

# **Supporting Information**

## **Fascaplysin Derivatives Are Potent Multi-target Agents Against Alzheimer's Disease: *in vitro* and *in vivo* Evidence**

Hanbo Pan<sup>1</sup>, Hongda Qiu<sup>2</sup>, Ke Zhang<sup>1</sup>, Panpan Zhang<sup>1</sup>, Weida Liang<sup>1</sup>, Mengxiang Yang<sup>1</sup>, Chenye Mou<sup>1</sup>, Miaoman Lin<sup>2</sup>, Ming He<sup>2</sup>, Xiao Xiao<sup>1</sup>, Difan Zhang<sup>1</sup>, Haixing Wang<sup>3</sup>, Fufeng Liu<sup>4</sup>, Yongmei Li<sup>2</sup>, Haixiao Jin<sup>5</sup>, Xiaojun Yan<sup>5</sup>, Hongze Liang<sup>2,\*</sup> and Wei Cui<sup>1,4,\*</sup>

<sup>1</sup> Ningbo Key Laboratory of Behavior Neuroscience, Zhejiang Province Key Laboratory of Pathophysiology, School of Medicine, Ningbo University, Ningbo, 315211, PR China

<sup>2</sup> School of Materials Science and Chemical Engineering, Ningbo University, Ningbo, 315211, PR China.

<sup>3</sup> Zhejiang Province Key Laboratory of Anesthesiology, Department of Anesthesiology, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou 325000, China.

<sup>4</sup> State Key Laboratory of Food Nutrition and Safety, College of Biotechnology, Tianjin University of Science & Technology, Tianjin 300457, China.

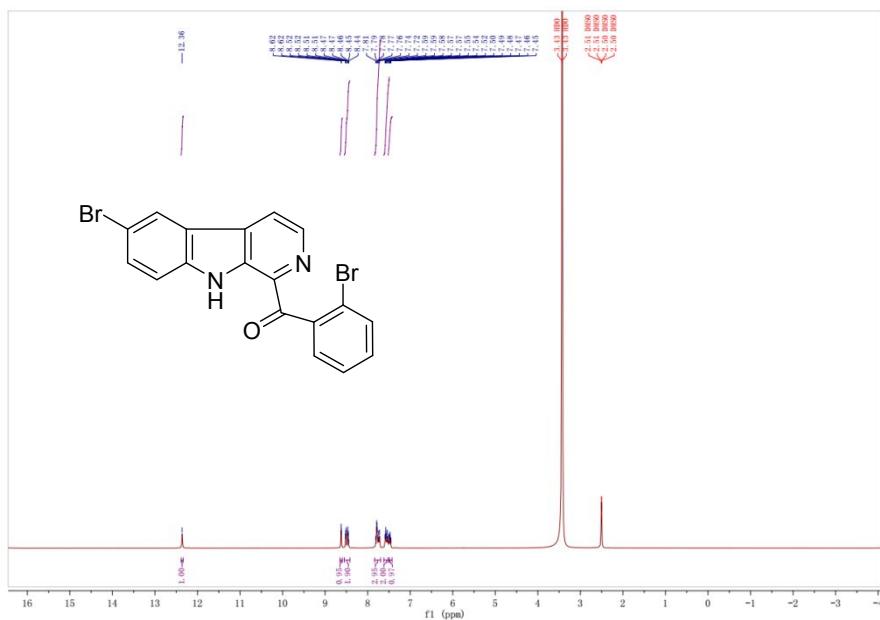
<sup>5</sup> Li Dak Sum Yip Yio Chin Kenneth Li Marine Biopharmaceutical Research Center, College of Food and Pharmaceutical Sciences, Ningbo University, Ningbo 315800, China.

\* Corresponding author: Prof. Hongze Liang, School of Materials Science and Chemical Engineering, Ningbo University, Zhejiang, China. Email: [lianghongze@nbu.edu.cn](mailto:lianghongze@nbu.edu.cn).

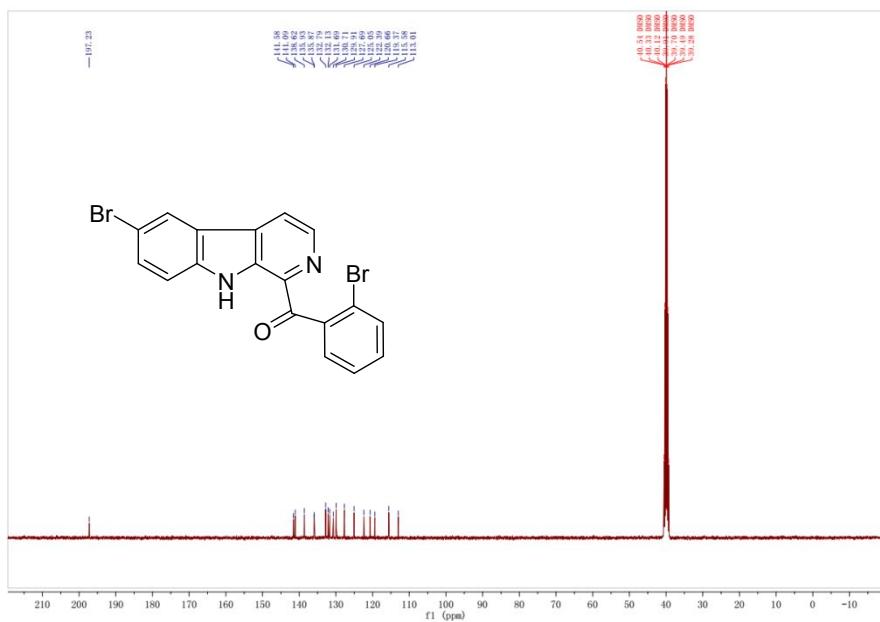
and Dr. Wei Cui, Department of Physiology, Medical School, Ningbo University. Zhejiang, China. Email: [cuiwei@nbu.edu.cn](mailto:cuiwei@nbu.edu.cn).

## Contents

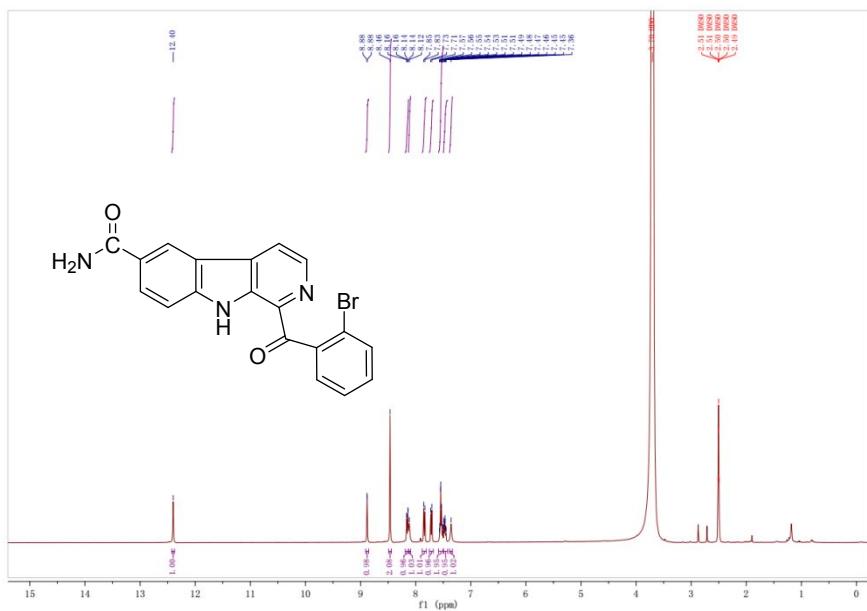
### 1. NMR spectroscopy of new compounds



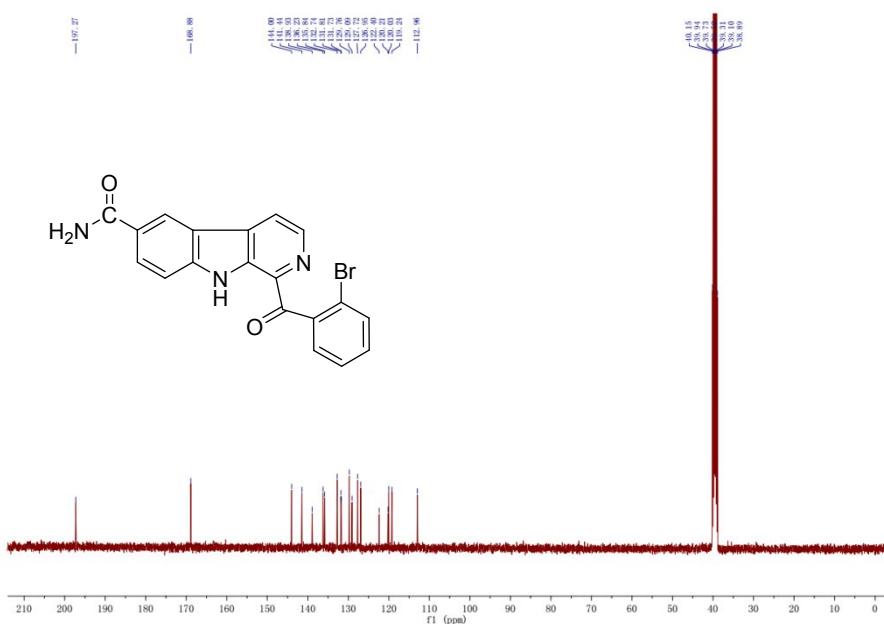
**Fig. S1** <sup>1</sup>H NMR of **1d**



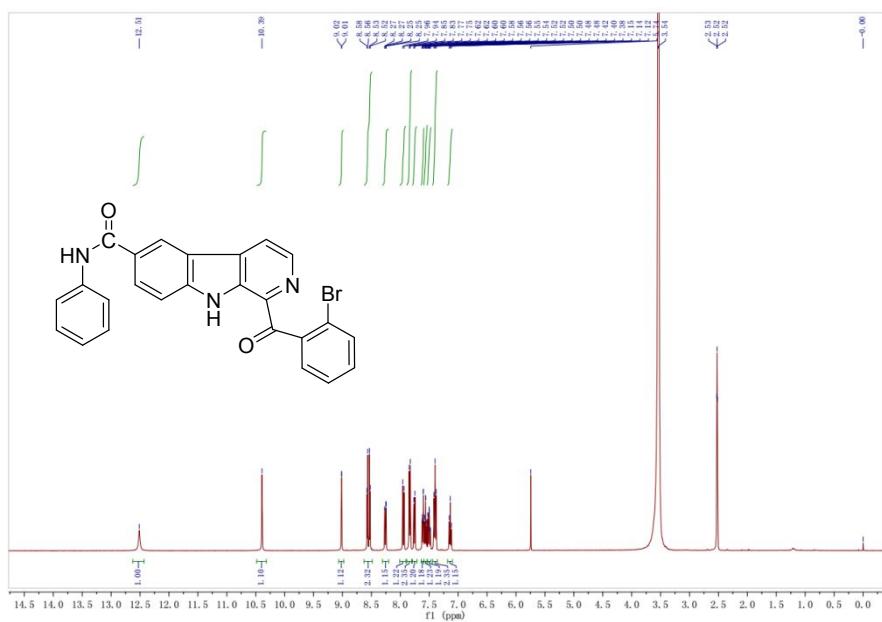
**Fig. S2** <sup>13</sup>C NMR of **1d**



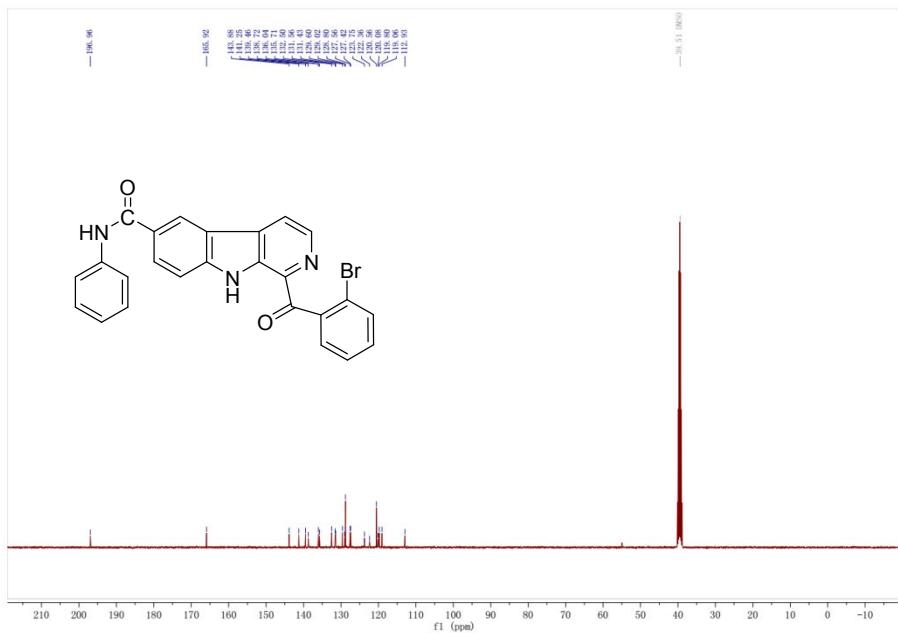
**Fig. S3**  $^1\text{H}$  NMR of **1f**



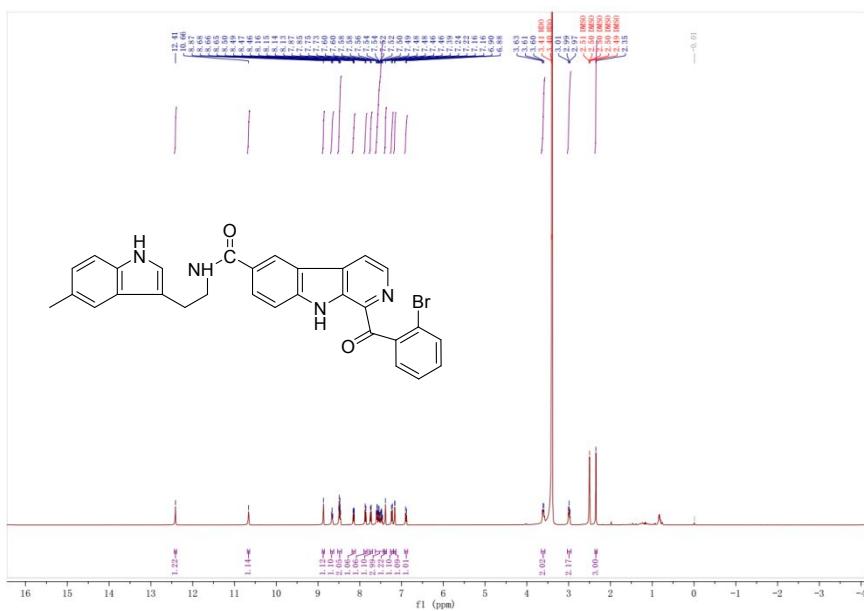
**Fig. S4**  $^{13}\text{C}$  NMR of **1f**



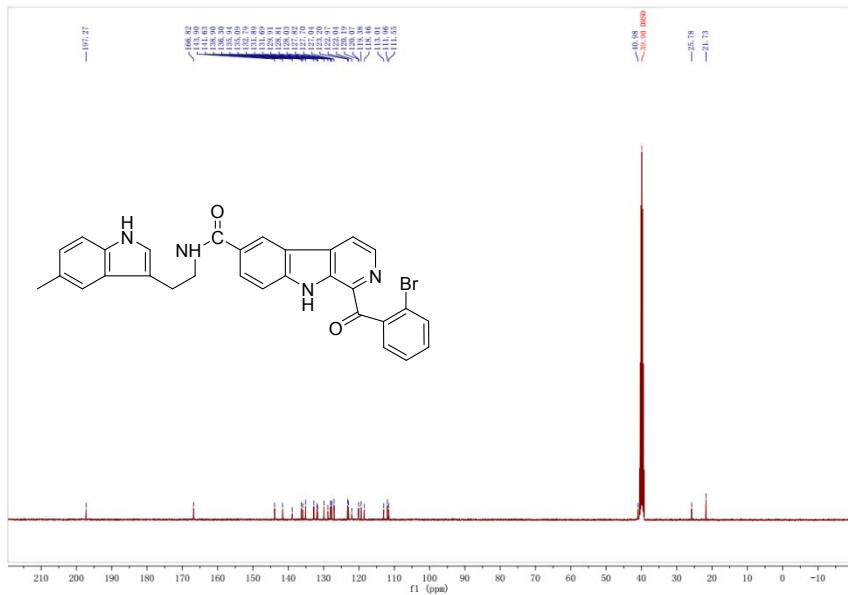
**Fig. S5**  $^1\text{H}$  NMR of **1g**



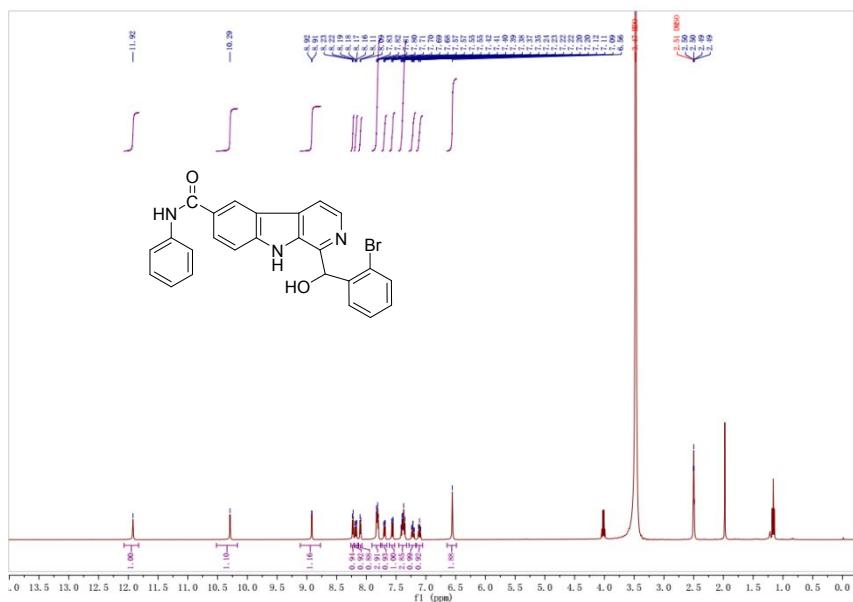
**Fig. S6**  $^{13}\text{C}$  NMR of **1g**



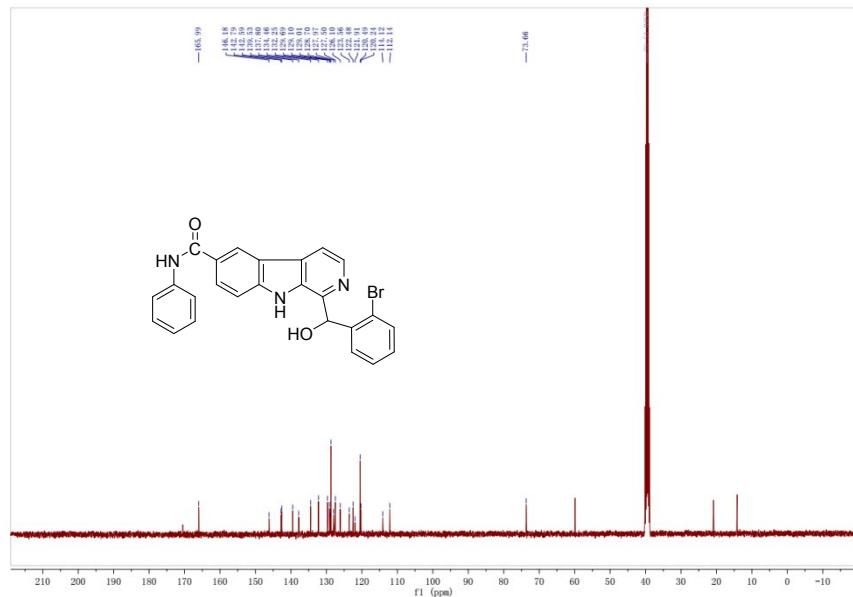
**Fig. S7**  $^1\text{H}$  NMR of **1h**



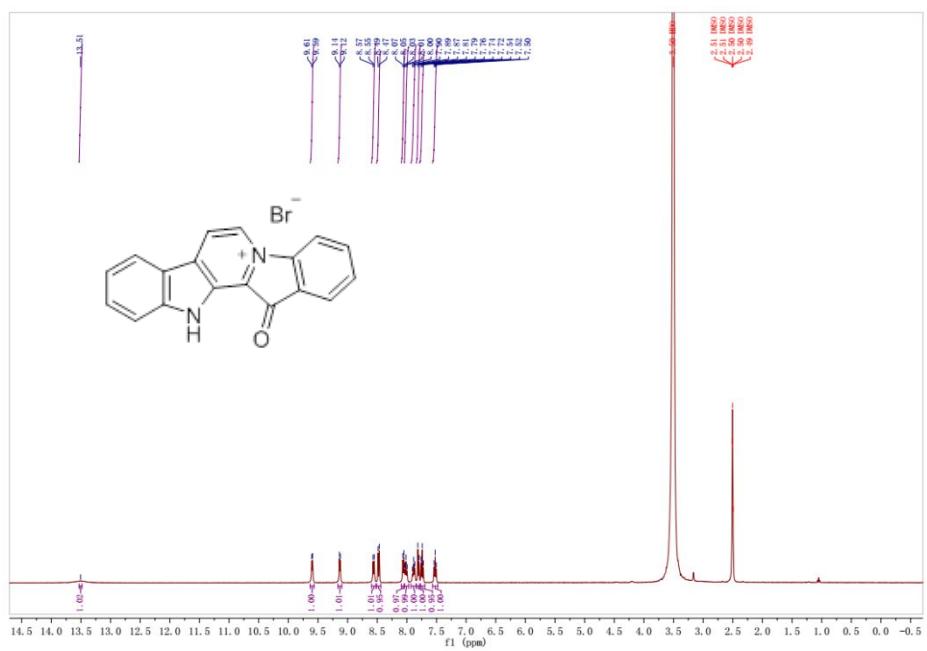
**Fig. S8**  $^{13}\text{C}$  NMR of **1h**



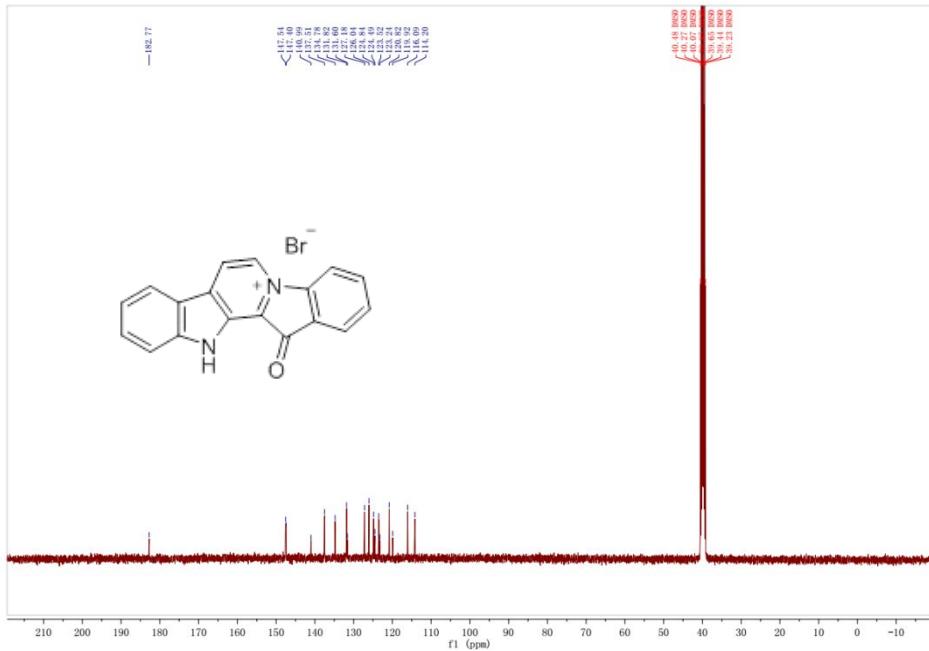
**Fig. S9** <sup>1</sup>H NMR of **1i**



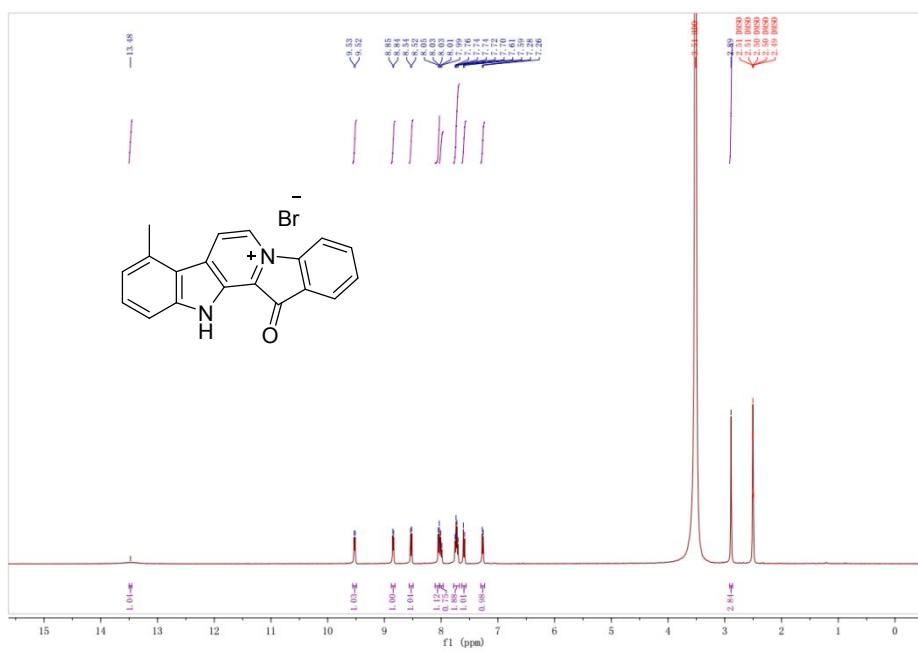
**Fig. S10** <sup>13</sup>C NMR of **1i**



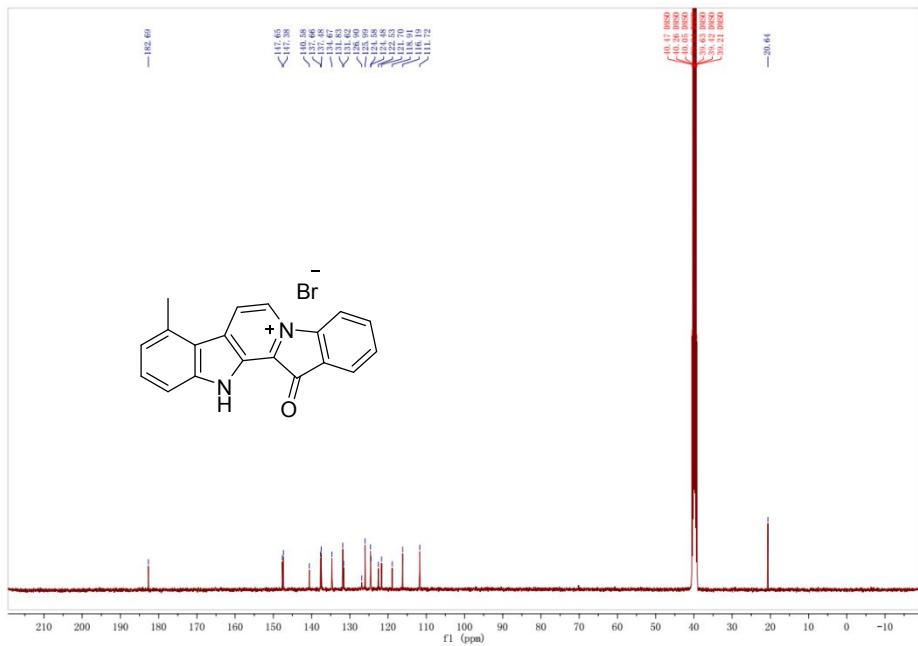
**Fig. S11**  $^1\text{H}$  NMR of **2a**



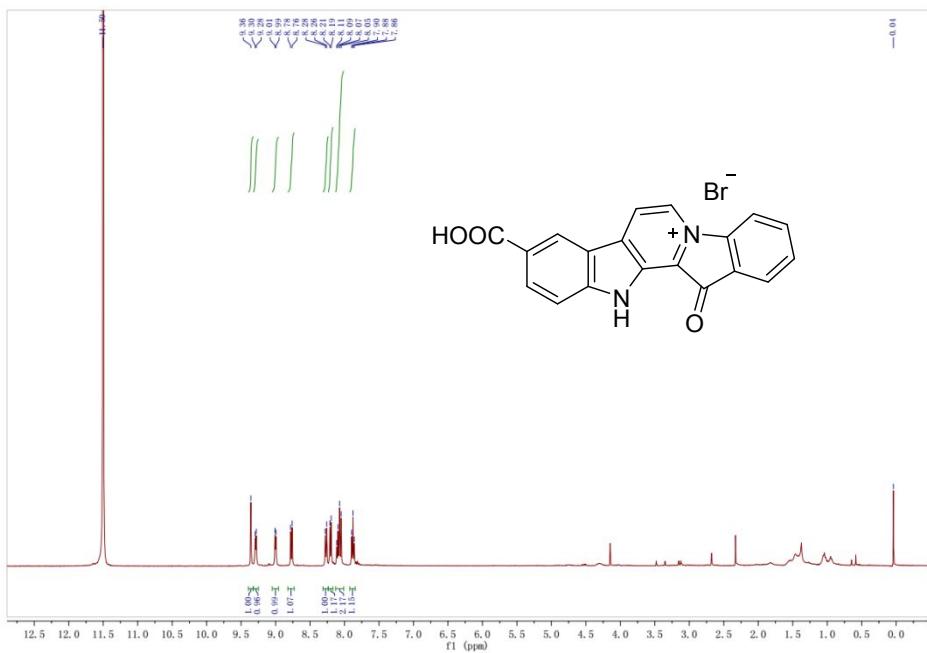
**Fig. S12**  $^{13}\text{C}$  NMR of **2a**



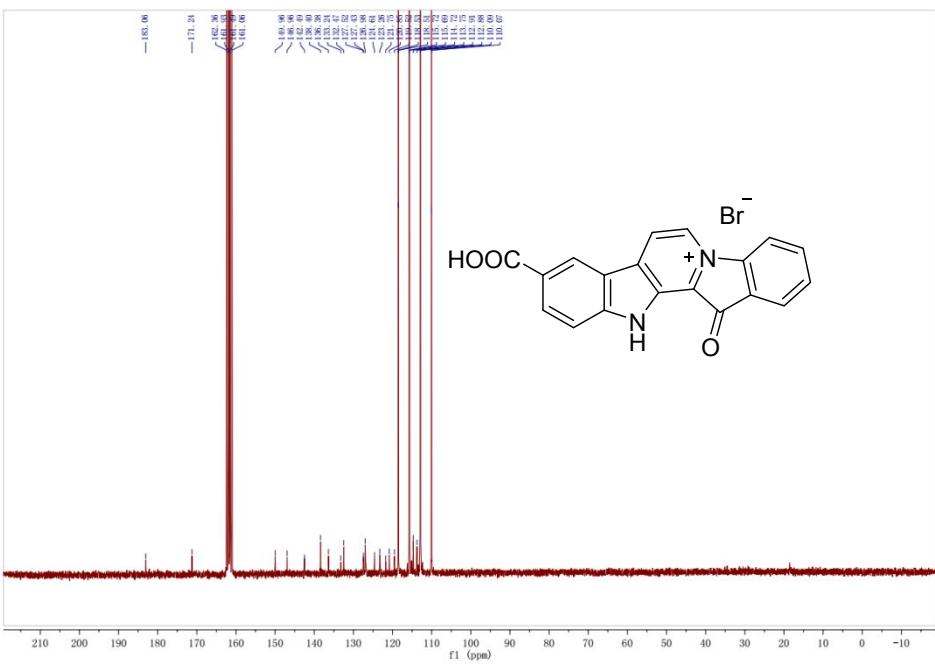
**Fig. S13**  $^1\text{H}$  NMR of **2c**



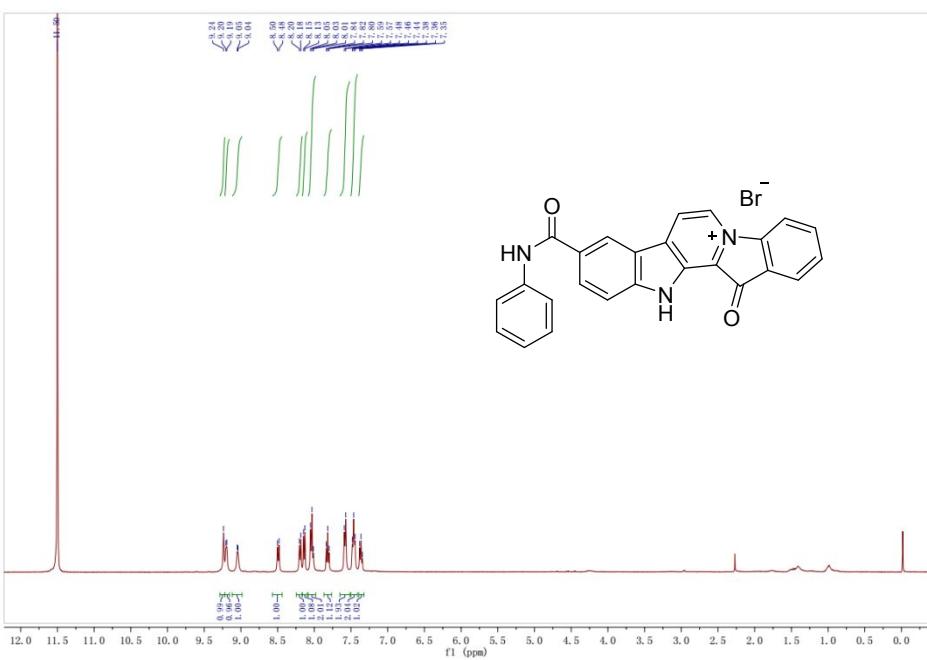
**Fig. S14**  $^{13}\text{C}$  NMR of **2c**



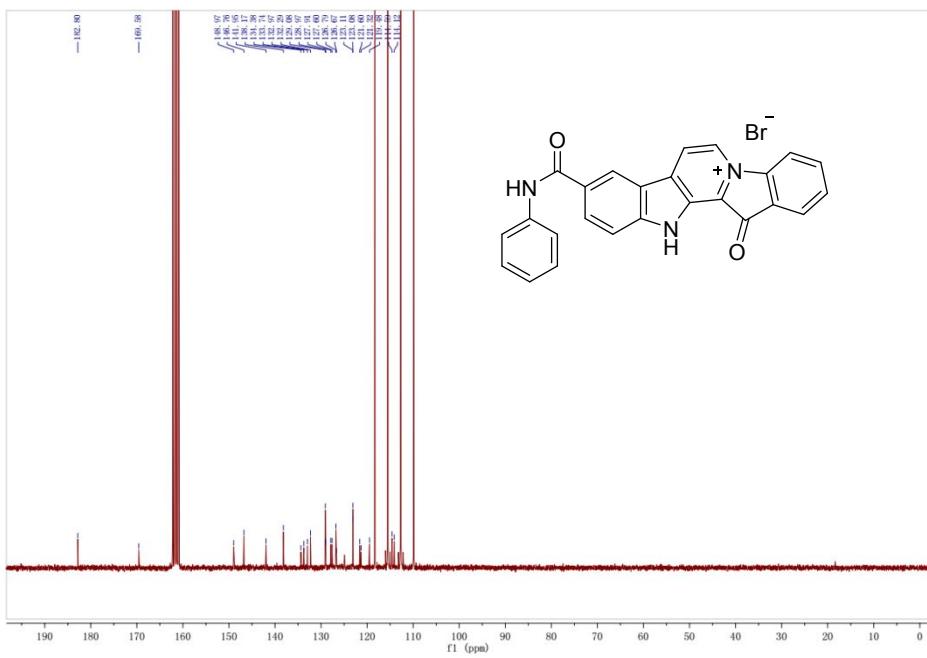
**Fig. S15**  $^1\text{H}$  NMR of **2e**



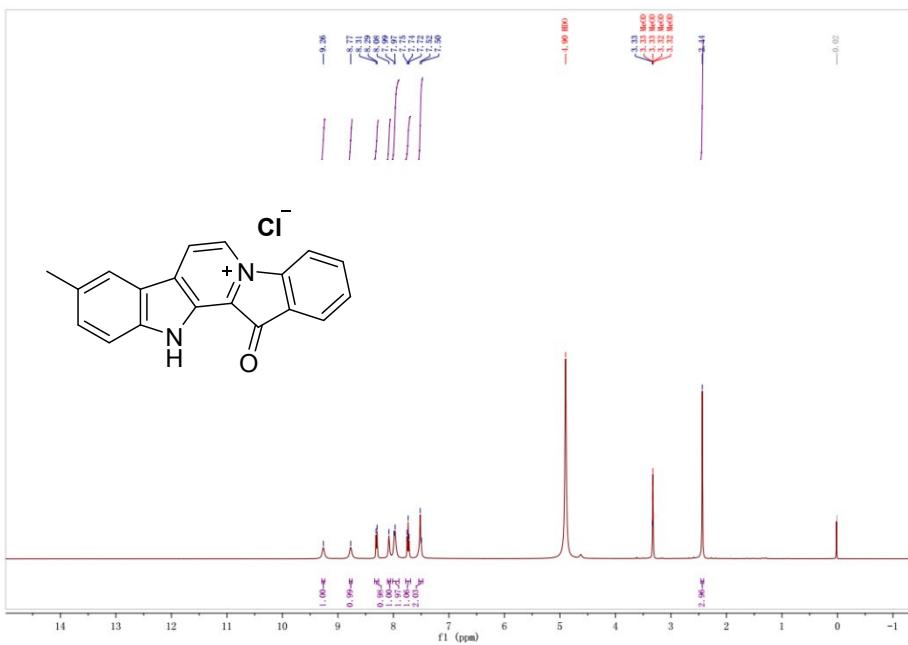
**Fig. S16**  $^{13}\text{C}$  NMR of **2e**



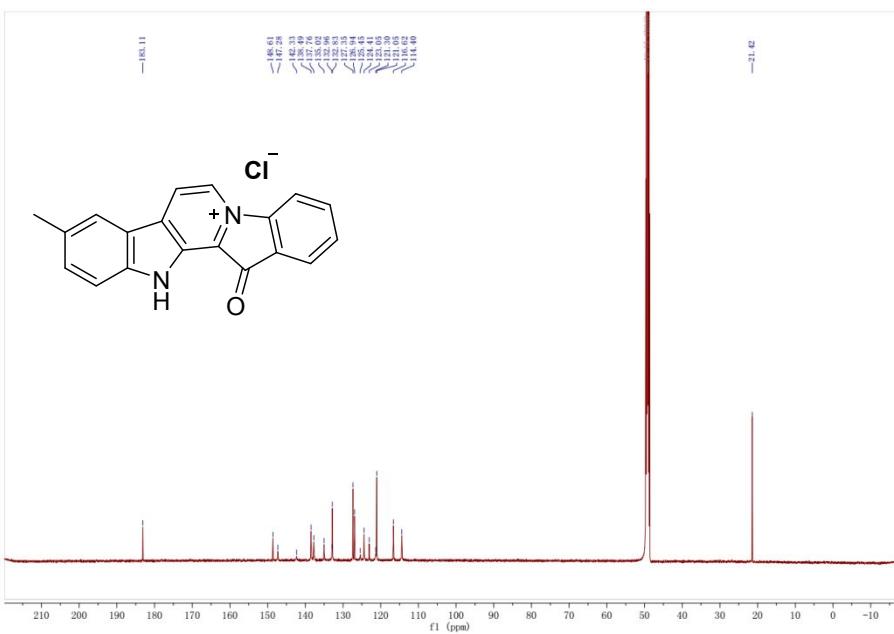
**Fig. S17** <sup>1</sup>H NMR of **2g**



**Fig. S18** <sup>13</sup>C NMR of **2g**



**Fig. S19**  $^1\text{H}$  NMR of **2i**



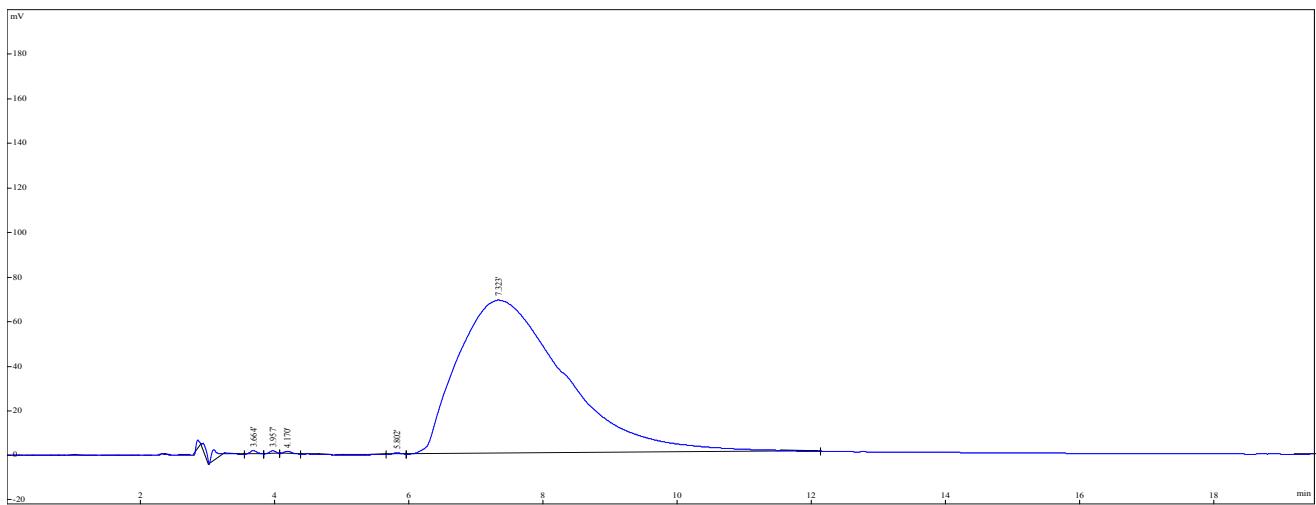
**Fig. S20**  $^{13}\text{C}$  NMR of **2i**

## **2. HPLC analysis of new compounds**

Purity analysis was conducted on a HPLC instrument (CXTH-LC-3000) using Welch Ultimate® Plus C18 column (5 µm, 4.6 mm X 250 mm), eluting at flow rate of 1 mL/min, and monitoring at wavelength of 280 nm.

**Table S1** HPLC analysis of new compounds

Entry	Compound	Mobile phase (MeOH/H <sub>2</sub> O, V/V)	Retention time (min)	Purity (%)
1	<b>1a</b>	90/10	7.323	99.54
2	<b>1b</b>	90/10	4.722	98.03
3	<b>1c</b>	90/10	7.633	99.28
4	<b>1d</b>	90/10	4.156	98.06
5	<b>1e</b>	40/60	3.963	99.36
6	<b>1f</b>	90/10	3.326	97.69
7	<b>1g</b>	90/10	3.956	99.63
8	<b>1h</b>	90/10	6.239	98.42
9	<b>1i</b>	90/10	4.691	99.35
10	<b>1j</b>	90/10	3.908	99.65
11	<b>1k</b>	70/30	9.537	99.06
12	<b>2a</b>	90/10	7.323	99.54
13	<b>2b</b>	90/10	6.157	98.96
14	<b>2c</b>	90/10	7.633	99.28
15	<b>2d</b>	90/10	7.172	97.86
16	<b>2e</b>	30/70	8.761	98.48
17	<b>2f</b>	30/70	8.732	99.18
18	<b>2g</b>	90/10	5.720	98.96
19	<b>2h</b>	90/10	6.239	98.42
20	<b>2i</b>	90/10	6.322	97.63

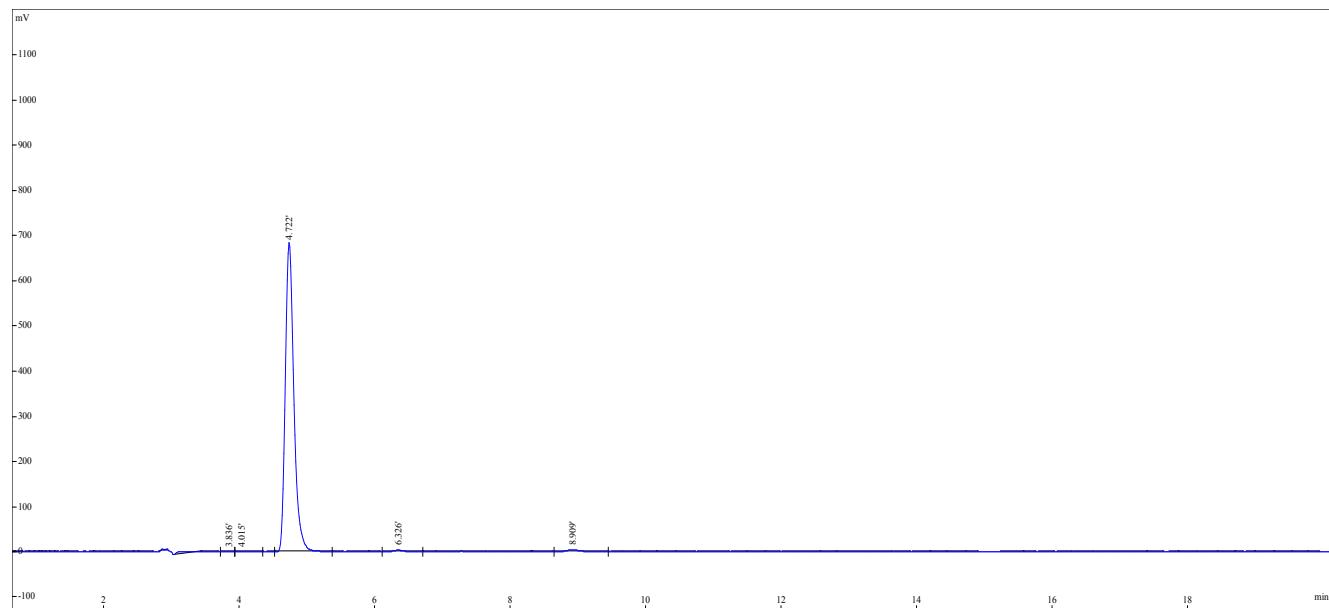


**Fig. S21** HPLC analysis of **1a**

**Table S2** HPLC analysis of **1a**

Peak	Retention time (min)	Area (%)	Area
1	3.664	0.15	11539
2	3.957	0.1104	8490
3	4.170	0.1175	9041
4	5.802	0.07683	5910
5	7.323	99.54	7657821
<b>Total</b>		100	7692801

**1b**

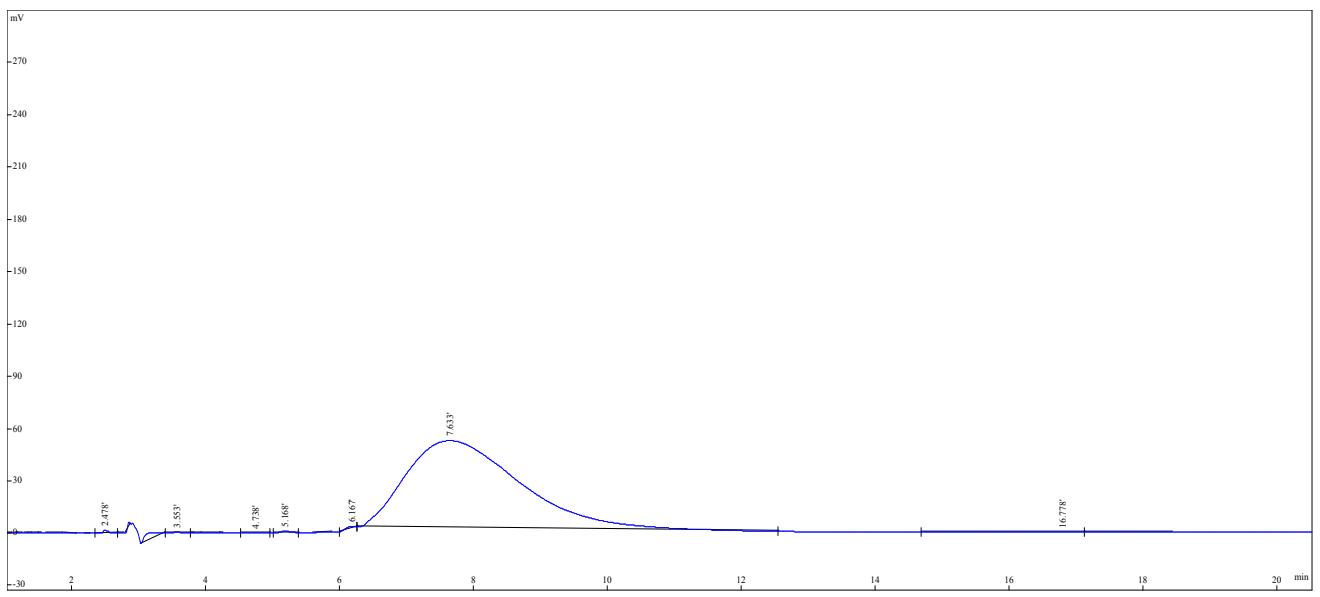


**Fig. S22** HPLC analysis of **1b**

**Table S3** HPLC analysis of **1b**

Peak	Retention time (min)	Area (%)	Area
1	3.836	0.09826	6032
2	4.015	0.03636	2232
3	4.722	98.03	6018046
4	6.326	0.7029	43149
5	8.909	1.127	69201
<b>Total</b>		100	6138660

**1c**

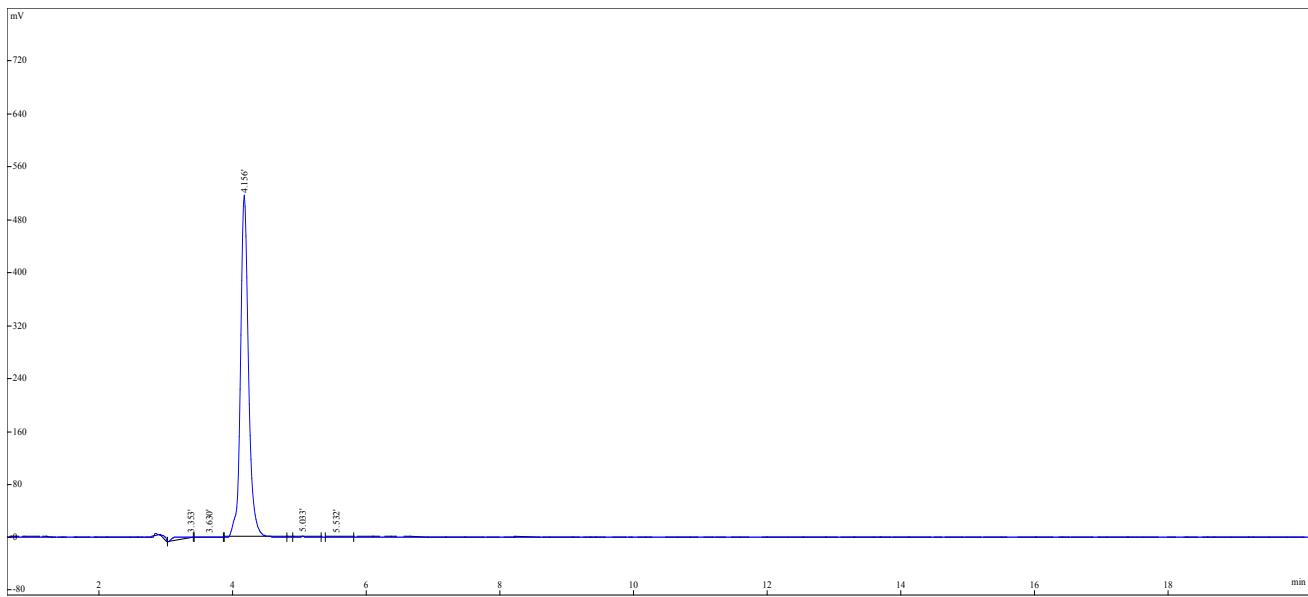


**Fig. S23** HPLC analysis of **1c**

**Table S4** HPLC analysis of **1c**

Peak	Retention time (min)	Area (%)	Area
1	2.478	0.1659	9889
2	3.553	0.06737	4015
3	4.738	0.03387	2019
4	5.168	0.1655	9862
5	6.167	0.2037	12141
6	7.633	99.28	5916623
7	16.778	0.08444	5032
<b>Total</b>		100	5959581

**1d**

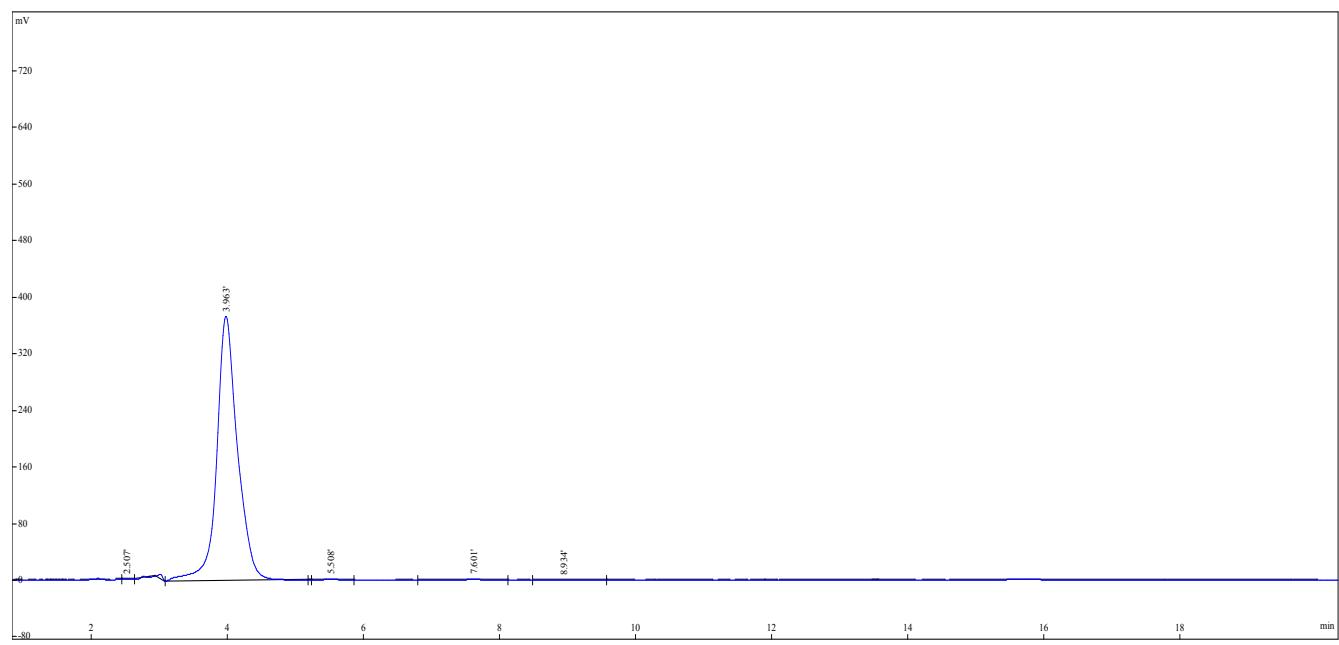


**Fig. S24** HPLC analysis of **1d**

**Table S5** HPLC analysis of **1d**

Peak	Retention time (min)	Area (%)	Area
1	3.353	1.496	65819
2	3.630	0.1741	7658
3	4.156	98.06	4313767
4	5.033	0.1958	8615
5	5.532	0.08148	3585
<b>Total</b>		100	4399444

**1e**

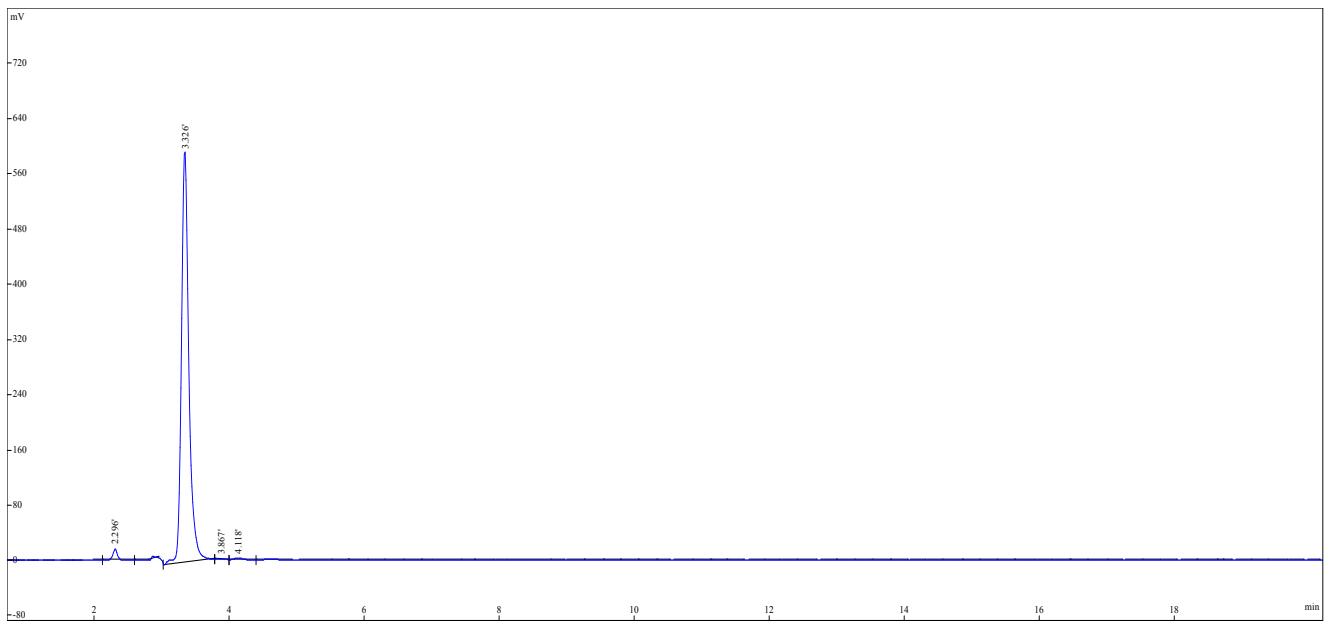


**Fig. S25** HPLC analysis of **1e**

**Table S6** HPLC analysis of **1e**

Peak	Retention time (min)	Area (%)	Area
1	2.507	0.05106	4148
2	3.963	99.36	8072203
3	5.508	0.1351	10979
4	7.601	0.2716	22069
5	8.934	0.1764	14334
<b>Total</b>		100	8123733

**1f**

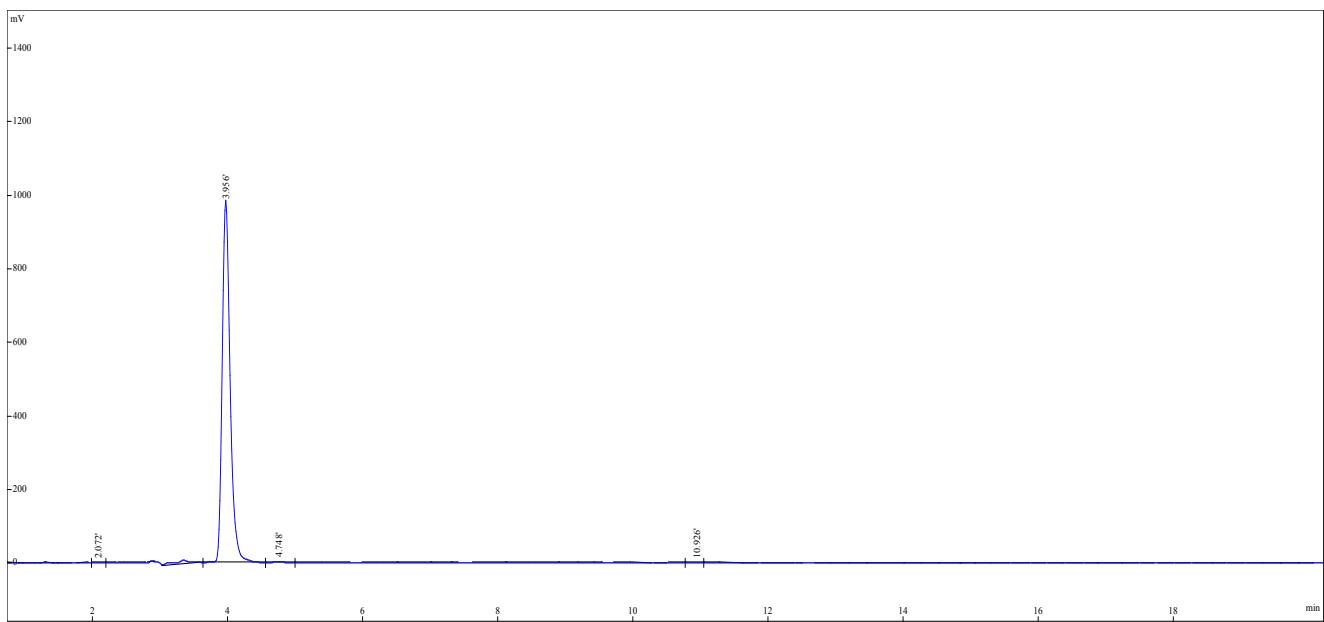


**Fig. S26** HPLC analysis of **1f**

**Table S7** HPLC analysis of **1f**

Peak	Retention time (min)	Area (%)	Area
1	2.296	1.692	79254
2	3.326	97.69	4576250
3	3.867	0.1094	5125
4	4.118	0.5031	23569
<b>Total</b>		100	4684198

**1g**

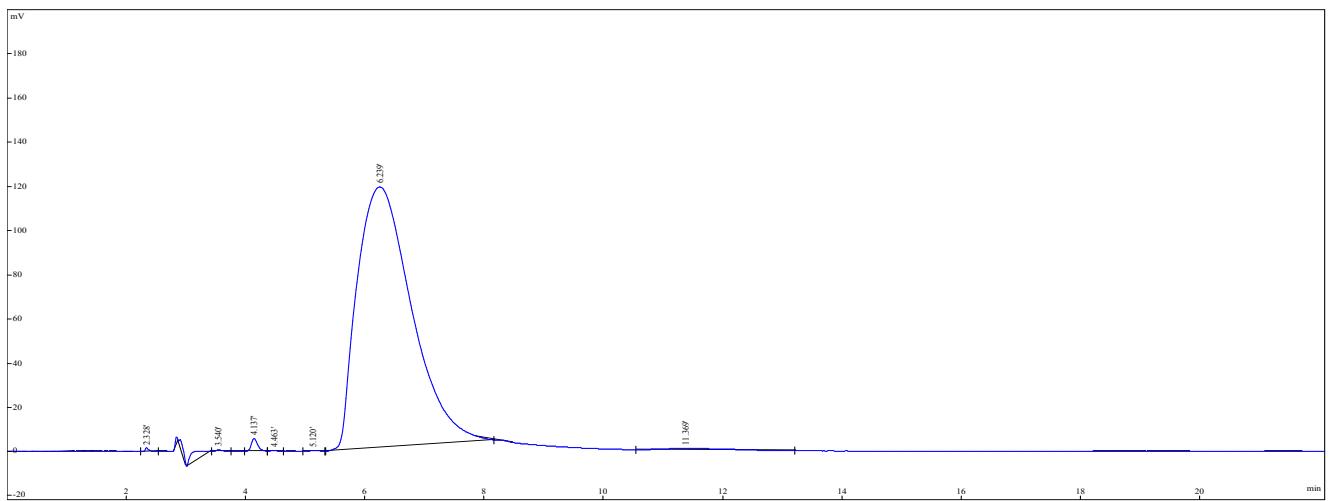


**Fig. S27** HPLC analysis of **1g**

**Table S8** HPLC analysis of **1g**

Peak	Retention time (min)	Area (%)	Area
1	2.072	0.05584	4519
2	3.956	99.63	8062622
3	4.748	0.312	25251
4	10.926	0.00283	229
<b>Total</b>		100	8092621

**1h**

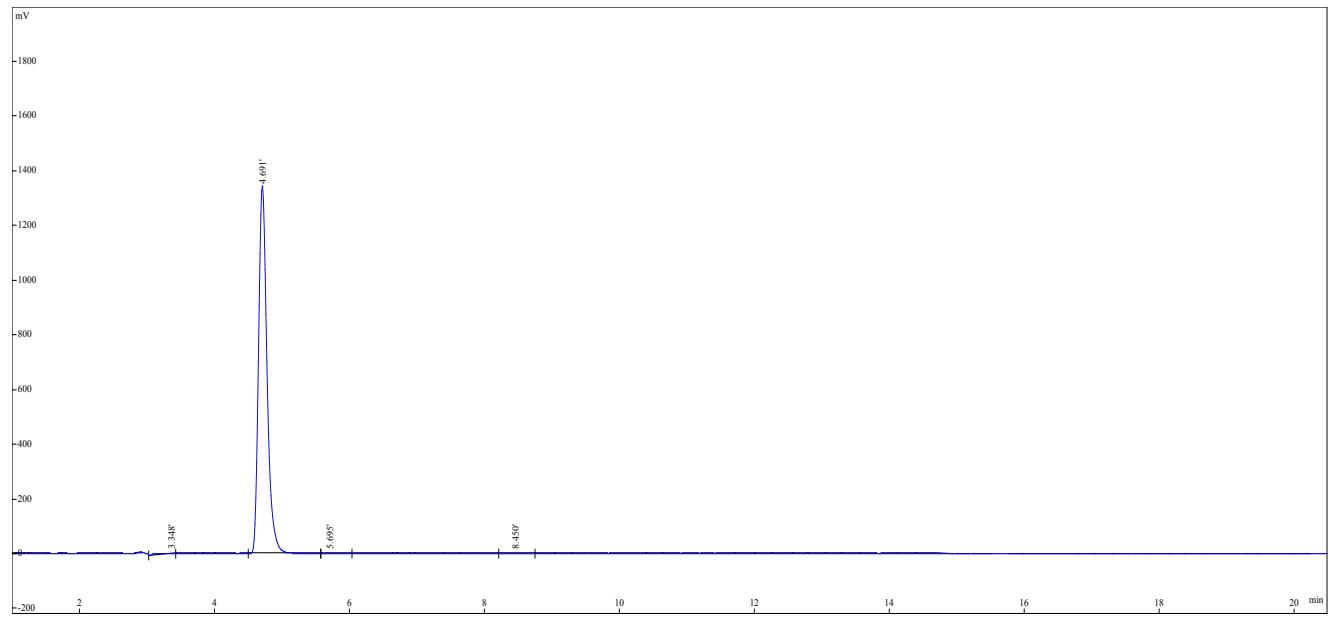


**Fig. S28** HPLC analysis of **1h**

**Table S9** HPLC analysis of **1h**

Peak	Retention time (min)	Area (%)	Area
1	2.328	0.1024	7763
2	3.540	0.05658	4289
3	4.137	0.5504	41729
4	4.463	0.03461	2624
5	5.120	0.04469	3388
6	6.239	98.42	7461362
7	11.369	0.7893	59832
<b>Total</b>		100	7580987

**1i**

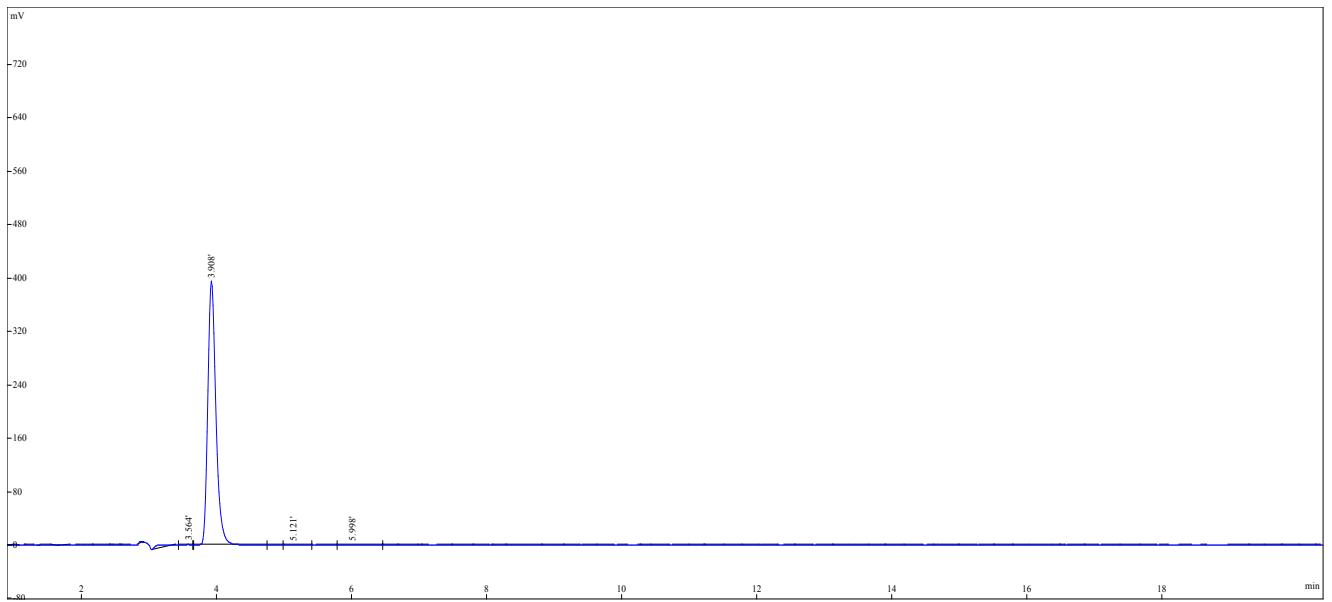


**Fig. S29** HPLC analysis of **1i**

**Table S10** HPLC analysis of **1i**

Peak	Retention time (min)	Area (%)	Area
1	3.348	0.6003	71200
2	4.691	99.35	11784526
3	5.695	0.03131	3714
4	8.450	0.01739	2063
<b>Total</b>		100	11861503

**1j**

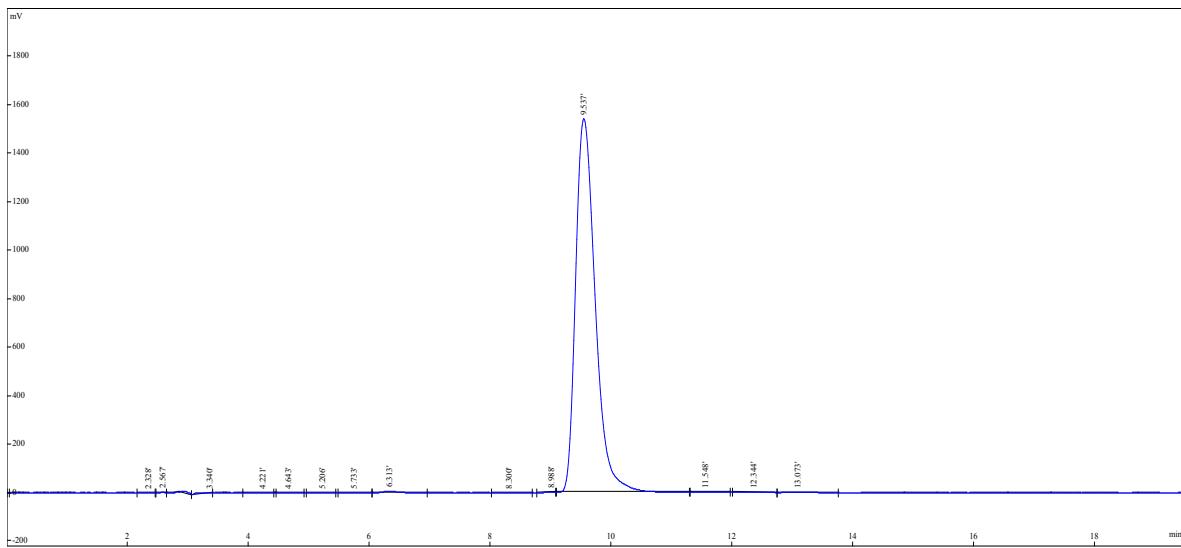


**Fig. S30** HPLC analysis of **1j**

**Table S11** HPLC analysis of **1j**

Peak	Retention time (min)	Area (%)	Area
1	3.564	0.1189	3940
2	3.908	99.65	3301052
3	5.121	0.1199	3972
4	5.998	0.1046	3466
<b>Total</b>		100	3312430

**1k**

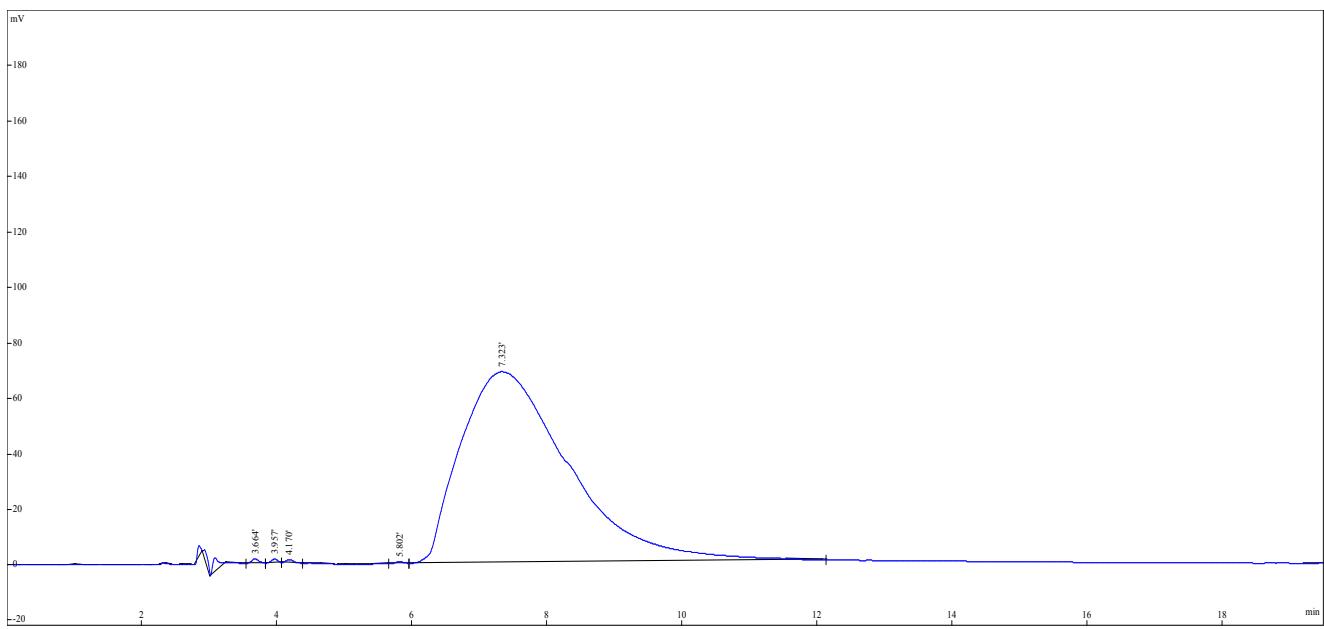


**Fig. S31** HPLC analysis of **1k**

**Table S12** HPLC analysis of **1g**

Peak	Retention time (min)	Area (%)	Area
1	0.019	0.008654	3037
2	2.328	0.01077	3780
3	2.567	0.01264	4435
4	3.340	0.2226	78103
5	4.221	0.01353	4748
6	4.643	0.01848	6485
7	5.206	0.00998	3502
8	5.733	0.02115	7423
9	6.313	0.2846	99855
10	8.300	0.03875	13599
11	8.988	0.01398	4904
12	9.537	99.06	34761632
13	11.548	0.04743	16642
14	12.344	0.09328	32734
15	13.073	0.1386	48628
<b>Total</b>		100	35089507

**2a**

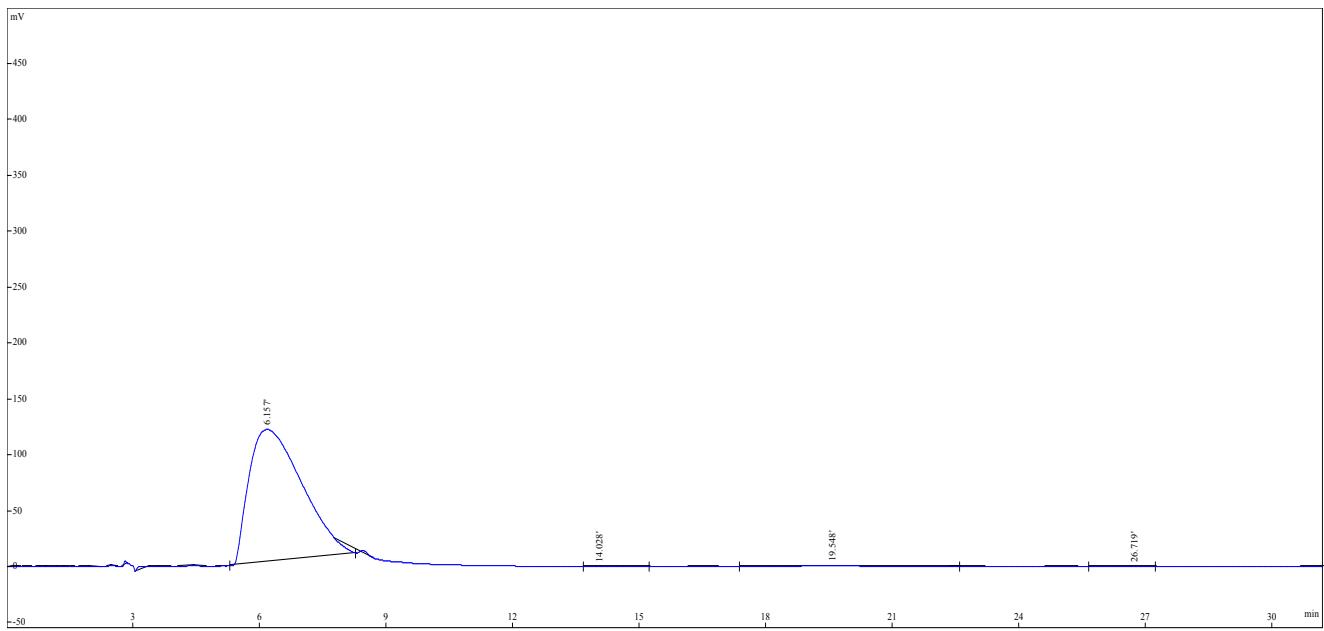


**Fig. S32** HPLC analysis of **2a**

**Table S13** HPLC analysis of **2a**

Peak	Retention time (min)	Area (%)	Area
1	3.664	0.15	11539
2	3.957	0.1104	8490
3	4.170	0.1175	9041
4	5.802	0.07683	5910
5	7.323	99.54	7657821
<b>Total</b>		100	7692801

**2b**

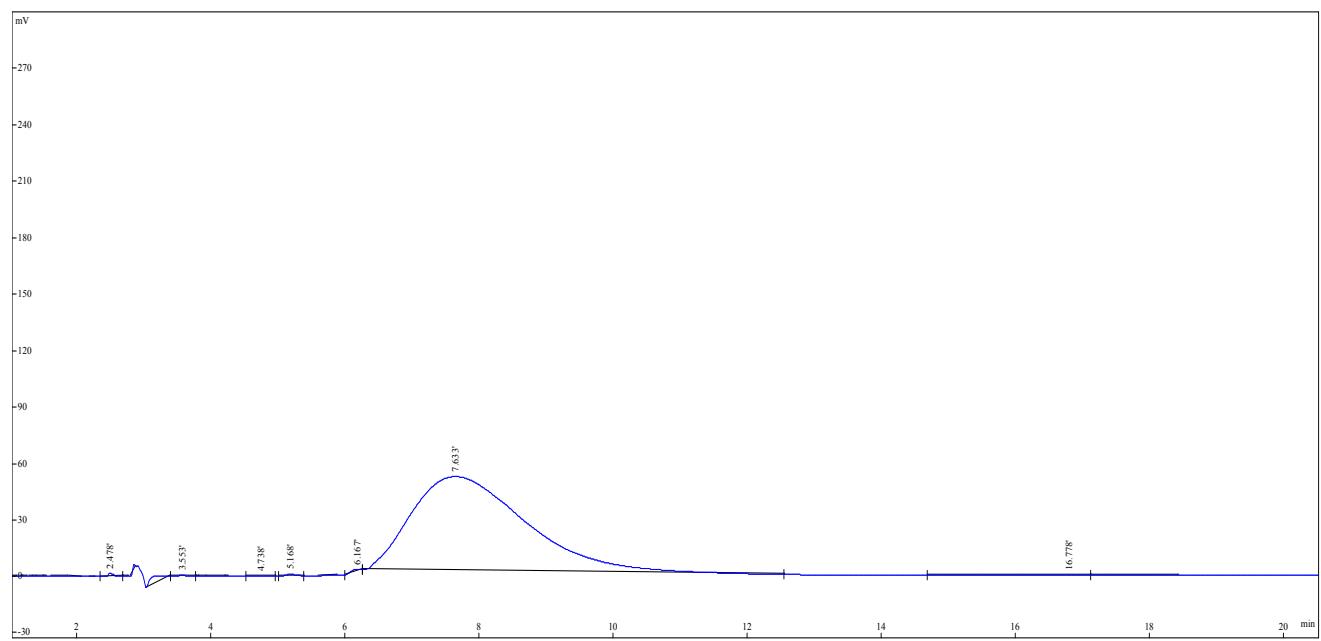


**Fig. S33** HPLC analysis of **2b**

**Table S14** HPLC analysis of **2b**

Peak	Retention time (min)	Area (%)	Area
1	6.157	98.96	10346263
2	14.028	0.04309	4506
3	19.548	0.9536	99703
4	26.719	0.05022	5251
<b>Total</b>		100	10455723

**2c**

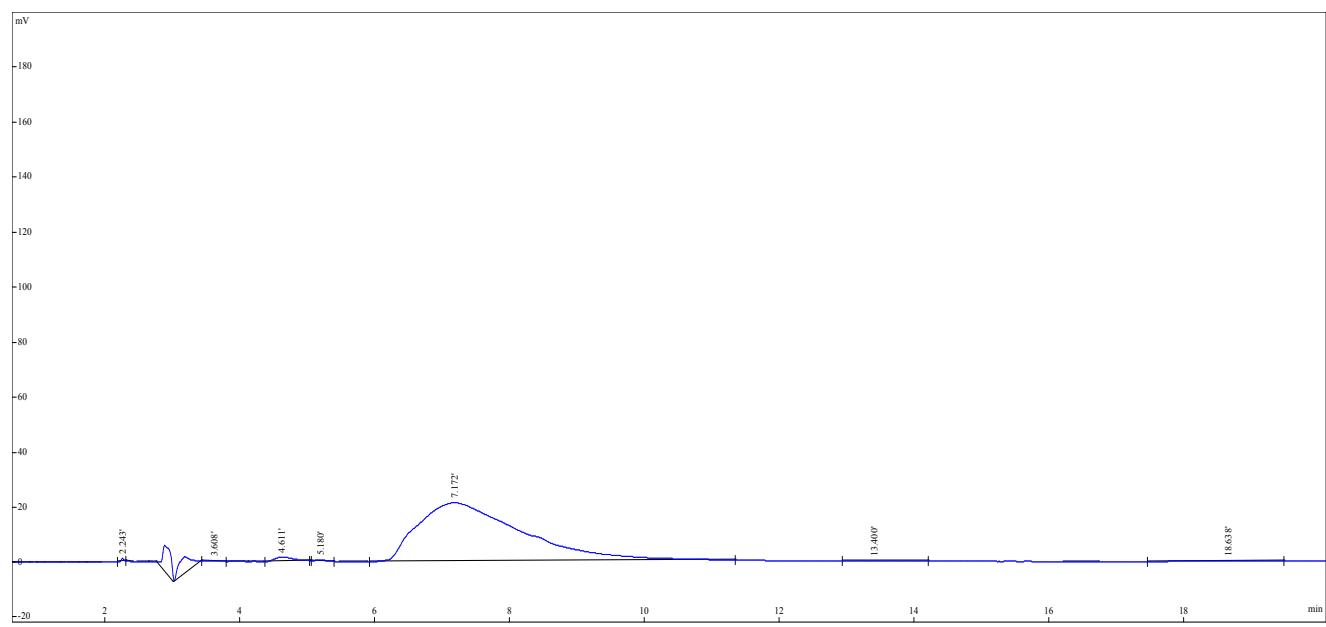


**Fig. S34** HPLC analysis of **2c**

**Table S15** HPLC analysis of **2c**

Peak	Retention time (min)	Area (%)	Area
1	2.478	0.1659	9889
2	3.553	0.06737	4015
3	4.738	0.03387	2019
4	5.168	0.1655	9862
5	6.167	0.2037	12141
6	7.633	99.28	5916623
7	16.778	0.08444	5032
<b>Total</b>		100	5959581

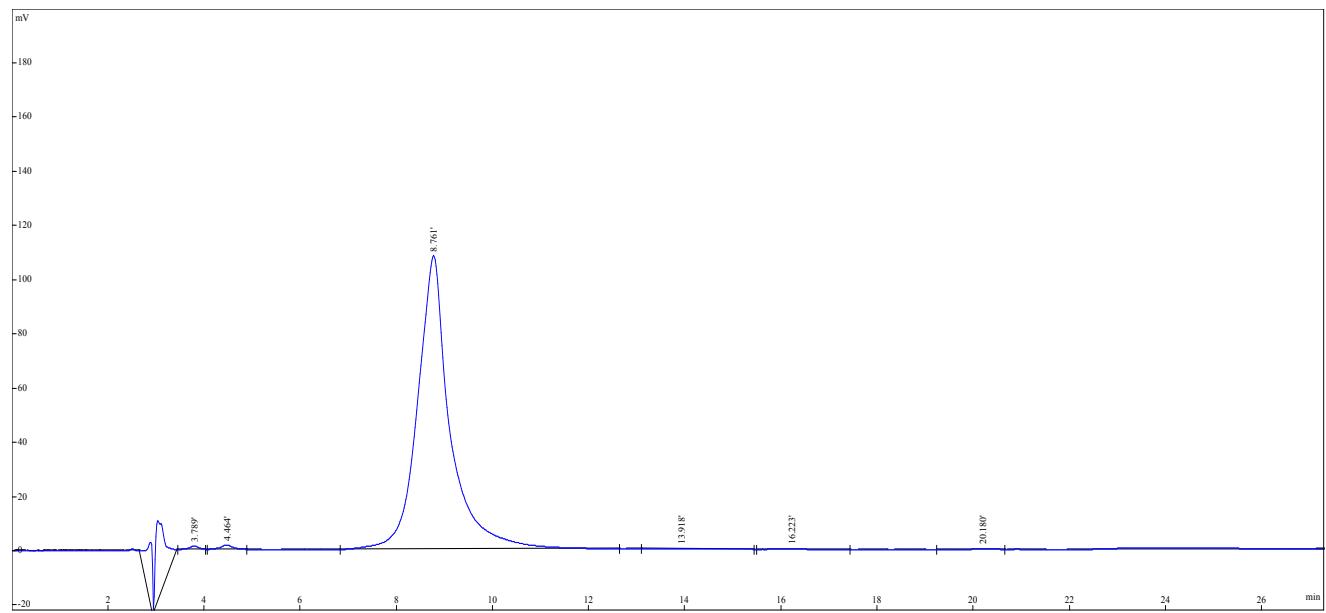
**2d**



**Fig. S35** HPLC analysis of **2d**

**Table S16** HPLC analysis of **2d**

Peak	Retention time (min)	Area (%)	Area
1	2.243	0.1564	3640
2	3.608	0.0907	2111
3	4.611	1.259	29308
4	5.180	0.1434	3338
5	7.172	97.86	2277763
6	13.400	0.1455	3387
7	18.638	0.3456	8045
<b>Total</b>		100	2327592

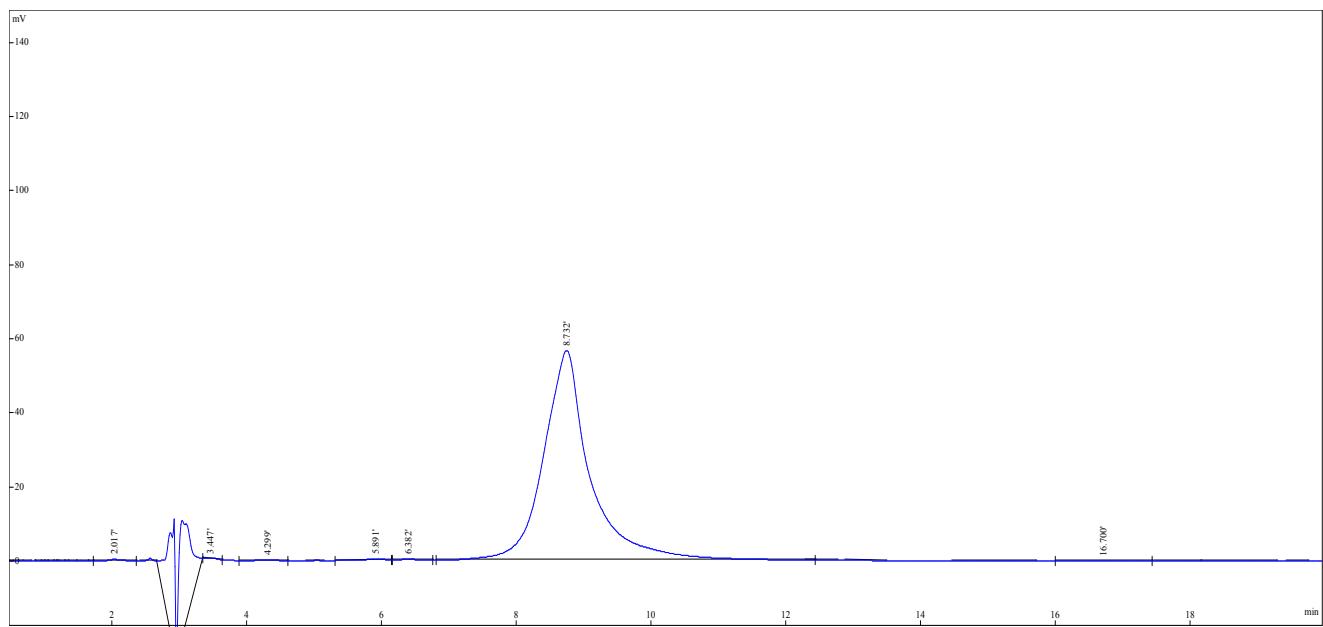


**Fig. S36** HPLC analysis of **2e**

**Table S17** HPLC analysis of **2e**

Peak	Retention time (min)	Area (%)	Area
1	3.789	0.3491	18141
2	4.464	0.5447	28305
3	8.761	98.48	5117525
4	13.918	0.3159	16414
5	16.223	0.1714	8907
6	20.180	0.1382	7182
<b>Total</b>		100	5196474

**2f**

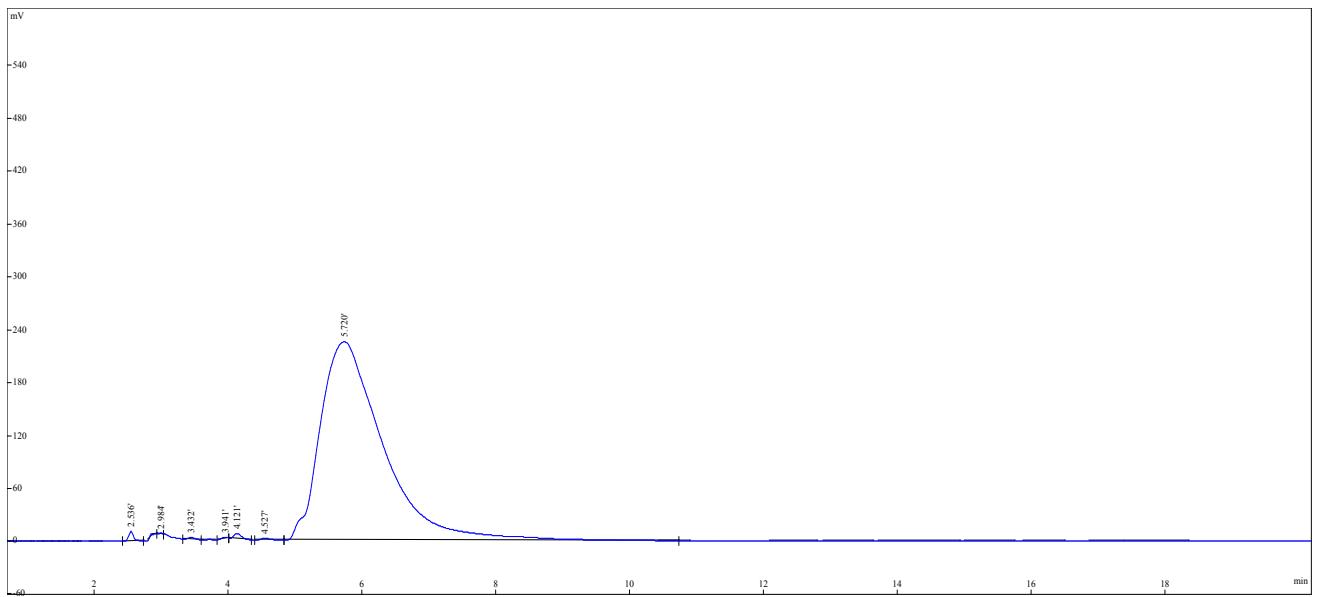


**Fig. S37** HPLC analysis of **2f**

**Table S18** HPLC analysis of **2f**

Peak	Retention time (min)	Area (%)	Area
1	2.017	0.2327	6368
2	3.447	0.1116	3053
3	4.299	0.1214	3322
4	5.891	0.154	4213
5	6.382	0.08256	2259
6	8.732	99.18	2714069
7	16.700	0.1234	3377
<b>Total</b>		100	2736661

**2g**

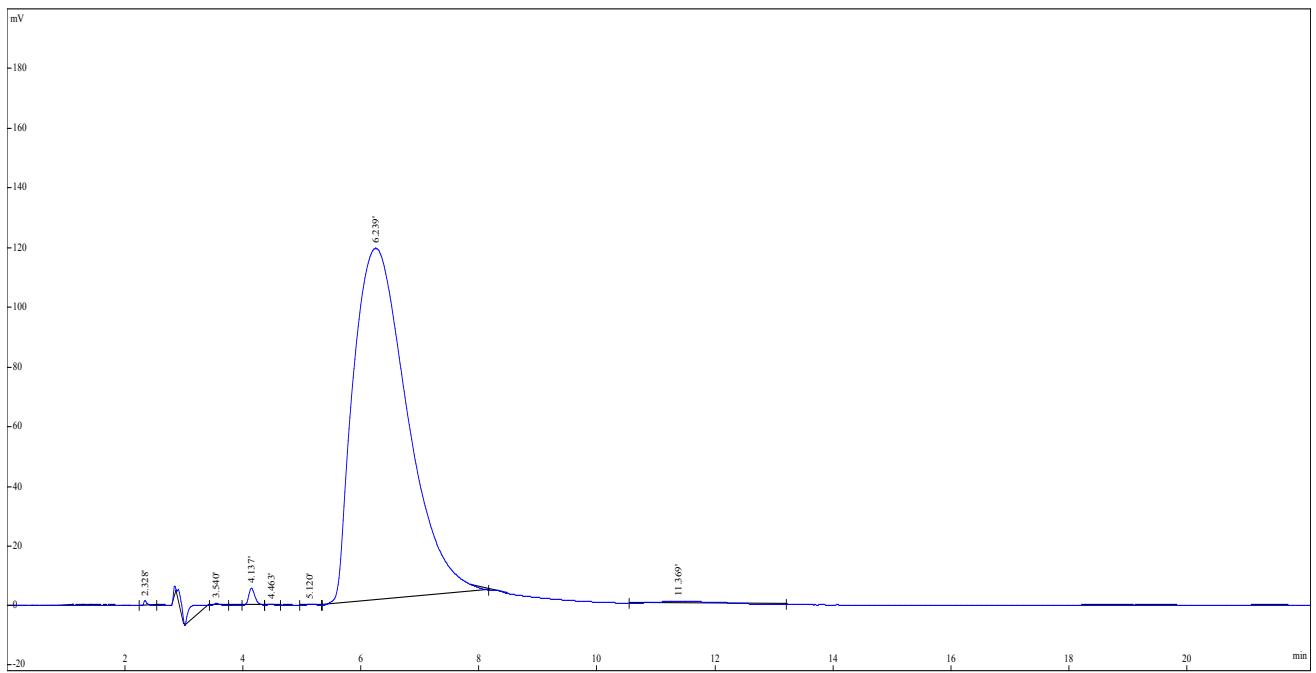


**Fig. S38** HPLC analysis of **2g**

**Table S19** HPLC analysis of **2g**

Peak	Retention time (min)	Area (%)	Area
1	2.536	0.3885	57323
2	2.984	0.03638	5368
3	3.432	0.103	15194
4	3.941	0.06517	9616
5	4.121	0.3027	44661
6	4.527	0.1457	21496
7	5.720	98.96	14601592
<b>Total</b>		100	14755250

**2h**

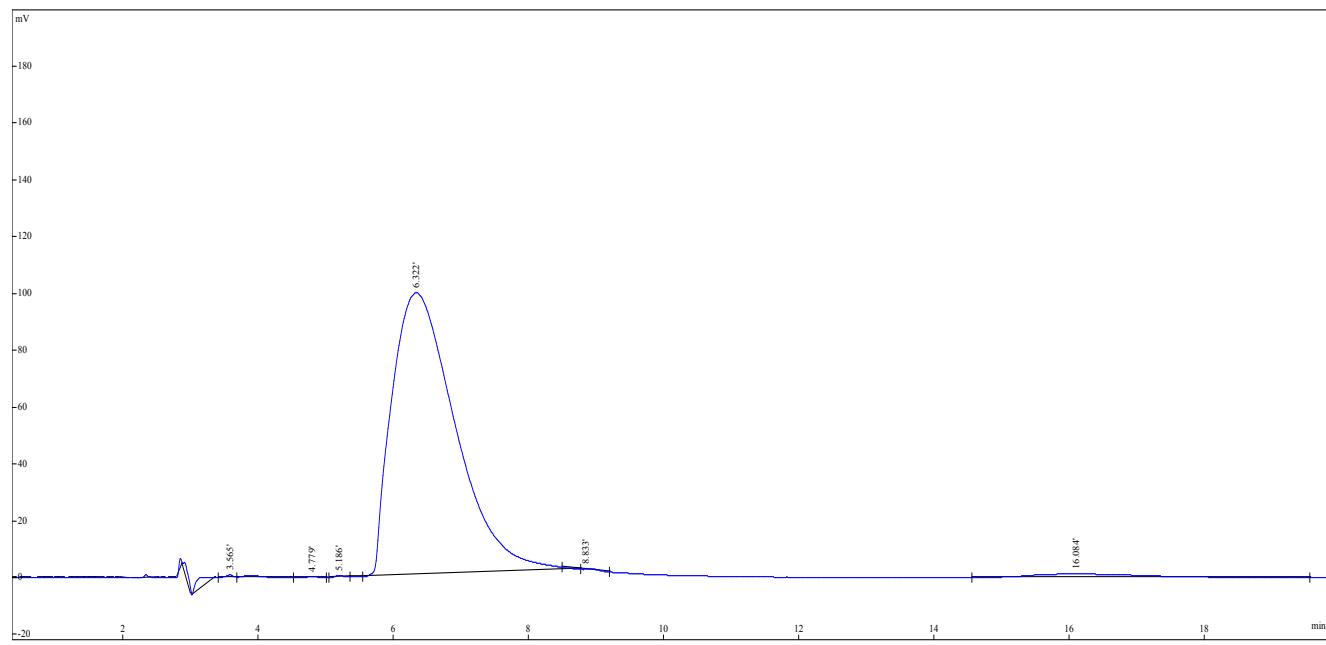


**Fig. S39** HPLC analysis of **2h**

**Table S20** HPLC analysis of **2h**

Peak	Retention time (min)	Area (%)	Area
1	2.328	0.1024	7763
2	3.540	0.05658	4289
3	4.137	0.5504	41729
4	4.463	0.03461	2624
5	5.120	0.04469	3388
6	6.239	98.42	7461362
7	11.369	0.7893	59832
<b>Total</b>		100	7580987

**2i**

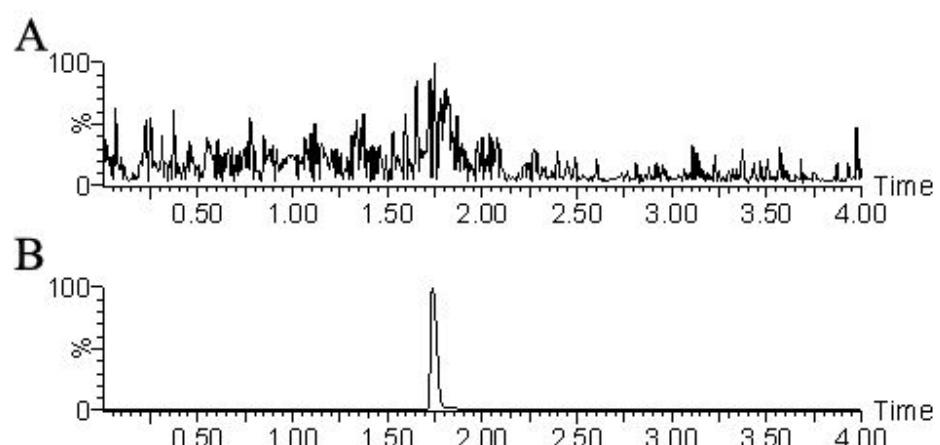


**Fig. S40** HPLC analysis of **2i**

**Table S21** HPLC analysis of **2i**

Peak	Retention time (min)	Area (%)	Area
1	3.565	0.08106	5393
2	4.779	0.04639	3086
3	5.186	0.06912	4598
4	6.322	97.63	6494777
5	8.833	0.06935	4613
6	16.084	2.104	139981
<b>Total</b>		100	6652448

**3. Pharmacokinetics studies showed compound 2b could penetrate BBB.**



**Fig. S41.** **2b** can reach the brain after tail vein injection. (A) UPLC-MS/MS chromatograms of brain homogenate extract from control mice. (B) UPLC-MS/MS chromatograms of brain homogenate extract from mice at 15 min after tail vein injection of 30 mg/kg **2b**.