Cancer Chemother Pharmacol (1992) 30: 401–406

Cancer Chemotherapy and Pharmacology

In vitro screening of crude extracts and pure metabolites obtained from marine invertebrates for the treatment of breast cancer*

John Stingl, Raymond J. Andersen, and Joanne T. Emerman

Departments of Anatomy (JS, JTE) and Chemistry (RJA), University of British Columbia, Vancouver, B. C., Canada

Received 15 November 1991/Accepted 2 March 1992

Summary. A total of 15 samples (crude extracts and pure secondary metabolites) obtained from marine invertebrates collected from the offshore waters of British Columbia, Papua New Guinea, and Sri Lanka have previously been shown to exert cytotoxic activity in the in vitro L1210 leukemic bioassay. We screened these metabolites for in vitro cytotoxic activity against the drug-sensitive breast-tumor cell lines MCF-7, T-47D, ZR-75-1, and MDA-MB-231; the multidrug-resistant and P-glycoprotein (Pgp)-positive breast-tumor cell lines MCF-7 Ad^r and MDA-$A1^r$; and normal and malignant human breast epithelial cells (HBEC) in primary culture. Eight samples exhibited significant [drug concentration resulting in a 50% decrease in cell growth as compared with controls (ED_{50}), <25 μg/ml] dose-dependent cytotoxicity against the drug-sensitive cell lines; the ED_{50} values were as low as 0.004 μg/ml. Five of the eight samples exhibited significant cytotoxicity against the multidrug-resistant cell lines; the ED_{50} values were as low as 0.0006 μg/ml. Incubation of MCF-7 Ad^r cells with varying concentrations of compounds in the presence of Adriamycin demonstrated that none of the compounds tested interfered with Pgp function. Results obtained using HBEC in primary culture showed a wide range of chemosensitivities for a given drug against tissue taken from different patients, demonstrating the uniqueness of the response of different individuals to chemotherapy.

Introduction

Currently, approximately 40 drugs are available for the treatment of cancer [24]. Combination chemotherapy for the treatment of breast cancer achieves response rates in the 50%–70% range, but complete remissions occur in only a small number of patients [15]. As a result, the development of new chemotherapeutic agents is critical.

Previous studies have shown that marine organisms represent a vast reservoir of new pharmacological agents with potentially unique modes of action [12, 20]. For example, the development of cytarabine, a widely used antileukemic chemotherapeutic agent, was based on leads provided by the isolation of 1-β-arabinofuranosyl derivatives of uracil from the Caribbean sponge *Cryptotethya crypta* [1, 14, 27]. Didemnin-B, a metabolite of the tunicate *Tridemnin solidum*, has entered phase II human cancer trials [25]. The bryostatins, a family of metabolites isolated from the bryozoan *Bugula neritina*, will soon be undergoing clincial trials at the National Cancer Institute (NCI) [25]. Our laboratory has evaluated seven crude extracts and eight pure secondary metabolites derived from marine organisms for their potential in the treatment of breast cancer. We have previously shown that these compounds are cytotoxic in the in vitro L1210 leukemic bioassay.

Past drug-screening programs have relied on the in vivo L1210 and P388 leukemic bioassays as primary screens, and as a result, a large number of drugs effective in the treatment of leukemias have been developed. However, relatively few drugs effective against solid tumors have resulted from this approach [4]. Therefore, we initially screened these compounds against four drug-sensitive breast-tumor cell lines. In addition, these compounds were screened against the two multidrug-resistant (MDR) cell lines MCF-7 Ad^r and MDA-$A1^r$ [11, 23]. These MDR cell lines are resistant to a number of drugs that are structurally and functionally unrelated. A major mechanism of MDR is associated with increased levels of the plasma membrane protein P-glycoprotein (Pgp) [13, 26]. Pgp functions as a pump that actively transports drugs out of the cell, thus resulting in a net lower intracellular drug concentration. Both the MCF-7 Ad^r and the MDA-$A1^r$ cell lines exhibit Pgp amplification (60-fold and 10- to 30-fold, respectively), increased Pgp mRNA expression, and decreased Adriamycin uptake [11, 23].

* This work was supported by grants from the National Cancer Institute of Canada and the B. C. Health Research Foundation, and by funds donated by Ms. J. Harowitz

Offprint requests to: J. Emerman, Department of Anatomy, University of British Columbia, 2177 Wesbrook Mall, Vancouver, B. C. V6T 1Z3, Canada

The MDR cell lines were included in the present drug-screening protocol to identify new drugs that might circumvent the MDR phenotype. Unfortunately, cell lines represent a very homogeneous and highly selected population of cells and thus do not represent the heterogeneous population seen in naturally occurring tumors. Therefore, we tested the cytotoxicity of these compounds against a panel of cell lines. In addition, we assayed two of the most cytotoxic compounds from the cell-line screenings against normal and malignant human breast epithelial cells (HBEC) in primary culture, which more closely resemble the counterpart cells in vivo. Normal HBEC were included in the screening protocol to detect tumor-specific agents. It has been shown that the selective activity of a chemotherapeutic drug against cancer cells in vitro relative to normal cells does result in antitumor activity in vivo [21].

Materials and methods

Isolation and purification of compounds. The procedures used for the isolation and purification of the geodiamolides [6], bromotopsentin [18], xestospongin E [16], bastidin-4 [17], imbricatine [2], chromodorolide A [7], jaspamide [5], glaciasterol A [22], and the *Garvea* extract [10] have previously been described. The crude extracts of the other organisms were prepared by homogenizing the organisms in methanol, filtering, and evaporating the methanol in vacuo.

Cell culture. The MCF-7, T-47D, ZR-75-1, and MDA-MB-231 cell lines, which originated from effusions from women with metastatic breast cancer, were obtained from the American Type Culture Collection (ATCC). The MDA-A1^r cell line was furnished by Dr. W. McGuire at the University of Texas, and the MCF-7 Ad^r cell line was obtained from Dr. K. H. Cowan at the National Institutes of Health (NIH). All cell lines were maintained in Ham's F12/Dulbecco's modified Eagle's medium [DME; 1:1 (v/v); Terry Fox Laboratory, Vancouver, B. C.) supplemented with 10 mM HEPES (H) and either 10% fetal bovine serum (FBS; Gibco Laboratories, Grand Island, N. Y.; for MDA-MB-231), 5% FBS and 5 μg insulin/ml (Sigma Chemical Co., St. Louis, Mo.; for MCF-7, T-47D, and ZR-75-1), 10% FBS and 1 μg Adriamycin/ml (ADR; Adria Laboratories, Mississauga, Ont.; for MDA-A1^r), or 5% FBS, 5 μg insulin/ml, and 0.5 μg ADR/ml (MCF-7 Ad^r).

Cultures were maintained in a humidified atmosphere consisting of 5% CO_2 and 95% air at 37° C and were subcultured weekly by treatment with saline-trypsin-versene (MCF-7, T-47D, ZR-75-1, MDA-MB-231, and MCF-7 Ad^r) or with calcium and magnesium-free phosphate-buffered saline (PBS) supplemented with 1 mM ethylenediaminetetraacetic acid (EDTA; MDA-A1^r). At the time of the experiment the cells were resuspended, and viable cells, determined by trypan-blue exclusion and counted on a hemacytometer, were seeded at 4×10^3 cells/well onto 96-well microtiter plates (Falcon, Becton Dickinson Co., N. J.) in 100 μl F12/DME/H supplemented with either 5% FBS and 5 μg insulin/ml (MCF-7, T-47D, ZR-75-1, and MCF-7 Ad^r) or 10% FBS (MDA-MB-231 and MDA-A1^r).

Normal tissue cells obtained from breast reductions and malignant tissue obtained from biopsies and mastectomies were prepared as described elsewhere [9]. Tissues were minced and dissociated in F12/DME/H supplemented with 2% bovine serum albumin (Gibco), 5 μg insulin/ml, 300 U collagenase/ml, and 100 U hyaluronidase/ml (Sigma) at 37° C for 18 h. The epithelial cell pellet was collected by centrifuging the cell suspension at 80 *g* for 4 min. The pellet was washed twice with F12/DME/H and viable cells were counted. Cells were resuspended and seeded at 1×10^4 cells/well onto collagen-coated 96-well microtiter plates in 100 μl F12/DME/H, 5 μg insulin/ml and 5% patients' sera.

Serum samples were collected in the mornings from patients who had fasted over the previous 8–12 h. Blood was received in nonheparinized tubes, incubated for 30 min at 37° C, and centrifuged at 100 *g*, and the serum was collected. If not used immediately, the serum was stored at –20° C.

Compound-testing protocol. On day 0, the cells were seeded onto the microtiter plates as described above. After 24 h, the medium was removed and 100 μl fresh medium containing serial dilutions of the agents to be tested was added to the wells. Cultures were incubated with the drugs for 48 h. The cells were then washed and cultured in drug-free medium for 48 h. A 48-h drug-incubation period was selected to ensure that all cells passed through at least one cell cycle, thus allowing phase-specific and cell-cycle-specific agents to exert their effects. The cell-cycle times of MCF-7, T-47D, ZR-75-1, MDA-MB-231, MCF-7 Ad^r, and MDA-A1^r cells are 30, 22, 44, 21, 23, and 18 h, respectively. A 48-h drug recovery period was selected to ensure that cytostatic agents could be distinguished from cytotoxic agents. After the recovery period, the cells were quantified by the tetrazolium-dye reduction (MTT) assay [3, 19].

In some experiments performed to detect agents that might circumvent the MDR phenotype, MCF-7 Ad^r cells were incubated in the concentration of Adriamycin that results in a 50% decrease in cell growth relative to controls for this line (ED_{50}, 5 μg/ml) as well as in different concentrations of the drug to be tested during the incubation period.

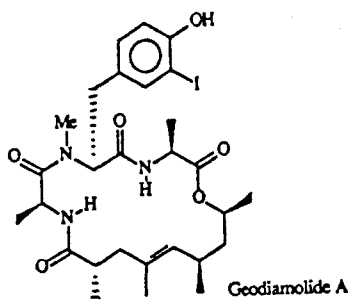
MTT assay. The medium was removed and 100 μl MTT (1 mg/ml, Sigma) dissolved in phenol-red-free F12/DME/H (Sigma) was added to each well. Following a 5-h incubation period at 37° C, 100 μl 20% formol in saline was added to each well and left for 30 min at room temperature to fix the cells to the plate. The formol-saline-MTT solution was then gently removed with a multichannel pipette (Titertek/ICN Flow, Costa Mesa, Calif.), and 100 μl isopropanol was added to each well to dissolve the formazan crystals. After 1 h, the plates were read on a 96-well microplate reader (model EL 311, Biotek Instruments Inc., Winooski, Vermont) at 540 nm. Absorbance values were converted to cell numbers via standard curves specific for each cell line. Cytotoxicity was determined by comparing drug-treated cultures with control cultures (which are assigned a value of 100%). The ED_{50} value was then determined from the dose-response curve.

Results

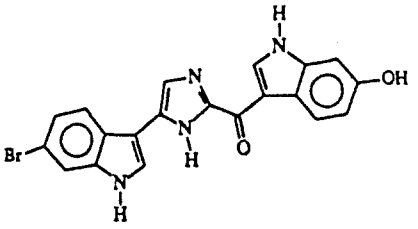
Compounds screened against drug-sensitive cell lines

Figure 1 illustrates the wide range of chemical structures of all of the pure samples tested in our in vitro screening protocol. Table 1 summarizes the ED_{50} values obtained for compounds screened against the MCF-7, T-47D, ZR-75-1, and MDA-MB-231 cell lines. Of the 15 samples screened, 8 demonstrated significant cytotoxicity (ED_{50}, <25 μg/ml) against either the MCF-7 or the T-47D cell line. Only these 8 samples were screened against ZR-75-1 and MDA-MB-231 cells; all 8 compounds were significantly cytotoxic against the former cell line, whereas 6 were significantly cytotoxic against the latter.

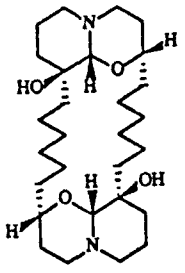
The ED_{50} values for Adriamycin were determined in all of these cell lines to provide a standard with which the samples could be compared. The ED_{50} values recorded for two pure samples, the geodiamolides and jaspamide, were comparable with or lower than those noted for Adriamycin. The values obtained for another sample, QCI-32, which is a crude extract, were 12-, 1.4-, 18-, and 0.47-fold those found for Adriamycin in screening against the MCF-7, T-47D, ZR-75-1, and MDA-MB-231 cell lines, respectively. ED_{50} values as low as 0.6 μg/ml were noted for the crude extract PNG-31A in the T-47D cell line; this value is



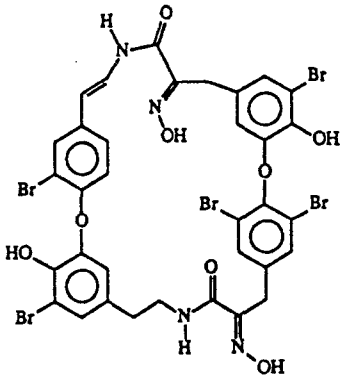
Geodiamolide A



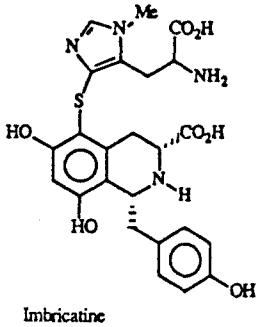
Bromotopsentin



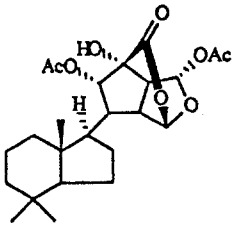
Xestospongin E



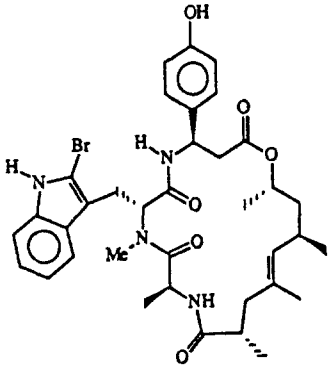
Bastidin-4



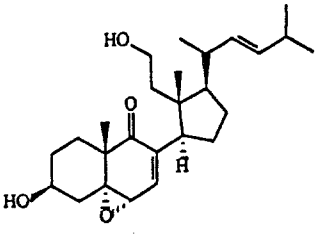
Imbricatine



Chromodorolide A



Jaspamide



Glaciasterol A

Fig. 1. Structures of pure metabolites isolated from marine organisms

10-fold that determined for Adriamycin. Values recorded for the five remaining compounds that exhibited significant cytotoxicity ranged from 0.58 to 33 μg/ml and were at least 9-fold those found for Adriamycin.

Compounds screened against drug-resistant cell lines

Table 2 summarizes the ED_{50} values obtained for the samples and for Adriamycin during screening against the Pgp-positive cell lines MCF-7 Ad^r and MDA-$A1^r$. Of the eight samples screened, six demonstrated significant cytotoxicity against either the MCF-7 Ad^r or the MDA-$A1^r$ cell

Table 1. ED_{50} values obtained for compounds screened against the drug-sensitive breast-cancer cell lines MCF-7, T-47D, ZR-75-1 and MDA-MB-231

Agent	ED_{50} values (μg/ml)			
	MCF-7	T-47D	ZR-75-1	MDA-MB-231
Geodiamolides	0.0068 ± .0003	0.044 ± .003	0.033 ± .024	0.004
Xestospongin E	>>50	>50	ND	ND
Bromotopsentin	>>100	32 ± 5	ND	ND
PNG-31A[a]	5.4 ± .5	0.6	7	24 ± 1
Bastidin-4	6.0 ± .6	0.58 ± .05	2.9 ± .1	2.6 ± .1
Imbricatine	>50	23 ± 9	ND	ND
Chromodorolide A	>>100	>>50	ND	ND
Garvea[a]	33 ± 9	19 ± 3	21 ± 1	23 ± 1
QCI-32[a]	1.2 ± .4	0.09 ± .02	0.09 ± .01	0.018 ± .001
QCI 4-16-89[a]	>100	50 ± 22	ND	ND
PNG-137A[a]	30 ± 3	13 ± 3	16 ± 3	>50
PNG-137B[a]	98 ± 2	35 ± 12	ND	ND
QCI-117[a]	>>100	>>100	ND	ND
Glaciasterol A	19 ± 1	21 ± 3	26 ± 1	>>50
Jaspamide	0.027 ± .004	0.019 ± .009	0.042 ± .002	0.024 ± .001
Adriamycin	0.10 ± .01	0.065 ± .009	0.005 ± .001	0.038 ± .006

Data represent mean values ± SEM. Only the compounds that exhibited significant cytotoxicity towards the MCF-7 and T-47D cell lines were screened against the ZR-75-1 and MDA-MB-231 cell lines. ND, Not done

[a] Crude extract

Table 2. ED_{50} values obtained for compounds screened against the multidrug-resistant breast-cancer cell lines MCF-7 Ad^r and MDA-$A1^r$

Agent	ED_{50} values (μg/ml)	
	MCF-7 Ad^r	MDA-$A1^r$
Geodiamolides	0.092 ± .015 (14)	0.0006 ± .0001 (0.15)
PNG-31A[a]	51 ± 2 (9.4)	41 ± 4 (1.7)
Bastidin-4	>>20 (>3.3)	>100 (>38)
Garvea[a]	27 ± 4 (0.82)	25 ± 3 (1.1)
QCI-32[a]	10 ± 4 (8.3)	1.3 ± .4 (72)
PNG-137A[a]	>50 (>1.7)	25 ± 2 (<0.5)
Glaciasterol A	18 ± 1 (0.95)	19 ± 2 (<0.38)
Jaspamide	0.77 ± .02 (29)	0.022 ± .005 (0.92)
Adriamycin	5 ± 2 (50)	7 ± 4 (184)

Data represent mean values ± SEM for 2–4 experiments. Values in parentheses indicate the magnitude of increase in resistance noted for the MDR daughter cell lines as compared with the corresponding drug-sensitive parent lines (ED_{50} of the resistant daughter cell line/ED_{50} of the sensitive parent line)

[a] Crude extract

line. MCF-7 Ad^r cells were 50 times more resistant to Adriamycin than was the parent MCF-7 cell line, whereas MDA-$A1^r$ cells were 184 times more resistant than was the parent line MDA-MB-231. Table 2 also compares the ED_{50} values found for compounds screened against the MDR cell lines MCF-7 Ad^r and MDA-$A1^r$ with those obtained using the drug-sensitive parent cell lines (ED_{50} drug-resistant/ED_{50} drug-sensitive).

Compounds screened against MCF-7 Ad^r cells in the presence of Adriamycin

To determine whether compounds whose ED_{50} ratios were less than or equal to 1 might circumvent the MDR phenotype by interfering with Pgp, MCF-7 Ad^r cells were incubated in the presence of the ED_{50} concentration (5 μg/ml) of Adriamycin and in varying concentrations of test compounds. Additive cell cytotoxicity was observed between Adriamycin and all compounds tested (results not shown). However, no synergy was observed between any of the samples and Adriamycin, suggesting that these compounds do not interfere with Pgp function.

Compounds screened against normal and malignant HBEC in primary culture

Table 3 summarizes the cytotoxicities of the geodiamolides and jaspamide as tested against malignant and normal HBEC in primary culture. No significant trend indicating tumor specificity was observed for either of these agents. However, broad variation was seen in the cytotoxicity of a given agent for different tissue samples: the ED_{50} values recorded for the geodiamolides ranged from 0.018 to 5.5 μg/ml and from 0.21 to >10 μg/ml in malignant and normal tissue, respectively, whereas the corresponding values determined for jaspamide ranged from 0.0005 to 3.8 μg/ml and from <0.05 to 0.5 μg/ml. Adriamycin ED_{50} values were included for reference in cases in which sufficient tissue was available.

Discussion

Past drug-screening programs have illustrated that the incorporation of solid tumors into a drug-screening protocol is essential, since agents that are effective in treating leukemias are not necessarily effective against solid tumors [4]. The crude extracts and pure metabolites evaluated herein had previously been screened and found to be cytotoxic in vitro in the leukemic L1210 bioassay.

Table 3. ED_{50} values obtained for the geodiamolides and jaspamide tested against normal and malignant breast epithelial cells in primary culture

Agent	ED_{50} values (μg/ml)							
	N1	N2	N3	Ca1	Ca2	Ca3	Ca4	Ca5
Geodiamolides	0.21	>10	>6	0.1	0.018	5.5	0.5	ND
PNG-73	<0.05	0.08	0.5	ND	ND	ND	0.0005	3.8
Adriamycin	ND	0.25	0.29	ND	ND	ND	ND	ND

N, Normal cells; Ca, malignant cells; ND, not done

In the present study, mammary carcinoma was used as a solid-tumor model. Breast-cancer cell lines were selected for initial screening because the cells are plentiful and easy to grow. However, cell lines result from cell selection and the populations are very homogeneous in their characteristics. Therefore, we used four drug-sensitive and two drug-resistant breast-tumor cell lines to represent the heterogeneity seen in naturally occurring breast tumors. In addition, primary cultures of epithelial cells from malignant breast tissue were incorporated into the screening protocol because they more closely resemble the counterpart cells in vivo. Epithelial cells from normal tissue were also included to determine the tumor specificity of these agents. As our results illustrate, there was broad variation in the biological activity of cytotoxic compounds tested on different cell lines and on different primary cultures.

Of the 15 compounds tested against the drug-sensitive cell lines MCF-7 and T-47D, 8 demonstrated significant activity. Agents with an ED_{50} value of up to 25 μg/ml were selected for further testing against the ZR-75-1 and MDA-MB-231 cell lines and the drug-resistant cell lines. We selected 25 μg/ml as our cutoff value because some of the compounds tested were crude extracts, and an excessively low cutoff point might have resulted in the exclusion of potentially toxic compounds from further testing. In addition, some of the compounds with ED_{50} values of around 25 μg/ml exhibited low in vivo host toxicity (unpublished results).

The geodiamolides (a mixture of two cyclodepsipeptides that are identical except that one is brominated and the other is iodinated) and jaspamide are very promising compounds, since they exhibited cytotoxicity comparable with that of Adriamycin when tested against the drug-sensitive cell lines and were several orders of magnitude more cytotoxic than Adriamycin when screened against the MDR cell lines. The observation that compounds such as the geodiamolides were much less cytotoxic against MCF-7 Ad^r cells as compared with the parent line MCF-7, whereas they were more cytotoxic against MDA-$A1^r$ cells than against the parent line MDA-MB-231, suggests either that cellular mechanisms in the MDA-$A1^r$ cells may enable the drugs to overcome the effects of Pgp overexpression or that these drugs are simply not affected by this efflux pump. Our failure to observe synergy between any of the samples and Adriamycin indicates that these drugs do not interfere with Pgp function. These compounds were also very cytotoxic against some of the malignant and normal HBEC in primary culture. The four crude extracts PNG-31A, BC-3, QCI-32, and PNG-137A also warrant further investigation since they demonstrated significant cytotoxicity in the preliminary cell-line screens; their full potential may not be realized until the pure active metabolites have been isolated.

For the drugs that demonstrated in vitro activity, we will determine the therapeutic index (median lethal dose/median effective dose) using mice bearing the transplantable Shionogi mammary carcinoma from our colony [7]. As the structures of the pure samples illustrate (Fig. 1), marine organisms represent a vast reservoir of new and interesting chemical families that potentially provide novel modes of action, better therapeutic indices, and fewer side effects and are effective against currently untreatable solid tumors.

Acknowledgements. The authors thank Ms. D. Wilkinson for her excellent technical assistance.

References

1. Bergmann W, Feeney RJ (1951) Contributions to the study of marine products. The nucleosides of sponges. J Org Chem 16: 981
2. Burgoyne DL, Miao S, Pathirana C, Andersen RJ, Ayer WA, Singer PP, Kokke WCMC, Ross DM (1991) The structure and partial synthesis of imbricatine, a benzyltetrahydroisoquinoline alkaloid from the starfish *Dermasterias imbricata*. Can J Chem 69: 20
3. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB (1987) Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res 47: 936
4. Chabner BA (1990) Screening methods in antineoplastic drug discovery. J Natl Cancer Inst 82: 1082
5. Crews P, Manes LV, Boehler M (1986) Jaspalkinotide, a cyclodepsipeptide from the marine sponge *Jaspis sp.* Tetrahedron Lett 27: 2797
6. de Silva ED, Andersen RJ, Allen TM (1990) Geodiamolides C to F, new cytotoxic cyclodepsipeptides from the marine sponge *Pseudaxinyssa* sp. Tetrahedron Lett 31: 489
7. Dumdei EJ, Silva ED de, Andersen RF, Iqbal Choudry M, Clardy J (1989) Chromodorolide A, a rearranged diterpene with a new carbon skeleton from the Indian Ocean nudibranch *Chromodoris cavae*. J Am Chem Soc 111: 2712
8. Emerman JT, Siemiatkowski J (1984) Effects of endocrine regulation of growth of a mouse mammary tumor on its sensitivity to chemotherapy. Cancer Res 44: 1327
9. Emerman JT, Fiedler EE, Tolcher AW, Rebbeck PM (1987) Effects of defined medium, fetal bovine serum, and human serum on growth and chemosensitivities of human breast cancer cells in primary culture: inference for in vitro assays. In Vitro Cell Dev Biol 23: 134
10. Fahy E, Andersen RJ (1987) Minor metabolites of the marine hydroid *Garvea annulata*. Can J Chem 65: 376

11. Fairchild CR, Ivy SP, Kao-Shan CS, Whang-Peng J, Rosen N, Isreal MA, Melera PW, Cowan CH, Goldsmith ME (1987) Isolation of amplified and overexpressed DNA sequences from Adriamycin-resistant human breast cancer cells. Cancer Res 47: 5141
12. Fautin DG (1988) In: Fautin DG, Biomedical importance of marine organisms. California Academy of Sciences, San Francisco, p vii
13. Juranka PF, Zastawny RL, Ling V (1989) P-glycoprotein: multidrug resistance and a superfamily of membrane-associated transport proteins. FASEB J 3: 2583
14. Lee WW, Benitez A, Goodman L, Baker BR (1960) Potential anticancer agents. Synthesis of the B-anomer of 9-(D-arabinofuranosyl)-adenine. J Am Chem Soc 82: 2648
15. Lippman ME (1987) Hormone stimulation and chemotherapy for breast cancer. J Clin Oncol 5: 331
16. Miao S (1991) Novel secondary metabolites from selected marine invertebrates. PhD Thesis, University of British Columbia
17. Miao S, Andersen RJ, Allen TM (1990) Cytotoxic metabolites from the sponge *Ianthella Basta* collected in Papua New Guinea. J Nat Prod 53: 1441
18. Morris SA, Andersen RJ (1989) Nitrogenous metabolites from the deep water sponge *Hexadella* sp. Tetrahedron Lett 31: 489
19. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55
20. Munro MGH, Luibrand RT, Blunt JW (1987) The search for antiviral and anticancer compounds from marine organisms. In: Scheuer PJ, Bioorganic marine chemistry. Springer, Berlin Heidelberg New York, p 93
21. Phillips RM, Bibby MC, Double JA (1990) A critical appraisal of the predictive value of in vitro chemosensitivity assays. J Natl Cancer Inst 82: 1457
22. Pika J, Tischler M, Andersen RJ (1992) Claciasterols A and B, 9,11-secosteroids from the marine sponge *Aplysilla glacialis*. Can J Chem (in press)
23. Schneider SL, Fuqua SAW, Speeg CV, Tandon A, McGuire W (1990) Isolation and characterization of an Adriamycin-resistant breast tumor cell line. In vitro Cellular & Developmental Biology 26: 621
24. Sikic BI (1991) Anticancer drug discovery. J Natl cancer Inst 83: 738
25. Suffness M, Thompson JE (1988) In: Fautin DG, Biomedical importance of marine organisms. California Academy of Sciences, San Francisco, p 151
26. Van der Bliek AM, Baas F, Van der Velde-Koerts T, Biedler JL, Meyers MB, Ozols RF, Hamilton TC, Joenje H, Borst P (1988) Genes amplified and overexpressed in human multidrug-resistant cell lines. Cancer Res 48: 5927
27. Whitley RJ, Soong SJ, Dolin R, Galasso G, Ch'ien LT, Alford CA, and the Collaborative Study Group (1977) Adenine arabinoside therapy of biopsy-proved herpes simplex encephalitis. N Engl J Med 297: 289