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A new dihydroxanthenone from a plant-associated strain of the fungus *Chaetomium globosum* demonstrates anticancer activity

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Abstract—Bioassay-guided fractionation of a cytotoxic EtOAc extract of the fungal strain, *Chaetomium globosum*, inhabiting the rhizosphere of the Christmas cactus, *Opuntia leptocaulis*, of the Sonoran desert afforded a new dihydroxanthenone, globosuxanthone A (**1**), a new tetrahydroxanthenone, globosuxanthone B (**2**), two new xanthones, globosuxanthone C (**3**) and D (**4**), 2-hydroxyvertixanthone (**5**), and two known anthraquinones (**6** and **7**). The structures of the new compounds **1**–**4** were elucidated by NMR and MS techniques, and the relative stereochemistry of **1** was determined by X-ray crystallographic analysis. Of the compounds encountered, **1** was found to exhibit strong cytotoxicity against a panel of seven human solid tumor cell lines, disrupt the cell cycle leading to the accumulation of cells in either G_2/M or S phase, and induce classic signs of apoptosis.

1. Introduction

Chaetomium is a large genus of the fungal family Chaetomiaceae which comprises over one hundred species.[1] Members of this filamentous fungal genus are commonly found in soil, air, and plant debris, and are encountered as causative agents of infections in humans.[2] As part of our ongoing studies to uncover potential anti-cancer agents from plant-associated microorganisms of the Sonoran desert,[3] an EtOAc extract derived from the fungal strain *Chaetomium globosum* Ames isolated from the rhizosphere of the Christmas cactus, *Opuntia leptocaulis* DC (Cactaceae), exhibited significant cytotoxicity against a panel of seven human cancer cell lines. Bioactivity-guided fractionation of this extract resulted in the isolation of a novel dihydroxanthenone which we named as globosuxanthone A (**1**), a tetrahydroxanthenone structurally related to **1** named globosuxanthone B (**2**), two new xanthones, globosuxanthones C (**3**) and D (**4**), 2-hydroxyvertixanthone (**5**), and the known anthraquinones, chrysazin (**6**) and 1,3,6,8-tetrahydroxyanthraquinone (**7**). Herein we report the isolation of **1**–**7**, structure elucidation of **1**–**4**, and initial evaluation of the anticancer activity of **1**.

Previous studies of soil-derived strains of *C. globosum* have resulted in the isolation of chaetomin,[4] chaetoglobosins A–B,[5] C–F,[6] G and J,[7] Q, R, and T,[8] 19-*O*-acetylchaetoglobosins B and D,[9] TAN-1142,[10] heptelidic acid,[11] dethio-tetra(methylthio)chetomin,[12] chaetoviridins A–D,[13] prenisatin,[14] PF1138 A and B,[15] chaetomanone and echinulin.[16] Recent studies on two endophytic strains of *C. globosum* have led to the isolation of globosumones A–C,[17] orsellinic acid,[17] orcinol,[17] trichodion,[17] chaetoglobosins C, F, E, and U,[18] and penochalasin.[18]

Keywords: Plant-associated fungus; *Chaetomium globosum*; *Opuntia leptocaulis*; Globosuxanthones A–D; Cytotoxicity; Cell-cycle disruptor.
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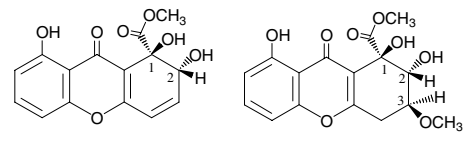
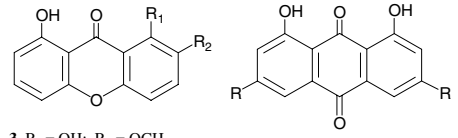
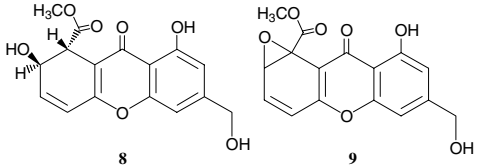


1 **2**



3 R_1 = OH; R_2 = OCH_3
4 R_1 = COOH; R_2 = H
5 R_1 = $COOCH_3$; R_2 = OH

6 R= H
7 R= OH

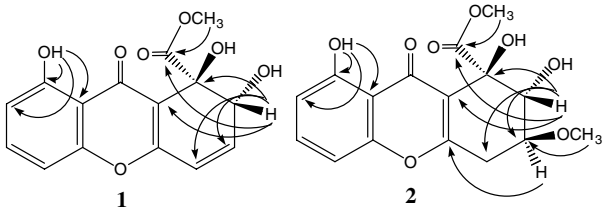


8 **9**

2. Results and discussion

The cytotoxic $CHCl_3$ fraction derived from an EtOAc extract of *C. globosum*, on gel permeation chromatography over Sephadex LH-20 followed by silica gel chromatography, led to the isolation of **1**–**7**. Globosuxanthone A (**1**) was obtained as a pale yellow crystalline solid that analyzed for $C_{15}H_{12}O_7$ by a combination of HRFABMS and ^{13}C NMR spectroscopy and indicated ten degrees of unsaturation. Its IR spectrum had absorption bands at 3440, 1734, 1654, and 1637 cm^{-1} suggesting the presence of hydroxyl, ester carbonyl, and conjugated carbonyl functions. ^1H NMR spectral data of **1** (in DMSO-d_6) revealed the presence of a hydrogen-bonded hydroxyl singlet (δ 12.40), three aromatic protons in an AMX spin system [δ 7.66 (t, J = 8.3 Hz), 7.07 (d, J = 8.3 Hz), and 6.81 (d, J = 8.3 Hz)], two olefinic protons [δ 6.62 (dd, J = 10.0, 4.3 Hz) and 6.49 (d, J = 10.0 Hz)], two hydroxyl groups [δ 5.85 (d, J = 8.4 Hz) and 5.70 (br s)], a proton attached to an oxygenated carbon [δ 4.26 (dd, J = 8.4, 4.3 Hz)], and a methoxy group (δ 3.58). On the basis of chemical shifts, coupling constants, and HMBC correlations (Fig. 1), the two olefinic protons, two hydroxyl groups, and the proton attached to an oxygenated carbon were assigned to a –CH=CH–CH(OH)–C(OH) spin system with Z configuration of the double bond. The ^{13}C NMR spectrum, while confirming the above functionalities in **1**, suggested the presence of a conjugated ketone carbonyl (δ 180.6), an ester carbonyl (δ 171.6), and two olefinic carbons (δ 114.6 and 159.8), of which one is oxygenated. In the HMBC spectrum of **1** (Fig. 1), the OCH_3 signal at δ_H 3.58 showed a correlation to the carbonyl carbon at δ_C 171.6 suggesting the presence of a methoxycarbonyl (CO_2CH_3) group. The presence of an HMBC correlation from H-2 (δ 4.26) to the ester carbonyl (δ 171.6) unambiguously placed the methoxycarbonyl group at C-1. Attempted derivatization of **1** under basic conditions yielded a product identified as 2-hydroxyvertixanthone (**5**),[19] the molecular formula ($C_{15}H_{10}O_6$) of which suggested that it was formed as a result of dehydration of **1**. Thus, globosuxanthone A (**1**) should have the same carbon skeleton as **5** with C-2 bearing a hydroxyl group. The relative configurations of the chiral centers C-1 and C-2 of **1** were determined to be 1R^*, 2R^* by single-crystal X-ray diffraction analysis (Fig. 2). Structure of globosuxanthone A was thus elucidated as 1R^*,2R^*,8-trihydroxy-1,2-dihydroxanthenone-1-carboxylic acid methyl ester (**1**). Prior to this report only two 1,2-dihydroxanthenones have been encountered in nature and both of these were from *Aspergillus* spp. AGI-B4 (**8**) isolated from *Aspergillus* sp. Y80118 has been reported to inhibit VEGF-induced endothelial cell growth,[20] while MS-347a (**9**) obtained from *Aspergillus* sp. KY52178 has been reported to be an inhibitor of myosin light chain kinase.[21]

Globosuxanthone B (**2**) was obtained as a colorless gum that analyzed for $C_{16}H_{16}O_8$ by a combination of HRFABMS and ^{13}C NMR spectroscopy and indicated nine degrees of unsaturation. Its IR spectrum had absorption bands at 3460, 1730, 1660, and 1590 cm^{-1} suggesting the presence of hydroxyl, ester carbonyl, and conjugated carbonyl groups. ^1H NMR spectral data of **2** indicated that it was structurally related to globosuxanthone A (**1**). The similarities included the presence of a hydrogen-bonded hydroxyl group, three aromatic protons in an AMX system, methyl ester group, and a proton attached to an oxygenated carbon atom. The major difference was found to be the absence of the two olefinic protons in **2**; instead it showed the presence of a CH_2 and an OCH_3 group. The HMBC correlation from OCH_3 protons (δ 3.53) to C-3 (δ 74.8) indicated the attachment of this group to C-3. Based on its structural similarity to **1**, the relative configurations of C-1 and C-2 of **2** were presumed to be R^*. The coupling constant observed for protons at C-2



1 **2**

Figure 1. Selected HMBC correlations for **1** and **2**.

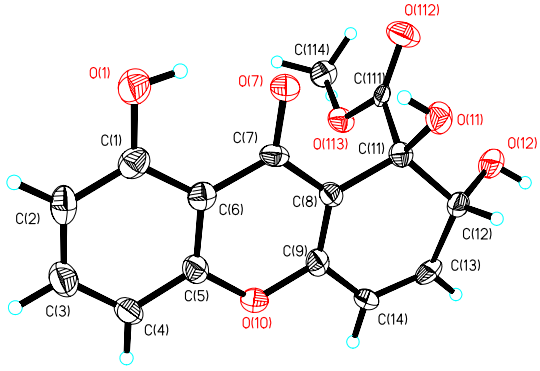


Figure 2. A perspective view of **1** by X-ray diffraction.

and C-3 ($J_{2,3}$ = 9.1 Hz) suggested that the relationship of these two protons is diaxial.[22,23] Therefore, the structure of globosuxanthone B was elucidated as 1*R**,2*R**,8-trihydroxy-3*S**-methoxy-1,2,3,4-tetrahydroxanthenone-1-carboxylic acid methyl ester (**2**). It is possible that **2** was formed from **1** during the extraction of the fungus with MeOH. In order to rule out this possible artifactual origin of **2**, compound **1** was heated with MeOH alone and in the presence of *p*-TSA and even after prolonged exposure to MeOH under these conditions, extensive TLC analysis indicated that **1** remained unchanged. Furthermore, the presence of only one diastereoisomer of **2** suggested that the C-3 methoxy group was introduced as a result of an enzymatic reaction.

Globosuxanthone C (**3**) was obtained as a yellow solid that analyzed for $C_{14}H_{10}O_5$ by a combination of HRFABMS and ^{13}C NMR spectroscopy and accounted for ten degrees of unsaturation. Its IR spectrum had absorption bands at 3400, 1654, 1631, and 1602 cm^{-1} suggesting the presence of hydroxyl and conjugated carbonyl groups. Characteristic UV spectral data and D_2O exchangeable ^1H NMR singlet at δ 11.97 indicated the presence of a 1-hydroxyxanthone moiety.[24] Remaining D_2O exchangeable singlet at δ 11.93 was placed at C-8, because of its low-field chemical shift due to hydrogen bonding with the carbonyl group. ^1H NMR spectrum showed signals for three adjacent aromatic protons in an AMX spin system at δ 7.57 (t, J = 8.3 Hz), 6.86 (d, J = 8.3 Hz), and 6.75 (d, J = 8.3 Hz), and two *ortho* coupled protons at δ 7.29 (d, J = 8.8 Hz) and 6.87 (d, J = 8.8 Hz). Remaining singlet at δ 3.92 for three protons was assigned for an OMe group and it was placed at C-2 with the help of HMBC correlations (Fig. 3). The structure of globosuxanthone C was thus elucidated as 1,8-dihydroxy-2-methoxyxanthone (**3**).

Globosuxanthone D (**4**) was obtained as a pale yellow solid, which analyzed for $C_{14}H_8O_5$ by a combination of HRFABMS and ^{13}C NMR spectroscopy and indicated eleven degrees of unsaturation. Its IR spectrum had absorption bands at 3400, 1695, 1645, and 1622 cm^{-1} suggesting the presence of hydroxyl, carboxylic acid carbonyl, and conjugated carbonyl groups. UV spectral data indicated it to be a xanthone derivative. ^1H NMR data suggested the presence of two AMX spin systems [δ 7.93 (t, J = 8.3 Hz), 7.72 (d, J = 8.3 Hz), and 7.41(d, J = 8.3 Hz)] and [δ 7.88 (t, J = 8.2 Hz), 7.10 (d, J = 8.2 Hz), and 6.85 (d, J = 8.2 Hz)]. Presence of a hydrogen-bonded hydroxyl group was indicated by the D_2O exchangeable singlet at δ 12.21 and based on its chemical shift it was placed at C-8. Remaining low-field singlet at δ 13.37 was assigned for the carboxylic acid proton. The foregoing spectral data together with HMBC correlations (Fig. 3) allowed the placement of the carboxylic acid group at C-1. The structure of globosuxanthone D was thus determined as 8-hydroxy-9-oxo-9*H*-xanthene-1-carboxylic acid (**4**). The known metabolites, 2-hydroxyvertixanthone (**5**), chrysazin (**6**), and 1,3,6,8-tetrahydroxyanthraquinone (**7**) were identified by comparison of their physical and spectral data with those reported in the literature.

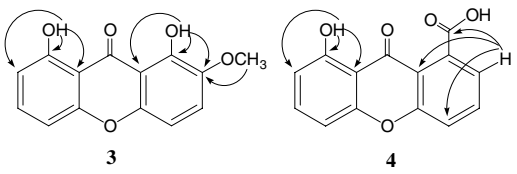


Figure 3. Selected HMBC correlations for **3** and **4**.

Compounds **1**–**7** were evaluated for in vitro cytotoxicity against a panel of seven human solid tumor cell lines and only globosuxanthone A (**1**) was found to have significant activity (Table 1). As an initial step in evaluating its potential as a lead molecule for drug development, we examined the reversibility of the cytotoxic activity of **1** using the mouse lung cancer cell line (3LL). Drug treatment for 4 h followed by wash-out and rescue with fresh medium demonstrated equivalent inhibition of cell growth/survival to that induced by continuous drug exposure for 72 h (Fig. 4). The irreversible nature of the cytotoxicity of **1** was confirmed by clonagenic assays (data not shown).[25]

To further characterize the anticancer activity of **1**, the cell cycle distribution of drug-treated cells was analyzed

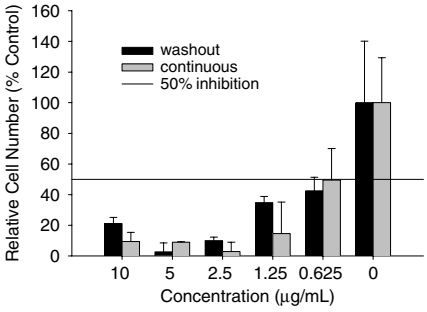


Figure 4. Globosuxanthone A (**1**) inhibits the proliferation/survival of Lewis Lung (3LL) tumor cells even after washout of the drug.

Table 1. Cytotoxicities (IC_{50}) of globosuxanthone (**1**) against a panel of seven human solid tumor cell lines[a]

Cell line[b]	NCI-H460	MCF-7	SF-268	PC-3	PC-3M	LNCaP	DU-145
1	3.6	1.3	1.1	0.65	1.1	1.5	1.2
Dox	0.01	0.07	0.04	ND	ND	ND	ND
Tax	0.01	0.01	0.02	ND	ND	ND	ND

[a] Results are expressed as IC_{50} values in μM; ND, not determined.

[b] Key: NCI-H460, non-small cell lung cancer; MCF-7, breast cancer; SF-268, CNS cancer (glioma); PC-3, hormone- (androgen) independent prostate adenocarcinoma; PC-3M, highly metastatic variant of PC-3; LNCaP, hormone-sensitive prostate cancer; DU-145, hormone-independent prostate cancer. Doxorubicin (Dox) and Taxol® (Tax) were used as positive controls.

by flow cytometry (Fig. 5). Drug exposure did not result in a well-defined block to progression, but rather accumulation of cells in both the S and G_2/M phases of the cycle. As seen in Figure 6, an increase in mitotic figures was not observed, indicating that drug-treated cells are not actually arrested in mitosis and that **1** does not appear to act directly as a spindle poison. In addition we found no evidence that **1** can form direct DNA adducts in vitro (data not shown), but we have not ruled out interaction with specific components of the DNA replication machinery at this point. Confocal microscopy of tumor cells treated with **1** did reveal many characteristic features of apoptosis including shrunken cell bodies as well as nuclear condensation and fragmentation (Fig. 6). Taken together, these preliminary biological findings suggest that globosuxanthone A (**1**) possesses promising anticancer activity, and merits further investigation. Indeed, we have completed a pilot study suggesting in vivo activity for **1**. Mice (C57/B16) were inoculated with 3LL (mouse Lewis Lung) tumor cells and **1** was administered as a single ip injection at 20 mg/kg on day 3 posttumor inoculation which resulted in ca. 50% reduction in mean tumor volume at 3 weeks compared to DMSO-treated mice. No evidence of systemic toxicity including weight loss was observed for the dose schedule examined. Due to the small number of animals used, however, inhibition of tumor growth did not reach statistical significance in this pilot study. We are continuing our studies to confirm these preliminary results and to elucidate the molecular mechanism(s) of action of globosuxanthone A (**1**).

3. Experimental

3.1. Chemistry

3.1.1. General procedures. Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO Dip-370 polarimeter using $CHCl_3$ or DMSO as solvent. IR spectra for KBr discs were recorded on a Shimadzu FTIR-8300 spectrometer. 1D and 2D NMR spectra were recorded in $CDCl_3$, acetone-d_6, and DMSO-d_6 using residual solvents as internal standards with a Bruker DRX-500 instrument at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. The chemical shift values (δ) are given in parts per million (ppm), and the coupling constants are in Hertz. Low resolution and high resolution MS were recorded, respectively, on Shimadzu LCMS QP8000α and JEOL HX110A spectrometers. The X-ray diffraction data were collected on a Bruker SMART 1000 CCD detector using graphite monochromated Mo-Kα radiation.

3.1.2. Fungal isolation, identification, and cultivation. The fungal strain was isolated from the rhizosphere of the Christmas cactus (*O. leptocaulis* DC.) growing in Tucson, Arizona, and was identified by Ms. Donna Bigelow (Division of Plant Pathology, Department of Plant Sciences, University of Arizona) as *C. globosum* by analysis of the ITS regions of the ribosomal DNA as described previously.[26] Excised roots of *O. leptocaulis* (1 cm long sections; ca. 5 g) were placed in 5 mL phosphate-buf-

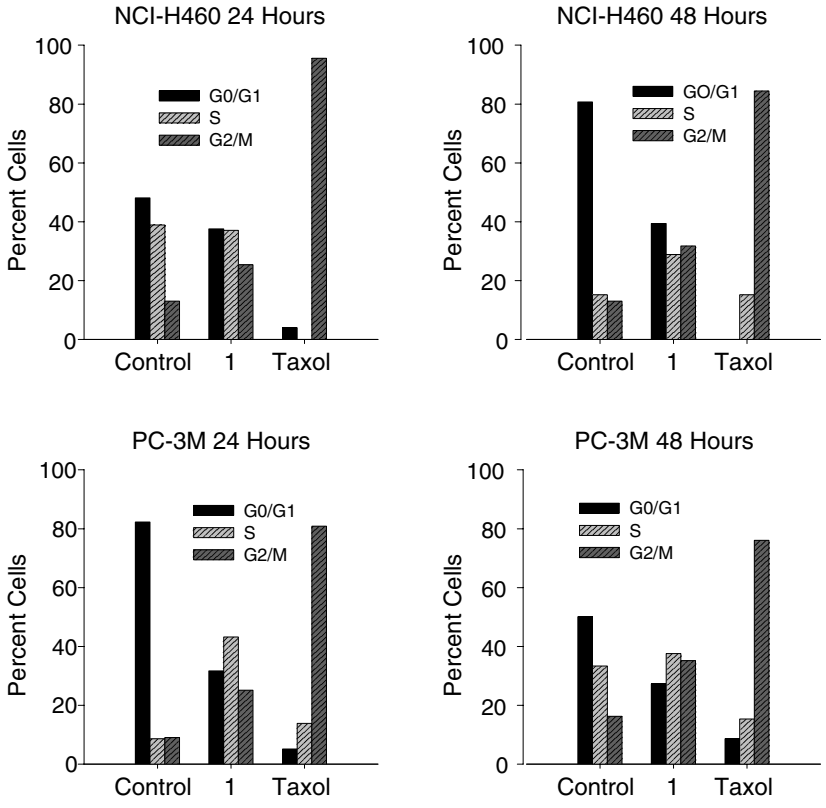


Figure 5. Globosuxanthone A (**1**) impairs cell cycle progression of NCI-H460 and PC-3M cells leading to accumulation in the G_2/M and S phases. Key: G_1-phase, pre-synthetic phase; G_2-phase, post-synthetic phase; S-phase, synthetic phase; M-phase, mitosis phase.

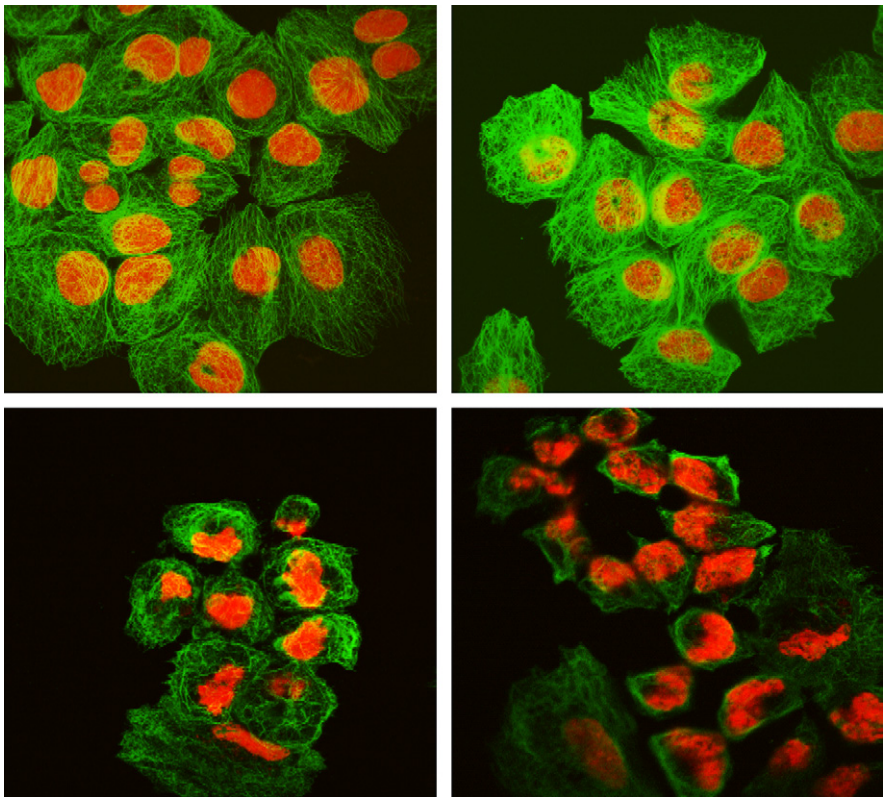


Figure 6. Confocal fluorescence microscopy of NCI-H460 cells treated with globosuxanthone (**1**). Control NCI-H460 cells demonstrate normal microtubule network organization (top left panel; green signal) and intact, homogeneously stained nuclei (red signal). As expected, exposure of these cells to Taxol® (9.5 nM) induced marked microtubule hyperpolymerization (top right panel). In contrast, exposure to **1** (3.6 μM for 24 h) resulted in classical evidence of apoptosis including shrunken cell bodies and nuclear condensation/fragmentation (bottom two panels).

fered saline (PBS, 0.1 M, pH 7.4) and microorganisms were detached from the roots by vortexing and sonication. A serial dilution of the suspension was placed on potato dextrose agar (PDA, Difco, Plymouth, MN) supplemented with chloramphenicol and streptomycin. After 4 days of incubation at 25 °C, single colonies were transferred to water agar containing the same antibiotics and after 3 days a pure culture of *C. globosum* was obtained by hyphal tipping. The strain is deposited in the Department of Plant Pathology and Southwest Center for Natural Products Research and Commercialization of the University of Arizona microbial culture collection under the accession number Opl-1-F17 (AH-45-00-F17). The organism was sub-cultured using Petri dishes with PDA and for long-term storage isolates were sub-cultured on PDA slants, overlaid with 40% glycerol, and stored at −80 °C. For isolation of bioactive compounds the fungus was cultured in 40 T-flasks (500 mL) each containing 135 mL of PDA coated on five sides of the T-flask (total surface area ca. 460 cm^2) for 28 days at 27 °C.

3.1.3. Extraction and isolation. Methanol (200 mL/T-flask) was added to all 40 T-flasks, briefly sonicated, and the resulting mixture was filtered through Whatman No. 1 filter paper and a layer of Celite 545. The filtrate was concentrated to one-fourth of its original volume and extracted with EtOAc (5 × 500 mL). Evaporation under reduced pressure afforded EtOAc extract (2.30 g), which was found to be cytotoxic. A portion (2.20 g) of the EtOAc extract was partitioned between hexane and 80% aqueous MeOH and the cytotoxic aqueous MeOH fraction was diluted to 60% aqueous MeOH by the addition of water and extracted with $CHCl_3$. Evaporation of solvents under reduced pressure yielded hexane (0.11 g) and $CHCl_3$ (1.68 g) fractions. A portion (1.00 g) of the cytotoxic $CHCl_3$ fraction was subjected to gel permeation chromatography over a column of Sephadex LH-20 (60.0 g) made up in hexane–CH_2Cl_2 (1:4) and eluted with hexane–CH_2Cl_2 (1:4) (500 mL), CH_2Cl_2–acetone (3:2) (250 mL), CH_2Cl_2–acetone (1:4) (250 mL), and finally with MeOH (250 mL). Forty-one fractions (20 mL each) were collected (F_1–F_{41}) of which fractions F_{10}–F_{26} were found to be cytotoxic. These fractions were combined and evaporated, and the resulting residue washed with MeOH to give globosuxanthone A (**1**) (190.7 mg). Fraction F_3 (23.9 mg) was separated by silica gel (1.0 g) column chromatography and elution with 10% hexane in CH_2Cl_2 followed by CH_2Cl_2 to give nine sub-fractions. Preparative TLC (silica gel, CH_2Cl_2) of the sub-fraction 4 yielded **3** as yellow solid (7.2 mg). Column chromatography of the fraction F_7 (26.6 mg) obtained above on silica gel (0.8 g) and elution with CH_2Cl_2 followed by CH_2Cl_2 containing increasing amounts of MeOH afforded eight sub-fractions. Sub-fraction eluted with 1% MeOH in CH_2Cl_2 on further purification by preparative TLC (silica gel, CH_2Cl_2–MeOH, 94:6) yielded **2** (8.2 mg). Fraction 28 (F_{28}; 25.6 mg) was separated by column chromatography on silica gel (1.5 g) using a gra-

dient of MeOH in CH_2Cl_2. Early fractions eluted with 2% MeOH in CH_2Cl_2 yielded **5** (7.3 mg). Middle fractions eluted with the same solvent system gave an additional quantity (13.8 mg) of **1**. A crystalline compound was separated from fraction 29 (F_{29}) and this when washed with CH_2Cl_2–MeOH (1:1) yielded **4** (3.2 mg). Fractions 38 and 39 (F_{38} and F_{39}) from the Sephadex column were combined to give **7** (7.3 mg). A portion (105 mg) of the hexane fraction obtained from the above solvent–solvent partitioning was chromatographed over a column of silica gel (4.0 g) made up in 20% hexane in CH_2Cl_2 and eluted with the same solvent followed by CH_2Cl_2. Ten milliliter fractions were collected. An early fraction eluted with 20% hexane in CH_2Cl_2 gave **6** (4.2 mg).

3.1.4. Globosuxanthone A (1). White solid; mp dec >233 °C; $[\alpha]_D^{25}$ −29.0 ° (c 0.2, DMSO); UV (MeOH) λ_{max} (log ε) 344 (4.77), 270 (5.42), 215 (5.24) nm; IR (KBr) υ_{max} 3440, 1734, 1655, 1638, 1595, 1560, 1475, 1446, 1278, 1190, 1168, 1085, 1035, 970, 818, 734 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ 12.4 (1H, s, OH-8), 7.66 (1H, t, J = 8.3 Hz, H-6), 7.07 (1H, d, J = 8.3 Hz, H-5), 6.81 (1H, d, J = 8.3 Hz, H-7), 6.62 (1H, dd, J = 10.0, 4.3 Hz, H-3), 6.49 (1H, d, J = 10.0 Hz, H-4), 5.85 (1H, d, J = 8.4 Hz, OH-2), 4.26 (1H, dd, J = 8.4, 4.3 Hz, H-2), 3.58 (3H, s, OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ 180.6 (C, C-9), 171.6 (C, $COOCH_3$), 159.8 (C, C-4a), 154.8 (C, C-5a), 141.3 (CH, C-3), 136.0 (CH, C-6), 119.7 (CH, C-4), 114.6 (C, C-9a), 111.2 (CH, C-7), 110.2 (C, C-8a), 107.3 (CH, C-5), 74.9 (C, C-1), 71.4 (CH, C-2), 51.7 (CH_3, OCH_3); HRFABMS m/z 305.2644 $[M+1]^+$ (calcd for $C_{13}H_{15}O_5$, 305.2644).

3.1.5. Globosuxanthone B (2). Colorless gum; $[\alpha]_D^{25}$ −38.3 ° (c 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 342 (3.35), 266 (4.99), 215 (4.78) nm. ^1H NMR (600 MHz, $CDCl_3$) δ 11.9 (1H, s, OH-8), 7.50 (1H, t, J = 8.3 Hz, H-6), 6.85 (1H, d, J = 8.3 Hz, H-5), 6.77 (1H, d, J = 8.3 Hz, H-7), 4.83 (1H,s, OH-2), 4.02 (1H, d, J = 9.1 Hz, H-2), 3.98 (1H, dd, J = 9.1, 5.6 Hz, H-3), 3.83 (3H, s, OCH_3), 3.53 (3H, s, OCH_3), 3.27 (1H, dd, J = 17.7, 5.6 Hz, H-4a), 2.96 (1H, s, OH-2), 2.76 (1H, dd, J = 17.7, 9.1 Hz, H-4b). ^{13}C NMR (125 MHz, $CDCl_3$) δ 182.1 (C, C-9), 172.1 (C, $COOCH_3$), 163.3 (C, C-4a), 160.7 (C, C-8), 156.1 (C, C-5a), 135.9 (CH, C-6), 117.3 (C, C-9a), 111.7 (CH, C-7), 110.2 (C, C-8a), 106.9 (CH, C-5), 76.8 (CH, C-2), 77.5 (C, C-1), 74.8 (CH, C-3), 57.9 (CH_3, OCH_3), 53.3 (CH_3, $COOCH_3$), 32.6 (CH_2, C-4); HRFABMS m/z 337.0938 $[M+1]^+$ (calcd for $C_{16}H_{17}O_8$, 337.0932).

3.1.6. Globosuxanthone C (3). Yellow solid; mp 142–144 °C; UV (MeOH) λ_{max} (log ε) 404 (4.37), 339 (4.73), 264 (5.38), 239 (5.22) nm; IR (KBr) υ_{max} 3055, 1654, 1631, 1602, 1577, 1490, 1463, 1319, 1440, 1280, 1238, 1218, 1085, 813, 725, 713 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 11.97 (1H, s, OH-1), 11.77 (1H, s, OH-8), 7.57 (1H, t, J = 8.3 Hz, H-6), 7.29 (1H, d, J = 8.8 Hz, H-3), 6.87 (1H, d, J = 8.8 Hz, H-4), 6.86 (1H, d, J = 8.3 Hz, H-5), 6.75 (1H, d, J = 8.3 Hz, H-7), 3.92 (3H, s, OMe-2). ^{13}C NMR (125 MHz, $CDCl_3$) δ 186.7 (C, C-9), 161.4 (C, C-8), 156.6 (C-5a), 150.2 (C, C-1), 149.7 (C, C-4a), 147.8 (C, C-2), 137.6 (CH, C-6), 121.3 (CH, C-3), 110.4 (CH, C-7), 108.1 (C, C-9a), 107.4 (C, C-8a), 107.1 (CH, C-5), 105.8 (CH, C-4); HRFABMS m/z 259.1041 $[M+1]^+$ (calcd for $C_{14}H_{11}O_5$, 259.1027).

3.1.7. Globosuxanthone D (4). Yellow solid, mp 245–246 °C; UV (MeOH) λ_{max} (log ε) 363 (4.45), 303 (4.62), 281 (4.59), 251 (5.16), 233 (5.19) nm; IR (KBr) υ_{max} 3060, 1695, 1645, 1622, 1597, 1489, 1462, 1437, 1412, 1271, 1223, 825, 765 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 13.37 (1H, s, COOH), 12.21 (1H, s, OH-8), 7.93 (1H, t, J = 8.3 Hz, H-3), 7.76 (1H, t, J = 8.2 Hz, H-6), 7.72 (1H, d, J = 8.3 Hz, H-2), 7.41 (1H, d, J = 8.3 Hz, H-4), 7.10 (1H, d, J = 8.2 Hz, H-5), 6.85 (1H, d, J = 8.2 Hz, H-7). ^{13}C NMR (125 MHz, $CDCl_3$) δ 186.7 (C, C-9), 169.4 (C, COOH), 160.7 (C, C-8), 155.4 (C, C-5a), 155.3 (C, C-4a), 137.7 (CH, C-6), 136.1 (CH, C-3), 122.5 (CH, C-4), 118.8 (CH, C-2), 115.9 (C, C-9a), 110.4 (CH, C-7), 108.3 (C, C-8a), 107.2 (CH, C-5); HRFABMS m/z 257.0447 $[M+1]^+$ (calcd for $C_{14}H_9O_4$, 257.0450).

3.1.8. 2-Hydroxyvertixanthone (5). Yellow solid; mp 244–246 °C; (lit.[19] 244–245 °C). ^1H NMR data were consistent with literature values;[19] APCIMS (+)ve mode m/z 287 $[M+H]^+$.

3.1.9. Chrysazin (6). Orange solid; mp 193-194 °C [lit.[27] 191–192 °C]; UV (MeOH) λ_{max} (log ε) 430 (4.33), 283 (4.31), 252 (4.60), 225 (4.94) nm. IR (KBr) υ_{max} 3400, 1626, 1601, 1469, 1442, 1284, 1267, 1209, 746 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 12.06 (2H, s, 2×OH), 7.83 (2H, d, J = 7.9 Hz, H-4 and H-5), 7.68 (2H, t, J = 7.9 Hz, H-3 and H-6), 7.29 (2H, d, J = 7.9 Hz, H-2 and H-7); APCIMS (+)ve mode m/z 241 $[M+1]^+$.

3.1.10. 1,3,6,8-Tetrahydroxyanthraquinone (7). Orange solid; mp 291 °C (dec) [lit.[28] 290 °C (dec)].[1] H NMR data were consistent with literature values;[19] APCIMS (+)ve mode m/z 273 $[M+H]^+$.

3.1.11. X-ray crystallographic data for globosuxanthone A (1). Crystal data for compound **1** at 170(2) K: $C_{15}H_{12}O_7$, M_r = 304.25, monoclinic, space group $P2_1$with a = 7.450 (3) Å, b = 7.775 (3) Å, c = 10.973 (4) Å, V = 633.0 (4) Å^3, Z = 2, D_{calc} = 1.596 mg/m^3. F_{000} = 316, λ (Mo Kα) = 0.71073 Å, μ = 0.129 mm^{-1}. Data collection and reduction: crystal size 0.211 × 0.16 × 0.12 mm, θ range 2.75–28.28°, 8225 reflections collected, 1669 independent reflections (R_{int} = 0.1070), final R indices ($I > 2\sigma(I)$): 0.0629, wR_2=0911 for 208 parameters, GOF = 1.021. Intensity data were measured on a Bruker SMART 1000 CCD diffractometer. Crystallographic data for the structure **1** in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 610092. Copies of the data can be obtained, free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [http:www.ccdc.cam.ac.uk/deposit; Fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

3.2. Biological studies

3.2.1. Cytotoxicity assays. A tetrazolium dye [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide; MTT]-based colorimetric assay was used to measure inhibition of the proliferation/survival of tumor cell lines in vitro as previously reported.[17,26] All test compounds were formulated in DMSO and applied to cells such that the final DMSO concentration was $\leqslant 0.1\%$. During bioassay-guided fractionation, the cytotoxicity of fractions was monitored using the NCI-H460 cell line.

3.2.2. Cell cycle analysis. NCI-H460 and PC-3M cells were harvested from flasks at 75–85% confluency, counted and plated into 60-mm dishes at a density of 2.5×10^5 cells/dish, and allowed to re-attach overnight. Compound **1** was prepared as a 26 mM stock solution in DMSO and applied to cells at final concentrations of 3.6 μM (NCI-H460) and 1.1 μM (PC-3M). The final concentrations of the positive control, Taxol®, were 9.5 nM (NCI-H460) and 0.6 nM (PC-3M). A control dish was treated with vehicle (DMSO) alone. Cells were incubated for 24 and 48 h in the continuous presence of the various treatments, at which times they were rinsed with PBS, harvested by trypsinization, resuspended in complete medium, pelleted by centrifugation, rinsed once with PBS, and resuspended in 1 mL of Krishan's buffer. Cells were analyzed for relative DNA content by flow cytometry using a Becton Dickinson FACScan instrument and cell cycle distribution calculated using ModFit software.

3.2.3. Confocal images. To complement flow cytometric data,NCI-H460 cell monolayers were established in a chamber slide and incubated overnight with Taxol® or globosuxanthone A (**1**). After treatment, the cells were fixed in cold methanol–acetone (1:1) and stained with propidium iodide (1 μg/mL) and fluorophore-conjugated mouse monoclonal antibody to β-tubulin. After staining, slides were rinsed with PBS, mounted, and confocal images acquired using a 40× oil objective (Nikon TE 300).

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