Supplementary Material

Antidiabetic Properties of Garciniacowone L, a New Xanthone with an Unusual 5,5,8a-Trimethyloctahydro-2*H*-1-benzopyran Moiety, and Other Xanthones from the Twig Extract of *Garcinia cowa* Roxb. ex Choisy

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1. Materials and Methods

1.1 Materials and instruments

The NMR spectra were recorded on an AVANCE NEO 500 MHz Bruker spectrometer. High-resolution mass spectra (HRMS) were measured on a Bruker microTOF mass spectrometer. Optical rotations were measured on a JASCO P-2000 polarimeter. IR spectra were recorded using a Thermo scientific, Nicolet iS50 FT-IR spectrometer, while UV spectra were recorded with a Perkin Elmer, lambda 850+ spectrophotometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 (5-40 µm, SiliCycle® Inc.) and silica gel 100 (63-200 µm, SiliCycle® Inc.), respectively. Precoated thin-layer chromatography (TLC) plates of silica gel 60 F254 (Merck, USA) were used for analytical purposes. The column chromatography (CC) used several types of silica gel, including silica gel C60 (0-20 mm; SiliCycle® Inc., Canada), silica gel G60 (60-200 mm; SiliCycle® Inc., Canada), Sephadex LH-20, and Sep-Pak® (RP-18) which were used for reversed-phase flash chromatography. The absorbance and fluorescence intensity were measured by a multimode microplate reader (Perkin Elmer, US). *α*-Glucosidase, *α*-amylase, substrate 4-nitrophenyl-*α*-D-glucopyranoside (*p*-NPG), Bovine serum albumin (BSA), fructose, and trichloroacetic acid were purchased from Sigma Aldrich (St Louis, USA). Cell culture media (DMEM) were purchased from Fisher Scientific International, Inc. (New Hampshire, USA). 3T3-L1 (ATCC number: CL-173™) and L6 myotube (ATCC number: CRL-1458™) were acquired from the American Type Culture Collection (USA).

1.2 α-Glucosidase inhibitory assay

The α-glucosidase inhibitory assay was performed using the same method as described in our previous reports (Phukhatmuen et al., 2020; Raksat et al., 2020). Briefly, various concentrations (0.1-1,000 µg/mL) of samples were prepared in 5% DMSO with phosphate buffer saline (PBS) (pH 6.8). Each solution (50 µL) was amalgamated with 50 µL of the *α*-glucosidase enzyme (0.35 U/mL) in a microcentrifuge tube and incubated at 37 °C for 10 min. Subsequently, 50 µL of 4-nitrophenyl-α-*D*-glucopyranoside (*p*-NPG) (1.5 mM) was added and the sample was further incubated for 20 min at 37 °C. Then, the reaction was stopped by adding 100 µL of Na_2CO_3 (1 M). After transferring the solution to 96 microplates well, the absorbance at 405 nm was measured with a microplate reader. The *α*-glucosidase inhibitory assay was performed in triplicate, and positive controls were acarbose, voglibose, and quercetin.

References

Phukhatmuen, P., Raksat, A., Laphookhieo, S., et al., 2020. Bioassay-guided isolation and identification of antidiabetic compounds from *Garcinia cowa* leaf extract. Heliyon. 6, e03625.

Raksat, A., Phukhatmuen, P., Yang, J., et al., 2020. Phloroglucinol benzophenones and xanthones from the leaves of *Garcinia cowa* and their nitric oxide production and α-glucosidase inhibitory activities. J. Nat. Prod. 83, 164-168.

1.3 α-Amylase inhibitory assay

The α-amylase inhibitory assay was modified from a previous report (Kusano et al., 2011). Briefly, soluble starch (500 mg) was dissolved in 25 mL of 0.4 M NaOH, heated at 100 °C for 5 min., and then cooled in ice water. The solution was then adjusted to pH 7 with 2 M of HCl. Distilled water was added to adjust the volume to 100 mL. Sample solutions were prepared by dissolving each sample in acetate buffer (pH 6.5) to make concentrations of 2, 0.2, and 0.02 mg/mL. Solutions of the substrate (40 µL) and sample (20 µL) were mixed in 96-well microplates, and the mixtures were pre-incubated at 37 °C for 3 min. 20 µL of amylase solution (50 µg/mL) was then added to each well, and the plate was incubated for 15 min. To terminate the reaction, 80 µL of 0.1 M HCl was added. Finally, 100 µL of iodine solution (1 mM) was added, and the absorbance was measured by a microplate reader at 650 nm. The α-amylase inhibitory assay was performed in triplicate. Acarbose, voglibose, and quercetin were used as positive controls.

Reference

Kusano, R., Ogawa, S., Matsuo, Y., et al., 2011. α-Amylase and lipase inhibitory activity and structural characterization of Acacia bark proanthocyanidins. J. Nat. Prod. 74, 119–128.

1.4 Glycation inhibitory assay

The procedure for the glycation inhibition assay was performed using the same procedure as in the previous report (Justino et al., 2016). In brief, samples (dissolved in DMSO) were incubated at 37 °C in a dark place for 72 h with BSA (100 µL, 50

mg/mL) and fructose (100 µL, 50 mg/mL). 20% Trichloroacetic acid was added and centrifuged at 10000 RPM for 10 min. The sediment was resuspended in PBS buffer and the fluorescence intensity was measured using a microplate reader at 350 nm ex/420 nm em. The glycation inhibition assay was performed in triplicate, and the standard control was quercetin.

Reference

Justino, A.B., Pereira, M.N., Vilela, D.D., et al., 2016. Peel of araticum fruit (*Annona crassiflora* Mart.) as a source of antioxidant compounds with α-amylase, α-glucosidase, and glycation inhibitory activities. Bioorg. Chem. 69, 167-182.

1.5 Glucose uptake assay

The glucose uptake assay was conducted using the same procedure as in the previous report with slight alteration (Phukhatmuen et al., 2020). Briefly, L6 myotubes (1×10^4 cells/well) were mixed with various concentrations of samples. The mixture was incubated at 37 °C in a 5% CO_2 incubator for 24 hr. The medium was removed and washed before further incubated in Kreb-Ringer bicarbonate buffer for 1 h. Serum-free phosphate buffer saline containing 0.2% bovine serum albumin was added and further incubated for 1 hr. Fluorescence intensity was measured at 485 nm ex/530 nm em after incubated cells with 2-deoxy-2-[(7-nitro-2, 1, 3-benzoxadiazol-4-yl) amino]-D-glucose for 20 min. The glucose uptake assay was carried out in triplicate, and metformin was used as the standard control.

Reference

Phukhatmuen, P., Raksat, A., Laphookhieo, S., et al., 2020. Bioassay-guided isolation and identification of antidiabetic compounds from *Garcinia cowa* leaf extract. Heliyon. 6, e03625.

1.6 Glucose consumption assay

The glucose consumption assay was performed using the same procedure as in our previous report (Phukhatmuen et al., 2020). Briefly, the culture 3T3-L1 cells 100 µL (density of 1×10^5 cells/well) were seeded in 96 well microplates together with various concentrations of samples. The mixture was incubated at 37 °C in a 5% CO_2 incubator for 24 h and then 10 µL of supernatant liquid was transferred to another 96-well plate. The remaining glucose concentration in the suspension was evaluated after 30 min by the illuminated reaction. The absorbance was measured at a wavelength of 495 nm. The glucose consumption assay was performed in triplicate, and metformin was used as standard control. Cell viability was carried out by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay, as previously described (Phukhatmuen et al., 2020).

Reference

Phukhatmuen, P., Raksat, A., Laphookhieo, S., et al., 2020. Bioassay-guided isolation and identification of antidiabetic compounds from *Garcinia cowa* leaf extract. Heliyon. 6, e03625.

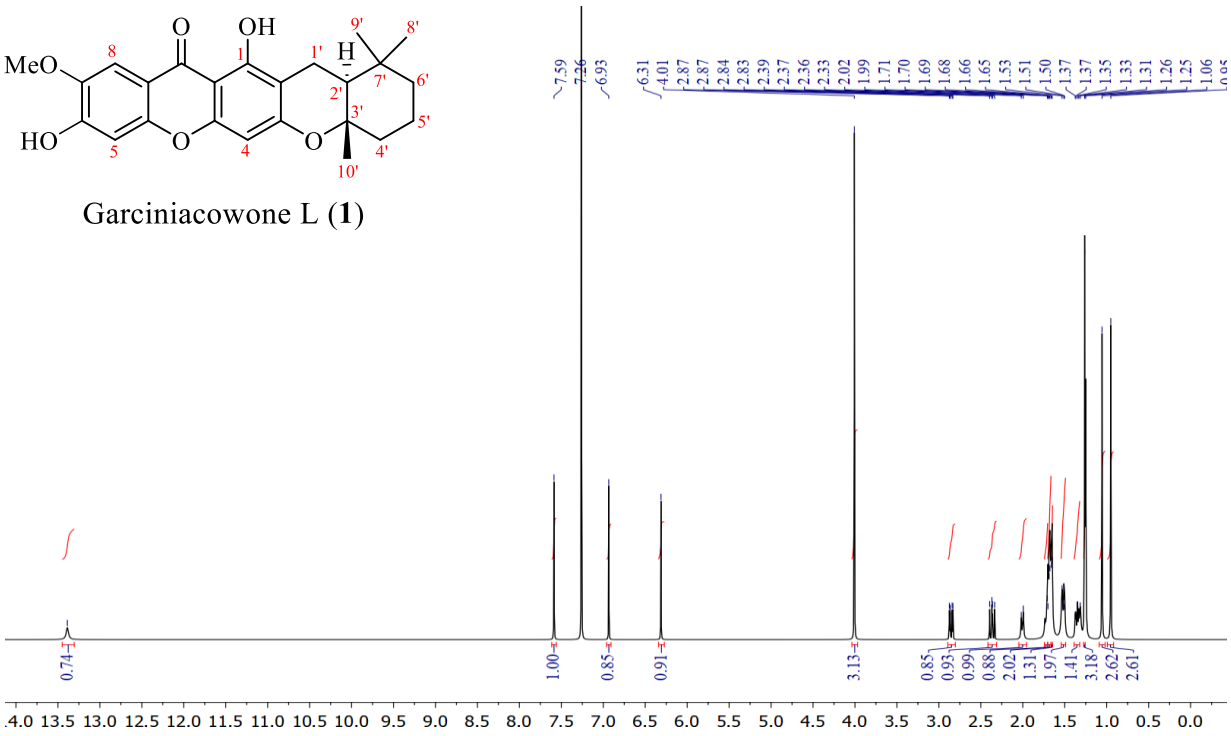


Figure S1. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of garciniacowone L (**1**)

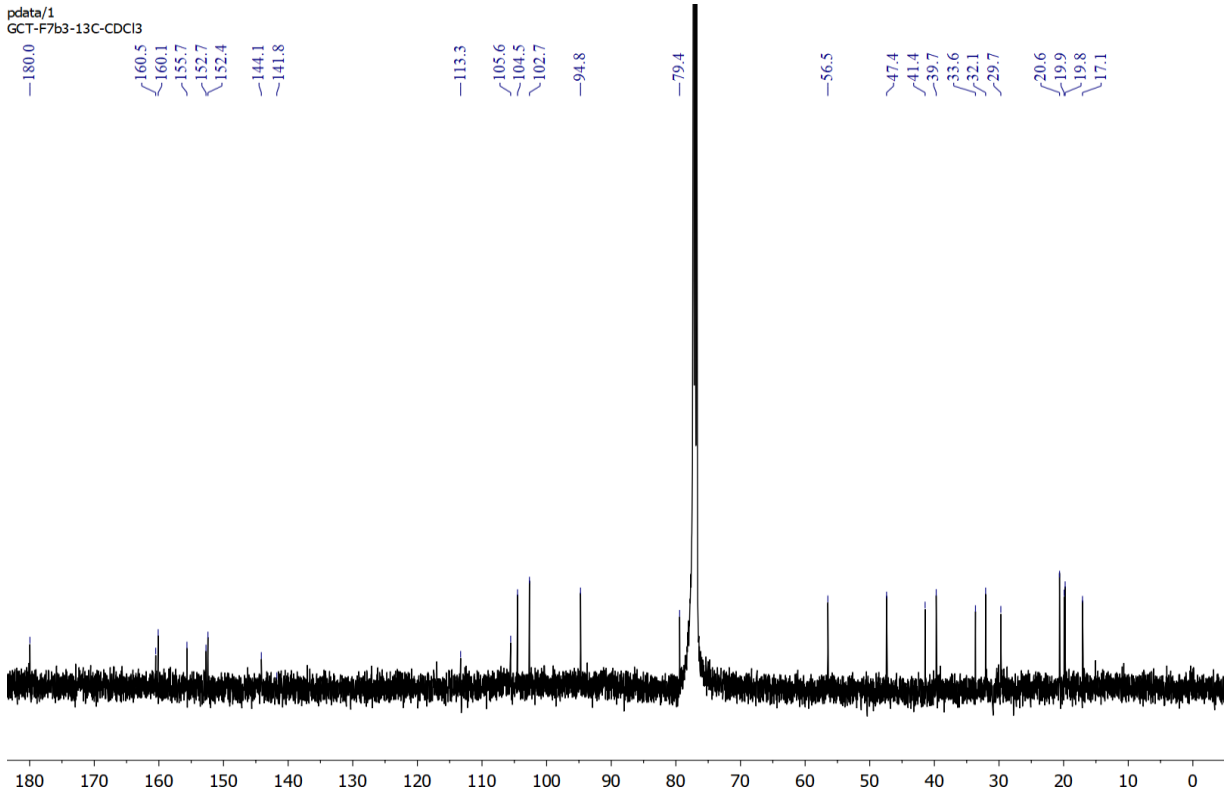


Figure S2. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of garciniacowone L (**1**)

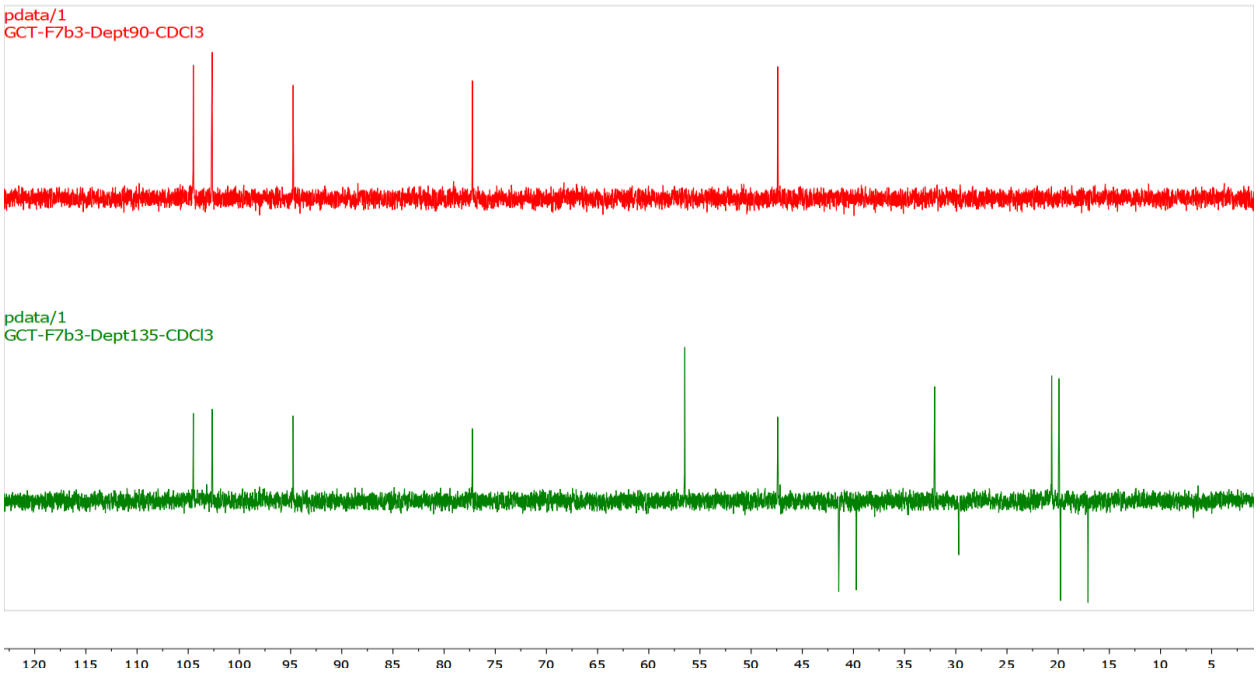


Figure S3. DEPT90 and DEPT135 spectrum (125 MHz, $CDCl_3$) of garciniacowone L (**1**)

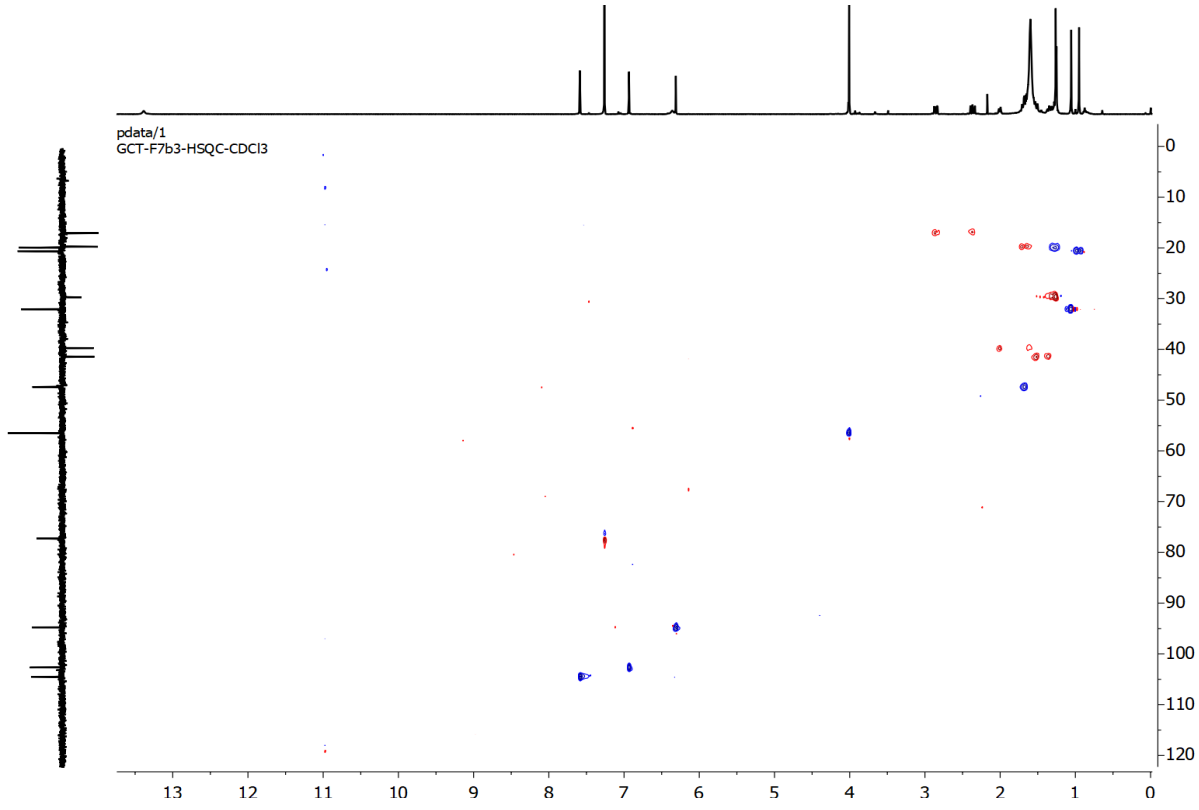


Figure S4. HSQC spectrum (500 MHz, $CDCl_3$) of garciniacowone L (**1**)

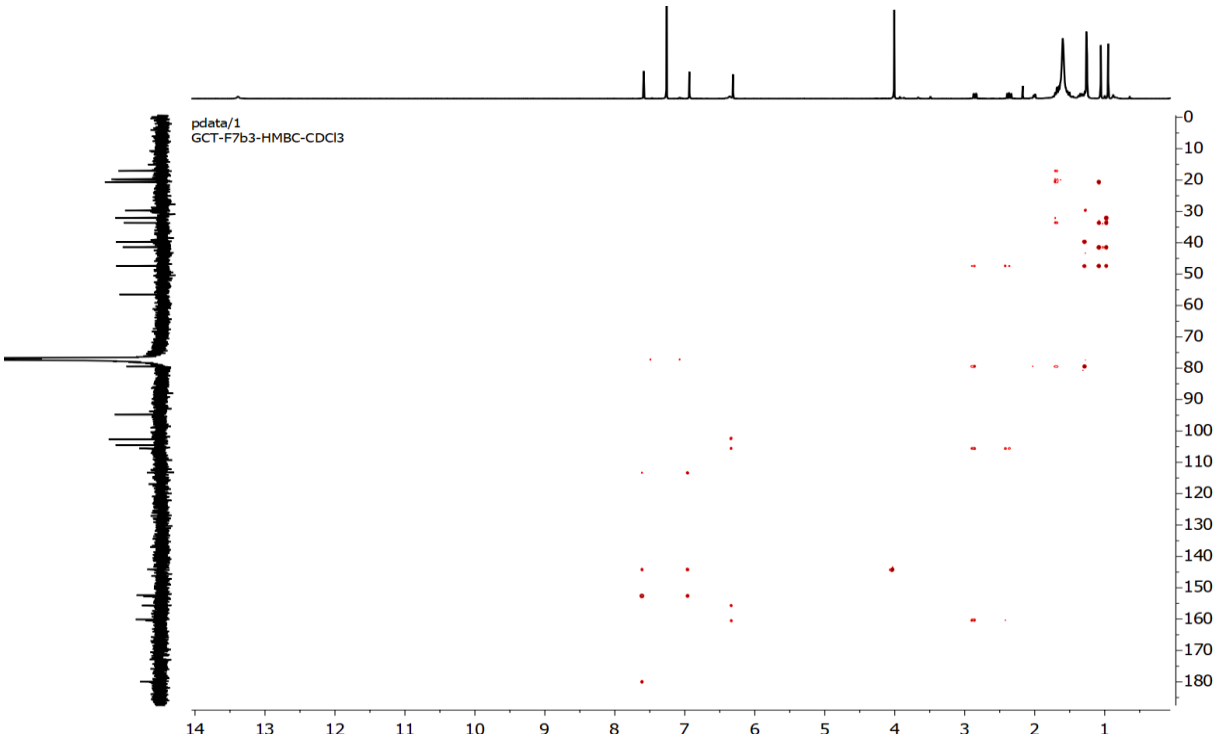


Figure S5. HMBC spectrum (500 MHz, $CDCl_3$) of garciniacowone L (**1**)

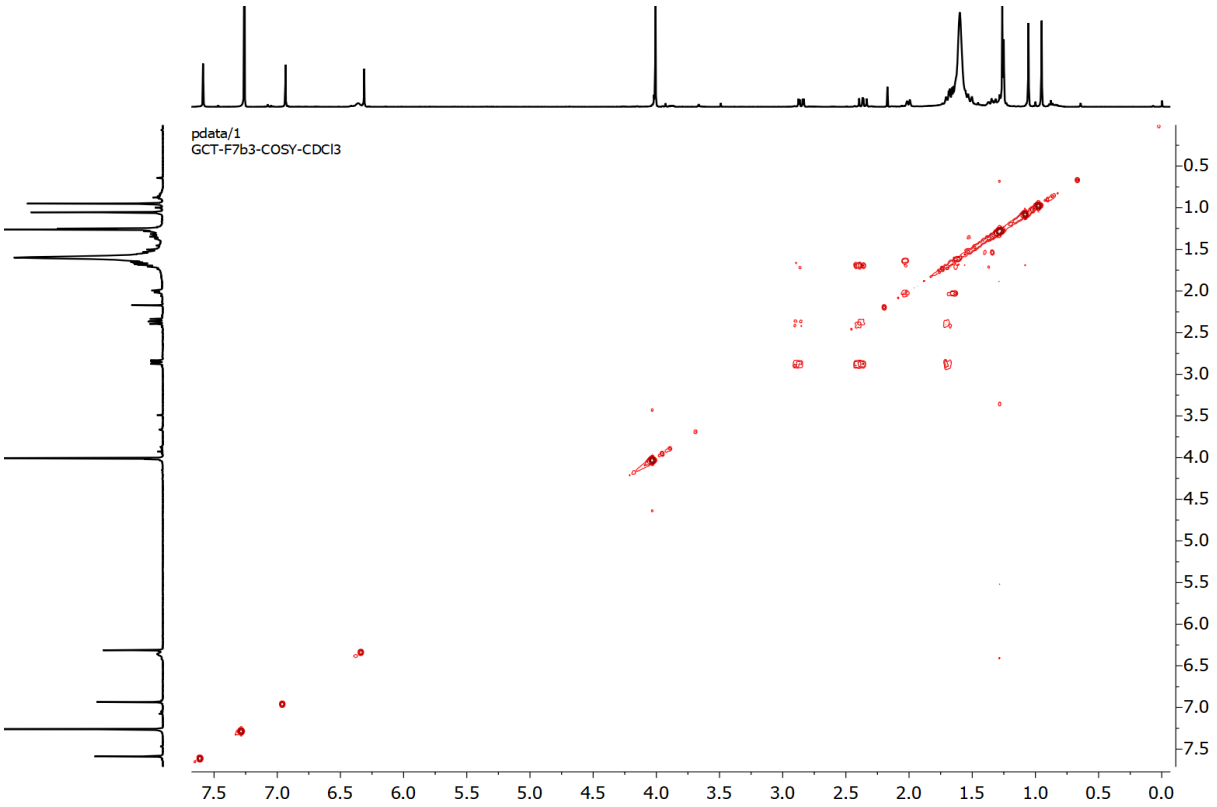


Figure S6. COSY spectrum (500 MHz, $CDCl_3$) of garciniacowone L (**1**)

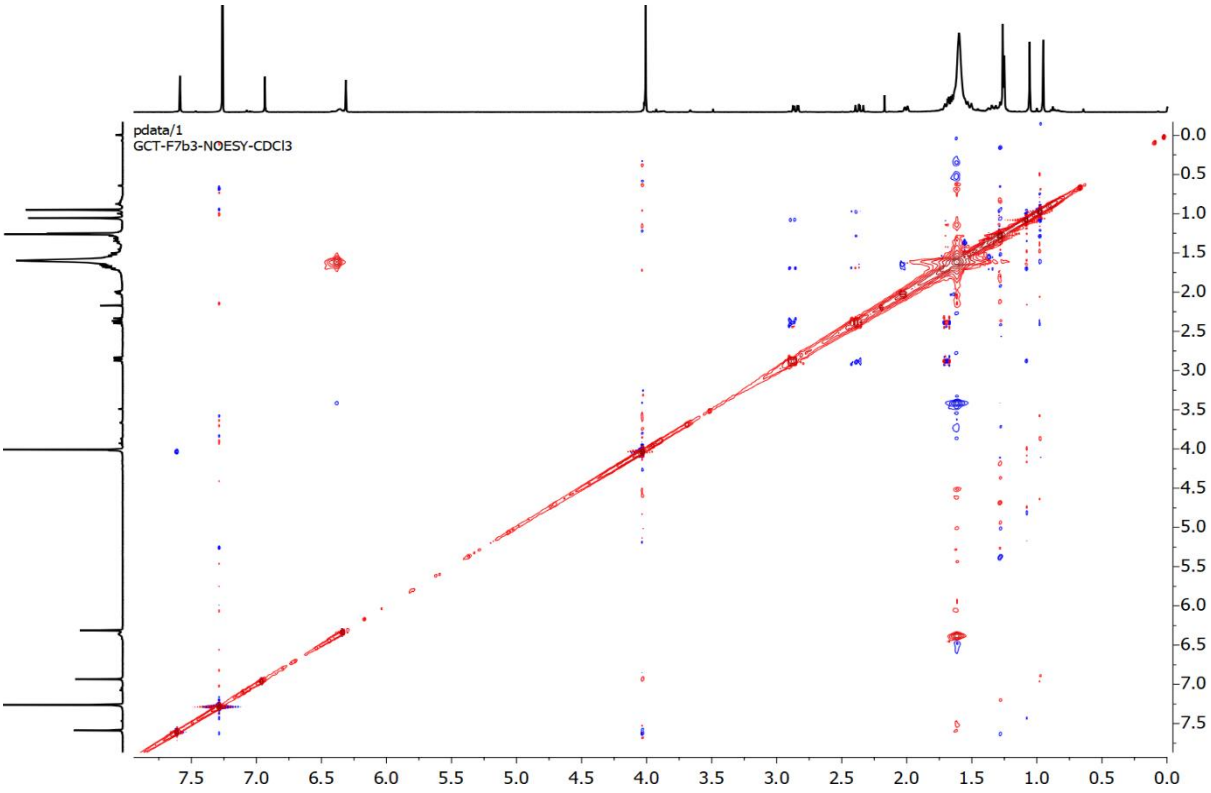
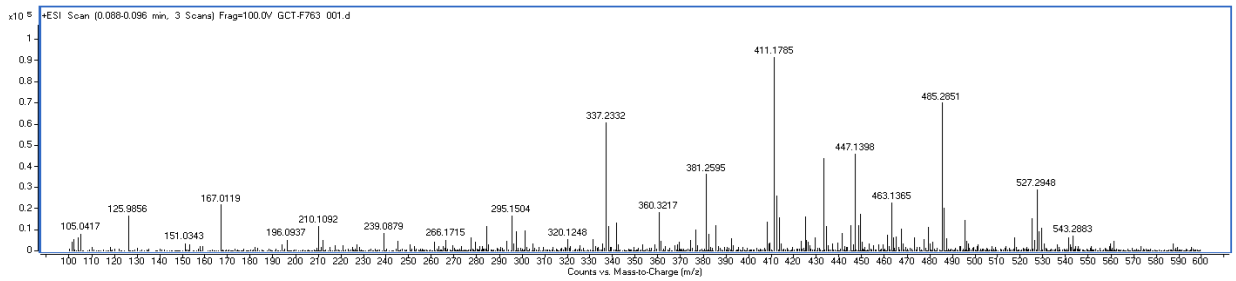


Figure S7. NOESY spectrum (500 MHz, $CDCl_3$) of garciniacowone L (**1**)



Formula (M)	Score (MFG)	Mass	Mass (MFG)	m/z (Calc)	Diff (ppm)	DBE	m/z
$C_{24}H_{26}O_6$	92.99	410.1712	410.1729	411.1802	4.18	12	411.1785
$C_{31}H_{22}O$	71.05	410.1712	410.1671	411.1743	-10.14	21	411.1785

Figure S8. HRESITOFMS spectrum of garciniacowone L (**1**)

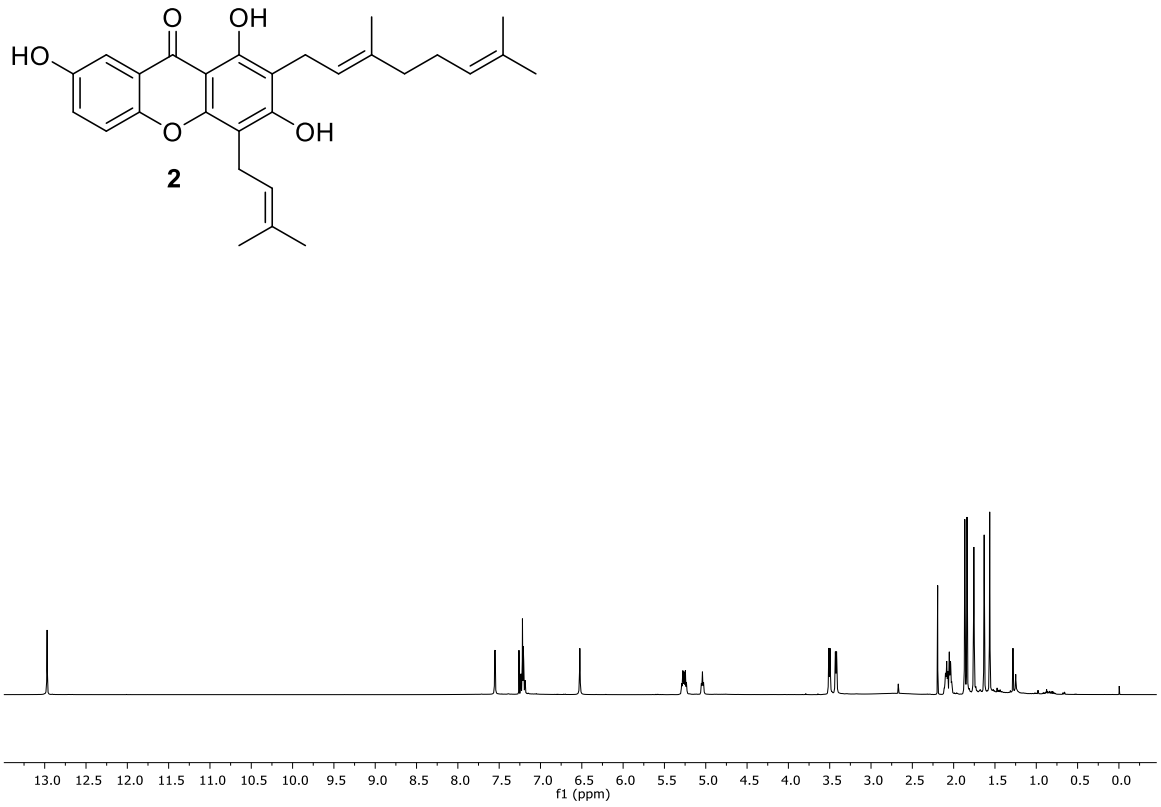


Figure S9. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of 2-geranyl-1,3,7-trihydroxy-4-(3,3-dimethylallyl)-xanthone (**2**)

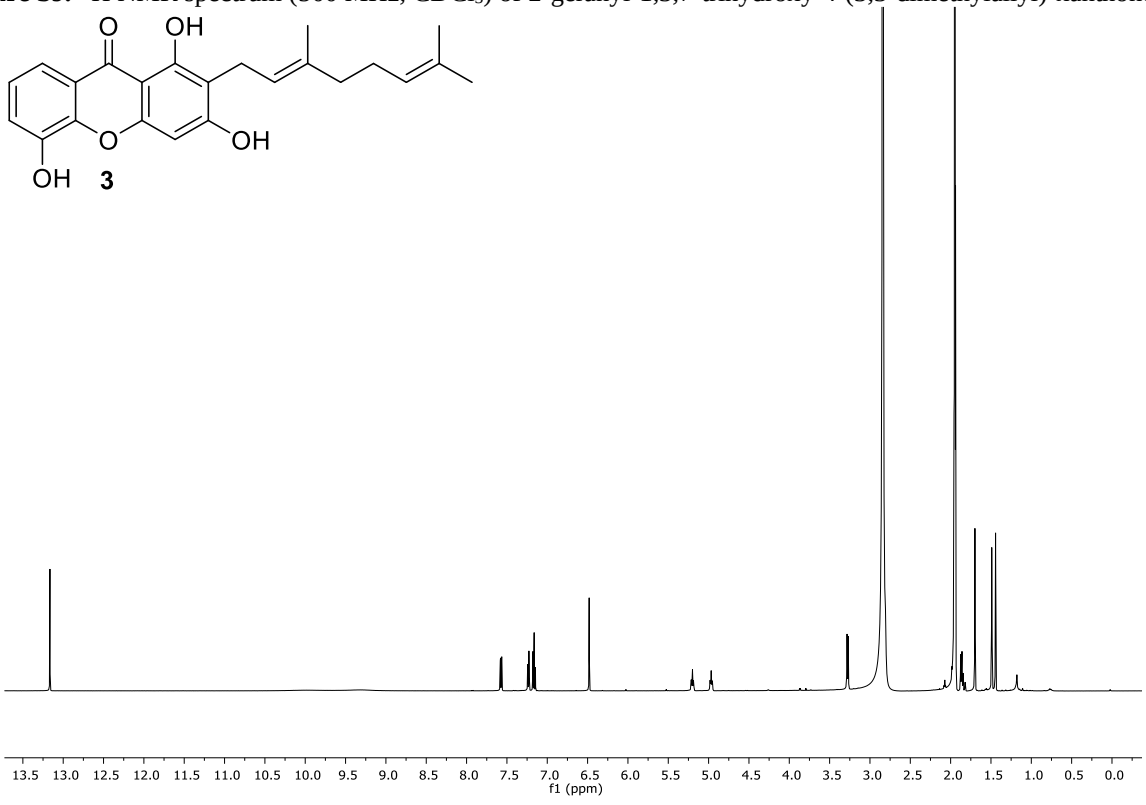
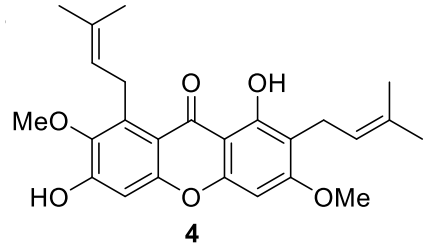


Figure S10. ^{1}H NMR spectrum (500 MHz, acetone-d_6) of mangostinone (**3**)



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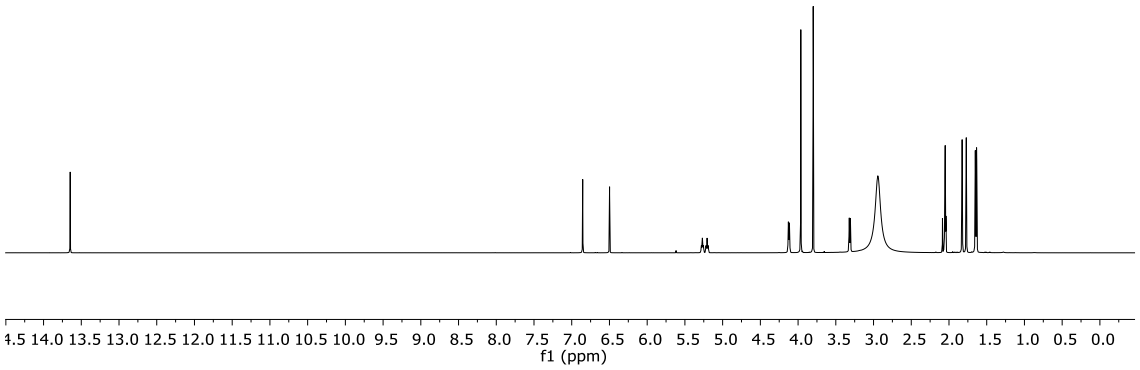
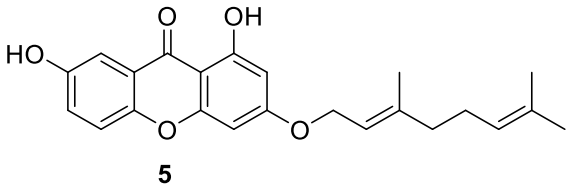


Figure S11. ^{1}H NMR spectrum (500 MHz, acetone-d_6) of β-mangostin (**4**)



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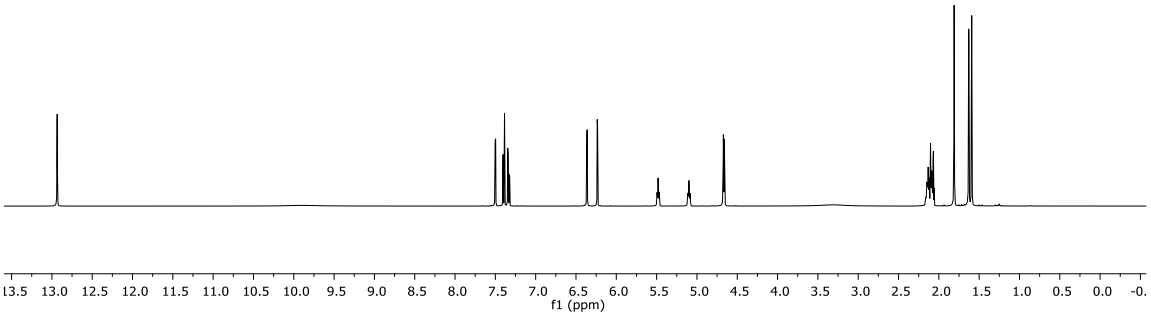

Figure S12. ^{1}H NMR spectrum (500 MHz, acetone-d_6) of cochinchinone G (**5**)

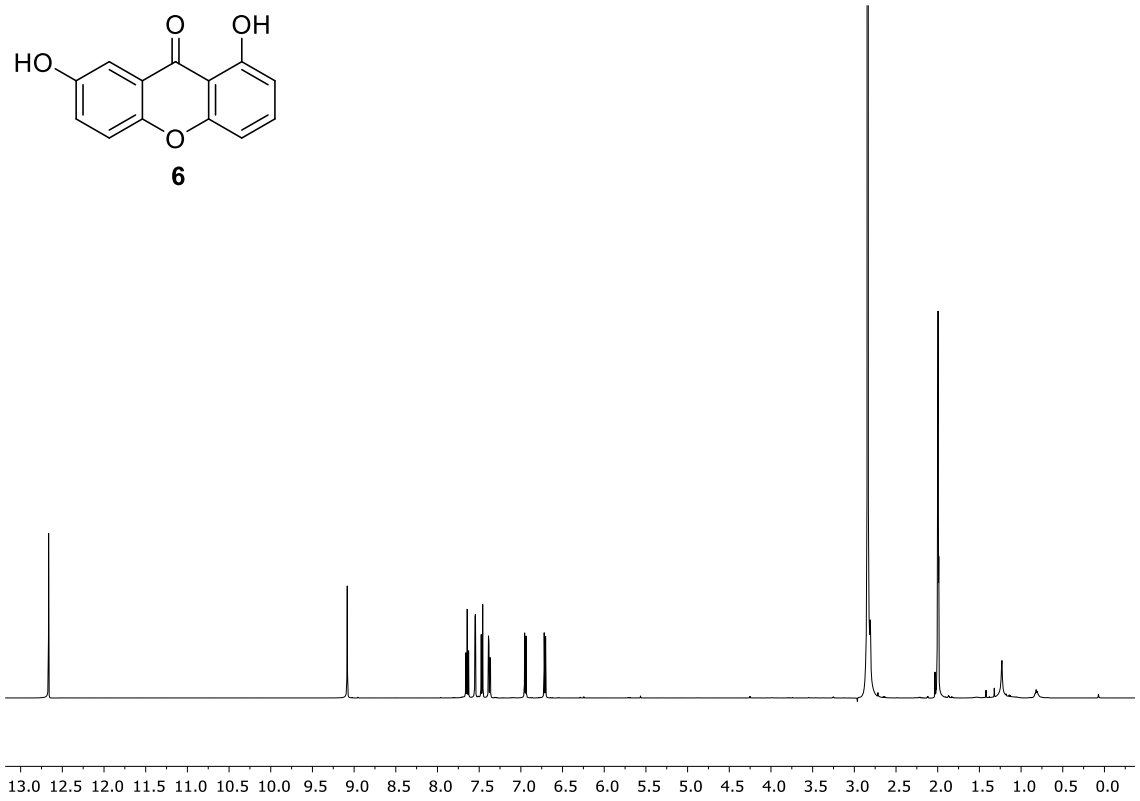

Figure S13. ^{1}H NMR spectrum (500 MHz, acetone-d_6) of 1,7-dihydroxyxanthone (**6**)

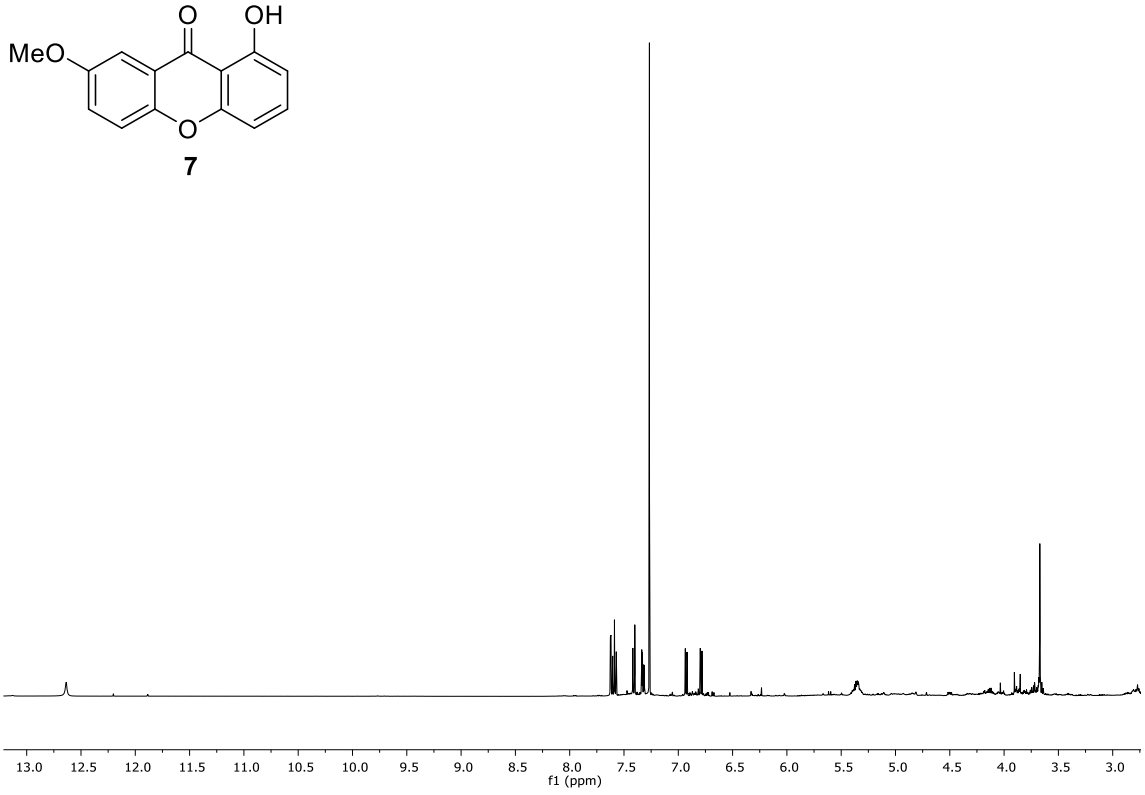

Figure S14. ^{1}H NMR spectrum (500 MHz, acetone-d_6) of 1-hydroxy-7-methoxy xanthone (**7**)

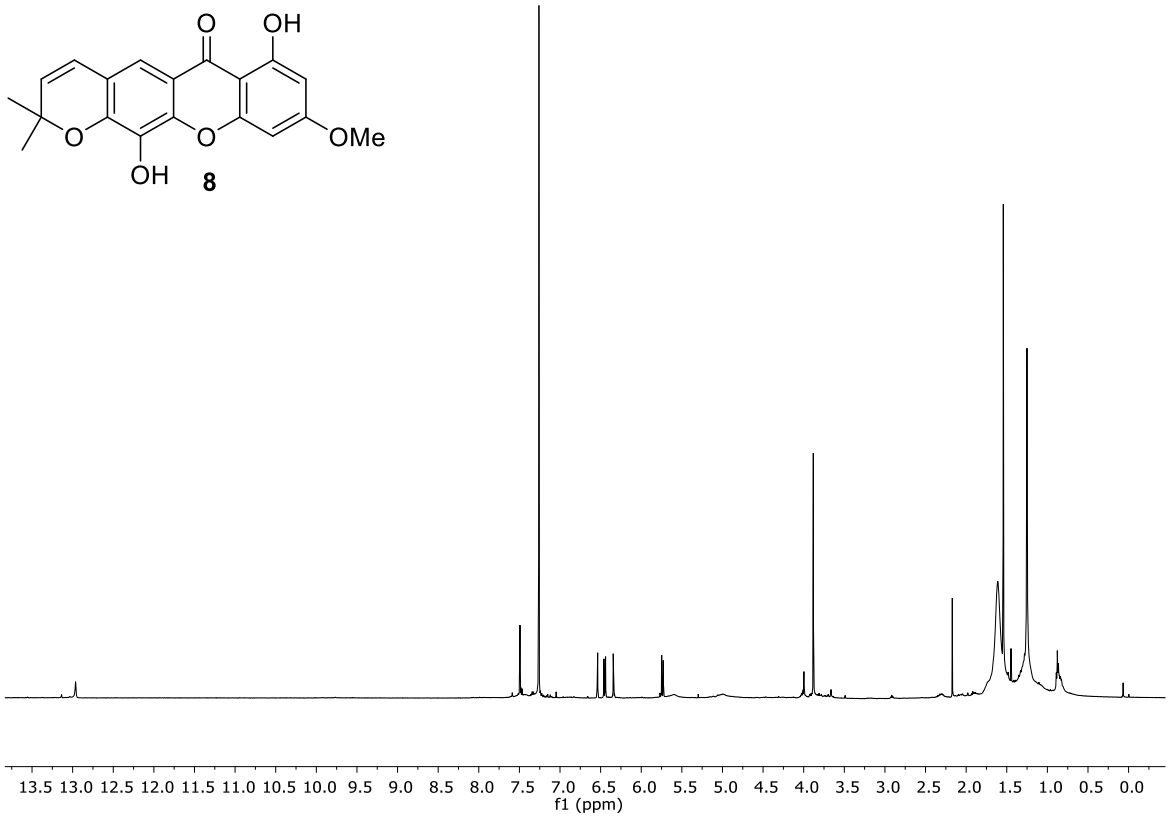

Figure S15. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of 1-hydroxy-7-methoxy xanthone (**8**)