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Synthesis and Biological Evaluation of Polyenylpyrrole Derivatives as Anticancer Agents Acting through Caspases-Dependent Apoptosis

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A class of polyenylpyrroles and their analogues were designed from a hit compound identified in a fungus. The compounds synthesized were evaluated for their cell cytotoxicity against human non-small-cell lung carcinoma cell lines A549. Two compounds were found to exhibit high cytotoxicity against A549 cells with IC_{50} of 0.6 and 0.01 μM, respectively. The underlying mechanisms for the anticancer activity were demonstrated as caspases activation dependent apoptosis induction through loss of mitochondrial membrane potential, release of cytochrome *c*, increase in B-cell lymphoma-2-associated X protein (Bax) level, and decrease in B-cell lymphoma-2 (Bcl-2) level. The two compounds were nontoxic to normal human lung Beas-2b cells (IC_{50} > 80 μM), indicating that they are highly selective in their cytotoxicity activities. Furthermore, one compound showed in vivo anticancer activity in human-lung-cancer-cell-bearing mice. These results open promising insights on how these conjugated polyenes mediate cytotoxicity and may provide a molecular rationale for future therapeutic interventions in carcinogenesis.

Introduction

Cancer, being one of the leading causes of death globally, is a disease of worldwide importance. Although anticancer drugs have played a major role in the success stories in cancer treatment, there are still many types of cancers where effective molecular therapeutics are nonexistent. Hence, there is an impetus to identify and develop more potent therapeutic agents for cancer.

Activation of apoptotic pathways is a key method by which anticancer drugs kill tumor cells.[1] It is well-known that anticancer drugs can stimulate apoptotic signaling through two major pathways. One is the death receptor (extrinsic) pathway involving death receptor and death ligand interaction, such as Fas receptor (Fas[a]) and other members of the tumor-necrosis factor (TNF) receptor family. These receptors activate caspase-8 and subsequently caspase-3, the major caspases participating in the execution phase of apoptosis.[2] Another apoptotic pathway is the mitochondrial (intrinsic) pathway, which is activated by the release of proapoptotic factors from mitochondria intermembrane space such as cytochrome *c*.[3] The released cytochrome *c* interacts with apoptotic protease activating factor 1 (Apaf-1) and activates caspase-9, which in turn proteolytically activates downstream caspase-3.[4] Activated caspase-3 cleaves many substrates, including poly ADP-ribose polymerase (PARP), a DNA repair enzyme that leads to inevitable cell death. Recently, novel molecules that induce mitochondrial pathways of caspase activation have been developed in cancer chemotherapy.[5] Our interest in investigating natural products for their potential therapeutic effects has recently spurred us to examine the influences of conjugated polyenes on anticancer properties.

Conjugated polyenes is an interesting class of widely occurring natural products, as they have been shown to possess excellent biological properties such as antibacterial, antifungal, and antitumor activities.[6] Presently, some conjugated polyenes that are sold commercially include rapamycin and fumigillin.[7] In 2006, Capon and co-workers published a report on the isolation and structure elucidation of several polyenylfurans and polyenylpyrroles from the soil microbe *Gymnoascus reessii*.[8] In that study, they discovered three new conjugated polyenes, 12*E*-isorumbrin **1e**, gymnoconjugatins A and B, alongside rumbrin and auxarconjugatin A **1b** which were isolated previously (Figure 1).[9] **1b** and **1e** were subsequently

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[a] Abbreviations: Bax, B-cell lymphoma-2-associated X protein; Bcl-2, B-cell lymphoma-2; Fas, Fas receptor; TNF, tumor-necrosis factor; Apaf-1, apoptotic protease activating factor 1; PARP, poly ADP-ribose polymerase; NS-1, nonstructural protein 1; DMP, Dess−Martin periodinane; DMSO, dimethylsulfoxide; THF, tetrahydrofuran; TEA, triethylamine; DMF, dimethylformamide; DIBAL, diisobutylaluminum hydride; IBX, iodoxybenzoic acid; NCS, *N*-chlorosuccimide; Pd_2dba_3, tris(dibenzylideneacetone)-dipalladium(0); TBAF, tetrabutylammonium fluoride; NMP, *N*-methyl-2-pyrrolidone; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; PI, propidium iodide; Z-VAD-fmk, carbobenzoxyvalylalanylaspartyl[*O*-methyl]fluoromethylketone; ROS, reactive oxygen species; NAC, *N*-acetylcysteine; DIDS, 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid; $DiOC_2(3)$, 3,3′-diethyloxacarbocyanine iodide; dATP, deoxyadenosine triphosphate; Bak, Bcl-2 homologous antagonist killer; PTP, permeability transition pore; IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane; Bid, Bcl-2 homology-3 interacting domain death agonist; sc, subcutaneous; TMS, tetramethylsilane; H_2DCFDA, dichlorodihydrofluorescein diacetate RPMI 1640, Roswell Park Memorial Institute 1640; PBS, phosphate buffered saline; PVDF, polyvinylidene fluoride.

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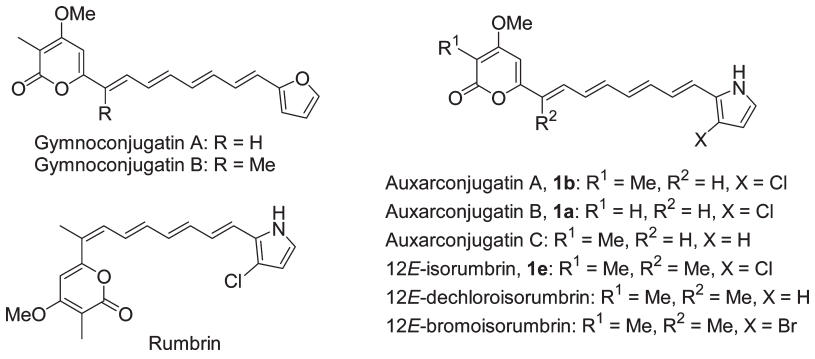
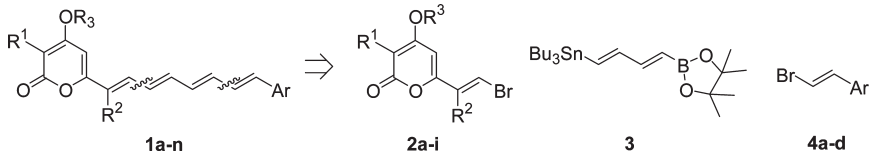


Figure 1. Structures of auxarconjugatin, 12*E*-isorumbrin, and related polyenes.

Scheme 1. Retrosynthesis of Auxarconjugatin and Its Analogues **1a**−**n**



found to possess potent cytotoxicity properties against non-structural protein (NS-1) cell line, while earlier studies by Yamagishi and co-workers[9a] have demonstrated that rumbrin was able to provide cytoprotection against cell death caused by calcium overload. Other related polyenylpyrroles that had been isolated previously include 12*E*-bromoisorumbrin, 12*E*-dechloroisorumbrin, auxarconjugatin B **1a**, auxarconjugatin C, and malbranpyrroles.[10] Unlike **1b** and **1e**, 12*E*-bromoisorumbrin, 12*E*-dechloroisorumbrin, and gymnoconjugatins A and B were absent of cytotoxicity activity, implying the importance of the 3-chloropyrrole moiety in effecting cytotoxicity in cancer cell lines.

Thus far, the main source of conjugated polyenes has been from the isolation of fungi or bacteria. The typically small quantities that can be obtained via these sources often limit the extent of biological work that can be carried out. To address this limitation as well as to provide access to structurally diverse analogues of these compounds, it would be useful to develop a synthetic strategy that allows conjugated polyenes to be synthesized expediently. To the best of our knowledge, there is presently only one reported synthesis of gymnoconjugatins A and B[11] and there is no literature describing the synthesis of polyenylpyrroles such as **1b** and **1e**. This, together with the promising cytotoxicity properties of **1b** and **1e**, prompted us to synthesize a class of polyenylpyrroles and their analogues where the 3-chloropyrrole is replaced with other 2- or 3-chlorosubstituted aromatic rings. Hence, we herein describe the synthesis of these polyenyl compounds and their in vitro and in vivo antitumor activity against human non-small-cell lung carcinoma cell lines A549. The mechanism of the active compounds' action on death of these cells was also examined.

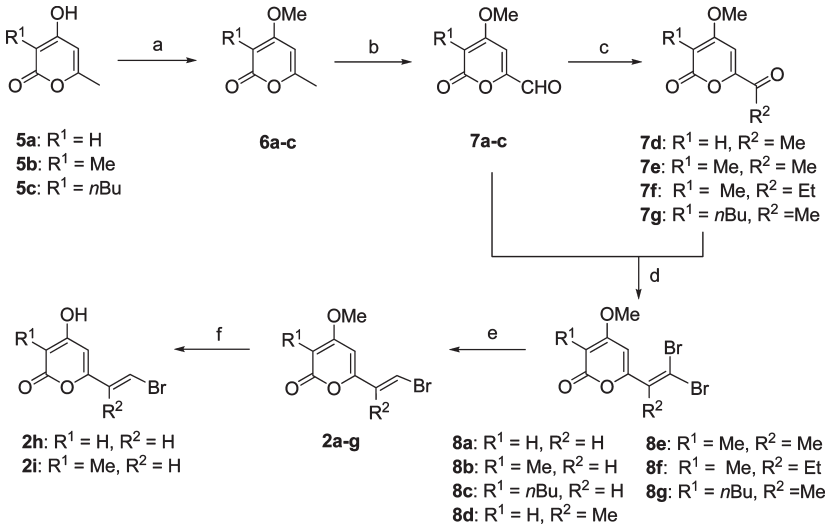
Results and Discussion

Chemistry. The retrosynthetic route of auxarconjugatin and its analogues **1** (Scheme 1) was modified from the synthesis of gymnoconjugatin.[11] Disconnection of the tetraene gave three fragments: pyrone **2**, the central butadiene connector **3**, and vinyl bromide **4**. It had been shown earlier that the hetero-bis-metallated butadiene **3** could be used for the synthesis of an extended polyene chain via sequential Stille and Suzuki coupling reactions.[12] With this strategy in mind, we proceeded with the synthesis of **1**.

Pyrones **2a**−**i** were prepared from the respective 4-hydroxy-6-methylpyran-2-ones **5a**−**c**[13] (Scheme 2). Methylation of the hydroxyl group on **5a**−**c** was achieved using dimethyl sulfate to afford **6a**−**c**. The oxidation of **6a**−**c** to **7a**−**c** was modified from a procedure reported earlier.[11] Instead of conventional heating in a sealed tube, we applied microwave irradiation which resulted in shorter reaction times with improved yields. To introduce diversity at the R^2 position for **2**, compounds **7a**−**c** were treated with MeMgBr or EtMgBr followed by oxidation of the resulting alcohol using Dess−Martin periodinane (DMP) to afford **7d**−**g**. Attempts to convert the aldehyde moiety on **7a** and **7b** directly to a vinyl iodide group via Takai olefination failed to provide the desired compound. Hence, to synthesize **2**, compounds **7a**−**g** were first converted to vinyl dibromides **8a**−**g** via Corey−Fuchs olefination followed by reduction using dimethylphosphite.[14] This afforded **2a**−**g** in excellent yields and *E*/*Z* ratio greater than 20:1. Further treatment of compounds **2a** and **2b** with a mixture of aqueous HBr and acetic acid gave the demethylated products **2h** and **2i**, respectively.

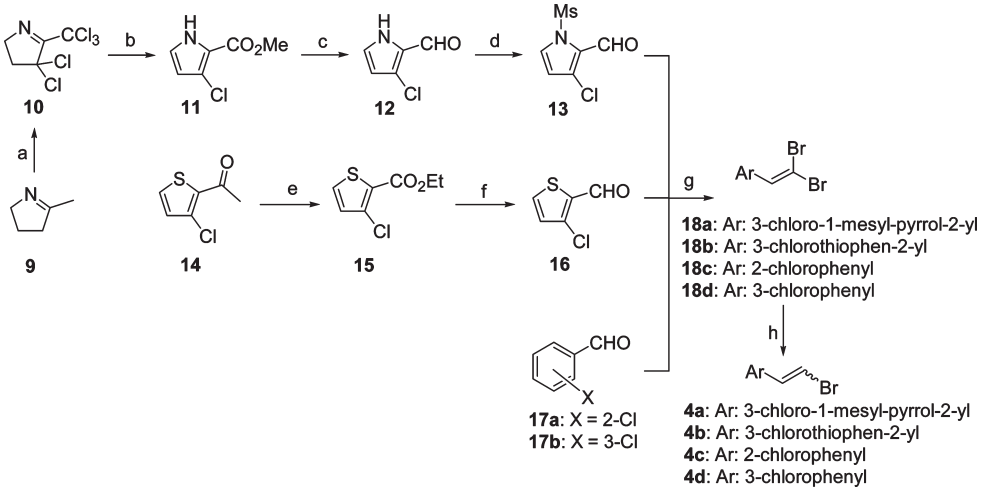
The second coupling partner, pyrrole **4a**, was synthesized from commercially available 2-methyl-1-pyrroline **9** (Scheme 3). The conversion of **9** to compound **11** was adapted from an earlier report.[15] Using THF instead of CCl_4 as a solvent for the chlorination of **9** led to a more than 200-fold increase in reaction rate to provide **10** which was used directly for the synthesis of **11** in excellent yield. Initial attempts to reduce **11** directly to the aldehyde **12** in a single step by using diisobutylaluminum hydride (DIBAL) failed, and the fully reduced alcohol was obtained as the major product. To obtain **12**, we therefore attempted to reduce **11** completely to the alcohol with $LiAlH_4$ and then oxidize the alcohol to the aldehyde **12** with DMP. Unfortunately, the addition of DMP led to the immediate decomposition of the alcohol. This could be attributed to the polymerization of pyrrole in the presence of the acetic acid which was formed as a byproduct of the DMP oxidation.[16] Addition of sodium bicarbonate[17] and pyridine[18] to neutralize the acetic acid byproduct did not resolve the problem. Thus, to circumvent this problem, we

Scheme 2. Synthesis of Pyrones **2a**−**i**[a]



[a] Reagents and conditions: (a) Me_2SO_4, K_2CO_3, DMSO, room temp; (b) SeO_2, dioxane, 150−160 °C; (c) (i) R^2MgBr in Et_2O, THF, room temp; (ii) DMP, CH_2Cl_2, room temp; (d) CBr_4, PPh_3, CH_2Cl_2, room temp; (e) dimethylphosphite, TEA, DMF, room temp; (f) aq HBr, AcOH, 90 °C.

Scheme 3. Synthesis of **4a**−**d**[a]



[a] Reagents and conditions: (a) NCS, THF, 55 °C; (b) (i) NaOMe, MeOH, 0 °C to room temp; (ii) aq HCl; (c) (i) $LiAlH_4$, THF, −20 °C to room temp; (ii) IBX, $NaHCO_3$, DMSO, room temp; (d) NaH, MsCl, THF, room temp; (e) (i) CuO, I_2, pyridine, EtOH, reflux; (ii) K_2CO_3, reflux; (f) (i) $LiAlH_4$, THF, 0 °C; (ii) DMP, CH_2Cl_2, room temp; (g) CBr_4, PPh_3, CH_2Cl_2, room temp; (h) dimethylphosphite, TEA, DMF, room temp.

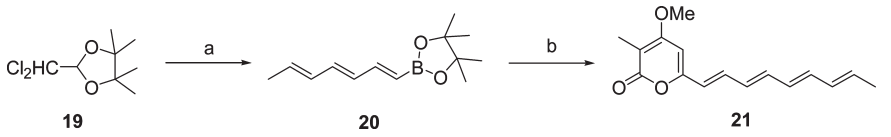
tried 2-iodoxybenzoic acid (IBX) which gratuitously gave **12** in moderate yields. The addition of excess sodium bicarbonate to the reaction mixture to neutralize the acidic conditions further improved the yield of **12**. With compound **12** in our hands, we proceeded to synthesize the corresponding vinyl iodide via Takai olefination. However, in the course of drying the vinyl iodide, polymerization occurred and a dark tar was obtained. This problem was subsequently resolved by first protecting **12** with a mesyl group whose electron-withdrawing property served to stabilize the pyrrole for subsequent transformations.

Earlier studies have shown that the 3-chloropyrrole group plays an important role in the cytotoxicity effects of **1b** and **1e**. When the chloro group was substituted with a bromo or hydrogen or when the 3-chloropyrrole moiety was substituted with a furan ring, the activity was drastically reduced.[11,19] To study other ring systems besides pyrrole, we replaced 3-chloropyrrole with other 2- or 3-chloro substituted aromatic rings. Compound **15** was prepared by the oxidation of 2-acetyl-3-chlorothiophene **14** (Scheme 3).[20] Reduction of **15** with $LiAlH_4$ followed by oxidation with DMP gave compound **16**. Corey−Fuchs olefination of **13**, **16**, **17a**, and **17b** gave the corresponding vinyl dibromides **18a**−**d**, and subsequent reduction of **18a**−**d** with dimethylphosphite afforded **4a**−**d**. Compound **4a** and **4b** were obtained as a ∼2:1 mixture of *E* and *Z* stereoisomers, but for **4c** and **4d**, the *E* isomer was obtained in greater than 9:1 ratio.

To establish if the cytotoxic effects of **1a** would be affected if the 3-chloropyrrole group was replaced by a methyl group (Scheme 4), compound **21** was synthesized. This synthesis involved the Takai olefination of 2,4-hexadienal with **19**[21] to afford **20** which was then reacted with **2b** via Suzuki coupling to provide **21**.

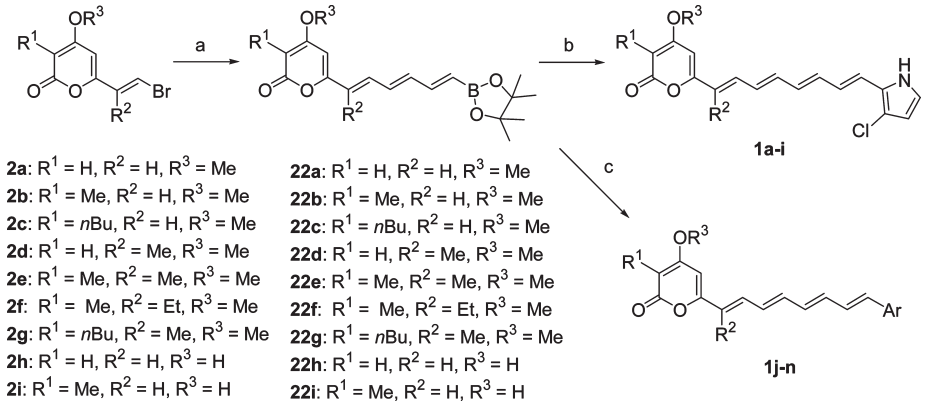
Pyrones **2a**−**i** was treated with **3** via Stille coupling to afford trienes **22a**−**i**. Suzuki coupling of **22a**−**i** with **4a** followed by treatment with tetrabutylammonium fluoride (TBAF) to remove the mesyl group afforded **1a**−**i** (Scheme 5). Compounds **1j**−**n**, bearing other aromatic rings besides 3-chloropyrrole,

Scheme 4. Synthesis of **21**[a]



[a] Reagents and conditions: (a) 2,4-hexadienal, $CrCl_2$, LiI, THF, room temp; (b) **2b**, Pd_2dba_3, $AsPh_3$, aq KOH, THF, room temp.

Scheme 5. Synthesis of **1a**−**n**[a]

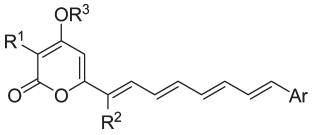


2a: R^1 = H, R^2 = H, R^3 = Me
2b: R^1 = Me, R^2 = H, R^3 = Me
2c: R^1 = *n*Bu, R^2 = H, R^3 = Me
2d: R^1 = H, R^2 = Me, R^3 = Me
2e: R^1 = Me, R^2 = Me, R^3 = Me
2f: R^1 = Me, R^2 = Et, R^3 = Me
2g: R^1 = *n*Bu, R^2 = Me, R^3 = Me
2h: R^1 = H, R^2 = H, R^3 = H
2i: R^1 = Me, R^2 = H, R^3 = H

22a: R^1 = H, R^2 = H, R^3 = Me
22b: R^1 = Me, R^2 = H, R^3 = Me
22c: R^1 = *n*Bu, R^2 = H, R^3 = Me
22d: R^1 = H, R^2 = Me, R^3 = Me
22e: R^1 = Me, R^2 = Me, R^3 = Me
22f: R^1 = Me, R^2 = Et, R^3 = Me
22g: R^1 = *n*Bu, R^2 = Me, R^3 = Me
22h: R^1 = H, R^2 = H, R^3 = H
22i: R^1 = Me, R^2 = H, R^3 = H

[a] Reagents and conditions: (a) **3**, Pd_2dba_3, $AsPh_3$, NMP, room temp; (b) (i) **4a**, Pd_2dba_3, $AsPh_3$, aq KOH, THF, room temp; (ii) TBAF, THF, room temp; (c) **4a**−**d**, Pd_2dba_3, $AsPh_3$, aq KOH, THF, room temp.

Table 1. Cytotoxicity of Conjugated Polyenes against Human Lung Cancer A549 Cells[a]



compd	R^1	R^2	R^3	Ar	IC_{50} (μM)
auxarconjugatin B, **1a**	H	H	Me	3-chloropyrrol-2-yl	0.6
auxarconjugatin A, **1b**	Me	H	Me	3-chloropyrrol-2-yl	1.2
1c	*n*-Bu	H	Me	3-chloropyrrol-2-yl	5.0
1d	H	Me	Me	3-chloropyrrol-2-yl	2.5
12*E*-isorumbrin, **1e**	Me	Me	Me	3-chloropyrrol-2-yl	5.2
1f	Me	Et	Me	3-chloropyrrol-2-yl	5.6
1g	*n*-Bu	Me	Me	3-chloropyrrol-2-yl	0.01
1h	H	H	H	3-chloropyrrol-2-yl	>20
1i	Me	H	H	3-chloropyrrol-2-yl	>20
1j	H	H	Me	3-chlorothiophen-2-yl	>20
1k	Me	H	Me	3-chlorothiophen-2-yl	>20
1l	Me	H	Me	2-chlorophenyl	>20
1m	Me	H	Me	3-chlorophenyl	>20
1n	Me	H	Me	3-chloro-1-mesylpyrrol-2-yl	>20
21	Me	H	Me	H	>20

[a] IC_{50} value expressed as the mean value of triplicate wells from at least three experiments.

were synthesized in a similar manner. Interestingly, the ^1H NMR of crude **1a**−**n** showed that the *E* stereoisomer of the respective compound was present in 75−85%. As the *E* stereoisomers of **4a**, **4b**, and **4c**,**d** were present in about 66%, 66%, and >90%, respectively, this indicated that isomerization could have occurred during the coupling process. Table 1 shows the 15 auxarconjugatin analogues **1a**−**n** and **21** synthesized.

Biological Results. Cytotoxicity. Compounds **1a**−**n** and **21** were evaluated for their cytotoxicities against the human lung cancer cell line A549 after 48 h of treatment. As shown in Table 1, the two most potent compounds are **1a** and **1g** with IC_{50} values of 0.6 and 0.01 μM respectively, indicating that these compounds are more potent against A549 cell lines than antitumor drugs like Gleevec (IC_{50} = 2−3 μM) and cisplatin (IC_{50} = 64 μM).[22] The loss of activity in compounds **1j**−**n**, where other chloro-substituted aromatic rings were present instead of 3-chloropyrrole, supported our hypothesis that the latter group played an important role in effecting cytotoxicity. In addition, the lack of cytotoxicity in compounds **1h** and **1i** also illustrated the importance of a methyl group at the R^3 position.

To further explore the selectivity of the compounds against A549 cells, the respective compound **1** with IC_{50} value less than 1 μM was further examined against Beas-2b cells which were derived from normal human bronchial epithelial cells.

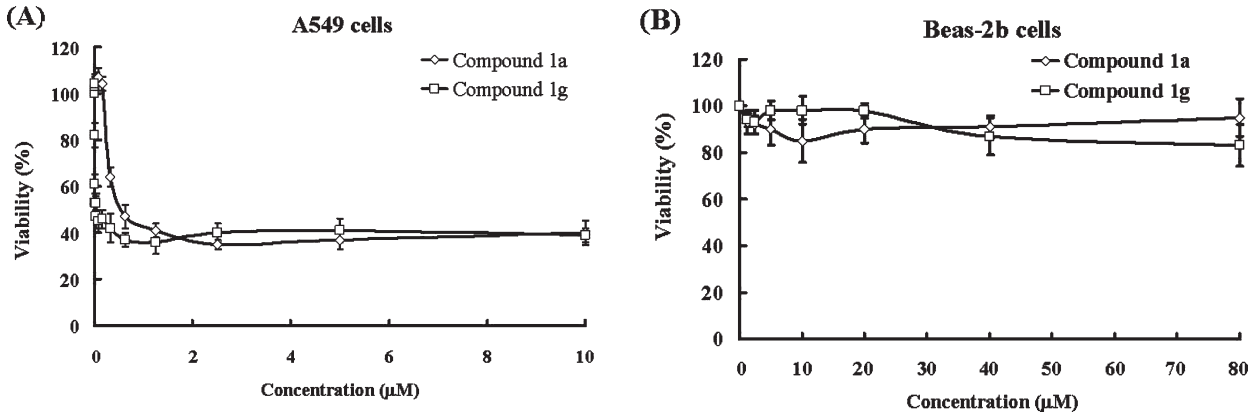


Figure 2. Compounds **1a** and **1g** were noncytotoxic to normal human lung cells. (A) A549 cells (5×10^3 cells) and (B) normal human lung cells Beas-2b (5×10^3 cells) were seeded in 96-well plates and treated with compounds **1a** and **1g** (0−80 μM) or vehicle (0.1% DMSO) for 48 h. Cell viability was measured by proliferation assay. The data are expressed as the mean ± SE of three separate experiments.

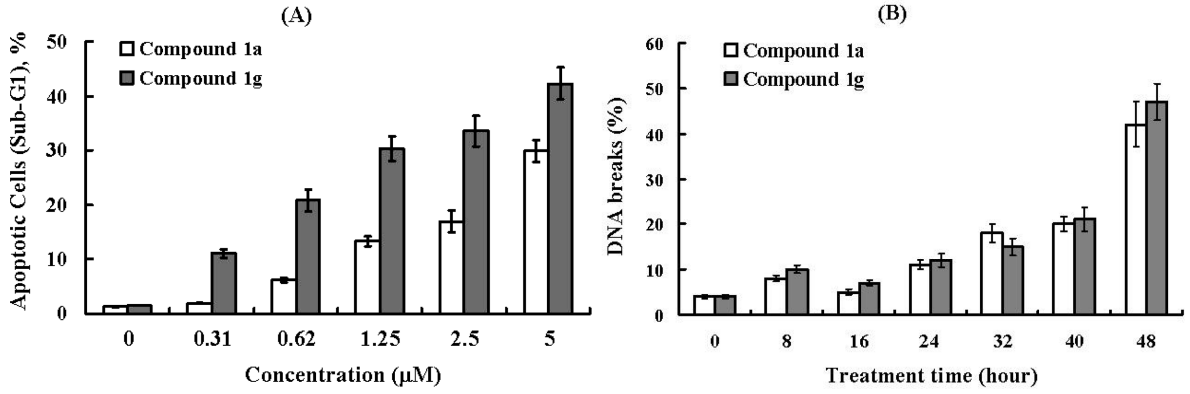


Figure 3. Compounds **1a** and **1g** induced apoptosis in human lung cancer cells. (A) A549 cells were treated with compounds **1a** or **1g** (0−5 μM) or vehicle (0.1% DMSO) for 48 h. The cell population in the sub-G1 phase was determined by flow cytometry after PI-staining of nuclei. The data are expressed as the mean ± SE of three separate experiments. (B) A549 cells were treated with **1a** or **1g** (5 μM) for various time points, and the DNA breaks were analyzed by flow cytometry based TUNEL assay. One of three repeated experiments is shown.

As can be seen in Figure 2, we found that compounds **1a** and **1g**, despite being very potent against A549 cells (0.6 and 0.01 μM, respectively) (Figure 2A), were found to be noncytotoxic toward Beas-2b cells at up to 80 μM (Figure 2B). These results indicated that compounds **1a** and **1g** have the potential to be developed as anticancer agents because of their high selectivity against A549 cells.

Apoptosis Induction in Human Non-Small-Cell Lung Carcinoma. To gain further insight into the mode of action of these compounds, two assays targeting hallmarks of apoptosis, namely, the cell cycle analysis and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, were performed. A549 cells were treated with **1a** or **1g** (0.31−5 μM) for 48 h, and the cell cycle distribution was determined by flow cytometry after propidium iodide (PI) staining of the nuclei. The results obtained (Figure 3A) show that the cells in sub-G1 phase increased in a concentration-dependent manner after treatment with **1a** or **1g**. These results are also consistent with the data obtained from the cytotoxicity assay in that **1g** is more potent against A549 cells than **1a**. The concentrations of the compounds **1a** and **1g** required to elicit hypoploidy are higher than the IC_{50} values for cell proliferation assays. This is because the concentration at nanomolar range may inhibit cell proliferation but the concentration at micromolar range may induce cell apoptosis. To confirm the proapoptotic activity of compounds **1a** and **1g**, DNA breaks phenomenon was analyzed by flow cytometry based TUNEL assay in **1a**- and **1g**-treated A549 cells. We found that at 5 μM, **1a** and **1g** induced DNA breaks from 24 h treatment and then dramatically increased cell population with DNA breaks. The TUNEL positive cells for **1a**- and **1g**-treated A549 cells increased to 42% and 47%, respectively (Figure 3B). In addition, autophagic cell death was not observed in **1a**- and **1g**-treated A549 cells (Supporting Information).

Caspases Activation in Apoptosis. To investigate whether compounds **1a** and **1g** induced apoptosis via caspases-dependent pathway, the effects of pan caspase inhibitor carbobenzoxyvalylalanylaspartyl[*O*-methyl]fluoromethylketone (Z-VAD-fmk) on compounds **1a** and **1g** treated cells were tested. As shown in Figure 4, Z-VAD-fmk dramatically blocked sub-G1 phase increase in **1a**- and **1g**-treated cells. In contrast, reactive oxygen species (ROS) inhibitors NAC and DIDS (*N*-acetylcysteine (NAC), antioxidant; 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid (DIDS), anion channel inhibitor which blocks ROS release from mitochondria) did not reduce the sub-G1 phase increase in **1a**- and **1g**-treated cells (Figure 4). These results imply that compounds **1a** and **1g** induced A549 apoptosis through a caspases-dependent pathway. The role of oxidative stress in apoptosis has been controversial. Although ROS have been generally regarded to be proapoptotic in nature by showing a protective effect of antioxidants on apoptosis in various cell types,[23] it has been

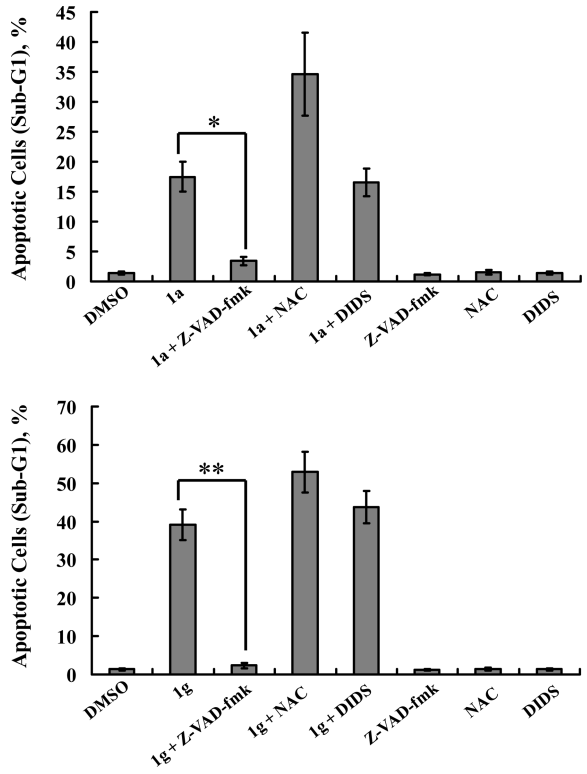


Figure 4. Compounds **1a** and **1g** induce caspases-dependent apoptosis in human lung cancer cells. A549 cells were treated with **1a** or **1g** (5 μM) or vehicle (0.1% DMSO) in the presence or absence of Z-VAD-fmk (20 μM), NAC (10 mM), or DIDS (10 μM) for 36 h. The cell population in the sub-G1 phase was determined by flow cytometry after PI-staining of nuclei. The data are expressed as the mean ± SE of three separate experiments.

recently suggested that ROS may also play an antiapoptotic and protective role.[24] Our results also clearly indicate that antioxidative property of NAC can exert a proapoptotic effect in compounds **1a** and **1g** induced A549 apoptosis.

Induction of Mitochondrial Death Pathway. Mitochondria play an important role in cell death by changing its outer and inner membrane permeability and thus leading to cytochrome *c* release and caspases activation.[25] To explore whether compounds **1a** and **1g** induced apoptosis via the mitochondrial signaling pathway, the mitochondrial membrane potential alteration was determined using a mitochondria-specific fluorescence dye, 3,3′-diethyloxacarbocyanine iodide ($DiOC_2(3)$). A549 cells treated with 5 μM **1a** or **1g** were found to demonstrate a loss of fluorescence intensity with time (Figure 5A), indicating that **1a** and **1g** induced a loss of mitochondrial membrane potential. Activation of the mitochondrial death pathway can also be identified by the release of mitochondrial cytochrome *c*. After cytochrome *c* is released from the mitochondria, it can bind to deoxyadenosine triphosphate (dATP) and Apaf-1 and activate caspase-9 and caspase-3.[26] We thus investigated the release of cytochrome *c* from the mitochondria into the cytosol by Western blotting. Cytosolic cytochrome *c* was detected by varying the exposure of A549 cells to **1a** and **1g**, and the levels of cytochrome *c* that remained in the mitochondria was observed to decrease concomitantly (Figure 5B). Cytochrome *c* release from mitochondria is a critical step in apoptosis, and earlier investigations had shown that ionizing radiation (IR) and etoposide induced the release of cytochrome *c* from mitochondria in two distinct stages.[27] At the early stage, low levels of cytochrome *c* are released from mitochondria and activate caspases 9 and 3. In contrast, the late stage cytochrome *c* release resulted in a drastic loss of mitochondrial cytochrome *c* and was associated with a reduction

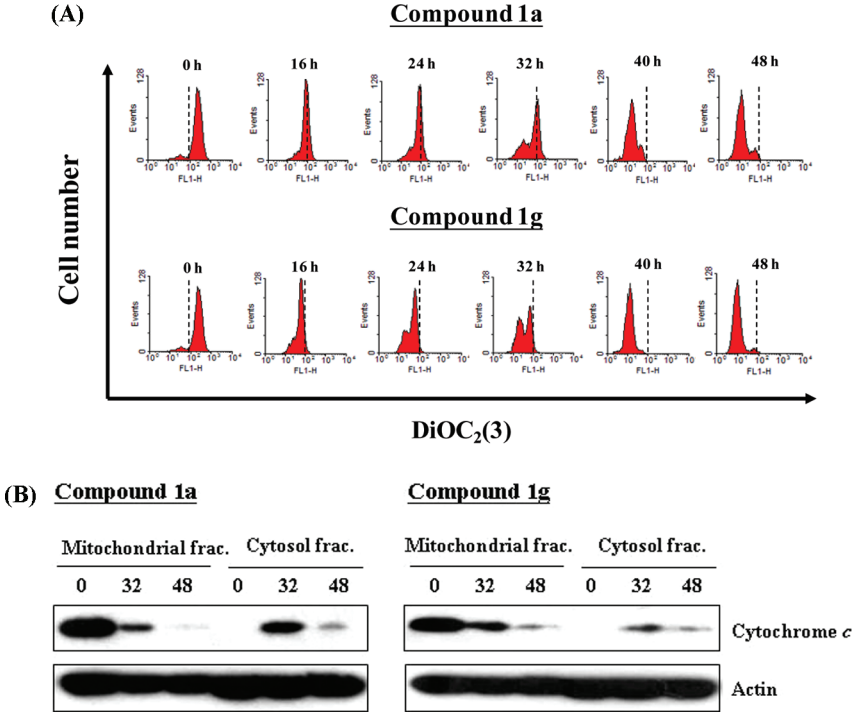


Figure 5. (A) Compounds **1a** and **1g** induce mitochondria membrane potential lost and cytochrome *c* release. A549 cells were treated with **1a** or **1g** (5 μM) or vehicle (0.1% DMSO) for the time as indicated, followed by $DiOC_2(3)$ staining. The mitochondrial membrane potential was analyzed by flow cytometry. One of three repeated experiments is shown. (B) Compound **1a** or **1g** (5 μM) or vehicle (0.1% DMSO) for 36 or 48 h. The cytochrome *c* in mitochondrial fraction and cytosolic fraction was measured by Western blot. One of three repeated experiments is shown.

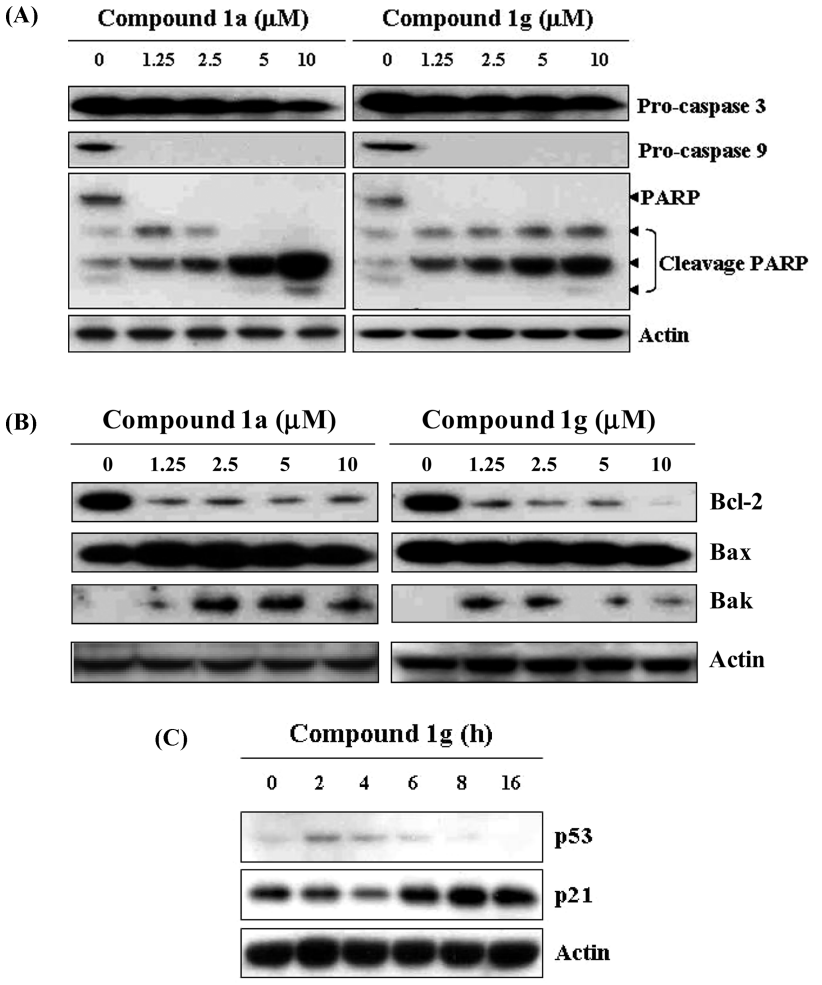


Figure 6. (A, B) Effects of compounds **1a** and **1g** on caspases activation and mitochondrial protein expression. A549 cells were treated with **1a** or **1g** (0−10 μM) or vehicle (0.1% DMSO) for 36 h. (A) The expression of pro-caspase 3, pro-caspase 9, PARP, and actin was measured by Western blot. One of three repeated experiments is shown. (B) The expression of Bcl-2, Bax, Bak, and actin were measured by Western blot. One of three repeated experiments is shown. (C) Effects of compounds **1g** on the expression of p53 and p21 protein expression. A549 cells were treated with compound **1g** (5 μM) or vehicle (0.1% DMSO) for 0−16 h. The expression of p53, p21, and actin were measured by Western blot. One of three repeated experiments is shown.

of the ATP levels and mitochondrial transmembrane potential. After accumulation, this protein is progressively degraded by caspase-like proteases.[28] Using immunoblotting, we did not detect earlier cytochrome *c* release before 32 h. This could probably be due to the very small amount of cytochrome *c* released. However, compound **1g** induced decrease of mitochondrial cytochrome *c* was similar to **1a** but the amount of cytosolic cytochrome *c* that was induced by **1g** was lesser than that induced by **1a**. This may be attributed to the greater cytosolic degradation of cytochrome *c* in **1g**-treated cells compared to **1a**-treated cells.

Caspases are known to cleave into a shorter active form upon activation.[29] As shown in Figure 6A, **1a** and **1g** (1.25−10 μM) induced a decrease of procaspase-3 and procaspase-9 in a concentration-dependent manner. The activation of caspase-3 was further confirmed by detecting the degradation of PARP which is cleaved by active caspase-3 during apoptosis. In contrast, we did not observe the cleavage of caspase-8 (data not shown). Bcl-2 family proteins including Bcl-2, Bax, and Bcl-2 homologous antagonist killer (Bak) are the critical regulatory proteins for mitochondrial mediated cell death.[30] The amount of antiapoptotic protein, Bcl-2, was observed to decrease dramatically after treatment with **1a** or **1g** (Figure 6B). In contrast, the expression of proapoptotic protein, Bak, increased in the presence of **1a** or **1g**. The other proapoptotic protein, Bax, was not affected by **1a** or **1g** (Figure 6B). These data confirm that **1a** and **1g** induced cell death through the mitochondrial death pathway.

Apoptotic signals that are transduced in response to stresses converge mainly on the mitochondria. Upon stimulation by death signals, a series of biochemical events is induced that results in the permeabilization of the outer mitochondrial membrane and release of cytochrome *c* and other proapoptotic molecules. A transmembrane channel, called the permeability transition pore (PTP), is formed at the contact sites between the inner mitochondrial membrane (IMM) and outer mitochondrial membrane (OMM).[31] Bcl-2 family proteins are shown to interact with the PTP complex proteins.[32] Apoptotic signals activate proapoptotic Bcl-2 members such as Bax, Bak, and Bcl-2 homology-3 interacting domain death agonist (Bid), resulting in a disturbed balance between pro- and antiapoptotic Bcl-2 family proteins. As a consequence, OMM integrity is lost because of the oligomerization of proapoptotic Bcl-2 members in the OMM.[33] This results in the permeabilization of the OMM, loss of mitochondrial membrane potential, and the release of proteins from the intermembrane space.

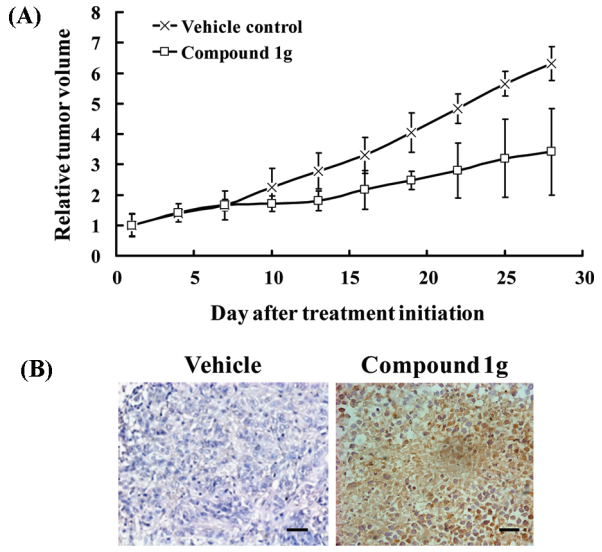


Figure 7. (A) Inhibition of human lung cancer xenografts growth in vivo by compound **1g**. A549 tumor-bearing mice were administered sc with vehicle control (×) or 3 mg/kg compound **1g** (□) on days 0−4 for 5 days. The figure shows the relative tumor volume of vehicle and compound **1g**-treated groups. (B) Immunohistochemical staining with activated caspase-3 was performed in tumor tissues from control and 3 mg/kg **1g**-treated mice (on day 28 after inoculation). Scale bar, 100 μm.

Bcl2 family gene transcription is regulated by a number of transcription factors. Previous report demonstrated that both Bcl2 and Bax are transcriptional targets for the tumor suppressor protein p53. P53 suppresses the activation of the *Bcl-2* promoter by the Brn-3a POU family transcription factor.[34] The expression of Bax has been found to be up-regulated at the transcriptional level by p53,[35] and the Bax gene promoter was shown to contain four p53-binding sites that could be specifically transcriptionally transactivated by p53.[36] Our data revealed that protein levels of p53 and p21 were increased after 2 and 8 h treatment with compound **1g** (Figure 6C). These suggest that p53 activation may be involved in our system and regulates Bcl-2 family protein expression.

Antitumor Activity In Vivo. To evaluate the antitumor activity of compound **1g** in vivo, human lung cancer xenografts were established by subcutaneous (sc) injection of approximately 1×10^7 A549 cells on the backs of nude mice. After the tumor has reached about 100 mm^3 in size, the mice were randomized into vehicle control and treatment groups (six animals each) and were given a daily sc injection of either 0 (vehicle control group) or 3 mg/kg **1g** (treatment groups) for 5 successive days. Results obtained show that **1g** inhibited the growth of tumor (Figure 7A). The human lung tumor tissues with in vivo **1g** treatment also displayed an increase in activated caspase-3 protein expression (relative to the vehicle control group) as evidenced by immunohistochemistry (Figure 7B). Histological examination showed that there were no observable histological changes in various normal tissues including brain, liver, and kidney in the **1g**-treated mice. Additionally the changes in body weight of the **1g**-treated mice were similar to that of the vehicle treated mice (data not shown).

Conclusion

Induction of apoptosis is considered a possible mechanism of most of the chemotherapeutic agents, and targeting the apoptosis signaling pathway is a promising strategy for the development of novel chemotherapeutic molecules.[37] In our efforts to develop potential chemotherapeutic agents from natural products, we have herein provided the first reported synthesis of a class of polyenylpyrrole natural products and their analogues. The compounds were evaluated for the cell cytotoxicity against human lung cancer cells A549. Two compounds, **1a** and **1g**, displayed potent effects in the inhibition of tumor cell proliferation. Flow cytometric analysis revealed that compounds **1a** and **1g** increase the sub-G1 cell population and DNA breaks suggesting apoptosis. Induction of apoptosis by these compounds in A549 cells followed the steps commonly observed for the intrinsic pathway involving mitochondrial damage. In vivo study revealed that compound **1g** demonstrated anticancer activities in tumor bearing mice. Further studies are presently ongoing to develop the compounds as a potential anticancer therapeutic.

Experimental Section

Chemistry. General Procedures. All chemical reagents and solvents were obtained from Sigma Aldrich, Merck, Lancaster, or Fluka and were used without further purification. The microwave-assisted reactions were performed using the Biotage initiator microwave synthesizer. Analytical thin layer chromatography was carried out on precoated silica plates (Merck silica gel 60, F254) and visualized with ultraviolet light or stained with phosphomolybdic acid stain. Flash column chromatography was performed with silica (Merck, 70−230 mesh). The purities of the compounds were determined via HPLC using a Shimadzhu LCMS-IT-TOF system with a Phenomenex Luma C18 column (50 mm × 3.0 mm, 5 μm). Detection was conducted at 254 nm, and integration was obtained with a Shimadzhu LCMS solution software. Compounds used in the biological assays have purities of at least 95%. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker ACF 300 or AMX 500 Fourier transform spectrometer. Chemical shifts were reported in parts per million (δ) relative to the internal standard of tetramethylsilane (TMS). The signals observed were described as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet). The number of protons (n) for a given resonance was indicated as nH. Mass spectra were performed on a Finnigan/MAT LCQ mass spectrometer under electron spray ionization (ESI) or electron impact (EI) techniques. The purities of the tested compounds were established by HPLC and were found to be >95% pure.

General Procedure for the Synthesis of 6a−c. To a mixture of K_2CO_3 (1.73 g, 12.5 mmol) and the corresponding pyrone **5** (5.00 mmol) in DMSO (10 mL) was added dimethyl sulfate (0.693 g, 5.50 mmol). The mixture was stirred at room temperature for 1 h and poured into water (60 mL). The mixture was extracted with EtOAc, and the combined organic extract was washed with saturated NaCl solution, dried over $MgSO_4$, concentrated, and purified by column chromatography.

4-Methoxy-6-methyl-2*H*-pyran-2-one (6a). The residue was purified using flash chromatography (EtOAc/hexane = 2:1) to afford **6a** (0.588 g, 86%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.74 (d, J = 1.3 Hz, 1H), 5.36 (d, J = 1.9 Hz, 1H), 3.75 (s, 3H), 2.16 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2, 164.8, 161.9, 100.2, 87.2, 55.7, 19.7. HRMS (EI): calcd for $C_7H_8O_3$, 140.0473; found 140.0472.

3-Butyl-4-methoxy-6-methyl-2*H*-pyran-2-one (6c). The residue was purified using flash chromatography (EtOAc/hexane = 1:2) to afford **6c** (0.725 g, 74%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.97 (s, 1H), 3.82−3.81 (d, J = 3.2 Hz, 3H), 2.39−2.34 (m, 2H), 2.21 (d, J = 3.8 Hz, 3H), 1.41−1.40 (m, 2H), 1.32−1.28 (m, 2H), 0.89−0.85 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.8, 165.4, 160.8, 105.6, 94.9, 56.1, 30.1, 22.9, 22.6, 20.2, 13.9. HRMS (EI): calcd for $C_{11}H_{16}O_3$, 196.1099; found 196.1098.

General Procedure for the Synthesis of 7a−c. SeO_2 (1.11 g, 10.0 mmol) was added to the corresponding pyrone **6** (2.00 mmol) in 1,4-dioxane (4 mL). The reaction mixture containing **6b** or **6c** was heated at 160 °C for 15 min while **6a** was heated at 150 °C for 15 min using microwave irradiation in a sealed tube. Then the mixture was allowed to cool, saturated $NaHCO_3$ solution was added, and the mixture was extracted with CH_2Cl_2. The combined organic extract was dried over $MgSO_4$, concentrated, and purified by column chromatography.

4-Methoxy-6-oxo-6*H*-pyran-2-carbaldehyde (7a). The residue was purified using flash chromatography (EtOAc/hexane/CH_2Cl_2 = 1:3:6) to afford **7a** (0.188 g, 61%) as a pale brown solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.46 (s, 1H), 7.14 (d, J = 1.9 Hz, 1H), 6.01 (d, J = 1.9 Hz, 1H), 3.89 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 184.3, 169.0, 161.2, 153.7, 112.6, 94.6, 57.1. HRMS (EI): calcd for $C_7H_6O_4$, 154.0266; found 154.0265.

5-Butyl-4-methoxy-6-oxo-6*H*-pyran-2-carbaldehyde (7c). The residue was purified using flash chromatography (EtOAc/hexane/CH_2Cl_2 = 1:6:12) to afford **7c** (0.319 g, 76%) as a pale brown solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.55 (s, 1H), 6.99 (s, 1H), 3.96 (s, 3H), 2.50−2.46 (t, J = 7.6 Hz, 2H), 1.47−1.41 (m, 2H), 1.35−1.31 (m, 2H), 0.91−0.88 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 183.3, 163.3, 162.3, 152.4, 115.9, 101.8, 56.7, 29.7, 23.9, 22.6, 13.8. HRMS (EI): calcd for $C_{11}H_{14}O_4$, 210.0892; found 210.0893.

General Procedure for the Synthesis of 7d−g. 3 M MeMgBr or 3 M EtMgBr in Et_2O (0.733 mL, 2.20 mmol) was added dropwise to the corresponding pyrones **7a−c** (2.00 mmol) in THF. The mixture was allowed to stir at room temperature for 30 min before quenching with saturated NH_4Cl solution. The mixture was extracted with CH_2Cl_2, and the combined organic extract was washed with saturated NaCl solution and dried over $MgSO_4$. The solvent was removed under reduced pressure to afford a brown residue. Dess−Martin periodinane (DMP) (1.02 g, 2.40 mmol) was added to the residue dissolved in CH_2Cl_2 (5 mL), and the reaction mixture was stirred at room temperature for 1 h. Subsequently, saturated $NaHCO_3$ solution (5 mL) and 15% $Na_2S_2O_3$ solution (5 mL) were added and the mixture was allowed to stir for an additional 15 min. Then the mixture was extracted with CH_2Cl_2 and the combined organic extract was dried over $MgSO_4$, concentrated and purified by column chromatography.

6-Acetyl-3-butyl-4-methoxy-2*H*-pyran-2-one (7g). The residue was purified using flash chromatography (EtOAc/hexane = 1:3) to afford **7g** (0.327 g, 73%) as a pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99 (s, 1H), 3.89 (s, 3H), 2.46 (s, 3H), 2.43−2.40 (t, J = 7.6 Hz, 2H), 1.41−1.36 (m, 2H), 1.31−1.23 (m, 2H), 0.85−0.82 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.4, 163.9, 162.7, 153.1, 114.1, 98.0, 56.5, 29.6, 25.7, 23.6, 22.5, 13.7. HRMS (ESI) $[M + Na]^+$: calcd for $C_{12}H_{16}O_4Na$ 247.0946; found 247.0942. HRMS (ESI) $[M + H]^+$: calcd for $C_{12}H_{16}O_4Na$, 247.0946; found 247.0942.

General Procedure for the Synthesis of 8a−g. CBr_4 (0.464 g, 1.40 mmol) in CH_2Cl_2 (4 mL) was added to a solution of the corresponding pyrone **7** (1.00 mmol) and PPh_3 (0.734 g, 2.80 mmol) in CH_2Cl_2 (8 mL). The mixture was stirred at room temperature for 30 min, and thereafter, the solvent was removed under reduced pressure and the residue was purified by column chromatography.

6-(2,2-Dibromovinyl)-4-methoxy-2*H*-pyran-2-one (8a). The residue was purified using flash chromatography (EtOAc/CH_2Cl_2 = 1:40) to afford **8a** (0.285 g, 92%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.09, (s, 1H), 6.35 (d, J = 2.6 Hz, 1H), 5.53 (d, J = 1.9 Hz, 1H), 3.81 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 162.8, 155.4, 128.5, 103.5, 97.3, 89.9, 56.1. HRMS (EI): calcd for $C_8H_6O_3Br_2$, 307.8684; found 307.8678.

3-Butyl-6-(1,1-dibromoprop-1-en-2-yl)-4-methoxy-2*H*-pyran-2-one (8g). The residue was purified using flash chromatography (EtOAc/hexane = 1:6) to afford **8g** (0.338 g, 89%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.39 (s, 1H), 3.85 (s, 3H), 2.39−2.36 (t, J = 7.6 Hz, 2H), 2.10 (s, 3H) 1.44−1.38 (m, 2H), 1.33−1.25 (m, 2H), 0.87−0.85 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.6, 164.1, 157.7, 135.2, 108.0, 98.1, 94.3, 56.3, 29.8, 23.2, 22.6, 22.5, 13.8. HRMS (ESI) $[M + Na]^+$: calcd for $C_{13}H_{17}Br_2O_3$ 378.9544; found 378.9532.

General Procedure for the Synthesis of 2a−g. To a solution of the corresponding pyrone **8** (0.80 mmol) and triethylamine (0.364 g, 3.60 mmol) in DMF (1.5 mL) was added dimethylphosphite (0.352 g, 3.20 mmol). The reaction mixture was stirred at room temperature for 1 h. Water was added to the mixture and extracted with Et_2O. The combined organic extract was washed with saturated NaCl solution, dried over $MgSO_4$, concentrated under reduced pressure, and purified by column chromatography.

(*E*)-6-(2-Bromovinyl)-4-methoxy-2*H*-pyran-2-one (2a). The residue was purified using flash chromatography (EtOAc/hexane = 1:2) to afford **2a** (0.181 g, 98%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30−7.27 (d, J = 13.9 Hz, 1H), 6.63−6.61 (d, J = 13.3 Hz, 1H), 5.83 (d, J = 2.5 Hz, 1H), 5.49 (d, J = 2.6 Hz, 1H), 3.80 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 163.1, 156.3, 128.2, 116.3, 101.4, 89.5, 56.0. HRMS (EI): calcd for $C_8H_7O_3Br$, 229.9579; found 229.9585.

(*E*)-6-(1-Bromoprop-1-en-2-yl)-3-butyl-4-methoxy-2*H*-pyran-2-one (2g). The residue was purified using flash chromatography (EtOAc/hexane = 1:8) to afford **2g** (0.232 g, 96%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (d, J = 1.3 Hz, 1H), 6.20 (s, 1H), 3.89 (s, 3H), 2.42−2.39 (t, J = 7.6 Hz, 2H), 2.06 (d, J = 1.3 Hz, 1H), 1.46−1.39 (m, 2H), 1.35−1.28 (m, 2H), 0.90−0.87 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2, 163.9, 157.2, 131.8, 114.8, 108.4, 93.5, 56.2, 30.0, 23.2, 22.6, 15.7, 13.9. HRMS (EI): calcd for $C_{13}H_{17}O_3Br$, 300.0361; found 300.0357.

Synthesis of Methyl 3-Chloro-1*H*-pyrrole-2-carboxylate (11). 2-Methyl-1-pyrroline **9** (0.831 g, 10.0 mmol) was added to a suspension of *N*-cholorosuccinimide (10.7 g, 80.0 mmol) in THF (25 mL), and the reaction mixture was heated at 55 °C for 20 min. Then the reaction mixture was cooled to room temperature, water was added, and the mixture was extracted with hexane. The combined organic extract was concentrated under reduced pressure to afford **10** which was directly used for the next step. Compound **10** was dissolved in MeOH (10 mL) and cooled to 0 °C. 3 M NaOMe in MeOH (20 mL, 60.0 mmol) was added dropwise over 5 min, and thereafter, the reaction mixture was warmed to room temperature and stirred for an additional 30 min. The mixture was acidified using 2 M HCl and extracted with EtOAc. The combined organic extract was washed with saturated NaCl solution, dried over $MgSO_4$, concentrated and the residue purified using flash chromatography (EtOAc/hexane = 1:4) to afford **11** (1.50 g, 94%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.56 (br, 1H), 6.86 (s, 1H), 6.23 (s, 1H), 3.89 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.9, 122.0, 119.2, 118.2, 111.9, 51.6. HRMS (EI): calcd for $C_6H_6NO_2Cl$, 159.0087; found 159.0088.

Synthesis of 3-Chloro-1*H*-pyrrole-2-carbaldehyde (12). 2 M $LiAlH_4$ in THF solution (4.80 mL, 9.60 mmol) was added dropwise to **11** (1.28 g, 8.00 mmol) in THF (15 mL) at −20 °C. The reaction mixture was warmed to 0 °C and stirred for 30 min before quenching with EtOAc (5 mL). Water (20 mL) and 2 M aqueous NaOH (20 mL) were added to the reaction mixture, and the solid formed was filtered and washed with EtOAc. The filtrate was extracted with EtOAc and the combined organic extract was washed with saturated NaCl solution and then concentrated to obtain the crude alcohol product. 2-Iodoxybenzoic acid (5.60 g, 20.0 mmol) was dissolved in DMSO (20 mL) before the addition of $NaHCO_3$ (4.03 g, 48.0 mmol) and the crude alcohol product. The mixture was stirred at room temperature for 16 h and quenched with 0.5 M aqueous NaOH (150 mL). The solid was filtered and washed with EtOAc, and the filtrate was extracted with EtOAc. The combined organic extract was washed with saturated NaCl solution, dried over $MgSO_4$, concentrated and the residue was purified using flash chromatography (EtOAc/hexane = 1:3) to afford **12** (0.777 g, 75%) as a pale brown solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.7 (br, 1H), 9.63 (s, 1H), 7.10−7.08 (m, 1H), 6.29−6.28 (m, 1H); ^{13}C

NMR (125 MHz, $CDCl_3$) δ 177.8, 127.7, 126.3, 125.3, 111.6. HRMS (EI): calcd for $C_5H_4NO_2Cl$, 128.9981; found 129.9977.

Synthesis of 3-Chloro-1-(methylsulfonyl)-1*H*-pyrrole-2-carbaldehyde (13). 60% NaH in mineral oil (0.132 g, 3.30 mmol) was added to **12** (0.388 g, 3.00 mmol) in THF portionwise. After evolution of hydrogen gas had ceased, methanesulfonyl chloride (0.378 g, 3.30 mmol) was added and the reaction mixture was stirred at room temperature for 20 min. Subsequently, the solvent was removed and the residue was purified by flash chromatography (EtOAc/hexane = 1:3) to afford **13** (0.573 g, 92%) as a pale brown solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.83 (s, 1H), 7.58−7.57 (d, J = 3.2 Hz, 1H), 6.37 (d, J = 3.2 Hz, 1H), 3.68 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.8, 132.5, 129.2, 127.2, 112.2, 42.9. HRMS (EI): calcd for $C_6H_6NO_3ClS$, 206.9757; found 206.9755.

General Procedure for the Synthesis of 18a−d. CBr_4 (0.345 g, 1.04 mmol) in CH_2Cl_2 (2 mL) was added to a solution of PPh_3 (0.545 g, 2.08 mmol) and **13**, **16**, **17a**, or **17b** (0.800 mmol) in CH_2Cl_2 (4 mL). The mixture was stirred at room temperature for 30 min, concentrated, and purified by column chromatography.

3-Chloro-2-(2,2-dibromovinyl)-1-(methylsulfonyl)-1*H*-pyrrole (18a). The residue was purified using flash chromatography (EtOAc/hexane = 1:10) to afford **18a** (0.194 g, 85%) as a pale brown solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (s, 1H), 7.19−7.18 (d, J = 3.2 Hz, 1H), 6.33−6.32 (d, J = 3.8 Hz, 1H), 3.13 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 126.1, 124.7, 122.1, 119.3, 113.4, 98.5, 42.9. HRMS (EI): calcd for $C_7H_6NO_2Br_2ClS$, 360.8175; found 360.8177.

General Procedure for the Synthesis of 4a−d. To a solution of **18** (0.735 mmol) and triethylamine (0.334 g, 0.331 mmol) in DMF (1.5 mL) was added dimethylphosphite (0.323 g, 0.294 mmol), and the reaction mixture was stirred at room temperature for 1 h. Thereafter, water was added to the mixture and extracted with Et_2O. The combined organic extract was washed with saturated NaCl solution, dried over $MgSO_4$, concentrated under reduced pressure, and purified by column chromatography.

2-(2-Bromovinyl)-3-chloro-1-(methylsulfonyl)-1*H*-pyrrole (4a). The residue was purified using flash chromatography (EtOAc/hexane = 1:10) to afford a pale brown solid **4a** (0.147 g, 97%) as a mixture of E/Z isomers in ~2:1 ratio. ^{1}H NMR (500 MHz, $CDCl_3$) (mixture of E/Z isomers) δ 7.41−7.38 (d, J = 13.9 Hz, 1H), 7.19−7.18 (d J = 3.2 Hz, 1H), 7.16−7.13 (m, 1.8H), 6.77−6.76 (d, J = 7.6 Hz, 0.4H), 6.34−6.33 (d, J = 3.2 Hz, 0.4 H), 6.30−6.29 (d, J = 3.8 Hz, 1H), 3.14 (s, 3H), 3.08 (s, 1.3H); ^{13}C NMR (125 MHz, $CDCl_3$) (mixture of E/Z isomers) δ 125.2, 124.2, 122.8, 122.6, 122.0, 121.6, 118.8, 117.5, 115.5, 113.6, 113.3, 112.6, 42.8, 42.7. HRMS (EI): calcd for $C_7H_7NO_2BrClS$, 282.9069; found 282.9069.

General Procedure for the Synthesis of 22a−i. Pd_2dba_3 (5.5 mg, 6.0 μmol) and $AsPh_3$ (7.3 mg, 24 μmol) were added to a mixture of the respective compounds **2** (0.20 mmol) and **3** (0.169 g, 0.36 mmol) in NMP (1 mL) and allowed to stir at room temperature for 6 h. Then water was added and the mixture was extracted with Et_2O. The combined organic extract was washed with saturated NaCl solution, dried over $MgSO_4$, concentrated, and the residue obtained was dissolved in CH_3CN (15 mL) and washed with pentane (15 mL × 5) to remove the tributyltin bromide byproduct. Following that, CH_3CN was removed under reduced pressure and the residue was purified using a short column (~5 cm) of silica gel (EtOAc/hexane = 1:2).

4-Methoxy-6-((1*E*,3*E*,5*E*)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)hexa-1,3,5-trienyl)-2*H*-pyran-2-one (22a). 22a (48.2 mg, 73%) was obtained as an orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16−7.11 (dd, J = 10.7 Hz, 15.1 Hz, 1H), 7.06−7.00 (dd, J = 10.7 Hz, 17.8 Hz, 1H), 6.53−6.48 (dd, J = 10.8 Hz, 14.5 Hz, 1H), 6.43−6.38 (dd, J = 10.7 Hz, 14.5 Hz, 1H), 6.10−6.07 (d, J = 15.2 Hz, 1H), 5.84 (d, J = 1.9 Hz, 1H), 5.72−5.68 (d, J = 17.7 Hz, 1H), 5.45−5.44 (d, J = 2.5 Hz, 1H), 3.79 (s, 3H), 1.26 (s, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 163.8, 158.3, 148.4, 139.5, 135.4, 133.9, 123.5, 101.5, 88.9, 83.3, 55.9, 24.7. HRMS (ESI) $[M + Na]^+$: calcd for $C_{18}H_{23}O_5BNa$, 353.1536; found 353.1522.

3-Butyl-4-methoxy-6-((2*E*,4*E*,6*E*)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)hepta-2,4,6-trien-2-yl)-2*H*-pyran-2-one (22g). 22g (48.8 mg, 61%) was obtained as an orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20−7.10 (m, 2H), 6.73−6.68 (dd, J = 12.0 Hz, 15.1 Hz, 1H), 6.62−6.57 (dd, J = 10.7 Hz, 14.5 Hz, 1H), 6.21 (s, 1H), 5.74−5.70 (d, J = 17.7 Hz, 1H), 3.91 (s, 3H), 2.46−2.43 (t, J = 7.6 Hz, 2H), 2.01 (s, 3H), 1.49−1.44 (m, 2H), 1.38−1.29 (m, 2H), 1.25 (s, 12H), 0.93−0.90 (t, J = 7.6 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.5, 164.3, 159.3, 148.9, 139.3, 131.1, 131.2, 127.6, 107.9, 93.1, 83.3, 56.0, 30.2, 24.7, 23.2, 22.6, 13.9, 12.6. HRMS (ESI) $[M + Na]^+$: calcd for $C_{23}H_{33}O_5BNa$, 423.2319; found 423.2334.

General Procedure for the Synthesis of 1a−i. Pd_2dba_3 (2.7 mg, 3.0 μmol) and $AsPh_3$ (4.6 mg, 15 μmol) was added to THF (1 mL) followed by **4a** (56 mg, 0.195 mmol) and 1.8 M aqueous KOH (0.167 mL, 0.300 mL). The respective compound **22** (0.150 mmol) was dissolved in THF (0.5 mL) and added dropwise to the reaction mixture over 5 min with stirring. The reaction mixture was stirred for 20 min at room temperature and quenched with saturated NH_4Cl. EtOAc was added, and the mixture was washed thrice with water followed by saturated NaCl solution. The organic extract was dried over $MgSO_4$ and concentrated under reduced pressure. The residue obtained was dissolved in THF (1 mL), and 1 M TBAF in THF (0.300 mL, 0.300 mmol) was added. The mixture was stirred at room temperature for 30 min, and thereafte, EtOAc was added and the mixture was washed thrice with water followed by saturated NaCl solution. The organic extract was dried over $MgSO_4$, concentrated under reduced pressure, and purified by column chromatography.

Auxarconjugatin B (1a). The residue was purified using flash chromatography (acetone/hexane/CH_2Cl_2 = 1:5:10 to afford **1a** (36.8 mg, 74%) as a red solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.45 (br, 1H), 7.07−7.02 (dd, J = 11.4 Hz, 15.1 Hz, 1H), 6.90−6.89 (m, 1H), 6.79−6.69 (m, 2H), 6.63−6.58 (dd, J = 11.4 Hz, 14.5 Hz, 1H), 6.54−6.37 (m, 3H), 6.30−6.27 (d, J = 15.1 Hz, 1H), 6.23−6.22(d, J = 1.9 Hz, 1H), 6.14−6.13 (m, 1H), 5.59−5.58 (d, J = 2.5 Hz, 1H), 3.81 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.7, 162.6, 158.4, 138.8, 136.8, 135.1, 131.1, 130.5, 126.0, 124.7, 121.6, 120.8, 120.5, 111.9, 109.1, 100.6, 88.4, 56.3. HRMS (ESI) $[M - H]^-$: calcd for $C_{18}H_{15}NO_3Cl$, 328.0740; found 328.0738.

3-Butyl-6-((2*E*,4*E*,6*E*,8*E*)-9-(3-chloro-1*H*-pyrrol-2-yl)nona-2,4,6,8-tetraen-2-yl)-4-methoxy-2*H*-pyran-2-one (1g). The residue was purified using flash chromatography (acetone/hexane/CH_2Cl_2 = 1:8:16) to afford **1g** (49.2 mg, 82%) as a red solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.45 (br, 1H), 7.06−7.04 (d, J = 10.7 Hz, 1H), 6.90−6.89 (m, 1H), 6.80−6.69 (m, 3H), 6.64−6.44 (m, 4H), 6.13−6.12 (m, 1H), 3.94 (s, 3H), 2.33−2.30 (t, J = 7.3 Hz, 2H), 2.05 (s, 3H), 1.40−1.34 (m, 2H), 1.30−1.23 (m, 2H), 0.89−0.86 (t, J = 7.3 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.0, 163.1, 159.0, 138.5, 136.3, 131.6, 131.1, 127.9, 126.0, 125.8, 124.8, 120.5, 120.4, 111.8, 109.1, 105.1, 93.7, 56.7, 29.7, 22.7, 22.0, 13.8, 12.3. HRMS (ESI) $[M + Na]^+$: calcd for $C_{23}H_{26}NO_3ClNa$, 422.1499; found 422.1510.

Reagents Used in Biological Assays. Roswell Park Memorial Institute 1640 (RPMI 1640) medium was obtained from GIBCO BRL (Gaithersburg, MD). $DiOC_2(3)$ was obtained from Molecular Probes (Eugene, OR). Anti-caspase-3, anti-caspase-8, anti-caspase-9, anti-PARP, anti-Bcl-2, anti-Bax, anti-Bak, anti-cytochrome *c*, and anti-actin antibody were purchased from Chemicon (Temecula, CA). Anti-p53 and anti-p21 antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Stock solution was kept at −20 °C and freshly diluted to the desired concentrations with medium

immediately before use (the final concentration of DMSO in culture medium was 0.1%).

In Vitro Growth Inhibition Study. Human non-small-cell lung carcinoma A549 cells and normal human bronchial epithelial cell line Beas-2b cells were obtained from ATCC (Manassas, VA). Cells were seeded at a density of 5000 cells in 100 μL of RPMI 1640 medium with 10% (v/v) fetal bovine serum per well in 96-well flat-bottom plates and incubated for 24 h at 37 °C in a 5% CO_2 incubator. Test compounds were dissolved in DMSO, further diluted with the culture media to obtain an optimal range of concentrations for treatments in triplicate per concentration, and incubated at 37 °C in a CO_2 incubator for 48 h. All treatment media contained the final DMSO concentration of 0.1%. Alamar Blue assay was used to determine the cytotoxicity of the test compounds. The procedure was conducted following the protocol described in the manufacturer's instructions (AbD Serotec, Oxford, U.K.).

Cell Cycle Determination. Aliquots of 5×10^5 cells was fixed in 70% ethanol on ice for at least 2 h and centrifuged. The pellet was incubated with RNase (200 μg/mL) and PI (10 μg/mL) at room temperature for 30 min. DNA content and cell cycle distribution were analyzed using a Becton Dickinson FACScan Plus flow cytometer. Cytofluorometric analysis was performed using a CellQuestR (Becton Dickinson, San Jose, CA) on a minimum of 10 000 cells per sample.

TdT End-Labeling Assay. Apoptosis was assayed by a TdT-mediated dUTP nick-end labeling assay. After treatments, 5×10^5 cells were fixed in 1% paraformaldehyde and 70% ethanol and then washed with phosphate buffered saline (PBS) and resuspended in 50 μL of TdT reaction solution containing 0.2 M sodium cacodylate, 25 mM Tris-HCl, 5 mM $CoCl_2$, 0.25 mg/mL bovine serum albumin, 10 units of terminal transferase, and 0.5 nM DIG-dUTP (all from Chemicon International, Inc.). The TdT end-labeling reactions were carried out at 37 °C for 30 min. The cells were rinsed with cold PBS, resuspended in 100 μL of fluorescein antibody solution (2.5 μg/mL anti-DIG conjugate fluorescein, 4× saline sodium citrate buffer, 0.1% Triton X-100, and 5% nonfat dry milk), and incubated in the dark for 30 min. Subsequently, the cells were rinsed in PBS containing 0.1% Triton X-100 and then treated with 1 mL of propidium iodide (PI, 5 μg/mL in PBS) and RNase A (100 μg/mL) for 30 min in the dark. An increase in fluorescence was determined by the FACScan system.

Measurement of Mitochondrial Membrane Potential ($\triangle\psi_m$). A reduction in mitochondrial membrane potential following treatment with compound **1** was monitored by flow cytometry. The $\triangle\psi_m$ was estimated by staining 5×10^5 cells with 50 nM $DiOC_2(3)$ (Molecular Probes), a cationic lipophilic dye, for 15 min at 37 °C in the dark and then washed with cold PBS. Cells were subjected to flow cytometry (excitation 488 nm; emission 525 nm; recorded in FL-1). The fluorescence of $DiOC_2(3)$ is oxidation-dependent and correlates with $\triangle\psi_m$. Cells without treatment were processed as the control. Mitochondrial damage was recognized by the presence of cells displaying "low" uptake of $DiOC_3$.

Western Blot Analysis. After treatment, cells were washed three times with PBS and lysed in 100 μL of sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 5% 2-mercaptoethanol, 0.002% bromophenol blue, and 10% glycerol). The samples were boiled for 5 min and then centrifuged at 12 000 rpm for 10 min. Protein extracts were subjected to electrophoresis on an SDS−polyacrylamide gel and then transferred onto polyvinylidene fluoride (PVDF) membrane. The blots were blocked for 1 h in PBST (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% Tween-20) containing 5% nonfat dried milk and then probed overnight with an appropriate dilution of the primary antibody. Reactions were detected with a suitable secondary antibody conjugated to horseradish peroxidase and an enhanced chemiluminescence kit (Millipore).

Cytosolic Cytochrome *c* Release. Cells with or without treatment were harvested by centrifugation at 600*g* for 10 min. After being washed once with ice-cold PBS, cell pellets were resuspended in buffer A (20 mM HEPES−KOH, pH 7.5, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM Na-EDTA, 1 mM Na-EGTA, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride containing 250 mM sucrose). After being chilled on ice for 30 min, the cells were disrupted by 15 strokes of a glass homogenizer. The homogenate was centrifuged twice to remove unbroken cells and nuclei (750*g*, 10 min, 4 °C). The supernatant was then obtained by centrifugation at 10000*g* for 60 min at 4 °C. The resulting pellets were identified as the mitochondrial fraction, and supernatants were identified as cytosolic fraction. All steps were performed on ice or 4 °C. Cytochrome *c* release into the cytosolic fraction for each condition was assessed by Western blot analysis.

Antitumor Activity in Vivo. Xenograft mice were used as a model system to study the cytotoxicity effect of compound **1g** in vivo. Female congenital athymic BALB/c nude (nu/nu) mice were purchased from National Sciences Council (Taipei, Taiwan), and all procedures were performed in compliance with the standard operating procedures of the Laboratory Animal Center of National Ilan University (Ilan, Taiwan). All experiments were carried out using 6−8 week old mice weighing 18−22 g. The animals were sc implanted with 1×10^7 A549 cells into the back of mice. When the tumor reached 80−120 mm^3 in volume, animals were divided randomly into control and test groups consisting of six mice per group (day 0). Daily sc administration of compound **1g**, dissolved in a vehicle of 20% Tween 80 in normal saline (v/v), was performed from days 0 to 4 far from the inoculated tumor sites (>1.5 cm). The control group was treated with vehicle only. The mice were weighed three times a week up to days 21−28 to monitor the effects, and the same time the tumor volume was determined by measurement of the length (L) and width (W) of the tumor. The tumor volume on day n (TVn) was calculated as TV (mm^3) = $(L \times W^2)/2$. The relative tumor volume on day n (RTVn) versus day 0 was expressed according to the following formula: RTVn = TVn/TV0. Xenograft tumors as well as other vital organs of treated and control mice were harvested and fixed in 4% formalin, embedded in paraffin, and cut in 4 mm sections for histological study.

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Supporting Information Available: Additional details of experimental procedures and ^1H, ^{13}C, and HRMS data of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Kaufmann, S. H.; Earnshaw, W. C. Induction of apoptosis by cancer chemotherapy. *Exp. Cell Res.* **2000**, *256*, 42–49. (b) Fulda, S.; Debatin, K. M. Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. *Oncogene* **2006**, *25*, 4798–4811.
(2) Thorburn, A. Death receptor-induced cell killing. *Cell. Signalling* **2004**, *16*, 139–144.
(3) Kuwana, T.; Newmeyer, D. D. Bcl-2-family proteins and the role of mitochondria in apoptosis. *Curr. Opin. Cell Biol.* **2003**, *15*, 691–699.
(4) Jiang, X.; Wang, X. Cytochrome C-mediated apoptosis. *Annu. Rev. Biochem.* **2004**, *73*, 87–106.
(5) Bose, J. S.; Gangan, V.; Prakash, R.; Jain, S. K.; Manna, S. K. A dihydrobenzofuran lignan induces cell death by modulating mitochondrial pathway and G2/M cell cycle arrest. *J. Med. Chem.* **2009**, *52*, 3184–3190.
(6) Thirsk, C.; Whiting, A. Polyene natural products. *J. Chem. Soc., Perkin Trans. 1* **2002**, 999–1023.
(7) (a) Schreiber, S. L. Chemistry and biology of the immunophilins and their immunosuppressive ligands. *Science* **1991**, *251*, 283–287. (b) Rosen, M. K.; Schreiber, S. L. Natural products as probes in the study of cellular functions. Investigation of immunophilins. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 384–400. (c) Katznelson, H.; Jamieson, C. A. Control of nosema disease of honeybees with fumagillin. *Science* **1952**, *115*, 70–71. (d) McCowen, M. C.; Callender, M. E.; Lawlis, J. F., Jr.

Fumagillin (H-3), a new antibiotic with amebicidal properties. *Science* **1951**, *113*, 202–203.
(8) Clark, B. R.; Capon, R. J.; Lacey, E.; Tennant, S.; Gill, J. H. Polyenylpyrroles and polyenylfurans from an Australian isolate of the soil ascomycete *Gymnoascus reessii*. *Org. Lett.* **2006**, *8*, 701–704.
(9) (a) Yamagishi, Y.; Matsuoka, M.; Odagawa, A.; Kato, S.; Shindo, K.; Mochizuki, J. Rumbrin, a new cytoprotective substance produced by *Auxarthron umbrinum*. I. Taxonomy, production, isolation and biological activities. *J. Antibiot.* **1993**, *46*, 884–887. (b) Yamagishi, Y.; Shindo, K.; Kawai, H. Rumbrin, a new cytoprotective substance produced by *Auxarthron umbrinum.* II. Physicochemical properties and structure determination. *J. Antibiot.* **1993**, *46*, 888–891. (c) Hosoe, T.; Fukushima, K.; Takizawa, K.; Miyaji, M.; Kawai, K.-I. Three pyrrolyloctatetraenyl-alpha-pyrones from Auxarthron conjugatum. *Phytochemistry* **1999**, *52*, 459–463.
(10) (a) Clark, B. R.; Lacey, E.; Gill, J. H.; Capon, R. J. The effect of halide salts on the production of *Gymnoascus reessii* polyenylpyrroles. *J. Nat. Prod.* **2007**, *70*, 665–667. (b) Yang, Y.-L.; Liao, W.-Y.; Liu, W.-Y.; Liaw, C. C.; Shen, C.-N.; Huang, Z.-Y.; Wu., S.-H. Discovery of new natural products by intact-cell mass spectrometry and LC-SPE-NMR: malbranpyrroles, novel polyketides from thermophilic fungus *Malbranchea sulfurea*. *Chem.—Eur. J.* **2009**, *15*, 11573–11580.
(11) Coleman, R. S.; Walczak, M. C. Total synthesis of gymnoconjugatins A and B. *J. Org. Chem.* **2006**, *71*, 9841–9844.
(12) Coleman, R. S.; Walczak, M. C. Tandem Stille/Suzuki−Miyaura coupling of a hetero-bis-metalated diene. rapid, one-pot assembly of polyene systems. *Org. Lett.* **2005**, *7*, 2289–2291.
(13) (a) Cervello, J.; Marquet, J.; Moreno-Mañas, M. Copper and cobalt mediated regioselective alkylation of polyketide models: methyl 3,5-dioxohexanoate and triacetic acid lactone. *Tetrahedron* **1990**, *46*, 2035–2046. (b) Nagawade, R. R.; Khanna, V. V.; Bhagwat, S. S.; Shinde, D. B. Synthesis of new series of 1-aryl-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid as potential antibacterial agents. *Eur. J. Med. Chem.* **2005**, *40*, 1325–1330.
(14) Abbas, S.; Hayes, C. J.; Worden, S. The "Hirao reduction" revisited: a procedure for the synthesis of terminal vinyl bromides by the reduction of 1,1-dibromoalkenes. *Tetrahedron Lett.* **2000**, *41*, 3215–3219.
(15) Abbaspour Tehrani, K.; Borremans, D.; De Kimpe, N. Synthesis of 2-acyl-3-chloropyrroles: application to the synthesis of the trail pheromone of the ant *Atta texana*. *Tetrahedron* **1999**, *55*, 4133–4152.
(16) Jolicoeur, B.; Chapman, E. E.; Thompson, A.; Lubell, W. D. Pyrrole protection. *Tetrahedron* **2006**, *62*, 11531–11563.
(17) (a) Trost, B. M.; Gunzner, J. L.; Dirat, O.; Rhee, Y. H. Callipeltoside A: total synthesis, assignment of the absolute and relative configuration, and evaluation of synthetic analogues. *J. Am. Chem. Soc.* **2002**, *124*, 10396–10415. (b) Harris, J. M.; O'Doherty, G. A. An olefination approach to the enantioselective syntheses of several styryllactones. *Tetrahedron* **2001**, *57*, 5161–5171.
(18) (a) Rühmann, A.; Wentrup, C. Synthesis of a photoactivatable 9-*Z*-oleic acid for protein kinase C labeling. *Tetrahedron* **1994**, *50*, 3785–3796. (b) Caprio, V.; Brimble, M. A.; Furkert, D. P. Synthesis of the novel 1,7,9-trioxadispiro[4.1.5.2]-tetradecane ring system present in the spirolides. *Tetrahedron* **2001**, *57*, 4023–4034.
(19) Clark, B. R.; Lacey, E.; Gill, J. H.; Capon, R. J. The effect of halide salts on the production of *Gymnoascus reessii* polyenylpyrroles. *J. Nat. Prod.* **2007**, *70*, 665–667.
(20) Yin, G.; Gao, M.; Wang, Z.; Wu, Y.; Wu, A. Direct conversion of aromatic ketones to arenecarboxylic esters via carbon−carbon bond-cleavage reactions. *Bull. Chem. Soc. Jpn.* **2008**, *81*, 369–372.
(21) Wuts, P. G. M.; Thompson, P. A. Preparation of halomethaneboronates. *J. Organomet. Chem.* **1982**, *234*, 137–141.
(22) Zhang, W.; Gao, W. Y.; Turner, S.; Ducatman, B. S. Gleevec (STI-571) inhibits lung cancer call growth (A549) and potentiates the cisplatin effect in vitro. *Mol. Cancer* **2003**, *2*, 1–9.
(23) Perez-Cruz, I.; Carcamo, J. M.; Golde, D. W. Vitamin C inhibits FAS-induced apoptosis in monocytes and U937 cells. *Blood* **2003**, *102*, 336–343.
(24) Vaquero, E. C.; Edderkaoui, M.; Pandol, S. J.; Gukovsky, I.; Gukovskaya, A. S. Reactive oxygen species produced by NAD(P)H oxidase inhibit apopotosis in pancreatic cancer cells. *J. Biol. Chem.* **2004**, *279*, 34643–34654.
(25) (a) Bose, J. S.; Gangan, V.; Prakash, R.; Jain, S. K.; Manna, S. K. A dihydrobenzofuran lignan induces cell death by modulating mitochondrial pathway and G2/M cell cycle arrest. *J. Med. Chem.* **2009**, *52*, 3184–3190. (b) Croninge, E.; Meeuwsen-De Boer, G. J.; De Graaf, S. S. N.; Kamps, W. A.; De Bont, E. S. J. M. Vincristine induced apoptosis in acute lymphoblastic leukemia cells: a mitochondrial pathway regulated by reactive oxygen species. *Int. J. Oncol.* **2002**, *21*, 1339–1345.
(26) Adams, J. M.; Cory, S. Apoptosomes: engines for caspase activation. *Curr. Opin. Cell Biol.* **2002**, *14*, 715–720.
(27) Chen, Q.; Gong, B.; Almasan, A. Distinct stages of cytochrome *c* release from mitochondria: evidence for a feedback amplification loop linking caspase activation to mitochondrial dysfunction in genotoxic stress induced apoptosis. *Cell Death Differ.* **2000**, *7*, 227–233.
(28) Bobba, A.; Atlante, A.; Giannattasio, S.; Sgaramella, P.; Calissano, P.; Marra, E. Early release and subsequent **c**aspase-mediated degradation of cytochrome c in apoptotic cerebellar granule cells. *FEBS Lett.* **1999**, *457*, 126–130.
(29) Park, J. B.; Schonen, N. *N*-Caffeoyltyramine arrests growth of U937 and Jurkat cells by inhibiting protein tyrosine phosphorylation and inducing caspase-3. *Cancer Lett.* **2003**, *202*, 161–171.
(30) Pepper, C.; Hoy, T.; Bently, D. P. Bcl_2/Bax ratios in chronic lymphocytic leukaemia and their correlation with in vitro apoptosis and clinical resistance. *Br. J. Cancer* **1997**, *76*, 935–938.
(31) (a) Saelens, X.; Festjens, N.; Vande Walle, L.; van Gurp, M.; van Loo, G.; Vandenabeele, P. Toxic proteins released from mitochondria in cell death. *Oncogene* **2004**, *23*, 2861–2874. (b) van Gurp, M.; Festjens, N.; van Loo, G.; Saelens, X.; Vandenabeele, P. Mitochondrial intermembrane proteins in cell death. *Biochem. Biophys. Res. Commun.* **2003**, *304*, 487–497.
(32) Martinou, J. C.; Green, D. R. Breaking the mitochondrial barrier. *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 63–67.
(33) Kuwana, T.; Mackey, M. R.; Perkins, G.; Ellisman, M. H.; Latterich, M.; Schneiter, R.; Green, D. R.; Newmeyer, D. D. Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. *Cell* **2002**, *111*, 331–342.
(34) Budhram-Mahadeo, V.; Morris, P. J.; Smith, M. D.; Midgley, C. A.; Boxer, L. M.; Latchman, D. S. p53 suppresses the activation of the Bcl-2 promoter by the Brn-3a POU family transcription factor. *J. Biol. Chem.* **1999**, *274*, 15237–15244.
(35) Miyashita, T.; Krajewski, S.; Krajewska, M.; Wang, H. G.; Lin, H. K.; Liebermann, D. A.; Hoffman, B.; Reed, J. C. Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. *Oncogene* **1994**, *9*, 1799–1805.
(36) Miyashita, T.; Reed, J. C. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell* **1995**, *80*, 293–299.
(37) (a) Hu, W.; Kavanagh, J. J. Anticancer therapy targeting the apoptotic pathway. *Lancet Oncol.* **2003**, *4*, 921–729. (b) Thompson, C. B. Apoptosis in the pathogenesis and treatment of disease. *Science* **1995**, *267*, 1456–1462.