# *Supporting Information*

# Discovery and Characterization of 1*H*-1,2,3-Triazole Derivatives as Novel Prostanoid EP4 Receptor Antagonists for Cancer Immunotherapy

*Jun-Jie Yang†, Wei-Wei Yu†, Long-Long Hu, Wen-Juan Liu, Xian-Hua Lin, Wei Wang, Qiansen Zhang, Pei-Li Wang, Shuo-Wen Tang, Xin Wang, Mingyao Liu, Weiqiang Lu*, and Han-Kun Zhang**

Drug Discovery Unit, Shanghai Key Laboratory of Regulatory Biology, Institute of Biomedical Sciences and School of Life Sciences, East China Normal University, 500 Dongchuan Road, Shanghai 200241, China

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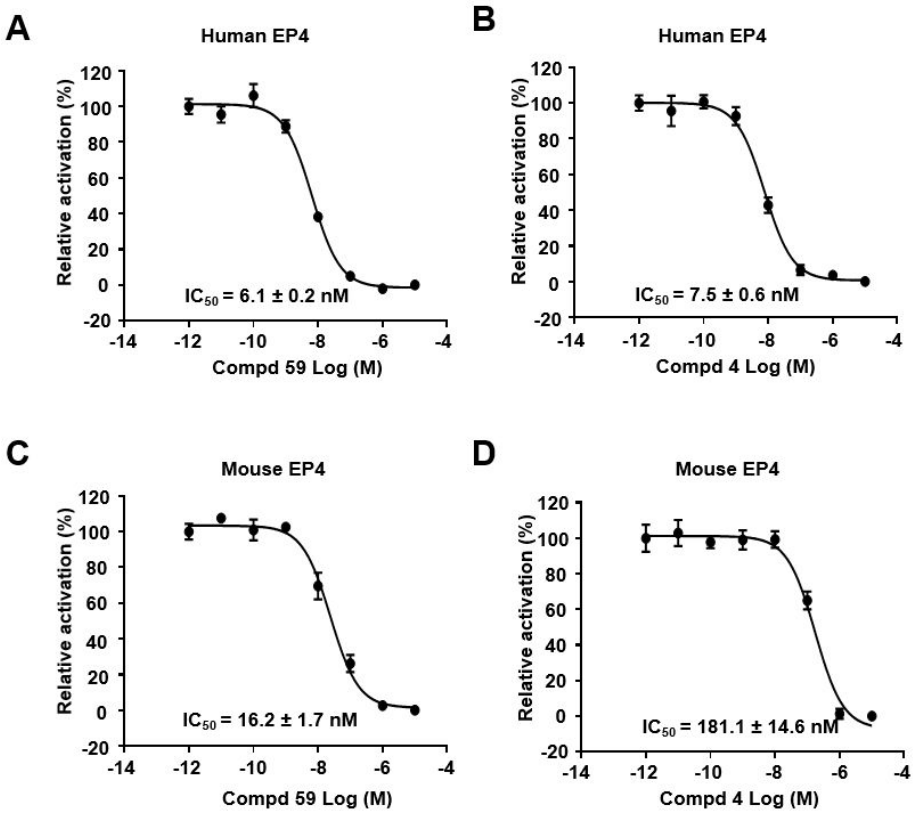


**Figure S1.** The antagonistic effects of Compounds **59** and **4** on human EP4 in calcium flux assay. (A, B) Dose-response curve of Compounds **59** and **4** in CHO-G$\alpha_{16}$ cells overexpressing human EP4 receptor. (C, D) Dose-response curve of Compounds **59** and **4** in CHO-G$\alpha_{16}$ cells overexpressing mouse EP4 receptor. Data are presented as mean ± SEM of three independent experiments. n = 3 per group.

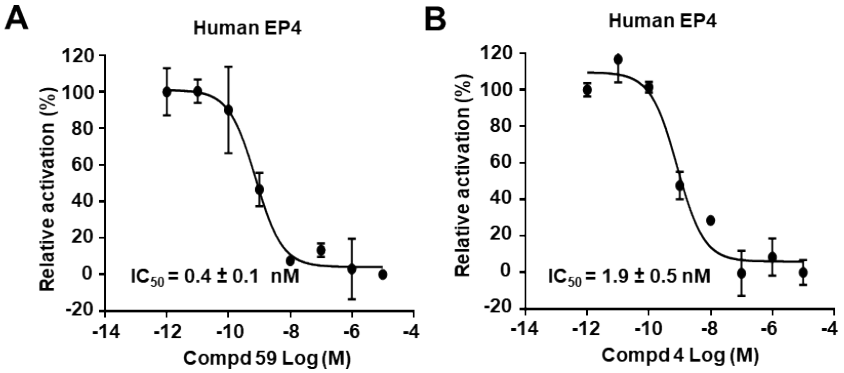



**Figure S2.** The inhibitory effects of Compounds **59** and **4** on human EP4 in β-arrestin recruitment assay. (A, B) Dose-response curve of Compounds **59** and **4** in HEK293 cells co-expressing human EP4 -LgBit and SmBit-β-arrestin.

## β-Arrestin recruitment assay.

A NanoBiT® β-arrestin recruitment assay of EP4 was developed (unpublished data). In brief, 2 ×$10^4$ co-expressing of EP4-LgBit and SmBit-β-arrestin HEK293 cells were seeded into white 96-well plates (Costar, USA) and cultured overnight. After incubation with substrate furimazine at room temperature for 30 min, cells were subsequently treated with different concentrations of compounds **59** or **4** for additional 15 min. Then cells were simulated with endogenous EP4 agonists $PGE_2$ (10 nM) and luminescence was continuously detected for 30 min by using a hybrid multi-mode microplate reader Cytation 5 (BioTek, USA).

**Table S1**: Cytotoxic Activity of Selected Compounds.

| Compd. | Cytotoxicity Activity | | | | |
|---|---|---|---|---|---|
| | CT26 $IC_{50}$, μM | Panc02 $IC_{50}$, μM | EMT-6 $IC_{50}$, μM | LLC $IC_{50}$, μM | HEK293 $IC_{50}$, μM |
| **Gemcitabine** | 0.016 ± 0.0001 | 0.073 ± 0.0003 | - | 0.0053 ± 0.0002 | 0.0051 ± 0.0001 |
| **34** | >100 | >100 | >100 | >100 | >100 |
| **43** | >100 | >100 | >100 | >100 | >100 |
| **45** | >100 | >100 | >100 | >100 | >100 |
| **48** | >100 | >100 | >100 | >100 | >100 |
| **52** | >100 | >100 | >100 | >100 | >100 |
| **56** | >100 | >100 | >100 | >100 | >100 |
| **57** | >100 | >100 | >100 | >100 | >100 |
| **58** | >100 | >100 | >100 | >100 | >100 |
| **59** | >100 | >100 | >100 | >100 | >100 |
| **61** | 40.5 ± 0.1 | 50.1 ± 0.7 | 30.2 ± 0.5 | 35.8 ± 0.2 | >100 |
| **62** | ND[a] | ND | ND | ND | ND |
| **64** | ND | ND | ND | ND | ND |
| **65** | ND | ND | ND | ND | ND |

CT26, Panc02, EMT-6, LLC, HEK293 were treated with 100 μM selected compounds

for 72 h. Then cell viability was determined by CCK8 assay. All the data are presented as mean ± SEM of three independent experiments. [a]ND: not determined.

**Cell viability.** CT26 ($2.5 \times 10^4$ cells/mL), Panc02 ($8.0 \times 10^4$ cells/mL), EMT6 ($2.0 \times 10^4$ cells/mL), LLC ($2.5 \times 10^4$ cells/mL), and HEK293 ($3.0 \times 10^4$ cells/mL) cells were seeded into 96-well plates (Costar, USA) overnight, and then treated with different concentrations of tested compounds for 72 h. Cell viability was determined using Cell Counting Kit-8 (CCK8) (7seabiotech, China). CCK-8 solution was added into the 96-well plate in 37 °C for 0.5-4 h after drug treatment (0.1% DMSO; negative control) according to the manufacturer's manual, and the absorbance was measured at 450 nm with Versa Max Microplate Reader (Molecular Devices, USA).

**Table S2**. The primer sequences of Q-PCR

| Gene | Direction | Primer sequences (5'-3') |
|---|---|---|
| *β-ACTIN* | Forward | GTACGCCAACACAGTGCTG |
| | Reverse | CGTCATACTCCTGCTTGCTG |
| *iNOS* | Forward | ATGCCGCCGCTCTAATACTT |
| | Reverse | GGAAAGGCCCAAGCCATCAT |
| *IL-6* | Forward | GGGACTGATGCTGGTGACAA |
| | Reverse | ACAGGTCTGTTGGGAGTGGT |
| *CXCL1* | Forward | CCATCCAGAGCTTGACGGTG |
| | Reverse | TGGGGGTTGAGGCAAACTTC |
| *IL-4Rα* | Forward | ACACCAATGTGTCCGACGAA |
| | Reverse | CTGCAGGGTTGTCCTCTCTG |
| *IL-10* | Forward | AAGGGTTACTTGGGTTGCCA |
| | Reverse | GCCTGGGGCATCACTTCTAC |
| *COX2* | Forward | CATCCCCTTCCTGCGAAGTT |
| | Reverse | CATGGGAGTTGGGCAGTCAT |
| *ARG1* | Forward | ACATTGGCTTGCGAGACGTA |
| | Reverse | ATCACCTTGCCAATCCCCAG |
| *IL-1β* | Forward | TGCCACCTTTTGACAGTGATG |
| | Reverse | AAGGTCCACGGGAAAGACAC |

## Structure Determination of Compounds 15, 13, and 21

The structure of compounds **15** and **21** was determined by their corresponding intermediates **11a** and **11g**, respectively. The structure of ester **13** was identified by its isomer **13b**. Structure was solved by Xiao-Li Zhao, Ph.D., Shanghai Key Laboratory of Green Chemistry and Chemical Processes, and Department of Chemistry, East China Normal University, Shanghai 200062, China.

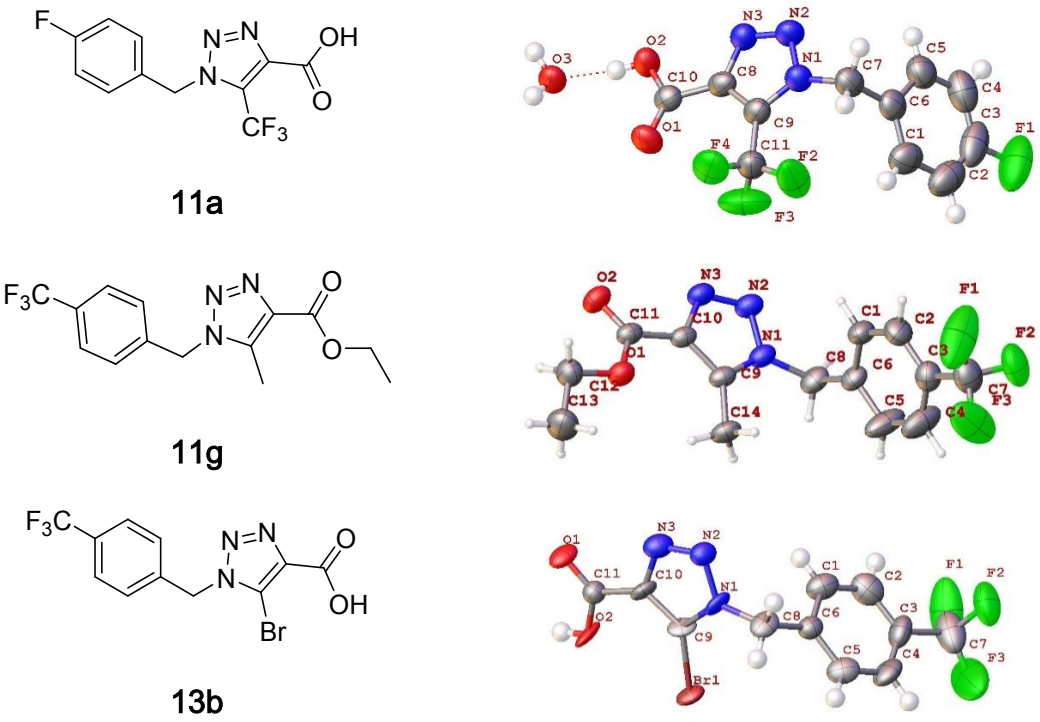



**Figure S2.** Crystal structure of **11a**, **11g** and **13b**

**Table S3A.** Crystal data and structure refinement for **11a**

| | |
|---|---|
| Identification code | 11a |
| Empirical formula | $C_{11}H_9F_4N_3O_3$ |
| Formula weight | 307.21 |
| Temperature/K | 100.01(10) |
| Crystal system | monoclinic |
| Space group | $P2_1/n$ |
| a/Å | 7.36990(10) |
| b/Å | 16.2416(3) |
| c/Å | 10.7978(2) |
| $\alpha$/° | 90 |
| $\beta$/° | 101.712(2) |
| $\gamma$/° | 90 |
| Volume/Å$^3$ | 1265.58(4) |
| Z | 4 |
| $\rho_{calc}$ g/cm$^3$ | 1.612 |
| $\mu$/mm$^{-1}$ | 1.396 |
| F(000) | 624 |
| Crystal size/mm$^3$ | 0.38 × 0.26 × 0.22 |
| Radiation | CuKα ($\lambda$ = 1.54184) |
| 2Θ range for data collection/° | 9.982 to 134.054 |

| | |
|---|---|
| Index ranges | $-8 \leq h \leq 8, -19 \leq k \leq 19, -12 \leq l \leq 12$ |
| Reflections collected | 27246 |
| Independent reflections | 2220 [$R_{(int)}$ = 0.0873, $R_{(sigma)}$ = 0.0350] |
| Data/restraints/parameters | 2220/0/194 |
| Goodness-of-fit on $F^2$ | 1.037 |
| Final R indexes [I>=2$\sigma$ (I)] | $R_1$ = 0.0330, $wR_2$ = 0.0824 |
| Final R indexes [all data] | $R_1$ = 0.0377, $wR_2$ = 0.0850 |
| Largest diff. peak/hole / e $Å^{-3}$ | 0.25/-0.21 |

**Table S3B.** Crystal data and structure refinement for **11g**

| | |
|---|---|
| Identification code | 11g |
| Empirical formula | $C_{14}H_{14}F_3N_3O_2$ |
| Formula weight | 313.28 |
| Temperature/K | 293(2) |
| Crystal system | monoclinic |
| Space group | $P2_1/c$ |
| a/Å | 13.1372(4) |
| b/Å | 11.8491(3) |
| c/Å | 10.1560(4) |
| $\alpha$/° | 90 |
| $\beta$/° | 103.698(4) |
| $\gamma$/° | 90 |
| Volume/Å$^3$ | 1535.96(9) |
| Z | 4 |
| $\rho_{calc}$ g/cm$^3$ | 1.355 |
| $\mu$/mm$^{-1}$ | 1.013 |
| F(000) | 648 |
| Crystal size/mm$^3$ | 0.46 × 0.38 × 0.32 |
| Radiation | CuK$\alpha$ ($\lambda$ = 1.54184) |

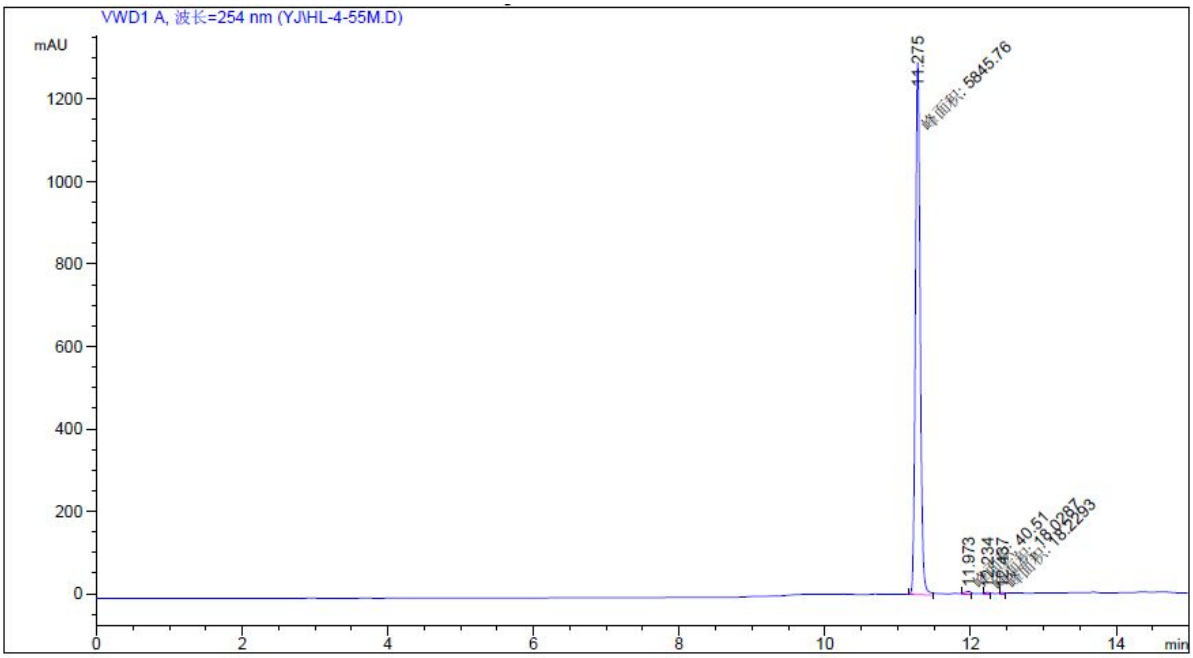
| | |
|---|---|
| 2Θ range for data collection/° | 10.186 to 134.16 |
| Index ranges | $-15 \leq h \leq 15, -14 \leq k \leq 14, -12 \leq l \leq 12$ |
| Reflections collected | 31270 |
| Independent reflections | 2731 [$R_{(int)}$ = 0.1108, $R_{(sigma)}$ = 0.0409] |
| Data/restraints/parameters | 2731/70/229 |
| Goodness-of-fit on $F^2$ | 1.081 |
| Final R indexes [I>=2σ (I)] | $R_1$ = 0.0496, $wR_2$ = 0.1404 |
| Final R indexes [all data] | $R_1$ = 0.0561, $wR_2$ = 0.1466 |
| Largest diff. peak/hole / e $Å^{-3}$ | 0.19/-0.15 |

**Table S3C.** Crystal data and structure refinement for **13b**

| | |
|---|---|
| Identification code | 13b |
| Empirical formula | $C_{11}H_7BrF_3N_3O_2$ |
| Formula weight | 350.11 |
| Temperature/K | 100.00(10) |
| Crystal system | triclinic |
| Space group | P-1 |
| a/Å | 4.8139(2) |
| b/Å | 6.2811(2) |
| c/Å | 20.6190(7) |
| $\alpha$/° | 83.193(3) |
| $\beta$/° | 86.789(3) |
| $\gamma$/° | 79.241(3) |
| Volume/Å$^3$ | 607.80(4) |
| Z | 2 |
| $\rho_{calc}$ g/cm$^3$ | 1.913 |
| $\mu$/mm$^{-1}$ | 5.072 |
| F(000) | 344 |
| Crystal size/mm$^3$ | 0.28 × 0.26 × 0.18 |
| Radiation | CuK$\alpha$ ($\lambda$ = 1.54184) |

| | |
|---|---|
| 2Θ range for data collection/° | 8.644 to 134.128 |
| Index ranges | $-5 \leq h \leq 5, -7 \leq k \leq 7, -24 \leq l \leq 24$ |
| Reflections collected | 7236 |
| Independent reflections | 2064 [$R_{(int)}$ = 0.1538, $R_{(sigma)}$ = 0.0942] |
| Data/restraints/parameters | 2064/18/182 |
| Goodness-of-fit on $F^2$ | 1.109 |
| Final R indexes [I>=2σ (I)] | $R_1$ = 0.0694, $wR_2$ = 0.1833 |
| Final R indexes [all data] | $R_1$ = 0.0781, $wR_2$ = 0.1949 |
| Largest diff. peak/hole / e $Å^{-3}$ | 1.55/-2.41 |

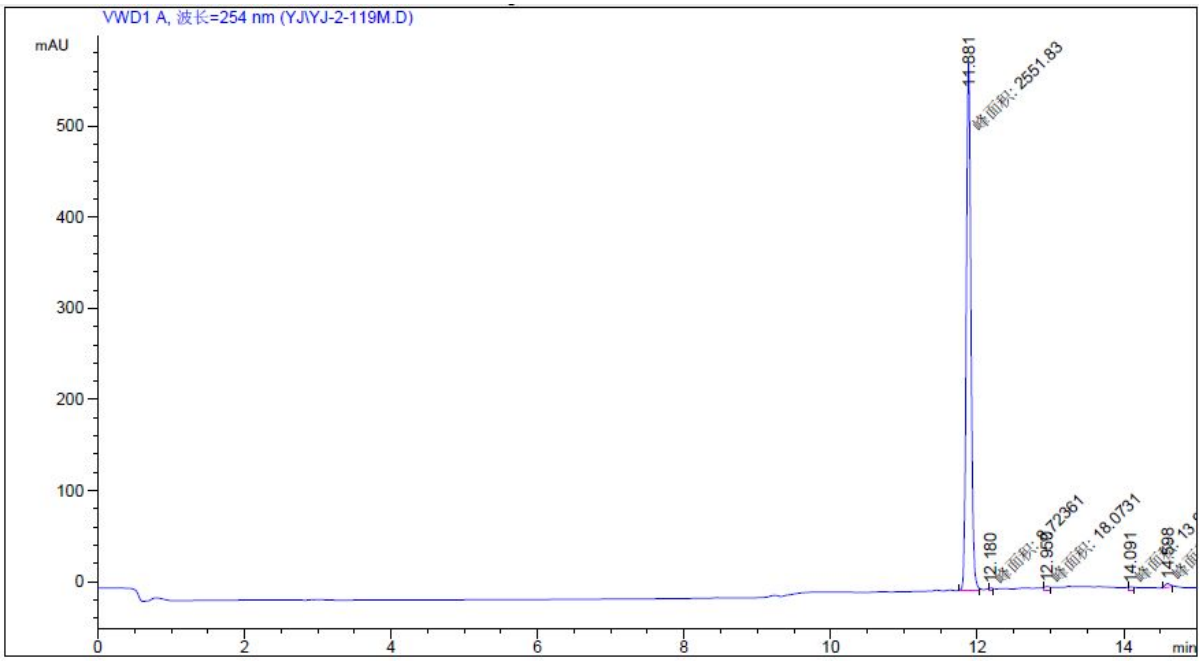
## HPLC trace of compound 15




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.275 | 5845.76367 | 98.7038 |
| 2 | 11.973 | 40.51000 | 0.6840 |
| 3 | 12.234 | 18.02865 | 0.3044 |
| 4 | 12.437 | 18.22928 | 0.3078 |

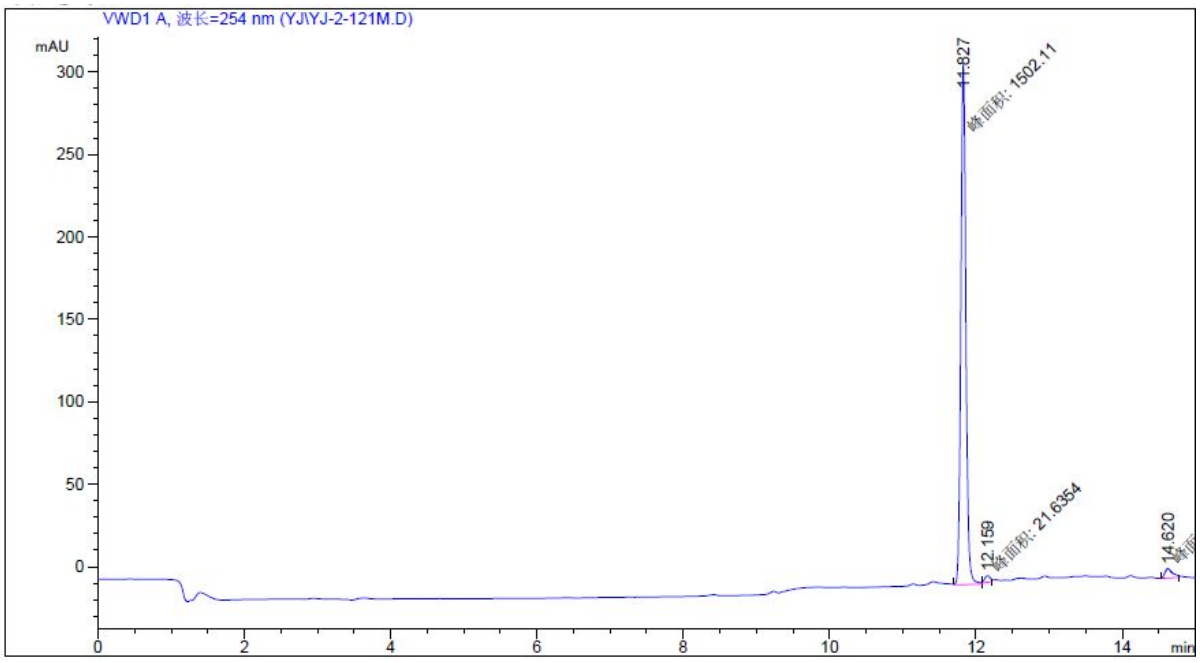
## HPLC trace of compound 16




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.881 | 2551.83179 | 97.6267 |
| 2 | 12.180 | 8.72361 | 0.3337 |
| 3 | 12.950 | 18.07315 | 0.6914 |
| 4 | 14.091 | 13.97124 | 0.5345 |
| 5 | 14.598 | 21.26625 | 0.8136 |

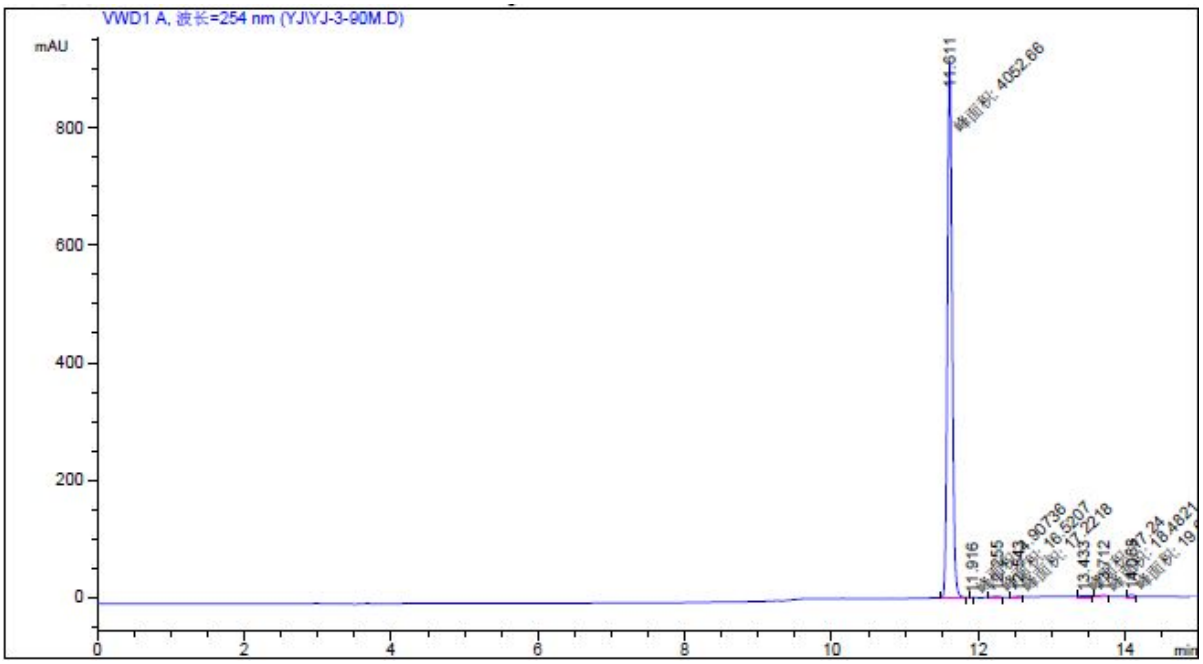
HPLC trace of compound 17




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.827 | 1502.11194 | 95.9551 |
| 2 | 12.159 | 21.63539 | 1.3821 |
| 3 | 14.620 | 41.68489 | 2.6628 |

HPLC trace of compound 18

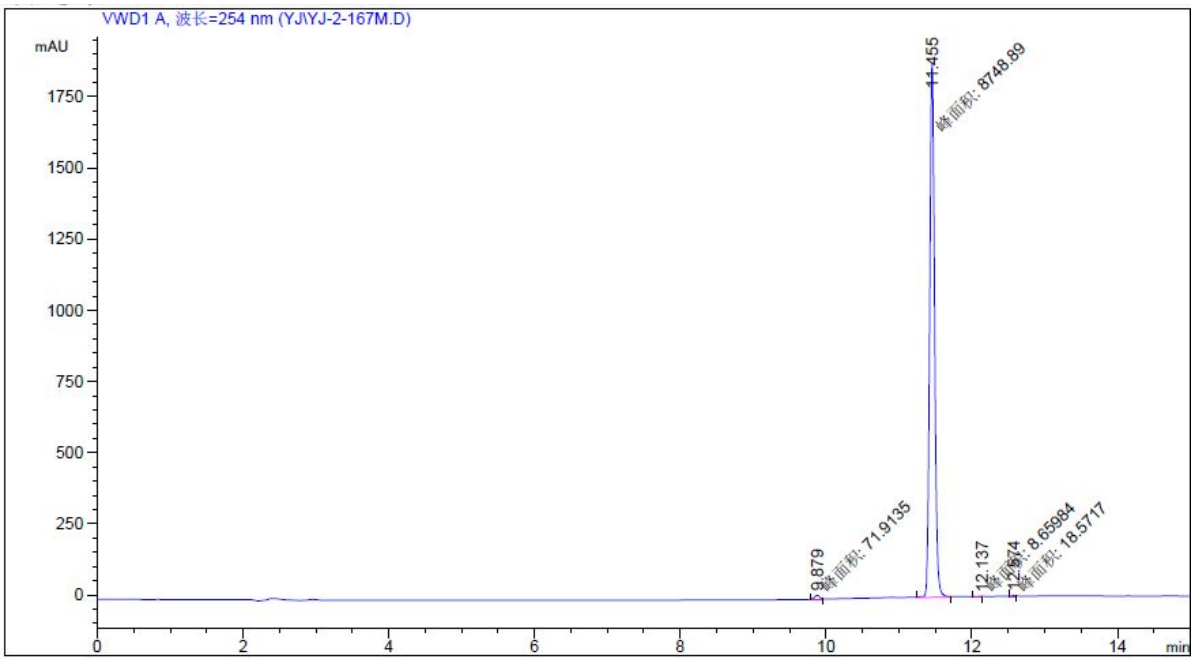



## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.611 | 4052.66479 | 97.7315 |
| 2 | 11.916 | 4.90736 | 0.1183 |
| 3 | 12.255 | 16.52071 | 0.3984 |
| 4 | 12.543 | 17.22178 | 0.4153 |
| 5 | 13.433 | 17.24001 | 0.4157 |
| 6 | 13.712 | 18.48209 | 0.4457 |

| | | | |
|---|---|---|---|
| 7 | 14.088 | 19.69880 | 0.4750 |

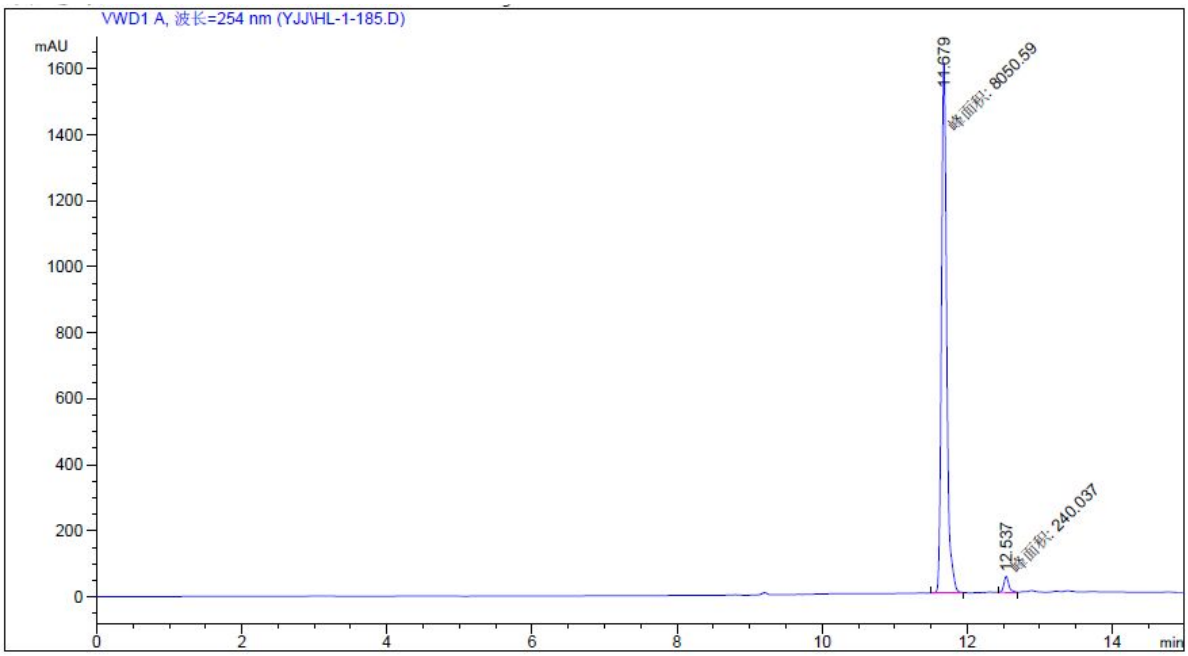
## HPLC trace of compound 19




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 9.879 | 71.91354 | 0.8128 |
| 2 | 11.455 | 8748.88672 | 98.8795 |
| 3 | 12.137 | 8.65984 | 0.0979 |
| 4 | 12.574 | 18.57173 | 0.2099 |

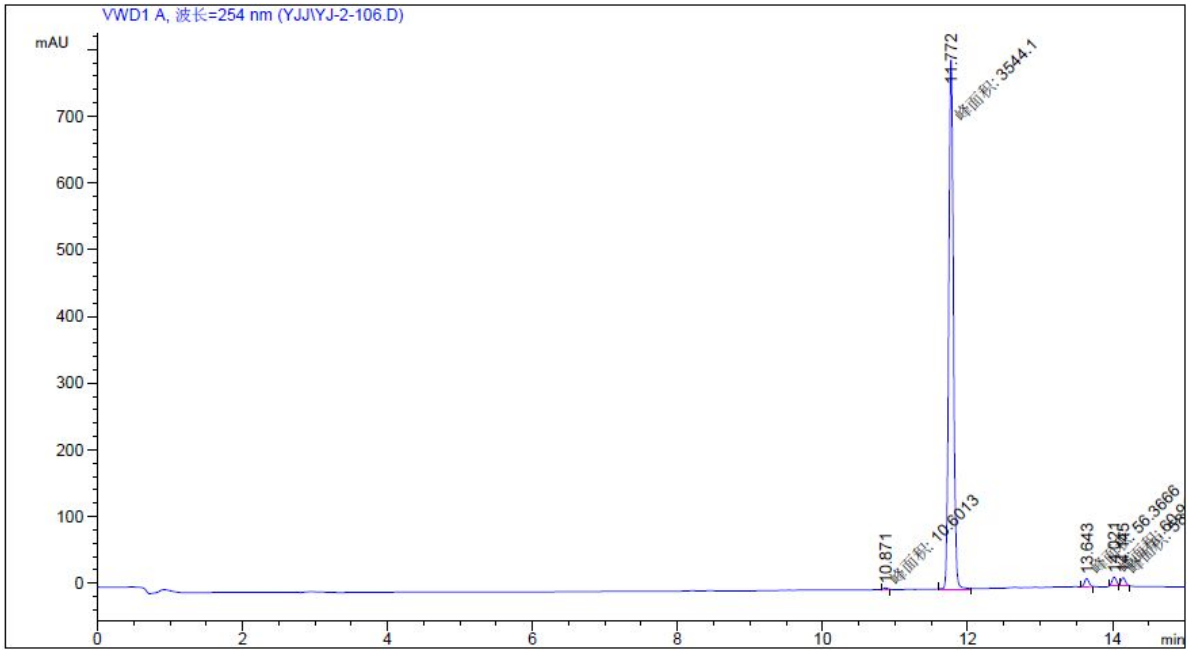
## HPLC trace of compound 20




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.679 | 8050.58838 | 97.1047 |
| 2 | 12.537 | 240.03699 | 2.8953 |

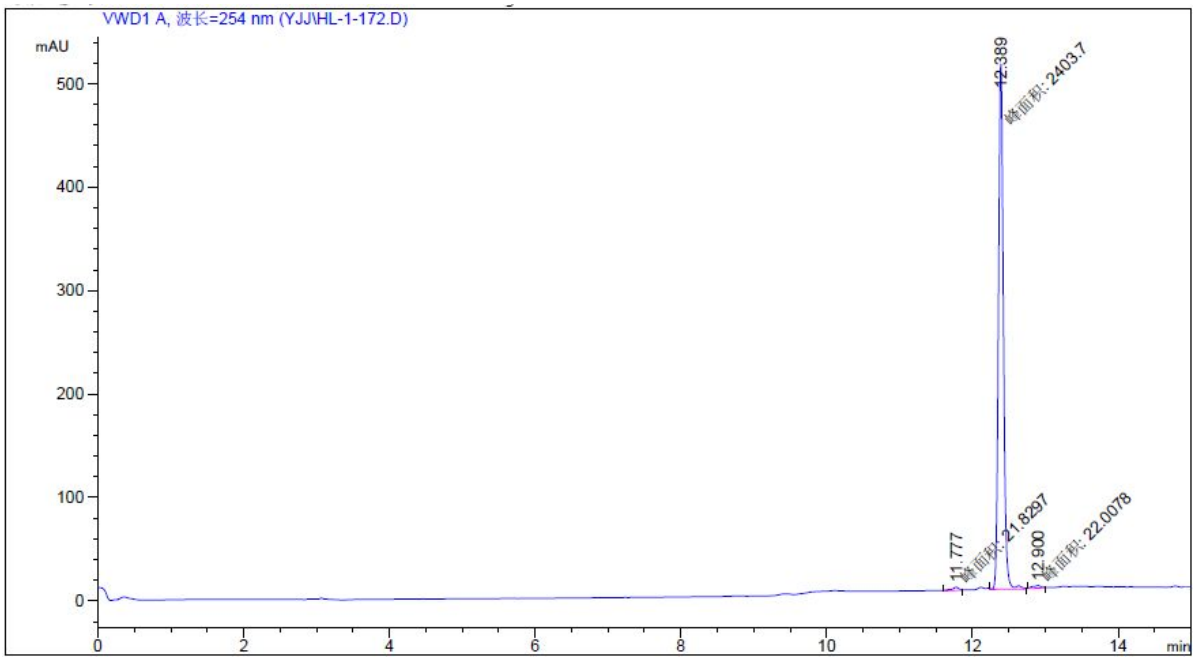
## HPLC trace of compound 21




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 10.871 | 10.60129 | 0.2842 |
| 2 | 11.772 | 3544.09863 | 95.0090 |
| 3 | 13.643 | 56.36662 | 1.5111 |
| 4 | 14.021 | 60.91159 | 1.6329 |
| 5 | 14.145 | 58.29802 | 1.5628 |

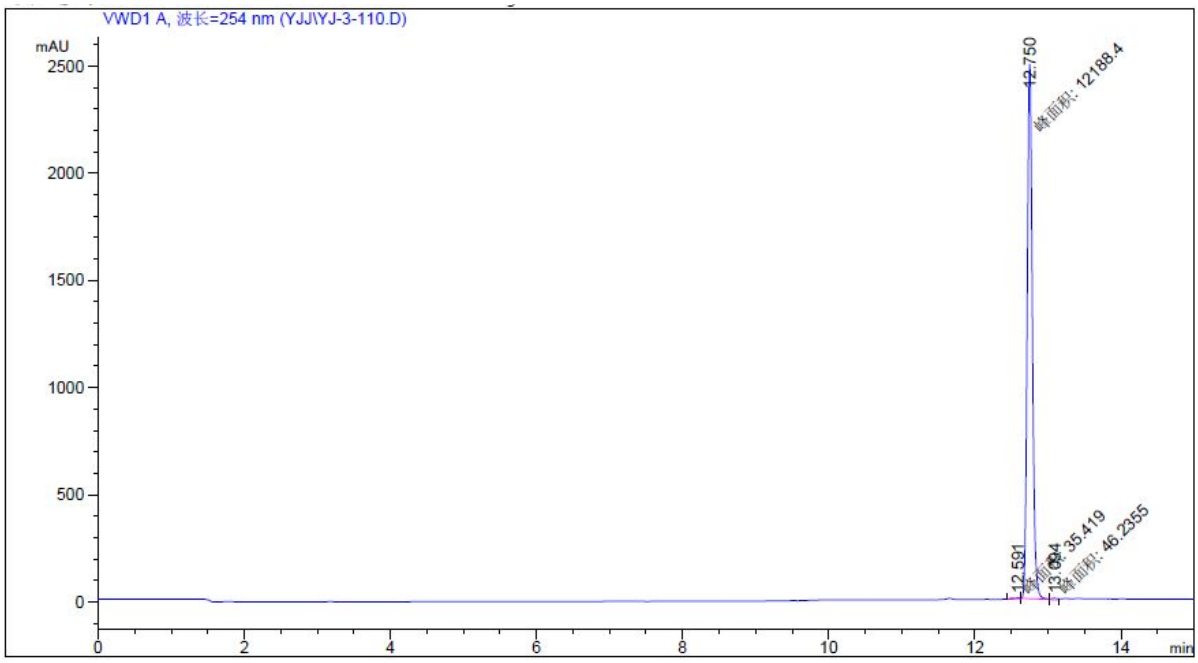
## HPLC trace of compound 22




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.777 | 21.82972 | 0.8919 |
| 2 | 12.389 | 2403.70068 | 98.2089 |
| 3 | 12.900 | 22.00776 | 0.8992 |

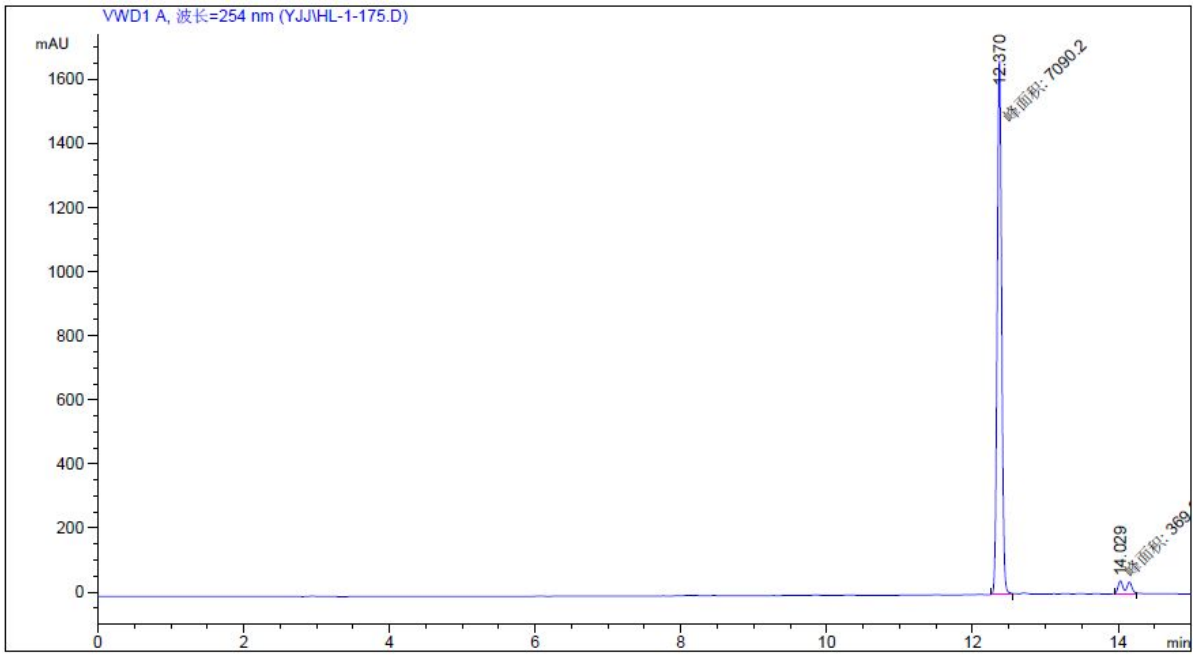
## HPLC trace of compound 23




### Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 12.591 | 35.41896 | 0.2887 |
| 2 | 12.750 | 121884 | 99.3345 |
| 3 | 13.094 | 46.23549 | 0.3768 |

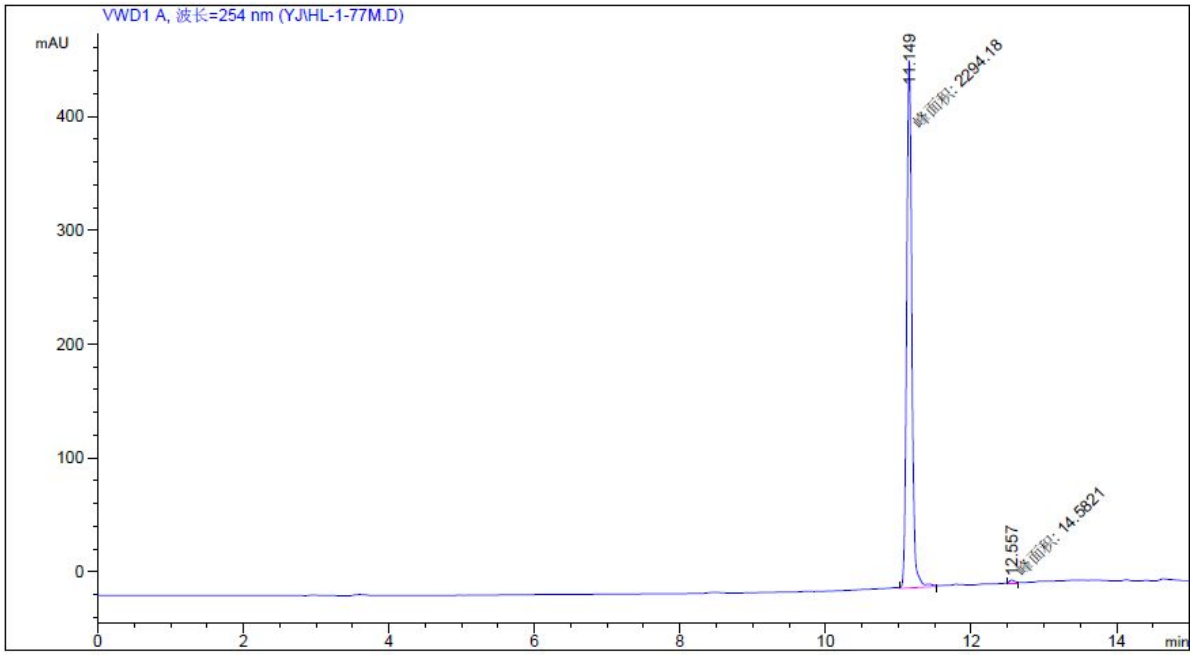
## HPLC trace of compound 24




### Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 12.370 | 7090.19971 | 95.0527 |
| 2 | 14.029 | 369.03293 | 4.9473 |

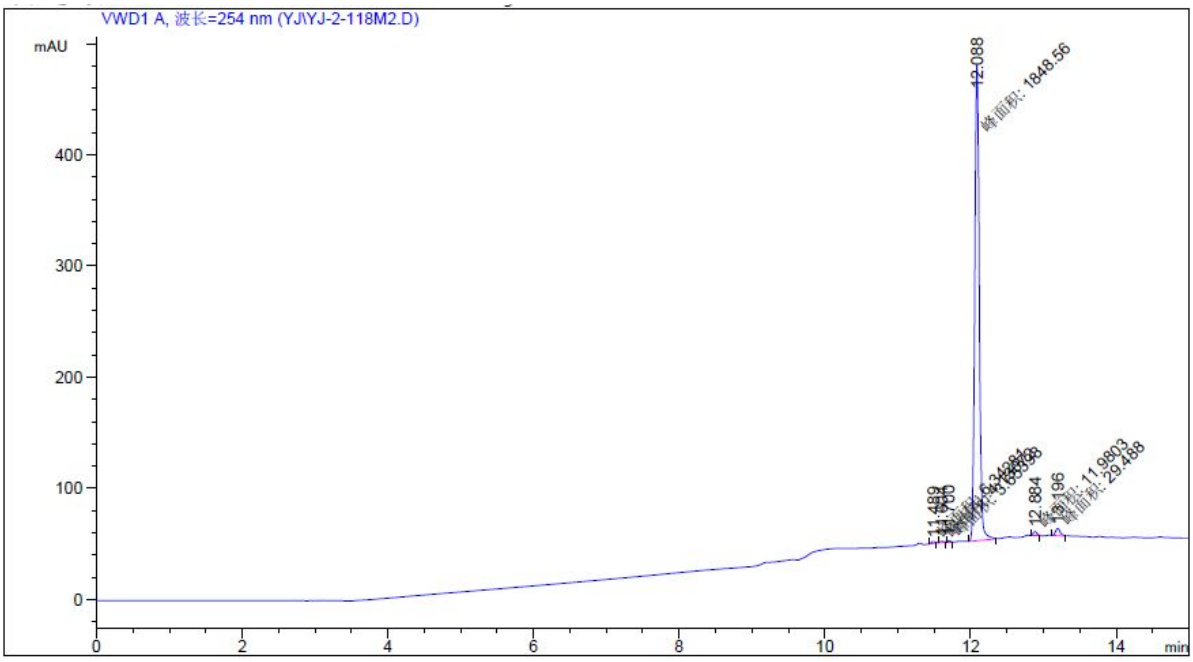
## HPLC trace of compound 25




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.149 | 2294.17651 | 99.3684 |
| 2 | 12.557 | 14.58215 | 0.6316 |

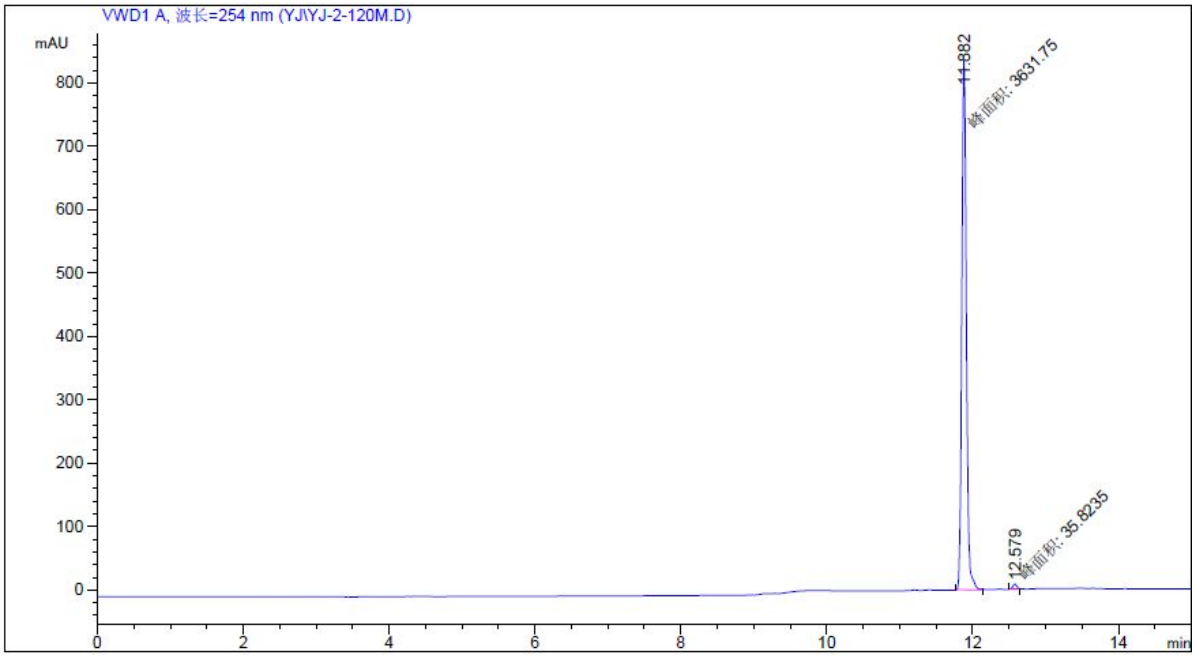
## HPLC trace of compound 26




### Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.489 | 6.34281 | 0.3331 |
| 2 | 11.604 | 4.13072 | 0.2169 |
| 3 | 11.700 | 3.65398 | 0.1919 |
| 4 | 12.088 | 1848.55896 | 97.0803 |
| 5 | 12.884 | 11.998032 | 0.6292 |
| 6 | 13.196 | 29.48799 | 1.5486 |

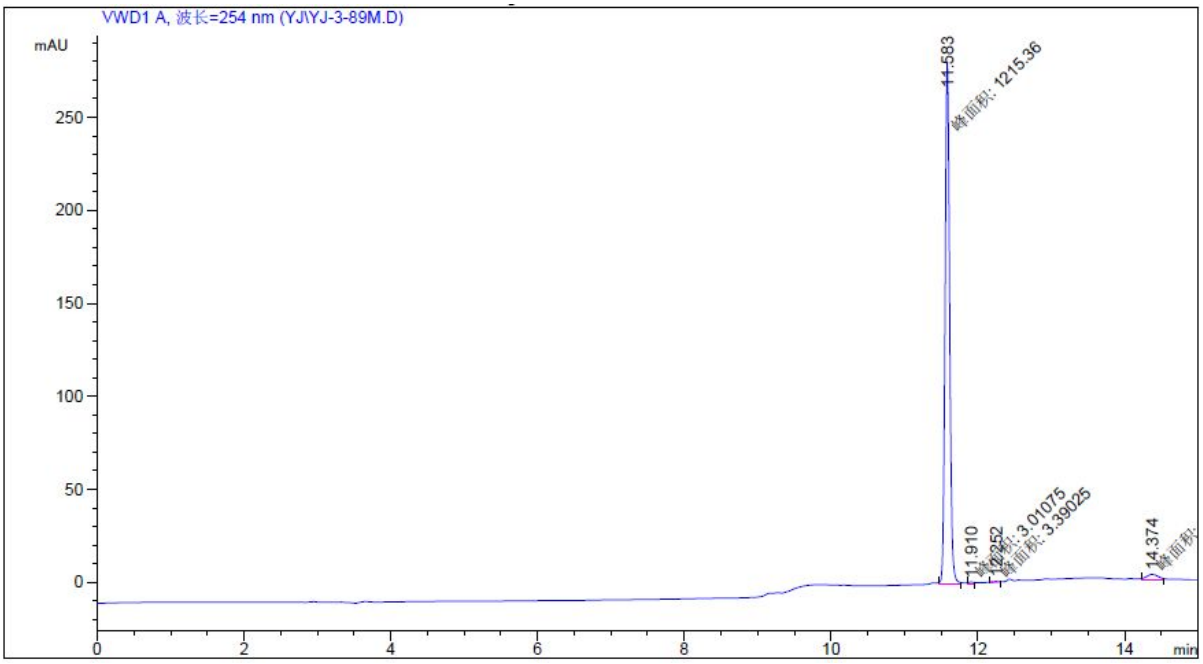
## HPLC trace of compound 27




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.882 | 3631.75439 | 99.0232 |
| 2 | 12.579 | 35.82351 | 0.9768 |

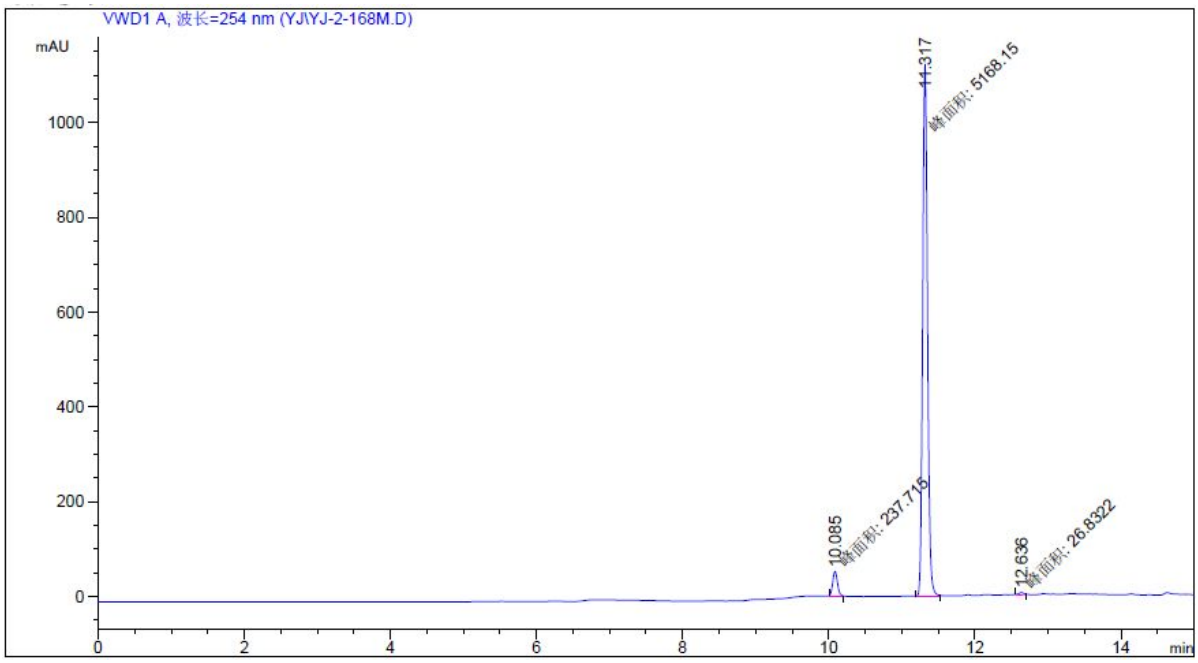
## HPLC trace of compound 28




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.583 | 1215.35913 | 97.2175 |
| 2 | 11.910 | 3.01075 | 0.2408 |
| 3 | 12.252 | 3.39025 | 0.2712 |
| 4 | 14.374 | 28.38466 | 2.2705 |

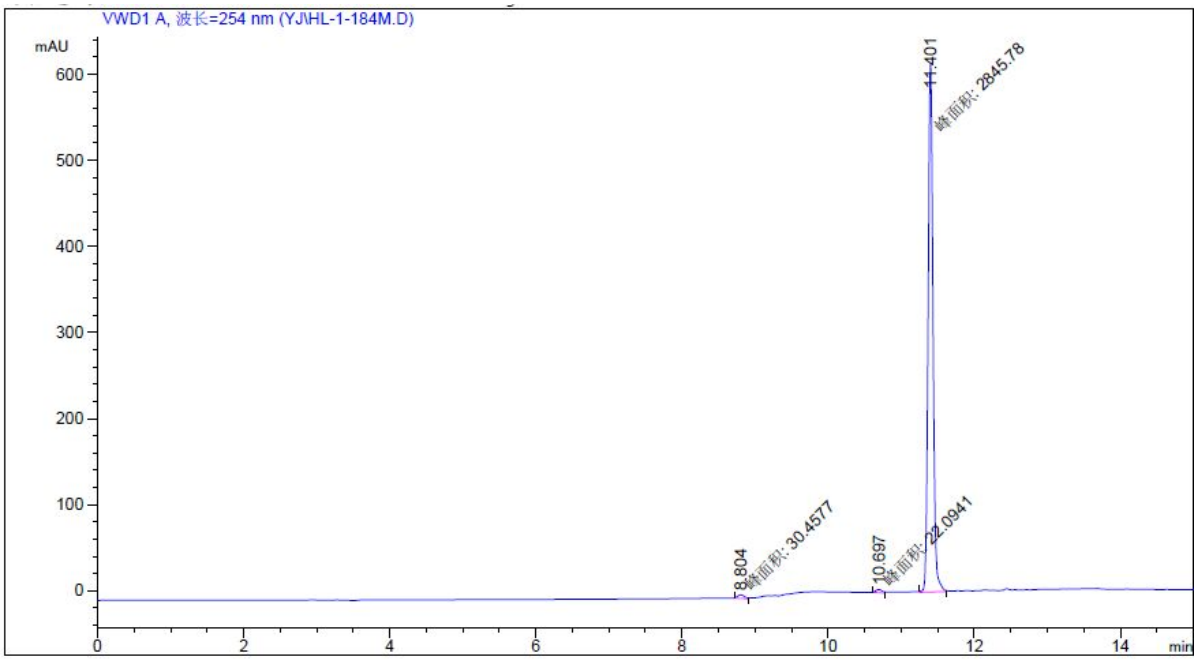
## HPLC trace of compound 29




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 10.085 | 237.71516 | 4.3756 |
| 2 | 11.317 | 5168.15039 | 95.1305 |
| 3 | 12.636 | 26.83220 | 0.4939 |

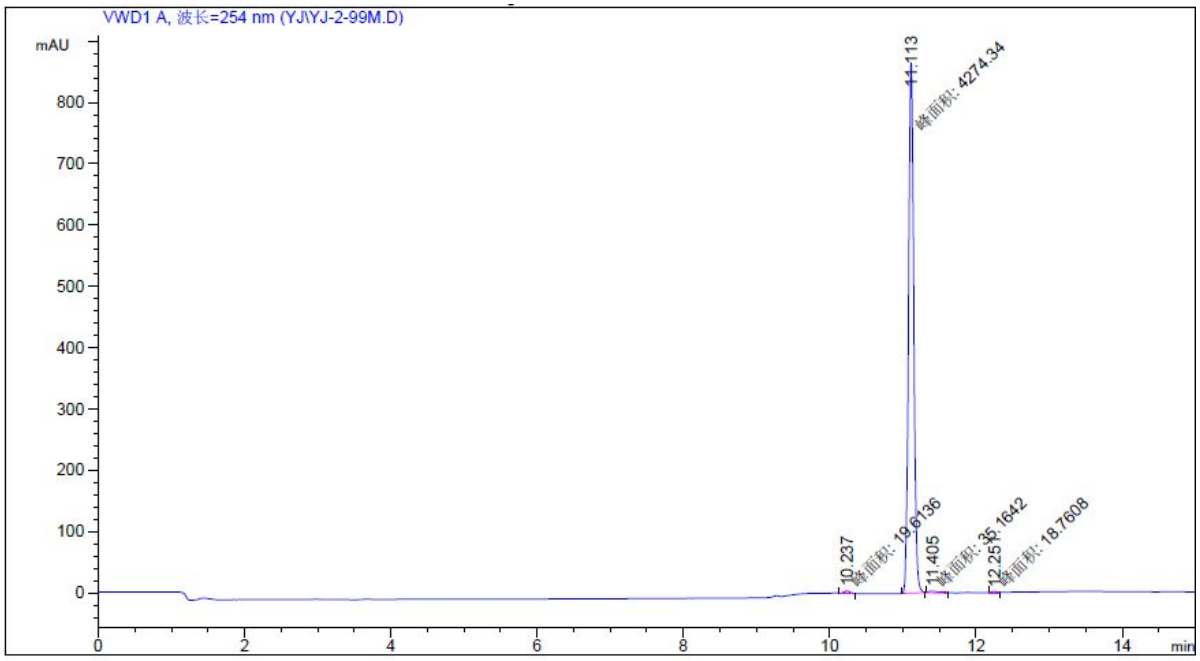
HPLC trace of compound 30




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 8.804 | 0.1138 | 1.0509 |
| 2 | 10.697 | 0.1014 | 0.7623 |
| 3 | 11.401 | 0.0773 | 98.1868 |

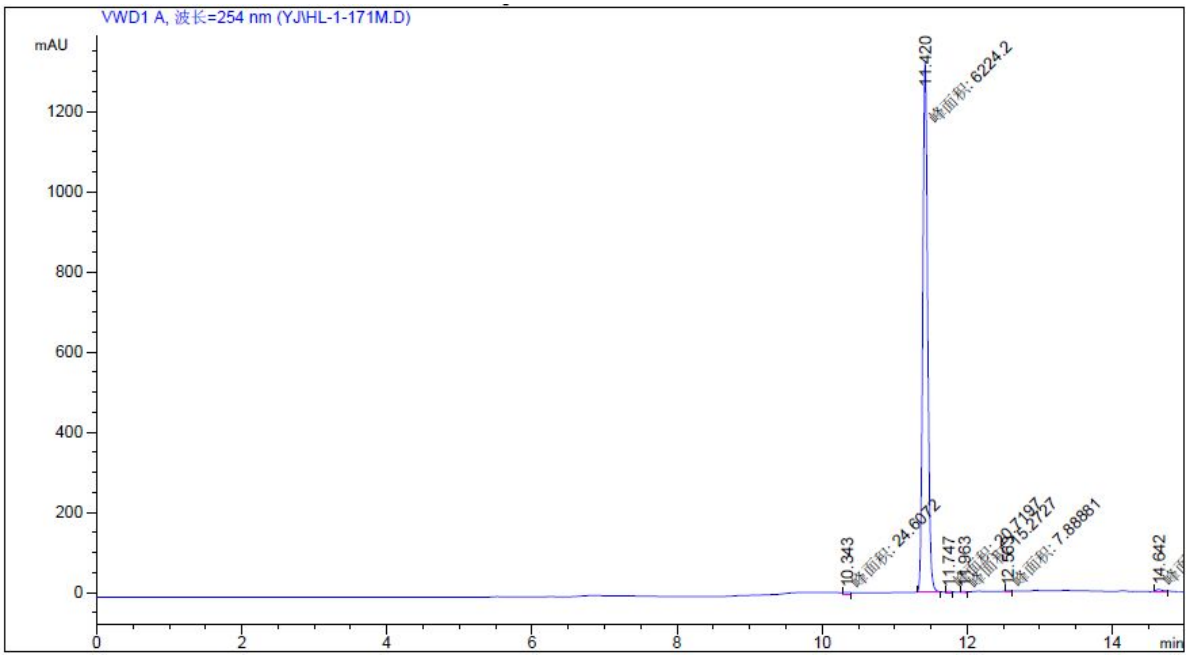
HPLC trace of compound 31




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 10.237 | 19.61359 | 0.4511 |
| 2 | 11.113 | 4274.33545 | 98.3086 |
| 3 | 11.405 | 35.16422 | 0.8088 |
| 4 | 12.251 | 18.76078 | 0.4315 |

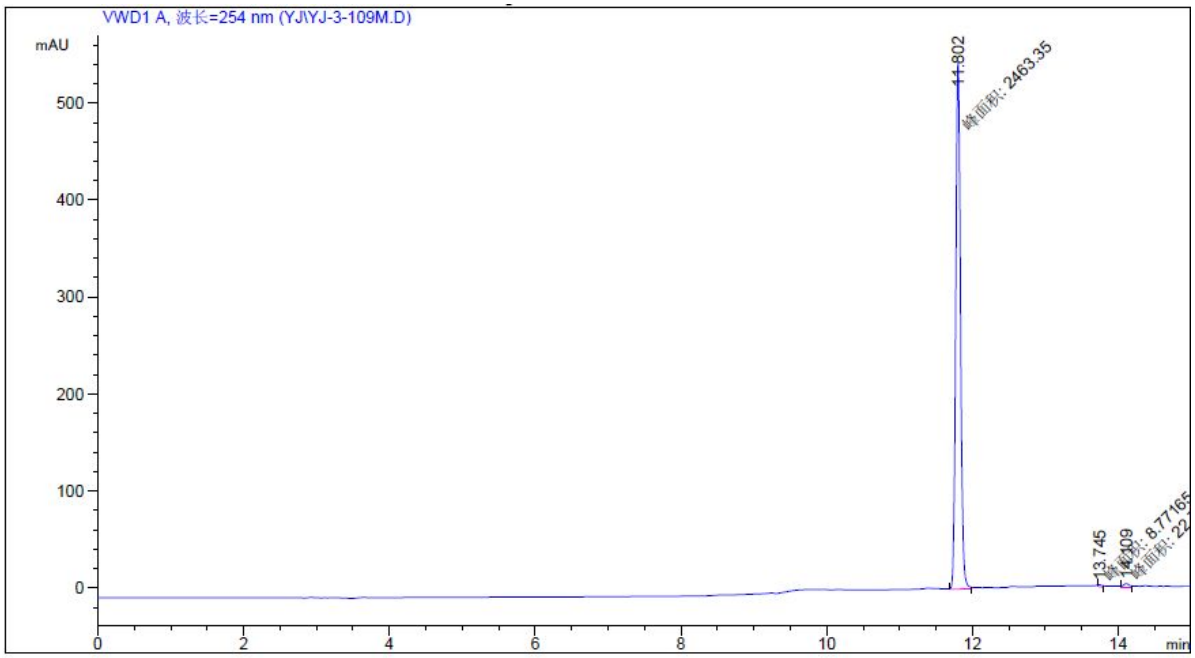
## HPLC trace of compound 32




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 10.343 | 24.60720 | 0.3882 |
| 2 | 11.420 | 6224.20264 | 98.1952 |
| 3 | 11.747 | 20.71974 | 0.3269 |
| 4 | 11.963 | 15.27273 | 0.2409 |
| 5 | 12.563 | 7.88881 | 0.1245 |
| 6 | 14.642 | 45.91381 | 0.7244 |

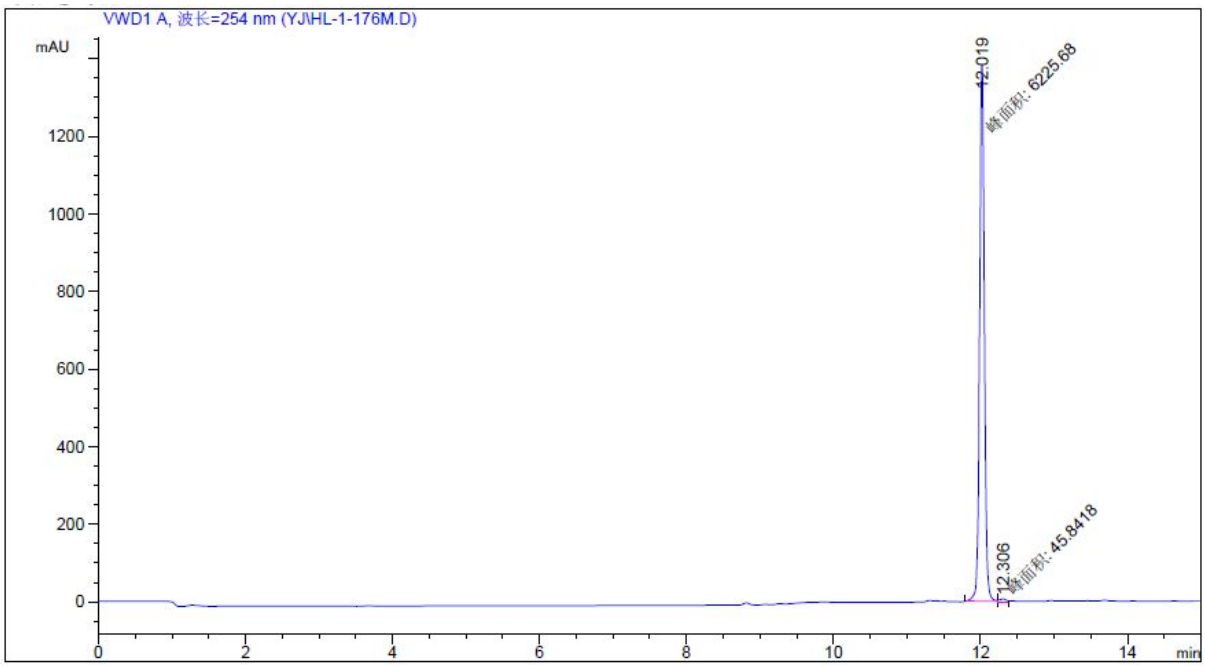
## HPLC trace of compound 33




### Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.802 | 2463.35254 | 98.7379 |
| 2 | 13.745 | 8077165 | 0.3516 |
| 3 | 14.109 | 22.71547 | 0.9105 |

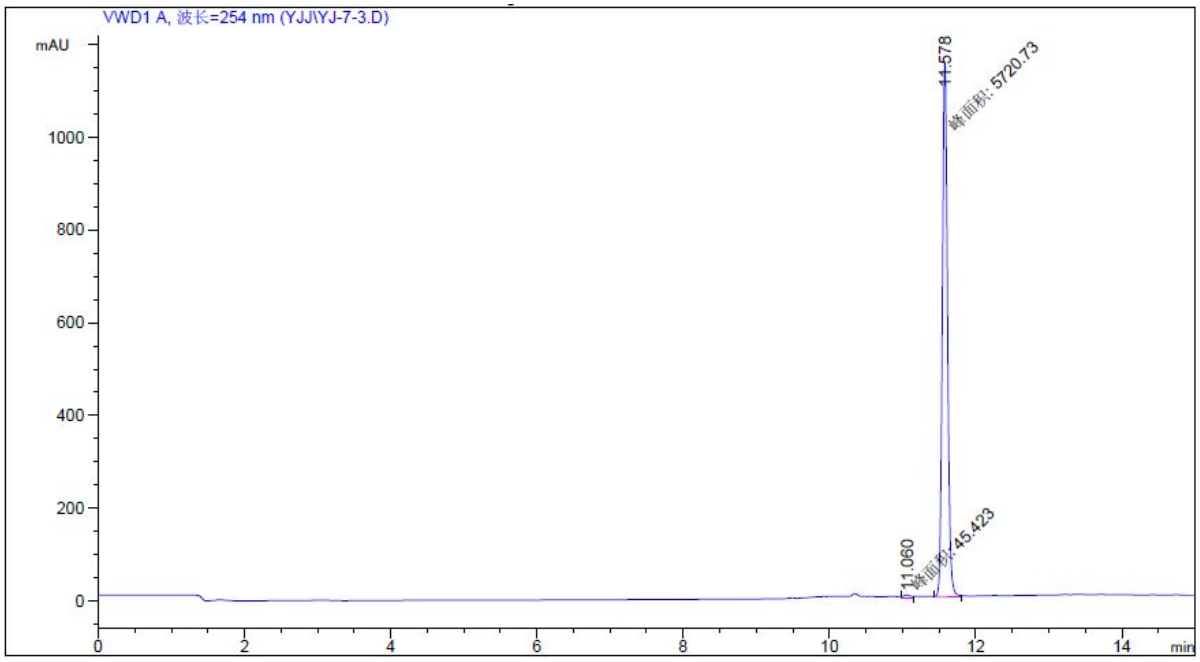
## HPLC trace of compound 34




### Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 12.019 | 6225.67969 | 99.2690 |
| 2 | 12.306 | 45.84183 | 0.7310 |

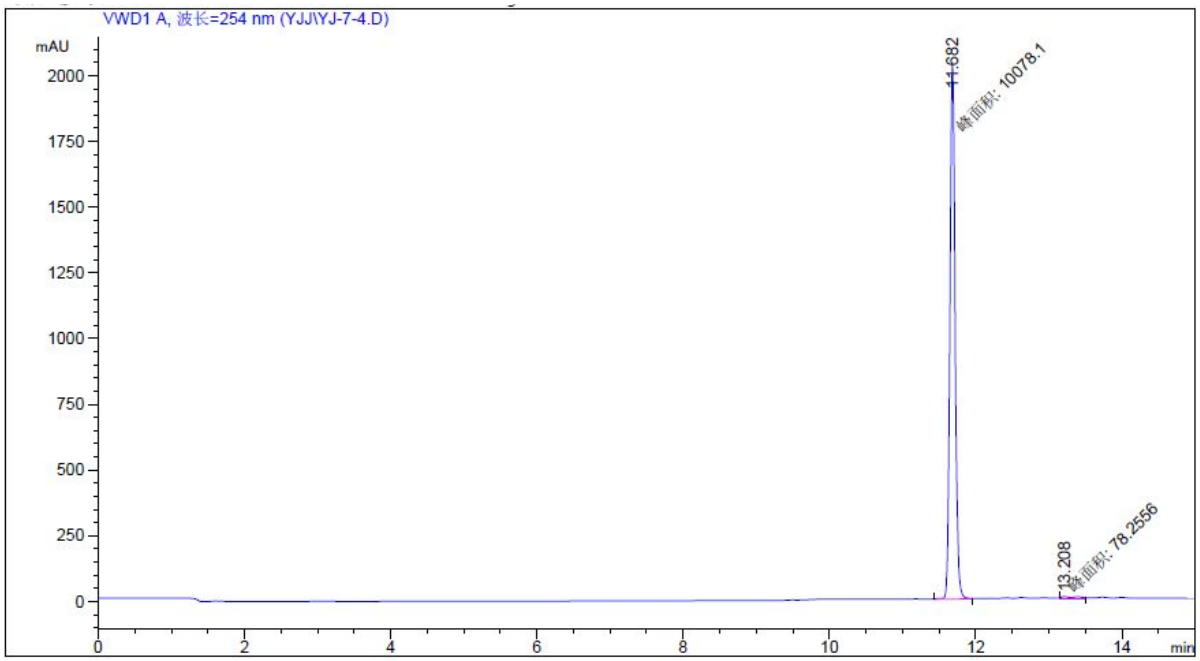
## HPLC trace of compound 35




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.060 | 45.42304 | 0.7878 |
| 2 | 11.578 | 5720.73291 | 99.2122 |

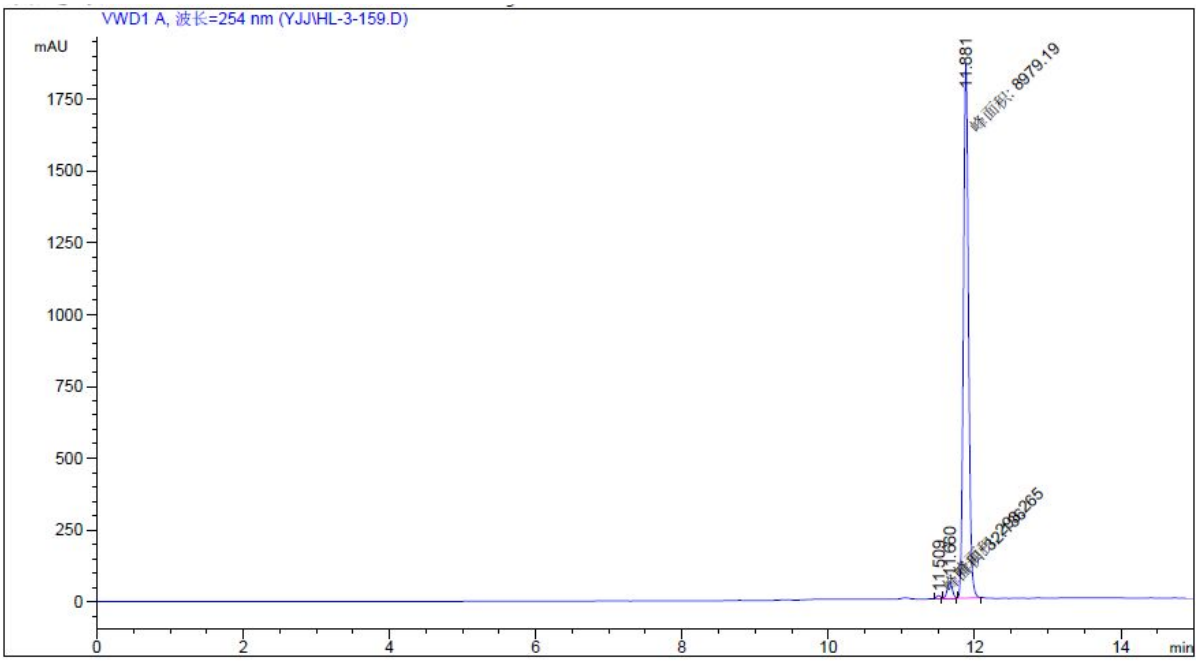
## HPLC trace of compound 36




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.682 | 2035.68823 | 99.2295 |
| 2 | 13.208 | 6.06097 | 0.7705 |

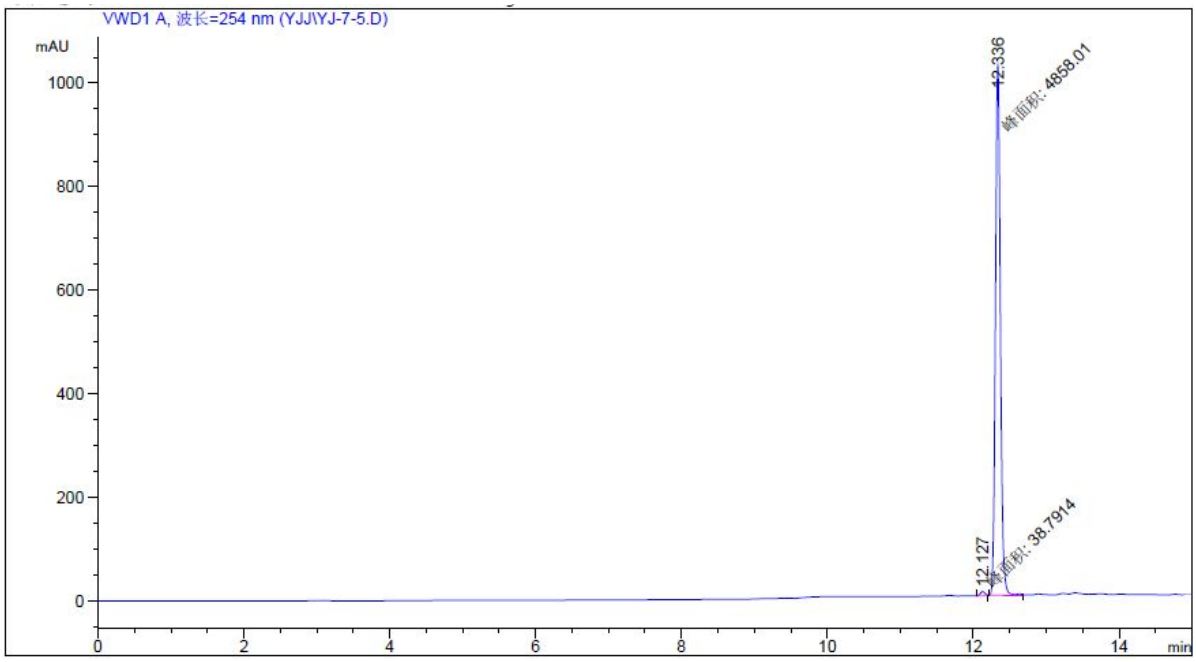
## HPLC trace of compound 37




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.509 | 32.13597 | 0.3454 |
| 2 | 11.660 | 293.26526 | 3.1518 |
| 3 | 11.881 | 8979.18555 | 96.5028 |

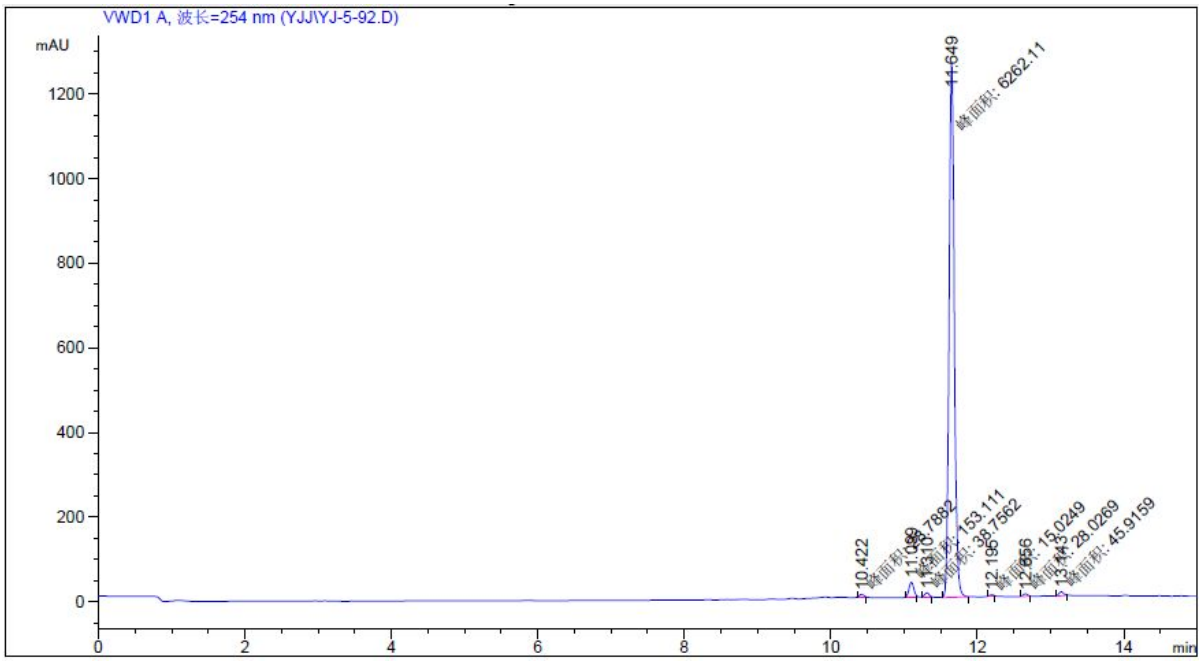
## HPLC trace of compound 38




### Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 12.127 | 38.79142 | 0.7922 |
| 2 | 12.336 | 4858.00977 | 99.2078 |

## HPLC trace of compound 39

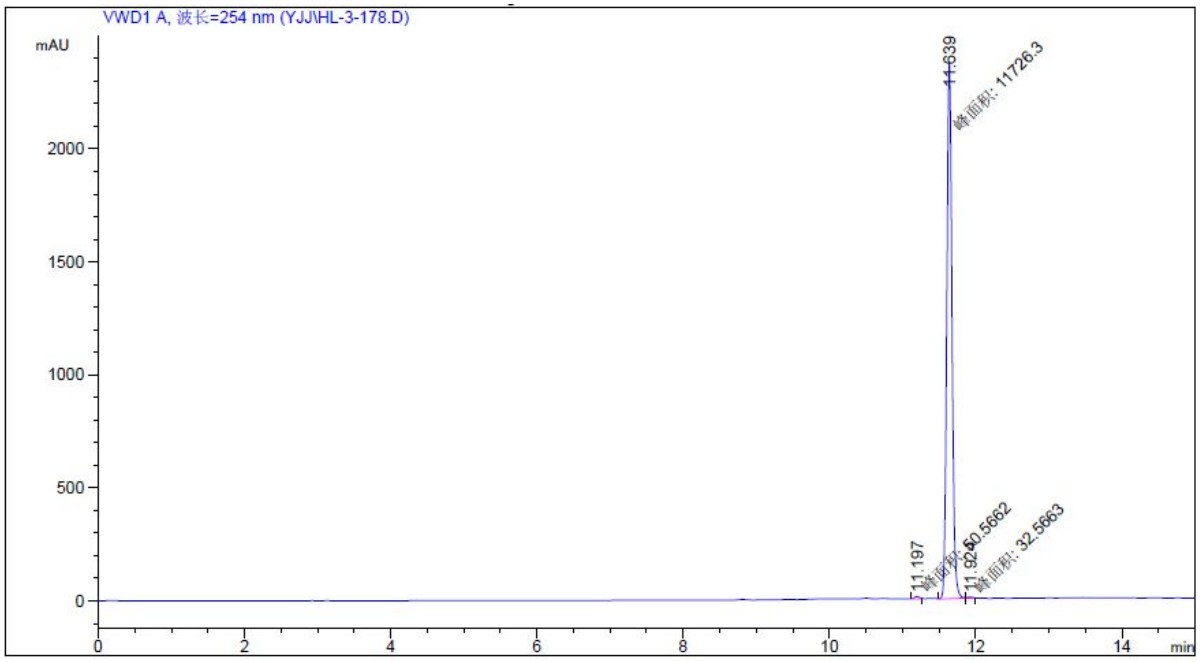



### Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 10.422 | 28.78823 | 0.4381 |
| 2 | 11.099 | 153.11110 | 2.3298 |
| 3 | 11.310 | 38.75622 | 0.5897 |
| 4 | 11.649 | 6262.10938 | 95.2886 |
| 5 | 12.195 | 15.02488 | 0.2286 |
| 6 | 12.656 | 28.02688 | 0.4265 |

| | | | |
|---|---|---|---|
| 7 | 13.143 | 45.91587 | 0.6987 |

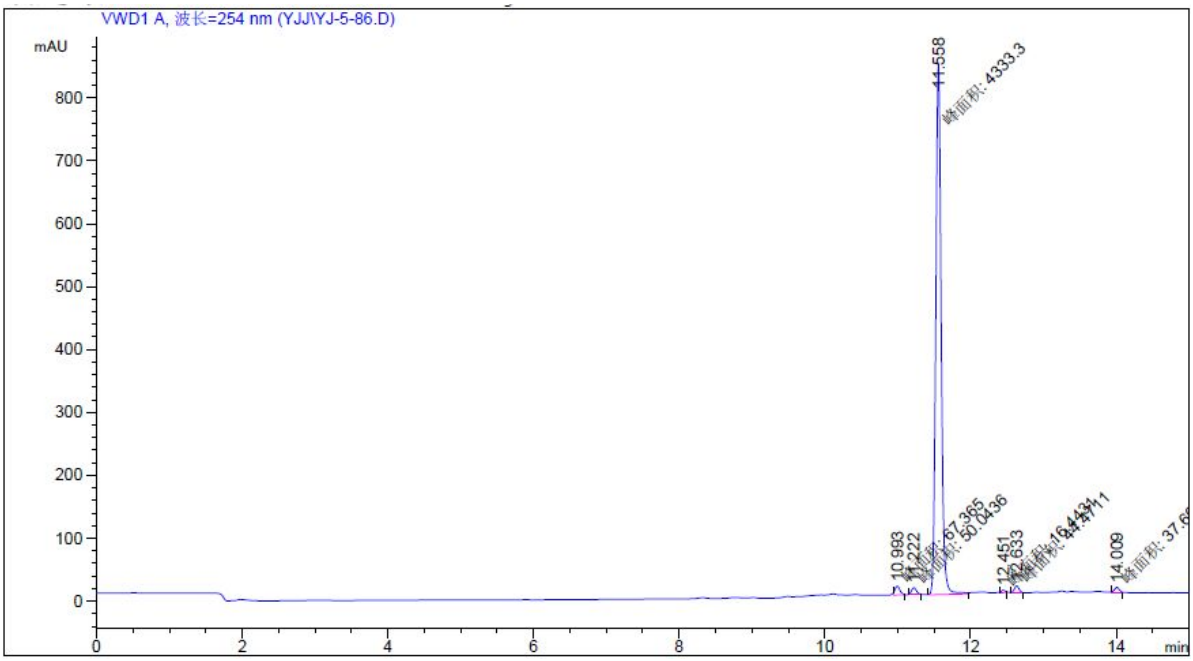
## HPLC trace of compound 40




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.197 | 50.56622 | 0.4282 |
| 2 | 11.639 | 11726.3 | 99.2960 |
| 3 | 11.924 | 32.56635 | 0.2758 |

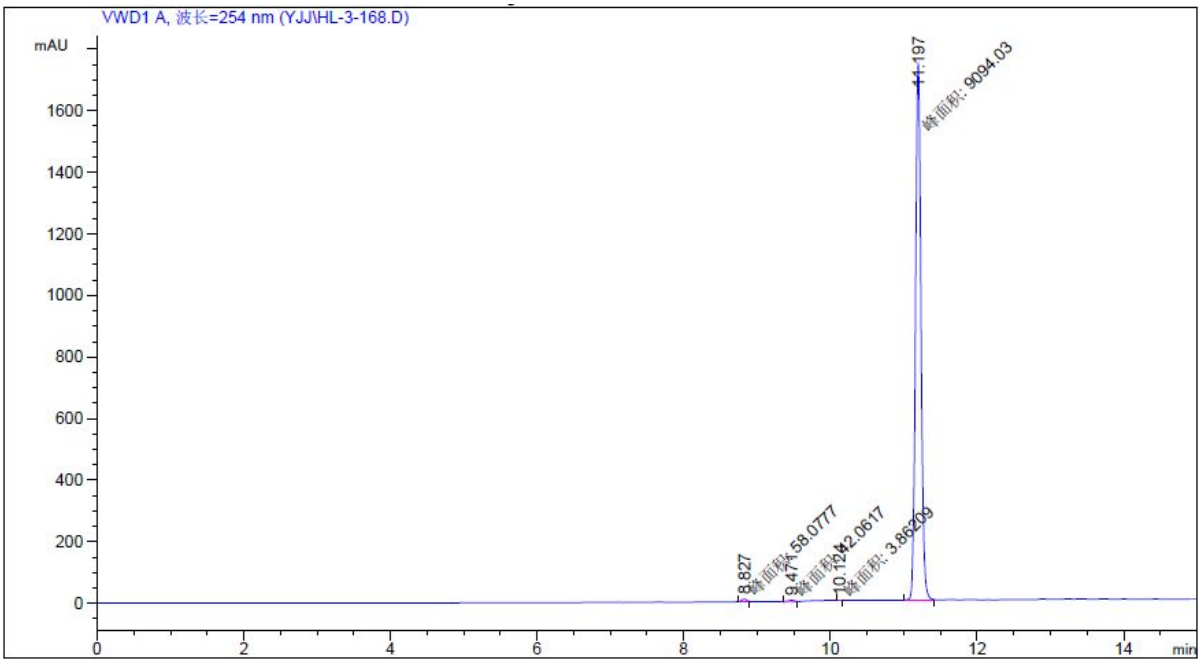
## HPLC trace of compound 41




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 10.993 | 67.36498 | 1.4808 |
| 2 | 11.222 | 50.04362 | 1.1000 |
| 3 | 11.558 | 4333.29736 | 95.2522 |
| 4 | 12.451 | 16.44314 | 0.3614 |
| 5 | 12.633 | 44.47114 | 0.9775 |
| 6 | 14.009 | 37.66735 | 0.8280 |

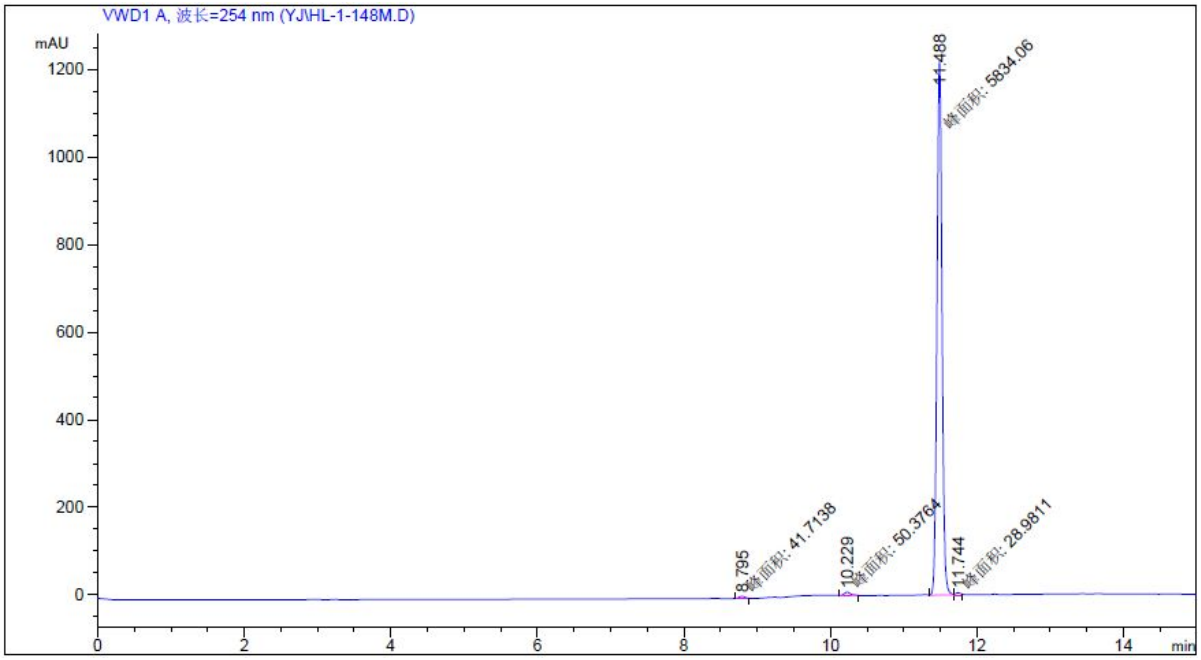
## HPLC trace of compound 42




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 8.827 | 58.07767 | 0.6314 |
| 2 | 9.471 | 42.06168 | 0.4573 |
| 3 | 10.124 | 3.86209 | 0.0420 |
| 4 | 11.197 | 9094.02637 | 98.8693 |

## HPLC trace of compound 43




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 8.795 | 41.71376 | 0.7005 |
| 2 | 10.229 | 50.37642 | 0.8459 |
| 3 | 11.488 | 5834.06494 | 97.9669 |
| 4 | 11.744 | 28.98110 | 0.4867 |

HPLC trace of compound 44

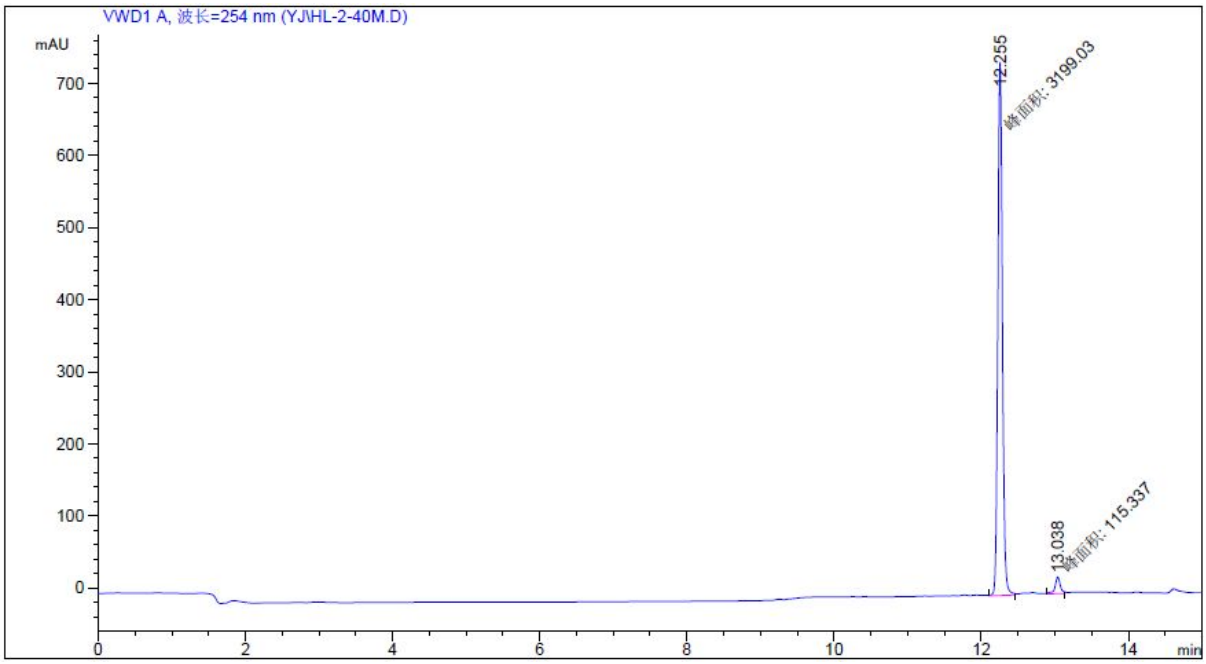



Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 12.255 | 3199.03394 | 96.5201 |
| 2 | 13.038 | 115.33668 | 3.4799 |

HPLC trace of compound 45

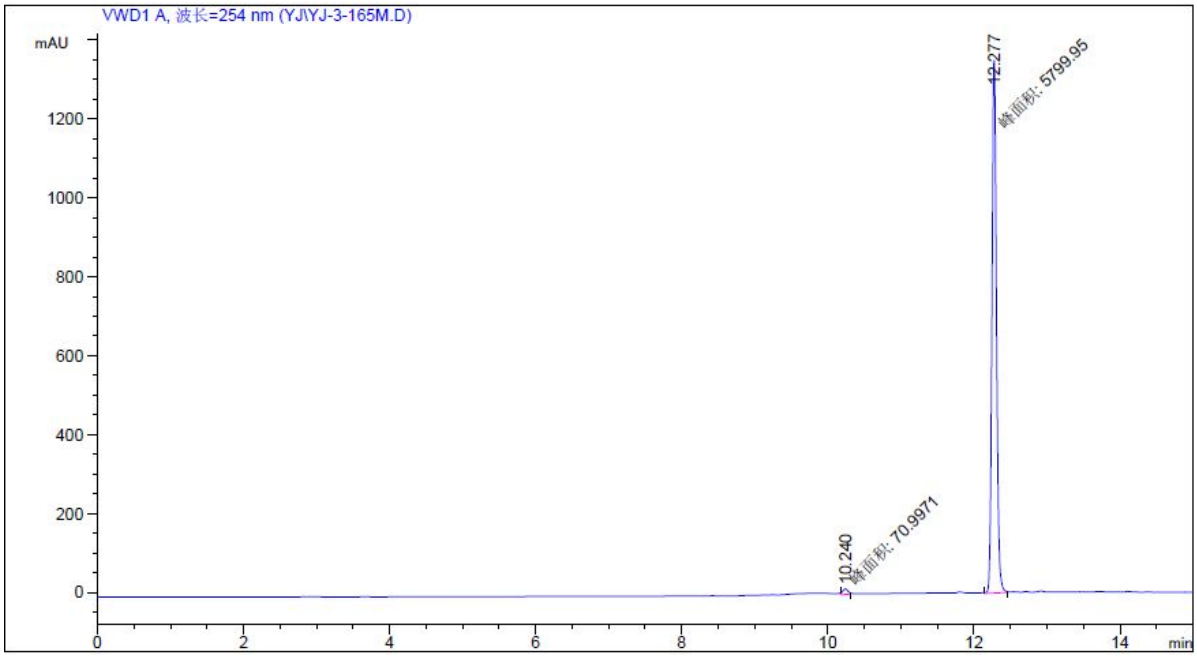
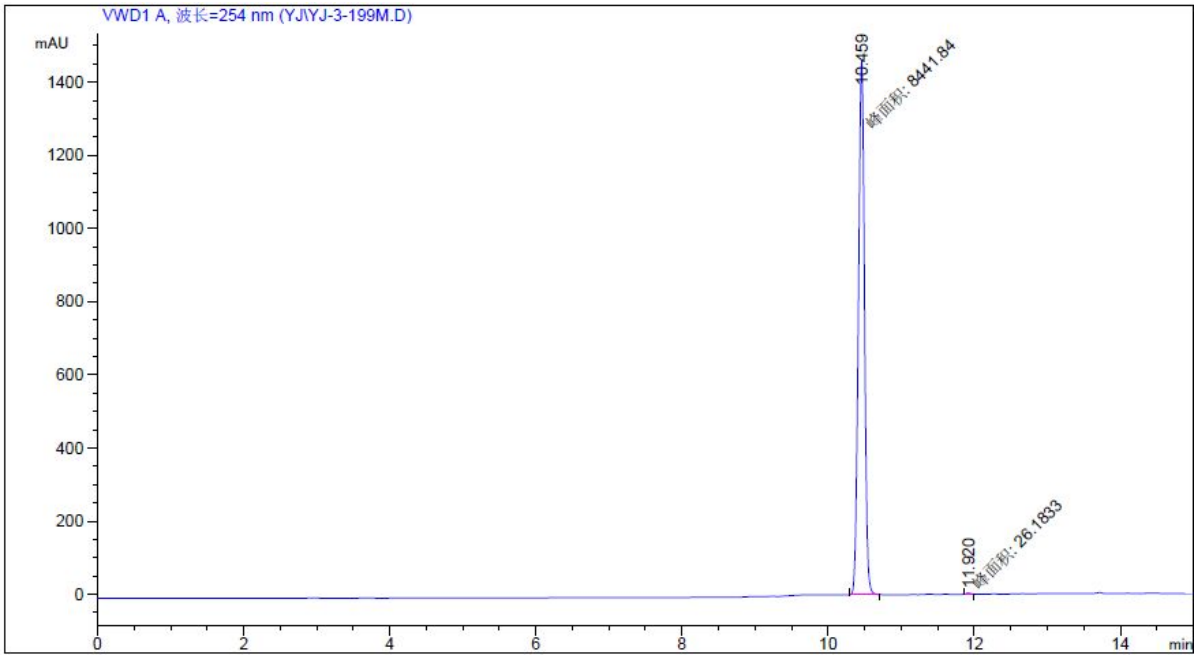



Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 10.240 | 70.99714 | 1.2093 |
| 2 | 12.277 | 5799.94727 | 98.7907 |

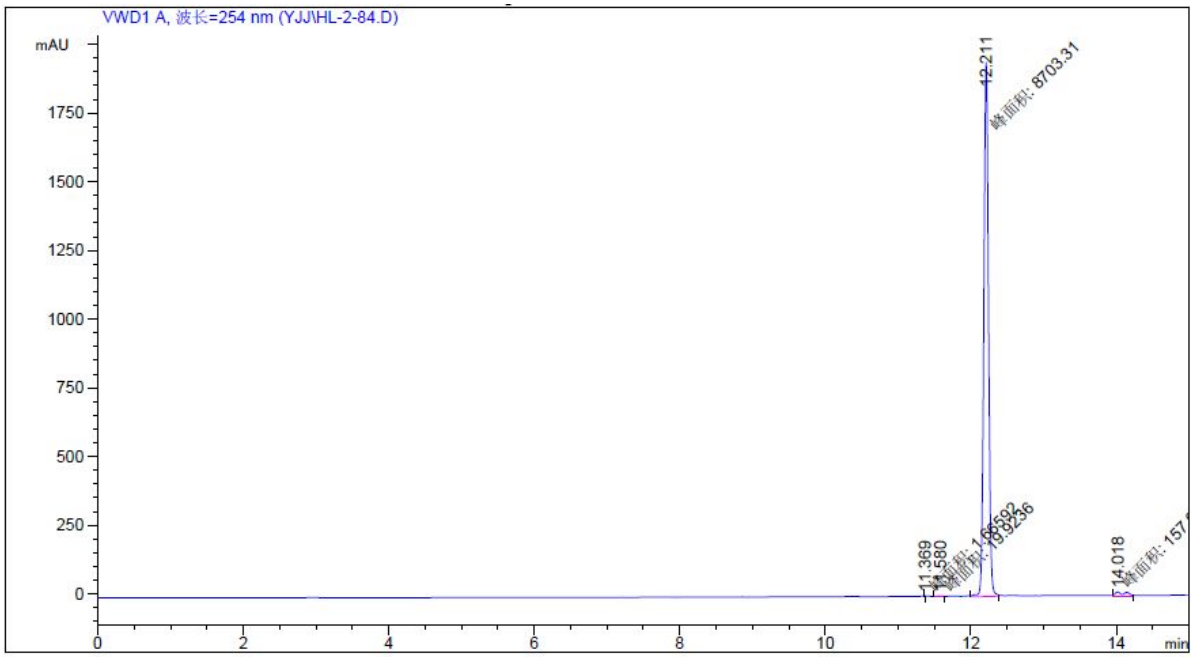
## HPLC trace of compound 46




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 10.459 | 8441.84180 | 99.6908 |
| 2 | 11.920 | 26.18334 | 0.3092 |

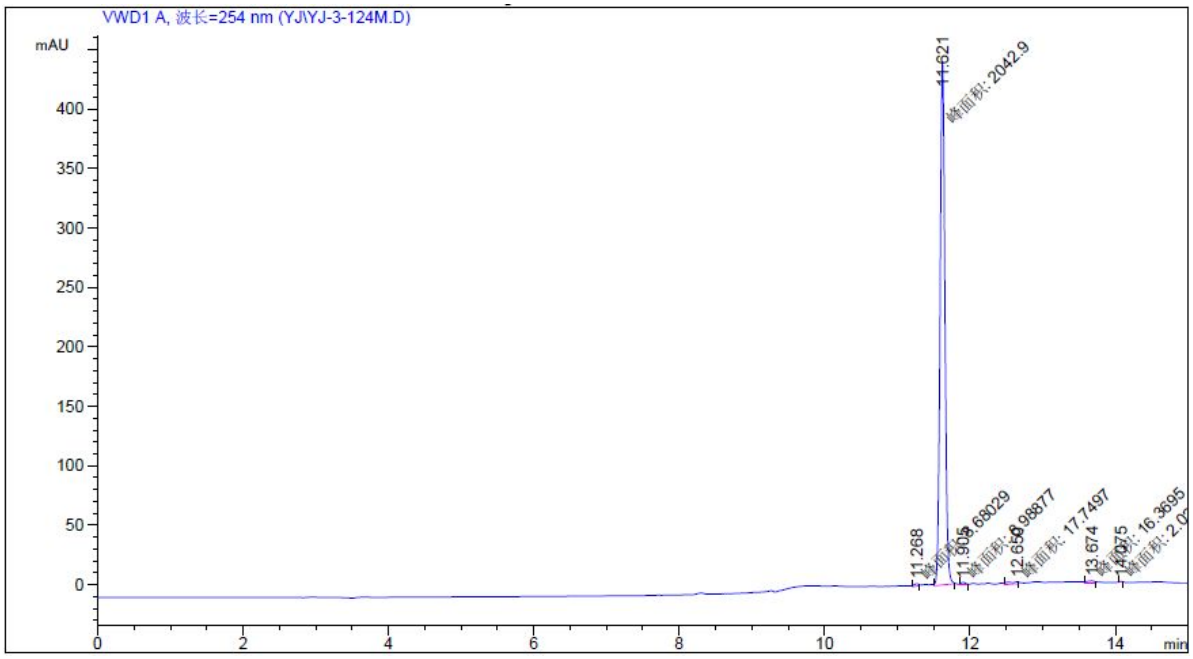
## HPLC trace of compound 47




### Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.369 | 1.66592 | 0.0188 |
| 2 | 11.580 | 19.92364 | 0.2243 |
| 3 | 12.211 | 8703.31348 | 97.9889 |
| 4 | 14.018 | 157.03271 | 1.7680 |

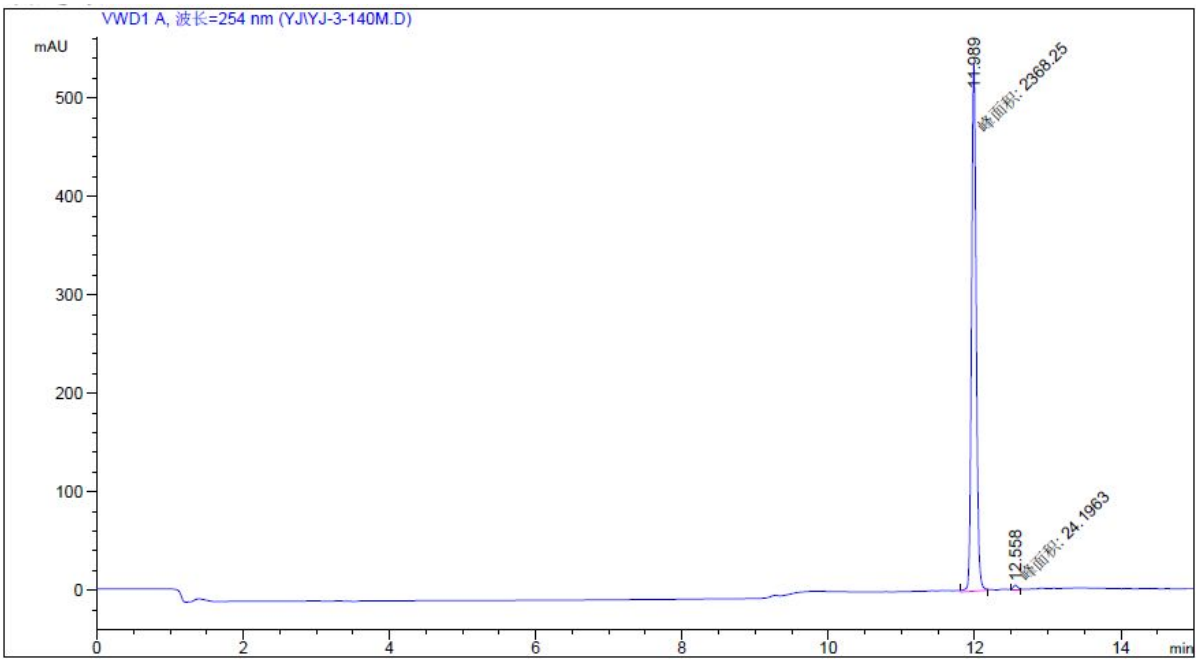
## HPLC trace of compound 48




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.268 | 8.68029 | 0.4140 |
| 2 | 11.621 | 2042.90112 | 97.4336 |
| 3 | 11.905 | 8.98877 | 0.4287 |
| 4 | 12.650 | 17.74973 | 0.8466 |
| 5 | 13.674 | 16.36952 | 0.7807 |
| 6 | 14.075 | 2.02157 | 0.0964 |

## HPLC trace of compound 49




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.989 | 2368.24561 | 98.9886 |
| 2 | 12.558 | 24.19634 | 1.0114 |

HPLC trace of compound 50

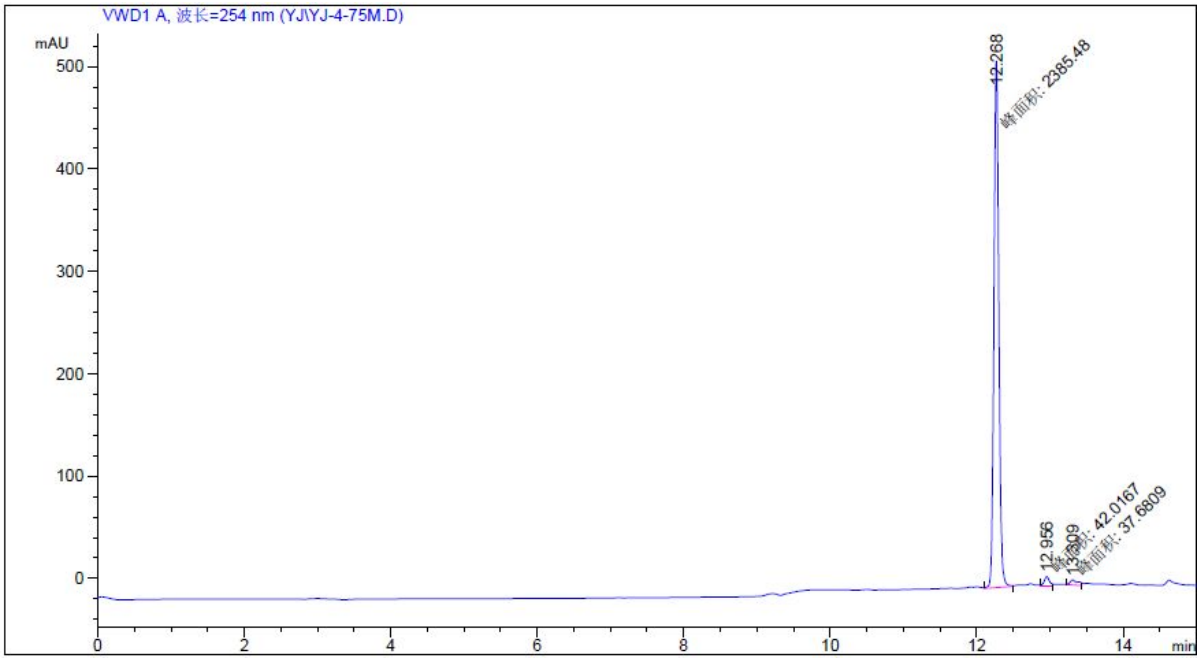
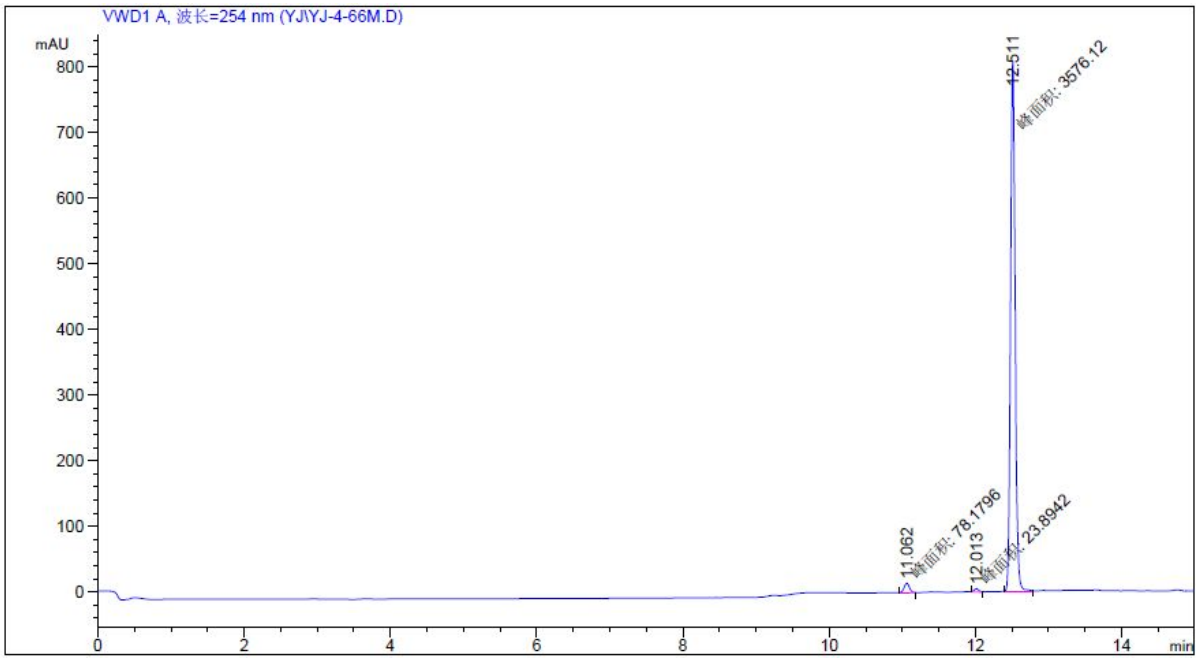



Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 12.268 | 2385.48289 | 96.7671 |
| 2 | 12.956 | 42.01667 | 1.7044 |
| 3 | 13.309 | 37.68092 | 1.5285 |

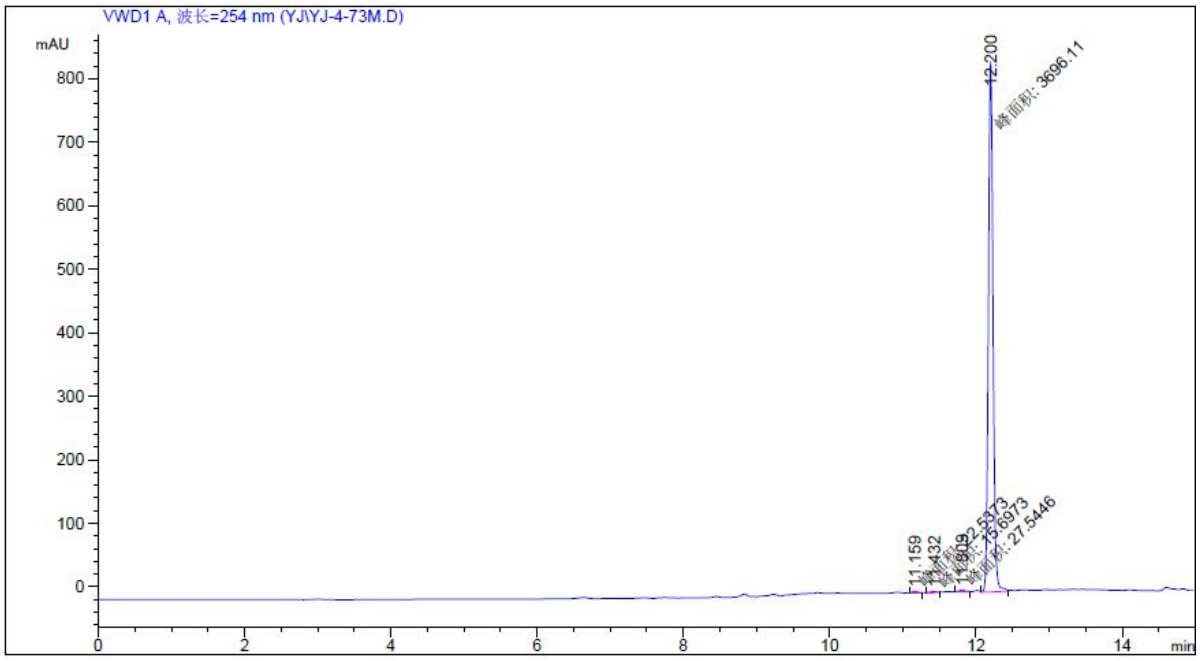
## HPLC trace of compound 51




### Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.062 | 78.17957 | 2.1255 |
| 2 | 12.013 | 23.89422 | 0.6496 |
| 3 | 12.511 | 3576.11914 | 97.2249 |

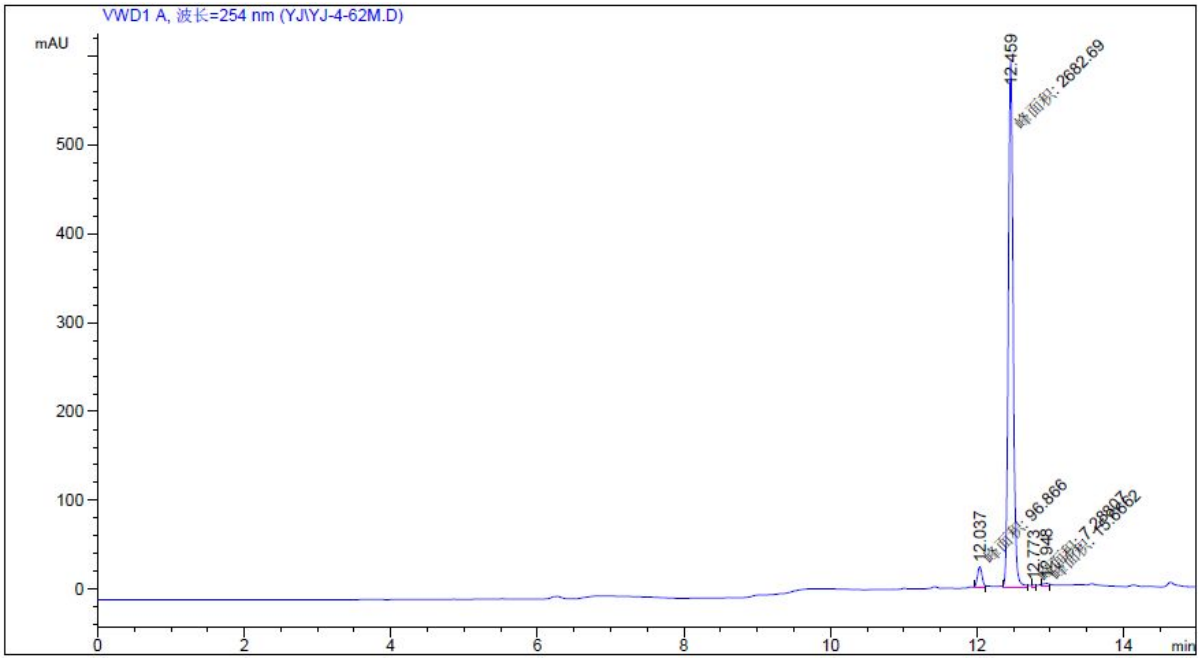
## HPLC trace of compound 52




### Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.159 | 22.53733 | 0.5991 |
| 2 | 11.432 | 15.69731 | 0.4173 |
| 3 | 11.809 | 27.54461 | 0.7322 |
| 4 | 12.200 | 3696.11377 | 98.2514 |

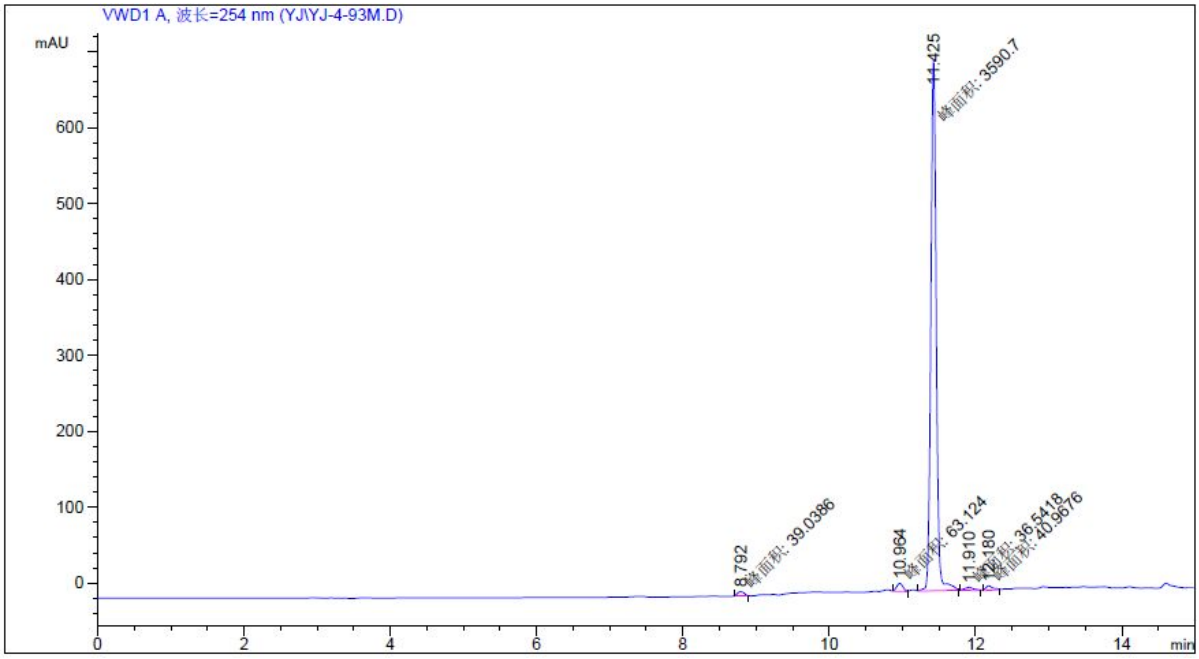
## HPLC trace of compound 53




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 12.037 | 96.86595 | 3.4564 |
| 2 | 12.459 | 2682.69385 | 95.7245 |
| 3 | 12.773 | 7.28807 | 0.2601 |
| 4 | 12.948 | 15.66621 | 0.5590 |

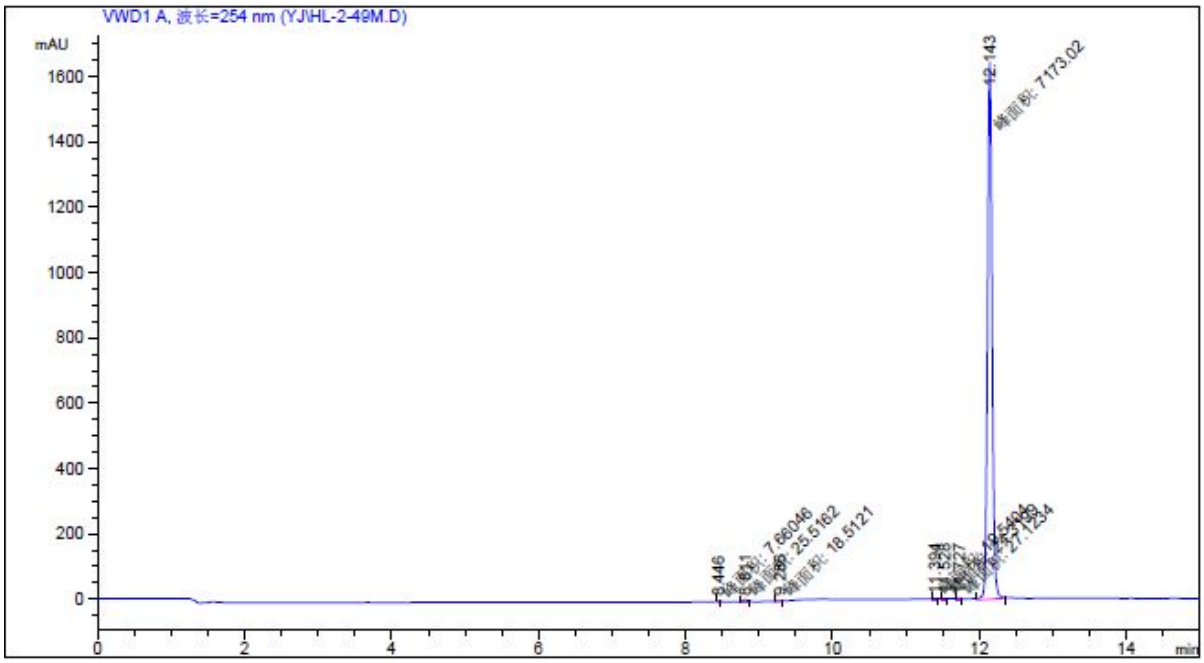
## HPLC trace of compound 54




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 8.792 | 39.03863 | 1.0354 |
| 2 | 10.964 | 63.12403 | 1.6742 |
| 3 | 11.425 | 3590.69995 | 95.2346 |
| 4 | 11.910 | 36.54180 | 0.9692 |
| 5 | 12.180 | 40.96756 | 1.0866 |

HPLC trace of compound 55

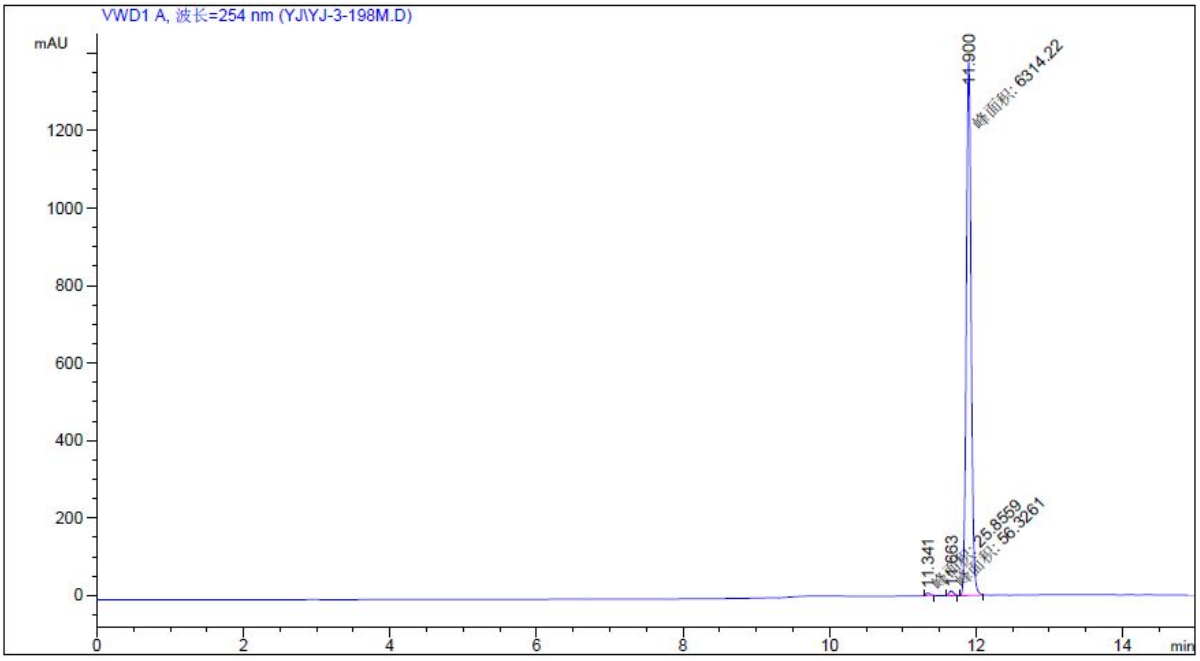



## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 8.446 | 7.66046 | 0.1050 |
| 2 | 8.811 | 25.51624 | 0.3497 |
| 3 | 9.286 | 18.51205 | 0.2537 |
| 4 | 11.394 | 19.54040 | 0.2678 |
| 5 | 11.528 | 24.31993 | 0.3333 |
| 6 | 11.727 | 27.12337 | 0.3718 |

| | | | |
|---|---|---|---|
| 7 | 12.143 | 7173.02197 | 98.3186 |

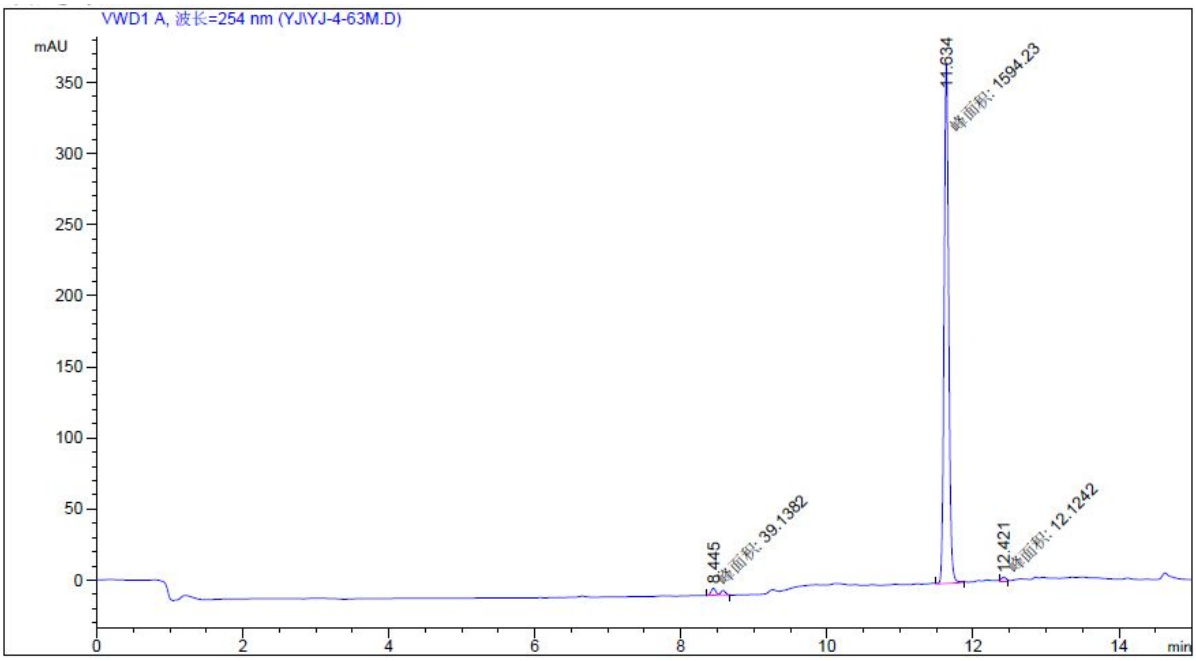
## HPLC trace of compound 56




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.341 | 25.85585 | 0.4042 |
| 2 | 11.663 | 56.32615 | 0.8806 |
| 3 | 11.900 | 6314.21631 | 98.7152 |

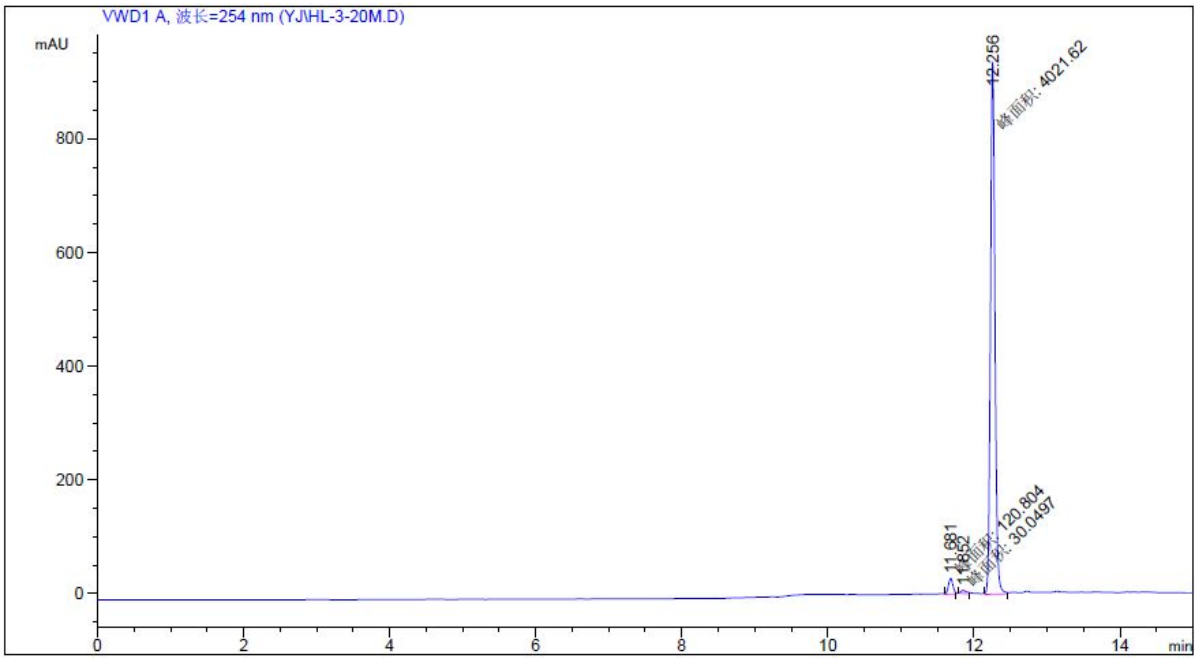
## HPLC trace of compound 57




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 8.445 | 39.13825 | 2.3785 |
| 2 | 11.634 | 1594.22925 | 96.8847 |
| 3 | 12.421 | 12.12417 | 0.7368 |

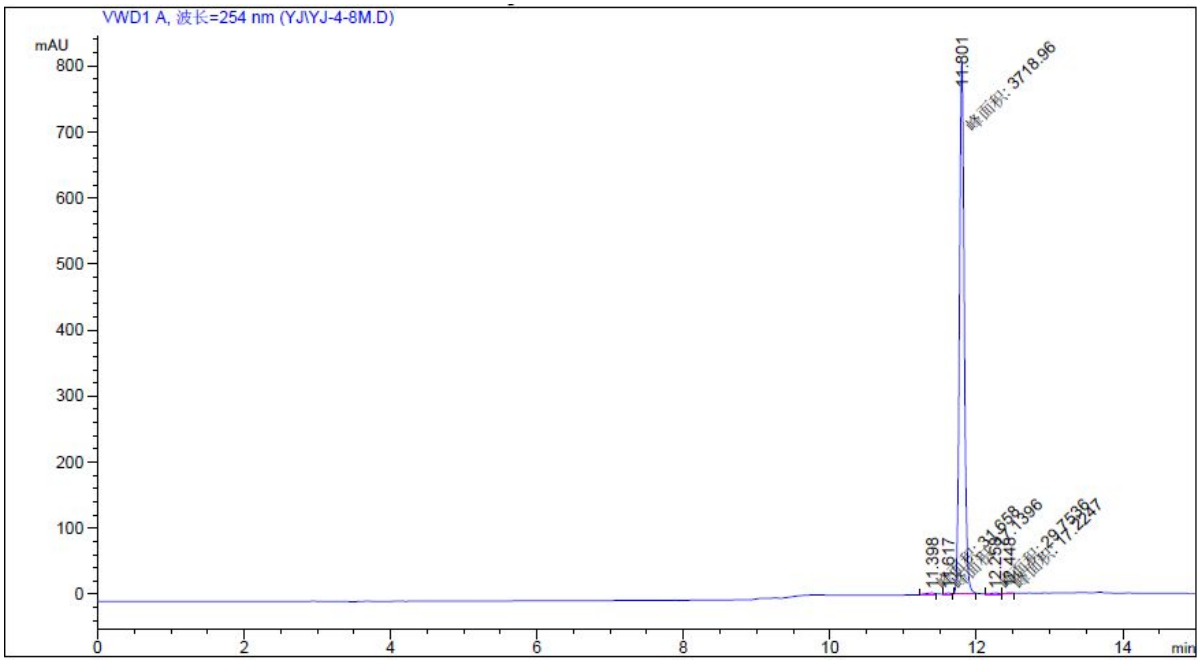
## HPLC trace of compound 58




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.681 | 120.80442 | 2.8953 |
| 2 | 11.852 | 30.04974 | 0.7202 |
| 3 | 12.256 | 4021.61743 | 96.3845 |

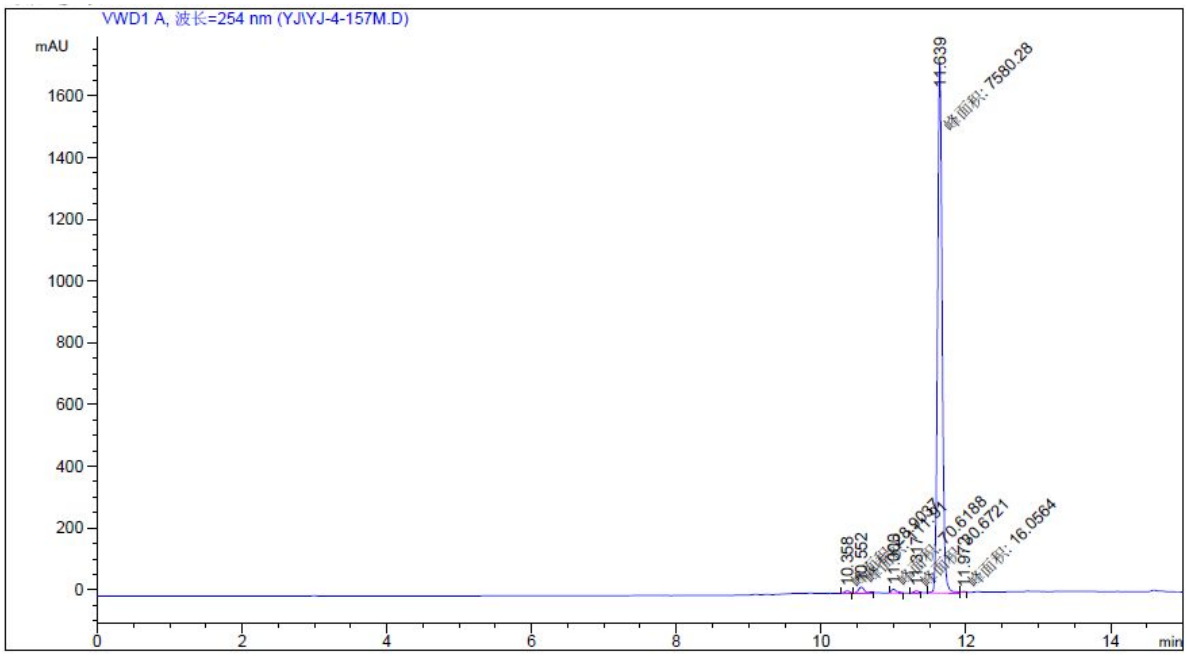
## HPLC trace of compound 59




### Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.398 | 31.65802 | 0.8299 |
| 2 | 11.617 | 17.13957 | 0.4493 |
| 3 | 11.801 | 3718.96338 | 97.4893 |
| 4 | 12.258 | 29.75363 | 0.7800 |
| 5 | 12.448 | 17.22470 | 0.4515 |

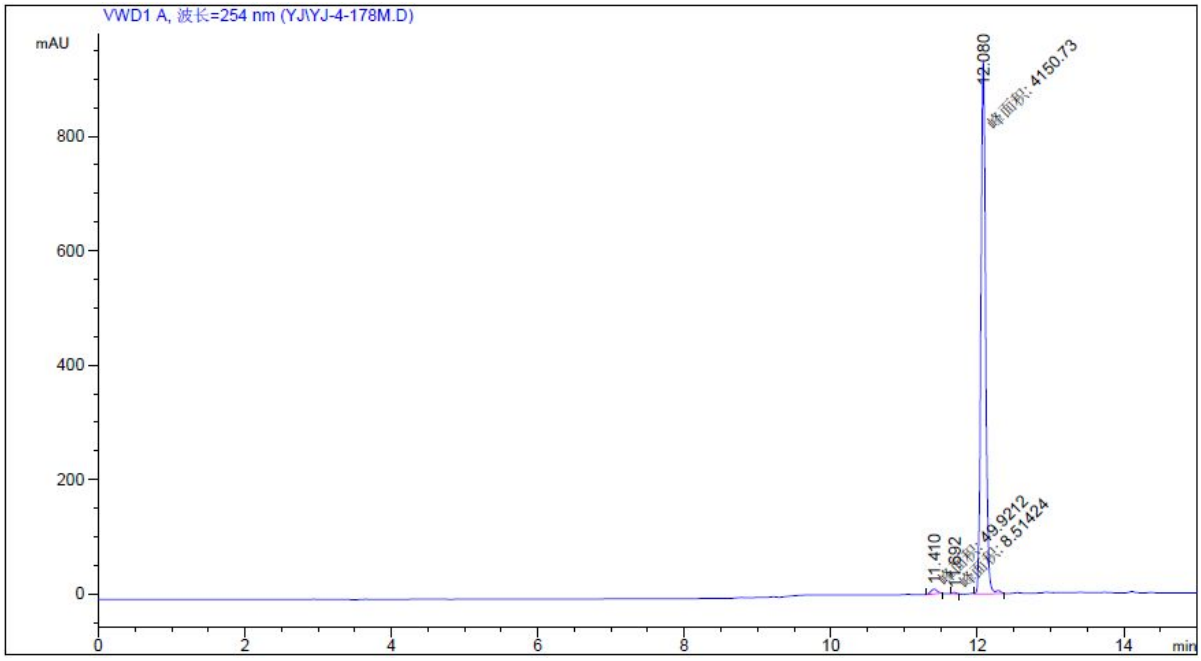
HPLC trace of compound 60




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 10.358 | 29.90369 | 0.3687 |
| 2 | 10.552 | 111.91039 | 1.4277 |
| 3 | 11.000 | 70.61876 | 0.9009 |
| 4 | 11.317 | 30.67211 | 0.3913 |
| 5 | 11.639 | 7580.27686 | 96.7065 |
| 6 | 11.972 | 16.05639 | 0.2049 |

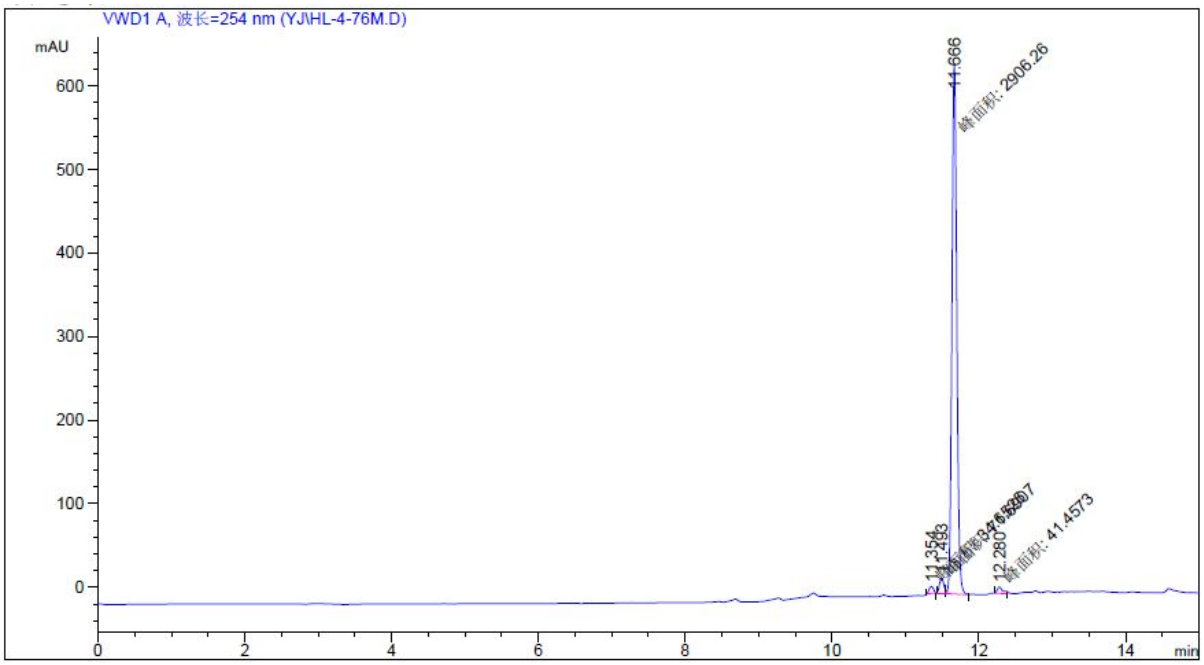
## HPLC trace of compound 61




### Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.410 | 49.92123 | 1.1860 |
| 2 | 11.692 | 8.51424 | 0.2023 |
| 3 | 12.080 | 4150.72998 | 98.6117 |

HPLC trace of compound 62




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.354 | 34.65250 | 1.1346 |
| 2 | 11.493 | 71.69067 | 2.3474 |
| 3 | 11.666 | 2906.25879 | 95.1605 |
| 4 | 12.280 | 41.45732 | 1.3574 |

HPLC trace of compound 63

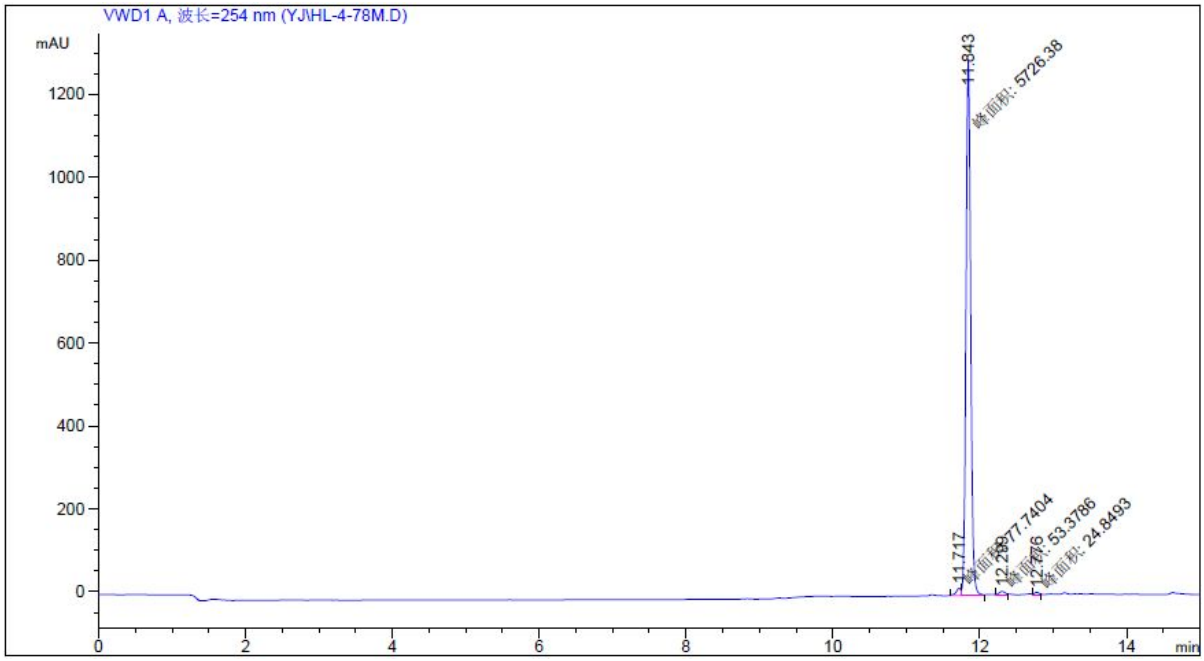
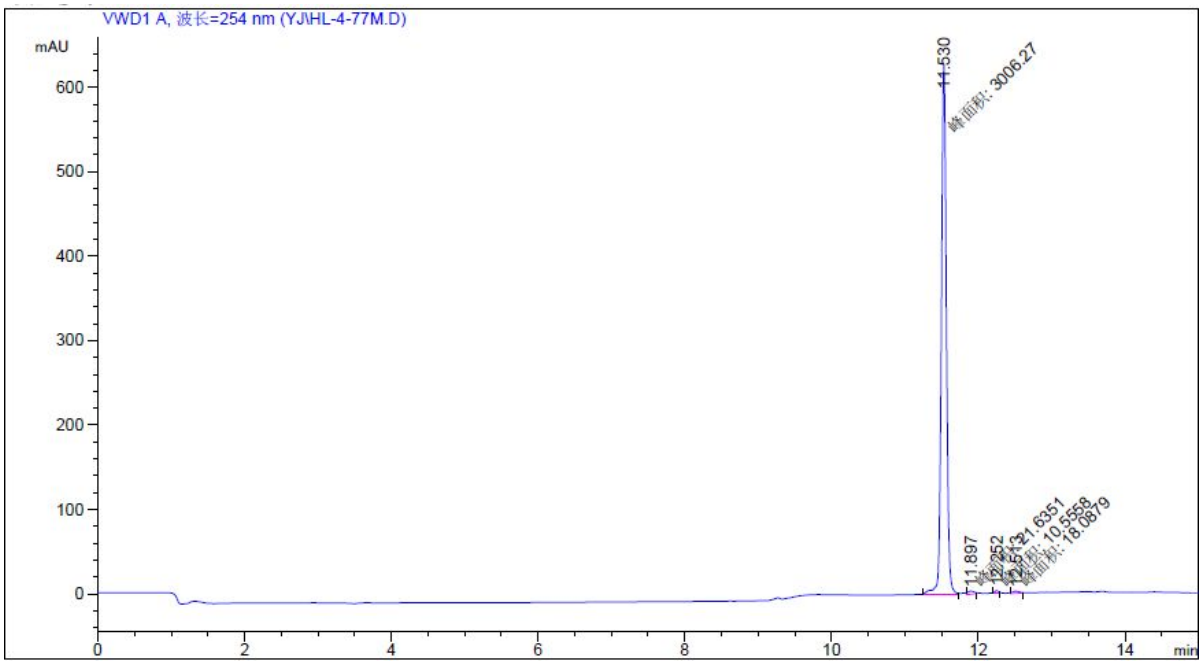



Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.717 | 77.74043 | 1.3216 |
| 2 | 11.843 | 5726.37598 | 97.3485 |
| 3 | 12.299 | 53.37857 | 0.9074 |
| 4 | 12.776 | 24.84932 | 0.4224 |

HPLC trace of compound 64




## Table Peak

| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.530 | 3006.27002 | 98.3550 |
| 2 | 11.897 | 21.63510 | 0.7078 |
| 3 | 12.252 | 10.55579 | 0.3454 |
| 4 | 12.513 | 18.08790 | 0.5918 |

HPLC trace of compound 65

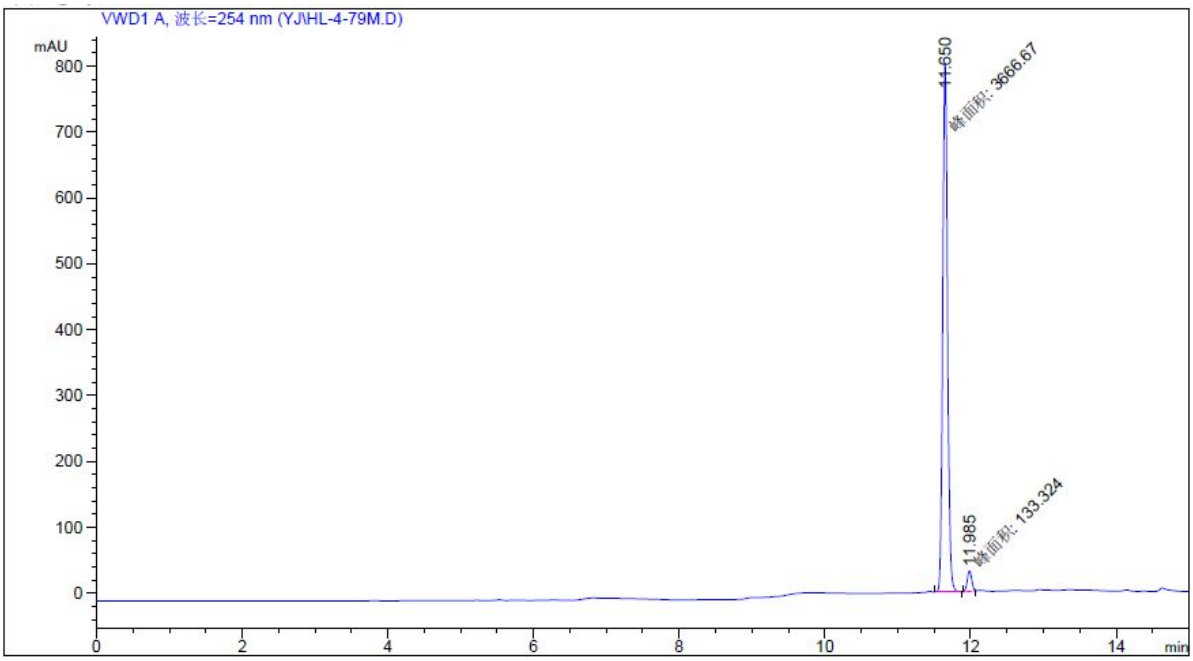
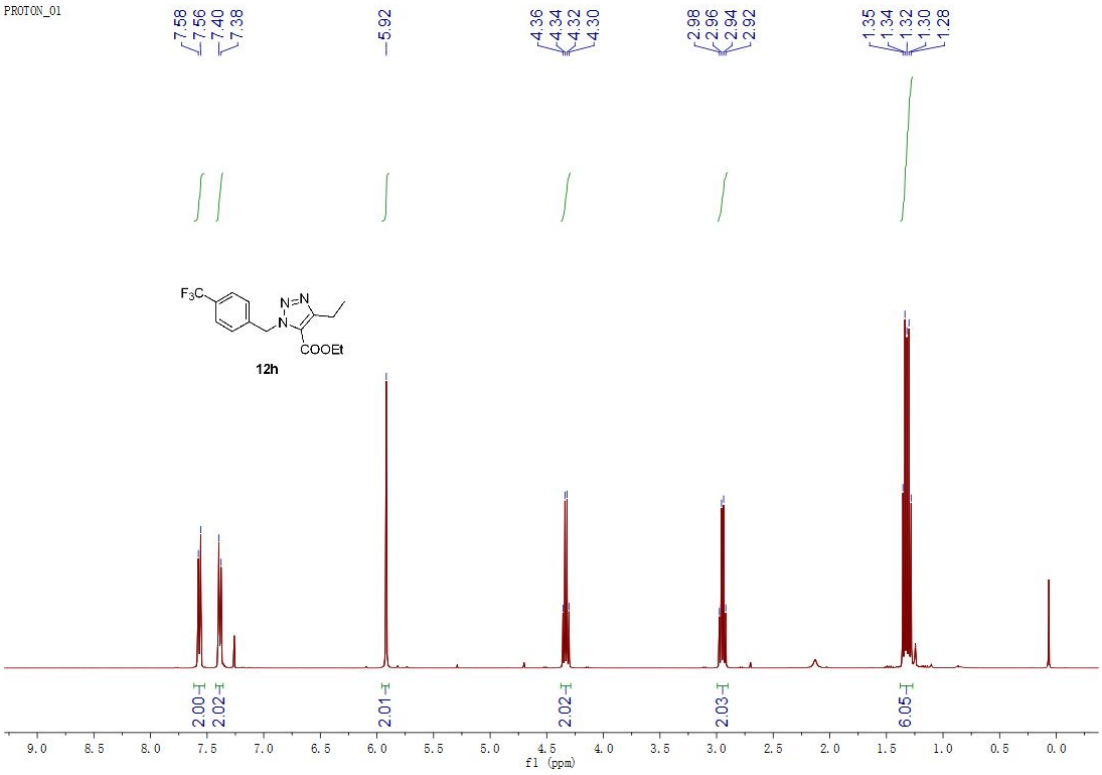



Table Peak

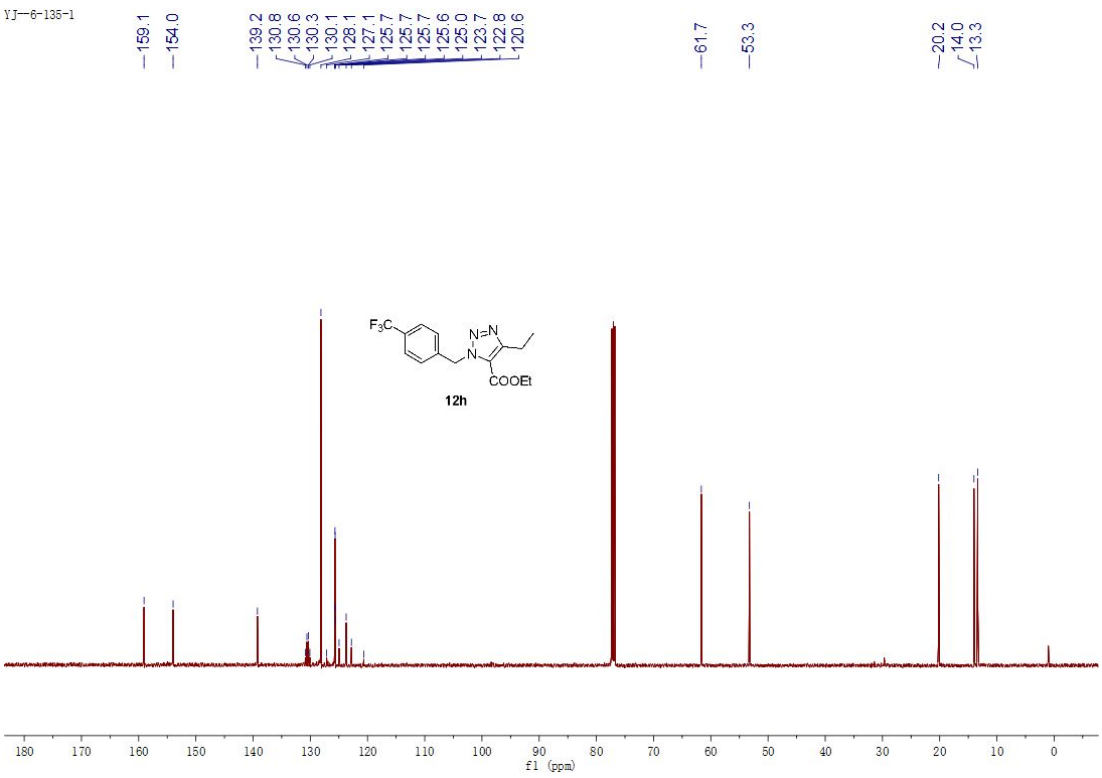
| Peak# | Ret. Time | Area | Area% |
|---|---|---|---|
| 1 | 11.650 | 3666.66675 | 96.4915 |
| 2 | 11.985 | 133.32355 | 3.5085 |

## Characterization of Structural Isomers by HMBC

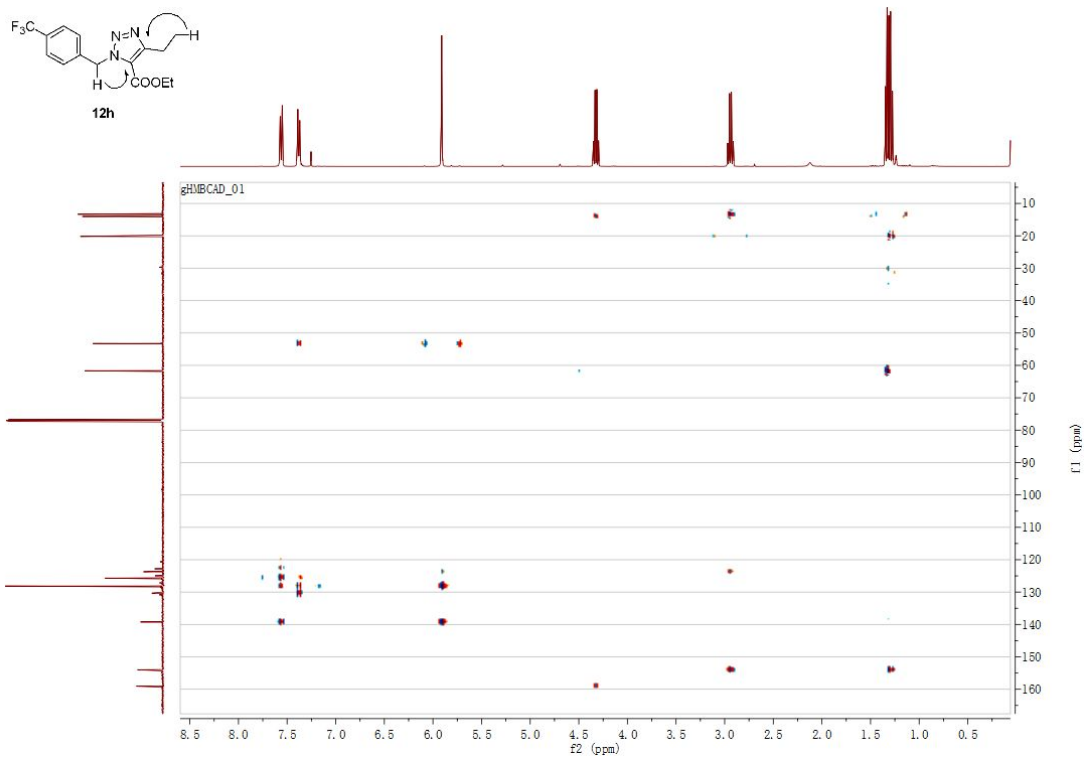
## $^{1}$H NMR of 12h



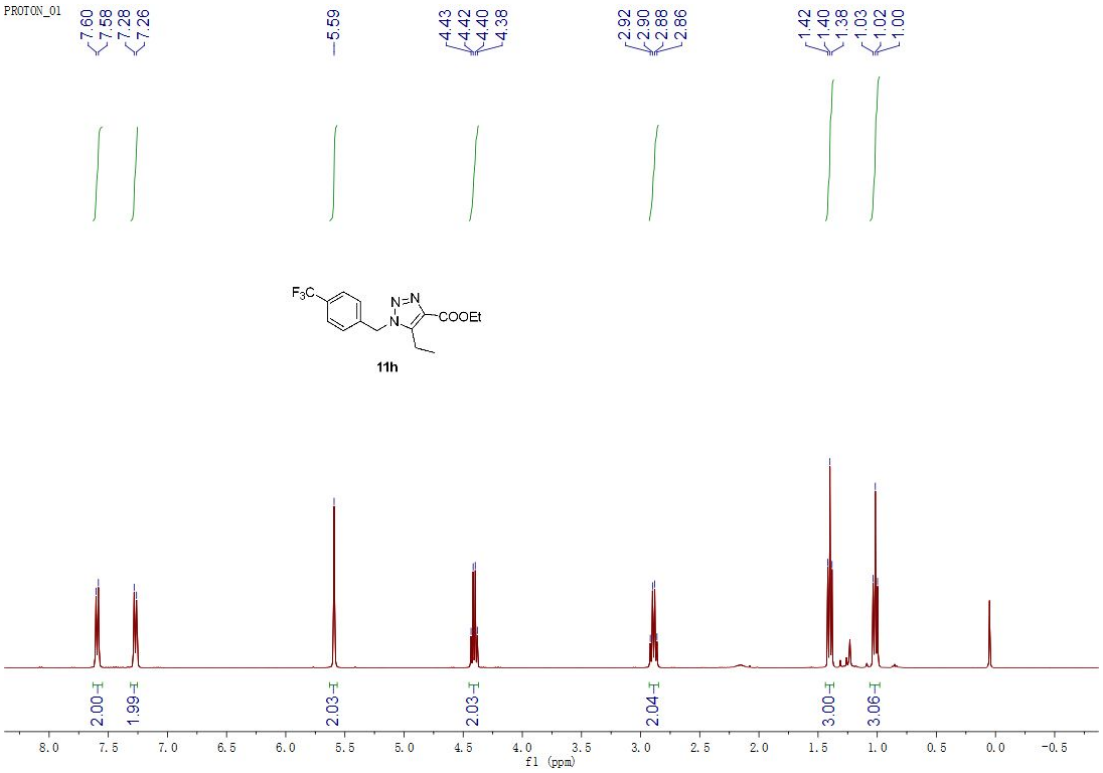

## $^{13}$C NMR of 12h


YJ--6-135-1
159.1
154.0
139.2
130.8
130.6
130.3
130.1
128.1
127.1
125.7
125.7
125.7
125.6
125.0
123.7
122.8
120.6
61.7
53.3
20.2
14.0
13.3
F3C
N=N
N
COOEt
12h
f1 (ppm)

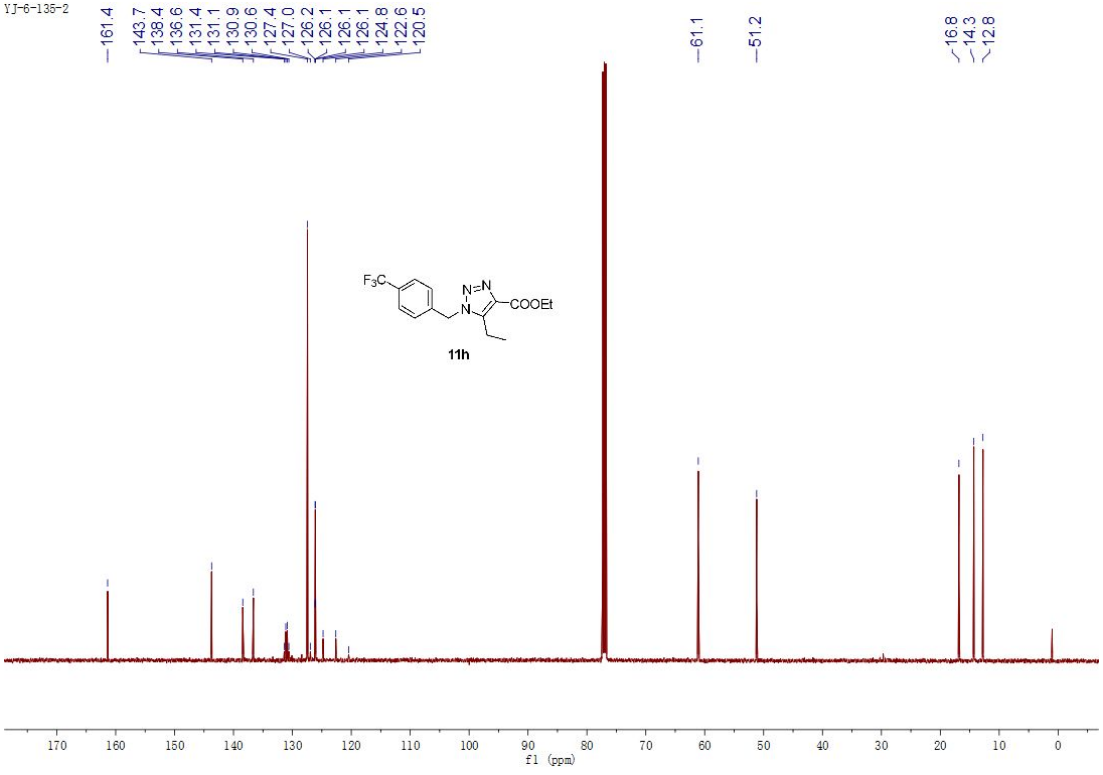
## HMBC of 12h


F3C
N=N
N
H
H
COOEt
12h
gHMBCAD_01
f2 (ppm)
f1 (ppm)

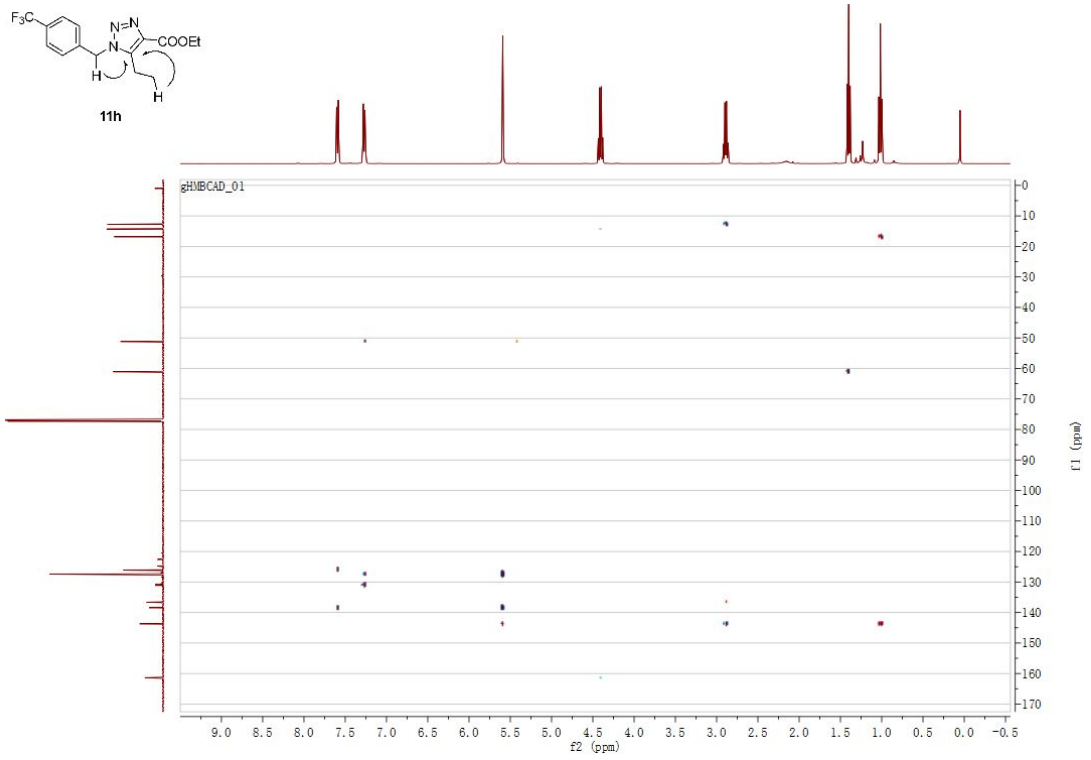
## $^{1}$H NMR of 11h



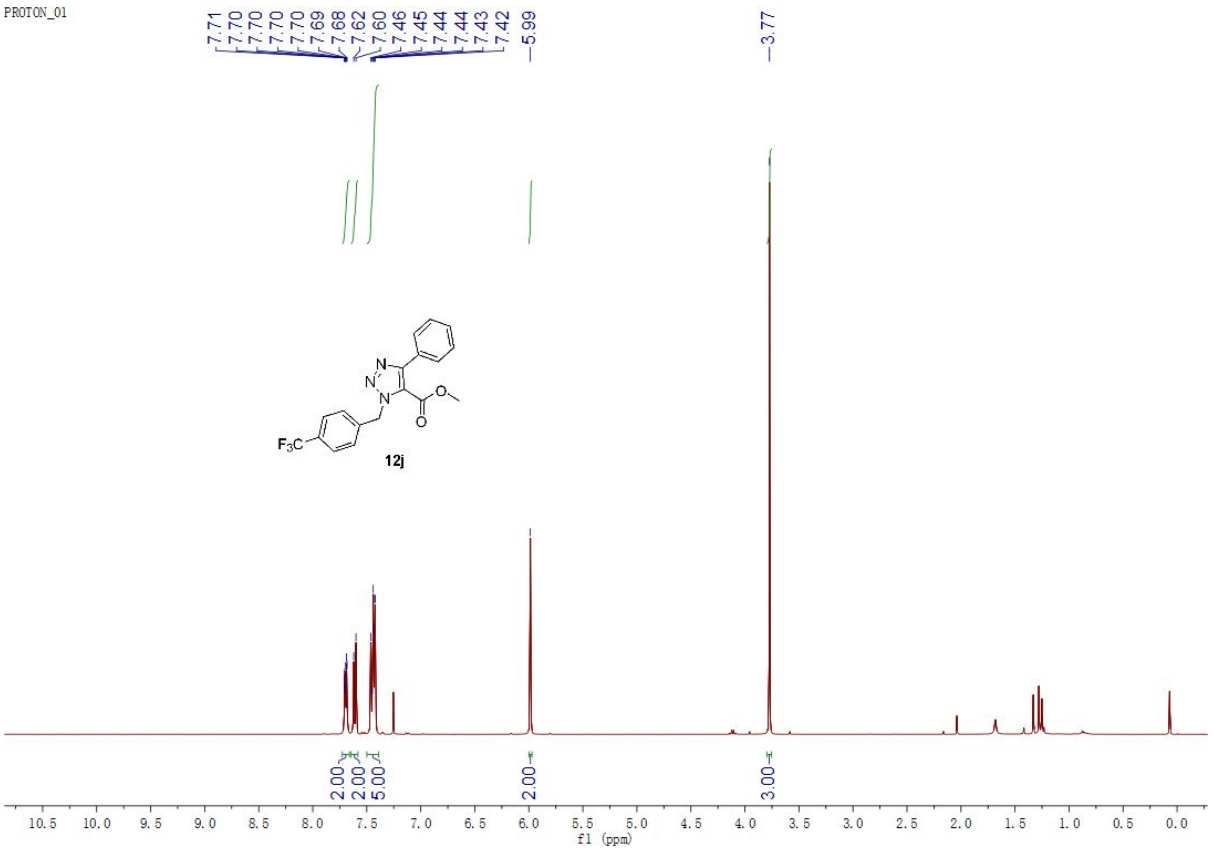

## $^{13}$C NMR of 12h



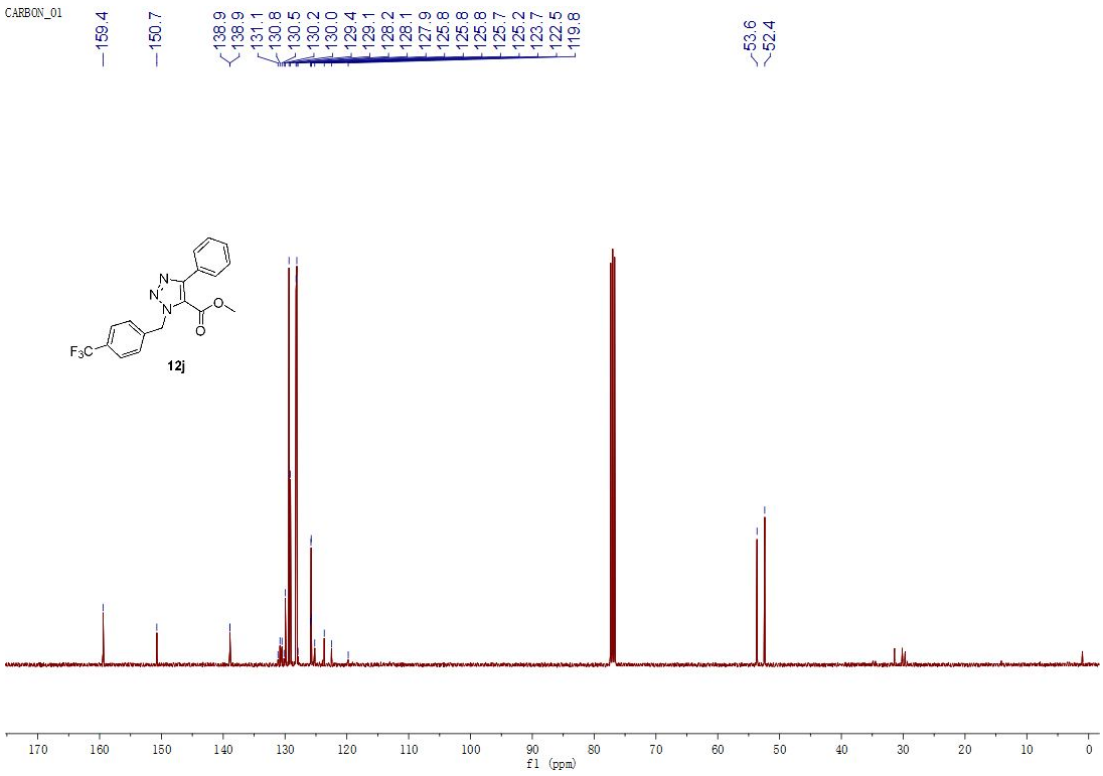
## HMBC of 11h



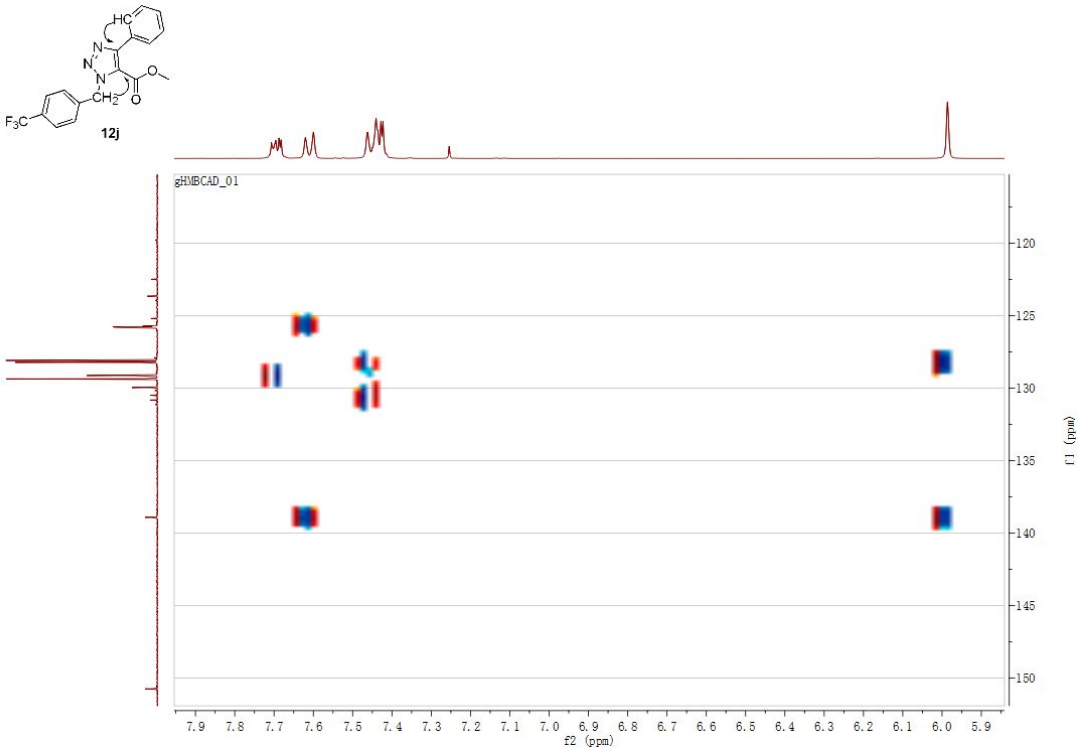
## $^{1}$H NMR of 12j



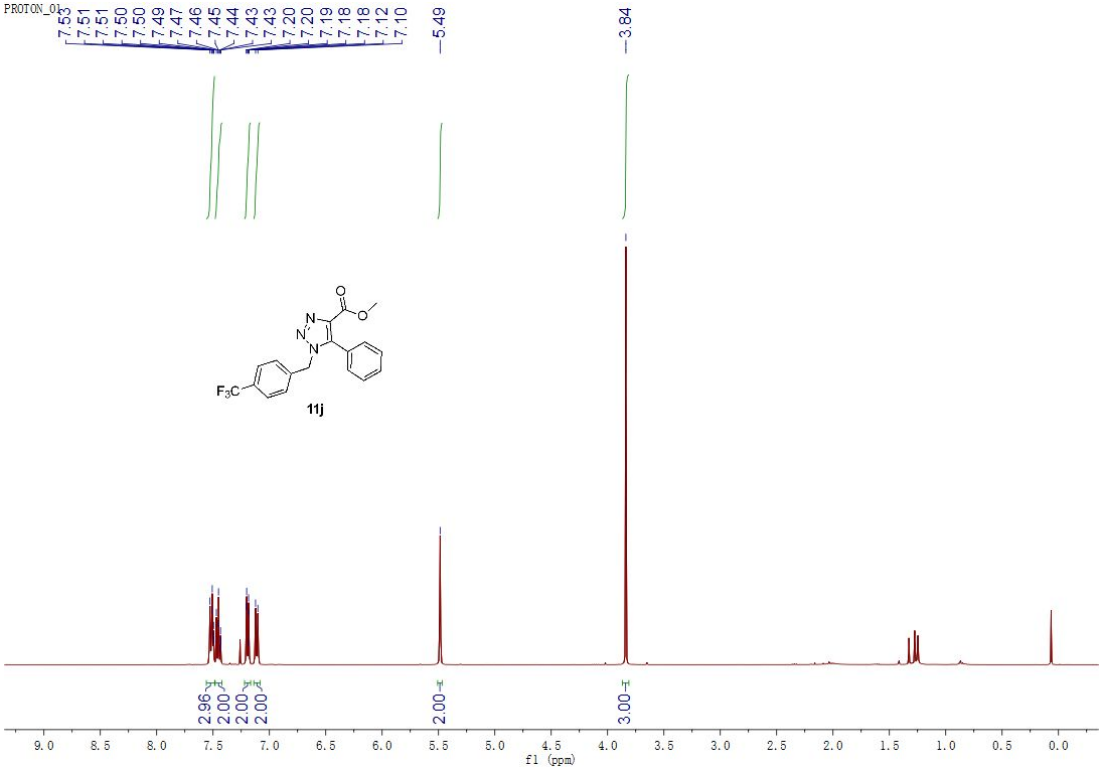

## $^{13}$C NMR of 12j


CARBON_01
159.4
150.7
138.9
138.9
131.1
130.8
130.5
130.2
130.0
129.4
129.1
128.2
128.1
127.9
125.8
125.8
125.8
125.7
125.2
123.7
122.5
119.8
53.6
52.4
F3C
12j
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

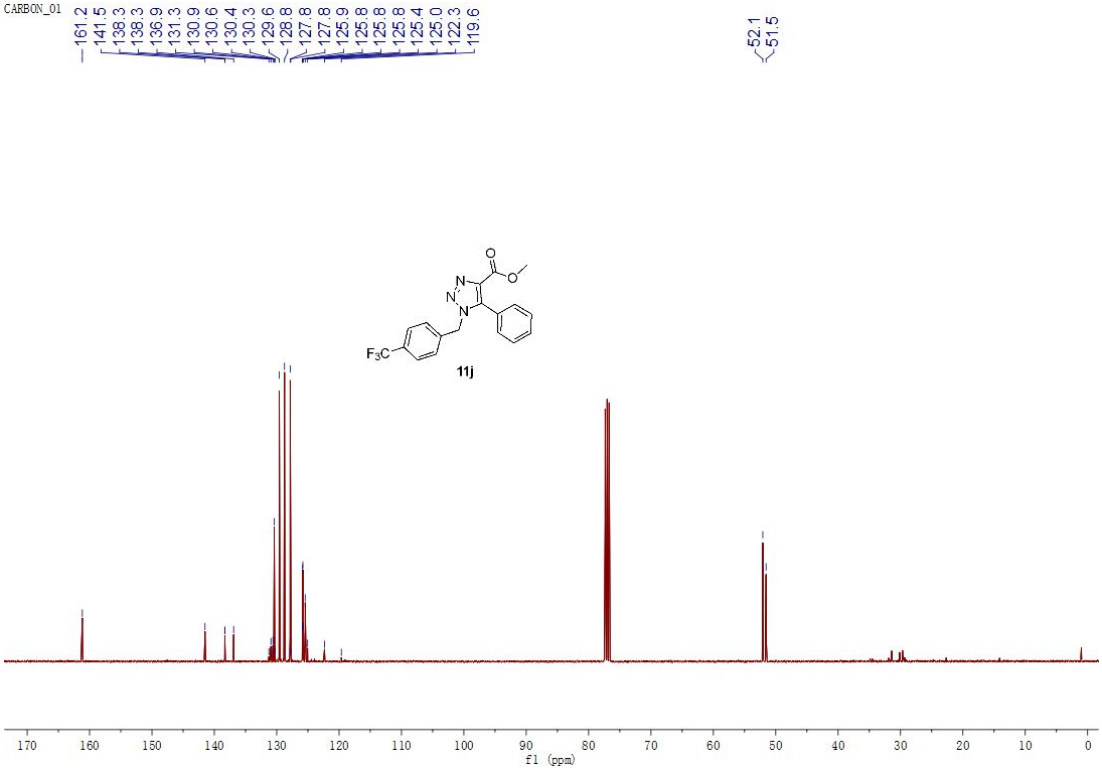
## HMBC of 12j


12j
gHMBCAD_01
f2 (ppm)
f1 (ppm)

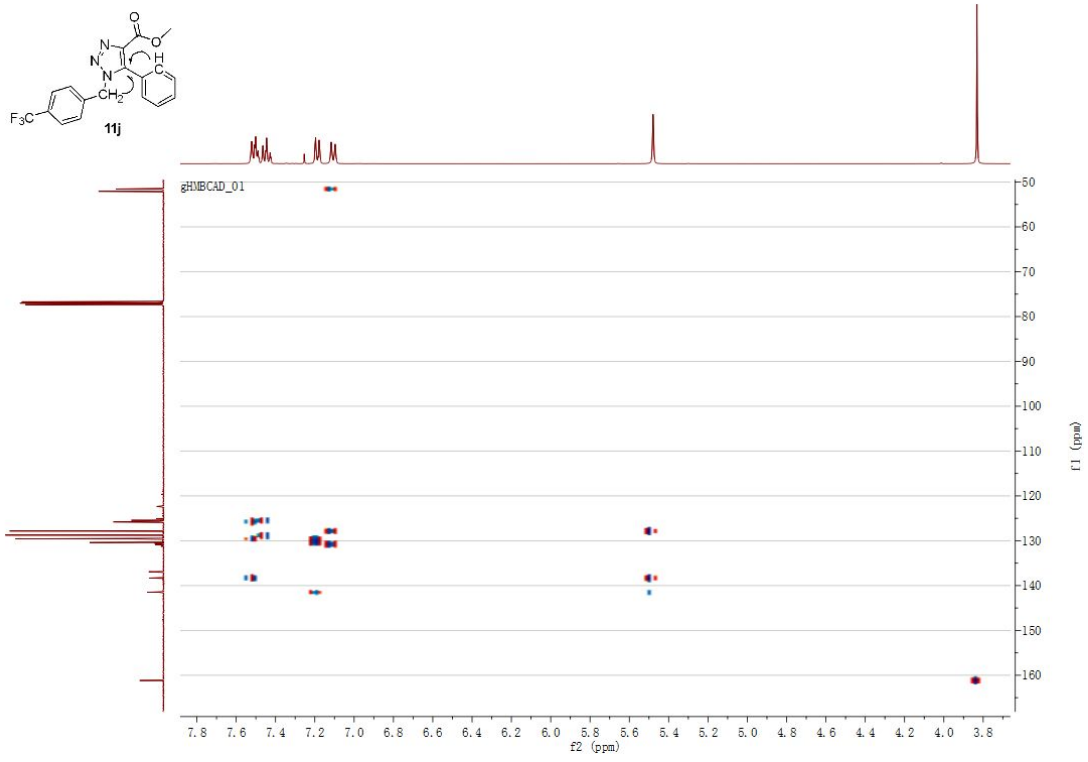
## $^{1}$H NMR of 11j



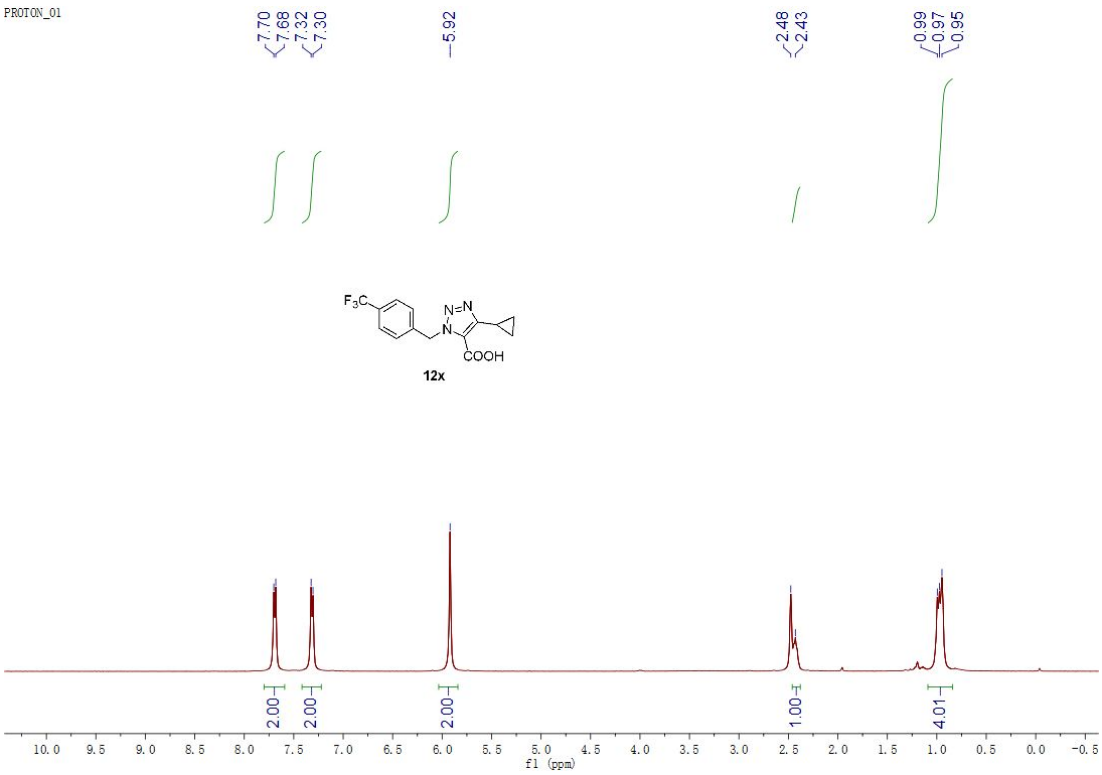

## $^{13}$C NMR of 11j



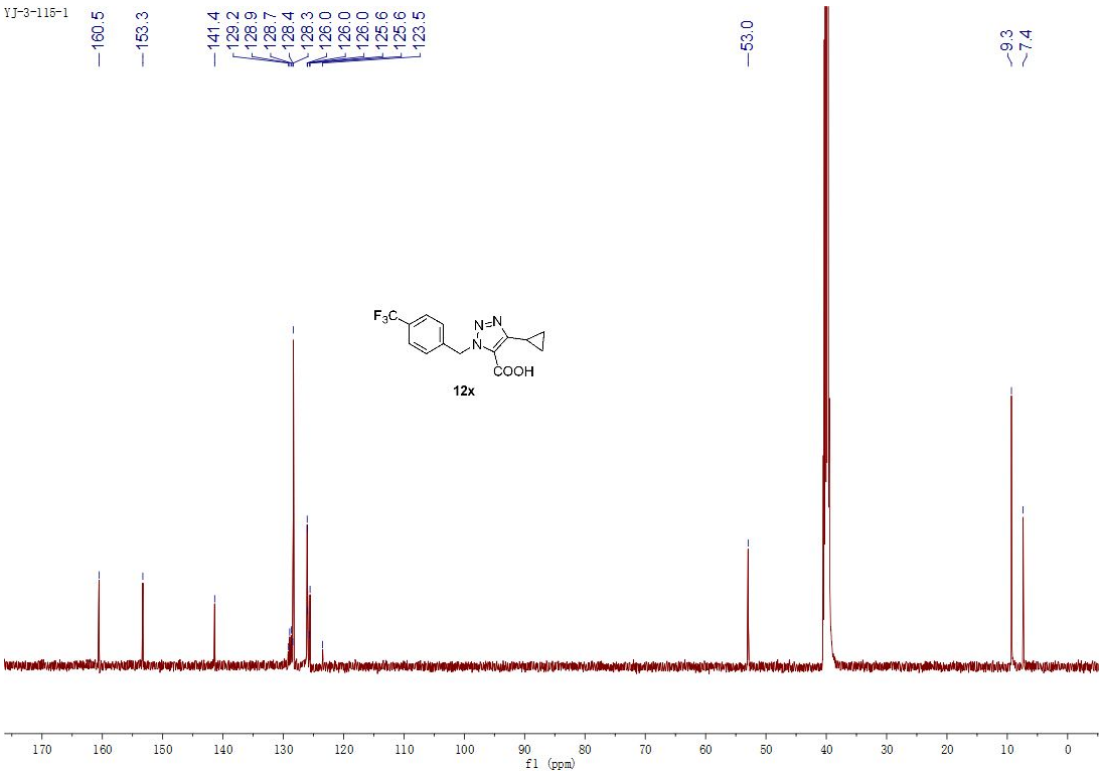
## HMBC of 11j



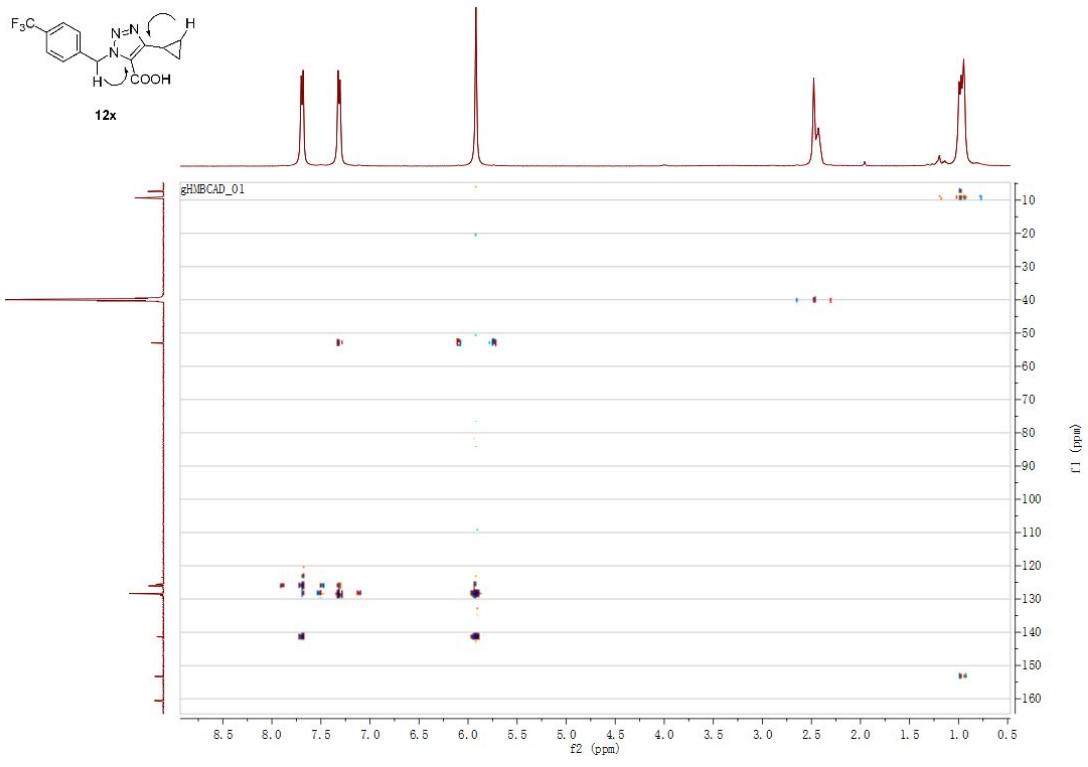
## $^{1}$H NMR of 12x



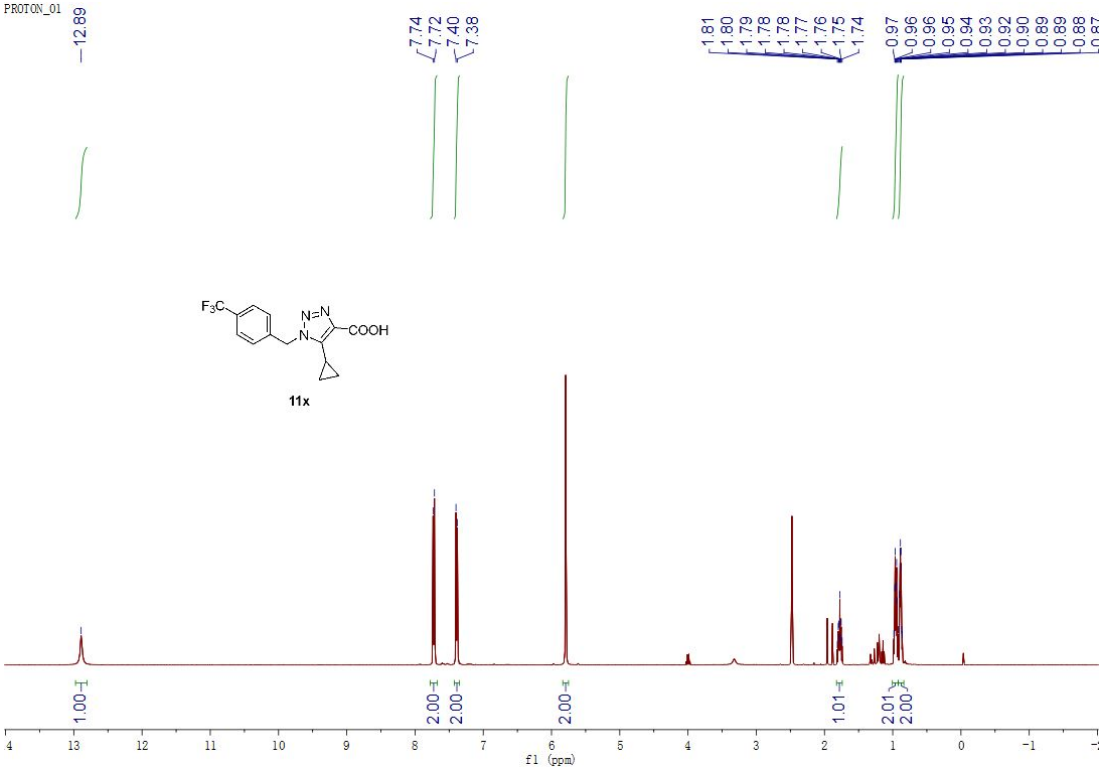

## $^{13}$C NMR of 12x


YJ-3-115-1
160.5
153.3
141.4
129.2
128.9
128.7
128.4
128.3
126.0
126.0
126.0
125.6
125.6
123.5
53.0
9.3
7.4
F3C
N=N
N
COOH
12x
f1 (ppm)

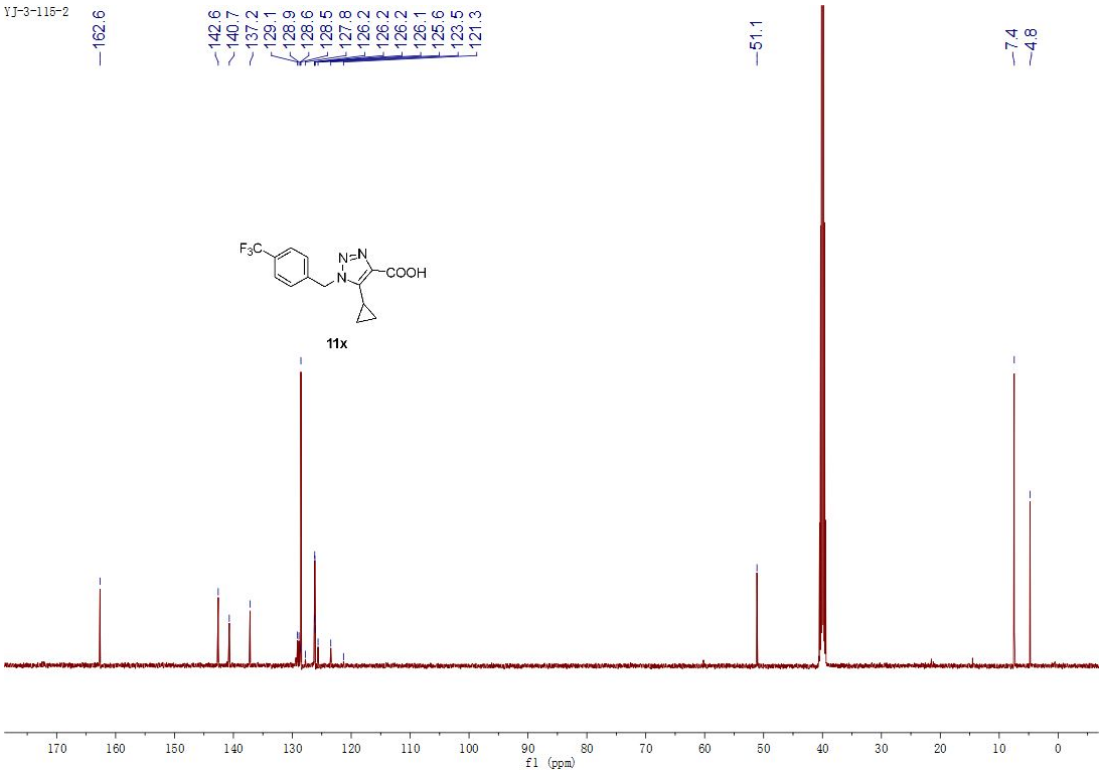
## HMBC of 12x


F3C
N=N
N
H
H
COOH
12x
gHMBCAD_01
f1 (ppm)
f2 (ppm)

## $^{1}$H NMR of 11x




## $^{13}$C NMR of 11x



## HMBC of 11x