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Cytotoxic cardenolide glycosides of *Roupellina (Strophanthus)* boivinii from the Madagascar rainforest¹

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Abstract

Bioassay-guided fractionation of an ethanol extract of *Roupellina (Strophanthus) boivinii* from the rainforest of Madagascar afforded the six new cardenolide glycosides boivinides **1–6**, as well as the four known cardenolide glycosides, digitoxigenin 3-O-[β -D-glucopyrananosyl-(1 \rightarrow 4)- α -L-acofriopyranoside], corotoxigenin 3-O- β -D-boivinoside, 17 β H-corotoxigenin 3-O- β -D-sarmentoside, and uzarigenin 3-O- α -L-rhamnoside. The structures of these compounds were elucidated by various 1D and 2D NMR techniques. All new compounds showed significant antiproliferative activity against A2780 human ovarian cancer cell line, with boivinide A being the most active at IC₅₀ = 0.17 μ M.

In our continuing search for bioactive natural products from the Madagascar rainforests as a part of International Cooperative Biodiversity Group (ICBG) program, 1 we obtained an ethanol extract of the plant *Roupellina boivinii* (Baill.) Pichon, (family Apocynaceae); this plant is also known as *Strophanthus boivinii* Baill. The extract, designated MG 2309PE, exhibited good antiproliferative activity against the A2780 ovarian cancer cell line (IC $_{50}=11\,\mu\text{g/mL}$), and hence was selected for fractionation to isolate its active components.

The Apocynaceae family is native mainly to Africa, with a few species in Asia. This family has 424 genera and 2100 species with 35–40 species of flowering plants in the *Roupellina/Strophanthus* genus.² These medicinal plants are known to contain lignans,³ cytotoxic alkaloids⁴ and cardenolide glycosides.⁴ Cardiac glycosides have also been isolated from the monarch butterfly (*Danaus plexippus* L.) which feeds on the plants of the milkweed (Apocynaceae) family.⁵ Cardenolide glycosides are an important class of natural products that can be used as drugs as well as toxins, and plants of the Apocynaceae family or their extracts have been used as arrow poisons, emetics or heart tonics since 1500 BC. Cardenolide glycosides are used in the treatment of congestive heart failure, but their toxicity limits their extensive use. The cytotoxicity and structure characterization of various cardenolides glycosides has been extensively studied. However, only two investigations to study the chemistry and the cardenolide glycosides of *Strophanthus boivinii* have been reported,^{6,7} and no work has been reported under the name *Roupellina boivinii*.

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Liquid-liquid partitioning of the ethanol extract of *R. boivini* yielded an active methanol-soluble fraction with $IC_{50} = 0.61 \,\mu g/mL$ and an active dichloromethane-soluble fraction with $IC_{50} = 3.7 \,\mu g/ml$ in the A2780 bioassay. Both of the active fractions were separately passed through a short reversed phase column with MeOH/H₂O as the mobile phase and further purified by HPLC over a C_{18} column to yield six new compounds designated boivinides A-F (1 – 6) and the four known cardenolide glycosides digitoxigenin 3-*O*-β-D-glucopyrananosyl-(1→4)-α-L-acofriopyranoside, 5-α-corotoxigenin-β-D-boivinoside, 6 17-α-corotoxigenin-β-D-sarmentoside, 6 and uzarigenin 3-*O*-α-L-rhamnoside. 9

Boivinide A (1) was obtained as a white amorphous solid. Positive ion LC-MS gave a molecular ion peak at m/z 749.4 [M+K⁺], consistent with a molecular composition of $C_{36}H_{54}O_{14}$. The 1H NMR spectrum suggested the presence of an aldehyde group at δ_H 10.01 (s, H-19), one methoxy group at δ_H 3.62 (s), and methyl groups at δ_H 0.93 (s, H-18) and δ_H 1.63 (d, H-6', J = 6.0 Hz), suggesting the presence of a deoxy sugar moiety in the compound. Two signals for anomeric protons at δ_H 4.77 (d, H-1', J = 7.2 Hz) and δ_H 5.20 (d, H-1", J = 8.0 Hz) confirmed the presence of two sugar moieties. The ^{13}C NMR spectrum of 1 contained 36 signals, confirming the composition $C_{36}H_{54}O_{14}$. The signals were assigned as one methoxy, two methyls, eleven methylenes, seventeen methines and five quaternary carbons. The 1H and ^{13}C NMR signals in C_5D_5N showed typical signals for an α , β -unsaturated γ -lactone unit, with peaks at δ_C 176.0 (C-20), δ_C 74.0 (C-21) and δ_H 5.03 and 5.27 (br d, J = 18.0 Hz, H-21), δ_C 118.1 (C-22) and δ_H 6.12 (br s, H-22), and δ_C 174.8 (C-23).

A consideration of the NMR spectra of 1 as discussed below and comparison with literature data indicated that the aglycone portion of 1 was that of corotoxigenin. 10 A detailed analysis of its COSY, 1D and 2D TOCSY spectra, and HSQC and HMBC correlations enabled the proton-carbon pairs to be connected to each other and the ${}^{1}J_{\text{CH}}$ correlations to be determined (Table 1). Thus HMBC correlations from H-17 (δ_{H} 2.75, s) to C-12, C-13, C-14, C-15, C-16, C-20, C-21 and C-22, as well as H-22 (δ_H 6.12, s) to C-17, indicated the point of attachment of the lactone ring system to ring D. H-18 (δ_H 0.93, s) exhibited a very strong correlation to C-12 ($\delta_{\rm C}$ 39.5), C-13 ($\delta_{\rm C}$ 49.9), C-14 ($\delta_{\rm C}$ 84.1) and C-17 ($\delta_{\rm C}$ 50.1). These correlations confirmed the C/D ring fusion at C-13 and C-14. The fusion of the A/B rings was confirmed by the correlation of H-19 (δ_H 10.01, s) to C-1 (δ_C 31.7), C-5 (δ_C 43.3), C-9 (δ_C 48.9); H-5 $(\delta_{\rm H}\,1.19,\,{\rm m})$ to C-1, C-3 $(\delta_{\rm C}\,76.9)$, C-4 $(\delta_{\rm C}\,36.4)$, C-6 $(\delta_{\rm C}\,29.0)$, C-7 $(\delta_{\rm C}\,27.4)$, C-9 and C-10 $(\delta_C 55.5)$. Similarly, the B/C ring fusion was confirmed by correlation of H-8 $(\delta_H 1.81)$ to C-6, C-7, C-11(δ_C 22.5), C-13, C-14 and H-9 (1.20, m) to C-1, C-5, C-7, C-8 (δ_C 43.2), C-10, C-11, C-12 and C-19 ($\delta_{\rm C}$ 209.1). The ROESY correlations of H-19 ($\delta_{\rm H}$ 10.01, s) to H_B-1 ($\delta_{\rm H}$ 2.39 m), H_{β} -2 (δ_H 2.20, m), H_{β} -4 (δ_H 2.04, m), H_{β} -11 (δ_H 1.22, m) and H-5 (δ_H 1.19, m) to H_{α} -1 $(\delta_{\rm H}\,0.90,\,{\rm m}),\,{\rm H}$ -3 $(\delta_{\rm H}\,3.97,\,{\rm br}\,{\rm s}),\,{\rm H}_{\alpha}$ -6 $(\delta_{\rm H}\,1.42,\,{\rm m}),\,{\rm H}$ -9 $(\delta_{\rm H}\,1.20,\,{\rm m})$ indicated a trans fusion of the A and B rings. The trans relationship between H-8 and H-9 and the cis fusion of the C/ D rings was established by observing correlations of H-8 (δ_H 1.81) to H-19 (δ_H 10.01, s) and H_{B} -11 (δ_{H} 1.22, m) as well as correlations of H-9 (δ_{H} 1.20, m) to H-5 (δ_{H} 1.19) and H-18 (δ_{H} 0.93, s). The ROESY spectrum indicated crosspeaks from H-17 to H-21, H-22 and H-16 as well as from H-18 to H-21 and H-22, but not to H-17, and so the lactone ring was assigned a β configuration. The ¹H and ¹³C chemical shifts for the sugar units were assigned as in Table 1, and indicated that the sugars had β-linkages. The aglycone portion of the compound was connected using COSY, TOCSY and HMBC spectra. The point of attachment of the sugar moieties to the aglycone portion of the molecule was determined from strong HMBC correlation of H-1' to H-3 (Fig. 1) indicating a 3→1' connectivity. The stereochemistry at this position was confirmed by a ROESY correlation of H-3 to H-1' (Fig. 1). The two sugars were linked from the 4'-position of the first sugar to the 1"-position of the second, and the H-4' and H-1" protons were oriented cis to each other. The chemical shifts of the sugar portion matched the literature values for the sugar portion of a β -D-glucopyranosyl- $(1\rightarrow 4)$ - β -D-digitaloside.

¹¹ The structure of boivinide A was thus established as the new compound corotoxigenin 3-O-[β -D-glucopyranosyl-($1\rightarrow 4$)- β -D-digitalopyranoside] (1). ¹²

Compound 2 was also obtained as a white amorphous solid. LC-MS gave a molecular ion peak at m/z 695.4 [M+H⁺], consistent with the molecular formula $C_{36}H_{54}O_{13}$. The ¹³C NMR spectrum (C₅D₅N, Table 1) showed signals for thirteen oxygenated carbons, and the ¹H NMR spectrum showed signals for an aldehyde proton group at δ_H 10.05 (s, H-19), one methoxy group at δ_H 3.62 (s), one methyl group at δ_H 0.92 (s, H-18) and a methyl doublet at 1.71 (d, H-6'), indicating the presence of a deoxy sugar moiety in the compound. Two anomeric proton signals were seen at δ_H 5.48 (d, H-1') and δ_H 5.04 (d, H-1"). The ¹³C spectrum of compound 2 contained a total of 36 signals: one methoxy, two methyls, twelve methylenes, sixteen methines and five quaternary carbons were consistent with a cardenolide structure. Further analysis of COSY, 1D and 2D TOCSY spectra established that the aglycone portion was the same as that of boivinide A (1). ROESY correlations of H-19 to H_B-1, H_B-2, H_B-4, and H_B-11, and of H-5 to H_{α} -1, H-3, H_{α} -6, and H-9 indicated a trans orientation of H-5 and H-19. HMBC correlations for the sugar portion showed the presence of similar sugar moieties to those in 1, but with the replacement of one oxygenated carbon with a CH₂ group. HMBC, ROESY and 1D TOCSY spectra indicated that the aglycone-linked sugar of 2 was different from that of 1. The assignments of the sugars in 2 were made as follows: $\delta_{\rm C}/\delta_{\rm H}$ 96.3 (C-1')/5.48 (dd, J=9.6 Hz), and δ_C/δ_H 106.6 (C-1")/5.0 (br d, J = 7.6 Hz); δ_C/δ_H 39.7 (C-2')/1.98 and 2.31; δ_C/δ_H δ_{H} 68.3 (C-3')/4.79 (m), δ_{C}/δ_{H} 78.8 (C-3")/3.99 (m); H-4' appeared as a doublet of doublets at $\delta_{\rm H}$ 3.73 (dd, J = 9.2, 2.8 Hz/C-4' $\delta_{\rm C}$ 84.4); H-5" was a multiplet at $\delta_{\rm H}$ 4.26 (C-5" $\delta_{\rm C}$ 78.9); H-6' was a prominent broad doublet at $\delta_{\rm H}$ 1.71 (J = 6.4 Hz, C-6' $\delta_{\rm C}$ 19.3), H-6" was a methylene with δ_H 4.40 and 4.52, and a 3'-methoxy group appeared as a singlet at δ_H 3.62. Both sugars were in the β anomeric form, and these conclusions were confirmed by comparison of the ${}^{1}H$ and ¹³C NMR spectra of 2 with the corresponding spectra of the carbohydrate portion of oleandrigenin- β -D-glucopyranosyl- $(1\rightarrow 4)$ - β -D-sarmentoside. ¹⁰ The structure of boivinide B (2) was thus established as corotoxigenin 3-O- $[\beta$ -D-glucopyranosyl- $(1\rightarrow 4)$ - β -Dsarmentopyranoside].

Compound 3 was also isolated as an amorphous substance, and HRFABMS indicated a molecular composition of $C_{36}H_{56}O_{12}$. Its 1H and ^{13}C NMR spectra showed that this compound also had a cardenolide skeleton. However, a triplet at δ_H 3.17 (t, J = 9.6 Hz) indicated that the lactone at C-17 had the α -orientation. 13 , 14 An additional methyl peak at δ_H 1.03 (s, H-19) and the absence of an aldehyde proton peak in the ^{1}H NMR spectrum confirmed that the aglycone of 3 was 5α , $^{17}\alpha$ -uzarigenin, and this was confirmed by comparison of the ^{13}C NMR spectrum of the aglycone with that of $^{5\alpha}$, $^{17}\alpha$ -uzarigenin. 15 Comparison of chemical shifts of the sugars with those of 2 indicated that 3 had the same sugar moieties as 2, and its structure was thus established as $^{5\alpha}$, $^{17}\alpha$ -uzarigenin 3 - 0 -[5 -D-glucopyranosyl-(1 - 4)- 5 -D-sarmentopyranoside].

Compound **4** was isolated as a white powder, and HRFABMS indicated a molecular formula of $C_{35}H_{52}O_{13}$. Compared to compound **3**, the 1H NMR spectrum of **4** had a signal for the proton of an aldehyde at δ_H 10.01 (s, H-19). 2D NMR studies confirmed that the aglycone was corotoxigenin. 10 The absence of a prominent methoxy singlet and comparison of its $^3J_{HH}$ coupling constants and carbon NMR data to the sugar portion of digitoxigenin-3-O-[β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-boivinopyranoside] 16 indicated that **4** was the new cardenolide glycoside corotoxigenin 3-O-[β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-boivinopyranoside].

Compound **5** had the molecular formula of $C_{35}H_{52}O_{14}$. ¹H NMR and ¹³C NMR spectra revealed that the only difference between compounds **4** and **5** was the absence of the peak for the C-19. aldehyde carbonyl group at δ_C 210.4 and the presence of a signal at δ_C 180.9 for a carboxylic acid carbonyl group. These spectroscopic data combined with its elemental

composition indicated that compound **5** was the new cardenolide glycoside 5-*epi*cannogeninic acid 3-O-[β -D-glucopyranosyl-($1\rightarrow 4$)- β -D-boivinopyranoside].

Compound **6** had the molecular formula $C_{38}H_{56}O_{15}$, and its aglycone portion was the same as that of compounds **1**, **2**, and **4**, as judged by its NMR spectra. The presence of a glucopyranoside unit was indicated by its ^{1}H and ^{13}C NMR spectra, and this was confirmed by 1D TOCSY. However, additional peaks appeared at δ_{C} 170.0 and δ_{C}/δ_{H} 21.4/2.08. HMBC correlations connected the first sugar as follows: δ_{C}/δ_{H} 101.1 (C-1')/4.82 (d, J = 8.4 Hz), δ_{C}/δ_{H} 72.9 (C-2')/5.83, indicating that it was highly deshielded; δ_{C}/δ_{H} 83.7 (C-3')/3.62 (d, J = 2.8 Hz), H-4' appeared as a multiplet at δ_{H} 4.47 (C-4', δ_{C} 75.4); H-6' was a prominent broad doublet at δ_{H} 1.60 (J = 6.0 Hz, C-6' δ_{C} 17.4), and the 3'-methoxy group appeared as a singlet at δ_{H} 3.49. The acetoxy group (δ_{C} 170.0, 21.1, δ_{H} 2.08, s) was attached at position C-2' (δ_{C}/δ_{H} 72.9/5.83). The relative stereochemistry of the first sugar was confirmed by ROESY crosspeaks from H-1' to H-3, H-3' and H-5' and H-4' to H-1". The sugar moiety was thus assigned as 3-*O*-[β-D-glucopyranosyl-(1→4)-2-*O*-acetyl-β-D-digitalopyranoside]. ¹⁷

This extract also yielded the four known cardenolide glycosides digitoxigenin 3-O-[β -D-glucopyrananosyl-($1\rightarrow 4$)- α -L-acofriopyranoside] (S7), corotoxigenin 3-O- β -D-boivinoside (S8), 17 α -corotoxigenin 3-O- β -D-sarmentoside (S9), and uzarigenin 3-O- α -L-rhamnoside (S10). The structures of these compounds were confirmed by comparison of their proton and carbon chemical shifts to the literature values. ^{6,8,9}

All ten compounds were tested for growth inhibition using the A2780 human ovarian cancer cell line. The six new cardenolides exhibited strong antiproliferative activity with $IC_{50} = 0.17$ μM for boivinide A. The results are listed in Table 3. The major factor in determining bioactivity among this group of compounds is the stereochemistry at C-17; compounds 3 and S9 with an α -orientation of the unsaturated lactone ring were an order of magnitude less active than compounds with a β -orientation of this ring.

Experimental Section

General Experimental Procedures

UV spectra were obtained on a Shimadzu UV-1210 series, and IR spectra were measured on a MIDAC M-series FTIR spectrophotometer. NMR spectra were obtained on a Varian INOVA 400 spectrometer at 400 MHz and JOEL Eclipse 500 spectrometer instrument for ^1H NMR spectra and ^{13}C NMR spectra; chemical shifts are given in ppm. High resolution mass spectra were obtained on a JEOL JMS-HX-110 instrument in the positive ion mode, and low resolution spectra were obtained on a Finnigan LTQ LC/MS^n instrument in either the positive or negative ion mode after sample elution with MeOH from a C_{18} column. HPLC was carried out using a Shimadzu LC-10AT instrument with analytical (5 μm , 250 \times 10 mm) and preparative (8 μm , 250 \times 10 mm) C_{18} Varian Dynamax columns coupled with a UV diode array detector.

Antiproliferative Bioassays

Antiproliferative activity against the A2780 human ovarian cancer cell line was determined at Virginia Polytechnic Institute and State University as previously described. ¹⁸ The A2780 cell line is a drug-sensitive human ovarian cancer cell line. ¹⁹

Plant Material

Samples of the roots, bark, wood, and leaves of *Roupellina boivinii* (Baill.) Pichon were collected on May 18, 2004 in the vicinity of the Baie des Dunes Orangéa, Antsiranana, Madagascar, at an elevation of 2 m. The collection coordinates were 12°14′26″S, 49°22′17″E. Collection was made by N. M. Andrianjafy et al., and given collection number ANM 430. The

herbarium specimen was from a tree of 5 m, diameter at breast height 12 cm. It had a white latex and green elongated spindle-shaped fruit. Its vernacular name is Tangeniala. Duplicates of the voucher specimens have been deposited at herbaria of the Centre National d'Application des Recherches Pharmaceutiques, Madagascar (CNARP); the Parc Botanique et Zoologique de Tsimbazaza, Madagascar (TAN); at the Missouri Botanical Garden, St. Louis, Missouri (MO); and at the Muséum National d'Histoires Naturelles, Paris, France (P).

Extraction and Isolation

The dried root (350 g), bark (350 g) and wood (369 g) of Strophanthus boivinii were separately ground and extracted with EtOH; the resulting extracts were designated MG2309 (14.1 g) MG2310 (10.9 g), and MG2311 (7.0 g) respectively, of which 3.3 g, 2.2 g, and 1.9 g respectively were made available for this work. The combined EtOH extracts of the above three parts of the plant, designated MG2309PE (3.0 g) was suspended in aqueous MeOH (MeOH:H₂O, 9:1, 100 mL) and extracted with hexanes (3×100 mL). The aqueous layer was then diluted to 50% MeOH (v/v) and extracted with CH₂Cl₂ (3× 180 mL). The aqueous MeOH fraction displayed the highest activity (IC₅₀ = 0.61 μ g/mL) followed by the CH₂Cl₂-soluble fraction with IC₅₀ = 3.7 µg/mL. About 634 mg of the MeOH fraction was partitioned between BuOH and H₂O which yielded 232 mg of an active BuOH fraction with IC₅₀ = 4.2 μ g/mL. The BuOH fraction was filtered through a short C₁₈ reversed phase column with MeOH/H₂O (6/4) as the mobile phase. The resulting filtrate was further separated using C_{18} Varian Dynamax column [8 μ m, 250×10 mm, 10 mL/min] with MeOH/H₂O (6/4) as the mobile phase to yield two new compounds, 1.5 mg of boivinide D (4) and 3.0 mg of boivinide E (5). The remaining MeOH fraction was chromatographed over a C₁₈ open column, eluting with 30% MeOH:H₂O to 100% MeOH, to yield five fractions. Only fractions III and IV were active, with IC_{50} 0.13 and 0.48 $\mu\text{g/mL}$ respectively. Fraction III was loaded on a C_{18} Varian Dynamax column [5 $\mu\text{m},\,250\,\times$ 10 mm, 1.8 mL/min, isocratic elution with 40% MeOH: H₂O (0.1% formic acid) for 40 min followed by 50% MeOH:H₂O (0.1% formic acid) for 50 min and twenty-five subfractions were collected. Fractions 16 and 18 were pure and new compounds and were named as boivinide B (2) (4.5 mg, t_R 59 min) and boivinide A (1) (5.9 mg, t_R 62 min). Fraction IV was loaded on a C₁₈ Varian Dynamax column [8 μm, 250 × 10 mm, 10 mL/min, isocratic elution with 50% MeOH:H₂O (0.05% formic acid) for 90 min]. Two pure subfractions were collected; one was a new compound, boivinide F (6) (3.5 mg, t_R 39 min) and the other was the known compound digitoxigenin 3-O-[β -D-glucopyrananosyl-($1\rightarrow 4$)- α -L-acofriopyranoside] (S7) (4.9 mg, t_R 49 min). The CH₂Cl₂ fraction was filtered through a short C₁₈ column with elution by 60% MeOH:H₂O and the resulting filtrate was further purified by C₁₈ HPLC using 60% MeOH: H₂O to yield one new compound, boivinide C (3) (15.0 mg) and the three known compounds **S8-S10**.6,8,9

Boivinide A (1)—white amorphous solid; UV (MeOH) λ_{max} (log ε) 217 nm; IR ν_{max} 3409, 2870, 1743, 1616, 1068, 884 cm⁻¹; ¹H NMR (400 MHz, C₅H₅N) in ppm: 0.93 (s, 3H, H-18), 1.63 (d, 3H, J=6.0 Hz, H-6'), 2.75 (m, H-17), 4.25 (m, 1H, H-4'), 4.38 (m, 1H, H_α- 6"), 3.97 (m, 1H, H-3), 4.59 (m, 1H, H_β- 6"), 4.77 (d, 1H, J=7.2 Hz, H-1'), 5.03, 5.27 (d, each, 1H, J=18.0 Hz, H-21), 5.20 (d, 1H, J=8.0 Hz, H-1"), 6.12 (br s, 1H, H-22), 10.01 (s, 1H, H-19); ¹³C NMR (100 MHz, C₅D₅N) see Table 1; LC-MS m/z 749.4 [M+K]⁺ (calcd for C₃₆H₅₄O₁₄K⁺, 749.3)

Boivinide B (2)—white amorphous solid; UV (MeOH) λ_{max} (log ε) 215 nm; IR ν_{max} 3400, 2873, 1739, 1616, 1162, 1071, 1024, 679 cm⁻¹; ¹H NMR (400 MHz, C₅H₅N) in ppm: 0.92 (s, 3H, H-18), 1.71 (d, 3H, J = 6.4 Hz, H-6'), 2.72 (m, 1H, H-17), 3.73 (dd, 1H, J = 2.8, 9.2 Hz, H-4'), 3.92 (m, 1H, H-3), 4.44 (m, 1H, H_α-6"), 4.52 (m, 1H, H_β-6"), 5.04 (d, 1H, J = 7.6 Hz, H-1"), 5.00, 5.04 (d, each, 1H, J = 4.0 Hz, H-21), 5.48 (d, 1H, J = 9.6 Hz, H-1'), 6.12 (br s,

1H, H-22), 10.05 (s, 1H, H-19); 13 C NMR (100 MHz, C_5D_5N), see Table 1; LC-MS m/z 695.4 [M+H] $^-$ (calcd for $C_{36}H_{55}O_{13}^-$, 695.4)

Boivinide C (3)—white amorphous solid; 1 H NMR (500 MHz, CD₃OD) in ppm: 0.89 (s, 3H, H-18), 1.26 (d, 3H, J = 6.6 Hz, H-6'), 3.18 (dd, 1H, J = 5.2, 9.1 Hz, H-17), 3.19 (br d, 1H, J = 3.4 Hz, H-4'), 3.66 (dd, 1H, J = 5.5, 12.0 Hz, H_α- 6"), 4.03 (m, 1H, H-3), 3.86 (dd, 1H, J = 2.0, 12.0, H_β- 6"), 4.29 (d, 1H, J = 8.0 Hz, H-1"), 4.85 (dd, 1H, J = 2.0, 10 Hz, H-1'), 4.92, 4.96 (dd, each, 1H, J = 2.0, 18.5 Hz, H-21), 5.88 (br s, 1H, H-22), 9.98 (s, 1H, H-19); 13 C NMR (125 MHz, CD₃OD), see Table 2; HRFABMS m/z 703.3665 [M+Na]⁺ (calcd for C₃₆H₅₆O₁₂Na⁺, 703.3664).

Boivinide D (4)—white amorphous powder; 1 H NMR (500 MHz, CD₃OD):δ0.81 (s, 3H, H-18), 1.24 (d, 3H, J = 6.0 Hz), 2.18 (dd, 1H, J = 5.2, 9.1 Hz, H-17), 3.19 (br d, 1H, J = 3.4 Hz, H-4′), 3.66 (dd, 1H, J = 5.5, 12.0 Hz, H_α- 6″), 4.03 (m, 1H, H-3), 3.87 (dd, 1H, J = 2.0, 12.0, H_β- 6″), 4.29 (d, 1H, J = 8 Hz, H-1″), 4.87 (dd, 1H, J = 2.0, 10 Hz, H-1′), 4.92, 5.02 (dd, each, 1H, J = 2.0, 18.5 Hz, H-21), 5.88 (br s, 1H, H-22), 13 C NMR (125 MHz, CD₃OD), see Table 2; HRFABMS m/z 703.3327 [M+Na]⁺ (calcd for C₃₅H₅₂NaO₁₃⁺, 703.3301)

Boivinide E (5)—white amorphous powder; ¹H NMR (500 MHz, CD₃OD): δ0.81 (s, 3H, H-18), 1.24 (d, 3H, J = 6.0 Hz), 2.18 (dd, 1H, J = 5.2, 9.1 Hz, H-17), 3.19 (br d, 1H, J = 3.4 Hz, H-4′), 3.66 (dd, 1H, J = 5.5, 12.0 Hz, H_α- 6″), 4.03 (m, 1H, H-3), 3.87 (dd, 1H, J= 2.0, 12.0, H_β- 6″), 4.29 (d, 1H, J = 8 Hz, H-1″), 4.87 (dd, 1H, J = 2.0, 10 Hz, H-1′), 4.92, 5.02 (dd, each, 1H, J = 2.0, 18.5 Hz, H-21), 5.88 (br s, 1H, H-22), ¹³C NMR (125 MHz, CD₃OD), see Table 2; HRFABMS m/z 719.3251 [M+Na]⁺ (calcd for C₃₅H₅₂NaO₁₄⁺, 719.3250)

Boivinide F (6)—yellow amorphous solid; UV (MeOH) λ_{max} (log ε) 216 nm; IR ν_{max} 3450, 2871, 1747, 1709, 1372, 1236, 1069, 985, 659 cm⁻¹; ¹H NMR (400 MHz, C₅H₅N) in ppm: 0.91 (s, 3H, H-18), 1.60 (d, 3H, J=6.0 Hz, H-6'), 2.75 (m, 1H, H-17), 3.90 (m, 1H, H-3), 4.38 (m, 1H, H_α- 6"), 4.46 (d, 1H, J=2.0 Hz, H-4'), 4.6 (m, 1H, H_β- 6"), 4.82 (d, 1H, J=8.4 Hz, H-1'), 5.01 (d, 1H, J=18.4 Hz, H-21), 5.04 (d, 1H, J=18.0 Hz, H-21), 5.16 (d, 1H, J=7.6 Hz, H-1"), 6.14 (br s, 1H, H-22), 10.04 (s, 1H, H-19); ¹³C NMR (100 MHz, C₅D₅N), see Table 1; HRFABMS m/z 775.3570 [M+Na]⁺ (calcd for C₃₈H₅₆O₁₅Na, 775.3512).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Key HMBC and ROESY correlations of compound **1**

2

4 R = CHO **5** R = COOH

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 1 Ha NMR and 13 Cb NMR Data of Compounds 1, 2 and 3.

	2.		20		pc	
position	1 H (J, Hz)	13 C	¹ H (J, Hz)	13 C	¹ H (J, Hz)	13 C
	0.9 m. 2.39 m	31.7	0.83 m. 2.4 m	31.8		38.3
7	1.56 m, 2.20 m	31.3	1.60 m, 2.38 m	31.6		31.8
3	3.97 br s	76.9	3.92 br s	76.2	4.03 m	73.3
4	2.04 m, 1.42 m	36.5	1.35 m, 1.86 m	36.7		35.6
ς.	1.19 m	43.3	1.2 m	43.4	1.18 m	45.6
9	1.89 m, 1.42 m	29	1.94 m, 1.40 m	29.2		28.8
7	2.48 m, 1.24 m	27.4	2.35 m, 1.26 m	28.3	1.05 m	29.9
8	1.81 m	43.2	1.8 m	43.3		42.2
6	1.2 m	48.9	2.75 m	51.6		49.1
10		51.5		49		36.9
11	1.64 m, 1.22 m	22.5	1.62 m	22.6		21.4
12	1.23 m, 1.34 m	39.5	1.98 m, 2.31 m	39.7	2.02 m	30.5
13		49.9		50.2		51.2
14		84.1		84.6		87.0
15	1.98 m, 1.81 m	32.8	1.82 m, 1.98 m	32.9		31.7
16	2.06 m, 1.96 m	28.2	2.10 m, 1.98 m	27.6		25.3
17	2.75 m	50.1	2.72 m	52.2	3.17 d (9.6)	49.7
18	$0.93 \mathrm{s}$	16.2	$0.92 \mathrm{s}$	16.4	0.89 s	18.9
19	10.01 s	209.1	10.05 s	209.3	1.03 s	12.6
207	0000	1/0	0000	1/0.7		1/0.8
21	5.03 d, 5.27 d(18.0)	4/	5.00 d (1.6), 5.04 d (4.0)	74.1	4.82, 4.96 dd (2.0, 18.5)	75.7
27	6.12 s	118.1	6.12 s	118.2	5.88 br s	116.9
) ' -	4 77 d (7 2)	10.5	5 48 4 (9.6)	96.4	4 85 dd (2 0 10 0)	97.6
2,	4.47 m	71.6	1.98 m. 2.31 m	39.7	(5:0) 22 (5:0)	32.2
3,	3.58 dd (10.0, 12.4)	85.7	4.79	68.3	4.26	76.5
4,	4.25 ^e	76.4	3.73 dd (9.2, 2.8)	84.4	3.19 br d (3.4)	73.4
5,	3.77 d (6.8)	70.8	4.44	69.4	3.91 ^e	70.3
,9	1.63 d (6.0)	18.1	1.71 d (6.4)	19.3	1.26 d (6.6)	17.2
OMe	3.62 s	59.3	3.62's	50.1	3.24 s	57.2
1"	5.20 d (8.0)	105.7	5.04 d (7.6)	106.6	4.29 d (8.0)	102.2
2"	3.96^{e}	76.5	3.97 ^e	75.6		74.9
3"	4.23 ^e	78.6	3.99^{e}	78.8		78.4
4"	4.18^{e}	72.2	4.26^{e}	71.9		71.9
5"	3.95 ^e	62	4.26^{e}	78.9		78.0
.9	4.38 m, 4.59 m	63.4	4.4 m, 4.52 m	63	3.66 dd (5.5,12.0),	0 23
					3.80 dd (2.0, 12.0)	0.50

 $^{^{}a}\delta$ (ppm) 500 MHz; s: singlet; br s: broad singlet; d doublet; m: multiplet;

 $^{^{}b}\delta$ (ppm) 125 MHz;

 $^{^{}c}$ in C5D5N;

 $d_{
m in}$ MeOH-d4;

 $^{^{\}it e}$ overlapped and unresolved signals, values obtained from HSQC and 1D TOCSY experiment

NIH-PA Author Manuscript Table 2

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 $^{1}\mathrm{H}^{a}$ and $^{13}\mathrm{C}^{b}$ NMR Data of Compounds 4, 5 and 6 NIH-PA Author Manuscript

	P		7		٠	
position	1 H (J, Hz)	13 ^C	1 H (J, Hz)	13 ^C	$\mathbf{H}^{1}(J,Hz)$	13°C
1	2.42 m	37.0	2.42 m	37.8	2.41 m, 0.98 m	31.8
2		29.6	,	30.8	1.98 m, 1.45 m	$\frac{31.5}{2}$
w <i>z</i>	4.03 m	77.7	4.03 m	77.8 36.8	3.90 m	77.9
t v	1.30 **	32.1	1 3.7 33	30.0	2.03 III, 1.30 III 1 24 m	30.0 42.4
2	11.00.11	286	1.32 III	28.0	1.24 III 1.30 m. 1.30 m.	45.4 4.86
		31.6		33.2	1.83 m. 1.83 m	29.2
. ∞	1.35 m	43.8	1.30 m	42.6	1.22 m	43.4
6	2.72 m	49.6	2.72 m	49.6	2.70 m	49.1
10		52.8		53.4		52.1
11		22.9		24.6	1.63 m, 1.33 m	22.6
12		40.4		41.3	1.21 m, 1.34 m	39.7
13		50.7		50.7		50.3
† ·		0.00		0.00	69 - 00	0.4°C
5.		32.8		33.1	1.99 m, 1.82 m	32.9
16		27.9		28.6	1.97 m, 2.15 m	27.6
17	2.81 dd (5.2, 9.1)	51.9	2.81 dd (5.2, 9.1)	51.2	2.75 m	51.7
18	0.81 s	16.1	0.81 s	16.6	0.94 s	16.4
19 20	10.01 s	210.4		180.9	10.04 s	209.3
2.5	4.92. 5.02 dd (2.0. 18.5)	75.3	4.92 dd. 5.02 dd (2.0.	75.9	5.01 d (18.0), 5.29 d (18.4)	75.9
i			18.5)			
22	5.88 br s	117.9	5.88 br s	117.9	6.14 s	118.3
23		177.2		177.2		174.9
1,	4.87 dd (2.0, 10.0)	97.5	4.87 dd (2.0, 10.0)	97.1	4.82 d (8.4)	100.7
2,		35.0		35.0	5.83 m	72.4
3,		66.5		66.4	3.64 d (2.8)	83.4
,4	3.19 br d (3.4)	75.9	3.19 d (3.4)	75.9	4.46 d (2.0)	75.1
5,		70.1		70.0	3.77 d (6.4)	71.2
,9	1.24 d (6.6)	17.2	1.24 d (6.6)	17.2	1.6 d (6.0)	18.0
OMe					3.49 s	58.5
000						170.0
;	6			4	2.08 s	21.5
J",	4.29 d (8.0)	102.4	4.29 d (8.0)	102.3	5.16 d (7.6)	105.5
1	4.5I ⁻	6.4.	4.51	7.4.7	4.02	t:0/
3"		77.9		78.1	4.26^{e}	78.7
4"		71.8		71.9	4.19^{e}	72.2
5"	3.43^{e}	78.1	3.45 ^e	78.5	3.98^{e}	79.2
9,,	3.87 dd (5.5, 12.0),	63.0	3.87 dd (5.5, 12.0),			
	3.67 dd (2.0, 12.0)		3.67 dd (2.0, 12.0)	63.0	4.38 m, 4.6 m	63.0

 $^{^{}a}\delta$ (ppm) 500 MHz; s: singlet; br s: broad singlet; d doublet; m: multiplet;

 $^{b}_{\delta}$ (ppm) 125 MHz;

 $c_{\rm in}$ C5D5N;

 d_{in} CD30D;

 $^{\varrho}$ overlapped peaks and unresolved signals, values obtained from HSQC and 1D TOCSY experiment.

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 Table 3

 Antiproliferative activity of compounds 1–10 against the A2780 human ovarian cancer cell line.

Compound	IC_{50} (μM)
1	0.17
2	0.66
3	2.9
4	0.29
5	0.28
6	0.54
S7	0.15
S8	0.15
S9	3.7
S10	0.15