

The Bryostatin 1 A-Ring Acetate is Not the Critical Determinant for Antagonism of Phorbol Ester-Induced Biological Responses

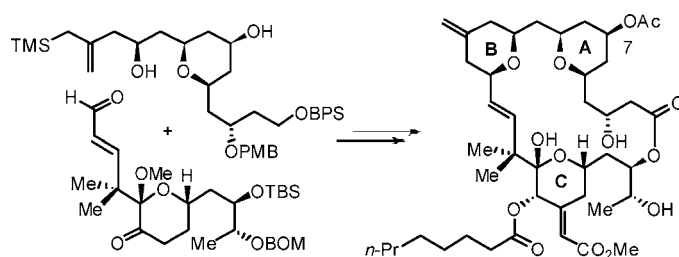
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ABSTRACT



The contribution of the A-ring C₇ acetate to the function of bryostatin 1 has been investigated through synthesis and biological evaluation of an analogue incorporating this feature into the bryopyran core structure. No enhanced binding affinity for protein kinase C (PKC) was observed, relative to previously characterized analogues lacking the C₇ acetate. Functional assays showed biological responses characteristic of those induced by the phorbol ester PMA and distinctly different from those observed with bryostatin 1.

The protein kinase C (PKC) family of kinases plays a central role in the regulation of crucial cellular events such as proliferation, differentiation, and apoptosis. Furthermore, alterations in the expression or activity of PKC isoforms have been linked to various human diseases such as cancer,¹ diabetes,² and Alzheimer's disease.³ PKC has thus emerged as an attractive target for drug development. Attempts at targeting the catalytic domain of PKC have been hindered by issues of selectivity since the human genome encodes approximately 500 protein kinases, each containing the highly

conserved ATP binding pocket.⁴ The novel and classical PKC isozymes possess cysteine-rich C1 domains which recognize endogenous 1,2-diacylglycerols (DAGs) ultimately leading to the activation of the enzyme. Targeting the C1 domain rather than the catalytic domain provides a potential strategic advantage in drug selectivity since only a small number of other enzymes are known to contain a C1 domain, e.g., the chimaerins, RasGRPs, PKDs, Unc-13s, and the diacylglycerol kinases.⁵

The first known exogenous ligands for the PKC C1 domain were the tumor promoting phorbol esters such as phorbol 12-myristate-13-acetate (PMA, **2**) (Figure 1), leading to the hypothesis that their tumor promoting activity was in part

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(1) Griner, E. M.; Kazanietz, M. G. *Nat. Rev. Cancer* **2007**, *7*, 281–294.

(2) Way, K. J.; Katai, N.; King, G. L. *Diabetic Med.* **2001**, *18*, 945–959.

(3) Alkon, D. L.; Sun, M.-K.; Nelson, T. J. *Trends Pharmacol. Sci.* **2007**, *28*, 51–60.

(4) Manning, G.; Whyte, D. B.; Martinez, R.; Hunter, T.; Sudarsanam, S. *Science* **2002**, *298*, 1912–1934.

(5) (a) Blumberg, P. M.; Kedei, N.; Lewin, N. E.; Yang, D.; Czifra, G.; Pu, Y.; Peach, M. L.; Marquez, V. E. *Curr. Drug Targets* **2008**, *9*, 641–652. (b) Teicher, B. A. *Clin. Cancer Res.* **2006**, *12*, 5336–5345. (c) Kazanietz, M. *Biochim. Biophys. Acta* **2005**, *1754*, 296–304. (d) Gould, C. M.; Newton, A. C. *Curr. Drug Targets* **2008**, *9*, 614–625.

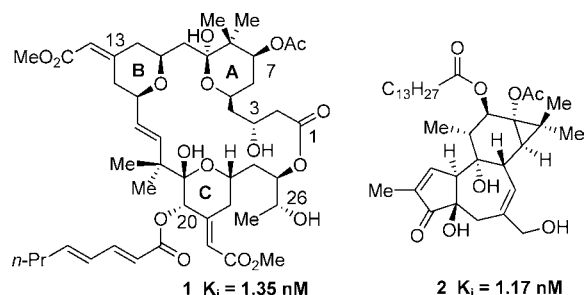


Figure 1. Structures of bryostatin 1 and PMA.

linked to PKC activation.⁶ Bryostatin 1 (**1**), isolated by Pettit from the marine bryozoan *Bugula neritina*, is also a potent activator of PKC.⁷ However, unlike most phorbol esters, bryostatin 1 is not tumor promoting and antagonizes many of the responses induced by the phorbol esters. Bryostatin 1 has potent activity toward a number of cancer cell lines, displays immunostimulant effects,⁸ and shows promise in the treatment of Alzheimer's disease.^{9,3} Recently, bryostatin 1 has also been shown to induce repair of neuronal damage resulting from stroke.¹⁰

In an effort to identify the mechanisms responsible for the unique activity of bryostatin 1, we are attempting to determine, through chemical synthesis, the structural features of bryostatin that are responsible for its unique biological profile. Recently, we have prepared and evaluated analogues (Merle **21**–**23**¹¹ and Merle **28**,¹² vide infra) which now limit the functionality largely responsible for the unique biological profile exhibited by bryostatin 1 to that present in the C₇–C₉ region of the A-ring.¹³ In this paper, we report studies which rule out the A-ring C₇ acetate as being the critical determinant of the unique biological responses characteristic of bryostatin 1, through synthesis and biological evaluation of analogue Merle **27**. The preparation of this new bryopyran is outlined in Scheme 1.

Analogue Merle **27** was prepared using a convergent pyran annulation strategy to unite preformed A- and C-rings with concomitant formation of the B-ring, as shown in Scheme 1. Preparation of the A-ring pyran began with pyran **3**,

prepared via pyran annulation as previously described.¹¹ The exomethylene was used to introduce the requisite C₇ oxygenation by ozonolysis followed by NaBH₄ reduction (94%) to give the C₇ alcohol **4** as a single diastereomer. Temporary protection of the alcohol as its TMS derivative¹⁴ was followed by Bunnelle reaction¹⁵ to give the desired hydroxy allylsilane **5** in 71% yield.

Pyran annulation between **5** and C-ring enal **6** using our standard conditions of TMSOTf in ether gave the desired tricyclic adduct, as a single diastereomer, in 92% isolated yield. Protection of the C₇ OH using TBSOTf and 2,6-lutidine gave **7** in 98% yield.

Aldol condensation with methyl glyoxylate gave a diastereomeric mixture of aldol products (92%) which underwent elimination (93%) by treatment with Ac₂O and DMAP in pyridine at 60 °C. The resulting enoate **8** was then subjected to Luche reduction¹⁶ followed by acylation with octanoic anhydride to give **9**. Selective removal of the BPS (*tert*-butyldiphenylsilyl) group at C₁ using TBAF and HOAc in DMF (89%) was followed by oxidation to the acid **10** (quantitative yield over 2 steps) by sequential Parikh–Doering¹⁷ and Pinnick¹⁸ oxidations. Removal of both TBS groups (at C₇ and C₂₅) using HF·Py then gave the corresponding dihydroxy acid.

Attempted macrolactonization of this dihydroxy acid using the Yamaguchi protocol¹⁹ afforded a macrolactone in which the C₇ alcohol had *also* been acylated by 2,4,6-trichlorobenzoyl chloride, in excellent (79%) yield. To preclude the undesired acylation of C₇, this hydroxyl was selectively protected by reaction with TESCl and DMAP to give **11**; the Yamaguchi reaction then afforded the desired macrolactone **12**. Removal of the TES group followed by acetylation gave the desired C₇ acetate derivative **13** (95% over 2 steps). Finally, protecting group removal (DDQ then LiBF₄ in aqueous CH₃CN) gave analogue Merle **27** (87% over 2 steps). The C₇ trichlorobenzoate derivative obtained inadvertently was also carried through a parallel sequence to give analogue **14**.

Analogue Merle **27** was found to bind to PKC with slightly lower affinity than that of Merle **21**–**23** ($K_i = 3.0 \pm 0.6$ nM with PKC α). Assays for differential response were conducted using leukemia U937 cells.²⁰ In this assay, PMA inhibits cell proliferation and induces attachment. Bryostatin 1 has much less effect on either response and blocks the effect of the phorbol ester when both agents are administered together. The results are shown in Figures 2 and 3. Results

(6) Castagna, M.; Takai, Y.; Kaibuchi, K.; Sano, K.; Kikkawa, U.; Nishizuka, Y. *J. Biol. Chem.* **1982**, *257*, 7847–7851.

(7) Pettit, G. R.; Herald, C. L.; Douobek, D. L.; Herald, D. L. *J. Am. Chem. Soc.* **1982**, *104*, 6846–6848.

(8) Koutcher, J. A.; Motwani, M.; Zakian, K. L.; Li, X.-K.; Matei, C.; Dyke, J. P.; Ballon, D.; Yoo, H.-H.; Schwartz, G. K. *Clin. Cancer Res.* **2000**, *6*, 1498–1507.

(9) (a) Etcheberrigaray, R.; Tan, M.; Dewachter, I.; Kuiperi, C.; Van der Au, Wera, I.; Wera, S.; Qiao, L.; Bank, B.; Nelson, T. J.; Kozikowski, A. P.; Van Leuven, F.; Alkon, D. L. *Proc. Natl. Acad. Sci., U.S.A.* **2004**, *101*, 11141–11146.

(10) Sun, M.-K.; Hongpaisan, J.; Nelson, T. J.; Alkon, D. L. *Proc. Natl. Acad. Sci., U.S.A.* **2008**, *105*, 13620–13625.

(11) Keck, G. E.; Kraft, M. B.; Truong, A. P.; Li, W.; Sanchez, C. C.; Kedei, N.; Lewin, N.; Blumberg, P. M. *J. Am. Chem. Soc.* **2008**, *130*, 6660–6661.

(12) Keck, G. E.; Poudel, Y. B.; Welch, D. S.; Kraft, M. B.; Truong, A. P.; Stephens, J. C.; Kedei, N.; Lewin, N. E.; Blumberg, P. M. *Org. Lett.* **2009**, *11*, 593–596.

(13) Here we adopt permanent identifiers (“Merle numbers”) for these analogues which will remain invariant from publication to publication.

(14) Stereochemistry at C₇ was confirmed at this point by observation of NOEs between the axial proton at C₇ and those at C₅ and C₉.

(15) Bunnelle, W. H.; Narayanan, B. A. *Organic Syntheses*; Wiley & Sons: New York, 1993; Collect. Vol. 8, pp 602–605.

(16) (a) Luche, J. L. *J. Am. Chem. Soc.* **1978**, *100*, 2226–2267. (b) Wender, P. A.; Baryza, J. L.; Bennet, C. E.; Bi, F. C.; Brenner, S. E.; Clarke, M. O.; Horan, J. C.; Kan, C.; Lacote, E.; Lipka, B.; Nell, P. G.; Turner, T. M. *J. Am. Chem. Soc.* **2002**, *124*, 13648–13649.

(17) Parikh, J. R.; Doering, W. v. E. *J. Am. Chem. Soc.* **1967**, *89*, 5505–5507.

(18) Bal, B. S.; Childers, W. E., Jr.; Pinnick, H. W. *Tetrahedron* **1981**, *37*, 2091–2096.

(19) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989–1993.

(20) Vrana, J. A.; Saunders, A. M.; Srikumar, P. C.; Grant, S. *Differentiation* **1998**, *63*, 33–42.



tion on, at most, two carbons.²¹ Efforts to determine, through synthesis, the role of substituents at each of these carbons are in progress.

It should be noted that Wender and Verma have recently reported the synthesis of the C₇ acetate **16** and a number of other C₇ derivatives, including the C₇–exomethylene compound **15**, using the B-ring acetal platform developed

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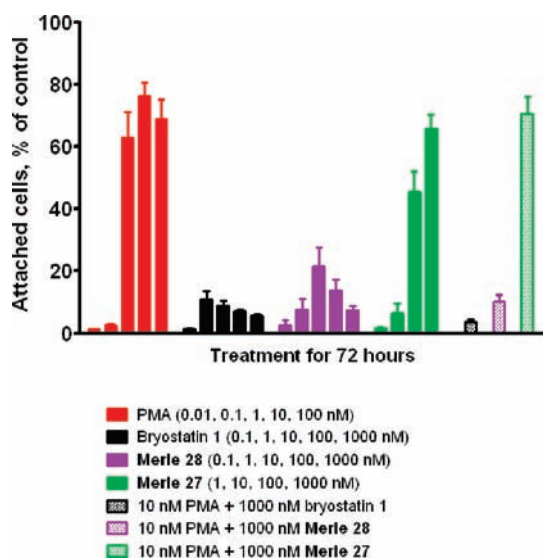


Figure 2. Effect of Merle 27 on U937 cell attachment.

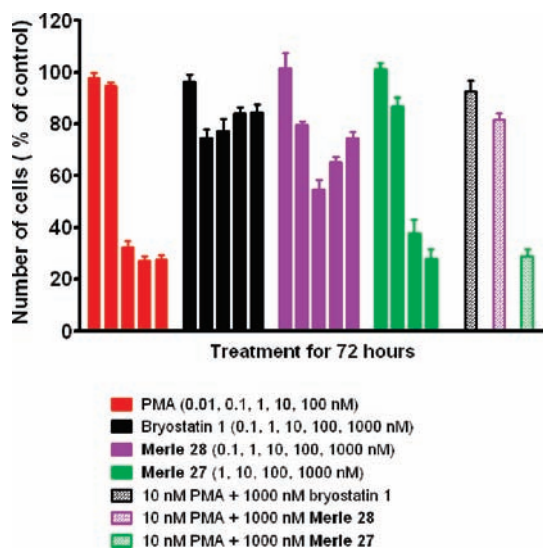


Figure 3. Effect of Merle 27 on U937 cell proliferation.

previously by that group.²² These authors also utilized our pyran annulation approach for construction of the A-ring bearing the versatile C₇ exomethylene group. Using this platform, the acetate **16** was found to have $K_i = 13 \pm 3.8$ nM against a mixture of PKC isozymes isolated from rat brain, while the exomethylene compound **15** had $K_i = 5.3$ nM.²³ (Note Figure 4.)

Interestingly, of the 12 C₇-functionalized derivatives reported by Wender and Verma, the highest affinity for PKCs was shown by the C₇-exomethylene derivative **15**, i.e., the analogue incorporating the C₇ functionality introduced

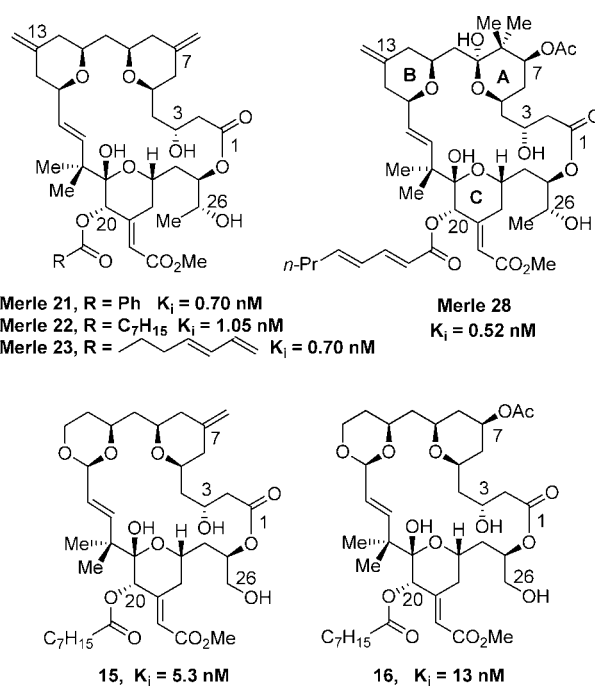


Figure 4. Structures of other recent analogues.

directly by the pyran annulation reaction. All other modifications at C₇ examined gave materials with decreased affinity for PKCs. The same is true of the C₇ acetate Merle 27 and the C₇ trichlorobenzoate **14** ($K_i = 16.8 \pm 4.2$ nM with PKC α) prepared by us using the bryopyran platform, which are less potent than the exomethylene compounds Merle 21–23. Thus, on both platforms, the exomethylene compounds are approximately 2.5–3-fold more potent in terms of binding than the compounds incorporating the natural C₇ acetate functionality.

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Supporting Information Available: Experimental procedures, assay results, and spectral data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(22) (a) Wender, P. A.; Verma, V. A. *Org. Lett.* **2008**, *10*, 3331–3334. (b) For an overview of bryostatin analogue synthesis by the Wender group, see: Wender, P. A.; Baryza, J. L.; Hilinski, M. K.; Horan, J. C.; Kan, C.; Verma, V. A. *Beyond Natural Products: Synthetic Analogues of Bryostatin 1*. In *Drug Discovery Research: New Frontiers in the Post-Genomic Era*; Huang, Z., Ed.; Wiley-VCH: Hoboken, NJ, 2007; pp 127–162.

(23) These authors suggest that the carbonyl oxygen of this acetate substituent may be involved in hydrogen bonding to a residue in the C1 domain.