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

Gisela P. Concepcion¹, Andrea Roxanne J. Anas¹,², and Miguel A. Azcuna¹*  
¹The Marine Science Institute, University of the Philippines, Diliman, Quezon City 1101, Philippines  
²Graduate 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; CDCl₃ - 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; IC₅₀ - 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 - meticillin-resistant *Staphylococcus aureus*; NCI - National Cancer Institute; FABMS - fast atom bombardment mass spectrometry; IC₅₀ - 50 percent inhibition concentration; 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; MRSA - meticillin-resistant *Staphylococcus aureus*; NCI - National Cancer Institute;

**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 extracellular...
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 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, eucheainascidins, 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). Eucheainascidins, tetrahydroisoquinolines isolated from the Caribbean ascidian *Ecteinascidia turbinata*, showed antitumor activity against leukemia cells, with eucheainascidins 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 eucheainascidins are the reniermycins, which were isolated from several genera of sponges (*e.g.*, *Reneira* sp. and *Xestospongia* sp.) (Suwanborirux et al. 2005, Frincke and Faulkner 1992). Reniermycins have potent anticancer activity, owing to their ability to downregulate protein tyrosine phosphatase receptor type K (PTPPRK) 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 okadaii*, 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). Erilubin 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 *Dolabella 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 *Didemnum 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, Broggi et al. 2003).

Bryostatin 1 (10), a macroamide 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-
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).
<table>
<thead>
<tr>
<th>Compound</th>
<th>Natural Product Source/Derivative</th>
<th>Target/Mechanism of Action</th>
<th>Approval/Clinical (Year)</th>
<th>Status (Phase)</th>
<th>Sponsor</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>APPROVED</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>brentuximab vedotin</td>
<td>dolastatin 10 analogue</td>
<td>tubulin</td>
<td>2011</td>
<td>approved</td>
<td>Seattle Genetics</td>
<td>Gerwick and Moore 2012, Deng et al. 2013</td>
</tr>
<tr>
<td><strong>CLINICAL TRIALS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LU-103793</td>
<td>dolastatin 15 analogue</td>
<td>tubulin</td>
<td>1996</td>
<td>II discontinued</td>
<td>De Arruda et al. 1995</td>
<td></td>
</tr>
<tr>
<td>squalamine lactate</td>
<td><em>Squalus</em> sp.</td>
<td>angiogenesis inhibitor</td>
<td>2001</td>
<td>II</td>
<td>Genaera</td>
<td>Herbst et al. 2003</td>
</tr>
<tr>
<td>LAF389</td>
<td>bengamide analogue</td>
<td>MetAp1 and 2</td>
<td>2002</td>
<td>I discontinued</td>
<td>Novartis</td>
<td>Singh et al. 2008</td>
</tr>
<tr>
<td>LY355703</td>
<td>analogue of cryptophycin</td>
<td>tubulin</td>
<td>2002</td>
<td>II</td>
<td>Eli Lilly</td>
<td>Edelman et al. 2003</td>
</tr>
<tr>
<td>discodermolide</td>
<td><em>Discodermia dissoluta</em></td>
<td>tubulin</td>
<td>2002</td>
<td>I discontinued</td>
<td>Novartis</td>
<td>Mita et al. 2003</td>
</tr>
<tr>
<td>HTI-286</td>
<td>hemiasterlin analog</td>
<td>tubulin</td>
<td>2002</td>
<td>I discontinued</td>
<td>Wyeth</td>
<td>Loganzo et al. 2003</td>
</tr>
<tr>
<td>kahalalide F</td>
<td><em>Bryopsis</em> sp.</td>
<td>disrupts lysosomal structure</td>
<td>2002</td>
<td>II</td>
<td>PharmaMar</td>
<td>Rademaker-Lakhai et al. 2005</td>
</tr>
<tr>
<td>ILX651</td>
<td>dolastatin 15 analogue</td>
<td>tubulin</td>
<td>2003</td>
<td>II</td>
<td>ILX Oncology</td>
<td>Ebbinghaus et al. 2005</td>
</tr>
<tr>
<td>LAQ824</td>
<td>psammaplin analogue</td>
<td>histone deacetylase</td>
<td>2003</td>
<td>I</td>
<td>Novartis</td>
<td>De Bono et al. 2008</td>
</tr>
<tr>
<td>KRN 7000</td>
<td>agelasphin 9a analogue</td>
<td>immunostimulant, activation of NKT cells</td>
<td>2003</td>
<td>I</td>
<td>Kirin</td>
<td>Ishikawa et al. 2005</td>
</tr>
<tr>
<td>staurosporine</td>
<td><em>Streptomyces staurosporeus</em></td>
<td>protein kinase C</td>
<td>2004</td>
<td>I</td>
<td>Pfizer</td>
<td>Monnerat et al. 2004</td>
</tr>
<tr>
<td><strong>PRECLINICAL TRIALS</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>largazole (11)</td>
<td><em>Symlocia</em> sp.</td>
<td>histone deacetylases</td>
<td></td>
<td></td>
<td>Hong and Luesch 2012, Bowers 2008</td>
<td></td>
</tr>
<tr>
<td>renieramycin</td>
<td><em>Xestospongia</em> sp.</td>
<td>downregulates PTPRK</td>
<td></td>
<td></td>
<td>Charupant et al. 2009</td>
<td></td>
</tr>
</tbody>
</table>
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), pyroles (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.

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

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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 \(\mu\)M, respectively. The 14-methoxyxestoquinone (14) and 15-methoxyxestoquinone (15) were cytotoxic to the same cell line at 28 \(\mu\)M. 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).

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).

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).

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

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Structural Class</th>
<th>Bioactivity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>adociaquinones (12,13)</td>
<td>quinone</td>
<td>topoisomerase II inhibitor</td>
<td>Concepcion et al. 1995</td>
</tr>
<tr>
<td>xestoquinones (14,15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>makaluvamine N</td>
<td>pyrroloiminoquinone</td>
<td>topoisomerase II inhibitor</td>
<td>Venables et al. 1997</td>
</tr>
<tr>
<td>neoamphimedine (17)</td>
<td>pyridoacridine</td>
<td>DNA intercalator and catenator in the presence of topoisomerase II</td>
<td>De Guzman et al. 1999</td>
</tr>
<tr>
<td>deoxyamphimedine (18)</td>
<td>pyridoacridine</td>
<td>DNA intercalator and catenator in the presence of ROS</td>
<td>Marshall et al. 2009</td>
</tr>
<tr>
<td>lissoclinotoxins (21,22)</td>
<td>alkaloid</td>
<td>PI3-K/AKT/mTOR pathway</td>
<td>Davis et al. 2003</td>
</tr>
<tr>
<td>aldisines (19,20)</td>
<td>alkaloid</td>
<td>growth signaling MEK pathway</td>
<td>Tasdemir et al. 2001</td>
</tr>
<tr>
<td>topsentiassterol sulfate E fibrosterols (26-28)</td>
<td>sulfated sterol</td>
<td>PKCζ pathway</td>
<td>Whitson et al. 2009</td>
</tr>
<tr>
<td>carteriosulfates (36-38)</td>
<td>sulfated acid</td>
<td>GSK-3β inhibitor</td>
<td>McCulloch et al. 2009</td>
</tr>
<tr>
<td>stelletins (29,30)</td>
<td>triterpene</td>
<td>p21 and p53 signaling pathway</td>
<td>Tasdemir et al. 2002b</td>
</tr>
<tr>
<td>microcionamides (31,32)</td>
<td>cyclic peptide</td>
<td>apoptosis and cell death pathway</td>
<td>Davis et al. 2004</td>
</tr>
<tr>
<td>heptylprodigiosin (33)</td>
<td>tripyrrole</td>
<td>apoptosis and cell death pathway</td>
<td>Lazaro et al. 2002</td>
</tr>
<tr>
<td>chondropsins (34,35)</td>
<td>macrolide</td>
<td>Wnt signaling pathway</td>
<td>Coombs et al. 2010</td>
</tr>
</tbody>
</table>
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. 10E-hymenialdisine (19) and 10Z-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).

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 debromo-hymenialdisine 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 PKCζ isoform can induce cell proliferation by activating other transcription factors (e.g., Fos, Jun, AP-1) (Weinberg 2007e).

Spheciospongei 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_{50} 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...
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 \(\mu\)M, 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).

Clathria (*Thalysias*) abietana, a marine sponge from Tiga- tabon Island, Zamboanga in Southern Mindanao, Philippines, gave two linear peptides cyclized via a cystine moiety, and the C-terminus was blocked by a 2-phenylethylamine 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 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 \(\mu\)M, as compared to the positive control rifampicin (MIC 1.52 nM) (Philippine Patent No. 1-2004-000049) (Davis et al. 2004).

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).

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 denitri ficans* from an unidentified tunicate from Zamboanga del Norte, Mindanao, Philippines. It produced a red pigment hep-
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 F423, 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 CDCl₃, which were active against meticillin-susceptible Staphylococcus aureus (MSSA) and meticillin-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 ³²P-labeling assay with IC₅₀ values of 12.5, 6.8, and 6.8 µM, respectively. The long chain fatty acid component of the cartieriosulfonic acids is critical for GSK-3β inhibition and is also a
good activator of Wnt signaling via β-catenin phosphorylation (McCulloch et al. 2009).

\[
\text{chondropsin A (34); R=OH} \\
\text{(Cantrell et al. 2000)}
\]

\[
\text{73-deoxychondropsin A (35); R=H} \\
\text{(Coombs et al. 2010)}
\]

\[
\text{carteriosulfonic acid A (36)} \\
\text{B (37)} \\
\text{C (38)} \\
\text{(McCullough et al. 2009)}
\]

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.

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.

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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.

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