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Advances in Exploring the Therapeutic Potential of Marine Natural Products

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Abstract

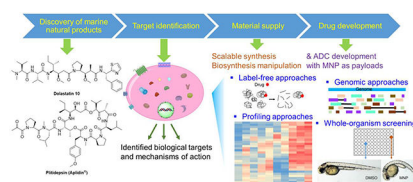
Marine natural products represent novel and diverse chemotypes that serve as templates for the discovery and development of therapeutic agents with distinct mechanisms of action. These genetically encoded compounds produced by an evolutionary optimized biosynthetic machinery are usually quite complex and can be difficult to recreate in the laboratory. The isolation from the source organism results in limited amount of material; however, the development of advanced NMR technologies and dereplication strategies has enabled the structure elucidation on small scale. In order to rigorously explore the therapeutic potential of marine natural products and advance them further, the biological characterization has to keep pace with the chemical characterization. The limited marine natural product supply has been a serious challenge for thorough investigation of the biological targets. Several marine drugs have reached the markets or are in clinical trials, where those challenges have been overcome, including through the development of scalable syntheses. However, the identification of mechanisms of action of marine natural products early in the discovery process is potentially game changing, since effectively linking marine natural products to potential therapeutic applications in turn triggers motivation to tackle challenging syntheses and solve the supply problem. An increasing number of sensitive technologies and methods have been developed in recent years, some of which have been successfully applied to marine natural products, increasing the value of these compounds with respect to their biomedical utility. In this review, we discuss advances in overcoming the bottlenecks in marine natural product research, emphasizing on the development and advances of diverse target identification technologies applicable for marine natural product research.

Graphical abstract

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Declaration of Interest

The authors declare the following competing financial interest(s): H.L. is a co-founder of Oceanix Pharmaceuticals, Inc., which has licensed patents and patent applications related to marine natural products mentioned in this review and is negotiating additional licenses (apratoxins, largazole).



Keywords

marine natural products; first-in-class pharmacological agents; target identification approaches; material supply strategies

1. Introduction

Historically, natural products served as important drug leads against a wide array of human diseases. From 1981 to 2014, among all of the 1211 approved small-molecule therapeutic agents, natural product related drugs could account for almost 65% of the marketed drugs, which were either used unaltered or developed based on the naturally occurring structures. [1] In recent years, the interest in marine sources has intensified, in part due to the awareness of the tremendous biodiversity in the marine environment viewed as a guarantee for new chemistry and biomedical potential, and in part due to the drastic advances in both research techniques and strategies to address the key issues hampering marine natural product-based drug discovery and development[2,3] Initial genome sequencing of marine natural product-producing microorganisms suggest that most marine natural products (MNPs) remain to be discovered.[4] MNPs have been well recognized as powerful bioweapons to fill the pipeline of drug leads for the pharmaceutical industry. As MNPs possess unique structural architectures covering broad chemical space, they continuously serve as promising templates to inspire drug development against a variety of diseases. Besides their clinical utility, MNPs are also applied as probes in advancing basic biomedical research for the study of biological pathways and exploring unconventional biological space for drug discovery. This review is not intended to be comprehensive, but highlights accomplishments in the development of advanced technologies to explore the biological targets of MNPs and the representative achievements using MNPs to target diverse therapeutically relevant biological space.

To continue to fuel the drug pipeline with MNPs (Chapter 2) and successfully compete with complementary drug discovery approaches, MNP drug discovery research requires addressing three major challenges: (1) how to identify novel molecules; (2) how to solve the supply issues and (3) how to correlate bioactivity with underlying target/MOA. Advances have been made in all these areas (Figure 1). The increased investigation and annotation of marine biological (genetic) diversity has been yielding a wealth of genome sequence information indicating biosynthetic potential that mostly remains to be translated into chemicals, as many compounds are only predicted based on biosynthetic gene cluster (BGC) sequence data. However, a steady number of chemically diverse structures has been characterized, in large part due to improvements of analytical capabilities and development of dereplication techniques, including nanoscale NMR[5] and molecular networking,[6] respectively, which will not be discussed here. More relevant to this review, advances in

exploring the therapeutic potential of existing MNPs are intimately tied to solving the supply problem to enable a rigorous biological evaluation and catalyze development efforts (Chapter 3). All MNPs possess biological functions and may have biomedical potential. However, there has to be an incentive to develop scalable methods. The incentive is the bioactivity linked to a direct therapeutic target (binding partner) or at least tractable mechanism of action tied to a disease process and that can provide biomarkers for preclinical and clinical studies. Novel target identification methods and techniques to capture MOA with minimal amount of material are potential game changers as they can be implemented early in the discovery process, even before a synthesis route has been developed (Chapter 4). The diversity of MNPs allows for the discovery of potential first-in-class therapeutic agents with common drug targets (proteins) and unusual biological targets like lipid membrane/sterols and provides fundamental options for the development of effective antibody-drug conjugates (ADCs) (Chapter 5). The following chapters should illustrate representative achievements to provide multifaceted insight into the current stages of research to advance MNPs and connect them with their therapeutically relevant biological targets.

2. Drugs and Drug Candidates from the Ocean

Through decades of intense efforts in both academia and the pharmaceutical industry, ten marine-derived drugs (Figure 2) have successfully reached the market, among which five are for the treatment of cancer, including cytarabine (Cytosar-U®), trabectedin (ET-743, Yondelis®), eribulin mesylate (Halaven®) and the antibody-drug conjugates (ADCs) brentuximab vedotin (Adcetris®) and polatuzumab vedotin (Polivy®), three are for the treatment of hypertriglyceridemia (fatty acid type drugs; Lovaza®, Vascepa® and Epanova®), two are for anti-virus treatment, including vidarabine (Vira-A®) and iota-carrageenan (Carragelose®) and one is for the amelioration of severe chronic pain, namely ziconotide (Prialt®).

Until now, over twenty MNPs as drug candidates have been investigated in different phases of clinical trials and a plethora of drug leads are under extensive preclinical development for various applications. [7,8] Newman and Cragg's recent review on drugs and drug candidates from marine sources should be consulted by interested readers. [8] Due to their potency, some MNPs also serve as payloads for ADCs, as demonstrated for the marketed brentuximab vedotin and polatuzumab vedotin (Figures 2 and 13). MNPs increasingly inspire the development of novel pharmacotherapeutics for various applications. Here we briefly highlight several structurally and functionally diverse molecules reaching different stages of clinical trials to further display the rich arsenal of bioweapons offered by the marine environment.

The cyclic depsipeptide plitidepsin (Aplidin®, first-in-class, Figure 3) was originally isolated from the marine tunicate *Aplidium albicans* and is known to interact with eukaryotic elongation factor 1A2 (eEF1A2) in tumor cells. It has reached Phase III clinical trial for the treatment of relapsed/refractory multiple myeloma in combination with dexamethasone. [9,10]

Tetrodotoxin (Tectin®, Figure 3), probably the most well-known marine toxin, is a guanidine derivative with a highly oxygenated carbon skeleton from puffer fish (specifically the liver, ovaries and skin) of the *Tetraodontidae* family, which was believed to accumulate via the marine food chain and/or derive from certain endo-symbiotic bacteria, such as *Vibrio* spp. and *Pseudomonas* spp.[11] Mechanism studies indicated tetrodotoxin exerted its effect by competitively blocking voltage-gated sodium channels (VGSCs) in a highly selective manner. Tetrodotoxin binding to VGSCs in nerve cell membranes prevents depolarization and propagation of action potentials and leads to the loss of sensation.[12] More recently, it has been extensively used as a chemical tool to functionally characterize VGSCs and has been applied as an analgesic agent against pathologic pain.[11,13] Two Phase III safety and efficacy studies for management of moderate to severe inadequately controlled cancer-related pain were completed in 2012 and results are awaiting to be reported.[8]

Plinabulin (NPI-2358, Figure 3) is a synthetic analog of halimide, a naturally-occurring aromatic alkaloid derived from the marine fungus *Aspergillus* sp. CNC139, which was collected from the waters off the Philippine Islands (halimide is also known as phenylahistin, isolated from terrestrial strains, *Aspergillus ustus* NSC-F037 and NSC-F038).[14,15] Targeting near the colchicine binding site on tubulin monomer and thus inhibiting tubulin polymerization, this molecule could lead to destabilization of the tumor vascular network, reduce tumor blood flow, and thereby cause tumor regression. [16,17] Plinabulin is now under investigation in Phase III trials to assess its application in combination with docetaxel in patients with advanced non-small cell lung cancer due to its function as a vascular-disrupting agents and its apoptotic effect on tumor cells.

Salinosporamide A (Marizomib, Figure 3) was isolated from marine bacteria *Salinispora tropica* and *Salinispora arenicola* and identified as a potent proteasome inhibitor.[18] Ever since the success of bortezomib, the first approved therapeutic proteasome inhibitor as anticancer agent, the proteasome has received growing interest as a target for the development of anticancer agents.[19] More importantly, in 2012, another proteasome inhibitor carfilzomib (Kyprolis®), an analog of epoxomicin (initially isolated from an *Actinomycetes* strain),[20] was approved as an anticancer drug, suggesting the potential of natural proteasome inhibitors as therapeutic agents. Salinosporamide A entered phase I clinical trials for the treatment of multiple myeloma only three years after its discovery. [19,21] To examine the safety, pharmacokinetics and pharmacodynamics of salinosporamide A, a phase II clinical trial in patients with relapsed/ refractory multiple myeloma has been completed.[22]

Ralaniten acetate (EPI-506, Figure 3) is an experimental nonsteroidal antiandrogen (NSAA), which is a prodrug of MNP EPI-002 and a derivative of MNP EPI-067. It targets the androgen receptor (AR) N terminus (NTD) and can block the AR signaling and has entered phase II clinical trial for treatment of metastatic castration-resistant prostate cancer (mCRPC).[23]

3. Research Highlights in Overcoming the Supply Problem to Advance Marine Natural Products for Therapeutic Applications

The successful translation of MNPs into commercial drugs further demonstrates the potential of MNPs as promising leads for the development of therapeutic agents. Even though there are bottlenecks obstructing the development process, significant advances have been made to overcome the related issues in MNP research. The isolation of therapeutically promising MNPs from natural sources is often not reproducible and even serendipitous, so the material supply of MNPs is always a critical problem, which would affect the structure elucidation and prevent the comprehensive biological investigation of the MNPs. With the development of various research strategies in synthesis and biosynthesis, the limited supply issue has been greatly improved. For example, total synthesis, especially large-scale synthesis of complex MNPs, has provided plentiful material supply and valid chemistry evidence for thorough biological investigation to promote the MNP research. For example, the large-scale total synthesis of largazole (Figure 4), a class I histone deacetylase (HDAC) inhibitor isolated from marine cyanobacteria originally believed to be *Symploca* sp.[24–26] and later reclassified as *Caldora penicillata*,[27,28] has been established and decagrams of the target compound was obtained as final product in 21% overall yield over eight steps.[29] Eribulin mesylate (Halaven®, Figure 3) is a synthetic analog of MNP halichondrin B, which was originally isolated from sponge *Halichondria okadai* in 1986. It is a microtubule-depolymerizing drug approved by FDA for the treatment of metastatic breast cancer.[30] The supply problem for eribulin mesylate was solved by developing scalable synthetic methods, even though over 60 steps are required.[31,32] (+)-Discodermolide (Figure 4) was first isolated from marine sponge *Discodermia dissoluta* in 1990[33] and it was reported to stabilize microtubules.[34,35] (+)-Discodermolide was proceeded into phase I clinical trial in 2002, the results further confirmed the activity of (+)-discodermolide as a microtubule stabilizer. Since there is minimal success in supplying enough material from other approaches, the material of (+)-discodermolide for thorough clinical studies is from large-scale total synthesis. Thus, intense efforts have been made by different research groups and companies and a number of synthetic methods have been developed.[36–42] Smith and his co-workers have established and optimized the total synthesis of (+)-discodermolide and meanwhile completed the gram-scale synthetic compound production to enable convenient supply for clinical investigations. [43–46] In addition, Smith *et al.* designed and developed the total synthesis of spongistatin 1 (Figure 4),[47,48] a MNP originally isolated from a sponge of the genus *Spongia*. [49] Spongistatin 1 is one of the most potent tumor cell growth inhibitors,[48] and it has been reported to inhibit mitosis and microtubule assembly.[50] Spongistatin 1 was reported to represent a promising therapeutic agent for the treatment of leukemic tumor cells which exhibit chemoresistance due to overexpression of X-linked inhibitor of apoptosis protein (XIAP).[51]

With the utilization of compounds readily isolated from natural sources, semisynthesis also serves as a pivotal solution to the limited supply of MNPs. For instance, an enantiospecific and concise semisynthetic route of MNP puupehedione (Figure 4) that was originally isolated from *Verongid* sponge,[52] was described.[53] The semisynthesis was achieved from the natural product sclareolide within seven steps. Puupehediones exhibit a variety of

biological activities including antiangiogenic, antitumor, antimicrobial and immunomodulatory effects. [54] Sufficient supply of puupehedione through semisynthesis allows for complete and in-depth study to extend the biomedical applications of puupehediones. Moreover, heterologous expression of biosynthetic gene clusters represents an emerging approach to enable MNP material supply. Successful heterologous production of various compounds have been reported, including polyketides, nonribosomal peptides and hybrid nonribosomal peptide–polyketides and isoprenoids,[55] although scalability is not necessarily straightforward. An interesting example regarding the heterologous expression of MNPs is haliangicin (Figure 4), the first marine myxobacterial antibiotic from the halophilic myxobacterium *Halicmgium ochraceum* SMP-2.[56] The biosynthetic gene cluster (BGC) of haliangicin was identified first and then expressed heterologously in *Myxococcus xanthus* to afford the haliangicin in a highly efficient manner, resulting in a 10-fold increase in the production and 3-fold increase in the growth rate. [57]

Besides, mariculture is another solution to the MNP supply problem. Bergman *et al.* reported the extended culture at sea of three sponge species *Negombata magnifica*, which produces latrunculin B (Figure 4), a cell-permeable actin polymerization inhibitor and confirmed this technology to be an appropriate approach for the accumulation of MNPs.[58] Apart from the approaches mentioned above, fermentation is also applied to provide MNP supply for scientific research and clinical investigations. Although the proteasome inhibitor salinosporamide A (Figure 3) has been synthesized, its ongoing clinical investigations mainly rely on large-scale saline fermentation for continuous supply.

4. Advances in Characterization of Target and Mechanism of Action

Although MNPs are clearly excellent starting points for the development of pharmacotherapeutics, one major challenge is to annotate the bioactivity and unveil the underlying mechanism to translate them into suitable biomedical applications. Among all the established target identification methods, the classical strategy to address this problem is to design a probe with reporter/affinity tag, where the natural product serves as a warhead. However, these strategies require extensive efforts to design feasible probes, conduct structure-activity relationship (SAR) studies, optimize natural product structures and validate resulted hits, among which are likely false positive targets.[59,60] To overcome these limitations, numerous label-free approaches were developed to probe the biological targets of various compounds without requirement of chemical modification and could be performed in high-throughput manner.[61,62] These strategies not only save efforts in chemical synthesis and structural modification, but also avoid the risk of altering the activity and molecular behavior of natural products as well as obviate the immediate need for SAR studies prior to target identification campaigns. Moreover, all the strategies mentioned below enable the identification of multiple targets for a certain compound, which would certainly expedite the determination of mechanisms of action (on-target) and the elucidation of potential side effects (off-target) or relevant pleiotropic pharmacology. More importantly, it is very difficult to identify the biological target and mechanism of action (MOA) of a compound with very small amount of material, which is very common in the case of MNPs. This bottleneck can be overcome by the following and other emerging approaches.

4.1 Label-free Approaches

4.1.1 Drug Affinity Responsive Target Stability (DARTS)—Drug affinity responsive target stability (DARTS) was developed to identify the molecular targets of small-molecule drugs and study protein-ligand interactions (Figure 6A). It is based on the principle that a protein becomes more stabilized and less susceptible to proteolysis when it is ligand/drug-bound than when it is ligand/drug-free. This reduction in the protease susceptibility is specific to the target protein(s) regardless of affinity. In addition, DARTS can utilize any small molecule in its native form without further chemical modifications or SAR studies.[63] Generally, protein or whole-cell lysates are obtained before incubation with small-molecule drugs or vehicle control, followed by proteolysis and then immunoblot to identify drug targets. [64] The feasibility of DARTS was demonstrated using human Jurkat cells after treatment of didemnin B (Figure 5). Didemnin B is an analog of plitidepsin (Aplidin®, Figure 3) and it was reported to inhibit protein synthesis by targeting the guanine nucleotide-binding elongation factor EF-1 α . [65] DARTS revealed a much stronger protected band corresponding to EF-1 α in the didemnin B treated Jurkat cell proteolyzed extracts compared to that in the vehicle treated group, [66] suggesting the practical applicability of DARTS for complex MNP target identification.

4.1.2 Cellular Thermal Shift Assay (CETSA)—Drug-target interaction can also be assessed by a cellular thermal shift assay (CETSA), which takes advantage of the biophysical principle of ligand-induced thermal stabilization of target proteins, producing a thermal shift to a higher temperature in its melting curve (Figure 6B).[67] In this method, cells are cultured with or without drug treatment and the resulting aliquots are heated to different temperatures to induce protein denaturation, remaining soluble proteins are then extracted and quantified. Quantification of the soluble target proteins at each temperature is achieved using Western blot to generate the protein thermal melting curve, which enables determination of thermal stability and identification of ligand-induced thermal shifts.[67] For example, an acetylenic alcohol, (3*S*)-icos-4*E*-en-1-yn-3-ol (Figure 5), was isolated from the marine sponge *Cribrochalina vasculum* and it was reported to induce tumor-specific apoptosis in non-small cell lung cancer cells. [68] CETSA of this acetylenic alcohol revealed that this alcohol can specifically bind with the insulin growth factor-1 receptor β (IGF-1R β), demonstrating the therapeutic potential of this compound for the treatment of cancer, especially non-small cell lung carcinoma (NSCLC). [68,69] To study the effect of drugs on the thermal profile of proteins on a proteome-wide scale, thermal proteome profiling (TPP) was developed by combing CETSA with multiplexed quantitative mass spectrometry (Figure 6B).[70] TPP exhibits promising applicability in identifying both therapeutically relevant targets and off-target protein binding to discover off-target toxicity or pleiotropic pharmacology. [71] Moreover, comparison of the thermal profiles obtained after drug treatment of intact cells with cell extracts enables to distinguish direct ligand/drug-target binding from downstream effectors, which could serve as a means to establish mechanistic biomarkers. However, since CETSA and TPP are both dependent on the extraction of proteins in the detergent-free buffer, they are not suitable for proteins not soluble under these conditions, such as membrane proteins.[61,70] To overcome this critical limitation, Savitski and Drewes *et al.* developed an optimized TPP approach to extend its application for ligand-membrane protein interactions with the involvement of a mild detergent during cell

extraction.[72] Denaturation temperature shift was observed for ATP-binding transmembrane proteins during their following assessment.

4.1.3 Thermal Stability Shift-based Fluorescence Difference in Two-dimensional Gel Electrophoresis (TS-FITGE)—Another label-free method to identify targets from a proteome-wide scale is TS-FITGE (thermal stability shift-based fluorescence difference in two-dimensional gel electrophoresis) (Figure 6C). Similar to CETSA, TS-FITGE detects the thermal stability shift of the target protein upon ligand engagement; but unlike CETSA, TS-FITGE takes advantage of the intra-gel quantification of each protein spot resulting from 2D gel electrophoresis to reveal the thermal stability change, with following inter-gel image analysis for hit confirmation. [73] Using this method, the protein kinase C α (PKC α) was confirmed to be the target of bryostatin 1 (Figure 5), a MNP from the marine bryozoan *Bugula neritina*,[74] suggesting the applicability of TS-FITGE to identify membrane proteins as active biological targets-. [74] Bryostatin 1 is demonstrated to be a potent activator of protein kinase C (PKC),[75] and it entered phase II clinical trials for assessment of its effect on various diseases, including cancer, HIV and Alzheimer's disease (AD). [8] Moreover, using TS-FITGE, nucleophosmin (NPM) was identified as a new protein target of hordenine, which is a natural product that can induce *in vitro* translation. [73]

4.1.4 Other Approaches—In addition to the label-free approaches applied for the target identification of natural products from marine species, several more methods have been developed to complement the biological target identification process applied to different compounds so far. Even though the generality and applicability of these methods in the target identification of MNPs have not been verified yet, they are amenable approaches to be applied to MNPs, especially because they only require a small amount of material.

SPROX (stability of proteins from rates of oxidation) takes advantage of mass spectrometry to measure protein thermodynamic stability change for target identification (Figure 6D).[62] It uses hydrogen peroxide in the presence of a denaturant in different concentrations to oxidize proteins[76] and measures thermodynamic changes of protein-folding reactions to detect ligand-target interactions. [77] Using SPROX, two known proteins, cyclophilin A and UDP-glucose-4-epimerase, were identified as biological targets of cyclosporine A, which is an immunosuppressive medication in organ transplantation.[77]

Another approach to identify targets for small-molecule drugs is a platform named SEC-TID (size-exclusion chromatography for target identification) (Figure 6E). SEC-TID is developed on the basis of size-exclusion chromatography in 384-well format to resolve drug-target interactions. [78] In this method, small-molecule drugs are mixed with recombinant or purified protein targets first, drug-target interactions are then resolved using size-exclusion column chromatography. Subsequently, small-molecule drugs bound to their targets are quantified using LC-MS.[62] This approach was utilized to elucidate the target of the Wnt inhibitor XAV939 as tankyrase 1. [79] Moreover, novel molecular interaction between the tumor-vascular disrupting agent vadimezan/ASA404 and farnesyl pyrophosphate synthase, as well as the interaction between the diuretic mefruside and carbonic anhydrase XIII, were identified using this strategy.

The emerging awareness of polypharmacology led to the establishment of an applicable approach for drug target identification, namely TICC (target identification by chromatography coelution) (Figure 6F). TICC monitors the interactions of small-molecule compounds with protein targets using non-denaturing HPLC coupled to LC-MS. TICC was developed based on the rationale that upon binding to a protein, a shift would occur to the chromatographic retention time of a compound. The identification of this characteristic shift leads to the deconvolution of the biological targets.[80] A cytoplasmic delta (24)-sterol C-methyltransferase, Erg6p, was identified as the biologically relevant secondary target of antifungal compound 4513-0042. Moreover, a possible off-target of the dopamine receptor agonist A77636 was identified as well. [80] TICC is suitable for the detection of protein targets with low abundance and low affinity, but it can only detect noncovalent hydrophobic protein-ligand interactions.[81]

The label-free approaches for target identification avoid chemical modification of the test compounds, which is especially beneficial for MNPs that are usually characterized by complex structures and challenging synthesis of functional tagged compounds for affinity chromatography.

4.2 Genomic Approaches

Besides the traditional target-/cell-based high-throughput screening strategies, which only focus on selected targets/cellular properties, modern screening approaches incorporate genome-wide analysis to correlate MNPs with biological targets or at least mechanisms in an unbiased fashion.[82]

4.2.1 Gene Dosage Screens—Systematically modulating gene product levels via cDNA overexpression and/or si/sh/miRNA-mediated mRNA knockdown has been increasingly utilized to elucidate the targets and mechanisms of natural product action. Gene dosage screens on a genomic scale in mammalian cells have been first undertaken with apratoxin A (Figure 7), a MNP produced by cyanobacterium *Moorea bouillonii*. [83] Genomic cDNA overexpression indicated that apratoxin A interrupts fibroblast growth factor receptor (FGFR)-mediated and signal transducer and activator of transcription (STAT) 3 signaling pathway.[84] Later apratoxin A was demonstrated to be an inhibitor of cotranslational translocation of proteins destined for the secretory pathway, such as receptor tyrosine kinases and growth factors, with its direct target identified as the central subunit of the protein translocation channel Sec61.[85–87] In addition, the protein target of aurilide (Figure 7), a polyketide-peptide hybrid isolated from the sea hare *Dolabella auricularia*, [88] was demonstrated to be prohibitin 1 (PHB1) using an affinity matrix prepared by immobilizing an aurilide-based biotin probe on avidin beads. Treatment of aurilide was reported to induce a mitochondria morphological change from tubular to fragmented forms, similar to the effects of siRNA-mediated *PHB1* knockdown, which further validates the inactivation of PHB1 by aurilide.[89] On the other hand, in a recent report, gene *ATP1A1* was identified to regulate the cytotoxicity of aurilide B (Figure 7), an analog of aurilide from marine cyanobacterium *Lyngbya majuscula* Gomont (Oscillatoriaceae), via a pooled and barcoded short-hairpin RNA (shRNA) library screen.[90]

An advanced genetic approach utilizing reducing or increasing gene dosage for target identification is chemogenomic profiling.[91] One practical strategy is named haploinsufficiency profiling (HIP), which identifies targets and pathways directly affected by the drug based on the observation that lowering a single gene dosage from two copies to one copy would cause increased sensitivity to drug treatment in diploid yeast.[92,93] The homozygous deletion profiling (HOP) is a complementary approach to identify functionally related genes that buffer the targeted pathway. [94] In this strategy, both copies of a certain gene would be deleted. Manzamine A (Figure 7) is an alkaloid isolated from sponges *Haliclona* sp.,[95] *Pellina* sp.,[96] and *Xestospongia* sp.[97] and has been demonstrated to exhibit various bioactivities, including antimalarial,[98] antibacterial[96] and anticancer[99] activities. It is also demonstrated to decrease the formation of single cells, abolish cell migration and sensitize pancreatic cancer cells to apoptosis induced by TRAIL (TNF-related apoptosis inducing ligand).[100] Chemogenomic profiling conducted in *S. cerevisiae* indicated that manzamine A (Figure 7) prevents autophagy in pancreatic cancer cells by targeting vacuolar ATPase.[101] Moreover, Andersen and Boone *et al.* reported a compendium composed of a number of chemical-genetic interaction profiles, resulting from exposure of yeast haploid deletion mutants to a small library of natural product extracts and pure compounds to identify hypersensitivity.[102] Extracts or pure compounds with similar MOAs were clustered together using a method utilizing hierarchical clustering and factorgram to reveal association of a compound or gene with more than one group. As a result, the biological target of papuamide B (Figure 7), a cytotoxic cyclic depsipeptide with anti-HIV activity originally isolated from sponge *Theonella mirabilis*,[103] was identified as phosphatidylserine (PS).[102]

Besides the successful application of gene dosage manipulation methods in the target identification process of MNPs, other genomic/transcriptomic screening technologies have been developed to decipher complex ligand/drug-target interactions.

4.2.2 Functional Signature Ontology (FUSION)—To properly develop therapeutic agents targeting disease-related mechanisms, an approach named FUSION (functional signature ontology) was recently developed to identify biological targets and MOA for MNPs (Figure 8A). A certain set of genes will display characteristic expression signatures from screening of siRNAs, miRNA mimics and MNPs. These signatures were assembled together into a similarity matrix to generate FUSION maps, which link bioactive compounds to specific biological processes.[104] During the assessment, several endogenous reporters of a specific biological process or disease will be selected first to quantify the perturbation after treatment. With this strategy, the putative MOA and potential therapeutic applications for several MNPs were successfully unraveled. In addition, this strategy is further highlighted by the successful application in target identification for didemnin B (Figure 5). The FUSION strategy indicated that didemnin B exerted its effects through activation of mTORC1.[105] Subsequent investigations regarding the MOA of didemnin B turned out to be consistent with published results, which demonstrated that didemnin B induced apoptosis by targeting both PPT1 (palmitoyl-protein thioesterase 1) and eEF1A1 (eukaryotic elongation factor 1A1).[106, 107]

4.2.3 RNA Sequencing (RNA-seq)—RNA-seq is a high-throughput strategy of transcriptome profiling using deep-sequencing approaches (Figure 8B).[108] It measures the expression of numerous genes at the same time and provides insights into multiple biological processes. RNA-seq can not only detect known transcripts, but also investigate the entire transcriptome in a high-throughput and quantitative manner.[108,109] In addition, RNA-seq is demonstrated to exhibit superior resolution to expose the fine transcriptome structure with a single nucleotide resolution.[109] The advancement of RNA-seq has been adopted in MNP research for potential target identification and especially mechanism studies. For example, the polysaccharide from the red alga *Gracilariopsis lemaneiformis* (PGL) was reported to significantly decrease proliferation and change morphology of lung cancer cells. The intrinsic mechanism and the genes modulated by PGL were revealed through transcriptome analysis using RNA-seq. The sequencing results indicate that PGL affects the apoptosis- and cell cycle-related gene expression, consistent with reported apoptosis and cell cycle arrest caused by PGL treatment.[110] Honaucin A (Figure 7), a MNP isolated from cyanobacterium *Leptolyngbya crossbyana*,[111] was demonstrated to activate Nrf2-ARE (nuclear erythroid 2-related factor 2-antioxidant response element) signaling pathway to exert antiinflammatory activity after high-throughput transcriptomic analysis by RNA-seq.[112]

4.2.4 DrugTargetSeqR—DrugTargetSeqR was developed to identify biological targets of drugs and other bioactive small-molecule compounds. It takes advantage of the repeated drug resistance related mutations in cancer identified by high-throughput sequencing and additionally incorporates CRISPR/Cas9-based genome editing and computational mutation discovery to reveal the targets of compounds of interest (Figure 8C). It was also developed to overcome some limitations inherited in gene manipulation methods, since the gene knockdown and phenotype correlation often fail due to differences between cumulative biological effects of protein knockdown and fast-acting chemical modulators.[113] To perform DrugTargetSeqR, resistant cancer cells are generated by treating the cells with test compounds, which will be further isolated to get rid of the MDR clones. The selected resistant cell clones and the parental cancer cells are then analyzed by RNA-seq. Mutations that specifically arise in response to drug treatment are identified. CRISPR/Cas9-based genome editing is adopted to determine the genes responsible for the recurring resistance of a specific drug, followed by biological investigation to validate the target. This approach was applied to ispinesib and YM155, two anticancer agents that entered phase II clinical trials, [114,115] to unveil mechanisms likely to cause drug resistance. Through integration with reporter gene assays or phenotypic screens, this approach displays potential to be applicable for more diverse compounds including non-cytotoxic agents.[113]

4.2.5 Genome Mining to Explore Biological Targets—Along with the advance achieved in exploring the biological targets of MNPs using different genomic approaches in disease-relevant cell types, there are parallel advances facilitating genome mining of MNP producing organisms/bacteria as a new method to identify natural products with therapeutically relevant biological targets, particularly antibiotics. Genome mining toward specific targets is a new approach (Figure 8D) developed to forecast the biological function of natural products without a priori knowledge of these compounds utilizing presumptive

resistance genes encoding target-modified proteins with orphan biosynthetic gene clusters. Using this strategy, Moore *et al.* discovered antibiotic producing gene clusters that produce fatty acid synthase (FAS) inhibitors belonging to the thiotetronic acid family from the marine actinomycete *Salinispora*. The gene cluster function was validated by direct cloning as well as heterologous expression. This methodology could be utilized to identify new gene clusters responsible for antibiotics production and could provide a helpful genome mining tool to discover new antibiotics.[116] Moreover, ARTS (Antibiotic Resistant Target Seeker) was developed to allow for efficient genome mining using Internet resources. ARTS incorporates target-directed genome mining and prediction of gene clusters for antibiotic production to serve as an information platform to identify potential or reported gene cluster targets.[117] In a recent report, Müller *et al.* described the discovery of topoisomerase inhibitors from myxobacteria following a self-resistance guided strategy derived from genome mining. Genes encoding biosynthesis of a topoisomerase inhibitor were identified first and subsequent activation of this gene cluster led to the discovery of a new group of compounds named pyxidicyclines.[118] Müller *et al.* further claimed the potential of target-driven genome mining in identifying new functional gene clusters and discovering new natural products in myxobacteria.[118] The successful application of target-directed genome mining in both marine bacterium and myxobacteria indicates the potential of this approach in predicting and identifying natural products with specific MOAs and demonstrates its practical value in different organisms, which would further enlarge the scope of novel chemical entity discovery from terrestrial and marine sources.

4.3 Profiling Methods

4.3.1 Antibiotic mode of action profile (BioMAP)—The NCI-60 human tumor cell line screen has served as a reliable platform for the discovery of new compounds with potent anticancer activity and MOA identification through biological response pattern comparison. [119] Similarly, BioMAP (Figure 10A) was established based on the hypothesis that antibiotics from the same structure class would generate similar biological activity profiles across a panel of bacterial pathogens, which can be used to classify unknown compounds by structural class. BioMAP provides a new profiling strategy for the discovery of new antibiotics with unique biological fingerprints. For example, arromycin (Figure 9) was isolated from a marine-derived actinobacterial library after BioMAP characterization and it was demonstrated to exhibit an antibacterial profile that did not match those of the training set included in this profiling. [120] Arromycin processes an unprecedented carbon skeleton for naphthoquinone antibiotics, it is the first reported naphthoquinone scaffold with a 2,4,4-trimethylcyclohexenyl C ring. Even though the exact MOA of arromycin's antibacterial activity remains unclear, its discovery validates the feasibility of BioMAP screen for the identification of structurally novel compounds as starting points for further medicinal chemistry investigation. [120]

4.3.2 Target-based profiling—The increasing prevalence of phenotypic screening has greatly promoted method development to assist in the target identification processes. Meanwhile, direct target-based profiling methods (Figure 10B) still play a pivotal role in discovering new compounds targeting potential therapeutic targets and determining the biological role of MNPs. Representative target-based profiling platforms were developed on

the basis of prevalent therapeutic targets, including kinases, G protein-coupled receptors (GPCRs) and proteases. Kinases play a crucial role in cell regulation including signal transmission, cell differentiation, proliferation, metabolism and apoptosis. [121] High-throughput kinase profiling allows a parallel approach by interrogating compounds against numerous targets in a single screen.[122] As an effort to discover new kinase inhibitors, a series of marine invertebrate extracts were screened against a panel of protein kinases. As a result, isoarenarol (Figure 9) and arenarol (Figure 9) from sponge *Dysidea arenaria* Bergquist were identified to be potent inhibitors of several therapeutically important kinases. [123] GPCRs represent the most common signal transduction system and the most intensively studied therapeutic targets since they are involved in diverse fundamental physiological functions. The dysfunction of GPCRs is implicated in diseases such as cancer, diabetes, hypertension and heart failure. [124] From profiling against a panel of 241 GPCR targets (agonist, antagonist and orphan), brintonamide D (Figure 9) was identified as a GPCR modulator, which can function as a C-X-C chemokine receptor type 7 (CXCR7) agonist as well as an antagonist of C-C chemokine receptor type 10 (CCR10), oxytocin receptor (OXTR), somatostatin receptor type 3 (SSTR3) and tachykinin receptor 2 (TACR2). Among all the GPCR targets, brintonamide D exhibits more than 10-fold potency toward CCR10 as an antagonist compared to other hits. [124] Recently, the discovery of amantamide (Figure 9) was reported and it was characterized as a GPCR agonist that can selectively activate the activity of CXCR7.[125] Proteases represent potential drug targets for various diseases including cancer, cardiovascular disorders as well as diseases resulting from parasites and viruses. [126] Target-based screening against a panel of proteases has led to the identification of several protease inhibitors from marine cyanobacteria. For instance, grassystatin A (Figure 9) has been reported to selectively inhibit aspartic protease cathepsin E and, to a lesser extent, cathepsin D after screening against a diverse set of 59 proteases. [127] Lyngbyastatin 7 (Figure 9) was found to exhibit elastase inhibitory activity, protect bronchial epithelial cells against elastase-induced antiproliferation and abrogate the elastase-triggered induction of pro-inflammatory cytokine expression. [128,129]

4.3.3 High-Content Screening (HCS)—High-content screening (HCS) is an image-based screening strategy that incorporates high-throughput techniques to collect quantitative data from complex biological systems (Figure 10C). HCS technology has been integrated into all the different aspects of drug discovery, including primary compound screening, SAR screening and evaluation of ADME (absorption, distribution, metabolism and excretion) properties. It has been considered as a mainstream technology in the pharmaceutical industry. [130] And it is certainly adopted by academia to discover new compounds with interesting biological activities, such as in the MNP field. Roch *et al.* reported a newly developed live cell-imaging platform for the discovery of new MNPs and investigation of their antimalaria effects on *Plasmodium falciparum*, which is regarded as the most aggressive malaria parasite. [131] They have taken advantage of this platform to discover new parasite inhibitors from a MNP library. The screening results identified bromophycolide A (Figure 9), isolated from red alga *Callophycus serratus*,[132] to exhibit potent antimalarial activities in the parasite while little cytotoxicity on the blood cells of the host. [131]

With the advancement of HCS, the combination of high-throughput screening technologies with imaging technique accompanied with automated microscopy has led to the establishment of CP (cytological profiling). CP utilizes imaging with automated microscopy in a high-throughput manner to quantify cells with a variety of descriptors. [133] This approach was applied to prioritize MNP extracts or fractions with interesting biological activity before further purification and following structure elucidation, leading to the discovery microferrioxamines A–D (Figure 9) as iron siderophores.[134] Moreover, using CP as a strategy to the discovery of MNPs with a specific MOA from a microbially derived extract library revealed two constituents from a marine sediment sample, as mitotic arrest inducers. These two constituents were then identified as XR334 and nocapyrone L (Figure 9), which function through disruption of tubulin dynamics and calcium channel function, respectively.[135]

To further explore the potential of HCS and enhance the probability to discovery novel compounds, an upgraded screening platform termed Compound Activity Mapping was developed by incorporating image-based CP information with metabolomics analysis.[136] This new technology can predict the constituents and MOA of bioactive components for very complex natural product extract or fraction library. For proof of principle, this strategy was applied to a marine bacterial sediment extract library for the discovery of quinocinnolinomycins A–D (Figure 9), which were identified to cause ER (endoplasmic reticulum) stress and induce the unfolding of proteins.

4.3.4 Whole-Organism Screening—With the aim of obtaining insight into the *in vivo* biological activity and to provide more predictable information about the potential therapeutic uses of a natural product, whole-organism models have been employed in medium/high-throughput screening at an early stage of marine biodiscovery.[137] Two representative examples utilizing roundworm (*Caenorhabditis elegans*) and zebrafish (*Danio rerio*), the most conventionally used simple model organisms (beyond yeast), are described here to indicate the pivotal role of an *in vivo* model in biomedical research and pharmaceutical development.

Caenorhabditis elegans has become a widely used animal model increasingly incorporated into various stages of drug discovery to reveal the biomedical relevance of natural products/synthetic molecules.[138,139] Using a *C. elegans*-based model (Figure 10D) for *Vibrio alginolyticus* infection, 256 extractions derived from sponge (*Haliclona spp.*) associated bacteria were screened to identify bioactive compounds that inhibit the virulence of *Vibrio* species and rescue the affected worms.[140] The initial screening identified the extract of *Alcaligenes faecalis* as complex nature product mixture capable of rescuing *V. alginolyticus* without significant toxicity. The following purification efforts led to the identification of a diketopiperazine (**1**, Figure 9) as the active constituent. The subsequent *in vitro* assays indicated that this compound solely inhibited the virulence of *V. alginolyticus* by suppressing quorum sensing without affecting its proliferation. *In vivo* assays further showed the newly discovered molecule could reduce the intestinal colonization of *Vibrio* species, improve pharyngeal pumping (food intake) of *C. elegans* and disrupt the formation of biofilm.

To date, numerous compounds have been discovered with readily available bioactivity using zebrafish as model.[141] This system proved particularly valuable for identifying novel neuroactive molecules against central nervous system diseases. As traditional *in vitro* assays fail to model brain complexity, and *in vivo* mice/rats assays were less compatible for high-throughput screening (high expense and ethical issues), zebrafish behavior-based screening (Figure 10E) is considered as an approach with predictive power.[142,143] Through profiling a library of xyloketal and isoprenyl phenyl ethers derived from marine mangrove fungus, a compound (**2**, Figure 9) was identified as a potent inhibitor of locomotor activity in larval zebrafish. Several derivatives were synthesized and evaluated at whole organism level using a pentylenetetrazole (PTZ)-induced epilepsy model in zebrafish. One parent compound together with three modified derivatives possessed the ability to attenuate PTZ-induced locomotor hyperactivity, revealing their potential for development as anti-epilepsy drug candidates. In addition, largazole (Figure 3) was validated as an antiangiogenic agent in a hypoxia-inducible factor (HIF)-dependent manner in human cells *in vivo* in zebrafish using a genetic model with activated HIF.[144]

4.4 Multidimensional Screening Platforms

In addition to the strategy incorporating genetic/chemical perturbation, isogenic cell lines and genetic zebrafish model systems have been employed together to pursue druggable targets/active constituents from a library of siRNAs (targeting 7784 genes)/commercial compounds (4720 small molecules) and MNPs simultaneously affecting multiple cancer pathways. [144] As oncogenic KRAS and hypoxia-inducible factor (HIF) pathways shared significant overlap in terms of gene expression signatures, molecules targeting both pathways were hypothesized to benefit cancer treatment in improving efficacy and lowering the risk of resistance. Two MNPs, dolastatin 10 (microtubule-depolymerizing agent) and largazole (class I HDAC inhibitor), were rediscovered as hits in modulating both KRAS and HIF pathways by displaying differential cytotoxicity between parental HCT116 and HCT116^{HIF-1 α -/-/HIF-2 α -/-} and HCT116^{WT KRAS} cells. For proof-of-concept studies, largazole was chosen for further *in vitro* and *in vivo* evaluation. At the transcriptional, protein expression, cellular as well as whole-organismal level, largazole was proven to exert antiproliferative and anti angiogenic effects in a HIF-dependent manner. Largazole treatment phenocopied the *colgate* (*hdac1*) mutant, validating HDAC as the target in zebrafish and specifically abrogated HIF-induced angiogenesis in *vhl* mutants. This multidimensional screening platform not only enabled the identification of molecules/targets with desired selectivity, but also offered molecular insights with an *in vivo* dimension in a rapid and targeted fashion.

All the approaches discussed in Chapter 4 that are applicable for the target identification of MNPs are summarized in Figure 11.

5. Marine Natural Products as First-in-Class Pharmacological Agents

All the approaches (Figure 11) described in Chapter 4 are amenable to protein target identification and most drugs including MNP-derived drugs target proteins. In many cases, revealing the biological target of a molecule is first attempted by recognition of structural

features resembling known pharmacophores. For example, largazole[25,26] (Figure 3) was recognized as a prodrug and the active species, largazole thiol, is proposed to interact with Zn²⁺-dependent HDAC isoforms since the thiol motif is similar to that of other HDAC inhibitors, such as FK228.[25,26] Compounds containing amino-hydroxy-piperidone (Ahp) moiety frequently display serine protease inhibitory activity such as lynghyastatin 7 (Figure 9), while molecules with embedded statine unit [(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid, Sta] like grassystatins A (Figure 9) are characteristic aspartic protease inhibitors. In addition to the protein targeted MNPs as first-in-class pharmacological agents, the incredible diversity of MNP has enabled the production and discovery of compounds targeting non-protein biological entities.

5.1 Marine Natural Products Targeting DNA Functionality

Some of the drugs listed in Chapter 2 target DNA at the level of replication and transcription and other drugs through intercalation. For example, cytarabine (Cytosar-U®, Figure 2) and vidarabine (Vira-A®, Figure 2) can both interfere with DNA synthesis,[145] while trabectedin (ET-743, Yondelis®, Figure 2) has been reported to interfere with activated transcription[146] and induce double-strand DNA breaks. [147] Moreover, aaptamine (Figure 12), a natural product commonly isolated from marine sponge *Aaptos*, exhibits potent DNA binding activity, which could be utilized for fragment-based drug design. [148] In addition, the transcription factor forkhead box O1 (FOXO1) was reported to negatively regulate the epidermal growth factor receptor (EGFR) signaling pathway. Stabilization of the FOXO1-DNA complex by small-molecule compounds could overcome drug resistance in anti-EGFR-based lung cancer therapy. High-throughput screening against a marine natural product library identified four hits as FOXO1-DNA stabilizers to selectively bind to the FOXO1 DNA-binding domain (DBD). The four hits are tanzawaic acid D (fungus *Penicillium steckii*, Figure 12), hymenidin (sponge *Stylissa carteri*, Figure 12), cribrostatin 6 (sponge *Cribrochalina vasculum*, Figure 12) and barbamide (Figure 12).

5.2 Marine Natural Products Targeting Unusual Biological Space

The diversity of MNPs allows for the discovery of compounds with novel structural characteristics, as well as the identification of compounds with unique MOAs, thus enabling the discovery of MNPs targeting unusual biological space. For example, theonellamide A (Figure 12) was originally isolated from the marine sponge *Theonella* sp.[149] and this bicyclic dodecapeptide was reported to exert antifungal activity while in the meantime display moderate cytotoxicity toward mammalian cells. In-depth study regarding the interaction of theonellamide A with the membrane revealed that theonellamide A binds to the membrane surface through interaction with sterols and the accumulation of theonellamide A could cause morphological change of the local membrane. With this unique characteristic, theonellamide-based probes would enable visualization of sterol-containing domains in living cells, which could further unveil the dynamic changes in membrane morphology.[150] Yoshida *et al.*[151] reported the chemogenomic profiling of theonellamide F (Figure 12), a MNP originally isolated from sponge *Theonella* sp..[152] The results indicated that theonellamide F would induce overproduction of 1,3-β-D-glucan in a Rho1-dependent manner. Thus, theonellamides were further characterized as sterol-

binding compounds to cause membrane damage and activate Rho1-dependent 1,3- β -D-glucan synthesis.

In addition to the lipid membrane/sterol targeting compounds, MNPs provide an opportunity to discover promising therapeutic agents with unique biological targets. Recently, a new natural product, seriniquinone (Figure 12), was isolated from the marine bacterium *Serinicoccus* and it was demonstrated to exhibit selective activity over a set of melanoma cell lines within the NCI-60 screen panel. Seriniquinone is the first compound reported to target dermcidin overexpressed in melanoma cells. Emerging data suggest that dermcidin plays a growing role in stabilizing cancer, targeting dermcidin by seriniquinone provides a new cell-specific approach to initiate autophagy and apoptotic cell death.[153]

5.3 Antibody Conjugation as Enabling Technology for Marine Natural Products

MNPs have been productive resources for cytotoxic compounds as drug leads. However, their exquisite potency may cause undesired off-target cytotoxicity. Antibody-targeted chemotherapy is a therapeutic strategy that utilizes a cytotoxic agent linked to a monoclonal antibody (mAb) that can specifically recognize a tumor-associated antigen, the complex used in this strategy is ADCs, which has become a representative class of therapeutic agents for the treatment of cancer. The mAb delivers the highly cytotoxic agent to tumor cells over normal healthy cells to achieve specificity and targeted cancer cell killing.[154,155] Currently, five ADCs have been approved by FDA in clinical application, including Mylotarg® (gemtuzumab ozogamicin) for the treatment of acute myeloid leukemia (AML), [156] Adcetris® (brentuximab vedotin) for the treatment of relapsed or refractory Hodgkin lymphoma (HL) and systemic anaplastic large cell lymphoma (ALCL),[157] Besponsa® (inotuzumab ozogamicin) for the treatment of B-cell acute lymphoblastic leukemia (ALL), [158] Kadcyla® (ado-trastuzumab emtansine) for the treatment of metastatic breast cancer[159] and Polivy® (polatuzumab vedotin-piiq) in combination with bendamustine and a rituximab product for the treatment of relapsed or refractory diffuse large B-cell lymphoma (DLBCL).[160] Considering the successes of Adcetris® (brentuximab vedotin) and Polivy® (polatuzumab vedotin-piiq), the ADC conjugates of monomethyl auristatin E (MMAE), a derivative of dolastatin 10 (Figure 13), a natural product isolated from marine cyanobacterium *Caldora penicillata*,[27] more efforts were made to seek for potent cytotoxins for the treatment of cancer in ADC format. For example, microtubule-targeting agent eribulin has been developed as ADC payload with humanized anti-human folate receptor alpha (FRA) antibody farletuzumab. This ADC has been reported to be a promising treatment for FRA-positive cancer.[161] Recently, a highly toxic proteasome inhibitor, carmaphycin B (Figure 13), a natural product isolated from the marine cyanobacterium *Symploca* sp.,[162] was engineered to function as an ADC warhead. This investigation resulted a potent cytotoxic analog of carmaphycin B with a suitable linker for conjugation as ADC and reveal additional underlying SAR principles of this compound class for further optimization (Figure 13).[155]

6. Conclusion

The advances in MNP research over the past years assisted with the sample prioritization to focus to identify novel structures and the spectroscopic characterization of novel MNPs, but also enabled effective ways to solve the MNP supply. However, the biological characterization of novel (and oftentimes structurally complex) MNPs is usually a challenging problem, especially since usually only small quantities are isolated and not all compounds would be subjected to total synthesis or scale-up efforts, unless a promising bioactivity or novel target has been identified. Various technologies have been developed to surpass this problem.

The utilization of label-free approaches in identifying the biological targets of MNPs has saved the efforts to modify the MNP, which is an advantage considering the commonly small amount of isolated compounds and usually complex structures. Genomic approaches provide options to investigate the biological role of MNPs from a genome-wide aspect, especially target-directed genome mining of producing organisms is expected to become increasingly productive when seeking to identify antibiotic targets. The more general profiling methods further facilitate the target identification process through direct target identification and phenotypic screening. In addition to the intensively studied therapeutic target proteins such as enzymes and GPCRs, MNPs have been reported to target unusual biological space, including lipid membrane components and proteins with unique biological functionality (Figure 14). The development of MNP-derived ADCs further demonstrates the potential of MNPs in different forms. Taken together, all the approaches discussed could be applied to identify the biological role of MNPs and we expect wider applications of these approaches to discover MNPs with unique MOAs. We anticipate that, through the increased development of assay systems to assess the biological roles of MNPs, we will witness the discovery of more first-in-class or most-potent-in-class drugs from marine sources.

According to the record in the MarinLit database, over 30,000 compounds have been reported from marine sources. Even though a decrease in the discovery rate of novel natural products has been observed while the rate and absolute number of the discovery of natural products have increased in the past years, the development of innovative discovery methods will continue to produce compounds with novel chemical characteristics.[163] More importantly, the advancement of target identification technologies guarantees in-depth investigation of MNPs that have been reported but the biological role of which has not been fully understood yet. The improved biological annotations of these MNPs would further facilitate the identification of potential drug candidates/ leads. Therefore, we expect that marine sources will continuously provide a rich supply for promising first-in-class therapeutic agents with new structural and biological characteristics.

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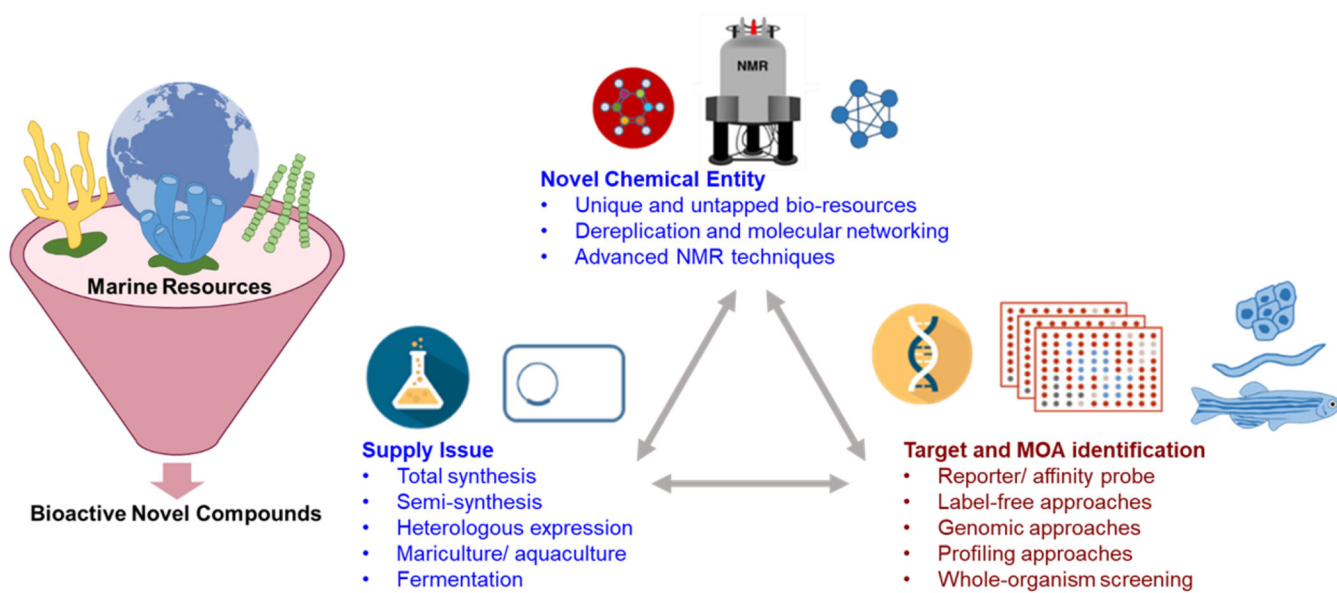


Figure 1. Three bottlenecks and corresponding research highlights in marine natural product drug discovery.

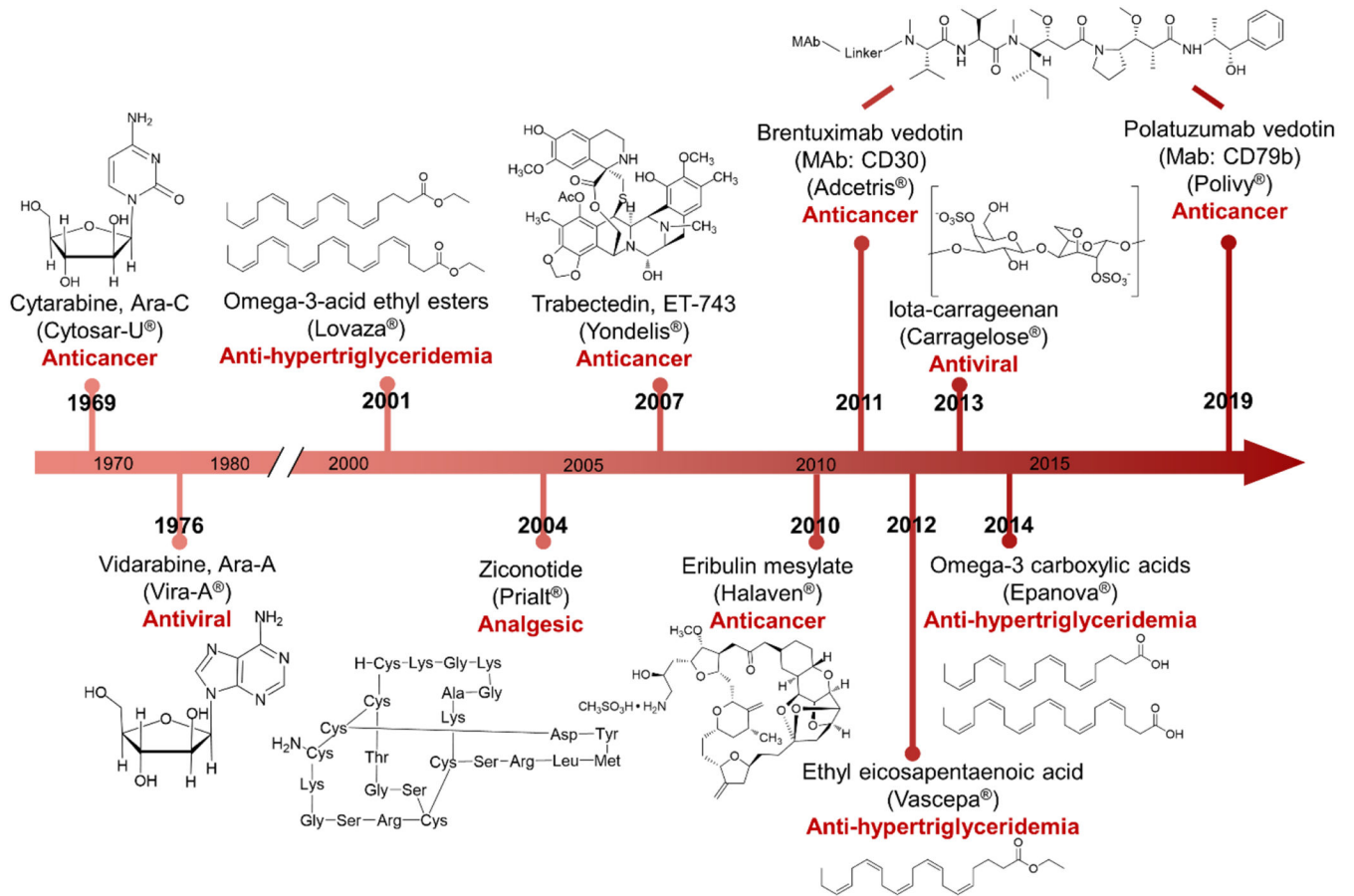


Figure 2.
Timeline for drugs from the ocean.

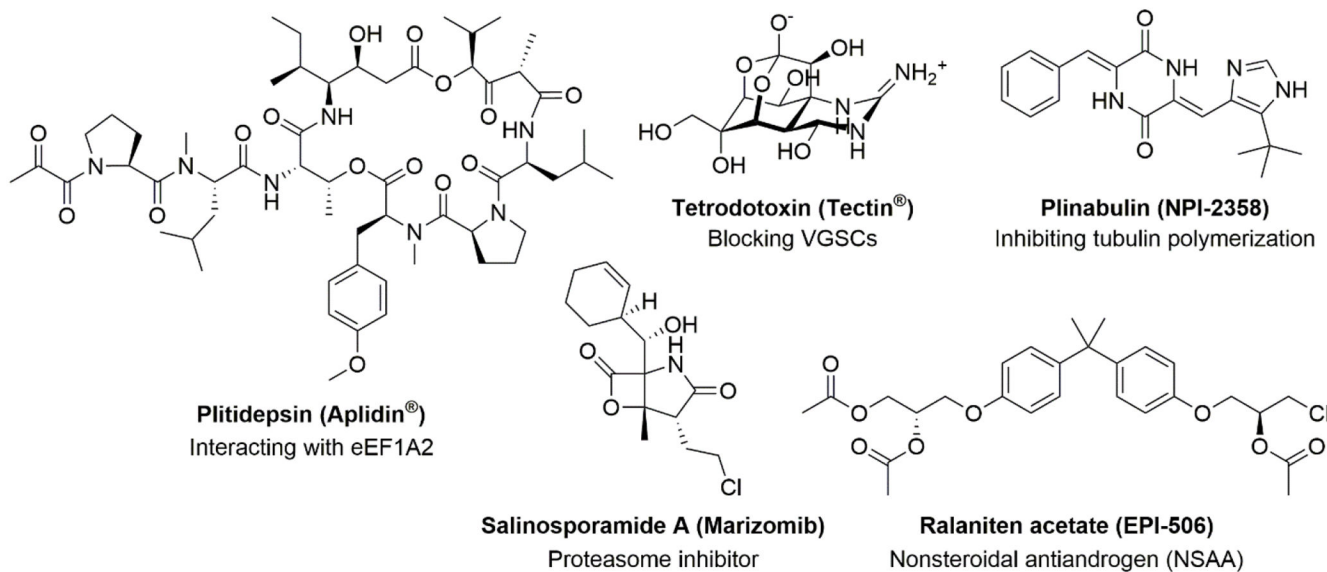


Figure 3.
Drug candidates from the ocean reaching clinical trials.

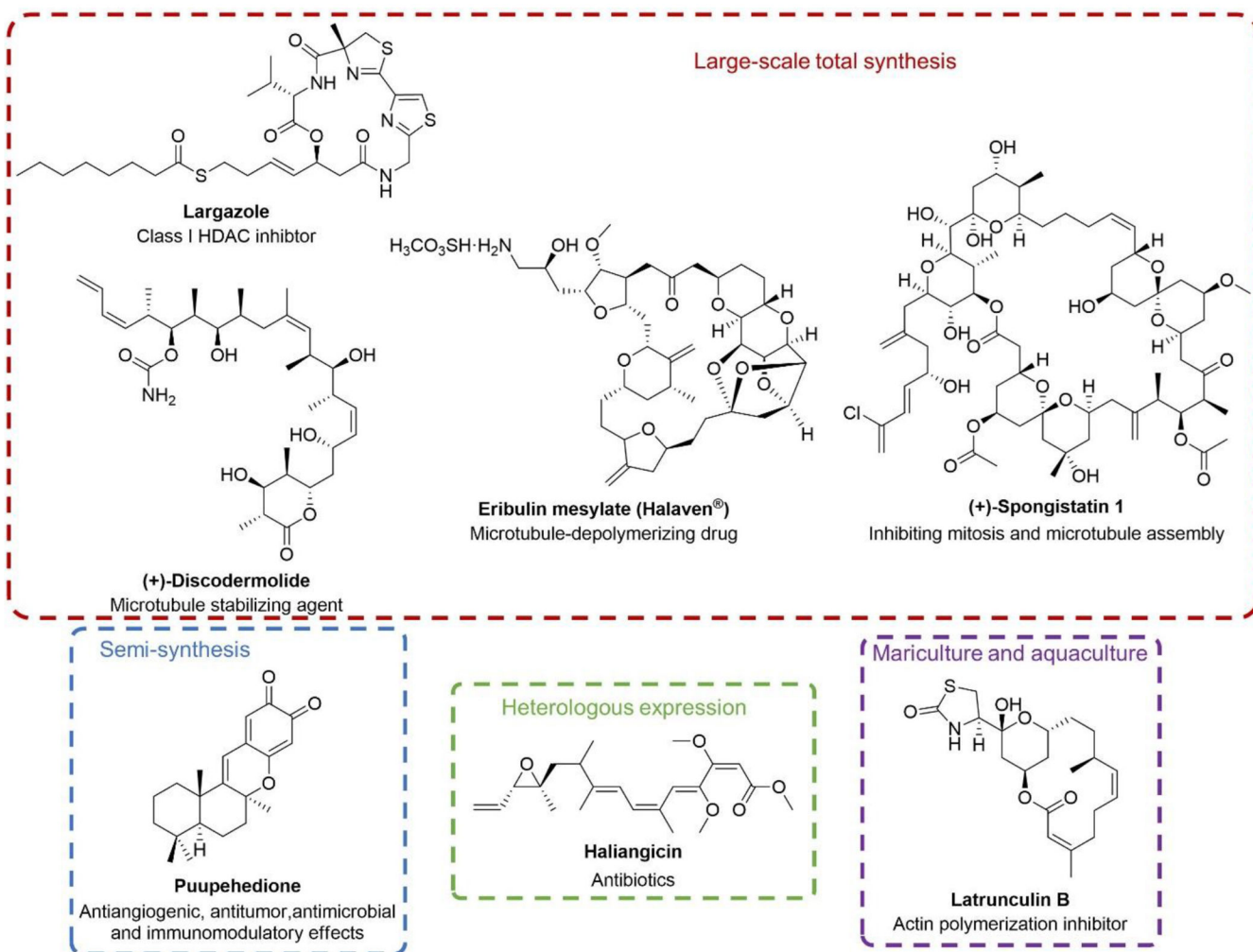


Figure 4. Examples of MNPs where the supply problem was solved by development of scalable total syntheses (largazole, eribulin mesylate (Halaven®), (+)-discodermolide and (+)-spongistatin 1), semi-synthesis (puupehedione), heterologous expression (haliangicin), and mariculture (latrunculin B).

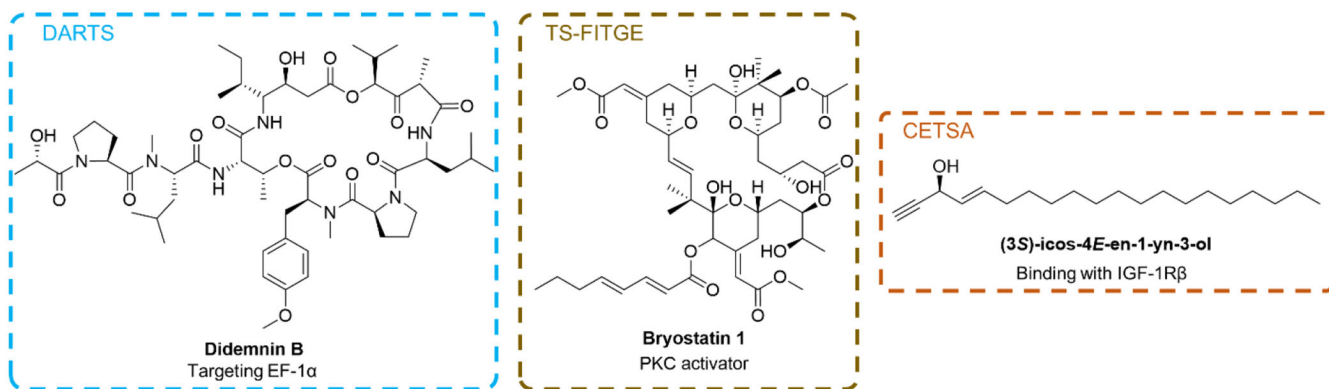


Figure 5. Structures of MNPs where label-free approaches were used for target identification or validation including didemnin B (DARTS), bryostatin 1 (TS-FITGE) and (3*S*)-icos-4*E*-en-1-yn-3-ol (CETSA).

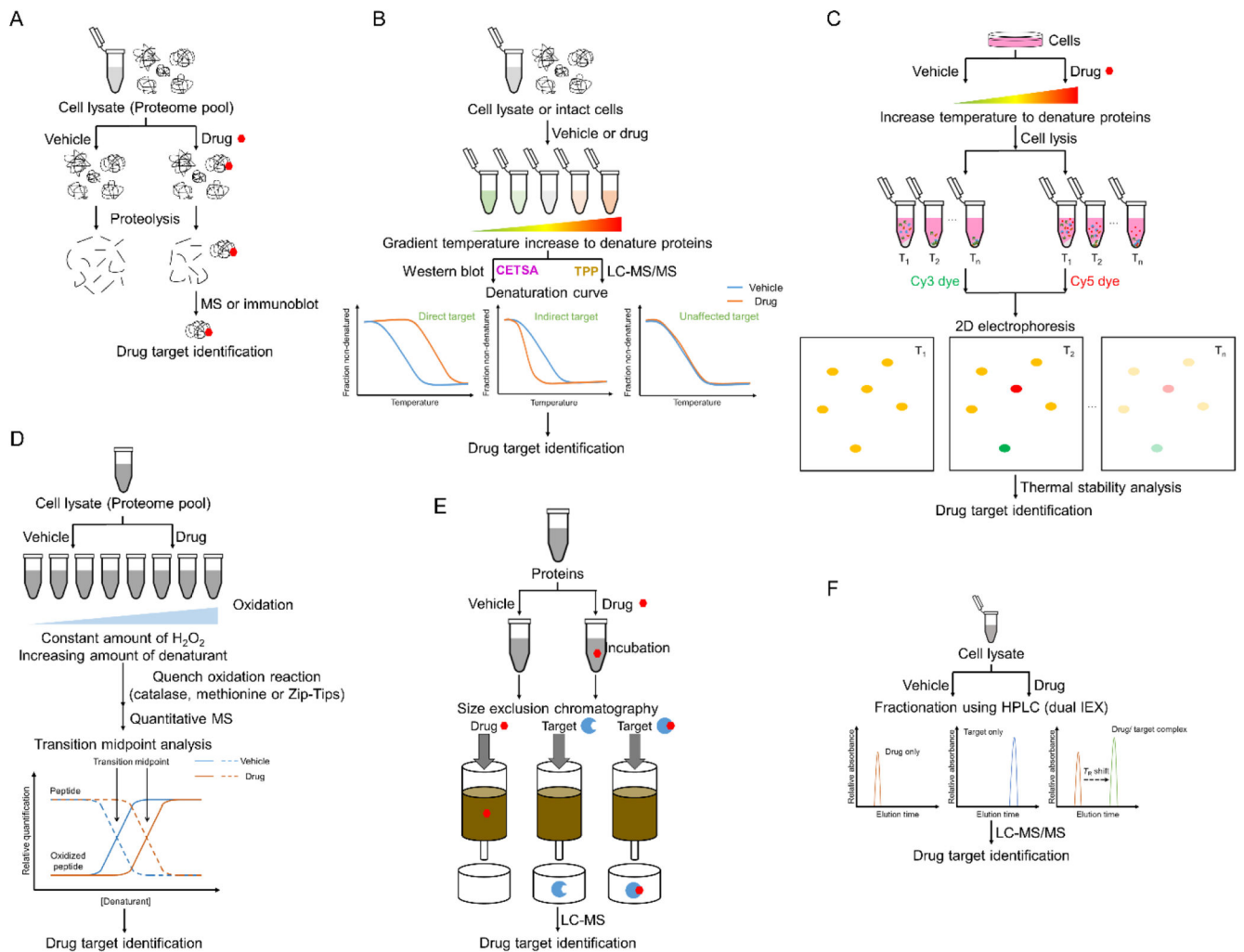


Figure 6. Illustrative schemes of label-free technologies. A. Drug affinity responsive target stability (DARTS); B. Cellular thermal shift assay (CETSA); C. Thermal stability shift-based fluorescence difference in two-dimensional gel electrophoresis (TS-FITGE); D. Stability of proteins from rates of oxidation (SPROX); E. Size-exclusion chromatography for target identification (SEC-TID); F. Target identification by chromatography co-elution (TICC).

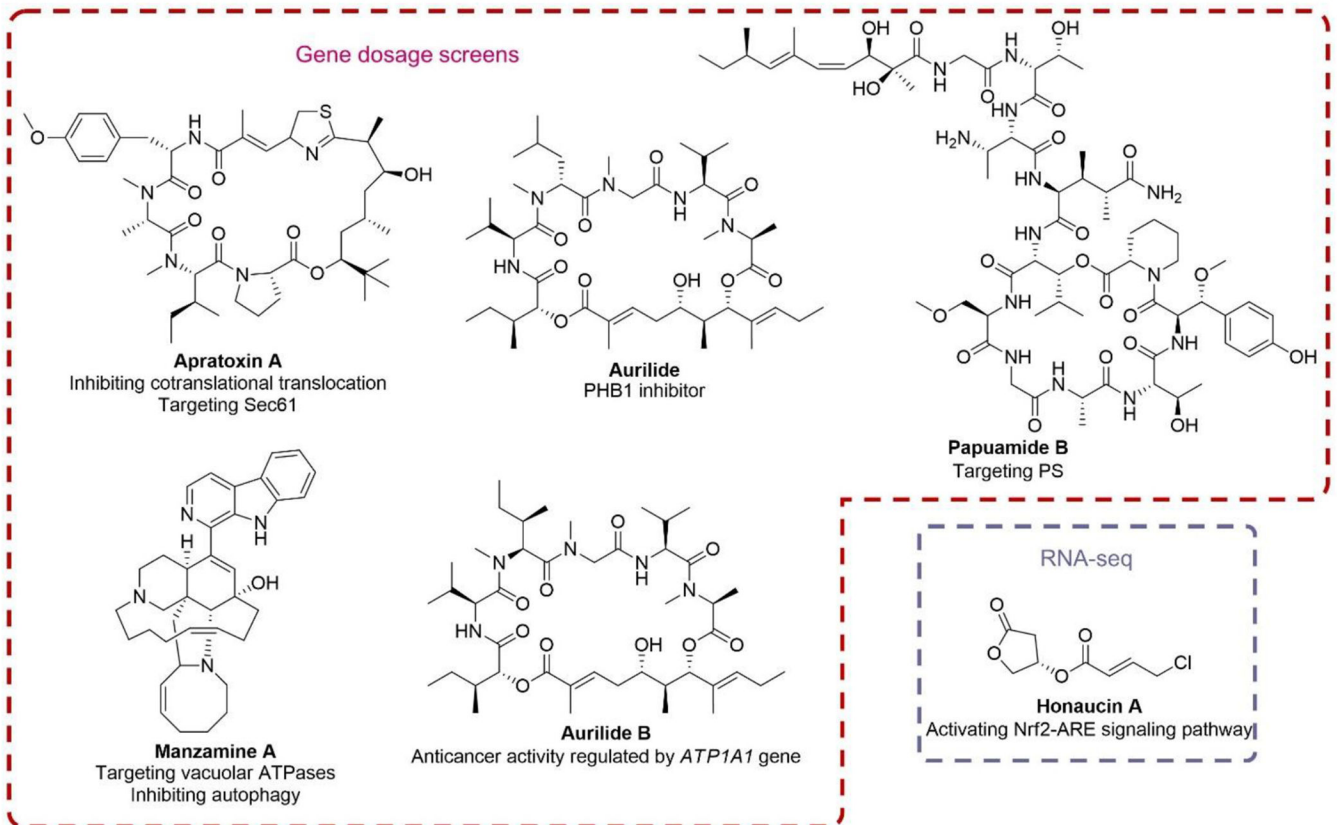


Figure 7. Structures of MNPs that were subjected to various genomics based mechanism studies, including gene dosage screens (apratoxin A, aurilide, aurilide B, manzamine A and papuamide B), RNA-seq (honaucin A).

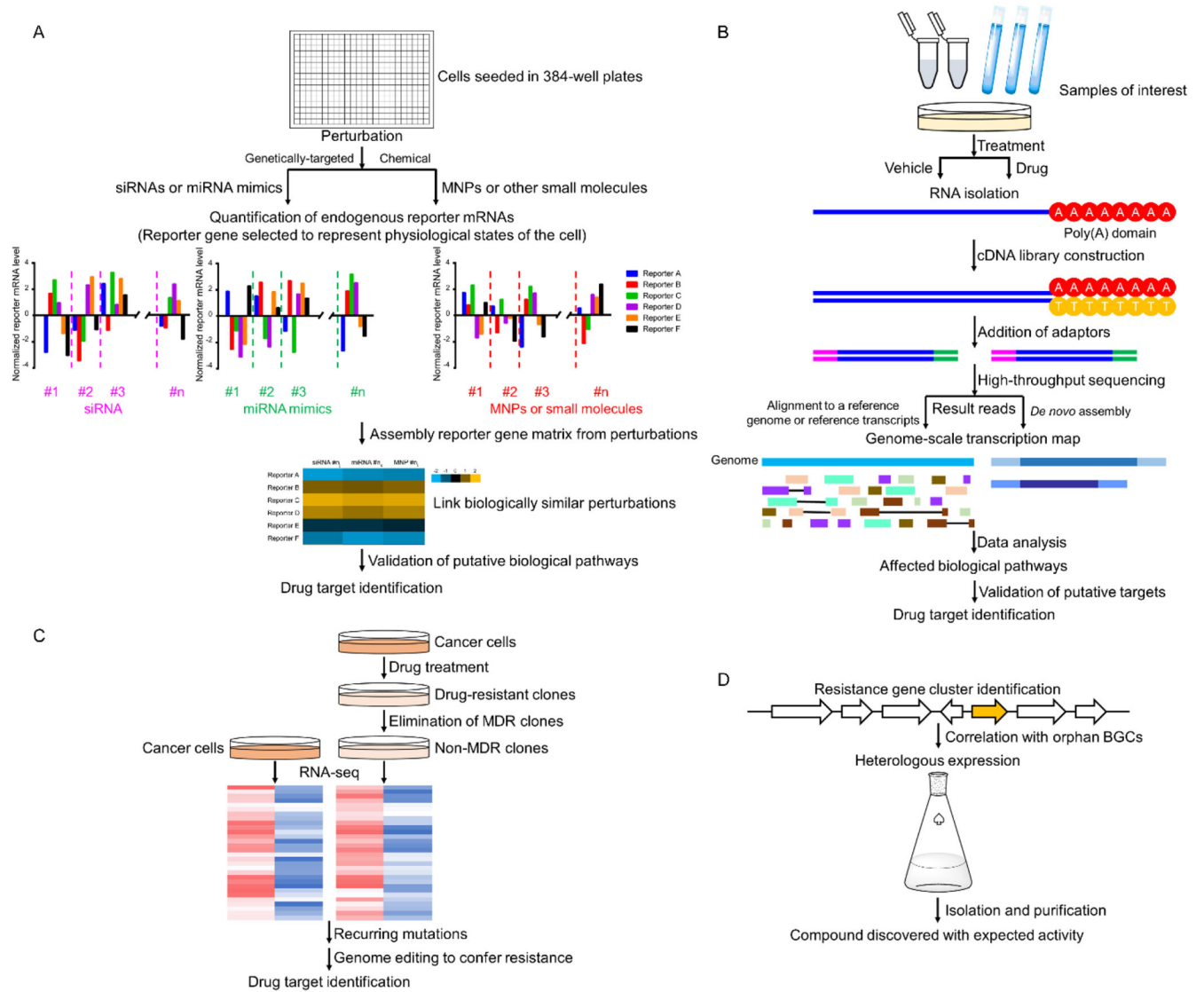


Figure 8. Flowcharts for genomic target identification approaches either in disease relevant cell types (A–C) or the producing organism (D). A: Functional signature ontology (FUSION); B: DrugTargetSeqR; C: RNA-sequencing (RNA-seq); D: Target-directed mining of bacterial genomes encoding MNP production.

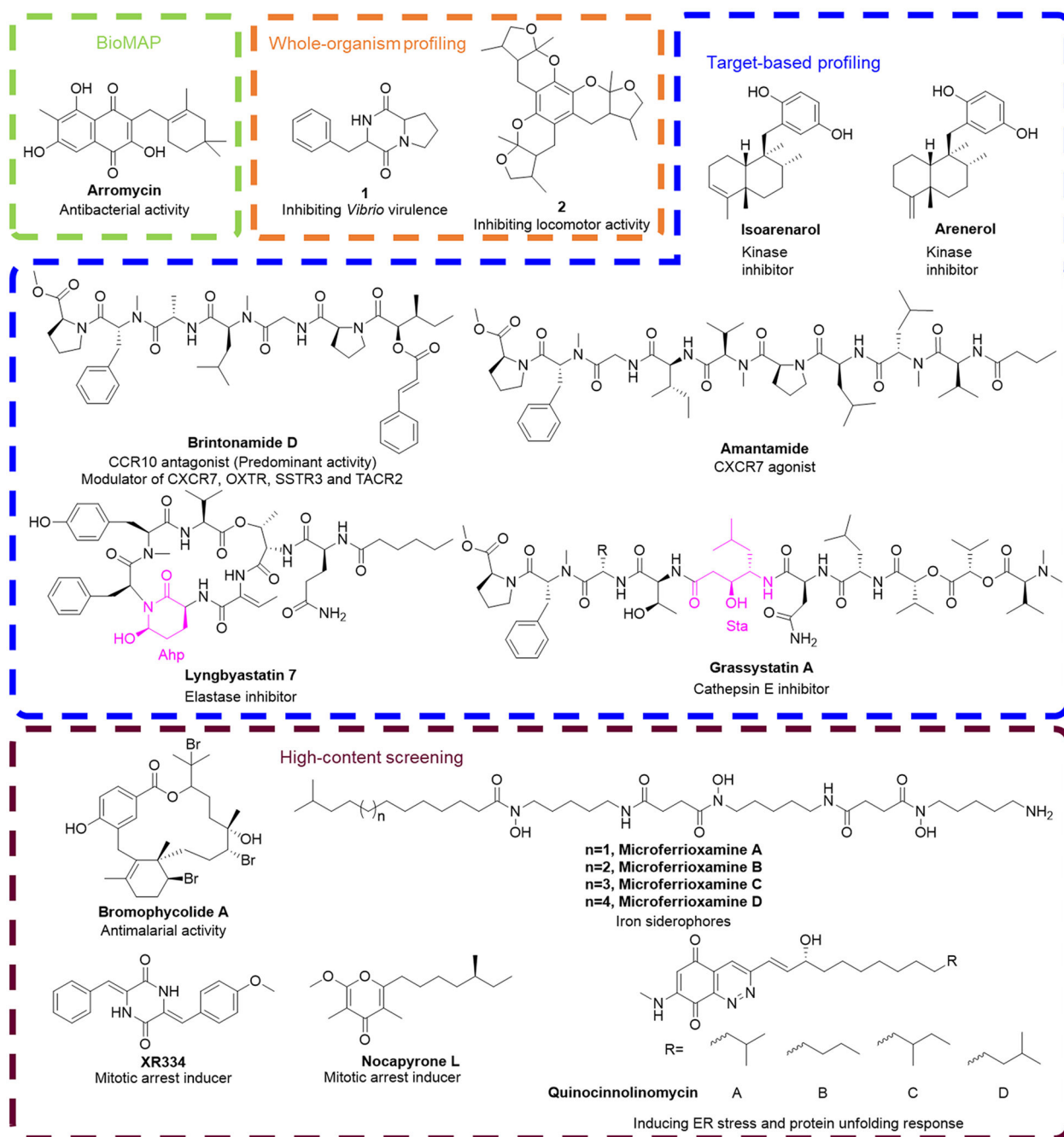


Figure 9. Structures of selected MNPs subjected to profiling methods, BioMAP (arromycin), target-based profiling (isoarenarol, arenerol, brintonamide D, amantamide, grassystatin A and lyngbyastatin 1), high-content screening (bromophycolide A, microferrioxamine A–D, XR334, nocapyroneL and quinocinnolinomycins A–D) and whole-organism screening (diketopiperazine **1** and compound **2**). Pharmacophores for protease inhibitors are highlighted.

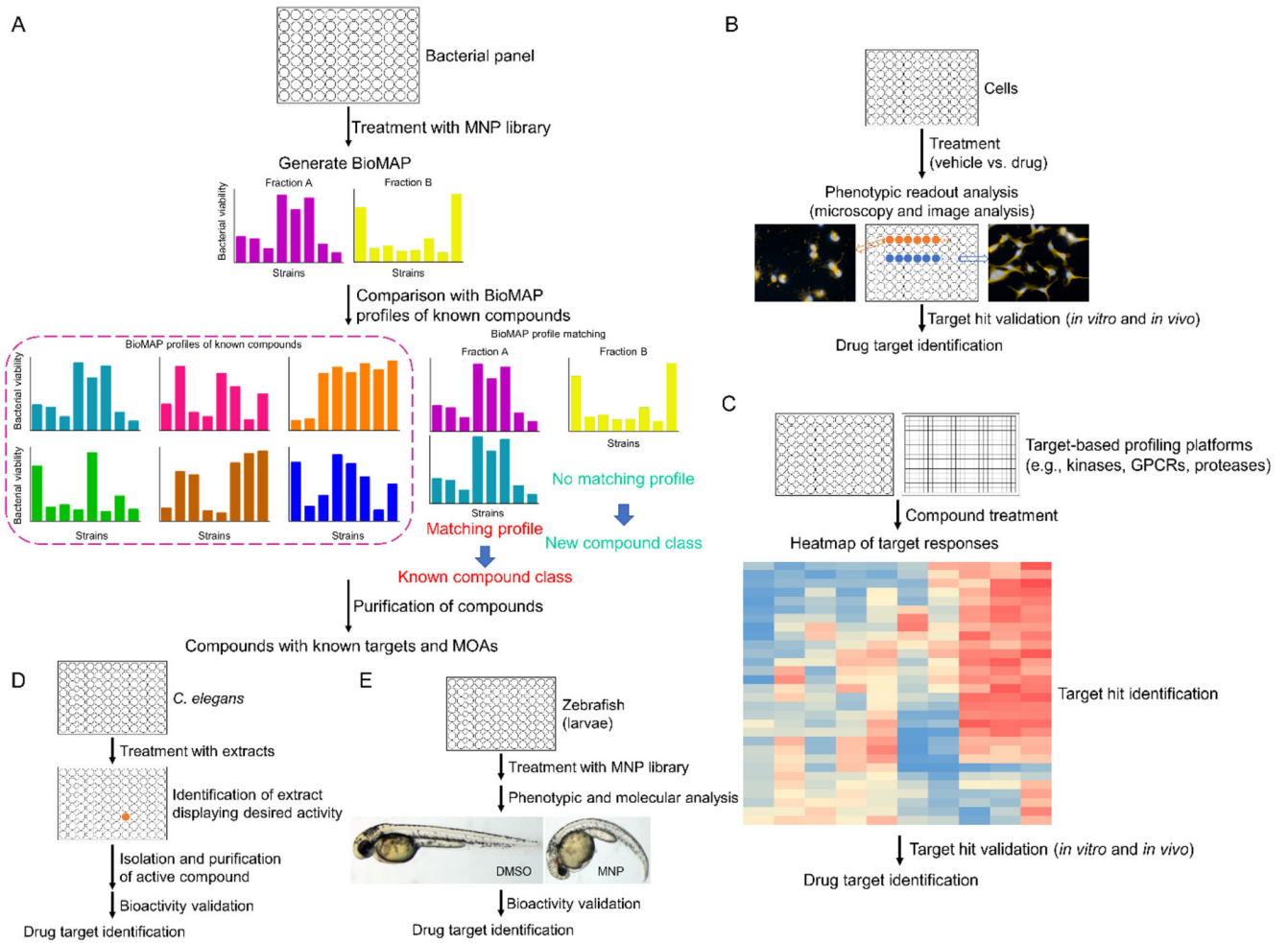


Figure 10. Schematic figure for profiling methods. A: BioMAP; B: High-content screening (HCS); C: Target-based profiling; D: *C. elegans*-based screening system; E: Zebrafish-based screening for impact on development and behavior.

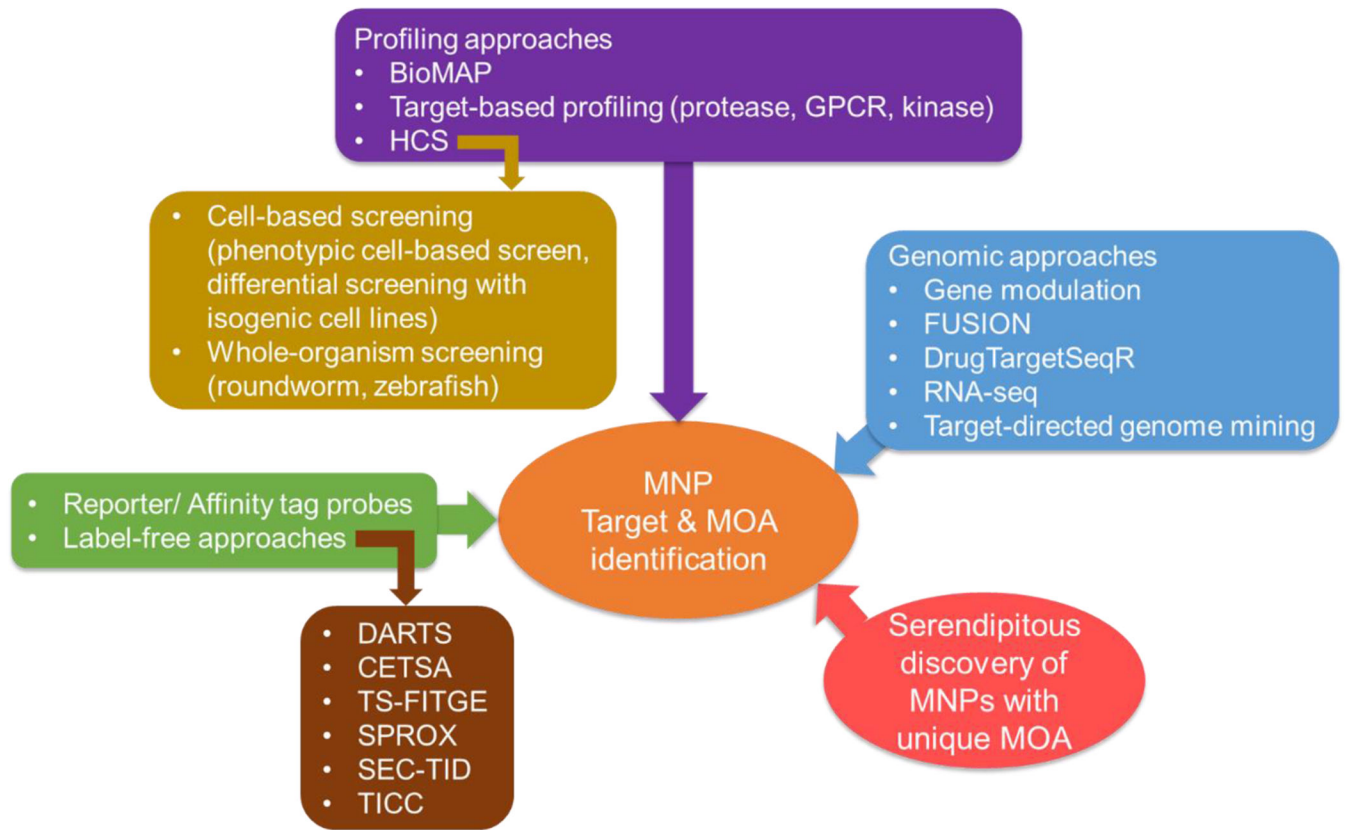


Figure 11.
Summarized approaches and advances in identifying targets and MOAs of MNPs.

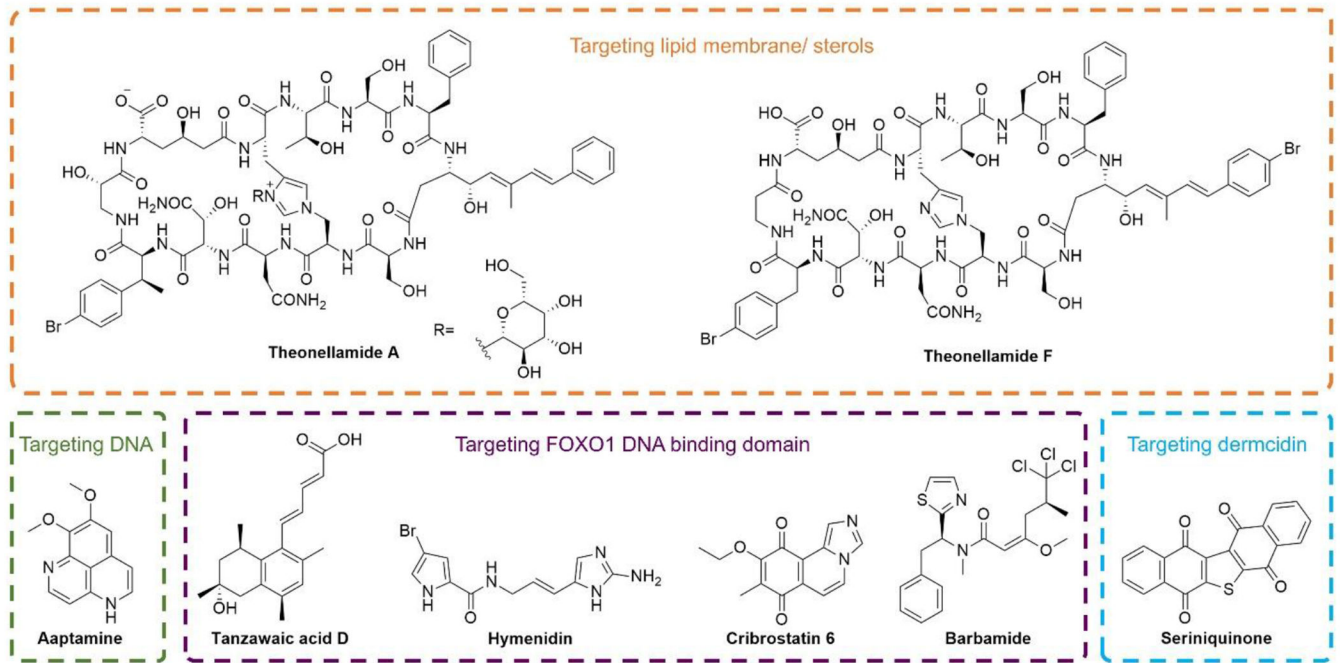


Figure 12. Structures of MNPs with wide-ranging biological targets including DNA (aptamine), FOXO1 DNA-binding domain (tanzawaic acid D, hymenidin, cribostratin 6 and barbamide), lipid/sterol (theonellamides A and F) and new protein target dermcidin (seriniquinone).

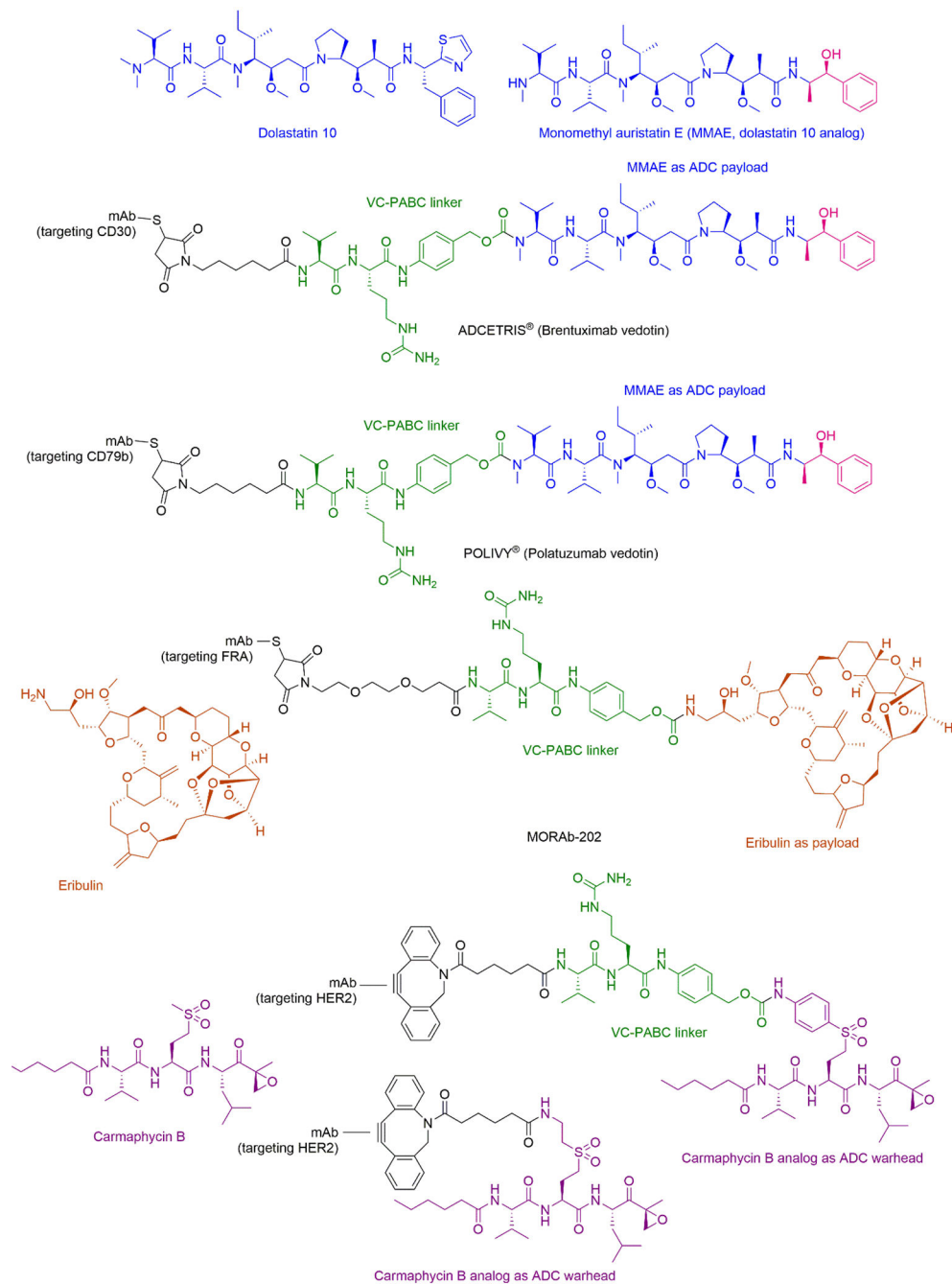


Figure 13.
Structures of dolastatin 10/MMAE, eribulin, carmaphycin B and their derived ADCs.

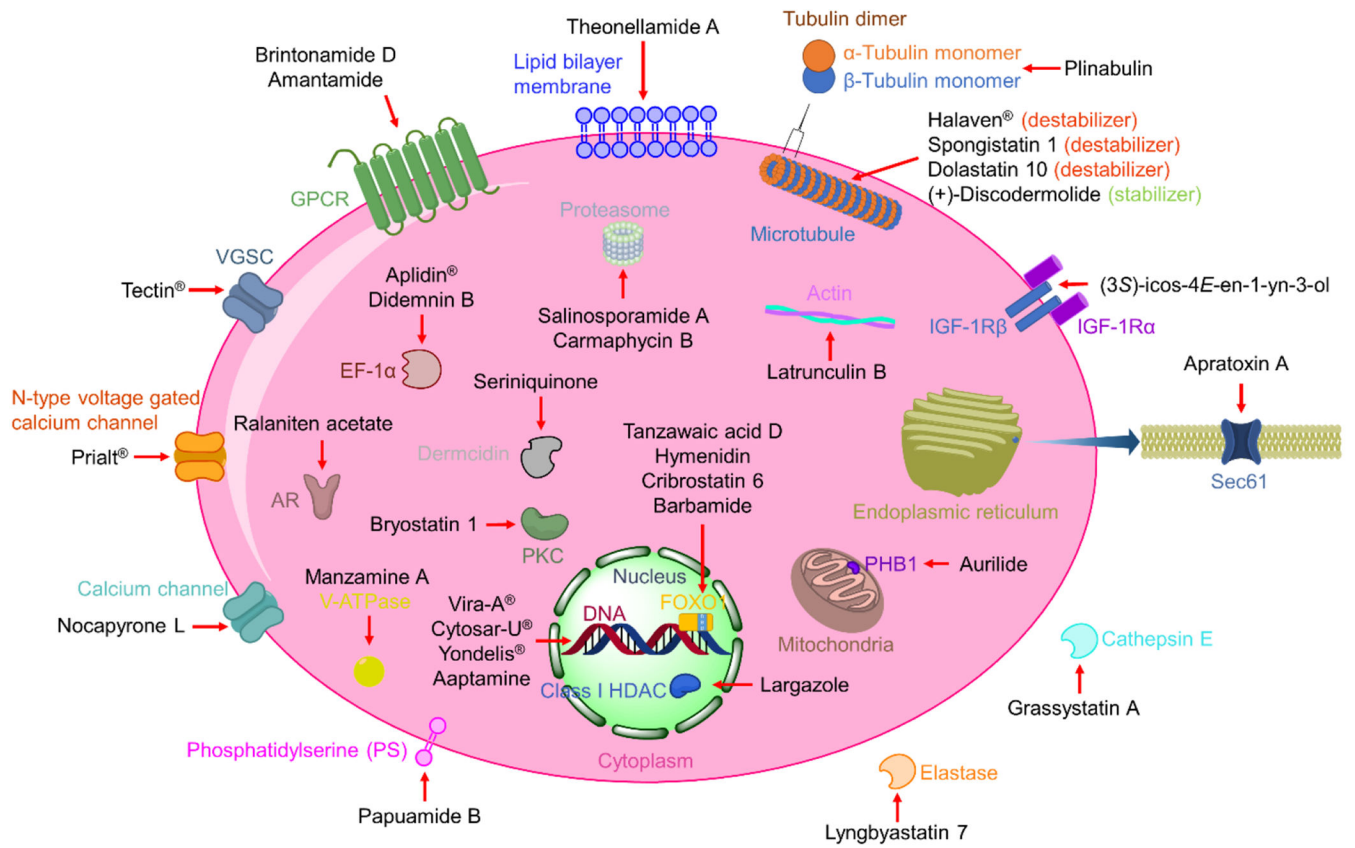


Figure 14. Representative MNPs and their cellular (and extracellular) targets.