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Digest

Chemistry and biology of ophiobolin A and its congeners

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ABSTRACT

Ophiobolin A is a fungal secondary metabolite that was found to have significant activity against apoptosis-resistant glioblastoma cells through the induction of a non-apoptotic cell death, offering an innovative strategy to combat this aggressive cancer. The current article aims to make the bridge between the anti-cancer effects of ophiobolin A and its unique reaction with primary amines and suggests that pyrrolylation of lysine residues on its intracellular target protein(s) and/or phosphatidylethanolamine lipid is responsible for its biological effects. The article also discusses chemical derivatization of ophiobolin A to establish first synthetically generated structure-activity relationship. Finally, the reported total synthesis efforts toward the ophiobolin class of sesterterpenes are discussed and identified as a fertile area for improvement in pursuit of these molecules as anticancer agents.

Natural sources and biological activities of ophiobolins

Ophiobolins are a group of sesterterpenes that have been isolated as secondary phytotoxic metabolites produced by fungal pathogens of several crops, such as rice, maize, and sorghum. Ophiobolin A (**1**, Figure 1), the first member of this group of naturally occurring compounds, also called cochliobolin, was isolated and characterized by Canonica et al.[1] as a phytotoxin produced by the rice pathogen *Helminthosporium orizae* (or *Cochliobolus orizae*, or *Drechslera orizae*, or *Bipolaris orizae*). It was also obtained independently from the same fungus, also named *Ophiobolus miyabeanus* (aka *Cochliobolus miyabeanus*) by Ohkawa and Tamura[2] and Nozoe et al.[3] Ophiobolin A and its congeners share the same 5-8-5 octadipentacarbotriclycic ring with fusicoccins and cotylenins, a group of diterpenoids produced by *Fusicoccum amygdali*, the causal agent of almond and peach diseases[4,5] and by *Cladosporium* sp. 501–7 W.[6,7]

After ophiobolin A, several congeners were isolated in the late 1960s. These include ophiobolin B (**2**) from *Bipolaris oryzae*,[8] ophiobolin C (**3**) from *Bipolaris zizanie*,[9] ophiobolin D (**4**) from *Cephalosporium caerulens*,[10,11] and ophiobolin F (**5**) from *Bipolaris maydis*.[12] Successively, a lactonized form of ophiobolin A (**6**) was obtained from *C. miyabeanus*,[13] 3-anhydroophiobolin A (**7**) and 6-epimers (**8** and **9**) from a species of *Helminthosporium*. Metabolites **7**, **8** and **9** were found to inhibit photosynthesis, with **1** being the most effective.[14] Successively, **7**, **8** and **9** were isolated together with **1** from the culture of *Bipolaris sorghicola* (or *Drechslera sorghicola*), a fungal pathogen of Johnsongrass (*Sorghum halepense* L.). When tested on several plants, using a leaf spot assay, **1** and **8** were more phytotoxic than their anhydro derivatives **7** and **9** against sorghum, sicklepod, and maize.[15] Ophiobolins G and H (**10** and **11**) were obtained from *Aspergillus ustus* and found to exhibit antibacterial activity against *Bacillus subtilis* (Gram +) but did not have any activity against Gram- bacteria.[15,16] Ophiobolin I and its 25-hydroxy congeners (**12** and **13**) were isolated together with **1**, **3**, **7**, **8**, **9** from *D. sorghicola* and *Drechslera maydis*, the causal agent of Southern corn leaf blight. All these ophiobolins induced characteristic lesions on host plants and **8** showed selective toxicity against corn.[17]

Successively, 6-*epi*-ophiobolin I, ophiobolin J and its 8-deoxy congener (**14-16**) were obtained from *D. orizae*.[18] Ophiobolin K and its 6-epimer (**17** and **18**) were isolated from *A. ustus*, with **17** exhibiting nematocidal activity against *Caenorhabditis elegans*.[19] Ophiobolin L and its 6-epimer (**19** and **20**), and ophiobolin B lactone (**21**) were found to

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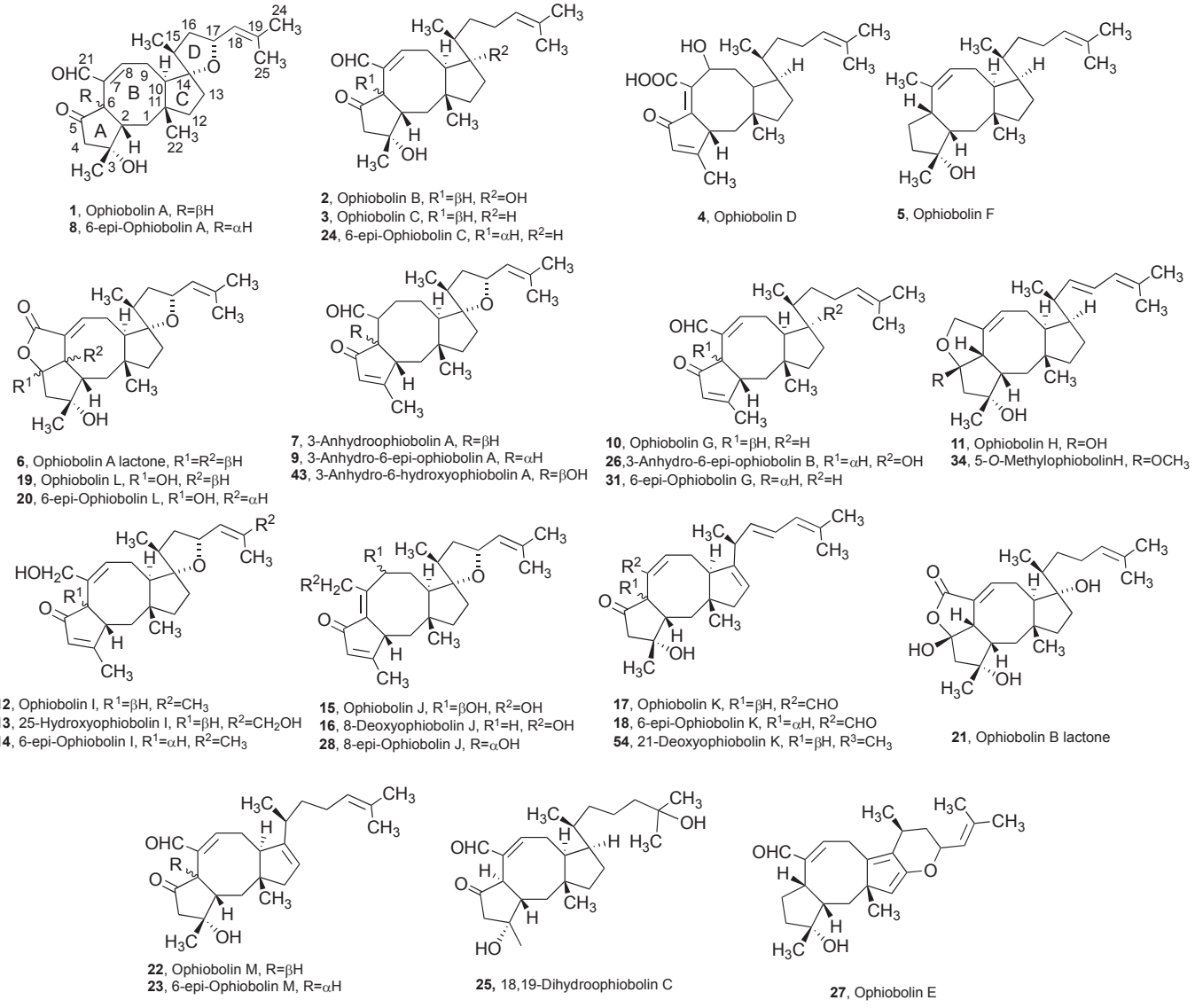

Figure 1. Structures of ophiobolins **1** **28**, **31**, **34**, **43** and **54** (Table 1).

be produced together with **1**, **2** and **7** by *Cochliobolus heterostrophus*, but no biological activity was reported.[20] From the cultures of the same fungus, ophiobolin M, 6-*epi*-ophiobolin M, 6-*epi*-ophiobolin C and 18,19-dihydroophiobolin C (**22-25**) were isolated together with some other known ophiobolins. When tested for nematocidal activity, metabolites **3** and **22** were found to be most potent compounds inhibiting *C. elegans*.[21] Successively, from *C. heterostrophus* race O, 6-*epi*-3-anhydroophiobolin B (**26**) was obtained, together with six already known metabolites **1**, **2**, **7** **9** and **12**. Among these, **1** produced potent cytotoxic and marginal antimalarial activities.[22]

More recently, during the research carried out on *Drechslera gigantea*, **1** and its three congeners, **8**, **9** and **12**, were isolated from the solid fungal culture. Structure-activity relationship (SAR) studies carried out assaying **1** and its three congeners on the host weed showed that the hydroxy group at C-3, the stereochemistry at C-6, and the aldehyde group at C-7 are structural features important for the phytotoxicity.[23] Successively, from the same culture filtrates, new metabolites, ophiobolin E and 8-*epi*-ophiobolin J (**27** and **28**), were obtained together with known **2** and **15**.[24] When assayed on four weeds by the leaf-puncture assay, **2** and **15** proved to be toxic to *Bromus* sp. and *Hordeum marinum* leaves.[24] The availability of a large amount of ophiobolin A made it possible to obtain crystals of **1** by slow evaporation from a benzene solution and its crystalline structure was determined by X-ray analysis.[25] The fungus *D. gigantea* was found to be a suitable source for obtaining ophiobolin A in large amounts. More recently, another prolific fungal source *B. maydis* was identified, where ophiobolin A yields depended on the use of light to affect its biosynthesis.[26]

Halorosellinic acid (**29**, Figure 2) was isolated from the marine fungus *Halorosellinia oceanica* BCC 5149. Metabolite **29** displayed antimalarial activity against *Plasmodium falciparum* (KB and BC-1 cell lines) with an IC_{50} of 13 μg/mL and week antibacterial activity at 200 μg/mL against *Mycobacterium tuberculosis*.[27] Although ophiobolin N has not yet been identified in a natural source, its 6-epimer (**30**) was produced, together with 6-*epi*-ohiobolin G (**31**) by the marine fungus *Emericella variecolor* together with six already known ophiobolins. Only **17** showed significant cytotoxic activity against various tumor cell lines, including adriamycin-resistant mouse leukemia cells P-388, with IC_{50} values of 0.27–0.65 μM.[28] More recently **17** and its 6-epimer **18** were also isolated from *Aspergillus calidoustus*.[29]

Five new ophiobolins, named 5,6 -ophiobolin H, its 5-*O*-methyl derivative (**32** and **33**), 5-*O*-methylophiobolin H (**34**), (6α)-21,21,*O*-dihydroophiobolin G and its 18,19-dihydro-18,19-dihydroxy congener (**35** and **36**), were isolated from the fungus *A. ustus* obtained from the

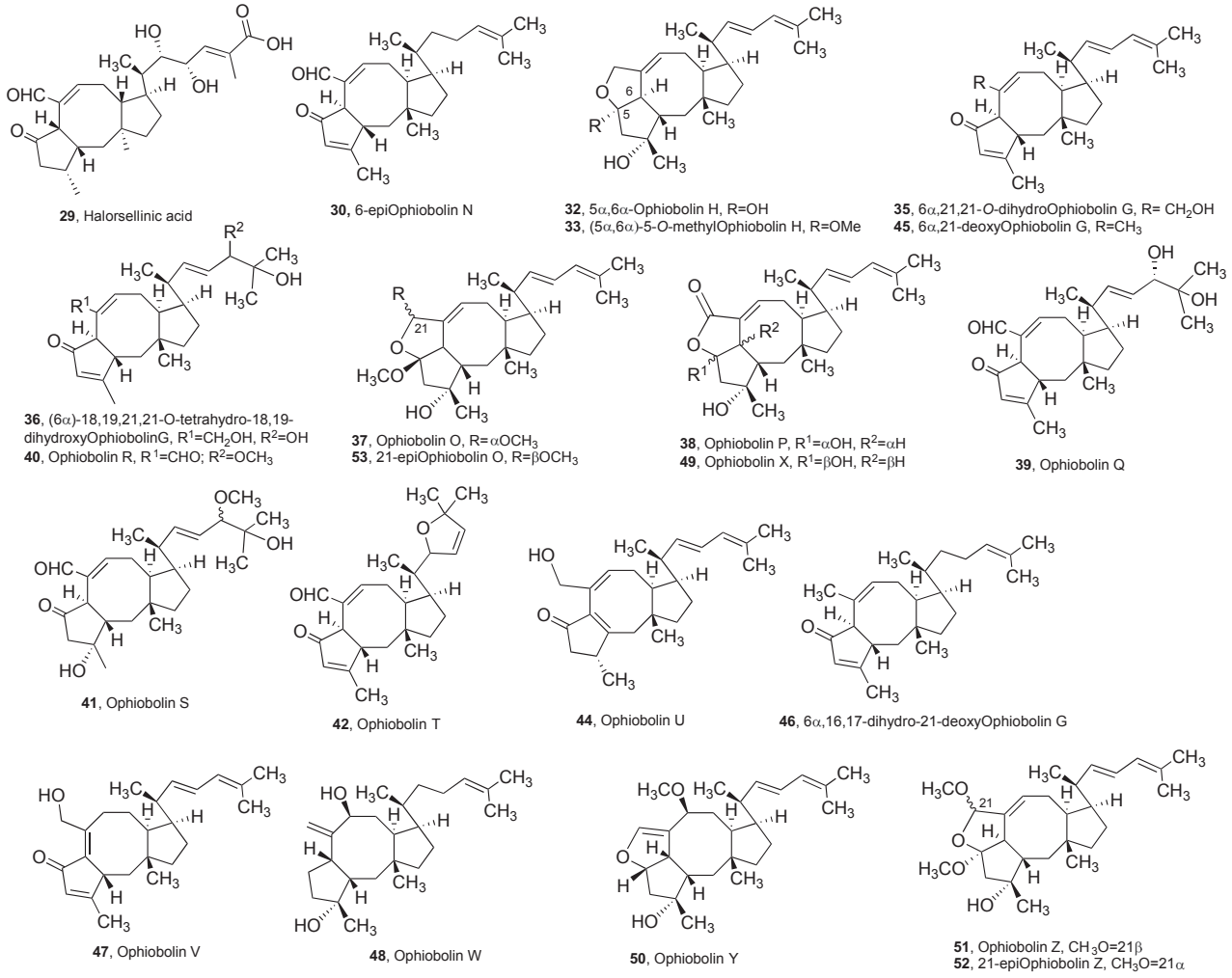

Figure 2. Structures of ophiobolins **29, 30, 32, 33, 35, 36-42**and **44-53** (Table 1).

Mediterranean sponge *Suberites domuncula*. They did not display cytotoxic activity when tested against murine L5178Y lymphoma cells.[30] Ophiobolin O (**37**) was found in *A. ustus* and showed antiproliferative activity against human breast cancer MCF-7 cells.[31] Ophiobolins P, Q, R and T (**38-42**) were isolated from an endolichenic fungus *Ulocladium* sp. Ophiobolin T exhibited cytotoxic, while ophiobolins P and T antibacterial activities against *B. subtilis*.[32] Successively, 3-anhydro-6-hydroxyophiobolin A (**43**) was obtained from *B. oryzae* and was found to display strong antimicrobial activity against Bacille *Calmette–Guerin*, *B. subtilis*, *Staphylococcus aureus*, and potent antiproliferative activity against HepG2 and K562 cell lines.[33] Ophiobolin U (**44**) was isolated from *Aspergillus* section *Usti* together with other known ophiobolins. Ophiobolins **1**, **2**, **3** and **17** displayed toxicity towards leukemia cells with the induction of apoptosis at nanomolar concentrations.[34]

The (6α)-21-deoxyophiobolin G, (6α)-16,17-dihydro-21-deoxyophiobolin G, ophiobolin V and ophiobolin W (**45-48**) were found to be produced together with previously known metabolites **32** and **44** by *A. ustus* obtained from the marine green alga *Codium fragile*.[35] Ophiobolins **32** and **44** exhibited inhibitory activities against *Escherichia coli*. Metabolite **44** also showed activity against *S. aureus* and toxicity against brine shrimp (*Artemia salina*).[35] Most recent additions to the ophiobolin family are ophiobolins X, Y, Z, ophiobolin Z C-21 epimer, 21-*epi*-ophiobolin O and 21-deoxyophiobolin K (**49-54**) that were isolated from the mangrove fungus *A. ustus*, with some of them exhibiting cytotoxic activity against 6 human cancer cell lines.[36]

For a more detailed review of the biological properties of the members of the ophiobolin family of sesterterpenes, the reader is referred to an earlier report by Au et al.[37] and a more recent comprehensive review by Tian et al.[38] These excellent sources cover biological properties of most of the above ophiobolins (known at the time of the publication date of the review), such as their role in plants, animals and microorganisms, and the mode of action studies. Here, our focus is chemical and biological studies of ophiobolin A as related to its potential of overcoming cancer cell resistance to therapy. We start by discussing the mode of action studies, carrying on with the reported synthetic modifications and conclude by discussing total chemical synthesis of three ophiobolins, which can be used to generate synthetic analogues with deep-seated structural modifications, inaccessible through natural product derivatization efforts.

Ophiobolin A as a promising anticancer agent

Cellular effects of ophiobolin A

The anticancer effects of ophiobolin A (**1**) have been the subject of numerous studies. It has been investigated by the National Cancer Institute (NCI) in the 60-cell line panel giving the mean concentrations resulting in 50% growth inhibition (GI_{50}) of 72 nM (ranging from 16 to 400 nM) and mean concentrations resulting in 50% lethality (LC_{50}) about 10 times higher, i.e. 629 nM. The mechanistic investigations of its cellular effects have been conducted in different cell types, including breast cancers, melanoma and glioma, although leukemia cells appear to be slightly more sensitive in comparison to other cancer types based on the NCI 60-cell line screen. In leukemic cells, **1** appeared firstly to

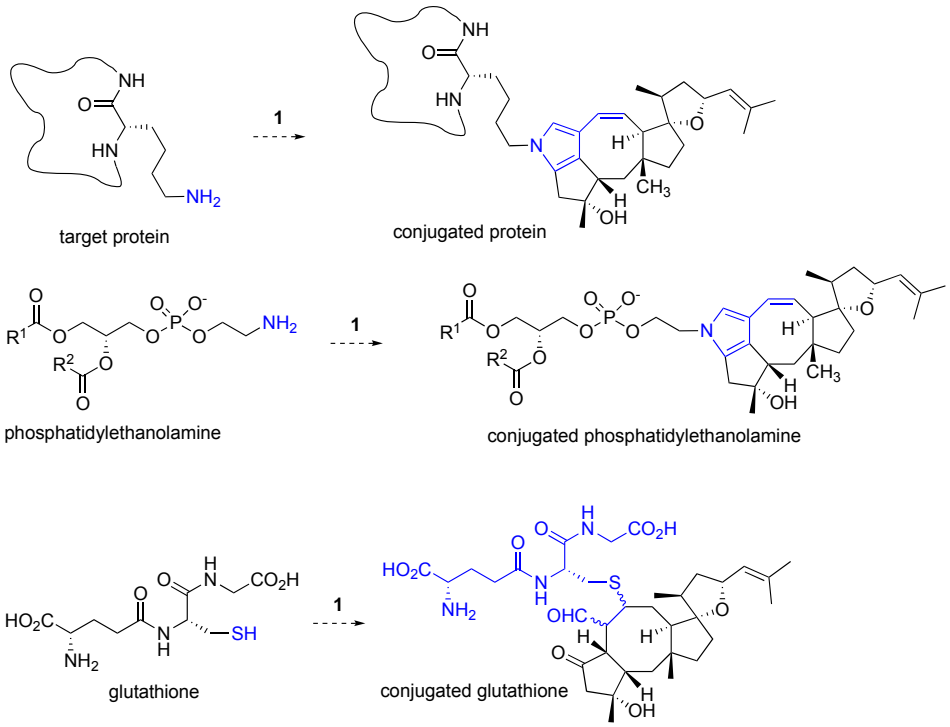

Figure 3. Proposed chemical reactivity of **1** in biological systems.

trigger apoptotic cell death,[39] a feature that was also observed later with respect to the MDA-MB-231 breast cancer model. In breast cancer cells treated with **1**, Bhatia *et al.* observed cell cycle blockage associated with a decrease in ribosomal protein S6, extracellular signal-regulated kinase (ERK) and retinoblastoma protein (Rb) phosphorylation leading consequently to apoptotic cell death.[40] However, we and others have shown that **1** does not induce apoptosis in glioblastoma (GBM) cells, but rather triggers a paraptosis-like cell death associated with endoplasmic reticulum vacuolization and unfolded protein response (UPR).[41,42] Paraptosis is a form of non-apoptotic cell death characterized by massive cytoplasmic vacuolization that begins with the physical enlargement of mitochondria and the endoplasmic reticulum (ER).[43,44] It is characterized by the absence of apoptotic morphology, DNA fragmentation, caspase activation or poly(ADP-ribose) polymerase (PARP) cleavage. Although paraptosis is prevented by inhibitors of transcription, indicating that it is programmatic in nature, apoptosis inhibitors are ineffective in inhibiting paraptosis, identifying it as a distinct biochemical process.[43,44] Paraptosis was shown to be a relevant cell death process in the brain tissue of GBM patients.[45]

Based on a systematic comparative study of cell death mechanisms triggered by **1** in 8 cancer cell models including glioma, breast cancers, rhabdomyosarcoma and cervical cancers, Morrison et al. highlighted that most of the cellular models (6/8) indeed displayed ER swelling associated with the expression of ER stress response proteins and/or Ca^{2+} release. Depending on the cellular model used, treated cells also displayed altered mitochondria with respect to the amount, branching, functionality, reactive oxygen species (ROS) and loss of membrane potential.[46] These results are consistent with the study of Rodolfo et al.[47] conducted on melanoma cells revealing mitochondrial network fragmentation, ROS production, increase in autophagy markers and finally apoptosis after treatment with **1**. In this study, the proteomic comparison between treated and untreated melanoma cells revealed significant changes in the expression of proteins involved in mitochondrial transport and protein folding, such as mitochondrial heat shock proteins and peptidylprolyl isomerase.[47] Interestingly, calreticulin, a well-known Ca^{2+} binding and ER resident marker, was increased upon treatment with **1**.[47] In glioma cells, activating transcription factor 4 (ATF4), CCAAT-enhancer-binding protein homologous protein (CHOP) and inositol-requiring enzyme 1α (IRE1α) expression were also increased as a consequence of the ER stress leading to polyubiquitinylation. However, in glioma cells, these features ultimately lead to the paraptosis-like cell death and not apoptosis.[42] It is noteworthy, thiol antioxidants prevented **1**-mediated ER stress in these cells (see molecular effects section below).

Additional considerations making **1** a promising anticancer agent stem from the results obtained in multidrug resistant cancer models expressing ATP-binding cassette (ABC) transporters or being tumor protein (p53) null. In these experiments **1** displayed similar IC_{50} values than on the non-multidrug resistant (MDR) parental cell lines.[48] Finally, **1** was also shown to decrease markedly the proportion of invasive mesenchymal cancer stem cells (CSCs; CD44+/CD24-) at 200 nM as well as mammosphere formation in four breast cancer models.[49] These effects seem to be related to the ability of **1** to impair Kirsten rat sarcoma virus (K-ras) nanoclustering in a calmodulin dependent manner as previously shown with respect to salinomycin used as a reference cancer stem cell inhibitor.[49] Cancer stem cells represent the top of the cancer cell hierarchy and display self-renewal capacities similar to stem cells. Being highly resistant to chemotherapies, they are thought to be responsible for patient relapses. Therefore, the decrease of cancer stem cells mediated by **1** could potentially reduce the risk of cancer recurrence.

Molecular effects of ophiobolin A

In 1984 and 1985, Leung et al. demonstrated for the first time that **1** is an inhibitor of the calmodulin-dependent cyclic nucleotide phosphodiesterase, both on bovine brain calmodulin and in plants, such as maize. This effect is irreversible, time-dependent and calcium-dependent.[50,51] In 1998, Au and Leung identified specific lysine residues in calmodulin that play key roles in inhibitory activity of **1** through a mutagenesis study.[52] However, the *in vitro* growth inhibitory effects of **1** do not correlate with calmodulin mRNA expression level in the NCI 60-cell line panel,[46] suggesting possible other targets. Two kinds of chemical reactivity have been shown to possibly occur in mammalian

Table 1
Fungal sources and biological activities of ophiobolins.

ophiobolin	fungal source	biological activity	reference
1	*Bipolaris oryzae* *Drechslera gigantea* *Aspergillus* section *Usti*	phytotoxic cytotoxic	1, 2, 3, 14, 23, 34
2	*B. oryzae* *A. section Usti*	phytotoxic cytotoxic	8, 23, 34
3	*Bipolaris zizanie* *A. section Usti*	nematocidal cytotoxic	9, 34, 21
4	*Cephalosporium caerulens*		10, 11
5	*Bipolaris maydis*		12
6	*Cochliobolus miyabeanus*		13
7	*Heminthosporium* sp. *Bipolaris sorghicola*	phytotoxic	14, 15
8	*B. sorghicola*	phytotoxic	14, 15
9	*B. sorghicola*	phytotoxic	14, 15
10	*Aspergillus ustus*	antibacterial cytotoxic	15, 16, 36
11	*A. ustus*	antibacterial cytotoxic	15, 36
12	*Drechslera maydis* *Drechslera sorghicola*	phytotoxic	17
13	*D. sorghicola*	phytotoxic	17
14	*Drechslera oryzae*		18
15	*D. oryzae*	phytotoxic	18, 23
16	*D. oryzae*		18, 23
17	*A. ustus* *Emericella variecolor* *A. section Usti*	nematocidal cytotoxic	19, 28, 34, 36
18	*Aspergillus ustus* *Aspergillus calidoustus* *Ulocladium* sp.	nematocidal cytotoxic	29, 33, 36
19	*Cochliobolus heterostrophus*	cytotoxic	20
20	*C. heterostrophus*	cytotoxic	20
21	*C. heterostrophus*	cytotoxic	20
22	*C. heterostrophus*	cytotoxic	21
23	*C. heterostrophus*		21
24	*C. heterostrophus*		21
25	*C. heterostrophus*		21
26	*C. heterostrophus*	cytotoxic	22
27	*Drechslera gigantea*		24
28	*D. gigantea*		24
29	*Halorosellinia oceanica*	antimalarial antibacterial	27
30	*Emericella variecolor*		28
31	*E. variecolor* *Ulocladium* sp. *A. ustus*	cytotoxic	28, 32, 36
32	*A. ustus*	antibacterial	30, 35
33	*A. ustus*		30, 36
34	*A. ustus*		30, 36
35	*A. ustus* *U.* sp.		30, 32, 36
36	*A. ustus*		30
37	*A. ustus*	cytotoxic cytotoxic	31, 36
38	*U.* sp. *A. ustus*	antibacterial	32, 36
39	*U.* sp. *A. ustus*	cytotoxic	32, 36
40	*U.* sp.		32
41	*U.* sp.		32
42	*U.* sp.	cytotoxic antibacterial	32
43	*B. oryzae*	antimicrobial cytotoxic	33
44	*A.* section *Usti* *A. ustus*	antibacterial zootoxic	34, 35, 36
45	*A. ustus*		35
46	*A. ustus*		35
47	*A. ustus*		35
48	*A. ustus*		35
49	*A. ustus*		36
50	*A. ustus*		36
51	*A. ustus*	cytotoxic	36
52	*A. ustus*	cytotoxic	36
53	*A. ustus*		36
54	*A. ustus*		36

cells and explain, at least partly, the cellular effects described above.

The first relates to the pyrrolylation of primary amines, demonstrated using a simple chemical model studies (Figure 5, bottom).[53] This can occur on lysine residues of several targets (Figure 3, top), including calmodulin, but also on phosphatidylethanolamine (Figure 3 middle).[54] Importantly, the inhibition of K-ras nanoclustering by **1** appeared dependent on its calmodulin inhibition properties according to the results obtained with calmodulins mutated on various lysine residues.[49] On the other hand, pyrrole **1** adducts on phosphatidylethanolamine switch its polar head group to a bulky hydrophobic residue, leading to lipid bilayer destabilization and leakiness.[54] The relevance of phosphatidylethanolamine-**1** adducts with respect to its *in vitro* anti-cancer activity is supported by the relative resistance of cancer cells when they are depleted of phosphatidylethanolamine through inactivation of genes involved in its biosynthesis, i.e. ethanolamine kinase, phosphoethanolamine cytidyl transferase or 1,2 diacylgycerol ethanolamine phosphotransferase.[54] These enzymes take part in the main synthetic pathway of phosphatidylethanolamine, namely the Kennedy pathway occurring within the ER. Alternatively, phosphatidylethanolamine can be synthesized in the mitochondria from phosphatidylserine through decarboxylation or via two other minor pathways in the ER.[55] Taking into account that (i) phosphatidylethanolamine is abundant in the inner leaflet of membranes and specifically in the inner mitochondrial membrane where it is required for respiration and (ii) it chaperons membrane-associated proteins involved in the ER stress to their folded state,[55] the effects of **1** on ER and/or mitochondria described in the previous section appear consistent. Despite the fact that Ras nanoclusters, which are lipid-dependent platforms enriched in phosphatidylserine, cholesterol, phosphatidic acid, phosphatidyl inositol 4,5-bisphosphate (PIP2) and phosphatidyl inositol (3,4,5)-trisphosphate PIP3,[56] modifications to phosphatidylethanolamine which accounts for ~20% of the total membrane lipids could impact Ras clustering indirectly. This could occur in conjunction with **1**-mediated inhibition of calmodulin, whose activity is also required for K-ras clustering and further signalling.

The second type of chemical reactivity of **1** is related to the Michael adduct formation with thiols, including glutathione (Figure 3 bottom).[42] Accordingly, thiol antioxidants prevent **1**-meditated vacuolization and ER stress.[42] In the absence of these defence mechanisms, **1** could react with sulfhydryl groups of proteins resulting in their misfolding, which is ultimately followed by cell death.[42] Whether the cell death is apoptotic, paraptotic or even of another kind would depend on the cell type.[46]

In vitro anti-cancer effects of other ophiobolins

Ophiobolin O (**37**) is the second best studied ophiobolin mainly investigated for combatting breast cancers.[57–59] It has been shown to induce cell cycle arrest of MCF-7 cells in G1 phase associated with (i) a decrease in cyclin D, cyclin E and cyclin-dependent kinase 2 (CDK2) protein expression levels and (ii) an increase in p-cyclin D1, p21 and p27 protein expression levels,[59] followed by an apoptotic cell death.[57,58] These effects observed at the concentration of 15 μM may be mediated by a strong decrease of phosphorylation of both protein kinase B (PKB) and glycogen synthase kinase 3 β (GSK3β).[59] The role of GSK3β in **37**-induced effects has been further supported by GSK3β silencing experiments showing partial impairment of cell cycle blockage induced by **37**. Additionally, molecular docking suggests possible direct

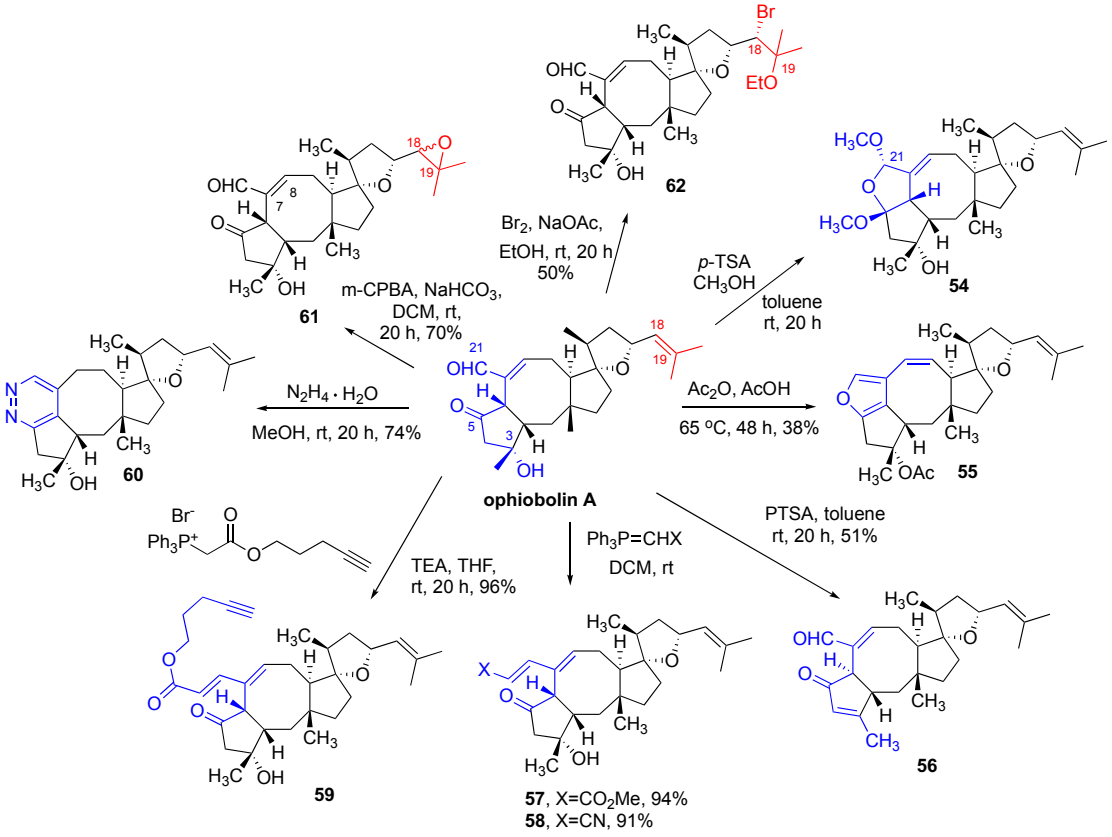

Figure 4. Derivatization of **1** at positions C5-C21 (blue) and C18-C19 (red).

binding of **37** on GSK3β.[56] It is noteworthy that **37** also decreases the expression level of the MDR1 efflux pumps at nontoxic concentrations (0.1 μM), leading to increased efficacy of chemotherapeutic agents, such as adriamycin both *in vitro* and *in vivo*.[58]

While most of the other ophiobolins have been evaluated for their cytotoxic activity against various cancer cell lines (see Table 1 and the detailed IC_{50} results in the review by Tian et. al.[38]), the detailed cellular and molecular effects have not been investigated in depth. Briefly, ophiobolins **2**, **3** and **17** display IC_{50} values below 1 μM, which should make them of interest as well.[34,60,38] Ophiobolin **2** displayed effects on CSC (CD44 + /CD24-) similar to **1,** while **3** lacked this kind of activity.[49]

In vivo anti-cancer effects of ophiobolins

In vivo studies of ophiobolins' anti-tumoral effects remain limited to date. The acute LD_{50} doses of **1** in mice range from 12 mg/kg by intravenous route to 238 mg/kg when injected subcutaneously.[37] It was shown to increase survival of mice bearing B16F10 melanoma lung pseudometastases when administered at 10 mg/kg ip 3 times per week for three weeks.[48] Similar results were obtained later in a human orthotopic glioblastoma model.[56] However, the NCI experiments failed to demonstrate **1**-mediated survival benefits in the L1210 leukemia models even with daily ip administration of 10 mg/kg for 9 days (nontoxic therapeutic scheme). To solve solubility issues and improve tumor targeting Morrison et al.[61] have developed the first formulation of **1** in mesoporous silica particles, but no *in vivo* use has been published to date. The only other ophiobolin that has been tested *in vivo* is **37**. It has been shown to reduce subcutaneous breast tumor growth in mice administered at 5 to 20 mg/kg intravenously every 4 days.[62] Additionally, it increased the therapeutic benefits of adriamycin when used in combination (5 mg/kg).[58]

Semisynthetic studies of ophiobolin A

Ophiobolin **1**, isolated from *D. gigantea* in significant quantities,[48] was subjected to a variety transformations as described by us.[53] It was found that **1** readily forms complex acetals, such as **54**, engaging the C-5 and C-21 carbonyl groups (Figure 4). Notably, derivative **54** was formed as a single stereoisomer. This part of the molecule can also give rise to aromatic rings, such furan in **55** and pyridazine in **60** when reacted with acetic anhydride and hydrazine, correspondingly. The C-3 hydroxyl is relatively labile and can undergo dehydration when treated with a strong acid to give **56**. The aldehyde group at C-21 is more reactive than ketone C-5 and can be engaged in a Wittig reaction to form α,β-unsaturated derivatives **57-59**. The C-18,C-19 olefin is more electron rich than its counterpart at C-7,C-8 and can be converted to an epoxide in **61** and a bromoethoxide moiety in **62**. Evaluation of these semisynthetic analogues for cytotoxic effects revealed the critical role played by the C-5,C-21 dicarbonyl functionality in the antiproliferative effects of ophiobolin A and its analogues. Indeed, activity was retained in analogues **61** and **62**, but eradicated in compounds **55, 57-60**. Compound **56** retained some activity, likely due to the presence of the C-5,C-21-dicarbonyl moiety. However, the potency was reduced probably because of diminished reactivity of the C-5 carbonyl conjugated with the C-3,C-4 olefin.

The bromoalkoxylation reaction was utilized for the introduction of an alkyne functionality for click chemistry (Figure 5, top). Thus, **63** obtained by bromopropargylation of **1** was coupled to the immunofluorescent coumarin dye **64** to obtain probe **65**. The latter was utilized in the development of a single dish gradient screening of small molecule localization.[59] Finally, to show the chemical feasibility of pyrrole formation engaging the C-5 and C-21 carbonyl groups, as referred to in the above discussion of biological significance of this reaction, **1** was reacted with primary amines in an organic solvent as well as under

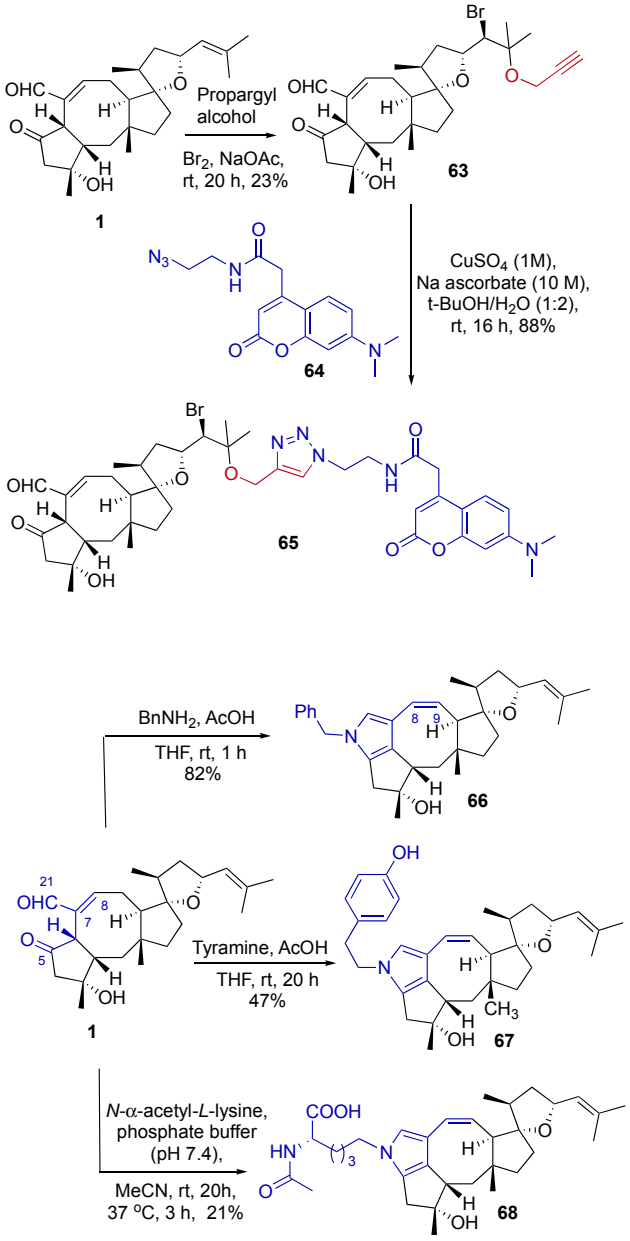

Figure 5. Preparation of a fluorescent analogue **65** (top) and pyrrolylation of **1** (bottom).

physiologically relevant conditions to obtain pyrroles **66-68**. This process was facile and pyrrole **66** was obtained under mild acid catalysis at room temperature in less than an hour in a good yield.[53]

Total syntheses of ophiobolins A, C and N

Total synthesis of (+)-ophiobolin A by Nakada and co-workers

This synthesis originally reported in 2011 is the first and remains the only synthesis of **1** to date.[63,64] Enzymatically prepared compound **69** (Figure 6) underwent selective reduction of the free carboxyl group to give alcohol **70**, which through a series of functional group transformations was converted to acetate **71**. Reductive ozonolysis, protection of the resulting primary alcohol as ethoxyethyl ethyl ether, removal of the acetate, oxidation of the alcohol, Wittig olefination and reduction of the ester then produced allylic alcohol **72**. Treatment of compound **72** with mesyl chloride gave an intermediate allylic chloride, which through the Still's reaction introduced an allyl silane that underwent iodination to yield the first coupling partner **73**. The other coupling partner was prepared from known oxazolidinone **74**. The latter was subjected to iodolactonization to give iodide **75**, which was converted to alcohol **76** and then protected as silyl ether **77**. The coupling of **73** and **77** was achieved by conversion of iodide **73** to an organolithium derivative, which upon treatment with **77** gave rise to hemiacetal **78** as a mixture of two diastereomers. The intramolecular Hosomi-Sakurai reaction of **78** was a subject of considerable experimentation and finally the investigators settled on the use of BF_3-OEt_2 in the ether-toluene (1:1) solvent that produced the spiro compound **79** possessing the desired stereochemistry. Further functional group transformation gave intermediate **80**, which was further converted to advanced aldehyde **81** (Figure 6).

Compound **82** (Figure 7), prepared through a sequence of straightforward transformations, was coupled with **81** utilizing the Utimoto protocol. In this reaction the bulky aldehyde in **81** was coupled through the intermediate boron enolate in **82** to give aldol product **83**. The utilization of the Burgess reagent then provided the unsaturated product **84**. The hydrogenation of the olefin in **84** and MeLi addition to the ketone in **85** was followed by a series of functional group transformations to yield **87** through the intermediacy of tertiary alcohol **86**. Oxidation of primary alcohol in **87** gave rise to hemiacetal **88**. This was followed by MeLi addition to generate a secondary alcohol, oxidation of the latter and protection of the tertiary alcohol as TMS ether furnished ketone **89**. Compound **89** was further converted to enol triflate **90**, which underwent methoxycabonylation and reduction with DIBAL to yield allylic alcohol **91**. Further functional group transformation gave rise to ring-closing metathesis precursor **92**, which in the crucial step catalyzed by Hoveyda-Grubbs II catalyst in the presence of 10 eq of benzoquinone afforded the 8-membered ring in **93**. Benzylation and desilylation of **93** then led to **94**. The synthesis was completed by oxidation of the primary alcohol, Wittig olefination to install the trisubstituted olefin, removal of the benzyl protection, and oxidation of the diol to furnish the desired natural product **1**. The key steps in the construction of the ophiobolin skeleton included the construction of the C,D spiro junction using the Hosomi-Sakurai steroselective cyclization, joining of the A-ring to the C,D-rings utilizing the Utimoto protocol, and the construction of the B-ring using the ring-closing olefin metathesis process. Overall, this tour de force synthesis was an impressive achievement. However, the length of the synthesis, consisting of 47 steps, will be prohibitive to provision of large quantities of **1** or its close analogues.

Total synthesis of (+)-ophiobolin C by Kishi and co-workers

The first total synthesis of any ophiobolin was reported by Kishi and co-workers in 1989.[65] These investigators synthesized ophiobolin C (**3**) in enantiopure form starting from camphor bromide **95** (Figure 8). Using a previously reported procedure, **95** was converted to exocyclic alkene **96**. Ozonolysis, protection of the primary alcohol as a THP acetal, oxidation to the α,β-unsaturated ketone and reduction with $NaBH_4$ gave allylic alcohol **97**, which esterified to **98**. Compound **98** then underwent tandem Brook and Claisen rearrangement, which after hydrolysis resulted in acid **99**. A series of functional group transformations led to alcohol **100**. This was treated with previously repoted vinyl lithium reagent **101** giving secondary alcohol **102**. Hydrolysis with aqueous HF, iododesilylation with ICl/TBAF, removal of THP acetal and oxidation of the resulting alcohol then led to vinyl iodide **103**. The key step in the synthesis was ring-B closure through an intramolecular coupling

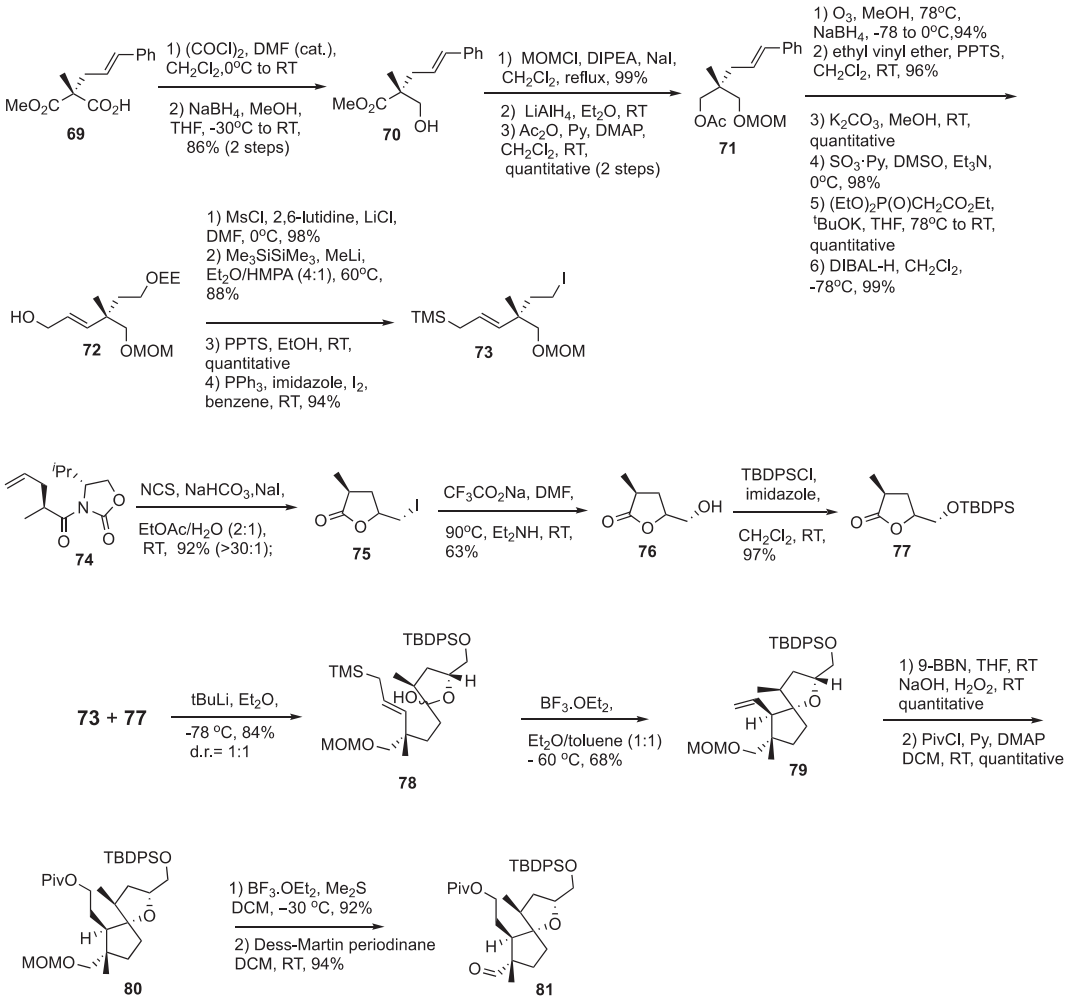

Figure 6. Synthesis of advanced intermediate **81.**

mediated by Ni(II)/Cr(II) pair. At this stage it was necessary to transpose allylic alcohol to C21 position and reduce the enone double bond. This was achieved through epoxidation of the exocyclic olefin, thiocarbonate formation and reduction with n-Bu_3SnH/AIBN. With the basic skeleton of **3** in place, the remaining transformations were not troublesome ultimately leading to the natural product (Figure 8). As described earlier, ophiobolin **3** is also of interest as a potential anticancer agent due to its high antiproliferative potency. Again, due to the length of this synthesis, consisting of 38 steps, it is unlikely that it could be utilized for the procurement of **3** in quantity or analogue synthesis.

Total synthesis of (-)-6-*epi*-ophiobolin N by Maimone and co-workers

The shortest synthesis of any ophiobolin was recently reported by Maimone and coworkers.[66] The investigators reported a total synthesis of (-)-6-*epi*-ophiobolin N utilizing a radical cascade cyclization. The key steps in the synthesis are shown in Figure 9. The radical cyclization precursors **106** and **107** were synthesized through a series of straightforward steps from farnesol. After a lot of experimentation, specifically related to the choice of the thiol component, which is believed to donate the final hydrogen atom, the researchers settled on benzothiophene-based TADDOL monothiol to produce **108** and **109** as mixtures of diastereomers. Corey-Chaykovsky epoxidation followed by a reductive ring opening of the epoxide with excess of lithium naphthalenide and dehalogenation furnished **111**. Finally, oxidation of both alcohols to carbonyls and an attempted removal of TBS ether protection, which resulted in dehydration, gave **30**. While this is a very short synthesis, it is plagued by diastereoselectivity problems and resulted in (-)-**30**, the enantiomer of the natural product. Furthermore, even the natural (+)-6-*epi*-ophiobolin N which has no reported activity. Clearly, the investigators did not set out to prepare ophiobolin **30**, but this is what they obtained from their radical cyclization procedure. It is thus unclear how applicable this strategy is to the synthesis of a particular desired ophiobolin or its analogues.

Conclusion

Glioblastoma is one of the top ten causes of cancer-related deaths. The standard treatment for glioblastoma patients includes surgery followed by radiotherapy with concomitant chemotherapy with temozolomide. Nevertheless, the prognosis is poor and the median survival is only 14.6 months. Despite a significant pace of drug discovery research, the median survival of glioblastoma patients has not changed over the past 10 years and the glioblastoma clinic is in dire need of conceptually new treatment strategies. Glioblastoma cells are generally highly

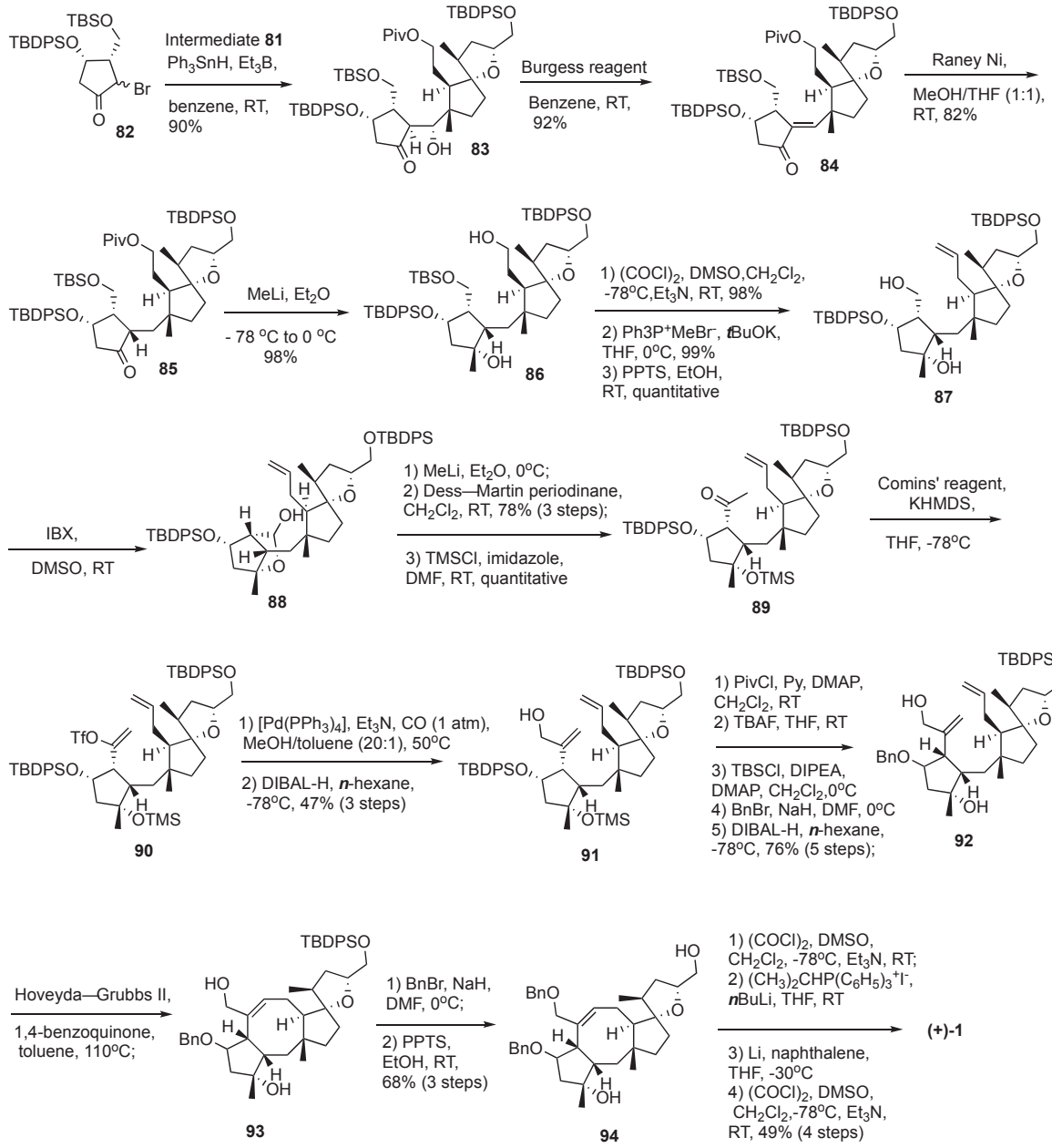

Figure 7. Completion of the synthesis of (+)-ophiobolin A by Nakada and co-workers.

resistant to the classical pro-apoptotic therapeutic approaches and thus anti-glioblastoma agents working through alternative non-apoptotic mechanisms are in great demand. Induction of paraptosis in apoptosis-resistant GBM cells with ophiobolin A was the first demonstration of a specific induction of this form of cell death in glioblastoma cells with a small molecule. Because paraptosis was found to be a relevant cell death process in the brain tissue of glioblastoma patients,[45] ophiobolin A, exhibiting initial promising *in vivo* effects in a mouse glioblastoma xenograft model, is an important lead in the pursuit of anti-glioblastoma agents with an innovative mode of action.

The first synthetically generated structure-activity relationship support the critical role played by the C5,C21-dicarbonyl functionality in the antiproliferative effects of ophiobolin A and its analogues. In addition, the unique reaction of ophiobolin A with primary amines suggests the possibility of pyrrolylation of lysine residues on its intracellular target protein(s) and/or phosphatidylethanolamine as discussed above. This finding should instigate further research efforts to study the biological significance of the Paal-Knorr pyrrole formation reaction and gain valuable insight into protein modification with natural products via this underexplored chemical mechanism.

The best source of ophiobolin A is currently through the fermentation of fungal strains *D. gigantea* and *B. maydis*. Unfortunately, the reported total syntheses of ophiobolins are currently incapable of procuring the large amounts of ophiobolins or even their analogues containing deep-seated structural modifications. This is an area where further efforts are required in pursuit of ophiobolin A and its analogues as promising anticancer agents.

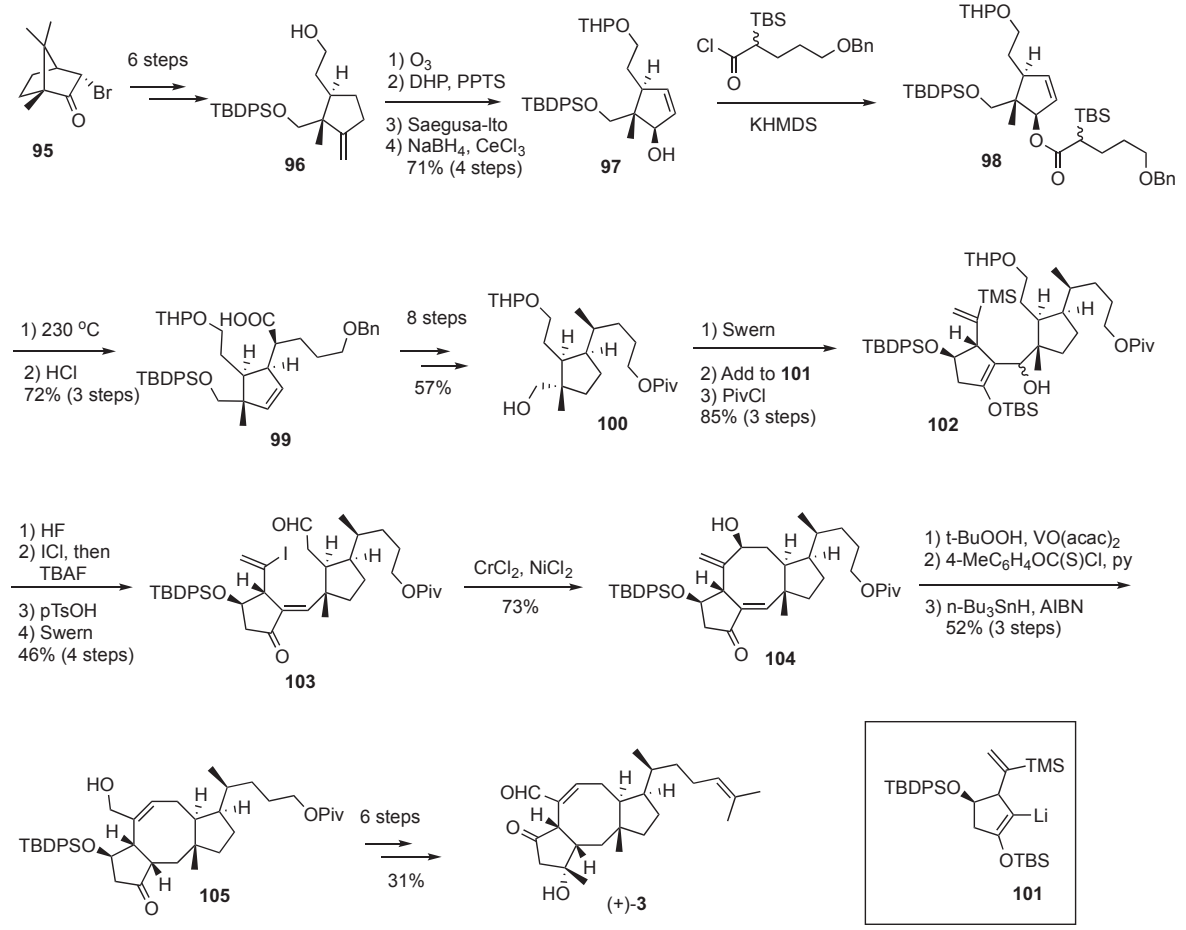

Figure 8. Total synthesis of (+)-ophiobolin C by Kishi and co-workers.

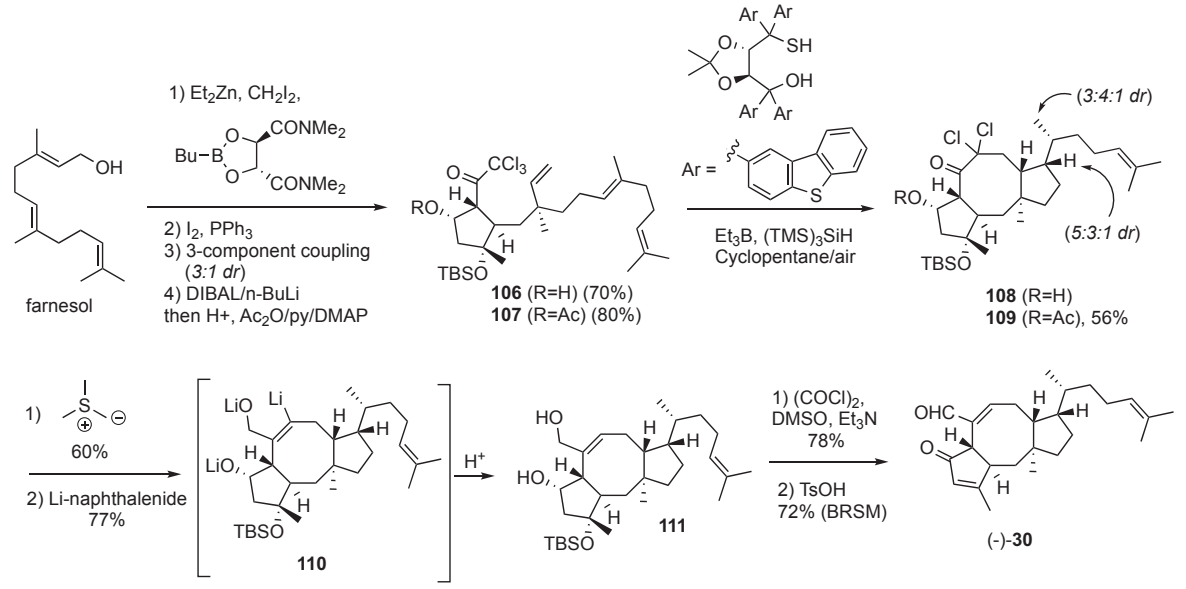

Figure 9. Total synthesis of (-)-6-*epi*-ophiobolin N by Maimone and co-workers.

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