

Potential of marine natural products against drug-resistant bacterial infections



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Natural products have been a rich source of compounds with structural and chemical diversity for drug discovery. However, antibiotic resistance in bacteria has been reported for nearly every antibiotic once it is used in clinical practice. In the past decade, pharmaceutical companies have reduced their natural product discovery projects because of challenges, such as high costs, low return rates, and high rediscovery rates. The largely unexplored marine environment harbours substantial diversity and is a large resource to discover novel compounds with novel modes of action, which is essential for the treatment of drug-resistant bacterial infections. In this Review, we report compounds derived from marine sources that have shown in-vivo and in-vitro efficacy against drug-resistant bacteria. Analysis of the physicochemical properties of these marine natural products with activity against drug-resistant bacteria showed that 60% of the compounds have oral bioavailability potential. Their overall distribution pattern of drug characteristics agrees with the observation that marketed antibacterial drugs have a polar distribution, with a lower median calculated logP. The aim of this Review is to summarise the diversity of these marine natural products, with a special focus on analysis of drug bioavailability. Such biologically active compounds, with high degrees of bioavailability, have the potential to be developed as effective drugs against infectious diseases.

Introduction

Even though antibiotic resistance in bacteria is a natural phenomenon, its spread among pathogenic bacteria is an increasing problem in medicine. The problem is even more threatening as there are few, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria. The existence and development of antibiotic resistance in bacteria were originally the result of the competition between various micro-organisms in the environment as certain microbes produced antibiotics to compete with their competitors.¹ The use of antibiotics in medicine, which is often too frequent and inappropriate, contributes to the fast spread of antibiotic resistance.² Methicillin-resistant *Staphylococcus aureus* (MRSA) has become one of the most frequently reported nosocomial pathogens worldwide, and is responsible for more than 11000 deaths annually in the USA alone.³ In 2017, there was an estimated 558000 new cases worldwide of tuberculosis with resistance to rifampicin, the most effective first-line drug, of which 490000 had multidrug-resistant tuberculosis.⁴ New drugs are needed because of decreases in drug efficacy and the need to combat antibiotic resistance. Antibiotic resistance in bacteria is not based on one or a few mechanisms. All possible mechanisms of antibiotic resistance can be found in bacteria (panel). Antibiotic resistance can be categorised into intrinsic and acquired resistance mechanisms. Intrinsic resistance appears when the target of a certain antibiotic is absent, such as penicillin-binding protein (Pbp) in *Mycoplasma* spp resistant to β -lactam antibiotics.⁵ Acquired resistance is normally obtained by reduced permeability, antibiotic efflux, modification of the antibiotic target by genetic mutation, post-translational modification, or inactivation of the antibiotic by hydrolysis or modification. The acquisition of the staphylococcal resistance cassette chromosome mec (SSCmec) is an example of the acquisition of a gene homologous to the original target.

The SSCmec carries the *mecA* gene, which encodes the α -lactam-insensitive Pbp2a. This protein enables cell wall synthesis to occur despite the native Pbp being inhibited in the presence of an antibiotic.⁶

Oceans cover 70% of the earth's surface and because of the various locations, temperature, and salinity, they contain a much more extensive phylogenetic diversity than that of the terrestrial environment.^{7,8} The association between the great genetic and ecological diversity of marine organisms make the oceans a unique and rich source of active compounds for the pharmaceutical

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Panel: List of known mechanisms of antibiotic resistance in bacteria

Intrinsic resistance

- Absence of the target of a certain antibiotic (eg, absence of penicillin-binding protein in *Mycoplasma* spp causes resistance to β -lactam antibiotics)
- Absence of a susceptible target of a specific antibiotic (eg, insensitive allele of *fabI* in *Pseudomonas* spp encoding an additional enoyl-ACP reductase causes resistance to triclosan)
- Inaccessibility of a target (eg, unique structure of the outer membrane of Gram-negative bacteria causes resistance to vancomycin)

Acquired resistance

- Reduced permeability (eg, reduction in porin expression in Enterobacteriaceae spp and *Pseudomonas* spp causes resistance to carbapenems)
- Increased efflux (eg, increased expression of efflux pumps in *Staphylococcus aureus* causes high levels of multi-drug resistance)
- Resistance by target mutation (eg, mosaic Pbp genes encoding penicillin-insensitive enzymes in *Streptococcus pneumoniae* causes resistance to penicillin)
- Resistance by target modification (eg, methylation of A2503 in the 23S rRNA by chloramphenicol-florfenicol resistance methyltransferase results in resistance to many antibiotics, such as phenicols, pleuromutins, streptogramins, lincosamides, and oxazolidinones)
- Resistance by direct modification of antibiotics (eg, thousands of enzymes in bacteria can degrade or modify β -lactams, aminoglycosides, phenicols, and macrolides, to become resistant to these antibiotics)

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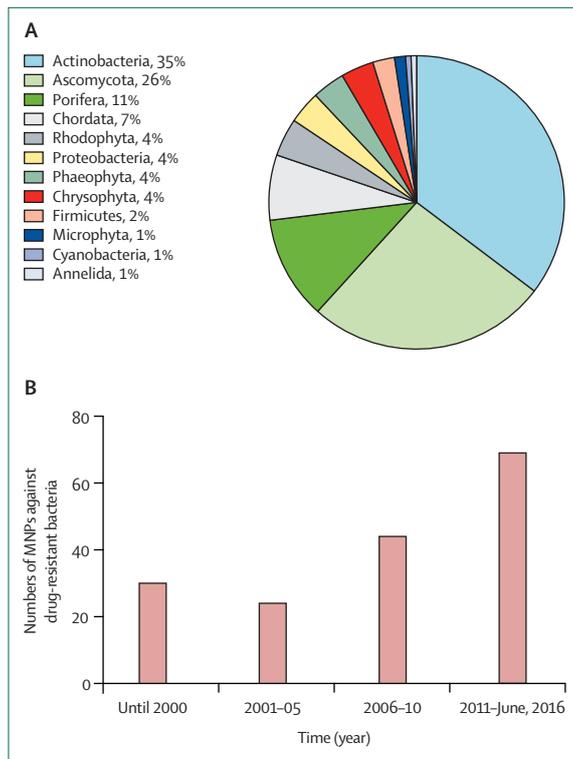


Figure 1: MNPs active against drug-resistant bacteria
(A) Percentage distribution (phylum-wise) of the 167 MNPs active against drug-resistant bacteria in the marine environment. (B) Discovery of the 167 MNPs against drug-resistant pathogens over the past 20 years. Data includes the compounds discovered until June, 2016. MNPs=marine natural products.

industry.⁹⁻¹¹ In this Review, we first describe the potential of marine natural products (MNPs) in the treatment of infections caused by drug-resistant bacteria, including MRSA, methicillin-resistant *Staphylococcus epidermidis*, vancomycin-resistant enterococci, penicillin-resistant *Streptococcus pneumoniae*, rifampin-resistant and methicillin-resistant *S aureus*, and multidrug-resistant *S aureus* and methicillin-sensitive *S aureus* (MSSA). The discovery of MNPs against drug-resistant bacteria has consistently increased during the past 15 years (figure 1). We consider the bioavailability of these MNPs by discussing their physical and chemical properties. In our previous Review, we have also discussed MNPs against drug-resistant fungal, viral, and parasitic infections.¹²

MNPs with activity against drug-resistant bacteria

To examine the potential of marine natural products as effective agents against drug-resistant bacteria, we conducted a comprehensive literature search covering the period up to June 2016. In total, 167 marine natural products have been reported to show activities against drug-resistant bacteria in in-vitro or in-vivo models. The structures and biological activities of 20 compounds are summarised in the table.

Two cyclohexapeptides, desotamide and desotamide B, have been isolated from the deep South China Sea derived from the microbe *Streptomyces scopuliridis* SCSIO ZJ46 (figure 2). Desotamide and desotamide B exhibited antibacterial activities against the Gram-positive pathogenic bacteria *S aureus* ATCC 29213, *S pneumoniae* NCTC 7466, and methicillin-resistant *S epidermidis* shhs-E1 (a clinical isolate) with minimum inhibitory concentrations (MICs) of 23 μM for *S aureus* ATCC 29213, 18 μM for *S pneumoniae* NCTC 7466, and 46 μM for methicillin-resistant *S epidermidis* shhs-E1. However, desotamide and desotamide B did not show cytotoxicity against four human tumour cell lines, including SF-268, MCF-7, NCI-H460, and HepG-2 (half maximal inhibitory concentration [IC_{50}] $>100 \mu\text{M}$). Structure-activity relationship studies showed that the Trp moiety of the compounds desotamide and desotamide B is essential for the antibacterial activity.¹³ Anthracimycin, a MNP isolated from *Streptomyces* species (strain CNH365), was obtained in 2013 from near-shore marine sediments found near Santa Barbara, CA, USA (figure 2).

Anthracimycin has potent antibacterial activity against the anthrax causative microbe *Bacillus anthracis* (strain UM23C1-1), with a MIC of 0.079 μM .¹⁴ Further biological studies showed in-vitro and in-vivo anti-staphylococcal activities against a broad panel of *S aureus* strains, including MSSA, MRSA, and vancomycin-resistant *S aureus*, with MIC values of 0.63 μM or more.¹⁵ At subinhibitory concentrations, anthracimycin decelerated the growth of MRSA and augmented the bactericidal effect of the human cathelicidin. Anthracimycin showed negligible toxicity against human carcinoma cells, with an IC_{50} of 175.9 μM .¹⁵ In a murine peritonitis model of infection, anthracimycin protected mice from MRSA-induced mortality, at a single dose of 1 mg/kg or 10 mg/kg.¹⁵ Mode of action studies, with an optimised macromolecular synthesis assay to quantitate incorporation of radiolabelled precursors, generated evidence that anthracimycin causes the disruption of DNA and RNA synthesis at around the MIC of MRSA.¹⁵

A new indole alkaloid, neofiscalin A, was isolated from the marine sponge-associated fungus *Neosartorya siamensis* (KUFA 0017), collected from the coral reef of the Similan Islands, Phang Nga Province, Southern Thailand (figure 1). Neofiscalin A showed potent antibacterial activity against Gram-positive bacterial pathogens, including MRSA and vancomycin-resistant *Enterococcus faecalis*, with a MIC of 17 μM and no cytotoxicity against a human brain capillary endothelial cell line.¹⁶

Fractionation of the extract from the marine fungus *Stachybotrys* species MF347 (of Sordariomycetes, of Hypocreales), isolated from a driftwood sample collected at Heligoland, North Sea, Germany, led to the identification of three spirocyclic drimanes stachyin B, stachyocin A, and stachyocin B, and the sesquiterpene ilicicolin (figure 2). These four compounds displayed antibacterial activity in vitro against MRSA with IC_{50}

	Class	Source	Activity (MIC)	Cytotoxicity (IC ₅₀)
Desotamide	Cyclohexapeptide	Deep South China Sea-derived microbe <i>Streptomyces scopuliridis</i> SCSIO ZJ46	Methicillin-resistant <i>Staphylococcus epidermidis</i> (46 µM)	SF-268, MCF-7, NCI-H460, and HepG-2 cell lines (>100 µM)
Desotamide B	Cyclohexapeptide	Deep South China Sea-derived microbe <i>S scopuliridis</i> SCSIO ZJ46	Methicillin-resistant <i>S epidermidis</i> (46 µM)	SF-268, MCF-7, NCI-H460, and HepG-2 cell lines (>100 µM)
Anthracimycin	Polyketide	Marine <i>Streptomyces</i> sp CNH365	MRSA (≥0.63 µM); vancomycin-resistant <i>Staphylococcus aureus</i> (≥0.63 µM)	Human carcinoma cells (>100 µM)
Neofiscalin A	Indole alkaloid	Marine sponge-associated fungus <i>Neosartorya siamensis</i> KUFA 0017	MRSA (17 µM); vancomycin-resistant <i>S aureus</i> (17 µM)	Human brain capillary endothelial cell line hCMEC/D3 (>42 µM)
Stachyin B	Alkaloid	Marine fungus <i>Stachybotrys</i> sp MF347	MRSA (1.75 µM)	Mouse fibroblasts cell line NIH-3T3 (13.01 µM); carcinoma cell line HepG2 (14.27 µM)
Stachyocin A	Alkaloid	Marine fungus <i>Stachybotrys</i> sp MF347	MRSA (3.71 µM)	Mouse fibroblasts cell line NIH-3T3 (30 µM); carcinoma cell line HepG2 (50 µM)
Stachyocin B	Alkaloid	Marine fungus <i>Stachybotrys</i> sp MF347	MRSA (3.94 µM)	Mouse fibroblasts cell line NIH-3T3 (>50 µM); carcinoma cell line HepG2 (>50 µM)
Ilicicolin	Sesquiterpene	Marine fungus <i>Stachybotrys</i> sp MF347	MRSA (0.74 µM)	Mouse fibroblasts cell line NIH-3T3 (>50 µM); carcinoma cell line HepG2 (>50 µM)
JBIR-97/98	Polyketides	Marine fungus <i>Engyodontium album</i> LF069	MRSA (0.19 µM)	Mouse fibroblasts cell line NIH-3T3 (14 µM)
Engyodontochone A	Polyketide	Marine fungus <i>E album</i> LF069	MRSA (0.17 µM)	Mouse fibroblasts cell line NIH-3T3 (11 µM)
JBIR-99	Polyketide	Marine fungus <i>E album</i> LF069	MRSA (0.25 µM)	Mouse fibroblasts cell line NIH-3T3 (13.2 µM)
Engyodontochone B	Polyketide	Marine fungus <i>E album</i> LF069	MRSA (0.24 µM)	Mouse fibroblasts cell line NIH-3T3 (14.4 µM)
MC21-A	Biphenyldiol	Marine bacterium <i>Pseudoalteromonas phenolica</i> O-BC30	MRSA (2–4 µM)	Human normal fibroblast, rat pheochromocytoma, and Vero cells (>100 µM)
Etamycin	Cyclic peptide	Marine actinomycete strain CNS-575	MRSA (1–2 µM)	HeLa cells (>40 µM)
cis-3,4-dihydrohydrohamacanthin B	bis-indole alkaloid	Sponge <i>Topsentia pachastrelloides</i>	MRSA (25.6 µM)	Mammalian HEK 293T cells (103 µM)
Nosiheptide	Thiopeptide	Marine <i>Streptomyces</i> sp CNT-373	MRSA (0.02–0.2 µM); vancomycin-resistant <i>E faecium</i> (0.1 µM)	Mammalian cells (>20 µM)
PM181104	Cyclic peptide	Sponge-associated actinobacterium <i>Kocuria</i> sp MTCC 5269	MRSA (0.02 µM); vancomycin-resistant <i>E faecium</i> (0.04 µM); vancomycin-resistant <i>E faecalis</i> (0.005 µM)	..
Citraglycon A	Polycyclic xanthenes	Marine <i>Streptomyces caelestis</i>	MRSA (15.4 µM)	HeLa cells (>77 µM)
2,4-diacetylphloroglucinol	Phenol	Red alga-associated <i>Pseudomonas</i> sp AMSN	MRSA (1.2–4.8 µM)	No cytotoxicity to mice
Hedistin	Peptide	Marine annelid <i>Nereis diversicolor</i>	MRSA (3–6 µM)	No cytotoxicity against <i>Nereis</i> coelomocytes

MRSA=metacillin resistant *Staphylococcus aureus*

Table: Marine natural products with activity against drug-resistant bacteria by compound

values of 1.75 µM for stachyin B, 3.71 µM for stachyocin A, 3.94 µM for stachyocin B, and 0.74 µM for ilicicolin.¹⁷ The anti-MRSA activity of these four compounds is similar to the anti-staphylococcal activity of the reference drug chloramphenicol (IC₅₀ 2.46 µM).¹⁷ Furthermore, these four compounds inhibited the growth of *Bacillus subtilis* and *S epidermidis* with IC₅₀ values of 1.02–4.22 µM. Stachyin B showed weak cytotoxicity against the mouse fibroblasts cell line NIH-3T3 and the carcinoma cell line HepG2 with IC₅₀ values of 13.01 µM for NIH-3T3 and 14.27 µM for HepG2. The sesquiterpene ilicicolin was less cytotoxic than stachyin B against the NIH-3T3 cell line (IC₅₀ 30 µM) and has no cytotoxicity against the HepG2 cell line (IC₅₀ >50 µM).¹⁷ Stachyocin A and stachyocin B were devoid of in vitro cytotoxicity against both cell lines (IC₅₀ >50 µM).¹⁷

Four antibiotic polyketides—JBIR-97/98, engyodontochone A, JBIR-99, and engyodontochone B—have been isolated from mycelia and culture broth of the fungus *Engyodontium album* strain LF069 obtained from sponge tissue of *Cacospinga scalaris* sampled at the

Limski Fjord, Croatia (figure 2). These four antibiotic polyketides showed antibacterial activity against MRSA (clinical isolate DSM 18827) that was ten times stronger than the anti-MRSA activity of the reference antibiotic chloramphenicol, with IC₅₀ values of 0.19 µM for JBIR-97/98, 0.17 µM for engyodontochone A, 0.25 µM for JBIR-99, and 0.24 µM for engyodontochone B.¹⁸ Cytotoxicity evaluation of these four antibiotic polyketides against NIH 3T3 showed weak cytotoxic effects with IC₅₀ values 14 µM for JBIR-97/98, 11 µM for engyodontochone A, 13.2 µM for JBIR-99, and 14.4 µM for engyodontochone B, which are similar to those of the positive control.¹⁸

A bactericidal compound, MC21-A, was purified from the methanol extract of the marine bacterium *Pseudoalteromonas phenolica* sp O-BC30T.^{19,20} The chemical structure of MC21-A was determined to be 3,3',5,5'-tetrabromo-2,2'-biphenyldiol (figure 2). MC21-A exhibited potent antibacterial activity against ten clinical isolates of MRSA, in addition to other Gram-positive pathogens such as *E serolicida*, *E faecium*, and *E faecalis*.

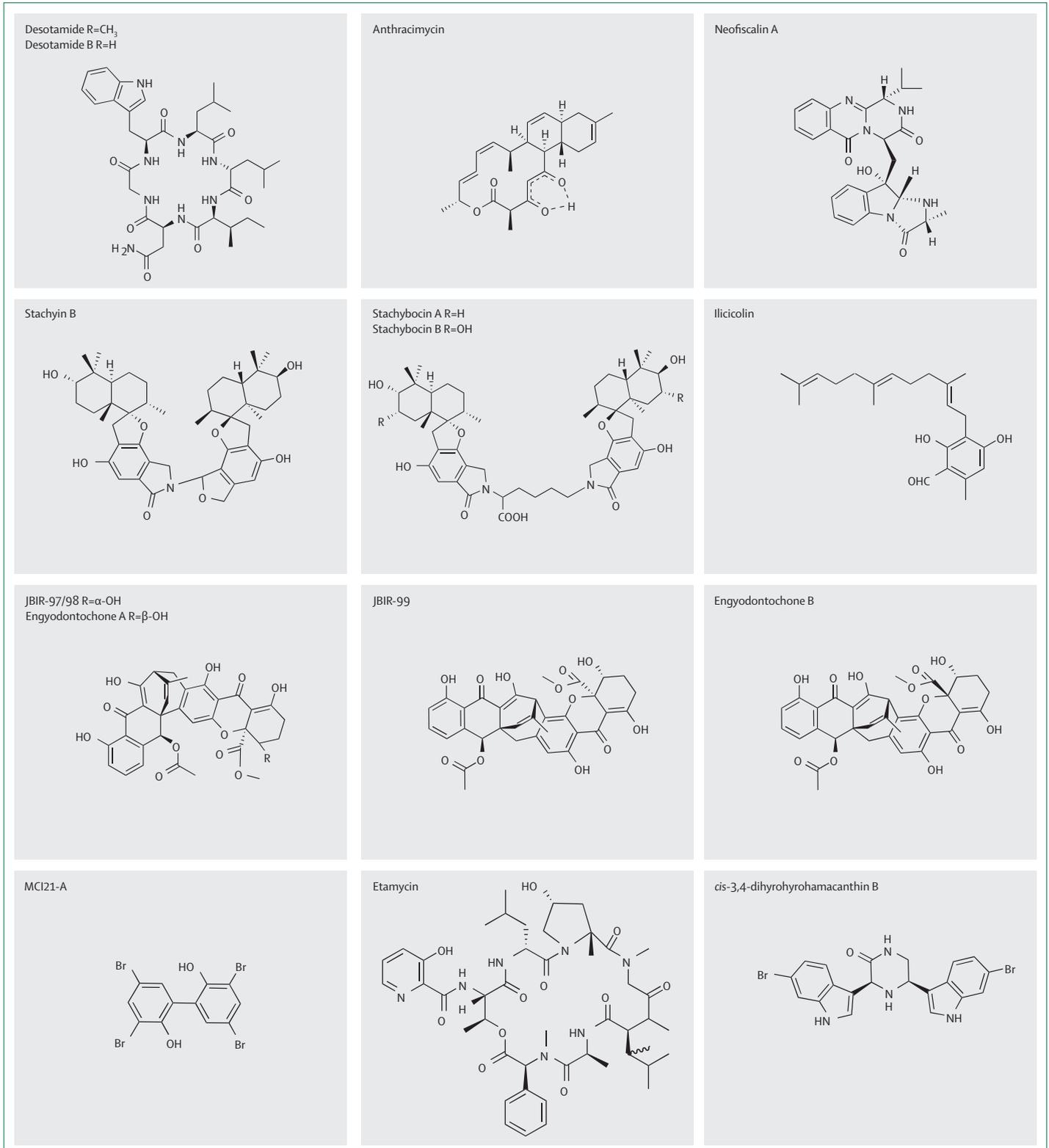


Figure 2: Chemical structures of marine natural products

The anti-MRSA activity of MC21-A (MICs 2–4 μM) was comparable to the anti-MRSA activity of the reference drug vancomycin (MICs <0.2–1.4 μM).²⁰ A time-kill study showed that MC21-A kills the bacteria with a higher rate than does vancomycin.²⁰ Mode of action studies, with a SYTOX Green staining experiment, suggested that MC21-A permeabilised the cell membrane of MRSA.²⁰ Additionally, MC21-A did not show cytotoxicity at a concentration of 100 μM to human normal fibroblast, rat pheochromocytoma, and Vero cells.^{19,20}

The streptogramin antibiotic etamycin has been isolated from actinomycete species (strain CNS-575) isolated from the coast of Fiji (figure 2). Etamycin showed potent antibacterial activity against community associated MRSA and health-care acquired MRSA with MIC values as low as 1–2 μM . Etamycin was not cytotoxic to HeLa cells at a concentration of 20 times MIC. Furthermore, in-vivo studies in a murine systematic infection model showed that 20 mg/kg of etamycin significantly protected mice infected with MRSA (strain Sanger 252) from mortality during the 72 h monitoring period.²¹ By comparison with the first-line MRSA antibiotic vancomycin, etamycin showed favourable time-kill kinetics.²¹

Pyruvate kinase (PK) in *S aureus* is an important target for the development of new antibacterial drugs, and was found to be one of the most highly connected hub proteins in MRSA. The bis-indole alkaloid *cis*-3,4-dihydroxyhamacanthin B was obtained from the sponge *Topsentia pachastrelloides* collected from South Africa, and determined as a potent MRSA PK inhibitor at low nanomolar concentration, with IC₅₀ value of 16 nM. The PK inhibitory activity of *cis*-3,4-dihydroxyhamacanthin B was found to be selective for MRSA PK at least 166 times over human PK isoforms. In addition, this bis-indole alkaloid showed promising antibacterial activity in vitro against *S aureus* strains RN4220 and MRSA252 with a MIC of 25.6 μM , with selectivity index (cytotoxicity concentration 50% [CC₅₀] per MIC) >4.²² Evaluation of cytotoxicity against the mammalian HEK 293T cells showed that *cis*-3,4-dihydroxyhamacanthin caused 24% cell death at a concentration of 103 μM , with CC₅₀ values of over 103 μM .²¹ Taken together, the selectivity indices of X4-dihydroxyhamacanthin (for the antibacterial activity and the cytotoxicity) indicate that this compound exhibited potent and selective inhibitory activity against bacteria versus mammalian cells.²² Further studies reported the synthesis of novel bis-indoles, derived from this naturally occurring marine alkaloid, that showed more potent anti-MRSA activities with MIC values as low as 1.5 μM .²³

Chemical and biological investigations of marine-derived actinomycete extract libraries led to the discovery of the thiopeptide nosiheptide from a *Streptomyces* spp (strain CNT-373) isolated from a marine sediment collected in Fiji (figure 3). Nosiheptide showed very potent antibacterial activity against a broad panel of

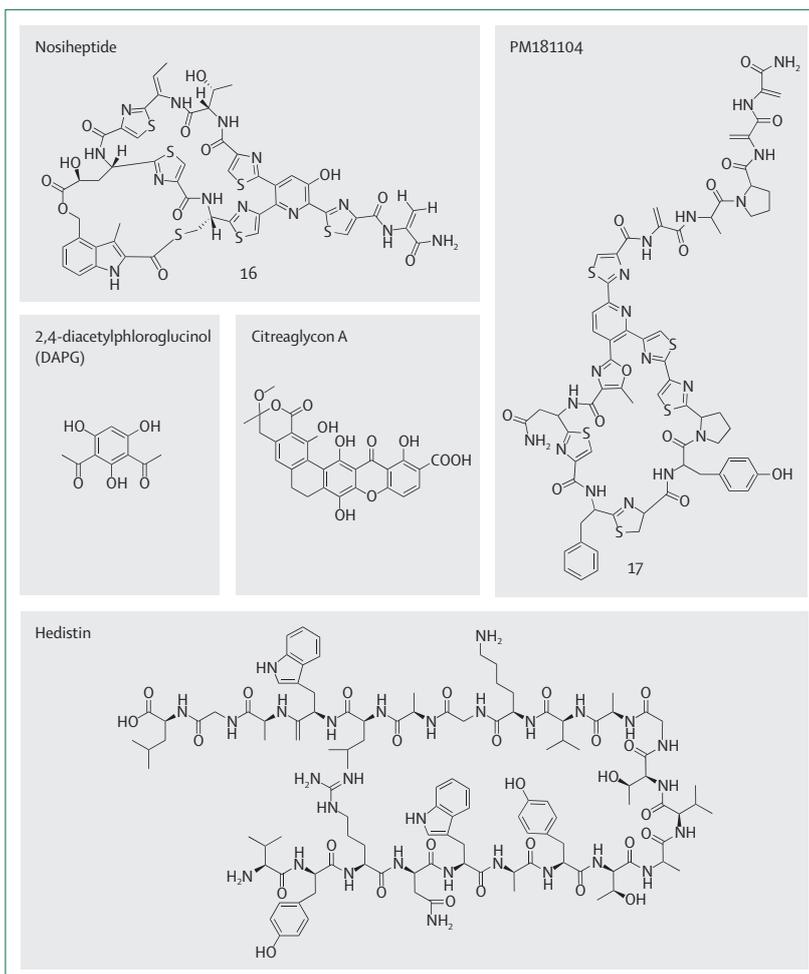


Figure 3: Chemical structures of marine natural products

S aureus including several MRSA strains with MIC values from 0.02 μM to 0.2 μM , with no cytotoxicity against mammalian cells at concentrations over 100 times MIC. Additionally, nosiheptide showed high antibacterial activity against vancomycin-resistant *E faecium* strains with a MIC of 0.1 μM , and against the contemporary hypervirulent BI/NAP1/027 strain of *Clostridioides difficile* with a MIC of 6.5 nM. Compared with the antibiotic vancomycin, nosiheptide showed significant prolonged post-antibiotic effect (exceeded 9 h) against both health-care acquired MRSA and community associated MRSA strains.²⁴ Moreover, in-vivo studies in a murine model of MRSA infection where mice were infected with health-care acquired MRSA strain Sanger 252, showed that nosiheptide has significantly protected mice from mortality ($p < 0.03$).²⁴

Bioassay-guided fractionation of the extract of a marine sponge-associated actinobacterium strain of the genus *Kocuria* (MTCC 5269) led to the identification of the novel thiazolyl cyclic-peptide antibiotic PM181104 (figure 3). Antibacterial screening of PM181104 showed

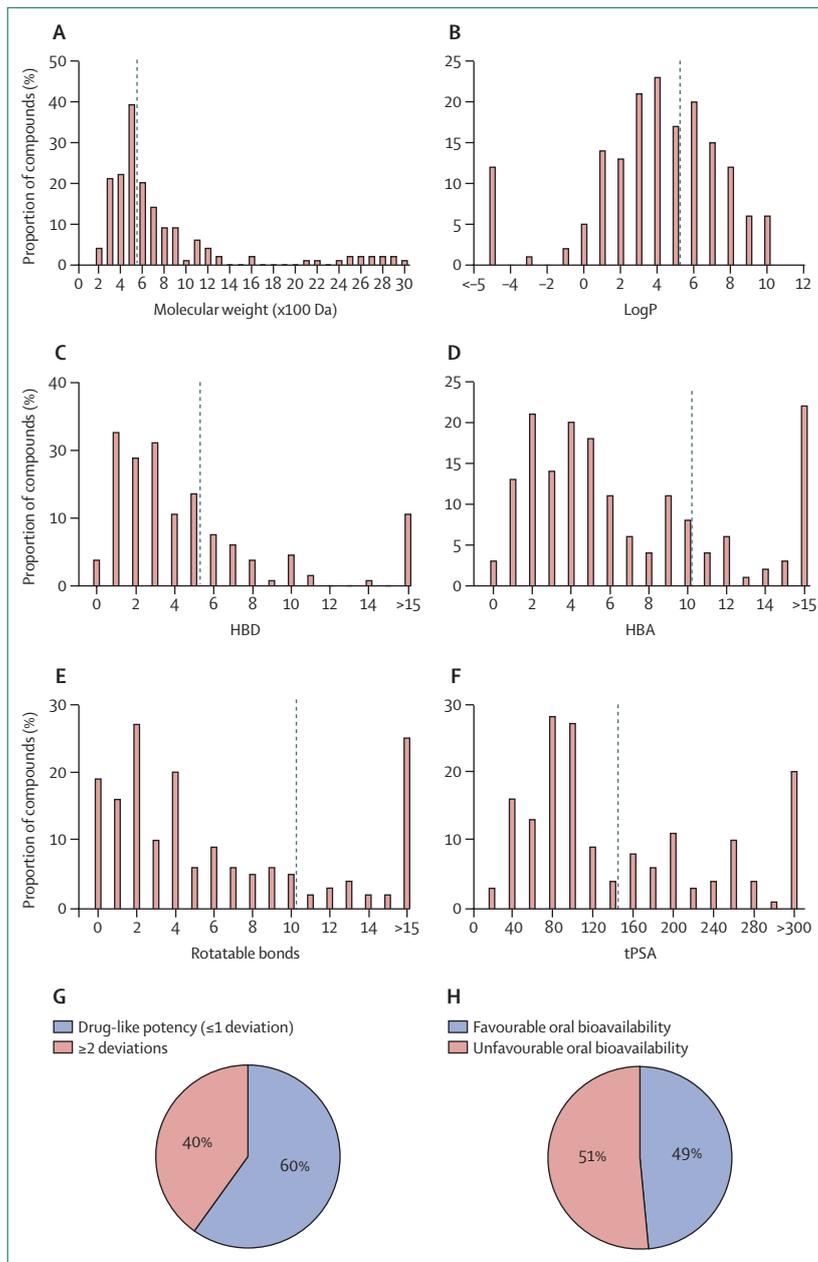


Figure 4: Distributions of Lipinski descriptors and Veber descriptors for drug-likeness prediction
 Analysis of physicochemical properties for antibacterial marine natural product by: (A) molecular weight, (B) calculated logP, (C) HBD, (D) HBA, (E) number of rotatable bonds, (F) tPSA, (G) compliance with Lipinski's rule of five, and (H) overall oral bioavailability prediction. The green line indicates the maximum desirable value for oral bioavailability defined by Lipinski's rule of five: molecular weight ≤ 500 Da, $\log P \leq 5$, HBD ≤ 5 , and HBA ≤ 10 . The blue area is the Lipinski-compliant region with none or one deviation, and the red area is the Lipinski-non-compliant region (≥ 2 deviations). HBD=hydrogen bond donors. HBA=hydrogen bond acceptors. tPSA=topological polar surface area.

See Online for appendix

extremely potent activity against a panel of Gram-positive pathogenic bacteria, including MRSA strains, vancomycin-resistant *E faecium*, and vancomycin-resistant *E faecalis*, at concentrations starting from single digit nanomolar concentrations, with MIC values from 2.6 nM up to 1.4 μM .²⁵ In-vivo studies of PM181104

against MRSA and vancomycin-resistant *E faecalis* infections in a BALB/c murine septicemia model, showed antibacterial activities with 100% effective dose of 2.5 mg/kg and 5 mg/kg against MRSA, and 10 mg/kg against vancomycin-resistant enterococci.²⁵

The polycyclic compound citreaglycon A was identified from a marine-derived *Streptomyces caelestis* obtained from the coastal water of the Red Sea (figure 3). Citreaglycon A inhibited the growth of MRSA (strain ATCC43300) with a MIC value of 15.4 μM . Unlike the other two antibacterial compounds isolated from the same marine organism, citreaglycon A had no in-vitro cytotoxicity against HeLa cells at the tested concentrations ($\text{IC}_{50} > 77 \mu\text{M}$).²⁶

Bioassay-guided fractionation of the extract of *Pseudomonas* spp strain AMSN isolated from the surface of the marine red alga *Ceratodictyon spongiosum* led to the isolation of the anti-MRSA compound 2,4-diacetylphloroglucinol (figure 3). Evaluation of the antibacterial activity of 2,4-diacetylphloroglucinol showed high anti-MRSA activity with MICs of 0.3–1.0 $\mu\text{g/mL}$ (1.2–4.8 μM) against ten clinical isolates of MRSA.²⁷ Bacteriolytic assay experiments showed that 2,4-diacetylphloroglucinol lysed both the Gram-positive bacterium MRSA and the Gram-negative bacterium *Vibrio parahaemolyticus*. 2,4-diacetylphloroglucinol was not toxic to mice at doses up to 100 mg/kg.^{27,28}

A new peptide, hedistin, with molecular weight of 2521 Da, was identified from the marine annelid *Nereis diversicolor* and exhibited promising antibacterial activities against different Gram-positive bacteria, including MRSA with MIC values of 3–6 μM and minimal bactericidal concentration of 12 μM , with no cytotoxicity against *Nereis* spp coelomocytes.²⁹ Additional MNPs with activity against drug-resistant bacterial infections are listed in the appendix.

Analysis of drug-like physicochemical properties

The drug-likeness of any compound is associated with its acceptable aqueous solubility and intestinal permeability. By calculating the physicochemical properties, it is possible to predict the oral bioavailability of each compound. However, some compounds with structural features that allow the drugs to act as substrates for naturally occurring transporters should be excluded from the rule.³⁰ Lipinski's so-called rule of five considers orally active compounds and defines four simple physicochemical parameter ranges (molecular weight ≤ 500 , $\log P \leq 5$, hydrogen bond donor [HBD] ≤ 5 , and hydrogen bond acceptor [HBA] ≤ 10) associated with 90% of orally active drugs that have achieved phase 2 clinical status.³⁰ If a compound does not meet rule of five requirements, there is a high probability that oral activity problems will be encountered. However, meeting the rule of five requirements is no guarantee that a compound is drug-like.³¹ Topological polar surface area (tPSA) is an additional descriptor that has been shown to correlate

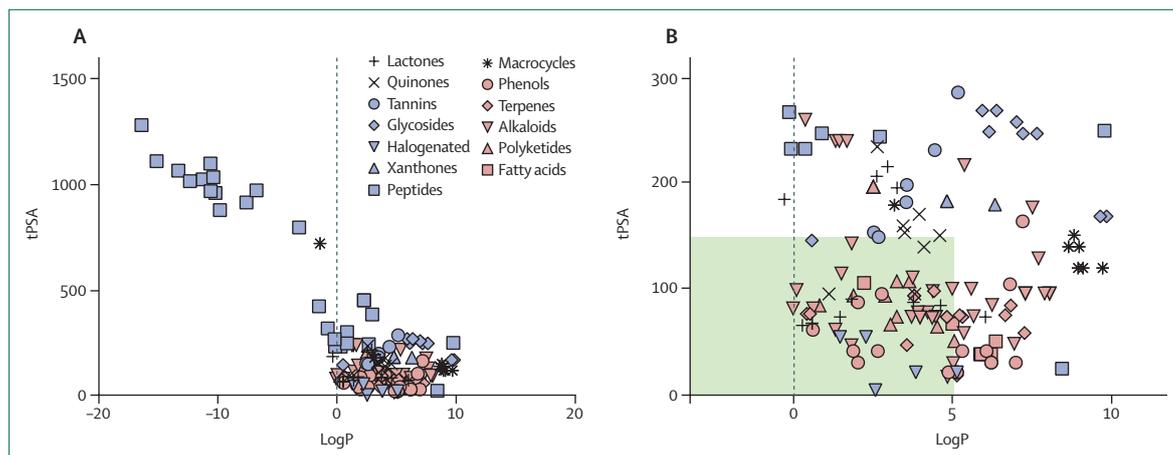


Figure 5: Distributions of tPSA values plotted against logP

(A) The whole distribution plot of different major classes of antibacterial compounds. (B) A partial plot of major classes of antibacterial compounds with the logP values between -3 and 12 and tPSA values between 0 to 300. The green area represents favourable oral bioavailability (logP < 5 and tPSA < 140).

with membrane transport, and therefore allows prediction of transport properties of drugs. It was shown that tPSA of $140 \times 10^{-10} \text{m}$ or less and number of rotatable bonds of ten or fewer (ie, Veber's oral bioavailability rule) is an efficient and selective criterion for a drug-like molecule.³² The physicochemical properties (molecular weight, logP, HBD, HBA, rotatable bond, and tPSA) of the 167 MNP compounds in this Review were calculated by Instant JChem version 1710.0 from ChemAxon Ltd (Budapest, Hungary) of the molecules and projected onto a drug-like cut-off threshold of Lipinski's rules (figure 4) and Veber's oral bioavailability rule (figure 5).

The molecular weight profile of the 167 MNP showed a wide range from 124.14 Da to 2955.62 Da. Most compounds are distributed between 400 Da and 500 Da (figure 4A). Among the higher molecular weight compounds, clusters were observed around 600 Da and 700 Da, exemplified by MC21-A with a molecular weight of 501.79 Da and engyodontochone A with a molecular weight of 616.58 Da. However, about 49% of the 167 compounds had a molecular weight greater than 500 Da, 8% of which were greater than 2000 Da. The logP histogram of the 167 MNP followed a normal distribution with the maximum around 4, and 65% of the compounds had a favourable logP value less than 5 (figure 4B). Notably, compounds with extremely large molecular weights were also found to show very low logP values (-1.37 to -16.35), in agreement with the observation that the marketed antibacterial drugs have a more polar distribution, with a lower median calculated logP.³³ The distribution of the calculated HBD (figure 4C) and HBA (figure 4D) showed a similar pattern within the range of HBD of 5 or less and HBA of 10 or less. Of 167 compounds, 72% were within the Lipinski-compliant HBD and 77% within the HBA regions. Overall, 57 of the 167 compounds had no deviation to Lipinski's rule of five, and 43 compounds had one

deviation. Around 60% of the 167 compounds showed drug-like potential, indicating they could be administered orally (figure 4G). A comparison of antibacterial and non-antibacterial compounds has shown that the physicochemical properties required for antibacterial activity might lie in a different region of property space (ie, key properties that should be considered for compounds to have oral bioavailability), which were on average large molecular weight and polar distribution.^{33,34} Taking rotatable bonds and polar surface area into account, 80 compounds were in the favourable oral bioavailability region (figures 4E, 4F, 4H). The four antibiotic polyketides (JBIR-97/98, engyodontochone A, JBIR-99, and engyodontochone B) with one Lipinski deviation were predicted to have unfavourable oral bioavailability because of their high tPSA values. The plot of logP versus tPSA for major compound classes provides a deeper insight into requirements for antibacterial compounds (figure 5A and 5B). As previously mentioned, the peptide compounds populate the upper left region with a higher polarity and tPSA. Notably, most of the polyketide compounds, except for the engyodontochones, fall within the oral bioavailability space defined by Lipinski's rules and Veber descriptors. This result shows a similar pattern to the analysis by Boucher and colleagues,³⁵ that Gram-positive antibacterials have a lower limit for polarity with an increase of molecular weight.

Conclusion and outlook

Growing resistance among Gram-positive and Gram-negative pathogens, especially the so-called ESKAPE pathogens (*E faecium*, *S aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) that cause substantial morbidity and mortality in hospitals and communities continues to threaten global health care.^{36,37} Compared with human beings, the high adaptability, genetic plasticity, and rapid

Search strategy and selection criteria

We searched PubMed and Google Scholar for publications in English published without a start date restriction and up to June, 2016, with the terms “drug-resistance”, “marine natural products”, and “marine natural products against drug-resistant bacteria”. Cross-referencing and related articles were used to expand the search criteria. Searches were further supplemented with publicly available information and reports from US Centers for Disease Control and Prevention and WHO websites.

replication ability allows microbes to inhabit almost all possible environments.^{38,39} This power is the primary factor that drives the development of antibiotic resistance. Moreover, genetic analysis of microbial metabolic pathways indicates that microbes developed both β -lactam antibiotics and β -lactamase enzymes against those antibiotics more than 2 billion years ago, which means microbes have had experience creating and defeating antibiotics for 20 million times longer than human beings have known that antibiotics exist.^{40,41} Control of unnecessary use of antibiotics is still important, not because it is a solution to drug resistance, but because it will limit selective pressure on bacteria and affect the rate of spread of bacterial resistance.⁴²

Meanwhile, despite advances made in the technology and basic knowledge of many infectious diseases, a decline in the discovery and development of new antibiotics to deal with this problem has occurred. The cause of the decline of antibiotic development is complicated. First, the high cost required for developing drugs has been and still is one of the most important factors. In 2013, development costs were estimated to be around US\$1.395 billion per approved agent.⁴³ Second, the need to limit the use of new, broad-spectrum antimicrobials to avoid the spread of drug resistance has caused increasing challenges to drug development as they bring less financial return than other drugs. Finally, the increasing difficulties in the discovery of new lead compounds largely influences the pace in the fight against drug resistant infections.

Natural products have continued to enter clinical trials or to provide lead compounds, especially as anticancer and antimicrobial agents.^{44–46} However, difficulties associated with the high rates of repeated isolation of known compounds represents a major bottleneck in natural product drug discovery. MNPs display a large range of chemical structures and biological activities for the discovery of leads for the development of drugs to treat various human diseases. Since 2008, more than 1000 new compounds have been discovered each year.⁴⁷ Because of the unique marine environment, the novel structures, biosynthetic pathways, and biological activities, these compounds are a promising source for drug development. Among all the MNPs in this Review, marine microbes

(particularly marine actinomycetes and fungi) produce the most abundant MNPs that are active against drug-resistant bacteria. Exploitation of these metabolically rich phyla with specific approaches could increase the rate of drug discovery. Specifically, compared with drugs from other sources, the common supply problem for large-scale production from these compounds can be overcome with large-scale fermentation procedures. Another advantage of MNPs as potential drug candidates is that most of them have the property of drug-likeness. Such biologically active compounds with high degrees of bioavailability have high potential to be developed as effective drugs against infectious diseases in the near future.

Contributors

ML and EME initiated the Review. URA, ML, TAO, and EME developed the scope of the manuscript. EME and ML did the literature search and prepared the first draft. URA, ML, and EME designed the figures and interpreted the findings. URA, TAO, MSD, and RJQ reviewed the data and draft. All authors subsequently modified the manuscript jointly. URA is the guarantor of the final version, which was read and approved by all the authors.

Declaration of Interests

We declare no competing interests.

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