Published in final edited form as:

J Nat Prod. 2016 March 25; 79(3): 598–606. doi:10.1021/acs.jnatprod.5b01131.

Antileishmanial and Cytotoxic Activity of Some Highly Oxidized Abietane Diterpenoids from the Bald Cypress, *Taxodium distichum*

C. Benjamin Naman^{†,§}, Anthony D. Gromovsky^{†,⊥}, Cory M. Vela^{†,||}, Joshua N. Fletcher^{†,▽}, Gaurav Gupta^{‡,○}, Sanjay Varikuti[‡], Xiaohua Zhu[†], Emilia M. Zywo[†], Heebyung Chai[†], Karl A. Werbovetz[†], Abhay R. Satoskar[‡], and A. Douglas Kinghorn^{*,†}

[†]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, Ohio 43210, United States

[‡]Department of Pathology, College of Medicine, The Ohio State University, 320 West 10th Avenue, Columbus, Ohio 43210, United States

Abstract

Two new compounds, including a *para*-benzoquinone ring-containing abietane (**1**), a *para*-benzoquinone ring-containing 7,8-*seco*-abietane (**2**), and 14 other known highly oxidized abietane diterpenoids (**3–16**), were isolated from an extract prepared from the cones of *Taxodium distichum*, collected in central Ohio. The active subfraction from which all compounds isolated in this study were purified was tested in vivo using *Leishmania donovani*-infected mice, and was found to dose-dependently reduce the parasite burden in the murine livers after iv administration of this crude mixture at 5.6 and 11.1 mg/kg. The structures of **1** and **2** were established by detailed 1D- and 2D-NMR experiments, HRESIMS data, and electronic circular dichroism studies. Compounds **3** and **4** were each fully characterized spectroscopically and also isolated from a natural source for the first time. Compounds **2–16** were tested in vitro against *L. donovani* promastigotes and *L. amazonensis* intracellular amastigotes. Compound **2** was the most active against *L. amazonensis* amastigotes (IC₅₀ = 1.4 μM), and **10** was the most potent against *L. donovani* promastigotes (IC₅₀ = 1.6 μM). These compounds may be suggested for further studies such as in vivo experimentation either alone or in combination with other *Taxodium* isolates.

*Corresponding Author. Tel: 1-614-247-8094. Fax: 1-614-247-8119. kinghorn.4@osu.edu..

[§]Present address: Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, San Diego, California 92037, United States

[⊥]Present address: Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio 44195, United States

^{||}Present address: Department of Pharmacy, Moffitt Cancer Center, 12902 USF Magnolia Drive Tampa, FL 33612, United States

[▽]Present address: Tate & Lyle, Hoffman Estates, IL 60192, United States

[○]Present address: Department of Biochemistry and Immunology, Ribeirão Preto Medical School, University of São Paulo, 14049-900 Ribeirão Preto, São Paulo, Brazil

Dedicated to Professors John Blunt and Murray Munro, of the University of Canterbury, for their pioneering work on bioactive marine natural products

Supporting Information. UV, IR, ¹H NMR, ¹³C NMR, ¹³C DEPT 135, ¹H-¹H COSY, HSQC, HMBC, and NOESY spectra for compounds **1–4**, along with characterization data for compounds **5–16**, ECD spectra for compounds **1** and **2**, and molecular model coordinates for **2** are available free of charge via the Internet at <http://pubs.acs.org>.

Notes. The authors declare no competing financial interest.

Abstract

The bald cypress, *Taxodium distichum* L. Rich. (Cupressaceae), is a widely distributed deciduous conifer that grows abundantly in the Southeastern United States, but has been introduced elsewhere ornamentally since at least 1640.¹⁻³ There exists some taxonomic confusion about the genus *Taxodium* in general, with the taxa *T. distichum* (bald cypress), *T. imbricatum* (pond cypress), and *T. mucronatum* (Montezuma or Mexican bald cypress) being regarded as combinations of one, two, or three species in the literature, and having been the subject of debate for centuries.²⁻⁴ However, as the earlier outcome of research on the morphology and some more recent studies on the DNA sequences from these taxa have suggested, these are regarded herein as a single organism, *T. distichum*, at the species rank.^{3,5}

Varieties of *T. distichum* have been used historically and for a multitude of purposes ranging from construction material to traditional medicine.^{6,7} The Aztec and other Mexican cultures used its tree resin medicinally for wound cleaning, ulcers, toothache, gout, and cutaneous diseases.^{7,8} In the Middle Ages, Islamic scholars documented in Arabic vast amounts of European culture and knowledge, including medicine, which ultimately allowed its preservation beyond the Dark Ages.^{9,10} A much more recent re-translation to English of subsets from this literature has noted the use of the leaves and seeds of *Taxodium* to treat malaria and liver disease.¹⁰ Several earlier phytochemical investigations of *T. distichum* have been reported, but were recently reviewed comprehensively by other authors and will not be presented here.⁶ It is noteworthy, however, that no prior report on this plant has described laboratory studies for the potential therapy of leishmaniasis or any other parasitic disease.

Leishmaniasis is the clinical manifestation of infection by parasites in the genus *Leishmania*, and is designated by the World Health Organization (WHO) as a neglected tropical disease.¹¹ Leishmaniasis can be classified as visceral, cutaneous, or mucocutaneous, based on its clinical characteristics and the particular species of infective parasite. Visceral leishmaniasis, caused most typically by *L. donovani* or *L. infantum*, is estimated to account for 0.2 to 0.4 million new infections and 20,000 to 40,000 deaths worldwide each year.¹²⁻¹⁴ The existing therapeutic options for treating leishmaniasis have several drawbacks, including increasingly drug-resistant parasitic infections, and the high cost, long duration of treatment,

inconvenient dosage delivery methods, and toxicity.¹⁵ Furthermore, large proportions of the affected populations lack access to healthcare options that would allow, for example, a daily injectable treatment.¹⁶ Thus, there is a great need for affordable, non-toxic, and orally available new antileishmanial drugs.

In a continuing effort to discover potential new drugs from higher plants for the therapy of leishmaniasis, the screening of a chloroform partition of *T. distichum* cone extract demonstrated in vitro antileishmanial activity of $IC_{50} = 2.1 \mu\text{g/mL}$ against *L. donovani* promastigotes, one morphological form of the parasite. Bioactivity-guided isolation using the *L. donovani* promastigote test as a gatekeeper was thus conducted of this leishmanicidal chloroform partition, and was performed by repeated column chromatography with silica gel, preparative TLC, and RP-C₁₈ HPLC. This led to the purification of a new abietane quinone (**1**), a new 7,8-*seco*-abietane (**2**), and 14 other known highly oxidized abietane diterpenoids (**3-16**), of which compounds **3** and **4** are here reported for the first time as natural products. The structures of all new isolated compounds were established by the interpretation of their spectroscopic and spectrometric properties, and the known compounds were identified by comparison of their experimental analytical data to published values. Thus, compounds **5-16** were identified as the known natural products microstegiol (**5**),¹⁷ taxoquinone (**6**),^{18,19} royleanone (**7**),^{20,21} horminone (**8**),²² 7-*O*-methylhorminone (**9**),²³ taxodione (**10**),^{18,19} 12-hydroxy-6,7-*seco*-abieta-8,11,13-triene-6,7-dial (**11**),²⁴ ferruginol (**12**),^{25,26} sugiol (**13**),^{27,28} 6 α -hydroxysugiol (**14**),²⁹ 6-hydroxy-5,6-dehydrosugiol (**15**),³⁰ and 14-deoxycoleon U (**16**),³¹ while compound **3** has only been described earlier as a synthetic material,³²⁻³⁴ and **4** was only reported previously as an intermediate product in the total synthesis of compound **5**.³⁵

RESULTS AND DISCUSSION

Compound **1** was determined to have the molecular formula C₂₁H₃₀O₄ based on the sodiated molecular ion peak at m/z 369.2031 [$M + Na$]⁺ (calcd for C₂₁H₃₀O₄Na, 369.2036) in the HRESIMS. The ¹H NMR spectrum exhibited several signals characteristic of a deshielded isopropyl group [δ_H 3.15 (1H, sept., $J = 7.1$ Hz, H-15), 1.23 (3H, d, $J = 7.1$ Hz, CH₃-16), and 1.20 (3H, d, $J = 7.1$ Hz, CH₃-17)], which is typical for an abietane diterpenoid with an oxidized C-ring.^{36,37} The ¹³C NMR spectrum suggested the occurrence of a tetra-substituted *para*-benzoquinone moiety [δ_C 187.4 (C-14), 184.0 (C-11), 150.4 (C-12), 148.7 (C-9), 145.4 (C-8), and 125.0 (C-13)], and was similar to that of the known natural product, taxoquinone (**6**), which was also isolated in this study. The molecular formula of **1** suggested that it contains one more methylene group when compared to **6**. The primary difference in the NMR spectra of these compounds was the methoxy group observed in **1** [δ_H 3.49 (3H, s, CH₃-7-OMe); δ_C 58.1 (C-7-OMe)], which could be assigned to C-7 because of a correlation observed in the ¹H-¹³C HMBC spectrum to C-7. Additional correlations observed in the ¹H-¹³C HSQC, ¹H-¹³C HMBC, ¹H-¹H COSY, and ¹H-¹H NOESY spectra allowed for the complete structural determination and assignment for this compound, as shown by selected correlations used in Figure 1. The UV, ECD, and IR spectra, and $[\alpha]^{20}_D$ data were consistent with the close structural relationship of **1** and **6**, which permitted the absolute configuration to be assigned as 5*S*, 7*S*, 10*S*, the same as for **6**. The C-7 epimer of **1** was also

isolated in this study as a known natural product, 7-*O*-methylhorminone (**9**), which allowed for exclusion of any different configuration for this molecule. Thus, compound **1** was assigned as 7-*O*-methyltaxoquinone [(4*b,S*,8*a,S*,10*S*)-3-hydroxy-2-isopropyl-10-methoxy-4*b*,8,8-trimethyl-4*b*,5,6,7,8,8*a*,9,10-octahydrophenanthrene-1,4-dione].

Compound **2** was determined to have the molecular formula C₂₁H₃₀O₅ based on the sodiated molecular ion peak in the HRESIMS at *m/z* 385.1985 [M + Na]⁺ (calcd 385.1985). The IR spectrum of **2** exhibited characteristic absorptions of three carbonyl groups (1722, 1663, and 1633 cm⁻¹). The splitting pattern observed in the ¹H NMR spectrum indicated the presence of an alkenyl isopropyl group typical of oxidized abietane diterpenoids [δ_{H} 2.99 (1H, septd, *J* = 6.9, 0.9 Hz, H-15), 1.16 (3H, d, *J* = 6.9 Hz, CH₃-16), and 1.15 (3H, d, *J* = 6.9 Hz, CH₃-17)]. The isopropyl methine proton also demonstrated long-range *J*-coupling with a peak at δ_{H} 6.39 (1H, d, *J* = 0.9 Hz, H-14). The ¹³C NMR spectrum displayed three carbonyl shifts at low field, of which one was non-conjugated (δ_{C} 210.4, C-6) and two were both conjugated and of typical shifts for a *para*-benzoquinone moiety [δ_{C} 189.4 (C-8) and 183.7 (C-12)]. The overlay of ¹³C-DEPT135 experimental data demonstrated three additional alkyl methyl groups for a total of five in the molecule, plus a methoxy group substituent, four quaternary carbons, a tertiary oxygenated sp² carbon, three methines, and four methylene groups that included one with an unusual splitting pattern and chemical shifts in the HSQC spectrum [δ_{H} 4.30 and 4.07 (2H, ABq, *J* = 18.1 Hz) and δ_{C} 81.4 (C-7)]. The proton spectrum also indicated the presence of an enolic hydroxy group at δ_{H} 7.70 (1H, s, OH-11) when taken together with the lack of any HSQC signal. The HMBC correlations (Figure 1) of the alkenyl (H-14), isopropyl (H-15), and enolic (OH-11) protons suggested that these all belong to a 2,3,5-trisubstituted 1,4-benzoquinone moiety. This group was connected to the six-carbon saturated A ring, typical of abietane diterpenoids, by the combined HMBC correlations observed to C-9 (δ_{C} 125.9) from the quinoid protons H-14 and OH-11, a methylene proton [δ_{H} 1.86 – 1.80 (1H, m, H-1)], an axial methyl group [δ_{H} 1.22 (3H, s, H-20)], and an axial methine proton typical of a bridgehead position [δ_{H} 3.62 (1H, s, H-5)]. As suggested by HMBC correlations from the AB quartet to C-5 (δ_{C} 54.7) and the methoxy group (δ_{C} 59.4), then confirmed by the ¹J_{C,C} couplings observed from a 1,1-ADEQUATE experiment (Figure 1), the remaining functionality was determined to be a 2-methoxyacetyl side chain at C-5. This latter type of functional group has not been often observed among natural products, but, for example, was found recently in koshikamide B, a polypeptide marine natural product produced by the sponge *Theonella* sp.³⁸ The NOESY correlations between the axial methyl protons (H-20) and the AB quartet (H-7) suggested a *trans* relationship between H-5 and CH₃-20, as would be expected from the typical biosynthesis of the abietane diterpenoids. The absolute configuration was determined by analysis of the spatial exciton coupling observed in the ECD spectrum. Both the UV peak from 300-260 nm, resulting from the *para*-benzoquinone chromophore, and the UV peak from 240-200 nm, produced by the non-conjugated carbonyl moiety, demonstrated positive Davydov splitting in the ECD spectrum. This splitting occurred due to the exciton chirality coupling previously described for any two chromophores in spatial proximity, where the sign of the splitting results from the angle between the two chromophores and the intensity is quadratically proportional to the distance between them in space.³⁹ The positive splitting observed in the ECD spectrum of **2** was consistent with a 5*S*, 10*S* absolute configuration that

is typical of abietane diterpenoids, and was visualized in the energy-minimized molecular model of **2** (Figure 2). Compound **2** is thus a 7,8-*seco*-abietane diterpenoid with three carbonyl groups, and was assigned as (1'*S*,2'*S*)-6-hydroxy-4-isopropyl-2'-(2-methoxyacetyl)-1',3',3'-trimethyl-[1,1'-bi(cyclohexane)]-3,6-diene-2,5-dione, and given the trivial name, taxotriene.

The molecular formula of compound **3** was determined as C₂₀H₂₆O₂ based on the protonated molecular ion peak at *m/z* 299.1991 [M + H]⁺ (calcd for C₂₀H₂₇O₂, 299.2006) in the HRESIMS. The ¹H NMR spectrum exhibited several signals characteristic of a deshielded isopropyl group [δ_{H} 3.35 (1H, septd, *J* = 6.9, 0.8 Hz, H-15), 1.35 (3H, d, *J* = 6.9 Hz, CH₃-17), and 1.31 (3H, d, *J* = 6.9 Hz, CH₃-16)], which is typical for an abietane diterpenoid with an oxidized C-ring. The ¹³C NMR spectrum, however, suggested the presence of a naphthalene moiety [δ_{C} 147.5 (C-12), 135.1 (C-13), 134.3 (C-11), 132.8 (C-5), 131.8 (C-10), 129.8 (C-9), 127.9 (C-8), 126.8 (C-6), 126.0 (C-7), 121.0 (C-14)]. Furthermore, the ¹H NMR spectrum of **3** was found to contain some similar features to that of another known natural product isolated in this study, microstegiol (**5**). For example, a deshielded methyl group at δ_{H} 2.43 (3H, s, CH₃-20) was observed to have a correlation in the ¹H-¹³C HSQC spectrum with δ_{C} 20.0 (C-20) as well as correlations in the ¹H-¹³C HMBC spectrum to C-5, C-6, and C-10, which suggested that it is connected to C-5 on the naphthalene ring (Figure 1). Additionally, the splitting of peaks in the aromatic region of the ¹H NMR and correlations in the ¹H-¹³C HMBC spectrum suggested protonation at C-6 and C-7, as well as C-14 of the naphthalene ring [δ_{H} 7.47 (1H, d, *J* = 8.3 Hz, H-7), 7.34 (1H, br s, H-14), and 7.08 (1H, d, *J* = 8.3 Hz, H-6)]. One final signal was observed at low-field in the ¹H NMR spectrum, but this proton lacked a correlation in the ¹H-¹³C HSQC spectrum and was assigned as a deshielded phenolic hydroxy group [δ_{H} 6.34 (1H, s, OH-12)]. The remaining functionality of an isohexane ether moiety bridging C-10 and C-11 into an eight-membered ring could be deduced by the observation of geminal methyl groups [δ_{H} 1.64 (3H, s, CH₃-19) and 1.15 (3H, s, CH₃-18); δ_{C} 27.7 (C-18) and 26.5 (C-19)] with shared correlations in the ¹H-¹³C HMBC spectrum to C-3 and C-4 [δ_{C} 85.2 (C-4) and 33.5 (C-3)], as well as three methylene groups connected by sequential correlations observed in the ¹H-¹H COSY spectrum. The ¹³C NMR shift of C-4 was typical of an oxygenated carbon, which suggested that the ether linkage is between C-4 and C-11. Thus, the core skeleton of **3** was determined to be a rearranged abietane diterpenoid with the B- and C-rings oxidized in the form of a naphthalenol moiety, which was equivalent to that of a previously synthesized molecule.^{32–34} The present investigation represents the first report of **3** being obtained as a natural product, with the first full spectroscopic assignments for this compound also being obtained. Although compound **3** exhibits only a slight structural modification from microstegiol (**5**), it is more closely related to a recently described natural product, viroxocin,⁴⁰ which differs from **3** by the presence of a carbonyl group at C-1. Interestingly, another natural product related to viroxocin and **3** was reported earlier, with the intermediate oxidation state of C-1, but was never assigned a trivial name.⁴¹ Thus, compound **3** was assigned as 1-deoxyviroxocin (10-isopropyl-2,2,6-trimethyl-2,3,4,5-tetrahydronaphtho[1,8-*bc*]oxocin-11-ol).

Compound **4** was determined to have the molecular formula $C_{20}H_{26}O$, which is one less oxygen atom compared to compound **3**, based on the protonated molecular ion peak at m/z 283.2060 $[M + H]^+$ (calcd for $C_{20}H_{27}O$, 283.2057). The 1H NMR spectrum of **4** exhibited some similarities to that of **3**, including peaks for an isopropyl group bonded to a quaternary sp^2 -hybridized carbon [δ_H 3.02 (1H, br sept, $J = 6.9$ Hz, H-15), 1.16 (3H, d, $J = 6.9$ Hz, CH_3 -17), and 1.13 (3H, d, $J = 6.9$ Hz, CH_3 -16)] and also a deshielded methyl group [δ_H 2.33 (3H, s, CH_3 -20); δ_C 20.6 (C-20)]. However, the ^{13}C NMR spectrum of **4** was significantly different from that of **3**, and shared many common features with that of the other rearranged abietane diterpenoid isolated in this study, the known natural product microstegiol (**5**). While microstegiol has a tertiary hydroxy group at C-11 with a ^{13}C NMR shift of δ_C 84.5, compound **4** lacked this peak and instead had a less deshielded methine signal [δ_H 3.65 (1H, s, H-11); δ_C 58.4 (C-11)]. With this in mind, the core skeleton of a rearranged abietane diterpenoid as an 11,12-dihydro-12-oxonaphthalene functionality [δ_C 203.3 (C-12), 143.1 (C-13), 139.1 (C-14), 138.8 (C-9), 138.3 (C-10), 135.6 (C-5), 129.0 (C-8), 128.9 (C-6), 126.1 (C-7)] became evident. This was supported by the correlations observed from H-11 in the HMBC spectrum to C-4, C-8, C-10, C-12, C-13, C-18, and C-19. Additionally, the aromatic region of the 1H NMR spectrum of **4** indicated the same substitution pattern as for **3** and **5**, but with significantly different chemical shifts than for these substances [δ_H 7.07 (1H, d, $J = 7.8$ Hz, H-6), 7.05 (1H, br s, H-14), and 7.03 (1H, d, $J = 7.8$ Hz, H-7)]. Analogous signals and correlations (Figure 1) allowed for the construction of an isohexane moiety as in both **3** and **5**, but without oxygenation at C-11, thus suggesting the final structure of **4** as shown. The 1H and ^{13}C NMR data of **4** were consistent with reported values for ($\pm$)-11-dehydroxymicrostegiol, which has been previously published only as a synthetic intermediate in the total synthesis of microstegiol (**5**).³⁵ The present investigation represents the first report of **4** being obtained as a natural product, with the first full spectroscopic assignments for this compound also being obtained. The isolation of **4** as a racemic mixture may suggest its tautomerization at C-11 and C-12 to form an achiral analogue, but it is noteworthy that this compound is stable in solution at room temperature. It is, however, possible that the acidic nature of silica gel enabled the transformation of these molecules in a surface chemistry type of equilibrium.

All isolates were purified from a subfraction of the methanolic extract of *T. distichum* cones that was determined to have in vitro ($IC_{50} = 3.3 \mu g/mL$) promastigotocidal and dose-dependent in vivo (Figure 3) antileishmanial activity against *L. donovani*. Accordingly, compounds **2-16** were evaluated for their in vitro activity against extracellular promastigotes of *L. donovani*, as well as intracellular *L. amazonensis* amastigotes (Table 1). Several compounds were also tested for in vitro cytotoxicity to HT-29 human colorectal carcinoma cells as a representation of mammalian cell toxicity and to establish a selectivity index in an effort to better evaluate the antileishmanial activity. Compound **1** was excluded from biological evaluation because it was obtained in too small a quantity for this purpose. Although *L. amazonensis* is not a causative agent of visceral leishmaniasis, as *L. donovani* is, the intracellular amastigotocidal activity is typically considered to be more relevant to the human disease state than bioassays using either axenic amastigotes or promastigotes.⁴² *L. amazonensis* itself is responsible for cutaneous leishmaniasis in regions of the New World. Compound **2** was the most active substance isolated against *L. amazonensis* intracellular

amastigotes ($IC_{50} = 1.4 \mu M$), and **10** was the most potent promastigotocide against *L. donovani* ($IC_{50} = 1.6 \mu M$). It is worth noting that the active compound **10** was found to be the most abundant constituent isolated, and was observed in many active subfractions by either isolation or a combination of TLC and 1H NMR spectroscopy.

The distribution of **10** across many chromatographic fractions may be due in part to the high concentration of this constituent in *T. distichum* cones. Additionally, this may have resulted from the generation of **10** by the previously reported aerial oxidation of the more polar analogue, taxodone (**17**), which was also described earlier as a constituent of the cones of *T. distichum* but was not isolated in the present study.¹⁸ Compound **10** has been described in the past as a potent antileishmanial agent against *L. donovani* promastigotes in vitro after it was isolated from other plant species, including *Clerodendrum eriophyllum* Gürke (Verbenaceae) and *Cupressus sempervirens* L. (Cupressaceae).^{43,44} More recently, this compound has been reported to be an inhibitor of human and trypanosomal farnesyl diphosphate synthase (FPPS),⁴⁵ which could explain the biological activity observed both in this study and in others, but putatively for **2** as well based on their structural similarity. Many of the compounds isolated from the active fraction were tested but were inactive ($IC_{50} > 10 \mu M$) in the antileishmanial bioassays. It is possible that some molecules produced either synergistic bioactivity, or solubilizing, or toxicity-reducing effects in the in vitro and in vivo evaluations of the parent fraction. For example, ferruginol (**12**) has been reported to be a bioavailable antioxidant and gastroprotective agent.^{46,47} Thus, these compounds may be suggested for further in vitro studies for combination effects, or potential in vivo experimentation of the active compounds either alone or in combination with other *Taxodium* isolates. Moreover, the in vivo activity of a refined *T. distichum* cone extract enriched in oxidized abietane diterpenoids could also be pursued in crude form as a potential new botanical drug product for the therapy of visceral leishmaniasis.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined on a Fisher-Johns melting point apparatus (Fisher Scientific, Pittsburgh, PA, USA). A Perkin Elmer model 343 polarimeter (Perkin Elmer, Waltham, MA, USA) was used to measure optical rotations at 20 °C. Ultraviolet spectra were recorded using a Hitachi U-2910 UV/vis double-beam spectrophotometer (Hitachi High-Technologies America, Schaumburg, IL, USA). Electronic circular dichroism (ECD) spectra were recorded on a JASCO J-810 spectropolarimeter (JASCO Inc., Easton, MD, USA). Infrared (IR) spectra were obtained on a Nicolet 6700 FT-IR spectrometer (Thermo Scientific, Waltham, MA, USA). A Bruker Avance DRX-400 spectrometer (Bruker, Billerica, MA, USA) was used to record NMR data at 300 K using standard Bruker pulse sequences. HRESIMS were recorded using a Waters Q-TOF micro mass spectrometer (Waters, Milford, MA, USA) in the positive-ion mode, with NaI being used for mass calibration. Preparative TLC was conducted on pre-coated 20 cm × 20 cm, 500 or 1000 μm thickness silica gel plates (UV254, glass backed, Sorbent Technologies, Atlanta, GA, USA). Preparative HPLC was performed using a Luna 5 μm C₁₈ 100A column (5 μm , 250 mm × 21.2 mm i.d., Phenomenex, Torrance, CA, USA) at ambient temperature, along with a Hitachi HPLC

system (Hitachi High-Technologies America), comprising an L-2200 autosampler, an L-2400 UV detector, and two PrepPumps. Flow cytometry was conducted using a Fluorescence-Activated Cell Sorter (FACS) Calibur flow cytometer (BD Biosciences, San Jose, CA, USA). All solvents used for column chromatography were purchased from Fisher Scientific (Fair Lawn, NJ, USA). HPLC-grade acetonitrile and methanol were purchased from WorldWide Life Sciences Division (Bristol, PA, USA). HPLC-grade water was obtained by filtration using a Milli-Q Direct water purification system (Billerica, MA, USA). Deuterated NMR solvents were purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA). Cell culture media and supplements used for HT-29 cytotoxicity studies were obtained from Life Technologies, Inc. (Grand Island, NY, USA). Solvents, solutions, and standards for antileishmanial studies were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Plant Material

The fallen cones of *Taxodium distichum* were collected from ornamental trees adjacent to Mirror Lake (39°59.535' N; 83°00.496' W) on The Ohio State University campus in Columbus, Ohio, United States in July 2010 by two of the authors (C.M.V. and J.N.F.) with the permission of the campus grounds-keeping staff. The plant was identified and vouchered (OS Accession # 445030) at the Museum of Biological Diversity Herbarium, The Ohio State University, Columbus, OH, USA by the herbarium manager and curator of vascular plants, Dr. Mesfin Tadesse.

Extraction and Isolation

The air-dried cones of *T. distichum* (2.4 kg) were pulverized and extracted three times overnight with 12 L 90% MeOH/H₂O at room temperature. The extract was concentrated in vacuo (208 g crude macerate) and subjected to the Wall-modified Kupchan partitioning scheme to afford a tannin-depleted chloroform partition (76 g).⁴⁸ The chloroform partition, TDCD3, was active against *L. donovani* promastigotes in vitro (IC₅₀ = 2.1 µg/mL), so a 25 g aliquot was subjected to step-gradient vacuum liquid chromatography (VLC) on a column packed with silica gel. From the VLC column, subfraction F2 (13.7 g; eluted with 3:1 hexanes-EtOAc) was the only sample to demonstrate in vitro activity in the same bioassay (IC₅₀ = 3.3 µg/mL). Accordingly, 6.6 g of F2 were separated by open column silica gel chromatography. Fractions F2.1 (2.8 g) and F2.2 (1.4 g) were each eluted by 10:1 hexanes-EtOAc and were promastigotocidal (IC₅₀ = 0.8 and 1.0 µg/mL, respectively). Since these fractions demonstrated similar TLC behavior, they were combined as F2.1'. An open silica gel column was used to further separate fraction F2.1' by isocratic elution with 20:1 hexanes-acetone. Fractions F2.1'.3 and F2.1'.6 were observed to develop crystals upon solvent removal, which were separated, respectively, as crystals (F2.1'.3C and F2.1'.6C) or mother liquors (F2.1'.3M and F2.1'.6M). The crystalline fraction F2.1'.3C was rinsed with hexanes, dissolved in acetone, and recrystallized with hexanes/acetone to afford compound **6** (7.5 mg). The crystalline fraction F2.1'.6C was chromatographed by HPLC using a Luna C₁₈ preparative column with 77% MeOH/H₂O as an isocratic elution solvent at 12 mL/min to yield compound **13** (t_R = 30.5 min). Of the eight subfractions collected (F2.1'.1-8), including the two mentioned as mother liquors, the first seven each showed

promastigotocidal activity with IC_{50} values between 0.5 and 2.1 $\mu\text{g/mL}$, and were all selected for further fractionation individually, because they had distinct TLC profiles.

Fraction F2.1'.1 (0.22 g) was separated with silica gel column chromatography, and elution by 20:1 hexanes-EtOAc produced three crude subfractions and further elution by 100% EtOAc afforded a fourth subfraction. Subfraction F2.1'.1 (0.1 g) was subjected to preparative HPLC using the above-mentioned C_{18} column and 80% MeOH/ H_2O at 16 mL/min to obtain compound **4** (2.2 mg; t_R = 51.5 min), **5** (2.5 mg; t_R = 45 min), and a complex subfraction (11 mg; t_R = 68.25 min). This crude subfraction was added to 18.5 mg of a similarly prepared sample from F2.1'.2. The resultant combined fraction was applied to a silica gel preparative TLC plate and developed four times with 2:1 hexanes-toluene to yield compounds **3** (5.5 mg; R_f = 0.83), **4** (6.6 mg; R_f = 0.21), and **7** (8.4 mg; R_f = 0.7). Subfraction F2.1'.1.2 was analyzed by TLC and ^1H NMR spectroscopy, and then set aside for combination with a later fraction (see below). Subfraction F2.1'.3 (79 mg) was refined by preparative HPLC using a Luna C_{18} column and 88% MeOH/ H_2O at a flow rate of 16 mL/min to yield compound **16** (7.4 mg; t_R = 19.25 min).

Fraction F2.1'.2 (1.0 g) was subjected to silica gel column chromatography using elution by 30:1 hexanes/EtOAc to produce four subfractions. Subfraction F2.1'.2.1 (0.355 g) was separated by preparative HPLC on a C_{18} column (85% MeOH/ H_2O , 16 mL/min) to afford compound **10** (0.156 g; t_R = 22.4 min) and another crude subfraction (83.1 mg; t_R = 20.5 min). This subfraction was combined with F2.1'.1.2 and applied to a preparative TLC silica plate that was developed twice with 10:1 toluene-EtOAc to yield compounds **1** (3.6 mg; R_f = 0.74) and **9** (8.2 mg; R_f = 0.81), and another 86.5 mg of **10** (R_f = 0.9). Subfraction F2.1'.2.3 (0.189 g) was purified by preparative HPLC using the same C_{18} column (85% MeOH/ H_2O , 16 mL/min) to furnish compound **12** (35 mg; t_R = 30.5 min).

The mother liquor fraction F2.1'.3M (0.5 g) was separated over a silica gel column and eluted with step gradients of 30:1 and 10:1 hexanes-EtOAc to produce five and three subfractions, respectively. Subfraction F2.1'.3.4 (90 mg) was applied to a preparative TLC silica plate and developed four times with 10:1 hexanes-EtOAc to afford more of compound **6** (14.2 mg; precipitate) and a further subfraction (46.6 mg; R_f = 0.66), which was separated by preparative HPLC with a C_{18} column eluted with 70% MeOH/ H_2O at a flow rate of 16 mL/min to yield an additional 2.3 mg of compound **6** (t_R = 73 min), as well as **8** (10.6 mg; t_R = 56 min).

Fraction F2.1'.5 (0.4 g) was chromatographed over silica gel with 5:1 hexanes-EtOAc to produce six subfractions. F2.1'.5.4 (46 mg) was purified by preparative HPLC using a C_{18} column (77% MeOH/ H_2O , 16 mL/min) to yield compound **2** (8.7 mg; t_R = 21 min). The fractional mother liquor F2.1'.6M (0.42 g) was separated by column chromatography using silica gel to produce eight subfractions. From subfraction F2.1'.6M.1 (9 mg), another 1.9 mg of compound **10** was obtained by RP-HPLC, in the manner indicated above. Preparative HPLC using a C_{18} column (77% MeOH/ H_2O , 12 mL/min) to further fractionate F2.1'.6M.3 (94 mg) yielded another 1.0 mg of compound **10**, as well as compounds **14** (9.0 mg; t_R = 25.5 min) and **15** (15.5 mg; t_R = 35-39 min). Fraction F2.1'.7 (0.17 g) was subjected to

preparative HPLC on a C₁₈ column with 75% MeOH/H₂O at a flow rate of 16 mL/min to yield compound **11** (12.6 mg; *t_R* = 14.5 min).

7-O-Methyltaxoquinone (1)—Yellow amorphous solid; [α]_D²⁰ +73 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (4.55), 272 (4.27) nm; ECD (MeOH) λ_{max} ([θ]) 200 (−50019), 273 (58112), 309 (−8303) nm (° M^{−1} m^{−1}); IR (film) ν_{max} 3369, 2960, 2930, 2871, 1770, 1727, 1641, 1604, 1460, 1392, 1378, 1327, 1261, 1164, 1105, 1088, 947, 757, 668 cm^{−1}; ¹H and ¹³C NMR, see Table 1; HRESIMS *m/z* 369.2031 [M + Na]⁺ (calcd for C₂₁H₃₀O₄Na, 369.2036).

Taxotrione (2)—Brown oil; [α]_D²⁰ +44 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.17), 267 (4.06) nm; ECD (MeOH) λ_{max} ([θ]) 200 (−15102), 220 (9656), 250 (−6334), 276 (−6132), 303 (5542) nm (° M^{−1} m^{−1}); IR (film) ν_{max} 3323, 2963, 2935, 2874, 1722, 1653, 1633, 1465, 1365, 1334, 1293, 1212, 755, 669 cm^{−1}; ¹H and ¹³C NMR, see Table 1; HRESIMS *m/z* 385.1985 [M + Na]⁺ (calcd for C₂₁H₃₀O₅Na, 385.1985).

1-Deoxyviroxocin (3)—Amorphous solid; UV (MeOH) λ_{max} (log ϵ) 239 (4.68), 290 (3.64), 336 (3.25) nm; IR (film) ν_{max} 3499, 2965, 2931, 2871, 1708, 1453, 1416, 1370, 1314, 1224, 1210, 1171, 1115, 997, 943, 877, 823, 790, 759, 678 cm^{−1}; ¹H NMR and ¹³C NMR, see Table 1; HRESIMS *m/z* 299.1991 [M + H]⁺ (calcd for C₂₀H₂₇O₂, 299.2006).

(±)-11-Dehydroxymicrostegiol (4)—Amorphous solid; [α]_D²⁰ 0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (4.67), 238 (4.45) nm; ECD (MeOH) λ_{max} ([θ]) 200 (0) nm (° M^{−1} m^{−1}); IR (film) ν_{max} 3425, 2966, 2933, 2873, 1719, 1459, 1370, 1216, 1171, 1116, 756, 669 cm^{−1}; ¹H NMR and ¹³C NMR, see Table 1; HRESIMS *m/z* 283.2060 [M + H]⁺ (calcd for C₂₀H₂₇O, 283.2057).

Molecular Modeling

Compound coordinates were built using the Spartan 2010 Version 1.1.0 computer software (Wavefunction Inc., Irvine, CA, USA). After initial geometry cleanup, the energy minimization was performed using Density Functional Theory (DFT) molecular dynamics with the hybrid functional B3LYP/6-31G* simulated in vacuum conditions.

In Vitro Cytotoxicity Test Protocol

Compounds were tested against HT-29 human colorectal carcinoma cells, according to a published protocol.⁴⁹ Briefly, HT-29 cells were placed into 96-well plates (9500 cells/190 μ L) and exposed to different concentrations of drugs or test samples in triplicate for 72 h. Cells treated only with 10% DMSO in H₂O and those treated with paclitaxel (taxol) in the same carrier were kept as negative and positive controls, respectively. After application of sulforhodamine B, the sample-induced cytotoxicity was quantified by fluorescence detection. Table Curve2Dv4 software using non-linear regression (log inhibitor vs. response on a variable slope) was used to determine IC₅₀ values (concentrations that resulted in 50% inhibition of cell survival).

In Vitro Killing of *L. donovani* Promastigotes Test Protocol

Compounds were tested against extracellular *L. donovani* promastigotes, according to a previously described protocol.^{50,51} Briefly, an earlier developed type of transgenic red fluorescent protein expressing DsRed2 *L. donovani* (strain LV 82) promastigotes,⁵² was grown in Medium 199 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin. The parasites were obtained from previously infected STAT4 knockout (immunocompromised) mice. The growth of fluorescent *L. donovani* promastigotes was selected by adding 50 µg/mL nourseothricin (clonNAT) to the growth medium during alternate passages. These promastigotes were placed into 24-well culture plates (1×10⁶ cells/mL/well) and exposed to different concentrations of drugs or test samples in duplicate for 72 h at 23 °C. Untreated promastigotes and those treated for 1 h with 1 mg/mL saponin (from *Quillaja saponaria*) were kept as negative and positive controls for the experiment, respectively. The antileishmanial drug, miltefosine, was used as a standard in this study. The sample-induced killing of *L. donovani* promastigotes was quantified by flow cytometry, and IC₅₀ values were determined by GraphPad Prism software (GraphPad Software, La Jolla, CA, USA). IC₅₀ calculations were made using non-linear regression (log inhibitor vs. response on a variable slope).

In Vitro Killing of *L. amazonensis* Amastigotes Test Protocol

Compounds were tested against intracellular *L. amazonensis* amastigotes according to a published method with some modifications.⁵³ Briefly, transgenic promastigotes that express β-lactamase were cultured in RPMI 1640 GlutaMAX with HEPES, 50 units/mL penicillin, 50 µg/mL streptomycin, 0.1 mM adenosine, 10 µg/mL folate, 1× RPMI vitamins (Gibco), and 10% heat-inactivated FBS [pH 6.9], as published.^{53,54} These parasites were placed into 96-well culture plates at a ratio of 5:1 for 24 h at 34 °C with peritoneal macrophages obtained from CD-1 mice (1 × 10⁵ cells/well) in RPMI 1640 GlutaMAX (Gibco) containing 10% FBS, 50 units/mL penicillin, and 50 µg/mL streptomycin [pH 7.4]). Infected macrophages were then washed twice with Hanks balanced salt solution (HBSS) to remove any remaining extracellular promastigotes. The infected macrophages were incubated for 72 h at 34 °C with different concentrations of samples according to the published protocol.⁵⁴ After washing the contents of each well with 200 µL PBS, the β-lactamase activity of the intracellular amastigotes was determined by application of 100 µL PBS containing the indicator dye nitrocefin (100 µM) and 0.1% Triton-X-100 lysis solution. The plates were incubated for 4 h at 37 °C before the absorbance of each well was measured at 490 nm using a SPECTRAmax PLUS 384 spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). IC₅₀ values were calculated using the SoftMax Pro software (Molecular Devices) employing the four parameter dose-response curve. Amphotericin B was used as the positive control.

In Vivo Antileishmanial Test Protocol

The crude fraction TDCD3F2 was tested by iv administration using *L. donovani* (strain LV 82) infected BALB/c mice, according to a previously published method,⁵⁵ through an Ohio State University Institutional Animal Care and Use Committee (IACUC) approved protocol (#2010A0048-R1; test sample approved under amendment AM-7). These experiments were performed in accordance with all local and national guidelines and regulations. Briefly, mice

that weighed an average of 18 g each were infected by tail vein injection of parasites and were treated after 14 d by 100 μ L iv injections of DMSO (10% v/v) in phosphate-buffered saline (PBS; 90% v/v) as a negative control, the crude fraction of *Taxodium distichum* cone extract [TD3F2 (5.6, 11.2, or 22.2 mg/kg in 10% DMSO/PBS)], or sodium stibogluconate (SSG; 70 mg/kg in 10% DMSO/PBS) as a positive control. Each treatment group comprised three animals, and the mice were sacrificed two weeks after sample injection. The liver and spleen of each euthanized mouse were collected and weighed before tissue samples of each organ were taken for impressions on glass slides followed by MeOH fixation and Giemsa staining. The number of amastigotes per 1000 host cell nuclei was determined by examination of these slides under a light microscope. The parasite burden was calculated in Leishman Donovan units (LDU; = organ weight (g) $\times$ number of amastigotes per 1000 host cell nuclei). Statistical analysis was conducted using GraphPad Prism 5.00 (GraphPad Software, La Jolla, CA, USA) by unpaired *t*-tests between the LDU values of control and test groups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

This study was supported by NIH grant RC4 AI092624 (to A.R.S. and A.D.K.) and U.S. Army/Department of Defense grant W81XWH-13-2-0168 (to A.R.S.). Dr. Mesfin Tadesse is acknowledged for identifying and vouchering the herbarium sample of the plant material used in this study. We thank Dr. Craig A. McElroy, College of Pharmacy, The Ohio State University, for facilitating the acquisition of the NMR and mass spectra. C.B. Naman acknowledges and appreciates financial support provided by the Jack L. Beal Graduate Scholarship in Medicinal Chemistry and Pharmacognosy from the College of Pharmacy, The Ohio State University, as well as pre-doctoral fellowships from the American Foundation for Pharmaceutical Education (AFPE) and an NIH Chemistry-Biology Interface Training Program (T32 GM08512).

REFERENCES

1. Vines, RA. Trees of East Texas. University of Texas Press; Austin: 1907.
2. Farjon, A. A Monograph of Cupressaceae and Sciadopitys. Linklater, R., editor. Royal Botanic Gardens; Kew, Richmond, Surrey, UK: 2005. p. 123-132.
3. Watson FD. Taxon. 1985; 34:506–509.
4. Lickey EB, Walker GL. Southeast. Natural. 2002; 1:131–148.
5. Adams RP, Arnold MA, King AR, Denny GC, Creech D. Phytologia. 2012; 94:159–168.
6. Su Z, Yuan W, Wang P, Li S. Pharm. Crop. 2013; 4:1–14.
7. Standley, PC. Contributions from the United States National Herbarium. Vol. 23. Trees and Shrubs of Mexico. Washington, DC: 1920.
8. Villamar, AA.; Asseleih, LMC.; Rodarte, ME. Atlas de las Plantas de la Medicina Tradicional Mexicana. I. Instituto Nacional Indigenista; Mexico City: 1994. p. 61-62.
9. Cruz-Coke RM. Rev. Méd. Chile. 2007; 135:1076–1081. [PubMed: 17989868]
10. Sharaf M. An English-Arabic Dictionary of Medicine, Biology, and Allied Sciences.
11. WHO. Sustaining the Drive to Overcome the Global Impact of Neglected Tropical Diseases. Crompton, DWT., editor. World Health Organization; Geneva: 2013.
12. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, den Boer M, the WHO Leishmaniasis Control Team. PLoS One. 2012; 7:e35671. [PubMed: 22693548]
13. McCall L-I, Zhang W-W, Matlashewski G. PLoS Pathog. 2013; 9:e1003053. [PubMed: 23300451]
14. Kaye P, Scott P. Nat. Rev. Microbiol. 2011; 9:604–615. [PubMed: 21747391]

15. den Boer M, Argaw D, Jannin J, Alvar J. Clin. Microbiol. Infect. 2011; 17:1471–1477. [PubMed: 21933305]
16. Andrews KT, Fisher G, Skinner-Adams TS. Int. J. Parasitol. Drugs Drug Resist. 2014; 4:95–111. [PubMed: 25057459]
17. Ulubelen A, Topcu G, Tan N, Lin L-J, Cordell GA. Phytochemistry. 1992; 31:2419–2421.
18. Kupchan SM, Karim A, Marcks C. J. Am. Chem. Soc. 1968; 90:5923–5924. [PubMed: 5679178]
19. Kupchan SM, Karim A, Marcks C. J. Org. Chem. 1969; 34:3912–3918. [PubMed: 5357534]
20. Edwards OE, Feniak G, Los M. Can. J. Chem. 1962; 40:1540–1546.
21. Carreño MC, García Ruano JL, Toledo MA. Chem. Eur. J. 2000; 6:288–291. [PubMed: 11931108]
22. Janot M-M, Potier P. Ann. Pharm. Fr. 1964; 22:387–395. [PubMed: 14185545]
23. Jonathan LT, Che C-T, Pezzuto JM, Fong HHS, Farnsworth NR. J. Nat. Prod. 1989; 52:571–575. [PubMed: 2778450]
24. Yanagawa T, Hirose Y. J. Japan Wood Res. Soc. 1969; 15:344.
25. Brandt CW, Neubauer LG. J. Chem. Soc. 1939:1031–1037.
26. Crystallographic O. J. Am. Chem. Soc. 1942; 64:928–935.
27. Huzii G, Tikamori T. J. Pharm. Soc. Japan. 1939; 59:124–130.
28. Brandt CW, Thomas BR. J. Chem. Soc. 1952; 1952:2442–2443.
29. Kuo YH, Chang BH, Lin YT. J. Chin. Chem. Soc. 1975; 22:49–52.
30. Ulubelen A, Topcu G. J. Nat. Prod. 1992; 55:441–444.
31. Phytochemistry. 1983; 22:2005–2009.
32. Sexmero Cuadrado MJ, de la Torre MC, Lin L-Z, Cordell GA, Rodríguez B, Perales A. J. Org. Chem. 1992; 57:4722–4728.
33. Karanatsios JSS, Eugster CH. Helv. Chim. Acta. 1966; 49:1151–1172.
34. Conti F, Eugster CH, von Philipsborn W. Helv. Chim. Acta. 1966; 7:2267–2274.
35. Taj RA, Green JR. J. Org. Chem. 2010; 75:8258–8270. [PubMed: 21069992]
36. Miguel del Corral JM, Gordaliza M, Salinero MA, San Feliciano A, del Corral JMM, Feliciano AS. Magn. Reson. Chem. 1994; 32:774–781.
37. Rodríguez B. Magn. Reson. Chem. 2003; 41:741–746.
38. Araki T, Matsunaga S, Nakao Y, Furihata K, West L, Faulkner DJ, Fusetani N. J. Org. Chem. 2008; 73:7889–7894. [PubMed: 18811200]
39. Harada, N.; Nakanishi, K.; Berova, N. Comprehensive Chiroptical Spectroscopy, Vol. 2: Applications in Stereochemical Analysis of Synthetic Compounds, Natural Products, and Biomolecules. Berova, N.; Polavarapu, PL.; Nakanishi, K.; Woody, RW., editors. John Wiley & Sons; New York: 2012. p. 115-166.
40. Rungsimakan S, Rowan MG. Phytochemistry. 2014; 108:177–188. [PubMed: 25256822]
41. Zhang Y, Lu Y, Zhang L, Zheng Q-T, Xu L-Z, Yang S-L. J. Nat. Prod. 2005; 68:1131–113. [PubMed: 16038567]
42. De Rycker M, Hallyburton I, Thomas J, Campbell L, Wyllie S, Joshi D, Cameron S, Gilbert IH, Wyatt PG, Frearson JA, Fairlamb AH, Gray DW. Antimicrob. Agents Chemother. 2013; 57:2913–2922. [PubMed: 23571538]
43. Machumi F, Samoylenko V, Yenesew A, Derese S, Midiwo JO, Wiggers FT, Jacob MR, Tekwani BL, Khan SI, Walker LA, Muhammad I. Nat. Prod. Commun. 2010; 5:853–858. [PubMed: 20614808]
44. Zhang J, Rahman AA, Jacob MR, Khan SI, Tekwani BL, Ilias M, Jain S. Res. Rep. Med. Chem. 2012; 2:1–6.
45. Liu Y-L, Lindert S, Zhu W, Wang K, McCammon JA, Oldfield E. Proc. Natl. Acad. Sci. USA. 2014
46. Wei Y, He J, Qin H, Wu X, Yao X. Biomed. Chromatogr. 2009; 23:1116–1120. [PubMed: 19444795]
47. Areche C, Theoduloz C, Yáñez T, Souza-Brito ARM, Barbastefano V, de Paula D, Ferreira AL, Schmeda-Hirschmann G, Rodríguez JA. J. Pharm. Pharmacol. 2008; 60:245–251. [PubMed: 18237473]

48. Wall ME, Wani MC, Brown DM, Fullas F, Olwald JB, Josephson FF, Thornton NM, Pezzuto JM, Beecher CWW, Farnsworth NR, Cordell GA, Kinghorn AD. *Phytomedicine*. 1996; 3:281–285. [PubMed: 23195084]
49. Pan L, Kardono LBS, Riswan S, Chai H, Carcache de Blanco EJ, Pannell CM, Soejarto DD, McCloud TG, Newman DJ, Kinghorn AD. *J. Nat. Prod.* 2010; 73:1873–1878. [PubMed: 20939540]
50. Naman CB, Gupta G, Varikuti S, Chai H, Doskotch RW, Satoskar AR, Kinghorn AD. *J. Nat. Prod.* 2015; 78:552–556. [PubMed: 25629555]
51. Singh N, Gupta R, Jaiswal AK, Sundar S, Dube A. *J. Antimicrob. Chemother.* 2009; 64:370–374. [PubMed: 19525291]
52. Lezama-Dávila CM, Isaac-Márquez AP, Kapadia G, Owens K, Oghumu S, Beverley S, Satoskar AR. *Biol. Pharm. Bull.* 2012; 35:1761–1764. [PubMed: 23037165]
53. Delfín DA, Morgan RE, Zhu X, Werbovetz KA. *Bioorg. Med. Chem.* 2009; 17:820–829. [PubMed: 19058972]
54. Zhu X, Pandharkar T, Werbovetz K. *Antimicrob. Agents Chemother.* 2012; 56:1182–1189. [PubMed: 22143523]
55. Peine KJ, Gupta G, Brackman DJ, Papenfuss TL, Ainslie KM, Satoskar AR, Bachelder EM. *J. Antimicrob. Chemother.* 2014; 69:168–175. [PubMed: 23956375]

Figure 1. Selected spectroscopic correlations observed for compounds **1-4**. Single-sided arrows represent ^1H - ^{13}C HMBC correlations, double-sided arrows represent ^1H - ^1H NOESY correlations, blue bonds represent ^1H - ^1H COSY correlations, and red bonds represent ^1H - ^{13}C ADEQUATE correlations (only tested for compound **2**).

Figure 2. Result of energy minimization of a computer-generated molecular model of **2**. A positive angle between the benzoquinone and carbonyl chromophores is highlighted.

Figure 3.

Results of in vivo testing of fraction TDCD3F2 using *L. donovani* infected mice. A. Parasite burden observed in spleen cells. B. Parasite burden observed in liver cells. Error bars represent the standard deviation from the mean for each group ($n = 3$). LDU = organ weight (g) $\times$ number of *L. donovani* amastigotes per 1000 host cell nuclei; control = 10% DMSO in phosphate buffered saline; SSG = sodium stibogluconate, a standard antileishmanial drug. Results of unpaired *t*-tests are labeled with connecting bars (* *p*-value < 0.05; ** *p*-value < 0.005).

Table 1Results of in Vitro Biological Testing on Compounds 2-16^a

compound	<i>L. donovani</i> promastigotes [IC ₅₀ µg/mL (µM)] ^b	HT-29 cytotoxicity [IC ₅₀ µg/mL (µM)]	promastigote selectivity index	<i>L. amazonensis</i> amastigotes [IC ₅₀ µg/mL (µM)] ^c	amastigote selectivity index
2	2.5 (6.9)	1.6 (4.4)	0.6	0.52 (1.4)	3.2
10	0.50 (1.6)	0.8 (2.5)	1.6	4.5 (14.3)	0.2
12	13.1 (45.7)	11.3 (39.4)	0.9	4.4 (15.4)	2.5
14	7.8 (24.6)	11.4 (36.0)	1.5	5.4 (17.1)	2.1
16	> 20 (> 60)	4.4 (13.3)	< 0.27	4.3 (13.0)	1.0

^aCompound **1** was not isolated in sufficient quantity for biological testing, and compounds **3-9**, **11**, **13**, and **15** were inactive (IC₅₀ > 10 µg/mL) in the antileishmanial assays conducted.

^bMiltefosine used as a standard (IC₅₀ = 3.7 µM).

^cAmphotericin B used as a standard (IC₅₀ = 0.086 µM). Selectivity Index (SI) = IC₅₀(cytotoxicity)/IC₅₀(antileishmanial activity).

Table 2

¹H and ¹³C NMR Spectroscopic Data of Compounds 1-4^a

position	1		2		3		4	
	δ_{H} , mult. (J Hz)	δ_{C}	δ_{H} , mult. (J Hz)	δ_{C}	δ_{H} , mult. (J Hz)	δ_{C}	δ_{H} , mult. (J Hz)	δ_{C}
1	α , 1.08, m ^b	36.2	α , 1.85, m	32.7	α , 2.92, ddd (13.2, 5.5, 2.3)	26.7	α , 2.96, m	26.0
	β , 2.73, dtd (13.3, 3.5, 1.4)		β , 2.56, d (14.1)		β , 3.92, td (13.6, 4.9)		β , 2.67, m	
2	α , 1.52, m ^b	18.8	α , 1.62, m	20.8	α , 2.17, m	23.2	α , 1.87, m	20.5
	β , 1.70, qt (13.3, 3.5)		β , 1.62, m		β , 1.67, m		β , 1.49, m	
3	α , 1.45, m	41.2	α , 1.89, m	34.1	α , 1.60, m	33.5	α , 1.36, m	43.4
	β , 1.17, m		β , 1.09, s		β , 1.23, m		β , 1.24, m	
4		33.3		34.1		85.2		37.4
5		49.1	3.62, s	54.7		132.8		135.6
6	α , 1.04, m ^b	24.9		210.4	7.08, d (8.3)	126.8	7.07, d (7.8)	128.9
	β , 1.50, m ^b							
7	α , 2.32, ddd (13.1, 8.1, 1.6)	75.0	α , 4.07, ½ ab q (18.1)	81.4	7.47, d (8.3)	126.0	7.03, d (7.8)	126.1
	β , 4.43, dd (9.1, 8.1)		β , 4.30, ½ ab q (18.1)					
8		145.4		189.4		127.9		129.0
9		148.7		125.9		129.8		138.8
10		38.9		41.2		131.8		138.3
11		184.0		150.3		134.3	3.65, s	58.4
12		150.4		183.7		147.5		203.3
13		125.0		149.2		135.1		143.1
14		187.4	6.39, d (0.9)	135.4	7.34, br s	121.0	7.05, br s	139.1
15	3.15, sept (7.1)	24.4	2.99, septd (6.9, 0.9)	26.8	3.35, septd (6.9, 0.8)	28.0	3.02, br sept (6.9)	26.5
16	1.23, d (7.1)	20.0	1.16, d (6.9)	21.3	1.31, d (6.9)	22.5	1.13, d (6.9)	22.2
17	1.20, d (7.1)	20.0	1.15, d (6.9)	21.1	1.35, d (6.9)	22.6	1.16, d (6.9)	22.0
18	0.918, s	33.3	0.81, s	30.4	1.15, s	27.7	1.17, s	27.5
19	0.92, s	21.7	0.83, s	28.0	1.64, s	26.5	0.61, s	24.4
20	1.33, s	19.4	1.22, s	27.8	2.43, s	20.0	2.33, s	20.6
11-OH			7.7, s					
12-OH	7.09, s				6.34, s			
7-O-Me	3.49, s	58.1	3.46, s	59.4				

^a¹H NMR recorded at 400 MHz; ¹³C at 100 MHz; in CDCl₃ and at 300 K. Assignments supported with 2D NMR spectra.^bSignal partially obscured or overlapping.