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Natural products of marine origin and their perspectives in the discovery of new anticancer drugs

Jakeline Trejos Jimenez, Mária Šturdíková, Ernest Šturdík[a]

Institute of Biotechnology and Food Science, Slovak University of Technology
SK-812 37 Bratislava, Slovak Republic
[a]Institute of Biochemistry, Nutrition and Health, Slovak University of Technology
SK-812 37 Bratislava, Slovak Republic

jakeline.jimenez@stuba.sk

Abstract

Marine invertebrates and a growing number of marine actinomycetes are the sources of novel, bioactive secondary metabolites. This review is presented as a short survey of recent investigations in the marine pharmacology and discovery of new anticancer drugs. The implications of this research towards the development of marine secondary metabolites as a sustainable source of new drugs are discussed.

Keywords: anticancer drugs, secondary metabolites, marine actinomycetes

Introduction

Natural products have played important role in treatment and prevention of human diseases during thousands of years. Remedies based on natural substances come from different sources, among them terrestrial plants and microorganisms, sea macro- and micro-organisms, as well as terrestrial invertebrates and vertebrates (Jones et al., 2006).

In the course of last 25 years marine organisms have been proven to be rich resources of wide range of worthy compounds for medicine. The above mentioned natural products are found as secondary metabolites of marine invertebrates, particularly sponges, bryozoans, tunicates and ascidians. The potential of new drugs discovery, natural products attract scientists of various disciplines, e.g. organic chemistry, bioorganic chemistry, pharmacology and biology. Nowadays more than a dozen of marine alkaloids are involved in different phases of clinical trials for treatment of human tumours (Newman et al., 2004). The analysis

of the origin of drugs developed between 1981 and 2002 showed, that natural products or remedies derived from natural products stand for 28% of all chemical entities introduced to the market (Newman et al., 2004). Bearing in mind that the secondary metabolites from the natural sources were developed in the context of living systems, they are often perceived as "biologically more friendly with major similarity to drugs" as completely synthetic molecules, becoming optimal candidates for the development of new medicines (Chang et al., 2002).

Marine micro-organisms, particularly actinomycetes, have evolved the greatest genomic and metabolic diversities. Therefore, the effort should be directed towards exploring marine actinomycetes as a source for the discovery of novel secondary metabolites (Kin, 2006). Actinomycetes do not only exist in the oceans but they are also widely distributed in different marine ecosystems. The exploitation of marine actinomycetes as a source for novel secondary metabolites production is in its infancy. There is a tremendous potential for the isolation of novel secondary metabolites from marine actinomycetes. In this respect, future success relies upon our ability to isolate novel actinomycetes from marine environments. Although isolation strategies directed towards new marine-derived actinomycetes have been lacking, some progress has recently been made in this area (Kin, 2006).

Marine natural anticancer products

Since the 1970's, more than 15,000 structurally diverse natural products with an astounding array of bioactivities have been discovered from marine microbes, algae and invertebrates (MarinLit Database, 2002). Unfortunately, the utility of marine natural products as a potentially sustainable drug source is hampered by several significant limitations. Compounds are often isolated in extremely low yields and it may be difficult to synthesize them economically. Source organisms (especially invertebrates and/or their symbionts) can be difficult to culture or may not produce the compound of interest under the given culture conditions, and wild collection of macro-organisms can be detrimental to the environment (Faulkner, 2000, Haefner, 2003). Despite these disadvantages, interest in the development of marine compounds as potential drugs is thriving and there are several dozen marine natural products (or derivatives thereof) that are in clinical or preclinical trials for the treatment of cancer (Table 1), inflammation, and other diseases (Newman and Cragg, 2004, Mayer a Gustafson, 2003). Didemnin B, isolated from a tunicate over 20 years ago, was the first marine natural product to enter human clinical trials against cancer and led the way for a

plethora of drug candidates isolated from marine organisms (Rinehart et al., 1981). The supply of marine metabolites being tested in the clinical trials is currently provided by several means: open aquaculture of the invertebrates (ET-743 and bryostatin), total synthesis (ziconotide, discodermolide, dolastatin 10, dehydrodidemnin, hemiasterlins), semi-synthesis (halichondrin B derivative, ET-743), and fermentation of producing microbes (thiocoraline) (Rinehart et al., 1981). In general, fermentation has been the most successful method for production of natural products (penicillin, clavulanic acid, erythromycin, *etc.*), especially if the compounds proceed through clinical trials and are needed on a commercial scale (> kg) (Demain, 2000). The analysis of structural types present in Table 1 suggests that more than half are polyketides and/or peptide metabolites, with predicted origins stemming from modular polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS). Our rapidly growing knowledge of modular biosynthetic systems may allow these gene clusters to be cloned and over-expressed in more amenable bacterial hosts. This approach could potentially provide a virtually unlimited supply of compounds and alleviate the need for culturing of the source macro-organisms. Although many marine natural products appear to arise from multi-functional enzymes that are also present in terrestrial systems, many also possess a myriad of functional groups not previously described from terrestrial metabolites. These unique structural differences are inevitably reflected in novel biosynthetic pathways with genes encoding completely new enzymatic activities (Li and Piel 2002, Chang at al., 2002,). Despite these differences, the presence of conserved regions of "terrestrial" biosynthetic genes can be used for probing analogous pathways in marine systems both in silico by analyzing sequenced genes and genomes for secondary metabolic enzymes and pathways. Some structures of marine natural anticancer product in clinical or preclinical trials are shown in Fig.1.

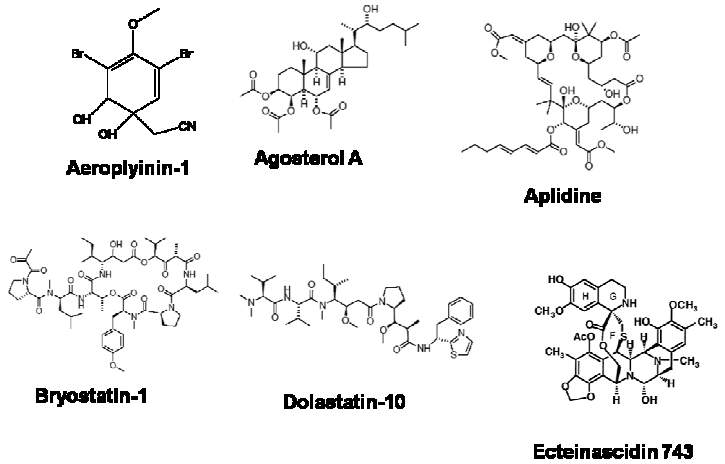

Fig.1 Structures of some marine natural anticancer product in clinical or preclinical trials

Table 1 Marine natural anticancer product in clinical or preclinical trials

Compound	**Probable biosynthetic route or compound type**	**Source organism**	**Clinical phase status**
Ecteinascidin 743 (Yondelis™)	NRPS[1]	*Ecteinascidia turbinata* (tunicate)	Approved drug 2007
Bryostatin 1	Mixed PKS/NRPS	*Bugula neritina* (bryozoan)	III
Dolastatin 10	NRPS[1]	*Dolabella auricularia* (mollusc, and later found in cyanobacteria	II
Dehydrodidemnin B (Aplidine™)	NRPS[1]	*Aplidium albicans* (tunicate)	II
Kahalalide F	NRPS[1]	*Elysia rufescens/Bryopsis* sp. (mollusc/green alga)	II
Squalamine	Aminosteroid	*Squalus acanthias* (shark)	II
Halichondrin B	PKS[2]	*Lissodendoryx* sp. (sponge)	I
Discodermolide	PKS[2]	*Discodermia dissoluta* (sponge)	I
Hemiasterlin derivative (HTI-286)	NRPS[1]	*Cymbastella* sp. (sponge)	I
Bengamide derivative (LAF389)	Mixed PSK/NRPS	*Jaspis* sp. (sponge)	I
Agelasphin derivative (KRN-7000)	Glycosphing olipid	*Agelas mauritianus* (sponge)	I
Laulimalide	PKS[2]	*Cacospongia mycofijiensis* (sponge)	preclinical
Vitilevuamide NRPS	NRPS[1]	*Didemnum cucliferum* and *Polysyncraton lithrostrotum* (tunicates)	preclinical
Diazonamide	NRPS[1]	*Diazona angulata* (tunicate)	preclinical
Eleutherobin	Diterpen glycoside	*Eleutherobia* sp. and *Erythropodium caribaeorum (soft corals)*	preclinical
Sarcodictyin	Terpene	*Sarcodictyon roseum* (sponge)	preclinical
Peloruside A	PKS[2]	*Mycale hentscheli* (sponge)	preclinical
Salicylhalimides A	PKS[2]	*Haliclona* sp. (sponge)	preclinical
ES-285 (Spisulosine)	Alkyl amino alcohol	*Spisula polynyma* (mollusc)	preclinical
Thiocoraline	NRPS[1]	*Micromonospora marina* (bacteria)	preclinical

1- nonribosomal peptide synthase

2- polyketide synthase

Table 2 Anticancer drugs of marine origin and their mechanisms of action. (Mayer and Gustafson, 2006)

Compound	Organizm	Chemical group	Experimental or clinical model	Mechanizm of action
Aeroplysinin-1	Sponge	Alkaloid	Quail chorioallantoic membrane assay	Induction of apoptosis on proliferating endothelial cells
Agosterol A	Sponge	Steroid	[I125]-azido agosterol A photolabelled HU epidermoid carcinoma cell sublines	PGP N-terminal fragment with high affinity in absence of glutathione
			HU adenocarcinoma and colon carcinoma cell lines	Induction of resistance and concomitant lack of MAP Kinase activation and apoptosis
Aplidine	Ascidian	Depsipeptide	HUVECs, HU ovarian carcinoma and angiogenesis assay	Inhibition of angiogenesis by affecting endothelial cells directly
Chondropsin A	Sponge	Macrolide	NCI 60-tumour cell line panel	In vitro inhibition of V-ATPase enzymes
Dehydrothrysiferol	Seaweed	Triterpene	HU breast tumour cell lines	Enhanced apoptosis induction in negative estrogen receptor breast cancer cells
Diazonamide A	Ascidian	Peptide	HU breast, prostate and lung tumour cell lines	Disruption of mitosis and cellular microtubules with inhibition of GTP hydrolysis
Dictyostatin-1	Sponge	Polyketide	HU lung, breast and uterine cell lines	Induction of tubulin polymerisation and active in P-glycoprotein expressing cells
Didemnin B	Ascidian	Depsipeptide	Molecular dynamics simulations	Binding to human elongation factor eEF1A and protein translation inhibition
Dolastatin 10	Mollusc	Peptide	Direct photo-affinity labelling	Binds to amino-terminal peptide of β-tubulin containing cysteine 12
Ecteinascidin-743	Ascidian	Isoquinoline alkaloid	HU melanoma cell lines	Telomere dysfunction increases susceptibility to ET-743
Laurenditerpenol	Alga	Diterpene	Breast tumour cell-based reporter assay	Inhibition of transcription factor hypoxia-inducible factor 1 activation
Lissoclinolide	Ascidian	Fatty acid	NCI 60 tumour cell line panel	G2/M cell cycle arrest
Neoamphimedine	Sponge	Alkaloid	HA and HU tumour cell lines	Induction of topoisomerase IIα-mediated catenation of DNA
Peloruside A	Sponge	Macrolide	HA and HU tumour cell lines	Tubulin binding site different from paclitaxel
Smenospongorine	Sponge	Seskviterpene	HU leukaemia cell line	Induced differentiation, haemoglobin production, glycophorin A and p21 expression

Secondary metabolites of marine actinomycetes with anticancer, antimicrobial and other bioactivities

Actinomycetes are economically and biotechnologically most valuable prokaryotes. They are responsible for the production of about a half of the discovered bioactive secondary metabolites (Berdy, 2008), notably antibiotics (Berdy, 2005, Strohl, 2004), antitumor compounds (Cragg, et al., 2005), immunosuppressive agents (Mann, 2001) and enzymes (Oldfield et al., 1998). Because of the excellent track record of actinomycetes in this regard, a significant effort has been focused on the successful isolation of novel actinomycetes from terrestrial sources for drug screening programs in the past fifty years. Recently, the rate of discovery of new compounds from terrestrial actinomycetes has decreased, whereas the rate of re-isolation of known compounds has increased. Thus, it is crucial for new groups of actinomycetes from unexplored or underexploited habitats to be pursued as sources of novel bioactive secondary metabolites. Although the diversity of life in the terrestrial environment is extraordinary, the greatest biodiversity is localized in oceans (Donia and Hamann, 2003). More than 70% of our planet's surface is covered by oceans and life on Earth originated from the sea. The experts estimate, that in some marine ecosystems, such as deep sea floor and coral reefs, the biological diversity is higher than in tropical rainforests (Haefner, 2003). As marine environmental conditions are extremely different from terrestrial ones, it is surmised that marine actinomycetes have different characteristics from those of terrestrial counterparts and, therefore, might produce different types of bioactive compounds. The living conditions to which marine actinomycetes had to adapt during the evolution range from extremely high pressure (with a maximum of ~1100 atmospheres) and anaerobic conditions at temperatures just below 0 °C on the deep sea floor, to high acidic conditions (pH as low as 2.8) and temperatures of over 100 °C near hydrothermal vents at the mid-ocean ridges. It is likely that this is reflected in the genetic and metabolic diversity of marine actinomycetes, which remains largely unknown. Indeed, the marine environment is a virtually untapped source of novel actinomycete diversity (Bull et al., 2005, Stach, et al., 2003) and, therefore, of new metabolites as well (Jensen, et al., 2005, Fiedler et al., 2005, Magarvey et al., 2004). However, the distribution of actinomycetes in the sea is largely unexplored and the presence of indigenous marine actinomycetes in oceans remains elusive. This is partly due to the lack of effort spent in exploring marine actinomycetes, whereas terrestrial actinomycetes have been, until recently, a successful source of novel bioactive metabolites. Furthermore,

scepticism regarding the existence of indigenous populations of marine actinomycetes arises from the fact that the terrestrial bacteria produce resistant spores that are known to be transported from land into sea, where they can remain available but dormant for many years (Bull et al., 2000). Thus, it has been frequently assumed that actinomycetes isolated from marine samples are merely of terrestrial origin (Udwary et al., 2007).

Actinomycetes are an exceptionally prolific source of secondary metabolites, accounting for more than a half of all microbial antibiotics discovered so far (Berdy, 2005). Remarkably, the vast majority of these compounds are derived from the single actinomycete genus *Streptomyces*, raising the intriguing possibility that additional chemically prolific taxa await their discovery. Further incentive to explore actinomycetes as a source of novel secondary metabolites comes from the genome sequences of *Streptomyces coelicolor* (Bentley et al., 2002) and *Streptomyces avermitilis* (Omura et al., 2001), both of which revealed many unanticipated biosynthetic gene clusters, thus demonstrating that even well studied taxa have the potential of yielding new metabolites. Such genomic-based information has been used not only to predict the chemical structures of previously unobserved metabolites but also in order to develop fermentation methods that enhance their production (Lautru et al., 2005, Song et al., 2006, Bok et al., 2006, Gross et al., 2007). Bioinformatics-based approaches to natural product discovery have also been used successfully at the industrial level, where genome scanning has led to the discovery of significant new chemical entities (Zazopoulos et al., 2003, McAlpine et al., 2005). These methods have the great potential of eliminating the redundant isolation of previously described compounds while allowing detailed fermentation studies or molecular cloning experiments to be focused on strains that possess a high probability of producing new chemical structures.

Genomics has already been particularly useful to microbial natural products studies because actinomycete secondary metabolites such as polyketides, non-ribosomal peptides, and hybrids thereof are often biosynthesized by large, multifunctional synthases that in an assembly line process sequentially assemble small carboxylic acid and amino acid building blocks into their products (Fischbach a Walsh, 2006). The biosynthetic genes responsible for the production of these metabolites are almost invariably tightly packaged into operon-like clusters that include regulatory elements and resistance mechanisms (Martin, 1992). In the case of modular polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) systems, the repetitive domain structures associated with these mega-synthases generally

follow a co-linearity rule (Staunton and Weissman, 2001), that, when combined with bioinformatics and biosynthetic precedence, can be used to predict the chemical structures of new polyketide- and peptide-based metabolites. Marine-derived actinomycetes have become a focus in the search for novel secondary metabolites (Fenical and Jensen, 2006). Among the strains cultured from marine samples is the genus *Salinispora*, which was recently described as the first seawater-requiring marine actinomycete (Maldonado et al., 2005). This genus is widely distributed in tropical and subtropical ocean sediments and currently comprises the two formally described species, *Salinispora tropica* and *Salinispora arenicola*, and a third species for which the name *Salinispora pacifica* has been proposed (Jensen and MafInas, 2006). These actinomycetes are proving to be an exceptionally rich source of structurally diverse secondary metabolites, which are produced in species-specific patterns (Jensen et al., 2007). In the case of *S. tropica*, the compounds observed to date from this bacterium include the potent proteasome inhibitor salinosporamide A (Feling et al., 2003), which is currently in phase I human clinical trials for the treatment of cancer; and unprecedented halogenated macrolides - sporolides A and B (Buchanan et al., 2005). Although the exploitation of marine actinomycetes as a source for discovery of novel secondary metabolites is at an early stage, numerous novel metabolites have been isolated in the past few years. Table 3 shows some examples of novel secondary metabolites isolated from marine actinomycetes in recent years.

Actinomycetes can be isolated from soil and marine sediments. Since collecting soil is relatively inexpensive, much is known about the distribution and abundance of terrestrial actinomycetes. Although soils have been screened by the pharmaceutical industry for about 50 years, only a miniscule fraction of the surface of the globe has been sampled, and only a small fraction of actinomycete taxons have been discovered (Baltz 2005, Baltz 2007). The use of specific enrichments and selections can reduce the burden of screening by avoiding common actinomycetes, and can be coupled with high-throughput fermentation, an approach that holds much promise, but is receiving little attention.

Table 3 Novel bioactive metabolites produced by marine actinomycetes (Lam, 2006)

Compound	Source	Activity
Abyssomicins	Verrucosispora sp.	Antibacterial
Aureoverticillactam	Streptomyces aureoverticillatus	Anticancer
Bonactin	Streptomyces sp.	Antibacterial; antifungal
Caprolactones	Streptomyces sp.	Anticancer
Chandrananimycins	Actinomadura sp.	Antibacterial; anticancer; antifungal
Chinikomycins	Streptomyces sp.	Anticancer
Chloro-dihydroquinones	Novel actinomycete	Antibacterial; anticancer
Diazepinomicin (ECO-4601)	Micromonospora sp.	Antibacterial; anticancer; anti-inflammatory
3,6-disubstituted indoles	Streptomyces sp.	Anticancer
Frigocyclinone	Streptomyces griseus	Antibacterial
Glaciapyrroles	Streptomyces sp.	Antibacterial
Gutingimycin	Streptomyces sp.	Antibacterial
Helquinoline	Janibacter limosus	Antibacterial
Himalomycins	Streptomyces sp.	Antibacterial
IB-00208	Actinomadura sp.	Anticancer
Komodoquinone A	Streptomyces sp.	Neuritogenic activity
Lajollamycin	Streptomyces nodosus	Antibacterial
Marinomycins	'Marinispora'	Antibacterial; anticancer
Mechercharmycins	Thermoactinomyces sp.	Anticancer
MKN-349A	Nocardiopsis sp.	Unknown biological activity
Trioxacarcins	Streptomyces sp.	Antibacterial; anticancer; antimalarial

Conclusion

Without a doubt, new advances in biotechnological methods focused on improving the search for medicines are going to be observed in the near future. We should mention among these, the exploitation of new habitats and the progress in the cultivation of micro-organisms, including symbionts. Development of new screening strategies, the investigation of mechanisms of action and the establishment of the bases are expected to expand the

production of new anticancer drugs, with possible contribution of new genetic techniques and the optimizing of corresponding biosynthetic pathway(s).

We could say that the originality and the variety of structures with marine origin discovered during the last 30 years, regarding their potent biological activities represent very attractive compounds, not only applied directly as medicines, but also providing possible prototypes for synthetic modifications. The search for the pharmacological targets as well as the synthesis of modified natural compounds will keep on moving biotechnologists and chemists in the process of new drugs' discovery.

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