Published in final edited form as:

Bioorg Med Chem Lett. 2011 August 15; 21(16): 4750–4752. doi:10.1016/j.bmcl.2011.06.068.

Synthesis of C-1 Homologues of Pancratistatin and their Preliminary Biological Evaluation

Sergey Vshyvenko^a, Jon Scattolon^a, and Tomas Hudlicky^{a,*}

Anntherese E. Romero^b and Alexander Kornienko^{b,*}

^aChemistry Department and Centre for Biotechnology, Brock University, 500 Glenridge Avenue, St. Catharines, Ontario L2S 3A1, Canada

^bChemistry Department, New Mexico Institute of Mining and Technology, 801 Leroy Place, Socorro, New Mexico 87801

Abstract

The synthesis of two C-1 analogues of pancratistatin has been accomplished in 17 steps from bromobenzene. The key steps involved the enzymatic dihydroxylation, regioselective opening of epoxyaziridine **9** with the alane derived from **8**, a solid-state silica-gel-catalyzed intramolecular opening of aziridine to produce phenanthrene **13** whose oxidative cleavage and recyclization provided the full skeleton of the Amaryllidaceae constituents. The new analogues **5** and **6** exhibited promising activity in several human cancer cell lines.

Pancratistatin **1a**, discovered by Pettit in 1984,¹ exhibits potent cytotoxicity against a variety of human cancer cell lines, Figure 1.² Its low availability from natural sources and lack of water solubility, however, limit its potential for development into a therapeutic agent. Together with its congener, narciclasine **2a** and the corresponding 7-deoxy analogues, this natural product represents the most promising Amaryllidaceae anticancer constituent and has been the subject of numerous biological studies. Pancratistatin's low availability from natural sources and the lack of water solubility, however, limit its potential for development into a therapeutic agent. The published SAR studies reveal that the 7-hydroxy group is an important part of the cytotoxic pharmacophore and compounds **1b** and **2b** are about ten times less potent than their 7-hydroxy analogues in the same assays.³ Over the years many unnatural or truncated derivatives of the Amaryllidaceae constituents have been prepared by Pettit,⁴ McNulty,⁵ Kornienko,⁶ Chapleur,⁷ Marion,⁸ Banwell,⁹ Gonzalez,¹⁰ and us,¹¹ and subjected to biological evaluation. In addition, many creative syntheses have been reported for these natural products.¹²

Based on the extensive studies it would appear that the essential pharmacophore of **1a** must retain the C-7 hydroxyl as well as the full amino inositol motif. The space at C-1 appears amenable to modifications, as evidenced by the high activity of C-1 benzoate ester prepared and tested by Pettit.^{4c} Based on this observation we have prepared several C-1 analogues of 7-deoxypancratistatin and subjected these to biological evaluation. The most active compounds proved to be the hydroxymethyl and acetoxymethyl derivatives **3** and **4** respectively, exhibiting activities similar to those of **1b**.^{12a} Because **1a** is more potent than **1b**, we therefore assumed that the corresponding analogues containing the required C-7 hydroxyl should also possess higher activity and perhaps better solubility than that of the natural product itself. In this paper we report the synthesis and preliminary evaluation of pancratistatin analogues **5** and **6**.

*thudlicky@brocku.ca . *akornien@nmt.edu .

The synthesis of **5** and **6** was executed, with some modifications, in a manner similar to that used for the preparation of the corresponding analogues of 7-deoxypancratistatin. The acetylene fragment **8**, possessing a masked 7-hydroxyl group, was prepared as shown in Scheme 1. Hydroxy aldehyde **5** was prepared from piperonal by a literature procedure¹³ involving the metalation of the corresponding imine with *n*BuLi, conversion to boronate ester, oxidation with hydrogen peroxide, and hydrolysis in 71% overall yield.

Methylation provided aldehyde **6**,¹⁴ which was converted to acetylene **8** via Corey-Fuchs protocol as shown.

Conversion of **8** to its alane was accomplished as shown in Scheme 2. Addition of the epoxyaziridine **9** to the alane produced acetylene **10**, which was hydrogenated under Lindlar's conditions to provide the cis-olefin **11**. The intramolecular aziridine opening was accomplished by heating the TBS-protected ether **12** adsorbed on silica-gel, according to a solid phase protocol developed in our laboratory for aziridine opening.¹⁵ It was discovered serendipitously that traces of quinoline (*ca.* 5%) adsorbed on the silica surface led to an improved yield. Phenanthrene **13** was attained in 74% yield and was then subjected to oxidative cleavage and recyclization to produce, after acylation, hemiaminal **16**, possessing the complete skeleton of the natural product. Oxidation of this material to phenanthridone **17** was followed by reductive detosylation to **18**^{15c} and generation of the free hydroxyl at C-7 in **19** by a modification of Trost's procedure, Scheme 2.¹⁶

To complete the synthesis of the required derivatives, **19** was subjected to desilylation to afford alcohol **20**, which on treatment with HCl and methanol furnished the fully hydroxylated analogue **5**, Scheme 3. Alternatively, a partial deprotection with TFA provided the C-1 acetoxymethyl analogue **6**.¹⁷

The hydroxymethyl and acetoxymethyl analogues **5** and **6**, together with their 7-deoxy counterparts **3** and **4**, were evaluated in a small panel of cancer cell lines diversely representing several types of human malignancy (Table 1). Narciclasine, which is about six times as potent as pancratistatin in the NCI 60 cell line screen,² was used as a reference compound because of its better availability. As expected, the antiproliferative potencies on the 7-hydroxy compounds were higher, again underscoring the beneficial effect of the 7-hydroxy substituent. For example, both **5** and **6** were about ten times more potent than their 7-deoxy analogues **3** and **4** against the prostate DU-145 cells and this difference in activity is similar to that between **1a** and **1b**.^{2,3} In addition, the double-digit nanomolar potency of the acetoxymethyl analogue **6** is noteworthy and it approaches that of narciclasine. This finding is consistent with the previous observations that the presence of large hydrophobic C-1 substituents does not lead to a substantial decrease in activity and in some cases actually leads to activity enhancement.^{12a,4c}

In conclusion, these results bode well for the possibility of a library construction with focus on the C-1 space. Of interest will be especially the evaluation of C-1 benzoxymethyl analogue and the comparison of its activity with the C-1 benzoate of pancratistatin, a compound which exhibited greater activity than pancratistatin itself, as reported by Pettit.^{4c} In addition, compounds **5** and **6** will be prepared on medium scale and subjected to a pharmacokinetics evaluation.

Acknowledgments

The authors are grateful to the following agencies for financial support of this work: Natural Sciences and Engineering Research Council of Canada (NSERC) (Idea to Innovation and Discovery Grants); Canada Research Chair Program, Canada Foundation for Innovation (CFI), TDC Research, Inc., TDC Research Foundation, and

Brock University. AER and AK thank the National Institutes of Health (P20 RR016480) for financial support and Professor Snezna Rogelj for her kind assistance with the cell culture.

References

1. Pettit GR, Gaddamidi V, Cragg GM, Herald DL, Sagawa Y. *J. Chem. Soc., Chem. Commun.* 1984:1693.
2. Kornienko A, Evidente A. *Chem. Rev.* 2008; 108:1982. [PubMed: 18489166]
3. Moser M, Banfield SC, Rinner U, Chapuis J-C, Pettit GR, Hudlicky T. *Can. J. Chem.* 2006; 84:1313.
- 4(a). Pettit GR, Melody N, O'Sullivan M, Thompson MA, Herald DL, Coates B. *Chem. Commun.* 1994:2725.(b) Pettit GR, Freeman S, Simpson MJ, Thompson MA, Boyd MR, Williams MD, Pettit GR III, Doubek DL. *AntiCancer Drug Design.* 1995; 10:243.(c) Pettit GR, Melody N, Herald DL. *J. Org. Chem.* 2001; 66(8):2583. [PubMed: 11304174] (d) Pettit GR, Melody N, Herald DL, Knight JC, Chapuis J-C. *J. Nat. Prod.* 2007; 70:417. [PubMed: 17346078] (e) Pettit GR, Ducki S, Eastham SA, Melody N. *J. Nat. Prod.* 2009; 72:1279. [PubMed: 19522518]
- 5(a). McNulty J, Mao J, Gibe R, Mo R, Wolf S, Pettit GR, Herald DL, Boyd MR. *Bioorg. Med. Chem. Lett.* 2001; 11:169. [PubMed: 11206451] (b) McNulty J, Larichev V, Pandey S. *A Bioorg. Med. Chem. Lett.* 2005; 15:5315.(c) McNulty J, Nair JJ, Griffin C, Pandey S. *J. Nat. Prod.* 2008; 71:357. [PubMed: 18225861] (d) McNulty J, Nair JJ, Little JRL, Brennan JD, Bastida J. *Bioorg. Med. Chem. Lett.* 2010; 20:5290. [PubMed: 20655219] (e) McNulty J, Nair JJ, Singh M, Crankshaw DJ, Holloway AC. *Bioorg. Med. Chem. Lett.* 2010; 20:2335. [PubMed: 20189386]
- 6(a). Manpadi M, Kireev AS, Magedov IV, Altig J, Tongwa P, Antipin MY, Evidente A, van Otterlo WAL, Kornienko A. *J. Org. Chem.* 2009; 74:7122. [PubMed: 19743883] (b) Kireev AS, Nadein ON, Agustin VJ, Bush NE, Evidente A, Manpadi M, Ogasawara MA, Rastogi SK, Rogelj S, Shors ST, Kornienko A. *J. Org. Chem.* 2006; 71:5694. [PubMed: 16839151]
7. Ibn-Ahmed S, Khaldi M, Chrétien F, Chapleur Y. *J. Org. Chem.* 2004; 69:6722. [PubMed: 15387596]
8. Marion, F.; Annereau, J-P.; Fahy, J. 2010. WO 2010012714 (A1)
9. Matveenko M, Banwell MG, Joffe M, Wan S, Fantino E. *Chemistry & Biodiversity.* 2009; 6:685. [PubMed: 19479847]
10. de la Sovera V, Bellomo A, Gonzalez D. *Tetrahedron Lett.* 2011; 52:430.
- 11(a). Rinner U, Siengalewicz P, Hudlicky T. *Org. Lett.* 2001; 4:115. [PubMed: 11772104] (b) Hudlicky T, Rinner U, Gonzalez D, Akgun H, Schilling S, Siengalewicz P, Martinot TA, Pettit GR. *J. Org. Chem.* 2002; 67:8726. [PubMed: 12467383] (c) Rinner U, Hillebrenner HL, Adams DR, Hudlicky T, Pettit GR. *Bioorg. Med. Chem. Lett.* 2004; 14:2911. [PubMed: 15125958]
12. For a recent compilation of total syntheses of Amaryllidaceae constituents see: (a) Collins J, Rinner U, Moser M, Hudlicky T, Ghiviriga I, Romero A, Kornienko A, Ma D, Griffith C, Pandey S. *J. Org. Chem.* 2010; 75:3069. [PubMed: 20373760] (b) Yadav JS, Satheesh G, Murthy CVSR. *Org. Lett.* 2010; 12:2544. [PubMed: 20441205] (c) Dam JH, Madsen R. *Eur. J. Org. Chem.* 2009:4666.
13. Garcia A, Castedo L, Domínguez D. *Tetrahedron.* 1995; 51:8585.
14. Lorient M, Jean-Pierre Robin J-P, Brown E. *Tetrahedron.* 1984; 49:2529.
- 15(a). Rinner U, Hudlicky T, Gordon H, Pettit GR. *Angew. Chem. Int. Ed.* 2004; 43:5342.(b) Hudlicky T, Rinner U, Finn KJ, Ghiviriga I. *J. Org. Chem.* 2005; 70:3490. [PubMed: 15844982] (c) Collins J, Drouin M, Sun X, Rinner U, Hudlicky T. *Org. Lett.* 2007; 10:361. [PubMed: 18163635]
16. Trost BM, Pulley SR. *J. Am. Chem. Soc.* 1995; 117:10143. [Note: In this paper LiI is listed as a reagent rather than LiCl, which was actually used.].
17. The full experimental and spectral data for all compounds reported in this manuscript will be published in detail in an upcoming full paper.

Figure 1.
Amaryllidaceae constituents and their C-1 analogues.

Scheme 1.

Scheme 2.

Scheme 3.

Table 1

Activity of C-1 analogues with narciclasine as a standard [IC₅₀ (μM)^a].

Cancer type	Cell Line	Narciclasine 2a	3	4	5	6
Pancreatic	CRL-1687/BXPC-3	0.05 ± 0.01	0.77 ± 0.01	0.34 ± 0.05	0.22 ± 0.01	0.07 ± 0.01
Prostate	HTB-81/DU-145	0.05 ± 0.04	1.10 ± 0.20	0.72 ± 0.27	0.09 ± 0.01	0.06 ± 0.01
Lung	HTB-177/NCI H460	0.04 ± 0.01	0.40 ± 0.01	0.53 ± 0.01	0.09 ± 0.01	0.07 ± 0.01
Breast	HTB-22/MCF-7	0.04 ± 0.01	0.86 ± 0.06	1.81 ± 1.20	0.24 ± 0.10	0.52 ± 0.47

^aConcentration required to reduce the viability of cells by 50%, after 48 h of treatment with indicated compounds, relative to DMSO control; ± SD from two independent experiments, each performed in 4 replicates, determined by MTT assay.