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## Marine drugs: Biology, pipelines, current and future prospects for production

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1 *Review – CORRECTED MANUSCRIPT R1*

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3  
4 **Marine Drugs: Biology, Pipelines, Current**  
5 **and Future Prospects for Production**

6  
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18  
19 **Abstract**

20  
21 **The marine environment is a huge reservoir of biodiversity and represents an**  
22 **excellent source of chemical compounds, some of which have large economical**  
23 **values. In the urgent quest for new pharmaceuticals, marine-based drug discovery**  
24 **has progressed significantly over the past several decades and we now benefit**  
25 **from a series of approved marine natural products (MNPs) to treat cancer and pain**  
26 **while an additional collection of promising leads are in clinical trials. However, the**  
27 **discovery and supply of MNPs had always been challenging given their low**  
28 **bioavailability and structural complexity. Their manufacture for pre-clinical and**  
29 **clinical development but also commercialization mainly relies upon marine source**  
30 **extraction and chemical synthesis, which are associated with high costs,**  
31 **unsustainability and severe environmental problems. In this review, we discuss**  
32 **how metabolic engineering now raises reasonable expectations for the**  
33 **implementation of microbial cell factories, which may provide a sustainable**  
34 **approach for MNP-based drug supply in the near future.**

35  
36 **Keywords:** Marine drugs, pharmaceuticals, production, metabolic engineering, biotechnology

## 40 1. Introduction

41

42 As with land plants, fungi and bacteria, marine organisms represent an extremely large resource of  
43 valuable natural products for humans. The bioactive properties of many of these molecules find  
44 applications in a wide range of industrial sectors such as nutrition, biotechnologies, cosmetics, and  
45 health (Sigwart et al., 2021). Among the large series of marine natural products (MNPs), those  
46 displaying a pharmaceutical potential are of great interest as part of our modern pharmacopeia  
47 (Ghareeb et al., 2020). Marine organisms continue to be a source of unique chemical entities with  
48 valuable biomedical potentialities (Blunt et al., 2018). This is illustrated nicely by the recent report on  
49 turbinicin, a marine microbe sourced polyketide displaying potent antifungal activity, particularly  
50 towards pathogenic yeasts that represent a worrisome problem for human health in terms of virulence  
51 and drug resistance (Zhang et al., 2020; Zhao et al., 2020).

52 From an economic viewpoint, the world market of MNPs is considerable with a recent report  
53 estimating the market size for marine-derived pharmaceuticals will reach up to USD 10b in 2021 with an  
54 expected annual growth rate of more than 8% from 2020 to 2026 (Daniotti and Re, 2021 ; ID:  
55 5123389). As observed for the main anticancer drugs derived from plants, enormous costs of  
56 production lead to high cost therapeutics. For instance, the monthly cost for treating breast cancer or  
57 liposarcoma with eribulin now reaches USD 10,000 while that of brentuximab for the management of  
58 lymphoma exceeds USD 30,000 (Howard et al., 2015 ; Gordon et al., 2018).

59 From a structural point of view and with few exceptions, MNPs of pharmaceutical importance derive  
60 from the so-called secondary metabolism of marine organisms. This means that their biosynthetic  
61 routes are diverse in terms of length, starting precursor and characterization level. MNPs also display  
62 complex structures that potentially render their total chemical syntheses *de novo* impractical. As a  
63 consequence, production strategies relying on marine macroorganism harvesting or culturing coupled  
64 with extraction procedures remain sometimes the only alternative for the production of these  
65 compounds. In this regard, while their supply can be often implemented with laboratory scale cultures  
66 for bacterial, fungal or microalgal sources, this is much more complex to consider for invertebrate  
67 sources where secondary metabolites usually naturally accumulate in only very small quantities. Most of  
68 the time, the concentrations of the promising MNPs in marine invertebrates may be sufficient for their  
69 chemical characterization and preliminary activity evaluation, however, they are far from providing a  
70 sustainable supply for pre-clinical and clinical trials, requiring quantities in a gram range, and even less  
71 in the perspective of a future commercialization where annual demands usually reach several kilograms  
72 (Gomes et al., 2016). For ecological issues, it is inconceivable to think that the supply of these MNPs  
73 with promising properties could only depend on wild harvest of marine invertebrates (Heffernan, 2020).

74 Importantly, similar production limits have been observed for plant-derived pharmaceuticals in past  
75 decades (Courdavault et al., 2021). This has promoted the stepwise emergence of alternative strategies

76 of supply based on metabolic engineering approaches that began to appear alongside the emergence of  
77 the synthetic biology era in the early 2000's (Kotopka et al., 2018 ; Nielsen, 2019). This type of strategy  
78 relies on the heterologous expression of biosynthetic genes from the source organism in a recipient  
79 bacterium and/or yeast to allow the *de novo* synthesis of the expected drugs by the transformed  
80 microbe. Likewise, in line with the pioneering success of the antimalarial semi-synthetic artemisinin  
81 (Paddon et al., 2013), genetically engineered microorganisms have emerged as suitable cell factories  
82 for the production of plant-derived drugs. In this context, we discuss here how all the background  
83 gained in recent years concerning the biotechnological production of plant drugs using engineered  
84 microbes will undoubtedly pave the way for innovative MNP manufacturing alternatives in the near  
85 future.

## 86

## 87 **2. Trends in marine drugs: sources, chemical classes, biological activities**

## 88 **and current pipeline**

89

90 Over five decades of sustained research, more than 30,000 compounds of marine origin have been  
91 identified within a diverse palette of biological sources, including both prokaryotic (eubacteria,  
92 cyanobacteria) and eukaryotic organisms (fungi, dinoflagellates, algae, sponges, cnidarians, bryozoans,  
93 mollusks, ascidians, and echinoderms) (Alves et al., 2018; Caroll et al., 2021). Sponges (porifera) and  
94 cnidarians (mainly corals and anemones) for invertebrates as well as marine fungi and actinomycetes  
95 for microorganisms have always been the prominent MNP sources in term of quantity and structural  
96 diversity (Hu et al., 2015). With the exception of some peptides (e.g. conotoxin), fatty acids (e.g.  
97 omega-3-acid ethyl esters) and polysaccharides (e.g. fucoidan, carrageenans, alginates, ulvans, chitins,  
98 and chitosans), all others classes of biologically active MNPs arise from the so-called "specialized  
99 metabolism" (or secondary metabolism ) pathways which means that most of the interesting MNPs are  
100 biosynthesized through clade-specific metabolic pathways. The major classes of marine secondary  
101 metabolites include alkaloids, terpenoids, polyketides, acetogenins, non-ribosomal peptides, and  
102 glycosides (Lu et al., 2021). Figure 1 summarises examples of the structural diversity of MNPs that  
103 include approved therapeutics, clinical candidates and bioactive structures that are the focus on  
104 ongoing studies. Although these chemical classes are also primarily found in the portfolio of  
105 metabolites produced by terrestrial natural sources (mainly plants, fungi and bacteria), recent advances  
106 shed light on shared but also on differential features between terrestrial and marine secondary  
107 metabolites. While displaying a global lower solubility, MNP scaffolds are substantially larger, containing  
108 more long chains and large rings with fewer oxygen but more nitrogen atoms compared to terrestrial  
109 natural products. Another distinguishing general trait of MNPs is that a fair proportion of compounds  
110 contain bromine and to a lesser extent chlorine, associated with the high concentration of halides in



111 seawaters and the presence of particular halogenation enzymes (e.g. vanadium bromoperoxidase) in a  
112 broad range of marine organisms (Shang et al., 2018; Voser et al., 2022).

113 About a quarter of the MNPs isolated so far have shown interesting biological activity including  
114 antiproliferative, antimicrobial, anti-inflammatory, anti-coagulation, analgesic, neuroprotective, and  
115 cardioprotective properties (Hu et al., 2015). To date, fourteen MNP-based drugs are clinically approved  
116 by various worldwide health organizations and are currently used to cure/treat some of the main  
117 medical pathologies including cancer, cardiovascular diseases, pain, and viral infections (Dyshlovoy and  
118 Honecker, 2020). A timeline of their discovery is depicted in Figure 2 and their main characteristics are  
119 compiled in Table 1. Beyond these proven pharmaceutical successes, we can anticipate that the number  
120 of approved MNP-derived drugs will keep increasing since 33 marine-derived pharmaceutical  
121 compounds are currently in clinical trials (5 in phase III, 12 in phase II, and 16 phase I, Table 2). In  
122 addition, it is highly likely that MNP research will continue to boom insofar as the recent developments  
123 in analytical technology, spectroscopy, machine learning, and high-throughput screening of activities all  
124 support the discovery of more than 1,000-1,500 new structures from marine sources annually (Ghareeb  
125 et al., 2020; Wilson et al., 2020, Carroll et al., 2021).

126 As these biomolecules are sourced from nature, bioprospecting researchers in this field need to be  
127 aware of the moral and legal obligations on them to ensure equitable benefit sharing with the country  
128 from which the biological/genetic material was sourced. In October 2014, parties to the Convention on  
129 Biological Diversity (CBD) adopted the supplementary agreement (known as the Nagoya Protocol) which  
130 provides a legal framework regarding access and fair and equitable sharing of benefits arising from the  
131 utilization of genetic resources. One of the founding aims of the Nagoya Protocol is to put an end to  
132 biopiracy, with researchers being required to establish access and benefit-sharing (ABS) agreements  
133 before being granted access to a country's national jurisdiction. In a marine context, this jurisdiction  
134 covers territorial seas and exclusive economic zones (EEZ), out to a 200 nautical mile boundary  
135 (<https://www.cbd.int/abs/>). Thus it is a legal imperative for any bioprospecting in these environments  
136 that, in addition to the usual collection permits, ABS agreements are put in place. To date, these close-  
137 to-shore and EEZ marine environments are the locations of the overwhelming majority of  
138 bioprospecting efforts. There are however ongoing deep-sea bioprospecting efforts, searching the water  
139 column and sediment for novel biomolecules. Exploration in ocean territory beyond any national  
140 jurisdiction boundaries is explicitly not covered by the Nagoya Protocol and so the concepts of access  
141 and benefit-sharing are somewhat vague. The closest regulatory framework is the United Nations  
142 Convention on the Law of the Sea (UNCLOS), which contains references to the sustainable use of  
143 marine living resources. There is a need for the development of sharing agreements for products arising  
144 from open ocean research, beyond bilateral/multilateral agreements, to perhaps extend so that benefits  
145 are shared by nations.

146

### 147 3. How approved MNP-based pharmaceuticals are manufactured?

148

149 The currently approved MNP-derived pharmaceuticals cover diverse chemical classes encompassing  
150 nucleosides, peptides, fatty acids, macrolides, depsipeptides, antibody-drug conjugates, hydroxamic  
151 acids, and alkaloids. As a consequence, their production process and cost differ sharply from one  
152 compound to another. In this section, we summarize how pharmaceuticals companies currently ensure  
153 the supply of these important medicines at an industrial scale.

154 The process for production of the lipid-regulating omega-3-acid ethyl esters (Lovaza<sup>®</sup> and Vascepa<sup>®</sup>)  
155 (Figure 2) is among the simplest and cheapest since triglyceride biosynthesis inhibitors are made from  
156 fish body oils that are purified and esterified (Cholewski et al., 2018). The anticancer drugs cytarabine  
157 (Cytosar-U<sup>®</sup>) and nelarabine (Arranon<sup>®</sup>/Atriance<sup>®</sup>), and the antiviral compound vidarabine (Vira-A<sup>®</sup>),  
158 are analogs of the marine nucleosides spongothymidine and spongouridine (Figure 1), which were  
159 originally isolated from the Caribbean sea sponge *Tectitethya crypta* (Figure 2). Due to their fairly  
160 simple chemical structures, these DNA/RNA polymerase inhibitors are currently manufactured by  
161 classical chemical synthesis (Shelton et al., 2016). The powerful analgesic agent ziconotide (Prialt<sup>®</sup>)  
162 (Figure 2) is  $\omega$ -contoxin MVIIA (Figure 1), a hydrophilic peptide originally isolated from the venom of  
163 the Pacific fish-hunting marine mollusk *Conus magus* (Figure 2). This N-type calcium channel blocker  
164 (Gao et al., 2021) is produced by solid phase peptide synthesis (Schroeder and Craik, 2012). The  
165 anticancer agent eribulin mesylate (Halaven<sup>®</sup>) (Figure 2) corresponds to a simplified analogue of the  
166 marine polyether macrolide, halichondrin B (Figure 1), which was originally isolated from the marine  
167 sponge *Halichondria okadai* (Figure 2). This microtubule-targeted anticancer drug is nowadays  
168 considered as the most complex synthetic drug and above all a perfect demonstration of how far the  
169 limits of total synthesis of natural products have been pushed on an industrial scale (Bauer, 2016).  
170 Another relatively new and efficient class of marine drug-derived pharmaceuticals corresponds to  
171 antibody-drug conjugates (ADC), combining a cytotoxic agent of marine origin with a selective  
172 monoclonal antibody. Four examples are currently approved to treat specific types of cancer including  
173 brentuximab vedotin (Adcetris<sup>®</sup>), enfortumab vedotin (PADCEV<sup>™</sup>), polatuzumab vedotin (Polivy<sup>™</sup>), and  
174 belantamab mafodotin (Blenrep<sup>™</sup>) (Figure 2). These MNP-derived ADC commonly consist of (i) a  
175 monoclonal antibody specific to surface antigens present on particular tumor cell types, (ii) a cleavable  
176 or non-cleavable linker, (iii) and a microtubule disrupting MNP derivative (either monomethyl auristatin  
177 E, MMAE, or monomethyl auristatin F, MMAF) (Joubert et al., 2020). Monoclonal antibodies are  
178 classically produced by mammalian (Chinese hamster ovary) cells while linkers are prepared with  
179 commercially available chemicals. MMAE and MMAF are synthetic analogs of the highly cytotoxic  
180 dolastatin 10 (Figure 1) that was originally isolated from the Indian Ocean sea hare *Dolabella auricularia*  
181 (Figure 2). To date, MMAE and MMAF are manufactured by classical chemical synthesis (Zhou et al.,  
182 2017). The anticancer MNPs trabectedin (Yondelis<sup>®</sup>) (Figure 1) and its derivative lurbinectedin

183 (Zepzelca™) are tetrahydroisoquinoline alkaloids – trabectedin is ecteinascidin 743 while lurbinectin is a  
184 modified analog of ET 736, both natural products having been originally identified in the Caribbean sea  
185 squirt *Ecteinascidia turbinata* (Figure 2). Although recent advancements provide new scalable total  
186 synthesis protocols, the production of these two complex alkaloids still comprises semisynthetic steps  
187 performed on the precursor cyanosafracin B produced by fermentation of the bacterium *Pseudomonas*  
188 *fluorescens* (Cuevas and Francesch, 2009; He et al., 2019). Finally, Plitidepsin (Aplidin®) (Figure 1) is a  
189 cyclic depsipeptide anticancer drug isolated from the marine tunicate *Aplidium albicans* (Figure 2). Since  
190 its approval, this eEF1A2 inhibitor is manufactured by total chemical synthesis (Alonso-Álvarez et al.,  
191 2017).

#### 192 193 **4. Anticipating the development of efficient production processes for future** 194 **approved MNPs**

195  
196 More than 30 MNP-derived pharmaceutical are currently in clinical trials (Table 2), about a hundred  
197 are in preclinical development each year, and the discovery of new compounds with interesting  
198 biomedical potentialities and novel mechanisms of action continues with little sign of slowing down  
199 (Mayer et al., 2010 ; Newman and Cragg, 2016 ; Mayer et al., 2021 ; Daniotti and Re, 2021). As a  
200 consequence, whether for preclinical and clinical development or marketing, it seems important to be  
201 able to anticipate efficient processes of sustainable supply for promising MNP-derived drugs. In this  
202 respect, all the background accumulated in the past decades that have allowed implementing such  
203 industrial production strategies for many different classes of natural products from highly diversified  
204 sources may combine to benefit the supply of new marine drugs.

205 As established with eribulin mesylate (Halaven®), advances in chemical synthesis continuously  
206 stretch the boundaries of our capacities of manufacturing pharmaceuticals with increasingly complex  
207 structures (Bauer 2016). We can thus expect that this powerful process of supply may continue to stand  
208 out as a prominent strategy to produce future approved MNP-derived drugs at an industrial scale.  
209 However, we have to admit that this production process is still poorly environment-friendly and very  
210 costly insofar the methods require a lot of different reagents and deliver overall low yields. Hence,  
211 especially in the case of new MNPs of great potential but displaying structures far too complex for a  
212 profitable production by chemical synthesis, alternative biotechnological approaches could be  
213 considered for which we summarize some of these key features hereafter.

214 For instance, concerning MNPs originally biosynthesized by a single cultivable marine microbe such  
215 as a bacterium or a fungus, axenic macroscale cultures (in a bioreactor) could be a relevant strategy  
216 (Quian et al, 2020). This type of production process has proved its worth for the industrial scale supply  
217 of many different antibacterial (erythromycin, vancomycin, streptomycin, penicillins, cephalosporins),  
218 antifungal (griseofulvin, amphotericin B) antiparasitic (avermectins, fumagillin), and immunosuppressant

219 (tacrolimus, cyclosporine, mycophenolic acid) natural products from a broad palette of terrestrial  
220 bacterial and fungal sources (Panter et al., 2021). In line with this statement, Zhao and colleagues  
221 recently identified a highly oxidized type II polyketide from the marine actinomycetes *Micromonospora*  
222 sp., turbinmicin, which displays antifungal activity by inhibiting the vesicle-mediated trafficking pathway  
223 of many life-threatening fungi (Zhao et al., 2020 ; Zhang et al., 2020). This promising new antifungal  
224 marine drug demonstrated mouse-model efficacy toward multiresistant yeasts and molds. We can thus  
225 anticipate that, in case of clinical success, axenic macrocultures would represent the most efficient  
226 process strategy to reach an industrial scale production of turbinmicin.

227 It is also important to remember that, in some specific cases, complementary semi-synthesis steps  
228 can be developed to complete the scaffold of some complex microbial secondary metabolites, as  
229 illustrated for the anticancer MNPs trabectedin (Yondelis®) and lurbinectedin (Zepzelca™) that are  
230 semisynthetic derivatives of cyanosafraicin B (produced by axenic cultures of *P. fluorescens*) (Cuevas  
231 and Francesch, 2009). A similar approach is currently achieved for the industrial production of the  
232 antifungals caspofungin (Cancidas®), micafungin (Mycamine®), and anidulafungin (Ecalta®) that are  
233 obtained following additional semisynthstic steps on the precursor pneumocandin B<sub>0</sub> (produced by  
234 axenic cultures of the ascomycetes mold *Glarea lozoyensis*) (Balkovec et al., 2014). It is highly likely  
235 that such semisynthetic processes would continue to be a procedure of choice for the industrial  
236 production of many different classes of clinically used secondary metabolites, as observed for some  
237 plant-derived anticancer drugs (Courdavault et al., 2020).

238 By contrast, production processes for complex MNPs that are originally biosynthesized by marine  
239 invertebrates are more difficult to implement. As currently performed for several drugs or their  
240 precursors that are extracted from terrestrial medicinal plants, random sampling of the source material  
241 from sessile marine organisms directly from the natural environment is not acceptable. Aside from  
242 ecological impact considerations, the overall naturally-available bulk mass would usually be insufficient  
243 to supply an industrial demand. Most importantly, such a practice is known to directly endanger  
244 biodiversity (Heffernan, 2020). By analogy to what has been achieved in the context of the mono-  
245 culture-based production of some plant drugs such as anticancer vinca-alkaloids (vincristine, vinblastine,  
246 vinorelbine) from the Madagascar periwinkle *Catharanthus roseus* or the antimalarial artemisinin from  
247 the sweet wormwood *Artemisia annua*, aquaculture of medicine-producing marine invertebrates has  
248 progressively been considered since the 1980's (Benkendorff, 2009). This trend is a logical extension of  
249 current commercial aquaculture practices, including for instance corals and shells (for tourism souvenir  
250 gifts), sponges (personal hygiene and cleaning), seaweeds (production of proteins, lipids and  
251 carbohydrates for food and cosmetics) and shellfish farming (mainly oysters for food or mother-of-  
252 pearls, clams, nutshells and mussels) (Naylor et al., 2021). To date, there are two main aquaculture  
253 methods including mariculture (also termed "in situ" aquaculture) and captive breeding (also referred to

254 as “*ex situ*” aquaculture) and both types of aquaculture display advantages and drawbacks from a MNP  
255 production perspective (Leal et al., 2013 ; García-Poza et al., 2020).

256 On the one hand, *in situ* techniques represent a low cost and simple methodology that relies entirely  
257 on the natural environment to supply essential light and nutrients for marine species growth.  
258 Importantly, it is nowadays well established in the literature that the production of a plethora of  
259 bioactive secondary metabolites initially identified in sponges, cnidarians, bryozoans, mollusks,  
260 ascidians, and echinoderms does not exclusively rely on the intrinsic biosynthetic pathways evolved by  
261 these marine metazoan species, but rather derives from associated micro-organisms. (Gomes et al.,  
262 2016). In other words, there is evidence that culturing marine species in their natural habitats through  
263 *in situ* aquaculture promotes holobiont (this means for instance a marine invertebrate associated to  
264 different microorganisms) homeostasis and this equilibrium requires the correct orchestration of the  
265 different metabolic fluxes necessary for the production and accumulation of specific MNPs (Leal et al.,  
266 2013 ; McCauley et al., 2020). However, in practice, monitoring growth conditions is fairly limited and  
267 the developing marine invertebrates are usually threatened by environmental stresses (predation,  
268 eutrophication, thermal anomalies) (Sheridan et al., 2013). As a consequence, and in the perspective of  
269 production of a specific MNP, both the biotic or abiotic stresses could have potential uncontrolled effects  
270 on the regulation of MNP biosynthetic pathways. Most importantly, the annual production of a specific  
271 metabolite by *in situ* aquaculture may be impeded by climatic variations or even natural disasters  
272 leading to a significant reduction in bulk mass necessary for extraction.

273 In contrast, since a proximate source of natural seawater is not mandatory, *ex situ* practices have  
274 also been considered in the framework of future industrial production of valuable MNPs. Captive  
275 breeding, although more expensive to implement, prevents occurrence of perturbing abiotic and biotic  
276 factors, but also allows the monitoring of optimal developmental conditions that are considerably  
277 species specific. All these previously mentioned aspects have thus favored an improved control of MNP  
278 production. However, with the exception of corals, cultivation of whole marine organisms *ex situ* has  
279 been globally less successful compared to mariculture. In addition, it is important to keep in mind that  
280 while *ex situ* aquaculture allows the fine tuning of growth conditions, this practice may change the  
281 holobiont composition and causes either the disruption of important microorganisms when cultured in  
282 their natural sites or promotes uncontrolled development of undesired microorganisms. As a  
283 consequence, although farming has been undertaken for preliminary studies of several compound  
284 classes including the halichondrins, ecteinascidins and mycalamides, experimentation aimed at  
285 producing valuable MNPs by captive breeding have never been reported hitherto (Leal et al., 2013 ;  
286 Gomes et al., 2016).

287 On the collective basis of these issues, there is an urgent need to implement alternative supply paths  
288 for future medically important classes of MNPs to prevent recurrent shortages and to limit the impact of  
289 their production on nature. In such a perspective, we discuss in the coming section, how the use of

290 engineered microbes could progressively stand out as a promising production strategy to overcome  
291 potential sourcing limitations of complex MNPs belonging to a broad palette of secondary metabolite  
292 classes, as recently emerged for plant-derived pharmaceuticals (Kotopka et al., 2018 ; Nielsen, 2019 ;  
293 Courdavault et al., 2021).

294

## 295 **5. Marine drug production: a metabolic engineering perspective**

296

297 The pioneering landmark production of the antimalarial artemisinin in yeast successfully achieved in  
298 the 2000's has inspired many subsequent developments in the field. Indeed, a boom in microbial  
299 engineering procedures for the production of different types of complex plant natural products (PNPs)  
300 has been observed in the past decades, particularly among the major families of polyphenols, terpenes,  
301 taxanes, indole alkaloids, opioids, cannabinoids, and tropane alkaloids (Courdavault et al., 2021). On  
302 this basis, now is the time to reflect on these outstanding achievements to guide future research in this  
303 domain to open new windows for sustainable production processes of complex pharmaceutical MNPs  
304 (Figure 3).

305 A rapid overview of the literature illustrates that the capacity to develop optimized microbial cell  
306 factories heterologously producing PNPs has accelerated thanks to very recent technological  
307 advancements. These consist in (i) a marked acceleration in the elucidation of the biosynthetic  
308 pathways of secondary metabolites (Lau and Sattely, 2015 ; Medema and Osbourn, 2016 ; Caputi et al.,  
309 2018 ; Nett et al., 2020 ; Dugé de Bernonville et al., 2020), (ii) an improved rationalization of the  
310 reconstruction stages of the biosynthetic pathway in the host microbe (Luo et al., 2019 ; Srinivasan and  
311 Smolke, 2020, Guirimand et al., 2021 ; Srinivasan and Smolke, 2021), (iii) the identification of new  
312 routes for optimizing the microbial production to reach industrial titers (Pyne et al., 2020 ; Zhang et al.,  
313 2020; Carqueijeiro et al., 2020).

314 As an obvious starting point, reconstructing biosynthetic pathways in heterologous hosts to create a  
315 MNP cell factory requires a comprehensive knowledge of the enzymatic reactions involved in the  
316 biosynthesis of the targeted compound in the native host organisms (Figure 3A). In bacteria and fungi,  
317 this step is facilitated by the genomic clustering of genes encoding enzymes from the biosynthetic  
318 pathways of most the secondary metabolites (Keller, 2019 ; Chevette et al., 2020 ; Lee et al., 2021 ;  
319 Zhang et al., 2019). However, in the context of a valuable MNP produced by an holobiont such as for  
320 instance a marine invertebrate associated to various microorganisms, the research is more challenging  
321 since the elucidation of the complete biosynthetic pathway requires the identification of highly specific  
322 enzymes encoded by genes present in multiple species and belonging to distinct superfamilies  
323 containing several tens to hundreds of members such as reductases, oxidases, methyltransferases, and  
324 cytochrome P450s among others.



325 Fortunately, the arrival of massive omics resources and tools could accelerate such investigations  
326 (Hautbergue et al., 2018 ; Dugé de Bernonville et al., 2020 ; Ramzi et al., 2020) (Figure 3B). In that  
327 sense, an emerging and powerful strategy relies on identifying a first set of candidate genes through  
328 metabolomic and transcriptomic analysis. More specifically, various metabolomics approaches can now  
329 lead to the accurate detection of plausible reaction intermediates while recent advancements in  
330 molecular networking provide new efficient opportunities in the processing of the acquired  
331 metabolomics data (Fox Ramos et al., 2019 ; Stuart et al., 2020). Once compounds are connected and  
332 a reaction sequence has been postulated *in silico* (reflecting for instance a metabolic branch),  
333 bioinspired organic chemistry and literature data may then help in identifying a type of enzymatic  
334 activity required for each step. At this stage, the availability of the holobiont metagenome and massive  
335 transcriptomics data are of primary importance since searching the specific functional conserved  
336 domains may lead to define first sets of candidate genes.

337 At this step, identifying the respective genes encoding specific enzymes involved in each catalytic  
338 reaction of the investigated pathways requires further prioritization procedures (Figure 3C). The various  
339 possibilities include first the screen for homology to orthologous gene sequences of already  
340 characterized biosynthesis steps in other models. A BLAST search for protein sequences from multiple  
341 species coupled with reconstruction of phylogenies statistically fitted on evolutionary models represent a  
342 basic approach to this aim (Farrow et al., 2018). Another possibility consists in prioritizing candidate  
343 genes by searching physical gene clusters. Some specific branches of the studied biosynthetic pathway,  
344 notably those achieved by microflora species associated to the holobiont, can indeed involve enzymes  
345 encoded by genes physically clustered in the same genomic region (Keller, 2019 ; Chevrette et al.,  
346 2020). Once again in this context, the knowledge of the metagenome sequence from the investigated  
347 holobiont is mandatory and the search for gene cluster is particularly efficient with more complete  
348 genome sequences composed of very long DNA sequence assemblies (McCauley et al., 2020 ; Robinson  
349 et al., 2021). It is interesting to mention that such a global strategy has already begun to bear fruit  
350 since it has allowed deciphering of the biosynthetic origin of the anticancer drug trabectedin (Rath et  
351 al., 2011; Schofield et al., 2015). Above all, the most powerful and global approach that have recently  
352 emerged to rationally prioritize gene sequences in metabolic engineering relies on gene co-expression  
353 analysis (Dugé de Bernonville et al., 2020 ; Ramzi et al., 2020). Briefly, these computational strategies  
354 are based on the hypothesis that metabolites accumulated specifically in an organism or a holobiont,  
355 even in a tissue-specific manner, should be produced through a chain of enzymes displaying a similar  
356 spatio-temporal expression pattern (Ambrosino et al., 2019 ; Paul et al., 2019). Several major  
357 approaches can now be used for browsing such co-expression data and guiding identification of  
358 biosynthetic enzymes. This includes the statistical comparison of contrasted samples (e.g. MNP  
359 accumulating sample versus a non-accumulating sample) to identify differentially expressed genes  
360 (DEG), the construction of gene co-expression networks (GCNs) to provide a more exhaustive view of

361 co-expression relationships among genes (Liesecke et al., 2018), and on the use of unsupervised  
362 machine learning (UML) that assists in grouping similarly expressed genes into a specific expression  
363 cluster (Prihoda et al., 2021). All these concepts have been extensively reviewed elsewhere (Dugé de  
364 Bernonville et al., 2020 ; Ramzi et al., 2020).

365 While new computational approaches now facilitate the identification of candidate biosynthetic genes  
366 by browsing both genomic and transcriptomic data, a laborious but essential step consists of validating  
367 the correct activity of each predicted genes/enzymes (Figure 3D). To this aim, biochemical  
368 characterization of recombinant proteins heterologously expressed in bacteria or yeasts still represents  
369 a basic but reliable approach. These functional assays are coupled with metabolomic techniques to  
370 detect product formation. Importantly, this approach is sometimes impeded by unknown, unpurifiable,  
371 or commercially unavailable reaction substrates. In this specific case, this requires the use of bulk mass  
372 extraction from the marine source. It is noteworthy that the validation of multiple genes from the same  
373 metabolic branch can be achieved following biosynthetic precursor feeding of the host microbe  
374 (expressing bacteria or fungi) co-overexpressing simultaneously several candidate genes. In this  
375 respect, tremendous advancement has been recently achieved since scalable processes for the  
376 expression of large numbers of full-length biosynthetic genes in a same vector are now accessible  
377 (Clevenger et al., 2017 ; Harvey 2018).

378 Once the complete set of biosynthetic genes identified are biochemically validated, engaging the  
379 reconstruction of the pathway in a microbial chassis becomes possible (Figure 3E). A key factor of  
380 success to this aim is the choice of an appropriate heterologous host. Indeed, the high number of  
381 enzymatic steps, complex species, tissue, differential subcellular localization of intermediates or  
382 enzymes, and interconnected regulation networks in the MNP-producing holobiont could dramatically  
383 challenge the whole pathway reconstruction in the microbial host. Furthermore, the choice of carbon  
384 sources for feeding and the ease of final product purification must be considered to ensure the  
385 sustainability of the production process. On this basis, two main microbial models are usually preferred  
386 including the bacterium *Escherichia coli* and the baker's yeast *Saccharomyces cerevisiae* (Carqueijeiro et  
387 al., 2020 ; Guirimand et al., 2021). The specific requirement of post-translational protein modifications  
388 and the presence of membrane-bound proteins condition the selection of the host organism towards the  
389 use of a prokaryotic (*E. coli*) or eukaryotic chassis (*S. cerevisiae*). Fortunately, extensive synthetic  
390 biology toolboxes are available for both microbial models such as genomic integrative constructs,  
391 including CRISPR-Cas9 technologies, and auto-replicative plasmids (Cravens et al., 2019). These  
392 versatile genetic tools now allow rapid heterologous pathway prototyping. The bacterium *E. coli* displays  
393 advantages in metabolic engineering such as a well-understood native metabolism and fast doubling  
394 time. However, this microbial model shows significant physiological differences with eukaryotic cells  
395 including the lack of subcellular compartmentation and protein post-translational modification  
396 mechanisms, which can alter the metabolic flux of the heterologous pathway and reduce product titer.



397 By contrast, the yeast *S. cerevisiae* displays a canonical eukaryotic cell architecture allowing the  
398 expression of membrane-bound proteins, as usually observed in biosynthetic pathways (e.g.  
399 cytochrome P450). However, there is still very few information on the heterologous expression of  
400 enzymes from marine invertebrates, in particular relating to post-translational modifications such as  
401 protein glycosylation (Zhu et al., 2019). An important and recent advancement in this field is the  
402 development of minimal synthetic genome organisms that only include documented genetic elements  
403 (Richardson et al., 2017 ; Fredens et al., 2019 ; Glass et al., 2017 ; Lu et al., 2019 ; Lloyd et al., 2018).  
404 These microbial chassis have the advantage to significantly decrease the risk of metabolic flux shifting.

405 While recent breakthroughs now facilitate the development of a prototypical microbial strain  
406 producing the  $\mu\text{g}$ -low mg range per liter, optimizing MNP synthesis in the microbial host up to industrial  
407 scale represents a last but considerable step (Figure 3F). While global approaches usually provide the  
408 lead for the main backbone compounds, production of the most effective bioactive molecule will require  
409 multiple iterative refinements using the Design-Built-Test-Learn cycle (Figure 3G). The mining of the  
410 synthesis pathways of natural biological products is indeed a primary application of system biology,  
411 however, the latter has also a great potential in the transformation and optimization of the metabolic  
412 network of microbial cell factories. In this regard, genome-scale metabolic network model, metabolic  
413 network optimization based on omics technologies now sustains optimization strategies for the efficient  
414 synthesis of active natural products in microbial cell factories (Srinivasan and Smolke, 2019). Whereas  
415 optimization of media and culture conditions as well as implementation of bioreactors can increase the  
416 production titers of the desired MNP, recent advances provide complementary solutions and strategies  
417 for improving production yields in the prototypical engineered microbes (Pyne et al., 2019 ; Zhang et  
418 al., 2020; Carqueijeiro et al., 2020) (Figure 3E). For instance, it is now well documented that optimizing  
419 the microbe native metabolism, notably by increasing production of enzyme co-factors, is a first efficient  
420 way to revise metabolic fluxes in the microbial host. In addition, it was found that the timely induction  
421 of transgene expression and/or product capture during yeast culture can lead to an improvement of  
422 host tolerance to the produced drug. Interestingly, low heterologous gene expression can now be  
423 solved by increasing gene copy number using the CRISPR/Cas9 technology (Kulagina et al, 2021).  
424 Finally, recent advancements also firmly demonstrate that the identification and expression of more  
425 active orthologues is indispensable to reach high production levels (Srinivasan and Smolke, 2020).  
426 Taken together, all these optimizations may be required to prevent undesired accumulation of metabolic  
427 intermediates and to reroute critical native precursors into the desired MNP biosynthetic pathways  
428 without overly impacting cellular fitness. This is an essential prerequisite to follow to reach an industrial  
429 scale production.

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## 433 6. Concluding remarks

434  
435 As recently successfully achieved for some bacterial, fungal and PNP-derived drugs (Lee et al.,  
436 2021), the prospect of exploiting the potential offered by microbial metabolic engineering for developing  
437 alternative supply processes for promising MNP-derived pharmaceuticals is now within our grasp (Figure  
438 4). While no MNPs have been produced by metabolic engineering to date, it will be important, in the  
439 near future, to carry out the first proof-of-concept studies in this field, notably by attempting to  
440 implement pioneering prototypical microbial strains in which biosynthetic pathways of several lead MNP  
441 are reconstructed. However, to this aim, it is highly likely that one will have to face some new  
442 difficulties of using systems biology methods to synthesize MNPs compared to PNPs. For MNPs that are  
443 biosynthesized (at least in part for a complex pathway) by marine invertebrates, the implementation  
444 would be requiring extensive research into the specialized metabolic routes in marine macroorganisms.  
445 In addition, there is no reported experience on the heterologous expression of marine invertebrate  
446 enzymes in bacterial or yeast platforms. Indeed, while the reconstruction of a whole PNP biosynthetic  
447 pathway in microbial chassis is feasible, what about the subcellular targeting and activity of MNP  
448 enzymes, the metabolic fluxes, the toxicity of MNP intermediates or final products in these recipient  
449 cells? It behooves the research community to pursue production of a MNP in this way, particularly one  
450 with a complex scaffold, which currently suffers from a high cost of production, and for which the  
451 biosynthetic pathways are fully elucidated (Table 3). It is anticipated that such implementations will  
452 benefit from the recent advancements made concerning the production of PNP in microbes, notably for  
453 MNPs belonging to chemical classes for which benchmark and successful examples were achieved for  
454 PNPs (Carqueijeiro et al., 2020). For instance and in such a perspective, producing trabectedin by  
455 microbial metabolic engineering could be supported by the recent development of a yeast platform for  
456 the synthesis of tetrahydroisoquinoline alkaloids at a gram per liter scale (Pynes et al., 2020). These  
457 technological developments seem ripe for future advancement in marine drugs and raise unprecedented  
458 expectations for the implementation of engineered cell factories, which may provide in a near future a  
459 sustainable approach for MNP pharmaceutical supply.

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**Table 1. The currently approved MNP-derived pharmaceuticals.**

<b>Drug Name</b>	<b>Chemical class</b>	<b>Marine origin</b>	<b>Disease area</b>	<b>Trademark</b>	<b>Company</b>
<b>Omega-3-acid ethyl esters</b>	Omega-3 fatty acids	Many fish species	Hyper-triglyceridemia	Lovaza®	Glaxo Smith Kline
<b>Eicosapentaenoic acid ethyl ester</b>	Omega-3 fatty acids	Many fish species	Hyper-triglyceridemia	Vascepa®	Amarin
<b>Cytarabine</b>	Nucleoside	<i>Tectitethya crypta</i> (Sponge)	Cancer	Cytosar-U®	Pfizer
<b>Vidarabine</b>	Nucleoside	<i>Tectitethya crypta</i> (Sponge)	Antiviral	Vira-A®	Mochida Pharmaceutical Co.
<b>Nelarabine</b>	Nucleoside	<i>Tectitethya crypta</i> (Sponge)	Cancer	Arranon® / Atriance®	GlaxoSmithKline
<b>Ziconotide</b>	Peptide	<i>Conus magus</i> (Mollusk)	Pain	Prialt®	Jazz Pharmaceuticals
<b>Plitidepsin</b>	Depsipeptide	<i>Aplidium albicans</i> (Tunicate)	Cancer	Aplidin®	Pharmamar
<b>Trabectedin</b>	Alkaloid	<i>Ecteinascidia turbinata</i> (Tunicate)	Cancer	Yondelis®	Pharmamar
<b>Lurbinectedin</b>	Alkaloid	<i>Ecteinascidia turbinata</i> (Tunicate)	Cancer	Zepzelca™	Pharmamar
<b>Eribulin mesylate</b>	Macrolide	<i>Halichondria okadai</i> (Sponge)	Cancer	Halaven®	Eisai Inc.
<b>Brentuximab vedotin</b>	ADC (MMAE)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	Adcetris®	Seagen
<b>Enfortumab vedotin</b>	ADC (MMAE)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	PADCEV™	Astellas Pharma / SeaGen
<b>Polatumab vedotin</b>	ADC (MMAE)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	Polivy™	Genetech / Roche
<b>Belantamab mafodotin</b>	ADC (MMAF)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	Blenrep™	GlaxoSmith Kline

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**Table 2. The current pipeline of MNP-derived pharmaceuticals.**

Drug Name	Chemical class	Marine origin	Disease area	Trademark	Company
<b>PHASE III</b>					
<b>Lurbinedin</b>	Alkaloid	<i>Ecteinascidia turbinata</i> (Tunicate)	Cancer	Zepzelka™	PharmaMar
<b>Tetrodotoxin</b>	Alkaloid	<i>Pseudoalteromonas</i> sp., <i>Pseudomonas</i> sp., <i>Vibrio</i> sp.	Pain	Halneuron™	Wex Pharmaceutical Inc.
<b>Plinabulin</b>	Diketopiperazine	<i>Aspergillus</i> sp. (Fungi)	Cancer	-	BeyondSpring Pharmaceuticals
<b>Plitidepsin</b>	Depsipeptide	<i>Aplidium albicans</i> (Tunicate)	Coronavirus	Aplidin®	PharmaMar
<b>Marizomib</b>	β-lactone-γ-lactam	<i>Salinospora tropica</i> (Bacteria)	Cancer	-	Triphase
<b>PHASE II</b>					
<b>Bryostatin</b>	Macrolide	<i>Bugula neritina</i> (Bryozoan)	Alzheimer's Disease	-	Neurotrope BioScienc
<b>Plocabulin</b>	Polyketide	<i>Lithoplocamia lithiostoides</i> (Sponge)	Cancer	-	PharmaMar
<b>GTS-21</b>	Alkaloid	<i>Nemertines</i> sp. (Worm)	Cognitive disorders	-	-
<b>AGS-16C3F</b>	ADC (MMAF)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	Agensys / Astellas Pharm
<b>Tisotumab Vedotin</b>	ADC (MMAE)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	HuMax®-TF-ADC	GenMab
<b>Ladiratumzumab vedotin</b>	ADC (MMAE)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	Seattle Genetics
<b>Telisotuzumab vedotin</b>	ADC (MMAE)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	Abbvie
<b>Enapotamab vedotin</b>	ADC (MMAE)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	Genmab
<b>Disitimab Vedotin</b>	ADC (MMAE)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	RemeGen
<b>CAB-ROR2</b>	ADC (MMAE)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	BioAtla
<b>CX-2029</b>	ADC (MMAE)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	AbbVie / CytomX Therapeutics
<b>W0101</b>	ADC (Auristatin variant)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	Pierre-Fabre
<b>PHASE I</b>					
<b>STRO-002</b>	ADC (Taltobulin)	<i>Hemiasterella minor</i> (Sponge)	Cancer	-	Sutro Biopharm
<b>Griffithsin</b>	Lectin	<i>Griffithsia</i> sp. (Red alga)	HIV	-	Center for Predictive Medicine
<b>FOR-46</b>	ADC (MMAF)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	Fortis Therapeutics
<b>ALT-P7</b>	ADC (MMAE)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	3SBio / Alteogen
<b>MRG003</b>	ADC (MMAE)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	Shanghai Miracogen Inc.
<b>RC-88</b>	ADC (MMAE)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	RemeGen
<b>SGN-B6A</b>	ADC (MMAE)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	Seattle Genetics
<b>SGN-CD228A</b>	ADC (MMAE)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	Seattle Genetics
<b>Cofetuzumab pelidotin</b>	ADC (Auristatin variant)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	AbbVie
<b>PF-06804103</b>	ADC (Auristatin variant)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	Pfizer / AbbVie
<b>ZW-49</b>	ADC (Auristatin variant)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	Zymeworks / BeiGene
<b>ARX-788</b>	ADC (Amberstatin 269)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	Ambrex / Zhejiang Medicine
<b>XMT-1536</b>	ADC (Dolaflexin)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	Mersana Therapeutics
<b>A-166</b>	ADC (Duostatin 5)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Cancer	-	Klus Pharma
<b>STI-6129</b>	ADC (Duostatin 5)	<i>Dolabella auricularia</i> (Mollusk) / <i>Symploca</i> sp. (Cyanobacteria)	Amyloidosis	-	Sorrento
<b>MORAb-202</b>	ADC (halichondrin B)	<i>Halichondria okadai</i> (Sponge)	Cancer	-	Eisai

711 **Table 3. Current knowledge on the biosynthetic pathways of the main marine drugs.**  
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<b>Drug Name</b>	<b>Biosynthetic pathway</b>	<b>Reference(s)</b>
<b>Omega-3-acid ethyl esters</b>	Elucidated	Lu et al., 2019
<b>Eicosapentaenoic acid ethyl ester</b>	Elucidated	Lu et al., 2019
<b>Spongouthyridine</b>	Unknown	-
<b>Spongouridine</b>	Unknown	-
<b>Nelarabine</b>	Unknown	-
<b><math>\omega</math>-contoxin MVIIA</b>	Elucidated	Jin et al., 2019
<b>Plitidepsin</b>	Elucidated	Xu et al., 2012
<b>Trabectedin</b>	Elucidated	Schofield et al., 2015
<b>Halichondrin B</b>	Unknown	-
<b>Dolastatin 10</b>	Unknown	-
<b>Tetrodotoxin</b>	Unknown	Makarova et al., 2029
<b>Plinabulin</b>	Unknown	-
<b>Salinosporamide A</b>	Elucidated	Eustáquio et al., 2009
<b>Bryostatin</b>	Elucidated	Slocum et al., 2018
<b>Plocabulin</b>	Unknown	-
<b>GTS-21</b>	Unknown	-
<b>Griffithsin</b>	Elucidated	Fuqua et al., 2015
<b>Hemiasterlin</b>	Unknown	-

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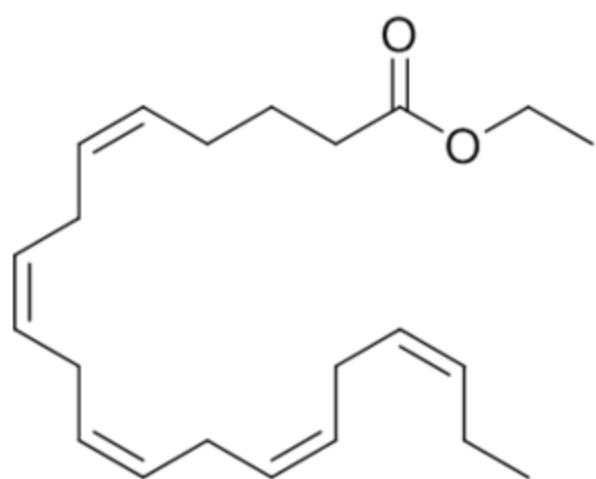
## 716 **Figure legends**

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718 **Figure 1. The structures of bioactive MNPs that are either themselves or the inspiration for**  
719 **approved therapeutics and clinical candidates.**

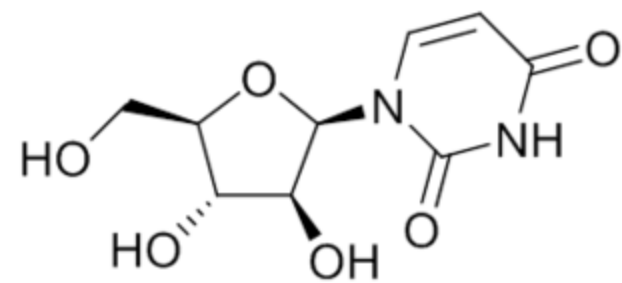
720  
721 **Figure 2. Timeline depicting the successive approval of the currently used MNP-derived**  
722 **pharmaceuticals.** Some prominent examples of marine source organisms are indicated. For instance, the  
723 anticancer drugs cytarabine and nelarabine, as the antiviral compound vidarabine are analogs of a couple of  
724 marine nucleosides, spongothymidine and spongouridine, which were originally isolated from the Caribbean  
725 sea sponge *Tectitethya crypta*. The painkiller ziconotide is an  $\omega$ -conopeptide from the venom of the Pacific  
726 fish-hunting marine mollusk *Conus magus*. The anticancer agent eribulin mesylate corresponds to a simplified  
727 analogue of the marine polyether macrolide, halichondrin B, which was originally isolated from the sea  
728 sponge *Halichondria okadai*. Antibody-drug conjugate (ADC) combining a cytotoxic agent of marine origin  
729 with a selective monoclonal antibody such as brentuximab vedotin, enfortumab vedotin, polatuzumab  
730 vedotin, and belantamab mafodotin. These ADC's notably include in their structures a microtubule disrupting  
731 MNP derivative, i.e. either monomethyl auristatin E or monomethyl auristatin F that are synthetic analogs of  
732 the highly cytotoxic dolastatin 10 that was historically isolated from the Indian Ocean sea hare *Dolabella*  
733 *auricularia*. The anticancer MNPs trabectedin and its derivative lurbectedin are tetrahydroisoquinoline  
734 alkaloids originally identified in the Caribbean sea squirt *Ecteinascidia turbinata*. Plitidepsin is a cyclic  
735 depsipeptide anticancer drug historically isolated from the marine tunicate *Aplidium albicans*.

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737 **Figure 3. A benchmark procedure to implement MNP production in microbial cell factories. A.**  
738 **Theoretical pathway reconstruction.** Powerful metabolomics profiling approaches lead to the accurate  
739 detection of plausible reaction intermediates while recent advancements in molecular networking provide new  
740 efficient opportunities in the processing of the acquired metabolomics data. **B. Identifying a first set of**  
741 **candidate genes.** Once compounds are connected and a reaction sequence has been postulated in silico,  
742 bioinspired organic chemistry and literature data may then help in identifying a type of enzymatic activity  
743 required for each step. At this stage, the availability of the holobiont metagenome and massive  
744 transcriptomics data are of primary importance since searching the specific functional conserved domains  
745 may lead to define first sets of candidate genes. **C. Prioritization procedures.** The various possibilities  
746 include the screen for homology to orthologous gene sequences of already characterized biosynthesis steps  
747 in other models or the search of physical gene clusters. The most powerful and global approach that have  
748 recently emerged to rationally prioritize gene sequences in metabolic engineering relies on gene co-  
749 expression analysis. **D. Validating the correct activity of each predicted genes/enzymes.** To this aim,  
750 biochemical characterization of recombinant proteins heterologously expressed in bacteria or yeasts still  
751 represents a basic but reliable approach. **E. Pathway reconstruction in a microbial chassis.** A key factor  
752 of success to this aim is the choice of an appropriate heterologous host (a bacterium or a yeast for instance).  
753 In such a perspective, extensive synthetic biology toolboxes are available for both microbial models such as  
754 genomic integrative constructs, including CRISPR-Cas9 technologies, and auto-replicative plasmids. **F. MNP**  
755 **synthesis in the microbial host.** Main strategies include optimization of media and culture conditions but  
756 also implementation of bioreactors, screening of enzyme variant with improved activities, adaptation of the  
757 host metabolism, and optimization of enzyme targeting. **G. Optimizing MNP synthesis with multiple**  
758 **iterative refinements based on the Design-Built-Test-Learn cycle.**

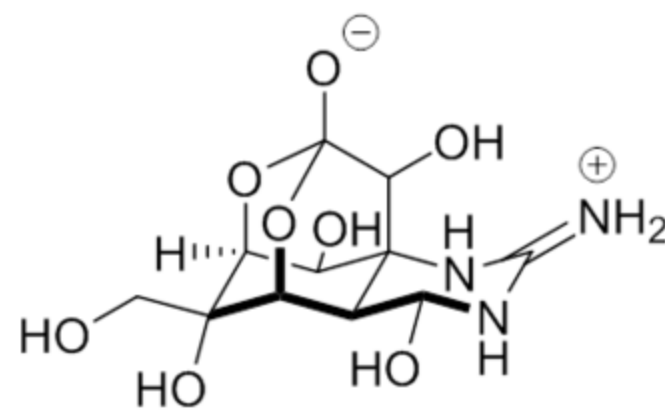
759  
760 **Figure 4. Current (A) and future (B) application fields of metabolic engineering and microbial**  
761 **cell factories.**



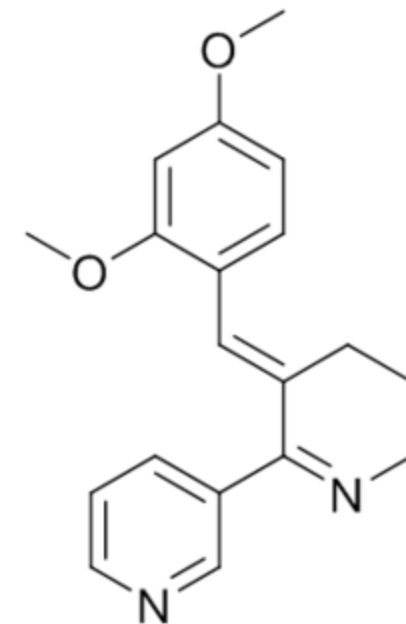
**omega-3-acid ethyl ester**



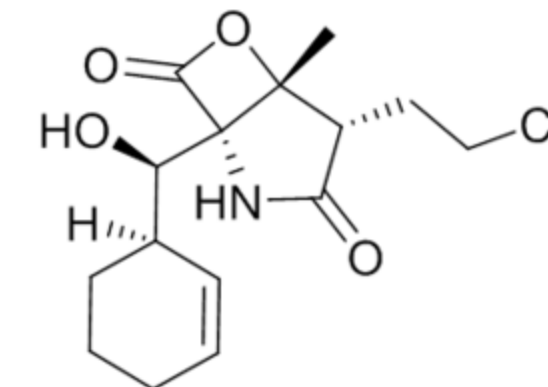
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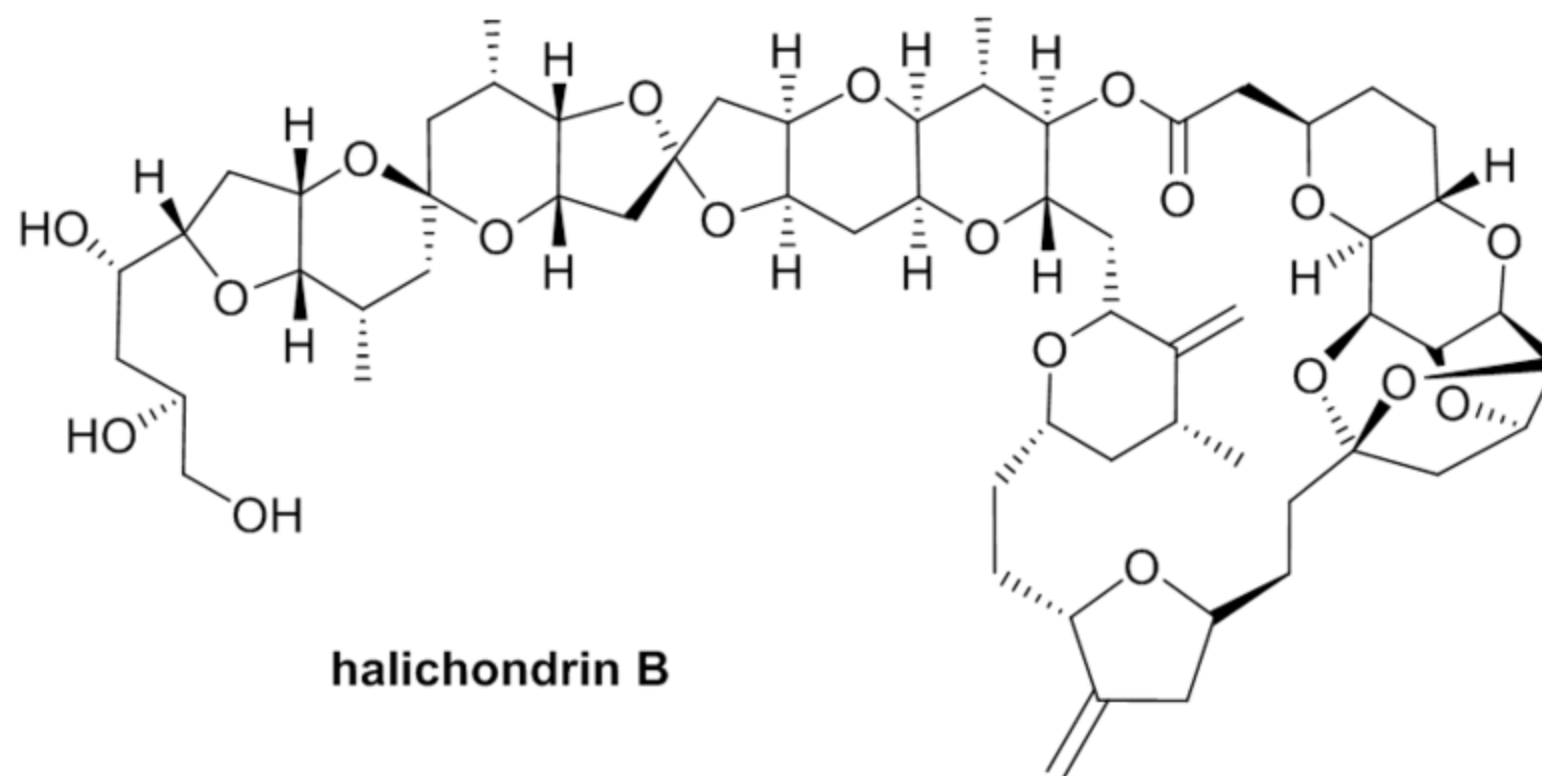
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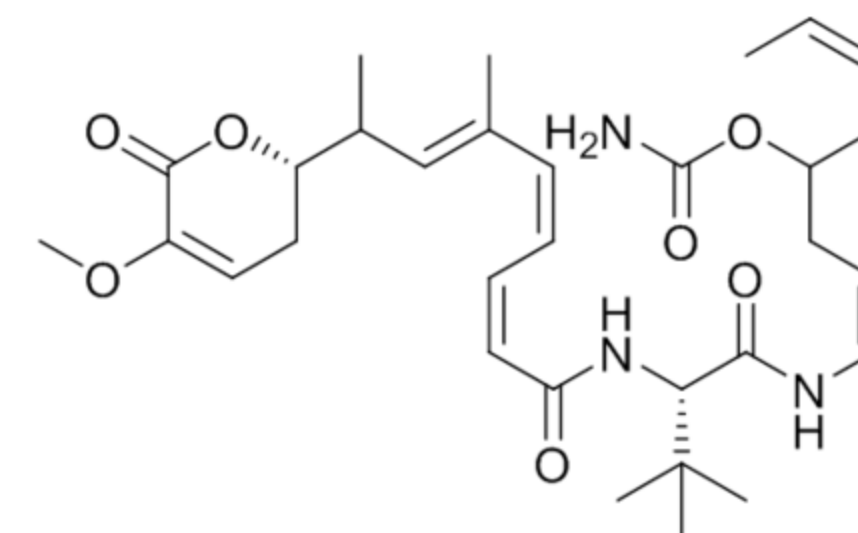
**GTS-21**



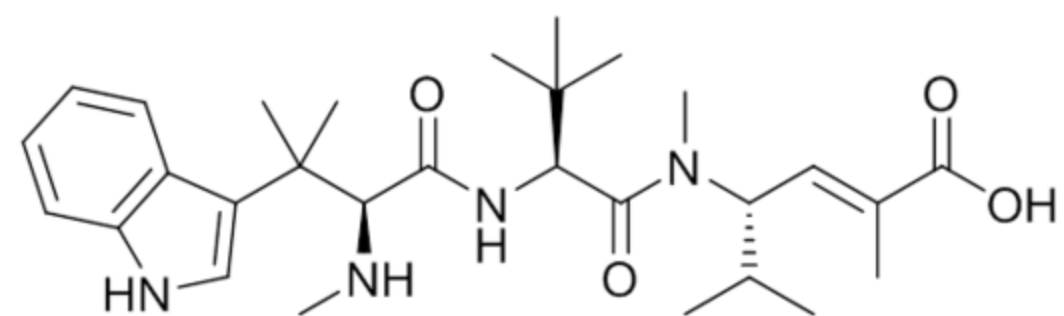
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**halichondrin B**



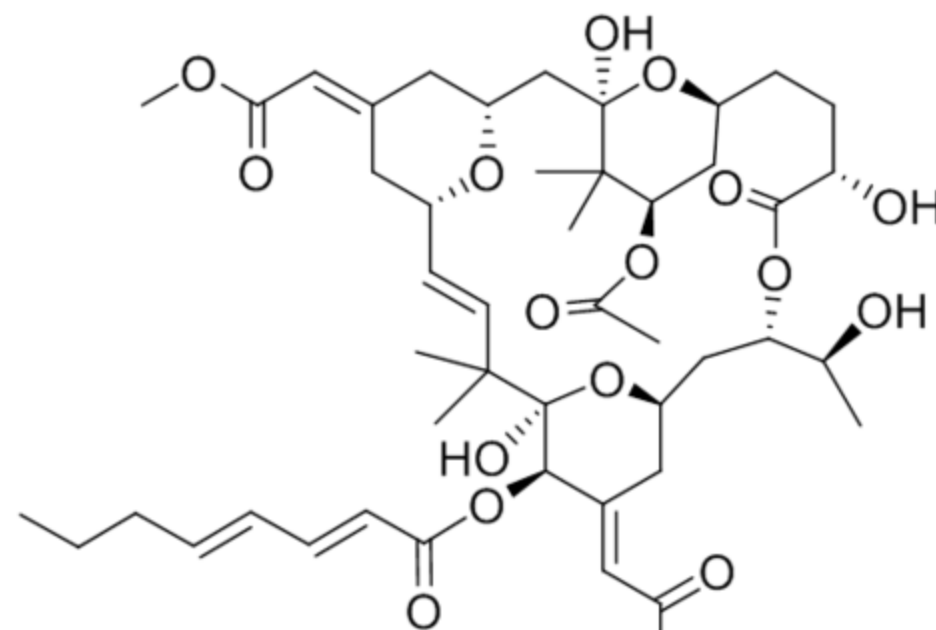
**plocabulin**



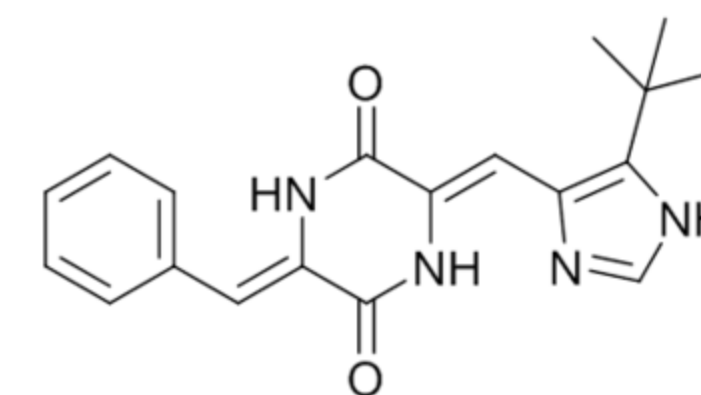
**hemiasterlin**



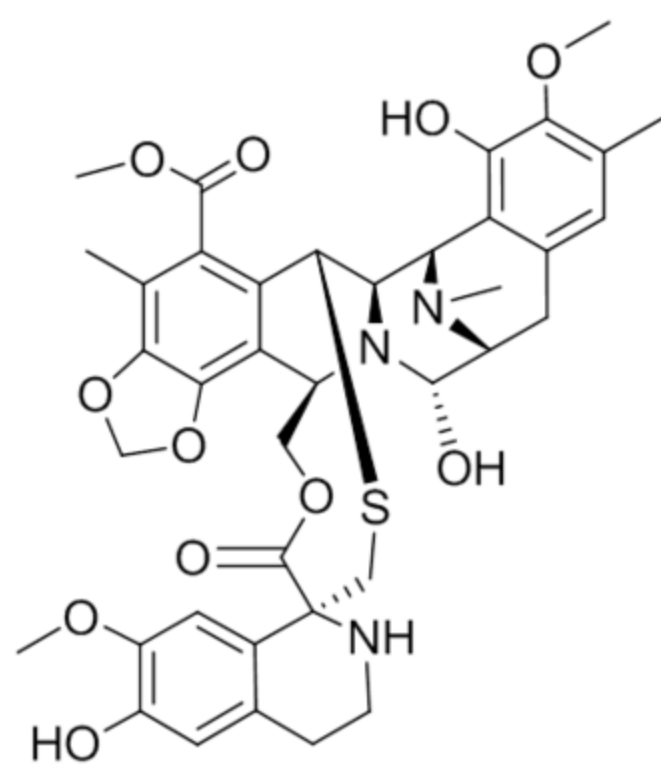
**ω-conotoxin MVIIA**



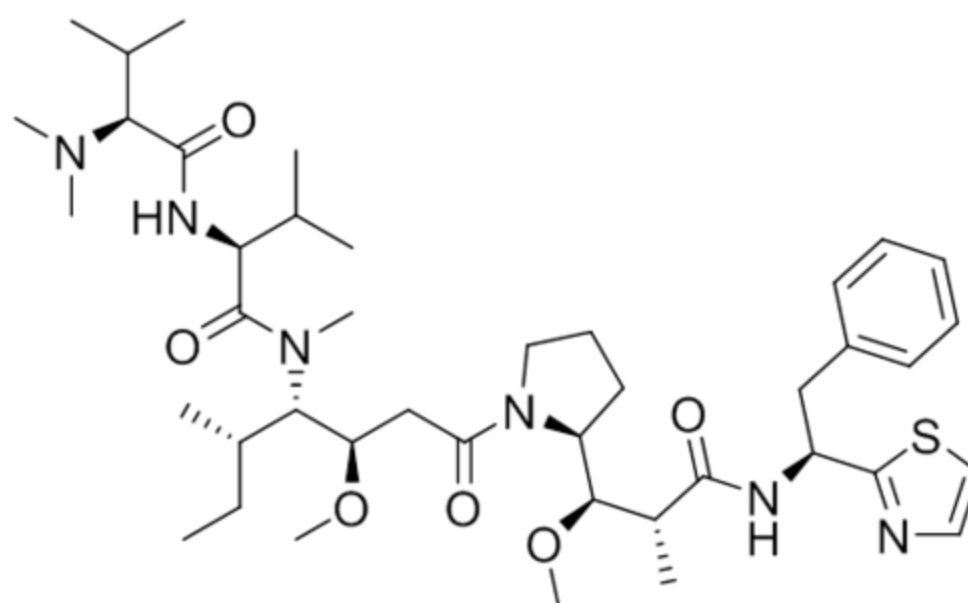
**bryostatin 1**



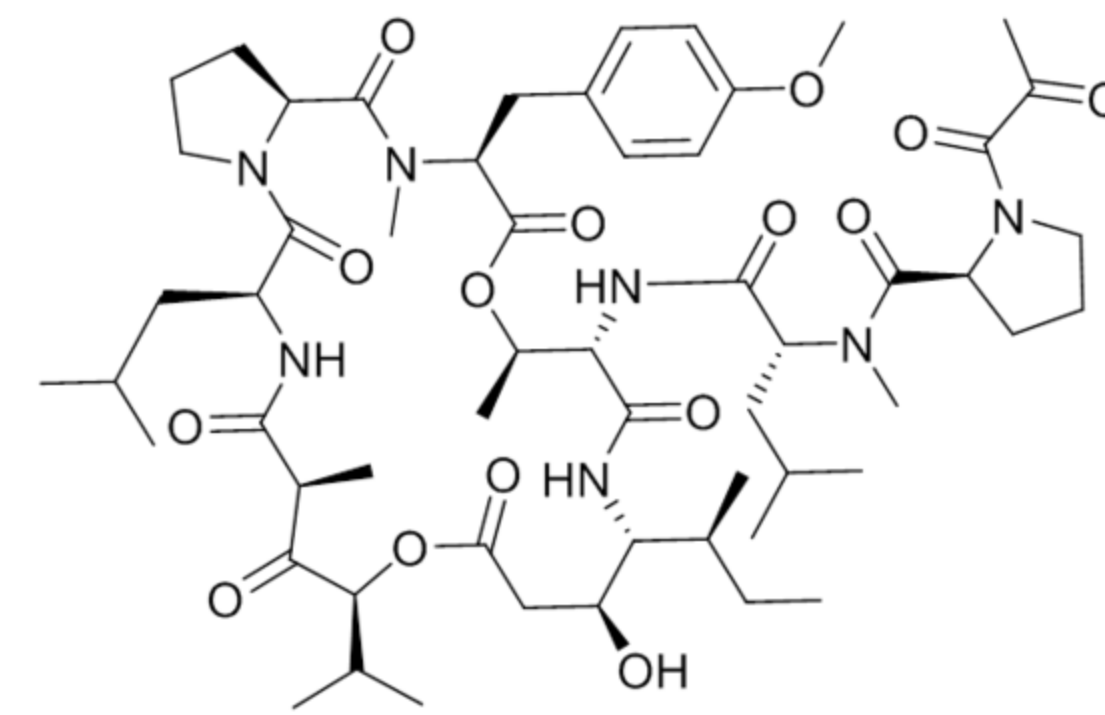
**plinabulin**



**trabectedin**

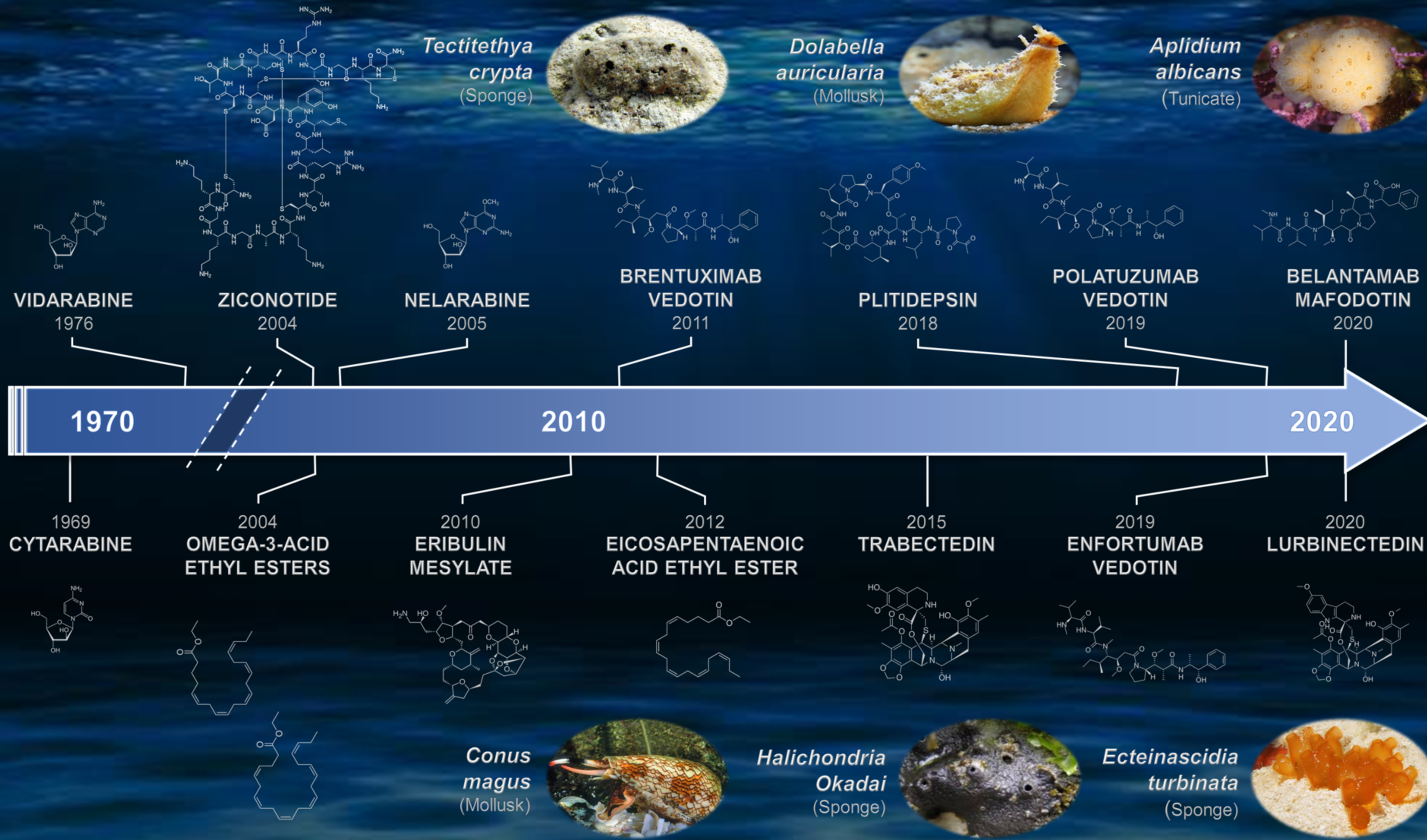


**dolastatin 10**



**plitidepsin**





*Tectitethya crypta*  
(Sponge)



*Dolabella auricularia*  
(Mollusk)



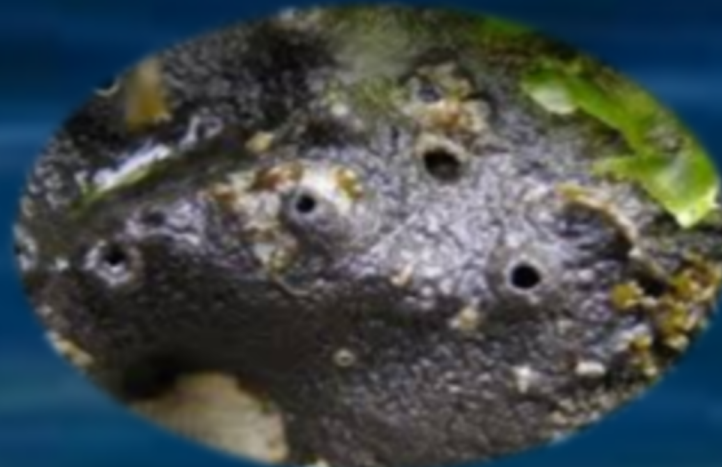
*Aplidium albicans*  
(Tunicate)



*Conus magus*  
(Mollusk)



*Halichondria Okadai*  
(Sponge)



*Ecteinascidia turbinata*  
(Sponge)



**VIDARABINE**  
1976

**ZICONOTIDE**  
2004

**NELARABINE**  
2005

**BRENTUXIMAB  
VEDOTIN**  
2011

**PLITIDEPSIN**  
2018

**POLATUZUMAB  
VEDOTIN**  
2019

**BELANTAMAB  
MAFODOTIN**  
2020

**CYTARABINE**  
1969

**OMEGA-3-ACID  
ETHYL ESTERS**  
2004

**ERIBULIN  
MESYLATE**  
2010

**EICOSAPENTAENOIC  
ACID ETHYL ESTER**  
2012

**TRABECTEDIN**  
2015

**ENFORTUMAB  
VEDOTIN**  
2019

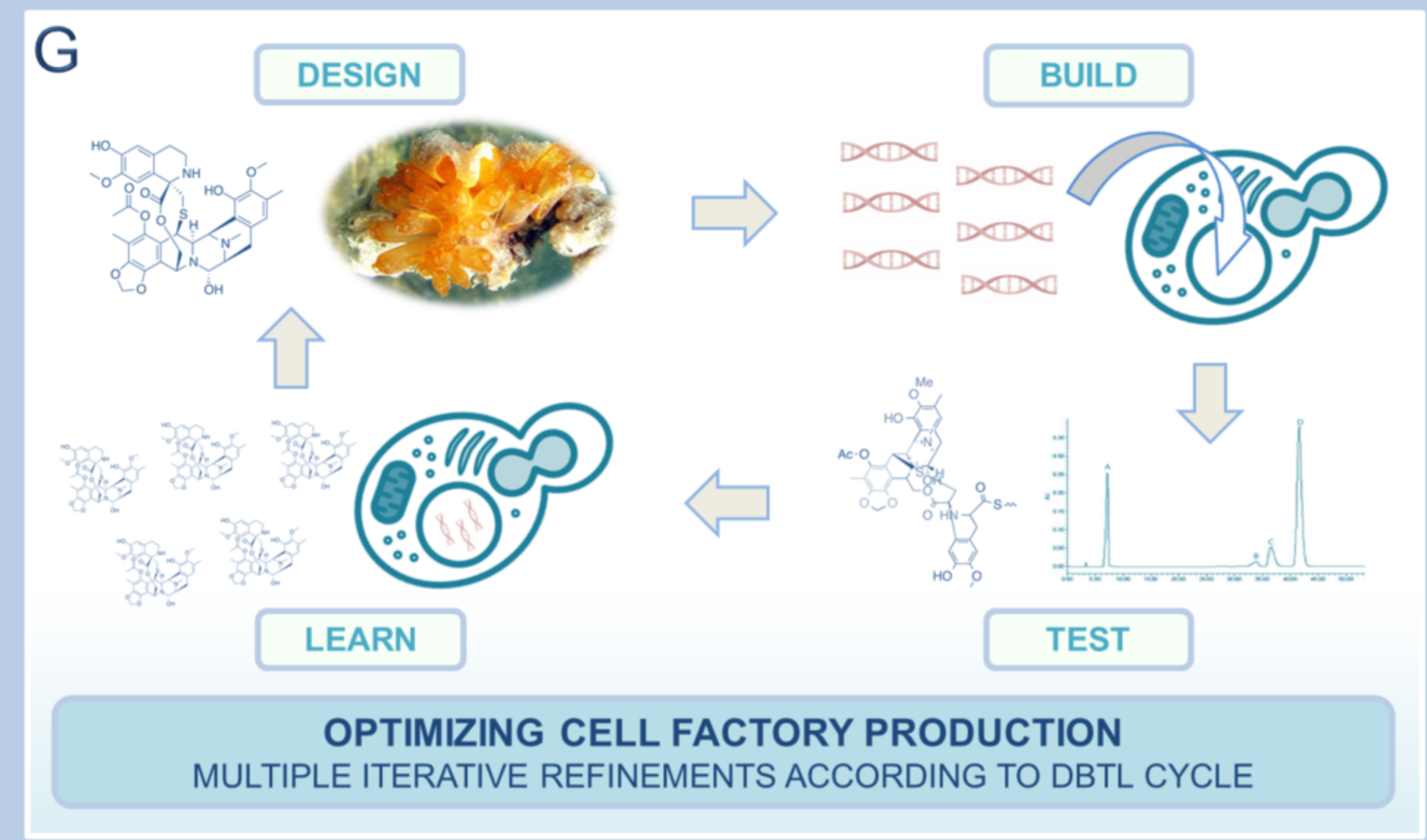
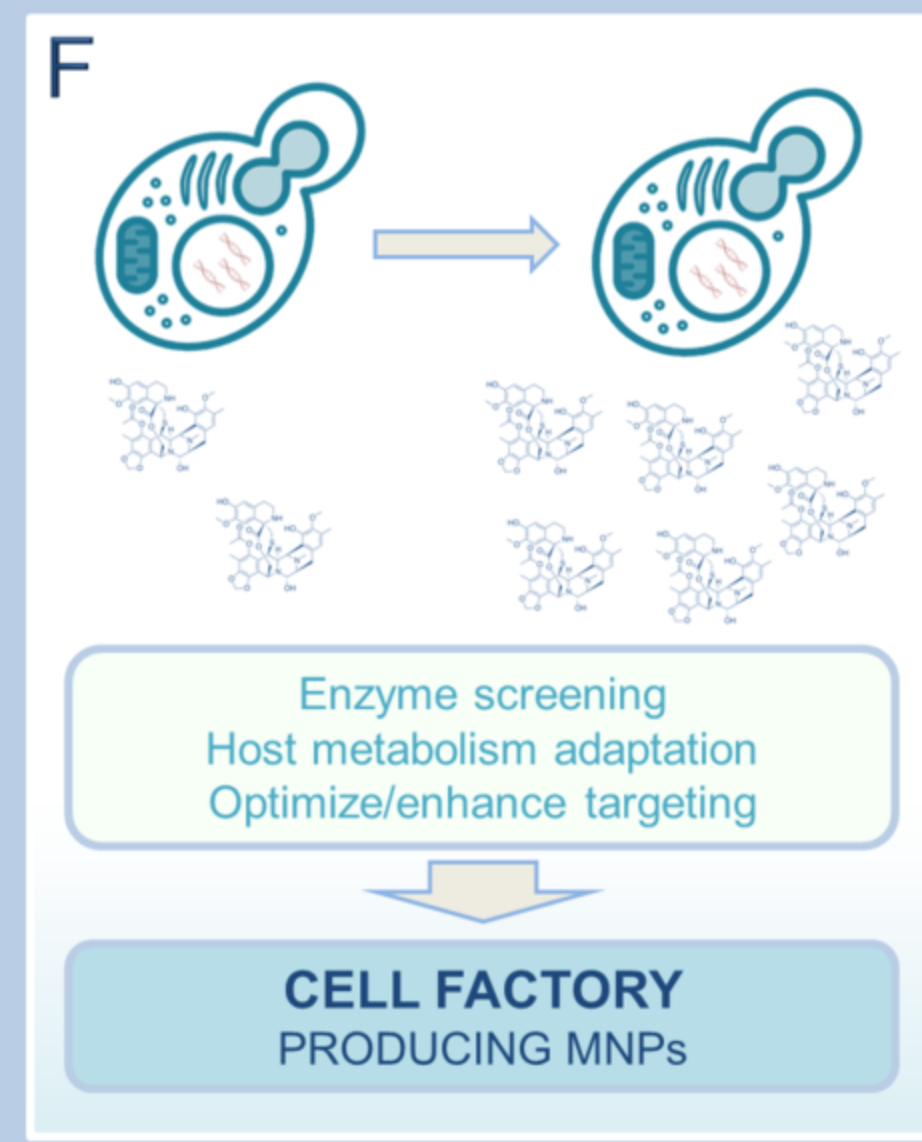
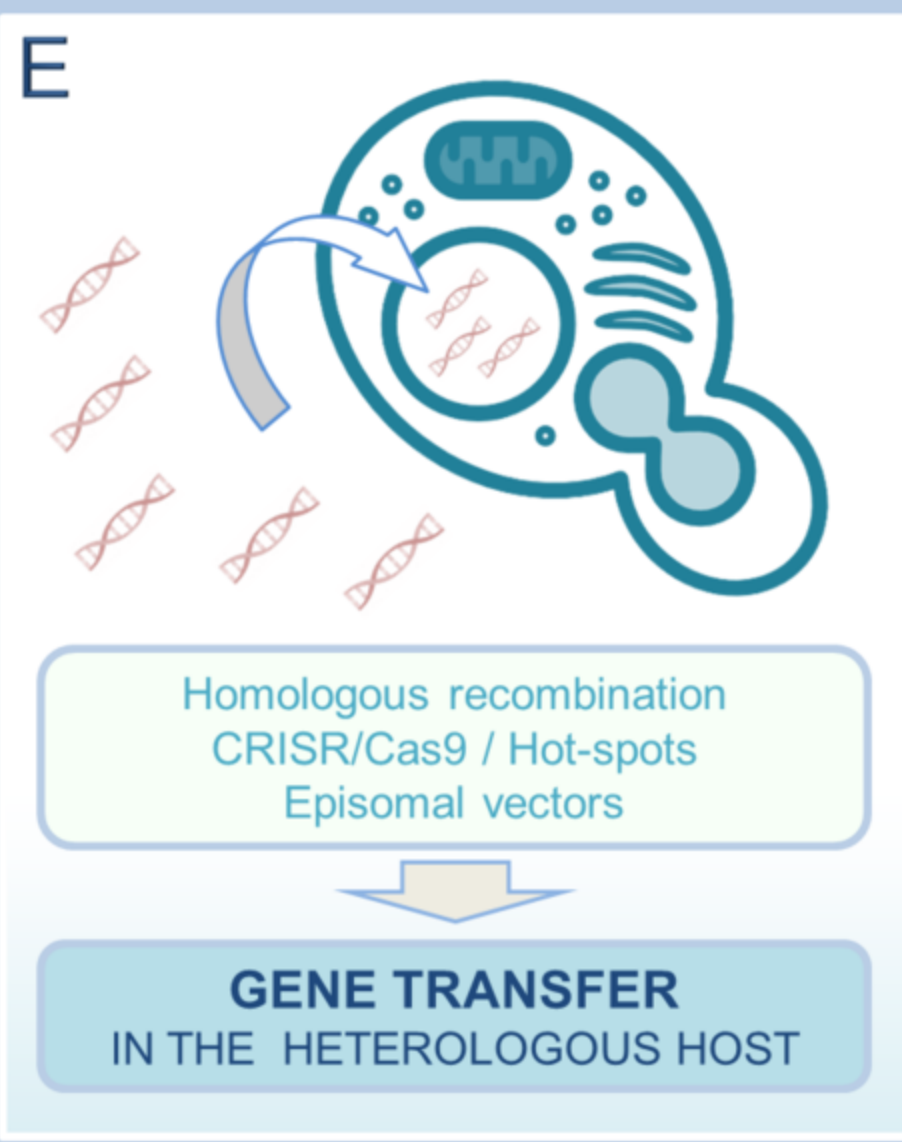
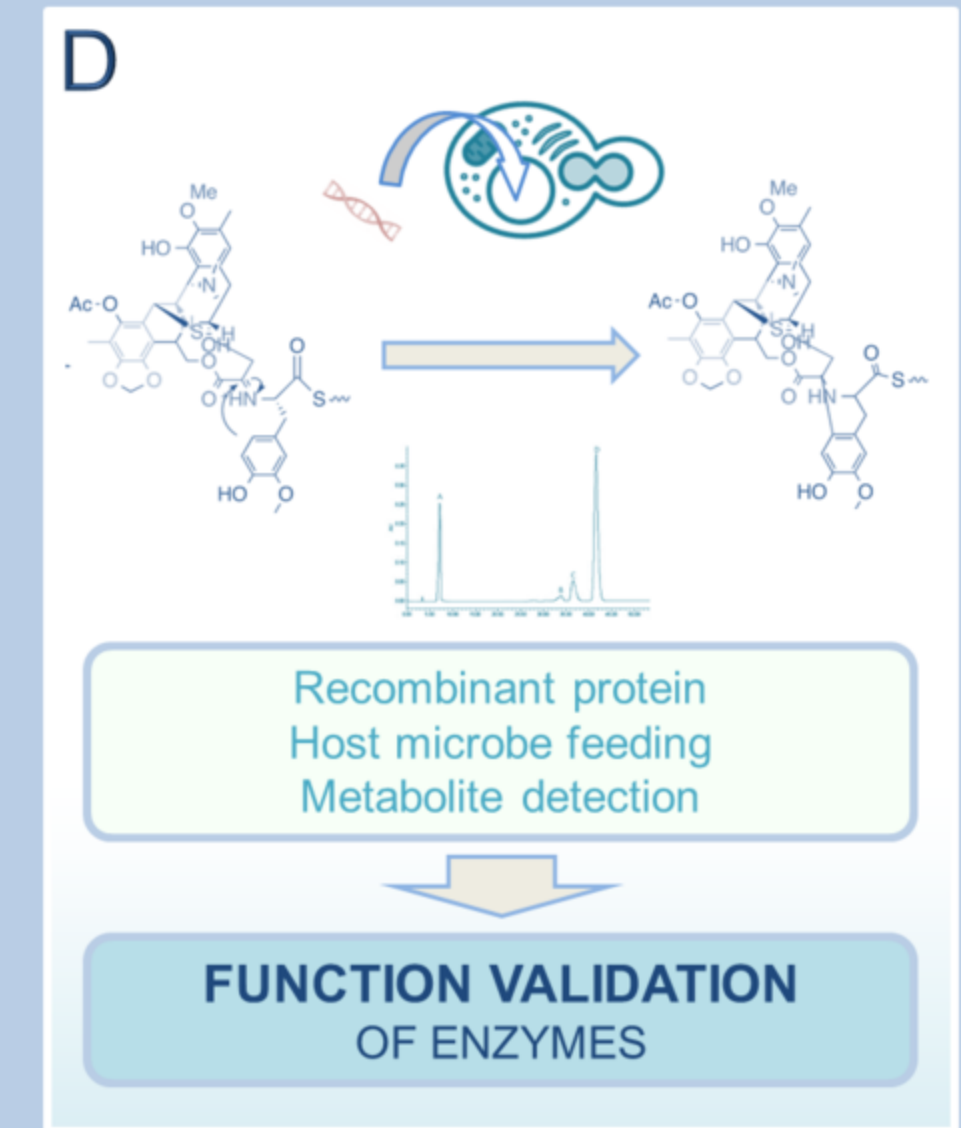
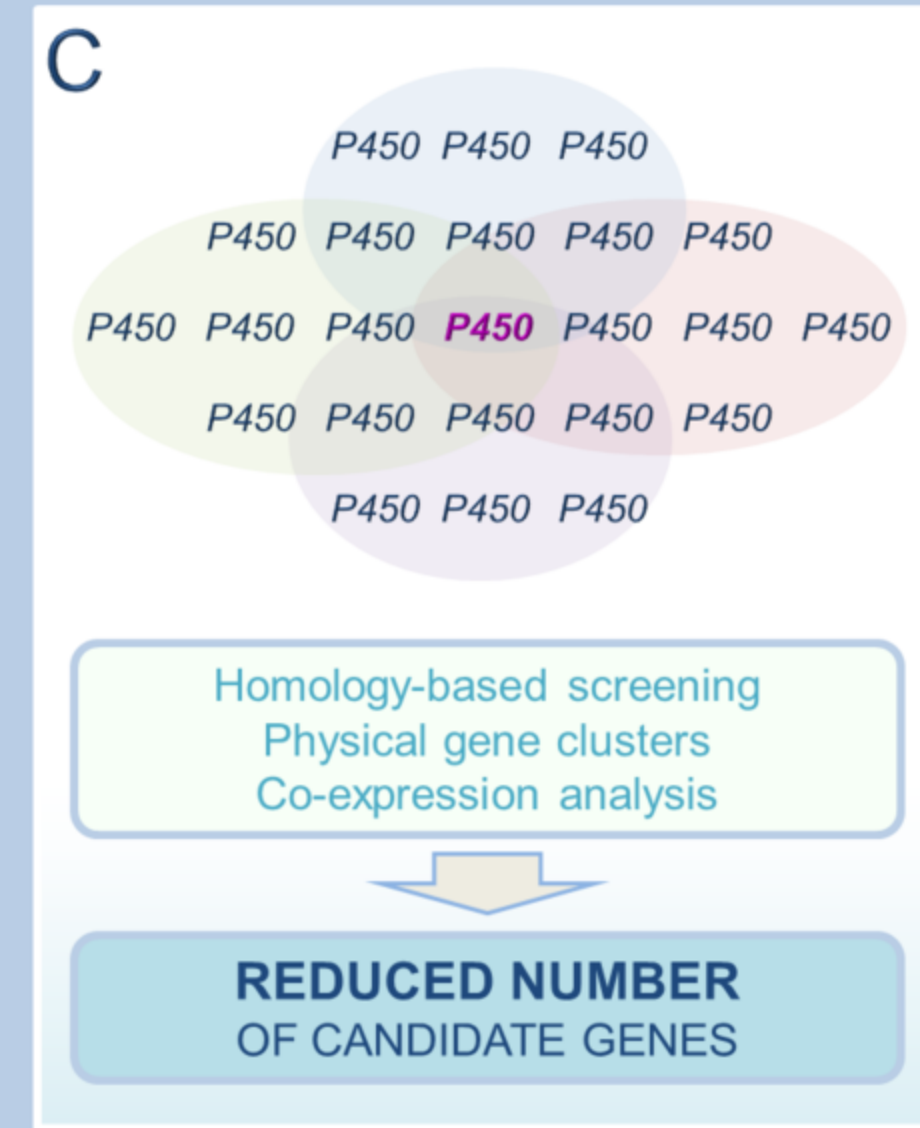
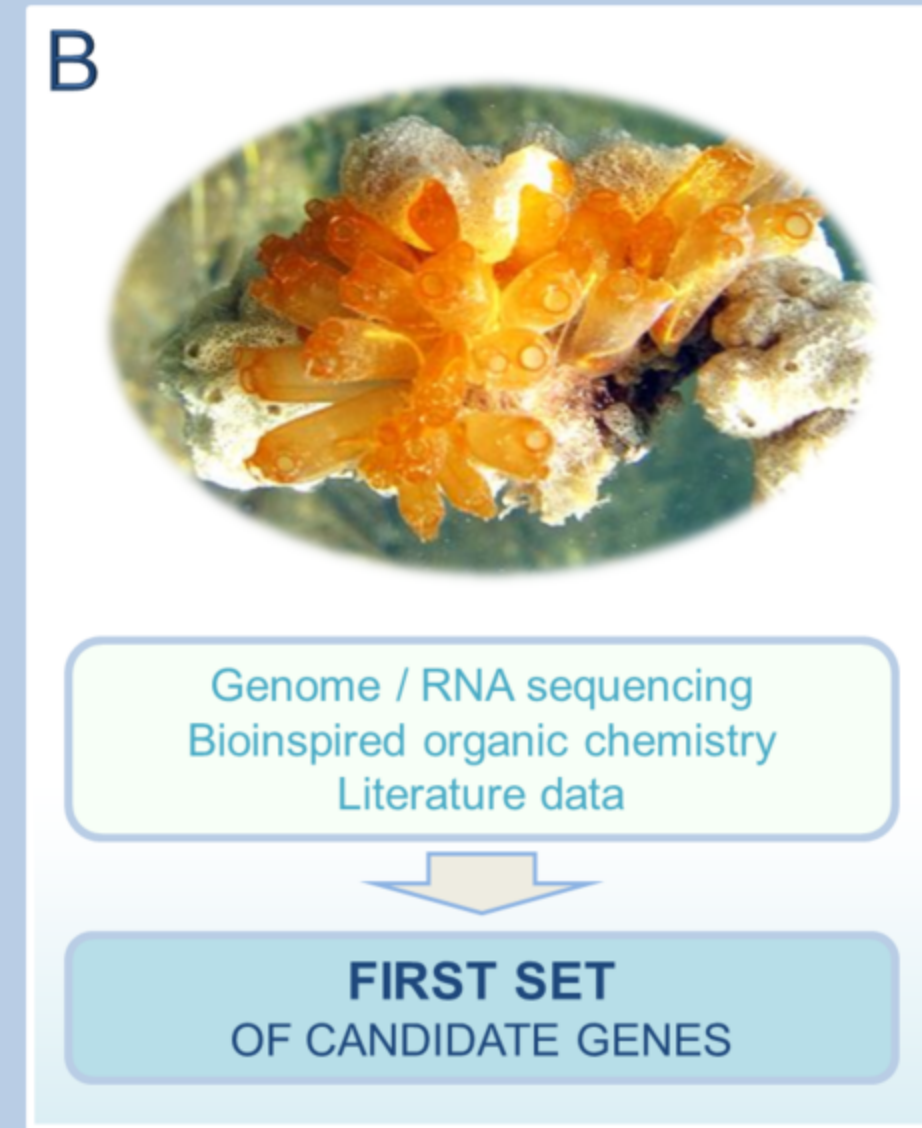
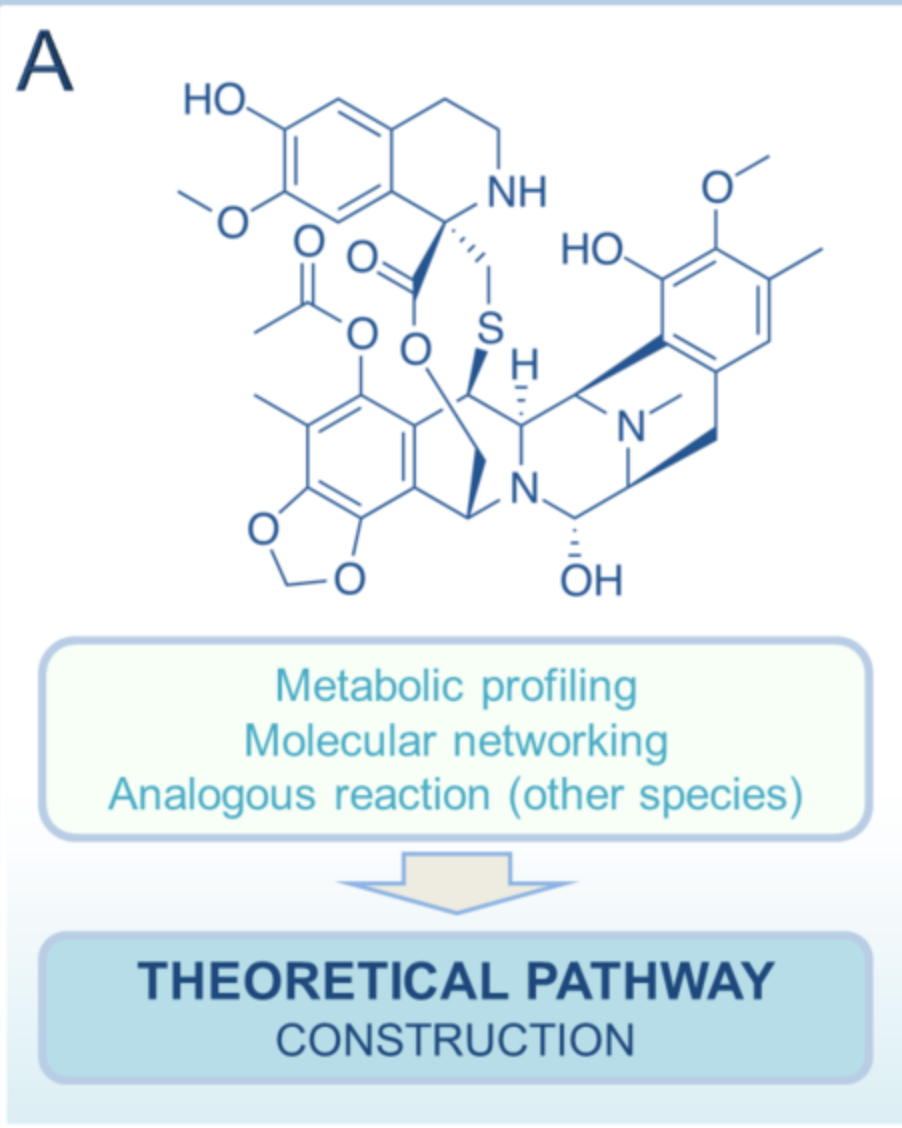
**LURBINECTEDIN**  
2020

1970

2010

2020



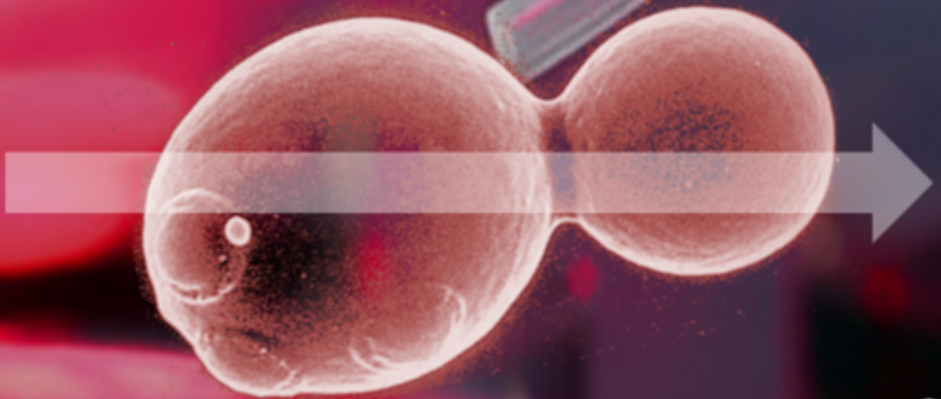




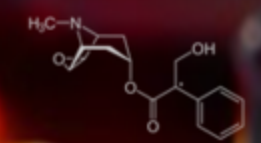
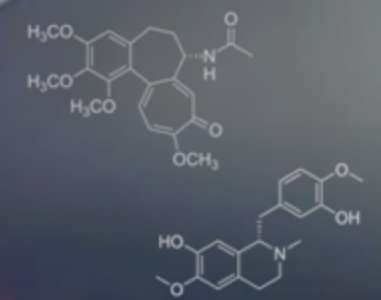
# A RED/WHITE BIOTECHNOLOGIES

## MICROBIAL CELL FACTORIES

CARBON SOURCES



SPECIALIZED METABOLITES

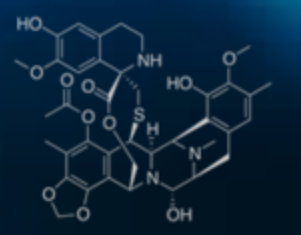
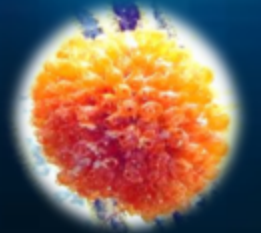
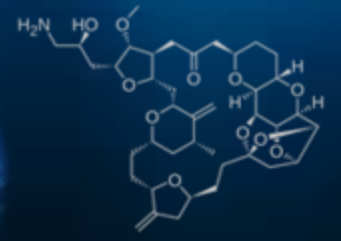
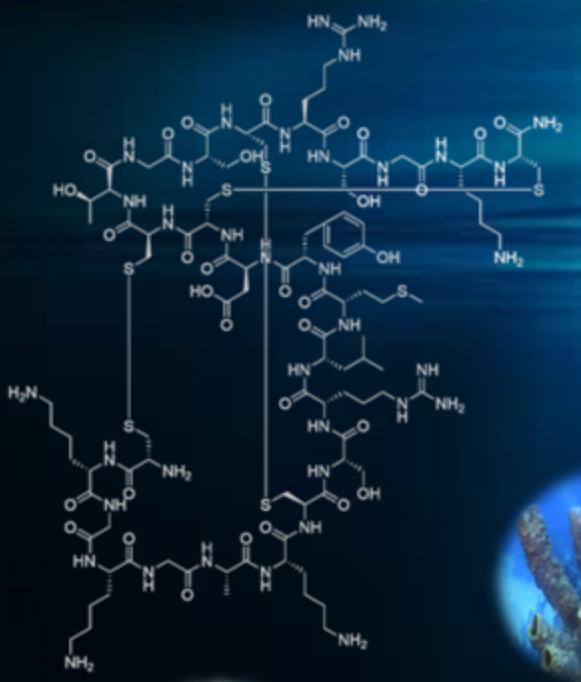


METABOLIC ENGINEERING  
SYNTHETIC BIOLOGY  
SYSTEM BIOLOGY



# B BLUE BIOTECHNOLOGIES

## MARINE NATURAL PRODUCTS



## MARINE RESOURCES

- Bacteria
- Green algae
- Red algae
- Ascidians
- Cyanobacteria
- Porifera
- Bryozoans
- Echinoderms
- Brown algae
- Mollusks
- Dinoflagellates
- Cnidarians
- Fungi