Electronic Supporting Information

A Biocatalytic Approach towards the Preparation of Natural Deoxyanthraquinones and their Impact on Cellular Viability

Anshul Rajput, Arijit De, Amit Mondal, Kiran Das, Biswanath Maity, Syed Masood Husain*

I. General Remarks

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. 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, brs = broad singlet, d = doublet, dd = doublet of a doublet, ddd = doublet of a doublet of doublet, t = triplet, dt = doublet of a triplet, m = multiplet. Electrospray ionization (ESI) mass spectrometry (MS) experiments were performed on an Agilent 6530 Accurate-Mass Q-TOF LC/MS system (Agilent Technologies). For determination of the enantiomeric excess (ee) the chiral phases Chiralcel OD-H (Daicel Inc., 250 × 4.6 mm, 5 µm), Chiralcel OJ-H (Daicel Inc., 250 × 4.6 mm, 5 µm), Chiralpak IC (Daicel Inc., 250 × 4.6 mm, 5 µm), Chiralcel OZ-H (Daicel Inc., 250 × 4.6 mm, 5 µm) and Chiralpak AS-H (Daicel Inc., 250 × 4.6 mm, 5 µm) were used on Agilent Technologies 1260 Infinity HPLC system equipped with OpenLAB CDS v2.3 software.

II. Isolation and biological activities of natural deoxyanthraquinones

Table S1. Summary of deoxyanthraquinone bioactivities and their isolation

Deoxyanthraquinone	Biological Activities	Isolation	Ref.
Chrysophanol (**1**)	antifungal, antibacterial, anticancer, hepatoprotective, neuroprotective, anti-inflammatory, antiulcer, antiviral, antimicrobial, antidiabetic and antiobesity activity	*Rheum rhabarbarum, Cassia* species, *Penicillium islandicum Sopp*	[1-3]
Aloe-Emodin (**2**)	anticancer, antivirus, antiinfluenzal, antiinflammatory, antibacterial, antiproliferative, antiparasitic, neuroprotective, hepatoprotective	Cassia *occidentalis*, *Rheum palmatum* L., *Aloe vera*, and *Polygonum multiflorum* Thunb.	[4-5]
3-Methoxychrysazin (**3**)	antifungal, mosquitocidal	*Photorhabdus temperata* of entomopathogenic nematodes *Heterorhabditis* spp.	[6, 7]
Rhein (**4**)	antifungal, antibacterial, laxative, hepatoprotective, nephroprotective, antioxidant, anticancer, antimicrobial, lipid-lowering activity, anti-inflammatory, and osteoarthritis treatment activity	*Rheum palmatum* L., *Cassia tora* L., *Polygonum multiflorum* Thunb., and *Aloe barbadensis* Miller	[8-9]
1,3,8-Tri-hydroxy anthraquinone (**5**)	antimicrobial, neuroprotective, anti-neuroinflammatory	*Photorhabdus temperate,* Endophytic fungus *Nigrospora* sp.	[7,10]
Dantron (**6**)	laxative, antioxidant, fungicide, neuroprotective, antiproleferative	*Rheum palmatum* L., *Xyris semifuscata* plant, *Pyrrhalta luteola* larvae	[11-13]
1-O-methyl chrysophanol (**7**)	antihyperglycemic (anti-diabetic)	*Amycolatopsis thermoflava* strain SFMA-103	[14]

III. Cloning, Expression of Enzymes

Gene synthesis and expression vector

The synthesis of codon-optimized gene encoding ARti (NCBI GenBank accession: CRG89873.1) was ordered from Biomatik Company (Biomatik, Ontario, Canada). The gene was cloned into pET-19b vector using 5'-NdeI and 3'-XhoI yielding (N)*his*$_{10}$-tagged ARti.[15] Plasmid encoding Glucose dehydrogenase (GDH) was obtained from Prof. Werner Hummel (University of Bielefeld, Germany).

> *ARti* (Anthrol reductase)[15]

Organism: *Talaromyces islandicus* WF-38-12

Codon-optimized Protein sequence of anthrol reductase:

MGHHHHHHHHHHSSGHIDDDDKHMMADSPYIPGRLDGKVALVTGSGRGIGAAIAV
ELGRRGAKVVVNYANAQDSAENVVAEIKSLGSDALALKADIRQVPQITKLMDDVVE
HFGGLDIVCSNSGVVSFGHVGDVTEEEFDRVFSLNTRGQFFVAREAYHHLNKGGRII
LMSSNTAKDFSVPKHSLYSGSKGAIDSFVRVMSKDCGIKKITVNAVAPGGTVTDMFH
AVAQHYIPDGHKYSPEELQQMAAHASPLTRNGYPVDIARVVCFLASKEGEWVNGK
VITLDGGAA

Transformation of plasmid to E. coli cells

Transformation of plasmid DNA to competent *E. coli* BL21 (DE3) cells was performed by applying a heat shock at 42 °C for 50 s. The transformed cells were grown overnight on SOB-agar medium containing 100 µg/mL ampicillin.

Media and growth conditions

One clone was picked and dispersed in 5 mL of LB-media (Lennox), followed by incubation overnight (37 °C, 220 rpm). Ampicillin (100 µg·mL^{-1}) was added as required.

Cultivation and expression

ARti: The overnight cultures were diluted to 500 mL of medium each (ampicillin 100 µg·mL^{-1}) and incubated at 37 °C, 160 rpm. IPTG (0.2 mM) was added to the mid-log phase (OD_{600} = 0.6) was reached, and cultures were incubated for 20 h at 18 °C, 160 rpm.

GDH: The overnight cultures were diluted to 500 mL of medium each (ampicillin 100 µg·mL^{-1}) and incubated at 37 °C, 160 rpm. IPTG (0.2 mM) was added to the mid-log phase (OD_{600} = 0.6) was reached, and cultures were incubated for 4 h at 37 °C, 160 rpm.

Workup and storage

ARti: The harvested *E. coli* cells were resuspended in lysis buffer (20 mM Tris-HCl, pH 8.0; 2.5 mL per harvested cells of 500 mL medium).

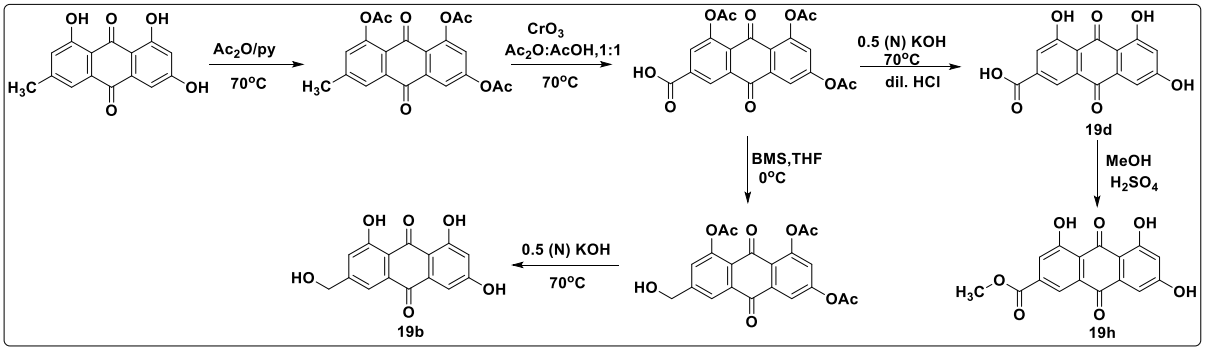
GDH: The harvested *E. coli* cells were resuspended in lysis buffer (50 mM HEPES, pH = 8.0; 2.5 mL per harvested cells of 500 mL medium).

The cells were disrupted by sonication (8 × 10 sec, Vibra-Cell Processors, model number VCX500, Sonics), followed by centrifugation (30 min, 12000×*g*, 4 °C). Glycerol (20% v/v) was added, and the crude enzyme preparation was frozen at –20 °C.

IV. Synthesis of anthraquinone substrates 19a-h

All the anthraquinones were synthesized using reported methods: emodin (isolated from commercially available *Rheum emodi* plant extract) **19a**[16], citreorosein **19b**[17], lunatin **19c**[18], emodic acid **19d**[15], 1-methyl emodin **19e**[15], tetrahydroxy emodin **19f**[15], trihydroxy emodin **6**[15], 1-methyl lunatin **19g**[19], methyl ester of emodic acid **19h**[15],

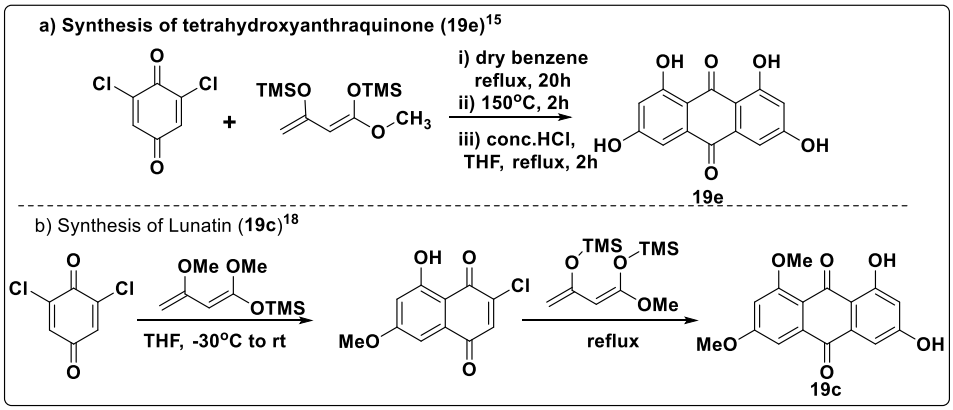
A. Synthesis of citreorosein (19b), emodic acid (19d) and methyl ester of emodic acid (19h): Substrates **19b, 19d, 19h** were synthesized starting from emodin (**19a**) in 3 to 4 steps according to scheme 1 using previously reported procedure (Scheme S1).



Scheme S1. Synthesis of citreorosein (**19b**), emodic acid (**19d**), methylester of emodic acid (**19h**)

B. Synthesis of lunatin (19c), tetrahydoxy anthraquinone (19e): Anthraquinones **19c, 19e:**

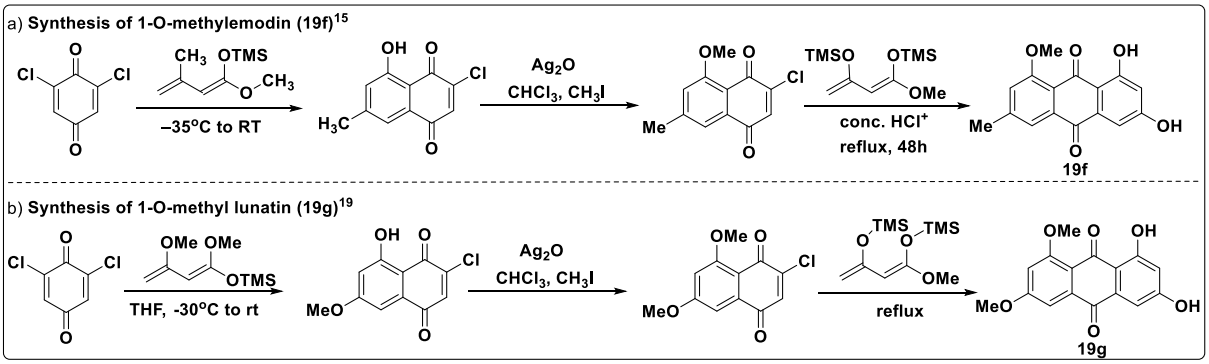
They were prepared from 2,6-dichlorobenzoquinone and various mixed vinyl ketene via consecutive Diels-Alder reactions according to the previously reported procedure. (Scheme S2)



Scheme S2. a) tetrahydroxyanthraquinone synthesis b) Synthesis of lunatin

C. Synthesis of 1-O-methylemodin (19f) and 1-O-methyllunatin (19h):

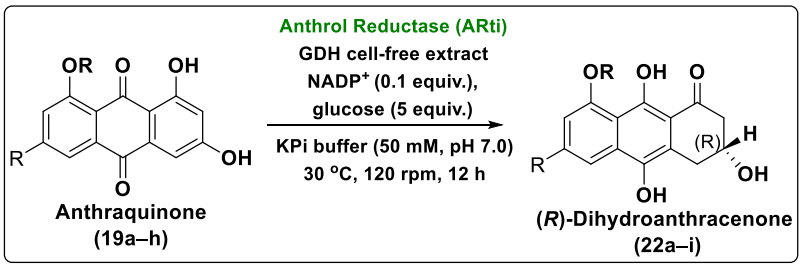
Both **19f** and **19g** were synthesized according to the scheme S3 using previously reported procedure.[4]



Scheme S3. a) 1-O-methylemodin synthesis b) Preparation of 1-O-methylunatin

V. Chemoenzymatic reduction of anthraquinones 19a-i by ARti

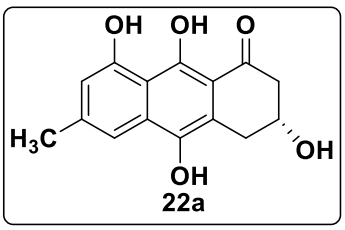
The stereo- and regioselective reduction of anthraquinones (**19a-i**) were carried out using the previously reported procedure[15] and all the analytical data for (*R*)-Dihydroanthracenone (**22a-i**) are consistent with the reported lit.[15,19]



Scheme S4: stereo- and regioselective reduction of anthraquinones

General Procedure for chemoenzymatic reduction of anthraquinones: To a 50 mL round bottom flask, $NADP^+$ (0.015 mmol, 0.1 equivalent), glucose (0.75 mmol, 5 equivalent) and GDH (30 U) was added into degassed buffer (30 mL, 50 mM KPi, pH 7.0) at room temperature. After that anthraquinone (**19a-h**) (0.15 mmol in dmso, 10% w/v) was added followed by $Na_2S_2O_4$ (3.0 mmol, 20 equivalent). ARti_his (500 μL) was added to the reaction mixture and kept stirring (100 rpm) for 12 h at room temperature. Then ethyl acetate (3 X 10 mL) the reaction mixtures were subjected to vortex and centrifugation to completely separate the organic and aqueous layers. The combined organic layer was dried over anhydrous Na_2SO_4, evaporate to dryness at rotary evaporator to afford product which is characterized by ^1H NMR. After that the crude product was subjected to next step to obtain the various 3-deoxyanthraquinones.

(*R*)-3,8,9,10-tetrahydroxy-6-methyl-3,4-dihydroanthracen-1(2H)-one (22a)



$C_{15}H_{14}O_5$: 274.27 g/mol

Conversion: 93%

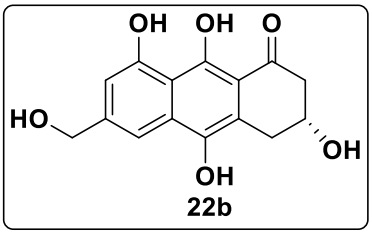
Yield: 74%

TLC: (hexane/EtOAc, 1:1 v/v): R_f = 0.29;

^{1}H NMR (400 MHz, acetone-*d*6) δ [ppm]: 2.44 (s, 3H), 2.80 (ddd, *J* = 17.1 Hz, *J* = 7.1 Hz, *J* = 1.1 Hz, 1H), 3.0 (dd, *J* = 17.1 Hz, *J* = 2.9 Hz, 1H), 3.07 (dd, *J* = 16.4 Hz, *J* = 6.8 Hz, 1H), 3.26 (dd, *J* = 16.4 Hz, *J* = 3.6 Hz, 1H), 4.37 (bs, 1H), 4.42– 4.48 (m, 1H), 6.69 (s, 1H), 7.47 (s, 1H), 7.64 (s, 1H), 9.78 (s, 1H), 15.94 (s, 1H).

HPLC: [Flow rate: 1 mL/min; Typical injection volume: 5 µL; Isocratic: 95% n-Hexane, 5% Isopropanol; DAD: 280 nm (bandwidth = 4 nm); Column: Chiralcel OD-H, 5 µm, 4.6 mm (ϕ) x 250 mm (L) mm, Temperature: 20 °C.]: Retention time (R_t), (*R*-**22a**) = 62.55 min.; >99% ee (determined by comparison to rac-**22a**, R_t [(*S*)-**22a**] = 52.27 min, R_t [(*R*)-**22a**] = 63.23 min.

(*R*)-3,8,9,10-tetrahydroxy-6-(hydroxymethyl)-3,4-dihydroanthracen-1(2H)-one (22b)



22b

$C_{15}H_{14}O_6$: 290.27 g/mol

Conversion: 100%

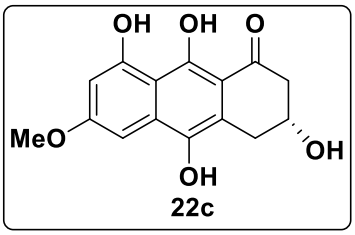
Yield: 76%

TLC (MeOH: $CHCl_3$, 1:9 v/v): R_f = 0.2.

^{1}H NMR (400 MHz, acetone-*d*$_6$): δ (ppm) 2.82 – 2.78 (m, 1H), 3.01 (dd, *J* = 17.1, 3.3 Hz, 1H), 3.11 (dd, *J* = 16.3, 6.7 Hz, 1H), 3.28 (dd, *J* = 16.4, 3.7 Hz, 1H), 4.38 (dd, *J* = 10.8, 4.8 Hz, 1H), 4.46 (m, 1H), 4.75 (d, *J* = 5.6 Hz, 2H), 6.84 (s, 1H), 7.67 (s, 1H), 7.69 (s, 1H), 9.81 (s, 1H), 15.90 (s, 1H).

HPLC: [Flow rate: 0.5 mL/min; Typical injection volume: 5 µL; Isocratic: 85% n-Hexane, 15% Isopropanol; DAD: 280 nm (bandwidth = 4 nm); Column: Chiralpak IC, 5 µm, 4.6 mm (ϕ) x 250 mm (L) mm, Temperature: 20 °C.]: Retention time (R_t), (*R*-**22b**) = 48.04 min.; >99% ee (determined by comparison to rac-**22b**, R_t [(*S*)-**22b**] = 41.23 min, R_t [(*R*)-**22b**] = 48.66 min)

(*R*)-3,8,9,10-tetrahydroxy-6-methoxy-3,4-dihydroanthracen-1(2H)-one (22c)



$C_{15}H_{14}O_6$: 290.27 g/mol

Conversion: 82%

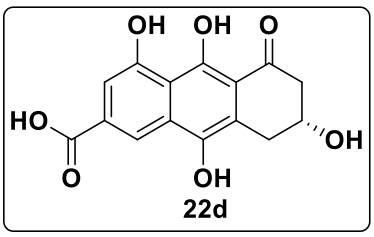
TLC: (hexane/EtOAc, 1:1 v/v): R_f = 0.32.

Yield: 64%

^{1}H-NMR: (400 MHz, acetone-d_6) δ [ppm]: 2.77 (dd, J = 17.1 Hz, J = 7.2 Hz, 1H), 2.91 –3.08 (m, 2H), 3.24 (dd, J = 16.4 Hz, J = 3.4 Hz, 1H), 3.91 (s, 3H), 4.36-4.45 (m, 2H), 6.45 (d, J = 2.7 Hz, 1H), 7.11 (d, J = 2.2 Hz, 1H), 7.65 (s, 1H), 9.99 (s, 1H), 16.18 (s, 1H).

HPLC: [Flow rate: 0.8 mL/min; Typical injection volume: 5 µL; Isocratic: 92% n-Hexane, 8% Isopropanol; DAD: 280 nm (bandwidth = 4 nm); Column: Chiralcel OD-H, 5 µm, 4.6 mm (ϕ) x 250 mm (L) mm, Temperature: 20 °C.]: Retention time (R_t), (*R*-**22c**) = 48.15 min.; >99% ee (determined by comparison to rac-**22c**, Rt[(*S*)- **22c**] = 39.21 min, R_t [(*R*)- **22c**] = 48.78 min.

(*R*)-4,7,9,10-tetrahydroxy-5-oxo-5,6,7,8-tetrahydroanthracene-2-carboxylic acid (22d)



$C_{15}H_{12}O_7$: 304.25 g/mol

Conversion: 100%

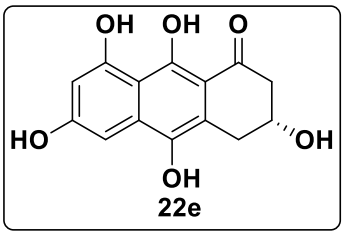
TLC (MeOH: $CHCl_3$, 1:4 v/v): R_f = 0.19.

Yield: 75%

^{1}H-NMR: **(400 MHz, dmso-d_6): δ (ppm)** 2.73 (dd, J = 16.9, 6.6 Hz, 1H), 2.98 – 3.04 (m, 2H), 3.14 (dd, J = 16.6, 3.3 Hz, 1H), 4.31 – 4.29 (m, 1H), 5.23 (s, 1H), 7.23 (s, 1H), 8.26 (s, 1H), 9.98 (s, 1H), 15.06 (s, 1H).

HPLC: [Flow rate: 1.2 mL/min; Typical injection volume: 10 µL; Isocratic: 85% n-Hexane, 15% Isopropanol; DAD: 280 nm (bandwidth = 4nm); Column: Chiralcel OZ-H, 5 µm, 4.6 mm(ϕ) x 250 mm (L), Temperature: 25 °C.]: Retention time (R_t), (*R*-**22d**) = 21.56 min.; >99% ee (determined by comparison to rac-**22d**, R_t (*S*-**22d**) = 17.05 min, R_t (*R*-**22d**) = 22.03 min).

(*R*)-3,6,8,9,10-pentahydroxy-3,4-dihydroanthracen-1(2H)-one (22e)



$C_{14}H_{12}O_6$: 276.24 g/mol

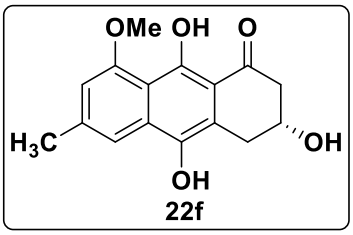
TLC (EA: Hexane, 1:1 v/v): R_f = 0.12.

Conversion: 99%

^{1}H NMR (400 MHz, acetone-d_6): δ (ppm) 2.66 (dd, *J* = 17.0, 7.0 Hz, 1H), 2.87 (dd, *J* = 13.8, 4.8 Hz, 1H), 2.90 (dd, *J* = 13.3, 3.9 Hz, 1H), 3.06 (dd, *J* = 16.3, 3.5 Hz, 1H), 4.21–4.23(m, 1H), 5.17 (d, *J* = 3.6 Hz, 1H), 6.34 (d, *J* = 2.2 Hz, 1H), 6.91 (d, *J* = 2.2 Hz, 1H), 8.39 (s, 1H), 9.96 (s, 1H), 10.28 (s, 1H), 16.01 (s, 1H).

HPLC: [Flow rate: 0.5 mL/min; Typical injection volume: 5 µL; Isocratic: 92.5% n-Hexane, 7.5% Isopropanol; DAD: 280 nm (bandwidth = 4nm); Column: Chiralcel OJ-H, 5 µm, 4.6 mm (ϕ) x 250 mm (L) mm, Temperature: 20 ºC.]: Retention time (R_t),(*R*-**22e**) = 259.41 min.; >99% ee (determined by comparison to rac-**22e**, R_t *(S*-**22e**) = 233.61 min, R_t (*R*-**22e**) = 260.76 min).

(*R*)-3,9,10-trihydroxy-8-methoxy-6-methyl-3,4-dihydroanthracen-1(2H)-one (22f)



$C_{16}H_{16}O_5$: 288.29 g/mol

TLC (EA: Hexane, 1:1 v/v): R_f = 0.29;

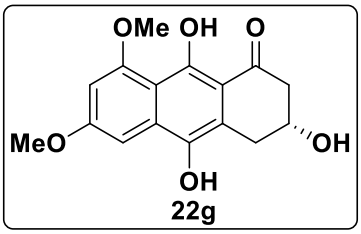
Conversion: 99%

Yield: 79%

^{1}H NMR (400 MHz, acetone-d_6): δ (ppm) 2.47 (s, 3H), 2.75 (ddd, *J* = 16.8, 7.6, 0.9 Hz, 1H), 2.96 (ddd, *J* = 16.8, 3.6, 1.1 Hz, 1H), 3.03 (dd, *J* = 16.2, 7.1 Hz, 1H), 3.30 (dd, *J* = 16.2, 3.0 Hz, 1H), 3.92 (s, 3H), 4.32 (d, *J* = 4.0 Hz, 1H), 4.39 – 4.37 (m, 1H), 6.81 (s, 1H), 7.43 (s, 1H), 7.57 (s, 1H), 14.65 (s, 1H).

HPLC: [Flow rate: 1 mL/min; Typical injection volume: 5 µL; Isocratic: 90% n-Hexane, 10% Isopropanol; DAD: 280 nm (bandwidth = 4nm); Column: Chiralpak AS-H, 5 µm, 4.6 mm (ϕ) x 250 mm (L) mm, Temperature: 20 ºC.]: Retention time (R_t), (*R*-**22f**) = 38.85 min.; >99% ee (determined by comparison to rac-**22f**, R_t (*S*-**22f**) = 41.82 min, R_t (*R*-**22f**) = 38.75 min).

(*R*)-3,9,10-trihydroxy-6,8-dimethoxy-3,4-dihydroanthracen-1(2H)-one (22g)



22g

$C_{16}H_{16}O_6$: 304.29 g/mol

TLC: (hexane/EtOAc, 1:1 v/v): R_f = 0.20.

Conversion: 80%

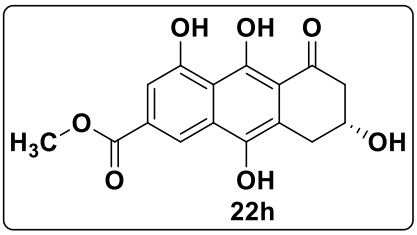
Yield: 72%

^{1}H NMR (400 MHz, dmso-d_6): *δ* (ppm) 2.72 (dd, *J* = 16.6 Hz, 7.6 Hz, 1H), 2.93 (dd, *J* = 17.2 Hz, 4 Hz, 1H), 2.95 (dd, *J* = 16.2 Hz, 7.4 Hz, 1H), 2.99 (dd, *J* = 16.2 Hz, 3.6 Hz, 1H), 3.92 (s, 6H), 4.33–4.34 (m, 1H), 6.52 (d, *J* = 2.3 Hz, 1H), 7.17 (d, *J* = 2.2 Hz, 1H), 7.44 (s, 1H), 14.70 (s, 1H).

HPLC: [Flow rate: 1 mL/min; Typical injection volume: 5 µL; Isocratic: 90% n-Hexane, 10% Isopropanol; DAD: 280 nm (bandwidth = 4nm); Column: Chiralpak AS-H, 5 µm, 4.6 mm (ϕ) x 250 mm (L) mm, Temperature: 20 ºC.]: Retention time (R_t), (*R*-**22g**) = 140.44 min.; >99% ee (determined by comparison to rac-**22g**, R_t (*S*-**22g**) = 142.77 min, R_t (*R*-**22g**) = 151.44 min).

---

methyl (*R*)-4,7,9,10-tetrahydroxy-5-oxo-5,6,7,8-tetrahydroanthracene-2-carboxylate (22h)



$C_{15}H_{14}O_7$: 318.28 g/mol

TLC (MeOH: $CHCl_3$, 1:9 v/v): R_f = 0.37.

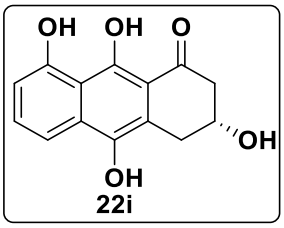
Conversion: 75%

Yield: 67%

^{1}H NMR (400 MHz, acetone-d_6): δ (ppm) 2.85 (dd, *J* = 16.9, 6.3 Hz, 1H), 3.07 (dd, *J* = 17.0, 2.9 Hz, 3.18 (dd, *J* = 16.3, 5.8 Hz, 1H),), 3.29 (dd, *J* = 16.5, 3.1 Hz, 1H), 3.94 (s, 3H), 4.52 (s, 1H), 7.29 (d, *J* = 1.5 Hz, 1H), 8.20 (s, 1H), 8.35 (d, *J* = 1.5 Hz, 1H), 9.87 (s, 1H), 15.56 (s, 1H).

HPLC: [Flow rate: 1 mL/min; Typical injection volume: 5 µL; Isocratic: 95% n-Hexane, 5% Isopropanol; DAD: 280 nm (bandwidth = 4nm); Column: Chiralcel OD-H, 5 µm, 4.6 mm (ϕ) x 250 mm (L) mm, Temperature: 20 ° C.]: Retention time (R_t),(*R*-**22h**) = 191.84 min.; >99% ee (determined by comparison to rac-**22h**, R_t (*S*-**22h**) = 174.40 min, R_t (*R*-**22h**) = 196.68 min)

(*R*)-3,8,9,10-tetrahydroxy-3,4-dihydroanthracen-1(2H)-one (22i)



$C_{14}H_{12}O_5$: 260.24 g/mol

TLC (EA: Hexane, 1:1 v/v): R_f = 0.21.

Conversion: 100%

Yield: 75%

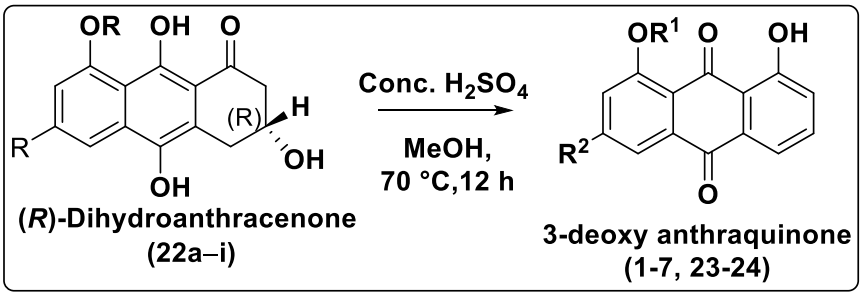
^{1}H NMR (400 MHz, acetone-d_6): δ (ppm) 2.81(dd, *J* = 16.8, 7.1 Hz, 1H), 3.03 (dd, *J* = 17.1, 3.3 Hz, 1H), 3.11 (dd, *J* = 16.4, 6.7 Hz, 1H), 3.28 (dd, *J* = 16.4, 3.5 Hz, 1H), 4.40 (d, *J* = 3.7 Hz, 1H), 4.45–4.51 (m,1H), 6.83 (d, *J* = 7.7 Hz, 1H), 7.55 (t, *J* = 7.8 Hz, 1H), 7.67 (d, *J* = 7.9 Hz, 1H), 7.77 (s, 1H), 9.86 (s, 1H), 15.89 (s, 1H).

HPLC: [Flow rate: 1 mL/min; Typical injection volume: 5 µL; Isocratic: 95% n-Hexane, 5% Isopropanol; DAD: 280 nm (bandwidth = 4nm); Column: Chiralcel OD-H, 5 µm, 4.6 mm (ϕ) x 250 mm (L) mm, Temperature: 20 °C.]: Retention time (R_t), (*R*-**22i**) = 65.74 min.; >99% ee (determined by comparison to rac-**22i**, R_t (*S*-**22i**) = 56.93 min, R_t (*R*-**22i**) = 65.39 min).

VI. General procedure for synthesis of various racemic dihydroanthracenones (*rac*-22a-i)[15]:

To a 50 ml round bottom flask in 10 mL degassed water the anthraquinone (**19a-h**) (74 µmol) in methanol (10% v/v) was added in argon atmosphere. $Na_2S_2O_4$ (20 equiv., 1.48 mmol) and $NaBH_4$ (20 equiv., 1.48 mmol) were added portion-wise at ice-cold temperature. After 20 min, 1 N HCl was added and the reaction mixture was extracted in ethyl acetate and concentrated on rotary evaporator. The crude reaction mixture was subjected for the column chromatography to obtain racemic dihydroanthracenones **22a-I** and were utilized to determine the enantiomeric excess using chiral HPLC.

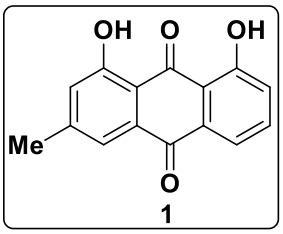
VII. Dehydration of dihydroanthracenones to deoxyanthraquinones



Scheme S5: 3-deoxyanthraquinones synthesis

General Procedure: Crude **(***R*)-dihydroanthracenone (**22a-i**) (0.15 mmol) was dissolved in methanol (10 mL) (EtOAc in case of rhein **4**) and five drops of conc. H_2SO_4 added into the reaction mixture and it refluxed at 70 ^oC for 12h. The reaction was monitor by checking TLC, after full consumption of the starting material, the reaction mixture was cooled to room temperature, evaporate the solvent at reduced pressure and subjected to purify using column chromatography as 20-30% ethyl acetate in hexane as eluent to isolate yellow solid as only one component in high yield. Product was characterized by NMR spectroscopy and Mass spectrometry.

1,8-dihydroxy-3-methylanthracene-9,10-dione (chrysophanol) 1



$C_{15}H_{12}O_4$: 254.24 g/mol

TLC (EA: Hex, 3: 7 v/v): R_f = 0.57.

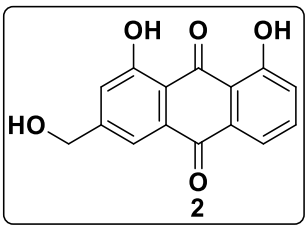
Yield: 75%

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.46 (s, 3H), 7.08 (s,1H), 7.27 (d, *J* = 8.5 Hz, 1H), 7.65 (m, 2H), 7.80 (d, *J* = 7.5 Hz, 1H), 11.99 (s, 1H), 12.10 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 22.4, 113.9, 116.0, 120.1, 121.5, 124.5, 124.7, 133.4, 133.8, 137.1, 162.6, 162.9, 182.1, 192.7.

Exact Mass $[M+H]^+$: 255.0657 (calculated), 255.0654 (found).

1,8-dihydroxy-3-(hydroxymethyl)anthracene-9,10-dione (aloe emodin) 2



$C_{15}H_{10}O_5$: 270.24 g/mol

TLC (EA: Hex, 3:7 v/v): R_f = 0.7

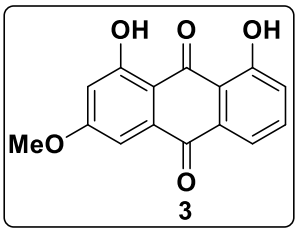
Yield: 76%

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 4.63 (d, *J* = 5.6 Hz, 2H), 5.6 (t, *J* = 5.6 Hz, 1H), 7.39 (s, 1H), 7.69 (d, *J* = 8.2 Hz, 1H), 7.71 (s, 1H), 7.72 (d, *J* = 7.6 Hz, 1H), 7.79 (t, *J* = 7.9 Hz, 1H)), 11.92 (s, 2H).

^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 62.5, 114.9, 116.4, 117.6, 119.8, 121.2, 124.9, 133.6, 133.8, 137.8, 154.1, 161.8, 1621.1, 182.0, 192.1.

Exact Mass $[M+H]^+$: 271.0601 (calculated), 271.0603 (found).

1,8–dihydroxy–3–methoxyanthracene–9,10–dione (3-methoxy chrysazin) 3



$C_{15}H_{10}O_5$: 270.24 g/mol

TLC: (EA/Hex, 1:9 v/v): *Rf* = 0.30

Yield: 65%

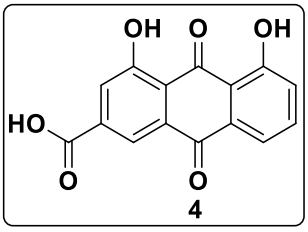
^{1}H NMR: (400 MHz, $CDCl_3$) *δ* [ppm]: 3.94 (s, 3H), 6.69 (d, *J* = 2.3 Hz, 1H), 7.28 (d, *J* = 9.5 Hz, 1H), 7.37 (d, *J* = 2.4 Hz, 1H), 7.64 (t, *J* = 7.9 Hz, 1H), 7.81 (d, *J* = 7.5 Hz, 1H), 12.12 (s, 1H), 12.23 (s, 1H).

^{13}C NMR: (100 MHz, $CDCl_3$) *δ* [ppm]: 56.1, 106.7, 108.4, 110.3, 115.8, 120.0, 124.7, 133.5,

135.2, 136.5, 162.4, 165.4, 166.8, 181.7, 191.2.

Exact Mass [M+H]$^{+}$: 271.0601(calculated), 271.0601 (found).

4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (Rhein) 4



$C_{15}H_8O_6$: 284.22 g/mol

TLC (MeOH: $CHCl_3$, 3:17 v/v): R_f = 0.24;

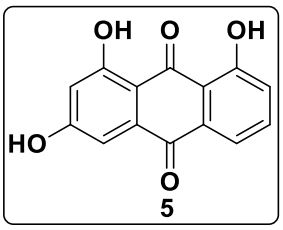
Yield: 80%

^{1}H NMR (400 MHz, dmso-d_6): δ (ppm) 7.39 (d, *J* = 8.3 Hz, 1H), 7.71 (d, *J* = 7.25 Hz, 1H), 7.74 (s, 1H), 7.81 (t, *J* = 8.0 Hz, 1H), 8.10 (s, 1H), 11.91 (s, 1H).

^{13}C NMR (100 MHz, dmso-d_6): δ (ppm) 116.2, 118.6, 118.8, 119.4, 124.1, 124.6, 133.2, 133.8, 137.6, 138.5, 161.1, 161.4, 165.5, 181.0, 191.3.

Exact Mass [M+H]: 285.0399 (calculated), 285.0392 (found).

1,3,8-trihydroxyanthracene-9,10-dione (trihydroxy anthraquinone) 5



$C_{14}H_8O_5$: 256.21 g/mol

TLC (EA: Hex, 3:7 v/v): R_f = 0.58.

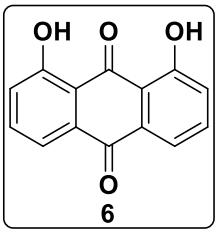
Yield : 79%

^{1}H NMR (400 MHz, acetone-d_6): δ (ppm) 6.66 (d, *J* = 2.4 Hz, 1H), 7.25 (d, *J* = 2.4 Hz, 1H), 7.31 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.79 – 7.72 (m, 2H), 10.30 (brs, 1H), 12.12 (s, 1H), 12.14 (s, 1H).

^{13}C NMR (100 MHz, acetone-d_6): δ (ppm) 108.9, 109.8, 110.5, 116.7, 120.3, 125.2, 134.5, 136.6, 137.6, 163.1, 166.4, 166.6, 182.0, 192.1.

Exact Mass $[M+H]^+$: 257.0444 (calculated), 257.0436 (found).

1,8-dihydroxyanthracene-9,10-dione (dantron) 6



$C_{14}H_8O_4$: 240.21 g/mol

TLC (EA: Hex, 3: 7 v/v): R_f = 0.58.

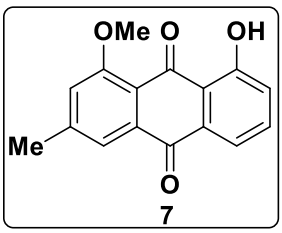
Yield: 78%

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.29 (d, *J* = 8.3 Hz, 1H), 7.68 (t, *J* = 7.9 Hz, 1H), 7.83 (d, *J*=7.9 Hz, 1H), 12.05 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 116.0, 120.2, 124.8, 133.7, 137.4, 162.7, 181.8, 193.2.

Exact Mass [M+H]: 241.0495 (calculated), 241.0493 (found).

8-hydroxy-1-methoxy-3-methylanthracene-9,10-dione (1-O-methyl chrysophanol) 6



$C_{16}H_{12}O_4$: 268.26 g/mol

TLC (EA: Hex, 3: 7 v/v): R_f = 0.58.

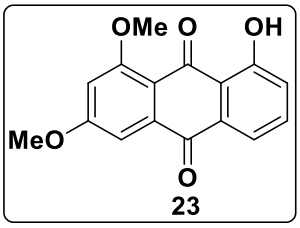
Yield: 76%

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.52 (s, 3H), 4.07 (s, 3H), 7.16 (s,1H), 7.28 (d, *J*= 8.5 Hz, 1H), 7.60 (t, *J*= 7.9 Hz, 1H), 7.80-7.74 (m, 2H), 13.04 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 22.6, 56.7, 117.2, 118.6, 118.7, 118.9, 121.2, 124.9, 132.9, 135.6, 135.8, 161.2, 162.6, 183.2, 188.9.

Exact Mass $[M+H]^+$: 269.0814 (calculated), 269.0808 (found).

8-hydroxy-1,3-dimethoxyanthracene-9,10-dione (1,3-dimethoxy chrysazin) 23



$C_{16}H_{12}O_5$: 284.26 g/mol

TLC (EA: Hex, 1: 1 v/v): R_f = 0.79.

Yield: 69%

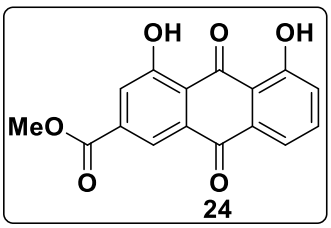
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 3.96 (s, 3H), 4.00 (s, 3H), 6.74 (d, *J* = 2 Hz, 1H), 7.25 (d, *J* = 8.2 Hz, 1H), 7.42 (d, *J* = *2* Hz, 1H), 7.56 (t, *J* = 8.2 Hz, 1H), 7.72 (d, *J* = 7.6 Hz, 1H), 13.11 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 56.2, 56.7, 104.1, 104.8, 115.2, 116.9, 118.9, 125.0, 132.7, 135.4, 137.7, 162.5, 163.1, 165.5, 182.7, 187.8.

Exact Mass $[M+H]^+$: 285.0763 (calculated), 285.0757 (found).

---

methyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate 24



$C_{16}H_{10}O_6$: 298.25 g/mol

TLC (MeOH: $CHCl_3$, 1: 9 v/v): R_f = 0.59.

Yield: 66%

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 3.99 (s, 3H), 7.31 (d, 1H, *J* = 2.4 Hz), 7.71 (d, 1H, *J* = 2.4 Hz), 7.8 (d, 1H, *J* = 1.6 Hz), 7.91 (d, 1H, *J* = 1.6 Hz), 8.39 (s, 1H),11.94 (s, 1H), 12.0 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 53.1, 115.9, 118.3, 120.4, 120.5, 125.0, 125.5, 133.6, 134.0, 137.9, 137.9, 162.9, 165.0, 181.0, 192.9.

Exact Mass $[M+H]^+$: 299.0556 (calculated), 299.0551 (found).

VIII. Cellular Viability studies

Cell culture: The human normal kidney (HEK293) cell line was procured from National Centre for Cell Science (NCCS), Pune, India. The cells were cultured in DMEM media, supplemented with 10% FBS, 100 U mL-1 penicillin, and 100 µg mL-1 streptomycin. The cells were grown at 37^{o} C under an atmosphere of 5% CO_2.

MTT assay: To measure the cellular toxicity of the test compounds, control cells and those treated with various concentrations of test compounds for 36 h time period were determined by the MTT reduction assay. Cells were first seeded in 48 well plates at density of $5x10^4$/well, cultured with DMEM supplemented with 10% FCS. Post 24h, medium was replaced and cells were exposed to the test compounds in serum free DMEM for 36 h. Cell viability were then documented by MTT assay. MTT solution was freshly prepared at concentration of 1 mg/mL in culture medium without phenol red and 200 µL of MTT solution was given into each well post replacing the old culture medium. Cells were then kept at 37 °C for 2 h with 5% CO_2, 95% air condition. Next, 200 µl of DMSO was added into each well 2 h after for the solubilisation of the formazan crystals. The optical density of the wells was next determined using a plate reader (Biotek Instrument) at a wavelength of 550 nm.

Statistical Analysis: Data were analysed by Student's test or two-way ANOVA. Statistical analyses were performed using Origin 6.1 software. Results were considered significantly different at $p<0.05$. Values are expressed as means ± SEM.

IC50 calculation method

Half-maximal inhibitory concentration (IC_{50}) of various compounds were determined by plotting scattered graph on MS Excel software with their different cell viability values at that concentration at 48 hours. On curve linear regression equation was displayed after addition of trendline. Then IC_{50} values of various compounds were calculated using the following formula:

$$Y = a + bX$$

where ***X*** is the explanatory variable (IC_{50} concentration), ***Y*** is the dependent variable (Y will be 50 as we are calculating IC_{50}), ***b*** is the slope of the line, and ***a*** is the intercept.

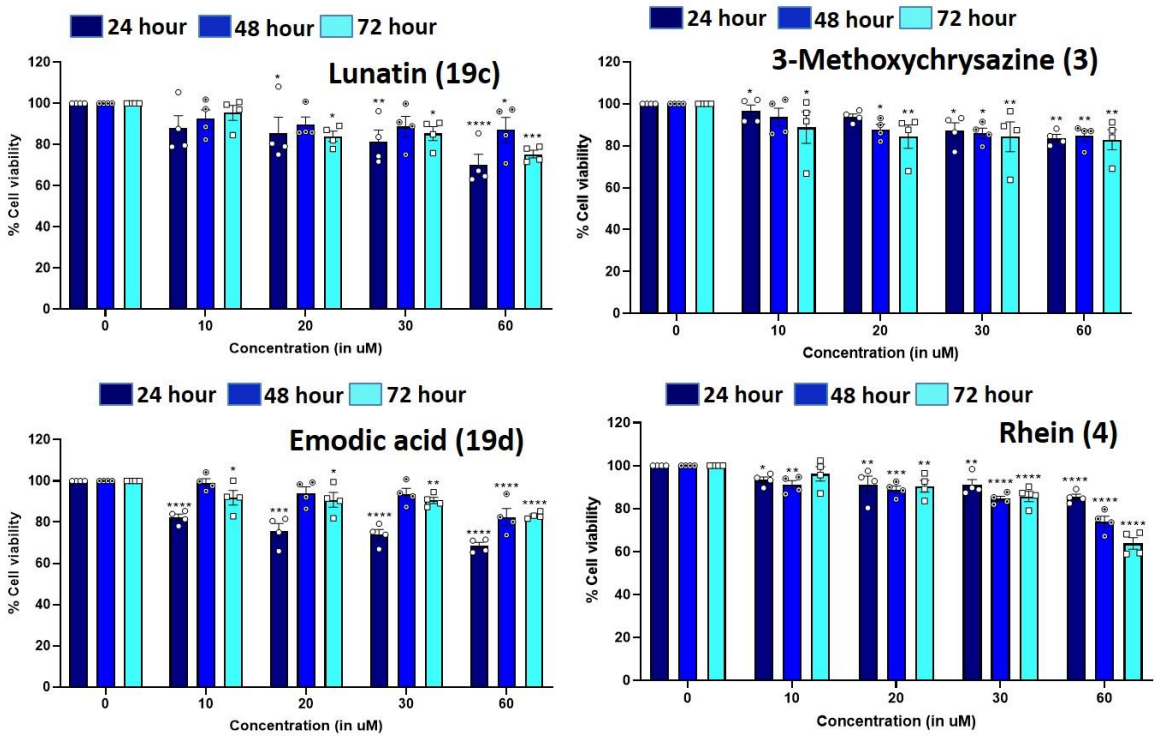


Figure S1. Cytotoxicity of the test compounds. Survival of normal human kidney HEK293 cells at different time points following incubation with test compounds with mentioned concentration as measured by MTT assays. Results are means ± S.E. of multiple experiments (n=3, *p<0.05; **p<0.01, **p<0.001 compared with control at 0 h time point).

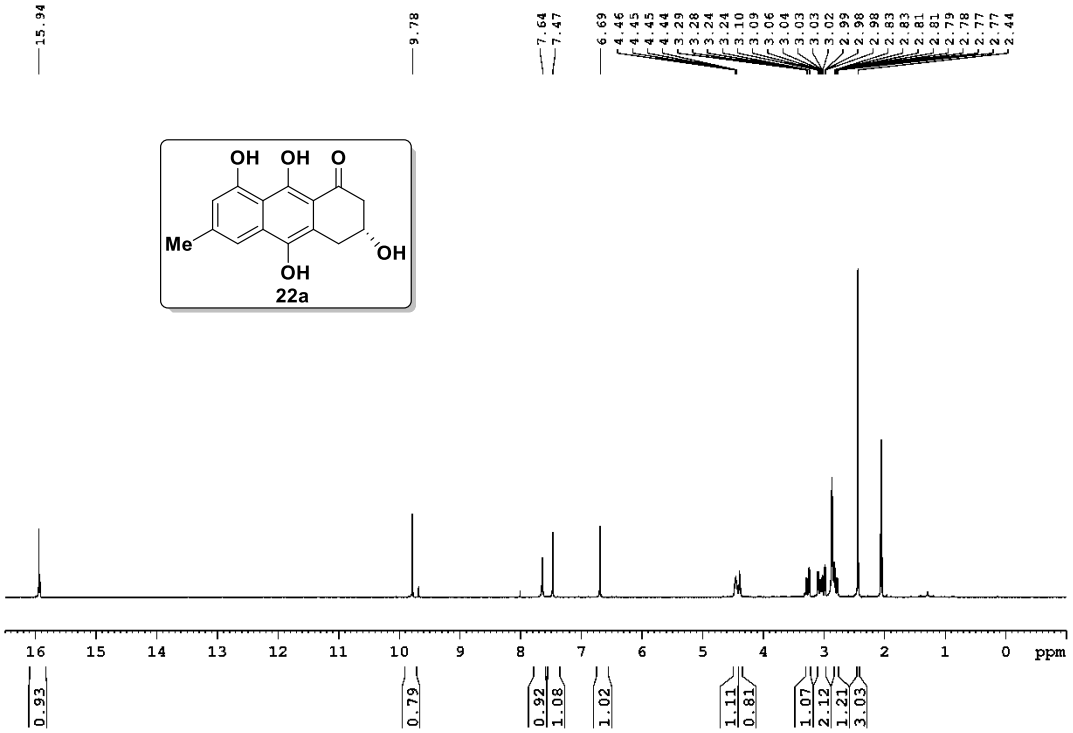
Comparative of IC_{50} values with doxorubicin in HEK293 cell lines.

Compound	IC_{50} (µM)	Compound	IC_{50} (µM)
Emodin (**19a**)	171.14	Chrysophanol (**1**)	59.24
Citreorosein (**19b**)	253.98	Aloe-Emodin (**2**)	70.55
Lunatin (**19c**)	116.82	3-Methoxychrysazin (**3**)	92.86
Emodic acid (**19d**)	198.4	Rhein (**4**)	184.66
Doxorubicin (standard)	52.99		

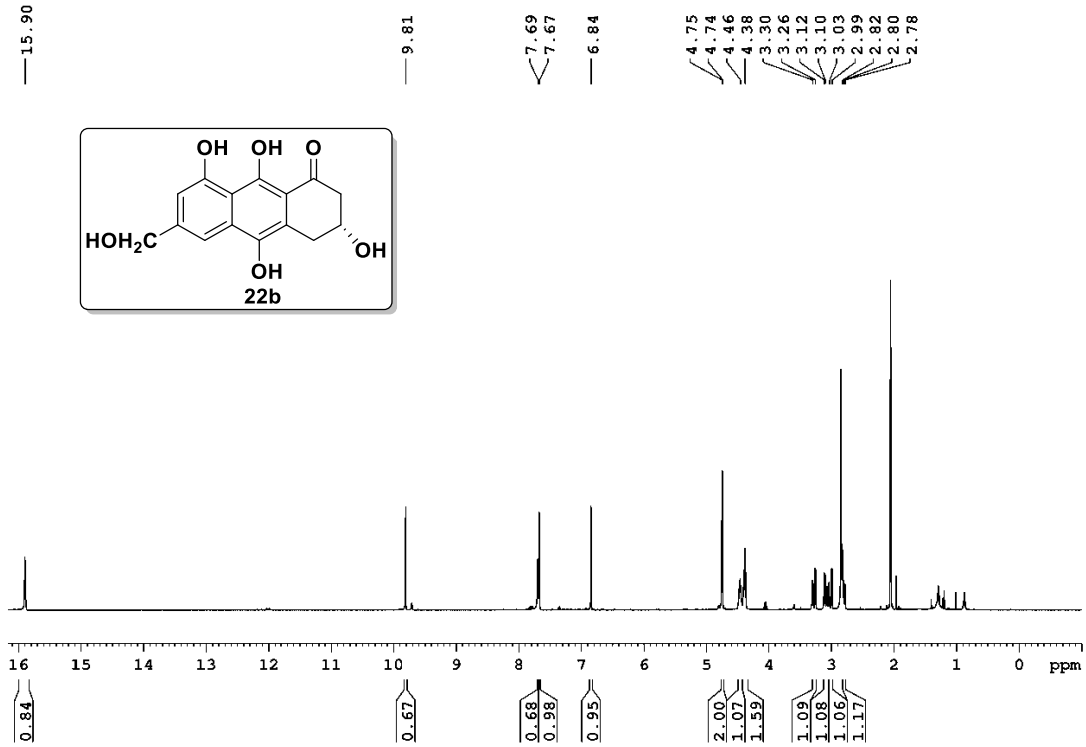
Table S2. IC_{50} values of assayed compounds and Doxorubicin in HEK293 cell line.

NMR Spectra

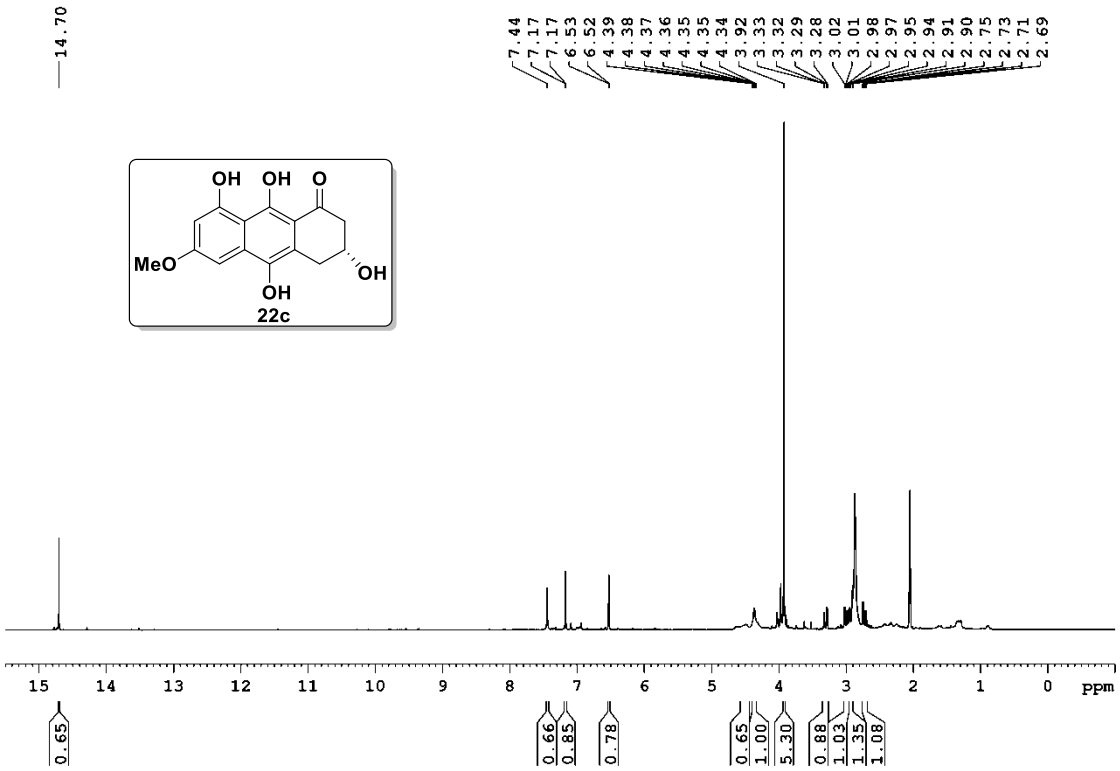
^{1}H NMR (400 MHz, acetone-d_6)



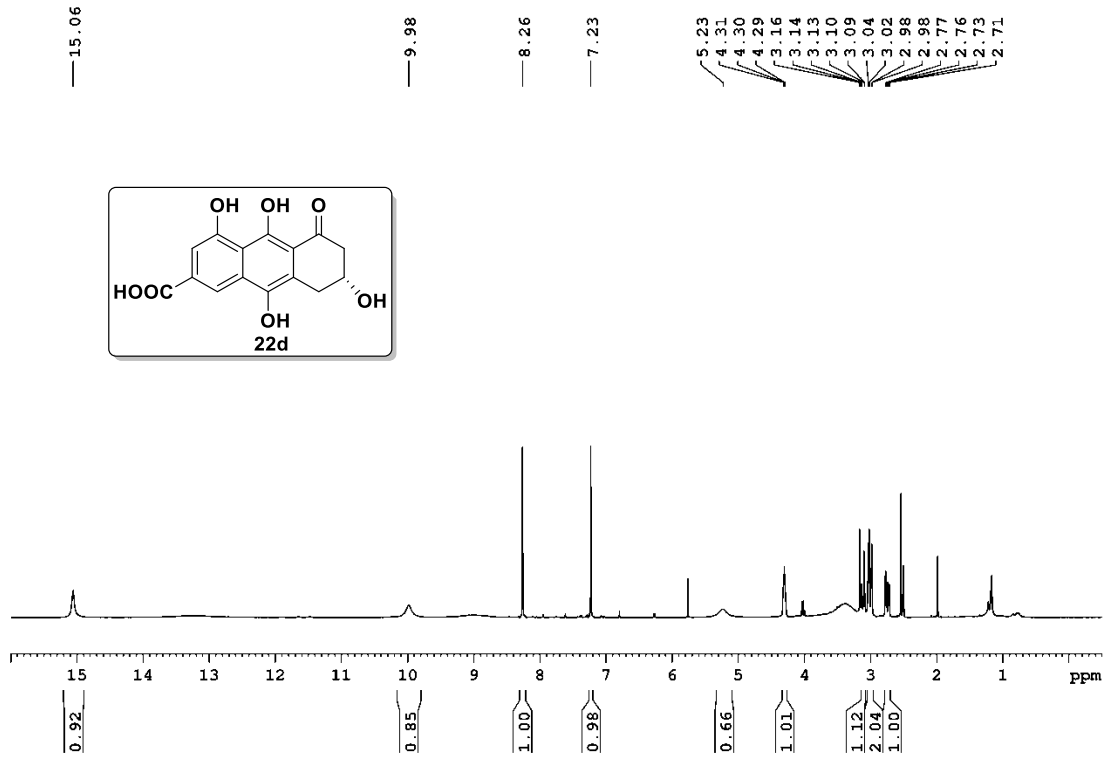
^{1}H NMR (400 MHz, acetone-d_6)



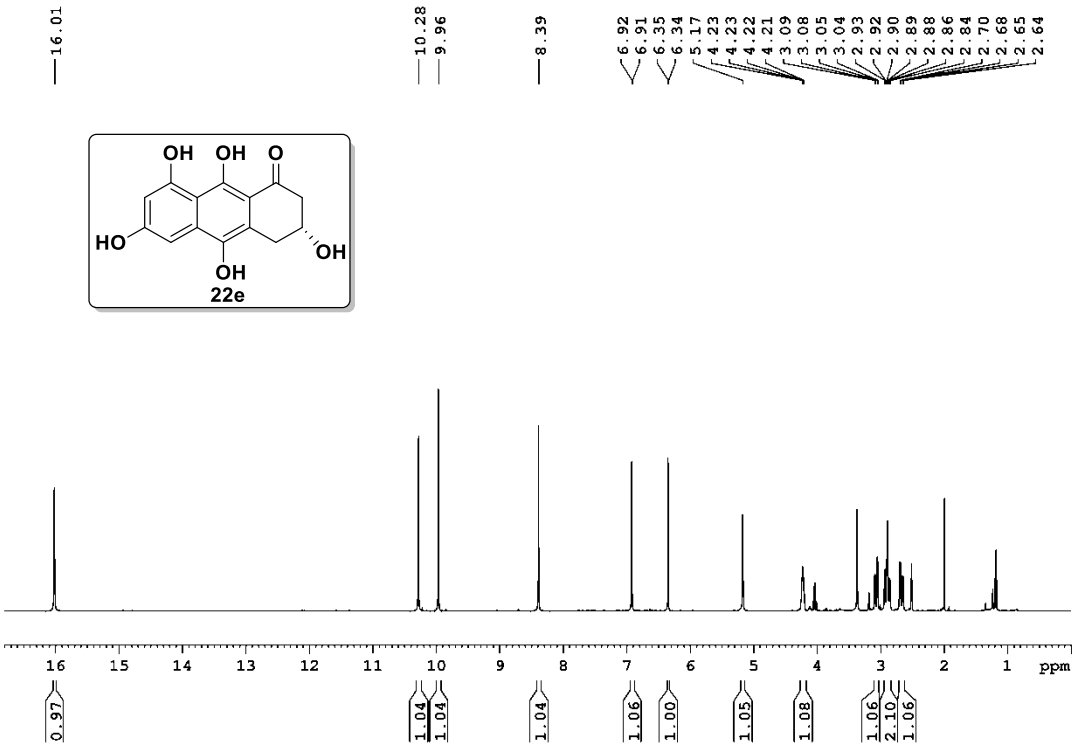
^{1}H NMR (400 MHz, acetone-d_6)



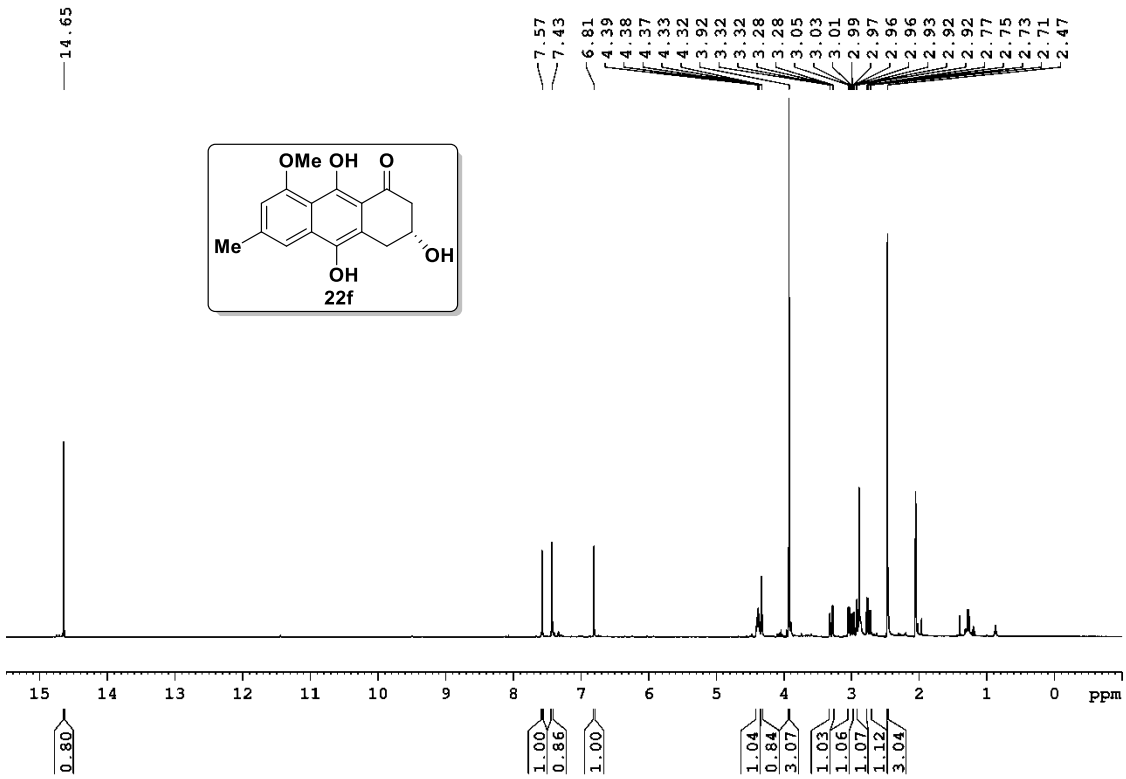
^{1}H NMR (400 MHz, DMSO-d6)



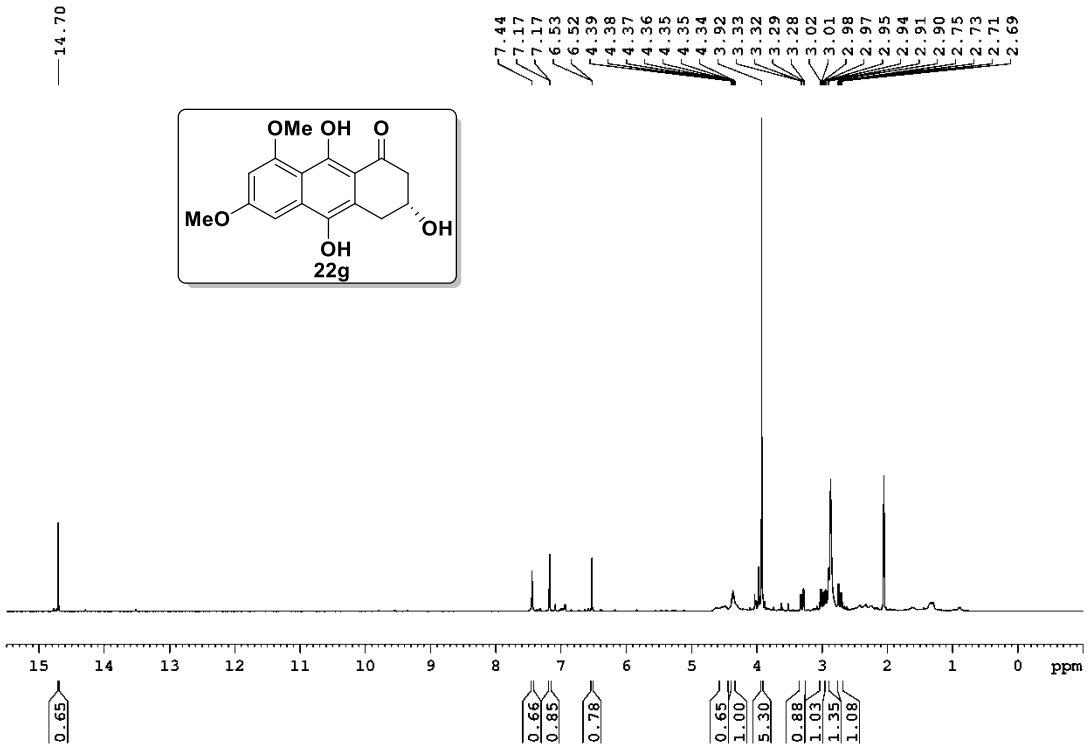
^{1}H NMR (400 MHz, acetone-d_6)



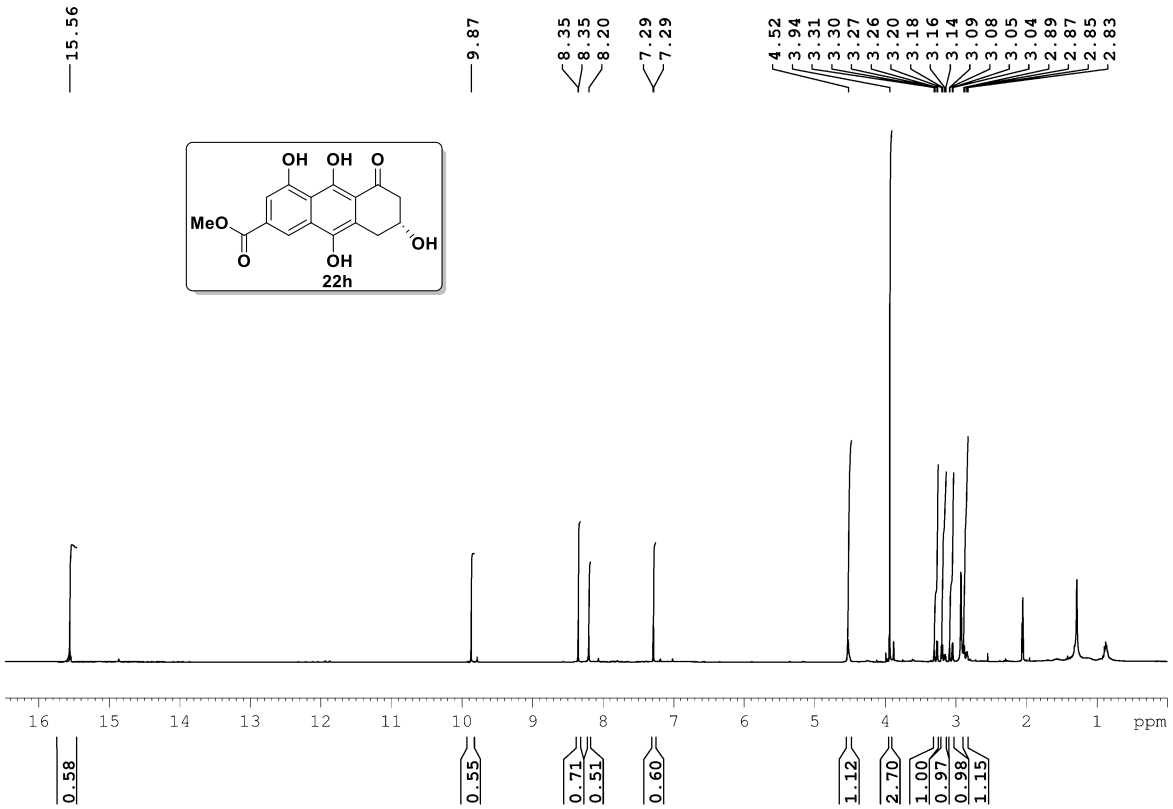
^{1}H NMR (400 MHz, acetone-d_6)



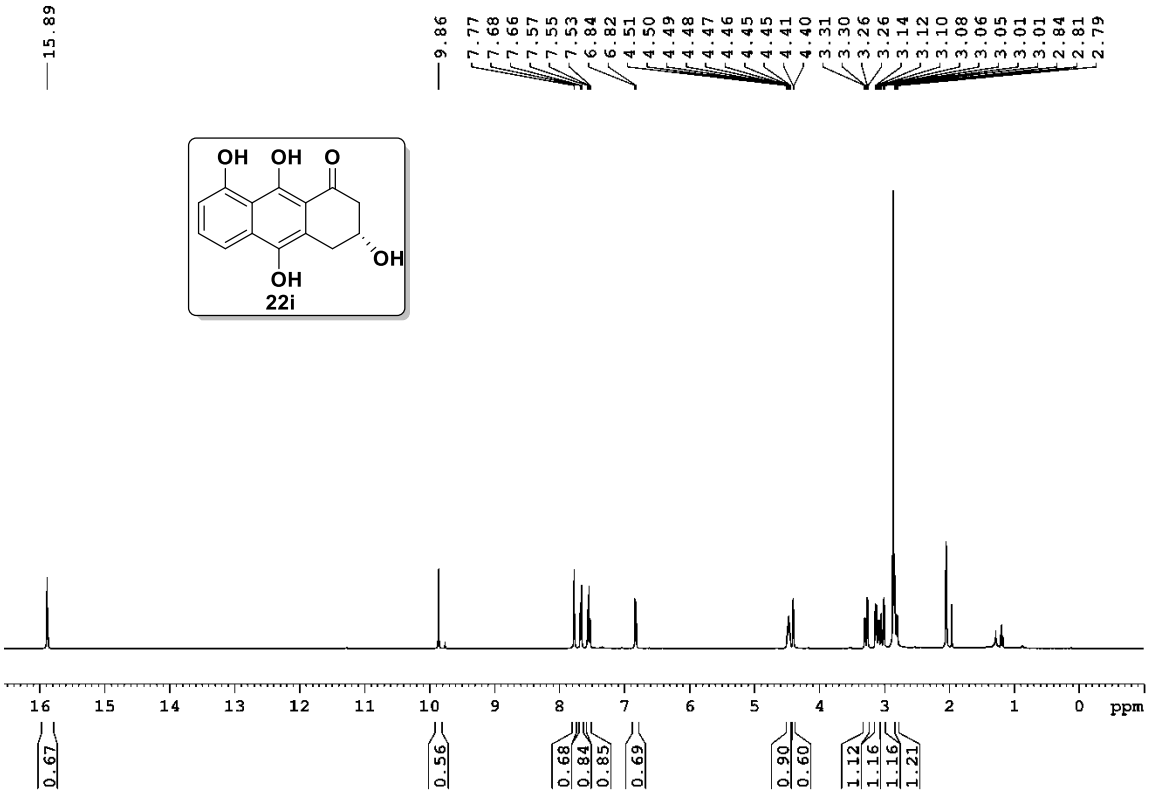
^{1}H NMR (400 MHz, acetone-d_6)



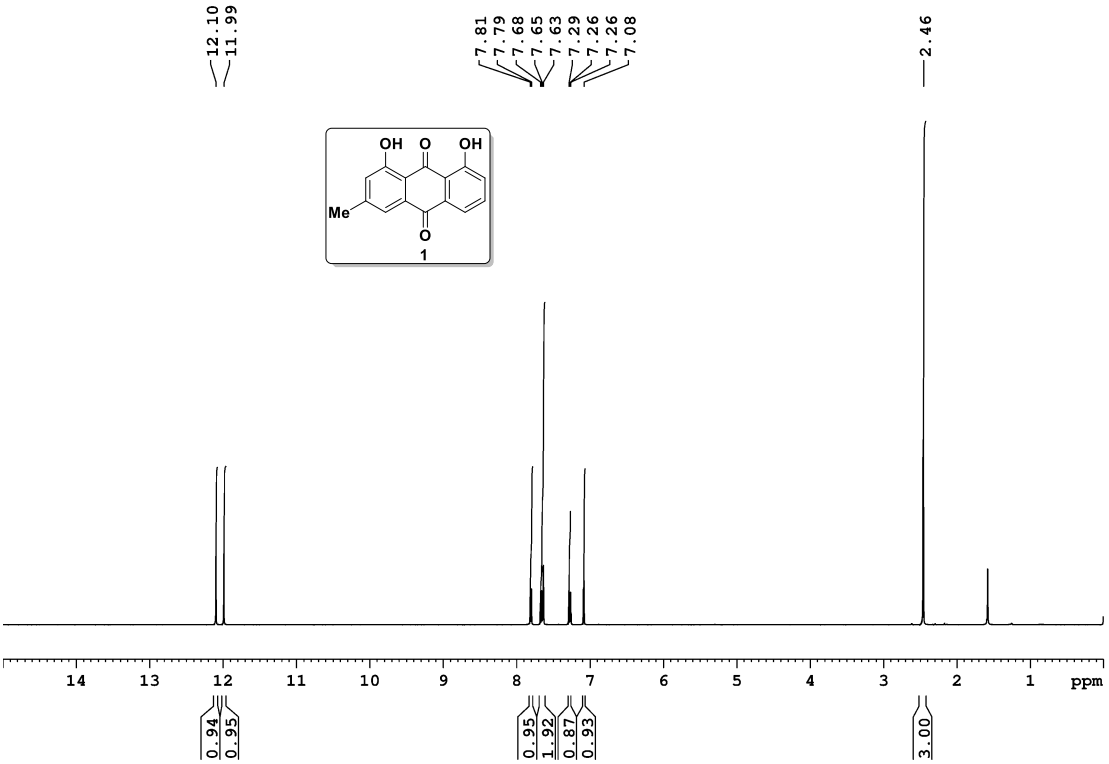
^{1}H NMR (400 MHz, acetone-d_6)



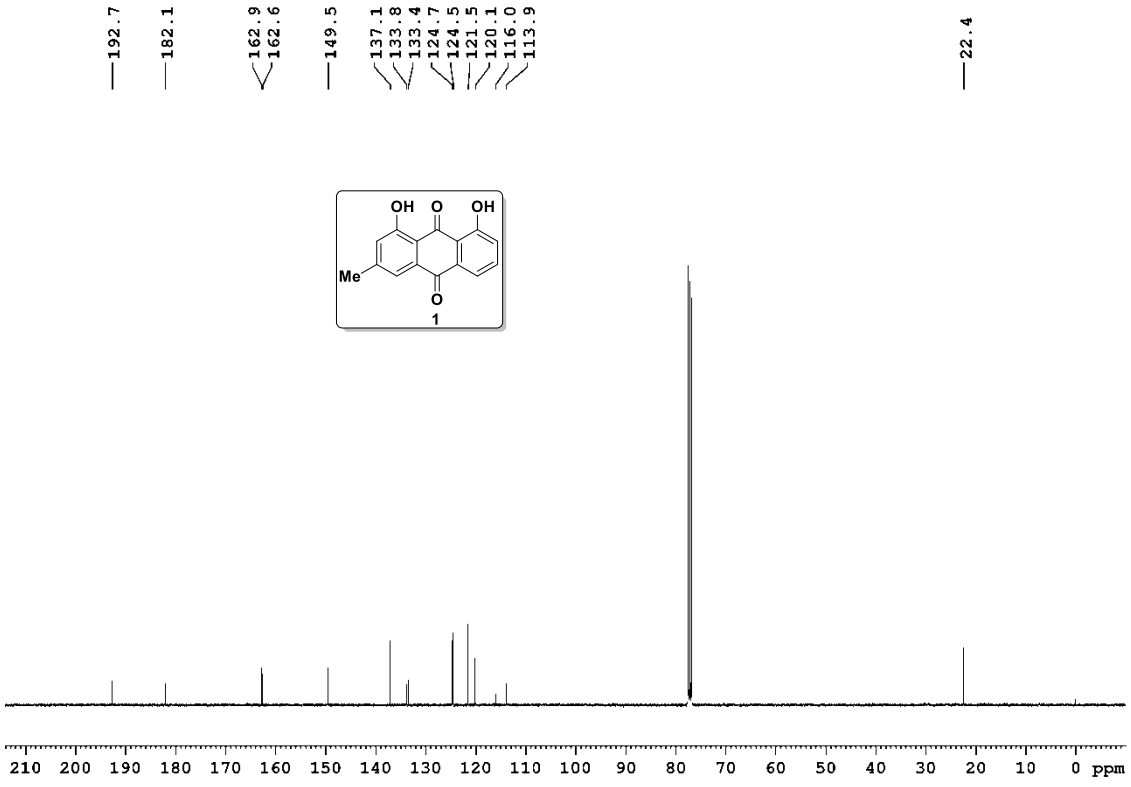
^{1}H NMR (400 MHz, acetone-d_6)



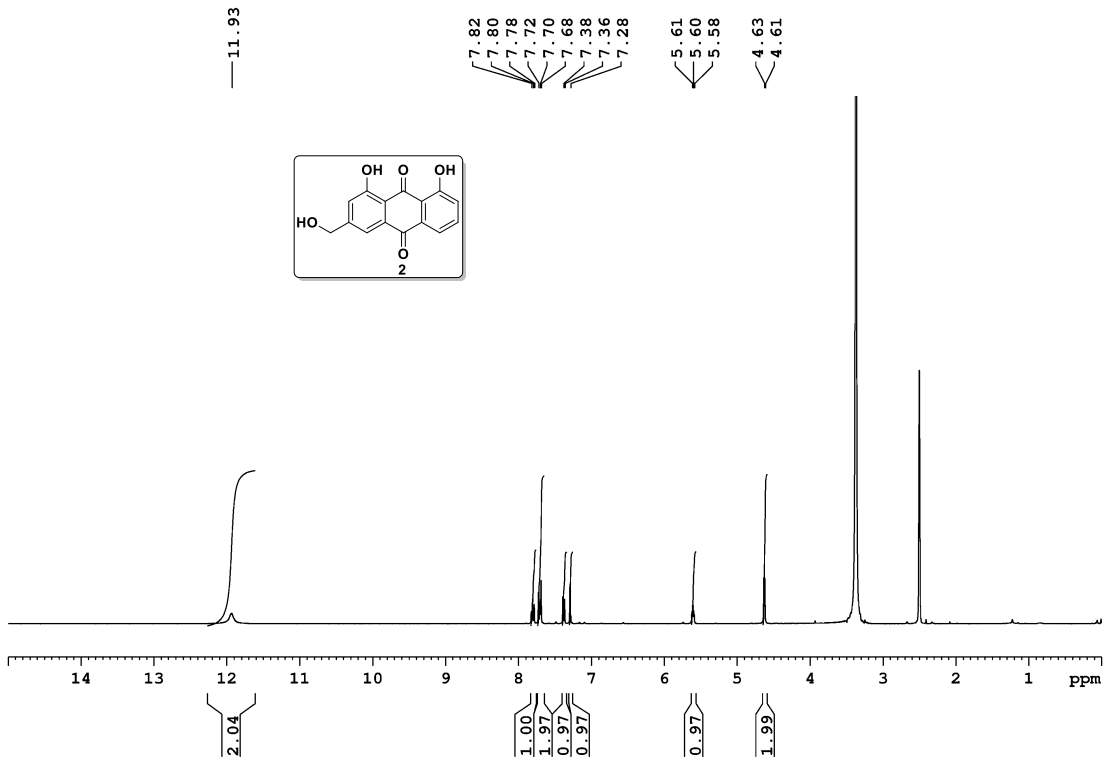
^{1}H NMR (400 MHz, $CDCl_3$)



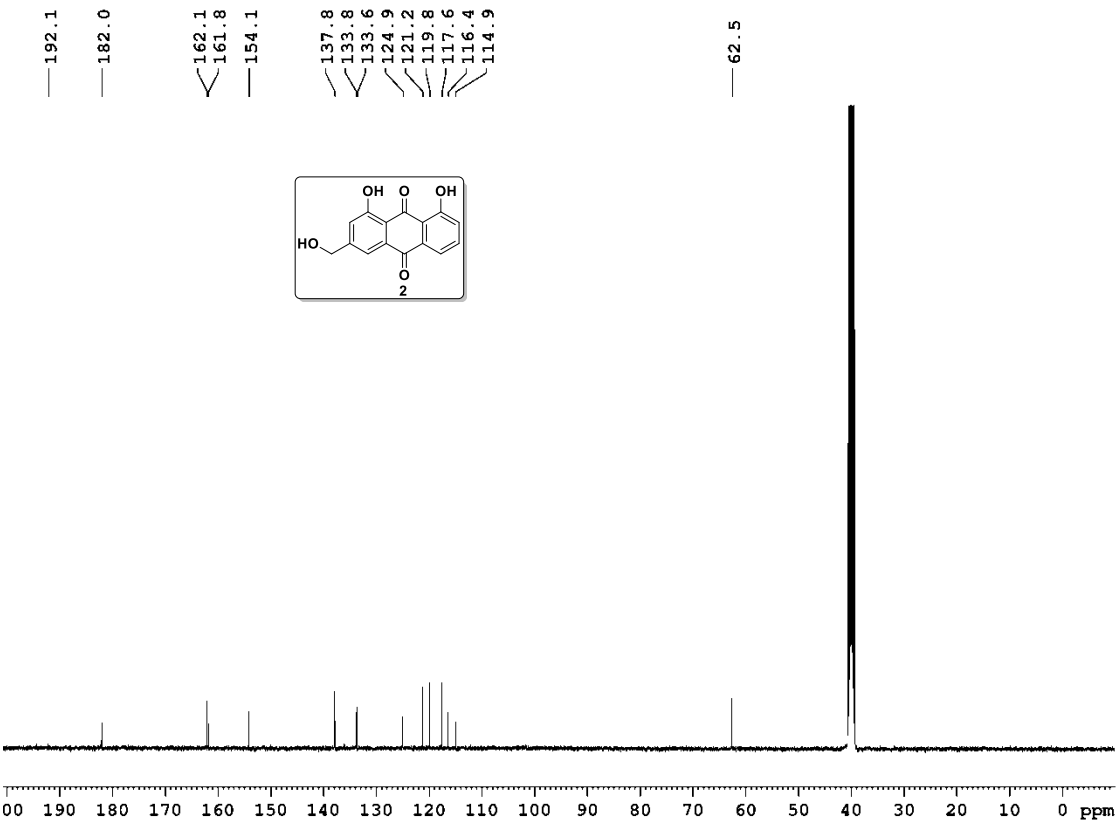
^{13}C NMR (100 MHz, $CDCl_3$)



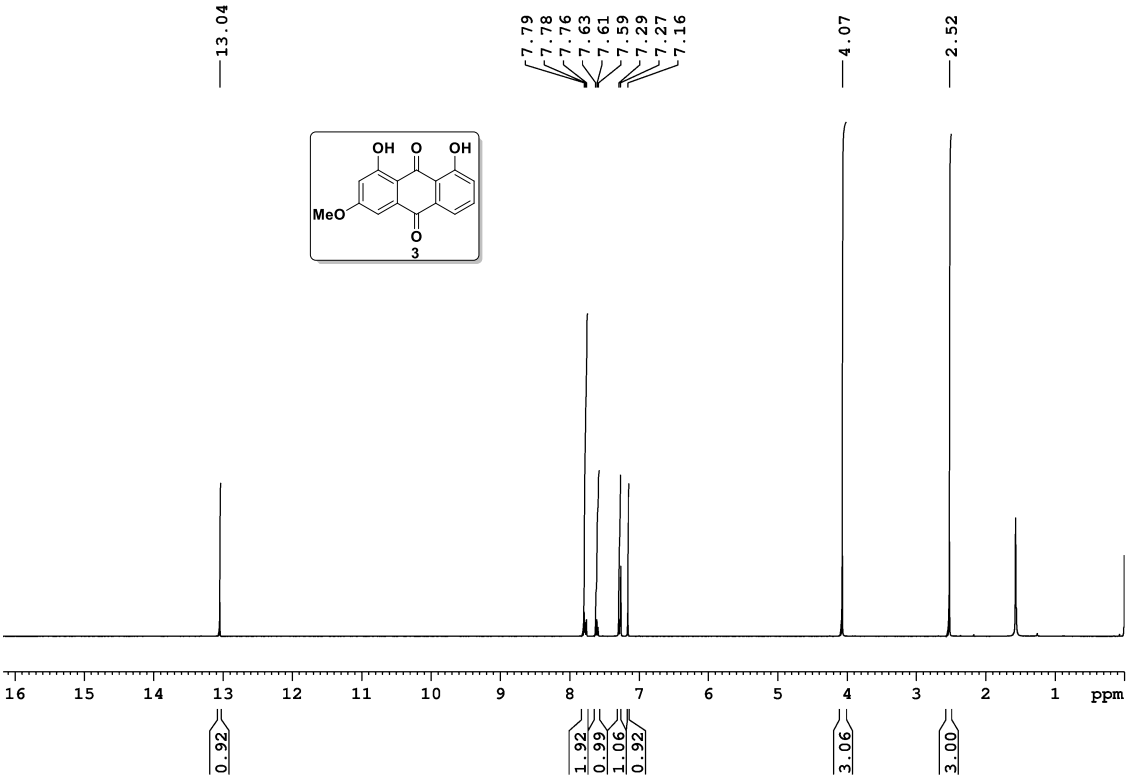
^{1}H NMR (400 MHz, dmso-d_6)



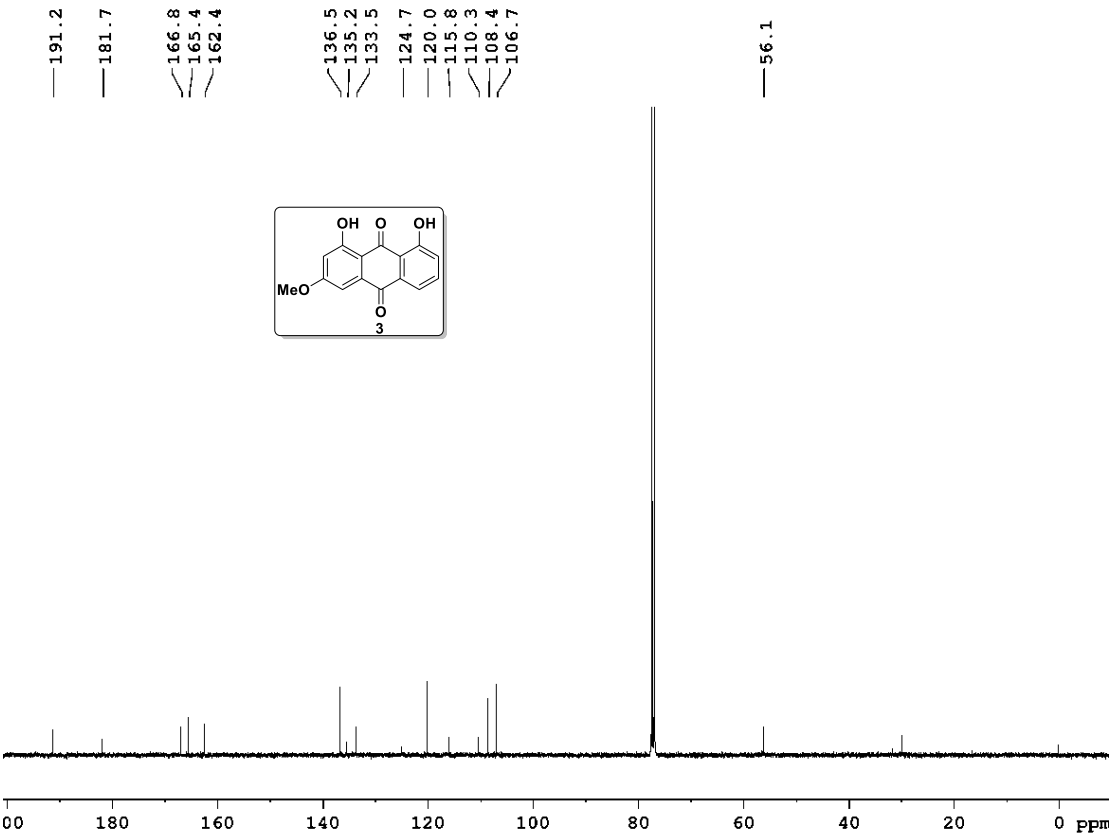
^{13}C NMR (100 MHz, dmso-d_6)



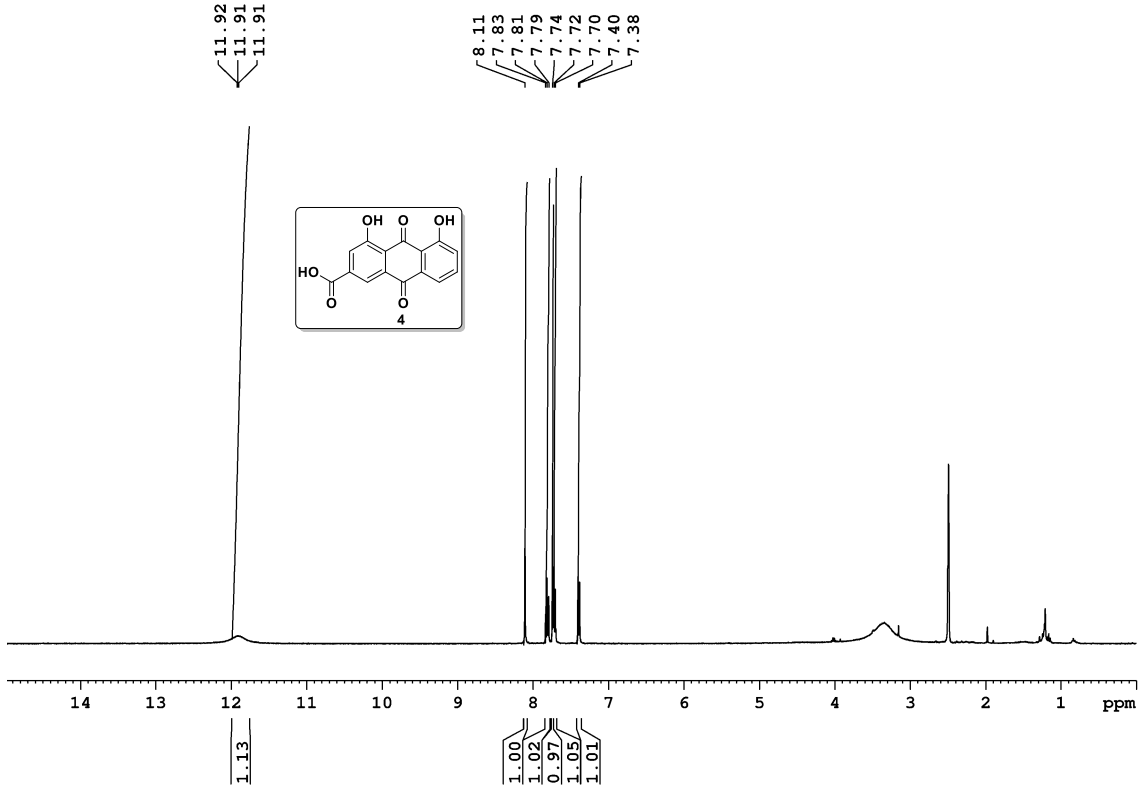
^{1}H NMR (400 MHz, $CDCl_3$)



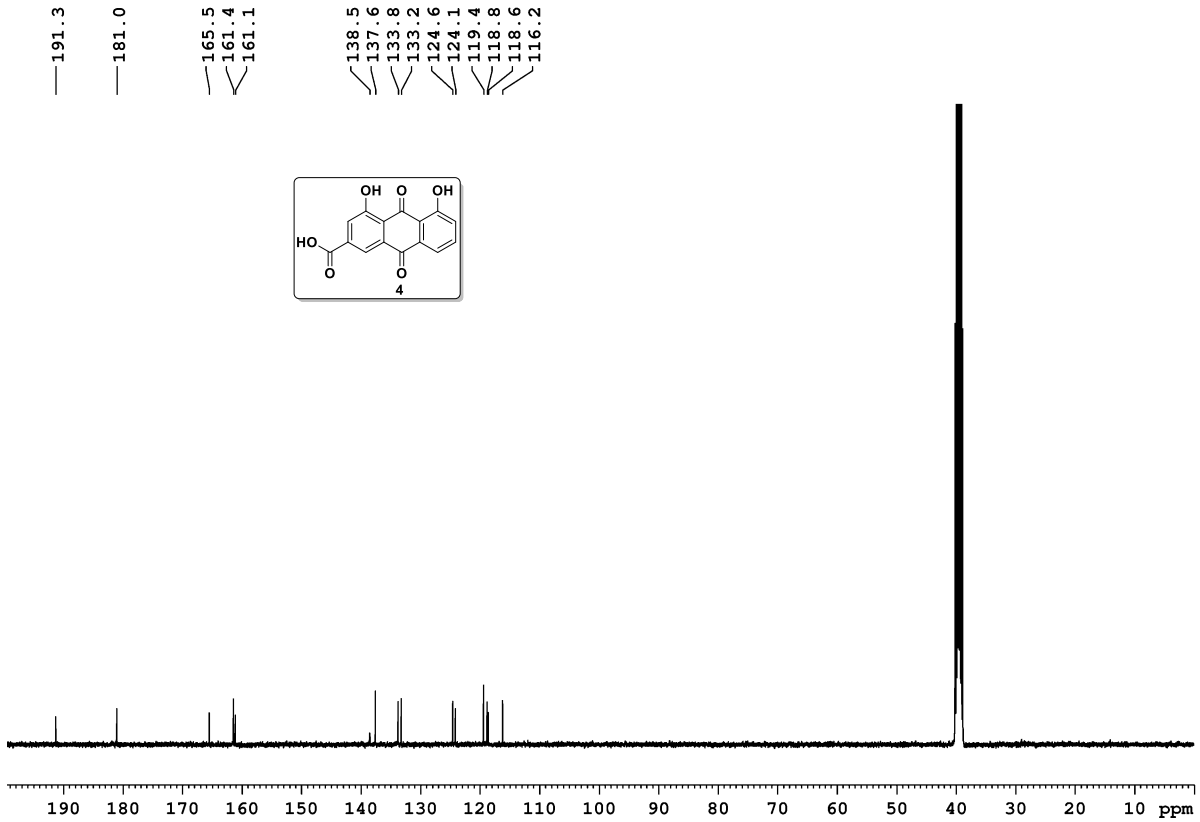
^{13}C NMR (100 MHz, $CDCl_3$)



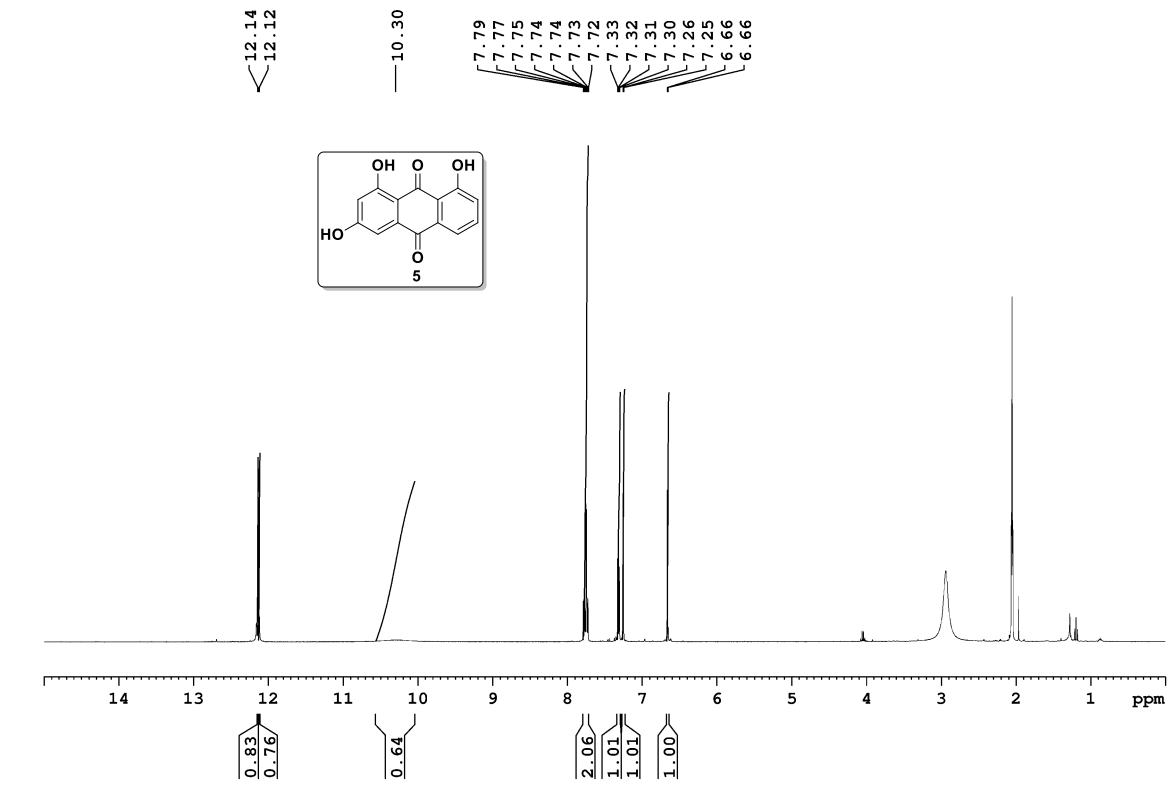
^{1}H NMR (400 MHz, dmso-d_6)



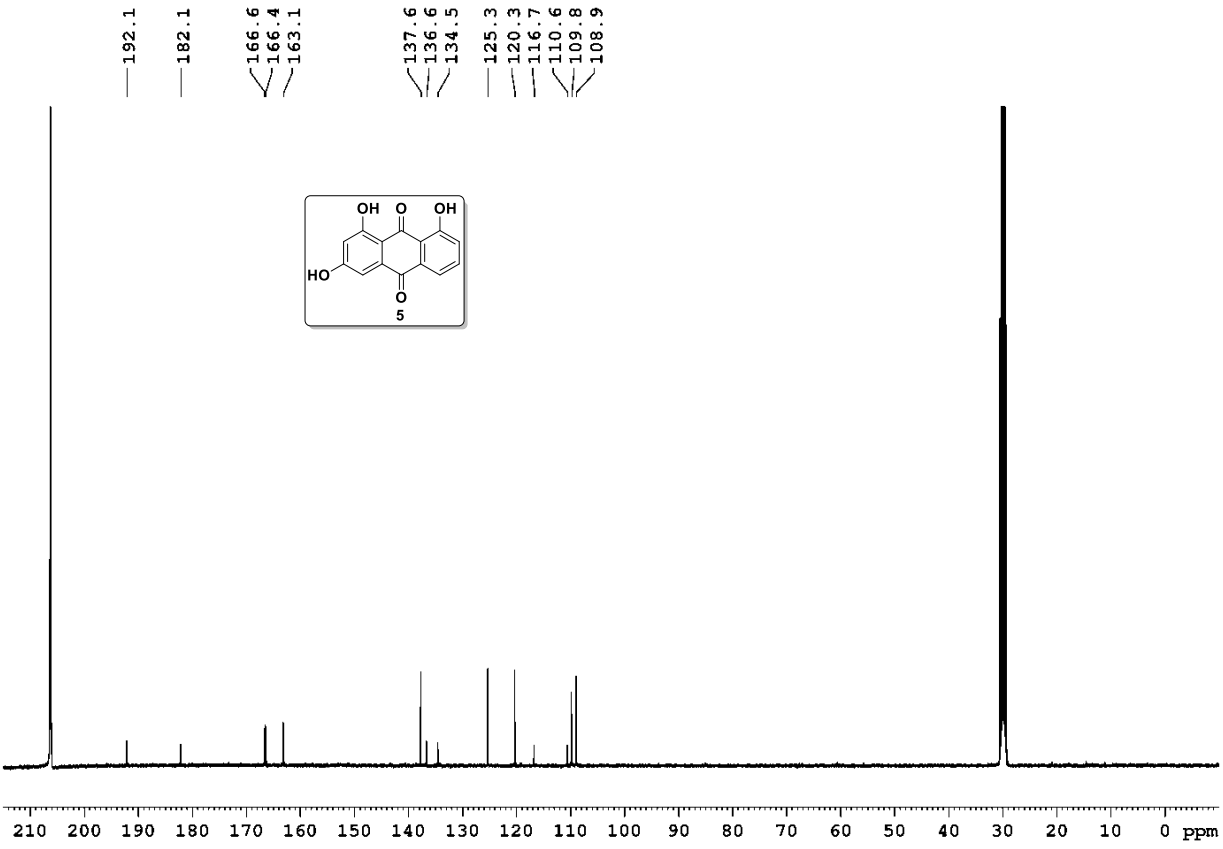
^{13}C NMR (100 MHz, dmso-d_6)



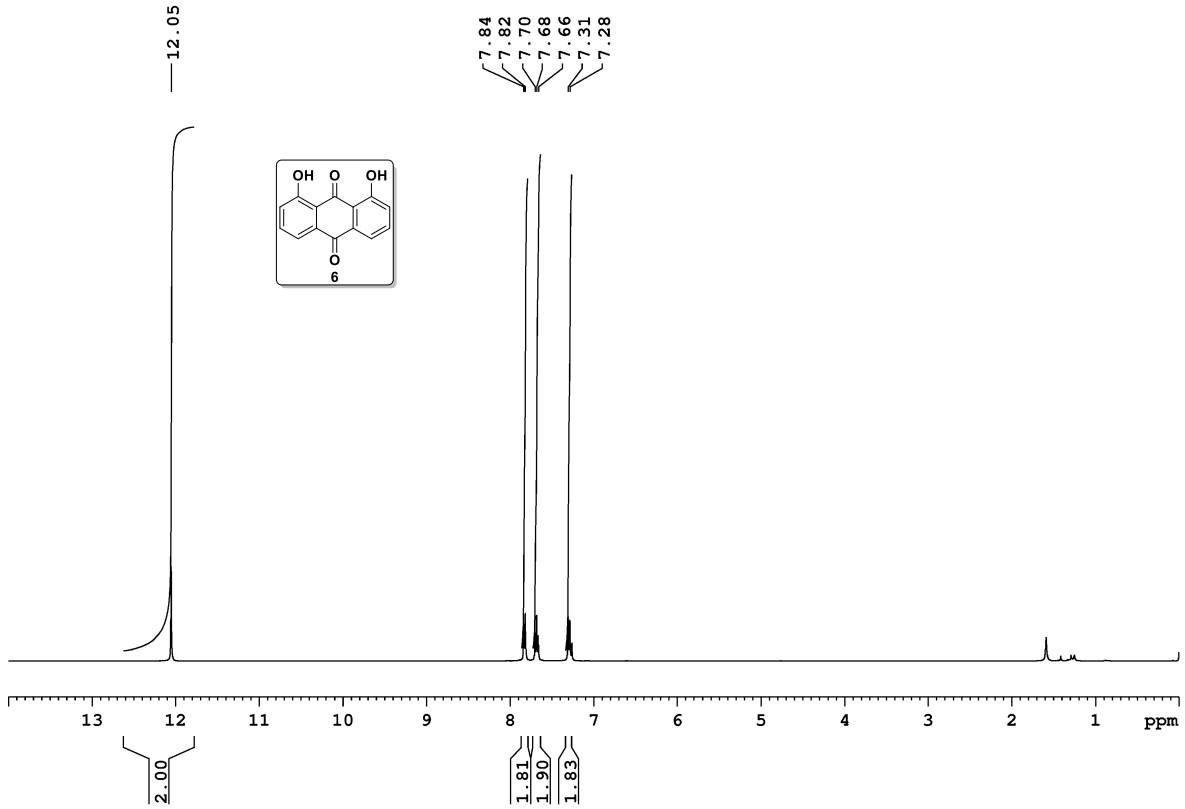
^{1}H NMR (400 MHz, acetone-d_6)



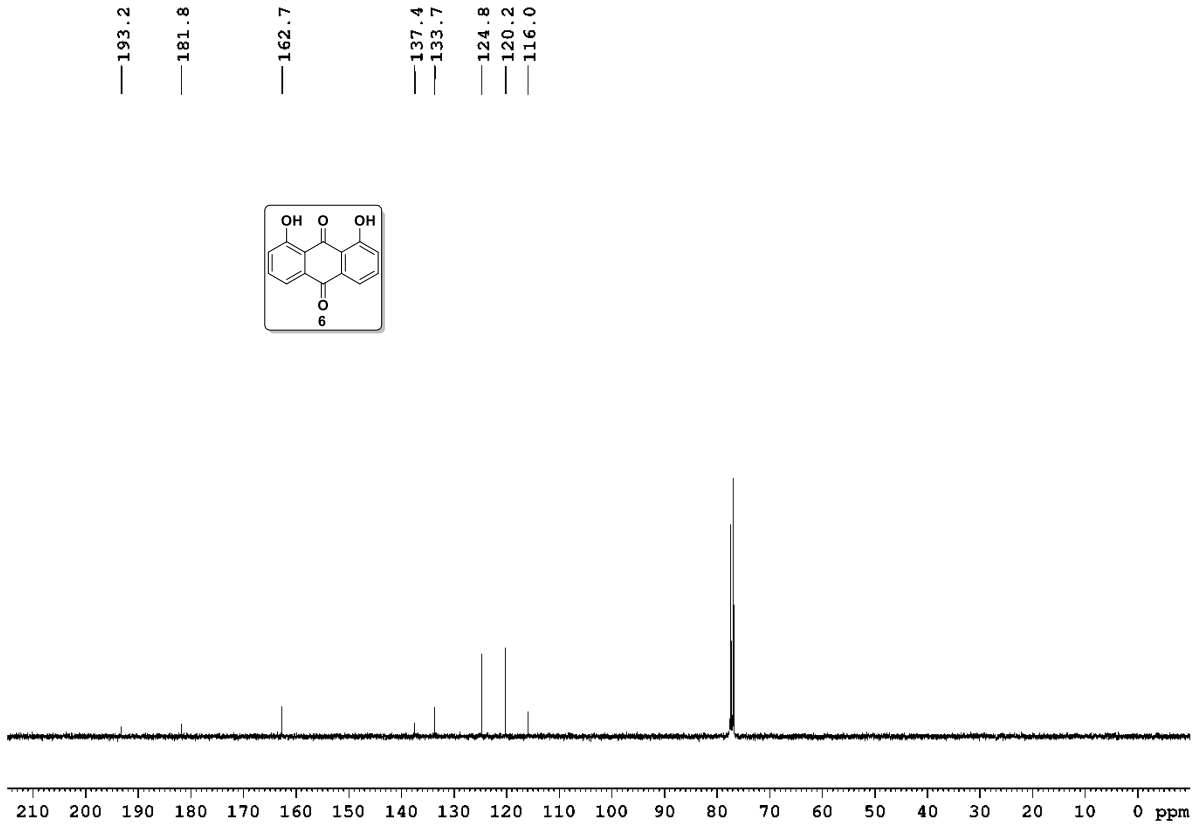
^{13}C NMR (100 MHz, acetone-d_6)



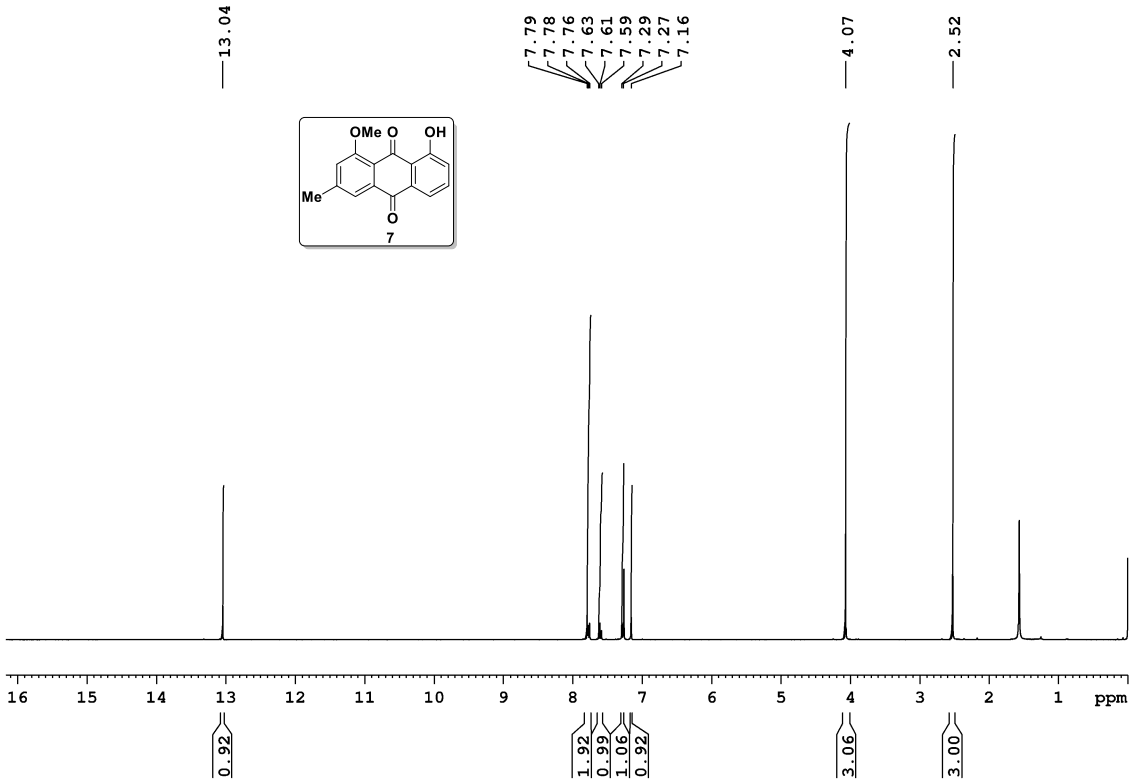
^{1}H NMR (400 MHz, $CDCl_3$)



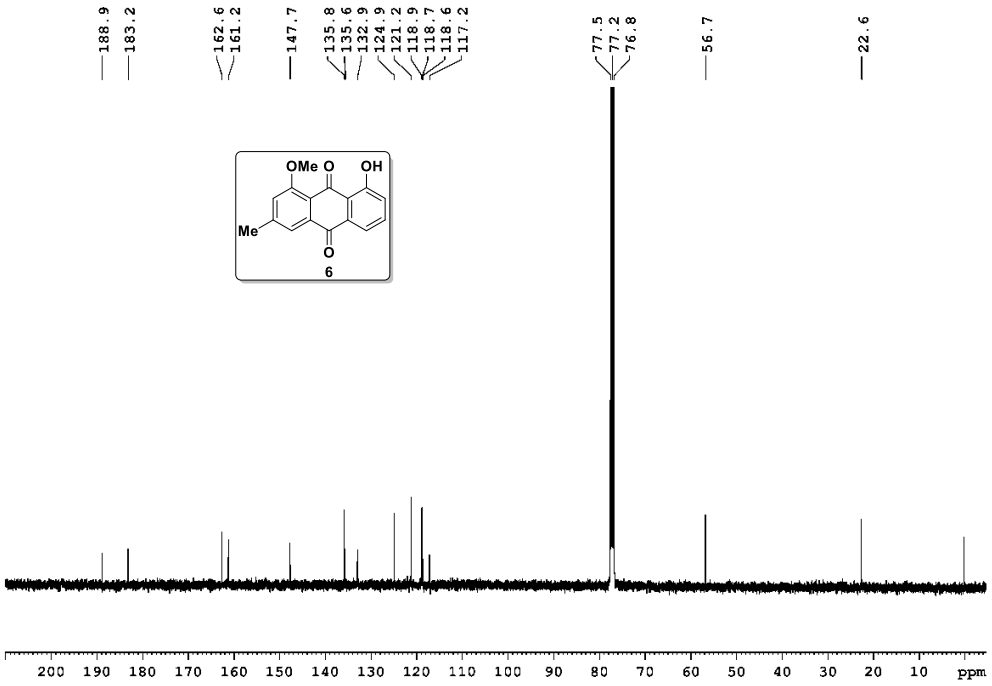
^{13}C NMR (100 MHz, $CDCl_3$)



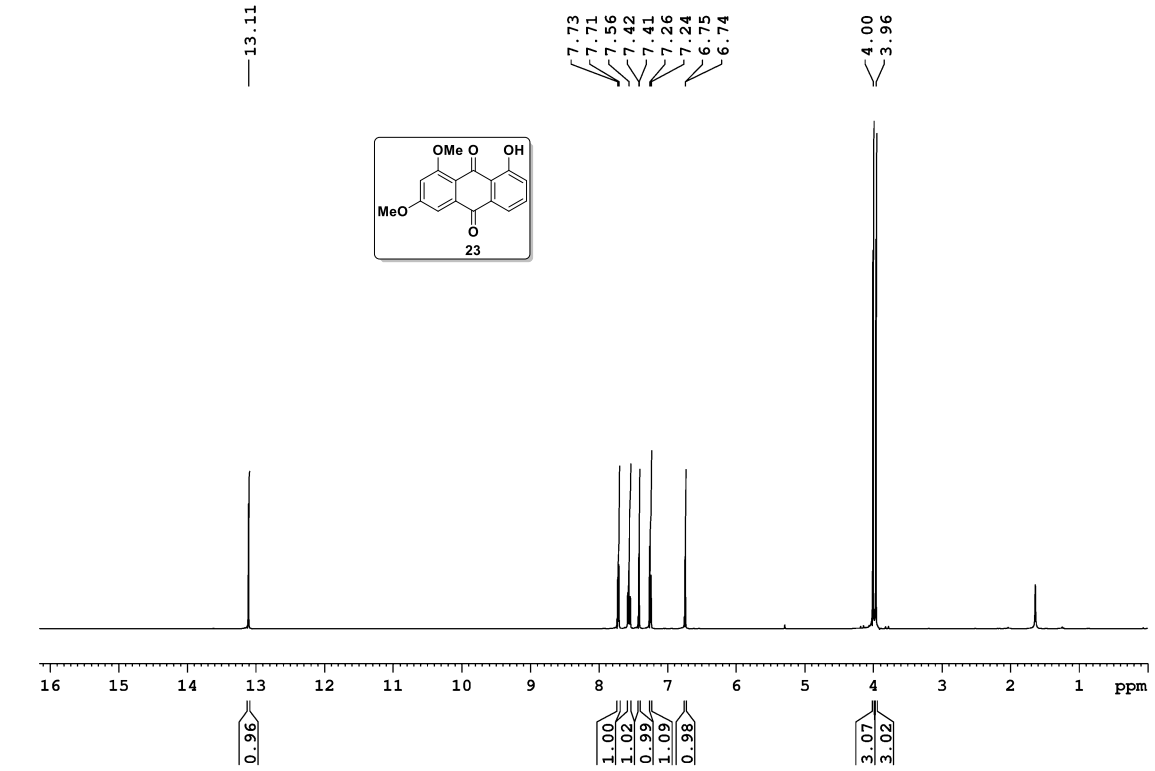
^{1}H NMR (400 MHz, $CDCl_3$)



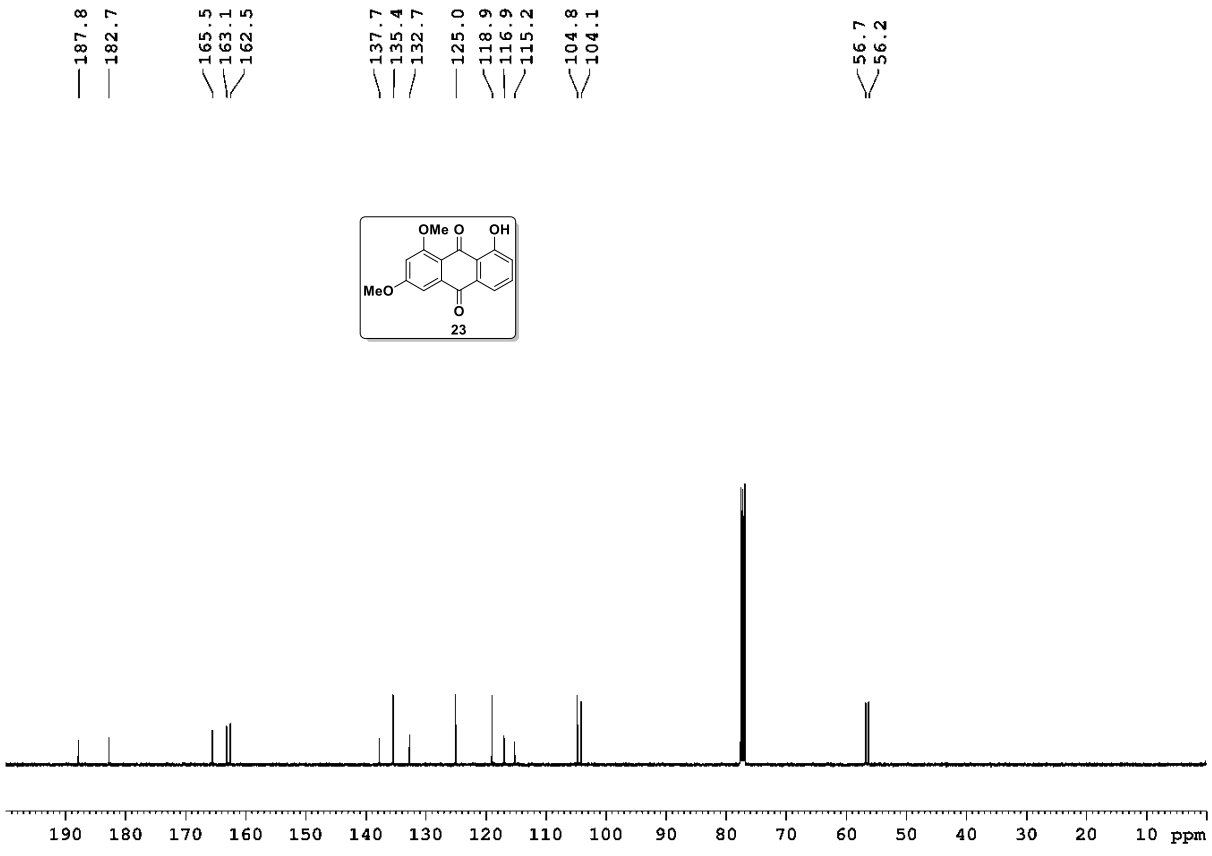
^{13}C NMR (100 MHz, $CDCl_3$)



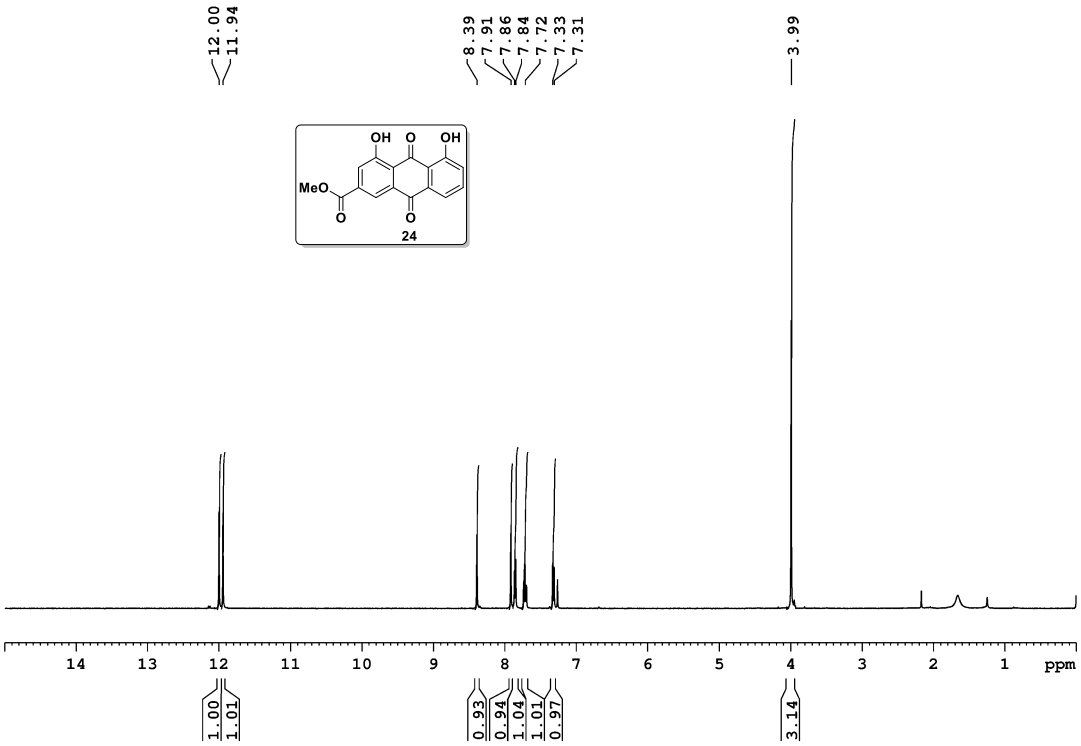
^{1}H NMR (400 MHz, $CDCl_3$)



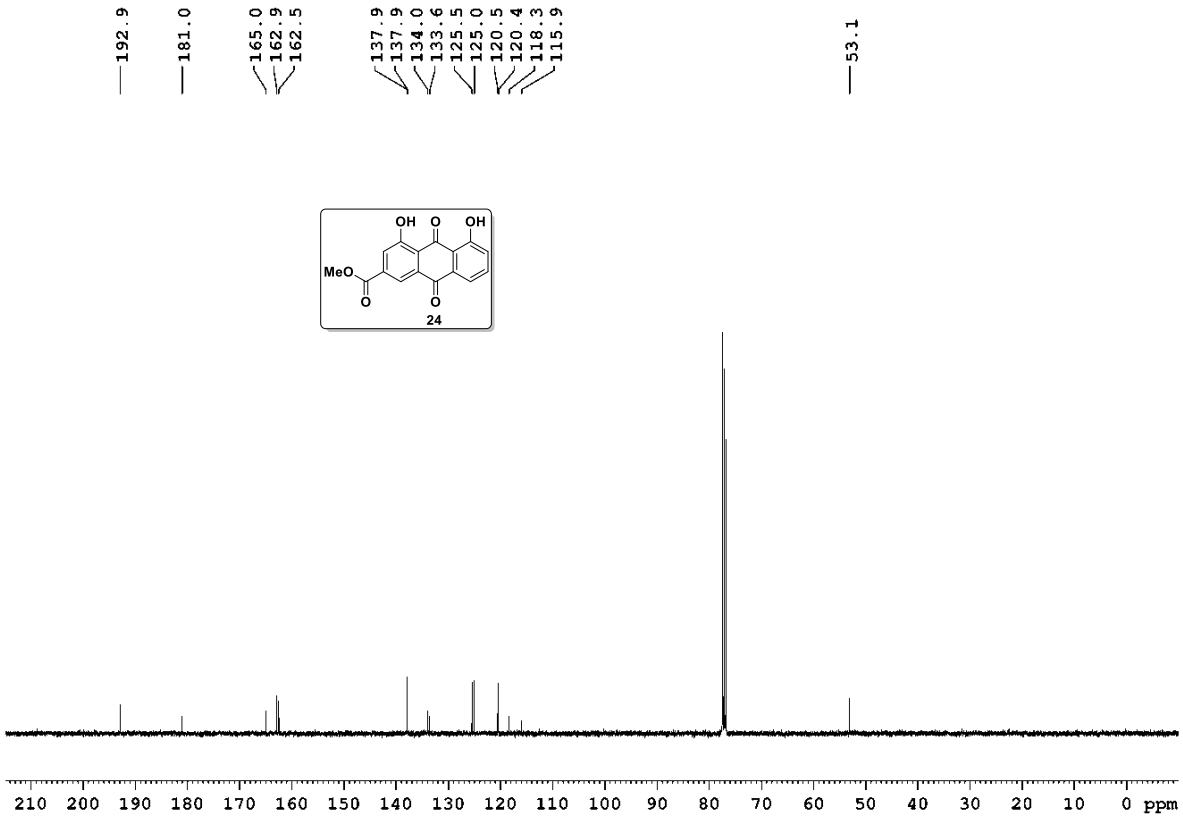
^{13}C NMR (100 MHz, $CDCl_3$)



^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (100 MHz, $CDCl_3$)



IX. References

1. Prateeksha, M. A. Yusuf, B. N. Singh, S. Sudheer, R. N. Kharwar, S. Siddiqui, A. M. Abdel-Azeem, L. F. Fraceto, K. Dashora and V. K. Gupta, *Biomolecules*, 2019, **9**, 2.
2. S. Su, J. Wu, Y. Gao, Y. Luo, D. Yang and P. Wang, *Biomed. Pharmacother.*, 2020, **125**, 110002.
3. L. Xie, H. Tang, J. Song, J. Long, L. Zhang and X. Li, *J. Pharm. Pharmacol.*, 2019, **71**, 1475–1487.
4. X. Dong, Y. Zeng, Y. Liu, L. You, X. Yin, J. Fu and J. Ni, *Phytoher. Res.*, 2020, **34**, 270–281.
5. R. M. Coopoosamy and M. L. Magwa, *African J. Biotechnol.*, 2006, **5**, 1092–1094.
6. J. Y. Ahn, J. Y. Lee, E. J. Yang, Y. J. Lee, K. B. Koo, K. S. Song and K. Y. Lee, *J. Asia. Pac. Entomol.*, 2013, **16**, 317–320.
7. E. J. Yang, S. H. Kim, K. Y. Lee and K. S. Song, *J. Microbiol. Biotechnol.*, 2018, **28**, 12–21.
8. Q. Chen, R. Pi and J. Chen, *Current Traditional Medicine*, 2016, **2**, 59–69.
9. Y. X. Zhou, W. Xia, W. Yue, C. Peng, K. Rahman and H. Zhang, *Evidence-based Complement. Altern. Med.*, 2015, **2015**.
10. R. Huang, T. Wang, X. S. Xie, K. X. Ma, X. W. Fang and S. H. Wu,*Chem. Nat. Compd.*, 2016, **52**, 697–699.
11. M. Masi and A. Evidente, *Toxins,* 2020, **12,** 714.
12. Rossi S, Tabolacci C, Lentini A, Provenzano B, Carlomosti F, Frezzotti S, Beninati S. *Anticancer Res*. 2010, **30**, 445-449.
13. V. Verebová, J. Beneš and J. Staničová, *Molecules*, 2020, **25**, 5666.
14. C. Chandrasekhar, H. Rajpurohit, K. Javaji, M. Kuncha, A. Setti, A. Z. Ali, A. K. Tiwari, S. Misra and C. G. Kumar, *Drug Chem. Toxicol.*, 2021, **44**, 148–160.
15. S. K. Singh, A. Mondal, N. Saha and S. M. Husain, *Green Chem.* 2019, **21**, 6594–6599.
16. B. Tripathi, R. Bhatia, A. Pandey, J. Gaur, G. Chawala, S. Walia and E. H. Choi, J. Chem. 2014, 1–9.
17. A. Mondal, N. Saha, A. Rajput, S. K. Singh, B. Roy and S. M. Husain, Org. Biomol. Chem., 2019, **17**, 8711–8715.
18. N. Saha, A. Mondal, K. Witte, S. K. Singh, M. Müller and S. M. Husain, *Chem. Eur. J.* 2018, **24**, 1283–1286
19. A. Mondal, A. De, and S. M. Husain, *Org. Lett.* 2020, **22**, 8511-8515.