Reviews

Challenges and Rewards of Research in Marine Natural Products Chemistry in Brazil[#]

Roberto G. S. Berlinck,*[,†] Eduardo Hajdu,[‡] Rosana M. da Rocha,[§] Jaine H. H. L. de Oliveira,[†] Isara L. C. Hernández,[†] Mirna H. R. Seleghim,[†] Ana Claudia Granato,[†] Érika V. R. de Almeida,[†] Cecília V. Nuñez,[†] Guilherme Muricy,[‡] Solange Peixinho,[⊥] Claudia Pessoa,[∥] Manoel O. Moraes,[∥] Bruno C. Cavalcanti,[∥] Gislene G. F. Nascimento,[Δ] Otavio Thiemann,[○] Marcio Silva,[○] Ana O. Souza,[◇] Celio L. Silva,[◇] and Paulo R. R. Minarini[◇]

Instituto de Química de São Carlos, Universidade de São Paulo, CP 780, CEP 13560-970, São Carlos, SP, Brazil, Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, s/n, 20940-040, Rio de Janeiro, RJ, Brazil, Departamento de Zoologia, Universidade Federal do Paraná, Centro Politécnico, 81531-990, CP 19020, Curitiba, Paraná, Brazil, Departamento de Biologia, Universidade Federal da Bahia, Salvador, BA, Brazil, Laboratório de Oncologia Experimental, Universidade Federal do Ceará, Fortaleza, CE, Brazil, Faculdade de Ciências da Saúde, Universidade Metodista de Piracicaba, Piracaba, SP, Brazil, Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos, SP, Brazil, and Departamento de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil

Received September 25, 2003

Brazil is blessed with a great biodiversity, which constitutes one of the most important sources of biologically active compounds, even if it has been largely underexplored. As is the case of the Amazon and Atlantic rainforests, the Brazilian marine fauna remains practically unexplored in the search for new biologically active natural products. Considering that marine organisms have been shown to be one of the most promising sources of new bioactive compounds for the treatment of different human diseases, the 8000 km of the Brazilian coastline represents a great potential for finding new pharmacologically active secondary metabolites. This review presents the status of marine natural products chemistry in Brazil, including results reported by different research groups with emphasis on the isolation, structure elucidation, and evaluation of biological activities of natural products isolated from sponges, ascidians, octocorals, and Opistobranch mollusks. A brief overview of the first Brazilian program on the isolation of marine bacteria and fungi, directed toward the production of biologically active compounds, is also discussed. The current multidisciplinary collaborative program under development at the Universidade de São Paulo proposes to establish a new paradigm toward the management of the Brazilian marine biodiversity, integrating research on the species diversity, ecology, taxonomy, and biogeography of marine invertebrates and microorganisms. This program also includes a broad screening program of Brazilian marine bioresources, to search for active compounds that may be of interest for the development of new drug leads.

Introduction

From a historical perspective, Bergmann's reports on sponge sterols[1] and nucleosides[2] are considered the starting point of marine natural products chemistry. However, Tahara's studies on the nature of Tetraodontidae fish poison (tetrodotoxins),[3] the investigation of sterols from the kingdom Animalia by Dorée[4] and Henze,[5] and the study of carotenoids from marine animals by Lederer[6] cannot be overlooked. But it was only during the 1960s that continuous research of marine natural products began, mainly due to the advances in the instrumentation for structure elucidation and isolation techniques as well as the availability of scuba for collection of marine organisms. Highlights of the achievements in marine natural products have been recently reviewed and illustrate the structural complexity and potent biological activities of many natural products isolated from different organisms of the marine environment.[7] Although Brazil has the second most extensive coastline after Australia, the development of the chemistry of marine organisms from Brazil has been hampered for many years because the main focus of Brazilian natural products chemists has been directed to the study of medicinal plants and plant chemotaxonomy.

Challenges

Brazilian Geography: The Impact on Marine Biodiversity. Brazil has a coast about 8000 km in length, adjacent to over 800 000 km^2 of continental shelf, spreading from 4° N at Cape Orange to 34° S at Chuí. A division of this vast area into sectors has been proposed based on primarily geologic and geographic criteria.[8,9] We have

[#] Based on the Matthew Suffness Award lecture presented at the 43rd Annual Meeting of the American Society of Pharmacognosy and the 3rd Monroe Wall Symposium, New Brunswick, NJ, July 27−31, 2002. Dedicated to the 50 years of the Centro de Biologia Marinha of the Universidade de São Paulo.

* To whom correspondence should be addressed. Tel: +55-16-2739954. Fax: +55-16-2739952. E-mail: rgsberlinck@iqsc.usp.br.
[†] Instituto de Química de São Carlos, Universidade de São Paulo.
[‡] Museu Nacional, Universidade Federal do Rio de Janeiro.
[§] Departamento de Zoologia, Universidade Federal do Paraná.
[⊥] Departamento de Biologia, Universidade Federal da Bahia.
[∥] Laboratório de Oncologia Experimental, Universidade Federal do Ceará.
[Δ] Faculdade de Ciências da Saúde, Universidade Metodista de Piracicaba.
[○] Instituto de Física de São Carlos, Universidade de São Paulo.
[◇] Departamento de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto.

10.1021/np0304316 CCC: $27.50
Published on Web 01/23/2004

adopted Knoppers' et al.[10] most recent revision and will comment upon the following five sectors: north, northeast, east, southeast, and south.

The Brazilian continental shelf varies from only 8 km width off Salvador (Bahia) to over 300 km off the mouth of the Amazon River (Pará). In general, it is narrower in the northeastern and eastern sectors and wider in the northern, southeastern, and southern sectors. The shelf break, where the continental shelf meets the continental slope, is shallower in the three upper sectors (40−80 m depth) and deeper in the southeast and south (100−160 m depth).[10]

The northern sector, situated between 4° N and 4° S, is characterized mainly by the Amazon River discharge, which constitutes nearly 20% of the world's freshwater input to the oceans. The most prominent ecosystems in the area are mangroves, but reef fishes are known to occur over rich sponge bottoms near 50 m deep.[11] Extensive sandy beaches occur in the southern portion of this sector, along the coast of the state of Ceará. Another important geomorphologic feature occurs offshore. The Manoel Luis Reefs (00°52′ S, 44°15′ W), a Maranhão State conservation park, is probably Brazil's least known reef complex.

Parallels 4° S and 13° S encompass the northeastern sector of the Brazilian coastal zone. Substrate types and dominant associated organisms have been mapped in great detail for parts of this sector,[12] revealing a conspicuous feature remarkable among other tropical shelves: an extensive, over 4000 km long, carbonaceous cover.[8] This cover is predominantly algal in nature, derived from hard, laminated, ramifying and globular calcareous algae, which confers some stability to the substrate, thus enabling a diverse flora and fauna to develop. Fringe reefs are also common along most of this sector,[13] and associated with these, a rich coral reef fauna has developed, where many taxonomic groups are less numerous than their Caribbean counterparts, but, nevertheless, endemism can be remarkable.[14−16] River runoff is not a conspicuous feature over most of this sector, apart from that originated by the São Francisco River, Brazil's third largest. Important offshore features are the das Rocas Atoll (3°52′ S, 33°49′ W), a Brazilian Biological Reserve, and the Fernando de Noronha Archipelago (3°51′ S, 32°25′ W), a Marine National Park. Both are UNESCO's Biosphere Heritage Reserves. Despite being situated slightly to the north of this sector, they are included here on the basis of overall geomorphologic and biotic affinity. Both offshore conservation areas, as well as segments of the coast, are becoming increasingly known for their rich biodiversity.

Eastern coastal Brazil is situated between 13° S and 22° S and is geomorphologically very similar to the northeastern sector. The main difference relates to a series of important rivers (such as the Jequitinhonha, Mucuri, and Doce). The most remarkable geomorphologic features in this area are the occurrence of the 100 km wide Royal Charlotte Bank, at 16° S, the 240 km wide Abrolhos Bank, at 18° S, and the Vitória-Trindade seamounts chain, at 21° S, streching over 1000 km to the east of Vitoria City (Espírito Santo State). From the perspective of a biological resources inventory, this is considered Brazil's least known sector of the continental shelf, despite its well-advertised, complex reef system on the Abrolhos Bank.[17−19] The Abrolhos Marine National Park (18°00′ S, 38°40′ W), situated 70 km offshore, comprises five islands disposed as an arch, possibly associated with a former volcanic crater, as well as several reef banks in the adjacent area.

The southeastern sector is situated between parallels 22° S and 29° S. The carbonaceous substrates to the north are replaced by rocky coasts and mostly muddy or sandy bottoms. Rocky coasts in this area are formed by granite and basalt rocks resulting from erosion of the Serra do Mar mountain chain, which lies parallel to the coastline. Upwelling can be intense in the area, thus increasing productivity, as well as bringing a subtropical faunal element closer to the surface. Mangroves become less conspicuous until they fully disappear. No coral reefs occur in the area, and the last "coralline oasis" is situated in the Cabo Frio region,[17] a major biogeographic borderline, associated with Brazil's most intense upwelling phenomena. This area is a transitional biogeographic domain known as the Paulista Biogeographic Province,[20] and its biotic component is considered Brazil's best known.[8] New species abound when detailed taxonomic work is undertaken, such as that conducted in parallel with the bioprospecting reported in this article.[21] The southern limit of this sector is dictated by the 20° C isothermal winter temperature.[10]

The south sector, from 29° S to 34° S, is mainly one long sandy beach (the world's largest). The taxonomic knowledge on the benthic fauna of this sector is nearly as detailed as it is for the southeastern sector. The largest densities of benthic macro invertebrates have been reported from the central portion of the continental shelf (80−150 m depth).[8]

From this very brief description of Brazil's coastal and continental shelf features, one can depict a challenging array of ecosystems mirrored by a diversified assembly of environmental settings, which permits the existence of a rich marine biodiversity. This is challenging, because only a portion of this diversity is known. For this reason, bioprospecting for marine natural products has to proceed in conjuction with the marine biodiversity inventory. In this way, chemists have direct access to information on species names, distribution, abundance, and perhaps even phylogenetic frameworks on which to build hypotheses of occurrence of particular classes of compounds. Conversely, taxonomists have a further reason for undertaking their frequently underrated basic research programs, which may yield easier acceptance of their unique goals by society and funding agencies.

The resources available for indentifying new leads against long-lasting human diseases are of 10 orders of magnitude greater than those available for conducting taxonomic inventories. Combining both activities is thus part of the challenge to be overcome.[22]

Rewards

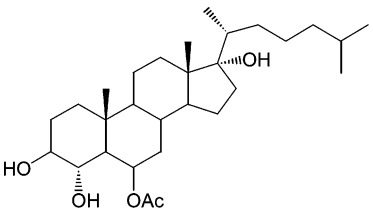
Marine Natural Products Chemistry in Brazil. The Chemistry of Marine Invertebrates. The first report on the chemistry of a marine organism from the Brazilian coastline dealt with the isolation of cholesterol from the sea urchin *Echinometra lucunter* in 1963.[23] At that time, Professor Bernard Tursch, originally from the Université Libre de Bruxelles (Brussels, Belgium), spent a two-year sabbatical at the Núcleo de Pesquisas de Produtos Naturais of Universidade Federal do Rio de Janeiro (NPPN-UFRJ). Participating in a pioneer team of natural product chemists, including Professors Carl Djerassi and Benjamin Gilbert, Prof. Tursch started the study of Brazilian marine invertebrates. After his return to Brussels, the research in Brazil was continued by Professor Alphonse Kelecom, a former Ph.D. student of Prof. Tursch. Prof. Kelecom's contribution to the chemistry of marine natural products in Brazil was seminal. His research team, initially at SARSA Pharmaceuticals, then briefly at NPPN-UFRJ, and subsequently at the Departamento de Biologia Geral of the Universidade Federal Fluminense, not only investigated

a number of marine invertebrates and marine algae crude extracts but also supported two of the major groups currently conducting marine natural products chemistry research in Brazil. The reader should refer to Prof. Kelecom's recent reviews for a comprehensive account of marine natural products research in Brazil.[24,25] Since Kelecom's reviews cover literature reports up to 1994, we present here an update of studies on Brazilian marine organisms.

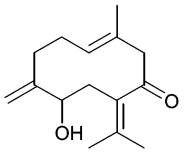
Professor Rosângela de A. Epifanio's research group at the Instituto de Química of Universidade Federal Fluminense has focused on chemical ecology of marine invertebrates. They have reported a new 17-hydroxysterol, punicin (**1**), from the gorgonian *Lophogorgia punicea*,[26] whose structure was established by analysis of spectroscopic data. Subsequently, the same group reported the isolation of a new germacrane sesquiterpene, germacra-1(10),4(15),7(11)-trien-5-ol-8-one (**2**), from the gorgonian *Phyllogorgia dilatata*.[27] One known (**3**) and the new heterogorgiolide (**4**) sesquiterpenes have been isolated as fish-feeding deterrents from the gorgonian *Heterogorgia uatumani*. Both **3** and **4** displayed feeding-deterrent activity against natural populations of carnivorous fishes at 0.5 and 0.8 mg/cm^3, respectively.[28] The known 11β,12β-epoxypukalide (**5**) has been isolated as a fish-feeding deterrent from the gorgonian *P. dilatata*.[29] Variabilin (**6**), isolated from the sponge *Ircinia strobilina*, was also reported as a fish-feeding deterrent.[30]

Two new didemnin derivatives, tamandarins A (**7**) and B (**8**), have been isolated from an ascidian belonging to the genus *Didemnum*. Both **7** and **8** were identified by analysis of spectroscopic data. The absolute stereochemistry of both compounds was established by chemical degradation and analysis of Marfey derivatives. Depsipeptides **7** and **8** displayed more potent cytotoxic activity than didemnin B (**9**) against BX-PC3 pancreatic carcinoma, DU-145 prostatic cancer, and UMSCC10b head and neck carcinoma cancer cells.[31] Two new sterol glycosides, riiseins A (**10**) and B (**11**), have been isolated from the octocoral *Carijoa* (*Telesto*) *riisei*, both of which have been identified by analysis of spectroscopic data.[32] The riiseins displayed cytotoxic activity against HCT-116 human colon adenocarcinoma with IC_{50} values of 2.0 μg/mL.

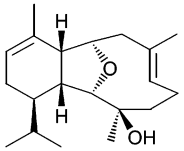
The chemical defenses of the gorgonian *Lophogorgia violacea* against generalist fish carnivores have been investigated.[33] Lophotoxin (**12**) was identified as the most potent feeding deterrent, and was isolated with deoxylophotoxin (**13**), 13-acetoxy-11β,12β-epoxypukalide (**14**), and two new furanocembranolides, 7-acetoxy-8-hydroxylophotoxin (**15**) and 3-methoxy-8-hydroxylophotoxin (**16**), all of which appear to contribute in an additive manner to the overall feeding deterrence observed.[33] Although the authors ruled out the possibility of **16** being an artifact of isolation



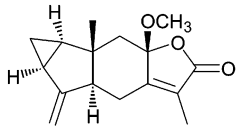
1



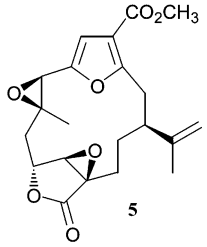
2



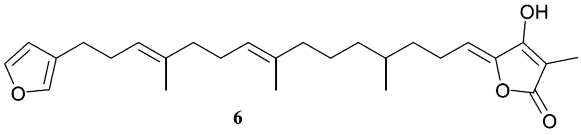
3



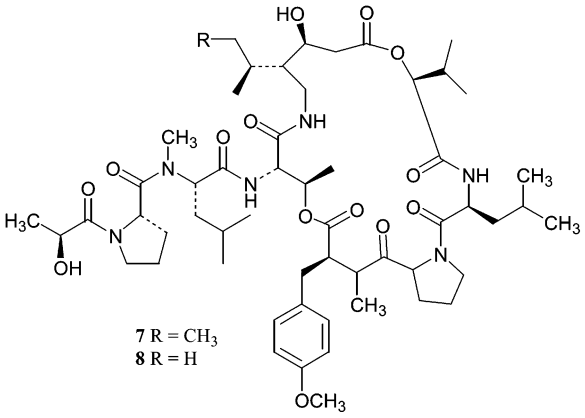
4



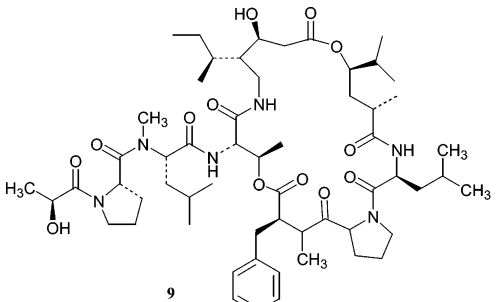
5



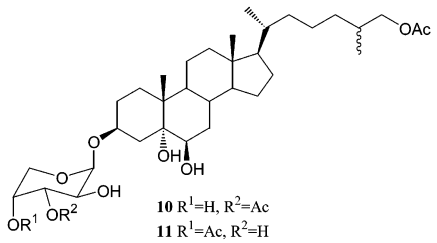
6



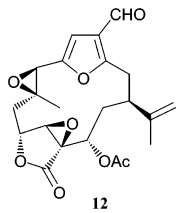
7 R = CH_3
8 R = H



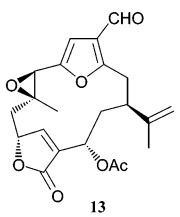
9



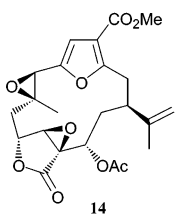
10 R^1=H, R^2=Ac
11 R^1=Ac, R^2=H



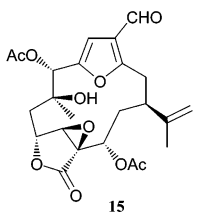
12



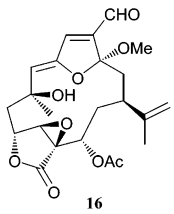
13



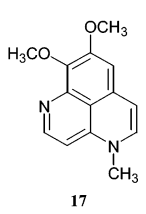
14



15



16



17

by addition of methanol on lophotoxin (**12**), one may envisage that **15** may be the product of addition of acetic acid (as a contaminant of ethyl acetate) on **12**. A new aaptamine derivative, 4-methylaaptamine (**17**), has been isolated from a sponge of the genus *Aaptos*. The new 1*H*-benzo[*de*][1,6]naphthyridine derivative **17** displayed antiviral activity against HSV-1 at 2 μg/mL.[34]

Professor Angelo da Cunha Pinto's research group at the Instituto de Química of the Universidade Federal do Rio de Janeiro studied the occurrence of halogenated chamigrene terpenes from the sea hare, *Aplysia dactylomela*, and reported the occurence of the known prepacifenol epoxide **18**, johnstonol (**19**), pacifidene (**20**), and the diol **21**, previously isolated from *Aplysia californica* and from a marine Rhodophyceae.[35] This group reported a series of a comprehensive NMR studies of the chamigrene skeleton.[36−38]

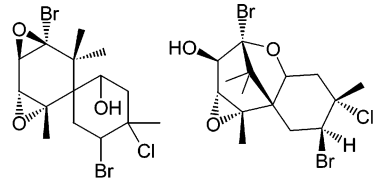
Research by our group started in 1996, when we conducted the first screening of marine sponge crude extracts with various bioassays. The methanol-soluble crude extract of the sponge *Amphimedon viridis* (formerly *Haliclona viridis*) displayed potent cytotoxic and hemolytic activities. A bioassay-guided fractionation procedure yielded a mixture of halitoxins (**22**), contamined with amphitoxins (**23**). Halitoxins were isolated initially from *Amphimedon compressa* (formerly *Haliclona rubens*) and related Haplosclerida sponges.[39,40] We decided to perform a study on the biological activities of the halitoxin complex of *A. viridis*. Our results demonstrated strong hemolytic activity against mice erythrocytes, with an ED_{50} of 2.2 ng/mL as well as potent lethality to mice, which died 35 min after intravenous injection, with a LD_{50} of 1.4 mg/kg. The halitoxin complex also promoted lysis of sea urchin eggs at 2.8 μg/mL and caused rapid blockade of potential conductance in the nerves of the blue crab, *Callinectes danae*. This effect could be partially reversed upon nerve washing, but full recovery of the control spike and resting potential was not possible.[41] We also observed strong icthyotoxic and molluscicidal activities of the *A. viridis* halitoxin complex.[42] Other pharmacological evaluations demonstrated that 3-alkylpyridinium oligomers and polymers isolated from different marine sponges display different and potent biological activities. Related toxins isolated from *Callyspongia fibrosa* displayed inhibition of the epidermal growth factor.[43] Alkylpyridinium oligomers and polymers obtained from the aqueous extract of the Mediterranean sponge *Reniera sarai* inhibited cholinesterase and showed cytotoxic and hemolytic activities.[44,45] Halitoxins obtained from *Callyspongia ridleyi* displayed several changes in membrane transport, such as an irreversible membrane potential depolarization, decreased input resistance, and inhibited evoked action potentials when applied to cultured dorsal root ganglion neuron. The halitoxins also showed an increase in cation conductance and transient increases in intracellular calcium, suggesting that the toxins could release calcium from intracellular stores.[46] The pore-forming action of these polymers was identified when the toxins were applied to artificial lipid bilayers composed of phosphatidylcholine and cholesterol. Halitoxins also evoked channel-like activity in lipid bilayers.[46] Halitoxins of *R. sarai* strongly inhibited acetyl cholinesterase (AChE) in vitro. In vivo experiments on male Wistar rats with the application of intravenous lethal doses of the toxin showed signs of hypoperfusion. Arterial blood pressure fell to mid-circulatory levels, and breathing stopped after a few breaths. At sublethal doses, the toxin caused an increase of residual volume, prolongation of expiration, and bradycardia. Histopathological examination revealed that the plugs in lung circulation may cause the death of experimental animals due to cardiorespiratory failure.[47]

A complex of halitoxins and amphitoxins isolated from the Red Sea *A. viridis* showed selective activity against specific bacteria rather than being a broad-spectrum antibiotic. The polymeric complex was highly active against eight strains of marine bacteria, whereas six different bacterial strains associated with the sponge *A. viridis* were resistant to these compounds.[48] The alkylpyridinium compounds also displayed moderate antifouling activity.[49] Sponge-derived alkylpyridinium oligomers and polymers are considered nuisance compounds because they have demonstrated a very broad range of nonspecific activity in several bioassays.[50] Since monomeric, dimeric, and polymeric 3-alkylpyridinium related compounds are chemotaxonomic markers of marine sponges belonging to the order Haplosclerida,[51,52] we may consider that Haplosclerid sponges acquired a highly effective mechanism of defense through natural selection.

We have also isolated a new purine, 1,3-dimethylisoguanine (**24**), from this same sponge, *A. viridis*.[53] Professor Chris Ireland's group reported the isolation of the same compound from the same sponge at the same time.[54] In our bioassays, 1,3-dimethylisoguanine increased the contractions obtained by transmural electrical stimulation in the guinea pig longitudinal muscle/myenteric plexus in a dose-dependent manner, while Mitchell et al.[54] observed that **24** displayed cytotoxic activity against an ovarian cancer cell line with an IC_{50} of 2.1 μg/mL. Although the results of both research groups agree on the structure of **24**, the ^{1}H and ^{13}C NMR data reported independently contained some inconsistencies. Therefore, we decided to confirm our structure proposal by X-ray crystallographic analysis.[55] In the solid state, 1,3-dimethylisoguanine occurred in a definite tautomeric form linked to three molecules of water through hydrogen bonds, which also stabilized the crystalline packing.

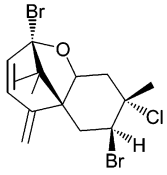
Three additional purines have been isolated from the ascidian *Symplegma rubra*: the new 6-methoxy-7-methyl-8-oxoguanine (**25**) as well as the known 8-oxoadenine (**26**) and 3-methylxanthine (**27**). Compounds **25**−**27** were identified by analysis of spectroscopic data and by comparison with literature data.[56] Compound **25** is closely related to heteromine C (**28**), isolated from *Heterostemna brownii* (Asclepiadaceae).[57] Both 8-oxoadenine (**26**) and 3-methylxanthine (**27**) were previously known as products of human metabolism.[58,59] An interesting question raised by one of the referees who evaluated the *Symplegma rubra* manuscript was: what is the actual tautomeric form of 8-oxoadenine, since four tautomeric formulas are possible? Both experimental and theoretical data indicated that structure **26** represents the thermodynamically most favorable tautomeric form of 8-oxoadenine.[60,61] Although modified purines are of relative common occurrence in marine sponges,[62,63] there are only a few additional examples of such compounds isolated from ascidians.[64]

The ascidian *Didemnum granulatum* is distributed along the Brazilian coastline and is found in the Red Sea and around Japan. A single and unstable compound, **29**, has been previously isolated from the pink *Didemnum granulata* (=*D. granulatum*?) from the Red Sea.[65] During a screening of ca. 1300 extracts from marine invertebrates and microorganisms from different biota, including the Bristish Columbia coastline, and samples from Papua New Guinea, Indonesia, and Brazil, the crude methanol extract of *D. granulatum* displayed potent inhibition of the G_2 cell cycle checkpoint.[67a] It is well-known that cell checkpoints

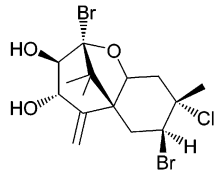


18

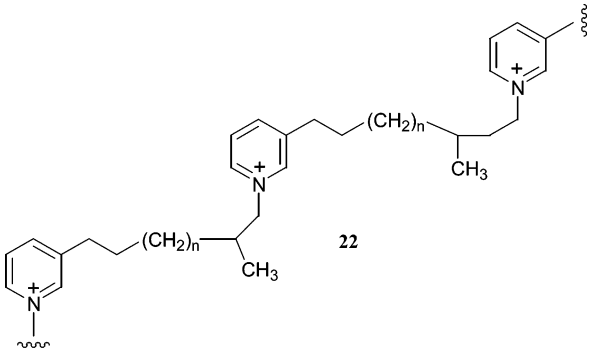
19



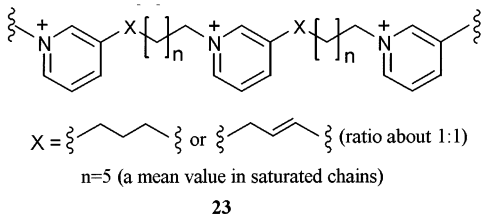
20



21

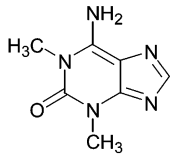


22

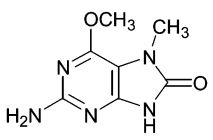


n=5 (a mean value in saturated chains)

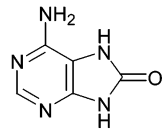
23



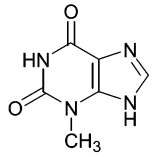
24



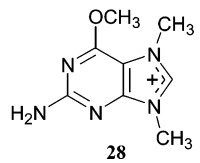
25



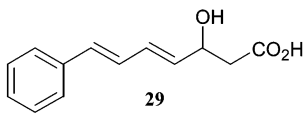
26



27



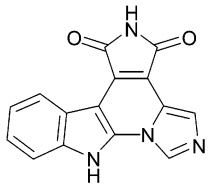
28



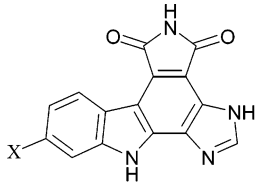
29

correspond to specific, activated feedback mechanisms that temporarily arrest cell cycle progression.[66] They operate during the G_1 checkpoint after mitosis in order to prevent anomalous DNA from being replicated, and during the G_2 checkpoint in order to avoid the segregation of abnormal chromosomes in mitosis. Therefore, the inhibition of cell checkpoints is considered an efficient mechanism to prevent DNA integrity. Checkpoint anomalies are commonly observed in tumor cells. Compounds that inhibit the G_2 checkpoint may enhance the effectiveness of DNA-damaging agents in tumors lacking the p53 tumor suppressor gene.[66,67] Although a number of nonselective natural cell cycle inhibitors are known, mainly from terrestrial and marine microbes,[68,69] marine invertebrates,[70] and terrestrial plants,[71] only a few selective cell cycle inhibitors have been reported. These include purine and staurosporine derivatives.[72]

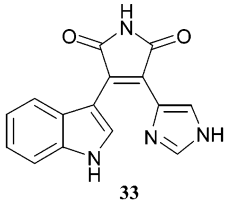
Bioassay-guided fractionation of the *D. granulatum* crude extract yielded isogranulatimide (**30**) as the only active G_2 cell cycle checkpoint inhibitor.[73] The complete structure elucidation of isogranulatimide was hampered by the small amount of **30** isolated. Additionally, the use of trifluoroacetic acid in the HPLC eluent promoted the protonation of **30** and delocalization of the imidazole π electrons and consequently enhanced the T_1 relaxation time of carbons near and those belonging to the imidazole ring. Therefore, we were unable to observe the ^{13}C NMR signals of carbons C-2, C-13, C-14, and C-16 of isogranulatimide (**30**). Although the HRFABMS data were indicative of the molecular formula of isogranulatimide as a "cyclized didemnimide",[74] we were unable to decide if the structure of the natural cell cycle inhibitor corresponded to isogranulatimide (**30**) or to granulatimide (**31**). The structural assignment was established by total synthesis of both compounds, confirming the structure of the natural product as isogranulatimide.[73] Since the final step of the synthesis involved a photocyclization, two questions were raised: (a) is granulatimide also present in the tissues of the ascidian *D. granulatum*, and (b) is isogranulatimide a natural product or an artifact of isolation resulting from the cyclization of didemnimide A (**33**) under sunlight?



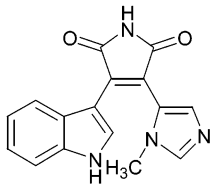
30



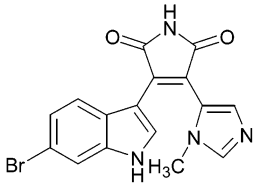
31 X = H
32 X = Br



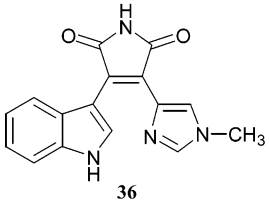
33



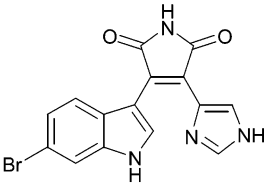
34



35



36



37

To address such questions, a new collection of *D. granulatum* was obtained. The animal was immediately stored

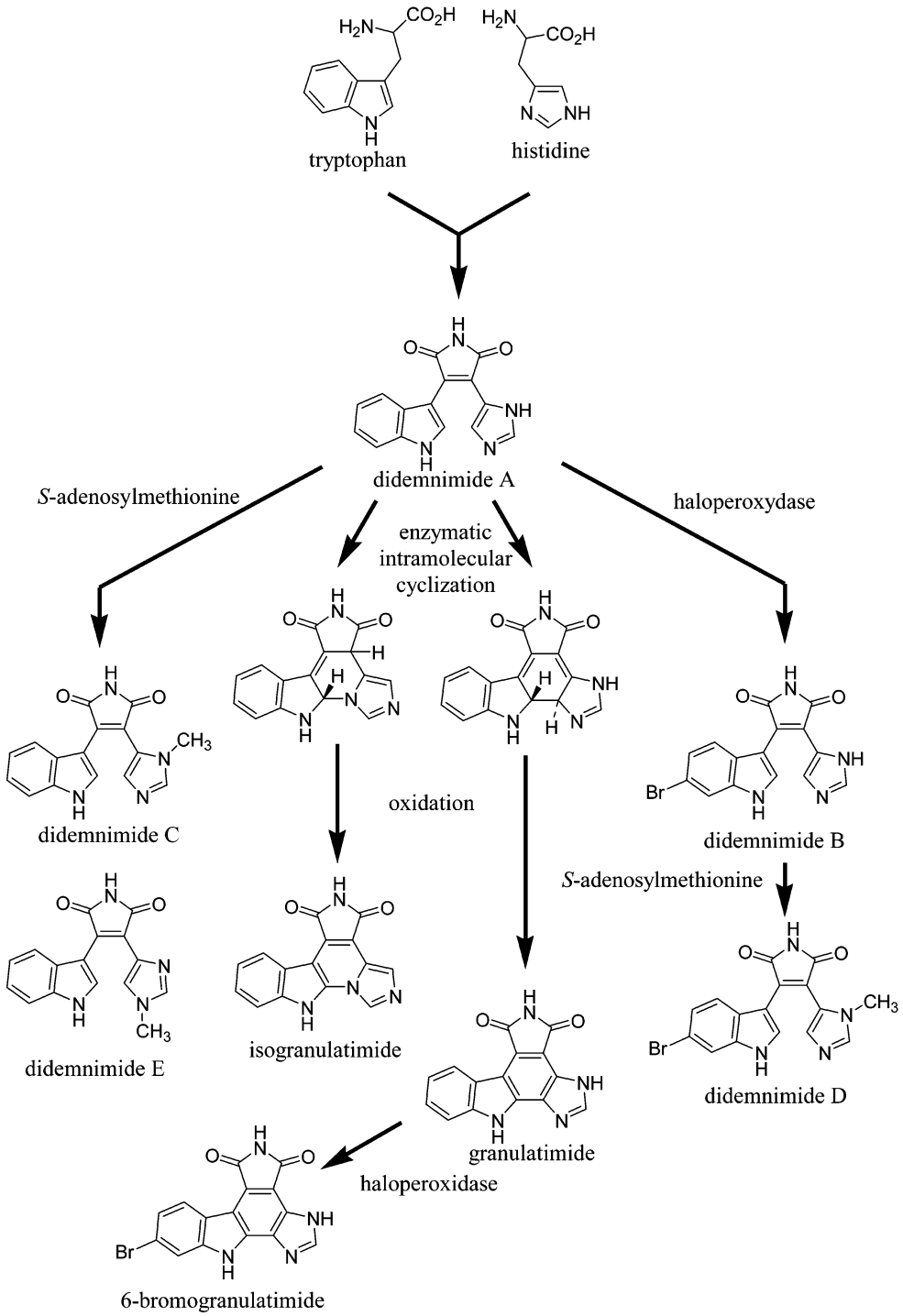


Figure 1. Postulated biogenetic pathway of the didemnimides and granulatimides.

in MeOH in dark bottles. After removal of the MeOH, the ascidian was re-extracted with DMF. A subsequent stepwise separation of the DMF crude extract by solvent partitioning and by chromatography on XAD-7, silica gel, and finally C_{18} RP-HPLC yielded natural granulatimide (**31**) and 6-bromogranulatimide (**32**).[75] The isolation of isogranulatimide, granulatimide, 6-bromogranulatimide, and didemnimides A (**33**), C (**34**), D (**35**), and E (**36**) from *D. granulatum*[73] suggests a common biogenetic pathway for this family of alkaloids (Figure 1). It is interesting to note that the synthetic photocyclization of didemnimide A yielded only granulatimide, while isogranulatimide is produced from didemnimide A via a thermal reaction.[76] Since the thermal conversion of didemnimide A to isogranulatimide requires high temperature for several hours, the formation of **30** from **33** is likely to be enzymatic, not photocyclization of didemnimide A via ambient sunlight. Indeed, normal handling of didemnimide A in ambient light conditions did not yield granulatimide in isolatable quantities. The *D. granulatum* extract contained no detectable amount of didemnimide B (**37**), a possible precursor to 6-bromogranulatimide (**32**). Therefore, 6-bromogranulatimide and granulatimide cannot be considered as isolation artifacts, although it is conceivable that the biosynthesis of granulatimide may arise via a photochemical reaction involving ambient sunlight followed by an oxidative step, either enzymatic or not (Figure 1).

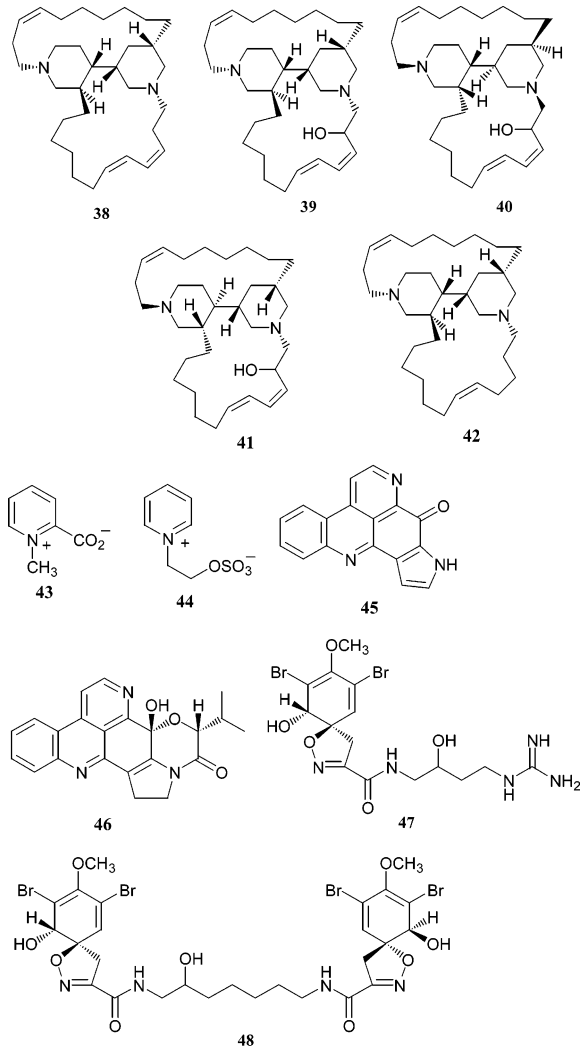
Another question is the true origin of these alkaloids, whether they are of microbial origin or produced by the ascidian. We are currently investigating the cellular local-

ization of the granulatimides and didemnimides in *D. granulatum*. The preliminary results obtained so far suggest that the compounds are located in *D. granulatum* cells, and we have been unable to detect any cells of a possible associated microorganism.[77]

Both isogranulatimide (**30**) and granulatimide (**31**) display selective G_2 checkpoint inhibitory activity at concentrations of 10 and 3 μM,[67a,78] respectively. However, the IC_{50} values for **31** and **30** were in the range 1.0−1.8 μM. The uncyclized precursor to the granulatimide type alkaloids, namely, didemnimide A (**33**), was inactive in the G_2 checkpoint inhibition assay. Other uncyclized structurally related synthetic derivatives have been prepared and were also inactive.[78] Therefore, the planar structure adopted by the granulatimide type alkaloids is essential for G_2 checkpoint inhibition, verified by molecular modeling studies.[79] At higher concentrations, a significant decrease in the number of mitotic cells was observed. This phenomenon is due to the drug toxicity. Indeed, isogranulatimide (**30**) was shown to be mildly cytotoxic (IC_{50} = 40 μM) to MCF-7 breast cancer cells. Isogranulatimide enhances the activity of γ-rays as a DNA-damaging agent, killing cells lacking p53 function. The combination of isogranulatimide (**30**) applied at a concentration of 35 mM with 6 Gy of γ-irradiation resulted in a doubling of cell death when compared to cells treated with isogranulatimide alone.[67a] Currently, the mechanism of action of the granulatimides is being investigated.[78]

As mentioned above, marine sponges belonging to the order Haplosclerida are a rich source of alkylpiperidine and alkylpyridine alkaloids.[51,52] The sponge *Arenosclera brasiliensis*, which is endemic to Brazil and has been only recently described, is almost completely devoid of associated fauna.[80] The crude methanol extract of *A. brasiliensis* displayed potent antimicrobial activity against different strains of pathogenic bacteria and *C. albicans*,[81] as well as cytotoxic activity. Fractionation of this crude extract yielded four new alkylpiperidine alkaloids: haliclonacyclamine E (**38**) as well as arenosclerins A (**39**), B (**40**), and C (**41**). Structure elucidation of compounds **38**−**41** was challenging because of poor resolution of the ^{1}H NMR spectrum due to signal superposition and line broadening. The structures could be solved by joint analysis of several two-dimensional NMR experiments, including HSQC-TOCSY, and ROESY and NOESY for assignment of relative stereochemistry.[82] Arenosclerins A−C (**39**−**41**) occur as diastereomers, as observed for other alkylpiperidine alkaloids from Haplosclerid sponges.[51,82] Halichondramine (**42**), recently isolated from a *Halichondria* sp.,[83] possesses the same relative stereochemistry as haliclonacyclamine E (**38**) and arenosclerin A (**39**). The alkaloids **38**−**41** displayed considerable cytotoxicity against human HL-60 (leukemia), L929 (fibrosarcoma), B16 (melanoma), and U130 (colon) cancer cell lines, as well as antibiotic activity against both common and resistant strains of pathogenic bacteria and *Candida albicans*.[84] Considering these and previous biological activities reported for related alkaloids,[51,84] it seems likely that such compounds constitute a component of the chemical defense of Haplosclerida sponges.

Chemical investigation of the crude extract from a sponge belonging to the genus *Aaptos* led to the isolation of two pyridinium betaines, **43** and **44** (homarine).[85] While homarine is of wide occurrence in the kingdom Animalia, pyridinium betaine **43** was previously reported from the marine sponge *Agelas dispar*.[86] The isolation of **43** from two taxonomically distant marine sponges corroborates previous assumptions that such betaines should be regarded rather as primary metabolites.[86] Additionally, 1*H*-benzo-[*de*][1,6]-naphthyridine alkaloids have not been found in the sponge *Aaptos* sp., the object of our investigation, a fact that raised a question about the occurrence of aaptamine-like alkaloids as chemotaxonomic markers.[87]

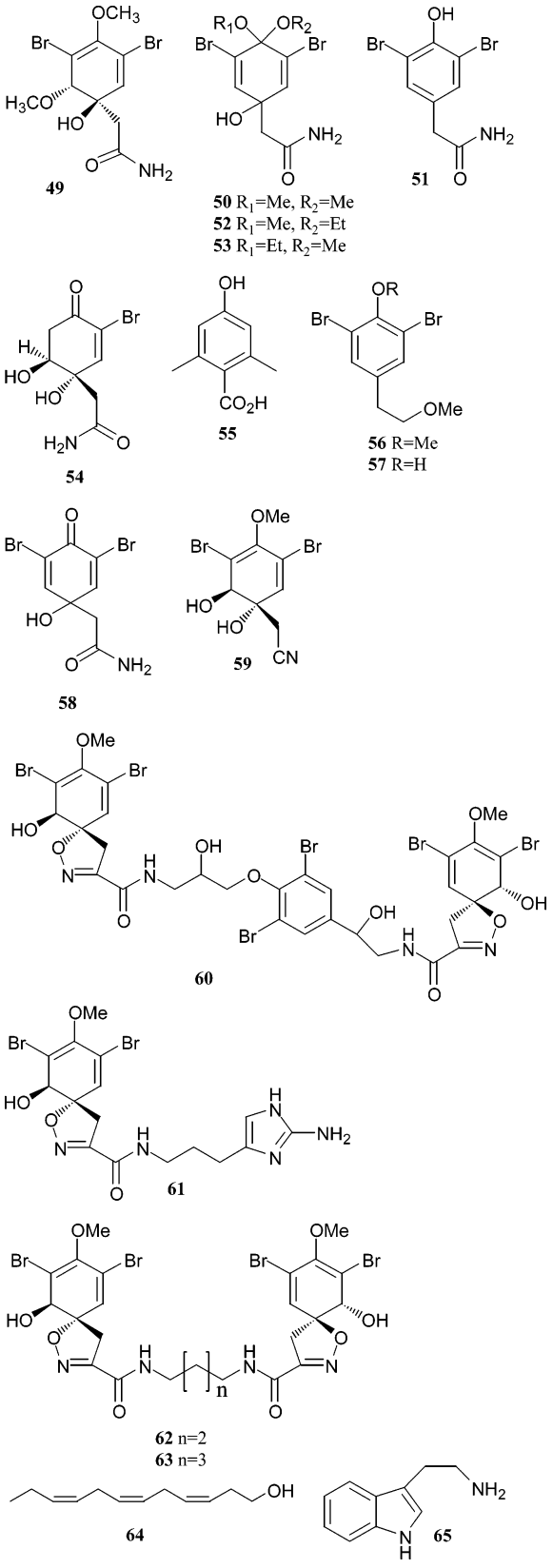


Sebastianines A (**45**) and B (**46**) are polycyclic heteroaromatic alkaloids with a pyridoacridine skeleton that have been isolated from a cytotoxic crude extract of the ascidian *Cystodytes dellechiajei*. Both compounds were identified by analysis of spectroscopic data and also by the preparation of *N*-9-methylsebastianine A.[88] Sebastianines **45** and **46** were screened against four human colon tumor (HCT) cell lines comprised of p53 and p21 knockouts as well as the parental cell line of each. Both alkaloids showed a similar pattern indicating a p53-dependent mechanism. The structure of sebastianine A was recently confirmed by total synthesis.[89] Pyridoacridine alkaloids have been isolated mainly from ascidians, but also from marine sponges, from a sea anemone, and from a marine mollusk belonging to the order Prosobranchia.[90,91] Salomon et al.[91] proposed that the occurrence of pyridoacridine-related alkaloids in animals belonging to phylogenetically distant taxa seems to be an indication that "where a bioactive metabolite can be produced in an energetically favorable manner from simple precursors, the same or similar biosynthetic pathways might have evolved in different phyla", rather than produced by associated microorganisms. A similar statement has been recently proposed by Skyler and Heathcock,[92] who suggested a "pyridoacridine family tree", on the basis of

which these authors predicted the existence and future isolation of new, biogenetically related compounds.

Recently we became aware that the Brazilian coastline harbors a high number of species of marine sponges belonging to the order Verongida.[93] Therefore, we have been interested in thoroughly investigating the chemistry of these sponges, since they very often produce bioactive bromotyrosine compounds as chemotaxonomic markers. The first species investigated was *Aplysina caissara*, which yielded three known and two new compounds, namely, caissarines A (**47**) and B (**48**).[94] Both compounds have been identified by analysis of spectroscopic data. Subsequently, we came across a 1979 study by Kelecom and Kannengiesser, who were unable to find any bromotyrosine-derived compound in specimens of the sponge *Verongida* sp. (lately identified as *Aplysina fulva*) collected in different geographic regions of Brazil.[95] Intrigued by these unexpected results, we decided to also perform a study on different populations of this sponge, collected at different sites. A partial study of a specimen of *A. fulva* collected in Baía de Todos os Santos (Salvador, Bahia State) yielded the new aplysinafulvin (**49**) along with the known 2-(3,5-dibromo-1-hydroxy-4,4-dimethoxy-2,5-cyclohexadien-1-yl)ethanamide (**50**). We are currently investigating the chemistry of this same sponge species collected in Arraial do Cabo (north coastline of Rio de Janeiro state), from which we have thus far isolated compound **50**, 2-(3′,5′-dibromo-4′-hydroxyphenyl)acetamide (**51**), isolated only once before from the marine sponge *Verongia archeri* (=*Aplysina archeri*),[96] **52** and **53**, which are artifacts of isolation, the new 2-(3-bromo-1,6-dihydroxy-4-oxo-cyclohex-2-enyl)acetamide (**54**), and the also new, but unbrominated, 4-hydroxy-2,6-dimethylbenzoic acid (**55**). Other related compounds are being isolated and identified. Considering that compounds **50**−**55** have been isolated in minute amounts from *A. fulva*, it is not surprising that Kelecom and Kannengiesser did not detect these compounds in 1979.[95] Curiously, however, we have not been able to detect any of the high molecular weight compounds previously isolated from *A. fulva*.[97] We have also isolated **56** and **57**, new compounds from another Verongida sponge, *Verongula gigantea*.

Although the secondary metabolism of sponges belonging to the order Verongida has been investigated since the early 1970s, recently some interesting questions concerning the true origin of the bromotyrosine-derived compounds have been addressed. A recent study suggested that debromoverongiaquinol (**58**) and aeroplysinin-1 (**59**) are formed through the enzymatic degradation of isofistularin-1 (**60**) and aerophobin-2 (**61**), after injury of *A. aerophoba* in an aquarium.[98] Debromoverongiaquinol is also obtained by exposure of aeroplysinin-1 in alkaline seawater or by extracting frozen-stored sponge specimens.[98,99] Specimens of *A. aerophoba* collected in shallow waters of the Mediterranean Sea yielded both **58** and **59**, while deep water specimens yielded only dibromoverongiaquinol **58**. The biosynthetic capability of the deep water sample of *A. aerophoba* to produce aeroplysinin-1 (**59**) is recovered after a few hours in an aquarium. Analysis of the sponge tissues demonstrated that exposed cells contain greater amounts of **59**, while internal, protected tissues have increased amounts of **58**.[99] Extracts obtained from *A. aerophoba* with damaged tissues exhibit a very strong deterrent activity against the fish *Thalassoma bifasciatum*. Both **58** and **59** display cytotoxic, algicidal, and antibacterial activity, while **60** and **61** are biologically inactive in such bioassays, suggesting that **58** and **59** are formed by the sponge under stress.[98,100] However, a recent investigation contested the results previously discussed, since no chemical transformation of the secondary metabolites of *Aplysina insularis* and *A. archeri* after tissue damage was observed.[101] These latter authors suggested that the preceding observations "may be the result of differential tissue extraction efficiency, hydrolysis from insoluble precursors or the heterogeneous distribution of metabolites in sponge tissue".



Our own results in which we have obtained a different chemical profile of *A. fulva* collected in different geographic areas separated by 2000 km differ in the chemistry observed from the same species collected in the Caribbean.[97] It is known that *A. aerophoba* and *A. cavernicola* harbor a high density of diverse bacteria, including *Plactomyces* sp., *δ-Proteobacteria* sp., *γ-Proteobacteria* sp., and *Bacteroides* sp., as well as Cyanophyceae and microalgae.[102,103] These bacteria inhibit the growth of terrestrial Gram-negative and Gram-positive bacteria, including antibiotic-resistant strains.[103] The possibility that the bromotyrosine-derived compounds usually observed in Verongida sponges could be biosynthesized by symbiotic and/or by associated bacteria cannot be ruled out.[104] However, early studies demonstrated that aerothionin (**62**) and homoaerothinin (**63**) are stored in spherulous cells of *Aplysina fistularis*.[105] Biosynthetic experiments performed with *A. fistularis* also demonstrated the incorporation of labeled precursors into **58** and **59**.[106] The overall scenario is further complicated by the fact that, very recently, closely related bromotyrosine-derived compounds have been isolated from a sponge belonging to the genus *Oceanapia* (order Petrosida, Demospongiae)[107] and also from a marine alga.[108] Also, we have recently isolated a bromotyrosine-derived compound from a new species of marine sponge, *Pachychalina* sp., belonging to the order Haplosclerida. Therefore, the chemistry and biosynthesis of the secondary metabolites of Verongida sponges merits further investigation.

Unpublished results obtained from additional investigations of marine sponges and ascidians include the isolation of the new alcohol dodeca-3*Z*,6*Z*,9*Z*-trien-1-ol (**64**),[109] *para*-hydroxybenzaldehyde and benzylamine from the ascidian *Botryllus giganteum* (Stolidobranchia, Styelidae), serotonin (**65**) from the sponge *Cliona delitrix*,[110] and a mixture of antiinflammatory glycerol esters of fatty acids from the sponge *Chondrilla nucula*.[111]

We have been interested in investigating not only bioactive compounds from marine sponges and ascidians but also the chemistry of their respective associated fauna, such as nudibranchs. Nudibranchs are shell-less mollusks that either produce or accumulate secondary metabolites, presumably for chemical defense. The investigation of *Doris* aff. *verrucosa* collected near the Centro de Biologia Marinha of the Universidade de São Paulo afforded (9-[5′-(methylthio)-*β*-D-xylofuranosyl]adenine (xylosyl-MTA) (**66**) from the mantle of the nudibranch.[85] The occurrence of xylosyl-MTA in the mantle of this animal strongly suggests that it is the same nudibranch species described in the Mediterranean Sea.[112] We have been unable to detect any other compound in the mantle extract of *D.* aff. *verrucosa*. GC−MS analysis of the sterol fraction from the nudibranch and its prey, the sponge *Hymeniacidon* aff. *heliophila*, revealed the occurrence of only common sterols. Recently, we obtained nine individuals of the nudibranch *Tambja eliora*. Fractionation of *T. eliora* MeOH crude extract yielded tambjamines A (**67**) and D (**68**)[113] as the major metabolites. Curiously, we isolated both **67** and **68** as their respective salts. We have been able to establish the site of protonation in tambjamines by $^{1}H-^{13}C$ HSQC, HMBC, and $^{1}H-^{15}N$ HMBC NMR methods.

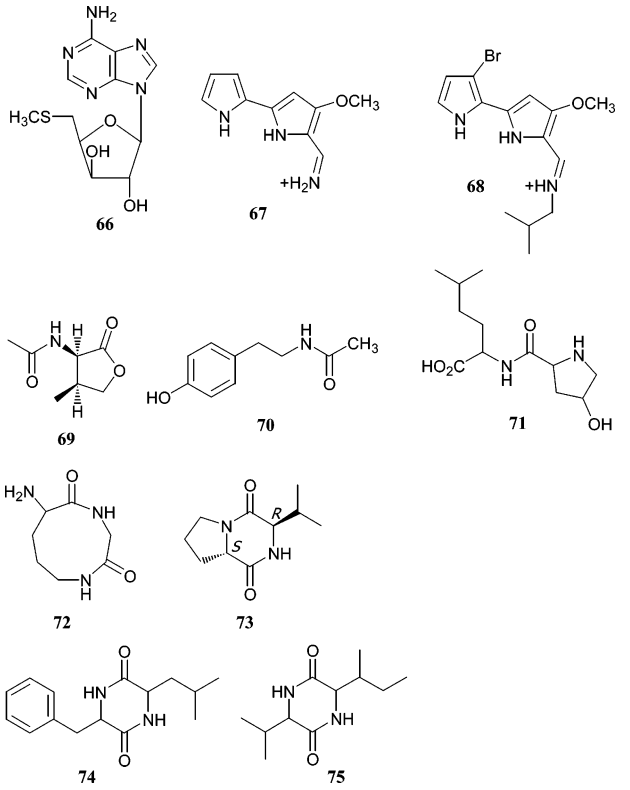
The Chemistry of Marine Microorganisms. Although terrestrial microbes have long been investigated as a source of bioactive natural products, marine microorganisms have been explored to a much lesser extent. Several research groups and pharmaceutical companies only recently have become interested in the potential of marine microbes as an untapped source of bioactive natural products. This is because it is assumed that the field of marine microbiology is still a new research field and that new microbiological methods need to be developed in order to enable the isolation and growth of marine microorganisms in artificial media. While these assumptions are partly true, recent achievements have shown that it is possible to conduct screening programs based solely on marine microbial strains. A number of excellent reviews on marine microbial-derived natural products have been published including those focusing specifically on chemistry and biological activities,[114] biosynthesis,[115] synthesis,[116] chemical ecology,[117] and chemistry and microbiology.[118]

The investigation of marine microorganisms along the Brazilian coastline to date has been minimal. The first survey was performed by Professor Tom Booth (during a sabbatical leave from the University of Manitoba, Winnipeg, Canada), who described the occurrence of various groups of marine fungi, including lignicolous fungi (50), foliicolous fungi (21), rhizosphere fungi (10), algicolous fungi (17), chytrids and thraustochytrids (18), and nematode-trapping fungi (2).[119] More recently, the mycobiota of the sandy soil of Ipanema Beach, Rio de Janeiro, Brazil, have been investigated. Altogether, 144 sand samples were collected at four different sites along the sea coast. Totals of 4285 yeast colonies and 6956 colonies of filamentous fungi were isolated using conventional media and techniques. Representatives of the filamentous fungi corresponding to a total of 1334 colonies were identified and assigned to 34 genera and 170 species. The genera of highest incidence were *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, *Paecilomyces*, *Cladosporium*, and *Acremonium*.[120]

Studies on the chemistry of marine microorganisms in Brazil started with our first collection of marine sediments in 1999. Among several strains isolated, we obtained three actinomycetes and one marine fungus. The actinomycetes were identified as *Streptomyces carpaticus* and *S. acrymicini*. Analysis of the 16S rDNA sequence of the third actinomycete strain indicated that this strain was a new Streptomycete species, which was subsequently named *Streptomyces cebimarensis* sp. nov.[121] The fungus isolated from marine sediments was identified as *Scolecobasidium arenarium*.

Chemical investigation of the ethyl acetate crude extract of the growth media of *S. cebimarensis* led to the isolation of *N*-acetyl-*γ*-hydroxyvaline lactone (**69**).[122] This amino acid derivative is closely related to acyl-homoserine lactone derivatives (acyl-HSL), which have been found in various strains of Gram-negative bacteria. Acyl-HSL are chemical mediators, known as quorum-sensing signals for the recognition of the bacterial population density.[123] Acyl-HSL derivatives are the key signaling factors for the control of bacterial aggregation, which is involved, for example, in tissue infection by *Pseudomonas aeruginosa*, in the antibiotic production by the plant-associated bacterium *P. aureofasciens*, and in the light emission by marine bacteria belonging to the genus *Vibrio*.[123,124] We are currently investigating the actual role of **69** in *S. cebimarensis*.[125]

Chemical investigation of the ethyl acetate crude extract obtained from the culture media of *S. acrimycini* led to the isolation of *N*-acetyltyramine (**70**), the new linear dipeptide leucyl-4-hydroxyproline (**71**), and the novel cyclic dipeptide 6-amino-[1,4]diazonane-2,5-dione (**72**).[126] All compounds have been identified by analysis of spectroscopic data. The cyclic core of **72** has been reported previously only once in the marinobactins, which are a group of amphiphilic siderophores recently isolated from *Marinobacter* sp.[127]



In addition to compounds **70**−**72** isolated from *S. acrimycini*, three diketopiperazines have been isolated from the fungi *S. arenarium*. The first one, *cyclo*[Pro-Val] (**73**), has been isolated several times from different animal and microbial sources.[128] The second one, *cyclo*[Leu-Phe] (**74**), is the direct biosynthetic precursor of albonoursin, previously isolated from *Streptomyces noursei* and *Streptomyces albulus*,[129] but has not been reported as a secondary metabolite. The third one is the new diketopiperazine *cyclo*[Ile-Val] (**75**), which was identified by analysis of MS and NMR data.

The results reported herein constitute the initial achievements of the first Brazilian program on natural products from marine microorganisms.

Current Integrated and Multidisciplinary Collaborative Program on the Chemistry of Bioactive Marine Natural Products. Our current research program started in 2002, when a new collaborative team was established with the aim of finding new biologically active compounds from marine invertebrates and microorganisms. A group of four bioassays was selected in order to evaluate and prioritize the choice of crude extracts to be investigated: anticancer, antibacterial against resistant strains of pathogenic bacteria, antituberculosis, and inhibitory to the enzyme adenosine phosphoribosyl transferase of *Leishmania tarentolae*. The reasons for choosing this group of bioassays is self-evident. Cancer is a growing public health problem whose estimated worldwide new incidence is over six million cases per year and is considered the second *causa mortis* factor all over the world.[130] In Brazil, it is estimated that 100 thousand people die of cancer per year.[131] All around the world the financial costs of cancer are immense, both to the individual and to society as a whole. Therefore, it is of seminal importance to provide new drug leads that may be developed into new cancer medicines. Natural products extracts continue to be a most promising source of new drug leads for cancer. They contain an exceptional diversity of chemotypes, suitable for high-throughput screening and further development by combinatorial synthesis, molecular modeling, and structure versus activity studies.[132]

The discovery of the first antibiotics in the first half of the 20th century left society and the scientific community unprepared for the emergence of antibiotic-resistant bacteria. This resistance has spread rapidly, and the infections caused by *Staphylococcus aureus* and other resistant strains of pathogenic bacteria are currently a considerable problem. Even vancomycin, which was the last resort for the treatment of infections by methillicin-resistant *S. aureus*, recently has been rendered ineffective. Clearly, the emergence and clinical significance of drug-resistant bacterial infection has created an urgent need for the rapid and continued development of new classes of antibiotics that can keep pace with the changing face of bacterial antibiotic susceptibility.

Tropical diseases, such as malaria, leishmaniasis, and Chagas' disease, are a major health problem in developing countries such as Brazil, China, India, and the African continent, affecting over 500 million people all over the world. Only a few natural and synthetic compounds have proven to be effective in treating such diseases. In addition to the frequent occurrence of acquired resistance, many of the currently available drugs present a considerable degree of toxicity, such as in the case of antimonium organometallic complexes used for the treatment of leishmaniasis.[133] Moreover, the cost of such drugs is a major problem for most of the population suffering from these infectious diseases. Therefore, there is an urgent need to find less toxic and low-cost new drugs for the treatment of tropical parasitic diseases.

Finally, the recent resurgence of tuberculosis is a worldwide health problem of major concern. Brazil experiences more than 90 000 identified infections/year, with ca. 5000 deaths/year. Brazil ranks tenth among the countries of the world in reported tuberculosis infections (about 129 000). Considering that one-third of the world's population is infected with *Mycobacterium tuberculosis*, the need for new therapeutic agents is truly beyond urgent.[134]

To date, only limited screening evaluations of extracts of Brazilian marine invertebrates have been reported.[135] During the last year we have conducted our first screen with more than 300 crude extracts obtained from marine sponges, ascidians, bryozoans, and octocorals (gorgonians) against MCF-7 (human breast cancer), B16 (murine melanoma), and HCT-8 (colon) cancer cells, resistant and nonresistant strains of *S. aureus* and other microorganisms, inhibition of the virulent strain *Mycobacterium tuberculosis* H37Rv, and inhibition of adenosine phosphoribosyl transferase of *L. major* (L-APRT). The overall results showed that marine sponges afforded the highest number of active extracts, followed by ascidian and octocoral crude extracts. Considering the small number of octocoral and bryozoan crude extracts tested (less than 15 species for each of these two groups of invertebrates), it is worth mentioning the high rate of activity found in octocoral crude extracts. The small percentage of actives in the enzymatic L-APRT inhibition bioassay may reflect its highly specific nature. Only marine sponge crude extracts showed activity against resistant strains of *S. aureus*.

In parallel, marine-derived fungal crude extracts were also submitted to the same bioassays and also to ^{1}H NMR, TLC, and photodiode array detector-HPLC analyses. The results showed that ca. 10% of marine-derived fungi exhibited cytotoxic, antibiotic, and antimycotic activities, corroborating results observed previously that marine-derived and strictly marine fungal strains have the poten-

tial for the production of bioactive secondary metabolites. Detailed reports discussing the results of our screening programs will be published in due course.

Our multidisciplinary collaborative program aims to establish a new paradigm toward the management of the Brazilian marine biodiversity, integrating research on species diversity, ecology, taxonomy, and biogeography of marine microorganisms and invertebrates. Concomitantly, a broad screening program is under development in order to explore rationally the marine resources of the Brazilian coastline and improve the ability to find new bioactive compounds that may be of interest in the development of new drug leads.

Acknowledgment. The authors are gratefully indebted to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for providing generous funding grants and to the Conselho de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for scholarships. R.G.S.B. thanks Profs. M. da Glória Blumer Soares Moreira, J. C. de Freitas, and E. Trajano, past and present directors of the Centro de Biologia Marinha of the Universidade de São Paulo, for use of many facilities for invertebrate and microbial collections. The collaborative effort with Prof. R. J. Andersen, Dr. D. E. Williams, Prof. M. Roberge, and M. Leblanc (University of British Columbia, Vancouver, Canada) and Prof. C. M. Ireland, Dr. M. K. Harper, and Dr. T. S. Bugni (Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT) through the NIH-NCI NCDDG program is greatly appreciated. Prof. B. Copp (Department of Chemistry, University of Auckland, Auckland, New Zealand) and Prof. P. Northcote (School of Chemical and Physical Sciences, Victoria University of Wellington, Wellington, New Zealand) are also acknowledged for their assistance in measuring MS and NMR data, as well as for many fruitful discussions.

References and Notes

(1) Bergmann, W. *J. Mar. Res.* **1949**, *8*, 137−141.
(2) (a) Bergmann, W.; Burke, D. C. *J. Org. Chem.* **1955**, *20*, 1501−1507. (b) Bergmann, W.; Feeney, R. J. *J. Am. Chem. Soc.* **1950**, *72*, 2809−2810. (c) Bergmann, W.; Feeney, R. J. *J. Org. Chem.* **1951**, *16*, 6, 981−987. (d) Bergmann, W.; Watkins, J. C.; Stempien, H. F. *J. Org. Chem.* **1957**, *22*, 1308−1313.
(3) Tahara, Y. *Biochem. Z.* **1909**, *30*, 255−275.
(4) Dorée, C. *Biochem. J.* **1909**, *4*, 72−106.
(5) Henze, M. *Hoppe-Seyler's Z. Physiol. Chem.* **1909**, *55*, 427−432.
(6) Lederer, E. *Bull. Soc. Chim. Biol.* **1938**, *20*, 567−634.
(7) Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 1−6.
(8) Lana, P. da C.; Camargo, M. G. de; Brogim, R. A.; Isaac V. J. *O Bentos da Costa Brasileira*; Femar: Rio de Janeiro, 1996.
(9) Ab'Saber, A. N. *Litoral do Brasil. Brazilian Coast*; Metalivros: São Paulo, 2001.
(10) Knoppers, B.; Ekau, W.; Figueiredo, A. G., Jr.; Soares-Gomes, A. In *Biologia Marinha*; Pereira, R. C., Soares-Gomes, A., Eds.; Interciência: Rio de Janeiro, 2002; pp 353−361.
(11) Collette, B. B.; Rützler, K. *Proc. 3rd Int. Coral Reef Symp.* 1977; pp 305−310.
(12) Kempf, M. *Mar. Biol.* **1970**, *5*, 213-214.
(13) Martins, L. R.; Coutinho, P. N. *Earth Sci. Rev.* **1981**, *17*, 87−107.
(14) Hechtel, G. J. In *Aspects of Sponge Biology*. Harrison, F. W., Cowden, R. R., Eds.; Academic Press: New York, 1976; pp 237−259.
(15) Schuhmacher, H. *Arrecifes Coralinos*; Ediciones Omega: Barcelona, 1978.
(16) Vermeij, G. J. *Biogeography and Adaptation. Patterns of Marine Life*; Harvard University Press: Cambridge, MA, 1978.
(17) Laborel, J. Madreporaires et Hydrocoralliaires Récifaux des Côtes Brésiliennes. Ph.D. Thesis, Université d'Aix-Marseille, France, 1967; 312 pp.
(18) Leão, Z. M. A. N.; Araújo, T. M. F.; Nolasco, M. C. *Proc. 6th Int. Coral Reef Symp.* **1988**, *3*, 339−347.
(19) Castro, C. B.; Pires, D. O. *Bull. Mar. Sci.* **2001**, *69*, 357−371.
(20) Palacio, F. J. *Bol. Inst. Ocean.* (*São Paulo*) **1982**, *31*, 69−92.
(21) Hajdu, E.; Berlinck, R. G. S.; Freitas, J. C. In *Biodiversidade do Estado de São Paulo, Brasil: Síntese do Conhecimento ao Final do Século XX*; Joly, C. A., Bicudo, C. E. M., Eds.; FAPESP: São Paulo, 1999; Vol. 3; pp 20−30.
(22) (a) Hajdu, E.; Muricy, G.; Berlinck, R. G. S.; Freitas, J. C. In *Biodiversity in Brazil: A First Approach, Proceedings of the Workshop Methods for the Assessment of Biodiversity in Plants and Animals*; Bicudo, C. E. M., Menezes, N. A., Eds.; CNPq: Brasília, 1996; pp 157−172. (b) Rocha, R. M.; Berlinck, R. G. S. In *The Biology of Ascidians, Proceeding of the First International Symposium on the Biology of Ascidians*; Sawada, H., Yokosawa, H., Lambert, C. C., Eds.; Springer-Verlag: Tokyo, 2001; pp 264−270.
(23) Tursch, B.; Barreto, H.; Sharapin, N. *Bull. Soc. Chim. Belg.* **1963**, *72*, 807−808.
(24) Kelecom, A. *Ciênc. Cult.* (*São Paulo*) **1997**, *49*, 321−330.
(25) Kelecom, A. *J. Braz. Chem. Soc.* **1998**, *9*, 101−118.
(26) Epifanio, R. D.; Maia, L. F.; Pinto, A. C.; Fenical, W. *J. Braz. Chem. Soc.* **1998**, *9*, 187−192.
(27) Martins, D. L.; Epifanio, R. D. *J. Braz. Chem. Soc.* **1998**, *9*, 586−590.
(28) Maia, L. F.; Epifanio, R. D.; Eve, T.; Fenical, W. *J. Nat. Prod.* **1999**, *62*, 1322−1324.
(29) Epifanio, R. D.; Martins, D. L.; Villaca, R.; Gabriel, R. *J. Chem. Ecol.* **1999**, *25*, 2255−2265.
(30) Epifanio, R. D.; Gabriel, R.; Martins, D. L.; Muricy, G. *J. Chem. Ecol.* **1999**, *10*, 2247−2254.
(31) Vervoort, H.; Fenical, W.; Epifanio, R. D. *J. Org. Chem.* **2000**, *65*, 782−792.
(32) Maia, L. F.; Epifanio, R. D.; Fenical, W. *J. Nat. Prod.* **2000**, *63*, 1427−1430.
(33) Epifanio, R. D.; Maia, L. F.; Fenical, W. *J. Braz. Chem. Soc.* **2000**, *11*, 584−591.
(34) Coutinho, A. F.; Chanas, B.; Souza, T. M. L.; Frugrulhetti, I. C. P. P.; Epifanio, R. D. *Heterocycles* **2002**, *57*, 1265−1272.
(35) Pitombo, L. F.; Kaiser, C. R.; Pinto, A. C. *Bol. Soc. Chil. Quim.* **1996**, *41*, 433−436.
(36) Kaiser, C. R.; Pitombo, L. F.; Pinto, A. C. *Spectrosc. Lett.* **1998**, *31*, 573−585.
(37) Kaiser, C. R.; Pitombo, L. F.; Pinto, A. C. *Spectrosc. Lett.* **2000**, *33*, 457−467.
(38) Kaiser, C. R.; Pitombo, L. F.; Pinto, A. C. *Magn. Reson. Chem.* **2001**, *39*, 147−149.
(39) Baslow, M. H.; Turlapaty, P. *Proc. West. Pharmacol. Soc.* **1969**, *12*, 6.
(40) Schmitz, F. J.; Hollenbeak, K. H.; Campbell, D. C. *J. Org. Chem.* **1978**, *43*, 3916−3922.
(41) Berlinck, R. G. S.; Ogawa, C. A.; Almeida, A. M. P.; Sanchez, M. A. A.; Malpezzi, E. L. A.; Costa, L. V.; Hajdu, E.; Freitas, J. C. *Comp. Biochem. Physiol.* **1996**, *115C*, 155−163.
(42) Berlinck, R. G. S.; Freitas, J. C. Unpublished results.
(43) Davies-Coleman, M. T.; Faulkner, D. J.; Dobowchik, G. M.; Roth, G. P.; Polson, C.; Fairchild, C. *J. Org. Chem.* **1993**, *58*, 5925−5930.
(44) Sepcic, K.; Guella, G.; Mancini, I.; Pietra, F.; DallaSerra, M.; Menestrina, G.; Tubbs, K.; Macek, P.; Turk, T. *J. Nat. Prod.* **1997**, *60*, 991−996.
(45) Malovrh, P.; Sepcic, K.; Turk, T.; Macek, P. *Comp. Biochem. Physiol.* **1999**, *124C*, 221−226.
(46) Scott, R. H.; Whyment, A. D.; Foster, A.; Gordon, K. H.; Milne, B. F.; Jaspars, M. *J. Membr. Biol.* **2000**, *176*, 119−131.
(47) Bunc, M.; Sepcic, K.; Turk, T.; Suput, D. *Pflug. Arch. Eur. J. Phys.* **2000**, *440* (Suppl.), R173−R174.
(48) Kelman, D.; Kashman, Y.; Rosenberg, E.; Ilan, M.; Ifrach, I.; Loya, Y. *Aquat. Microb. Ecol.* **2001**, *24*, 9−16.
(49) Faimali, M.; Sepcic, K.; Turk, T.; Geraci, S. *Biofouling* **2003**, *19*, 47−56.
(50) Patil, A. D.; Freyer, A. J.; Taylor, P. B.; Carté, B.; Zuber, G.; Johnson, R. K.; Faulkner, D. J. *J. Org. Chem.* **1997**, *62*, 1814−1819.
(51) Andersen, R. J.; van Soest, R. W. M.; Kong, F. In *The Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Pergamon Press: New York, 1996; Vol. 10, pp 301−355.
(52) Almeida, A. M. P.; Berlinck, R. G. S.; Hajdu, E. *Quím. Nova* **1997**, *20*, 170−185.
(53) Chehade, C. C.; Dias, R. L. A.; Berlinck, R. G. S.; Ferreira, A. G.; Costa, L. V.; Rangel, M.; Malpezzi, E. L. A.; Freitas, J. C.; Hajdu, E. *J. Nat. Prod.* **1997**, *60*, 729−731.
(54) Mitchell, S. S.; Whitehill, A. B.; Trapido-Rosenthal, H. G.; Ireland, C. M. *J. Nat. Prod.* **1997**, *60*, 727−728.
(55) Gambardella, M. T. P.; Dias, R. L. A.; Chehade, C. C.; Berlinck, R. G. S. *Acta Crystallogr.* **1999**, *55C*, 1585−1587.
(56) Lindsay, B. S.; Almeida, A. M. P.; Smith, C. J.; Berlinck, R. G. S.; Rocha, R. M.; Ireland, C. M. *J. Nat. Prod.* **1999**, *62*, 1573−1575.
(57) Lin, Y.-L.; Lee, H.-P.; Ou, J.-C.; Kuo, Y.-H. *Heterocycles* **1996**, *43*, 781−786.
(58) Madyastha, K. M.; Sridhar, G. R. *J. Chem. Soc., Perkin Trans. 1* **1999**, 677−680.
(59) Van Gennip, A. H.; De Bree, P. K.; Van der Heiden, C.; Wadman, S. K.; Haverkamp, J.; Vliegenthart, J. F. G. *Clin. Chim. Acta* **1973**, *45*, 119−127.
(60) Cho, B. P.; Evans, F. E. *Nucleic Acids Res.* **1991**, *19*, 1041−1047.
(61) Cysewski, P.; Vidal-Madjar, C.; Jordan, R.; Olinski, R. *J. Mol. Struct.* **1997**, *397*, 167−177.
(62) Faulkner, D. J. *Nat Prod. Rep.* **2002**, *19*, 1−48.
(63) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Princep, M. R. *Nat. Prod. Rep.* **2003**, *20*, 1−48.
(64) (a) Itaya, T.; Hozumi, Y.; Kanai, T.; Ohta, T. *Tetrahedron. Lett.* **1998**, *39*, 4695−4696. (b) Lindsay, B. S.; Battershill, C. N.; Copp, B. R. *J. Nat. Prod.* **1999**, *62*, 638−639. (c) Itaya, T.; Hozumi, Y.; Kanai, T.; Ohta, T. *Chem. Pharm. Bull.* **1999**, *47*, 1297−1300. (d) Copp, B. R.; Wassvik, C. M.; Lambert, G.; Page, M. J. *J. Nat. Prod.* **2000**, *63*, 1168−1169. (e) Pearce, A. N.; Babcock, R. C.; Lambert, G.; Copp, B. R. *Nat. Prod. Lett.* **2001**, *15*, 237−241.

(65) Isaacs, S.; Kashman, Y.; Benayahu, Y. *J. Nat. Prod*. **1994**, *57*, 648−649.
(66) (a) Pietenpol, J. A.; Stewart, Z. A. *Toxicology* **2002**, *181*, 475−481. (b) Nyberg, K. A.; Michelson, R. J.; Putnam, C. W.; Weinert, T. A. *Annu. Rev. Genet*. **2002**, *36*, 617−656. (c) Clarke, D. J. *J. Cell. Biochem*. **2003**, *88*, 95−103. (d) Sampath, D.; Plunkett, W. *Curr. Opin. Oncol*. **2001**, *13*, 484−490. (e) Pietenpol, J. A. *Toxicology* **2001**, *164*, 35−36. (f) Wassmann, K.; Benezra, R. *Curr. Opin. Genet. Devel*. **2001**, *11*, 83−90. (g) Walworth, N. C. *Curr. Opin. Cell Biol*. **2000**, *12*, 697−704. (h) Hixon, M. L.; Gualberto, A. *Front. Sci*. **2000**, *5*, D50−D57. (i) Kelly, T. J.; Brown, G. W. *Annu. Rev. Biochem*. **2000**, *69*, 829−880.
(67) (a) Roberge, M.; Berlinck, R. G. S.; Xu, L.; Anderson, H.; Lim, L. Y.; Curman, D.; Stringer, C. M.; Friend, S. H.; Davies, P.; Vincent, I.; Haggarty, S. J.; Kelly, M. T.; Britton, R.; Piers, E.; Andersen, R. J. *Cancer Res*. **1998**, *58*, 5701−5706. (b) Meek, D. W. *Pathol. Biol*. **2000**, *48*, 246−254.
(68) (a) Kanzaki, H.; Yanagisawa, S.; Kanoh, K.; Nitoda, T. *J. Antibiot*. **2002**, *55*, 1042−1047. (b) Sakai, Y.; Tsujita, T.; Akiyama, T.; Yoshida, T.; Mizukami, T.; Akinaga, S.; Horinouchi, S.; Yoshida, M.; Yoshida, T. *J. Antibiot*. **2002**, *55*, 863−872. (c) Ueki, M.; Teruya, T.; Nie, L.; Usami, R.; Yoshida, M.; Osada, H. *J. Antibiot*. **2001**, *54*, 1093−1095. (d) Kakeya, H.; Kageyama, S.; Nie, L.; Onose, R.; Okada, G.; Beppu, T.; Norbury, C. J.; Osada, H. *J. Antibiot*. **2001**, *54*, 850−854. (e) Honda, Y.; Ueki, M.; Okada, G.; Onose, R.; Usami, R.; Horikoshi, K.; Osada, H. *J. Antibiot*. **2001**, *54*, 10−16. (f) Tsuchiya, E.; Yukawa, M.; Miyakawa, T.; Kimura, K. I.; Takahashi, H. *J. Antibiot*. **2001**, *54*, 84−90. (g) Takatsu, T.; Ohtsuki, M.; Muramatsu, A.; Enokita, R.; Kurakata, S. *J. Antibiot*. **2000**, *53*, 1310−1312. (h) Kim, Y. B.; Ki, S. W.; Yoshida, M.; Horinouchi, S. *J. Antibiot*. **2000**, *53*, 1191−1200. (i) Ishii, T.; Hayashi, K.; Hida, T.; Yamamoto, Y.; Nozaki, Y. *J. Antibiot*. **2000**, *53*, 765−778. (j) Yoshimura, S.; Sato, B.; Kinoshita, T.; Takase, S.; Terano, H. *J. Antibiot*. **2000**, *53*, 615−622. (k) Watanabe, H.; Watanabe, H.; Usui, T.; Kondoh, M.; Osada, H.; Kitahara, T. *J. Antibiot*. **2000**, *53*, 540−545. (l) Asai, A.; Hasegawa, A.; Ochiai, K.; Yamashita, Y.; Mizukami, T. *J. Antibiot*. **2000**, *53*, 81−83. (m) Hosokawa, N.; Yamamoto, S.; Uehara, Y.; Hori, M.; S-Tsuchiya, K. S. *J. Antibiot*. **1999**, *52*, 485−490. (n) Stead, P.; Affleck, K.; Sidebottom, P. J.; Taylor, N. L.; Drake, C. S.; Todd, M.; Jowett, A.; Webb, G. *J. Antibiot*. **1999**, *52*, 89−95. (o) Osada, H. *J. Antibiot*. **1998**, *51*, 973−982. (p) Nakajima, H.; Hori, Y.; Terano, H.; Okuhara, M.; Manda, T.; Matsumoto, S.; Shimomura, K. *J. Antibiot*. **1996**, *49*, 1204−1211. (q) Cui, C. B.; Kakeya, H.; Osada, H. *J. Antibiot*. **1996**, *49*, 832−835. (r) Cui, C. B.; Kakeya, H.; Okada, G.; Onose, R.; Osada, H. *J. Antibiot*. **1996**, *49*, 527−533. (s) Cui, C. B.; Kakeya, H.; Osada, H. *J. Antibiot*. **1996**, *49*, 534−540. (t) Matsuura, N.; Onose, R.; Osada, H. *J. Antibiot*. **1996**, *49*, 361−365. (u) Cui, C. B.; Ubukata, M.; Kakeya, H.; Onose, R.; Okada, G.; Takahashi, I.; Isono, K.; Osada, H. *J. Antibiot*. **1996**, *49*, 216−219. (v) Cui, C. B.; Kakeya, H.; Okada, G.; Onose, R.; Ubukata, M.; Takahashi, I.; Isono, K.; Osada, H. *J. Antibiot*. **1995**, *48*, 1382−1384. (w) Rani, B. R.; Cui, C. B.; Ubukata, M.; Osada, H. *J. Antibiot*. **1995**, *48*, 1179−1181. (x) Shimada, Y.; Ogawa, T.; Sato, A.; Kaneko, I.; Tsujita, Y. *J. Antibiot*. **1995**, *48*, 824−830. (y) Katagiri, M.; Hayashi, M.; Matsuzaki, K.; Tanaka, H.; Omura, S. *J. Antibiot*. **1995**, *48*, 344−346. (z) Horiguchi, T.; Hayashi, K.; Tsubotani, S.; Iinuma, S.; Harada, S.; Tanida, S. *J. Antibiot*. **1994**, *47*, 545−556.
(69) (a) Akinaga, S.; Nomura, K.; Gomi, K.; Okabe, M. *J. Antibiot*. **1993**, *46*, 1767−1771. (b) Abe, K.; Yoshida, M.; Horinouchi, S.; Beppu, T. *J. Antibiot*. **1993**, *46*, 728−734. (c) Abe, K.; Yoshida, M.; Naoki, H.; Horinouchi, S.; Beppu, T. *J. Antibiot*. **1993**, *46*, 735−740. (d) Osada, H.; Koshino, H.; Kudo, T.; Onose, R.; Isono, K. *J. Antibiot*. **1992**, *45*, 189−194. (e) Nishikawa, K.; Shibasaki, C.; Uchida, T.; Takahashi, K.; Takeuchi, T. *J. Antibiot*. **1991**, *44*, 1237−1246. (f) Kuramochimotegi, A.; Kuramochi, H.; Takahashi, K.; Takeuchi, T. *J. Antibiot*. **1991**, *44*, 429−434. (g) Yoshida, M.; Hoshikawa, Y.; Koseki, K.; Mori, K.; Beppu, T. *J. Antibiot*. **1990**, *43*, 1101−1106. (h) Conradt, P.; Dittmar, K. E. J.; Schliephacke, H.; Chliephacke, H.; Trowitzschkien, W. *J. Antibiot*. **1989**, *42*, 1158−1162. (i) Takamiya, K.; Yoshida, E.; Takahashi, T.; Okura, A.; Okanishi, M.; Komiyama, K.; Umezawa, I. *J. Antibiot*. **1988**, *41*, 1854−1861.
(70) (a) Stevenson, C. S.; Capper, E. A.; Roshak, A. K.; Marquez, B.; Eichman, C.; Jackson, J. R.; Mattern, M.; Gerwick, W. H.; Jacobs, R. S.; Marshall, L. A. *J. Pharmacol. Exp. Ther*. **2002**, *303*, 858−866. (b) Hood, K. A.; West, L. M.; Rouwe, B.; Northcote, P. T.; Berridge, M. V.; Wakefield, S. J.; Miller, J. H. *Cancer Res*. **2002**, *62*, 3356−3360. (c) Erba, E.; Bassano, L.; Di Liberti, G.; Muradore, I.; Chiorino, G.; Ubezio, P.; Vignati, S.; Codegoni, A.; Desiderio, M. A.; Faircloth, G.; Jimeno, J.; D'Incalci, M. *Br. J. Cancer*. **2002**, *86*, 1510−1517. (d) Cvetkovic, R. S.; Figgitt, D. P.; Plosker, G. L. *Drugs* **2002**, *62*, 1185−1192. (e) Edler, M. C.; Fernandez, A. M.; Lassota, P.; Ireland, C. M.; Barrows, L. R. *Biochem. Pharmacol*. **2002**, *63*, 707−715. (f) Martinez, E. J.; Corey, E. J.; Owa, T. *Chem. Biol*. **2001**, *8*, 1151−1160. (g) Curman, D.; Cinel, B.; Williams, D. E.; Rundle, N.; Block, W. D.; Goodarzi, A. A.; Hutchins, J. R.; Clarke, P. R.; Zhou, B. B.; Lees-Miller, S. P.; Andersen, R. J.; Roberge, M. *J. Biol. Chem*. **2001**, *276*, 17914−17919. (h) Erba, E.; Bergamaschi, D.; Bassano, L.; Damia, G.; Ronzoni, S.; Faircloth, G. T.; D'Incalci, M. *Eur. J. Cancer* **2001**, *37*, 97−105. (i) Fukuoka, K.; Yamagishi, T.; Ichihara, T.; Nakaike, S.; Iguchi, K.; Yamada, Y.; Fukumoto, H.; Yoneda, T.; Samata, K.; Ikeya, H.; Nanaumi, K.; Hirayama, N.; Narita, N.; Saijo, N.; Nishio, K. *Int. J. Cancer* **2000**, *88*, 810−819. (j) Soni, R.; Muller, L.; Furet, P.; Schoepfer, J.; Stephan, C. *Biochem. Biophys. Res. Commun*. **2000**, 275, 877−884. (k) Dassonneville, L.; Wattez, N.; Baldeyrou, B.; Mahieu, C.; Lansiaux, A.; Banaigs, B.; Bonnard, I.; Bailly, C. *Biochem. Pharmacol*. **2000**, *60*, 527−537. (l) Bergamaschi, D.; Ronzoni, S.; Taverna, S.; Faretta, M.; De Feudis, P.; Faircloth, G.; Jimeno, J.; Erba, E.; D'Incalci, M. *Br. J. Cancer* **1999**, *79*, 267−277. (m) Izbicka, E.; Lawrence, R.; Raymond, E.; Eckhardt, G.; Faircloth, G.; Jimeno, J.; Clark, G.; Von Hoff, D. D. *Ann. Oncol*. **1998**, *9*, 981−987. (n) Bergamaschi, D.; Faretta, M.; Ronzoni, S.; Taverna, S.; DeFeudis, P.; Bonfanti, M.; Guidi, G.; Faircloth, M.; Jimeno, J.; Dincalci, M.; Erba, E. *Eur. J. Histochem*. **1997**, *41*, 63−64. (o) Zidane, M.; Pondaven, P.; Roussakis, C.; Quemener, B.; More, M. T. *Comp. Biochem. Physiol*. **1996**, *115C*, 47−53. (p) Hung, D. T.; Chen, J.; Schreiber, S. L. *Chem. Biol*. **1996**, *3*, 287−293. (q) Lopp, A.; Pihlak, A.; Paves, H.; Samuel, K.; Koljak, R.; Samel, N. *Steroids* **1994**, *59*, 274−281. (r) Mackanos, E. A.; Pettit, G. R.; Ramsdell, J. S. *J. Biol. Chem*. **1991**, *266*, 11205−11212.
(71) (a) Ansah, C.; Gooderham, N. J. *Toxicol. Sci*. **2002**, *70*, 245−251. (b) Ye, R. Q.; Bodero, A.; Zhou, B. B.; Khanna, K. K.; Lavin, M. F.; Lees-Miller, S. P. *J. Biol. Chem*. **2001**, *276*, 4828−4833. (c) Khaoustov, V. I.; Ozer, A.; Smith, J. R.; Noda, A.; Mearns, M.; Krishnan, B.; Slagle, B. L.; Yoffe, B. *Lab. Invest*. **1995**, *73*, 118−127.
(72) Newman, D. J.; Cragg, G. M.; Holbeck, S.; Sausville, E. A. *Curr. Cancer Drug Targ*. **2002**, *2*, 270−308.
(73) Berlinck, R. G. S.; Britton, R.; Piers, E.; Lim, L.; Roberge, M.; Rocha, R. M.; Andersen, R. J. *J. Org. Chem*. **1998**, *63*, 9850−9856.
(74) Vervoort, H. C.; Fenical, W.; Keifer, P. A. *J. Nat. Prod*. **1999**, *62*, 389−391.
(75) Britton, R.; de Oliveira, J. H. H. L.; Andersen, R. J.; Berlinck, R. G. S. *J. Nat. Prod*. **2001**, *64*, 254−255.
(76) Piers, E.; Britton, R.; Andersen, R. J. *J. Org. Chem*. **2000**, *65*, 530−535.
(77) Berlinck, R. G. S.; Custódio, M. R.; Seleghim, M. E. R. Unpublished results.
(78) Britton, R. The Isolation, Structure Determination and Total Synthesis of Potential Cancer Chemotherapeutic Agents from Natural Sources. Ph.D. Thesis, University of British Columbia, 2002.
(79) Camargo, A. J.; de Oliveira, J. H. H. L.; Trsic, M.; Berlinck, R. G. S. *J. Mol. Struct*. **2001**, *559*, 67−77.
(80) Muricy, G.; Ribeiro, R. *Beaufortia* **1999**, *49*, 47−60.
(81) Muricy, G.; Hajdu, E.; Araujo, F. V.; Hagler, A. N. *Sci. Mar*. **1993**, *57*, 427−432.
(82) Torres, Y. R.; Berlinck, R. G. S.; Magalhães, A.; Schefer, A. B.; Ferreira, A. G.; Hajdu, E.; Muricy, G. *J. Nat. Prod*. **2000**, *63*, 1098−1105.
(83) Chill, L.; Yosief, T.; Kashman, Y. *J. Nat. Prod*. **2002**, *65*, 1738−1741.
(84) Torres, Y. R.; Berlinck, R. G. S.; Nascimento, G. G. F.; Fortier, S. C.; Pessoa, C.; Moraes, M. O. *Toxicon* **2002**, *40*, 885−891.
(85) Granato, A. C.; Berlinck, R. G. S.; Schefer, A. B.; Magalhães, A.; Ferreira, A. G.; de Sanctis, B.; Freitas, J. C.; Migotto, A. E.; Hajdu, E. *Quim. Nova* **2000**, *23*, 594−599.
(86) Cafieri, F.; Fattorusso, E.; Taglialatela-Scafati, O. *J. Nat. Prod*. **1998**, *61*, 1171−1173.
(87) Bergquist, P. R.; Cambie, R. C.; Kernan, M. R. *Biochem. Syst. Ecol*. **1991**, *19*, 289−290.
(88) Torres, Y. R.; Bugni, T. S.; Berlinck, R. G. S.; Ireland, C. M.; Magalhães, A.; Ferreira, A. G.; Rocha, R. M. *J. Org. Chem*. **2002**, *67*, 5429−5432.
(89) Legentil, L.; Bastide, J.; Delfourne, E. *Tetrahedron Lett*. **2003**, *44*, 2473−2475.
(90) (a) Molinski, T. F. *Chem. Rev*. **1993**, *93*, 1825−1838. (b) Davidson, B. S. *Chem. Rev*. **1993**, *93*, 1771−1791. (c) Ding, Q.; Chichak, K.; Lown, J. W. *Curr. Med. Chem*. **1999**, *6*, 1−27. (d) Selvi, S. T.; Mohan, P. S. *Indian J. Chem*. **1999**, *38B*, 1118−1120. (e) Groundwater, P. W.; Munawar, M. A. *Adv. Heterocycl. Chem*. **1998**, *70*, 89−161.
(91) Salomon, C. E.; Deerinck, T.; Ellisman, M. H.; Faulkner, D. J. *Mar. Biol*. **2001**, *139*, 313−319.
(92) Skyler, D.; Heathcock, D. H. *J. Nat. Prod*. **2002**, *65*, 1573−1581.
(93) Pinheiro, U. Revisão Taxonômica de *Aplysina* Nardo, 1834 (Aplysinidae, Verongida, Porifera) na Costa Brasileira, M.Sc. Thesis, Universidade de São Paulo, 2002.
(94) Saeki, B. M., Granato, A. C., Berlinck, R. G. S., Magalhães, A., Schefer, A. B., Ferreira, A. G., Pinheiro, U. G.; Hajdu, E. *J. Nat. Prod*. **2002**, *65*, 796−799.
(95) Kelecom, A.; Kannengiesser, G. J. *An. Acad. Bras. Ciênc*. **1979**, *51*, 633−637. The authors acknowledge Prof. Kelecom for providing the actual taxonomy of the sponge concerned.
(96) Chib, J. S.; Stempien, M. F., Jr.; Mierzwa, R. A.; Ruggieri, G. D.; Nigrelli, R. F. *J. Pharm. Sci*. **1978**, *67*, 264−265.
(97) Ciminiello, P.; Constantino, V.; Fattorusso, E.; Magno, S.; Mangoni, A. *J. Nat. Prod*. **1994**, *57*, 705−712.
(98) Weiss, B.; Elbrächter, M.; Kirchner, M.; Proksch, P. *Biochem. Syst. Ecol*. **1996**, *24*, 1−12.
(99) Ebel, R.; Brenzinger, M.; Kunze, A.; Gross, H. J.; Proksch, P. *J. Chem. Ecol*. **1997**, *23*, 1451−1462.
(100) Koulman, A.; Proksch, P.; Ebel, R.; Beekman, A. C.; Van Uden, W.; Konings, A. W. T.; Pedersen, J. A.; Pras, N.; Woerdenbag, H. J. *J. Nat. Prod*. **1996**, *59*, 591−594.
(101) Puyana, M.; Fenical, W.; Pawlik, J. R. *Mar. Ecol. Prog. Ser*. **2003**, *246*, 127−135.
(102) Vacelet, J. *J. Microsci. Biol. Cell*. **1975**, *23*, 271−288.
(103) Hentschel, U.; Schmid, M.; Wagner, M.; Fieseler, L.; Gernet, C.; Hacker, J. *FEMS Microbiol. Ecol*. **2001**, *35*, 105−113.
(104) Friedrich A. B.; Merkert, H.; Fendert, T.; Hacker, J.; Proksch, P.; Hentschel, U. *Mar. Biol*. **1999**, *134*, 461−470.

(105) Thompson, J. E.; Barrow, K. D.; Faulkner, D. J. *Acta Zool*. **1983**, *64*, 199−210.
(106) Carney, J. R.; Rinehart, K. L. *J. Nat. Prod*. **1995**, *58*, 971−985.
(107) Nicholas, G. M.; Newton, G. L.; Fahey, R. C.; Bewley, C. A. *Org. Lett*. **2001**, *3*, 1543−1545.
(108) Meragelman, K. M.; McKee, T. C.; Boyd, M. R. 43rd Annual Meeting of the American Society of Pharmacognosy and 3rd Monroe Wall Symposium, July 27−31, 2002, New Brunswick, NJ, abstract O27.
(109) Glassy solid; UV (MeOH) λ_{max} (ϵ) 274 (100); IR (film) ν_{max} 3246, 2920, 2851, 1656, 1610, 1585, 1567, 1460, 1406, 1218, 1175, 1108 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.69 (2H, t, J = 7.0 Hz, H_2-1), 2.30 (2H, m, H_2-2), 5.35 (1H, m, H-3), 5.34 (1H, m, H-4), 2.78 (1H, m, H_2-5), 5.30 (1H, m, H-6), 5.28 (1H, m, H-7) 2.78 (2H, m, H_2-8), 5.32 (1H, m, H-9), 5.32 (1H, m, H-10), 2.04 (2H, dd, J = 7.0 and 7.3 Hz, H_2-11), 0.93 (3H, t, J = 7.5 Hz, H_3-12); ^{13}C NMR (DMSO-d_6, 100.1 MHz) δ 64.9 (t, C-1), 27.2 (t, C-2), 126.0 (d, C-3), 129.1 (d, C-4), 25.2 (t, C-5), 127.9 (d, C-6), 127.6 (d, C-7), 25.0 (t, C-8), 126.8 (d, C-9), 131.4 (d, C-10), 19.9 (t, C-11), 14.0 (q, C-12); HRFABMS *m/z* 181.15899, calcd for $C_{12}H_{21}O$, 181.15141.
(110) Serotonin has been previously isolated from the holothurian *Pentacter crassa* (see: Gregson, R. P.; Marwood J. F.; Quinn R. J. *Experientia* **1981**, *37*, 930−931) and more recently from the sponge *Hyrtius reticulatus* (Salmoun, M.; Devijver, C.; Daloze, D.; Braekman, J. C.; van Soest, R. W. M. *J. Nat. Prod*. **2002**, *65*, 1173−1176).
(111) Desiderá, C.; Bensuaski, A. C.; Freitas, J. C.; Berlinck, R. G. S. Unpublished results.
(112) Cimino, G.; Sodano, G. *Top. Curr. Chem*. **1993**, *167*, 77−115.
(113) Carté, B.; Faulkner, D. J. *J. Org. Chem*. **1983**, *48*, 2314−2318.
(114) (a) Faulkner, D. J. *Nat. Prod. Rep*. **2002**, *19*, 1−48. (b) Fenical, W.; Jensen, P. R. In *Fungi in Marine Environments*; Hyde, K. D., Ed.; *Fungal Diversity Research Series*, 2002; Vol. 7, pp 293−315. (c) Bernan, V. S. In *Marine Chemical Ecology*; McClintock, J. B., Baker, B. J., Eds.; CRC Press: Boca Raton, 2001; pp 567−592. (d) Holler, U.; Wright, A. D.; Matthee, G. F.; König, G. M.; Draeger, S.; Aust, H. J.; Schulz, B. *Mycol. Res*. **2000**, *104*, 1354−1465. (e) Verbist, J.; Sallenave, C.; Ponchus, Y. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam; 2000; Vol. 24, pp 979−1092. (f) Jaspars, M. *Chem. Ind*. **1999**, 51−55. (g) Biabani, M. A. F.; Laatsch, H. *J. Prakt. Chem. Ztg*. **1998**, *340*, 589−607. (h) Bernan, V. S.; Greenstein, M. *Adv. Appl. Microbiol*. **1997**, *43*, 57−90. (i) Pietra, F. *Nat. Prod. Rep*. **1997**, *14*, 453−464. (j) Scheuer, P. J. *J. Nat. Toxins* **1996**, *5*, 181−189. (k) Scheuer, P. J. *Adv. Exp. Mar. Biol*. **1996**, *391*, 1−8. (l) Carté, B. K. *Bioscience* **1996**, *46*, 271−286. (m) König, G. M.; Wright, A. D. *Planta Med*. **1996**, *62*, 193-211. (n) Liberra, K.; Lindquist, U. *Pharmazie* **1995**, *50*, 583−588. (o) Faulkner, D. J. *Chem. Br*. **1995**, 680−684. (p) Kobayashi, J.; Ishibashi, M. *Chem. Rev*. **1993**, *93*, 1753−1769. (q) Fenical, W. *Chem. Rev*. **1993**, *93*, 1673−1683. (r) Holmes, C. F. B.; Boland, M. P. *Curr. Opin. Struct. Biol*. **1993**, *3*, 934−943.
(115) (a) Moore, B. S. *Nat. Prod. Rep*. **1999**, *16*, 653−674. (b) Garson, M. J. *Chem. Rev*. **1993**, *93*, 1699−1733. (c) Nakagawa, A.; Omura, S. *J. Antibiot*. **1996**, *49*, 717−741.
(116) (a) Norcross, R. D.; Paterson, I. *Chem. Rev*. **1995**, *95*, 2041−2114. (b) Kobayashi, J.; Ishibashi, M.; Hideuki, S. *Synth. Org. Chem. Jpn*. **1992**, *50*, 772−785.
(117) (a) Faulkner, D. J.; Harper, M. K.; Haygood, M. G.; Salomon, C. E.; Schmidt, E. W. In *Drugs from the Sea*; Fusetani, N., Ed.; Karger: Basel, 2001; pp 107−119. (b) Armstrong, E.; Boyd, K. G.; Burgess, J. G. *Biotechnol. Annu. Rev*. **2000**, *6*, 221−241. (c) Osinga, R.; Tramper, J.; Wijffels, R. H. *Mar. Biotechnol*. **1999**, *1*, 509−532. (d) Potin, P.; Bouarab, K.; Kupper, F.; Kloareg, B. *Curr. Opin. Microbiol*. **1999**, *2*, 276−283. (e) Haygood, M. G.; Schmidt, E. W.; Davidson, S. K.; Faulkner, D. J. *Mol. Microbiol. Biotechnol*. **1999**, *1*, 33−43. (f) Haygood, M. G.; Davidson, S. K. In *New Developments in Marine Biotechnology*, *Proc. Int. Mar. Biotech. Conf*.; Le Gal, Y., Halvorson, H. O., Eds.; Plenum: New York, 1998; pp 281−284. (g) Faulkner, D. J.; He, H. Y.; Unson, M. D.; Bewley, C. A.; Garson, M. J. *Gazz. Chem. Ital*. **1993**, *123*, 301−307.
(118) (a) Jensen, P. R.; Fenical, W. *J. Ind. Microbiol. Biotechnol*. **1996**, *17*, 346−351. (b) Jensen, P. R.; Fenical, W. *Annu. Rev. Microbiol*. **1994**, *48*, 559−584. (c) Fenical, W.; Jensen, P. R. In *Marine Biotechnology. Pharmaceutical and Bioactive Natural Products*; Attaway, D. H., Zaborsky, O. R., Eds.; Plenum Press: New York, 1993; pp 419−457.
(119) Booth, T. *Rev. Microbiol*. (*São Paulo*) **1979**, *10*, 123−138.
(120) Sarquis, M. I. D.; de Oliveira, P. C. *J. Basic Microbiol*. **1996**, *36*, 51−58.
(121) Hernandez, I. L. C.; Berlinck, R. G. S.; Godinho, M. J. L.; Manfio, G. P.; de Oliveira, V. M.; Kroppenstedt, R. Unpublished results.
(122) Hernandez, I. L. C.; Godinho, M. J. L.; Magalhães, A.; Schefer, A. B.; Ferreira, A. G.; Berlinck, R. G. S. *J. Nat. Prod*. **2000**, *63*, 664−665.
(123) Strauss, E. *Science* **1999**, *284*, 1302−1304.
(124) Beppu, T. *Trends Biotechnol*. **1995**, *13*, 264−269.
(125) Hernández, I. L. C., Padilla, G.; Berlinck, R. G. S. Unpublished results.
(126) Hernández, I. L. C.; Camargo, G. S. P.; Macedo, M. L.; Berlinck, R. G. S.; Ferreira, A. G.; Sponchiado, S. R. P.; Godinho, M. J. L.; Barata, M. *J. Braz. Chem. Soc*., submitted.
(127) (a) Martinez, J. S.; Zhang, G. P.; Holt, P. D.; Jung, H.-T.; Carrano, C. J.; Haygood, M. G.; Butler, A. *Science* **2000**, *287*, 1245−1247. (b) Xu, G.; Martinez, J. S.; Groves, J. T.; Butler, A. *J. Am. Chem. Soc*. **2002**, *124*, 13408−13415.
(128) (a) Adamczeski, M.; Reed, A. R.; Crews, P. *J. Nat. Prod*. **1995**, *58*, 201−208. (b) Jayatilake, G. S.; Thornton, M. P.; Leonard, A. C.; Grimwade, J. E.; Baker, B. J. *J. Nat. Prod*. **1996**, *59*, 293−296. (c) Schmitz, F. J.; Vanderah, D. J.; Hollenbeak, K. H.; Enwall, C. E. L.; Gopichand, Y.; Sengupta, P. K.; Hossain, M. B.; van der Helm, D. *J. Org. Chem*. **1983**, *48*, 3941−3945. (d) Stierle, A. C.; Cardellina II, J. H.; Singleton, F. L. *Experientia* **1988**, *44*, 1021.
(129) Kanzaki, H.; Yanagisawa, S.; Nitoda, T. *J. Antibiot*. **2000**, *53*, 1257−1264.
(130) (a) http://www.cancer.org. (b) http://www.nci.nih.gov.
(131) Anonymous. *Ciência Hoje* (*São Paulo*) **1998**, *24*, 40−41.
(132) Newman, D. J.; Cragg, G. M.; Snader, K. M. *J. Nat. Prod*. **2003**, *66*, 1022−1037.
(133) Chan-Bacab, M. J.; Peña-Rodriguez, L. M. *Nat. Prod. Rep*. **2001**, *18*, 674−688.
(134) (a) Stead, W. W. *Clin. Chest Med*. **1997**, *18*, 76−165. (b) Daniel, T. M.; Bates J. H.; Downes K. A. ARS Press: Washington, 1994; pp 13−23. (c) Ayvazian, L. F. In *History of Tuberculosis. Tuberculosis−a Comprehensive International Approach;* Reichman L. B., Hershfield E. S., Eds.; Marcel Dekker: New York, 1993; pp 1−13.
(135) (a) Rangel, M.; de Sanctis, B.; de Freitas, J. C.; Polatto, J. M.; Granato, A. C.; Berlinck, R. G. S.; Hajdu, E. *J. Exp. Mar. Biol. Ecol*. **2001**, *262*, 31−40. (b) Monks, N. R.; Lerner, C.; Henriques, A. T.; Farias, F. M.; Schapoval, E. E. S.; Suyenaga, E. S.; da Rocha, A. B.; Schwartzmann, G.; Mothes, B. *J. Exp. Mar. Biol. Ecol*. **2002**, *281*, 1−12. (c) Jimenez, P. C.; Fortier, S. C.; Lotufo, T. M. C.; Pessoa, C.; Moraes, M. E. A.; Moraes, M. O.; Costa-Lotufo, L. V. *J. Exp. Mar. Biol. Ecol*. **2003**, *287*, 93−101.

NP0304316