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Fungal metabolite ophiobolin A as a promising anti-glioma agent: In vivo evaluation, structure–activity relationship and unique pyrrolylation of primary amines

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ABSTRACT

Glioblastoma, the most common form of malignant primary brain tumor, is characterized by resistance to apoptosis, which is largely responsible for the low effectiveness of the classical chemotherapeutic approaches based on apoptosis induction in cancer cells. Previously, a fungal secondary metabolite ophiobolin A was found to have significant activity against apoptosis-resistant glioblastoma cells through the induction of a non-apoptotic cell death, thus, offering an innovative strategy to combat this type of cancer. The current work describes the results of a preliminary evaluation of ophiobolin A in an in vivo glioblastoma model and its chemical derivatization to establish first synthetically generated structure–activity relationship. The synthetic work has also led to the discovery of a unique reaction of ophiobolin A with primary amines suggesting the possibility of pyrrolylation of lysine residues on its intracellular target protein(s).

Glioblastoma (GBM) is one of the top ten causes of cancer-related deaths.[1] The standard treatment for GBM patients includes surgery followed by radiotherapy with concomitant chemotherapy with temozolomide.[2,3] Nevertheless, the prognosis is poor and the median survival is only 14.6 months.[3] About half of GBM patients harbor an unmethylated MGMT promoter and they respond poorly to temozolomide chemotherapy.[4,5] To date, no alternative treatment exists for this group of patients. The development of molecularly targeted therapy has been a focus of current GBM research and recently more than 50 such targeted agents entered clinical trials.[6] To date, however, these have failed to improve clinical outcomes.[6–8] Despite a significant pace of drug discovery research, the median survival of GBM patients has not changed over the past 10 years and the GBM clinic is in dire need of conceptually new treatment strategies.

GBM cells are generally highly resistant to the classical pro-apoptotic therapeutic approaches.[9] Although a great deal of research has been aimed at rendering cancer cells more susceptible to therapy-induced apoptosis,[10] over the past decade a growing number of non-apoptotic cell death pathways have been revealed and compounds that induce anoikis, autophagy, regulated necrosis, mitotic catastrophe, paraptosis, parthanatos, methuosis, and lysosomal membrane permeabilization, are being investigated in clinical trials, with several agents already approved for the treatment of various cancers.[11] Our labs have been pursuing the identification of new anti-GBM agents working through alternative non-apoptotic mechanisms,[12–20] and in the first demonstration of a specific induction of paraptosis in GBM cells with a small molecule, we discovered that ophiobolin A (Fig. 1), a fungal metabolite from *Bipolaris* species, exhibited promising activity against apoptosis-resistant GBM cells through the induction of paraptosis.[12] Paraptosis is a form of non-apoptotic cell death characterized by a process of vacuolization that begins with the physical enlargement of mitochondria and the endoplasmic reticulum (ER).[21,22] It is accompanied by the absence of apoptotic morphology, DNA fragmentation, caspase activation or poly(ADP-ribose) polymerase (PARP) cleavage.[21,22] Although this cell death is prevented by inhibitors of protein synthesis and transcription, indicating that it is programmatic in nature, apoptosis inhibitors are ineffective in

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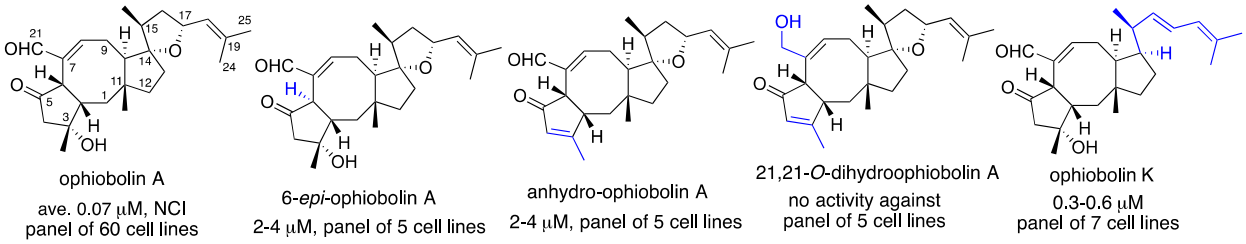

Figure 1. SAR of natural ophiobolin A congeners.

preventing paraptosis, identifying it as a distinct biochemical process. Moreover, paraptosis was found to be a relevant cell death process in the brain tissue of GBM patients.[23]

Ophiobolin A was evaluated in the NCI panel of 60 cell lines, where it registered an average GI_{50} value of 70 nM. Correlations of the differential cellular sensitivities reveal a possibly novel mechanism of action compared with >763 K compounds in the NCI database. Furthermore, non-proliferating keratinocytes displayed 5–10× lower sensitivity to ophiobolin A than cancer cells and this agent was shown not to be a substrate for MDR-related efflux pumps.[24] Ophiobolin A is a member of a large family of over 25 natural ophiobolins that have been isolated from various species of phytopathogenic fungi.[25] A number of them have been evaluated for cytotoxicity against cancer cells, providing the initial structure–activity relationship (SAR) data. These data identify the C5,C21-dicarbonyl functionality as critical for anticancer activity in this family of natural products. Indeed, changes that may affect the reactivity of this functionality, such as those represented by 6-*epi*- and anhydro-ophiobolin A (Fig. 1), result in a significant drop in activity.[26] Moreover, the elimination of one of these carbonyl groups from the molecule, such as that in 21,21-*O*-dihydroophiobolin A, leads to a complete loss of activity.[26] In contrast, changes in the C15–C25 fragment, such as that found in ophiobolin K, are tolerated.[27,28]

Despite its promise in the treatment of GBM, ophiobolin A has not been evaluated in an in vivo GBM model and its ability to cross the blood–brain barrier (BBB) has not been tested. Furthermore, no synthetic studies aimed at ophiobolin A derivatization have been reported to our knowledge and, thus, no synthetically generated SAR are available. Finally, the specific types of reactions that the reactive C5,C21-dicarbonyl functionality can undergo and that could be important in the biological environment have not been reported. The present investigation fills these gaps in the literature and describes a study aimed to address the above questions.[29]

A pilot in vivo experiment in an orthotopic mouse GBM model was performed (IP, 10 mg/kg ophiobolin A), 3× per week for a total of 21 days) revealing both statistically significant survival of mice bearing orthotopic U251-LUC tumors (Fig. 2, left) and tumor growth reduction (Fig. 2, right). Although conducted in a pilot manner without the optimization of the administration route, dose or treatment regimen, the initial positive results bode well for the successful development of ophiobolin A-based analogs or prodrugs with efficacious in vivo activity in GBM. Additionally, the ability of ophiobolin A to cross the blood–brain barrier (BBB) was demonstrated by injecting this metabolite intravenously into the lateral tail vein of a tumor bearing mouse and detecting it in an intact form 15 min post administration in the brain tumor extract (data not shown).

Synthetic derivatization of ophiobolin A began with the study of the reactivity of the C5,C21-dicarbonyl functionality (Fig. 3). It was found that in the presence of acid, ophiobolin A reacted with alcohols to form bis-acetals **1**–**3**. Acetals **1** and **2** were isolated as single diastereomers at C5 and C21, whereas **3** consisted of a mixture of C21-epimers. In addition, ophiobolin A readily formed aromatic rings, such as pyridazine **4** and furan **5** upon reactions with hydrazine and acetic anhydride, respectively. Furthermore, the C21-aldehyde was converted into α,β-unsaturated esters **6** and **7**, and nitrile **8**, when treated with stabilized Wittig reagents. Lastly, dehydration with the formation of C3,C4-alkene **9** was observed when the natural product was treated with acid in the absence of alcohols.

Next, the reactivity of the C18,C19-alkene was probed (Fig. 4). Thus, treatment with *m*-CPBA produced a mixture of C18-epimeric epoxides **10**. The reaction occurred selectively with the electron-rich C18,C19-alkene in the presence of the electron-deficient C7, C8-olefin. Following a dated report[30] illustrating the possibility of a selective halo-methoxylation of the electron-rich C18,C19-alkene, ophiobolin A was subjected to the treatment with bromine in a variety of alcohols as a solvent to obtain C18-bromo-C19-alkoxy derivatives **11**–**13**. In the case of ethoxy and 5-hexynoxy derivatives **11** and **12** only C18-α epimers were produced, whereas the major C18-α propargyl derivative **13** was accompanied by

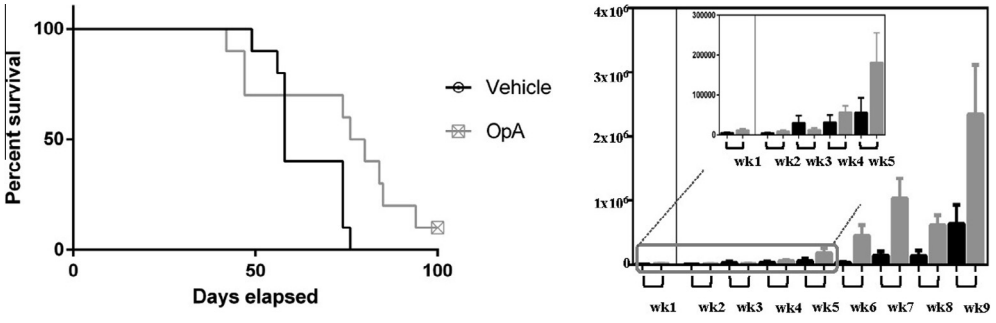

Figure 2. (Left) Survival of ophiobolin A-treated mice bearing orthotopic U251-LUC tumors. Mice inoculated with 1×10^6 U251-LUC cells were treated with 10 mg/kg ophiobolin A 3× per wk for a total of 21 days. Mice with no signs of GBM were sacrificed on day 100 after inoculation and were considered long term survivors. (Right) Temporal imaging of tumor growth after inoculation with U251-luciferase-expressing human GBM tumor cells. Relative growth in response to 10 mg/kg ophiobolin A (black columns) or 0.9% saline vehicle control (grey columns).

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