Supporting Information

Microbial glycosylation of tanshinone IIA by *Cunninghamella elegans* AS 3.2028

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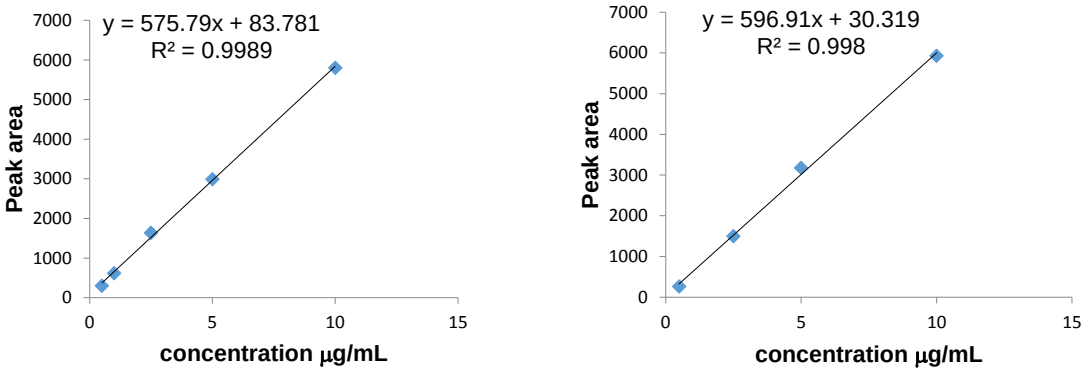


Figure S1. Calibration curves for solubility test of TIIA and **1**.

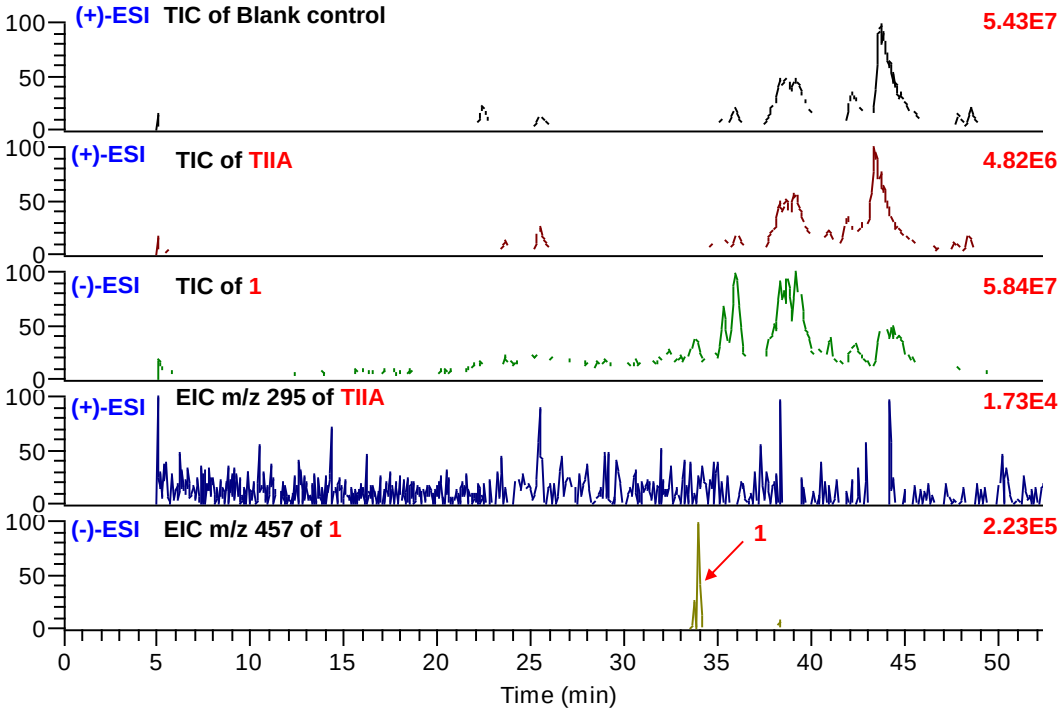


Figure S2. Total ion chromatograms (TIC) and extracted ion chromatograms (EIC) of TIIA and **1** in mice plasma after oral administration.

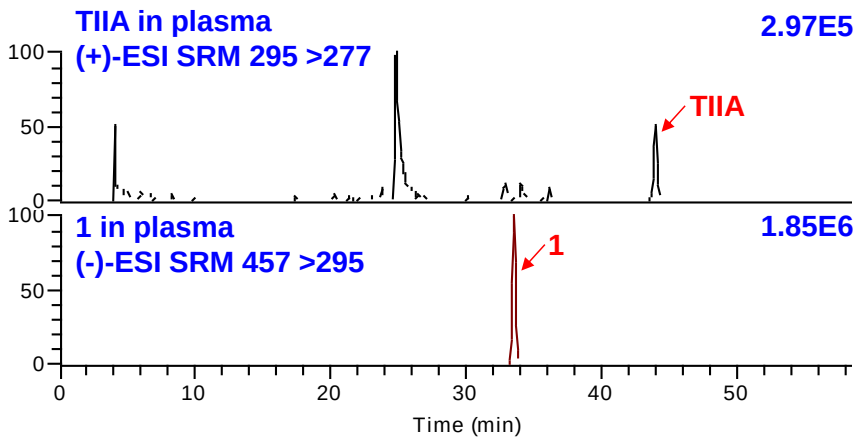
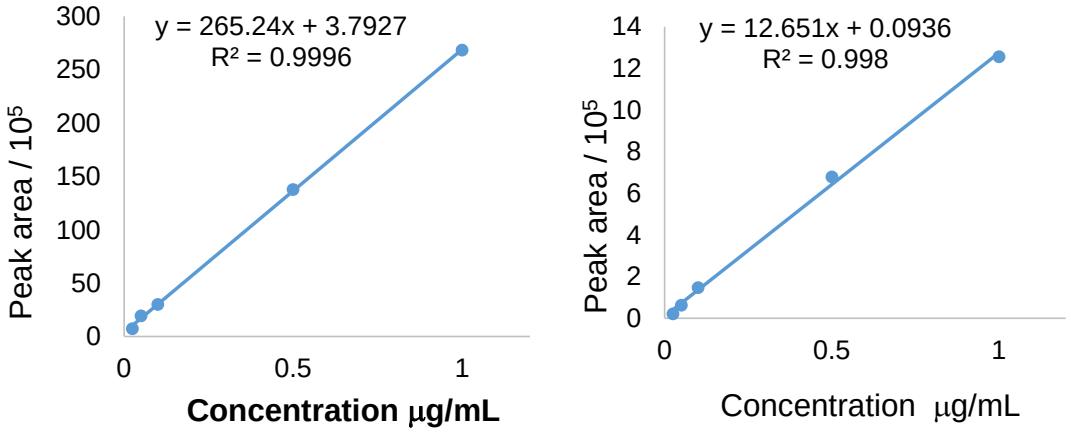


Figure S3. Selected reaction monitoring (SRM) chromatograms of TIIA and **1** in mice plasma after oral administration.



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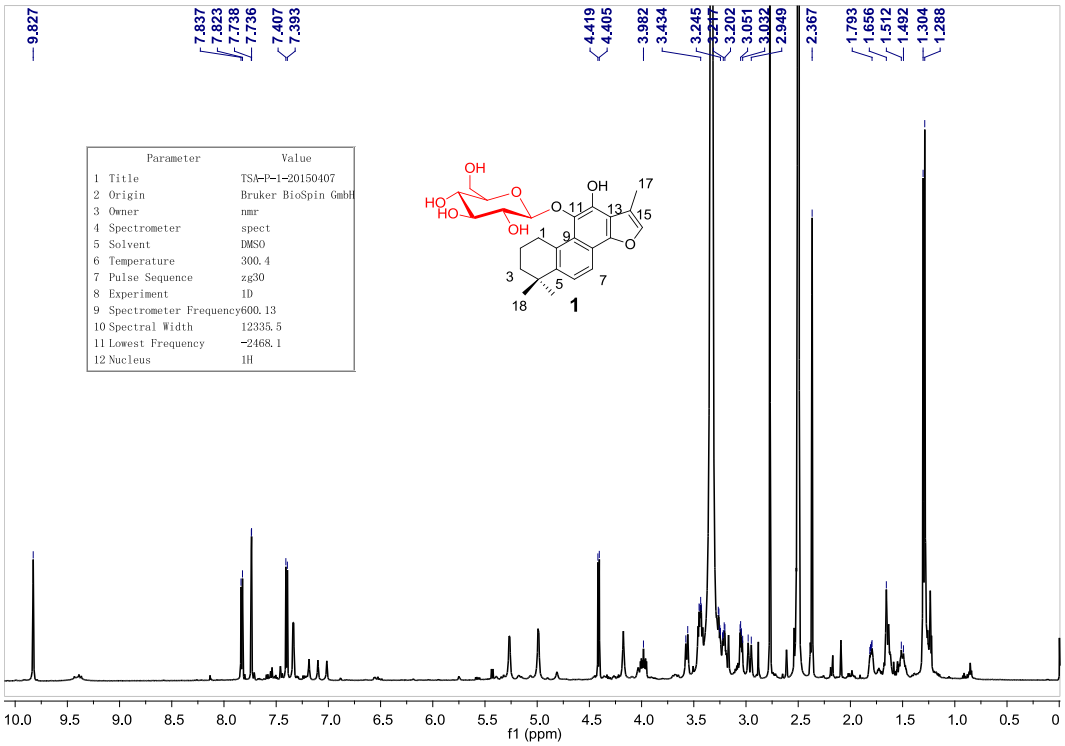


Figure S5. ^{1}H NMR spectrum of **1** (600 MHz, DMSO-d_6).

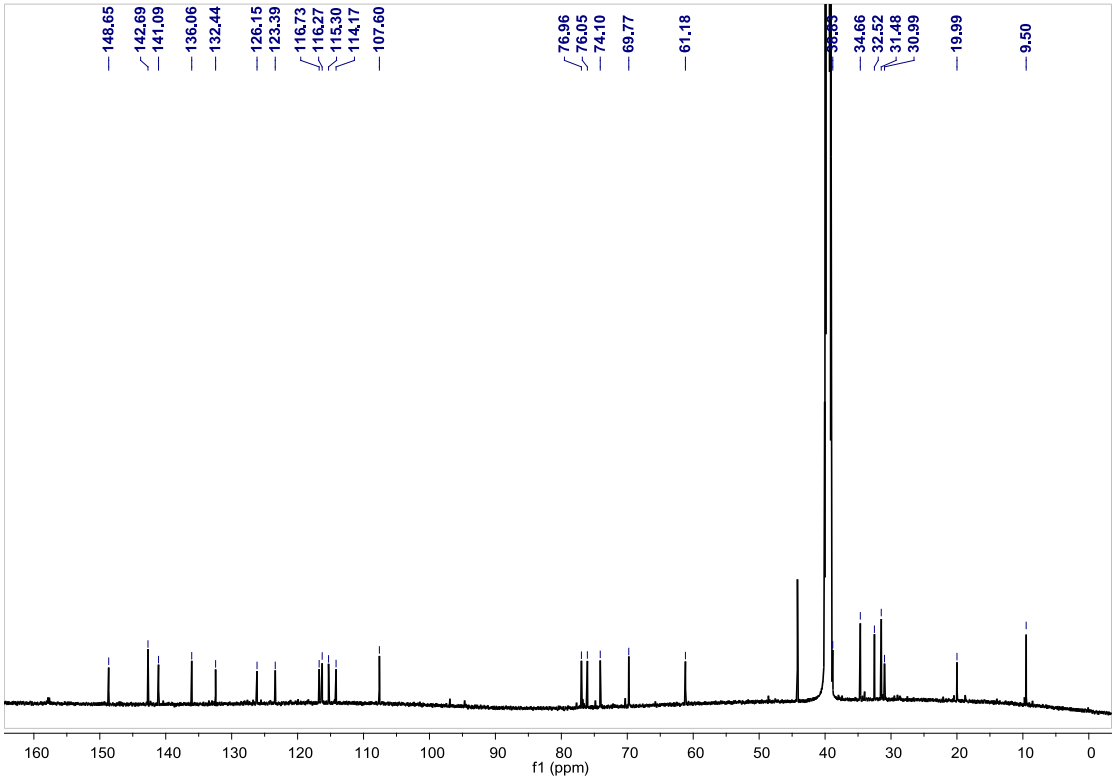
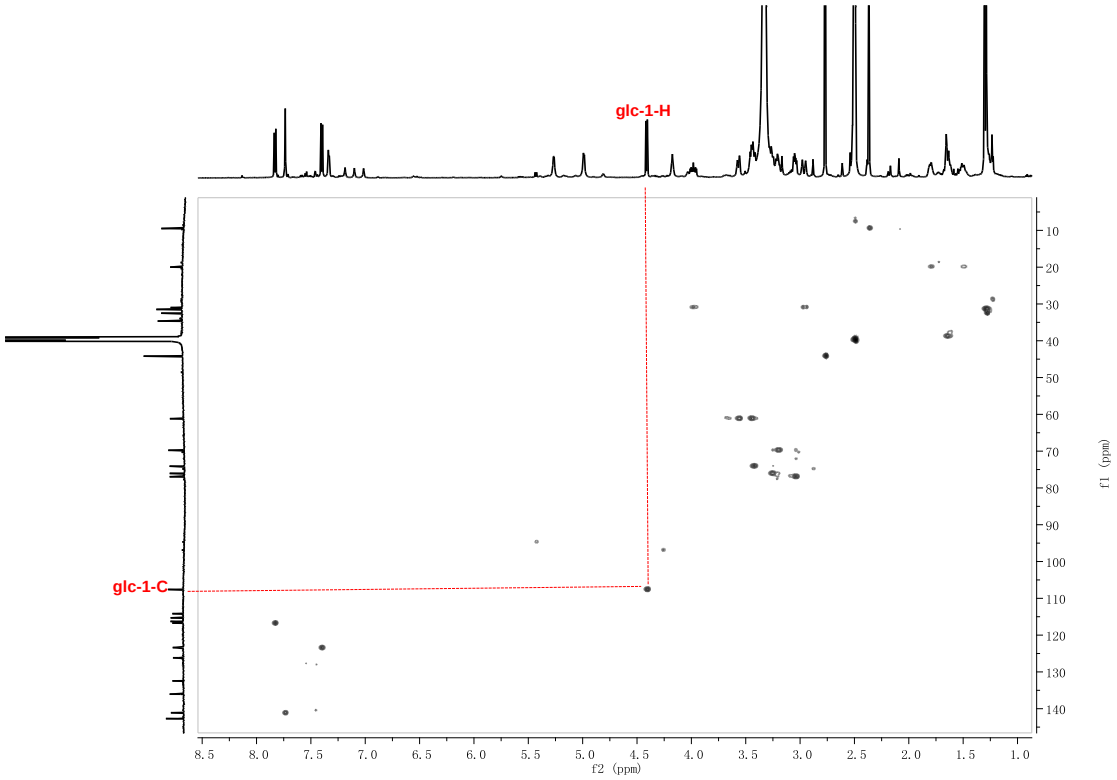


Figure S6. ^{13}C NMR spectrum of **1** (150 MHz, DMSO-d_6).



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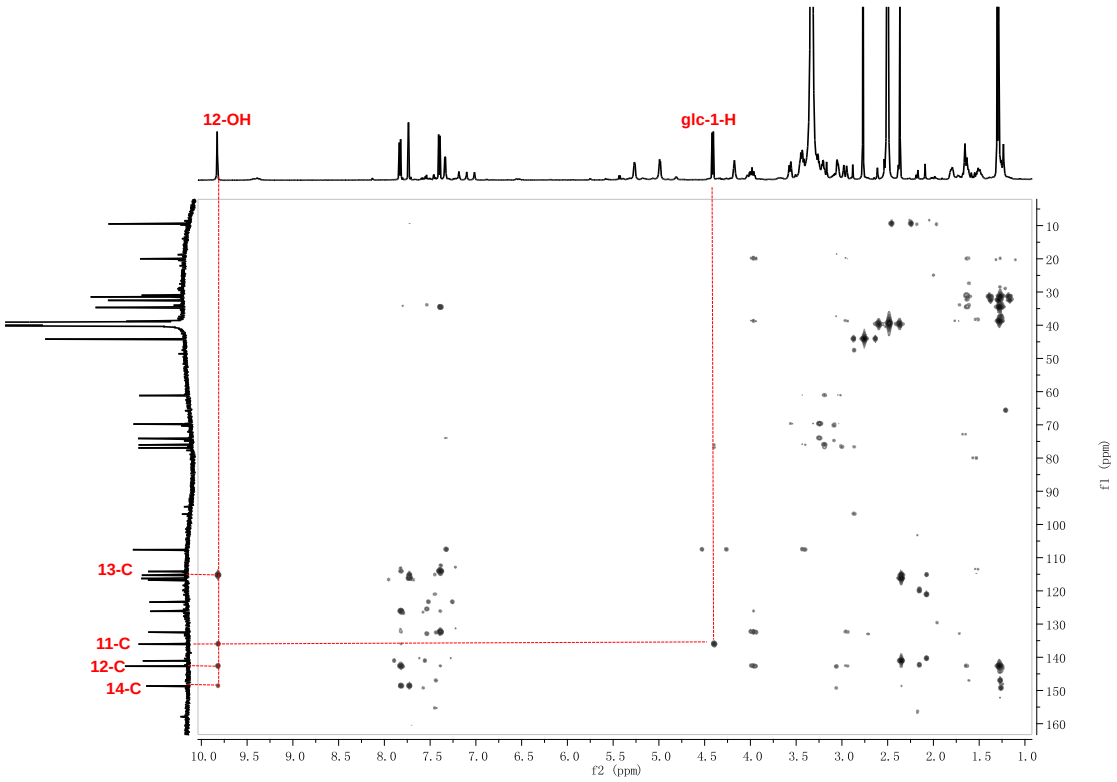

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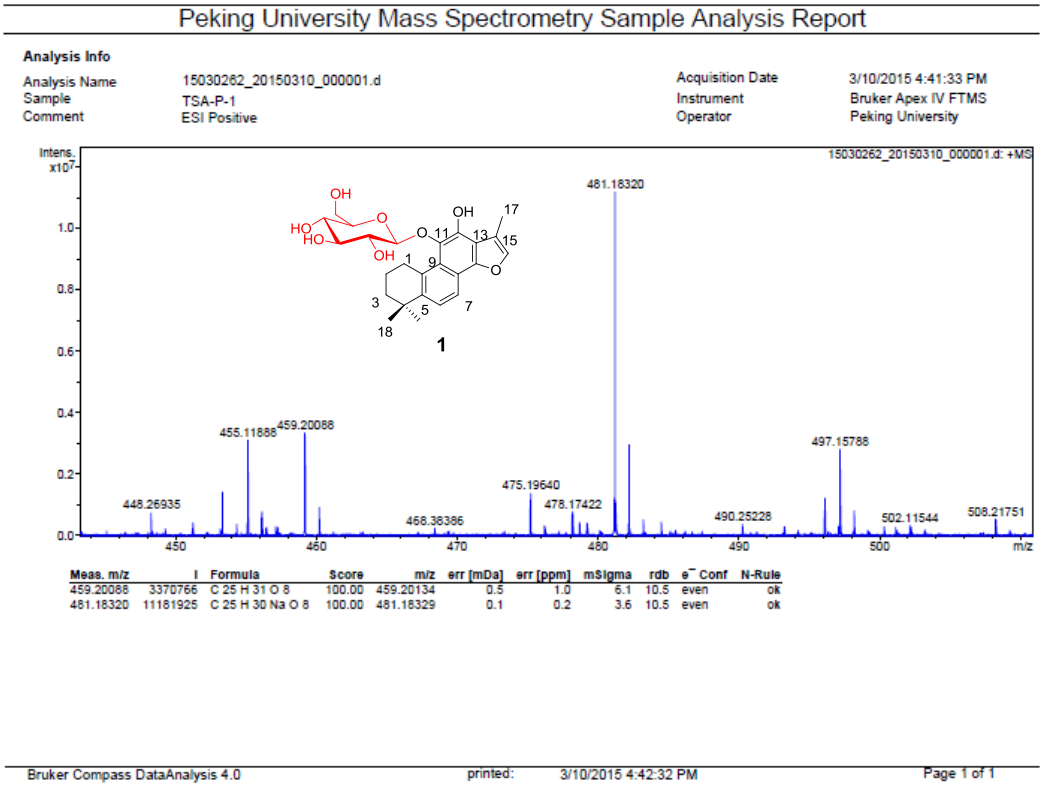

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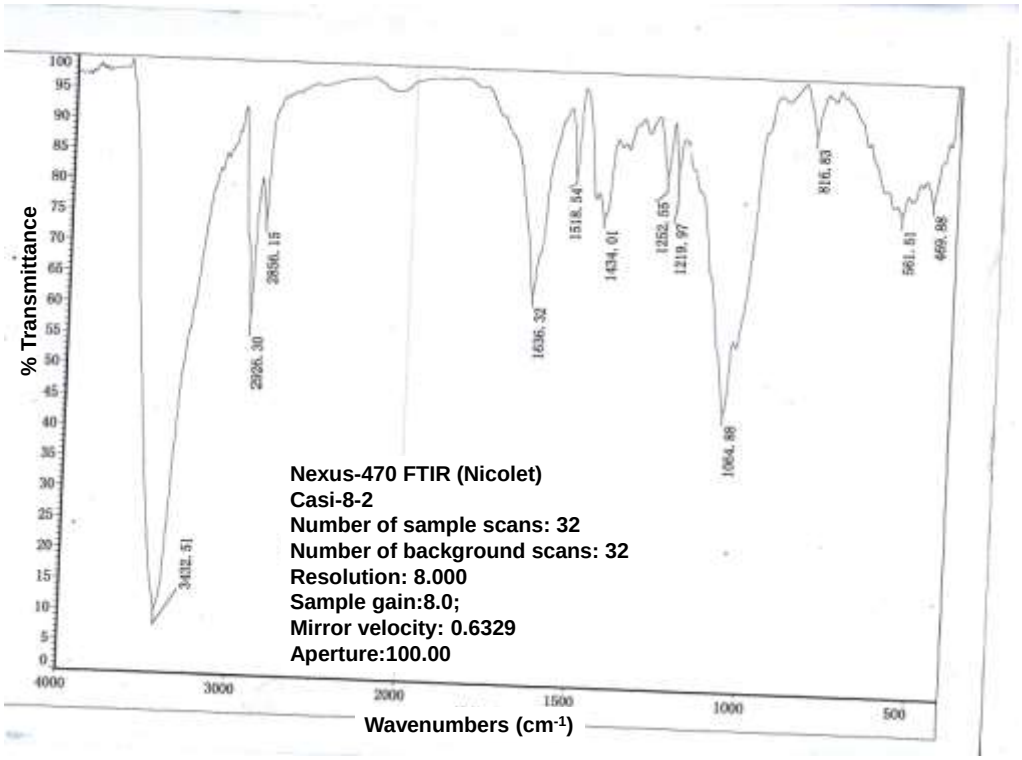
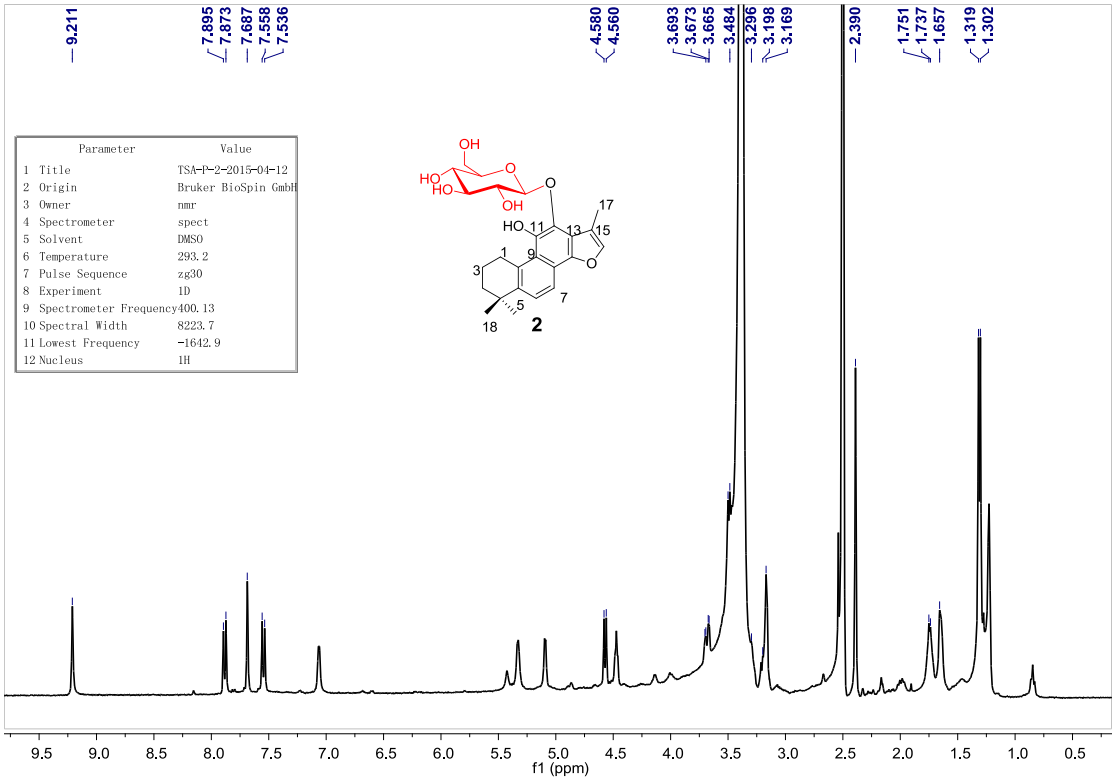


Figure S10. IR spectrum of **1**.



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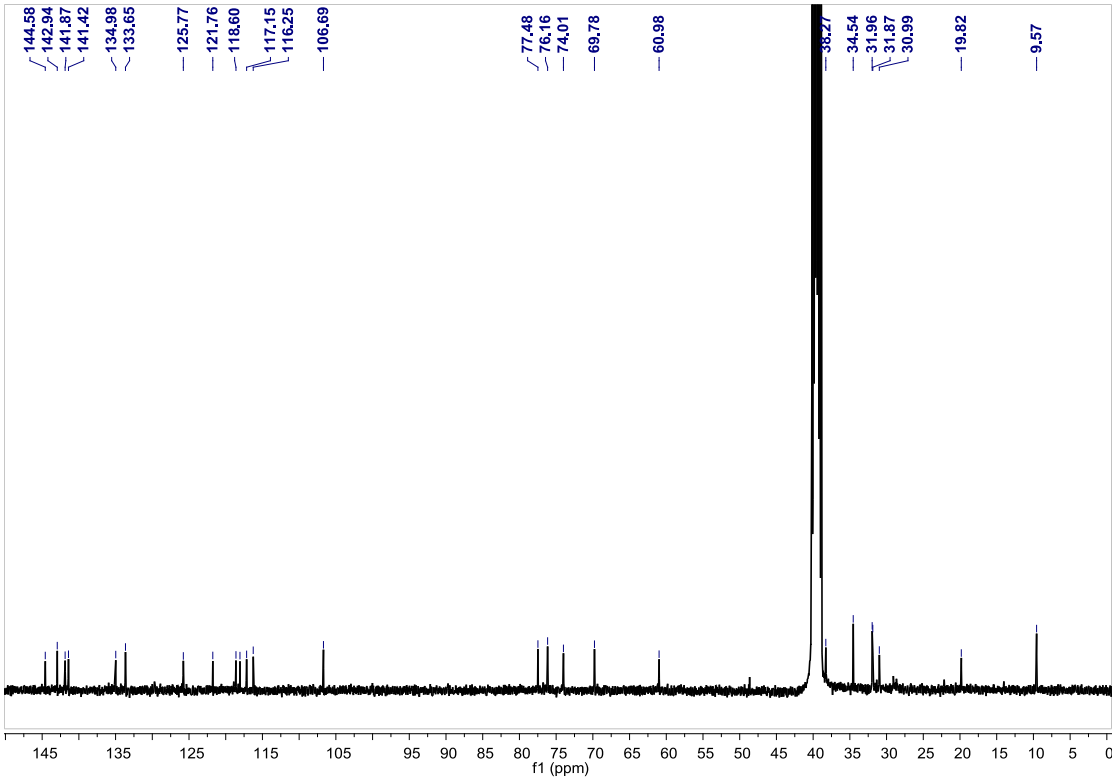
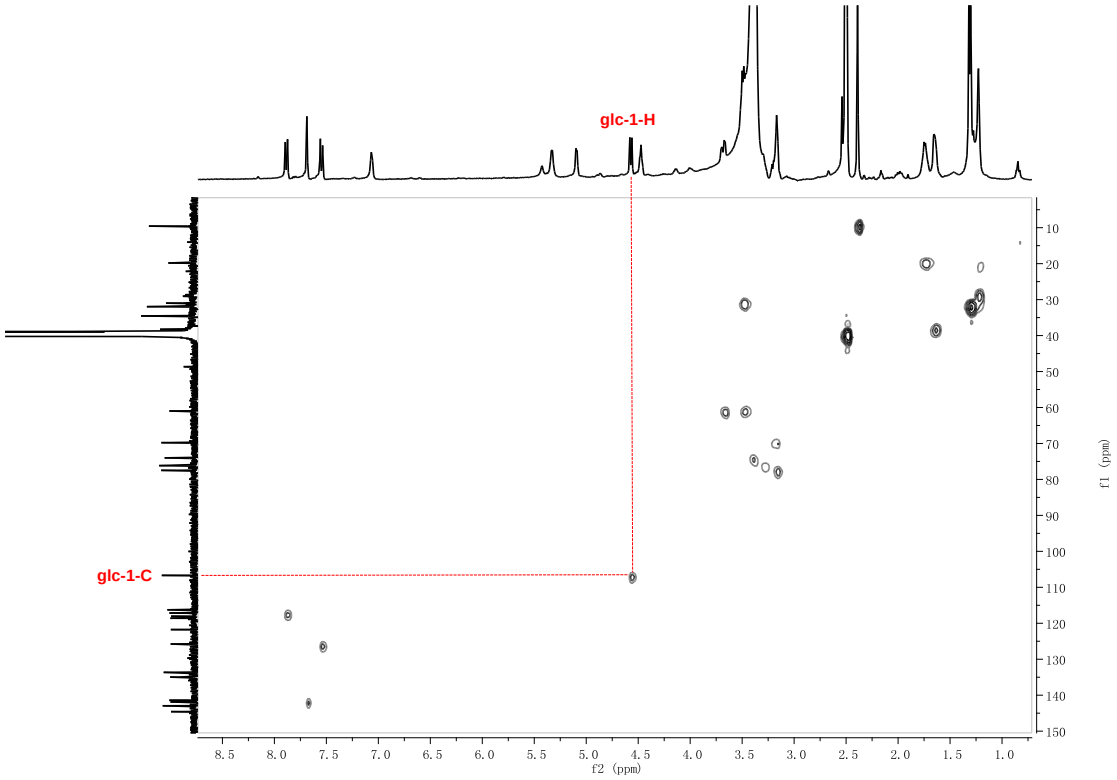


Figure S12. ^{13}C NMR spectrum of **2** (100 MHz, DMSO-d_6).



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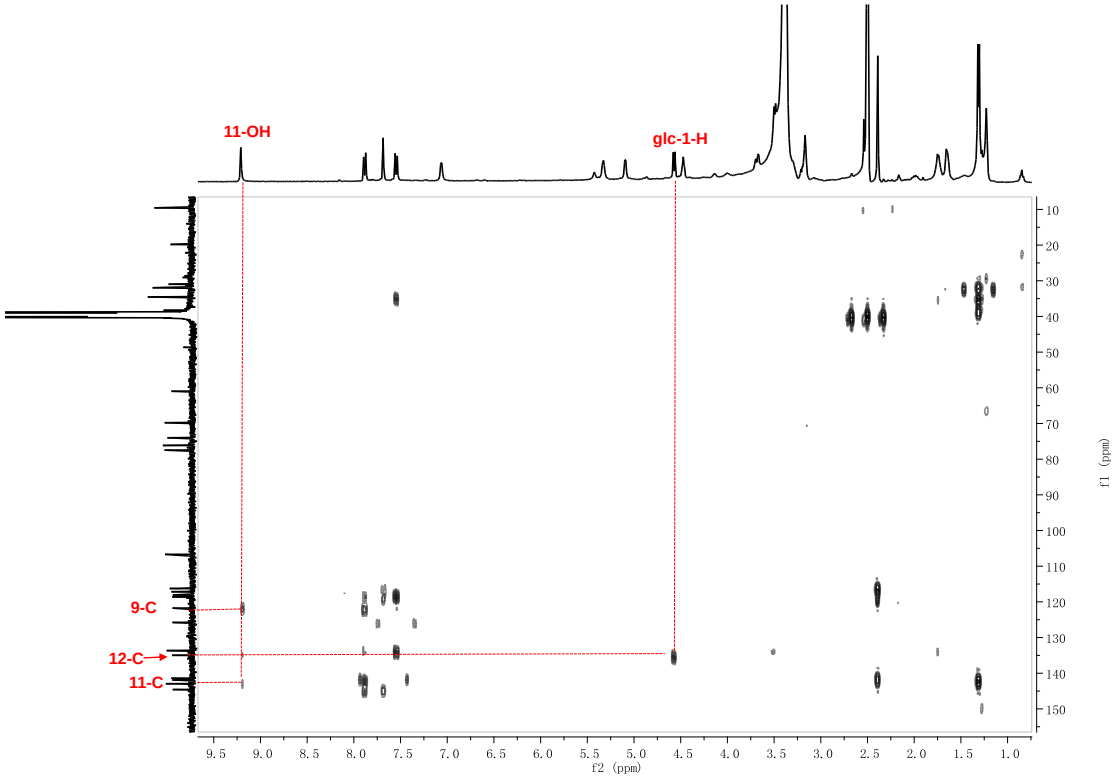
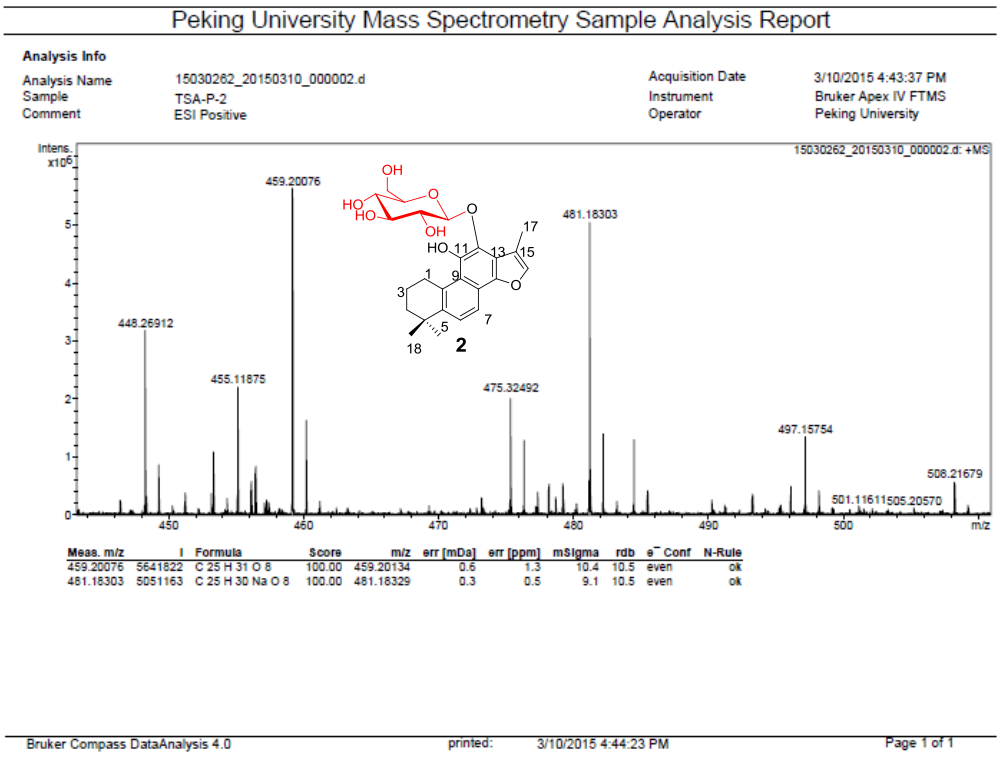


Figure S14. HMBC spectrum of **2** (400 MHz, DMSO-d_6).



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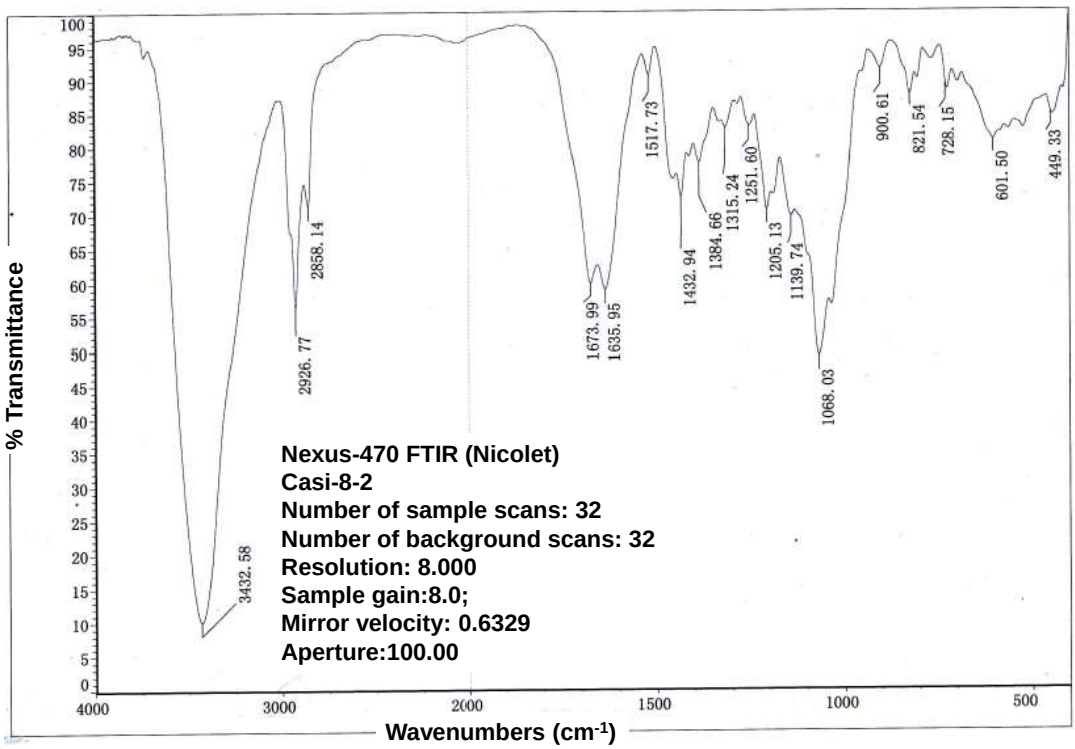

Figure S16. IR spectrum of **2**.

2. Tables

Table S1. Area of indicated concentrations of TIIA and **1**.

C/μg mL^{-1}	Area	
	TIIA	**1**
0.5	301.9	264
1	620.4	-
2.5	1637.3	1497.6
5	2994.8	3175.4
10	5804.6	5928.6
100	59238.8	51540.2
In aqueous water	Not detected	Not detected
In 30% MeOH-H_2O	29.4	449.5
In 50% MeOH-H_2O	1049.4	50543.6

C, concentration; Area, peak area in HPLC chromatogram (λ = 254 nm).

Table S2. Solubility of TIIA and **1**.

	TIIA/μg mL^{-1}	**1**/μg mL^{-1}
H_2O	Not detected	Not detected
30% MeOH-H_2O	Under limit of quantitation	0.7
50% MeOH-H_2O	1.7	84.6

3. Experimental section

3.1 Apparatus and reagents

NMR spectra were recorded at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR on a Bruker AVANCE III-400 spectrometer in MeOH-d_4, using TMS as the reference. HRESIMS spectra were obtained on a Bruker APEX IV FT-MS spectrometer. Silica gel (200-300 mesh) for column chromatography was from Qingdao Marine Chemical Corporation (Qiaodao, China). HPLC grade methanol, acetonitrile and formic acid (Mallinkrodt, Phillipsburg, NJ, USA) were used. High-purity nitrogen (99.9%) and helium (99.99%) were from Gas Supple Center of Peking University Health Science Center (Beijing, China). Solid phase extraction (SPE) cartridges (Oasis HLB, 20 cc, 1 g) (Waters, Etten-Leur, Netherlands) were used. Tanshinone IIA was from Nanjing Zelang Co. Ltd. (Nanjing, China). Its structure was characterized by NMR spectroscopy, and the purity was determined to be above 95% by HPLC analysis.

The fungal strain *Cunninghamella elegans* Lendner AS 3.2028 was purchased from China General Microbiological Culture Collection Center (Beijing, China), and was preserved on the agar slope culture medium below 4 ℃.

Seven-week-old female ICR mice were obtained from the Laboratory Animal Center of Peking University Health Science Center. They were kept in an environmentally controlled room (23 ± 2 °C, 55 ± 10% humidity) with a 12-hr light/dark cycle and allowed free access to food and water. The mice were fasted for 12 h before experiments. The animal facilities and protocols were approved by Animal Care and Use Committee of Peking University Health Science Center.

3.2 Cultivation and biotransformation

The fungal strain was incubated at 250 mL Erlenmeyer flasks with 100 mL of potato medium at room temperature on a rotary shaker for 1 day to the exponential phase. Then 1 mg of TIIA in 1 mL of methanol was added into the culture medium. The culture was extracted with an equal volume of ethyl acetate after one day incubation. The organic phase was evaporated to dryness, dissolved in 1 mL of methanol, and injected for HPLC analysis.

Scale-up biotransformation was carried out in 1000 mL of Erlenmeyer flasks

each containing 400 mL of liquid culture medium. Due to poor solubility in the culture medium, 32 mg of TIIA was dissolved in 20 mL of DMSO, and 2.4 mL was added to each flask (0.8 mL added at 24 h, 36 h, and 42 h, respectively). After additional 12 h incubation, the supernatant was extracted with equal volume of ethyl acetate for two times. The organic phase was collected and evaporated to dryness. The pigment was removed by solid phase extraction eluted with MeOH-H_2O (H_2O, 20 mL; 50% MeOH, 20 mL; 80% MeOH, 20 mL; MeOH, 30 mL). The 80% MeOH (20 mL) and MeOH (30 mL) eluates were collected and combined, and were purified by semi-preparative HPLC using an Allsphere ODS-2 colum (10 × 250 mm, i.d. 5 μm, Grace) eluted with MeOH-H_2O (80:20, v/v) at a flow rate of 2 mL/min. The detection wavelength was 254 nm. Compounds **1** (8 mg) and **2** (1.5 mg) were eluted at 19 min and 21 min, respectively.

3.3 Hydrolysis and IC-PAD Analysis of 1 and 2

To identify the sugar residues of compounds **1** and **2**, they were hydrolyzed and then analyzed by IC-PAD, as we had previously reported.[1] Compounds **1** and **2** (each 1.0 mg) was dissolved in 5 M trifluoroacetic acid (aqueous solution, 3 mL), and heated in a water bath at 90 ℃ for 3 h. After extraction with CH_2Cl_2 (3 mL × 3), the water-soluble layer was evaporated to dryness, and reconstituted in 2 mL of water. The samples were filtered through a 0.22-μm membrane before IC-PAD analysis.

IC-PAD analysis was carried out on an ICS3000 ion chromatography instrument (Thermo-Dionex Inc., USA) equipped with an ED-3000 electrochemical detector. Samples were separated on a Dionex CarboPac PA20 column (3 × 150 mm) protected with a CarboPac PA20 guard column (3 × 30 mm). The linear gradient elution program was as follows: 0−8 min, 15 mM NaOH; 8.1−15 min, 15 mM NaOH + 150 mM NaOAc; 15.1−17 min, 200 mM NaOH. The flow rate was 0.45 mL/min, and the temperature was 30 °C. The injection volume was 5 μL for each sample. Data were processed by Chromeleon 2.2 software.

[1] W. Song, L. Si, S. Ji, H. Wang, X.M. Fang, L.Y. Yu, R.Y. Li, L.N. Liang, D. Zhou, M. Ye, *J. Nat. Prod*. 2014, **77**, 1632−1643.

3.4 HPLC/DAD/ESI-MS^n analysis for the biotransformed products of TIIA

HPLC analysis was performed on an Agilent 1100 HPLC instrument equipped with a quaternary pump, a diode-array detector, an auto sampler, and a column compartment (Agilent, Waldbronn, Germany). Samples were separated on an Atlantis dC_{18} column (4.6 × 250 mm, i.d. 5 μm, Waters) protected with a Zorbax SB-C_{18} guard column (4.6 × 12.5 mm, i.d. 5 μm, Agilent). The mobile phase consisted of methanol (A) and water containing 0.1% (v/v) formic acid (B). A linear gradient elution program was used as follows: 0 min, 60% A; 6 min, 80% A; 16 min, 83% A; 21 min, 83% A; 26 min, 100% A; 30 min, 100% A; 35 min, 100% A. The flow rate was 1.0 mL/min. The HPLC effluent was introduced into the mass spectrometer with a splitting ratio of 4:1. A Finnigan LCQ advantage ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) was connected to the HPLC system via an ESI interface. The mass spectrometer was operated in the negative ion mode. High-purity nitrogen (N_2) and high-purity helium (He) were used as the nebulizing gas and collision gas, respectively. Source-dependent parameters were as follows: sheath gas (N_2), 50 arb; auxiliary gas (N_2), 10 arb; ion spray voltage, 3.50 kV; capillary temperature, 350 °C; source fragmentation voltage, 10 V; tube lens offset voltage, -36 V. LC/MS data were processed with Xcalibur^{TM} 2.0.7 software (ThermoFisher, CA, USA).

3.5 Solubility test

The solubility test was conducted according to the saturation shake-flask method.[13] Briefly, 2 mg of pure solid samples of **1** and TIIA were added into 10 mL of 50% MeOH-H_2O solution in a conical flask, respectively. The solutions were stirred at room temperature for more than 6 hours. After 18 hours of sedimentation, the saturated solutions were filtered for HPLC analysis.

3.6 Biological activity evaluation

HepG2 human hepatocellular carcinoma cells stably transfected with Nrf2/ARE luciferase reporter were used to evaluate the effects of TIIA, **1** and **2** on Nrf2 transcriptional activity. Briefly, the cells were treated with test samples and incubated for 6 h. Then the luciferase activities were measured using the Luciferase Assay System (Promega, WI, USA) on a Centro LB 960 microplate luminometer (Berthold, Germany). Tertiary butylhydroquinone (tBHQ) was used as the positive control. The

results are representatives of at least three independent replicates.

3.7 Animal treatment and samples determination.

Female ICR mice (25g) were obtained from the Laboratory Animal Center of Peking University Health Science Center. All animals were fasted for 12 hours before experiments. The animals were randomly divided into 3 groups (n = 2 for each group). One group received 0.3 mL of 0.25% sodium carboxy-methyl cellulose (CMC-Na) solution as blank control, and the other two groups were respectively orally administrated with TIIA and **1** (suspended in 0.3 mL of 0.25% CMC-Na solution) at the same mole dose of 0.17 mmol/Kg (TIIA, 50 mg/Kg; **1**, 79 mg/Kg). For each group, the two mice were sacrificed at the time points of 1 and 2 h, respectively. The blood samples were collected into heparinized centrifuge tubes and immediately centrifuged at 9000 rpm to obtain the plasma. Then the plasma at two time points were combined, and 600 μL of plasma samples were mixed with three-fold volumes of methanol-acetonitrile (2:1, v/v) to precipitate protein. After centrifuged at 9000 rpm for 10 min, the supernatants were dried in vacuum at 40 °C, dissolved in 200 μL of methanol, and filtered through a 0.22 μm membrane for HPLC/DAD/ESI-MS^n and LC/SRM-MS analysis.

*HPLC/DAD/ESI-MS^n analysis for TIIA and **1** in plasma samples*. HPLC analysis was performed on an Agilent 1100 HPLC instrument equipped with a quaternary pump, a diode-array detector, an auto sampler and a column compartment (Agilent, Waldbronn, Germany). Samples were separated on an Atlantis dC_{18} column (4.6 × 250 mm, i.d. 5 μm, Waters) protected with a Zorbax SB-C_{18} guard column (4.6 × 12.5 mm, i.d. 5 μm, Agilent). The mobile phase consisted of acetonitrile (A) and water containing 0.1% (v/v) formic acid (B). A linear gradient elution program was used as follows: 0 min, 19% A; 8 min, 28% A; 16 min, 28% A; 17 min, 35% A; 21 min, 50% A; 26 min, 50% A; 38 min, 75% A; 48 min, 95%; 53 min, 95%. The flow rate was 1.0 mL/min. The HPLC effluent was introduced into the mass spectrometer with a splitting ratio of 4:1. A Finnigan LCQ advantage ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) was connected to the HPLC system via an ESI interface. The mass spectrometer was operated in the positive ion mode. High-purity nitrogen (N_2) and high-purity helium (He) were used as the nebulizing gas and collision gas, respectively. Source-dependent parameters were as follows: sheath gas (N_2), 50 arb; auxiliary gas (N_2), 10 arb; ion spray voltage, 3.50 kV;

capillary temperature, 350 °C; source fragmentation voltage, 10 V; tube lens offset voltage, -36 V. LC/MS data were processed with Xcalibur^{TM} 2.0.7 software (ThermoFisher, CA, USA).

*LC/SRM-MS analysis for TIIA, **1** and their metabolites in plasma samples*. The system consisted of a Finnigan Surveyor LC instrument connected to a Finnigan TSQ Quantum triple quadrupole mass spectrometer via ESI interface (ThermoFisher, CA, USA). The HPLC conditions were the same as those for HPLC/DAD/ESI-MS^n analysis. The ESI source was operated in the positive ion mode. High purity nitrogen was used as the sheath (50 arb) and auxiliary (5 arb) gas; high purity argon was used as the collision gas (1.5 mTorr). Ion source parameters were as follows: spray voltage, 4.0 kV; capillary temperature, 330 °C; tube lens offset, -35 V. The mass spectrometer was operated in selected reaction monitoring (SRM) mode. The SRM ion pairs were set according to MS^n data obtained in HPLC/DAD/ESI-MS^n analysis.