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Anti-inflammatory and Antimalarial Meroterpenoids from the New Zealand Ascidian *Aplidium scabellum*

Susanna T. S. Chan,[†] A. Norrie Pearce,[†] Ana H. Januario,[‡] Michael J. Page,[§] Marcel Kaiser,[⊥,∥] Rene J. McLaughlin,[▽] Jacquie L. Harper,[▽] Victoria L. Webb,[#] David Barker,[†] and Brent R. Copp*[,†]

[†]School of Chemical Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand

[‡]Núcleo de Pesquisa em Ciências Exatas e Tecnológicas da Universidade de Franca, Av. Dr. Armando Salles de Oliveira 201, Parque Universitário, CEP 14404-600, Franca, SP, Brazil

[§]National Institute of Water & Atmospheric Research (NIWA) Ltd., P.O. Box 893, Nelson, New Zealand

[⊥]Swiss Tropical and Public Health Institute, Socinstrasse 57, P.O. Box CH-4002, Basel, Switzerland

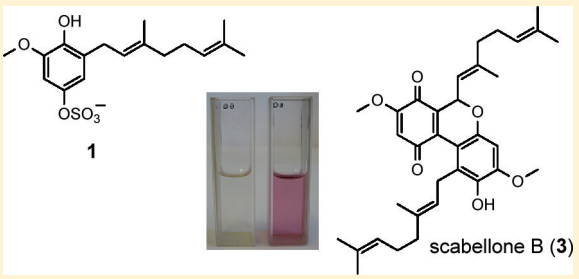
[∥]The University of Basel, CH-4003 Basel, Switzerland

[▽]Malaghan Institute of Medical Research, P.O. Box 7060, Wellington South, New Zealand

[#]NIWA, Private Bag 14-901, Kilbirnie, Wellington, New Zealand

Supporting Information

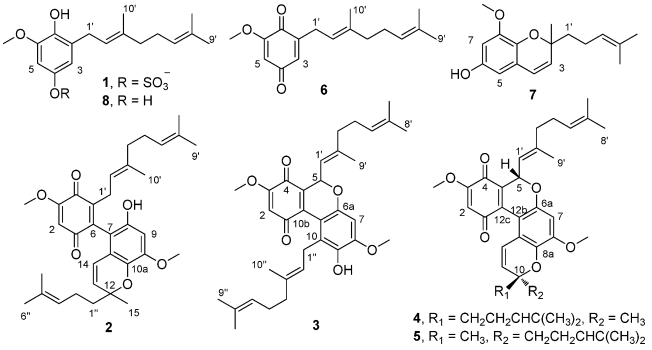
ABSTRACT: Bioassay-directed fractionation of an extract of the New Zealand ascidian *Aplidium scabellum* has afforded the anti-inflammatory secondary metabolite 2-geranyl-6-methoxy-1,4-hydroquinone-4-sulfate (**1**) and a family of pseudodimeric meroterpenoids scabellones A (**2**)−D (**5**). The benzo[*c*]chromene-7,10-dione scaffold contained within scabellones A−D is particularly rare among natural products. The structures were elucidated by interpretation of NMR data. Scabellone B was also identified as a moderately potent, nontoxic inhibitor of *Plasmodium falciparum*.



Ascidians of the genus *Aplidium* (Order Enterogona, Family Polyclinidae) are recognized as a valuable source of bioactive marine natural products.[1] As part of our ongoing program aimed at discovering new anti-inflammatory natural products produced by New Zealand and Antarctic ascidians of the genus *Aplidium*,[2−4] it was found that a crude organic extract of the encrusting species *Aplidium scabellum* (Michaelsen, 1924) collected off Rabbit Island, Hauraki Gulf, New Zealand, exhibited modest ability to inhibit the respiratory burst of stimulated human neutrophils.

Bioassay-directed fractionation resulted in the isolation of five new secondary metabolites, 2-geranyl-6-methoxy-1,4-hydroquinone-4-sulfate (**1**) and scabellones A−D (**2**−**5**), as well as the known metabolites verapliquinone A (**6**)[5,6] and 8-methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-1-benzo-pyran-6-ol (**7**).[7] The identities of **6** and **7** were confirmed by comparison of spectroscopic data with those found in the literature.[5−7] The isolation, structure elucidation, and biological evaluation of the five new metabolites are presented.

Specimens of the gray, encrusting ascidian were extracted with MeOH and fractionated by reversed-phase C_{18} to give two fractions. The first fraction was further purified by reversed-phase cyanopropyl flash column chromatography to give 2-geranyl-6-methoxy-1,4-hydroquinone-4-sulfate (**1**), verapliquinone A (**6**), and chromenol **7**. The second fraction was subjected to a combination of silica, diol, and reversed-phase C_2 flash column chromatography to yield scabellones A−D (**2**−**5**).



High-resolution negative-ion ESIMS data observed for the pseudomolecular ion of **1** were consistent with a molecular formula of $C_{17}H_{24}O_6S$. All 17 carbon resonances required by this formula were accounted for in the ^{13}C NMR spectrum. In the ^1H NMR spectrum (CD_3OD), resonances attributable to two aromatic protons (δ_H 6.75, 6.54), two olefinic protons (δ_H 5.29, 5.09), three moderately deshielded methylene signals (δ_H

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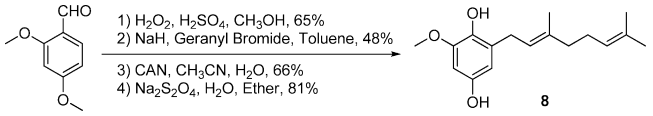
3.27, 2.08, 2.00), one methoxyl signal (δ_H 3.83), and three allylic methyl singlets (δ_H 1.69, 1.65, 1.58) were observed. The presence of a highly oxygenated benzene ring and geranyl side chain were deduced from combined analysis of ^{1}H, ^{13}C, COSY, NOESY, HSQC, and HMBC NMR data. These structural fragments were reminiscent of those observed for quinol **8**, the semisynthetic reduction product of verapliquinone A (**6**), previously reported from a Brittany, France collection of *Aplidium* sp.[5] While the molecular formula of **1** suggested the presence of a sulfate group, attempts at desulfation led to degradation prompting the synthesis of 2-geranyl-6-methoxy-1,4-hydroquinone (**8**)[5,6] to enable comparative NMR analysis to establish the position of sulfation in **1**. Commercially available 2,4-dimethoxybenzaldehyde was subjected to Baeyer–Villiger oxidation to give 2,4-dimethoxyphenol,[8] which was subsequently coupled with geranyl bromide to yield the desired 2-geranyl-4,6-dimethoxyphenol. Oxidation of 2-geranyl-4,6-dimethoxyphenol with CAN afforded synthetic 2′*E*-verapliquinone A (**6**),[5,6] with subsequent reduction using sodium dithionite affording the desired 2-geranyl-6-methoxy-1,4-hydroquinone (**8**) (Scheme 1).

Scheme 1. Synthesis of 2-Geranyl-6-methoxy-1,4-hydroquinone (8)



Direct comparison of the ^{1}H and ^{13}C chemical shifts of the aromatic ring signals of **1** and **8** identified an upfield shift of C-4 and downfield shifts of C-3 and C-5 in the ^{13}C NMR spectrum (of **1** vs **8**) and downfield shifts of H-3 and H-5 in the ^{1}H NMR spectrum which were consistent[9] with the placement of the sulfate group at the C-4 position as shown.

Scabellone A (**2**) was isolated as an optically inactive brown oil with a molecular formula of $C_{34}H_{42}O_6$, established using (+)-HRESI mass spectrometry. Two substructures, a 5-substituted chromenol (using **7** numbering) and a 3-substituted 2′*E*-verapliquinone A fragment, were identified by analysis of ^{1}H, ^{13}C, COSY, NOESY, HSQC, and HMBC NMR data and by direct comparison with the data observed for **7** and **6**, accounting for all the atoms required by the ESIMS molecular formula. Although no HMBC or NOESY correlations were observed to provide direct evidence for the linking of the two substructures, the only logical connection was between C-6 and C-7 as shown for **2**. A lack of optical rotation (at multiple wavelengths) and no detectable CD spectrum indicated scabellone A was isolated as a racemate.

Scabellone B (**3**) was isolated as an optically inactive purple oil. A molecular formula of $C_{34}H_{42}O_6$ was established by (+)-HRESI mass spectrometry. Analysis of ^{1}H, ^{13}C, COSY, NOESY, HSQC, and HMBC NMR data allowed the identification of four substructures of the molecule: a 1,1-disubstituted oxygeranyl chain, a regular 1-substituted geranyl chain, a 2,3,5-trisubstituted 1,4-benzoquinone ring, and a 2,3,4,6-tetrasubstituted phenol. Direct comparison of NMR data with those of **8** and scabellone A (**2**) also supported the presence of these fragments.

The composition of the first substructure, an oxygeranyl chain, was established by interpretation of COSY, NOESY, HSQC, and HMBC data. The COSY spectrum of **3** identified correlations extending from an oxymethine proton at δ_H 6.00 (d, J = 9.4 Hz, H-5) to an olefinic proton at δ_H 5.28 (d, J = 9.4 Hz, H-1′) to a methyl group at δ_H 1.93 (3H, s, H_3-9′). A second spin-system extended from a methylene resonance at δ_H 1.94 (H_2-3′), to a second methylene group at δ_H 1.98 (H_2-4′), to an olefinic proton at δ_H 4.93 (H-5′), which in turn was correlated to two methyl groups (δ_H 1.59, 1.50). HMBC correlations from the methyl group at δ_H 1.93 to a methylene carbon resonance at δ_C 39.8 (C-3′), and from the methylene protons at δ_H 1.94 to a carbon resonance at δ_C 116.9 (C-1′) joined these two fragments, establishing the presence of a 1,1-disubstituted oxygeranyl chain fragment. The presence of a second, regular 1-substituted 2″*E*-geranyl chain was identified by analysis of the NMR data and direct comparison with the data observed for **8**.

The two remaining highly substituted fragments of the molecule were deduced to be 2,3,5-trisubstituted 1,4-benzoquinone and 2,3,4,6-tetrasubstituted phenol rings by analysis of ^{13}C, NOESY, and HMBC NMR data. HMBC correlations observed from the proton resonance at δ_H 5.80 (H-2) to ^{13}C resonances at 182.6 (C-1), 157.8 (C-3), 178.7 (C-4), and 137.6 (C-10b) and from a methoxyl group (δ_H 3.80) to C-3 defined the presence of the quinonoid ring system, while a similar set of correlations from the remaining aromtic proton resonance at 6.40 (H-7) to ^{13}C resonances at δ_C 150.0 (C-8), 139.2 (C-9), 151.4 (C-6a), and 111.0 (C-10a) and from the second methoxyl group (δ_H 3.89) to C-8 defined the second ring as a tetrasubstituted phenol.

Interfragment HMBC correlations (Figure 1) observed between quinonoid proton H-2 and phenol ring resonance

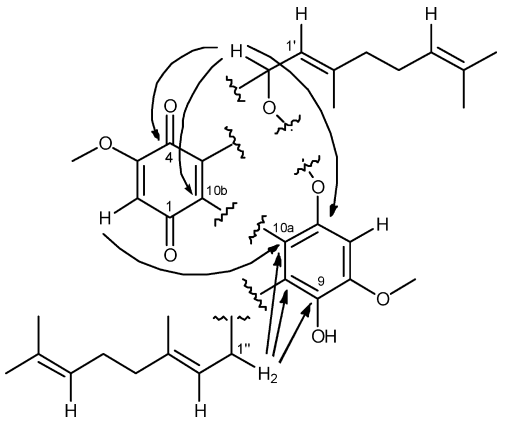


Figure 1. Interfragment HMBC correlations observed for scabellone B (**3**).

δ_C 111.0 (C-10a) and between the oxymethine signal at δ_H 6.00 (H-5) and ^{13}C resonances at δ_C 178.7 (C-4), 151.4 (C-6a), and 137.6 (C-10b) established the connectivity between the 1,1-disubstituted oxygeranyl chain and the hydroquinone–quinone core. The second geranyl chain (C-1″-C-10″) was placed at C-10 (δ_C 126.8) as HMBC correlations from the diastereotopic methylene protons at δ_H 3.57 and 3.36 of the chain (H_2-1″) were observed to carbon resonances δ_C 139.2 (C-9), 126.8 (C-10), and 111.0 (C-10a) of the hydroquinone ring, completing the planar structure of scabellone B (**3**) as shown. A lack of detectable optical rotation and no CD spectrum led to the conclusion at **3** was isolated as a racemate.

Scabellone C (**4**) was obtained as an optically inactive purple oil that gave a pseudomolecular ion at m/z 545.2889 in the (+)-HRESIMS from which a molecular formula of $C_{34}H_{40}O_6$

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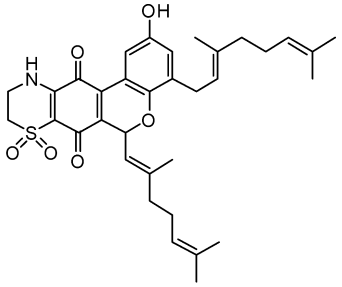
was derived. Similarities in the NMR data observed for scabellone C and scabellones A (**2**) and B (**3**) were evident, identifying the presence of a 5-substituted chromenol fragment (as in **2**) and a 1,1-quinonoid-oxy-disubstituted geranyl chain (as per the fragment defined by C-1−C-5, C-10b, C-1′−C-9′ of **3**). HMBC correlations observed from both the quinonoid proton at δ_H 5.89 (H-2) and the oxymethine signal at δ_H 6.04 (H-5) to carbon resonances at δ_C 179.0 (C-4) and 133.9 (C-12c) identified the connection between the quinone ring and the 1,1-disubstituted 2′*E*-oxygeranyl chain. Additional correlations between both the aromatic proton at δ_H 6.44 (H-7) and H-5 to a carbon resonance at δ_C 151.6 (C-6a) established the connectivity between the 1,1-substituted oxygeranyl chain and the 5-substituted chromenol substructures and, therefore, completed the structure of scabellone C (**4**) as shown.

The molecular formula derived from (+)-HRESIMS for the optically inactive scabellone D (**5**) was $C_{34}H_{40}O_6$, the same as that observed for scabellone C (**4**). Analysis of ^{1}H, ^{13}C, COSY, NOESY, HSQC, and HMBC NMR data readily identified the same substructural fragments and interfragment connectivities as those observed for **4**. Close comparison of NMR data observed for scabellones C (**4**) and D (**5**) revealed differences in chemical shifts centered around C-10, with C-11 in **5** being modestly deshielded (+0.8 ppm) while shielding was observed for C-1″ (−2.6 ppm) and C-2″ (−0.8 ppm). These differences were interpreted as establishing scabellone D as a diastereomer of scabellone C, depicted with arbitrary relative configuration. In both cases, scabellones C and D also failed to exhibit optical rotation at several wavelengths, nor were CD spectra detectable, indicating both compounds were isolated as racemates.[10]

The tricyclic 6*H*-benzo[*c*]chromene-7,10-dione core of scabellone B (**3**) is rare among natural products, being contained within the structures of apoptosis-inducing ascidian metabolites thiaplidiaquinones A (**9**) and B,[11] the sponge-sourced indoleamine-2,3-dioxygenase inhibitor exiguamine A,[12] the microbial metabolite juglochroman B,[13] and the cytotoxic plant metabolite microphyllaquinone,[14] while the tetracyclic core of scabellones C (**4**) and D (**5**) has been reported just once before, contained within the hexacyclic teak wood metabolite tecomaquinone I.[15]



Thiaplidiaquinone A (**9**)

Quinol sulfate **1**, scabellone B (**3**), chromenol **7**, and quinol **8** were found to inhibit the superoxide production by PMA-stimulated human neutrophils in vitro,[2] with IC_{50}'s of 21, 125, 92, and 0.2 μM, respectively. To determine the effect of the different treatments on cell survival, drug-treated neutrophils were stained with the fluorescent markers for necrosis (propidium iodide) and apoptosis (Annexin V-FITC) and analyzed by flow cytometry. Treatment with quinol **8** was found to induce cell death via apoptosis (see the Supporting Information). In contrast, **1** and **3** had no effect on neutrophil viability. Together, these data indicated that **1** and **3** were in fact inhibiting neutrophil superoxide production whereas the potent anti-inflammatory activity of **8** was the result of programmed cell death. Prenylated hydroquinones related to **8** are well-known to exhibit broad ranging biological activities[1,4,16] while their corresponding quinones are noted for their ability to induce p53-independent apoptosis in transformed cell lines.[6]

Scabellone B (**3**) and quinol **8** were also evaluated against the neglected disease parasites targets *Trypanosoma brucei rhodesiense*, *T. cruzi*, *Leishmania donovani*, and *Plasmodium falciparum*. While **8** was found to be pan-panel active, likely due to its general toxicity, **3** exhibited selectivity toward *Plasmodium falciparum* (K1 chloroquine-resistant strain) with IC_{50} 4.8 μM and only poor cytotoxicity (L6 rat myoblast cell line, IC_{50} 65 μM).[17]

The discovery that quinol sulfate 1 exhibits moderately potent and selective ability to inhibit neutrophil respiratory burst suggests that meroterpenoid sulfates could indeed hold promise for the development of new anti-inflammatory agents. The core benzo[*c*]chromene-7,10-dione scaffold of scabellones A−D is rare among natural products and has previously been associated with antiproliferative or apoptosis-inducing biological properties. Our finding of selective antimalarial activity for **3**, combined with no detectable apoptosis toward human neutrophils, highlights this structural class as a novel lead for the development of new treatments for malaria. Efforts directed toward the biomimetic synthesis and structure−antimalarial activity relationship of these new metabolites are in progress, the results of which will be reported in due course.

EXPERIMENTAL SECTION

General Procedures. Optical rotations were recorded using a 0.1 dm cell in CH_2Cl_2 solvent. Ultraviolet−visible and circular dichroism spectra were run as methanol solutions. NMR spectra were recorded at either 600, 400, or 300 MHz for ^{1}H nuclei and 150, 100, or 75 MHz for ^{13}C nuclei. Residual solvent signals were used as reference (CD_3OD: δ_H 3.30, δ_C 49.05; $CDCl_3$: δ_H 7.25, δ_C 77.0). ^{1}H NMR data is reported as position (δ), relative integral, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, br = broad), coupling constant (J, Hz), and the assignment of the atom. ^{13}C NMR data are reported as position (δ) and assignment of the atom.

Analytical reversed-phase HPLC was run using a C_8 column (3 μm, 7 × 33 mm) and eluting with a linear gradient of H_2O (0.05% TFA) to MeCN over 13.5 min at 2 mL/min. Reversed-phase flash column chromatography was carried out on C_{18}, C_8, C_2 or CN stationary support with a particle size of 40−63 μm. Silica gel column chromatography was carried out on silica media with either 40−63 μm or 15−40 μm particle size. All solvents used were distilled analytical grade of better. Chemical reagents used were purchased from standard chemical suppliers.

Extraction and Isolation. Specimens (collection no. MNP9123) of the gray, encrusting *Aplidium scabellum* were collected from the reef at the southern end of Rabbit Island (36 20.9395S, 175 30.308E, 15 m), Great Barrier Island, New Zealand. The ascidian was identified by M.P., and a voucher specimen is kept at NIWA Wellington under collection code MNP9123.

Frozen ascidian specimens were freeze-dried (dry weight 200.92 g), extracted with methanol (5 × 200 mL), and filtered, and solvent was removed in vacuo. The green crude extract (7.74 g) was subjected to C_{18} flash chromatography with a steep gradient from water to methanol, fractionated at 0%, 25%, 50%, 75%, and 100% methanol in water. The third fraction (50% MeOH in water) was then subjected to reversed-phase cyanopropyl flash column chromatography eluting with water. The first fraction collected yielded 2-geranyl-6-methoxy-1,4-hydroquinone-4-sulfate (**1**) (4.0 mg, 0.002%, t_R 5.62 min). The fifth

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fraction (100% MeOH) from the crude C_{18} column was subjected to silica flash chromatography, eluting with a gradient of dichloromethane through to 10% methanol in dichloromethane, and two fractions (dichloromethane and 1% methanol/dichloromethane) were subjected to further purification. The 100% dichloromethane fraction was subjected again to silica flash chromatography where a purple-colored fraction (hexane/ethyl acetate, 9:1) was collected and by analysis of ^{1}H NMR data ($CDCl_3$) deduced to be a mixture of two compounds. Reversed-phase C_2 flash column chromatography with a steep gradient from water to methanol, afforded brown-red 8-methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-1-benzopyran-6-ol (**7**) (0.8 mg, 0.0004%) and scabellone B (**3**) (0.44 mg, 0.0002%), in the fractions eluted with water/methanol (1:1) and (1:4), respectively. The 1% methanol/dichloromethane fraction was subjected to silica flash chromatography with a gradient from hexane to ethyl acetate. A brown-colored fraction (hexane:ethyl acetate, 4:1) was collected and further subjected to reversed-phase C_2 flash column chromatography with a steep gradient from water to methanol. Compounds of interest were found in the 70% methanol and 100% methanol fractions. Diol flash column chromotography with dichloromethane as the eluent was used on the 70% methanol fraction and afforded scabellone A (**2**) (0.49 mg, 0.0002%). The 100% methanol fraction was subjected to silica flash chromatography (15−40 μm) where scabellone C (**4**) (0.85 mg, 0.0004%) and scabellone D (**5**) (0.53 mg, 0.0003%) were isolated (hexane/ethyl acetate, 9:1).

Additional biomass (dry weight 40.11 g, crude extract 2.53 g) was extracted using the same isolation procedure to afford more 8-methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-1-benzopyran-6-ol (**7**) (0.73 mg, 0.0018%) and, from the same fraction, verapliquinone A (**6**) (1.27 mg, 0.003%).

2-Geranyl-6-methoxy-1,4-hydroquinone-4-sulfate (1): brown oil; UV (MeOH) λ_{max} (log ε) 210 (4.00), 282 (3.19), 354 (2.00) nm; IR ν_{max} 3401 1669, 1640, 1495, 1433, 1229, 1207 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) δ 6.75 (1H, d, J = 2.1 Hz, H-5), 6.54 (1H, d, J = 2.1 Hz, H-3), 5.29 (1H, t, J = 6.6 Hz, H-2′), 5.09 (1H, t, J = 6.6 Hz, H-6′), 3.83 (3H, s, OCH_3), 3.27 (2H, d, J = 7.3 Hz, H_2-1′), 2.08 (2H, m, H_2-5′), 2.00 (2H, m, H_2-4′), 1.69 (3H, s, H_3-10′), 1.65 (3H, s, H_3-9′), 1.58 (3H, s, H_3-8′); ^{13}C NMR (CD_3OD, 150 MHz) δ 148.6 (C-6), 146.4 (C-4), 141.2 (C-1), 136.7 (C-3′), 132.2 (C-7′), 129.2 (C-2), 125.5 (C-6′), 123.9 (C-2′), 114.1 (C-3), 103.6 (C-5), 56.6 (OCH_3) 41.0 (C-4′), 29.1 (C-1′), 27.9 (C-5′), 25.9 (C-9′), 17.8 (C-8′), 16.3 (C-10′); (-)-ESIMS m/z 355 [M − H]$^-$; (−)-HRESIMS m/z 355.1219 [M − H]$^-$ (calcd for $C_{17}H_{23}O_6S$, 355.1221).

Scabellone A (2): brown oil; $[\alpha]^{20}_D$ 0, $[\alpha]^{20}_{578}$ 0, $[\alpha]^{20}_{546}$ 0, $[\alpha]^{20}_{436}$ 0 (c 0.005, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 207 (4.58), 230 sh (4.48), 269 (4.27), 315 (3.81), 450 sh (3.11) nm; R_f (50% ethyl acetate/hexane) 0.44; IR ν_{max} (ATR) 3447, 1677, 1634, 1436, 1226 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.43 (1H, s, H-9), 6.01 (1H, s, H-2), 5.92 (1H, d, J = 10.0 Hz, H-14), 5.59 (1H, d, J = 10.0 Hz, H-13), 5.07 (1H, t, J = 6.6 Hz, H-3″), 5.00 (1H, t, J = 6.6 Hz, H-6′), 4.90 (1H, t, J = 6.6 Hz, H-2′), 3.85 (3H, s, 3-OCH_3), 3.85 (3H, s, 10-OCH_3), 3.10 (1H, m, H-1′a), 2.97 (1H, m, H-1′b), 2.10 (2H, m, H_2-2″), 1.95 (2H, m, H_2-5′), 1.86 (2H, m, H_2-4′), 1.74 (1H, m, H-1″a), 1.65 (1H, m, H-1″b), 1.64 (3H, s, H_3-6″, obscured by water), 1.64 (3H, s, H_3-9′, obscured by water), 1.55 (3H, s, H_3-8′), 1.55 (3H, s, H_3-5″), 1.44 (3H, s, H_3-15), 1.36 (3H, s, H_3-10′); ^{13}C NMR ($CDCl_3$, 150 MHz, carbon resonances deduced from ^{1}H−^{13}C HMBC and HSQC NMR data) δ 186.4 (C-1), 181.5 (C-4), 158.3 (C-3), 149.4 (C-10), 145.7 (C-8), 145.7 (C-5), 138.4 (C-6), 138.1 (C-3′), 136.2 (C-10a), 131.3 (C-13), 131.5 (C-4″), 131.2 (C-7′), 124.1 (C-6′), 124.0 (C-3″), 120.4 (C-14a), 120.1 (C-14), 118.0 (C-2′), 109.1 (C-7), 107.6 (C-2), 101.4 (C-9), 77.5 (C-12),56.2 (3-OCH_3), 56.2 (10-OCH_3), 39.6 (C-1″), 39.2 (C-4′), 26.6 (C-1′), 26.3 (C-5′), 25.4 (C-15), 25.4 (C-9′), 25.4 (C-6″), 22.2 (C-2″), 17.3 (C-8′), 17.3 (C-5″), 15.6 (C-10′); (+)-ESIMS m/z 547 (100) [M + H]$^+$; (+)-HRESIMS 547.3020 [M + H]$^+$ (calcd for $C_{34}H_{43}O_6$, 547.3054).

Scabellone B (3): purple oil; $[\alpha]^{20}_D$ 0, $[\alpha]^{20}_{578}$ 0, $[\alpha]^{20}_{546}$ 0, $[\alpha]^{20}_{436}$ 0 (c 0.004, CH_2Cl_2); (MeOH) λ_{max} (log ε) 209 (4.57), 238 sh (4.25), 303 (3.94), 340 sh (3.78), 358 sh (3.59), 546 (3.32) nm; UV−vis (MeOH/KOH) λ_{max} 212 nm (log ε 4.55), 303 (4.01), 546 (3.44); R_f (100% dichloromethane) 0.38; IR ν_{max} 3484, 1658, 1592, 1481, 1441, 1223 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.40 (1H, s, H-7), 6.00 (1H, d, J = 9.3 Hz, H-5), 5.80 (1H, s, H-2), 5.48 (1H, br s, OH), 5.28 (1H, d, J = 9.3 Hz, H-1′), 5.06 (1H, m, H-2″), 5.01 (1H, t, J = 6.6 Hz, H-6″), 4.93 (1H, t, J = 6.6 Hz, H-5′), 3.89 (3H, s, 8-OCH_3), 3.80 (3H, s, 3-OCH_3), 3.57 (1H, m, H-1″a), 3.36 (1H, dd, J = 17.5, 9.1 Hz, H-1″b), 1.98 (4H, m, H_2-4′, H_2-5″), 1.94 (2H, m, H_2-3′), 1.93 (3H, s, H_3-9′), 1.87 (2H, m, H_2-4″), 1.62 (3H, s, H_3-9″), 1.59 (3H, s, H_3-8′), 1.56 (3H, s, H_3-10″), 1.50 (3H, s, H_3-7′), 1.49 (3H, s, H_3-8″); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 182.6 (C-1), 178.7 (C-4), 157.8 (C-3), 151.4 (C-6a), 150.0 (C-8), 144.3 (C-2′), 139.2 (C-9), 137.6 (C-10b), 137.1 (C-3″), 131.7* (C-6′), 131.6* (C-7″), 130.8 (C-4a), 126.8 (C-10), 124.2 (C-2″), 123.8 (C-6″), 123.6 (C-5′), 116.9 (C-1′), 111.0 (C-10a), 107.3 (C-2), 98.4 (C-7), 67.6 (C-5), 56.1 (3-OCH_3), 56.1 (8-OCH_3), 39.8* (C-4″), 39.7* (C-3′), 26.5 (C-1″), 26.3* (C-4′), 26.2* (C-5″), 25.6* (C-8′), 25.5* (C-9″), 17.6* (C-7′), 17.5* (C-8″), 17.2 (C-9′), 16.5 (C-10″); (+)-ESIMS m/z 547 (100) [M + H]$^+$; (+)-HRESIMS m/z 547.3063 [M + H]$^+$ (calcd for $C_{34}H_{43}O_6$, 547.3054).

Scabellone C (4): purple oil; R_f (20% ethyl acetate/hexane) 0.33; $[\alpha]^{20}_D$ 0 (c 0.005, CH_2Cl_2); $[\alpha]^{20}_{578}$ 0, $[\alpha]^{20}_{546}$ 0, $[\alpha]^{20}_{436}$ 0 (c 0.007, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 205 (3.86), 243 (3.71), 294 sh (3.29), 338 (3.35), 558 (2.82) nm; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.44 (1H, s, H-7), 6.10 (1H, d, J = 9.9 Hz, H-12), 6.04 (1H, d, J = 9.1 Hz, H-5), 5.89 (1H, s, H-2), 5.58 (1H, d, J = 9.9 Hz, H-11), 5.35 (1H, d, J = 9.1 Hz, H-1′), 5.15 (1H, t, J = 6.7 Hz, H-3″), 4.94 (1H, t, J = 6.7 Hz, H-5′), 3.86 (3H, s, 8-OCH_3), 3.84 (3H, s, 3-OCH_3), 2.21 (1H, m, H-2″a), 2.11 (1H, m, H-2″b), 1.99 (2H, m, H_2-4′), 1.94 (2H, m, H_2-3′), 1.93 (3H, s, H_3-9′), 1.93 (1H, m, H-1″a), 1.73 (1H, m, H-1″b), 1.68 (3H, s, H_3-6″), 1.60 (3H, s, H_3-5″), 1.59 (3H, s, H_3-8′), 1.51 (3H, s, H_3-7′), 1.49 (3H, s, H_3-13); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 185.4 (C-1), 179.0 (C-4), 158.3 (C-3), 153.0 (C-8), 151.6 (C-6a), 144.3 (C-2′), 138.3 (C-8a), 133.9 (C-12c), 131.7 (C-6′), 131.7 (C-4″), 131.4 (C-4a), 126.6 (C-11), 124.2 (C-3″), 123.8 (C-12), 123.6 (C-5′), 120.2 (C-12a), 117.0 (C-1′), 107.6 (C-12b), 107.1 (C-2), 101.2 (C-7), 77.8 (C-10), 67.7 (C-5), 56.2 (3-OCH_3), 56.2 (8-OCH_3), 41.1 (C-1″), 39.7 (C-3′), 26.2 (C-4′), 25.7 (C-6″), 25.6 (C-8′), 24.5 (C-13), 23.2 (C-2″), 17.7 (C-7′), 17.7 (C-5″), 17.2 (C-9′); (+)-ESIMS m/z 545 (100) [M + H]$^+$; (+)-HRESIMS m/z 545.2889 [M + H]$^+$ (calcd for $C_{34}H_{41}O_6$, 545.2898).

Scabellone D (5): purple oil; R_f (20% ethyl acetate/hexane) 0.44; $[\alpha]^{20}_D$ 0, $[\alpha]^{20}_{578}$ 0, $[\alpha]^{20}_{546}$ 0, $[\alpha]^{20}_{436}$ 0 (c 0.007, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 203 (3.82), 242 (3.65), 295 sh (3.20), 338 (3.29), 552 (2.74) nm; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.43 (1H, s, H-7), 6.07 (1H, d, J = 10.0 Hz, H-12), 6.02 (1H, d, J = 9.6 Hz, H-5), 5.88 (1H, s, H-2), 5.55 (1H, d, J = 10.0 Hz, H-11), 5.33 (1H, m, H-1′, obscured by solvent), 5.11 (1H, t, J = 6.8 Hz, H-3″), 4.93 (1H, t, J = 6.8 Hz, H-5′), 3.85 (3H, s, 8-OCH_3), 3.82 (3H, s, 3-OCH_3), 2.20 (1H, m, H-2″a), 2.14 (1H, m, H-2″b), 1.99 (2H, m, H_2-4′), 1.92 (2H, m, H_2-3′), 1.92 (3H, s, H_3-9′), 1.88 (1H, m, H-1″a), 1.80 (1H, m, H-1″b), 1.62 (3H, s, H_3-6″), 1.57 (3H, s, H_3-5″), 1.58 (3H, s, H_3-8′), 1.50 (3H, s, H_3-7′), 1.53 (3H, s, H_3-13); ^{13}C NMR ($CDCl_3$, 150 MHz, deduced from HMBC and HSQC) δ 184.9 (C-1), 178.7 (C-4), 158.0 (C-3), 152.5 (C-8), 151.3 (C-6a), 144.0 (C-2′), 137.9 (C-8a), 133.6 (C-12c), 131.5 (C-6′), 131.5 (C-4″), 131.1 (C-4a), 127.4 (C-11), 124.3 (C-3″), 123.7 (C-5′), 123.6 (C-12), 120.5 (C-12a), 117.0 (C-1′), 107.2 (C-2), 107.1 (C-12b), 101.0 (C-7), 77.4 (C-10), 67.7 (C-5), 56.5 (3-OCH_3), 56.5 (8-OCH_3), 38.5 (C-1″), 39.4 (C-3′), 26.1 (C-4′), 25.8 (C-6″), 25.9 (C-13), 25.8 (C-8′), 22.4 (C-2″), 17.6 (C-7′), 17.6 (C-5″), 17.2 (C-9′); (+)-ESIMS m/z 545 (100) [M + H]$^+$; (+)-HRESIMS 545.2882 [M + H]$^+$ (calcd for $C_{34}H_{41}O_6$, 545.2898).

2-Geranyl-4,6-dimethoxyphenol. 2,4-Dimethoxyphenol (**10**)[8] (0.52 g, 3.38 mmol) was dissolved in dry toluene (25 mL), and sodium hydride (0.15 g, 3.73 mmol) was added. The reaction mixture was stirred for 1 h at room temperature under nitrogen, and then geranyl bromide (0.73 g, 3.38 mmol) was added dropwise at 0 °C. The mixture was stirred for an additional 3 h before it was poured into ice−water and acidified with 2 M acetic acid. The acidic solution was extracted with diethyl ether (100 mL). The organic layer was washed with saturated aqueous sodium bicarbonate, followed by saturated

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aqueous sodium chloride, and dried over anhyd $MgSO_4$ and solvent removed in vacuo. Purification by silica gel column chromatography eluting with ethyl acetate (0−20%) in hexane and subsequently 100% dichloromethane gave the product as a yellow oil (0.47 g, 48%): R_f (100% dichloromethane) 0.82; IR ν_{max} (ATR) 3546, 2914, 1611, 1224, 1053 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.35 (1H, d, J = 2.6 Hz, H-5), 6.30 (1H, d, J = 2.6 Hz, H-3), 5.33 (1H, m, H-2′), 5.29 (1H, m, OH), 5.10 (1H, m, H-6′), 3.84 (3H, s, 4-OCH_3), 3.74 (3H, s, 6-OCH_3), 3.35 (2H, d, J = 7.2 Hz, H_2-1′), 2.10 (2H, m, H_2-5′), 2.04 (2H, m, H_2-4′), 1.72 (3H, s, H_3-10′), 1.67 (3H, s, H_3-9′), 1.59 (3H, s, H_3-8′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.8 (C-4), 146.7 (C-6), 137.4 (C-1), 136.5 (C-3′), 131.4 (C-7′), 127.5 (C-2), 124.3 (C-6′), 122.0 (C-2′), 105.4 (C-3), 96.7 (C-5), 56.0 (6-OCH_3), 55.7 (4-OCH_3), 39.7 (C-4′), 28.1 (C-1′), 26.7 (C-5′), 25.6 (C-9′), 17.6 (C-8′), 16.1 (C-10′); (+)-ESIMS m/z 291 $[M + H]^+$; (+)-HRESIMS m/z 291.1952 $[M + H]^+$ (calcd for $C_{18}H_{27}O_3$, 291.1955).

2-Geranyl-6-methoxy-1,4-benzoquinone (synthetic verapliquinone A) (6).[5,6] A solution of cerium ammonium nitrate (0.96 g, 1.76 mmol) solution in a 1:2 mixture of CH_3CN:H_2O (15 mL) was added dropwise at 0 °C to a solution of 2-geranyl-4,6-dimethoxyphenol (**11**) (0.26 g, 0.88 mmol) in CH_3CN (5 mL) with stirring. The mixture was stirred overnight at 0 °C, poured into a 10% sodium chloride solution, and then extracted with diethyl ether. The organic extract was dried over anhyd $MgSO_4$ and solvent evaporated in vacuo. Purification by silica gel column chromatography eluting with ethyl acetate (0−10%) in hexane gave the product as a bright yellow oil (0.16 g, 66%): R_f (10% ethyl acetate/hexane) 0.22; IR ν_{max} (ATR) 2914, 1678, 1230 cm^{-1}; ^1H NMR data agreed with that previously reported;[6] ^{13}C NMR ($CDCl_3$, 100 MHz) δ 187.7 (C-4), 182.2 (C-1), 158.9 (C-6), 146.4 (C-2), 140.1 (C-3′), 132.8 (C-3), 131.8 (C-7′), 123.9 (C-6′), 117.7 (C-2′), 107.1 (C-5), 56.3 (OCH_3), 39.6 (C-4′), 27.1 (C-1′), 26.4 (C-5′), 25.7 (C-9′), 17.7 (C-8′), 16.1 (C-10′); (+)-ESIMS m/z 297 $[M+Na]^+$; (+)-HRESIMS m/z 297.1459 $[M + Na]^+$ (calcd for $C_{17}H_{22}NaO_3$, 297.1461).

2-Geranyl-6-methoxy-1,4-hydroquinone (8). A solution of sodium dithionite (3.05 g, 17.52 mmol) in water (7 mL) was added to a stirred solution of 2-geranyl-6-methoxy-1,4-benzoquinone (**6**) (0.16 g, 0.58 mmol) in diethyl ether (15 mL). The solution was stirred for 30 min. The organic layer was dried over anhyd $MgSO_4$ and solvent removed in vacuo to obtain the product as an off-white gum (0.13 g, 81%): R_f (100% dichloromethane) 0.46; IR ν_{max} (ATR) 3264, 2914, 1649, 1215, 1189 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 6.26 (1H, d, J = 2.8 Hz, H-5), 6.15 (1H, d, J = 2.8 Hz, H-3), 5.28 (1H, m, H-2′), 5.09 (1H, m, H-6′), 3.78 (3H, s, OCH_3), 3.23 (2H, d, J = 7.6 Hz, H_2-1′), 2.09 (2H, m, H_2-5′), 2.01 (2H, m, H_2-4′), 1.69 (3H, s, H_3-10′), 1.64 (3H, s, H_3-9′), 1.58 (3H, s, H_3-8′); ^{13}C NMR (CD_3OD, 100 MHz) δ 151.0 (C-4), 149.3 (C-6), 138.0 (C-1), 136.6 (C-3′), 132.3 (C-7′), 129.8 (C-2), 125.4 (C-6′), 124.1 (C-2′), 108.4 (C-3), 98.6 (C-5), 56.4 (OCH_3), 41.0 (C-4′), 29.0 (C-1′), 27.8 (C-5′), 25.9 (C-9′), 17.8 (C-8′), 16.2 (C-10′); (+)-ESIMS m/z 299 $[M + Na]^+$; (+)-HRESIMS m/z 299.1611 $[M + Na]^+$ (calcd for $C_{17}H_{24}NaO_3$, 299.1618).

Biological Assays. The neutrophil respiratory burst assay, details of which have been reported elsewhere,[2] utilized superoxide dismutase as a positive control with an IC_{50} of 0.78 μM. Details of the whole organism parasite assay protocols have been reported elsewhere.[18] Parasite strains and cell lines and positive controls for the assays were *Trypanosoma brucei rhodesiense*, STIB 900 strain, trypomastigotes stage (positive control melarsoprol, IC_{50} 0.01 μM), *Trypanosoma cruzi*, Tulahuen C4 strain, amastigotes stage (positive control benznidazole, IC_{50} 1.35 μM), *Leishmania donovani*, MHOM-ET-67/L82 strain, amastigote/axenic stage (positive control miltefosine, IC_{50} 0.52 μM), *Plasmodium falciparum*, K1 strain, IEF stage (positive control chloroquine, IC_{50} 0.20 μM), L6 rat skeletal myoblast cell line for cytotoxicity (positive control podophyllotoxin, IC_{50} 0.01 μM).

Flow Cytometry. Treated and untreated human neutrophils were washed in chilled annexin binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$). Neutrophils were incubated 15 min in the dark with FITC labeled annexin V (AnV-FITC, BD Pharmingen, NJ), washed with annexin binding buffer and resuspended in 200 μL of FACS buffer (0.1% BSA, PBS) containing 250 ng/mL propidium iodide (PI, Sigma-Aldrich, Auckland, NZ). Cells were then analyzed by flow cytometry (FACSCalibur; Becton Dickinson) to identify live cells (AnV−/PI−), apoptosing cells (AnV+/PI−), necrotic cells (AnV−/PI+), and dead cells (AnV+/PI+).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details, NMR spectra for **1**−**8**, and cytometry plots. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: b.copp@auckland.ac.nz.

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