Anticancer Potential of Compounds from the Brazilian Blue Amazon

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Key words
Brazilian Economic Exclusive Zone, cytotoxicity, marine natural products, marine pharmacology, mechanisms of action

received June 16, 2020
accepted after revision September 1, 2020
published online November 3, 2020

Bibliography
Planta Med 2021; 87: 49–70
DOI 10.1055/a-1257-8402
ISSN 0032-0943

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

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ABSTRACT

"Blue Amazon" is used to designate the Brazilian Economic Exclusive Zone, which covers an area comparable in size to that of its *green* counterpart. Indeed, Brazil flaunts a coastline spanning 8000 km through tropical and temperate regions and hosting part of the organisms accredited for the country's megadiversity status. Still, biodiversity may be expressed at different scales of organization; besides species inventory, genetic characteristics of living beings and metabolic expression of their genes meet some of these other layers. These metabolites produced by terrestrial creatures traditionally and lately added to by those from marine organisms are recognized for their pharmaceutical value, since over 50% of small molecule-based medicines are related to natural products. Nonetheless, Brazil gives a modest contribution to the field of pharmacology and even less when considering marine pharmacology, which still lacks comprehensive in-depth assessments toward the bioactivity of marine compounds so far. Therefore, this review examined the last 40 years of Brazilian natural products research, focusing on molecules that evidenced anticancer potential–which represents ~ 15% of marine natural products isolated from Brazilian species. This review discusses the most promising compounds isolated from sponges, cnidarians, ascidians, and microbes in terms of their molecular targets and mechanisms of action. Wrapping up, the review delivers an outlook on the challenges that stand against developing groundbreaking natural products research in Brazil and on a means of surpassing these matters.

Introduction

Brazil holds one of the largest coastlines for a country in the world, with an extension of 8000 km crossing tropical and temperate regions [1]. Despite the efforts of global inventory programs on marine biodiversity, like the Census of Marine Life, it is estimated that over 90% of the species found in the oceans lack proper description [2, 3], and Brazil is no exception. During the past 3 decades, an increasing number of programs aimed at informing on Brazilian biodiversity have emerged, including the Program for Assessing the Sustainable Potential of Living Resources of the Exclusive Economic Zone (REVIZEE; https://www.mma.gov.br/biodiversidade/

ABBREVIATIONS

ABCG2	ABC transporter G family member 2
BCRP	breast cancer resistance protein
BGC	biosynthetic gene clusters
BRL3A	rat liver epithelial cells
DS	disulfide dermatan sulfates
GAGs	sulfated glycosaminoglycans
GNPS	Global Natural Product Social Molecular Networking
HS	heparan sulfate
IAP	inhibitory apoptosis protein
KS	keto synthases
LAAs	lipidic alpha-amino acids
NOTCH2	neurogenic locus notch homolog protein 2
NSCLC	nonsmall cell lung cancer
PKS	polyketide synthase
PRDX1	peroxiredoxin-1
ROS	reactive oxidative species
SAR	structure-activity relationships
SPSPA	Saint Peter and Saint Paul Archipelago
US-FDA	United States Food and Drug Administration

biodiversidade-aquatica/zona-costeira-e-marinha/programa-revizee) launched in 1994; the FAPESP Research Program on Biodiversity Characterization, Conservation, Restoration, and Sustainable Use (BIOTA; http://www.biota.org.br/), which celebrated 20 y in 2019; and, more recently, the National System of Research on Biodiversity (SISBIOTA; http://cnpq.br/sisbiota/apresentacao/). All these efforts contributed to the description of several unknown and endemic species; however, despite all these efforts, Brazil is still a long way from a proper characterization of its biodiversity and ecosystem functioning [4, 5]. Good examples of unique Brazilian marine ecosystems are the reef system at the Amazon River mouth dominated by large sponges [6] and the Abrolhos Bank Reef, located in the south of Bahia state, housing the largest and richest reefs of the South Atlantic, including 6 endemic coral species [7, 8].

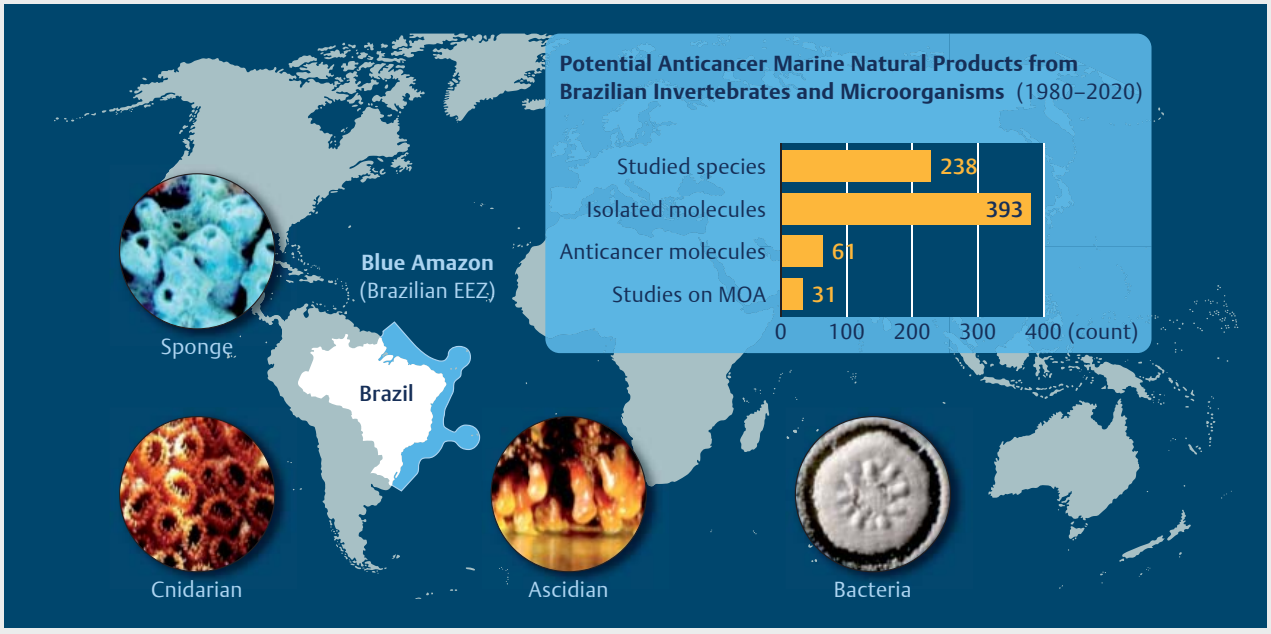
While recognized as a megadiverse country, Brazil has a timid contribution to the field of marine biotechnology. A recent review discussed a countrywide initiative launched by the Brazilian Government to create the National Research Network in Marine Biotechnology (BiotecMar, www.biotecmar.sage.coppe.ufrj.br) by uniting research groups with different expertise but a common aim: developing the marine bioeconomy through innovative research [9]. Research in this area, especially related to natural marine products, which was initiated in the 1960s, yielded a significant increase in the number and impact of the findings reported, especially over the past 2 decades. Still, it remains mainly restricted to universities and research institutes, and the results are still in early stages when considering product generation [10–12].

From a pharmacological perspective, chemical diversity opens up a multitude of possibilities, enthusing the discovery and development of drugs to treat all sorts of ailments. Indeed, the value of natural products as pharmaceuticals is extensively recognized, since over 50 % of all small molecule-based medicines are, to some degree, related to a natural product [13]. Especially for anticancer therapy, in recent years, compounds from marine sources have already shown a great impact, as tackling such disease is the purpose of 8 out of the 13 approved marine-based drugs. Each of these medicines conveys remarkable courses of development, which have, moreover, instructed scientists on overcoming issues related to supply and toxicity [14].

Bioactivity-guided fractionation protocols that routinely lead to the isolation of novel compounds from crude chemical extracts have expanded the chemical space of natural products [15]. Although the rates of discovery of new scaffolds are not increasing as predicted by theoretical estimates, it is generally accepted that there is still room for isolation of a vast number of new molecules with interesting biological properties, especially when prioritizing the access of unique genetic resources and using innovative strategies [16]. In this sense, Brazilian biodiversity is an attractive underexplored source where the impact of high-quality groundbreaking research can potentially reveal a myriad of new bioactive molecules. Indeed, the number of isolated compounds has steadily increased over the last 2 decades, but it is quite obvious that biological resources are still underexplored, especially with regard to drug development [11, 12]. Microorganisms, including fungi, cyanobacteria, and bacteria, have seduced the natural products community worldwide and in Brazil alike, receiving significant attention as the reputed true producers of most bioactive compounds isolated from marine invertebrates [12].

Studies with microorganisms brought not only unique metabolites and the means for a sustainable production of compounds but also the possibility to mine genomes as a worthy alternative path for drug discovery [17]. Genome mining techniques uncover a diversity of “orphan” or “silent” routes that are a majority of BGCs from which the expected chemistry is untraceable by traditional fractionation chemical protocols, or that expression is downregulated under the growth conditions applied in the laboratory for bacterial cultivation [18–20]. Nevertheless, the isolation of the whole chemical universe predicted by genome mining is probably not an attainable endeavor, while best attempts to understand this complex chemical diversity are through combining the DNA-centered tools with other omics, especially those on the very end of the information chain: the metabolomics [21]. In this case, different strategies may be outlined targeting the largest possible number of substances within the same analytical technique, while keeping in mind that a majority of these compounds remain to be identified [21]. Such rationale is widely used to identify or indicate one or a group of metabolites related to a specific effect, such as an organism’s response to an environmental condition [22]. Nonetheless, integrative approaches of omics data are gaining more attention as a powerful ally to optimize efforts in the identification of potential drug candidates in complex natural matrices.

There are few comprehensive reviews on marine natural products isolated from Brazilian organisms highlighting chemical diversity; however, those merely list the pharmacological activities of isolated compounds [10–12, 23]. In this context, to the best of our knowledge, there is no previous publication addressing marine compounds with anticancer potential in terms of molecular



▶ **Fig. 1** World map highlighting Brazil and the Brazilian Economic Exclusive Zone (EEZ). The bar graph inset depicts a numerical overview of the achievements in the field of marine natural products in Brazil, narrowing down to those molecules that evidenced anticancer potential and, moreover, have been studied for their mechanism of action.

targets and mechanisms of action. Therefore, a literature search was conducted, initially considering reports on isolated molecules from invertebrates and microorganisms collected in the Brazilian Economic Exclusive Zone, which was then followed by identifying those studies describing anticancer potential and mechanism of action of those compounds. With this in mind, the most promising compounds isolated from sponges, cnidarians, ascidians, and microbes, whether in association with invertebrates or recovered from marine sediments, were herein considered. Once omics approaches were applied, whether to advance the identification of potential anticancer compounds in a complex mixture or to improve the productivity of the active molecule based on the knowledge of biosynthetic gene clusters, a discussion of these results was included.

As shown in ▶ **Fig. 1**, molecules that evidenced anticancer potential, including cytotoxic and antimetastatic activities, represent approximately 15% of the total number of marine natural products isolated from Brazilian species, while half of these compounds have been studied to some degree regarding their mechanism of action. These reports have revealed a variety of phenotypic events and cellular targets (▶ **Table 1**), including some novel and relevant achievements to the anticancer drug discovery body of knowledge, which can be appreciated in the following section.

The last section of this review explores the main challenges that stand in front of innovative research in natural products in Brazil and the perspectives to overcome issues particular to the Brazilian scientific community as well as common issues worldwide. These encompass avoidance of natural product redundancy and enhancement of the biotechnological value of somewhat "old" molecules that lack sufficient biological characterization.

Exploring Marine Invertebrates and Associated Microbiota from Brazilian Oceans as Sources of Potential Anticancer Compounds

Sponges

Sponges are sessile invertebrates that belong to the phylum Porifera and the most primitive multicellular animals to present efficient defense mechanisms against predators [24]. The competition for space with other sessile and predatory organisms is believed to have been one of the factors for natural selection of the means to produce a wide variety of secondary metabolites [25]. For this reason, sponges appear as very promising marine organisms in the search for bioactive compounds with anticancer, antiviral, anti-inflammatory, antibiotic, and other biological proprieties [26], and the Porifera account for the most studied animal taxa in marine drug discovery [11].

In the early 1950s, the biomedical interest in sponges was aroused by an important discovery carried out by Yale researchers Werner Bergmann and Robert Feeney, credited by many authors as the debut of the field of marine natural products: the arabinonucleosides from the marine sponge *Tectitethia crypta* (de Laubenfels, 1949) (Tethydae). These nucleosides were the basis for the synthesis of the first drug of marine origin with anticancer activity [27]. Launched in 1969, cytarabine (also known as Ara-C) is a chemotherapy medication currently employed in the routine treatment of patients with hematological cancers, such as leukemia and lymphoma [28]. The next sponge-derived anticancer marine drug would then be approved in 2010. Eribulin

▶ **Table 1** Bioactivity and mechanism of action of compounds with anticancer potential obtained from marine organisms collected along the Brazilian coast and oceanic islands.

Isolated compound	Source	Collection site	Studies on bioactivity	Reference
Sponges				
Haliclonacyclamine E, arenosclerins (A, B and C)	*Arenosclera brasiliensis* Muricy & Ribeiro, 1999 (callyspongiidae)	João Fernandinho Beach, Búzios, RJ	Cytotoxicity against HL-60, L929, B16, and U138 cells. Cytoskeleton alterations.	[40, 41]
Geodiamolides (A, B, H, and I)	*Geodia corticostylifera* Hajdu, Muricy, Custodio, Russo & Peixinho, 1992 (Geodiidae)	Toque-Toque Island, São Sebastião, SP	Antiproliferative activity against T47D and MCF7 cells. Cytoskeleton alterations on actin backbone. Geodiamolide H: decreased migration and invasion of Hs578T cells probably due to modifications in actin cytoskeleton. Nontumoral epithelial breast cell line (MCF-10A) remained unaltered after treatment.	[46, 47]
8bβ-hydroxyptilocaulin Ptilocaulin	*Monanchora arbuscula* (Duchassaing &Michelotti, 1864) (Crambeidae)	Marine State Park of Pedra da Risca do Meio, Fortaleza, CE	Cytotoxicity against HL-60, MDA-MB-435, HCT-8, and SF-295 cells. Apoptosis induction.	[49, 50]
Haliclonacyclamine F, arenosclerins (D and E), madangamine F, ingenamine G	*Pachychalina alcaloidifera* Pinheiro, Berlinck & Hajdu, 2005 (Niphatidae)	São Sebastião Channel, SP	Cytotoxic activities against SF 295, MDA-MB-435, HCT-8, and HL-60 cells.	[53]
Two dihydrofurans (6-desmethyl-6-ethylspongosoritin A and Spongosoritin A) and 3 6-membered peroxides (plakortides)	*Plakortis angulospiculatus* (Carter, 1879) (Plakinidae)	National Marine Park of Fernando de Noronha and Tamandaré, PE	Cytotoxicity against HCT-116 and PC-3M cell lines. Cell cycle modifications depending on structural characteristics: dihydrofurans induce G_0/G_1 arrest and 6-membered peroxides (plakortides) deliver a G_2/M arrest.	[49, 56]
Cnidarians and associated microorganisms				
18-acetoxipregna-1,4,20-trien-3-one	*Carijoa riisei* (Duchassaing & Michelotti, 1860) (Clavulariidae)	São Sebastião, SP	Cytotoxicity against SF-295, MDA-MB-435, HCT-8, and HL-60.	[49]
3-*O*-methyl-amphidinolide P	*Stragulum bicolor* van Ofwegen & Haddad, 2011 (Clavulariidae)	Caponga Beach, CE	Cytotoxicity against HCT 116.	[66]
Punicinols (A, B, C, D, and E)	*Leptogorgia punicea* (Milne Edwards & Haime, 1857) (Gorgoniidae)	Aranhas Island, SC	Cytotoxic activity against A549. A synergistic effect of these compounds with paclitaxel was observed.	[67]
Bc2	*Bunodosoma caissarum* Corrêa in Belém, 1987 (Actiniidae)	Florianópolis, SC	Cytotoxicity against U87 and A172, either wild type or p53 mutant. Pore formation on cell membrane. Cytotoxicity occurs potentiated when combined with approved chemotherapeutic agents (AraC, doxorubicin, and vincristine).	[68, 69]
6β-Carboxyl- 24(*R*)-(8 → 6)-*abeo*-ergostan-3β,5β-diol and 2 lipidic alpha-amino acids (LAAs) in mixture	*Palythoa variabilis* (Duerden, 1898) (Sphenopidae)	Pedra Rachada Beach, Paracuru, CE	Ergostan: cytotoxicity against HCT 116. LAAs: cytotoxicity against SF-295, HCT-8, and HL-60. Apoptosis induction on HL-60 cells.	[73, 75, 77]
Chromomycins (A_5, A_6, A_7, and A_8)	*Streptomyces* sp. BRA-384 isolated from *Palythoa caribaeorum* Duchassaing & Michelotti, 1860 (Sphenopidae)	Pedra Rachada Beach, Paracuru, CE	Cytotoxicity against 501mel and WM293A, RD, RH30, MCF-7, HCT 116, and PC-3M. Chromomycins A_5 and A_6 bind to TBX2 transcription factor.	[76, 78]
Ascidians and associated microorganisms				
Sebastianines (A and B)	*Cystodytes dellechiajei* (Della Valle, 1877) Polycitoridae)	São Sebastião Channel, São Sebastião, SP	Cytotoxicity against HCT 116 $p53^{+/+}$, HCT 116 $p53^{-/-}$, HCT 116 $p21^{+/+}$, and HCT 116 $p21^{-/-}$ cells. Indication of a p53-dependent mechanism of cell death.	[94]

cont.

▶ **Table 1** *Continued*

Isolated compound	Source	Collection site	Studies on bioactivity	Reference
Granulatimide Isogranulatimide	*Didemnum granulatum* Tokioka, 1954 (Didemnidae)	São Sebastião Channel, São Sebastião, SP Araçá Beach, São Sebastião, SP Arvoredo Marine Biological Reserve, Florianópolis, SC	G_2-checkpoint arrest in MCF-7 mp53 cells. Inhibition of kinases Chk1 and Cdk1.	[95, 96]
Mixture of methyl esters (methyl myristate, methyl palmitate, and methyl stearate) and mixture of glyceryl ethers [1,2-propanediol, 3-(heptadecyloxy), batyl alcohol, and 1,2- propanediol, 3-[(methyl-octadecyl)oxy]]	*Didemnum psammatodes* (Sluiter, 1895) (Didemnidae)	Flexeiras Beach, Trairi, CE	Cytotoxicity against leukemia cell lines HL-60, Molt-4, CEM, and K562. Indication of induction of programmed and accidental cell death on HL-60 cell line.	[113]
Mixture of 2-hydroxy-7-oxostaurosporine and 3-hydroxy-7-oxostaurosporine	*Eudistoma vannamei* Millar, 1977 (Polycitoridae)	Taíba Beach, São Gonçalo do Amarante, CE Ponta Grossa Beach, Icapuí, CE	Cytotoxicity against HL-60, Molt-4, Jurkat, K562, HCT-8, MDA MB-435, and SF-295 cell lines. Cytotoxicity against PBMC. Induction of G_2 arrest in HL-60 cells.	[103]
Penicillic acid	*Aspergillus* sp. EV-10 associated to *E. vannamei* Millar, 1977 (Polycitoridae)	Taíba Beach, São Gonçalo do Amarante, CE	Cytotoxicity against HCT-8 and MDA-MB-435 cell lines.	[108]
Antracyclinones (4,6,11-trihydroxy-9-propyltetracene-5,12-dione and 10β-carbomethoxy-7,8,9,10-tetrahydro-4,6,7α,9α,11-pentahydroxy-9-propyltetracene-5,12-dione)	*Micromonospora* sp. BRA-006 associated to *E. vannamei* Millar, 1977 (Polycitoridae)	Taíba Beach, São Gonçalo do Amarante, CE	Cytotoxicity against HCT-8 cell line.	[111]
Dithiolpyrrolone	*Streptomyces* sp. BRA-010 associated to *E. vannamei* Millar, 1977(Polycitoridae)	Taíba Beach, São Gonçalo do Amarante, CE	Cytotoxicity against HCT 116, OVCAR-8, NCI-H358, PC-3M, HL-60, and SF-295. Induction of polynucleated cells, inhibition of cytokinesis, and apoptosis in PC-3M cells. Indication of impairment of cytokinesis motor proteins.	[112]
Tamandarins (A and B)	*Didemnum* sp.	Mamucabinhas Beach, Tamandaré, PE	Inhibition of colony formation of BX-PC3, DU145, and UMSCC10b cells lines. Inhibition of protein synthesis. Indication of a didemnin-like mechanism of action.	[114]
Dermatan sulfate [(IdoA2-GalNAc)n, O-sulfated at C2 of the IdoA and at C4 of the GalNAc]	*Styela plicata* (Lesueur, 1823) (Styelidae)	Praia da Urca, Rio de Janeiro, RJ	Inhibition of LS180 cells adhesion to P-selectin *in vitro* and *in vivo*. Attenuation of lung metastasis in mice injected with MC-38 GFP or B16-BL6 cells. Indication of antimetastatic effect dependent on P-selectin.	[115]
Dermatan sulfate [(IdoA2-GalNAc)*n*, O-sulfated at C2 of the IdoA and at C6 of the GalNAc] and heparan sulfate [(αGlcN−αIdoA-βGlcA)*n*, sulfated at C2 of the IdoA and β-GlcA and at C6 of the N-acetylated α-GlcN]	*Phallusia nigra* Savigny, 1816 (Ascidiidae)	Angra dos Reis, RJ	Dermatan sulfate: inhibition of LS180 cells adhesion to P-selectin *in vitro* and *in vivo*. Attenuation of lung metastasis in mice injected with MC-38 GFP or B16-BL6 cells. Indication of antimetastatic effect dependent on P-selectin. Heparan sulfate: inhibition of LS180 cells adhesion to P-selectin.	[115, 118]
Sediment-associated microorganisms				
Gliotoxin, Acetylgliotoxin G	*Dichotomomyces cejpii* BRF082	Pecém's offshore port terminal, CE	Cytotoxicity against HCT 116 cell line.	[122]
Malformins (A and C)	*Aspergillus niger* BRF074	Pecém's offshore port terminal, CE	Cytotoxicity against HCT 116 cell line.	[130]

cont.

▶ **Table 1** *Continued*

Isolated compound	Source	Collection site	Studies on bioactivity	Reference
Fumitremorgin C	*Aspergillus* sp. BRF030	Mucuripe Beach, Fortaleza, CE	Cytotoxicity against HCT 116 cell line.	[134]
Chromomycins (A_2 and A_3)	*Streptomyces* sp. BRA-090	Paracuru Beach, CE	Cytotoxicity against HCT 116, HL-60, OVCAR-8, PC-3M, and MALME-3M. Chromomycin A_2: autophagy induction.	[138]
Prodigiosin	*Pseudoalteromonas* sp. BRA-007	Taíba Beach, CE	Cytotoxicity against HCT-8, HL-60, MDA-MB435, and SF-295. Selective cytotoxic activity against cell lines overexpressing the tyrosine kinase receptor ErbB-2.	[139]
Nonylprodigiosin, cyclononilprodigiosin	*Actinomadura* sp. BRA-177	Saint Peter and Saint Paul Archipelago, PE	Cytotoxicity against SK-Mel-147, HCT 116, and MCF-7 cell lines.	[145]
Diketopiperazines [cyclo(L-Phe-L-Pro) and cyclo(L-Trp-L-Pro)]	*Streptomyces* sp. BRA-199	Saint Peter and Saint Paul Archipelago, PE	Cyclo(l-Phe-l-Pro): cytotoxic against HCT 116, OVCAR-8, and SF- 295 cell lines. Cyclo(l-Trp-l-Pro): cytotoxic against OVCAR-8 cell line.	[150]

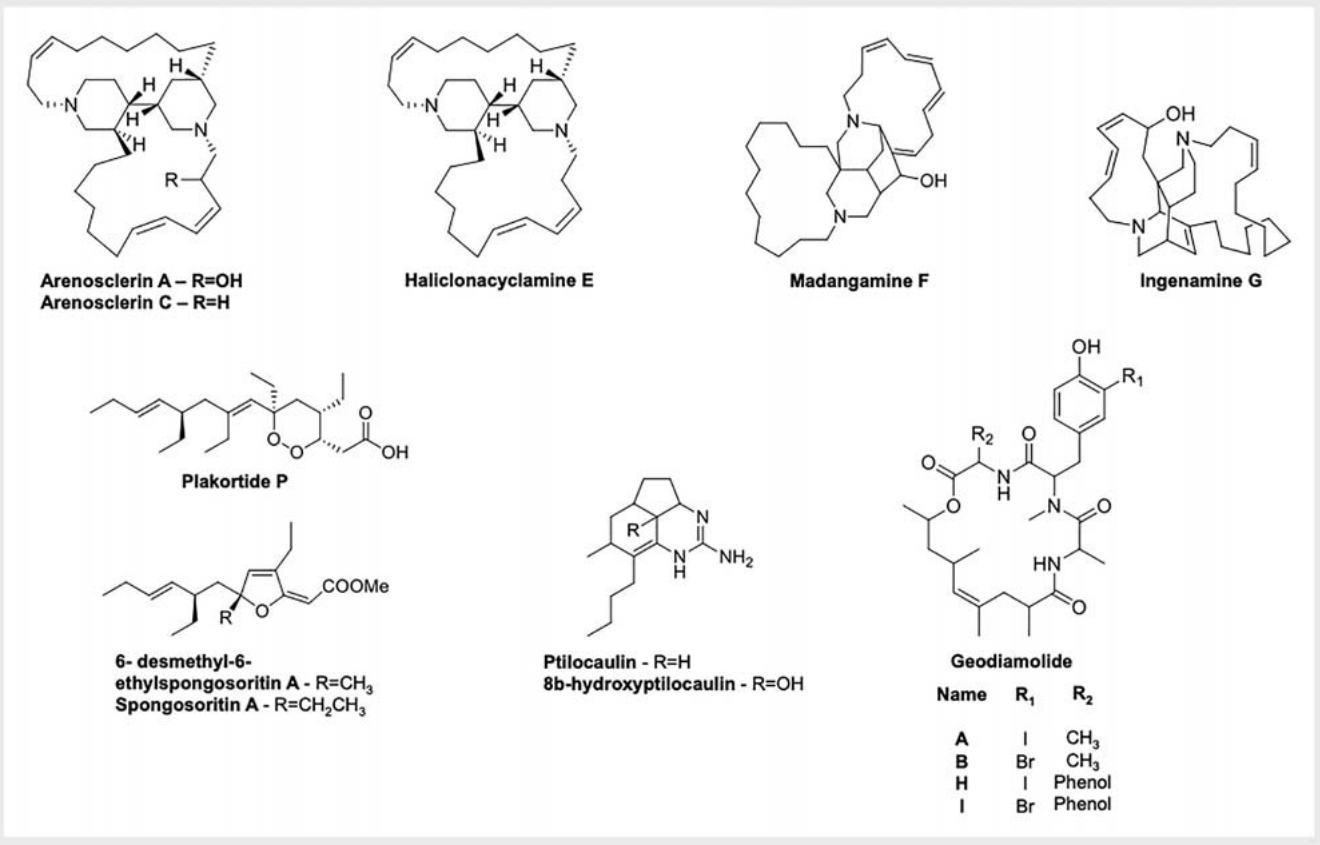
The references listed are solely of molecules with anticancer potential obtained from marine species collected in the Brazilian Economic Exclusive Zone. **Tumor cell lines origin according to tissue:** A172, glioblastoma; A549, lung; B16, melanoma; B16-BL6, melanoma; BX-PC3, pancreas; CEM, leukemia; DU145, prostate; HCT 116, colon; HCT-8, colon; HL-60, leukemia; Hs578T, triple-negative breast cancer; K562, leukemia; L929, fibrosarcoma; LS180, colon; MALME-3M, metastatic melanoma; MC-38 GFP, colon; MCF-7, breast; MDA-MB-435, melanoma; Molt-4, leukemia; OVCAR-8, ovary; PC-3M, metastatic prostate; RD, rhabdomyosarcoma; RH30, rhabdomyosarcoma; SF-295, glioblastoma; SK-Mel-147, melanoma; T47D, breast; U138, colon; U87, glioblastoma; UMSCC10b, metastasis of laryngeous squamous cells; WM293A, melanoma; 501mel, melanoma. **Nontumor cells origin according to tissue**: MCF-10A, epithelial breast; PBMC, peripheral blood mononuclear cells.

mesylate, a structurally simplified synthetic analog of the tubulin inhibitor halichondrin B, isolated from the marine sponge *Halichondria okadai* (Kadota, 1922) (Halichondriidae) by Hirata and Uemura [29], was developed into Halaven® and is currently used for the treatment of metastatic breast malignancies and inoperable liposarcoma [30]. In phase I clinical trial is E7974, a synthetic analog of the marine sponge natural product hemiasterlin that has been made available to patients with refractory solid tumors [31].

In Brazil, Porifera diversity comprises approximately 5.3% of the 8553 valid species known worldwide [32], which corresponds to 443 species, mostly from the Demospongiae class [33]. It is, however, possible that a much larger number of species are still unknown due to areas that remain completely unexplored along the Brazilian coast. The localities reported as the most biodiverse for the occurrence of sponge species are Salvador, with 72 species identified, followed by Recife (68), Potiguar Basin (65), Fernando de Noronha Archipelago (59), São Sebastião (55) and Arraial do Cabo (52) [33].

Early investigations of marine sponges as resources for biomolecules with cytotoxic activity can be attested in Berlinck and collaborators (1996). This study led to the isolation of halitoxin complex from *Amphimedon viridis* Duchassaing & Michelotti, 1964 (Niphatidae), as observed from other Haplosclerida sponges, and described different biological activities, including cytotoxicity, all related to the lytic properties of these molecules [34]. Rangel and collaborators [35] followed, assessing hemolytic, cytotoxic, and neurotoxic activities in 24 different sponge species from the southeastern Brazilian coast. The authors reported that nearly 30% of the sponge extracts tested showed moderate to strong inhibition of the development of sea urchin eggs [35]. A few years later, a screening of 40 extracts of marine sponges and ascidians evaluated their antiproliferative potential on human breast cancer cells (T47D) [36]. Seven extracts from *Amorphinopsis sp., Arenosclera brasilensis, Cystodytes dellechiajei, Cliona aff. celata, Didemnum sp., Hadromerida*, and *Scopalina ruetzleri* (Wiedenmayer, 1977) (Scopalinidae) showed antiproliferative effects with IC_{50} ≤ 30 µg/mL and produced strong effects on microtubules' organization and on the cell cycle progression of T47D human breast cancer cells [36]. Among endemic sponge genus in the Brazilian Blue Amazon with cytotoxic effects, *A. brasiliensis, Geodia corticostylifera, Monanchora arbuscula, Pachychalina alcaloidifera*, and *Plakortis angulospiculatus* have been further studied by different research groups and will be discussed here.

A. brasiliensis inhabits shallow waters in the coast of Rio de Janeiro State, Southeastern Brazil [37], and the crude extract was shown to have antimitotic proprieties in early stages of the development of sea urchin eggs, inducing anomalies at the highest tested concentrations [35]. Considering genotoxicity, this crude extract showed a potential to protect DNA from various chemically-induced damage, suggesting an antimutagenic activity [38]. Furthermore, acetone (AreAc) and ethanol (AreEt) extracts of *A. brasiliensis* were evaluated in a qualitative *Salmonella* reverse mutation test. While AreAc showed significant toxicity against test strains, AreEt revealed a protective activity against DNA lesions, agreeing with an antimutagenic effect [39]. Tetracyclic alkylpiperidine alkaloids named arenosclerins A, B, and C, as well as haliclonacyclamine E (▶ **Fig. 2**), were isolated from the extract of *A. brasiliensis* and presented cytotoxic activity against human HL-60 (leukemia), L929 (fibrosarcoma), B16 (melanoma), and



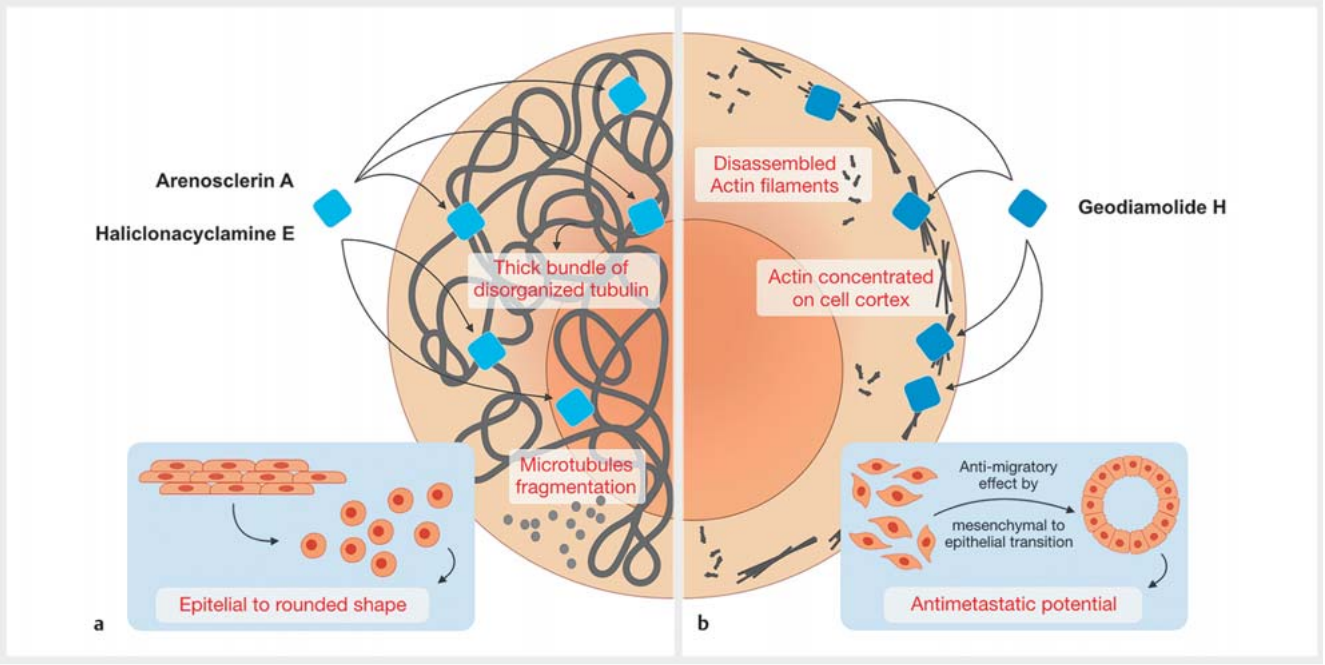
▶ **Fig. 2** Compounds with anticancer potential isolated from marine sponges from Brazilian Blue Amazon.

U138 (colon) cancer cells at concentrations between 1.5 and 7.0 µg/ml [40,41]. In T47D (breast) cancer cells, arenosclerin A and haliclonacyclamine E produced noteworthy effects against microtubule integrity and cell cycle progression, indicating these compounds may induce their cytotoxicity through disassembly of the cytoskeleton (▶ **Fig. 3a**) [36].

In the 1980s, studies with administration of radioactively-labeled subunits suggested that precursor molecules of a wide variety of cyclic alkylpiperidine alkaloids, such as the 3-alkylpyridine precursor or arenosclerins, were derived from a polyketide chain, that is, synthesized by successive addition of acetate units according to the function of enzymes of the PKS class [42]. Based on this premise, Trindade-Silva and collaborators applied massive and parallel amplicon sequencing to *A. brasiliensis*, allowing the exploration of type I PKS as well as hybrid BGCs diversity housed in its complex microbiome [43]. A phylogenetic reconstruction of 235 recovered KS contigs was performed to uncover a great diversity of type I PKS families presented in this sponge microbiome, including a novel and *A. brasiliensis* exclusive KS clade. However, such clade could not be addressed to the still undescribed arenosclerins BGC.

The crude extract from *G. corticostylifera*, a marine sponge also collected in Rio de Janeiro [44], was found to be highly toxic against sea urchin embryos, inducing cell lysis even before inhibiting cell division [35]. This effect was connected to the capacity of such extract in inducing the formation of ionic pores in the cell membrane, which also led to the release of hemoglobin from erythrocytes and depolarization of nerve and muscle membranes, leading to the death of treated mice through respiratory arrest [45]. The cyclic peptides geodiamolides A, B, H and I (▶ **Fig. 2**) isolated from *G. corticostylifera* presented antiproliferative activity against breast cancer cell lines (T47D and MCF7) through inducing actin cytoskeleton alterations. In turn, primary human fibroblasts and BRL3A were not affected following treatment with these peptides, thus suggesting selectivity of such compounds for malignant cells [46]. Geodiamolide H was additionally shown to revert the malignant phenotype of the breast carcinoma cells Hs578T, inducing polarized spheroid-like structures in a 3D environment. Moreover, this marine depsipeptide also inhibited migration and invasion of Hs578T cells, seemingly through disruption of actin cytoskeleton (▶ **Fig. 3b**), while leaving nontumor breast cells (MCF10A) unaffected [47].

M. arbuscula is a shallow-water marine sponge distributed in the Tropical Western Atlantic [48] for which the crude methanolic extract showed antibacterial and cytotoxic activities. This extract yielded a myriad of guanidine alkaloids, namely isoptilocaulin, mirabilin B, 8bβ-hydroxyptilocaulin, ptilocaulin, and a mixture of the 8β- and 8α-epimers of 8-hydroxymirabilin [49,50]. Compounds 8bβ-hydroxyptilocaulin and ptilocaulin (▶ **Fig. 2**) presented IC_{50} values in the range of 7.9 to 61.5 µM, and 5.8 to 40.0 µM, respectively, over a mini panel of human tumor cell lines. Ptilocaulin was further tested in HL-60 leukemia cells, revealing the induc-



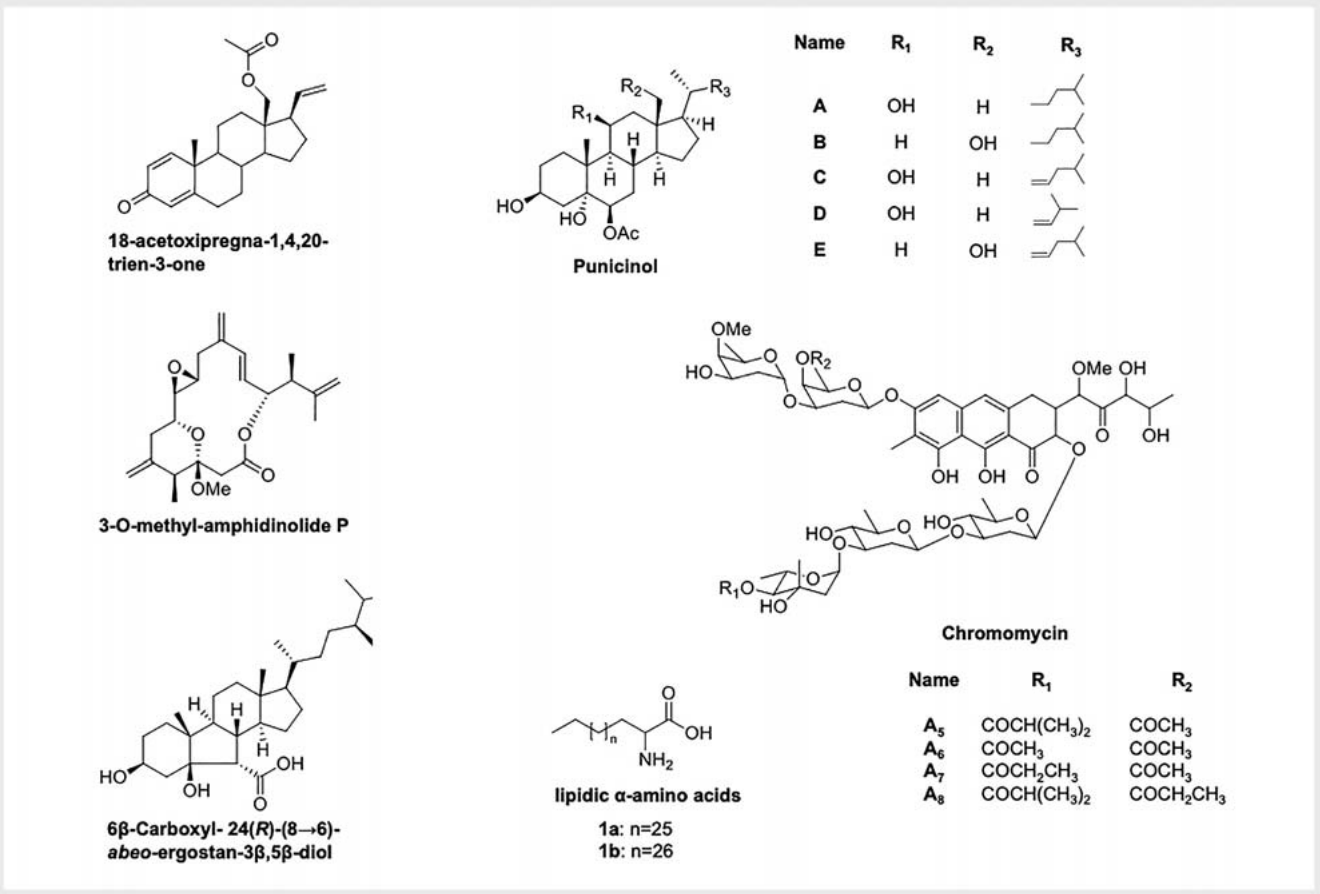
▶ **Fig. 3** Schematic model of the mechanisms of action of compounds isolated from marine sponges *Arenosclera brasiliensis* (left, **a**) and *Geodia corticostylifera* (right, **b**). Arenosclerin A and haliclonacyclamine E, isolated from the first sponge, cause tubulin disorganization and fragmentation, inducing the formation of thick bundles of tubulin and change in the epithelial cell morphology to a rounded shape. Geodiamolide H, obtained from the later species, causes accumulation of actin filaments in the cellular membrane, actin fragmentation, and mesenchymal to epithelial transition, which reduces cellular migration and antimetastatic potential.

tion of cell death by apoptosis as a possible mechanism of action for this guanidine compound [50].

P. alcaloidifera [51] is a shallow-water marine sponge from the São Sebastião channel and its environs (Tropical Southwestern Atlantic). The chemical investigation of MeOH crude extract led to the isolation of 6 new nitrogenous metabolites, including ingenamine G, as well as a mixture of new cyclostellettamines G, H, I, K, and L with the previously known cyclostellettamines A–F [52]. Four bis-piperidine alkaloids (madangamine F, [▶ **Fig. 2**], haliclonacyclamine F, and arenosclerins D and E) were further isolated from this and displayed cytotoxic activity against SF 295 (human CNS), MDA-MB435 (human breast), HCT 8 (colon), and HL60 (leukemia) cancer cell lines [53]. The most prominent alkaloid isolated from *P. alcaloidifera* was ingenamine G (▶ **Fig. 2**), which showed cytotoxicity against human proliferating lymphocytes (IC_{50} 15.0 µg/mL) and genotoxicity, inducing strand breaks on DNA, which was correlated with the mutagenic and carcinogenic activity of the molecule [54].

P. angulospiculatus was described in shallow waters of the Fernando de Noronha Archipelago and Tamandaré (Northeastern Brazilian coast, Pernambuco State) [55]. Fractionation of the crude extract afforded the isolation of 1 new polyketide, along with 5 known polyketides, which were tested for antileishmanial, antitrypanosomal, antineuroinflammatory, and cytotoxic activities [49]. Among the isolated compounds, plakortide P showed antiparasitic activity [49]. Further studies have been done combining aspects of compound isolation to understand the SAR and associated biological activity in a complex panel of natural products isolated from marine sponges in the *Plakortis* genus [56]. Therein, 3 new plakortides, along with known natural products (spongosoritin A and plakortide P, ▶ **Fig. 2**), were isolated from *P. angulospiculatus* collected off the northeast coast of Brazil and showed cytotoxic activities against HCT 116, PC-3M, and MRC-5 cell lines, with IC_{50} values ranging from 0.2 to 10 µM, and the ability to hamper different phases of the cell cycle [56]. The plakortides were divided into 2 groups according to the mode of action observed by these compounds: while dihydrofurans induced a G0/G1 arrest, 6-membered peroxides delivered a G2/M arrest and an accumulation of mitotic figures [56]

The occurrence of a rich microbiome associated with Brazilian marine sponges has been revealed through many investigations [43, 57]. Cultivation efforts have led to the isolation of a collection of 98 heterotrophic bacteria from the sponge *A. brasiliensis*, of which approximately 28% displayed antibiotic activity [58]. One strain, *Pseudovibrio denitrificans* Ab134, was further shown to produce bromotyrosine-derived alkaloids, which have been previously isolated exclusively from marine sponges [59]. Nevertheless, compounds obtained from fungal and bacterial communities associated with marine sponges have been evaluated majorly for anti-inflammatory, antibiotic, antiviral, and cytotoxic activities; however, the observed cytotoxicity on cancer cells was not very stimulating, and most of isolated compounds were further studied for antiviral properties [60–63]. Indeed, a role of microbial sponge symbionts in the production of cytotoxic compounds that can be potentially applied in anticancer therapies remains to be better studied and evaluated.



▶ **Fig. 4** Compounds with anticancer potential isolated from marine cnidarians from Brazilian Blue Amazon.

Cnidarians

Many Cnidaria species are crucial for coral reef building and balance. The animals of this Phylum live exclusively in marine environments and are among the most prolific groups of producers of cytotoxic molecules. The Blue Amazon shelters over 50 species, and nearly half of these are described as endemic to the Brazilian coast [63, 64]. However, cnidarians assessed for cytotoxic activity include only a few species belonging to the Anthozoa class, which will be described hereafter.

Two species of octocoral were studied for cytotoxicity, and their isolated compounds showed weak potency. *Carijoa riisei* from São Paulo was reported to produce the steroid 18-acetoxy-pregna-1,4,20-trien-3-one (▶ **Fig. 4**), which showed activity against the cell lines SF295 (glioblastoma), MDA-MD435 (breast cancer), HCT 8 (colon cancer), and HL60 (leukemia) [65]. The 3-*O*-methyl derivative of amphidinolide P (▶ **Fig. 4**) obtained from *Stragulum bicolor,* collected at Caponga beach, Ceará, was cytotoxic on colon cancer (HCT 116) cells [66].

A series of new polyoxygenated sterols was isolated from the gorgonian *Leptogorgia punicea* from Aranha Islands, Santa Catarina. These 5 punicinols (A–E, ▶ **Fig. 4**) depicted cytotoxicity against a lung cancer (A549) cell line. While punicinols A and B displayed moderate cytotoxic, C–E were 3 to 7 times more potent. Such a difference in bioactivity was attributed to the absence of the double bond at the side chain of later punicinols [67].

The sea anemone *Bunodosoma caissarum* from Florianopolis, on the southern Brazilian coast, was reported to produce toxin Bc2, which is cytotoxic against tumor cells [68]. Bc2 acts as a cytolysin, forming pores on the targeted cell membrane, thus producing cytotoxic and cytolytic effect. Cytolysins depict remarkable stability in a water-soluble state or as an integral membrane pore. These cytolytic toxins can induce cancer cell death alone or when associated with anticancer agents [69]. The association of subcytotoxic concentration of Bc2 with anticancer drugs potentiated the effects of chemotherapeutics such as Ara C, doxorubicin, and vincristine against glioblastoma cell lines U87 and A172 *in vitro* [68].

Zoanthids from the *Palythoa* genus and their associated bacteria are a rich source of cytotoxic molecules. Their chemically and genetically rich profiles were assessed through *P. variabilis* and *P. caribaeorum* along the Brazilian coast [70]. The MS-based metabolomics followed by GNPS [71] analysis revealed the presence of many chemical compounds, including mycosporine and related amino acid derivatives, zoanthid alkaloids, ecdysteroids, phosphatidylcholine derivatives, indole diterpenes, and sulphonoceramides. A major influence of geographical location was observed on the chemical divergences among samples when compared to

species distinction. Interestingly, analysis of the microbial community by metagenome DNA sequencing showed that *P. variabilis* hosts more alphaproteobacteria and deltaproteobacteria, whereas gammaproteobacteria preferentially associates to *P. caribaeorum*. However, no integrative analysis of metabolomics and metagenomics was performed.

Altogether, 30 compounds have been isolated from *Palythoa* species or their associated bacteria [72–75], from which 7 evidenced cytotoxic activity against tumor cells [73, 75, 76]. Two LAAs (▶ **Fig. 4**) with long alkyl chains [75] and 1 sterol, the *β*-norergostan-3*β*-5*β*-diol-6*β*-carboxyl acid (▶ **Fig. 4**) [73], isolated from *P. variabilis* displayed cytotoxicity against cancer cells *in vitro*. The results on the LAAs highlighted some interesting novelties. This was the first report on the occurrence of this group of molecules in a natural source, while alkyl chains of the isolated molecules were shown to be even longer than their typical synthetic analogs. Additionally, this was the first study on the cytotoxic activity of LAAs. IC_{50} values for the isolated compounds were found in ng/mL magnitudes against glioblastoma (SF-295), colon cancer (HCT 8), and leukemia (HL-60) cell lines. A further study compared the cytotoxicity of natural and synthetic LAAs, shedding light on their structure activity relationship [77]. This investigation revealed that cytotoxicity of these substances increases proportionally to the alkyl chain; once the naturally occurring LAAs possessed longer alkyl chains, they were, thus, more potent than any of their 14 synthetic counterparts. Finally, Wilke and collaborators (2010) described LAAs as elicitors of programmed cell death in HL-60 cells.

The actinobacteria *Streptomyces* sp. BRA384 was selected among 9 isolated strains associated to *P. caribaeorum* collected at Ceará State due to a highly cytotoxic ethanol extract against HCT 116 cancer cell line [76], from which 3 new dextrorotatory chromomycins (A_6, A_7, and A_8), along with chromomycin A_5 (CA_5) (▶ **Fig. 4**), were isolated. Chromomycins are a promising class of anticancer candidates, and all 4 chromomycins obtained were highly cytotoxic against a tumor cell line mini panel, showing IC50 values in nM range. CA_5 was the most effective one across all tested cells, displaying 10-, 200-, and 300-fold higher potency than doxorubicin on metastatic prostate cancer, metastatic melanoma, and colon cancer cells, respectively [76]. Chromomycins are typically known to bind DNA, causing inhibition of replication and transcription and further induction of programmed cell death. In addition to the DNA-binding properties, CA_5, through a target-directed approach, was shown to bind the transcription factor TBX2, which impacts the cytotoxic activity of this compound [78]. The TBX2 transcription factor is overexpressed in several types of cancer and contributes to increased cell proliferation and bypass of senescence and, therefore, has been considered a potential target for new anticancer therapies (▶ **Fig. 5**).

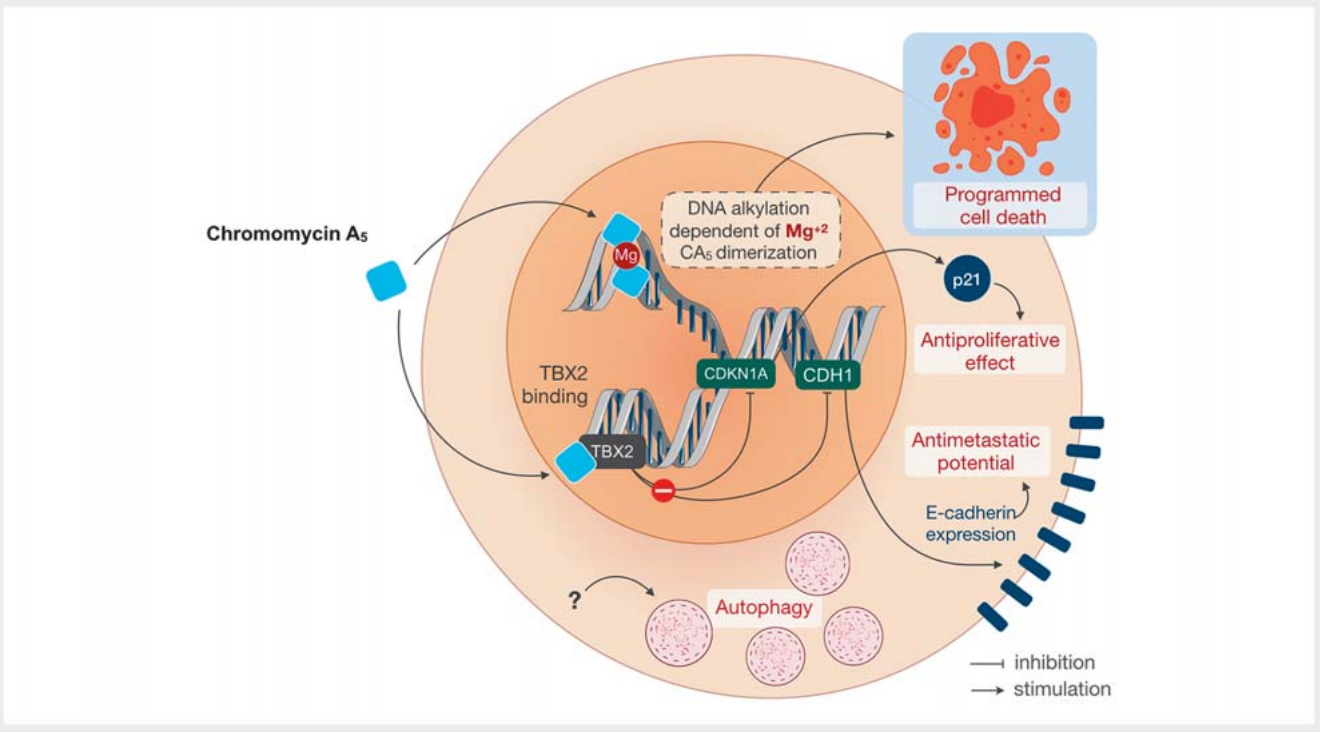
Ascidians

Ascidian typically describe the sessile, filter-feeding, tunic-wrapped invertebrates from the class Ascidiaceae, the most representative taxa for phylum Chordata, subphylum Tunicata. Therefore, these organisms may also be referred to by the broader term “tunicate”: The ascidians are a diverse and abundant group that present themselves in solitary or colonial forms widespread mainly among shallower waters in marine environments [79].

From the natural products perspective, ascidians are among the best-studied groups and evidence has shown them, and their associated microorganisms, to abound in inventive chemistry with interesting bioactivity [80, 81]. Three ascidian-sourced molecules have made it all the way to the clinics and figure among the list of drugs available for cancer treatment (reviewed by [14]). Trabectedin (ET-743), a peculiar kind of DNA alkylator, is an alkaloid obtained from *Ecteinascidia turbinata* Herdman, 1880 (Perophoridae) and the active principle of Yondelis, a chemotherapeutic agent used for treating soft tissue sarcoma since 2007 [82, 83]. Lurbinectedin, an analogue thereof, has just recently been approved for the treatment of metastatic small cell lung cancer as Zepzelca [84]. The cyclic depsipeptide plitidepsin (aplidin, dehydrodidemnin B), isolated from *Aplidium albicans* (Milne Edwards, 1841) (Polyclinidae), is a quite unusual inhibitor of protein synthesis that makes up Aplidin, approved in late 2018 for the treatment of multiple myeloma [14, 85, 86].

The Brazilian coast and islands are home to a diversity of ascidian species [87–91]. Particularly, the southeastern region of Brazil has distinguished itself within the Atlantic Ocean as one of 3 regions with peak species richness and as one in 8 regions with high endemicity regarding this group [92]. Analogously, a higher number of southeastern ascidians have been examined for the chemistry they host or surveyed for bioactivity. Seleghim et al. [93] screened 99 extracts obtained from ascidians (from which 20 were derived from then unidentified species) collected predominantly from sites along the coastline of São Paulo and Rio de Janeiro States–but also Bahia–and revealed that 60% of these extracts presented bioactivity in at least one of the 5 assays employed. Another study conducted by Prado et al. [36] assessed 16 extracts from ascidians from the southeastern Brazilian coast and reported that one obtained from *Cystodytes dellechiajei* induced *in vitro* antiproliferative effects against breast cancer cells through disruption of their cytoskeleton. Continuous studies with this extract led to the isolation of the pyridoacridine alkaloids sebastianines A and B (▶ **Fig. 6**), named as a reference to the site of species collection, the São Sebastião Channel, São Paulo [94]. These compounds displayed cytotoxicity against p53 or p21 knockout HCT 116 cells; however, cells expressing p53 were slightly more sensitive to sebastianines.

A pair of polyheteroaromatic alkaloids, granulatimide and isogranulatimide (▶ **Fig. 6**), were obtained from *Didemnun granulatum*, collected around São Sebastião, São Paulo, and were shown to induce G2-arrest in the cell cycle of breast cancer MCF-7 cells. Further studies have shown them to strongly inhibit the kinases Chk1 and Cdk1, which are important players in the G2-M transition and promising target for cancer treatment (▶ **Fig. 7**). As a matter of fact, these molecules were revealed through a rational search using a high-throughput assay directed at identifying G2 checkpoint modulators and were the first examples of this new class of cell cycle inhibitors specific for the G2 phase [95, 96], and were later shown to be stored in bladder cells in the ascidian tunic, suggesting a protective role to the host [97]. In a subsequent re-investigation of the crude extract of *D. granulatum*, yet another derivative, 6-bromogranulatimide [98] was isolated.

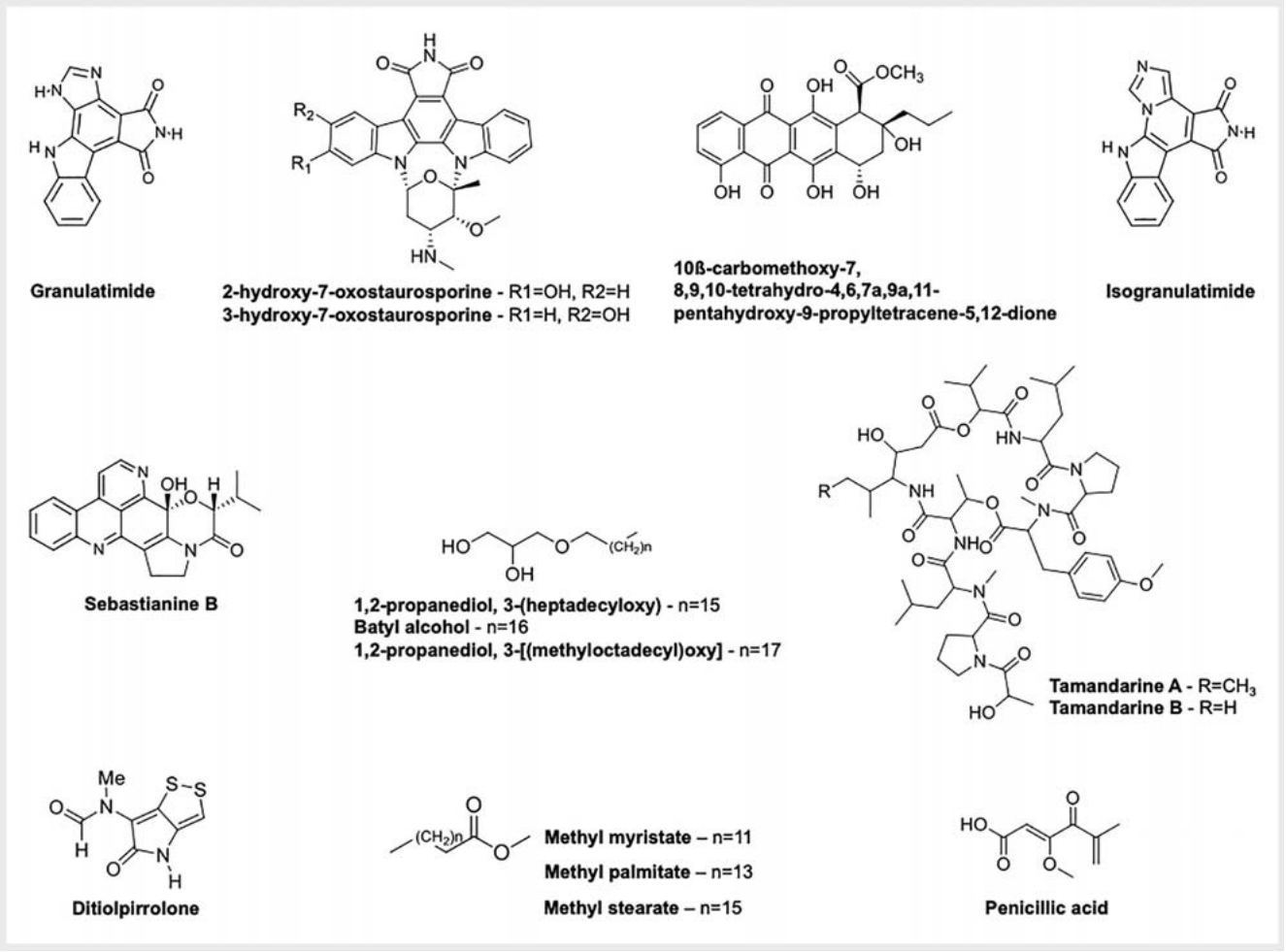


▶ **Fig. 5** Schematic model of the mechanisms of action of chromomycin A_5 (CA_5) isolated from the actinobacteria *Streptomyces* sp. BRA384 associated to the zoanthid *Palythoa caribaeorum*. CA_5 forms Mg^{+2} dependent dimers that bind to double strand DNA, thus inhibiting DNA replication and transcription and inducing programmed cell death. CA_5 inhibits the T-box 2 transcription factor (TBX2), inducing antiproliferative and antimetastatic effects by allowing the expression of cyclin-dependent kinase 1 (p21) and e-cadherin, respectively. CA_5 also induces autophagy.

Nevertheless, ascidians from the Northeast coast also revealed pharmacological potential, as shown by Jimenez et al. [99], where 6 among 10 extracts analyzed displayed some kind of cytotoxic activity, notably that obtained from *Eusdistoma vannamei*. Subsequent studies with this species, the most abundant one on the coast of Ceará State, led to the identification of purine and pyrimidine derivatives [100, 101], a tyrosine peptide derivative [102], and, remarkably, 2 novel alkaloids, 2-hydroxy-7-oxostaurosporine and 3-hydroxy-7-oxostaurosporine (▶ **Fig. 6**) [103], which presented high selectivity towards cancer cells and induced potent G2-arrest at nM concentrations in a leukemia cell line. Interestingly, Schupp and collaborators [104, 105] reported the isolation of 12 staurosporine derivatives from *E. toealensis* Millar, 1975 (Polycitoridae) collected in Micronesia. These compounds have also been shown to have, generally, antiproliferative effects against leukemia cells at a nM order [106]. Staurosporines form a group of highly cytotoxic natural compounds and synthetic derivatives structured around an indolocarbazole skeleton. The inaugural molecule, staurosporine, was isolated from the fermentation broth of soil actinobacteria *Streptomyces staurosporeus*, drafted from a screening program directed at identifying inhibitors of protein kinase C [107]. Recently, midostaurin (Rydapt), a multi-target-protein kinase inhibitor semi-synthetic derivative of staurosporine, has been approved by the USFDA to treat acute myeloid leukemia in patients carrying a specific mutation, FLT3, in combination with typical chemotherapy (US FDA, 2017).

Further investigations on *E. vannamei* looked into the associated fungi [108], leading to the isolation of penicillic acid (▶ **Fig. 6**) from the cultures of *Aspergillus* sp. EV10 strain. Bacteria associated with the ascidian [109, 110] yielded novel however moderately cytotoxic anthracyclinones (▶ **Fig. 6**) produced by the *Micromonospora* sp. BRA006 strain [111], and an anticytokenesis dithiolpyrrolone (▶ **Fig. 6**) isolated from the growth broth of the *Streptomyces* sp. BRA010 strain [112]. The latter compound, differently from most natural products that prevent cytokinesis, does not act on tubulin but seemingly on motor proteins that initiate this process (▶ **Fig. 8**), thus disclosing a chemical scaffold with a rather uncommon but assuring mode of action to be considered in the anticancer drug discovery trail.

Another study with *Didemnum* genus identified 14 compounds from the ethanolic extract of *D. psamatodes* collected at the coast of Ceará, among which a mixture of 3 methyl esters (methyl myristate, methyl palmitate, and methyl stearate, ▶ **Fig. 6**) and the mixture of 3 glyceryl ethers–(1,2-propanediol, 3-(heptadecyloxy), batyl alcohol, and 1,2- propanediol, 3-[(methyloctadecyl) oxy] (▶ **Fig. 6**)–were moderately cytotoxic against 4 leukemia cell lines. Additionally, inhibition of DNA synthesis and elicitation of programmed and accidental cell death by the methyl esters on HL-60 cells was observed [113]. Furthermore, tamandarins A and B (▶ **Fig. 6**), cyclic depsipeptides that bear great structural similarity to the didemnins and are thus suggested to have a similar mechanism of action, have been isolated from a *Didemnum* sp. collected in Tamandaré, on the coast of Pernambuco, also in the

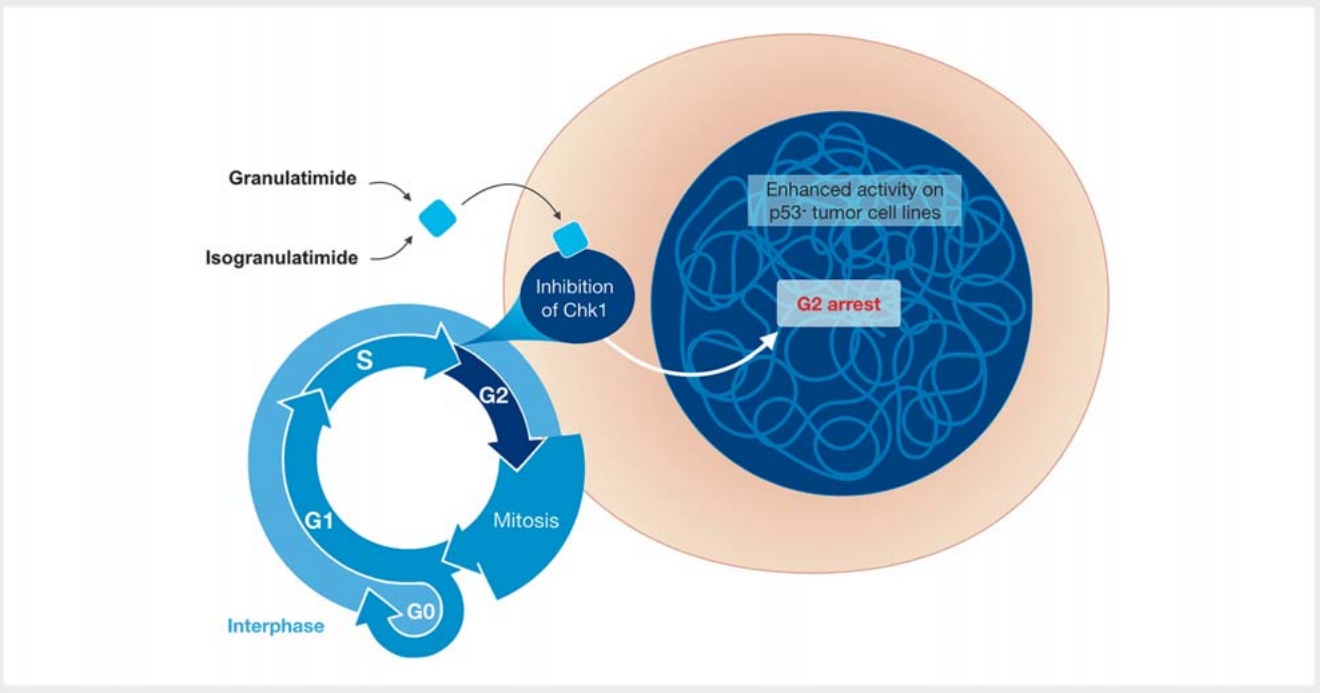


▶ **Fig. 6** Compounds with anticancer potential isolated from ascidians from Brazilian Blue Amazon.

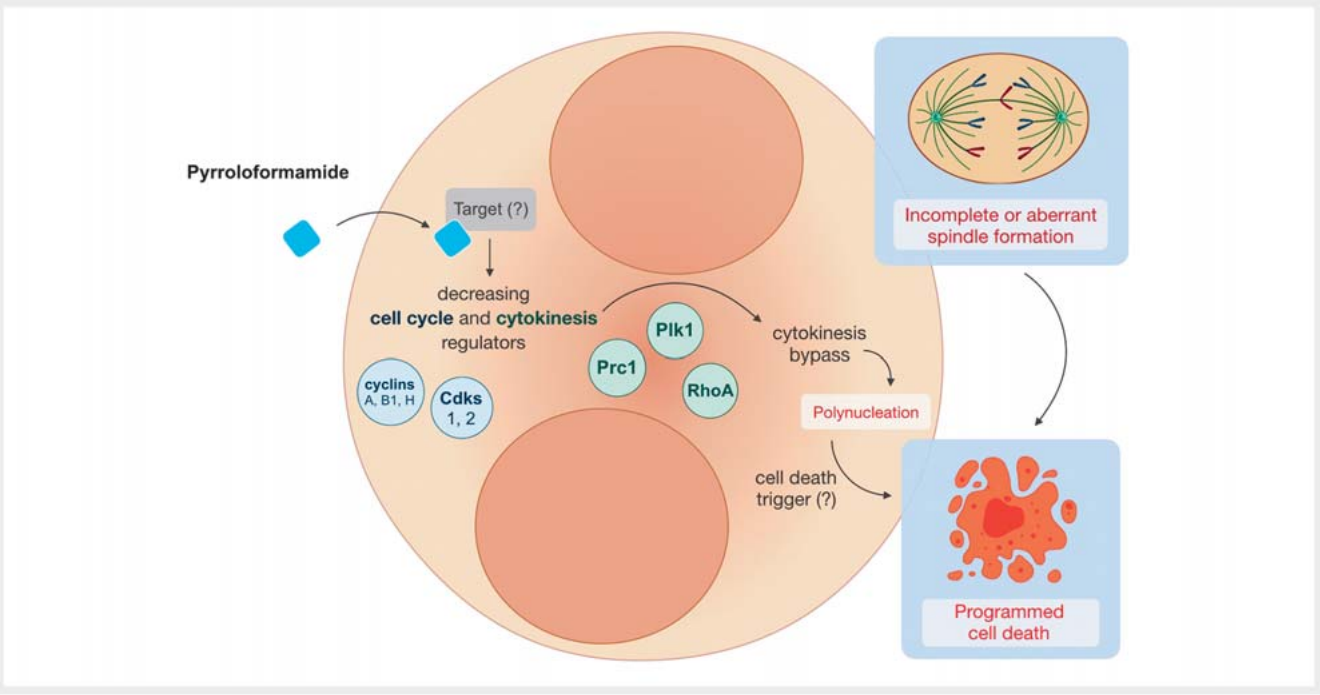
northeast of Brazil. Tamandarin A proved to be highly cytotoxic and slightly more active than didemnin B in the colony-forming clonogenic assay against human tumor cell lines, with mean IC_{50} in the nM range [114]. The authors also report this compound to be a strong inhibitor of protein biosynthesis, offering additional shreds of evidence that support a didemnin-like activity of these molecules.

Considering the macromolecules, *Styela plicata* and *Phallusia nigra*, solitary species collected in Rio de Janeiro State, were shown to produce biologically active GAGs that have shown anticoagulant, antithrombotic, and antimetastatic activities [115–117]. DS with different sulfation patterns–2,4-O-sulfated (2,4-DS) and 2,6-O-sulfated (2,6-DS) to their core structure (IdoA2-GalNAc)n–were obtained from the internal organs of the aforementioned species, respectively, and were shown to inhibit binding of human adenocarcinoma LS-180 cells to immobilized P-selectin at comparable potencies, which were, in turn, 2-fold higher than that of mammalian DS. P-selectin is an endogenous glycoprotein responsible for cell-cell adhesion and plays a role in pathogenic processes such as inflammation and metastasis. Indeed, the ascidian DSs were further shown to attenuate metastasis in *in vivo* models using mouse colon carcinoma cells stably expressing GFP (MC-38GFP) and in mouse melanoma cells (B16-BL6), however with less efficiency [118]. Another ascidian GAG, this time a peculiar HS with a high content of 2-sulfated β-glucuronic acid isolated from the viscera of *P. nigra*, displayed an 11-fold increased potency, when compared to mammalian heparin, in reducing the activity of P-selectin. Moreover, such HS was rendered nearly inactive as an anticoagulant, thus offering a more efficient and selective alternative to heparin-based antimetastatic therapy [119].

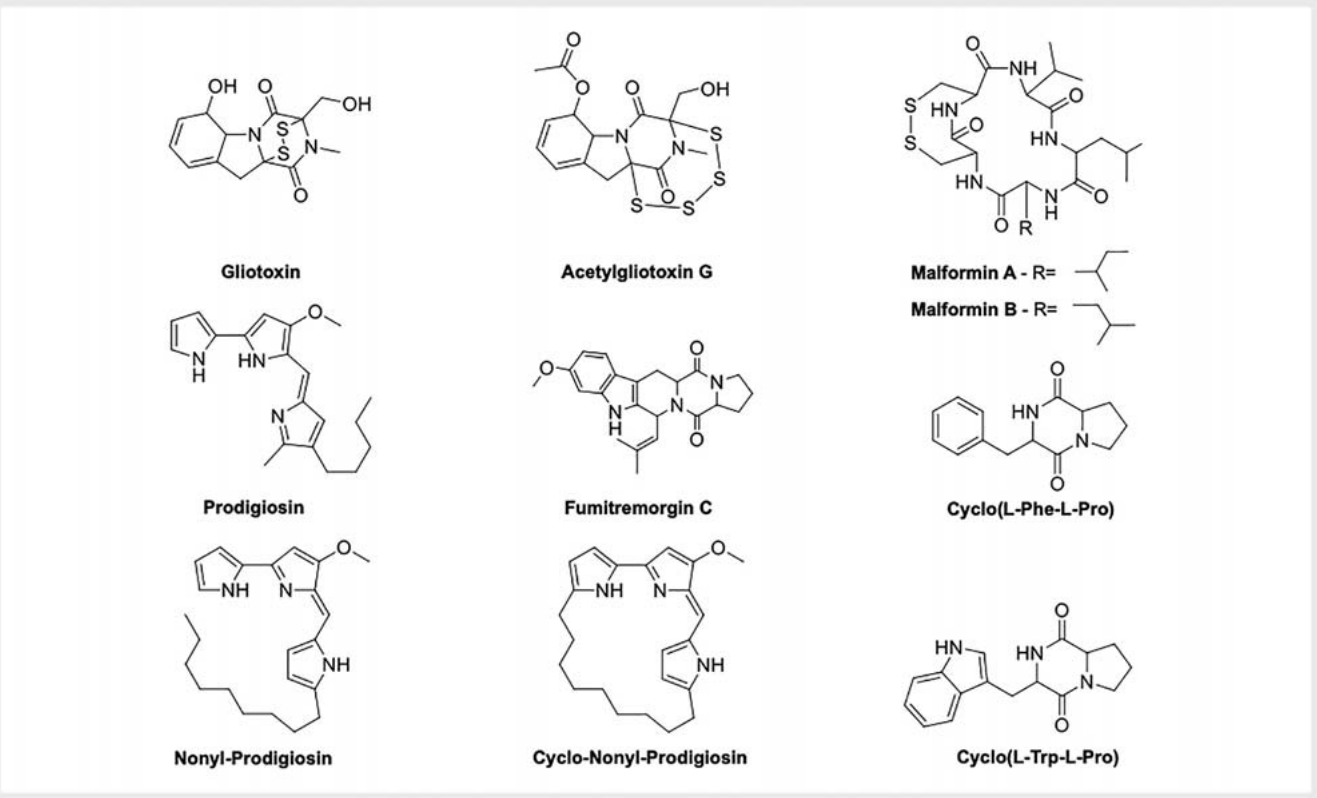
Studies based on molecular networks of ascidian-associated microbiota have emerged as an interesting approach to the identification of cytotoxic molecules. In this sense, ascidians, along with sponges and sediments from Rocas Atoll, a unique environment in the equatorial Atlantic Ocean hosting a large number of endemic species, have been assessed for the evaluation of metabolomic diversity and pharmacological potential of the inhabiting microbiota. From the 80 bacterial strains recovered, 39% were recovered from ascidians, 36% from sponges, and 25% from sediment samples. Many chemical classes of compounds, such as diketopiperazines, lipopeptides, staurosporines, surugamides, sphinganines, erythromycins, TAN antibiotics, and rifamycins, were annotated within the extracts using GNPS-based



▶ **Fig. 7** Schematic model of the mechanism of action of granulatimide and isogranulatimide isolated from the ascidian *Didemnum granulatum*. These compounds inhibit checkpoint kinase 1 (Chk1) and induce cell cycle arrest in G2 phase predominantly in cells with impaired p53 function.



▶ **Fig. 8** Schematic model of the mechanism of action of pyrroloformamide isolated from the actinobacteria *Streptomyces* sp. BRA010 associated to the ascidian *Eudistoma vannamei*. This compound induces bypass of cytokinesis due to inhibition of motor proteins polo-like kinase (Plk), protein regulator of cytokinesis (Prc), and Ras homologue gene family member A (RhoA). Additionally, pyrroloformamide modulates cyclin dependent kinases (Cdks) 1 and 2 and cyclins A, B1, and H. Cells exposed to pyrroloformamide show polynucleation, impaired spindle formation, and programmed cell death features.



▶ **Fig. 9** Compounds with anticancer potential isolated from marine sediment-associated bacteria from Brazilian Blue Amazon.

molecular networking [120]. Further analysis using the tool DEREPLICATOR+ [121] of highly cytotoxic extracts obtained from *Streptomyces* sp. BRB298 and BRB302, strains isolated from a yet unidentified ascidian, allowed the annotation as new novonestmycin derivatives, glycosylated macrolides with remarkable cytotoxic activity against cancer cells, with IC_{50} values reported in the subnanomolar range. These data reinforced the value of omics-based strategies in the search of anticancer compounds from marine sources.

Further Accessing Brazilian Marine Environments: Studies with Sediment-associated Microbiota

Despite the relative scarcity of data, a consistent increase in studies aimed at bioprospecting the pharmacological potential of microorganisms associated with Brazilian marine sediments can be observed in recent years. Ióca and collaborators [12] reported that merely 3% of the total natural products isolated from microbial sources comes from marine sediments, which mainly comprise peptides, followed by terpenes. Despite this small number when compared to natural products retrieved from plant and soil microorganisms, the structural diversity and richness of microbial marine natural products added to their unique activity and distinctive mechanisms of action sufficiently justifies the continuous investigation of such a source of compounds.

Sediments from 2 harbor areas in Ceará State, on the Northeastern coast of Brazil, have been investigated for fungi producing biologically active compounds. From sediment collected at Pecém's offshore port terminal, 48 fungal strains were recovered and their extracts evaluated for cytotoxicity against HCT 116 cells, from which that obtained from *Dichotomomyces cejpii* BRF082 was identified as the most promising. It was then shown that the strain produced a series of sulfur-containing diketopiperazines, from which gliotoxin and acetylgliotoxin G (▶ **Fig. 9**) were cytotoxic against HCT 116 cell line [122]. Although this study did not explore the mechanisms underlying the observed antiproliferative activity, there are many other reports on gliotoxin cytotoxic properties revealing a multifaceted signaling pathway linked to their activity against different cancer cells [123–125]. This molecule has demonstrated potential in targeting the Wnt/β-catenin pathway [123], farnesyltransferase and geranylgeranyltransferase [126], and the NOTCH2 [125, 127]. Besides, gliotoxin was shown to activate JNK and Bim-mediated apoptosis through a RhoA-ROCK-MKK4/MKK7-dependent pathway [128] and to exert antiangiogenic activity through disruption of the HIF-1α/p300 complex in prostate cancer cell lines and xenograft models [129].

Another strain recovered from the sediment samples from Pecém's offshore port terminal, *Aspergillus niger* BRF074, yielded a new furan ester derivative containing an unprecedented nitrogenated skeleton, the cyclopeptides malformins A and C (▶ **Fig. 9**), and several diketopiperazines. The furan ester derivative showed cytotoxic activity against HCT 116 tumor cell line [130], but the mechanisms of action or target were not investi-

gated. The aforementioned study did not further assess the bioactivity of malformins A and C; however, these cyclic pentapeptides are acknowledged for their cytotoxic activity in several other cancer cell lines [131–133]. Still, malformin C demonstrated significant acute toxicity that may limit its use as chemotherapeutic agent [132].

From another *Aspergillus* sp. (strain BRF030) recovered from sediments from the port of Mucuripe, also in the State of Ceará, 2 compounds with cytotoxic activity against HCT 116 cells were isolated: fumitremorgin C and 12,13-dihydroxyfumitremorgin C [134]. Fumitremorgin C (▶ **Fig. 9**) is an indolyl diketopiperazine alkaloid that was the first identified inhibitor of BCRP [135]. The BCRP, also named ABCG2, is a membrane protein half-molecule ABC transporter, responsible for pumping out a wide range of chemotherapeutic agents and, thus, it functions as a key player in the multidrug-resistance phenotype of cancer cells. Fumitremorgin C reversed chemoresistance to distinct chemotherapeutic agents including mitoxantrone, topotecan, and doxorubicin in colon cancer [136] and almost completely reversed the chemoresistance to mitoxantrone in breast cancer that overexpresses BCRP [137]. However, despite its elevated inhibitory potency, its clinical use was abolished due to neurotoxic side effects [135].

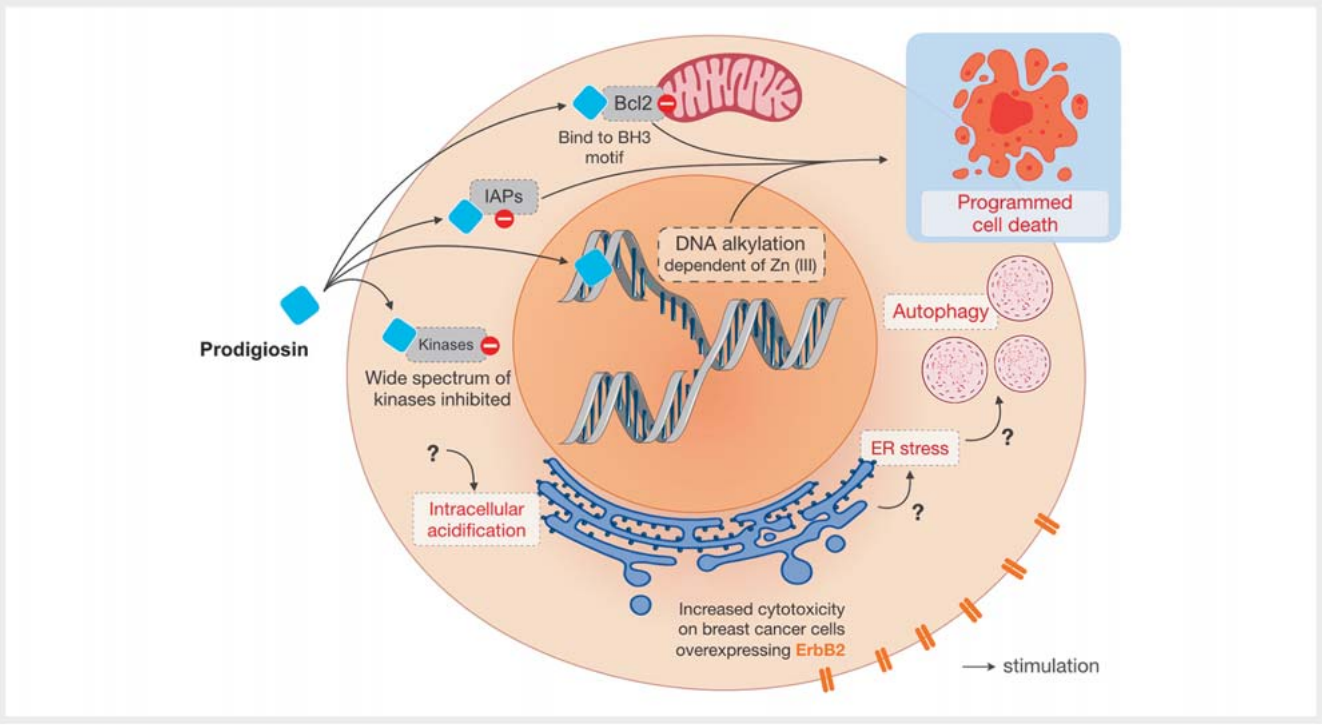
Marine bacteria recovered from sediments collected in the coast of Ceará have also been assessed. Three chromomycins, typically known as DNA intercalators (above mentioned and discussed in the section *"Cnidarians"*), were isolated from *Streptomyces* sp. BRA090, also recovered from dredged sediments from the port of Mucuripe. Chromomycin A_2 displayed cytotoxicity in the nM-range against a 7-cell lines panel and induced autophagy in a metastatic melanoma cell model [138].

Moreover, the tripyrrole red pigment prodigiosin, a member of the prodiginine class of natural products recognized for their anticancer potential, was isolated from the growth broth of *Pseudoalteromonas* sp. BRA007 (M23), a strain obtained from Taíba Beach, Ceará state. This study described the cytotoxicity of prodigiosin in a 4-tumor cell line panel and, remarkably, a nearly 100-fold selectivity towards a human breast epithelial cell line, HB4a, stably transfected with cDNA for the receptor tyrosine kinase ErbB-2, in comparison to the parental cell line [139]. Prodigiosin, which is known to induce apoptosis in cancer cells through an intricate, multi-target but not fully characterized mechanism, has been shown to reduce GSK-3β/NAG-1 [140] and JNK/p38/RAD51 [141], as well as to downregulate the expression of members of the IAP family of proteins (▶ **Fig. 6**) [142]. This compound demonstrated cytotoxicity against a wide range of human cancer cell lines and, to a lesser extent, to nonmalignant cells. The wide variety of mechanisms related to cytotoxicity of prodigiosin include induction of DNA damage; acidification of intracytoplasmic compartment; and modulation of kinases pathways [142, 143]. Additionally, it has been shown that prodigiosin is able to induce intense cell stress such as autophagy and endoplasmic reticulum stress on tumor cells, which could also trigger cell death [144]

Furthermore, the cytotoxic strain *Actinomadura* sp. BRA177, recovered from SPSPA, a set of islets and rocks distant 590 nmi from continental Brazil, in the equatorial Atlantic Ocean, yielded prodiginine derivatives, such as nonylprodigiosin and cyclononylprodigiosin (▶ **Fig. 9**), that displayed antiproliferative activity against tumor and nontumor cells [145]. Shotgun sequencing of BRA177 genome revealed 22 biosynthetic gene clusters related to the production of ribosomally- (lantipeptides) and nonribosomally-derived (nonribosomal peptide synthetase) bioactive peptides, terpenes, siderophores, and polyketides, including the one responsible for the production of the isolated prodiginines [145]. These particular prodiginines were isolated in 1969 and 1970, respectively, from *Actinomadura madurae* [146, 147] and, adjoined by amply studied prodigiosin, are members of a family of red-pigmented tripyrroles. Their bioactivities have not been broadly addressed so far (▶ **Fig. 10**). However due to structural resemblance to prodigiosin, it is believed that prodiginines may share similar modes of action [143]. Still, prodigiosin and the synthetic prodiginine derivative obatoclax mesylate–which has completed phase II clinical trials for the treatment of various cancers–were shown to bind the BH3 domain of Bcl-2 protein, a protagonist in antiapoptotic signaling [148, 149].

Indeed, the SPSPA has shown additional favorable evidence to validate the assessment of the inhabitant marine microbial diversity for their pharmacological potential. Among culturable actinobacteria isolated from sediments collected therein, 268 strains were isolated and 94 were tested for cytotoxicity of their extracts, from which 26 produced cytotoxic extracts. Chemical analysis by HPLC-MS/MS suggested the production of known cytotoxic compounds, such as staurosporines and piericidins and, interestingly, saliniketals and rifamycins [150]. The latter class of compounds are typical natural products synthesized by bacteria of the *Salinispora* genus. Indeed, the *Salinispora* have attracted much attention as these obligate marine bacteria house unique biosynthetic pathways and, therefore, are a prolific spring of natural products [151]. Specifically, the species *S. tropica* is the producer of marizomib, a b-lactone-g-lactam proteasome inhibitor that is currently undergoing phase III clinical trials for the treatment of glioblastoma and multiple myeloma [14]. Following this hint, further studies then confirmed the occurrence of *Salinispora* sp. at the SPSPA [81], which was the first report of this genus in Brazilian waters, and compared the metabolomics profile of strictly marine actinobacteria *Salinospora arenicola* and *S. pacifica* among strains occurring in Brazilian and Portuguese islands [152]. By using the spectral library search from GNPS, the authors showed that *S. arenicola* strains isolated from Brazilian waters are able to produce the molecular families of staurosporine, desferrioxamine, rifamycin, ferroxamine, and saliniketal, typical compounds to the metabolome of *S. arenicola*. Through inspection of the molecular networking, a new saliniketal analog with a difference of a methyl group was found [152].

Another strain recovered from the SPSPA that gave a cytotoxic extract, *Streptomyces* sp. BRA199, was subjected to a bioassay-guided fractionation to yield piericidin A and 3 diketopiperazines [150]. Although the first compound was not particularly assessed therein for bioactivity, piericidins are widely known as potent cytotoxins, originally isolated from actinobacteria, especially from *Streptomyces* sp. Due to their structural resemblance to coenzyme Q, it was proposed that piericidins act as their antagonists. Indeed, they are specific and effective NADH-ubiquinone oxidoreductase (complex I) inhibitors in the mitochondrial electron transport chain [153]. Moreover, piericidin A directly interacts



▶ **Fig. 10** Schematic model of the mechanism of action of prodigiosin isolated from the bacteria *Pseudoalteromonas* sp. BRBA007 from marine sediment. Prodigiosin induces a milieu of cell perturbations, including DNA alkylation, inhibition of kinases, and apoptosis, through modulations of key players such as IAPs and Bcl2. In agreement with these multiple targets, cells exposed to prodigiosin display several phenotypic features of ER-stress and programmed cell death. Interestingly, this compound shows increased cytotoxicity on breast cancer cells overexpressing ErbB2.

with the protein PRDX1, co-localizing with that in the nucleus. This promotes increased expression of PRDX at mRNA and protein levels, further inhibiting key genes involved in the progression of renal cancer and reducing the generation of ROS in renal cancer cell lines, promoting apoptosis [154].

In turn, the diketopiperazines (▶ **Fig. 9**) obtained from SPSPA strain BRA199 were assayed against HCT 116, OVCAR-8, and SF-295, where cyclo(L-Phe-L-Pro), first isolated from *Lactobacillus plantarum*, displayed moderate cytotoxicity to all cell lines. It is worth to mention that diketopiperazines are ubiquitously synthesized across living organisms. Although they are commonly isolated from fungi, especially from the genera *Aspergillus* and *Penicillium* [155], these compounds also occur in bacteria, plants, and animals [156]. There are different chemical scaffolds described for diketopiperazines; the most common one and that with further therapeutic usefulness is the 2,5-diketopiperazine, a cyclodipeptide whose core structure has been often employed in drug design to overcome poor pharmacokinetics proprieties of various current active principles. Their anticancer potential may be illustrated by plinabulin, a synthetic analog of the marine fungal diketopiperazine halimide, isolated, in turn, by an *Aspergillus* sp. associated with a *Halimeda* sp. algae, for which the mechanism of action consists of promoting vascular disruption and tubulin-depolymerizing. Currently, plinabulin is undergoing the last stage of clinical development for the treatment of NSCLC [157, 158].

It is worth mentioning that much evidence has led natural product researches to consider the associated microorganisms as the actual producers of cytotoxic compounds isolated from marine invertebrates. The growing indications–most of which are generated by studies applying omics approaches–that this may imply a majority of cases, even if only a few have been compellingly confirmed, opens the way to vastly explore free-living microorganisms, such as those from sediments, in search of bioactive molecules. In Brazil, although the marine microbiota have been assessed for a much shorter time and suffer even more from the lack of sufficient occurrence and taxonomic information, this has shown to be a rapidly evolving field and a promising source of pharmacologically relevant compounds.

Concluding Remarks and Perspectives

Within natural products science, it is common to associate innovation with the discovery of original carbon skeletons with novel biological properties. In this sense, the probability of finding new chemical structures rises with the biodiversity of the studied samples and, additionally, the number of assays in the screening platform [16, 159]. One key factor to increase the natural product chemical space is the prospection of novel taxonomical space, which, in principle, would allot megadiverse countries like Brazil an especially privileged position. However, translation of the predicted chemical diversity into isolated molecules amenable to biological assays is one of the biggest challenges in the process of finding a new pharmacological hit. Pondering the results discussed in this review, it is clear that Brazilian taxonomical space

is still mostly unexplored, revealing–literally–an ocean of possibilities to find new chemical entities.

One important issue in the discussion of sustainable use of marine biodiversity, either in Brazil or elsewhere, is the ownership of the natural resources and the establishment of fair and equitable sharing of resulting benefits, as predicted by the 1992 Convention of Biological Diversity and the Nagoya Protocol from 2010 [160]. Although Brazil was one of the prompt signatories of the Nagoya Protocol, it has not yet ratified the commitment. Still, Brazil is among the countries with the most restrictive laws regulating the access to genetic resources. Law number 13.123/2015 and decree 8.772/2016 regulate basic and applied research with native organisms in Brazil and, in that scope, created a National System for Governance of Genetic Heritage and Associated Traditional Knowledge (SisGen) [161]. In this context, it can be speculated that such restrictive laws and subsequent bureaucracy to apply for the necessary permits, aligned with incessant funding challenges, may contribute to limiting the development of the field of marine biotechnology in Brazil.

Still, this review reveals key contributions of Brazilian science to anticancer research related to marine natural products, which encompasses studies on the mechanisms and targets of known chemical scaffolds. While this can be a bit disappointing considering all the chemical diversity anticipated from the sizeable number of species distributed in our oceans, it represents an important contribution to the field. Indeed, the scientific community is aware that a huge gap remains in attributing ecological or biomedical properties to known natural products and, regardless of structural novelty, understanding their bioactivities can bring innovative knowledge with impacts toward human health [16, 162]. One important example is the recent description of the transcription factor TBX2 as a target of the chromomycins, which are actually 70-y-old molecules that have undergone clinical trials back in the 1960s [163]). However, at that time, this useful information was not available to be used in the selection of patients, which could have changed the outcome of those clinical trials. Through COMPARE analysis of the respective outcomes on NCI-60 cell line panel, the cytotoxic activity profiles of chromomycin A_3 and trabectedin revealed some similarities, which, in turn, is suggestive of a common mechanism of action. In fact, trabectedin was the first compound able to displace an oncogenic transcription factor from its target promoters with high specificity [164, 165].

Undoubtedly, the observed contributions have only been possible due to collaborative studies that address marine biodiversity in the broadest sense. Currently, there are several networks running in Brazil combining diversified omics strategies and biological assessments supporting the next steps and further consolidating Brazilian marine natural products investigations. Undeniably, Brazilian science and innovation, conducted mostly by academics, has never seen sufficient funding. Still, during the past 2 decades, the country was benefiting from growing and significant improvements on research infrastructure. Lately, however, a drastic reduction of already lesser funding has been threatening Brazilian science and technology, assigning a vulnerable position to these only recent gains and investments.

In such a scenario, a drop in the number scientists is expected to accompany the funding reduction, which should affect various fields. Natural products research, in particular, which is inherently tied to geography and to the national restrictive laws to assess biodiversity, may endure yet another hardship. Nevertheless, Brazilian science can still collect on well-developed human resources, skilled in biological and pharmacological evaluations, in genomics approaches and, moreover, in classical chemical techniques that allow for isolation, purification, and structural determination of organic molecules. These competencies will be evermore essential. In this sense, a measurable effect, at this moment, is the upsurge in academic spinoff companies. This is a clear result of good postgraduate training and evolution of technological maturity, even if the product to be developed is not yet a new anticancer drug.

Acknowledgements

The authors are grateful to FAPESP (2015/17177-6; 2017/09022-8, 2017/17648-4), ArboControl Brasil Project (FNS/UnB TED74/2016 and TED42/2017), and National Institute of Science and Technology–INCT-BioNat (FAPESP #2014/50926-0 and CNPQ #465637/2014-0).

Conflict of Interest

The authors declare that they have no conflict of interest.

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