Tedanolide C: a potent new 18-membered ring cytotoxic macrolide isolated from the Papua New Guinea marine sponge *Ircinia* sp.

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Figure S1. 400 MHz ¹H NMR spectrum of Tedanolide C (**1**).

Figure S2. 400 MHz DQF-COSY spectrum of Tedanolide C (1).

Figure S4. 400 MHz GHSQC spectrum of Tedanolide C (**1**).

Figure S5. 400 MHz GHMBC spectrum of Tedanolide C (**1**).

Figure S6. 500 MHz GHETLOC spectrum of Tedanolide C (1).

Figure S7. 500 MHz G-BIRD_R-HSQMBC spectrum of Tedanolide C (1).

General Experimental Procedures. UV spectra were obtained in MeOH on a diode array spectrophotometer. IR spectra were recorded on a FTIR spectrophotometer. Optical rotations were measured on a digital polarimeter. NMR spectra were acquired in CD₃OD, CDCl₃ and CD₃COCOD₃. An instrument (400 and 500 MHz for ¹H and 100 and 125 MHz for ¹³C NMR) with a 3 mm probe was used. High resolution mass spectra were obtained using a FTICR mass spectrometer equipped with an actively shielded 9.4 Tesla superconducting magnet, an external ESI source, and a 50W CO₂ CW laser. All calculations have been carried out in our investigation using the Gaussian03 programming suite and computational resources of the University of Utah at the Center for High Performance Computing (www.chpc.utah.edu).

Mass spectrometry. The sodiated molecular ion of m/z 621 was isolated using correlated sweep and then dissociated using a Sustained Off-Resonance Irradiation with Collision Induced Dissociation (SORI-CID).¹⁹ With ultra-high resolution achieved routinely by FTMS, the fragment ion peaks having the same nominal mass (m/z 293) have been resolved to the baseline. Based on ppm mass measurement accuracy (see Table S1), all the fragment ions detected in the SORI-CID experiment were assigned with unique elemental compositions allowing us to rule out a number of alternative fragmentation pathways for some fragment ions. However, there is still more than one fragmentation scheme corresponding to one single elemental composition. Further stage tandem mass spectrometry FTMS³ was, therefore, performed on the major fragment ions to deduce the plausible fragmentation pathways. An FTMS SORI-CID experiment on the deuterated molecular ion (with six deuterium atoms) was also conducted in deuterium solvents to narrow the alternative fragmentation pathways based on the number of deuteriums retained on the fragment ions. Figure S8 illustrated the proposed fragmentation pathways for some major fragments based on all the data obtained. The main fragment ions detected in the FTMS SORI-CID experiments were those arising from the double cleavage of the macrolide ring.

It was not possible to deduce the exact structures of a few fragments as the fragment peak detected might represent a mixture of isomeric fragments. One example is the fragment ion with m/z 351, which could result from another alternative scheme (not shown) due to the symmetry of the molecular structure. Nevertheless, we have tried to explain fragmentations using mechanisms that are known to be commonly observed for low energy dissociation of ions produced by ESI, and the fragmentation pathways given in figure S8 are the best and consistent representation we can provide and can be most reasonably postulated to occur.

Figure S8. Fragmentation pathway for the $[M+Na]^+$ ion observed in the ESI FTMS SORI-CID mass spectrum of tetranolide C (**1**).

¹⁹ McDonald, L.A.; Barbieri, L.R.; Carter, G.T.; Kruppa, G.; Feng, X.; Lotvin, J.A.; Siegel, M.M. *Anal. Chem.* **2003**, 75, 2730-2739

Table S1. Proposed analysis of FTMS2 of m/z 621 $[M+Na]^+$ for tetanolide C (**1**).

Experimental Mass	Theoretical Mass	Δ (mDa)	Δ (ppm)	Intensity	Relative Intensity (%)	Elemental Formula	Assignment
621.32423	621.32508	-0.85	-1.37	0.32	32	$C_{31}H_{50}O_{11}Na^{1+}$	$[M+Na]^{1+}$
551.31887	551.3196	-0.73	-1.32	0.05	5	$C_{28}H_{48}O_9Na^{1+}$	see figure S8
521.23548	521.23627	-0.79	-1.52	1.00	100	$C_{28}H_{38}O_{10}Na^{1+}$	see figure S8
481.24036	481.2408	-0.44	-0.91	0.11	11	$C_{23}H_{38}O_9Na^{1+}$	see figure S8
451.23001	451.23024	-0.23	-0.51	0.36	36	$C_{22}H_{36}O_8Na^{1+}$	see figure S8
433.21967	433.21967	0.00	0	0.17	17	$C_{22}H_{34}O_7Na^{1+}$	see figure S8
393.22471	393.22476	-0.05	-0.13	0.18	18	$C_{20}H_{34}O_6Na^{1+}$	$[M+Na]^{1+}-C_{11}H_{16}O_5$
381.18826	381.18837	-0.11	-0.29	0.09	9	$C_{18}H_{30}O_7Na^{1+}$	$[M+Na]^{1+}-C_{13}H_{20}O_4$
379.13622	379.13634	-0.12	-0.32	0.04	4	$C_{17}H_{24}O_8Na^{1+}$	$[M+Na]^{1+}-C_{14}H_{26}O_3$
351.1413	351.14142	-0.12	-0.34	0.47	47	$C_{16}H_{24}O_7Na^{1+}$	see figure S8
309.13086	309.13086	0.00	0	0.07	7	$C_{14}H_{22}O_6Na^{1+}$	$[M+Na]^{1+}-C_{17}H_{28}O_5$
293.17163	293.17233	-0.70	-2.39	0.10	10	$C_{15}H_{26}O_4Na^{1+}$	$[M+Na]^{1+}-C_{16}H_{24}O_7$
293.13592	293.13594	-0.02	-0.07	0.78	78	$C_{14}H_{22}O_5Na^{1+}$	$[M+Na]^{1+}-C_{17}H_{28}O_6$
275.12552	275.12538	0.14	0.51	0.04	4	$C_{14}H_{20}O_4Na^{1+}$	$[M+Na]^{1+}-C_{17}H_{30}O_7$
263.12542	263.12538	0.04	0.15	0.14	14	$C_{13}H_{20}O_4Na^{1+}$	see figure S8
193.08364	193.08351	0.13	0.67	0.20	20	$C_9H_{14}O_3Na^{1+}$	see figure S8
163.07318	163.07295	0.23	1.41	0.02	2	$C_8H_{12}O_2Na^{1+}$	see figure S8

Biological assay descriptions

Cell lines and Cell Culture. All cell cultures were grown at 37 °C, 5% CO₂ and maximal humidity (by equilibration) and cultured according to standard protocols.²⁰ The HCT 116 strains were donated by Dr. Bert Vogelstein (Johns Hopkins University) and grown in McCoy's 5A media supplemented with 10% v/v heat-inactivated fetal bovine serum (FBS), 1% v/v MEM (Minimal Essential Media) sodium pyruvate, 1% v/v penicillin-streptomycin and 1% v/v L-glutamine. Cells were counted using a Beckman Coulter counter.

Cell Proliferation Assay. The ability of tedanolid C (**1**) to inhibit cellular growth was determined using an MTT assay.²¹ For the HCT 116 cell lines, 4000 cells per well were aliquoted in 96-well plates in 200 μ L of media and allowed to adhere overnight. Serial dilutions of drug were then added to wells in quadruplicate. Control wells were treated with vehicle (DMSO). After 48 h of incubation at 37 °C and 5% CO₂, the media was removed and 90 μ L fresh media was added along with 10 μ L of 2.5 mg/mL aqueous 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT). The cells were then incubated for 4 h as above. The media was then removed and 100 μ L DMSO added to each well. The amount of formazan resulting from MTT metabolism was measured by observing absorbance at 540 nm using a Multiskan plate reader. Using the absorbance in the vehicle treated lanes as 100% survival (1.00 fractional survival) the fractional survival of each treatment was calculated. The amount of compound needed to cause a 50% inhibition in growth (IC₅₀) was determined using the GraphPad Prism.²²

Flow Cytometry. In each experiment, 5 $\times$ 10⁵ HCT-116 cells in 5 mL of media were placed on aliquoted 25 cm² flasks and allowed to adhere overnight. Each flask was then treated with compound **1** (0.1 and 0.2 μ g/mL) or vehicle (DMSO) and incubated for either 24 or 48 h. Media were collected and the cells washed with 2 mL versene. The versene wash was pooled with the corresponding media and the cells incubated at room temperature for 5 min with 1 mL trypsin-EDTA. Trypsinization was quenched with 4 mL of media and the media-trypsin was pooled with the corresponding media-versene in 15 mL centrifuge tubes. Cells were pelleted by centrifugation at 305g for 5 min. The supernatant was gently removed and the cell pellets were resuspended in cold PBS to a final concentration of 1-2 $\times$ 10⁶ cells per mL. Ice-cold methanol was then slowly added dropwise to a volume of 2X the volume of PBS with gentle vortexing. The fixed cells were stored at 4°C for 24 h and 48 h. The cells were then submitted to the University of Utah Flow Cytometry Resource Facility for propidium iodine staining and FACS analysis.

²⁰ ATCC, 2002. American Type Culture Collection <http://www.atcc.org>.

²¹ Carmichael, J.; DeGraff, W.; Gazdar, A.; Minna, J.; Mitchell, J. *Cancer Res.* **1987**, *47*, 936-942.

²² *GraphPad Prism 3.0*; GraphPad Software: San Diego, 2002.

Figure S9. Lowest energy three-dimensional conformer of Tedanolide C (**1**) as determined from molecular dynamics using AMBER.

Figure S10. Chemical structures of Tedanolide C (**1**), Tedanolide (**2**) and 13-deoxytedanolide (**3**)

Figure S11. The effect of Tedanolide C (**1**) on cell cycle progression in HCT-116 cells as compared to vehicle. The treatments are as follows : (a) control, 24 h ; (b) 0.2 μ g/mL (**1**), 24 h ; (c) control, 48 h; d) 0.2 μ g/mL (**1**), 48 h.