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Studies Directed Towards the Refinement of the Pancratistatin Cytotoxic Pharmacophore

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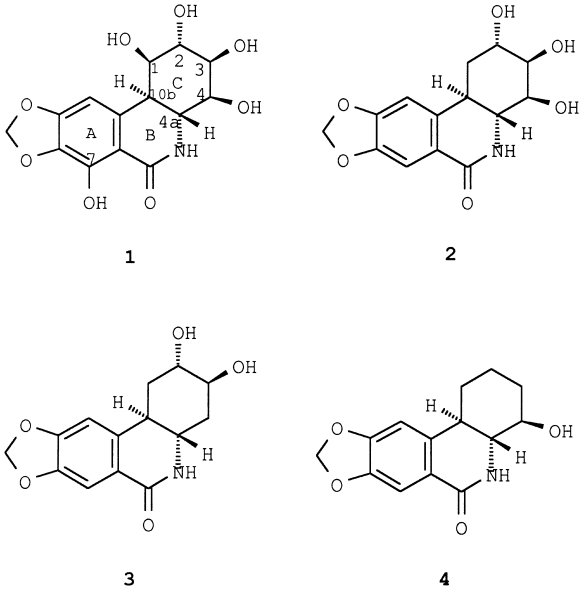
Abstract—Two deoxy-analogues of the anticancer/antiviral agent pancratistatin containing functionality complementary to the minimum structural pharmacophore were synthesized and subjected to anticancer screening. One of the analogues exhibited selective inhibition of certain tumor cell lines but was significantly less potent than the natural products. The minimum structural pharmacophore has now been refined from eight to three possible structures.

The *Amaryllidacae*[1] alkaloid pancratistatin **1**, isolated in small quantities from the Hawaiian *Hymenocallis littoralis*[2] along with some of its closely related natural congeners including notably *trans*-dihydrolycoricidine **2** (Scheme 1), has demonstrated antiviral activity as well as potent cytotoxicity against certain tumor cell lines.[3,4] The promising pharmacological profile of the compounds, in addition to the complex polyoxygenated phenanthridone skeleton, has attracted considerable synthetic interest, and pancratistatin has been the subject of several elegant total syntheses.[5] Despite the potent activity of **1** and **2**, no systematic study has been undertaken to define minimum structural requirements of the pharmacophore or to identify the biological target of these compounds. Structural similarity to the related alkaloid narciclasine has led to the suggestion that the compounds may be inhibitors of protein biosynthesis,[3,4] although more definitive studies have been hampered in part by the limited supply of these alkaloids.

Limited structure–activity correlations of the natural compounds **1** and **2** and a few minor natural and semisynthetic analogues have defined some of the structural requirements of the cytotoxic pharmacophore. Compounds **1** and **2** shared a similar potency and profile of cytotoxicity activity against the NCI panel of 60 human tumor cell lines, indicating that the C-1 and C-7 hydroxyl substituents are not requirements of the pharmacophore.[6] In addition, the C-7 hydroxyl was not required for antiviral[3] activity. On the other hand, the *trans*-B/C ring junction stereochemistry was found to be



Scheme 1.

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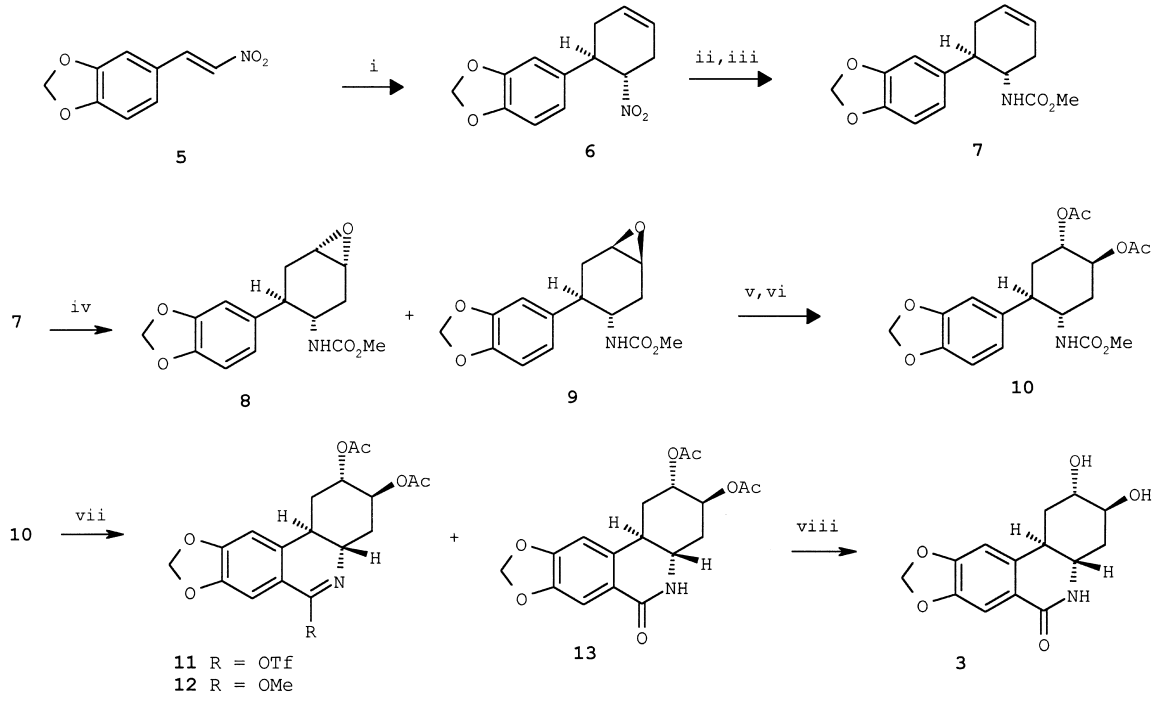
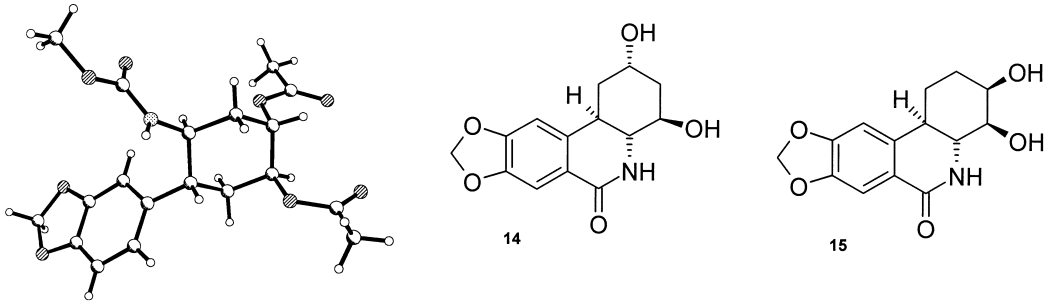
crucial for potent cytotoxicity in both the natural series[6] and in a fully oxygenated synthetic derivative.[7a] Lastly, oxygenated *seco*-analogues (B/C ring opened) of these compounds were devoid of biological activities.[7b] These studies indicate that the conformation of the C-ring, or more specifically, the correct spatial orientation of one or more of the hydroxyl substituents at positions C-2, C-3, and C-4 are essential for recognition. At present, compound **2** is the structurally simplest analogue known that exhibits potent cytotoxicity. Based on this information, eight possible structures could embody the overall minimum pharmacophore; one triol (i.e., compound **2**), three diols (**2**α,**3**β; **2**α,**4**β; or **3**β,**4**β), three monoalcohols (**2**α; **3**β; or **4**β) or the fully deoxygenated cyclohexane derivative. As a means of systematically evaluating the role of the three hydroxyl groups at C-2, C-3, and C-4 in 2, we divided the functionality into pairs of *complementary analogues*. We define these as pairs of analogues of the active compound having the same structural skeleton with differing functionality but between them covering all of the functional groups on the minimum known pharmacophore. Compounds **3** and **4** may therefore be considered complementary analogues to the presently known minimum pharmacophore in **2**. We previously reported the synthesis of compound **4**[8] using a stereoselective nitroaldol approach. We now report the preparation of the complementary analogue **3**, utilizing a Diels–Alder approach to the core structure, as well as the cytotoxic screening of both of these analogues.

The most direct synthetic route to diol **3** is outlined in Scheme 2. Diels–Alder reaction of nitroalkene **5**[9] with butadiene using a modification of the method reported by Bryce[10] provided the cyclohexene derivative **6** and allowed us to secure the necessary future *trans*-B/C ring junction early in the synthesis. In our hands, the reaction proceeded faster and gave cleaner product under Lewis acid catalysis ($ZnCl_2$) in a sealed bomb. Chemoselective reduction of the nitro group was best achieved



Scheme 2. Reagents and conditions: (i) Butadiene sulfone, PhMe, 127 °C, $ZnCl_2$, 12 h, 85%; (ii) Al–Hg, THF/H_2O, 22 °C, 12 h, then (iii) $ClCO_2Me$ (1.5 equiv), CH_2Cl_2, Et_3N, 0 °C, 5 h, 96% from **6**; (iv) MCPBA (3.0 equiv), CH_2Cl_2 0 °C, 5 h, 93%; (v) $PhCO_2Na$, H_2O, 100 °C, 12 h, then (vi) Ac_2O/Py, 0 °C, 12 h, 55–62% from **9**; (vii) Tf_2O (5.0 equiv), DMAP (3.0 equiv), CH_2Cl_2, 0–15 °C, 15 min; then 2 M HCl (aq), dioxane, 20 °C, 18 h; then Ac_2O/Py, 0 °C, 6 h, 85%; (viii) NaOMe (2.1 equiv), THF:MeOH (1:3), 0–18 °C, 12 h, 96%.



Scheme 3. X-ray structure of **10** ($R_1 = 0.0855$).

using aluminium amalgam in THF, with the resulting amine being immediately protected as the methoxycarbonyl derivative giving cyclohexene **7** in 96% overall yield from **6**.[11] Epoxidation with MCPBA gave the α- and β-epoxides **8** and **9** (ratio 2.7:1) in 93% yield. The epoxides were readily separable on flash silica and independently subjected to a surprisingly stereospecific ring opening using the method reported by Magnus and co-workers.[5c] Reaction of the α-epoxide **8** with refluxing aqueous sodium benzoate followed by treatment with acetic anhydride in pyridine provided the diacetoxy derivative **10** in 55% yield. Similar opening of the β-epoxide **9** and likewise protection as the diacetate gave the same diol derivative **10** in 62% yield. Under these conditions, the conformationally biased diastereomeric epoxides are opened exclusively by *axial* attack. Thus in **8**, opening occurs from the *axial* direction by attack at C-3 while the diastereomeric epoxide **9** is opened by *axial* attack at C-2 yielding the same 2,3-*diaxial* diol. The relative stereochemistry in product **10** was confirmed by a single crystal X-ray structural determination (Scheme 3). The crystal structure clearly shows the chair conformation of the cyclohexane ring of **10** locked by the equatorial aryl and methoxycarbonylamino substituents as well as the *diaxial*-2,3-diacetoxy substituents.[12] All four substituents on the cyclohexane ring are therefore set up with the correct relative stereochemistry desired in analogue **3**. Cyclization of **10** to give the phenanthridone skeleton was effected in low yield (5–15%) using the method of Banwell.[13] Under these conditions two intermediate products were also isolated in addition to the desired product **13**. These were readily identified as **11** and **12** in comparison with the results of the Magnus work.[5c] Further investigation showed that the cyclization reaction with Tf_2O and DMAP was extremely rapid, complete in 15 min at 0 °C, giving **12** which slowly converts to **13** on standing. Quenching the reaction after 15 min and acidic hydrolysis of the mixture led also to partial loss of the acetate groups requiring a final re-acetylation step. The cyclization process is very sensitive to traces of water, however under the optimized cyclization/hydrolysis/reprotection protocol a 74–90% yield of the cyclized protected diol **13** could be realized with no detectable trace of the side products. Removal of the acetate groups completed the synthesis of the desired diol **3**.

Initial screening of complementary analogues **3** and **4** against the P-388 mouse leukemia cell line gave interesting results. The mono alcohol derivative **4** displayed marginal inhibition ($ED_{50} = 40.1\ \mu g/mL$) while the diol **3** proved to be relatively potent ($ED_{50} = 0.45\ \mu g/mL$), although less potent than pancratistatin itself. Further comparative assaying of compound **3** against the NCI 60 panel human tumor cell-line assay was undertaken simultaneously with authentic pancratistatin. The results confirmed that **3** was in fact overall two to three magnitudes less potent than pancratistatin although selective inhibition of some of the cell lines was indicated. Compound **3** inhibited proliferation of the non-small cell lung cancer line NCI-H226 ($ED_{50} = 0.65\ \mu g/mL$), as well as two of the leukemia cell lines; CCRF-CEM ($ED_{50} = 0.55\ \mu g/mL$) and HL-60(TB) ($ED_{50} = 0.89\ \mu g/mL$). Overall, compound **3** was not sufficiently potent to generate a useful mean-graph for COMPARE analysis[14] (Scheme 3)

The present study helps further define the minimum cytotoxic pharmacophore shared by pancratistatin **1** and *trans*-dihydrolycoricidine **2**. In addition to the necessary conformationally locked *trans*-fused B/C ring junction, at least two correctly positioned hydroxyl groups appear to be needed in the C-ring for potent cytotoxic activity. The results with the complementary analogues **3** and **4** rule out any monoalcohol analogue as a minimum pharmacophore and the modest potency of **3** suggests the importance of either, or both, of the C-2 and C-3 hydroxyl groups, in addition to the C-4 hydroxyl as a minimum pharmacophore. We tentatively conclude that the minimum structural pharmacophore must reside in either of the diols **14** or **15** (Scheme 3) or may in fact be that depicted in the trihydroxy containing natural product **2**. The synthesis of analogues **2**, **14** and **15** is currently under investigation in our laboratories.

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