Efficacy of Selected Natural Products as Therapeutic Agents against Cancer[⊥]

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With emerging sophistication in the exploration of ocean environment, a number of marine bioactive products have been identified with promising anticancer activity. Many of these are in active phase I or phase II clinical trials or have been terminated because of adverse side effects, mainly hematological in nature. Nonetheless, the information derived has aided enormously in providing leads for laboratory synthesis with modifications in the parent structure affecting compound solubility, absorption, and toxicity, resulting in less severe toxicity while achieving maximum efficacy in smaller doses. We describe herein, a few of the compounds obtained from marine and terrestrial sources [bryostatin 1 (**1**), dolastatin 10 (**2**), auristatin PE (**3**), and combretastatin A4 (**4**)] that have been extensively investigated in our laboratory and continue to be investigated for their sensitization effects with other cytotoxic agents in several different site-specific tumors employing murine models or human subjects.

Introduction

Nature abounds with a rich potential heritage of therapeutic resource that has been exploited for effective and beneficial use against many human cancers, either in prevention strategy or as therapeutic armamentaria to kill tumor cells. Many of these naturally occurring nondietary, non-nutritive compounds have evolved to counteract natural predators and for self-defense. Some of these agents are derived from terrestrial plants, whereas others are obtained from microorganisms, marine organisms, and animals.[1,2] Despite the availability and synthesis of a wide spectrum of agents derived from knowledge-based high-throughput combinatorial chemistry, limited effects were observed when translated into the clinics. As part of the National Cancer Institute's Natural Products Program, a number of novel agents with antitumor activity against solid tumors derived from marine resources have been identified. Many of these agents derived from natural products including marine organisms have entered into phase I and II clinical trials, particularly for use in combination chemotherapy.[3,4] Herein we present a concise review of preclinical and published clinical trials that have emerged from the lead author's own laboratory [bryostatin 1 (**1**), dolastatin 10 (**2**), auristatin-PE (**3**), combretastatin A4 phosphate (**4**)] and from elsewhere, which attests to the efficacy of marine- and terrestrial-derived natural products in anticancer therapy and has opened new avenues for further investigations of their efficacy and development against human cancer in phase I and II trials. Figure 1 depicts the chemical structures of these compounds as described in the text.

Bryostatin 1

Bryostatin 1 (**1**) is a member of a novel group of macrocyclic lactones, the bryostatins, isolated from the marine invertebrate *Bugula neritina*, often found as moss-like colonies attached as a foulant to structures such as dock sides, pilings, buoys floats, and vessel hulls, as well as on rocks and shells, and sometimes on seaweeds, mangrove roots, seagrasses, and algae in many parts of the world.[5] Bryostatin 1 (**1**) has been endowed with an important pharmacological property of being a potent inhibitor of protein kinase C (PKC), which is involved in the phosphorylation of serine and threonine residues, and actually counteracts tumor promotion induced by phorbol esters.[6] In addition, it has other coveted multifaceted activities including potentiation of the body's own natural cancer fighting weapons such as interferons, interleukin 2, and killer T cells, growth inhibition, and induction of differentiation, and exhibits significant antitumor activity in preclinical models against a wide spectrum of cancer cell lines. In addition, bryostatin 1 (**1**) has been shown to synergize the antitumor effects of various chemotherapeutic agents, such as cytosine arabinoside, gemcitabine, vincristine, cisplatin, and paclitaxel.[7–9] Bryostatin 1 (**1**) has also been associated with a novel mechanism that inhibits the production of components of the matrix metalloproteinases family, down-regulation of multidrug-resistance 1 (MDR1) gene expression, and also modulation of Bcl-2 and p53 gene expression in favor of apoptosis.[10]

A number of studies reported from the lead author's laboratory have significantly contributed to the understanding of the role of bryostatin 1 in hematological malignancies. Notable among these were the initial findings that bryostatin 1 (**1**) is capable of inducing differentiation in the acute lymphoblastic leukemia cell line-Reh, to a monocytoid B cell stage.[11] Similar findings were observed in chronic lymphocytic leukemia (CLL) cells as deduced from significantly increased coexpression of two hairy cell associated surface antigens, CD22 and CD11c, by flow cytometry indicative of differentiation of CLL cells to a hairy cell stage.[12] Further investigations revealed that bryostatin 1 (**1**) induces ubiquitin COOH-terminal hydroxylase and fyn kinase; these molecular alterations contribute at a cellular level to bryostatin 1 (**1**)-induced differentiation.[13,14] Other mechanism-based contributions from our laboratory have shown that bryostatin 1 (**1**) down-regulates Bcl-2 expression through enhanced Bcl-2 protein degradation, leading to the activation of the ubiquitin-proteosome pathway and decreased Bcl-2 mRNA.[15] Additionally, we also reported that modulation of the Bax:Bcl-2 ratio by bryostatin 1 (**1**) was important for susceptibility to drug-induced apoptosis.[16] As a further corollary to laboratory studies, phase I and II studies were conducted in relapsed non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL) to study the maximum tolerated dose, major toxicities, and possible antitumor activity of bryostatin 1 (**1**).[17,18] Generalized myalgia was concluded as the dose-limiting toxicity (DLT) in phase I studies, whereas corroborating with our previous in vitro findings, bryostatin 1 (**1**) was found to be effective in inducing differentiation of CLL, to a hairy cell phenotype.[18] Subsequently, several preclinical studies done in our laboratory using mammary and pancreatic tumor xenografts, as well as orthotopic implantation, sc

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tumors of human CLL, and diffuse large cell lymphoma in SCID mouse xenografts, led to an improved understanding of the action of bryostatin 1 (**1**) at the molecular level as well as the sequence of treatment to obtain maximum benefits of synergy following the use of two agents for therapy.[19–23] One such study reported from our laboratory, involving WSU-CLL-bearing SCID mice, showed that the anticancer activity of bryostatin 1 (**1**) is highly dose- and schedule-specific.[19] The best results were obtained when 2-chlorodeoxyadenosine was given at 30 mg/kg/injection/day after bryostatin 1 (**1**) at 5 μg/kg/injection/day. Reversal of administration or 2-chloro- deoxyadenosine alone was not active at all. Bryostatin 1 (**1**) on its own was active, but not as good as the combination. The only reported cure of WSU-CLL-bearing SCID mice (5/5) was achieved with auristatin PE (**2**) (1.5 mg/kg iv) followed by bryostatin 1 (**1**) (75 μg/kg ip) every second day, repeated three times.[19]

Phase I trials using different infusion schedules of bryostatin 1 (**1**) have been reported.[24,25] However, based on conflicting results of objective responses observed in phase I studies in a broad spectrum of tumors, a number of phase II studies of bryostatin 1 (**1**) using various infusion regimens were conducted in both solid and hematological malignancies.[17,25–28] To date, phase II studies of bryostatin 1(**1**) have failed to demonstrate any clinically meaningful activity. Despite the lack of significant clinical benefit observed when used as a single agent, additional upcoming results revealed synergistic action between bryostatin 1 (**1**) and conventional cytotoxic drugs, which triggered several phase I studies of bryostatin 1 (**1**) in combination with cytotoxic agents.[29–32] According to a recently published result by Ajani et al.[31] in a multicenter phase II study of sequential paclitaxel and bryostatin 1 (**1**) (NSC 339555) treatment in patients with untreated, advanced gastric or gastroesophageal junction adenocarcinoma, a superior response rate was observed compared to what would have been expected of paclitaxel as a single agent. Another recently published phase I study conducted by El Rayes et al.[32] involved combination of bryostatin 1 (**1**) and gemcitabine, which seemed to be well-tolerated with limited grade 3 toxicity. Their objective was to determine the recommended dose for phase II trials of bryostatin 1 (**1**), and the authors have concluded that the recommended dose of **1** in combination with full doses of gemcitabine was 35 μg/m^2.

Thus, overall, despite the promising results in preclinical and early clinical studies with bryostatin 1 (**1**), phase II studies have failed to show a significant benefit as a single agent, although some clinical studies are in progress to date. The reasons for this lack of efficacy are unclear, but as suggested by Amador et al.,[3] this might be related to pharmacological factors. Likewise, Jorgensen et al.[33] emphasized the necessity for carefully scheduling drug combinations for efficacious cell killing. Additional studies are warranted to improve the understanding of bryostatin 1 (**1**) pharmacokinetics and pharmacodynamic effects in tumor tissues, which will aid in fully elucidating further development and therapeutic potential of this agent.

Dolastatins

The dolastatins were originally isolated in the 1970s by Pettit et al.[34] from the sea hare, *Dolabella auricularia*, and later identified as secondary potent antimitotic and cytostatic bioactive metabolites resulting from consumption of marine cyanobacteria of the genus *Symploca* as a dietary source. The dolastatins are essentially a class of small oligopeptides (pentapeptides) with four unique amino acid residues besides valine (dolavaline, dolaisoleucine, dolaproline, and dolaphenine). Within this family, the linear peptide dolastatin 10 (**2**) and a seven-unit depsipeptide, dolastatin 15, were chosen for development in view of potent and promising antiproliferative activities at picomolar or low nanomolar concentrations.[1] The structure, isolation, and synthesis of natural (−)-dolastatin 10 (**2**) have been reported.[35,36] Mechanistically, they strongly inhibit microtubule assembly, tubulin-dependent GTP hydrolysis, and the binding of vinca alkaloids to tubulin, causing cell cycle arrest in metaphase.[37,38] This effect was more potent than paclitaxel or vinblastine.[39] In addition to alterations in the molecular dynamics of tubulin assembly, dolastatin 10 (**2**) also exhibits potent pro-apoptotic effects in some drug-resistant cancer cell lines despite being identified as a novel member of the MDR phenotype that confers resistance to cytotoxic chemotherapeutics at least in part, due to overexpression of P-glycoprotein.[40,41]

Mirsalis et al. conducted preclinical toxicity studies of dolastatin 10 (**2**) following a single intravenous bolus dose in order to arrive at a safe starting dose and dose schedule for phase I clinical trials.[42] Dolastatin 10 (**2**) entered phase I clinical trials and successfully progressed to phase II trials. Accordingly, the results from the first phase I clinical trial reported by Pitot et al.[43] recommended a phase II starting dose of 400 μg/m^2 for patients who have undergone either two or fewer prior chemotherapy regimens or 325 μg/m^2 for patients pretreated with more than two prior chemotherapy regimens. Another phase I study, reported by Madden et al. concluded that escalated dolastatin 10 (**2**) dosing with cytokine support is warranted.[44] According to their observation, stabilization of tumor growth was observed in four patients, but no objective responses were seen. Unfortunately, due to some potential pitfalls and accompanying side effects, with ~40% of patients developing moderate peripheral neuropathy, and insignificant activity in patients with hormone refractory metastatic adenocarcinoma and recurrent platinum-sensitive ovarian carcinoma, dolastatin 10 (**2**) was discontinued from clinical trials as a single agent. Further preclinical studies were carried out in the lead author's laboratory to evaluate therapeutic synergism of dolastatin 10 (**2**) alone, and in combination with bryostatin 1 (**1**) on a human diffuse large cell lymphoma line (WSU-DLCL2) in vitro, and in a SCID mouse xenograft model.[45] Although the combinations were highly active, no cure was observed. Nonetheless, its novel mechanism of action, high potency, and positive therapeutic index in preclinical models stimulated the development of a water-soluble analogue, auristatin PE (**3**) (TZT-1027; soblidotin).

Auristatin PE (TZT-1027; Soblidotin)

The auristatins (**3**) are novel synthetic analogues of dolastatin 10 (**2**) and differ structurally by the absence of the thiazole ring from the original dolaphenine residue, resulting in a terminal benzylamine moiety (Figure 1). Functionally, they maintain potent antitumor activity and are associated with less toxicity than their parent compound, making them an ideal substitute for the parental compound. Moreover, in addition to its efficacy in the inhibition and disruption of the microtubule assembly, auristatin PE (**3**) has a dual action in blocking blood supply to tumor vasculature[46–48] and reportedly is less affected by overexpression of any of P-glycoprotein or multidrug resistance associated protein or breast cancer resistance protein.[49] Other cellular mechanistic studies revealed its interaction with microtubule-induced Bcl-2 phosphorylation, leading to apoptosis of tumor cells.[50] Otani et al. evaluated the unique antitumor vascular activity of auristatin-induced tumoral vascular collapse and tumor cell death in an advanced murine colon 26 adenocarcinoma tumor model as well as in cultured human umbilical vein endothelial cells (HUVEC).[47] They reported tolerable doses of TZT-1027 [auristatin PE (**3**)]-induced tumor-selective hemorrhage mainly in the peripheral area of the tumor mass followed by induction of apoptosis of the tumor cells, tumor tissue necrosis, and tumor regression. Thus, TZT-1027 (**3**) combines both a conventional antitumor activity and also distinct antitumor vascular activity to make it a potentially attractive tool for cancer therapy.

Shnyder et al. investigated and compared the efficacy and the mechanism of action of dolastatin 10 (**2**) and auristatin PE (**3**) following intravenous administration of the drugs in subcutaneous

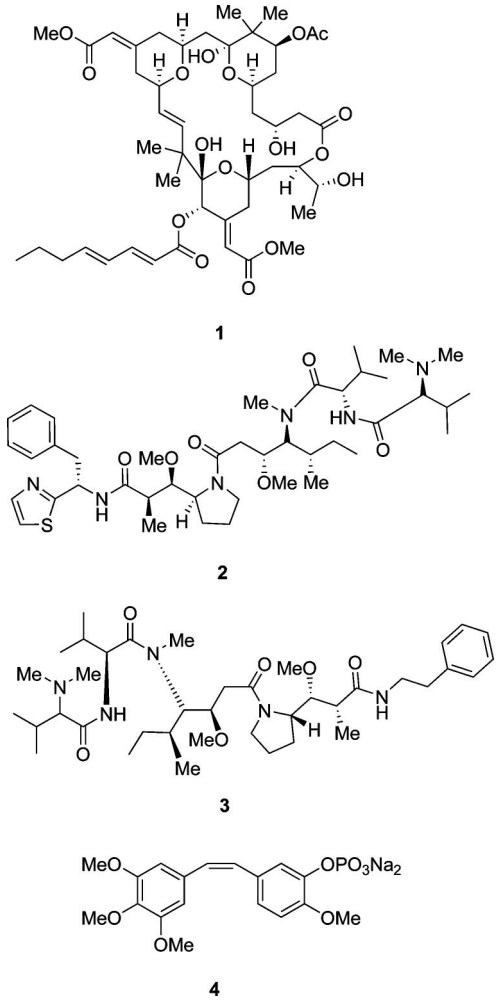

Figure 1. Molecule structure of bryostatin 1, dolastatin 10, auristatin PE, and combretastatin A4 phosphate.

xenografts of two human colon adenocarcinoma models, DLD-1 and COLO 205.[51] Their results showed that auristatin PE (**3**) was significantly more effective in vivo against both tumors than dolastatin 10 (**2**), with antitumor effects mediated through vascular shutdown as envisaged earlier. These results strongly supported that auristatin PE (**3**) has good potential as an anticancer agent in the treatment of colon cancer. The efficacy of auristatin PE (**3**) against Waldenstrom's macroglobulinemia (WM), an uncommon lymphoproliferative disease that remains incurable with current treatment protocols, was evaluated in our laboratory utilizing a WSU-WM SCID mouse xenograft model, as well as investigating the in vivo mechanism of action in this cell line.[52] It was inferred from in vivo results that dolastatin10 (**2**) was inactive, while auristatin PE (**3**) was highly active.[52] Another significant study reported from our laboratory revealed a synergistic interaction between auristatin PE (**3**) and other drugs against human diffuse large cell lymphoma.[45] In a study reported by Watanabe et al. TZT-1027 (**3**) exhibited potent antitumor activities in tumor models in which vincristine and docetaxel failed to show effectiveness.[49,50] Other investigators reported in vitro and in vivo antitumor activity against auristatin PE (**3**)-sensitive MCF-7 and auristatin PE (**3**)-resistant R-27 cells and found remarkable in vivo antitumor activity against R-27 cell-induced tumors.[48] This may be attributed to the effect of auristatin PE (**3**) in selective blockage of tumor blood flow in tumors derived from R-27 cells. Other preclinical studies have shown that TZT-1027 (**3**) could induce apoptosis within 24 h in human leukemia HL-60 cells and other solid tumor cell lines such as human prostate carcinoma cells DU145 and human mammary carcinoma cells MCF-7.[53] TZT-1027 (**3**) showed good antitumor activity against human MX-1 and LX-1 xenografts without causing serious body weight reduction, resulting in tumor regression.[53]

Auristatin PE (**3**) has also been evaluated in four phase I studies (Table 1), and no significant evidence of antitumor activity was noted in patients with advanced tumors.[54–57] Phase II studies have recently started. Greystoke et al. reported the results of their phase I study of intravenous auristatin PE (**3**) administration on day 1 and day 8 of a three-week cycle in combination with carboplatin given on day 1 alone in patients with advanced solid tumors.[56] According to them, the recommended phase II dose for auristatin PE (**3**) was 1.6 mg/m^2 and carboplatin AUC 5. Pharmacokinetic analysis showed no evidence of interaction between these agents. Tamura et al. conducted a phase I study in Japan, and in summarizing their results they concluded that in Japanese patients, the maximal tolerated dose (MTD) was 1.5 mg/m^2, lower than the value of 2.4 mg/m^2 in European patients.[57] Moreover, antitumor activity was observed at low doses, and auristatin PE (**3**) was well-tolerated at the MTD, without grade 3 nonhematological toxicities or neutropenia up to grade 2, suggesting that its further investigation in phase II studies are warranted.

Two recent reports have been published on phase II studies. In one study reported by Riely et al., auristatin PE (**3**) administered weekly to patients with advanced non-small-cell lung cancer following treatment with platinum-based chemotherapy failed to show any anticancer activity.[58] The second study by Patel et al., in patients with advanced or metastatic soft-tissue sarcomas with prior exposure to anthracycline-based chemotherapy, failed to demonstrate any confirmed response following intravenous auristatin PE (**3**) administration.[59]

Combretastatin A4

Isolated from stem wood of the South African tree *Combretum caffrum*, combretastatin A4 (CA4) is a low molecular weight vascular disruptive agent (VDA), a new class of cancer chemotherapy designed to induce rapid and selective vascular shutdown in tumors.[60–63] In essence, following therapy with VDAs, tumor blood vessel growth is inhibited, and tumor cell death and necrosis occurs as a result of prolonged deprivation of oxygen and nutrient supplies, which are pivotal for tumor growth and survival.[64,65] Thus, compared to the antiangiogenic method, the vascular disrupting tactic therefore seems to be cytotoxic rather than cytostatic. The underlying mechanism of action of CA4 in tumor tissue involves disruption of the endothelial cytoskeleton by acting at the colchicine binding site of the subunit of endothelial tubulin, causing depolymerization of microtubules and disorganization of actin and tubulin.[66] This results in conformational changes leading to occlusion of blood vessels and a marked reduction in tumor blood flow. This is reportedly advantageous for the host on the basis of previous information showing that tumor-related endothelial cells are much more sensitive to the activity of tubulin binding agents than normal endothelial cells.[64,65] Because of limited water solubility of combretastatin A4, a water-soluble pro-drug combretastatin A4 phosphate [CA4P; (**4**)] has been synthesized;[67] this compound is cleaved to its natural and active form by endogenous phosphatases.

CA4P (**4**) pro-drug (Oxigene Inc. Boston, MA) has undergone extensive preclinical evaluation in human tumor xenografts and orthotopically transplanted tumors in murine models, demonstrating that the pro-drug CA4P (**4**) causes profound disruption of the tumor blood vessel network at well-tolerated doses.[68–75] A subsequent study by Dark et al. concluded that for VDAs to be most effective, they should be combined with conventional chemotherapeutic agents and/or other treatment options, so that the entire tumor cell population can be completely exterminated.[73] It was later experimentally confirmed that chemotherapy following VDA administration increased the efficacy of antitumor activity of VDAs.[68,76–79]

The effect of CA-4 (**4**) against a panel of malignant human B-lymphoid cell lines [early pre-B acute lymphoblastic leukemia

Table 1. Summary of Clinical Trials with CA4P and Auristatin PE

schedule	toxicity	reference
CA4P		
18–90 mg/m^2 q^3 wks	minimal cumulative side effects; DLTs were tumor pain, acute coronary syndrome, and shortness of breath	Dowlati et al.[84]
5–114 mg/m^2 d1, 7, 14 q28 days[2]	most common toxicity was cardiovascular; DLTs included reversible ataxia, vasovagal syncope, and motor neuropathy	Rustin et al.[82]
d1–5 q^3 wks	bowel ischemia, tumor pain, vagal syncope motor neuropathy, reversible ataxia, cardiac ischemia, dyspnoe	Stevenson et al.[83]
carboplatin with 27–45 mg/m^2 CA4P d1 q21 days	myelosuppression consistent with carboplatin treatment; also fatigue, tumor pain, tingling in the extremities	Bilenker et al.[85]
auristatin PE		
d1, 8 q^3 wks	neutropenia, pain infusion arm, peripheral neuropathy, fatigue, ileus	de Jonge et al.[55]
d1, 8 q^3 w+ carbo AUC4-5		Greystoke et al.[56]
d1, q^3 wks		Schoffski et al.[54]
d1, 8 q^3 wks	no grade 3 nonhematological toxicity or neutropenia up to grade 2	Tamura et al.[57]

(Reh), diffuse large cell lymphoma (WSU-DLCL2), chronic lymphocytic leukemia (WSU-CLL), and Waldenstrom's macroglobulinemia (WSU-WM)] has been evaluated in our laboratory wherein a concentration-dependent growth inhibition in all the tested cell lines was observed.[80] Preliminary studies showed that mitotic catastrophe was the predominant mechanism by which CA-4 (**4**) induced cell death rather than apoptosis. This was later confirmed by another study reported from our laboratory showing that CA4P (**4**) induces mitotic catastrophe and arrest in WSU-CLL cells, mostly in the M phase This, was independent of p53 and independent of chk1 and cdc2 phosphorylation pathways, while apoptosis was a secondary mechanism of death in a small proportion of cells through activation of caspases-9 and PARP cleavage. The two mechanisms of cell death, i.e., mitotic catastrophe and apoptosis, were independent of each other as shown in our model.[81]

Four phase I trials of CA4P (**4**) in humans have been published (Table 1).[82–85] All of these studies used a different dosing schedule (weekly, 3-weekly, and daily for 5 days every 3 weeks) and showed some signs of clinical activity, demonstrating that CA4P (**4**) was safe, well-tolerated, and lacking hematological toxicity. In addition to establishing acceptable doses and treatment schedules, these phase I trials attempted to evaluate the biologic activity of CA4P (**4**) by assessing tumor blood flow using magnetic resonance imaging (MRI) and positron emission tomography (PET), and demonstrated that CA4P (**4**) reduced blood flow in a variety of tumors at doses lower than the MTD. In the phase I study by Stevenson et al. comprising 37 patients, one patient with metastatic sarcoma had a partial response, and 14 patients showed stable disease.[83] A patient with an anaplastic thyroid cancer had a complete response 30 months after treatment.[83]

Additional studies wherein CA4P (**4**) has been administered in combination with carboplatin to patients with advanced cancer have also been reported.[85] The combination was well-tolerated, with confirmed DCE-MRI measurements of the reduction in tumor blood flow 4–6 h after treatment.[85] CA4P (**4**) is currently also being evaluated for the treatment of anaplastic thyroid carcinoma, and in combination with external-beam radiotherapy for the treatment of various other types of malignant disease, and also in combination with a radiolabeled anticarcinoembryonic antigen antibody for the treatment of colon carcinoma.

In conclusion, the reported active agents derived from either marine or terrestrial sources have a unique chemistry that offers us valuable information for their use as lead compounds for further chemical synthesis of more potent chemotherapeutic drugs against a variety of cancers. Finally, the search for more active natural products as therapeutic agents in cancer should continue until a novel and very effective compound is found.

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Note Added after ASAP Publication: The version posted on Feb 27, 2008, contained errors in the structures in Figure 1. The current version shows the correct structures.

References and Notes

(1) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2004**, *67*, 1216–1238.
(2) Schwartsmann, G.; Da Rocha, A. B.; Mattei, J.; Lopes, R. *Expert Opin. Investig. Drugs* **2003**, *12*, 1367–1383.
(3) Amador, M. L.; Jimeno, J.; Paz-Ares, L.; Cortes-Funes, H.; Hidalgo, M. *Ann Oncol.* **2003**, *14*, 1607–1615.
(4) Simmons, T. L.; Andrianasolo, E.; McPhail, K.; Flatt, P.; Gerwick, W. H. *Mol. Cancer Ther.* **2005**, *4*, 333–342.
(5) Pettit, G. R.; Fujii, Y.; Hasler, J. A.; Schmidt, J. M. *J. Nat. Prod.* **1982**, *45*, 272–276.
(6) Watters, D. J.; Parsons, P. G. *Biochem. Pharmacol.* **1999**, *58*, 383–388.
(7) Mohanty, S.; Huang, J.; Basu, A. *Clin. Cancer Res.* **2005**, *11*, 6730–6737.
(8) Beck, F. W.; Eilender, D. S.; Dandashi, M. H.; Siddiq, F.; Snell, D. C.; Godmere, M. A.; Al-Katib, A. M.; Mohammad, R. M. *Int. J. Mol. Med.* **2004**, *14*, 113–119.
(9) Ali, S.; Aranha, O.; Li, Y.; Pettit, G. R.; Sarkar, F. H.; Philip, P. A. *Cancer Chemother Pharmacol.* **2003**, *52*, 235–246.
(10) Maki, A.; Diwakaran, H.; Redman, B.; al-Asfar, S.; Pettit, G. R.; Mohammad, R. M.; al-Katib, A. *Anticancer Drugs* **1995**, *6*, 392–397.
(11) al-Katib, A.; Mohammad, R. M.; Khan, K.; Dan, M. E.; Pettit, G. R.; Sensenbrenner, L. L. *J. Immunother. Emphasis Tumor Immunol.* **1993**, *14*, 33–42.
(12) al-Katib, A.; Mohammad, R. M.; Dan, M.; Hussein, M. E.; Akhtar, A.; Pettit, G. R.; Sensenbrenner, L. L. *Exp. Hematol.* **1993**, *21*, 61–65.
(13) Mohammad, R. M.; Maki, A.; Pettit, G. R.; al-Katib, A. M. *Enzyme Protein* **1996**, *49*, 262–272.
(14) Li, Y.; Mohammad, R. M.; al-Katib, A.; Varterasian, M. L.; Chen, B. *Leuk Res.* **1997**, *21*, 391–397.
(15) Wall, N. R.; Mohammad, R. M.; Reddy, K. B.; Al-Katib, A. M. *Int. J. Mol. Med.* **2000**, *5*, 165–671.
(16) Wall, N. R.; Mohammad, R. M.; Al-Katib, A. M. *Leuk. Res.* **1999**, *23*, 881–888.
(17) Varterasian, M. L.; Mohammad, R. M.; Eilender, D. S.; Hulburd, K.; Rodriguez, D. H.; Pemberton, P. A.; Pluda, J. M.; Dan, M. D.; Pettit, G. R.; Chen, B. D.; Al-Katib, A. M. *J. Clin. Oncol.* **1998**, *16*, 56–62.
(18) Varterasian, M. L.; Mohammad, R. M.; Shurafa, M. S.; Hulburd, K.; Pemberton, P. A.; Rodriguez, D. H.; Spadoni, V.; Eilender, D. S.; Murgo, A.; Wall, N.; Dan, M.; Al-Katib, A. M. *Clin. Cancer Res.* **2000**, *6*, 825–828.
(19) Mohammad, R. M.; Katato, K.; Almatchy, V. P.; Wall, N.; Liu, K. Z.; Schultz, C. P.; Mantsch, H. H.; Varterasian, M.; al-Katib, A. M. *Clin. Cancer Res.* **1998**, *4*, 445–453.
(20) Mohammad, R. M.; Dugan, M. C.; Mohamed, A. N.; Almatchy, V. P.; Flake, T. M.; Dergham, S. T.; Shields, A. F.; Al-Katib, A. A.; Vaitkevicius, V. K.; Sarkar, F. H. *Pancreas* **1998**, *16*, 19–25.
(21) Mohammad, R. M.; Al-Katib, A.; Pettit, G. R.; Vaitkevicius, V. K.; Joshi, U.; Adsay, V.; Majumdar, A. P.; Sarkar, F. H. *Clin. Cancer Res.* **1998**, *4*, 887–894.
(22) Al-Katib, A. M.; Smith, M. R.; Kamanda, W. S.; Pettit, G. R.; Hamdan, M.; Mohamed, A. N.; Chelladurai, B.; Mohammad, R. M. *Clin. Cancer Res.* **1998**, *4*, 1305–1314.
(23) Wang, H.; Mohammad, R. M.; Werdell, J.; Shekhar, P. V. *Int. J. Mol. Med.* **1998**, *1*, 915–923.
(24) Clamp, A.; Jayson, G. C. *Anticancer Drugs* **2002**, *13*, 673–683.

(25) Marshall, J. L.; Bangalore, N.; El-Ashry, D.; Fuxman, Y.; Johnson, M.; Norris, B.; Oberst, M.; Ness, E.; Wojtowicz-Praga, S.; Bhargava, P.; Rizvi, N.; Baidas, S.; Hawkins, M. J. *Cancer Biol Ther.* **2002**, *1*, 409–416.
(26) Philip, P. A.; Rea, D.; Thavasu, P.; Carmichael, J.; Stuart, N. S.; Rockett, H.; Talbot, D. C.; Ganesan, T.; Pettit, G. R.; Balkwill, F.; Harris, A. L. *J Natl. Cancer Inst.* **1993**, *85*, 1812–1818.
(27) Blackhall, F. H.; Ranson, M.; Radford, J. A.; Hancock, B. W.; Soukop, M.; McGown, A. T.; Robbins, A.; Halbert, G.; Jayson, G. C. *Br. J. Cancer* **2001**, *84*, 465–469.
(28) Haas, N. B.; Smith, M.; Lewis, N.; Littman, L.; Yeslow, G.; Joshi, I. D.; Murgo, A.; Bradley, J.; Gordon, R.; Wang, H.; Rogatko, A.; Hudes, G. R. *Clin. Cancer Res.* **2003**, *9*, 109–114.
(29) Dowlati, A.; Lazarus, H. M.; Hartman, P.; Jacobberger, J. W.; Whitacre, C.; Gerson, S. L.; Ksenich, P.; Cooper, B. W.; Frisa, P. S.; Gottlieb, M.; Murgo, A. J.; Remick, S. C. *Clin. Cancer Res.* **2003**, *9*, 5929–5935.
(30) Roberts, J. D.; Smith, M. R.; Feldman, E. J.; Cragg, L.; Millenson, M. M.; Roboz, G. J.; Honeycutt, C.; Thune, R.; Padavic-Shaller, K.; Carter, W. H.; Ramakrishnan, V.; Murgo, A. J.; Grant, S. *Clin. Cancer Res.* **2006**, *12*, 5809–5816.
(31) Ajani, J. A.; Jiang, Y.; Faust, J.; Chang, B. B.; Ho, L.; Yao, J. C.; Rousey, S.; Dakhil, S.; Cherny, R. C.; Craig, C.; Bleyer, A. *Invest. New Drugs* **2006**, *24*, 353–357.
(32) El-Rayes, B. F.; Gadgeel, S.; Shields, A. F.; Manza, S.; Lorusso, P.; Philip, P. A. *Clin. Cancer Res.* **2006**, *12*, 7059–7062.
(33) Jorgensen, H. G.; Allan, E. K.; Mountford, J. C.; Richmond, L.; Harrison, S.; Elliott, M. A.; Holyoake, T. L. *Exp. Hematol.* **2005**, *33*, 1140–1146.
(34) Pettit, G. R.; Kamano, Y.; Fujii, Y.; Herald, C. L.; Inoue, M.; Brown, P.; Gust, D.; Kitahara, K.; Schmidt, J. M.; Doubek, D. L.; Michel, C. *J. Nat. Prod.* **1981**, *44*, 482–485.
(35) Pettit, G. R.; Singh, S. B.; Hogan, F.; Lloyd-Williams, P.; Herald, D. L.; Burkett, D. D.; Clewlow, P. J. *J. Am. Chem. Soc.* **1989**, *111*, 5463–5465.
(36) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tuinman, A. A.; Boettner, F. E.; Kizu, H.; Schmidt, J. M.; Baczynskyj, L.; Tomer, K. B.; Bontems, R. J. *J. Am. Chem. Soc.* **1987**, *109*, 6883–6885.
(37) Mitra, A.; Sept, D. *Biochemistry* **2004**, *43*, 13955–13962.
(38) Bai, R.; Roach, M. C.; Jayaram, S. K.; Barkoczy, J.; Pettit, G. R.; Luduena, R. F.; Hamel, E. *Biochem. Pharmacol.* **1993**, *45*, 1503–1515.
(39) Bai, R. L.; Pettit, G. R.; Hamel, E. *J. Biol. Chem.* **1990**, *265*, 17141–17149.
(40) Aherne, G. W.; Hardcastle, A.; Valenti, M.; Bryant, A.; Rogers, P.; Pettit, G. R.; Srirangam, J. K.; Kelland, L. R. *Cancer Chemother. Pharmacol.* **1996**, *38*, 225–232.
(41) Bai, R.; Friedman, S. J.; Pettit, G. R.; Hamel, E. *Biochem. Pharmacol.* **1992**, *43*, 2637–45.
(42) Mirsalis, J. C.; Schindler-Horvat, J.; Hill, J. R.; Tomaszewski, J. E.; Donohue, S. J.; Tyson, C. A. *Cancer Chemother. Pharmacol.* **1999**, *44*, 395–402.
(43) Pitot, H. C.; McElroyzfn, E. A., Jr.; Reid, J. M.; Windebank, A. J.; Sloan, J. A.; Erlichman, C.; Bagniewski, P. G.; Walker, D. L.; Rubin, J.; Goldberg, R. M.; Adjei, A. A.; Ames, M. M. *Clin. Cancer Res.* **1999**, *5*, 525–351.
(44) Madden, T.; Tran, H. T.; Beck, D.; Huie, R.; Newman, R. A.; Pusztai, L.; Wright, J. J.; Abbruzzese, J. L. *Clin. Cancer Res.* **2000**, *6*, 1293–1301.
(45) Mohammad, R. M.; Pettit, G. R.; Almatchy, V. P.; Wall, N.; Varterasian, M.; Al-Katib, A. *Anticancer Drugs* **1998**, *9*, 149–156.
(46) Natsume, T.; Watanabe, J.; Tamaoki, S.; Fujio, N.; Miyasaka, K.; Kobayashi, M. *J. Cancer Res.* **2000**, *91*, 737–747.
(47) Otani, M.; Natsume, T.; Watanabe, J. I.; Kobayashi, M.; Murakoshi, M.; Mikami, T.; Nakayama, T. *J. Cancer Res.* **2000**, *91*, 837–844.
(48) Hashiguchi, N.; Kubota, T.; Koh, J.; Yamada, Y.; Saikawa, Y.; Otani, Y.; Watanabe, M.; Kumai, K.; Kitajima, M.; Watanabe, J.; Kobayashi, M. *Anticancer Res.* **2004**, *24*, 2201–2208.
(49) Watanabe, J.; Minami, M.; Kobayashi, M. *Anticancer Res.* **2006**, *26*, 1973–1981.
(50) Watanabe, J.; Natsume, T.; Fujio, N.; Miyasaka, K.; Kobayashi, M. *Apoptosis* **2000**, *5*, 345–353.
(51) Shnyder, S. D.; Cooper, P. A.; Millington, N. J.; Pettit, G. R.; Bibby, M. C. *J. Oncol.* **2007**, *31*, 353–360.
(52) Mohammad, R. M.; Limvarapuss, C.; Wall, N. R.; Hamdy, N.; Beck, F. W.; Pettit, G. R.; Al-Katib, A. *Int. J. Oncol.* **1999**, *15*, 367–372.
(53) Kobayashi, M.; Natsume, T.; Watanabe, J.; Fujio, N.; Mikami, T.; Miyasaka, K.; Tsukagoshi, S. *Nippon Yakurigaku Zasshi* **1999**, *114*, 230P–235P.
(54) Schoffski, P.; Thate, B.; Beutel, G.; Bolte, O.; Otto, D.; Hofmann, M.; Ganser, A.; Jenner, A.; Cheverton, P.; Wanders, J.; Oguma, T.; Atsumi, R.; Satomi, M. *Ann Oncol.* **2004**, *15*, 671–679.
(55) de Jonge, M. J.; van der Gaast, A.; Planting, A. S.; van Doorn, L.; Lems, A.; Boot, I.; Wanders, J.; Satomi, M.; Verweij, J. *Clin. Cancer Res.* **2005**, *11*, 3806–3813.
(56) Greystoke, A.; Blagden, S.; Thomas, A. L.; Scott, E.; Attard, G.; Molife, R.; Vidal, L.; Pacey, S.; Sarkar, D.; Jenner, A.; De-Bono, J. S.; Steward, W. *Ann Oncol.* **2006**, *17*, 1313–1319.
(57) Tamura, K.; Nakagawa, K.; Kurata, T.; Satoh, T.; Nogami, T.; Takeda, K.; Mitsuoka, S.; Yoshimura, N.; Kudoh, S.; Negoro, S.; Fukuoka, M. *Cancer Chemother. Pharmacol.* **2007**, *60*, 285–293.
(58) Riely, G. J.; Gadgeel, S.; Rothman, I.; Saidman, B.; Sabbath, K.; Feit, K.; Kris, M. G.; Rizvi, N. A. *Lung Cancer* **2007**, *55*, 181–185.
(59) Patel, S.; Keohan, M. L.; Saif, M. W.; Rushing, D.; Baez, L.; Feit, K.; DeJager, R.; Anderson, S. *Cancer* **2006**, *107*, 2881–2887.
(60) Gaya, A. M.; Rustin, G. J. *Clin Oncol. (R. Coll. Radiol.)* **2005**, *17*, 277–290.
(61) Siemann, D. W.; Chaplin, D. J.; Horsman, M. R. *Cancer* **2004**, *100*, 2491–2499.
(62) Tozer, G. M.; Kanthou, C.; Parkins, C. S.; Hill, S. A. *Int. J. Exp. Pathol.* **2002**, *83*, 21–38.
(63) Hinnen, P.; Eskens, F. A. *Br. J. Cancer* **2007**, *96*, 1159–1165.
(64) Chaplin, D. J.; Dougherty, G. J. *Br. J. Cancer* **1999**, *80* (Suppl 1), 57–64.
(65) Thorpe, P. E. *Clin. Cancer Res.* **2004**, *10*, 415–427.
(66) Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garia-Kendall, D. *Experientia* **1989**, *45*, 205–211.
(67) Pettit, G. R.; Temple, C., Jr.; Narayanan, V. L.; Varma, R.; Simpson, M. J.; Boyd, M. R.; Rener, G. A.; Bansal, N. *Anti-Cancer Drug Des.* **1995**, *10*, 299–309.
(68) Siemann, D. W.; Mercer, E.; Lepler, S.; Rojiani, A. M. *Int. J. Cancer* **2002**, *99*, 1–6.
(69) Galbraith, S. M.; Maxwell, R. J.; Lodge, M. A.; Tozer, G. M.; Wilson, J.; Taylor, N. J.; Stirling, J. J.; Sena, L.; Padhani, A. R.; Rustin, G. J. *J Clin. Oncol.* **2003**, *21*, 2831–2842.
(70) Horsman, M. R.; Murata, R.; Breidahl, T.; Nielsen, F. U.; Maxwell, R. J.; Stodkiled-Jorgensen, H.; Overgaard, J. *Adv. Exp. Med. Biol.* **2000**, *476*, 311–323.
(71) Tozer, G. M.; Prise, V. E.; Wilson, J.; Locke, R. J.; Vojnovic, B.; Stratford, M. R.; Dennis, M. F.; Chaplin, D. J. *Cancer Res.* **1999**, *59*, 1626–1634.
(72) Horsman, M. R.; Ehrnrooth, E.; Ladekarl, M.; Overgaard, J. *Int. J. Radiat. Oncol. Biol. Phys.* **1998**, *42*, 895–898.
(73) Dark, G. G.; Hill, S. A.; Prise, V. E.; Tozer, G. M.; Pettit, G. R.; Chaplin, D. J. *Cancer Res.* **1997**, *57*, 1829–1834.
(74) Li, L.; Rojiani, A.; Siemann, D. W. *Int J. Radiat. Oncol. Biol. Phys.* **1998**, *42*, 899–903.
(75) Grosios, K.; Holwell, S. E.; McGown, A. T.; Pettit, G. R.; Bibby, M. C. *Br. J. Cancer* **1999**, *81*, 1318–1327.
(76) Nelkin, B. D.; Ball, D. W. *Oncol. Rep.* **2001**, *8*, 157–160.
(77) Li, L.; Rojiani, A. M.; Siemann, D. W. *Acta Oncol.* **2002**, *41*, 91–97.
(78) Pedley, R. B.; Hill, S. A.; Boxer, G. M.; Flynn, A. A.; Boden, R.; Watson, R.; Dearling, J.; Chaplin, D. J.; Begent, R. H. *Cancer Res.* **2001**, *61*, 4716–4722.
(79) Yeung, S. C.; She, M.; Yang, H.; Pan, J.; Sun, L.; Chaplin, D. *J. Clin. Endocrinol. Metab.* **2007**, *92*, 2902–2909.
(80) Nabha, S. M.; Wall, N. R.; Mohammad, R. M.; Pettit, G. R.; Al-Katib, A. M. *Anticancer Drugs* **2000**, *11*, 385–392.
(81) Nabha, S. M.; Mohammad, R. M.; Dandashi, M. H.; Coupaye-Gerard, B.; Aboukameel, A.; Pettit, G. R.; Al-Katib, A. M. *Clin. Cancer Res.* **2002**, *8*, 2735–2741.
(82) Rustin, G. J.; Galbraith, S. M.; Anderson, H.; Stratford, M.; Folkes, L. K.; Sena, L.; Gumbrell, L.; Price, P. M. *J. Clin. Oncol.* **2003**, *21*, 2815–2822.
(83) Stevenson, J. P.; Rosen, M.; Sun, W.; Gallagher, M.; Haller, D. G.; Vaughn, D.; Giantonio, B.; Zimmer, R.; Petros, W. P.; Stratford, M.; Chaplin, D.; Young, S. L.; Schnall, M.; O'Dwyer, P. J. *J Clin. Oncol.* **2003**, *21*, 4428–4438.
(84) Dowlati, A.; Robertson, K.; Cooney, M.; Petros, W. P.; Stratford, M.; Jesberger, J.; Rafie, N.; Overmoyer, B.; Makkar, V.; Stambler, B.; Taylor, A.; Waas, J.; Lewin, J. S.; McCrae, K. R.; Remick, S. C. *Cancer Res.* **2002**, *62*, 3408–3416.
(85) Bilenker, J. H.; Flaherty, K. T.; Rosen, M.; Davis, L.; Gallagher, M.; Stevenson, J. P.; Sun, W.; Vaughn, D.; Giantonio, B.; Zimmer, R.; Schnall, M.; O'Dwyer, P. J. *Clin. Cancer Res.* **2005**, *11*, 1527–1533.

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