SUPPORTING INFORMATION

Synthesis, characterization and in vivo evaluation of desmethyl anethole trithione phosphate prodrug for ameliorating cerebral ischemia-reperfusion injury in rats

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Supporting Information

Contents

I. The LC analysis method

Table S1. The LC analysis method (The analysis condition)

The LC analysis condition	
Instrument	LC-MS/MS-12 (TQ5500, Triple quad)
Sample	Rat plasma
Internal standard	Tolbutamide
LC analysis	ESI: positive; MRM detection; OS:Q1/Q3 Masses: 224.9/159.7 Da; IS: Q1/Q3 Masses: 269.2/106.1 Da

Table S2. The LC analysis method (the separation condition)

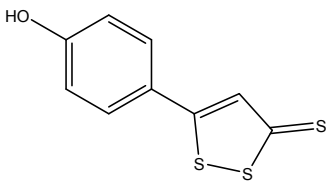
The separation condition	
Mobile phase A	0.1%Formic acid in water
Mobile phase B	0.1%Formic acid in acetonitrile
Weak wash	0.1%FA in MeOH/Water(v:v=1:1)
Strong wash	MeOH:ACN:IPA:0.1%FA in water(v:v:v:v,1:1:1:1)

The separation	Time (min)	Moblie phase B (%)
	0.01	20
	0.20	20
	0.50	55
	1.10	55
	1.30	90
	1.5	90
	1.51	20
	1.60	20
Column	ACQUITY UPLC BEH C18 1.7um (2.1*50mm)	
Oven	40℃	
Flow rate	0.50mL/min	
Retention time	OS:1.03 min, IS: 0.96 min	

II. General information

All reactions were carried out in air unless otherwise stated. Column chromatography was performed using silica gel (200-300 mesh). 1H NMR and 13C NMR spectra were recorded on Bruker-AV (400 and 100 MHz, respectively) instrument internally referenced to tetramethylsilane (TMS) or chloroform signals. Mass spectra were measured on Bruker-Micro OTOF QII GC-MS instrument (EI). High-resolution mass spectra were recorded at Chengdu Institute of Biology. The structure of the compound was further confirmed by comparing the ^{1}H NMR, ^{13}C NMR and HRMS data of the compound with the literature. Reagents were used as received or prepared by our laboratory.

III. Characterization data of ATX



Chemical Formula: $C_9H_6OS_3$
Exact Mass: 225.95808

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.49 (s, 1H), 7.78 (d, J = 8.4 Hz, 2H), 7.69 (s, 1H), 6.90 (d, J = 8.5 Hz, 2H).

^{13}C NMR (101 MHz, DMSO) δ 214.52, 174.35, 162.10, 133.66, 129.05, 122.50, 116.76.

HRMS (ESI, m/z): calcd for $C_9H_6OS_3$(M+H$^+$) 226.96538, found 226.96526

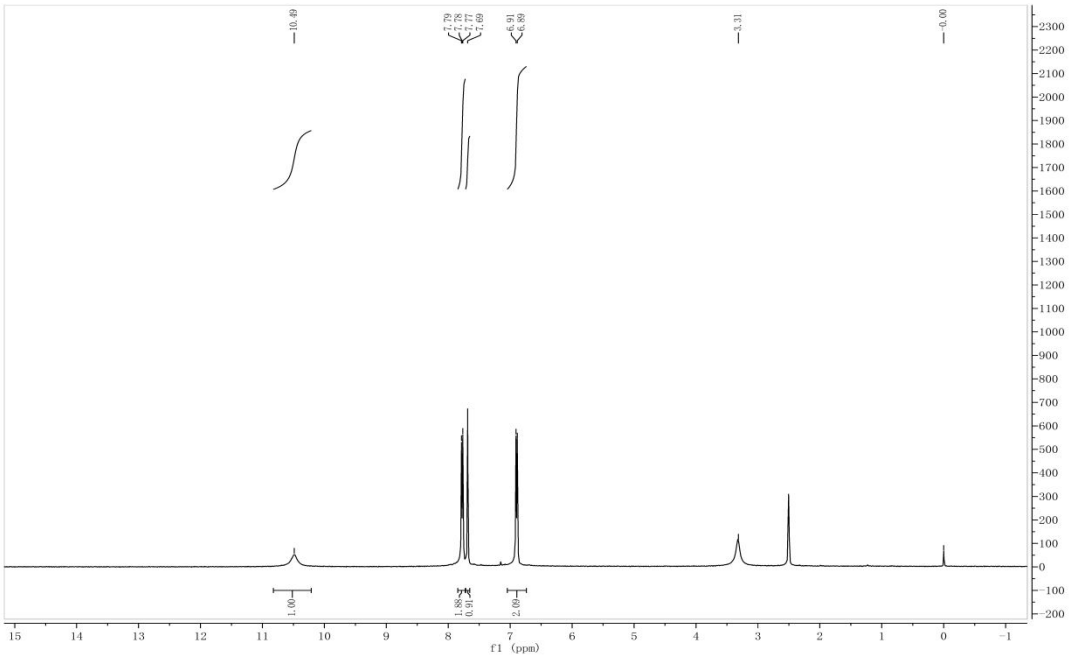


Figure S1. Proton NMR for ATX

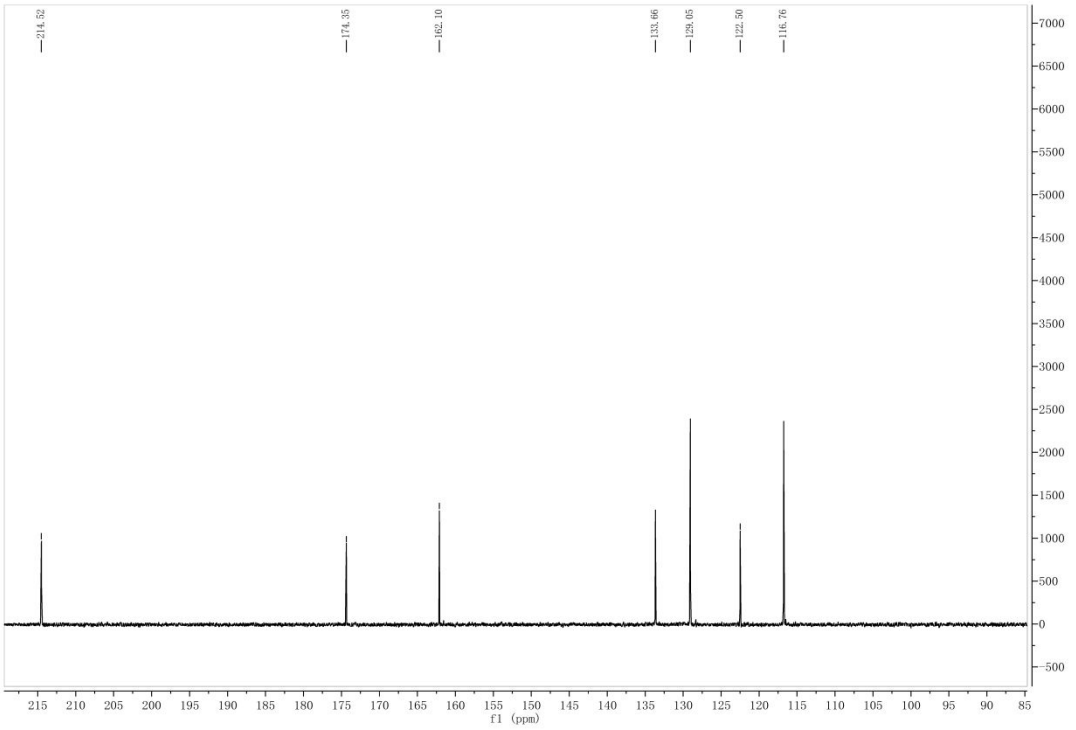


Figure S2. Carbon NMR for ATX

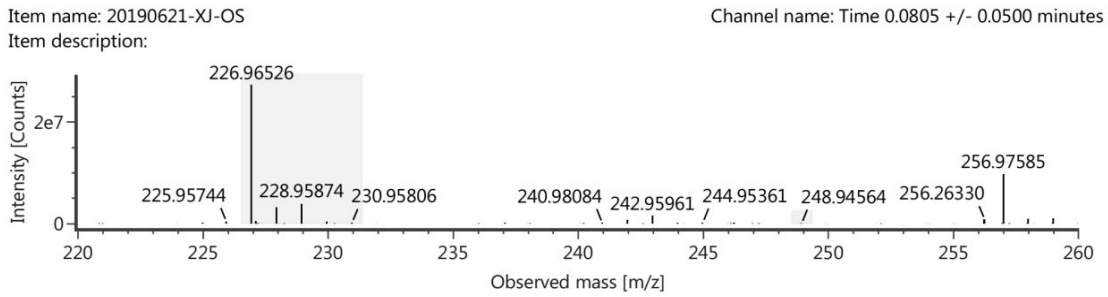
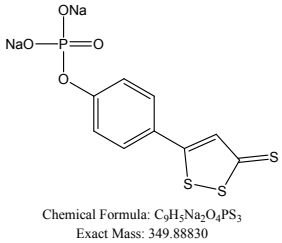


Figure S3. mass spectra for ATX

Ⅳ. **Characterization data of ATXP**



Chemical Formula: $C_9H_5Na_2O_4PS_3$
Exact Mass: 349.88830

^{1}H NMR (400 MHz, D_2O) δ 7.59 (d, J = 8.3 Hz, 2H), 7.46 (s, 1H), 7.18 (d, J = 8.3 Hz, 2H). ^{13}C NMR (101 MHz, D_2O) δ 214.13, 176.33, 158.13, 158.07, 134.85, 128.79, 124.75, 120.77, 120.71.

HRMS (ESI, m/z): calcd for $C_9H_5Na_2O_4PS_3$($M+H^+$) 350.89030, found 350.89575.

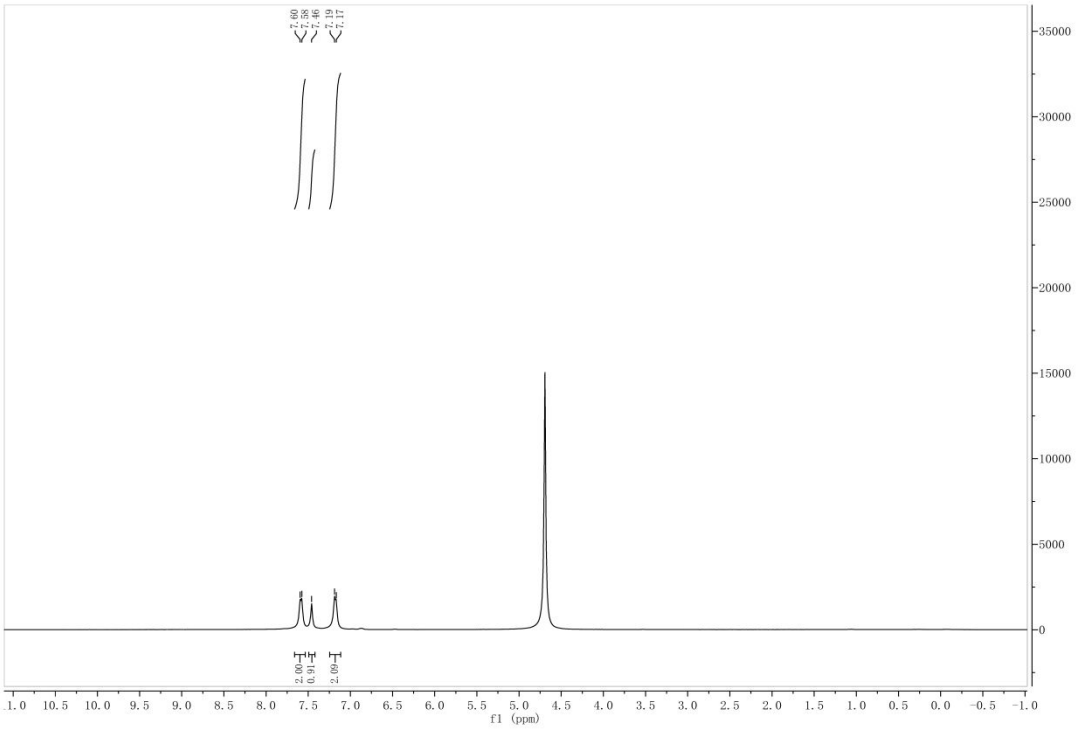

7.60
7.58
7.46
7.19
7.17
35000
30000
25000
20000
15000
10000
5000
0
2.00
0.91
2.09
f1 (ppm)

Figure S4. Proton NMR for ATXP

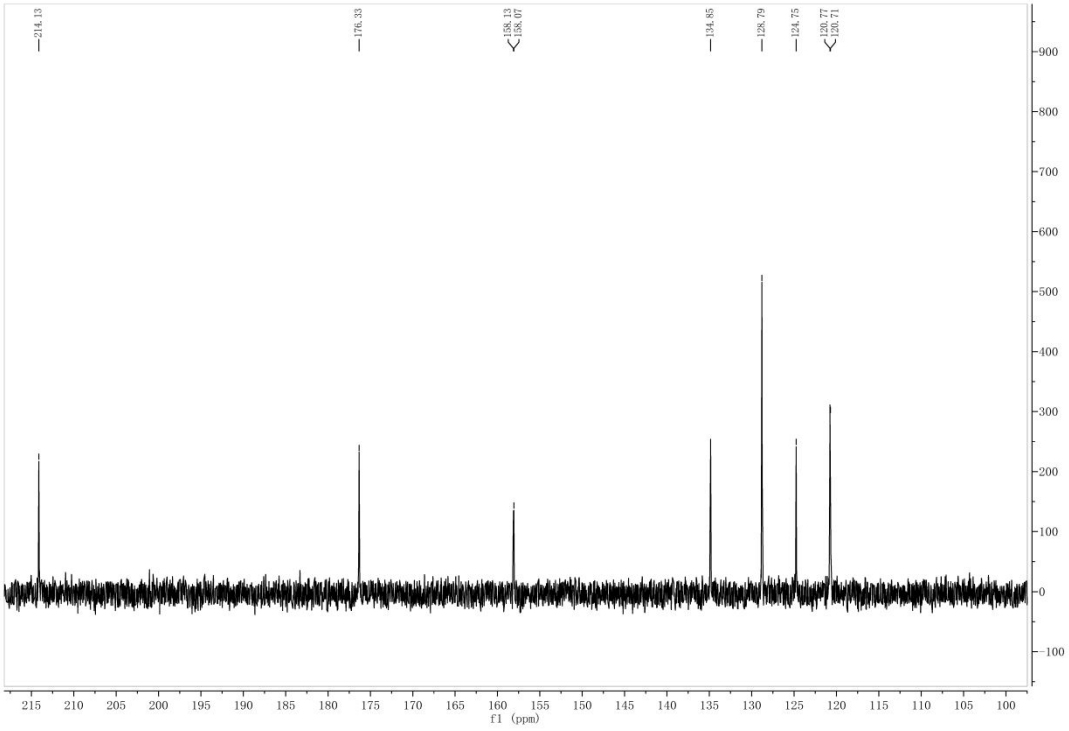


Figure S5. Carbon NMR for ATXP

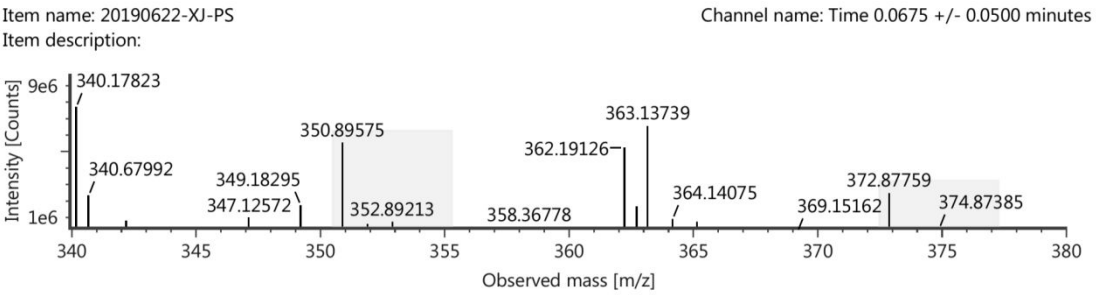


Figure S6. mass spectra for ATXP

V. Determination of the purity by HPLC

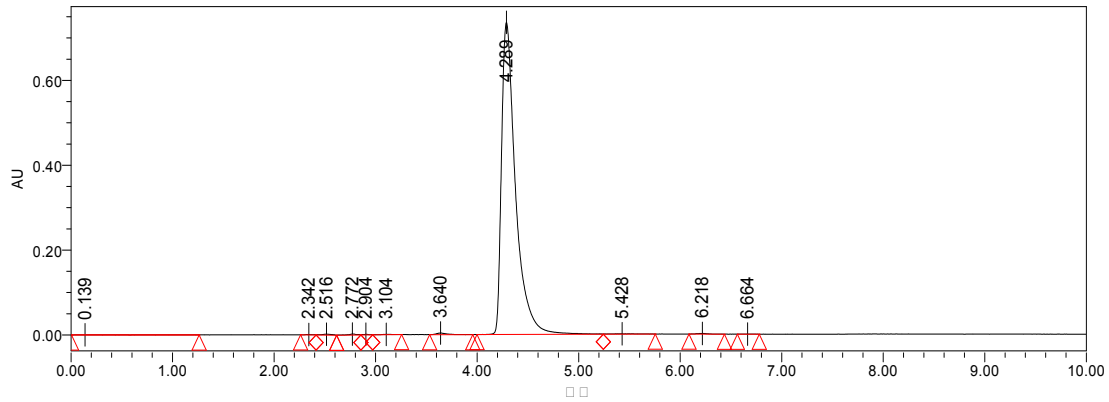


Figure S7. Purity of ATXP analyzed by HPLC

Table S3. HPLC analysis for ATXP

Number	retention time (min)	Peak area	% Area	Peak height
1	0.139	18260	0.25	488
2	2.342	6168	0.09	1115
3	2.516	17423	0.24	2373
4	2.772	16452	0.23	2515
5	2.904	6262	0.09	1591
6	3.104	7052	0.10	909
7	3.640	26667	0.37	3796
8 (ATXP)	4.289	7064692	98.17	735774
9	5.428	16567	0.23	857
10	6.218	13424	0.19	1571
11	6.664	3222	0.04	408

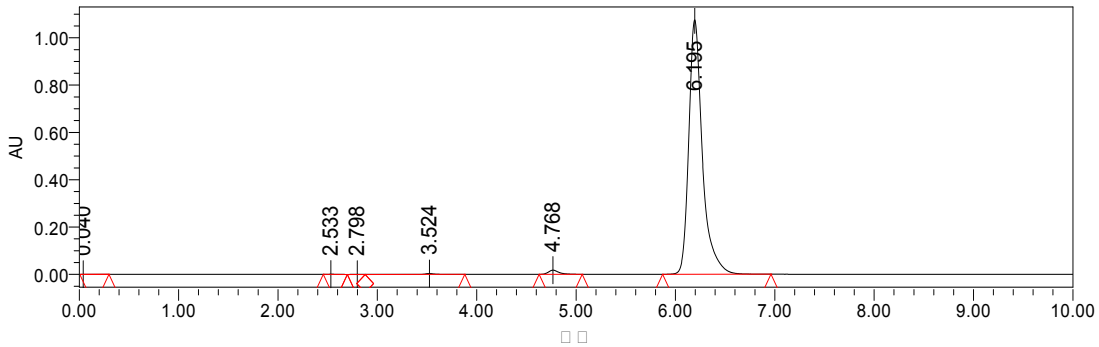


Figure S8. Purity of ATX analyzed by HPLC

Table S4. HPLC analysis for ATX

Number	retention time (min)	Peak area	% Area	Peak height
1	0.040	4313	0.04	535
2	2.533	7663	0.07	1392
3	2.798	7450	0.07	1011
4	3.524	48792	0.47	3815
5	4.768	131788	1.26	17791
6 (ATX)	6.195	10247523	98.09	1075724