A Quarter Century of Marine Biodiscovery in Algoa Bay, South Africa

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ABSTRACT: Algoa Bay, the largest crenulate bay on the southeastern coast of South Africa, is currently one of the most well-studied marine ecosystems in southern Africa. A plethora of endemic marine invertebrates inhabits the benthic reefs on the western edge of the Bay in close proximity to South Africa's sixth largest city. Over the past 25 years, South African marine natural products chemists, together with international collaborators from the US National Cancer Institute and other US institutions, have focused their attention on Algoa Bay's benthic marine invertebrates as a potential source of new anticancer compounds. This review commemorates a quarter of a century of marine biodiscovery in Algoa Bay and presents the structures and bioactivities of 49 new and 36 known specialized metabolites isolated from two molluscs, eight ascidians, and six sponges. Thirty-nine of these compounds were cytotoxic to cancer cells *in vitro* with 20 exhibiting moderate to potent cytotoxicity. Six other compounds exhibited antimicrobial activity. Foremost among the potential anticancer compounds is mandelalide A (**38**) from the Algoa Bay ascidian *Lissoclinum* species.

Mandelalide A
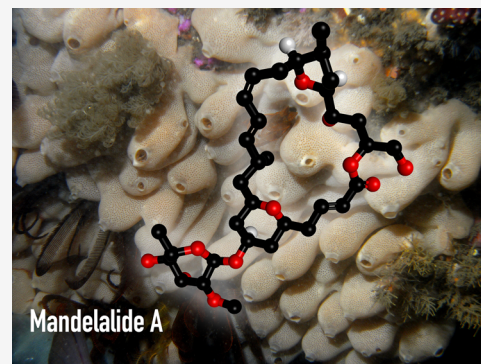
■ INTRODUCTION

The search for natural products with medicinal potential from South African marine organisms began with large-scale collections of marine invertebrates from reefs off the southern Cape coast by Pettit (Arizona State University, USA) during the 1970s.[1,2] Targeting the discovery of new anticancer drugs, Pettit and co-workers isolated and identified two cohorts of novel marine natural products with potent, selective *in vitro* cancer cytotoxicity: the cephalostatins from the marine tubeworm *Cephalodiscus gilchristi*,[3,4] and the spongiostatins from the "wall sponge" *Spirastrella spinispirulifer*.[5] Despite an increasing global interest in marine organisms as a source of new pharmaceuticals, the medicinal potential of South Africa's vast, and largely endemic, marine resources remained unexplored during the 1980s.[2] However, at the beginning of the following decade, South African-based marine natural products chemistry research found a sustainable foothold at Rhodes University.[6]

A quarter of a century ago, Algoa Bay (Figure 1) became the primary research focus area of the Rhodes University marine natural products chemists following the first collections of the sea hare *Aplysia dactylomela* from the intertidal zone at Cape Recife in March 1998.[7] The choice of Algoa Bay as the main study site for Rhodes University marine natural products researchers is fourfold. First is the unique and rich biodiversity of the filter-feeding marine invertebrates inhabiting western Algoa Bay's benthic, nearshore (−4 to −20 m) reefs, which are of particular interest to chemists searching for new biologically active marine natural products. Second, valuable marine

Figure 1. Map of Algoa Bay, South Africa. Inset: The position of Algoa Bay on the southern coast of South Africa in relation to the Agulhas Current and the continental shelf (Agulhas Bank).

invertebrate taxonomic expertise is available at Nelson Mandela University (NMU), previously the University of Port Elizabeth, and the Elwandle Node of the South African

Environmental Observation Network (SAEON). Third, ready assistance is available to natural products chemists to collect marine invertebrates via scuba, initially through the NMU Department of Zoology's Research Diving Unit and more recently with remotely operated vehicles (ROVs), operated by SAEON. Fourthly, the proximity (120 km) of Algoa Bay to Rhodes University facilitates resource-efficient investigation and preservation of fresh, intact marine samples.

OVERVIEW OF THE ALGOA BAY MARINE ECOSYSTEM

Algoa Bay is the furthest east and the largest of several, eastward-facing, crenulate bays that feature prominently in the topography of the southeastern Cape coast of South Africa (Figure 1).[8] Crenulate bays have a log-spiral shape perpendicular to the main wave direction and occur when a sediment-constrained stretch of coastline separates two erosion-resistant headlands.[9] The two rocky headlands Cape Recife and Cape Padrone (separated by approximately 70 km) mark the western and eastern extremities of the mouth of Algoa Bay. The Swartkops and Sundays Rivers drain the semiarid hinterland of Algoa Bay and provide limited sediment input into the Bay. The large Alexandria and Woody Cape sand dunes dominate the eastern edges of Algoa Bay, while the Nelson Mandela Metropole, incorporating the large industrial city of Gqeberha (formerly Port Elizabeth), lines the western periphery of the Bay (Figure 1).

The sea floor of Algoa Bay is relatively shallow and slopes gently toward the south–southeast at an angle of 0.15°, to reach a maximum depth of 73 m at the entrance to the Bay.[8,10] In common with the majority of the global ocean seabed, the sea floor of Algoa Bay mostly comprises unconsolidated marine sediments.[11] However, exposed Table Mountain Group sandstone bedrock, protruding through these marine sediments, gives rise to a complex array of shallow (−10 to −30 m) benthic rocky reefs, confined to the western inshore region around Cape Recife and in the mouth of the bay, particularly around Riy Bank and a small area surrounding Bird Island.[8] A diverse plethora of filter-feeding marine invertebrates inhabits these benthic reefs.[12] Recently, Truter et al.[11] comprehensively surveyed the epi-benthic diversity of the extensive unconsolidated marine sediments in Algoa Bay. A total of 106 epibenthic species, dominated by polychaete worms, anthozoans, hydrozoans, and crinoids, were found at the 13 monitoring stations set up across the Bay. This was one of the first comprehensive surveys of epibenthic diversity in the Southern Hemisphere and suggested that depth, bottom current speed, temperature, and differences in the sediment substrate were the major physical drivers responsible for the variation in the epibenthic diversity observed at the different stations.[11] Filter-feeding sponges, ascidians, and octocorals, common on the inshore reefs of Algoa Bay, are a rich source of bioactive specialized metabolites and are of greater interest than cnidarians, crinoids, and hydroids to marine natural products chemists with a marine biodiscovery agenda.

Algoa Bay is situated in a dynamic oceanographic transition zone between the large, warm, southwestward flowing Agulhas Current (the predominant oceanographic feature of the region) and the southeastern African continental shelf at a point where the shelf begins to widen to form the Agulhas Bank (Figure 1 inset).[10] The Bay's wide mouth allows free exchange of onshore and offshore water, and a combination of the episodic meandering of the Agulhas Current, prevailing

wind conditions, and various currents within the Bay dictates the circulation of seawater into, out of, and around the Bay.[13−15] Cold water upwelling events along the edge of the Agulhas Current[13] and at the two headlands,[16] coincident with the dominant easterly winds in summer, can drive cold water into Algoa Bay.[14,17] In addition to cold water upwelling events, bodies of warm water from the upper layers of the Agulhas Current can also penetrate into the shallow and nearshore regions of Algoa Bay,[10,15] affecting the water temperature profile and currents within the Bay.[14] In the nearshore zone, wind and wave action cause the northward transport of sediment in Algoa Bay,[18] while prevailing winds, in combination with wave action, drive complex nearshore current systems that play an important role in the dispersal of the eggs and larvae of marine organisms.[19]

MARINE BIODISCOVERY IN ALGOA BAY

In common with the earliest marine bioprospecting off the southern African coast, the search for potential new anticancer treatments has been the driving force behind marine biodiscovery in Algoa Bay over the past quarter century. Rhodes University marine natural products chemists, in collaboration with marine biologists at NMU and the Coral Reef Research Foundation (CRRF) from Palau, Micronesia, made three extensive scuba collections of marine invertebrates and algae from the reefs on the western edge of Algoa Bay over the period 1998−2000. The three marine invertebrate and algal collections complied with the terms of a collection agreement and a Memorandum of Understanding reached between Rhodes University, the United States National Cancer Institute NCI, and South Africa's marine and coastal management authorities.[20] The agreements stipulated equitable benefit sharing from any commercial products derived by the NCI from discoveries made due to the collection of Algoa Bay invertebrates.[21]

The NCI's natural products extraction facilities in Frederick, Maryland, prepared aqueous and organic solvent extracts from the 852 marine invertebrates and algae collected in Algoa Bay (1998−2000) and screened all the extracts in the NCI 60 human tumor cell line screen. Only one of the Algoa Bay marine invertebrate extracts (from a *Pseudodistoma* species of an ascidian) exhibited sufficient cytotoxicity in this preliminary screening program to justify further chemical investigation by researchers at the NCI.[22] The weak cytotoxicity[23] of the purified active component of the *Pseudodistoma* extract precluded its further development as an anticancer lead compound. Nonetheless, marine invertebrate material collected during this collaboration with the NCI subsequently underpinned numerous other marine natural products chemistry research projects at Rhodes University, the results of which are presented in this review.

Interestingly, the NCI's Program for Natural Products Discovery has recently shown that further solid phase, chromatographic prefractionation of natural product extracts significantly increases the probability of uncovering therapeutic lead compounds in large-scale high-throughput screening (HTS) programs.[24] The NCI's Natural Product Extract Repository, situated in Frederick, Maryland, houses one of the world's largest collections of natural product extracts (>230 000 extracts).[21] Prefractionation of the Algoa Bay marine invertebrate and algal extracts in storage at the NCI's Natural Product Repository is currently underway, and the prefractionated material will be freely available in the near

future for further drug discovery screening (O'Keefe, personal communication).

Underwater photographs of collected marine invertebrates obtained over two decades via the Rhodes/NMU/NCI/CRRF collaboration have provided an important visual record of the marine invertebrate biodiversity on the reefs at Riy Bank and surrounding Cape Recife. The CRRF also facilitated the taxonomic identification of the majority of the marine invertebrates collected in Algoa Bay by leading international invertebrate taxonomists. The South African Institute for Aquatic Biodiversity (SAIAB) in Makhanda has curated the voucher specimens and the collection details of >1500 Algoa Bay marine invertebrates collected during 25 years of marine natural products research at Rhodes University. This marine invertebrate material remains a valuable resource for ongoing and future marine invertebrate biodiversity studies in Algoa Bay.

An Inter-Institutional Marine Anti-Cancer and Anti-AIDS Drug Discovery Program between Rhodes University and Scripps Institution of Oceanography (SIO) in La Jolla, California, was initiated in 2001.[25] This program formed part of a competitive National Cooperative Drug Discovery Group (NCDDG) research grant secured from the NCI.[26] The South African component of the NCDDG was supported by the South African National Research Foundation (NRF) and the Department of Environmental Affairs and Tourism (Marine and Coastal Management Coordination Branch). As part of this program, additional samples of marine invertebrates were collected in Algoa Bay in 2002 in collaboration with researchers from SIO. The invertebrate samples remained in frozen storage at Rhodes University, and the resources provided through the NCDDG grant enabled in-house marine extract preparation on-site. The NCDDG collaboration also facilitated provision of the Algoa Bay marine invertebrate extracts prepared at Rhodes University to the drug discovery HTS at Bristol Myers Squibb (BMS) in the USA. Although none of the marine invertebrate extracts was active in the BMS HTS programs, the extracts provided material for ancillary marine natural products chemistry research projects at Rhodes University. Algoa Bay is an area of high endemic marine ascidian diversity.[27] Accordingly, Rhodes University and NMU researchers carried out several, targeted scuba collections of ascidians (2004−2015). Ascidian material, collected as part of this collaboration, led to the discovery of several new bioactive marine natural products with potent cancer cytotoxicity, as described below.

Symbiotic marine microorganisms can play a significant role in the biosynthesis of the specialized metabolites associated with their marine invertebrate hosts.[28−31] Investigations of the biosynthetic source of the pyrroloiminoquinone metabolites occurring in Algoa Bay latrunculid sponges have been ongoing for nearly a decade and are summarized here. Recently, there have been reports of extracts of an Algoa Bay sponge and sponge symbionts producing specialized metabolites of unknown chemical structure with antibiotic activity.[32,33] In addition, several reports have emerged describing the isolation of bioflocculant glycoproteins with undetermined chemical structures from bacteria isolated from Algoa Bay sediments.[34−39] In the absence of definitive chemical structures for the metabolites responsible for the antibiotic and bioflocculant activities, this research is not discussed further in this small-molecule chemistry-focused review.

Our understanding of the natural product molecular diversity produced by Southern Africa's vast plethora of largely endemic marine invertebrates, algae, and micro-organisms is still in its infancy.[1,2] Quantifiable cytotoxicity to various cancer cell lines and inhibition of cancer molecular targets have dominated the marine biodiscovery research in Algoa Bay over the past quarter of a century. However, there is also an intrinsic value in exposing the marine molecular diversity that may not exhibit immediate biomedical potential in the limited assays applied or may possess an unknown ecological role. Accordingly, the first marine natural products investigations in Algoa Bay focused on two marine molluscs, known to sequester specialized metabolites from their diet of red algae and marine octocorals, respectively. These investigations provided an important snapshot of the marine natural product biodiversity in Algoa Bay and are included here as an introduction to the marine biodiscovery research program that subsequently unfolded in Algoa Bay. The molecular diversity and potential biomedical applications of secondary metabolites isolated from Algoa Bay ascidians and sponges are presented chronologically within the two respective sections of this review. The cytotoxicity and mechanism of action of the mandelalides from the ascidan *Lissoclinum* sp., in particular, are highlighted as the most significant discovery thus far from the ongoing marine biodiscovery program in Algoa Bay.

■ BIOACTIVE METABOLITES FROM ALGOA BAY MARINE MOLLUSCS

The natural product investigations of Algoa Bay marine molluscs have focused on shell-less gastropod molluscs from the subclass Heterobranchia (formerly Opisthobranchia). The loss of a shell makes these molluscs susceptible to predation, and to counter this vulnerability, many sea hares and nudibranchs (sea slugs) have evolved an ability to sequester toxic metabolites from their diet and store them in their outer mantle tissue as a form of acquired chemical defense.[40−42] A small number of nudibranchs are also able to biosynthesize their chemical defense metabolites *de novo*.[42] Many shell-less molluscs exhibit aposematic coloration to warn potential predators of their toxicity and are thus relatively easily located on benthic reefs. Natural product investigations of the sequestered chemistry of shell-less marine molluscs provide a direct chemical link to their prey species and afford a useful overview of the range of bioactive specialized metabolites available in a marine ecosystem.

Aplysia dactylomela. *A. dactylomela* (Figure 2) is a large (ca. 100−400 mm), circumtropical sea hare that occurs sporadically in South African intertidal zones from Algoa Bay in the south to Mozambique in the north.[7] Natural products chemists have been studying the specialized metabolites sequestered by *Aplysia* sea hares for nearly 60 years.[43] In common with other sea hares, *A. dactylomela* sequesters bioactive natural products from its diet of red algae, especially *Laurencia* and *Plocamium* species.[40] Sea hares protect themselves from predation through several different modes of chemical defense including the transfer of diet-derived bioactive natural products from their gut, via a large digestive gland, to defensive secretory glands in their mantle tissues.[43]

Extracts of the digestive glands from four specimens of *A. dactylomela*, collected in the intertidal zone at Cape Recife in March 1998, afforded four new halogenated sesquiterpenes: algoane (**1**), 1-deacetoxyalgoane (**2**), 1-deacetoxy-8-hydroxyalgoane (**3**), and ibhayinol (**4**) (Figure 3).[7,44] Two known
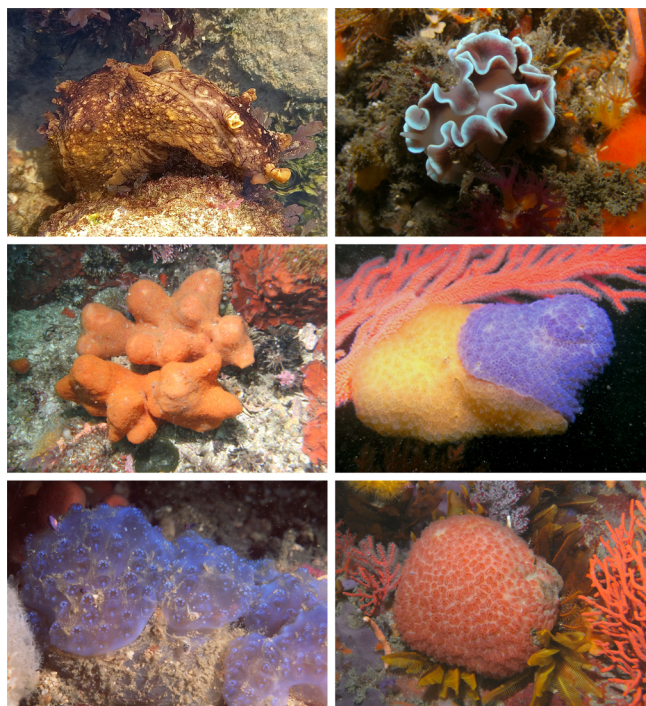
Figure 2. Underwater photographs taken in Algoa Bay of (left to right from the top) sea hare *A. dactylomela*; nudibranch *L. millecra*; ascidian *Pseudoditoma* sp., a blue/yellow colormorph of ascidian *D. skoogii* attached to the gorgonian *L. palma*; ascidian *Polycitor* sp. (Photo CRRF); and ascidian *S. globosum*.
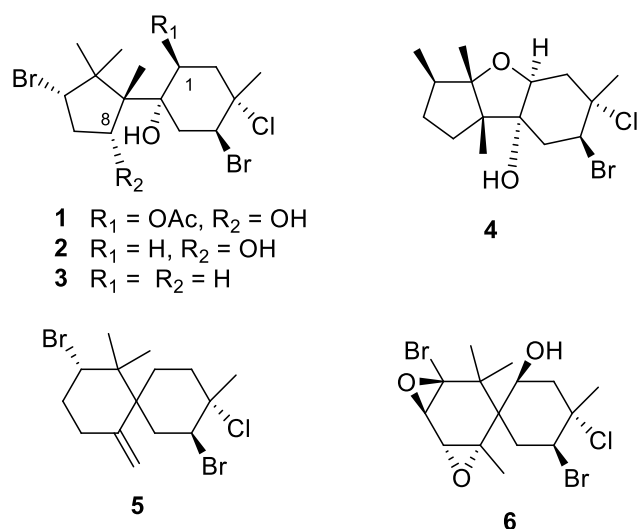
Figure 3. Sequestered metabolites from Algoa Bay specimens of the sea hare *A. dactylomela*.

compounds of red algal origin, nidificene (**5**) and prepacifenol epoxide (**6**) (Figure 3), were also identified in the Algoa Bay *A. dactylomela* extracts.[7] The biological activity of the Algoa Bay *A. dactylomela* metabolites was not explored.

Leminda millecra. The arminacean, or "frilled" nudibranch, *L. millecra* (Figure 2), is endemic to South Africa and is the sole member of the family Lemindidae and genus *Leminda*.[45] *L. millecra* occurs at depths of 10−40 m from the Cape Peninsula to KwaZulu Natal and is relatively abundant on the western reefs of Algoa Bay. An acetone extract of *L.*

millecra specimens collected from Algoa Bay in October 1998 afforded nine new natural products: toluquinones (**7**, **8**), toluhydroquinones (**9**−**13**), algoafuran (**14**), cubebenone (**15**), and four known sesquiterpene metabolites, millecrones A (**16**) and B (**17**), isofuranodiene (**18**), and 8-hydroxycala-menene (**19**) (Figure 4).[46]

Compounds **16** and **17** were first isolated and identified several years earlier from specimens of *L. millecra* collected off the Eastern Cape's Wild Coast, 500 km northeast of Algoa Bay.[47] The sesquiterpene structural scaffolds of **16** and **17** are reminiscent of sesquiterpene metabolites produced by soft corals (class Anthozoa, subclass Octocorallia).[48] Pika and Faulkner supported an octocoral dietary source of these two compounds by identifying soft coral spicules (including those from *Alcyonium foliatum*, *A. valdivae*, and *Capnella thrysoidea*) in the gut contents of the Wild Coast specimens of *L. millecra*.[47] During our large-scale collections of marine invertebrates from Algoa Bay with the CRRF, we had observed *L. millecra* feeding on octocorals (including soft corals and gorgonians or sea fans). We used gas chromatography (GC) to screen for volatile sesquiterpenes in 18 different Algoa Bay octocoral extracts (both soft corals and gorgonians) previously prepared by the NCI. Through this GC analysis, we confirmed that *L. millecra* sequesters **15** and **17** from the gorgonian *Leptogorgia palma* and **16** from two *Alcyonium* soft corals, one of which was identified as *Alcyonium fauri*.[46] The dietary source of the other metabolites sequestered by *L. millecra* in Algoa Bay remains unknown.

Squamous cell esophageal cancer (SCOC) is particularly prevalent in poor rural communities in South Africa.[49,50] In a preliminary screening program, **7** and **9** exhibited weak[23] *in vitro* cytotoxicity to the SCOC cell line WCHO1. Further investigation of the mechanism of cancer cytotoxicity of **7** and **9** revealed that these compounds induced cell cycle arrest and apoptosis through the initial release of reactive oxygen species followed by triggering of the JNK/c-Jun signaling pathway in the cancer cells.[49] Although the *in vitro* cytotoxicity of **9** (IC$_{50}$ = 9.5 μM to WHCO1 esophageal cancer cells) was comparable with that of cisplatin (IC$_{50}$ = 16.5 μM), a standard treatment for esophageal cancer in South Africa, the paucity of available **9**, either from laboratory synthesis or the original marine source, prevented further *in vivo* studies in animal models.[49]

■ BIOACTIVE METABOLITES FROM ALGOA BAY MARINE ASCIDIANS

Ascidians (subphylum Tunicata, class Ascidiacea), commonly referred to as tunicates or sea squirts, are particularly diverse and abundant marine invertebrate filter feeders on the inshore reefs of Algoa Bay.[27] An estimated 3000 ascidian species occur globally, and about ca. 60% of these species live in colonies, the community structure favored by ascidians occurring in warmer waters including Algoa Bay.[51] Ascidians and their associated symbiotic bacteria are prolific sources of bioactive metabolites with pharmaceutical potential.[28,30,52] The Algoa Bay ascidians have similarly proved to be a productive reservoir of new bioactive natural products.[53−55]

***Pseudodistoma* Species.** The colonial ascidian *Pseudodistoma* sp. (Figure 2) was collected from Table Top Reef in October 1998 by the CRRF. Its organic extract exhibited cytotoxic activity in the NCI's 60-human tumor cell lines screen.[22] Bioassay-guided fractionation of this extract afforded four new nitrogen-containing metabolites: pseudodistamine (**20**), (3*E*,5*E*)-tetradeca-3,5-dien-2*R*-amine (**21**),
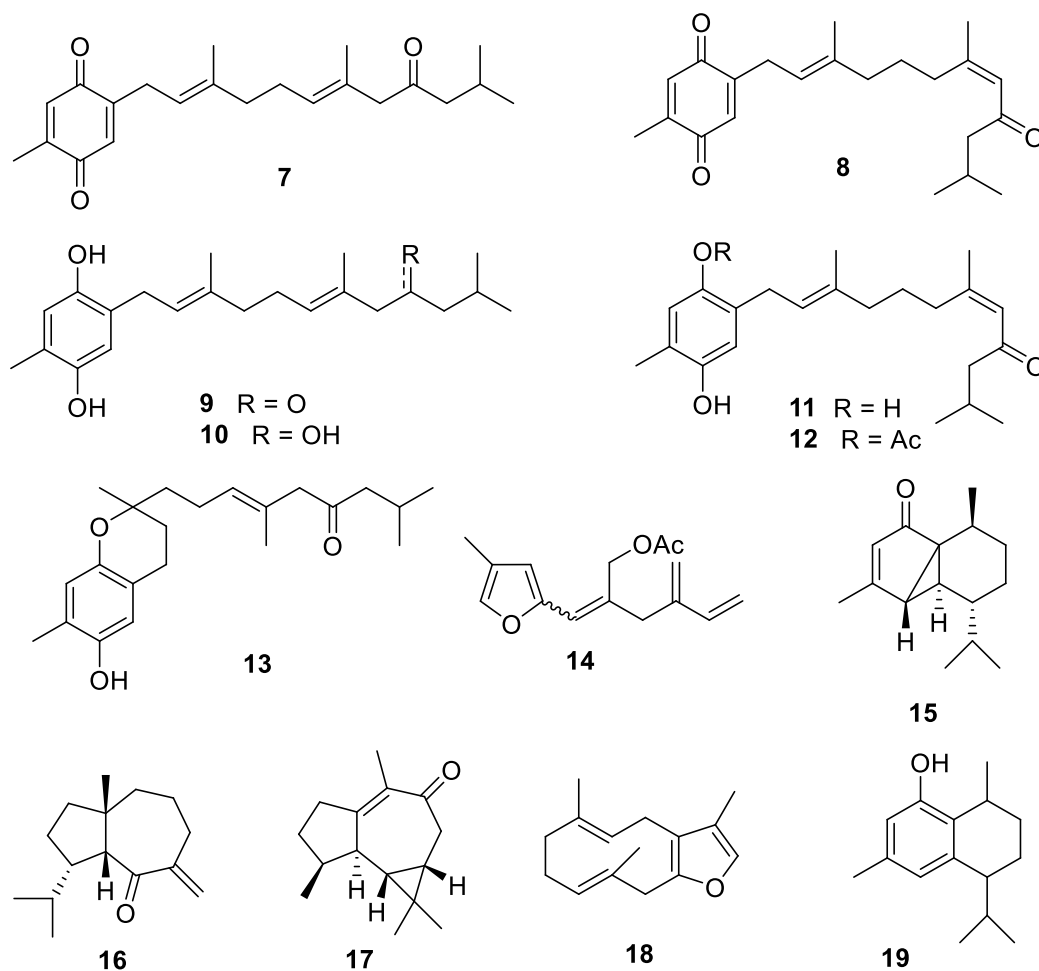
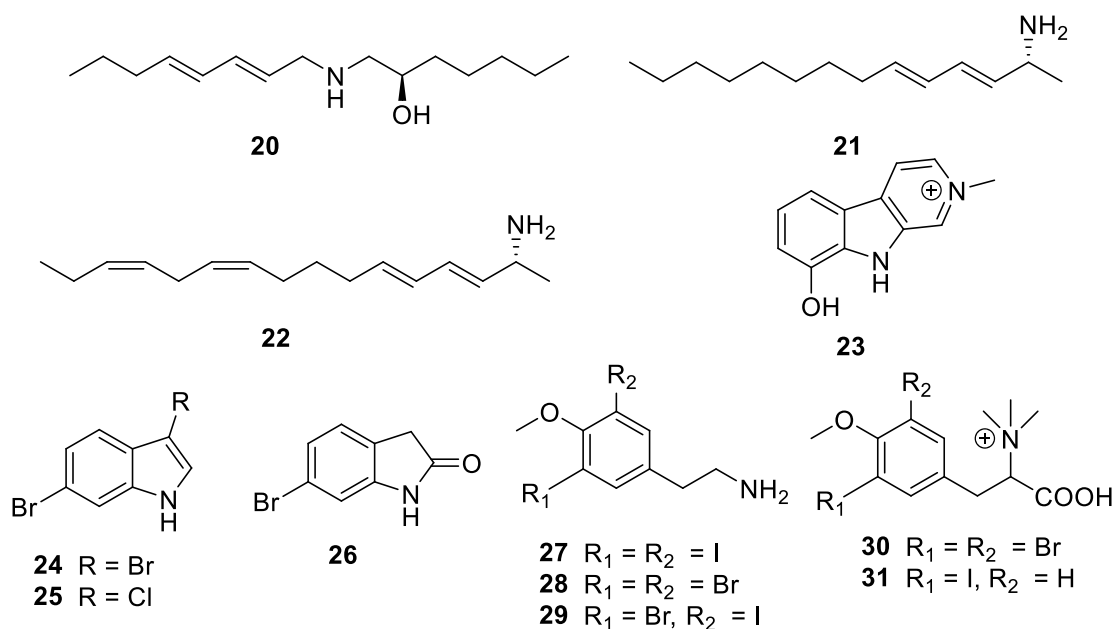**Figure 4.** Sequestered metabolites from the nudibranch *L. millecra*.

Figure 5. Nitrogen-containing metabolites from six species of Algoa Bay ascidians.

(3*E*,5*E*,10*Z*,13*Z*)-hexadeca-3,5,10,13-tetraen-2*R*-amine (**22**), and 8-hydroxy-2-methyl-*β*-carboline (**23**) (Figure 5). The

tricyclic pyrido[3,4-*b*]indole ring system of *β*-carbolines is found in many natural and synthetic cytotoxic compounds.[56]

Only **21** [IC$_{50}$ 30 μM, LOX (melanoma), A549 (non-small cell lung), SNB-19 (CNS), and OVCAR-3 (ovarian) human tumor cell lines] was found to be responsible for the cytotoxicity of the Algoa Bay ascidian extract.[22] With this mild biological effect, the acyl amine was deemed inactive[23] and unsuitable for further development as a potential anticancer lead compound.

Halogenated Metabolites from Five Species of Algoa Bay Ascidians. A methanol extract of the Aplousobranch ascidian *D. skoogi* (Figure 2) collected from White Sands Reef in Algoa Bay in April 2011 yielded three known halogenated indole and oxindole specialized metabolites, 3,6-dibromoindole (**24**), 6-bromo-3-chloroindole (**25**), and 6-bromo-2-oxindole (**26**) (Figure 5).[57] Only the former compound had been previously isolated from an ascidian. All three indole metabolites were only marginally cytotoxic to the metastatic MDA-MB-231 breast cancer cell line (IC$_{50}$ 118, 73, and 74 μM, respectively).[57]

Despite the importance of the tetra-iodinated hormone thyroxine in humans, iodinated natural products are rare in nature, with only ca. 3% of the more than 5000 known halogenated specialized metabolites isolated from bacteria, fungi, plants, and marine organisms reported to contain iodine substituents.[58] The first iodinated natural product to be isolated from a South African marine organism was the known compound 3,5-diiodo-4-methoxyphenethylamine (**27**, Figure 5) from the Aplousobranch ascidian *Aplidium monile* collected from Bell Buoy Reef in Algoa Bay.[59] Utilizing a hyphenated liquid chromatography mass spectrometry technique, viz., liquid chromatography-inductively coupled plasma-mass spectrometry/electrospray ionization-mass spectrometry (LC-ICP-MS/ESI-MS), Bromley et al. were able to confirm the presence of **27** in two other Algoa Bay Aplousobranch ascidians, *Polycitor* sp. (Figure 2) and a *Leptoclinides* sp.[59] Further analysis of the LC-ICP-MS/ESI-MS data revealed that known metabolite 3,5-dibromo-4-methoxyphenethylamine (**28**) and a new metabolite, 3-bromo-5-iodo-4-methoxyphenethylamine (**29**), were also present in these three ascidian extracts (Figure 5). The presence of the known 3,5-dibromotetramethyltyrosine (**30**) and the new 3-iodotetramethyltyrosine (**31**) (Figure 5) in extracts of an unidentified *Didemnum* species was similarly proposed from LC-ICP-MS/ESI-MS evidence.[59] The paucity of these halogenated compounds in the ascidian extracts precluded further bioactivity testing.

Synoicum globosum. The Aplousobranch ascidian *S. globosum* (Figure 2) was collected from White Sands Reef in Algoa Bay in July 2004.[60] An extract of this ascidian exhibited antimicrobial activity against a panel of four clinically relevant pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA), *S. epidermis* and gentamycin- and vancomycin-resistant *Enterococcus faecalis*, and *Escherichia coli* species.[60] Bioassay-guided fractionation of the *S. globosum* extract afforded the two known rubrolides E (**32**) and F (**33**)[61] and four new brominated analogues of these two compounds: 3'-bromorubrolide E (**34**), 3'3''-dibromorubrolide E (**35**), 3''-bromorubrolide F (**36**), and 3''-bromorubrolide F (**37**) (Figure 5).[60] Further investigations of other *Synoicum* species from the Indian and Pacific Oceans have extended the rubrolide series.[62,63] While all rubrolide congeners displayed varying levels of antimicrobial activity against all four bacterial pathogens, the methoxylated rubrolide F congeners were several times less potent against MRSA than the non-methoxylated rubrolide E congeners.[60] Bracegirdle et al. demonstrated a loss of antibacterial activity associated with

hydration of the C-5/C-6 butenolide exomethylene moiety in new rubrolides V and W recently isolated from a New Zealand *Synoicum* species.[63] Barbosa et al.[64] evaluated rubrolides for herbicidal applications, while more recently, Gudzuhn et al.[65] evaluated rubrolides as biofilm inhibitors against the emerging MDR pathogen *S. maltophilia* associated with cystic fibrosis.
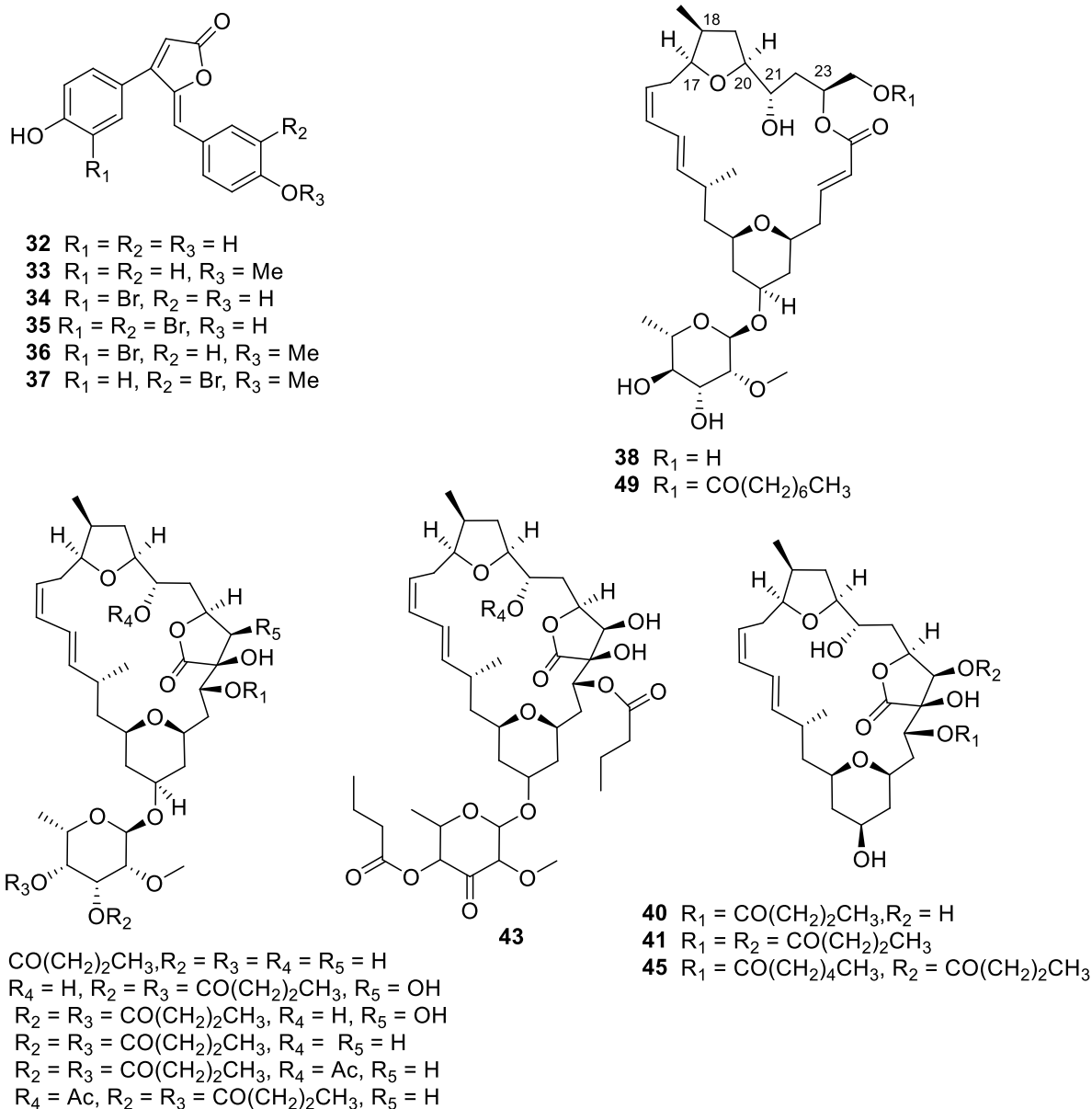
***Lissoclinum* Species.** An organic extract of specimens of the colonial ascidian *Lissoclinum* sp. (Figure 6), collected from
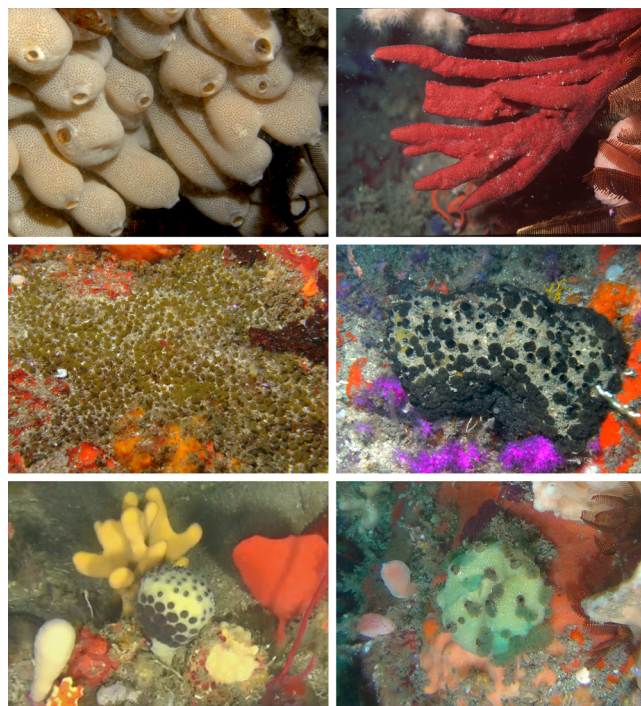
Figure 6. Underwater photographs taken in Algoa Bay of (left to right from the top) ascidian *Lissoclinum* sp., sponges *Axinella* sp. (photo CRRF), *C. bellae, T. favus, T. pedunculata* (center, photo SAIAB), and *T. michaeli* (center, photo SAIAB).

White Sands Reef in Algoa Bay in 2004, exhibited significant initial *in vitro* cytotoxicity to NCI-H460 lung cancer cells (IC$_{50}$ = 0.7 μgmL^{-1}).[53] Bioassay-guided chromatography of this extract afforded the four novel macrolide lactones mandelalides A−D (**38**−**41**, Figure 7).[53] The rhamnosylated mandelalide A (**38**) comprises a 24-membered macrolactone core, with 14 stereogenic centers, *cis*-fused tetrahydrofuran and tetrahydropyran rings, and a *cis,trans*-diene motif. Mandelalide B (**39**) contains an unprecedented 23-membered, all-carbon macrocyclic backbone with talose glycosylation, while mandelalides C (**40**) and D (**41**) are oxidized aglycones. The structural elucidation and investigation of biological activity of these natural products were challenging given the paucity of compounds isolated (<1 mg). Nonetheless, relative configurations of the macrocycles and monosaccharides in the natural products were assigned by ROESY NMR experiments, and extensive *J*-based configuration analysis using NMR data obtained in two different solvents confirmed the rhamnose and talose moieties in **38** and **39**, respectively. A 2-*O*-methyl-α-L-rhamnose structure was established for **38** upon its hydrolysis, subsequent permethylation and silylation of the sugar-containing hydrolysate, and comparison with similarly derivatized D- and L-rhamnose standards using GC-MS. This assignment of the rhamnose absolute configuration in **38** was

32 $R_1 = R_2 = R_3 = H$
33 $R_1 = R_2 = H, R_3 = Me$
34 $R_1 = Br, R_2 = R_3 = H$
35 $R_1 = R_2 = Br, R_3 = H$
36 $R_1 = Br, R_2 = H, R_3 = Me$
37 $R_1 = H, R_2 = Br, R_3 = Me$

38 $R_1 = H$
49 $R_1 = CO(CH_2)_6CH_3$

39 $R_1 = CO(CH_2)_2CH_3, R_2 = R_3 = R_4 = R_5 = H$
42 $R_1 = R_4 = H, R_2 = R_3 = CO(CH_2)_2CH_3, R_5 = OH$
44 $R_1 = R_2 = R_3 = CO(CH_2)_2CH_3, R_4 = H, R_5 = OH$
46 $R_1 = R_2 = R_3 = CO(CH_2)_2CH_3, R_4 = R_5 = H$
47 $R_1 = R_2 = R_3 = CO(CH_2)_2CH_3, R_4 = Ac, R_5 = H$
48 $R_1 = R_4 = Ac, R_2 = R_3 = CO(CH_2)_2CH_3, R_5 = H$

43

40 $R_1 = CO(CH_2)_2CH_3, R_2 = H$
41 $R_1 = R_2 = CO(CH_2)_2CH_3$
45 $R_1 = CO(CH_2)_4CH_3, R_2 = CO(CH_2)_2CH_3$

Figure 7. Specialized metabolites from Algoa Bay ascidians *S. globosum* and *Lissoclinum* sp.

enabled by the well-defined monosaccharide solution structure and glycosidic bond conformation, which encouraged attempts to relay these absolute configurational assignments to the macrocycle. The obvious retention in relative configuration between the macrocycles of **38** and **39** also allowed a proposed absolute configuration of the macrocycle and monosaccharide in **39**.[53] Both mandelalides A and B were potently cytotoxic to NCI-H460 human lung cancer cells (IC_{50} 12 and 44 nM, respectively), while insufficient amounts of mandelalides C and D were available for this preliminary cytotoxicity testing.[53]

The unique array of multiple chiral centers in the macrolactone of **38** and its potent *in vitro* cancer cell toxicity, plus the dearth of this compound available for mechanism of action studies, prompted broad interest in the total synthesis of this natural product. The research groups of Ye[66] and Fürstner[67] first drew attention to NMR spectral discrepancies between the data reported for the originally reported natural product mandelalide structure and their synthetic products, with the Ye group simultaneously reporting a revised structure

for the natural product based on synthesis of alternative diastereomers. The corrected mandelalide A structure (**38**)[66] exhibited inversion of the five stereocenters in the northern hemisphere of the mandelalide A macrocycle (C17, 18, 20, 21, and 23), indicating inaccurate translation of the relative configuration between the southern and northern hemisphere of the macrocycle in the originally proposed natural product structure. The reassigned macrocycle configuration, with the tetrahydrofuran moiety and two adjacent chiral centers inverted, was subsequently deemed correct for **39**–**41** as well, based on quantum mechanical NMR chemical shift predictions,[68] although no synthesis of these butyrolactone-containing mandelalides has been reported to date. Within four years, five prominent international synthetic chemistry research groups had synthesized, and tested, the corrected absolute structure of **38**.[66,67,69−71] Two additional synthetic strategies were reported in 2019.[72,73]

The cytotoxicity reported for synthetic **38** against 11 different cancer cell lines (including H1229 lung cancer) was

disappointingly at variance[4,66,67] with the potent cytotoxicity originally reported for the natural product.[53] While Fürstner et al.[67] erroneously attributed this discrepancy in cytotoxicity to an impure/contaminated natural product used for testing, this broader testing of synthetic **38** against cancer cell lines with varied metabolic backgrounds provided early clues to the biological mechanism of the mandelalides. A re-collection of *Lissoclinum* sp. in 2013 had increased the amount of **39** and **40** available for structure–activity relationship (SAR) studies, in addition to affording a new congener, mandelalide E (**42**, Figure 7), containing a dibutyrylated talose moiety.[54] These three natural products were tested against HeLa, NCI-H460, HCT116 colon cancer, and U87-MG glioblastoma cell lines, alongside synthetic **38** provided by Professor Tao Ye.[54] The SAR studies indicated that glycosylation of the macrolide is necessary for cytotoxicity and that esterification of the monosaccharide substituent leads to ca. 100-fold loss of activity. Importantly, McPhail and co-workers[54] demonstrated that potent (low nanomolar) cytotoxicity of natural and synthetic mandelalide A to NCI-H460 lung cancer cells was dependent on high seeding density of cancer cells in the *in vitro* assay. NCI-H460 cancer cells were generally resistant to mandelalide A when exposed to this compound at low cell density.

The synthesis of **38** by Smith and co-workers using a high-yielding anion relay chemistry (ARC) strategy[71,74] provided additional confirmation of potent toxicity against NCI-H460 and HeLa cells for **38**, ample material for subsequent biological mechanism studies, and the opportunity to access additional mandelalide A analogues. The 2013 re-collection of the *Lissoclinum* ascidian had also enabled characterization of additional mandelalides F–L (**43–49**, Figure 7), from an even more extensive mandelalide molecular family.[55] SARs were explored by considering three structural prototypes based on mandelalides A (A-type with a standard macrolactone bond), B (B-type with a butyrolactone containing macrocycle), and C (C-type with a hydroxybutyrolactone-containing macrocycle) (Figure 7). Members of each group differed in the patterns of acetate, butanoate, and octanoate esterification of the respective parent compound. A broader SAR study[55] using natural and synthetic products confirmed the earlier observations of the importance of the monosaccharide moiety for activity[54] and established **38**, **49**, and **39** as the most active compounds against NCI-H460 (EC$_{50}$ 11, 9.8, and 44 nM, respectively) and HeLa (EC$_{50}$ 9.9, 2.8, and 16 nM, respectively) cell lines. McPhail, Ishmael, Smith, et al.[55] also established that mandelalide cytotoxicity depends on the basal metabolic phenotype of cells and is associated with an oxidative phenotype or compromised adaptive survival response. They also used real-time analysis of oxygen consumption rate in living cells, measurement of mitochondrial complex V activity in isolated mitochondria, and analysis of caspases-3 and -7 activation to show that mandelalides induce apoptotic cell death, likely via inhibition of the mitochondrial ATP synthase (mitochondrial complex V). Finally, one-dose testing of **38** and **39** in the NCI60 human tumor cell line panel provided an antiproliferative profile that was consistent with well-characterized ATP synthase/Complex V inhibitors oligomycin A and apoptolidin A. Recently, Ishmael et al. have provided further insights into the pharmacology of **38**.[75] They provided conclusive evidence that this compound is an indirect or secondary activator of adenosine monophosphate-activated protein kinase (AMPK).[75] Ishmael et al. also

reported that **38** was potently cytotoxic (IC$_{50}$ 0.38–0.85 nM) against a panel of five human glioblastoma cell lines over a prolonged period (3–6 days), thus underlining the ongoing significance of this compound as a possible preclinical anticancer therapeutic candidate.

Obtaining sufficient **38** for preclinical development is problematic and not easily resolved. Given the paucity of mandelalides isolated from the *Lissoclinum* ascidian, harvesting of this ascidian in Algoa Bay is neither practical nor environmentally acceptable. Many bioactive natural products isolated from ascidians are biosynthesized by ascidian microbial symbionts and not by the ascidian itself.[28,30] A metagenomics analysis of the *Lissoclinum* sp. revealed mandelalide biosynthetic genes in the genome of "*Candidatus* Didemnitutus mandela" (family Opitutaceae, phylum Verrucomicrobia), a symbiont associated with the reproductive zooid component of the ascidian.[76] The highly reduced genome (2.17 Mbp, 94.2% complete) of this organism indicates an obligate symbiont that is currently unculturable in the absence of the tunicate. A scaled-up laboratory synthesis of **38** is, therefore, the most expeditious route to secure sufficient amounts of this compound for any further preclinical development of this compound.[75]
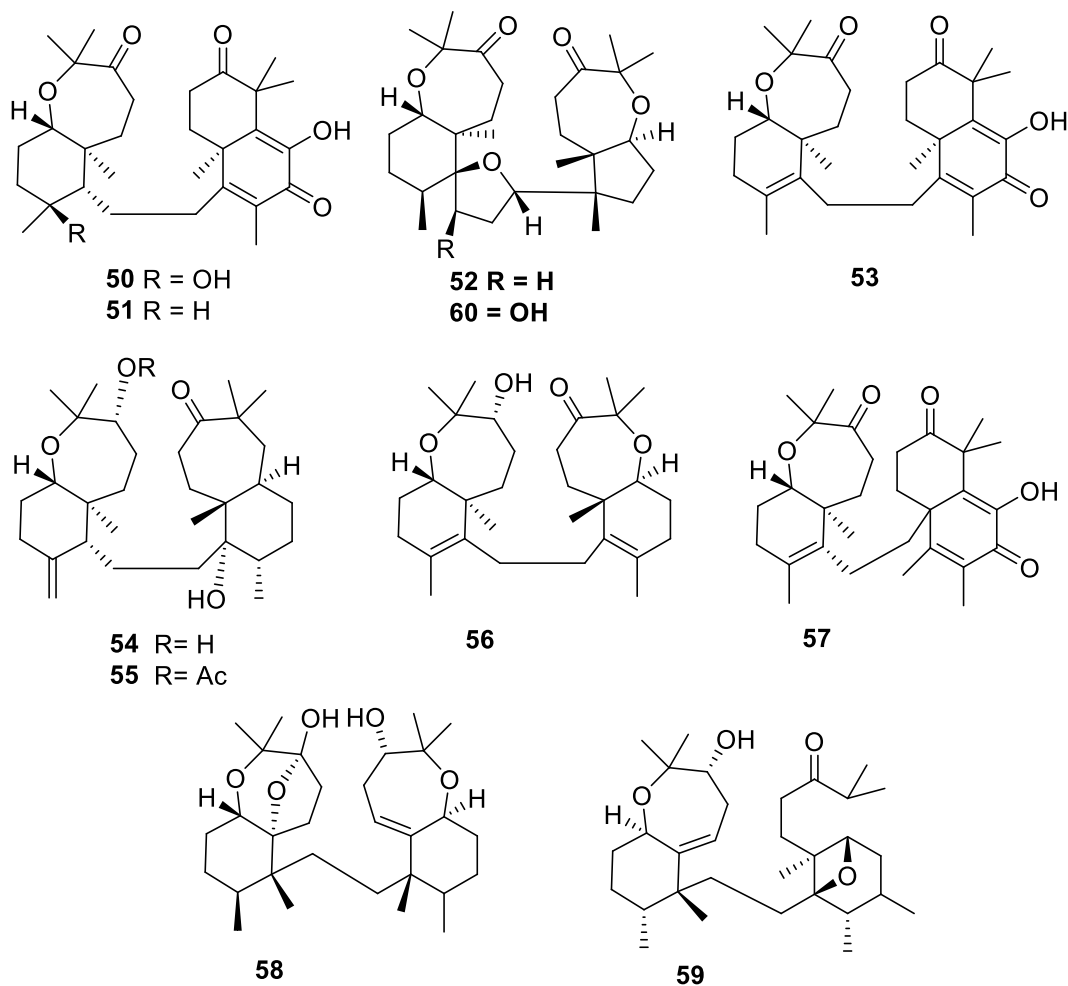
■ BIOACTIVE METABOLITES FROM ALGOA BAY MARINE SPONGES

Bioactive natural products have been isolated and identified from six species of Algoa Bay marine sponges, an *Axinella* sp. and five latrunculid species: *Tsitsikamma favus*, *T. pedunculata*, *T. michaeli*, *Cyclacanthia bellae*, and *Strongylodesma algoaensis*. Marine sponges of the family Latrunculiidae (order Poecilosclerida) are sources of highly cytotoxic pyrroliminoquinone metabolites.[77–79] Globally, Algoa Bay is recognized as an area of high latrunculid sponge endemism,[79] and considerable effort has been directed toward unravelling the chemotaxonomic diversity of the species that occur in this region of the South African coast.

***Axinella* Species.** Insufficient and disordered vascularization within regions of some rapidly growing solid tumors can induce hypoxia (reduced cellular oxygen levels) in these tumors.[80–82] Hypoxia-inducible-factor (HIF-1), a transcription factor essential for promoting cancer cell survival under hypoxic conditions, also triggers "immune escape" signaling events that disrupt the body's immune response to the cancer cells that make up these tumors.[81] Therefore, HIF-1 is recognized as a viable anticancer drug target, and cell-based reporter assays are used effectively to monitor the response of HIF-1 to potential inhibitors.

Over a period of several years, Nagle and co-workers screened 10 560 marine invertebrate and algal extracts from the Natural Products Open Repository of the NCI in a T47D breast tumor cell-based reporter assay designed to detect potential HIF-1 inhibitors.[80] Approximately 1% of these extracts were active in the T47D breast tumor HIF-1 primary screen, with the majority (>50%) originating from marine sponges. An unidentified sponge, *Axinella* sp., collected off Grootbank Reef in Algoa Bay in March 2000 (Figure 6) was one of the 109 extracts that inhibited hypoxia (1% O$_2$)-induced HIF-1 activation in T47D breast cancer cells with pronounced cytotoxicity.[80,83] Nagle and co-workers subsequently isolated the known related triterpenes, sodwanone A (**50**), sodwanone B (**51**), and yardenone (**52**), and seven new analogues of these compounds, 10,11-dihydrosodwanone B

Figure 8. Sodwanone and yardenone metabolites from the Algoa Bay marine sponge *Axinella* sp.

(**53**), 3-*epi*-sodwanone K (**54**), 3-*epi*-sodwanone K 3-acetate (**55**), sodwanones T–W (**56–59**), and 12*R*-hydroxy-yard-enone (**60**), from this extract (Figure 8).[83] Interestingly, *A. weltneri*, collected in Sodwana Bay, KwaZulu Natal, South Africa, was the original source of the sodwanone series of compounds,[84] while a Red Sea sponge, *Pitlocaulis spiculifer*, from the same Axinellidae family as *A. welteneri*, was the original source of the yardenones.[85]
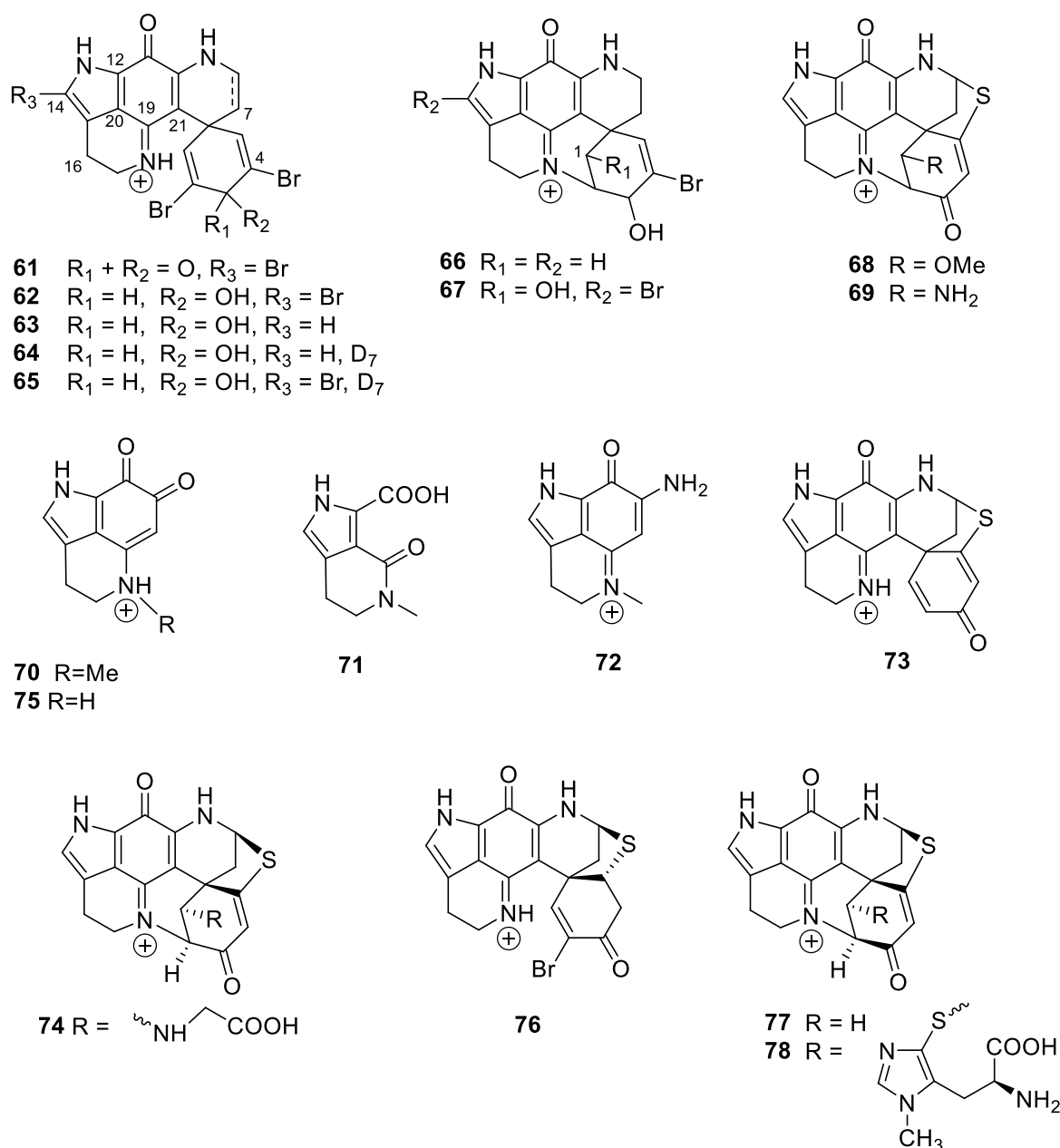
Sodwanone V was the only compound that inhibited hypoxia-induced HIF-1 activation in both T47D breast and prostate tumor cells (IC$_{50}$ 15 μM). Four other compounds, **50**, **53**, **54**, and **56**, were less effective as inhibitors of hypoxia-induced HIF-1 activation in the former cell line (IC$_{50}$ 20–25 μM).[83] There has been no further development of any of these compounds as potential anticancer drugs.

■ PYRROLOIMINOQUINONE METABOLITES FROM FIVE SPECIES OF ALGOA BAY LATRUNCULID SPONGES

Latrunculid sponges are a rich source of cytotoxic pyrroliminoquinone metabolites. More than one hundred pyrroliminoquinone compounds have thus far been isolated from the 83 latrunculid sponge species known to occur in cold and temperate coastal shallow and deep-water environments in both the southern and northern hemispheres.[79] The first species of a new genus of latrunculid sponge, *Tsitsikamma*

favus[86] (Figure 6), was discovered on subtidal reefs in South Africa's Tsitsikamma National Park, 185 km west of Algoa Bay. *T. favus* afforded the first pyrroloiminoquinone metabolites to be isolated from an African latrunculid sponge.[87] Since the discovery of *T. favus*, the subtidal reefs of South Africa's Agulhas ecoregion, including those in Algoa Bay, have proved to be a latrunculid sponge biodiversity hotspot.[88–91] The initial investigations of the bioactive pyrroloiminoquinone metabolites from Algoa Bay latrunculid sponges focused on three species: *T. pedunculata*, *Strongylodesma algoanesis*, and *Latrunculia bellae*.[92] *L. bellae* was subsequently reassigned to the endemic South African genus *Cyclacanthia*.[88] Recently a taxonomic review of the genus *Tsitsikamma* has recognized two subgenera.[91] The "cushion-shaped" species (e.g., *T. favus*) have been placed in the subgenus *Tsitsikamma*, while the "stalked" or peduculate species (e.g., *T. pedunculata* and *T. michaeli*) are now contained in the subgenus *Clavicaulis*.[90,91] The original taxonomic names assigned to *T. favus*, *T. pedunculata*, and *T. michaeli*, as recorded in the chemistry literature, are retained here to avoid confusion.

Specimens of *T. pedunculata* (Figure 6) collected from Thunderbolt Reef in Algoa Bay yielded the known compounds 14-bromodiscorhabdin C (**61**), 14-bromo-3-dihydrodiscorhabdin C (**62**), and 3-dihydrodiscorhabdin C (**63**) together with four new minor metabolites, 3-dihydro-7,8-dehydrodiscorhabdin C (**64**), 14-bromo-7,8-dehydro-3-dihydrodiscorhabdin C

61 $R_1 + R_2 = O$, $R_3 = Br$
62 $R_1 = H$, $R_2 = OH$, $R_3 = Br$
63 $R_1 = H$, $R_2 = OH$, $R_3 = H$
64 $R_1 = H$, $R_2 = OH$, $R_3 = H$, D_7
65 $R_1 = H$, $R_2 = OH$, $R_3 = Br$, D_7

66 $R_1 = R_2 = H$
67 $R_1 = OH$, $R_2 = Br$

68   R = OMe
69   R = NH_2

70   R=Me
75   R=H

71

72

73

74 R = $\overset{\sim}{}$NH—COOH

76

77   R = H
78   R =

Figure 9. Pyrroloiminoquinone metabolites from Algoa Bay latrunculid sponges *T. pedunculata*, *T. michaeli*, *C. bellae*, and *S. algoaensis*.

(**65**), discorhabdin V (**66**), and 14-bromo-1-hydroxydisco-rhabdin V (**67**) (Figure 9).[92] Compounds **61** and **62** were previously isolated from *T. favus*.[87] A recent liquid chromatography tandem mass spectrometry (LCMS[2]) molecular networking analysis by Kalinski et al.[93] of 43 specimens from six different species of South African and sub-Antarctic latrunculid sponges included four species, viz., *T. pedunculata*, *C. bellae*, *T. favus*, and a new species, *T. michaeli*[90] (Figure 6), collected from Bell Buoy, Evans Peak, and Riy Banks Reefs in Algoa Bay. More than 200 known and unknown pyrroloiminoquinone and related metabolites were detected in the organic extracts of the latrunculid sponges. Putative chemical structures of novel makaluvamine-discorhabdin adducts and previously unknown hydroxylated discorhabdin I analogues were proposed from the collision-induced dissociation (CID) mass data.[93] The comparative networking analysis confirmed species-specific pyrroloiminoquinone specialized metabolite

profiles in each of the six latrunculid species including identifying two "regiospecific" chemotypes of *T. michaeli*, separately inhabiting two different reefs. The tribrominated discorhabdin C dominated metabolite profile of *T. michaeli* chemotype I (Riy Banks Reef) was consistent with that of *T. favus* chemotype I and differed only in the absence of tsitsikammamines.[93] The chemical profile of *T. michaeli* chemotype II (Evans Peak Reef) incorporated a series of brominated and hydroxylated discorhabdin C analogues which were absent from chemotype I. The pyrroloiminoquinone metabolite profiles of both chemotypes of *T. michaeli* differed from that of *T. pedunculata*, with the latter characterized by mono- and dibrominated discorhabin V type pyrroloiminoquinones. Kalinski et al. consequently suggested that the reported co-occurrence of *T. favus* discorhabdin-type metabolites in the original natural products investigation of *T. pedunculata* by Antunes et al.[92] may have been as a result of

contamination with specimens of the pedunculated and morphologically similar *T. michaeli* chemotype I.[93]

A collection of *C. bellae* from Riy Banks Reef in the mouth of Algoa Bay afforded the new discorhabdins 1-methoxydiscorhabdin D (**68**) and 1-aminodiscorhabdin D (**69**) and five known metabolites: damirone B (**70**), makaluvic acid A (**71**), makaluvamine C (**72**), and discorhabdins G* and N (**73** and **74**) (Figure 9).[92] Interestingly, Kalinski et al.[93] recently drew attention to the similarities in the pyrroloiminoquinone metabolite profiles between the Algoa Bay *C. bellae* specimens and the specimens of the deep-water sub-Antarctic latrunculid sponge *L. apicalis* collected ca. 1000 km south of Algoa Bay. However, the relative abundance of **72** and damirone C (**75**) in the *C. bellae* extracts clearly distinguished them from the *L. apicalis* extracts.[93] The recent LCMS[2] molecular networking studies of latrunculid sponges in Algoa Bay[93,94] and elsewhere[95] have demonstrated the value of this approach to unravel the complex pyrroloiminoquinone profiles of, and chemotaxonomic relationships between, marine sponge species within the family Latrunculidae.

Specimens of *S. algoaensis* collected from a rock pool in the intertidal zone near Gqeberha yielded the known compound **62** and discorhabdins A (**76**), D (**77**), and H (**78**) (Figure 9).[92] The absolute configuration of **78** was established by electronic circular dichroism (ECD).[96] Sixteen of the 17 metabolites isolated from Algoa Bay latrunculid sponges were screened for human colon tumor (HCT-116) cytotoxicity. With the exception of **67** and **71** (IC$_{50}$ 12.5 and 28 μM), all the remaining pyrroloiminoquinone compounds exhibited *in vitro* HCT-116 cytotoxicity with IC$_{50}$ values < 3.2 μM. Of the compounds tested in this assay **61−65**, **68**, **69**, **73**, and **77** were moderately toxic to HCT-116 cancer cells, with only **76** (IC$_{50}$ 7 nM) exhibiting potent cytotoxicity (Table 1).[92]

Table 1. EC$_{50}$ and LC$_{50}$ Values (nM) for the Ascidian-Derived Mandelalides[55,75] (NT = Not Tested)

	Cancer Cell Lines			
compound	HeLa cervix (EC$_{50}$)	NCI-H460 lung (EC$_{50}$)	U87-MG glioblastoma (LC$_{50}$)	HCT116 colon (LC$_{50}$)
38	9.9	11	0.38	NT
49	2.8	9.8	61	54
39	16	44	inactive	inactive
41	660	>1000	NT	NT
43	50	270	NT	NT
45	330	>1000	NT	NT
46	>1000	910	NT	NT
47	>1000	790	NT	NT

T. favus is a relatively common latrunculid sponge species occurring on the reefs in Algoa Bay. The bis-pyrroloiminoquinone (pyrrolo[4,3,2-*de*]quinolone) metabolites, tsitsikammamines A (**79**) and B (**80**) (Figure 10), originally isolated from *T. favus*,[87] neither are confined to the genus *Tsistikamma* nor occur in the other two *Tsitsikamma* species found in Algoa Bay.[92,93] An *N*-methylated analogue of **80**, tsitsikammamine C (**81**), was isolated from an Australian latrunculid sponge, *Zyzzia* sp.,[97] while the deep-water Antarctic latrunculid sponge *Latrunculia biformis* recently afforded **79** and its Δ^{16} analogue (**82** Figure 10).[95]

Kalinski et al. performed LCMS[2] molecular networking of extracts from more than 50 individual *T. favus* sponges
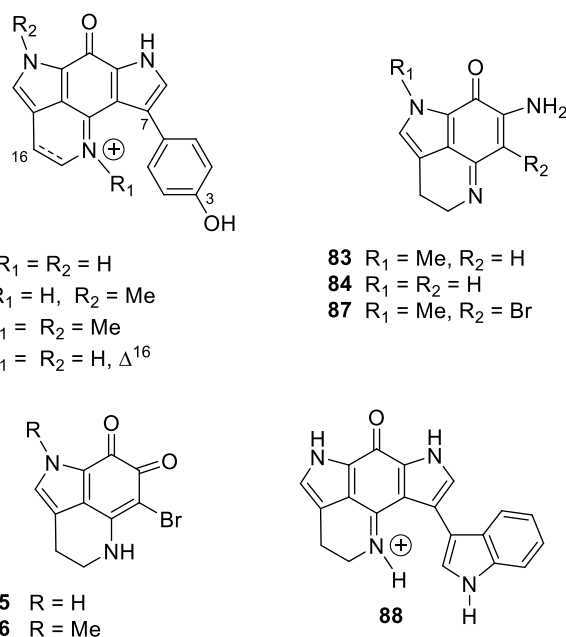
Figure 10. Pyrroloiminoquinone metabolites from the Algoa Bay latrunculid sponge *T. favus* and closely related compounds isolated from an Australian latrunculid sponge *Zyzzia* sp., sub-Antarctic latrunculid sponge *L. apicalis*, and the ascidian *Clavelina* sp.

collected by scuba and ROV from Evans Peak Reef in Algoa Bay over a period of 15 months and found two distinct *T. favus* chemotypes at this single collection site.[94] The LCMS[2] data implied the presence of 48 different pyrroloiminoquinone metabolites in these extracts, of which seven were isolated and identified, including the known **65**, **80**, makaluvamines A, I, and O, and makaluvone (**83−86**) and the new makaluvamine Q (**87**) (Figure 10).[93] *T. favus* chemotype 1 was dominated by **80**, with putative mass spectrometric evidence for the presence of **79** and several dehydro, hydroxylated, and brominated tsitsikammamine analogues. The mass spectrometric data also showed the presence of **65** and other related discorhabdin C type analogues in chemotype I. Conversely, only trace amounts of the tsitsikammines and no discorhabins were evident in *T. favus* chemotype II, which was dominated by makaluvamines and related pyrrolo-*ortho*-quinolines with a relatively high abundance of phenylalanine, a known precursor in all the putative pyrroloiminoquinone biosyntheses proposed thus far for these compounds.[77,79,94]

The first example of a specialized metabolite possessing a bis-pyrroloiminoquinone structural scaffold was wakayin (**87**, Figure 10) isolated from a marine ascidian, *Clavelina* species.[98] In common with wakayin, **79** strongly intercalates DNA and inhibits topoisomerase I.[92] A possible target binding mode for **79** was recently proposed from its computational docking into the active sites of topoisomerase I and II and indoleamine 2,3 dioxygenase.[95] All seven of the pyrroloiminoquinone compounds isolated from *T. favus* by Kalinski et al. exhibited topoisomerase I inhibition and DNA intercalation activity, with **85** and **86** the strongest inhibitors of topoisomerase I, and **87** displaying the highest affinity for DNA.[94]

Sponge-associated microbes can account for a large proportion (ca. 50%) of the sponge biomass, and they have been shown, in some instances, to be responsible for the biosynthesis of specialized metabolites previously attributed to

Table 2. LC$_{50}$ Values (nM) for the Sponge-Derived Pyrroloiminoquinone Metabolites[92,105] (NT = Not Tested)

	Cancer Cell Lines	
compound	HCT116 colon	WHC-01 esophageal
61	77	NT
62	645	NT
63	323	NT
64	197	NT
65	222	NT
68	232	NT
69	119	NT
73	327	NT
76	7	17
77	595	NT

their sponge host.[99] The debate around the biosynthetic origin of pyrroloiminoquinone metabolites was reignited by the isolation of the marine pyrroloiminoquinone metabolite **83** from a laboratory-cultured terrestrial myxomycete, *Didymium bahiense*,[100] and the distribution of bis-pyrroloiminoquinones in three different latrunculid sponges and a phylogenetically unrelated marine ascidian.[79] Accordingly, over the past decade Dorrington and co-workers have worked to establish whether the bis-pyrroloiminoquinone metabolites in Algoa Bay specimens of *T. favus* are biosynthesized by the sponge, or one or more of its microbial symbionts, or are the end-products emerging from a combination of both biosynthetic sources. Walmsley et al. applied denaturing gradient gel electrophoresis (DGGE) combined with clonal and deep sequencing of microbial 16S rRNA gene amplicons to nine individual specimens of *T. favus* collected from Evans Peak Reef in Algoa Bay over a period of one year and showed that *T. favus* has a distinct microbial population dominated by a novel β-proteobacterium species.[101] From an examination of the operational taxonomic unit (OTU$_{0.03}$) of the β-proteobacterium in five Algoa Bay and one sub-Antarctic latrunculid sponge species (*L. apicalis*) Matcher et al. subsequently reported that each latrunculid sponge species hosts a single conserved and phylogenetically related β-proteobacterium strain.[102] Sequence analysis of the 16S rRNA genes from the latrunculid β-proteobacteria identified them as lithoautotrophic ammonia-oxidizing bacteria from the Nitrosomonadacea, a family of bacteria that utilize ammonia as their primary energy source. Nitrogen is a scarce resource in the marine environment, and these bacteria may play an important role in nitrogen cycling, including providing a source of bioavailable nitrogen to their sponge hosts.[102] Matcher et al. further proposed that the β-proteobacterium is a specialized sponge symbiont that has coevolved with its latrunculid sponge hosts and together with a phylogenetically closely related Spirochaetae bacterium, also found in both the *Tsitsikamma* and *Cyclacanthia* species, may play a role in pyrroloiminoquinone biosynthesis.[102]

Daniels et al.[103] have recently provided valuable new insights into the biosynthetic pathway to pyrroloiminoquinones. Their discovery of an unanticipated posttranslational modification process to initiate the biosynthesis of pyrroloiminoquinones, involving the addition of tryptophan from tryptophanyl-tRNA to the C-terminus of a ribosomal protein by peptide aminoacyl-tRNA ligase (PEARL), has potentially important ramifications to the ongoing exploration of the biosynthesis of pyrroloiminoquinones in latrunculid sponges.

■ CONCLUSION

South Africa's bays are threatened coastal ecosystems requiring protection and ongoing conservation management and protection.[14,104] The large industrial city of Gqeberha, with a population of over 1 million people, has an increasingly negative environmental impact on the vulnerable inshore marine reefs of Algoa Bay through industrial pollution, sewerage outflows, dredging, and anchor scours from increased shipping and commercial fishing.[11,14] In 2007, SAEON identified Algoa Bay as a Sentinel Site for Long-Term Ecological Research. The steady accumulation of oceanographic, biophysical, and biodiversity data from Algoa Bay over the past 15 years has placed this region of the South African coast among the best monitored coastal areas in Africa and the southern hemisphere.[12]

The marine biodiscovery program in Algoa Bay has contributed significantly to our understanding of Algoa Bay's rich and endemic marine invertebrate biodiversity and has led to a greater appreciation of the potential medicinal value of the marine invertebrate fauna on the inshore reefs on the western

Table 3. Important Milestones in the Chemical and Biological Investigation of the Mandelalides

Year	Mandelalide milestones
2004	Initial collection of ascidian *Lissoclinum* sp. from White Sands Reef, Algoa Bay
2011	Organic extract of *Lissoclinum* sp. exhibits significant *in vitro* cytotoxicity to NCI-H460 lung cancer cells.
2012	McPhail et al. publish the chemical structures of mandelalides A−D and the potent cytotoxicity of mandelalides A and B to NCI-H460 lung cancer and mouse Neuro-2A neuroblastoma cells.[53]
2013	Re-collection of *Lissoclinum* species from White Sands Reef, Algoa Bay
2014	First total synthesis and stereochemical reassignment of mandelalide A published by Xu and Ye et al; original report of mandelalide A cytotoxicity questioned[66]
2015	Second total synthesis of revised mandelalide A structure published by Fürstner et al., also questioning originally reported cytotoxicity of mandelalide A[67]
2016	McPhail et al. publishes the chemical structure of mandelalide E and explains the variance in cancer cell cytotoxicity, viz., seeding density of cancer cells.[54]
	Three further syntheses of mandelalide A published by Altmann et al.,[69] Carter et al.,[70] and Smith et al.[74]
2017	McPhail, Ishmael, Smith, et al. publish the chemical structures of mandelalides F−L, and SAR studies confirm the importance of the monosaccharide moiety for activity. Induction of apoptotic cell death via inhibition of the mitochondrial ATP synthase (complex V) is proposed.[55]
	A metagenomics study of *Lissoclinum* sp. by Kwan et al. reveals the presence of mandelalide biosynthetic genes in the genome of an obligate bacterial symbiont *Candidatus* sp. associated with the ascidian's larvae.[76]
2018	Synthesis of mandelalide A and L by Smith et al. using a high-yielding anion relay chemistry strategy[71]
2019	Cheong and Carter et al. synthesize the northern fragment of mandelalide A.
2022	Ishmael et al. demonstrate that mandelalides A, B, and L are indirect or secondary inhibitors of adenosine monophosphate-activated protein kinase (AMPK) and potently inhibit of a panel of human glioblastoma cells.

periphery of Algoa Bay. Over the past quarter of a century, 49 new and 36 known natural products with diverse chemical structures and bioactivities have been isolated from 16 Algoa Bay marine invertebrates. Although no new pharmaceuticals have yet emerged from the marine biodiscovery program in Algoa Bay and elsewhere in South Africa, the advantages of international collaborations in furthering marine biodiscovery in South Africa are well documented, both here and elsewhere.[2,4] Of the 20 marine specialized metabolites isolated from Algoa Bay marine invertebrates with moderate (100−1000 nM) and potent (<100 nM) cytotoxicity[23] to selected cancer cell lines (Table 1 and 2), the mandelalides hold out significant promise. Ongoing investigation of the mechanism of action of these potently cytotoxic compounds, and the development of more efficient strategies for their laboratory synthesis, augurs well for the future potential drug development of these compounds. A timeline summarizing milestones in the mandelalide investigation is presented in Table 3.

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

Dedicated to Dr. Mary Garson, The University of Queensland, for her pioneering work on bioactive natural products.

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