Discovery and synthesis of (+)/(–)-muyoquinone A, a pair of rare cyclopropane bridge-containing pentacyclic benzoquinone dimer with cytotoxic activity

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1. General Experimental Procedures.

Optical rotations were obtained using a Rudolph Autopol V automatic polarimeter at 20°C (Rudolph Research Analytical, Hackettstown, USA). IR analysis was performed using a Nicolet 5700 FT-IR spectrometer (FT-IR microscope transmission, Thermo Electron Corporation, Madison, WI, USA). UV spectra were recorded by a V-670 UV-visible/NIR spectrophotometer (JASCO, Tokyo, Japan). NMR spectra were recorded by an Agilent Technologies DD2-500 spectrometer a Bruker A VIIIHD-600 spectrometer (Bruker Corp. Karlsruhe, Germany) and a Bruker AVANCE NEO 700 spectrometer (Bruker Corp. Karlsruhe, Germany) ; HR-ESIMS data were measured by an Agilent Technologies 6250 Accurate-Mass Q-TOF LC/MS spectrometer (Agilent Technologies, Ltd., Santa Clara, CA, USA) and a Thermo Scientific Q-Exactive Fouce LC/MS spectrometer (Thermo Fisher Scientific, USA). X-ray diffraction analysis was conducted on an XtaLAB Synergy R, HyPix diffractometer at 100K using Cu Kα radiation. Preparative HPLC was performed on a Shimadzu LC-6AD instrument with SPD-20 detector (Shimadzu, Tokyo, Japan), using YMC-Pack ODS-A column (250 × 10 mm, 5 μm, YMC co., Ltd, Kyoto, Japan). ODS (45-70 μm, Merck, Darmstadt, Germany) and silica gel (200-300 mesh, Qingdao Marine Chemical Inc. China) were used for column chromatography. Thinlayer chromatography (TLC) was conducted on glass precoated with silica gel GF254 (Qingdao Marine Chemical Inc. China).

The chromatographic methanol and acetonitrile were produced by Mreda Company (other liquid reagents used were purchased from Shanghai Titan Technology Co., Ltd. unless otherwise specified, the grade was analytically pure and used directly without treatment). 2-hydroxy-3, 4-dimethoxybenzaldehyde, potassium persulfate, 4-dimethylaminopyridine, imidazole, tert-butyldimethylchlorosilane, ultra-dry tetrahydrofuran, n-butyllithium, 2, 3, 4-trimethoxybenzaldehyde, m-chloroperoxybenzoic acid, trifluoroacetic acid, tetrabutylammonium fluoride and cerium ammonium nitrate are all purchased from J&K Scientific. Anhydrous magnesium sulfate, sodium bisulfite, sodium hydroxide, potassium hydroxide, sodium chloride, sodium carbonate, ethyl acetate, n-hexane, dichloromethane purchased from Beijing Yinokai Technology Co., LTD., the grade is analytical pure.

2. Plant material.

The fresh leaves of *Tylophora ovata* were collected in Guangxi Province, China. The plant was identified by Prof. Song-Ji Wei of Guangxi College of Chinese Traditional Medicine. A voucher specimen (specimen No. S2505) was deposited in the herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, China.

3. Fungal material.

M. laterale was cultured and purified from a fresh leaf of T. ovata using PDA plating medium.

The strain was identified by Beijing Sunbiotech Co., Ltd based on the ITS (MT658037.1). It was named WET-14 and stored in Professor Liu's culture collection at the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College.

4. Fermentation, Extraction and Isolation.

M. laterale was cultured on a solid PDA medium at 28 °C for 3 days, then transferred to modified Martin broth conical flask and incubated on a rotary shaker (110 rpm) for 4 days at 28 °C. The culture liquid (10 mL) was transferred into the solid rice medium (100 g of rice and 100 mL of water in each 1000 mL flask). We prepared 100 flasks of rice medium. After culture for 30 days at room temperature, ethanol was used to extract for three times. The supernatants were evaporated under reduced pressure to yield the extract (1192 g). Then the extract was suspended in water and partitioned with petroleum ether and ethyl acetate, successively. The EtOAc fraction (51.5 g) was separated by a silica gel column and then ten fractions (W1-W10) were obtained by using CH_2Cl_2-MeOH (200:1-5:1) as mobile phase. Fraction W4 (18 g) was separated over an ODS column and eluted with gradient MeOH in water (10, 30, 50, 70 and 100%) to yield subfractions Z1-Z7. Subfraction Z6 (6.7 g) was separated by a silica gel column and then eight fractions (g1-g8) were obtained by using CH_2Cl_2-MeOH (100:1-10:1) as mobile phase. Fraction g2 was purified by RP-HPLC (C_{18} column) to produce compound **1** (8.5 mg, 64% CH_3CN, t_R = 32 min).

Muyoquinone A (**1**): orange crystal; (+)-**1a** ($[\alpha]_D^{20}$+117.0, c=0.10, CH_3OH) and (−)-**1b** ($[\alpha]_D^{20}$-112.0, c=0.10, CH_3OH); UV (CH_3CN) λ_{max} (log ε) 207 (4.27), 293 (4.07) nm; IR ν_{max} 2955, 2933, 2872, 1667, 1594, 1455, 1284cm^{-1}; CD (CH_3CN) λ_{max} ($\Delta\varepsilon$): 218 (-0.49), 246 (+0.19), 286 (+0.45) nm; ^{1}H NMR and ^{13}C NMR data, see Table 1; (+)-HRESIMS *m/z* 471.20078 $[M+H]^+$ (calcd. for $C_{26}H_{30}O_8$, 471.20134).

5. X-ray Crystallographic Analysis of compound 1.

Crystallographic data (excluding structure factors) of compound **1** (Cu Kα radiation) have been deposited with the Cambridge Crystallographic Data Center as supplementary publication numbers. The data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Crystal Data for $C_{26}H_{30}O_8$ (M =470.50 g/mol): triclinic, space group P-1 (no. 2), a = 9.2609(2) Å, b = 12.1917(4) Å, c = 12.2211(3) Å, α = 114.996(2)°, β = 97.054(2)°, γ = 107.271(2)°, V = 1144.02(6) Å^3, Z = 2, T = 100.00(10) K, μ(Cu Kα) = 0.837 mm^{-1}, D_{calc} = 1.366 g/cm^3, 13307 reflections measured (8.332° ≤ 2θ≤ 146.974°), 4477 unique (R_{int} = 0.0271, R_{sigma} = 0.0230) which were used in all calculations. The final R_1 was 0.0379 ($I > 2\sigma(I)$) and wR_2 was 0.1037 (all data). CCDC number :2091018.

Table S1. X-ray crystallographic data of natural (±)-Muyoquinone A

Identification code	(±)-Muyoquinone A
Empirical formula	$C_{26}H_{30}O_8$
Formula weight	470.50
Temperature/K	100.00(10)
Crystal system	triclinic
Space group	P-1
a/Å	9.2609(2)
b/Å	12.1917(4)
c/Å	12.2211(3)
α/°	114.996(2)
β/°	97.054(2)
γ/°	107.271(2)
Volume/Å^3	1144.02(6)
Z	2
ρ_{calc}g/cm^3	1.366
μ/mm^{-1}	0.837
F (000)	500.0
Crystal size/mm^3	0.26 × 0.24× 0.12
Radiation	CuKα (λ = 1.54184)
2θ range for data collection/°	8.332 to 146.974
Index ranges	$-11 \leq h \leq 11$, $-15 \leq k \leq 15$, $-11 \leq l \leq 14$
Reflections collected	13307
Independent reflections	4477 [R_{int} = 0.0271, R_{sigma} = 0.0230]
Data/restraints/parameters	4477/0/313
Goodness-of-fit on F^2	1.067
Final R indexes [I>=2σ (I)]	R_1 = 0.0379, wR_2 = 0.1017
Final R indexes [all data]	R_1 = 0.0401, wR_2 = 0.1037
Largest diff. peak/hole / e Å^{-3}	0.31/-0.22

Table S2. X-ray crystallographic data for synthetic (+)-Muyoquinone A

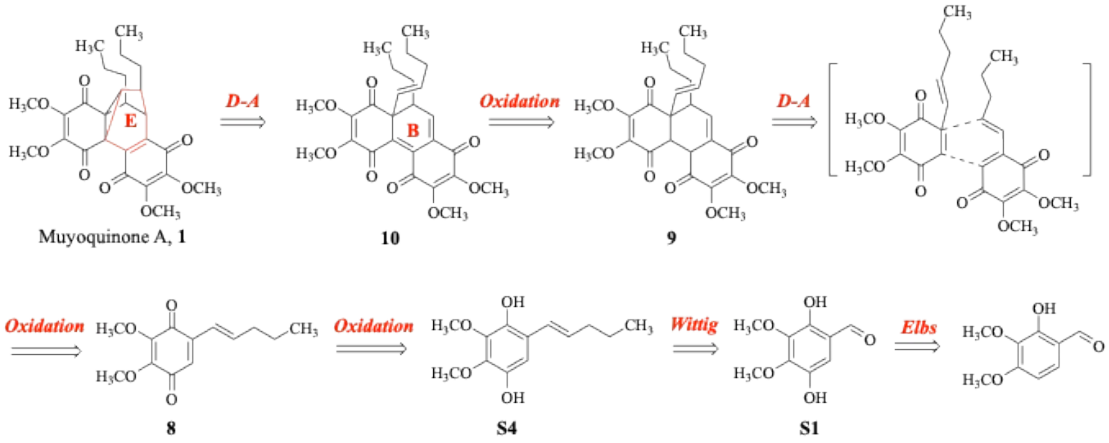
Identification code	(+)-Muyoquinone A
Empirical formula	$C_{26}H_{30}O_8$
Formula weight	470.50
Temperature/K	100.00(10)
Crystal system	monoclinic
Space group	Pc
a/Å	17.8130(3)
b/Å	31.9544(5)
c/Å	8.12990(10)
α /°	90
β /°	91.874(2)
γ /°	90
Volume/Å^3	4625.09(12)
Z	8
ρ_{calc}g/cm^3	1.351
μ /mm^{-1}	0.828
F(000)	2000.0
Crystal size/mm^3	0.35 × 0.03 × 0.02
Radiation	CuKα(λ = 1.54184)
2Θ range for data collection/°	7.434 to 133.192
Index ranges	-21 ≤ h ≤ 21, -38 ≤ k ≤ 38, -9 ≤ l ≤ 9
Reflections collected	64328
Independent reflections	14787 [R_{int} = 0.0719, R_{sigma} = 0.0481]
Data/restraints/parameters	14787/56/1249
Goodness-of-fit on F^2	1.100
Final R indexes [I>=2 σ (I)]	R_1 = 0.0924, wR_2 = 0.2466
Final R indexes [all data]	R_1 = 0.0957, wR_2 = 0.2489
Largest diff. peak/hole / e Å^{-3}	0.85/-0.43
Flack parameter	0.00(10)

Table S3. X-ray crystallographic data for synthetic (±)-Muyoquinone A

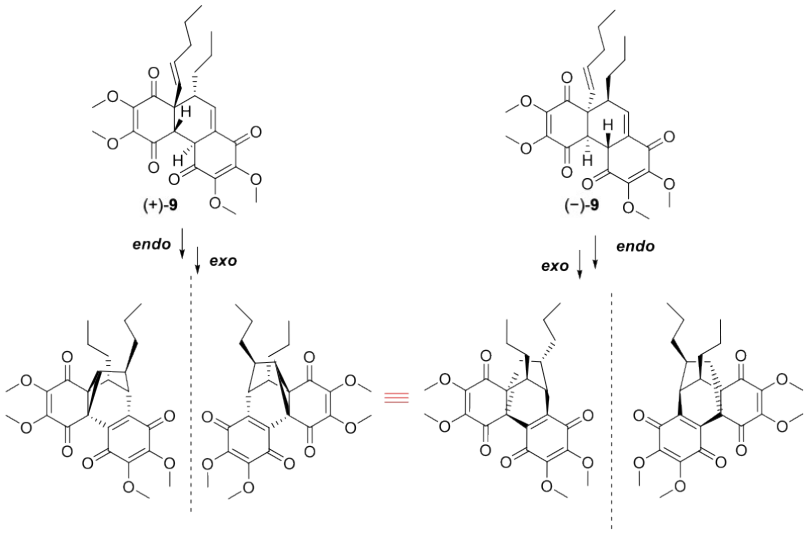
Identification code	(±)-Muyoquinone A
Empirical formula	$C_{26}H_{30}O_8$
Formula weight	470.50
Temperature/K	100.00(10)
Crystal system	triclinic
Space group	P-1
a/Å	9.2598(2)
b/Å	12.1943(3)
c/Å	12.2161(3)
α /°	115.022(2)
β /°	97.0123(19)
γ /°	107.239(2)
Volume/Å^3	1143.96(5)
Z	2
ρ_{calc}g/cm^3	1.366
μ /mm^{-1}	0.837
F(000)	500.0
Crystal size/mm^3	0.15 × 0.1 × 0.03
Radiation	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	8.336 to 153.748
Index ranges	-11 ≤ h ≤ 11, -15 ≤ k ≤ 14, -14 ≤ l ≤ 15
Reflections collected	23732
Independent reflections	4518 [R_{int} = 0.0321, R_{sigma} = 0.0211]
Data/restraints/parameters	4518/0/313
Goodness-of-fit on F^2	1.058
Final R indexes [I>=2σ(I)]	R_1 = 0.0347, wR_2 = 0.0930
Final R indexes [all data]	R_1 = 0.0386, wR_2 = 0.0962
Largest diff. peak/hole / e Å^{-3}	0.30/-0.23

6. Retrosynthetic analysis of compound 1

Based on the construction strategy and key reaction design of the hexacyclic skeleton mentioned in the previous literature, we take one configuration (+)-muyoquinone A as an example to perform a retrosynthetic analysis (Scheme S1). The cage-like skeleton of (+)-muyoquinone A contains two cyclohexene fragments (rings B and E), which are constructed by two steps of Diels-Alder reactions. The formation of ring E promotes the assembly of the cage-like skeleton, while the formation of ring B completes the polymerization between two molecules. Firstly, the cage-like skeleton of (+)-muyoquinone A is formed from intermediate **10** through a single intramolecular D-A reaction. Meanwhile, intermediate **9** is easily oxidized to form cis-1,3-butadiene in ring B. Subsequently, the cis-1,3-butadiene on one molecule of intermediate **8** undergoes a single intermolecular D-A reaction with the double bond on the quinone ring of another molecule of **8** to form the phenanthrene skeleton of muyoquinone A. Finally, the complex hexacyclic skeleton of muyoquinone A is simplified to a 1,4-benzoquinone substituted with a vinyl group. The quinone structure is directly formed by oxidation of intermediate **S4** while the vinyl group is generated through a simple Wittig reaction starting from an aldehyde.



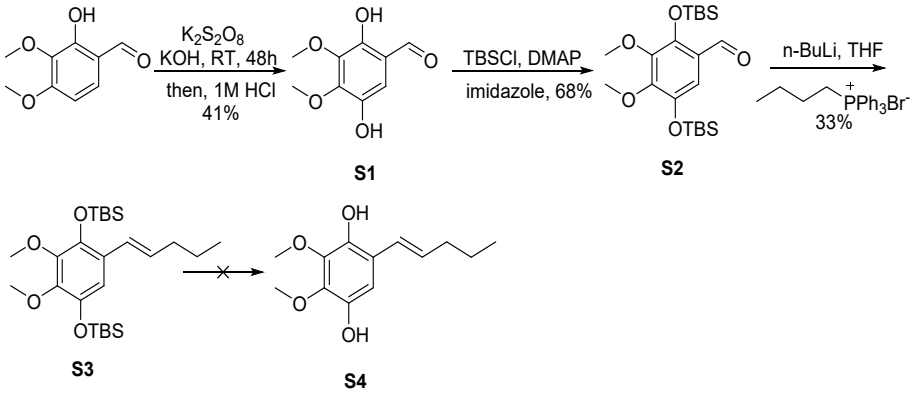
Scheme S1. Reverse synthesis analysis of muyoquinone A (**1**).



Scheme S2. Four theoretical configurations resulting after the two-step D-A reactions.

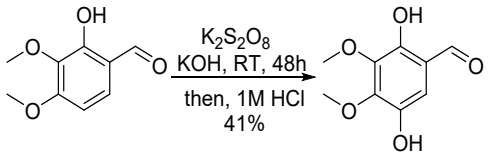
7. Synthesis procedures

In order to provide sufficient sample quantity for further biological analysis, the readily available 2-hydroxy-3,4-dimethoxybenzaldehyde was used as a starting material to form the structural fragment of para-dihydroxyphenol through a one-step Elbs oxidation reaction (Scheme S2). The intermediate **S1** was protected on the dihydroxyphenol hydroxyl group with TBS and then obtained via Wittig reaction to yield an intermediate **S3** with a trans double bond side chain. However, during the separation process, **S4** became unstable on silica gel and gradually converted into a red substance, resulting in decreased yield and affecting the implementation of the next step reaction. Fortunately, it was discovered during the experimental process that when the ortho-hydroxyl group was substituted by methyl groups, its stability increased and it could still be oxidized by cerium ammonium nitrate to form quinone-like structure. Therefore, substrate replacement and certain modifications were made to the route.



Scheme S3. Exploration of the synthetic route of muyoquinone A (**1**).

7.1 Synthesis of S1

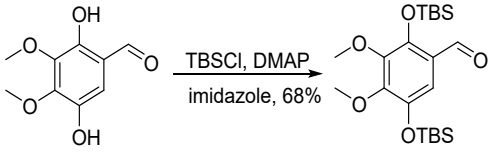


S1

Intermediate S1: The substrate 2-hydroxy-3, 4-dimethoxybenzaldehyde (5.0g, 27.4mmol) was placed in a 250 mL round-bottomed flask, completely dissolved with 6% KOH solution in an ice water bath, and then added (7.42g, 27.4mmol) to the solid in batches under agitation. After reaction for 12 hours, the reaction solution was acidified with 1M hydrochloric acid to pH=6.5, and then the reaction solution was extracted with ethyl acetate (100×3 times) to remove the unfinished substrate. After adding 1M hydrochloric acid to the water phase to pH=1.0, heating reflux at 100℃ for 2 hours. After the reaction system was cooled to room temperature, it was extracted with ethyl acetate (100 mL×3) to recover the organic phase. After adding salt water and washing, anhydrous magnesium sulfate is dried, and the coarse product is concentrated by rotating evaporation. The crude product was separated by silica gel column chromatography (n-hexane: ethyl acetate = 5:1) to obtain the intermediate S1 (colorless crystal, 2.22g, 41%). Rf = 0.55 (n-hexane: ethyl acetate = 1:1).

^{1}H NMR (700 MHz, $CDCl_3$) δ_H 11.03 (s, 1H), 9.71 (s, 1H), 6.85 (s, 1H), 5.53 (s, 1H), 4.12 (s, 3H), 3.94 (s, 3H). ^{13}C NMR (175 MHz, $CDCl_3$) δ_C 195.5, 151.0, 147.2, 141.9, 139.5, 115.8, 111.7, 61.5, 61.1.

7.2 Synthesis of S2



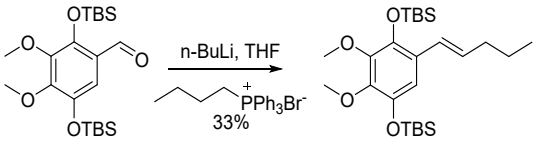
S1 **S2**

Intermediate S2: The intermediate **S1**(0.83 g, 4.2 mmol), tert-butyldimethylchlorosilane (2.50 g, 17 mmol), imidazole (0.63 g, 9.2 mmol) and 4-dimethylaminopyridine (0.26 g, 2.1 mmol) were successively added into 250 mL round-bottomed flask. Add 100 mL of methylene chloride and stir at room temperature for 12 hours. After the reaction was monitored by TLC plate until the substrate completely disappeared, the reaction liquid was extracted three times with water (100 mL×3), the organic phase was recovered, and the anhydrous magnesium sulfate was washed with salt water, dried, and the crude product was concentrated by rotary evaporation. Intermediate **S2** (colorless oil liquid, 1.24g, 68%) was obtained by silica gel column chromatography (n-hexane: ethyl acetate = 50:1).

^{1}H NMR (700 MHz, DMSO-d_6) δ_H 10.20 (s, 1H), 6.90 (s, 1H), 3.86 (s, 3H), 3.76 (s, 3H), 0.98

(d, J = 4.1 Hz, 18H), 0.19 (s, 6H), 0.17 (s, 6H).

7.3 Synthesis of S3

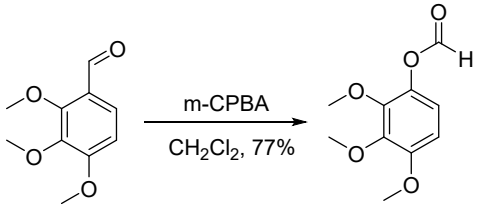


S2 **S3**

Intermediate S3: Anhydrous butyltriphenylphosphine bromide (1.10g, 2.32mmol) was put into a 100 mL three-neck bottle, and 40 mL ultra-dry tetrahydrofuran was added under nitrogen protection in a dry ice-acetone bath. The reaction system temperature was maintained at -78℃, and n-butyllithium (2.17mL, 3.48mmol) was slowly added under the mixture. The reaction liquid is bright yellow emulsion. Continue to stir at -78℃ for 30 min, and then gradually restore the reaction system to room temperature and continue to react for 1 hour, the reaction liquid becomes orange-red clarified solution. The reaction system was reduced to -78℃ again, and after holding for 10 min, the intermediate **S2** (0.50g, 1.17mmol) was slowly added, and the reaction liquid gradually turned into orange-yellow emulsion. After the reaction was continued at this temperature for 30 min, the reaction was restored to room temperature and continued to stir until the reaction point disappeared. The quenching reaction was performed by adding 10 mL water drop by drop under the ice bath, and the reaction liquid was gradually clarified. The reaction liquid was extracted with ethyl acetate (100 mL×3), the organic phase was recovered and washed with salt water, and the coarse product was dried with anhydrous magnesium sulfate. The crude product was separated by silica gel column chromatography (n-hexane: ethyl acetate = 50:1) to obtain intermediates **S3**(light yellow oily liquid, 0.17g, 33%).

^{1}H NMR (700 MHz, $CDCl_3$) δ_H 6.67 (s, 1H), 6.61 (dt, J = 15.9, 1.6 Hz, 1H), 5.95 (dt, J = 15.9, 6.8 Hz, 1H), 3.78 (d, J = 24.8 Hz, 6H), 2.16 (qd, J = 7.0, 1.6 Hz, 2H), 1.48 (m, 2H), 1.00 (d, J = 2.5 Hz, 18H), 0.94 (t, J = 7.4 Hz, 3H), 0.16 (d, J = 16.5 Hz, 12H)。 ^{13}C NMR (175 MHz, $CDCl_3$) δ_C 145.2, 144.3, 143.4, 140.8, 123.0, 125.3, 125.1, 112.0, 60.9, 60.7, 35.5, 26.2, 25.9, 22.6, 18.8, 18.5, 14.0.

7.4 Synthesis of S5

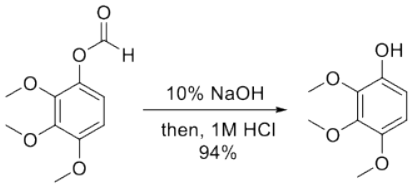


S5

Intermediate **S5**: Substrate 2, 3, 4-trimethoxybenzaldehyde (100.0 g, 0.51 mol) was added to a 2 L round-bottomed flask and dissolved with 500 mL dichloromethane. In the ice water bath at 0℃, m-chloroperoxybenzoic acid (152.5 g, 1.2 mol) was added in batches, and the reaction temperature was maintained at 0℃-5℃ for 30min. Gradually bring the reaction back to room temperature and continue stirring for 12 hours to obtain a white suspension. Filter the sediment. Continue to wash the precipitate with 1000 mL methylene chloride to recover the organic phase. The organic phase was extracted twice with 500 mL 10% (v/w) sodium bisulfite aqueous solution,500 mL 5% sodium carbonate aqueous solution, and 500 mL water. Brine washing, anhydrous sodium sulfate drying, filtration, rotary evaporation concentrated intermediate **S5** (yellow oil, 77.4g, 77%). Rf = 0.36 (n-hexane: ethyl acetate = 5:1).

^{1}H NMR (700 MHz, $CDCl_3$) δ_H 8.25 (s, 1H), 6.79 (d, J = 9.0 Hz, 1H), 6.63 (d, J = 9.0 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (175 MHz, $CDCl_3$) δ_C 159.7, 152.2, 145.6, 143.2, 136.9, 116.5, 106.6, 77.3, 77.2, 77.0, 61.3, 61.1, 56.3.

7.5 Synthesis of 2

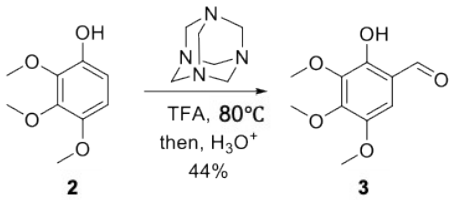


S5 **2**

Intermediates 2: The intermediates S5(76.0g, 0.36mol) were put into a 1L round-bottomed flask, dissolved with 400 mL methanol and placed in an ice bath at 0℃, slowly added into 200 mL 10% sodium hydroxide solution, stirred at 0℃ for 20 min and then gradually heated to 40℃, and monitored by TLC plate after 1 hour of agitation. After the substrate point completely disappeared, the reaction bottle was re-placed in the ice bath, and 1M hydrochloric acid neutralization reaction solution was slowly added to pH = 6. The reaction solution was extracted by ethyl acetate for three times (500 mL×3), the organic phase was recovered and washed with salt water, and the coarse product was dried by anhydrous magnesium sulfate. The crude product was separated by silica gel column chromatography (n-hexane: ethyl acetate = 5:1) to obtain intermediates 1 (light yellow oily liquid, 64.7 g, 94%).

^{1}H NMR (700 MHz, $CDCl_3$) δ_H 6.62 (d, J = 8.9 Hz, 1H), 6.55 (d, J = 8.9 Hz, 1H), 5.46 (s, 1H), 3.95 (s, 2H), 3.89 (s, 2H), 3.80 (s, 3H). ^{13}C NMR (175 MHz, $CDCl_3$) δ_C 147.1, 143.5, 142.4, 140.6, 108.7, 107.7, 61.4, 61.0, 56.7.

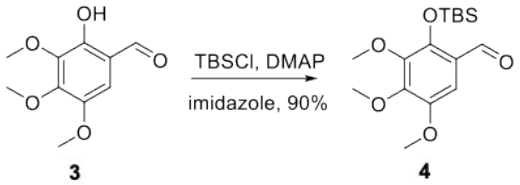
7.6 Synthesis of 3



Intermediates 3: The intermediates 2 (51.7g, 0.28mol) were put into a 1L round-bottomed flask, 400 mL trifluoroacetic acid was added, and then ulotriptin (39.4g, 0.28mol) was added under stirring, then the reaction liquid was heated to 80℃, and the reflux was condensed overnight. Then the reaction was restored to room temperature, adding 200 mL ice water at room temperature and stirring for 1 hour. The reaction solution was extracted three times with ethyl acetate (500 mL×3), the organic phase was washed with salt water, and the anhydrous magnesium sulfate was dried to obtain the crude product. The crude product was separated by silica gel column chromatography (n-hexane: ethyl acetate = 5:1) to obtain intermediates 3 (light yellow oily liquid, 25.6 g, 44%). Rf: 0.75 (n-hexane: ethyl acetate = 1:1).

^{1}H NMR (500 MHz, DMSO-D_6) δ_H 10.06 (s, 1H), 6.97 (s, 1H), 5.69 (s, 1H), 3.83 (s, 3H), 3.74 (s, 3H), 3.72 (s, 3H). ^{13}C NMR (125 MHz, DMSO-D_6) δ_C 191.4, 150.6, 149.6, 146.6, 141.9, 117.7, 106.4, 61.4, 61.2, 56.5.

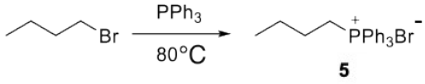
7.7 Synthesis of 4



Intermediates 3: The intermediates 3 (22.3 g, 0.11 mol), tert-butyldimethylchlorosilane (71.1 g, 0.21 mol), imidazole (64.2 g, 0.53 mol), 4-dimethylaminopyridine (0.8 g, 11.8 mmol) were successively added into 1 L round-bottomed flask. Add 500 mL dichloromethane and stir at room temperature for 12 hours. After the reaction was monitored by TLC plate until the substrate completely disappeared, the reaction liquid was extracted three times with water (500 mL×3), the organic phase was recovered, and the anhydrous magnesium sulfate was washed with salt water, dried, and the crude product was concentrated by rotary evaporation. Intermediate 4 (light yellow oily liquid, 30.85g, 90%) was obtained by silica gel column chromatography (n-hexane: ethyl acetate = 20:1). Rf = 0.55 (n-hexane: ethyl acetate = 20:1).

^{1}H NMR (700 MHz, $CDCl_3$) δ_H 10.33 (s, 1H), 7.08 (s, 1H), 3.97 (s, 3H), 3.85 (s, 3H), 3.81 (s, 3H), 1.01 (s, 9H), 0.20 (s, 6H). ^{13}C NMR (175 MHz, $CDCl_3$) δ_C 189.2, 149.5, 148.6, 148.2, 145.0, 122.8, 103.8, 61.3, 61.0, 56.2, 26.0, 26.0, 25.8, 25.8, 25.8.

7.8 Synthesis of 5



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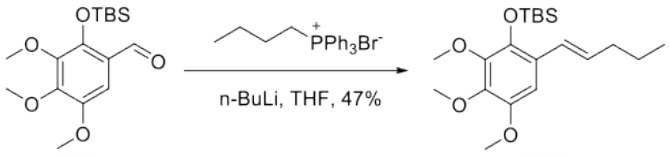
Intermediates 5: To a stirred solution of n-bromobutane (30g, 218.7mmol) and triphenylphosphonium (52.2g, 198.9mmol) in toluene (120 mL). The mixture was allowed to stir at 80°C for 48 h before the resultant mixture was washed with ethyl acetate to obtain a white solid (36g). R_f = 0.43(dichloromethane: methanol= 10:1).

^{1}H NMR (500 MHz, methanol-d4): δ_H 7.76-7.91(m, 15H), 3.31-3.49 (m, 2H), 1.58-1.68 (m, 4H), 0.95-0.98 (m, 3H).

7.9 Synthesis of 6

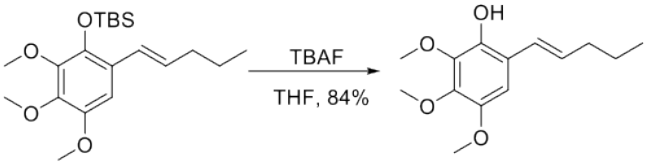


4 6

Intermediates **6**: The anhydrous butyltriphenylphosphine bromide (36.0 g, 57.9 mmol) was put into 1000 mL three-neck bottle, and 500 mL ultra-dry tetrahydrofuran was added under nitrogen protection in dry ice-acetone bath. The reaction system temperature was maintained at -78℃, and n-butyllithium (92.6 mL, 92.6 mmol) was slowly added under agitation. The reaction liquid is bright yellow emulsion. Continue to stir at -78℃ for 30 min, and then gradually restore the reaction system to room temperature and continue to react for 1 hour, the reaction liquid becomes orange-red clarified solution. The reaction system was reduced to -78℃ again, and after holding for 10 min, the intermediate **4** (27.4 g, 84.2mmol) was slowly added, and the reaction liquid gradually turned into orange-yellow emulsion. After the reaction was continued at this temperature for 30 min, the reaction was restored to room temperature and continued stirring until the reaction point disappeared. The quenching reaction was performed by adding 100 mL water drop by drop under the ice bath, and the reaction liquid was gradually clarified. The reaction liquid was extracted with ethyl acetate (500 mL×3), the organic phase was recovered and washed with salt water, and the coarse product was dried with anhydrous magnesium sulfate. The crude product was separated by silica gel column chromatography (n-hexane: ethyl acetate = 50:1) to obtain intermediates **6** (light yellow oily liquid, 10.4 g, 47%).

^{1}H NMR (500 MHz, DMSO-D_6) δ_H 6.81 (s, 1H), 6.58 (dt, J = 16.0, 1.6 Hz, 1H), 6.15 (dd, J = 15.8, 6.9 Hz, 1H), 3.76 (s, 3H), 3.72 (s, 3H), 3.69 (s, 3H), 2.18 – 2.10 (m, 2H), 1.45 (m, 2H), 0.97 (s, 9H), 0.91 (t, J = 7.4 Hz, 3H), 0.14 – 0.07 (m, 6H). ^{13}C NMR (125 MHz, DMSO-D_6) δ_C 147.6, 144.5, 141.7, 139.3, 129.6, 124.5, 123.7, 103.5, 60.6, 60.4, 55.9, 34. 9, 25.8, 21.9, 18.3, 13.7.

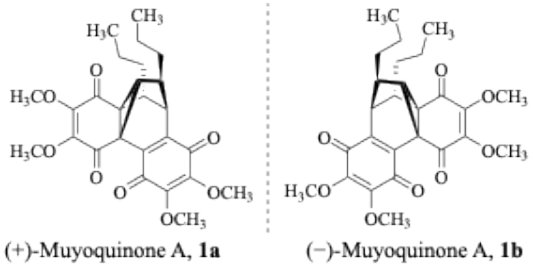
7.10 Synthesis of 7



Intermediates **7**: Intermediates **6** (10.0g, 27.4mmol) were put into 500 mL round bottom flask, 200 mL ultra-dry tetrahydrofuran was added, tetrabbutylammonium fluoride (30 mL, 30 mmol) was added under 0℃ ice bath, and the reaction was detected by TLC plate. After the substrate point completely disappeared, 50 mL was added for water quenching reaction. The reaction liquid (200 mL×3) was extracted with ethyl acetate, the organic phase was recovered, and the crude product was dried with anhydrous magnesium sulfate after brine washing. Intermediates **7** (colorless oil liquid, 6.45g, 84%) were isolated by silica gel column chromatography (n-hexane: ethyl acetate = 10:1). Rf = 0.52 (n-hexane: ethyl acetate = 5:1).

^{1}H NMR (700 MHz, $CDCl_3$) δ_H 6.60 (s, 1H), 6.52 (d, J = 15.9 Hz, 1H), 6.11 (dt, J = 16.1, 7.0 Hz, 1H), 3.86 (d, J = 0.9 Hz, 3H), 3.80 (d, J = 1.0 Hz, 3H), 3.75 (s, 3H), 2.13 (q, J = 7.2 Hz, 2H), 1.42 (h, J = 7.5 Hz, 2H), 0.88 (t, J = 7.4 Hz, 3H). ^{13}C NMR (175 MHz, $CDCl_3$) δ_C 146.6, 141.1, 140.5, 140.4, 131.2, 123.9, 118.8, 104.7, 61.3, 61.1, 56.6, 35.6, 22.8, 13. 9.

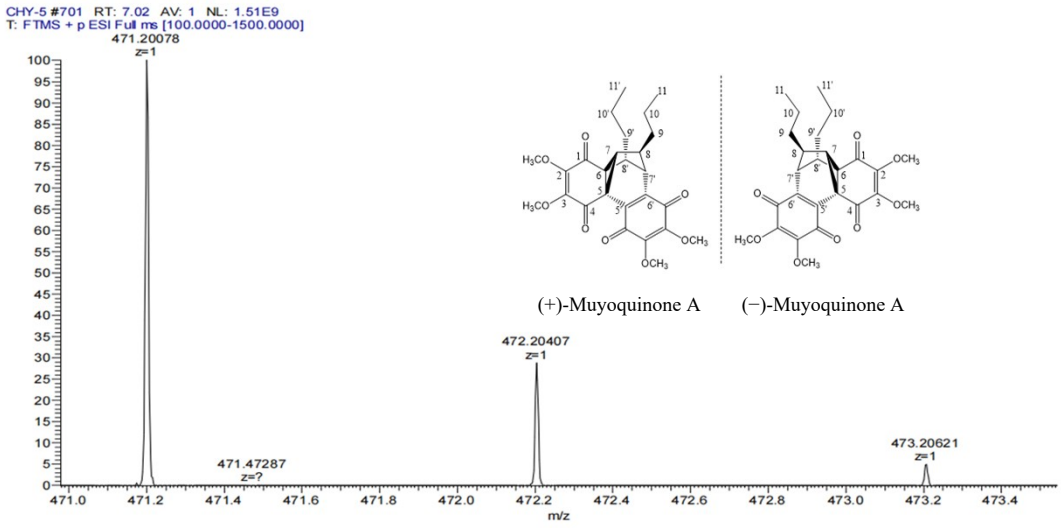
7.11 Synthesis of muyoquinone A



Muyoquinone A: Add ammonium cerium nitrate (27.2g, 49.4mmol) aqueous solution into 500 mL round-bottomed flask containing 25 g silica gel powder, stir until the silica gel is liquid, add 200 mL dichloromethane, and add **7** (5.0g, 19.8mmol) dichloromethane solution of intermediate drop by drop. The solution in the reaction bottle was rapidly transformed from orange yellow to red. After the reaction for 5 min, the organic phase was extracted and filtered, and the red oily liquid was concentrated by rotating evaporation. After the red liquid was dissolved with methanol and left for 7 days, muyoquinone A (241.1 mg, 2.58%) was separated by preparation of liquid phase (C_{18} semi-preparation column, mobile phase was 65% acetonitrile -35% water).

^{1}H NMR (700 MHz, $CDCl_3$) δ_H 4.09 (s, 3H), 4.05 (s, 3H), 4.03 (s, 3H), 4.00 (s, 3H), 3.59 (t, J = 4.5 Hz, 1H), 2.78 (dt, J = 11.4, 4.2 Hz, 1H), 2.41 (dd, J = 2.4, 1.2 Hz, 1H), 2.26 (m, 1H), 1.32 (m, 1H), 1.24 (d, J = 7.5 Hz, 2H), 1.14 (m, 2H), 1.02 (m, 2H), 0.84 (t, J = 7.3 Hz, 3H), 0.77 (t, J = 7.3 Hz, 3H), 0.46 (m, 1H).^{13}C NMR (175 MHz, $CDCl_3$) δ_C 188.0, 186.6, 180.5, 179.4, 149.0, 147.7, 145.7, 143.4, 137.2, 136.5, 61.3, 61.3, 61.2, 61.2, 46.3, 46.1, 42.5, 38.7, 36.9, 34.5, 29.6, 27.8, 20.9, 20.9, 14.1, 14.1.

8. HRMS, UV, IR and NMR spectra of compounds



m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition	
471.20078	471.20134	-1.2	11.5	C26 H31 O8	M+H

Fig. S1. HRESIMS data of natural muyoquinone A (**1**).

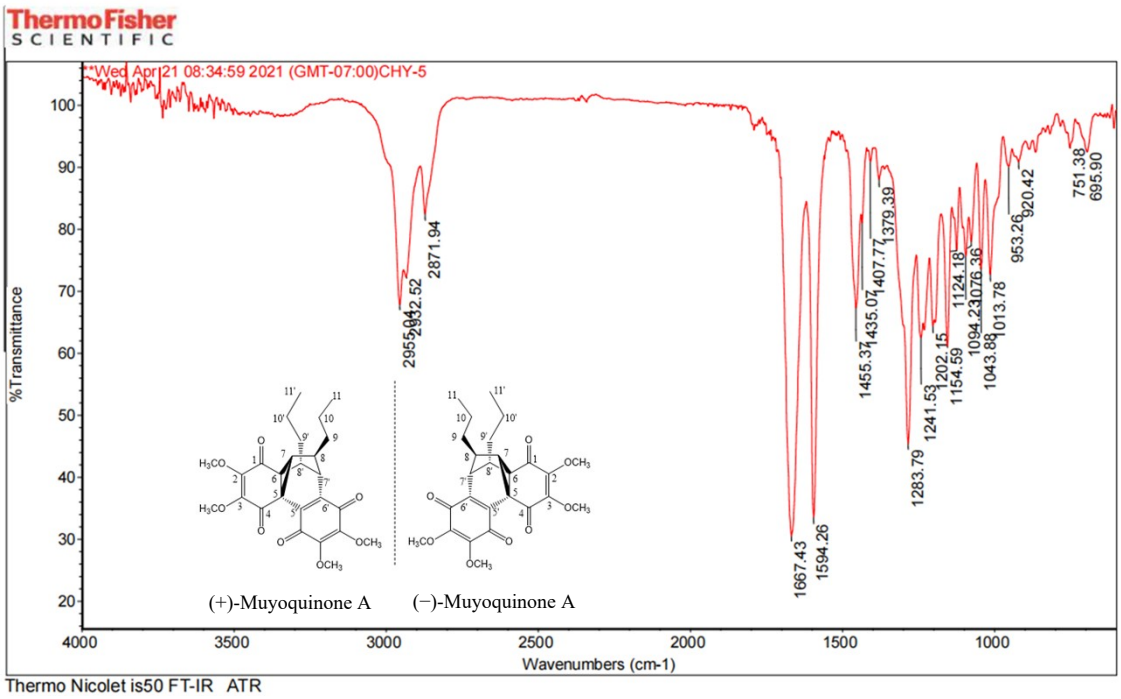


Fig. S2. IR spectrum of natural muyoquinone A (**1**).

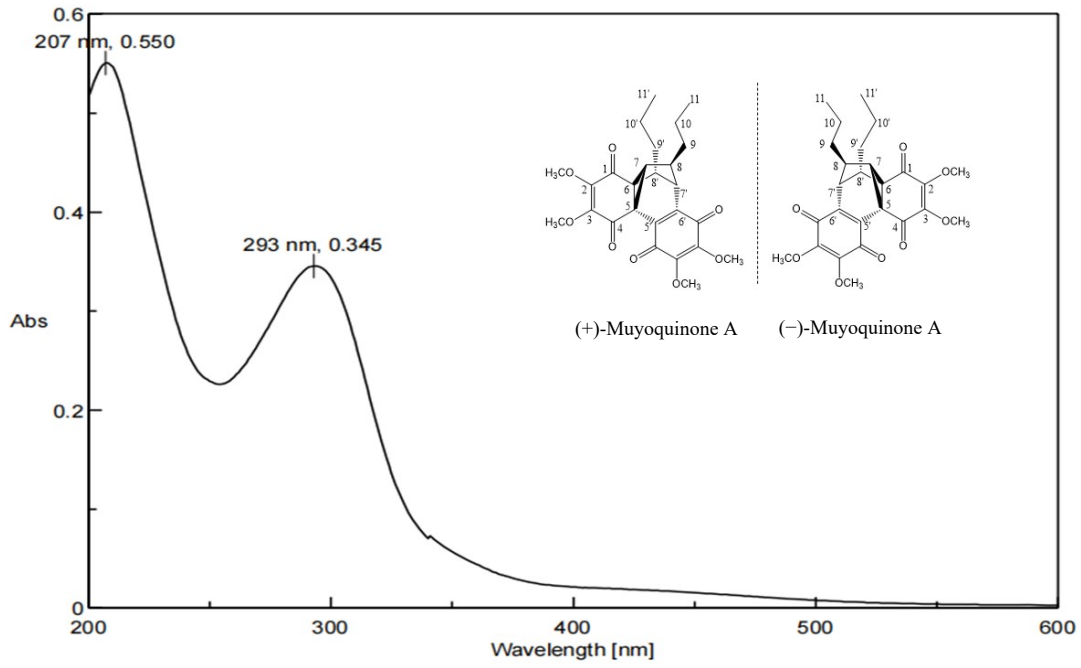


Fig. S3. UV spectrum of natural muyoquinone A (**1**) in CH_3CN.

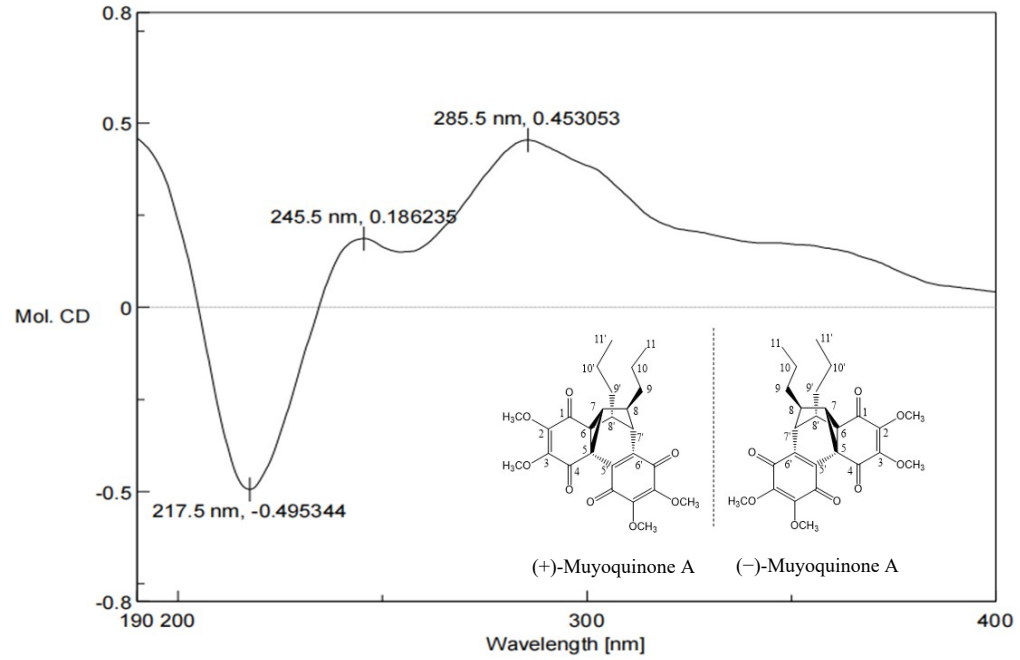


Fig. S4. CD spectrum of natural muyoquinone A (**1**) in CH_3CN.

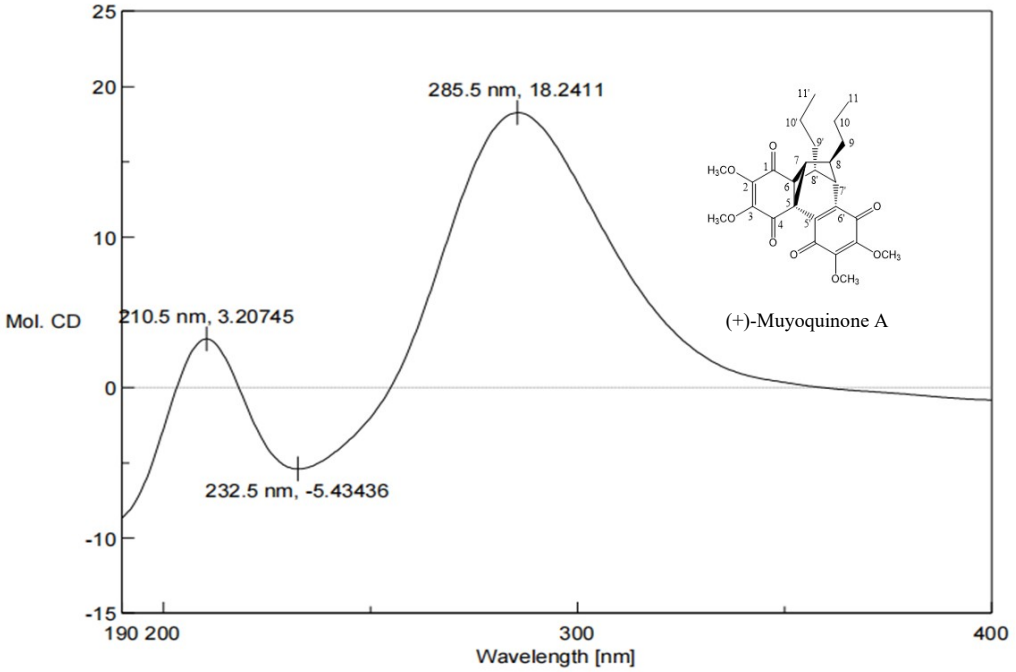


Fig. S5. CD spectrum of natural (+)-muyoquinone A in CH_3CN.

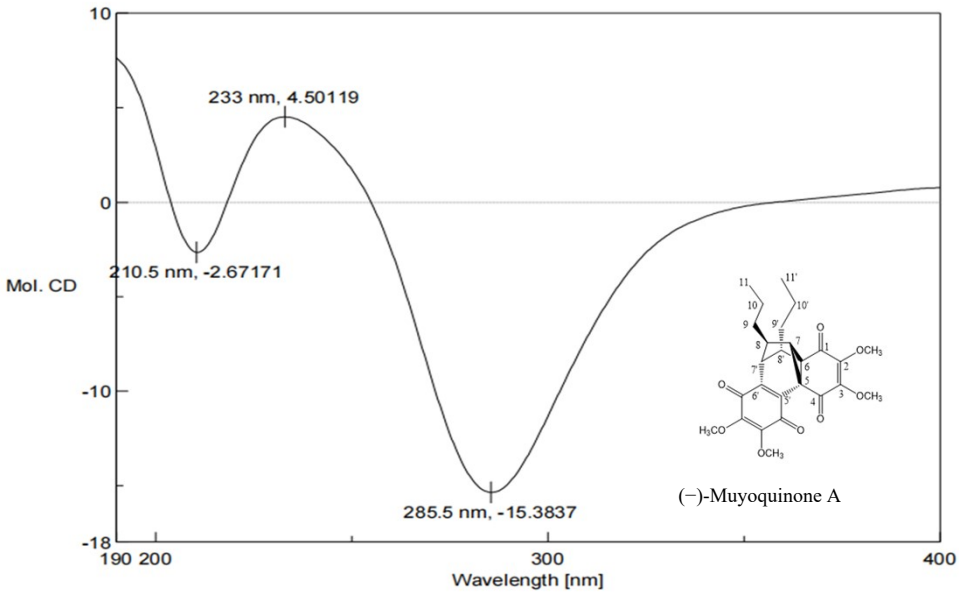


Fig. S6. CD spectrum of natural (−)-muyoquinone A in CH_3CN.

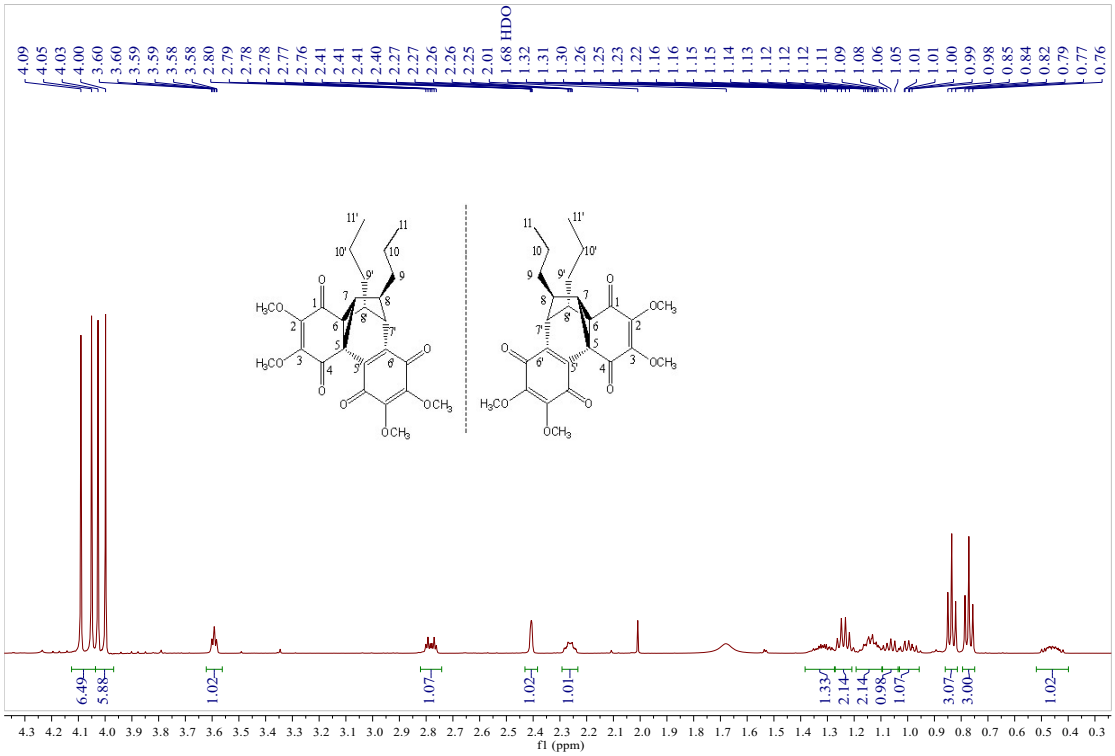


Fig. S7. ^{1}H NMR spectrum of natural muyoquinone A (**1**) (500 MHz, $CDCl_3$).

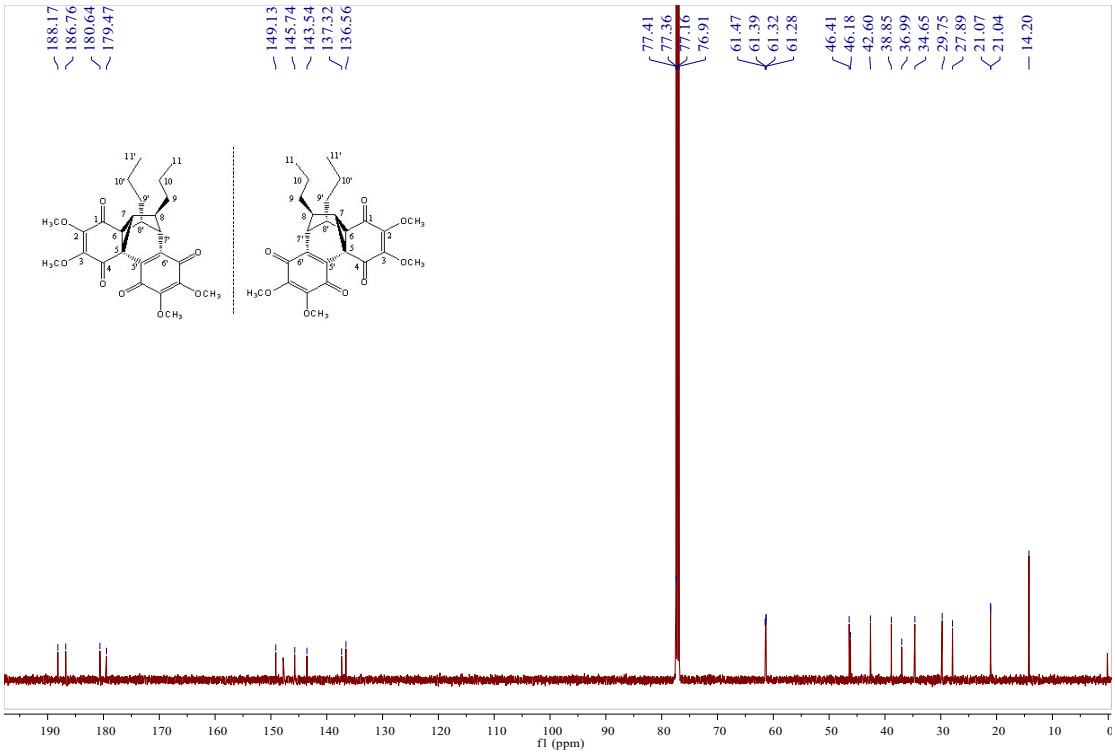


Fig. S8. ^{13}C NMR spectrum of natural muyoquinone A (**1**) (126MHz, $CDCl_3$).

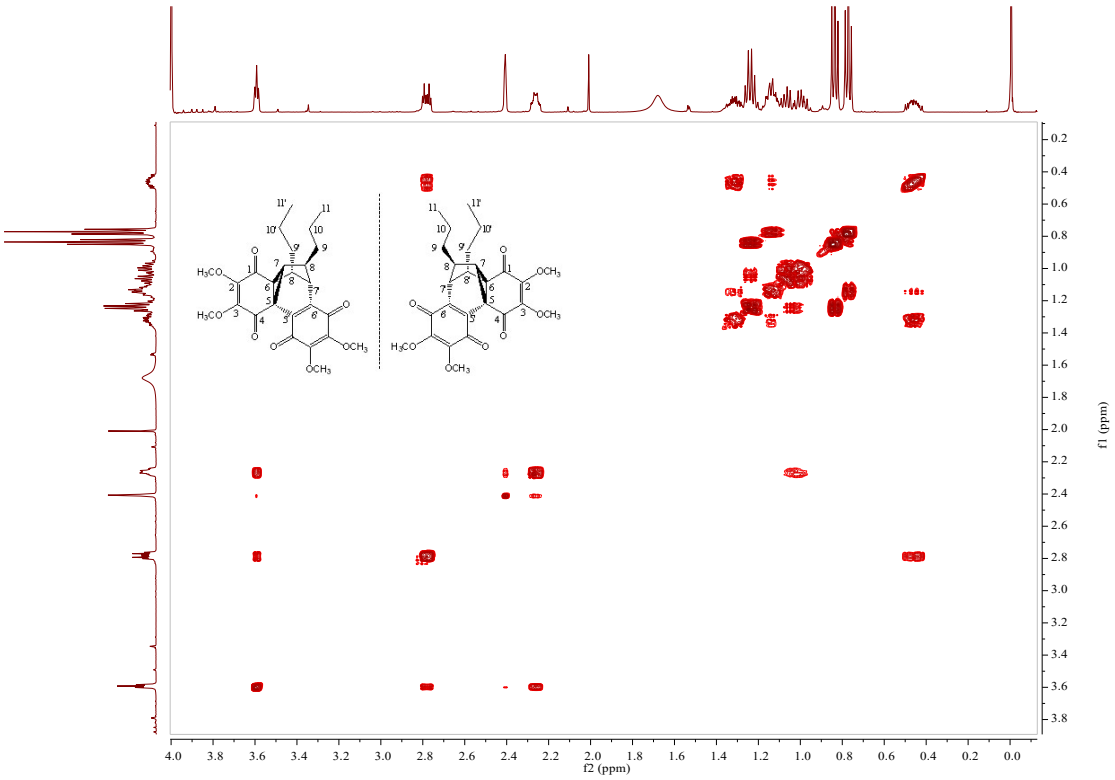


Fig. S9. ^{1}H-^{1}H COSY spectrum of natural muyoquinone A (**1**) (500MHz, $CDCl_3$).

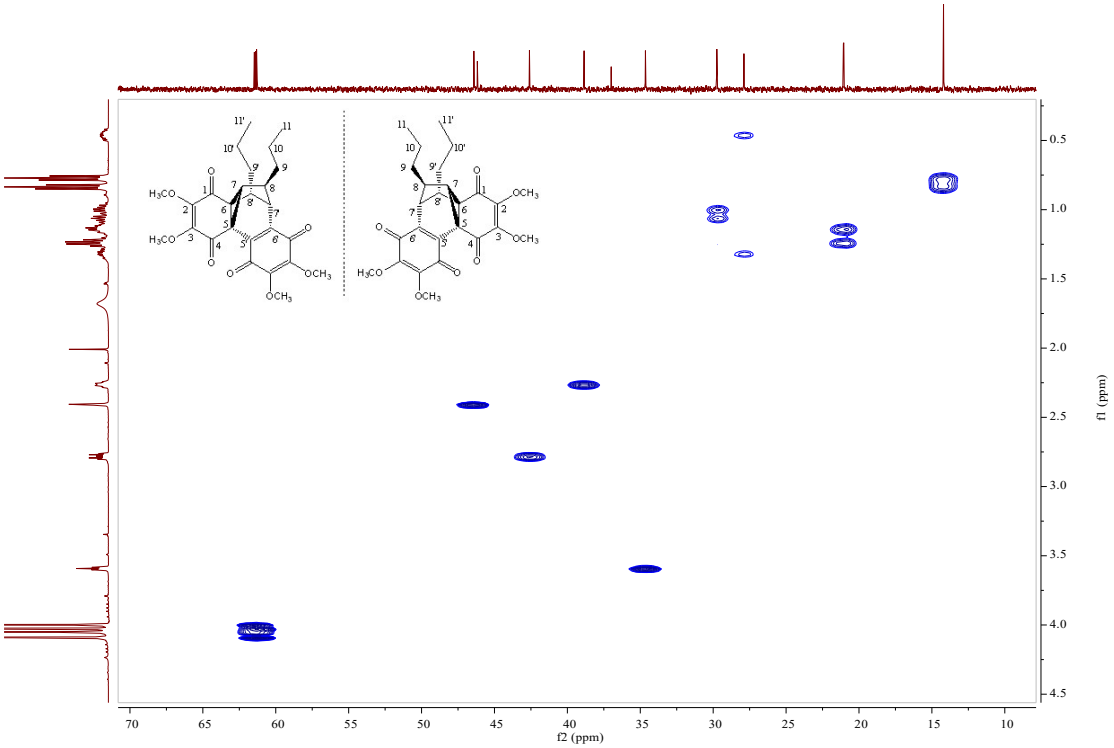


Fig. S10. HSQC spectrum of natural muyoquinone A (**1**) (500MHz, $CDCl_3$).

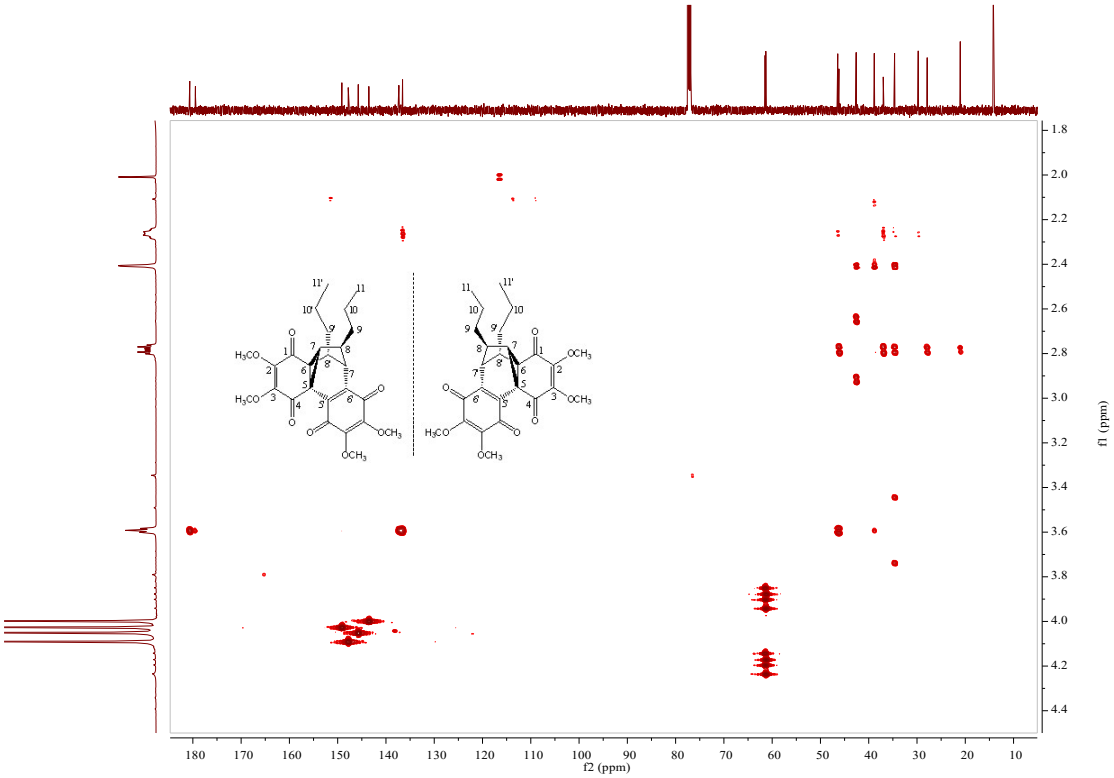


Fig. S11. HMBC spectrum of natural muyoquinone A (**1**) (500MHz, $CDCl_3$).

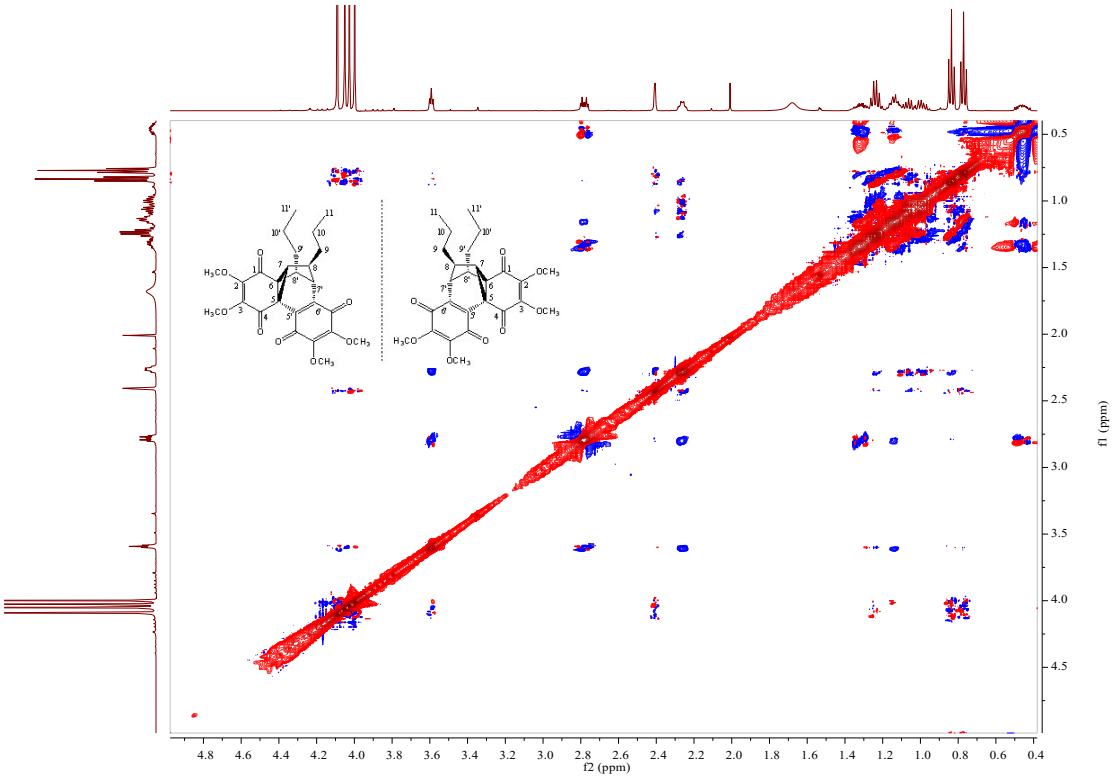


Fig. S12. ROESY spectrum of natural muyoquinone A (**1**) (500MHz, $CDCl_3$).

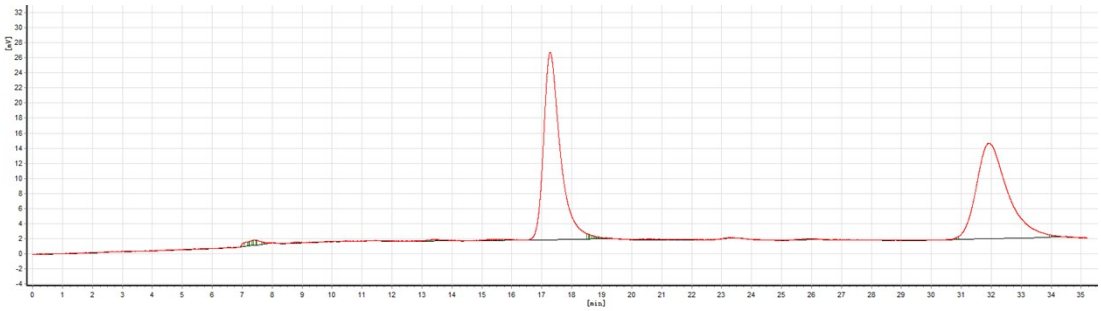


Fig. S13. HPLC separation trace of a natural racemic muyoquinone A (**1**).

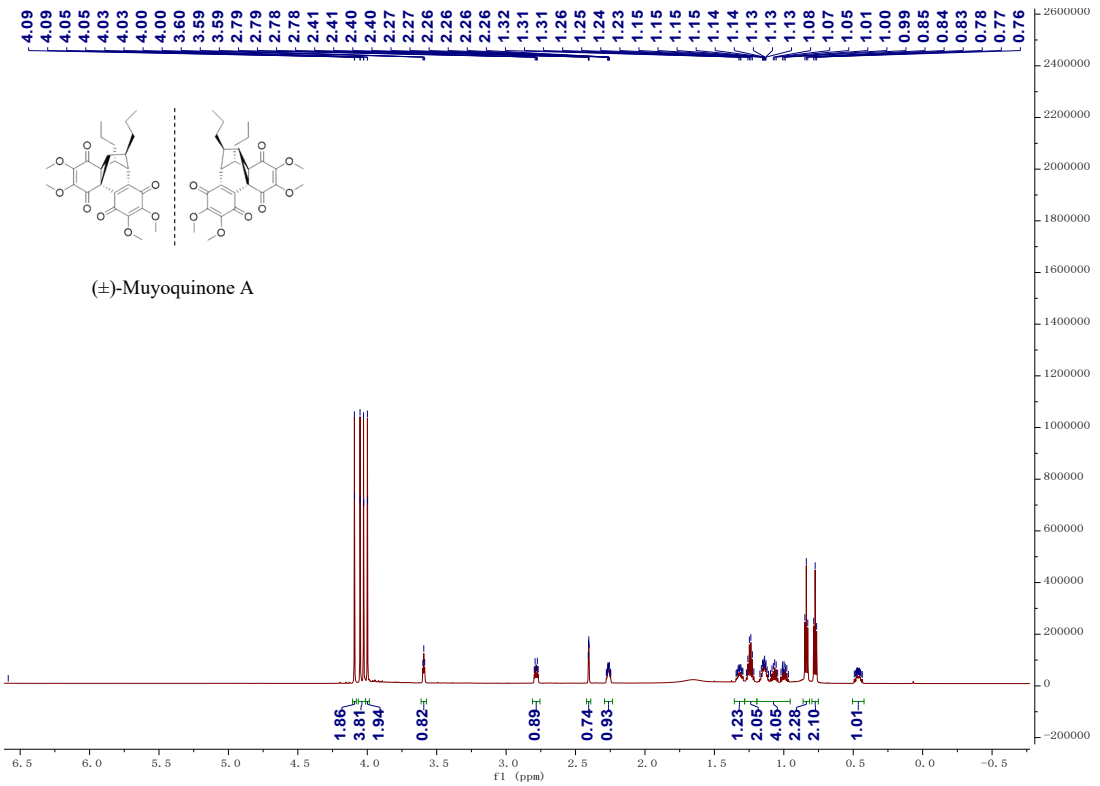


Fig. S14. ^{1}H NMR spectrum of muyoquinone A (**1**) (700 MHz) in $CDCl_3$

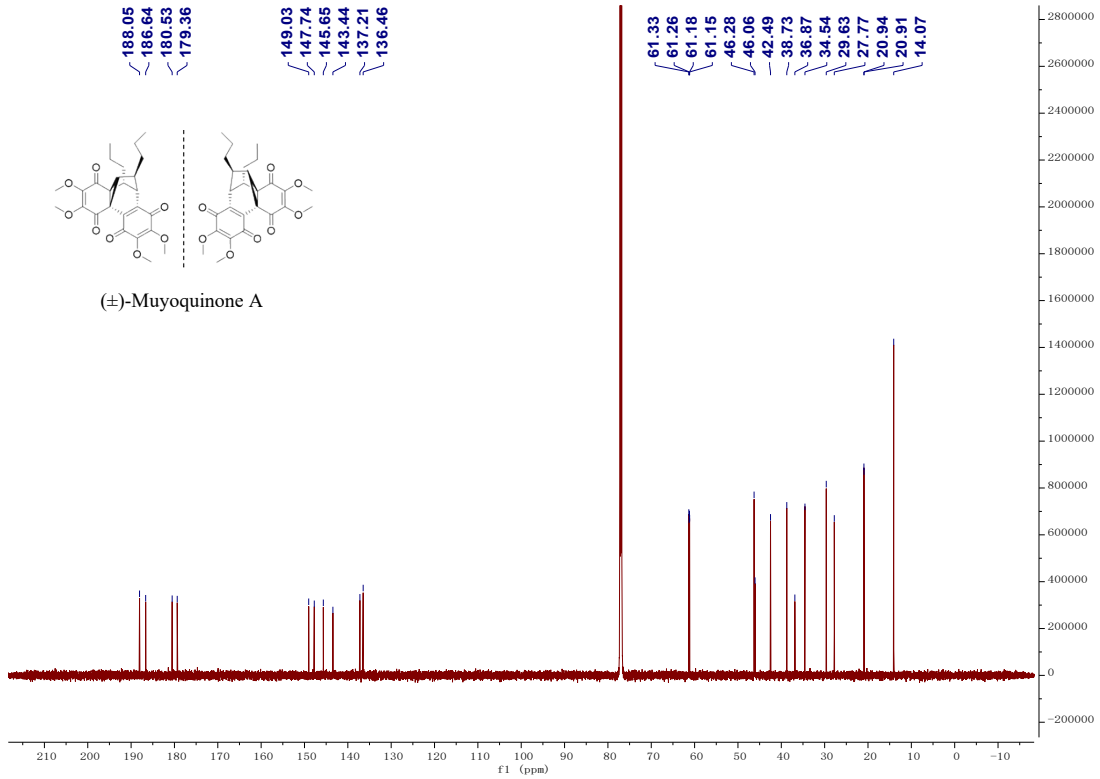


Fig. S15. ^{13}C NMR spectrum of muyoquinone A (**1**) (175 MHz) in $CDCl_3$

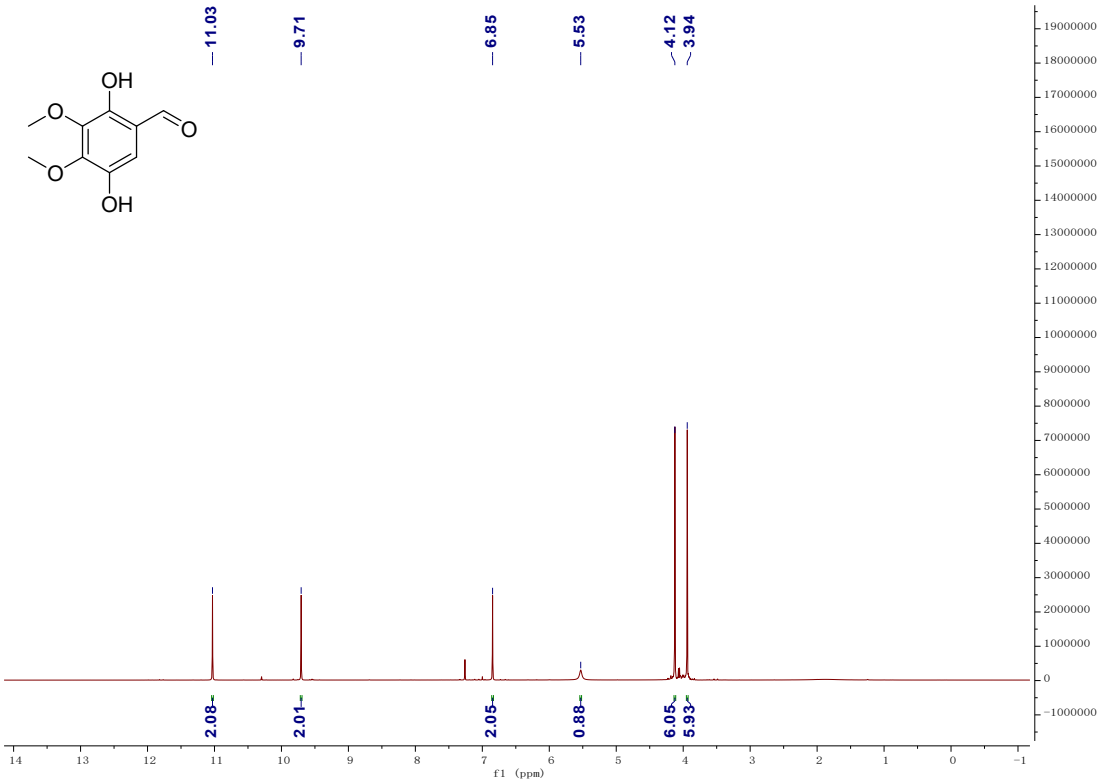


Fig. S16. ^{1}H NMR spectrum of **S1** (700 MHz) in $CDCl_3$

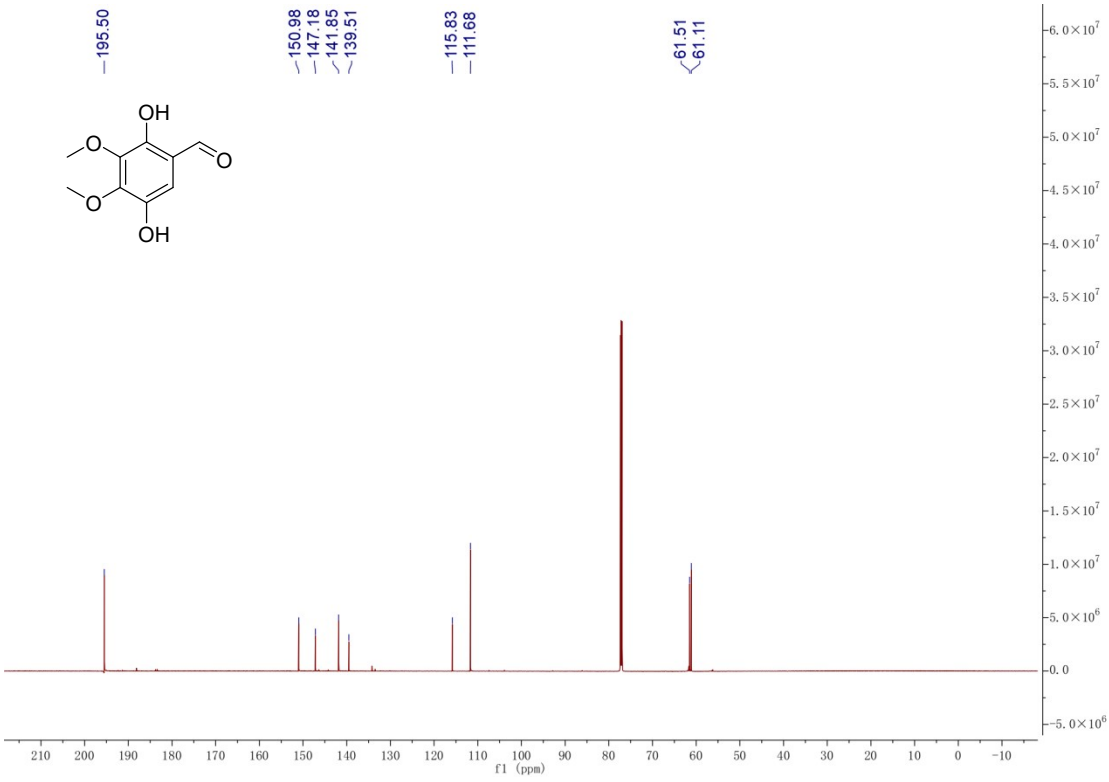


Fig. S17. ^{13}C NMR spectrum of **S1** (175 MHz) in $CDCl_3$

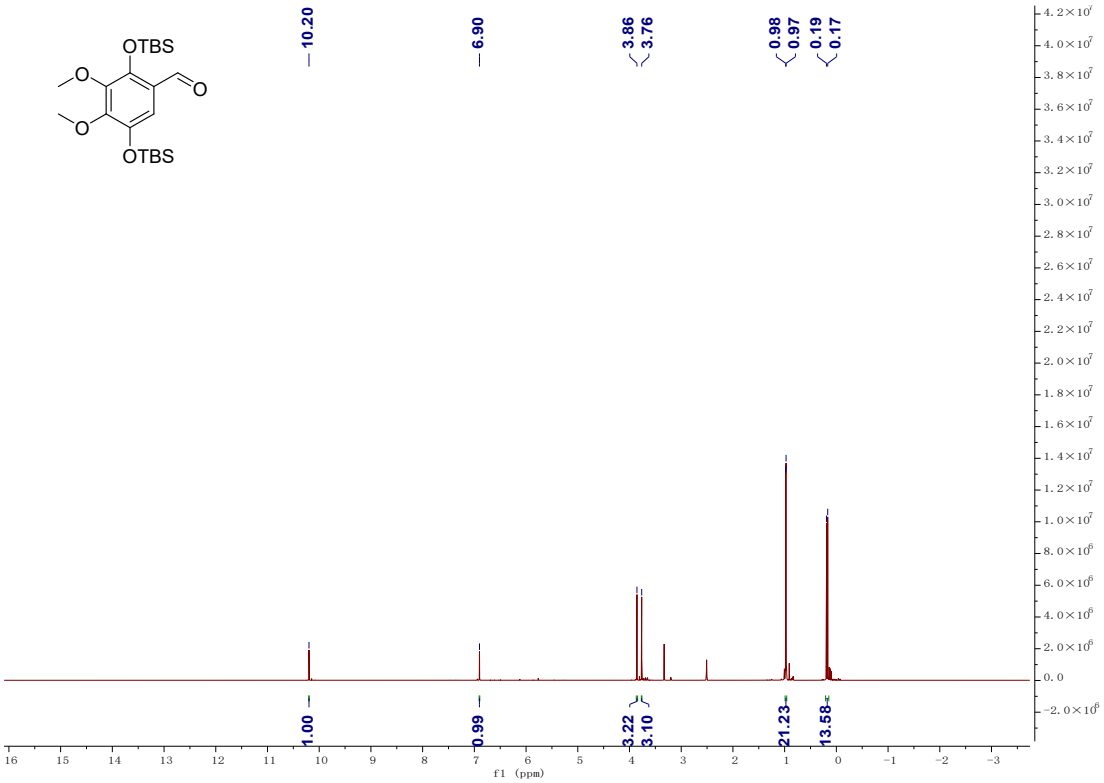


Fig. S18. ^{1}H NMR spectrum of **S2** (700 MHz) in $CDCl_3$

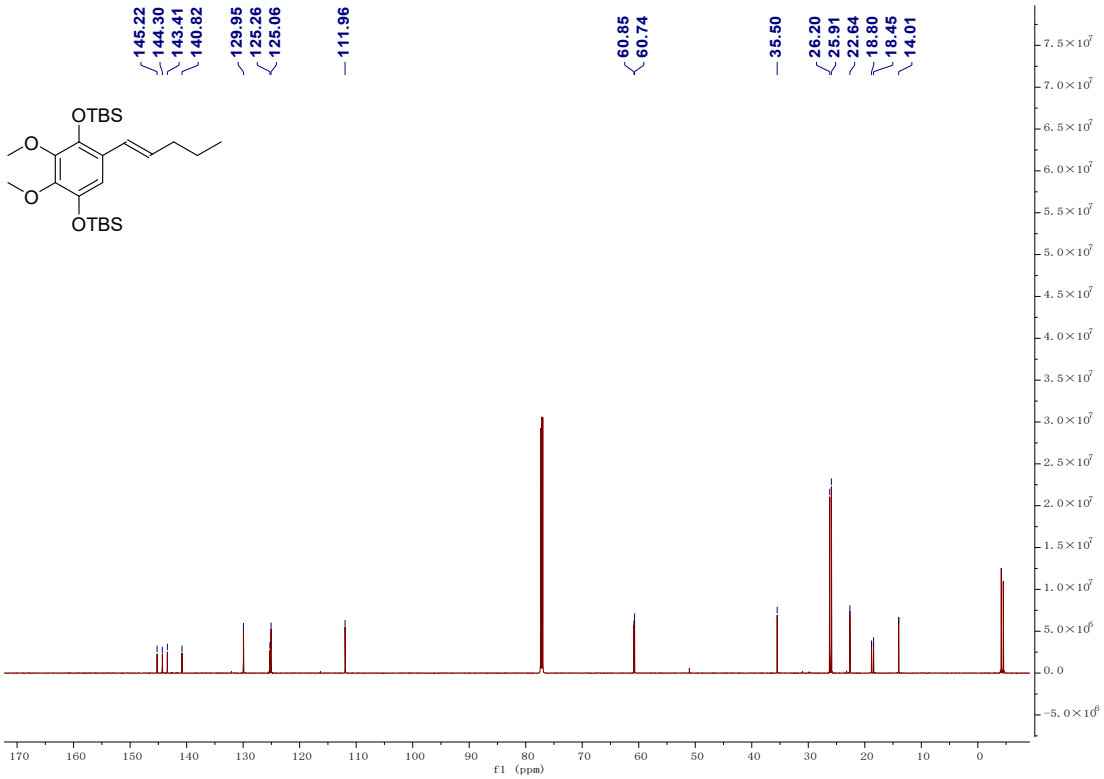


Fig. S19. ^{13}C NMR spectrum of **S3** (175 MHz) in $CDCl_3$

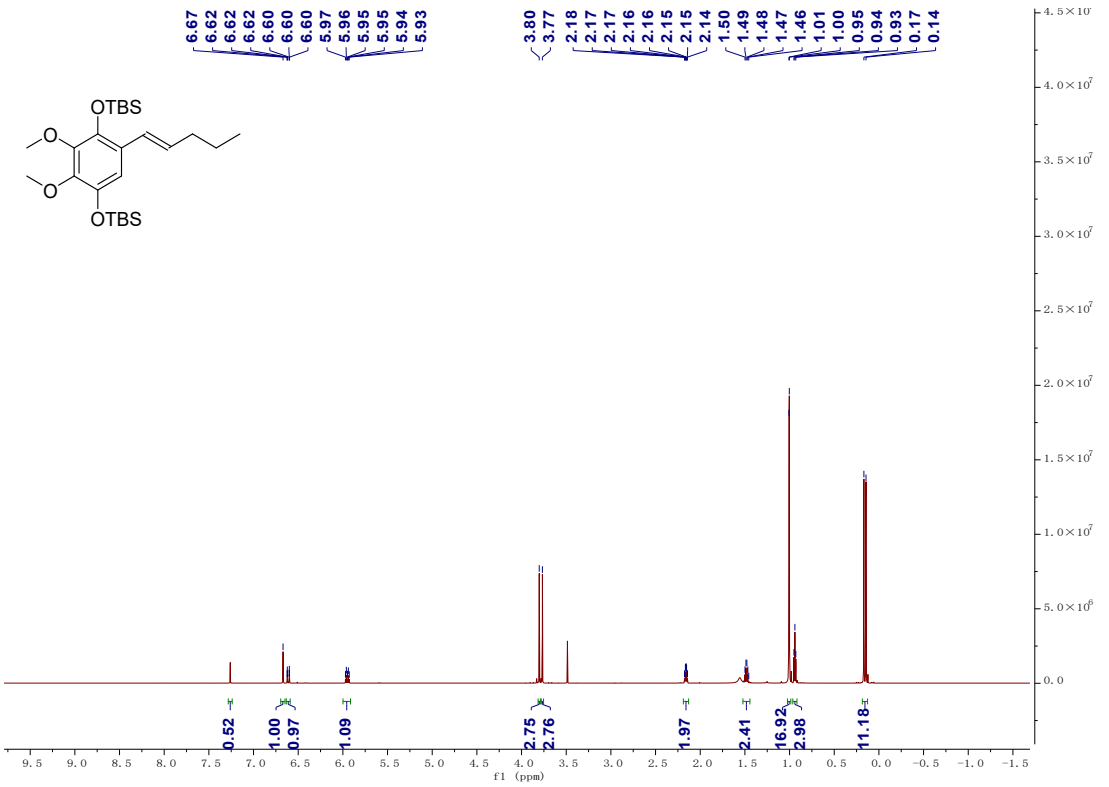


Fig. S20. ^{1}H NMR spectrum of **S3** (175 MHz) in $CDCl_3$

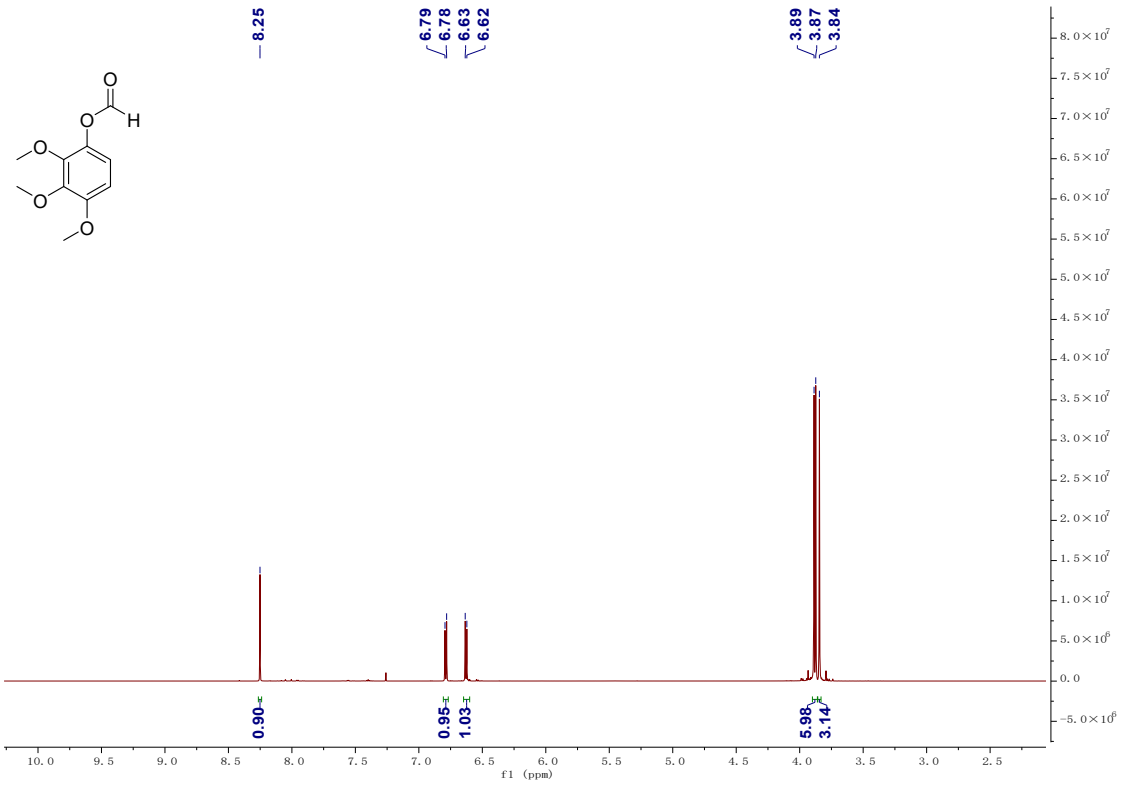


Fig. S21. ^{1}H NMR spectrum of **S5** (175 MHz) in $CDCl_3$

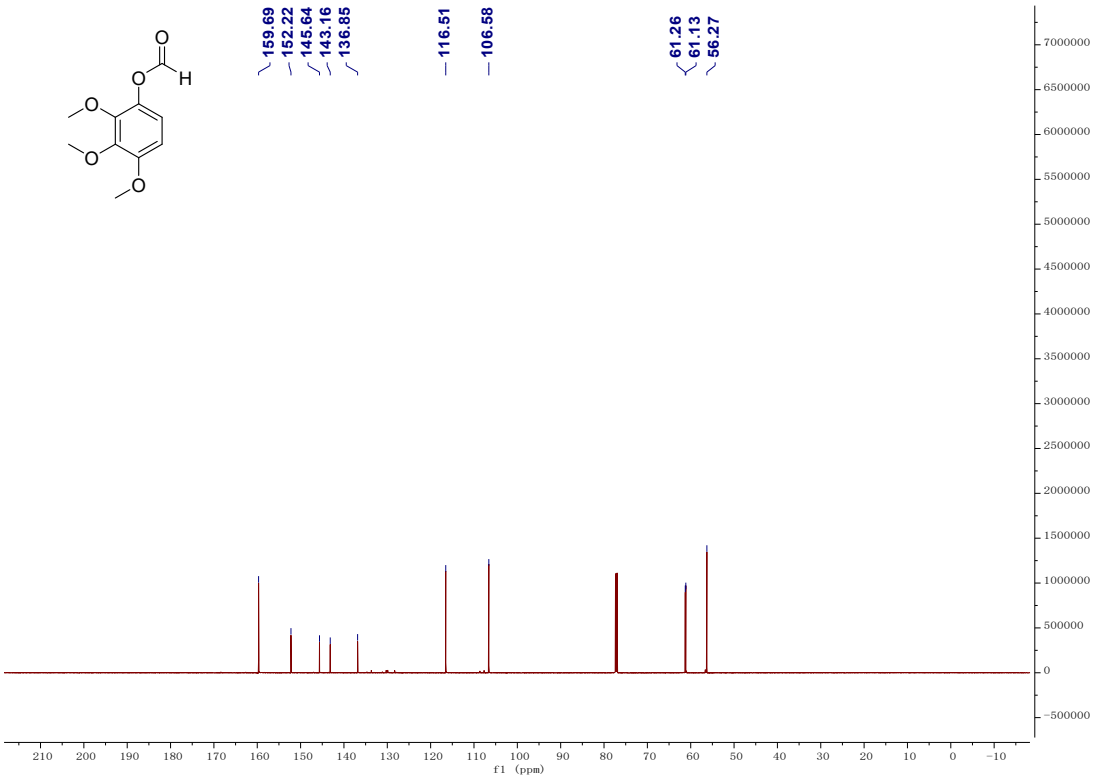


Fig. S22. ^{13}C NMR spectrum of **S5** (175 MHz) in $CDCl_3$

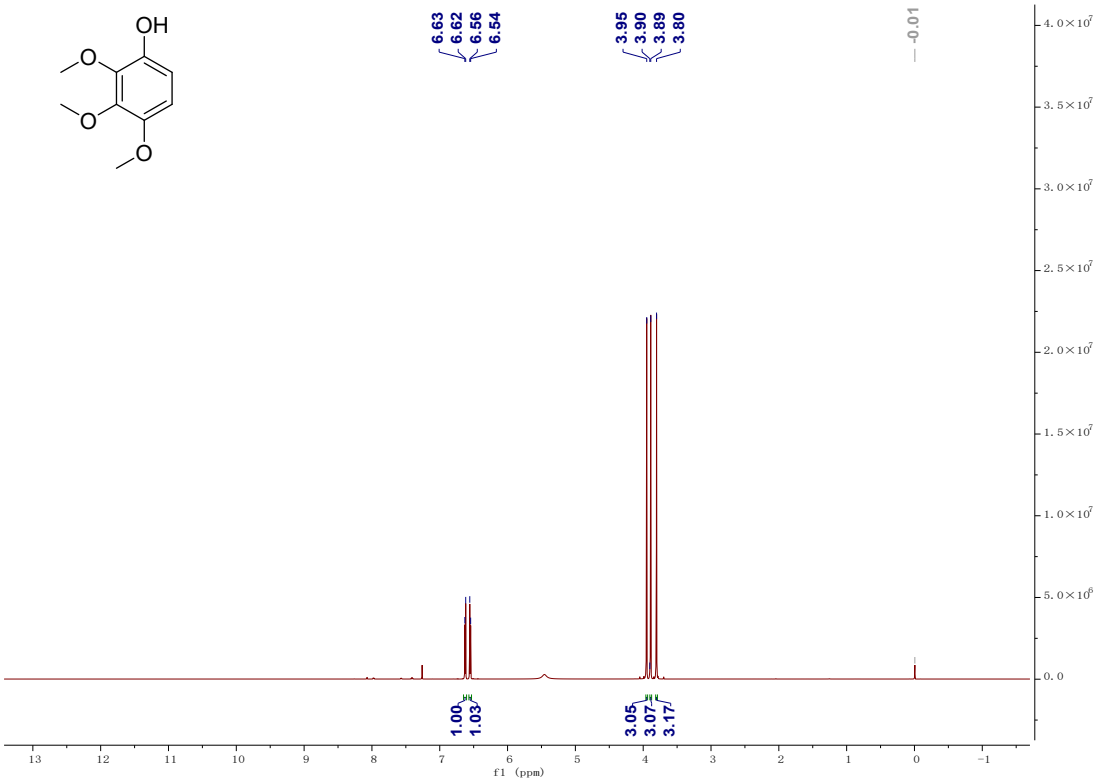


Fig. S23. ^{1}H NMR spectrum of **3** (700 MHz) in $CDCl_3$

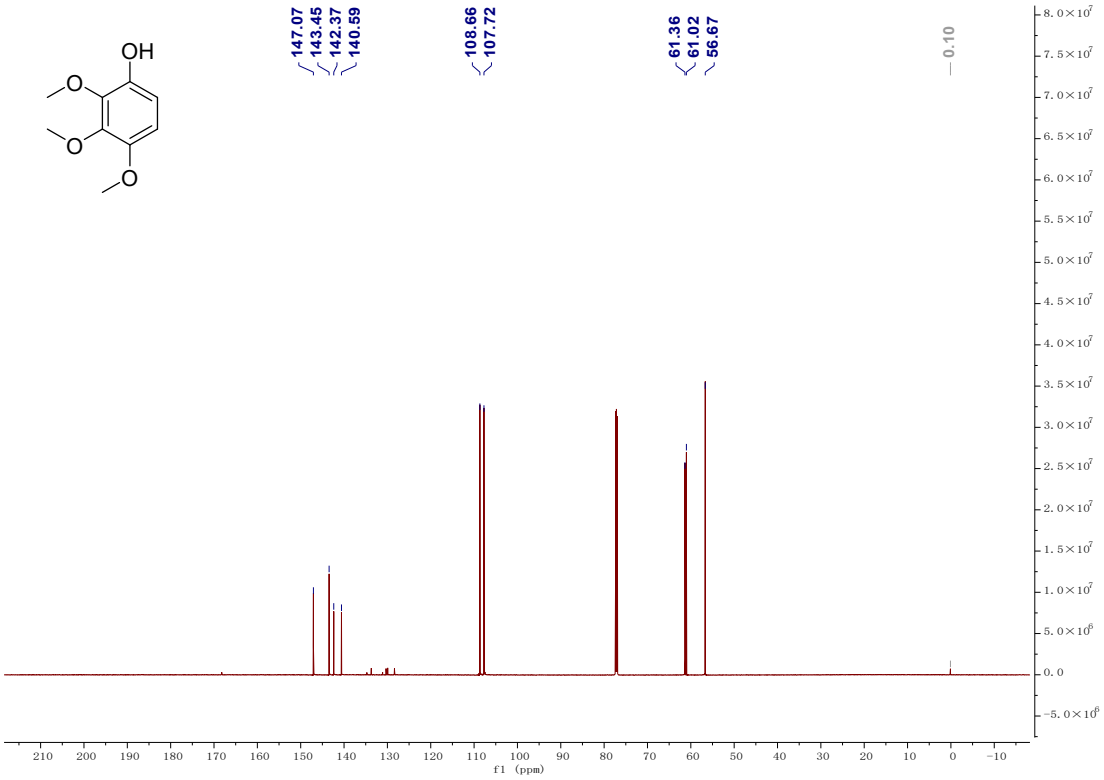


Fig. S24. ^{13}C NMR spectrum of **3** (175 MHz) in $CDCl_3$

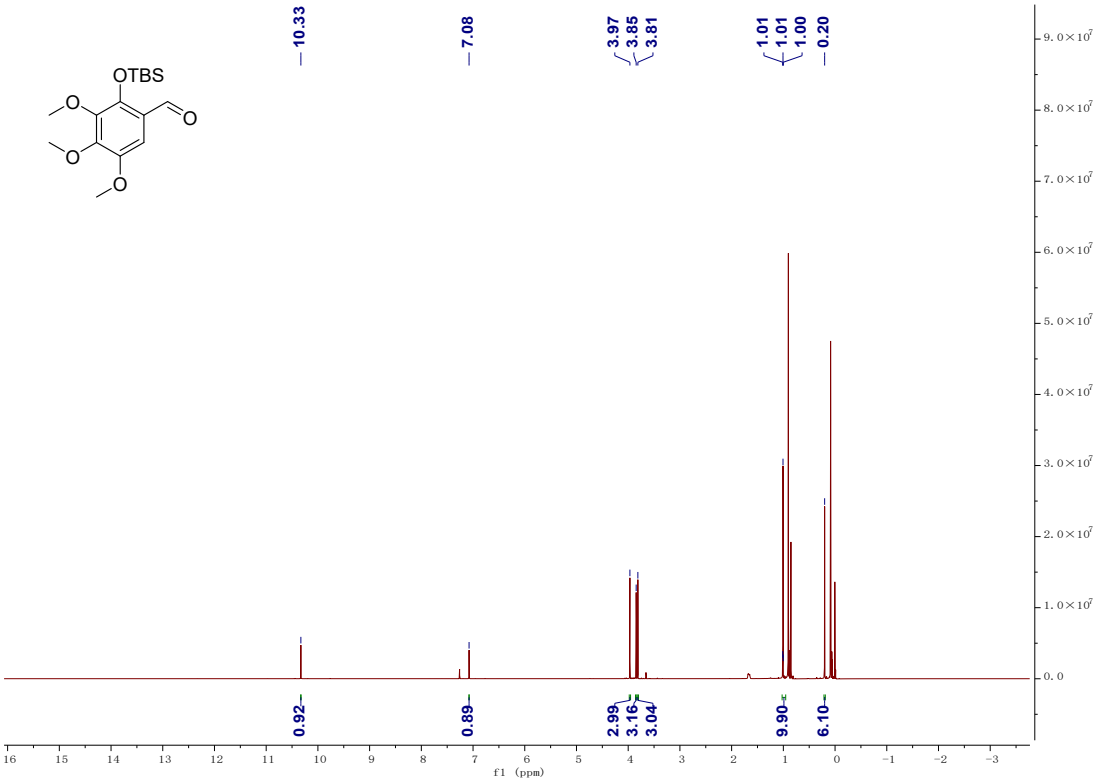


Fig. S25. ^{1}H NMR spectrum of **4** (500 MHz) in $CDCl_3$

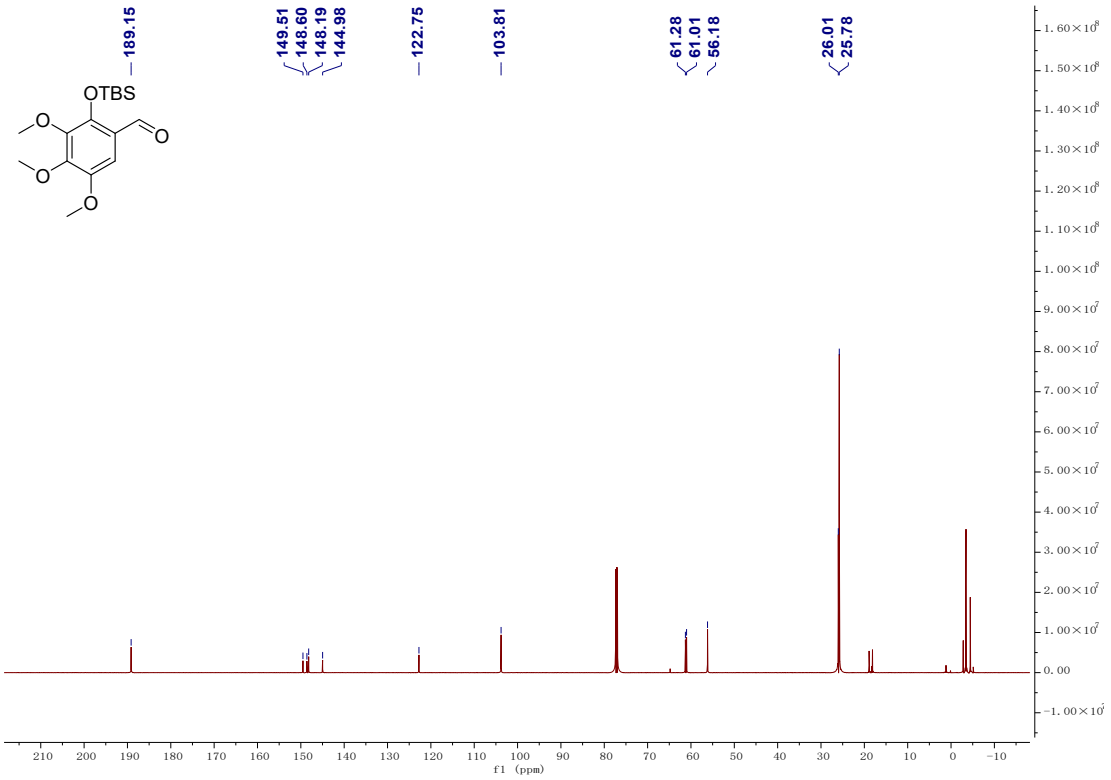


Fig. S26. ^{13}C NMR spectrum of **4** (125 MHz) in $CDCl_3$

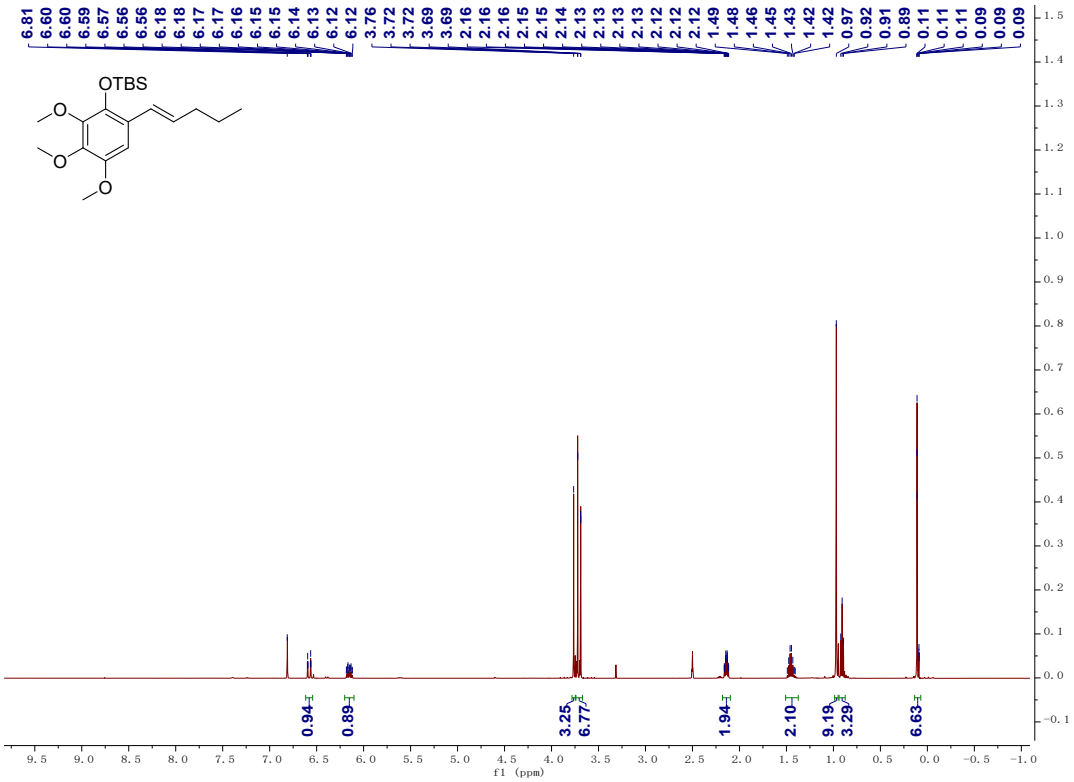


Fig. S27. ^{1}H NMR spectrum of **6** (500 MHz) in DMSO-D_6

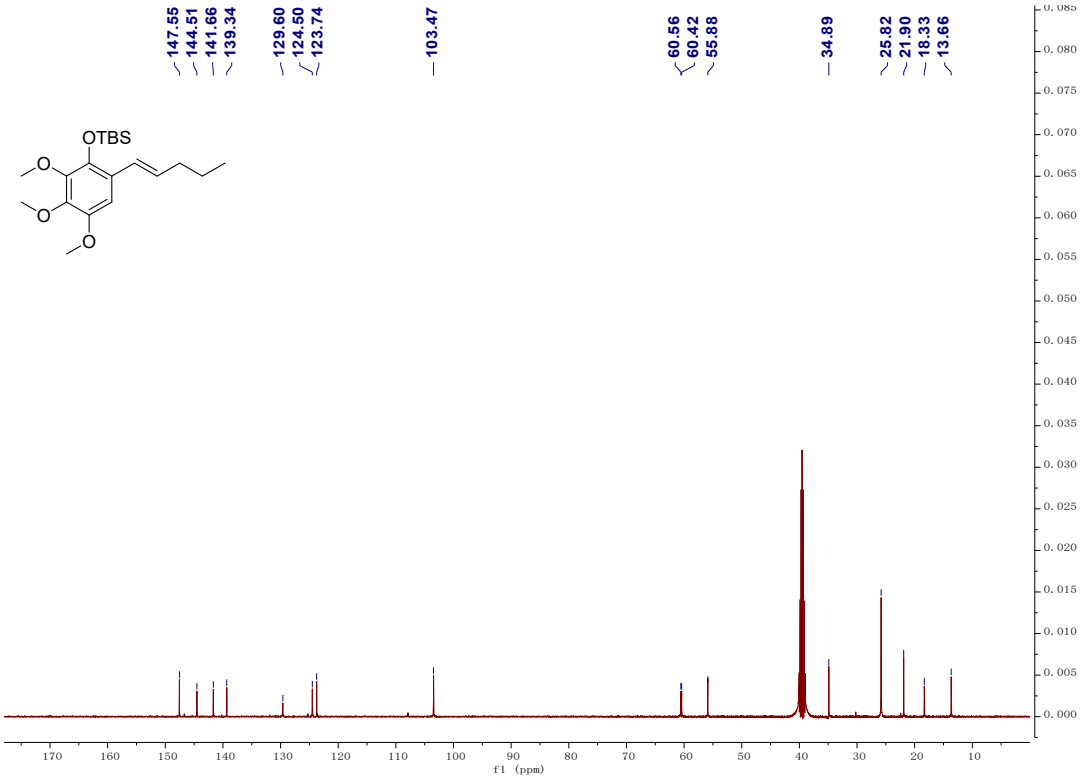


Fig. S28. ^{13}C NMR spectrum of **6** (125 MHz) in DMSO-D_6

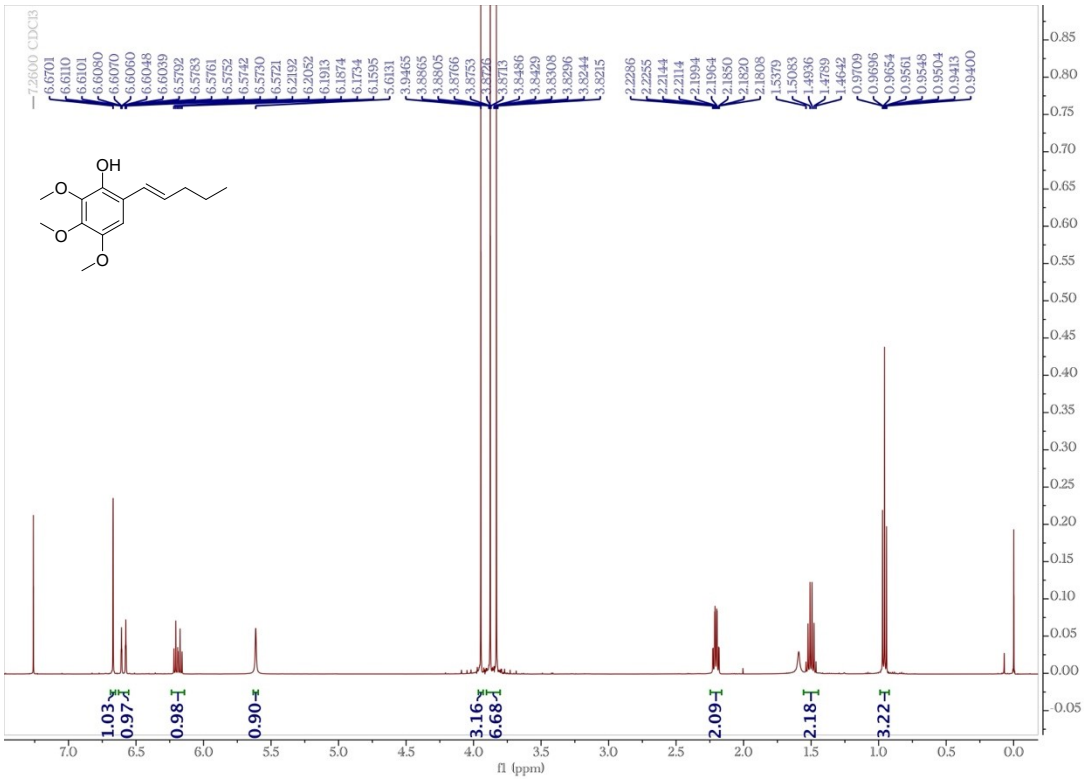


Fig. S29. ^{1}H NMR spectrum of **7** (700 MHz) in $CDCl_3$

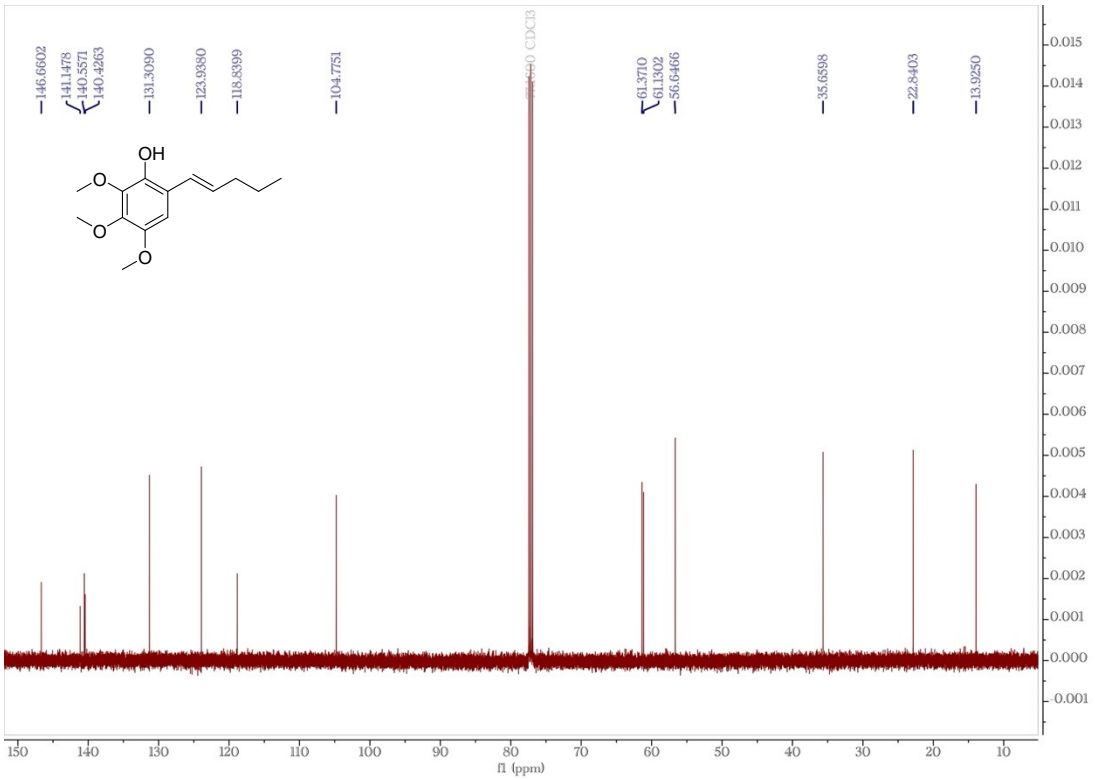


Fig. S30. ^{13}C NMR spectrum of **7** (175 MHz) in $CDCl_3$

9. Bioassay of compound 1

The cytotoxic activities of compound **1**, **1a** and **1b** was measured using the MTT method. The cell lines (human liver carcinoma HepG2, human colorectal carcinoma HCT-116, human gastric carcinoma HGC27, human glioma cell U251, mouse melanoma cell B16F10) were cultured in the RRMI 1640 medium containing 10% fetal bovine serum and 100 U/mL penicillin at 37 °C. After incubating the cells in a 96-well plate for 24h, the compounds were added to give final concentrations of 0.1, 1, and 10 μM, respectively, and experiments were carried out in triplicate. Then 100 μL of MTT (0.5 mg/mL) was added to each well after 72 h of culturing. Four hours later, 200 μL of DMSO was added to dissolve the formazan crystals. Absorbances at 570 nm were measured. The inhibition rates were calculated by the following formula: Inhibition rate = 1- the mean OD of the medicated group/the mean OD of the solvent control group. Taxol awas used as positive control. The IC_{50} was listed in table 2. The dose-response curves are as follows.

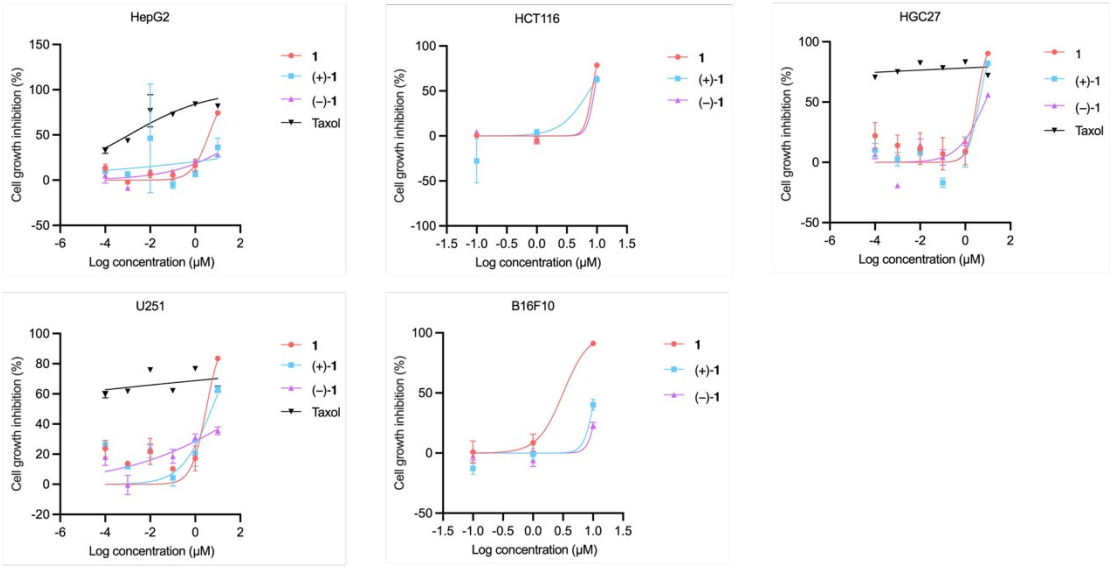


Fig. S31. Dose-response curves of compound **1** against five tumor cell lines (human liver carcinoma HepG2, human colorectal carcinoma HCT-116, human gastric carcinoma HGC27, human glioma cell U251, mouse melanoma cell B16F10)

10. Results of Gibbs free energy calculations

Table. S4. Gibbs free energy of each intermediate in the (±)-Muyoquinone A synthesis route

G=E+ZPE+ΔH-T*S				
			298	627.51
	E	G_{corr}	G(a.u.)	G (kcal/mol)
H_2	-1.1682	-0.0017	-1.1699	-734.1199
8	-805.8270	0.21703	-805.6100	-505528.3383
(±)-**9**	-1611.7	0.46648	-1611.2335	-1011065.1606
(±)-**10**	-1610.488	0.44238	-1610.0458	-1010319.8096
(±)-Muyoquinone A	-1610.524	0.44789	-1610.0763	-1010338.9990