Electronic Supporting Information

Chemoenzymatic Reduction of Citreorosein and its Implications for Aloe-Emodin and Rugulosin C (Bio)synthesis

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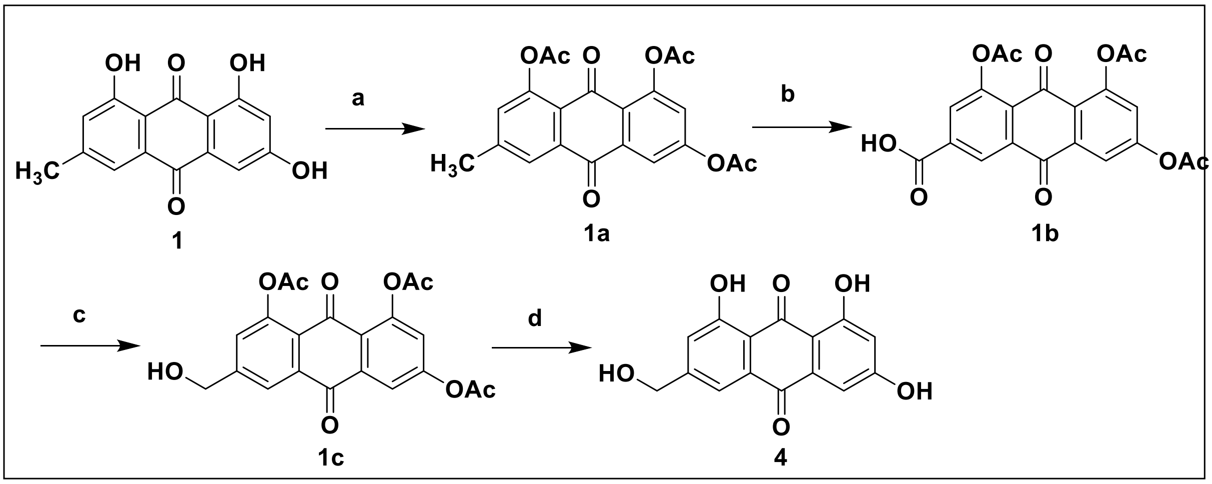
I. General Materials and Methods.

All commercial reagents were obtained from Sigma-Aldrich Chemical Co. and Sisco Research Laboratories, India. Reactions were monitored by thin-layer chromatography (TLC, 0.25 mm E. Merck silica gel plates, $60F_{254}$) and the plates were visualized by using UV light. Column chromatography was performed on silica gel 60–120/230–400 mesh obtained from S. D. Fine Chemical Co., India. 10% Oxalic acid impregnated silica gel was prepared by adding silica gel (230–400 mesh size, 10 g) to a solution of oxalic acid (10 g) in H_2O (200 mL), filtered the resulting suspension to dryness under reduced pressure and then activating at 125 °C overnight, and finally cooling under argon. Yields refer to chromatographically pure materials; conversions were calculated from the ^{1}H NMR spectra of the crude products. ^{1}H NMR spectra were recorded on Bruker 400 Ultra Shield instruments using deuterated solvents. Proton coupling constants (*J*) are reported as absolute values in Hz. ^{13}C NMR spectra were recorded on Bruker 400 Ultra Shield instruments operating at 100 MHz. Chemical shifts (δ) of the ^{1}H and ^{13}C NMR spectra are reported in ppm with a solvent resonance as an internal standard. For ^{1}H NMR: chloroform 7.26, acetone-d_6 2.05, DMSO-d_6 2.50; for ^{13}C NMR: chloroform-d_1 77.16, acetone-d_6 29.84, DMSO-d_6 39.52. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, dd = doublet of a doublet, ddd = doublet of a doublet of doublet, t = triplet, dt = doublet of a triplet, q = quartet, quint = quintet, m = multiplet, br = broad, ar = aromatic. Electrospray ionization (ESI) mass spectrometry (MS) experiments were performed on an Agilent 6530 Accurate-Mass Q-TOF LC/MS system (Agilent Technologies). Optical rotations were measured on a DigiPol 781 M6U Automatic Polarimeter. CD spectroscopy was carried out on a Jasco J-1500 CD Spectrometer (Jasco International Co.). UV spectroscopy and activity measurements were performed on Cary 300 UV/Vis spectrophotometer (Agilent Technologies).

II. Bacterial Culture, Enzyme Expression and Purification.

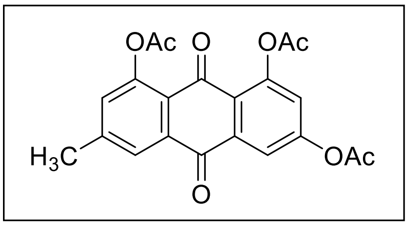
The strains *E. coli* DH5α and BL21 (DE3) (Sigma-Aldrich) were used for cloning and expression, respectively. Recombinant plasmids (pET19b) each containing one of T_4HNR_his, MdpC_his, and PHAR_his genes were obtained from Prof. Michael Müller (University of Freiburg, Germany). Cloning details of T_4HNR,[1] MdpC[2] and PHAR[3] in the pET19b vector has been published elsewhere. Glucose dehydrogenase (GDH) plasmid was generously provided by Prof. Werner Hummel (University of Bielefeld, Germany). Competent *E. coli* BL21 (DE3) cells were transformed with plasmid by applying a heat shock at 42 °C for 45 seconds and grown overnight on SOB-agar medium containing 100 µg/mL ampicillin. One clone from a colony was picked and dispersed in 5 mL of LB-media (Lennox) containing ampicillin (100 $\mu g \cdot mL^{-1}$), followed by incubation overnight (37 °C, 160 rpm). For expression of T_4HNR_his, MdpC_his, and GDH, each overnight culture was diluted to 500 mL of medium and incubated at 37 °C, 160 rpm. Once the culture reached the mid-log phase ($OD_{600\ nm}$ = 0.6), IPTG (0.2 mM) was added and cultures were further incubated for 4 h at 37 °C, 160 rpm. For expression of PHAR_his, the cultures were incubated for 20 h at 18 °C, 160 rpm after the addition of IPTG. Cells were harvested (by centrifugation at 12000 x *g*, 4 °C for 15 minutes) and resuspended in resuspension buffer. For T_4HNR_his, MdpC_his and GDH, the harvested *E. coli* cells were resuspended in HEPES buffer (50 mM, pH = 7.5; 2.5 mL per 500 mL culture medium). For PHAR_his, the harvested *E. coli* cells were resuspended in Tris-HCl buffer (50 mM Tris-HCl, 0.5 mM dithiothreitol, 10 % glycerol, 5 mM imidazole, pH = 7.5; 2.5 mL per harvested cells of 500 mL medium). The cells were disrupted by sonication (6 times 10 sec, Vibra-Cell Processors, model no. VCX500, Sonics), followed by centrifugation (12000 x *g*, 4 °C for 40 minutes). The supernatant was supplemented with 20% v/v glycerol and stored at –20 °C as crude enzyme until use. T_4HNR_his, MdpC_his, and PHAR_his were purified by Ni-NTA affinity chromatography. Non-specifically bound proteins were washed off with buffer containing 20 and 50 mM imidazole (prepared in the resuspension buffer used earlier for workup). Elution of pure proteins was performed with buffer containing 250 mM imidazole. The eluted fractions were desalted by gel filtration (Econo-Pac 10DG desalting gel column, Bio-Rad). The purified proteins were concentrated by ultrafiltration (Vivaspin 15R centrifugal filter units, 10 kDa nominal molecular weight limit, Sartorius). The concentration of the protein was measured by UV absorption at 280 nm (NanoVue, GE Healthcare).

III. Substrate synthesis



Scheme S1. Synthesis of citreorosein (**4**) from emodin (**1**)[4] Reagents and conditions: a) Ac_2O/py, 70 ºC, 4 h, 98%; b) CrO_3, Ac_2O/AcOH, 70 ºC, 24 h, 80%; c) BMS, dry THF, 0 ºC, 15 min, 48%; d) 0.5 N KOH, 70 ºC, 45 min, 88%.

6-methyl-9,10-dioxo-9,10-dihydroanthracene-1,3,8-triyl triacetate (1a)



$C_{21}H_{16}O_8$: 396.084 g/mol

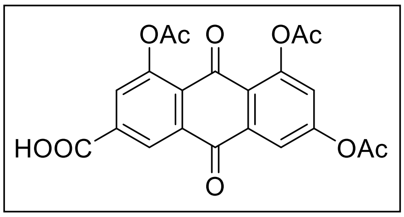
Emodin (**1**) (1.0 g, 3.70 mmol) was dissolved in a solution of pyridine (20.0 mL) and acetic anhydride (2.2 mL, 18.5 mmol, 5.0 equiv.). Then, the reaction mixture was heated at 70ºC for 4h. The reaction was monitored by TLC. After completion, the reaction mixture was cooled to room temperature and poured onto crushed ice to afford 1, 3, 8-triacetyl emodin (TAEM), **1a** (1.40 g, 98% yield) as a pale-yellow solid as crystal after filtration.

TLC (cyclohexane/ethyl acetate, 7:3 v/v): R_f = 0.42;

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.35 (s, 3H, CH_3), 2.43 (s, 6H, OCH_3), 2.50 (s, 3H, OCH_3), 7.22 (q, ^4J = 0.65 Hz, 1H, H-7), 7.23 (d, ^4J = 2.4 Hz, 1H, H-4), 7.95 (d, ^4J = 2.4 Hz, 1H, H-2), 8.01 (q, ^4J = 0.6 Hz, 1H, H-5).

^{13}C NMR (100 MHz, $CDCl_3$): δ 21.2, 21.2, 21.8, 118.2, 126.2, 130.9, 134.0, 135.79, 146.18, 150.31, 151.6, 154.7, 168.22, 169.15, 169.78, 180.50, 182.49.

4,5,7-triacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (1b):



$C_{21}H_{14}O_{10}$: 426.33 g/mol

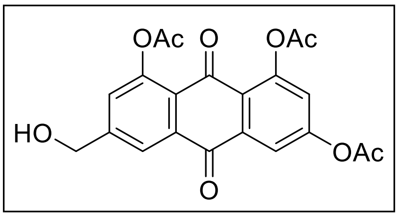
1a (1.4 g, 3.53 mmol) was dissolved in a mixture of acetic acid (30.0 mL) and acetic anhydride (50.0 mL). Then, the solution of chromium trioxide (3.53 g, 35.3 mmol, 10.0 equiv.) dissolved, in acetic acid (20.0 mL) was gradually added to the solution of **1a** and the reaction mixture was stirred at 70°C for 24 h. Then, the reaction mixture was cooled to room temperature, extracted with ethyl acetate, washed with water and dried over anhydrous sodium sulphate. The extracted organic layer was concentrated on rotary evaporator and purified by the column chromatography (silica gel 230-400 mess size, CH_2Cl_2: MeOH 1:19) to afforded 4,5,7-triacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (TAEA, **1b**) (1.2 g, 80% yield).

TLC (cyclohexane/ethyl acetate, 1:1 v/v): R_f = 0.8.

^{1}H NMR (400 MHz, acetone-d_6): δ 2.37 (s, 3H, OCH_3), 2.41 (s, 3H, OCH_3), 2.43 (s, 3H, OCH_3), 7.43 (d, ^4J = 2.4 Hz, 1H, H-8), 7.97 (d, ^4J = 2.4 Hz, 1H, H-6), 8.08 (d, ^4J = 1.7 Hz, 1H, H-1), 8.75 (d, ^4J = 1.7 Hz, 1H, H-3).

^{13}C NMR (100 MHz, acetone-d_6): *δ* 20.9, 21.0, 21.0, 119.0, 124.3, 124.8, 126.4, 129.3, 131.6, 135.5, 136.5, 137.0, 151.5, 152.7, 156.2, 165.3, 168.9, 169.4, 169.6, 180.5, 181.3.

6-(hydroxymethyl)-9,10-dioxo-9,10-dihydroanthracene-1,3,8-triyl triacetate (1c)



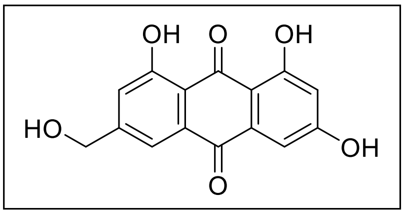
$C_{21}H_{16}O_9$: 412.35 g/mol

1b (400.0 mg, 0.94 mmol) was dissolved in anhydrous THF (40.0 mL), followed by the addition of BMS (24.0 mL, 2 M in THF) at 0°C for 15 min. The reaction mixture was monitored by TLC. After completion, the reaction was stopped by adding water, and the mixture was extracted with diethyl ether, washed with saturated brine solution and dried over anhydrous sodium sulphate. The extracted organic layer was concentrated on rotary evaporator, followed by purification using column chromatography (silica gel, CH_2Cl_2: MeOH 1: 24) to afford **1c** (186.0 mg, 48% yield).

TLC ($CHCl_3$/MeOH, 9:1 v/v): R_f = 0.

^{1}H NMR (400 MHz, acetone-d$_6$): *δ* 2.36 (s, 3H, OCH_3), 2.39 (s, 3H, OCH_3), 2.40 (s, 3H, OCH_3), 4.75 (t, ^3J= 5.7 Hz, 1H, aliphatic–OH), 4.85 (d, ^3J = 5.6 Hz, 2H, CH_2), 7.39 (d, ^4J = 2.4 Hz, 1H, H-4), 7.53–7.54 (m, 1H, H-7), 7.93 (d, ^4J = 2.4 Hz, 1H, H-2), 8.17–8.18 (m, 1H, H-5).
^{13}C NMR (100 MHz, acetone-*d*$_6$): *δ* 21.0, 21.1, 63.38, 118.9, 123.1, 124.3, 124.6, 124.9, 128.6, 135.2, 136.6, 151.5, 151.9, 152.6, 155.9, 168.9, 169.4, 169.6, 180.6, 181.9.

1,3,8-trihydroxy-6-(hydroxymethyl)anthracene-9,10-dione (citreorosein, 4)



$C_{15}H_{10}O_6$: 286.23 g/mol

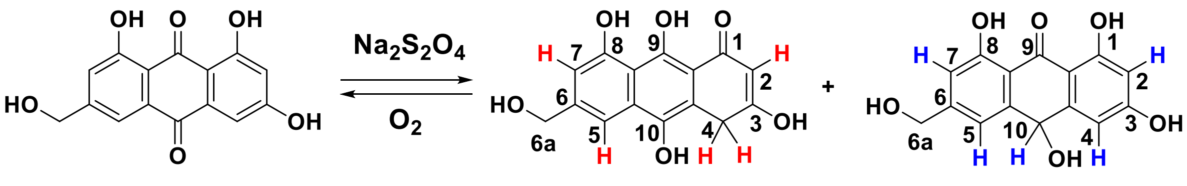
1c (200.0 mg, 0.485 mmol) was dissolved in 0.5(N) KOH solution (50 mL) and the reaction mixture was stirred at 70ºC for 45 min. After completion of reaction as monitored through TLC, acidification was done with 10 % HCl solution and the reaction mixture was extracted with ethyl acetate (3 X 40 mL), washed with brine solution and dried over anhydrous sodium sulphate. The extracted organic layer was concentrated on rotary evaporator and purified by the column chromatography (silica gel, CH_2Cl_2: MeOH 1: 19) to afforded citreorosein, **4** (122 mg, 88% yield).
TLC (MeOH: $CHCl_3$, 1:9 v/v): R_f = 0.36.

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 4.57 (s, 2H, CH_2), 6.51 (d, ^4J = 2.4 Hz, 1H, H-2), 7.04 (d, ^4J = 2.4 Hz, 1H, H-4), 7.17 (s, 1H, H-7), 7.55 (s, 1H, H-5), 12.00 (s, 2H, OH-1, OH-8).

^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 62.4 (CH_2), 108.3(C-2), 109.2 (C-4), 109.3 (C-9a), 114.4 (C-8a), 117.4 (C-5), 121.1 (C-7), 133.2 (C-5a), 135.4 (10a), 153.2 (C-6), 161.9 (C-8), 164.9 (C-3), 166.1 (C-1), 181.6 (C-10), 190.0 (C-9).

IV Characterization of citreorosein hydroquinones (10a/10b)

Reduction of Citreorosein (4) by sodium dithionite ($Na_2S_2O_4$) to citreorosein hydroquinones (10a/10b)



To investigate the reduction of **4** by sodium dithionite, the anthraquinone (**4**) was treated as follows: 6.0 mg (20.9 µmol) of **4** was dissolved in 800 µL of acetonitrile-d_3 and 800 µL of argon-flushed water. Phase separation occurred on the addition of 70.0 mg (402.0 µmol) of $Na_2S_2O_4$. The mixture was shaken for 3 minutes and the organic phase was directly subjected to NMR analysis which showed **10a** and **10b** in a ratio of 2:1 at room temperature.

3,8,9,10-tetrahydroxy-6-(hydroxymethyl)anthracene-1(4*H*)-one (10a).

^{1}H NMR (400 MHz, acetonitrile-d_3 + water from extraction): δ 3.57 (s, 1 H, H-4), 4.61 (s, 1 H, H-10), 5.27 (s, 1 H, H-2), 6.60 (d, ^4J = 1.2, Hz 1 H, H-7), 7.39 (s, 1H, H-5).

^{13}C NMR (100 MHz, acetonitrile-d_3 + water from extraction): δ 34.9 (C-4), 64.1 (C-6a), 99.8 (C-2), 107.2 (C-7), 109.1 (C-5), 158.3 (C-8), 148.2 (C-9) 143.1 (C-10).

HRMS (ESI-TOF) m/z: $[M+H]^+$: Calculated for $C_{15}H_{14}O_6$ 289.0707: Found 289.0721.

All the ^{1}H attached with carbon are assigned from HSQC (^{1}H-^{13}C) experiment.

1,3,8,10-tetrahydroxy-6-(hydroxymethyl)anthracen-9(10*H*)-one (10b).

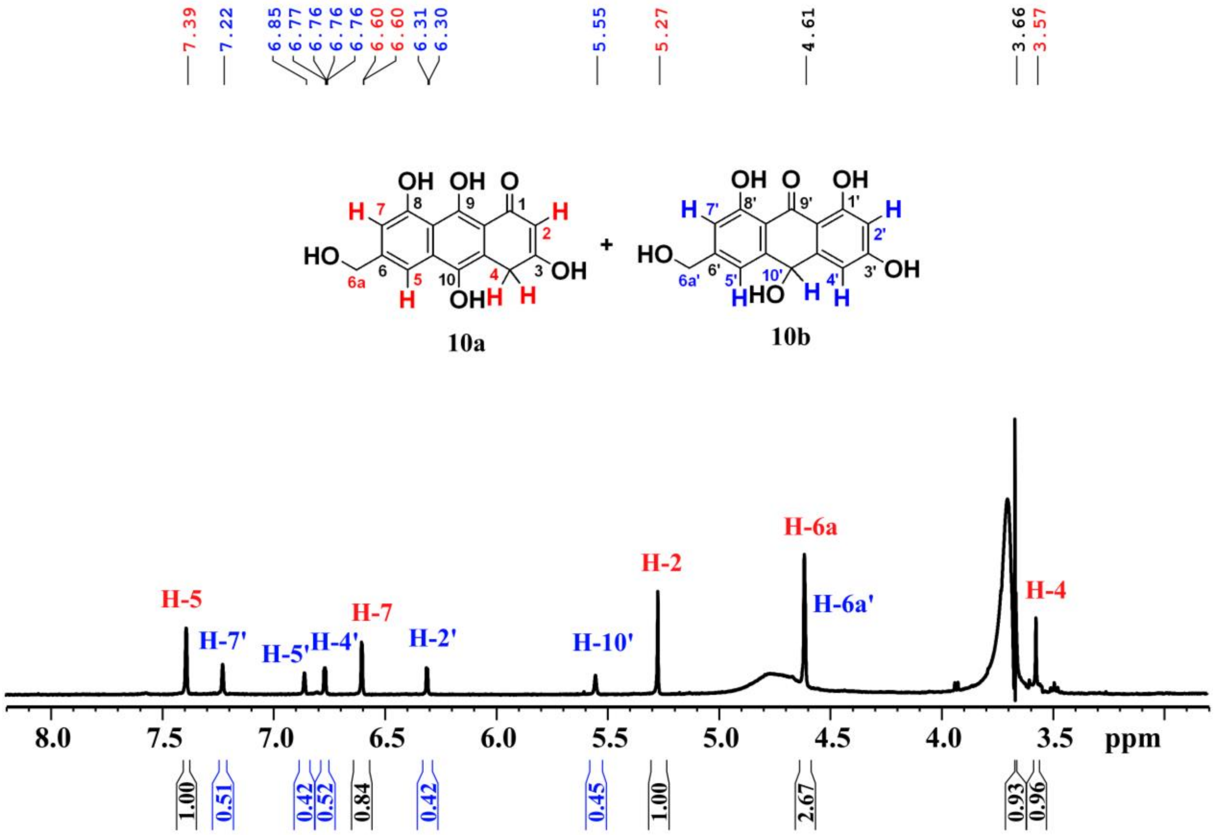
^{1}H NMR (400 MHz, acetonitrile-d_3 + water from extraction): δ 4.61 (s, 1 H, H-6a), 5.55 (s, 1 H, H-10), 6.30 (d, ^4J = 2.3 Hz, 1 H, H-2), 6.76 (s, ^4J = 1.1 Hz, 1 H, H-7), 6.85 (s, 1H, H-5), 7.22 (s, 1 H, H-5).

^{13}C NMR (100 MHz, acetonitrile-d_3 + water from extraction): δ 63.1 (H-6a), 65.7 (C-10), 102.2 (C-2), 106.2 (C-2) 107.9 (C-4), 130.2 (C-5).

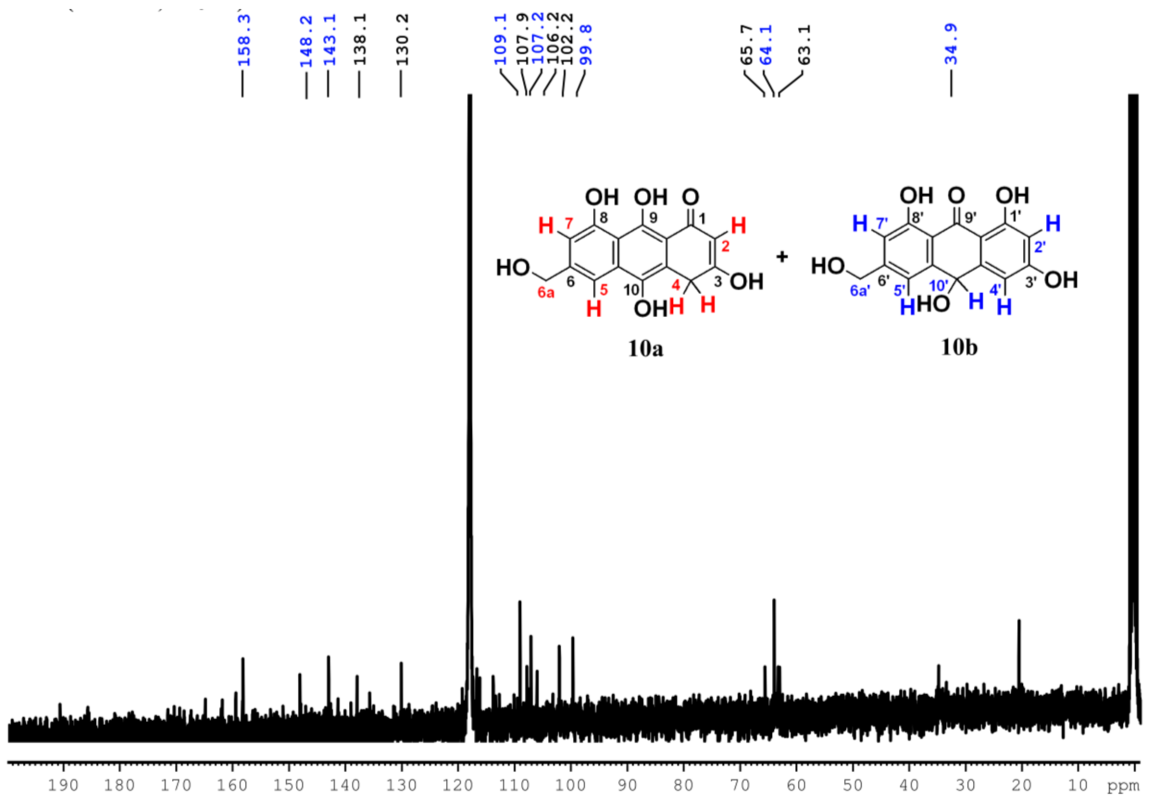
HRMS (ESI-TOF) m/z: $[M+H]^+$: Calcd for $C_{15}H_{14}O_6$ 289.0707: Found 289.0721.

All the ^{1}H attached with carbon are assigned from HSQC (^{1}H-^{13}C) experiment.

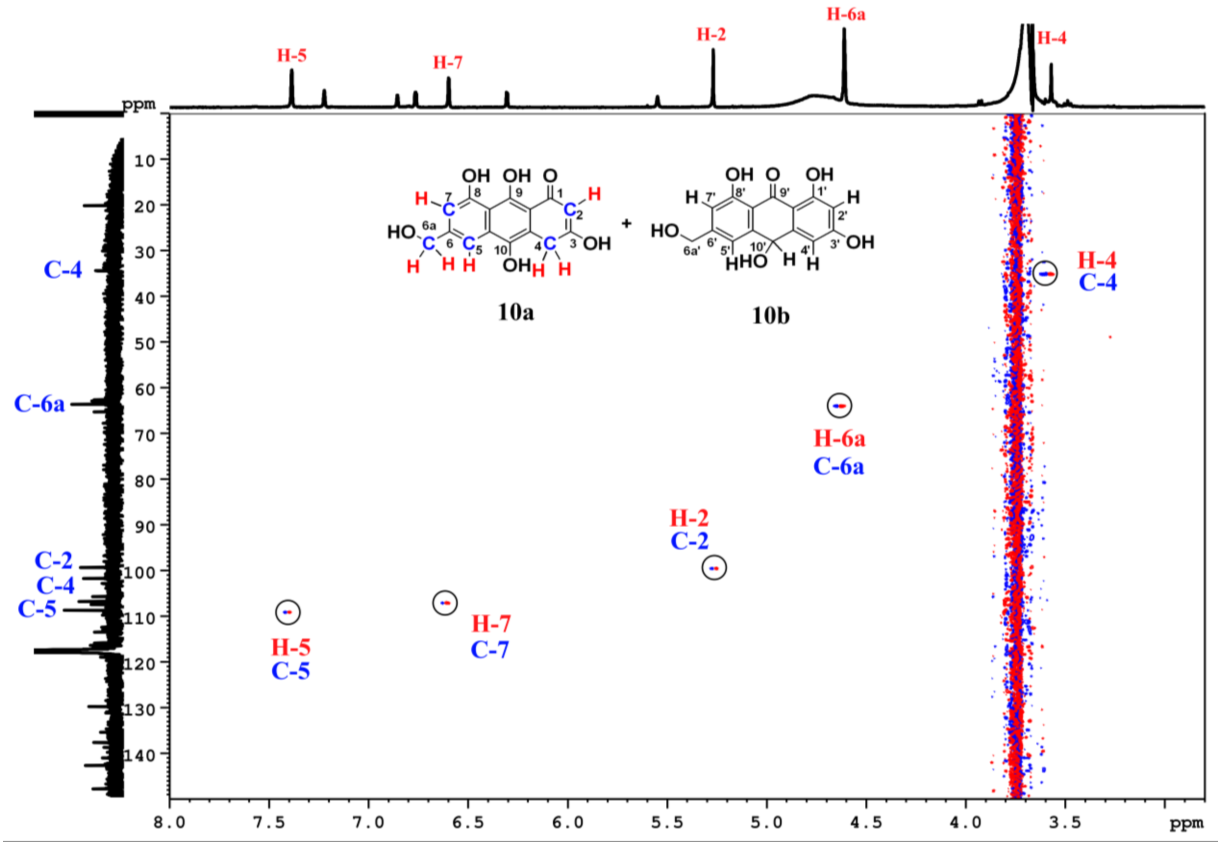
^{1}H NMR (400 MHz, CD_3CN)



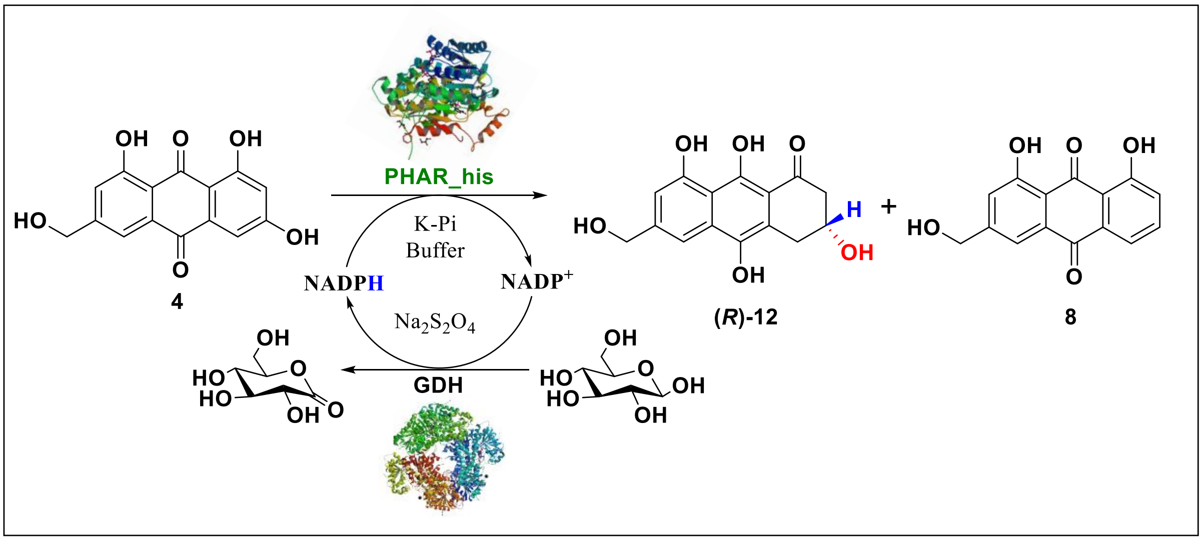
^{13}C NMR (100 MHz, CD_3CN)



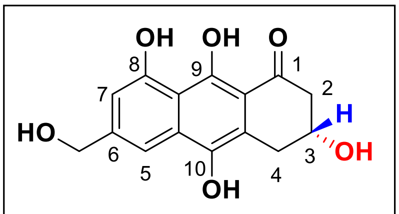
HSQC NMR (^{1}H-^{13}C in CD_3CN)



V. Chemoenzymatic Reduction of Citreorosein (4)



(*R*)-3,8,9,10-tetrahydroxy-6-(hydroxymethyl)-3,4-dihydroanthracen-1(2*H*)-one (12):



$C_{15}H_{14}O_6$: 290.27 g.mol^{-1}

Potassium phosphate buffer (50 mM, 1 mM EDTA, 1 mM DTT, pH 7; 100 mL) was degassed under reduced pressure for 20 minutes to remove molecular oxygen, followed by stirring under argon atmoshphre. Under argon counter flow, D-glucose (314.8 mg, 1.75 mmol, 5 equiv.), $NADP^+$ (27.5 mg, 34.9 µmol, 0.1 equiv.), $Na_2S_2O_4$ (1216.7 mg, 6.99 mmol, 20 equiv.), and citerorosein (**4**; 100 mg, 349.3 µmol) in DMSO (10 mL, 10% v/v), GDH (200 U), and PHAR_*his* (4 mL, 2.1 mg/mL) were added to the buffer and the mixture was stirred under argon atmosphere for 24 h. The solution was extracted with EtOAc (3x50 mL), dried over Na_2SO_4, and the solvent was removed under reduced pressure. Flash column chromatography (silica gel; DCM/MeOH, 90:10) afforded the title compound **12** (75.0 mg, 74%) as an orange solid. Conversion: > 99 % (^{1}H NMR in acetone-d_6)

TLC (MeOH: $CHCl_3$, 1:9 v/v): R_f = 0.2. Yield: 75.0 mg (74%),

^{1}H NMR (400 MHz, acetone-d_6): δ 2.80 (dd, ^2J = 17.0 Hz, ^3J = 7.0 Hz, 1H, H-4), 3.01 (dd, ^2J = 17.1 Hz, ^3J = 3.3 Hz, 1H, H-4), 3.09 (dd, ^2J = 16.3 Hz, ^3J = 6.7 Hz, 1H, H-2), 3.28 (dd, ^2J = 16.4 Hz, ^3J = 3.7 Hz, 1H, H-2), 4.39 (dd, ^2J = 10.8 Hz, ^2J = 4.8 Hz, 1H), 4.46 (m, 1H, H-3), 4.75 (d, ^3J = 5.6 Hz, 2H), 6.84 (s, 1H, ArH), 7.67 (s, 1H, ArH), 7.69 (s, 1H, OH-10), 9.81 (s, 1H, OH-8), 15.90 (s, 1H, OH-9).

^{13}C-NMR (100 MHz, acetone-d_6): δ 32.6 (C-4), 46.8 (C-2), 64.7 ($\underline{C}H_2$), 66.0 (C-3), 110.0 (C-5), 110.3 (C-7), 110.6, 112.4, 117.8, 133.9, 142.1, 148.1 (C-10), 159.0 (C-8), 160.0 (C-9), 205.1 (C-1).

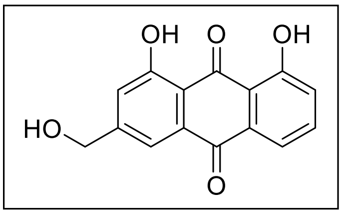
Assignment of protonated carbons is done by 2D-HSQC (^{1}H-^{13}C), COSY (^{1}H-^{1}H) experiments.

HRMS (ESI-TOF) m/z: $[M+H]^+$: Calcd for $C_{15}H_{15}O_6$ 291.0863: Found 291.0868.

CD (c 50 µM, 1,4-dioxane): λ [nm] (mdeg) 215 (–1.39), 228 (–1.15), 240 (–0.45), 250 (–1.10), 259 (–1.44), 270 (–1.34), 300 (–0.09), 310 (0.41), 320 (0.60), 348 (0.22), 360 (0.35), 380 (0.78), 419 (1.53), 450 (1.09), 497 (0.09).

$[\alpha]_D^{27}$ = +22.4 (c = 0.025, acetonitrile).

1,8-dihydroxy-3-(hydroxymethyl)anthracene-9,10-dione (aloe-emodin, 8).



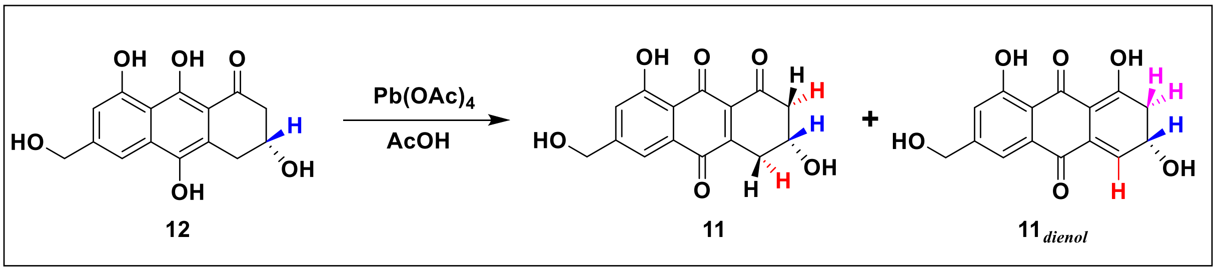
The formation of the title compound aloe-emodin (**8**) (9 mg, 8.0 %) was observed after workup and it was isolated as a pure compound using column chromatography and DCM as an eluent during the purification of **12**.

TLC (cyclohexane/ethyl acetate, 7:3 v/v): $R_f = 0.7$,

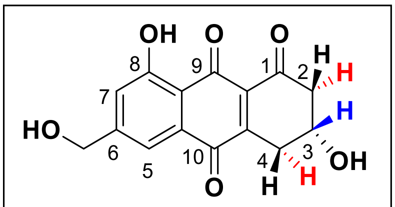
^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 4.63 (d, $J = 5.6$ Hz, 2H), 5.62 (t, $J = 5.6$ Hz, 1H), 7.29 (s, 1H), 7.39 (d, $J = 8.2$ Hz, 1H), 7.69 (s, 1H), 7.72 (d, $J = 7.6$ Hz, 1H), 7.81 (t, $J = 7.9$ Hz, 1H)), 11.92 (s, 1H), 11.98 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 62.1, 114.5, 116.0, 117.1, 119.4, 120.7, 124.5, 133.2, 133.4, 137.4, 153.7, 161.4, 161.6, 181.5, 191.7.[32]

VI. Charaterization of 3,4-Dihydro Citreorosein (11) and its tautomer (11*dienol*)



3,4-Dihydrocitreorosein (11)



11

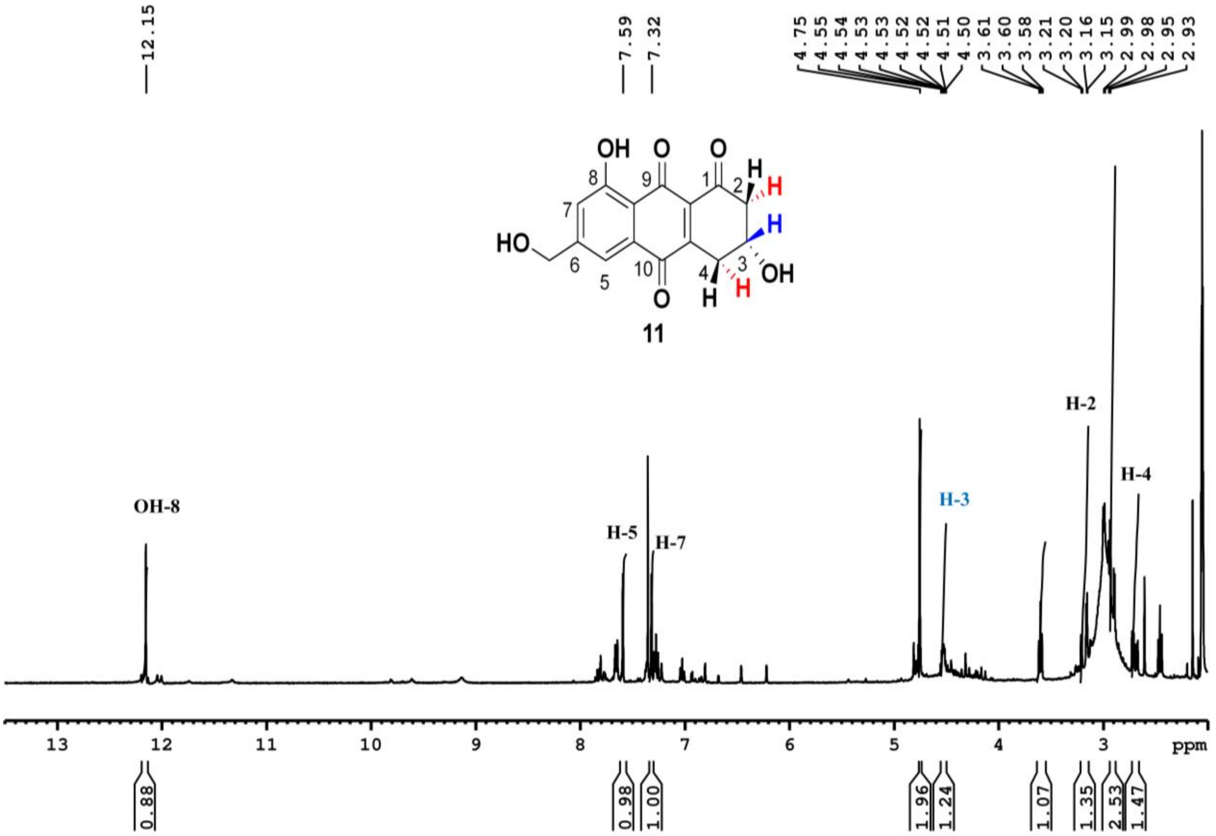
$C_{15}H_{12}O_6$: 288.25 g.mol^{-1}

To an ice-cold suspension of **12** (15.0 mg, 51.6 µmol, 1.0 equiv.) in acetic acid (0.15 mL) was added lead tetraacetate (22.0 mg, 51 µmol, 1.0 eqiv) at 0 ºC. The reaction was monitored through TLC (MeOH/$CHCl_3$ 1:9). After 20 minutes colour of the reaction mixture was turned into orange. To this ice-cold water was added and the reaction mixture was extracted with ethyl acetate (3x10 mL), followed by removal of solvent under reduced pressure. Flash column chromatography (0.2 N oxalic acid impregnated silica gel 230-400; acetone/benzene, 3:17) afforded the mixture of **11** and **11*dienol*** (8.0 mg, 54%) as an orange solid. **TLC** (MeOH/$CHCl_3$, 1:9 v/v): R_f = 0.4,

^{1}H NMR of 11 (400 MHz, acetone-d_6): δ 2.70 (ddd, ^2J = 15.1 Hz, ^3J = 7.0 Hz, ^4J = 1.1 Hz, 1H, H-2), 2.95 (ddd, ^2J = 18.3 Hz, ^3J = 10.1 Hz, ^4J = 2.3 Hz, 2H, H-2/H-4), 3.19 (dd, ^2J = 19.8 Hz, ^3J = 4.1 Hz, l H, H-4), 4.58–4.48 (m, 1H, H-3), 4.75 (s, 2H, OH-6), 7.32 (s, 1H, H-7), 7.59 (s, 1H, H-5), 12.15 (s, 1H, OH-8).

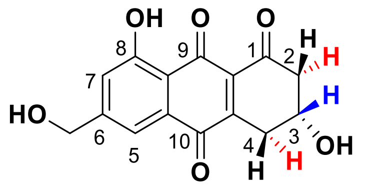
HRMS (ESI-TOF) m/z: $[M+H]^+$: Calcd for $C_{15}H_{13}O_6$ 289.0707: Found 289.0721.

^{1}H NMR (400 MHz, acetone-d_6)



1D-^{1}H-^{1}H Selective Gradient TOtal Correlation Spectroscopy Experiment

1D-TOCSY (400 MHz, acetone-*d₆*): Selective gradient excitation at freq: 2.70 ppm, 2.95 ppm, 3.19 ppm, 4.54 ppm.



11

This study confirms the spin system of keto tautomer **11** (First intermediate).

Pulse programme: seldigpzs, Acquisition time [AQ]= 4.08 Sec, Dwell time [DW]= 62.4 μsec, Pre-scan delay [DE]= 6.5 μsec.

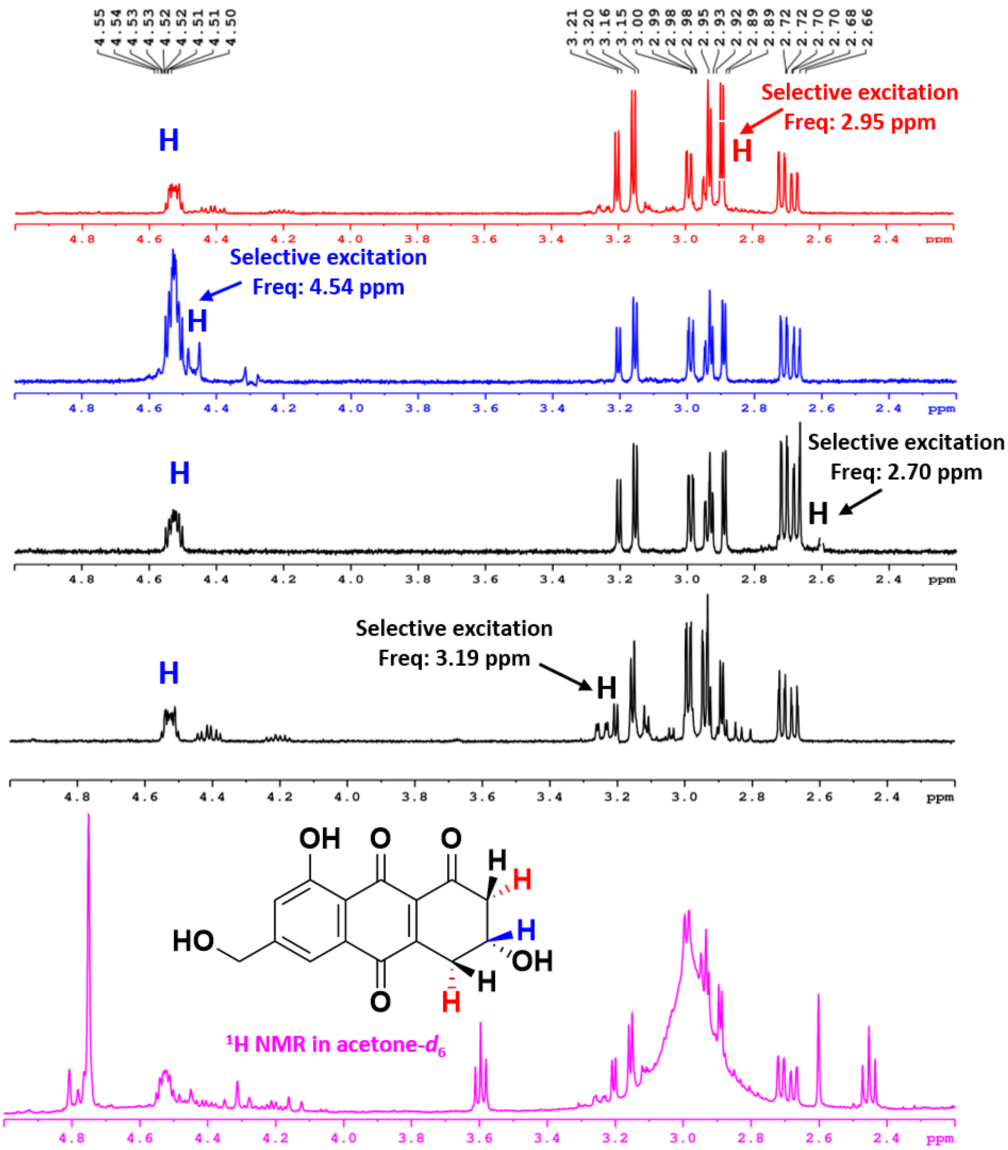
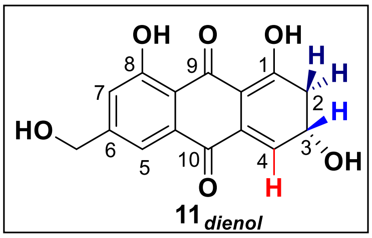


Figure S1. 1D-^{1}H-^{1}H Selective Gradient TOtal Correlation Spectra (11).

1D-TOCSY (400 MHz, acetone-d_6): Selective gradient excitation at freq: 4.80 ppm, 6.92 ppm.



This study confirms the spin system of enol tautomer **11***dienol* (second intermediate).

[Pulse programme: seldigpzs, Acquisition time [AQ]= 4.08 Sec, Dwell time [DW]= 62.4 μsec, Pre-scan delay [DE]= 6.5 μsec].

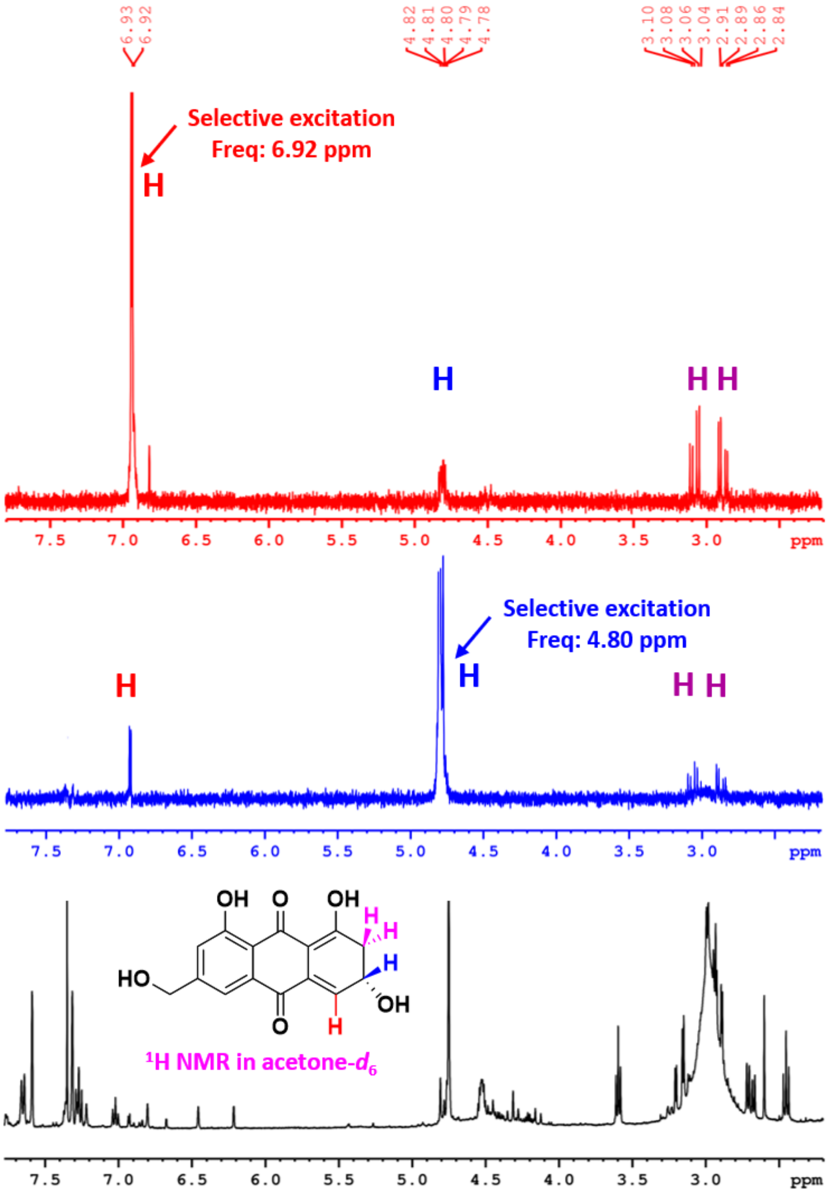


Figure S2: 1D-^{1}H-^{1}H TOtal Correlation Spectra (1D-TOCSY, 11$_{dienol}$).

VII. Measurement of conversion from dihydro citreorosein (11) into aloe-emodin (8) with time through ¹H NMR study.

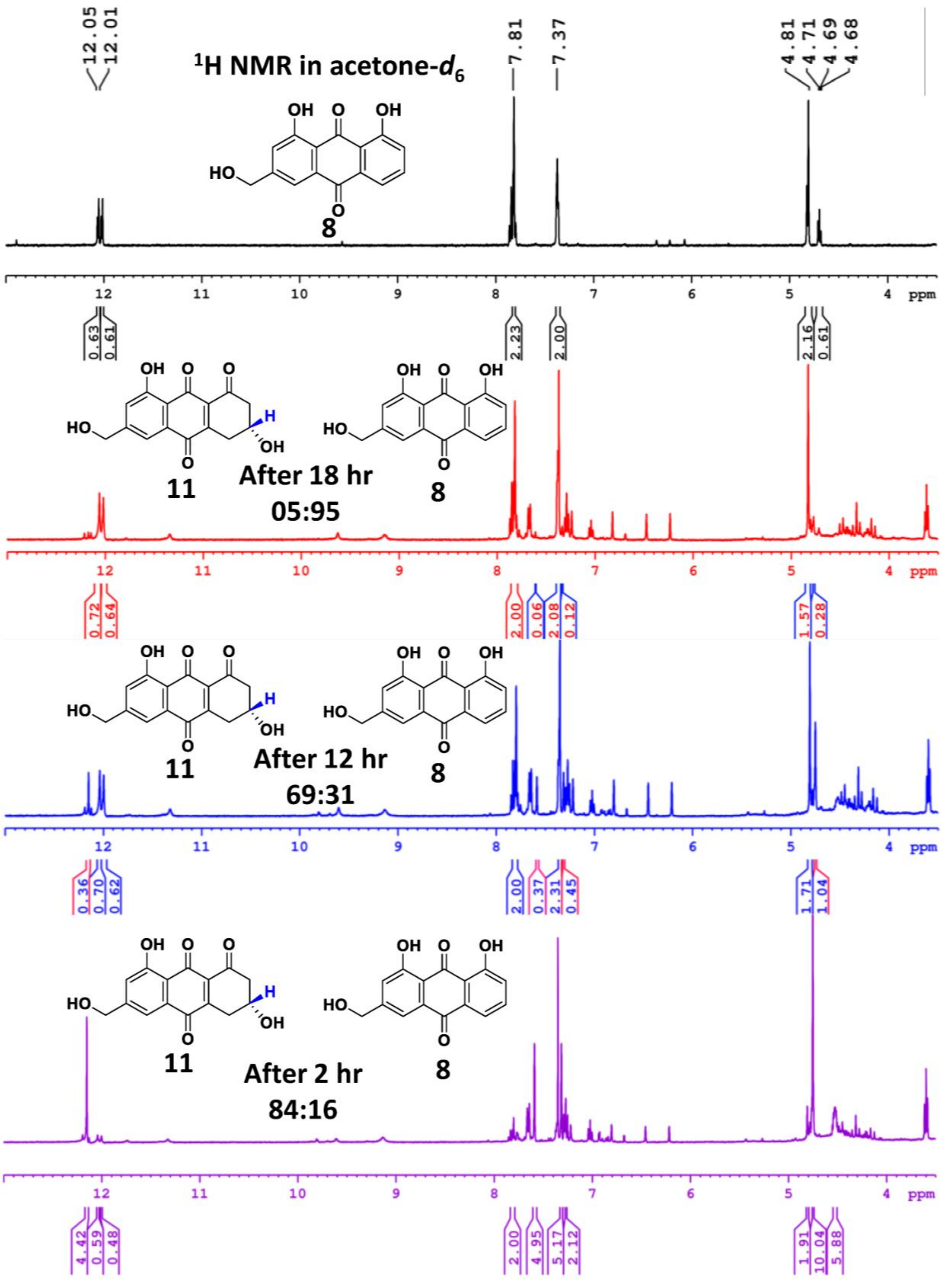
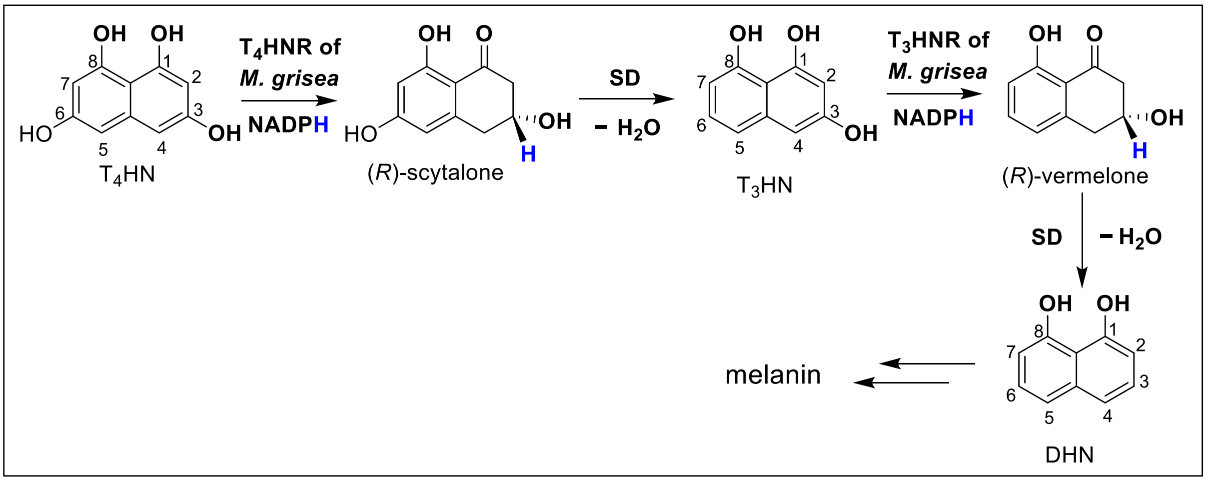


Figure S3. Measurement of spontaneous conversion of 11 into 8 with time through ¹H NMR study.

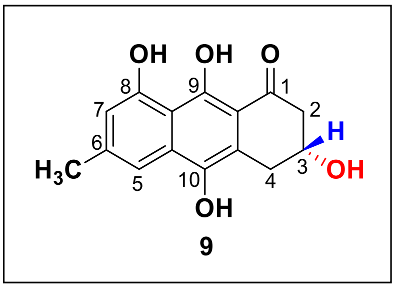
VIII. Biosynthesis of fungal DHN-melanin starting from polyketide based T_4HN



Scheme S2. Native reactions catalyzed by T_4HNR and T_3HNR of *M. grisea* and scytalone dehydratase (SD)[5–6]

IX. Chemoenzymatic Reduction of emodin (1)

(*R*)-3,8,9,10-Tetrahydroxy-6-methyl-3,4-dihydroanthracen-1(2*H*)-one (9)[2]



$C_{15}H_{14}O_5$: 274.27 g·mol^{-1}

Potassium phosphate buffer (50 mM, 1 mM EDTA, 1 mM DTT, pH 7; 100 mL) was degassed under reduced pressure for 20 minutes to remove molecular oxygen, followed by stirring under argon atmoshphere. Under argon counterflow, D-glucose (166.68 mg, 0.93 mmol, 5 equiv.), $NADP^+$ (15.76 mg, 18.50 µmol, 0.1 equiv.), $Na_2S_2O_4$ (643.8 mg, 3.70 mmol, 20 equiv.), and emodin (**1**; 50.0 mg, 185.02 µmol) in DMSO (5 mL, 10% v/v), GDH (100 U), and MdpC_*his* (3 mL, 2.8 mg/mL) were added to the buffer and the mixture was stirred under argon atmosphere for 24 h. The solution was extracted with EtOAc (3x30 mL), dried over Na_2SO_4, and the solvent was removed under reduced pressure. Flash column chromatography (silica gel; hexane/EtOAc, 1:4) afforded the title compound **9** (38.2 mg, 75%) as a yellow solid. **TLC:** (cyclohexane/EtOAc, 1:1 v/v): $R_f = 0.29$.

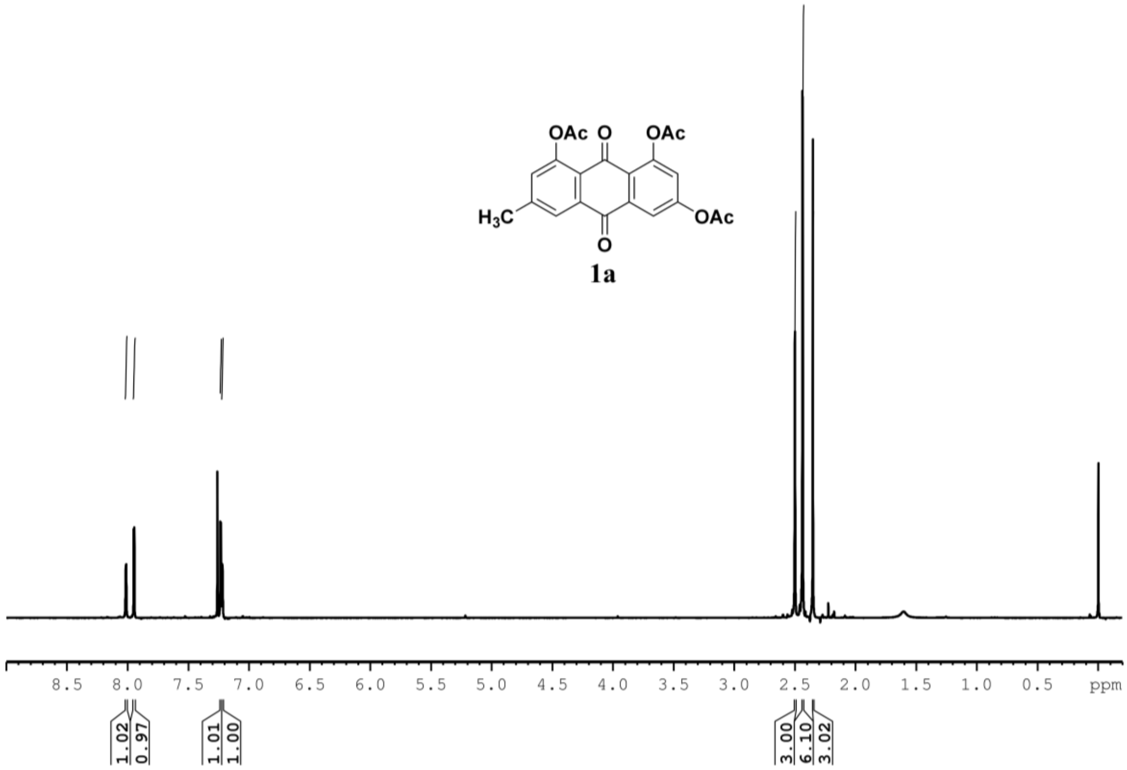
^{1}H NMR (400 MHz, acetone-*d*$_6$): δ 2.44 (s, 3H, CH_3), 2.80 (ddd, $^2J = 17.1$ Hz, $^3J = 7.1$ Hz, $^4J = 1.1$ Hz, 1H, H-2), 3.0 (dd, $^2J = 17.1$ Hz, $^3J = 2.9$ Hz, 1H, H-2), 3.07 (dd, $^2J = 16.4$ Hz,$^3J = 6.8$ Hz, 1H, H-4), 3.26 (dd, $^2J = 16.4$ Hz, $^3J = 3.6$ Hz, 1H, H-4), 4.37 (bs, 1H, OH-3), 4.42– 4.48 (m, 1H, H-3), 6.69 (s, 1H, H-7), 7.47 (s, 1H, H-5), 7.64 (s, 1H, OH-10), 9.78 (s, 1H, OH- 8), 15.94 (s, 1H, OH-9).

^{13}C NMR (100 MHz, acetone-*d*$_6$): δ 21.5 (CH_3), 31.7 (C-4), 45.8 (C-2), 65.0 (C-3), 109.1 (8a/9a), 110.7 (8a/9a), 112.5 (C-5/C-7), 112.6 (C-5/C-7), 116.8 (C-10a), 133.1 (C-5a), 140.7 (C-10), 142.8 (C-6), 158.0 (C-8/C-9), 159.3 (C-8/C-9), 204.0 (C-1).

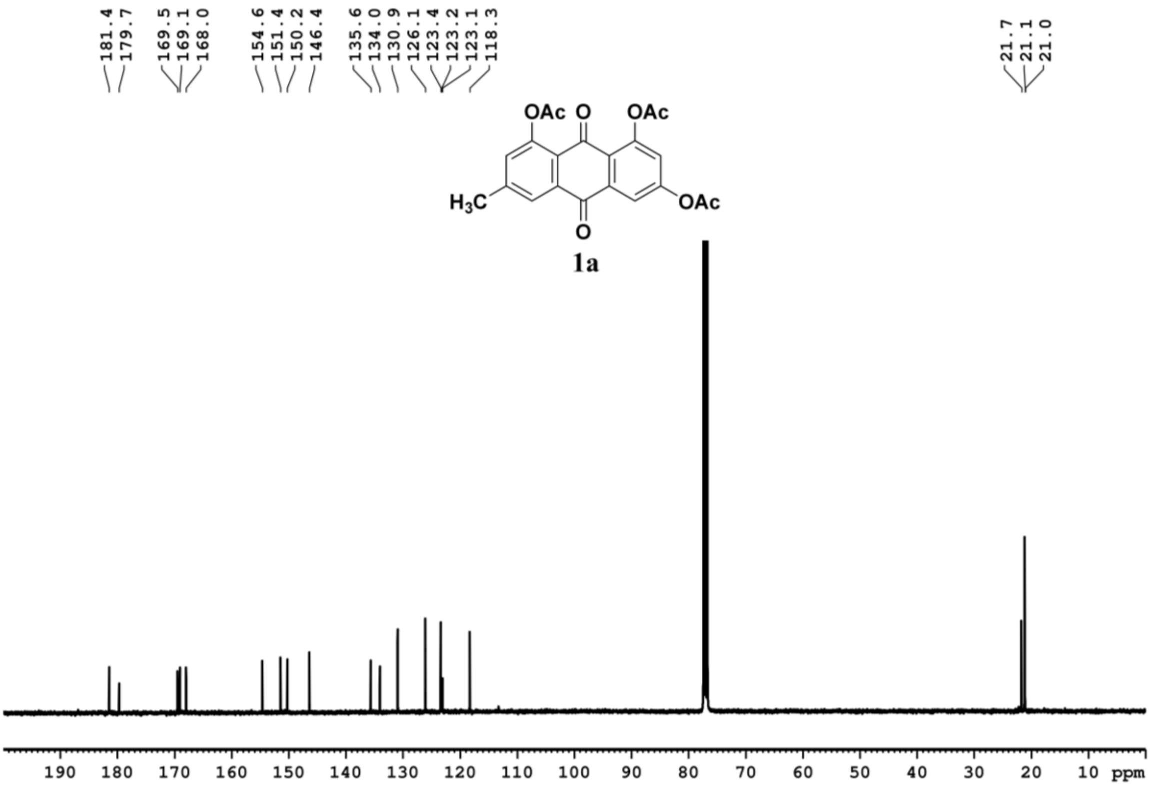
CD (*c* 50 µM, 1,4-dioxane): λ [nm] (mdeg) 220 (0.13), 224 (–1.14), 231 (–1.90), 240 (–0.73), 245 (–0.41), 260 (–1.31), 267 (–1.76), 280 (–0.44), 288 (–0.16), 295 (–0.21), 323 (0.27), 350 (0.00), 418 (0.19).[26]

X. NMR Spectra

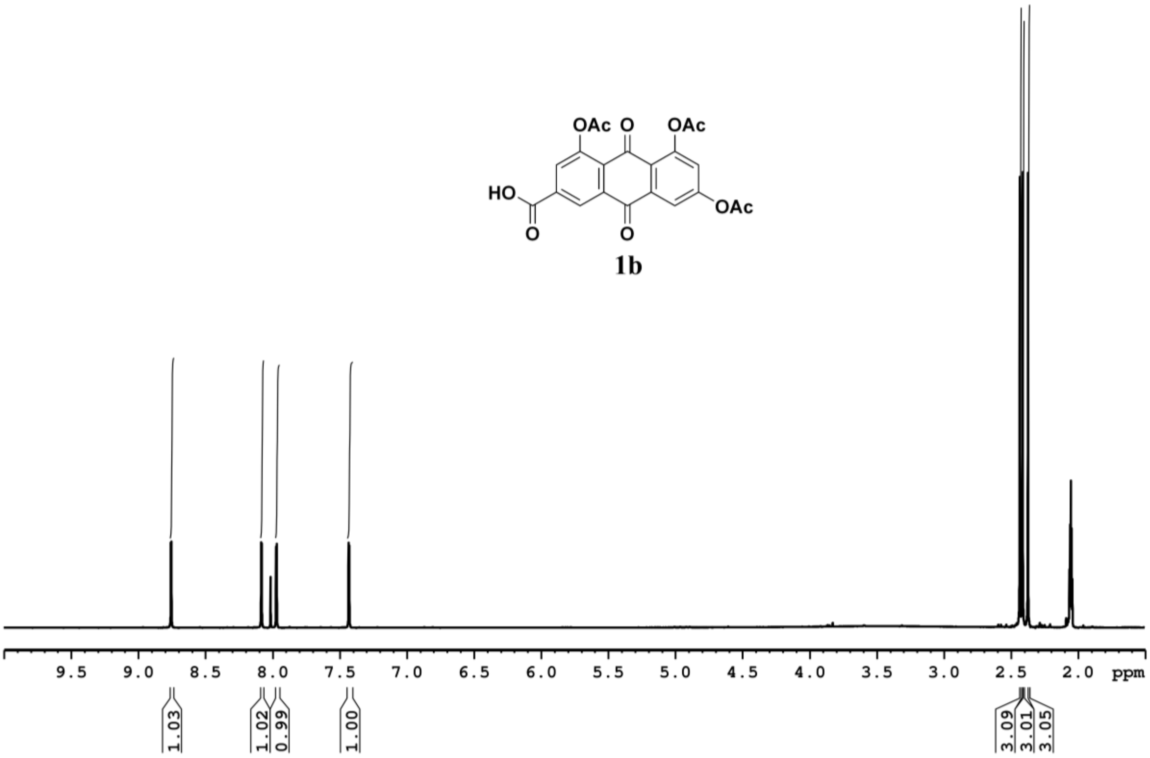
[1]H NMR (400 MHz, $CDCl_3$)



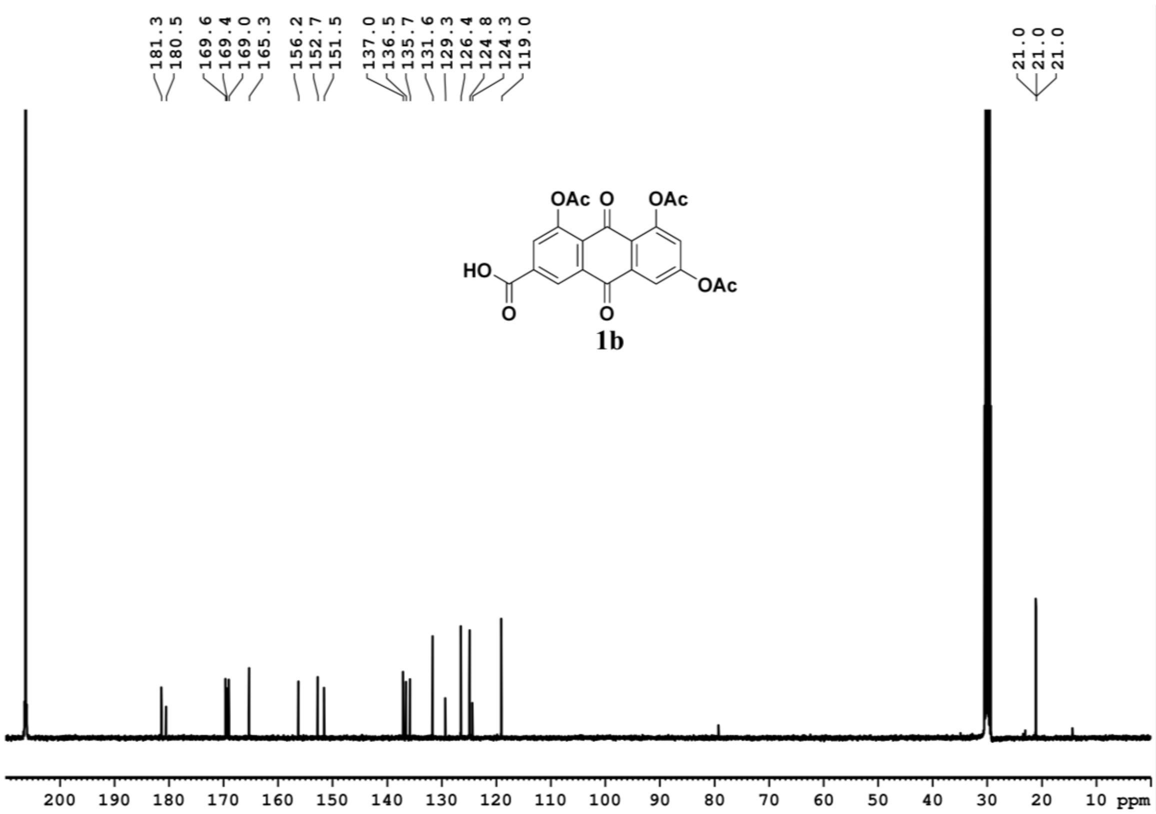
[13]C NMR (100 MHz, $CDCl_3$)



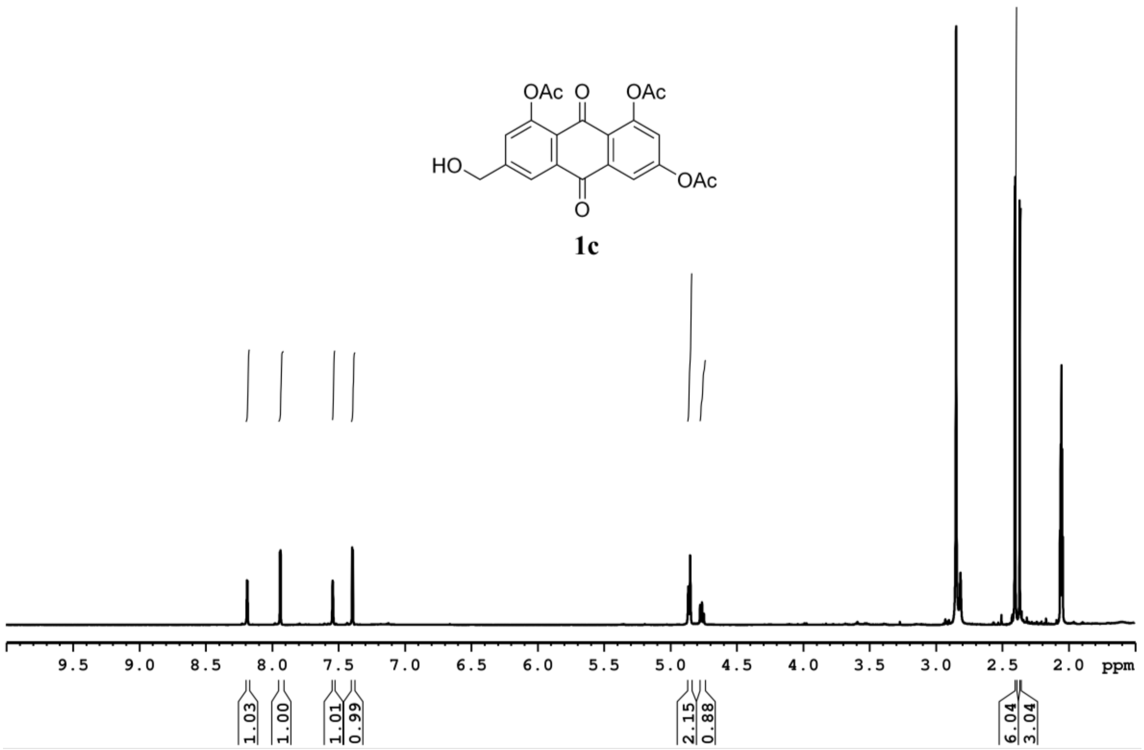
^{1}H NMR (400 MHz, acetone-d_6)



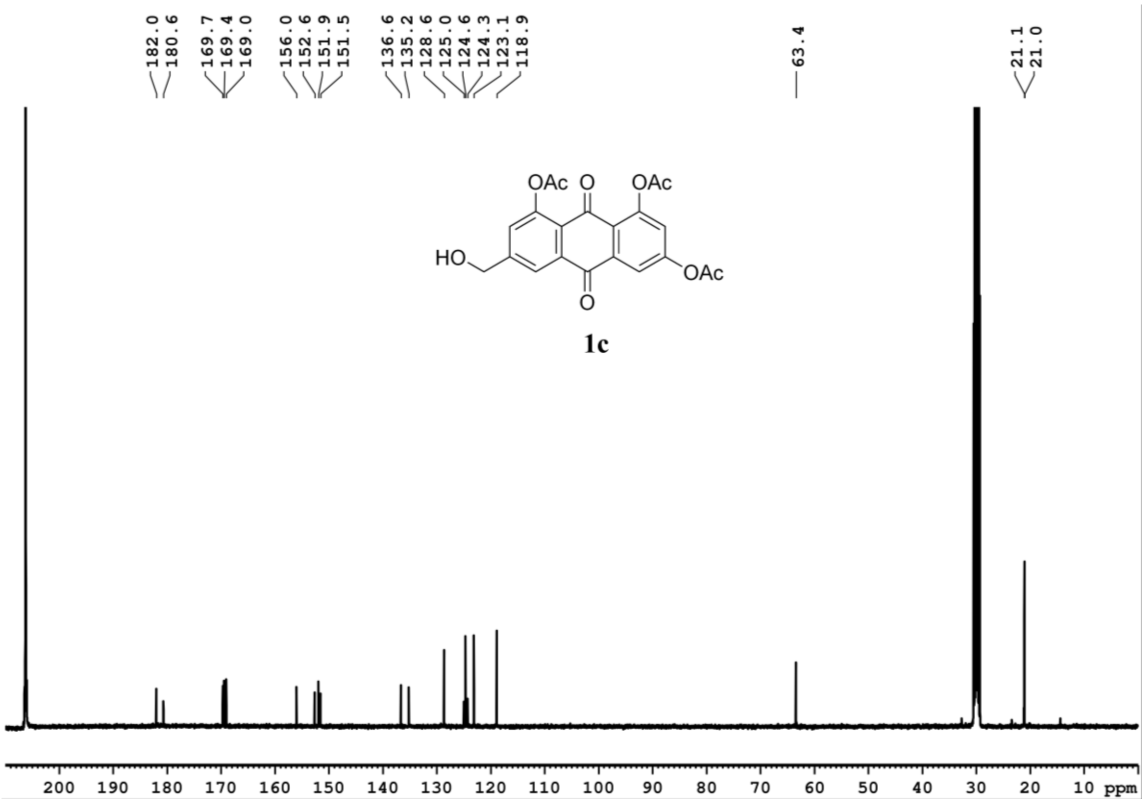
^{13}C NMR (100 MHz, acetone-d_6)



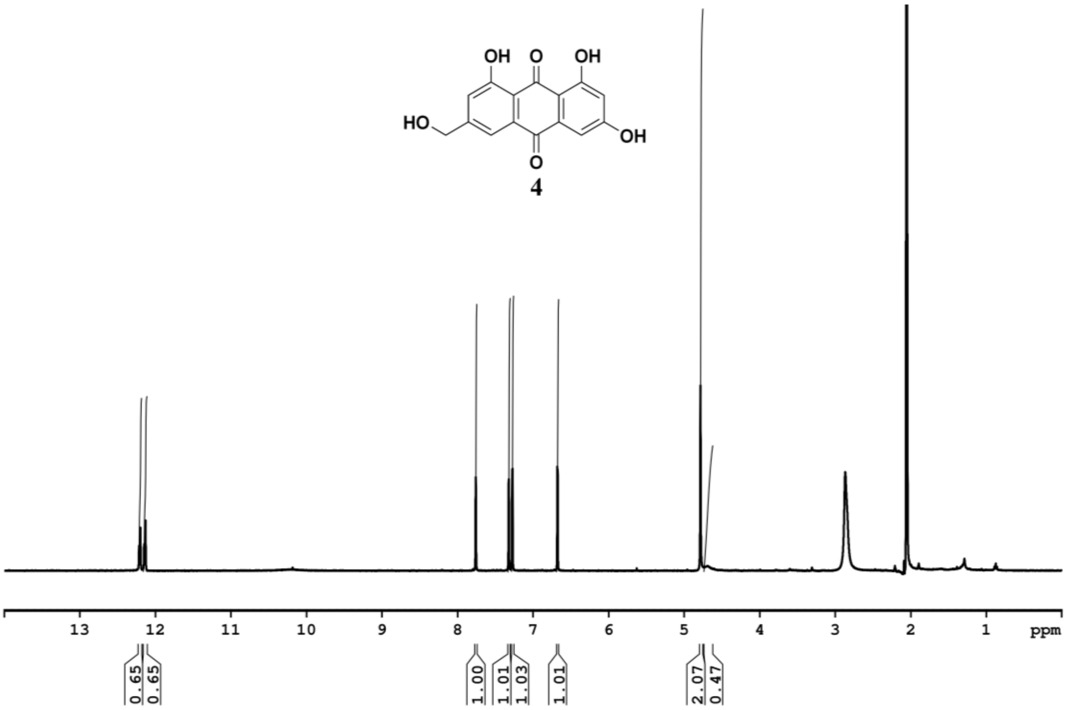
^{1}H NMR (400 MHz, acetone-d_6)



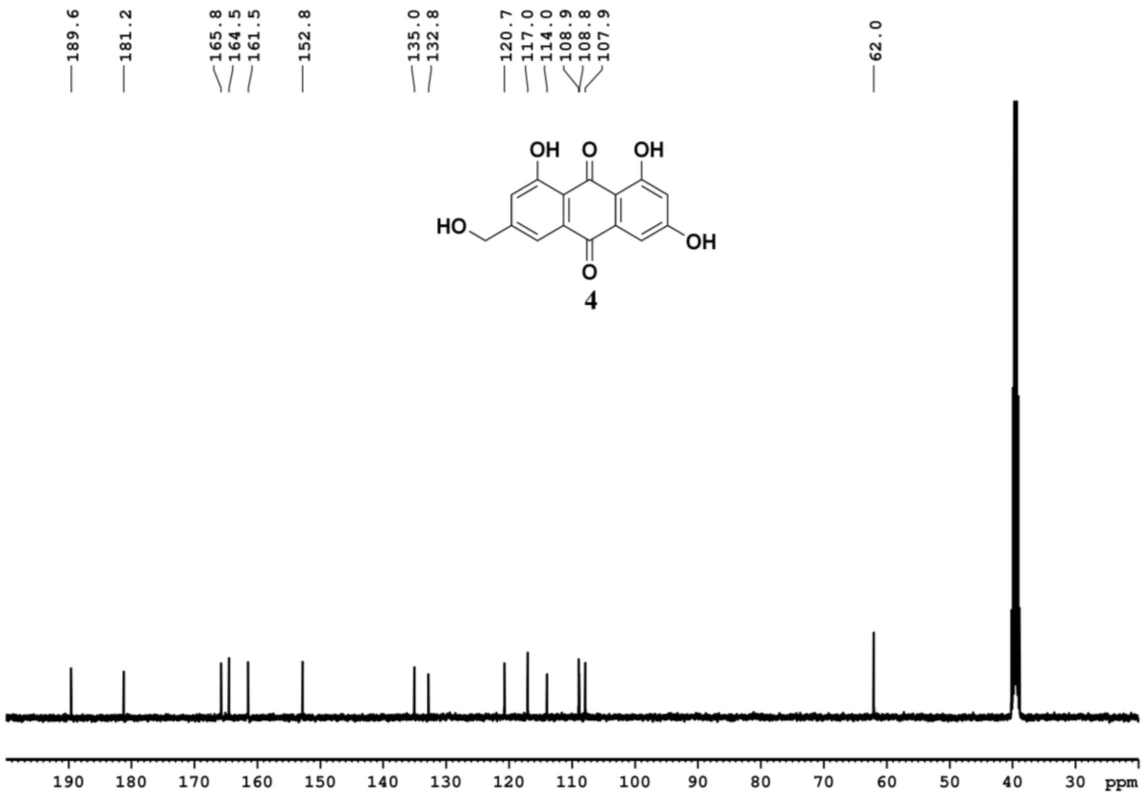
^{13}C NMR (100 MHz, acetone-d_6)



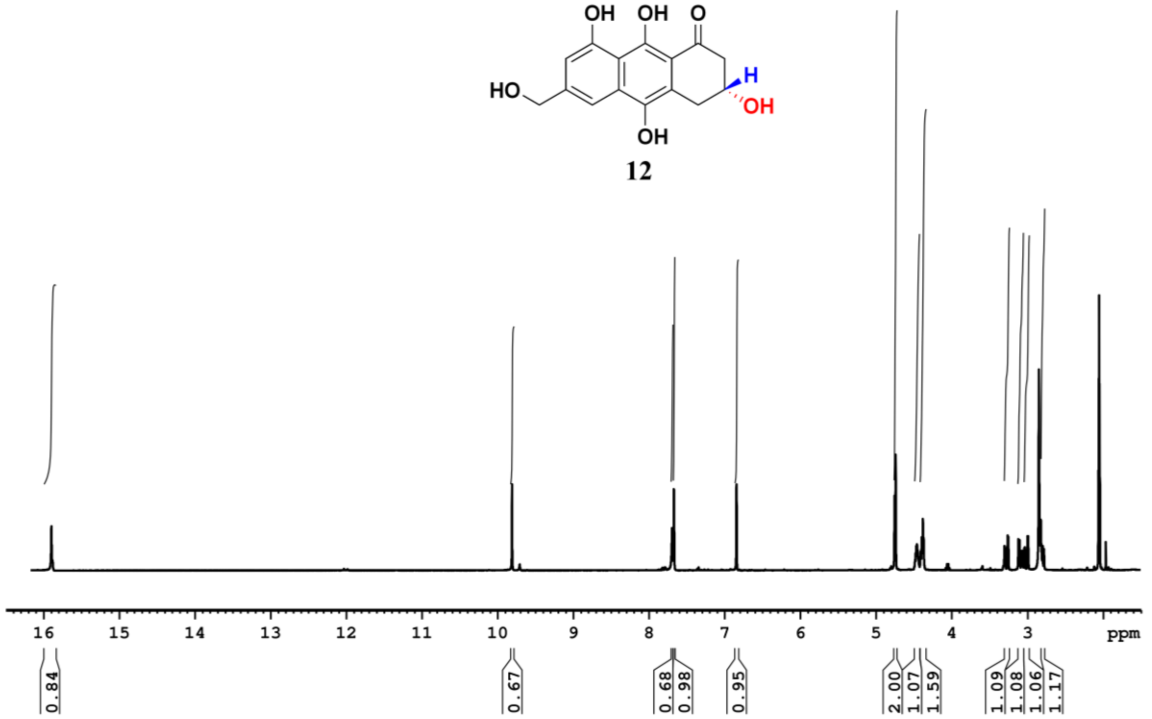
^{1}H NMR (400 MHz, DMSO-d_6)



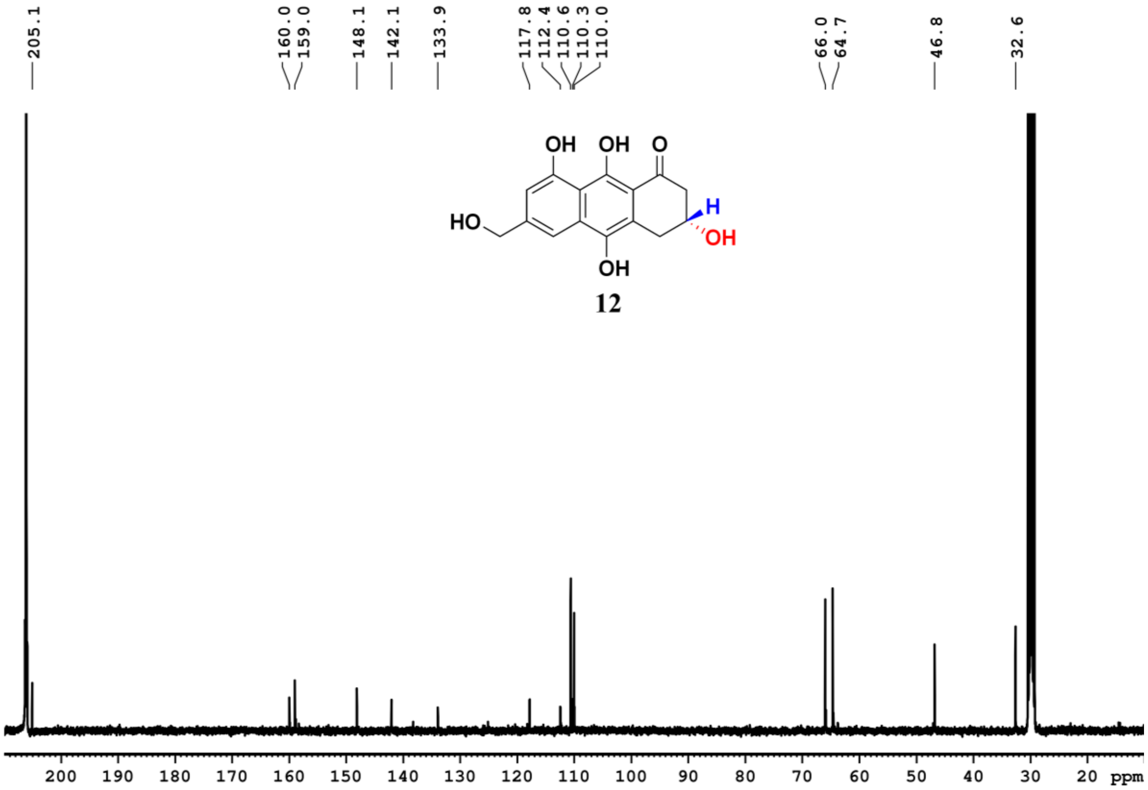
^{1}H NMR (400 MHz, DMSO-d_6)



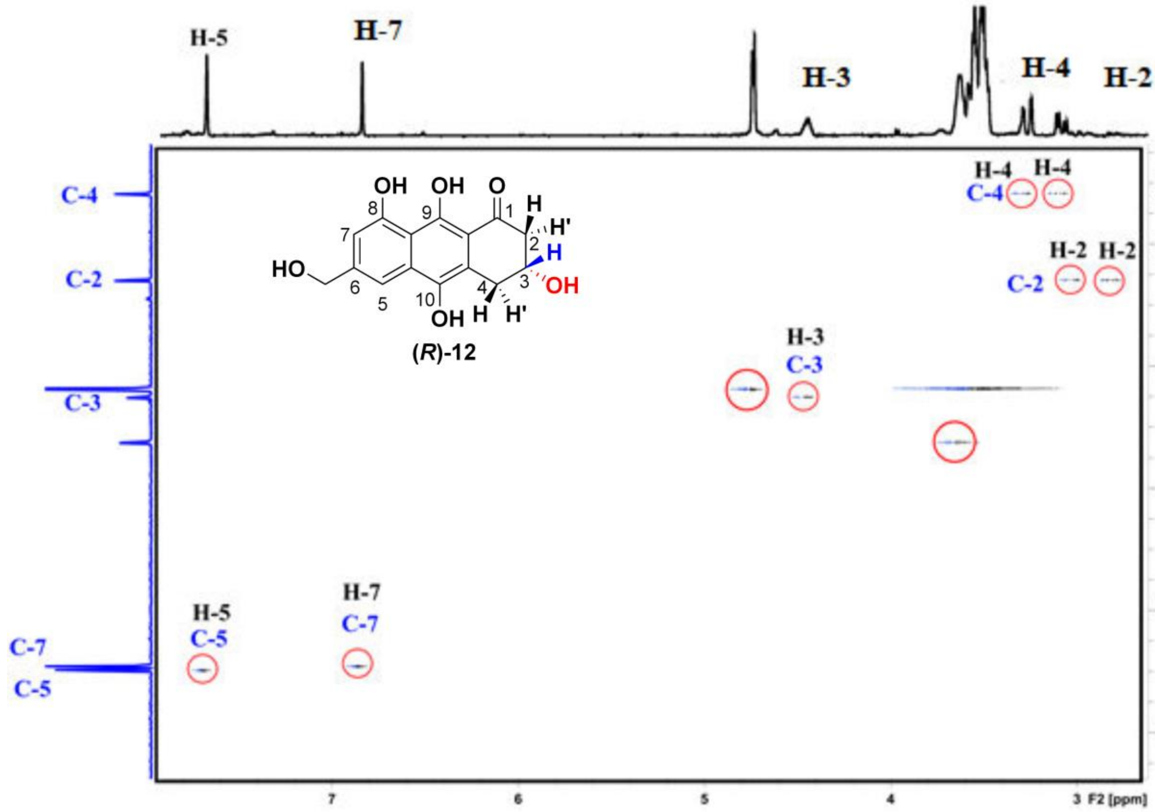
^{1}H NMR (400 MHz, acetone-d_6)



^{13}C NMR (100 MHz, acetone-d_6)

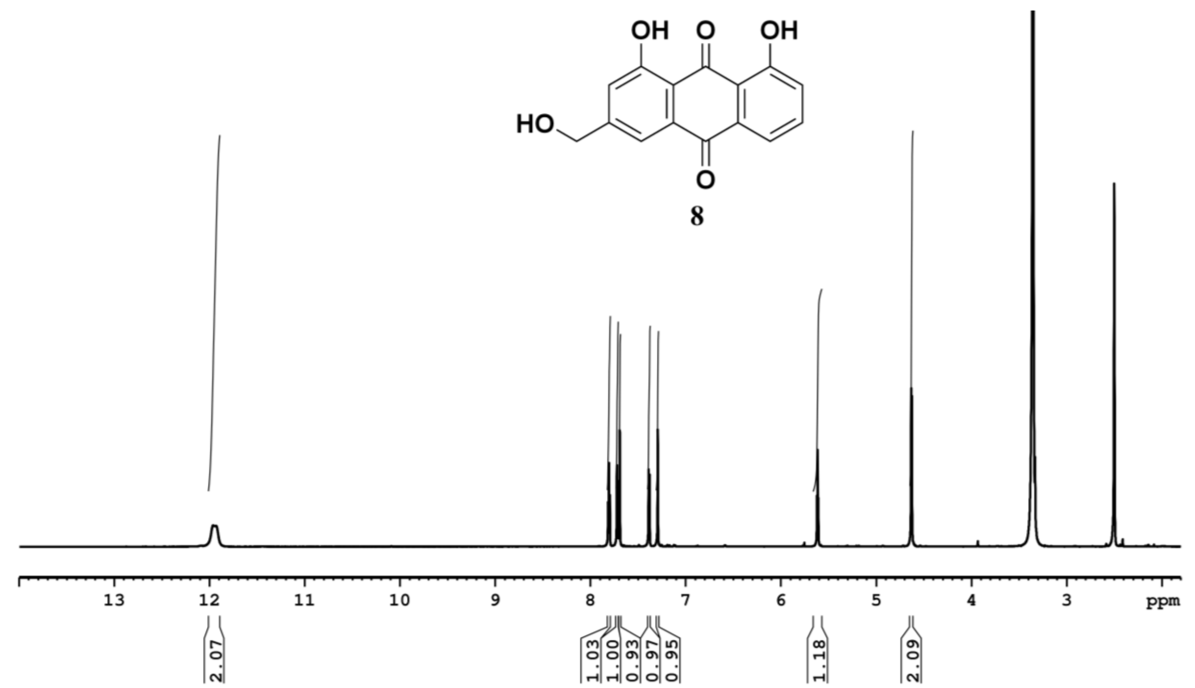


HSQC (^{1}H-^{13}C)

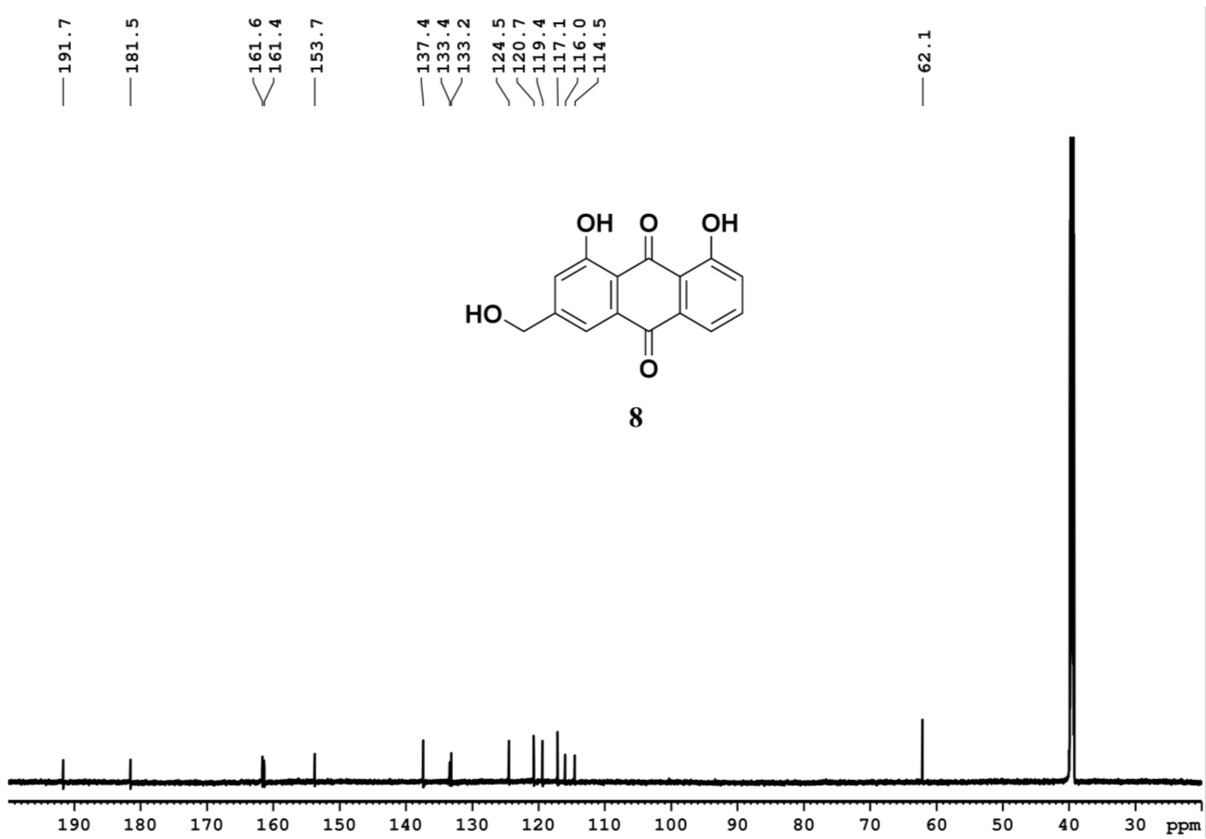


H-5
H-7
H-3
H-4
H-2
C-4
C-2
C-3
C-7
C-5
OH
OH
O
OH
OH
HO
H
H'
H
H'
(R)-12
H-4
C-4
H-2
C-2
H-3
C-3
H-5
C-5
H-7
C-7
F1 [ppm]
F2 [ppm]

^{1}H NMR (400 MHz, DMSO-d_6)



^{13}C NMR (100 MHz, DMSO-d_6)



XI. CD Spectra

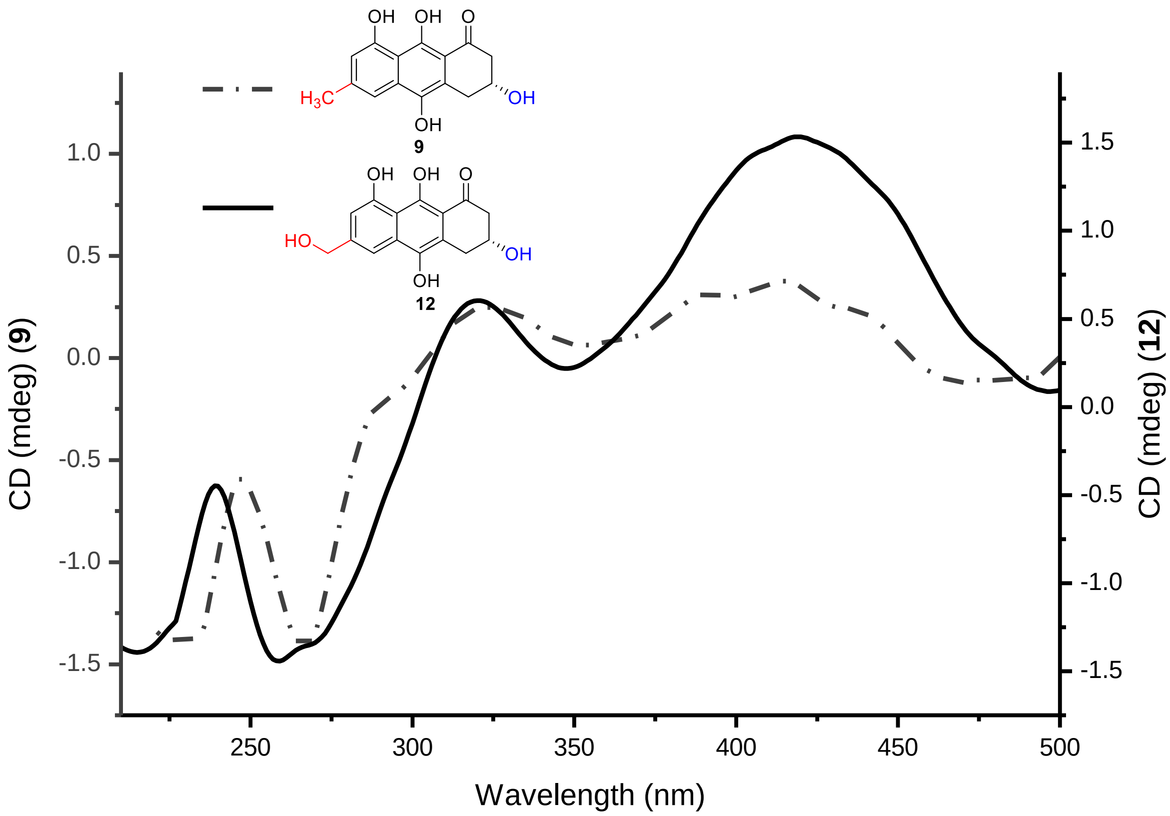


Figure S4. Comparison of CD spectra of 12 with 9.

XII. References

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