Electronic Supplementary Material (ESI) for Analyst.

Fluorescence Probe for Real-Time Malonaldehyde Detection in epilepsy model

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1. Supporting tables

Table S1. Abbreviation Index

Abbreviations	Full name
Ala	Alanine
CCK-8	Cell Counting Kit-8
CLSM	Confocal Laser Scanning Microscopy
CTAB	Cetrimonium Bromide
DL	detection limit
DMEM	Dulbecco's Modified Eagle Medium
DMSO	dimethyl sulfoxide
EA	ethyl acetate
FBS	fetal bovine serum
FQY	fluorescence quantum yield
GC	gas chromatography
Gly	Glycine
GSH	Glutathione
Hcy	Homocysteine
HPLC	high-performance liquid chromatography
KA	kainic acid
MDA	malondialdehyde
MS	mass spectrometry
PBS	Phosphate Buffer Saline
PC12	Pheochromocytoma cells-12
PE	petroleum ether
ROS	reactive oxygen species
TBA	thiobarbituric acid
TLC	thin—layer chromatography
Val	Valine

Table S2. Chemicals Index

Chemicals	Source
2-(3,5,5-trimethylcyclohex-2-en-1-ylidene) malononitrile	Energy Chemical
4-amino-3-nitrobenzaldehyde	Energy Chemical
ethanol	XiLONG SCIENTIFIC
pyridine	Sinopharm Chemical Reagent Co.,Ltd
iron powder	Energy Chemical
hydrochloric acid(HCl)	Sinopharm Chemical Reagent Co.,Ltd
petroleum ether(PE)	China Petrochemical Corporation
ethyl acetate(EA)	China Petrochemical Corporation
dimethyl sulfoxide(DMSO)	XiLONG SCIENTIFIC
benzaldehyde	Energy Chemical
formaldehyde	Energy Chemical
acetaldehyde	Energy Chemical
n-propanal	Energy Chemical
p-chlorobenzaldehyde	Energy Chemical
NaCl	Energy Chemical
KCl	Energy Chemical
$CaCl_2$	Energy Chemical
$ZnCl_2$	Energy Chemical
$FeCl_3$	Energy Chemical
CH_3COONa	Energy Chemical
Na_2CO_3	Energy Chemical
Na_2SO_3	Energy Chemical
$NaHSO_3$	Energy Chemical
Na_2S	Energy Chemical
HClO4	Energy Chemical
Alanine (Ala)	Energy Chemical
Homocysteine(Hcy)	Energy Chemical
Glutathione(GSH)	Energy Chemical
Glycine(Gly)	Energy Chemical
Valine(Val)	Energy Chemical
H_2O_2	Sinopharm Chemical Reagent Co.,Ltd
Sodium Hydroxide(NaOH)	XiLONG SCIENTIFIC
Cetrimonium Bromide(CTAB)	Energy Chemical
Rhodamine B	Energy Chemical
Cell Counting Kit-8	Sigma-Aldrich
kainic acid(KA)	Sigma-Aldrich
malondialdehyde (MDA)	Energy Chemical
Hoechst 33342 Live Cell Stain (100X)	Beyotime
L-carnosine	Sigma-Aldrich

3. Supporting Figures

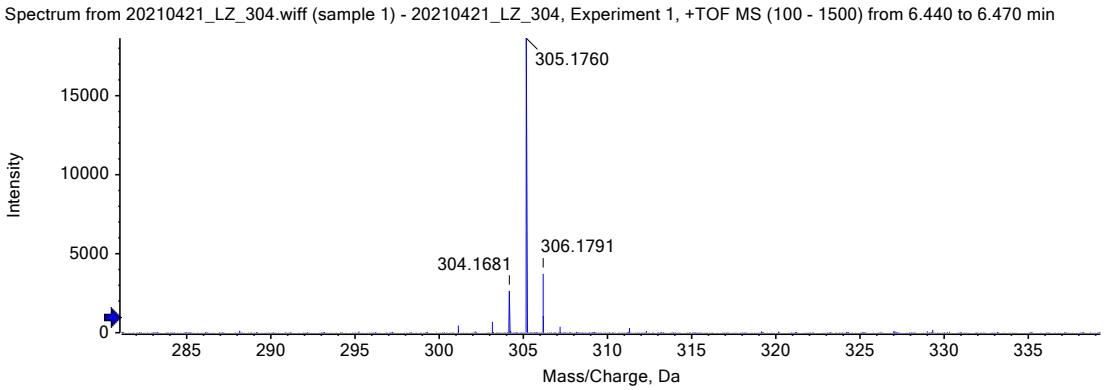

Figure S1. The mass spectrometry (MS) value calculated for $C_{19}H_{20}N_4$ $[M+H]^+$ 305.1761 and found 305.1760.

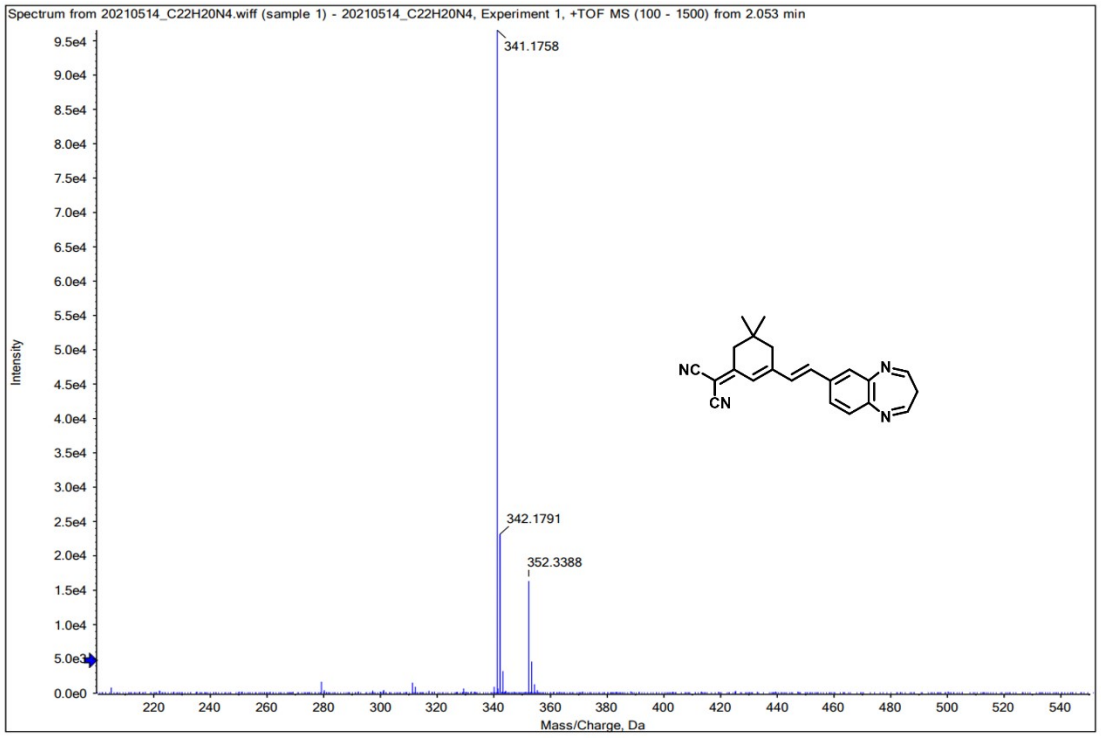

Figure S2. The MS of MDP was reacted with MDA. The new compound structure was shown in picture $C_{22}H_{20}N_4$ $[M+H]^+$ 341.1761 and found 341.1758.

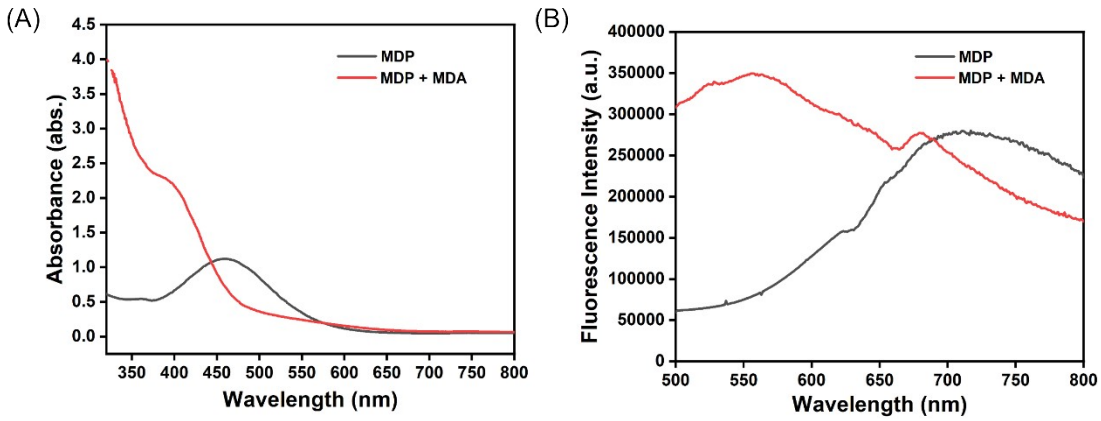

Figure S3. (A) The absorption spectra of MDP (10 μM) reacted without or with MDA (150 μM) in 10 mM PBS buffer (pH = 7.4, 1% DMSO, 2 mM CTAB) at 37 °C. (B) Fluorescence emission spectra of MDP (10 μM) were recorded after reacting with MDA (150 μM) in 10 mM PBS buffer for 30 minutes at 37 °C. mean ± s.d., n = 3 independent samples, λ_{ex} = 452 nm, d_{em}= d_{ex} = 10 nm.

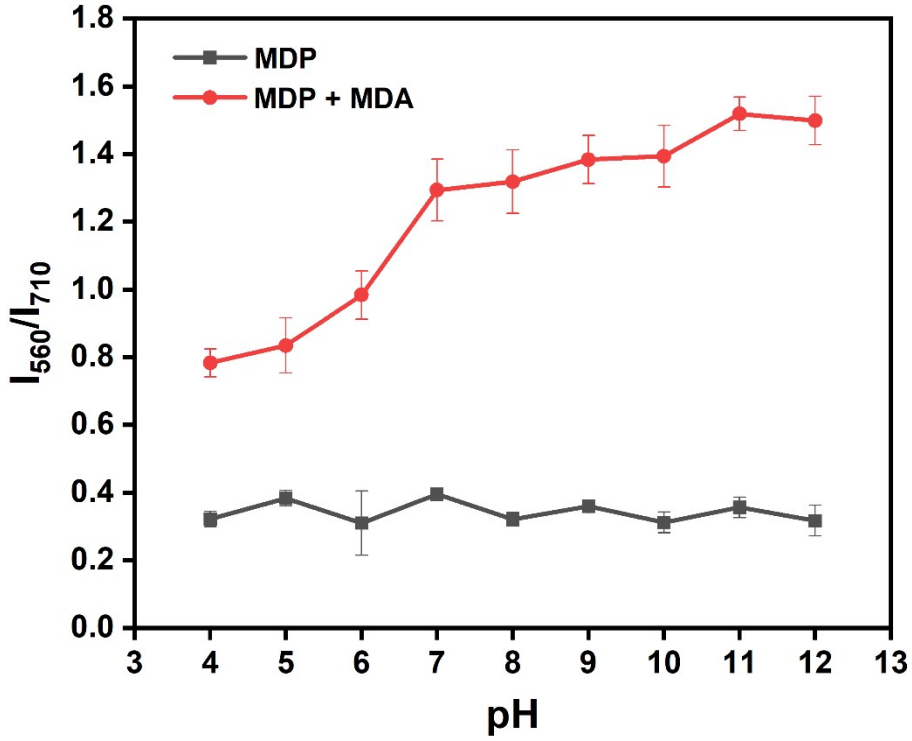

Figure S4. Fluorescence intensity ratio at I_{560}/I_{710} of **MDP** (10 μM) after incubation with and without MDA (100 μM) in different pH value PBS buffer (1% DMSO, 2 mM CTAB) at 37 °C. λ_{ex} = 452 nm, d_{em}= d_{ex} = 10 nm.

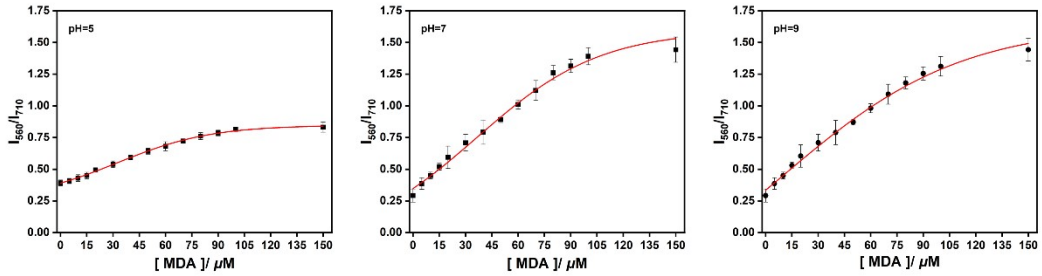

Figure S5. The fluorescence intensity ratio (I_{560}/I_{710}) was recorded of MDP (10 μM) with the various concentrations of MDA (0 – 150 μM) in different pH value PBS buffer (10 mM, pH 5, 7, 9, containing 1% DMSO. λ_{ex} = 452 nm, slit: 10 nm/10 nm. The data represents the average of three independent experiments.

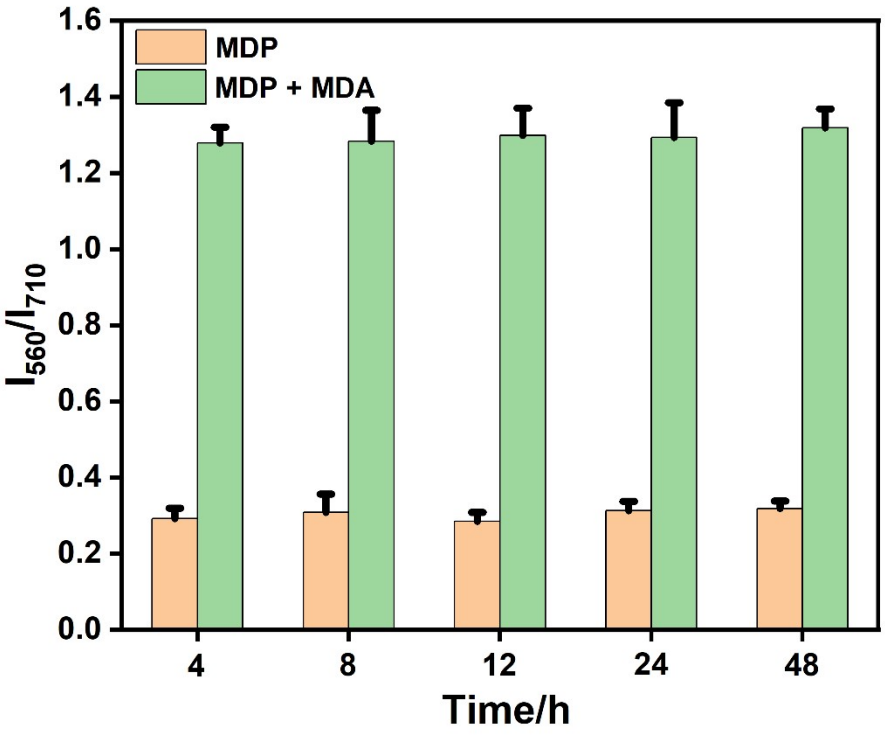

Figure S6. Fluorescence intensity ratio at I_{560}/I_{710} of **MDP** (10 μM) after incubation with and without MDA (100 μM) in PBS buffer (pH = 7.4, 1% DMSO, 2 mM CTAB) at 37 °C for different time. The time point: 4, 8, 12, 24, 48 h. λ_{ex} = 452 nm, d_{em}= d_{ex} = 10 nm.

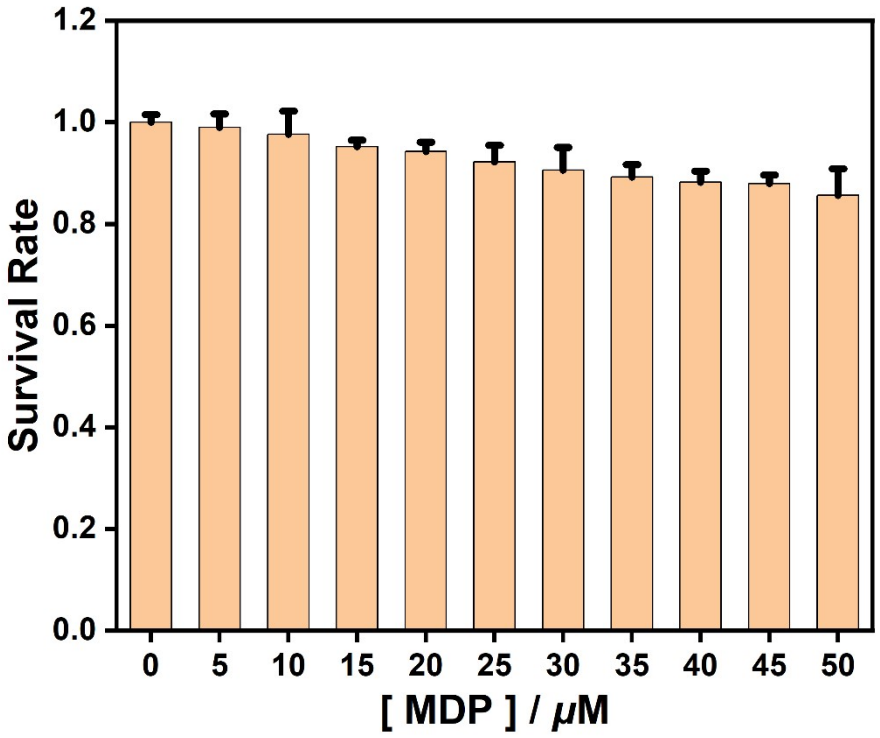

Figure S7. Cell viability of PC12 cells treated with MDP (0, 5, 10, 15, 20, 25, 30, 35, 40, 45,50 μM).

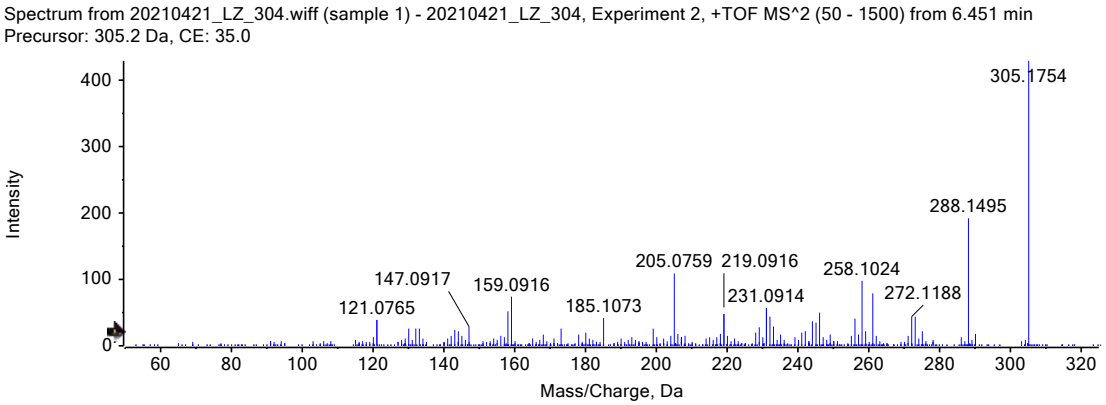

Figure S8. 30 min after the probe was injected into the tail vein, we euthanized the mice, retrieved their brain tissues, and added them to 1 mL of PBS buffer for homogenization. The homogenate was then centrifuged at 3500 rpm for 10 min to remove the supernatant. Subsequently, we added 1 mL of acetonitrile to precipitate excess proteins and impurities, followed by another centrifugation at 3500 rpm for 10 min. Finally, 200 μL of the supernatant was collected. The MS value calculated for $C_{19}H_{20}N_4$ $[M+H]^+$ 305.1761 and found 305.1760.

3. ^{1}H NMR and ^{13}C NMR

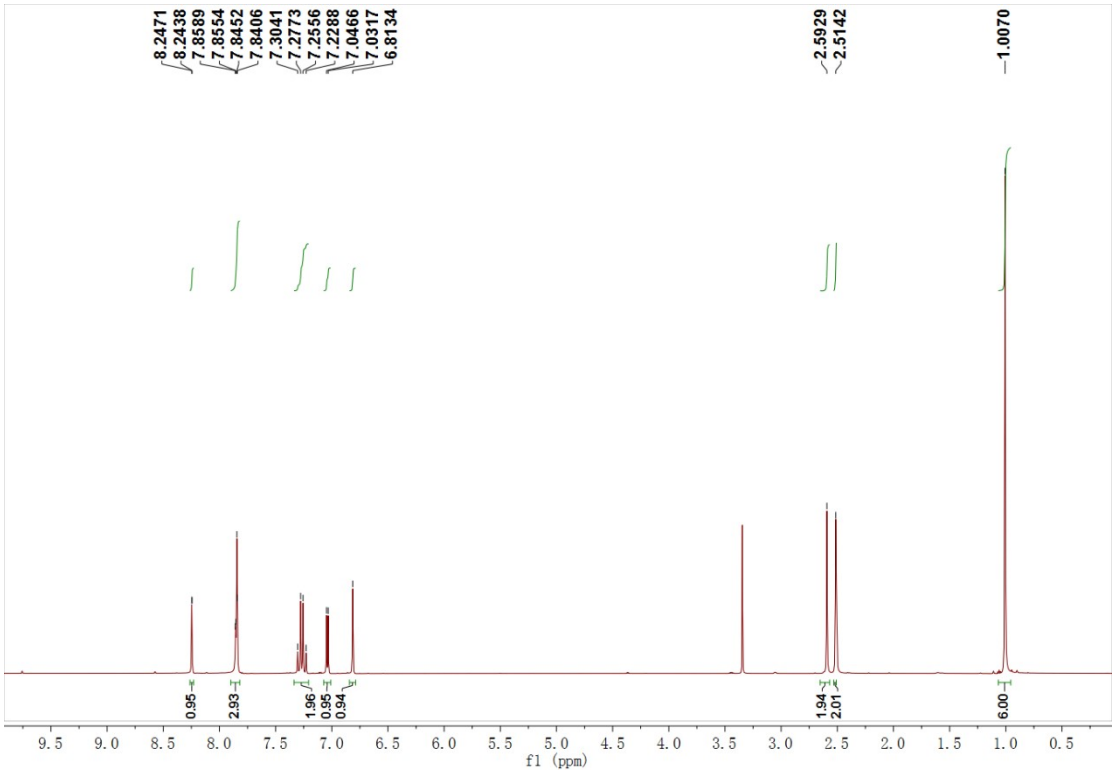

Figure S9. ^{1}H NMR spectrum of compound **1** (DMSO-*d6*).

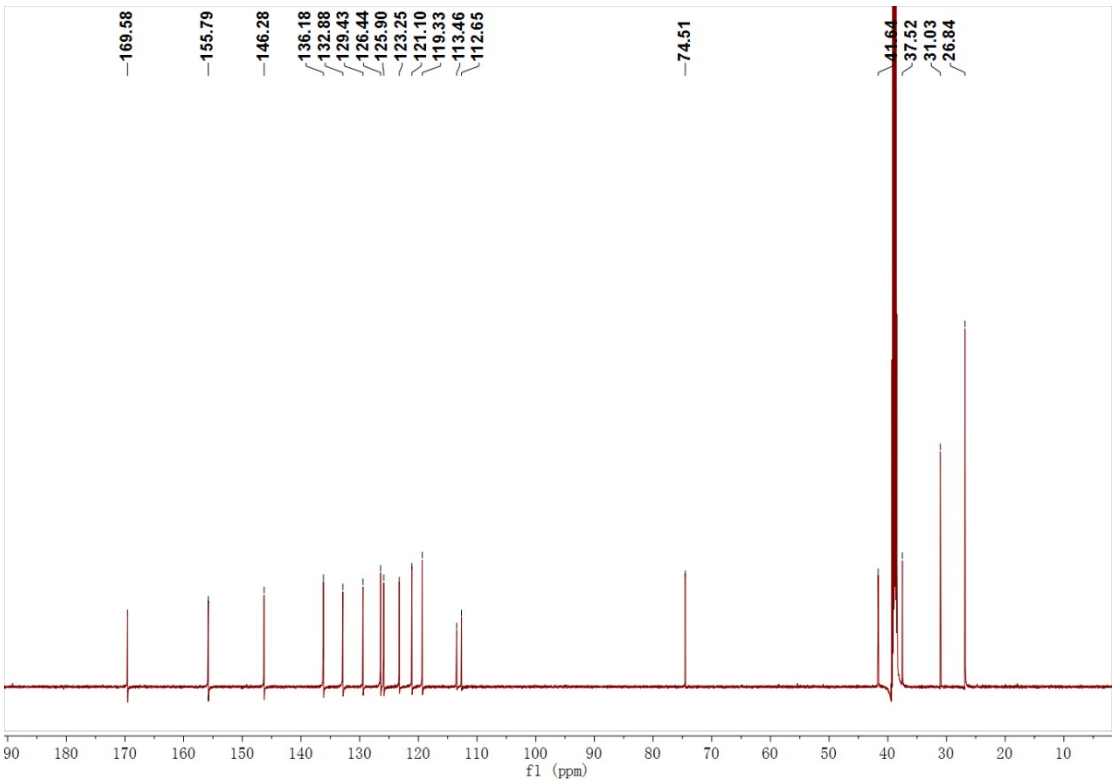

Figure S10. ^{13}C NMR spectrum of compound **1** (DMSO-*d6*).

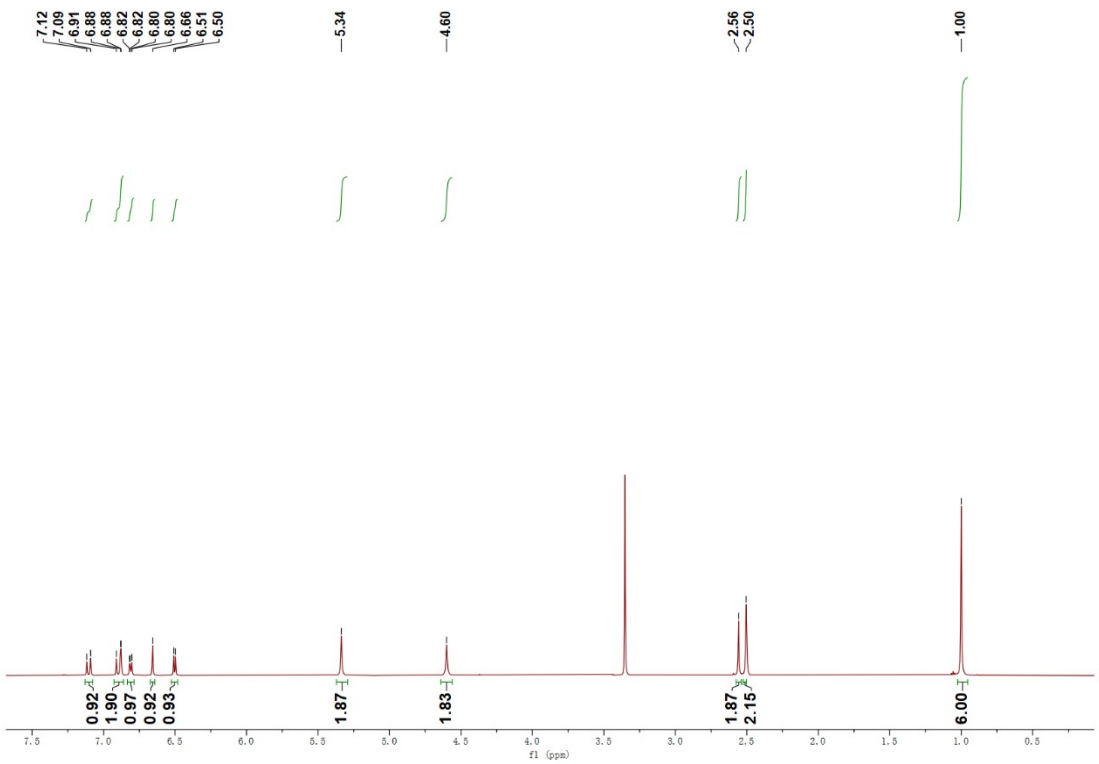

Figure S11. ^{1}H NMR spectrum of **MDP** (DMSO-d_6).

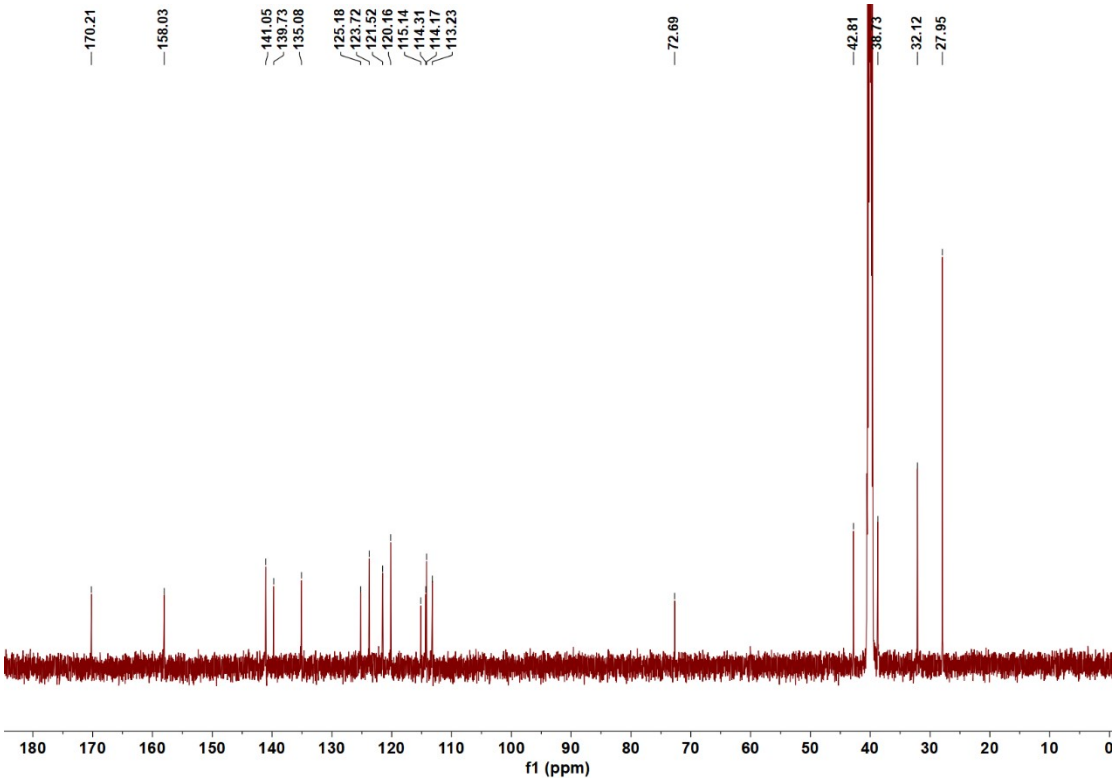

Figure S12. ^{13}C NMR spectrum of **MDP** (DMSO-d_6).