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

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A class of polyenylpyroroles 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 IC50 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 (IC50 > 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 polynenes 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. 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) 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. 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.

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. 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. Our interest in investigating natural products for their potential therapeutic effects has recently spurred us to examine the influences of conjugated polynenes on anticancer properties.

Conjugated polynenes 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. Presently, some conjugated polynenes that are sold commercially include rapamycin and fumigillin. In 2006, Capon and co-workers published a report on the isolation and structure elucidation of several polynylfurans and polyenylpyrroles from the soil microbe Gymnascus reessii. In that study, they discovered three new conjugated polynenes, 12E-isoruminbr 1e, gyronocjugatins A and B, alongside rumbrin and auxcarconjugatin A 1b which were isolated previously (Figure 1). 1b and 1e were subsequently
found to possess potent cytotoxicity properties against non-
structural protein (NS-1) cell line, while earlier studies by
Yamagishi and co-workers have demonstrated that rumbrin
was able to provide cytotoxicity against cell death caused
by calcium overload. Other related polyenylpyrroles that had
been isolated previously include 12E-bromoisorumbrin, 12E-
dehloroisorumbrin, auxarconjugatin B, auxarconjugatin C,
and malbranpyrroles. Unlike dechloroisorumbrin, auxarconjugatin
E was absent of cytotoxicity activity, implying the im-
portance 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 gymnoconju-

gatins A and B, and there is no literature describing the

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Results and Discussion

Chemistry. The retrosynthetic route of auxarconjugatin and
and its analogues 1 (Scheme 1) was modified from the
synthesis of gymnoconjugatin. Disconnection of the tetra-
ene 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. With this strategy in
mind, we proceeded with the synthesis of 1.

Pyrones 2a–i were prepared from the respective 4-hydroxy-
6-methylpyrano-2-ones 5a–c (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. 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 period-

inane (DMP) to afford 7d–g. Attempts to convert the alde-
hyde 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 fol-

lowed by reduction using dimethylphosphite. Hence, to synthesize 2, compounds 7a–g were first converted
to vinyl dibromides 8a–g via Corey–Fuchs olefination fol-

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. 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 diisobu-
tylaluminum 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. Addition of sodium bicarbonate and
pyridine to neutralize the acetic acid byproduct did not
resolve the problem. Thus, to circumvent this problem, we


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

Scheme 1. Retrosynthesis of Auxarconjugatin and Its Analogues 1a–n

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Scheme 2. Retrosynthesis of 4-Hydroxy-6-methylpyrano-2-ones 5a–c

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Scheme 3. Retrosynthesis of 2-Methyl-1-pyrroline 9

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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-drawing 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 LiAlH4 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 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,
were synthesized in a similar manner. Interestingly, the $^1$H 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 synthesized.

**Table 1. Cytotoxicity of Conjugated Polyenes against Human Lung Cancer A549 Cells**

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<tr>
<th>compd</th>
<th>R$^1$</th>
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<th>R$^3$</th>
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<td>Me</td>
<td>Me</td>
<td>H</td>
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$^a$IC$_{50}$ value expressed as the mean value of triplicate wells from at least three experiments.

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.
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 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\textsubscript{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 benzoxycyvalalanylalaspartyl(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′-disothiocyanato-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,\textsuperscript{23} it has been...
Recently suggested that ROS may also play an antiapoptotic and protective role. 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. 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 (DiOC₃(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. 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. 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 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.

**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 DiOC₃(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.
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 less 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 \(\mu\)M) 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 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 \(\mu\)M) 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 was 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 \(\mu\)M) 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.](image-url)
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. The expression of Bax has been found to be up-regulated at the transcriptional level by p53, and the Bax gene promoter was shown to contain four p53-binding sites that could be specifically transcriptionally transactivated by p53. 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 × 10^6 A549 cells on the backs of nude mice. After the tumor has reached about 100 mm³ 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 or 3 mg/kg 1g-treated mice (on day 28 after inoculation). Scale bar, 100 μm.

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. 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 Shimadzu LCMS-IT-TOF system with a Phenomenex Luna C18 column (50 mm × 3.0 mm, 5 μm). Detection was conducted at 254 nm, and integration was obtained with a Shimadzu LCMS solution software. Compounds used in the biological assays have purities of at least 95%. 1H NMR and 13C 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 K₂CO₃ (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 MgSO₄, concentrated, and purified by column chromatography.

**4-Methoxy-6-methyl-2H-pyrrole-2-one (6a).** The residue was purified using flash chromatography (EtOAc/hexane = 1:2) to afford 6a (0.588 g, 86%) as a white solid. 1H NMR (500 MHz, CDCl₃) δ 7.47 (d, J = 1.3 Hz, 1H), 5.36 (d, J = 1.9 Hz, 1H), 3.75 (s, 3H), 2.16 (s, 3H); 13C NMR (125 MHz, CDCl₃) δ 171.2, 164.8, 161.9, 100.2, 87.2, 55.7, 19.7. HRMS (EI): calcd for C₇H₁₀O₂, 140.0473; found 140.0472.

**3-Butyl-4-methoxy-6-methyl-2H-pyrrole-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. 1H NMR (500 MHz, CDCl₃) 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); 13C NMR (125 MHz, CDCl₃) 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 C₁₁H₁₃O₂, 196.1099; found 196.1098.

**Conclusion**

The tumor volume of A549 tumor-bearing mice was determined (vehicle control group or 3 mg/kg 1g-compound) in tumor tissues with in vivo treated mice (data not shown).

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).

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.

**4-Methoxy-6-methyl-2H-pyrrole-2-one (6a).** The residue was purified using flash chromatography (EtOAc/hexane = 1:2) to afford 6a (0.588 g, 86%) as a white solid. 1H NMR (500 MHz, CDCl₃) δ 7.47 (d, J = 1.3 Hz, 1H), 5.36 (d, J = 1.9 Hz, 1H), 3.75 (s, 3H), 2.16 (s, 3H); 13C NMR (125 MHz, CDCl₃) δ 171.2, 164.8, 161.9, 100.2, 87.2, 55.7, 19.7. HRMS (EI): calcd for C₇H₁₀O₂, 140.0473; found 140.0472.
General Procedure for the Synthesis of 7a–c. SeO₂ (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 NaHCO₃ solution was added, and the mixture was extracted with CH₂Cl₂. The combined organic extract was dried over MgSO₄, concentrated, and purified by column chromatography.

4-Methoxy-6-oxo-6H-pyran-2-carbaldehyde (7a). The residue was purified using flash chromatography (EtOAc/hexane/CH₂Cl₂ = 1:3:6) to afford 7a (0.188 g, 61%) as a pale brown solid. ¹H NMR (500 MHz, DMSO-d₆)  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); ¹³C NMR (125 MHz, DMSO-d₆) δ 184.3, 169.0, 161.2, 153.7, 112.6, 94.6, 57.1. HRMS (EI): calcd for C₇H₆O₄, 154.0265; found 154.0265.

5-Butyl-4-methoxy-6-oxo-6H-pyran-2-carbaldehyde (7c). The residue was purified using flash chromatography (EtOAc/hexane/CH₂Cl₂ = 1:6:12) to afford 7c (0.319 g, 76%) as a pale brown solid. ¹H NMR (500 MHz, DMSO-d₆)  δ 9.55 (s, 1H), 7.07 (m, 1H), 6.20 (s, 1H), 3.89 (s, 3H), 2.42 (t, J = 7.3 Hz, 2H), 1.47–1.41 (m, 2H), 1.35–1.31 (m, 2H), 0.91–0.88 (t, J = 7.3 Hz, 3H); ¹³C NMR (125 MHz, DMSO-d₆) 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 C₁₇H₂₅O₄Na, 300.0357; found 300.1357.

Synthesis of Methyl 3-Chloro-1H-pyrrole-2-carboxylate (11). 2-Methyl-1-pyrrole-9 (0.831 g, 10.0 mmol) was added to a suspension of Na₂CO₃ (10.7 g, 80.0 mmol) in THF (25 mL). The reaction mixture was heated at 80 °C for 30 min before quenching with EtOAc and stirred for an additional 30 min. The reaction mixture was cooled to room temperature, and the mixture was extracted with hexane. The combined organic extract was concentrated under reduced pressure to afford 11 (2.00 g, 94%) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ 3.34–3.30 (m, 1H), 2.15–2.05 (m, 1H), 1.80–1.70 (m, 1H), 1.30–1.20 (m, 1H), 0.85–0.82 (t, J = 7.3 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 160.9, 122.0, 119.2, 118.2, 111.9, 51.6.

General Procedure for the Synthesis of 8a–g. 3 M EtMgBr or 3 M MeMgBr in Et₂O (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 NH₄Cl solution. The mixture was acidified using 2 M HCl and extracted with hexane. The combined organic extract was washed with saturated NaCl solution and dried over MgSO₄. The solvent was removed under reduced pressure, and purified by flash chromatography (EtOAc/hexane = 1:3) to afford 8a (0.285 g, 92%) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ 1.90–1.82 (m, 2H), 1.39–1.35 (m, 2H), 1.33–1.25 (m, 2H), 0.87–0.85 (t, J = 7.3 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 164.6, 161.4, 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 C₁₁H₁₁BrO₃, 378.5546; found 378.5544.

5-Butyl-4-methoxy-6-oxo-6H-pyran-2-carbaldehyde (7d). The residue was purified using flash chromatography (EtOAc/hexane/CH₂Cl₂ = 1:3:6) to afford 7d (0.181 g, 98%) as a white solid. ¹H NMR (500 MHz, CDCl₃) 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); ¹³C NMR (125 MHz, CDCl₃) δ 170.5, 163.1, 156.3, 128.2, 116.3, 101.4, 89.5, 56.0. HRMS (EI): calcd for C₆H₁₁O₂Br, 229.9579; found 229.9585.

General Procedure for the Synthesis of 8d–g. 3 M MeMgBr or 3 M EtMgBr in Et₂O (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 NH₄Cl solution. The mixture was acidified using 2 M HCl and extracted with hexane. The combined organic extract was concentrated under reduced pressure to afford 10 which was directly used for the next step. Compounds 10 and 11 were 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 reaction mixture was acidified using HCl and extracted with ethyl acetate. The combined organic extract was washed with saturated NaCl solution, dried over MgSO₄, concentrated, and purified by flash chromatography (EtOAc/hexane = 1:3) to afford 11 (1.50 g, 94%) as a yellow solid. ¹H NMR (500 MHz, CDCl₃) δ 3.34–3.30 (m, 1H), 2.15–2.05 (m, 1H), 1.39–1.35 (m, 2H), 1.33–1.25 (m, 2H), 0.85–0.82 (t, J = 7.3 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 160.9, 122.0, 119.2, 118.2, 111.9, 51.6. HRMS (EI): calcd for C₁₃H₁₄NO₄, 230.0361; found 230.0357.

Synthesis of 3-Chloro-1H-pyrrole-2-carboxylate (12). 2 M LiAlH₄ 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 concentrated. The combined organic extract was washed with saturated NaCl solution and then concentrated to obtain the crude alcohol product. 2,4-Dioxobutyric acid (5.60 g, 20.0 mmol) was dissolved in DMSO (20 mL) before the addition of NaHCO₃ (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 MgSO₄, concentrated, and purified by flash chromatography (EtOAc/hexane = 1:3) to afford 12 (0.877 g, 75%) as a pale brown solid. ¹H NMR (500 MHz, CDCl₃) δ 7.57 (br, 1H), 7.15–7.07 (m, 1H), 6.29–6.28 (m, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 170.2, 163.1, 156.3, 128.2, 116.3, 101.4, 89.5, 56.0. HRMS (EI): calcd for C₁₄H₁₁ClO, 229.9579; found 229.9585.
NMR (125 MHz, CDCl₃) δ 177.8, 127.7, 126.3, 125.3, 111.6. HRMS (EI): calcd for C₁₄H₂₁NO₂Cl, 289.9981; found 290.0002.

Synthesis of 3-Chloro-1-(methylsulfonyl)-1H-pyrrole-2-carboxaldehyde (13). A mixture of 1H-pyrrole (0.152 g, 3.30 mmol) was added to 12 (0.388 g, 3.00 mmol) in THF portionwise. After evolution of hydrogen gas had ceased, methanesulfonyle 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 white solid. ¹H NMR (500 MHz, CDCl₃) δ 9.93 (s, 1H), 7.58–7.57 (d, J = 3.2 Hz, 1H), 6.37–6.32 (d, J = 3.8 Hz, 1H), 13.9, 12.6. HRMS (ESI) [M-H⁺]: calcd for C₁₈H₂₃O₅BNa, 353.1536; found 353.1522.

3-Butyl-4-(methoxy)-6-((2E,4E,6E,8E)-7-(4,4,5,5-tetramethyl-1,3-dioxaborolan-2-yl)hepta-2,4,6,8-tetraen-2-yl)-2H-pyran-2-one (22). 22 (48.8 mg, 61%) was obtained as an orange solid. ¹H NMR (500 MHz, CDCl₃) 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), 3.79 (s, 3H), 1.26 (s, 1H), 1.13 (s, 3H), 1.04 mmol) in CH₂Cl₂ (2 mL) was added to a solution of PPh₃ (0.300 mmol) and NaOH (0.300 mmol) in Et₂O. The combined organic extract was washed thrice with water followed by saturated NaCl solution. The organic extract was washed with THF and dried over MgSO₄ 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 the mixture was washed thrice with water followed by saturated NaCl solution. The organic extract was dried over MgSO₄ and concentrated under reduced pressure, and purified by column chromatography.

AUXACONJUGATIN B (1a). The residue was purified using flash chromatography (acetonitrile/hexane/CH₂Cl₂ = 1:5:0) to afford 1a (36.8 mg, 74%) as a red solid. ¹H NMR (500 MHz, DMSO-d₆) 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 (d, 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), 1.3C NMR (125 MHz, DMSO-d₆) 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 C₁₈H₁₅NO₃Cl, 328.0740; found 328.0738.

4-Methoxy-6-((1E,3E,5E)-6-((4,4,5,5-tetramethyl-1,3-dioxaborolan-2-yl)hexa-1,3,5-trienyl)-2H-pyran-2-one (22a). The residue was purified using flash chromatography (acetonitrile/hexane/CH₂Cl₂ = 1:16) to afford 1g (49.2 mg, 82%) as a red solid. ¹H NMR (500 MHz, DMSO-d₆) 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). ¹³C NMR (125 MHz, DMSO-d₆) 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 C₁₃H₂₆O₄N₂Na⁺, 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). DiOC₂(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.
in buffer A (20 mM HEPES–KOH, pH 7.5, 10 mM KCl, 1.5 mM MgCl₂, 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 (750g, 10 min, 4°C). The supernatant was then obtained by centrifugation at 10000g 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 Ig in vivo. Female congenital athymic BALB/c nude (nu/nu) mice were purchased from National Science 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 × 10⁶ A549 cells into the back of mice. When the tumor reached 80–120 mm³ in volume, animals were divided randomly into control and test groups consisting of six mice per group (day 0). Daily sc administration of compound Ig, 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 thrice 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 (mm³) = (L × W²)/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, ¹³C, and HRMS data of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References


(11) Coleman, R. S.; Walczak, M. C. Total synthesis of gymnoconjuga


(21) Wuts, P. G. M.; Thompson, P. A. Preparation of halomethanebor


(26) Adams, J. M.; Cory, S. Apoptosomes; engines for caspase activa

