

Design, Synthesis and Discovery of andrographolide derivatives against Zika virus infection

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Table S1. Anti-Zika activity (EC₅₀) and cytotoxicity (CC₅₀) to SNB-19 and Vero cell lines

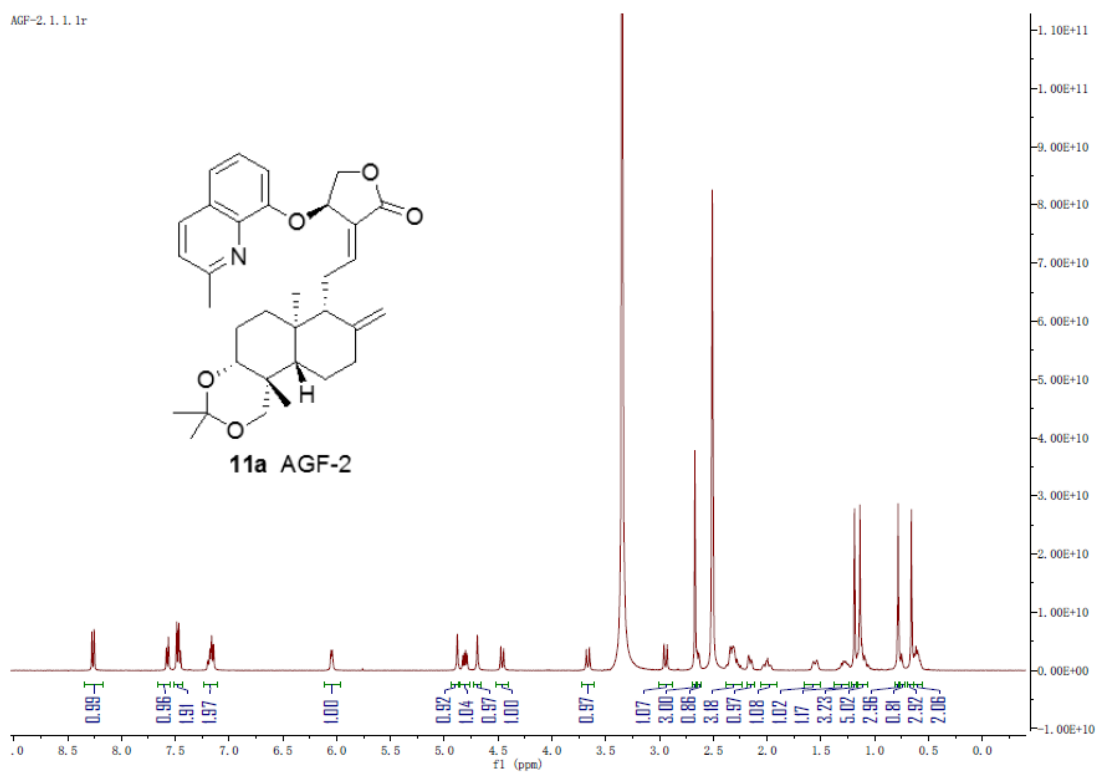
compd	EC ₅₀ (μM)	SNB-19 cell line ^a		Vero cell line ^b	
		CC ₅₀ (μM)	SI	CC ₅₀ (μM)	SI
2	25.9 ± 1.5	21.1 ± 1.1	0.82	22.0 ± 0.4	0.85
3	1.3 ± 0.1	22.7 ± 1.1	17.5	20.8 ± 0.5	16.1
4	- ^c	49.5 ± 0.1	/ ^d	27.5 ± 0.1	/
5	-	21.9 ± 0.1	/	12.8 ± 0.1	/
6	-	61.2 ± 0.3	/	20.7 ± 0.3	/
7	11.0 ± 0.2	60.1 ± 1.1	5.5	22.8 ± 0.2	2.1
8	-	46.5 ± 0.6	/	26.2 ± 0.2	/
9	12.5 ± 0.4	67.7 ± 1.1	5.4	37.7 ± 0.1	3.0
11a	8.5 ± 0.4	78.4 ± 1.0	9.2	55.9 ± 0.2	6.6
11b	16.6 ± 0.3	66.5 ± 1.0	4.0	65.3 ± 0.3	3.9
12a	25.8 ± 1.1	>100	>3.9	99.8 ± 0.7	3.9
12b	-	>100	/	61.4 ± 0.2	/
13a	-	65.9 ± 0.2	/	46.5 ± 0.1	/
13b	-	>100	/	50.61 ± 0.03	/
14a	-	51.7 ± 0.2	/	46.7 ± 0.2	/
14b	-	>100	/	72.4 ± 0.7	/
15a	-	39.3 ± 0.4		55.9 ± 0.2	/
15b	-	63.5 ± 0.2	/	58.6 ± 3.4	/
16a	13.3 ± 0.5	>100	>7.5	>100	>7.5
16b	7.8 ± 0.4	85.2 ± 1.0	10.9	82.5 ± 2.2	7.5
17a	-	39.3 ± 0.2	/	51.9 ± 0.3	/
17b	4.5 ± 0.2	88.7 ± 1.1	19.7	85.0 ± 1.6	18.9
18a	24.6 ± 0.9	72.0 ± 1.0	2.9	57.4 ± 0.3	2.3
18b	-	>100	/	64.7 ± 0.3	/

^aincubation for 24 hours. ^bincubation for 48 hours. ^c“-” means “not active”. ^d“/” represents “not available”.

NMR spectra

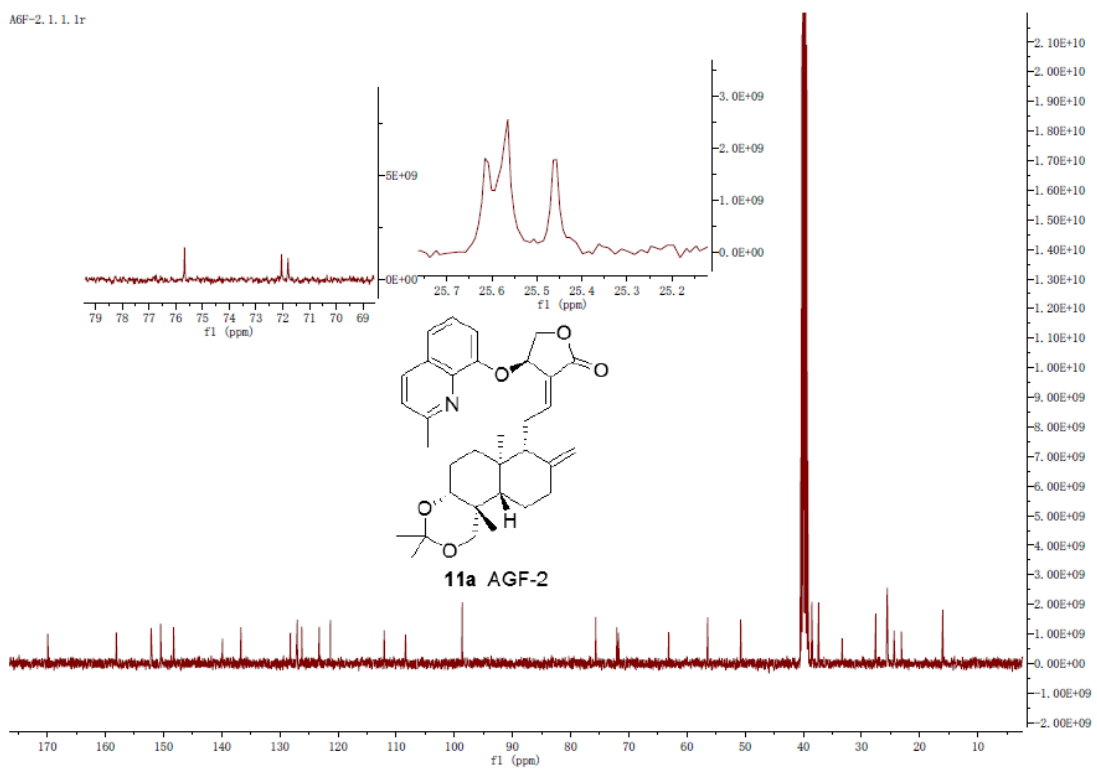
^1H NMR of **11a**

AGF-2, 1.1.1r



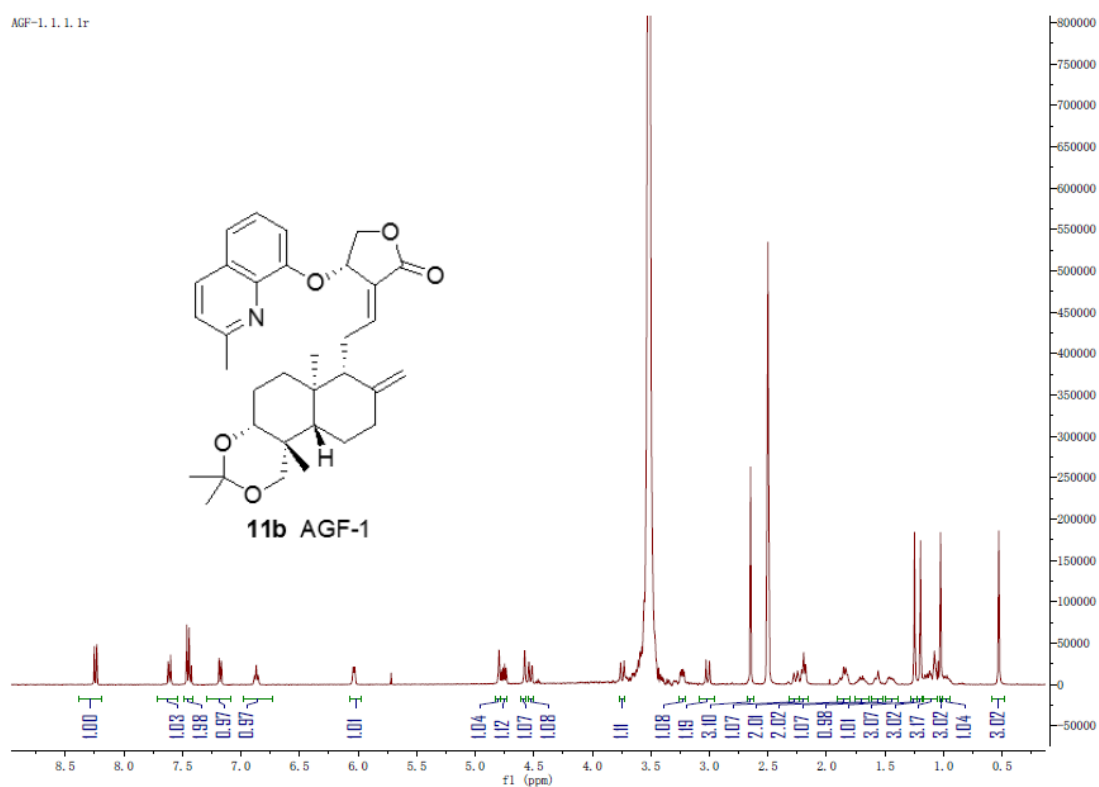
^{13}C NMR of **11a**

AGF-2, 1.1.1r



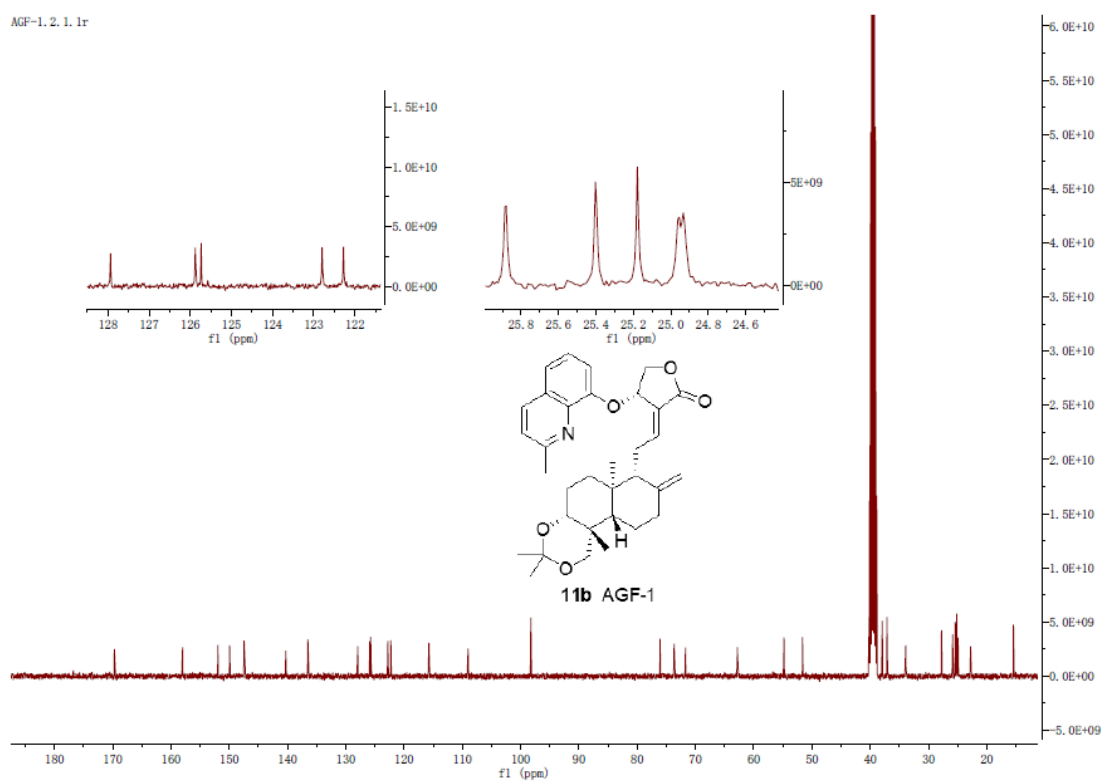
¹H NMR of **11b**

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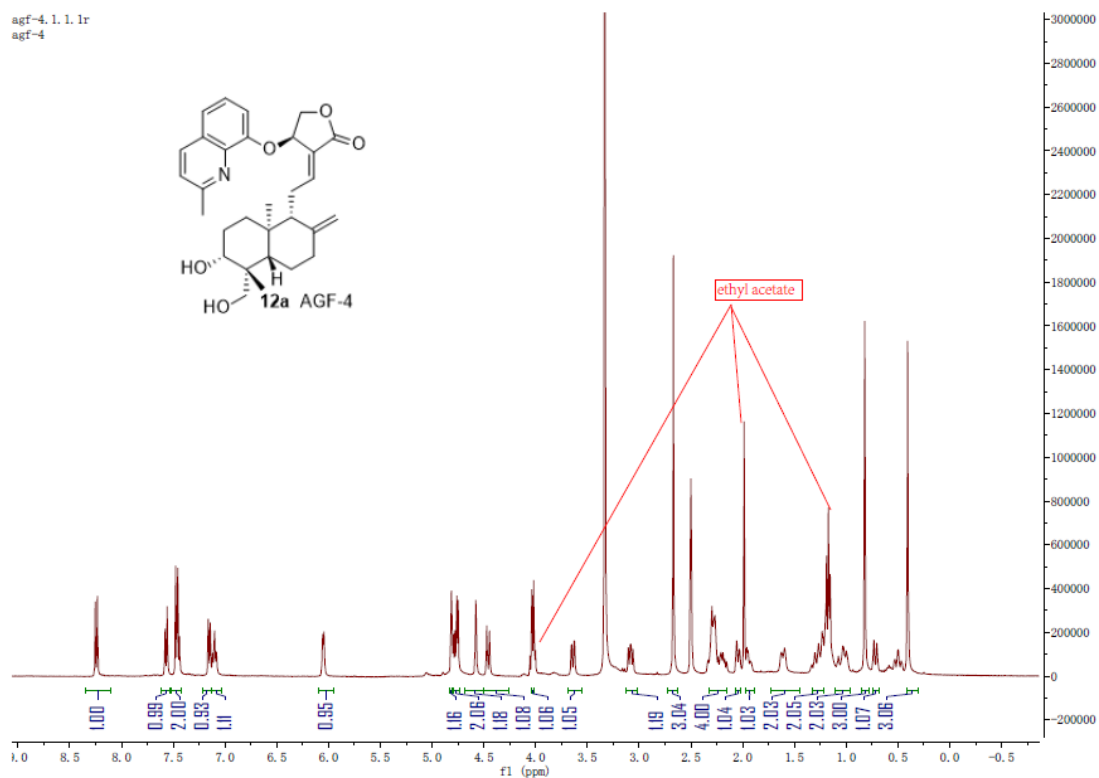


¹³C NMR of **11b**

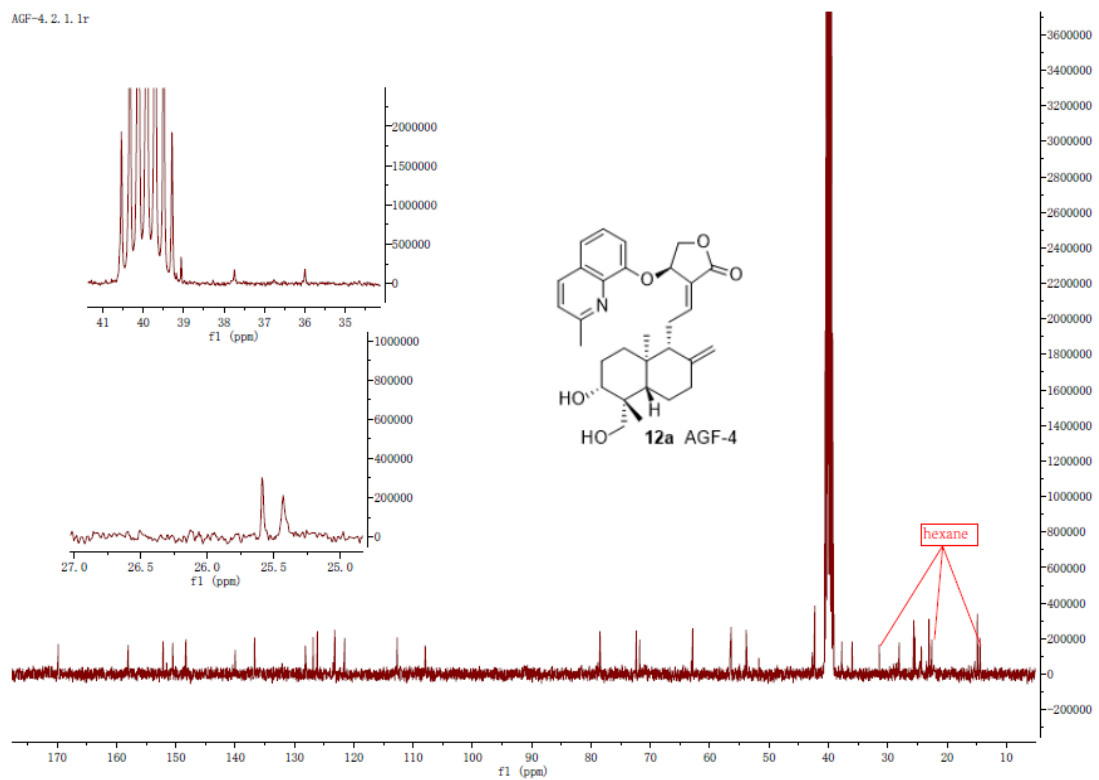
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¹H NMR of 12a

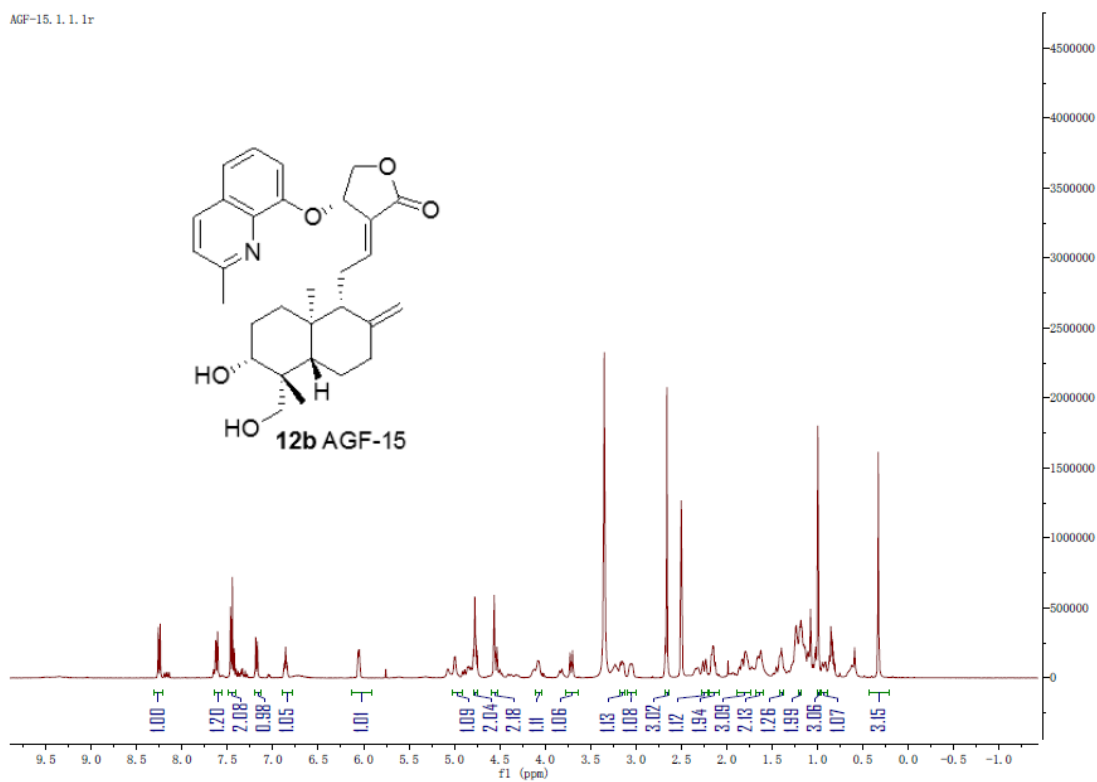


¹³C NMR of 12a



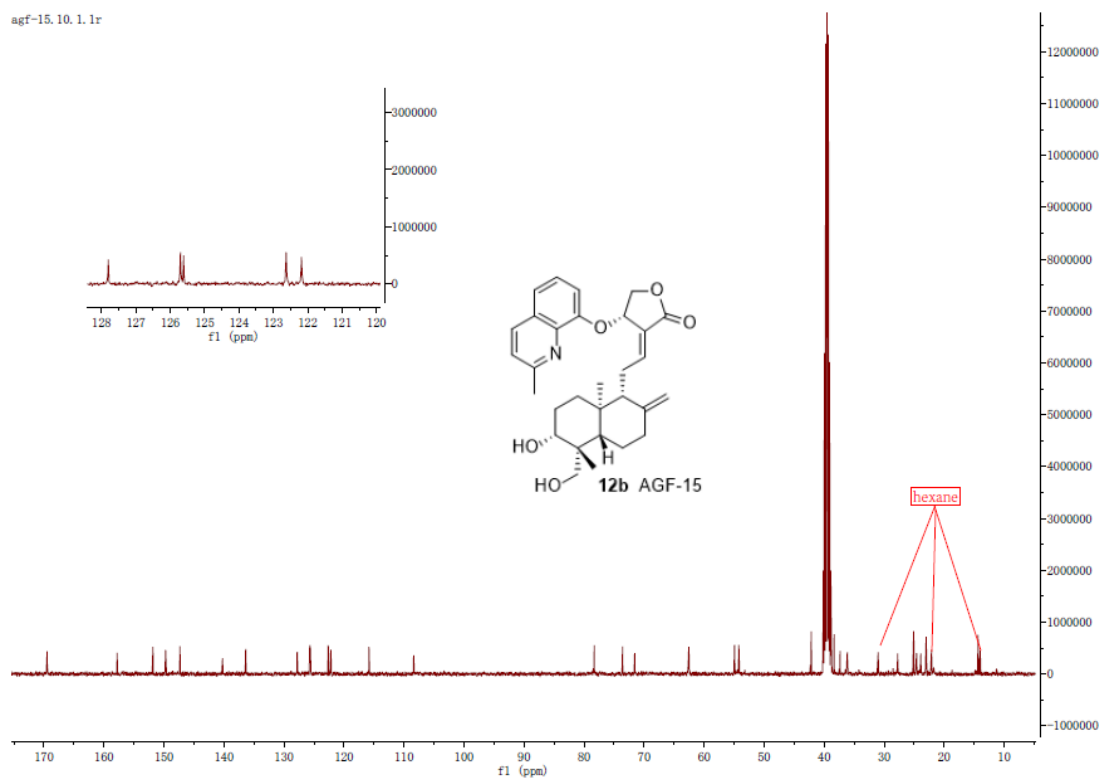
¹H NMR of **12b**

AGF-15. 1. 1. 1r



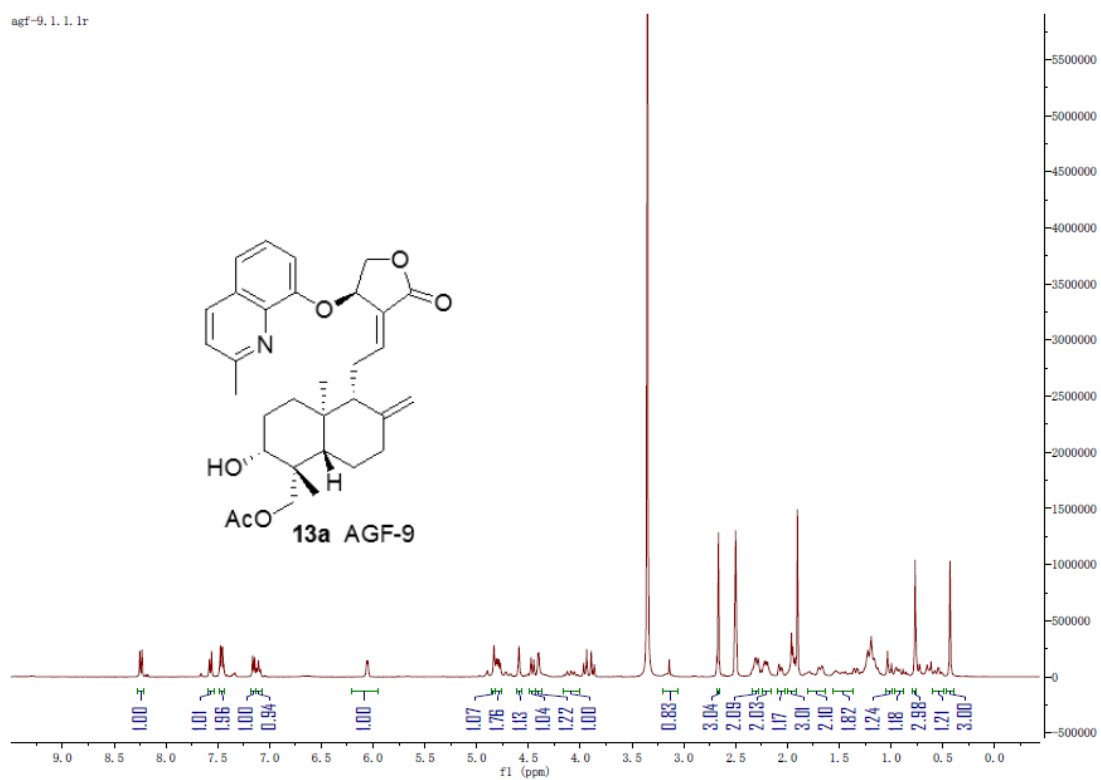
¹³C NMR of **12b**

agf-15. 10. 1. 1r



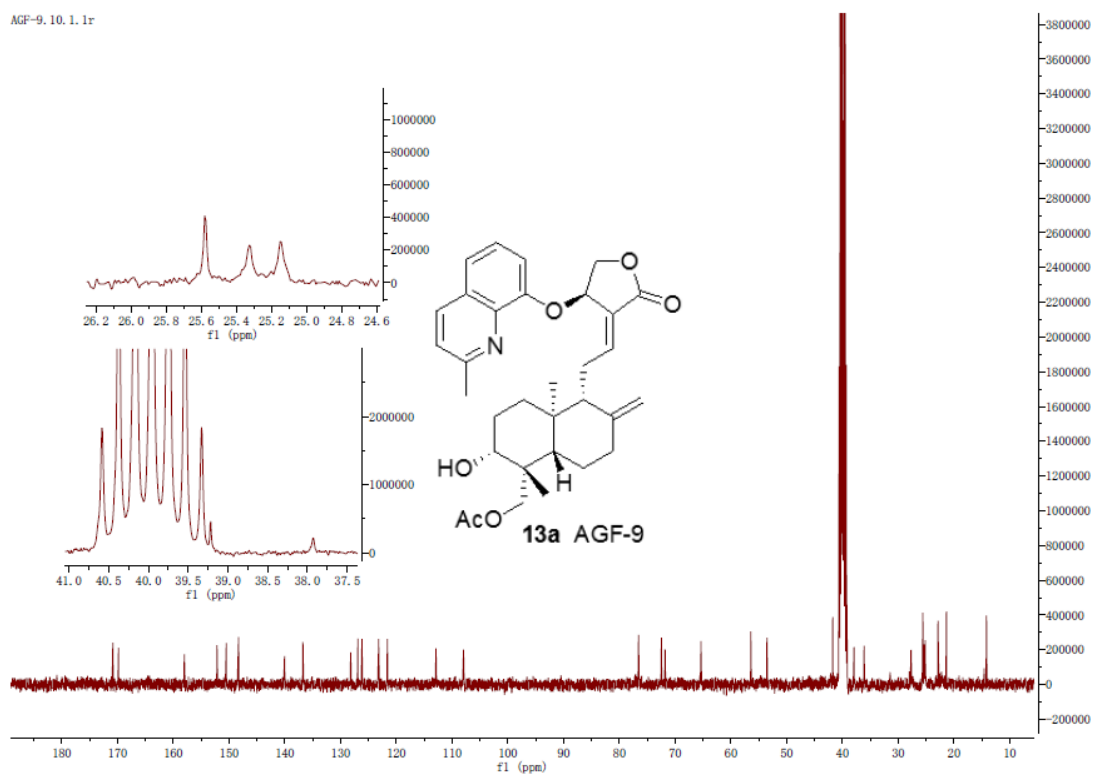
¹H NMR of **13a**

agf-9.1.1.1r



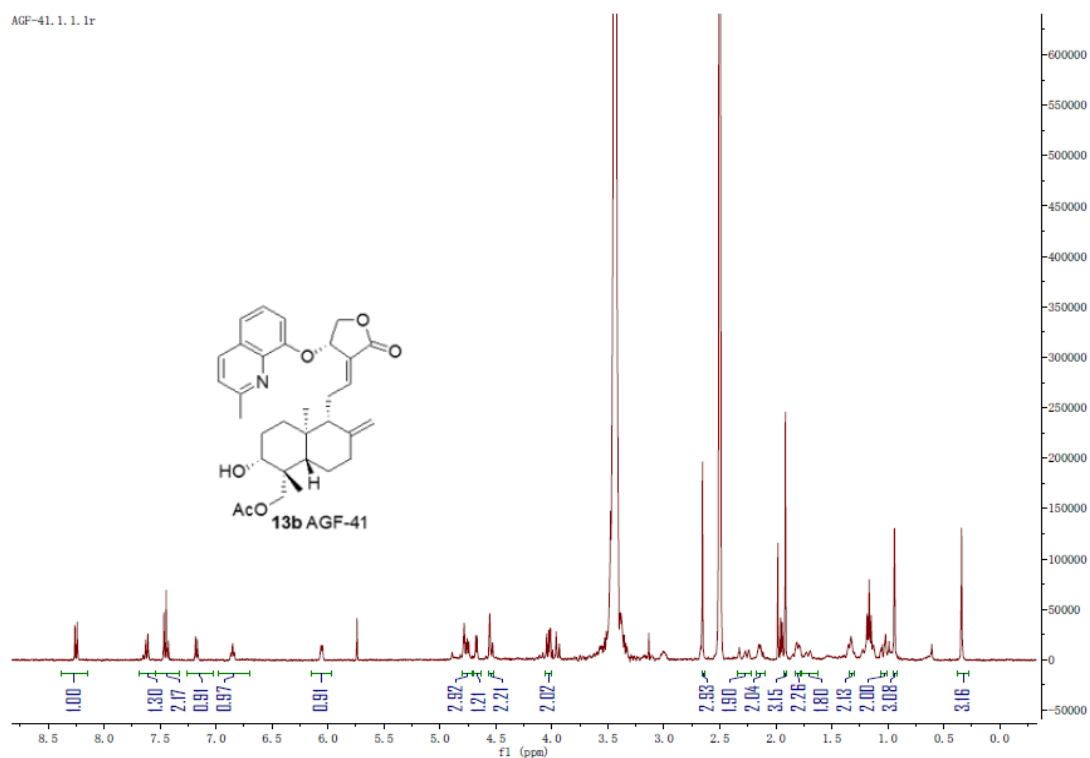
¹³C NMR of **13a**

AGF-9.10.1.1r



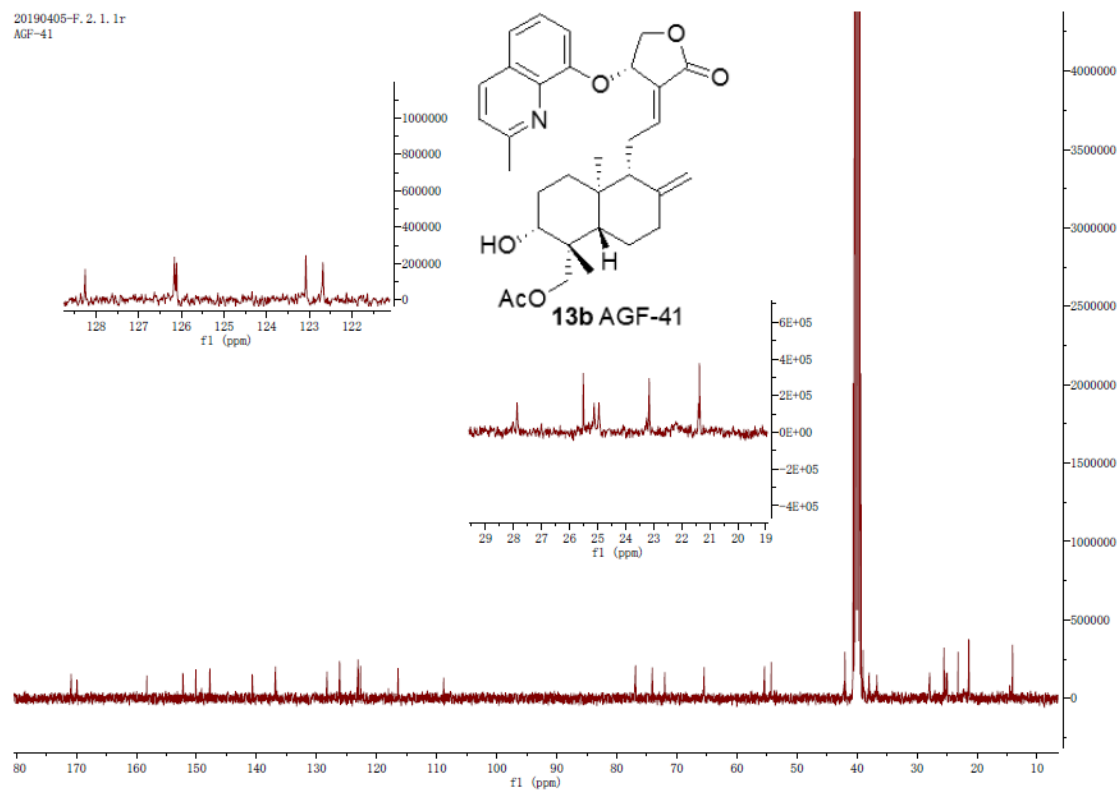
¹H NMR of **13b**

AGF-41. 1. 1. 1r



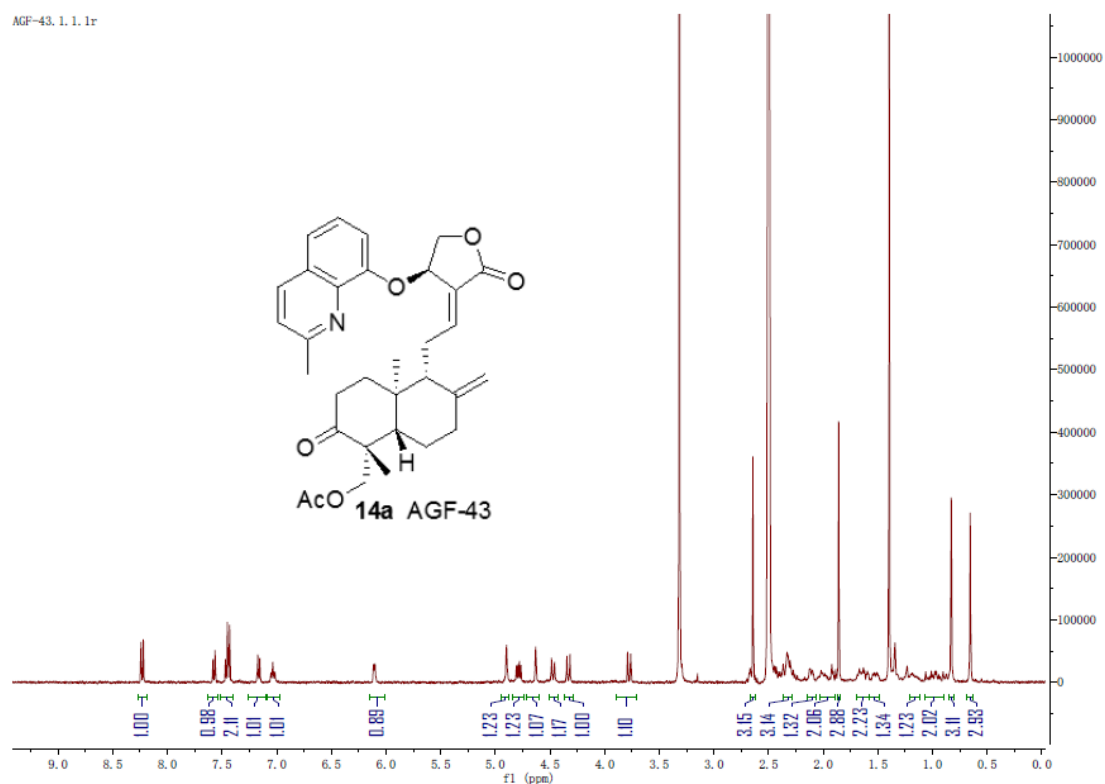
¹³C NMR of **13b**

20190405-F. 2. 1. 1r
AGF-41



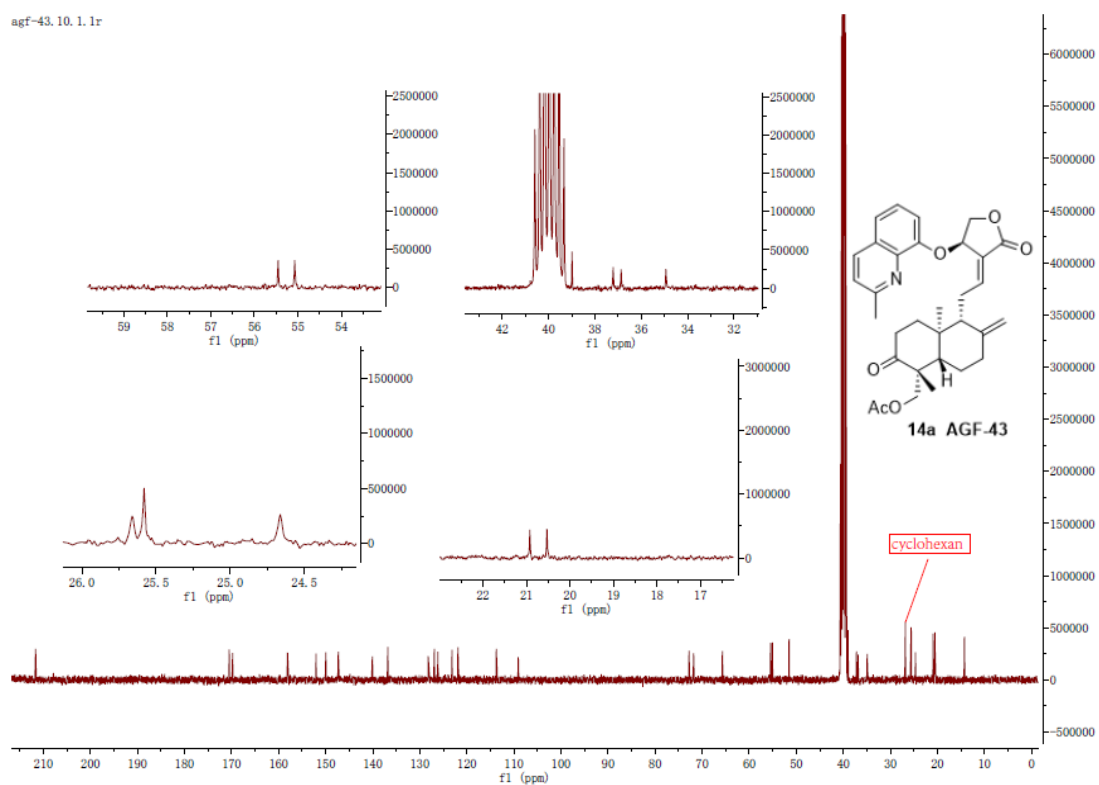
¹H NMR of 14a

AGF-43. 1. 1. 1r



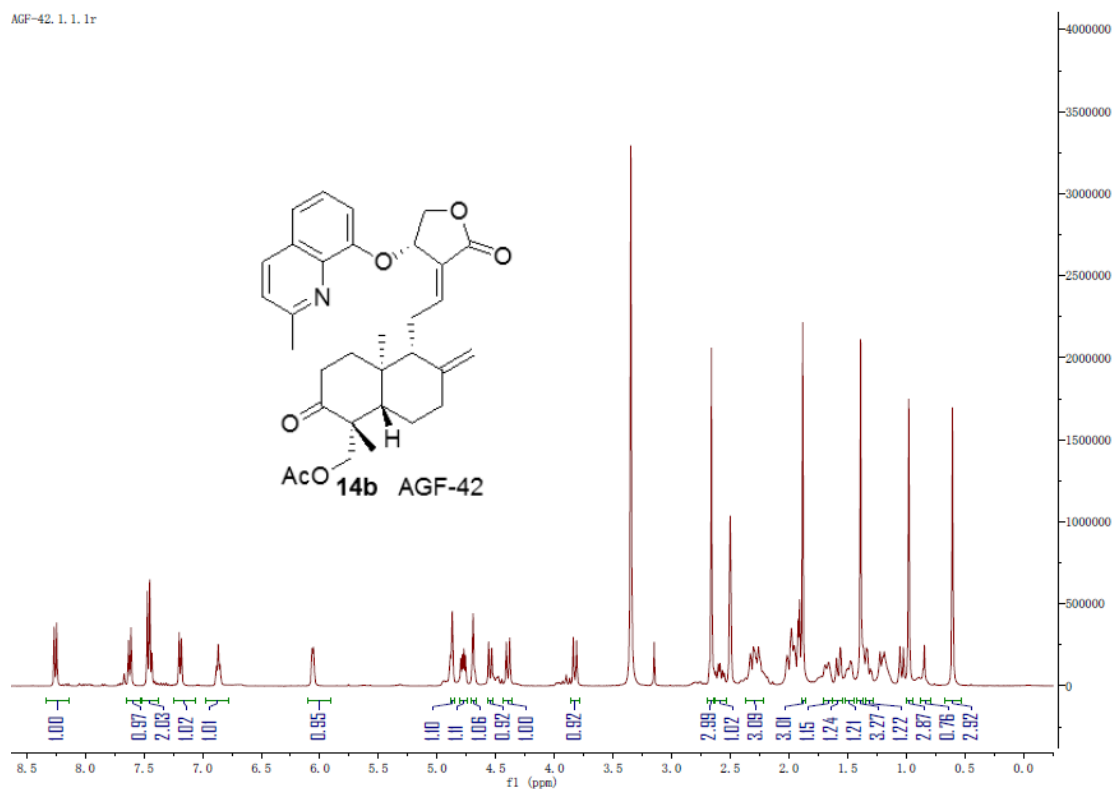
¹³C NMR of 14a

agf-43. 10. 1. 1r



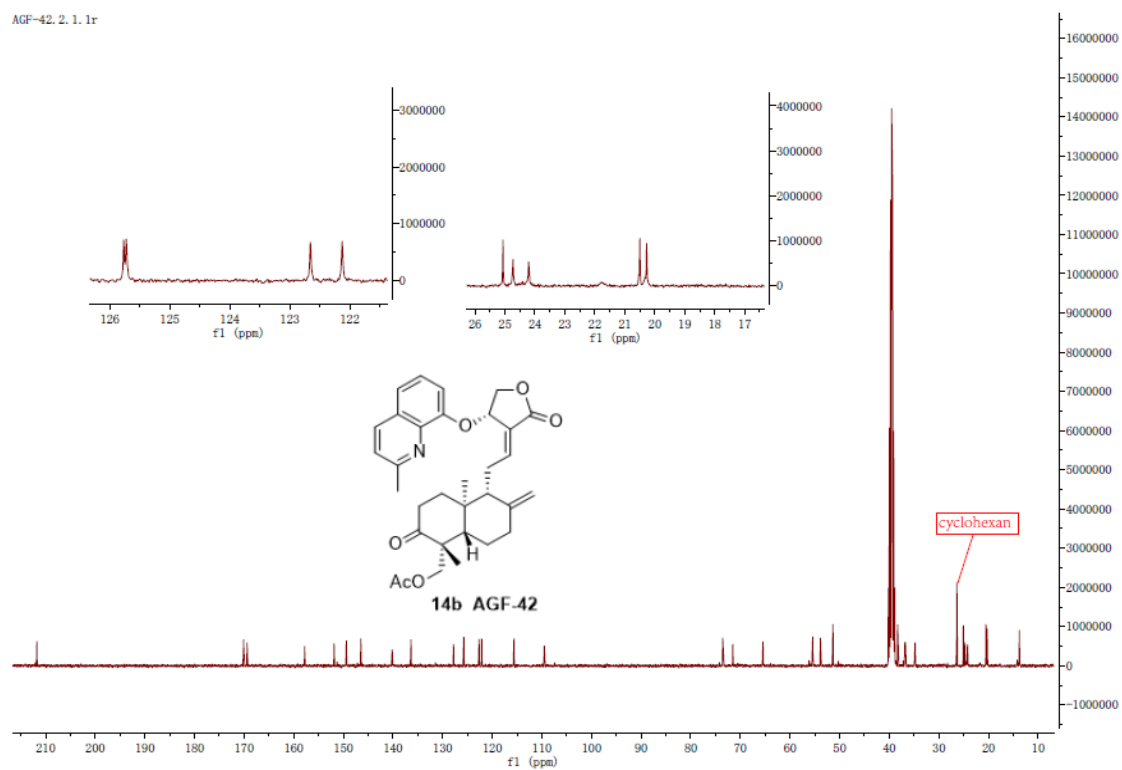
¹H NMR of **14b**

AGF-42. 1. 1. 1r



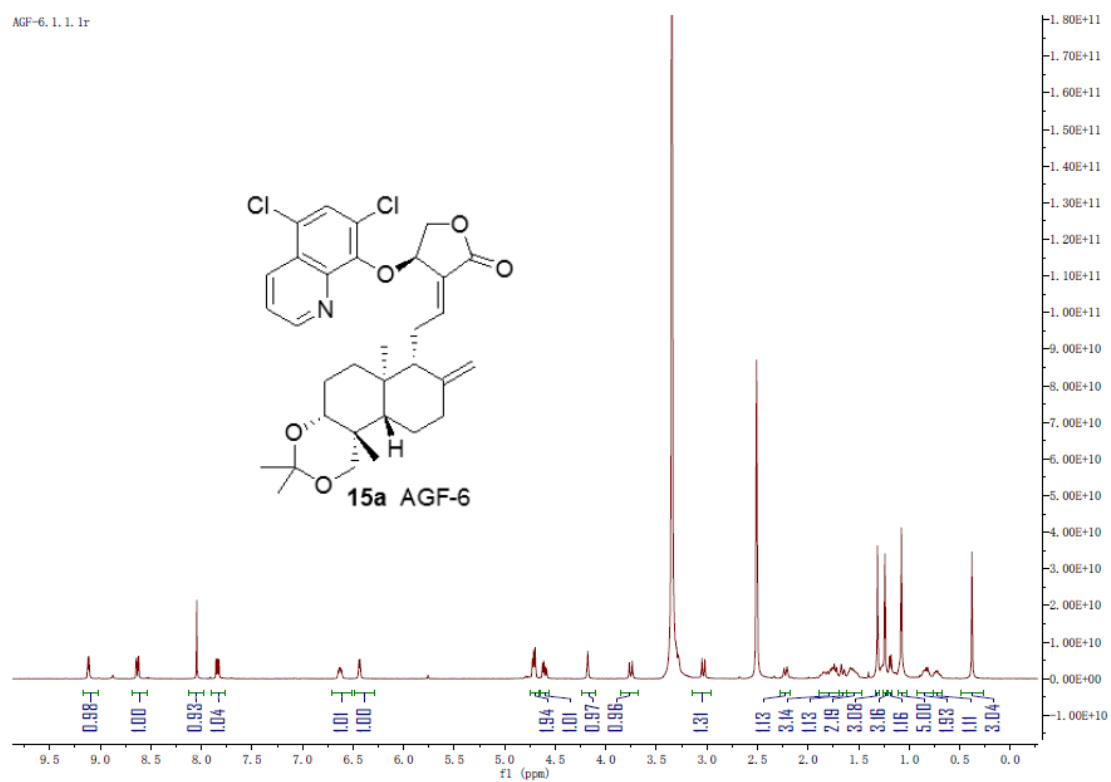
¹³C NMR of **14b**

AGF-42. 2. 1. 1r



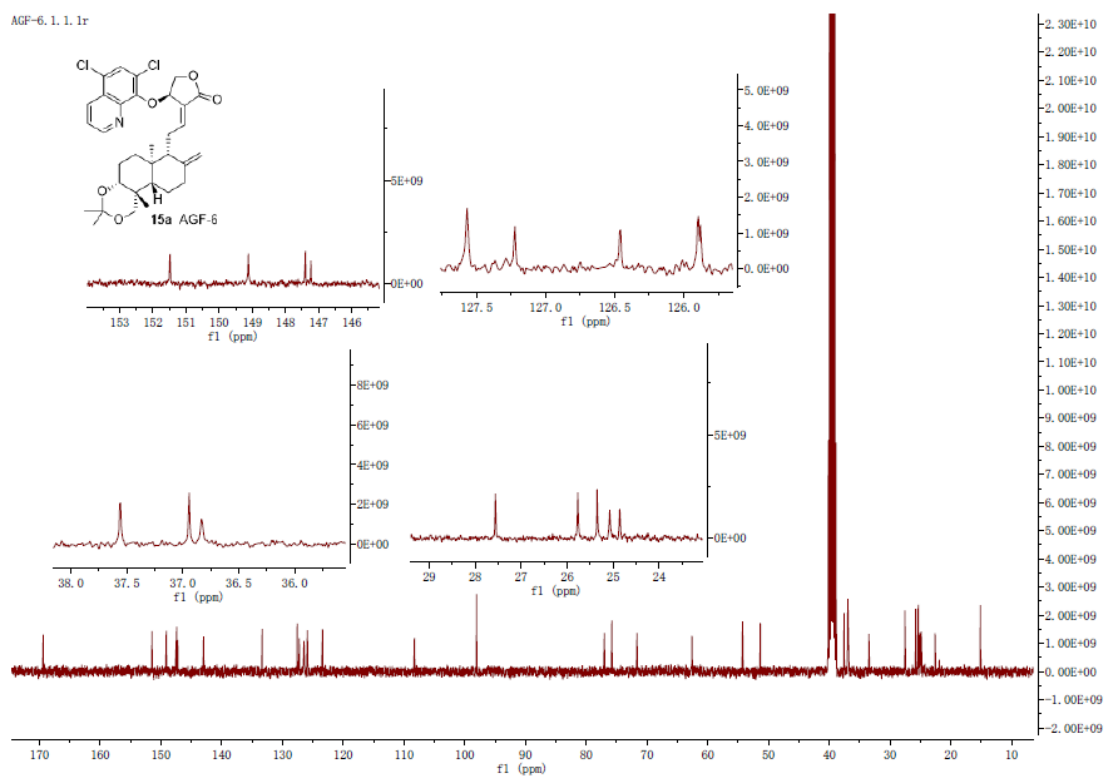
¹H NMR of 15a

AGF-6. 1. 1. 1r



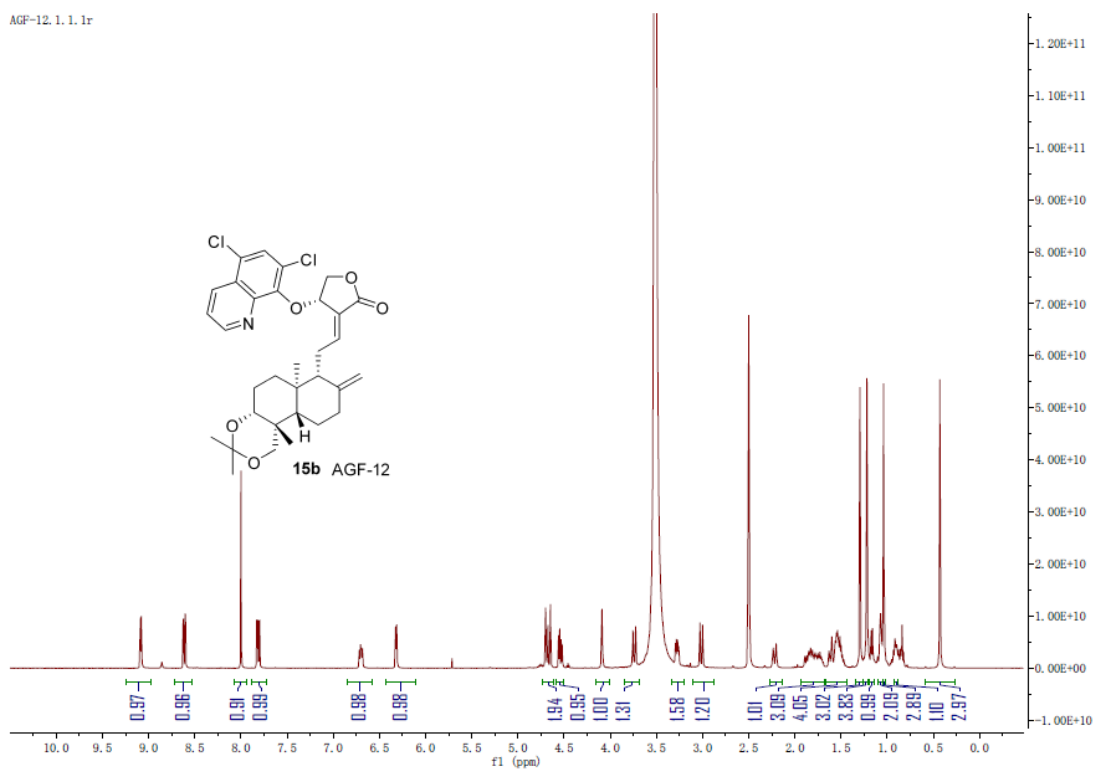
¹³C NMR of 15a

AGF-6. 1. 1. 1r



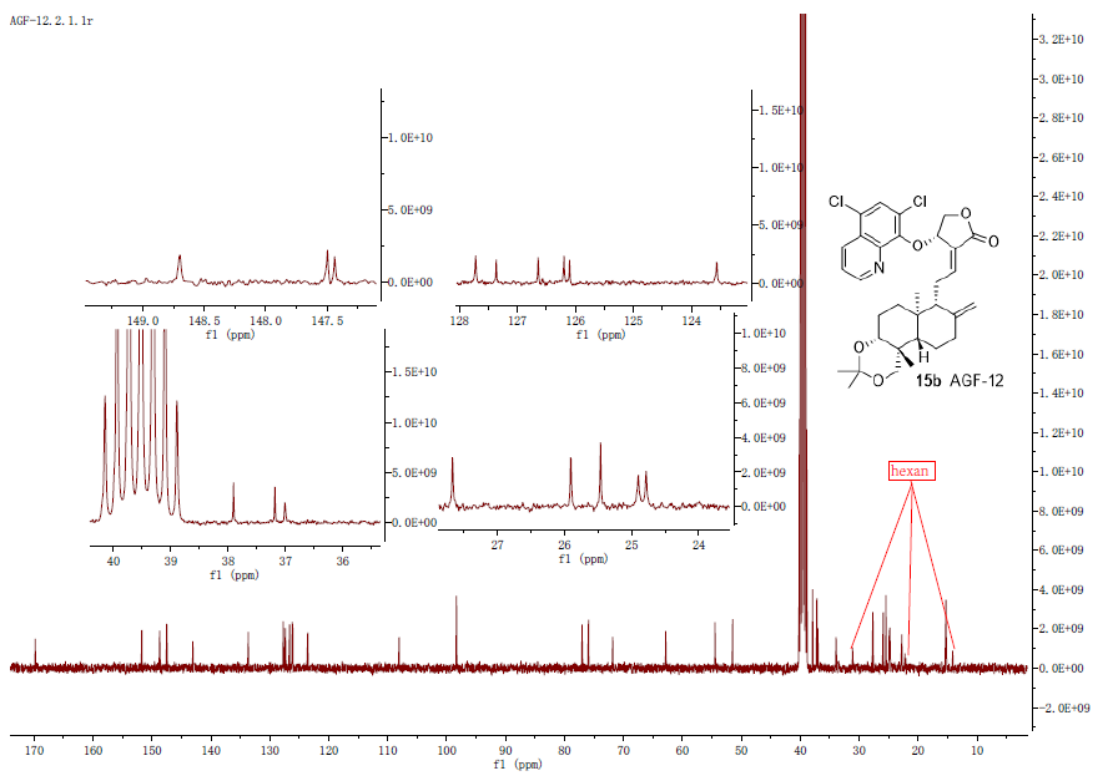
¹H NMR of **15b**

AGF-12. 1. 1. 1r



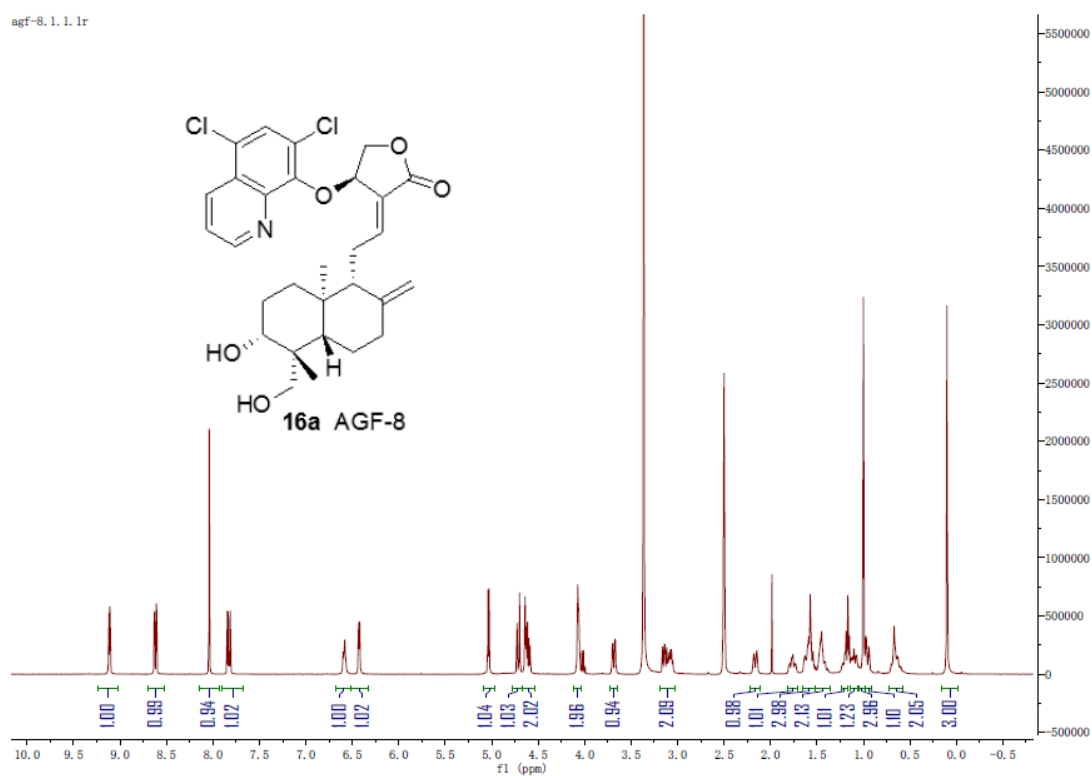
¹³C NMR of **15b**

AGF-12. 2. 1. 1r



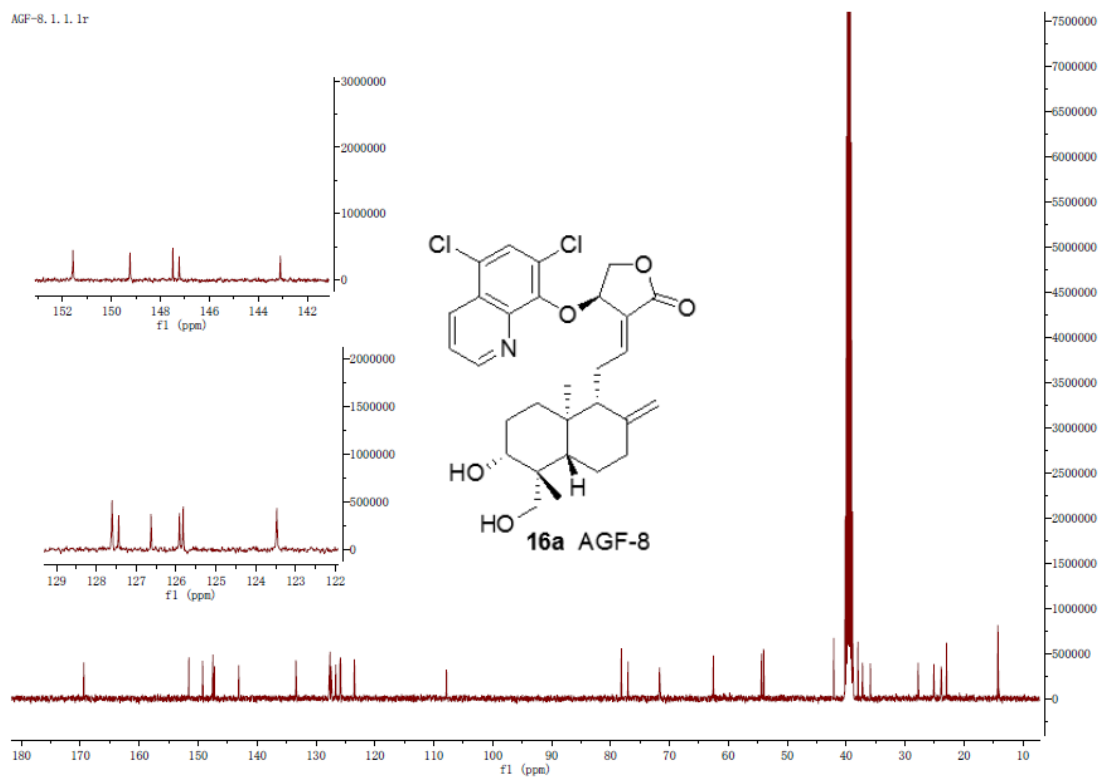
¹H NMR of 16a

agf-8.1.1.1r



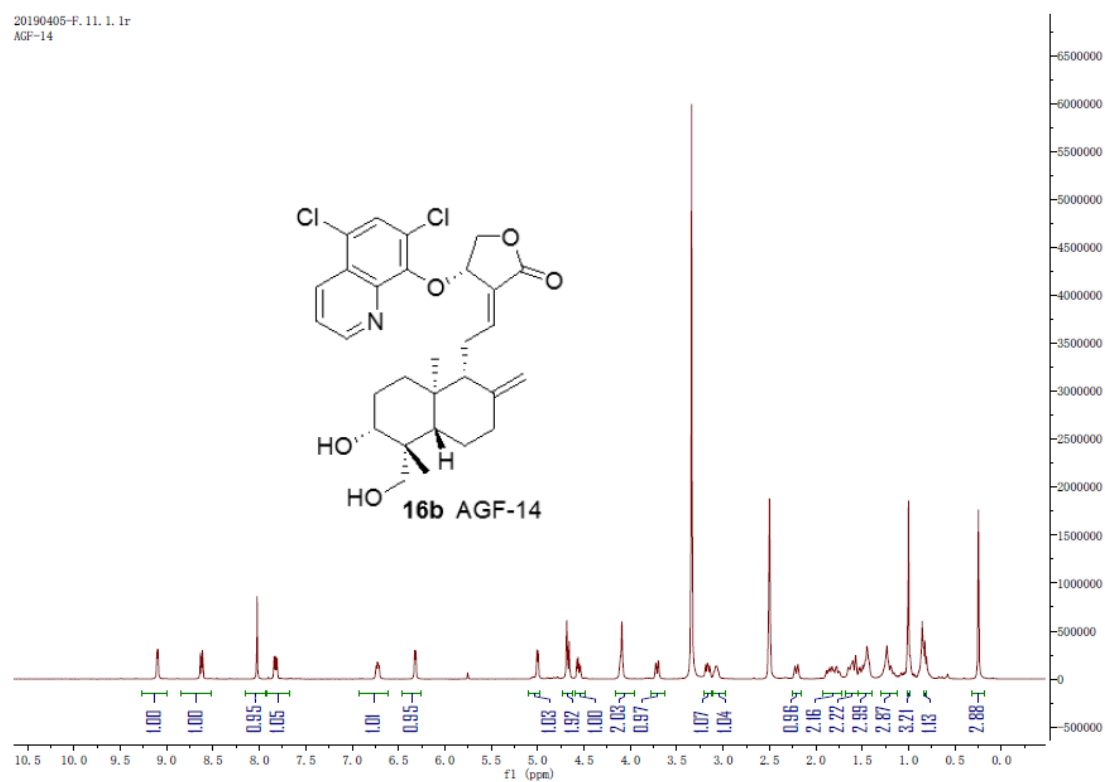
¹³C NMR of 16a

AGF-8.1.1.1r



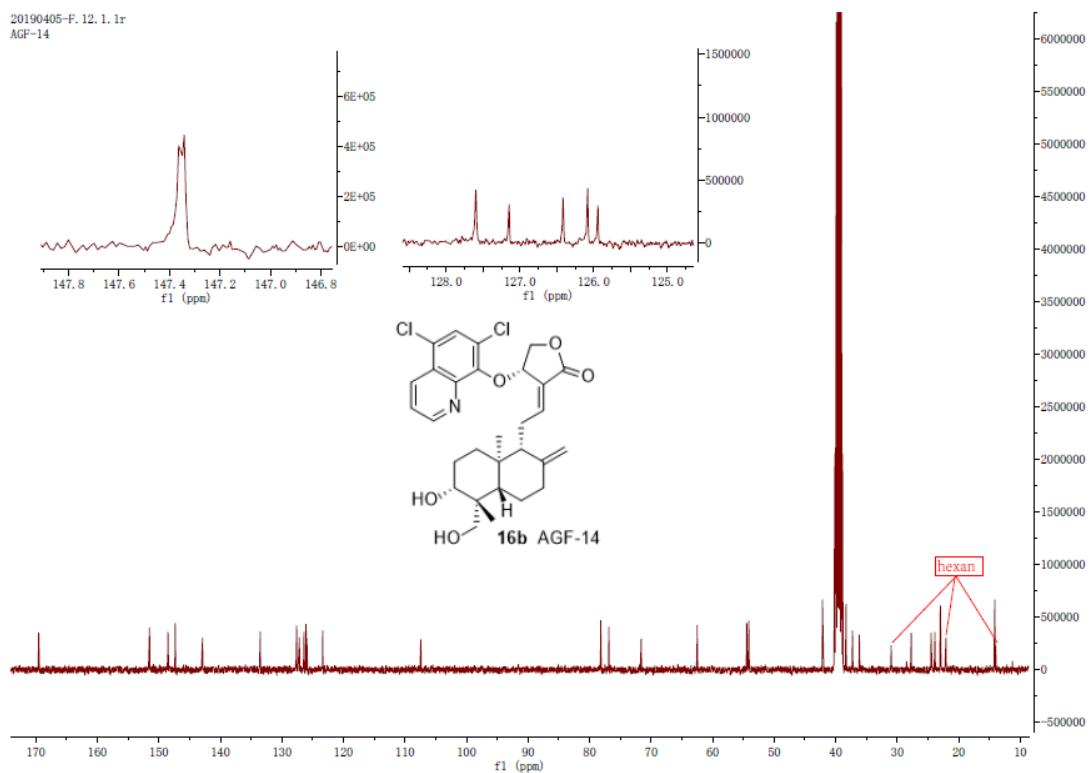
¹H NMR of 16b

20190405-F. 11. 1. 1r
AGF-14



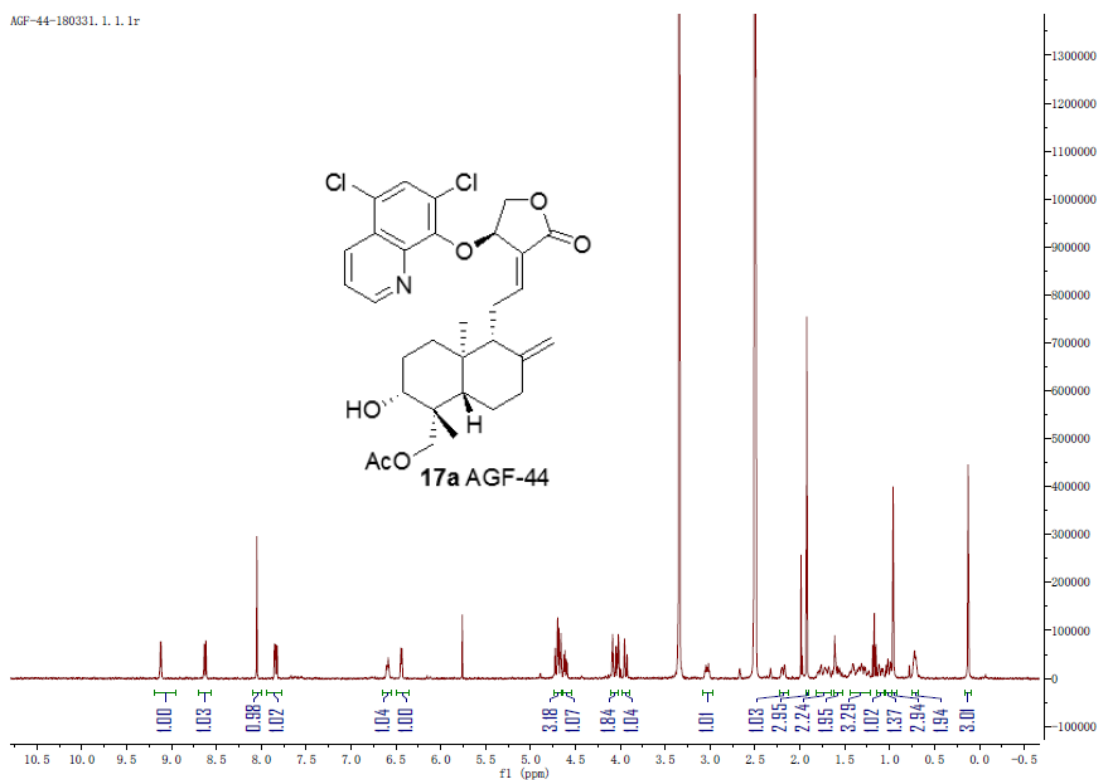
¹³C NMR of 16

20190405-F. 12. 1. 1r
AGF-14



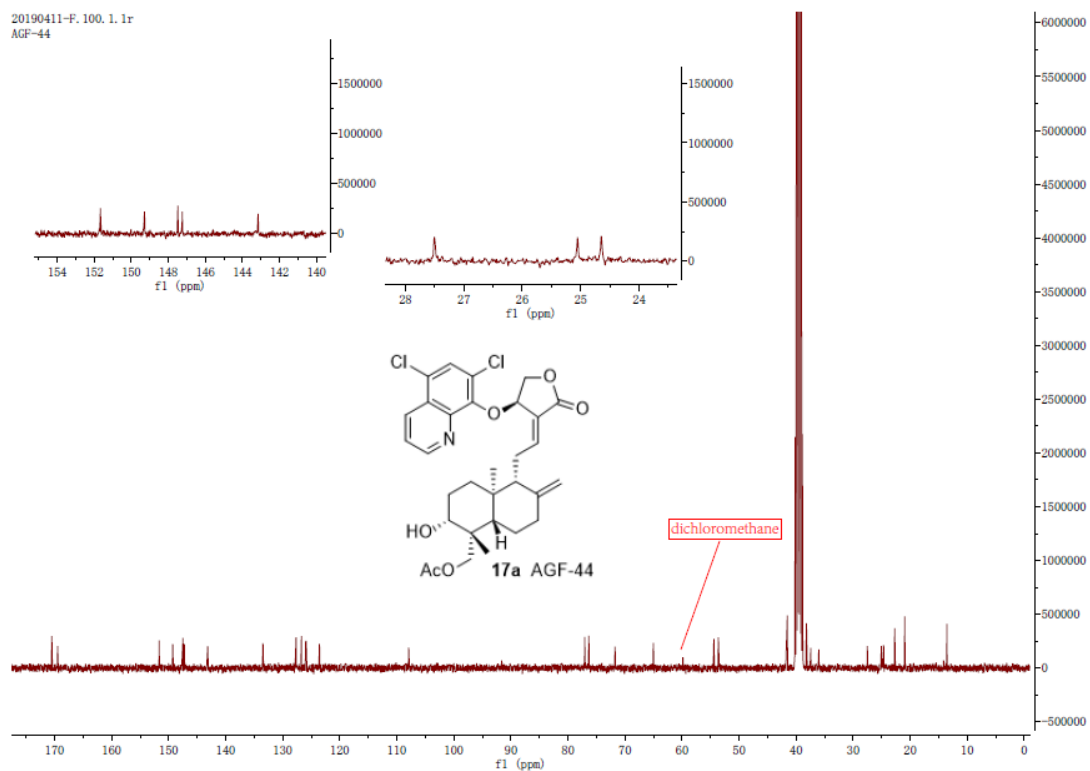
¹H NMR of 17a

AGF-44-180331.1.1.1r



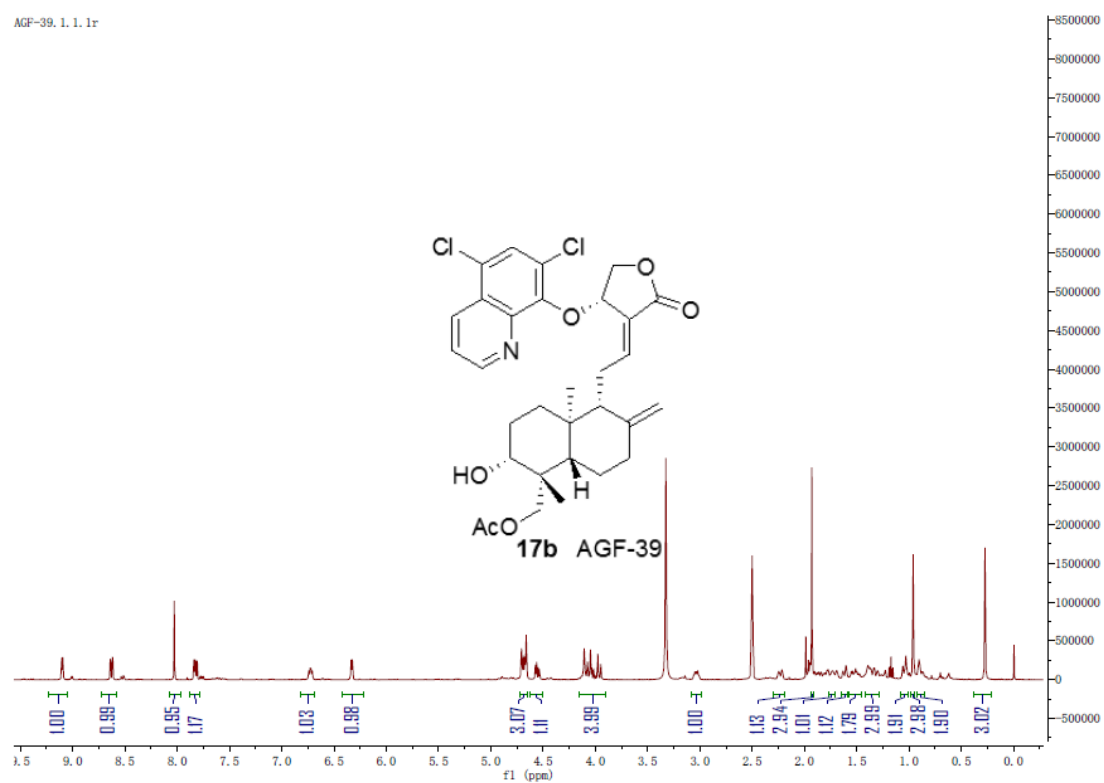
¹³C NMR of 17a

20190411-F.100.1.1r
AGF-44



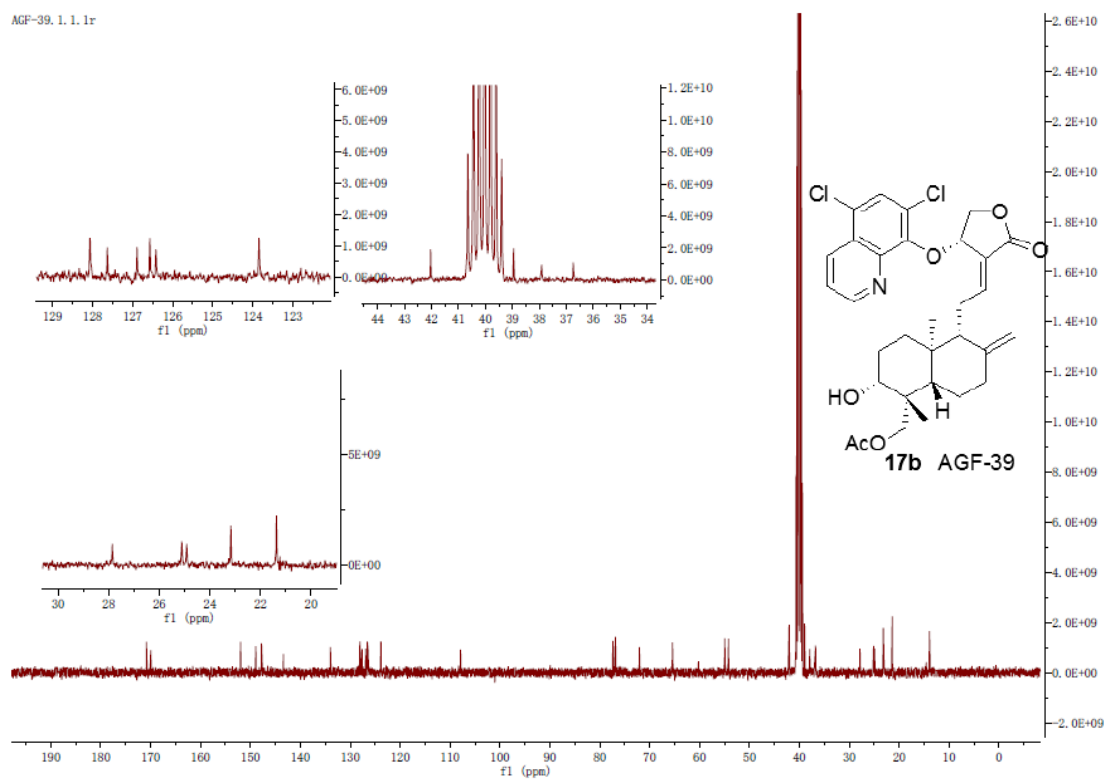
¹H NMR of **17b**

AGF-39. 1.1.1r



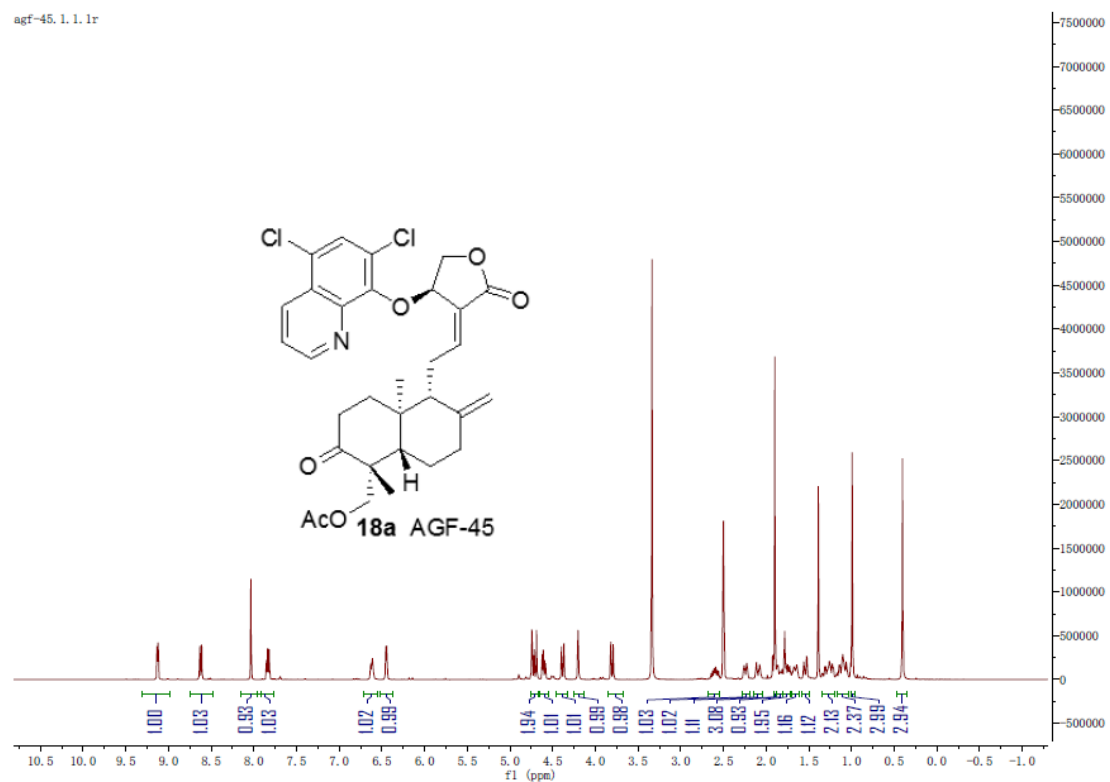
¹³C NMR of **17b**

AGF-39. 1.1.1r



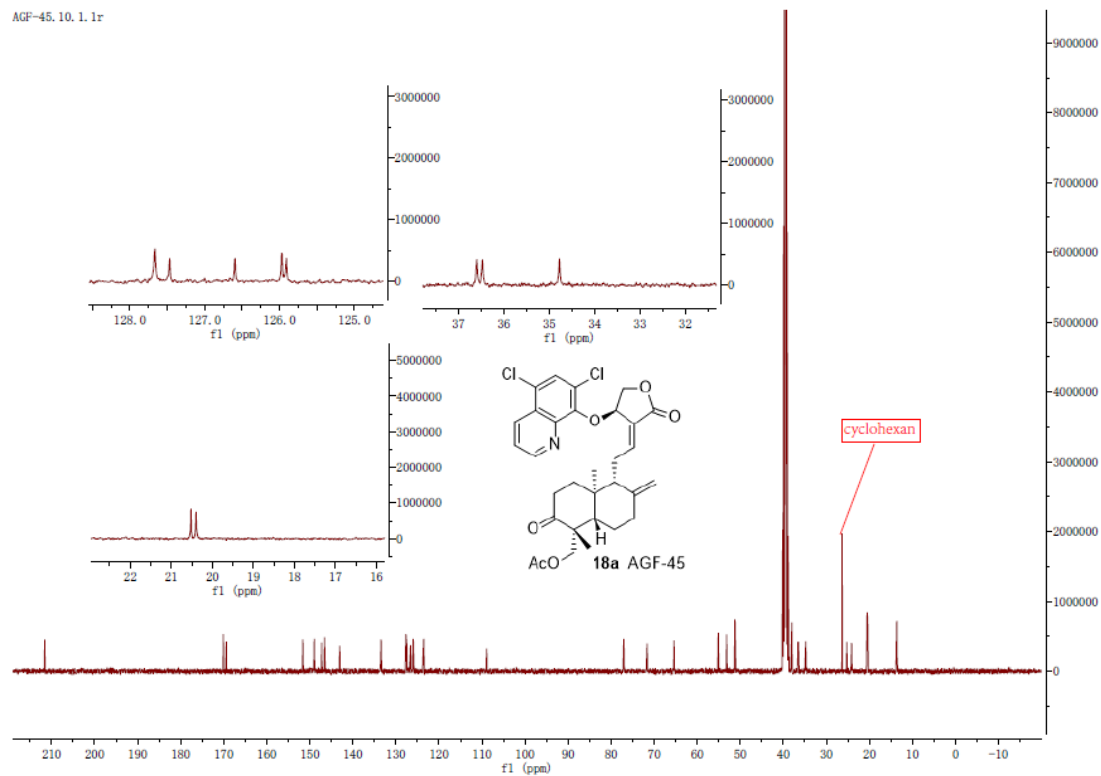
¹H NMR of **18a**

agf-45. 1. 1. 1r



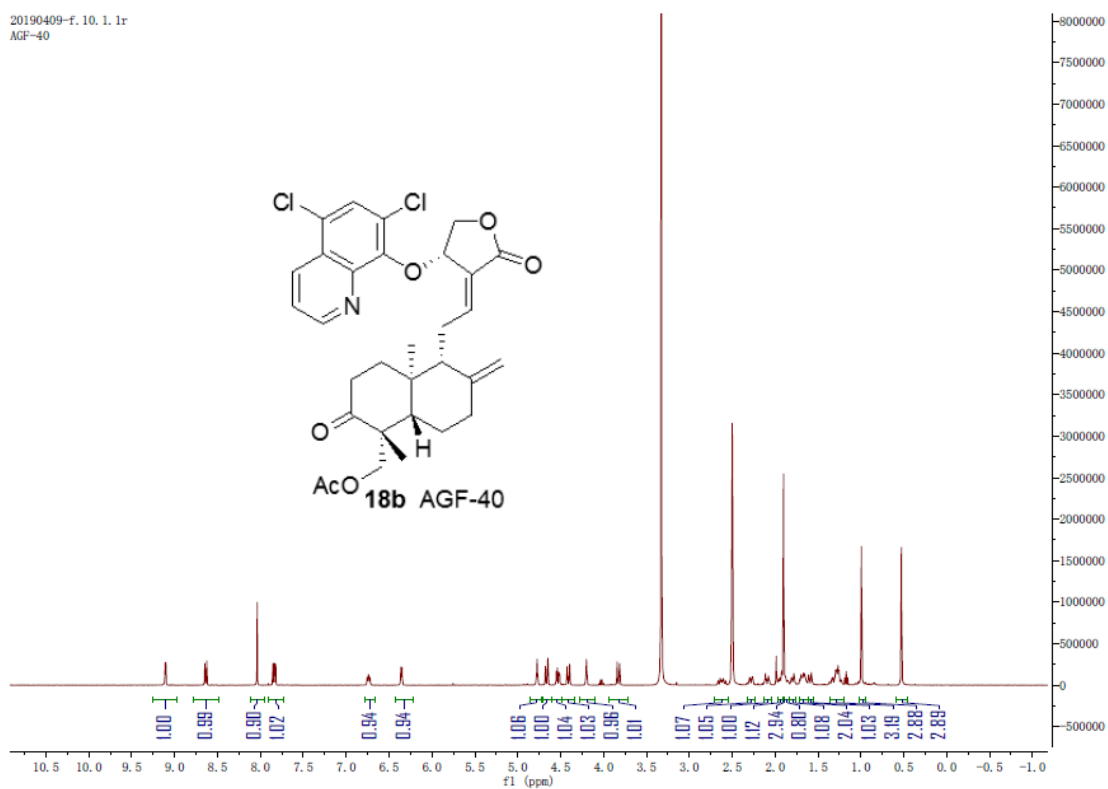
¹³C NMR of **18a**

AGF-45. 10. 1. 1r



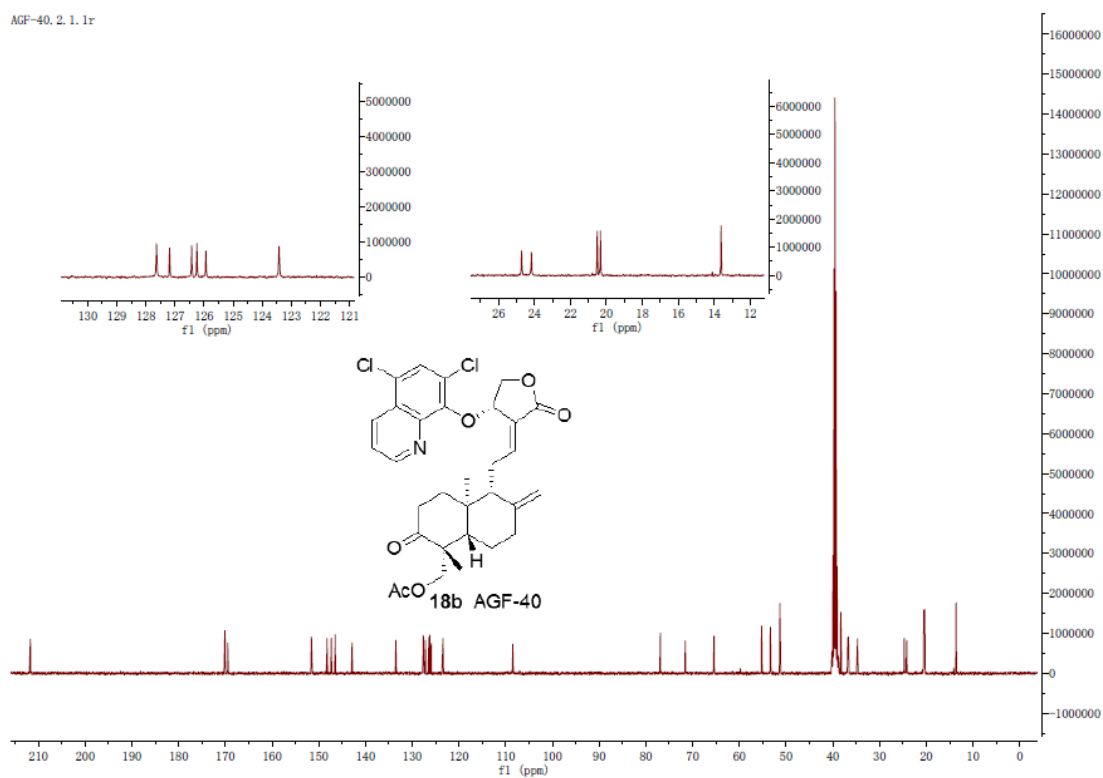
¹H NMR of **18b**

20190409-f. 10. 1. 1r
AGF-40



¹³C NMR of **18b**

AGF-40. 2. 1. 1r



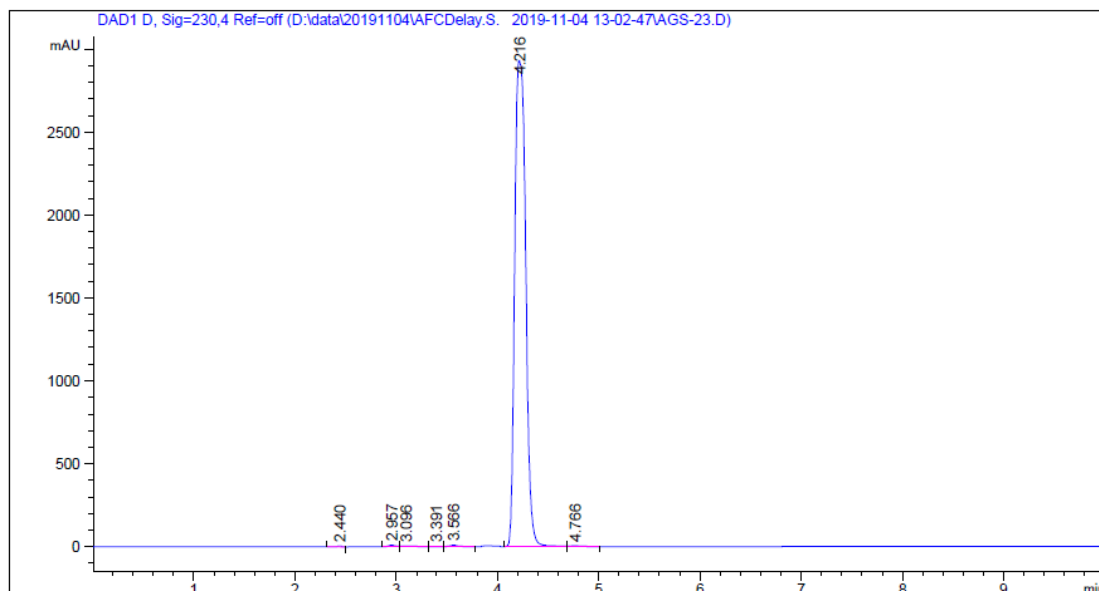
Details for compound dilutions and viral inoculation for screening

SNB-19 cells were seeded overnight into 96-well plates to achieve 20,000-30,000 cells the next day. In 96-well compound dilution plates, 300 μ l of 1X complete DMEM media (DMEM, 10% FBS, 1% PSA 1% NEAA to column 1 and 200 μ l of 1X complete DMEM media to columns 2-12. Individual compounds in a volume of 0.9 μ l at 10mM concentration was added to each well in column 1, for a total of 8 compounds per 96-well plate. Each well in column 1 was mixed 5 times; then, 100 μ l was removed from column 1 and added to column 2 and mixed. This process was repeated for each dilution across columns 1-12 to achieve an 11-fold dilution range of 30 μ M-0.001 μ M (30 μ M, 10 μ M, 3 μ M, 1 μ M, 0.3 μ M, 0.1 μ M, 0.03 μ M, 0.01 μ M, 0.003 μ M, 0.001 μ M). Column 12 was the no compound control. Day old media from SNB-19 cells was removed by aspiration and 100 μ l of each drug dilution was added to the previously seeded SNB-19 cells. After a 1 hour incubation at 37 °C and 5% CO₂, high titer Zika virus (strain PRVABC59) was added to cells at MOI = 1, and incubated for 24 hours.

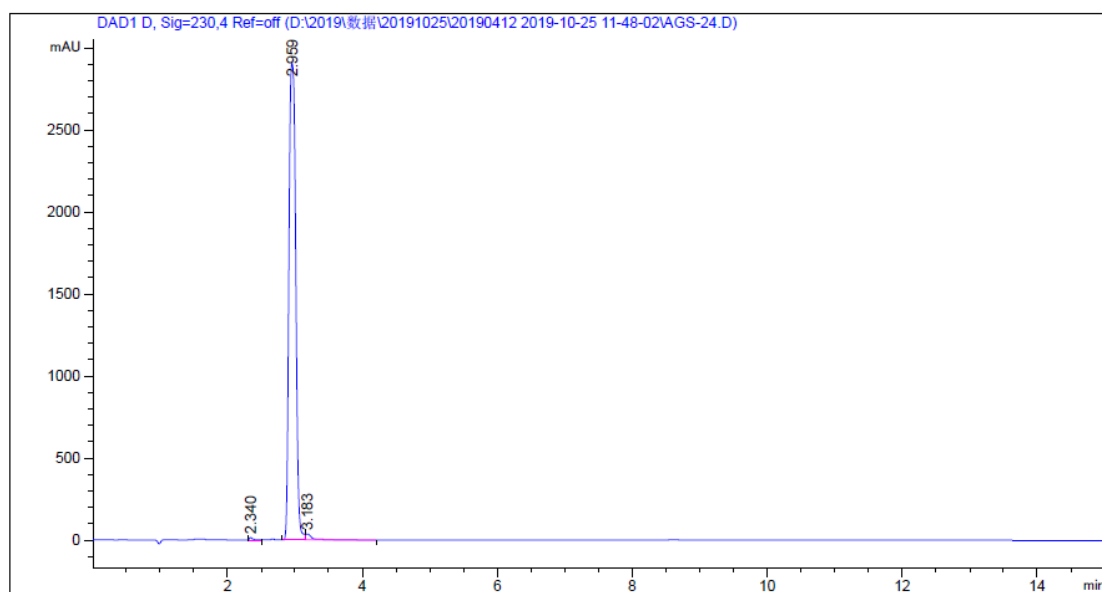
HPLC analysis spectra

HPLC analysis condition: Sunfire C18 column (4.6 × 250 mm, 5 μm), elution by 90% methanol with 10% purified water (for compounds of **2**, **3**, **5**, **8**, **11a**, **11b**, **14a**, **14b**, **15a**, **15b**, **18a**, **18b**) or 80% methanol with 20% purified water (for compounds of **4**, **6**, **7**, **9**, **12a**, **12b**, **13a**, **13b**, **16a**, **16b**, **17a**, **17b**), rate = 1.0 ml/min, detection wavelength of 230 nm.

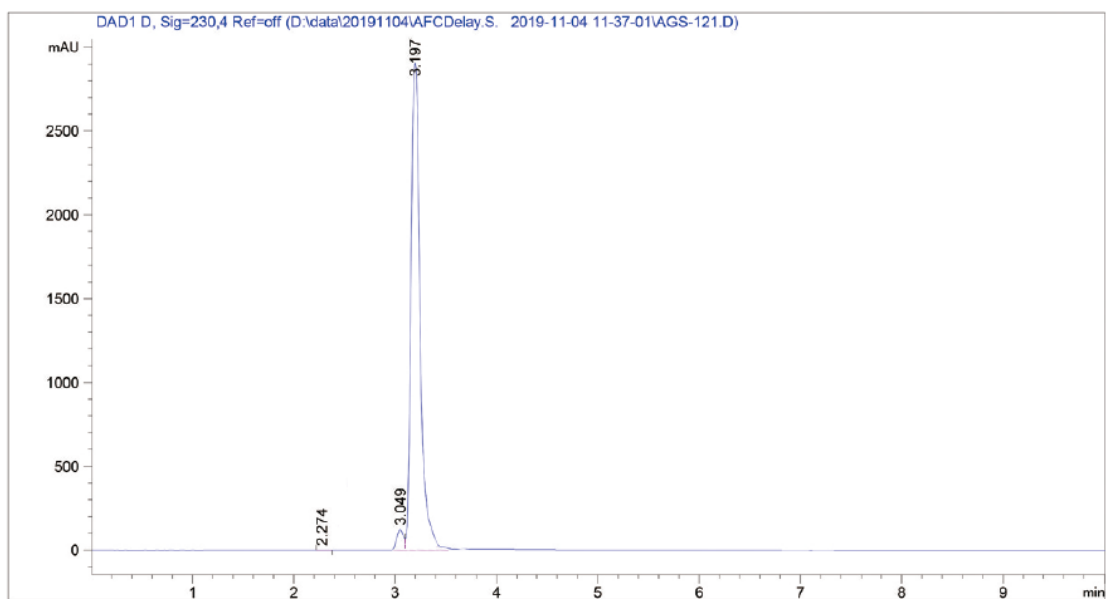
HPLC spectrum of **2** (purity >98%)



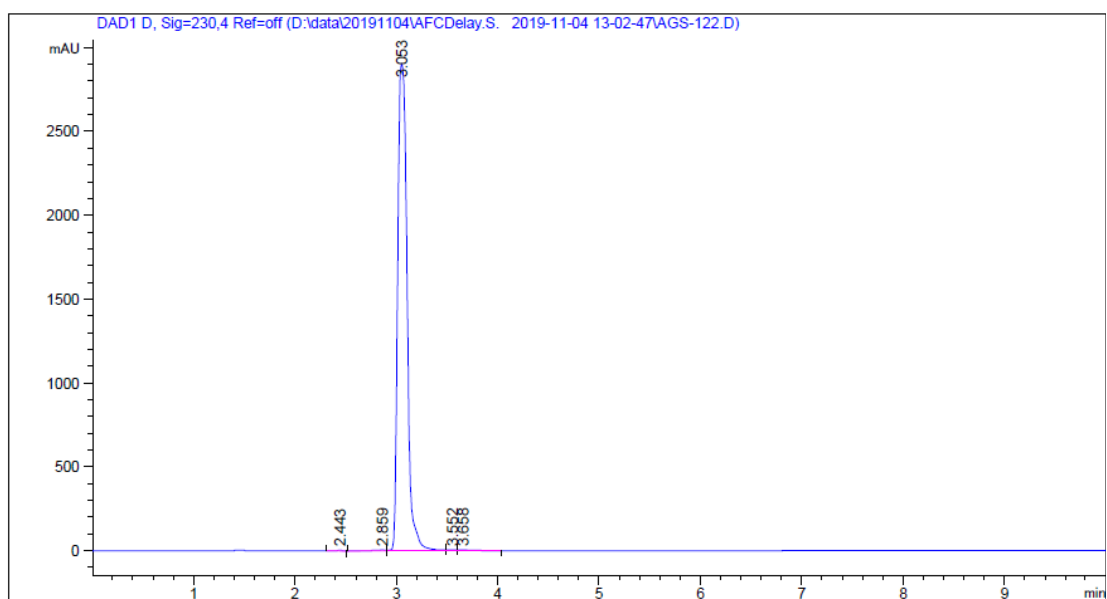
HPLC spectrum of **3** (purity >98%)



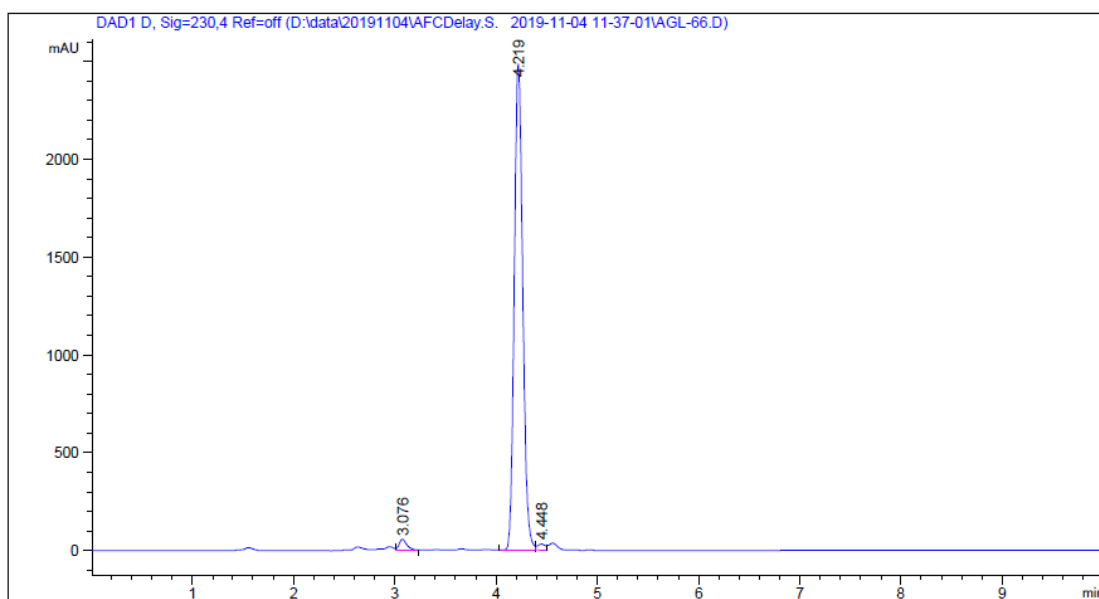
HPLC spectrum of **4** (purity >95%)



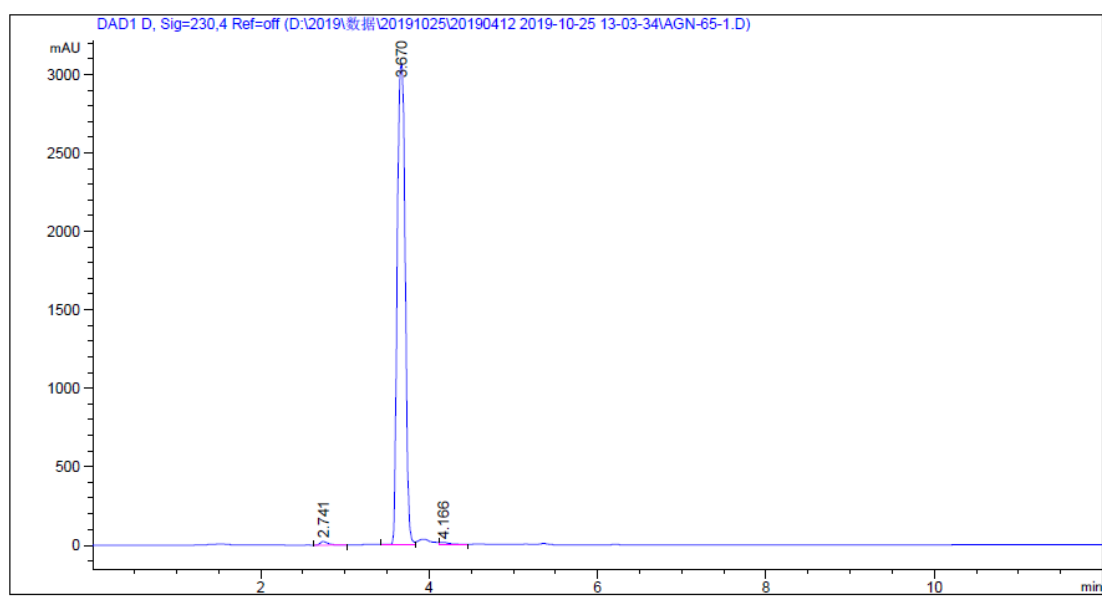
HPLC spectrum of **5** (purity >98%)



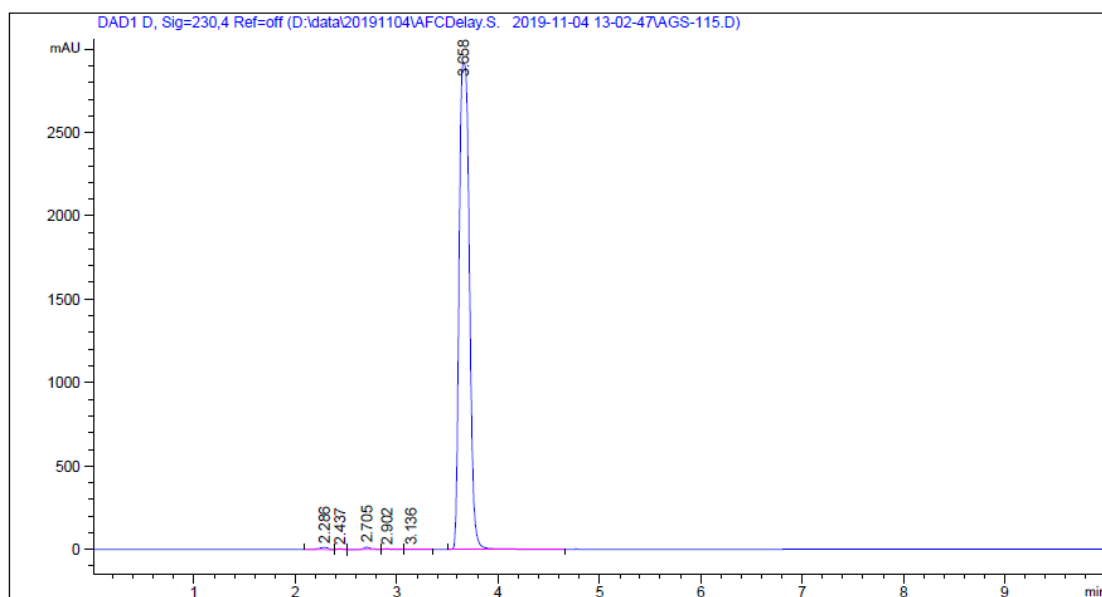
HPLC spectrum of **6** (purity >95%)



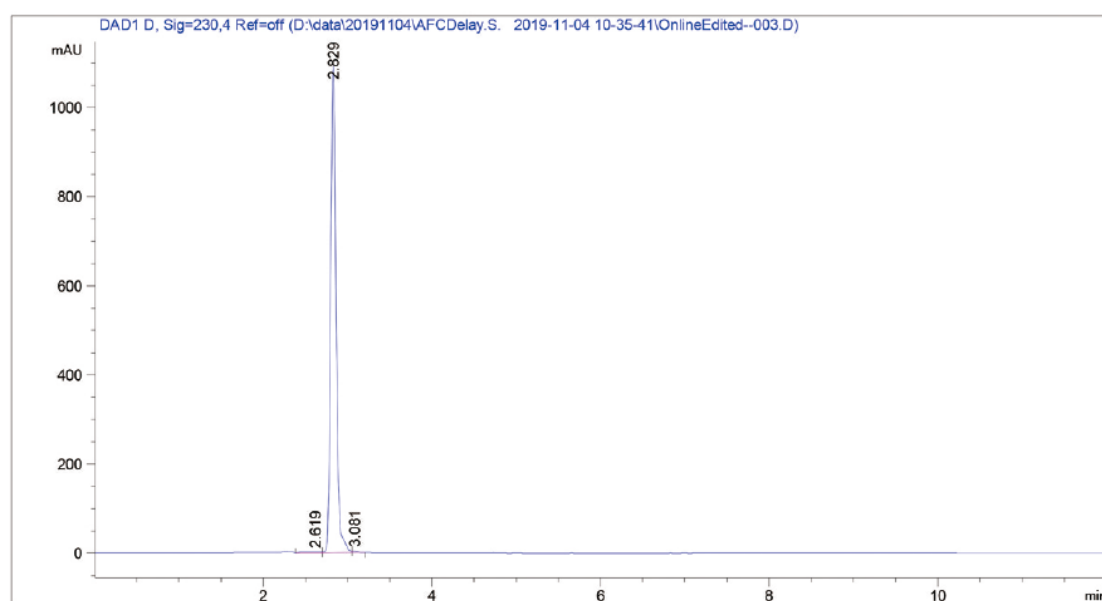
HPLC spectrum of **7** (purity >95%)



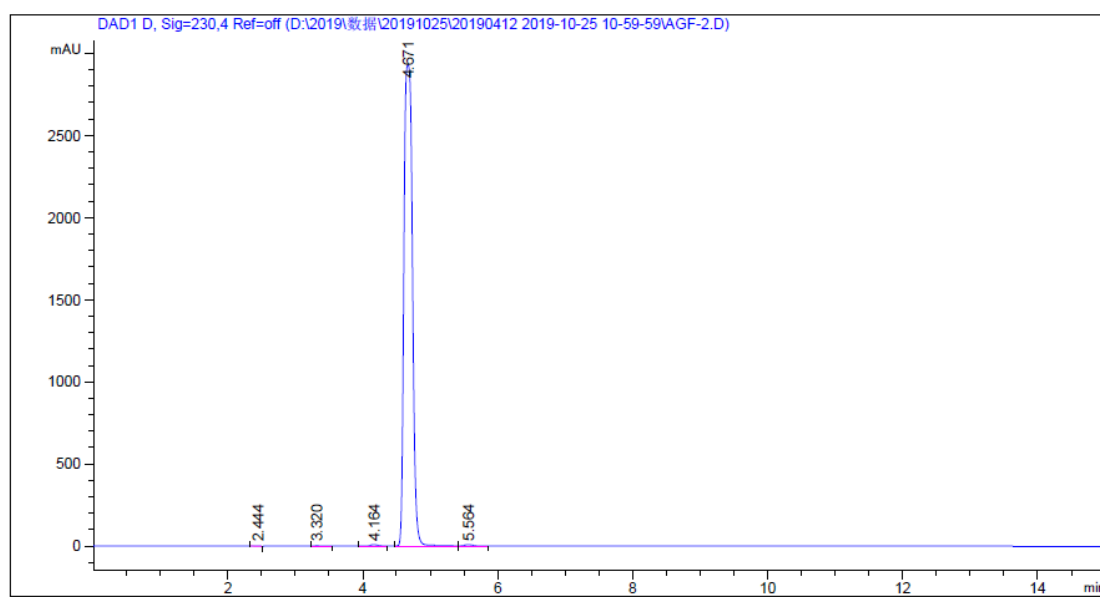
HPLC spectrum of **8** (purity >97%)



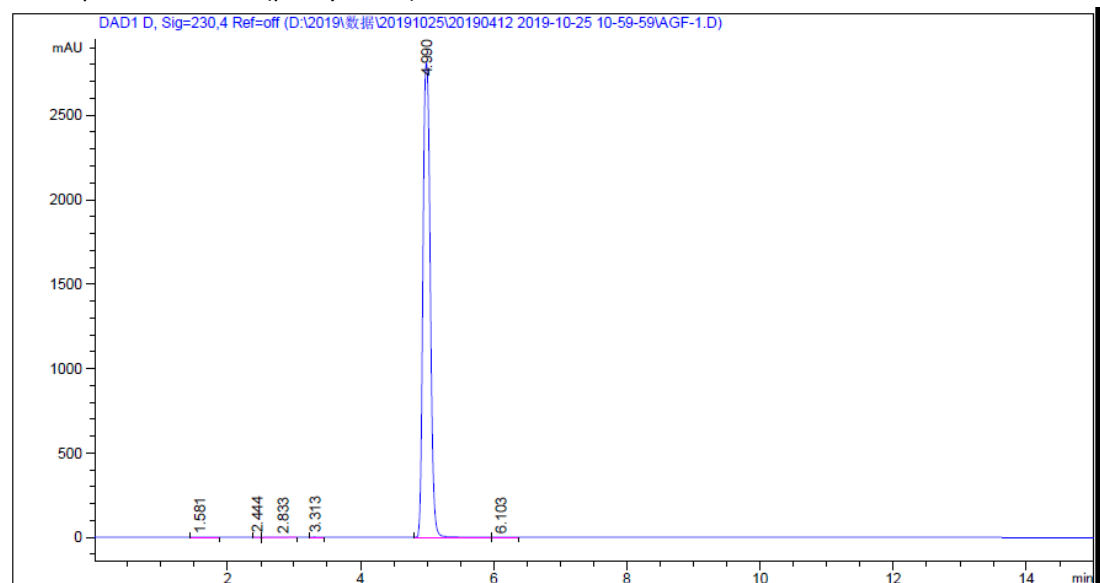
HPLC spectrum of **9** (purity >97%)



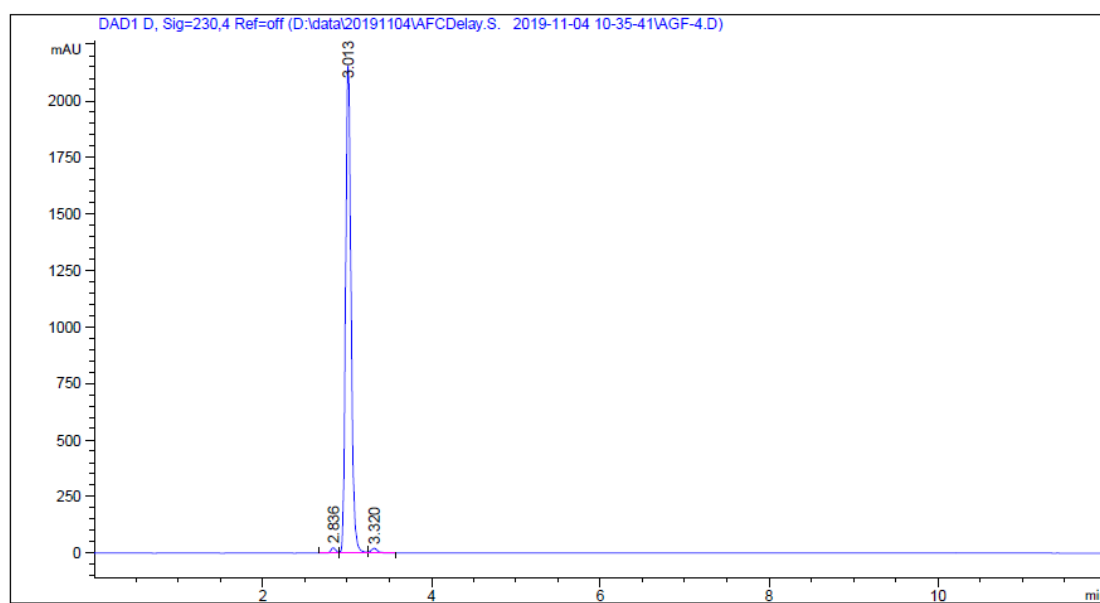
HPLC spectrum of **11a** (purity >98%)



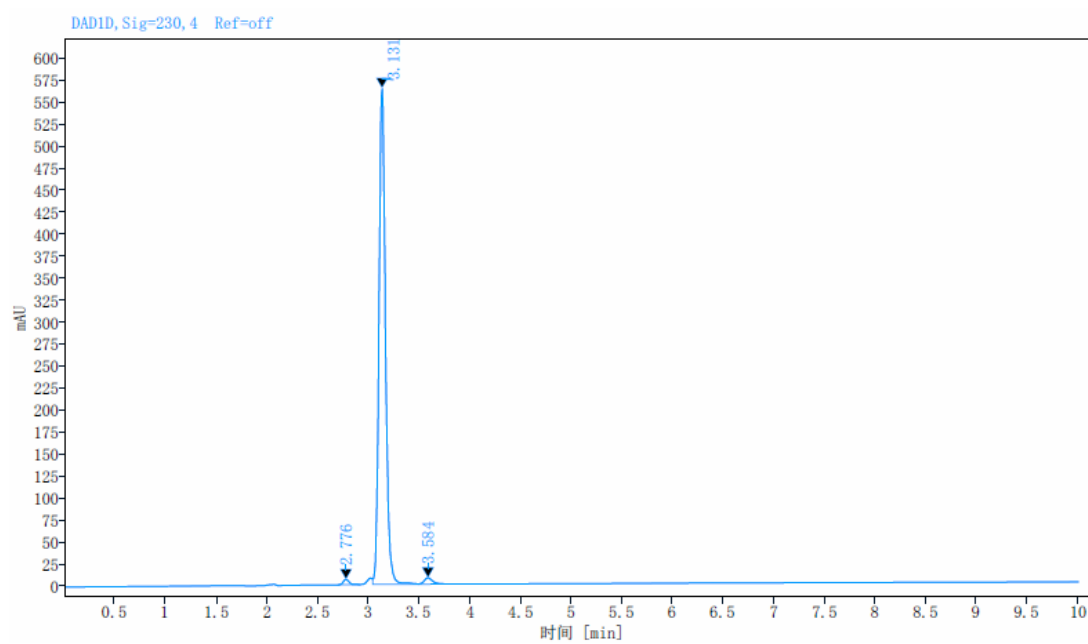
HPLC spectrum of **11b** (purity >98%)



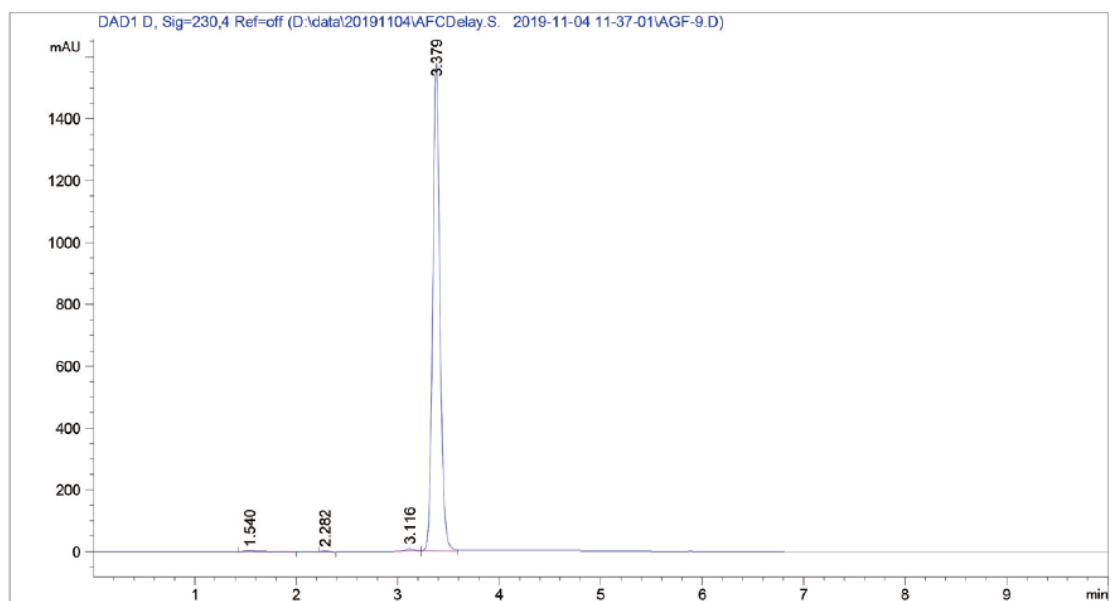
HPLC spectrum of **12a** (purity >97%)



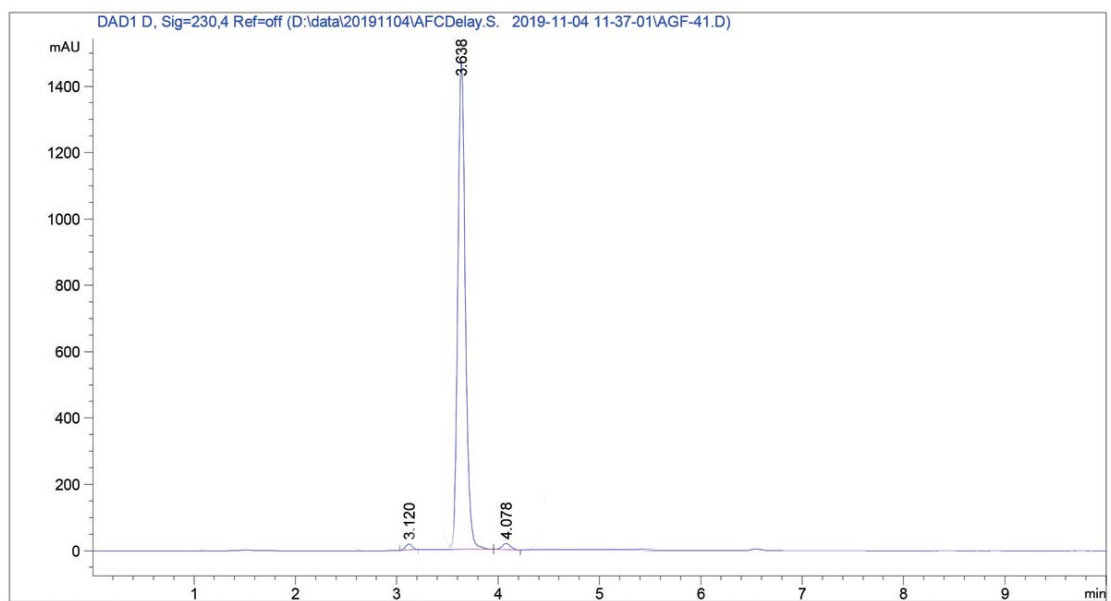
HPLC spectrum of **12b** (purity >95%)



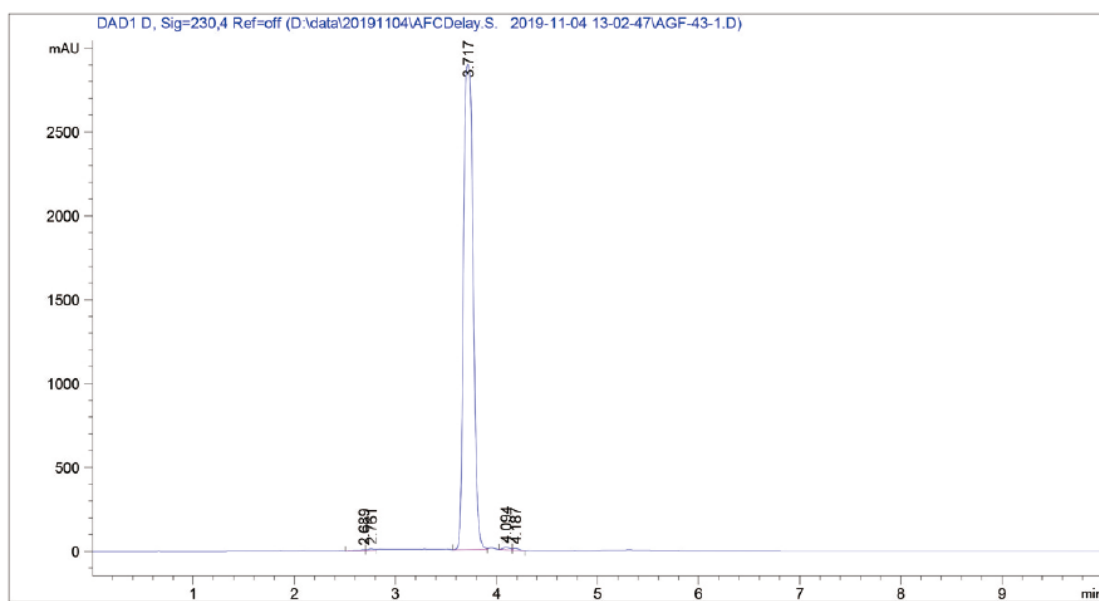
HPLC spectrum of **13a** (purity >98%)



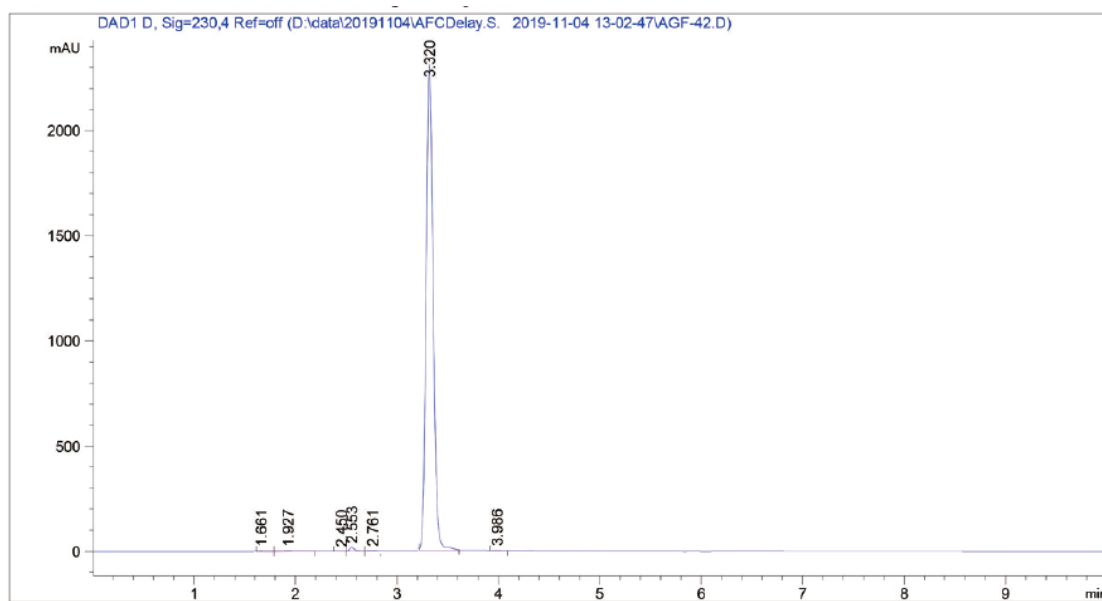
HPLC spectrum of **13b** (purity >98%)



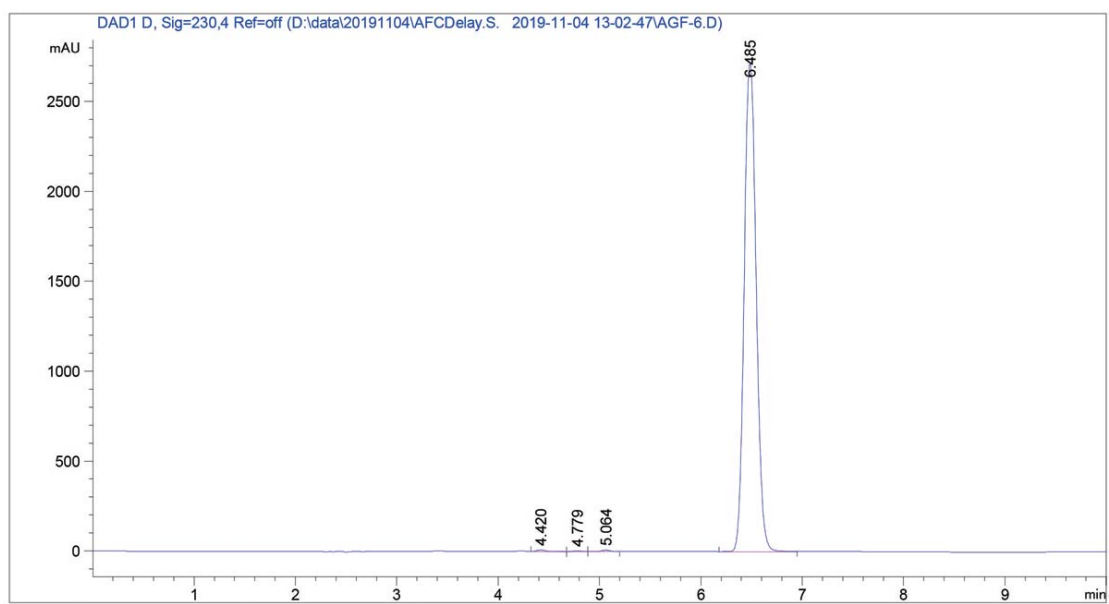
HPLC spectrum of **14a** (purity >98%)



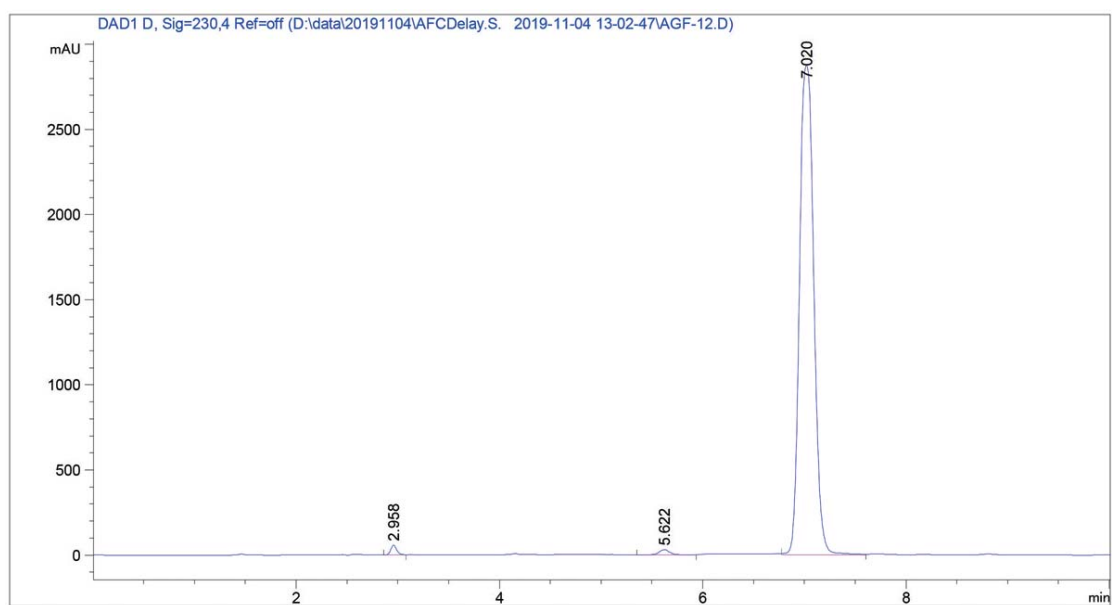
HPLC spectrum of **14b** (purity >95%)



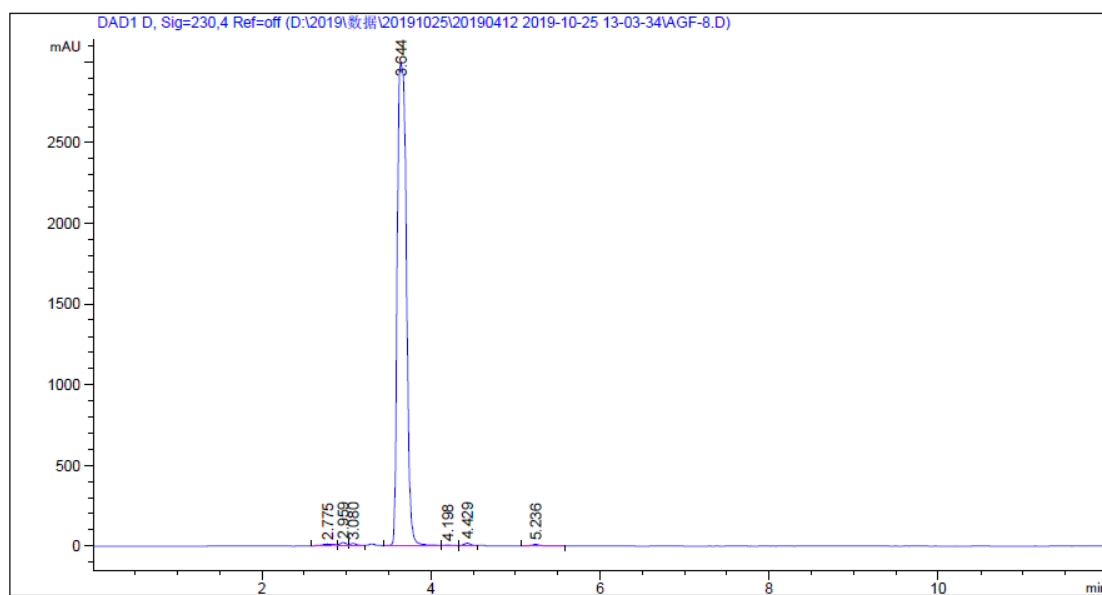
HPLC spectrum of **15a** (purity >98%)



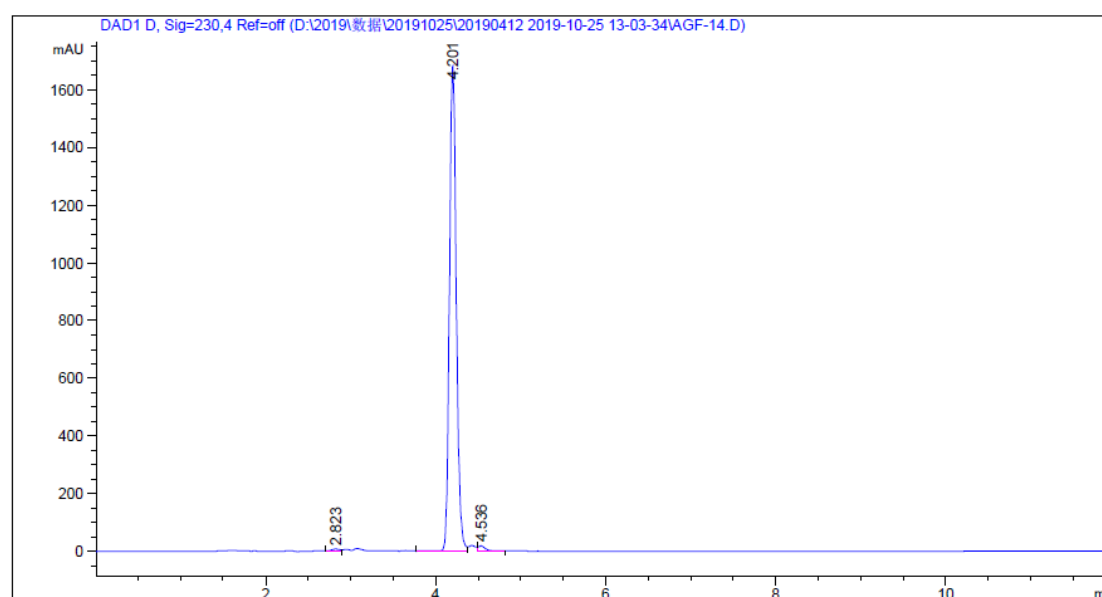
HPLC spectrum of **15b** (purity >95%)



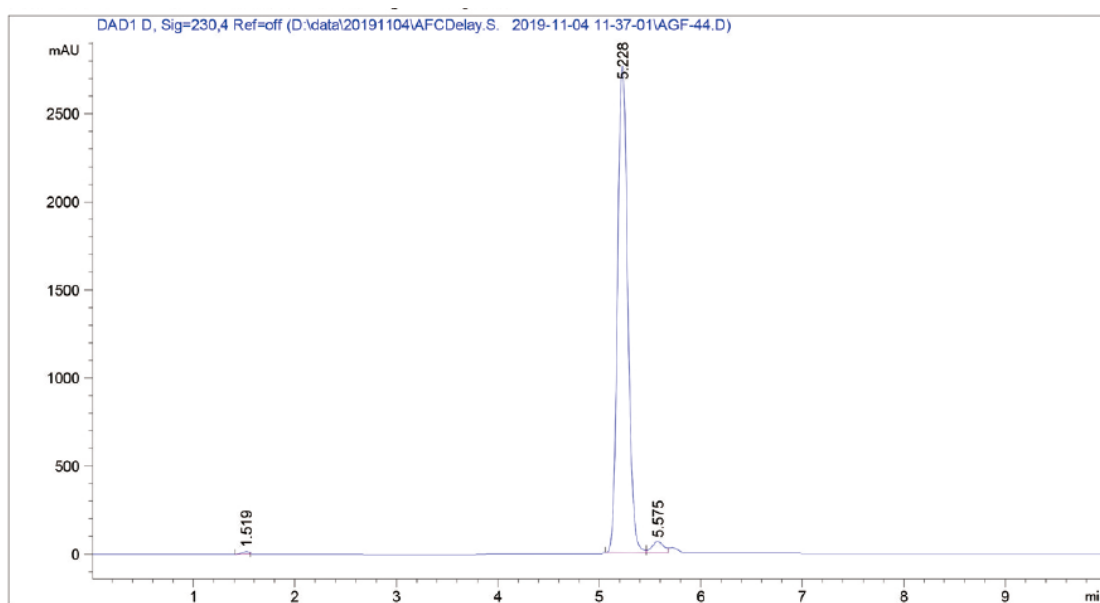
HPLC spectrum of **16a** (purity >96%)



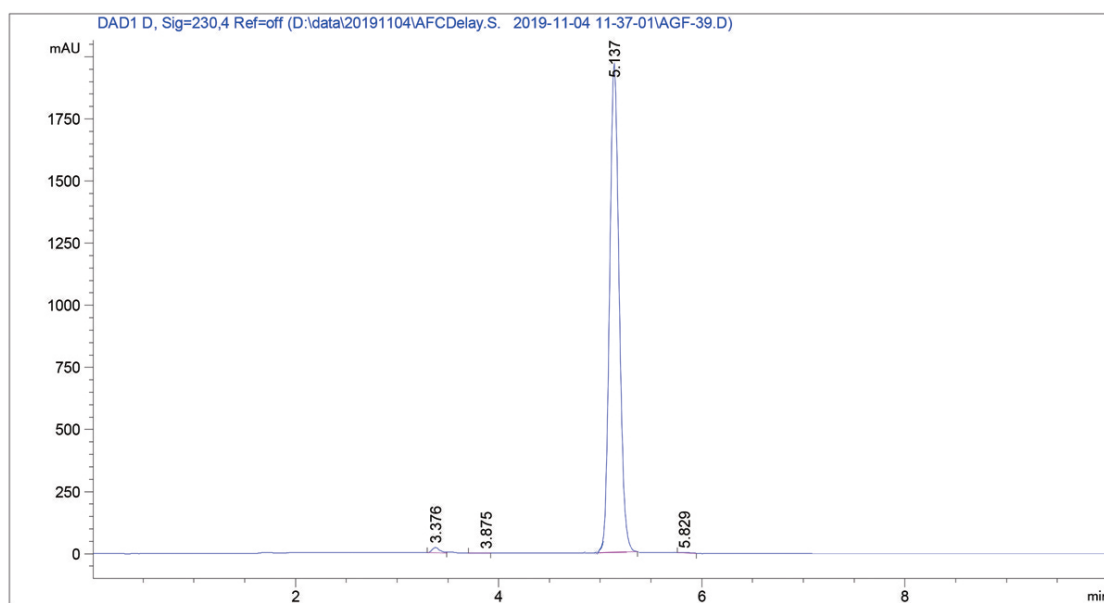
HPLC spectrum of **16b** (purity >96%)



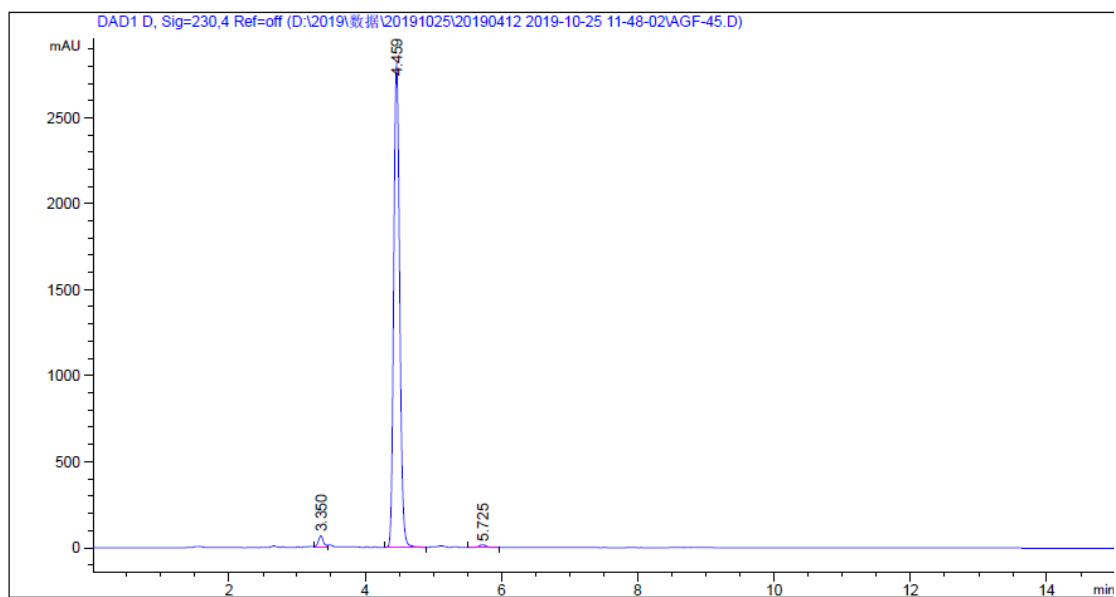
HPLC spectrum of **17a** (purity >95%)



HPLC spectrum of **17b** (purity >98%)



HPLC spectrum of **18a** (purity >97%)



HPLC spectrum of **18b** (purity >98%)

