栓状cytotoxic cembranolides from Okinawan soft coral, Lobophytum sp.

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New cytotoxic cembranolides from an Okinawan soft coral, *Lobophytum* sp.

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**Abstract:**
Three new cembranolides (1–3) were isolated from an Okinawan soft coral, *Lobophytum* sp., together with the known cembranolide diterpenoids (4–9). Their structures were determined by extensive analysis of spectroscopic data (1D and 2D NMR, IR, and MS), molecular modeling, and comparison with data reported elsewhere. All compounds contain an α-methylene-γ-lactone ring adjacent to a cembrane, and some of them (1, 6–8) have an epoxide ring as well. The new metabolites were evaluated for cytotoxicity against HeLa, A459, B16-F10, and RAW 264.7 cells and anti-inflammatory effect in LPS-stimulated inflammatory RAW 264.7 macrophage cells.

**Key words:** Cembranolide; Diterpene; Cytotoxicity; Anti-inflammatory; *Lobophytum*

**1. Introduction**
Marine organisms are biologically diverse and so are their metabolites, showing a wide spectrum of bioactivities [1]. The soft coral genus, *Lobophytum*, is a good source of cembrane-type metabolites [2] containing a 14-carbon rings that are structurally diverse and show various biological activities. This soft coral is also a source of diterpenoids, lipids, steroids, tocopherols, triterpenoids, and zoanthamine-type alkaloids [3]. Over 250 cembrane-type diterpenoids have been isolated from the genus *Lobophytum* and these exhibit various pharmacological activities, such as cytotoxic [4–11], anti-viral [12, 13], anti-bacterial [14, 15], anti-inflammatory [16–18], HIV-inhibitor [13], and neutrophil elastase-release activity [19–21]. A cembrane-type lactone, lobophytolide, was first isolated from *Lobophytum cristagalli* [22]. The cembrane lactones are generally five-, six-, or seven-membered lactone rings [3].
that occasionally bear either an unsaturation or an exo-double bond. The lactone rings of cembranolides account for most of their bioactivity.

Cembranolide structural diversity and pharmacological potential inspired us to work further on the soft coral (Lobophytum) to find novel secondary metabolites. We examined an Okinawan soft coral, Lobophytum sp., and isolated three new cembranolides (1–3) along with the known cembranolide diterpenoids 4–9 [23–26]. Herein, we report the isolation, structure determination, and cytotoxicities of these new metabolites.

2. Experimental section
2.1. General experimental procedures
Optical rotation was measured using a JASCO P-1010 Polarimeter. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III 500 spectrometer in CDCl3. Chemical shifts and coupling constants were given as δ and Hz, respectively, and 1H and 13C chemical shifts were referenced to the residual solvent peaks (δH = 7.26 and δC = 77.24). Infrared (IR) spectra were recorded on a JASCO FT/IR-6100 Fourier Transform Infrared Spectrometer. High-resolution mass spectra (HRMS) were obtained on an LTQ Orbitrap hybrid mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with a nanospray ionization (NSI) source. Open column chromatography was performed on Kieselgel 60 (70–230 mesh, Merck). High-performance liquid chromatography (HPLC) was performed using a COSMOSIL Si60 HPLC column (5SL, φ 10 × 250 mm). Analytical thin-layer chromatography (TLC) was performed using Kieselgel 60 F254 DC-fertigplatten (Merck). All solvents were reagent grade.

2.2. Animal materials
The soft coral was collected during low tide from the coast of Irabu Island, Okinawa, Japan, in March 2013, and identified as a Lobophytum sp. A voucher specimen was deposited at University of the Ryukyus (Specimen no. 13033105).

2.3. Extraction and isolation
The soft coral Lobophytum sp. (110.0 g, wet weight) was collected by hand, transported to the lab, and extracted with acetone (500 mL×3). After filtration, extracts were concentrated under reduced pressure to yield a crude acetone extract. The crude extract was partitioned between H2O/EtOAc (1:1, 200 mL×2). The EtOAc part was evaporated under vacuum to give a crude extract (1.38 g) that inhibited the growth of Gram-positive bacteria (Staphylococcus
aureus) and Gram-negative bacteria (Escherichia coli). The active crude extract was first chromatographed over silica gel to give seven fractions (Hexane/EtOAc/MeOH gradient). The 2nd (362.7 mg) and 3rd (413.3 mg) fractions were subjected to further purification by considering their $^1$H NMR spectra. An aliquot (200 mg) of the 2nd fraction was purified by HPLC (a COSMOSIL Si60 column) using hexane/EtOAc (7:3) to afford a new cembranolide 1 (13.0 mg) and the known cembranolides 4 (11 mg), 5 (44.3 mg), 6 (6.4 mg), and 7 (16.6 mg). An aliquot (86.1 mg) of the 3rd fraction was purified by HPLC using hexane/EtOAc (7:3) to afford new cembranolides 2 (2.9 mg), 3 (2.0 mg), and the known cembranolides 8 (22.4 mg) and 9 (1.8 mg).

2.4. Spectroscopic data

2.4.1. Compound 1
Colorless oil; $\{\alpha\}_{D}^{25.5} \text{–} 31.42$ (c 0.070 CH$_3$OH); FT/IR $v_{\text{max}}$ (film) 1769, 1739, 1668, 1455, 1370 and 1231 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR data are listed in Table 1; HRNSIMS $m/z$ 375.2169 [M+H]$^+$ (calcd. for C$_{22}$H$_{31}$O$_5$, 375.2166).

2.4.2. Compound 2
Colorless oil; $\{\alpha\}_{D}^{25.6} \text{–} 7.5$ (c 0.11 CH$_3$OH); FT/IR $v_{\text{max}}$ (film) 3453, 1769, 1668, 1455 and 1016 cm$^{-1}$; $^1$H and $^{13}$C NMR (CDCl$_3$) data are listed in Table 1; HRNSIMS $m/z$ 3333.2054 [M+H]$^+$ (calcd. for C$_{20}$H$_{27}$O$_4$, 333.2060).

2.4.3. Compound 3
Colorless oil; $\{\alpha\}_{D}^{25.8} \text{–} 9.5$ (c 0.12 CH$_3$OH); FT/IR $v_{\text{max}}$ (film) 3452, 1769, 1731, 1715, 1651, 1455 and 962 cm$^{-1}$; $^1$H and $^{13}$C NMR (CDCl$_3$) data are listed in Table 1; HRNSIMS $m/z$ 375.2161 [M+H]$^+$ (calcd. for C$_{22}$H$_{31}$O$_5$, 375.2166).

2.5. Calculation of molecular mechanics
Implementation of the MM2 force field [27] in ChemBioOffice Ultra 12.0 software was used to calculate molecular models.

2.6. Anti-bacterial assay
The paper disc diffusion method [28] was used to evaluate the antibacterial activity of EtOAc crude extracts of the soft coral Lobophytum sp. The bacterial strains were provided by the Biological Resource Center (NRBC), Japan and cultured in an agar medium containing polypeptone (10 g/L), yeast (2 g/L), MgSO$_4.7$H$_2$O (1 g/L), and agar (15 g/L) prepared in distilled water. The medium was autoclaved and transferred into Petri dishes. The bacterial inoculum was evenly spread on the agar medium. Each methanolic solution of EtOAc crude extracts was perfused (50 µg/25 µL) to a sterilized disc (φ 8 mm, Toyo Roshi Kaisha, Ltd.,
After removal of the solvent, the disc containing EtOAc crude extracts was placed on the seeded bacterial lawn on the agar surface. The plate was incubated for 2 days at 30 °C and then the inhibition zone sizes were measured.

2.7. Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade. DMEM was purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). Casein, Griess reagents, 70% perchloric acid and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) were also purchased from Sigma-Aldrich. DMEM without phenol red was purchased from Thermo Fisher Scientific (Massachusetts, USA). Fetal bovine serum (FBS) was purchased from HyClone, Victoria, Australia. Streptomycin/penicillin was purchased from Funakoshi Co. Ltd. (Tokyo, Japan). All other chemicals mentioned hereafter were obtained from either Wako Pure Chemical Industries Ltd. (Osaka, Japan) or Kanto Chemical Co. Inc. (Tokyo, Japan).

2.8. Cell Culture

HeLa cell, human lung cancer cell (A549), melanoma cell (B16-F10), and murine macrophage cell (RAW 264.7) were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin (10,000 U/mL and 100 μg/mL). All cells were incubated at 37 °C in a humidified atmosphere containing 5% CO₂. In the bioassay, cells were maintained at 80% confluency.

2.9. Cell viability

The MTT assay was used to examine the cytotoxicity of compounds 1–3. All cells in exponential growth phase (HeLa, A459, B16-F10, and RAW 264.7) were seeded at a density of 10⁵ cells/mL in a 96-well plate. Cells were cultured for 12 h to permit attachment to the wells. Then, the culture medium was removed from the wells and 100 μL fresh culture medium containing different concentrations (5, 10, 25, 50, 100 and 200 μM) of compounds 1–3 was added. After a 48 h incubation, 10 μL MTT solution (0.5 mg/mL in phosphate buffer saline [PBS]) was added to each well followed by incubation at 37 °C for 4 h. To stop the reduction reaction and to dissolve the purple formazan, 100 μL SDS solution (10% in Milli-Q water) was added. A microplate reader (Thermos Scientific Multiskan Go) was used to measure the absorbance of each well at 570 nm. Cytotoxicity results were calculated, compared to the control, and expressed as cell viability (%).

2.10. Nitrite Assay

RAW 264.7 cells were used for the nitrate assay. Cells in exponential growth phase were plated on a 24-well plate in DMEM medium at a density of 5 × 10⁵ cells/500 μL and cultured for 12 h to attach to the wells. Then, the medium was removed and wells were washed with
PBS followed by addition of 500 μL culture medium containing: (i) control: 495 μL DMEM (without phenol red) and 5 μL LPS (Lipopolysaccharide); and (ii) treatment: 490 μL DMEM (without phenol red), 5 μL LPS and 5 μL compounds (1–3) solution (0.124, 0.25, 0.5, 1.0 and 2.0 mM). After a 24 h incubation, 100 μL cultured supernatant were transferred from the 24-well plate to a 96-well plate. An equal volume of Griess reagent (0.1% N-(1-naphtyl)-ethylenediamine and 1% sulfanilamide in 5% orthophosphoric acid) was added and incubated at room temperature for 10 min. A microplate reader (Thermos Scientific Multiskan Go) was used to measure the absorbance of each well at 540 nm. Nitrite concentration was determined from a standard curve of sodium nitrite.

2.11. Statistical Analysis
Data were expressed as mean ± SD. Statistical significance (p < 0.01) was analyzed by Student’s t-tests.

3. Results and Discussion
3.1. Structure elucidation and characterization of compounds 1–3

The soft coral, Lobophytum sp., was collected from Iriabu Island, Okinawa, and extracted with acetone. After evaporation of the acetone under reduced pressure (40 °C), the thick acetone extract was partitioned between ethyl acetate and water. The ethyl acetate soluble portion (50 μg/disk) inhibited the growth of Gram-negative bacteria (Escherichia coli) and Gram-positive bacteria (Staphylococcus aureus) with inhibition zones 12 and 18 mm, respectively. Repeated chromatography over SiO₂ followed by normal phase HPLC purification (ethyl acetate/hexane) of the active crude extract resulted in the isolation of new metabolites [(1, 0.021%, wet weight), (2, 0.012%) and (3, 0.008%)] along with known metabolites [(4, 0.018%), (5, 0.073%), (6, 0.01%), (7, 0.027%), (8, 0.097%) and (9, 0.007%)]. The known metabolites were identified by comparison of their NMR data with those reported elsewhere [23–26].

The molecular formula of 1 was determined as C₂₂H₃₀O₅ by high-resolution nanospray-ionization mass (HRNSIMS) [m/z 375.2169 (M+H)⁺, calcd. for C₂₂H₃₁O₅, 375.2166] containing eight degrees of unsaturation. The infrared (IR) absorption bands at 1769, 1739 and 1668 cm⁻¹ indicated the presence of carbonyl and olefinic groups in the molecule. The ¹H and ¹³C nuclear magnetic resonance (NMR) data (Table 1 and supplementary materials) suggested that 1 is a cembranolide diterpene derivative. The NMR spectra revealed 22 carbon signals consisting of two ester carbonyls [δC 169.8 (C-16) and δC 170.8 (-OAc)], six olefinic carbons [δC 130.6 (C-4), δC 129.3 (C-5) (δH 5.17 t, J=7.5 Hz), δC 124.1 (C-7) (δH 4.96 d, J=8.2 Hz), δC 136.4 (C-8), δC 133.6 (C-15), and δC 125.5 (C-17) (δH 6.50 s, 5.80 s)] including
a terminal olefine, four oxygenated carbons [δ_C 60.1 (C-12), δ_C 65.7 (C-13) (δ_H 2.73 d, J=8.9 Hz), and δ_C 73.0 (C-2) (δ_H 5.11 dt, J=2.4, 8.9 Hz), δ_C 80.8 (C-14) (δ_H 4.13 dd, J=2.4, 8.9 Hz)] including an epoxide group, one methine [δ_C 41.7 (C-1) (δ_H 3.41 m)], five methylenes [δ_C 39.8 (C-3) (δ_H 2.38 m, 2.25 m), δ_C 24.6 (C-6) (δ_H 2.13 m, 2.30 m), δ_C 39.8 (C-9) (δ_H 2.30 m, 1.91 m), δ_C 24.5 (C-10) (δ_H 2.13 m, 2.30 m), and δ_C 52.4 (C-11) (δ_H 1.26 m, 2.10 m)], an acetylmethyl [δ_C 21.1 (δ_H 2.00 s)], and three methyls [δ_C 17.1 (C-18) (δ_H 1.67 s), δ_C 15.8 (C-19) (δ_H 1.67 s), and δ_C 16.8 (C-20) (δ_H 1.30 s)].

Out of the eight degrees of unsaturation in 1, five were counted for three π-bonds and two ester carbonyls (Table 1). Thus, the remaining three degrees of unsaturation could be assigned to a tricyclic ring system in 1. Interpretation of correlation spectroscopy (COSY), three major spin systems were established [a: −CH(13)−CH(14)−CH(1)−CH(2)−CH(3), b: −CH(5)−CH(6)−CH(7), and c: −CH(9)−CH(10)−CH(11)] (Figure 2).

The heteronuclear multiple-bond-connectivity (HMBC) correlations of olefinic methyls (H3-18/C-3, -4, -5 and H3-19/C-7, -8, -9) and methyl (H3-20/C-11, -12, -13) easily completed a 14-membered cembrane ring. Additional HMBC correlations from an oxymethylene (H-2/C-16) and exomethylene (H2-17/C-1, -15, -16) finally completed a lactone ring attachment in the 14-membered cembrane. The acetoxy group was assigned at C-14 (HMBC H-14/OAc). To complete the tricyclic ring in 1, an epoxide ring was assigned between C-12/C13 (HMBC H-13/OAc). Thus, the planar structure of 1 was established as a tricyclic ring having a 14-membered cembrane, an α-methylene-γ-lactone, and a trisubstituted epoxide ring.

Based on relatively upfield δ_C values of CH3-18 and CH3-19 (< 20 ppm) [29, 30], the two double bonds at C-5 and C-7 were assigned as E geometry. Nuclear overhauser enhancement spectroscopy (NOESY) correlation between H-1 and H-2 indicated a cis-fused junction of the two rings (α-methylene-γ-lactone and 14-membered rings). In addition, the NOESY correlations among protons H-1/H-13, -14, -17; H-2/H-3, -14; H-14/H-2, -13, -20 and H3-20/H-13, -14 implied that these protons reside on the same face of the molecule (Figure 3). Moreover, the coupling constant (J= 8.9 Hz) at H-13, H-14 and H-2 supported that the vicinal protons were either in an anticoplanar or eclipse relationship. The relationship should be correct because these signals showed a strong NOESY correlation with each other.
The molecular formula of 2 was established as C$_{20}$H$_{28}$O$_{4}$ by HRNSIMS [m/z 333.2054 (M+H)$^+$, calcd. for C$_{20}$H$_{29}$O$_{4}$, 333.2060] with seven degrees of unsaturation. The IR absorption bands at 1769 and 1668 cm$^{-1}$ indicated the presence of carbonyl and olefinic groups. The NMR data (Table 1) suggested that 2 was also a cembranolide diterpene derivative having 20 carbon signals: an ester carbonyl [δ$_C$ 170.1 (C-16)], eight olefinic carbons [δ$_C$ 123.2 (C-3) (δ$_H$ 5.13 d, J=9.5 Hz), δ$_C$ 144.9 (C-4), δ$_C$ 123.4 (C-7) (δ$_H$ 4.90 t, J=7.2 Hz), δ$_C$ 134.4 (C-8), δ$_C$ 129.3 (C-10) (δ$_H$ 5.44 ddd, J=16.0, 7.5, 7.5 Hz), δ$_C$ 131.6 (C-11) (δ$_H$ 5.29 d, J=16.0 Hz, δ$_C$ 134.6 (C-15), and δ$_C$ 122.7 (C-17) (δ$_H$ 6.42 d, J=3.3 Hz, 5.79 d, J=3.0 Hz)] including a terminal olefine, three oxygenated carbons [δ$_C$ 76.6 (C-2) (δ$_H$ 5.02 t, J=9.5 Hz), δ$_C$ 84.4 (C-12), and δ$_C$ 65.5 (C-14) (δ$_H$ 4.01 t, J=6.7 Hz)], one methine [δ$_C$ 54.5 (C-1) (δ$_H$ 2.70 m)], four methylenes [δ$_C$ 39.6 (C-5) (δ$_H$ 2.31 m, 2.09 m), δ$_C$ 24.1 (C-6) (δ$_H$ 2.23 m), δ$_C$ 42.5 (C-9) (δ$_H$ 2.63 m), and δ$_C$ 44.0 (C-13) (δ$_H$ 2.48 dd, J=15.2, 6.7 Hz; 1.90 d, J=15.2 Hz)], and three methyls [δ$_C$ 15.8 (C-18) (δ$_H$ 1.73 s), δ$_C$ 17.3 (C-19) (δ$_H$ 1.64 s), and δ$_C$ 25.6 (C-20) (δ$_H$ 1.40 s)].

Both 1 and 2 showed comparable NMR data. However, the presence of two additional sp$^2$ carbons along with the lack of an oxygenated methine and acetyl carbons were clearly observed in the $^{13}$C NMR spectrum of 2. Four π-bonds and one ester carbonyl counted for five degrees of unsaturation. Thus, 2 might be a bicyclic molecule. Interpretation of COSY and HMBC correlations revealed a planar structure of 2 (Figure 2). Unlike 1 ($\Delta^{4,5}$), a strong COSY correlation (H-2/H-3) indicated that the double bond was indeed at C-3 in 2 ($\Delta^{3,4}$). Thus, the planar structure of 2 was established as a bicyclic ring consisting of a 14-membered cembrane and an α-methylene-γ-lactone ring.

Like 1, E geometries of the double bonds at C-3 and C-7 were assigned in 2, and a cis-fused ring junction between α-methylene-γ-lactone and 14-membered ring was assigned by NOESY correlation (H-1 and H-2). The coupling constant (J=16.0 Hz) between H-10 and H-11 allowed the E geometry of the double bond at C-10. Additional NOESY correlations (H-1/H-2, -13$_b$/-14, -17; H-2/H-1, -14, -18; H-14/H-1 -2, -13$_b$/-20 and H$_3$-20/H-13, -14) implied that these protons exist on the same plane of the molecule (Figure 3). The vicinal coupling (J= 9.5 Hz at H-2 and J= 6.7 Hz at H-14) are also support the strong NOESY correlations of H-2 and H-14 protons are cis orientation.

Compound 3 had the same molecular formula C$_{22}$H$_{30}$O$_{5}$ as 1 [HRNSIMS, m/z 375.2161 (M+H)$^+$, calcd. for C$_{22}$H$_{31}$O$_{5}$, 375.2166] with eight degrees of unsaturation. The IR absorption bands (1769, 1715 and 1668 cm$^{-1}$) of 3 suggested the presence of an α-methylene-γ-lactone and an ester moiety as in 1. The $^1$H and $^{13}$C NMR data (Table 1) showed 22 carbon signals as in 1. The NMR data of 3 compared with those of 1 showed close similarity with
those of 2. The COSY correlations supported major spin systems [a: −CH$_2$(14)−CH(1)−CH(2)−CH(3); b: −CH$_2$(5)−CH$_2$(6)−CH(7), and c: −CH(9)−CH(10)−CH(11)] (Figure 2). Like 1 and 2, the HMBC correlations (H$_3$-18/C-3, -4, -5; H$_3$-19/C-7, -8, -9; H$_3$-20/C-11, -12, -13; H-7/C-5) completed a 14-membered cembrane ring. Further, HMBC cross-peaks from methylenes (H$_2$-13/C-1, -12; H$_2$-14/C-1, -2), an oxymethine (H-2/C-16), and an exomethylene (H$_2$-17/C-1, -15, -16) finally completed an α-methylene-γ-lactone attachment in the 14-membered ring in 3. An HMBC correlation (H-8/OAc) confirmed the acetoxy group attachment at C-8. Thus, the planar structure of 3 was established (Figure 2).

As in 1 and 2, relatively upfield values of CH$_3$-18 (δ$_C$ 16.3) and CH$_3$-19 (δ$_C$ 10.4) indicated E geometry of the double bonds at C-3 and C-7 and coupling constant (J=15.5 Hz) between H-10 and H-11 allowed the E geometry of the double bond at C-10 in 3. Based on NOESY correlation between H-1 and H-2, a cis-fused ring junction between α-methylene-γ-lactone and 14-membered ring was concluded as in 1 and 2. In addition, NOESY correlations between H-2/H-14a; H-14a/H-1, -2, -9 and H-9/H-20 implied that these protons remain on the same face of the molecule (Figure 3).

The new isolates were evaluated for cytotoxicity against HeLa, A459, B16-F10, and RAW 264.7 cells (Table 2). The new compounds 1–3 exhibited moderate to mild cytotoxicity (Figures 4–6). The anti-inflammatory activity of 1–3 was also evaluated in LPS-stimulated RAW 264.7 macrophage cells at non-cytotoxic concentrations (Figure 7). These compounds suppressed NO production in a dose-dependent manner, indicating that they had an anti-inflammatory effect. NO production inhibition was relatively minor (IC$_{50}$, at 24 h, for 1–3 were 10.67, 13.92 and 14.02 µM, respectively) compared with alcyonolide congeners (2–8 µM) [31] and carotenoids (6.25–25 µM), such as fucoxanthin and fucoxanthinol [32].

4. Conclusion
Three new cembranolide diterpenoids (1–3) and the known diterpenoids (4–9) were isolated from the Okinawan soft coral, Lobophytum sp. Their structures were established by spectroscopic analysis (NMR, IR, and MS) and compared with those reported previously. The new isolates showed cytotoxicity against HeLa, A459, B16-F10, and RAW 264.7 cells and an anti-inflammatory effect in LPS-stimulated RAW 264.7 macrophage cells. Among them, 1 exhibited moderate cytotoxicity [IC$_{50}$ 5.99–10.83 µM] and anti-inflammatory activity [IC$_{50}$ 7.75 µM]. The epoxide group in 1 might be responsible for its mild bioactivities. Absolute stereochemistry of the asymmetric centers of these compounds remains to be solved.
Decomposition of compound 2 have prevented us to determine its absolute stereochemistry by modified Mosher’s method.

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Conflicts of Interest: The authors declare no conflict of interest.

Supplementary Materials:
Supplementary materials can be assessed at: http://www.***.com/

References


Table 1. $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR data for 1–3 in CDCl$_3$.

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Table 2. Cytotoxicity effect of compounds 1–3.

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Figure 1. Structures of compounds 1–9
Figure 2. Partial structures of 1–3 based on COSY (bold line) and key HMBC correlations (arrow).

Figure 3. Computer-generated models of 1–3 using MM2 force calculations and key NOESY correlations.
**Figure 4.** Cytotoxicity of 1 against HeLa, A459, B16-F10, and RAW 264.7 cells. Data represent the mean ± standard error of three replicates.

**Figure 5.** Cytotoxicity of 2 against HeLa, A459, B16-F10 and RAW 264.7 cells. Data represent the mean ± standard error of three replicates.
Figure 6. Cytotoxicity of 3 against HeLa, A459, B16-F10 and RAW 264.7 cells. Data represent the mean ± standard error of three replicates.

Figure 7. Inhibition of NO production in LPS-induced RAW 264.7 macrophage cells. Data represent the mean ± standard error of three replicates.
Graphical abstract