Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Fluorogenic Probes for Mitochondria and Lysosomes via Intramolecular Photoclick Reaction

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Contents

General Methods

Materials and instrumentation

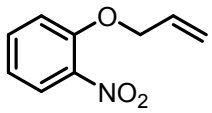
All solvents such as ethanol, dimethyl sulfoxide (DMSO), and reagents used for synthesis and spectroscopic experiments were from standard suppliers in reagent grade. All chemicals used for synthesis were purchased from Admas Energy Chemical (Shanghai, China) and Aladdin (Shanghai, China). Unless otherwise specified, the chemicals did not need further purification.

The NMR spectra were measured by Bruker DRX-400 spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR at 298 K using tetramethylsilane as the internal standard. All reactions were monitored by TLC on silica gel (Yantai, China) under UV light (λ = 254 nm, 302 nm, 365 nm). The absorption spectra were recorded by an Agilent Cary 60 UV–vis spectrophotometer. The emission spectra have been recorded on Hitachi F-7000 spectrometer. Mass spectra were carried out on Thermo Finnigan LCQ Advantage using methanol solution.

Synthesis

The synthesis method of the compounds was performed with reference to the literature.[1-4]

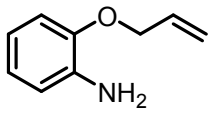
Compound 1: 1-(allyloxy)-2-nitrobenzene



2-nitrophenol (2.78 g, 20 mmol), 3-bromoprop-1-ene (3.94 g, 20 mmol) and potassium carbonate (2.77 g, 20 mmol) were dissolved in acetone (100 mL). The suspension was refluxed for 6 h. After cooling to room temperate, water (200 mL) was added and the mixture was extracted with ethyl acetate and washed by brine.[1] The solvent was removed in vacuum to obtain compound **1** as pale-yellow oily matter (6.25 g, Yield: 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, *J* =8.0 Hz, 1H), 7.53 (m, *J* =16.0 Hz,1H), 7.09 (s, 1H), 7.04 (m, *J* = 12.0 Hz, 1H), 6.06 (m, *J* = 4.0 Hz, 1H), 5.51 (d, *J* =16.0 Hz,1H), 5.35 (d, *J* = 12.0 Hz, 2H), 4.70 (d, *J* = 4.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.88, 140.10,

134.01, 131.73, 125, 120.47, 118.27, 114.91, 69.99. ESI-MS m/z, calcd for $C_9H_9NO_3^+$ $[M+H]^+$: 180.18; found, 179.69.

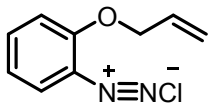
Compound 2: 2-(allyloxy)aniline



1-(allyloxy)-2-nitrobenzene (1.79 g, 10 mmol) and tin (II) chloride dihydrate (9.02 g, 40 mmol) were dissolved into ethanol (25 mL), then concentrated HCl (5.5 mL) were added, the mixture was heated and refluxed for 8 h. Water (40 mL) was added and the pH was adjusted to 8.0 with 4.0 M NaOH solution, then the mixture was extracted with ethyl acetate. The resulting precipitate was removed by filtration and rinsed with ethyl acetate.[1] The solvent of organic phase was removed in vacuum to obtain Compound **2** as black oily matter (1.19 g, Yield: 80%).

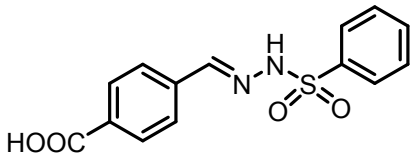
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (m, *J* = 8.0 Hz, 2H), 6.12 (m, *J* = 8.0 Hz, 2H), 7.04 (m, *J* = 12.0 Hz, 1H), 5.45 (d, *J* = 20.0 Hz, 1H), 5.32 (d, *J* = 12.0 Hz,1H) , 4.60 (d, *J* = 8.0 Hz, 2H), 3.81 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.28, 136.51, 133.61, 121.42, 118.40, 117.41, 115.26, 112.12, 69.25. ESI-MS m/z, calcd for $C_9H_1NO^+$ $[M+H]^+$: 150.19; found, 149.07.

Compound 3: 2-(allyloxy)benzenediazonium chloride



Compound **2** (0.90 g, 5 mmol) was dissolved in a solution of ethanol-water mixture (2:1, 12 mL), cooled to 0 °C in an ice bath, then concentrated hydrochloric acid (1 mL) was slowly added, returned to 0 °C, an sodium nitrite aqueous (5 mmol, 1 mL) was added and stirred for about 10 min to afford reddish brown solution to synthesize compound **4** without purification.[2]

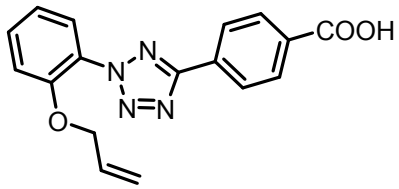
Compound 4: (E)-4-((2-(phenylsulfonyl)hydrazono) methyl) benzoic acid



4-carboxybenzaldehyde (1.49 g, 10 mmol) and benzenesulfonyl hydrazide (1.72 g, 10 mmol) were dissolved in ethanol (50 mL). The solution was stirred at room temperature for 3 h. After the addition of water, the white precipitate was collected by filtration.[2] Then water was added to the filtrate, the remaining precipitate was collected by filtration to afford compound **4** as a white powder (2.85 g, Yield: 95%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.06 (s, 1H), 11.76 (s, 1H), 7.99 (s, 1H), 7.94 (d, *J* = 8.0 Hz, 2H), 7.90 (d, *J* = 8.0 Hz, 2H), 7.69 (s, 1H), 7.67 (m, *J* = 4.0 Hz, 2H), 7.62 (m, *J* = 12.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.27, 146.40, 139.39, 138.78, 138.01, 133.62, 133.59, 132.21, 130.20, 129.78, 129.51, 128.18, 127.60, 127.26. ESI-MS m/z, calcd for $C_{14}H_{12}N_2O_4S^+$ $[M+H]^+$: 305.32; found, 304.17.

Compound 5: 4-(2-(2-(allyloxy)phenyl)-2*H*-tetrazol-5-yl)benzoic acid

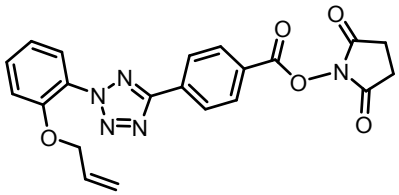


Compound **4** (1.51 g, 5 mmol) was dissolved in cooled pyridine in cold ethanol (-10 °C), then the freshly prepared compound **3** (1.01 g, 5 mmol) solution was slowly added dropwise. The reaction was stirred overnight at room temperature. A portion of the pyridine was removed in vacuum, and the mixture was washed with 4.0 M HCl solution followed by ethyl acetate. The ethyl acetate layer was dried with Na_2SO_4.[2] The solvent was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate = 1:1) to give compound **5** as a brown powder (1.29 g, Yield: 80%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, *J* = 8.0 Hz, 2H), 8.31 (d, *J* = 8.0 Hz, 2H), 7.66 (d, *J* = 4.0 Hz, 1H), 7.57 (m, *J* = 16.0 Hz, 1H), 7.19 (m, *J* = 8.0 Hz, 2H), 5.98 (m, *J* = 4.0 Hz, 1H), 5.39 (d, *J* = 16.0 Hz, 1H), 5.27 (d, *J* = 16.0 Hz, 1H), 4.69 (d, *J* = 4.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.99, 163.91, 152.52, 132.05, 132.01, 130.85, 127.05, 126.94, 126.66, 121.03, 117.87, 114.36. ESI-MS m/z, calcd for $C_{17}H_{14}N_4O_3^-$ $[M-H]^-$: 321.32; found, 320.86.

Compound 6:

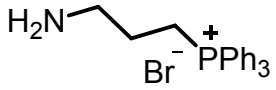
2,5-dioxopyrrolidin-1-yl-4-(2-(2-(allyloxy)phenyl)-2H-tetrazol-5-yl)benzoate



Compound **5** (0.64 g, 2 mmol) and N-hydroxy-succinimide (NHS) (3 mmol) were dissolved in dichloromethane (25 mL) at 0 °C, then 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was added, the mixture was stirred overnight at room temperature.[1] The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (petroleum ether/ethyl acetate = 2:1) on silica gel to give a white powder (0.67 g, Yield: 80%).

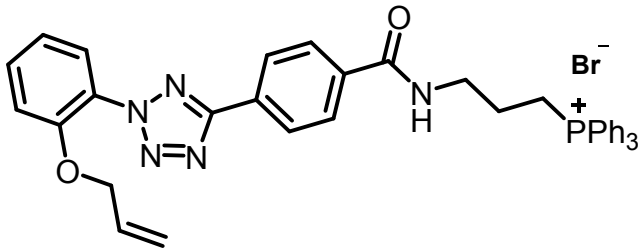
^{1}H NMR (100 MHz, $CDCl_3$) δ 8.44 (d, *J* = 4.0 Hz, 2H), 8.32 (d, *J* = 8.0 Hz, 2H), 7.66 (m, *J* = 8.0 Hz, 1H), 7.57 (m, *J* = 16.0 Hz, 1H), 7.19 (d, *J* = 8.0 Hz, 2H), 5.95 (m, *J* = 28.0 Hz, 1H), 5.38 (d, *J* = 16.0 Hz, 1H), 5.27 (d, *J* = 8.0 Hz, 1H), 4.69 (d, *J* = 4.0 Hz, 2H), 2.96 (s, 4H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 169.12, 163.54, 161.43, 152.50, 133.39, 132.12, 131.98, 131.22, 127.28, 126.90, 126.56, 121.03, 117.88, 114.35, 69.77, 25.72. ESI-MS m/z, calcd for $C_{21}H_{17}N_5O_5^+$ $[M+H]^+$: 420.40; found, 420.09.

Compound 7: (3-aminopropyl) triphenylphosphonium



3-Bromopropionamide (0.31 g, 2 mmol) and triphenylphosphine (0.52 g, 2 mmol) were dissolved in methanol (50 mL) and stirred at room temperature for 5 h.[3] The solvent was removed under reduced pressure to get the product as white powder (0.34 g, Yield: 86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85 (m, 4H), 7.40 (d, *J* = 4.0 Hz, 8H), 7.25 (m, *J* = 20.0 Hz, 5H), 3.61 (t, *J* = 12.0 Hz, 2H), 2.92 (s, 2H), 2.11 (m, *J* = 24.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 137.10, 133.69, 131.94, 129.25, 38.09, 31.60, 30.49. ESI-MS m/z, calculated for $C_{21}H_{22}NP^+$ [M-Br]$^+$: 319.39; found, 320.86.

Compound Mt-Tet: (3-(4-(2-(2-(allyloxy)phenyl)-2H-tetrazol-5-yl)benzamido)propyl) triphenylphosphonium



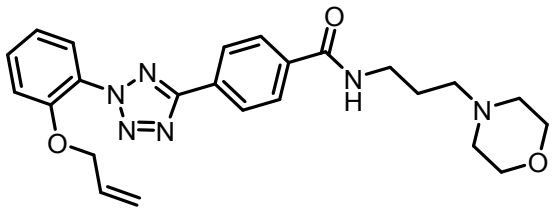
Compound **5** (0.33 g, 1 mmol), N,N-Diisopropylethylamine (DIPEA, 0.13 g, 1 mmol) and O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluorophosphate (HBTU, 1.90 g, 0.5 mmol) were dissolved in dichloromethane, and stirred at room temperature for about 30 min. A catalytic amount of DMAP was added and the solution was stirred for another 30 min. Then Compound **7** (0.48 g, 1.2 mmol) was added and the solution was stirred at room temperature overnight. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel (dichloromethane /methanol = 5:1) to give a white powder (0.51 g, Yield: 73%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (d, *J* = 8.0 Hz, 2H), 8.05 (d, *J* = 8.0 Hz, 2H), 7.78 (m, *J* = 16.0 Hz, 3H), 7.66 (m, *J* = 8.0 Hz, 15H), 7.15 (t, *J* = 16.0 Hz, 2H), 5.96 (m, *J* = 40.0 Hz,

1H), 5.36 (d, *J* = 16.0 Hz, 1H), 5.24 (d, *J* = 8.0 Hz, 1H), 4.66 (s, 2H), 4.14 (m, *J* = 20.0 Hz, 1H), 3.34 (t, *J* = 20.0 Hz, 2H), 1.28 (t, *J* = 12.0 Hz, 2H); ^{13}C NMR (100 MHz,$CDCl_3$) δ 167.43, 164.1, 152.49, 135.32, 135.29, 135.02, 133.33, 133.23, 132.04, 131.92, 130.69, 130.57, 130.31, 127.93, 127.16, 126.90, 126.74, 121.01, 118.27, 117.88, 117.42, 114.41, 77.23, 69.81, 39.82, 22.34, ESI-MS m/z, calculated for $C_{38}H_{35}N_5O_2P^+$ $[M-Br]^+$: 624.25; found, 624.15.

Compound Ly-Tet:

4-(2-(2-(allyloxy)phenyl)-2H-tetrazol-5-yl)-N-(3morpholinopropyl)benzamide

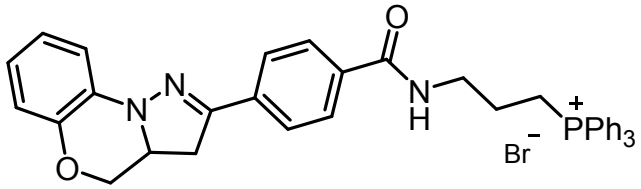


Compound **5** (0.33 g, 1 mmol), N,N-Diisopropylethylamine (DIPEA, 0.13 g, 1 mmol) and O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluorophosphate (HBTU, 1.90 g, 0.5 mmol) were dissolved in dichloromethane, and the solution was stirred at room temperature for about 30 min. A catalytic amount of DMAP was added and the solution was stirred for another 30 min. Then N-(3-aminopropyl)morpholine (0.58 g, 4 mmol) was added and stirred at room temperature overnight. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate = 1:1) to give a white powder (0.43 g, Yield: 80%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, *J* = 8.0 Hz, 2H), 8.21 (s, 1H), 7.99 (d, J = 8.0 Hz, 2H), 7.65 (s, 1H), 7.56 (d, *J* = 4.0 Hz, 1H), 7.17 (m, *J* = 12.0 Hz, 2H), 5.98 (m, 1H), 5.39 (m, 1H), 5.27 (m, 1H), 4.68 (m, *J* = 8.0 Hz, 2H), 3.74 (m, *J* = 8.0 Hz, 4H), 3.64 (m, *J* = 8.0 Hz, 2H) , 2.62 (m, *J* = 4.0 Hz, 2H), 2.55 (s, 4H); ^{13}C NMR (100 MHz,$CDCl_3$) δ 167.14, 163.99, 152.49, 136.09, 132.03, 130.17, 127.09, 126.90, 121.02, 117.81, 114.36, 69.75, 66.58, 58.17, 55.48, 53.63, 53.25, 40.21, 24.00, ESI-MS m/z, calcd for $C_{24}H_{28}N_6O_3^+$ $[M+H]^+$: 450.22; found, 449.01.

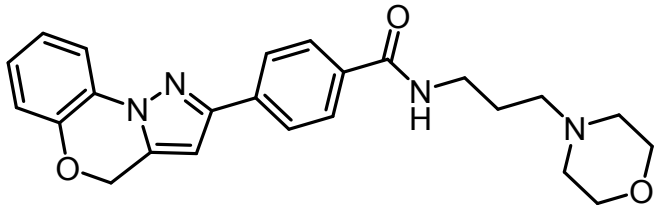
Compound Mt-Pyr:

4-(3a,4-dihydro-3H-benzo[b]pyrazolo[1,5-d][1,4]oxazin-2-yl)-N-(3-morpholinopropyl)benzamide



The compound **Mt-Tet** (35 mg, 0.05 mmol) was irradiated with a hand-held 302 nm UV lamp for 30 min in 50 mL solvent of acetonitrile/PBS = 1:1 (v/v). The solvent was removed in vacuo and further purified by silica gel column (dichloromethane/methanol = 2:1) to give an orange powder (25 mg, 74%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (d, J = 50.7 Hz, 1H), 8.35 (s, 2H), 8.08 (m, 2H), 7.94 (m, 2H), 7.85 (d, J = 5.3 Hz, 2H), 7.78 (dd, J = 22.8, 9.8 Hz, 10H), 7.58 (dd, J = 18.6, 9.0 Hz, 1H), 7.47 (m, 1H), 7.28 (m, 1H), 6.94–6.82 (m, 1H), 4.36 (m, 1H), 4.20 (s, 1H), 3.89 (d, J = 18.0 Hz, 2H), 2.91 (m, 1H), 2.80 (s, 1H), 2.11 (m, 1H), 1.87 (d, J = 31.9 Hz, 2H), 1.64 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.81, 167.42, 149.66, 144.83, 136.33, 135.87, 131.39, 130.39, 130.09, 126.38, 125.95, 124.70, 123.25, 122.15, 121.98, 120.09, 117.44, 100.00, 60.22, 31.61, 30.30, 21.22, 14.55. ESI-MS m/z, calculated for $C_{38}H_{35}N_3O_2P^+$ [M-Br]$^+$: 596.69; found, 596.20.

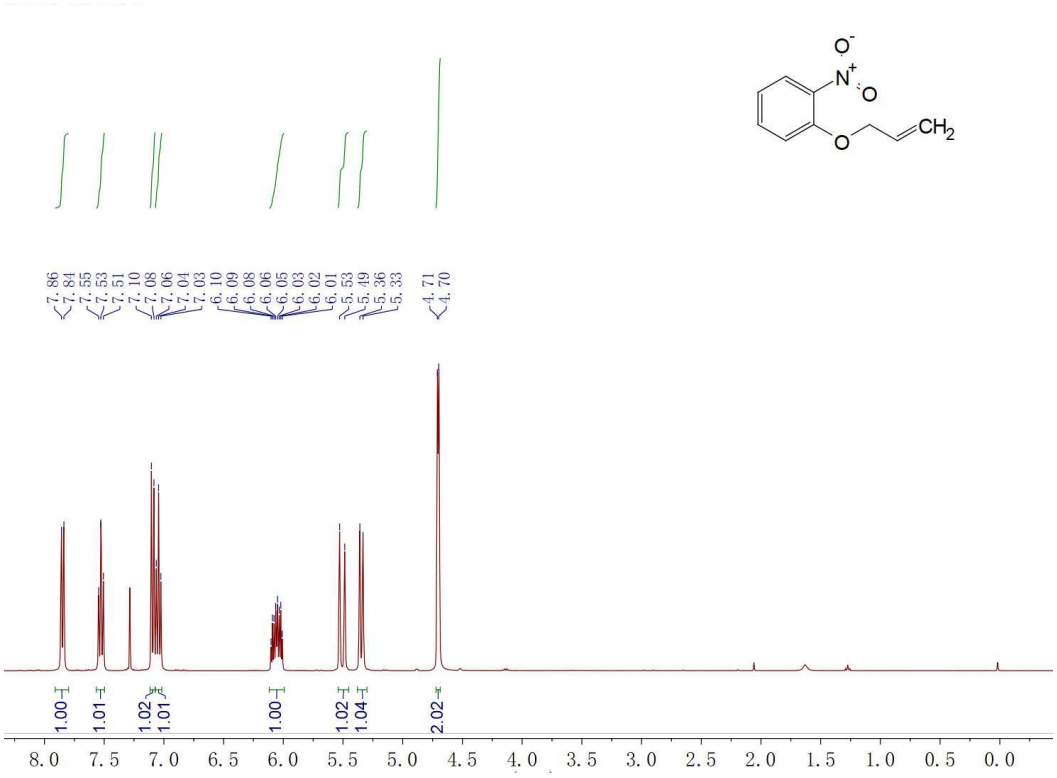
Compound Ly-Pyr: (3-(4-(3a,4-dihydro-3H-benzo[b]pyrazolo[1,5-d][1,4]oxazin-2-yl)benzamido)propyl)triphenylphosphonium



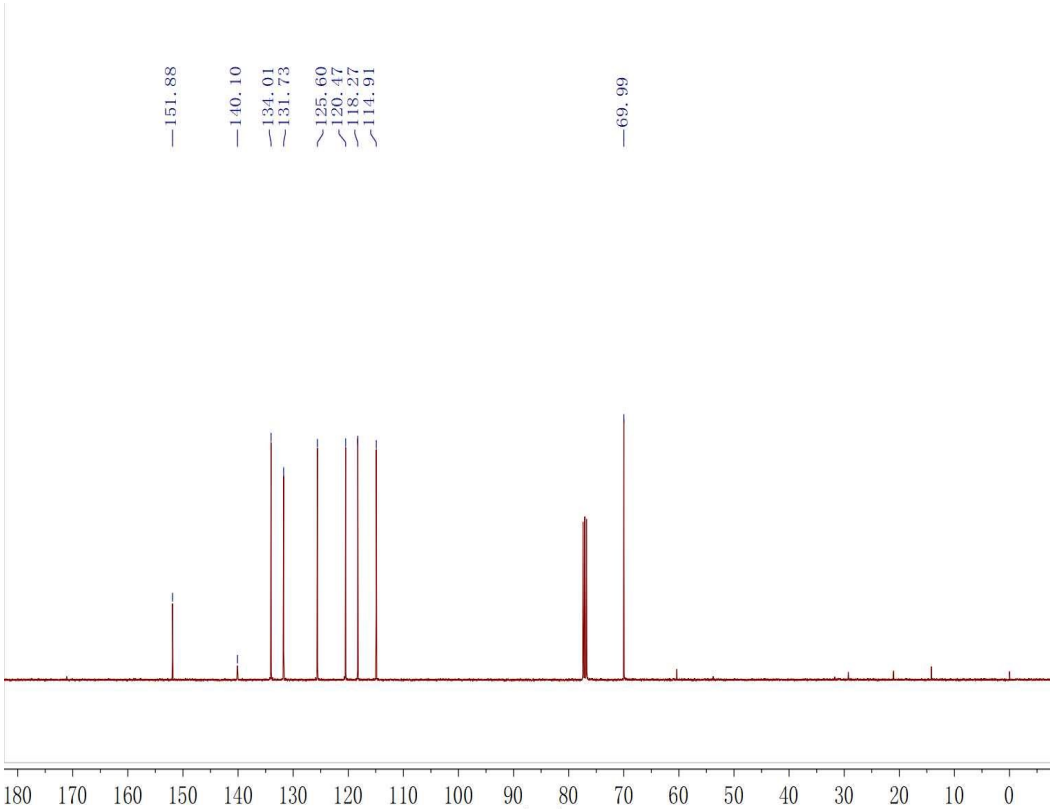
The compound **Ly-Tet** (36 mg, 0.8 mmol) was irradiated with a hand-held 302 nm UV lamp for 30 min in 50 mL solvent of acetonitrile/PBS = 1:1 (v/v). The solvent was removed in vacuo and the aqueous layer was extracted with ethyl acetate and further purified by silica gel column (petroleum ether/ethyl acetate = 1:2) to give a yellow powder

(28 mg, 83%). 1H NMR (400 MHz, DMSO-d_6) δ 8.78 (s, 1H), 7.94 (d, J = 7.9 Hz, 2H), 7.77 (d, J = 7.8 Hz, 2H), 7.45 (d, J = 7.7 Hz, 1H), 7.05 (m, 3H), 4.31 (d, J = 10.7 Hz, 1H), 4.21 (s, 1H), 3.82 (s, 4H), 3.53 (m, 2H), 3.22 (d, J = 4.9 Hz, 2H), 3.18 (d, J = 4.6 Hz, 1H), 1.94 (s, 4H), 1.23 (s, 2H), 0.84 (d, J = 6.3 Hz, 2H). 13C NMR (100 MHz, DMSO-d_6) δ 165.90, 163.94, 152.69, 142.47, 137.01, 133.20, 129.30, 128.67, 127.76, 126.87, 126.19, 121.44, 117.61, 115.05, 107.34, 69.35, 66.53, 56.44, 53.70, 38.28, 26.25. ESI-MS m/z, calcd for $C_{24}H_{28}N_4O_3^+$ $[M+H]^+$: 421.51; found, 421.18.

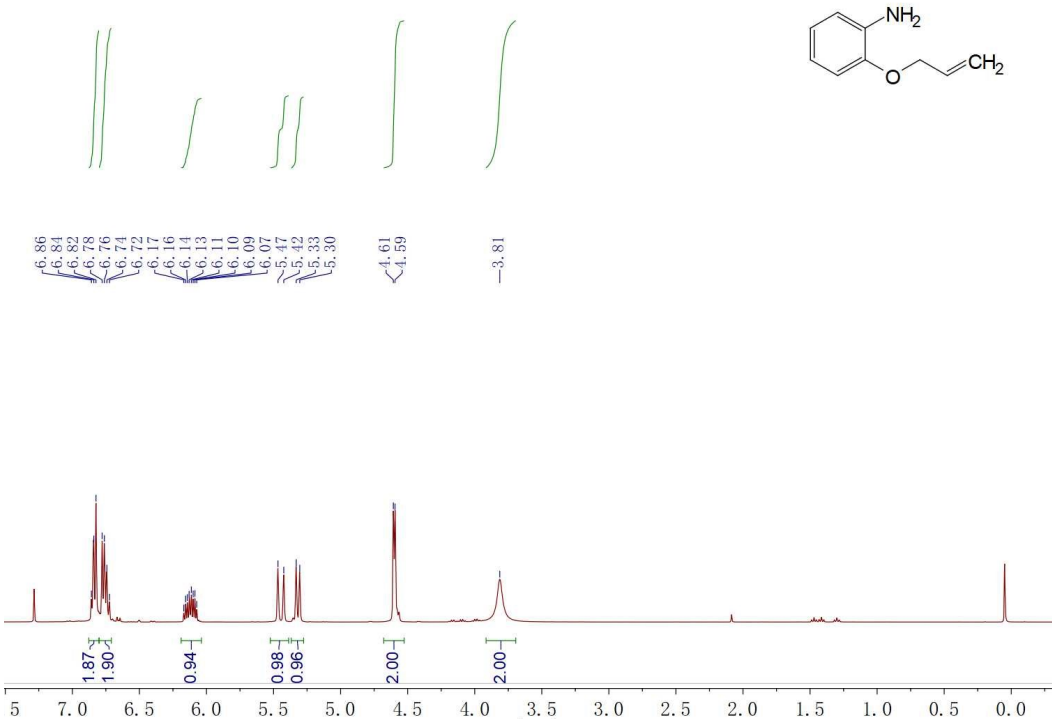
NMR spectra of nine compounds



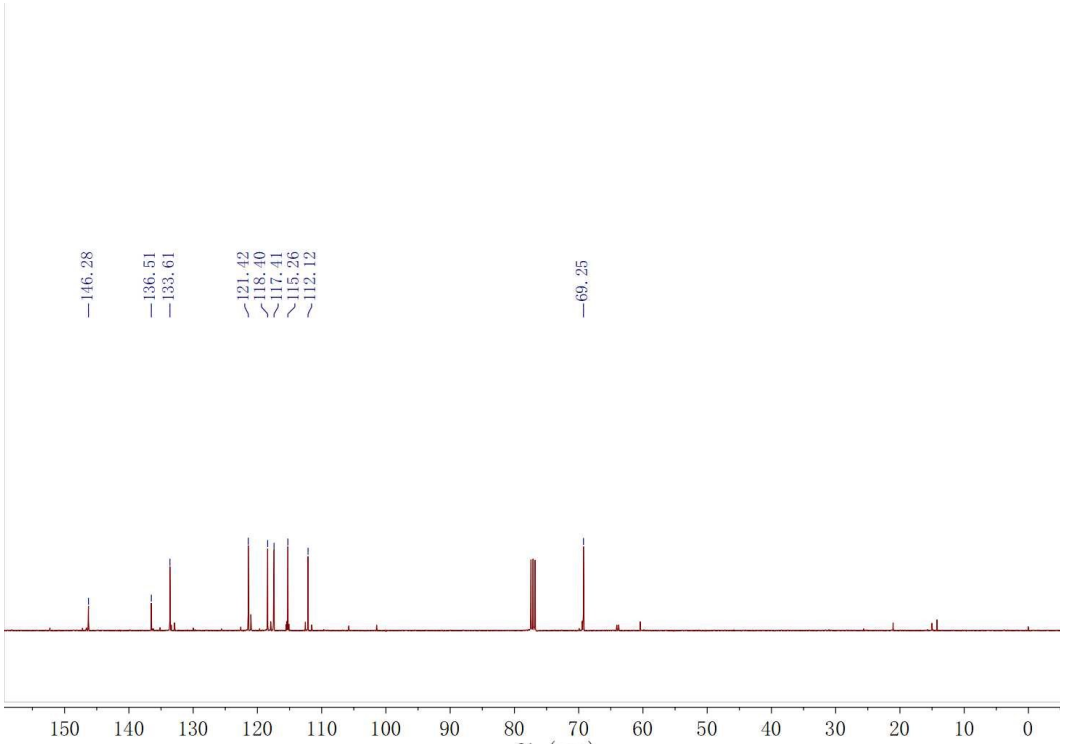
^{1}H NMR spectrum (400.1 MHz, $CDCl_3$) of compound **1**.



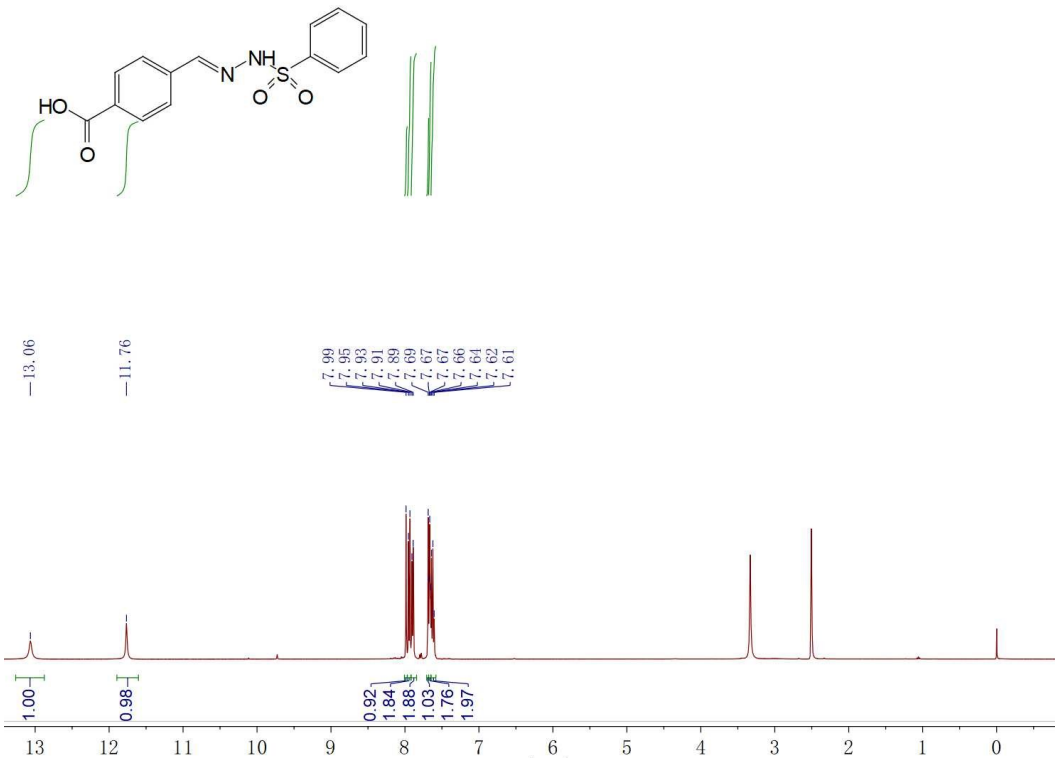
^{13}C NMR spectrum (400.1 MHz, $CDCl_3$) of compound **1**.



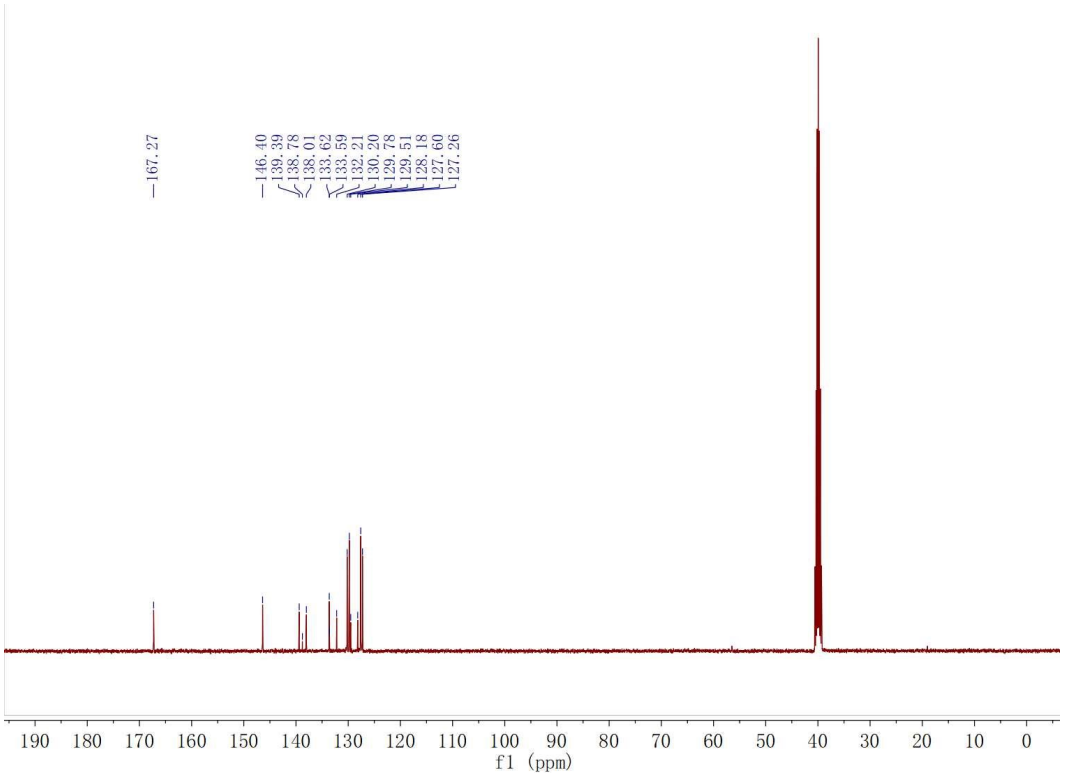
^{1}H NMR spectrum (400.1 MHz, CDCl3) of compound **2**.



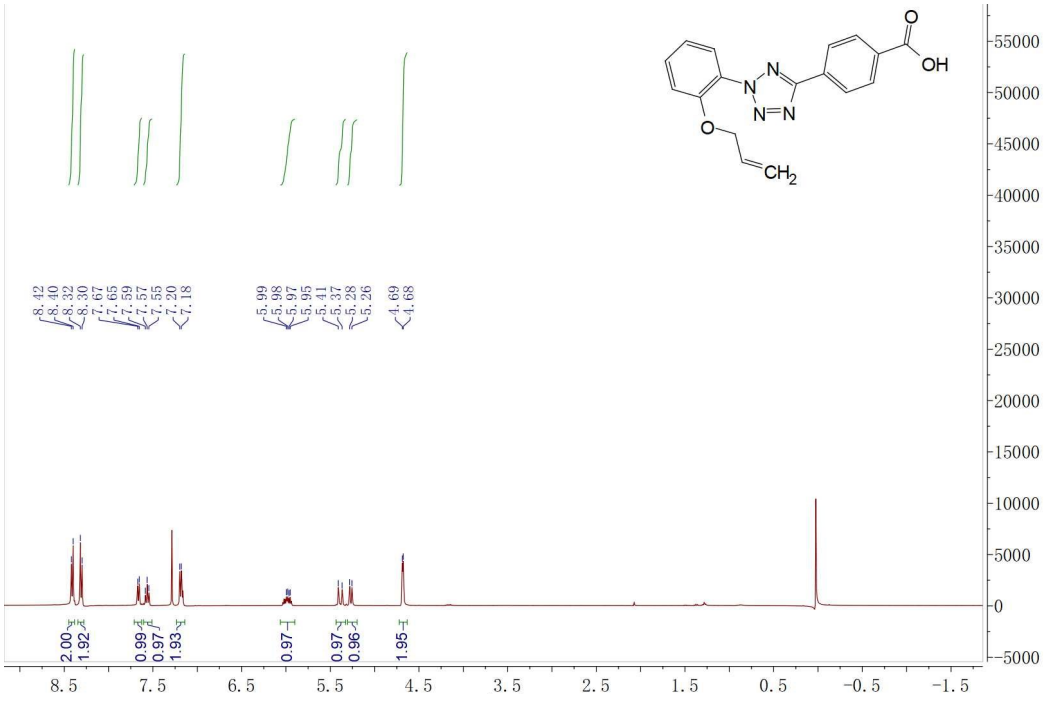
^{13}C NMR spectrum (400.1 MHz, $CDCl_3$) of compound **2**.



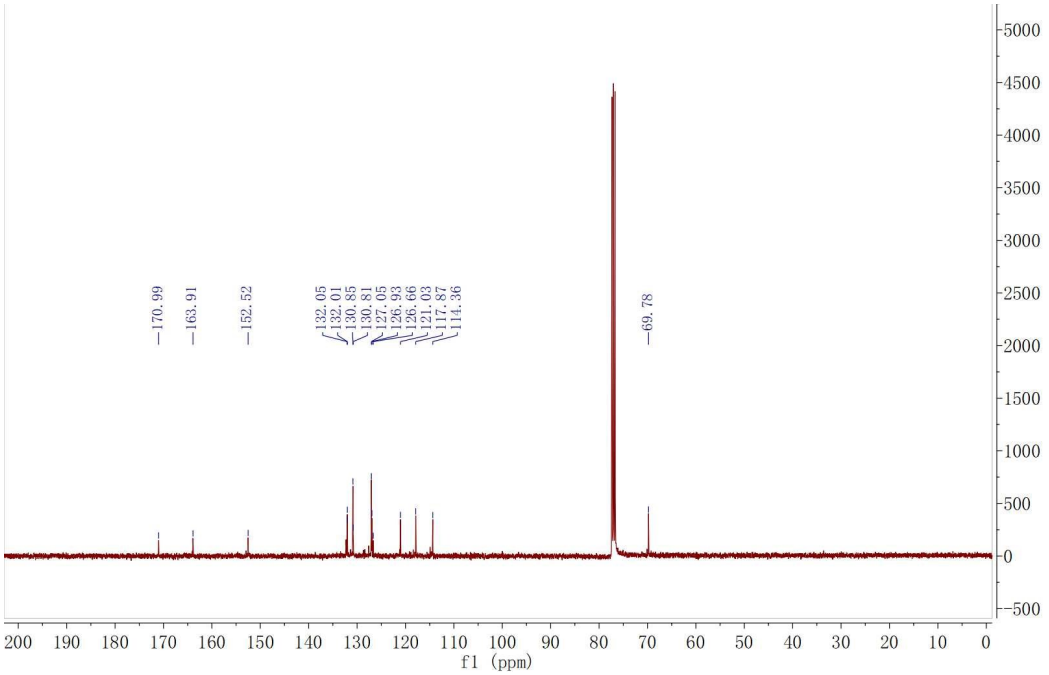
^{1}H NMR spectrum (400.1 MHz, DMSO-d_6) of compound **4**.



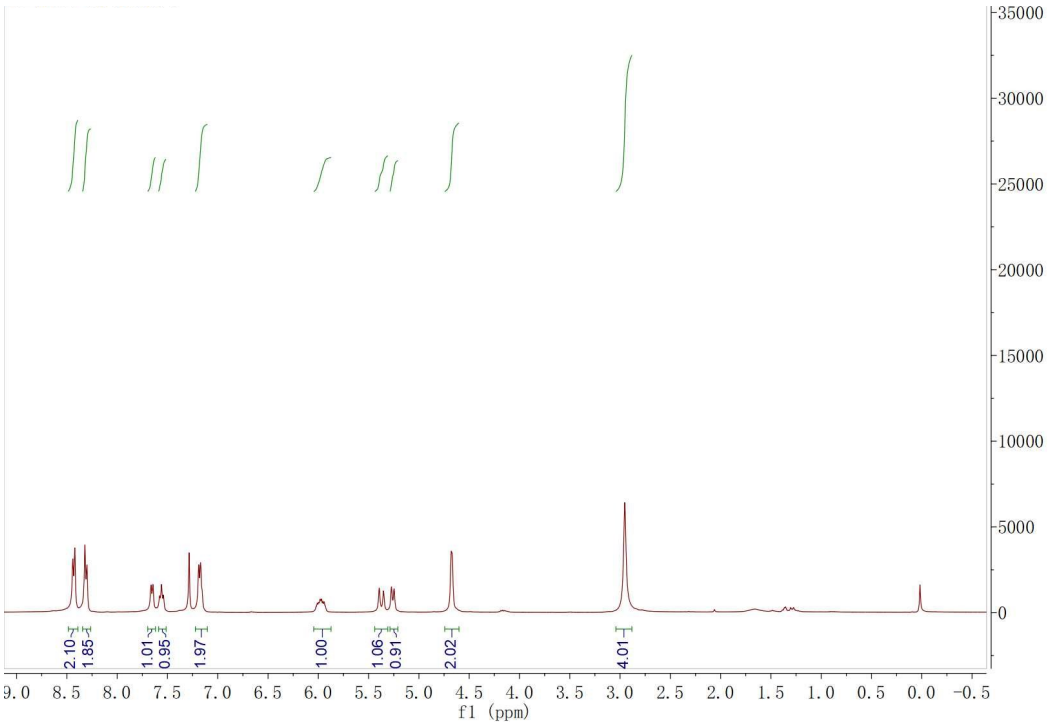
^{13}C NMR spectrum (400.1 MHz, DMSO-d_6) of compound **4**.



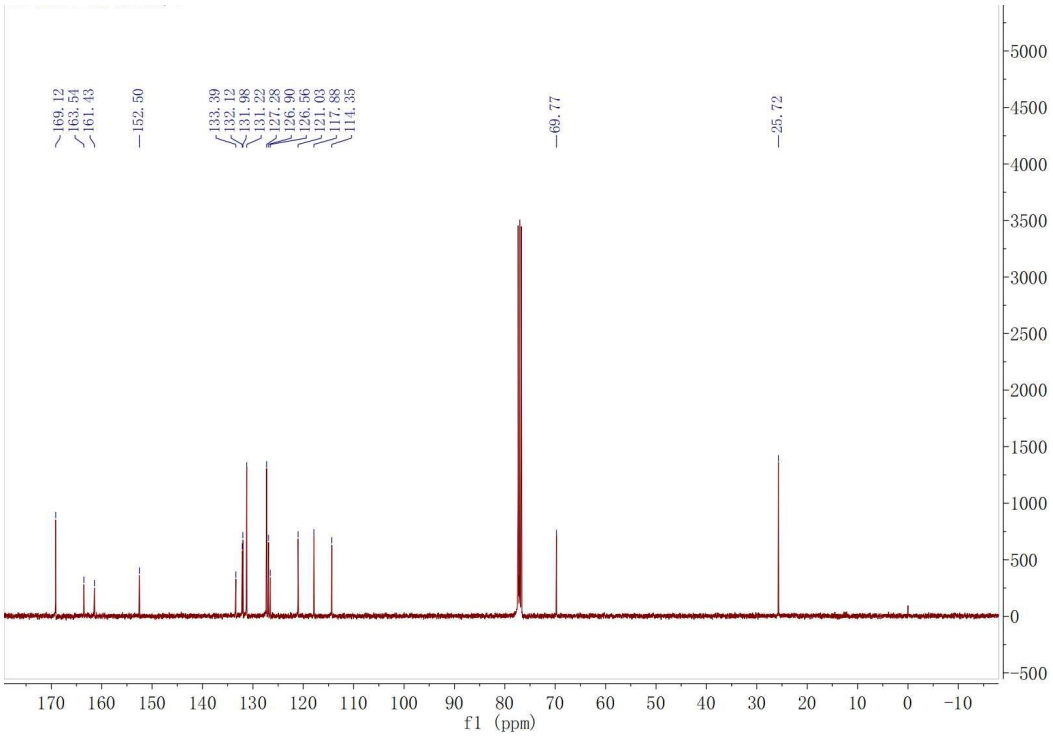
^{1}H NMR spectrum (400.1 MHz, $CDCl_3$) of compound **5**.



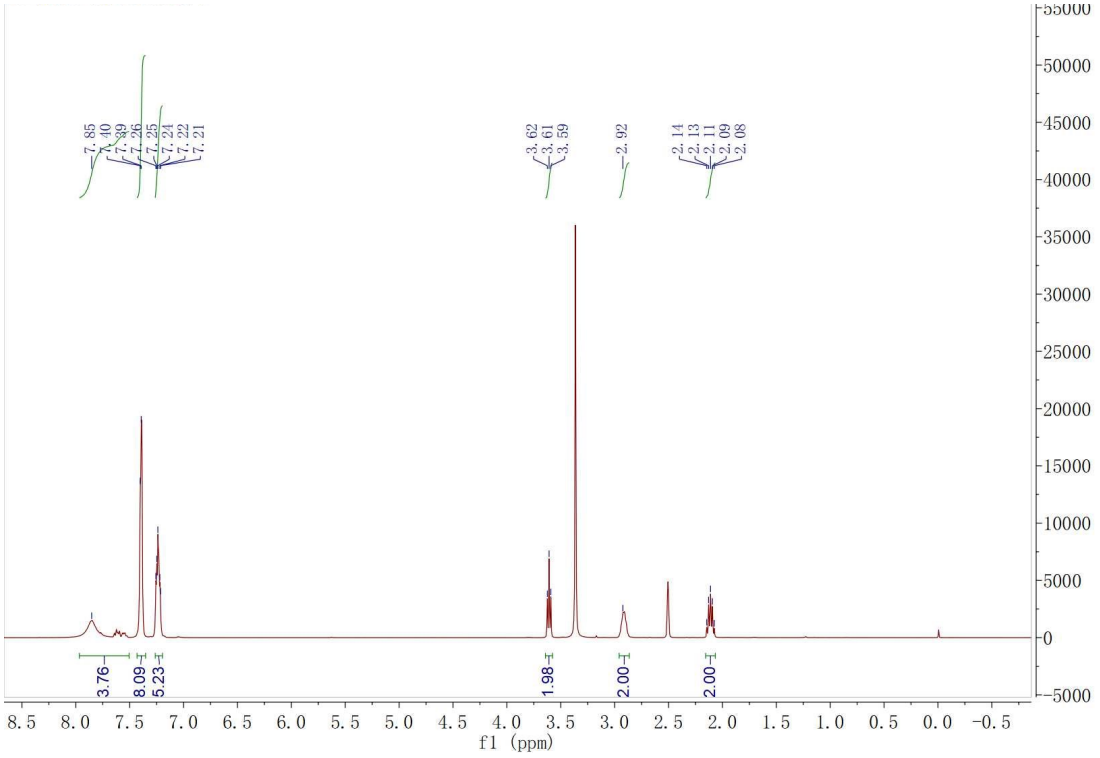
^{13}C NMR spectrum (400.1 MHz, $CDCl_3$) of compound **5**.



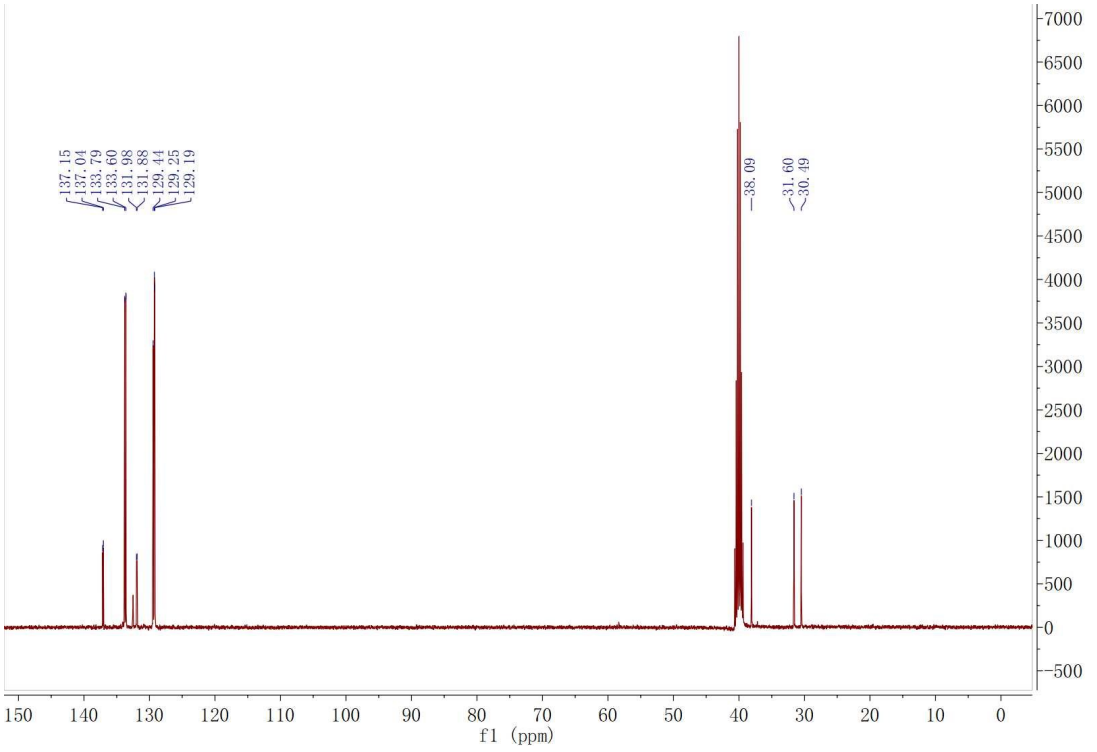
^{1}H NMR spectrum (400.1 MHz, $CDCl_3$) of compound **6**.



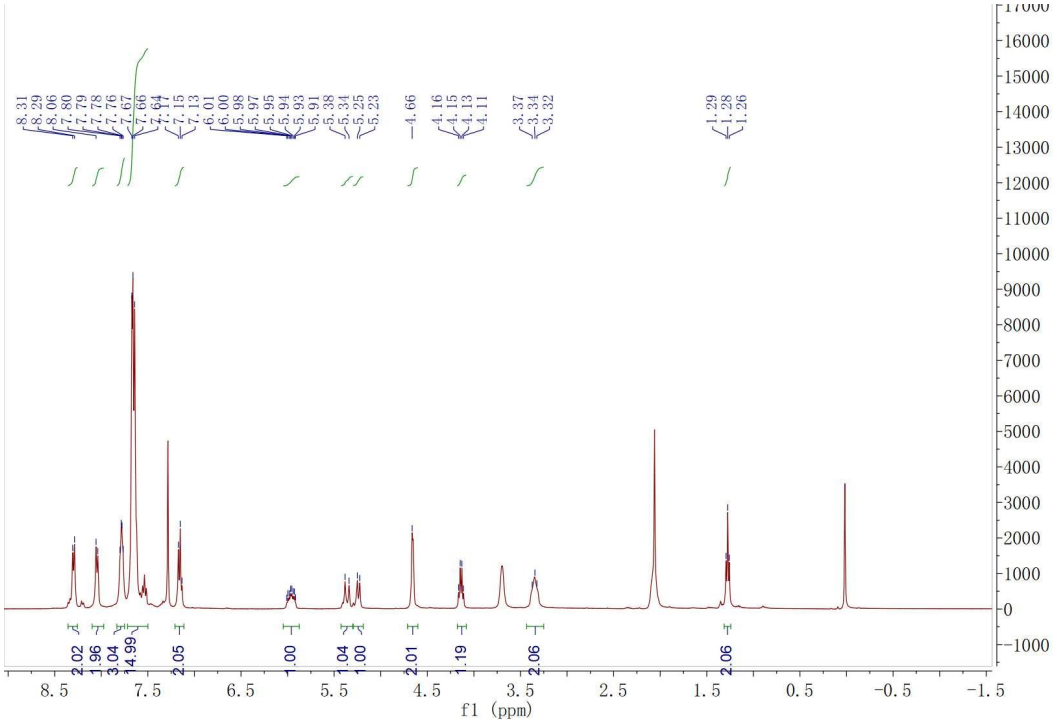
^{13}C NMR spectrum (400.1 MHz, $CDCl_3$) of compound **6**.



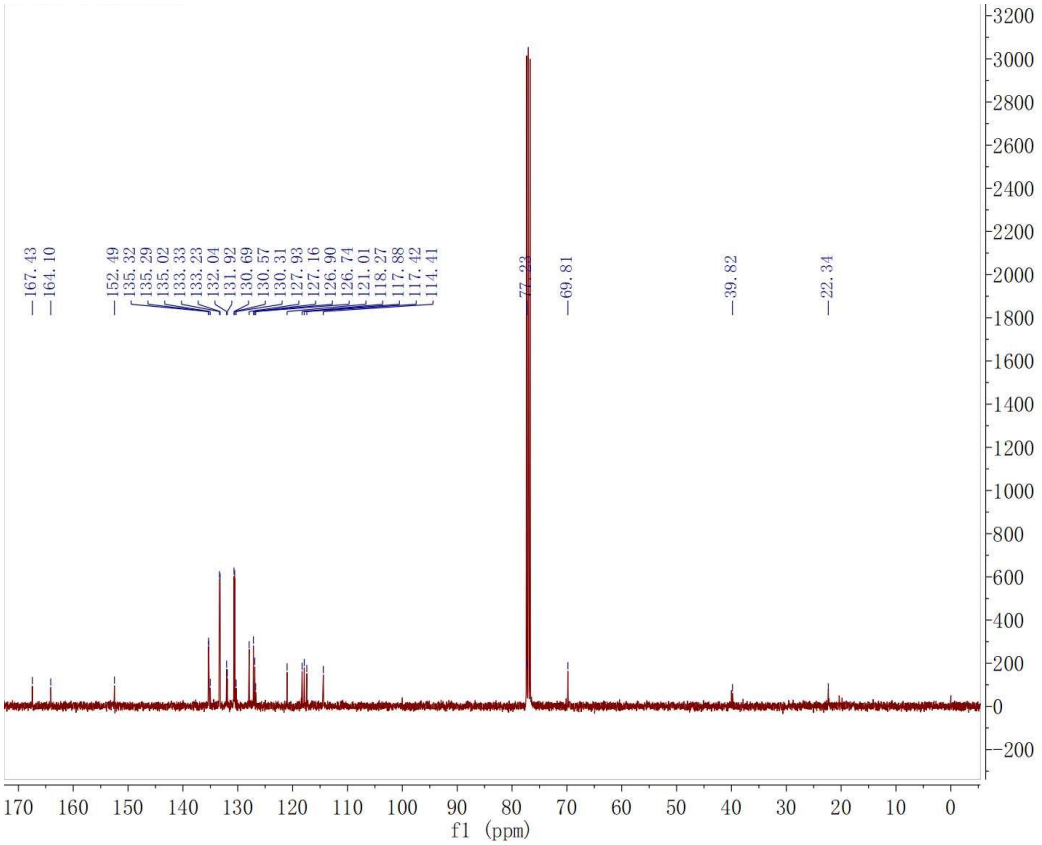
^{1}H NMR spectrum (400.1 MHz, DMSO-d_6) of compound **7**.



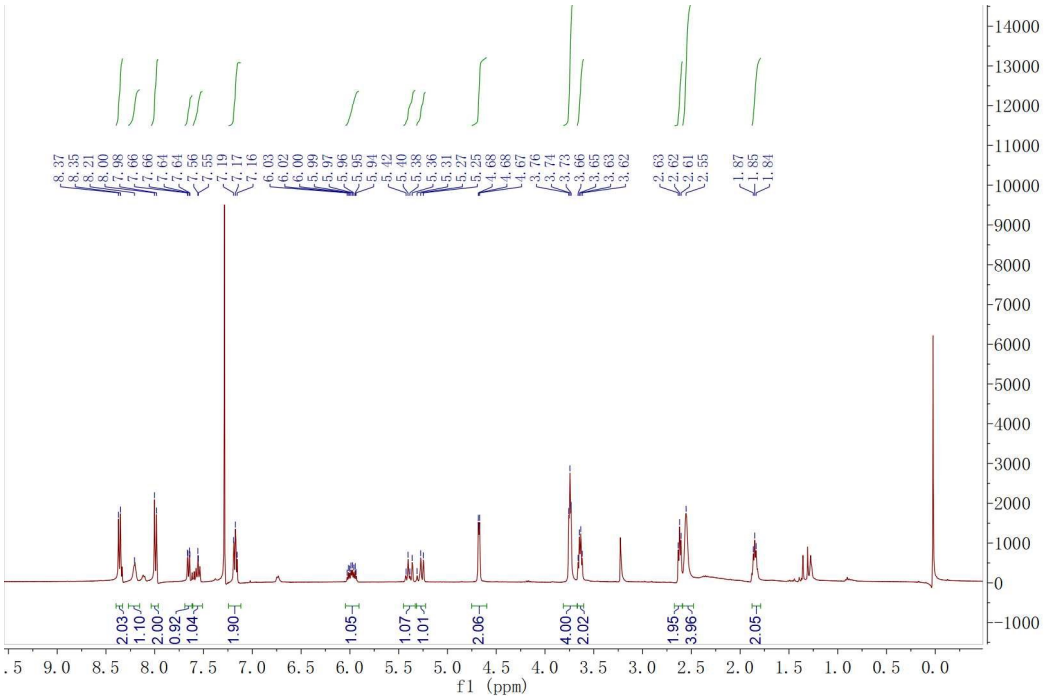
^{13}C NMR spectrum (400.1 MHz, DMSO-d_6) of compound **7**.



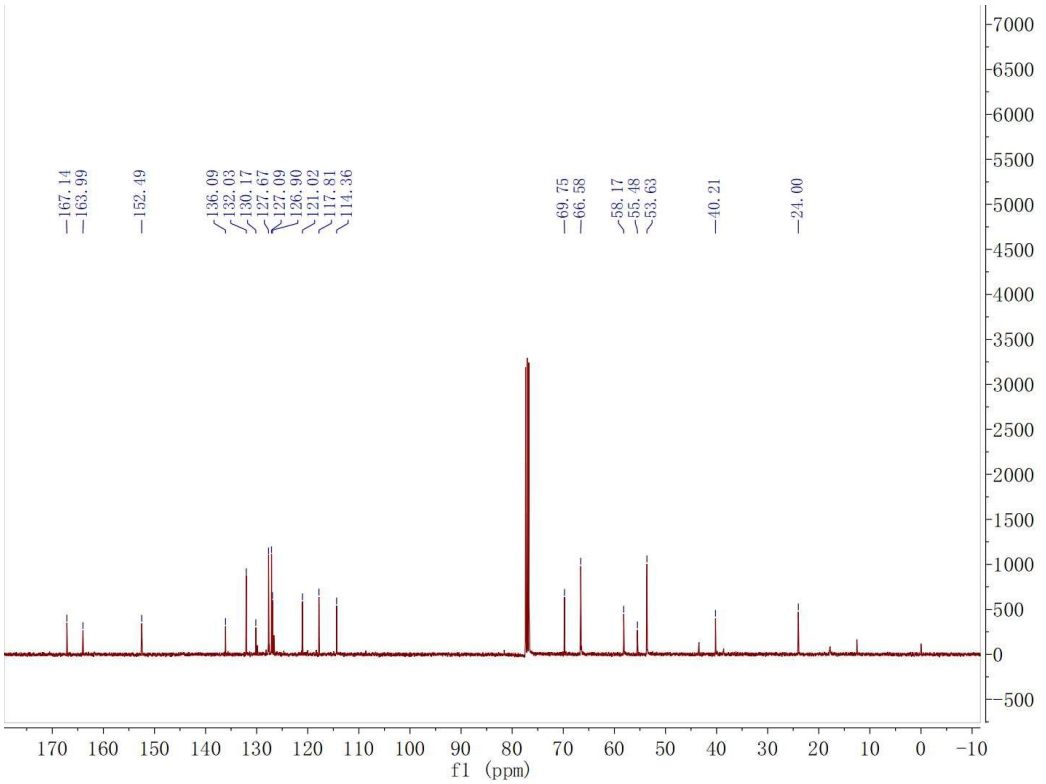
^{1}H NMR spectrum (400.1 MHz, $CDCl_3$) of **Mt-Tet**.



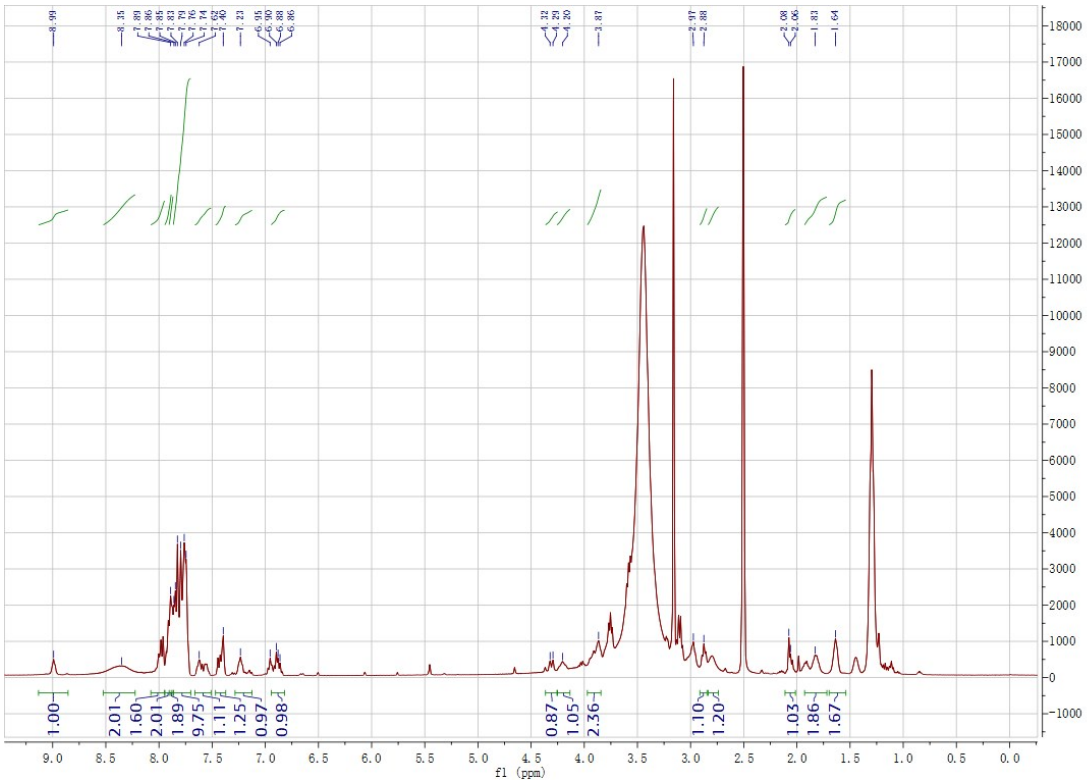
^{13}C NMR spectrum (400.1 MHz, $CDCl_3$) of **Mt-Tet**.



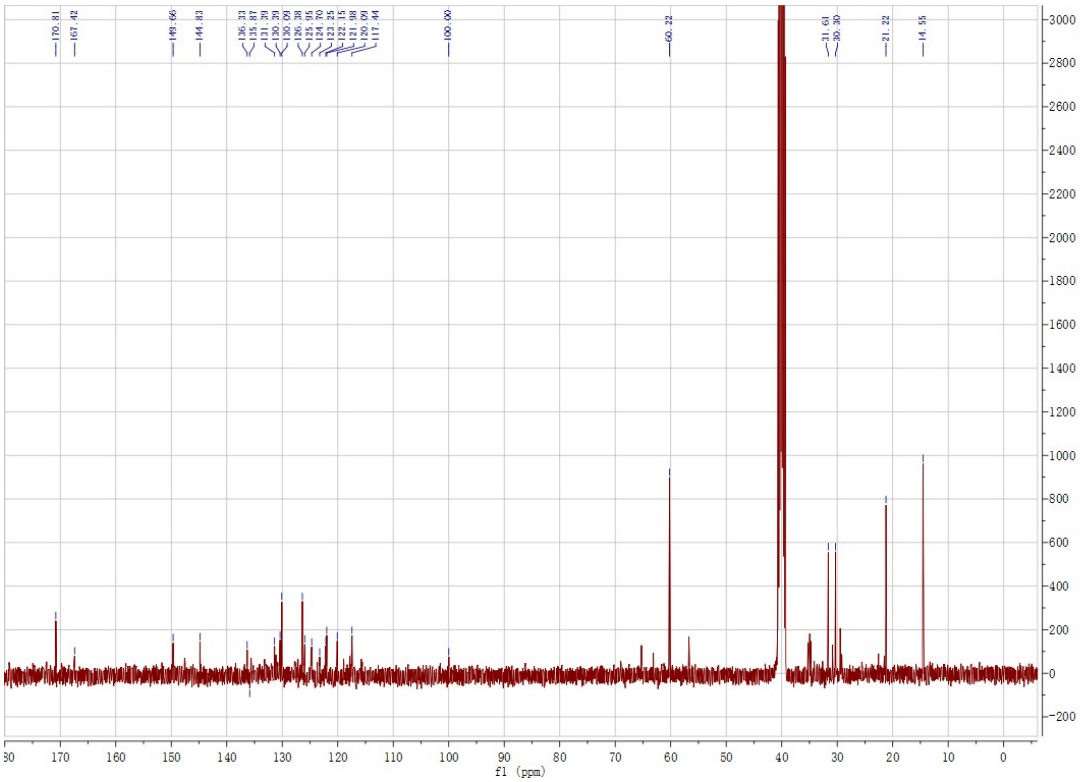
^{1}H NMR spectrum (400.1 MHz, $CDCl_3$) of **Ly-Tet**.



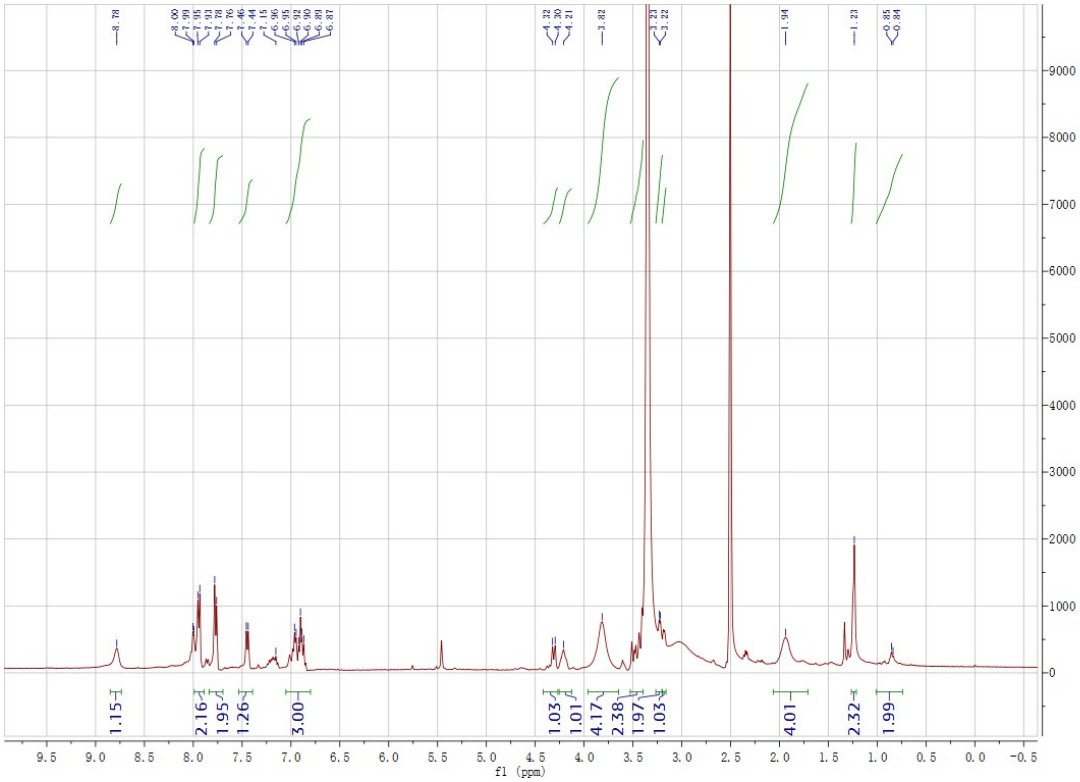
^{13}C NMR spectrum (400.1 MHz, $CDCl_3$) of **Ly-Tet**.



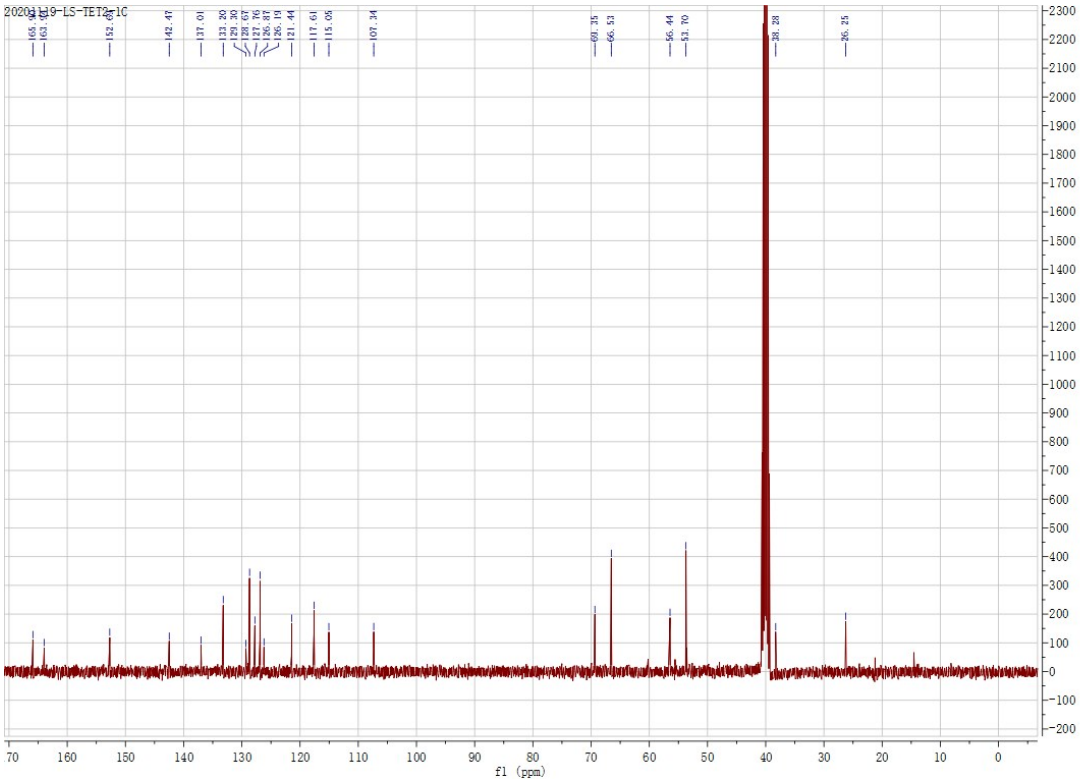
^{1}H NMR spectrum (400.1 MHz, $CDCl_3$) of **Mt-Pyr**.



^{13}C NMR spectrum (400.1 MHz, $CDCl_3$) of **Mt-Pyr**.

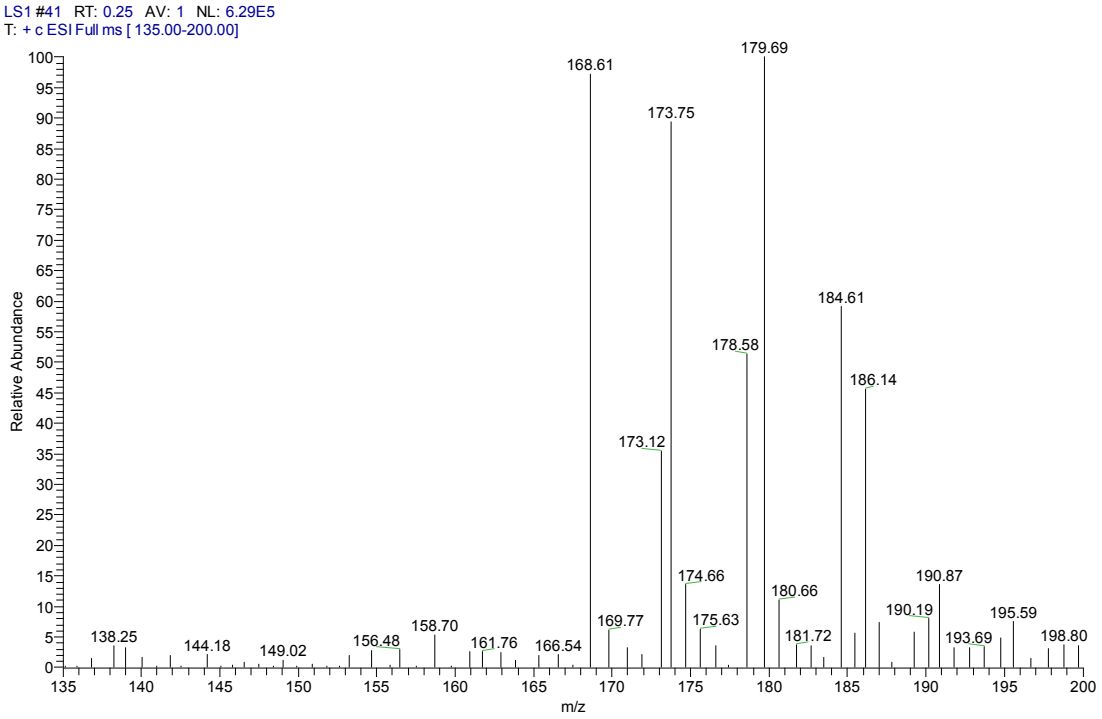


^{1}H NMR spectrum (400.1 MHz, $CDCl_3$) of **Ly-Pyr**.

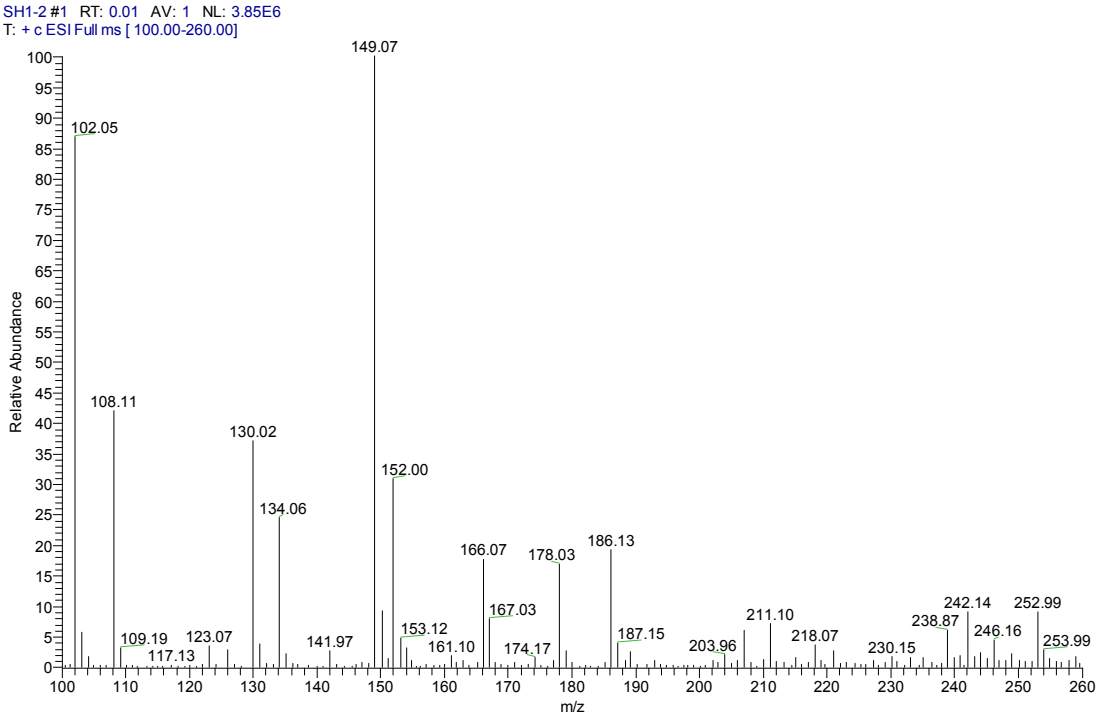


^{13}C NMR spectrum (400.1 MHz, $CDCl_3$) of **Ly- Pyr**.

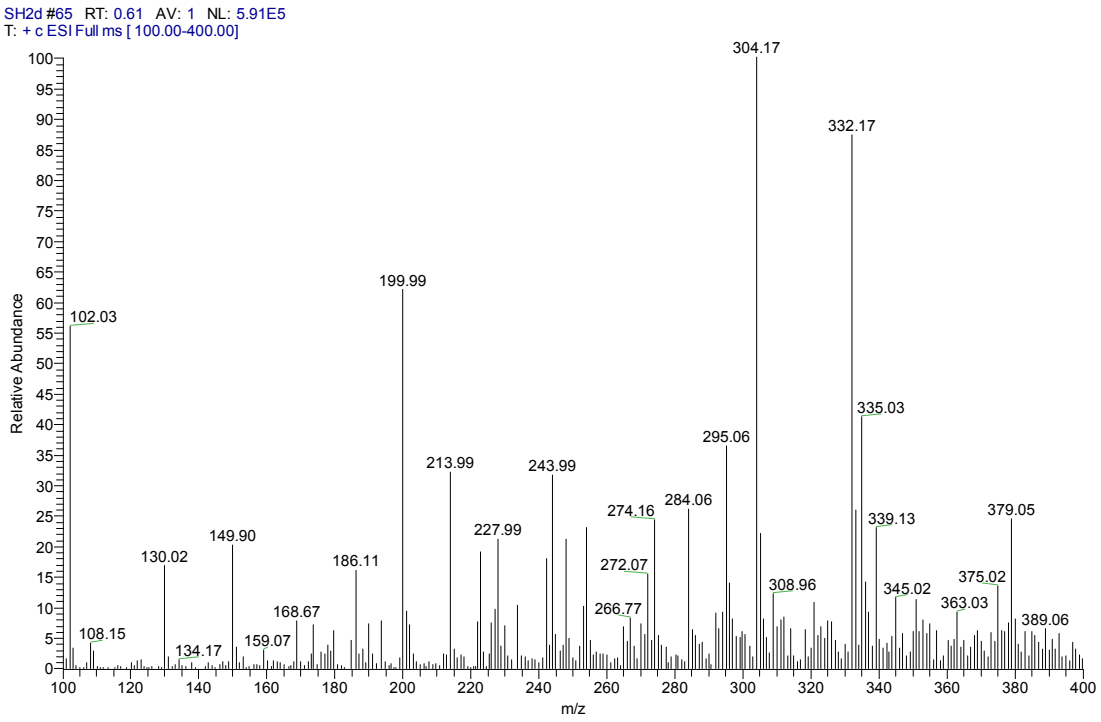
ESI mass spectrum



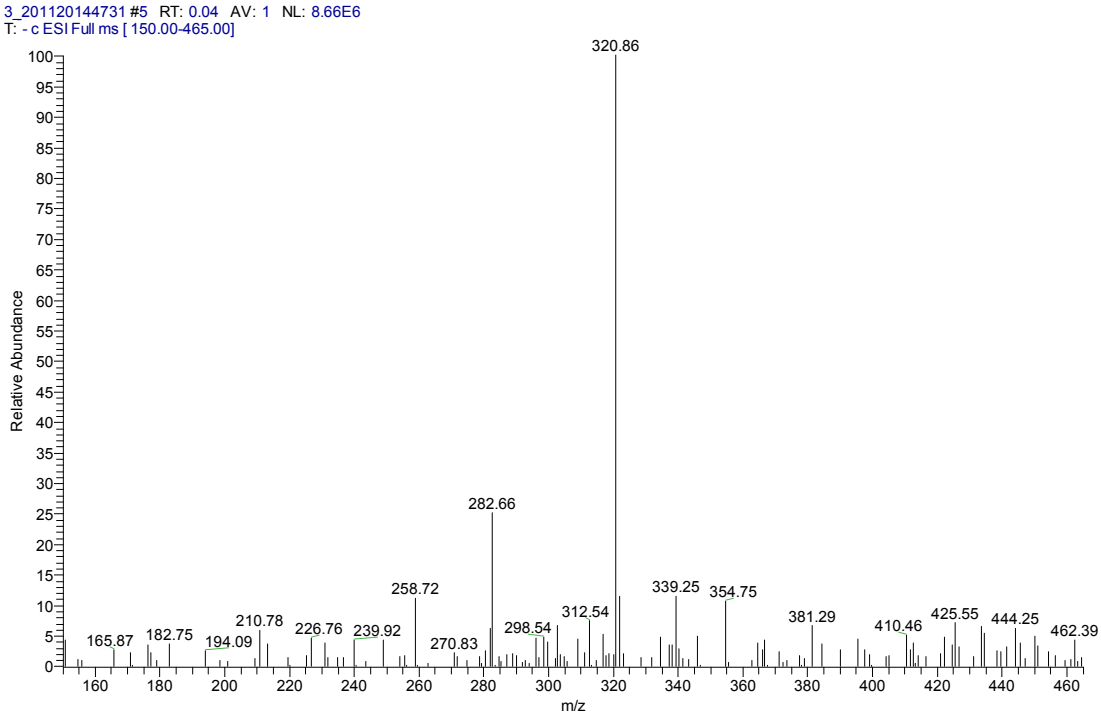
ESI mass spectrum of compound **1**.



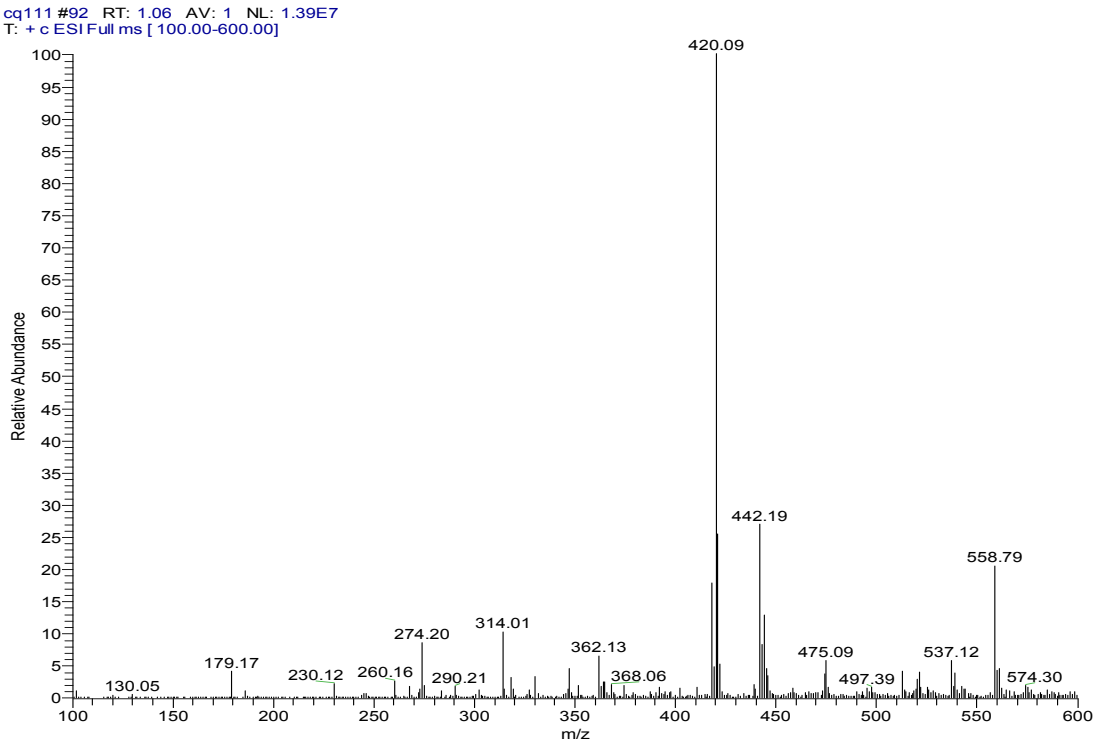
ESI mass spectrum of compound **2**.



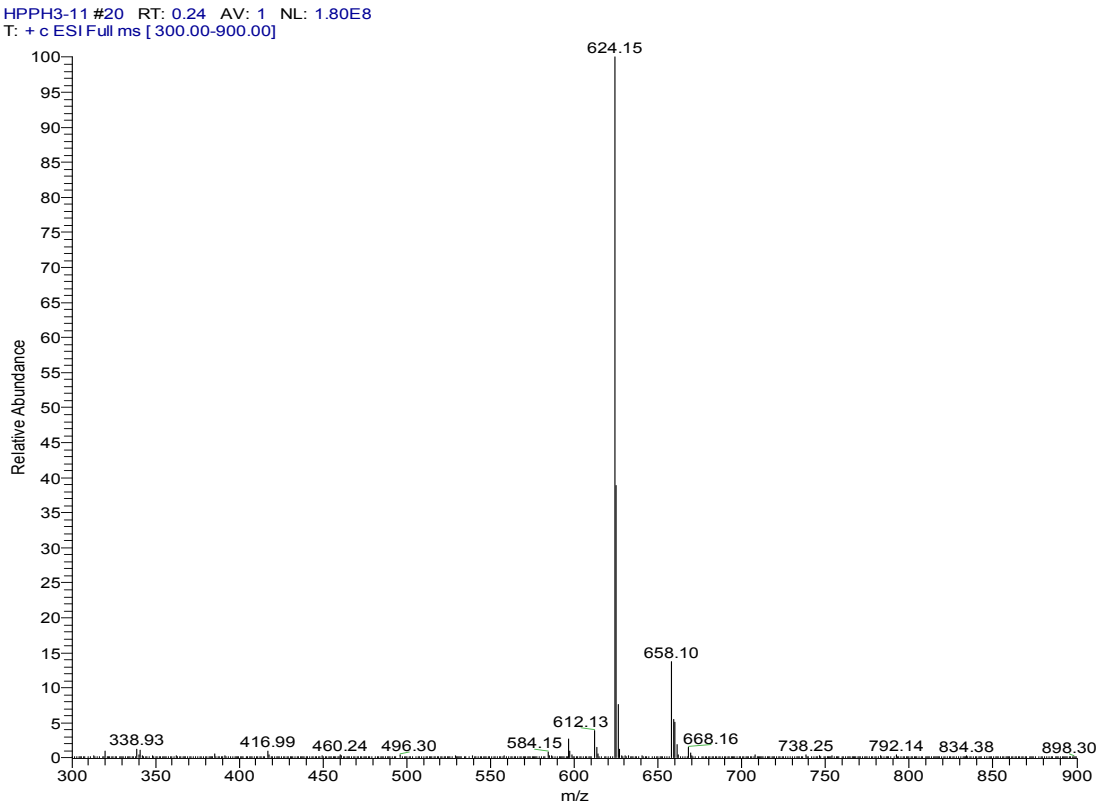
ESI mass spectrum of compound **4**.



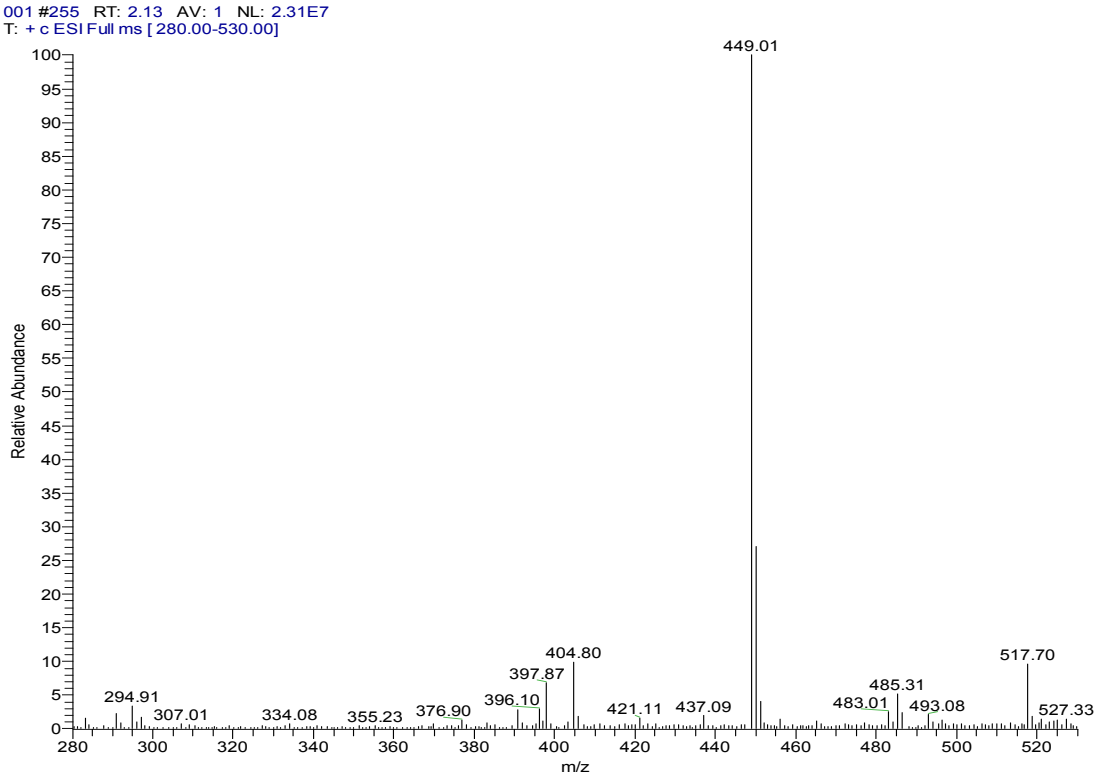
ESI mass spectrum of compound **5**.



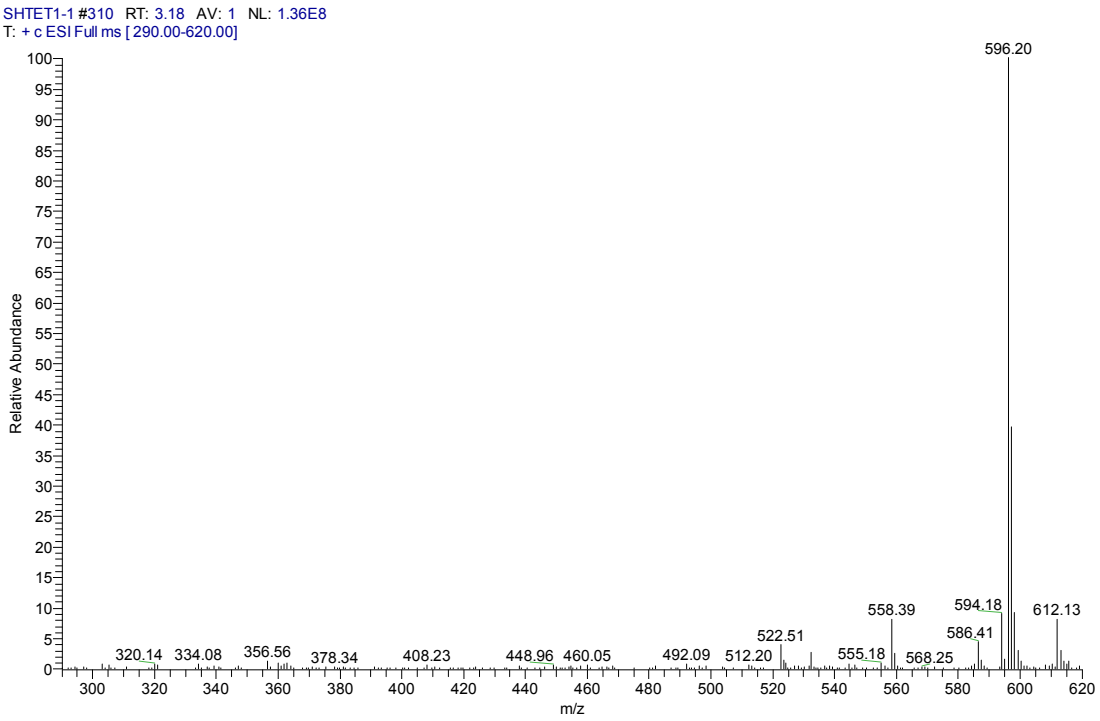
ESI mass spectrum of compound **6**.



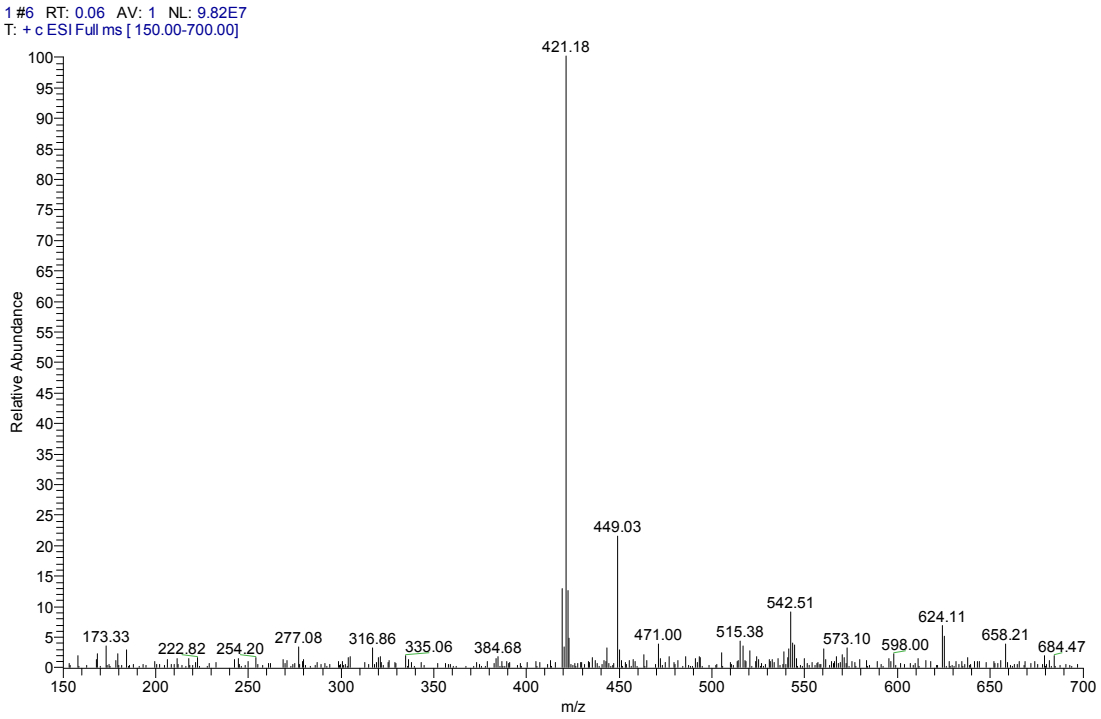
ESI mass spectrum of **Mt-Tet**.



ESI mass spectrum of **Ly-Tet**.



ESI mass spectrum of **Mt-Pyr**.



ESI mass spectrum of **Ly-Pyr**.

HPLC monitoring

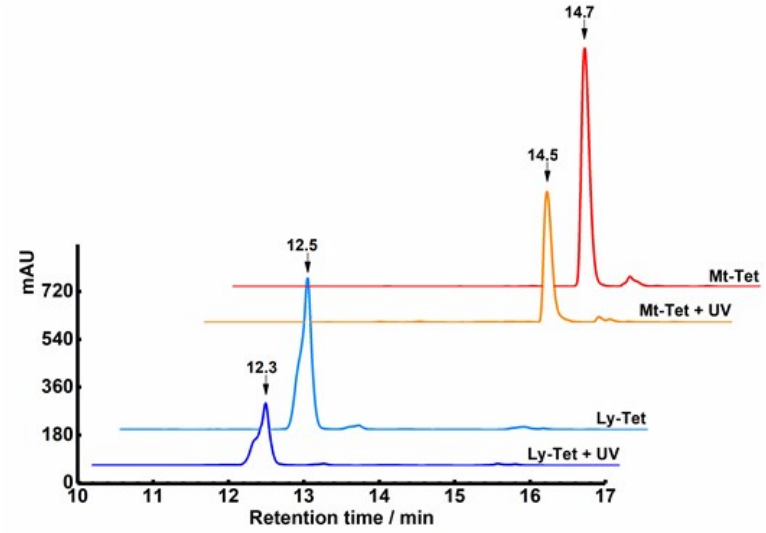


Figure S1. HPLC monitoring of the transformation of Mt-Tet to Mt-Pyr and Ly-Tet to Ly-Pyr with a hand-held 302 nm UV lamp for 5 min in CH_3CN/PBS = 1:1 (v/v). HPLC analysis was performed on an Agilent 1260 Infinity HPLC, equipped with a 4.6X250 mm, 5 µm, 100 Å C18 Aquasil column. The gradient was started at 95% H_2O (0.03% CF_3COOH) – 5% acetonitrile with a flow rate of 1 mL/min. The gradient was maintained for 4 min and then changed to 80% H_2O – 20% acetonitrile over 1 min, 50% H_2O – 50% acetonitrile over 25 min, 5% H_2O – 95% acetonitrile over 3 min, 95% H_2O – 5% acetonitrile over 1 min.

Cell viability assay

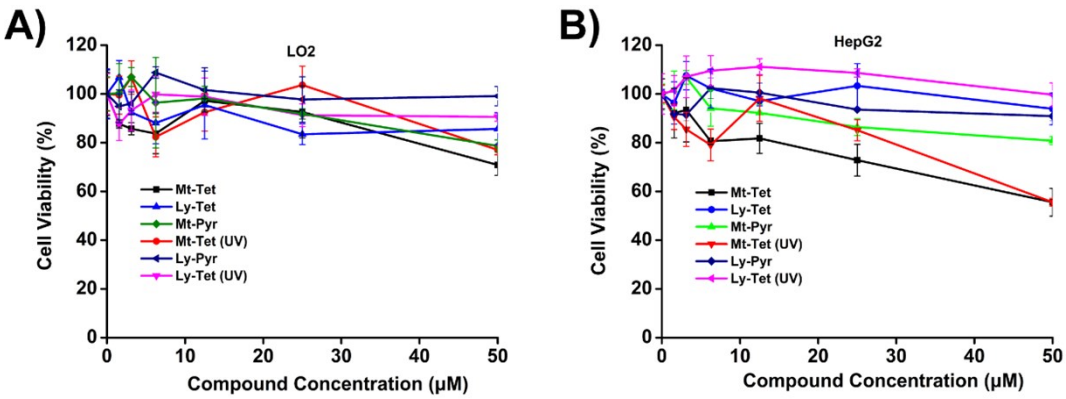


Figure S2. Cell viability assay of (A) LO2 and (B) HepG2 cells treated with **Mt-Tet**, **Ly-Tet**, **Mt-Tet** (302 nm UV), **Ly-Tet** (302 nm UV), **Mt-Pyr** and **Ly-Pyr** for 24 h at varied concentrations.

Fluorescence imaging and colocalization of compound 5

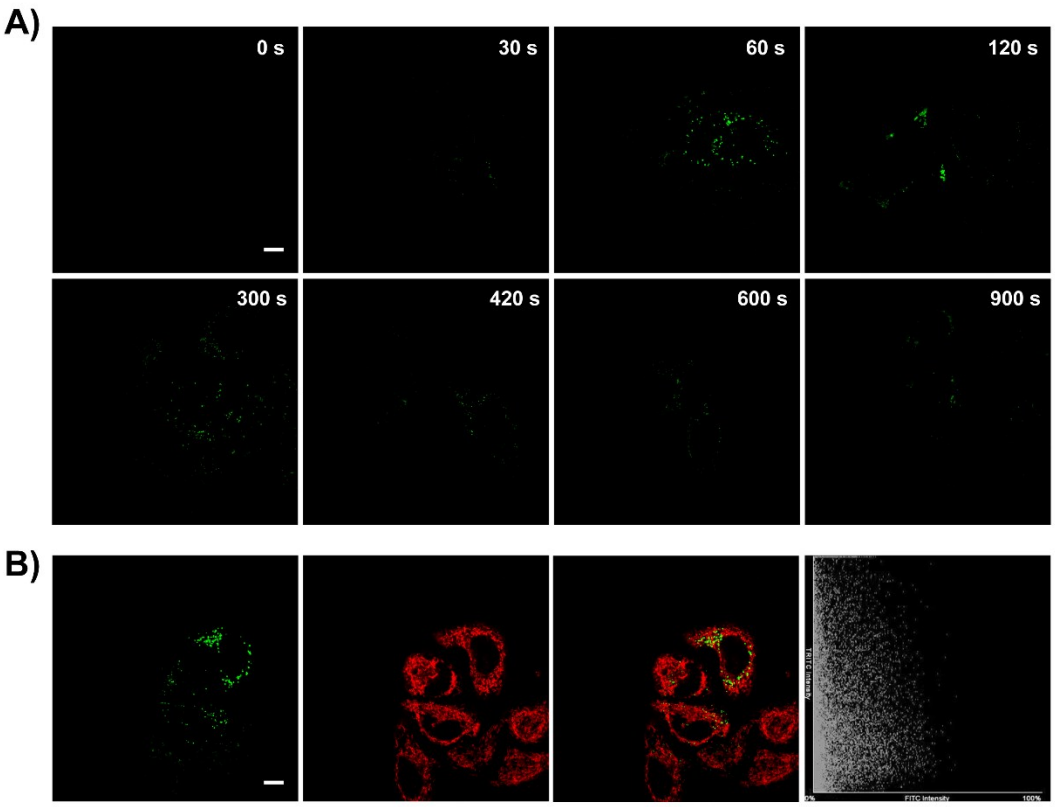


Figure S3. (A) Effect of UV exposure times (0, 30, 60, 120, 300, 420, 600 and 900 s) on the fluorescence imaging of compound **5 (**10 μM**)**. (B) Colocalization assay of compound **5** and MitoTracker® Red CMXRos. Scale bar: 10 μm.

Photostability study of MitoTracker and LysoTracker

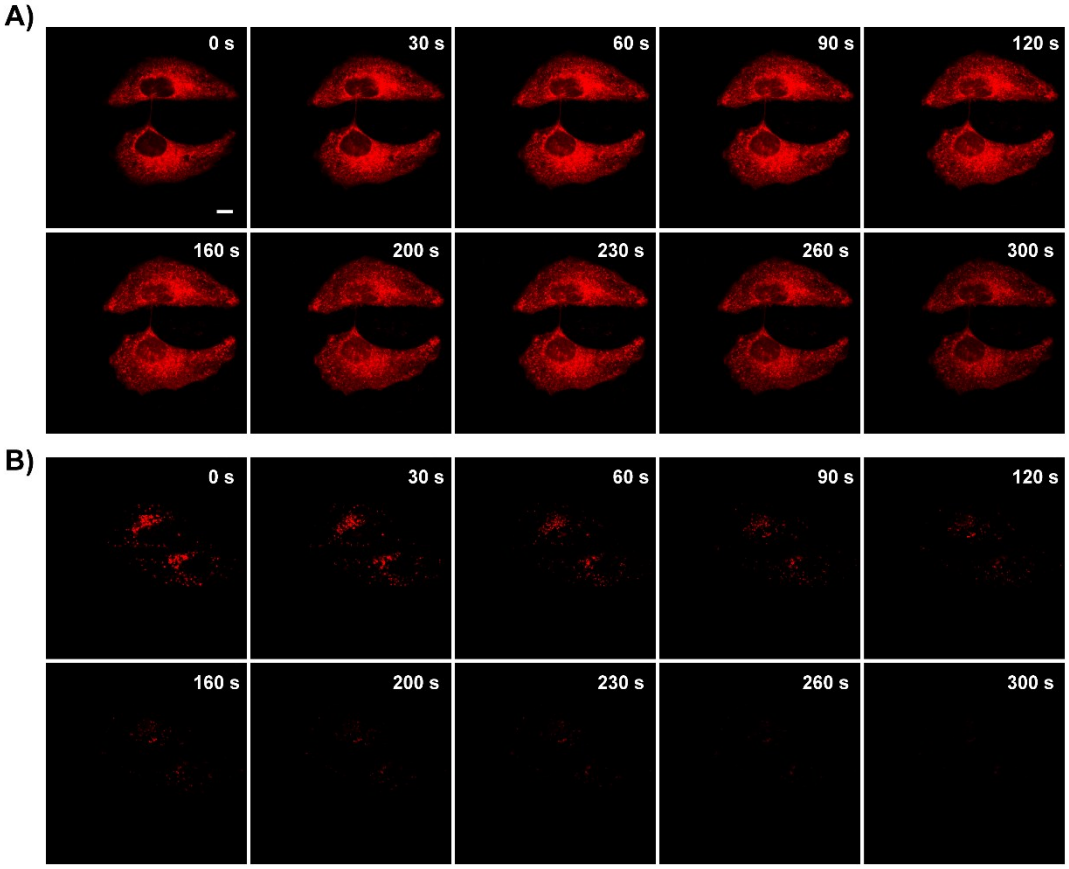


Figure S4. Fluorescence images of HepG2 cells treated with MitoTracker® Red CMXRos and LysoTracker™ Red DND-99 acquired at different times under successive excitation (561 nm laser). Scale bar: 10 μm.

Table S1. Photophysical properties of the probes

	compound **5**	compound **5** + UV	Mt-Tet	Mt-Pyr	Ly-Tet	Ly-Pyr
λ_{ex} (nm)	375	375	352	352	340	340
λ_{em} (nm)	-	557	-	570	-	560
ε (M^{-1} cm^{-1})	1.54×10^4	2.51×10^4	5.65×10^3	3.01×10^4	1.15×10^4	2.51×10^4
QY (%)	ND	9.14	ND	20.08	ND	27.90

References

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2. M. He, J. Li, S. Tan, R. Wang and Y. Zhang, Journal of the American Chemical Society, 2013, 135, 18718-18721.
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