

# Natural Product Reports

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## Marine natural products†

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Covering: January to the end of December 2022

This review covers the literature published in 2022 for marine natural products (MNPs), with 645 citations (633 for the period January to December 2022) referring to compounds isolated from marine microorganisms and phytoplankton, green, brown and red algae, sponges, cnidarians, bryozoans, molluscs, tunicates, echinoderms, the submerged parts of mangroves and other intertidal plants. The emphasis is on new compounds (1417 in 384 papers for 2022), together with the relevant biological activities, source organisms and country of origin. Pertinent reviews, biosynthetic studies, first syntheses, and syntheses that led to the revision of structures or stereochemistries, have been included. An analysis of NP structure class diversity in relation to biota source and biome is discussed.

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## 1 Introduction

The annual review of marine natural products was first published in 1984 by John Falkner and the 2024 edition marks its 40th anniversary. This review is of the literature for 2022 and describes 1417 new compounds from 384 papers, compared to 1425 new compounds in 416 papers reported for 2021.<sup>1</sup> In addition, 24 known NPs were reported from a marine source for the first time and 70 known MNPs had their structures revised. We have also introduced a new artefact category this year and this includes three compounds. Only new MNP structures or previously reported compounds where there has been a structural revision, or a newly established stereochemistry are shown in this review. The review also covers previously reported MNPs with significant new bioactivities or ones that have been synthesised for the first time, but their structures are generally not shown. A † symbol on the identifying diagram number is used to distinguish structures where the absolute configuration has

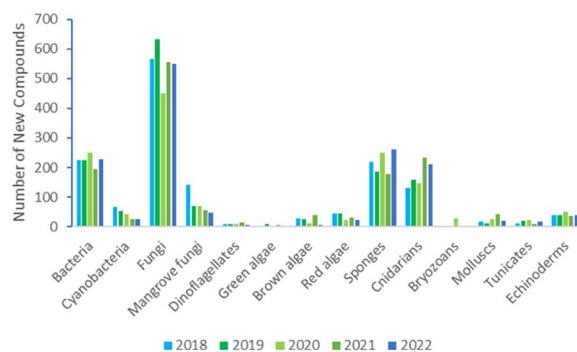


Fig. 1 Trends in new MNPs. The bars represent the total number of new MNPs reported each year over the last five years.

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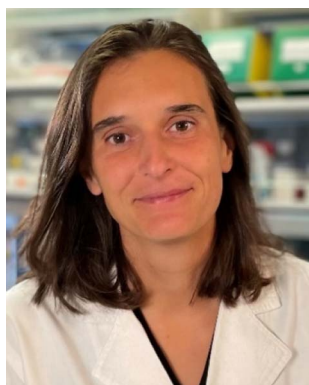
Anthony R. Carroll

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Brent R. Copp

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Tanja Grkovic

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been determined for all stereogenic centres, axes and/or planes in a compound. Reports of new MNPs that were identified based solely on a combination of gene cluster information, MS/MS data and/or Global Natural Products Social (GNPS)-based molecular networking, with compounds not isolated and no NMR data recorded, are excluded from the review. Only a selection of highlighted structures (89) is shown in the review. Compound numbers for structures not highlighted in the review are *italicised*, and all structures are available for viewing, along with their names, taxonomic origins, collection locations, and biological activities, in an associated ESI document.† Access to the curated MNP data held in the MarinLit database<sup>2</sup> provides all the structural and literature data used to prepare this review. The 10 year anniversary of RSC running MarinLit is also being celebrated in 2024. This review welcomes Tanja Grkovic from the National Cancer Institute, USA as a new author into the team.

Trends in the number of new MNPs reported annually over the semi-decade have returned to a pre-pandemic level. Decreasing trends in reporting of cyanobacterial, mangrove fungal and algal metabolites continues. However, this is



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*Rob Keyzers carried out his BSc(Hons) and PhD studies at Victoria University of Wellington. His thesis research, carried out under the guidance of Assoc. Prof. Peter Northcote, a former contributor to this review, focused on spectroscopy-guided isolation of sponge metabolites. He then carried out post-doctoral research with Mike Davies-Coleman (Rhodes University, South Africa) and Raymond Andersen (University of British Columbia, Canada) before a short role as a flavour and aroma chemist at CSIRO in Adelaide, Australia. He was appointed to the faculty at his alma mater in 2009 where he is currently an Associate Professor.*



Michèle R. Prinsep

*Michèle Prinsep received her BSc (Hons) and PhD degrees from the University of Canterbury, where she studied the isolation and structural elucidation of biologically active secondary metabolites from sponges and bryozoans under the supervision of Professors Blunt and Munro. She undertook post-doctoral research on cyanobacteria with Richard Moore at the University of Hawaii before returning to New Zealand to take up a lectureship at the University of Waikato, where she is currently an Associate Professor.*



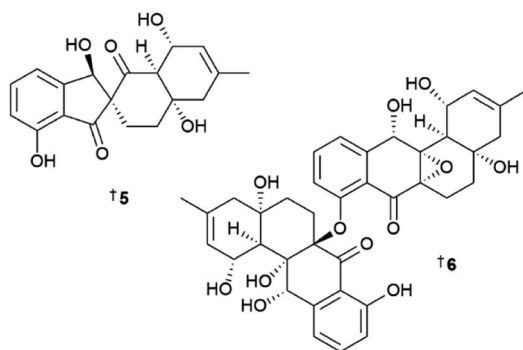
compensated by increases in the reporting of sponge and cnidarian MNPs (Fig. 1).

A paper describing the use of machine learning in combination with fast DU8+ hybrid density functional theory/parametric computations termed DU8ML provides an important additional tool for MNP chemists to accurately assign structures based on interpretation of NMR data. This new method has been applied to reassign the structures of previously published NPs, including a number from marine sources. We anticipate that implementing DU8ML routinely at the time of manuscript preparation and peer review should help to reduce the proliferation of incorrect structures appearing in the literature.<sup>3</sup>

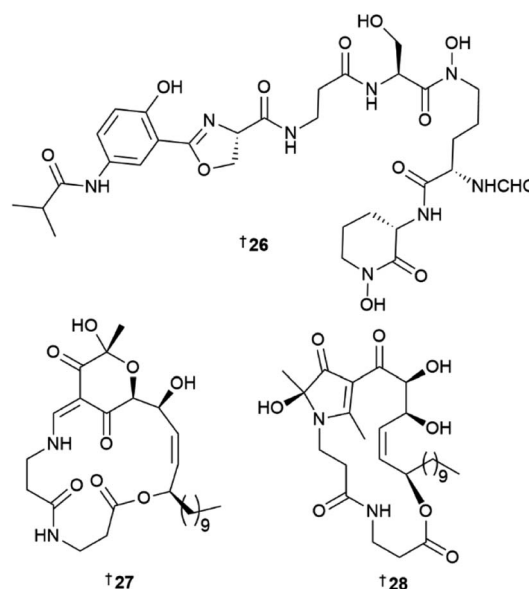
## 2 Marine microorganisms and phytoplankton

### 2.1 Marine-sourced bacteria

Actinobacteria were the most common source of bacterial compounds with 182 new NPs reported in 2022. A new  $\beta$ -carboline, marinacarboline glucuronide **1** was isolated from a sponge-derived *Actinoalloteichus cyanogriseus*.<sup>4</sup> The structure determination of the NP, including the absolute configuration, was aided by single-crystal X-ray diffraction (XRD) analysis. A deep-sea water-derived strain, *Actinomadura* sp. yielded seven new angucyclinone-class polyketides kumemicinones A–G **2–8** which included three novel structures, the 4-hydroxyspiro[4.5]deca-1,6-dione-containing **5** and two ether-bridged dimers **6** and **7**.<sup>5</sup> In a comprehensive study of the NP metabolome of a deep-sea sediment-derived *Amycolatopsis* sp., 22 compounds were reported, including four new structures, a linear peptide agrotetrate A **9**, spermidine derivative **10**, the furan **11** and agrocusin A **12**.<sup>6</sup> Six new chromones, amycolachromones A–F **13–18** were isolated from a deep-sea sediment-derived collection of *Amycolatopsis* sp.<sup>7</sup> while a new 2,5-piperazinedione analogue, georgenione A **19** was reported from *Georgenia* sp.<sup>8</sup> Continuing work on *Gephyromycinifex aptenodytis* derived from the gut microbiota of the Antarctic emperor penguin, *Aptenodytes forsteri* yielded an additional new angucyclinone analogue, 2-hydroxy-frigocyclinone **20**.<sup>9</sup> An aromatic glycoside **21** was identified from a sediment-derived collection of *Nocardiosis synnemataformans*<sup>10</sup> and four new fluvirucin-type macrolactams, fluvirucins B7–B10 **22–25** were isolated from a sponge-derived rare actinomycete *Nonomuraea* sp.<sup>11</sup>



Using metabolomic-guided microbial strain prioritisation and genome-mining strategies, a new 5-aminosalicylate containing siderophore, pseudonochelin **26** was isolated from a sponge-derived *Pseudonocardia* sp.<sup>12</sup> Isotopic feeding studies with labelled *p*-aminobenzoate enabled annotation of the putative biosynthetic pathway and functional gene assignments for **26**. Pseudonochelin showed moderate activity against methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA), and a one log reduction of bacterial cell burden in MRSA-infected mice at an intraperitoneal dose of 160 mg kg<sup>-1</sup>. Four new macrolides, kongjuemycins A **27** B1 **28**, and B2–B3, **29**, **30** were isolated from a coral-derived *Pseudonocardia kongjuensis*.<sup>13</sup> While the structure of **27** has an unusual 2-hydroxy-2-methyl-4-methine-pyran-3,5-dione six membered *O*-heterocycle, in **28–30** it was substituted by a 2-hydroxy-2,5-dimethyl-pyrrol-3-one five membered *N*-heterocycle. *Saccharomonospora* sp. yielded a new indole dimer saccharobisindole **31**, asteric acid analogue **32** and the quinolone **33**.<sup>14</sup>



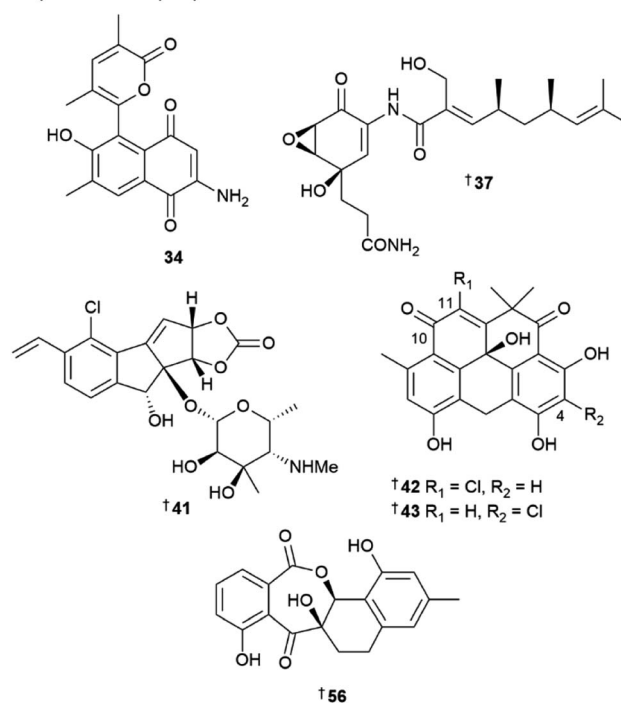
Seven new MNPs were isolated from the genus *Salinispora* in 2022. *Salinispora arenicola* yielded a new aminoquinone polyketide salinisporamine **34**, and two lactones salinorcinol **35** and salinacetamide **36**.<sup>15</sup> The structure of proton-deficient **34** could not be assigned by NMR spectroscopy alone and elucidation was aided by XRD analysis. The structures of **35** and **36** have previously been reported from a mutated rifamycin producer *A. mediterranei* but never as NPs from a wild-type bacterial culture. *S. pacifica* yielded a new manumycin-type polyketide pacificamide **37**.<sup>16</sup> The candidate biosynthetic gene cluster (BGC) *pac* for **37** was identified and bioinformatic analysis showed that closely related BGC were present in other bacterial genera, opening further potential for the identification of this rare class of MNP. A mass spectrometry (MS)-guided screening method yielded three new phosphotriesters salinipostins L–N **38–40** from *S. tropica* with potent serine hydrolase inhibitory activity.<sup>17</sup>



As in previous years, the genus *Streptomyces* was the major source of new compounds from marine bacteria with 140 new MNPs reported in 2022. Eneidyne-targeted genomic signature-based PCR screening of a bacterial DNA library of over 1000 strains resulted in the identification of a new tetracyclic NP, jeju-carboside A **41** from a sediment-derived *Streptomyces* sp.<sup>18</sup> The unusual structure of **41** contains a carbonate-bearing, chlorinated cyclopenta[*a*]indene skeleton and 3-methyl-4-methylamino-4,6-dideoxy-*D*-gulose. Upon comparison of the BGC of other nine-membered eneidyne NPs, a putative biosynthetic pathway for jeju-carboside was proposed. This involved cycloaromatisation of a nine-membered cyclic eneidyne precursor. Notably, the initial yield of **41** in yeast malt extract liquid medium supplemented with sea salt was only 0.008 mg mL<sup>-1</sup> and resulted in a total of 360 L of culture required to isolate 3 mg of the compound. To supply an adequate amount for biological testing, subcultures of the producing strain were screened for increased production of **41** and the titre was increased 150-fold to 1.25 mg mL<sup>-1</sup>. Jeju-carboside A did not show any cytotoxic, antioxidant, or anti-inflammatory activity, demonstrating that the eneidyne moiety is essential for the activity observed for this chemotype. Two chlorinated, pentacyclic polyketides, chlororesistoflavins A **42** and B **43**, were reported from a marine sediment-derived *Streptomyces* sp., with **42** showing potent activity against MRSA (MIC 0.25 µg mL<sup>-1</sup>) which was eight-fold higher than that of the C-4 chloro isomer **43** (MIC 2.0 µg mL<sup>-1</sup>).<sup>19</sup> The two compounds also had remarkably different ECD spectra, with **42** displaying a Cotton effect near the n-π\* transition of the C-10 carbonyl group which was opposite to that observed in other resistoflavins. This difference was attributed to allylic-1,3 strain imposed by the chlorine substitution at the C-11 position of the cyclohexadiene ring in **42**. Both new NPs were shown to transform into their respective C-10 phenolic resistomycin analogues upon prolonged exposure to UV light. A deep-sea hydrothermal vent-derived *Streptomyces* sp. yielded three new actinopyrone analogues, actinoketone **44** and actinopyrones E **45** and F **46**, as well as three known MNPs, actinopyrone D **47**, PM050463 **48**, and PM050511 **49**, the absolute configurations for which have been revised upon re-examination of the Mosher ester results and analysis of the BGC assembly data.<sup>20</sup> The same strain also yielded polyethers, *seco*-salinomycins A-E **50–54**, and mini-pyrone **55**.<sup>21</sup>

A novel ring C-expanded angucyclinone oxemycin A **56** and seven new ring C-fragmented analogues **57–63** have been isolated from a sediment-derived *Streptomyces* sp.<sup>22</sup> Other sediment-derived *Streptomyces* collections have yielded nine hexa-substituted benzothioate glycosides, suncheonosides E-M **64–72**, and four other benzothioate MNPs **73–76**,<sup>23</sup> nine anti-fungal polyene macrolides filipins VI–XIV **77–85**,<sup>24</sup> and four polyketides with a unique 6/5/5 tricyclic ring system, streptoglycerides E–H **86–89**.<sup>25</sup> In addition, a xanthone sathahipmycin **90** was reported from a sediment-derived *Streptomyces* sp. and showed potent antibacterial activity, moderate anti-plasmodial activity, and weak antiproliferative activity against five human

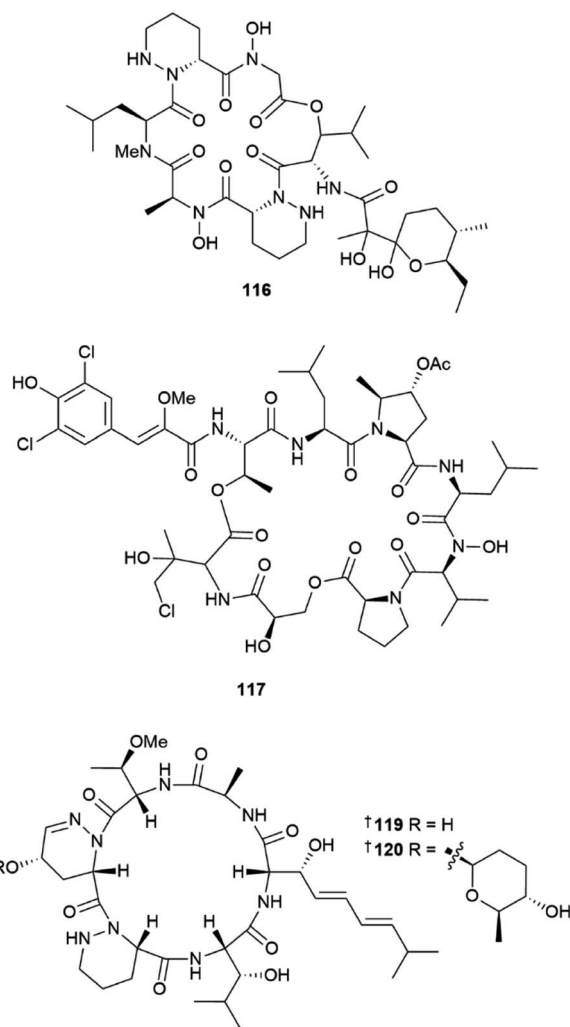
tumour cell lines (HTCLs).<sup>26</sup> Butenolide **91** was reported as a NP for the first time from a sediment-derived collection of *Streptomyces* sp.<sup>27</sup> and a deep-sea sediment-derived *S. chumphonensis* yielded six aromatic acids **92–97**, and three oxime-containing leucine derivatives **98**, **99**, and **100**. Compound **100** was reported as a MNP for the first time.<sup>28</sup> Two dimeric benzoic polyene acids, youssoufenenes A2 **101** and A3 **102** were reported from a *dtla* activated mutant of a *S. youssoufiensis* strain.<sup>29</sup> A sediment-derived *Streptomyces* strain yielded 11 naphthalenic macrolides, hygrocins K–U **103–113** and one phenylpropanamide derivative, streptobenzenepropanamide A **114**.<sup>30</sup>



Two peptides, polyoxyperuin A *seco* acid **115** and polyoxyperuin A **116** were reported from a marine sediment-derived *Streptomyces* sp.<sup>31</sup> The putative BGC *pop* that is responsible for the production of the compounds, was identified, as well as the regulatory elements of the BGC. This enabled a strain that was engineered to overexpress the transcriptional activator to produce the compounds in significantly increased yield. The cyclic analogue **116** had potent antibacterial activity, while the ring opened **115** was inactive. Another sediment-derived *Streptomyces* sp. yielded chlorinated depsipeptides, streptocinnamides A **117** and B **118**.<sup>32</sup> that each contain *m,m*-dichloro-*p*-hydroxy-*cis*- $\alpha$ -methoxycinnamic acid as well as two rare amino acids, 3-hydroxy-4-chlorovaline and 4-acetoxy-5-methyl-proline. Streptocinnamide A showed potent antibacterial activity against *Micrococcus* sp. with a MIC of 4 ng mL<sup>-1</sup>. Pyridapeptide A **119**, a cyclohexapeptide containing the rare hexahydropyridazine-3-carboxylic acid and 5-hydroxytetrahydropyridazine-3-carboxylic acid residues, together with an additional four new glycosylated analogues pyridapeptides B–E **120–123**, were reported from a sponge-



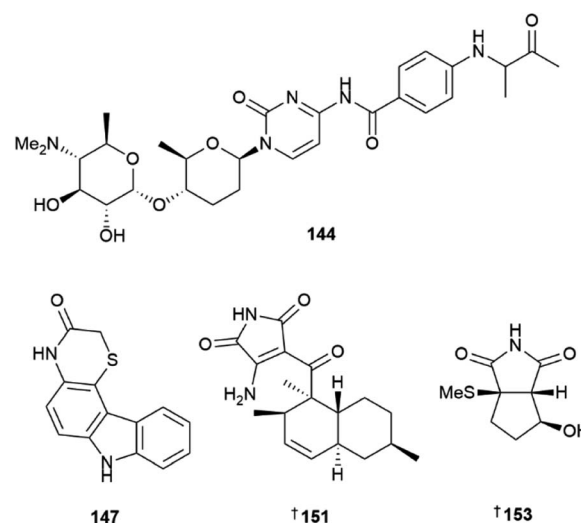
derived *Streptomyces* sp.<sup>33</sup> Bioinformatic analysis identified the putative BGC *pdp* to be responsible for the assembly of the compounds, including the production of the glycosidic bonds and the assembly of the sugar chains. Only **122** and **123** that contained a tetrasaccharide chain, showed weak to moderate antiproliferative activity against a panel of five HTCLs. Seven siderophore-related compounds **124–130** were isolated from a sponge-associated *S. diastaticus*.<sup>34</sup> Other *Streptomyces*-sourced peptide NPs included the epoxy cinnamoyl-containing epoxinamide **131**,<sup>35</sup> teanamides A **132** and B **133**,<sup>36</sup> xanthostatin B **134**,<sup>37</sup> cystargamides C **135** and D **136** (ref. 38) and levesquamide B **137**,<sup>39</sup> as well as four diketopiperazines **138–140** (ref. 40) and **141**.<sup>41</sup>



Three pyrimidine nucleosides streptocytosine P **142**, and cytosaminomycins F **143**, and G **144** were reported from a sediment-derived strain of *Streptomyces* sp.<sup>42</sup> Cytosaminomycin G **144**, with an amine instead of an amide bond between the *p*-aminobenzoic acid and the terminal side chain, represents the first such structural modification within the group. A sediment-derived *Streptomyces* sp. yielded two pyrazine alkaloids, actinopolymorphols E **145** and F **146** with **146** showing weak

antibacterial activity against *Kocuria rhizophilia*.<sup>43</sup> Four carbazoles, thiocarbazomycins A **147** and B **148**, chlocarbazomycin E **149**, and brocarbazomycin A **150** were reported from a coral-associated *Streptomyces diacarni*.<sup>44</sup> Compounds **147** and **148** possess a rare thiomorpholinone group.

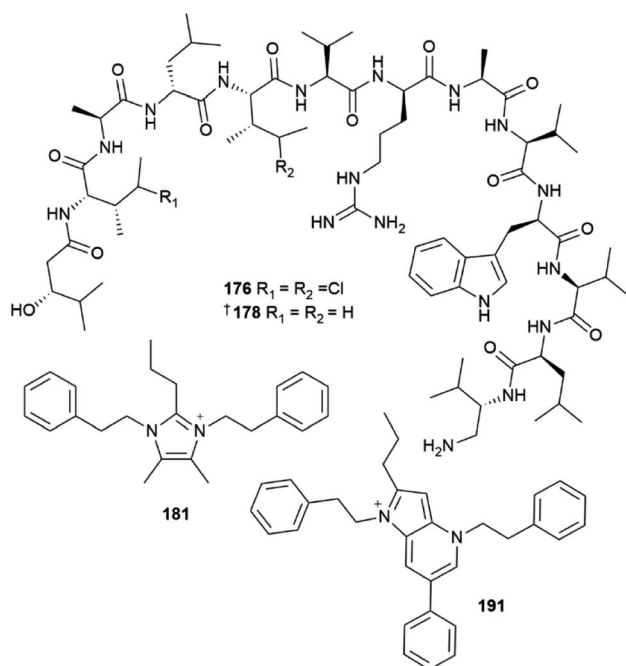
Microbial co-culture of two actinomycete strains, *Streptomyces* sp. and *Achromobacter* sp. derived from the gut microbiota of the isopod *Ligia exotica*, led to the identification of two 3-amino-1*H*-pyrrole-2,5-dione-containing alkaloids, ligiamycins A **151** and B **152**.<sup>45</sup> While the *Streptomyces* sp. strain was found to be the producer of the NPs, the yield of **151** was increased 24 times when co-cultured with *Achromobacter* sp., demonstrating the importance of using ecologically relevant microbial interactions when designing co-culture experiments. A methylsulfide substituted cyclopenta[*c*]pyrrole-1,3-dione bacillimide **153** and a pyrrole-carboxamide containing bacillapyrrole **154** were reported from a sediment-derived *S. bacillaris*.<sup>46</sup> Other *Streptomyces*-sourced alkaloids included the streptindoles A–D **155–158**,<sup>47</sup> streptocarbazoles F–H **159–161**,<sup>48</sup> penzonemycins A **162** and B **163** and demethylmycemycin A **164**,<sup>49</sup> as well as an indole, streptoprenylindole D **165**, co-isolated with a diterpene, 15-hydroxycyclooctatin **166**.<sup>50</sup> Piercidins A5 **167** and G1 **168** were isolated from a sediment-derived *Streptomyces* sp.<sup>51</sup> and two pyrrolisosquiterpenes, glaciapyrroles D **169** and E **170** were reported from a deep-sea sediment-derived *Streptomyces* sp.<sup>52</sup> An ethyl acetate extract of the culture broth of two *Streptomyces* sp. strains yielded three flavonoid-like glycosides, actinoflavosides B–D **171–173**.<sup>53</sup> While their structures are similar to plant-sourced flavonoids, they are distinguished by additional alkylation at C-5 and the rare ristosamine amino sugar moiety. Two trehalose lipids, tsukalipids A **174** and B **175** were reported from a sediment-derived *Tsukamurella pseudospumae*.<sup>54</sup>



The phylum Bacteroidetes yielded 24 new MNPs in 2022. A sponge-derived *Aquimarina* sp. contained five antibacterial peptides, aquimarins A–E **176–180**.<sup>55</sup> These MNPs bear an unusual amino group at the C-terminus and isoleucine residues chlorinated at the  $\gamma$ -position for **176** and **177**. Full structural



assignment of **176–180**, was carried out using conventional spectroscopic and spectrometric methods and the confirmation of the structures of **178** and **179** was complemented *via* total synthesis. The structures of three other, minor analogues, aquimarins F–H were only proposed based on the analysis of mass spectral fragmentation data and their structures are not included. Aquimarins A–D showed activity against a panel of Gram-positive bacteria. They most potently inhibited *M. tuberculosis* growth with IC<sub>50</sub> values of 45 and 23 nM for **176** and **177** respectively. Fourteen bacteria-sourced, imidazolium-containing MNPs were reported in 2022. This is a remarkable statistic since prior to 2022, the positively charged 1,3-difunctionalised imidazolium structural motif had only been reported once in MNPs.<sup>2</sup> A red alga-derived *Tenacibaculum discolor* yielded eight imidazolium alkaloids discolins A–H **181–188**, together with two 1,2,3,5-alkylated pyridinium structures dispyridine A **189** and dispyridine **190**, 1*H*-pyrrolo[3,2-*b*]pyridinium alkaloids dispyrrolopyridines A **191** and B **192**, and the dispyrrole **193**.<sup>56,57</sup> Notably, dispyrrolopyridine A **191** showed potent antibacterial activity, moderate antifungal activity, and weak nematocidal activity. Hydroxamate-containing siderophores, tenacibactins K–M **194–196** were isolated from a coral-derived *Tenacibaculum* sp., with **196** showing moderate activity against rat embryonic fibroblasts and the P388 murine leukemia cell line.<sup>58</sup>



The phylum Firmicutes yielded 18 new MNPs in 2022. Eight tris-thiazole-containing cyclic peptides, bathiapeptides A–G **197–204** were reported from a biofilm-derived *Bacillus* sp.<sup>59</sup> A putative NRPS-encoding BGC *bat* was proposed to be responsible for the biosynthesis of the compounds. The cyclic hexapeptides **197–201** showed weak to moderate antiproliferative activity against four HTCLs, whereas the pentacyclic **202** and the two

linear bathiapeptides **203** and **204** were inactive. Six additional imidazolium alkaloids bacillimidazole A–F **205–210** were reported from a sponge-associated *Bacillus* sp.,<sup>60</sup> and a new glycosylated indole alkaloid pityriacitrin D **211** was isolated from *B. siamensis*.<sup>54</sup> Other MNPs reported from the phylum Firmicutes included bacillamide F **212**, a non-ribosomal peptide isolated from a sponge-derived *B. atrophaeus*,<sup>61</sup> and (±)-bacillipyrrole A **213** and bacillipyrzazine A **214** sourced from a Mariana Trench sediment-derived *B. subtilis*.<sup>62</sup>

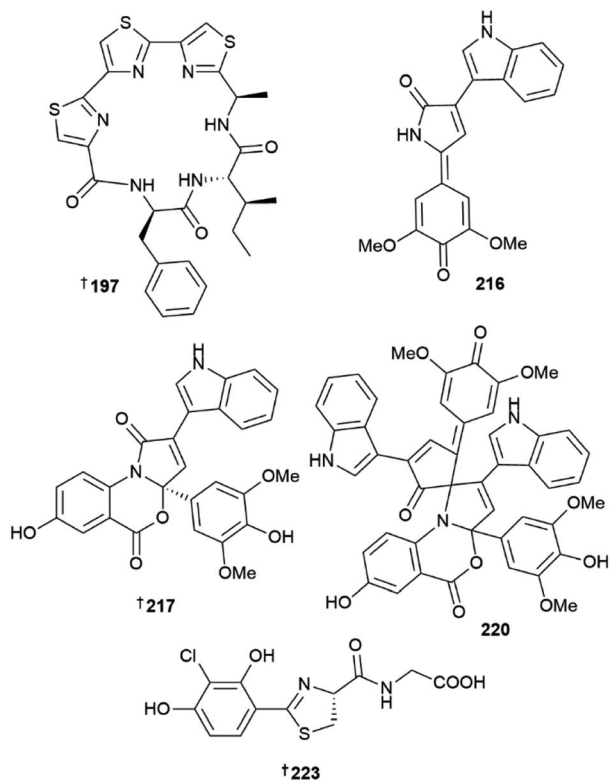
The phylum Pseudomonadota yielded 22 new MNPs in 2022. Eight novel, 3-pyrroline-2-one-containing alkaloids, sinacidins A **215**, and B **216**, sinartyptins A–C **217–219**, and racemic mixtures of sinamicins A–C **220–222** were identified from the  $\alpha$ -proteobacterium *Phaeobacter inhibens*.<sup>63</sup> Structural assignment of **216**, as well as **221** and **222** which were obtained in low yield and analysed as a mixture, was aided by microcrystal electron diffraction (microED). At the time, this was only the second report of this technique being used for the structural elucidation of a new NP. *P. inhibens* was grown in a sea-salt-containing medium enriched with tryptophan and sinapic acid, common microalgal metabolites present in the natural setting where these bacteria are found. The authors were able to demonstrate biosynthetic incorporation of isotopically-labelled tryptophan into the new bacterial NPs, and showed **215–217**, and **220** to have weak algacidal activity against *Emiliania huxleyi*, suggesting they have a defensive role in the producing organism. Teredinibactin **223** and dechloroteredinibactin A **224** were isolated from a shipworm associated bacterium *Teredinibacter turnerae*.<sup>64</sup> Dechloroteredinibactin A **223** represents the first instance of halogen incorporation in a phenolate-thiazoline siderophore and was shown to form complexes with copper, iron, and molybdenum in aqueous solution.

Other proteobacteria-sourced MNPs included eight new butanolides, deoxyenhygrolides C–J **225–232** isolated from the myxobacterium *Plesiocystis pacifica*,<sup>65</sup> marinoquinolones A **233** and B **234** and marinobactoinic acid **235** reported from a coral-derived *Marinobacterium* sp.,<sup>66</sup> and a lipopolysaccharide **236** from a deep-sea collection of *Idiomarina zobellii*.<sup>67</sup>

As with previous years, a small number of MNPs published in the literature from marine bacteria did not have adequate spectrometric and spectroscopic data to support the proposed structures.<sup>68–71</sup> Two structural revisions of bacterial MNPs were reported in 2022; the absolute configurations of the cyclic peptides ogipeptin A **237** and tumescenamide A **238** were corrected *via* total synthesis.<sup>72,73</sup> Other total syntheses of bacterial NPs included cyanogramides A and C,<sup>74</sup> (±)-nesteretal A,<sup>75</sup> lucentamycin A together with barmumycin, oxotomaymycin and oxoprothracarcin,<sup>76</sup> rakicidin F,<sup>77,78</sup> anthracimycin and anthracimycin B,<sup>79</sup> xiamycins C–F,<sup>80</sup> seongsanamide E,<sup>81</sup> salimabromide,<sup>82</sup> (+)-nocardiozine B,<sup>83</sup> the indolocarbazole alkaloid ZHD-0501,<sup>84</sup> (±)-spiroindimicins A, D, G and H,<sup>85</sup> bahamaolide A,<sup>86</sup> aqabamycin G,<sup>87</sup> and elmonin and pratenone A.<sup>88</sup> Reviews focused on marine bacterial NPs published during 2022 included publications on the biosynthesis of microbial terpenoids,<sup>89</sup> polyketides and non-ribosomal peptides,<sup>90</sup>



ribosomal peptides,<sup>91</sup> and the anthracyclines.<sup>92</sup> A comprehensive review of the biologically active NPs from the genus *Micromonospora* together with their mode of action, biosynthetic pathways and chemical syntheses was summarised,<sup>93</sup> as was the potential of marine sponges as sources of diverse bacteria.<sup>94</sup>



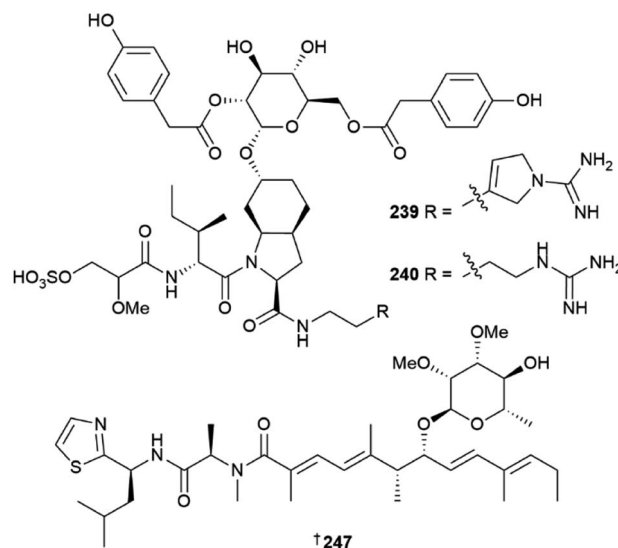
Interestingly, most bacterial MNPs reported in 2022 were identified as a result of either genome- or metabolomics-guided studies of single bacterial strains. While some papers followed the bioassay-guided isolation paradigm, surprisingly few reported new NPs that were responsible for the observed extract bioactivity. Overall, sediment followed by marine invertebrate collections were the primary sources of new MNP producing bacterial strains. Unique ecological niches such as the deep sea,<sup>5,6,8,67</sup> Mariana Trench,<sup>62</sup> hydrothermal vents,<sup>20,21</sup> and the gut microbiota of the Antarctic emperor penguin<sup>9</sup> were also explored. In 2022, the biosynthetic potential of the open ocean microbiome was assessed by analysing more than 1000 seawater samples which resulted in the identification of over 40 000 putative BGCs, most of which were new,<sup>95</sup> demonstrating there are many new niches and opportunities for new bacterial MNPs to be explored.

## 2.2 Cyanobacteria

Thirty new cyanobacterial NP structures were reported in 2022, with the majority belonging to either the peptide or a mixed PKS/NRPS biosynthetic origin. Two aeruginosin-type linear peptides, varlaxins 1046A 239 and 1022A 240 were isolated from *Nostoc* sp.<sup>96</sup> The compounds were shown to be potent inhibitors

of porcine and human trypsins, with 239 one of the most potent cyanobacterial trypsin inhibitors found. Notably, the 1-amidino-3-(2-aminoethyl)-3-pyrroline amino acid moiety in 239 was shown to enhance activity and selectivity in three human trypsin isoforms over a hundred-fold compared to 240 that contained a 4-amidinobutylamide moiety. Other new cyanobacterial-sourced peptides included the linear peptides amantamide B 241 from *Oscillatoria* sp., identified via a MS/MS-based molecular networking approach,<sup>97</sup> acetylene-containing odookeanynes A 242 and B 243 from *Okeania* sp.,<sup>98</sup> as well as the cyclic depsipeptides triproamide 244 and pemukainalides A 245 and B 246 isolated from *Symploca hydroides*.<sup>99</sup>

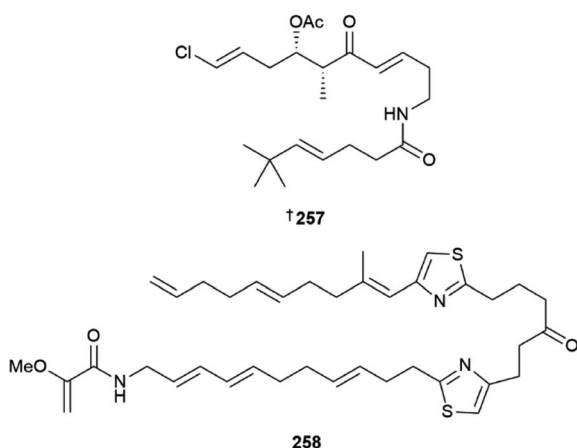
A novel peptide-polyketide hybrid NP, iezoside 247, was reported from the marine benthic species *Leptochromothrix valpauliae*.<sup>100</sup> Structurally novel features of 247 include an odd number of carbon atoms along the polyketide backbone, a  $\beta$ -branched methyl group at C-17, and a 2,3-*O*-dimethyl- $\alpha$ -1-rhamnose substitution positioned between an  $\alpha,\beta,\gamma,\delta$ -unsaturated amide and a conjugated diene group. Confirmation of the absolute configuration of 247 containing eight stereogenic centres, was achieved via total synthesis in over 16 steps and in a 4.4% overall yield. The compound showed potent anti-proliferative activity against HeLa cells (IC<sub>50</sub> of 6.8 nM) and caused spindle-type morphological changes and cell cycle delay, which led to it being identified as an inhibitor of the sarco/endoplasmic reticulum Ca<sup>2+</sup>-ATPase membrane protein. Luquilloamides A-G 248–254 of a mixed PKS/NRPS biosynthetic origin were reported from *Oscillatoria* sp.,<sup>101</sup> while a novel strain of *Hormosilla* sp. yielded anaenamides C 255 and D 256.<sup>102</sup>



The acyclic polyketide beru'amide 257 was isolated from *Okeania* sp.<sup>103</sup> Configurational assignment was achieved via total synthesis, and the compound showed moderate activity against *Trypanosoma brucei rhodesiense* (IC<sub>50</sub> 1.2  $\mu$ M) and weak activity against the HeLa cell line. Caldorazole 258, reported from *Caldora* sp., is a new polyketide with two thiazole rings and an *O*-methyl-enolpyruvamide end terminus. It showed potent antiproliferative



activity towards three HTCLs and was found to inhibit mitochondrial electron transport.<sup>104</sup> Heterologous expression of the columbamide BGC from *Moorena bouillonii* in *Anabena* yielded several known columbamide NPs as well as five chlorinated acyl amide analogues, including columbamide K **259**.<sup>105</sup> The gene cluster for this PKS/NRPS hybrid pathway is 28 kb in length and was not well tolerated in the host, limiting the isolated yields of the new NPs. Due to low mass of compounds available, complete NMR structural characterization was only achieved for columbamide K **259** while columbamides I, J, L and M were only characterised by high resolution MS, mass spectral fragmentation and <sup>1</sup>H NMR data, so their structures are not included here. An azirine-containing NP, dysidazirine carboxylic acid **260** was isolated from a novel *Caldora* sp.<sup>106</sup> Three eudesmane-type sesquiterpenes **261–263** were isolated from media extracts of *Scytonema* sp.<sup>107</sup> While these sesquiterpene NPs are typically reported from fungi, no attempt was made to show the presence of the compounds in the cyanobacterial cell mass.



Reviews focused on marine cyanobacteria included a comprehensive review of the distribution, NP chemistry, and ecology of *Moorena producens*,<sup>108</sup> and the anti-infective potential of the NPs reported from this genus.<sup>109</sup> Cytotoxic, anti-proliferative and antineoplastic activities<sup>110</sup> and anti-inflammatory, antioxidant, antimicrobial, antiviral and anticancer activities of cyanobacterial NPs were also reviewed.<sup>111</sup> In addition, the use of cyanobacteria and their toxins against fungal and oomycete phytopathogens was reviewed.<sup>112</sup>

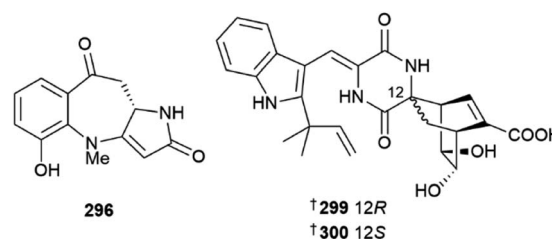
Total syntheses of bastimolides A and B,<sup>113,114</sup> and the 10-aza-9-oxakalkitoxin analogue of kalkitoxin<sup>115</sup> were reported in 2022. Other notable work on cyanobacterial NPs included a report on the biosynthesis of the carbon skeleton of nocuolin,<sup>116</sup> synthesis of coibamide A mimetics with improved cellular bioactivity,<sup>117</sup> and an evaluation of antitrypanosomal activity of gallinamide and analogues.<sup>118</sup>

### 2.3 Marine-sourced fungi (excluding from mangroves)

Cultures of *Acremonium* species led to isolation of sorbicillinoid derivatives **264–266**,<sup>119</sup> steroid **267**,<sup>120</sup> chlorinated orsellinic aldehyde derivatives **268–270** and brominated orsellinic acid **271**, the last of which is a known synthetic compound but new NP.<sup>121</sup>

*Albifimbria verrucaria* was the source of a modified  $\gamma$ -lactone **272** (ref. 122) and the genus *Alternaria* yielded a range of metabolites including the meroterpenoids tricycloalternarenes O–R, **273–276**,<sup>123</sup> dibenzo- $\alpha$ -pyrone derivatives **277–279**,<sup>124</sup> sulfated dibenzopyrones **280** and **281**,<sup>125</sup> phomalone derivatives **282–284** (ref. 126) and perylenequinone derivatives **285–287**.<sup>127</sup> Picoline-derived meroterpenoids, amphichoterpenoids D **288** and E **289** (C-10 epimers)<sup>128</sup> and  $\alpha$ -pyrones **290** and **291** (ref. 129) were obtained from *Amphichorda felina*, the last two *via* genome mining and heterologous expression and an *Athrinium* species yielded carboxamides **292** and **293** and polyketide **294**.<sup>130</sup>

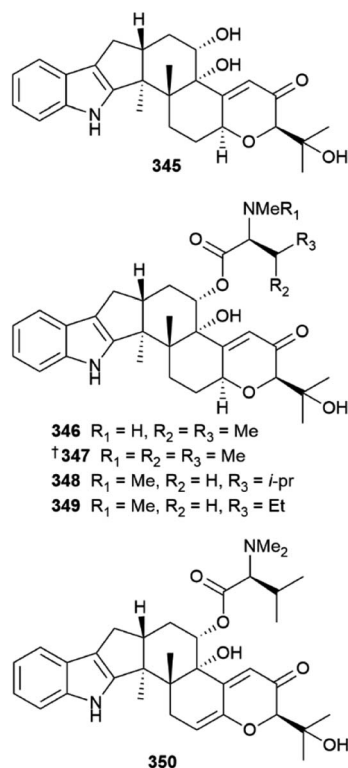
As has been the case in previous years, the genus *Aspergillus* was overwhelmingly the most common source of fungal metabolites this year. A gorgonian-derived strain of *Aspergillus candidus* was the source of the pyrrolinone-fused benzazepine alkaloids asperazepanones A **295** and B **296**. Asperazepanone A **295** was first isolated as a racemate but further studies indicated that only (+)-**295** was a NP. Total syntheses of both metabolites were achieved by employing an intramolecular Friedel–Crafts reaction and **296** exhibited potent LPS-induced expression of both TNF- $\alpha$  and IL-6.<sup>131</sup> Co-culture of *A. candidus* and *Beauveria felina* yielded drimane sesquiterpenes **297** and **298** that were mixtures of stereoisomers.<sup>132</sup> Chevalinulins A **299** and B **300**, alkaloids with an unprecedented spiro[bicyclo[2.2.2]octane-diketopiperazine] skeleton, were isolated from *A. chevalieri* sourced from deep-sea cold-seep sediment,<sup>133</sup> whilst another deep-sea-derived strain of *A. chevalieri* yielded indole diketopiperazine alkaloids **301** and **302**.<sup>134</sup> Other deep-sea-derived *Aspergillus* strains yielded indole alkaloids **303** and **304** (ref. 135) and polyketides **305**,<sup>135</sup> **306** and **307**.<sup>136</sup> Cytochalasins **308** and **309** were isolated from a culture of *A. flavipes*, although the possibility that they may be artefacts of isolation could not be excluded<sup>137</sup> and *A. flavus* strains yielded triterpene **310** (ref. 138) and  $\alpha$ -cyclopiiazonic acid alkaloid **311**.<sup>139</sup>



*N*-Methylated cyclic peptides, asperflomide **312** and asperfosamide **313** were isolated from *A. flocculosus*<sup>140</sup> and *A. fumigatus* strains were the source of a steroid **314**, a 2-oxo-furanone derivative **315**, indole alkaloids **316** and **317** (as an inseparable mixture), **318**, pseurotin A derivative **319**,<sup>141</sup> alkaloids **320–324**, and penibenzophenone E **325**.<sup>142</sup> Gorgonian-derived *Aspergillus hiratsukae* strains yielded  $\alpha$ -pyrone meroterpenoids **326–331**,<sup>143</sup> cyclic peptide **332**, ecdysteroid derivative **333** and sesquiterpene lactone **334**,<sup>144</sup> and nitrobenzoyl sesquiterpenoids **335–338**,<sup>145</sup> cyclohexadepsipeptides **339**, **340** (ref. 146) and sesquiterpenoids **341**, **342–344** (ref. 147) were obtained from sponge-derived



*Aspergillus* strains. Isolation of indole diterpene amino acid conjugates **345**–**350** from *A. noonimiae* suggested that the corresponding BGC in the fungus contains an NRPS-like modifying enzyme able to incorporate multiple lipophilic amino acids.<sup>148</sup>



Culture of *Aspergillus ochraceopetaliformis* yielded polyketide **351** (ref. 149) and cyclic tripeptide **352**,<sup>150</sup> whilst co-cultivation of deep sea-derived *A. ochraeus* with a terrestrial soil-derived *Penicillium* yielded prenylated indole alkaloids **353** and **354**.<sup>151</sup> *Aspergillus* strains derived from deep-sea sediment yielded a variety of metabolites including phenols **355**, **356** (C-8 epimers), **357**, **358**,<sup>152</sup> aromatic polyketides **359**–**361** and isoquinoline alkaloids **362**, **363**,<sup>153</sup> **364**–**377**,<sup>154</sup> acremolin alkaloid **378**,<sup>155</sup> and cyclopropane acids **379**–**382**.<sup>156</sup> Sponge-derived strains were the source of 2,5-diketopiperazines **383**–**385**,<sup>157</sup> isocoumarin **386**, propylpyridinium anthraquinone **387**, resorcinol derivative **388**,<sup>158</sup> and dipyrroloquinones **389**, **390**,<sup>159</sup> whilst notoamide alkaloids **391**–**396**,<sup>160</sup> prenylated notoamide-type alkaloids **397**–**406**,<sup>161</sup> butenolides **407**–**411** and *p*-hydroxybenzaldehyde derivative **412** (ref. 162) were obtained from gorgonian-derived strains. Pulvinone derivatives, aspulvinones S–V **413**–**416** were isolated from green alga-derived *A. terreus*,<sup>163</sup> a coral-derived strain of *A. unguis* was the source of polyketides **417** and **418**, (the latter a known synthetic compound and terrestrial metabolite but a new MNP).<sup>164</sup> A shrimp-derived *A. unguis* strain yielded ergostane-type sterols **419**–**422**,<sup>165</sup> nitrogenous metabolites variotin B **423** and coniosulfide E **424**,<sup>166</sup> and phenolic polyketides **425**, **426**,<sup>167</sup> whilst various seawater-derived *Aspergillus* strains yielded phenolic

polyketide **427** (ref. 167) aromatic bisabolene sesquiterpenoids **428**–**431** and benzaldehyde derivative **432** (the last a known synthetic compound but new NP)<sup>168</sup> and cyclohexapeptides **433**–**436**.<sup>169</sup> Other *Aspergillus* strains derived from sediment yielded nucleoside derivatives **437**, **438**,<sup>170</sup> austocystin analogues **439**, **440**,<sup>171</sup> diketopiperazines **441**–**444** (ref. 172) and indole diketopiperazine alkaloids **445**–**448**,<sup>173</sup> and gorgonian-derived strains were the source of indole diketopiperazine hybrids **449**–**452**,<sup>174</sup> benzodipyran comprising eurotiumide G enantiomers **453**, **454** (configurations revised to 1*S*,3*R*,4*R* and 1*R*,3*S*,4*S* respectively), **455**–**462** (all isolated as racemates but separated by chiral HPLC),<sup>175</sup> asperbenzophenone A **463** and versicolamide C **464**.<sup>176</sup>

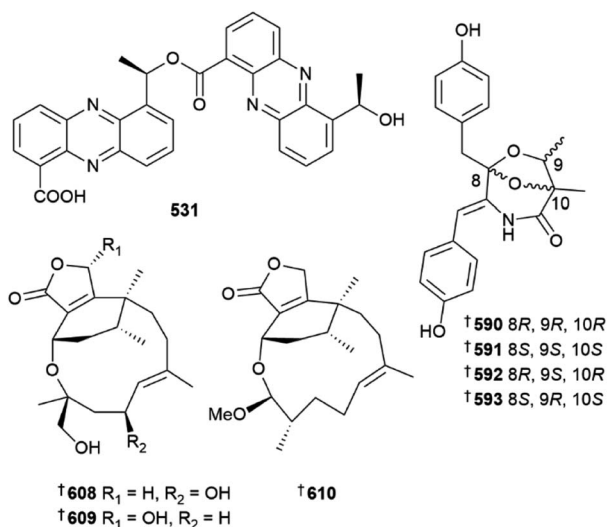
Re-profiling of a fungal library derived from the gastrointestinal tract of commercially sourced mullet by various means including miniaturised 24-well plate cultivation (MATRIX), chemical profiling and precursor directed biosynthesis, identified an *Aspergillus* strain as a chrysosporazine producer and led to the isolation of chrysosporazines T, U, T1 and U1, **465**–**468**.<sup>177</sup> Other *Aspergillus* strains yielded phenolic glucosides **469**, **470**,<sup>178</sup> dipeptides **471**, **472**,<sup>179</sup> the unusual steroid-sorbicillinoid adduct **473**,<sup>180</sup> the unsaturated fatty acids, pantheric acids D–F **474**–**476**,<sup>181</sup> *p*-terphenyl derivatives **477**–**481** (ref. 182) and depsidone **482**.<sup>183</sup>

Destruxin hexadepsipeptides **483** and **484** were isolated from a culture of *Beauveria felina*<sup>184</sup> and *Byssoschlamys spectabilis* was the source of terpenoids **485**–**490** and polyketides **491**–**493**, the last a known synthetic but new NP.<sup>185</sup> A precursor-directed biosynthetic approach to culturing of a *Chrysosporium* species led to isolation of a range of metabolites. Supplementation with a range of precursors yielded neochrysosporazines A–L **494**–**505**, chrysosporazines R **506** and S **507**, neochrysosporazines M–Q **508**–**512** and the known terrestrial but new MNP, hancockamide C **513**. A SAR study of the new analogues indicated key structural requirements for reversal of P-glycoprotein efflux mediated doxorubicin resistance in a HTCL.<sup>186</sup> *Cladosporium* strains derived from deep-sea sediment were the sources of indole derivatives **514** and **515**,<sup>187</sup> sulfur and peroxy bridged macrolide **516** and iodinated dimeric naphtho- $\gamma$ -pyrone **517** (ref. 188) and oxygenated fatty acids, *seco*-patulolides **518**–**520**.<sup>189</sup> Co-culture of a *Cosmospora* species with phytopathogenic fungus *Magnaporthe oryzae* yielded isochromanones **521** and **522**, the latter as an inseparable mix of epimers<sup>190</sup> and a culture of *Curvularia verruculosa* was the source of cytochalasin derivatives **523**–**525**, the last a known synthetic compound but new NP.<sup>191</sup> *Cystobasidium laryngis* derived from deep-sea sediment yielded the diphenazines phenazostatins E–J **526**–**531**, of which phenazostatin J **531** exhibited potent cytotoxicity to six HTCLs and potent antineuroinflammatory activity *via* inhibition of NO production whilst phenazostatins E–I were inactive in these assays.<sup>192</sup> An ascidian-derived strain of *Diaporthe* was the source of a range of metabolites including xanthenes **532**–**538**,<sup>193</sup> monoterpenes **539**, **540**, (C-3 epimers) **541** and  $\alpha$ -pyrone **542**.<sup>194</sup>

A large number of gabosine and/or chlorogentisyl alcohol metabolites **543**–**561** were obtained from an *Epicoccum* species,<sup>195</sup> polyketides **562**, **563**, (C-14 epimers) and **564** were isolated from *Eutypella scoparia*<sup>196</sup> and a tetrahydrocarbazol-1-one analogue, exophilone **565** was obtained from *Exophiala oligosperma*.<sup>197</sup> *Fusarium* strains yielded indole alkaloids **566**–**570**,<sup>198</sup> diketopiperazines



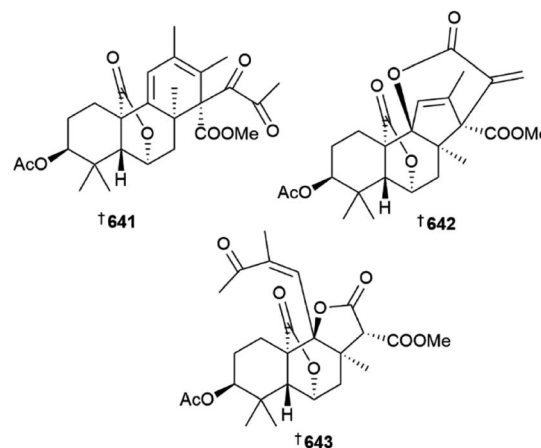
571, 572, polyketides 573–575 and isochromanone 576 (ref. 199) and prenylated glycine derivatives 577 and 578.<sup>200</sup> The macrolides halosmysins B 579 and C 580 were isolated from a Halosphaeriaceae strain,<sup>201</sup> cerebrosides 581 and 582 were obtained from *Hortaea werneckii*<sup>202</sup> and *Lecanicillium fusisporum* was the source of 3-acyl tetramic acid derivatives 583–589.<sup>203</sup> Culture of *Leptosphaerulina chartarum* derived from deep-sea sediment yielded the enantiomeric hydroxybenzyl dimers leptochartamides A 590/591 and B 592/593, which contain a dioxo-azabicyclo[3.2.1]octane core. Total synthesis of each enantiomeric pair was achieved in nine steps.<sup>204</sup> Polyketides 594 and 595 and dendrochol B derivatives 596 and 597 were isolated from a culture of *Lopadostoma puzarii*,<sup>205</sup> *Metarhizium* strains yielded a *N*-butenone spiroquinazoline alkaloid 598,<sup>206</sup> macrolides 599, 600 (as an equilibrating mixture of C-2 epimers), 601 and aromatic glycosides 602 and 603,<sup>207</sup> and supplementation of a *Monascus albidus* culture with amino acids led to isolation of  $\gamma$ -lactams; enantiomers 604/605 and C-3 epimers 606/607.<sup>208</sup>



Neocucurbins A–C, 608–610, phomactins featuring a polyoxygenated-hetero 5/6/12 or 5/6/13 fused tricyclic ring system were isolated from a strain of *Neocucurbitaria unguis-hominis* derived from deep-sea sediment, along with their open chain derivatives neocucurbins D–G 611–614 which contain a 5/6 fused bicyclic ring system.<sup>209</sup> The same *N. unguis-hominis* strain also yielded neocucurbols A–H, 615–622, phomactin diterpene derivatives with either a 6/6/5/5/6 polycyclic ring system (615–618) or a 6/8/6 tricyclic ring system (619–622).<sup>210</sup> Genome mining identified a BGC from *Neosartorya pseudofischeri* and determined that it encodes for the biosynthesis of pseudofisnin A 623, a 1-benzazepine-containing compound. The biosynthetic pathway was elucidated through *in vivo* and *in vitro* experiments.<sup>211</sup> Culture of *Ochroconis humicola* resulted in isolation of *N*-(2-phenylacetyl)benzamide 624, a known synthetic compound but new NP<sup>212</sup> and culture of various *Paraconiothyrium* strains yielded bergamotane sesquiterpenoid derivatives 625–629,<sup>213</sup> 630–637 (ref. 214) and polyketide 638.<sup>215</sup>

The *Penicillium* genus has again been extensively studied as a source of new metabolites. Culture of *Penicillium aculeatum*

yielded sulfonyl metabolites 639 and 640 (ref. 216) and *P. antarcticum* was the source of meroantartines A–C 641–643, meroterpenoids with unique 6/5/6/6, 6/5/6/5/6 and 6/5/6/5 skeletons respectively, all of which exhibited moderate inhibition of P-glycoprotein and resensitisation of resistant cancer cells to docetaxel.<sup>217</sup>

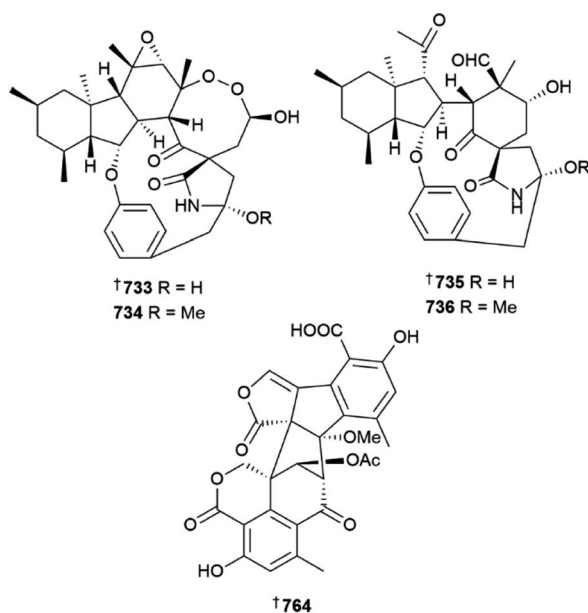


Further meroterpenoids were obtained from *P. chermesinum* (644–647)<sup>218</sup> and *P. chrysogenum* (648–650),<sup>219</sup> (the latter group *via* mutation with diethyl sulfate) and indole diterpenoids, paspalines C–D 651, 652 and paxillines B–D 653–655,<sup>220</sup> *N*-acetyl-D-glucosamine derivatives 656, 657,<sup>221</sup> citreoviridins J–O 658–663,<sup>222</sup> pentacyclic alkaloid 664,<sup>223</sup> dipeptide 665 (ref. 224) and eremophilane-type sesquiterpenes 666–677 (ref. 225) were obtained from various other *Penicillium* strains. Heterologous expression of a nonreducing polyketide synthase (NR-PKS) gene from *P. crustosum* yielded 3-orsellinoxipropanoic acid 678 and indicated the role of the gene as an orsellinic acid (OA) synthase and a transferase,<sup>226</sup> the 13-membered macrolides cyclopiumolides A 679 and B 680 were obtained from a *P. cyclopium* culture.<sup>227</sup> *P. oxalicum* cultures were the source of polyketide-amino acid hybrids 681 and 682 (ref. 228) and polyketides 683 and 684,<sup>229</sup> while azaphilones 685 and 686 were isolated from a *P. sclerotiorum* strain.<sup>230</sup> A deep-sea coral-derived strain of *P. steckii* yielded a number of tanzawaic acid derivatives 687–696 (ref. 231) and polyketides 697–704.<sup>232</sup> A culture of *P. sumatraense* resulted in the isolation of 3-hydroxybutyric acid and glycolic acid derivatives 705–709, (706 a known synthetic but new NP)<sup>233</sup> and cultures of various other *Penicillium* strains yielded dike-topiperazine alkaloid 710 and polyketides 711 and 712,<sup>234</sup> further tanzawaic acids 713–726 (719 and 720 known terrestrial fungal metabolites but new MNPs),<sup>235</sup> citrinin dimers 727–730 (ref. 236) and meroterpenes 731, and 732.<sup>237</sup> The decahydrofluorene alkaloids pyrrospirones K–Q 733–739 were isolated from a soft coral-derived *Penicillium* strain and of these, 733 and 734 possessed a 6/5/6/8/5/6/13 polycyclic skeleton whilst 735 and 736 possessed a 6/5/6/5/6/13 polycyclic skeleton.<sup>238</sup> Co-culture of two deep-sea-derived *Penicillium* strains resulted in isolation of sesquiterpenes 740 and 741 from one strain and organic acid 742 from the other<sup>239</sup> and other sediment-derived



*Penicillium* strains were the source of meroterpenoid **743** (ref. 240) and alkaloids **744**, **745**,<sup>240</sup> **746**, **747**.<sup>241</sup>

Polyketide derivatives **748–752**, were isolated from a *Pestalotiopsis* strain<sup>242</sup> and tyrosine derivative **753** and tereazine derivatives **754** and **755** were obtained from *Phoma herbarum*.<sup>243</sup> *Pseudopithomyces maydicus* was the source of aromatic polyketides **756–760** (ref. 244) and a strain of *Pyrenochaetopsis* yielded pyrenosetin C (for which the configuration was revised to **761**) and related compounds **762** and **763**.<sup>245</sup> Talaverrucin A **764**, a heterodimeric oxaphenalenone with a unique 6/6/6/5/5/5/6 fused ring system, was isolated from an Antarctic sponge-derived strain of *Talaromyces*.<sup>246</sup> Other *Talaromyces* strains yielded a diverse range of metabolites including thioester-containing benzoate derivatives **765–770**,<sup>247</sup> sulfur containing spiro alkaloid **771**, unusual diacid **772** and alkaloids **773–777**,<sup>248</sup> aromatic polyketides **778–782**,<sup>249</sup> tripeptides **783** and **784**, containing an *N-trans*-cinnamoyl group,<sup>250</sup> chlorinated unsaturated alcohol **785** and coumarin derivative **786**,<sup>251</sup> decalin derivatives **787** and **788**,<sup>252</sup> meroterpenoid **789** (ref. 253) and oligophenalenone **790** and **791** and xanthoradone **792** dimers.<sup>254</sup> Culture of a *Trametes* strain yielded spiromeroterpenoids **793** and **794** (ref. 255) and cultures of various *Trichoderma* strains were the source of trichothecanes **795–798**,<sup>256</sup>  $\alpha$ -pyrone **799** and decalin **800**,<sup>257</sup> sesquiterpene glycoside **801** and sorbicillinoid glycosides **802** and **803**,<sup>258</sup>  $\beta$ -carboline alkaloids **804–807** (**805** and **806**, known synthetics but new NPs),<sup>259</sup> and polyketides **808**, **809**,<sup>260</sup> **810–814**.<sup>261</sup>



A machine learning-augmented density functional theory method (DU8ML) was utilised to revise the stereochemistry of tersone E to **815**, which is identical to the terrestrial fungal metabolite citridone A.<sup>3</sup> Structural revision of penipacids A–E, originally isolated from *Penicillium paneum*, from amidines to hydrazones **816–820** through total synthesis also raised the possibility that they may be Schiff base adduct artefacts produced through conjugation with a putative NP precursor, the hydrazine *N*-aminoanthranilic acid with diacetone alcohol

under extraction conditions.<sup>262</sup> Total synthesis of trichomide D also resulted in revision of the stereochemistry to **821**.<sup>263</sup>

A convergent synthetic strategy was utilised in the total synthesis of aspergillolide<sup>264</sup> and a biomimetic route was employed in the synthesis of asperfloketal A which also provided evidence for the biosynthetic hypothesis proposed.<sup>265</sup> Asymmetric total synthesis of asnovolins A and E utilised anionic fragment coupling to construct the sterically crowded skeleton<sup>266</sup> and solid peptide synthesis was employed to assemble the cyclic pentapeptides, versicotides E and F.<sup>267</sup> Indole diterpenoids (+)-shearinines G and D were prepared *via* a convergent route.<sup>268</sup> The C-2 reverse prenyltransferase, NotF was characterised and it was then used in the first synthesis of eurotiumin A,<sup>269</sup> synthesis of (–)-eurothiocin A was achieved in 14 linear steps from commercially available starting materials,<sup>270</sup> the triazole penipanoid A was prepared from 4-methoxyphenyl acetic acid<sup>271</sup> and total synthesis of the 13-membered macrolide (–)-melearoride A was achieved *via* a route including a Julia–Kocienski olefination.<sup>272</sup> Synthesis of the polyketide penicyclone A was accomplished in ten steps using a double Grignard reaction,<sup>273</sup> synthesis of raistrickindole A was achieved *via* two approaches to the diketopiperazine subunit,<sup>274</sup> a divergent strategy was employed in the synthesis of polyketides heterocornol A and B<sup>275</sup> and an Ir(III)-catalysed alkylation of acetophenone in aqueous medium was utilised in the total synthesis of cytosporones A and C.<sup>276</sup>

The cyclodepsipeptides, scopularides A and B displayed potent larvicidal activity against mosquito (*Culex pipiens*) larvae,<sup>277</sup> alkaloids *epi*-azonalenin A and azonalenin were shown to inhibit angiogenesis by inhibition of inflammation and apoptosis<sup>278</sup> and cyclodepsipeptide isaridin E was shown to downregulate the PI3K/Akt signalling pathway and thus possesses antiplatelet and antithrombotic effects.<sup>279</sup> The alkaloid meleagrins had a protective effect in mice against pulmonary fibrosis induced by bleomycin<sup>280</sup> and epidithiodiketopiperazine *N*-methylpretrichodermamide B displayed potent inhibition of P-glycoprotein and was able to resensitise drug resistant cells to docetaxel.<sup>281</sup> Indolyl alkaloid oxaline and anthraquinone isorhodoptilometrin exerted weak anti-neuroinflammatory effects on murine cell lines<sup>282</sup> and the polyketide citrinin exhibited weak anti-parasitic effects against *Trichomonas vaginalis*.<sup>283</sup>

Whole genome sequencing of a sponge-derived strain of *Aspergillus niger* indicated the presence of 69 BGCs for a wide array of secondary metabolites, highlighting the biosynthetic potential of the strain.<sup>284</sup> Refactoring transcription factors in the biosynthetic pathway to the *A. terreus* metabolite terrein greatly enhanced production in mutant strains.<sup>285</sup> Density Functional Theory (DFT) calculations were utilised in study of the asperterpenol/preasperterpenoid biosynthetic pathways in *Aspergillus* and led to proposal of a reaction cascade for construction of the molecular core which is consistent with experimental observations.<sup>286</sup> The biosynthetic pathway to the homodimer phomoxanthone A from a *Diaporthe* fungus was elucidated and indicated that a cytochrome P450 enzyme was implicated in regioselective oxidative coupling to produce the dimer.<sup>287</sup> Elucidation of the biosynthesis of the cyclase



spiromaterpene A from a *Spiromastix* strain involved use of heterologous expression, chemical characterisation and incubation experiments.<sup>288</sup>

Ion mobility can be a useful tool to separate and distinguish coeluting molecules by mass spectrometry and was successfully employed in the case of the aphidicolane diterpenoids.<sup>289</sup> There were many reviews on marine fungi and topics pertinent to them produced in 2022. A few of note include one on bioactive metabolites from extremophilic marine fungi<sup>290</sup> and another on bioactive metabolites from deep-sea-derived strains,<sup>291</sup> one on One Strain Many Compounds (OSMAC) and epigenetic approaches to cryptic metabolites from marine organisms which cited many fungal examples,<sup>292</sup> a review on the use of fungal–fungal co-culture to generate chemical diversity<sup>293</sup> and one covering halogenated metabolites from marine fungi with pharmacological activity.<sup>294</sup>

## 2.4 Fungi from mangroves

Fungi isolated from mangrove roots and their surrounding sediments continue to be a source of derivatives of commonly encountered NP structure classes associated with terrestrial fungi.<sup>295</sup> None of the 47 new mangrove fungi-derived MNPs reported across 13 papers in 2022 contain novel ring systems and only three of the 45 compounds tested showed biological activity above the threshold criteria adopted in this review. Those MNPs that were isolated as single enantiomers (34) had their absolute configurations determined through DFT computational comparisons with experimentally determined ECD data 87% of the time.

Drimane sesquiterpenes **822–825** were reported from a sediment-derived *Aspergillus* sp.<sup>296</sup> and a butyrolactone **826** was isolated from *Aspergillus terreus*.<sup>297</sup> *Daldinia eschscholtzii* was the source of a simple macrocyclic ether, eschscholin B **827**, benzoic acid derivatives dalditones A **828** and B **829**, and naphthalene derivatives **830** and **831**.<sup>298</sup> Sorbicinol derivatives **832**, **833**, **834** and two dimeric sorbicinols **835** and **836** were isolated from sediment-derived *Hypocrea jecorina*.<sup>299</sup> Sediment-derived *Nigrospora camelliae-sinensis* yielded two simple diketopiperazines nigrosporamide A **837** and B **838**.<sup>300</sup> A thiodiketopiperazine, adametizine C **839** and alkane derivatives **840–844** were reported from sediment-derived *Penicillium ludwigii*. Adametizine C showed weak anti-inflammatory activity.<sup>301</sup> A simple  $\alpha$ -pyran **845** and tetrahydrofuran **846** were sourced from *Penicillium polonicum*,<sup>302</sup> while sesquiterpenes containing either a benzofuran (citreobenzofurans D–F **847–849**) or tetrahydronaphthalenone moiety (phomenones A **850** and B **851**), were found in a sediment-derived *Penicillium* sp. collected in Wenchang, China.<sup>303</sup> Phomenone B was the only compound possessing an epoxide and it showed moderate antibacterial activity towards *Bacillus subtilis* but was inactive towards four other bacterial strains. None of the related compounds showed any antibacterial activity. The roots of the Chinese mangrove *Xylocarpus granatum* yielded a *Penicillium* sp. endophyte that contained two isocoumarins penicimarins L **852** and M **853** but neither compound showed antioxidant activity.<sup>304</sup> Two prenylated indole diketopiperazines, penicilamides A **854** and B **855**, and three simple polyketides penicinones A–C **856–858**, were

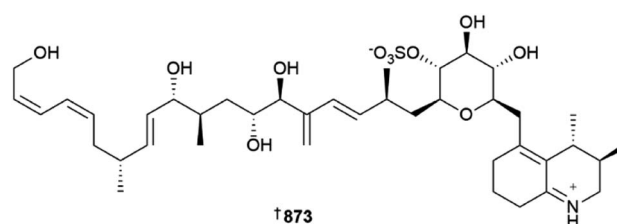
reported from a sediment-derived *Penicillium* sp. The NPs were screened for cytotoxic activity and only penicinone A had weak activity towards one HTCL.<sup>305</sup> Two cyclodecadesepsipeptides, phaeosphamides A **859** and B **860** from *Phaeosphaeriopsis* sp. growing in the rhizosphere of the Chinese mangrove *Bruguiera gymnorrhiza* were tested for cytotoxicity against five HTCLs but only **859** weakly arrested AGS cells in the G2 phase through induction of apoptosis.<sup>306</sup> *Phomopsis asparagi* obtained from the roots of the red mangrove, *Rhizophora mangle* contained cytochalasin derivatives, phomoparagins A–C **861–863**,<sup>307</sup> while *Talaromyces* sp. contained talarobenzofurans A–C **864**, **865**, **866** that are either thioester or carboxylic acid derivatives of a dihydrobenofuran, and two  $\alpha$ -pyrones, talaropyrones A **867**, and B **868**. None of these MNPs possessed antibacterial or  $\alpha$ -glucosidase activity.<sup>308</sup>

## 2.5 Dinoflagellates

The number of new compounds reported from dinoflagellates and other microalgae plummeted in 2022; the number of newly reported MNPs (only six) was approximately half that of the average for the preceding nine years.<sup>1</sup> As is the norm for dinoflagellates, polyketide-derived compounds remain the main biosynthetic class of metabolites reported. Voratins A–C **869–871** are spiro-cyclic compounds from *Effrenium voratum*, a symbiotic dinoflagellate associated with the coral *Alveopora japonica*.<sup>309</sup> while an anti-fungal amphidinol congener **872** was reported from an Irish isolate of *Amphidinium carteri*.<sup>310</sup>

Ovataline **873** is a linear carbohydrate-bearing polyketide isolated from cultures of a Korean strain of *Ostreopsis ovata*, the absolute stereo-structure of which was assigned using standard methodologies. Encouragingly, **873** is non-toxic against brine shrimp and human RWPE-1 epithelial prostate cells, but it moderately suppresses RWPE-1 type II  $5\alpha$ -reductase involved in benign prostatic hyperplasia, making it a useful lead for prostate cancer therapies. Ovataline is also the first non-toxic compound reported from strains of *O. ovata* that also produce the potent toxins palytoxin-like ovatoxins and ostreols.<sup>311</sup>

The final new compound reported from dinoflagellates was a saxitoxin congener **874** from *Alexandrium pacificum*,<sup>312</sup> the structure of a maitotoxin congener was only partially characterised and proposed using MS and NMR data and consequently is not shown here.<sup>313</sup>



The synthesis of the structures proposed for iriomoteolide-1a and 1b, and three possible diastereomers, has been achieved yet none of the spectroscopic data for the synthetic materials matches that reported for the natural isolates, hence the structures of these MNPs require revision.<sup>314</sup> Amphidinolide C2 has been synthesised for the first time.<sup>315</sup>



Notable dinoflagellate-related reviews published in 2022 include a summary of the taxonomy, distribution, genetics and toxin content of *Gambierdiscus* spp.,<sup>316</sup> a compilation of microalgal compounds that influence  $\beta$ -amyloid and  $\tau$ -protein formation and hence may have application in Alzheimer's disease treatments,<sup>317</sup> and an assessment of the methodology used to establish toxicity and regulatory limits of algal biotoxins, along with the implications of climate change on production levels of these important environmental metabolites.<sup>318</sup>

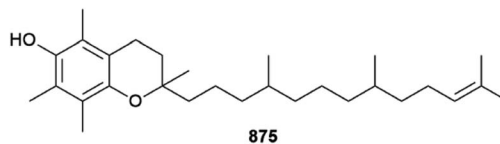
A study has shown not all brevetoxin metabolites (BTXs) bind to voltage-gated sodium channels, the commonly accepted mechanism of action for these biotoxins. Rather, aldehyde-containing BTXs are more immunotoxic, with the conclusion from the study being that different BTX pharmacophores can elicit different toxicity profiles.<sup>319</sup> Another study has evaluated the levels of paralytic shellfish toxins (PSTs; in saxitoxin equivalents) in Arctic food webs during the anomalously warm 2019 period. Notably, high levels of PSTs in benthic clams and the Pacific walrus who predate upon them were detected, at levels high enough to cause observable physiological effects if consumed by humans in comparable amounts.<sup>320</sup> GNPS has been used to profile the chemical diversity of MNPs present in *Gambierdiscus* spp. from the Philippines, indicating a high level of biosynthetic potential in the strains studied,<sup>321</sup> while a new LCMS-based method has been developed to detect PSTs, including C-11 hydroxyl ("M"-class) and benzoate ("GC"-class) saxitoxin analogues that current methods do not pick up.<sup>322</sup>

A stable isotope labelling study using <sup>13</sup>CO<sub>2</sub> has indicated that a polyketide pathway is involved in polyunsaturated fatty acid biosynthesis in *Tisochrysis lutea*, with remarkably fast production of the fatty acids meaning <sup>13</sup>C incorporation into early components of the pathway is undetectable.<sup>323</sup> The anaerobic transformation of domoic acid (*Pseudonitzschia* spp.) by a consortium of benthic microbes (Dalian, China) was profiled, indicating the role other microorganisms could play in the fate and effects of this important biotoxin in marine environments.<sup>324</sup> In a separate study, the biotic and abiotic factors that control toxin production in *Ostreopsis* spp. were examined. Both the ovatoxins and the liguriatoxins were rapidly degraded following exposure to sunlight or other bacteria, which calls into question their effective role in presentation of dermatitis and toxic inhalation following blooms of *Ostreopsis*.<sup>325</sup>

Found in marine sediments, 4-methyl steranes are "molecular fossils" that have been used to unravel eukaryotic evolutionary history. An accepted paradigm in steroidal biosynthesis is that 4 $\beta$ -methyl steranes are formed exclusively from isomerism of their 4 $\alpha$ -stereoisomers within sedimentary deposits. On the other hand, 4 $\alpha$ -methyl steranes are believed to be formed from oxidative demethylation of a triterpenoid precursor. A study of methyl sterane biosynthesis in the coral endosymbiont *Breviolum minutum* has uncovered a methyltransferase that can produce both 4 $\alpha$ - and 4 $\beta$ -methyl steranes, as opposed to the sterol methyl oxidases that were assumed to produce the previously known demethylated compounds. This mechanism of formation of 4-methyl steranes is present not only in dinoflagellates but also in higher eukaryotes of various phyla, identifying a previously unknown source of these biomarkers and could provide further insight into our understanding of the evolution of eukaryotic life on earth.<sup>326</sup>

### 3 Green algae

Although the Chlorophyta (green algae) are always a minor contributor to the number of new MNPs each year,<sup>1</sup> 2022 was exceptional in that only one new compound was reported. 11' $\alpha$ -Tocomonoenol **875** is a common terrestrial plant metabolite, but its presence in green alga *Tetraselmis* sp. means it has been detected from the marine environment for the first time.<sup>327</sup>



Other structures were claimed but they are inconsistent with the spectroscopic data provided and are not shown here.<sup>328,329</sup>

A review of the biotechnological application of edible green alga *Ulva lactuca* has been published.<sup>330</sup> A combined MS-based metabolomics and bioactivity profiling approach using supervised discriminatory statistical methods has been used to highlight various fatty acids, terpenoids and phenolics as the main bioactive components within *Ulva fasciata* and *Spirulina platensis* from the Mediterranean Sea.<sup>331</sup>

The lipidome of a plant is an important element in the organism's resilience to environmental stresses. Betaine lipid diacylglycerol-*N,N,N*-trimethylhomoserine (DGTS) is exclusively found in green algae and non-flowering plants. A study of the effects of phosphorus deficiency upon the green alga *Chlorella kessleri* showed that various zwitterionic phospholipids were degraded to release cellular phosphorus, while DGTS synthesis was promoted at the same time. This remodelling of the lipidome under these conditions to replace phosphorus-containing lipids with an equivalent non-phosphorus containing zwitterionic metabolite can help explain how lower plants respond to conditions of phosphorus deficiency as an acclimation strategy.<sup>332</sup>

### 4 Brown algae

The number of new compounds reported from the Ochrophyta (brown algae) in 2022 was the fewest by a significant margin, being roughly one third of the rolling average over a ten-year period.<sup>1</sup> Somewhat surprisingly, all the newly reported compounds were terpenoid in origin. One meroterpenoid **876** was reported from a Japanese *Sargassum*.<sup>333</sup> A confusing situation has arisen relating to naming of several nor-diterpenoid compounds. A butenolide **877**, named sargassumin A, was reported from a South Korean (Jeju Island) *Sargassum micracanthum*; this manuscript was submitted in mid-2021 and accepted for publication in December of that year.<sup>334</sup> The name sargassumin A was then used for a different lactone **878** along with sargassumin B **879**, from a Jeju Island *Sargassum macrocarpum*, published by a different group with their manuscript submitted and accepted well after the former paper was published.<sup>335</sup> Butenolide sargassumin C **880** was subsequently reported from *S. micracanthum* and is related to **877**,<sup>336</sup> although this publication shows the authors were obviously aware of the others publication and therefore they did not use the



name sargassumin B. Given the timing precedent, the *S. macrocarpum* metabolites should be given a different trivial name, although this may create more confusion in the field than it solves. Two sterols **881** and **882** were also reported from an Australian (Tasmania) *Cystophora xiphocarpa*.<sup>337</sup> Several other structures were claimed but are inconsistent with the spectroscopic data provided and their structures are not shown here.<sup>338,339</sup>

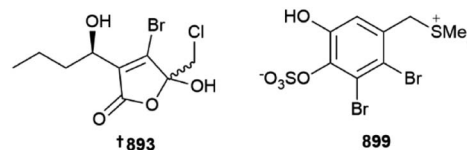
Only one first total synthesis of a brown algal MNP was reported in 2022, that of carotenoid loroxanthin.<sup>340</sup> Reviews focusing on brown algae and their metabolites published in 2022 include summaries of the anticancer activity of various eckols and fucoxanthin, respectively,<sup>341,342</sup> phlorotannins as sedative aids for promoting sleep,<sup>343</sup> and catalogues of the compounds found from species *Ecklonia stolonifera*,<sup>344</sup> and the genera *Dictyota* and *Dictyopteria*, respectively.<sup>345,346</sup>

An *in silico* study of various brown algal phlorotannins as SARS-COV-2 protease inhibitors identified several lead compounds, which were then validated as weak to moderate inhibitors using *in vitro* bioassays.<sup>347</sup> One study has examined the effects of temperature and light on lipid production in two different *Streblonema* algae, with the concentrations of various polar glycerol-derived metabolites varying significantly following exposure to low or high light intensities.<sup>348</sup> The structure of a tetraprenylated chromane has been revised to **883** following in-depth J-based configurational analysis coupled with comparison of experimental and calculated chiro-optical properties.<sup>349</sup> A seasonal study of the volatiles of both fresh and dried Croatian *Dictyota dichotoma* has indicated that the main volatile components are short chain lipids or are diterpenoid in origin, with distinct variation throughout the months of the study facilitating statistical differentiation of the sample treatments.<sup>350</sup>

## 5 Red algae

Acetogenins are common metabolites of red algae, with nine C<sub>15</sub> variants being reported from investigations of various *Laurencia* species from Japan **884** (Katsura)<sup>351</sup> and Greece **885–892**, (Tinos Island),<sup>352</sup> respectively. The tongalides **893–898** are halogenated butenolides isolated from an Antarctic (Bonaparte Point) *Delisea* sp. Although all six isolates were inactive against the ESKAPE panel of clinically relevant microbial pathogens, they are related to the co-isolated known compound Z-acetoxyfimbrolide A, which was responsible for the antibacterial activity of the algal extract, showing the importance of the exocyclic alkene functionality for bioactivity.<sup>353</sup> An Irish *Vertebrata lanosa* was the source of six brominated amino acid derivatives **899–904** along with a glycolipid **905**; **899** is the first algal compound to incorporate a dimethylsulfonium moiety on a phenolic ring.<sup>354</sup> Other structures were put forward although these were inconsistent with the spectroscopic data provided and are not shown here.<sup>355</sup>

Several first total syntheses of red algal metabolites were published in 2022, including those of C<sub>15</sub> acetogenins dehydroitomanallene B,<sup>356</sup> Z-**906** and E-ocellenyne **907**, which also established their absolute configurations,<sup>357</sup> sesquiterpenoid laurencenone C<sup>358</sup> and thysiferol-type triterpenoid saiyacenol A.<sup>359</sup>



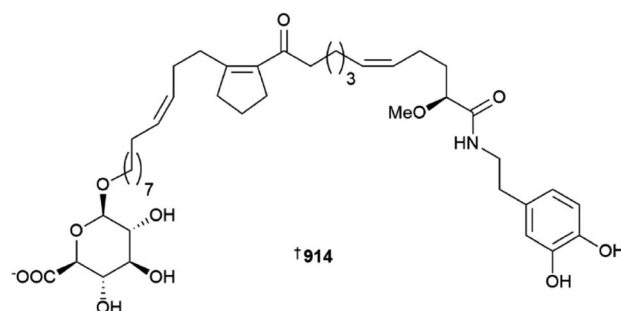
A review of the metabolites from the possibly invasive genus *Acanthophora* and of *Asparagopsis*, respectively, have been published,<sup>360,361</sup> as has a summary of red algal compounds with skin whitening cosmeceutical potential.<sup>362</sup>

The role of carotenoids in the adaptation of the intertidal red alga *Neopyropia yezoensis* towards daily desiccation has been investigated. Upregulation of carotenoid biosynthesis was observed once the thalli of the alga reached the critical 60% dehydration point, presumably to act as antioxidants against damage caused in the air.<sup>363</sup>

Domoic acid is an important diatom-derived neurotoxin, although it was originally discovered from the red alga *Chondria armata*. Analysis of the *C. armata* BGC responsible for domoic acid production showed that the *rad* genes responsible for its production are organised in a similar fashion to those found in the diatom *Pseudonitzschia multiseries*, including the presence of a key cytochrome P450 that is absent in other red algal BGCs that encode for the related, but chemically simpler, neurotoxin kainic acid. Detailed investigation of the domoic acid biosynthesis in *C. armata* suggests a slightly different route to its formation when compared to that found in the diatom. Overall, this study has shown that domoic acid production in *C. armata* incorporates aspects of algal and microbial biosynthesis and hints at a complex evolutionary history.<sup>364</sup> A timely review of the biosynthesis of domoic and kainic acids have also been published.<sup>365</sup>

## 6 Sponges

Various glycerolipids and butenolides have been isolated from *Pericharax heteroraphis* **908**, **909** and *Scopalina hapalia* **910–913**, respectively; the synthesis of the *Pericharax* metabolites was also achieved.<sup>366,367</sup> The toporosides A–D **914–917** are unusual cyclopentene-containing fatty acid amides from a deep-sea (dredged at 476–519 m) *Stelodoryx toporoki*. Three of the isolates prevent TNF- $\alpha$ -induced damage to rat cardiac cells at non-toxic concentrations; based upon SAR analysis, the C-11 carbonyl is important for cardio-protective activity.<sup>368</sup> A series of alkynynols **918–934** and polyacetylenes **935–937** have been reported from *Cribochalina* and *Petrosia* sponges, respectively,<sup>369,370</sup> while plaki-lactone J **938** is an ethyl-branched polyketide  $\gamma$ -lactone and its absolute configuration was determined using VCD data.<sup>371</sup>



As always, many peptidic MNPs were reported from sponges in 2022. Such isolates include subarmigeride A **939** from an Indonesian *Suberea*,<sup>372</sup> various friomaramides **940**, **941** and shagamides **942–947** from an Antarctic *Inflatella*,<sup>373</sup> ectyoplasin **948** from a Mexican *Ectyoplasia*,<sup>374</sup> cyclopsammocinamide A **949** and B **950** which are of the opposite enantiomeric series to known cyclocinamide A,<sup>375</sup> and a new aciculitin **951** from a *Poecillastra* dredged from a Japanese sea-mount.<sup>376</sup> Two separate samples of the prolific genus *Theonella* yielded three theonellapeptolides **952–954** and theonellamide/theopalauamides **955–957**, respectively.<sup>377,378</sup>

A Chinese (Yongxing Island) *Axinella* was the source of four peptides named axinellasin A–D **958–961**, found as two pairs of diastereomeric methionine oxides. The isolation of the peptides was facilitated by use of precursor ion-directed supercritical fluid chromatography targeting sulfoxide-containing metabolites. All four compounds were inactive against five HTCLs but were active as immunosuppressive agents at 10  $\mu$ M *via* inhibition of B and T cell proliferation. The total synthesis of axinellasin D was also achieved.<sup>379</sup>

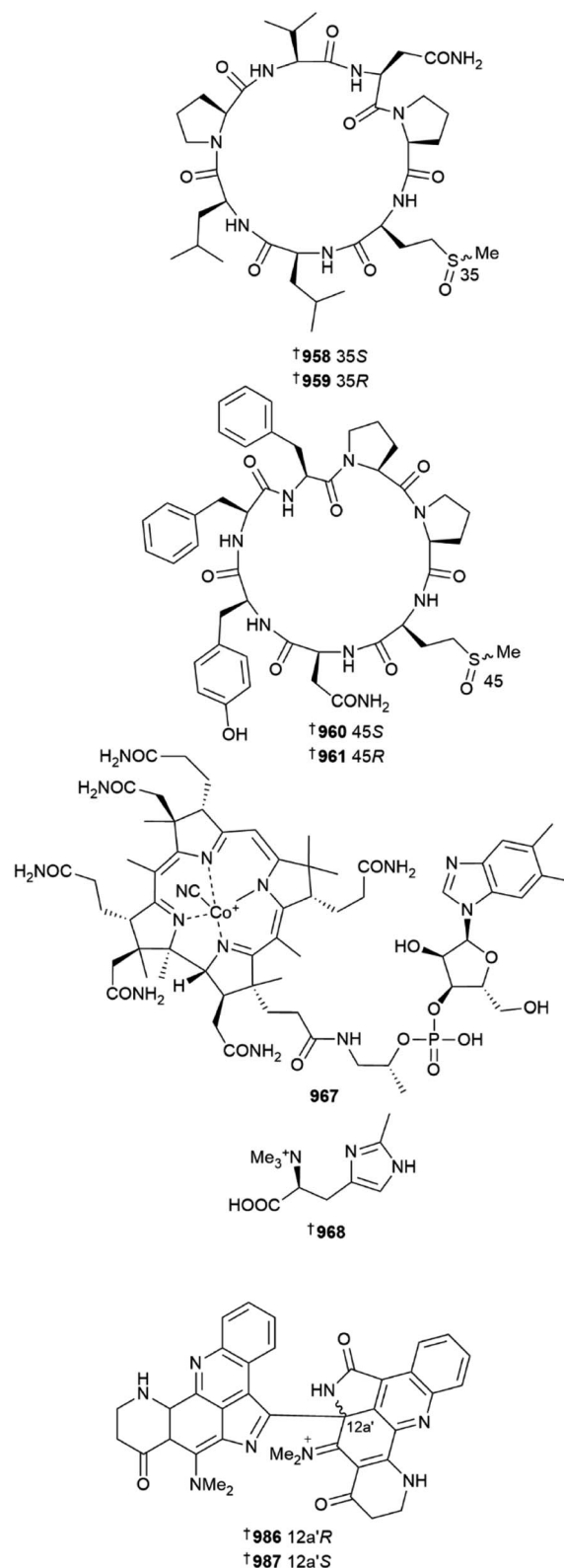
Three enigmazole congeners **962–964** were reported from a *Homophymia* sponge, one of which (enigmazole E) is an artefact of isolation; this was also the only report of a new sponge-derived macrolide in 2022.<sup>380</sup> Two diketopiperazines **965** and **966** were reported from a *Haliclona baeri* specimen.<sup>381</sup>

A known terrestrial vitamin B<sub>12</sub> derivative, cyanocobalamine **967**, was isolated from a deep-sea (809 m) *Characella pachastrelloides* dredged from an Irish submarine canyon. This species has previously been the source of unique anti-inflammatory glycolipopeptides. Trace amounts of B<sub>12</sub> were detected by LCMS in extracts of the sponge. In addition, an unusual and weakly cytotoxic amino acid, 6-methylhercynine **968** was isolated during the same study.<sup>382</sup>

Sponge-derived alkaloids include haliclorensins **969** and two haliclonyclamines **970** and **971** from a *Neopetrosia* and a *Callyspongia*, respectively,<sup>383,384</sup> while related zamamiphidin alkaloids **972** and **973** were isolated from a Japanese *Amphimedon* sponge.<sup>385</sup> An N-oxide congener of manzamine A **974** has been isolated from *Neopetrosia proxima*,<sup>386</sup> while *N. chaliniformis* yielded four indole-C-mannopyranosides, neopetrosins A–D **975–978**, most of which have weak hepatoprotective activity.<sup>383</sup>

Various aaptamines **979–985** have been reported from two separate collections of *Aaptos aaptos*.<sup>387,388</sup> A racemic mixture of an aromatic alkaloid, plakoramine A **986/987** was isolated from a *Plakortis* sponge (Kingdom of Tonga). Chiral separation of the enantiomers provided both metabolites, which are products of photochemical dimerisation of co-isolated and commonly found metabolite plakinidine B. Both enantiomers are weak inhibitors of Casitas B-lineage lymphoma proto-oncogene-b (CLB-B), but are inactive in the NCI 60-cell line panel, while surprisingly monomer plakinidine B is potently cytotoxic *vs.* the NCI panel but inactive against CLB-B. The ubiquitin ligase activity of CLB-B requires its dimerisation, hence it would be interesting to investigate the differential SAR of plakoramine and plakinidine B in this context.<sup>389</sup>

Pyrrolo-amide and -lactam alkaloids are commonly encountered in sponge extracts. Reports of sponge alkaloids of the class include a structural revision of mukanadin C **988**



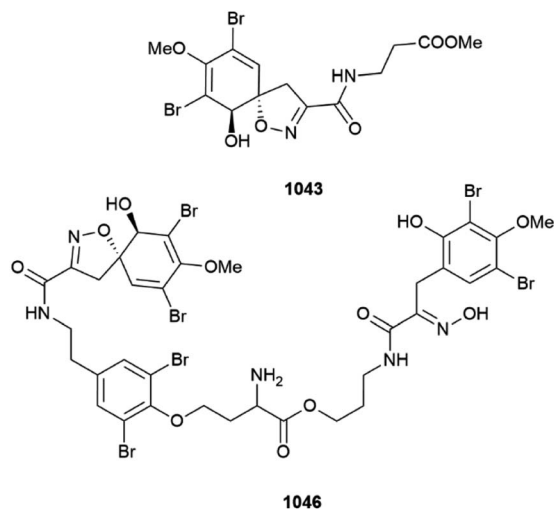
(*Agelas nakamurai*),<sup>390</sup> alongside cyclic **989–1010** and linear monomers and dimers **1011–1020** reported from *Axinella*, *Stylissa* and *Agelas* sponges.<sup>391–393</sup> Guanine-containing metabolites included **1021–1023**, and additionally **1024–1031** that are the first metabolites reported from genus *Ernstia*.<sup>394–396</sup> The sponge



genera *Suberea* and *Pseudoceratina* yielded eleven bromotyrosines **1032–1042** between them.<sup>397,398</sup>

A Western Australian (Ningaloo Reef) specimen of *Pseudoceratina verrucosa* was the source of several bromotyrosines, methyl purpurocerate A **1043** and B **1044**, possible artefacts of methanolic isolation from known purpuroceratic acids, along with purpuroceratic acid C **1045** and bromotyrosine trimer nialamide A **1046** and dimer B **1047**. A new method for determining the enantiomeric purity (% ee) of chiral bromotyrosines using a combination of chiral chromatography with ECD detection was established. The % ee of all the new isolates varied from almost full racemic mixtures (~5% ee) to almost enantiomeric purity (~84% ee).<sup>399</sup>

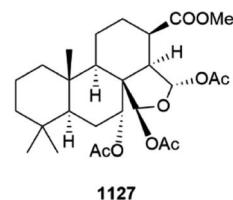
Unsurprisingly, most sponge-derived metabolites reported in 2022 were of terpenoid origin. Merosesterterpenoids were isolated from sponges of genus *Dactylospongia* **1048–1051**, *Dysidea* **1052–1055**, and *Spongia* **1056–1058**, respectively.<sup>400,401</sup> Note **1057**, named pelorol A, is a methylated congener of known pelorol, and so logically should be termed pelorol B.<sup>402</sup> Meroditerpenoid alkaloids **1059–1062** were discovered from a Mexican *Agelas citrina* sponge, with some showing antimicrobial properties.<sup>403</sup>



Various sesquiterpenoid structures were disclosed in 2022. The use of computational-aided structure elucidation facilitated the assignment of agelasidine G–I **1063–1065** from a specimen of *Agelas nakamurai*,<sup>390</sup> which was also the source of related sulfone cyclohexagelasidine A **1066** reported in a separate study.<sup>404</sup> A series of monomeric and dimeric bisabolene-class sesquiterpenoids, bubaridin A–F **1067–1073** and several theonellin congeners **1074–1076**, were obtained from a Futuna Island sponge of order Bubarida.<sup>405</sup> Other sesquiterpenoids were reported from *Acanthella* (**1077–1081**) and *Myrmekioderma* (**1082–1084**) sponges, respectively.<sup>406,407</sup> Isolation of a number of nitrogenous sesquiterpenoids **1085–1104** from a Chinese *Acanthella cavernosa* also required revision of two pairs of known compounds as **1105–1108**, respectively.<sup>408</sup>

Diterpenoid metabolites have been reported from specimens of dictyoceratid *Spongia* from China **1109–1121** and from Saudi Arabia **1122**, respectively.<sup>409–411</sup> A Japanese *Halichondria* yielded

aromatic peroxide amitorin **1123** that exhibits weak cytotoxicity,<sup>412</sup> while a different Saudi specimen of *Spongia* was the source of three spongian diterpenoids **1124–1126** that were inactive in various assays.<sup>413</sup> Spongionellol A **1127** is a further spongian diterpenoid from a dredged (Gulf of Sakhalin) *Spongionella* which exhibits weak to moderate cytotoxicity vs. seven HTCLs, in particular vs. prostate cancer cells, but was less active against non-cancerous lines with a mean selectivity index of 5.2. The mechanism of action of spongionellol A involves caspase-dependent apoptosis. In addition, **1127** inhibits drug efflux by P-glycoprotein multi-drug resistance protein-1 (MDR-1), a main detoxification effector in cancer cells that helps them avoid the effects of standard chemotherapeutics, making spongionellol an exciting anti-cancer lead compound.<sup>414</sup>



Marine sponges remain one of the main reservoirs of new sesterterpenoids reported annually. A *Hippospongia fistulosa* specimen was the source of four linear furospinulosin-type sesterterpenoids **1128–1131**, while two unstable ircinianin butenolides **1132** and **1133** were obtained from an Australian (Great Barrier reef) *Ircinia*.<sup>415,416</sup> *Phorbos* sponges from Howe Sound, British Columbia, continue to yield ansellone-class metabolites, including **1134–1138**, which have activity as HIV-1 latency reversal agents.<sup>417</sup> Isomalabaricane sesterterpenoids were reported from *Rhabdastrella globostellata* from China **1139–1147** and Vietnam **1148–1150**, respectively,<sup>418,419</sup> while scalaranes were found from specimens of *Phyllospongia* **1151–1154**, *Hyrtios* **1155–1162** and *Spongia* **1163–1174**; the *Phyllospongia* metabolites were already known but their structures revised in the current study.<sup>420–422</sup> New sterols were reported from *Halichondria* **1175**, *Neopetrosia* **1176**, **1177**, and *Spongia* **1178**, **1179**; the latter study also reported a fatty acid **1180**.<sup>411,423,424</sup> Other manuscripts reported a number of new metabolites that were either not isolated,<sup>425</sup> or the structures presented are inconsistent with the spectroscopic data provided and hence are dubious in nature and not shown as part of this review.<sup>426–430</sup>

Numerous first total syntheses of sponge metabolites were published in 2022. These include the syntheses of ethyl branched polyketides plakortone Q and plakdiepoxide,<sup>431</sup> and peptides stylissatin B,<sup>432</sup> cyclotheonellazole A,<sup>433</sup> and stylopeptide II, the last of which was prepared synthetically on the gram scale.<sup>434</sup> Hemicalide is an extremely potent (sub nM IC<sub>50</sub>) cytotoxin, although only the planar structure was originally published within a patent application. It contains 21 stereocenters with over two million possible stereoisomers. The potent bioactivity and structural complexity of hemicalide has prompted many syntheses of parts of the compound to try and establish the stereochemistry of the molecule, the latest of which has refined the structure as **1181**, leaving only eight



possible stereoisomers for the compound.<sup>435</sup> Two reviews of the struggle to identify this compound were also published in 2022.<sup>436,437</sup>

Five bromophenol NPs were synthesised for the first time,<sup>438</sup> as was pyridinium alkaloid *epi*-tetrahydrohalicyclamine B.<sup>439</sup> The structure of echinosulfonic acid D has been synthesised, one year after it was revised independently by three groups as reported in last year's review.<sup>1,440</sup> Aaptamine-type alkaloids suberitines A–D were synthesised for the first time,<sup>441</sup> as were pyrrole-containing metabolites denigrin D and E.<sup>442</sup> Somewhat surprising given its age, the first (racemic) synthesis of archetypical bromotyrosine compound psammaphysin A, which was first reported in 1982, was accomplished in 2022,<sup>443</sup> as was the synthesis of related spiroisoxazolines clavatadine D and E.<sup>444</sup> The first total syntheses of merosesquiterpenoid dysiherbol C and D,<sup>445</sup> dactyloquinone A,<sup>446</sup> and amino-sesquiterpenoids boneratamide A–C,<sup>447</sup> and two halichonic acids were reported.<sup>448</sup> Both the relative and absolute configurations of lamellodysidine A were confirmed following its synthesis.<sup>449</sup> Use of computer-assisted retro-synthetic analysis of the pupukeane class of sesquiterpenoid MNPs identified several routes with unusual disconnections beyond those already preceded for manufacture of the compounds.<sup>450</sup> Diterpenoids ansellone G and hamigeran F,<sup>451,452</sup> eight phorbaketal congeners,<sup>453</sup> aromatic sterol myrmenaphthol A, and *seco*-sterols glaciasterol B and 6-ketoaplidiasterol B have been synthesised for the first time.<sup>454,455</sup> Complex tropolone-containing triterpenoid gukulenin B has also been prepared.<sup>456</sup>

Reviews of MNPs from class Demospongiae,<sup>457</sup> the genera *Agelas*,<sup>458</sup> *Oceanapia*,<sup>459</sup> *Smenospongia*,<sup>460</sup> and the species *Dactylospongia elegans* have been published,<sup>461</sup> as have summaries of the development of the bengamides as antibiotics,<sup>462</sup> the quintessential alkaloid aaptamine,<sup>463</sup> and the scalaranes, a common class of sponge sesterterpenoid.<sup>464</sup>

High-throughput screening (HTS) of 3764 marine-derived fractions has identified macrolide leioldolide A as a potent anti-*Cryptosporidium* agent with high selectivity for the parasite over mammalian cell lines.<sup>465</sup> The cytotoxic mode of action of polyketide psymberin (a.k.a. irciniastatin A) was determined to be *via* inhibition of protein translation leading to upregulation of the p38 cellular stress response.<sup>466</sup> Manzamine A inhibits Sine oculus homeobox homolog 1 (SIX1) gene expression, which is highly expressed in osteoblasts and is involved in craniofacial development; this famous alkaloid may therefore have importance for development of new promoters of bone formation.<sup>467</sup> The marine alkaloid thiaplakortone B was found to suppress RANKL-induced osteoclast formation, and therefore may have value in reducing bone-reabsorption and hence be a lead for treatment of osteoporosis.<sup>468</sup> Promiscuous alkaloid aaptamine is a dual inhibitor of both acetyl- and butyryl-cholinesterase and therefore has potential as an Alzheimer's disease therapeutic lead.<sup>469</sup> HTS of 300 marine invertebrate extracts from Australia has revealed that the known guanidine-based, plant growth promoter asterubine binds to  $\alpha$ -synuclein, therefore making it a lead for Parkinson's disease; asterubine structurally resembles the  $\beta$ -amyloid aggregation inhibitors drugs tramiprosate and ALZ-801.<sup>470</sup> The same HTS campaign also identified

(+)-aerotherionin-1 and -2 as  $\alpha$ -synuclein inhibitors; interestingly both inhibit protein aggregation but while (+)-aerotherionin-1 is toxic to dopaminergic neuronal cells, -2 is not.<sup>471</sup> Bromotyrosine aerophobin-1 shows low toxicity in a zebrafish model of development but promoted osteogenesis at 100 nM so may have application in promoting bone regeneration.<sup>472</sup>

A report has described a virtual structure-based approach to highlight likely bioactive bromotyrosine metabolites. Following the *in silico* portion of the study, 12 alkaloids were then isolated using a molecular networking-guided approach from a *Pseudoceratina durissima* sponge with one (aeropylsinin-1) showing antibiotic activity, five showing toxicity to zebrafish and potentially having effects on the central nervous system, and four inhibiting the parasitic nematode *Haemonchus contortus*. This study highlights the value of using *in silico* predictive models with modern chemical screening methodologies to identify new lead compounds.<sup>473</sup> In other reports, the mode of anti-colorectal cancer action of merosesquiterpenoid ilimaquinone is *via* mitochondrial-mediated apoptosis, caused by activation of caspase-9/-3 and the subsequent DNA damage caused,<sup>474</sup> while spongian diterpenoid membranolid G irreversibly inhibits relaxation of supercoiled DNA *via* inhibition of topoisomerase 1B and it may therefore be a new anti-cancer lead compound.<sup>475</sup> The biosynthesis of macrolide lasonolide A (*Forcepia* sp.) has been accomplished using a metagenomic approach, suggesting the potent (nM) cytotoxin is made by the first member of new bacterial genus *Candidatus* Thermopylae lasonolidus, whose nearest taxonomic neighbour is *Pedospaera parvula*.<sup>476</sup>

Use of the DU8ML approach has resulted in the reassignment of two compounds from the Red Sea sponge *Negombata magnifica* as the common and known metabolites *epi*-loliolide and loliolide; the structures of the “new” compounds magnificines A and B were not shown in our previous review as they were not consistent with the spectroscopic data provided, and the revision shows that the structures were misassigned due to incorrect interpretation of MS data leading to erroneous molecular formulae being proposed.<sup>1</sup> In addition, the structure of cinerol A was also revised to **1182** as part of the same study; the amidine unit in the polycyclic aromatic system is “flipped” in the revision.<sup>3</sup>

A chemical ecological study of the Caribbean sponge *Agelas tubulata* has shown that specimens across multiple sites and depths (down to 61 m) in both Belize and the Grand Cayman Islands, including reciprocal transplanted samples, possessed different secondary metabolic profiles driven mainly by nine compounds, and using regression analysis that various metabolites showed targeted antibacterial activity against different pathogenic strains. The results of this study examine the disease resistance and resilience of sponges within tropical reef systems.<sup>477</sup>

Sponges are notorious for being difficult to taxonomically classify. A study to investigate isomalabaricane triterpenoids from the sponge genera *Jaspis*, *Geodia*, *Stelletta* and *Rhabdastrella* provided an opportunity to re-examine voucher specimens of the sponge sources of the compounds. Of the 21 vouchers re-examined, which came from 27 unique sponge sources



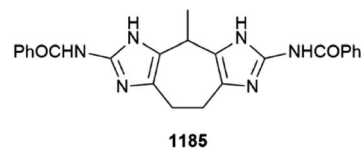
described in 44 articles, over 50% (11) were mis-identified; all of the sponges described as members of the *Jaspis* and *Stelletta* genera were in fact *Rhabdastrella globostellata* specimens. The authors of the study recommend stricter guidelines for identification of sponges and also for the storage of voucher specimens.<sup>478</sup>

## 7 Cnidarians

Two landmark publications have described for the first time the presence of BGCs for terpene biosynthesis in soft corals. In one of the studies,<sup>479</sup> sequencing of the metatranscriptome of *Erythropodium caribaeorum*, a source of eunicellane and briarane diterpenes, led to the identification of eight putative terpene synthase (TPS) genes. The majority of these genes were considered of coral origin, with the sequences found in all published octocoral genomes. The eleutherobin genes were clustered with the genes of tailoring enzymes including cytochrome P450 and acyltransferases. Expression in *E. coli* afforded soluble protein, that when incubated with geranylgeranyl pyrophosphate afforded klysimplexin R. Similar methodology identified TPS associated with the biosynthesis of cembrene C, the prototypical precursor of cembranoid MNPs. The second study<sup>480</sup> utilised an untargeted genome mining approach, querying publicly available genomes, to identify a set of 14 genes that harbored motifs typical of plant and microbial terpene cyclases. Expression in *E. coli* followed by precursor incubation studies identified enzymes producing cembrene A, cembrene C, elisabethatriene, a previously unreported xeniaphyllene scaffold and klysimplexin R, with four producing mixtures of sesquiterpenes. The cembrene A producing enzyme ErTC-2 from *Eleutherobia rubra*, was successfully crystallised. In a further demonstration of the utility of this newfound knowledge of soft coral terpene cyclase genes, the genome of *Capnella imbricata* was sequenced and queried, identifying a terpene cyclase homologue that was expressed and functionally validated as being a capnellane terpene cyclase. Both groups speculated on the timing of the origin of terpenes in soft corals, noting that they appear to coincide with the evolutionary divergence of hard (stony) corals and soft corals, supporting the premise that these MNPs represent a means of chemical defence.

To explore the pharmaceutical potential of a set of 5600 cnidarian NPs, a systematic approach was used to calculate and compare descriptors of chemical space, drug-likeness and predicted toxicity and to virtually screen the compound set against a panel of cancer-relevant protein targets.<sup>481</sup> The most favourable metabolites spanned 210–265 Da, were often sesquiterpenes and possessed moderate complexity. The structure of butanolide **1183** from *Cladiella conferta* has been previously reported as a synthetic compound.<sup>482</sup> It exhibited weak ability to inhibit the release of COX-2 from stimulated macrophage cells. A deep-sea collection of the soft coral *Duva florida* afforded the meroterpene tuaimenal A **1184**.<sup>483</sup> *In silico* docking experiments suggested it could bind to SARS-CoV-2 3CLpro, with a biochemical assay identifying it as a very weak inhibitor of the enzyme, with an IC<sub>50</sub> of 21 μM. A set of eight diacylated zoanthoxanthin derivatives, dentithecamides A–H **1185–1192**, were isolated from the hydroid *Dentitheca habereri*.<sup>484</sup> The

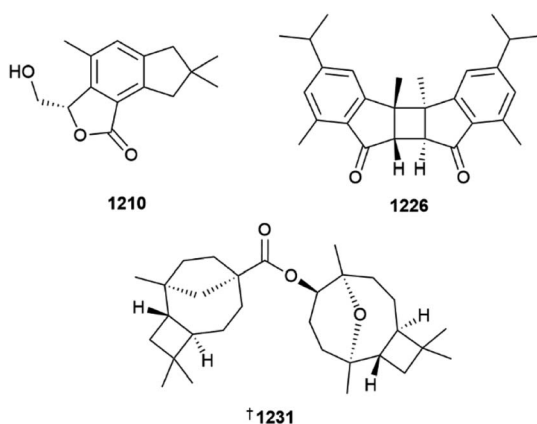
biosynthetic origin of the alkaloids could be *Parazoanthus* sp. zoanthids that the hydroid associates with. While not all compounds were tested, dentithecamide A was the most active (IC<sub>50</sub> 12 μM) at suppressing PAX3-FOXO1-driven transcription, a driver of soft tissue rhabdomyosarcoma in young people. While this level of activity is considered inactive by the criteria set for this review, the value is presented here to indicate that dentithecamide A is in fact an inhibitor, albeit weakly, of a transcription factor fusion which some view as undruggable. The zoanthamine alkaloid norzoanthamine demonstrates anti-osteoporotic activity in an *in vivo* model.<sup>485</sup>



Three norsesquiterpenes pathyspirolactone A **1193** and B **1194** and napalilactone **1195** were isolated from cultured specimens of *Paralemnalia thyrsoides*.<sup>486</sup> The structure and relative configuration proposed for pathyspirolactone A is identical to that reported for a synthetic compound *epi*-pathylactone A. Notable differences in chemical shift and coupling constants for the H-1 methine resonance between the two compounds were attributed to the unlikely scenario that the NP was a new conformational isomer that did not interconvert when heated. Absolute configuration was assigned to known co-metabolite napalilactone using XRD. Sesquiterpene alkaloid echinoflorine **1196** and lactones echinofloranolide A–C **1197–1199** were isolated from a South China Sea collection of *Echinogorgia flora*.<sup>487</sup> and their absolute configurations were assigned by standard methods. Four acetylated sesquiterpene lipids, crannenol A–D **1200–1203** were reported from an Irish deep-sea collection of *Acanella arbuscula*.<sup>488</sup> In addition to the related acetal and methylacetal analogues, parathyroidin K **1204** was isolated from a Malaysian collection of a soft coral from the genus *Lemnelia*.<sup>489</sup> Three rearranged sesquiterpenes lemnalemnane A–C **1205–1207**, were isolated from *Paralemnalia thyrsoides* and *Lemnalia* sp.<sup>490</sup> In a bioassay using transgenic zebrafish, lemnalemnanes A and B reduced the number of macrophages surrounding the neuromast, while lemnalemnane C exhibited strong angiogenesis effects. In addition to a highly oxidised sterol (alcyosterone discussed later), three illudane sesquiterpenes, alcyopterosins T–V **1208–1210** were purified from extracts of an Antarctic collection of *Alcyonium* sp.<sup>491</sup> Although inactive against a panel of ESKAPE microorganisms, alcyopterosin V was weakly active against *Clostridium difficile* (MIC 8.1 μM) and *Leishmania donovani* (IC<sub>50</sub> 7.0 μM) with low toxicity towards HEK293T and HepG2 cells and J774.A1 macrophages. Two studies have reported new examples of nardosinane-type sesquiterpenoids in addition to unsymmetrical bis-sesquiterpenoids. In the first study, investigation of the constituents of Xisha Is. collections of *Lemnalia* sp. afforded parathyroidins H–J **1211–1213**, linardosinenes D **1214** and E **1215** and nardosinoids A **1216** and B **1217**.<sup>492</sup> Bioactivity of the compounds ranged from weak to inactive against a variety of viruses, bacteria, and tumour cell lines. In the second study, specimens of *Litophyton nigrum*, also from the Xisha Is. region of



the South China Sea yielded, in addition to several known MNPs, bis-sesquiterpenoid linardosinene H **1218** and the nardosinane sesquiterpene linardosinene I **1219**.<sup>493</sup> Structure confirmation and assignment of absolute configuration of the previously reported sesquiterpene **1220** was achieved by XRD analysis. Of the three compounds, only linardosinene I exhibited biological activity, being a very weak inhibitor of PTP1B, while all compounds were inactive towards a panel of four HTCLs. Subergorgines A–E **1221–1225** are C-6/N-linked suberosanone-purine hybrids reported from extracts of *Subergorgia suberosa*.<sup>494</sup> Defining the head-to-head and head-to-tail orientations, respectively, of the indanone-dimers weizhouochrones A **1226** and B **1227**, isolated from *Anthogorgia ochracea*, required analysis of an extensive set of experimental data including NMR residual dipolar couplings, residual chemical shift anisotropy, computational chemistry including DP4+ probability analysis and computer-assisted 3D structure elucidation.<sup>495</sup> In the case of DP4+ analysis, the study provided more evidence that successful assignment of relative configuration using this workflow requires the use of both <sup>1</sup>H and <sup>13</sup>C NMR chemical shift data. Weizhouochrone A was racemic, while the optical purity status of the co-metabolite could not be determined due to its instability. In addition to seven xenicane-type diterpenoids described later, an extract of *Sinularia hirta* afforded norcaryophyllenes sinuhirtins F **1228** and G **1229**.<sup>496</sup> The structure of oxyfungiformin **1230**, originally isolated from *Capnella fungiformis*, was confirmed by total synthesis, with the study also establishing its absolute configuration.<sup>497</sup> A biomimetic synthetic approach was used to define the structure and absolute configuration of the disesquiterpene rumphellolide J **1231**.<sup>498</sup> The target was prepared by ester formation between two building blocks of (+)-rumphellaic acid A, starting from caryophyllene oxide, and 4β,8β-epoxycaryophyllan-5-ol, derived from (–)-caryophyllene.



A new example of a prenylemane-type diterpene, lobatate **1232**, was isolated from the soft coral *Sinularia nanolobata* collected from Ximao Is., South China Sea.<sup>499</sup> A total of nine diterpenes were purified from extracts of *Clavularia inflata* comprised of five dolabellanes clavularinlides A–E **1233–1237** and four racemic elemane alkaloids, clavulacylides A–D each of which were separated by chiral HPLC prior to characterisation.<sup>500</sup> The structures of clavulacylides A and C were assigned as being **1238–1241**, bearing a *trans* C-1 Me/C-6 H relationship. The congeners clavulacylides B and D were

originally proposed to bear a *cis* Me/H relationship, based upon the claimed observation of NOESY correlations. A subsequent study however, by Novitskiy and Kutateladze<sup>3</sup> that made use of machine learning DU8+ hybrid density functional theory calculations suggested that the structures were more likely to be the *trans* substituted stereoisomers **1242–1245**. Closer examination of the ESI of the original isolation report failed to indicate the presence of the claimed NOESY correlations, leading to the conclusion that the structures of clavulacylides B and D should indeed be revised to those shown. A Xisha Is. collection of *Clavularia viridis* was the source of 12 examples of dolabellane diterpenoids, including clavurools A **1246** and B **1247**, peroxide-containing clavuperoxyllides A **1248** and B **1249**, clavurools C–E **1250–1253**, furan-ring containing clavufuranolides A–C **1254–1256** and epoxide clavirolide I **1257**.<sup>501</sup> In addition to confirming the structure and assigning the absolute configuration of clavurool E and clavirolide I by the use of XRD, absolute configuration was also established for the known co-metabolite clavudiol A **1258**.

New examples of capnosane diterpenes, sarboettgerins A–E **1259–1263** were isolated from Weizhou Is., South China Sea collections of *Sarcophyton boettgeri*.<sup>502</sup> XRD was used to confirm the structure and assign absolute configuration to sarboettgerin A. The structurally-related sinuhumilol A **1264**, a non-cytotoxic capnosane, was purified from *Sinularia humilis* collected at Ximao Is., also in the South China Sea.<sup>503</sup> In a relatively rare example, diterpenes belonging to two different bicyclic skeletons, mililatensol A **1265** (sarsolenane) and mililatensols B **1266** and C **1267** (capnosanes), were reported from a collection of *Sarcophyton mililatensis*.<sup>504</sup>

Unusual tricyclic diterpenoids sinuaustones A **1268** and B **1269** and a spartane-type diterpenoid isolobophytumin E **1270** were isolated from a Hainan collection of *Sinularia australiensis*.<sup>505</sup> The structures and absolute configurations of both sinuaustones were secured by XRD. Mercury lamp irradiation of a methanolic solution of co-metabolite lobophytumin A afforded a mixture containing the three new NPs, suggesting they either derive from photochemical reactions in nature or could be considered artefacts of isolation. Biselisabethoxanes A **1271** and B **1272** are unusual dimeric diterpenoids, with the former representing amphilectane and elisapterane diterpenes linked by an ether, while the latter appears to be derived from hetero-Diels–Alder cycloaddition between two amphilectane-type diterpenes.<sup>506</sup> The structure and absolute configuration of biselisabethoxane A was secured by XRD. Despite being given every opportunity to exhibit biological activity against a diverse panel of targets, including *Mycobacterium tuberculosis* and *Plasmodium falciparum*, cytotoxicity, neurodegenerative and neuroinflammation, cyclin-dependent kinases and antiviral assays, no activity was observed for either MNP.

A comprehensive review of the structures, naming, and NMR assignments of pseudopterosin and *seco*-pseudopterosin has been published, highlighting inconsistencies in the primary literature.<sup>507</sup> The introduction of primary amino groups onto Gram-positive only antibiotics of the pseudopterosin aglycone family has been demonstrated to convert the compounds into broad spectrum antibiotics with activity towards Gram-negative



bacteria.<sup>508</sup> The structure of the intriguing *Pseudopterogorgia elisabethae* metabolite (+)-aberraronone **1273** has been confirmed by synthesis, with the core 5-5-5 fused ring system constructed using a Au-catalysed cascade.<sup>509</sup> The structure of the final NP was further confirmed by XRD. The synthetic material exhibited a relatively strong specific rotation ( $[\alpha]_{\text{D}} +85.0$  ( $c = 0.4$ ,  $\text{CHCl}_3$ )) compared to the isolated NP ( $[\alpha]_{\text{D}} +2.2$  ( $c = 1.4$ ,  $\text{CHCl}_3$ )), suggesting the NP was isolated as either the racemate or a nearly equal scalemic mixture.

Many cembranoid-related diterpenes were reported from cnidarians in 2022. Xishaglaucumins A–J **1274–1283** were isolated from a Xisha Is. South China Sea collection of *Sarcophyton glaucum*.<sup>510</sup> XRD was used to define the structure and absolute configuration of the known co-metabolites sarcophytol Q **1284**, iso-sarcophytol Q **1285**, and 13-acetoxy-7,8-epoxycembra-1(15),3,11-trien-2,16-olide **1286**. Amongst a set of five cembranoids, sarcomililats C–G **1287–1291**, purified from *Sarcophyton mililatis*, sarcomililatol D was the only compound to inhibit (weakly) TNF- $\alpha$ .<sup>511</sup> XRD analysis of a sixth cembranoid **1292** from the coral led to its structure revision. There are only a few reports of investigation of the NP chemistry of *Sarcophyton boettgeri*; a new study has led to the characterisation of sarco-boettgerols A–C **1293–1295** and 12-*epi*-humilisin D **1296** from a Ximao Is. collection.<sup>512</sup> Eight cembranoids, sarcophytembranoids A–H **1297–1304**, were isolated from a Ximao Island collection of the soft coral *Sarcophyton trocheliophorum*; the study also reported three terpenoid compounds **1305–1307** as MNPs for the first time.<sup>513</sup>

Biosynthetically, the metabolites ximaonanobatin A–I **1308–1316**, isolated from *Sinularia nanolobata*, likely all derive from the common precursor cembrene A.<sup>514</sup> The structure of ximaonanobatin A was secured by XRD. Three terpenoids, comprised of two cembranoids **1317** and **1318** and casbane-type diterpene **1319** were isolated from a Ximao Is. collection of *Sinularia pedunculata*; all were inactive in a TNF- $\alpha$  release assay and did not inhibit the enzyme PTP1B.<sup>515</sup> An unusual 13,15-ether bridged cembranoid, odosinularol **1320**, was reported from extractions of a collection of *Sinularia* sp. collected from the Odo Coast, Okinawa.<sup>516</sup> In addition to the venerable furanocembranoid pukalide, a new congener, 11-hydroxy- $\Delta^{12(13)}$ -pukalide **1321**, also isolated from an Okinawan collection of *Sinularia* sp.<sup>517</sup> No activity was observed against a bacterial phytopathogen and the MNP was inactive towards brine shrimp. A bisepoxy derivative of cembrene A **1322**, and two chlorine-containing cembranoids **1323** and **1324** were purified from extracts of *Sinularia* sp. collected at Turtle Is., Taiwan.<sup>518,519</sup> The structures and absolute configurations of the former two compounds were secured by XRD. Furanone-containing cembranoids sarcoconvolutums F **1325** and G **1326**, isolated from *Sarcophyton convolutum*, were proposed to be biosynthetically-derived from sarcophine by a series of steps including oxidation and dehydration.<sup>520</sup> Additional examples of furanone cembranoids were also reported, including ehrenbergols F **1327** and G **1328**, isolated from a Vietnamese collection *Sarcophyton ehrenbergi*,<sup>521</sup> sarcoeleganolides C–G **1329–1333** from South China Sea samples of *S.*

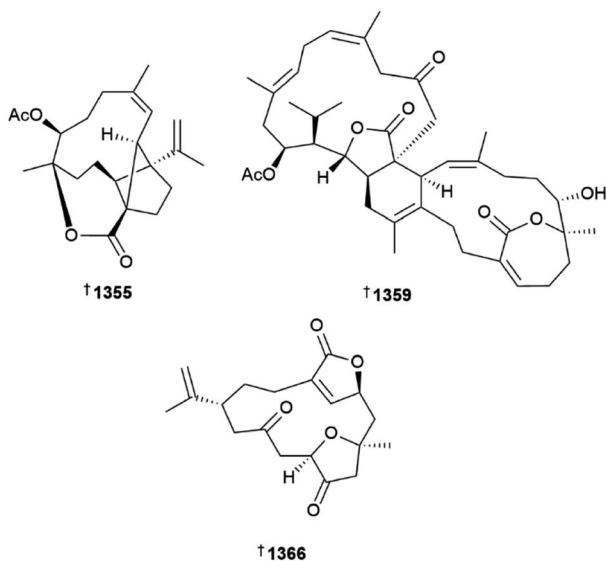
*elegans*,<sup>522</sup> and sarcoeseolides A–D **1334–1337** from a Red Sea collection of *S. roseum*.<sup>523</sup> Cembranoid pyran **1338** from a Red Sea collection of *Sarcophyton trocheliophorum* was inactive when tested against a panel of HTCLs and *Leishmania major*.<sup>524</sup> Two studies reported new examples of 8,20-lactonised cembranoids, including cinerenolides A–C **1339–1341** from *Sarcophyton cinereum*<sup>525</sup> and tortuolides A **1342** and B **1343** isolated from *S. tortuosum*.<sup>526</sup> The latter study also described a diterpene *epi*-sarcophytonolide Q **1344**. Nine  $\text{C}_{19}$ -norcembranoids comprised of sinudenoids A–E **1345–1349**, isolated from *Sinularia densa*,<sup>527</sup> and sinuscalides A–D **1350–1353**, from *S. scabra*,<sup>528</sup> were isolated from the respective soft corals collected from Xisha Island, South China Sea. The latter study also led to the determination of the structure of the diterpenoid sinuscatone A **1354**. Sinuscalide A exhibited weak antiviral activity towards enterovirus 71 and all the new MNPs from *S. scabra* inhibited RANKL-induced osteoclastogenesis at a single dose of 10  $\mu\text{M}$ .

An unusual tricyclic cembranoid cinerelolide **1355** was reported from a Taiwanese collection of *Sarcophyton cinereum*.<sup>529</sup> Its absolute configuration was assigned by comparison of experimental and calculated specific rotation values. In addition, a weakly cytotoxic sarsolenone analogue **1356** was characterised along with known congeners sarsolenone **1357** and 7-deacetylsarsolenone **1358**. Structure elucidation of **1356** prompted re-examination of the latter two MNPs, leading to revision of their relative configurations and assignment of absolute configurations. Seven biscembranoids were reported from either open water collections of *Sarcophyton sereni* from Xisha Island, or from *S. trocheliophorum* that was originally collected from the coast of Pingtung, Taiwan but had been subjected to aquaculture. Bistochelides H–L **1359–1363** were isolated from *S. sereni* specimens with the structure and absolute configuration of bistochelide H being secured by XRD.<sup>530</sup> Bistochelides H and J were moderate inhibitors of RANKL-induced osteoclastogenesis.

The remaining two biscembranoids, sarcotrochelides A **1364** and B **1365** were identified using a molecular networking-directed study of extracts of *S. trocheliophorum*.<sup>531</sup> Both biscembranoids were inactive in testing for anti-inflammatory responses related to superoxide anion or elastase release from induced human neutrophils. First syntheses of cembranoid MNPs molestin E,<sup>532</sup> (+)-ineleganolide and (–)-sinulochmodin C,<sup>533</sup> and scabrolide F **1366** have been reported, with the latter study establishing the absolute configuration of the molecule.<sup>534</sup> The structure assigned to scabrolide B has been called into question as the result of a total synthesis of the purported structure.<sup>535</sup> An asymmetric total synthesis of havellockate afforded a product that exhibited opposite sign and different magnitude of specific rotation to those reported for the isolated NP, leaving the authors unsure if there was an error in the original measurement or whether they had synthesised the enantiomer of the NP.<sup>536</sup> A racemic synthesis of the cembranoid rameswaralide, originally isolated from *Sinularia dissecta*, has been reported.<sup>537</sup> Further investigation of the biological properties of cembranoid MNPs has identified that

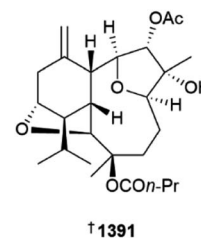


crassolide induces cell death in human non-small-cell lung cancer cells by reactive oxygen species accumulation leading to cell cycle arrest at G2/M,<sup>538</sup> and sinularin is also cytotoxic towards glioblastoma multiforme *via* a mechanism involving ROS generation leading to mitochondria-mediated apoptosis.<sup>539</sup>



Details regarding the isolation of new briarane diterpenes from *Briareum stechei* have been reported over three publications. Specimens grown in aquaculture were the source of congeners briastecholides D–F **1367–1369**,<sup>540</sup> a Taiwanese collection was the source of briastecholides K **1370** and L **1371** (ref. 541) while Okinawan specimens afforded briastecholides G–J **1372–1375**.<sup>542</sup> Between them, these studies also led to the confirmation of structure and assignment of absolute configuration by XRD analysis of brianodin A **1376**, and excavatolides E **1377**, and M **1378**. Additional examples of briarane diterpenoids, briavioids A–C **1379–1381** were reported from cultured specimens of *Briareum violaceum*.<sup>543</sup> The structure and relative configuration of the former metabolite was secured by XRD analysis. As noted earlier, for some cembranoid MNPs, briarane excavatolide C also exhibits cytotoxicity, this time towards bladder cancer cell lines, *via* a mechanism involving modulation of oxidative stress.<sup>544</sup> Two publications from the same research group described new xenicane diterpenes, coniferains A–D **1382–1385**, isolated from a Taiwanese collection of *Cladiella conifera*.<sup>545,546</sup> The structure of coniferain A matched that reported in 2010 for australin F, prompting reexamination of NMR data leading in turn to reassignment of the structure of the latter to **1386**. Litophynols C **1387** and D **1388** were isolated from a Ximao Island collection of *Cladiella krempfi*.<sup>547</sup> Their structures and absolute configurations were established by a combination of single crystal XRD analysis and chemical conversion. Clakrefelin A **1389**, also isolated from a Ximao Island collection of *Cladiella krempfi*, represents a des-butyrate ester variant of australin E.<sup>548</sup> Reaction of clakrefelin A with butyrylchloride afforded a product identical to australin E **1390**, single crystal

XRD analysis of which further confirmed the structure and established the absolute configuration of both NPs. An extract of *Sinularia ornata* was the source of ximaoornatins A–C **1391–1393**.<sup>549</sup> The structures likely derive from oxidation and ring-closures of a eunicellin diterpene precursor. Also purified from the extract was litophynin K **1394**.



In addition to two norcaryophyllenes noted earlier, *Sinularia hirta* was also the source of eight xenicane-related diterpenes sinuhirfuranones A–C **1395–1397**, sinuhirtins C–E **1398–1400** and sinuhirtones A **1401** and B **1402**.<sup>496</sup> Absolute configuration was assigned to the known xenicane 13-*epi*-9-deacetylxenicin **1403** by a combination of XRD analysis and modified Mosher's methodology, and a number of acylated derivatives were prepared semi-synthetically, identifying many derivatives with weak to moderate cytotoxicity towards a panel of four HTCLs.<sup>550</sup> All of the examples of a library of lipophilic semisynthetic analogues of the xenicane plumisclerin A were effectively inactive as cytotoxins.<sup>551</sup> The synthesis of racemic alcyonolide has been reported using an inverse electron demand hetero-Diels–Alder reaction to construct the core of the NP.<sup>552</sup>

A series of 12 sterols were reported from cnidarians, including *seco*-sterol cladiellasterol A **1404** from *Cladiella krempfi*,<sup>548</sup> epoxides **1405** (ref. 553) and **1406** (ref. 554) from *Sinularia variabilis* and *Isis hippuris*, respectively, ketones and enones **1407–1410** from *Lobophytum pauciflorum*,<sup>555</sup> alcyosterone **1411** from *Alcyonium* sp.<sup>491</sup> and dienones **1412–1415** from *Dendronephthya* sp.<sup>556</sup> In addition to these new NPs, samples of previously reported MNPs hippuristerones A **1416** and I **1417** were isolated from the *I. hippuris* specimens as well as nanjiol A **1418** from the *Dendronephthya* sp. sample and their respective absolute configurations assigned by single crystal XRD analysis. A gram-scale total synthesis of pinnisterol E **1419** from ergosterol has been reported.<sup>455</sup>

A Kunitz peptide purified from the venom of the sea anemone *Anemonia sulcata* activates G protein-coupled inward-rectifier potassium channels with only minor effect on voltage-gated K<sub>v</sub>1.6 channels.<sup>557</sup> Hcr 1b-2, a peptide from *Heteractis crispa*, exhibits interactions with a diverse set of voltage-gated ion channels including those for potassium, sodium and calcium, making it of interest as a tool to pharmacological studies.<sup>558</sup> Analysis of the transcriptomes from six species of clownfish hosting anemones, including *Cryptodendrum adhaesivum*, *Stichodactyla haddoni*, *Heterodactyla hemprichii*, *Heteractis crispa*, *Macroactyla dorensis* and *Entacmaea quadricolor* identified a large number of toxins with reported hemolytic and hemorrhagic properties.<sup>559</sup>



## 8 Bryozoans

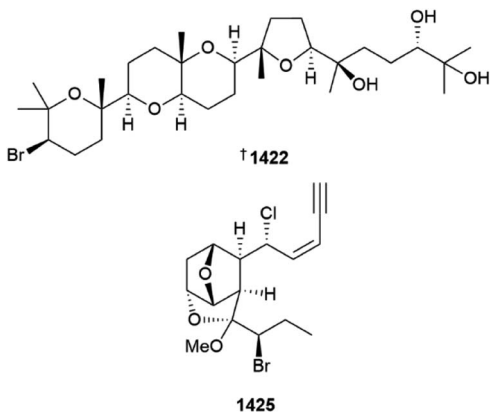
Once again in 2022, there were very few papers published on metabolites from bryozoans. An undescribed bryozoan of the genus *Calyptotheca* was the source of the steroid sulfates **1420** and **1421** (ref. 560) and the  $\beta$ -carboline alkaloids, orthoscuticellines A–E were synthesised *via* a straightforward route from tryptamine *via* pavettine (1-vinyl- $\beta$ -carboline) which co-occurs in the producing organism (*Orthoscuticella ventricosa*) so is likely also the biosynthetic precursor.<sup>561</sup>

## 9 Molluscs

A weakly cytotoxic, 20-residue lysine-rich peptide was isolated from the hemolymph of the bivalve mollusc *Arca inflata* and demonstrated the ability to induce apoptosis in colorectal carcinoma cells by a mechanism involving activation of the p38-MAPK pathway.<sup>562</sup> *In vivo* activity towards CRC HT-29 cells in a xenograft model was observed.

In a study designed to evaluate the antifeedant/deterrent properties of nudibranchs, extracts of different body parts of 30 species collected in Australian waters were evaluated for antifeedant activities against three generalist fish predators and toxicity towards brine shrimp.<sup>563</sup> No clear relationship was observed between palatability and toxicity.

Asymmetric total synthesis has established that the sea hare metabolite aplysiol B **1422** and the red algal metabolite laurenmariannol are in fact identical, with the structure shown.<sup>359</sup> Two brominated sesquiterpenes dactylomelanins A **1423** and B **1424** were isolated from a Vietnamese collection of the seahare *Aplysia dactylomela*.<sup>564</sup> The absolute configurations of both molecules were assigned using DP4+ and calculated ECD analysis. Two new halogenated acetogenins **1425** and **1426** were reported from Okinawan collections of *Aplysia dactylomela*.<sup>565</sup> The metabolites contain a terminal enyne-moiety, more commonly contained in MNPs reported from *Laurencia* species of red algae.



The structures and absolute configurations of azuriaplysin A **1427** and B **1428**, isolated from the sea hare *Aplysia kurodai*, were confirmed and established *via* total synthesis starting from farnesol.<sup>566</sup> Azuriaplysin B and its enantiomer exhibited weak cytotoxicity. Endo-peroxide containing  $\gamma$ -

pyrones ocellatuperoxides A–F **1429–1440**, were isolated as their respective racemates.<sup>567</sup> Absolute configurations were ascribed to the structures following separation using chiral HPLC and configurations assigned using standard methods. Racemate **1433/1434** exhibited weak cytotoxicity towards the A549 cell line, which upon further examination determined the activity was due solely to the (+)-enantiomer. Gene expression studies suggested a mechanism of action related to regulation of cell proliferation and migration. Proline betaine **1441** was reported from a marine organism, the seahare *Elysia crispata*, for the first time.<sup>568</sup> Two sterol disulfates, **1442** and **1443**, were isolated from a Vietnamese collection of the marine spider conch *Lambis lambis*.<sup>569</sup> A new bioinspired route for the synthesis of aplaminal has been reported, enabling the discovery of a new phenolic analogue with increased cytotoxic potency.<sup>570</sup> A late-stage diversification methodology has been used to synthesise, and confirm the structure of ocellatusone C, recently reported from *Placobranchus ocellatus*.<sup>571</sup> Exploration of the antifouling activity of the cyclic depsipeptide dolastatin 16 has identified smaller fragments with activity in a barnacle cyprid settlement assay, but the level of potency is significantly reduced compared to the NP.<sup>572</sup> RNA-Seq and shotgun proteomic studies of venomous gastropods of the genus *Vexillum* has identified them as a source of conotoxin-like, short chain, cysteine containing cyclic peptides.<sup>573</sup>

The pharmacological potential, and possible future trends in engineering methods to improve the properties, of conotoxins isolated from *Conus regius* have been reviewed.<sup>574</sup> Transcriptomic sequencing of *Conus quercinus* led to the identification of  $\alpha$ -CTx QuIA, a new conotoxin which demonstrates preferential antagonism of neuronal  $\alpha 3\beta 2$  nAChR and  $\alpha 6/\alpha 3\beta 4$  nAChR's making it of some interest.<sup>575</sup> Genomic analysis of the fish hunting cone snail *Conus striatus* identified a novel  $\alpha$ M-superfamily sequence SIIID which was prepared by solid-phase synthesis.<sup>576</sup> Disulfide bond formation gave three isomers with one being more active in patch-clamp studies and demonstrating selective inhibition of  $\alpha 7$  nAChR's. Exploration of the structure–activity relationship of the  $\alpha$ -conotoxin AuIB, originally isolated from *Conus aulicus*, has identified a variant with the Pro7 residue changed to arginine and an alternative disulfide connectivity that is a potent inhibitor of GABA<sub>B</sub>R-coupled Ca<sub>v</sub> 2.2 channels and exhibits *in vivo* analgesic activity.<sup>577</sup> Comparative evaluation of  $\mu$ -conotoxins SxIIIC, SmIIIA, and KIIIA in patch-clamp and receptor-based studies has identified that the number of residues in loop 3 can influence the peptides ability to inhibit human voltage-gated sodium hNa<sub>v</sub> 1.7 channels.<sup>578</sup> Five peptides were purified from the venom of *Conus marmoreus* – notably, none of the peptides contained disulfide bonds, and they were inactive towards a panel of rat nAChR subtypes.<sup>579</sup>

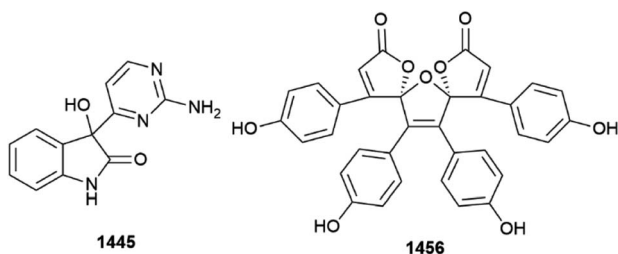
Five additional publications claim to report new mollusc-derived MNPs, however the structures are inconsistent with spectroscopic evidence and are not included in this review.<sup>580–584</sup>



## 10 Tunicates (ascidians)

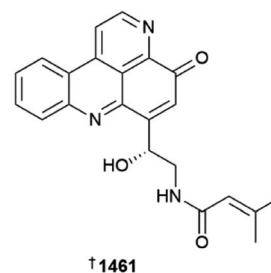
A new congener of the azadecalin family of alkaloids, lepadin L **1444** was isolated from a Mediterranean Sea collection of the ascidian *Clavelina lepadiformis*.<sup>585</sup> In comparative testing, the related analogue lepadin A was found to exhibit weak levels of cytotoxicity towards a panel of HTCLs, while lepadin F was inactive. Using <sup>1</sup>H NMR spectroscopy and a zebrafish embryo development assay for guidance, investigation of an Antarctic collection of *Synoicum* sp. afforded australindolones A–D (**1445–1448**), and in addition to the known meridianins A–G, a new example, meridianin H **1449**.<sup>586</sup> Australindolones A–D represent C-2/C-3 oxidation products of meridianins G, C, D and F respectively. With the observation of small magnitude laevorotatory specific rotations ( $[\alpha]_D -7$  to  $-12$ ), and the finding that australindolone B crystallised as a racemate, the authors were unable to determine whether the NPs were in fact racemic or scalemic mixtures. Meridianins A–G were the more active NPs in the zebrafish embryo assay, causing a range of developmental defects, while the australindolones were considerably less active.

New examples of bis-spiroketal-containing butenolides, prunolides D–I **1450–1455**, and *cis*-prunolide C **1456** and a  $\beta$ -carboline sulfamate, pityriacitrin C **1457** were isolated from an Australian collection of *Synoicum prunum*.<sup>587</sup> The structure of *cis*-prunolide C is notable as being the first congener, in what is now an expanding set of prunolides, to feature a *cis*-configuration bis-spiroketal at its core. Also notable is that the structure of pityriacitrin C is closely related to pityricitrins A and B, isolated from the pathogenic yeast *Malassezia furfur*. In an extensive set of biological evaluations related to the discovery of novel methods to limit the progression of Parkinson's disease, the prunolides formed  $\alpha$ -synuclein protein–ligand complexes as judged by mass spectrometry and inhibited  $\alpha$ -synuclein aggregation in an *in vitro* assay, with prunolide B also found to reduce the number of tyrosine hydroxylase positive dopamine neurons that contained phosphorylated  $\alpha$ -synuclein aggregates in an embryonic mouse midbrain model.



In a search for new antimycobacterial compounds, lissoclinotoxin F sulfoxide **1458** was isolated from an Indonesian collection of *Lissoclinum* sp.<sup>384</sup> While it and known co-metabolite lissoclibadin 2 were barely active (MIC 10  $\mu$ M) and with poor to low selectivity *vs.* HEK293 cells, a third metabolite from the extract, lissoclinotoxin F, was weakly active against *M. tuberculosis* with moderate selectivity (SI = 19) and did inhibit bacterial growth in infected macrophages. The compound exhibited some degree of selectivity towards mycobacteria *vs.* other Gram-positive and Gram-negative bacteria. The unusual

cyclopentanone end group of the carotenoid roretziaxanthin **1459** was proposed to be derived by the action of bacterial symbionts in *Halocynthia roretzi* on the Flavobacterium-sourced carotenoid rubixanthin.<sup>588</sup> The absolute configuration of (+)-(*R*)-tiruchanduramine **1460** has been determined by enantioselective total synthesis.<sup>589</sup> The synthetic material exhibited only mM  $\alpha$ -glucosidase inhibiting properties, with one synthetic model compound being more active (IC<sub>50</sub> 7.5  $\mu$ M). Bioinspired syntheses of cystodytins A–K have been reported, leading to assignment of 12*R* absolute configuration for cystodytins D–I **1461–1466** and K **1467**, and clarifying that the fatty acid present as an ester at C-12 of cystodytins H and I is oleic acid.<sup>590</sup>



The first total syntheses of (–)-cylindricine H **1468** (ref. 591) and iheyamine B **1469** (ref. 592) have been reported, leading to assignment of their respective absolute configurations.

Further investigation of ascidian-derived NPs continues to reveal new and intriguing biological activities. The previously noted abilities of the meridianins to inhibit glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) were the basis for evaluation in a mouse model of depression caused by chronic stress where a mixture of the alkaloids showed some beneficial alteration in behaviour.<sup>593</sup> GSK3 $\beta$  inhibitors have the potential to act as treatments for diabetes – optimisation of meridianin C led to the identification of new sulfonamide analogues that modelling suggested had retained kinase binding, that exhibited increased glucose uptake in L6 cells and had significantly improved *in vivo* bioavailability.<sup>594</sup>

In the search for new anticancer therapy adjuvants and immune-based anticancer candidates from Mediterranean marine organisms, lepadin H was found to be an activator of innate immune cells.<sup>595</sup> Testing of a library of cadiolide analogues, including alkyne-containing synthetic intermediates, identified a number of compounds with the ability to inhibit quorum sensing in *Vibrio harveyi*, and that could inhibit biofilm formation by *Escherichia coli* RP347 at concentrations that had no effect on planktonic growth of the microorganism.<sup>596</sup> Conversely, some of the compounds were found to inhibit the growth of planktonic *Pseudomonas aeruginosa* PAO1 while also acting to promote biofilm formation by the microorganism. The mechanism by which didemnin B inhibits translation has been studied using cryoelectron microscopy and single molecule fluorescence, identifying that the cyclic decapeptide is able to trap GTPase elongation factor-1 alpha (eEF1A) in an intermediate state during amino acid-tRNA selection, ultimately preventing tRNA accommodation in the ribosome binding site and eEF1A release.<sup>597</sup>



The mandelalides are cytotoxic macrolactones known to inhibit mitochondrial ATP synthase, leading to cellular depletion of ATP. This mechanism of action have been studied in more detail, identifying a consequential activation of AMP-activated protein kinase (AMPK), which normally acts to maintain cellular ATP balance in response to energy stress.<sup>598</sup> When evaluated for cytotoxicity towards a panel of glioblastoma cell lines, mandelalide A was potently (nM) active towards some cell lines and essentially inactive towards others, presumably due to their degree of glycolytic metabolic phenotype, when run in a standard three day (72 h) end-point assay format. When the assays were performed using an extended six day run time, the survival pathways were overwhelmed, and cytotoxicity was restored to all cell lines.

## 11 Echinoderms

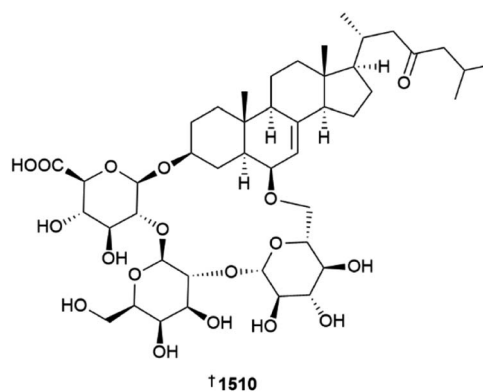
A Red Sea, Egypt collection of the brittle star *Ophiocoma dentata* afforded two sesquiterpene ethers **1470** and **1471**.<sup>599</sup> The metabolites isolated from starfish are usually either ceramides/cerebrosides or steroid derivatives. A total of six new lipids, ceramides **1472–1474** and cerebrosides **1475–1477**, were isolated from extracts of a Far Eastern collection of the orange cookie starfish *Ceramaster patagonicus*.<sup>600</sup> Although several of the MNPs were evaluated against a panel of four HTCLs and for the ability to inhibit colony formation by MDA-MB-231 human breast cancer cells, the compounds were either inactive or failed to reach a level of activity that the authors of this review consider active.

Two simple sterol derivatives, **1478** and **1479**, were isolated from Red Sea collections of the starfish *Acanthaster planci*,<sup>601</sup> with four disulfated steroids **1480–1483** being reported from a Far Eastern deep sea trawling collection of *Pteraster marsipus*.<sup>602</sup> Steroids **1480** and **1481** were characterised as a mixture. Two polyhydroxylated sterols, including the glycoside reguloside D **1484** and cholestane **1485** isolated from a Vietnamese collection of *Pentacaster regulus* exhibited weak inhibition of LPS-induced NO production in RAW264.7 cells.<sup>603</sup>

The C-3/C-26 diglycosides **1486** and **1487**, purified from Xisha Is. specimens of *Culcita novaeguineae*, were found to be inactive when tested against three HTCLs *in vitro*.<sup>604</sup> In addition to eight triterpene glycosides, pacificosides D–K **1488–1495**, extracts of the starfish *Solaster pacificus* also afforded the known related glycoside cucumarioside D, previously reported from the sea cucumber *Eupentacta fraudatrix*.<sup>605</sup> Variable levels of cytotoxicity towards a normal human cell line and three HTCLs were observed, with all compounds also exhibiting haemolytic properties. Pacificoside A and cucumarioside D were the most active of the purified compounds at inhibiting the neoplastic transformation of non-cancerous mouse epidermal cells when exposed to chemical (EGF, TPA) or ionising radiation (X-ray or UVB) carcinogens. A Vietnamese collection of the sea cucumber *Stichopus herrmanni* was the source of sulfated hydrocarbons herrmananes A **1496** and B **1497**, both of which were inactive towards a panel of five HTCLs.<sup>606</sup> A Troitsa Bay, Japan collection of *Paracaudina chilensis* afforded the triterpene pentaosides chilensosides A, A<sub>1</sub>, B, C and D **1498–1502**, all of which, except

for the last, exhibited haemolytic properties towards erythrocytes with weak to no activity towards a panel of five HTCLs.<sup>607</sup> Additional examples of triterpene glycosides, chitonoidosides I, J, K, K<sub>1</sub> and L **1503–1507** are hexaosides with all but chitonoidoside K<sub>1</sub> exhibiting haemolytic properties and variable levels of cytotoxicity towards three HTCLs.<sup>608</sup>

A chemoenzymatic synthesis of LLG-5, a neuritogenic ganglioside first isolated from the starfish *Linckia laevigata* has been reported, achieved in 10 steps with an overall yield of about 12%.<sup>609</sup> The methodology is flexible enough to allow preparation of a variety of analogues. The structures of the complex steroidal C-3/C-6 trisaccharide linked starfish metabolites luzonicosides A **1508** and D **1509** and sepositoside A **1510** have been confirmed by total synthesis, with absolute configuration assigned.<sup>610</sup>



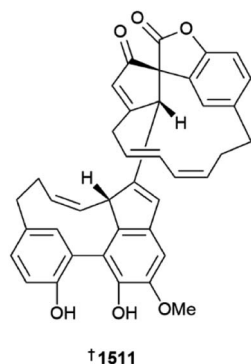
A plausible biosynthetic precursor to the echinochrome family of naphthoquinone sea urchin pigments is 2-acetyl-1,3,6,8-tetrahydroxynaphthalene, synthesis of which has now been directly linked by genetic and biochemical studies to a unique class of nonreducing aromatic polyketide synthases.<sup>611</sup> Phylogenetic analysis identified the presence of the gene SpPks1 and its homologues are widespread amongst echinoderms and close relatives the acorn worms, providing further support for the importance of this structural class to the evolutionary fitness of these organisms. The anti-inflammatory activities of echinoderm metabolites in general have been reviewed.<sup>612</sup> The biological activities of specifically quinonoid sea urchin pigments, including echinochrome A, have also been reviewed,<sup>613</sup> with a number of new studies reporting a variety of additional biological activities for echinochrome A including regulation of inflammation related to myocardial dysfunction,<sup>614</sup> reduced blood pressure in a rat model of preeclampsia,<sup>615</sup> as a treatment for menopausal dry mouth,<sup>616</sup> suppression of melanin synthesis,<sup>617</sup> and maintenance and regeneration of intestinal epithelium cells.<sup>618</sup> The ecological roles played by anthraquinone pigments have been widened by the finding that host selection between crinoids and the symbiotic snapping shrimp *Synalpheus stimpsonii* is mediated by the pigments.<sup>619</sup> In addition to antioxidant properties, othiol appears to play a central role in the control of cell proliferation in the early stages of embryo development in the sea urchin *Paracentrotus lividus*.<sup>620</sup>



The multi-step biosynthesis of sterols in animals involves the enzyme lanosterol synthase to achieve cyclisation of a squalene epoxide to form lanosterol. Sea cucumbers lack this enzyme, relying instead upon two oxidosqualene cyclase enzymes that appear to have evolved from an ancestral lanosterol synthase.<sup>621</sup> With the common occurrence of steroidal glycosides in sea cucumbers, and the knowledge that saponins act as membrane disruptors, the authors noted the ability of the marine organisms to produce larger quantities of lathosterol and related sterols that appear to confer protection from saponin self-poisoning. Further biological evaluation of sea cucumber metabolites has identified the simple fatty acid decanoic acid as being active in a number of Parkinson's disease related models and assays,<sup>622</sup> desulfated echinoside A has much reduced haemolytic activities and can still act to lower lipid levels *in vitro* and *in vivo*,<sup>623</sup> and the saponin stichloroside C2 is cytotoxic towards triple-negative breast cancer cells *via* mechanisms that include inhibition of epithelial-mesenchymal transition and the induction of apoptosis *via* the MAPK signalling pathway.<sup>624</sup>

## 12 Miscellaneous

A new member of the zosterabispheone family of diarylheptanoid dimers, zosterabispheone C **1511** was reported from Northern Germany collections of the seagrass *Zostera marina*.<sup>625</sup> The structure contains an unusual spiro-fused  $\gamma$ -lactone moiety, proposed to be biosynthetically derived by rearrangement of co-metabolite zosterabispheone A. Weak cytotoxicity towards the HCT-116 cell line was observed.



Two new tetrodotoxin-related NPs have been characterised, with 9-*epi*-tetrodotoxin isolated from *Takifugu flavipterus* as a mixture of hemilactal **1512** and 10,8-lactone **1513** forms, and spiro-cyclic guanidine Tb-242B **1514** isolated from *Dichotomyctere ocellatus*.<sup>626</sup> Tb-242B was found to convert in neutral buffer to the previously reported 9-epimer NP Tb-242A, whereas the latter did not convert to the former under the same conditions. 9-*epi*-Tetrodotoxin did not exhibit any toxic effects in mice, identifying the stereochemistry at C-9 as being important for the biological activity of tetrodotoxin. The first asymmetric syntheses of four spiro-cyclic guanidines related to tetrodotoxin, Tb-210B, Tb-226, Tb-242C, and Tb-258, have been reported, with the methodology utilising a common intermediate.<sup>627</sup> LC-MS analysis of flatworms collected on the New South Wales, Australia coastline has identified most to

contain tetrodotoxin analogues, with only *Stylochus cf. mcgrathi* containing tetrodotoxin itself.<sup>628</sup> Imaging MS was attempted – using a fixing/dehydration method revealed no tetrodotoxin-like signals, while freeze-dried specimens of *Stylochus* sp. only revealed the most dominant metabolite, 11-deoxy-TTX, which was mainly located in the rostral intestinal region.

The mechanism of antibacterial and antibiofilm activity of the 20-residue peptide fragment capitellacin, derived from whole genome sequencing and gene expression studies of the polychaete worm *Capitella teleta*, is attributed to detergent-like membrane destabilisation.<sup>629</sup> A mixture of eight amino alcohol-containing betaines were isolated from the stinging fireworm *Hermodice carunculata*.<sup>630</sup> Although structures of the NPs were presented in the publication, the reliance placed upon LC-MS/MS fragment analysis and NMR spectroscopy of the mixture, means the decision has been made to not show the structures in this review.

Nereistoxin, 4-dimethylamino-1,2-dithiolane, is a structurally simple insecticidal nAChR antagonist originally isolated from the annelid worm *Lumbriconereis heteropoda*. A set of analogues, comprised of quaternised *N*-methyl-nereistoxin, 5-dimethylamino-1,3-dithiane and its quaternised *N*-methyl derivative, were prepared and evaluated against three mammalian nAChR's.<sup>631</sup> The quaternised analogues, while displaying lower affinity for  $\alpha 4\beta 2$  nAChRs, bound with higher affinities than their secondary amine variants to *Torpedo* muscle nAChR and rat  $\alpha 7$  brain receptors.

Genome mining of the nematode *Anisakis* sp. has led to the identification of eight new antimicrobial peptides, the anisaxins, which exhibit preferential activity towards Gram-negative bacteria, with a membranolytic mechanism that leads to membrane bulging and lipid extraction.<sup>632</sup> Only mild cytotoxicity to a mammalian cell line was observed but the peptides were unstable in serum.

Methoxy-bromodiphenyl ethers (MeO-BDEs) are susceptible to CYP-450 oxidative demethylation by hepatic microsomes of the red snapper *Lutjanus campechanus*, with the phenolic products being similar to those formed by oxidation of BDE flame retardants.<sup>633</sup> The phenols are in turn susceptible to glucuronidation and sulfation excretion mechanisms by the same fish species.

## 13 Conclusion

The analysis of NP chemical diversity through cheminformatics techniques has benefited from the availability of extensive databases housing a wide range of NP structures and their meta data. We previously used principal component analysis of physicochemical properties to analyse MNPs and drugs and this showed that MNPs from most marine phyla are physicochemically different from each other.<sup>634</sup> An alternative method to assess NP diversity uses Tanimoto indices derived from pre-defined substructure fragments.<sup>635</sup> A 2017 study using a Tanimoto index cut-off of 0.4 between pairs of compounds highlighted that the discovery of novel NPs as a proportion of total NPs isolated annually is becoming less frequent.<sup>636</sup> However, a paired Tanimoto index can be a blunt instrument if one is



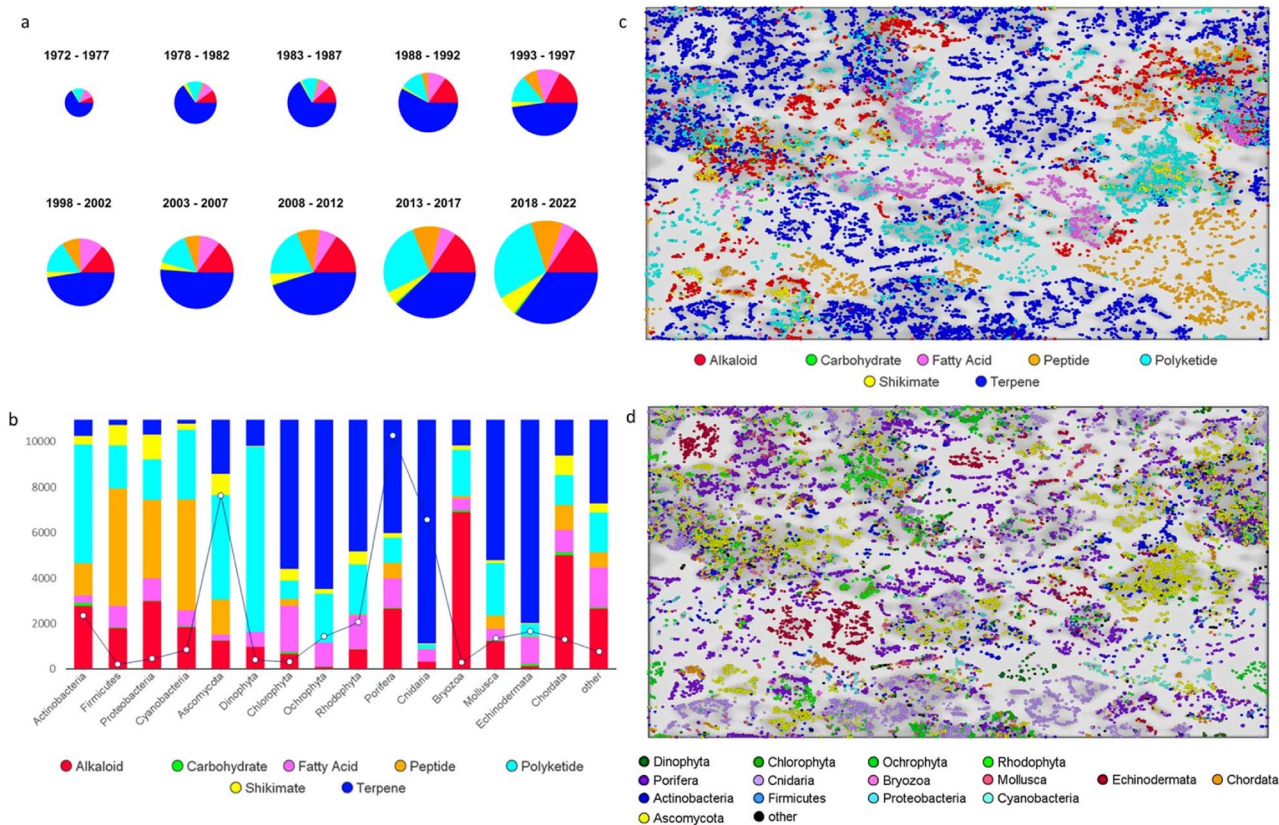


Fig. 2 MNP structure classes. (a) Semi-decadal time series (pie chart size reflects the total MNPs reported (38 357)), (b) proportion of MNPs in different structure class in the major marine phyla. The line graph indicates total MNPs in each phylum, SOM ( $200 \times 200$  neurons) for MNPs colour coded by (c) structure class and (d) phylum.

trying to establish if compounds have similar or different molecular frameworks since pairs of NPs with Tanimoto indices  $>0.7$  can often belong to different structure classes, while compounds sharing the same biosynthetic origin can have indices  $<0.4$ .

Rather than simply relying on the paired Tanimoto similarities, the matrix of paired similarities of compounds in large data sets can be mapped using self-organising maps (SOM) that use artificial neural networks to group objects with similar attributes based on features such as the Tanimoto index. For chemical diversity analysis, this more powerfully discriminates molecules that have similar Tanimoto indices into clusters of compounds that are more likely to share common structural frameworks (including both rings and sidechains). MNP structure class diversity *vs.* phyla were last reported in the 2004 edition of this review.<sup>637</sup> Another study in 2020 looked at structure class diversity across all taxa but was limited in the scope of its analysis.<sup>638</sup> We have now used SOMs derived from analysis of extended connectivity-based fingerprints to map MNP structure class diversity. Firstly, most MNPs ( $n = 38\,357$ ) found in Marinlit up to the end of 2022 were categorised using NP classifier, a deep neural network-based structural classification tool that is available as part of the GNPS toolbox hosted at the Scripps Institute for Oceanography in the USA.<sup>639</sup> NP classifier categorises NPs at three levels: pathway, superclass and class.

A time series (Fig. 2a) shows the change in the proportion of structure classes (pathway classification: alkaloid, carbohydrate, fatty acid, peptide, polyketide, shikimate, and terpene) reported each semi-decade since the early 1970s. Over the last 10 years, a shift to more polyketide derived MNPs compared to terpenoid MNPs has occurred. This shift is largely because marine fungi (and to a lesser extent Actinobacteria) have recently become prolific sources of new MNPs. The proportion of different structure classes within individual phyla supports this trend (Fig. 2b).

Applying a SOM ( $200 \times 200$  neurons using the skelspheres descriptor in Osiris DataWarrior<sup>640</sup> (DW)) and colour coding the map according to structure classes shows how effective it is at grouping structurally related molecules (Fig. 2c). By comparison, colour coding the map according to phylogeny shows that specific groups of compounds within structure classes are phylogenetically separated (Fig. 2c) (the grey scale background on the SOM shows areas of higher similarity are darker). Structure subclass groupings are further illustrated when the terpenoid pathway (representing  $\sim 41\%$  of all MNPs) is colour coded by terpene super-classes (monoterpene, sesquiterpene, diterpene, sesterterpene, triterpene, steroid and polyisoprene) (Fig. 3). Interestingly, there are discrete differences in the occurrence of terpenes within different phyla (Fig. 3a) and limited overlap of MNPs within discrete super-classes *vs.* phyla



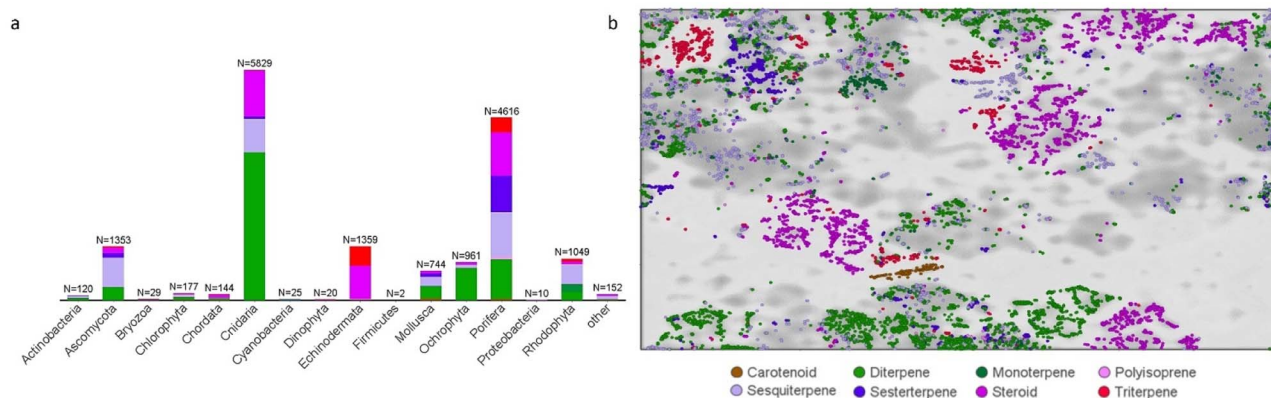


Fig. 3 Distribution of terpene MNPs by (a) phylum and (b) in a SOM (200 × 200 neurons).

(Fig. 2d and 3b). Cnidarians (producers of the most terpene NPs) mainly yield diterpenes, echinoderms contain triterpene and steroid NPs and sesterterpenes are almost exclusively produced by sponges.

Marine fungi are the main source of peptides and have yielded 78% of all diketopiperazine MNPs, while linear and cyclic oligopeptides and depsipeptides have mostly been isolated from sponges (510), cyanobacteria (354), fungi (278), actinobacteria (208) and tunicates (123). Polyketides represent 23% of MNPs and marine fungi contribute 44% of this total.

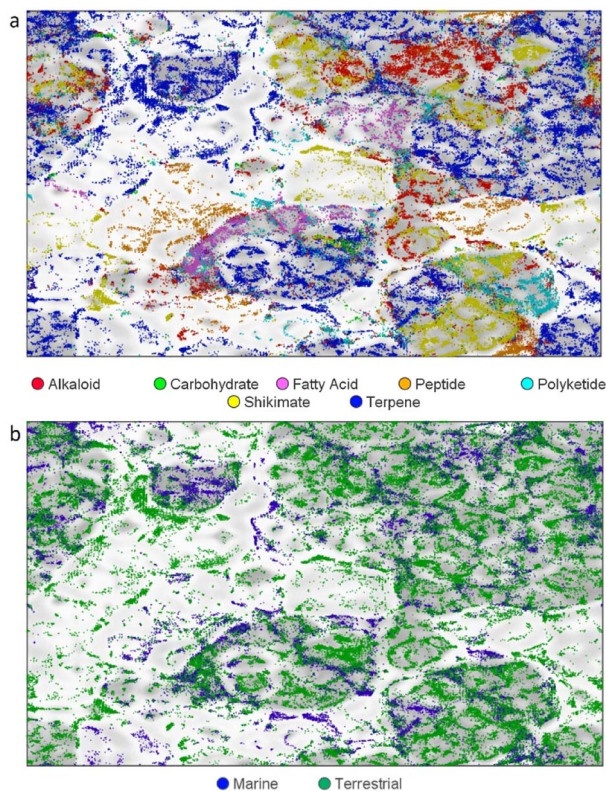


Fig. 4 SOM (200 × 200 neuron) of 172 320 NPs (114 257 plant NPs, 19 706 terrestrial microbe NPs, 38 357 MNPs) (a) colour coded by structure class, (b) colour coded by biome.

More than half of these fungal MNPs are simple aromatic phenols, xanthenes, naphthoquinones, anthraquinones, benzopyrones or benzofurans. Polyketide macrolides are mostly reported from actinobacteria (385), sponges (140), fungi (196) cyanobacteria (81) and dinoflagellates (53). Polyether molecules are produced by dinoflagellates (99), red algae (70), sponges (31) and sequestered by molluscs (26). Diphenyl ethers (DPE) are found in brown algae (157), fungi (134) and sponges (52). While the fungi-derived diphenyl ethers are mainly acyl/alkyl resorcinol derivatives, the sponge DPEs are polybrominated phenols and the brown algae DPEs are phlorotannins. Endoperoxides are almost exclusively found in sponges (see the ESI† for more figures showing the MNP SOM filtered by super-class and/or class categories).

Additional structure class diversity comparisons were undertaken amongst a wider range of NPs including terrestrial microbial NPs from NPAtlas<sup>641</sup> ( $n = 19\,706$ ) and plant NPs from the Universal NP Database (UNPD)<sup>642</sup> ( $n = 114\,257$ ) compared to the MNPs ( $n = 38\,357$ ). All 172 320 NPs were assigned to structure classes using NP classifier and the dataset was analysed to generate a SOM (200 × 200 neuron) using the skelspheres descriptor in DW. Colour coding by structure class again highlighted discrete areas of pathway specific diversity (Fig. 4a). Colour coding the NPs by biome (Fig. 4b) also showed segregation of many NPs as specifically terrestrial or marine in origin. A 2022 study showed that NPs reported from marine and terrestrial microorganisms are structurally closely related and the SOM mirrored this result (see Fig S4 and S5 in the ESI†).<sup>295</sup>

Cyanobacteria are the exception with greater differences in their structural diversity between freshwater and marine species (see Fig S6 ESI†). Sponges are regularly reported to contain NPs derived from microorganisms and several studies that have investigated the origins of some NPs in sponge tissues have shown that they are produced by symbionts.<sup>643–645</sup> These investigations use genome mining techniques to support the microbial origins of some sponge MNPs. However little overlap exists between newly published sponge and marine microorganism chemical diversity, particularly for alkaloid, terpene, and polyketide NPs. Peptide NPs reported from sponges, bacteria and fungi are more closely related. A caveat to using NP



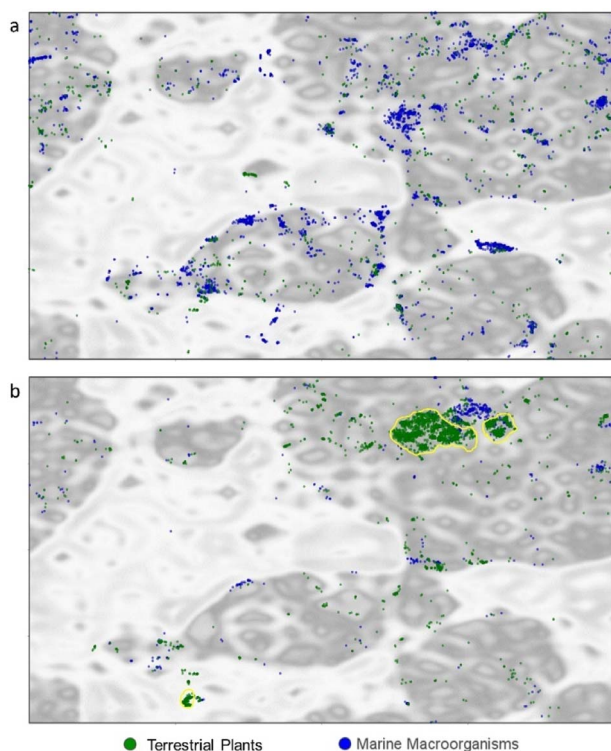


Fig. 5 SOM (200 × 200 neuron, plant and marine macroorganisms only,  $n = 140\,351$ ) (a) non-terpene structure classes with little overlap ( $n = 5834$ ) bromotyrosins, histidine, guanidine, imidazole and pyrrole NPs, (b) tryptophan-derived NPs. ( $n = 4874$ , plants 4110 marine microorganism 764). Monoterpene indole alkaloid clusters are circled in yellow.

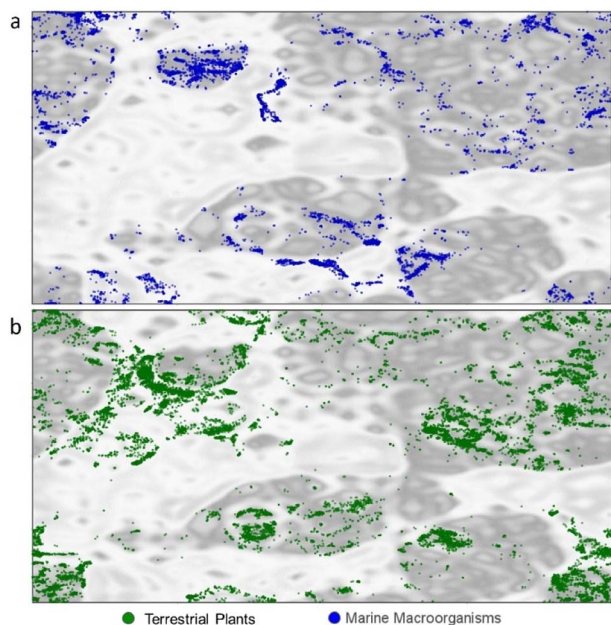


Fig. 6 Chemical diversity comparison of diterpene, sesterterpene, triterpene, steroid and meroterpene NPs ( $n = 45\,043$ ) between (a) terrestrial plants (33 604) and (b) marine macroorganisms (11 439) using a 200 × 200 SOM.

databases is that they often only include the first reported source of NPs and so there may be more examples of MNPs first reported from marine macroorganisms being later found in marine microorganisms. One might expect new MNPs isolated from cultured marine microorganisms to overlap with known macroorganism MNPs if the symbiotic microbiomes of host organisms are being efficiently accessed but the lack of overlap suggests otherwise.

Marine macroorganism and plant-derived NPs are structurally different. Bromoindole, bromotyrosine, guanidine, imidazole, purine, pyrazine, pyridine, pyridoacridine, and pyrrole alkaloids are mostly produced by marine macroorganisms (85%) and are structurally different from plant alkaloids in the categories (Fig. 5a). Likewise, acetogenins, 4-pyrone, DPES, endoperoxides, isonitriles, isothiocyanates, oximes, linear polyunsaturated compounds and polyethers are also predominately reported from marine species (Fig. 5a). Plant tryptophan-derived alkaloids are mainly monoterpene indole alkaloids (75%), but in marine macroorganisms they are more structurally diverse and occupy different areas of chemical space (Fig. 5b).

The terpene super-classes: diterpene, sesterterpene, triterpene and steroid all possess compounds that are separated into either plant or marine macroorganism-derived groups (Fig. 6). Marine specific classes occupying unique region of the SOM are briarane, cembrane, eunicellin, spongiane and xenicane diterpenes, cholestane steroids, sesterterpenes, many meroterpenes and lanostane triterpenes. These groups of terpenes represent ~30% of all MNPs (12 474) and the combined total of uniquely marine structures represents more than 50% of MNPs isolated from macroorganisms.

This structure class analysis further highlights the unique chemical diversity of MNPs. Even within well studied and categorised groups of NP structure classes, there is a significant difference in the chemical diversity reported from marine macroorganisms compared to plants, while marine and terrestrial microorganism NPs (except cyanobacteria) share much in common with each other.

## 14 Conflicts of interest

There are no conflicts to declare.

## 15 Acknowledgements

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