Anticancer compounds from Philippine marine organisms act on major pathways in cancer

Gisela P. Concepcion[1], Andrea Roxanne J. Anas[1,2], and Miguel A. Azcuna[1*]

[1]The Marine Science Institute, University of the Philippines, Diliman, Quezon City 1101, Philippines
[2]Graduate School of Environmental and Human Science and Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468-8503, Japan

Philippine marine biodiversity ranks among the richest in the world and is still largely unexplored. Such biodiversity can have profound applications in the search for compounds with anticancer properties. Our increased understanding of the molecular mechanisms in cancer has enabled us to demonstrate exactly how these compounds work. In the past two decades, compounds with diverse structures from Philippine marine organisms, ascidians, and associated microorganisms were reported. These compounds are cytotoxic to a variety of cancer cell lines and act on specific molecular targets in major cell signaling pathways implicated in cancer. To address the challenges associated with developing marine natural products for the pharmaceutical drug pipeline, future directions will focus on optimizing the culture of marine organisms and symbionts, utilizing biosynthetic genes in microbial hosts, and developing the concept of treating cancer with combinatorial therapy.

Definition of terms: A2780wt - human ovarian cancer cell line; A2780AD - drug resistant human ovarian cancer cell line; AMOR - Antibody and Molecular Oncology Research; ATP - adenosine triphosphate; CHO - Chinese ovarian hamster; $CDCl_3$ - deuterated chloroform; CDK - cyclin-dependent kinase; CDKI - cyclin-dependent kinase inhibitor; CRA - commercial research agreement; DEPT - distortionless enhancement by polarization transfer; DISC - death inducing signaling complex; DNA - deoxyribonucleic acid; ER - estrogen receptor; ESI-LC-MS – electrospray ionization liquid chromatography mass spectrometry FADD - Fas associated protein with death domain; FDA - Food and Drug Administration; G-phase - growth phase; GTP - guanosine triphosphate; GSK- glycogen synthase kinase; HAT - histone acetyltransferase; HDAC - histone deacetylase; HCT-116 - human colon cancer cell line; HMBC - heteronuclear multiple bond correlation; HMQC - heteronuclear multiple quantum coherence; HPDG - heptylprodigiosin; HPLC - high pressure liquid chromatography; HR-MS - high resolution mass spectrometry; HR-FABMS - high resolution fast atom bombardment mass spectrometry; IC_{50} - 50 percent inhibition concentration; M-phase - mitotic phase; MABA - microplate Alamar blue assay; MCF-7 - estrogen dependent human breast cancer cell line; MDA-MB-231 - multi-drug resistant human breast cancer cell line; MDA-MB-468 - PTEN-deficient human breast cancer cell line; MDA-MB-435S - human breast carcinoma cell line; MIC - minimum inhibition concentration; MNP - marine natural products; MRD - minimal residual disease; MRSA - methicillin-resistant *Staphylococcus aureus*; NCI - National Cancer Institute;

*Corresponding author
Email Address: mazcuna@upmsi.ph
Submitted: September 26, 2013
Revised: February 10, 2014
Accepted: February 19, 2014
Published: May 31, 2014
Editor-in-charge: Fabian M. Dayrit
Reviewers: Fabian M. Dayrit, Tatsufumi Okino, Nina Rosario L. Rojas, and Rosalia C. M. Simmen

KEYWORDS
Philippine marine biodiversity, cancer, anticancer compounds, marine natural products, cell cycle, DNA, cancer molecular targets, drug discovery

NCDDG - National Cooperative Drug Discovery Group; NF- κB - nuclear factor κB; NKTI-RITM - National Kidney and Transplant Institute - Research Institute for Tropical Medicine; NOESY - nuclear Overhauser effect spectroscopy; PIC - prior informed consent; PKCζ - protein kinase C zeta; PMS-ICBG - Philippine Mollusk Symbiont - International Cooperative Biodiversity Group; PTPRK - protein tyrosine phosphatase receptor type K; PTEN - phosphatase and tensin homolog; ROESY - rotating frame Overhauser effect spectroscopy; ROS - reactive oxygen species; RTK - receptor tyrosine kinase; TEM - transmission electron microscopy; TLC - thin layer chromatography; S-phase - synthetic phase; SKBR3 - Her2-positive human breast cancer cell line; TOPO - topoisomerase; UP - University of the Philippines; UP MSI – University of the Philippines Marine Science Institute; UV - ultraviolet; VIS - visible; Wnt - wingless-related integration site; 2D-NMR - two dimensional nuclear magnetic resonance; 4T1 – mouse mammary cancer cell lines

1. Introduction

The relentless search to discover new species and attempts to understand new habitats have led to the realization that biodiversity is a manifestation of chemical and biomolecular diversity. This rich diversity has been the basis for discovering useful drugs and therapeutic agents from nature throughout human history and civilization. The majority of anti-infective and anticancer drugs today are derived from nature (Newmann and Cragg 2012). Terrestrial biodiversity has long been studied, and recent decades have shown fewer new classes of terrestrial compounds being discovered. Marine biodiversity, however, still remains largely unexplored. Over the past few decades, marine invertebrates and microorganisms have been shown to yield novel compounds with unique chemical structures and significant biological activities (Joseph and Sujatha 2011, Kelecom 2002, Ireland et al. 1988). The oceans constitute an untapped pharmacological resource, and this makes the conservation of marine biodiversity important not only to ecology but also to biomedical research and human health (Roberts et al. 2002).

The chemical diversity of isolated compounds reflects the biodiversity of the source organisms which have evolved to adapt to their environment. Secondary metabolites from marine organisms exhibit cytotoxic and growth inhibitory activity, indicating that highly regulated biological mechanisms and ecological interactions exist among organisms in the competitive marine environment. Sedentary, soft-bodied sponges and ascidians produce toxic compounds to compete for space, and to prevent parasitism, fouling, and predation. They also produce growth inhibitors to regulate growth in response to the level of nutrients available in the environment. Protective compounds guard against physicochemical stress and damaging agents (*e.g.,* ROS and high energy UV radiation). Biology and ecology provide valuable leads to the discovery of potentially useful compounds from these marine organisms with applications to health and biomedicine, particularly to cancer and cancer prevention (Ireland et al. 2003).

Philippine marine biodiversity is evaluated as one of the richest in the world (Carpenter and Springer 2005). For several years, the Marine Natural Products Laboratory of the University of the Philippines Marine Science Institute (UP MSI) and the Department of Medicinal Chemistry, University of Utah, have been involved in the investigation and documentation of Philippine marine biodiversity and the isolation of anticancer compounds from ascidians, marine sponges, and other marine invertebrates and associated microorganisms. The collection of marine organisms from various biogeographic regions of the Philippines had proper permits from the Philippine Government, principally as a subcontract from the United States National Cancer Institute National Cooperative Drug Discovery Group (US NCI NCDDG) grant entitled, "Anticancer Agents from Unique Natural Products Sources" (Ireland et al. 2003, NCDDG report 1999).

The majority of the samples collected in the NCDDG project were marine invertebrates, particularly sponges. Sponges are found to be the most important source of anticancer compounds and, in a few examples, have been shown to make use of small molecules for external defense (Thoms and Schupp 2007, Proksch 1994). Some sponges are ready hosts for microbial symbionts and epibionts, which have been visualized microscopically to be residing in the sponge surface, extracellularly or intracellularly (Lee et al. 2001). Microorganisms can provide an additional source of secondary metabolites in sponge tissues (Schröder et al. 2006).

Underlying sponge biology is the plasticity of tissues which consist of a few cell types, the most notable being the stem cells known as archaeocytes. Stem cells are likely to make use of signaling pathways involving small molecules to trigger growth, proliferation or differentiation as the sponge's mechanism for adaptation (Wiens et al. 2007, Müller 2006). The sponge and human genomes share more than 70% kinase homology (Srivastava et al. 2010, King et al. 2008, Sullivan et al. 2006). Furthermore, metazoan developmental and structural gene orthologs have been found to be well integrated into the expression profiles at every stage of sponge development (Conaco et al. 2012). This supports the idea that sponge biology is a good model for human cell biology, particularly for cancer cells, since both possess the property of genetic plasticity (Koziol et al. 1998).

2. Molecular targets in cancer

Cancer is a leading cause of death in the developed countries (World Health Organization 2008). It is also the second leading cause of death in developing countries (Jemal et al. 2011, World Health Organization 2008). Cancer cells acquire capabilities not possessed by normal cells (Hanahan and Weinberg 2011, 2000). The underlying molecular mechanisms, *e.g.*, unregulated cell cycling and growth signaling, are now more clearly understood. A cell possesses an intricate circuitry involving protein-protein and protein-gene regulatory networks that allow the cell to respond with immense sensitivity to extracellu-

lar and intracellular signals. Different types of cancers have different key proteins in the circuitry that are mutated. Key proteins behave aberrantly and disrupt the normal processes in the cell, resulting in uncontrolled growth and proliferation, and aggressive invasion of cancer cells (Hanahan and Weinberg 2011, 2000).

2.1. Classical molecular targets in cancer

Compounds acting on the classical targets of cancer—DNA and the enzymes DNA topoisomerase 1 and 2 (TOPO 1 and 2) in the S (synthetic) phase, and microtubules/tubulins in the M (mitotic) phases of the cell cycle - remain among the most widely used anticancer drugs in the clinics today. DNA interactive agents, *e.g.* DNA antimetabolites, alkylators, intercalators, and crosslinkers, are generally the most effective but are non-specific. Therefore, they are extremely debilitating due to toxic side effects (Gurova 2009, Silverman 1992). TOPO inhibitors are highly cytotoxic. However, specific TOPO inhibitors that do not intercalate or interact with DNA directly are less likely to cause DNA mutations that lead to a second primary tumor. The antimitotic drug paclitaxel (Taxol®) is considered a "miracle" drug for many cancers (Kingston 2007, Ginsberg 2003). Instead of inhibiting microtubule formation, Taxol® stimulates the development of microtubules. When treated with Taxol®, cells churn out so many microtubules that they are unable to coordinate cell division. As an anticancer drug, it has a unique chemistry and mode of action, causing regression in mammalian tumor xenografts (Ginsberg 2003). Newer regimens administering lower doses of the classical drugs over longer periods are now being adopted to reduce toxicity, particularly to the immune system. Synergistic combinations of drugs applied simultaneously or sequentially make use of several-fold lower doses of drugs compared with single drug applications. The advantages of this regimen are lower toxicity, greater efficacy or effective killing, and less drug resistance (Koh and Nishio 2002). Anticancer drugs that act on the M (mitotic) phase of the cell cycle are used in combination with S phase drugs. The challenge remains to develop more drugs that target mitotic proteins involved in cytoskeleton activity, since much fewer M-phase drugs exist today compared to S-phase drugs.

2.2. New molecular targets in cancer

The "hallmarks" of cancer are biological capabilities progressively acquired in the multistep development of human tumors. Barriers to the regulation of cell growth and proliferation are overcome by cancer cells via specific mechanisms made possible by polygenic mutations. The hallmarks continue to postulate a strong basis for understanding the biology of cancer. In recent cancer research, newfound observations modified the original hallmarks of cancer (Hanahan and Weinberg 2011, 2000). Through progressive mutations resulting from genome instability and under chronic inflammatory conditions, cancer cells acquire capabilities, *e.g.,* unregulated cell cycling and growth signaling, evasion of growth suppressors and apoptosis, sustained angiogenesis, tissue invasion and metastasis, replicative immortalization, metabolic reprogramming, and immune evasion or escape, with mechanisms supported by a unique tumor microenvironment.

Cancer cells sustain chronic proliferation, deregulate signals, and attain full control of the fate of their cells. Among the major pathways is the transmission of enabling signals that are largely influenced by growth factors that bind cell-surface receptors with intracellular receptor tyrosine kinase (RTK) domains. Mutated RTKs transmit growth signals uncontrollably even in the absence of growth factors in the extracellular space. RTKs have an intracellular autocatalytic domain where substrates are phosphorylated. The signal is transmitted and amplified downstream through cytoplasmic serine-threonine kinases, resulting in the entry into the nucleus of the specific transcription factors. This leads to activated transcription or gene expression of cell cycling enzymes and proteins (Hanahan and Weinberg 2011, 2000).

The cell cycle describes a series of integrated events, *e.g.*, growth and proliferation, and the cyclin-dependent kinases (CDKs) are the critical areas of the cell cycle machinery. The activated CDKs allow the cell to move sequentially through phases in the cell cycle. The CDKs are positively regulated by cyclins and negatively regulated by CDK inhibitors (CDKIs) (Schwarz and Shah 2005). Identifying the cell cycle and CDKs as targets for cell therapy has been based on the prevalence of their functional perturbations based on underlying progressive genetic mutations found associated with human malignancies. Also, cell cycle arrest by CDK inhibition could induce apoptosis. Targeting CDKs would limit a cell's capability to cycle and could facilitate induction of apoptosis (Chen et al. 1999, Hartwell and Kastran 1994, Harper and Elledge 1996).

Richardson et al. (2003) reported that the 26S proteasome is also a relatively new and unexpected therapeutic target in cancer. Since the proteasome is an essential component of cellular metabolism, developing specific inhibitors of the 26S proteasome is being explored for cancer therapy. Pharmacophores connected to short peptides are considered good candidates as proteasome inhibitors (Mitchell 2003). Tumor development is also dependent on the inflammatory and fibroblastic environments which surround the malignant cell (Weinberg 2014, Balkwill et al. 2005). Targeting the inflammatory parts of the tumor rather than the malignant cell offers new perspectives to cancer therapy (Lin and Karin 2007).

Another molecular target in cancer treatment is the DNA-histone complex (Lane and Chabner 2009). Recent studies in cancer, which involved a significant change in functions of oncogenes and tumor suppressor genes, revealed the epigenetic regulation of genes as a major mechanism in carcinogenesis (Pan et al. 2007, Smith et al. 2007). The basic amino acids of histones can be post-translationally modified with methyl, acetyl, and phosphate groups. The acetylation-deacetylation of histone is

regulated by histone acetyltransferase (HAT) and histone deacetylases (HDAC), which are key enzymes involved in the transcriptional regulation of genes (Bhalla and List 2004, Zhang et al. 2003, Jenuwein and Allis 2001). Deregulation of the aforementioned enzymes was found in certain human cancers (Bhalla and List 2004, Gayther et al. 2000, Marks et al. 2000, Mahlknecht and Hoelzer 2000). Histone deacetylase (HDAC) activity was shown to cause compaction of the DNA-histone complex, blocking gene transcription and inhibiting differentiation (Lane and Chabner 2009). HDAC inhibitors promoted growth arrest, differentiation, and apoptosis of cancer cells, with minimal effects on normal tissue. HDAC inhibitors demonstrated antitumor activity in clinical trials and have significant potential in combination therapy (Lane and Chabner 2009, Pan et al. 2007, Mahlknecht and Hoelzer 2000).

3. Important anticancer compounds from marine organisms in other parts of the world

Several compounds from marine organisms have become promising drug candidates to fight cancer (Table 1). Compounds from marine organisms that first entered the drug pipeline include marine arabinonucleosides, adriamycin (doxorubicin), aplidine, ecteinascidins, dolastatin, and bryostatin. The arabinonucleosides ara-A (**1**) and ara-U (**2**) are synthetic analogues of two different spongouridine nucleosides from a *Tethya crypta* sponge. These nucleosides contained arabinose sugars instead of ribose sugars. Ara-A (**1**) or vidarabine is an antiviral drug that inhibits viral DNA synthesis (Doering et al. 1966, Privat de Garilhe and de Rudder 1964, Bergmann and Feeney 1951). These compounds along with the analogue ara-C (**3**) or cytarabine (**3**) served as models for anticancer drugs (Sagar et al. 2010, Mayer et al. 2010).

Some marine-derived compounds with anticancer activity have reached FDA approval and are currently being marketed under specific brand names (Table 1). Adriamycin (**4**) or doxorubicin is an anticancer drug that is a DNA intercalator. Adriamycin was first isolated from a *Streptomyces* bacterium in the Adriatic sea (Arcamore et al. 1969). Ecteinascidins, tetrahydroisoquinolines isolated from the Caribbean ascidian *Ecteinascidia turbinata*, showed antitumor activity against leukemia cells, with ecteinascidin 743 (**6**) and 729 (**7**) showing potent activities (Rinehart et al. 1990). Compound **6** alkylates DNA at guanine residues and interferes with DNA replication and transcription (Cragg 1999, Sakai et al. 1996, Fenical 1996). It was approved in 2009 under the brand name Yondelis/Trabectidin®. Another class of tetrahydroisoquinolines that are structurally similar to ecteinascidins are the renieramycins, which were isolated from several genera of sponges (*e.g., Reneira* sp. and *Xestospongia* sp.) (Suwanborirux et al. 2005, Frincke and Faulkner 1992). Renieramycins have potent anticancer activity, owing to their ability to downregulate protein tyrosine phosphatase receptor type K (PTPRK) in HCT-116 and MDA-MB-435 cancer cell lines (Charupant et al. 2009). Recently, the antimitotic compound, eribulin mesylate (**8**), an analogue of halichondrin B, first isolated from the sponge *Halichondria okadai*, was approved for clinical application after it was proven to increase the lifespan of cancer patients who had developed resistance to anthracycline and taxane drugs (Cortes and Lorca 2011, Huyck et al. 2011, Morris 2010). Eribulin mesylate and halichondrin B bind to tubulin and arrest the cell cycle at mitosis (Towle et al. 2001).

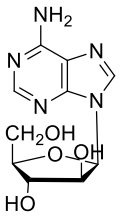
The occurrence, roles and functions of some secondary metabolites that have been documented in the natural environment have become evidence to suggest that host-microbial symbioses are important. Microbial symbionts are associated with diverse taxonomic groups, *e.g.,* ascidians, bryozoans, and sponges (Schmidt 2008, Lee et al. 2001). Dolastatin 10 (**9**), a linear peptide first isolated by Petit and co-workers from the sea hare *Dollabella auricularia,* was also found in the marine cyanobacterium *Symploca* sp. VP642 by Luesch and co-workers (Fennel et al. 2003, Luesch et al. 2001, Pettit et al. 1998, Pettit et al. 1997, Sone et al. 1997). It showed remarkable antitumor activity *in vivo* against PS leukemia and B16 melanoma cells and effected curative responses against human melanoma xenografts in nude mice at a dose range of 3.25 – 26 μg/kg (Pettit 1997). Dolastatins inhibit polymerization of tubulin dimers by inhibiting GTP hydrolysis (Downing 2000). Monomethyl auristatin E, an analogue of dolastatin 10, was linked to an antibody that can target CD30, a cell membrane protein present on Hodgkin's lymphoma cells. The duo was found to be highly effective and well tolerated, and was named brentuximab vedotin (Katz et al. 2011). Accelerated FDA approval was granted for this agent in August 2011 for use in Hodgkin's lymphoma and anaplastic large cell lymphoma (Deng et al. 2013, Gerwick and Moore 2012).

Compounds that reach the clinical trials stage are required to satisfy stringent standards before becoming approved. Dehydrodidemnin B or aplidine (**5**) is a cyclic depsipeptide isolated by the Rinehart Group from the ascidian *Tridemnun solidum*. It was the first marine natural product administered to humans. Aplidine (**5**) acts by inducing oxidative stress and triggering the pro-apoptotic receptor Fas (CD-95) to induce mitochondrion-mediated apoptosis (Gajate et al. 2003, Garcia-Fernandez et al. 2002, Grubb et al. 2001). It entered clinical trials in 1986, however, therapeutic blood levels in patients could not be attained (Molinski et al. 2009, Rinehart 2003, Broggini et al. 2003).

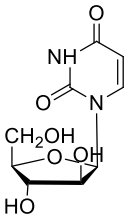
Bryostatin 1 (**10**), a macrolide lactone from the bryozoan *Bugula neritina*, was eventually traced to the *Candidatus Endobugula sertula* symbiont as the true origin of bryostatins. It inhibited tumor production by preventing the binding of tumor promoters to Swiss 3T3 mouse cells (Da Rocha et al. 2001, Haygood et al. 1999, Pettit et al. 1982). Compound **10** binds to Protein Kinase C (PKC), and prolonged exposure precludes PKC activity, which is overstimulated in various types of cancer. Phase I clinical trials established dose limiting toxicity as myalgia, which occurred in over 60% of patients (Varterasian et al. 1998).

Largazole (**11**), a cyclic depsipeptide from the cyanobacte-

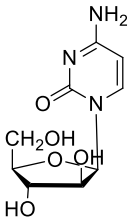
rium *Symploca* sp., was shown to be a potent HDAC inhibitor. This depsipeptide has highly differential growth-inhibitory activity and was found to preferentially target transformed over non-transformed cells (Hong and Luesch 2012). Largazole is in fact a pro-drug, which must be converted to its active form, free-thiol. Largazole and its active form were shown to have anti-proliferative effects on a panel of human malignant cell lines (Bowers et al. 2008).



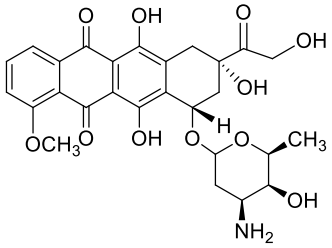
ara-A (**1**)
(Bergmann and Feeney 1951)



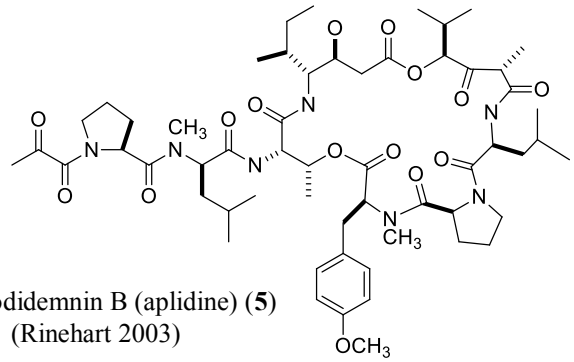
ara-U (**2**)
(Bergmann and Feeney 1951)



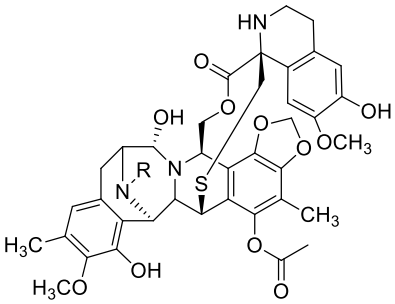
ara-C (cytarabine) (**3**)
(Sagar et al. 2010; Mayer et al. 2010)



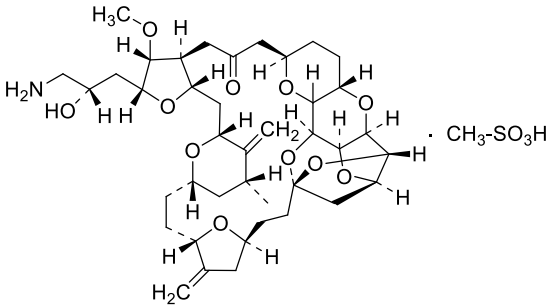
adriamycin or doxorubicin (**4**)
(Arcamone et al. 1969)



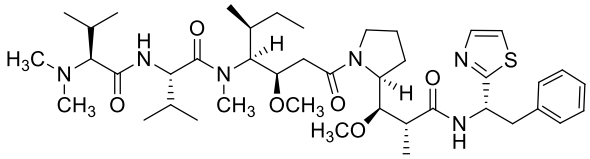
dehydrodidemnin B (aplidine) (**5**)
(Rinehart 2003)



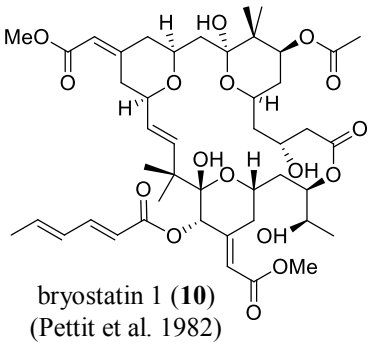
ecteinascidin 743, R=CH_3 (**6**)
729, R= H (**7**)
(Rinehart et al. 1990)



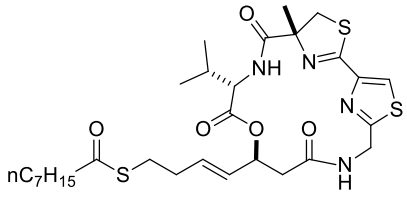
eribulin mesylate (**8**)
(Huyck et al. 2010)



dolastatin 10 (**9**)
(Pettit et al. 1997)



bryostatin 1 (**10**)
(Pettit et al. 1982)



largazole (**11**) from
(Hong and Luesch, 2012)

Table 1. Anticancer compounds from marine organisms in clinical development.

Compound	Natural Product Source/ Derivative	Target/Mechanism of Action	Approval/ Clinical (Year)	Status (Phase)	Sponsor	Reference
APPROVED						
ET 743 (**6**)	*Ecteinascidia turbinata*	DNA alkylation	2009	approved	PharmaMar	Rinehart et al. 1990, Cragg 1999, Sakai et al. 1996, Fenical 1996
E7389 eribulin mesylate (**8**)	halichondrin B analogue	tubulin	2010	approved	Eisai	Huyck et al. 2011, Tan et al. 2009, Vahdat et al. 2009
brentuximab vedotin	dolastatin 10 analogue	tubulin	2011	approved	Seattle Genetics	Gerwick and Moore 2012, Deng et al. 2013
CLINICAL TRIALS						
aplidine (**5**)	*Aplidium albicans*	protein synthesis	1994	II	PharmaMar	Gajate et al. 2003, Garcia-Fernandez et al. 2002, Grubb et al. 2001, Molinski et al. 2009, Rinehart 2003, Broggini et al.2003
dolastatin 10 (**9**)	*Dolabella auricularia*	tubulin	1995	II	NCI	Fennel et al. 2003, Luesch et al. 2001, Pettit et al. 1998, Pettit 1997, Sone et al. 1997, Downing 2000
LU-103793	dolastatin 15 analogue	tubulin	1996	II discontinued		De Arruda et al. 1995
bryostatin 1 (**10**)	*Bugula neritina*	protein kinase C	1998	II	NCI	Da Rocha et al. 2001, Haygood et al. 1999, Pettit et al. 1982, Varterasian et al. 1998
squalamine lactate	*Squalus* sp.	angiogenesis inhibitor	2001	II	Genaera	Herbst et al. 2003
LAF389	bengamide analogue	MetAp1 and 2	2002	I discontinued	Novartis	Singh et al. 2008
LY355703	analogue of cryptophycin	tubulin	2002	II	Eli Lilly	Edelman et al. 2003
discodermolide	*Discodermia dissoluta*	tubulin	2002	I discontinued	Novartis	Mita et al. 2003
HTI-286	hemiasterlin analog	tubulin	2002	I discontinued	Wyeth	Loganzo et al. 2003
kahalalide F	*Bryopsis sp.*	disrupts lysosomal structure	2002	II	PharmaMar	Rademaker-Lakhai et al. 2005
ILX651	dolastatin 15 analogue	tubulin	2003	II	ILX Oncology	Ebbinghaus et al. 2005
LAQ824	psammaplin analogue	histone deacetylase	2003	I	Novartis	De Bono et al. 2008
KRN 7000	agelasphin 9a analogue	immunostimulant, activation of NKT cells	2003	I	Kirin	Ishikawa et al. 2005
staurosporine	*Streptomyces staurosporeus*	protein kinase C	2004	I	Pfizer	Monnerat et al. 2004
salinosporamide A	*Salinispora tropica* CNB-440	proteasome inhibitor	2005	I	Nereus	Fenical et al. 2009
PRECLINICAL TRIALS						
largazole (**11**)	*Symploca* sp.	histone deacetylases				Hong and Luesch 2012, Bowers 2008
renieramycin	*Xestospongia* sp.	downregulates PTPRK				Charupant et al. 2009

4. Anticancer compounds isolated from Philippine marine organisms and their molecular targets

The National Cooperative Drug Discovery Group (NCDDG) project began in 1995 with the objective of collecting marine invertebrate samples from various geographic regions in the Philippines and discovering new anticancer agents. In compliance with the bioprospecting laws of the Philippines, a Commercial Research Agreement (CRA) was signed between the University of Utah, the University of the Philippines, and the Department of Agriculture. Prior Informed Consent (PIC) certificates were obtained from the municipalities where marine samples were collected. Local government units and residents of the municipalities were informed of the research that was being conducted and its importance to human society. A Memorandum of Understanding and Material Transfer Agreement were also signed between the two academic institutions. Several subsequent projects have continued the thrust of marine natural products research and drug development in the Philippines. These include the AMOR program, NKTI-RITM project, Conus/Turrid project, PharmaSeas program, and the PMS-ICBG program.

In the last 20 years, several bioactive compounds have been isolated from Philippine marine organisms. The structures of these compounds have remarkable diversity. They include cyclic peptides (Davis et al. 2004), quinones (Sandoval et al. 2004, Venables et al. 1997, Concepcion et al. 1995), pyridoacridines (De Guzman et al. 1999), pyrroles (Lazaro et al. 2002), complex alkaloids (Lazaro et al. 2006, Davis et al. 2003), macrolides (Pimentel et al. 2003), terpenes (Tasdemir et al. 2002b), sterol sulfates (Bugni et al. 2002), and others. These compounds are listed in Table 2. They were shown to be bioactive against sever-

Table 2. Summary of bioactive compounds isolated from Philippine marine organisms and associated bacteria.

Compounds	Structural Class	Bioactivity	Reference
bistratamides (**39**,**40**)	cyclic peptide	cytotoxic to human colon cancer HCT-116 cells	Foster et al. 1992
adociaquinones and xestoquinones (**12-15**)	quinone	cytotoxic to HCT-116 cells	Concepcion et al. 1995
makaluvamine N	pyrolloiminoquinone	topoisomerase II-inhibitor	Venables et al. 1997
bolinaquinone	sesquiterpene hydroxyquinone	cytotoxic to HCT-116 and CHO xrs-6 cells	De Guzman et al. 1998
neoamphimedine (**17**)	pyridoacridine	cytotoxic to normal CHO AA8 cells cytotoxic to KB tumors and HCT-116 in mice	De Guzman et al. 1999
agelasine	terpenoid	inhibited *M. tuberculosis* and drug resistant strains (izoniazid ATCC 358222, rifampicin ATCC 35838, ethambutol ATCC 35837, ethionamide ATCC 35830) *in vitro*	Mangalindan et al. 2000
aldisines (**19**,**20**)	alkaloid	cytotoxic to human tumor LoVo cells	Tasdemir et al. 2001
perophoramidine	alkaloid	cytotoxic to HCT-116 cells	Verbitski et al. 2002
bromotryptophans	bromoindole	cytotoxic to HCT-116 cells	Tasdemir et al. 2002a
heptylprodigiosin (**33**)	tripyrrole	anti-malaria activity against chloroquine-sensitive strain *Plasmodium falciparum* 3D7	Lazaro et al. 2002
isomalabaricanes	triterpene	cytotoxic to p21-deficient HCT-116 cells	Tasdemir et al. 2002b
p-sulfooxyphenylpyruvic acid	sulfated	minimal activity to epidermoid carcinoma A431 cells	Bugni et al. 2002
lissoclinotoxins (**21**,**22**)	alkaloid	cytotoxic to PTEN-deficient human breast cancer MDA-MB-468 cells	Davis et al. 2003
xestospongin B	macrolide	platelet aggregation inhibitor *in vivo*	Pimentel et al. 2003
kalihinols	diterpene	inhibits bacterial folate synthesis	Bugni et al. 2004
microcionamides (**31**,**32**)	cyclic peptide	cytotoxic to human breast cancer MCF-7 and SKBR-3 cells	Davis et al. 2004
cribostatin 7 reneirone	isoquinoline quinones	cytotoxic to HCT-116 cells	Sandoval et al. 2004
crambescidin	guanidine alkaloid	anti-malaria activity against *Plasmodium falciparum* 3D7	Lazaro et al. 2006
speciosterol sulfates (**23-26**)	sterol sulfates	inhibits NF-κB activation in human chondrocytes	Whitson et al. 2008
fibrosterol sulfates (**27**,**28**)	sulfated sterol	inhibits PKCζ	Whitson et al. 2009
deoxyamphimedine (**18**)	pyridoacridine	cytotoxic to human ovarian cancer A2780wt and A2780AD cells	Marshall et al. 2009
chondropsins (**34**,**35**)	macrolide	cytotoxic to LOX (melanoma) and OVCAR-3 (ovarian) human tumor cell lines	Coombs et al. 2010, Cantrell et al. 2000

al types of cancer cells, including some drug resistant cell lines (Marshall et al. 2009). There is great probability that more bioactive compounds will be discovered from marine organisms in the Philippines and there is hope that new mechanisms of action to inhibit tumor growth will be unveiled.

4.1 Isolation and cell-based molecular target testing of compounds: General strategies

A general strategy was adopted for discovering these new bioactive compounds (Figure 1). In the early years, marine extracts were tested for selective cytotoxicity in a panel of cancer cell lines. Cytotoxic extracts containing nitrogenous compounds (detected by chloro-o-toluidine spray reagent on thin layer chromatography) were prioritized for purification, extracted with methanol, and partitioned following the procedure of Kupchan et al. (1973), C18 adsorption for de-salting, step gradient column chromatography and reverse phase HPLC. Since 1995, Wyeth-Ayerst (Oncology Department), in collaboration with the University of Utah and NIH, implemented screens for inhibitors of protein kinases, cell cycle, transcription, and translation, and other molecular targets. Aside from using a panel of human tumor cell lines, Wyeth-Ayerst included clinically resistant cell lines in their screening assays. Bioactive compounds against the drug-resistant cell lines could exhibit mechanisms of action different from compounds that are tested on typical cancer cells. The NCDDG crude and semi-pure extracts were tested for cytotoxicity in cancer cell lines (NCDDG Report 1999).

Priority compounds were those with IC_{50} values in the low µM to nM range indicating high potency and those with a sharp sigmoidal dose response (fractional survival) suggesting well defined molecular targeting and potential for a wide therapeutic window. Priorities were identified using molecular target assays performed at Wyeth-Ayerst and other laboratories. The structures of pure compounds were extensively elucidated by HR-ESIMS/HR-FABMS, LC-MS, and NMR spectroscopy, *e.g.,* ^{1}H-^{13}C- and 2D-NMR, DEPT, HMBC, HMQC, NOESY, and ROESY.

4.2. Compounds from Philippine marine organisms acting on classical molecular targets in cancer

4.2.1. Adociaquinones and xestoquinones

Most anticancer drugs act during the S and M phases of the cell cycle. Compounds that act as TOPO 2 inhibitors prevent DNA relaxation, which is required for DNA replication, transcription, recombination and other functions. Compounds like vinca alkaloids and taxanes act in the M phase of the cell cycle,

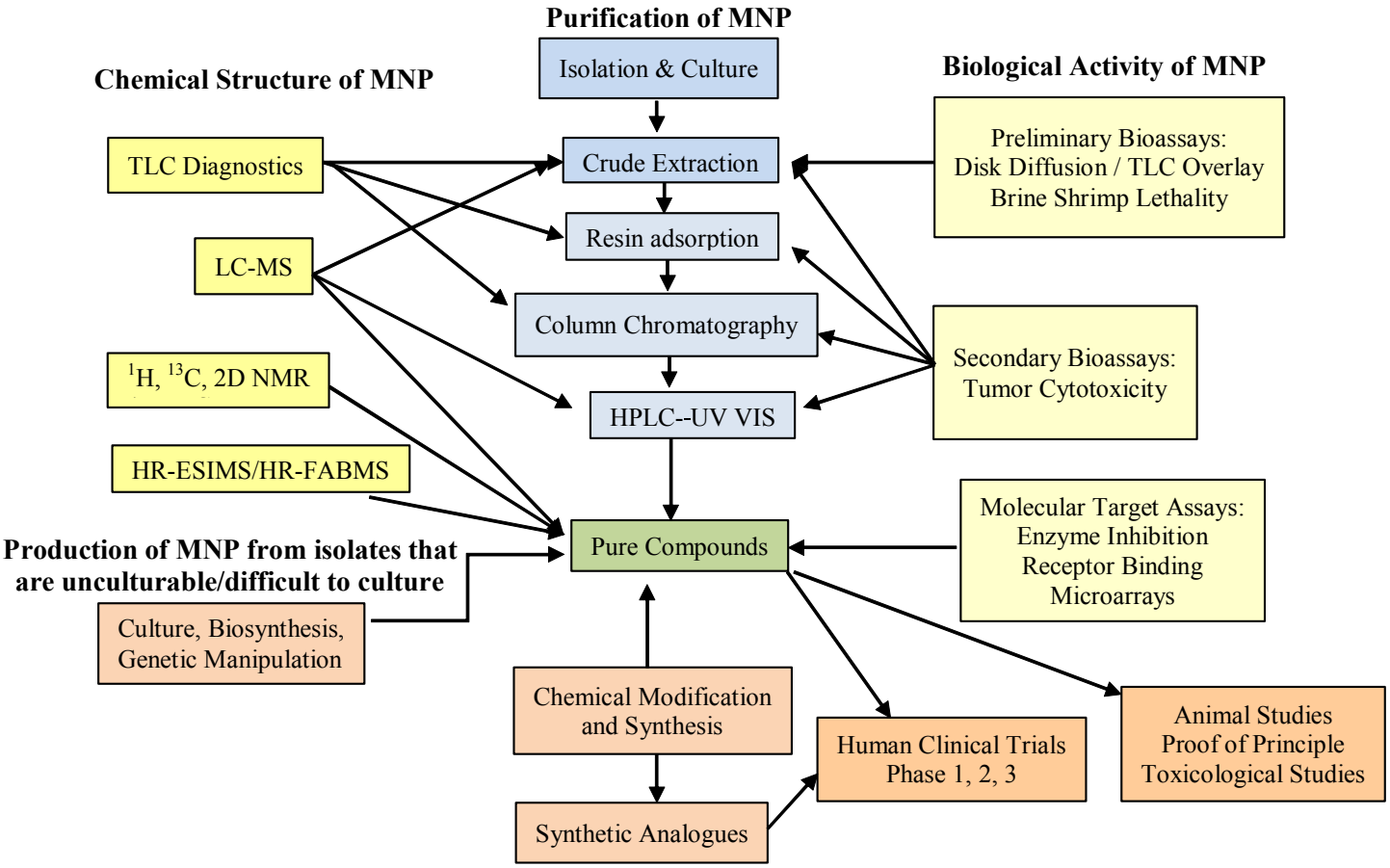
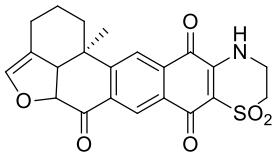


Figure 1. General strategy for discovering new anticancer compounds in the University of Utah - University of the Philippines Marine Natural Products (MNP) project.

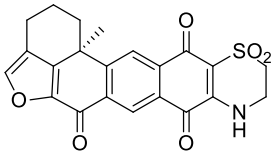
inhibiting tubulin polymerization and depolymerization, respectively (Morris and Fornier 2008). The S and M phases are separated by the G_1 and G_2 phases. Interestingly, only a few drug leads have been found to act during the G_1 and G_2 phases of the cell cycle (Coombs et al. 2010, Davis et al. 2003, Lazaro et al. 2002).

TOPO 2 alters DNA topology by virtue of a DNA strand double break. Higher levels of this enzyme were detected in tumor cells. TOPO 2 has been used as a molecular target for anticancer agents in clinical use (Hsiang and Liu 1988, Liu 1989). FDA-approved TOPO 2 inhibitors include etoposide, teniposide, doxorubicin, idarubicin, edarubicin, and mitoxantrone (Thakur 2011).

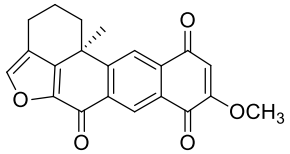
Adociaquinones and xestoquinones were isolated from a *Xestospongia* sp. sponge in Bolinao, Pangasinan, Philippines. These pentacyclic quinones showed significant cytotoxicity. Adociaquinones A (**12**) and B (**13**) showed cytotoxicity to HCT-116 colorectal cancer cells at IC_{50} values of 24 and 21 µM, respectively. The 14-methoxyxestoquinone (**14**) and 15-methoxyxestoquinone (**15**) were cytotoxic to the same cell line at 28 µM. In addition, these compounds showed cytotoxicity to CHO xrs-6 cells (Concepcion et al. 1995). The CHO xrs-6 cells are more susceptible to DNA double strand breaks than the normal CHOs (Swaffer et al. 1994). Since many TOPO 2 poisons are DNA intercalators, the ability of adociaquinone B (**13**) to intercalate with DNA was measured. However, adociaquinone B (**13**) did not displace ethidium bromide from calf thymus DNA, thereby suggesting that it is not a DNA intercalator (Concepcion et al. 1995).



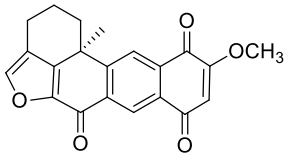
adociaquinone A (**12**)
(Concepcion et al. 1995)



adociaquinone B (**13**)
(Concepcion et al. 1995)



14-methyloxyxestoquinone (**14**)
(Concepcion et al. 1995)



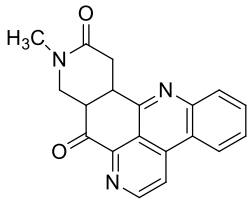
15-methyloxyxestoquinone (**15**)
(Concepcion et al. 1995)

4.2.2. Amphimedines

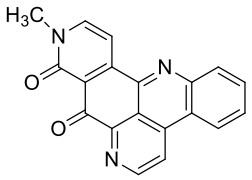
Pyridoacridines are compounds that can intercalate with DNA and interfere with DNA topoisomerases. Amphimedine (**16**), neoamphimedine (**17**), and deoxyamphimedine (**18**) are chemically similar, but minor structural differences result in differing abilities to intercalate with DNA. This DNA intercalation results in catenation and subsequent DNA damage (Marshall et al. 2009). Amphimedine was first reported from a Pacific sponge *Amphimedon* sp. in Guam by Schmitz et al. (1983). Neoamphimedine (**17**), first isolated from a *Xestospongia* sp. sponge in Surigao, Philippines, was shown to catenate DNA in the presence of TOPO 2 (De Guzman et al. 1999). It showed significant cytotoxicity to HCT-116 cells and KB cells compared with amphimedine (**16**) (Marshall et al. 2003).

Neoamphimedine (**17**) was comparable to etoposide in reducing the size of tumor xenografts of the HCT-116 colorectal cancer cell line and the KB nasopharyngeal cancer cell line in nude mice; on the other hand, amphimedine (**16**) had no tumor reduction effect (Marshall et al. 2009).

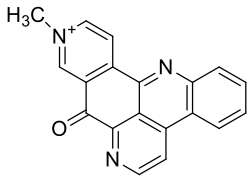
Deoxyamphimedine (**18**) differs slightly in structure from neoamphimedine (**17**). It is positively charged and has a high tendency to intercalate into DNA. Compound **18** damaged DNA *in vitro* without the presence of topoisomerases by generation of reactive oxygen species (ROS). The activity of **18** was decreased in low oxygen, with removal of a reducing agent, and in the presence of antioxidants (Marshall et al. 2009).



amphimedine (**16**)
(Schmitz et al. 1983, Marshal et al. 2009)



neoamphimedine (**17**)
(de Guzman et al. 1999)



deoxyamphimedine (**18**)
(Marshall et al. 2009)

4.3. Compounds from Philippine marine organisms acting on new molecular targets in cancer

Compounds isolated from Philippine marine organisms were also found to act on new cancer targets and pathways, *e.g.,* in the G (gap) phases of the cell cycle when DNA damage is repaired or when apoptosis is initiated (Davis et al. 2003, Lazaro et al.

2002). Other compounds act on major cell cycle pathways: growth factor and hormone signaling, cytokines and related survival factors, anti-growth factors, apoptosis and cell death, and extracellular matrix proteins (Table 3).

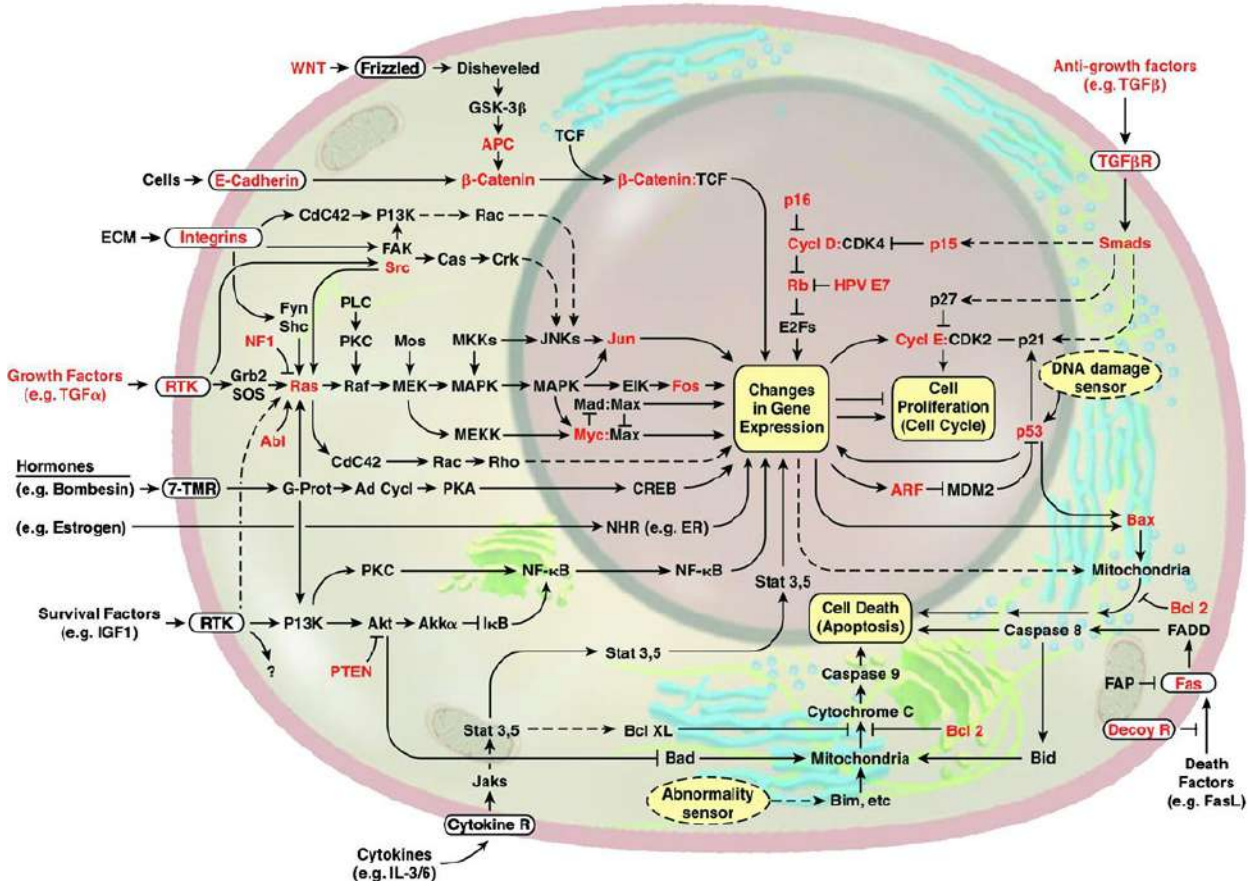


Figure 2. The various signaling pathways in the cell. The emergent integrated circuit of the cell by Hanahan and Weinberg (2000). Signaling pathways are represented as intergrated circuits composed of proteins – *e.g.,* kinases and phosphatases as transistors. Reprinted from Cell *vol.* 100, Hanahan, D. and Weinberg, R.A. The Hallmarks of Cancer, pp. 57-70, Copyright (2000), with permission from Elsevier.

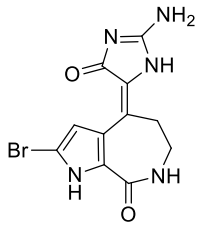
Table 3. Compounds from Philippine marine organisms with identified molecular targets against cancer.

Compounds	Structural Class	Bioactivity	Reference
adociaquinones (**12**,**13**) xestoquinones (**14**,**15**)	quinone	topoisomerase II inhibitor	Concepcion et al. 1995
makaluvamine N	pyrroloiminoquinone	topoisomerase II inhibitor	Venables et al. 1997
neoamphimedine (**17**)	pyridoacridine	DNA intercalator and catenator in the presence of topoisomerase II	De Guzman et al. 1999
deoxyamphimedine (**18**)	pyridoacridine	DNA intercalator and catenator in the presence of ROS	Marshall et al. 2009
lissoclinotoxins (**21**,**22**)	alkaloid	PI3-K/AKT/mTOR pathway	Davis et al. 2003
aldisines (**19**,**20**)	alkaloid	growth signaling MEK pathway	Tasdemir et al. 2001
topsentiasterol sulfate E fibrosterols (**26**-**28**)	sulfated sterol	PKCζ pathway	Whitson et al. 2009
carteriosulfates (**36**-**38**)	sulfated acid	GSK-3β inhibitor	McCulloch et al. 2009
stelletins (**29**,**30**)	triterpene	p21 and p53 signaling pathway	Tasdemir et al. 2002b
microcionamides (**31**,**32**)	cyclic peptide	apoptosis and cell death pathway	Davis et al. 2004
heptylprodigiosin (**33**)	tripyrrole	apoptosis and cell death pathway	Lazaro et al. 2002
chondropsins (**34**,**35**)	macrolide	Wnt signaling pathway	Coombs et al. 2010

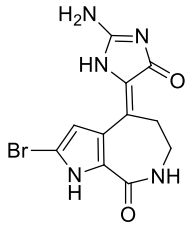
4.3.1. Growth factor and hormone signaling

Raf/MEK/MAPK proteins are involved in cellular signaling processes. The Ras-MAPK cascade is involved in transmitting extracellular signals into the cytosol and nucleus. Activated Raf-1 phosphorylates and activates the dual specificity kinase, MEK (MAP kinase kinase). Consequently, activated MEK-1 phosphorylates and activates MAPKs (mitogen activated protein kinases), which can translocate to the nucleus and modulate cell proliferation and differentiation (Lavoie et al. 1996, Kolch 2000).

The sponge *Stylissa massa* (PS97-1-21) from Surigao, Philippines afforded eight known alkaloids. 10*E*-hymenialdisine (**19**) and 10*Z*-hymenialdisine (**20**) gave significant enzyme inhibition against the Raf/MEK1/MAPK kinase signaling cascade by selectively inhibiting the phosphorylation of MAPK by MEK-1 with IC_{50}s of 3 and 6 nM, respectively (Tasdemir et al. 2001). Compounds (**19**) and (**20**) were tested for their ability to inhibit the growth of LoVo and Caco-2 human colon tumor cell lines. LoVo cells are sensitive to growth inhibition by Ras farnesyl protein transferase inhibitors (FTIs) (Lerner et al. 1997). Ras is a protein that is abnormally active in cancer cells, and FTIs inhibit Ras from functioning properly by disrupting the farnesyltransferase enzyme that is critical to the formation of the pre-Ras protein. Without the farnesyl arm, Ras is unable to attach to the cell membrane and transfer signals to membrane receptors (Weinberg 2007a). The sensitivity of LoVo cells to FTIs is attributed to the presence of mutant activated K-Ras. Mutant K-Ras causes the activation of the Raf/MEK-1/MAPK signaling cascade that emanates from Ras. Inhibitors of Raf or MEK-1 should inhibit LoVo growth. The Caco-2 cells contain wild-type K-Ras, which correlates with these cells' resistance to the growth inhibitory effects of FTIs (Lerner et al. 1997).



10*E*-hymenialdisine (**19**)
(Tasdemir et al. 2001)



10*Z*-hymenialdisine (**20**)
(Tasdemir et al. 2001)

Compounds **19** and **20** inhibited the growth of LoVo cells with IC_{50} of 586 and 710 nM, respectively, but were shown to be less effective in Caco-2 cells. Hamilton FTI-286, a known Ras-inhibitor, was used as a reference in this assay and inhibited the growth of LoVo cells with IC_{50} of 50 nM. Meijer et al. (2000) reported that hymenialdisine inhibits glycogen synthase kinase-3β (GSK-3β), cyclin-dependent kinase (CDK1), and cyclin kinase (CK1) at nM levels. These protein kinases are involved in cellular signaling. Furthermore, CDK1 and CK1 act as checkpoints in the cell cycle. The inhibition of these protein kinases can have profound effects on cellular processes. Also, Curman et al. (2001) reported the inhibition of the G2-DNA checkpoint (Chk), Chk1, and Chk2 kinases by hymenialdisine and debromohymenialdisine at μM levels.

4.3.2. Cytokines and related survival factors signaling

Cytokines are growth factors that stimulate parts of the hematopoietic system. Receptors for cytokines form complexes with tyrosine kinases of the Jak class, which phosphorylate STATs (signal transducers and activators of transcription). The STATs form dimers and migrate into the nucleus. Here, they function as transcription factors of genes involved in cell growth and survival (Weinberg 2007b). The PTEN (Phosphate and Tensin Homolog), a phosphatase enzyme, is a negative regulator of the PI3-K/AKT/mTOR pathway. This pathway is important in suppressing apoptosis and promoting cell growth when survival signals are present. In cancer cells, this pathway can become deregulated either from PI3 hyperactivation or PTEN inactivation (Weinberg 2007c). Mutations and deletions in PTEN frequently occur in advanced lung and ovarian cancers (Wang et al. 1998, Rasheed et al. 1997). The MDA-MB-435S ($PTEN^{+/+}$) human breast carcinoma cell line possesses a wild-type PTEN protein and is specific to PI3-K/ AKT/mTOR cellular signaling pathway.

Lissoclinotoxins E (**21**) and F (**22**) are dimeric alkaloids from an unidentified Philippine didemnid ascidian in the Batanes Islands, Philippines. Lissoclinotoxin E (**21**), the *trans*-isomer as the main metabolite, and lissoclinotoxin F (**22**), the *cis*-isomer as the minor metabolite, have IC_{50} of 2.3 and 1.5 μg/mL, respectively, against the PTEN-deficient ($PTEN^{-/-}$) human breast carcinoma cell line, MDA-MB-468 (Davis et al. 2003). Compounds **21** and **22** are cytotoxic against MDA-MB-435S human breast carcinoma cell line with IC_{50} values of 2.1 and 4.2 μg/mL, respectively. However, compound **21** is not selective between the two cell lines, while compound **22** gave approximately three-fold greater potency against the PTEN-deficient cell line (Davis et al. 2003).

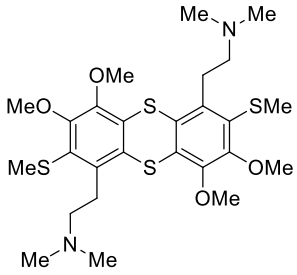
The nuclear factor kappa B (NF-κB) protein complex is involved in the transcription of DNA. NF-κB is present in several cells (*e.g.,* human chondrocytes) and activates the expression of many genes (*e.g.,* anti-apoptotic and mitogenic genes) (Weinberg 2007d). Protein kinase C functions by controlling other proteins via signal transduction cascades. The activated PKCξ isoform can induce cell proliferation by activating other transcription factors (*e.g.,* Fos, Jun, AP-1) (Weinberg 2007e).

Spheciosterol sulfates A-C (**23-25**) (Whitson et al. 2008), 4β-hydroxy-14α-methyl sterols, and topsentiasterol sulfate E (**26**) (Fusetani et al. 1994) were isolated from the marine sponge *Spheciospongia sp*. (CDO-00-12-141) from Cagayan de Oro, Philippines. The compounds inhibited NF-κB activation with EC_{50} values of 12-64 μM using primary human chondrocytes. Compounds **23-26** inhibited PKCζ with IC_{50s} of 1.59, 0.53, 0.11 and 1.21 μM, respectively (Whitson et al. 2008).

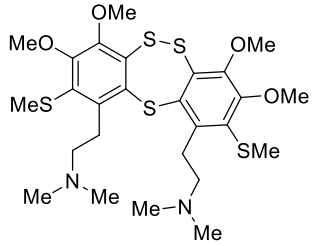
Fibrosterol sulfates A (**27**) and B (**28**) are sulfated sterol dimers isolated from a *Lissodendoryx (Acanthodoryx) fibrosa* sponge from Coron Island Palawan, Philippines (Whitson et al. 2008). Compounds **27** and **28** inhibited PKCζ with IC_{50} values of 16.4 and 5.6 µM, respectively (Whitson et al. 2009).

4.3.3. Anti-growth factors signaling

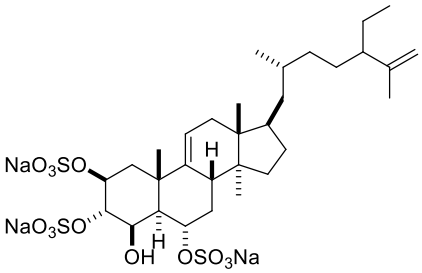
The p21 protein is a potent cyclin-dependent kinase inhibitor (CKI). The expression of p21 is induced by p53 protein. The genes of the p53 protein are among the most commonly mutated



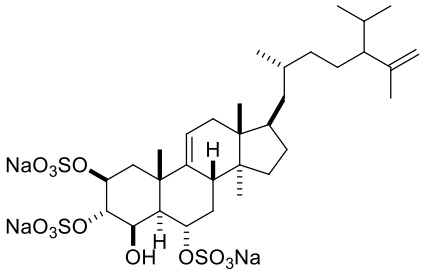
lissoclinotoxin E (**21**)
(Davis et al. 2003)



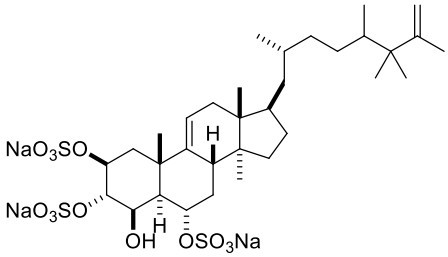
lissoclinotoxin F (**22**)
(Davis et al. 2003)



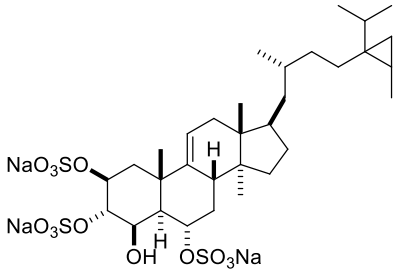
spheciosterol sulfate A (**23**)
(Whitson et al. 2008)



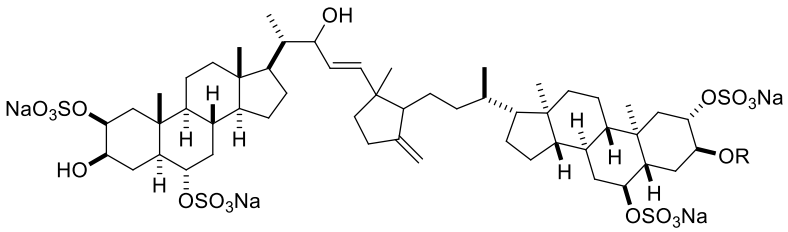
spheciosterol sulfate B (**24**)
(Whitson et al. 2008)



spheciosterol sulfate C (**25**)
(Whitson et al. 2008)



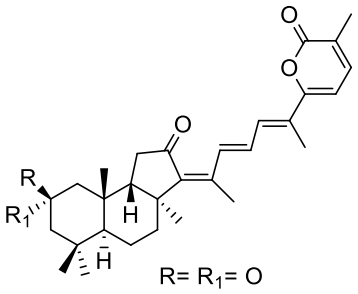
topsentiasterol sulfate E (**26**)
(Fusetani et al. 2008)



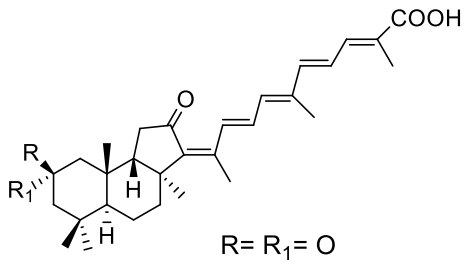
fibrosterol sulfate A (**27**); R=H
(**28**); R=SO_3Na
(Whitson et al. 2009)

in human cancers. The overexpression of p21 arrests the cell cycle by inhibiting many cyclin-dependent kinases. The p21-dependent cell cycle arrest allows for cellular repair before continuation of the cell cycle. Without this checkpoint, mutated cells have the chance to proliferate unchecked (Weinberg 2007f).

Stelletins isolated from the marine sponge *Rhabastrella globostellata* in Guimputlan in Mindanao, Philippines, were tested against isogenic colorectal cancer cells, wild-type HCT-116, and the corresponding p21-deficient mutant cell line. Stelletin B (**29**) and stelletin E (**30**) gave selective cytotoxicity toward $p21^{WAF1/Cip1}$-deficient HCT-116 colorectal cancer cell line with IC_{50} values of 0.043 and 0.039 µM, respectively. The other compounds were inactive. Compounds **29** and **30** did not show differential cytotoxicity in wild type (WT) and p53-deficient HCT-116 cell lines. The mechanism of action in both compounds may be p21-dependent and p53-independent, or the cells lacking the p21 genes may be more sensitive to these compounds than the wild type cells and thus preferentially undergo cell death/apoptosis (Tasdemir et al. 2002b).



stelletin B (**29**)
(Tasdemir et al. 2002)



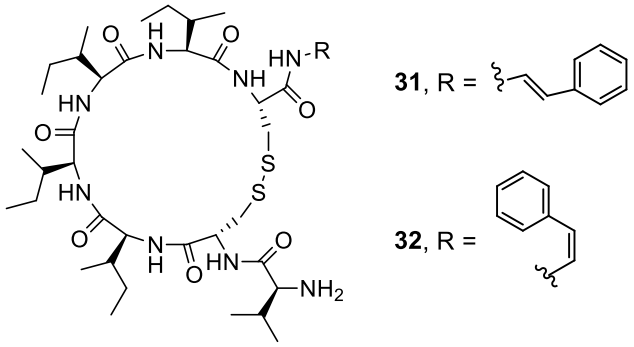
(-)stelletin E (**30**)
(Tasdemir et al. 2002)

4.3.4. Apoptosis and cell death signaling

Resistance to cell death is one of the hallmarks of cancer, and the evasion of apoptosis is one way for cancer cells to continue proliferation (Hanahan and Weinberg 2011). The apoptosis machinery is composed of both upstream and downstream effector components. Cancer cells avoid apoptosis by creating mutations or deletions in the TP53 gene responsible for encoding the p53 protein, a critical damage sensor in the apoptosis-inducing circuitry system. Another strategy for avoiding apoptosis is to increase the expression of anti-apoptotic regulators or to down-regulate pro-apoptotic factors (Hanahan and Weinberg 2011). Anticancer therapy studies have shown that apoptosis-inducing stresses result in changes in cancer cells like DNA damage and signaling imbalances (Adams and Cory 2007).

Clathria (Thalysias) abietana, a marine sponge from Tigtabon Island, Zamboanga in Southern Mindanao, Philippines, gave two linear peptides cyclized via a cystine moiety, and the C -terminus was blocked by a 2-phenylethylenamine moiety. The microcionamides A (**31**) and B (**32**) are cytotoxic toward human breast tumor cell lines MCF-7 and SKBR-3, with IC_{50} comparable to those of the positive control doxorubicin (**4**) (MCF-7: 257 nM; SKBR-3: 33 nM).

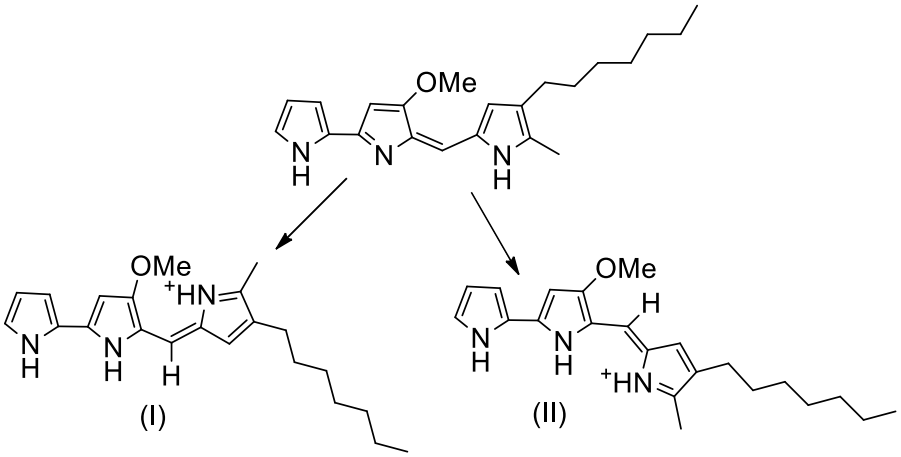
Compound **31** has IC_{50} of 125 and 98 nM for MCF-7 and SKBR-3 cells, respectively. The minor compound **32** was active against the aforementioned cell lines with IC_{50} values of 177 and 172 nM, respectively. The MCF-7 cells' morphology after 24 h of compound treatment showed the hallmarks of apoptosis, *e.g.*, cytoplasmic blebbing and condensation, shrinkage, loss of cell-to -cell contact, and formation of membrane bound vesicles (Davis et al. 2004). Extensive DNA fragmentation, a biomarker of apoptotic cells, and Hoescht staining of MCF-7 and SKBR-3 cells confirmed the apoptosis phenomena. Compounds **31** and **32** also exhibited anti-tuberculosis activity against *Mycobacterium tuberculosis $H_{37}Ra$* with MIC values of 5.7 µM, as compared to the positive control rifampicin (MIC 1.52 nM) (Philippine Patent No. 1-2004-000049) (Davis et al. 2004).



microcionamide A (**31**)
B (**32**)
(Davis et al. 2004)

One of the obstacles in dealing with compounds from collected sponges and other marine invertebrates is the limited amount of starting material and pure compounds obtained from them. To address this problem, the culture of marine microorganisms was explored at the Marine Natural Products Laboratory at UP MSI.

The marine microbial isolate Z143-1 is a *Pseudovibrio denitrificans* from an unidentified tunicate from Zamboanga del Norte, Mindanao, Philippines. It produced a red pigment hep-



heptylprodigiosin (**33**)
(Sertan-de Guzman et al. 2007)

Figure 3. Heptylprodigiosin (HPDG) (33) isolated from marine microbial isolate Z143-1, a *Pseudovibrio denitrificans* strain from an unidentified tunicate collected by SCUBA in Zamboanga del Norte in Mindanao, Philippines. Possible conformers (I) *α*- and (II) *β*-HPDG. HPDG from Z143-1 has *β*-conformer in $CDCl_3$ (Sertan-de Guzman et al. 2007).

tylprodigiosin (HPDG) (**33**) (Figure 3), or 16-methyl-15-heptylprodiginine, that was active against *Staphylococcus aureus*. The morphological, physiological, and biochemical characteristics of the Z143-1 bacterial isolates have similarities with those of the *Pseudovibrio denitrificans* type strain and *Pseudovibrio denitrificans* F_{423}, except for their ability to ferment glucose. Z143-1 has white translucent colonies in marine agar at an early stage and turns red as the culture ages, a characteristic not observed with the other two *P. denitrificans* strains. Z143-1 is a gram negative, rod-shaped, motile, polarly flagellated, and oxidatively metabolizing chemoorganotrophic microorganism capable of denitrification. It can hydrolyze gelatin and enulin, metabolize acid from glucose, and produce indole (Sertan-de Guzman et al. 2007).

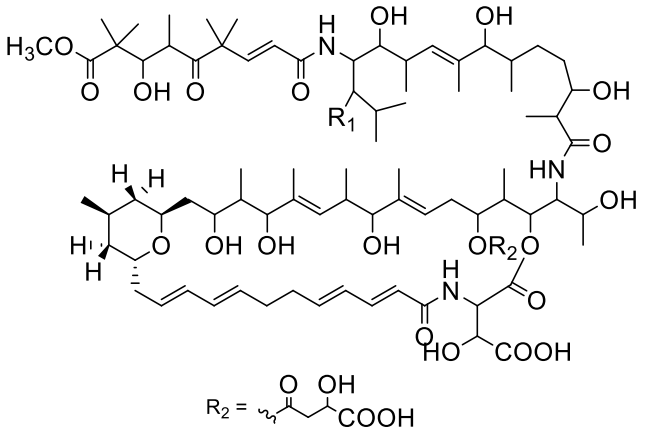
Rizzo et al. (1999) reported on the α- and β-conformers in **33**. Sertan-de Guzman et al. (2007) reported on the pure β-rotamers in $CDCl_3$, which were active against methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA). Also, Lazaro et al. (2002) reported the anti-malaria properties of this compound. As a potential drug for development, their various biological activities were explored in the laboratory, *e.g.,* tumor cytotoxicity, immunosuppressant activity, and apoptotic effects, as well as for their potential use in molecular target and mechanism-based studies, and for incorporation into drug delivery systems with antibody targeting, for use in animal studies, for the preparation of analogues, and for use with biosynthetic genes being isolated (Monge et al. 2007, Soto-Cerato et al. 2007). Compound **33** displayed cytotoxicity and apoptotic activity in human Jurkat T cell leukemia (S-Jurkat). It triggered apoptosis in a caspase 3-dependent and CD95 receptor-mediated manner. It also inhibited the protection mechanism of the anti-apoptotic proteins Bcl2 and Bcl-xl (Ranches et al. 2013). A preliminary study using BALB/c xenografted with a BALB/c-derived mouse mammary cancer cell line 4T1 was undertaken for heptylprodigiosin; this study will be the subject of another paper.

4.3.5. Extracellular matrix proteins

Wnts are a conserved family of secreted acylated and glycosylated protein hormones. Wnts regulate diverse signaling pathways from the cell surface to the nucleus, using canonical and non-canonical pathways. The canonical pathway involves an accumulation of β-catenin in the cytosol to activate transcription factors in the nucleus, while the non-canonical pathway makes use of calcium and of other proteins to effect changes in the cell nucleus (Gordon and Nusse 2006). The impacts of Wnts to cell processes are broad and comprise the following: cell proliferation, fate determination, morphology, polarity, and motility. In addition, Wnts regulate stem cell proliferation and may control the fate of cancer stem cells. The Wnt signaling pathways are tightly controlled by positive and negative regulators during the development and adult life of humans and are misregulated in many cancers. Chondropsin A (**34**) and its 73 deoxy analogue (**35**) reduce the secretion of active Wnt3A (Coombs et al. 2010, Cantrell et al. 2000). Chondropsins target V-ATPase, thus blocking vesicular acidification which inhibits Wnt processing and secretion. Blocking vesicular acidification potently inhibits Wnt/planar cell polarity signaling and Akt activation and mTOR activity. Consequently, this also inhibits the Wnt/β-catenin signaling pathway. The Wnt, as acidification inhibitors, may contribute to their pleiotropic effects and potential anticancer efficacy (Coombs et al. 2010).

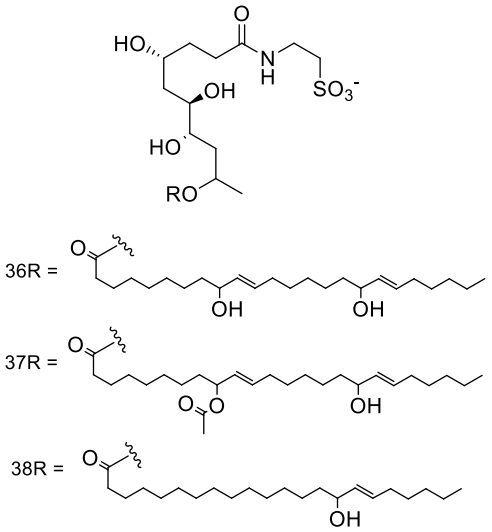
The enzyme GSK-3β plays a role in the Wnt/β-catenin signaling pathway by phosphorylating and marking β-catenin for subsequent degradation (Doble and Woodgett 2003). A *Carteriospongia* sp. sponge (PSO-04-3-79) from Sorsogon, Philippines, yielded oxygenated fatty acid derivatives with low μM GSK-3β inhibition. Carteriosulfonic acids A-C (**36-38**) inhibited GSK-3β in a ^{32}P-labeling assay with IC_{50} values of 12.5, 6.8, and 6.8 μM, respectively. The long chain fatty acid component of the carteriosulfonic acids is critical for GSK-3β inhibition and is also a

good activator of Wnt signaling via β-catenin phosphorylation (McCulloch et al. 2009).



chondropsin A (**34**); R=OH
(Cantrell et al. 2000)

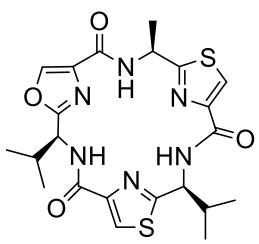
73-deoxychondropsin A (**35**); R=H
(Coombs et al. 2010)



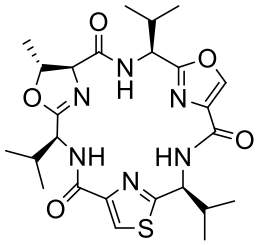
carteriosulfonic acid A (**36**)
B (**37**)
C (**38**)
(McCullough et al. 2009)

5. Challenges and collaborations in marine drug discovery

There are many challenges to be met in marine drug discovery, principally the supply problem. Studies on promising compounds from marine invertebrates require the collection of vast amounts of the source organisms in order to establish their chemical structures and bioactivities. Even more material is needed if the compounds are to be used in pre-clinical and clinical trials. Farm culture of source organisms like sponges and ascidians can be a viable option if the compounds are produced at medium to high concentrations (Sipkema et al. 2005). Another strategy is to exploit the symbiotic bacteria of marine invertebrates. The PharmaSeas and PMS-ICBG programs were successful in isolating bioactive compound-producing microorganisms from sponges and mollusks. In cases where isolation of the symbiotic bacteria on media is difficult or impossible, sequencing the symbiont's biosynthetic genes that produce the bioactive compound can be an alternative option. The *Prochloron* symbiont in the ascidian *Lissoclinum bistratum* provides an example of this strategy. Bistratamides C **(39)** and D **(40)** were isolated from a *Lissoclinum bistratum* ascidian in Bolinao, Pangasinan, Philippines (Foster et al. 1992). Compounds **39** and **40** exhibited moderate cytotoxicity against HCT-116 cells. The *Prochloron* symbiont was difficult to culture in the laboratory, and so bistratamides could not be isolated directly from cultures of the symbiont. Subsequently, Schmidt (2008) showed that the biosynthetic pathway for the bistratamides was ribosomal protein synthesis, which could be attributed to the *Prochloron* symbiont in the ascidian. In this case, even though the symbiont could not be directly cultured, the biosynthesis of bioactive compounds in other microbial hosts, (*e.g.,* in *E. coli*) or chemical synthesis can be pursued to ensure a steady supply of bioactive compounds.



bistratamide C (**39**)
(Foster et al. 1992)



bistratamide D (**40**)
(Foster et al. 1992)

The second challenge pertains to the need for anticancer drugs that are highly specific to cancer cells and their mutations. Combinatorial synergy of compounds acting on different molecular targets has been explored to improve efficacy and reduce drug resistance. A synergistic effect was observed with HPDG (**33**) and adociaquinone B (**13**) against MCF-7 cells (Bojo et al. 2010). These compounds may act on various molecular targets such as DNA topoisomerases, cell growth and survival signaling, and apoptosis targets. The specificity can be enhanced by conjugating them directly to antibodies that target tumor surface markers or by incorporation into stealth liposomes or other delivery agents linked to an antibody that targets a pancarcinomic cell surface antigen. Gemtuzumab ozogamicin, a conjugate of calicheamycin and anti-CD33 antibody, was the first antibody-targeted therapeutic agent approved by the FDA (Hamann et al. 2002). Tumor surface antigens, such as receptors and glycoproteins, are potential targets. The binding of the targeting agent, the size and properties of the drug delivery system, and the amount of drug that it will carry will be considered in future studies.

6. Summary and conclusion

The future direction points towards more anticancer drugs from marine organisms. Cellular pathways that are unique to cancer should be targeted, and the therapeutic potential of the compounds should be tested *in vivo*. Structure-activity relationship studies should be pursued to examine the molecular structure properties of compounds and the corresponding effects on their interactions. This implies establishing a strong medicinal chemistry program in the Philippines. Our vision is to build multidisciplinary, coordinated research programs in the marine biomedical field. We would like to continue to explore the marine biodiversity of the Philippines as a source of small molecule drugs for the treatment of serious diseases like cancer.

ACKNOWLEDGMENTS

We acknowledge Prof. Chris M. Ireland and the NCDDG Group, Department of Medicinal Chemistry, University of Utah and the Marine Natural Products Group, The UP Marine Science Institute, for the research collaboration, with funding from the US NIH NCI NCDDG Grant # U19 CA67786, CFDA # 93.395 Philippine Subcontract # 9910042-00 (G.P.C.). We thank the Philippine Department of Science and Technology (DOST) (G.P.C.) and the International Foundation for Science (IFS) (G.P.C.) for complementary funding. We especially thank Dr. Lilibeth Salvador-Reyes for her constructive comments to improve the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

CONTRIBUTIONS OF AUTHORS

MAA and ARA contributed equally to writing the manuscript and preparing the figures and tables. GPC provided guidance to MAA and ARA, and contributed to writing and editing the manuscript.

REFERENCES:

Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 2007; 26: 1324-1337.

Arcamore F, Cassinelli G, Fantini G, Grein A, Orezzi P, Pol C, Spalla C. Adriamycin, 14-hydroxydaimomycin, a new antitumor antibiotic from *S. Peucetius var. caesius*. Biotechnol Bioeng 1969; 11: 1101-1110.

Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell 2005; 7: 211-217.

Bergmann W, Feeney RJ. Contributions to the study of marine products. XXXII. The nucelosides of sponges. I. J Org Chem. 1951; 16: 981-987.

Bhalla K, List A. Histone deacetylase inhibitors in myelodysplastic syndrome. Best Pract Res Cl Ha 2004; 17: 595–611.

Broggini M, Marchini SV, Galliera E, Borsotti P, Taraboletti G, Erba E, Sironi M, Jimeno J, Faircloth GT, Giavazzi R, D'Incalci M. Aplidine, a new anticancer agent of marine origin, inhibits vascular endothelial growth factor (VEGF) secretion and blocks VEGF-VEGFR-1(*flt-1*) autocrine loop in human leukemia cells MOLT-4. Leukemia 2003; 17: 52-59.

Bojo ZP, Deano CD, Jacinto SD, Concepcion GP. Synergistic *in vitro* cytotoxicity of adociaquinone B and heptyl prodigiosin against MCF-7 breast cancer cell line. Phil Sci Lett 2010; 3: 48-59.

Bowers A, West N, Taunton J, Schreiber SL, Bradner JE, Williams RM. The total synthesis and biological mode of action of largazole: A potent class I histone deacetylase (HDAC) inhibitor. J Am Chem Soc 2008; 130: 11219-11222.

Bugni TS, Singh MP, Chen L, Arias DA, Harper MK, Greenstein M, Maiese WM, Concepcion GP, Mangalindan, GC, Ireland CM. Kalihinols from two *Acanthella cavernosa* sponges: inhibitors of bacterial folate biosynthesis. Tetrahedron 2004; 60: 6981-6988.

Bugni TS, Concepcion GP, Mangalindan GC, Harper MK, James RD, Ireland CM. *p*-sulfooxyphenylpyruvic acid from the red macro alga *Ceratodictyon spongiosum* and its sponge symbiont *Haliclona cymaeformis*. Phytochemistry 2002; 60: 361-363.

Cantrell CL, Gustafson KR, Cecere MR, Pannell LK, Boyd MR. Chondropsins A and B, novel tumor cell growth-inhibitory macrolide lactams from the marine sponge *Chondropsis* sp. J Am Chem Soc 2000; 122: 8825-8829.

Carpenter KE, Springer VG. The center of the center of marine shore fish biodiversity: the Philippine islands. Environ Biol Fish 2005; 72: 467-480.

Charupant K, Suwanborirux K, Daikuhara N, Yokoya M, Ushijima-Sugano R, Kawai T, Owa T, Saito N. Microarray-based transcriptional profiling of renieramycin M and jorunnamycin C, isolated from Thai marine organisms. Mar Drugs 2009; 7: 483-494.

Chen YN, Sharma, SK, Ramsey TM, Jiang L, Martin MS, Baker K, Adams PD, Bair KW, Kaelin WG Jr. Selective killing of transformed cells by cyclin/cyclin-dependent kinase 2 antagonists. Proc Natl Acad Sci USA 1999; 96: 4325-4329.

Conaco C, Neveu P, Zhou H, Arcila ML, Degnan SM, Degnan BM, Kosik KS. Transcriptome profiling of the demosponge *Amphimedon queenslandica* reveals genome-wide events that accompany major life cycle transitions. BMC Genomics 2012; 13: 209.

Concepcion GP, Foderaro TA, Eldredge GS, Lobkovsky E, Clardy J, Barrows LR, Ireland CM. Topoisomerase II-mediated DNA cleavage by adocia- and xestoquinones from the Philippine sponge *Xestospongia* sp. J Med Chem 1995; 38: 4503-4508.

Coombs GS, Yu J, Canning CA, Veltri CA, Covey TM, Cheong

JK, Utomo V, Banerjee N, Zhang ZH, Jadulco RC, Concepcion GP, Bugni TS, Harper MK, Mihalek I, Jones CM, Ireland CM, Virshup DM. WLS-dependent secretion of WNT3A requires Ser209 acylation and vacuolar acidification. J Cell Sci 2010; 123: 3357-3368.

Cortes J, Lorca R. Eribulin mesylate: a promising new antineoplastic agent for locally advanced metastatic breast cancer. Future Oncol 2011; 7: 355-364.

Cragg GM. Natural products as a source of molecular diversity: prospects for the twenty-first century. IBC's 5th Annual Conference on Natural Products Discovery. 1999, May 13 -14.

Curman D, Cinel B, Williams DE, Rundle N, Block WD, Goodarzi AA, Hutchins JR, Clarke PR, Zhou B-B, Lees-Miller SP, Andersen RJ, Roberge M. Inhibition of the G2 DNA damage checkpoint and of protein kinases Chk1 and Chk2 by the marine sponge alkaloid debromohymenialdisine. J Biol Chem 2001; 276: 17914-17919.

Da Rocha AB, Lopes RM, Schwartsmann G. Natural products in anticancer therapy. Curr Opin Pharmacol 2001; 1: 364-369.

Davis RA, Mangalindan GC, Bojo ZP, Antemano RR, Rodriguez NO, Concepcion GP, Samson SC, de Guzman D, Cruz LJ, Tasdemir D, Harper MK, Feng X, Carter GT, Ireland CM. Microcionamides A and B, bioactive peptides from the Philippine sponge *Clathria (Thalysias) abietina*. J Org Chem 2004; 69: 4170-4176.

Davis RA, Sandoval IT, Concepcion GP, Da Rocha RM, Ireland CM. Lissoclinotoxins E and F, novel cytotoxic alkaloids from a Philippine didemnid ascidian. Tetrahedron 2003; 59: 2855-2859.

De Arruda M, Cocchiaro CA, Nelson CM, Grinnell M, Janssen B, Haupt A, Barlozzari T. LU103793 (NSC D-669356): A synthetic peptide that interacts with microtubules and inhibits mitosis. Cancer Res 1995; 55: 3085-3092.

De Bono JS, Kristeleit R, Tolcher A, Fong P, Pacey S, Karavasilis V, Mita M, Shaw H, Workman P, Kaye S, Rowinsky EK, Aherne W, Atadja P, Scott JW, Patnaik A. Phase I pharmacokinetic and pharmacodynamic study of LAQ824, a hydroxamate histone deacetylase inhibitor with a heat shock protein-90 inhibitory profile, in patients with advanced solid tumors. Clin Cancer Res 2008; 14: 6663-6673.

De Guzman FS, Carte B, Troupe N, Faulkner DJ, Harper MK, Concepcion GP, Mangalindan GC, Matsumoto SS, Barrows LR, Ireland CM. Neoamphimedine: a new pyridoacridine topoisomerase II inhibitor which catenates DNA. J Org Chem 1999; 64: 1400-1402.

De Guzman FS, Copp BR, Mayne CL, Concepcion GP, Mangalindan GC, Barrows LR, Ireland CM. Bolinaquinone: a novel cytotoxic sesquiterpene hydroxyquinone from a Philippine *Dysidea* sponge. J Org Chem 1998; 63: 8042-8044.

Deng C, Pan B, O'Connor OA. Brentuximab vedotin. Clin Cancer Res 2012; 19: 22-27.

Doble BW, Woodgett JR. GSK-3: tricks of the trade for a multi-tasking kinase. J Cell Sci 2003; 116: 1175-1186.

Doering A, Keller J, Cohen S. Some effects of D-arabinosyl nucleosides on polymer syntheses in mouse fibroblasts. Cancer Res 1966; 26: 2444-2450.

Downing KH. Structural basis for the interaction of tubulin with proteins and drugs that affect microtubule dynamics. Annu Rev Cell Dev Biol 2000; 16: 89-111.

Ebbinghaus S, Rubin E, Hersh E, Cranmer LD, Bonate PL, Fram RJ, Jekunen A, Weitman S, Hammond LA. A phase I study of the dolastatin-15 analogue tasidotin (ILX651) administered intravenously daily for 5 consecutive days every 3 weeks in patients with advanced solid tumors. Clin Cancer Res 2005; 11: 7807-7816.

Edelman MJ, Gandara DR, Hausner P, Israel V, Thornton D, DeSanto J, Doyle LA. Phase 2 study of cryptophycin 52 (LY355703) in patients previously treated with platinum based chemotherapy for advanced non-small cell lung cancer. Lung Cancer 2003; 2: 197-199.

Fenical, WF, Jensen, PR, Palladino, MA, Lam, KS, Lloyd, K, Potts, BC. Discovery and development of the anticancer agent salinosporamide A (NPI-0052). Bioorg Med Chem 2009; 17: 2175-2180.

Fenical W. The biodiversity and medicine cabinet status of new drugs from marine organisms. Oceanography 1996; 9: 1-27.

Fennell BJ, Carolan S, Pettit GR, Bell A. Effects of the antimitotic natural product dolastatin 10, and related peptides, on the human malarial parasite *Plasmodium falciparum*. J Antimicrob Chemoth 2003; 51: 833-841.

Foster MP, Concepcion GP, Caraan GB, Ireland CM. Bistratamides C and D. Two new oxazole-containing cyclic hexapeptides isolated from a Philippine *Lissoclinum bistratum* ascidian. J Org Chem 1992; 5: 6672-6675.

Frincke JM, Faulkner DJ. Antimicrobial metabolites of the sponge *Reniera* sp. J Am Chem Soc 1982; 104: 265-269.

Fusetani N, Takabashi M, Matsunaga S. Topsentiasterol sulfates, antimicrobial sterol sulfates possessing novel side chains, from a marine sponge, *Topsentia* sp. Tetrahedron 1994; 50: 1765-7710.

Gajate C, An F, Mollinedo F. Rapid and selective apoptosis in human leukemic cells induced by aplidine through a Fas/CD95- and mitochondrial-mediated mechanism. Clin Cancer Res 2003; 9: 1535-1545.

Garcia-Fernandez LF, Losada A, Alcaide V, Alvarez AM, Cuadrado A, Nakayama KI, Fernandez-Sousa JM, Muñoz A, Sanchez-Puelles JM. Aplidine induces the mitochondrial apoptotic pathway via oxidative stress mediated JNK and p38 activation and protein kinase C. Oncogene 2002; 21: 7533-7544.

Gayther SA, Batley SJ, Linger L, Bannister A, Thorpe K, Chin SF, Daigo Y, Russell P, Wilson A, Sowter HM, Delhanty JD, Ponder BA, Kouzarides T, Caldas C. Mutations truncating the EP300 acetylase in human cancers. Nat Genet 2000; 24: 300-303.

Gerwick WH, Moore BS. Lessons from the past and charting the future of marine natural products drug discovery and

chemical biology. Chem Biol 2012; 19: 85-99.
Ginsberg J. The discovery of Camptothecin and Taxol®. National Historic Chemical Landmarks Program Commemorative Booklet. American Chemical Society 2003.
Gordon MD, Nusse R. Wnt signaling: multiple pathways, multiple receptors, and multiple transcription factors. J Biol Chem 2006; 281: 22429-22433.
Grubb DR, Ly JD, Vaillant F, Johnson KL, Lawen A. Mitochondrial cytochrome c release is caspase-dependent and does not involve mitochondrial permeability transition in didemnin B-induced apoptosis. Oncogene 2001; 20: 4085 –4094 .
Gurova, K. New hopes from old drugs: revisiting DNA-binding small molecules as anticancer agents. Future Oncol 2009; 5: 1685-1704.
Hamann PR, Hinman LM, Hollander I, Beyer CF, Lindh D, Holcomb R, Hallett W, Tsou H-R, Upeslacis J, Shochat D, Mountain A, Flowers DA, Bernstein I. Gemtuzumab ozogamicin, a potent and selective anti-CD33 antibody−calicheamicin conjugate for treatment of acute myeloid leukemia. Bioconjugate Chem 2002; 13: 47-58.
Hanahan D, Weinberg RA. The hallmarks of cancer: The next generation. Cell 2011; 144: 646-672.
Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100: 57-70.
Harper JW, Elledge SJ. Cdk inhibitors in development and cancer. Curr Opin Genet Dev 1996; 6: 56-64.
Hartwell LH, Kastan MB. Cell cycle control and cancer. Science 1994; 266: 1821:1828.
Haygood MG, Schmidt EW, Davidson SK, Faulkner DJ. Microbial symbionts of marine invertebrates: opportunities for microbial biotechnology. J Mol Microbiol Biotechnol 1999; 1: 33-43.
Herbst RS, Hammond LA, Carbone DP, Tran HT, Holroyd KJ, Desai A, Williams JI, Bekele BN, Hait H, Allgood V, Solomon S, Schiller JH. A phase I/IIA trial of continuous five-day infusion of squalamine lactate (MSI-1256F) plus carboplatin and paclitaxel in patients with advanced non-small cell lung cancer. Clin Cancer Res 2003; 9: 4108.
Hong J, Luesch H. Largazole: From discovery to broad-spectrum therapy. Nat Prod Rep 2012; 29: 449-456.
Hsiang YH, Liu LF. Identification of mammalian DNA topoisomerase I as an intracellular target of the anticancer drug camptothecin. Cancer Res 1988; 48: 1722-1726
Huyck TK, Gradishar W, Manuguid F, Kirkpatrick P. Fresh from the pipeline: eribulin mesylate. Nat Rev Drug Discov 2011; 10: 173-174.
Ireland CM, Roll DM, Molinski TF, McK.ee TC, Zabriskie TM, Swersey JC. Biomedical importance of marine organisms. Calif Acad Sci, San Francisco. 1988; 13: 41-57.
Ireland CM, Aalbersberg W, Andersen RJ, Ayral-Kaloustian S, Berlinck RGS, Bernan V, Carter G, Churchill ACL, Clardy J, Concepcion GP, De Silva ED, Discafani C, Fojo T, Frost P, Gibson D, Greenberger LM, Greenstein M, Harper MK, Mallon R, Loganzo F, Nunes M, Poruchynsky MS, Zask A. Anticancer agents from unique natural products sources. Pharm Biol 2003; 41: 15-38.
Ishikawa A, Motohashi S, Ishikawa E, Fuchida H, Higashino K, Otsuji M, Iizasa T, Nakayama T, Taniguchi M, Fujisawa T. A phase I study of α-galactosylceramide (KRN7000)-pulsed dendritic cells in patients with advanced and recurrent non-small cell lung cancer. Clin Cancer Res 2005; 11: 1910-1917.
Joseph B, Sujatha S. Pharmacologically important natural products from marine sponges. J Nat Prod 2011; 4: 5-12
Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69–90.
Jenuwein T, Allis CD. Translating the histone code. Science 2001; 293: 1074–1080.
Katz J, Janik JE, Younes A. Brentuximab vedotin (SGN-35). Clin Cancer Res 2011; 17: 6428-6436.
Kelecom A. Secondary metabolites from marine microorganisms. An Acad Bras Cienc 2002; 74: 151-170.
King N, Westbrook MJ, Young SL, Kuo A, Abedin M, Chapman J, Fairclough S, Hellsten U, Isogai Y, Letunic I, Marr M, Pincus D, Putnam N, Rokas A, Wright KJ, Zuzow R, Dirks W, Good M, Goodstein D, Lemons D, Wanqing L, Lyons JB, Morris A, Nichols S, Richter DJ, Salamov A, Sequencing JGI, Bork P, Lim WA, Manning G, Miller WT, McGinnis W, Shapiro H, Tjian R, Grigoriev IV, Rokhsar D. The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. Nature 2008; 451: 720-727.
Kingston DG. The shape of things to come: structural and synthetic studies of taxol and related compounds. Phytochemistry 2007; 68: 1844-1854
Koh Y, Nishio K, Saijo N. Mechanisms of action of cancer chemotherapeutic agents: topoisomerase inhibitors. In: Alison MR, ed.; The Cancer Handbook 1st Edition. New York: Wiley Press, 2002: 1313-1322.
Kolch W. Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. Biochem J 2000; 351: 289-305.
Koziol C, Borojevic R, Steffen R, Müller WEG. Sponges (Porifera) model systems to study the shift from immortal to senescent somatic cells: the telomerase activity in somatic cells. Mech Ageing Dev 1998; 100: 107-120.
Kupchan SM, Britton RW, Ziegler MF, Siegel CW. Bruceantin, a new potent antileukemic simaroubolide from *Brucea antidysenterica*. J Org Chem 1973; 38: 178-179.
Lane AA, Chabner BA. Histone deacetylase inhibitors in cancer therapy. J Clin Oncol 2009; 27: 5459-5468.
Lavoie JN, L'Allemain G, Brunet A, Moller R, Pouyssegur J. Cyclin D1 expression is regulated positively by the p42/p44 MAPK and negatively by the p38/HOGMAPK pathway. J Biol Chem 1996; 271: 20608-20616.
Lazaro JEH, Nitcheu J, Predicala RZ, Mangalindan GC, Nesslany F, Marzin D, Concepcion GP, Diquet B. Heptylprodigiosin, a bacterial metabolite, is antimalarial *in vivo* and non-mutagenic *in vitro*. J Nat Toxins 2002; 11: 367-377.
Lazaro JEH, Nitcheu J, Mahmoudi N, Ibana JA, Mangalindan

GC, Black GP, Howard-Jones AG, Moore CG, Thomas DA, Mazier D, Ireland CM, Concepcion GP, Murphy PJ, Diquet B. Antimalarial activity of crambescidin 800 and synthetic analogues against liver and blood stage of *Plasmodium* sp. J Antibiot 2006; 59: 583-590.

Lee YK, Lee, J-H, Lee, HK. Microbial symbiosis in marine sponges. J Microbiol 2001; 39: 254-264.

Lerner EC, Zhang TT, Knowles DB, Qian Y, Hamilton AD, Sebti SM. Inhibition of the prenylation of K-Ras, but not H- or N-Ras, is highly resistant to CAAX peptidomimetics and requires both a farnesyltransferase and a geranylgeranyltransferase I inhibitor in human tumor cell lines. Oncogene 1997; 15: 1283-1288.

Lin WW, Karin MA. A cytokine-mediated link between innate immunity, inflammation and cancer. J Clin Invest 2007; 117: 1175-1183.

Liu LF. DNA topoisomerase poisons as antitumor drugs. Annu Rev Biochem 1989; 58: 351-375.

Loganzo F, Discafani CM, Annable T, Beyer C, Musto S, Hari M, Tan X, Hardy C, Hernandez R, Baxter M, Singanallore T, Khafizova G, Poruchynsky MS, Fojo T, Nieman JA, Ayral-Kaloustian S, Zask A, Andersen RJ, Greenberger LM. HTI-286, a synthetic anlogue of the tripeptide hemiasterlin, is a potent antimicrotubule agent that circumvents p-glycoprotein-mediated resistance *in vitro* and *in vivo*. Cancer Res 2003; 63: 1838-1845.

Luesch H, Moore RE, Paul VJ, Mooberry SL, Corbett TH. Isolation of dolastatin 10 from the marine cyanobacterium *Symploca* species VP642 and total stereochemistry and biological evaluation of its analogue symplostatin 1. J Nat Prod 2001; 64: 907-910.

Mahlknecht U, Hoelzer D. Histone acetylation modifiers in the pathogenesis of malignant disease. Mol Med 2000; 6: 623 -644.

Mangalindan GC, Talaue MT, Cruz LJ, Franzblau SG, Adams LB, Richardson AD, Ireland CM, Concepcion GP. Agelasine F from a Philippine *Agelas* sp. sponge exhibits *in vitro* antituberculosis activity. Planta Med 2000; 66: 364-365.

Marks PA, Richon VM, Rifkind RA. Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. J Natl Cancer Inst 2000; 92: 1210–1216.

Marshall KM, Andjelic CD, Tasdemir D, Concepcion GP, Ireland CM, Barrows LR. Deoxyamphimedine, a pyridoacridine alkaloid, damages DNA via the production of reactive oxygen species. Mar Drugs 2009; 7: 196-209.

Marshall KM, Matsumoto SS, Holden JA, Concepcion GP, Tasdemir D, Ireland CM, Barrows LR. The antineoplastic and novel topoisomerase II-mediated cytotoxicity of neoamphimedine, a marine pyridoacridine. Biochem Pharmacol 2003; 66: 447-458.

Mayer AMS, Glaser KB, Cuevas C, Jacobs RS, Kem W, Little RD, McIntosh JM, Newman DJ, Potts BC, Shuster DE. The odyssey of marine pharmaceuticals: a current pipeline perspective. Trends Pharmacol Sci 2010; 31: 255–265.

McCulloch MW, Bugni TS, Concepcion GP, Coombs GS, Harper MK, Kaur S, Mangalindan GC, Mutizwa, MM, Veltri CA, Virshup DM, Ireland CM. Carteriosulfonic Acids A-C, GSK-3β Inhibitors from a *Carteriospongia* sp. J Nat Prod 2009; 72: 1651–1656.

Meijer L, Thunnissen AM, White AW, Garnier M, Nikolic M, Tsai LH, Walter J, Cleverley KE, Salinas PC, Wu YZ, Biernat J, Mandelkow EM, Kim SH, Pettit GR. Inhibition of cyclin-dependent kinases, GSK-3β and CK1 by hymenialdisine, a marine sponge constituent. Chem Biol 2000; 7: 51-63.

Mita C, Hammond LA, Lockhart AC, Shah M, Curtright J, Chen T, Dugan M, Cooper W, Chaffin M, Sharma S, Rowinsky EK, Rothenberg ML. A phase I and pharmacokinetic study of XAA296A (discodermolide) administered once every three weeks in adult patients with advanced solid malignancies. Clin Cancer Res 2003; 9(Suppl), A252 (abstr).

Mitchell BS. The proteasome - an emerging target in cancer. New Eng J Med 2003; 348: 2597-2598.

Monge M, Vilaseca M, Soto-Cerato V, Montaner B, Giralt E, Perez-Tomas R. Proteomic analysis of prodigiosin-induced apoptosis in a breast cancer mitoxantrone-resistant (MCF-7 MR) cell line. Invest New Drugs 2007; 25: 21-29.

Molinski T, Dalisay DS, Lievens SL, Saludes JP. Drug development from marine natural products. Nat Rev Drug Discov 2009; 8: 69-85.

Monnerat C, Henriksson R, Le Chevalier T, Novello S, Berthaud P, Faivre S, Raymond E. Phase I study of PKC412 (N-benzoyl-staurosporine), a novel oral protein kinase C inhibitor, combined with gemcitabine and cisplatin in patients with non-small-cell lung cancer. Ann Oncol 2004; 15: 316-323.

Morris PG. Advances in therapy: eribulin improves survival for metastatic breast cancer. Anti-Cancer Drugs 2010; 2: 885-889.

Morris PG, Fornier MN. Microtubule active agents: beyond the taxane frontier. Clin Cancer Res 2008; 14: 7167-7172.

Müller WEG. The stem cell concept in sponges (Porifera): metazoan traits. Semin Cell Dev Bio 2006; 17: 481-491.

National Cooperative Drug Discovery Group (NCDDG) Report: Anticancer agents from unique natural products sources. 1999.

Newman DJ, Cragg GM. Natural products as sources of new drugs over the period 1981-2010. J Nat Prod 2012; 75: 311-335

Pan L, Lu J, Huang B. HDAC inhibitors: a potential new category of anti-tumor agents. Cell Mol Immunol 2007; 4: 337-343.

Pettit GR, Srirangam JK, Barkoczy J, Williams MD, Boyd MR, Hamel E, Pettit R, Hogan F, Bai R, Chapuis JC, McAllister SC, Schmidt JM. Antineoplastic agents 365. Dolastatin 10 SAR probes. Anticancer Drug Des 1998; 13: 243–277.

Pettit GR. The dolastatins. Fortschr Chem Org Naturst 1997; 70: 1-79.

Pettit GR, Herald, CL, Doubek DL, Herald DL. Isolation and

structure of bryostatin 1. J Am Chem Soc 1982; 104: 6846 -6848.

Pimentel SMV, Bojo ZP, Roberto AVD, Lazaro JEH, Mangalindan GC, Florentino LM, Lim-Navarro P, Tasdemir D, Ireland CM, Concepcion GP. Platelet aggregation inhibitors from Philippine marine invertebrate samples screened in a new microplate assay. Mar Biotechnol 2003; 5: 395-400.

Privat de Garilhe M, de Rudder J. Effect of 2 arabinose nucleosides on the multiplication of herpes virus and vaccinia viruses in cell culture. CR Acad Sci (Paris) 1964; 259: 2725-2728.

Proksch P. Defensive role for secondary metabolites from marine sponges and sponge-feeding nudibranchs, Toxicon 1994; 32: 639-655.

Rademaker-Lakhai JM, Horenblas S, Meinhardt W, Stokvis E, de Reijke TM, Jimeno JM, Lopez-Lazaro L, Martin JA, Beijnen JH, Schellens JHM. Phase I clinical and pharmacokinetic study of kahalalide F in patients with advanced androgen refractory prostate cancer. Clin Cancer Res 2005; 11: 1854-1862.

Ranches GD, Ruda A, Vollmar A, Tun JO, Concepcion GP. Heptylprodigiosin induces apoptosis in Jurkat leukemia T cells via CD95 death receptor and evades anti-apoptotic Bcl-2 and Bcl-x proteins. Phil Sci Lett 2013; 6: 153-167.

Rasheed BKA, Stenzel TT, McLendon RE, Parsons R, Friedman AH, Friedman HS, Bigner DD, Bigner SH. PTEN gene mutations are seen in high-grade but not in low-grade gliomas. Cancer Res 1997; 57: 4187-4190.

Richardson PG, Barlogie B, Berenson J, Singhal S, Jagannath S, Irwin D, Rajkumar VS, Srkalovic G, Alsina M, Alexanian R, Siegel D, Orlowski RZ, Kuter D, Limentani SA, Lee S, Hideshima T, Esseltine D-L, Kauffman M, Adams J, Schenkein DP, Anderson KC. A phase 2 study of bortezomib in relapsed, refractory myeloma. New Eng J Med 2003; 348: 2609-2617.

Rinehart K. Antitumor compounds from tunicates. Med Res Rev 2003; 1: 1-27.

Rinehart KL, Holt TG, Fregeau NL, Stroh JG, Keifer PA, Sun F, Li LH, Martin DG. Ecteinascidins 729,743, 745, 759A, 759B, and 770: Potent antitumor agents from the Caribbean tunicate *Ecteinascidia turbinata.* J Org Chem 1990; 55: 4515-4516.

Rizzo V, Morelli A, Pinciroli V, Sciangula D, D'Alessio R. Equilibrium and kinetics of rotamer interconversion in immunosuppressant prodigiosin derivatives in solution. J Pharm Sci 1999; 88: 73-78.

Roberts CM, McClean CJ, Veron JEN, Hawkins JE, Allen GE, McAllister DE, Mittermeier CG, Schueler FW, Spalding M, Wells F, Vynne C, Werner TB. Marine biodiversity hotspots and conservation priorities for tropical reefs. Science 2002; 295: 1280-1284.

Sagar S, Kaur M, Minneman KP. Antiviral lead compounds from marine sponges. Mar Drugs 2010; 8: 2619-2638.

Sakai R, Jares-Erijman EA, Manzanares I, Elipe MVS, Rinehart KL. Ecteinascidins: putative biosynthetic precursors and absolute stereochemistry. J Am Chem Soc 1996; 118: 9017-9023.

Sandoval IT, Davis RA, Bugni TS, Concepcion GP, Harper MK, Ireland CM. Cytotoxic isoquinoline quinones from sponges of the genus *Petrosia.* Nat Prod Res 2004; 18: 89-93.

Schmidt EW. Trading molecules and tracking targets in symbiotic interactions. Nat Chem Biol 2008; 4: 466-473.

Schmitz FJ, Agarwal SK, Gunasekera SP, Schmidt PG, Shoolery JN. Amphimedine, a new aromatic alkaloid from a pacific sponge, *Amphideon* sp. Carbon connectivity determination from natural abundance carbon 13-carbon-13 coupling constants. J Am Chem Soc 1983; 105: 4835-4836.

Schröder HC, Breter HJ, Fattorusso E, Ushikima H, Wiens M, Steffen R, Batel R, Müller WEG. Okadaic acid, an apoptogenic toxin for symbiotic/parasitic annelids in the demosponge *Suberites domuncula.* Appl Environ Microb 2006; 72: 4907-4916.

Schwarz GK, Shah MA. Targeting the cell cycle: A new approach to cancer therapy. J Clin Oncol 2005; 23: 9408-9421.

Sertan-de Guzman A, Predicala RZ, Bernardo EB, Neilan BA, Elardo SP, Mangalindan GC, Tasdemir D, Ireland CM, Barraquio WL, Concepcion GP. *Pseudovibrio denitrificans* strain Z143-1, a heptylprodigiosin producing bacterium isolated from a Philippine tunicate. FEMS Microbiol Lett 2007; 277: 188–196.

Silverman RB. Classes of drugs that interact with DNA. In Silverman RB, ed. The Organic Chemistry of Drug Design and Drug Action. San Diego: Academic Press, 1992: 235-269.

Singh R, Sharma M, Joshi P, Rawat DS. Clinical status of anticancer agents derived from marine sources. Anti-Cancer Agents in Medicinal Chemistry 2008; 8: 603-617.

Sipkema D, Osinga R, Schatton W, Mendola D, Tramper J, Wikffels RH. Large-scale production of pharmaceuticals by marine sponges: Sea, cell, or synthesis? Biotechnol Bioeng 2005; 90: 201-222.

Smith LT, Otterson GA, Plass C. Unraveling the epigenetic code of cancer for therapy. Trends Genet 2007; 23: 449-456.

Sone H, Kigoshi H, Yamada K. Isolation and stereostructure of dolastatin I, a cytotoxic cyclic hexapeptide from the Japanese sea hare *Dollabella auricularia.* Tetrahedron 1997; 53: 8149-8154.

Soto-Cerato V, Viñals F, Lambert JR, Kelly JA, Perez-Tomas R. Prodigiosin induces the proapoptotic gene NAG-1 via glycogen synthase kinase-3beta activity in human breast cancer cells. Mol Cancer Ther 2007; 6: 362-369.

Srivastava M, Simakov O, Chapman J, Fahey B, Gauthier MEA, Mitros T, Richards GS, Conaco C, Dacre M, Hellsten U, Larroux C, Putnam NH, Stanke M, Adamska M, Darling A, Degnan SM, Oakley TH, Plachetzki DC, Zhai Y, Adamsk M, Calcino A, Cummins SF, Goodstein DM, Harris C, Jackson D, Leys SP, Shu S, Woodcroft BJ, Vervoort M, Kosik KS, Manning G, Degnan BM, Rokhsar DS. The *Amphimedon queenslandica* genome and the evolution of animal complexity. Nature 2010; 466: 720-726.

Sullivan JC, Reitzel AM, Finnerty JR. A high percentage of introns in human genes were present early in animal evolution: evidence from the basal metazoan *Nematostella vectensis*. Genome Inform 2006; 17: 219-229.

Suwanborirux K, Amnuoypol S, Plubrukarn A, Pummangura S, Kubo A, Tanaka C, Saito N. Chemistry of renieramycins. Part 3. (I) Isolation and structure of stabilized renieramycin type derivatives possessing antitumor activity from Thai sponge *Xestospongia* species, pretreated with potassium cyanide. J Nat Prod 2003; 66: 1441-1446.

Swaffer DS, Ireland CM, Barrows LR. A rapid mechanism-based screen to detect potential anti-cancer agents. Anticancer Drugs 1994; 5: 15-23.

Tan AR, Rubin EH, Walton DC, Shuster DE, Wong, YN, Fang F, Ashworth, S, Rosen, LS. Phase I study of eribulin mesylate administered once every 21 days in patients with advanced solid tumors. Clin Cancer Res 2009; 15: 4213-4219.

Tasdemir D, Bugni TS, Mangalindan GC, Concepcion GP, Harper MK, Ireland CM. Cytotoxic bromoindole derivatives and terpenes from the Philippine marine sponge *Smenospongia* sp. Z Naturforsch 2002a; 57: 914-922.

Tasdemir D, Mangalindan GC, Concepcion GP, Verbitski SM, Rabindran S, Miranda M, Greenstein M, Hooper JNA, Harper MK., Ireland CM. Bioactive isomalabaricane triterpenes from the marine sponge *Rhabdastrella globostellata*. J Nat Prod 2002b; 65: 210-214.

Tasdemir D, Mallon R, Greenstein M, Feldberg LR, Kim SK, Collins K, Wojciechowicz D, Mangalindan GC, Concepcion GP, Harper MK, Ireland CM. Aldisine alkaloids from the Philippine sponge *Stylissa massa* are potent inhibitors of mitogen-activated protein kinase kinase-1 (MEK-1). J Med Chem 2001; 45: 529-532.

Thakur DS. Topoisomerase II inhibitors in cancer treatment. Internat J Pharmaceut Sci Nanotech 2011; 3: 1173-1181.

Thoms C, Schupp PJ. Chemical defense strategies in sponges: a review. Porifera Research: Biodiversity, Innovation and Thoms C, Schupp PJ. Chemical defense strategies in sponges: a review. Porifera Research: Biodiversity, Innovation and Sustainability 2007; 28: 627-637.

Towle MJ, Salvato KA, Budrow J, Wels BF, Kuznetsov G, Aalfs KK, Welsh S, Zheng W, Seletsky BM, Palme MH, Habgood GJ, Singer LA, DiPietro LV, Wang Y, Chen JJ, Quincy DA, Davis A, Yoshimatsu K, Kishi Y, Yu MJ, Littlefield BA. *In vitro* and *in vivo* anticancer activities of synthetic macrocyclic ketone analogues of halichondrin B. Cancer Res 2001; 61: 1013-1021.

Vahdat LT, Pruitt B, Fabian CJ, Rivera RR, Smith DA, Tan-Chiu E, Wright J, Tan AR, da Costa NA, Chuang E, Smith J, O'Shaughnessy J, Shuster DE, Meneses NL, Chandrawansa K, Fang F, Cole PE, Ashworth S, Blum JL. Phase II study of eribulin mesylate, a halichondrin B analog, in patients with metastatic breast cancer previously treated with an anthracycline and a taxane. J Clin Oncol 2009; 27(18): 2954-2961.

Varterasian ML, Mohammad RM, Eilender DS, Hulburd K, Rodriguez DH, Pemberton PA, Pluda JM, Dan MD, Pettit GR, Chen BDM, Al-Katib AM. Phase I study of bryostatin 1 in patients with relapsed non-hodgkin's lymphoma and chronic lymphocytic leukemia. J Clin Oncol 1998; 16: 56-62.

Venables DA, Concepcion GP, Matsumoto SS, Barrows LR, Ireland CM. Makaluvamine N: a new pyrroloiminoquinone from *Zyzzya fuliginosa*. J Nat Prod 1997; 60: 408-410.

Verbitski SM, Mayne CL, Davis RA, Concepcion GP, Ireland CM. Isolation, structure determination, and biological activity of a novel alkaloid, perophoramidine, from the Philippine ascidian *Perophora namei*. J Org Chem 2002; 67: 7124-7126.

Wang SI, Parsons R, Ittmann M. Homozygous deletion of the PTEN tumor suppressor gene in a subset of prostate adenocarcinomas. Clin Cancer Res 1998; 4: 811-815.

Weinberg RA. In: Weinberg RA, 2nd ed. The Biology of Cancer. New York: Garland Science, 2014: 441-444.

Weinberg RA. In: Weinberg RA, 2nd ed. The Biology of Cancer. New York: Garland Science, 2007a: 150-153.

Weinberg RA. In: Weinberg RA, 2nd ed. The Biology of Cancer. New York: Garland Science, 2007b: 185.

Weinberg RA. In: Weinberg RA, 2nd ed. The Biology of Cancer. New York: Garland Science, 2007c: 346-349.

Weinberg RA. In: Weinberg RA, 2nd ed. The Biology of Cancer. New York: Garland Science, 2007d: 193-194.

Weinberg RA. In: Weinberg RA, 2nd ed. The Biology of Cancer. New York: Garland Science, 2007e: 120.

Weinberg RA. In: Weinberg RA, 2nd ed. The Biology of Cancer. New York: Garland Science, 2007f: 325-328.

Whitson EL, Bugni TS, Chockalingam PS, Concepcion GP, Harper MK, He M, Hooper JNA, Mangalindan GC, Ritacco F, Ireland CM. Spheciosterol sulfates, PKCζ inhibitors from a Philippine sponge *Spheciospongia* sp. J Nat Prod 2008; 71: 1213-1217.

Whitson EL, Bugni TS, Prdiya S, Chockalingam PS, Concepcion GP, Feng X, Jin G, Harper MK, Mangalindan GC, McDonald LA, Ireland CM. Fibrosterol sulfates from the Philippine sponge *Lissodendoryx* (Acanthodoryx) *fibrosa*: sterol dimers that inhibit PKCζ. J Org Chem 2009; 74: 5902-5908.

Wiens M, Korzhev M, Perovic-Ottstadt S, Luthringer B, Brandt D, Klein S, Müller WEG. Toll-like receptors are part of the innate immune defense system of sponges (Demospongiae: Porifera). Evolutionary origin of toll-like receptors in Metazoa. Mol Biol Evol 2007; 24: 792-804.

World Health Organization. The Global Burden of Disease. 2004 Update. Geneva:World Health Organization; 2008.

Zhang XD, Gillespie SK, Borrow JM, Hersey P. The histone deacetylase inhibitor suberic bishydroxamate: a potential sensitizer of melanoma to TNF-related apoptosis-inducing ligand (TRAIL) induced apoptosis. Biochem Pharmacol 2003; 66: 1537-1545.