Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Imaging of formaldehyde fluxes in epileptic brains with a two-photon fluorescence probe

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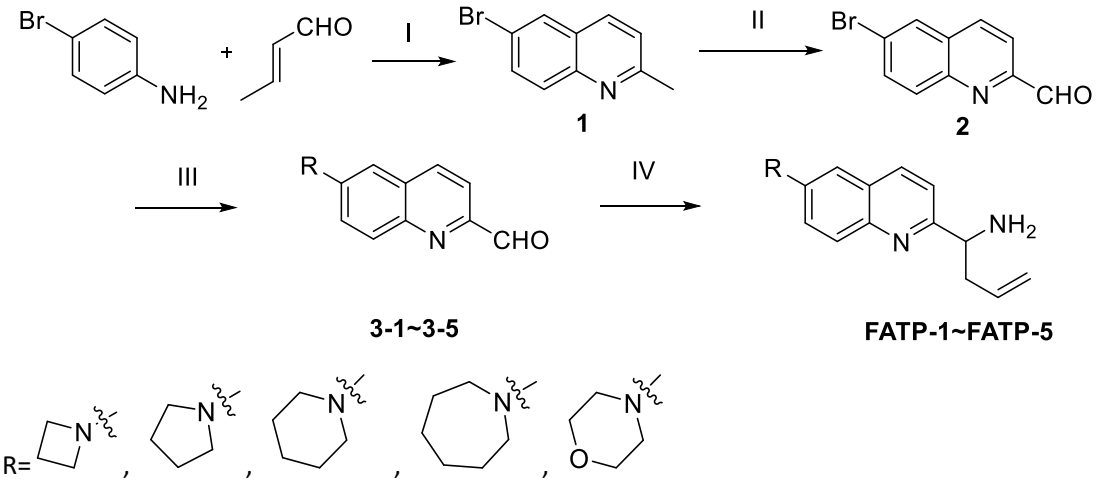
Table of content

1. Materials and equipment

All chemicals were available commercially and used without further purification. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DRX-600. Fluorescence measurements were performed on Hitachi Fluorescence Spectrophotometer F-7000. UV–vis spectra were recorded on Shimadzu UV-2550 spectrometer. Mass spectra were obtained by Mass Spectrometry Facility at Nanjing University. The type of Fourier transform infrared spectrometer was NEXUS870. The imaging experiments were carried out by a two-photon confocal fluorescence microscope (Leica TCS SP8 MP, Nanjing University). All the fluorescent images of mice were recorded by an IVIS Lumina XR *in-vivo* imaging system.

Formaldehyde standard solution (10 mg/ml) was firstly diluted into a stock solution (100 mM) for further testing, other analytes including alanine, benzaldehyde, methylglyoxal, cysteine, Glutamic acid, glycine, glutathione, H_2O_2, oxalaldehyde, Na_2S, $MgCl_2$, $CaCl_2$, *p*-chlorobenzaldehyde, $NaHCO_3$ were dissolved by dilution. Probe **FATP-1** was dissolved in MeCN to get a 1 mM stock solution. The final concentration of **FATP-1** was settled at 20 μM with 1% CH_3CN in PBS buffer (10 mM, pH 7.4). After incubation with the various analytes for 2 h at 37 ℃ in PBS buffer, the emission spectrum was measured and scanned from 450 nm to 650 nm at 1200 nm/min, and both excitation and emission slit widths were 10 nm, λ_{ex} =371 nm.

2. Synthesis of fluorescent probes FATP-1~FATP-5



Reagents and conditions:

(I) 4-Bromoaniline (10 g, 58 mmol) was dissolved in a solution of HCl (6 M, 260 mL), the mixture was stirred at room temperature for 1 h after addition of Crotonaldehyde (10.2 g, 174 mmol), the reaction was further refluxed at 100 ℃ for 2 h. After cooling down to room temperature, saturated sodium hydroxide solution was added to neutralize the aqueous. The above solution was extracted with ethyl acetate, washed twice with saturated NaCl solution, and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (PE: EA = 6:1 v/v) to obtain **1** as a yellow solid (5 g, 50%

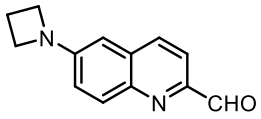
yield).

(II) A mixture of selenium dioxide (5 g, 36 mmol) in dioxane/water (200 mL/20 mL) was heated at 80 ℃ for 30 min, compound **1** (5 g, 23 mmol) was then added and the mixture was stirred at 80 ℃ for 4 h. After cooling down to room temperature, the mixture was filtered through diatomite, then the filter residue was flushed many times by a small amount of methylene chloride. The filtrate was concentrated under reduced pressure to afford crude product that purified by column chromatography on silica gel (PE: EA = 6:1 v/v) to afford the corresponding aldehyde **2** as a yellow solid (1.5 g, 30% yield).

(III) Cesium carbonate (2.7 g, 8.28 mmol), 2,2'-bis (diphenylphosphino)-1,1'-binaphthyl (0.119 g, 0.019 mmol) and palladium(II) acetate (0.057 g, 0.0255 mmol) was dissolved in a solution of toluene (5 mL), then compound **2** (1.5 g, 5.67 mmol) and different imines (RH, 12.3 mmol) was added and the reaction was further refluxed at 100 ℃ for 6 h. After cooling down to room temperature, the solution was filtered with diatomite, extracted with ethyl acetate, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (PE: EA = 6:1 v/v) to obtain the desired compound **3-1~3-5** (~ 10% yield).

(IV) Potassium allyltrifluoroborate (0.393 g, 2.66 mmol) was dissolved in a 7 N solution of NH_3 in MeOH (4 mL) and stirred at room temperature for 15 min. Dissolved compound **3** (300 mg, 1.5 mmol) in 7 N solution of NH_3 in MeOH (3 mL), and then the solution was added to the above reaction which was further stirred for another 16 h. The reaction was poured into saturated $NaHCO_3$ (100 mL), extracted with EtOAc and the extraction solution was dried with anhydrous sodium sulfate and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography using PE/EA (1:1 v/v) as eluent to obtain probe **FATP-1~FATP-5**. (15-17% yield).

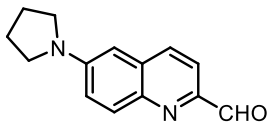
Synthesis of 3-1 6-(azetidin-1-yl)quinoline-2-carbaldehyde



3-1

Light yellow oil, 11% yield. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.99 (s, *J* = 0.8 Hz, 1H), 8.18 (d, *J* = 8.5 Hz, 1H), 7.99 (d, *J* = 9.1 Hz, 1H), 7.81 (d, *J* = 8.5 Hz, 1H), 7.16 (dd, *J* = 9.1, 2.6 Hz, 1H), 6.69 (d, *J* = 2.5 Hz, 1H), 4.04 (t, *J* = 7.3 Hz, 4H), 2.43 – 2.38 (m, 2H).

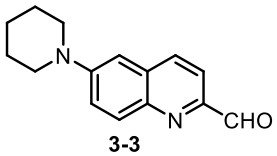
Synthesis of 3-2 6-(pyrrolidin-1-yl)quinoline-2-carbaldehyde



3-2

Yellow oil, 11% yield. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.97 (s, 1H), 8.16 (d, *J* = 8.5 Hz, 1H), 7.99 (d, *J* = 9.2 Hz, 1H), 7.79 (d, *J* = 8.5 Hz, 1H), 7.40 (dd, *J* = 9.3, 2.7 Hz, 1H), 6.82 (d, *J* = 2.7 Hz, 1H), 3.45 – 3.42 (t, 4H), 2.05 – 2.01 (m, 4H).

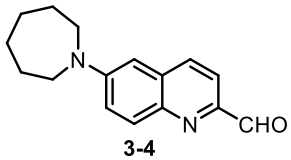
Synthesis of 3-3 6-(piperidin-1-yl)quinoline-2-carbaldehyde



3-3

Yellow oil, 10% yield. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.01 (s, 1H), 8.23 (d, *J* = 8.5 Hz, 1H), 7.98 (d, *J* = 9.4 Hz, 1H), 7.83 (d, *J* = 8.5 Hz, 1H), 7.73 (dd, *J* = 9.4, 2.8 Hz, 1H), 7.24 (d, *J* = 2.8 Hz, 1H), 3.44 (t, *J* = 5.0 Hz, 4H), 1.67 – 1.60 (m, *J* = 4.8 Hz, 6H).

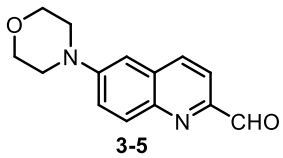
Synthesis of 3-4 6-(azepan-1-yl)quinoline-2-carbaldehyde



3-4

Light yellow oil, 9% yield. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.97 (s, 1H), 8.15 (d, *J* = 8.6 Hz, 1H), 7.97 (d, *J* = 9.4 Hz, 1H), 7.78 (d, *J* = 8.5 Hz, 1H), 7.55 (dd, *J* = 9.5, 2.9 Hz, 1H), 7.01 (d, *J* = 2.9 Hz, 1H), 3.65 (t, *J* = 6.1 Hz, 4H), 1.83 – 1.78 (m, 4H), 1.49 (m, *J* = 2.7 Hz, 4H).

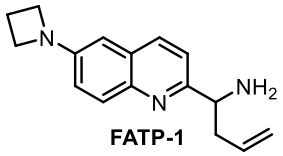
Synthesis of 3-5 6-(piperidin-1-yl)quinoline-2-carbaldehyde



3-5

Yellow oil, 10% yield. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.03 (s, *J* = 0.8 Hz, 1H), 8.30 – 8.28 (m, 1H), 8.05 – 8.03 (m, 1H), 7.87 (d, *J* = 8.5 Hz, 1H), 7.78 (dd, *J* = 9.4, 2.8 Hz, 1H), 7.30 (d, *J* = 2.8 Hz, 1H), 3.81 – 3.79 (m, 4H), 3.40 – 3.37 (m, 4H).

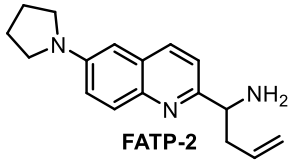
Synthesis of FATP-1 1-(6-(azetidin-1-yl)quinolin-2-yl)but-3-en-1-amine



FATP-1

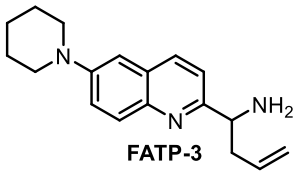
Light yellow oil, 16% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 (t, *J* = 1.6 Hz, 2H), 7.23 (d, *J* = 8.5 Hz, 1H), 6.90 (d, *J* = 9 Hz, 1H), 6.47 (s, 1H), 5.75 – 5.68 (m, 1H), 5.03 (dd, *J* = 29.5, 13.6 Hz, 2H), 4.13 – 4.11 (m, 1H), 3.90 (t, *J* = 7.2 Hz, 4H), 2.58 – 2.54 (m, 1H), 2.40-2.30 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6). δ 159.55, 159.51, 149.78, 141.88, 135.11, 134.50, 129.66, 128.63, 119.53, 118.00, 117.76, 103.85, 56.65, 52.49, 42.98, 16.84. HR MS (ESI): m/z found $[M+H]^+$ for $C_{16}H_{20}N_3$ 254.1657, found 254.1653.

Synthesis of FATP-2 1-(6-(pyrrolidin-1-yl)quinolin-2-yl)but-3-en-1-amine



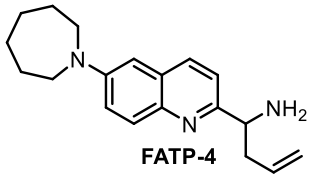
FATP-2 Yellow oil, 15% yield. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.99 (d, J = 8.5 Hz, 1H), 7.78 (d, J = 9.1 Hz, 1H), 7.42 (d, J = 8.5 Hz, 1H), 7.20 (*dd*, J = 9.2, 2.7 Hz, 1H), 6.71 (d, J = 2.7 Hz, 1H), 5.82-5.75 (m, 1H), 5.01 (d, J = 17.2 Hz, 1H), 4.96 (dd, J = 10.3, 2.2 Hz, 1H), 4.00 (dd, J = 7.6, 6.0 Hz, 1H), 3.35-3.33 (m, 4H), 2.39-2.34 (m, 2H), 2.01–1.99 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.66, 145.96, 140.66, 134.65, 133.88, 129.60, 129.35, 119.37, 119.08, 118.91, 103.41, 55.78, 47.85, 41.69, 25.57. HR MS (ESI): m/z found $[M+H]^+$ for $C_{17}H_{22}N_3$ 268.1814, found 268.1812.

Synthesis of FATP-3 1-(6-(piperidin-1-yl)quinolin-2-yl)but-3-en-1-amine



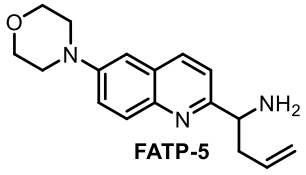
FATP-3 Yellow oil, 16% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85 (d, J = 8.5 Hz, 1H), 7.81 (d, J = 9.3 Hz, 1H), 7.40 (dd, J = 9.3, 2.7 Hz, 1H), 7.23 (d, J = 8.5 Hz, 1H), 6.93 (d, J = 2.6 Hz, 1H), 5.70-5.66 (m, 1H), 5.05 (d, J = 17.1 Hz, 1H), 5.00 (d, J = 10.2 Hz, 1H), 4.25 – 4.20 (m, 1H), 3.19 (t, J=5.5 Hz, 4H), 2.61-2.57 (m, 1H), 2.47-2.42 (m, 1H), 1.70-1.66 (m, 4H), 1.57-1.54 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.31, 149.59, 142.26, 136.52, 135.19, 129.36, 128.57, 122.93, 119.98, 117.24, 109.08, 57.32, 50.14, 43.26, 25.67, 24.37. HR MS (ESI): m/z found $[M+H]^+$ for $C_{18}H_{24}N_3$ 282.1970, found 282.1968.

Synthesis of FATP-4 1-(6-(piperidin-1-yl)quinolin-2-yl)but-3-en-1-amine



FATP-4 Light yellow oil, 16% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (t, J = 8.3 Hz, 2H), 7.27-7.21 (m, 2H), 6.68 (d, J = 2.8 Hz, 1H), 5.75-5.68 (m, 1H), 5.08 (d, J = 18 Hz 1H), 5.02 (d, J = 10.1 Hz, 1H), 4.20 – 4.18 (m, 1H), 3.51-3.49 (t, J = 6 Hz, 4H), 2.63-2.58 (m, 1H), 2.47-2.42 (m, 1H), 1.78 (brs, 4H), 1.50-1.49 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$). δ 155.18, 147.01, 140.57, 134.76, 133.64, 129.68, 129.51, 119.35, 119.08, 118.58, 103.25, 55.66, 49.56, 41.41, 27.69, 26.98. HR MS (ESI): m/z found $[M+H]^+$ for $C_{19}H_{26}N_3$ 296.2127, found 296.2118.

Synthesis of FATP-5 1-(6-morpholinoquinolin-2-yl)but-3-en-1-amine



FATP-5 Orange oil, 17% yield, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.89 (dd, J = 15.4, 8.9 Hz, 2H), 7.39 (dd, J = 9.2, 2.7 Hz, 1H), 7.31 (d, J = 8.5 Hz, 1H), 6.95 (d, J = 2.5 Hz, 1H), 5.77 – 5.70 (m, 1H), 5.05 (dd, J = 26.5, 13.6 Hz, 2H), 4.16 (dd, J = 8.1, 5.2 Hz, 1H), 3.88-3.81 (t, J = 4.7 Hz, 4H), 3.24-3.17 (t, J = 4.8 Hz, 4H), 2.61-2.57 (m, 1H), 2.42-2.37 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.32, 149.09, 143.23, 135.35, 135.05, 129.83, 128.38, 122.05, 119.67, 118.07, 109.14, 66.87, 56.74, 49.57, 43.08. HR MS (ESI): m/z found $[M+H]^+$ for $C_{17}H_{22}N_3O$ 284.1763, found 284.1782.

3. Determination of the fluorescence quantum yield

The fluorescence quantum yield Φ_u was estimated from the absorption and fluorescence spectra of **FATP-1~FATP-5** according to the equation, where using the ethanol solution of rhodamine B (10 μM, Φ = 0.69, λ_{ex} = 365 nm) for the reference. The fluorescence quantum yield was calculated using equation as follows:

$$\Phi_u = [(A_s F_u n^2)/ (A_u F_s n_0^2)]\Phi_s.$$

Φ_s is the quantum yield of reference substance, A_s and A_u represent the absorbance of the reference and testing solution at the excitation wavelength and controlled to be lower than 0.05 at the same time, F_s and F_u refer to the integrated emission band areas, n and n_0 are the solvent refractive indexes of sample and reference, respectively.

4. The limit of detection (LOD) of FATP-1

The emission spectrum of free **FATP-1** in PBS buffer (10 mM, pH = 7.40, containing 1% MeCN) was collected for 20 times to confirm the background noise σ. The linear regression curve was then fitted according to the data in the range of FA from 0 to 10 equiv. and obtained the slope of the curve, then the detection limit was calculated using the following equation:

$$\text{The limit of detection (LOD)} = 3\sigma/k$$

Where σ is the standard deviation of eleven blank measurements, and k is the slope of the linear equation. The detection limit (3σ/k) was then determined to be 0.3 μM.

5. The measurement of lipophilicity

Lipophilicity was presented as log $P_{o/w}$ values, which were determined by the flask-shaking method. An aliquot of a stock solution of the sample in aqueous NaCl (0.9% w/v and saturated with octanol) was added to an equal volume of octanol (saturated with 0.9% NaCl, w/v), and the mixture was shaken overnight at 60 rpm to allow partitioning at 298 K. After the sample was centrifuged at 3000 rpm for 10 min, the probe content of the organic and aqueous phases was determined by UV absorbance (254 nm). Log *P* was defined as the logarithmic ratio of probe concentrations in the organic and aqueous phases.

6. Cell viability assay

The cytotoxicity of **FATP-1** was evaluated using the Cell Counting Kit-8 assay (APE × BIO). Cells in log phase were harvested and placed in 96-well plates (5000 cells/well). After 12 hours, cells were treated with different concentrations (0.1-500 μM) of **FATP-1**. After 48 hours of incubation, cells were washed with PBS and incubated in 100 μL of fresh medium containing 10 μL of CCK8 dye for more than 2 hours. After 10 min shaking on shaking table, plates were read in Infinite® M200 Pro Multimode Microplate Reader (Tecan, Switzerland) at the wavelength of 450 nm (reference wavelength 650 nm). The IC_{50} value was defined as the concentration of compound required to inhibit cell viability by 50%, compared to cells treated with the maximum amount of CH_3CN (1%) and considered to be 100% viable.

7. Cell culture

Live SH-SY5Y human neuroblastoma cells were cultured in Dulbecco's Modified Eagle's medium (DMEM, PAN Biotech, Germany) supplemented with 10% fetal bovine serum (Life

Technologies, USA), sodium pyruvate, non-essential amino acids and 100 U/mL penicillin/100 μg/mL streptomycin, and grown at 37 ℃ in a 5% CO_2 incubator.

8. Fluorescent imaging studies

C. elegans were pretreated with different concentration of FA (500-1000 μM, 2 h), THF (10 mM, 2 h), $NaHSO_3$ (200 μM, 2 h), MeOH (10%, 2h), Fe^{2+} (100 μM, 2 h), H_2O_2 (100 μM, 2 h), KA (400 μM, 12 h), Glu (2 mM, 12 h), NaHS (80 μM, 12 h), GSK-LSD1 (1 μM, 12 h) and TCP (20 μM, 12 h) at 20℃, respectively. After washing three times, *C. elegans* were then incubated with 20 μM **FATP-1** for another 30 min. Then *C. elegans* were imaging by a two-photon confocal fluorescent microscope (Leica TCS SP8 MP). The excitation wavelength was 750 nm, and the emission wavelength was 540-600 nm. Fluorescence intensity of *C. elegans* was obtained by the confocal fluorescent microscope (Leica TCS SP8 MP) and analyzed on the Zen (ZEISS, Blue version) by calculating the means of total fields of view in corresponding channels.

9. Flow cytometry analysis with FATP-1

SH-SY5Y cells were pretreated with NaHS (100 μM, 12 h), Glu (2 mM, 12 h), KA (500 μM, 12 h), GSK-LSD1 (1 μM, 12 h), TCP (20 μM, 12 h), MeOH (2%, 2 h), THF (10 mM, 2 h), Fe^{2+} (100 μM, 2 h), KO_2 (200 μM, 2 h), or $NaHSO_3$ (200 μM, 30 min), after washing three times, the cells were then incubated with 10 μM **FATP-1** for another 30 min before performing flow cytometry analysis. The fluorescence excitation was 405 nm, and the fluorescence emission was 555-651 nm. Fluorescence intensity of cells was collected on an Attune NxT 4 flow cytometer (Thermo Fisher Scientific) and analyzed on the Flowjo V10 by calculating the means of cells in corresponding channels.

10. *In vivo* fluorescence imaging with FATP-1

All mice were purchased from the Model Animal Research Centre of Nanjing University (Nanjing, China). All mice were maintained under specific pathogen-free conditions and used in accordance with the animal experimental guidelines set by the Institute of Animal Care and Use Committee. This study has been approved by the Institutional Animal Care and Use Committee of Nanjing University.

5-weeks-old mice were divided into three groups mice (wildtype control group, combination KA with GSK-LSD1 treated group, and KA-induced epileptic group). *In vivo* and *Ex vivo* fluorescence images of relative FA levels in mice brains at 5, 15, 30, 45, and 60 min after *i.v.* injection with **FATP-1** (0.5 mg/kg) were recorded by using the IVIS Spectrum imaging system. KA (6 mg/kg, 12 h) was intraperitoneal (*i.p.*) injected into a mouse to cause epilepsy. At the same time, GSK-LSD1 (1.5 mg/kg, 12 h) was intraperitoneal *(i.p.*) injected into the mouse to inhibit the production of HCHO. The fluorescence mission was collected between 475-600 nm and excitation at 430 nm. Fluorescence intensity of *in vivo* and *ex vivo* mouse brains were obtained by IVIS Lumina XR in vivo imaging system from PerkinElmer and analyzed on the Living Image Software by calculating average radiant efficiency (p/sec/cm^2/sr/(μW/cm^2)).

11. Frozen sectioning

Mice were deeply anesthetized with isoflurane and killed. The fresh brain was taken out and frozen directly in liquid nitrogen. The compound embedded at the optimal cutting temperature

(O.C.T) was performed and the microtome (Leica CM1950) was frozen for sectioning. The thickness of each slice is about 10 μM. After drying, the slices were stored at -80 ℃. The slices were taken out and restored to room temperature, and then fluorescence images were recorded by a fluorescence microscope. Fluorescence tasks were collected between 540-600 nm and excited at 371 nm.

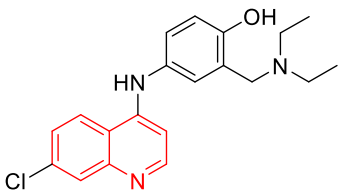
12. The two-photon absorption cross-section (σ_2) value of FATP-1

The two-photon absorption cross-section value can be determined from the relation:

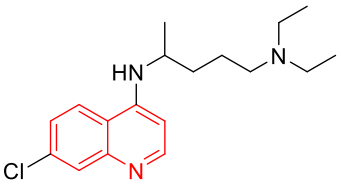
$$\sigma_{2s} = \frac{F_{2s}(\lambda_{reg})\ C_r \varphi_r(\lambda_{reg})}{F_{2r}(\lambda_{reg})\ C_s \varphi_s(\lambda_{reg})} \sigma_{2r}$$

Where F_2 is the fluorescence intensity, λ_{reg}, is the fluorescence registration wavelength. Indices *r* and *s* stand, respectively, for the rhodamine B and the sample under study, Φ is the fluorescence quantum yield.

13. Supplementary Figures

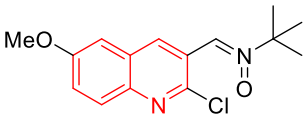


Amodiaquine



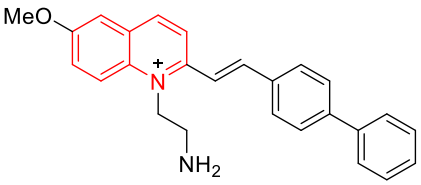
Chloroquine

neuroprotective strategy for Parkinson's disease (PD)
PNAS **2015**, 112, 8756-8761



neuroprotective agent

J. Med. Chem. **2019**, 62, 4, 2184-2201



brain-imaging agents

Angew Chem Int Ed Engl **2004**, 43, 6331-6335

Scheme S1. Quinoline-derivatives used as the potential neuroprotective or imaging agents.

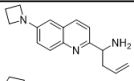
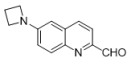
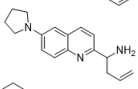
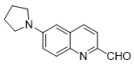
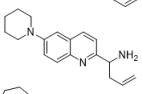
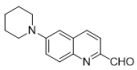
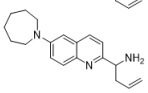
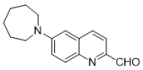
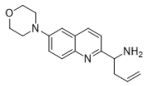
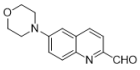
Probe	Probe structure	Log P	λ_{abs} (nm)	$\lambda_{ex}/\lambda_{em}$ (nm)	Stokes Shift	Φ_1	ε_1(*10^4 M^{-1} cm^{-1})	Fluorophore	$\lambda_{ex}/\lambda_{em}$ (nm)	Φ_2	(Φ_2/Φ_1)	ε_2(*10^4 M^{-1} cm^{-1})	Increasing ratio[a]
FATP-1		2.43	249	355/500	145	0.23	1.5		371/571	0.72	3.13	1.2	6.4-fold
FATP-2		2.40	258	386/507	121	0.21	3.4		381/567	0.54	2.57	3.5	1.7-fold
FATP-3		2.30	254	346/506	160	0.21	2.1		346/512	0.51	2.42	1.7	1.4-fold
FATP-4		1.83	262	400/470	70	0.20	1.1		400/508	0.60	3.00	1.1	1.2-fold
FATP-5		2.09	252	355/531	176	0.42	2.2		355/526	0.66	1.57	1.5	1.3-fold

Table S1. Spectroscopic data for formaldehyde probes (**FATP-1~5**) and their corresponding fluorophores. Log *P*, Log value of the partition coefficient (P); λ_{abs}, absorption maximum; $\lambda_{ex}/\lambda_{em}$, excitation/emission maximum; Φ, quantum yield; ε, extinction coefficient; [a]The increased ratio of fluorescence intensity.

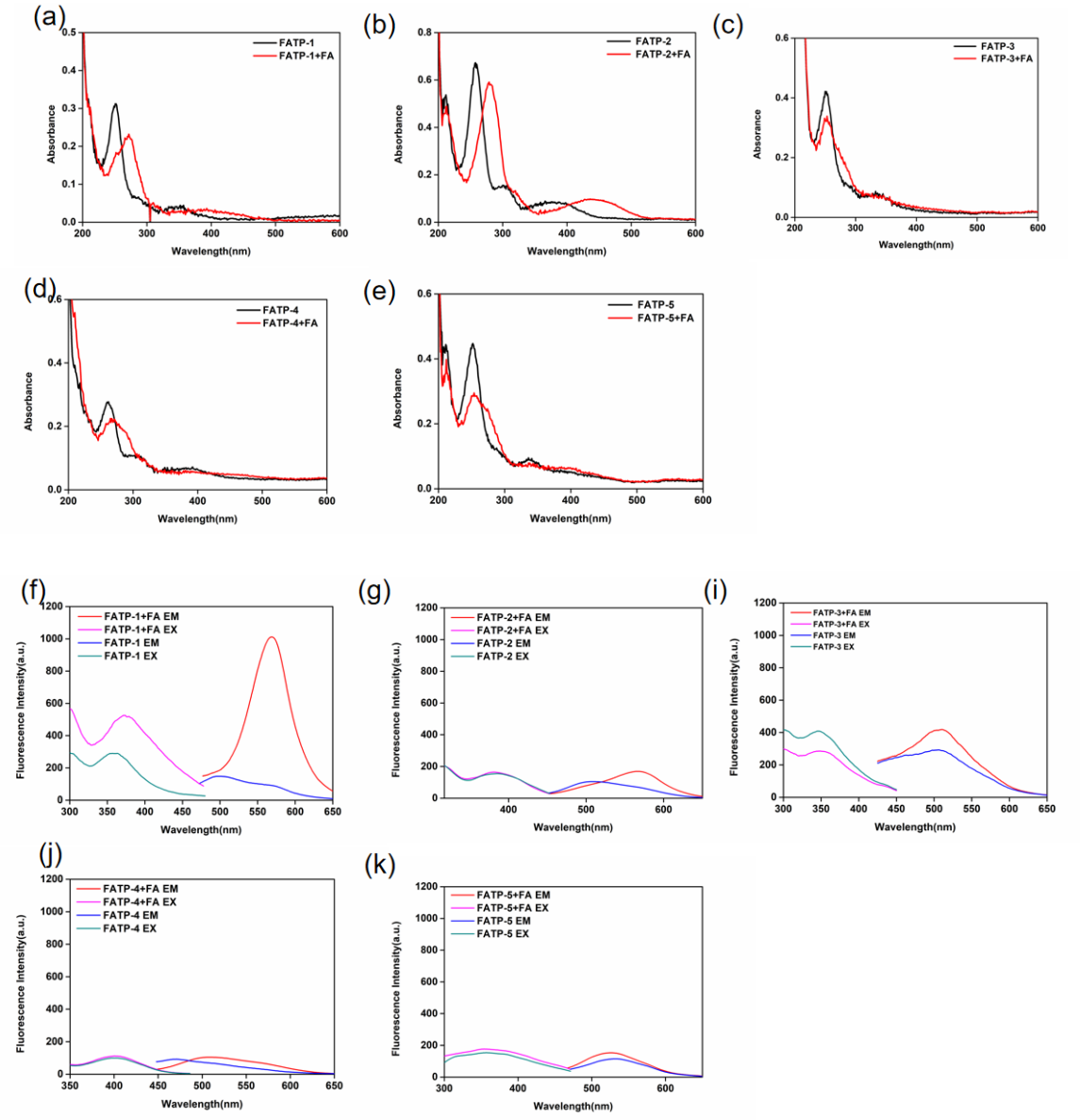


Figure S1. (a-e) The absorption spectrum of **FATP-1~FATP-5** (20 μM) and **FATP-1~FATP-5** (20 μM) with FA (2 mM) in PBS buffer (10 mM, pH 7.4, containing 1% MeCN) at 37℃ for 120 min. (f-k) The Excitation and Emission spectrum of **FATP-1~FATP-5** (20 μM) and **FATP-1~FATP-5** (20 μM) with FA (2 mM) in PBS buffer (10 mM, pH 7.4, containing 1% MeCN) at 37℃ for 120 min.

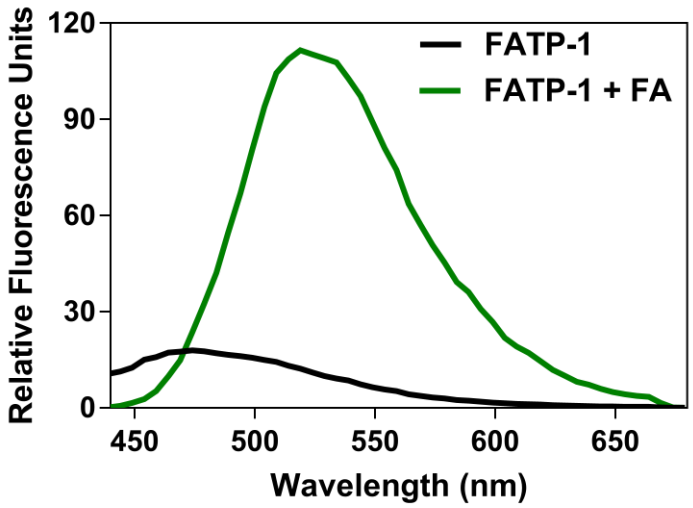


Figure S2. Two-photon action spectra of 10 μM **FATP-1** without or with FA upon excitation at 750 nm. The two-photon absorption cross section (σ_2) value of **FATP-1** upon excitation at 750 nm is about 28.32±0.68 GM, while the two-photon absorption cross section value of **FATP-1**+FA (σ_2 = 78.44±2.89 GM) gives a greater increase.

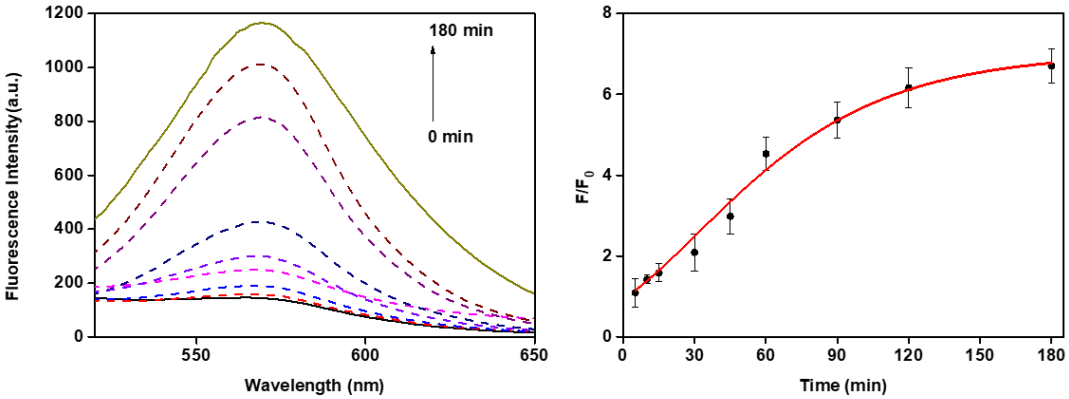


Figure S3. (Left) Fluorescence spectra of **FATP-1** (20 μM) in PBS buffer (10 mM, pH 7.4, containing 1% MeCN) after treatment with FA (2 mM) for 0-180 min. (Right) The changes of fluorescence intensity ratio (F/F_0) of **FATP-1** (20 μM) at 571 nm after incubation with FA (2 mM) in PBS buffer (10 mM, pH 7.4, containing 1% MeCN) with different time (0-180 min). All the data represent the average of three independent experiments.

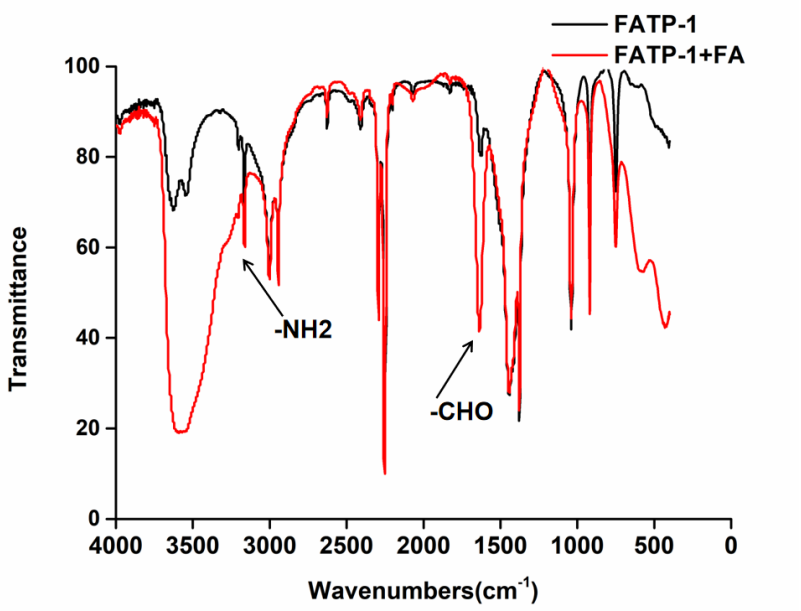
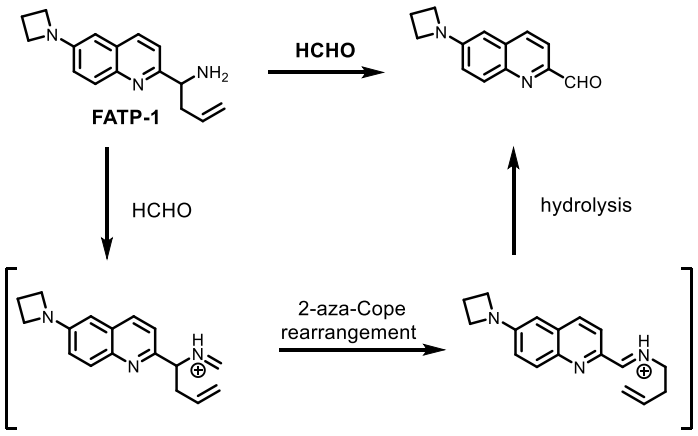


Figure S4. The IR spectra of **FATP-1** before (black) and after (red) treatment with FA. A signal -CHO- group (nearly in 1690-1715 cm^{-1}) and the $-NH_2$ group (around 3300-3500 cm^{-1}) were clearly observed.



Scheme S2. The proposed sensing mechanism of FATP-1 for monitoring FA.

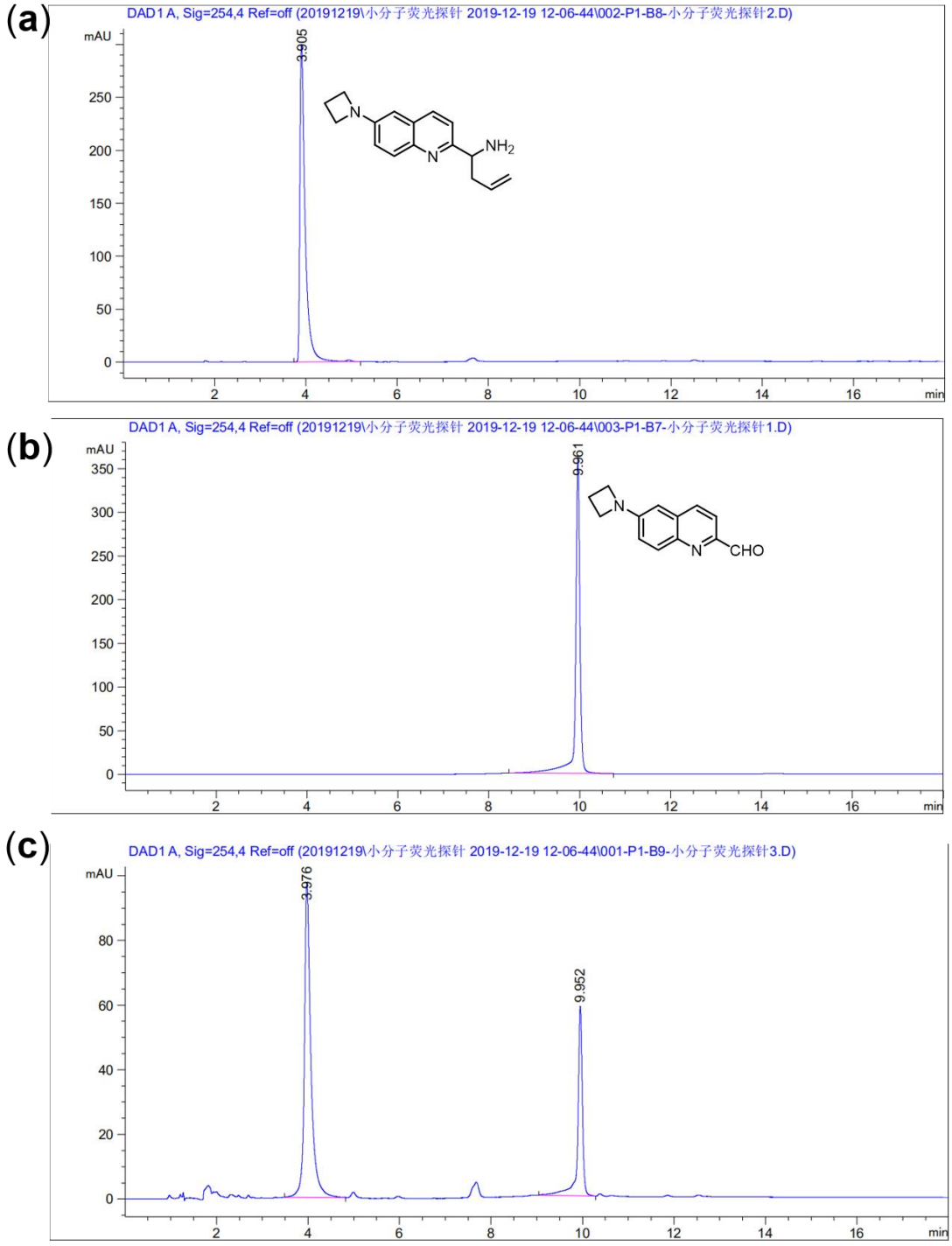


Figure S5. HPLC analysis of **FATP-1** before and after incubation with FA. (a) HPLC chromatograms of **FATP-1** in MeCN. (b) HPLC chromatograms of compound **3-1** in MeCN. (c) HPLC chromatograms of **FATP-1** incubated with FA (1 mM) in PBS buffer for 2 h at 37 ℃. All the experiments were conducted using Agilent 1260 Infinity II liquid chromatography with Ultimate XB-C18 column, 5 μm, 150*4.6 mm. HPLC runs used a linear gradient from 40% MeCN/60% 40 mM NH_4Ac to 60% MeCN/40 mM NH_4Ac over 20 min. The column temperature is 30 ℃. UV detector wavelength is set at 254 nm.

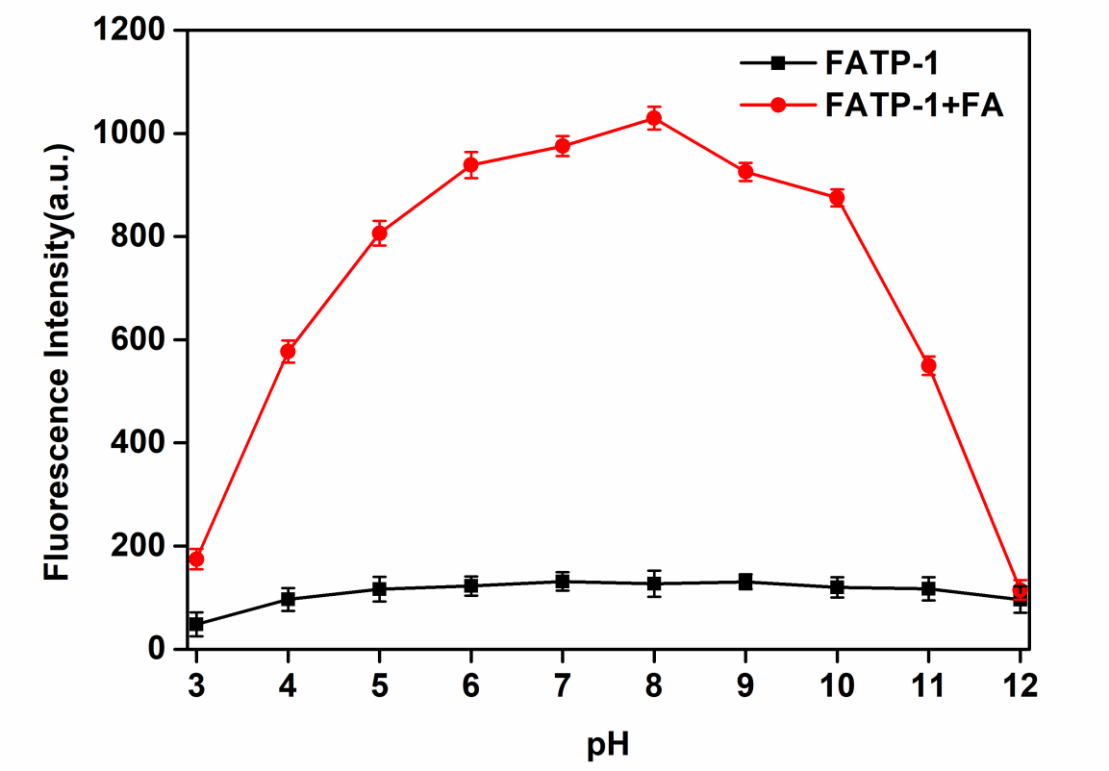


Figure S6. Fluorescence intensity changes of **FATP-1** at 571 nm in the absence and presence of FA in PBS buffer with various pH conditions. (Black: **FATP-1** (20 μM); Red: **FATP-1**+FA (2 mM); Time 120 min). All the data represent the average of three independent experiments.

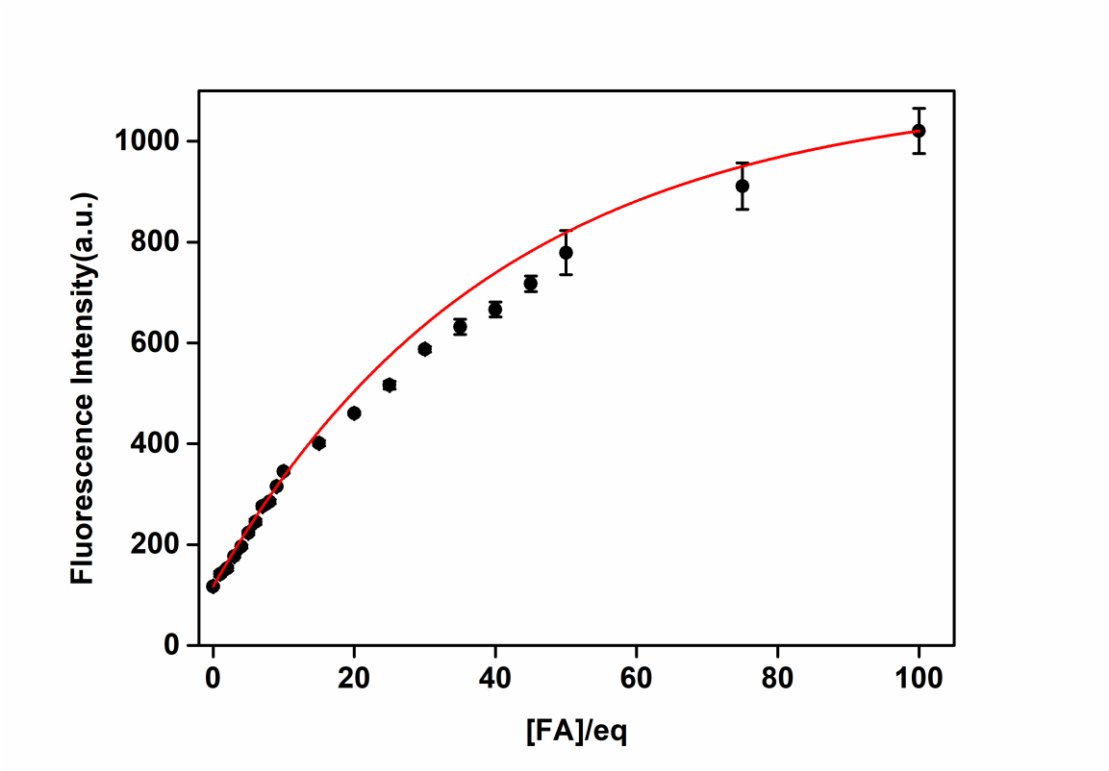


Figure S7. The changes of fluorescence intensity of **FATP-1** (20 μM) at 571 nm after incubation with different concentrations of FA (0-2 mM) in PBS buffer for 120 min. All the data represent the average of three independent experiments.

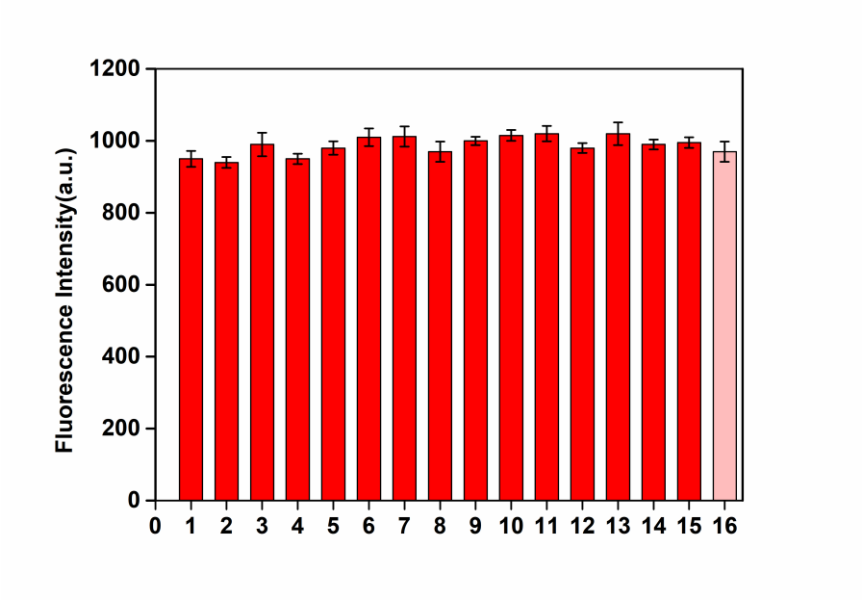


Figure S8. Fluorescence intensity of **FATP-1** at 571 nm in the presence of both FA (2 mM) and the various relevant analytes (2.0 mM) in PBS buffer (10 mM, pH 7.4, 1.0% MeCN) at 37℃. Relevant analytes: (1) H_2O; (2) alanine; (3) benzaldehyde; (4) methylglyoxal; (5) cysteine; (6) Glutamic acid; (7) glycine; (8) glutathione; (9) H_2O_2; (10) oxalaldehyde; (11) Na_2S; (12) $MgCl_2$; $CaCl_2$; (14) *p*-chlorobenzaldehe; (15) $NaHCO_3$; (16) FA. All the data represent the average of three independent experiments.

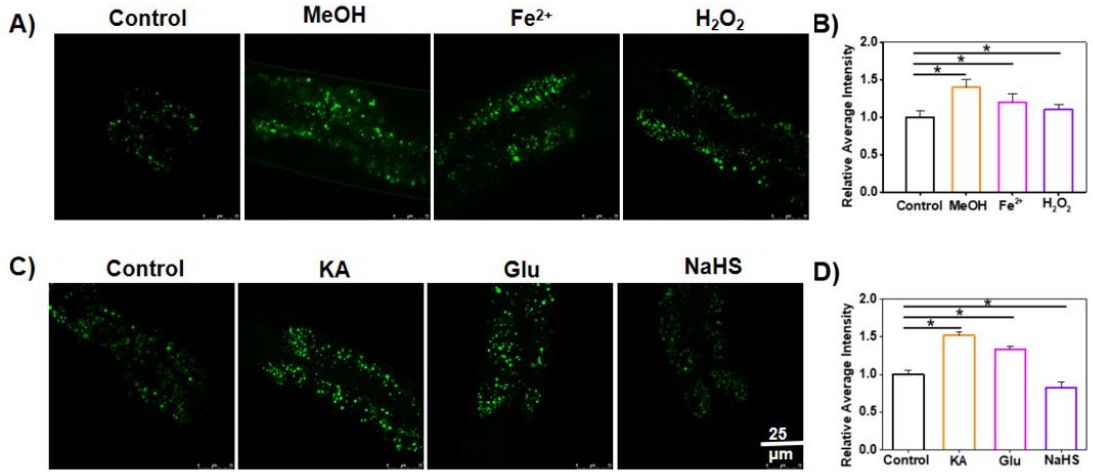


Figure S9. (A) *C. elegans* were pretreated with 10% MeOH, Fe^{2+}(100 μM), and H_2O_2(100 μM) at 20°C for 2 h, then washed and incubated with **FATP-1** (20 μM) for another 30 min before imaging. Green channel: collected at 540-600 nm, λ_{ex}=750 nm, scale bar = 25 μm. (B) The relative ratio of fluorescence intensity shown in (A) was quantified. (C) *C. elegans* were pretreated with KA (400 μM), Glu (2 mM), and NaHS (80 μM) at 20°C for 12 h, then washed and incubated with **FATP-1** (20 μM) for another 30 min before imaging. Green channel: collected at 540-600 nm, λ_{ex}=750 nm, scale bar = 25 μm. (D) The relative ratio of fluorescence intensity shown in (C) was quantified. The error bars were ±SD (n=3). Statistical analyses performed with an Ordinary one-way ANOVA test to the control with H_2O or DMSO treatment, $^{*}P < 0.05$.

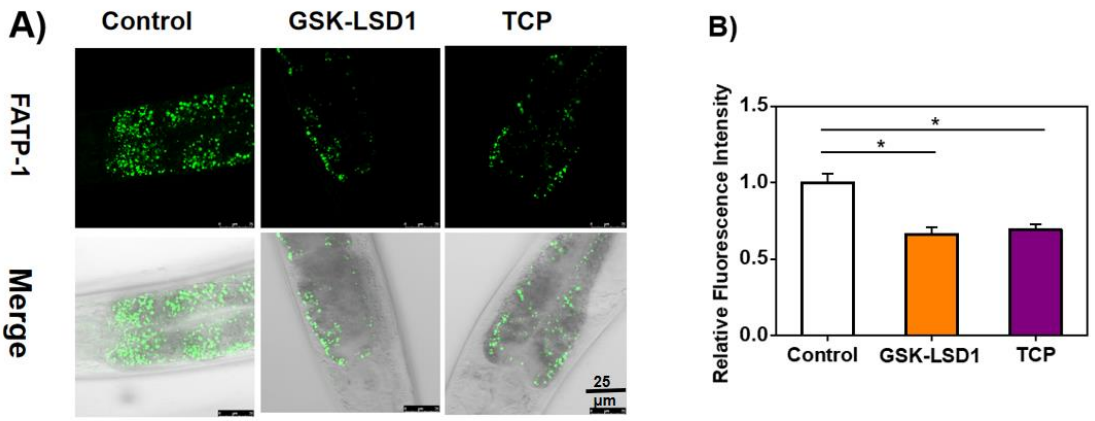


Figure S10. (A) *C. elegans* were pretreated with GSK-LSD1 (1 μM), TCP (20 μM) at 20 ℃ for 12 h, then washed and incubated with **FATP-1** (20 μM) for another 30 min before imaging. Green channel: collected at 540-600 nm, λ_{ex}=750 nm, scale bar = 25 μm. (B) The relative ratio of fluorescence intensity shown in (A) was quantified. The error bars were ±SD (n=3). Statistical analyses performed with an Ordinary one-way ANOVA test to the control with DMSO treatment, $^{*}P < 0.05$.

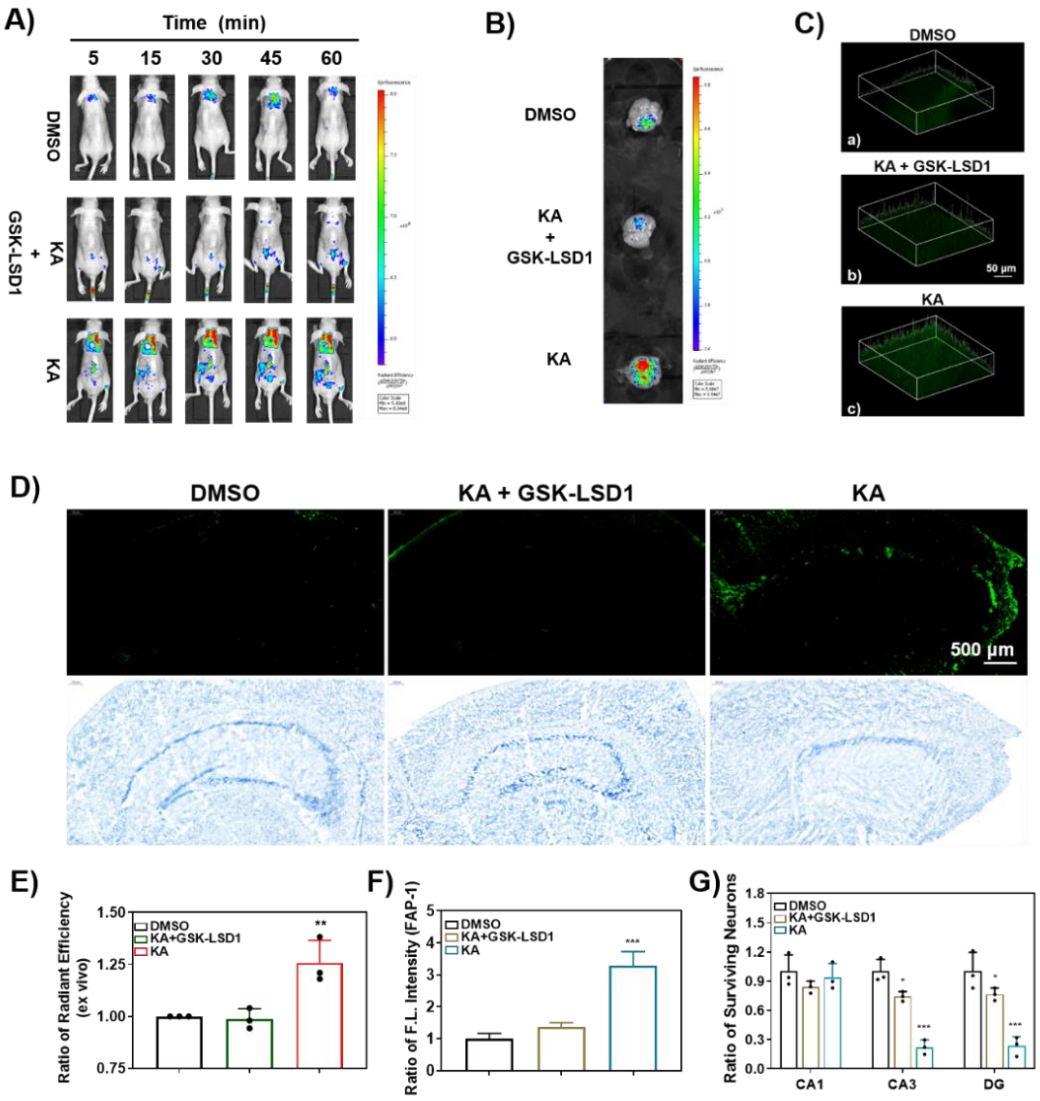


Figure S11. (A) Fluorescence images of three groups mice [wide-type (WT), combination KA with GSK-LSD1 and KA-induced epileptic mice] at 5, 15, 30, 45, and 60 min after *i.v.* injection with FATP-1 (0.5 mg/kg). KA (6 mg/kg, 12 h) was intraperitoneal (*i.p.*) injected into mouse to cause epilepsy. At the same time, GSK-LSD1 (1.5 mg/kg, 12 h) was intraperitoneal (*i.p.*) injected into mouse to inhibit the production of HCHO. (B) Ex vivo fluorescence images of relative HCHO levels in mice brains 60 min post-injection of FATP-1. (C) The two-photon fluorescence images of 2.5D volume-rendered in brain tissues from (B). Green=FATP-1 channel. Fluorescence excitation filter MP 750 nm, emission filter 475-600 nm. Scale bar= 50 μm. (D) Fluorescent imaging and Nissl's staining for evaluating neuronal damage in the whole hippocampal region (HIP), and CA1, CA3, dentate gyrus (DG) sub-regions after KA administration. Scale bar =500 μm. (E) Quantification of images in (B). Note that fluorescence signals in KA-induced epilepsy mice were significantly higher

than those in the healthy WT group. Treatment with GSK-LSD1 would help to inhibit the overexpressed HCHO. (F) Quantification of images in (D). (G) Quantification of images of the CA1, CA3, and DG sub-regions of hippocampus in (D). The relative ratio of fluorescence intensity of the brains shown in A and B was quantified using the IVIS Spectrum imaging system. The data are expressed as the mean ± S.D (n=3). Statistical analyses performed with Ordinary one-way or two-way ANOVA test to the control with DMSO treatment, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Figure S12. The TP fluorescence images of 3D volume-rendered and different depths of maximum hippocampal surface in brain tissues from Fig. 3C. Green = **FATP-1** channel. Fluorescence excitation filter MP 750 nm, emission window: 450-600 nm. Scale bar = 100 μm.

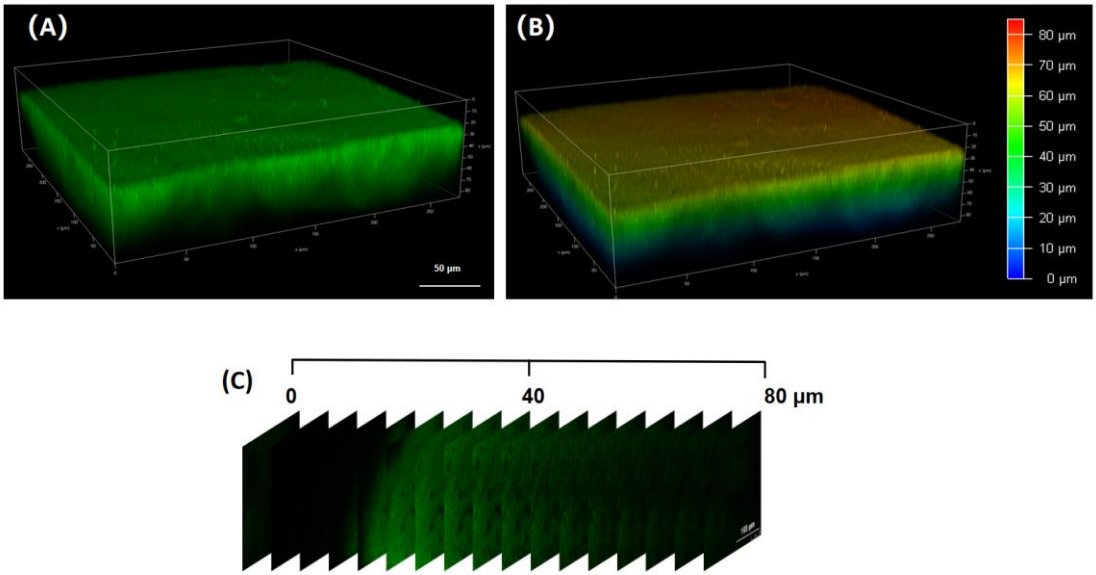


Figure S13. (Top left) The TP fluorescence images of 3D volume-rendered of maximum hippocampal surface in KA (12 h)-induced epileptic mice brain tissues from Fig. 3B. Green, **FATP-1** channel. Excitation, TP 750 nm. Emission window, 450-600 nm. Scale bar = 50 μm. (Top right) Fluorescence heat maps at different depths of the brain sections shown in (left). (Down) Z-stack images of the 3D spheroid from 0–40 μm under excitation at 750 nm. Scale bar = 100 μm.

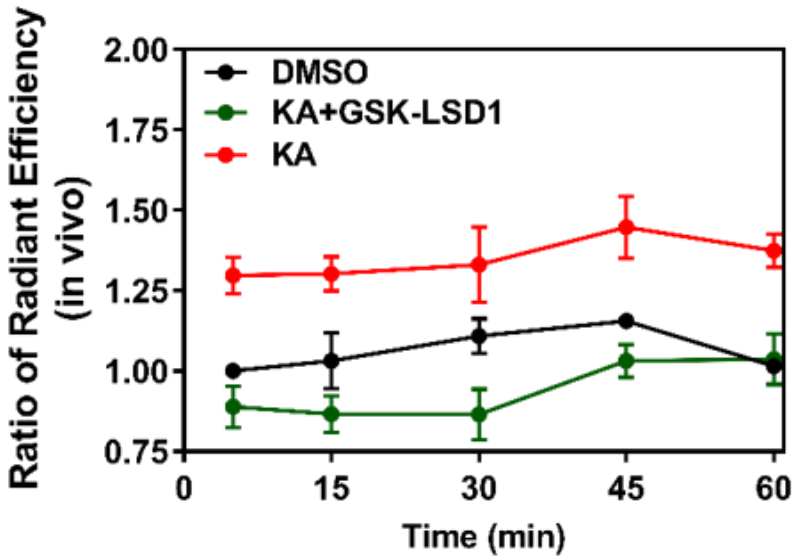


Figure S14. Quantification of images in Figure 3A. Note that fluorescence signals in KA-induced epilepsy mice were significantly higher than those in the healthy WT group. Treatment with GSK-LSD1 would help to inhibit the overexpressed FA. All the data represent the average of three independent experiments.

14. NMR and MS Spectra

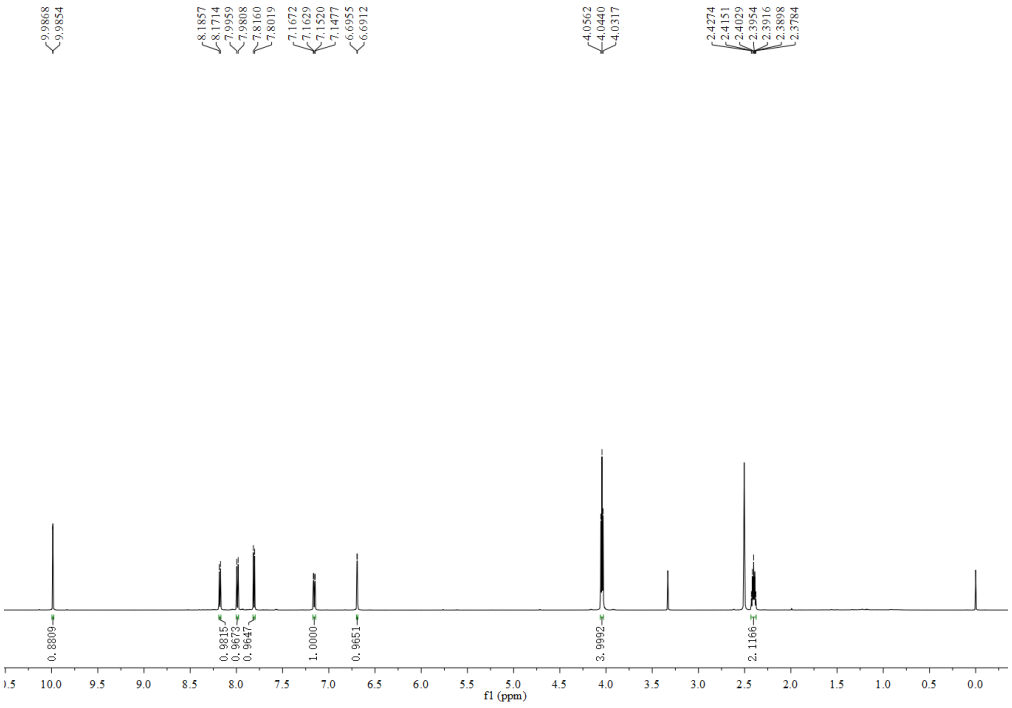


Figure S15. ^{1}H NMR of compound **3-1** (600 MHz, in DMSO-d_6).

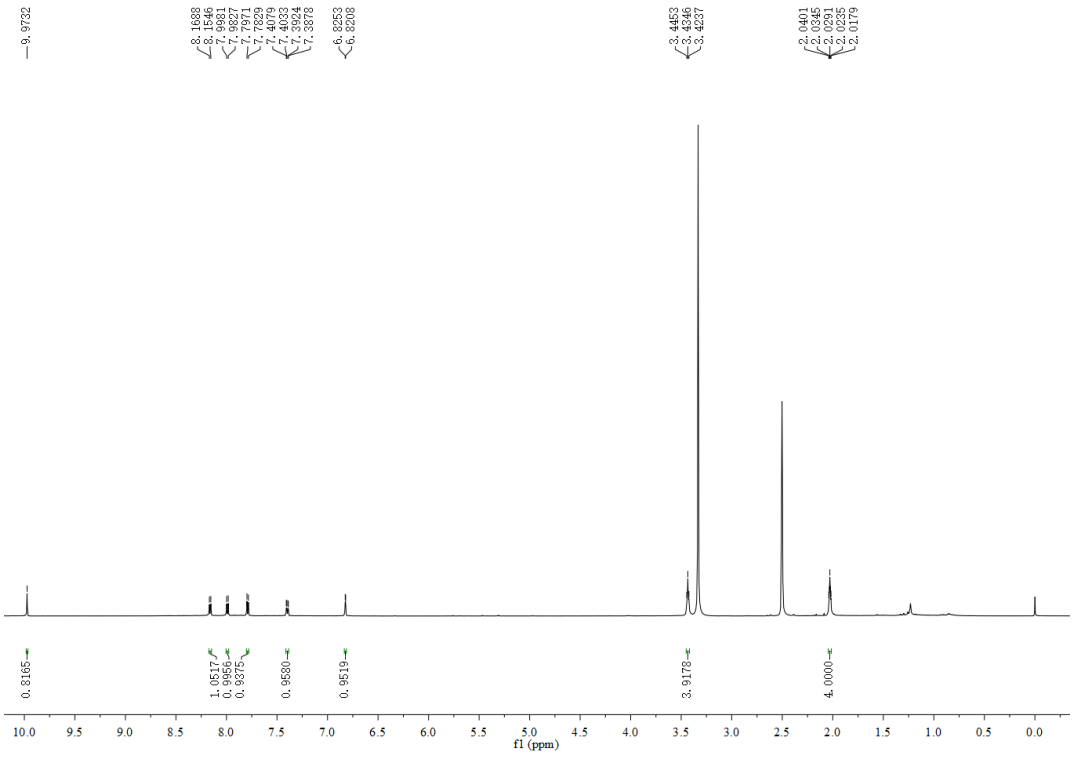


Figure S16. ^{1}H NMR of compound **3-2** (600 MHz, in DMSO-d_6).

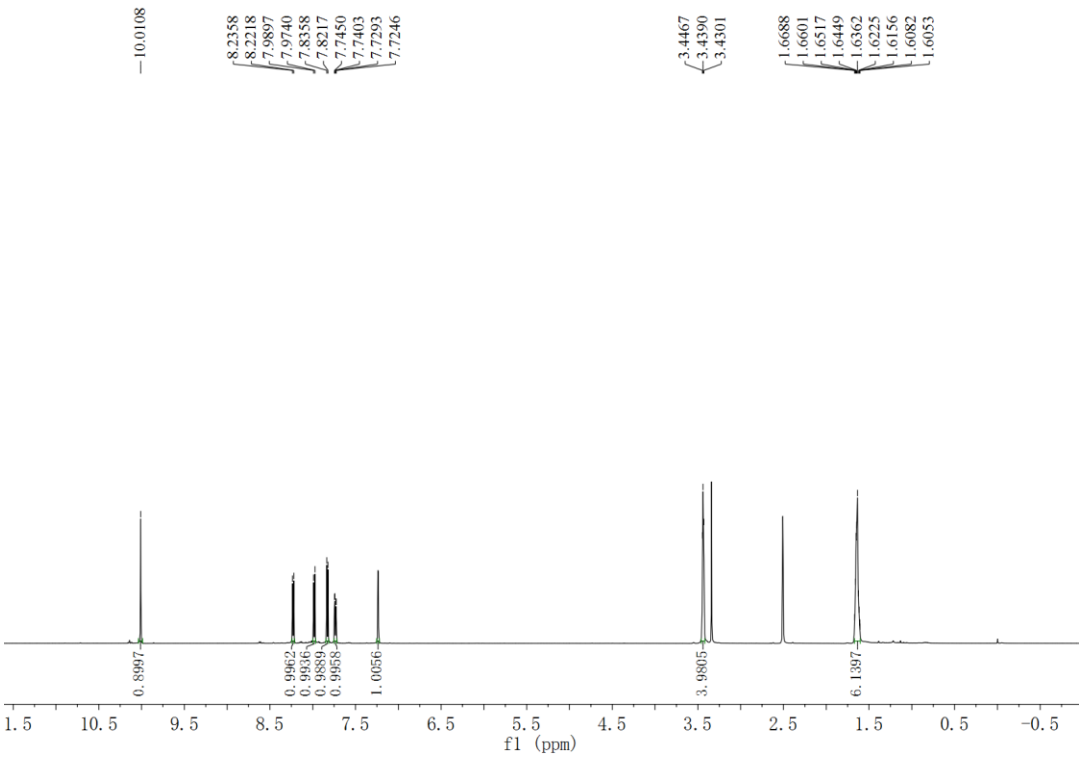


Figure S17. ^{1}H NMR of compound **3-3** (600 MHz, in DMSO-d_6).

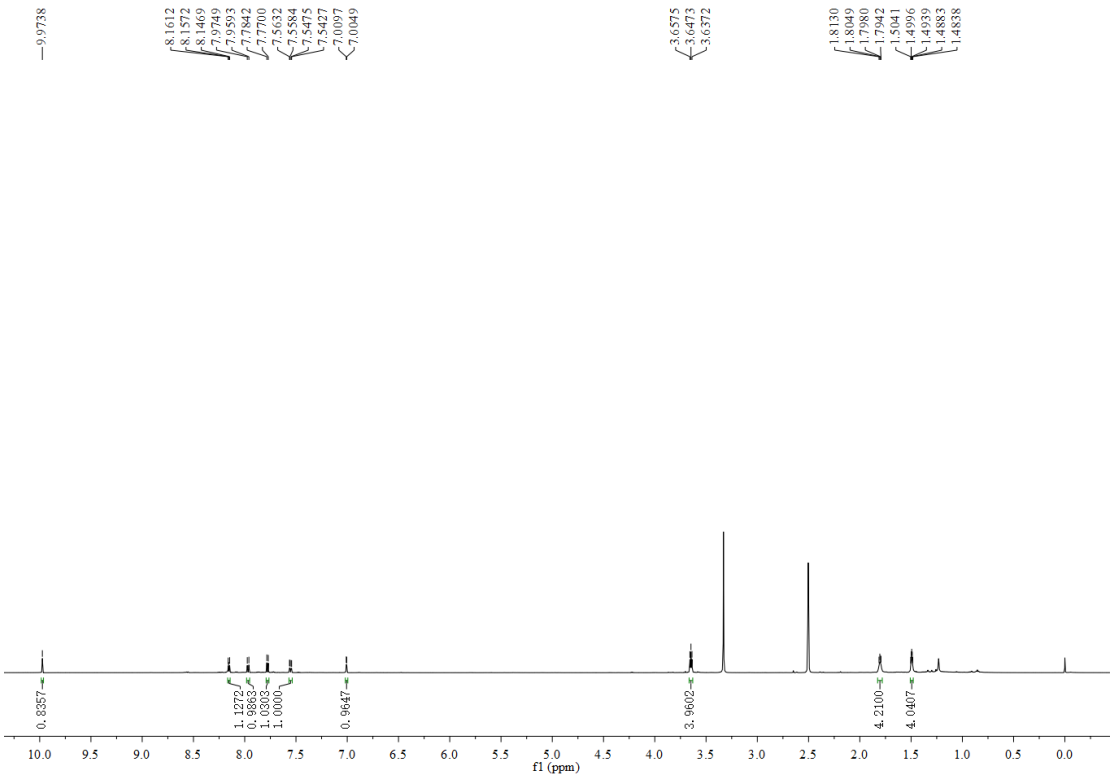


Figure S18. ^{1}H NMR of compound **3-4** (600 MHz, in DMSO-d_6).

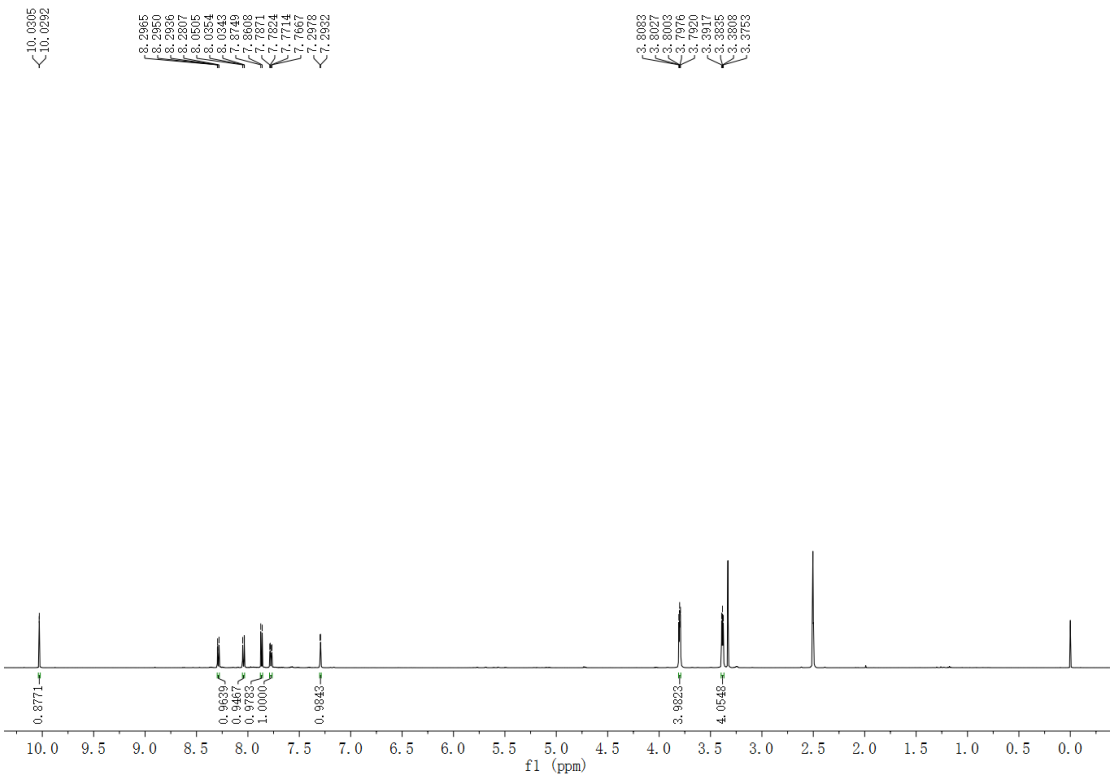


Figure S19. ^{1}H NMR of compound **3-5** (600 MHz, in DMSO-d_6).

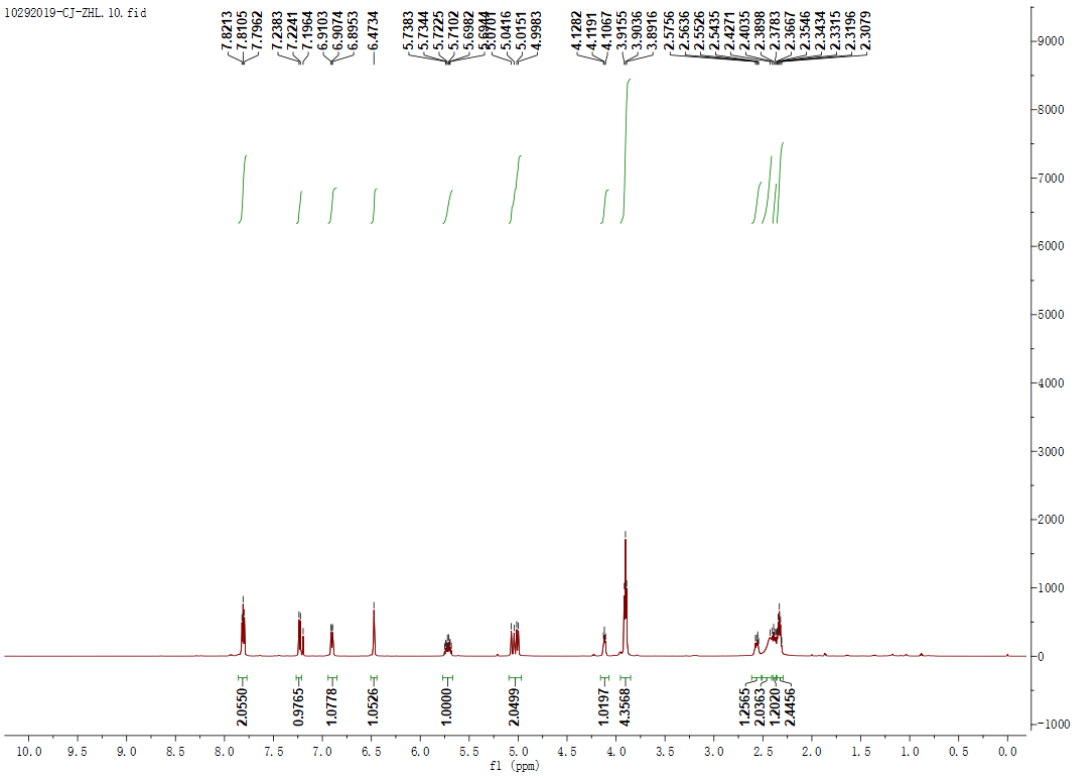


Figure S20. ^{1}H NMR of compound **FATP-1** (600 MHz, in $CDCl_3$).

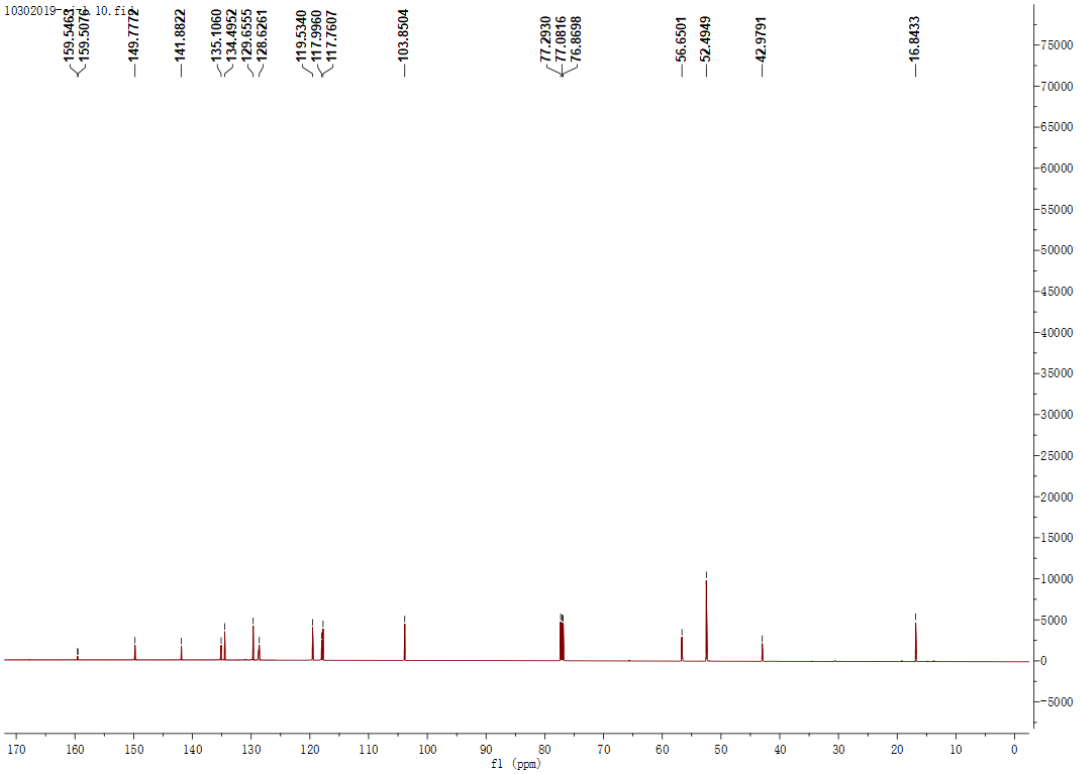


Figure S21. ^{13}C NMR of compound **FATP-1** (151 MHz, in $CDCl_3$).

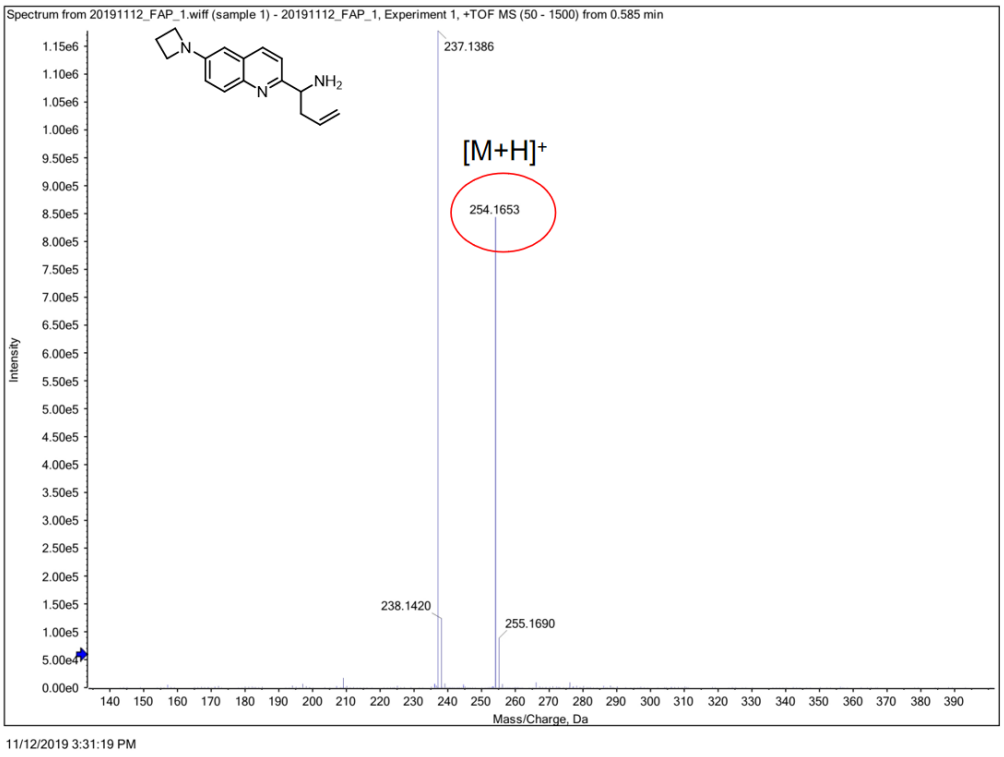


Figure S22. The ESI-MS spectrum of compound **FATP-1** (calculated for $C_{16}H_{20}N_3$ [M+H]+ 254.1657, found 254.1653).

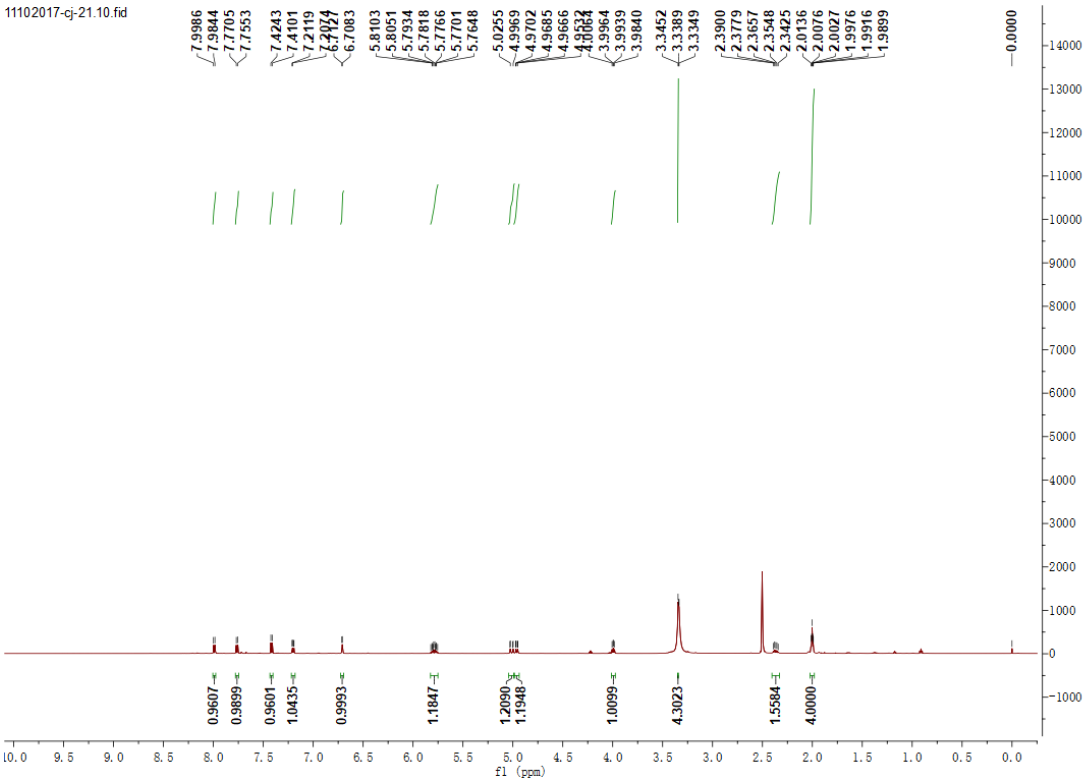


Figure S23. ^{1}H NMR of compound **FATP-2** (600 MHz, in DMSO-d_6).

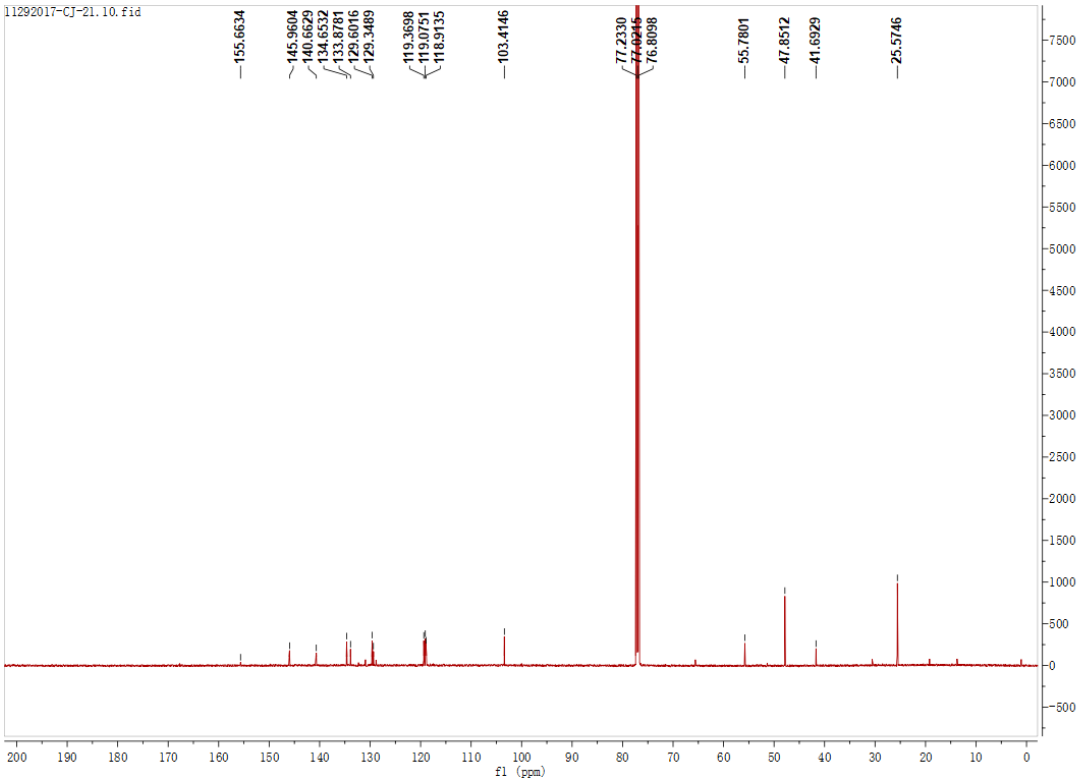


Figure S24. ^{13}C NMR of compound **FATP-2** (151 MHz, in DMSO-d_6).

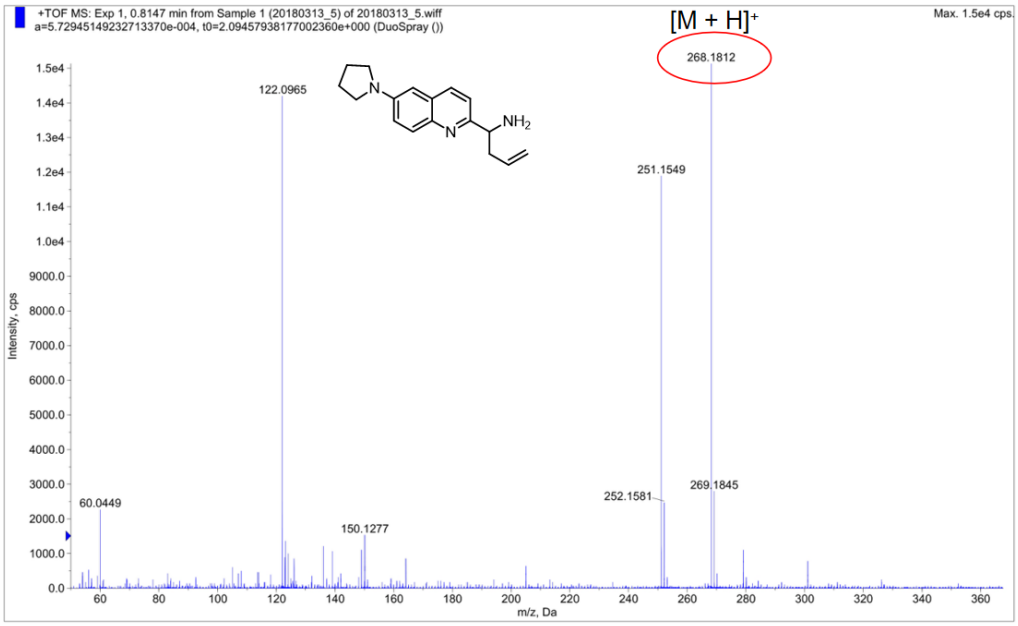


Figure S25. The ESI-MS spectrum of compound **FATP-2** (calculated for $C_{17}H_{22}N_3$ [M+H]+ 268.1814, found 268.1812)

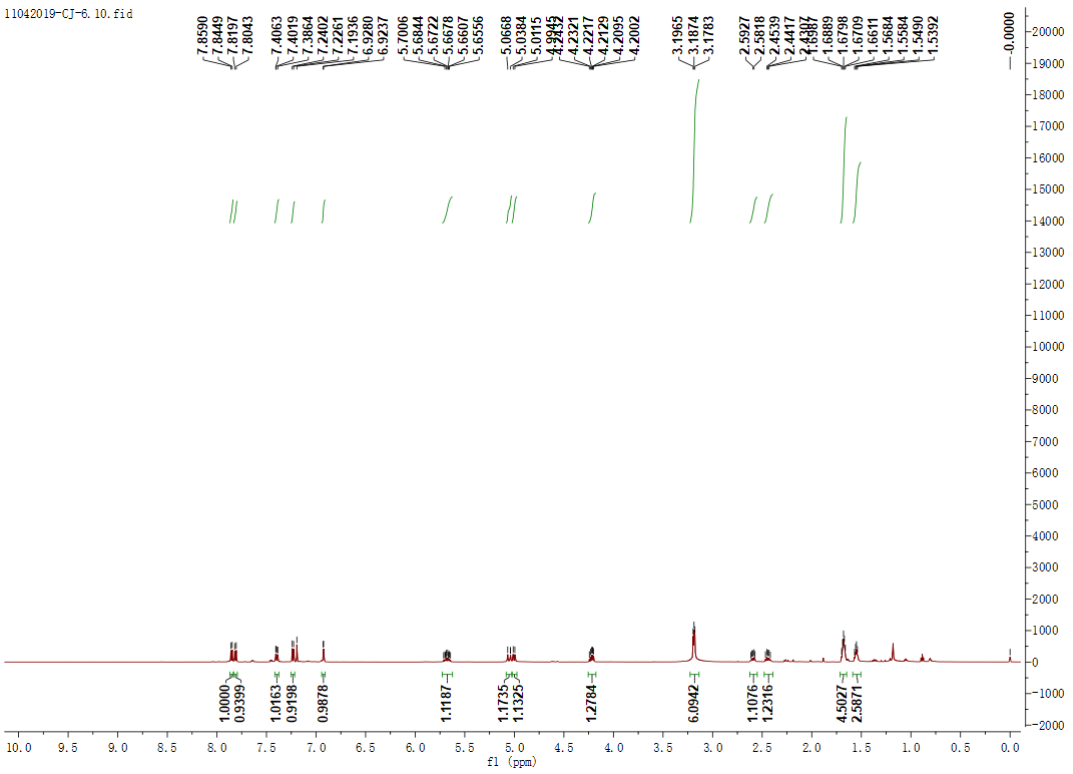


Figure S26. ^{1}H NMR of compound **FATP-3** (600 MHz, in $CDCl_3$).

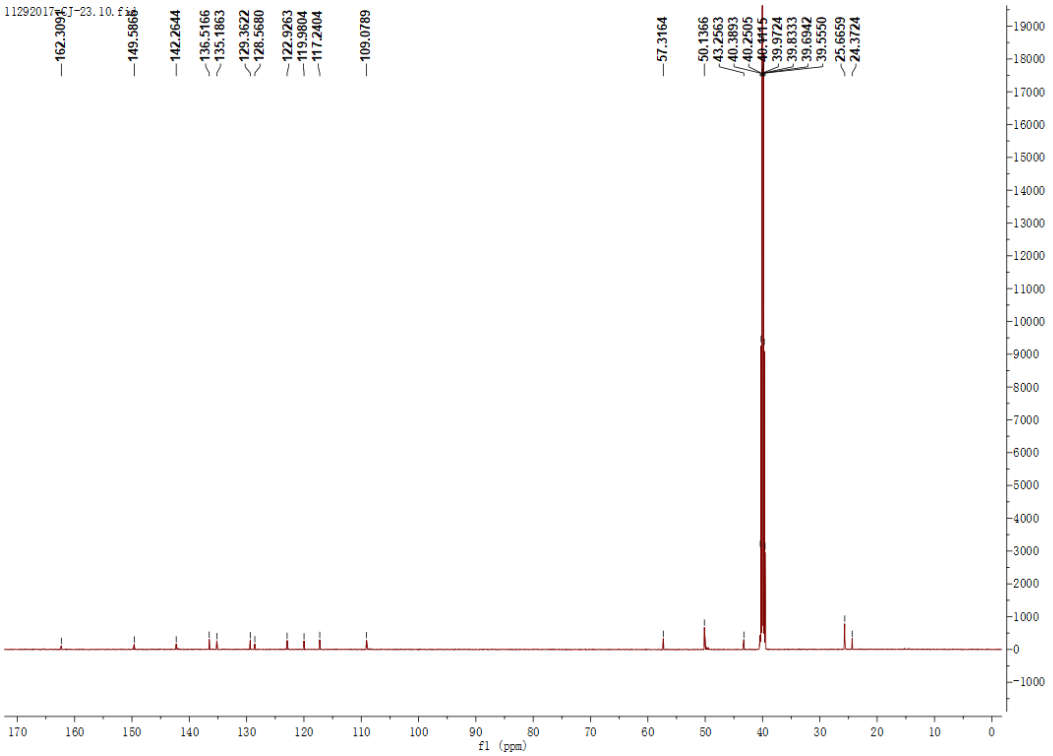


Figure S27. ^{13}C NMR of compound **FATP-3** (151 MHz, in DMSO-d_6).

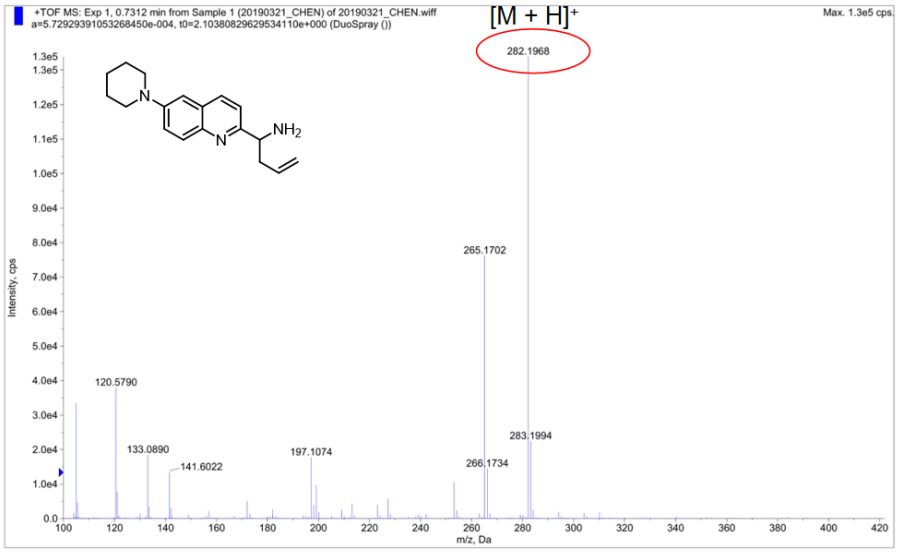


Figure S28. The ESI-MS spectrum of compound **FATP-3** (calculated for $C_{18}H_{24}N_3$ [M+H]+ 282.1970, found 282.1968).

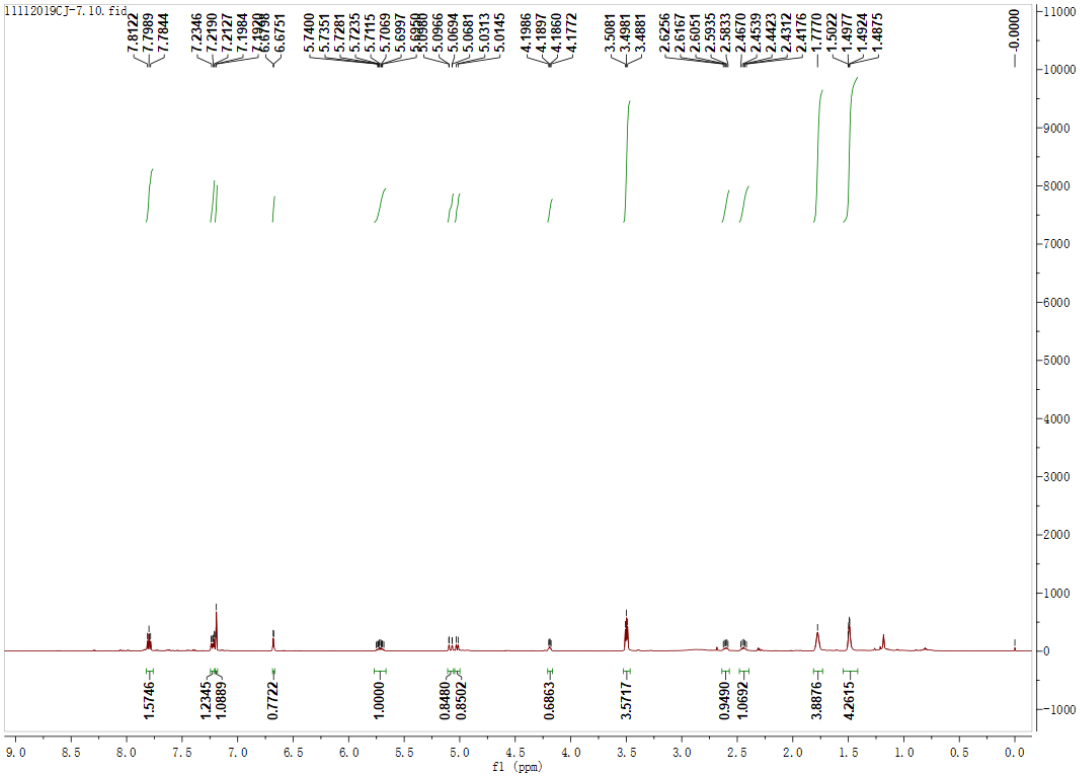


Figure S29. ^{1}H NMR of compound **FATP-4** (600 MHz, in $CDCl_3$).

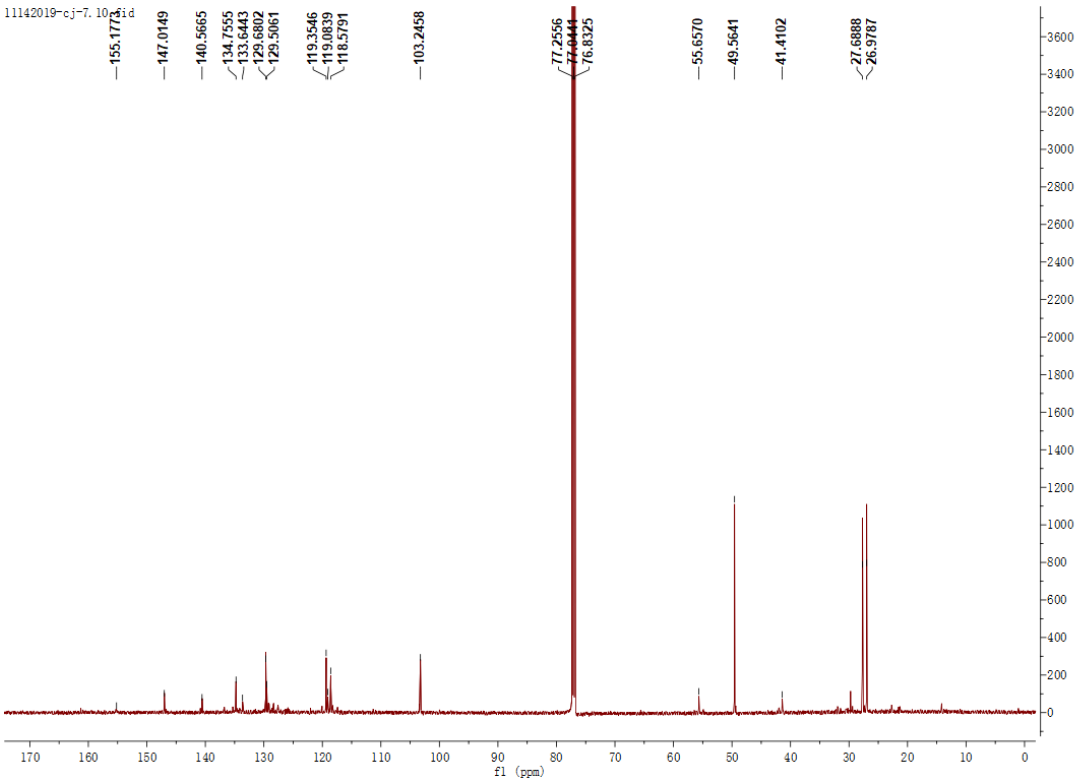


Figure S30. ^{13}C NMR of compound **FATP-4** (151 MHz, in $CDCl_3$).

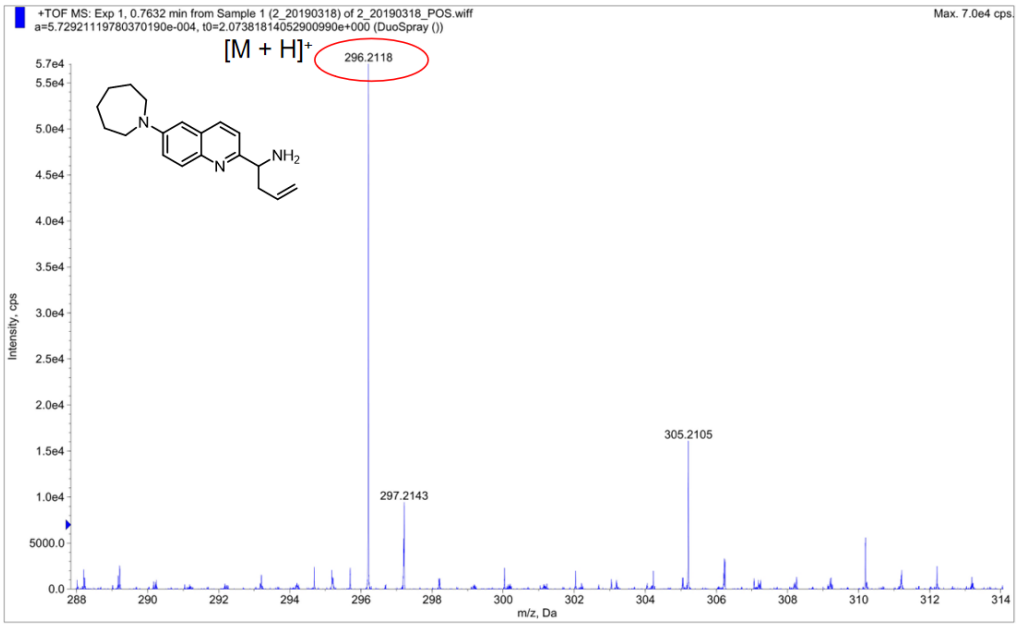


Figure S31. The ESI-MS spectrum of compound **FATP-4** (calculated for $C_{19}H_{26}N_3$ [M+H]+ 296.2127, found 296.2118).

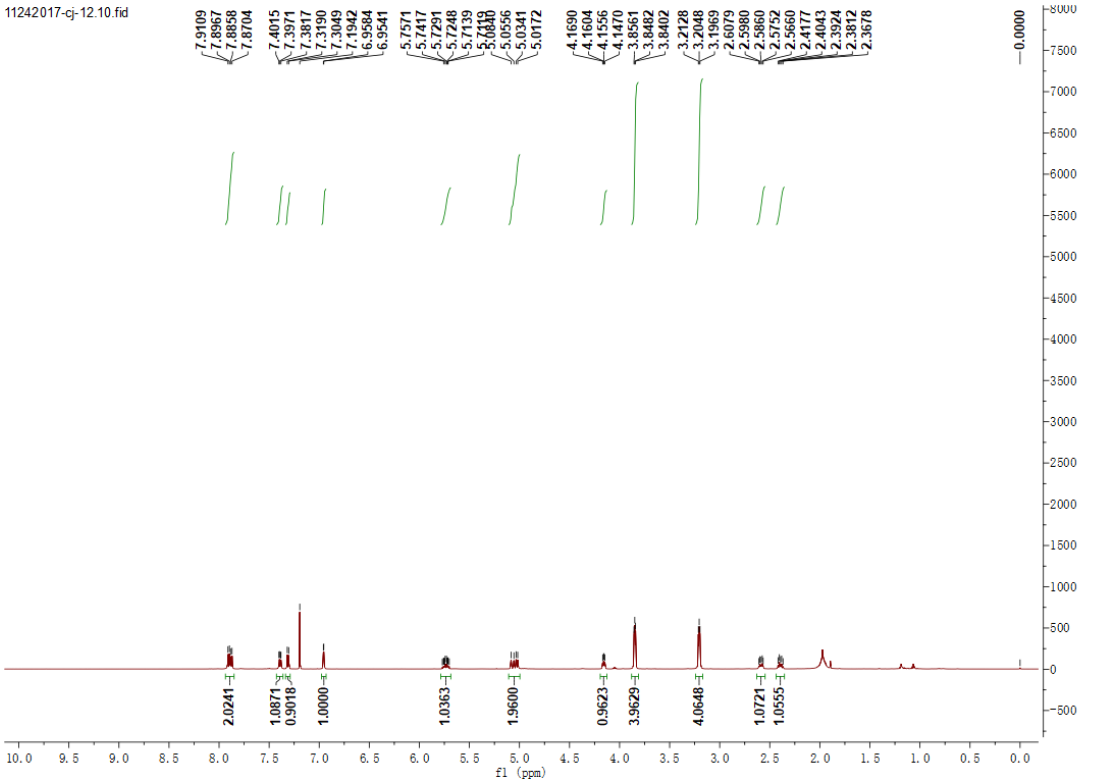


Figure S32. ^{1}H NMR of compound **FATP-5** (600 MHz, in $CDCl_3$).

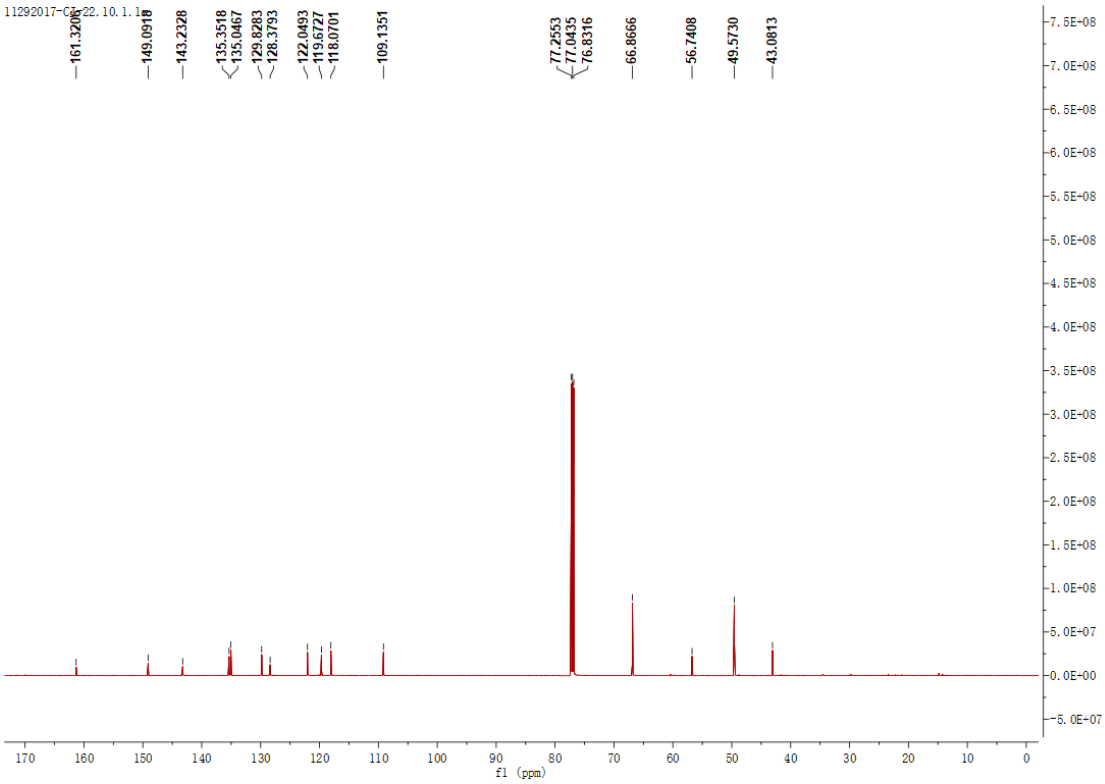


Figure S33. ^{13}C NMR of compound **FATP-5** (151 MHz, in $CDCl_3$).

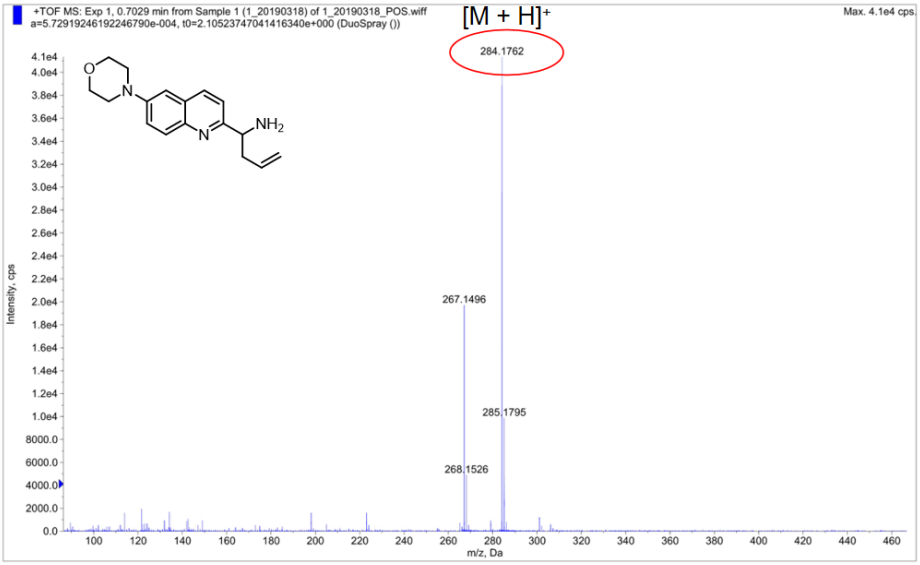


Figure S34. The ESI-MS spectrum of compound **FATP-5** (calculated for $C_{17}H_{22}N_3O$ [M+H]+ 284.1763, found 284.1762)