Supplementary information

Anti-infective activity of *Salviae miltiorrhizae* against *Staphylococcus aureus* by attenuating accessory gene regulator system-mediated virulence

Jia-Ming Jiang[a,1], Gan Chen[a,1], Yu-Yu Chen[a], Shi-Jie Wan[a], Si-Min Chen[a], Han-Gui Ren[a], Zhi-Xiu Lin[b], Hui Feng[c], Hong Zhang[a,*], and Hong-Xi Xu[d,*]

[a] School of Pharmacy, Shanghai University of Traditional Chinese Medicine, Shanghai, P.R. China

[b] School of Chinese Medicine, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, P.R. China

[c] Shanghai Guanghua Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, P.R. China

[d] Shuguang Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, P.R. China

[1] These two authors contributed equally to this work.

* Corresponding author. E-mail: xuhongxi88@gmail.com; Tel.: +86-21-51323089; Fax: +86-21-51323089 (Hong-Xi Xu); E-mail: zhnjau19851010@163.com (Hong Zhang).

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Part 1 Additional materials and methods

UPLC-PDA-QTOFMS analysis

The fractions or compounds were analyzed by using LC-MS/MS with a Waters ACQUITY UPLC HSS T3 column (2.1 mm × 100 mm I.D., 1.7 μm). The mobile phase A was water with 0.1% formic acid, and the mobile phase B was acetonitrile with 0.1% formic acid. The elution gradient and flow rate were set as follows: 0-5 min: 5% B to 15% B, 5-15 min: 15% B to 22% B, 15-20min: 22% B to 28% B, 20-30min: 28% B to 65% B, 30-35min: 65% B to 90% B with the flow rate of 0.4 mL/min. The column was maintained at 40°C. All fractions or compounds were analyzed using the data-dependent acquisition (DDA) mode. The top five ions were selected for MS/MS from a single MS survey scan.

The desolvation gas was set to 800 L/h at temperature of 400 °C, the cone gas was set to 50 L/h, and the source temperature was set to 120 °C. The capillary voltage was set to 3000 V. Spectra were acquired in centroid and postive mode. Argon was employed as the collision gas. In DDA mode, optimal parameters were set as follows: MS/MS collision energy: 20 V to 50 V, scan time: 0.1 s, data format: centroid. The SYNAPT G2-Si HDMS system was calibrated using sodium formate clusters and operated in resolution mode. The molecular masses of the precursor ion and the product ions were accurately determined using leucine-enkephalin as a reference compound (m/z 556.2771) in the LockSpray mode at a concentration of 50 pg/μL at an infusion flow rate of 5 μL/min.

Part 2 Supplementary Tables

Table S1. Primers used in this study

Primers	Sequence (5'-3')
16S rRNA-F	CCATAAAGTTGTTCTCAGTT
16S rRNA-R	CATGTCGATCTACGATTACT
hla-F	TATTAGAACGAAAGGTACCA
hla-R	ACTGTACCTTAAAGGCTGAA
RNAIII-F	TTCACTGTGTCGATAATCCA
RNAIII-R	GGAAGGAGTGATTTCAATGG
psmα-F	TATCAAAAGCTTAATCGAACAATT C
psmα-R	CCCCTTCAAATAAGATGTTCATAT C
agrA-F	TGATAATCCTTATGAGGTGCTT
agrA-R	CACTGTGACTCGTAACGAAAA
agrB-F	CCCATTCCTGTGCGACTTAT
agrB-R	TTGAATGAATTGGGCAAATG
agrC-F	CATTCGCGTTGCATTTATTG
agrC-R	CCTAAACCACGACCTTCACC
agrD-F	ACATTGGTAACATCGCAGCTTA
agrD-R	CGTGTAATTGTGTTAATTCTTTTGG

Part 3 Supplementary Figures

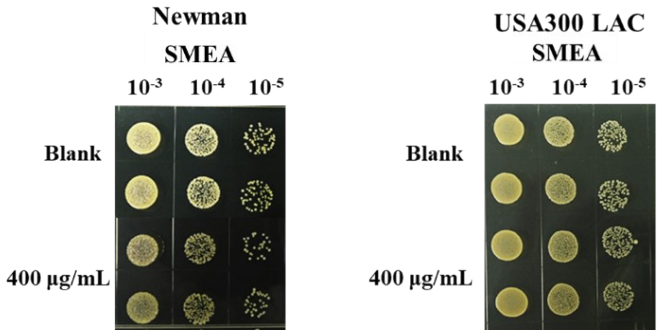

Fig. S1. SMEA is not toxic to *S. aureus*. Mid-log culture (OD_{600} = 0.3) was treated with SMEA (400 μg/mL) for 3 h at 37 °C. The resulting cultures were serially diluted with 9 volumes of PBS and plated on TSA.

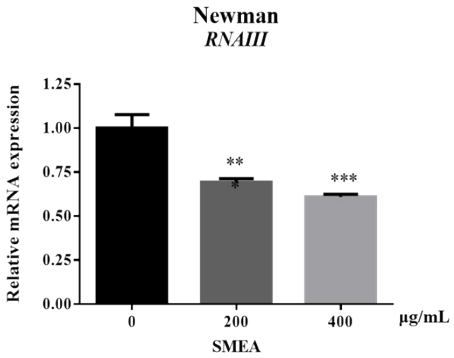

Fig. S2. SMEA inhibits the transcription of *RNAIII* in *S. aureus* Newman strain.

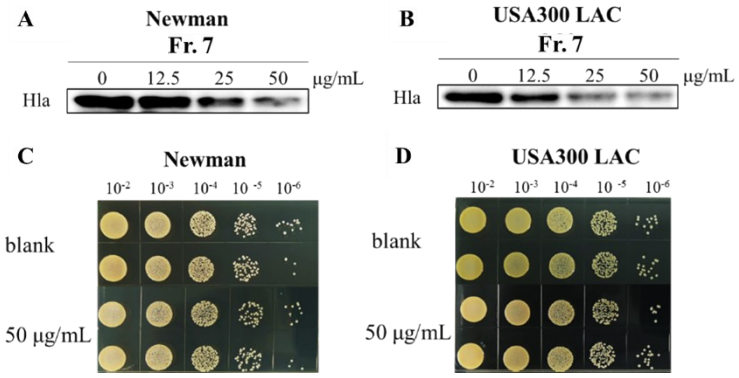

Fig. S3. Fr. 7 decreases the virulence expression in *S. aureus*. Western blotting analysis showing that Fr. 7 inhibits the production of Hla in both Newman (A) and USA300 LAC (B) strains. SMEA is not toxic to *S. aureus* Newman (C) and USA300 LAC (D) strains. Mid-log culture (OD_{600} = 0.3) was treated with Fr. 7 (50 μg/mL) for 3 h at 37 °C. The resulting cultures were serially diluted with 9 volumes of PBS and plated on TSA.

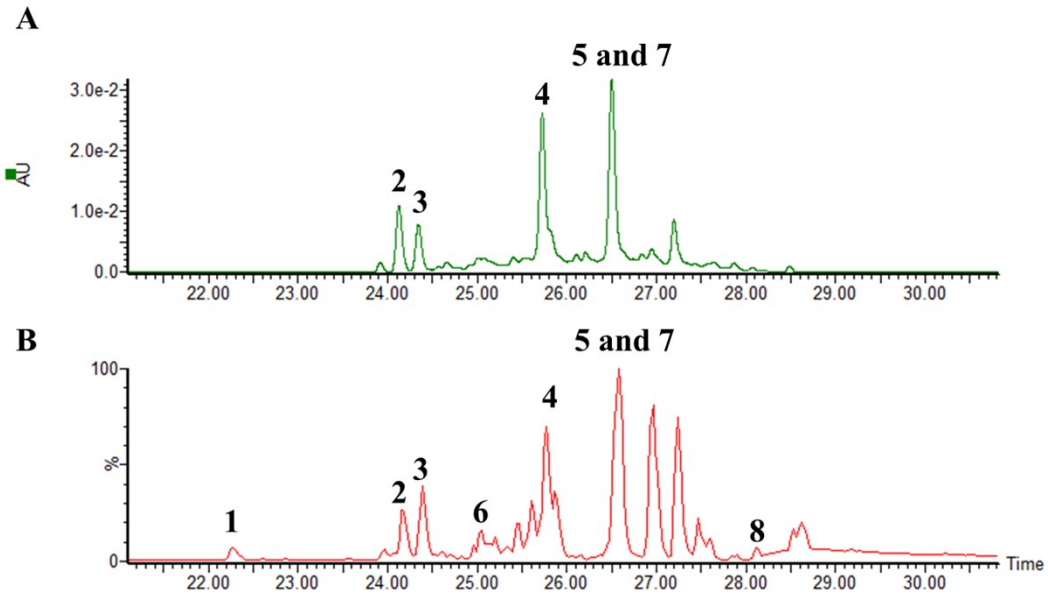

Fig. S4. UHPLC-PDA-QTOF-MS analysis of active fraction Fr. 7. (A) UV chromatogram of Fr. 7. (B) Total ion chromatogram of Fr. 7.

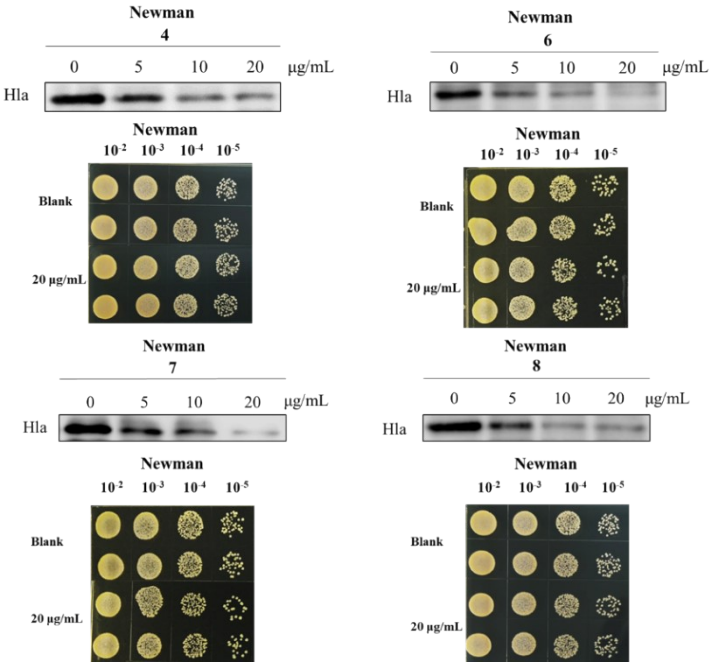

Fig. S5. Compounds **4**, **6**, **7**, **8** obviously inhibit the Hla expression in *S. aureus* at 32 μM.

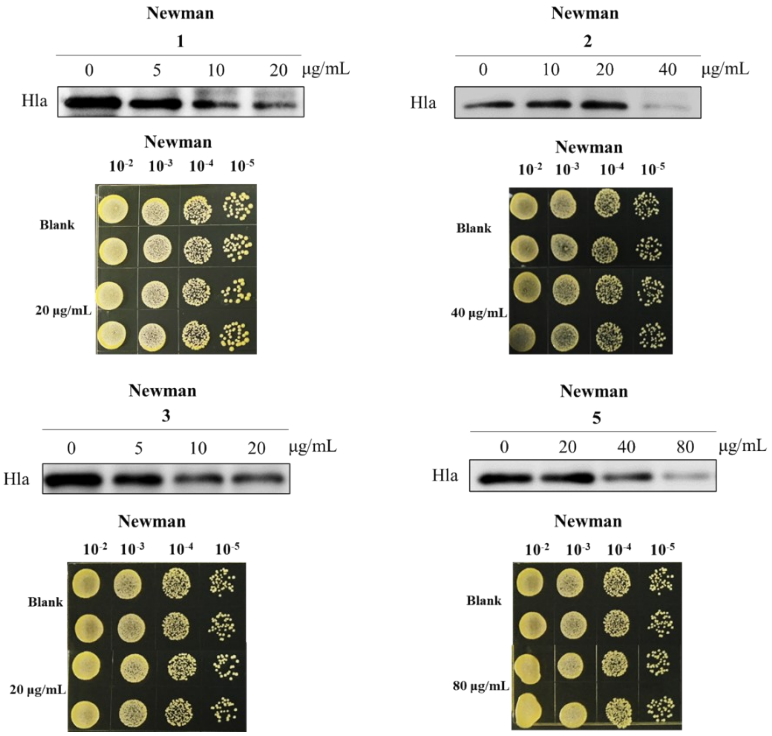

Fig. S6. Compounds **1**, **2**, **3**, **5** show limited inhibition of the Hla expression in *S. aureus*.

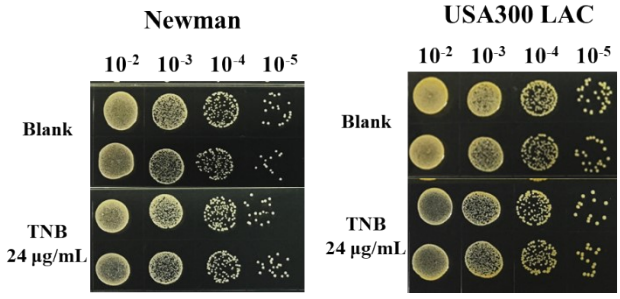

Fig. S7. TNB is not toxic to *S. aureus*. Mid-log culture (OD_{600} = 0.3) was treated with TNB (24 μg/mL) for 3 h at 37 °C. The resulting cultures were serially diluted with 9 volumes of PBS and plated on TSA.

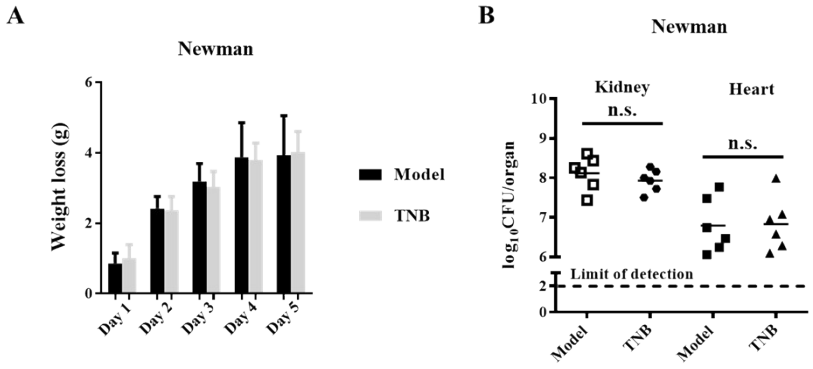

Fig. S8. Protective effects of tanshinone IIB (5 mg/kg/d) on mice (n = 6) intravenously challenged with 4×10^6 CFU of bacteria. (A) Mean body weight loss during the course of experiment. (B) Statistical analysis of enumeration of colony forming units. Mann-Whitney test, two-tailed. Horizontal bars indicate observational means and dashed line indicates limit of detection.

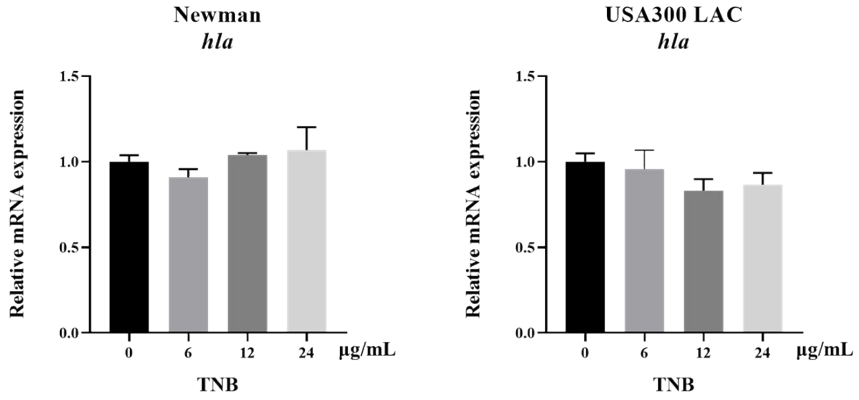

Fig. S9. TNB has little effect on the transcriptional level of *hla* in the Newman and USA300 LAC strains.

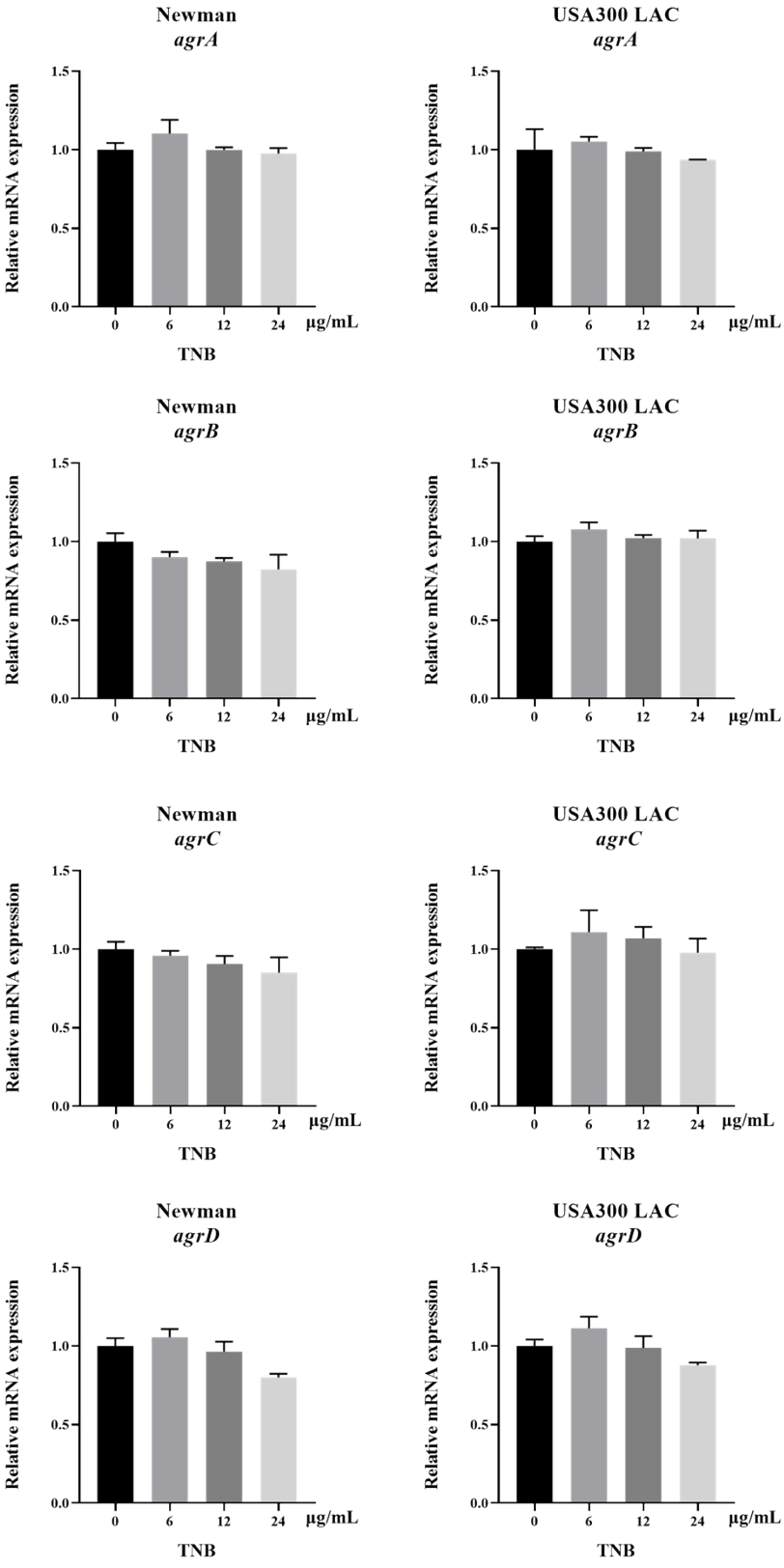
Newman
agrA
USA300 LAC
agrA
Newman
agrB
USA300 LAC
agrB
Newman
agrC
USA300 LAC
agrC
Newman
agrD
USA300 LAC
agrD
Relative mRNA expression
1.5
1.0
0.5
0.0
0
6
12
24
μg/mL
TNB

Fig. S10. TNB has little effect on the transcriptional level of *agrA-D* in the Newman and USA300 LAC strains.

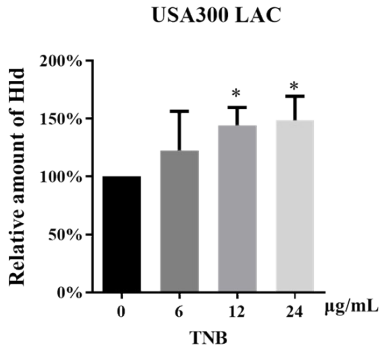

Fig.S11. TNB increases the expression of Hld. *P < 0.05, in comparison with control, one-way ANOVA.

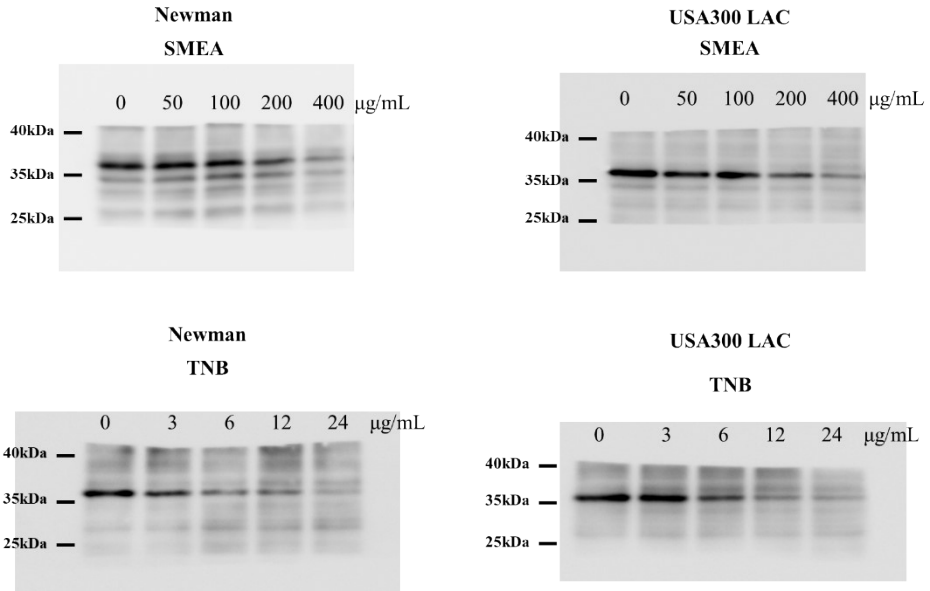

Fig.S12. Full-length blots of Hla protein were presented when Newman, and USA300 LAC were treated with different concentrations of SMEA or TNB. One representative image of each protein is showed and experiments were performed at least three times.

Part 4 NMR and HRESIMS spectra for compounds 1-8

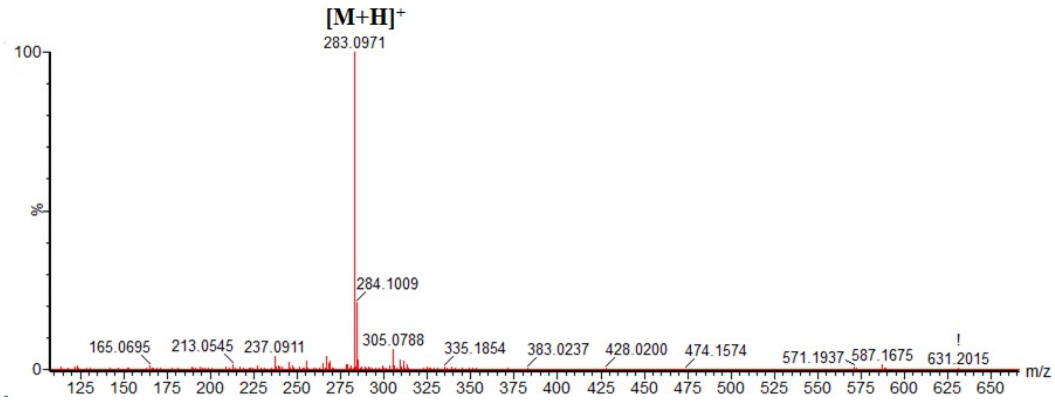

Fig. S13. HRESIMS spectrum of **1**

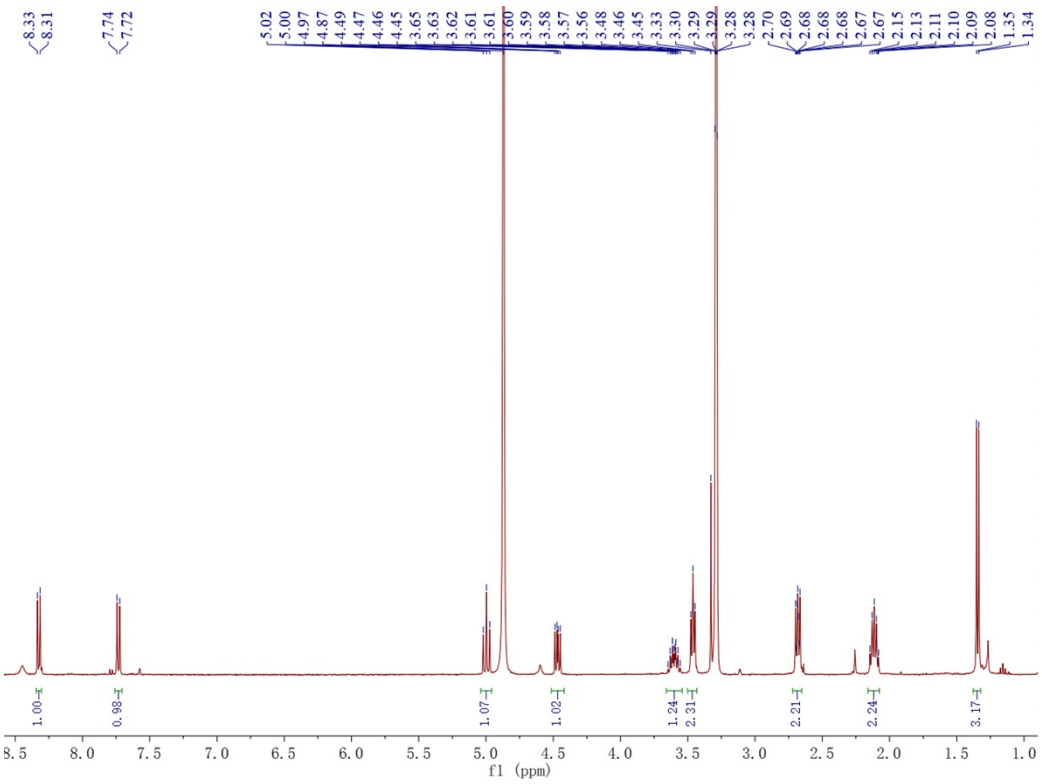

Fig. S14. ^{1}H NMR spectrum of **1** (in CD_3OD)

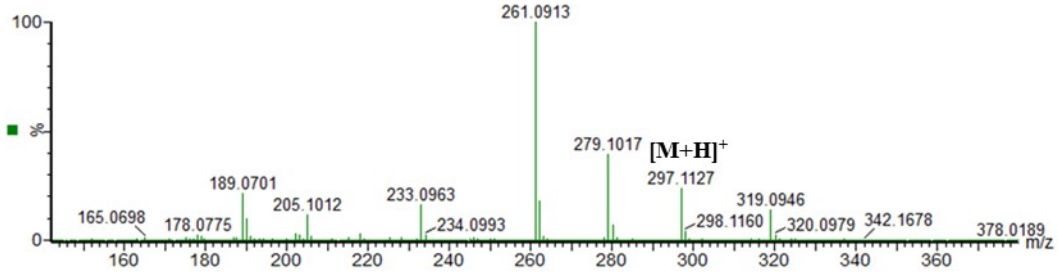

Fig. S15. HRESIMS spectrum of **2**

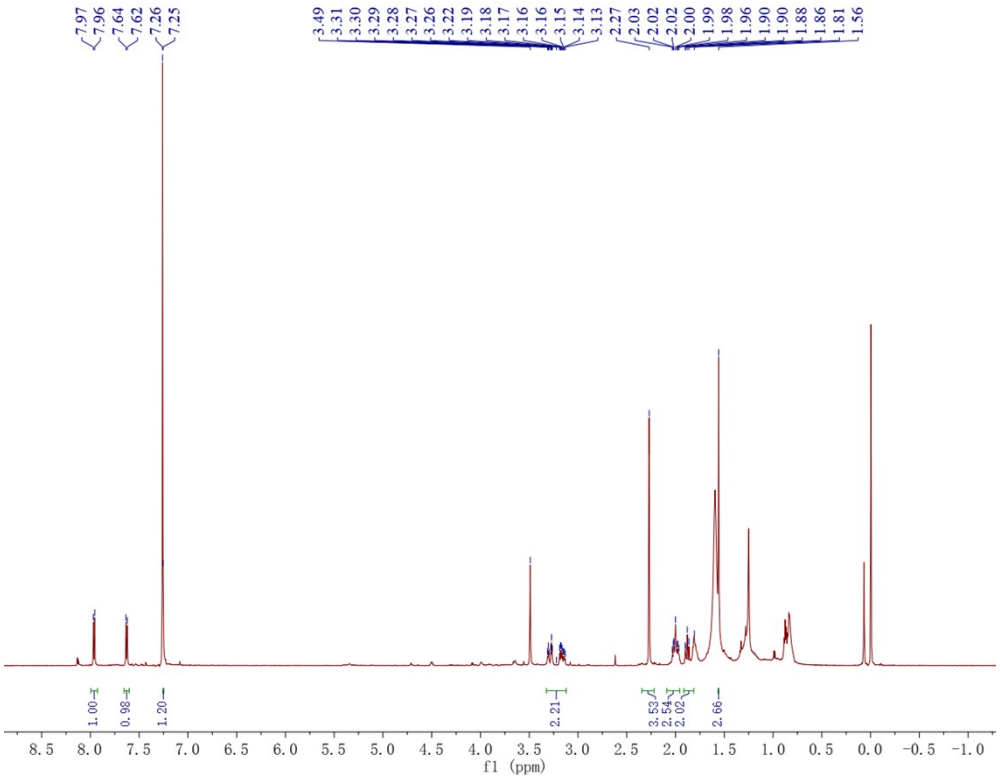

Fig. S16. ^{1}H NMR spectrum of **2** (in $CDCl_3$)

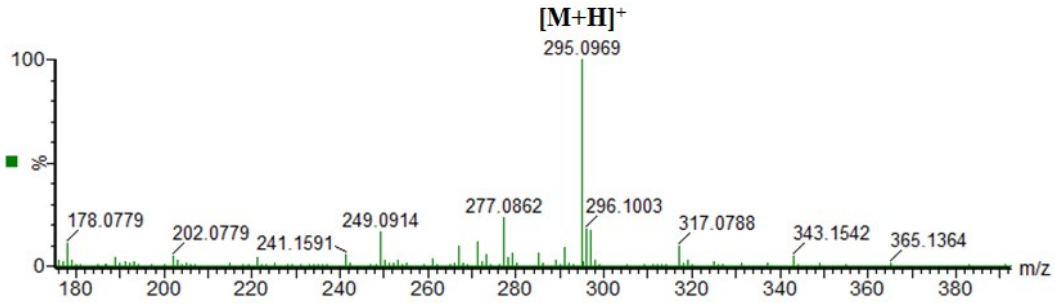

Fig. S17. HRESIMS spectrum of **3**

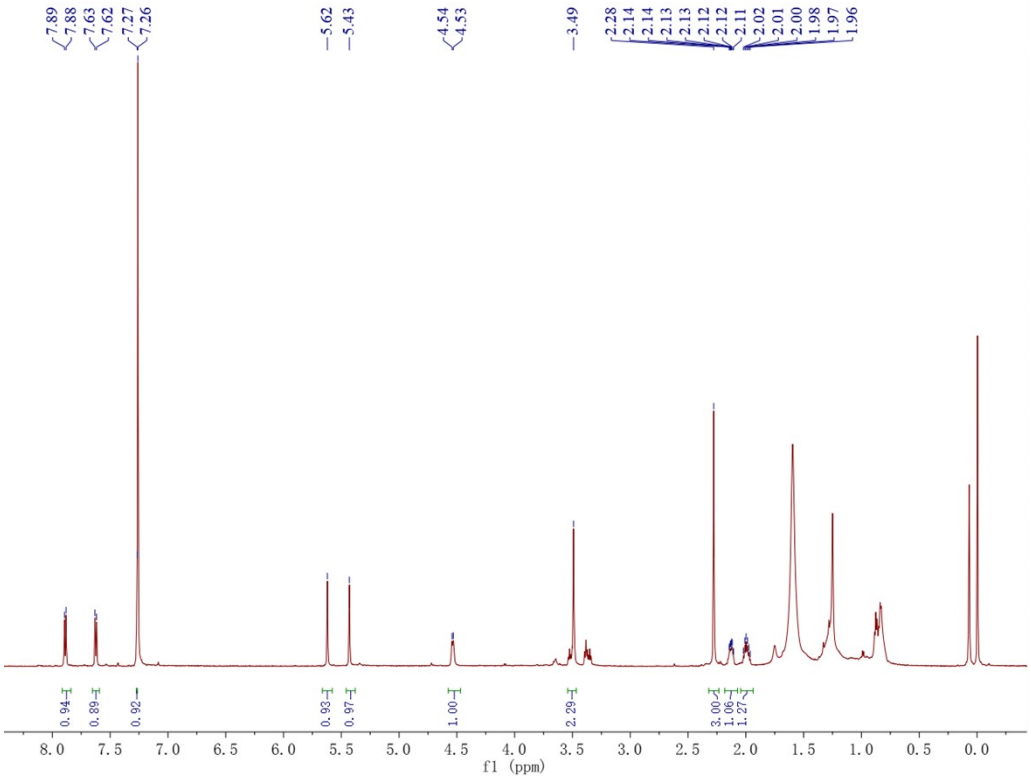

Fig. S18. ^{1}H NMR spectrum of **3** (in $CDCl_3$)

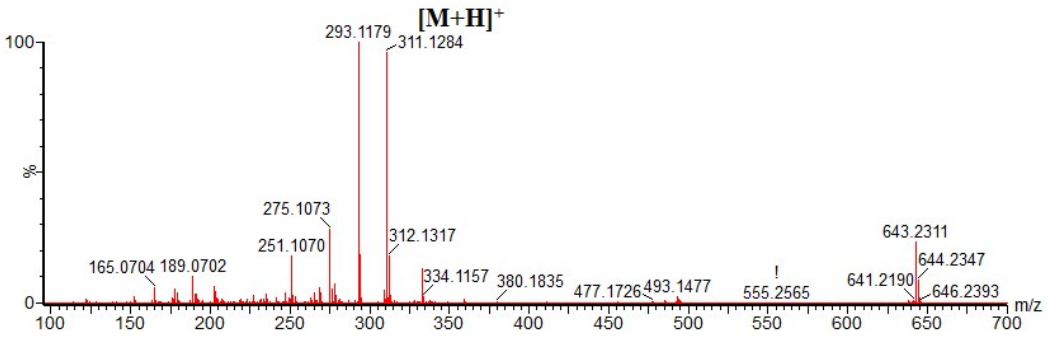

Fig. S19. HRESIMS spectrum of **4**

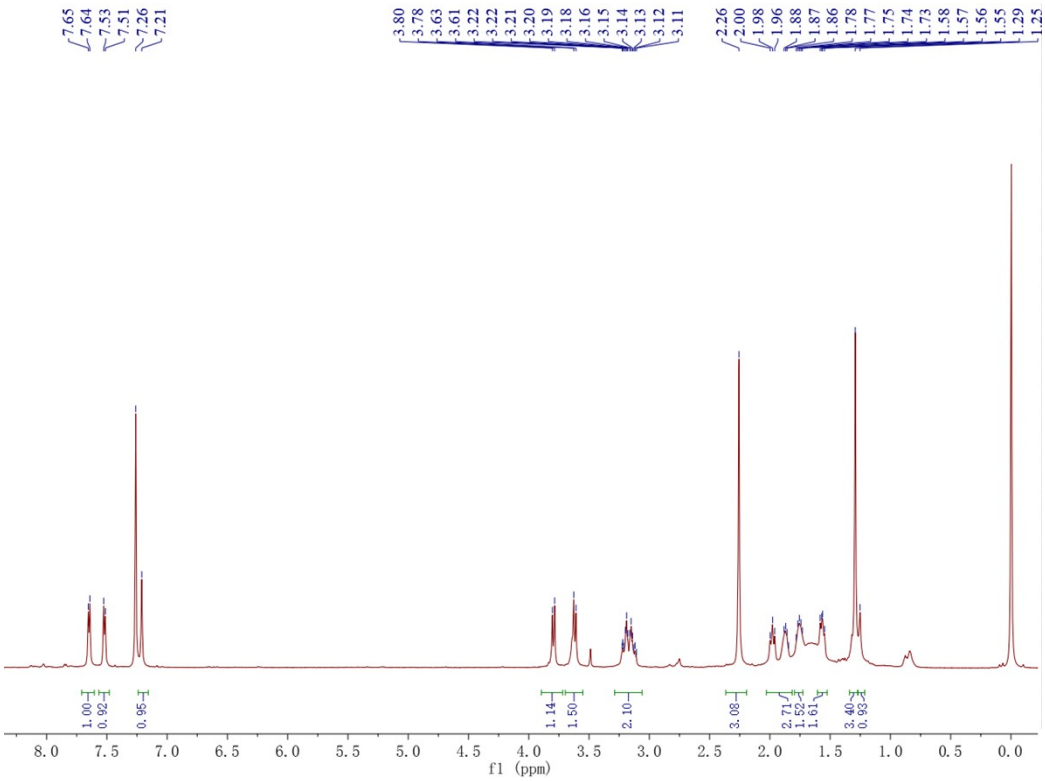

Fig. S20. ^{1}H NMR spectrum of **4** (in CD_3OD)

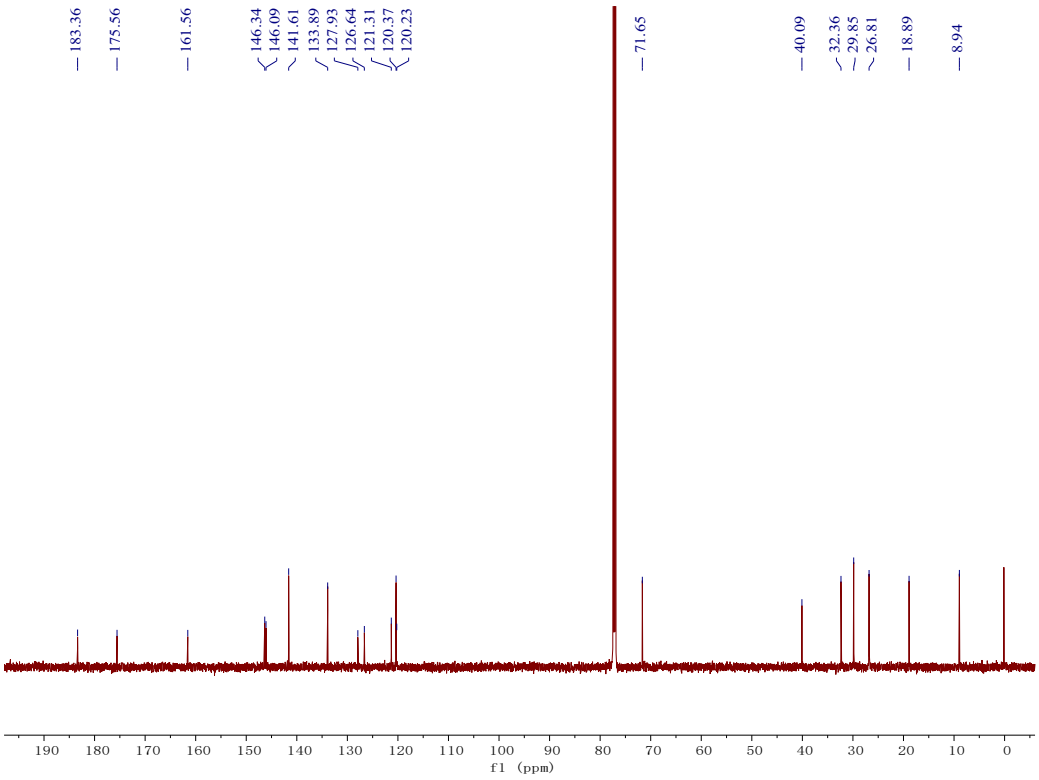

Fig. S21. ^{13}C NMR spectrum of **4** (in $CDCl_3$)

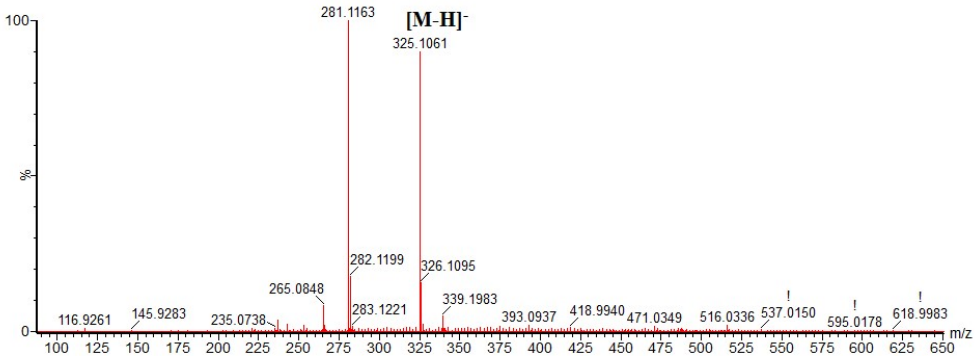

Fig. S22. HRESIMS spectrum of **5**

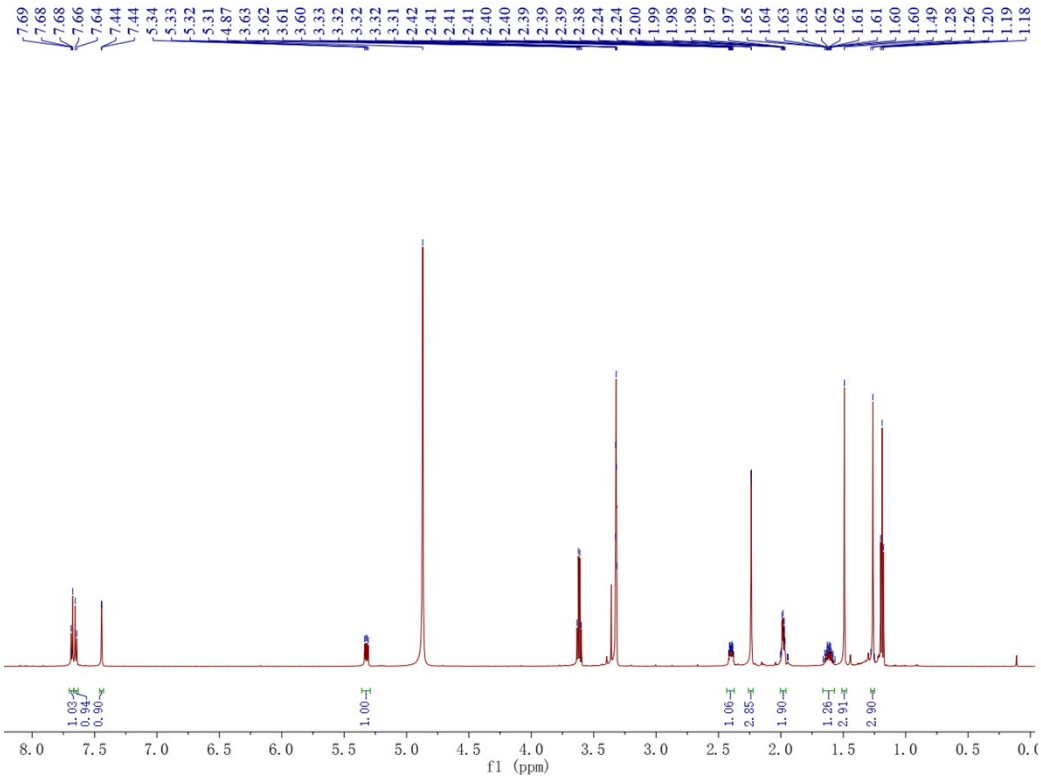

Fig. S23. ^{1}H NMR spectrum of **5** (in CD_3OD)

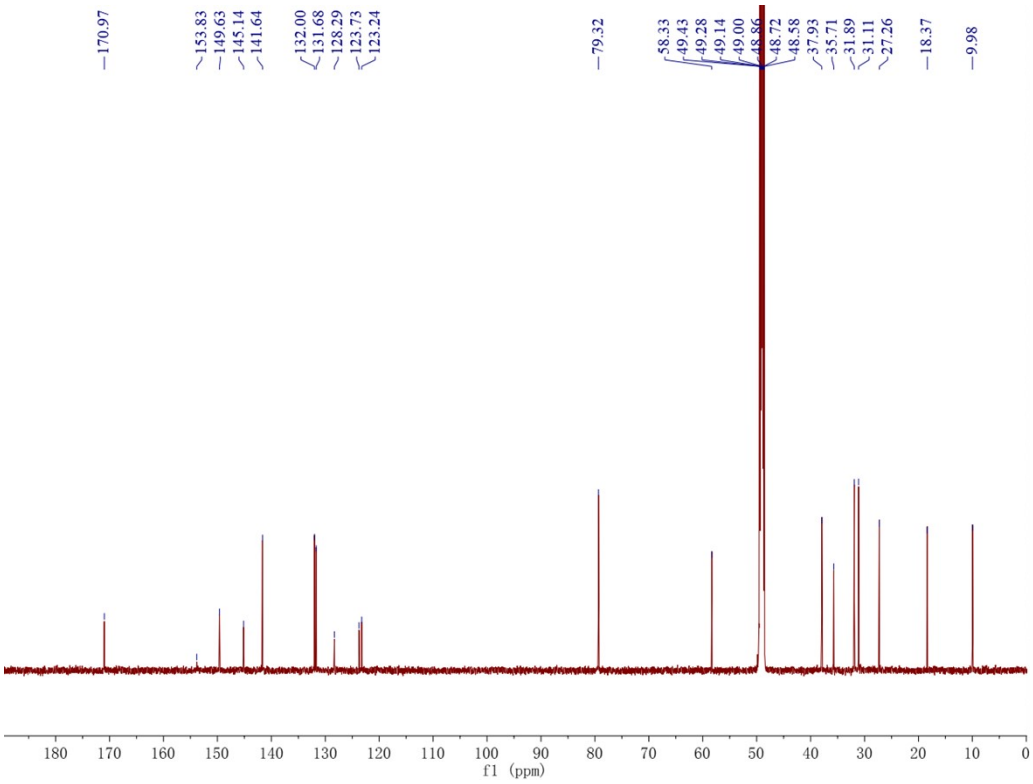

Fig. S24. ^{13}C NMR spectrum of **5** (in CD_3OD)

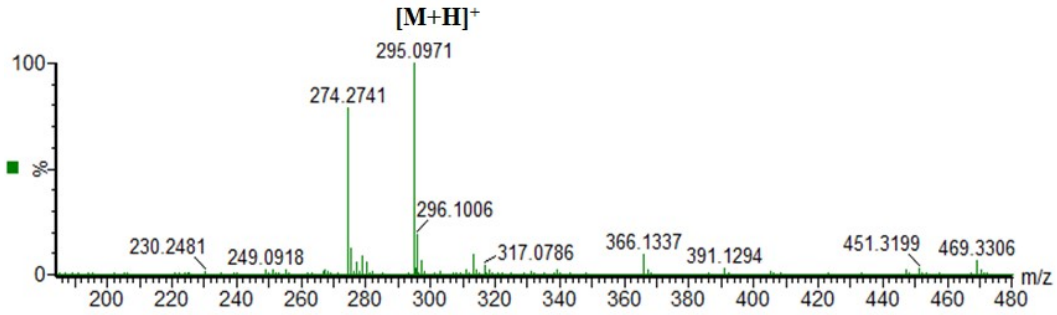

Fig. S25. HRESIMS spectrum of **6**

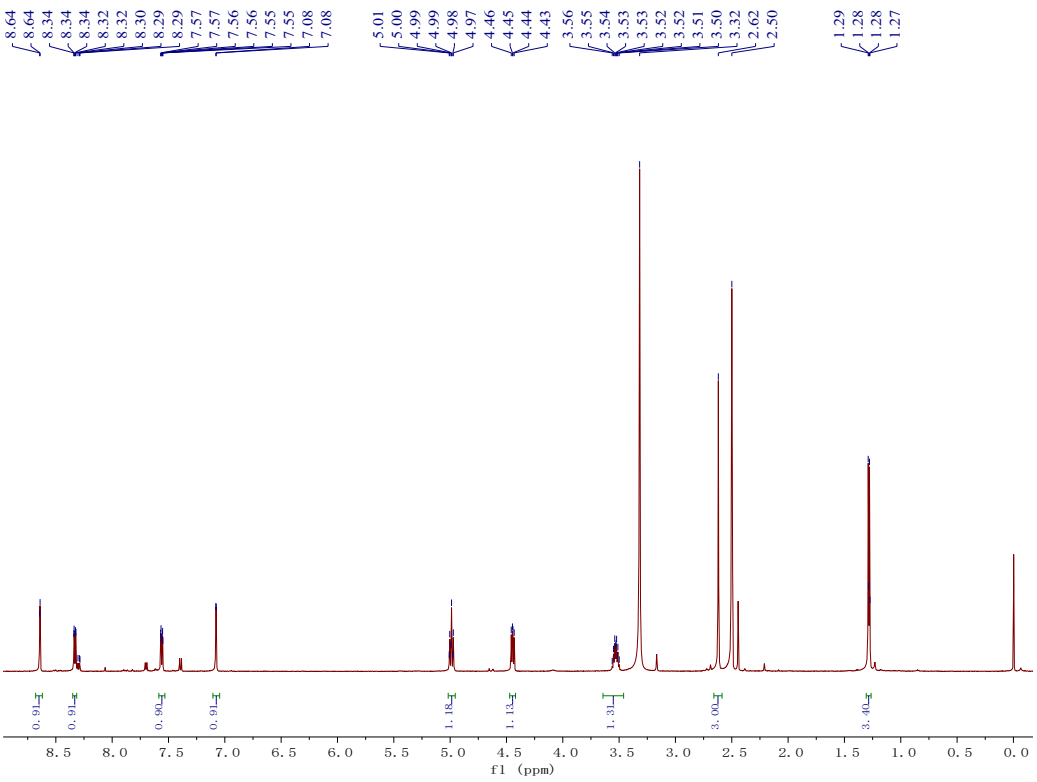

Fig. S26. ^{1}H NMR spectrum of **6**(in DMSO-d_6)

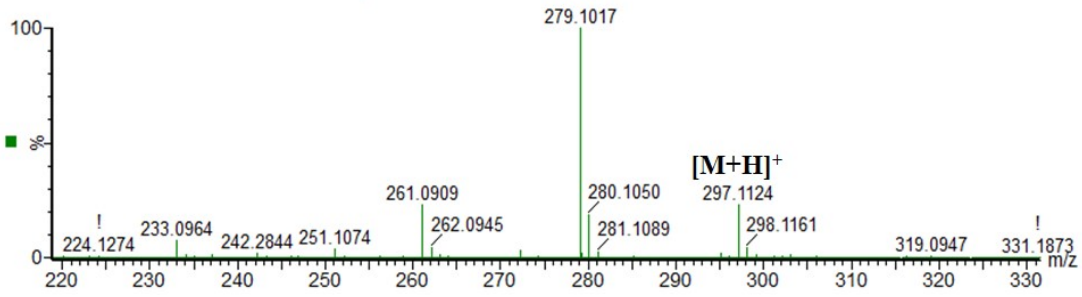

Fig. S27. HRESIMS spectrum of **7**

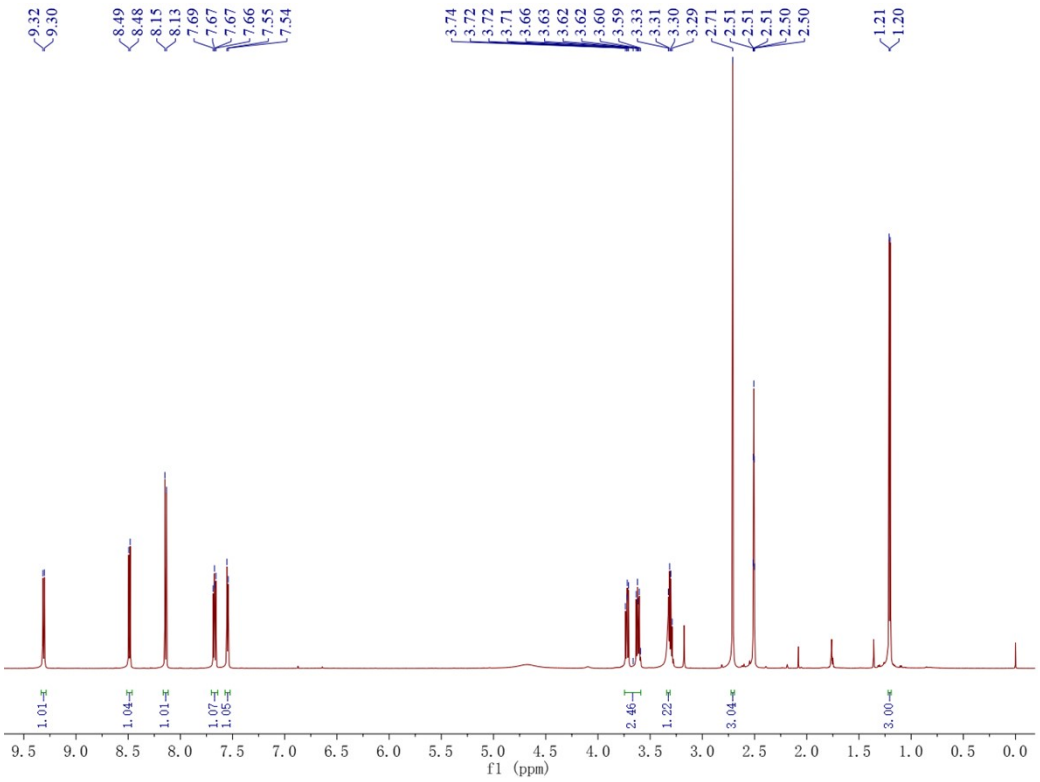

Fig. S28. ^{1}H NMR spectrum of **7** (in DMSO-d_6)

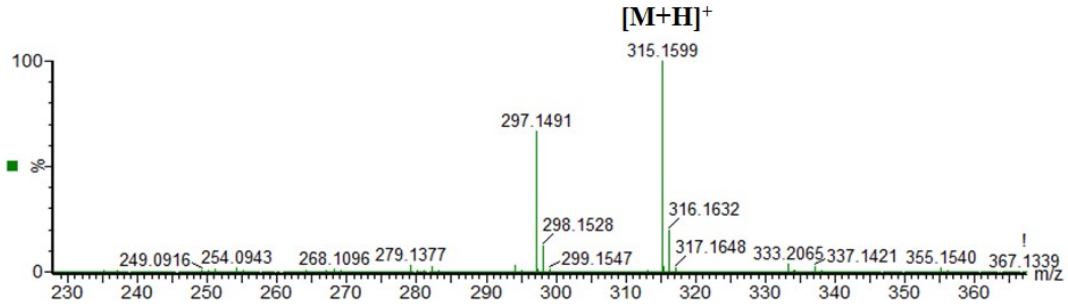

Fig. S29. HRESIMS spectrum of **8**

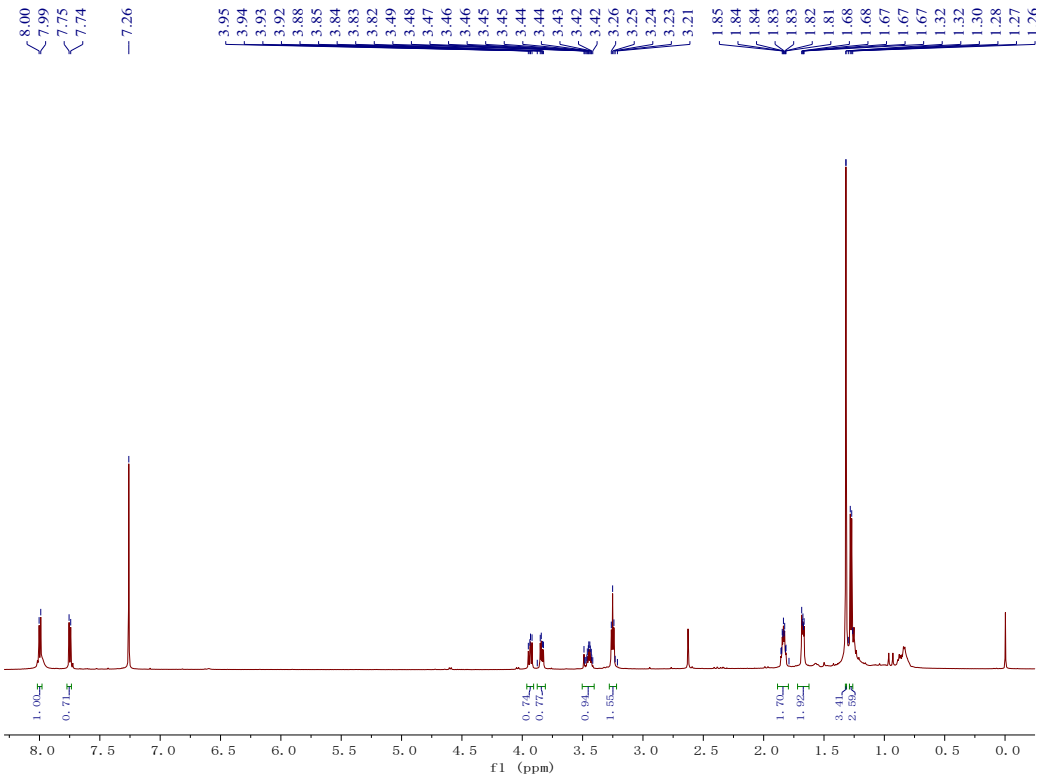

Fig. S30. ^{1}H NMR spectrum of **8** (in $CDCl_3$)

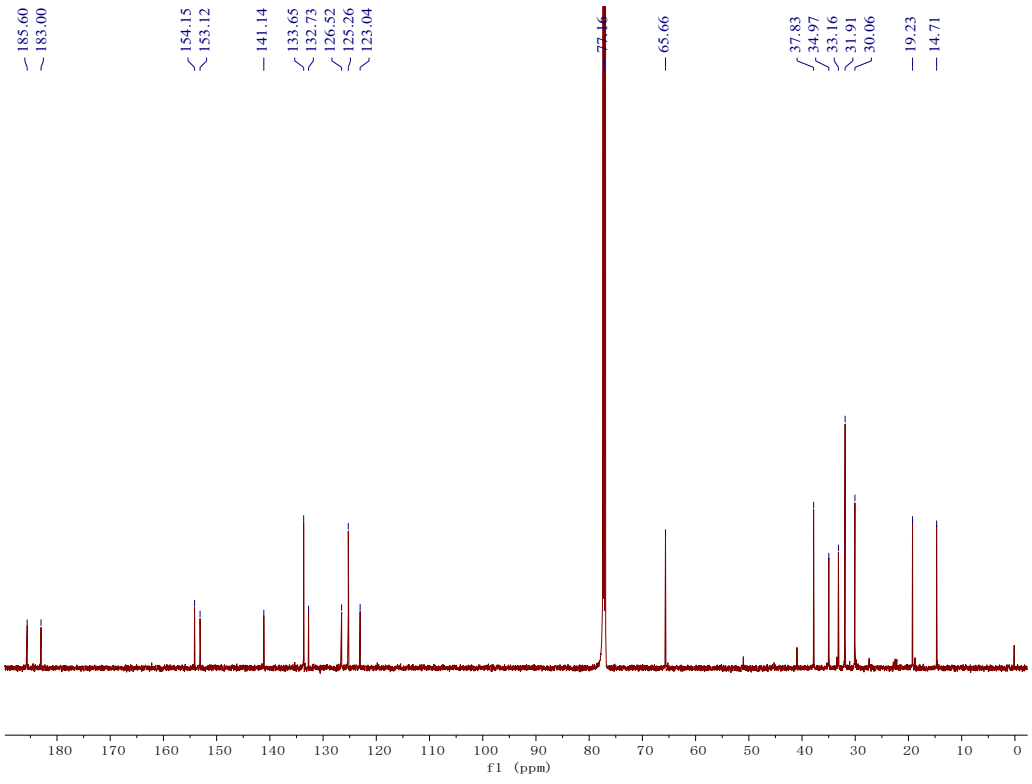

Fig. S31. ^{13}C NMR spectrum of **8** (in $CDCl_3$)