

Marine Natural Products as Anticancer Agents: Therapeutic Treasures from the Deep

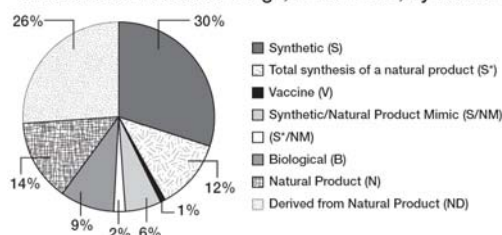
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ABSTRACT

All available anticancer drugs, 1940s-2002, by source:



A recent analysis by Newman and associates revealed that natural products, natural product-derived drugs, or synthetic mimic compounds comprised 52% of all NCEs launched onto the market between 1981 and 2002. In the area of cancer, the percentage of small molecule NCEs from natural sources has remained at 62% over the same time frame. In 2006, 67% of cancer chemotherapeutic agents on the market were natural products or small molecules based upon natural product leads.

Natural products have been the source of therapeutics since the advent of traditional medicine and healing, and remain a dominant source to date. The World Health Organization (WHO) approximates that 80% of the world's inhabitants depend mainly on traditional medicine for their primary health care.¹ Medicines of natural product origin are collected from a variety of sources, including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates.² The value of natural products becomes particularly obvious when considering the rate of introduction of new chemical entities (NCEs) with significant structural diversity, including templates for semi-synthetic and total synthetic modification. The number of diseases treated or prevented by natural products and the frequency of their use in treating disease further substantiate their utility. Natural products and related synthetic mimics or analogues are

used to treat 87% of all categorized human diseases, and the discovery of novel agents is often used to better understand targets and pathways in disease processes.^{3,4} Cancer is one such disease that has many naturally-derived therapies and for which tools are necessary to elucidate its complex progression pathways. Improvements in the area of cancer therapy are vital, considering that cancer is forecasted to be the major cause of death in the 21st century.¹

The fervent interest in natural products is driven by their facile application to therapeutics. The considerable quantity of natural product compounds in drug discovery stems from both the uniqueness and the intricacy of their carbon skeletons.³ This structural diversity is revealed by the existence of a greater number of chiral centers, increased steric complexity, greater molecular rigidity, and a higher number of oxygen atoms when compared to

¹ Ravelo, A. G.; Estevez-Braun, A.; Chavez-Orellana, H.; Perez-Sacau, E.; Mesa-Siverio, D. *Curr. Top. Med. Chem.* **2004**, *4*, 241-265.

² Newman, D. J.; Cragg, G. M.; Snader, K. M. *Nat. Prod. Rep.* **2000**, *17*, 215-234.

³ Chin, Y.-W.; Balunas, M. J.; Chai, H. B.; Kinghorn, A. D. *AAPS J.* **2006**, *8*, 239-253.

⁴ Gullo, V. P.; McAlpine, J.; Lam, K. S.; Baker, D.; Petersen, F. J. *Ind. Microbiol. Biotechnol.* **2006**, *33*, 523-531.

synthetic molecules. Moreover, the ratio of aromatic ring atoms to total heavy atoms is lower in natural products, while the number of solvated hydrogen-bond donors and acceptors is higher than in synthetic drugs. The novelty of these structures is exemplified by the fact that less than one-fifth of the ring systems found in natural products are represented in current drugs.⁵ Moreover, natural products are often proposed to be drug-like and biologically friendly because they have been elaborated within living systems.³ Generally, natural products can be looked upon as a collection of privileged structures selected by evolutionary pressure to interact with a vast array of biological targets with specific purposes.⁵

Compared with the study of terrestrial natural products, the study of marine natural products is still in its infancy. A 2006 report revealed that over the past few decades, during which the study of marine natural products has begun in earnest, approximately 16,000 novel marine natural products have been discovered. These discoveries and related studies have been published in over 15,000 publications and protected by more than 300 patents,⁶ with a steady increase in publication rate observed since 1965.⁷

Initial discoveries from the marine environment can be traced back to the early 1950s. It was during this decade that Bergmann discovered and subsequently identified a series of arabinosyl nucleosides, including spongothymidine and spongouridine, from the Caribbean sponge *Cryptotethia crypta*. This discovery provided the pharmacophore used in the synthesis of a close analogue of spongouridine known as cytosine arabinoside, commercialized by Upjohn (now Pharmacia) as Ara-C (Cytarabine) in 1969 to treat leukemia.^{8,9} Ara-C derives its activity by intracellular conversion to arabinosyl cytosine triphosphate through the action of deoxycytidine kinase. This modified molecule then has the abilities to incorporate into cellular DNA and inhibit DNA polymerase, and is useful in the treatment of acute myelocytic leukemia and non-Hodgkin's lymphoma.^{9,10}

Prior to the development of reliable SCUBA diving techniques, the collection of marine organisms was limited to those from intertidal and shallow subtidal environments, accessible by wading and snorkeling.⁹ The advent of SCUBA diving in the 1960s allowed researchers to explore depths ranging from 10 to 130 feet. The 1960s were marked by research of marine natural products purely for their chemical structures,¹¹ while any biological activity

was investigated as an afterthought.^{8,12}

Beginning with the 1970 inauguration of a series of symposia on food and drugs by the Marine Technology Society in Youngken, the biomedical potential of marine natural products became apparent. The discovery in the late 1960s and early 1970s that some gorgonians contain large quantities of prostaglandins also contributed to the rapid growth of interest in the field.⁹ By 1975, three parallel tracks in marine natural products chemistry explored marine toxins, marine biochemicals, and marine chemical ecology.⁸ Between 1977 and 1987, nearly 2500 new metabolites were reported from a variety of marine organisms.¹¹

The 1980s saw an increasingly collaborative effort between marine chemists and pharmacologists, resulting in the first marine metabolites to enter preclinical studies and clinical trials, as well as many useful biochemical tools for exploring cellular processes at the molecular level.⁹ Research focused on central nervous system membrane-active toxins, ion channel effectors, tumor promoters, anticancer, antiviral, and anti-inflammatory agents.¹⁰ These earliest efforts by marine biologists and naturalists resulted in the identification of unique toxins, often excreted as defense mechanisms by the organisms.¹² Studies of marine toxins were dominated by Japanese researchers, with the majority of toxins belonging to the polyether structural class. The ladder-like skeleton of the polyether toxins was established in 1981 by an X-ray crystallographic study of brevetoxin B, from the dinoflagellate *Gymnodinium breve*.^{8,13} Brevetoxin B is responsible for the noxious phenomenon known as red tide.

The early 1990s were characterized by more sophisticated collaborations between natural product chemists, molecular pharmacologists, biochemists and cell biologists.⁹ A series of agency-supported programs, such as the National Cooperative Drug Discovery Program of the National Cancer Institute (NCI), recognized that the rich chemistry of marine organisms was not translating into useful drug leads due to poorly developed connections between academia and industry and sought to forge productive collaborations.¹² Discoveries during the 1990s were also spurred by advances in molecular and biochemical pharmacology, recombinant DNA technologies and genomic analyses, leading to renewed interest and further efforts to explore the marine world.⁹

Ecological and evolutionary pressures have led to the development of complex marine creatures coexisting in a competitive environment. The evolutionary results of such stresses are chemical adaptations, known as secondary metabolites, manifested as unique chemical functionalities. One characteristic trait is the common incorporation of covalently bound halogen atoms, mainly chlorine and bromine, presumably due to their ready availability in sea

⁵ Koehn, F. E.; Carter, G. T. *Nat. Rev. Drug Discov.* **2005**, *4*, 206-220.

⁶ Rawat, D. S.; Joshi, M. C.; Joshi, P.; Atheaya, H. *Anticancer Agents Med. Chem.* **2006**, *6*, 33-40.

⁷ Blunt, J. W.; Copp, B. R.; Hu, W.-P.; Munro, M. H.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2007**, *24*, 31-86.

⁸ Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2004**, *67*, 1216-1238.

⁹ Carte, B. K. *Bioscience* **1996**, *46*, 271-286.

¹⁰ Ireland, C.; Copp, B.; Foster, M.; McDondald, L.; Radisky, D.; Swersey J. *Biomedical potential of marine natural products*; Plenum Press: New York, 1993; Vol. 1.

¹¹ Cragg, G. M.; Newman, D. J. *Expert Opin. Investig. Drugs* **2000**, *9*, 2783-2797.

¹² Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2004**, *67*, 1216-1238.

¹³ Andrianasolo, E. H. Oregon State, 2005.

water.¹² Marine natural product literature from the 1970s, for example, is dominated by reports of halogenated metabolites derived from red algae.⁹ Several other structural motifs are uniquely or predominantly marine, some of which continue to evade synthesis, such as the monoterpene isolated from the sea hare *Aplysia californica*, reported by Faulkner in 1973.^{14,15} The sulfamate group, shown in haplosamate A (Figure 2), and the carbonimidic dichloride functionality have only been found in nature as metabolites of marine sponges. Likewise, sesquiterpene or diterpene isonitriles, isothiocyanates, thiocyanates and formamides are predominantly produced by sponges. In addition, unusual cyclic peptides from tunicates and sesterterpenes from sponges represent uniquely marine and complex functionalities.¹⁴ The intricate chemical scaffolds isolated from marine extracts have inspired many hypotheses about their biosynthetic pathways and have stimulated research to elucidate such pathways.

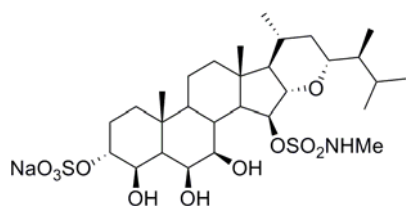


Figure 2. Structure of haplosamate A

The isolation and identification of marine natural products is an intricate and complex process. It has been stated that the oceans contain nearly 300,000 described species. It is estimated, however, that this number represents only a small percentage of the total number of species, the majority of which have yet to be discovered and described. The actual number of oceanic species may approach one to two million.^{12,16} In addition, the oceans, which cover 70% of the earth's surface, contain tremendous biodiversity, with organisms representing all but two of the 28 major animal phyla.¹⁷ An examination of the sources of marine natural products by phyla over the last few years reveals an increased interest in microorganisms, a fact supported by the observation that 45% of the accumulated literature on marine microorganisms has been published since 2000.⁷ If all marine phyla are compared in terms of NCI screening for cytotoxic compounds, the most prolific producers of potential anticancer therapeutics are in the porifera, bryozoa and chordata.¹⁸ Although the marine ecosystem is

rich and diverse, natural resources are limited and the exploration of collectable marine organisms is thought to near completion within the next twenty years.¹⁹

The locations in which these marine natural products are found have been limited, with the majority of species diversity found at the land-sea interface. This slender strip is comprised of a high concentration of species and is among the most biodiverse and productive environments on earth. Deep ocean thermal vent colonies represent another of these rich environments. Although open ocean waters generally lack nutrients and have been compared to deserts in terms of biomass and species diversity, recent research indicates the existence of substantial microbial diversity in pelagic water. Less than one percent of the earth's surface, including the narrow ocean fringe and the deep sea vents, is thought to be home to the majority of the marine world's biologically productive species.¹²

Traditional screens for natural products require sensitive and robust assays that can be run in high throughput. Continual improvement of screening formats, reagent production, robotics and data management have enabled the mechanism-based high-throughput screening of marine natural products.²⁰ Screening of natural product extract libraries, however, can be problematic due to the complex mixtures of predominantly uncharacterized compounds, which can mask biological activity of otherwise potent compounds and give false readouts. A potential way to avoid such interference is to prefractionate the extracts, which will prevent undesired compounds from masking activity and allow detection of very small amounts of compound.^{5,20} This prefractionation, however, increases the concentration of nuisance compounds and desired compounds alike.²⁰ HTS of crude extracts (>100 compounds), semi-pure mixtures (5-10 compounds), or single purified products are all options. However, a heterogeneous sample that elicits a biological response must undergo rounds of chemical purification and additional assays to identify the active component. The speed of structural identification and elucidation, coupled with the suitability of the screens are two factors that make HTS of natural products a troublesome process.⁵

The isolation and identification of analytical amounts of marine natural products require minimal raw material. However, further development of these novel compounds requires significantly more material, and progress is often hindered by supply issues. The concentrations of many highly active compounds in marine invertebrates are often miniscule, amounting to less than 10⁻⁶% of the wet weight upon harvesting.²¹ There are many synergistic options for sustainable use of marine natural products, such as controlled harvesting, chemical synthesis, aquaculture of

¹⁴ Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 1-6.

¹⁵ Faulkner, D. J.; Stallard, M. O.; Fayos, J.; Clardy, J. *J. Am. Chem. Soc.* **1973**, *95*, 3413-3414.

¹⁶ Malakoff, D. *Science* **1997**, *277*, 486-488.

¹⁷ Schwartzmann, G.; Brondani da Rocha, A.; Berlinck, R.; Jimeno, J.

Lancet Oncol. **2001**, *2*, 221-225.

¹⁸ Munro, M.; Blunt, J. W.; Dumdei, E. J.; Hickford, S.; Lill, R. E.; Li, S.;

Battershill, C. N.; Duckworth, A. R. *J. Biotechnol.* **1999**, *70*, 15-25.

¹⁹ Amador, M. L.; Jimeno, J.; Paz-Ares, L.; Cortes-Funes, H.; Hidalgo, M. *Ann. Oncol.* **2003**, *14*, 1607-1615.

²⁰ Butler, M. S. *J. Nat. Prod.* **2004**, *67*, 2141-2153.

²¹ Proksch, P.; Edrada, R. A.; Ebel, R. *Appl. Microbiol. Biotechnol.* **2002**, *59*, 125-134.

the source organism, *in vitro* production through cell culture of the source, and transgenic production.²² It is important to note that large-scale harvesting of marine organisms is neither practical nor ecologically acceptable and is not an option for many organisms of limited supply.²³ Total synthesis has the potential for supplying sufficient quantities of natural products; however, the complex molecular structures of many marine agents are considerably challenging.^{5,24,24} Chemistry is also being used in conjunction with natural product chemistry in carrying out semi-synthesis as well as in the generation of natural product libraries. As an alternative to the demand for new natural products, chemists are now enlisting solid-phase combinatorial synthesis to construct libraries of natural product analogues and related compounds.¹

Several impressive total syntheses of marine natural products have been accomplished in recent years. Palytoxin,²⁵ a very potent neurotoxin, is one complex example of a marine natural product that was structurally elucidated and synthesized as a single isomer in 1994 by Y. Kishi. This landmark total synthesis was accompanied by the development of several important reactions to control the stereochemistry of 64 stereogenic centers and it remains a remarkable accomplishment in organic synthesis.^{26,27} P. Baran has also made significant contributions to the synthesis of marine natural products, especially in the areas of chemoselectivity and novel methodology.²⁸

In the area of cancer therapeutics, E. J. Corey and co-workers published the first enantioselective total synthesis of Ecteinascidin-743 (Figure 3) in 1996. The opportunity to move this exceptionally potent marine tetrahydroisoquinoline alkaloid into advanced clinical trials was enabled through Corey's convergent and concise total synthesis.²⁹ The total synthesis of diazonamide A by K. C. Nicolaou has produced enough of the marine natural product to investigate its low nanomolar cytotoxicity against a panel of human cancer cell lines. This synthesis also verified the utility of total synthesis in structure elucidation and offered examples of novel chemical reactions and reactivity through its two divergent strategies.^{30,31} Palmerolide A, a recently discovered marine cytotoxin, was synthesized by J. K. De Brabander and colleagues in 2007. This enamide-bearing polyketide is

difficult to isolate from natural sources and, therefore, its total synthesis has enabled further investigation of its promising anti-tumor activity as well as elucidation of the actual structure responsible for its bioactivity.³²

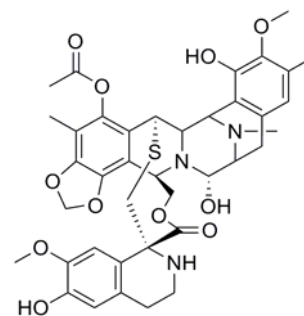


Figure 3. Structure of ecteinascidin-743 (ET-743).

Marine-derived anticancer therapeutics exhibit their activity through several mechanisms of action on a diverse range of biological targets. In a recent assessment of the cytotoxic and antitumor properties of 150 marine natural products isolated from 2003-2004 it was noted that only 31 of the structurally defined marine natural products had known mechanisms of action, while the other 119 remained unknown.³³ Common anticancer targets include signal transduction, angiogenesis, apoptosis, cell cycle, DNA synthesis, mitochondrial respiration, mitosis and multidrug efflux, among others.^{21,34}

Future efforts in the area of marine natural products as anticancer agents are concentrated on the development of new technologies. Genetic engineering is one such technology, which allows for the production of a desired metabolite in a heterologous host.^{23,35} Thus, genetic engineering will allow marine organisms to provide inspiration for new compounds and will exclude the need for excessive harvesting.¹⁴ The exploration of new regions such as the deep-sea vents occurring along ocean ridges, including the East Pacific Rise and the Galapagos Rift, as well as extreme habitats, home to extremophiles, are exciting because of their potential for untapped resources.¹¹ Additionally, polymer therapeutics may enhance the value of marine toxins by allowing specific targeting of a tumor without harming the surrounding healthy tissue.¹⁸ There is great potential in the emerging field of marine natural products as chemotherapeutics.

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²² Pomponi, S. A. *J. Biotechnol.* **1999**, *70*, 5-13.

²³ Nuijen, B.; Bouma, M.; Manada, C.; Jimeno, J.; Schellens, J.; Bult, A.; Beijnen, J. *Anticancer Drugs* **2000**, *11*, 793-811.

²⁴ Fortman, J. L.; Sherman, D. H. *ChemBiochem* **2005**, *6*, 960-978.

²⁵ Moore, R. E. *Prog. Chem. Org. Nat. Prod.* **1985**, *48*, 81-202.

²⁶ Armstrong, R. W.; Beau, J. M.; Cheon, S. H.; Christ, W. J.; Fujioka, H.; Ham, W. H.; Hawkins, L. D.; Jin, H.; Kang, S. H. *J. Am. Chem. Soc.* **1989**, *111*, 7525-7530.

²⁷ Suh, E. M.; Kishi, Y. *J. Am. Chem. Soc.* **1994**, *116*, 11205-11206.

²⁸ Baran, P. S.; Maimone, T. J.; Richter, J. M. *Nature* **2007**, *446*, 404-408.

²⁹ Corey, E. J.; Gin, D. Y.; Kania, R. S.; *J. Am. Chem. Soc.* **1996**, *118*, 9202-9203.

³⁰ Nicolaou, K. C.; Bella, M.; Chen, D. Y.-K.; Huang, X.; Ling, T.; Snyder, S. A. *Angew. Chem. Int. Ed.* **2002**, *41*, 3495-3499.

³¹ Nicolaou, K. C.; Rao, P. B.; Hao, J.; Reddy, M. V.; Rassias, G.; Huang, X.; Chen, D. Y.-K.; Snyder, S. A. *Angew. Chem. Int. Ed.* **2003**, *42*, 1753-1758.

³² Jiang, X.; Liu, B.; Lebreton, S.; De Brabander, J. K. *J. Am. Chem. Soc.* **2007**, *129*, 6386-6387.

³³ Mayer, A.; Gustafson, K. R. *Eur. J. Cancer* **2006**, *42*, 2241-2270.

³⁴ Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57-70.

³⁵ O'Hanlon, L. H. *J. Natl. Cancer Inst.* **2006**, *98*, 662-663.