Study on Marine Natural Products: Bryostatin and its Analogue as Anti-Cancer Agent

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Abstract- Bryostatins are a unique family of novel chemotherapeutics of marine origin isolated from Bugula neritina. Although the biochemical basis of their therapeutic activity is unknown, these macrolactones have a high affinity for protein kinase C (PKC) isozymes, compete for the phorbol-ester binding site on PKC, and stimulate kinase activity in vitro and in vivo. Computer modeling has generated a new class of bryostatin analogs that retain the putative recognition domain of bryostatins but are simplified by removing and modifying the C1–C14 middle domain. The solution structures of the two synthetic analogs were determined by NMR spectroscopy and found to be very similar to the previously reported structures of bryostatins 1 and 10. Furthermore, these structures seem to indicate that the stereochemistry of the C3 hydroxyl group plays an important role in the shape of the macrolactone. All analogs bound strongly to a mixture of PKC isozymes, and several showed significant in vitro growth inhibitory activity against human cancer cell lines. Collectively, this work provides important steps toward the development and understanding of simplified, synthetically accessible analogs of bryostatins as potential chemotherapeutic agents.

Keywords: Bryostatins, analogue, marine-derived, PKC

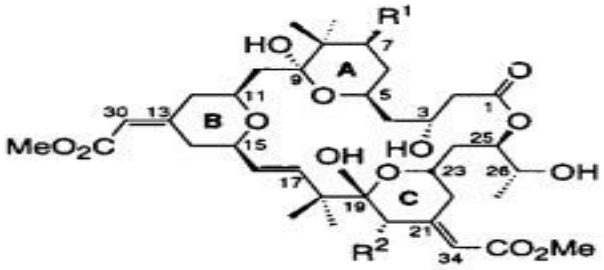
INTRODUCTION

Cancer is a large group of diseases that can start in almost any organ or tissue in the body when abnormal cells grow out of control, exceed their normal limits, invade adjacent parts of the body and spread to other organs. Cancer is the second leading cause of death worldwide and is responsible for approximately 9.6 million deaths, or one in six. Since 1968, bryostatins in extracts of the marine moss Bugula neritina have been studied for their cellular activity and therapeutic potential. B. neritina is the only source of this unique family of macrolides. Bryostatins are complex polyketides based on the bryopyran ring system and are similar to bacterial secondary metabolites. The low natural abundance of bryostatins and the difficulties associated with their isolation have hindered the elucidation of their molecular mode of action and the acceleration of their clinical development. For example, the initial isolation of bryostatin 1 from B. neritina required 500 kg wet animals to obtain enough material for structural characterization and bioanalysis. Subsequent isolations of this and other bryostatins gave yields ranging from 1023 to 1028%. In order to produce enough material for human therapeutic use, large-scale aquaculture of B. neritina is currently being investigated. Currently, however, the availability of these molecules for research and clinical trials is limited..

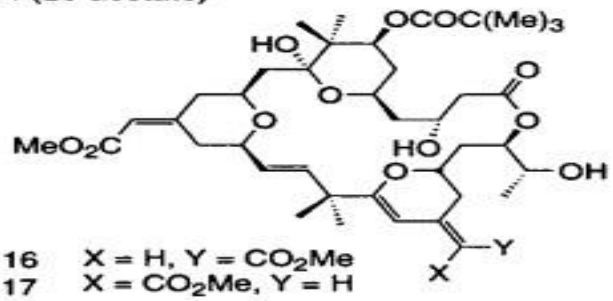
BRYOSTATIN AND IT'S ANALOGUES

Bryostatins are potent against a wide range of human cancer cell lines and significantly prolong in vivo lifespan in murine xenograft tumor models. Typically, the doses required in life are extremely small and activity is seen in concentration. Among other therapeutic responses, bryostatin promotes normal growth of bone marrow progenitor cells, provides cytoprotection against normally lethal doses of ionizing radiation, stimulates immune system responses that lead to production of T cells, tumor necrosis factors, interleukins and interferons.1 Bryostatin [1] has been found to promote normal growth of bone marrow progenitor cells, providing in vivo protection against normally lethal doses of ionizing radiation and acting as an immunostimulant by influencing the normal production of interleukin [2] and interferon. Bryostatin was also effective in inducing the transformation of chronic lymphocytic leukemia cells into hairy cell types by increasing p53 expression while decreasing bcl-2 expression, inducing cancer cell apoptosis and reversing multidrug resistance (MDR).[2] Important. , bryostatins have been shown to competitively inhibit the binding of plant phorbol

esters and endogenous diacylglycerols to protein kinase C (PKC) at low nanomolar or picomolar concentrations and stimulate the corresponding kinase activity in vitro. The most widely studied agent of the bryostatin group is bryostatin-1, a highly oxygenated macrolide with a unique polyacetate backbone. It was originally isolated from the marine organism Bugula neritina L. (Bugulidae), which often occurs as moss colonies attached to piers, piles, buoys, floats and ship hulls, or occurs in rocks and shells. Only several grams of highly pure bryostatin-1 were obtained from approximately 10,000 gallons of Bugula neritina. Bryostatin has been obtained from the moss Bugula neritina since the late 1960s, mainly from the Gulf of California and Gulf of Mexico regions, and from various places in the eastern and western Pacific, including Japan.



Entry	Bryostatin	R^1	R^2	K_i (x 10^{-9} M†)
1	1	O_2CCH_3		1.35 ± 0.17
2	2	OH		5.86 ± 1.13
3	3*			2.75 ± 0.05
4	4	$O_2CC(CH_3)_3$	$O_2CCH_2CH_2CH_3$	1.30 ± 0.19
5	5	$O_2CC(CH_3)_3$	O_2CCH_3	1.04 ± 0.10
6	6	$O_2CCH_2CH_2CH_3$	O_2CCH_3	1.18 ± 0.29
7	7	O_2CCH_3	O_2CCH_3	0.84 ± 0.07
8	8	$O_2CCH_2CH_2CH_3$	$O_2CCH_2CH_2CH_3$	1.72 ± 0.10
9	9	O_2CCH_3	$O_2CCH_2CH_2CH_3$	1.31 ± 0.00
10	10	$O_2CC(CH_3)_3$	H	1.56 ± 0.16‡
11	2 (13,30,2',3',4',5'-hexahydro)			9.61 ± 0.94
12	2 (13,30,21,34,2',3',4',5'-octahydro)			473 ± 96
13	4 (13,30-epoxide)			0.54 ± 0.07
14	1 (26-epi)			32.6 ± 6.6‡
15	4 (26-acetate)			>>100



16	16	X = H, Y = CO_2Me		118 ± 2‡
17	17	X = CO_2Me, Y = H		188 ± 7‡

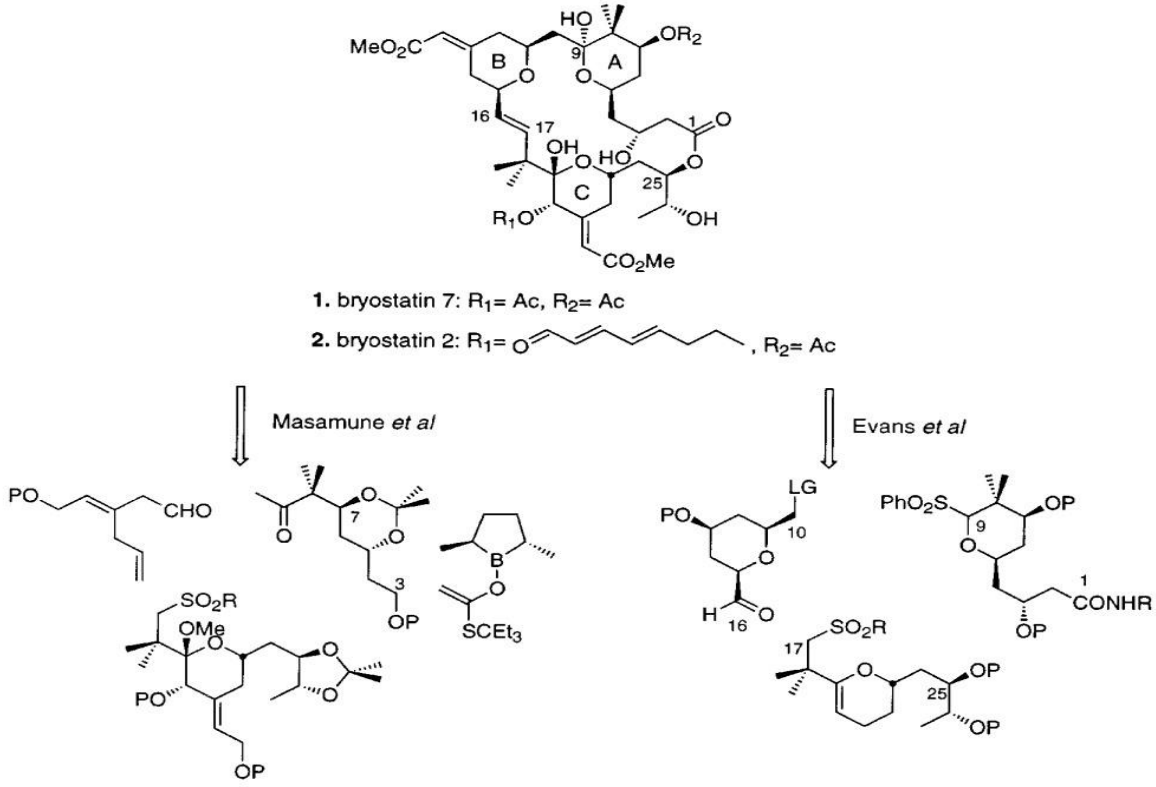
Protein Kinase C

PKC is a serine/threonine kinase family that has been shown to play a key role in the signaling pathway of various extracellular stimuli such as growth factors, hormones and neurotransmitters. PKC appears to play a critical role in the causation and potential treatment of cancer.[3] For example, PKC is the major intracellular target of potent tumor-promoting phorbol esters. In addition, different isozymes of PKC have been shown to be upregulated in cancer cells, which are believed to play an important role in two of the most common cellular events that defeat chemotherapy, namely tumor metastasis and multidrug resistance. Although bryostatins bind PKC competitively with phorbol

esters, they specifically elicit only some of the typical phorbol ester responses and block effects of phorbol esters that they do not initiate. Inhibited responses by phorbol ester include differentiation in HL-60 promyelocytic leukemia cells and Friend erythroleukemia cells, proliferation of JB6 mouse epidermal cells, arachidonic acid release in $C_3H_{10}T1/2$ cells42, and promotion of first-phase tumorigenesis in mouse skin.

Anticancer Property of Bryostatins

The two syntheses differ most significantly in their approach to functionalizing the C ring. Masamune and co-workers prepare a more fully functionalized C ring which is then coupled to the completed AB portion of the molecule. Evans' team chooses instead to take a relatively bare dihydropyran C ring through the synthesis and only completes the functionalization when the macrolactone has been formed.[11]



Structural Modification

Several preclinical studies have been reported in the past years that have helped to understand the role of bryostatin in various malignancies. PKC exists as 10 isoforms that are differentially regulated by DAG, Ca^{2+} , and phospholipids, and it is likely that when bryostatin-1 binds to the cysteine-rich domains of PKC, similar changes occur, although there may be specific differences between the different ones. [4] This hypothesis led to the synthesis of simplified analogs based on the modification of the A and B rings of bryostatin. As the structure-activity relationship (SAR) of bryostatins continues to be elucidated, simplified B-ring analogs have been synthesized. Synthesis of B-ring analogs lacking the A-ring showed that the B-ring ester can affect the selective activation of PKC isozymes. Late diversification through cross-metathesis led to the synthesis of five more analogues.[1]

Supply of bryostatins

The low natural abundance of bryostatins, the difficulty of their isolation, and the possibility of harming ecologically sensitive areas by sourcing them from other countries-natural sources for human therapeutic use. One approach involves the large-scale hydroculture of arugula neritina, which is currently being investigated.[5] However, even if this method were successful, a schematic representation of the homologous regions of the various PKC isozymes. It is limited to the production of naturally occurring

bryostatins and their biosynthetic precursors. Total synthesis also has the potential to produce these molecules quantitatively, and several approaches and two total syntheses have been reported. Masamune and co-workers produced the first synthesis of bryostatin [7], a member of the bryostatin family, and recently Evans and co-workers published the total synthesis of bryostatin [2,6].

The two syntheses share several features, mainly due to two relatively simple cleavages. in bryostatins, namely the C1-C25 esters and the C16-C17 olefin. Both strategies allow the formation of the C1-C25 bond by C1 acid activation and the formation of the C16-C17 bond by Julia coupling.9 Once these two bonds are removed, both retrosynthetic analyzes rely on further simplification of AB. through cuts in the ring section. either C10-C11 (Masamune) or C9-C10 (Evans).[7] Structure-activity relationships (SAR) of bryostatins. Here, Bryostatin 2 was converted to Bryostatin 1 and Bryostatin 12 by selective protection and deprotection involving the C26 hydroxyl group. without reducing cytosolic levels of PKC. [8]

Analog Design

Due to the limited availability of natural bryostatins and the difficulties associated with their selective modification, first-generation structure-activity studies were limited to natural products and their closely related derivatives.[13] A comparison of the binding affinities of PKC bryostatins 1 - 10 shows that changes in the R1 and R_2 groups have little effect.[6] Similarly, analogs obtained by reduction or epoxidation of C13–C30 alkene still retain significant affinity. In contrast, analogs where both the C13-C30 and C21-C34 alkenes are hydrogenated had clearly reduced affinity.[8,14] Removing the C19 hydroxyl group or inverting the stereochemistry at C26 has a greater effect on affinity. Finally, acetylation at C26 eliminated significant binding (entry 16). Taken together, these findings indicate that changes in the C4-C16 region have little effect on bryostatin binding, but changes in the C19-C26 region significantly impair binding.[15]

CONCLUSION

Bryostatins induce a series of unique biochemical responses and are promising candidates for cancer therapy. However, relatively little is known about their molecular mode of action. Structure-activity studies have been limited by the lack of material and the difficulties that arise in selectively changing the functionality of complex bryostatin. The aim of this study was to provide insight into the structural features of bryostatins that contribute to their activity, in this case by binding to PKC. In turn, this approach will help determine the information needed to design simpler and potentially better clinical candidates that could be made through practical synthesis. Since the role of these marine-derived macrocycles in mammalian biochemistry is determined in part by the spatial array of their recognition elements, a computer search was initially performed to identify possible common recognition elements in bryostatins and other compounds that compete for binding to bryostatins. for PKC. Several common activities have been identified and prioritized. The low natural abundance and clinical applicability of bryostatins have made the simplest modifications of their structures inaccessible, leaving many questions about the molecular basis of their cellular actions unresolved. Showing that simplified analogs can mimic the solution structure and binding behavior of bryostatins and their functional activity is an important step toward the development of truly simple, clinically superior agents based on the bryostatin backbone.

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