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SPECIAL FEATURE: REVIEW ARTICLE

Highlights of marine natural products having parallel scaffolds found from marine-derived bacteria, sponges, and tunicates

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Abstract

Marine-derived bacteria are a prolific source of a wide range of structurally diverse natural products. This review, dedicated to Professor William Fenical, begins by showcasing many seminal discoveries made at the University of California San Diego from marine-derived actinomycetes. Discussed early on is the 20-year journey of discovery and advancement of the seminal actinomycetes natural product salinosporamide A into Phase III anticancer clinical trials. There are many fascinating parallels discussed that were gleaned from the comparative literature of marine sponge, tunicate, and bacteria-derived natural products. Identifying bacterial biosynthetic machinery housed in sponge and tunicate holobionts through both culture-independent and culture-dependent approaches is another important and expanding subject that is analyzed. Work reviewed herein also evaluates the hypotheses that many marine invertebrate-derived natural products are biosynthesised by associated or symbiotic bacteria. The insights and outcomes from metagenomic sequencing and synthetic biology to expand molecule discovery continue to provide exciting outcomes and they are predicted to be the source of the next generation of novel marine natural product chemical scaffolds.

Introduction

A brief sketch of seminal discoveries by Professor Fenical that set the stage for exploring bioactive substances from marine-derived bacteria

It has become increasingly evident that there is significant overlap between the biosynthetic machinery of marine-derived bacteria vs. that of chemically prolific invertebrates, especially sponges and tunicates. A continuing stream of statements in both primary research papers and reviews outline the hypothesis that many invertebrate-derived compounds are seemingly produced by the action of an invertebrate microbiome. Thus, microbial symbionts may be critical to the production of many marine invertebrate natural products [1]. Relevant to this possibility are two significant findings. In 2015, a family of complex alkaloids, containing three tetrahydroisoquinoline moieties, originally isolated from the tunicate *Ecteinascidia turbinate*, was eventually found to be produced by the unculturable bacterial endosymbiont *Candidatus* Endoecteinascidia frumentensis, obtained directly from metagenomic DNA [2]. Similarly, in 2017 it was noted that the unculturable and ubiquitous endosymbiont *Candidatus* Entotheonella detected in sponges including *Theonella swinhoei* (themselves a source of a diverse set of molecular structures) possessed biosynthetic richness akin to that of soil actinomycetes [3]. Thus, building a broad foundation on the biosynthetic capabilities of unique libraries of marine-derived actinomycetes is a requisite for catalyzing future research to gain a firm understanding about overlapping invertebrate/bacterial biosynthetic machinery.

The overall goal in this review is to trace selected examples of intersections in the scaffolds (i.e., biosynthetic pathways) of complex small molecules from marine sponges and tunicates vs. marine-derived bacteria. In this section, our focus is on fundamentals of marine-derived actinomycetes and discussions about invertebrates will come later. An obscure study published in 1959 illustrated

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that dozens of marine-derived actinomycetes, as obligate halophytes, could be isolated, maintained in stable culture, and further studied [4]. However, for years there was virtually no follow-up work by others especially in terms of marine natural product compound discovery. As will be discussed next, many years passed until this situation changed. The path forward involved challenging risk-taking research at the University of California San Diego (UCSD) beginning in the early 1990s. Initially it took almost a decade of trial-and-error investigations to achieve the first notable outcomes [5]. A selection of some of the most striking results is highlighted by the cluster of eight structures collected in Fig. 1.

It is interesting to note that at UCSD Professor Fenical often asked the question, "where are the new horizons in marine bioorganic chemistry?" [6]. We deem that the selected structures and accompanying annotations shown in Fig. 1 provide some answers to this crisp question and are relevant to the discussions contained in the latter sections of this review. The contents of Fig. 1 span the time window 1999–2020. We invite all readers to examine each structural drawing, then ponder their significance (shown in each panel), and gain insights on how this assemblage has catalyzed the defining and subsequently expanding fundamental understanding of the chemistry and biology of marine actinomycete bacteria.

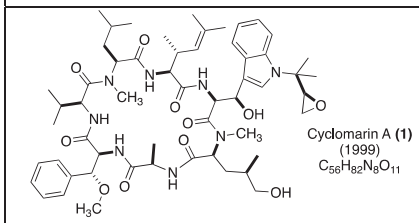
Here is a brief synopsis of selected inspirational molecules discovered at UCSD from actinomycetes. Cyclomarin (**1**), the first entry in Fig. 1, embodies a rather complex scaffold in terms of atom count ($C_{56}H_{82}N_8O_{11}$), chiral centers (12), and molecular weight (MW = 1043) [7]. It has bioactivity against organisms that cause malaria and tuberculosis is also significant. The Phase III anticancer candidate salinosporamide A (**2**) (Sal A, aka NPI-0052, marizomib) continuously isolated from *Salinispora tropica* in good yields and recently isolated in very low yields from *S. arenicola* is a compound that remains of high value more than two decades after its discovery (see Fig. 2 for a timeline) [8]. The sporolides A (**3**) and B, also from *S. tropica*, have a fascinating biogenesis including the involvement of a para-benzyne intermediate [9]. The six MRSA active marinopyrroles headed by the axial chiral atropisomer (−)-marinopyrrole A (**4**) are under intense study by many labs and the (±) form called maritoclax is commercially available [10]. At first glance ammosamide B (**5**), a relatively small achiral pigment (MW = 291) discovered in 2009 might seem unimportant, however, ammosamide A and B (**5**) possess potent activity against HCT-116 cancer cells (IC_{50} = 320 nM) [11]. Also, unlocking the mysteries about their biosynthesis is stretching molecular genetics tools to yield new hypotheses for the biosynthetic pathways of these and other amino acid containing natural products [12]. (+)-Merochlorin A (**6**) is a novel MRSA active tetracyclic chlorinated merosesquiterpene [13]. Even though X-ray analysis provided relative configuration assignments, it took an additional 7 years to finalize the absolute configurations of the members of this family through total synthesis [14]. (−)-Anthracimycin (**7**) is an antibiotic whose name celebrates its activity against *Bacillus anthracis* (MIC = 0.03 μg/mL) the anthrax bioterrorism weapon [15]. The complete structure of neaumycin B (**8**), a spectacularly potent cytotoxin (IC_{50} = 0.07 pM) against U87 human glioblastoma, was recently deduced by the UCSD team and updated two incomplete previous reports by others [16]. Interestingly, several actinomycetes strains are a source of this unique spiroketal containing polyketide.

The salinosporamide story—from a marine actinomycete-derived natural product to clinical trials

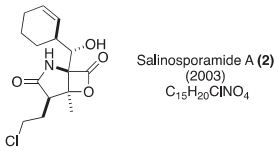
There are only a few marine natural products that have progressed through the advanced stages of clinical trials or gained FDA approval for therapeutic use. Sal A (**2**) (aka NPI-0052 or marizomib) [8] is an important member of this select group (see Fig. 3) because of its recent (2020) entry into Phase III clinical trials [17]. "Sal A" (structure shown in Fig. 1 with selected background information) is produced from a surprisingly complex biosynthetic pathway. Shown in Fig. 2 is the 20-year timeline from the isolation and characterization of **2** from a salt-water obligate marine actinomycete, *Salinispora* (strain CNB-392) to its entry into Phase III anticancer clinical trials.

We will not reiterate the many details shown in Fig. 2 but will instead focus on some additional factors. (1) "Sal A" (**2**) was initially targeted for additional SAR, biosynthetic, and experimental therapeutic investigations. This was motivated by the potent, selective results in the NCI's 60-cell-line panel with a mean GI_{50} < 10 nM and greater than a 4 log LC_{50} differential between resistant and susceptible cell lines. The greatest potency of **2** was observed against NCI-H226 non-small cell lung cancer, SF-539 brain tumor, SK-MEL-28 melanoma, and MDA-MB-435 melanoma all with LC_{50} values <10 nM [18]. (2) In 2005, a public (UCSD)–private partnership (Nereus) successfully launched preclinical evaluation culminating an Investigational New Drug application to initiate clinical testing on **2** [19, 20]. (3) Early on, the anticancer molecular target was identified when **2** was tested against purified 20S proteasome, it inhibited proteasomal chymotrypsin-like proteolytic activity with an IC_{50} value of 1.3 nM. This action is similar to that of omuralide [21], a known inhibitor of proteasome which has structural similarities but important structural deficiencies for bioactivity vs. **2**. (4) Even though many, somewhat lengthy, total syntheses have been published [22], a scale-up saline fermentation is being used to provide

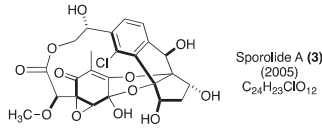
Compounds & Significance



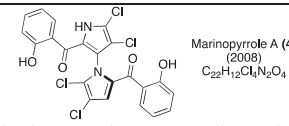
Cyclomarins A-D came from a 1990's effort to create an actinomycetes strain libraries from shallow-water San Diego sediments. One early lesson was that a non-salt-obligate *Streptomyces* sp. strain exclusively produced A-C in salt containing media. Eventually, it was discovered that only two of 46 *Salinispora arenicola* chemotypes produced cyclomarins A-D. Sustained interest in this class are due to their potent antimalarial (*P. falciparum* IC_{50} = 40 nM) and antituberculosis (IC_{50} = 3 μM) properties with action modes due to restricting the dynamics of target proteins during binding. These properties have stimulated creation of synthetic biology routes to prepare unnatural analogues (2010), and a steady stream of total syntheses (2004-2019).



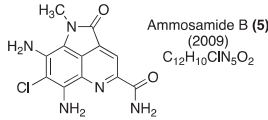
Salinosporamide A ("sal A") was discovered early in the UCSD risky and time exhaustive campaign to discover unique marine-derived actinomycete chemotypes. As noted for the cyclomarins, the project began in the 1990's with San Diego samples but quickly expanded to shallow & deep-water Caribbean environments. The cytotoxicity-guided isolation of "Sal A," from the halophilic members of a new marine genus *Salinispora* was first described in a 2003 landmark paper (cited 689 times by May 2020). This potent proteasome inhibitor, closely related to omuralide, (also a proteasome, inhibitor - Omura, *J. Antibiot.* **1991**), has entered (2020) Phase III clinical trials, as shown in Figure 2, using material obtained from scale-up fermentation.



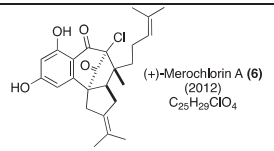
Additional work on the *Salinispora tropica*, strain CNB-392 (see Figure 2) culture extracts affording salinosporamide A yielded sporolides A & B. We view them as being significant even though no substantial experimental bioactivity has been reported to date. This pair of polyketides possess differing Cl regiochemistry (B has the aryl Cl meta to the CHOH). An unstable enediyne biosynthetic precursor (the presporolide core was synthesized by Yamashita in 2018) transformed via a non-enzymatic Masamune-Bergman cycloaromatization to an unsymmetrical para-benzyne captured by a Cl to give either A or B. The heptacyclic structures have 11 chiral centers and only two of the 24 carbons are present as a simple -CH_2-group. Additional milestones include total syntheses (2009) and characterization of biosynthetic genes (2008).



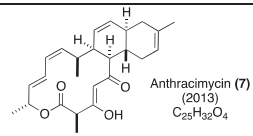
Investigations on a deep-water sediment-derived *Streptomyces* (CNQ-418) yielded six MRSA active marinopyrroles (axial chiral atropisomers): (−)-A, (−)-B, (−)-C, (−)-D, (−)-E, amd (±)-F. Defining (−)-A was challenging; the central core $C_{10}H_2N_2Cl_4$ deficient in H atoms, rendered NMR as useless. So, X-ray analysis on (−)-B enabled assignment of the structure and the M-configuration. There has been world-wide study of more than 42 unnatural analogues. The MRSA activity of A is killed by serum, but (±)-ACl_5 is less sensitive to such inactivation. Fascinatingly, (±)-A, renamed maritoclax. operates as a novel Mcl-1-specific inhibitor, and it is commercially available (Tocris and SelleckChem). (−)-A is the best marinopyrrole binder to Mcl-1 with its potential for melanoma treatment discovered in 2015. The Genome-2-Bio-Medicine Discovery center disclosed in 2020 that maritoclax promotes heart regeneration.



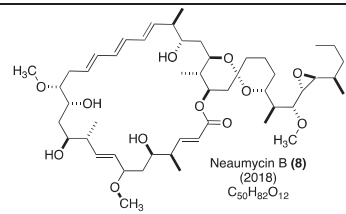
Ammosamides are relatively small achiral pigments (MW = 291) possessing significant activity against HCT-116 cancer cells (IC_{50} = 320nM) and an ability to target myosin, a cytokinetic protein. NMR-based analyses are complicated by the lack of H-atoms They were isolated from a deep-water sediment *Streptomyces* (CNR-698). The structural features present in its 16 analogues have motivated a broad array of investigations. There have been many total syntheses, but no ammosamides are under pre-clinical study. Alternatively, important biosynthetic questions are being explored. Both enzymatic and non-enzymatic processes appear responsible for their creation. Electrophilic reactions on C produce analogues: A, E, & B (which on standing goes to D). Biosynthetic gene clusters associated with RiPPs (**Ri**bosomally synthesized **p**osttranslationally modified **p**eptides) biosynthesis have been defined. Significantly, the $C_{10}N_2$ core is also present in PANC-1 active sponge compounds such as makaluvamine J, and aleutianamine.



(+)-Merochlorin A is a novel tetracyclic chlorinated merosesquiterpene isolated from a marine-derived *Streptomyces* (CNH-189). This unique molecule contains four contiguous chiral centers deduced through NMR and X-ray analyses, but only relative configurations were initially described. This family, (+)-A, (+)-B, (+)-C, (+)-D, (+)-E, and (+)-F features first in class structures significantly formed by the consecutive operation of C_{15} isoprene — polyketide biosynthetic gene clusters accompanied by action of halogenating enzymes. Sustained interest in (+)-A arises from its novel biosynthesis and the encouraging antimicrobial activity against MRSA (MIC = 2-4 mg/mL). However, incubation with human serum kills the *in vitro* activity (MRSA MIC > 64mg/mL), hopefully this could be overcome by future SAR evaluations (i.e. (±)-bromo merochlorin A is very active). The five total syntheses of **6** include an *in vivo* enzymatic scheme, three biomimetic routes to (±) A, and in 2019 preparation of (−)-A, to finalize *R/S* assignments for **6**.



(−)-Anthracimycin is an antibiotic with an attention-grabbing name coined in 2013 to emphasize its activity against *Bacillus anthracis* (MIC = 0.03μg/mL) whose spores are used as the anthrax bioterrorism weapon. Initially (−)-**7**, reported by the UCSD group, came from the marine-derived culture of a *Streptomyces* (CNH365). Recent work by others shows that additional terrestrial and marine *Streptomyces* produce both (−)-anthracimycin (**7**) & (−)-anthracimycin B. Strikingly these Gram-positive bacteria derived tetracyclics, biosynthesized from 10 acetate units subsequently cyclized to form the trans-decalin, are pseudo-enantiomeric vs. (+)-chlorotonil A, produced by from a Gram-negative myxobacterium. Draft genome sequences being sought could illuminate the mechanisms producing these enantiomeric polyketides. In 2018 (−) **7** was shown to be active against MRSA (MIC < 0.03μg/mL) and vancomycin resistant *E. faecium* (MIC < 0.03 μg/mL). Future work will presumably create anthracimycin analogs for pre-clinical study.



Neaumycin B, a spectacularly potent cytotoxin (IC_{50} = 0.07pM) against U87 human glioblastoma, was isolated at UCSD from a non-salt obligate marine-derived *Micromonospora.* (CNY-010). This 28-membered spiroketal containing macrolide is related to other widely studied large ring 6,6-spiroacetal containing polyketides including: the 26-membered oligomycins/rutamycins, the 24-membered dunaimycins/ossamycins and 22-membered cytovaricin. Preclinical evaluation of B is currently thwarted due to both compound stability and supply issues. A complete 3D-structural hypothesis was proposed through a complex brute force combination of genomic and NMR analyses. We predict that in the future many labs will revisit and extend understanding on the neaumycins through synthesis of natural & non-natural compounds.

Fig. 1 Inspirational molecules discovered during the pioneering campaign by the Fenical team to explore marine-derived actinomycetes

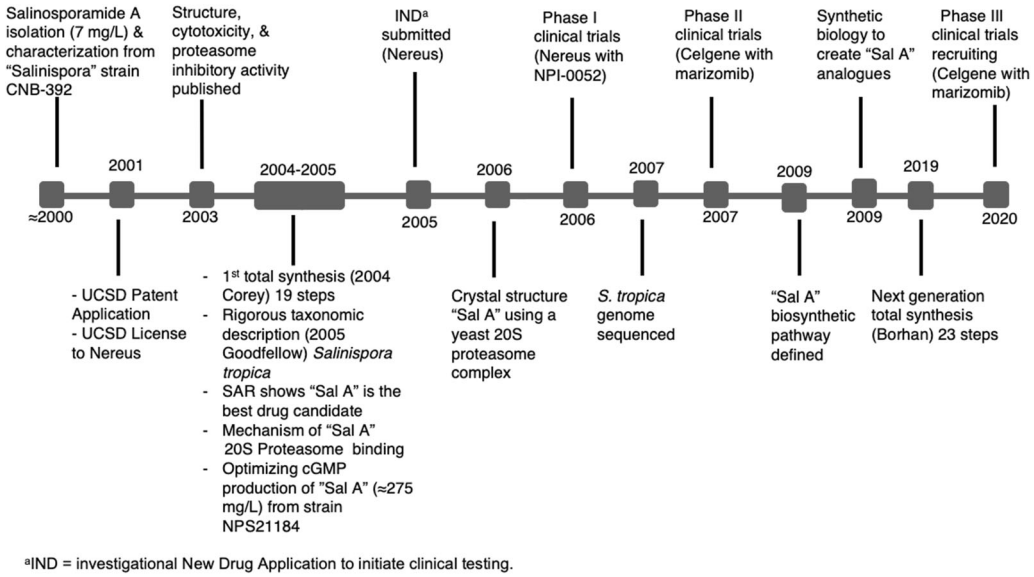


Fig. 2 Salinosporamide A (aka "Sal A", NPI-0052, marizomib)—an update on the discovery timeline from the initial isolation, structure elucidation, scale-up compound production, chemical biology studies, preclinical evaluation, through cancer clinical trials

natural **2** from strain NPS21184 via a current good manufacturing practice scheme [18]. (5) An important encouraging finding is that **2** can cross the blood-brain barrier, motivating the Phase III clinical trials in patients with glioblastoma [17]. In summary, an unusual marine-derived shallow and deep-water salt obligate actinomycete has provided **2** as a promising new treatment for glioblastoma, the cancer that killed Sen. John McCain.

Comments on the recent drug development progress for bioactive marine natural products derived from sponges, tunicates, or bacteria

In section "The salinosporamide story—from a marine actinomycete-derived natural product to clinical trials," we noted that the list is very short for marine natural products that have successfully progressed through advanced clinical trials or gained FDA approval for therapeutic use. This outcome is unusual because the idea of drugs from the sea has been on the table since the late 1960s [23]. There have been countless reviews that have examined the progress on this topic [24]. Nonetheless, some brief milestones include that in December 2004, the US Food and Drug Administration approved the first totally marine-derived drug, Prialt® to treat chronic and severe pain. This compound originally named ziconotide was isolated from the cone snail *Conus magus* [25–27]. By 2016, there were seven FDA or EMA approved small molecule drugs based on marine natural products [28], and as of 2020 the count is up to 12 [29]. Currently, there are many other promising scaffolds in the pipeline [29]. We believe that the most important current and future opportunities for marine inspired clinical therapeutics have and will continue to come from marine invertebrates (in particular sponges and tunicates) and bacteria. Examples of proof-of-concept outcomes will be discussed here, and the current successes illustrate that the past obstacles primarily associated with the supply problem during preclinical and clinical development can be overcome [30]. In this regard, the varying strategies that have been successful range from scale-up fermentation, total synthesis, invertebrate mariculture, and partial synthesis from precursors available from bacterial fermentation. As the exploration of the microbial origin of many invertebrate-derived natural products expands, strategies involving synthetic biology and the expression of key biosynthetic pathways are on the horizon. These roadblock busting strategies are often discussed in reviews and are briefly highlighted in some sections below.

The focus of our treatise next is to underscore the outstanding potential for drug development based on sponge, tunicate, and bacterial-derived natural products. Currently, 5 out of 12 marine-derived drugs in the FDA approved portfolio are from sponges (3) and tunicates (2) (Fig. 3). There are currently no approved therapeutics from marine bacteria, however, it is very important to note, as shown in Scheme 1, that a large percentage of sponge and tunicate overall mass is attributed to the associated microbiome of these organisms [31–34]. In the case of sponges, many have a significant microbial biomass (up to 35% of the total) and some of the most chemically rich taxa high in microbial abundance (HMA) sponges have been shown to have associations with both gram-positive and gram-negative bacteria. A similar situation can be seen for the tunicate holobiont—significant biomass of bacterial associates,

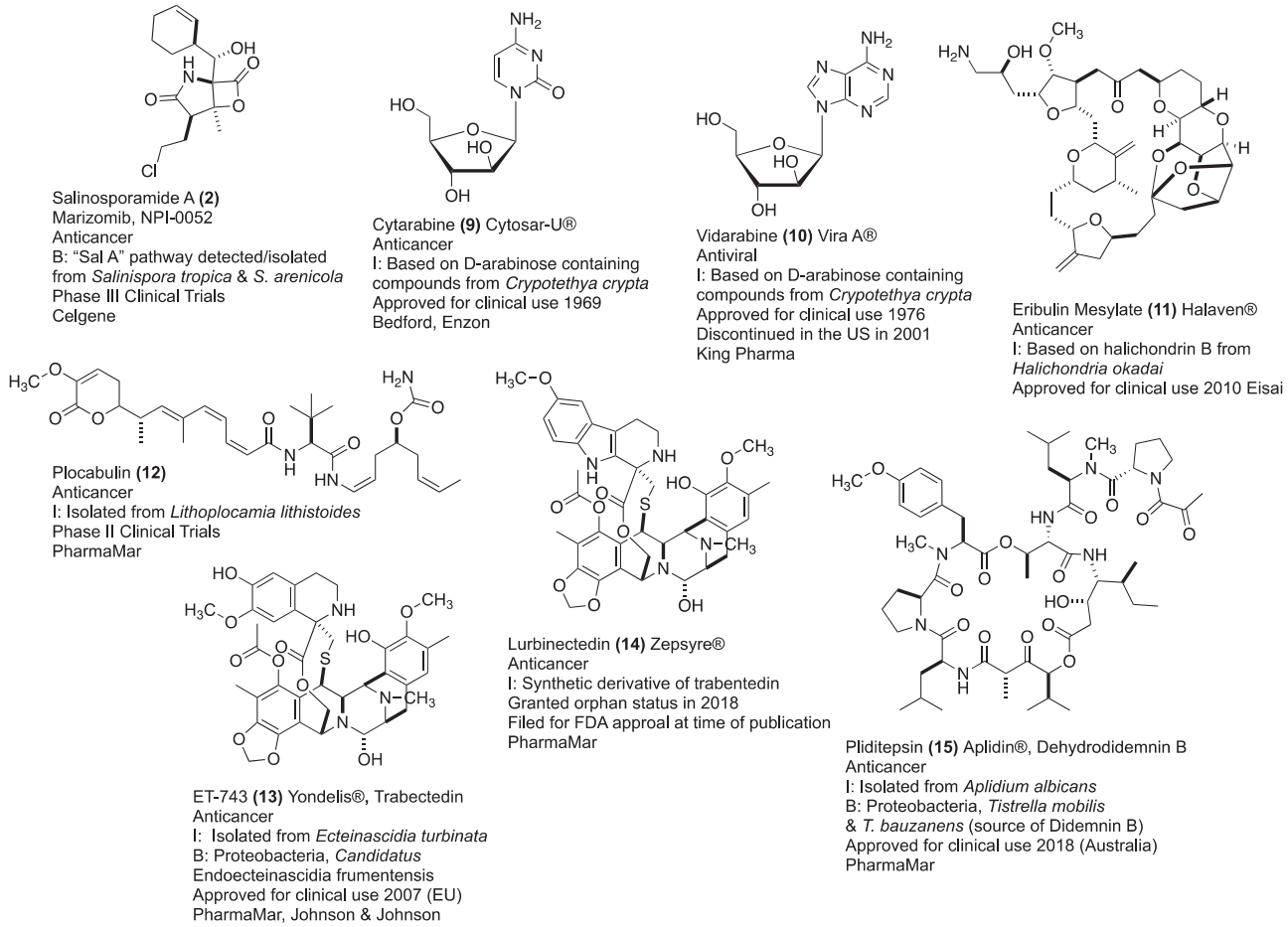
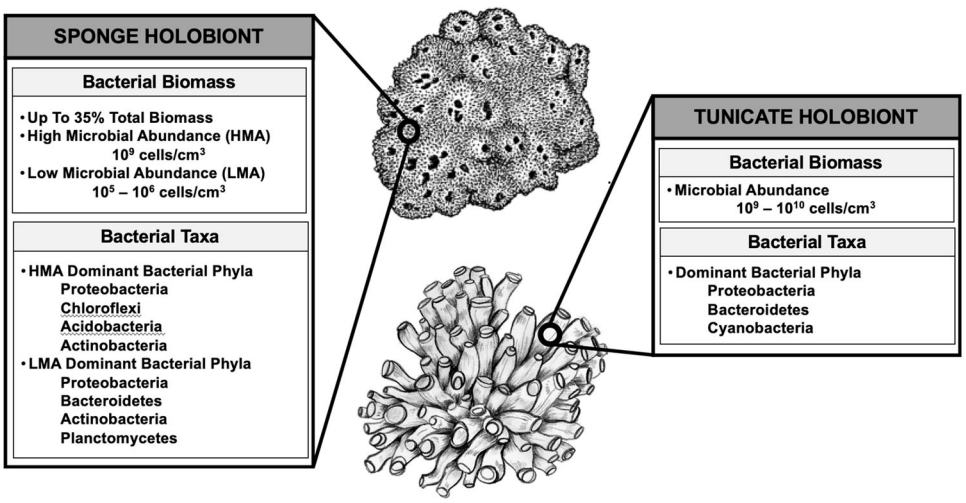


Fig. 3 Summary of therapeutics approved for clinical use or currently in Phase II/III clinical trials, inspired by or based on compounds isolated from sponges, tunicates, or marine-derived actinomycetes. I = invertebrate source; B = bacterial producer

Scheme 1 A selective view of the holobiont of sponges and tunicates—varying bacterial biomass and dominate bacterial taxa



dominated by gram-negative bacteria. We will discuss in greater detail below, the important role such bacteria play in the biosynthetic origin of these approved therapeutics.

A summary of therapeutics approved for clinical use or currently in Phase II/III clinical trials from sponges, tunicates, and bacteria is presented in Fig. 3. There are a larger number of marine natural products that have been examined in clinical trials and eventually dropped. Currently, there is no comprehensive review of such compounds, but important insights can be gleaned from a 2014

American Society of Pharmacognosy meeting lecture [35]. The assemblage of eight compounds in Fig. 3 includes representatives from all three of the taxa mentioned above. The tremendous developmental success for Sal A (**2**) with the preclinical work being driven by compound obtained from scale-up fermentation of the actinomycete *Salinispora* strain NPS21184 was discussed above (Fig. 2, section "The salinosporamide story—from a marine actinomycete-derived natural product to clinical trials"). The collection in Fig. 3 also includes the following sponge-derived (phylum *Porifera*) molecules. (1) Cytarabine (**9**) and vidarabine (**10**) are D-arabinose containing nucleosides that were isolated from *Cryptotethya crypta* and are considered by some as the first marine-derived approved pharmaceuticals [36]. Cytarabine is still used in treatments against various forms of leukemia and non-Hodgkin's lymphoma [37], while vidarabine was an antiviral drug that is active against poxviruses, herpes viruses, some rhabdoviruses, hepadnaviruses, and RNA tumor viruses [38]. However, with the advancement of less toxic and more metabolically stable antivirals vidarabine was discontinued in the US in 2001 [29]. (2) Eribulin mesylate (**11**) (aka Halaven®) consists of the macrocyclic lactone pharmacophore of the natural product halichondrin B, a compound that was isolated in extremely low yields from *Halichondria okadai* and is hypothesized to be produced by an associated bacterium [39, 40]. This polyketide is currently used to treat metastatic breast cancer and inoperable liposarcoma [41]. Lastly, (3) plocabulin (**12**) was originally isolated from *Lithoplocamia lithistoides* and is currently in Phase II clinical trials for patients with advanced malignancies [42, 43].

A total of three tunicate-derived (phylum *Chordata*) natural products are in clinical use or late stage clinical trials. (1) ET-743 (**13**) (aka trabectedin, Yondelis®), isolated from *Ecteinascidia turbinate*, is currently in use to treat soft-tissue sarcoma and ovarian cancer [44]. (2) Lurbinectedin (**14**) (aka Zepsyre®) is a synthetic derivative of ET-743 that has been shown to have a substantially higher tolerated dose than its natural product counterpart [45], as well as higher overall survival, progression-free survival, and overall response rates in Phase III clinical trials against ovarian cancer [46]. (3) Plitidepsin (**15**) (aka Aplidin®, dehydrodidemnin B) was originally isolated from *Aplidium albicans*, has been approved in Australia for use against multiple myeloma [47], and has exhibited activity against the human coronavirus HCoV-229E, suggesting it could be an effective agent against the current outbreak of COVID-19 [48]. The bioactive **15**, which was isolated from its tunicate host in high yields, is structurally related to didemnin B (**67**) from *Tistrella* bacteria [49, 50], and it is currently manufactured by total synthesis [51].

It is important to emphasize that during the discovery of some invertebrate-associated compounds, such as halichondrin B (sponge) and ET-743 (**13**) (tunicate), hypotheses were formulated and subsequently proven (see section "The possible bacterial biosynthetic origin of molecules isolated from sponges and tunicates—the case made by analyzing isolated yields, similar scaffolds, or results from culture-independent insights") that the true biosynthetic sources for some molecules are associated bacteria. A summary of a few general supporting observations that stimulate additional inquiry on this topic is: (1) when similar classes of compounds are extracted from invertebrates of diverse taxonomic origin; (2) when a large percentage of some marine invertebrates' overall mass is attributed to the associated microbiome (Scheme 1); and (3) when natural products isolated from invertebrates possess similar chemical scaffolds vs. that of bacterial natural products [52, 53].

The possible bacterial biosynthetic origin of molecules isolated from sponges and tunicates—the case made by analyzing isolated yields, similar scaffolds, or results from culture-independent insights

It is essential to further discuss four compounds shown above in Fig. 3 that are currently used as medicines and whose origins included sponges or tunicates. This list consists of: cytarabine (**9**), eribulin mesylate (**11**), ET-743 (**13**), and dehydrodidemnin B (**15**). Here is an important question: are their clues in the development trajectory of these compounds to imply that bacterial-derived pathways are functional in their biosynthesis? Another question—are these compounds present at extremely low concentrations from the invertebrate also implying a bacterial origin? Early on, Professor Fenical championed the bacterial origin theory stating in his 1993 review paper, "The importance of bacterial symbiosis is growing in recognition that bacteria may be the true producers of many compounds isolated from sponges, ascidians, and other marine invertebrates." [54]. The possibility that true biosynthesis occurs by symbionts is directly relevant for two of the aforementioned compounds, **11** and **13**. However, this does not apply to **9**, which is isolated in high yields from sponges and discovered by medicinal chemistry. Also, the situation for **15** is perhaps enigmatic, as it was isolated in high yields from the *Aplidium* (tunicate) and is almost identical to didemnin B (**67**), which was obtained in high yields from *Aplidium* and in variable yields from marine-derived *Tistrella* bacteria [49, 50].

The design of eribulin mesylate (**11**) arose from the discovery and elucidation of halichondrin B ($C_{60}H_{86}O_{19}$), a potent cytotoxin ($IC_{50} = 0.09$ ng/mL vs. B-16 melanoma cancer cells). Halichondrin B was isolated in miniscule

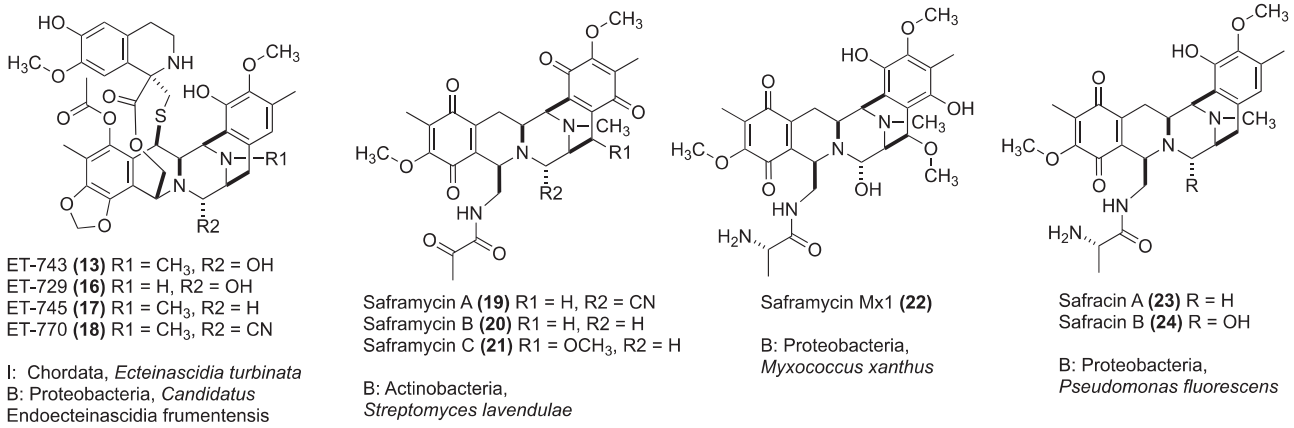


Fig. 4 Molecular structures of ecteinascidins (ETs) from the tunicate *Ecteinascidia turbinata* vs. those from diverse bacteria possessing similar structural features. I = invertebrate source; B = bacterial producer

yields from two disparate sponges, *Halichondria okadai* and *Lissodendoryx* sp. [39]. Its antitumor activity and pharmacophore were the inspiration for **11**. Problematic for the preclinical development of halichondrin B was that a scant of 300 mg was obtained from 2000 pounds of *Lissodendoryx*, which thwarted its extensive preclinical follow-up. Currently, it is believed that dinoflagellate symbionts in the sponge are the true biosynthetic source of halichondrin B [55], and consistent with this idea is that the known dinoflagellate polyether okadaic acid was isolated from two species of *Halichondria* sponges [56].

A similar story to that sketched above is evident for ET-743 (**13**) first isolated in very low yields from the tunicate *Ecteinascidia turbinate*. Reports of anti-cellular proliferation were first described as early as 1969 when it was found that an extract of the Caribbean tunicate killed tumor cells in vitro and was capable of inhibiting tumor growth in vivo [57]. Due to the low abundance of this compound in the tunicate, the structure responsible for the described cytotoxic activity would not be published until 1990 and preclinical follow-up using natural material was not possible [58, 59].

Early on it was hypothesized that **13** and its congeners (**16–18**) shown in Fig. 4 were bacterial in origin as they shared similarities to chemical scaffolds of previously reported bacterial compounds including: saframycin A–C (**19–21**) from *Streptomyces lavendulae* [60, 61], saframycin Mx1 (**22**) from *Myxococcus xanthus* [62], and safracin A and B (**23, 24**) from P*seudomonas fluorescens* [63]. In fact, safracin B (**24**) would later serve as the starting point in the semi-synthesis of Yondelis® (**13**) for clinical use [64]. Studies investigating the microbiome of *E. turbinate* in the Mediterranean [65] and Caribbean [66] found the γ-proteobacterium, *Ca*. E. frumentensis was the dominant member of the microbiome regardless of geographical location, furthering the bacterial origin hypothesis for **13**. Then in 2011, the first confirmation of the bacterial origin of **13** was reported [67]. This study reported the assembly of a 35 kb contig from the tunicate metagenomic DNA consisting of 25 genes from the core of the non-ribosomal peptide synthase (NRPS) portion of the gene cluster associated with **13** production. During this work it was not possible to assemble the entire **13** gene cluster, yet the GC content and codon preference of the contig suggested that this NRPS belonged to *Ca*. E. frumentensis [67]. In 2015, **13** was concluded to be produced by the unculturable bacterial endosymbiont *Ca*. E. frumentensis, obtained directly from metagenomic DNA [2]. This study also identified that *Ca*. E. frumentensis has an extremely reduced genome, indicating it is in the later stages of symbiosis, and that direct fermentation to supply **13** may not be possible. However, scale-up production through semi-synthesis by PharmaMar in partnership with Johnson & Johnson has provided a sustainable source of the compound.

It is important to underscore several points contained in the preceding narrative. To date there have been no meaningful examples of mariculture as a cost-effective route for sponge or tunicate-derived compound scale-up [68]. Alternatively, total synthesis of complex chemical scaffolds possessing multiple chiral centers present in many bioactive sponge and tunicate natural products has been successful in moving compounds through preclinical evaluation (best example is discodermolide [69]). This was a strategy used to create eribulin mesylate (**10**), and as noted above semi-synthesis continues to be successful in providing ET-743 (**13**). Not to be forgotten is that there is much interest in the hypothesis that bacteria are the true producers of many bioactive compounds in marine invertebrates, and as understanding and tools continue to evolve there may be practical alternatives to provide scale-up production of these desired compounds [52, 53].

The advancement of next generation sequencing technology has allowed additional tunicate-derived natural products to be concluded to be bacterial in origin and ten

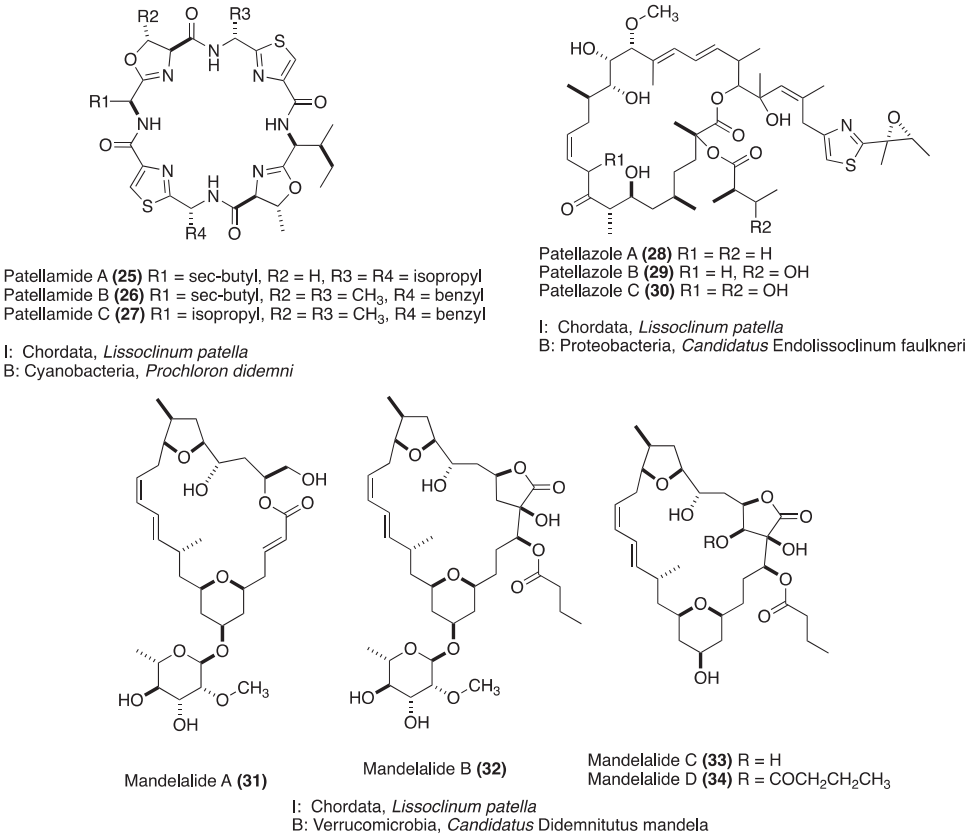


Fig. 5 Structures of tunicate (phylum *Chordata*) natural products determined to be bacterial in origin through culture-independent methods. I = invertebrate source; B = bacterial producer

examples are shown in Fig. 5. In 2005, the cytotoxic patellamides (**25**–**27**), originally isolated from *Lissoclinum patella* [70], were determined to be biosynthesised by the symbiotic cyanobacteria, *Prochloron didemnid* [71, 72]. Later, it was determined that the patellazoles (**28**–**30**), cytotoxic polyketides originally isolated from *L. patella* in 1988 [73, 74], were in fact biosynthesized by the symbiotic *Candidatus* Endolissoclinum faulkneri, a bacterium that belongs to the phylum Proteobacteria [75]. Lastly, in 2017, the biosynthetic gene cluster for the madelalides (**31**–**34**) was identified [76]. These mitochondrial ATP synthase inhibiting compounds, originally isolated from a *Lissoclinum* sp. [77–79], were determined to be produced from the symbiotic bacterium, *Candidatus* Didemnitutus mandela that belongs to the phylum *Verrucomicrobia*.

The cytotoxic onnamide A (**35**) and psymberin (**36**) shown in Fig. 6 were among the first sponge associated natural products to be identified as bacterial in origin through culture-independent methods [80–84]. The onnamides (**35**) were initially isolated from the sponge *T. swinhoei* and psymberin (**36**) from the sponges *Psammocinia* aff. *bulbosa* and *Ircinia ramose* [85–87]. However, the discovery of the biosynthetic origin for these compounds actually began with the structurally similar pederin and its initial isolation source, the terrestrial beetle *Paederus fuscipes* [88]. In 2004, the biosynthetic gene cluster for pederin was identified from the metagenome of *P. fuscipes* and determined that it originated from an uncultured *Pseudomonas*-like bacterium [89, 90]. Using the knowledge gained from the pederin biosynthetic gene cluster, researchers were able to probe the metagenome of the *T. swinhoei* and *P.* aff. *bulbosa* for homologous polyketide synthase domains. In 2004, they identified part of the biosynthetic gene cluster for onnamide A (**35**) from an unidentified bacterium in *T. swinhoei* [82], and in 2009 the nearly complete biosynthetic gene cluster for psymberin (**36**) was identified from an unknown bacterium in *P.* aff. *bulbosa* [84]. It was not until 2014, that the identity of the onnamide producing bacterium was determined to be the symbiont "*Candidatus* Entotheonella factor." This bacterium would prove to be the source of many *T. swinhoei* compounds including the structurally similar theopederin A (**37**) [91]. However, mycalamide A (**38**) from a *Mycale* sponge while similar in structure to **35**–**37** from the very different sponge *Theonella* would be determined to originate from a completely different taxa of symbiont (to be discussed below) [92, 93]. Unfortunately, to date the identity of the psymberin producing bacterium is still unknown.

Briefly discussed above is that the bacterial genus *Candidatus* Entotheonella detected in sponges has proved to be a massive repository of biosynthetic richness, similar to what has been previously observed in soil actinomycetes

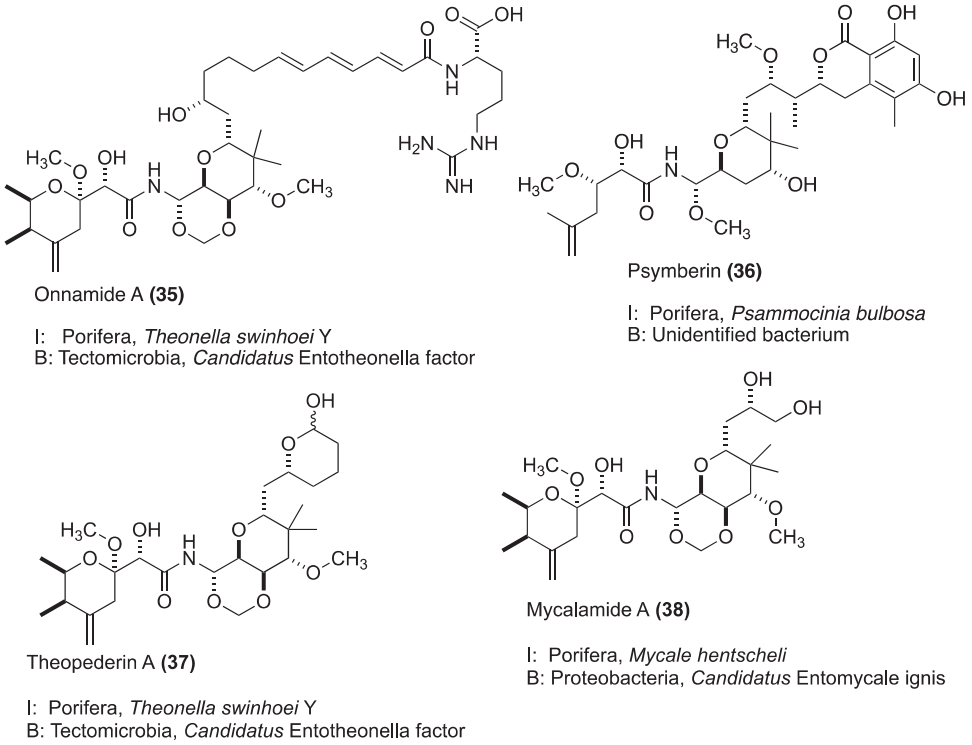


Fig. 6 Structures of sponge-derived (phylum *Porifera*) polyketide natural products determined to be bacterial in origin through culture-independent methods. I = invertebrate source; B = bacterial producer

(see section "Introduction"). Furthermore, these filamentous bacteria belong to the unique phylum Tectomicrobia and account for the majority of all known natural products isolated from *T. swinhoei*. [91, 94–99]. Distinct species of *Candidatus* Entotheonella have been shown to have symbiotic relationships with the different phenotypes of *T. swinhoei* (Y = yellow interior, W = white interior), perhaps accounting for the distinct chemotypes that have been previously observed in these sponges [100]. The *Candidatus* Entotheonella factor is the symbiont of *T. swinhoei* Y as shown in Fig. 7a and is responsible for the production of the onnamides (**35**) [85], theopederins (**37**) [101], polytheonamides (**39, 40**) [102], keramamides (**41**–**43**) [103], pseudotheonamides (**44**) [104], nazumamide A (**45**) [105], and the cyclotheonamides (**46, 47**) [106]. Representatives from each of these families of compounds are shown in Fig. 7a. The bacterial genus *Candidatus* Entotheonella serta is the symbiont of *T. swinhoei* W as shown in Fig. 7b and is responsible for the production of the swinholides (**48**–**50**) [107], misakinolides (**51**) [108], theopalauamide (**52**), and theonellamides (**53**–**60**) [109]. Representatives from each of these families of compounds are shown in Fig. 7b.

Although the chemically prolific bacterial genus *Candidatus* Entotheonella, detected in *T. swinhoei* sponges, are the most well studied, it is also present in other sponge genera discussed in Fig. 8. In 2014, the metagenome of the sponge *Discodermia calyx* was mined for the biosynthetic gene cluster responsible for the production of the highly cytotoxic protein phosphatase inhibitor calyculin A (**61**) [110, 111]. This research identified the complete biosynthetic gene cluster and determined that it belonged to a bacterium of the genus *Candidatus* Entotheonella. However, the team discovered a phosphotransferase tailoring enzyme within the biosynthetic gene cluster suggesting a diphosphate compound may be the true end product of the biosynthetic pathway. Using a flash freeze-lyophilization extraction method on freshly collected *D. calyx*, they were able to isolate this new natural product, phosphocalyculin A (**62**). Interestingly, **62** exhibited a greater than 1000-fold reduction in cytotoxicity when compared with **61**, suggesting that **62** is in fact the protoxin that *D. calyx* stores to avoid self-toxicity, but can quickly become the active agent in response to environmental stimuli [111]. The research discussed above highlights the powerful resource that metagenomic analysis can provide in the understanding of the chemical biology of sponge-derived natural products.

The preceding discussion illustrated that the bacterial genus *Candidatus* Entotheonella can be considered to be a "super-producer" of natural products. Culture-independent methods have also enabled the discovery of other sponge associated bacteria putatively responsible for the biosynthesis of complex natural products. As a striking example, in 2017, a metagenome exploration of *Dysideidae* sponges led to the identification of the biosynthetic gene clusters responsible for the production of cytotoxic polybrominated diphenyl ethers (PBDEs) (**63, 64**) [112]. This extended 2014 findings on the biosynthetic gene clusters responsible for the production of PBDEs from cultured γ-proteobacteria

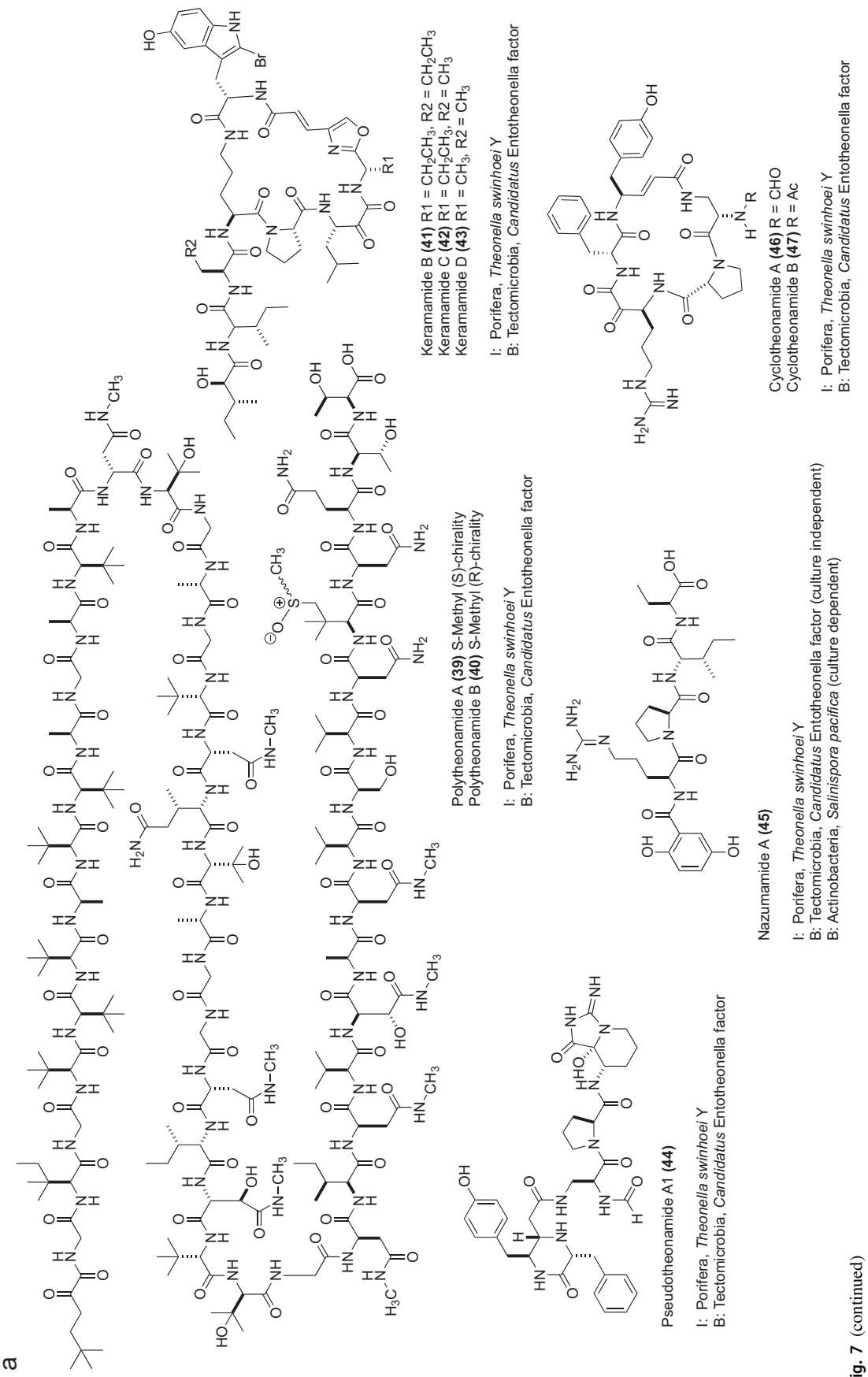
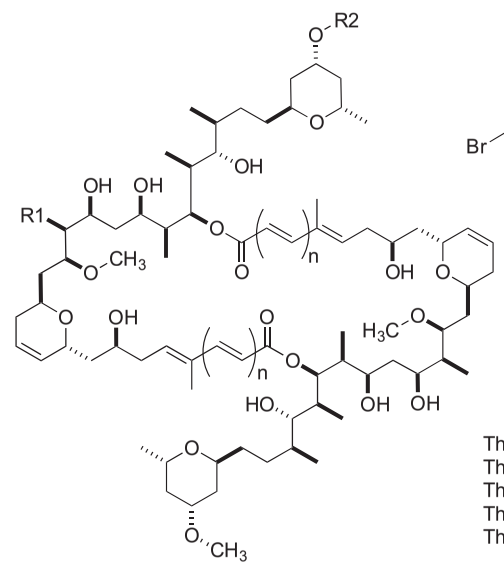


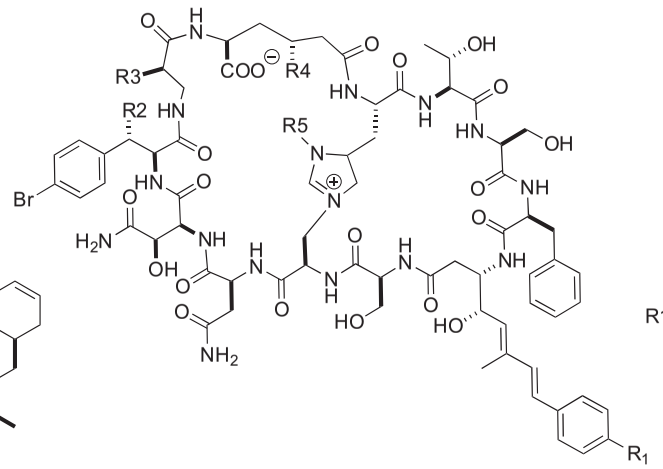
Fig. 7 (continued)

b



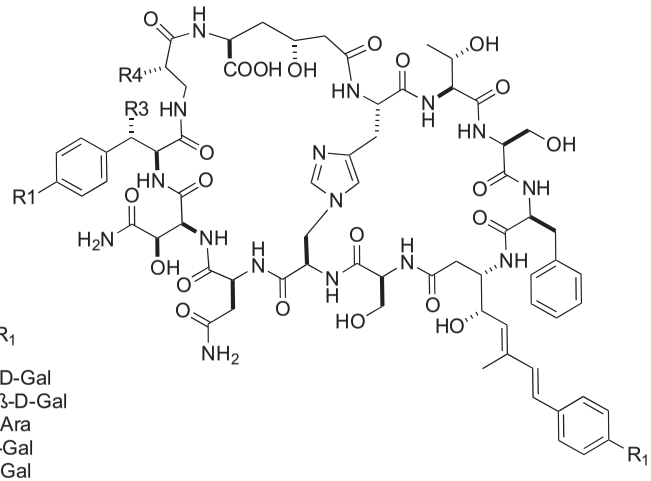
Swinholida A **(48)** R1 = CH_3, R2 = CH_3, n = 1
Swinholida B **(49)** R1 = H, R2 = CH_3, n = 1
Swinholida C **(50)** R1 =CH_3, R2 = H, n = 1
Misakinolide A **(51)** R1 = CH_3, R2 = CH_3, n = 0

I: Porifera, *Theonella swinhoei* WA & WB
B: Tectomicrobia, *Candidatus* Entotheonella serta



Theopalauamide **(52)** R1 = H, R2 = CH_3, R3 = OH, R4 = H, R5 = ß-D-Gal
Theonellamide A **(53)** R1 = H, R2 = CH_3, R3 = OH, R4 = OH, R5 = ß-D-Gal
Theonellamide D **(54)** R1 = Br, R2 = H, R3 = H, R4 = OH, R5 = ß-L-Ara
Theonellamide E **(55)** R1 = Br, R2 = H, R3 = H, R4 = OH, R5 = ß-D-Gal
Theonellamide G **(56)** R1 = H, R2 = H, R3 = OH, R4 = H, R5 = ß-D-Gal

I: Porifera, *Theonella swinhoei* WA & WB
B: Tectomicrobia, *Candidatus* Entotheonella serta



Theonellamide B **(57)** R1 = H, R2 = Br, R3 = CH_3, R4 = OH
Theonellamide C **(58)** R1 = Br, R2 = H, R3 = H, R4 = H
Theonellamide F **(59)** R1 = Br, R2 = Br, R3 = H, R4 = H
Theonellamide H **(60)** R1 = H, R2 = Br, R3 = H, R4 = H

I: Porifera, *Theonella swinhoei* WA & WB
B: Tectomicrobia, *Candidatus* Entotheonella serta

Fig. 7 **a** Structures of *Theonella swinhoei* Y (phylum *Porifera*) natural products determined to originate from "*Candidatus* Entotheonella factor" through culture-independent methods. I = invertebrate source; B = bacterial producer. **b** Structures of *Theonella swinhoei* W (phylum *Porifera*) natural products determined to originate from "*Candidatus* Entotheonella serta" through culture-independent methods. I = invertebrate source; B = bacterial producer

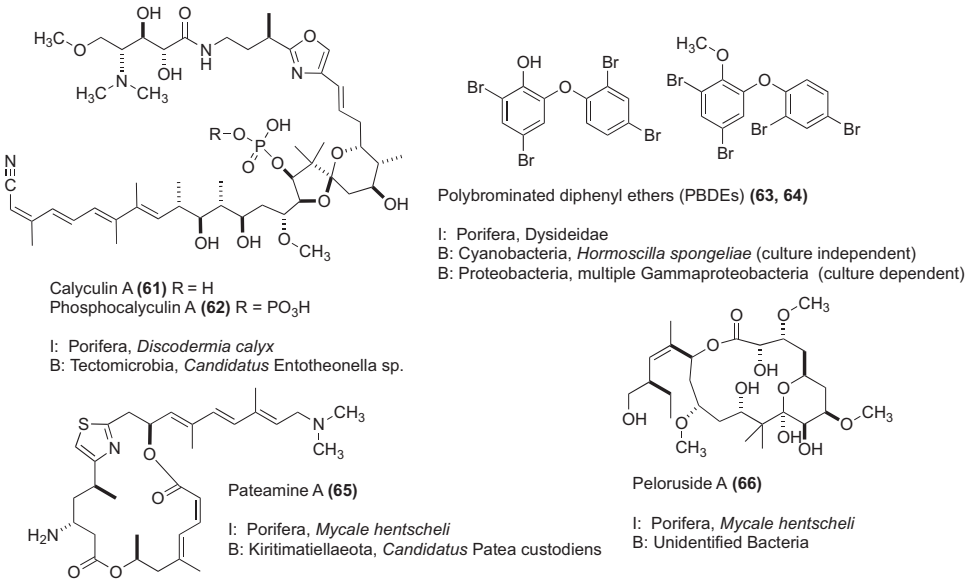


Fig. 8 Structures of sponge (phylum *Porifera*) natural products determined to be bacterial in origin through culture-independent methods. I = invertebrate source; B = bacterial producer

[113]. The next step was to close the loop by investigating the biosynthetic origin of PBDEs which are ubiquitous in *Dysideidae* sponges collected from coral reefs throughout the Indo-Pacific [114]. Interestingly, γ-proteobacteria were not concluded to be responsible for the production of PBDEs in *Dysideidae*, but instead originate from the sponge symbiotic cyanobacteria *Hormoscilla spongeliae*. Furthermore, the structural diversity of the PBDEs isolated from different *Dysideidae* samples appears correlated to the taxonomic clades of *H. spongeliae* strains [112].

The additional examples presented next, based on compounds shown in Figs. 6 and 8, further underscore that the biosynthetic production of complex sponge natural products can occur from various taxa of bacterial symbionts. One case involves mycalamide A (**38**) (Fig. 6) [115, 116], initially discovered in the 1990s from *Mycale hentscheli*. In 2020, two different groups independently exploring the metagenome [92, 93] of this sponge identified the biosynthetic pathway for **38** in the genome of *Candidatus* Entomycale ignis, a bacterium in the phylum Proteobacteria. Similarly, via additional metagenome guided research on *M. hentscheli*, the biosynthetic gene cluster for the translation initiation inhibitor pateamine A (**65**) (Fig. 8) [117, 118] was identified within the genome of *Candidatus* Patea custodiens, a bacterium in the phylum Kiritimatiellaeota. In addition, the biosynthetic gene cluster responsible for the production of the microtubule inhibitor peloruside A (**66**) [119, 120] was also identified within the metagenome of *M. hentscheli*. To date the taxonomy of the producing bacteria involved in the production of **66** has not been identified. Lastly, as part of the massive genetic diversity present in the metagenome of *M. hentscheli*, a polytheonamide-like ribosomally synthesized and posttranslationally modified peptide (similar in structure to **39** and **40**) was identified within the genome of *Candidatus* Caria hoplite, a bacterium in the phylum Proteobacteria [93].

The preceding commentary dealing with bacterial biosynthetic gene clusters from invertebrate metagenomes, especially sponge metagenomes, emphasizes the existence of an immense range of natural product genetic diversity. It is clear that future metagenomic-driven research on invertebrates, such as those producing compounds shown in Figs. 4–8, will further reveal undiscovered natural products possessing inspirational structures.

The possible bacterial biosynthetic origin of molecules isolated from sponges and tunicates—the case made by analyzing results of cultured biosynthetic production

In the section above, we discussed the structures of more than 60 complex molecules directly isolated from sponges and tunicates. The focus was on assessing their chemodiversity alongside insights of the bacterial-derived biosynthetic machinery involved in their creation. In the future, synthetic biology coupled with the functional biosynthetic gene clusters identified in the metagenomes could provide a supply of important natural products [121]. A current roadblock is that in many cases the bacteria producing the natural products are obligate symbionts and are incapable of independent growth outside of their invertebrate host [122]. It is useful to explore situations where molecules analogous to those produced by sponges and tunicates can be successfully obtained by direct bacterial culturing in parallel with actual compound isolation. We suggest that insights

obtained from such a survey could provide answers to the vexing question—what would it take to find new natural product chemical space? On the one hand, it is relevant to note that bacterial genome sequencing sometimes reveals that an individual strain can contain the machinery to produce more than 20 distinct molecules. This has stimulated many groups to contend that molecules encoded within the genome of bacterial isolates that are not obtained from culturing persist as "cryptic" entities whose pathways need to be turned-on [123, 124].

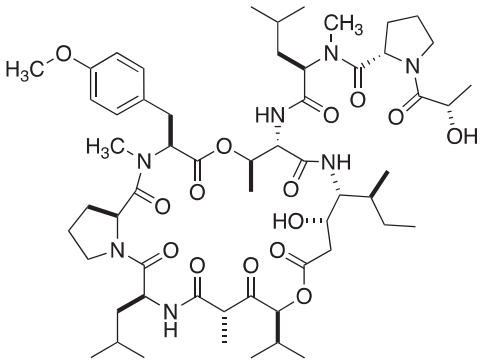
The situation with didemnin B (**67**) (Fig. 9a) provides an important case example. This compound was originally isolated in 1978 from a *Tridemnum* tunicate and exhibits a wide range of biological activity [125]. To date, nine congeners (didemnins A–E, G, X, Y, and nordidemnin B) have been isolated from extracts of *Tridemnum solidum*, and **67** possesses the most potent biological activities [126]. Relatively large amounts of didemnin B were isolated from the tunicate and even larger amounts were obtained by total synthesis. Unfortunately, high toxicity during Phase II clinical trials led to termination of further trials on **67**. However, as discussed in Fig. 3 the analogue dehydrodidemnin B (**15**) successfully progressed through clinical trials and is now approved for therapeutic use. Especially important for this discussion was the serendipitous isolation of **67** through laboratory culture of five strains of *Tistrella* four *mobilis*, and one *bauzanensis* (see Fig. 9a) [49, 50]. The unoptimized compound yields from the culturing of two *T. mobilis* strains YIT 12409 and KA081020-065 were modest and included **67** (YIT 12409 = 3.2 mg/L; KA081020-065 = 0.2 mg/L) as well as nordidemnin B (YIT 12409 = 0.5 mg/L; KA081020-065 = 0.1 mg/mL). A detailed annotation of the biosynthetic gene cluster for **67** is in hand for *Tistrella* sp. [50] and suggests that synthetic biology coupled with semi-synthesis could provide an alternative scale-up route to **67**, which for now is obtained by total synthesis.

There are four additional cases involving tunicate-associated natural products that have been isolated from cultured bacteria, and these are listed in Fig. 9a. For each, a member of the compound family was identified from their bacterial source prior to or simultaneously to being observed from invertebrate source. Initially, lissoclinolide (**68**) exhibiting antibiotic and antitumor activity was obtained from the terrestrial Actinobacteria, *Micropolyspora venezuelensis* in 1969, then >20 years later **68** was re-isolated from the tunicate *Lissoclinum patella* [127–129]. In 1976, the antibiotic enterocin (**69**) was identified from a terrestrial soil *Streptomyces* and in 1996 there are two reports of **69** being isolated, one from a marine *Streptomyces maritimus* and the other from the tunicate *Didemnum* [130–132]. Staurosporine (**70**) is a ubiquitous alkaloid commonly isolated from various terrestrial and marine *Streptomyces* and in 1992 was obtained from the tunicate *Eudistoma toealensis* [133, 134]. Lastly, the cytotoxic haterumalides was simultaneously isolated in 1999 from three disparate sources: (1) haterumalide NA (**71**) from *Serratia marcescens*, a terrestrial Proteobacteria, (2) **71** along with congeners haterumalides NB-NE (**72**–**75**) from an *Ircinia* sponge, and (3) **76** from a *Lissoclinum* tunicate [135–137].

There are a few cases where cultured bacterial isolates contained compounds also isolated from sponges. Two of these were discussed above and include nazumamide A (**45**) and PBDEs (**63**, **64**) (Figs. 7a and 8). The situation for **45** is somewhat unique, this *T. swinhoei* sponge-derived compound was identified by culture-independent methods from in the genome of the *T. swinhoei* symbiont, *Candidatus* Entotheonella [91, 105] and also from the culturing of the actinomycete *Salinispora pacifica* [138]. The biosynthetic machinery for the *Dysideidae* sponge-derived **63**, **64** was identified within the genome of cyanobacterial symbionts *H. spongeliae*, in addition to PBDE's isolated from other cultured γ-proteobacteria [112, 113].

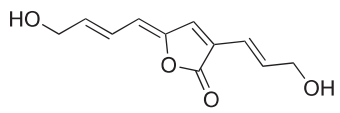
An additional 13 examples of sponge-derived compounds also isolated from cultured bacteria, shown in Fig. 9b, are discussed next. Toyocamycin (**77**) an antibiotic nucleoside was first isolated in 1965 from a terrestrial *Streptomyces* [139] and then decades later from the sponge *Jaspis johnstoni* [140]. Bacteriohopanetetrol (**78**) has been isolated from various taxa of bacteria, but its initial isolation was from the Proteobacteria *Acetobacter xylinum* in 1976 [141] and in 2001 it was identified in high yields in the sponge *Plakortis simplex* [142]. Manzamine A (**79**) exhibits a wide range of biological activity and since 1986 has been isolated in high yields from multiple taxa of sponges [143]. Later, it was isolated in miniscule and irreproducible yields from an actinobacteria *Micromonospora* strain [144]. The cytotoxic heterocycles bengamide E (**80**) and E′ (**81**), initially isolated from a *Jaspis* sponge [145], were later obtained in high and reproducible yields by culturing the terrestrial *Myxococcus virescens* [146]. The antifungal microsclerodermins were initially isolated from *Microscleroderma* and *Theonella* sponges [147, 148] and in 2013 microsclerodermin D (**82**) and L (**83**) were isolated from cultured *Sorangium cellulosum* and a *Jahnella* sp., bacteria belonging to the phylum Proteobacteria [149]. Lastly, a series of bromotyrosine-derived alkaloids isolated from various sponge sources were obtained but not always reproducibly from the Proteobacteria, *Pseudovibrio denitrificans* [150]. This included the cytotoxic fistularin-3 (**84**) [151], aerothionin (**85**), hydroxyaerothionin (**86**) [152], aplysinamisine II (**87**) [153], purealidin L (**88**) [154], and homopurpuroceratic acid (**89**) [155].

a



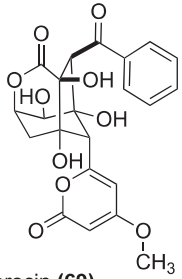
Didemnin B **(67)**

I: Chordata, *Tridemnum solidum*
B: Proteobacteria, *Tistrella mobilis* & *T. bauzanensis*



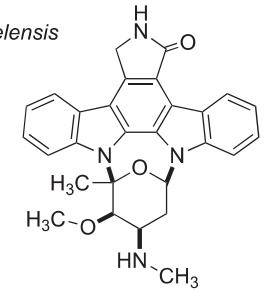
Lissoclinolide **(68)**

I: Chordata, *Lissoclinum* sp.
B: Actinobacteria, *Micropolyspora venezuelensis*



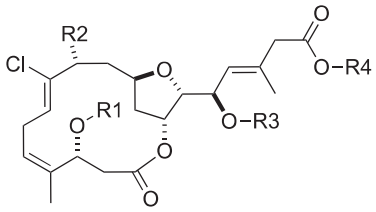
Enterocin **(69)**

I: Chordata, *Didemnum* sp.
B: Actinobacteria,
Streptomyces maritimus

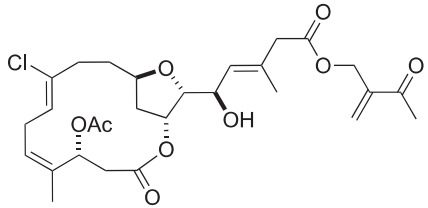


Staurosporine **(70)**

I: Tunicate, *Eudistoma toealensis*
B: Actinobacteria, *Streptomyces* sp.



Haterumalide NA **(71)** R1 = Ac, R2 = R3 = R4 = H
Haterumalide NB **(72)** R1 = Ac, R2 = R3 = H, R4 = ^{n}Bu
Haterumalide NC **(73)** R1 = Ac, R2 = OH, R3 = H, R4 = ^{n}Bu
Haterumalide ND **(74)** R1 = Ac, R2 = OH, R3 = R4 = H
Haterumalide NE **(75)** R1 = R2 = R3 = R4 = H

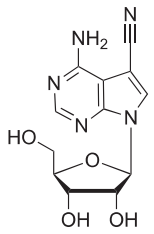


Haterumalide B **(76)**

I: Chordata, *Lissoclinum* sp.; Sponge, *Iricinia* sp.
B: Proteobacteria, *Serratia plymuthica* & *S. marcescens*

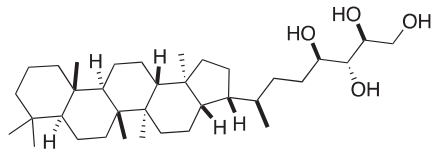
Fig. 9 (continued)

b



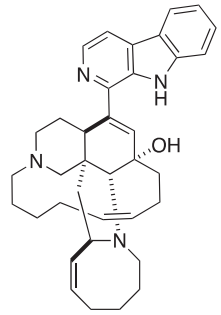
Toyocamycin **(77)**

I: Porifera, *Jaspis johnstoni*
B: Actinobacteria, *Streptomyces* sp.



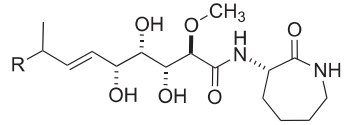
Bacteriohopanetetrol **(78)**

I: Porifera, *Plakortis simplex*
B: Proteobacteria, *Acetobacter* sp., *Rhodopseudomonas* sp. & Zymomonas sp.
B: Actinobacteria, *Frankia* sp.



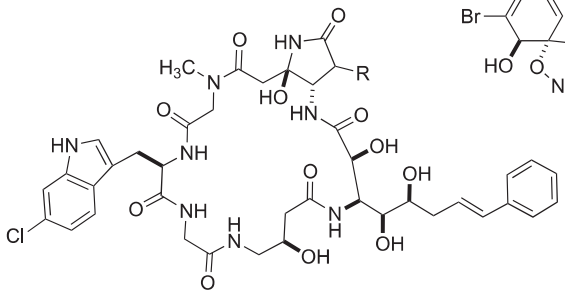
Manzamine A **(79)**

I: Porifera, *Acanthostrongylophora ingens*
B: Actinobacteria, *Micromonospora* sp.



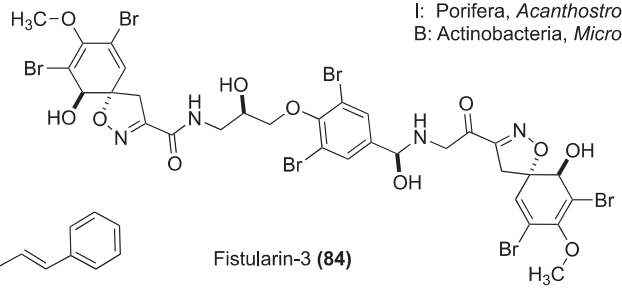
Bengamide E **(80)** R = CH_3
Bengamide E' **(81)** R = CH_2CH_3

I: Porifera, *Jaspis* sp.
B: Proteobacteria, *Myxococcus virescens*



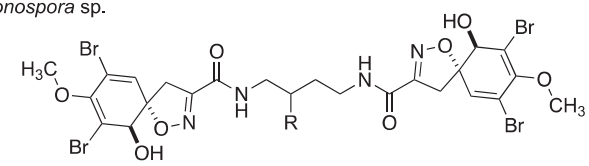
Microsclerodermin D **(82)** R = H
Microsclerodermin L **(83)** R = OCH_3

I: Porifera, *Microscleroderma* sp. & *Theonella* sp.
B: Proteobacteria, *Jahnella* sp. & *Sorangium cellulosum*



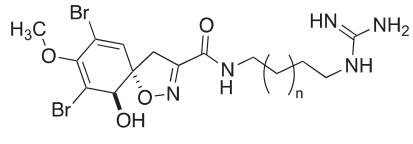
Fistularin-3 **(84)**

I: Porifera, *Aplysina* sp.
B: Proteobacteria, *Pseudovibrio denitrificans*



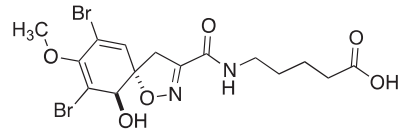
Aerothionin **(85)** R = H
Hydroxyaerothionin **(86)** R = OH

I: Porifera, *Aplysina* sp., *Psammaplysilla* sp., & *Pseudoceratina* sp.
B: Proteobacteria, *Pseudovibrio denitrificans*



Aplysinamisine II **(87)** n = 1
Purealidin L **(88)** n = 2

I: Porifera, *Aplysina* sp. & *Psammaplysilla* sp.
B: Proteobacteria, *Pseudovibrio denitrificans* sp.



Homopurpuroceratic acid B **(89)**

I: Porifera, *Pseudoceratina purpurea*
B: Proteobacteria, *Pseudovibrio denitrificans*

Fig. 9 **a** Structures of tunicate (phylum *Chordata*) and sponge (phylum *Porifera*) natural products determined to be bacterial in origin through culture-dependent methods. I = invertebrate source; B = bacterial producer. **b** Structures of sponge (phylum *Porifera*) natural products determined to be bacterial in origin through culture-dependent methods. I = invertebrate source; B = bacterial producer

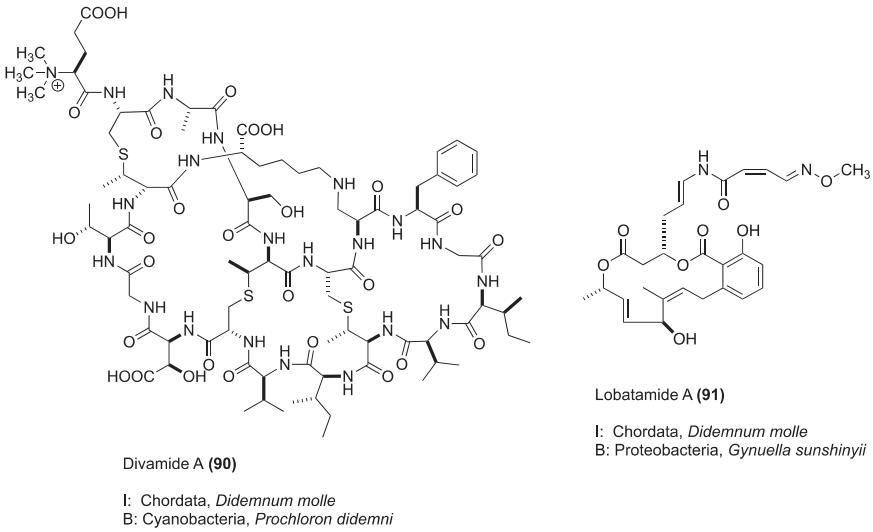


Fig. 10 Structures of tunicate (phylum *Chordata*) natural products determined to be bacterial in origin through both culture-independent and culture-dependent methods. I = invertebrate source; B = bacterial producer

The possible bacterial biosynthetic origin of molecules isolated from sponges and tunicates—the case made by analyzing of biosynthetic production from both culture-dependent and independent schemes

Harnessing the power of metagenomics, synthetic biology, and culturable isolates potentially provides a route to unlock access to diverse chemical scaffolds often only seen in the metagenomes of invertebrates. The following two cases based on the compounds shown in Fig. 10 highlight different strategies in which these tools can assist in the discovery of invertebrate-associated natural products.

The anti-HIV peptide divamide A (**90**) was isolated from the ubiquitous tunicate, *Didemnum molle*, however, only nanogram quantities were obtained hindering structural elucidation. Metagenomic sequencing was used to predict structural features and the symbiotic cyanobacteria *Prochloron didemnid* was identified as the biosynthetic source of this peptide. A proof-of-concept result was provided by expressing the divamide pathway in *E. coli* which provided better quantities of divamide A (**90**) and of 11 other congeners. This latter outcome facilitated structural elucidation and biological screening [156].

Lobatamide A (**91**) was initially isolated from the tunicate *Aplidium lobatum* in 1998. Over two decades later, genome mining focused on identifying the products of diverse oxidation and directed researchers to focus on the polyketide synthases-associated flavoprotein monooxygenases involved in the biosynthesis of oxygen containing polyketides. The biosynthetic modules identified from the plant-derived Proteobacteria, *Gynuella sunshinyii* [157], were identified as responsible for the oxygen incorporation in the biosynthesis of **91**, and also provided a biosynthetic hypothesis for insertion of oxygen atoms into the macrocyclic cores of sponge-derived salarin A and pateamine A (**65**). A 5 L culture provided an unspecified amount of **91** that was rigorously characterized by NMR and MS.

Prospects to gather future understanding on the involvement of bacteria in producing meaningful metabolites from sponges and tunicates

Scheme 1 and the accompanying annotations highlight bacterial diversity associated with the holobiont of sponges and tunicates. Also, the remarks in Figs. 3–10 summarize bacterial taxa possessing prolific machinery for natural product biosynthesis. Prior to the advancements and general availability of next generation sequencing it was often hypothesized, without buttressing experimental data, that sponge and tunicate-associated natural products were bacterial in origin especially when similar chemical scaffolds were isolated from both sources. This section focuses on three sets of our favorite sponge natural products that have similar scaffolds to the bacterial compounds as shown in Fig. 11. Most importantly, current knowledge is incomplete about the biosynthetic synergy between the invertebrates and bacteria that produce this collection of compounds. First, jasplakinolide (**92**) and its >20 congeners have been isolated from at least three different taxa of sponges [158, 159]. This family of compounds contain structural similarities to the chondramides (**93**–**96**) produced by the terrestrial bacteria *Chondromyces crocatus*, and the miuraenamides (**97**–**99**) produced by the marine bacteria *Paraliomyxa miuraensis* [160, 161]. Second, salicylihalamide A (**100**) isolated from the *Haliclona* sponge [162] is structurally similar to apicularen A (**101**) produced by the

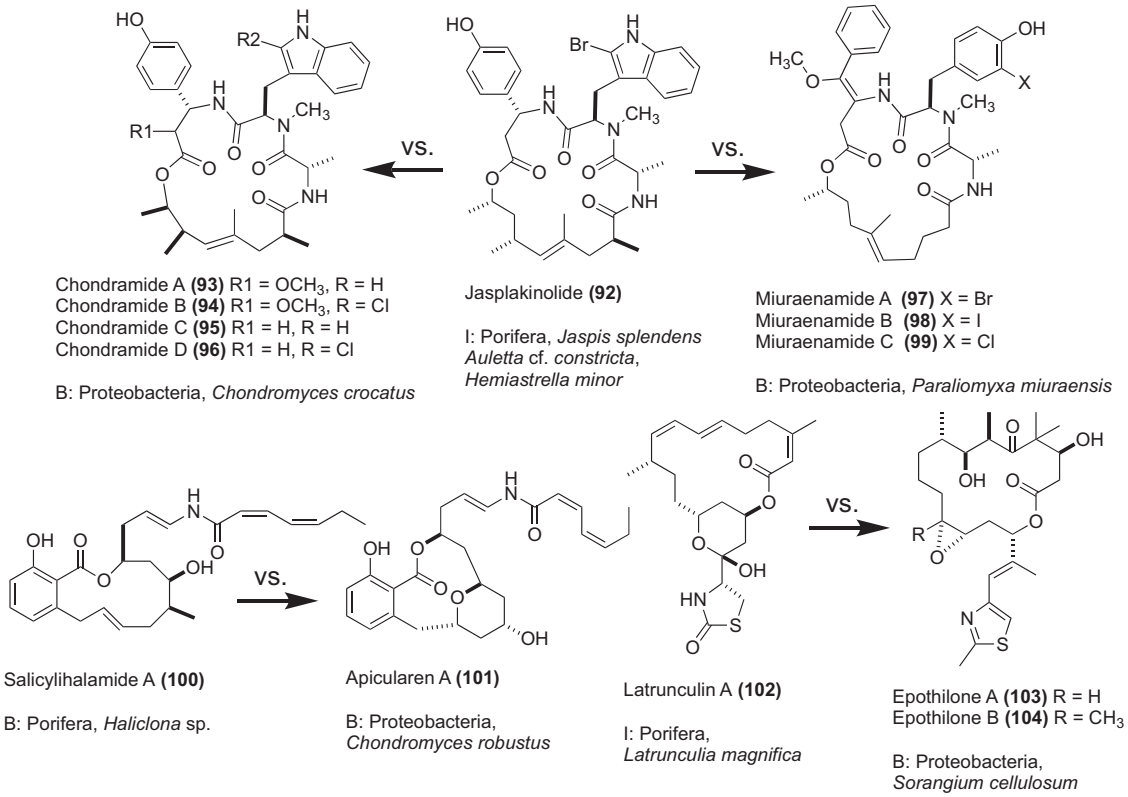


Fig. 11 Structures of sponge (phylum *Porifera*), tunicate (phylum *Chordata*), and bacterial metabolites with similar chemical scaffolds. I = invertebrate source; B = bacterial producer

terrestrial bacteria, *Chondromyces robustus* [163]. Last, latrunculin A (**102**), initially isolated from the sponge *Latrunculia magnifica* [164], shares structural similarities with the epothilones (**103**, **104**) produced by the terrestrial bacteria, *Sorangium cellulosum* [165].

Conclusions

An overarching goal in this review has been to illustrate the evolving overlap between the biosynthetic machinery of marine-derived bacteria vs. that of the chemically prolific sponges and tunicates. The ~28,500 marine natural products identified by the end of 2016 constitute an enormous assemblage of wide-ranging structural scaffolds. Annually, more than 1000 new compounds are described from Oceania and in 2018 the annual total tally was 1554 compounds: 222 from sponges, 12 from tunicates, and 240 from marine bacteria (with 69% of these from actinomycetes) [81]. A striking theme contained in both recent research papers and reviews outlines that several sponge/tunicate-derived bioactive compounds are seemingly produced by the action of the invertebrate microbiome.

Discussed in this review were examples describing biosynthetic outcomes producing an immense range of natural product structural diversity, potentially arising through synergy between gene clusters from sponge, tunicate, and bacteria metagenomes. At the top of the list of examples in the review are: (1) the molecular genetics-based discoveries from the sponge *T. swinhoei*, rich with the unculturable symbiont *Candidatus* Entotheonella, and (2) the use of complete genome sequences from the actinomycete *S. tropica* to direct further molecule discovery. These and many other case examples presented in this review dramatically illustrate the potential for selected sponges, tunicates, and marine-derived bacteria to provide an inexhaustible supply of novel natural products. Needed at this juncture is a firm understanding of the true nature of sponge/tunicate-microorganism symbiont interactions whose machineries produce novel metabolites in the natural environment that could potentially be followed-up by carrying out the natural product production in the laboratory. Such new understanding will undoubtedly reveal fresh paradigms in marine natural products research and this is underscored by the dramatic statement in a 2020 review, “We continue to draw the attention of readers to the recognition that a significant number of natural product drugs/leads are actually produced by microbes and/or microbial interactions with the host from whence it was isolated” [24]. We predict that in the future new insights will be obtained by focusing on parallel

scaffolds from sponges and tunicates vs. those of marine-derived bacteria. The toolbox for the future to discover new paradigms in marine natural products research must combine analytical spectrometry with the application of synthetic biology, genome mining, experimental therapeutics, and other transformative approaches.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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