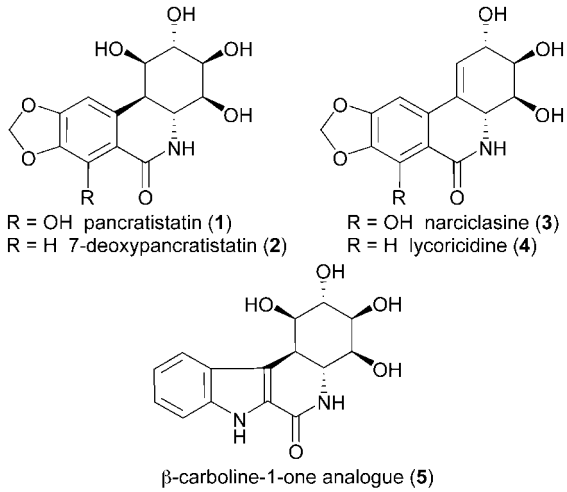
Synthetic Methods

A β-Carboline-1-one Mimic of the Anticancer *Amaryllidaceae* Constituent Pancratistatin: Synthesis and Biological Evaluation**

Uwe Rinner, Tomas Hudlicky, Heather Gordon, and George R. Pettit*

Pancratistatin (**1**) and narciclasine (**3**; Scheme 1), well-known constituents[1] of the *Amaryllidaceae* species, have been the subject of intense study, both in the realm of total synthesis[2] and with respect to their anticancer activities.[3] Both compounds contain a free phenolic hydroxy group that is part of the enolized β-ketoamide function. It is this functional group that accounts for the greater (10-fold or more) activity of



Scheme 1. *Amaryllidaceae* constituents containing the enolized β-ketoamide motif and their β-carboline-1-one analogue.

[*] Dr. U. Rinner, Prof. Dr. T. Hudlicky, Prof. Dr. H. Gordon
Department of Chemistry
Brock University
500 Glenridge Avenue, St. Catharines, Ontario L2S 3A1 (Canada)
Fax: (+1) 905-984-4841
E-mail: thudlicky@brocku.ca
Prof. Dr. G. R. Pettit
Department of Chemistry & Biochemistry and
the Cancer Research Institute
Arizona State University
Tempe, Arizona 85287 (USA)

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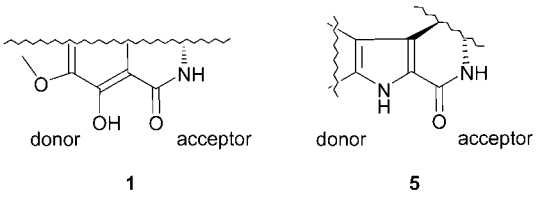
these compounds compared to that of their congeners 7-deoxypancratistatin (**2**) and lycoricidine (**4**), which lack the phenol group.[4]

Several research groups have focused their efforts on identifying the pharmacophore of these plant constituents.[5] To this end, a series of derivatives of **1** have been prepared in which functionalities in the aminoinositol moiety have been deleted or changed,[6] or in which the skeleton has been truncated to produce smaller derivatives.[5c] No modification of the aminoinositol ring has led to an increase in the activity of such derivatives. Truncated derivatives that retain the phenanthridone moiety show decreased activities compared to that of the parent compound. An account of the synthesis of a lactone analogue of **1** containing a carbohydrate motif appeared recently but no biological evaluation of the compound was reported.[7]

Pancratistatin has been proved active in antiviral screens[3f] and is highly active against various cancer cell lines in vitro and in vivo. However, it has poor bioavailability and a major effort has been made to develop more soluble analogues or prodrugs.[5a,8] We speculated that the potency of **1** and **3** may in part be due to the hydrogen-bonding donor–acceptor pairing of the β-ketoamide motifs present in these compounds but absent from the 7-deoxy congeners. We therefore decided to test the β-carboline-1-one analogue of **1**, compound **5**, in which such donor–acceptor pairing is extended through the vinyl indole ring, as shown in Scheme 2. The steric and, to some degree, the electronic



Scheme 2. Donor–acceptor pairing for hydrogen bonding in pancratistatin (**1**) and β-carboline-1-one analogue **5**.

properties of **1** and **5** are similar. We expected the β-carboline-1-one analogue to interfere with RNA transcription by the mechanism proposed for the activity of narciclasine (**3**), the only member of the *Amaryllidaceae* family for which data on the possible mode of action are available.[3c,d] In addition, β-carboline-1-ones and sterically constrained tryptamines have been found to have serotonin-regulating activity.[9]

Molecular models of these two compounds, pancratistatin (**1**) and its carboline-1-one mimic **5**, show interesting spatial similarities (Figures 1 and 2). Except for the electron density associated with the methylenedioxy bridge of **1**, the compounds seem to occupy almost identical space, as confirmed in Figure 2, which shows that the functionalities of the two compounds directly overlap.

Geometry optimizations of both pancratistatin (**1**) and its β-carboline-1-one analogue **5** were performed at the HF/6-31G* level of theory by using the Gaussian 03 package.[10] We calculated the optimal geometries of the compounds in vacuo. The initial orientations of the aminoinisitol hydroxy groups

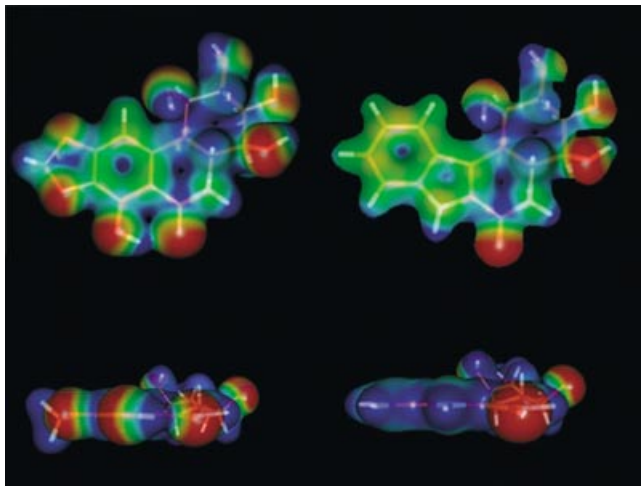


Figure 1. Comparison of the electrostatic potential energy surfaces of minimum energy conformations of pancratistatin (**1**; left) and β-carboline-1-one analogue **5** (right). The isodensity surfaces are color-coded according to the electrostatic potential: red −0.05, yellow 0.00, green 0.05, light blue 0.10, blue 0.15 e.

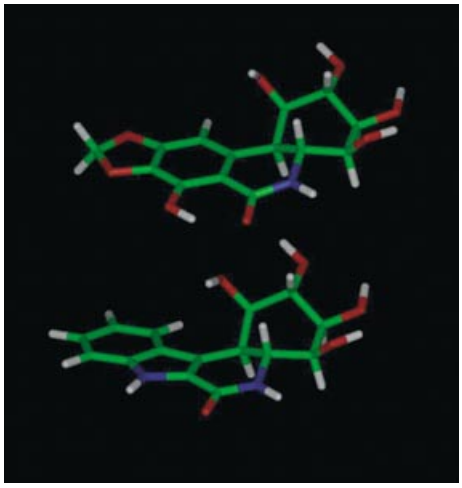


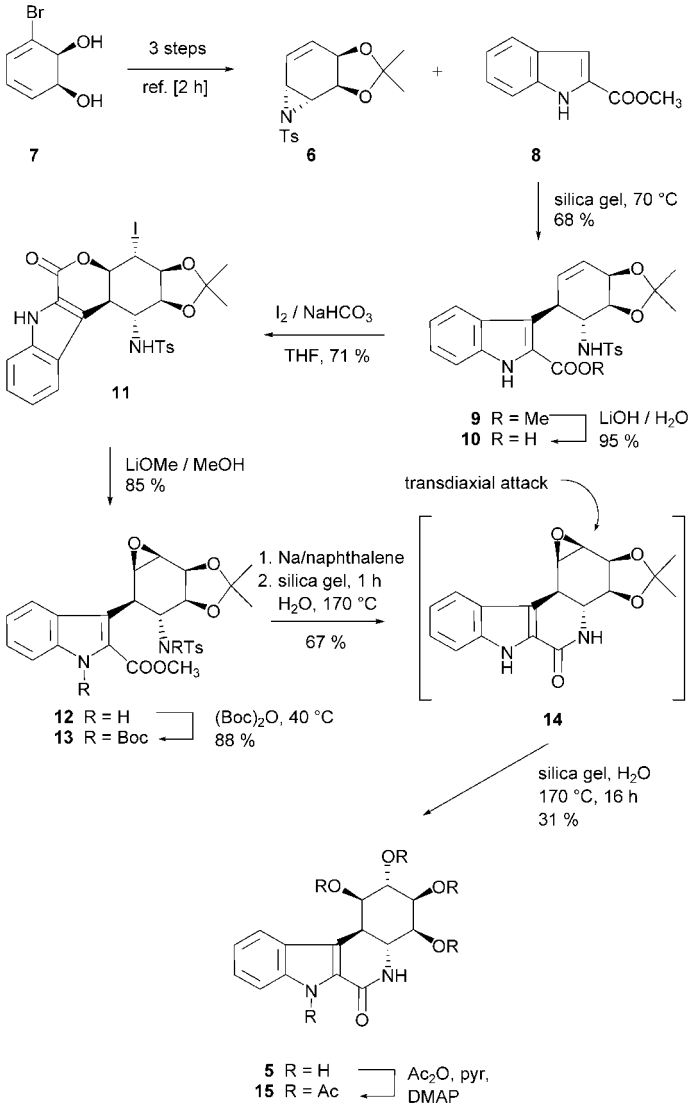
Figure 2. Direct overlap of the structures of pancratistatin (**1**, top) and β-carboline-1-one analogue **5** (bottom).

were obtained by carrying out a concerted dihedral search using the CHARMm molecular mechanics forcefield.[11] Not surprisingly, the lowest energy conformation of the phenolic hydroxy group under these conditions is that required to form an intramolecular hydrogen bond with the amide carbonyl oxygen atom.

The isodensity surfaces displayed in Figure 1 were constructed with the program Molden[12] and are color-coded according to the electrostatic potential. The geometry-optimized structures were superimposed based on the positions of the atoms of the shared aminoinositol moiety. Figure 1 clearly illustrates the difference between the electrostatic potential of the putative hydrogen-bond donor–acceptor pair of the β-ketoamide motif of **1** and that of the analogous portion of **5**. The hydrogen-bonding β-ketoamide motif has three distinct regions of negative electrostatic potential, while the equiv-

alent part of **5** has negative electrostatic energy only around the carbonyl oxygen atom.

Herein we report a concise synthesis of **5** that features a number of interesting transformations (Scheme 3), along with



Scheme 3. Synthesis of β-carboline-1-one analogue **5**. Boc = *tert*-butoxycarbonyl, DMAP = 4-dimethylaminopyridine, pyr = pyridine, Ts = tosyl.

the biological evaluation of the product in a series of cancer cell lines as a prelude to a more focused drug discovery effort aimed at the heterocyclic variants of **1**. We found that the synthesis of **5** is easier than the preparation or extraction of the natural alkaloids.

The synthesis began with vinylaziridine **6**,[2e,13] which was prepared in three steps from enzymatically derived diene-diol **7**,[14] and methyl indole-2-carboxylate (**8**), prepared from commercially available indole-2-carboxylic acid.[15] The two compounds were adsorbed on silica and heated at 70 °C for 48 h to provide tosylamide **9** cleanly in 68 % yield. This transformation was surprising. Indole itself has been reported to open aziridines under $InCl_3$ catalysis[16] and condensed smoothly with **6** under silica gel catalysis. However, we did not expect ester **8** to react well with **6** because of the decreased nucleophilicity of the 3-position of the indole as a result of conjugation to the vinyl urethane function. Lewis acid catalysis provided low yields of **9** compared to those obtained from the reactions on the silica surface.[17–19]

The methyl ester group of **9** was hydrolyzed ($LiOH/H_2O$, 12 h, RT) and the free acid **10** subjected to iodolactonization to produce lactone **11** as a single stereoisomer in 71 % yield.[20] This protocol allowed full control of the stereochemistry while avoiding the use of oxidizing agents or other epoxidation procedures that would have been detrimental to the fate of the indole core. Exposure of lactone **11** to LiOMe/MeOH gave epoxide **12** cleanly. This transformation could also be achieved by using a two-step procedure ($LiOH/H_2O$; CH_2N_2; 85 % yield). Detosylation of compounds of this type has been shown to proceed more smoothly when the tosylamide is first converted into an imide.[2e,5b] We converted **12** into the *bis*-Boc-protected material **13** (88 % yield), which was easily detosylated in 71 % yield by treatment with sodium naphthalide at −65 °C.

The final transformation of **13** into the β-carboline-1-one analogue **5** was accomplished in a one-flask sequence during which four separate events took place. A sample of **13** was dissolved in acetone and adsorbed on silica. The dry powder was suspended in H_2O, placed in a thick-walled pressure tube, and heated at 170 °C. After one hour the material had been quantitatively transformed into the free amide **14** by a thermal retro-ene reaction of the Boc-carbamate and internal amidation of the methyl ester. Thermolysis on wet silica gel is superior to the previously reported technique, in which the starting material was treated with 5 % aqueous sodium benzoate under reflux.[21] Separation of polar products from benzoic acid is often problematic on a small scale. Continued heating of **14** for an additional 16 h at 160 °C resulted in its conversion into **5**, which was obtained as a single stereoisomer through transdiaxial opening of the epoxide and thermolytic cleavage of the acetonide. Pure carboline-1-one derivative **5**, a sparingly soluble compound, was isolated by chromatography. The crude product was converted into its pentaacetate **15** for use in detailed NMR studies because **15** is more easily purified than **5**.

β-Carboline-1-one **5** and each of the key intermediates in this nine-step sequence were tested against a small panel of cancer cell lines. The results are shown in Table 1 as GI_{50} values (amount of substrate in $\mu g\,mL^{-1}$ cell solution necessary to stop the growth of 50 % of the cancer cells in 1 mL cell solution). The cutoff for activity in human cancer cell lines is considered to be $10\,\mu g\,mL^{-1}$. Some of the indole modifications produced compounds that meet this criterion for activity against certain cancer cell lines. Biological evaluation of **5** confirmed borderline activity in the murine P388 lymphocytic leukemia assay. Surprisingly, iodolactone **11** showed promising activities against pancreas and breast adenocarcinoma ($GI_{50} = 1.9$ and $4.3\,\mu g\,mL^{-1}$). It is possible that the mechanism of action of the iodolactone is different from that of pancratistatin and it may be beneficial to examine **11** as a completely different scaffold on which the design of further

Table 1: Evaluation of the activities of β-carboline-1-one and intermediates in the synthesis, as well as pancratistatin and 7-deoxypancratistatin, against Murine P388 lymphocytic leukemia and human cancer cell lines. GI_{50}-values in [$\mu g\,mL^{-1}$][a].

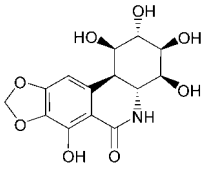
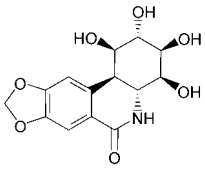
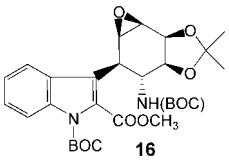
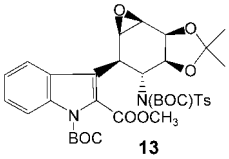
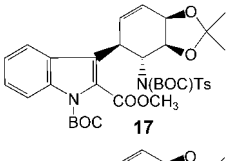
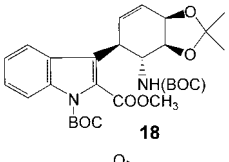
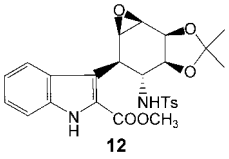
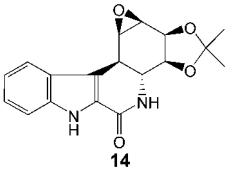
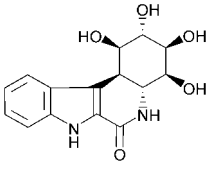
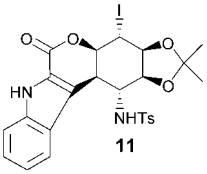
Compound	P388[b]	BXPC-3[c]	MCF-7[d]	KM20L2[e]
 **1**	0.02	0.03	–	0.045
 **2**	0.44	–	–	0.22
 **16**	22.8	>10	>10	>10
 **13**	12.8	>10	>10	>10
 **17**	11.8	8.6	10.5	>10
 **18**	4.6	>10	>10	>10
 **12**	>100	>10	>10	3.5
 **14**	>100	>10	>10	3.8
 **5**	18.3	>10	>10	>10

Table 1: (Continued)

Compound	P388[b]	BXPC-3[c]	MCF-7[d]	KM20L2[e]
 **11**	11.7	1.9	4.3	>10

[a] No significant activity against human SF268 (CNS glioblastoma), NCI-H460 (lung large cell), or DU-145 (prostate carcinoma) was detected. [b] P388, lymphocytic leukemia. [c] BXPC-3, pancreas adenocarcinoma (human). [d] MCF-7, breast adenocarcinoma (human). [e] KM20L2, colon adenocarcinoma (human).

derivatives could be based. *bis*-Boc derivative **13** and unsaturated analogues **17** and **18** are also active against murine P388 lymphocytic leukemia, with GI_{50} values (12.8, 11.8, and 4.6 $\mu g\,mL^{-1}$, respectively) one order of magnitude smaller than that of 7-deoxypancratistatin (GI_{50} = 0.44 $\mu g\,mL^{-1}$). These exciting results suggest new possibilities that should be examined in the next series of analogues.

Some guidelines for the design of new analogues emerged as a result of this particular study: 1) The presence of both the oxygen atoms in the methylenedioxy bridge of pancratistatin seems essential for high activity. We recently showed that deletion of the C8 methoxy group from the dimethoxy derivative of **1** leads to GI_{50} values 10- to 20-fold higher than that of the natural product.[5d] 2) The activity of compounds such as lactone **11** opens up the possibility that analogues can be structured around completely different scaffolds in future because such compounds can be further functionalized at a number of positions. 3) The synthesis of **5** is, with nine steps, the shortest existing preparation of pancratistatin analogues containing the aminoinositol motif. Some of the transformations that were employed will no doubt find further application in the design of heterocyclic analogues of pancratistatin. 4) The vinylaziridine **6** proved useful as a scaffold for the generation of diversity and will be further exploited in the design of new derivatives of the title compounds.

Future endeavors in this area will focus on heteroatom alterations in the aromatic portion of pancratistatin as it has already been shown that the aminoinositol moiety must remain intact for these compounds to retain activity. We will report our findings in due course.

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[1] The pancratistatin, narciclasine, and lycoricidine group of natural products has been collectively referred to as "*Amaryllidaceae* alkaloids" by most, if not all, synthetic chemists (Martin, Hudlicky, Keck, Polt, and others). Such nomenclature is not accurate as these compounds do not contain basic nitrogen atoms. Another term used in the literature is "isocarbostyril"

(Pettit), a term which is correct but not generally known in the synthetic community. To avoid further inaccuracies we will avoid both names in future publications and simply refer to these compounds as "constituents".

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