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Synthesis and antiproliferative activity of derivatives of the phyllanthusmin class of arylnaphthalene lignan lactones

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ABSTRACT

A series of arylnaphthalene lignan lactones based on the structure of the phyllanthusmins, a class of potent natural products possessing diphyllin as the aglycone, has been synthesized and screened for activity against multiple cancer cell lines. SAR exploration was performed on both the carbohydrate and lactone moieties of this structural class. These studies have revealed the importance of functionalization of the carbohydrate hydroxy groups with both acetylated and methylated analogues showing increased potency relative to those with unsubstituted sugar moieties. In addition, the requirement for the presence and position of the C-ring lactone has been demonstrated through reduction and selective re-oxidation of the lactone ring. The most potent compound in this study displayed an IC_{50} value of 18 nM in an HT-29 assay with several others ranging from 50 to 200 nM. In an effort to elucidate their potential mechanism(s) of action, the DNA topoisomerase IIa inhibitory activity of the most potent compounds was examined based on previous reports of structurally similar compounds, but does not appear to contribute significantly to their antiproliferative effects.

1. Introduction

The genus *Phyllanthus* has historically been a rich source of natural products possessing diverse chemical structures and biological activities.[1] Building in part upon this diversity, a recent effort to identify novel compounds with cytotoxic activity from *Phyllanthus poilanei* by Kinghorn and coworkers led to the isolation of two new natural products, phyllanthusmins D and E (Fig. 1),[2] along with the previously reported phyllanthusmins A–C.[3] The isolated phyllanthusmins displayed promising potent antiproliferative activity against various cancer cell lines, with phyllanthusmin D displaying the most potent activity with an IC_{50} value of 0.17 μM against HT-29 colon carcinoma cells and a semisynthetic analogue, 2″-acetylphyllanthusmin D, also exhibiting similarly potent activity in the same cell line (IC_{50} = 0.11 μM).[2]

From a structural perspective, the phyllanthusmins represent a subset of the arylnaphthalene lignan lactone class of natural products (Fig. 1A). Phyllanthusmins B–E are diphyllin glycosides possessing substituted arabinose units linked via a glycosidic bond to the C7 phenol of diphyllin, the aglycone portion of the molecule and a natural product itself.[4] Likewise, phyllanthusmin A is also built upon a diphyllin-like core, but possesses a hydroxy group at the C4 position rather than a methoxy substituent and, more importantly, does not contain the glycosidic linkage seen in other members of this group. When considering the promising activity of these compounds, it is interesting to note that diphyllin has also been shown to display cytotoxic,[5,6] antimicrobial,[7] and antiviral[8] activities. Other members of the arylnaphthalene lignan class and the closely related aryltetralin lignan class of natural products have also garnered significant interest due to the range of biological activities possessed by their constituents, including cytotoxic,[9–13] antioxidant,[14,15] antiviral,[16–18] anti-inflammatory,[19] cardioprotective,[20,21] insecticidal,[22] and neuroprotective[23] properties. The relationship between the arylnaphthalene and aryltetralin classes of compounds is of interest due to their deceptive structural similarities as seen in Fig. 1. The two classes differ, however, in the oxidation state of the B ring, imparting clear conformational differences that potentially lead to different modes of action. Despite this fact, both the aryltetralins, including etoposide and teniposide (Fig. 1B),

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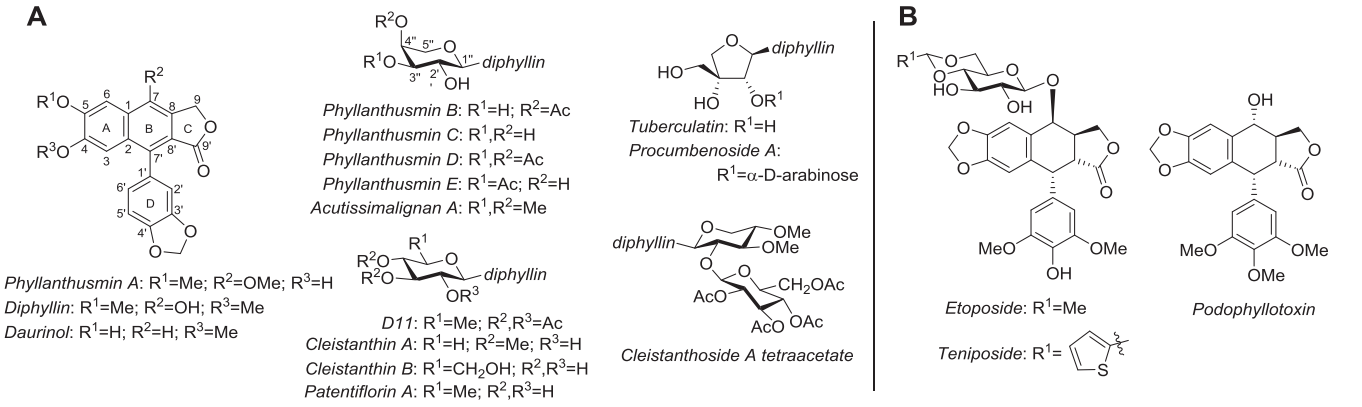

Fig. 1. Phyllanthusmins A–E along with structurally related arylnaphthalene lignan lactones (A) and aryltetralin lignan lactones (B).

and some arylnapthalenes like daurinol have been shown to possess inhibitory activity against the same biological target, DNA topoisomerase II, albeit via different interactions with the enzyme as either topoisomerase poisons or catalytic inhibitors, respectively.[24,25] In addition to mechanistic considerations, the development of etoposide and teniposide, two nearly identical clinically approved aryltetralin drugs, also points to the importance of optimization of the glycosidic moiety in these compounds as these drugs display unique pharmacological properties[26] and an entirely different mechanism of action than podophyllotoxin, the aglycone from which they are derived.[27]

Previous studies of several other structurally related arylnaphthalene compounds have also indicated the critical importance of the glycosidic sugar moiety in mediating general antiproliferative activities within the series of arylnaphthalene lignans.[12,28,29] In addition to phyllanthusmins B–E,[2] other diphyllin glycosides, including tuberculatin[5] and D11,[29] have also demonstrated more potent *in vitro* activity than diphyllin, their aglycone, in a variety of cancer cell lines. The impact of the glycosidic moiety can also be observed in the relative, although highly varied, antiproliferative activities of related natural products containing an array of substituted carbohydrate groups attached to diphyllin. The earliest examples of these are cleistanthins A[30] and B[31] (as well as cleistanthin A methyl ether)[11] which have been thoroughly investigated for their antiproliferative properties as well as their inherent toxicities in rats.[32–37] Acutissimalignan A, which possesses a functionalized arabinose moiety analogous to the isolated phyllanthusmins, was also recently found to possess highly potent activity against HT-29 cells following its isolation by the Kinghorn group with an IC_{50} value of 19 nM.[38,39] Other diphyllin glycosides that have also been found to be cytotoxic towards various cancer cell lines include patentiflorin A,[9] procumbenoside A,[5] and cleistanthoside A tetraacetate.[11,40–43]

Considering the potential influence of the carbohydrate group for antiproliferative activity, this moiety was identified as a useful starting point for the exploration of the structure-activity relationships in the phyllanthusmin class of natural products. Expanding on the structure activity relationship studies reported by Shi et al. and Zhao et al. for this class of compounds,[12,28] in this study we describe the synthesis of analogues containing various mono- and disaccharide units and the modification of the C-ring lactone in the aglycone portion of the molecules. The library of arylnaphthalene lignan glycosides generated during these studies has provided important structure-activity relationship (SAR) data across a series of cell lines previously not investigated with this class of compounds, including HT-29 (colon), MDA-MB 435/231 (breast), and OVCAR3 (ovarian cancer), as well as insight into the role of DNA topoisomerase IIα as a target for their antiproliferative activity.

2. Results and discussion

Based on the relatively straightforward retrosynthetic disconnection of diphyllin from the carbohydrate moiety via the glycosidic linkage, the synthesis of analogues of the phyllanthusmin class of natural products, like other diphyllin glycosides, was predicated on the ability to efficiently produce diphyllin in sufficient quantities for derivatization. Although numerous elegant recent approaches to the synthesis of similar arylnaphthalene core ring systems have been reported, they are either not specifically amenable to the synthesis of diphyllin or are potentially limited by scale-up cost.[44–51] The route of Charlton and coworkers,[52] however, has previously been employed[12,28,53–55] for the synthesis of diphyllin and related analogues based on the ease of access to the requisite starting materials and the overall efficiency of the route. Employing only minor modifications to the reported procedure, gram scale quantities of diphyllin (**5**) have been produced (Scheme 1). The most significant modification to the reported procedure was made based on initial difficulties observed during the isolation and purification of diphyllin following the final reduction of diester **4** using the reported sodium borohydride reduction conditions. Despite early work that indicated the potential for over-reduction of the desired lactone product in the presence of lithium aluminum hydride (LAH),[56] there was literature precedent indicating that LAH could be utilized under careful dropwise inverse addition[44,57] or portion-wise addition[55] conditions to affect this type of transformation. In the present study, portion-wise addition of 4 equivalents of LAH cleanly and efficiently resulted in the regioselective reduction of diester **4** within five minutes as observed by thin-layer chromatography. Upon workup, trituration of the crude product with methanol cleanly provided diphyllin. The overall yield of the five step sequence to prepare diphyllin ranged from 30 to 41% and required minimal chromatographic purification over the course of the synthesis. This method, therefore, facilitated the preparation of the aglycone in sufficient quantities for subsequent SAR studies.

With diphyllin in hand, a series of simple glycosyl bromides were prepared from the corresponding carbohydrates for glycosylation of the free phenol of the aglycone. The brominated substrates were immediately subjected to phase transfer glycosylation with diphyllin, following the same procedure implemented by Zhao and coworkers.[28] Utilizing this approach, the stereochemistry of the glycosidic linkage was established through neighboring group participation of the adjacent acetyl group, necessitating the presence of a C2″ equatorial alcohol in all starting materials to ultimately achieve the desired stereochemical control in the glycosylation reaction. For this reason, L-arabinose, D-xylose, D-glucose, D-galactose, D-arabinose, and lactose could be effectively