Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1687–1690

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SELECTIVE BINDING OF BRYOSTATIN ANALOGUES TO THE CYSTEINE RICH DOMAINS OF PROTEIN KINASE C ISOZYMES

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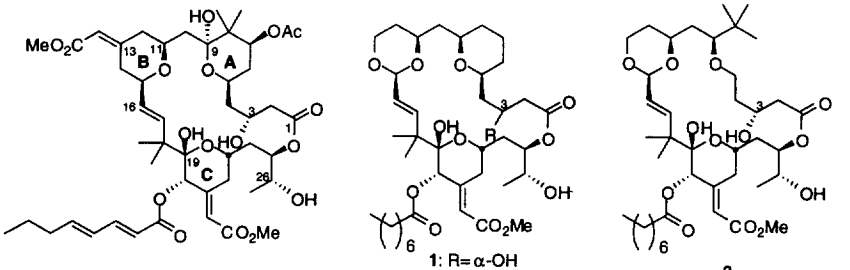
Received 24 February 1999; accepted 4 May 1999

Abstract: Designed bryostatin analogues are assayed for binding affinity to individual cysteine rich domains of several protein kinase C (PKC) isozymes. These analogues exhibit significant selectivity for the PKCδ-C1B peptide in terms of absolute affinity and the PKCδ-C1A peptide in terms of relative affinity when compared to phorbol-12,13-dibutyrate.

The bryostatins are a family of structurally novel, macrocyclic lactones isolated from marine bryozoa on the basis of their potent activity in the murine P388 in vitro cell line.[1] Currently, bryostatin 1 is in over 25 human clinical trials for the treatment of various types of cancer.[2] While their molecular mode of action remains unknown, the bryostatins are found to bind with high affinity to the C1A and C1B regions of the regulatory domain of protein kinase C (PKC) isozymes and to stimulate comparable kinase activity in vitro.[3]



Molecular mode of action studies on the bryostatins have been severely limited, in part because they are isolated in low yields after arduous separations from ecologically sensitive sources and in part because the limited material so obtained is needed for clinical evaluation. Recently, we reported the synthesis of first and second generation, simplified bryostatin analogues designed on the basis of computational, spectroscopic, and

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00263-2

structure activity data.[4] These analogues have been found to exhibit activity on par or better than the natural bryostatins in both in vitro human cancer cell line studies and PKC binding assays.

An important starting point for elucidating the mode of action of these analogues is to understand their role in the activation of PKC isozymes, established receptors for the bryostatins. These enzymes are individually implicated in a variety of cellular responses and could be of consequence in the activity of the bryostatins. For instance, although all PKC isozymes are upregulated immediately after administration of bryostatins or tumor promoting phorbol esters followed by an extended down-regulation, several studies suggest that PKCδ is protected against down regulation at high concentrations of bryostatin 1, even when phorbol myristate acetate is co-applied.[5] Significantly, independent studies have shown that over expression of PKCδ inhibits tumor cell growth and induces cellular apoptosis, whereas depleting cells of PKCδ can cause tumor promotion.[5d,6] Translocation assays of selectively mutated C1A or C1B domains of PKCδ have shown that tumor promoters depend on only the C1B domain of PKCδ to cause enzyme translocation to the cellular membrane, whereas non tumor promoters depend on both C1A and C1B domains being unaltered for full translocation.[7]

Table 1 K_i values for inhibition of the specific binding of [^{3}H]-PDBu by the bryostatin analogues (**1-3**)

PKC	bryostatin analogues K_i (nM)			K_d (nM)
C1 peptides	**1**	**2**	**3**	PDBu
α-C1B	125.8 (29.9)[a]	>500	>500	46.7
β-C1B	13.4 (3.5)	280.0 (37.9)	36.4 (5.9)	1.3
γ-C1A	>500	>500	>500	65.8
γ-C1B	>500	>500	>500	16.9
δ-C1A	65.9 (10.5)	494.4 (114.0)	85.4 (15.0)	272.6[b]
δ-C1B	2.5 (0.0)	64.2 (3.5)	6.3 (1.8)	1.0
ε-C1B	17.9 (0.3)	489.6 (52.9)	55.0 (0.3)	1.5
η-C1B	23.5 (3.5)	>500	70.2 (4.9)	0.9
θ-C1B	6.6 (0.0)	147.5 (17.0)	24.1 (2.8)	3.4

[a] Standard deviation of at least two separate experiments.

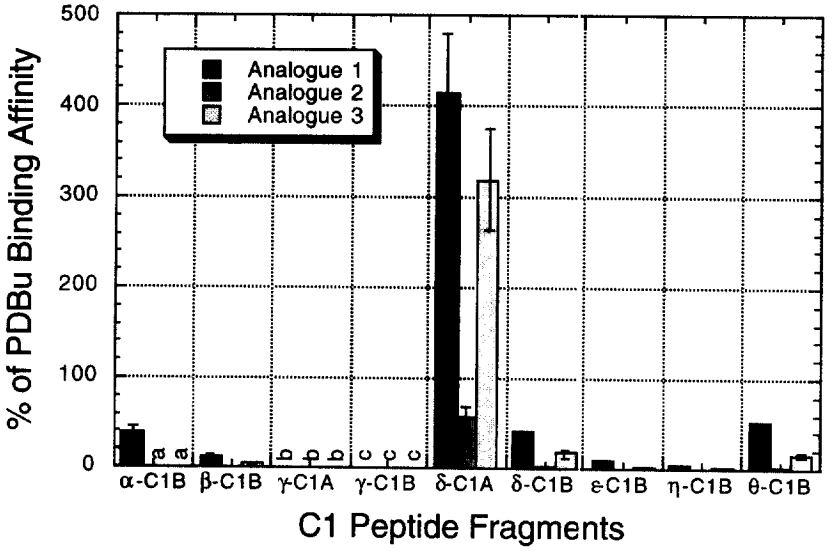
[b] K_i value calculated from K_d of PDBu (300 nM). The standard deviation of this value is 70.7 nM.

We have recently reported the solid-phase synthesis, folding and binding properties of individual C1A and C1B domains of all PKC isozymes.[8] Among other significant advantages, these readily available and highly pure PKC surrogates provide a facile and effective method to screen compounds for independent binding to either the C1A or C1B domains of all conventional and novel PKCs.

To date there has been no reported study based directly on the binding properties of bryostatin or its analogues to the individual C1A and C1B domains of the various PKC isozymes. The availability of synthetic bryostatin analogues and these C1 peptide surrogates provides an ideal opportunity to obtain this crucial mechanistic information. Toward this end, we conducted binding assays on our analogues in competition

with [^{3}H]-phorbol-12,13-dibutyrate (PDBu) using the individual C1 domains and a procedure reported previously.[8] The magnitude of binding affinity for all C1 domains was found to be greatest for analogue **1**, followed by the *tertiary*-butyl analogue **3**; the lowest affinity analogue in all cases was the C3-epi analogue **2** (Table 1). These relative affinities are in agreement with previous assays employing a PKC isozyme mix isolated from rat brain.[4] Surprisingly, each analogue showed similar isozyme and C1 domain selectivity despite the wide variation in their structures. All analogues displayed the highest affinity for the δ-C1B peptide fragment and slightly lower affinity for θ-C1B, β-C1B, ε-C1B and δ-C1A peptide fragments. Low affinity was observed between the analogues and both γ-C1 peptide fragments. The selectivity observed in this assay is similar in kind, although considerably greater in magnitude than that observed in whole isozyme assays conducted with bryostatin 1.[9] The observed δ-C1B binding selectivity might explain bryostatin's unique ability to protect this isozyme from down regulation and is of possible significance to the molecular mode of action of this exciting family of compounds.

Figure 1 Relative binding affinities of bryostatin analogues expressed as a percentage of PDBu binding



[a]<9.3%; [b]<13.2%; [c]<3.4%

In comparison with PDBu binding, the affinities of these analogues for the δ-C1A fragment are noteworthy (Figure 1). In the case of analogue **1**, the binding affinity to this fragment is over four times greater than that of PDBu. Binding of the further simplified analogue **3** is only slightly weaker (319% of PDBu binding). The binding affinity of analogues to all other peptide fragments was below 50% of relative PDBu binding due mainly to PDBu's high affinity for those fragments. Importantly, this relative affinity could be significant given the correlation of δ-C1A dependence on PKCδ translocation for non-tumor promoters and the putative role of PKCδ in apoptosis.

In conclusion, the binding affinity data obtained in this study reveals a strong PKCδ selectivity in absolute binding for all synthetic bryostatin analogues and a very strong relative δ-C1A binding affinity when compared to PDBu. It is noteworthy that the binding selectivity observed for these analogues coincides with an isozyme which when over expressed is known to suppress tumor growth, and that the highest relative binding coincides with a C1 domain that has been shown empirically to be important in a lack of a tumor promoting response. Current efforts are underway to further understand the mode of action of bryostatin and its analogues and to synthesize bryostatin analogues with enhanced PKCδ affinity and selectivity.

Acknowledgment: Support of this work through a grant (CA31845) provided by the National Institutes of Health (P.A.W.) and a grant-aid for scientific research (C) (No. 11660109) from the Ministry of Education, Science, Culture, and Sports, Japan (K.I.) are gratefully acknowledged. Fellowship support from the following sources is also gratefully recognized: Eli Lilly (B.L.), Hoffmann-La Roche (B.L.), and the Korean Science and Engineering Foundation (C.M.P.).

References and Notes

1. (a) Pettit, G. R.; Herald, C. L.; Doubek, D. L.; Herald, D. L.; Arnold, E.; Clardy, J. *J. Am. Chem. Soc.* **1982**, *104*, 6846. (b) Pettit, G. R. *J. Nat. Prod.* **1996**, *59*, 812.
2. Current information on the scope and status of bryostatin clinical trials can be found on the NCI web page at: http://cancernet.nci.nih.gov/prot/patsrch.shtml
3. (a) DeVries, D. J.; Herald, C. L.; Pettit, G. R.; Blumberg, P. M. *Biochem. Pharmacol.* **1988**, *37*, 4069. (b) Slater, S. J.; Ho, C.; Kelly, M. B.; Larkin, J. D.; Taddeo, F. J.; Yeager, M. D.; Stubbs, C. D. *J. Biol. Chem.* **1996**, *271*, 4627. (c) Kraft, A. S.; Smith, J. B.; Berkow, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1334.
4. (a) Wender, P. A.; De Brabander, J.; Harran, P. G.; Jimenez, J-M.; Koehler, M. F. T.; Lippa, B.; Park, C-M.; Shiozaki, M. *J. Am. Chem. Soc.* **1998**, *120*, 4534. (b) Wender, P. A.; De Brabander, J.; Harran, P. G.; Jimenez, J-M.; Koehler, M. F. T.; Lippa, B.; Park, C-M.; Siedenbiedel, C.; Pettit, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6624. (c) Wender, P. A.; Harran, P. G.; Hinkle, K. W.; Lippa, B.; Pettit, G. R. *Tetrahedron Lett.* **1998**, *39*, 8625.
5. (a) Szallasi, Z.; Smith, C. B.; Pettit, G. R.; Blumberg, P. M. *J. Biol. Chem.* **1994**, *269*, 2118. (b) Szallasi, Z.; Denning, M. F.; Smith, C. B.; Dlugosz, A. A.; Yuspa, S. H.; Pettit, G. R.; Blumberg, P. M. *Mol. Pharmacol.* **1994**, *46*, 840. (c) Szallasi, Z.; Kosa, K.; Smith, C. B.; Dlugosz, A. A.; Williams, E. K.; Yuspa, S. H.; Blumberg, P. M. *Mol. Pharmacol.* **1995**, *47*, 258. (d) Lu, Z. M.; Hornia, A.; Jiang, Y. W.; Zang, Q.; Ohno, S.; Foster, D. A. *Mol. Cell. Biol.* **1997**, *17*, 3418.
6. (a) Fukumoto, S.; Nishizawa, Y.; Hosoi, M.; Koyama, H.; Yamakawa, K.; Ohno, S.; Morii, H. *J. Biol. Chem.* **1997**, *272*, 13816. (b) Ghayur, B. T.; Hugunin, M.; Talanian, R. V.; Ratnofsky, S.; Quinlan, C.; Emoto, Y.; Pandey, P.; Datta, R.; Huang, Y. Y.; Kharbanda, S.; Allen, H.; Kamen, R.; Wong, W.; Kufe, D. *J. Exp. Med.* **1996**, *184*, 2399.
7. Bögi, K.; Lorenzo, P. S.; Szallasi, Z.; Acs, P.; Wagner, G. S.; Blumberg, P. M. *Cancer Res.* **1998**, *58*, 1423.
8. (a) Wender, P. A.; Irie, K.; Miller, B. L. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 239. (b) Irie, K.; Oie, K.; Nakahara, A.; Yanai, Y.; Ohigashi, H.; Wender, P. A.; Fukuda, H.; Konishi, H.; Kikkawa, U. *J. Am. Chem. Soc.* **1998**, *120*, 9159.
9. Kazanietz, M. G.; Lewin, N. E.; Gao, F.; Pettit, G. R.; Blumberg, P. M. *Mol. Pharmacol.* **1994**, *46*, 374, and references cited therein.