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A synthesis of 3-deoxydihydrolycoricidine: Refinement of a structurally minimum pancratistatin pharmacophore

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Abstract—The synthesis of 3-deoxydihydrolycoricidine, a key element toward elucidation of the pancratistatin anticancer pharmacophore, is described. Biological evaluation of this compound showed it to be significantly less active against tumor cells than pancratistatin. In addition to those features previously identified, the requirement of a 2,3,4-triol functionalized ring-C is now definitely established as the minimum pharmacophoric element for potent anticancer activity.

There has been considerable interest in both the chemistry and biological activity of the pancratistatin family of amaryllidaceae constituents over the past two decades. Pancratistatin **1** and the natural deoxy analog **2** (Fig. 1) were isolated from the Hawaiian *Pancratium littorale* (re-identified as *Hymenocallis littoralis*) and shown to exhibit potent cytotoxicity against human tumor cell lines.[1–3] Of equal importance was the novel, self-consistent pattern of differential cytotoxicity displayed by these natural products that did not match that of any other class of cytostatic agents in the NCI database indicating the possibility of a new target or mode of biological intervention.[3] These constituents have attracted wide synthetic interest in view of the complex poly-oxygenated phenanthridone skeleton and several successful total syntheses have been reported.[4]

We and others[7–9] have been engaged in a systematic, structural-based approach to unravel the anticancer pharmacophore in order to define the minimum structural requirements for potent cytotoxicity. An understanding of these requirements will also guide the preparation of biological probes that may illuminate the biological target that is operative. The anticancer activity demonstrated by the natural product **2** and other natural congeners[3] served to confirm that the C1 and C7-hydroxyl groups are not requirements for potent cytotoxicity and also demonstrated the requirement of a *trans*-fused b/c ring junction. Compound **2** remains the structurally minimum analog to exhibit the full cytotoxic profile, results that were recently confirmed.[5] The full deoxy synthetic analog **3** and complementary analogs **4**[6] and **5**[7] were prepared previously in our laboratory. While compounds **3** and **4** proved to be essentially inactive, analog **5** exhibited some cytotoxicity but was in general 1–2 magnitudes less active than compounds **1** and **2**.

From these results we determined that the hydroxyl group at C4 is required in conjunction with either, or both of the hydroxyl groups at C2 and C3.[7] The

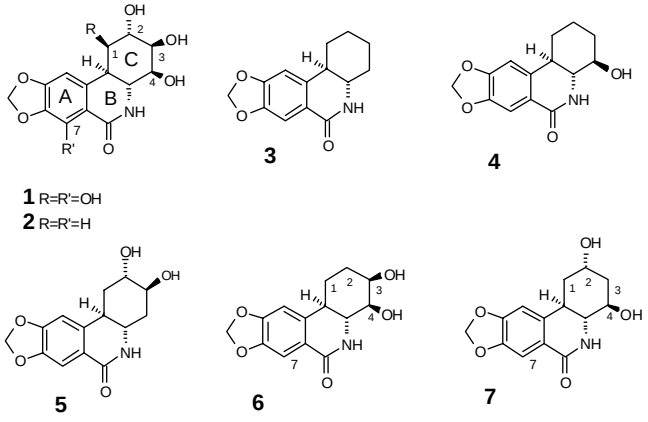

Figure 1. Systematic analog approach to refinement of the pancratistatin pharmacophore.

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minimum pharmacophore must therefore be present in either of the diols **6** or **7** or is in fact contained within the 2,3,4-triol **2**. The synthesis of diol **6** was recently described by Hudlicky and co-workers, and the compound was shown to be significantly less active than **2**.[8] Thus, diol **7** is the last remaining truncated analog that may exhibit the full spectrum of anticancer activity. In this Letter, we report the synthesis and biological evaluation of 3-deoxydihydrolycoricidine **7**.

The synthesis of compound **7** is outlined in Figure 2. Diels–Alder reaction of nitroalkene **8**[10] with Danishefsky's diene led to the *exo*-adduct **9** with high selectivity (*exo*:*endo* ratio 96:4).[11] Hydride reduction with sodium borohydride provided the C2-equatorial alcohol **10** as expected through axial hydride attack. Attempts to prepare the C2-axial alcohol directly with bulky hydride donors led only to mixtures of epimers. A modified Mitsunobu protocol on alcohol **10** with 2,4,6-trimethyl benzoic acid using our recently described phosphorane[12] led cleanly to the inverted ester **11** in 70% yield. Reduction of the nitro group with aluminum amalgam and protection gave the carbamate **12** (70%, both steps), which was cyclized using Banwell's procedure[13] to the tetracyclic derivative **13** (65% yield). A single crystal X-ray structural determination was carried out at this stage (see Fig. 3) confirming the relative stereochemistry as shown in **13**.

Hydrolysis of the C4 methyl ether proved troublesome and resisted all standard electrophilic and nucleophilic reagents. Hydrolysis was eventually realized using boron triiodide in dichloromethane giving the C4 alcohol **14** (15% isolated) along with recovered starting material. Reductive removal of the C2 benzoate then provided the desired 2,4-diol **7**.

Compound **7** was evaluated alongside authentic pancratistatin against MCF-7 (human breast cell carcinoma cells) and Jurkat (human B cell lymphoma) cells in serial dilution from 1 to 10 μM. No inhibition of these cell lines was seen with compound **7**, even at the highest concentration as determined by live cell counting at various periods of time. In addition, the well-established pattern of apoptotic induction observed following treatment with pancratistatin was not observed with compound **7** at 10 μM concentration. While native Pancratistatin induced efficient apoptosis in both of these cancer cell lines, as determined by nuclear condensation and annexin-V binding assay, compound **7** did not cause any of these characteristics in these cells.[14]

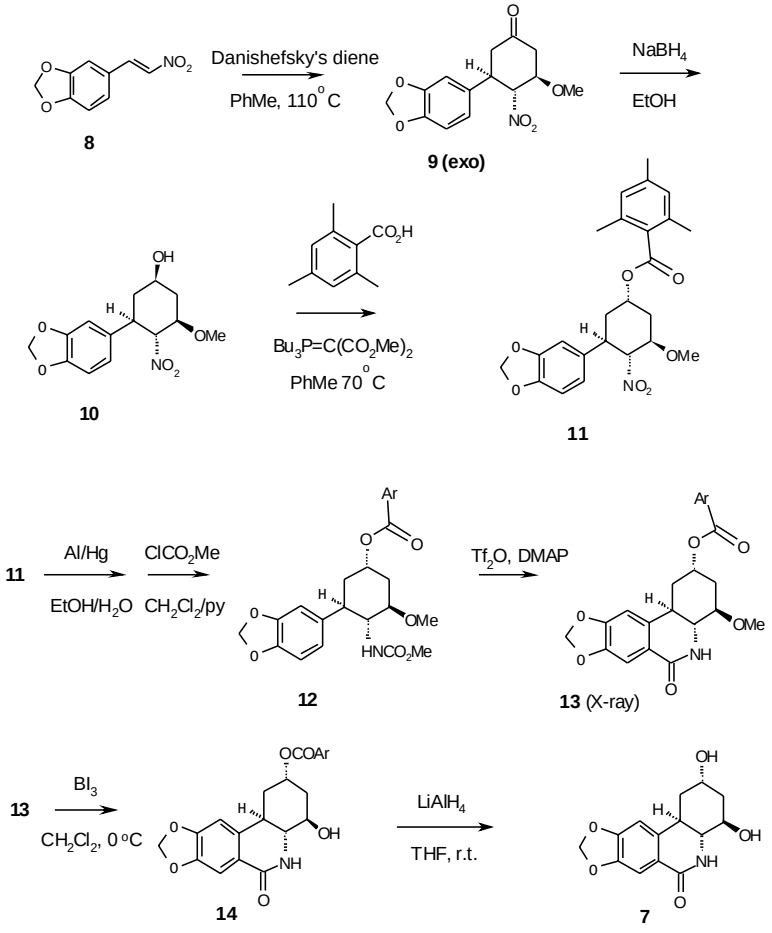

Figure 2. Synthesis of 3-deoxydihydrolycoricidine **7**.

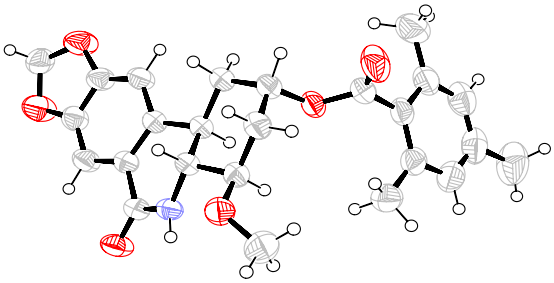

Figure 3. Crystal structure of compound **13**.

The present study confirms for the first time the definitive requirement of the 2,3,4-triol structural element in ring-C for potent cytotoxicity in the pancratistatin series of natural products. In conjunction with those features of the pharmacophore previously revealed,[3,6–8,15] a comprehensive view of the overall structural requirements in this important class of cell-line selective anticancer agents is now on hand. The modest activity reported for diols **5** and **6** in comparison to the inactivity of diol **7** points to a significant role for the C3-hydroxyl group in binding to the as yet unidentified target. Although not requirements for potent cytotoxicity, the pharmacophoric element in the pancratistatin series is moderated slightly by the incorporation of the C7 phenolic hydroxyl group[3] and, interestingly, through the inclusion of a β-benzyloxy substituent on the C1 position.[15] Since these features are not crucial, they highlight areas where further elaboration is possible on top of the intact pharmacophore.

It has not escaped our attention that compound **2** both exhibits the minimum cytotoxic pharmacophore *and* is the simplest of the natural deoxy derivatives isolated thus far in the pancratistatin series. The investment of energy and resources in biosynthetic pathways that evolve non-modulating small molecules appears to offer little evolutionary advantage and is a powerful pronouncement of the lead role that Natural products occupy in the central dogma of chemical-biology.[16] The features of the pharmacophore now revealed will guide the design of active labeled derivatives for identification of the biological target and clarification of the mechanism of action[17,18] of this valuable class of anticancer agent.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.08.024.

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14. Materials and methods: two human cancer cell lines, breast carcinoma (MCF-7), and human lymphoma cell line (Jurkat cells), were purchased from ATCC, Manassas, VA. These cells were grown and maintained in an incubator at 37 °C, 5% CO_2, and 95% humidity. Cells were cultured in RPMI-1640 media supplemented with 10% fetal bovine serum (FBS) and 10 mg/mL gentamycin (Life Technologies, Mississauga, ON, Canada). Cells were grown to 70% confluence and then treated with compound **7** at different concentrations (1, 2, 5, and 10 μM) for varying periods of time (24, 48, 72, and 96 h). Pancratistatin was used in parallel with these experiments at 1 μM

concentration. To examine the viability of cancer cells after treatment, a 10 μL cell suspension was added to 10 μL of 0.4% Trypan blue stain (Life Technologies). Using a Hemacytometer (Fisher Scientific, Horsham, PA) both dead (Trypan blue-positive) and live cells were counted. The results were calculated and tabulated as a percentage of dead cells using Microsoft® Excel 6.0 Software. To examine apoptotic morphology changes, the Jurkat cells were grown and treated as above, and then incubated with 10 μM final concentration of cell permeable Hoechst 33342 for 5 min at 37 °C and stained cells were examined by fluorescence microscopy (Leica DM IRB, Germany) at 400×.

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