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Synthesis and biological activity of some structural modifications of pancratistatin

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Abstract—Structurally modified derivatives of 7-deoxypancratistatin have been synthesized and evaluated in cancer cell line inhibition studies.

1. Introduction

Pancratistatin (**1**) and 7-deoxypancratistatin (**2**), along with their congeners narciclasine (**3**) and lycoricidine (**4**), Figure 1, belong to the Amaryllidaceae group of natural products that continues to attract high levels of attention.[1–5] Although **1** and **3** possess promising antitumor activities[6] much remains to be known about the precise mode of action of these natural products.[6c,d,7] Some of the recent insights include inhibition of the cell cycle from G_0/G_1 to S phase[6g] and the powerful antiparasite activity shown by pancratistatin.[6h,i] The search for more bioavailable derivatives as well as attempts to identify the pharmacophore[8] of **1** led to a number of truncated as well as unnatural derivatives in these series. Most notable efforts have been published from the groups of Pettit et al.[8a] and McNulty et al.,[8b] in addition to limited investigations from our group already published.[8c] Recently an unnatural derivative containing a sugar motif was reported by Fessner and co-workers with biological evaluation pending.[9]

The biological activity of truncated derivatives is limited, and the search continues to identify those elements in isocarbostyril **1** that are essential to the activity of the compounds against various cancer cell lines. Recently,

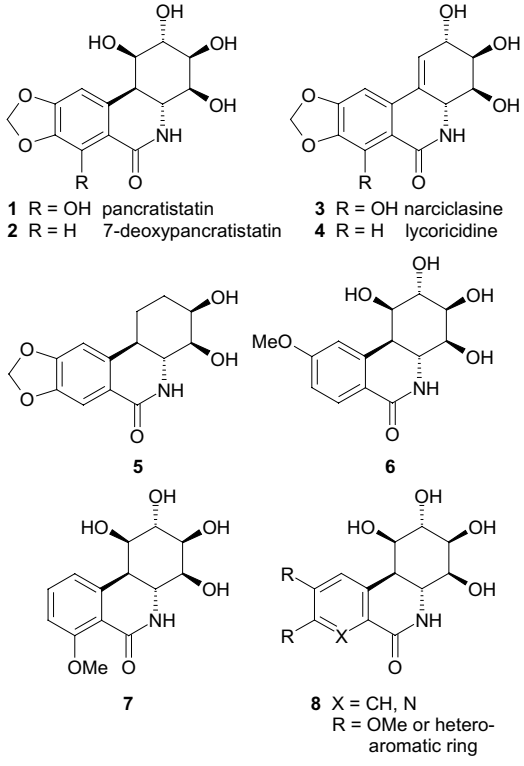

Figure 1. Important members of the Amaryllidaceae group and truncated or unnatural derivatives of **1** and **2**.

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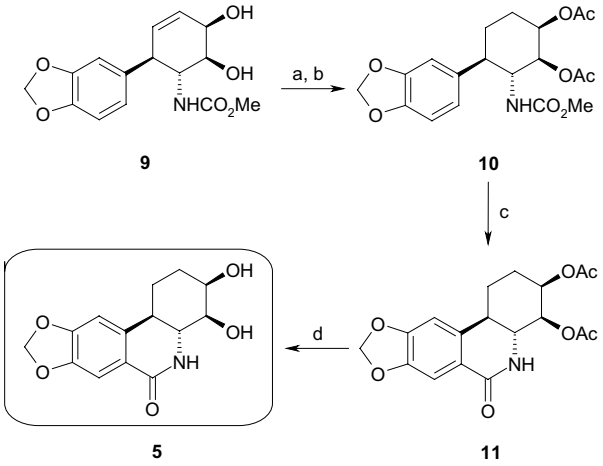
we established that the *cis*-isomer of **2** is inactive compared to the *trans*-fused natural product.[10] Little is known about the precise mode of action of pancratistatin and only limited results are available about the interaction of narciclasine with RNA.[6c,d] From currently available data it appears that at least one or two hydroxyl groups of the aminocyclitol ring are essential[8b] as is the full aromatic portion and the donor–acceptor hydrogen bonding system of the enolized β-ketoamide contained in **1**.

To probe this issue further we chose to synthesize truncated derivatives **5**, **6**, and **7** as well as compounds of type **8**, Figure 1, in which the piperonyl residue is replaced by various heteroaromatic nuclei presenting both size and donor–acceptor hydrogen bonding attributes of pancratistatin. In these compounds, the systematic replacement or deletion of functionality should provide further insight into the activity issues. Herein we report the synthesis and evaluation of **5** and **6**, Figure 1, along with some of the intermediates.

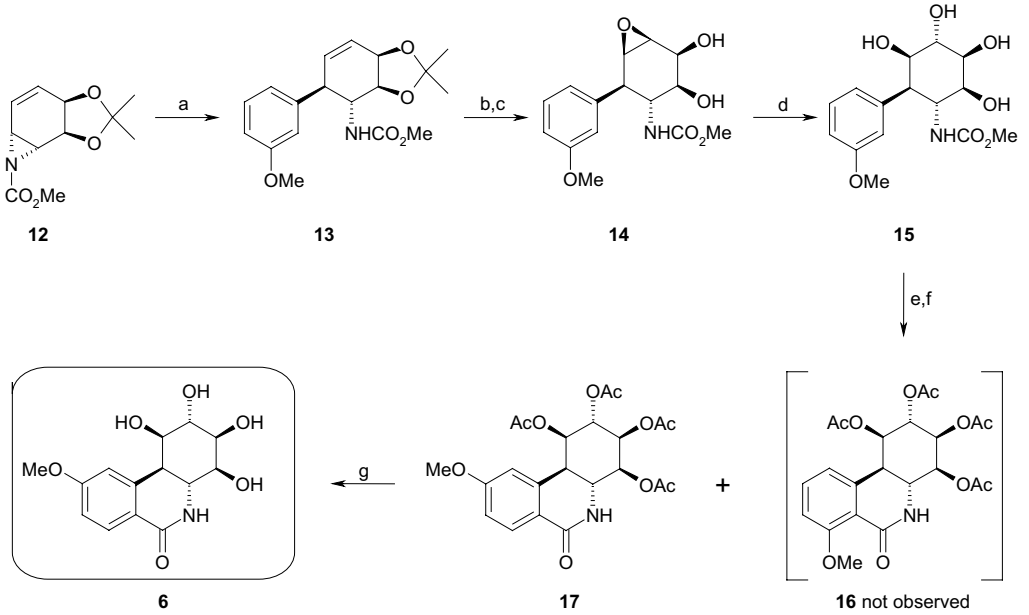
The synthesis of **5** was straightforward and was based on the interception and adaptation of a known procedure from our previous synthesis of 7-deoxypancratistatin, Scheme 1.[3d] Diol **9** was prepared as described previously[3d] and acetylated (98%) prior to its hydrogenation to **10**. Modified Bischler–Napieralski closure was effected with Tf_2O/DMAP[3d,11] to furnish amide **11** in 50% yield, which was treated with MeOH/MeONa to give **5** (85%).

Methoxy derivative **6** was prepared as shown in Scheme 2, following the strategy employed in the synthesis of 7-deoxypancratistatin, namely the opening of aziridine **12**



Scheme 1. Total synthesis of truncated derivative **5**. Reagents and conditions: (a) Ac_2O, py, 98%; (b) H_2, Pd/C, EtOH, 95%; (c) Tf_2O, DMAP, CH_2Cl_2, 4 °C, 50%; (d) $NaOCH_3$, CH_3OH, 70%.

with the lithio derivative of 3-bromoanisole to yield **13**.[2e] The synthesis proceeded well in analogy to previous experience to the stage of **15** where the Banwell et al. procedure[11] for closure was utilized. Based on the report by Magnus et al.[2h] in which similar closures generated 7:1 and 3:1 mixtures of regioisomers when C-7 position featured methoxy and acetoxy groups, respectively. We had hoped that both compounds **6** and **7** would become accessible by this strategy via the expected nonselective condensation with the intermediate isocyanate. To our surprise, only **17** was produced regioselectively in these closures and was transformed to **6** by deprotection of the acetate groups. We failed to detect the other isomer, **16**, in the reaction mixtures.



Scheme 2. Total synthesis of truncated derivative **6**. Reagents and conditions: (a) i. 3-bromo anisole, *n*-BuLi, THF, −78 °C; ii. CuCN, 2 h; iii. **12**, $BF_3{\cdot}Et_2O$, −78 °C to rt, 50%; (b) AcOH, THF, H_2O, 60 °C, 76%; (c) *t*-BuOOH, $VO(acac)_2$, C_6H_6, 60 °C, 22%; (d) NaOBz, H_2O, reflux, 54%; (e) Ac_2O, py, 88%; (f) i. DMAP, Tf_2O, CH_2Cl_2, 4 °C; ii. THF, HCl (2 M), 76%; (g) $NaOCH_3$, THF, rt, 85%.

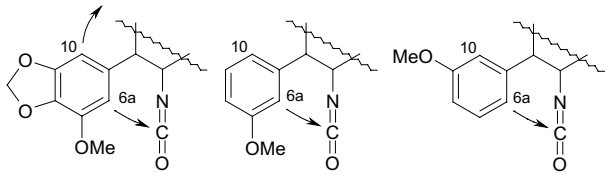


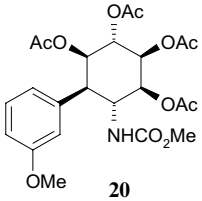
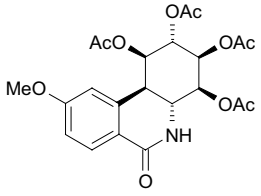
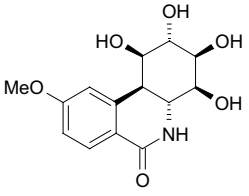
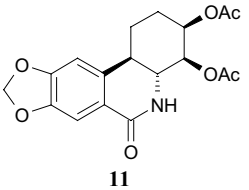
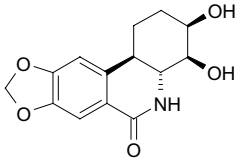
Figure 2. Regioselectivity of ring closure reactions.

In the intermediate **18**, Figure 2, employed by Magnus C-6a and C-10 are similarly activated toward reaction with the isocyanate, albeit with 7:1 or 3:1 selectivity for the more reactive *ortho* position at C-6a. It appears that in anisoles **19** the *para* attack is preferred (**19b**) based on steric rather than electronic arguments. It may be interesting to repeat the reaction with the free phenol in which the product of the closure from C-6a would be stabilized by an intramolecular hydrogen bond.

2. Biological evaluation

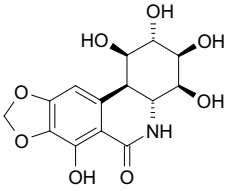
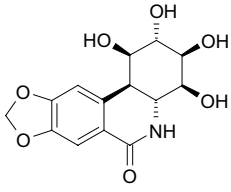
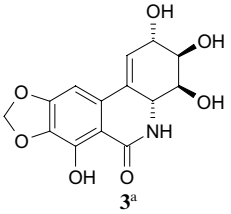
The cancer cell line growth inhibition activity of the new compounds with structural modifications of pancratistatin was tested against the natural isocarbostyril (**1**) and narciclasine (**3**) (Table 1). Only the compounds possessing the complete ring system and stereochemistry of pancratistatin, namely modification **6**, displayed significant activity, albeit some 10-fold less than 7-deoxy-

Table 1. Murine P388 lymphocytic leukemia and human cancer cell biology data for truncated derivative **6**, synthetic intermediates, and 7-deoxypancratistatin and pancratistatin for comparison

Compound	Murine P388 lymphocytic leukemia and human cancer cell results [μg/mL]						
	Murine P388 lymphocytic leukemia	Pancreas BXPC-3	Breast MCF-7	CNS SF-268	Lung NCI-H460	Colon KM20L2	Prostate DU-145
 **20**	19.3	>10	>10	>10	>10	>10	>10
 **17**	2.1	>10	4.6	>10	>10	>10	6.7
 **6**	4.3	4.9	4.4	3.3	2.8	3.6	2.6
 **11**	1.10	>10	>10	>10	>10	>10	>10
 **5**	1.39	>10	>10	>10	>10	>10	>10

(*continued on next page*)

Table 1. (*continued*)

Compound	Murine P388 lymphocytic leukemia and human cancer cell results [μg/mL]						
	Murine P388 lymphocytic leukemia	Pancreas BXPC-3	Breast MCF-7	CNS SF-268	Lung NCI-H460	Colon KM20L2	Prostate DU-145
 **1**[a]	0.039	0.028	0.032	0.017	0.048	0.062	0.016
 **2**[a]	0.44				0.29	0.22	
 **3**[a]	0.0012	0.026	0.019	0.021	0.032	0.021	0.011

[a] For the listed activities see Ref. 6h.

pancratistatin (100-fold less than **1**). Deletion of two of the four alcohol groups provided compounds 30-fold less active in the cancer cell inhibition. Compound **5**, its acetate **11** and compound **6** showed activities in P388 lines an order of magnitude lower than that of 7-deoxypancratistatin. The fully hydroxylated derivative **6** also displayed activities in all human cancer cell lines tested; compound **5** lacking the *trans*-diol unit was inactive in these lines.

3. Conclusion

It is clear from this and previous studies that enhanced activity in pancratistatin-type compounds cannot be expected in compounds that do not have the completely functionalized cyclitol ring or that lack the full phenanthridone nucleus. Future endeavors will focus on synthesis and evaluation of derivatives of type **8** in which the aminoinositol motif is preserved.

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