Supporting Information

Antiproliferative Butyrolactones from *Mezilaurus crassiramea*

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General experimental procedures

Optical rotations were determined on a Perkin Elmer 341 polarimeter (Na filter, $\lambda = 589$ nm). IR spectra were recorded as KBr pellets on a Bomem-Hartmann & Braun FT-IR spectrometer. $^1$H and $^{13}$C, 1D- and 2D-NMR spectra were obtained at room temperature in CDCl$_3$ (Cambridge Isotope Laboratories) on Bruker DPX-300 and Bruker Avance DRX500 spectrometers, operating at 300.13 MHz ($^1$H)/75.47 MHz ($^{13}$C) and 500.13 MHz ($^1$H) and 125.76 MHz ($^{13}$C), with an inverse probehead of 5 mm (BBI $^1$H-BB), respectively. HR-ESIMS spectra were acquired in the positive ion mode on an UltrOTOF-Q instrument (Bruker Daltonics). The column chromatographic procedures were performed on silica gel 60 (70-230 and 230-400 mesh; Merck) and Sephadex LH-20 (Amersham Pharmacia Biotech). Thin-layer chromatography was carried out on Merck silica gel 60 F$_{254}$ TLC aluminum sheets.

Extraction and isolation of compounds 1-3

Air-dried and finely powdered leaves (1.96 kg) of M. crassiramea were exhaustively extracted with EtOH (20 L) at room temperature. After concentration under vacuum, the EtOH extract was subsequently partitioned between MeOH-H$_2$O 9:1 (1 L) and hexane (3 x 300 mL), MeOH-H$_2$O 1:1 (1 L) and CH$_2$Cl$_2$ (3 x 300 mL), and MeOH-H$_2$O 1:1 (1 L) and EtOAc (3 x 300 mL), to afford the respective hexane (82.1 g), CH$_2$Cl$_2$ (71.7 g), and EtOAc (148.7 g) phases, the brine shrimp toxicity residing in the CH$_2$Cl$_2$ solubles. Part of the bioactive CH$_2$Cl$_2$ phase (10.0 g) was applied to a silica gel chromatographic column (70-230 mesh; 5 x 22 cm) eluted with hexane, hexane-CHCl$_3$ (1:1), CHCl$_3$, CHCl$_3$-EtOAc (8:2, and 1:1), EtOAc, and EtOAc-MeOH (95:5, 8:2, 1:1), to give 15 fractions of 500 mL each (I-XV mL). Testing for brine shrimp toxicity showed fractions VI (CHCl$_3$-EtOAc 8:2; 1.38 g), IX (CHCl$_3$-EtOAc 1:1; 452.3 mg), and XI (EtOAc; 563.0 mg) to be bioactive (LD$_{50}$ = 83.6 $\mu$g/mL, 3.23 $\mu$g/mL, and 28.4 $\mu$g/mL, respectively). An aliquot (1.30 g) of fraction VI was subjected to column chromatography (CC) on Sephadex LH-20 (2.7 x 18 cm, CHCl$_3$) to give 21 fractions of 20 mL each (Frs. 1-21). Part of Frs. 4-10 (351.4 mg) was further subjected to CC on silica gel (230-400 mesh; 2.5 x 35 cm) using a step gradient elution with hexane-EtOAc (9:1 to EtOAc) to afford 2 (hexane-EtOAc 8:2; 38.6 mg). An aliquot (300.0 mg) of fraction IX was subjected to CC on Sephadex LH-20 (2.7 x 18 cm, CHCl$_3$)
to give 1 (159.0 mg). Compound 3 (82.0 mg) was obtained after CC on Sephadex LH-20 (2.7 × 18 cm, CHCl₃) of an aliquot (300.0 mg) of fraction XI.

Specific optical rotation values, IR, NMR, and HR-ESIMS spectral data of compounds 1-3

3'-Acetylrubrenolide (1): Amorphous solid; [α]D₂₀ +20.86 (c 0.12, acetone); IR (KBr): νmax (cm⁻¹) (Fig. 9S): 3429, 1747, 1678, 1234; for 1D- and 2D-NMR spectral data, see Table 1 and Figs. 1S to 8S; HR-ESIMS (positive ion mode) (Fig. 10S): m/z 363.2141 [M + Na]⁺ (calcd. for C₁₉H₃₂O₅Na, m/z 363.2147).

2',3'-Diacetylrubrenolide (2): Amorphous solid; [α]D₂₀ +35.43 (c 0.10, acetone); IR (KBr): νmax (cm⁻¹) (Fig. 19S): 2927, 1743, 1678, 1222; for 1D- and 2D-NMR spectral data, see Table 1 and Figs. 11S to 18S; HR-ESIMS (positive ion mode) (Fig. 20S): m/z 405.22455 [M + Na]⁺ (calcd. for C₂₁H₃₄O₆Na, m/z 405.22437).

Rubrenolide (3): Amorphous solid; [α]D₂₀ +32.40 (c 0.11, acetone); IR (KBr): νmax (cm⁻¹) (Fig. 29S): 3352, 2923, 1747, 1207; for 1D- and 2D-NMR spectral data, see Table 1 and Figs. 21S to 28S; HR-ESIMS (positive ion mode) (Fig. 30S): m/z 321.20350 [M + Na]⁺ (calcd. for C₁₇H₃₀O₄Na, m/z 321.20330).
Fig. 1S $^1$H NMR spectrum (300.13 MHz, CDCl$_3$) of compound 1.

Fig. 2S Expansion of $^1$H NMR spectrum (300.13 MHz, CDCl$_3$) of compound 1.
Fig. 3S $^{13}$C NMR spectrum (75.47 MHz, CDCl$_3$) of compound 1.

Fig. 4S DEPT-135 NMR experiment (75.47 MHz, CDCl$_3$) of compound 1.
Fig. 5S HSQC experiment (300.13/75.47 MHz, CDCl₃) of compound 1.

Fig. 6S HMBC experiment (300.13/75.47 MHz, CDCl₃) of compound 1.
Fig. 7S Expansion of HMBC experiment (300.13/75.47 MHz, CDCl₃) of compound 1.

Fig. 8S ¹H-¹H COSY experiment (CDCl₃) of compound 1.
Fig. 9S IR spectrum of compound 1.
Fig. 10S HRESIMS (positive ion mode) of compound 1.
Fig. 11S $^1$H NMR spectrum (300.13 MHz, CDCl$_3$) of compound 2.

Fig. 12S Expansion of $^1$H NMR spectrum (300.13 MHz, CDCl$_3$) of compound 2.
**Fig. 13S** $^{13}$C NMR spectrum (75.47 MHz, CDCl$_3$) of compound 2.

**Fig. 14S** DEPT-135 experiment (75.47 MHz, CDCl$_3$) of compound 2.
**Fig. 15S** HSQC experiment (500.13/125.77 MHz, CDCl$_3$) of compound 2.

**Fig. 16S** HMBC experiment (500.13/125.77 MHz, CDCl$_3$) of compound 2.
Fig. 17S Expansion of HMBC experiment (500.13/125.77 MHz, CDCl$_3$) of compound 2.

Fig. 18S $^1$H-$^1$H COSY experiment (CDCl$_3$) of compound 2.
Fig. 19S IR spectrum of compound 2.

Fig. 20S HRESIMS (positive ion mode) of compound 2.
Fig. 21S $^1$H NMR spectrum (500.13 MHz, CDCl$_3$) of compound 3.
Fig. 22S Expansion of $^1$H NMR spectrum (500.13 MHz, CDCl$_3$) of compound 3.

Fig. 23S $^{13}$C NMR spectrum (75.47 MHz, CDCl$_3$) of compound 3.
Fig. 24S DEPT-135 (75.47 MHz, CDCl$_3$) experiment of compound 3.

Fig. 25S HSQC experiment (500.13/125.77 MHz, CDCl$_3$) of compound 3.
Fig. 26S HMBC experiment (500.13/125.77 MHz, CDCl$_3$) of compound 3.

Fig. 27S Expansion of HMBC experiment (500.13/125.77 MHz, CDCl$_3$) of compound 3.
Fig. 28S $^1$H-$^1$H COSY experiment (CDCl$_3$) of compound 3.
Fig. 29S IR spectrum of compound 3.

Fig. 30S HRESIMS (positive ion mode) of compound 3.
Brine shrimp lethality assay

The brine shrimp (*A. salina*) [San Francisco Bay Brand, Inc.] lethality test was performed with crude extract and phases, in triplicate, according to Meyer et al. [a], using quinidine sulfate dehydrate (≥ 97%, Sigma) as a positive control. LD<sub>50</sub> values in µg/mL were determined using probit analysis.

In vitro cytotoxic assay

Cytotoxicity of compounds 1-3 was measured *in vitro* by growth inhibition of six human cancer cell lines – namely, 786-0 (kidney carcinoma), MCF-7 (breast adenocarcinoma), PC-3 (prostate carcinoma), HT-29 (colon adenocarcinoma), UACC-62 (melanoma), and NCI/ADR-RES (ovarian multidrug-resistant), using the sulfurhodamine B (Sigma) assay, as described elsewhere [b]. The cell lines were provided by Dr. João E. de Carvalho (CPQBA, Universidade Estadual de Campinas, Campinas, Brazil). Doxorubicin hydrochloride (≥ 98%, Eurofarma) was used as a positive control. The purity of the isolated compounds tested was ≥ 95%. Each sample was tested in triplicate at four different concentrations. The following formula was used to calculate the growth percentage, according to Monks et al. [c]: 100 × [(T-T0)/(C-T0)]. Measurements were performed at T0 (at the beginning of incubation) and 48 h after incubation for both untreated (negative control, C) and treated (T) cells. T ≥ T0 and T < C characterized a cytostatic effect, while T < T0 indicated a cytocidal effect. GI<sub>50</sub> values were calculated from the differences in absorbance readings at 540 nm in untreated and treated cells [c], through non-linear regression analysis, using Origin 6.0 software (OriginLab).

References
