CHAPTER 9

MAJOR MILESTONES
Anthracyclines

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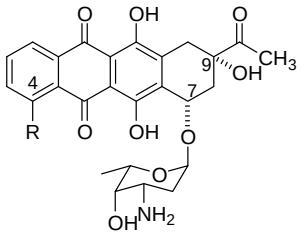
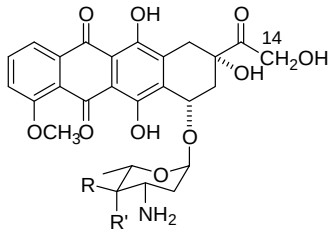
Anthracycline aminoglycosides[1] are microbial metabolites whose aglycones belong to the large family of natural products derived from polyketide biogenetic intermediates. The antitumor activity of a biosynthetic anthracycline was first reported in 1959.[2] Subsequently, useful pharmacological properties were found associated with the novel compounds produced by *Streptomyces peucetius* and related strains. Doxorubicin (**IIa**),[3] the 14-hydroxylated derivative of the main fermentation product daunorubicin (**Ia**), exhibited a wide spectrum of anticancer activity[4,5] and, since its first registration in the early seventies (the trademark of doxorubicin formulations in the US is Adriamycin®), has been one of the most widely used drugs in cancer chemotherapy. Hundreds of biosynthetic anthracyclines have been isolated[6] and the fascinating molecular biology of anthracycline biosynthesis has been studied.[7] On the industrial scale, doxorubicin is prepared by semisynthesis, starting from **Ia**, the reaction sequence comprising an electrophilic bromination followed by conversion of the 14-bromoketone to **IIa**.[3] Main steps of the mode of action of antitumor anthracyclines are the formation of a drug-DNA-topoisomerase II ternary complex, in which the enzyme is covalently linked to a broken DNA strand, followed by protein-associated double-strand breaks that trigger apoptotic cell death.[8,9] On the basis of a study on different semisynthetic analogs, it was concluded that the cytotoxic potency of anthracyclines might be the result of an interplay of different factors, namely level, persistence, and genomic localization of topoisomerase II-mediated DNA cleavage.[10]

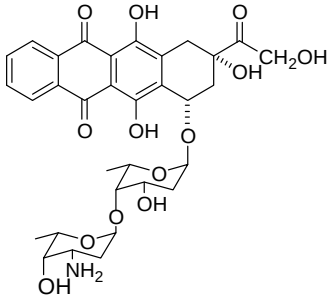
A number of important tumors of clinical importance, such as colon, lung, pancreatic and renal cancers and malignant melanoma, to cite only some examples of "naturally resistant" tumors, do not respond to **IIa** and to the other currently prescribed anthracycline drugs. Other diseases, such as gastric and small cell lung cancers, advanced ovary and breast tumors are only partially responsive and the benefit of drug treatment is often marginal. So far, only the classic multidrug resistance (MDR) phenotype, which is due to the presence of P-glycoprotein (PGP) in plasma membrane (a "pump" that can extrude a wide range of anticancer drugs) has been shown to contribute to resistance in clinical conditions.[11] Together with the onset of resistance, a major dose limiting factor in the repeated treatment with doxorubicin is the development of cardiotoxicity at cumulative dosages higher than 500 mg/m^2 .[12]



Ia: R=OCH_3
Ib: R=H



IIa: R=H, R'=OH
IIb: R=OH, R'=H



III

The successful therapeutic application of **IIa** stimulated a considerable research effort aimed at the development of better analogs by chemical modification of the parent drug or by total synthesis of new structurally related compounds.[13] As a result, although **IIa** remains one of the most effective agents in the medical treatment of a range of solid tumors, new members of this chemical group, such as 4-demethoxydaunorubicin (idarubicin, **Ib**) and 4′-epidoxorubicin (epirubicin, **IIb**), are presently used in medical practice and are currently known as second-generation anthracyclines. Idarubicin (Zavedos®) has been developed to the clinical stage because of its powerful activity in experimental leukemia models and reduced cardiotoxicity. A major advantage over the 4-methoxylated parent drug is its ability to partially overcome multi-drug resistance. Its major metabolite, idarubicinol, is as active as the parent compound.[14] Registration of **IIb** as a better tolerated doxorubicin analog in the U.S. was obtained recently, and the compound is marketed in this country as Ellence®.

Sabarubicin (**III**), containing the chemical functionalities considered to be appropriate for optimal activity, was obtained by total synthesis.[15] Activity of **III** was associated with the stimulation of p53-independent apoptosis. The new compound exhibited, especially at selected DNA sites, a more marked topoisomerase II mediated cleavage, accompanied by superior antitumor efficacy in the experimental systems when compared with doxorubicin. Very significant activity was found against a spectrum of human tumors such as breast, ovary and lung cancer xenografted in athymic nude mice, and the compound appeared markedly superior to doxorubicin in inhibiting tumor growth and in terms of an increased number of disease-free survivors among treated animals.[16,17] In human patients, sabarubicin exhibited a mean elimination half-life significantly shorter than, and a volume of distribution much smaller than, those of **IIa** or **IIb**.[18] An i.v. dosage of 80 to 90 mg/ m^2 every three weeks was used in non-small-cell lung cancer patients with advanced or metastatic disease.[19] Out of 22 evaluable patients, 2 partial responses and 8 minor responses (stable disease) were observed. In small-cell lung cancer, 7 partial responses and 1 stable disease were recorded in a group of 10 patients.[20] Significant response rates were observed in advanced or metastatic platinum/ taxane-resistant ovarian cancer[21] and in progressive hormone refractory prostate cancer.[22]

(1) Brockmann, H. *Fortshr. Chem. Organ. Naturst.* **1963**, *21*, 121-182.
(2) Arcamone, F.; et al. *Giorn. Microbiol,* **1961**, *9*, 83-.
(3) Review: Arcamone, F. *Doxorubicin Anticancer Antibiotics*, De Stevens, G. Ed., Academic Press, New York, **1981**.
(4) Bonadonna, G. et al. *Brit. Med. J.* **1969,** *3*, 503-506.
(5) Carter, S. K. *J. Nat. Cancer Inst.* **1975**, *55*, 1265-1274.
(6) Review: Arcamone, F.; Cassinelli, G. *Current Med. Chem.* **1998**, *5*, 391-419.
(7) Review: Hopwood, D. A. *Chem. Rev.* **1997**, *97*, 2465-2497.
(8) Gerwitz, D. A. *Biochem. Pharmacol.* **1992**, *57*, 727-741.
(9) Review: Pommier, Y. In *Cancer therapeutics, experimental and clinical agents*, Techter B. A., Ed.; Humana Press, Totowa, **1997**, pp. 153-174.
(10) Binaschi, M. et al. *Mol Pharmacol.* **1997**, *51*, 1053-1059.
(11) Nielsen, D.,;Maare, C.; Skovsgaard, T. *Gen. Pharmacol.* **1996**, *27*, 251-255.
(12) Ito, H. et al. *Proc. Acad. Sci. USA* **2000**, *87*, 4275-4279.
(13) Review: Arcamone, F. and Penco, S. In *Anthracycline and Anthracenedione-based Anticancer Agents*, Lown, J.W., Ed.; Elsevier, Amsterdam, **1988**, pp. 1-53.
(14) Borchmann, P. et al. *Int J Clin Pharmacol Ther.* **1997,** *3*, 80-83.
(15) Arcamone, F. et al. *J. Nat. Cancer Inst.* **1997**, *89*, 1217-1223.
(16) Pratesi, G. et al. *Clin. Cancer Res.* **1998**, *4*, 2833-2839.
(17) Pratesi, G. et al. *Biochem. Pharmacol.* **2001** *62*, 63-70.
(18) Bos, A.M.E. et al. *Cancer Chemother. Pharmacol.* **2001**, *48*, 361-369.

(19) Tjan-Heijnen, V.C.G. et al. *Ann. Oncol.* **2002**, *13*, *Suppl 5*, 148.
(20) Dickgreber N. J. et al. *Proc. Am. Soc. Clin. Oncol.* **2003.** *22*, 703 (abstract 2879).
(21) Caponigro, F. et al. *Invest. New Drugs* **2005**, *23*, 85-89.
(22) Fiedler, W. et al. *Eur. J. Cancer* **2006**, *42*, 200-204.

From Qing Hao, Herb of Antiquity, and the Advent of a Remarkable Class of Pharmaceutical Drugs Based on the Active Principle Qing Hao Su (Artemisinin)

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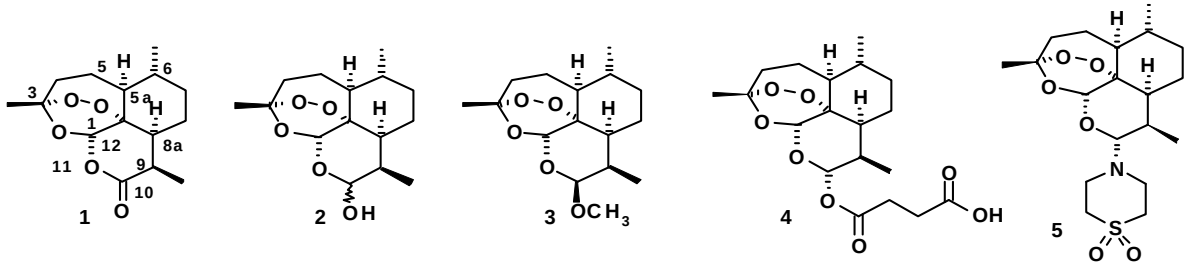
In response to the problem of drug-resistant falciparum malaria afflicting personnel from China and Vietnam involved in the US-Vietnam conflict, members from the Chinese National Science and Technology Commission and the People's Liberation Army participated in a meeting in May 1967 in Beijing to coordinate research on treatment. "Project 523" emerged from this, and eventually involved over 500 scientists from various research units across China. During the period 1967-1980, new chemotherapy (based on conventional drugs) for malaria and measures for controlling the mosquito vector were introduced. However, the most important achievement was the discovery of the active principle, qinghaosu, or "artemisinin," as it was subsequently named (**1**), from the traditional Chinese herb Qing Hao (*Artemisia annua* L.), and its conversion into the derivatives dihydro-artemisinin (DHA) (**2**), artemether (**3**) and artesunate (**4**).[1] Artemisone (**5**), representing a new class of artemisinins known as amino-artemisinins, is curative in clinical trials at one third the dose regimen of artesunate. It is characterized by low toxicity.



Isolation of artemisinin from *A. annua* was guided by assessment of bioactivity of extracts.[1] The first sample of crystalline artemisinin was obtained under the Project 523 program in 1973, using a light petroleum extract of the dried leaves. Structural elucidation, coupled with an evaluation of its chemistry, was carried out from 1974-1977 at the Institute of Chinese Materia Medica (Academy of Chinese Medicine), the Shanghai Institute of Materia Medica (IMM), the Shanghai Institute of Organic Chemistry, and the Beijing Institute of Biological Physics. The seminal paper describing the work was published as a one-page synopsis in the *Ke Xue Tong Bao* in 1977 by the "Cooperative Research Group on Qinghaosu".[2] The unique feature of **1** is the embedded 1,2,4-trioxane comprising the peroxide bridge linked via a carbon atom to a third non-peroxidic oxygen atom, which represents the first example of such a motif reported in a natural product.

The endoperoxide is the active pharmacophore, although the third non-peroxidic oxygen atom confers optimal activity.[3]

The insolubility of artemisinin posed problems during formulation into dosages suitable for treatment of malaria. Thus, the Project 523 group at the IMM reduced **1** with $NaBH_4$ in methanol to give the hemiacetal DHA (**2**),[1,2,4] which had superior antimalarial activity, but also was unsuitable for formulation. It was converted into **3** by treatment with methanol and an acid catalyst; this derivative was six times more active than **1**, and because of its solubility in oil, it could be administered by intramuscular injection. Clinical trials on malaria patients in China recorded a short-term cure rate of 100%, with faster parasite clearance times than with quinoline drugs. The final key derivative, **4**, was prepared from DHA and succinic anhydride at the Guilin Pharmaceutical Factory. As a bicarbonate formulation, it was used in clinical trials in 1978, and, like **3**, it was fast acting, and had low toxicity.

These artemisinins are now universally deployed for combatting malaria, being used in **1** combination therapies, so-called ACT, with longer half-life drugs.[5] However, metabolism of **3** and **4** to DHA (**2**) is facile, and the neurotoxicity, of DHA, is demonstrated in laboratory screens.[6] Whilst neurotoxicity has generally not been expressed in humans treated with these drugs, there are exceptions,[7] and debate over the problem continues.[8] Although an enormous number of other derivatives have been made, very few have met the requirements of economy, low toxicity and efficacy.[9] In our own program, we used medicinal chemistry principles to guide the preparation of derivatives with enhanced polarity and water-solubility and reduced facility of metabolism to DHA, with the aims of countering toxicity and improving the antimalarial efficacy,.[10] leading eventually to **5**, a 10-amino-artemisinin, which emerged as a clinical development candidate.[11] It elicits no significant neurotoxicity,[12] and has greater efficacy than **4**, up to nine-fold greater *in vitro*, and at least three-fold greater *in vivo*.[13,14] In a Phase IIa trial in Thailand, cure was achieved with a three-fold lower dose compared with that of **4**.[15] Furthermore, **5** is not metabolized to DHA, and, unlike the other artemisinins, it displays no clinically-relevant autoinduction of metabolizing enzymes.[15,16] Also, **5** is the first clinically successful artemisinin derivative developed outside of China.

The antimalarial efficacy aside, the advent of the artemisinins has sparked questions on the nature of their intracellular mechanisms, and has enormously stimulated an awareness of the importance of peroxide xenobiotics in modulation of cell physiology, and their considerable potential in the management and therapy of non-parasitic diseases. Thus, the discovery by the Chinese of artemisinin certainly ranks in importance with that of quinine, and represents one of the greatest developments in medicine in the latter third of the 20th Century.

Acknowledgements
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(1) Zhang, J.-F. Beijing 2006, Yang Cheng Evening News Publishing Company.
(2) Co-Operative Research Group On Qinghaosu 'A Novel Type of Sesquiterpene Lactone – Qinghaosu', *Ke Xue Tong Bao* **1977**, *22*, 142.
(3) Avery, M. A. et al. *J. Med. Chem.* **1996**, *39*, 1885-1897.
(4) Liu, J.- M. et al. *Acta Chimica Sinica* **1979**, *37*, 129-141.
(5) Olliaro, P. L. et al. *J. Postgrad. Med.* **2004**, *50*, 40-44; White, N.J. *J. Clin. Invest.* **2004**, *113*, 1084-1092.

(6) Brewer, T. G. et al.; *Am. J. Trop. Med. Hyg.* **1994**, *51*, 251-259; Nontprasert, A. et al. *Am. J. Trop. Med. Hyg.* **2000**, *62*, 409-412.
(7) Toovey, S. et al. *Trans. R. Soc. Trop. Med. Hyg.* **2004**, *98*, 261-7; Comments **2004,** 268-9; *Trans. Roy. Soc. Trop. Med. Hyg.* Correspondence **2005**, *99*, 313-317; Toovey, S. *Travel. Med. Infect. Diseases.* **2006**, *4*, 71-76; Campos, S. et al. *J. Pediatric Infect. Diseases* **2008**, in press; see http://www.jpid.org/abstracts/JPID%202008(1)/Artesunate.end. doc.
(8) Johann-Liang, R. et al. *Clin. Infect. Dis.* **2003,** *36*, 1626–7; Woodrow, C. J. et al.. *Postgrad. Med. J.* **2005,** *81*, 71-78; Toovey, S. *Lancet* **2006**, *367*, 111-112; Toovey, S. *Clinical Infect. Diseases* **2006**, *42*, 1214-1215; Toovey, S. *J. Am. Trop. Med. Hyg.* **2006**, *74*, 939-940; Toovey, S. *Toxicol. Lett.* **2006**, *166*, 95-104.
(9) Haynes, R. K. *Current Med. Chem.* **2006**, *13*, 509-537.
(10) Haynes, R. K. *Current Opinion in Infectious Diseases* **2001**, *14*, 719-772.
(11) Haynes, R. K. et al. *Angew. Chem.. Internat. Edit.* **2006**, *45*, 2082-2088.
(12) Schmuck, G. et al.. XVI International Congress for Tropical Medicine and Malaria: Medicine And Health In The Tropics; Marseille, France, 11-15 September 2005, Abstract Book, O-004, p. 26.
(13) Ramharter, M. et al. *Am. J. Trop. Med. Hyg.*, **2006**, *75*, 637–639.
(14) Vivas, L. et al. *J. Antimicrob. Chemother.* **2007**, *59*, 658 – 665.
(15) Krudsood, S. et al. XVI International Congress for Tropical Medicine and Malaria: Medicine And Health In The Tropics; Marseille, France, 11-15 September 2005, Abstract Book, P054, p. 142.
(16) Schmeer, K. et al. Presentation PO63, XVI International Congress for Trop. Med. and Malaria: Medicine And Health In The Tropics; Marseille, France, 11-15 September 2005, Abstract Book, O-063, p. 144.

The Bryostatins

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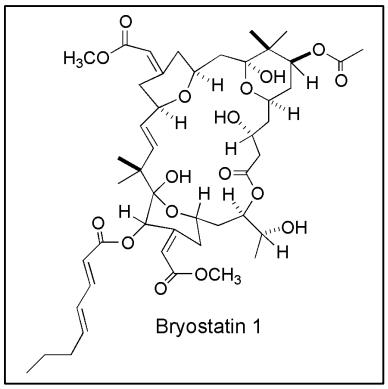
The Bryozoa phylum comprises colonial filter-feeders, each member of which (zooid) is enclosed in a separate unit (zooecium). In 1965-1966, in collaboration with colleagues in the US National Cancer Institute (NCI), one of us (GRP) began the first broad geographically based evaluation of marine invertebrates and vertebrates as potential new sources of structurally unique anticancer agents. In1968, we began to focus on a Gulf of Mexico bryozoan, *Bugula neritina* (family Bugulidae), [1,2] that provided extracts exhibiting potent *in vivo* activity in the NCI P388 murine lymphocytic leukemia. Parallel collections in the Gulf of California, and later collections off the coast of California gave further specimens, and extensive bioassay-directed separation of the California *Bugula neritina* extracts led to isolation of the first few milligrams of bryostatin 1, a macrocyclic lactone, whose structure was determined by X-ray crystallography and reported in 1982.[2] Subsequently, we have discovered twenty new bryostatins from *Bugula neritina* collections that range from the Gulf of Mexico, Gulf of California, and coast of California, to Japan (Gulf of Sagami). Later collections from two more remote areas in the Gulf of Japan gave bryostatin 10 in relatively abundant amounts (about 10^{-3}%) compared to10^{-6}% yields of bryostatin 1 and 10^{-8}% for some of the more rare bryostatins. This higher yield of bryostatin 10 could be important for its future development. [2]



Bryostatin 1 at ~50 μg/kg doses provided excellent to curative levels of activity against a variety of murine tumor systems, as well as against human cancer models in the nude or SCID models. [2] The potent anticancer activity of bryostatin 1 and the bryostatins was shown by Dr. Blumberg of NCI to be primarily based on initial activation followed by rapid down-regulation of protein kinase C (PKC), a very important family of isozymes in the cell transduction pathway involved in regulation, different-

iation, gene expression, and tumor promotion. In contrast to some PKC interactive compounds, bryostatin 1 proved to be a potent antitumor promoting agent. Furthermore, it has been shown to exhibit immunostimulatory properties, including stimulation of cytokine release, enhancement of T- and B-cell activation, and lymphokine-activated killer cell activity; to stimulate accessory cell population to produce granulocyte-monocyte colony-stimulating factor (GM-CSF), neutrophil phagocytic activity and degranulation; to down-regulate *mdr*-1 (multidrug resistance); to stimulate normal production of interleukin-2 (IL-2) and interferon; and to down-regulate *bcl*-2 (resistance to programmed cell death or apoptosis) and upregulate *bax* (induction of apoptosis).

Clinical development of bryostatin 1 began in 1990. Presently, over 90 human cancer clinical trials of bryostatin 1 have either been completed or are ongoing in the United States, England, and Canada under the auspices of the NCI, Cancer Research UK, and the National Cancer Institute of Canada, involving some 200 principal investigators in many different institutions. To date, a large number of patients have been treated with bryostatin 1 in phase I or phase II cancer clinical trials, and while some promising benefits ranging from complete responses and partial responses to stable disease have been observed, current clinical results clearly indicate that bryostatin 1 will be most useful in combination with other anticancer drugs exhibiting different mechanisms of action, such as gemcitabine for refractory cancer patients,[3] and fludarabine with the anti-CD20 monoclonal antibody rituximab for treatment of CLL and indolent lymphomas.[4] In parallel, it is necessary for the future clinical development to overcome the common dose-limiting side effects of myalgias. That, for example, would allow the full potential of the bryostatin 1/taxol combination[5] against esophageal carcinoma where this treatment has already received Orphan Drug approval. Bryostatin 1 has been found to stimulate *via* a PKC pathway, production of cyclooxygenase-2 (COX-2) derived prostaglandins that cause pain which can be blocked by all *Trans*-retinoic acid.[6] That advance should set the stage for much improved clinical trial design.

Among other promising indications for the bryostatins is the exciting potential for development as a CNS drug.[7] In preclinical investigations, bryostatin 1 has been found to promote important cognitive and antidepressant effects. That may involve activation of PKC isozymes involved in syntheses of proteins needed for long-term memory, re-establishing stress-based blocking of PKC and reduction of the neurotoxic amyloid accumulation of Alzheimer's disease.[7b,c]

Future developments will require the continuation of the current important advances in total syntheses[8a] and structural modification.[8b,c,d] Also of major importance, the as yet uncultivated polyketide synthase gene cluster from *Candidatus endobugula sertula*, the microbial symbiont of *Bugula neritina* believed to be the bryostatin source, has now been identified.[9,10] Expression of this biosynthetic gene cluster can potentially lead to scale-up production of the bryostatins employing biological methods.

(1) Pettit, G. R.; Day, J. F.; Hartwell, J. L.; Wood, H. B. *Nature* **1970**, *227*, 962-963.

(2) (a) Pettit, G. R.; Herald, C. L.; Hogan, F. In *Anticancer Drug Development*, Baguley, B., Ed.; Academic Press: San Diego, CA, 2002; pp 203-235. b) Newman, D. J. In *Anticancer Agents from Natural Products*, Cragg, G. M.; Kingston, D. G. I.; Newman, D. J.,Eds; CRC Press, LLC: Boca Raton, FL, 2005; pp 137-150.

(3) El-Rayes, B.F. et al. *Clin. Cancer Res.* **2006**, *12*, 7059-7062.

(4) Roberts, J. D. et al. *Clin. Cancer Res.* **2006**, *12*, 5809-5816.

(5) a) Ajani, J. A. et al. *Invest. New Drugs* **2006**, *24*, 353-357. b) Kortmansky, J.; Schwartz, G. K. *Cancer Invest.* **2003**, *21*, 924-936.

(6) DeLorenzo, M. S. et al. *Clin. Cancer Res.* **2003**, *9*, 5036-5043.

(7) a) Sun, M-K.; Alkon, D. L. *CNS Drug Rev.* **2006**, *12*, 1-8. b) Alkon, D. L. et al. *Trends in Pharm. Sci.* **2007**, *28*, 51-60. c) Alkon, D. L. et al. *Proc. Natl. Acad. Sci. U. S.* **2005**, *102*, 16432-16437.
(8) a) Manaviazar, S. et al. *Org. Lett.* **2006**, *8*, 4477-4480. b) Wender, P. A. et al. *DDR* **2007**, 127-162. c) Wender, P. A. Et al. *Org. Lett.* **2006**, *8*, 5299-5302. d) Trost, B.M. et al. *J. Amer. Chem. Soc.* **2007**, *129*, 2206-2207.
(9) Sudek, S. et al. *J. Nat. Prod.* **2007**, *70*, 67-74.
(10) Lopanik, N.et al. *J. Nat. Prod.* **2004**, *67*, 1412-1414.

The Discovery of Camptothecin

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As noted elsewhere in this volume, the therapeutic potential of the anticancer alkaloid camptothecin was the primary driving force in the relocation of Monroe Wall from the Philadelphia USDA office to establish the Natural Products Laboratory at Research Triangle Institute. This discovery was also the nucleus of his 40-plus-year collaboration with Mansukh Wani.

The journey of camptothecin (CPT) from tree to drug began in 1934 when then-uncharacterized seeds of *hsi shu* or tree of joy, *Camptotheca acuminata*, were sent to the US Department of Agriculture (USDA) by Dr. A.N. Steward, chair of the Department of Botany at the University of Nanking, China.[1] Seedlings of this rapidly growing tree were sent to several USDA stations in 1937 but the only surviving planting was at the US Plant Introduction Station at Chico, CA. The road may have stopped here - had USDA known the seeds were from *C. acuminata* they would not have been planted since Harvard botanist E.H. Wilson had already collected and sown seeds from Szechuan province with plants sent to USDA in 1912.

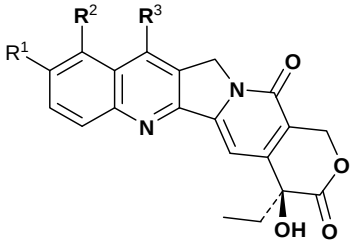
In 1950, *C. acuminata* leaves were sent to Wall, then at the USDA's Eastern Utilization Research and Development Division at Wyndmor, Pennsylvania, in his search for precursors to cortisone and other steroid hormones; the *Camptotheca* extracts were negative in this regard. But in 1957, a chance meeting between Wall and Jonathan Hartwell, Assistant Chief of the NCI Cancer Chemotherapy National Service Center (CCNSC), led Wall to send 1,000 extracts for cytotoxicity testing against mouse L1210 leukemia cells. *C. acuminata* was the only positive hit with *in vitro* antileukemic activity but USDA did not support Wall's further work on the extract, citing that treating cancer was outside their mission.

When Wall moved to RTI in 1960, USDA sent him new samples of *Camptotheca* obtained from branch cuttings of a 30-year-old tree. The new extracts were submitted to the CCNSC in 1962, and when Wall later hired Mansukh Wani as a postdoctoral fellow, the active compound, CPT, was isolated and reported in 1966 in the *Journal of the American Chemical Society* as a "novel alkaloidal leukemia and tumor inhibitor.[2]" Not well-appreciated in CPT history is that Susan Band-Horwitz, the scientist instrumental in discovering taxol's mechanism of action, was also one of the first to work on the subsequent cellular actions of CPT. [3]

A major obstacle in scale-up was overcome relatively quickly when NCI researchers found that *Camptotheca* seedlings exhibited as much cytotoxic activity as young or old trees. [4] After Wani, Wall, and colleagues elucidated the structure of CPT,[2] all seemed on track. The fortuitous Chico plantings of *Camptotheca* allowed NCI to underwrite the production of 50,000 seedlings to provide enough CPT for clinical trials.[1,4]. Initial studies against gastrointestinal tumors were going well but a major clinical trial at the Mayo Clinic was less successful. At the May 1971 American Association for Cancer Research Annual Meeting in Chicago, Mayo's medical

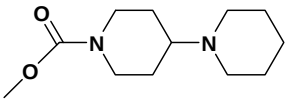
oncologist Charles Moertel prefaced his presentation stating, "I have come here not to praise camptothecin but to bury it. Camptothecin is a drug of protean and unpredictable toxicity that has no clinical value in the management of gastrointestinal cancer,[5]" as only 2 of 61 patients responded in his trial. Moertel concluded with a picture of a new crop of *Camptotheca acuminata* in California and the comment that, "the tree is not pretty and the roots are not even edible." NCI subsequently terminated the CPT program. However, we know now that attempts to make CPT more water soluble by forming the sodium salt destroyed its antitumor activity by opening its crucial pharmacophore, the lactone ring. Subsequently, at the slightly acidic pH of the urine, the lactone reconstituted and caused significant renal and bladder toxicity.

Two crucial events occurred nearly 15 years later that revived camptothecin. Leroy Liu's group, then at Johns Hopkins, demonstrated in 1985 that CPT killed tumor cells by a novel mechanism: the poisoning of DNA topoisomerase I, converting this important DNA unwinding enzyme into an intracellular cytotoxin by covalently trapping it on DNA. It is now understood that CPT



Topotecan $R^1 = OH$; $R^2 = CH_2N(CH_3)_2$; $R^3 = H$

Irinotecan $R^1 =$



$R^2 = H$
$R^3 = CH_2CH_3$

Camptothecin $R^1 = R^2 = R^3 = H$

mimics an endogenous process by which cells with spontaneous DNA aberrations are eliminated. Any antitumor agent acting by a novel mechanism is a gold mine for combination chemotherapy, as targeting non-overlapping mechanisms is key to slowing the emergence of drug resistance. Randall Johnson and colleagues at then-SmithKline & French Laboratories were working on an NCI NCDDG project with Liu and the Florida topoisomerase group of Ross and Rowe. Johnson was a former NCI scientist with interests in CPT dating back to the mid-1970s. With this new mechanistic information, his chemists synthesized a more water-soluble and active CPT that preserved the lactone, first calling it "hycamptamine" which then became topotecan (Hycamptin). Japan's Yakult Honsha Co. was also working on a water-soluble prodrug, CPT-11, which became known as irinotecan (Camptosar). Irinotecan has been the most successful of the two drugs, with excellent activity in gastrointestinal, lung, and ovarian cancers. However, topotecan has its own benefits as well, being the first CPT approved in an oral form (October 1997, for non-small cell lung cancer) with additional indications in cervical cancer for the intravenous form.

The intense, ongoing interest in these drugs is evident from the NCI Cancer Clinical Trials Database (http://www.cancer.gov/Search/SearchClinicalTrialsAdvanced.aspx) showing 160 and 67 trials of various forms of irinotecan and topotecan, respectively. Liu and others are also investigating non-CPT topoisomerase I poisons to build upon the foundations of chemotherapy revealed by this remarkable natural product.

(1) Perdue, Jr., R.E., Smith, R.L., Wall, M.E., Hartwell, J.L., and Abbott, B.J. (1970) U.S. Department of Agriculture Technical Bulletin No. 1415, *Camptotheca acuminata* Decasine (Nyssaceae). Source of Camptothecin, an Antileukemic Alkaloid.

(2) Wall, M. E., M. C. Wani, C. E. Cook, K. H. Palmer, A. T. McPhail and G. A. Sim, Plant antitumor agents. 1. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata. Journal of the American Chemical Society.* 1966; **88:** 3888-3890.
(3) Horwitz, S.B., Chang, C.K., and Grollman, A.P. Studies on camptothecin: effects on nucleic acid and protein synthesis. *Mol Pharmacol* 1971; **7**(6):632-644.
(4) Persinos G. A cancer drug odyssey. *World & I* 1993; **8**(11) 194-201.
(5) Personal communication, Dr. Saul Shepartz to M.C. Wani, May 1971

The Combretastatins

George R. Pettit and Kevin G. Pinney

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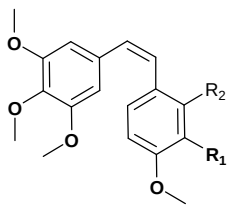
Tropical and subtropical trees of the family Combretaceae (about 600 species in 20 genera) represent a reserve of constituents with potentially useful biological properties. The genus *Combretum* contains 250 species, of which, 25 are used in the traditional medicine of Africa and India. The root bark of *C .caffrum* has been used by the Zulu of South Africa as a charm to harm an enemy, and recently it was learned that the Xhosa people of South Africa have been using *C. caffrum* for treatment of cancer and other ailments. However, prior to 1999, none of the latter anecdotal information was known to us,[1] and only the Indian *C. latifolium* appears to have been recorded as a folk medical treatment for cancer. Important events leading to the discovery of the combretastatins began with the first (1973) collection of *C. caffrum* for the NCI in Rhodesia (now Zimbabwe). Extracts were active against the murine P-388 lymphocytic leukemia (PS system), but efforts to isolate the active constituents from scale-up recollections failed. Using a 1979 *C. caffrum* collection and the astrocytoma bioassay, however, Pettit and colleagues isolated the first cancer cell growth inhibitor, (-)-combretastatin, and this research subsequently led to our discovery of 20 cancer cell growth inhibitory stilbenes, bibenzyls, dihydrophenanthrenes, and phenanthrenes produced by *C. caffrum*, and resulted in the development of the first well-established antiangiogenic/vascular-disrupting anticancer drug, sodium combretastatin A-4 phosphate (CA4P), which is now in broad human cancer clinical trials.



Combretastatin A1: R_1 = R_2 = OH
Combretastatin A4: R_1 =OH; R_2= H
CA1P: R_1 = R_2 =OP(O)ONa)$_2$
CA4P: R_1 = OP(O)ONa)$_2$;,R_2= H

The combretastatins, despite their relatively simple chemical structures, are characterized by remarkable biological activity as inhibitors of tubulin polymerization, while some exhibit potent *in vitro* inhibition against human cancer cell lines, *in vivo* efficacy as vascular disrupting agents (VDAs), and act as antiangiogenesis agents. Tumors receive their nutrients through existing vasculature using newly formed neovascularization (angiogenesis), and the combretastatin A-4 (CA4) and A-1 (CA1) phosphate prodrugs (CA4P[2] and CA1P[3]) selectively damage tumor neovasculature with induction of extensive blood flow shutdown in the metastatic tumor compared to normal tissues. In prodrug form, these VDAs [4,5] are largely protected from binding to tubulin, but enzymatic (non-specific phosphatases) cleavage to their corresponding phenol(s) restores their tubulin assembly inhibition, enabling them to bind to endothelial cell tubulin causing rapid morphology changes in the endothelial cells lining the microvessels; this results in disruption of blood flow, finally leaving new microvessels and mature and established vessels unable to deliver blood, thereby causing hypoxia and necrosis of the tumor. The

selectivity of the VDAs for the microvessels of tumors is believed to be caused by disrupting the endothelial cell function molecule, vascular endothelial cadherin (VE-cadherin), an important mediator of cell-cell contacts, thus blocking the endothelial signaling pathway needed for continuing functional endothelial cell structure and survival. This is primarily limited to the newly forming vessel endothelial cells, and not those already in normal/established smooth vessel walls. The result in new tumor neovessels is disruption of cell-cell junctions, cell migration, and anchorage, leading to apoptosis.[6]

In 1998, four Phase I human cancer clinical trials of CA4P were initiated, and Phase I, Ib, I/II, and II trials have continued through 2007. While preclinical data for CA4P indicated significant reduction in tumor blood flow resulting in tumor necrosis, a rim of viable tumor cells at the tumor periphery was observed that continued to receive nutrition from normal tissue, so later clinical trials have incorporated combination with other anticancer drugs such as paclitaxel (Taxol), Carboplatin, Avastin (antiangiogenic monoclonal), and/or fractionated external beam radiotherapy. Other CA4P combinations in development include the therapeutic antibody 131I-A5B7.[7] In July of 2003, the U.S. FDA awarded orphan drug status to CA4P for treatment of advanced anaplastic thyroid cancer (ATC), medullary, stage IV papillary and stage IV follicular thyroid cancer. The FDA also assigned fast-track designation; in late 2007, CA4P (trademark Zybrestat) was advanced to Phase II/III pivotal registration clinical trials against thyroid cancer, which is resistant to almost all forms of therapy, and it is likely going to be the first small molecule VDA to be approved for marketing. In May, 2006, it was also given FDA orphan drug status for the treatment of ovarian cancer in combination with Carboplatin, and paclitaxel for advanced platinum-resistant ovarian cancer. The mechanism of action of CA1P (Oxi-4503) differs from that of CA4P (formation of an *o*-quinone intermediate *in vivo*),[8] and it causes tumor regression as a single agent that also attacks the remaining tumor rim cancer cells.[3,5] In 2005, the first Phase I clinical trial of CA1P was initiated supported by the use of extensive blood studies, MRI and PET scans, to gain further insights into the mechanism of action.

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Myopic macular degeneration (MMD) and age-related macular degeneration (AMD) are progressive eye diseases leading to blindness caused by abnormal blood vessels growing from the choroids that infiltrate the retina and result in hemorrhaging and scarring that usually leads to central visual loss. In February 2007, stabilization of vision of all 23 patients in the first Phase II clinical trial of CA4P against MMD was reported, demonstrating the promise of VDAs in the treatment of these diseases.[9] Parallel ophthalmology clinical trials for AMD have been planned, as well as submission of an IND for use of a topical formulation for the treatment of other eye diseases arising from neovascularization, such as diabetic retinopathy and retinoblastoma.[10]

The combretastatins continue to inspire the synthesis of many structurally distinct mimics, encompassing distinct molecular skeletons (e.g., indole, benzofuran, dihydronaphthalene) and heteroatom bridge modified congeners, some of which have entered human cancer clinical trials. These synthetic efforts, together with the promising results of clinical studies with CA4P in the treatment of cancers and eye diseases, serve as testimony to the legacy that the combretastatins will leave in the history of compounds with important medicinal value.

(1) K. G. Pinney, C. Jelinek, K. Edvardsen, D. J. Chaplin, G. R. Pettit, In *Anticancer Agents from Natural Products*, Ed. by G. M. Cragg, D. G. I. Kingston, and D. J. Newman, Taylor and Francis, Boca Raton, FL, 2005, pp. 23-46.
(2) Pettit, G. R.; Rhodes, M. *Anti-Cancer Drug Des.* **1998**, *13*, 183-191.
(3) Pettit, G. R.; Lippert, J. W. III. *Anti-Cancer Drug Design* **2000**, *15*, 203-216.

(4) Kanthou, C.; Tozer, G. M. *Exper. Opin. Ther. Targets* **2007**, *11*, 1443-1457; Lippert, J. W. III. *Bioorg. Med. Chem.* **2007**, *15*, 605-615.
(5) Sheng, Y. et al. *Int. J. Cancer* **2004**, *111*, 604-610. (b) Salmon. H. W. et al. *Clin. Cancer Res.* **2006**, *12*, 4090-4094.
(6) Vincent, L. et al. *J. Clin. Invest.* **2005**, *115*, 2992-3006; Marlotti, A. et al. *Expert Opin. Investig. Drugs* **2007**, *16*, 451-465.
(7) Lankester, K. J. et al. *Int. J. Oncol.* **2007**, *30*, 453-460.
(8) Folkes, L. K. et al. *Chem. Res. Toxicol.* **2007**, *20*, 1885-1894.
(9) Michels, S. et al. *Expert Opin. Invest. Drugs* **2006**, *15*, 779-793.
(10) Escalona-Benz, E. et al. *Invest. Ophthalmol. Visual Sci.* **2005**, *46*, 8-11.

Cyanobacteria, an Extraordinary Source of Bioactive and Structurally Novel Natural Products, including the Cryptophycins

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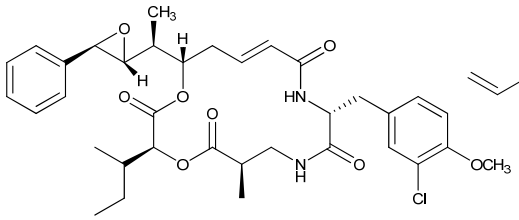
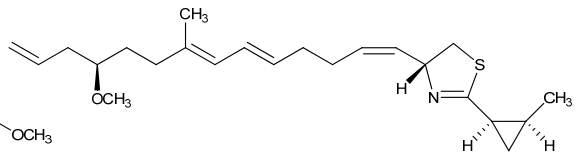
It was only a few decades ago that Richard (Dick) Moore moved to Hawaii and started his independent academic career by exploring the marine equivalents of 'pond scum' for bioactive molecules. The shallow reefs of Hawaii, like many tropical ecosystems, have a notable presence of red or mossy green stands of the filamentous cyanobacterium genus *Lyngbya*, also known as Mermaids Hair. What he discovered is that they are an incredible source of structurally diverse nitrogen-containing lipids, many of which have powerful biological activities. His earliest discoveries with marine cyanobacteria, both reported in 1977, were from Hawaiian *Lyngbya majuscula* and described the majusculamides A and B[1], as well as one of the first anticancer leads from the sea, debromoaplysiatoxin.[2] From these pioneering discoveries, he and his notable students at the time, such as Jon Mynderse, John Cardellina and others went on to explore the unique natural products of cyanobacteria from the central and south Pacific Ocean.



Cryptophycin A



Curacin A

However, about 1984, Moore recognized that to have a steady supply of these compounds as well as to investigate their biosynthetic pathways, he needed to culture cyanobacteria in the laboratory. This need motivated a 15-year collaboration with Greg Patterson, a multi-talented scientist with a profound interest in cyanobacterial culture, which culminated in more than 60 joint publications focused on the chemistry and biological activities of fresh water cyanobacteria. This move away from marine cyanobacteria was motivated in part by reports of strong biological properties associated with blooms of the freshwater species, and in part by the fact that they are much more easily cultured than their marine cousins. Their early successes kindled enthusiasm by other research groups to examine cultured freshwater cyanobacteria as sources of pharmaceutical leads, including a Merck effort headed by Bob Schwartz.[3] This later program was focused on anti-infective drug discovery, and resulted in 1990 in the discovery of a novel antifungal cyclic depsipeptide, cryptophycin, from cultured *Nostoc* sp. A few years later, the

Patterson-Moore collaboration re-discovered cryptophycin in a program focused on anticancer drug discovery. The cryptophycin family of compounds are exceptionally potent anti-proliferative agents by virtue of their binding to a site on tubulin that appears to overlap that of the vinca alkaloids.[4] These exciting discoveries led to an aggressive synthetic program which ultimately gave a cryptophycin analog that was evaluated in human clinical trials by Eli Lilly.[5] While the derivative taken into these initial trials failed, other derivatives of this potent cyanobacterial compound are reported to be under continuing examination. Meanwhile, with culturable blue green algae in hand, Dick was actively exploring the biosynthesis of these natural products, and ultimately, through collaboration with the David Sherman laboratory (Michigan), came to understand at the chemical and genetic levels how one of the flagship compounds of his career, cryptophycin A, was created.[6]

At the same time that Moore was beginning his examination of freshwater cyanobacteria, the author of this essay was initiating independent investigations of the unique chemistry of cyanobacteria from the Caribbean. These and other studies showed that the chemical diversity available from these organisms was far greater than previously believed, and from the author's laboratory, has resulted in more than 50 publications on this topic, including the discovery of such notable compounds as curacin A, jamaicamide A and hectochlorin.[7] A theme emerged from these studies that marine cyanobacterial metabolites commonly possess antitubulin or antiactin mechanisms of antiproliferative activity towards mammalian cells. Furthermore, in the author's laboratory some success was gained in culturing marine cyanobacteria, and this enabled detailed investigations of their biosynthesis. In recent years, the author's laboratory has also collaborated with David Sherman's to pioneer the description of cyanobacterial natural product biosynthesis at the molecular genetic level.[8]

Beginning in the late 1990's, Dick Moore returned for a final look at field collections of marine cyanobacteria, inspired in large part from the growing interest by Valerie Paul, then located in Guam, into the chemical ecology of these organisms.[9] This was a highly successful and productive collaboration, resulting in over 40 remarkable publications in 6 years. This productivity was also attributable to two of Dick's final students, Philip Williams and Hendrik Luesch, both of whom were co-authors on most of these papers. Dick clearly inspired both of these former students, as the former is now occupying Dick's academic position at the University of Hawaii, and the latter is an independent scientist at the University of Florida making important new discoveries of structurally novel and highly bioactive compounds from "Mermaid's Hair".[10]

(1) Marner, F.-J.et al. J. *J. Org. Chem.* **1977**, *42*, 2815-2819.
(2) Mynderse, J. S.et al. *Science* **1977**, *196*, 538-540.
(3) Schwartz, R. E.. et al. *J. Indust. Microbio.* **1990**, *5*, 113-123.
(4) Mooberry, S. L. et al. *Cancer Lett.* **1996**, *107*, 53-57.
(5) Stevenson, J. P. et al. *Clinical Cancer Res.* **2002**, *8*, 2524-2529.
(6) Magarvey, N. A. et al. *ACS Chem. Biol.* **2006**, *1*, 766-779.
(7) Gerwick, W. H. et al. In "The Alkaloids" G. Cordell, ed., Academic Press, San Diego, **2001**, *57*, 75-184.
(8) Chang, Z.; et al. *J. Nat. Prod.* **2004**, *67*, 1356-1367.
(9) Harrigan, G. G. et al. *J. Nat. Prod.* **1998**, *61*, 1075-1077.
(10) Taori, K.; Paul, V. J.; Luesch, H. *J. Am. Chem. Soc.* **2008**, *130*, 1806-1807.

Didemnins and Kahalides

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Didemnin B.

Didemnin B was isolated by Rinehart's group from of the tunicate *Tridemnum solidum* that demonstrated excellent antiviral activity and subsequent cytotoxic activity against P388 and L1210 murine leukemia cell lines. It was advanced into preclinical and clinical trials (Phases I and II) under the auspices of the NCI in the early 1980s as the first defined chemical compound *directly* from a marine source to go into clinical trials for any major human disease. In spite of many different treatment protocols and testing against many types of cancer, the compound was too toxic for use, and trials were officially terminated in the mid-1990s. Despite this setback, the experience gained was immensely helpful in aiding the trials of other natural product-derived agents/compounds. Thus Rinehart's group developed methods of large-scale isolation and purification and total syntheses that permitted significant SAR to be derived. MOA studies demonstrated binding to elongation factor 1-α (ef1-α), and rapamycin was reported to inhibit the didemnin-induced apoptosis of human HL-60 cells, perhaps by binding to the FK-506 binding protein(s). The latter result implied that didemnin B might modulate the FK-binding proteins as part of its immunomodulatory activity and thus lead to cell death via apoptosis.

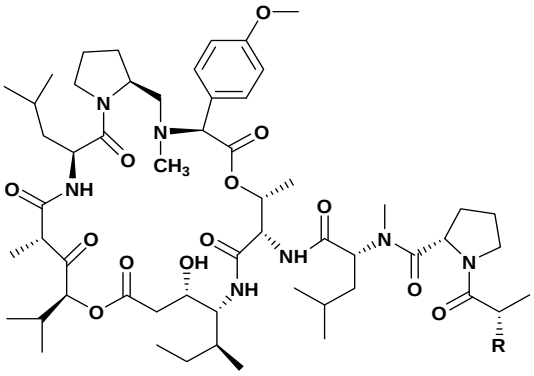
In a review of didemnins as cell probes and targets for syntheses,[1] Vera and Joullie argued that the dosing schedules used in the early clinical trials, *viz* a single bolus dose at the maximum tolerated dose (MTD), may have been non-optimal for demonstrating activity as a cytotoxin, rather than as an immunosuppressive/modulator. Although didemnin B was not successful, a very close chemical relative (aplidine) is currently in clinical trials, and in 2000 Rinehart published an overview of these compounds as part of a discussion of antitumor compounds from tunicates which should be consulted for further details.[2] In due course it may be most indicative to compare the dosing schedules and responses for didemnin B and aplidine (Aplidin™) in humans once the latter are fully reported in the literature.

Aplidine [3]

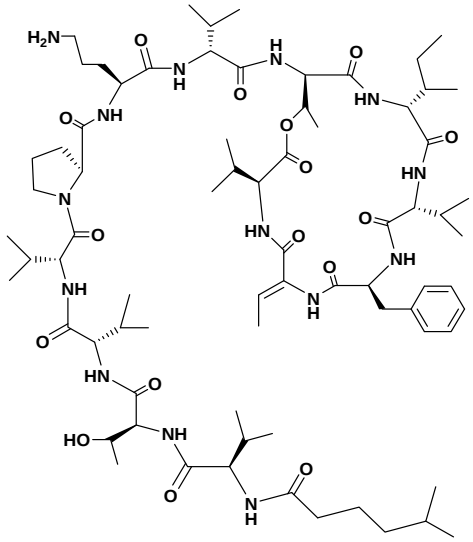
Aplidine, formally dehydrodidemnin B, was first reported in a patent application in 1989, and then referred to in the 1996 paper from Rinehart's group on SAR relationships amongst the didemnins.[4] The initial work on aplidine, its entry into Phase I and II trials and the preferred method of synthesis were described in detail through late 2004.[5] Significant numbers of Phase II clinical trials are now underway in Europe for acute lymphoblastic leukemia (ALL), lymphoma, multiple myeloma, prostate and bladder cancer. The precise MOA of this agent is not yet known, but it appears to block VEGF secretion and the corresponding VEGF-VEGF-Receptor-1 (also known as *flt-1*) autocrine loop in leukemic cells, and it has been confirmed that cells undergo apoptosis at levels of 5nM, below the blood levels achievable in man. A pharmacogenomic model has been developed leading to a molecular fingerprint for sensitivity to this agent using the "Oncochip" array,[5] and recent papers have reported that membrane cholesterol levels may influence both cellular binding and Rac1/JNK pathways, and that the level of $p27^{kip1}$ was inversely proportional to the sensitivity to aplidine.

It is very interesting that the conversion of the lactyl side chain to a pyruvyl side chain appears to significantly alter the toxicity profile when compared to didemnin B. Use of constrained

didemnin/aplidine derivatives implies that part of the biological activities reported may be due to interaction with protein-prolyl isomerases in a fashion analogous to that reported for FK506, cyclosporin and rapamycin interactions with similar enzymes, as all of the didemnin class of molecules (didemnn, aplidine and tamandarines) do exhibit immunomodulatory activities.



Didemnin B: R = OH
Aplidine: R = =O



Kahalalide F

Kahalalide F [3]

This cyclic depsipeptide was isolated from the Sacoglossan mollusk, *Elysia rufescens*, following grazing by the mollusk on a green macroalga, *Bryopsis* sp. Following isolation, it was discovered that the depsipeptide also occurs in the alga, but on a wet weight basis, the mollusk concentrated the depsipeptides significantly. It has been synthesized using solid phase peptide techniques and licensed to PharmaMar, entering Phase I clinical trials in Europe in December 2000 for the treatment of androgen-independent prostate cancer. There are a variety of mechanisms attributed to this compound. It targets lysosomes, thus suggesting selectivity for tumor cells such as prostate tumors, and has been shown to induce cell death via "oncosis" (the progression of cellular processes leading to necrotic cell death), possibly initiated by lysosomal membrane depolarization in both prostate and breast cancer cell lines. In 2005, it was reported that HepG2 cells demonstrated significant alterations in their membrane permeability at 300nM, and induction of necrosis-like cell death involving inhibition of Akt signaling and depletion of ErbB3 has been reported; thus an ErbB3 kinase inhibitor may well increase efficacy. In 2005, a PCT Application was filed by Hill *et al*. claiming production of kahalalide F and other derivatives from a *Vibrio* species isolated from *Bryopsis* and also *Elysia rufesens*. Thus there is a potential renewable source of these agents by use of fermentation.

Suggested readings:

(1) Vera, M.; Joullie, M. M. *Med. Res. Revs.* **2002,** 22, 102-145.
(2) Rinehart, K. *Med. Res. Rev.* **2000,** 20, 1-27.
(3) For a review see Henríquez, R. ; Faircloth, G. ; Cuvas, C. In *Anticancer Agents from Natural Products*; Cragg, G. M.; Kingston, D. G. I.; Newman D. J. Eds.; CRC Press: Boca Raton, 2005; pp 215 – 240.
(4) Sakai, R. et al. *J. Med. Chem.* **1996,** 39, 2819-2834.
(5) Jimeno, J.; Aracil, M.; Tercero, J. C. *J. Trans. Med.* **2006,** 4:3.

Discodermolide, a Potent Microtubule Stabilizing Compound from the Marine Sponge *Discodermia* spp.

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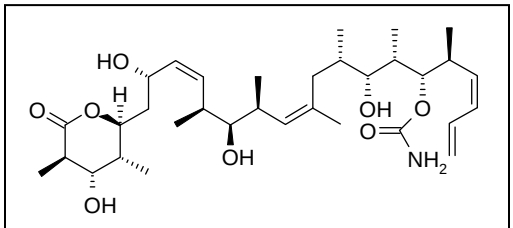
Discodermolide, **1**, is a highly functionalized polyketide that displays a unique biological profile. First isolated by Gunasekera and co-workers[1] of Harbor Branch Oceanographic Institution from the sponge *Discodermia dissoluta*, its planar structure was determined through extensive analysis of 1 and 2D-NMR spectra. The relative configuration of the 13 stereogenic centers was defined via x-ray diffraction studies, and the absolute stereochemistry was defined through total synthesis of the (-) antipode conducted by the Schreiber group.[2]



1 (+)-Discodermolide

Discodermolide was isolated based upon its *in vitro* cytotoxic properties towards the P388 murine leukemia cell line, and later demonstrated to be a potent immunosuppressive agent both *in vivo* and *in vitro* with activity comparable to that of the clinically proven immunomodulator, cyclosporine A.[3,4] Most notably, it has also been shown to be a potent inhibitor of numerous tumor cell lines with typical IC_{50} values in the low nM range, and it retains cytotoxicity against multi-drug resistant tumor cell lines that overexpress the P-glycoprotein transporter. It also showed no loss of sensitivity against two 1A9-derived cell lines bearing β-tubulin mutations that render them 20-fold less sensitive to paclitaxel.

(+)-Discodermolide was shown to block cell cycle progression at the G2/M phase in numerous tumor cell lines.[4] ter Haar and co-workers demonstrated that it rapidly induces polymerization of tubulin and can promote assembly of tubulin in the absence or presence of microtubule associated proteins (MAPS) and/or GTP.[5] The discodermolide induced-polymer differs from the paclitaxel-induced polymer in that it is completely stable at 0°C in the presence of high Ca^{2+} ion concentration. It was also shown to have a higher binding affinity for tubulin than paclitaxel, and that the binding of the two agents is mutually exclusive.[6] Experiments conducted by Horwitz, Smith and co-workers using a radiolabeled discodermolide analog bearing a benzophenone photoaffinity probe suggest that it occupies the paclitaxel binding site on tubulin, although its precise mode and orientation in the binding pocket remains undefined.[7]

Unlike the majority of other microtubule stabilizing agents, discodermolide works synergistically with paclitaxel both *in vitro* and *in vivo*, with the majority of microtubule instability parameters synergistically altered by the combination.[8,9] Similarly, arrest of the cell cycle at G2/M, as well as induction of apoptosis showed synergistic effects with the combination treatment. These data suggest strong potential for the utility of discodermolide/paclitaxel combination therapy as a novel chemotherapeutic regimen. Discodermolide has also been shown to be a potent inducer of accelerated senescence, a factor that has been proposed to contribute in part to its unique biological profile.[10] Very recently investigation as a potential neuroprotective agent has been proposed.[11] Research in this field is on-going.

The potent bioactivity and unique biological profile of discodermolide has led to it being the focus of numerous synthetic studies. To date, 12 unique syntheses have been reported and an excellent review has recently been published.[11] All of the syntheses reported to date use a

convergent approach in which three major fragments are coupled to form discodermolide. The synthesis of 1 g by the Smith group was critical to its progression into clinical trials.[12] Using synthetic methods developed by the Smith and Paterson groups along with modifications developed at Novartis, the Novartis production group led by Mickel synthesized 60 g for use in the clinical investigations.[13] This represented a monumental effort by over 43 scientists and was perhaps the first demonstration of the power of synthetic chemistry in providing clinical supplies of a complex polyketide. The production of analogs through these various synthetic schemes has allowed for significant structure activity relationships to be defined, and led to the synthesis of additional active analogs. It entered Phase I clinical trials in 2002 for the treatment of advanced solid malignancies, but these were halted in 2004 due to undisclosed toxicity issues. Given the recent data on its synergy with paclitaxel, hope remains that this promising compound can be reevaluated in the future as part of a combination therapy regime for the treatment of cancer.

(1) Gunasekera, S. P et al. *J. Org. Chem.* **1990**, *55*, 4912-4915.
(2) Nerenberg, J. B. Et al. *J. Am. Chem. Soc.* **1993**, *115*, 12621-12622.
(3) Longley R. E. et al. *Transplantation* **1991**, *52*, 650-656.
(4) Longley, R. E. et al. *Ann. N Y Acad. Sci.* **1993**, *696*, 94-107.
(5) ter Haar E. et al. *Biochemistry* **1996**, *35,* 243-250.
(6) Kowalski, R. J. et al. *Mol. Pharmacol*. 1997, 52, 613-622.
(7) Xia, S. et al. *Biochemistry* **2006**, *45*, 11762-11775.
(8) Martello L. A. et al. *Clin. Cancer Res.* **2000**, *6*, 1978-87.
(9) Honore, S. et al. *Cancer Res.* **2004**, *64*, 4957-4964.
(10) Klein L. E. et al. *Cell Cycle* **2005**, *4*, 501-507.
(11) Smith A. B. and Frieze B. S. *Tetrahedron* **2008,** *64*, 261-298.
(12) Smith A. B. 3rd et al. *Org. Lett*. **1999**, *1*, 1823-1826.
(13) Mickel, S. J. *Pure Appl. Chem.* **2007**, *79*, 685-700.

The Dolastatins

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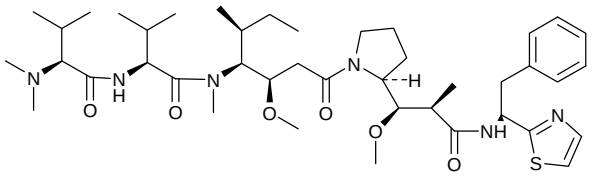
In 1965-1966, we began the first systematic investigation of marine invertebrates, vertebrates, and plants as a vast, untapped resource for discovery of promising new anticancer drugs exhibiting unprecedented structures that could provide leads to improvements in human cancer treatments. By 1968, our original expectations concerning certain marine animals as potential sources of new anticancer drugs were confirmed and reported in 1970.[1] Two years later, one of the most important early leads was found in the phylum Mollusca - specifically, a *Gastropoda* ("sea hare") species in the family Aplysiidae of the class aplysiomorpha, namely *Dolabella auricularia.*

In 1972, specimens of *D. auricularia*, were collected off the island of Mauritius. Extracts were found to more than double the life-span of mice with the P388 lymphocytic leukemia. This was a very high-priority lead that we pursued intensively, but it was not until we obtained a final 1,600 kg recollection ten years later that we were finally able to solve the extremely challenging isolation problems for the most active constituents, dolastatins 10-15, in 1984.[2] The simplest way we found to isolate the first few mgs of the key constituent, dolastatin 10, involved about 20,000 fractions, and some 23 separate chromatographic steps using various techniques. About a year was needed to solve the structure with the first milligram (amorphous powder), employing high-field NMR, high-resolution mass spectrometry, and finally total synthesis. The next

challenge was the unknown configuration of the nine chiral centers. Furthermore, to scale-up isolation for eventual clinical trials, we would have needed about 700 tons of the sea hare. For ecological and many other reasons, that was not an option. Dolastatin 10 had to be synthesized, and that required determination of the chirality. Thus we relied on our knowledge of the high-field NMR characteristics of the compound to direct the total synthetic approaches. Each of the total syntheses we completed required about 28 steps; they were not easy at the beginning, and it took 15 total syntheses to prepare the natural product, a definite improvement over the theoretical 512!



Dolastatin 10

Subsequently, enough dolastatin 10 was obtained by total synthesis for preclinical development (confirmed strong *in vitro* and *in vivo* anticancer activity, as well as inhibition of tubulin assembly),[2] and for clinical trials.[2] Eleven NCI phase II and 4 Phase I clinical trials have been completed, and no other trials are active at this time.[3] These trials (using about 400 $\mu g/m^2$) were designed prior to discovery of its tumor vascular disrupting agent activities (VDA) now being revealed for its close structural modifications.[4] One of these, soblidotin (auristatin PE; TZT-1027), is presently undergoing a number of phase II clinical trials in Japan, the U.S., and Europe.[5] Because of the VDA properties of dolastatin 10 and its analogs, future clinical trials designs should entail combinations[4,6] with other anticancer drugs, radiation, monoclonals (e.g., Avastin) and/or a chemically bonded monoclonal. Such clinical strategies are already proving successful with auristatin E, which has begun phase I clinical trials, as a desmethyl derivative linked to a monoclonal antibody,[7] and in combination with another small molecule VDA, combretastatinA-4 phosphate.

Meanwhile, we have been undertaking preclinical development of selected members of the dolastatin 11-19 series. Dolastatins 11 (actin active) and 15 are promising substances with a different antineoplastic profile than that shown by dolastatin 10. The only unit common to dolastatin 10 and dolastatin 15 is the dolavaline group. The dolastatin 15 analog, cemadotin (LU-103793),[8] has been undergoing phase II clinical trials in Europe and the U.S., while another analog, designated Tasidotin (a.k.a. Synthadotin or ILX-651), was already in clinical trials (Phase II) in 2005, progressing with schedules of 34.4 mg/m^2 - 46.8 mg/m^2.[9] Importantly, in an early trial, there was a complete response in a patient with melanoma metastatic to liver and bone at 15.4 $mg/m^2/d$, while two other melanoma patients had mixed responses at 27.3 $mg/m^2/d$ and another nine (of 36 total with advanced solid tumors) experienced stable disease.

Clearly, discovery of the dolastatins from the sea hare (and later in cyanobacteria[10]) has revealed a remarkable series of naturally occurring, unique, and powerfully bioactive peptides. Dolastatins 10 and 15 have already taken the lead in serving as templates for further structural modifications, and have led to two new anticancer drugs, each now in Phase II clinical trials. Certain members of the parent dolastatin series and their structural modifications are awaiting further preclinical development. The future, including extensions to the combination drug trials

mentioned above, bode well for witnessing further improvements in cancer treatments and as antifungal drugs.[11]

(1.) Pettit, G. R. et al. *Nature* **1970**, *227*, 962-963.

(2.) Flahive, E.; Srirangam, J. In *Anticancer Agents from Natural Products;* Cragg, G. M.; Kingston, D. G. I.; Newman, D. J., Eds.; CRC Press: Boca Raton, FL, 2005, pp 191-213.

(3.) http://www.cancer.gov/search/ResultsClinicalTrialsAdvanced.aspx?protocolsearchid=4528425; for leading references, see: (a) Perez, E. A.et al. *Invest. New Drugs* **2005**, *23*, 257-261. (b) von Mehren, M. et al. *Sarcoma* **2004**, *8*, 107-111. (c) Hoffman, M.A. et al. *Gynecol. Oncol.* **2003**, *89*, 95-98. (d) Vaishampayan, U. et al. *Clin. Cancer Res.* **2000**, *6*, 4205-4208. (e) Madden, T. et al. *Clin. Cancer Res.* **2000**, *6*, 1293-1301.

(4.) Watanabe, J. et al. *Anticancer Res.* **2006**, *26*, 1973-1982.

(5.) (a) Patel, S. et al. *Cancer* **2006**, *107*, 2881-2887. (b) deJonge, M. J. A. et al. *Clin. Cancer Res.* **2005**, *11*, 3806-3813.

(6.) Hashiguchi, N.; et al. *Anticancer Res.* **2004**, *24*, 2201-2208.

(7.) (a) Smith, L.M. et al. *Mol. Cancer Ther.* **2006**, *5*, 1474-1482. (b) Ma, D. et al. *Clin. Cancer Res.* **2006**, *12*, 2591-2596. (c) Jeffrey, S. C.; et al. *Bioconjugate Chem.* **2006**, *17*, 831-840. (d) Sutherland, M. S. K. et al. *J. Biol. Chem.* **2006**, *281*, 10540-10547.

(8.) (a) Marks, R. S. et al. *Amer. J. Clin. Oncol.* **2003**, *24*, 336-337. (b) Villalona-Calero, M. A. et al. *J. Clin. Oncol.* **1998**, *16*, 2770-2779.

(9.) (a) Mita, A. C. et al. *Clin. Cancer Res.* **2006**, *12*, 5207-5215. (b) Rasila, K. K. et al. *Curr. Opin. Invest. Drugs* **2005**, *6*, 631-638. (c) Ebbinghaus, S. et al. *Clin. Cancer Res.* **2005**, *11*, 7807-7816.

(10.) (a) Leusch, H. ; et al. *J. Nat. Prod.* **2001**, *64*, 907-910; (b) Nagle, L.M.; Gerwick. W. H.**-***J. Nat. Prod.* **2002**, *65*, 21-24.

(11) Pettit, R. K. Et al. *Antimicrob. Agents Chemother.* **1998**, *42*, 2961-2965.

Ecteinascidins- Potent Anticancer Agents from the Marine Tunicate *Ecteinascidia turbinata*

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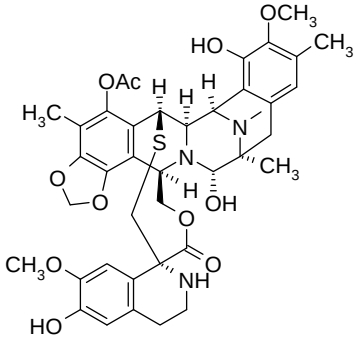
After almost 40 years of research and development, ET-743 (Yondelis™, trabectedin), derived from the ascidian *Ecteinascidia turbinata*, has been approved for marketing in Europe for the treatment of soft tissue sarcoma. This represents the first marine natural product (not an analog or derivative) approved for use in the treatment of cancer.



Ecteinascidin 743

Crude aqueous ethanol extracts of the tunicate *E. turbinata* were first reported to possess *in vivo* antitumor activity by Sigel *et al*. in 1969.[1] A number of research groups pursued the active compounds over the intervening years, with the first report of their purification being made by the Rinehart group.[2] The major compounds were named ecteinascidin 743 and 729 (ET-743 and ET-729), based upon mass spectrometric characteristics. The ecteinascidins were determined to be complex tris tetrahydroisoquinoline alkaloids using spectroscopic methods, and the structures were disclosed in back to back publications in 1990 by the Harbor Branch and University of Illinois groups.[3,4] The structure elucidation was hindered by the low quantities of the compounds present in the ascidian (approximately 0.00015% of wet weight), and poor sensitivity obtained in the NMR experiments. The introduction and availability of the 2D ^{1}H-detected heteronuclear correlation experiments HMQC and HMBC, was instrumental in allowing for the final structure

determination. A number of analogs have been reported by the Illinois group, and the compounds have been reviewed previously.[5, 6, 7]

The ecteinascidins show exceptional *in vivo* and *in vitro* activity against many tumor cell lines.[5, 6, 7] ET-743 has a unique and perhaps not yet fully understood mechanism of action. It binds in the minor groove of DNA with preference for GC-rich triplets, and forms covalent adducts with the N2-position of guanine through ET-743's C-21 carbinolamine moiety.[8] A series of hydrogen bonding interactions stabilize binding to the preferred DNA binding sequences. The covalent binding of ET-743 results in an opening of the minor groove of DNA and an unprecedented bending of DNA towards the major groove leading to its ability to interfere with transcriptional factors in a promoter-dependent way.[9] ET-743 also inhibits transcription-dependent nucleotide excision repair (NER) by trapping proteins responsible for NER, and driving cells towards apoptosis.[10]

The University of Illinois licensed ET-743 to PharmaMar SA, who later partnered with Johnson & Johnson Pharmaceutical R.& D. in its development as an anticancer treatment. Kilogram supplies of the material are produced through a semisynthetic process using cyanosafracin B as a starting material.[11] Numerous clinical trials have been conducted which have established a role for the use of ET-743 administered as a single agent for the treatment of advanced pretreated soft tissue sarcoma. ET-743 also shows promise in the treatment of pretreated ovarian and breast cancers. Preclinical evaluation of ET-743 in combination with cisplatin, paclitaxel or doxorubicin shows greater than additive effects, and clinical evaluation for the combination therapies is ongoing.

(1) Sigel, M. M. et al. *In Food-Drugs Fom the Sea*, proceedings, 1969; Youngken, H. W., Ed. Marine Technology Society; Washington DC **1970**; pp 281-295.
(2) Holt, T. G. Ph.D. Thesis, (University of Illinois, Urbana); 1986, *Chem. Abstr.* **1987**, 106, 193149u. Diss. Abstr, Int. B **1987**, *47*, 3771-3772.
(3) Wright, A. E. et al. *J. Org. Chem.* **1990**, *55*, 4508-4512.
(4) Rinehart, K. L. et al. *J. Org. Chem.***1990**, 55, 4512-4515
(5) Sakai, R. et al. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 11456-11460.
(6) Manzanares I. et al. *Curr. Med. Chem. – Anticancer Agents* **2001**, *1*, 257-276.
(7) Henriquez, R. ; Faircloth, G. ; Cuevas, C. In *Anticancer Agents from Natural Products*; Cragg, G. M.; Kingston, D. G. I.; Newman D. J. Eds.; CRC Press: Boca Raton, 2005; pp 215-265.
(8) Pommier Y. et al. *Biochem*. **1996**, *35*, 13303-13309.
(9) Zewail-Foote M., Hurley L. *J. Med. Chem*. **1999**, *42*, 2493-2497.
(10) von Mehren M. *Lancet Oncology* **2007**, *8*, 565-567
(11) Cuevas, C. et al. *Org. Lett.* **2000**, *2*, 2545-2548.

The Discovery, Structure and Biological Activity of Epibatidine

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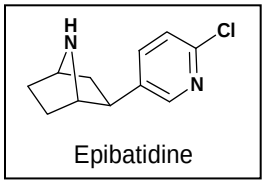
"…a glimpse of truth for which you have forgotten to ask." Joseph Conrad, 1897;
"Serendipity is usually the rule of the day" Thomas Eisner, 1999

Discovery and Structure: In 1974 John Daly and Charles Myers collected 8 populations (3-10 skins each) of the "poison frog" *Dendrobates tricolor*, most from lowland, some from upland Ecuador. Initially considered comprised of two related species (*D. tricolor* and *D. anthonyi*), then later classified as color morphs of *D. tricolor*, these were finally renamed one species,

Epipedobates tricolor. Routine toxicity screening of extracts using sc injection into mice, elicited a surprising Straub-tail (S-T) reaction from roughly one-third of the extracts. The rigid upward tail had never been seen before nor since with any of the hundreds of poison frog extracts investigated; it persisted undiminished after alkaloid partitioning, requiring substantial amounts of the alkaloid mixtures (100 mg; often 25% of the total extract) per injection, and overlaid the significant toxicity seen in all 8 collections studied. As Daly's notes record: "Initial agitation, then extreme labored breathing, jumping, becoming running convulsions in ca. 3 min. Straub-tail very marked, death, convulsions in 4 min." The S-T effect is considered diagnostic of opioid-induced analgesia, and activity was confirmed by the hot-plate assay. Daly's usual field taste test on a live frog indicated a "bitter metallic taste followed by a long-lasting (ca. 1 hr) warm sensation on tip of tongue." Many more collections of *E. tricolor* frogs frustratingly, had even less of the S-T agent, estimated now at 0.1 μg/frog (earlier estimates; ~1μg). In fact, one large collection at a banana plantation had *no* alkaloids at all. Now, in the context of the dietary hypothesis whereby it is posited that virtually all the skin alkaloids likely arise from a diet of arthropods, these frogs were likely eating a menu of fruit flies lacking the S-T-alkaloid containing arthropod. The S-T effect was absent in laboratory-raised *E. tricolor.*

Bioassay-guided fractionation gave active fractions containing a substance with nominal MS molecular weights of 208/210 (3:1), indicating the presence of one chlorine atom, shown not to be an artifact of isolation. Earlier quantitation attempts using GC with FID showed the 208/210 peak as barely detectible on the tail side of an overloaded major peak, and it was even considered to represent an impurity. Repeated attempts at chemical characterization using hydrogenations, acetylation and trimethylsilylation either lost the 208/210 material or achieved ambiguous results. Only the advent of HPLC and GC-MS gave reliably pure samples and MS data, finally proving that the S-T agent was indeed the 208/210 substance, and confirming the molecular ion [FAB-MS, thermospray and CI with NO-N_2, CH_4, iso-butane, and NH_3; HRMS: parent ion $C_{11}H_{13}N_2Cl$; major ions including chloroaromatic fragments with one or two nitrogens; characteristic base peak at *m/z* 69 (C_4H_7N); one exchangeable NH (CI/ND_3)].



Epibatidine

Given the long-term stability and tiny amount of active material left (est. 0.75mg), investigation was suspended for a decade. In 1989,extracts were examined with GC-FTIR, and the 208/210 peak indicated the likely presence of a chloropyridine moiety, agreeing with early UV data. A trial separation from the major contaminating tertiary amine alkaloids in the extract (5 μL scale) was achieved by acetylation and extraction with acid, leading to isolation of a pure 208/210 alkaloid *N*-Ac derivative. Then the entire 0.2 ml extract, the irreplaceable world's supply, was acetylated, purified and studied by ^{1}H-NMR to elucidate the structure. The name epibatidine was coined, indicating its origin from *Epipedobates tricolor*. Its structure was quickly confirmed by a synthesis by Corey.

Biological Activity: Though producing the characteristic S-T response of opioids, epibatidine was not blocked by the classical opioid antagonist naloxone, indicating operation *via* a non-opioid pathway. Nonetheless, it was clearly a potent analgesic, having 200-500 times the potency of morphine. The availability of synthetic material allowed the examination of both enantiomers for antinociceptive activity. Examination at a wide variety of neurotransmitter receptors showed epibatidine to have extra-ordinarily high affinity at nicotinic receptors, but virtually none at others (NMDA, GABA, serotonin, muscarinic) that have been implicated in various pain pathways. Furthermore, epibatidine was shown to be an agonist by eliciting sodium

flux in several cell lines, which could be blocked by the nicotinic antagonist mecamylamine, which also blocked the *in vivo* analgesic activity, but had no effect on morphine-induced analgesia, thus confirming the nicotinic receptor pathway. Curiously, two labs noted that the synthetic material appeared not to elicit a S-T response (up to 30 μg/kg) and postulated that the response may have been due to other factors. Later studies, however, showed positive responses at high concentrations, and that it was more potent (2 μg/kg) in knock-in mice bearing a gain-of-function mutation in the α4 nicotinic receptor subunit. Epibatidine produces long-term (60 min) analgesic effects compared with nicotine (2-10 min), and is 100-200 times more potent than nicotine, but its utility as a drug is limited by its high toxicity (onset of seizures) and poor therapeutic index (TI <5). However, its extremely high and extraordinarily selective affinity for nicotinic receptors made it an ideal choice as a pharmacologic probe, and radioligands were soon produced with ^{3}H, ^{125}I, ^{18}F and ^{11}C for binding and positron-emission tomographic applications. It interacts with all known subtypes of nicotinic receptors, and, at the time of its discovery, was the most potent ligand for nearly all, with subnanomolar affinities for all but the neuromuscular and homomeric α7 neuronal receptor, at which anatoxin-a is more potent. In transfected cells, it has a mild preference for β2-containing receptors and displays highest affinity at α2β2, but varies little across the series expressing α2-α4 and β2 or β4 sub-units, except for the α3β4 combination. It is a full agonist at most of the receptors at which it has been examined, except the homomeric receptors α7-α10, and has been used to both identify and help characterize several new subtypes of nicotinic receptors.

The unusually high potency and efficacy of epibatidine made it an ideal lead compound for the development of novel nicotinic ligands, and hundreds of variations on it and other agonist structures such as anatoxin-a and nicotine have been produced by companies and academic laboratories. Some have reached the clinic in the past decade. Indeed, nicotinic pharmacology has experienced a resurgence in activity due in no small measure to epibatidine, both as a lead compound, and as a research tool. Applications to a wide variety of pathologic states have been proposed, notably pain, anxiety, Alzheimer's dementia, Parkinson's disease, Tourette's syndrome, schizophrenia, and tobacco addiction. The development of epibatidine-derived and inspired compounds with increased selectivity holds great promise for the treatment of many of these conditions.

Epothilones

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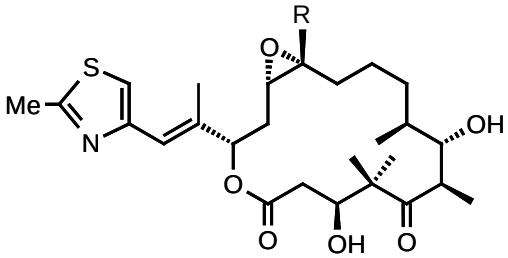
In 1987, during a screening of the common soil bacterium *Sorangium cellulosum*, strain So ce90 was identified as producer of a promising antifungal activity. From its culture extract two novel closely related macrolides, later named epothilone A and B, were isolated. Their activity against plant pathogenic fungi and immune suppressive activity were investigated for some time by industry. Later considerable cytotoxicity at the lower nm level was confirmed in the 60-cell line panel by the NCI. However, in spite of good patent protection,[1] there was no interest by pharma companies in a potential antitumor application, and the compounds were dropped from the GBF research program.



Epothilone A (R = H)
Epothilone B (R = Me)

Fortunately, the epothilones were later re-discovered during a screening of natural products for taxol mimics by Merck Sharp & Dohme in 1995.[2] Among the 7,000 extracts screened, one hit was observed for a *Sorangium cellulosum* strain (SMP44) obtained from John E. Peterson (Emporia State University, Kansas). The activity was traced back to two compounds identified as epothilone A and B. This immediately revealed the mode of action of epothilones: promotion of tubulin polymerization. Competition experiments indicated that both compounds, taxol and epothilone, share the same or overlapping binding sites in β-tubulin. Further, and most importantly, the activity of epothilones was hardly impaired by the resistance of cancer cells to taxol and other cytotoxic agents.[2]

After the absolute configuration of the epothilones had been revealed,[3] world-wide activities towards total synthesis started in late 1995. First syntheses of epothilone A were published by the groups of Danishefsky, Nicolaou and Schinzer a year later, followed by those of epothilone B. Meanwhile around 20 independent syntheses of the major natural epothilones have been published making these compounds some of the most often synthesized natural products in recent years. In addition to natural epothilones, many more structural analogs were synthesized to elucidate the structure/activity relationships.[9]

After elucidation of the biosynthesis of epothilones by feeding studies at GBF, the biosynthetic gene cluster was cloned from the strains So ce90 and SMP44 by groups from Novartis and KOSAN Biosciences, respectively. This opened access to a variety of structural modifications in the northern ring-segment as well as the side-chain by genetic engineering and heterologous expression. However, most of these new compounds were produced in minute amounts, and only few reached a level which has been obtained by classical mutation. Also the notorious problem of formation of the C13 homologues of the A/B-type could not be solved. Similarly, great hopes for structural optimization by molecular modeling of epothilones in the protein binding site has not materialized, so far. Various models have been proposed, and even a structure solved by electron crystallography and extensive computing was questioned later.[4]

After strain improvement by mutation and process optimization, 100 g of epothilone B had been produced at GBF by 1997, and semisynthesis programs were started at GBF and Bristol-Myers to optimize the pharmacological profile and therapeutic window. Two clinical candidates emerged from this work, epothilone B lactam, ixabepilone, [5] and 21-amino-epothilone B. First *in vivo* studies of epothilones in mouse xenograft models were published by Danishefsky's group at the Sloan Kettering Cancer Center indicating a rather narrow therapeutic window for epothilone B, the most active natural variant, while that for the less active 12,13-desoxy analog, epothilone D, was remarkably wide.[6] Both compounds were entered into clinical trials, as patupilone by Novartis, and as KOS-862 by KOSAN Biosciences.

From the beginning Schering AG relied on total synthesis for structure optimization. An epothilone B analog with 5-allyl and 15-benzothiazole substituents was introduced as sagopilone into clinical trials.[7] Recently, iso-fludelone, a synthetic analog of epothilone D with an extra 9,10-double bond, a 13-trifluoromethyl group and an isoxazole ring in the side chain, was presented as a clinical candidate, supposed to replace epothilone D and fludelone.[8]

In October, 2007, ixabepilone was approved by the FDA for the treatment of advanced breast cancer and brought to the market by BMS under the trade name IXEMPRA.

(1) Höfle G.; Bedorf N.; Gerth K.; Reichenbach H. (GBF) German Patent 4138042, filed Nov 11, **1991**; World Patent 9310121 filed Nov 19, **1992**.

(2) Bollag D. M.; McQuenay P. A.; Zhu J.; Lazarides E.; Hensens O.; Koupal L.; Liesch J.; Goetz M.; Woods C. *Cancer Res*. **1995**, *55***,** 2325.

(3) Höfle G.; Bedorf N.; Steinmetz H.; Schomburg D.; Gerth K.; Reichenbach H. *Angew. Chem. Int. Ed.* **1996**, *35*, 1567.

(4) Heinz D. H.; Schubert W.-D.; Höfle G. *Angew. Chem. Int. Ed*. **2005**, *44*, 1298.

(5) Borzilleri, R.M. et al. *J. Am. Chem. Soc.* **2000**, *122*, 8890.

(6) Chou T.-C.; Zhang X.-G.; Balog A.; Su D.-S.; Meng D.; Savin K.; Bertino J. R.; Danishefsky S. J. (1998) *Proc. Natl. Acad. Sci*. **1998**, *95*, 9642.

(7) Klar U.; Buchmann B.; Schwede W.; Skuballa W.; Hoffmann J.; Lichtner R. B. *Angew. Chem. Int. Ed Engl*. **2006**, *45*, 7942.

(8) Chou T.-C.; Dong H.; Rivkin A.; Yoshimura F.; Gabarda A. E.; Cho Y. S.; Tong W. P.; Danishefsky S. J. *Angew. Chem. Int. Ed*. **2003**, *42*, 4762; http://www.publichealth.pitt.edu/supercourse/SupercoursePPT/30011-31001/30581- 30781.pdf

(9) For reviews see e.g.: Höfle, G.; Reichenbach, H. In *Anticancer Agents from Natural Products*; Cragg, G. M.; Kingston, D. G. I.; Newman D. J. Eds.; CRC Press: Boca Raton, 2005; pp 413 – 450; Reichenbach, H.; Höfle, G. *Drugs R&D*, **2008**, 1 – 10; Altmann, K. H.; Höfle G.; Müller, R.; Mulzer, J. In *Progress* in the *Chemistry of Organic Natural Products* Kinghorn, A. D.; Falk H.; Kobayashi J.; Eds.; Springer-Verlag: Berlin, 2008, in print.

HTI-286, A Synthetic Analogue of the Antimitotic Natural Product Hemiasterlin

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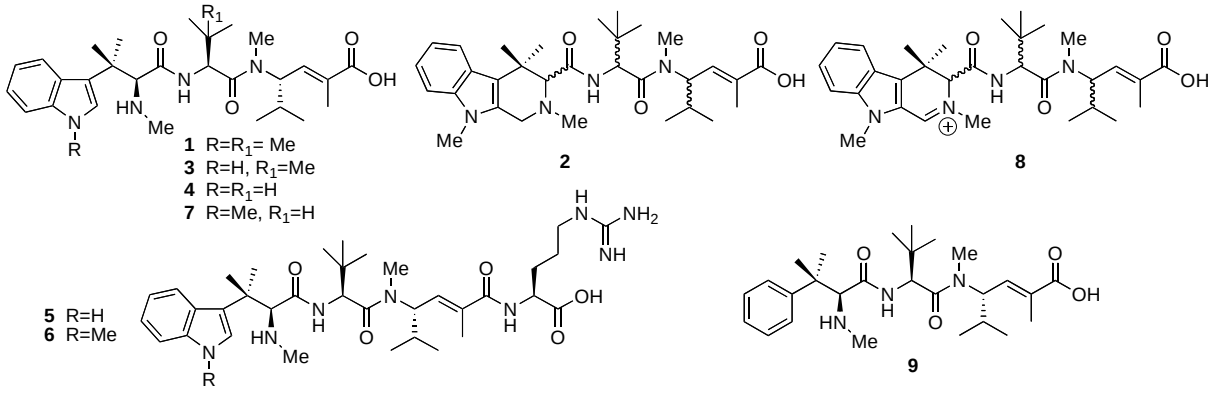
The hemiasterlins, criamides, and milnamides are a small family of tri- and tetrapeptide cytotoxins isolated from marine sponges. Hemiasterlin (**1**) was first isolated by Kashman in 1994 from the sponge *Hemiasterella minor* collected in South Africa.[1] At virtually the same time, Crews reported the isolation of milnamide A (**2**) from the sponge *Auletta* cf. *constricta* collected in Papua New Guinea.[2] In 1995, Andersen and Allen reported the isolation of **1**, hemiasterlins A (**3**) and B (**4**), and criamides A (**5**) and B (**6**) from the sponge *Cymbastela* sp. collected on reefs off Madang, PNG and they showed that all the amino acids in the hemiasterlins and criamides had the L configuration.[3,4] Boyd subsequently reported finding **1**, **3**, and hemiasterlin C (**7**) in an *Auletta* sp. collected in PNG[5] and Ireland reported isolating **1**, **2**, and the new compound milnamide D (**8**) from *Cymbastela* sp. collected in Milne Bay, PNG.[6]



Kashman and Crews reported that hemiasterlin and milnamide A showed in vitro cytotoxicity.[1,2] Andersen and Allen reported that hemiasterlin had an IC_{50} of 87 pM versus murine leukemia P388 and potent activity against a small panel of human cancer cell lines.[3] Roberge found that hemiasterlins blocked human mammary carcinoma MCF-7 cells in mitosis at the same concentrations that showed cytotoxicity.[7] Examination of the hemiasterlin mitotic arrest phenotype using immunofluorescence microscopy showed that the cells arrested at a metaphase-like stage with effects on the morphology of the mitotic spindle that were similar to those caused by tubulin-depolymerizing agents, such as vinblastine and nocodazole. Lassota at Wyeth and Bai *et al.* at NCI subsequently confirmed that hemiasterlin is a noncompetitive inhibitor of the binding of vinblastine to tubulin and inhibits competitively the binding of dolastatin 10.[8] They further demonstrated that hemiasterlin inhibits nucleotide exchange on β-tubulin, and that it induces the formation of tubulin aggregates with a ring-like structure and a diameter of about 40 nm. Overall, these effects resembled, but were not identical to, those of dolastatin and cryptophycin, which bind to the same site in tubulin.

Andersen and Piers reported the first total synthesis of hemiasterlin in 1997,[9] and they also prepared a small library of analogs.[10] The phenylalanine analog **9**, initially designated SPA110 (Synthetic Peptide Analog 110), was roughly 3 fold more potent than hemiasterlin in Roberge's cell-based antimitotic assay (SPA110 IC_{50} 0.08 nM; hemiasterlin IC_{50} 0.3 nM) and its synthesis was considerably shorter and more efficient.[10] On the basis of its relative ease of synthesis and enhanced potency, Wyeth Pharmaceuticals licensed SPA110 from UBC and they gave it the code number HTI-286 (Hemiasterlin Tubulin Inhibitor 286).

HTI-286 showed broad *in vitro* antiproliferative activity, independently of tumor origin, with an average IC_{50} of 2.5 ± 2.1 nM and it was more potent than paclitaxel (IC_{50} = 128 ± 369 nM) for all cell lines. Importantly, cells expressing P-glycoprotein, and consequently resistant to many drugs including paclitaxel, retained nearly complete sensitivity to HTI-286 and the KB-8-5 cell line, that has been selected for expression of drug efflux pumps by chronic drug exposure, also retained sensitivity to HTI-286. Wyeth showed that HTI-286 administered intravenously has *in vivo* efficacy against a variety of xenograft tumor models and was able to regress even large established tumors. HTI-286 had efficacy when administered orally and it displayed *in vivo* efficacy against tumors derived from cell lines with inherent or acquired multidrug resistance.[11]
A phase I clinical trial was carried out in 2002 with patients with metastatic or advanced-stage malignant solid tumors to assess the safety, tolerability, and pharmacokinetics of HTI-286.[12] Pain, hypertension and neutropenia were observed as dose-limiting toxicities. A phase II open-label study of HTI-286 as a single agent for the treatment of non-small cell lung cancer for

disease re-occurrence following platinum-based therapy was carried out by Wyeth. HTI-286 produced some favorable responses and a high level of stable disease in this study. The results have not been published and HTI-286 is awaiting further evaluation.

(1) Talpir, R., et al., *Tetrahedron Lett.* **1994**, *35*, 4453-4456.
(2) Crews, P., et al., *J. Org. Chem.* **1994**, *59*, 2932-2934.
(3) Coleman, J. E., et al., *Tetrahedron* **1995**, *51*, 10653-10662.
(4) Coleman, J. E., et al., *Acta Crystal.* **1996**, *52*, 1525-1527.
(5) Gamble, W. R., et al., *Bioorg. Med. Chem.* **1999**, *7*, 1611-1615.
(6) Chevallier, C., et al., *Org. Lett.* **2003**, *5*, 3737-3739.
(7) Anderson, H. J., et al., *Cancer Chemother. Pharmacol.* **1997**, *39*, 223-26.
(8) Bai, R., et al., *Biochemistry* **1999**, *38*, 14302-14310.
(9) Andersen, R. J., et al., *Tetrahedron Lett.* **1997**, *38*, 317-320.
(10) Nieman, J., et al., *J. Nat. Prod.* **2003**, *66*, 183-199.
(11) Loganzo, F., et al., *Cancer Res.* **2003**, *63*, 1838-1845.
(12) Ratain, M. J., et al., *Proc. Am. Soc. Clin. Oncol.* **2003**, *22*, 129.

Homoharringtonine

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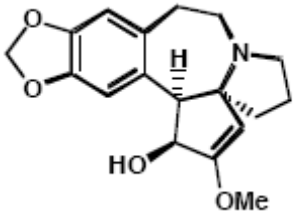
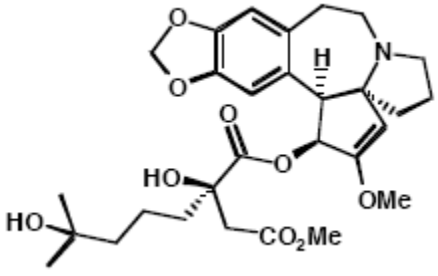
Cephalotaxus harringtonia, a small tree native to Japan and China, had been introduced as an ornamental, but had gained little popularity in the U.S. prior to 1960. Seed of *C. harringtonia* was included in the germplasm collection at the Northern Regional Research Laboratory (NRRL, now NCAUR) for investigation as a potential new crop. This slow growing tree was low on the priority list; however, Ivan Wolff and Cecil Smith included an extract of *C. harringtonia* seed as one of 200 extracts that were submitted to the antitumor screen of the National Cancer Institute (NCI) by NRRL around 1960. Initial test results were encouraging as the extract showed significant *in vivo* activity against L-1210 and P388 leukemia in mice, and Jonathan Hartwell (NCI) soon placed *Cephalotaxus* on the priority list for identification of the active components.

Antileukemic activity of the *Cephalotaxus* extract was traced to the alkaloid fraction. The Paudler group[1] reported cephalotaxine to be the major *Cephalotaxus* alkaloid in 1963; however, cephalotaxine was inactive. Re-collections of *Cephalotaxus* samples were difficult to obtain, and limited in amounts, and testing of fractions was slow; but by 1970 the cephalotaxine esters harringtonine, isoharringtonine and homoharringtonine were identified as the active principles.[2,3]



cephalotaxine



homoharringtonine

NCI requested quantities of these alkaloids sufficient for preclinical trials. However, there was no known source of *Cephalotaxus* plants in the US. Attempts to obtain material from China and Japan all failed, and growing quantities of the plant material from seed or cuttings would have taken many years. Robert Perdue, then of the USDA, Beltsville, MD, in cooperation with NCI,

was able to obtain 1000 lbs of *C. harringtonia* var. *harringtonia* cv. Fastigiata, 17 entire trees including roots, from a nursery in Oregon. Extraction of this collection at NCAUR yielded 330 g of mixed alkaloids and, ultimately, 16.6 g of homoharringtonine which was selected for preclinical studies.[4] Availability of plant material was less of a problem in China, and George R. Pettit, a member of the National Academy of Sciences Delegation to the People's Republic of China, reported that homoharringtonine was being prepared there for clinical trials in June 1974.[5] China has continued to be the major supplier of *Cephalotaxus* alkaloids.

Esterification of the more abundant cephalotaxine to harringtonine or homoharringtonine was a logical approach to the supply problem; however, this proved to be much more difficult in the laboratory than on paper. The difficulties in obtaining *Cephalotaxus* alkaloids in desired quantities, and the novel and relatively complex structures, led many to attempt synthetic approaches to cephalotaxine and its esters with varying levels of success.[6] Tomas Hudlicky and his group described a stereospecific total synthesis of homoharringtonine in 1983,[7] and reviewed synthetic work on these alkaloids in 1987.[8]

Homoharringtonine (HHT) has shown encouraging activity in preclinical and Phase I-II trials in patients with hematologic and some solid tumors. Early studies in China reported high response rates in patients with leukemia,[9] and recent studies in the United States and England have shown promising results in patients with chronic myeloid leukemia.[10,11] Itokawa, Wang and Lee reviewed the chemistry and pharmacology of homoharringtonine and related alkaloids in 2005.[12]

Homoharringtonine has been granted orphan drug status by the FDA, patents have been granted for semi-synthetic preparation of HHT and derivatives[13] and for treatments of chronic myelogenous leukemia that include HHT,[14] and HHT is being promoted by ChemGenex Pharmaceuticals under the trademark Ceflatonin®. These actions have encouraged expanded clinical trials and evaluation of the potential of this drug for treatment of various malignant diseases.

(1) Paudler, W. W.; Kerley, G. I.; McKay, J. *J. Org. Chem.* **1963**, *28*, 2194-2197.
(2) Powell, R. G.; Weisleder, D.; Smith C. R. Jr.; Rohwedder, W. K. *Tetrahedron Lett.* **1970**, 815-818.
(3) Powell, R.G.; Weisleder, D.; Smith, C. R. Jr. *J. Pharm. Sci.* **1972**, *61*, 1227-1230.
(4) Powell, R.G.; Rogovin, S. P.; Smith, C. R. Jr. *Ind. Eng. Chem. Prod. Res. Dev.* **1974**, *13*, 129-132.
(5) Pettit, G. R. *The China Quarterly* **1976**, *68*, 789-796.
(6) Smith, C. R. Jr.; Mikolajczak, K. L.; Powell, R. G. *Anticancer Agents Based on Natural Product Models*; Cassady, J. M.; Douros, J. D., Eds.; Academic Press: New York, 1980; Chapter 11, pp 391-416.
(7) Hiranuma, S.; Shibata, M.; Hudlicky, T. *J. Org. Chem.* **1983**, *48*, 5321-5326.
(8) Hudlicky, T.; Kwart, L. D.; Reed, J. W. *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; John Wiley & Sons: New York, 1987; Vol. 5, Chapter 5, pp 639-690.
(9) Kantarjian, H. M.; Talpaz, M.; Santini, V.;Murgo, A.; Chenson, B.; O'Brien, S. M. *Cancer* **2001**, *92,* 1591-1605.
(10) O'Brien, S.; Kantarjian, H.; Keating, M.; Beran, M.; Koller, C.; Robertson, L. E.; Hester, J.; Rios, M. B.; Andreeff, M.; Talpaz, M. *Blood* **1995**, *86*, 3322-3326.
(11) Marin, D.; Kaeda, J. S.; Andreasson, C.; Saunders, S. M.; Bua, M.; Olavarria, E.; Goldman, J. M.; Apperley, J. F. *Cancer* **2005**, *103*, 1850-1855.
(12) Itokawa, H.; Wang, X.; Lee, K-H. *Anticancer Agents from Natural Products*; Cragg, G. M.; Kingston, D. G. I.; Newman, D. J. CRC Press: Boca Raton, 2005, pp 47-70.
(13) Robin, J-P.; Blanchard, J.; Chauviat, L.; Dhal, R.; Marie, J-P.; Radosevic, N. U.S. Patent 7,169,774, 2007.
(14) Robin, J-P.; Mahon, F.; Maisonneuve, H.; Maloisel, F. Blanchard, J. U.S. Patent 6,987,103, 2006.

Maytansinoids/Ansamitocins

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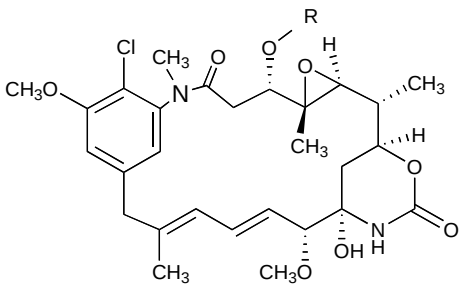
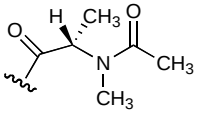
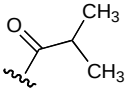
Maytansine, the parent compound of this group of potent antitumor agents, was discovered in 1972 by S. M. Kupchan and co-workers[1] by bioassay-guided fractionation of extracts of the Ethiopian shrub *Maytenus serrata*, under the auspices of the National Cancer Institute's (NCI) plant antitumor agent program. Its structure (**I**), determined by X-ray crystallography of a derivative, revealed it to belong to the family of ansamycin antibiotics of (generally) microbial origin. Since this initial report, about 50 additional members of this group of compounds have been isolated by several investigators from the same and closely related plants and from members of two unrelated plant families.[2] Significantly, the closely related ansamitocins (**II**), showing equally potent antitumor activity, were subsequently isolated from the Actinomycete, *Actinosynnema pretiosum*, by Japanese investigators.[3]



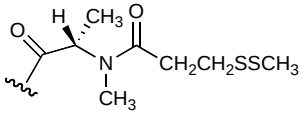
I : Maytansine, R =



II : Ansamitocin P-3, R =



III : DM1, R =



IV : Maytansinol, R = H

Its extraordinary potency (ED_{50} KB cells 10^{-4} – 10^{-5} μg/ml) against various types of tumors *in vitro* and *in vivo* made **I** a high-priority target for clinical development by the NCI. Extensive synthetic efforts led to three total syntheses of **I** by the groups of A. I. Myers, E. J. Corey and T. Goto by the early 1980s.[2] Studies on structure-activity relationships revealed that the presence of an ester group at C-3, with the natural configuration at this stereocenter, and of the cyclic carbinolamide structure with a free OH-group are essential for high level antitumor activity. Modifications of most other functional groups around the ring structure modulated activity, but did not abolish it. The mode-of-action of the maytansinoids has been traced to inhibition of tubulin-polymerization by binding to the β-subunit at a site overlapping the vincristine binding site, and different from the colchicine binding site.[2]

Following preclinical pharmacology studies,[4] **I** was entered into clinical trials by the NCI in 1975. Based on some partial responses in Phase I, Phase II trials of the compound alone and in combination with other agents were carried out from 1977-1984. These proved rather disappointing; of over 800 patients with more than 35 different tumor types, only 1 patient showed a complete response and 20 showed partial responses.[5] The failure of **I** in the clinic is probably due to dose-limiting toxicity.

Interest in the clinical potential of maytansinoids was, however, resurrected by work at Immunogen, Inc., Cambridge, MA, who developed a derivative of **I**, called DM1 (**III**), which can be conjugated to tumor-specific antibodies as a targeted delivery vehicle.[6] DM1 is

synthesized from ansamitocin P-3, obtained by fermentation, via maytansinol (**IV**). The disulfide is reduced and the resulting thiol then reacted with a thiol group on a linker attached to the antibody. Based on very promising initial results in animals[7] the technology has been developed further at Immunogen and also licensed to a number of other companies to generate and evaluate conjugates with their own, proprietary antibodies. Several of these immunoconjugates are in Phase I and II clinical trials. Folate-DM1 conjugates are also being evaluated as delivery vehicles to cancer cells expressing folate receptors.[8]

With a view towards possibly using the ansamitocin biosynthetic machinery to generate analogs of the natural products, Yu, Floss, and coworkers cloned, sequenced, and analyzed the ansamitocin biosynthetic gene cluster from *A. pretiosum*.[2] The genes are located in two clusters and encode 4 large proteins constituting a modular type I polyketide synthase (PKS), two sets of proteins responsible for formation of the PKS starter unit, 3-amino-5-hydroxybenzoic acid, and of a unique chain extension substrate, 2-methoxymalonyl-ACP, respectively, and 6 proteins catalyzing the post-PKS finishing reactions. This work established the detailed biosynthetic pathway to **II**. It was hoped that one of the post-PKS enzymes, the acyltransferase Asm19, could be useful for the more efficient preparation of DM1, based on the expectation that esterification of **III** to **II** would be the last step in the biosynthesis. This is, unfortunately, not the case; 3-*O* esterification occurs before epoxidation and N-methylation, and **III** is not a substrate of the enzyme.[9]

Using the tools of molecular biology, Leistner and co-workers recently provided strong evidence that the maytansinoids found in higher plants are not produced by the plant itself, but by an affiliated microorganism.[10] A candidate organism has been isolated and identified as a new species of *Kitasatospora* .[11]

(7) Kupchan, S. M. et al. *J., Am. Chem. Soc*. **1972**, *94*, 1354-1356.
(8) Reviews: (a) Cassady, J. M., et al. *Chem. Pharm. Bull*. **2004,** *52,* 1-26); (b) Yu, T.-W.; Floss, H. G. In *Antitumor Agents from Natural Products*, Cragg, G. M.; Kingston, D. G. I.; Newman, D. J. Eds.; Taylor & Francis: Boca Raton, Florida, 2005, pp 321-337.
(9) Higashide, E. et al. *Nature* (London) **1977,** *270,* 721-722.
(10) Issell, B. F.; Crooke, B. F. *Cancer Treatment Revs*. **1978,** *5,* 199-207.
(11) Annual Report to the FDA by DCT, NCI, on Maytansine, NSC153858, IND11857, February 1984.
(12) Widdison, W. C. et al. *J. Med. Chem*. **2006,** *49,* 4392-4408.
(13) Liu, C. et al. *Proc. Nat. Acad. Sci. USA* **1996,** *93,* 8618-8623.
(14) Reddy, J. A. et al. *Cancer Res*. **2007,** *67,* 6376-6382.
(15) Moss, S. J. et al. *J. Am. Chem. Soc*.**2002,** *124,* 6544-6545.
(16) Pullen, C. B. et al. *Phytochemistry* **2003,** *62,* 377-387.
(17) Groth, I. et al., *Int. J. Syst. Evol. Microbiol*. **2003,** *53,* 2033-2040.

NICOSAN^{TM}: An Anti Sickling Drug from Traditional Medicine

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NICOSAN^{TM} (formerly NIPRISAN or NIX-0699), a Traditional Medicine (TM) and a phytopharmaceutical is a formulation of the extracts of four plants *(Eugenia caryophyllum, Piper guinensis, Pterocarpus osun and Sorghum bicolor)*, that was being used by the Traditional Health Practitioners (THPs) in the villages in Nigeria as a decoction for sickle cell patients. The

Sickle Cell Disease (SCD) is a genetic hemoglobinopathy that primarily afflicts Africans, and now has been found in Turkish, Greek, Saudi Arabian, Egyptian, Iranian, Italian, Latin American, and Asiatic Indian populations.

Rev. P.O. Ogunyale, a Traditional Health Practitioner brought the product to the attention of Dr. Charles Wambebe, the then Director General of the National Institute for Pharmaceuticals Research and Development (NIPRD), Abuja, Nigeria. Toxicological and pharmaceutical studies at NIPRD of the freeze-dried extract in rats showed that it does not contain toxic material, and the product did not significantly alter the liver and kidney function in rats.[1, 2]

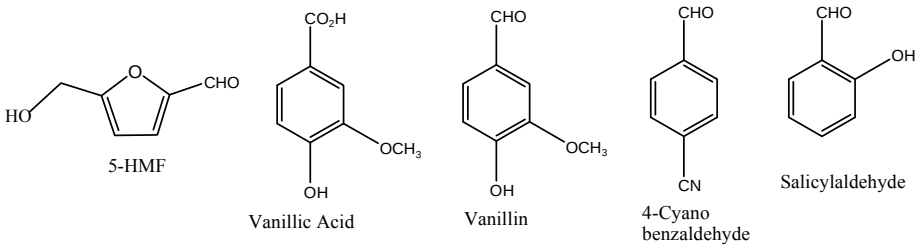
Further studies at NIPRD on gross behavior, pain, inflammation, and polymerization of hemoglobin (HbS) indicated that it is safe and has a profound activity against the polymerization of HbS. It was concluded that it may have the possibility to use for the management of SCD.[2]

After Phase I clinical trials, a randomized, double-blind, placebo-controlled, crossover Phase II clinical trial on patients[3,4] who met the criteria for homozygous SCD and had three painful or vaso-occulusive crises per year, found that the product was efficacious in reducing the painful episodes and increased school attendance of patients.

Dr. Ramesh Pandey, President & CEO of Xechem International licensed the product from NIPRD in July, 2002 for further development. While using various preparations of NICOSAN™, extensive variability in the biological activity was observed. To control the batch-to-batch variability, further research and development was carried out at the Xechem Research Laboratories in New Brunswick, New Jersey. Analytical HPLC methods using various detection systems and protocols for QA/QC and standardization of the batches were developed, followed by process development for scale-up production.

The anti-sickling effect of NICOSAN™ was confirmed by Prof. Toshio Asakura and his group at the Sickle Cell Disease Reference Laboratory of the NIH National Heart, Lung and Blood Institute located at the Children's Hospital of Philadelphia using *in vitro* assays, followed by testing in transgenic (Tg) sickle cell mice under acute hypoxic conditions.[5, 6]

The goal was set to gain the approval of the US Food and Drug Administration (FDA) for use of NICOSAN™ as a prescription drug. To accomplish this, compounds present in the NICOSAN™ were identified and checked for their anti-sickling effects. Some of the identified compounds are shown below. It was found that NICOSAN™ contains various aromatic aldehydes that combine with sickle hemoglobin and inhibit cell sickling by hydrating sickle erythrocytes at very low concentrations.[7, 8] The individual compounds did not have as good an activity as the whole mixture. Also, if the mixture was fractionated and fractions checked for anti-sickling effect,[8-11] they were less active and developed hemolytic toxicity, which suggests that NICOSAN™ components have a synergistic effect in the prophylactic management of the disease. Based on all the collected data, the FDA granted "Orphan Drug" designation to NICOSAN™ on September 2nd, 2003 for the prophylactic management of SCD. Similarly on August 30th, 2005 the European Medicine Evaluation Agency (EMEA) also designated NICOSAN™ as "Orphan Drug" for European Union (EU) countries.



The product received marketing approval from the Nigerian Regulatory Authorities, the National Agency for Food, Drug Administration and Control (NAFDAC), on July 3rd, 2006, and NICOSAN™ was launched in Nigeria on July 6th, 2006. NICOSAN™ is manufactured at the Abuja facilities of Xechem Pharmaceuticals Nigeria., Ltd,

(1) Awodogan, A. O. et al. *J Pharmaceutical Research and Development.* **1996,** *1*, 39-45.
(2) Gamaniel, T. et al. *J. Pharmaceutical Research and Development.* **1998,** *3*, 89-94.
(3) Wambebe, C. et al. *Curr Ther Res.* **2001,** *62*, 26-34.
(4) Wambebe, C. et al. *Phytomedicine.* **2001,** *8*, 252-261.
(5) lyamu, E. W. et al. *Brit J Haematol.,* **2002,** *118*, 337-343.
(6) lyamu, E. W. *et al.* Brit. J. Haematol. ***2003,*** 122, *1001-1008.*
(7) Gillette, P. N. *et al.* Fourth International Healthcare and Herbal Expo and Seminar, April 2-4, New Delhi, India, ***2004,*** *Source Book, pp. 141-149.*
(8) Gillette, P. N. et al. *27th Annual Meeting of the National Sickle Cell Disease Program, April 18-21, Los Angeles, California,* **2004**, Abst. P: 104.
(9) Tripathi, P. et al. *28th Annual Meeting of the National Sickle Cell Disease Programs, April 9-13, Cincinnati, Ohio,* **2005.**
(10) Pandey, R. C. et al. *In Vitro Biology Meeting, June 5-7, Baltimore, Maryland,* **2005.**
(11) Pandey, R. C. et al. *34th Annual SCDAA Conference, September 27th – 30th, Dallas, Texas,* **2006.**

Podophyllotoxin and Derivatives

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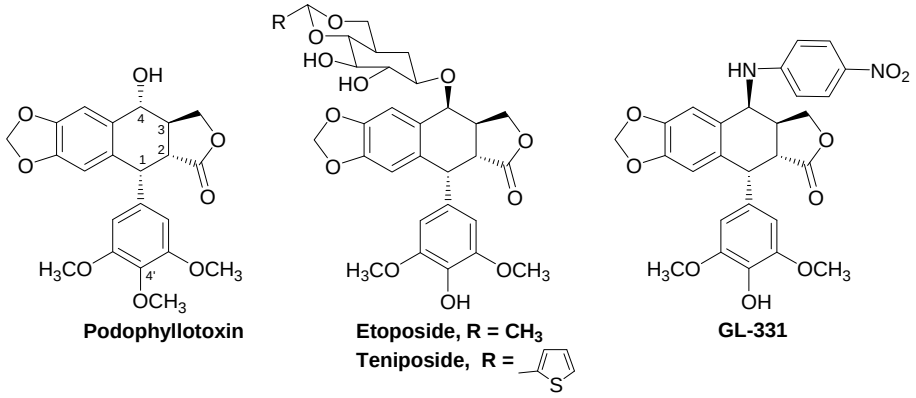
Podophyllum plants, including the American *P. peltatum* L. and Tibetan *P. emodi* Wall., were long prized as medicines by the native populations of North America and the Himalayas. The complex path from the plant (*Podophyllum*) to bioactive lead compound (podophyllotoxin) to clinically used drugs (etoposide and teniposide) and forward to new generation anticancer candidates (including GL-331 from the author's laboratories) illustrates the successful development of clinically useful drugs from natural sources.



In 1942, studies found that podophyllin, the alcoholic extract of *Podophyllum* rhizome, exhibited curative effects on the benign tumor *Condylomata acuminate*.[1] Further research showed that its major constituent, podophyllotoxin, inhibits assembly of the mitotic spindle[2] and was effective against tumors in mice.[3] However, the hopes for developing podophyllotoxin as a cancer chemotherapeutic drug were offset by its unacceptable side effects, particularly gastrointestinal toxicity.[4]

Studies on *Podophyllum* glycosides at Sandoz, Ltd. in the 1950s led to the opportune discovery of etoposide (**2**) and teniposide (**3**) in the late 1960s. These two compounds displayed significant antineoplastic effects.[5] In 1983, the FDA approved etoposide for the treatment of testicular cancer, and in 1992, teniposide was brought into the U.S. market. These drugs are currently used against many cancers, including small cell lung cancer, testicular cancer, lymphoma, leukemia, and Kaposi's sarcoma.[6]

Structurally, podophyllotoxin is an aryltetralinlactone cyclolignan with five rings: a methylene-dioxy ring A, tetrahydronaphthalene rings B and C, and lactone ring D, which together make a four-ring pseudoplane, and a pendant aryl ring E, which is attached pseudo-axially at C-1. Unique and important structural features are the configurations at the four asymmetric centers ($C_{1\text{-}4}$) and the highly strained *trans*-lactone D ring.

Mechanistically, podophyllotoxin binds reversibly to cellular tubulin, inhibits mitotic spindle formation, disturbs the dynamic equilibrium between microtubule assembly/disassembly, and eventually causes mitotic arrest. Epimerization at C-4 on the C-ring from α, as in podophyllotoxin, to β, as in etoposide, shifts the molecular target from tubulin to DNA topoisomerase II (topo II), an essential enzyme in DNA cleavage/religation.[6] Etoposide and related compounds inhibit the catalytic activity of topo II, and induce topo II-mediated double-strand DNA breakage, which eventually leads to cell death.[7] The structural preferences of topo II inhibitors over antimicrotubule agents have been roughly identified as: 1) 4β-configuration, 2) 4β-bulky substitution, and 3) 4'-demethylation (hydroxy rather than methoxy group at C-4' on the E-ring).[8]

Although etoposide and teniposide are commonly used anticancer drugs, they still suffer from problems such as acquired drug-resistance and poor water-solubility. To overcome these problems and generate new generations of clinical trial candidates, extensive research studies have continued.[9] In the author's laboratories, a notable synthetic modification was the introduction of nitrogen at the 4β-position.[10-13] From the many analogs synthesized, GL-331 emerged as a clinical trials candidate. GL-331 has a *p*-nitro anilino group rather than the sugar moiety in etoposide. Compared with etoposide, it causes more double-strand breaks and mitotic arrest and is also more potent against tumor cells.[11] Remarkably, it overcomes multidrug resistance in many cancer cell lines. GL-331 showed marked antitumor efficacy, with minimal side effects, in Phase I clinical trials against non-small and small cell lung, colon, and head/neck cancers. Phase II clinical trials against several cancers, especially etoposide-resistant, are being planned.[14]

The author's laboratories have also applied computer modeling techniques to aid continued chemical efforts to produce improved analogs. Comparative molecular field analysis (CoMFA) models[15] prompted synthesis of new compounds that were more active than etoposide in both cytotoxicity and topo II inhibition assays.[16] Variable selection *k* nearest neighbor modeling methodology was also applied,[17] and its high predictive ability should guide the rational design

of novel epipodophyllotoxin derivatives, and aid the search for bioactive structures from large databases (Aided by NIH Grant CA-17625 from the National Cancer Institute).

(1) Kaplan, I.W. *New Orleans Med. Surg. J.* **1942**, *94*, 388.
(2) Sullivan, B.J.; Wechsler, H.I. *Science* **1947**, *105*, 433.
(3) Hartwell, J.L.; Shear, M.J. *Cancer Res.* **1947**, 7, 716-717.
(4) Canel, C.; Moraes, R.M.; Dayan, F.E.; Ferreira, D. *Phytochemistry* **2000**, *54*, 115-120.
(5) Stähelin, H.; von Wartburg, A. *Cancer Res.* **1991**, *51*, 5-15.
(6) Zhang, Y. L.; Lee, K.H. *Chin. Pharm. J.* **1994**, *46*, 319-369.
(7) Berger, J.M.; Wang, J.C. *Curr. Opin. Struct. Biol.* **1996**, *6*, 84-90.
(8) Imbert, T.F. *Biochimie* **1998**, *80*, 207-222.
(9) Lee, K.H.; Xiao, Z. In *Antitumor Agents from Natural Products*; Cragg, G.M.; Kingston, D.G.I.; Newman, D.J. Eds., Taylor & Francis: New York, 2005, Chapter 5, pp 71-87.
(10) Lee, K.H.; Imakura, Y.; Haruna, M.; Beers, S.A.; Thurston, L.S.; Dai, H.J.; Chen, C.H.; Liu, S.Y.; Cheng, Y.C. *J. Nat. Prod.* **1989**, *52*, 606-613.
(11) Wang, Z.Q.; Kuo, Y.H.; Schnur, D.; Bowen, J.P.; Liu, S.Y.; Han, F. S.; Chang, J.Y.; Cheng, Y.C.; Lee, K.H. *J. Med. Chem.* **1990**, *33*, 2660-2666.
(12) Zhou, X.M.; Wang, Z.Q.; Chang, J.Y.; Chen, H.X.; Cheng, Y.C.; Lee, K.H. *J. Med. Chem.* **1991**, *34*, 3346-3350.
(13) Lee, K.H.; Beers, S.A.; Mori, M.; Wang, Z.Q.; Kuo, Y.H. Li, L.; Liu, S.Y.; Cheng, Y.C. *J. Med. Chem.* **1990**, *33*, 1364-1368.
(14) Lee, K.H. *Med. Res. Rev.* **1999**, *19*, 569-596.
(15) Cho, S.J.; Tropsha, A.; Suffness, M.; Cheng, Y.C.; Lee, K.H. *J. Med. Chem.* **1996**, *39*, 1383-1395.
(16) Ji, Z.; Wang, H.K.; Bastow, K.F.; Zhu, X.K.; Cho, S.J.; Cheng, Y.C.; Lee, K.H. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 607-612.
(17) Xiao, Z.; Xiao, Y.D.; Feng, J.; Golbraikh, A.; Tropsha, A.; Lee, K.H., *J. Med. Chem.* **2002**, *45*, 2294-2309.

Salinosporamide A and the Exploration of Marine Microorganisms

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In the early 1990s, with the failed development of diazonamide A and eleutherobin, I began to realize that the development of marine invertebrate-derived drugs was fraught with problems beyond my control. First and foremost was the recollection and resupply issue that had plagued marine natural products researchers for many years. Second was the complexity of these compounds, rendering them difficult to modify and synthesize. Although today some successes have been reported, the issue of supply of structurally-complex drug candidates remains a serious limitation. I sought a solution to this problem that would pave the way for successful development of new marine drugs.

Since reading of the historic discovery of penicillin and later actinomycin, I had always admired the complex structures and important biological activities found in metabolites produced by soil-derived microorganisms. In the areas of infectious diseases, cancer and to lower cholesterol, soil bacteria and fungi provided an unparalleled number of new drugs and many thousands of complex metabolites. From 1940 to 1995, with included the great "Antibiotic Era", hundreds of drugs were developed that had significantly eradicated pneumonia and other infectious diseases. But, the heyday of microbial drug discovery was to soon come to an end. By 1995, most of Big Pharma had abandoned this style of research. Diminished returns, perhaps because microbial

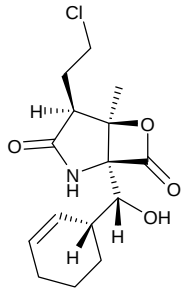
diversity had been exhausted, simply dictated that alternative methods to access chemical diversity be found. The method of choice was combinatorial chemistry.

As I thought about this turn of events, I questioned what was known about the chemistry of marine microorganisms. There were a few discoveries made by random screening, but by in large there had never been a comprehensive examination of the oceans! I found this to be difficult to understand, but it is easily explainable on the basis of pharma's lack of familiarity with the ocean itself. While it was known that marine habitats contain significant numbers of bacteria and fungi, the paradigm advanced at that time was that the ocean was a repository for terrestrial microbes. Further, it had been reported that less than 0.5% of the microbes present could be cultured. Under these conditions, why try?

Although concerned that I would make serious mistakes, I asked my then colleague Ken Nealson to educate me and assist me to start to explore marine bacteria. The beginning was very rough indeed and little was discovered. With the enormous microbial taxonomic diversity in the oceans, where could we begin? With a lesson from our terrestrial colleagues, we realized that microbial metabolites were rarely produced by the vast majority of bacteria taxa. Indeed, it was clear that only a few groups of terrestrial bacteria were responsible for the massive numbers of metabolites isolated. These groups were mainly the pseudomonads, the bacilli, and most importantly the filamentous actinomycetes. Based upon this history, we began to see if these groups were present in the ocean. There were discouraging concepts that filled the literature. It was argued that there were no true actinomycetes, and they had been observed in the ocean only because their spores were transported into the ocean by runoff and rivers.

Although we had earlier isolated several new compounds from marine microbes, it wasn't until 1990 when Paul Jensen joined my group that we began to develop an understanding of the sources for new marine microorganisms. We examined marine-derived fungi and were disappointed to find at least 100 known compounds for every novel structure type. We concluded that fungi were cosmopolitan and that well known taxa were found as the majority in marine sediments and other sources.

It was the routine utilization of 16S rDNA phylogenetic sequence methods for bacterial identification that allowed us to make significant advances. A new graduate student, Tracy Mincer, led us to use this technique, and to apply it to a large recent collection of actinomycete bacteria from the Bahamas. Phylogenetic classification of these organisms showed them to represent a completely new branch within the family Micromonosporaceae.[1] The unique organisms, when cultured, yielded extracts which showed significant bioactivities. More than 70% were able to inhibit the growth of cancer cells! Based upon this discovery, we immediately went forward to examine the metabolites responsible for this effect. One of the strains, later called *Salinispora tropica*, was found to produce an amazingly potent agent, salinoporamide A, that was ultimately found to be many times more active than omuralide as an inhibitor of the 20S proteasome.



Salinosporamide A

Paul Jensen and I felt that marine microorganisms would be a major new resource for drug discovery. As a consequence, he and I founded Nereus Pharmaceuticals in San Diego. Nereus promptly licensed salinosporamide A and embarked upon an aggressive developmenta campaign. Within less than four years, salinisporamide was approved for Phase I human trials. Currently, salinisporamide A is distinguished by exceptional *in vivo* efficacy and a unique mechanism of action that appears to support the ultimate development of this agent for the treatment of cancer.

The discovery of salinosporamide A unequivocally proved that the actinomycetes in the ocean are a unique resource for drug discovery. [3] Since the discovery of salinosporamide A, Paul Jensen and I have continued to explore marine actinomycetes. More than 15 new taxa have been discovered, and a sizable number of new bioactive agents have been isolated. The future seems bright for this new area, especially when one considers the growing need for new antibiotics effective against multiply drug resistant human pathogens. The growing incidence of methicillin-resistant *Staphylococcus aureus* (MRSA), which has now reached epidemic levels, provides expanding motivation to explore these unknown, but prolific resources. It is unfortunate that the pharmaceutical industry abandoned microbial drugs discovery prior to these discoveries. It does not appear that, for them, returning to microbial sources will be feasible.

(1) Mincer et al. **2002**. Widespread and persistent populations of a major new marine actinomycete taxon in ocean sediments, *Appl. Envir. Microbiol. 68(10),* 5005-5011.
(2) Chauhan, et al. **2005**. A novel orally active proteasome inhibitor induces apoptosis in multiple myeloma cells with mechanisms distinct from Bortezomib. *Cancer Cell, 8(5),* 407-419.
(3) Jensen and Fenical, **2007**. Marine Actinomycete Bacteria, Developing A New Resource for Drug Discovery. *Nat. Chem. Biol. 2,* 666-673.

Paclitaxel (Taxol™): Discovery and Development

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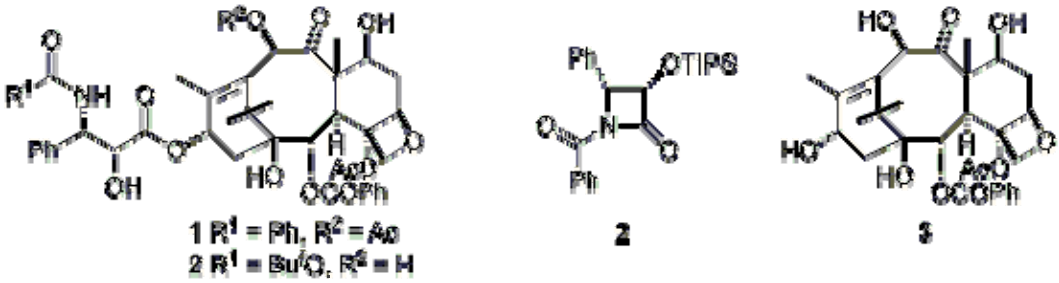
When Arthur Barclay, a botanist with the USDA, collected a sample of the bark, needles, roots and berries of the western (or Pacific) yew, *Taxus brevifolia,* from the Gifford Pinchot National Forest in Washington State on August 21, 1962, he could have had no idea that he was taking the first step in the development of one of the most important and influential anticancer drugs ever discovered. The collection was made as part of a systematic survey of plants and other organisms for potential anticancer compounds, instigated by Jonathan Hartwell, then the Chief of the NCI Natural Products Branch. At that time the isolation and structure elucidation of active compounds was carried out by a group of NCI-approved contractors, one of whom was Monroe

Wall, who had recently moved from the USDA to a new position at the Research Triangle Institute in North Carolina. After biological testing showed that the extracts had activity against the 9KB cell line, they were sent to Monroe in 1963 for isolation and structure elucidation.

The isolation of taxol was demanding because it was present only in very small quantities in the bark of the yew, but it became clear as the work progressed that this was an unusually promising compound. Writing to the NCI in 1966, Monroe said "I would like to ask whether you could arrange to have this sample…receive a special priority, as I regard it as one of the most important samples we have had in a long time."[1] Once taxol had been isolated, the problem of its structure elucidation remained, and this was especially challenging, since it could not be crystalized, and the NMR facilities available in the mid-1960's were far less powerful than those today. The structure elucidation was thus put on the back burner while Monroe worked at his other exciting lead, camptothecin, and it fell to his long time collaborator Mansukh Wani to make the key discovery that taxol could be cleaved into two pieces—the side chain and the baccatin core—by treatment with sodium methoxide. This then led to the preparation of crystalline derivatives of the two pieces, and their structure elucidation by X-ray crystallography by Andrew McPhail at nearby Duke University. The final structure (**1**) was published in a landmark paper in *JACS*.[2]



Although the structure of taxol was now known, and its *in vitro* activity against KB cells and *in vivo* activity against P388 lymphocytic leukemia were documented, it aroused what can best be described as "underwhelming enthusiasm" among the powers that be at the NCI. This is understandable, given its shortcomings as a drug candidate: it was obtained only in low yield from the thin bark of the western yew, it was very insoluble (and thus difficult to formulate), its only *in vivo* activity was against leukemia, for which other drugs existed, and it had an unknown mechanism of action. Its develop-ment thus languished until testing in the newly developed human tumor xenograft model in nude mice showed promising activity against several cancers, including the MX1 breast xenograft. Based on these new data, and with strong support from Matthew Suffness, who had recently joined the Natural Products Branch, taxol was approved for preclinical development in 1977.

Interest in taxol increased significantly when Susan Horwitz discovered that it acted by a previously unknown mechanism, by promoting the assembly of tubulin into microtubules.[3] Taxol entered Phase I clinical trails in 1983, and the initial results were discouraging, because the clinicians encountered toxicities due to the large amounts of the Cremophor™ emulsifying agent that was used. Fortunately the novelty of its mechanism provided the impetus to find a way around this problem, which was achieved by premedication with antihistamines and lengthening the infusion period. The first positive Phase II clinical results, showing activity against ovarian cancer, were published in 1989,[4] and this result turned taxol from an obscure lead compound into an instant celebrity. Once it became clear that taxol would be a commercial drug, the NCI invited pharmaceutical companies to take over its development. Since the only reported activity was against the relatively uncommon ovarian cancer, the pharmaceutical industry was reluctant to

commit to the investment that would clearly be needed to bring taxol into large-scale use, and in the end only Bristol-Myers Squibb (BMS) produced a competitive bid; it signed a Cooperative Research and Development Agreement (CRADA) with the NCI in 1990.

The transformation of taxol from an investigational drug to a widely used oncological agent required a large investment in securing a stable supply. Fortuitously, the excellent activity of taxol against breast cancer (sadly, much more common than ovarian cancer), was reported in 1991,[5] and the demand for taxol skyrocketed, giving BMS an enormous return on their investment. The supply problem was initially overcome by isolation of the drug by Hauser Chemical Research after large-scale harvesting of *T. brevifolia* bark, but in 1994 BMS converted to a semisynthetic process that involved coupling a β-lactam such as **2** with 10-deacetylbaccatin III (**3**), which could be obtained in reasonable yield from the needles of the much more common English yew, *T. baccata*.[6] This process was licensed from Robert Holton, who was a colleague of the author at Virginia Tech in the 1980s, but left for Florida State University before the patent work was done. BMS was also successful in trademarking the name Taxol® for their formulation of the drug, based on an obscure discontinued laxative of the same name, and so the name paclitaxel must now be used to refer to Wall's taxol. BMS has recently converted to a plant tissue culture method for production of paclitaxel.[7] Worldwide sales of paclitaxel and its relative docetaxel (**4**) totaled approximately US$4 billion in 2005,[8] and the drugs have brought relief, and in some cases cures, to multitudes of cancer patients.

The story of paclitaxel does not end with its introduction into clinical use, since. its use in combination chemotherapy is still a subject of active investigation. Many analogs of paclitaxel in addition to docetaxel have been developed, and the first of these are close to entering clinical use. Improved methods of drug delivery, such as the nanoparticle formulation Abraxane™, which has increased the therapeutic index of paclitaxel, are playing an important role.[9] For more information on these topics, readers are referred to a recent review.[10]

(1) Junod, T. *Life* **1992**, *15(5)*, 71-76.
(2) Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971,** *93*, 2325-2327.
(3) Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979,** *277*, 665-667.
(4) McGuire, W. P. et al. *Ann. Intern. Med.* **1989,** *111*, 273-279.
(5) Holmes, F. A. et al. *J. Nat. Cancer Inst.* **1991,** *83*, 1797-1805.
(6) Chauviere, G.; Guenard, D.; Picot, F.; Senilh, V.; Potier, P. *Acad. Sc. Paris, Serie II,* **1981,** *293*, 501-503.
(7) Leistner, E. *Pharm. Unserer Zeit* **2005,** *34*, 98-103.
(8) http://www.bioxelpharma.com/docs/en/financial/22.BIP_Q3_Full.pdf (accessed 03/17/08)
(9) Gradishar, W. J. *Expert Opin. Pharmacother.* **2006,** *7*, 1041-1053.
(10) Kingston, D. G. I.; Newman, D. J. *Curr. Opin. Drug Disc. Devel.* **2007,** *10*, 130-144.

The *Vinca* (*Catharanthus*) Alkaloids

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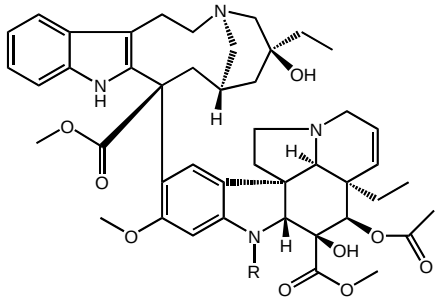
The discovery of the so-called "Vinca" alkaloids vincristine (leurocristine)(VCR) and vincaleukoblastine (vinblastine)(VLB) arose from studies in two different laboratories, the Eli Lilly and Company in the USA and the University of Western Ontario in Canada. Both groups were interested in this plant because of its reputation as being effective in treating diabetes. Svoboda obtained his sample of *C. roseus* from the Philippines, where this plant was claimed to be useful for treating diabetes. Nobel, Beer and Cutts [1] in Canada acquired the leaves of *Catharanthus roseus* (L.) G. Don from the West Indies, prepared an extract, injected it into rats, and found that the animals perished. The rats were found to have a *Pseudomonas* sp. infection

but *Pseudomonas* was not found in the extracts. It was subsequently found that the rats experienced a profound granulocytopenia and bone marrow suppression. Following bioassay-directed fractionation an uncharacterized alkaloid was found to be the active principle and was named vincaleukoblastine. Vincaleukoblastine was found to have activity against a transplantable mammary adenocarcinoma in mice and a transplantable sarcoma in rats. Further studies did not arise from the Noble et al. laboratories.



R = Me VLB; R = CHO VCR

At about the same time, Svoboda, Neuss and Gorman at the Eli Lilly Co. started a research program to identify new antitumor agents from plants. Svoboda's area of interest was Southeast Asia, and *Catharanthus roseus* was collected because it contained alkaloids and had a folklore reputation for use in diabetes [2,3]. It was the 40th plant that Svoboda acquired. All extracts were submitted for pharmacological screening, including a six-tumor *in vivo* battery of test targets (cytotoxicity was not in vogue at the time). Activity was pronounced vs. the P-1534 leukemia, and VCR and VLB were isolated and reported by Svoboda in *Lloydia.* [2,4] VCR was approved by FDA and marketed in 1963, less than five years after the crude extract showed antitumor activity in mice. A major problem with regard to production of VCR was the low yield (ca. 30 gm from 15 tons of dried leaves). Most of the plant material was obtained from Madagascar, Mozambique and India, with several plantations in the USA providing additional material. Since the only difference between VLB (a major alkaloid) and VCR (a minor alkaloid) was an N-methyl on VLB and an N-formyl on VCR, a challenge arose to convert VLB to VCR. This was subsequently carried out by scientists at Richter Gedeon in Hungary by a chromic acid oxidation at -60^{0}C. that resulted in a 70% conversion of VLB to VCR.[5] As a result, Eli Lilly and Company began purchasing "synthetic" VCR from Richter Gedeon for $1.4 million/kg [6].

Two additional semi-synthetic alkaloids related to VLB and VCR, vinorelbine and vindesine, were marketed.[7] A major importance of the discovery of VCR was its novel mechanism of action (at the time); blocking mitosis with metaphase arrest and specifically binding with tubulin and preventing its polymerization. Because of this VCR became an important component of combination chemotherapy.

(1) Noble, R. L.; Beer, C. T.; Cutts, J. H. *Ann. N.Y. Acad. Sci.* **1958**, 76(3): 882-894.
(2) Svoboda GH, Neuss, N, Gorman, M. *J. Amer. Pharm. Ass. Sci. Ed.* **1959**, 48(11): 659-666.
(3) Farnsworth, N. R. In *Biodiversity*; Wilson, E. O., Ed.; National Academy Press: Washington, D.C. **1988**; Chapter 9, pp 83-97.
(4) Svoboda GH. *Lloydia*. **1961**, 24(4): 173-178.
(5) Jovanovics, K, Szasz, K, Fekete, G, et al. U. S. Patent 3,899,493. **1975**.
(6) Svoboda, GH. Personal communication to NR Farnsworth. **1970**.
(7) Johnson, S. A. et al. Cancer Treat. Rev. **1996**, 22, 127; Guéritte, F.; Fahy, J. In *Anticancer Agents from Natural Products*; Cragg, G. M.; Kingston, D. G. I.; Newman, D. J., Eds.; Taylor and Francis, Boca Raton, FL, **2005**, pp. 123-135.