

# *Marine Mollusk-Derived Agents with Antiproliferative Activity as Promising Anticancer Agents to Overcome Chemotherapy Resistance*

*Maria Letizia Ciavatta,<sup>1</sup> Florence Lefranc,<sup>2</sup> Marianna Carbone,<sup>1</sup> Ernesto Mollo,<sup>1</sup> Margherita Gavagnin,<sup>1</sup> Tania Betancourt,<sup>3</sup> Ramesh Dasari,<sup>3</sup> Alexander Kornienko,<sup>3</sup> and Robert Kiss<sup>4</sup>*

<sup>1</sup>Consiglio Nazionale delle Ricerche (CNR), Istituto di Chimica Biomolecolare (ICB), Via Campi Flegrei 34, 80078 Pozzuoli, Italy

<sup>2</sup>Service de Neurochirurgie, Hôpital Erasme, Université Libre de Bruxelles (ULB), 1070 Brussels, Belgium

<sup>3</sup>Department of Chemistry and Biochemistry, Texas State University, San Marcos, TX 78666

<sup>4</sup>Laboratoire de Cancérologie et de Toxicologie Expérimentale Faculté de Pharmacie, Université Libre de Bruxelles (ULB), 1050 Brussels, Belgium

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**Abstract:** The chemical investigation of marine mollusks has led to the isolation of a wide variety of bioactive metabolites, which evolved in marine organisms as favorable adaptations to survive in different environments. Most of them are derived from food sources, but they can be also biosynthesized de novo by the mollusks themselves, or produced by symbionts. Consequently, the isolated compounds cannot be strictly considered as “chemotaxonomic markers” for the different molluscan species. However, the chemical investigation of this phylum has provided many compounds of interest as potential anticancer drugs that assume particular importance in the light of the growing literature on cancer biology and chemotherapy. The current review highlights the diversity of chemical structures, mechanisms of action, and, most importantly, the potential of mollusk-derived metabolites as anticancer agents, including those biosynthesized by mollusks and those of dietary origin. After the discussion of dolastatins and kahalalides, compounds previously studied in clinical trials, the review covers potentially promising anticancer agents, which are grouped based on their structural type and include terpenes, steroids, peptides, polyketides and nitrogen-containing compounds. The “promise” of a mollusk-derived natural product as an anticancer agent is evaluated on the basis of its ability to target biological characteristics of cancer cells responsible

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*Correspondence to:* Maria Letizia Ciavatta, Consiglio Nazionale delle Ricerche (CNR), Istituto di Chimica Biomolecolare (ICB), Via Campi Flegrei 34, 80078 Pozzuoli, Italy. E-mail: lciavatta@icb.cnr.it. Alexander Kornienko, Laboratoire de Cancérologie et de Toxicologie Expérimentale, Faculté de Pharmacie, Université Libre de Bruxelles (ULB), 1050 Brussels, Belgium. E-mail: a\_k76@txstate.edu. Robert Kiss, Department of Chemistry and Biochemistry, Texas State University, San Marcos, TX 78666. E-mail: rkiss2012@gmail.com

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for poor treatment outcomes. These characteristics include high antiproliferative potency against cancer cells *in vitro*, preferential inhibition of the proliferation of cancer cells over normal ones, mechanism of action via nonapoptotic signaling pathways, circumvention of multidrug resistance phenotype, and high activity *in vivo*, among others. The review also includes sections on the targeted delivery of mollusk-derived anticancer agents and solutions to their procurement in quantity. © 2016 The Authors Medicinal Research Reviews Published by Wiley Periodicals, Inc. Med. Res. Rev., 37, No. 4, 702–801, 2017

**Key words:** mollusk; cancer; preclinical evaluation; treatment; innovative mechanism of action; targeted delivery

## 1. INTRODUCTION

### A. Cancer Epidemiology and Treatment

As emphasized by Torre et al.<sup>1</sup> the occurrence of cancer is increasing because of the growth and aging of the population, as well as an increasing prevalence of established risk factors such as use of tobacco products, obesity, physical inactivity, and changing reproductive patterns associated with urbanization and economic development. About 14 million new cancer cases and more than 8 million deaths occurred in 2012 worldwide.<sup>1</sup>

In contrast to benign tumors, which with few exceptions<sup>2</sup> do not invade other sites in the body, cancer is a malignant tumor that is characterized by local tissue invasion and/or distant metastatic processes. At early stages of cancer development, surgery or surgery in combination with adjuvant radiotherapy are curative in most cases. Once the cancer is more developed and has invaded other tissues, systemic chemotherapy (oral or intravenous) is added to surgery and radiotherapy to eliminate isolated cancer cells and/or cancer cell subpopulations that have not been removed or destroyed. As a general rule (always with exceptions), the more advanced the cancer at the time of diagnosis, the more aggressive the adjuvant chemotherapy or polychemotherapy that will be applied. Apart from chemotherapy, a number of other treatments are often used, such as immunotherapy<sup>3–5</sup> and photodynamic therapy.<sup>6,7</sup> Doroshow and Kumar<sup>8</sup> state that more extensive molecular characterization of tumors and their supporting matrices are anticipated to become standard aspects of oncological practice, which will permit the design of the treatment type for each individual patient and its continuous reevaluation during the course of the treatment.

Paul Ehrlich, the founder of chemotherapy, who received the Nobel Prize for Physiology or Medicine a century ago, postulated the creation of “magic bullets” to be used as chemotherapeutic agents in the fight against human diseases.<sup>9</sup> Unfortunately, such magic bullets are generally ineffective in oncology. One reason is related to the fact that, for the most part, cancers are heterogeneous and consist of multiple cell subpopulations.<sup>10–12</sup> Combinations of various anticancer therapies (including polychemotherapies) involving drugs with different mechanisms of action are thus widely used.<sup>13</sup> In addition, chemotherapy and hormone therapy have been combined with immunotherapy for the treatment of various solid cancers.<sup>14,15</sup> The US Food and Drug Administration (FDA) recently approved the first “immunotherapy combo,” the Bristol–Myers Squibb’s combination of nivolumab and ipilumab, for the treatment of metastatic melanoma.<sup>16</sup>

### B. Cytotoxic Versus Targeted Anticancer Therapies

Most chemotherapies consist of the use of cytotoxic drugs or molecularly targeted agents. Each type of therapy could be effective in the treatment of patients whose cancer has reached an advanced clinical stage and often these therapies are used in combination. However, for certain cancer types, current chemotherapeutic standards seem still to rely mainly on cytotoxic drugs (postsurgery and/or radiotherapy). This is the case, for example, for pancreatic cancer,<sup>17</sup>

advanced stage small cell lung cancer (SCLC),<sup>18</sup> and glioblastoma (GBM).<sup>19</sup> In their review entitled “*An anticancer strategic dilemma: to kill or to contain,*” Perret and Uzzan<sup>20</sup> classify the use of cytotoxic compounds as “killing” tools and targeted therapies as containment tools, where the latter is aimed at the induction or extension of tumor dormancy.

Another difference between cytotoxic and targeted therapeutics consists of the way by which the compounds are discovered and developed. Many cytotoxic compounds are discovered from plants, fungi, or animals (especially from marine invertebrates) using bioactivity-guided isolation, while targeted agents are typically designed and synthesized by researchers. Bioassay guided isolation of natural extracts, when successful, leads to the discovery of novel peptides, proteins, polysaccharides, lectins, small molecules, or other agents whose growth inhibitory activity is first assayed *in vitro* in normal and cancer cell lines and then *in vivo* in various murine syngeneic and/or human xenografted models. During these pharmacological (and early toxicological) evaluations, it is rarely possible to decipher the mechanism(s) of anticancer action. Targeted therapies, on the other hand, mainly rely on the screening of libraries of compounds against a specific target protein that is usually intracellular. Researchers have also developed biological agents (such as antibodies and nucleic acid aptamers) to target specific proteins that are usually presented extracellularly and are typically involved in cancer cell biology and/or characteristic of the tumor microenvironment.

### C. Cancer Resistance to Chemotherapy

As will be seen later in the review, mollusk metabolites are evaluated based on the ability of these natural products to overcome cancer cell resistance to chemotherapy, a property which, in our view, makes a particular compound a promising anticancer agent. We thus summarize below some of the major mechanisms of cancer cell resistance to chemotherapy that generally lead to dismal prognoses. These discussed mechanisms are of most relevance to the compounds presented in the current review. It must however be emphasized that there exist many more types of cancer drug resistance, which are not mentioned herein. These, for example, include the involvement of noncoding RNAs and multiple repair mechanisms,<sup>21</sup> such as DNA base excision<sup>22,23</sup> and DNA double-strand break,<sup>24</sup> among others.

#### 1. The Multidrug Resistance (MDR) Phenotype

Chen et al.<sup>25</sup> emphasize that one of the common mechanisms for cancer cells to resist cytotoxic insults is the overexpression of the ATP-binding cassette (ABC) efflux transporters such as P-glycoprotein (P-gp/ABCB1), MDR-associated protein 2 (MRP2/ABCC2), and breast cancer resistance protein (BCRP/ABCG2). These mechanisms belong to the so-called MDR phenotype and limit the prolonged and effective use of chemotherapeutic drugs. For example, P-gp overexpression in cancer cells leads to the decreased uptake of the drug and intracellular drug accumulation, minimizing drug–target interactions.<sup>26</sup> As emphasized by Cui et al.,<sup>27</sup> the superfamily of human ABC transporters comprises seven subfamilies with 48 members, which exclude structurally and/or functionally unrelated drugs.<sup>26</sup> Dinic et al.<sup>26</sup> report that there are two types of MDR: intrinsic and acquired. These authors<sup>26</sup> further report that tumor microenvironment-induced selection pressure leads to the development of intrinsic MDR, while acquired resistance is a consequence of chronic chemotherapy administrations. Cort and Ozben<sup>28</sup> as well as Dinic et al.<sup>26</sup> state that natural product-based drugs are important in overcoming or reversing MDR in cancer therapy.

## 2. *The Resistance to Targeted Therapies*

Schmitt et al.<sup>29</sup> recently reviewed the preexisting subclonal resistance mutations to various molecularly targeted agents that lead to clinical failures in the treatment of cancer patients with targeted therapies. In addition, as mentioned earlier in this review and also discussed Schmitt et al.,<sup>29</sup> the problem of cancer heterogeneity leads to the inability of a single agent, whatever it may be, to kill all the subclones and the associated populations in a given cancer. Schmitt et al.<sup>29</sup> accordingly state that early detection of preexisting or emerging drug resistance could enable more personalized use of targeted cancer therapy, as patients could be stratified to receive the therapies that are most likely to be effective. Further, Kim<sup>30</sup> recently reviewed the mechanisms of resistance to targeted therapy, with a focus on acquired resistance involving mutations and amplification of genes in the same or parallel signaling pathways. This author also emphasizes that sequencing of primary tumors has revealed that therapy-resistant clones already exist prior to targeted therapy, demonstrating once again that tumor heterogeneity in primary tumors confers a mechanism for inherent therapy resistance. Pazarentzos and Bivona<sup>31</sup> also recently reviewed an important aspect of tumor resistance to targeted therapies involving the adaptive stress signaling process. They discussed the early adaptive changes by which tumor cells respond to the stress of a targeted therapy that may be crucial for tumor cell survival during treatment and the development of resistance.

An international task force of 180 scientists was recently assembled to explore the concept of a low-toxicity “broad-spectrum” therapeutic approach that could simultaneously target many key pathways and mechanisms.<sup>32</sup> Block et al.<sup>32</sup> report that using cancer hallmark phenotypes and the tumor microenvironment to account for the various aspects of relevant cancer biology, interdisciplinary teams reviewed each hallmark area and proposed a wide range of high-priority targets (74 in total) that could be modified to improve patient outcomes. For these targets, corresponding low-toxicity therapeutic approaches were then suggested, many of which were phytochemicals.<sup>32</sup>

## 3. *Cancer Stem Cells (CSCs)*

Several theories attempting to explain “the origin” of cancer(s) have been proposed. Some theories attribute cancer to problems in developmental biology and cell differentiation,<sup>33–35</sup> while others point to a normal cell undergoing tumorigenic transformation as a result of genetic mutations.<sup>36,37</sup> In fact, some of these ideas were published in the early 1900s,<sup>38,39</sup> half a century before the solving of the DNA structure.<sup>40,41</sup> Regardless of its origin, as emphasized by Rycaj and Tang,<sup>37</sup> an established clinical tumor is sustained by subpopulations of self-renewing cancer cells, operationally called CSCs that can intraclonally generate both tumorigenic and nontumorigenic cells. Pattabiraman and Weinberg<sup>42</sup> report that since their identification in 1994, CSCs have been demonstrated to be directly implicated in resistance to conventional anticancer therapeutics. Skrbo et al.<sup>11</sup> have also recently revisited the scientific background of the CSC theory. In their recent review, Pattabiraman and Weinberg<sup>42</sup> also propose ways to use the current knowledge of the complex biology of CSCs to design novel therapies to eliminate these cells. As detailed in Section 1.C.3, CSCs are implicated in both minimal residual disease (the major cause of cancer recurrence) and metastasis.<sup>43</sup> Dragu et al.<sup>44</sup> in their recent review discuss targeting CSC surface biomarkers, signaling pathways that regulate CSC self-renewal and differentiation, drug-efflux pumps involved in apoptosis resistance, and microenvironmental signals that sustain CSC growth, as well as manipulation of miRNA expression and induction of CSC apoptosis and differentiation as strategies for hampering CSC regeneration and cancer relapse. These authors<sup>44</sup> also report that several anti-CSC agents are under evaluation in preclinical and clinical studies, with most of them being

designed to be used in combination with traditional therapies. Lisanti et al.<sup>45</sup> are developing strategies to eradicate CSCs using mitochondria targeting<sup>46</sup> or through inhibition of protein synthesis.<sup>47</sup>

#### 4. Hypoxia

Span and Bussink<sup>48</sup> recently discussed that: (i) hypoxia is a heterogeneous effect with oxygen tensions ranging from 0.01% (anoxia) to 5%, (ii) hypoxia can be chronic, acute, or cycling, all with differential effects on tumor cells, and (iii) low oxygen tension often occurs in tumor cells as a result of several processes, for example, poor angiogenesis and increased oxygen consumption. Dhani et al.<sup>49</sup> accordingly report that hypoxia drives a complex compensatory response in cancer cells (and also in endothelial cells) leading to continued cell survival and induces genomic changes resulting in selection of hypoxia-adapted cells with the propensity to invade locally, metastasize, and recur following surgery or radiotherapy. Along the same lines, Paolicchi et al.<sup>50</sup> state that the hypoxic tumor microenvironment promotes metabolic changes, oncogene activation, epithelial to mesenchymal transition, and resistance to chemo- and radiotherapy, all of which are hallmarks of aggressive tumor behavior. All of these characteristics are orchestrated through the activation of the hypoxia-inducible factor 1 alpha (HIF1A), which is an independent marker of poor prognosis.<sup>50,51</sup> Accumulating evidence in recent years suggests that hypoxia inducible factor 2 (HIF-2) also contributes to chemo- and radio-resistance in solid tumors.<sup>52</sup>

In a recent review, Parks et al.<sup>53</sup> report that hypoxia promotes tumor growth by controlling nutrient import and acidic metabolite export. Eales et al.<sup>54</sup> state that hypoxic tumor areas usually contain some of the most malignant cells in a given cancer. Liang et al.<sup>51</sup> also state that the development of cancer therapies that target hypoxia is of vital importance and that one such targeting strategy is the design of hypoxia-activated prodrugs, which release chemotherapeutic agents within hypoxic tumor regions. This targeting strategy is accomplished by attaching a hypoxia-activated trigger to a chemotherapeutic agent such that, under oxygen-poor conditions, the agent (effector) is released into the tumor, while remaining intact in normal tissue leaving nonhypoxic cells unaffected.

#### 5. Resistance to Proapoptotic Stimuli

Apoptosis-related signaling pathways have been extensively reviewed by Galluzzi et al.<sup>55–57</sup> As stated by Simpson et al.,<sup>58</sup> the ability of a cell to survive in an anchorage-independent manner is a critical step in the development of metastatic potential. Such cells must be able to overcome anoikis, which is a type of cell death related to apoptosis and results from the loss of contact with neighboring cells or extracellular matrix.<sup>58,59</sup> Portt et al.<sup>59</sup> reviewed the various molecular signaling pathways by which cancer cells evade apoptosis.<sup>59</sup> These pathways involve, among others, activation or upregulation of mitogenic signaling pathways (Erk1/2, Akt, etc.), inactivation, or downregulation of certain proapoptotic pathways (Fas receptor, Bax, etc.), and the up-regulation of a number of antiapoptotic genes (Bcl-2, cFLIP, etc.). Portt et al.<sup>59</sup> report that these signaling pathways leading to antiapoptotic phenotypes are activated in response to not only cytotoxic and proapoptotic insults, but also stressful environment such as hypoxia.

Speirs et al.<sup>60</sup> state that an alternative approach to overcome the resistance of cancer cells to cytotoxic proapoptotic stimuli is to induce cell death pathways that are mechanistically distinct from apoptosis. These authors accordingly reviewed drugs that induce autophagic cell death or necrosis in cancer cells.<sup>60</sup> However, whether inducing autophagy is beneficial or detrimental for cancer cells still remains an actual subject of debate that was recently reviewed by Belaid et al.<sup>61</sup> Indeed, inhibiting (as opposed to inducing) autophagy in cancer cells also seems to be a promising approach to combat cancers associated with dismal prognoses.<sup>62–64</sup>

Furthermore, in addition to apoptosis, autophagy, and necrosis, there are multiple other cell death pathways that have been reviewed by Galluzzi et al.<sup>55–57</sup> Some examples include necroptosis, mitotic catastrophe, senescence, lysosomal membrane permeabilization, oncosis, parthanatos, pyroptosis, ferroptosis, and autosis.<sup>55–57</sup> We recently reviewed compounds that are able to induce these various death mechanisms in cancer cells.<sup>65</sup> For example, lysosomal inhibition could emerge as a new therapeutic strategy to overcome drug resistance in cancer.<sup>66</sup> Lysosomes are membrane-bound intracellular organelles that receive macromolecules delivered by endocytosis, phagocytosis, and autophagy for degradation and recycling. Later in the review in Section 4.C, we describe a mollusk-derived compound (belonging to the chemical family of kahalalides) that has reached clinical trials in oncology and that targets lysosomes in melanoma cells displaying marked resistance to apoptosis-related cytotoxic insults.<sup>67</sup>

GBM is the deadliest type of cancer characterized by pronounced resistance to proapoptotic stimuli.<sup>68</sup> The only chemotherapeutic drug that leads to real but still limited beneficial effects for GBM patients is the 30-year-old cytotoxic drug temozolomide.<sup>19,69</sup> Much effort has been applied to discovering ways to kill apoptosis-resistant GBM cells by activating nonapoptotic cell death signaling pathways, such as paraptosis<sup>70–72</sup> or methuosis,<sup>73</sup> but none of these attempts have yet successfully translated into effective treatments for GBM patients. Methuosis is a nonapoptotic cell death type associated with vacuolization of macropinosome and endosome compartments.<sup>74</sup> Paraptosis is a caspase-independent cell death type.<sup>65,75</sup> Lee et al.<sup>76</sup> recently reviewed the natural products capable of inducing paraptosis in cancer cells.

#### 6. *Tumor Metastasis*

Metastases are resistant to conventional therapies and remain the major cause of death from cancer.<sup>77</sup> Indeed, a great majority of cancer patients (~90%) die from tumor metastases<sup>78,79</sup> because metastatic cancers are resistant to almost any type of currently available treatment. Belaid et al.<sup>61</sup> accordingly state that the survival rates of patients with metastatic or recurrent cancers have remained virtually unchanged in the past 30 years.

The metastatic process is characterized by a complex series of interactions between cancer cells that detach from the primary cancer site, known as circulating cancer cells, and tissue microenvironment.<sup>80</sup> As was discussed in a previous section, circulating cancer cells must escape anoikis, and thus resist apoptosis, to be able to form secondary cancer sites (metastases). Of interest, less than 0.01% of circulating cancer cells will succeed in forming metastases<sup>81</sup> and initiating cell growth in secondary organs is the most challenging step in this process.<sup>82</sup> While some cancer types are capable of forming metastases in virtually every tissue in the body, the most frequent target organs of metastasis are bone, brain, liver, and lung.<sup>80</sup> The site preference is described by the “seed” (cancer cells) and “soil” (the tumor microenvironment) theory proposed more than a century ago by Stephen Paget,<sup>83</sup> which has been validated by extensive experimental as well as clinical data.<sup>80</sup> Part of the seed and soil theory is explained by multiple tumor–stromal interactions (which however represent only part of the tumor microenvironment as explained in the next section) that influence the preference for metastatic spread toward a given organ.<sup>80</sup> Fidler and Kripke<sup>77</sup> report that targeting these interactions, in addition to the cancer cells themselves, can produce synergistic therapeutic effects against existing metastases. However, Pienta et al.<sup>84</sup> state that describing metastasis in terms of a simple one-way migration of cells from primary to target organs is insufficient to cover the nuances of cancer spread. Pienta et al.<sup>84</sup> thus raise the question of whether cancer cells escape the confinement of their original habitat in the primary tumor or are they forced out by ecological changes in their home niche? These authors consequently propose an innovative concept of “diaspora,” which is a term used by social scientists to describe the scattering of people away from an established homeland.<sup>84</sup> They argue that invoking the ecological and population science concepts can help understand the biology of tumorigenesis and metastasis, and inspire new ideas for therapy.<sup>84</sup>

Another problem linked to metastases is the capability of metastatic cancer cell to evade therapies by entering dormancy and resuming proliferation years after primary cancer treatment.<sup>85</sup> Ghajar,<sup>86</sup> thus, advocates directing therapies toward the niches that harbor dormant disseminated tumor cells to sensitize them to cytotoxic agents.

Tumor-associated immune cells also play an important role in the promotion of tumor metastasis. Indeed, as discussed by Smith and Kang,<sup>87</sup> inflammation and infiltration of the tumor tissue by host immune cells, such as tumor-associated macrophages, myeloid-derived suppressor cells, and regulatory T cells, have been shown to support tumor growth, invasion, and metastasis. Smith and Kang<sup>87</sup> further describe that each step of tumor development, from initiation through metastatic spread, is promoted by the communication between tumor and immune cells via the secretion of cytokines, growth factors, and proteases that remodel the tumor microenvironment.

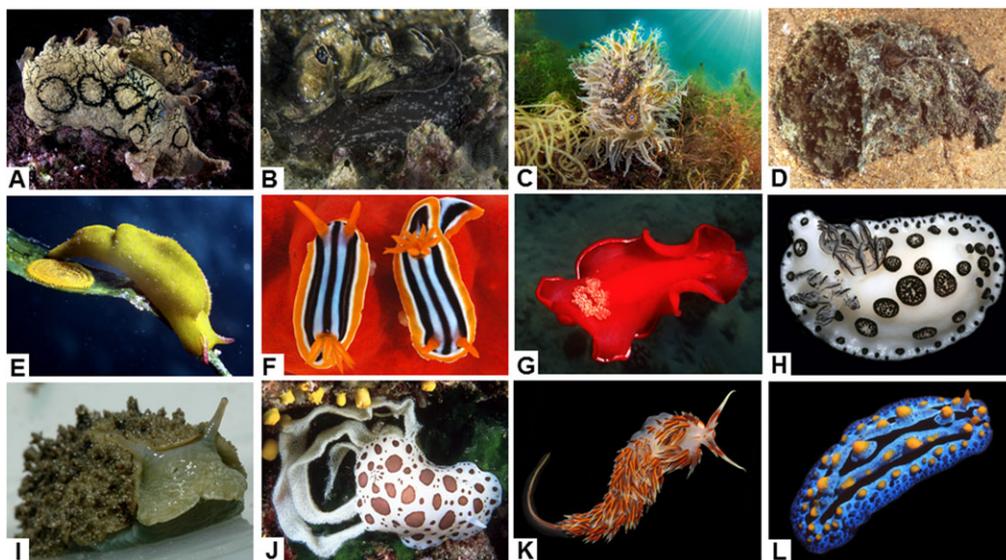
### 7. Tumor Microenvironment

Berns and Pandolfi<sup>88</sup> emphasize the major role played by stromal cells in the development of a cancer, citing the example of pancreatic cancer in which stromal cells and their deposited matrices can make up to 90% of the tumor mass. Cancer-associated fibroblasts actively interact with cancer cells and form a myofibroblastic microenvironment that promotes cancer growth and survival and supports malignancy.<sup>89</sup> It appears that specific oncogenes induce cancer-associated fibroblast phenotype.<sup>45</sup> Berns and Pandolfi<sup>88</sup> report that stromal components might contribute to drug resistance by creating a physical barrier limiting drug access, secreting growth-promoting or antiapoptotic factors, providing niches for CSCs, or by mediating immunosuppression. These authors accordingly argue that combining treatments that degrade the tumor stroma with immunomodulation could be effective given the remarkable success of immunomodulation in a number of tumors and the notion that the tumor microenvironment plays an important role in immune suppression.<sup>88</sup>

Another crucial point in cancer biology is the notion of cancer cell fueling by noncancer cells. For example, autophagic senescent fibroblasts metabolically promote tumor growth and metastasis by paracrine production of high-energy mitochondrial fuels.<sup>90</sup> Migration stimulating factor (MSF) reprograms myofibroblasts toward lactate production, fueling anabolic tumor growth.<sup>91</sup> Sotgia et al.<sup>92</sup> thus state that as metabolic symbiosis promotes drug resistance and may represent an escape mechanism during antiangiogenic therapy, new drugs targeting metabolic symbiosis may also be effective in cancer patients with recurrent and advanced metastatic diseases.

Tabassum and Polyak<sup>93</sup> compare tumorigenesis to a village. These authors indeed report that there is growing evidence that cancer cells behave as communities and different cancer subclones manifest cooperative behavior that can influence tumor progression.<sup>93</sup> It is in this context that the notion of “cancer cell cannibalism” should also be evoked. Homotypic cell cannibalism, a cell-death process regulated by the nuclear protein 1, opposes metastasis in pancreatic cancer.<sup>94</sup> On the other hand, cancer cell cannibalism also leads to increasing levels of aneuploidy in cancer cells, a cell behavior that could promote tumor progression.<sup>95</sup>

Another important component of tumor microenvironment is the tumor vasculature network. Indeed, as emphasized by Blazejczyk et al.,<sup>96</sup> endothelial cells accompany the malignant cancer cells at almost every stage of the metastatic process. This includes infiltration of tumor cells into the neighboring tissue, transmigration through endothelium (intravasation), survival in the blood stream, and extravasation followed by colonization of the target organ. The concept of antiangiogenic therapies in oncology raised great hopes after the pioneering articles by Folkman in the early 1970s.<sup>97,98</sup> However, 45 years



**Figure 1.** Illustration of selected mollusks under review. (A) *Aplysia dactylomela*. (B) *Aplysia punctata*. (C) *Bursatella leachii*. (D) *Dolabella auricularia*. (E) *Elysia subornata*. (F) *Glossodoris quadricolor*. (G) *Hexabranchus sanguineus*. (H) *Jorunna funebris*. (I) *Onchidium* sp. 2. (J) *Peltodoris atromaculata*. (K) *Phidiana militaris*. (L) *Phyllidia coelestis*. Table I provides taxonomical, geographical, and ecological information about these mollusks, whose pictures have been taken by one of us.

later, the antiangiogenic therapies have not led to improved clinical outcomes, especially of cancers associated with dismal prognoses.<sup>99</sup> In addition, once cancers develop resistance to antiangiogenic therapy, they may become more invasive or lead to the metastatic disease.<sup>99,100</sup> Rapisarda and Mellilo<sup>99</sup> accordingly discuss several studies, which indicate that inhibitors of vascular endothelial growth factor (VEGF) (and its receptors) can promote an invasive metastatic switch, in part by creating an increasingly hypoxic tumor microenvironment.

For further information on the role of tumor microenvironment in supporting cancer development, the reader is referred to the recent review articles on this topic.<sup>101,102</sup> The above discussion of the tumor microenvironment playing a big role in supporting tumor progression can lead to a great deal of pessimism for the development of successful therapies. However, it is important to mention that recently two marine-derived compounds that efficiently combat not only cancer cells but also the tumor microenvironment, plitidepsin (aplidin), and trabectedin (Yondelis®) reached late-stage clinical trials in oncology.<sup>103,104</sup>

Due to the success of plitidepsin and trabectedin, anticancer agents targeting tumor microenvironment are actively searched. In marine environment, sessile organisms, especially sponges, make use of bioactive metabolites to prevent the growth of competitors and foulers, and to deter predators from feeding.<sup>105–108</sup> Although such “chemical warfare” frequently represents the main defensive strategy of sessile marine invertebrates, slow moving marine mollusks, including species mentioned in the current review (see Fig. 1 in Section 4.A), are also able to sequester and reuse the chemical weapons from their prey, especially sponges and cnidarians, upon which they feed. In a sense, marine mollusks can thus become effective probes for the selection of bioactive metabolites that evolved in different groups of marine organisms with possible multitargeted effects on predators and competitors. Even though we are still far from having identified the molecular mechanisms behind the natural function of the metabolites from mollusks, it is reasonable to expect that they can interact with more than one molecular

target with both critical ecological and pharmacological interest, possibly contributing to the generation of an “antitumor microenvironment.” In other words, the compounds that inhibit the growth of competitors in marine environment can also affect the growth and evolution of cancerous cells in the tumor microenvironment as has been showcased with plitidepsin and trabectedin.

## 2. WHAT IS A “PROMISING ANTICANCER AGENT?”

The initial steps in anticancer drug discovery utilizing natural sources (plants, fungi, marine invertebrates) are conducted by chemists or biologists who are neither oncologists nor physicians. After having read hundreds of articles that have led to the writing of the present review, it became obvious to us that the term “promising anticancer agent” has different meanings depending on whether it is used by fundamental (chemists and cell biologists) or more applied researchers (pharmacologists and oncologists). The literature is replete with the use of this term by the fundamental researchers, who provide cancer cell killing data as the only basis for their judgment.

To label a particular compound as promising based on cytotoxicity data alone is to forget that a cancer in a patient is much more complex than a few isolated cancer cells in a plastic flask, growing alone outside of tumor microenvironment, and without the possibility to metastasize. This is, therefore, the reason why in the Introduction section we summarized the major characteristics of cancer cell biology and microenvironment representing a crucial obstacle to successful cancer treatment.<sup>109,113</sup> The biological characteristics that we utilize to label an anticancer compound as promising are described next.

### A. *In Vitro* Activity

#### 1. *Antiproliferative Activity*

Antiproliferative effects are most commonly expressed as IC<sub>50</sub> or GI<sub>50</sub> indexes (used interchangeably), representing a compound's concentration that reduces the growth of a given cell population (normal or cancerous) *in vitro* by 50% as compared to the (untreated) control condition. GI<sub>50</sub> values are generally calculated after having cultured the cell population of interest for 48 to 72 hr in the presence (or absence) of the compound of interest. This is commonly done with a colorimetric assay that compares the optical densities (ODs) of a treated cell population to the ODs of a control condition (untreated cells) arbitrarily scaled at 100%. The sole GI<sub>50</sub> value obtained by means of such a colorimetric assay (e.g., the popular MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay) does not represent actual cytotoxic effects, a mistake that is unfortunately so often reported in the literature, but rather only provides relative global growth inhibition information. Indeed, a colorimetric assay does not differentiate cell killing (cytotoxic effects), the inhibition of cell proliferation (cytostatic effects), cell detachment (anti-adhesive effects), or various combinations of these features. Once the GI<sub>50</sub> value has been determined by means of a colorimetric assay for a given compound on a given cell line, it is mandatory to perform complementary morphological and/or biochemical analyses in order to determine whether the compound is cytotoxic, cytostatic, antiadhesive, etc. Galluzzi et al. have comprehensively and regularly reviewed all the assays that are available in this domain, including those that can define the type of cell death.<sup>55-57</sup>

The US National Cancer Institute (hereafter referred to as the NCI) established colorimetric assays involving the analyses of compound-induced growth inhibitory effects in a panel of 60 cancer cell lines belonging to more than ten histopathological types.<sup>114</sup> The precise strategy

developed by the NCI is detailed by Shoemaker.<sup>115</sup> The NCI researchers use three parameters calculated from the colorimetric assays to determine whether a compound is cytotoxic or rather cytostatic. As mentioned above, an NCI-based GI<sub>50</sub> value corresponds to a global growth decrease by 50% induced by a compound on a given cell line after having cultured the cells with the drug for 48 hr in comparison to an untreated cancer cell population (control condition = 100%) grown during the same time.<sup>115</sup> An NCI-based LD<sub>50</sub> (or LC<sub>50</sub>) value corresponds to a global growth decrease by 50% induced by a compound on a given cell line after having cultured the cells with the drug for 48 hr in comparison to the initial number of cells in the untreated control condition.<sup>115</sup> A total growth inhibition (TGI) value is used to determine the concentration needed to kill 100% of the treated cells.<sup>115</sup> For example, based on the combined analysis of the NCI-based GI<sub>50</sub> and LD<sub>50</sub> values, we deduced cytotoxic effects induced by sphaeropsidin A, a rearranged pimarane diterpene of fungal origin, in various cancer cell lines.<sup>116</sup>

Simple morphological analyses, such as those performed with phase-contrast microscopy, can be quite useful in interpreting the data obtained with a colorimetric antiproliferative assay and, specifically differentiate cytotoxic from cytostatic effects. For example, such morphological analyses confirmed the cytotoxic nature of sphaeropsidin A that was originally deduced based on the NCI data.<sup>116</sup> In contrast, narciclasine, an isocarbostryl isolated from daffodils,<sup>117,118</sup> was cytostatic at its GI<sub>50</sub> concentration in melanoma cells as was concluded on the basis of morphological analyses.<sup>119</sup>

It must also be emphasized that colorimetric assays can lead to false-positive or false-negative data with respect to certain types of compounds.<sup>120</sup> In addition, for some compounds the growth curves can reach a plateau-phase in certain cancer cell lines.<sup>121</sup> In both cases, morphological analyses can be useful to validate the data generated by a colorimetric assay, not only in cancer, but also in normal cells.

The GI<sub>50</sub> indexes that precisely define the ranges “weakly active,” “active,” and “highly active” are not unanimously agreed upon in the literature. The instructions for authors of the *Journal of Natural Products* define a compound to be inactive if its GI<sub>50</sub> value is higher than 10 μM. The NCI also uses 10 μM as the highest dose for compounds tested in the 60 cell line panel. However, some compounds that work through nonapoptotic mechanisms (e.g., inducers of senescence or autophagy) can display GI<sub>50</sub> values higher than 10 μM. Thus, we took these issues into account in comments about the “promise” associated with a given anticancer compound.

## 2. Selectivity

The term “selective” is not a synonym for “bioselective” (see Section 2.A.3). A compound is “selective” when it displays *in vitro* growth inhibitory selectivity with respect to one or several histological groups of cancer cell lines out of a larger number of histological cancer types. The data obtained in the NCI 60 cell line panel are most useful in determining selectivity.<sup>114,115</sup> For example, using the NCI data we found that sphaeropsidin A displays *in vitro* selectivity against melanoma and kidney cancer cell lines.<sup>116</sup> This type of information is very important in the design of further *in vivo* experiments, including, for example, the use of nanomedicine approaches to target a specific organ in which a cancer develops.<sup>122–124</sup>

## 3. Bioselectivity

The term “bioselective” is used to refer to a compound that displays differential growth inhibitory effects between normal and cancer cell lines. In the current review, we label a compound as “bioselective” if it displays a GI<sub>50</sub> ratio of higher than 3 between normal and cancer cells, which is not highly discriminant but acknowledges the efforts applied by researchers of a given

article to perform this kind of analysis. The ratio of 3 is also indicative of the possibility to make more bioselective derivatives of the compound of interest.

#### 4. Characterization of the Mechanism(s) of Action

The NCI researchers have tested so far ~800,000 compounds and developed an algorithm (the COMPARE software)<sup>115</sup> that can assist in identifying a compound's mode of action. The COMPARE software compares the shapes of 60 growth inhibitory curves (one per each of the 60 cancer cell lines) of each of the ~800,000 compounds available in the NCI database to the growth curves determined for a compound of interest.<sup>115</sup> Each growth inhibitory curve is determined using five concentrations ranging from 0.001 to 10  $\mu$ M for a given compound. The COMPARE software thus provides a statistical probability value that the compound of interest acts as, for example, a tubulin inhibitor, protein synthesis inhibitor, or a topoisomerase II poison. If the statistical probability provided by the COMPARE software is weak ( $< 0.05$ ), it is potentially indicative of a novel mechanism of action. For example, the COMPARE tool assisted us in identifying a novel compound of interest (a novel trisubstituted harmine derivative) as being a potential protein synthesis inhibitor,<sup>125</sup> a fact that was later validated at the biochemical level. The NCI contributed to the characterization of the anticancer activity and/or the characterization of the mechanism(s) of action of major anticancer drugs that are listed in the review by Shoemaker.<sup>115</sup> The NCI 60 cell line panel also represents a powerful tool for studying the effects of a given compound at the level of mutations,<sup>126</sup> transcript and protein expression profiles,<sup>127</sup> microRNA expression profiles,<sup>128</sup> and global proteome analysis.<sup>129</sup>

In the Introduction section, we emphasized why proapoptotic cytotoxic agents are not promising in combating cancers associated with dismal prognoses. Thus, we do not label as “promising” an anticancer compound that kills cancer cells (*in vitro*) through proapoptotic mechanisms, with no *in vivo* demonstration of activity. We summed up in the Introduction the extraordinary complexity of the metastatic biological process. It is at first glance difficult to appreciate *in vitro* the potential antimetastatic effects of compounds that are evaluated in cancer cells that are grown isolated, without cancer-associated cells, in a 2D environment. We, nevertheless, identify promise in compounds displaying *in vitro*-specific antimetastatic properties such as effects on cancer cell adhesion, motility, migration, or invasion. We highlight compounds displaying effects on cell adhesion at the level of cell–cell and/or cell–extracellular matrix interactions, or affecting the adhesive machinery of cancer cells including, for example, the focal adhesion kinase (FAK)<sup>130</sup> and beta-1 integrins.<sup>131</sup> We also consider that a compound displays “*in vitro* antimetastatic effects” if it decreases cancer cell migration<sup>132</sup> and/or if it targets cytoskeleton or cytoskeleton components such as actin or tubulin.<sup>132</sup> Finally, we regard a compound as able to affect cell invasiveness if it inhibits proteases implicated in the metastatic cascade, such as cysteine cathepsins,<sup>133</sup> and/or if it inhibits migration in transwell migration assays. We highlighted in the Introduction the importance of the MDR phenotype in the resistance of cancer cells to chemotherapeutics, especially in relation to natural cytotoxic compounds. Thus, we emphasize compounds that have shown ability to bypass MDR processes. Lastly, we also highlighted in the Introduction section the major roles displayed by hypoxia and CSCs in cancer chemoresistance. We describe promising compounds that can be triggered by hypoxia or that have been shown to affect CSCs.<sup>133, 144</sup>

#### B. *In Vivo* Activity

As emphasized by Cekanova and Rathore,<sup>145</sup> cancer is the term used to describe over 100 diseases that share several common hallmarks. There are *in vivo* models for any type of cancer, which can be studied in a large variety of species ranging from *Drosophila* flies<sup>146, 147</sup> to zebrafish,<sup>147</sup> and rodents<sup>148–152</sup> to companion animals such as cats and dogs.<sup>145, 153, 154</sup>

However, most *in vivo* preclinical models involve rodents (mostly mice) and generally belong to two groups, cancers developing in immunocompetent rodents and human cancers xenografted in immunocompromised (“nude”) or immunodeficient (SCID) rodents. Cancers developing in immunocompetent mice are obtained by grafting procedures from preexisting solid tumors or cancer cell lines, genetic manipulations, or chemically inducing cancer, among other strategies. Cancers developing in immunocompromised or immunodeficient mice are xenografted into rodents from preexisting solid tumors, cancer cell lines, or cancer cells directly obtained from patients.

There are comprehensive reviews for each type of organ-specific cancer that analyze the advantages and disadvantages of each model. In the following sections, we highlight compounds that have been evaluated for therapeutic efficacy *in vivo* in various preclinical models of cancer and in clinical trials.

### 3. MARINE NATURAL PRODUCTS AS SOURCES OF NEW ANTICANCER DRUGS

Natural products, their potency, selectivity, and mechanisms of action have evolved in nature as critical adaptations, serving as protection from predators, inhibiting competitors, parasites, and pathogens, and influencing reproductive and alimentary behavior. Natural selection is thus the reason behind their importance as drug candidates.<sup>155</sup> Even though a detailed discussion of the possible natural functions of the bioactive metabolites is beyond the scope of this review, it is worth noting that chemoecological role of natural products is only available for a limited number of natural compounds. Chemical ecology, the discipline focusing on chemically mediated ecological interactions between organisms, is quite new.<sup>156,157</sup> Instead, the traditional man-centered perspective on natural products, which is focused on potential medicines, has roots in ancient history and still being a key driver of natural product research.

However, over the past few decades the role of natural products in drug discovery has suffered from several changes. After a boost over the last half of the 20th century, the field experienced a sharp decline as the pharmaceutical industry practically abandoned natural product research in the mid-1990s and shifted the focus toward building massive compound libraries via combinatorial chemistry.<sup>158–160</sup> Still, against all expectations, in the last 25 years, 70% of all approved drugs have been natural products or their derivatives, while only a few *de novo* combinatorial compounds have been approved as drugs.<sup>160–163</sup> A special issue on *Natural Product-Based Drug Discovery* was published in 2016 in *Medicinal Research Reviews*. In addition, reviews published on a regular basis by David J. Newman and Gordon M. Cragg are an excellent source of information on product-based cancer drug discovery.<sup>161,164–168</sup>

In the genomic era, with the advent of a series of novel technologies that speed up the rates of natural product discovery and assist in identifying novel mechanisms of action, there has been a steady reemergence of natural product-based drug discovery as reviewed by Harvey et al.<sup>169</sup> Some of these technologies involve a recapitulation of tumor ecosystems in 3D culture models<sup>134</sup> with the possibility to apply quantitative high-throughput screening,<sup>135</sup> patient-oriented screening,<sup>141,142</sup> the use of cut tissue slices,<sup>144</sup> or “organs-on-chips” approaches,<sup>170</sup> among others.

Traditionally, natural product-based drug discovery mainly concerned the study of compounds isolated from terrestrial organisms, including bacteria, fungi, and especially higher terrestrial plants (Tracheophyta), many of which employ some form of chemical defense to escape predation by herbivores.<sup>168,171–176</sup> In contrast, the history of marine natural products in the context of anticancer drug discovery is relatively young. Oceans and seas constitute 75% of the Earth surface in which nearly one million multicellular (plants and animals) and one billion

unicellular (distributed under 100 different phyla) species live. Oceans and seas have already yielded ~26,000 active compounds.<sup>177</sup> Blunt et al.<sup>177</sup> report that these ~26,000 compounds belong to ~9000 collections obtained worldwide since 1965.

Similar to their terrestrial counterparts, marine organisms have evolved to produce bioactive compounds to adapt to environmental conditions and especially to deter feeding by predators (defensive allomones). Mitsiades et al.<sup>178</sup> argued that compounds derived from marine organism may have evolved to be more potent than similar compounds from the above-water organisms to compensate for the increased diffusion and thus rapidly decreasing protective concentration gradient of these compounds under water. However, although such considerations are certainly appropriate for water-soluble polar compounds, low water solubility of many bioactive marine natural products, especially terpenes, instead prevents their dilution in the medium, allowing them to act at high doses during the contact with predators.<sup>179</sup> In some cases lipophilicity may thus be an important feature both in preventing the dilution of defensive chemical weapons in water and making bioactive compounds of interest in pharmacology capable of permeating lipophilic biomembranes.

The NCI estimates that more than 1% of marine natural products show antitumor properties as compared to the 0.01% among their terrestrial counterparts.<sup>180</sup> The marine-sourced anticancer and cancer pain control agents that have reached late preclinical and clinical development were recently reviewed by Newman and Cragg.<sup>166</sup> Gerwick and Moore<sup>110</sup> reported in 2012 that the success rate of discovery from the marine world (for any type of clinical indication) with seven clinically useful and approved drugs from 22,000 discovered molecular entities (i.e., one drug per 3140 natural products described) is thus approximately 1.7- to 3.3-fold better than the industry average, which is one in 5000–10,000 tested compounds.<sup>181, 185</sup>

Skropeta and Wei<sup>186</sup> reviewed in 2014 (for the period 2009–2013) 188 novel marine natural products from deep-water (from 50 to >5000 m) marine fauna including bryozoa, chordata, cnidaria, echinodermata, microorganisms, mollusca, and porifera. They report that 75% of the compounds they reviewed possess bioactivity, with almost half exhibiting low micromolar cytotoxicity toward a range of human cancer cell lines. Gerwick and Moore<sup>110</sup> also report that mollusks, sponges, and tunicates are the richest collected sources of clinically useful drugs. However, they emphasize a lot of evidence showing that the actual producers of the bioactive substances are associated microorganisms (mainly heterotrophic bacteria and cyanobacteria). In addition, a trophic transfer of the compounds from sponges to their specialist nudibranch predators is also described.<sup>187–189</sup>

The next section covers the anticancer potential of molecules isolated from the phylum Mollusca describing the *in vitro*, *in vivo* preclinical and clinical studies published in the literature (PubMed and Scopus databases). As emphasized by Molinski et al.,<sup>159</sup> drug discovery from marine natural products has enjoyed a renaissance in the past few years due to the recent marketing of the cancer pain control agent ziconotide (Prialt®) and the anticancer drug trabectedin (Yondelis®).

#### 4. MOLLUSK-DERIVED ANTICANCER AGENTS

Given the controversial theme of the biosynthetic origin of the metabolites isolated from mollusks, it must be emphasized that in the current review we chose to simply consider the studied mollusks as the natural sources of the compounds under investigation, and not their actual producers. Although some of the bioactive compounds under review are produced by the mollusks themselves, most of them are, in fact, of dietary origin. Noteworthy, the “mollusk-derived” metabolites that entered or that are in clinical trials are actually produced

by microbes. Consequently, the study of the molluscan chemistry has often resulted in the study of compounds produced by organisms at lower trophic levels.

#### *A. Mollusks as Natural Product Sources*

The phylum Mollusca shows great morphological, ecological, and chemical variability and it is subdivided in two subphyla, the Aculifera and the Conchifera. The Aculifera, which do not produce a complete shell, comprise the two classes of the Aplacophora, worm-like in form, and the Polyplacophora, with a dorsal shell divided into eight valves. The shelled Conchifera includes five classes: (i) the Monoplacophora with a cap-shaped shell; (ii) the Gastropoda including snails, slugs, and limpets; (iii) the Cephalopoda including octopuses, cuttlefish, and squids; (iv) the Bivalvia such as clams, oysters, scallops, and mussels; and (v) the Scaphopoda with a tubular shell.<sup>190</sup> Most of the chemical studies on marine natural products have been focused on the gastropods, which are traditionally divided in three major groups, the subclasses Prosobranchia, Pulmonata, and Opisthobranchia,<sup>188</sup> and more recently on the bivalves.<sup>191</sup>

Marine mollusks can be found from tropical seas and temperate waters to Artic-Antarctic regions, showing different morphologies and occupying a wide range of ecological niches.<sup>192</sup> They feed on a wide variety of benthic invertebrates and plants, often accumulating dietary metabolites from their prey to be reused against their own potential predators. Consequently, and as emphasized above, the study of the molluscan chemical diversity involves, at least in part, the exploration of the chemical composition of organisms at lower trophic levels.

Mollusks are also able to accumulate the dietary metabolites in localized anatomical structures.<sup>193,194</sup> This type of defensive strategy, which is particularly common in opisthobranchs, can be reinforced by the presence of visual aposematic patterns, which can be shared by groups of similarly colored species that share the cost of the education of predators within Müllerian mimetic circles.<sup>195</sup> Recently, it has been even proposed that a group of nudibranchs belonging to the family Chromodorididae forms a putative Müllerian mimetic circle based on a common chemosensory signal, the anticancer macrolide latrunculin A, which is among the compounds discussed in this review.<sup>189</sup> In Table I, we provide taxonomical, geographical, and ecological information for the mollusks under review and in Figure 1, we illustrate a selection of these organisms.

If reproducibility is a crucial issue in all sciences, it assumes a critical importance in cancer research. When the bioactive compounds are difficult or costly to synthesize, the extraction from natural sources may be the only realistic way to provide sufficient amounts of purified metabolites for preliminary pharmacological screenings. Consequently, the correct species classification and information about the collection sites becomes of utmost importance. Even appropriate taxonomic and geographic information, however, is not always sufficient. Intraspecific variations in secondary metabolites between individuals of a given species, in fact, are widely documented in the literature. In addition, given that the taxonomy evolves, sometimes it is difficult to track the source of a given metabolite in the available chemical literature. The so-called “chemotaxonomy” adds more confusion to the issue because a secondary metabolite can be of dietary origin, produced by symbionts, or de novo biosynthesized. This generate a sort of “labyrinth” from which we could get out only by providing, in the future, more information about the origin of the metabolites, more biosynthetic studies, more accurate GPS data of the sampling locations, and more complete information about the cases of synonymy and possible misidentification.

The current review highlights the diversity of chemical structures, mechanisms of action, and most importantly the assessment of “promise” of mollusk-derived metabolites as anticancer agents. We include, as mollusk-derived metabolites, compounds that were originally identified in mollusks. However, since the initial discovery of certain metabolites with anticancer effects

Table I. Taxonomical, Geographical, and Ecological Information for the Mollusks under Review

Reported name	Valid name	Class: Family	Photo in Figure 3	Compounds under review	Distribution	Feeding behavior
<i>Aplysia angasi</i> * Sowerby, 1869	<i>Aplysia dactylomela</i> (Rang, 1828)	Gastropoda: Aplysiidae	A	19; 22; 23; 24a-b; 29a-d; 39; 40	Circumtropical	Herbivore (on macroalgae)
<i>A. dactylomela</i> Rang, 1828		Gastropoda: Aplysiidae	–	42	Eastern Atlantic and Mediterranean	Herbivore (on macroalgae)
<i>Aplysia depilans</i> Gmelin, 1791		Gastropoda: Aplysiidae	–	41	Eastern Atlantic and Mediterranean	Herbivore (on macroalgae)
<i>Aplysia fasciata</i> Poiret, 1789		Gastropoda: Aplysiidae	–	21; 64a-h; 70; 71a-c	Pacific	Herbivore (on macroalgae)
<i>Aplysia kurodai</i> Baba, 1937		Gastropoda: Aplysiidae	–	27; 28	Indo-Pacific	Herbivore (on macroalgae)
<i>Aplysia oculifera</i> A. Adams & Reeve, 1850		Gastropoda: Aplysiidae	–			Herbivore (on macroalgae)
<i>Aplysia punctata</i> Cuvier, 1803		Gastropoda: Aplysiidae	B	16; 17; 18	European waters	Herbivore (on macroalgae)
<i>Austrodothis kerguelensis</i> * Bergh, 1884	<i>Doris kerguelensis</i> (Bergh, 1884)	Gastropoda: Dorididae	–	32a-f	Antarctic Ocean	Carnivore (on sponges)
<i>Bathymodiolus thermophilus</i> Kenk & B. R. Wilson, 1985		Bivalvia: Mytilidae	–	80a-b	Pacific (deep sea)	Suspensivore (also absorbs nutrients synthesized by chemosymbiotic bacteria)
<i>Bursatella leachii</i> Blainville, 1817		Gastropoda: Aplysiidae	C	73b-c; 75a-b	Indo-Pacific	Herbivore (on cyanobacteria)
<i>Cheylonotus semperi</i> Bergh 1886		Gastropoda: Velutinidae	–	82	Indo-Pacific	Carnivore (on tunicates)
<i>Chromodoris annae</i> Bergh, 1877		Gastropoda: Chromodorididae	–	67a	Indo/West Pacific	Carnivore (on sponges)

Continued

Table 1. Continued

Reported name	Valid name	Class: Family	Photo in Figure 3	Compounds under review	Distribution	Feeding behavior
<i>Chromodoris elisabethina</i> Bergh, 1877		Gastropoda: Chromodorididae	–		Indo/West Pacific	Carnivore (on sponges)
<i>Chromodoris kuiteri</i> Rudman, 1982		Gastropoda: Chromodorididae	–		Indo/West Pacific	Carnivore (on sponges)
<i>Chromodoris magnifica</i> Quoy & Gaimard, 1832		Gastropoda: Chromodorididae	–		Indo/West Pacific	Carnivore (on sponges)
<i>Chromodoris inornata</i> * Pease, 1871	<i>Chromodoris aspersa</i> (Gould, 1852)	Gastropoda: Chromodorididae	–	<b>35a-c; 36a-d; 37</b>	Indo/West Pacific	Carnivore (on sponges)
<i>Chromodoris lochi</i> Rudman, 1982		Gastropoda: Chromodorididae	–	<b>63a-b; 67a</b>	Indo/West Pacific	Carnivore (on sponges)
<i>Chromodoris obsoleta</i> * Ruppell & Leuckart, 1831	<i>Goniobranchus obsoletus</i> (Ruppell & Leuckart, 1830)	Gastropoda: Chromodorididae	–	<b>30a-d; 31a-g</b>	Indo-Pacific	Carnivore (on sponges)
<i>Coriocella nigra</i> Blairville, 1824		Gastropoda: Velutinidae	–	<b>77a-b</b>	Indo-Pacific	Carnivore (on tunicates)
<i>Dicathais orbita</i> Gmelin, 1791		Gastropoda: Muricidae	–	<b>81</b>	Coasts of Australia and New Zealand	Carnivore (on mollusks and crustaceans)
<i>Dolabella auricularia</i> Lightfoot, 1786		Gastropoda: Aplysiidae	<b>D</b>	<b>1a; 2a; 3; 4; 5; 6; 7; 8; 9; 10; 11; 12; 13a-b; 14a-b; 38a; 46; 52a-b; 56a-b; 57; 58a-b; 59a-d; 74</b>	Indo-Pacific	Herbivore (on macroalgae)
<i>Elysia nisbeti</i> Thompson, 1977		Gastropoda: Plakobranchidae	–	<b>20</b>	Caribbean	Herbivore (on macroalgae)

Continued

Table I. Continued

Reported name	Valid name	Class: Family	Photo in Figure 3	Compounds under review	Distribution	Feeding behavior
<i>Elysia patina</i> Ev. Marcus, 1980		Gastropoda: Plakobranchidae	-	<b>20</b>	Caribbean	Herbivore (on macroalgae)
<i>Elysia rufescens</i> Pease, 1871		Gastropoda: Plakobranchidae	-	<b>15a</b>	Pacific	Herbivore (on macroalgae)
<i>Elysia subornata</i> A. E. Verrill, 1901		Gastropoda: Plakobranchidae	<b>E</b>	<b>20</b>	Atlantic, Caribbean	Herbivore (on macroalgae)
<i>Glossodoris quadricolor</i> * Ruppell & Leuckart, 1828	<i>Chromodoris quadricolor</i> (Ruppell & Leuckart, 1830)	Gastropoda: Chromodorididae	<b>F</b>	<b>67b</b>	Red Sea	Carnivore (on sponges)
<i>Hexabranchius sanguineus</i> Ruppell & Leuckart, 1830		Gastropoda: Hexabranchidae	<b>G</b>	<b>60a-b, 61a, c-f, 62a-b</b>	Indo-Pacific and Red Sea	Carnivore (on sponges)
<i>Jorunna funebris</i> Kelaart, 1859		Gastropoda: Discodorididae	<b>H</b>	<b>84a, 85, 86</b>	Indo-Pacific and Red Sea	Carnivore (on sponges)
<i>Kelletia kelleitii</i> Forbes, 1850		Gastropoda: Buccinidae	-	<b>68a-b</b>	Eastern Pacific	Carnivorous scavenger
<i>Lamellaria</i> sp. Montagu, 1816		Gastropoda: Velutinidae	-	<b>76</b>	Palau	Carnivore (on tunicates)
<i>Leminda millecra</i> Griffiths, 1985		Gastropoda: Charcotiidae	-	<b>45</b>	Coasts of South Africa	Carnivore (on cnidarians)
<i>Mactromeris polynyma</i> Stimpson, 1860		Bivalvia: Mactrida)	-	<b>87</b>	Northeast Pacific	Suspensivore
<i>Onchidium</i> sp.1 Buchannan, 1800		Gastropoda: Onchidiidae	-	<b>55a-b</b>	New Caledonia	Herbivore (on microalgae)
<i>Onchidium</i> sp.2 Buchannan, 1800		Gastropoda: Onchidiidae	<b>I</b>	<b>65</b>	South China Sea	Herbivore (on microalgae)

Continued

Table I. Continued

Reported name	Valid name	Class: Family	Photo in Figure 3	Compounds under review	Distribution	Feeding behavior
<i>Peltochlamys atramaculata</i> Bergh, 1880		Gastropoda: Discodorididae	J	69a	Mediterranean and Atlantic	Carnivore (on sponges)
<i>Phidiana militaris</i> Alder & Hancock, 1864		Gastropoda: Facelinidae	K	83a-b	Indo-Pacific	Carnivore (on cnidarians)
<i>Philinopsis speciosa</i> Pease, 1860		Gastropoda: Aglajidae	-	50; 51a-b	Indo/West Pacific	Carnivore (on mollusks)
<i>Phyllidia coelestis</i> Bergh, 1905		Gastropoda: Phyllidiidae	L	25a-b	Indo/West-Pacific	Carnivore (on sponges)
<i>Pleurobranchus albiguttatus</i> Bergh, 1905		Gastropoda: Pleurobranchidae	-	33a-b, d, f, j; 34a-c	Indo/West-Pacific	Carnivore (on tunicates)
<i>Pleurobranchus forskalii</i> Ruppell & Leuckart, 1828		Gastropoda: Pleurobranchidae	-	33a-b, d, f, j; 34a; 53; 54	Indo/West-Pacific	Carnivore (on tunicates)
<i>Reticulidia fungia</i> Brunckhorst & Gosliner in Brunckhorst, 1993		Gastropoda: Phyllidiidae	-	26a-b	Indo-Pacific	Carnivore (on sponges)
<i>Roboastra tigris</i> Farmer, 1978		Gastropoda: Polyceridae	-	78a	Gulf of California	Carnivore (on mollusks)
<i>Stylocheilus longicauda</i> Quoy & Gaimard, 1825		Gastropoda: Aplysiidae	-	51a-b; 73a	Circumtropical	Herbivore (on cyanobacteria)
<i>Tambja abdere</i> Farmer, 1978		Gastropoda: Polyceridae	-	78a	Eastern Pacific	Carnivore (on bryozoans)
<i>Tambja ceutae</i> Garcia-Gomez & Ortea, 1988		Gastropoda: Polyceridae	-	78c	Eastern Atlantic	Carnivore (on bryozoans)

Continued

Table I. Continued

Reported name	Valid name	Class: Family	Photo in Figure 3	Compounds under review	Distribution	Feeding behavior
<i>Tambja elitora</i> Er. Marcus & Ev. Marcus, 1967		Gastropoda: Polyceridae	–	<b>78a</b>	Gulf of California	Carnivore (on bryozoans)
<i>Trimusculus costatus</i> Krauss, 1848		Gastropoda: Ellobiidae	–	<b>44</b>	Coasts of South Africa	Filter feeder
<i>Trimusculus peruvianus</i> G. B. Sowerby I, 1835		Gastropoda: Ellobiidae	–	<b>43</b>	Coasts of Chile and Peru	Filter feeder
<i>Turbo stenogyrius</i> P. Fischer, 1873		Gastropoda: Turbimidae	–	<b>79</b>	Indo/West Pacific	Herbivore
<i>Tylodina perversa</i> Gmelin, 1791		Gastropoda: Tylodimidae	–	<b>72</b>	Northeastern Atlantic and Mediterranean	Carnivore (on sponges)

The species names reported in the chemical literature are listed by alphabetical order, and marked by asterisks when synonymized. The distribution refers to the native range of the species, rather than new ranges where nonnative species have recently become established.

The species names and distribution was found at [1] World Register of Marine Species. Available from <http://www.marinespecies.org> at VLIZ; accessed April 8, 2016 [2]. MolluscaBase (2015); accessed at <http://www.molluscaBase.org> on April 8, 2016 [3]. Australian Museum Sea Slug Forum, Sydney; available at <http://www.seaslugforum.net>.

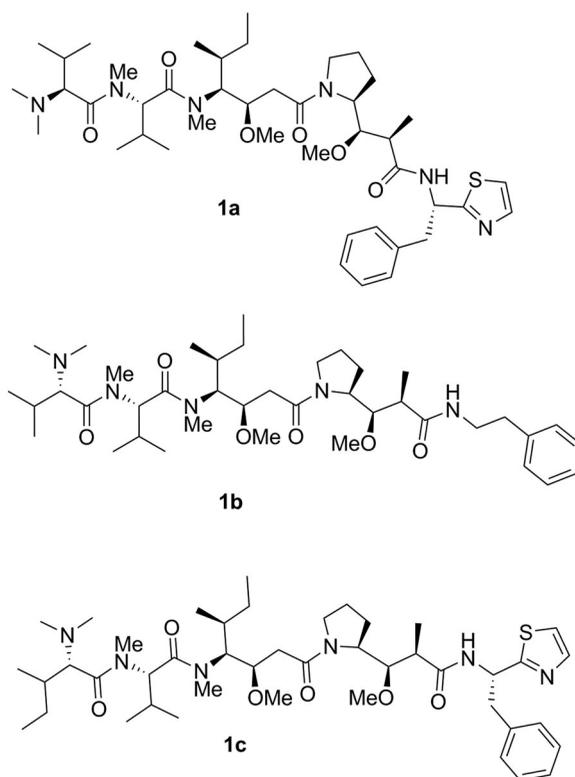


Figure 2. Chemical structures of dolastatin 10 (**1a**), soblidotin (TZT-1027; **1b**), and symplostatins 1 (**1c**).

in a given mollusk, it has been demonstrated in certain cases that these compounds are not biosynthesized by the mollusk but instead are of dietary origin. We thus provide in Table II all the compounds under review (alphabetically listed) that were initially discovered in mollusks and we also report the possible actual producer(s) of the compounds of interest when such information is available in the literature.

### B. Dolastatins

We describe below the cyanobacterial metabolites of the dolastatin type. However, it must be recalled that these types of compounds did not successfully complete clinical trials, usually exiting trials at or before the Phase II level. Thus, though we detail below the use of agents derived from dolastatins as potential anticancer agents, it should be noted that most of the Phase I trials did not proceed any further. Therefore, dolastatins can no longer be labeled as “promising anticancer drugs.”

Dolastatins (**1a-14b**; Figs. 2–6) embody a large family of active compounds originally isolated from *Dolabella auricularia* (Anaspidea mollusk, Table I; Fig. 1D). From the chemical point of view, dolastatins show high structural heterogeneity including linear and cyclic peptides, depsipeptides, peptides containing the thiazole and oxazole heterocycles, and macrolides. The low concentrations of dolastatins found in sea hares suggested a dietary origin and this was subsequently confirmed by direct isolation of dolastatin 10 from field collections of the marine cyanobacterium *Symploca*.<sup>196</sup> Also, it has been confirmed for a number of other dolastatins that they are not produced by sea hares,<sup>197</sup> but ingested and sequestered from the dietary

Table II. Compounds Under Review That Were Isolated from Mollusks Listed in Alphabetical Order

Compound		No.	Figure	Reported source(s) among mollusks (see Table I)	Possible actual producer(s)
3-Acetyl-11-(3-methylbutanoyl)-13-propanoyllikonapyrone		65	18	<i>Ondichidium</i> sp.2	—
3-Epi-aplykurodinone B		41	12	<i>Aplysia fasciata</i>	—
Aplaminal		70	20	<i>Aplysia kurodai</i>	—
Aplaminones		71a-c	20		—
Aplyronines		64a-h	19		—
(-)-Aplysin		21	7		<i>Laurencia tristicha</i> (red algae)
Aplysistatin		22	7	<i>Aplysia angasi</i>	—
Aplysqualenol A		39	12	<i>Aplysia dactylomela</i>	—
Aurilide		52a-c	14	<i>Dolabella auricularia</i>	<i>Lynghya</i> (cyanobacteria)
Aurilol		38a	11	<i>D. auricularia</i>	<i>Laurencia</i> (red algae)
Auripyrones		58a-b	16		—
Aurisides		56a-b	16	<i>D. auricularia</i>	Cyanobacteria
Bathymodiolamides		80a-b	23	<i>Bathymodiolus thermophilus</i>	—
C-21-hydroxylated sterol		43	12	<i>Trinuscultus peruvianus</i>	—
Caulerpyne		20	7	<i>Elysia subornata</i> , <i>E. patina</i> , <i>E. nisbeti</i>	<i>Caulerpa</i> spp. (green algae)
Cycloforskamide		54	15	<i>Pleurobranchus forskalii</i>	<i>Prochloron didemni</i> (cyanobacteria)
Dichlorolissoclimide, chlorolissoclimide		33 a,b	10	<i>Pleurobranchus albigitatus</i> , <i>P. forskalii</i>	<i>Lissoclinium</i> (tunicate)
Dolabelides		59a-d	16	<i>D. auricularia</i>	—
Dolabellin		74	22		—
Dolastatin 1		3	3		—
Dolastatin 3		4	3		—
Dolastatin 10		1a	2		<i>Lynghya majuscula</i> (cyanobacteria)
Dolastatin 11		5	4		<i>Symploca</i> sp. VP642, <i>S. hydroides</i> (cyanobacteria)
Dolastatin 12		6	4		<i>L. majuscula</i> , <i>Schizothrix calcicola</i> (cyanobacteria)
Dolastatin 13		7	4		—
Dolastatin 14		8	4		—
Dolastatin 15		2a	3		—
Dolastatin 16		9	5	<i>D. auricularia</i>	<i>Lynghya majuscula</i> , <i>Symploca</i> cf. <i>hydroides</i> (cyanobacteria)

Continued

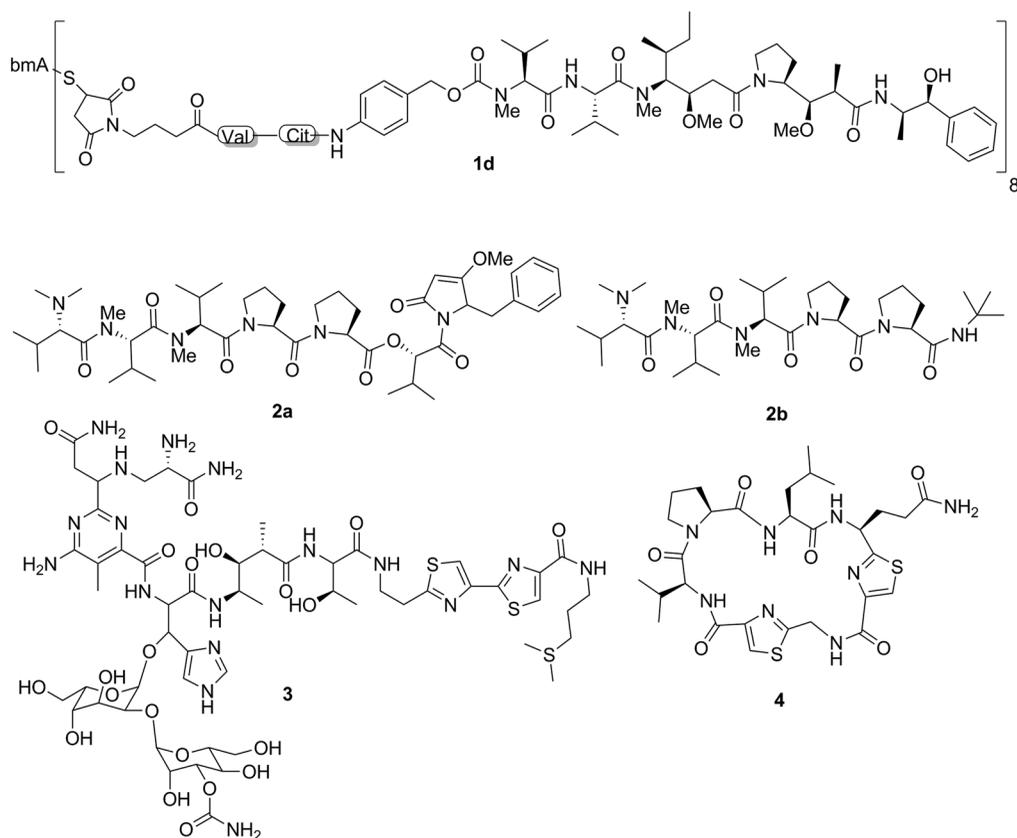
Table II. Continued

Name	Compound		Reported source(s) among mollusks (see Table I)	Possible actual producer(s)
	No.	Figure		
Dolastatin 17	10	5		—
Dolastatin 18	11	5		—
Dolastatin 19	57	16		—
Dolastatin D	12	5		—
Dolastatin G, Nordolastatin G	13 a,b	5		—
Dolastatin H, isodolastatin H	14 a,b	6		—
Doliculide	46	13		Cyanobacteria Sponges
Dorisenones and related spongian diterpenoids	30 a-d 31 a-g	8-9	<i>Chromodoris obsoleta</i>	
Elatol	23	7	<i>A. dactylomela</i>	<i>Laurencia</i> (red algae)
Endoperoxide sterol	42	12	<i>Aplysia depilans</i>	—
Enshuol	38b	11		<i>Laurencia</i> (red algae)
Halichondramides	62a-c	18	<i>Hexabranchnus sanguineus</i>	<i>Halichondria</i> sp. (sponges)
Halogenated monoterpenes	16	7	<i>Aplysia punctata</i>	<i>Plocamium</i> (red algae)
	17	7		
	18	7		
	19	7	<i>A. dactylomela</i>	—
Haterumaimides	33c-q/34a-c	10	<i>P. albiguttatus</i> , <i>P. forskalii</i>	<i>Lissochinum</i> (tunicates)
Hectochlorin	75a-b	22	<i>Bursatella leachii</i>	<i>L. majuscula</i> (cyanobacterium)
Hydroxyl-dehydroisofulvinol	69a	20	<i>Peltodoris atromaculata</i>	<i>Haliclona fubra</i> (sponges)
Inorolides	35a-c	10	<i>Chromodoris inornata</i>	<i>Hyrtios</i> (sponges)
Isofistularin-3	72	21	<i>Tyrodina perversa</i>	<i>Aplysina</i> (sponges)
Jorumycin	84a	24	<i>Jorunna funebris</i>	Sponges
Jorunnamycin C	85	24		
Kabirnamides	61a-f	17	<i>H. sanguineus</i>	<i>Halichondria</i> (sponges)
Kahalalide F	15a	6	<i>Elysia rufescens</i>	<i>Bryopsis pennata</i> (green algae)
Keenamides A	53	15	<i>P. forskalii</i>	—
Kelletinins	68a-b	20	<i>Kelletia kelleitii</i>	—
Kuanoniamine A	82	23	<i>Chelynotus semperi</i>	Tunicates
Kulokekahilides 1 and 2	51 a,b	14	<i>Philinopsis spectiosa</i>	<i>L. majuscula</i> (cyanobacteria)

Continued

Table II. Continued

Compound		No.	Figure	Reported source(s) among mollusks (see Table I)	Possible actual producer(s)
Name					
Kulohide I		50	13		–
Lamellarin D		76	22	<i>Lamellaria</i> sp.	Tunicates and/or sponges
Latrunculin A		67a	20	<i>Chromodoris lochi</i>	<i>Spongia mycifiensis</i> (sponges)
Latrunculin B		67b	20	<i>Glossodoris quadricolor</i>	<i>Latrunculia magnifica</i> (sponges)
Laulimalides		63a-b	18	<i>C. lochi</i>	<i>Hyattella</i> sp. (sponges)
Malungamides		73 a	21	<i>Stylocheilus longicauda</i>	<i>L. majuscula</i> (cyanobacteria)
		73 b,c	21	<i>B. leachii</i>	
		28	8	<i>Aplysia oculifera</i>	<i>Laurencia</i> , <i>Plocanium</i> (red algae)
Obtusane (epi-)					
Obtusol					
Isoobtusol		24 a,b	7	<i>A. dactylomela</i>	<i>Laurencia</i> (red algae)
Oculiferane		27	8	<i>A. oculifera</i>	<i>Laurencia</i> , <i>Plocanium</i> (red algae)
Onchidins		55a-b	15	<i>Onchidium</i> sp.1	–
Palmadorins		32a-f	9	<i>Austrodoris kerguelensis</i>	–
Parguerol and derivatives		29a-e	8	<i>A. dactylomela</i>	<i>Jania rubens</i> (red alga) and/or sponges
Phidianidines		83a-b	23	<i>Phidana militaris</i>	–
Prenylated hydroquinone		45	12	<i>Leminda millecra</i>	<i>Leptogorgia</i> (cnidarians)
Pupukeanane derivatives		25 a,b	7	<i>Phyllidia coelestis</i>	Sponges
Renieramycin M		86	24	<i>J. funebris</i>	Sponges
Reticulidins A and B		26 a,b	8	<i>Reticulia fungia</i>	Sponges
Secosterol		44	12	<i>Trimusculus costatus</i>	–
Sesterterpenoids		36a-d/37	11	<i>C. inornata</i>	<i>Hyrtios</i> (sponges)
Sphinxolide		66	19	Unidentified nudibranch	<i>Neosiphonia superstes</i> , <i>Reidispongia coreula</i> (sponges)
Spisulosine (ES-285)		87	24	<i>Mactromeris polymyxa</i>	–
Staurosporine analogues		77a-b	22	<i>Coriocella nigra</i>	–
Tambjamines D, E, and K		78a-c	22	<i>Tambja eliora</i> , <i>T. abdere</i> , <i>T. centae</i> , <i>Roboastra tigris</i>	Bryozoans
Thyrsiferol		40	12	<i>A. dactylomela</i>	<i>Laurencia thyrsifera</i> (red algae)
Turbostatin A		79	23	<i>Turbo stenogyris</i>	–
Tyridoleninone		81	23	<i>Dicathais orbita</i>	–
Ulapualides		60a-b	17	<i>H. sanguineus</i>	<i>Halichondria</i> (sponges)



**Figure 3.** Chemical structures of brentuximab vedotin (SGN-35; Adcetris®; **1d**), dolastatin 15 (**2a**), tasidotin (ILX-65; **2b**), dolastatin 1 (**3**), and dolastatin 3 (**4**). MAB in structure **1d** represents the monoclonal antibody portion of this ADC.

cyanobacteria. In some cases, the true producers of dolastatins have been demonstrated to be cyanobacteria of genus *Symploca* (Table II) recently revised into the new genus *Caldora* and species *penicillata*,<sup>198</sup> as well as *Lyngbya*.

As reported by Pettit et al.,<sup>199</sup> the genus *Dolabella* has interesting history. The Roman natural scientist Pliny the Elder comprehensively studied and then described (~60 A.D.) a potent Indian Ocean sea hare of the genus *Dolabella*. The Romans named the Mollusca of the family Aplysiidae as “sea hare” due to the similarity between the ears of a hare and the auriculate tentacles of these gastropods.<sup>199</sup> Extracts from the *Dolabella* sea hare were already known for their toxic properties during the reign of Nero.<sup>199</sup> Nicander (~150 A.D.) recognized the possibility of using *Dolabella* extracts to treat certain diseases.<sup>199</sup> Pettit et al.<sup>199</sup> thus argued that the dolastatin family of compounds they discovered (see below) most probably correspond to the potent *D. auricularia* constituents recognized by the above-named Romans. Among these largely diverse compounds, the linear peptides dolastatin 10 and 15 show the most remarkable picomolar GI<sub>50</sub> values in most *in vitro* cancer growth inhibition studies.<sup>200</sup>

Dolastatin 10 (**1a**; Fig. 2; Table II) was first isolated from *D. auricularia* collected from the Indian Ocean<sup>201</sup> and then discovered (with its methyl derivative, symplostatins 1) in the cyanobacterium *Symploca hydroides*.<sup>196</sup> Dolastatin 10 is a linear pentapeptide with four of residues being structurally unique: dolavaline (Dov), dolaisoleuine (Dil), dolaproine (Dap), and dolaphenine (Doe), in addition to valine (Fig. 2). Its absolute configuration was established by

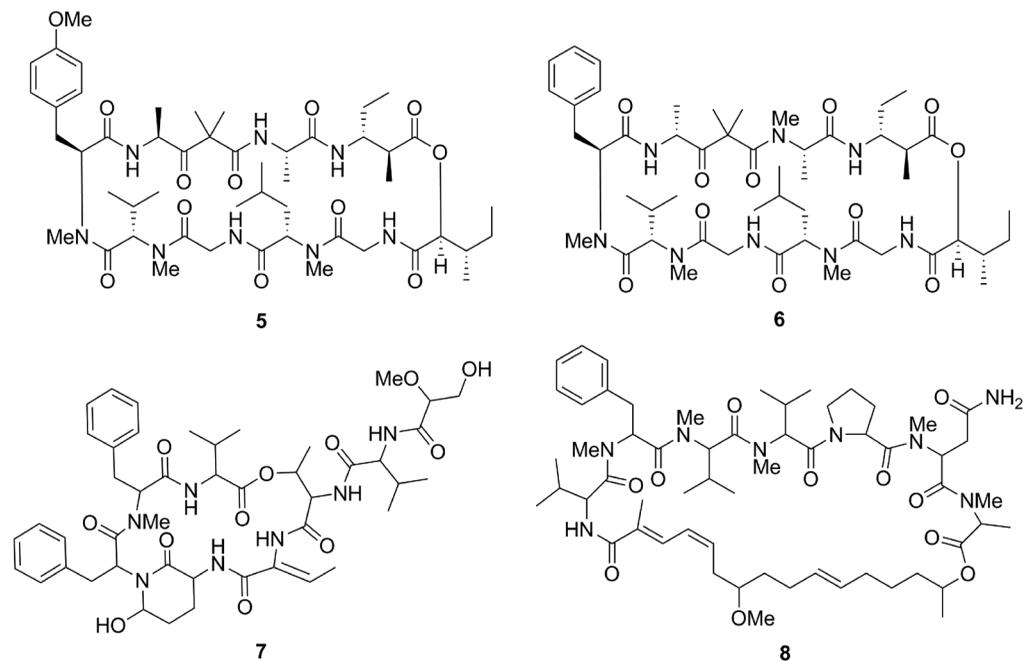


Figure 4. Chemical structures of dolastatin 11 (**5**), dolastatin 12 (**6**), dolastatin 13 (**7**), and dolastatin 14 (**8**).

synthesis by Pettit et al.<sup>202</sup> Singh et al.<sup>203</sup> comprehensively reviewed the historical development of dolastatin 10. Briefly, it entered Phase I clinical trials in the early 1990s under the auspices of the NCI. Then, several Phase II clinical studies (advanced and metastatic soft tissue sarcoma, advanced hepatobiliary cancers, pancreatic cancers, among others) did not reveal the efficacy of dolastatin 10 (**1a**) as a single anticancer agent due to dose-limiting side effects, such as neuropathy.<sup>203</sup>

Singh et al.<sup>203</sup> explained that although clinical trial results with dolastatin 10 were discouraging, these as well as preclinical studies offered the basis for structure–activity relationship (SAR) analyses and have led to the discovery of soblidotin (TZT-1027, **1b**; Fig. 2), a synthetic analogue of dolastatin 10,<sup>203,204</sup> in which the thiazole moiety of the dolaphenine was replaced by phenylalanine methyl ester. The *in vitro* growth inhibitory effects of soblidotin decline depending on the amount of P-gp expressed in cancer cells.<sup>205</sup> Soblidotin displayed *in vivo* anticancer activity in several models including the murine P388 leukemia, Colon26 colon cancer, Lewis Lung carcinoma, B16 melanoma and M5076 sarcoma models as well as human MX-1 breast cancer, and LX-1 and SBC-3 SCLC xenografts.<sup>205</sup> Soblidotin entered several Phase II clinical trials (advanced and metastatic soft tissue sarcomas, NSCLCs, among others) but it was also found ineffective as a single agent and its clinical development was no longer pursued.<sup>203</sup>

The GI<sub>50</sub> index of dolastatin 10 in the NCI 60 cell-line panel is ~0.2 nM and a COMPARE analysis with a cut-off correlation at  $p = 0.7$  is negative in the standard agent dataset. This agent is not able to overcome the MDR phenotype mediated by the P-glycoprotein (P-gp) in cancer cells<sup>206</sup> as is also the case with respect to its analogues soblidotin<sup>205</sup> and symplostatins (**1c**; Fig. 2).<sup>207</sup> Dolastatin 10 induces proapoptotic stimuli in cancer cells<sup>208,209</sup> as do soblidotin<sup>205,210</sup> and symplostatins.<sup>207</sup> Dolastatin 10 was rapidly identified as an inhibitor of tubulin polymerization.<sup>211</sup> In addition to the inhibition of tubulin polymerization, it inhibits tubulin-dependent GTP hydrolysis and the binding of vinblastine, maytansine, and vincristine to tubulin, although its binding site on tubulin is different from that of the vinca alkaloids.<sup>210</sup>

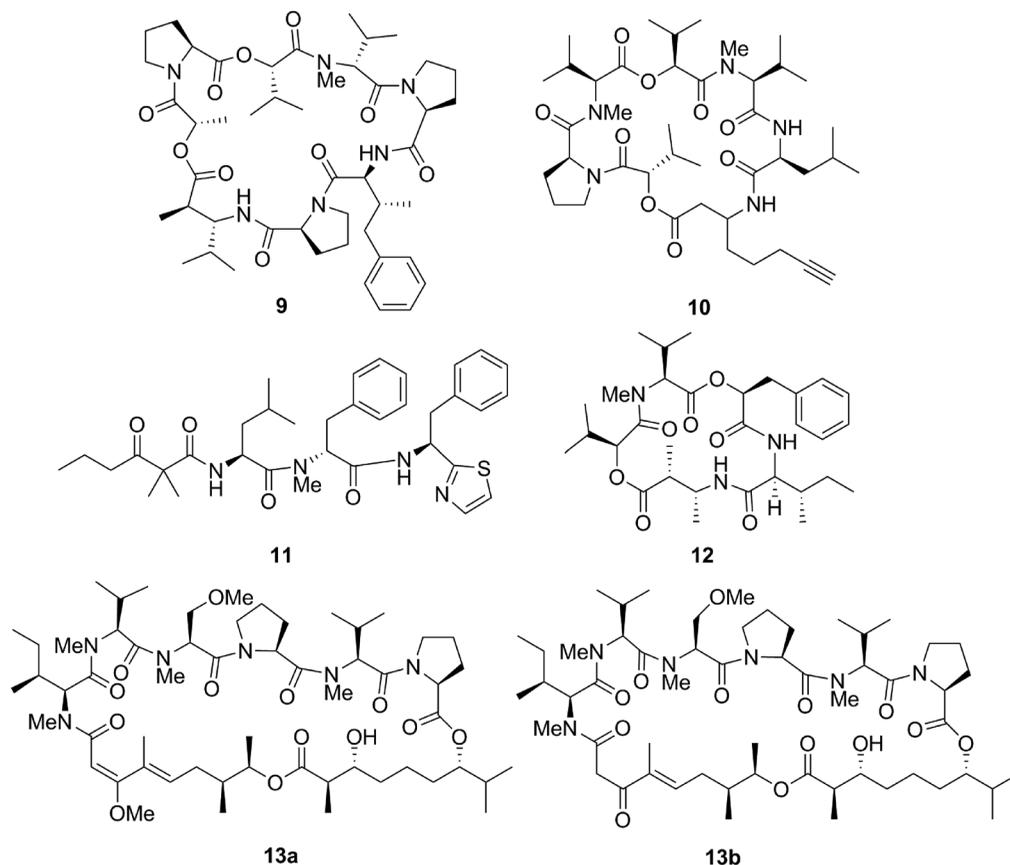
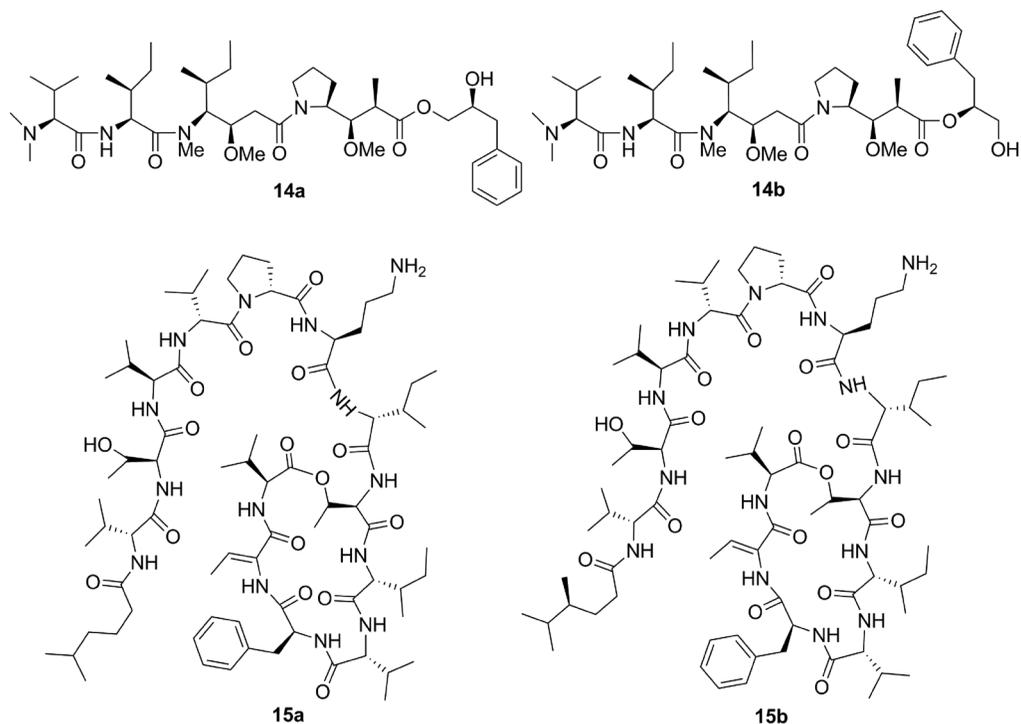


Figure 5. Chemical structures of dolastatin 16 (**9**), dolastatin 17 (**10**), dolastatin 18 (**11**), dolastatin D (**12**), dolastatin G (**13a**), and nordolastatin G (**13b**).

The tubulin-binding sites of colchicine, taxol, vinblastine, rhizoxin F, and maytansine are discussed by Prota et al.<sup>212</sup> and in the commentary by Field et al.<sup>213</sup>

Dolastatin 10 displays *in vivo* anticancer activity in various models, including, for example, MDR diffuse large cell lymphoma WSU-DLCL2,<sup>214</sup> SCLC NCI-H446<sup>215</sup>, and ovarian carcinoma<sup>216</sup> xenografts. Dolastatin 10 also displayed anticancer activity in various cancer models at the NCI: mouse P388 and L1210 leukemia, B16 melanoma and M5076 sarcoma as well as human LOX melanoma, and MX-1 breast cancer xenografts.

In addition to soblidotin, the modification of C-terminus of dolastatin 10 yielded a series of analogs called auristatins.<sup>217</sup> However, these were also ineffective in clinical trials.<sup>200</sup> An important discovery was made by Miyazaki et al.,<sup>218</sup> who found that the removal of one *N*-methyl group of dolavaline at the N-terminus of dolastatin 10 gives an analog showing only slightly attenuated potency. With this secondary amine at their N-terminus free, the auristatins became suitable for attachment of a linker to facilitate its conjugation to monoclonal antibodies, leading to the generation of highly potent and efficacious antibody drug conjugates (ADCs). This led, for example, to the FDA-approved ADC brentuximab vedotin (SGN-35, Adcetris®; **1d**; Fig. 3).<sup>219</sup> Vedotin is monomethyl auristatin E chemically conjugated to the chimeric anti-CD30 antibody.<sup>220</sup> It was approved for the treatment of relapsed Hodgkin lymphoma and systemic anaplastic large-cell lymphoma.<sup>221</sup> Over 30 ADCs in clinical trials currently employ auristatins as payloads. The reader is referred to recent reviews on this subject.<sup>166, 200, 219, 222</sup>



**Figure 6.** Chemical structures of dolastatin H (**14a**), isodolastatin H (**14b**), kahalalide F (**15a**), and elipsidepsin (**15b**).

After Pettit et al. published the structure of dolastatin 15 (**2a**; Fig. 3; Table II), also isolated from *D. auricularia*,<sup>223</sup> several groups developed its total synthesis.<sup>224,225</sup> The supply of synthetic dolastatin 15 has allowed its biological evaluation. Dolastatin 15 also interacts with tubulin and may bind in the vinca domain of tubulin, presumably in the same site as dolastatin 10.<sup>226</sup> It induces a loss of tension across the kinetochore pairs due to the disruption of normal microtubule assembly dynamics.<sup>227</sup> Beckwith et al.<sup>228</sup> reported that dolastatin 10 and 15 display *in vitro* antiproliferative activities that are three to four orders of magnitude more potent than vincristine, a clinically used antiproliferative agent, while dolastatin 15's NCI-based GI<sub>50</sub> is about ten times higher than that of dolastatin 10 (2 vs. 0.2 nM). Dolastatin 15 was also slightly inferior in terms of *in vivo* anticancer activity in a human ovarian carcinoma xenografted model.<sup>216</sup> Dolastatin 15 is a classical inducer of apoptosis in cancer cells and thus behaves as a conventional proapoptotic cytotoxic agent.<sup>229,230</sup> Furthermore, it is not able to overcome the MDR phenotype mediated by the P-glycoprotein (P-gp) in cancer cells.<sup>216</sup>

Like for dolastatin 10, a series of SAR studies have been undertaken with the focus on structural and stereochemical modification at the C terminus (Hiva-Dpy).<sup>217</sup> These studies led to the discovery of tasidotin, which was advanced to clinical trials. Tasidotin (ILX-651; **2b**; Fig. 3) is a peptide analog of dolastatin 15 in which the carboxyl-terminal ester group is replaced by the *tert*-butyl amide.<sup>231</sup> Tasidotin, which is orally active, reduces the shortening rate, the switching frequency from growth to shortening (catastrophe frequency), and the fraction of time the microtubules grow.<sup>232</sup> Tasidotin is a proapoptotic cytotoxic compound<sup>233</sup> and it is also a P-gp substrate<sup>234</sup> as all the other dolastatins. It showed promising *in vivo* anticancer activity in preclinical models of pediatric sarcomas,<sup>233</sup> LOX melanoma xenografts,<sup>234</sup> and xenograft models of breast cancer, ovarian cancer, prostate cancer, and colon cancer as reported in the

ILX-651 investigators brochure (Genzyme Corp., 2004) cited by Garg et al.,<sup>233</sup> but not in H460 NSCLC xenografts.<sup>234</sup> Tasidotin was also efficient *in vivo* in murine P388 leukemia model.<sup>233</sup> But as for soblidotin, the clinical development of tasidotin was not pursued beyond Phase II clinical trials because of lack of efficacy.

As mentioned above, all dolastatins seem to be proapoptotic agents unable to circumvent the MDR phenotype in cancer cells and they all displayed poor anticancer activity in Phase II clinical trials, leading to the discontinuation of their clinical evaluation.<sup>203</sup>

In 1981, Pettit et al.<sup>199</sup> claimed that dolastatin 1 (**3**; Fig. 3; Table II), which was isolated from *D. auricularia*, represented by that time the most potent anticancer agent discovered with an 88% life extension (at a dose of 11  $\mu\text{g}/\text{kg}$ ) in the murine P388 lymphocytic leukemia model, and a curative rate (33% at a dose of  $\sim 2 \mu\text{g}/\text{kg}$ ) in the murine B16 melanoma model. The NCI 60 cell-line panel had not been established yet at that time.<sup>115</sup>

Dolastatin 3 (**4**; Fig. 3; Table II), a cyclic peptide containing two thiazole rings, was isolated from a Japanese specimen of *D. auricularia* (Fig. 1D) and displayed the  $\text{GI}_{50}$  of  $< 1 \mu\text{M}$  in P388 murine leukemia cells.<sup>235</sup> Its full structure was confirmed by synthesis some years later.<sup>236</sup> This compound was also isolated from a Palauan collection of *Lyngbya majuscula*.<sup>237</sup> Dolastatin 3 induced *in vivo* a 78% life extension in the murine P388 lymphocytic leukemia model and a 52% life extension in murine colon carcinoma 38.<sup>237</sup>

Dolastatin 11<sup>238</sup> (**5**; Fig. 4; Table II), dolastatin 12<sup>238</sup> (**6**; Fig. 4; Table II), dolastatin 13<sup>239</sup> (**7**; Fig. 4; Table II), and dolastatin 14<sup>240</sup> (**8**; Fig. 4; Table II) are depsipeptides isolated from *D. auricularia*. Dolastatin 12 was also isolated from *L. majuscula/Schizothrix calcicola* cyanobacterial assemblages (Table II).<sup>241</sup> Dolastatin 11's mean  $\text{GI}_{50}$  was  $\sim 0.07 \mu\text{M}$  in the NCI 60 cell line panel and the COMPARE analysis with a cut-off correlation at  $p = 0.7$  was negative in the standard agent dataset. Dolastatin 12's  $\text{GI}_{50}$  values ranged from  $\sim 1$  (human NCI-H460 NSCLC) to  $\sim 30 \text{ nM}$  (human SF-295 CNS cancer) when assayed in five cancer cell lines.<sup>242</sup> Additionally, its  $\text{GI}_{50}$  values in mouse neuro-2a neuroblastoma<sup>243</sup> and P388 leukemia<sup>238</sup> cells were  $\sim 0.1$  and  $> 1 \mu\text{M}$ , respectively. Dolastatin 13's and 14's  $\text{GI}_{50}$  values were  $14 \text{ nM}$ <sup>239</sup> and  $20 \text{ nM}$ ,<sup>240</sup> respectively, in the murine P388 leukemia cell line.

While both dolastatin 10 and 15 affect tubulin as detailed above, dolastatin 11 targets actin and it displays about threefold higher growth inhibitory effects than the sponge-derived depsipeptide jasplakinolide.<sup>244</sup> Dolastatin 11 does not arrest cancer cells in mitosis, as do dolastatin 10 and 15, but induces massive rearrangement of the cellular actin filament network leading to dramatic cytoplasmic retraction and subsequently cell division arrest at the level of cytokinesis.<sup>244</sup> It binds actin at a site distinct from phalloidin,<sup>244</sup> which is a toxin from the mushroom *Amanita phalloides*<sup>245</sup> and one of the best known antiactin compounds.<sup>246</sup> Specifically, dolastatin 11 connects two long-pitch strands in F-actin to stabilize microfilaments.<sup>247</sup> Tubulin and actin are not only key players in normal and cancer cell division, but also in cancer cell invasion and metastasis.<sup>248</sup>

As reported above for dolastatin 11, dolastatin 12 also targets actin microfilaments.<sup>241</sup> A dolastatin 12 analog displayed only marginal or no *in vivo* anticancer activity against murine colon adenocarcinoma 38 and mammary adenocarcinoma 16/C and appeared to be toxic.<sup>241</sup>

Dolastatin 16 (**9**; Fig. 5; Table II) is a cyclic depsipeptide containing two new amino acids, dolamethylleuine, and dolaphenvaline collected from *D. auricularia* in Papua New Guinea.<sup>249</sup> The X-ray crystal structure of dolastatin 16 and syntheses of the new amino acid units dolamethylleuine and dolaphenvaline were published later.<sup>250</sup> Dolastatin 16 was also isolated from a Madagascan collection of *L. majuscula*<sup>251</sup> as well as from *Symploca cf. hydroides* collected off Guam.<sup>252</sup> Dolastatin 16's  $\text{GI}_{50}$  values were in low nanomolar ranges when assayed in a mini panel of four human solid cancer cell lines and in five leukemia cell lines.<sup>249</sup> Its mean  $\text{GI}_{50}$  in the NCI 60 cell line panel was  $\sim 0.3 \mu\text{M}$  and COMPARE

analyses provided relatively low correlations with dolastatin 10 ( $p = 0.76$ ) and dolastatin 15 ( $p = 0.71$ ).<sup>249,253</sup> The total synthesis of dolastatin 16 consisting of 23 steps was recently achieved.<sup>254</sup> Interestingly, Pettit et al.<sup>254</sup> report that the synthetic dolastatin 16, while otherwise identical (by X-ray crystal structure and spectral characteristics) with the natural product, did not reproduce the nanomolar cancer cell growth inhibition displayed by the natural isolate. Pettit et al.<sup>254</sup> reported that presumably this result can be attributed to the conformation(s) of the synthetic dolastatin 16 or to a chemically undetected component isolated with the natural product.

Additional dolastatins that have been isolated from *D. auricularia* are dolastatin 17<sup>255</sup> (**10**; Fig. 5), collected in Papua New Guinea specimens; dolastatin 18<sup>256</sup> (**11**, Fig. 5), collected in Indian Ocean specimens; as well as dolastatin D<sup>257</sup> (**12**; Fig. 5), dolastatin G<sup>258</sup> (**13a**; Fig. 5), nordolastatin G<sup>258</sup> (**13b**; Fig. 5), dolastatin H<sup>259</sup> (**14a**; Fig. 6) and isodolastatin H<sup>259</sup> (**14b**; Fig. 6) found in Japanese specimens.

Dolastatin 17 (**10**) is a cyclodepsipeptide with a novel acetylenic  $\beta$ -amino acid named dolayne (Doy), similar to that found in onchidin<sup>260</sup> (discussed in Section 4.D.2). Dolastatin 17's GI<sub>50</sub> values were in submicromolar ranges in the four cancer cell lines in which it was assayed.<sup>255</sup> Dolastatin 18 (**11**) is characterized by the presence of a thiazole ring in its structure. Its GI<sub>50</sub> values were in submicromolar ranges in the mouse P388 lymphocytic leukemia and the human NCI-H460 NSCLC cell lines.<sup>256</sup> Dolastatin 19 (**57**, for structure see Fig. 16), isolated from a different collection of *D. auricularia* specimens from the Gulf of California,<sup>261</sup> is a macrocyclic lactone that is strongly related to aurisides as illustrated in Section 4.D.3 (Table II). Dolastatin 19 displays *in vitro* growth inhibitory activity with GI<sub>50</sub> values of  $\sim 1 \mu\text{M}$  in breast MCF-7 and colon KM20L2 cancer cells.<sup>261</sup> Dolastatin D is a cyclic depsipeptide with a GI<sub>50</sub> value of  $\sim 4 \mu\text{M}$  in human HeLaS3 cancer cells.<sup>257</sup> Dolastatin G and nordolastatin G are cyclic depsipeptides with GI<sub>50</sub> values of  $\sim 1$  and  $\sim 5 \mu\text{M}$ , respectively, in human HeLaS3 cancer cells.<sup>258</sup> Dolastatin H and isodolastatin H, isolated from Western Indian Ocean specimens of *D. auricularia*, are linear peptides closely related to dolastatin 10.<sup>259</sup> Synthetic dolastatin H displayed a GI<sub>50</sub> value of 2 nM in human HeLaS3 cancer cells.<sup>259</sup> Isodolastatin H was evaluated *in vivo* against murine P388 leukemia and shown to exhibit antitumor activity that is slightly weaker (41% of life time extension as compared to control) than that of dolastatin 10 (55% of life time extension as compared to control).<sup>259</sup>

### C. Kahalalides

Many sacoglossan mollusks feed upon algae from which they acquire biologically active metabolites. For example, several species of *Elysia* sequester toxic compounds from *Bryopsis*.<sup>262</sup> In particular *Elysia rufescens* (Table I), *E. grandifolia*, and *E. ornata* were found to contain depsipeptides called kahalalides present also in *Bryopsis*, which are known to be responsible for the deterrent properties of the mollusks.<sup>263</sup> Davis et al.<sup>264</sup> report that sacoglossan mollusks are characterized by the ability to sequester functional chloroplasts from their algal diet through a process called kleptoplasty, enabling them to photosynthesize. These authors provided experimental evidence showing that a diverse bacterial assemblage is associated with *E. rufescens* and its mucus, with secreted mucus harboring higher bacterial richness than entire *E. rufescens* samples. Davis et al.<sup>264</sup> have thus shown that the most abundant bacterial groups affiliated with *E. rufescens* and its mucus are *Mycoplasma* spp. and *Vibrio* spp., respectively. They accordingly suggest that kahalalide F, found in *E. rufescens* (Table I), is possibly of bacterial origin.

Chemically, the size and composition of kahalalides are highly variable, ranging from a C31 tripeptide to a C77 tridecapeptide, and each peptide contains a different fatty acid chain.<sup>263</sup> Kahalalide family has reached more than 20 members, of which kahalalide F is the most

promising anticancer agent as detailed below. Gao and Hamann<sup>263</sup> comprehensively reviewed in 2011 the chemistry and biology of kahalalides.

Kahalalide F (**15a**; Fig. 6; Table II) is a cyclic peptide connected with an amidic bond to a short chain fatty acid. It was isolated from the herbivorous sacoglossan mollusk *E. rufescens* (Table I) living in the seas near Hawaii, and then from its green algal diet *Bryopsis pennata* (Bryopsidaceae).<sup>262</sup> Before describing its anticancer effects, we would like to draw the reader's attention to the following point. Some articles report on oncosis-like effects induced by kahalalide F in cancer cells, while others report on the induction of necrosis-like features. At first glance, it thus seems that kahalalide F could induce either oncosis or necrosis in cancer cells, while in fact this is not the case. Indeed, as clearly stated by Weerasinghe and Buja,<sup>265</sup> oncosis is a term that actually relates to a mode of cell injury and cell death, while necrosis relates to a cell population (tissue) degradation process following cell death. In other terms, necrosis should not be used to describe a specific death process occurring in single cells. Weerasinghe and Buja<sup>265</sup> comprehensively reviewed the oncosis-related characteristics at both morphological and biochemical levels and they define that oncotic cell death involves progressive membrane injury involving three stages that they detail in their review. These authors thus define oncosis as a form of cell death accompanied by cellular swelling, organelle swelling, blebbing, and increased membrane permeability caused by the failure of various ionic pumps in the plasma membrane, with increases in concentrations of cytosolic calcium and rearrangement of cytoskeletal proteins.<sup>265</sup>

The fact remains that (i) oncosis shares no common signaling pathways with apoptosis,<sup>265</sup> (ii) lysosomal targeting can lead to cell death types other than oncosis,<sup>266–268</sup> and (iii) altered cancerous lysosomes are involved in promoting cancer progression of metastatic disease.<sup>267</sup> Inducing oncosis in cancer cells would thus make it possible to overcome the resistance to proapoptotic stimuli displayed by cancer cells associated with aggressive biological behaviors.<sup>65</sup> Suárez et al.<sup>269</sup> accordingly observed that kahalalide's F cytotoxicity did not correlate with the expression level of the MDR1 efflux pump. In fact, García-Rocha et al.<sup>270</sup> already reported two decades ago that kahalalide F kills cancer cells via the targeting of lysosomes. Suárez et al.<sup>269</sup> then reported that kahalalide F induces oncosis in human prostate and breast cancer cells. Suárez et al.<sup>269</sup> thus suspected that kahalalide F does not induce apoptosis in cancer cells. This hypothesis was then experimentally confirmed by Janmaat et al.,<sup>271</sup> who observed that several markers of caspase-dependent apoptosis, such as phosphatidyl-serine externalization, cytochrome c release, and caspase-3 and poly-(ADP-ribose) polymerase cleavage were negative after exposure of cancer cells to kahalalide F. Also, inhibitors of caspases or cathepsins failed to protect cancer cells against kahalalide's F cytotoxicity. Janmaat et al.<sup>271</sup> identified ErbB3, a downstream molecule of the PI3K-Akt pathway, as an important determinant of the cytotoxic activity of kahalalide F *in vitro*. Appert-Collin et al.<sup>272</sup> have recently reviewed the roles of ErbB receptors in cancer cell migration and invasion, while Mayer and Arteaga<sup>273</sup> comprehensively reviewed the PI3K-Akt pathway as a target for cancer treatment.

Pardo et al.<sup>274</sup> reported that kahalalide F was found to be COMPARE-negative when it was tested in the NCI 60 cell line panel. Kahalalide's F GI<sub>50</sub> values in the NCI 60 cell line panel ranged between 0.2 and 10  $\mu\text{M}$ , with hormone-independent prostate cancer cells being most sensitive to this compound.<sup>274</sup> Ling et al.<sup>275</sup> report that kahalalide F was then shown to have a strong correlation between cytotoxicity and high c-erbB2 mRNA expression levels.<sup>275</sup>

Suárez et al.<sup>269</sup> reported that normal cells are 5–40 times less sensitive to kahalalide F than cancer cells. *in vivo*, kahalalide F demonstrated activity against human prostate hormone-independent xenograft models<sup>274, 276, 277</sup> and in the hollow fiber assay.<sup>263</sup>

Kahalalide F underwent several clinical trials in oncology and entered Phase II but without much success.<sup>278</sup> For example, Martín-Algarra et al.<sup>279</sup> evaluated the antitumor response of

kahalalide F in advanced malignant melanoma patients in a Phase II study; however, while kahalalide F was well tolerated by the patients, this trial was closed after the first stage because of the lack of an objective response. Iso-kahalalide F, a regioisomer of kahalalide F, also entered Phase II clinical trials for liver cancer, melanoma, and NSCLC patients, but failed to be effective as well.<sup>280</sup>

Elisidepsin (PM02734, Irvalec®; **15b**; Fig. 6) is a synthetic marine-derived cyclic peptide of the kahalalide F family that also entered clinical development.<sup>281–283</sup> Its GI<sub>50</sub> values ranged between 0.4 and ~9  $\mu\text{M}$  in a panel of 23 cancer cell lines from breast, colon, head and neck, lung, ovary, pancreas, prostate, and melanoma origins.<sup>281</sup> Herrero et al.<sup>284</sup> showed that elisidepsin induces necrosis-like cell death in yeast *Saccharomyces cerevisiae* used as a model system. They also demonstrated that the cell membrane and, in particular, cell membrane components like 2-hydroxy fatty acid-containing ceramides, are important for elisidepsin's activity. Elisidepsin-induced necrosis-like cell death was also experimentally shown by Váradi et al.<sup>285</sup> and also recently by Molina-Guijarro et al.,<sup>286</sup> who demonstrated that elisidepsin interacts directly with glycosylceramides in the plasma membrane of cancer cells. Kiraly et al.<sup>287</sup> observed that hypoxia reduces the efficiency of elisidepsin by inhibiting hydroxylation and altering the structure of lipid rafts. In addition, Teixido et al.<sup>288</sup> reported that acquired resistance to elisidepsin in cancer cells is associated with a switch to the epithelial-mesenchymal transition (EMT) state, which may be seen as a hallmark of cancer progression to the invasive and metastatic disease.<sup>289</sup> This compound displayed *in vivo* anticancer activity against human melanoma, liver, pancreas, breast, and prostate cancer xenografts.<sup>290</sup>

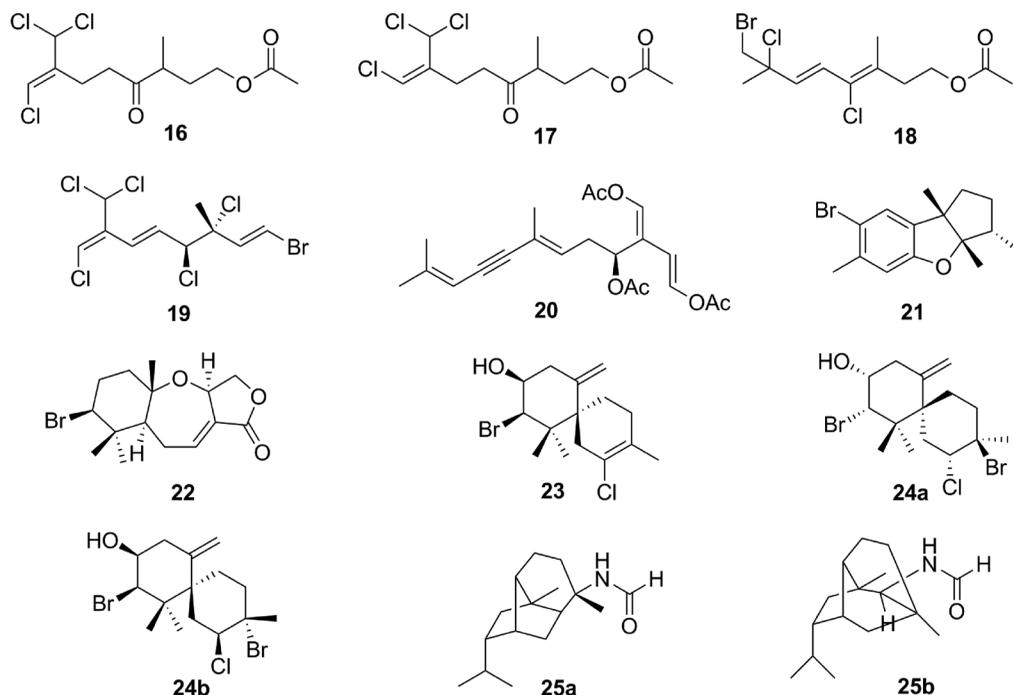
Clinical trials with elisidepsin have so far produced disappointing results. For example, while elisidepsin presented an acceptable safety profile, it was not recommended for further evaluation in advanced or metastatic gastroesophageal cancer due to the absence of activity.<sup>291</sup> Similar conclusions were reached by Goldwasser et al.<sup>282</sup> for other types of malignancy. Other members of the kahalalide family that display cytotoxic activity against cancer cells are described in the comprehensive review by Gao and Hamann.<sup>263</sup>

#### D. Potentially Promising Anticancer Agents Derived from Mollusks

Compounds here considered do not include proteins and other substances with molecular weight higher than 2000 amu. Although we made a significant effort to find mollusk-derived compounds showing *in vitro* growth inhibitory activity against cancer cell lines at a concentration of < 10  $\mu\text{M}$ , we are cognizant that some substances could have escaped our attention. Compounds under review are listed alphabetically in Table II along with their possible origin if described in the literature. The information on classification, distribution, and alimentary habits of the mollusks under review is summarized in Table I, while Figure 1 shows photographs of selected species. All the NCI data that we report below (if available) were obtained on the public NCI website at [https://dtp.cancer.gov/databases\\_tools/data\\_search.htm](https://dtp.cancer.gov/databases_tools/data_search.htm).

##### 1. Terpenes and Steroids

Unusual acetates of halogenated monoterpenes have been characterized from a Spanish population of *Aplysia punctata* (Fig. 1B; Table I). Among them, compounds **16–18** (Fig. 7; Table II under halogenated monoterpenes) displayed GI<sub>50</sub> values ranging between 4 and 10  $\mu\text{M}$  in four cancer cell lines.<sup>292</sup> Another linear halogenated monoterpene (**19**; Fig. 7; Table II) was isolated from two specimens of *Aplysia dactylomela* (Fig. 1A; Table I) collected from different sites along the Spanish coast.<sup>293</sup> This compound displayed growth inhibitory effects against HM02 (gastric carcinoma), HEP-G2 (liver carcinoma), and MCF-7 (breast carcinoma) cancer cells with the GI<sub>50</sub> values of ~3  $\mu\text{M}$ .<sup>293</sup>



**Figure 7.** Chemical structures of acetates of halogenated monoterpenes (compounds **16-19**), caulerpenyne (**20**), (-)-aplysin (**21**), aplystatin (**22**), elatol (**23**), obtusol (**24a**), isoobtusol (**24b**), 1-formamido-10(1→2)-abeopupukeanane (**25a**), and 2-formamidopupukeanane (**25b**).

Caulerpenyne (**20**; Fig. 7; Table II) is a sesquiterpene possessing a 1,4 diacetoxy-butadiene moiety found in algae of genus *Caulerpa* and in several sacoglossan mollusks (including *Elysia subornata*; Fig. 1E; Table I) that feed on these organisms.<sup>294,295</sup> The average  $GI_{50}$  for caulerpenyne is  $\sim 10 \mu\text{M}$  in various cancer cell lines assayed in academic laboratories.<sup>296-298</sup> The  $GI_{50}$  value reported by the NCI for caulerpenyne in the 60 cell line panel is  $\sim 40 \mu\text{M}$  and a COMPARE analysis with a cut-off correlation at  $p = 0.7$  is negative in the standard agent dataset. No selectivity was noted with respect to a given histological type of cancer cell lines in this panel. In the same manner, caulerpenyne does not display bioselectivity between normal (hamster fibroblasts, human keratinocytes, and melanocytes) and cancer cells.<sup>298,299</sup> Barbier et al.<sup>298</sup> reported that this compound induces aggregation of tubulin, which may be responsible for the inhibition of tubulin polymerization and bundling of residual microtubules. Bourdrion et al.<sup>300</sup> then showed that it does not bind to colchicine, taxol, and vinca-alkaloid binding domains. Caulerpenyne blocks the stimulation of mitogen-activated protein kinase (MAPK), which participates in the control of cell functions such as proliferation, differentiation, and death.<sup>298,301</sup>

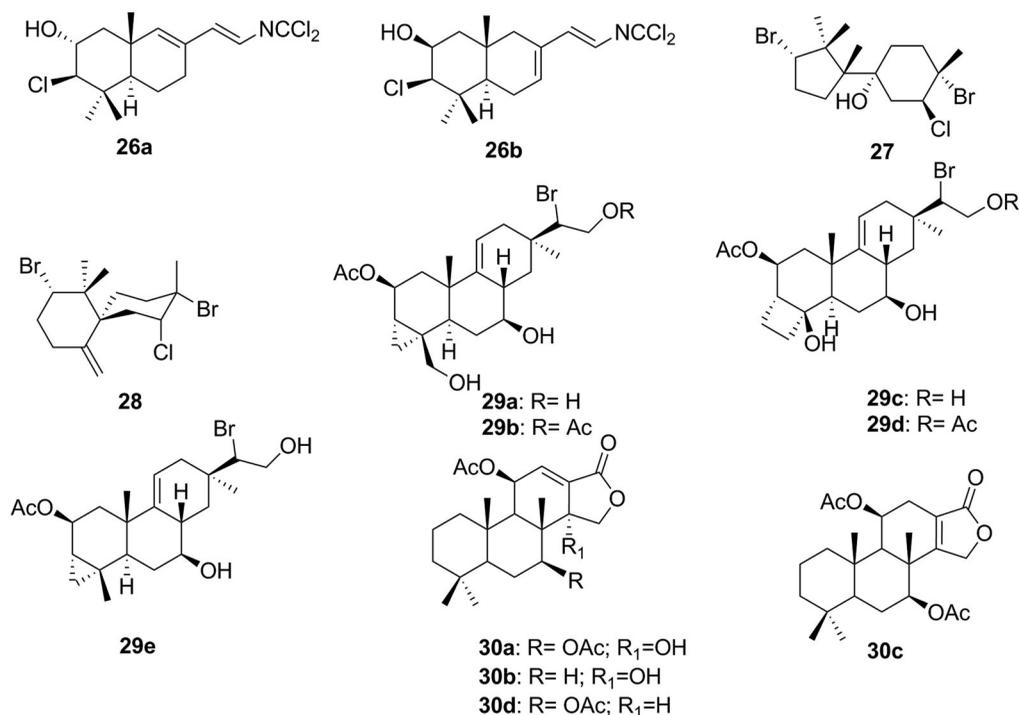
One of the first halogenated metabolites isolated from a marine source was (-)-aplysin (**21**; Fig. 7; Table II), which was found in the anaspidean mollusk *Aplysia kurodai* (Table I).<sup>302</sup> Although this algal dietary sesquiterpene was originally described as an antifeedant, later it was reported to induce growth inhibitory effects in various cancer cell lines with  $GI_{50}$  values ranging between 4 and  $8 \mu\text{M}$ .<sup>303</sup> The mean  $GI_{50}$  value reported by the NCI in the 60 cell line panel was  $\sim 30 \mu\text{M}$  and a COMPARE analysis with a cut-off correlation at  $p = 0.7$  was negative in the standard agent dataset. No selectivity was shown with respect to a given histological type of cancer cell lines in the NCI panel. Aplysin did not display *in vivo* anticancer activity in the mouse P388 leukemia model used by the NCI. However, it displayed marginal effects in the human

A549 NSCLC xenograft with 18% of tumor growth reduction as compared to the control.<sup>79</sup> Liu et al.<sup>79</sup> showed that aplysin acts as a sensitizer for tumor necrosis factor related apoptosis inducing ligand (TRAIL) induced apoptosis in cancer cells and that these effects occurred via the P38 MAPK/survivin pathway. It sensitized TRAIL-induced *in vivo* anticancer activity in the human A549 NSCLC xenograft model.<sup>79</sup> As reported by Liu et al.,<sup>79</sup> TRAIL has served as a biological agent for cancer treatment for decades. Yet, clinical trials have not shown significant survival benefit in cancer patients. Great hopes are thus placed on innovative compounds that would be able to significantly sensitize apoptosis-resistant cancer cells to TRAIL. Aplysin also sensitizes the cytotoxic effects of the alkylating drug temozolomide against human glioma cells by increasing miR-181 expression, which acts as a glioma tumor suppressor.<sup>304</sup> In addition, aplysin (**21**) induces cell cycle arrest and apoptosis in glioma cells through the inhibition of the PI3K/Akt signaling, which plays important roles in glioma cell survival.<sup>304</sup> Glioblastoma, the most malignant form of glioma, displays dismal prognoses because of major mechanisms of resistance to pro-apoptotic stimuli.<sup>68</sup>

A 2-propanol extract of the sea hare *Aplysia angasi* (also named *A. dactylomela*; Fig. 1A; Table I) displayed growth inhibitory activity against murine lymphocytic leukemia P388 cells.<sup>305</sup> The active compound, aplysistatin (**22**; Fig. 7; Table II), was obtained by bio-guided fractionation, and identified as a brominated tricyclic 6-7-5-fused sesquiterpene, in which the seven-membered ring contains a bridging oxygen atom and the five-membered ring is a lactone. Aplysistatin displayed the GI<sub>50</sub> value of ~8 μM against human KB cancer (an oral cancer cell line but cross-contaminated by HeLa cervix carcinoma cells<sup>115</sup>) and mouse P388 leukemia cells.<sup>305</sup>

Several halogenated chamigrene sesquiterpenes were isolated from *A. dactylomela* (Fig. 1A; Table I) collected off Canary Islands.<sup>293</sup> Among these metabolites of dietary algal origin are elatol (**23**; Fig. 7; Table II) and obtusol (**24a**; Fig. 7; Table II). Elatol was first isolated from *Laurencia elata*.<sup>306</sup> Lang et al.<sup>307</sup> report that several species of *Laurencia* (red algae) produce elatol as a major secondary metabolite, especially *Laurencia microcladia* from which elatol was obtained with a yield of ca. 10% (w/w) from the ethanolic extract of the algae. Isoobtusol (**24b**; Fig. 7; Table II) was isolated for the first time from *Laurencia obtusa*.<sup>308</sup> Elatol displays GI<sub>50</sub> values ranging between 1 and 10 μM against about ten cancer cell lines that were assayed by various academic laboratories.<sup>293,309,310</sup> While normal Vero (African green monkey kidney) cells displayed weaker sensitivity to elatol-induced growth inhibitory effects (GI<sub>50</sub> = 25 μM),<sup>309</sup> normal L929 murine fibroblasts displayed sensitivity similar to cancer cells (GI<sub>50</sub> = 1 μM).<sup>310</sup> Elatol cannot therefore be considered as a bioselective compound. It is a cytotoxic and proapoptotic compound in murine B16F10 melanoma cells through a decrease in Bcl-x and an increase in Bak, caspase-9, and p53 expression.<sup>310</sup> B16F10 melanoma cells are known to be very sensitive to proapoptotic stimuli.<sup>119,311</sup> It is therefore not surprising that elatol decreased *in vivo* the growth of B16F10 melanoma growing subcutaneously (s.c.) in syngeneic mice.<sup>310</sup> It would interesting to know how efficient elatol is against B16F10 melanoma cells that are intravenously transplanted in syngeneic mice and lead to aggressive lung pseudometastases.<sup>311</sup> Indeed, the murine B16F10 melanoma model is not at all representative of the great majority of human melanomas associated with dismal prognoses that display marked resistance to proapoptotic stimuli.<sup>67</sup> Obtusol and isoobtusol display weaker *in vitro* growth inhibitory activity than elatol.<sup>293,307,309</sup>

Two sesquiterpenes named 1-formamido-10(1→2)-abeopupukeanane (**25a**; Fig. 7; Table II under pupukeanane derivatives) and 2-formamidopupukeanane (**25b**; Fig. 7; Table II) were recently isolated and chemically characterized from the nudibranch *Phyllidia coelestis* (Fig. 1L; Table I).<sup>312</sup> As emphasized by Jaisamut and colleagues,<sup>312</sup> tubercle nudibranchs of the genus *Phyllidia* graze on sponges and can sequester specific sponge metabolites. For example,



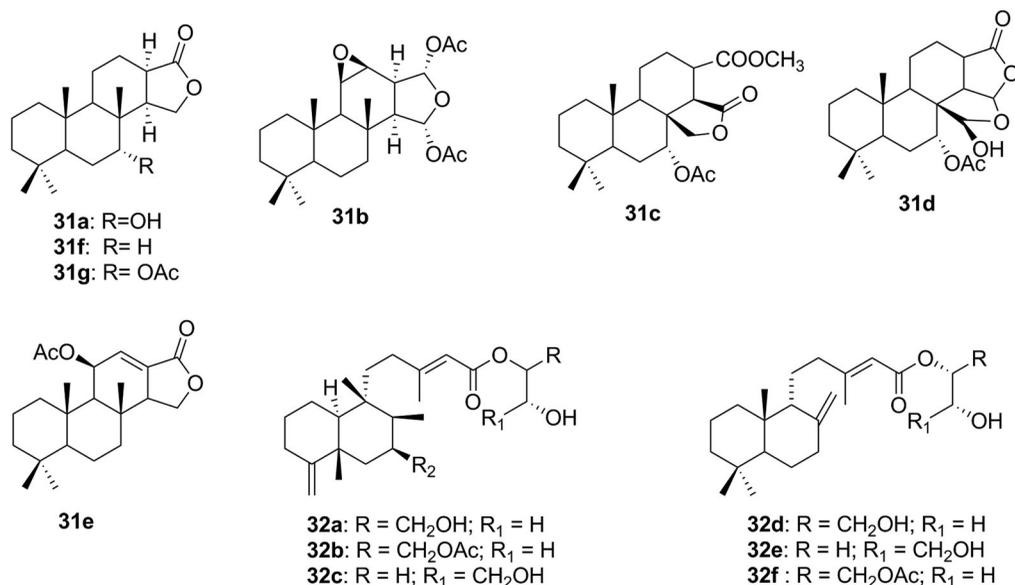
**Figure 8.** Chemical structures of reticulidins A (**26a**) and B (**26b**), oculiferane (**27**), *epi*-obtusane (**28**), parguerol (**29a**), parguerol-16-acetate (**29b**), isoparguerol (**29c**), isoparguerol-16-acetate (**29d**), deoxyparguerol (**29e**), and dorisone A (**30a**), B (**30b**), C (**30c**), and D (**30d**).

*P. varicosa* secretes in its mucus 9-isocyanopopukeanane that is lethal to crustaceans and arises from its sponge prey *Ciocalypta* sp. (ex. *Hymeniacion* sp.). Both compounds **25a** and **25b** display *in vitro* growth inhibitory activity in the range between  $\sim 0.1$  and  $\sim 7 \mu\text{M}$  in four distinct human cancer cell lines.<sup>312</sup> Also, both of them display some levels of bioselectivity. Specifically, they inhibited by 65 and 25%, respectively, the growth of human gingival fibroblasts when assayed at  $5 \mu\text{M}$ .<sup>312</sup>

Reticulidin A (**26a**; Fig. 8; Table II) and B (**26b**; Fig. 8; Table II) are two carbonimidic chlorinated sesquiterpenes that have been isolated from the Okinawan nudibranch *Reticulidia fungia* (Table I).<sup>313</sup> The *in vitro* GI<sub>50</sub> values displayed by compounds **26a** and **26b** were  $\sim 1 \mu\text{M}$  in KB cells, and  $\sim 2$  and  $\sim 0.3 \mu\text{M}$  in mouse L1210 leukemia cells, respectively.<sup>313</sup> As mentioned above, tubercle nudibranchs of the genus *Phyllidia* feed on their sponge prey from the *Ciocalypta* genus,<sup>312</sup> and thus, the possibility remains that the nudibranch *R. fungia* also feeds on sponges, which could belong to *Pseudoaxinyssa pitys*, *Stylotella aurantium*, and *Axinyssa* sp. genera.<sup>313</sup>

Two novel halogenated sesquiterpenes were isolated from the digestive glands of the Egyptian sea hare *Aplysia oculifera* (Table I).<sup>314</sup> These two compounds, oculiferane (**27**; Fig. 8; Table II) and *epi*-obtusane (**28**; Fig. 8; Table II), displayed GI<sub>50</sub> values ranging between  $\sim 2$  and  $\sim 8 \mu\text{M}$  in a minipanel of five human cancer cell lines including the PC-3 prostate, A549 NSCLC, MCF-7 breast, HepG2 liver, and HCT116 colon cancer models.<sup>314</sup>

Parguerol (**29a**; Fig. 8; Table II), parguerol-16-acetate (**29b**; Fig. 8; Table II), isoparguerol (**29c**; Fig. 8; Table II), isoparguerol-16-acetate (**29d**; Fig. 8; Table II), and deoxyparguerol (**29e**; Fig. 8; Table II) are tricyclic monobromoditerpenes isolated from *A. dactylorella* (Fig. 1A; Table I).<sup>315</sup> The algal origin of these metabolites could be strongly suggested as they were isolated from the digestive gland of this anaspidean mollusk.<sup>315</sup> Indeed, these metabolites were

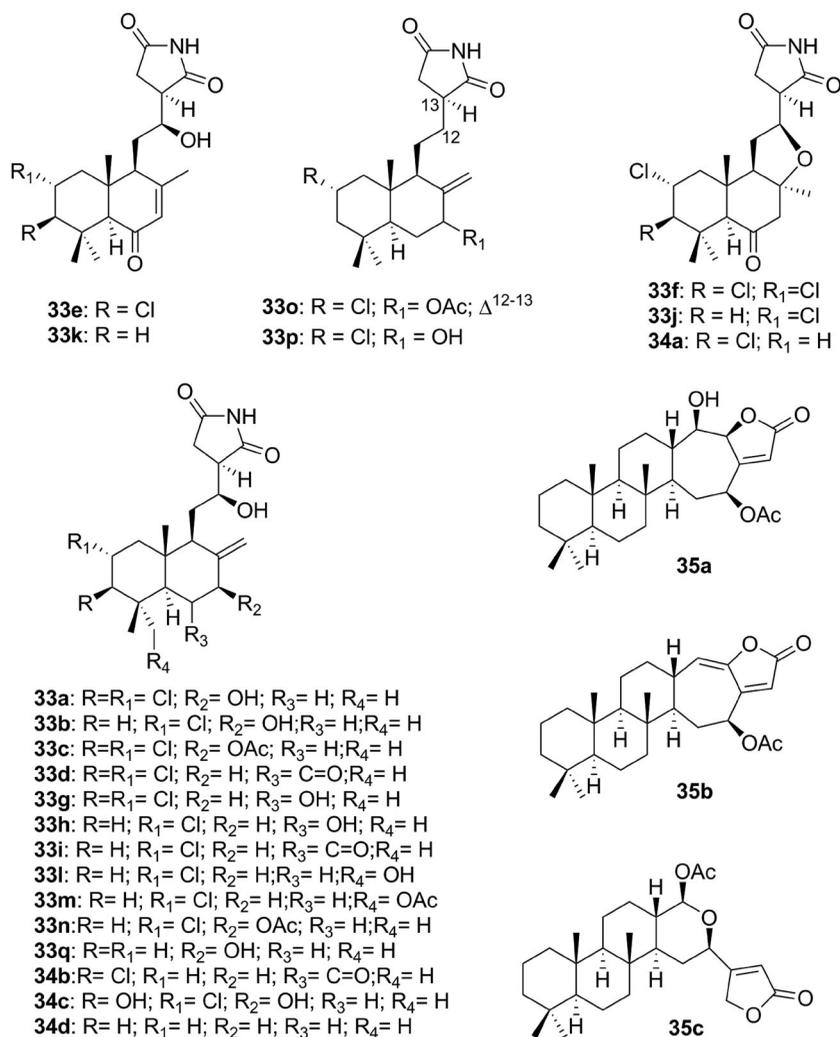


**Figure 9.** Chemical structures of the other spongian diterpenoids include 7 $\alpha$ -hydroxyspongian-16-one (**31a**), 15 $\alpha$ ,16 $\alpha$ -diacetoxy-11,12 $\beta$ -epoxyspongian (**31b**), 7 $\alpha$ -acetyxydendrillol-3 (**31c**), 7 $\alpha$ -acetoxy-17 $\beta$ -hydroxy-15,17-oxidospongian-16-one (**31d**), 11 $\beta$ -hydroxyspongi-12-en-16-one (**31e**), spongian-16-one (**31f**), 7 $\alpha$ -acetyxydendrillol-3 (**31g**), and palmadorins (A: **32a**; B: **32b**; D: **32c**; M: **32d**; N: **32e**; O: **32f**).

identified by Awad<sup>316</sup> in the red alga *Jania rubens* (L.) Lamx. collected from the Red Sea coast at Hurgada (Egypt) and it is known that some *Aplysia* species can feed on sponges.<sup>315</sup> It is interesting to note that the complex chemical mixtures obtained from *A. dactylomela* collected from la Parguera (Puerto Rico) versus the ones from Bimini (Bahamas) do not have any common components.<sup>315</sup> The five compounds displayed *in vitro* growth inhibitory activity in low micromolar ranges but they were tested on one cancer cell line only, the murine P388 leukemia,<sup>315</sup> which is highly sensitive to proapoptotic stimuli.<sup>317</sup> Awad<sup>316</sup> confirmed the data obtained by Schmitz et al.,<sup>315</sup> but once more on highly apoptosis-sensitive cancer cells, Ehrlich ascite carcinoma. Both Schmitz et al.<sup>315</sup> and Awad<sup>316</sup> showed that isoparguerol derivatives displayed slightly higher *in vitro* growth inhibition of cancer cells than those of parguerol.

A series of dorisenones (**30a-30d**; Fig. 8; Table II) and related spongian diterpenoids (**31a-31g**; Fig. 9; Table II) were isolated from the Japanese nudibranch *Chromodoris obsoleta* (also named *Goniobranchus obsoletus*; Table I).<sup>318</sup> Compounds **30a-30d** and **31a-31g** displayed GI<sub>50</sub> values ranging between submicromolar and low micromolar in mouse L1210 leukemia and human KB cells.<sup>318</sup> Compounds **31d** and **31g** were assayed *in vivo* in the P388 leukemia model but displayed no activity.<sup>318</sup>

A chemical study of the Western Antarctic Peninsula nudibranch *Austrodoris kerguelensis* (also named *Doris kerguelensis*; Table I) led to the characterization of a series of about 20 clerodane, labdane, and halimane diterpene glyceride esters, named palmadorins (palmadorin A to palmadorin Q).<sup>319,320</sup> Palmadorin A (**32a**), B (**32b**), D (**32c**), M (**32d**), N (**32e**), and O (**32f**) (Fig. 9; Table II) inhibit human erythroleukemia (HEL) cell proliferation with low micromolar GI<sub>50</sub> values, while palmadorin M was shown to inhibit JAK2, STAT5, and Erk1/2 activation in HEL cells, and cause apoptosis at 5  $\mu$ M. These HEL cells represent the AML-M6 model of acute erythroleukemia, a rare (~3% of cases) form of acute myeloid leukemia (AML), in which erythroblastic precursors cause the myeloproliferation.<sup>320</sup>



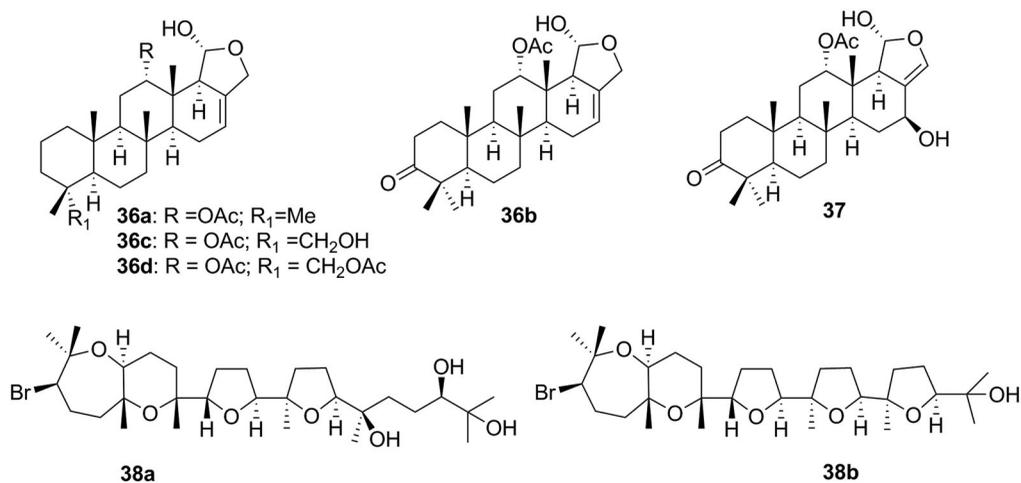
**Figure 10.** Chemical structures of dichlorolissoclimide (**33a**), chlorolissoclimide (**33b**), haterumaimides (A: **33c**; B: **33d**; C: **33e**; D: **33f**; E: **33g**; F: **33h**; G: **33i**; H: **33j**; I: **33k**; J: **33l**; K: **33m**; N: **33n**; O: **33o**; P: **33p**; Q: **33q**; L: **34a**; M: **34b**), 3β-hydroxychlorolissoclimide (**34c**), a lissoclimide-type alkaloid (**34d**), and inorolides A (**35a**), B (**35b**), and C (**35c**).

A series of interesting bioactive succinimide-containing labdane terpenoids have been isolated from ascidians of the genus *Lissoclinum* since 1991. Dichlorolissoclimide<sup>321,322</sup> (**33a**; Fig. 10; Table II) and chlorolissoclimide<sup>323</sup> (**33b**; Fig. 10; Table II) were reported from an ascidian collected in New Caledonia, whereas haterumaimides A-E<sup>324</sup> (**33c-33g**; Fig. 10; Table II), F-I<sup>325</sup> (**33h-33k**; Fig. 10; Table II), J-K<sup>326</sup> (**33l-33m**; Fig. 10; Table II), and N-Q<sup>327</sup> (**33n-33q**; Fig. 10; Table II) were all isolated from *Lissoclinum* ascidians collected off Hateruma Islands. In 2004, new haterumamide-type diterpenes, haterumamides L (**34a**; Fig. 10; Table II), M (**34b**; Fig. 10; Table II), and 3β-hydroxyllissoclimide (**34c**; Fig. 10; Table II) have been found in two Notaspidean mollusks, *Pleurobranchus albigitatus* and *P. forskalii*, along with chlorolissoclimide, dichlorolissoclimide, haterumamides B, D, and H by Fu et al.<sup>328</sup> As suggested by the authors,<sup>328</sup> these labdanes are presumably metabolites of a *Lissoclinum* species of the ascidian on which the mollusks feed.

Chlorolissoclimide (**33b**), dichlorolissoclimide (**33a**), and haterumaimide D (**33f**) displayed  $GI_{50}$  values of  $\sim 0.08$ ,  $\sim 0.08$ , and  $\sim 1 \mu M$  in the NCI 60 cell line panel, respectively, and **33a** and **33b** (but not **33f**) showed selectivity toward melanoma cell lines.<sup>328,329</sup> Chlorolissoclimide (**33b**) and dichlorolissoclimide (**33a**) also displayed marked growth inhibitory effects in the Corbett–Valeriotte soft agar disk diffusion assay, while they showed no solid tumor selectivity.<sup>328</sup> In contrast, while less active than **33b** and **33a**,  $3\beta$ -hydroxychlorolissoclimide (**33c**) displayed solid tumor selectivity (thus potential bioselectivity) in this soft agar assay.<sup>328</sup> The Corbett–Valeriotte soft agar disk diffusion assay detects differences in zones of inhibition between a solid tumor cell line (Colon38, ColonH116, LangH125) and either leukemia (L1210 or CEM) or normal (CFU-GM) cells.<sup>330</sup>

On the other hand,  $GI_{50}$  values for haterumaimides J (**33l**) and K (**33m**), haterumaimides C (**33e**), G (**33i**), and I (**33k**) range between 0.5–1 nM (**33l** and **33m**) and  $>20 \mu M$  (**33e**, **33i** and **33k**).<sup>327</sup> Uddin et al.<sup>327</sup> report that based on an SAR analysis, it appears that the presence of hydroxyl groups at C-6, C-7, C-12, and C-18; the chlorine atom at C-2; and the imido NH in ring C are very important for haterumaimide-induced *in vitro* growth inhibitory effects in P388 leukemia cells. A recent synthesis of lissoclimide-type compounds has been published by Gonzalez et al.<sup>331</sup> One of these synthesized compounds, specifically **34d**, displayed  $GI_{50}$  values of  $\sim 12 \mu M$  in human HeLa cervix epithelia carcinoma and Junket acute lymphoblastic leukemia cells, while the  $GI_{50}$  value of compound **34d** was  $\sim 80 \mu M$  in normal Vero (African green monkey [*Cercopithecus aethiops*] kidney) cells, suggesting bioselectivity properties for compound **34d**. The synthesis of chlorolissoclimide (**33b**) was published by Quinn et al in 2016.<sup>332</sup> Chlorolissoclimide (**33b**) and dichlorolissoclimide (**33a**) are active against murine P388 leukemia cells resistant to adriamycin.<sup>328,333</sup> Robert et al.<sup>334</sup> demonstrated that chlorolissoclimide (**33b**) and dichlorolissoclimide (**33a**) exert their growth inhibitory effects through the blockade of translation elongation by inhibiting translocation, leading to the accumulation of ribosomes on mRNA. At concentrations relevant to the marked protein synthesis inhibition, DNA synthesis and RNA transcription are slightly or not affected.<sup>334</sup> Robert et al.<sup>334</sup> also reported that chlorolissoclimide (**33b**) does not induce a loss of polysomes in contrast to other inhibitors of elongation, such as phyllanthoside and nagilactone C. These authors emphasize that (i) the ribosome recruitment phase of translation initiation is usurped in many human cancers, (ii) several inhibitors of elongation have been previously tested as anticancer agents in preclinical animal models and clinical trials, and (iii) the selectivity of these general inhibitors for cancer cells may stem from the fact that cancer cells have higher translation rates than do normal cells.<sup>334</sup> One protein synthesis inhibitor, the *Cephalotaxus* alkaloid homoharringtonine (an inhibitor of translation elongation), has been approved by the FDA for the treatment of adult patients with chronic myeloid leukemia displaying resistance and/or intolerance to two or more tyrosine kinase inhibitors.<sup>335</sup> The clinical data obtained with this compound have been recently reviewed by Heiblig et al.<sup>336</sup> and Kantarjian et al.<sup>337</sup>

Several sesterterpenoids were isolated from Japanese specimens of the nudibranch *Chromodoris inornata* (also named *C. aspersa*; Table I).<sup>338</sup> These compounds were assayed *in vitro* for growth inhibition against the human KB and the murine L1210 leukemia cell lines.<sup>338</sup> These compounds and their  $GI_{50}$  values are inorolide A (**35a**; Fig. 10; Table II;  $GI_{50} = \sim 7 \mu M$ ), inorolide B (**35b**; Fig. 10; Table II;  $GI_{50} = \sim 5 \mu M$ ), inorolide C (**35c**; Fig. 10; Table II;  $GI_{50} = \sim 4 \mu M$ ), deoxoscalarin (**36a**; Fig. 11; Table II;  $GI_{50} = \sim 3 \mu M$ ), and several of its analogues, namely deoxoscalarin-3-one (**36b**; Fig. 11; Table II under sesterterpenoids;  $GI_{50} = \sim 2 \mu M$ ), 21-hydroxydeoxoscalarin (**36c**; Fig. 11; Table II;  $GI_{50} = \sim 9 \mu M$ ), 21-acetoxydeoxoscalarin (**36d**; Fig. 11; Table II;  $GI_{50} = \sim 1 \mu M$ ), and 12-*O*-acetyl-16-*O*-deacetyl-12,16-episcalarolbutenolide (**37**; Fig. 11; Table II under sesterterpenoids;  $GI_{50} = \sim 5 \mu M$ ).<sup>338</sup> Some of these compounds have also been isolated from marine sponges from the *Hyrtios* genus.<sup>338</sup> We recently reviewed the anticancer activity associated with sesterterpenoids from different origins.<sup>339</sup>

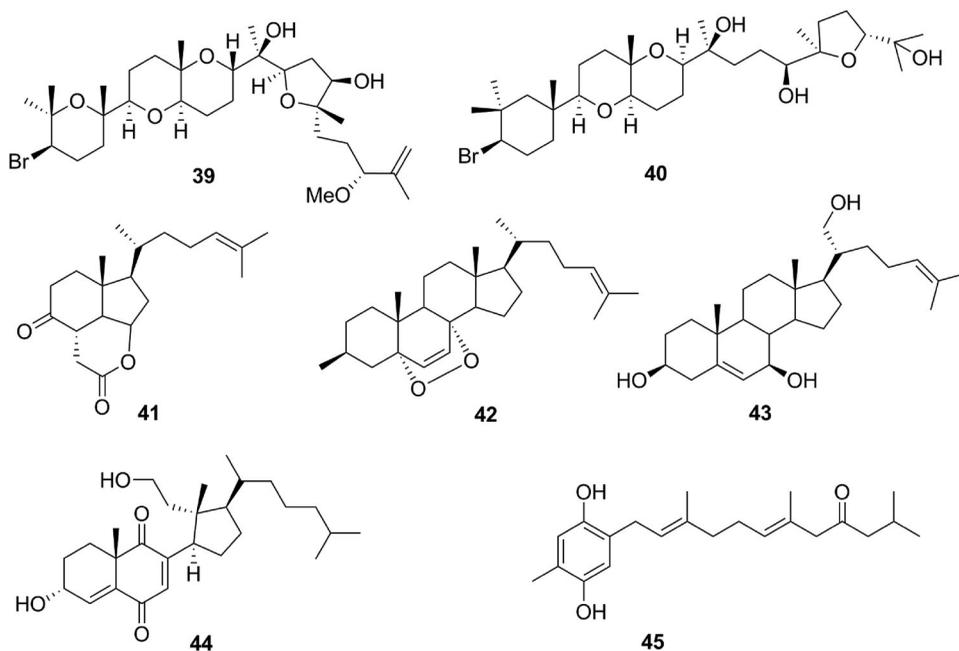


**Figure 11.** Chemical structures of deoxoscalarin (**36a**) and several of its analogues, that is, deoxoscalarin-3-one (**36b**), 21-hydroxydeoxoscalarin (**36c**), and 21-acetoxydeoxoscalarin (**36d**), 12-O-acetyl-16-O-deacetyl-12,16-episcalarolbutenolide (**37**), aurilol (**38a**), and enshuol (**38b**).

The bromotriterpene aurilol (**38a**; Fig. 11; Table II) was isolated from the digestive gland of the sea hare *D. auricularia* (Fig. 1D; Table I), with a GI<sub>50</sub> value of ~7 μM against human HeLa S3 cancer cells.<sup>340</sup> Aurilol is related to enshuol (**38b**; Fig. 11; Table II), a bromo triterpenic polyether with a dioxabicyclo(5.4.0) undecane ring system, isolated from the algae *Laurencia*, suggesting that **38a** is most likely dietary.<sup>340</sup> The complete synthesis of aurilol (**38a**) was accomplished in 2005 by Morimoto et al.<sup>341</sup>

The bromotriterpene polyether aplysqualenol A (**39**, Fig. 12; Table II) has been isolated from the Caribbean sea hare *A. dactylomela* (Fig. 1A; Table I).<sup>342</sup> It was submitted to the NCI one-dose primary assay in the cancer 60 cell line panel and found to display GI<sub>50</sub> values of ~0.4 μM in the human SNB-19 central nervous system cancer and the T-47D breast cancer cell lines.<sup>342</sup> The GI<sub>50</sub> values were >10 μM in the remaining cancer cell lines.<sup>342</sup> Vera et al.<sup>343</sup> discovered that aplysqualenol is a ligand for the light chain of dynein type 1 (DYNLL1), which is suggestive of potential development of small-molecule regulators of the dynein complex<sup>344</sup> with applications in cancer treatment.<sup>345,346</sup>

Thyrsiferol (**40**; Fig. 12; Table II) is a brominated triterpene polyether first isolated from the tropical marine red algae *Laurencia thyrsifera* J. Agardh (Rhodomelaceae),<sup>347</sup> and more recently also found in the sea hare *A. dactylomela* (Fig. 1A; Table I) from the South China Sea.<sup>348</sup> Mahdi et al.<sup>349</sup> stated that thyrsiferol displays potent *in vitro* growth inhibitory activity in the mouse P388 leukemia cell line, while Fernández et al.<sup>350</sup> found that this compound displayed moderate growth inhibitory activity in cell lines originating from solid tumors with GI<sub>50</sub> values of ~0.02 and ~17 μM in P388 and A549 NSCLC cancer cells, respectively.<sup>350</sup> It must be emphasized that P388 leukemia cells display dramatic sensitivity to proapoptotic stimuli,<sup>317</sup> while A549 NSCLC cells are moderately resistant.<sup>351</sup> These data thus point to the fact that thyrsiferol is indeed more active *in vitro* against apoptosis-sensitive cancer cells than their resistant counterparts. Mahdi et al.<sup>349</sup> showed that thyrsiferol inhibits hypoxia-induced HIF-1 activation in T47D human breast tumor cells and suppressed hypoxic induction of HIF-1 target genes (VEGF and GLUT-1) at the mRNA level.<sup>349</sup> They also reported that thyrsiferol suppressed mitochondrial respiration at complex I.



**Figure 12.** Chemical structures of aplysqualenol A (**39**), thysiferol (**40**), 3-*epi*-aplykurodinone B (**41**), an endoperoxide sterol (**42**), a C-21 hydroxylated sterol (**43**), a secosterol (**44**), and a prenylated hydroquinone (**45**).

The degraded sterol 3-*epi*-aplykurodinone B (**41**; Fig. 12; Table II) was isolated from a Spanish collection of *Aplysia fasciata* (Table I) and it displayed GI<sub>50</sub> values of ~8 μM in four cancer cell lines including mouse P388 leukemia, and human A549 NSCLC, HT-29 colon cancer, and SKMEI-28 melanoma.<sup>352</sup>

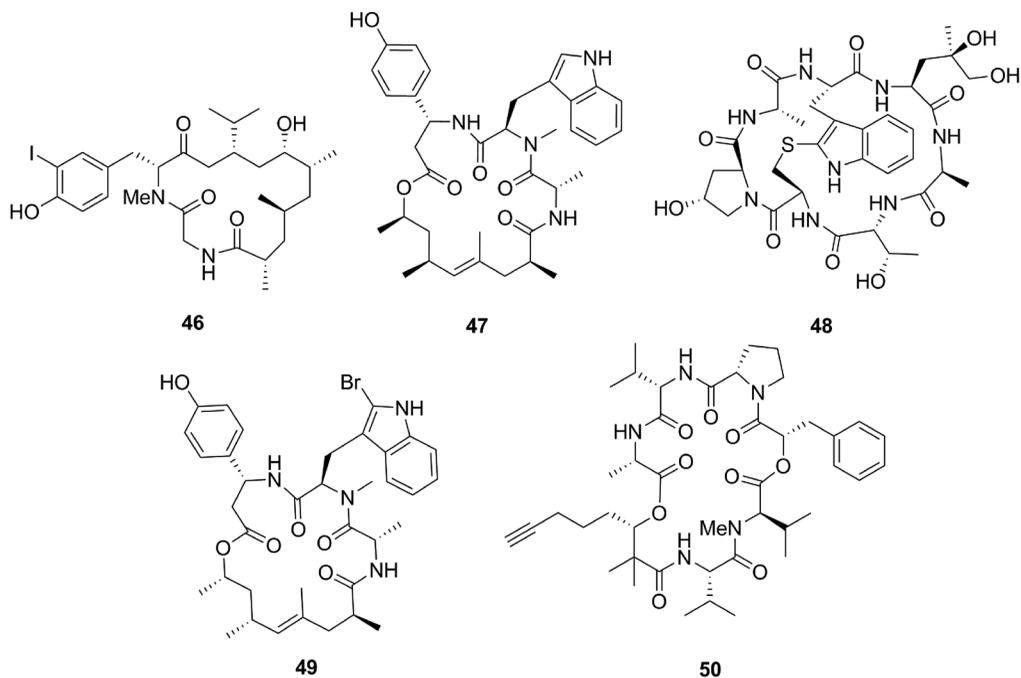
An endoperoxide sterol (**42**; Fig. 12; Table II) was isolated from the digestive gland of *Aplysia depilans* (Table I) and its structure confirmed later by synthesis.<sup>353</sup> This compound displays a GI<sub>50</sub> value of ~3 μM in human HCT-116 colorectal cancer cells.<sup>354</sup>

*Trimusculus* is a shelled pulmonate mollusk (Table I) living in the intertidal zone of rocky shores.<sup>355</sup> An unusual C-21 hydroxylated sterol (**43**; Fig. 12; Table II) was isolated from *T. peruvianus* (living on Chilean coasts, Table I), and displayed GI<sub>50</sub> values of ~6 μM against human HCT-116 and HT29 colon cancer cell lines.<sup>356</sup> More recently, a novel secosterol (**44**; Fig. 12; Table II) was isolated and characterized from a population of *T. costatus* (Table I) collected in South Africa.<sup>357</sup> Compound **44** displays the GI<sub>50</sub> value of ~3 μM in the WHCO1 esophageal cancer cell line.<sup>357</sup>

Among numerous metabolites isolated from the arminacean nudibranch *Leminda millecra* (Table I), including among others six sesquiterpenes and eight prenylquinones,<sup>358</sup> only one compound, prenylated hydroquinone **45** (Fig. 12; Table II), displayed moderate *in vitro* growth inhibitory activity with GI<sub>50</sub> values of ~6 and ~9 μM in the human WHCO1 and WHCO6 esophageal cancer cell lines, respectively.<sup>359</sup> The compounds described above could be from soft coral (*Alcyonium fauri*, e.g.) or gorgonian (*Leptogorgia palma*, e.g.) origin because these are the preys of *L. millecra*.<sup>358</sup>

## 2. Peptides and Depsipeptides

Metabolites of this class have been isolated from a wide range of mollusks belonging to different subclasses of gastropods and diverse orders of opisthobranchs as well as other benthic invertebrates.<sup>360,361</sup> These compounds are most likely produced by symbiotic cyanobacteria,



**Figure 13.** Chemical structures of dolicolide (**46**), jasplakinolide (**47**), phalloidin (**48**), chondroamide C (**49**), and kulolide 1 (**50**).

as they share very similar structures.<sup>362,363</sup> However, we will describe here all the substances reported from mollusks even if they have been later found to be of cyanobacterial origin, while clearly indicating the origin of each compound in Tables I and II.

Dolicolide (**46**; Fig. 13; Table II), which has been isolated from Japanese specimens of the anaspidian *D. auricularia* (Fig. 1D; Table I),<sup>364</sup> is a compound of mixed peptide–polyketide biogenesis characterized by the presence of an iodo-*N*-Me-tyrosine and one unit of glycine. This compound exhibits marked growth inhibitory effects ( $GI_{50} = \sim 2$  nM) against human HeLaS<sub>3</sub> cervix carcinoma cells.<sup>364</sup> Total enantioselective syntheses of dolicolide were reported first by Ishiwata and colleagues,<sup>365</sup> and then by Ghosh and Liu<sup>366</sup> and Matcha et al.<sup>367</sup> Dolicolide binds to actin and consequently arrests cancer cells at the G2/M phase of the cell cycle by interfering with normal actin assembly.<sup>368</sup> Like jasplakinolide (**47**; Fig. 13), a cyclodepsipeptide isolated from marine sponges of *Jaspis* species,<sup>366</sup> dolicolide causes the hyperassembly of purified actin into F-actin.<sup>368</sup> Dolicolide, like jasplakinolide, displaces phalloidin (**48**; Fig. 13), a cyclic peptide isolated from the mushroom *A. phalloides*,<sup>245</sup> from actin polymer.<sup>368</sup> Similar effects were also observed with other depsipeptides known as chondramides.<sup>368</sup> Chondramides, such as chondroamide C (**49**; Fig. 13), are isolated from myxobacteria of the genus *Chondromyces*.<sup>369</sup> Bai et al.<sup>368</sup> used a computer-driven shape descriptor analysis<sup>370</sup> to gain insight into a possible pharmacophore shared by dolicolide, jasplakinolide, phalloidin, and chondroamide C that would explain the apparent binding of this diverse group of substances at the same site on F-actin. They found that the segment of dolicolide that best overlapped with the three other compounds encompassed its phenyl and isopropyl side chains and the portion of the macrocycle between these substituents (Fig. 13). Matcha et al.<sup>367</sup> validated these findings by using unbiased computational docking.

Treatment of human MCF-7 and MDA-MB-231 breast cancer cells with dolicolide leads to inhibition of proliferation and impairs the migratory potential of these cancer cells.<sup>371</sup> Foerster

et al.<sup>372</sup> quite recently showed that dolicolide treatment of p53 wild-type cancer cells alters up to 13% of senescence-related genes at nontoxic concentrations. Like tubulin, actin is also implicated in the metastatic process.<sup>373–375</sup>

Cyclodepsipeptide kulolide 1 (**50**; Fig. 13; Table II) was isolated from the cephalaspidean mollusk *Philinopsis speciosa* (Table I) collected off Hawaii.<sup>376</sup> Kulolide 1 displays growth inhibitory activity in micromolar range against murine L-1210 ( $GI_{50} = \sim 1 \mu\text{M}$ ) and P388 ( $GI_{50} = \sim 3 \mu\text{M}$ ) leukemia cells.<sup>376</sup> It must be emphasized once more that L1210 and P388 leukemia cells are highly sensitive to proapoptotic stimuli.<sup>317</sup>

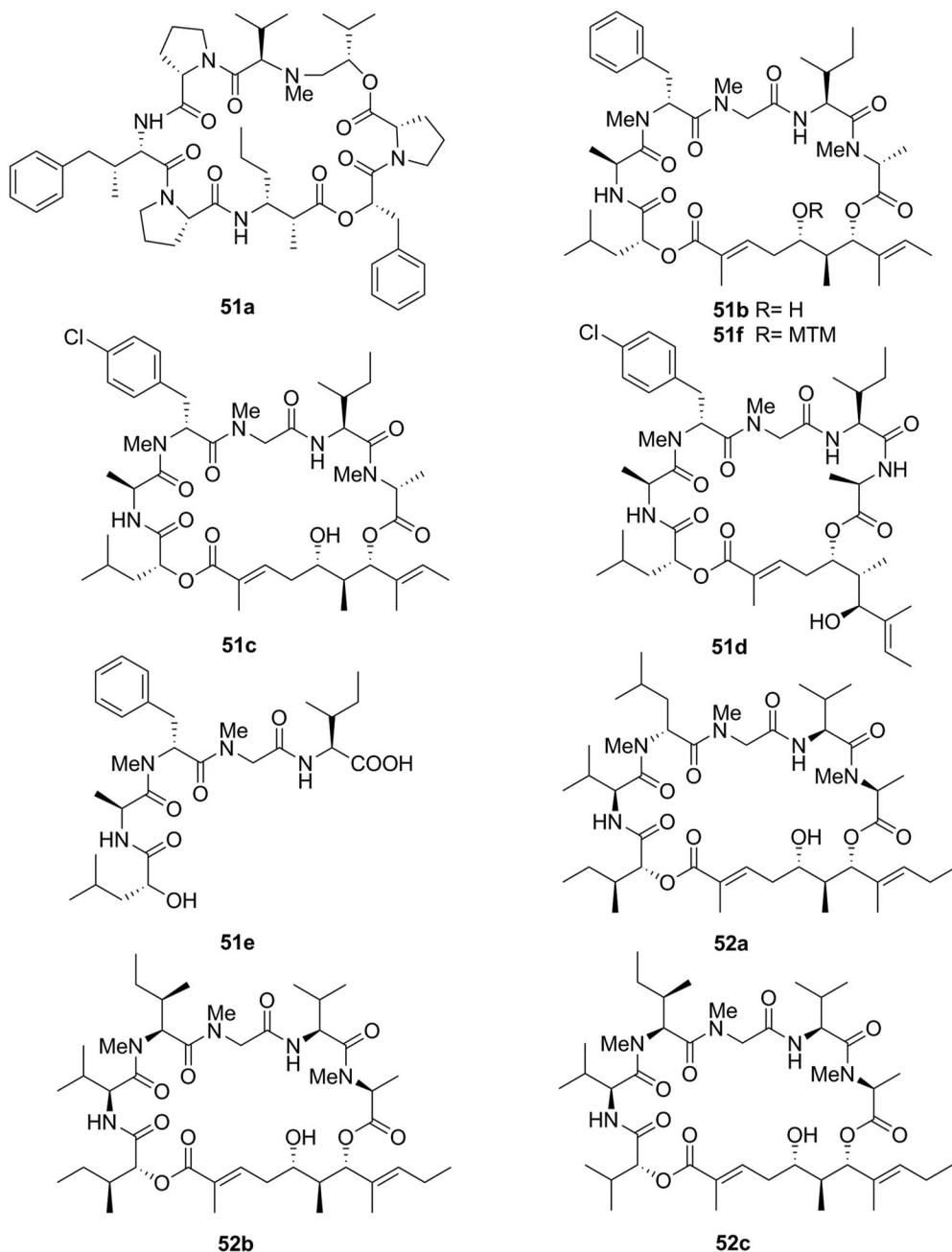
Kulokekahilide-1<sup>405</sup> (**51a**; Fig. 14; Table II) and kulokekahilide-2<sup>378</sup> (**51b**; Fig. 14; Table II) are cyclic depsipeptides isolated from *P. speciosa* (Table I), which is a carnivorous mollusk.<sup>378</sup> One of the preys of *P. speciosa* is the sea hare *Stylocheilus longicaudus*, which feeds on cyanobacteria.<sup>379</sup> The possibility thus remains that compounds isolated from *P. speciosa* could have a cyanobacterial origin.

Kulokekahilide-1 displays growth inhibitory effects with the  $GI_{50}$  of  $\sim 2 \mu\text{M}$  in murine P388 leukemia cells,<sup>377</sup> while kulokekahilide-2 displays higher potency in this cell line with a  $GI_{50}$  value of  $\sim 4 \text{ nM}$ .<sup>378</sup> In addition, kulokekahilide-2 displayed  $GI_{50}$  values of  $\sim 8$  and  $\sim 15 \text{ nM}$  in the human SK-OV-3 ovarian and the MDA-MB-435 breast cancer cell lines, respectively.<sup>378</sup> The mean  $GI_{50}$  value calculated for the three P388, SK-OV-3, and MDA-MB-435 cancer cell lines is thus  $\sim 9 \text{ nM}$ . Because the  $GI_{50}$  value of kulokekahilide-2 in A-10 (nontransformed rat [*Rattus norvegicus*] aortic cells) cell line is  $\sim 60 \text{ nM}$ ,<sup>378</sup> it behaves as a bioselective compound, at least within the few cell lines in which it has been assayed. In the study reported by Umehara et al.,<sup>380</sup> the mean  $GI_{50}$  value of kulokekahilide-2 was  $\sim 0.2 \text{ nM}$  in three other cancer cell lines, namely human A549 NSCLC, K562 chronic myelogenous leukemia, and MCF-7 breast cancer. In the study reported by Takada et al.,<sup>381</sup> the  $GI_{50}$  values for kulokekahilide-2 (**51b**) were  $\sim 19$  and  $\sim 4 \text{ nM}$  in murine P388 leukemia and human HeLa cervix carcinoma cells, respectively. Umehara et al.<sup>380</sup> used the CellTiter-Blue Cell Viability Assay with a 72-hr period of cell-drug incubation, while Takada et al.<sup>381</sup> used the MTT colorimetric assay incubating cells with the compound for 96 hr. The different methods to evaluate the growth inhibition could explain, at least partly, why kulokekahilide-2 appeared to be at least tenfold more potent in the study of Umehara et al.<sup>380</sup>

Among the series of kulokekahilide-2 analogues synthesized by Umehara et al.,<sup>380</sup> compounds **51c** (Fig. 14) and **51d** (Fig. 14) were about 100-fold more potent than kulokekahilide-2 with  $GI_{50}$  values of  $\sim 0.001$  and  $\sim 0.008 \text{ nM}$ , respectively. In contrast, compounds **51e** (Fig. 14) and **51f** (Fig. 14) were completely devoid of *in vitro* growth inhibitory activity in A549, K562, and MCF-7 cancer cell lines with  $GI_{50}$  values  $> 10 \mu\text{M}$ .<sup>380</sup> These authors thus conclude that the addition of halogen at the para position of the phenyl group in the 24-D-MePhe residue in kulokekahilide-2 remarkably increased *in vitro* growth inhibitory activity. The kulokekahilide-2 derivatives generated by Takada et al.<sup>381</sup> did not display higher *in vitro* growth inhibitory effects.

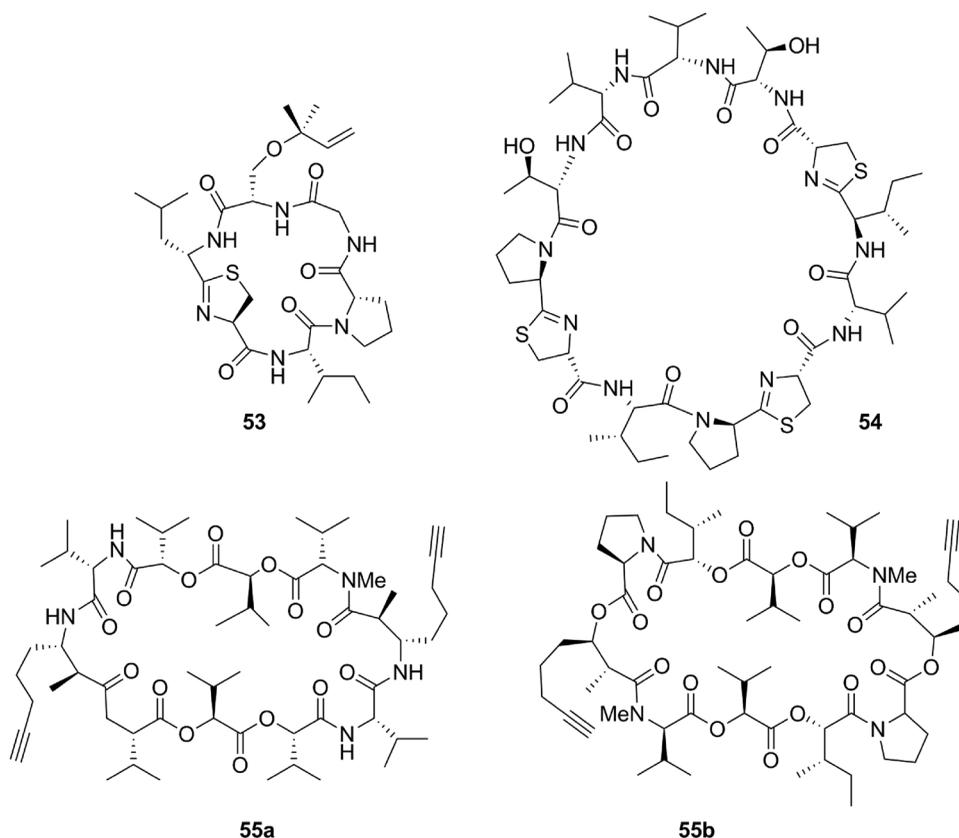
Suenaga et al.<sup>382</sup> reported the isolation of the cyclic depsipeptide aurilide (**52a**; Fig. 14; Table II) from Japanese specimens of *D. auricularia* (Fig. 1D; Table I). It must be noted that aurilides B (**52b**; Fig. 14) and C (**52c**; Fig. 14), which are closely related to aurilide, were isolated from a Papua New Guinea collection of the marine cyanobacterium *L. majuscula*.<sup>383</sup>

Aurilide was assayed in the NCI 60 cell line panel and this analysis revealed the mean  $GI_{50}$  value of  $\sim 0.01 \mu\text{M}$ , with selectivity toward ovarian, renal, and prostate cancer cell lines.<sup>384</sup> It also displayed marked *in vivo* anticancer activity in the NCI hollow fiber assay (detailed by Hollingshead et al.<sup>385</sup>), while it was inactive in a xenograft model (that was unfortunately not described in detail by Suenaga et al.<sup>384</sup>) because of high toxicity. Aurilide shows marked microtubule stabilization properties, while it does not seem to interact directly with tubulin; its mechanism of action thus appears to be distinct from that displayed by taxol.<sup>384</sup> Aurilide



**Figure 14.** Chemical structures of kulokekahilides 1 (**51a**) and 2 (**51b**), kulokekahilide-2 derivatives (**51c-51f**), aurilide (**52a**), and aurilides B (**52b**) and C (**52c**).

selectively binds to prohibitin 1 (PHB1) in the mitochondria and activates the proteolytic processing of optic atrophy 1 (OPA1) that results in mitochondria-induced apoptosis.<sup>386</sup> Because in the NCI testing aurilide showed no differences in its growth inhibitory effects in apoptosis-sensitive versus apoptosis-resistant cancer cell lines,<sup>384</sup> it thus appears that this compound can overcome various types of cancer cell resistance to proapoptotic stimuli. Semenzato et al.<sup>387</sup> emphasize that proapoptotic drugs targeting the mitochondrial Bcl-2 rheostat of apoptosis have



**Figure 15.** Chemical structures of keenamide A (**53**), cycloforskamide (**54**), onchidin (**55a**), and onchidin B (**55b**).

potential to selectively kill cancer cells. Semenzato et al.<sup>387</sup> thus argue that the study by Sato et al.<sup>386</sup> adds to the available anticancer strategies by identifying the target of aurilide in the PHB1/OPA1-dependent apoptotic cristae remodeling. The reader interested in the overview of the various types of resistance patterns to cytotoxic insults displayed by the 60 cancer cell lines used by the NCI is referred to the studies by Shoemaker<sup>115</sup> for an overall overview, Ikediobi et al.<sup>126</sup> for mutation analysis of 24 known cancer genes, Shankavaram et al.<sup>127</sup> for transcript and protein expression profiles, Blower et al.<sup>128</sup> for microRNA expression profiles, and Gholami et al.<sup>129</sup> for a global proteome analysis. Shankavaram et al.<sup>388</sup> also present the CellMiner tool, which is a relational database and query tool for the NCI 60 cancer cell line panel.

Keenamide A (**53**; Fig. 15; Table II) is a cyclic peptide isolated from the gastropod notaspidean *P. forskalii* (Table I) off Manado in Indonesia.<sup>389,390</sup> Its  $GI_{50}$  values range between 4 and 8  $\mu\text{M}$  in four cancer cell lines.<sup>389</sup>

Another macrocyclic peptide, cycloforskamide (**54**; Fig. 15; Table II), was recently isolated from *P. forskalii* (Table I), but off Ishigaki Island (Japan).<sup>390</sup> Cycloforskamide could be a symbiont-derived peptide conferring beneficial effects to the host organism because *P. forskalii* belongs to the family of opportunistic carnivorous mollusks.<sup>390</sup> Its  $GI_{50}$  value is  $\sim 6$   $\mu\text{M}$  in murine P388 leukemia cells.<sup>390</sup> Once more, let us emphasize the high chemosensitivity level of P388 leukemia cells, making therefore this model poorly representative of the

clinical cancer,<sup>317</sup> which is the reason for why the NCI abandoned this model several decades ago.<sup>115</sup>

The methanolic extract of the pulmonate mollusk *Onchidium* sp.1 (Table I) subjected to activity-guided fractionation using murine P388 leukemia and human KB oral cancer cells afforded cytotoxic onchidin (**55a**; Fig. 15; Table II).<sup>391</sup> Later, a new metabolite named onchidin B (**55b**; Fig. 15; Table II) was isolated from the same animal.<sup>392</sup> These two cyclic depsipeptides display GI<sub>50</sub> values of ~7 μM in P388 and KB cancer cells.<sup>392</sup>

### 3. Polyketides

Polyketides comprise a wide array of compounds, most commonly sharing a common feature, such as a macrolide ring. As for cyclic depsipeptides, the true origin of macrolidic compounds has been postulated to be microorganisms. For instance bryostatins are complex macrocyclic lactones isolated from bryozoans, whose real origin has been attributed to bacteria after the identification of putative biosynthetic genes in the uncultured symbiotic bacterium “*Candidatus Endobugula sertula*.”<sup>393,394</sup> As for peptides, we will discuss all polyketide compounds isolated from mollusks (Tables I and II).

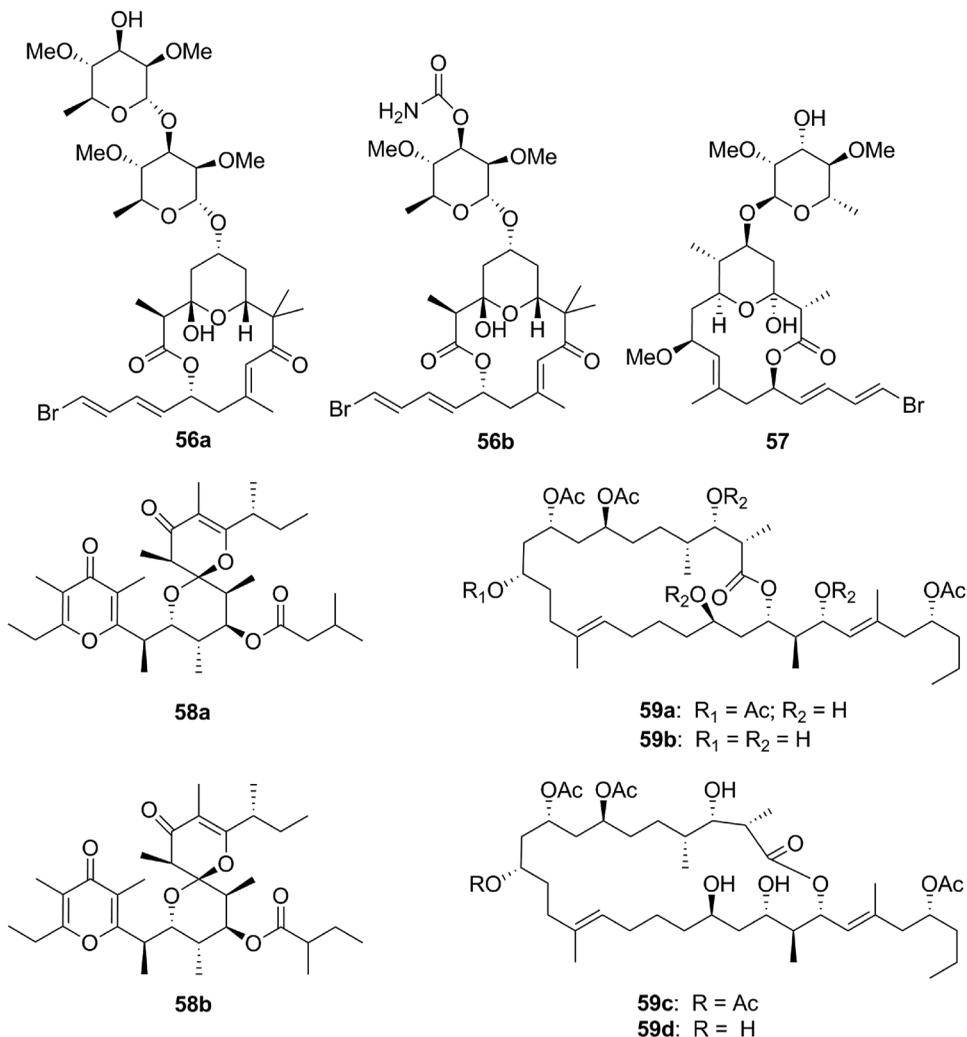
A bioguided fractionation of a Japanese collection of the sea hare *D. auricularia* (Fig. 1D, Table I) led to the isolation of two macrolide glycosides, auriside A (**56a**; Fig. 16; Table II) and auriside B (**56b**; Fig. 16; Table II).<sup>395</sup> From the chemical point of view aurisides have unique structures. Indeed, the aglycon possesses a new type of carbon backbone, 5,7,13-trihydroxy-3,9-dioxoheptadecanoic acid, and contains a bromine-substituted conjugated diene moiety, a 14-membered lactone, and a cyclic hemiacetal part.<sup>395</sup> Auriside A with the GI<sub>50</sub> of ~0.2 μM displays more potent growth inhibitory effects than auriside B (GI<sub>50</sub> = ~2 μM) in human HeLaS3 cervix cancer cells.<sup>395</sup> Various chemical syntheses of aurisides have been reported.<sup>396,397</sup> As mentioned earlier, dolastatin 19 (**57**) shares significant structural similarities with aurisides A (**56a**) and B (**56b**).

Suenaga et al.<sup>398</sup> isolated auripyrones A (**58a**; Fig. 16; Table II) and B (**58b**; Fig. 16; Table II) from the digestive gland of Japanese specimens of *D. auricularia* (Fig. 1D; Table I). These two compounds display the GI<sub>50</sub> of ~0.5 μM in human HeLaS3 cells.<sup>398</sup> Various chemical syntheses of auripyrones have been reported.<sup>399–402</sup>

Dolabelide A (**59a**; Fig. 16; Table II) and its acetyl derivative dolabelide B (**59b**; Fig. 16; Table II) are C-22 macrolides isolated from Japanese specimens of *D. auricularia* (Fig. 1D; Table I).<sup>403</sup> Their GI<sub>50</sub> values were ~8 and ~2 μM, respectively, in human HeLaS3 cervix cancer cells.<sup>403</sup> Dolabelides C (**59c**; Fig. 16; Table II) and D (**59d**; Fig. 16; Table II) were also isolated from Japanese specimens of *D. auricularia*, with GI<sub>50</sub> values of ~2 μM for both compounds in the HeLaS3 cancer cell line.<sup>404</sup> Various chemical syntheses of dolabelides have been reported.<sup>405,406</sup>

Ulapualide A (**60a**; Fig. 17; Table II) and ulapualide B (**60b**; Fig. 17; Table II) were isolated from the red-colored egg masses of the nudibranch *Hexabranhus sanguineus* (Fig. 1G; Table 3).<sup>407</sup> Though exposed and vulnerable, these eggs have only one known predator, the aeolid nudibranch *Favorinus japonicus*.<sup>407</sup> Ulapualides A and B could be some of the bioactive metabolites protecting these eggs from their predators.<sup>407</sup> Ulapualide A can also be found in sponges.<sup>408</sup> Ulapualides contain in their structures three contiguous oxazole rings, forming part of a macrolide ring with the attached lipid-like side chain that terminates in the *N*-methyl-*N*-alkenylformamide group.<sup>407</sup> The total synthesis of ulapualide A was reported in 1998.<sup>408</sup> Ulapualides A and B displayed GI<sub>50</sub> values of ~10 and ~30 nM, respectively, in murine L1210 leukemia cells.<sup>407</sup> Ulapualide A harbors potent actin-depolymerizing activity.<sup>409</sup>

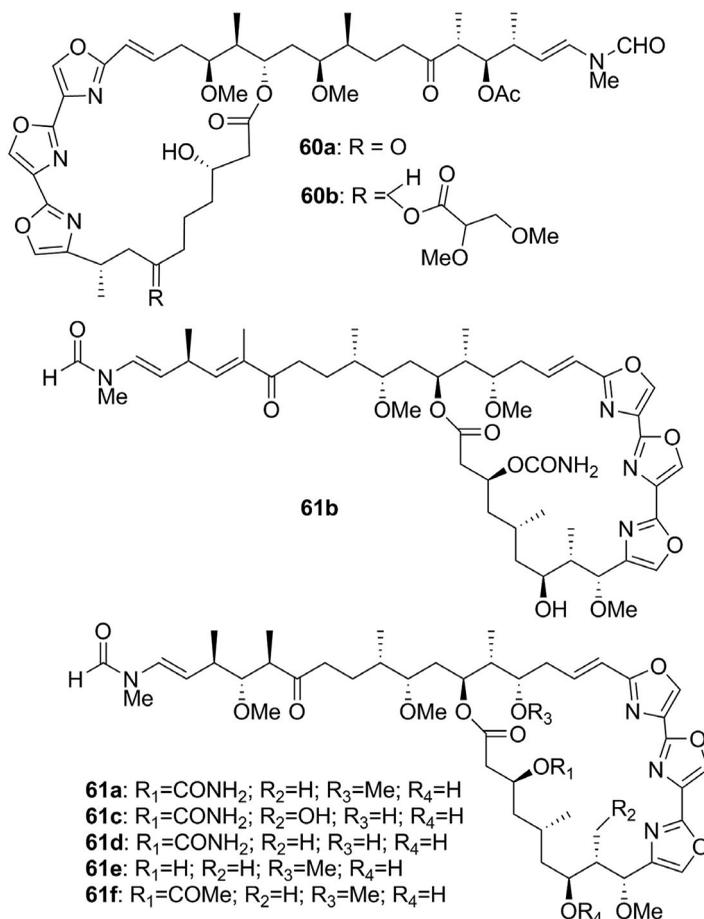
Another study on the egg masses of an unidentified nudibranch led to the characterization of kabiramide C (**61a**; Fig. 17; Table II), which contains a macrolide ring possessing contiguous



**Figure 16.** Chemical structures of aurisides A (**56a**) and B (**56b**), dolastatin 19 (**57**), auripyrone A (**58a**) and B (**58b**), and dolabelides A (**59a**), B (**59b**), C (**59c**), and D (**59d**).

trioxazole rings.<sup>410</sup> Compound **61a** was later isolated from the nudibranch *H. sanguineus* (Fig. 1G; Table I) and from two *Halichondria* sponges.<sup>411</sup> More recently, kabiramide C was found along with other kabiramides, including kabiramide G (**61b**; Fig. 17; Table II), in the sponge *Pachastrissa nux*.<sup>412</sup> Kabiramide C displays *in vitro* growth inhibitory effects that are tenfold higher in human MCF-7 breast cancer cells ( $GI_{50} = \sim 0.5 \mu\text{M}$ ) than in human fibroblasts ( $GI_{50} = \sim 8 \mu\text{M}$ ).<sup>412</sup> This compound thus appears to display a certain level of bioselectivity. The bioselectivity of kabiramide G is even more pronounced with  $GI_{50}$  values of  $0.02 \mu\text{M}$  in MCF-7 cancer cells and  $>2 \mu\text{M}$  in human fibroblasts.<sup>412</sup> Kabiramide C binds to actin and its actin complex, which is formed through a two-step binding reaction, is extremely stable, and long-lived.<sup>413</sup> Although kabiramide C binds to actin, it nevertheless displays a certain level of bioselectivity between normal and cancer cells as mentioned above.<sup>412</sup>

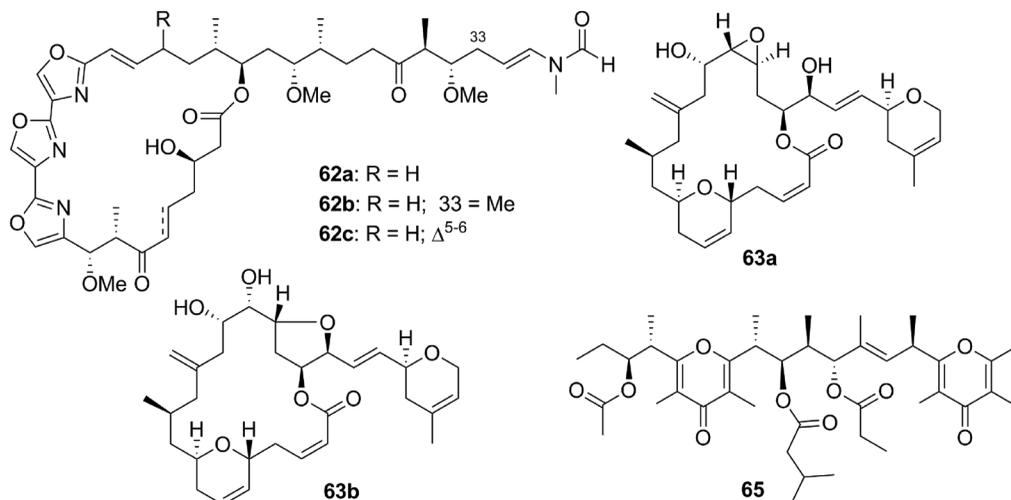
Some years later, Matsunaga et al.<sup>414</sup> isolated a series of new kabiramides (Table II) including A (**61c**; Fig. 17), B (**61d**; Fig. 17), D (**61e**; Fig. 17), and E (**61f**; Fig. 17), as well as



**Figure 17.** Chemical structures of ulapualides A (**60a**) and B (**60b**), and kabiramides C (**61a**), G (**61b**), A (**61c**), B (**61d**), D (**61e**), and E (**61f**).

dihydrohalichondramide (**62a**; Fig. 18) and 33-methylhalichondramide (**62b**; Fig. 18; Table II under halichondramides) from the egg masses of *H. sanguineus* (Fig. 1G; Table I).<sup>414</sup> Halichondramide (**62c**; Fig. 18) was isolated from the sponge *Halichondria sp.* collected in Palau.<sup>415</sup> Dalisay et al.<sup>416</sup> report that *H. sanguineus* is a specialist predator with a spongeverous diet: in an aquarium, the nudibranch was found to consume only *Halichondria* containing trisoxazole macrolides and not sponges of other species or genera. Dalisay et al.<sup>416</sup> thus argue that these observations provide strong evidence that *H. sanguineus* acquires its suite of trisoxazole macrolides from a selective sponge diet.

Dihydrohalichondramide and 33-methylhalichondramide displayed growth inhibitory effects in the murine L1210 leukemia cell line with GI<sub>50</sub> values of ~40 and ~60 nM, respectively.<sup>414</sup> Kabiramides A and B were active with GI<sub>50</sub> values of ~10 nM, and kabiramides D and E with GI<sub>50</sub> values of ~30 nM in the same cancer cell line.<sup>414</sup> Halichondramide and dihydrohalichondramide disrupt actin microfilaments.<sup>417</sup> Halichondramide displays the GI<sub>50</sub> value of <1 μM in human PC-3 prostate cancer cells.<sup>418</sup> This compound is cytostatic at low (~0.5 μM) and cytotoxic at higher concentrations (~2.5 μM).<sup>418</sup> Halichondramide displays both antiproliferative and antimigratory effects *in vitro*. It induces A549 (NSCLC) cell cycle arrest in the G<sub>2</sub>/2 phase with upregulation of p53 and GADD45α and downregulation of cyclin B1, cyclin A, CDC2,

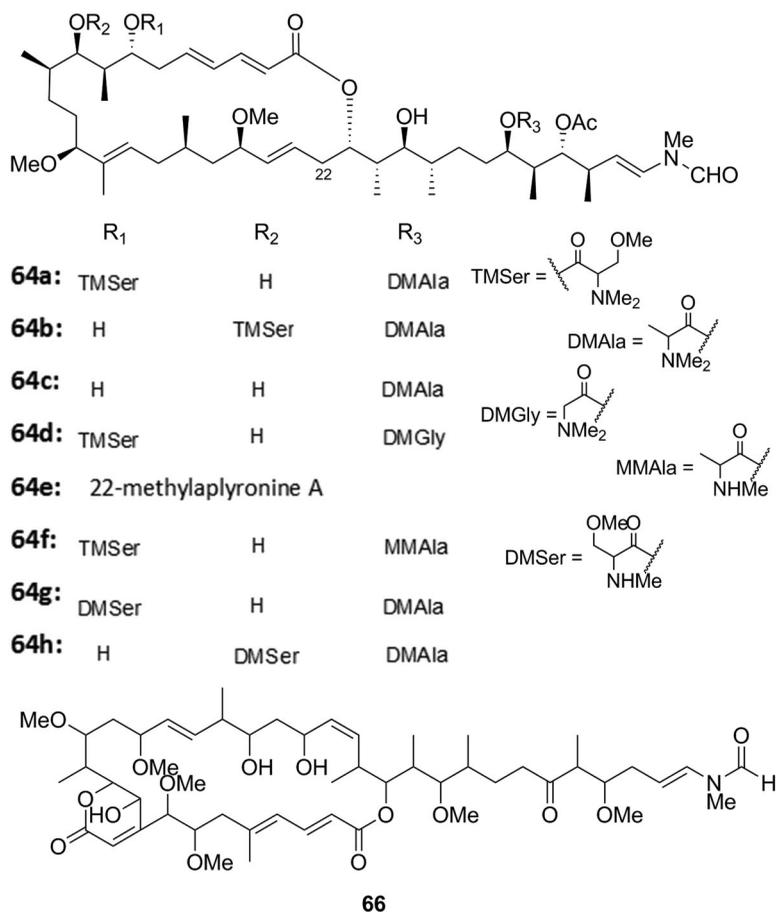


**Figure 18.** Chemical structures of dihydrohalichondramide (**62a**), 33-methylhalichondramide (**62b**), halichondramide (**62c**), laulimalide (**63a**) isolaulimalide (**63b**), and 3-acetyl-11-(3-methylbutanoyl)-13-propanoyllilikonapyrone (**65**).

and CDC25C.<sup>419</sup> The growth inhibitory effects induced by halichondramide in A549 NSCLC cancer cells were also associated with the suppression of mTOR and its downstream effector 4EBP1 and p70S6K; this process occurs by its effects on the regulation of mTOR upstream proteins, such as downregulation of Akt and p38 MAPK, and also upregulation of AMPK.<sup>419</sup> As already mentioned previously, the A549 NSCLC cell line displays various levels of resistance to proapoptotic stimuli.<sup>351</sup> In terms of antimigratory effects observed *in vitro*, halichondramide suppresses the expression of a potential metastatic biomarker, phosphatase of regenerating liver-3 (PRL-3), in human PC-3 prostate cancer cells.<sup>418</sup> The suppression of PRL-3 sequentially downregulates the expression of phosphoinositide 3-kinase (PI3K) subunits p85 and p110.<sup>418</sup> The antimigratory (antimetastatic) effects induced by this compound in PC-3 cancer cells were also correlated with the downregulation of matrix metalloproteases (MMPs) and the modulation of cadherin switches, N-cadherin and E-cadherin.<sup>418</sup> It is unfortunate that although halichondramide (NSC622258) exists in the NCI database, no data are available.

Laulimalide (**63a**; Fig. 18; Table II) and isolaulimalide (**63b**; Fig. 18; Table II) are macrolides that were isolated from the Indonesian sponge *Hyattella* sp. and its nudibranch predator, *Chromodoris lochi* (Table I).<sup>420</sup> Laulimalide displays growth inhibitory activity in low nanomolar ranges in about a dozen of cancer cell lines,<sup>420,421</sup> while isolaulimalide is much less potent with GI<sub>50</sub> values in low micromolar range.<sup>422</sup> Laulimalide is active in P-gp overexpressing cancer cells<sup>421,423</sup> and against cell lines resistant to paclitaxel or epothilones.<sup>423</sup> It has a unique binding site located on two adjacent  $\beta$ -tubulin units between tubulin protofilaments of a microtubule.<sup>212,424</sup> In addition, although laulimalide is a microtubule stabilizer as are the plant-derived metabolites taccalonolide and paclitaxel, laulimalide causes the formation of aberrant, but structurally distinct mitotic spindles in contrast to the effects induced by the other two molecules.<sup>425</sup> Kanakkanthara et al.<sup>426</sup> showed that increased expression of  $\beta$ II- and  $\beta$ III-tubulin isotypes in ovarian cancer cells confers resistance to laulimalide.<sup>426</sup> Downregulation of vimentin (an intermediate filament) expression in human ovarian carcinoma cells also confers resistance to laulimalide.<sup>427</sup>

Liu et al.<sup>421</sup> evaluated the *in vivo* anticancer activity of laulimalide in two xenograft models, human MDA-MB-435 breast cancer and the human HT-1080 fibrosarcoma models. They found



**Figure 19.** Chemical structures of aplyronines A (**64a**), B (**64b**) and C (**64c**), D (**64d**), E (**64e**), F (**64f**), G (**64g**), and H (**64h**), and of sphinxolide (**66**).

only minimal tumor growth inhibition; rather, severe toxicity accompanied by mortality was observed. In contrast, paclitaxel (positive control) led to the clear and dramatic tumor regression in both studies, without unacceptable toxicities.<sup>421</sup> Various teams are actively involved in the syntheses of various fragments of the laulimalide structure with the goal of reaching an efficient total synthesis of this compound.<sup>428–433</sup>

An investigation of the chemical composition of *A. kurodai* (Table I) led to the isolation and characterization of the macrolide family named aplyronines (Table II), specifically aplyronine A (**64a**; Fig. 19), B (**64b**; Fig. 19), C (**64c**; Fig. 19), D (**64d**; Fig. 19), E (**64e**; Fig. 19), F (**64f**; Fig. 19), G (**64g**; Fig. 19), and H (**64h**; Fig. 19).<sup>434–437</sup> Yamada et al.<sup>438</sup> and Kita and Kigoshi<sup>439</sup> reviewed the chemical and pharmacological characteristics of aplyronine A and its analogues.

The GI<sub>50</sub> values were 0.08, 0.5, 3, 10, and 22 nM for aplyronine D, A, B, H, and C, respectively, in the study by Ojika et al.,<sup>437</sup> who used human HeLa-S3 cancer cells. Yamada et al.<sup>434</sup> found GI<sub>50</sub> values of ~0.4 nM for aplyronine A, ~4 nM for aplyronine B, and ~20 nM for aplyronine C, respectively. The presence of various methylated amino acids (*N,N,O*-trimethylserine or *N,N*-dimethylalanine) at the position 22 seems to influence the *in vitro* growth inhibitory activity of these macrolides.<sup>437</sup> Aplyronine A is registered as NSC687160 in the NCI database and it displays the mean GI<sub>50</sub> value in the 60 cancer cell line panel of ~0.2 nM. While highly effective against cancer cells in terms of growth inhibition, it is interesting to note that

aplyronine A does not inhibit the 60 cancer cell lines in a homogeneous manner, but rather displays some selectivity toward certain cell lines (but not toward a given histopathological group). Indeed, six cancer cell lines, specifically HOP-92 (NSCLC), OVCAR-4 (ovarian cancer), TK-10 and UO-31 (renal cancer), and BT-549 and T47-D (breast cancer) display  $GI_{50}$  values that are 10- to 100-fold higher than the mean  $GI_{50}$ , indicating that these six cancer cell lines are 10- to 100-fold more resistant to aplyronine A. In sharp contrast, the MDR cancer cell line NCI/ADR-RES displays a  $GI_{50}$  concentration of  $\sim 0.2$  nM, indicating that aplyronine A is not a substrate for the P-gp efflux pump. Thus, aplyronine A does not behave *in vitro* as a nonspecific poisonous agent. In addition, aplyronine A displayed high *in vivo* anticancer activity (without obvious limiting toxicity) in various murine syngeneic tumor models, including P388 leukemia, Lewis lung carcinoma, Erhlich carcinoma, colon 26 carcinoma, and B16 melanoma.<sup>434</sup> The increases in life span ranged between 201% (B16 melanoma model) and 556% (Lewis lung carcinoma).<sup>434</sup> Thus, the life spans of the aplyronine A-treated tumor-bearing mice increased by twofold to more than fivefold as compared to those of the control (untreated) tumor-bearing mice. To our surprise, we did not find any reports describing aplyronine A's *in vivo* anticancer effects in human xenograft models.

We ran a COMPARE analysis for aplyronine A in the standard agent dataset of the NCI database and found  $p = 0.62$  as a correlation with vincristine sulfate,  $p = 0.70$  with maytansine and 0.87 with vinblastine sulfate. These three compounds are well-known tubulin inhibitors and both vincristine and vinblastine are routinely used to treat cancer patients whose cancer belongs to various histopathological types. Ado-trastuzumab emtansine (T-DM1) is a human epidermal growth factor receptor 2 (HER2) targeted antibody–drug conjugate composed of trastuzumab, a stable linker (4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate, MCC), and the cytotoxic agent DM1, which is mertansine, a derivative of maytansine.<sup>440</sup> T-DM1 underwent several successful Phase III clinical trials and it is now proposed as a treatment of choice in second line and beyond for patients with advanced HER2-expressing breast cancer.<sup>440</sup>

Aplyronine A was proposed recently as an inhibitor of the actin microfilaments. Hirata et al.<sup>441</sup> reported that aplyronine A depolymerizes F-actin and inhibits the polymerization of actin by forming a 1:1 complex with monomeric actin. These authors<sup>441</sup> concluded that, indeed, as expected, aplyronine A binds to a hydrophobic cleft composed of subdomains 1 and 3 of actin by intercalating its aliphatic tail into the actin molecule as do the other reported F-actin depolymerizing agents. Kita et al.,<sup>442</sup> after comparing the amount of abundant actin molecules with the amount of aplyronine A incorporated into the cells, came to the conclusion that the significant antitumor activities of this compound may not be accounted for only by its F-actin-severing properties. They elegantly demonstrated recently that, in fact, aplyronine A forms a 1:1:1 heterotrimeric complex with actin and tubulin and inhibits tubulin polymerization. Kita et al.<sup>443</sup> thus emphasize that aplyronine A represents a rare type of natural product that binds to two different cytoplasmic proteins to exert its highly potent anticancer activities.

Aplyronine A was reported to exert proapoptotic effects in cancer cells.<sup>444</sup> However, these data have been obtained in cancer cells that display very high sensitivity to proapoptotic stimuli (HL-60 leukemia and HeLa cervix carcinoma). In addition, the fact that this compound could induce proapoptotic effects in some cancer cell type is the logical consequence (and not at all a cause) of cell death occurring when crucial components such as microfilaments (actin) and microtubules (tubulin) are severely damaged by a given compound.

The impressive *in vivo* anticancer activity observed by Yamada et al.<sup>434</sup> in five distinct murine tumor models, as reported above, was obtained with doses ranging from 0.04 to 0.08 mg/kg. Several strategies are already underway to obtain aplyronine A by means of chemical syntheses.<sup>445,446</sup> Paterson et al.<sup>447,448</sup> claimed that aplyronine A has potential as a clinical candidate. However, the *in vivo* data reported by Yamada et al.<sup>434</sup> have never been

replicated, even though gram quantities of aplyronine A are currently available. The clinical trial site, <https://clinicaltrials.gov>, does not mention any ongoing trial with aplyronine A.

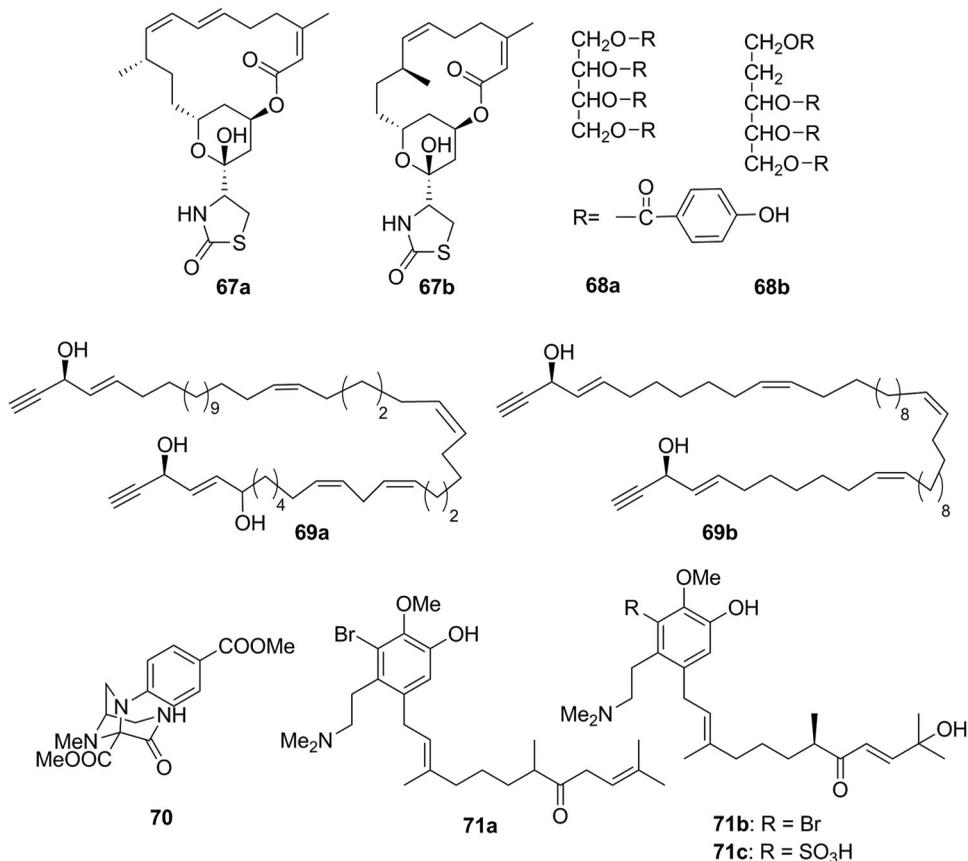
A lipophilic extract of the pulmonate mollusk *Onchidium* sp.2 (Fig. 11; Table I) collected from the South China Sea contained a series of polypropionates characterized by the presence of bis- $\gamma$ -pyrone rings and structurally related to ilikonapyrones, previously found in a different species of pulmonate.<sup>449</sup> The *in vitro* growth inhibition assay carried out on the metabolites isolated from this mollusk showed growth inhibitory activity for only 3-acetyl-11-(3-methylbutanoyl)-13-propanoylilikonapyrone (**65**; Fig. 18; Table II) in a minipanel of six human cancer cell lines: A549 NSCLC, MCF-7 breast cancer, PC-3 prostate cancer, Hs683 oligodendroglioma, U373 glioblastoma, and SKMEL-28 melanoma.<sup>449</sup> The GI<sub>50</sub> values were very similar and ranged from 3 to 9  $\mu$ M, without any correlation with the level of sensitivity of these cancer cell lines to proapoptotic stimuli, indicating therefore that this metabolite is active against cancer cells displaying resistance to proapoptotic stimuli.<sup>449</sup>

Sphinxolides bear structural resemblance to scytophycins, which are found in cyanobacteria, such as *Scytonema pseudohofmanni*.<sup>447,450</sup> Sphinxolide (**66**; Fig. 19; Table II) is a 26-membered macrolide that was first isolated from an unidentified Pacific nudibranch<sup>451</sup> and later reisolated from the New Caledonian marine sponges *Neosiphonia superstes* and *Reidispongia coreula*, along with the congeneric reidispongiolides.<sup>452,453</sup>

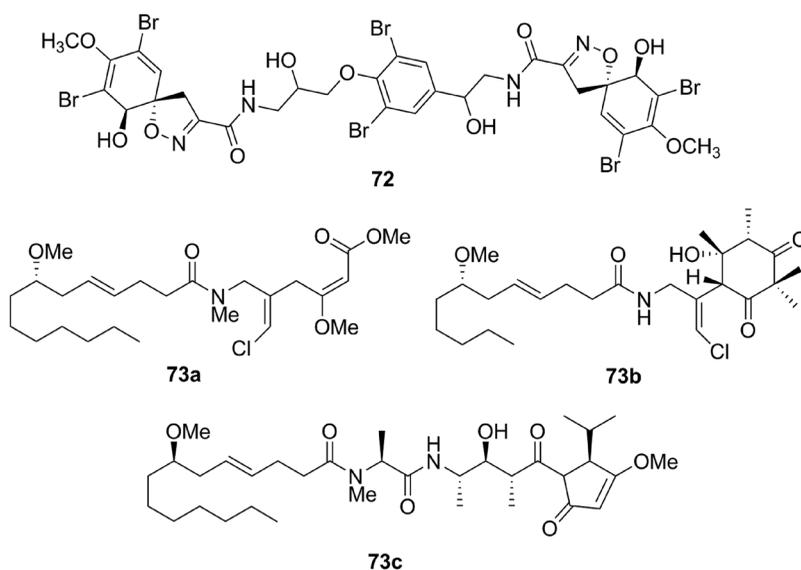
Like the related families of trisoxazole-containing marine macrolides (described above), sphinxolide is an actin-binding molecule that disrupts the actin microfilament organization.<sup>447</sup> As previously mentioned and clearly emphasized by Paterson et al.<sup>447</sup> and Allingham et al.,<sup>454</sup> actin microfilaments exert crucial roles in cancer cell division and metastasis and therefore actin-binding natural products could be used as leads for novel types of cancer chemotherapy. However, the actin microfilaments also exert crucial roles (as microtubules) on eukaryotic cell shape, cell motility, cell adhesion, and intracellular transportation.<sup>447,454</sup> Antiactin compounds seem at first glance more toxic than antitubulin ones if one takes into account that no antiactin compound is currently marketed as an anticancer drug.

Sphinxolide displays *in vitro* GI<sub>50</sub> value of  $\sim$ 0.02 nM in human KB cancer cells.<sup>451</sup> The actin-binding properties of sphinxolide in cancer cells lead to subsequent apoptosis,<sup>450</sup> as usually occurs with any compounds severely impairing the microfilament and/or the microtubule networks. Sphinxolide causes rapid loss of microfilaments in cultured cells, without affecting microtubule organization, and potently inhibits actin polymerization *in vitro* as well as the microfilament-dependent ATPase activity of purified actomyosin, indicating the direct effect on actin.<sup>450</sup> It is active *in vitro* against MDR cancer cells.<sup>450,454</sup> No *in vivo* studies have been performed for sphinxolide, at least to the best of our knowledge, and it is not registered in the NCI database.

Latrunculins A (**67a**; Fig. 20; Table II) and B (**67b**; Fig. 21; Table II) were first reported as spongian metabolites, but were subsequently isolated from different dorid mollusks. In particular, *Glossodoris quadricolor* (also named *Chromodoris quadricolor*; Fig. 1F; Table I) accumulates latrunculin B from *Latrunculia magnifica*,<sup>455</sup> whereas latrunculins A was found in *C. lochi* feeding on *Spongia mycifyjensis*.<sup>456</sup> In addition to *C. lochi*, compound **67a** has been recently also found in other four species belonging to the genus *Chromodoris*: *C. elisabethina*, *C. kuiteri*, *C. annae*, and *C. magnifica*, showing that it acts as both a feeding deterrent and a toxic chemical weapon.<sup>189</sup> These diet-derived toxins are characterized as 2-thiazolidinone macrolides and were shown to be strongly ichthyotoxic and cytotoxic.<sup>457</sup> Latrunculin A displayed GI<sub>50</sub> values of  $\sim$ 0.5  $\mu$ M in the study by Longley et al.,<sup>458</sup> who utilized three cancer cell lines: murine P388 leukemia, and human HT-29 colon cancer, and A549 NSCLC.<sup>458</sup> Although the P388 leukemia cells<sup>115,317</sup> are much more sensitive to proapoptotic stimuli than the A549 NSCLC ones,<sup>351</sup> latrunculin A displayed greater than a fivefold level of *in vitro* growth inhibitory effects against A549 NSCLC compared to P388 leukemia.<sup>458</sup> It thus seems that the marked



**Figure 20.** Chemical structures of latrunculins A (**67a**) and B (**67b**), kellelins I (**68a**) and II (**68b**), hydroxyl-dehydroisofulvinol (**69a**), fulvinol (**69b**), aplaminal (**70**), aplaminone (**71a**), neoaplaminone (**71b**), and neoaplaminone sulfate (**71c**).



**Figure 21.** Chemical structures of isofistularin-3 (**72**), and malyngamides O (**73a**), S (**73b**), and X (**73c**).

latrunculin's A growth inhibitory effects are not impaired by the levels of resistance of cancer cells to proapoptotic stimuli. Latrunculin A is registered in the NCI database as NSC613011. Its mean GI<sub>50</sub> value in the 60 cancer cell line panel is ~0.7 μM, and there is more than twofold log magnitude difference between the most sensitive and the most resistant cancer cell lines. Latrunculin A thus does not behave as a nonspecific poison. In addition, this compound is as active against MDR NCI/ADR-RES as it is against cells that do not display the MDR phenotype.

The *in vitro* growth inhibitory effects of latrunculins, including latrunculin A are related to their ability to reversibly bind actin monomers, forming 1:1 stoichiometric complexes with G-actin, and therefore disrupting actin polymerization.<sup>459,460</sup> As expected with antitubulin and/or antiactin compounds, apoptosis occurs in latrunculin A treated cancer cells. Konishi et al.<sup>461</sup> showed that latrunculin A induced apoptosis in cancer cells occurs via the activation of the caspase-3/caspase-7 pathway. We ran a COMPARE analysis on latrunculin A and found no correlation with  $p \geq 0.7$  with compounds in the standard agent database.

Konishi et al.<sup>461</sup> report that latrunculin A has strong anticancer effects in peritoneal dissemination models of MKN45 and NUGC-4 human gastric cancer in mice. The *in vivo* anticancer activity of latrunculin A was also evaluated in s.c. A549 NSCLC xenografts in which this compound increased by 46% the life span of the treated tumor-bearing mice as compared to the control.<sup>458</sup>

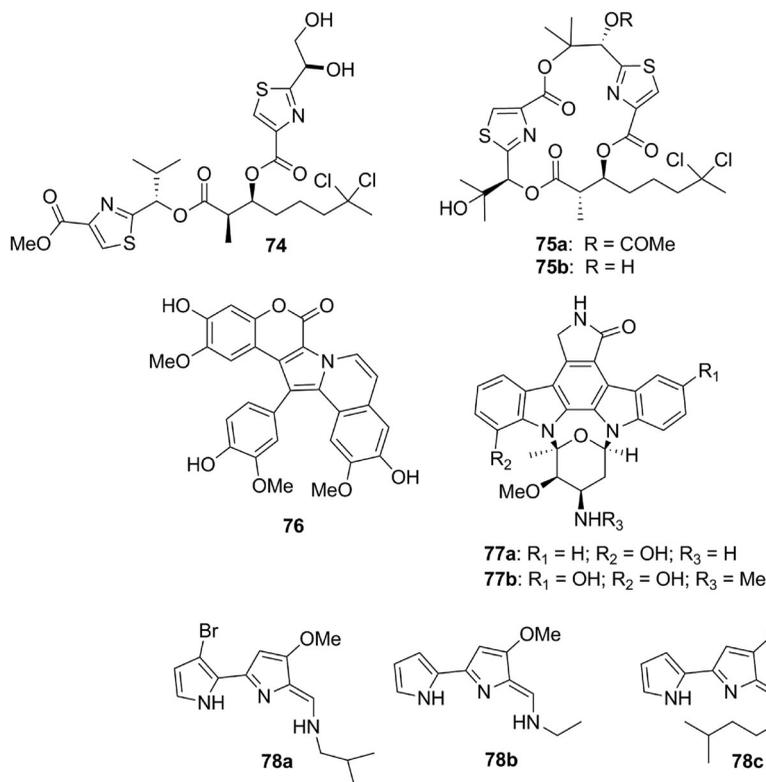
The prosobranch *Kelletia kelletii* is a hard-shelled mollusk (Table I) that contained among other compounds kelletinins I (**68a**; Fig. 20; Table II) and II (**68b**; Fig. 20; Table II), *p*-OH-benzoic acid tetraester of erithritol, and 2-deoxy-D-ribose, respectively. The two compounds displayed *in vitro* growth inhibitory effects with GI<sub>50</sub> values of ~0.07 μM in murine L1210 leukemia cells.<sup>462</sup>

*Peltodoris atromaculata* (Fig. 1J, Table I) is a Mediterranean nudibranch usually feeding on sponges of genus *Petrosia*, whose secondary metabolites are mainly long-chain polyacetylenes called petroformynes.<sup>463</sup> This mollusk sequesters the sponge metabolites and accumulates them in their digestive glands.<sup>463</sup> No reports about *in vitro* growth inhibitory effects against cancer cells have yet been published for this type of compounds, at least to the best of our knowledge. However, petroformynes strongly resemble related compounds from sponges, neopetroformynes, which display GI<sub>50</sub> values ranging between 0.1 and 0.7 μM in murine P388 leukemia cells.<sup>464</sup> Recently, a recollection of the same animal feeding on the sponge *Haliclona fulva* led to the isolation of new polyacetylenes, including hydroxyl-dehydroisofulvinol (**69a**; Fig. 20; Table II), which resembles fulvinol (**69b**; Fig. 20). Fulvinol (**69b**), isolated from the sponge *Reniera fulva*, displays a mean GI<sub>50</sub> value of ~2 μM in four cancer cell lines: murine P388 leukemia, A549 NSCLC, HT-29 colon cancer, and SKMEL-28.<sup>465</sup> Hydroxyl-dehydroisofulvinol, isolated from *P. atromaculata*, displayed a GI<sub>50</sub> value of ~3 μM against SKMEL28 melanoma cells.<sup>466</sup>

#### 4. Nitrogen Containing Compounds

Kuroda and Kigoshi<sup>467</sup> isolated aplaminal (**70**; Fig. 20; Table II), containing a triazabicyclo [3.2.1] octane framework from *A. kurodai*. This compound displays a GI<sub>50</sub> value of ~2 μM against human HeLaS3 cervix carcinoma cells.<sup>467</sup>

Aplaminone (**71a**; Fig. 20; Table II), neoaplaminone (**71b**; Fig. 20; Table II), and neoaplaminone sulfate (**71c**; Fig. 20; Table II) were also isolated from Japanese specimens of *A. kurodai*.<sup>468</sup> From the chemical perspective, these substances are constructed from a bromine-containing dopamine unit and a sesquiterpenoid part.<sup>468</sup> Aplaminone and neoaplaminone sulfate display GI<sub>50</sub> values of ~1 μM against human HeLa cervix cancer cells, while neoaplaminone has the GI<sub>50</sub> of ~1 nM in this cancer cell line.<sup>468</sup>



**Figure 22.** Chemical structures of dolabellin (**74**), hectochlorin (**75a**) and its deacetylated form (**75b**), lamellarin D (**76**), staurosporine analogues (**77a**, **77b**), and tambjamines D (**78a**), E (**78b**), and K (**78c**).

*Tylodina perversa* is a Mediterranean notaspidean mollusk whose dietary preference are sponges of genus *Aplysina*.<sup>469,470</sup> Brominated isoxazoline alkaloids constitute the metabolite pattern of these porifera with validated feeding deterrent properties against fishes.<sup>470</sup> Studies in natural and captivity conditions showed that *T. perversa* is able to sequester and accumulate these metabolites in different anatomical parts.<sup>470</sup> Among all isoxazoline alkaloids found in this mollusk, isofistularin-3 (**72**; Fig. 21; Table II) displayed the GI<sub>50</sub> value of  $\sim 9 \mu\text{M}$  against human HeLa cervix carcinoma cells.<sup>470</sup>

Malyngamides are *N*-substituted amides of long chain methoxylated fatty acids, characterized by the presence of a *trans* double bond and a 7*S* configuration of the oxygen-bearing carbon. To date, more than 30 malyngamides have been isolated from cyanobacteria and sea hares. Metabolites of this class have been reported to possess *in vitro* growth inhibitory properties against cancer cells and include malyngamide O (**73a**; Fig. 21; Table II), isolated from the anaspidean mollusk *Stylocheilus longicauda*,<sup>471</sup> as well as malyngamide S<sup>472</sup> (**73b**; Fig. 21; Table II) and malyndamide X<sup>473</sup> (**73c**; Fig. 21; Table II), both isolated from *Bursatella leachii* (Table I). These three compounds displayed GI<sub>50</sub> values ranging between  $\sim 4$  and  $\sim 8 \mu\text{M}$  in seven cancer cell lines including murine P388 leukemia and human A549 NSCLC, NCI-H187 (SCLC), HT-29 colon cancer, HL60 leukemia, KB and BC breast cancer models.<sup>471–473</sup>

An investigation of Indian Ocean specimens of the sea hare *D. auricularia* (Fig. 1D, Table I) led to the isolation of a bisthiazole metabolite, dolabellin (**74**; Fig. 22; Table II), which displayed the GI<sub>50</sub> value of  $\sim 10 \mu\text{M}$  in the human HeLaS3 cervix carcinoma cell line.<sup>474</sup>

Although previously isolated from the cyanobacterium *L. majuscula*, hectochlorin (**75a**; Fig. 22; Table II) and its deacetylated derivative (**75b**; Fig. 22; Table II) have also been found in the sea hare *B. leachii* (Fig. 1C, Table I).<sup>475</sup> Hectochlorin structurally resembles dolabellin (**74**, Fig. 22) as reported by Marquez et al.<sup>476</sup> In the study by Suntornchashweij et al.,<sup>475</sup> the *in vitro* growth inhibitory effects of compounds **75a** and **75b** were determined in the human KB, NCI-H187 SCLCL, and BC breast cancer cell lines. The deacetylated derivative of hectochlorin **75b** was the most active of these two compounds, displaying a mean GI<sub>50</sub> value of ~1 μM.<sup>475</sup> Marquez et al.<sup>476</sup> showed that hectochlorin is equipotent to jasplakinolide in its ability to promote actin polymerization, however unlike jasplakinolide, it is unable to displace a fluorescent phalloidin analogue from polymerized actin. Hectochlorin has no effect on the polymerization of purified tubulin.<sup>476</sup>

Marquez et al.<sup>476</sup> also report that hectochlorin displays a unique profile in the NCI 60 cancer cell line panel. Indeed, it showed the greatest potency against several cell lines in the colon, melanoma, ovarian, and renal cell line panel with the average GI<sub>50</sub> against the 60 cell line panel of ~5 μM.<sup>476</sup> In addition, the flat shape of the dose–response curves of heterochlorin is generally characteristic of compounds that are cytostatic rather than cytotoxic.<sup>476</sup> The chemical total synthesis of hectochlorin has been reported by Cetusic et al.<sup>477</sup>

More than three decades ago, Andersen and et al.<sup>478</sup> isolated lamellarins, aromatic alkaloids characterized by the presence of a fused-pyrrole ring, from the Palauan prosobranch mollusk *Lamellaria* sp. various. Since the first report, more than 50 lamellarins have been found mainly in *Didemnum* spp. ascidians as well as in various sponges.<sup>479–481</sup> Of all the lamellarins identified up to date, lamellarin D (**76**; Fig. 22; Table II) displays the highest growth inhibitory effects on cancer cells,<sup>481</sup> with GI<sub>50</sub> values ranging between 10 and 20 nM in a large panel of cancer cell lines.<sup>478</sup> In addition to the nuclear targeting involving topoisomerase I inhibition with the modulation of several apoptotic mediators,<sup>482,483</sup> lamellarin D accumulates rapidly inside the mitochondria, directly poisoning the mitochondrial topoisomerase I (Top1mt), and thus leading to the dysfunctional mitochondrial respiration in cells.<sup>484,485</sup> As suggested by the sensitivity of topoisomerase I mutated P388-CPT5 chemoresistant cells,<sup>482</sup> lamellarin's D dual mechanism may be effective against drug-resistant cancers offering an alternative to the conventional topoisomerase I inhibitors, irinotecan, and topotecan, which are inefficient as Top1mt inhibitors due to their inactivation in the mitochondrial pH environment.<sup>486</sup> In addition, lamellarin D is not sensitive to P-gp and its growth inhibitory effects are maintained in MDR cancer cells.<sup>487</sup> Due to its intriguing anticancer properties, lamellarin D has been the focus of considerable research and several syntheses and SAR studies of lamellarin derivatives have been reported in the last few years.<sup>481</sup> The fact nevertheless remains that despite this research activity, no *in vivo* efficacy has been demonstrated yet for lamellarins in general and for lamellarin D in particular, at least to the best of our knowledge. A bioguided fractionation of various extracts of the prosobranch mollusk *Coriocella nigra* resulted in the isolation of two members of the staurosporine<sup>488</sup> (selective kinase inhibitor) family: compounds **77a** (4'-*N*-demethyl-11-hydroxystaurosporine; Fig. 22; Table II under staurosporine analogues) and **77b** (3,11-dihydroxystaurosporine; Fig. 22; Table II).<sup>489</sup> Their GI<sub>50</sub> values in a minipanel of cancer cell lines ranged between 4 and 130 nM.<sup>489</sup>

Tambjamines are methoxyppyrrolic alkaloids whose chemical features resemble the bacterial prodigiosins.<sup>490–492</sup> All these compounds contain a common 4-methoxy-2,2'-bipyrrole chromophore.<sup>491,492</sup> Tambjamines A–D (tambjamine D; **78a**; Fig. 22; Table II) were isolated from the nembrothid nudibranchs *Roboastra tigris*, *Tambja eliora*, and *Tambja abdere* (Table I).<sup>490</sup> *Roboastra tigris* is a large carnivorous nudibranch that preys on two smaller nudibranchs, *T. eliora* and *T. abdere*.<sup>490</sup> Tambjamines A–D were also traced to a dietary source, the ectoproct (bryozoan) *Sessibugula translucens*, and are implicated in a chemical defense mechanism.<sup>490</sup> Indeed, when attacked by *R. tigris*, *T. abdere* produces a yellow mucus from

goblet cells in the skin and this defense secretion often causes *R. tigris* to break off its attack.<sup>490</sup> *Tambja eliora* does not produce a defensive secretion but attempts to escape from *R. tigris* by using a vigorous writhing motion.<sup>490</sup> In laboratory experiments, *R. tigris* prefers to eat *T. eliora* rather than *T. abdere*.<sup>490</sup> Tambjamines E (**78b**; Fig. 22; Table II), F, G, H, I and J were isolated from various bacteria and marine invertebrates.<sup>492</sup> Tambjamine K (**78c**; Fig. 22; Table II) was isolated from the Azorean nudibranch *Tambja ceutae*.<sup>493</sup>

The GI<sub>50</sub> values of tambjamine K (**78c**) were determined in four cancer cell lines and ranged between ~0.004 and 15 μM.<sup>493</sup> Tambjamine K displayed the GI<sub>50</sub> value of ~19 μM in mouse 3T3-L1 fibroblasts.<sup>493</sup> At first glance, it thus appears that tambjamine K (**78c**) is not a bioselective compound. However, it displays a ~4000-fold differential sensitivity between human Caco-2 colon cancer cells and HeLa cervix cancer cells.<sup>493</sup> Tambjamine K (**78c**) has unfortunately not been assayed by the NCI.

Tambjamine C has recently been demonstrated to be an efficient anion carrier, that is, a transmembrane anion transporter similar to prodigiosin, whereas tambjamine E does not share this property and tambjamine K has not yet been assayed as such, at least to the best of our knowledge.<sup>494</sup> Transmembrane anion transporters play important roles in cancer cell biology, including cancer cell migration, and they are distinctly expressed in various cancer cell types.<sup>495–499</sup> It is interesting to note that Caco-2 colon cancer cells, displaying high sensitivity to tambjamine K as mentioned above, have often been used to study the roles of various transmembrane transporters.<sup>500,501</sup> Tambjamine E targets DNA as also does tambjamine D,<sup>502</sup> which in addition displays genotoxic effects in normal cells.<sup>503</sup> It thus appears that tambjamine K certainly merits additional in depth pharmacological and toxicological preclinical evaluations as a potential anticancer agent.

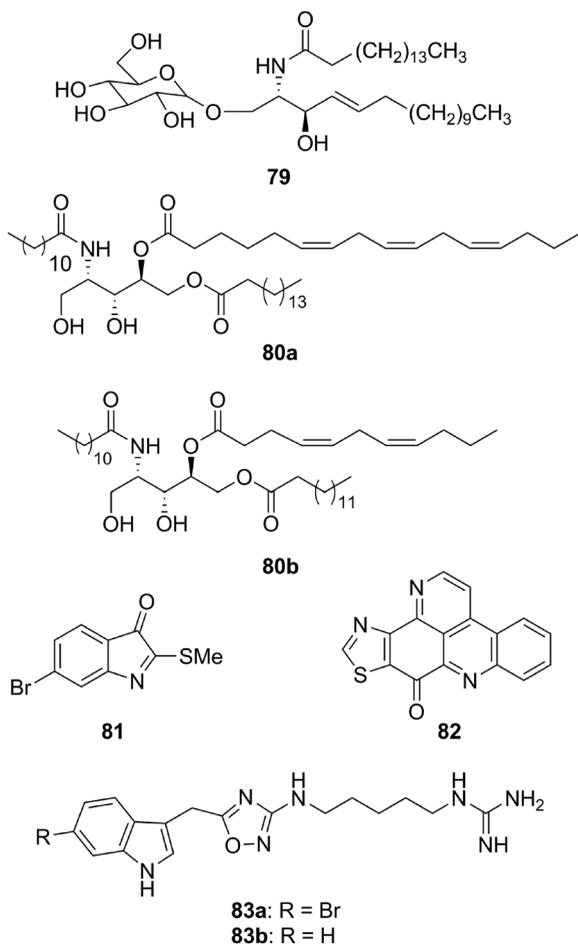
A chemical investigation of the Asian mollusk *Turbo stenogyrus* (Table I) led to the isolation of four cerebrosides, turbostatins A–D, whose GI<sub>50</sub> values ranged between ~0.2 and ~4 μM in murine and human cancer cell lines.<sup>504</sup> The chemical structure of turbostatin A (**79**; Table II) is illustrated in Figure 23.

Bathymodiolamides A (**80a**; Fig. 23; Table II) and B (**80b**; Fig. 23; Table II) are ceramides isolated from the deep vent mussel *Bathymodiolus thermophilus* (Table I). Their GI<sub>50</sub> values in human HeLa cervix cancer and MCF7 breast cancer cells were <1 μM.<sup>505</sup>

Tyrindoleninone (**81**; Fig. 23; Table II) is a brominated indole derivative (6-bromo-2-methylthioindolin-3-one) isolated from the egg mass extract of the gastropod *Dicathais orbita* (Table I).<sup>506</sup> The GI<sub>50</sub> values of this compound in human colon cancer cells are >100 μM.<sup>506</sup>

A series of pentacyclic alkaloids have been isolated from the prosobranch mollusk *Chelynotus semperi* (Table I) and its prey, an unidentified colonial tunicate.<sup>507</sup> Among these compounds, only kuanoniamine A (**82**; Fig. 23; Table II) showed weak growth inhibitory activity against KB cancer cells (GI<sub>50</sub> = ~4 μM).<sup>507</sup>

The indole alkaloids phidianidines A (**83a**; Fig. 23; Table II) and B (**83b**; Fig. 23; Table II) were isolated from *Phidiana militaris* (Fig. 1K; Table I), an aeolid nudibranch from the South China Sea.<sup>508</sup> The GI<sub>50</sub> values for these compounds range between ~0.4 and >100 μM in three cancer cell lines. They display no bioselectivity toward mouse 3T3-L1 fibroblasts and rat H9c2 cardiomyocytes as the GI<sub>50</sub> values toward these cells ranged between ~0.1 and ~5 μM.<sup>508</sup> Interestingly, while human HeLa cervix cancer cells display weak sensitivity to the growth inhibitory effects of tambjamine K,<sup>493</sup> they are much more sensitive to the growth inhibitory effects of phidianidines.<sup>508</sup> The exactly opposite features are observed with respect to Caco-2 colon cancer cells.<sup>493</sup> These data thus highlight distinct mechanisms of action for tambjamine K and phidianidines A and B as potential anticancer compounds and suggest that tambjamine K as well as phidianidines A and B are not nonspecific poisons. It would be very interesting to have an NCI profiling of these compounds. Phidianidines A and B act as selective

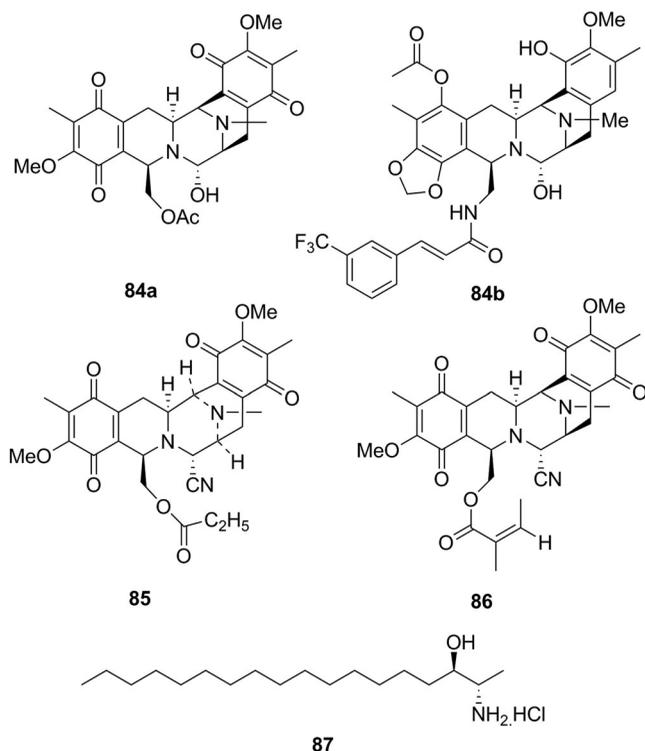


**Figure 23.** Chemical structures of turbostatin A (**79**), bathymodiolamides A (**80a**) and B (**80b**), tyrindoleninone (**81**), kuanoniamine A (**82**), and phidianidines A (**83a**) and B (**83b**).

and potent ligands with partial agonist activity against the  $\mu$  opioid receptor (versus  $\delta$ - and  $\kappa$ -opioid receptors).<sup>509</sup> Indeed, the  $\mu$  opioid receptor is involved in cancer progression.<sup>510</sup> They were also demonstrated to act as ligands of CXCR4, a chemokine receptor involved in cancer progression and the metastatic process.<sup>511</sup> Total syntheses of phidianidines A and B have been reported.<sup>509, 512–514</sup>

Fontana et al.<sup>515</sup> isolated jorumycin (**84a**; Fig. 24; Table II) from the mantle and mucus of the Pacific nudibranch mollusk, *Jorunna funebris* (Fig. 1H; Table I). Jorumycin displays  $\text{GI}_{50}$  values in low nanomolar or even subnanomolar ranges in various cancer cells including cells resistant to proapoptotic stimuli.<sup>515, 516</sup> The high potency of jorumycin and the clinical successes of ecteinascidin led to intensive chemical modifications of jorumycin's chemical structure and this campaign thus resulted in PM00104 (Zalypsis<sup>®</sup>, **84b**, Fig. 24).<sup>516</sup> Petek and Jones<sup>516</sup> recently reviewed the preclinical and clinical data related to PM00104, which has entered several ongoing Phase II clinical trials.

Jorumycin belongs to the class of dimeric isoquinoline alkaloids and it shares the same carbon framework as saframycin and ecteinascidin.<sup>515</sup> In fact, ecteinascidin (trabectedin) has three isoquinolines, while the other two alkaloids have only two isoquinolines per molecule, while the two isoquinolines are joined in similar manner in all three compounds.<sup>515</sup> The difference



**Figure 24.** Chemical structures of jorumycin (**84a**), PM00104 (Zalypsis<sup>®</sup>, **84b**), jorunnamycin C (**85**), renieramycin M (**86**), and ES-285 (spisulosine, **87**).

in structure between ecteinascidin and the jorumycin analog PM00104 could explain, at least partly, why these two drugs display slightly different DNA-binding properties and nucleotide excision repair dependencies.<sup>516</sup>

In 2007, studies on Thai *J. funebris* (Fig. 1H) specimens led to the isolation from the mantle, viscera, and egg-ribbons novel tetrahydroisoquinoline alkaloids, jorunnamycins A–C, along with several renieramycins.<sup>517</sup> It is interesting to note that certain renieramycins have also been isolated from the Thai sponge *Xestospongia* sp.<sup>518</sup> Indeed, *J. funebris* is carnivorous and feeds mainly on sponges such as *Xestospongia* sp., *Haliclona* sp., *Euplaccella* cf. *australis*, and *Oceanapia* sp.<sup>517</sup> Jorunnamycin C (**85**; Fig. 24; Table II) and renieramycin M (**86**, Fig. 24, Table II) differ only in the C-22 ester side chain. These two compounds display GI<sub>50</sub> values in the low nanomolar ranges against human colon (HCT-116) and breast (MDA-MB-435) cancer cells.<sup>518</sup> Charupant et al.<sup>518</sup> showed that the downregulation of protein tyrosine phosphatase receptor type K (PTPRK) could be one of the pathways through which jorunnamycin C (**85**) and renieramycin M (**86**) exert their anticancer effects, at least *in vitro*. PTPRK acts as a tumor suppressor gene product<sup>519</sup> and could be implicated in the progression of colon cancer.<sup>520</sup>

Sphingolipid-related compounds called spisulosines were isolated from the Nord-Atlantic clam *Spisula polynyma* (syn. *Mactromeris polynyma*; Table I).<sup>521,522</sup> Sphingolipids constitute a group of natural products characterized by the presence of a long chain 2-amino-1,3-diol scaffold or sphingoid base.<sup>522</sup> As emphasized by Abad et al.,<sup>522</sup> besides playing a structural role and regulating the physical properties of cell membranes, sphingolipid metabolites also participate in cell signaling and in the control of numerous cellular functions in mammals. Spisulosines constitute a group of 1-deoxysphingolipids derived from a central core lacking the C1-OH group present in dihydrosphingosine.<sup>522</sup> One of these spisulosines is ES-285 (**87**;

Fig. 24; Table II under Spisulosine), which resembles the natural sphingolipids in the C18 sphingoid backbone and in the (2*S*,3*R*) configuration of the amino and hydroxyl groups, respectively.<sup>522</sup> ES-285 is associated with GI<sub>50</sub> values ranging between low nanomolar and low micromolar in the NCI 60 cancer cell line panel.<sup>522-526</sup> ES-285 triggers an atypical cell death pathway because it does not affect pathways widely implicated in regulating cell survival and apoptosis, such as JNK, ERKs, or Akt, but it nevertheless activates caspase-3 and caspase-12.<sup>525</sup> Sánchez et al.<sup>526</sup> showed that ES-285 inhibits the growth of human PC-3 and LNCaP prostate cancer cells through intracellular ceramide accumulation and PKC $\zeta$  activation. This compound modulates RHO protein and ceramide signaling and consequently induces disassembly of the actin stress fibers,<sup>527</sup> but does not affect the microtubule network.<sup>525</sup>

ES-285 is COMPARE-negative, suggesting therefore a possible novel mechanism of anticancer action as compared to the other anticancer drugs.<sup>524</sup> It displayed significant *in vivo* anticancer activity in a hollow fiber model with s.c. implanted SK-HEP-1 hepatoma tumor cells, and xenografted models utilizing human PC-3 prostate and MRI-H-121 renal cancer cells.<sup>524</sup> ES-285 has entered several Phase I clinical trials since the late 2000s. Baird et al.<sup>524</sup> report that pharmacologically relevant concentrations of this drug were safely achieved in adult cancer patients, with pharmacogenomics studies indicating changes in the expression of genes of potential mechanistic relevance. Schöffski et al.<sup>528</sup> reported hepato- and neurotoxicity as schedule independent dose-limiting adverse events for ES-285 were noted. Vilar et al.<sup>529</sup> also observed liver enzyme elevations as dose limiting for ES-285 administration to cancer patients. Massard et al.<sup>530</sup> also recently conducted a Phase I clinical trial. To the best of our knowledge, there is no available information on its Phase II clinical trials.

## 5. TARGETED DELIVERY OF MOLLUSK-DERIVED ANTICANCER AGENTS

Some of the issues that have prevented the advancement of mollusk-derived anticancer agents to the clinic can be mitigated by employing delivery vehicles that can improve the drugs' ability to impart a strong therapeutic action while minimizing off-target effects. Nanomedicine, or the medical application of nanotechnology, has been at the forefront of the anticancer drug delivery field. Nanomedicines are designed to improve the drug's solubility in physiological fluids, improve the drug's biodistribution and pharmacokinetics, passively or actively target tumors, and protect the drug from premature degradation or excretion. Nanomedicines including liposomes, micelles, nanoparticles, nanoemulsions, nanocapsules, dendrimers, and macromolecular conjugates have been utilized to improve the performance of many anticancer agents.<sup>531</sup> These drug delivery systems can target tumors passively by taking advantage of the enhanced permeability and retention (EPR) effect which is a result of the high permeability of leaky angiogenic blood vessels and poor lymphatic function at the tumor.<sup>532,533</sup> In addition, nanomedicines can be actively directed to molecular targets overexpressed in tumors by inclusion of high affinity ligands such as antibodies, aptamers, or peptides on their structure.<sup>532,534,535</sup>

Nanomedicines can utilize a number of materials including biocompatible lipids, polyesters, oligosaccharides, polyethers, oligonucleotides, peptides, and even proteins. Among these materials, the polyether poly(ethylene glycol) (PEG) has been widely used in drug delivery applications due to its ability to shield hydrophobic biomaterials and increase the circulation time of macromolecules and nanocarriers.<sup>536</sup> Nanomedicines can also include activatable switches that maintain the drug in an inactive, nontoxic state until activated by low pH, reducing conditions, or proteolytic enzymes that are overexpressed in tumors.<sup>537</sup> Nanomedicines can enable delivery of high-drug payloads and have been reported to overcome MDR.<sup>531</sup> Several nanomedicine formulations of anticancer agents including Doxil<sup>TM</sup>, Myocet<sup>TM</sup>, DaunoXome<sup>TM</sup>, and Abraxane<sup>TM</sup> have been approved by the FDA for clinical use. Among the various types of nanomedicines,

surprisingly only few types of nanovectors (namely micelles, nanoparticles, and conjugates) have been explored with mollusk-derived anticancer agents. In all cases, these nanovectors were used as means to increase the solubility of or to minimize dose-limiting toxicity associated with these highly active anticancer agents by targeting tumors. Next, we summarize the reported design as well as results of *in vitro* and *in vivo* evaluation, as available, of nanovectors utilized for the targeted delivery of mollusk-derived anticancer agents.

### A. Lamellarins

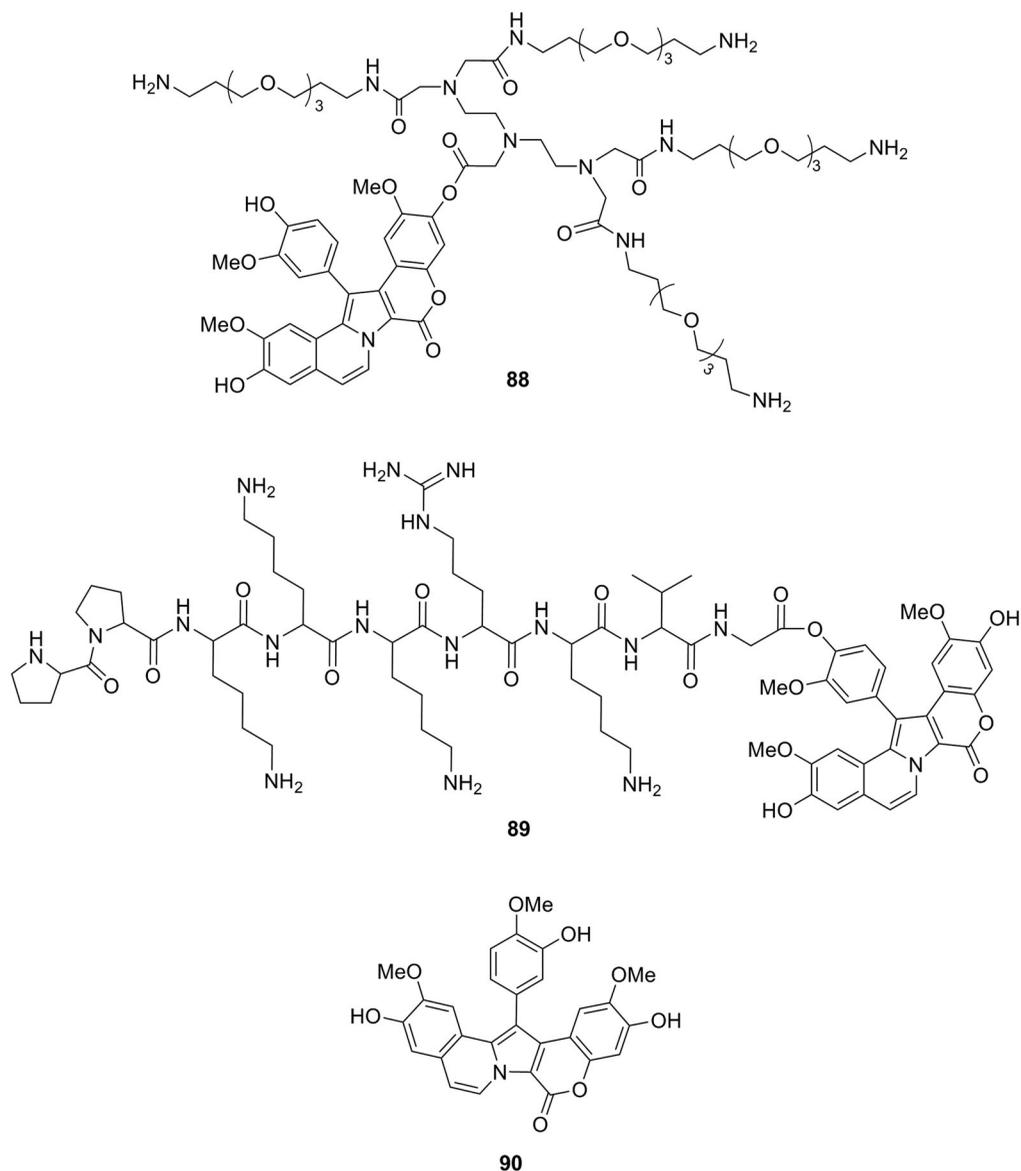
Macromolecular covalent conjugates of lamellarin D (**76**, Fig. 22; Table II) were prepared to enhance the solubility of this hydrophobic alkaloid. A multiarm PEG dendrimer conjugate of lamellarin D (**88**, Fig. 25) decreased the GI<sub>50</sub> value of the drug by 40% in MDA-MB-231 breast cancer cells, but showed slightly higher GI<sub>50</sub> in HT29 colon cancer cells and A549 lung cancer cells.<sup>538</sup> On the other hand, a nuclear localization sequence (NSL) oligopeptide conjugate of lamellarin D (**89**, Fig. 25) had lower GI<sub>50</sub> values in all three cancer cell lines.<sup>538</sup> Importantly, both conjugates **88** and **89** showed reduced growth inhibition in noncancerous BJ skin fibroblasts, demonstrating bioselectivity of these drug delivery systems toward cancer cells.<sup>538</sup> *in vivo* evaluation of these conjugates has not been reported.

Lamellarin N (**90**, Fig. 25), a hydrophobic compound ( $\log P = 3.37$ ) isolated from mollusks and later found in ascidians and sponges, was incorporated into polymeric micelles as a means to improve its aqueous solubility. Biodegradable micelles are self-assembled nanostructures made from amphiphilic block copolymers where the hydrophobic polymer segments form an inner core in which hydrophobic drugs can be entrapped, and the hydrophilic segments form a protective shell that interacts with the surrounding aqueous medium. Micelles of the amphiphilic block copolymers poly(ethylene glycol)-*b*-poly( $\epsilon$ -caprolactone) (PEG-*b*-PCL) and poly(ethylene glycol)-*b*-poly(D,L-lactide) (PEG-*b*-PLA) were used to encapsulate Lamellarin N.<sup>539</sup> Importantly, encapsulation increased the concentration of lamellarin N that could be suspended in water by up to 200-fold compared to the free drug.<sup>539</sup> Neither *in vitro* nor *in vivo* evaluation of these micelles was reported.

### B. Kahalalides

As previously mentioned, kahalalide F (**15a**, Fig. 6; Table II) is a depsipeptide that has been evaluated in Phase II clinical trials for the treatment of melanoma, liver cancer, and NSCLC. Unfortunately, these clinical trials failed to demonstrate anticancer response in patients. The activity of two diastereomeric cysteine analogues of kahalalide F (**91a** and **91b**, Fig. 26) conjugated to 20- and 40-nm gold nanoparticles was evaluated *in vitro*.<sup>540</sup> The gold-drug nanoscaled conjugate aimed to utilize the nanoparticles' ability to deliver a high payload of the peptide to the cells intracellularly, thereby increasing therapeutic efficacy. Studies in HeLa cervical carcinoma cells showed that although the cell growth inhibition of the 20-nm gold nanoparticle conjugates was not different than that of the free peptides, the 40-nm conjugates of **91a** and **91b** resulted in higher cell growth inhibition.<sup>540</sup> Similarly, higher nanoparticle uptake into HeLa cells was observed with 40-nm nanoconjugates as demonstrated with confocal fluorescence microscopy.<sup>540</sup> Neither *in vitro* nor *in vivo* therapeutic efficacy was evaluated with these nanoparticle systems.

Elisidepsin (**15b**, Fig. 6), a cyclic peptide of kahalalide F, also failed clinical trials due to poor therapeutic activity. An elisidepsin oral formulation based on 150-nm solid lipid nanoparticles of Precirol® ATO 5 was evaluated as a means to increase the oral bioavailability of this drug.<sup>541</sup> This delivery system was evaluated in Beagle dogs and compared to a cyclodextrin-based formulation.<sup>541</sup> Despite not being able to increase oral elisidepsin bioavailability above 1%, the

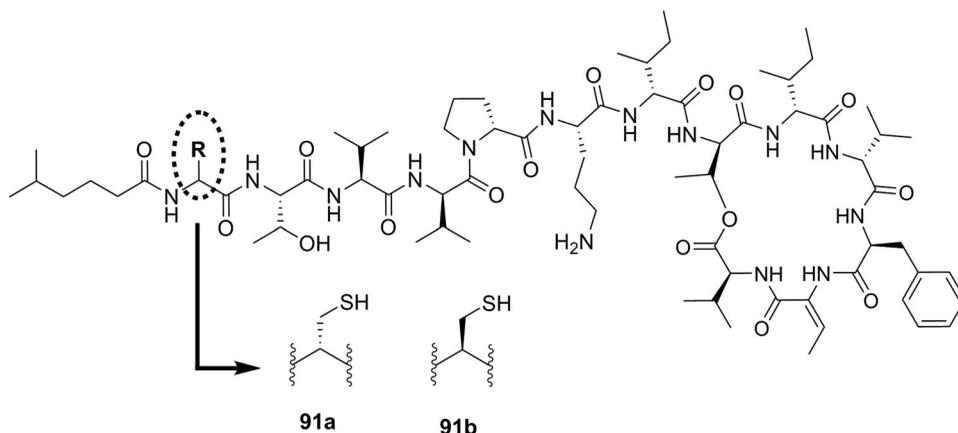


**Figure 25.** Chemical structures of multiarm PEG dendrimer of lamellarin D (**88**), nuclear localization sequence oligopeptide conjugate of lamellarin D (**89**), and lamellarin N (**90**).

nanoparticle formulation enhanced drug absorption and maintained the drug concentration in the blood within measurable levels over a longer timeframe than cyclodextrin.<sup>541</sup> The *in vivo* therapeutic efficacy of this oral delivery system was not reported.

### C. Dolastatins

Several dolastatins have been formulated into antibody conjugates as a means to increase their concentration at the tumors while minimizing off-target effects. The FDA-approved monomethyl auristatin E (MMAE) conjugate with anti-CD30 antibody (brentuximab vedotin) has been approved for the treatment of Hodgkin's lymphoma. As previously



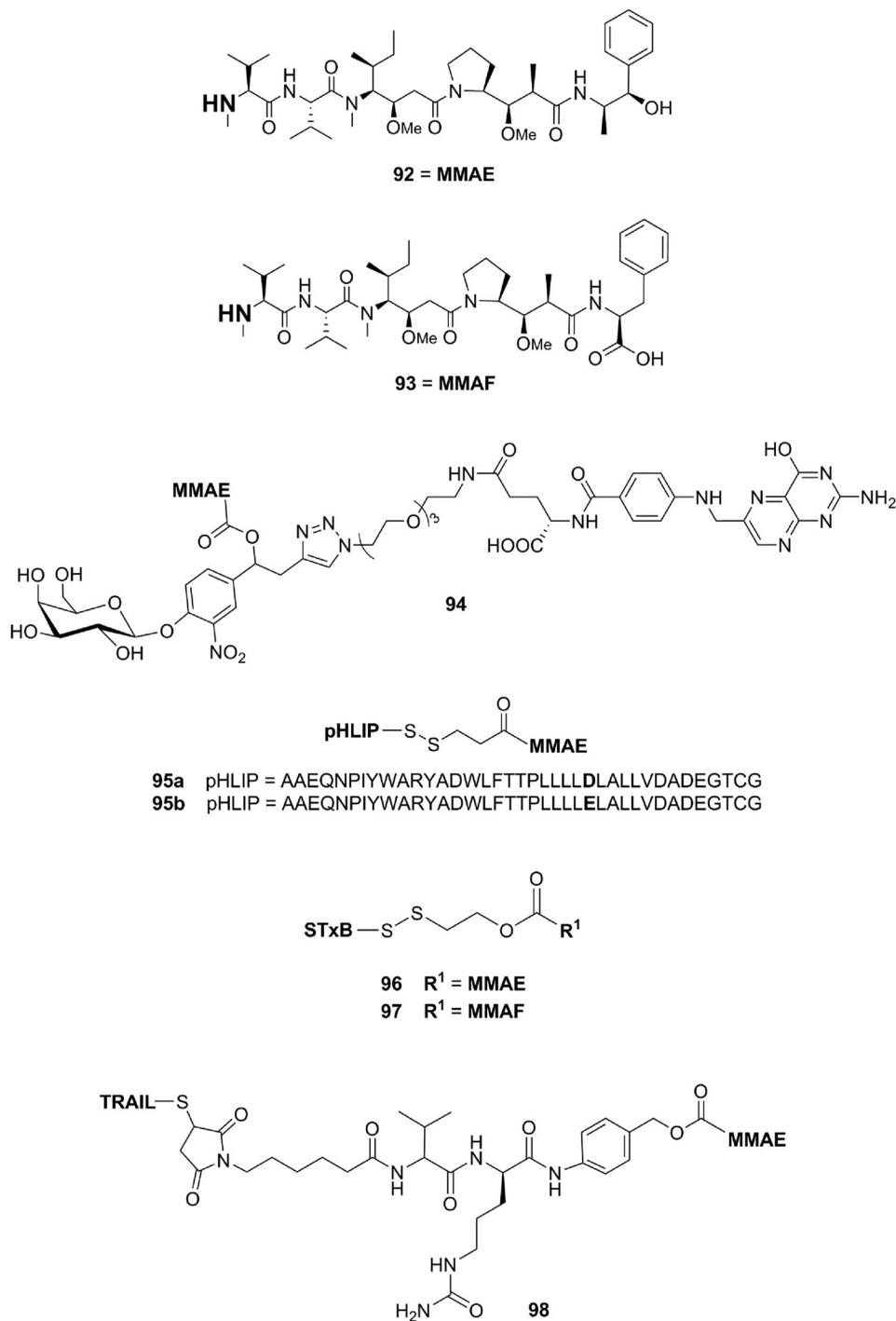
**Figure 26.** Chemical structures of D-cys (**91a**) and L-cys (**91b**) analogs of Kahalalide F that were conjugated to gold nanoparticles for intracellular delivery.

mentioned, antibody–drug conjugates have been previously reviewed<sup>166,200,219,222</sup> and will not be further discussed in this review. Most nanomedicine systems for the delivery of dolastatin 10 derivatives MMAE (**92**, Fig. 27) and monomethyl auristatin F (MMAF, **93**, Fig. 27). Here, we summarize current efforts to develop innovative targeted and activatable auristatin nanomedicines that are designed to protect healthy tissue from the drugs' action while achieving high anticancer effect.

A folate-directed conjugate of MMAE (**94**, Fig. 27) based on a self-immolative linker that could be activated by lysosomal  $\beta$ -galactosidase was developed by Legigan et al.<sup>542</sup> *in vitro* tests demonstrated over 20-fold higher growth inhibition by **94** in folate receptor-positive KB and HeLa cells compared to folate receptor-negative A549 cells.<sup>542</sup> Conjugate **94** also demonstrated very high anticancer activity, with a  $GI_{50}$  of  $\sim 0.2$  nM in KB cells. This system was not evaluated *in vivo*.

Burns et al.<sup>543</sup> demonstrated a pH-activated system for the delivery of MMAE based on pH (low) insertion peptide (pHLIP) conjugates (**95a** and **95b**, Fig. 27) that undergo conformational changes under the acidic pH typical of tumors. Specifically, at a pH of  $\sim 6$ , the pHLIP conjugates insert into the membrane and deliver their cargo directly into the cytoplasm of target cancer cells. MMAE was conjugated to the C-terminus of wild type (**95a**) and modified (**95b**) pHLIP via a disulfide bond that could undergo intracellular reduction for delivery of an N-modified MMAE derivative.<sup>543</sup> Studies with HeLa and MDA-MB-231 cancer cells demonstrated over 90% inhibition of cell growth after a 2-hr exposure to both drug conjugates.<sup>543</sup> Conjugate translocation and intracellular release of the drug occurred faster than passive diffusion of free drug. Finally, cell growth inhibition with the conjugates was over 11-fold higher at pH 5.0 versus pH 7.4.<sup>543</sup> A pilot *in vivo* study also showed that conjugate **97a** was able to target triple-negative MDA-MB-231 breast cancer xenografts in mice, although the *in vivo* antitumor effect was not investigated.<sup>543</sup>

Batisse et al.<sup>544</sup> developed delivery systems that targeted MMAE and MMAF to glycosphingolipid globotriaosylceramide (Gb3, also referred to as CD77) positive cancer cells.<sup>544</sup> The systems consisted of degradable conjugates of MMAE and MMAF and the cysteine-terminated B-subunit of the Shiga toxin (STxB) (**96** and **97**, respectively, Fig. 27).<sup>544</sup> The conjugates were designed to enable release of the active agents under reducing conditions.<sup>544</sup> *in vitro* studies in Gb3-positive and Gb3-negative HT29 colorectal cancer cells showed that the conjugates had approximately 100-fold higher anticancer activity on Gb3-positive cells ( $GI_{50}$  in nM range).<sup>544</sup> This is in contrast to free MMAE or MMAF, whose cytotoxicities were independent of Gb3



**Figure 27.** Chemical structures of monomethyl auristatin E (MMAE, **92**), monomethyl auristatin F (MMAF, **93**),  $\beta$ -galactosidase activatable folate-targeted conjugate of MMAE (**94**), wild type (**95a**) and modified (**95b**) pH (low) insertion peptide conjugates of MMAE, Shiga toxin conjugates of MMAE (**96**) and MMAF (**97**), and TRAIL-MMAE conjugate (**98**). In **94**, **95**, **96**, **97**, and **98**, MMAE and MMAF are conjugated to the carriers utilizing the monomethyl nitrogen that is bolded in structures **92** and **93**.

expression. In addition, the activity of the conjugates on Gb3-positive cells was either similar to (96) or higher (97) than that of the free drugs.<sup>544</sup> These conjugates have not yet been tested *in vivo*.

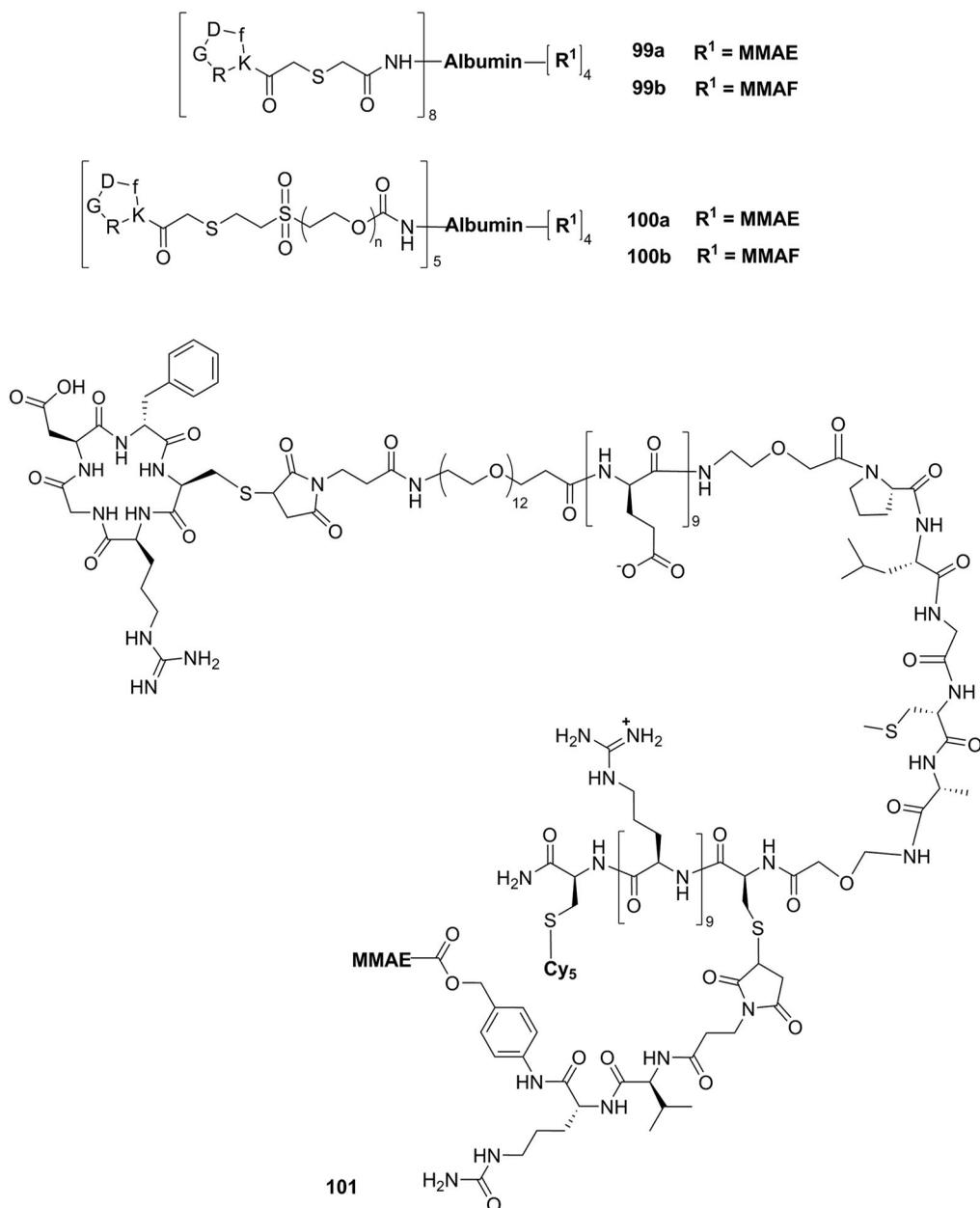
Pan et al.<sup>545</sup> developed a conjugate of MMAE and the TRAIL with the purpose of overcoming TRAIL resistance in cell lines expressing the TRAIL-targeted DR4 and DR5 death receptors. The conjugate termed N109C-vcMMAE was prepared by attachment of MMAE and TRAIL through a dipeptide (valine-citrulline) moiety (98, Fig. 27) that could be cleaved by lysosomal cathepsin to release active MMAE.<sup>545</sup> This conjugate showed the highest activity with a GI<sub>50</sub> of ~63 nM in the TRAIL-resistant MCF-7 cell line.<sup>545</sup> Studies demonstrated that conjugate internalization occurred by TRAIL-death receptor-mediated endocytosis, and that cell cycle arrest in the G2-M phase could be affected within 8 hr of exposure to the conjugate.<sup>545</sup> *in vivo* studies in a mouse NCI-H460 lung cancer xenograft model demonstrated the ability of the N109C-vcMMAE conjugate to target the tumors, potentially by taking advantage of the EPR effect.<sup>545</sup> The antitumor efficacy of the conjugate was not evaluated in this animal model.

Temming et al.<sup>546</sup> developed arginine-glycine-aspartic acid (RGD)-targeted albumin carriers for MMAE. Specifically, MMAE-human albumin conjugates 99a and 100a (Fig. 28) decorated with cyclic c(RGDfK) peptides were used to target and cause apoptosis of  $\alpha_v\beta_3$ -integrin-expressing angiogenic endothelial cells, thereby destroying the tumor's blood supply.<sup>546</sup> These conjugates also utilized a valine-citrulline linker to enable intracellular release of MMAE from the albumin carrier upon activation by lysosomal cathepsin.<sup>546</sup> The two carriers differed in the use of either a short alkyl chain (99a) or a poly(ethylene glycol) (PEG) spacer (100a) for attachment of the targeting RGD peptide. Both carriers showed high specificity toward, internalization into, and growth inhibition (GI<sub>50</sub> in nM range) of  $\alpha_v\beta_3$ -integrin-expressing human umbilical vein endothelial cells (HUVEC).<sup>546</sup> Preliminary evaluation in a C26 murine colon carcinoma mouse xenograft model showed that these carriers could preferentially accumulate on tumor blood vessels as well as inside of tumors within 24 hr of intravenous injection.<sup>546</sup> The antitumor effect of these systems was not evaluated *in vivo*.

This same group also evaluated RGD-targeted MMAF albumin conjugates.<sup>547</sup> As before, two conjugate designs were tested for each of the two drugs: one that used a PEG chain and another a short linker for attachment of the targeting RGD moiety.<sup>547</sup> Both MMAF conjugates (99b and 100b, Fig. 28) showed strong ability to reduce HUVEC cell growth, with GI<sub>50</sub> values of 22–77 nM compared to 65–420 nM for MMAE conjugates.<sup>547</sup> Similar trends were observed in C26 colon cancer cells, with GI<sub>50</sub> values of ~10–150 nM for MMAF conjugates and ~530–2070 nM for MMAE conjugates.<sup>547</sup> Noticeably, RGD-targeted MMAF conjugates 99b and 100b were more effective than free MMAF, demonstrating that the use of active targeting enabled intracellular delivery of this charged drug into the cells.<sup>547</sup> These systems were not evaluated *in vivo*.

Recently, the use of activatable cell-penetrating peptides (ACPP) consisting of a polycationic cell penetrating peptide (polyarginine) and a polyanionic (polyglutamate) peptide that are joined by a MMP-cleavable linker was used for the delivery of MMAE.<sup>548</sup> MMAE was covalently conjugated to the polyanionic portion of an ACPP using a linker that could be cleaved by lysosomal cathepsin B.<sup>548</sup> The ACPP was further targeted to  $\alpha_v\beta_3$ -integrin-expressing cells using cyclic RGD (101, Fig. 28). *in vitro* studies showed that the targeted peptide was readily uptaken by a number of human cancer cell lines.<sup>548</sup> In addition, *in vivo* studies in MDA-MB-231 mouse xenograft and in immunocompetent syngenic Py230 murine models demonstrated that the RGD-targeted MMAE-ACPP conjugate 101 controlled tumor growth more effectively than either free MMAE or an MMAE-ACPP conjugate targeted with a sham RAD peptide.<sup>548</sup>

The use of the MMAE-ACPP conjugate 101 as a radiosensitizer for combination chemo/radiotherapy was investigated by the same group.<sup>549</sup> RGD-targeted MMAE-ACPP



**Figure 28.** Chemical structures of c(RGDfK)-targeted conjugates of MMAE and MMAF to albumin using short alkyl chain linker (**99a** and **99b**) or a poly(ethylene glycol) spacer (**100a** and **100b**), and activatable cell-penetrating peptide conjugate of MMAE (**101**). In all of these structures, MMAE and MMAF are conjugated to the carriers utilizing the monomethyl nitrogen that is bolded in structures **92** and **93**.

conjugate **101** was shown to sensitize human colorectal and pancreatic cancer cells to radiation, resulting in increased DNA damage upon dual treatment.<sup>549</sup> Studies in mouse xenograft models with PANC-1 and HCT-116 tumors showed that the targeted conjugate paired with ionizing radiation provided enhanced tumor regression than radiation alone, free MMAE, or MMAE in combination with radiation.<sup>549</sup>

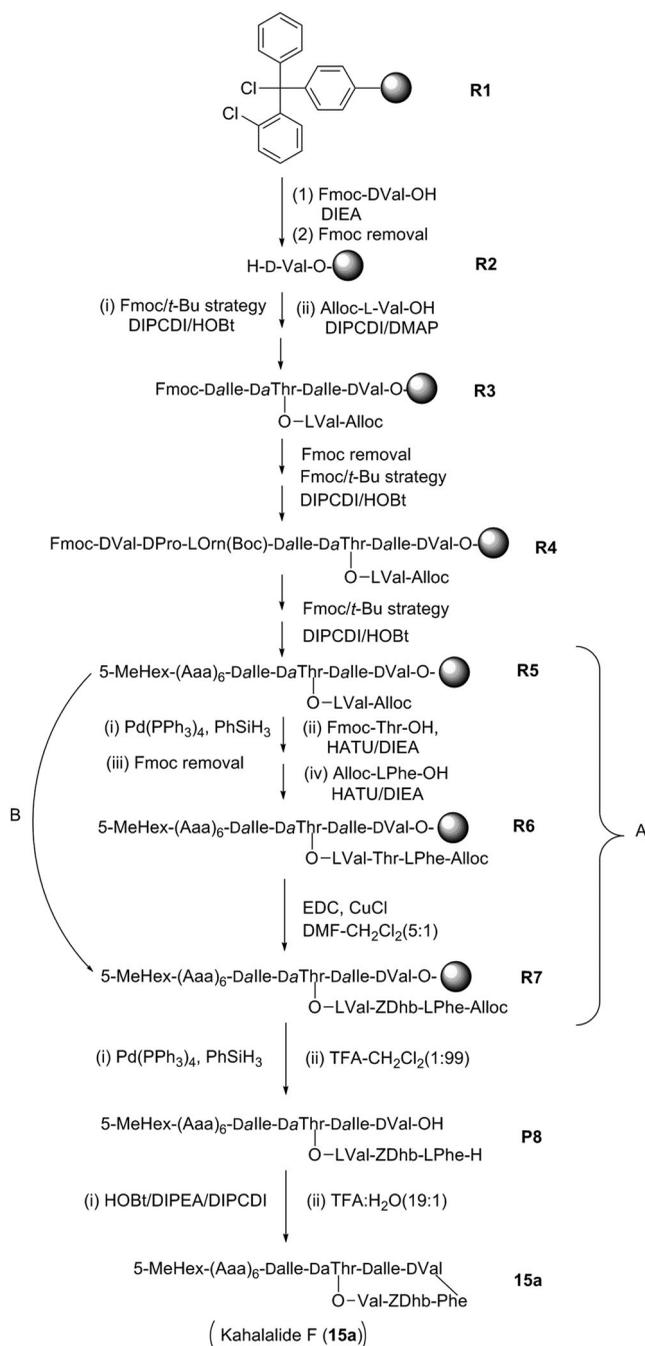
As described, nanomedicine formulations promise to improve the activity and safety of mollusk-derived anticancer agents by increasing the payload and specificity of these agents toward cancer cells. While not many types of nanomedicines have been explored as of yet for the targeted delivery of mollusk-derived anticancer agents, and even fewer evaluated for *in vivo* therapeutic efficacy, the few systems reported to date all showed improvements in solubility, targeting ability, or therapeutic efficacy compared to the free drugs. We therefore believe that there is a great opportunity for further development in this field that could enable clinical advance of these active therapeutic agents in the future.

## 6. THE PROBLEM OF SUPPLY OF MOLLUSK-DERIVED ANTICANCER AGENTS

Usually, chemists working in a marine natural product laboratory isolate only a few milligrams of a promising anticancer compound of novel structure. In the case of marine mollusks, bioactive metabolites are usually available in very small amounts—often less than 1 mg—due either to compound's low natural abundance in these organisms or the difficulty of sampling mollusk biomass. Only small amounts of pure compounds are required for preliminary *in vitro* screening in cancer cell lines. In contrast, the preclinical evaluation including studies in *in vivo* rodent models often require over 100 mg of a pure compound. Subsequently, grams to hundreds of grams are usually needed to progress satisfactorily toward clinical trials. Finally, when a compound is commercialized as a pharmaceutical, kilograms of the pure compound are required to supply drug production.<sup>220, 550, 551</sup> Due to this, for many years the major perception in the medical and in general scientific communities has been that marine active agents are not directly feasible for further development.<sup>552</sup> This was true indeed in the past. In recent years, however, the application and combination of novel techniques in harvest/culture and isolation processes, the increased knowledge of genomics, and major advances in chemical synthesis have enabled the large-scale production of several marine natural products.<sup>110, 220, 550, 551</sup>

What strategies can be utilized to scale up the production of mollusk-derived anticancer compounds? In principle, similar to other invertebrates, this may be performed by large-scale culture including mariculture (growth of the organism in its natural environment) and aquaculture (under artificial conditions). However, due to critical environmental parameters (temperature, pH, light cycle) and nutrition issues,<sup>553–555</sup> the cultivation or maintenance of mollusk specimens is very difficult and often impossible. Although large-scale cultivation has been widely applied to bivalves for food production<sup>556</sup> and significant advances have been achieved in the larviculture and grow out of marine snails for human consumption,<sup>557, 558</sup> the cultivation of mollusks for biotechnological applications is yet to be addressed. To date, the only successful example is the production of the sea slug *Aplysia californica*, which is the well-known model organism widely used in neuroscience.<sup>559</sup> In addition, if a compound comes from the diet including microorganisms,<sup>110</sup> the proper source has to be identified for its production.

Therefore, for almost all mollusk-derived products, the only feasible route to overcome the supply problem is chemical synthesis. Synthesis provides additional advantages; in fact, it may not only provide corroboration for the structural assignment, but also may lead to the production of valuable intermediates and analogues to be used for SAR studies.<sup>560</sup> Synthetic strategies also include the development of derivatives with more manageable properties, or the design of a pharmacophore of reduced complexity, which can be synthesized more easily. For example, the production of kahalalide F for its use in clinical trials was developed using solid-phase peptide chemistry techniques by PharmaMar and according to the same synthetic procedure, more than 150 analogues were prepared for SAR studies.<sup>263, 561, 562</sup> This synthesis reported by Jimenez et al.<sup>563</sup> is illustrated in Figure 29 and described next.



**Figure 29.** Synthesis of Kahalalide F utilized for its production for clinical trials using solid-phase peptide chemistry. Alloc, allyloxycarbonyl; DIEA, *N,N*-diisopropylethylamine; DIPCDI, *N,N'*-diisopropylcarbodiimide; DMAP, 4-dimethylaminopyridine; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; Fmoc, fluoromethyloxycarbonyl chloride; HATU, 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate, HOAT, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxybenzotriazole; Pd(PPh<sub>3</sub>)<sub>4</sub>, tetrakis (triphenylphosphine)palladium(0); PhSiH<sub>3</sub>, phenylsilane; t-Bu, tert-butyl; TFA, trifluoroacetic acid; (Z)-Dhb, a,b-didehydro-a-aminobutyric acid.

This solid-phase synthesis was based on an orthogonal protecting system employing chlorotriyl chloride as resin (Cl-TrtCl-resin, **R1**, Fig. 29). Resin **R1**, was converted to H-D-Val resin **R2** using Fmoc-D-Val-OH and DIEA followed by Fmoc removal. A sequential incorporation of D-*allo*-Ile, D-*allo*-Thr, and D-*allo*-Ile derivatives using Fmoc/*t*-Bu and DIPCDI/HOBT reagent mixture followed by esterification with *allo*-Val-OH using DIPCDI/DMAP produced tetrapeptide resin **R3**. This was then converted to decapeptide resin **R5** by chain elongation through a sequential addition of six amino acids. The N-terminus was then capped with 5-methylhexanoic acid using the Fmoc removal protocol, Fmoc/*t*-Bu strategy, and the coupling reagent mixture DIPCDI/HOBT. Resin **R7** was synthesized by two methods. In method A, tridecapeptide **R6** was prepared from **R5** by side chain elongation using first Pd(PPh<sub>3</sub>)<sub>4</sub> and PhSiH<sub>3</sub>, followed by treatment with FmocThr-OH and HATU/DIEA and Fmoc removal. Next, the reaction mixture was treated with Alloc-LPhe-OH and HATU/DIEA to yield **R6**. The preparation of **R6** was followed by selective synthesis of (Z)-Dhb on the resin by Fukase's conditions (EDC and CuCl) to give **R7**. In method B, the dipeptide (Alloc-Phe-(Z)-Dhb-OH) was directly added to **R5** using Pd(PPh<sub>3</sub>)<sub>4</sub>, PhSiH<sub>3</sub>, Alloc-LPhe-ZDhb-OH, and DIPCDI/HOAt to yield **R7**. Resin **R7** was converted to depsi-peptide resin **R8** by removal of Alloc group in **R7** with Pd(PPh<sub>3</sub>)<sub>4</sub> and PhSiH<sub>3</sub>, followed by peptide release from the resin using TFA/CH<sub>2</sub>Cl<sub>2</sub> (1:99) mixture. The macrocyclization of peptide **P8** with HOBT/DIPEA/DIPCDI and the removal of the side chain protecting groups using TFA/H<sub>2</sub>O (19:1) mixture afforded kahalalide F (**15a**; Fig. 6; Table II).

The synthetic methods performed to produce dolastatins<sup>217</sup> have led to a number of novel intermediate substances such as auristatins and to antibody–drug conjugates currently in Phases I to III clinical trials.<sup>166, 222, 564</sup>

For an increasing number of synthetically challenging anticancer compounds from mollusks the supply problem has been solved through semisynthesis by starting from a more accessible precursor—typically of microbial origin. An example is Zalypsis® (PM00104, **84b**), which was synthesized by workers at PharmaMar (Madrid, Spain) using methodologies related to the trabectedin synthesis from safracin B.<sup>565</sup> Moving to more chemically complex molecules, the challenges associated with their total synthesis are significant but not insurmountable. Today, chemists can synthesize organic compounds—natural and designed—of all types of structural architectures.<sup>566</sup> In the last few decades a surge in total synthesis endeavors around the world in constructing natural products of medicinal importance against cancer has led to a remarkable collection of achievements that covers a wide ranging landscape of molecular and stereochemical complexity and diversity.<sup>566</sup>

Of course, scalability of the entire synthetic process leading to the target compound remains a crucial feature.<sup>567</sup> Historically, the pharmaceutical industry's acceptance of structurally complex natural products or natural product derivatives supplied commercially through total synthesis has been low for a variety of reasons, but the situation is slowly changing. The accessibility of target active molecules by simple scalable routes is based on a series of factors including step count, level of complexity of the chemistry used, stereochemical control, chemical stability of each process and intermediate, overall yield, and operational complexities, among others. An increasing number of potential anticancer marine natural products are now prepared in a scalable fashion.<sup>567</sup> The most significant example of a large-scale total synthesis that has solved a major supply issue is that of eribulin mesylate (Halaven®) inspired by the marine natural product halichondrin B.<sup>568</sup> Eribulin is a macrocyclic ketone with 36 atoms, 19 of which are stereogenic centers, that represents the most structurally complex, nonpeptidic, and fully synthetic drug on the market today. However, it should be taken into consideration that the “supply issue” is only one of the crucial aspects in anticancer drug development. The so-called “valley of death” that is the gap between the discovery of the active principle and its entering clinical trials is a highly complex problem where numerous driving forces—scientific, financial,

and other economic and nontechnical factors—play a role.<sup>569</sup> Scientific critical tasks include drug production, of course, but also tumor physiology, drug pharmacokinetics, preclinical models, drug delivery, and clinical translation. The lack of common methods to address these aspects by industrial pharmaceutical companies (Pharma) and academic scientists (Academia) is a fundamental point. Only a greater Pharma-Academia collaboration could overcome the barrier of the valley of death in the development of new effective anticancer therapies.<sup>570</sup>

## 7. CONCLUSIONS: WHAT ARE THE MOST PROMISING MOLLUSK-DERIVED ANTICANCER COMPOUNDS?

Of note, no “recent” mollusk-derived compounds have attained late Phase II or Phase III clinical trials in oncology. However, we think that several “mollusk-derived” compounds merit further consideration.

In terms of *in vivo* activity, we think that a compound for which clear evidence of activity has been demonstrated in various models (including aggressive metastatic) has a greater probability to move toward clinical trials than a compound that has not been assayed *in vivo*, displays no activity *in vivo*, or whose *in vivo* activity has been demonstrated, for example, in ascitic models of mouse leukemia, which are by far too chemosensitive and not at all representative of the clinical situation.

It is interesting that several dolastatins (or their derivatives) displayed marked *in vivo* anticancer activity in multiple preclinical models (murine syngeneic as well as human xenograft models), but failed to demonstrate actual anticancer activity in Phase II clinical trials. It is important to keep in mind that tumor doubling times markedly differ between murine tumor models (days to weeks) and human cancers (weeks to years).<sup>571</sup> Dolastatins are tubulin inhibitors, which are known to be more effective against rapidly proliferating cells. Human orthotopic xenografts in most cases develop much more rapidly in immunocompromised or immunodeficient mice than their counterparts developing in humans. Furthermore, dolastatins are tubulin inhibitors without other reported targets in the literature (at least to the best of our knowledge). They are cytotoxic proapoptotic compounds and substrates of the P-gp, which makes them unable to overcome resistance to proapoptotic stimuli or circumvent the MDR phenotype. It is important to emphasize once again that cancers associated with dismal prognoses, including metastatic cancers, display resistance to proapoptotic stimuli.

According to data we report in the current review, we estimate that the following compounds are “promising” and should be pursued further: dolastatin 16 (**9**), chlorolissoclimide (**33b**), dolicolide (**46**), ulapaulide A (**60a**), kabiramide C (**61a**), halichondramide-related metabolites (**62a-62c**), aplyronine A (**64a**), sphinxolide (**66**), latrunculin A (**67a**), hectochlorin (**75a**), lamellarin D (**76**), and spisulosine (**87**). We urge the readers of the current review to perform experiments designed to address the major “biological cancer characteristics” we detailed in the Introduction to create a stronger basis for the selection of promising anticancer agents derived from marine mollusks.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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**Maria Letizia Ciavatta** studied Pharmacy at the University "Federico II" of Naples, getting graduation "cum laude" in 1988. Since 1999 she is a Researcher at Istituto di Chimica Biomolecolare (ICB) of the Italian Consiglio Nazionale delle Ricerche (CNR). Her research interests are focused on the exploration of the biodiversity in natural products, involving skills in the purification and structural characterisation of new compounds from both marine and terrestrial sources. She is coauthor of more than 70 peer-reviewed articles. Her profile is available on Research Gate.

**Florence Lefranc** obtained an M.D. degree in 1995 at the Université Libre de Bruxelles (ULB, Brussels, Belgium) and then she received her neurosurgeon diploma in 2002 (ULB). She received a Ph.D. in tumor cell biology in 2005 in ULB and she is currently neurosurgeon and chief of neuro-oncology at the Department of Neurosurgery at Hôpital Erasme (ULB, Brussels, Belgium). She was also a M.D. post-doctoral fellowship with the Fonds National de la Recherche Scientifique (FNRS, Belgium). Her main research area relates to the identification of biologicals or natural compounds aiming to combat glioma, and also to novel semisynthetic or synthetic compounds. She has authored or co-authored ~150 peer-reviewed articles and she is currently conducting several clinical trials with glioma and meningioma patients.

**Marianna Carbone** is Researcher at the Istituto di Chimica Biomolecolare of the Italian Consiglio Nazionale delle Ricerche. She received her PhD in Pharmaceutical Science from the University of Salerno working on the chemistry of marine mollusks. Her research activity is mainly focused on the isolation and structural characterization of secondary metabolites from marine invertebrates. Her studies include the evaluation of the relevant pharmacological properties of marine natural products, and the exploration of their ecological roles.

**Ernesto Mollo** is Researcher at the Istituto di Chimica Biomolecolare of the Italian Consiglio Nazionale delle Ricerche. He graduated with a Laurea degree in Biological Sciences from the University of Naples "Federico II". The primary focus of his work is on the study of the ecological roles of natural products from marine organisms and their phyletic and geographic distribution in nature. His research campaigns for the exploration of the molluscan biological and chemical diversity have been carried out on a global scale and led to the discovery of many novel bioactive metabolites.

**Margherita Gavagnin** received her doctoral degree in Organic Chemistry in 1983 from University of Naples. In 1985 she moved to the Institute of Biomolecular Chemistry (ICB), Italian National Council of Research (CNR) where she joined the group of Prof. G. Cimino and started working on the chemical ecology of marine opisthobranch molluscs. Currently, she is Research Director at

*ICB. Her researches are mainly focused to the definition of the structure of new bioactive molecules from marine organisms and plants. She published more than 150 papers on peer-reviewed journals.*

**Tania Betancourt** is Assistant Professor in the Department of Chemistry and Biochemistry and in the Materials Science, Engineering, and Commercialization Program at Texas State University in San Marcos, TX (USA). She obtained a Ph.D. in biomedical engineering from the University of Texas at Austin in 2007. Her laboratory's research focuses on the development of stimuli-responsive biomaterials and nanomedicines for cancer detection and treatment. She has coauthored 17 peer-reviewed articles and two book chapters. Her profile is available on Research Gate and Google Scholar.

**Ramesh Dasari** was born in Ambala, Nalgonda district, Telangana, India. He obtained his M.Sc. in organic chemistry from Osmania University, Hyderabad, India and Ph.D. in synthetic organic chemistry from Indian Institute of Chemical Technology (CSIR-IIT), Hyderabad, India. In 2012, he joined Texas State University as a Postdoctoral Research Associate. During his postdoctoral research (Oct 2012-present) at Texas State University with Prof. Alexander Kornienko, he has synthesized analogs of various structurally diverse natural products as part of their development as anticancer agents. He has published 33 peer reviewed papers.

**Alexander Kornienko** was born in a small Arctic town of Vorkuta, Russia. He studied at Mendeleev University in Moscow and then moved to the United States, where he received his Ph.D. in synthetic organic chemistry at Tufts University. After a two-year postdoctoral fellowship at the University of Montreal, working on the synthesis of novel aminoglycoside antibiotics, he started his independent career at New Mexico Tech in 2001 and then moved to Texas State University where is now Professor of Chemistry Throughout his career he published 86 refereed research papers mostly dealing with natural product-based cancer drug discovery.

**Robert Kiss** is Director of Research with the Fonds National de la Recherche Scientifique (FRS-FNRS, Belgium) at the Laboratoire de Cancérologie et de Toxicologie Expérimentale, Faculté de Pharmacie—Université Libre de Bruxelles (ULB, Brussels, Belgium). He obtained a Ph.D. in tumor cell biology at ULB in 1987. He has characterized, with the various research teams he has headed, the mechanisms of action of numerous novel anticancer drugs, with a particular attention to natural products. He has authored or coauthored more than 520 peer-reviewed articles. His profile is available on Research Gate.