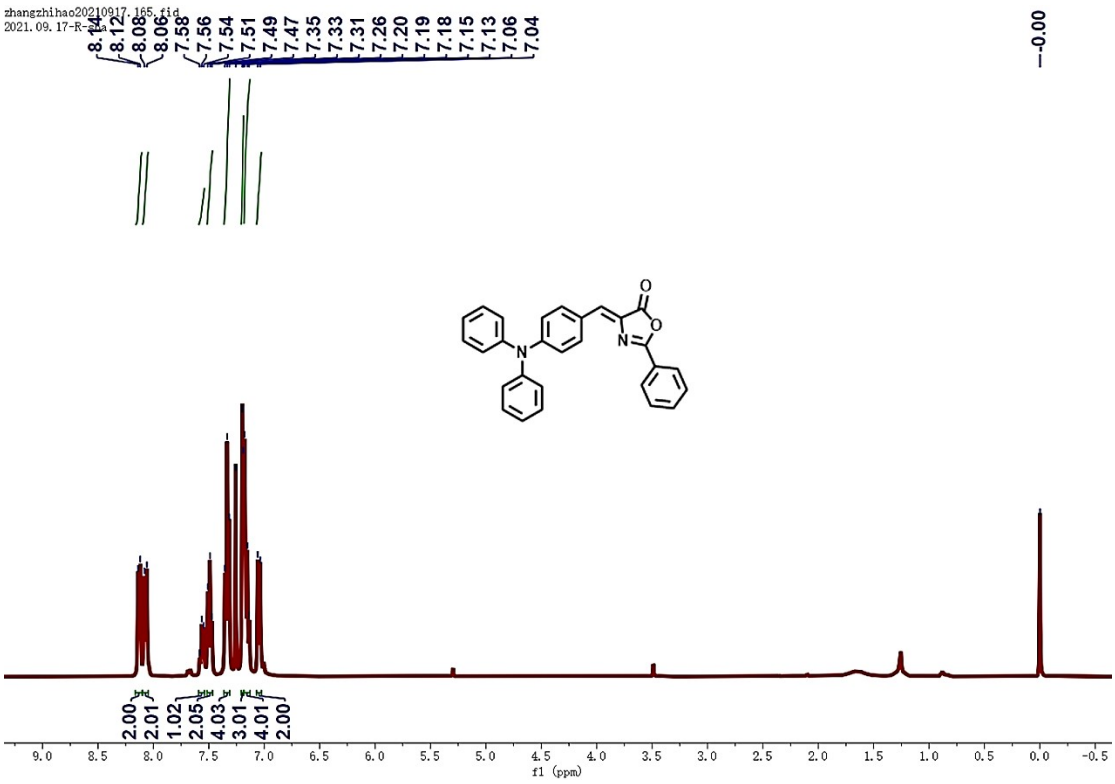

Figure S33. ^{1}H NMR spectrum of compound **2h (T-LD)** in $CDCl_3$.

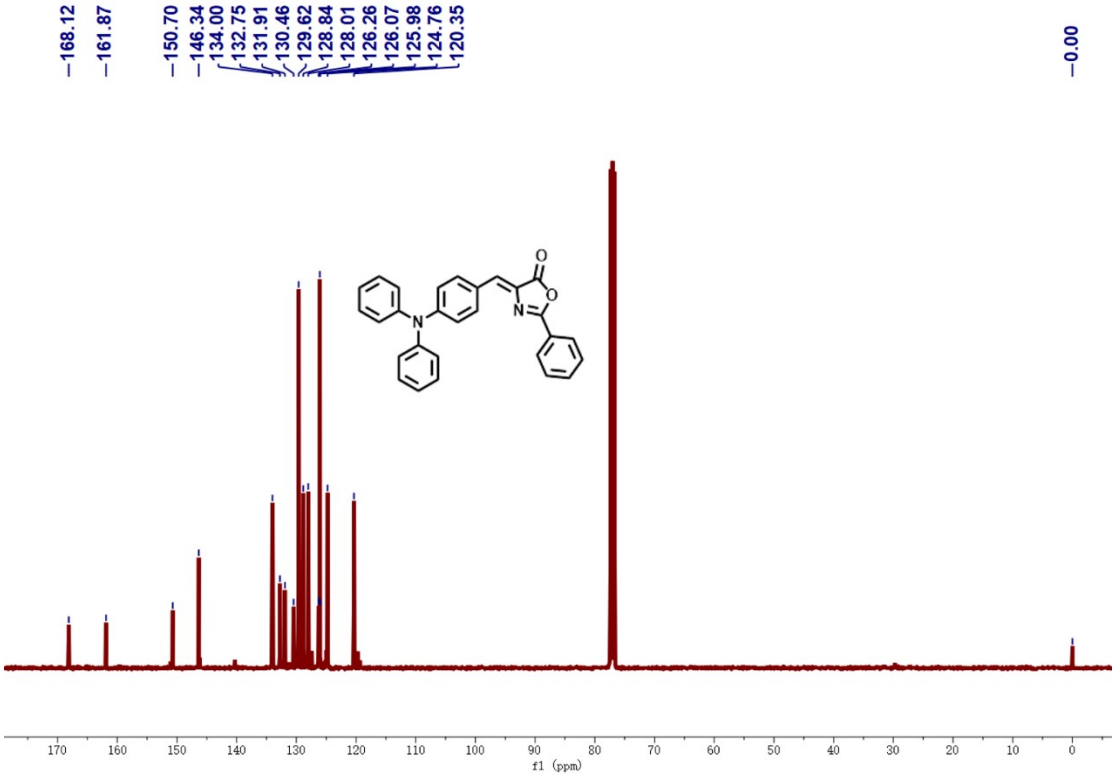

Figure S34. ^{13}C NMR spectrum of compound **2h (T-LD)** in $CDCl_3$.

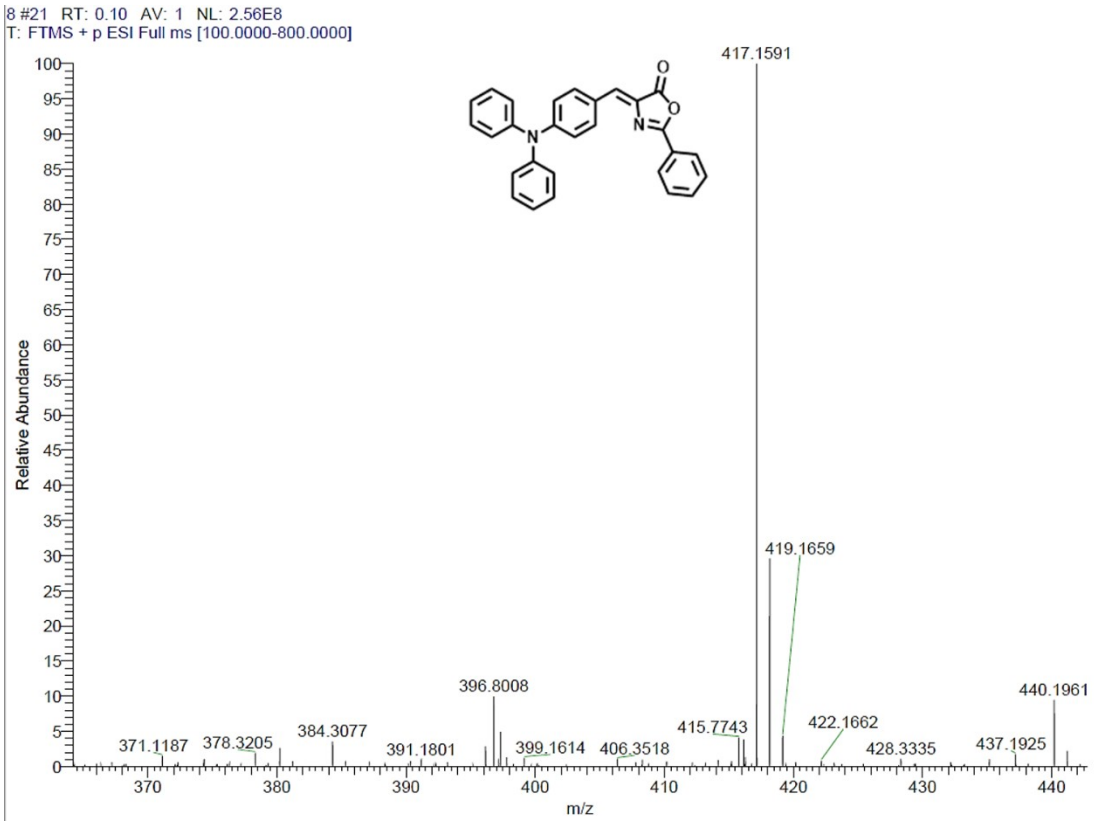

Figure S35. HR-MS spectrum of compound **2h (T-LD)** in CH_3OH.