Supporting Information

In vivo SAR and STR analysis of alkaloids from *Picrasma quassioides* identifies 1-hydroxymethyl-8-hydroxy-*β*-carboline as a novel natural angiogenesis inhibitor

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Supporting Information

S1 Extensive experimental methods

In vitro proliferation assay

Proliferation assay was performed as described.[30] HUVEC (3000 per well) were seeded in 96-well plate in the EGM-2 medium for 12 h for attachment. Then cells were treated for 48 h with EGM-2 medium containing vehicle 0.1% dimethyl sulfoxide (DMSO) and various concentrations of **3**. Cell growth was assessed using a cell-counting kit-8 (CCK-8, Dojindo, Japan) according to the protocol provided. The spectrophotometric absorbance of each well was measured by a multi-detection microplate reader (Synergy HT, BioTeks, USA) at a wavelength of 450 nm. Each treatment was performed in triplicate. The IC_{50} was calculated by GraphPad Prism 6statistical software (San Diego, CA, USA).

In vitro migration assay

HUVEC migration assay was performed in a Boyden chamber migration assay and a scratch-wound assay.

Boyden chamber migration assay: HUVEC (8000 per well) were seeded on 48-well chamber (8μm pore-size, AP48, Neuro Probe). The top chamber contained vehicle or various concentrations of **3**. Cells were allowed to migrate for 8 hours. Non-migrated cells were scraped with a cotton swab, and migrated cells were fixed with 100% methanol and stained with 0.05% crystal violet.

Scratch-wound assay: HUVECs in growth medium were seeded into 6-well plates precoated with 0.1% gelatin (Sigma-Aldrich, St. Louis, USA), and grown overnight to

confluence. The monolayer cells were wounded by scratching with 200μL pipette tips and washed with PBS to remove the non-adherent cells. EBM-2 medium containing 0.5% FBS together with various concentrations of **3** was then added into the wells. Cells receiving 0.1% DMSO only served as a vehicle control. Cells receiving 2 μM SU5416 served as a positive control. After 12 h incubation, cells were washed by PBS and fixed with methanol for 10 min and then stained with 0.05% crystal violet solution. Then the 0.05% crystal violet solution was washed by ddH2O.

Images were taken at 0 h and 12 h independently through an inverted microscope (Nikon, Japan). The migrated cells were counted manually. The values were observed from four randomly selected fields. The percentage of inhibition was expressed using control wells at 100%.

In vitro tube formation assay

Matrigel (growth factor reduced; BD Biosciences, USA) was thawed at 4 °C overnight. Each well of pre-chilled 96-well plates was coated with 100 μl of Matrigel, incubated and solidified at 37 °C for 120 min. HUVECs at the density of 2×10^4 per well in EGM-2 containing the indicated concentrations of **3** or 2 μM SU5416were placed onto the Matrigel layer and incubated for 12 h. The network formation was visualized and imaged under an inverted microscope (Nikon, Japan) at 100 × magnification. The tube length was quantified by Image Pro Plus 6.0 software. The values were observed from four randomly selected fields.

Display Report

Analysis Info

Analysis Name	D:\Data\MS\xiaowai\sd-lj\1204\KM-7_RD1_01_789.d	Acquisition Date	12/4/2014 3:17:42 PM
Method	tune_200-800_hcoona-pos-2.5min.m	Operator	gftang
Sample Name	KM-7	Instrument / Ser#	micrOTOF II 10257
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.6 Bar
Focus	Not active			Set Dry Heater	180 ℃
Scan Begin	50 m/z	Set Capillary	4000 V	Set Dry Gas	6.0 l/min
Scan End	1000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste

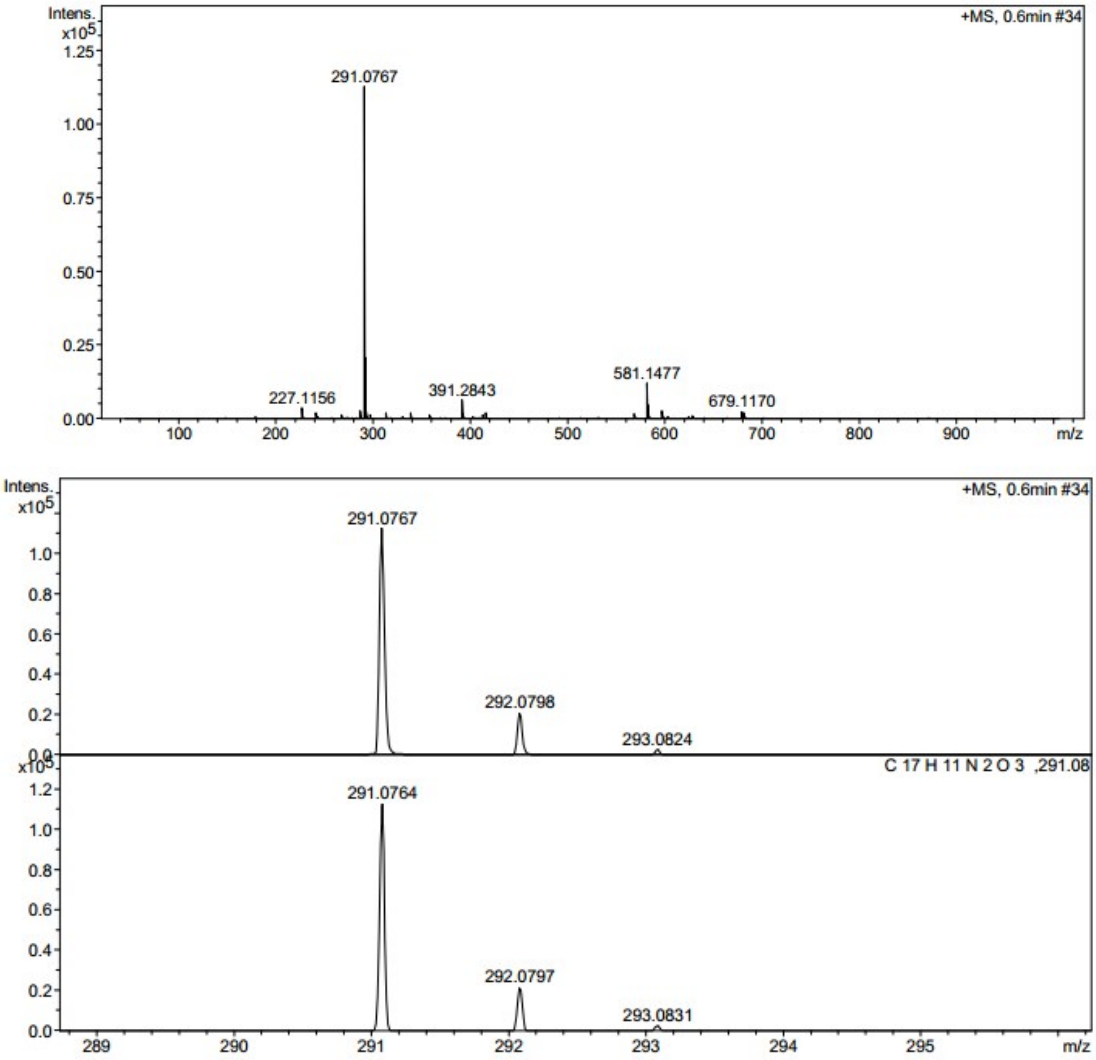

Fig. S2a HR-ESI-MS Spectrum of **1**

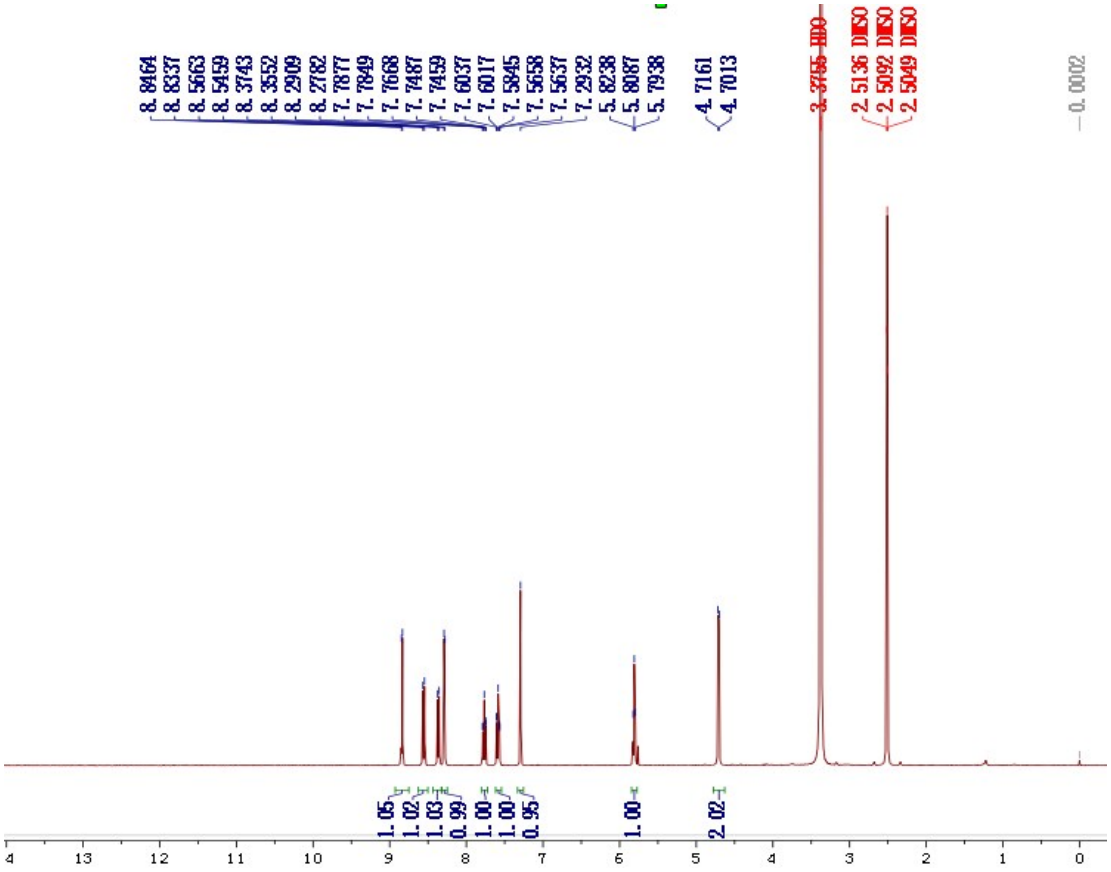

Fig. S2b ^{1}H NMR Spectrum of **1** in DMSO-d_6

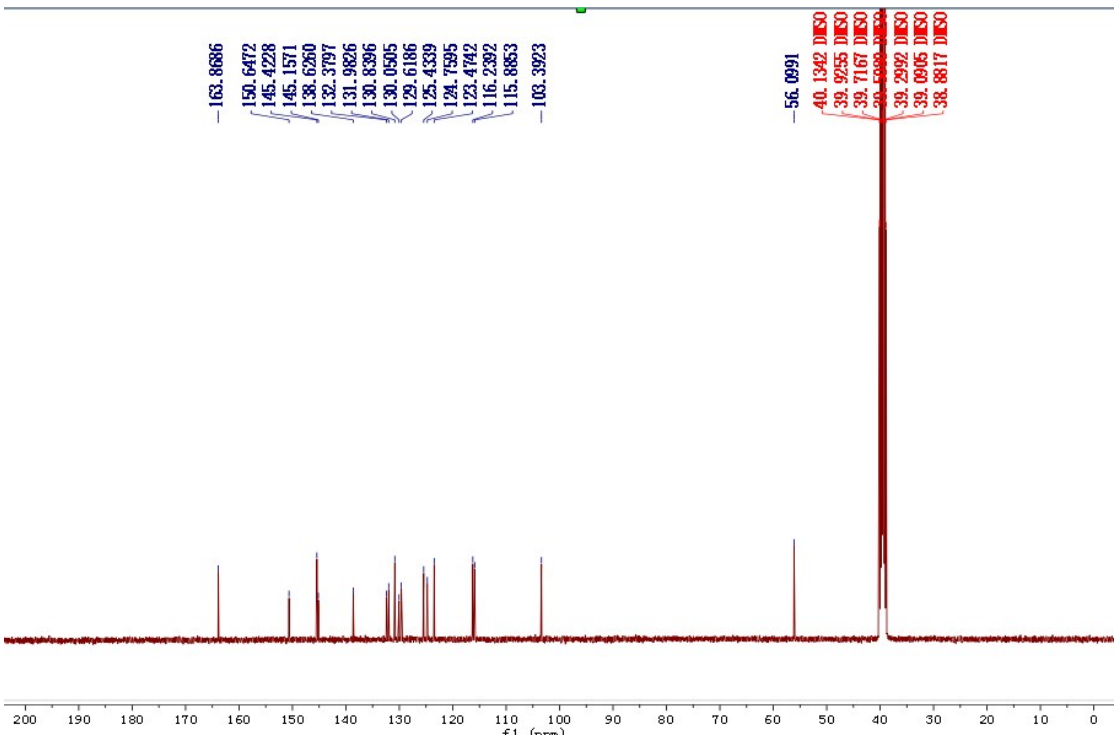

Fig. S2c ^{13}C NMR Spectrum of **1** in DMSO-d_6

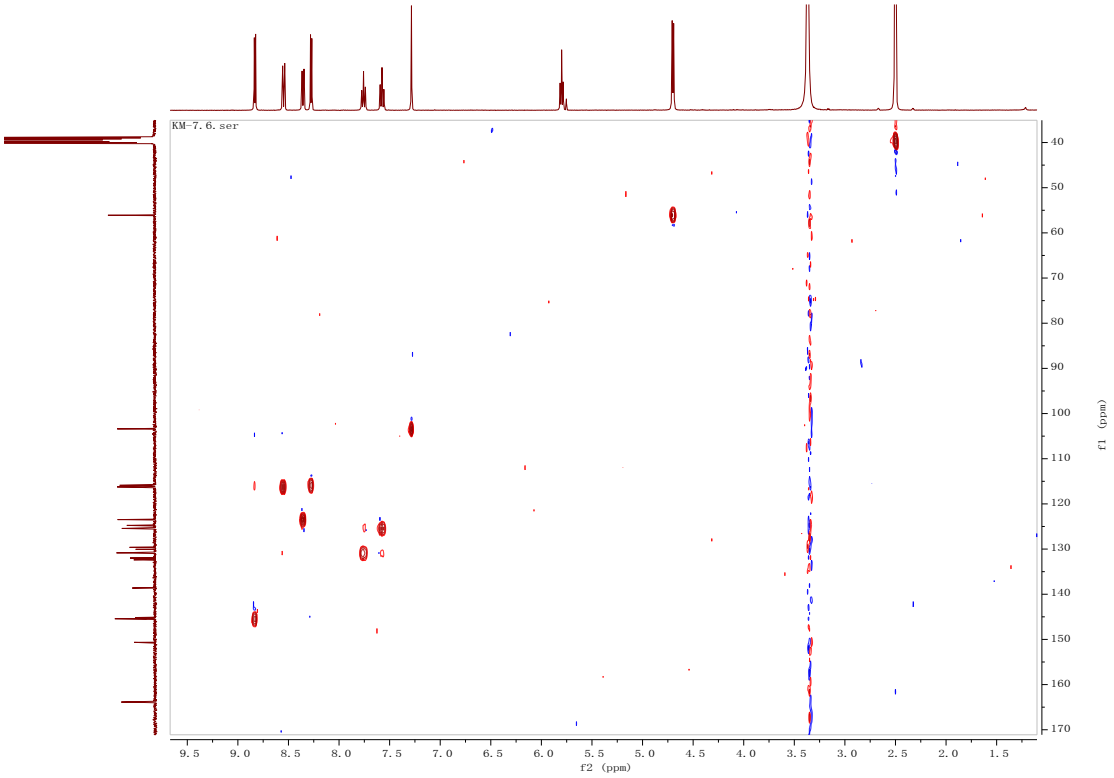

Fig. S2d HSQC Spectrum of **1** in DMSO-d_6

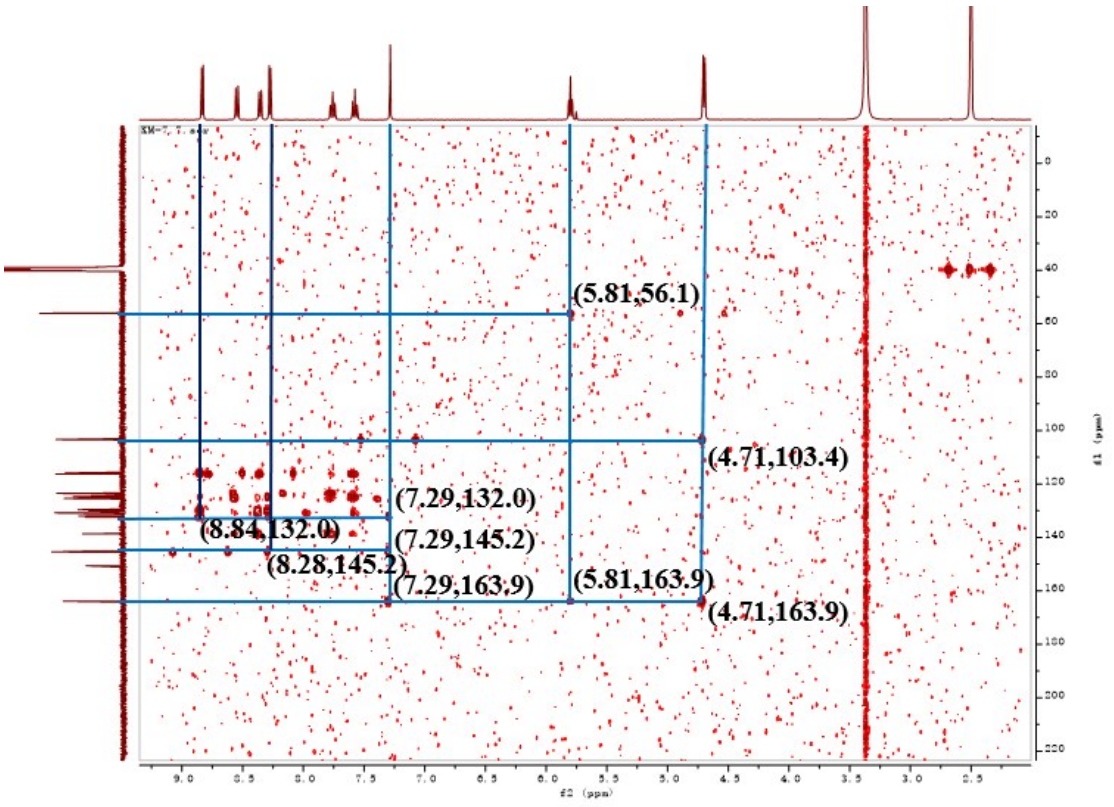

Fig. S2e HMBC Spectrum of **1** in DMSO-d_6

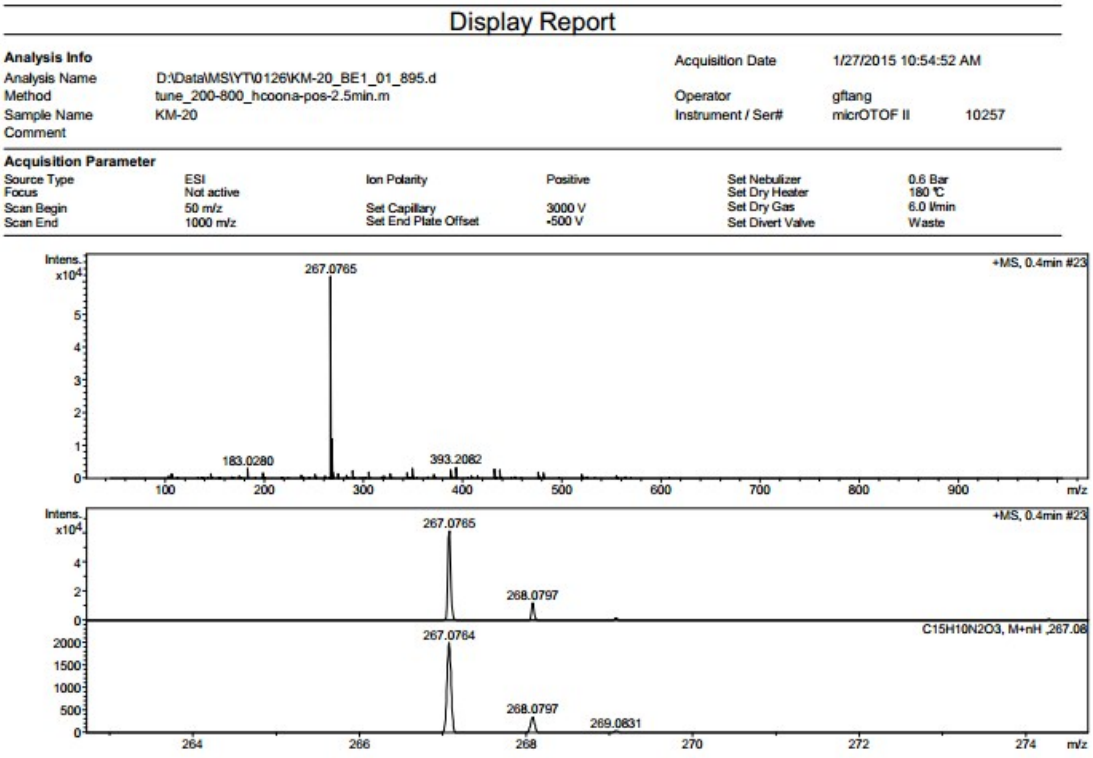

Fig. S3a HR-ESI-MS Spectrum of **2**

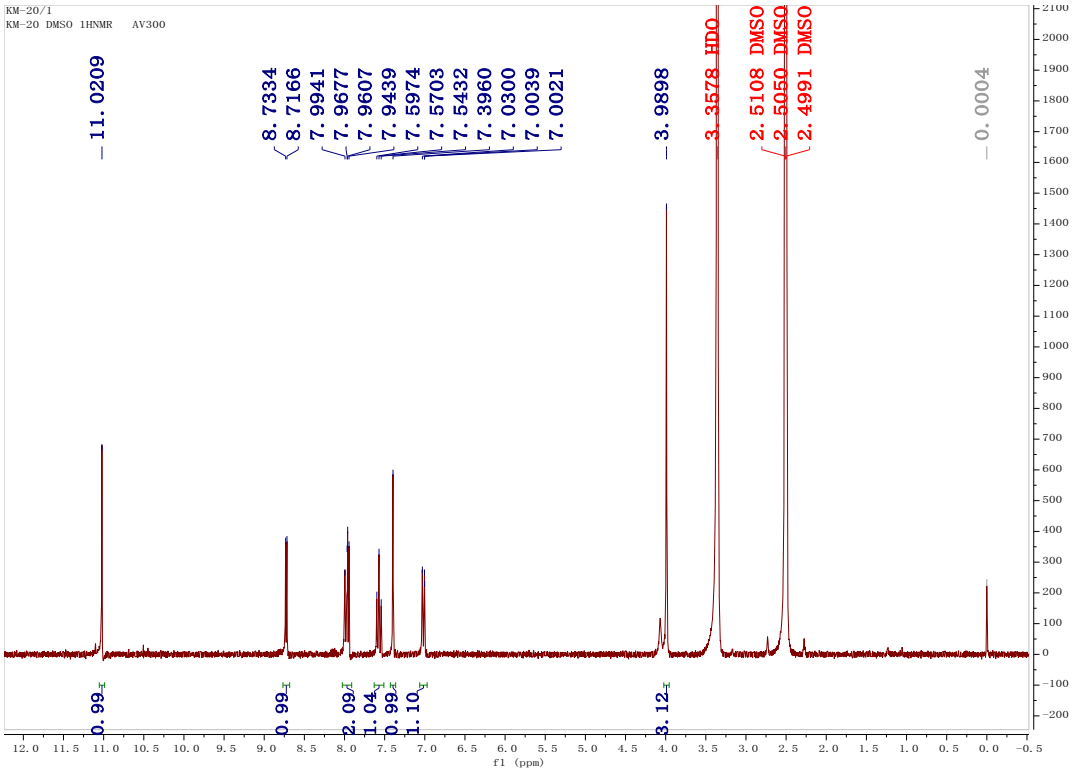

Fig. S3b ^{1}H NMR Spectrum of **2** in DMSO-d_6

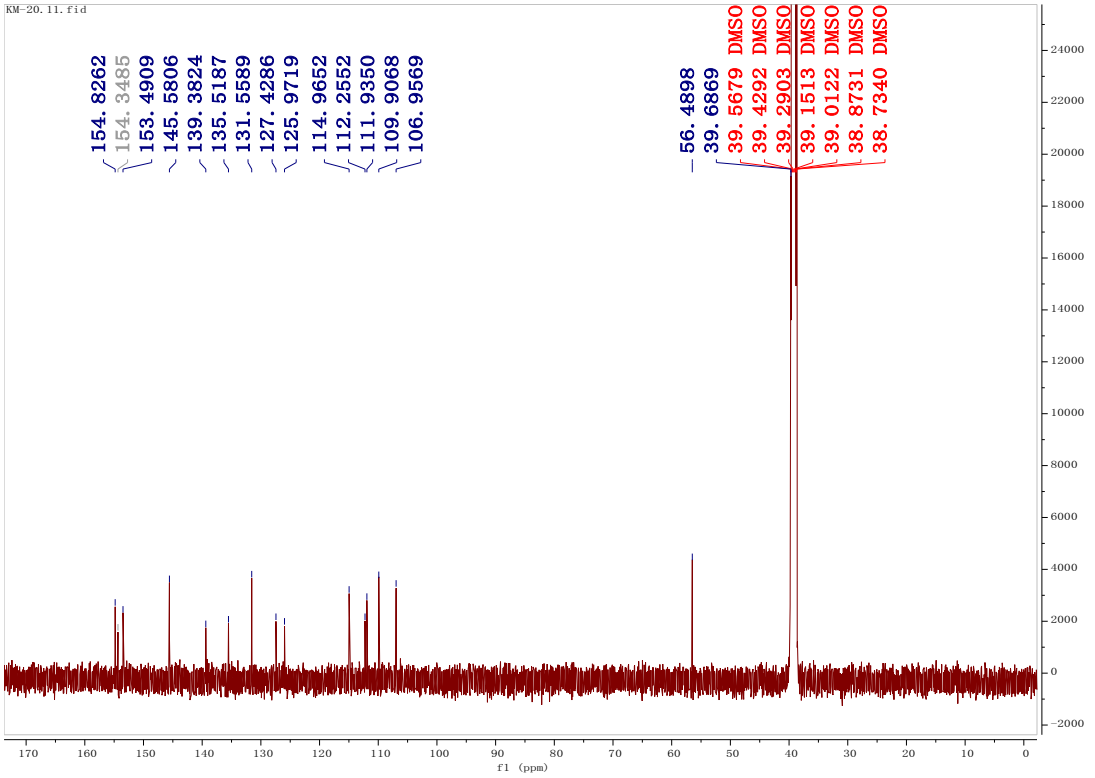

Fig. S3c ^{13}C NMR Spectrum of **2** in DMSO-d_6

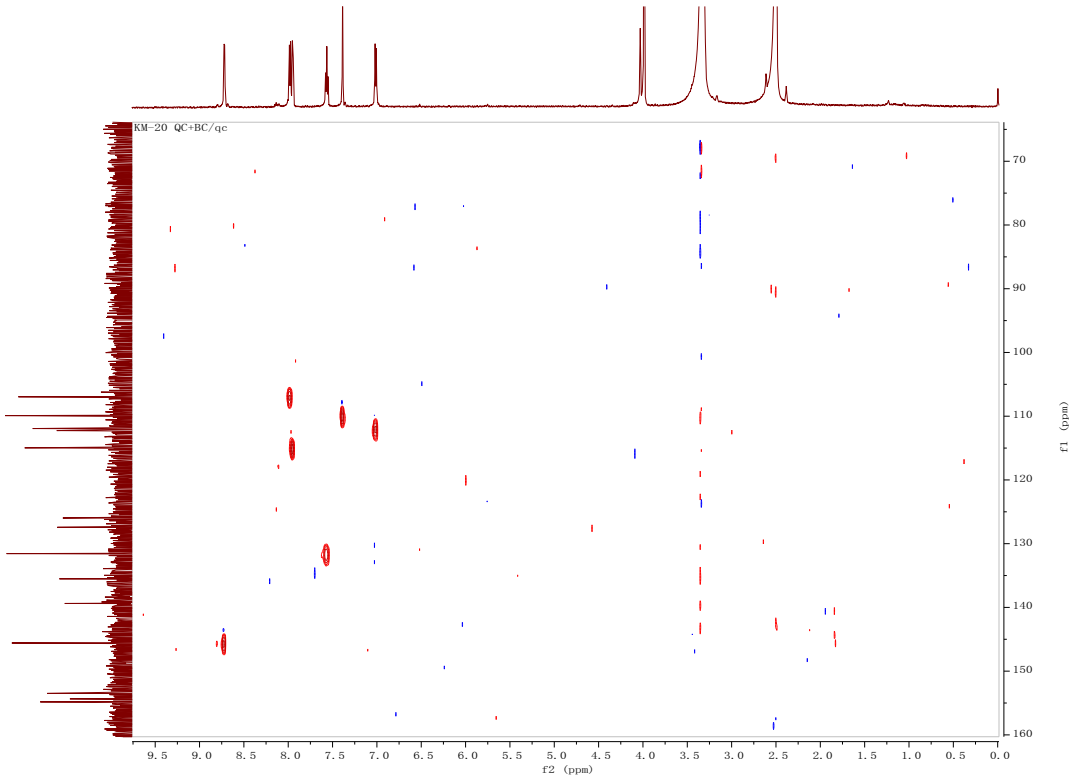

Fig. S3d HSQC Spectrum of **2** in DMSO-d_6

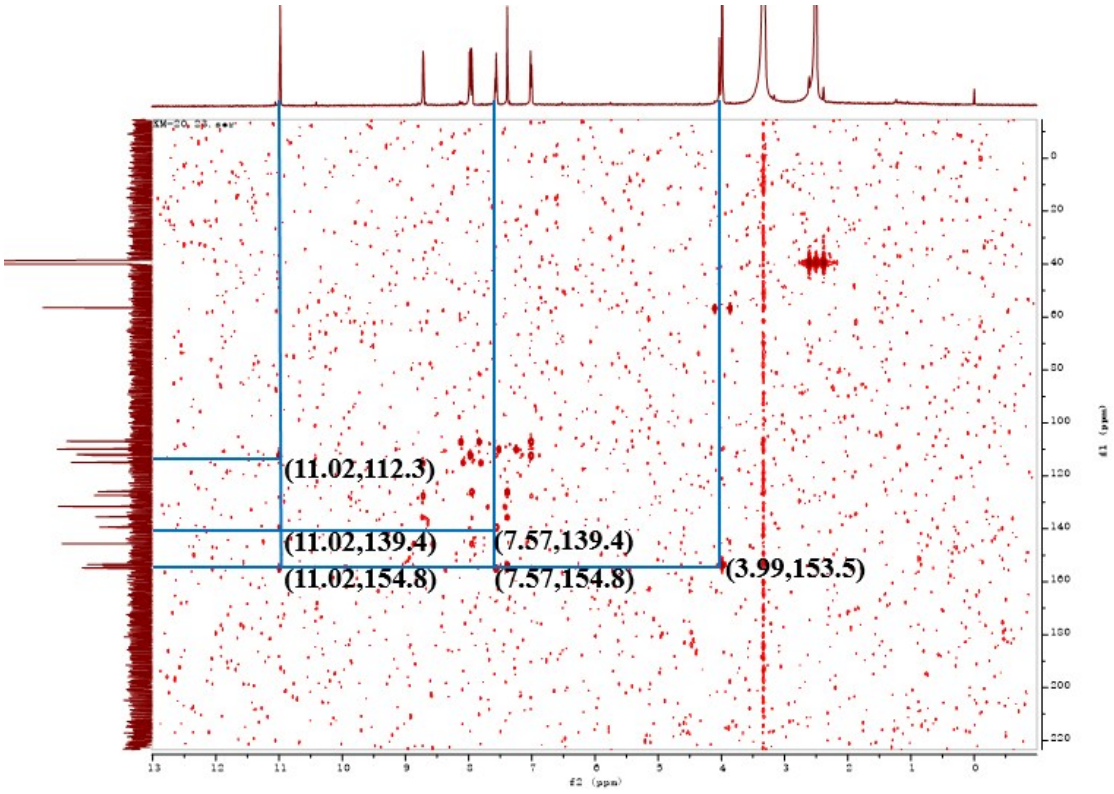

Fig. S3e HMBC Spectrum of **2** in DMSO-d_6

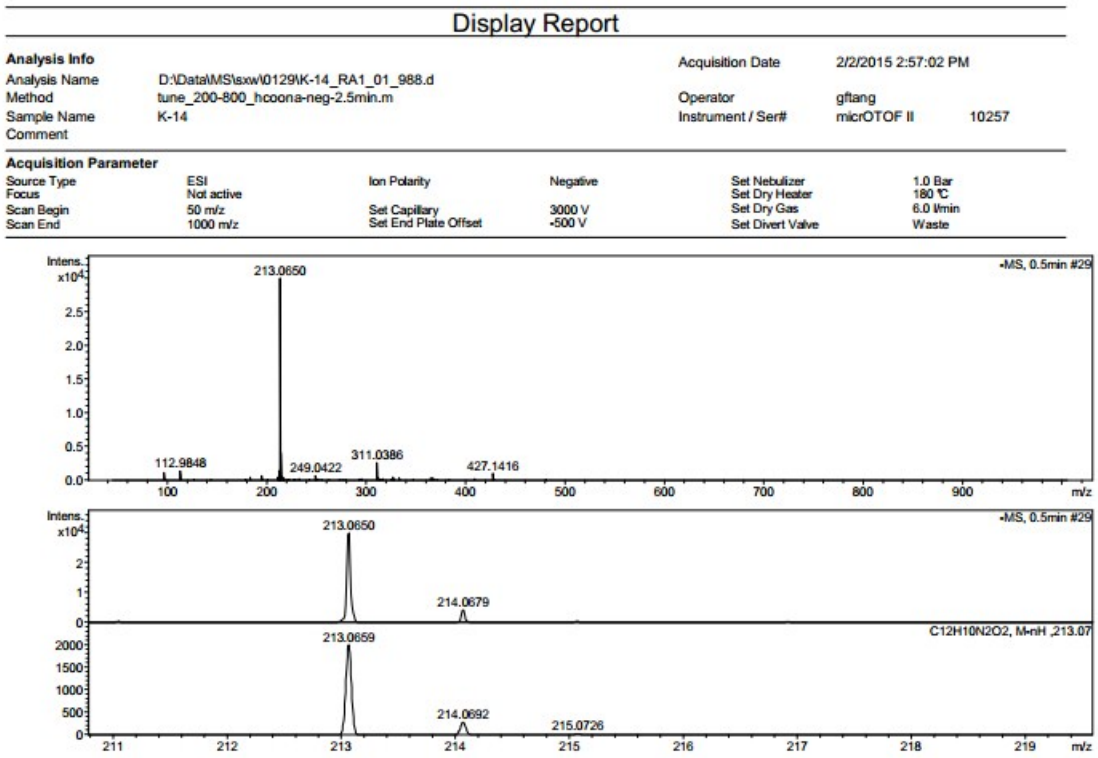

Fig. S4a HR-ESI-MS Spectrum of **3**

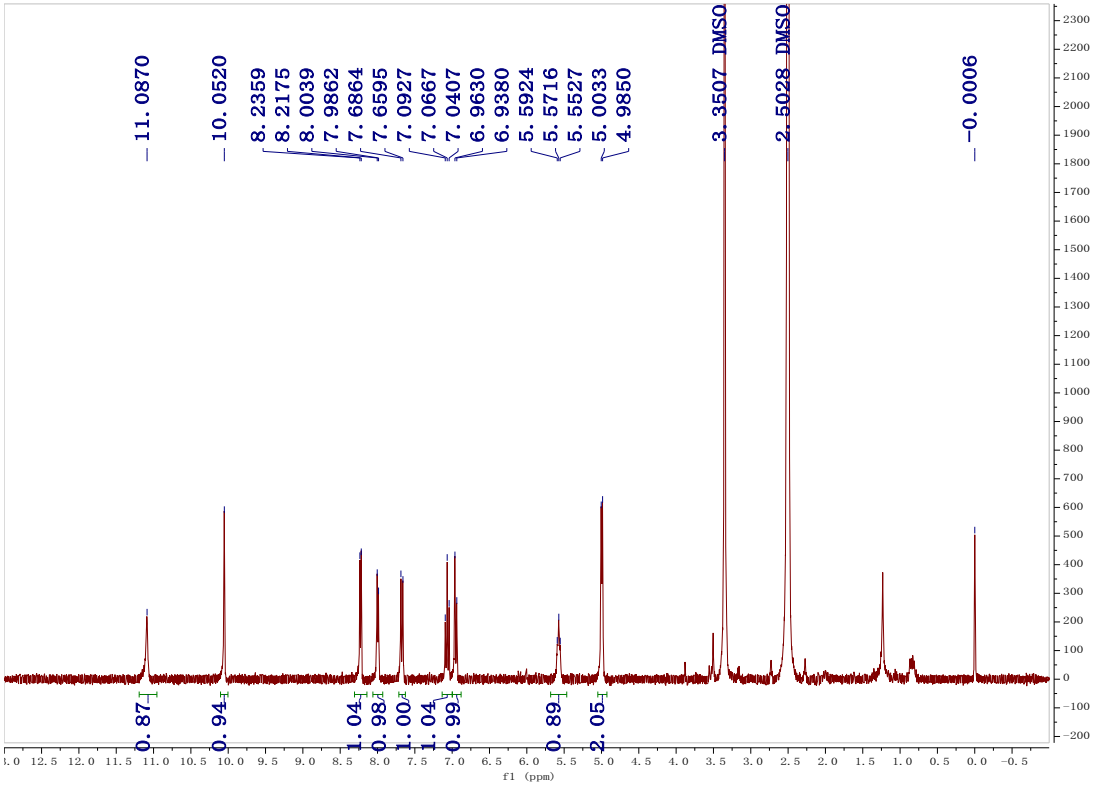

Fig. S4b ^{1}H NMR Spectrum of 3 in DMSO-d_6

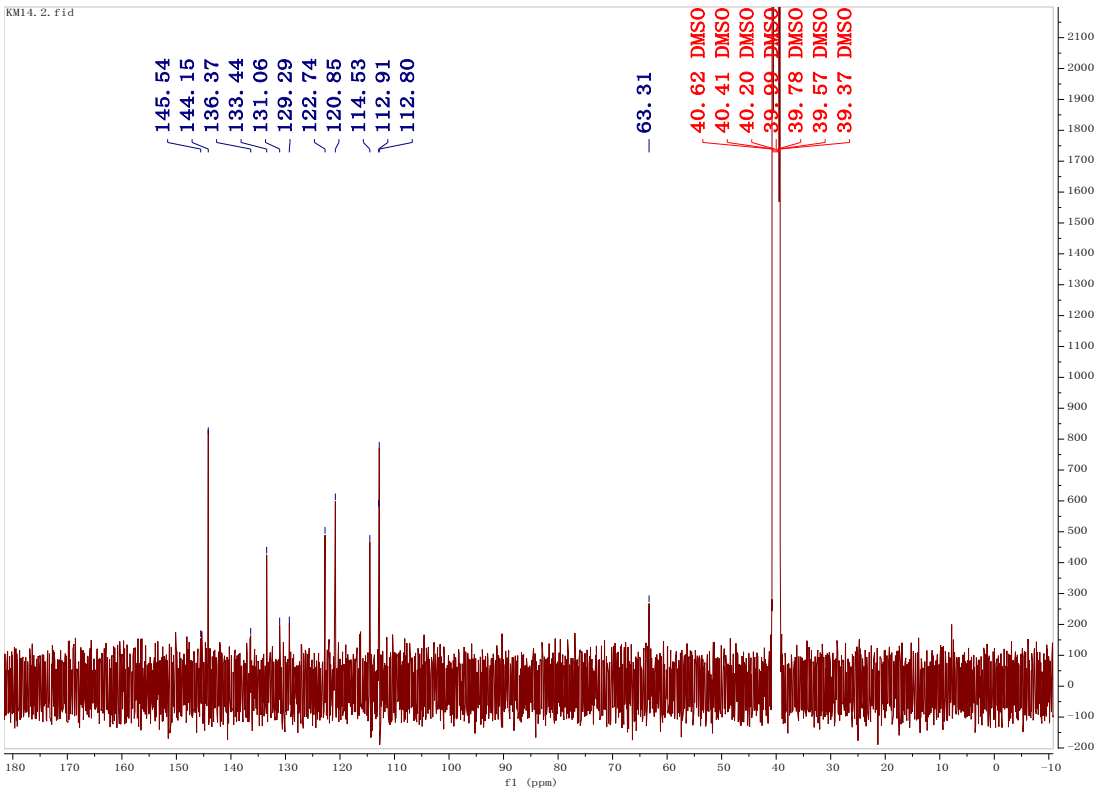

Fig. S4c ^{13}C NMR Spectrum of **3** in DMSO-d_6

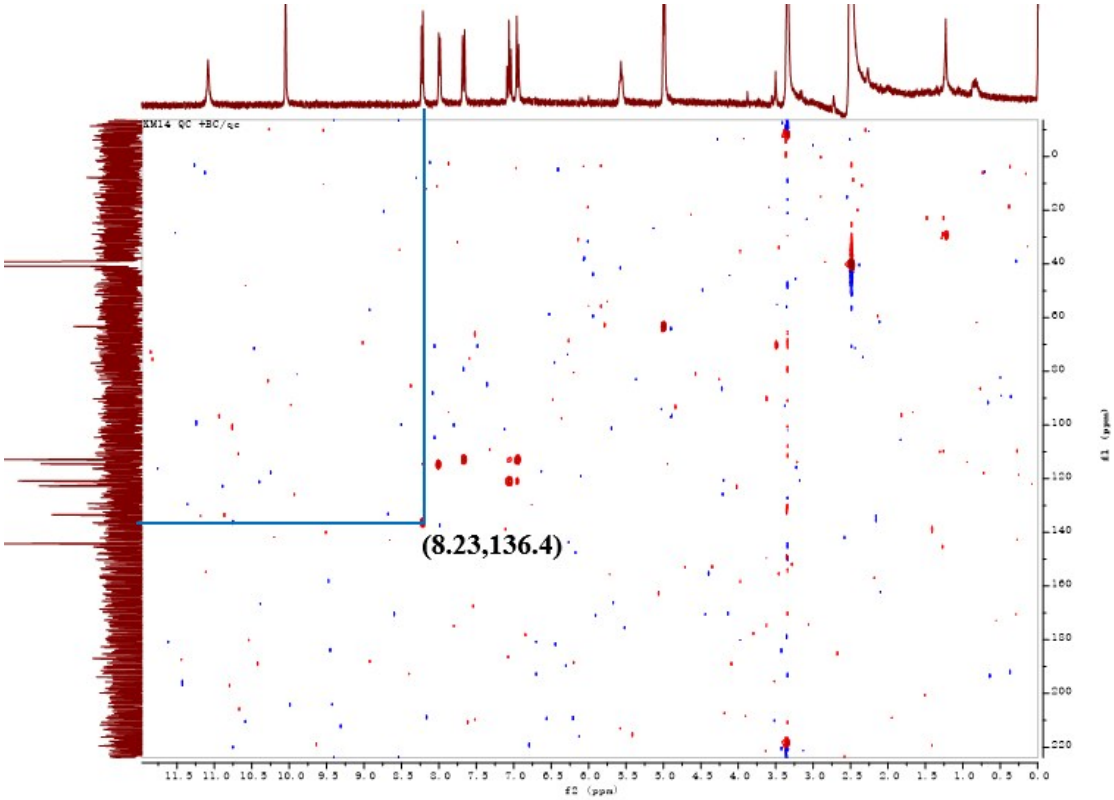

Fig. S4d HSQC Spectrum of **3** in DMSO-d_6

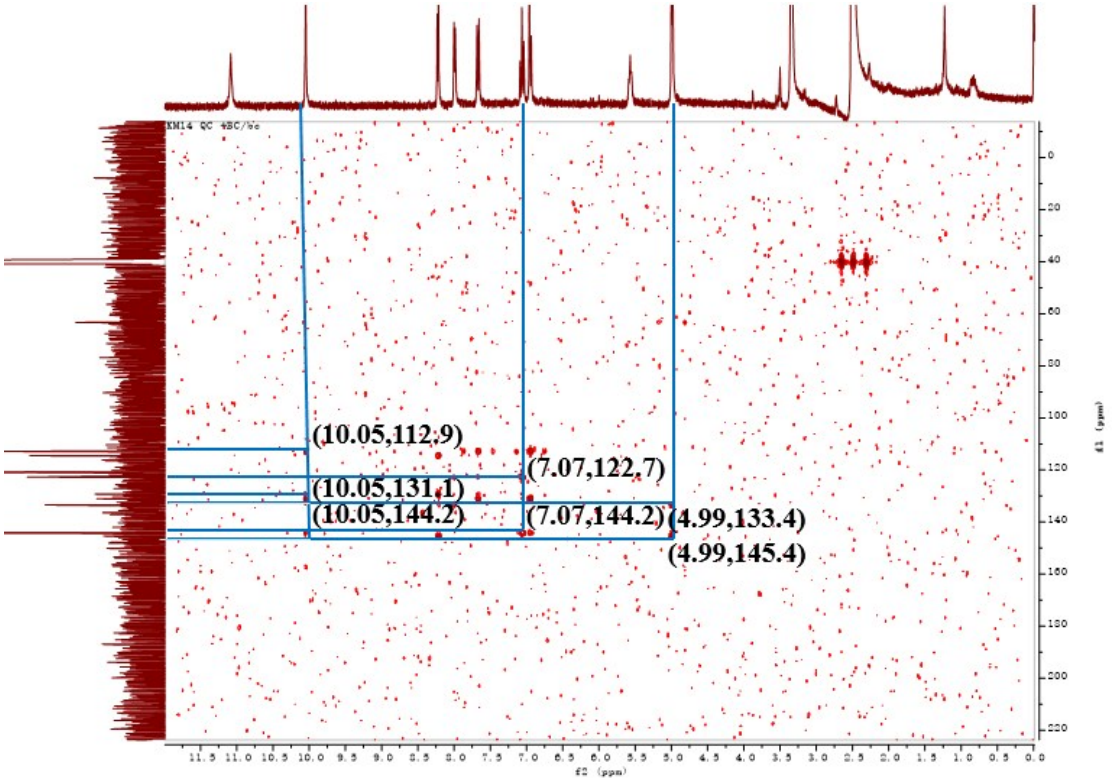

Fig. S4e HMBC Spectrum of **3** in DMSO-d_6

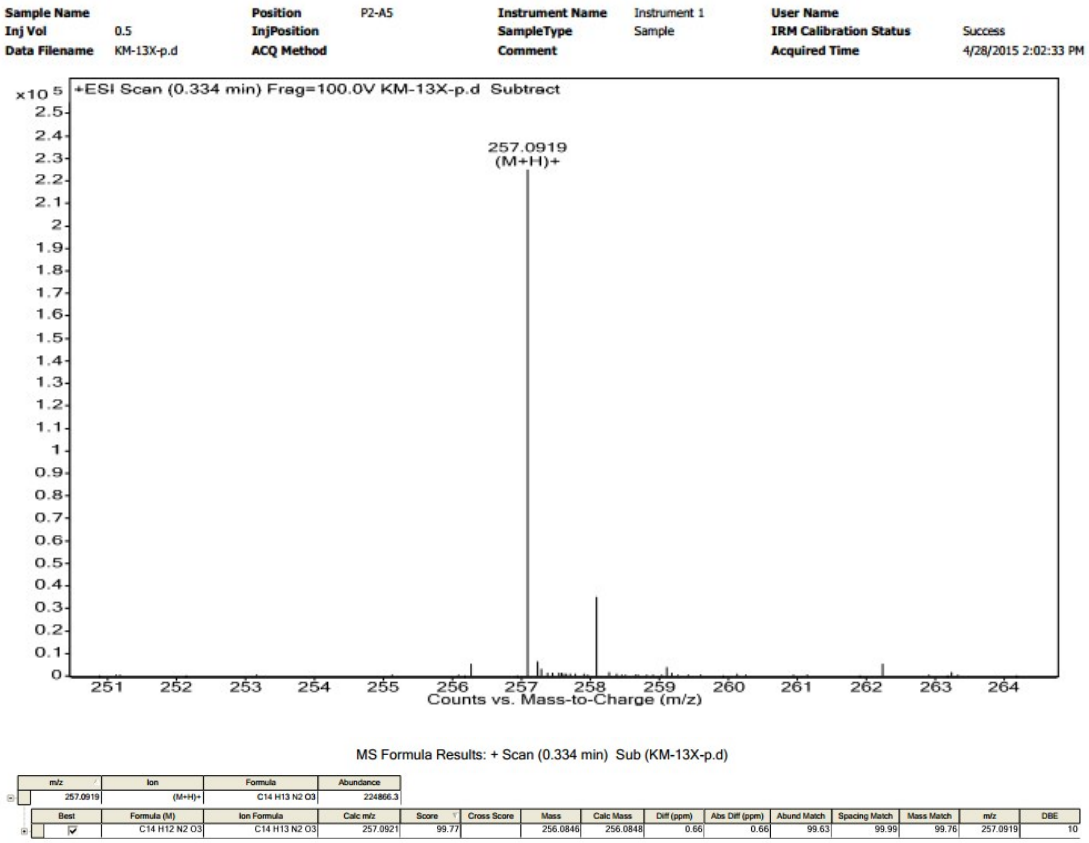

Fig. S5a HR-ESI-MS Spectrum of **4**

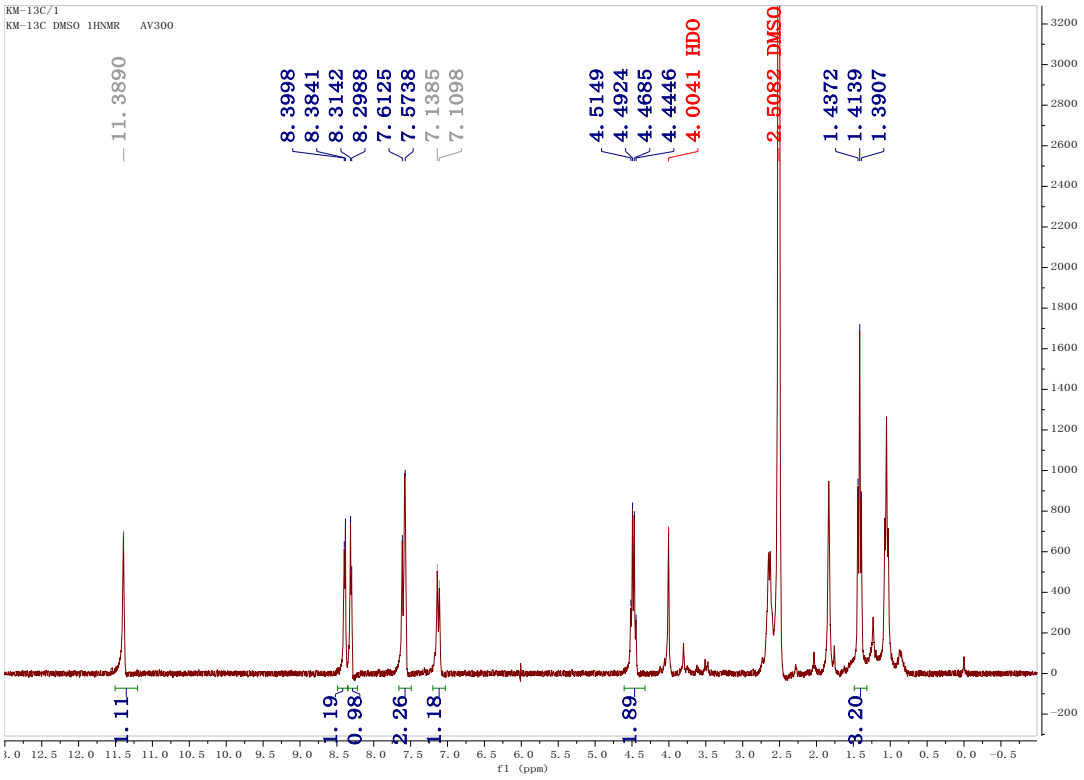

Fig. S5b ^{1}H NMR Spectrum of **4** in DMSO-d_6

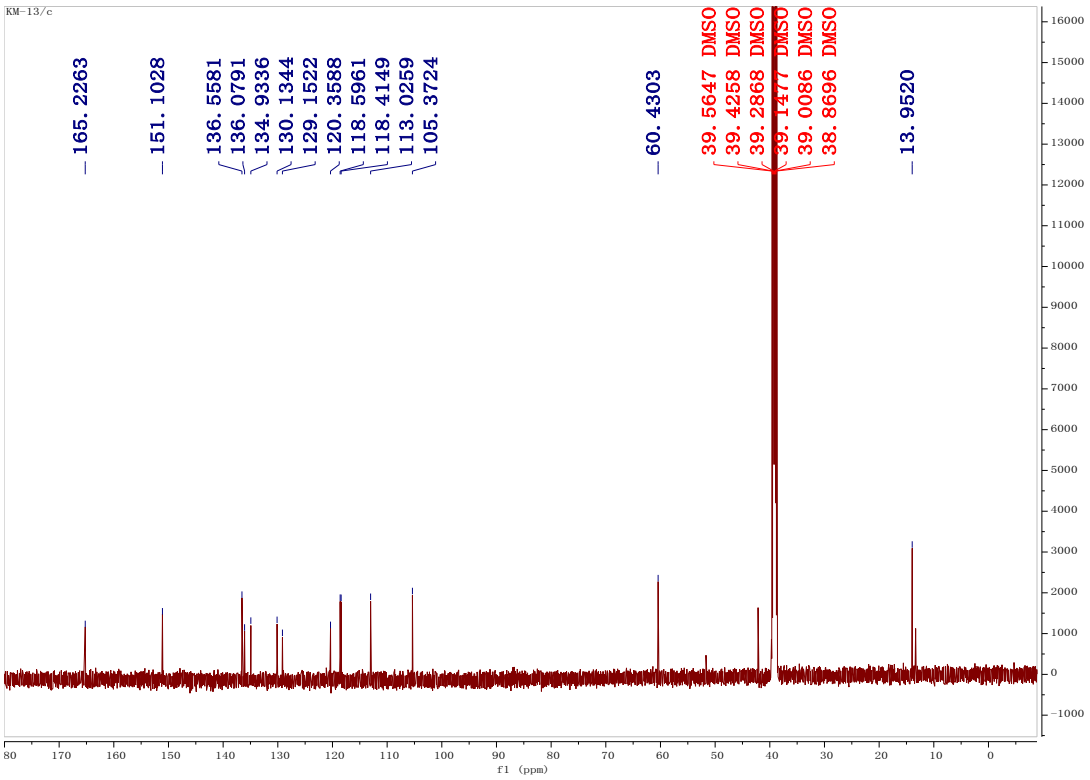

Fig. S5c ^{13}C NMR Spectrum of **4** in DMSO-d_6

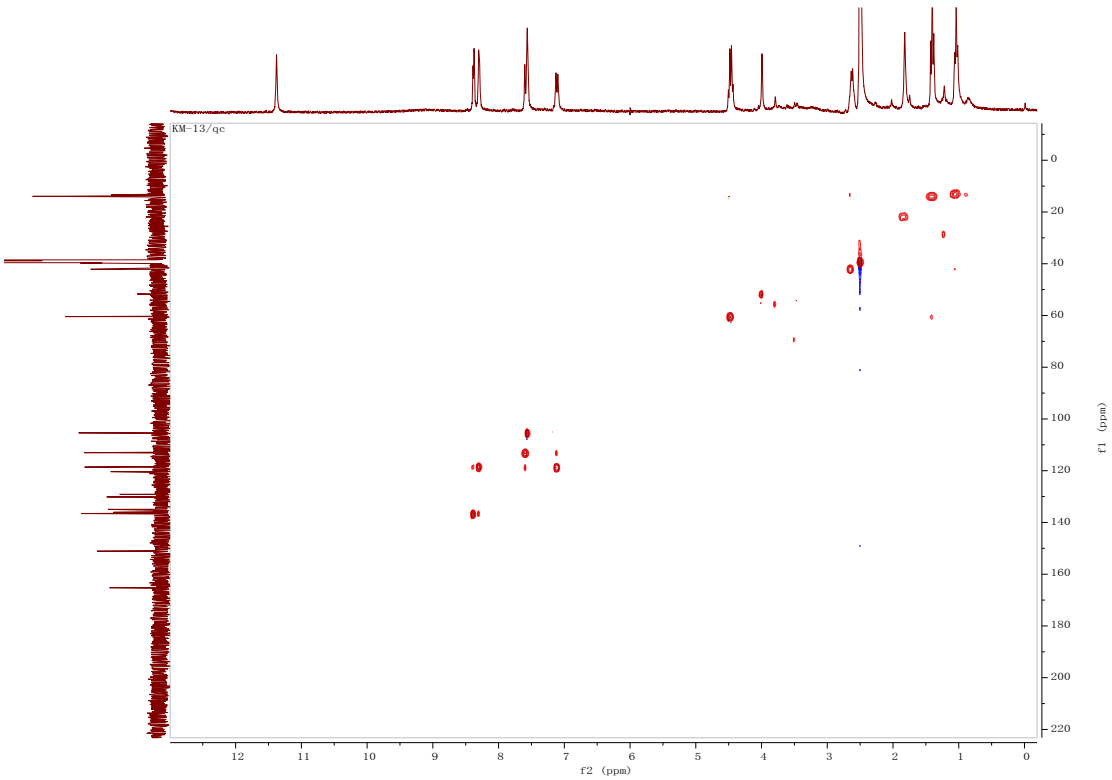

Fig. S5d HSQC Spectrum of **4** in DMSO-d_6

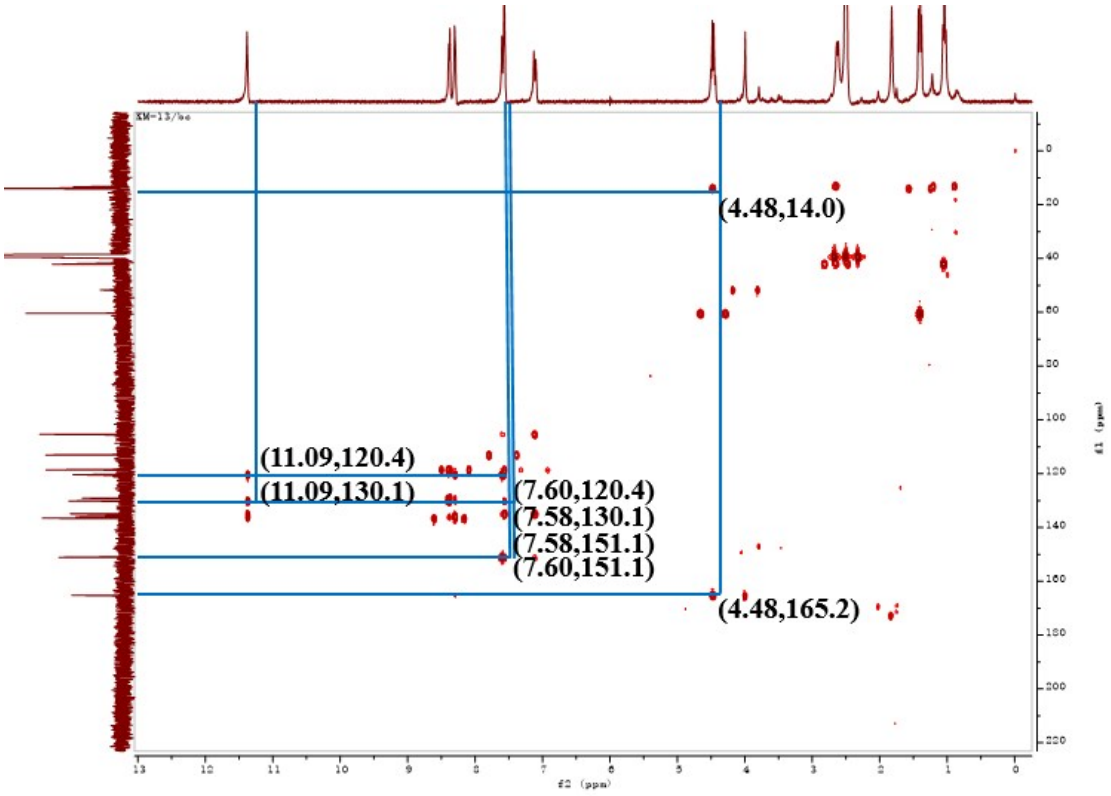

Fig. S5e HMBC Spectrum of **4** in DMSO-d_6

number	原编号	1	5	10	25	50	75	100	150	200	LD100
1	KM-7	100	100	n.d.	n.d.	n.d	n.d.	n.d.	n.d	n.d	N/A
2	KM-20	100	100	100	100	100	100	100	100	100	>200
3	KM-14	100	100	100	100 (+)	100 (++)	78.3 ± 7.6 (+++)	60.0 ± 15.0 (+++)	0	0	150
4	KM-13	100	100	100	100	100	100	93.3 ± 2.9	83.3 ± 2.9 (+)	0	200
5	XJ-1	100	95.0 ± 5.0	53.3 ± 7.6	0	0	0	0	0	0	25
6	KM-3	100	0	0	0	0	0	0	0	0	5
7	KM-31	100	100	100	100	100	100	100	100	100	>200
8	KM-50	100	68.3 ± 7.6 (+)	0	0	0	0	0	0	0	10
9	KM-1	100	100	100	100	100	100	100	100	100	>200
10	KM-12	100	100	100	100	n.d	n.d.	n.d.	n.d	n.d	N/A
11	KM-24	100	100	100	100	100	100	100	100	100	>200
12	KM-10	100	100	100	100	100	98.3 ± 2.9 (+)	46.7 ± 10.4(++)	0	0	150
13	KM-30	100	100	100	100	100	100	100	100	91.7 ± 7.6	>200
14	KM-29	100	100	100	100	100	100	100	100	100	>200
15	KM-44	100	100	100	100	100	100	100 (+)	100 (++)	100 (+++)	>200
16	KM-2	100	100	100	100	100	100	93.3 ± 7.6 (+)	0	0	150
17	XJ-9	100	100	0	0	0	0	0	0	0	10
18	KM-4	100	100	100	82.7 ± 12.6 (+)	0	0	0	0	0	50
19	KM-40	100	100	100	100	100	100	100	n.d	n.d	N/A
20	KM-11	100	100	100	100	100	100	100	100	100	>200
21	SU5416	100 (++)	0	0	0	0	0	0	0	0	5

Fig. S6 Anti-angiogenesis activity/lethal toxicity evaluation information

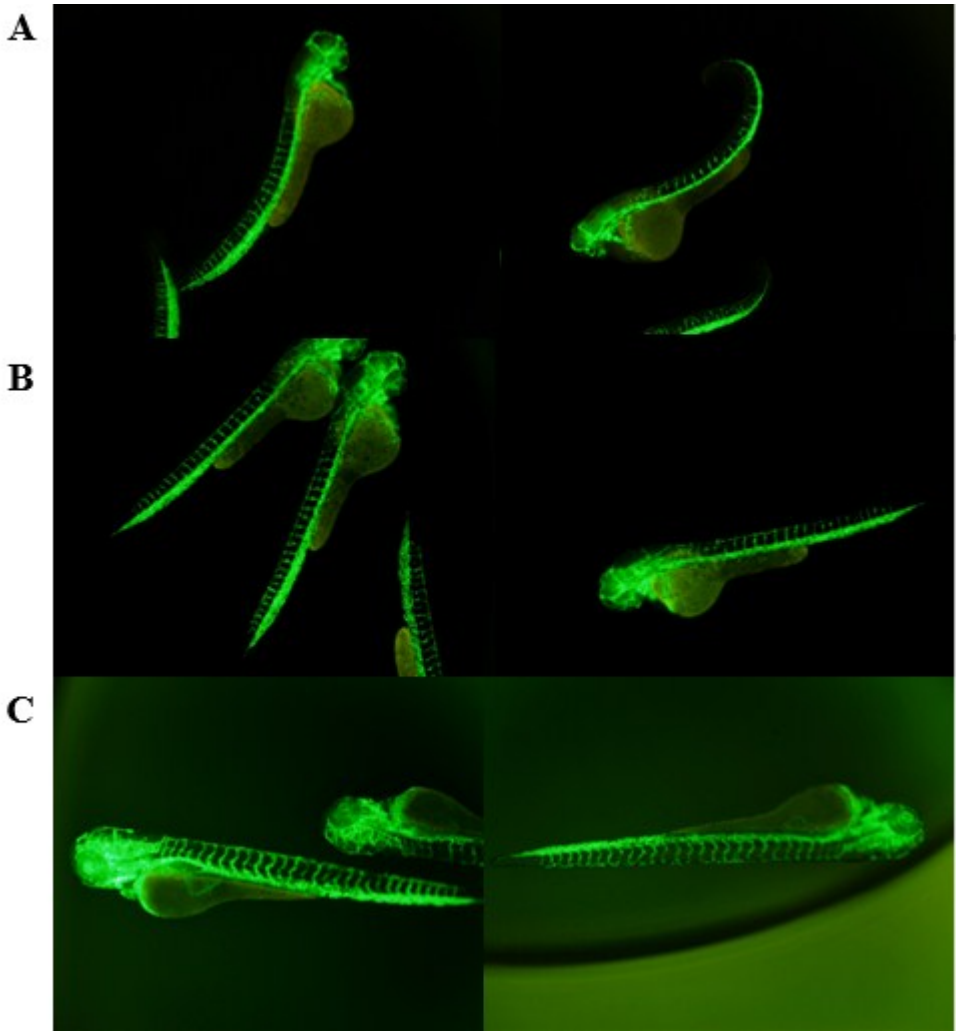

Fig. S7 Bioassay-guided fractionation results. A. 15 μg/ml pH 9 fraction (++); B. 15 μg/ml pH 6 fraction; C. 15 μg/ml acid-insoluble fraction.