Antimicrobial natural products: an update on future antibiotic drug candidates

Muhammad Saleem,*[a] Mamona Nazir,[a] Muhammad Shaiq Ali,[b] Hidayat Hussain,[c] Yong Sup Lee,[d] Naheed Riaz[a] and Abdul Jabbar[a]

Received 13th August 2009
First published as an Advance Article on the web 25th November 2009
DOI: 10.1039/b916096e

Covering: 2000 to 2008

Over the last decade, it has become clear that antimicrobial drugs are losing their effectiveness due to the evolution of pathogen resistance. There is therefore a continuing need to search for new antibiotics, especially as new drugs only rarely reach the market. Natural products are both fundamental sources of new chemical diversity and integral components of today's pharmaceutical compendium, and the aim of this review is to explore and highlight the diverse natural products that have potential to lead to more effective and less toxic antimicrobial drugs. Although more than 300 natural metabolites with antimicrobial activity have been reported in the period 2000–2008, this review will describe only those with potentially useful antimicrobial activity, *viz.* with MICs in the range 0.02–10 μg mL^{-1}. A total of 145 compounds from 13 structural classes are discussed, and over 100 references are cited.

1 Introduction

Antibiotics were considered to be 'miracle drugs' when they first became available half a century ago, but their popularity rapidly led to overuse. Over the last decade, it has become clear that antibiotics are losing their effectiveness as pathogens evolve resistance against them, a problem compounded by the fact that new drugs only rarely reach the market. Moreover, bacteria can acquire drug resistance in a multitude of ways, so getting around the resistance problem is not a straightforward matter. To address these issues, pharmaceutical companies have recently revived efforts to develop new antibiotics,[1] which is now a matter of urgency due to the appearance of antibiotic-resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA).

Natural products are both a fundamental source of new chemical diversity and an integral component of today's pharmaceutical compendium. However, many currently available antifungal and antibacterial agents have undesirable toxicity, and the widespread use of these drugs has led to rapid development of drug-resistant strains, which are the leading cause of failure in both clinical and agricultural applications.

Several thousand microbial products have so far been discovered, and many of these with potential for medicinal use await investigation.[2] The aim of this review is not only to list the natural molecules that are future antimicrobial candidates, but also to explore the diverse sources that may provide more effective and less toxic antimicrobial compounds. This review covers the literature between 2000 and 2008, and covers only those metabolites that have potentially useful antimicrobial activity, namely those with minimum inhibitory concentrations (MICs) in the range 0.02–10 μg mL^{-1}. These compounds, which total 145 from 13 structural classes, are arranged according to class and then source.

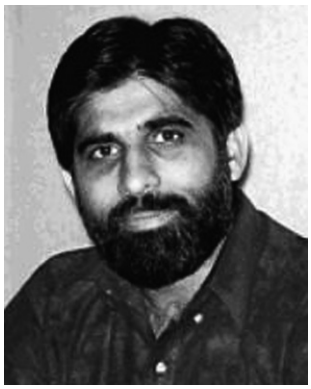
2 Alkaloids

Probably the first medicinally important alkaloid reported was morphine isolated from *Papver somniferum*. Relatives of many

[a]*Department of Chemistry, Baghdad-ul-Jadeed Campus, The Islamia University of Bahawalpur, 63000 Bahawalpur, Pakistan*
[b]*H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, 75270 Karachi, Pakistan*
[c]*Department of Chemistry, Universität Paderborn, Warburger Str. 100, 33098 Paderborn, Germany*
[d]*Department of Pharmaceutical Science, College of Pharmacy, Kyung-Hee University, Dongdaemun-Ku, 1, Hoegi-Dong, Seoul 130-701, Korea*

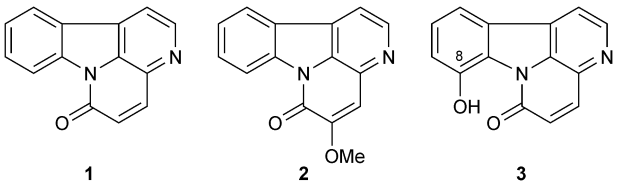
plant families are known to produce antimicrobial alkaloids,[3] and several alkaloids from natural sources are reported to possess potent antimicrobial properties and could therefore be a good substitute for existing drugs.

Canthin-6-one (**1**) is known from *Allium neapolitanum* and *Zanthoxylum chiloperone* var. *angustifolium*, and exhibited a broad spectrum of activities against *Aspergillus fumigatus*, *A. niger*, *A. terreus*, *Candida albicans*, *C. tropicalis*, *C. glabrata*, *Cryptococcus neoformans*, *Geotrichum candidum*, *Saccharomyces cerevisiae*, *Trichosporon beigelii*, *Trichosporon cutaneum* and *Trichophyton mentagrophytes* var. *interdigitale*, with MIC values between 1.66 and 10.12 μg mL^{-1}. 5-Methoxycanthin-6-one (**2**) was active against only *T. mentagrophytes* var. *interdigitale*, with an MIC value of 3.075 μg mL^{-1}.[4] In addition, compound **1** and 8-hydroxycanthin-6-one (**3**) from *Allium neapolitanum* inhibited *Mycobacterium smegmatis* ATCC 14468, *M. smegmatis* mc22700, *M. phlei* ATCC 11758, *Staphylococcus aureus* 1199B and *S. aureus* XU212 (all with an MIC of 8.0 μg mL^{-1}), whereas compound **3** was more active against *M. smegmatis* mc22700, with an MIC of 2.0 μg mL^{-1}.[5] The activity of **3** was greater against *M. smegmatis* mc22700 than *M. smegmatis* ATCC 14468, suggesting that the C-8 hydroxyl substituent may play a role in the inhibition of fatty acid synthetase I (FASI). Sephadex LH-20 was utilized for isolation of these compounds and structures were elucidated by 1D and 2D NMR techniques.



Muhammad Saleem

Muhammad Saleem graduated from The Islamia University of Bahawalpur, Pakistan, in 1996, and received his Ph.D. in 2001 from the HEJ Research Institute of Chemistry, University of Karachi, Pakistan. During his Ph.D., he worked on secondary metabolites of higher plants and marine algae under the supervision of Prof. Muhammad Shaiq Ali. He then joined the Pakistan Council of Scientific and Industrial Research Laboratories (PCSIR) Karachi and worked on applied pharmaceutical and cosmeceutical aspects of natural products. In 2003, Dr. Saleem carried out postdoctoral research at the Korea Institute of Science and Technology, Seoul, with Professor Yong Sup Lee. In 2004 he was appointed a visiting scientist at the same institute and worked on the development of new drug candidates from natural products. In 2005, he joined the Institute of Organic and Biomolecular Chemistry, University of Göttingen, Germany, where he searched for new drug candidates from microbial sources under the guidance of Prof. Dr. H. Laatsch. He returned to the Chemistry Department of the Islamia University of Bahawalpur in 2007, where is currently an Assistant Professor. His research interests are the development of new drug candidates, including novel antibiotics, anti-cancer compounds, neuroprotectants and anti-virals, from natural products.



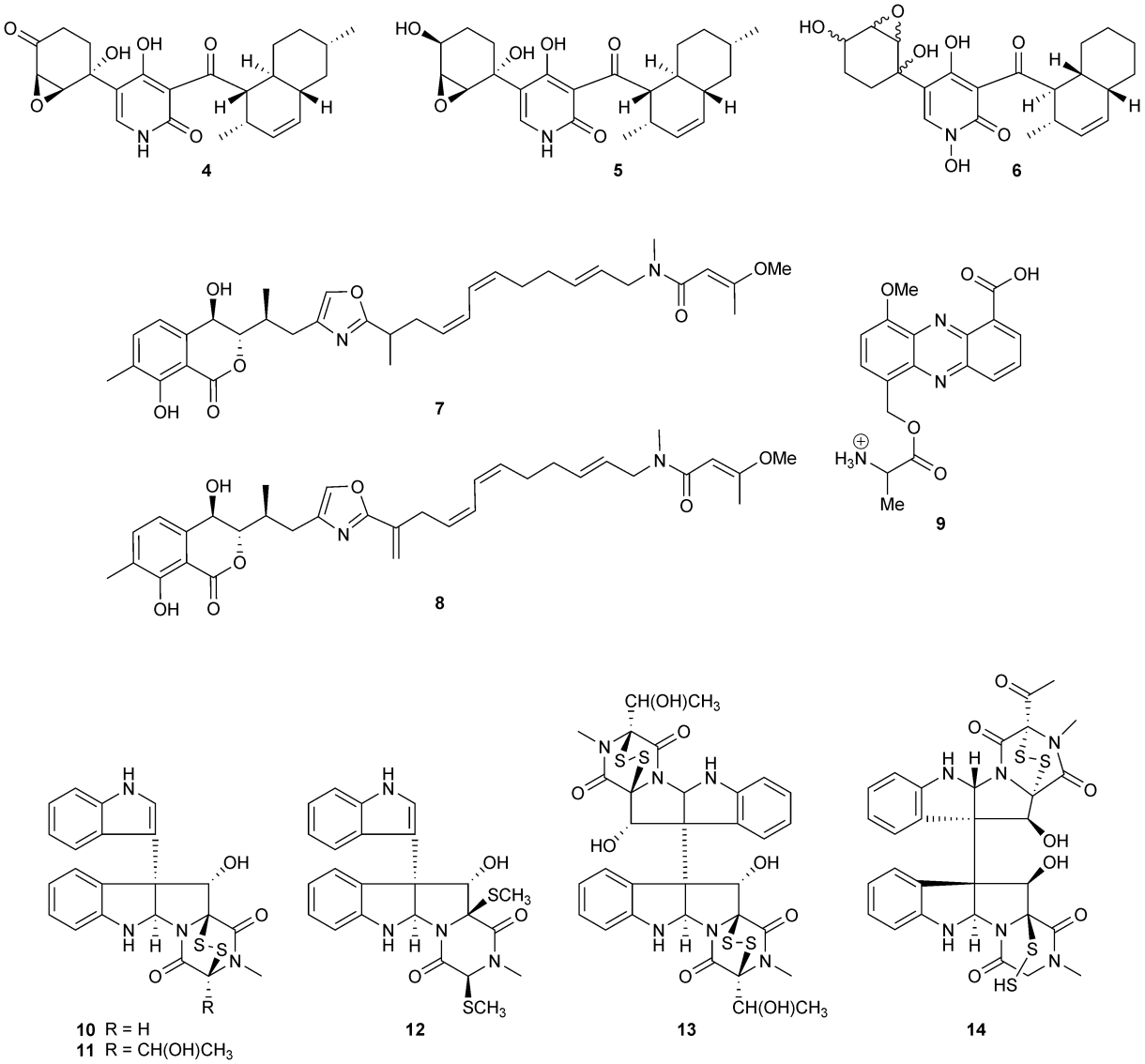
1 **2** **3**

In an experiment to determine the mechanism of action of canthin-6-one, Lagoutte *et al.* observed the accumulation of the derivatives of these compounds within lipid droplets, and that the relative amount of unsaturated alkyl chain fatty acids is enhanced, which they propose is due to the stimulation of desaturase enzyme systems.[6]

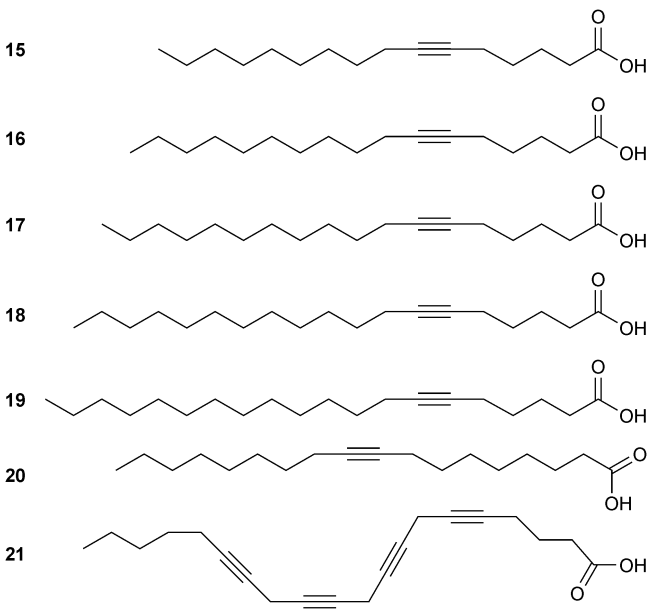
A cytotoxic compound, YM-215343 (**4**), found in the bacterial culture of *Phoma* sp. exhibited antifungal activity against the pathogenic fungi *C. albicans*, *C. neoformans* and *A. fumigatus*, with MIC values of 2–16 μg mL^{-1}. The structure of **4** was determined by several spectroscopic experiments, and found to be closely related to apiosporamide (**5**) and fischerin (**6**).[7] The mode of action of this compound has not been studied. Two alkaloids with an oxazole moiety, ajudazols A (**7**) and B (**8**), were identified as antimicrobial agents from *Chondromyces crocatus*. Both showed their potential against *Micrococcus luteus* (MIC 12.5 μg mL^{-1}). Ajudazol A (**7**) also showed minor activity against a few fungi and Gram-positive bacteria. It is suggested that these compounds block the electron flow in submitochondial particles (SMPs).[8] It is well known that the oxazole nucleus is rarely found in nature, but several simple substituted oxazoles have been isolated from plants and/or synthesized that exhibit antimicrobial activities.[9]

Phenazine antibiotics are synthesized by a number of bacteria from diverse genera including *Streptomyces*, *Pseudomonas*, *Pelagiobacter* and *Vibrio*.[10–12] These microbes produce a range of phenazine compounds that differ widely in antibiotic properties, according to the nature and position of side groups attached to the phenazine nucleus.[13] For example D-alanylgriseoluteic acid (AGA, **9**) is a potent antimicrobial phenazine compound produced by *Pantoea agglomerans* (*Erwinia herbicola*) Eh1087 and isolated from the culture supernatant.[14] Susceptibility tests against a range of microbes indicated that **9** exhibited a broad spectrum of antimicrobial activity, particularly against Gram-positive pathogens (many pneumococcal and multi-drug resistant isolates), with MICs of 0.06–0.75 μg mL^{-1}). It was further established that **9** induced an SOS response in *Escherichia coli* and slightly increased the frequency of GC–AT transition mutations. The potency and broad-spectrum activity of **9** warrant further investigation, and in future it could have an application as a topical agent.[15]

Zheng *et al.* isolated five epidithiodioxopiperazines, bionectins A (**10**), B (**11**) and C (**12**) and verticillins D (**13**) and G (**14**), from the mycelium of liquid fermentation cultures of the fungus *Bionectra byssicola* F120. Compounds **10**, **11** and **13** exhibited antibacterial activity against *S. aureus* including methicillin-resistant *S. aureus* (MRSA) and quinolone-resistant *S. aureus* (QRSA), with MIC values of 10–30 μg mL^{-1}, while **12** showed no antibacterial activity even at 100 μg mL^{-1}.[16] Verticillin G (**14**) was the most active and inhibited the growth of *S. aureus* including methicillin-resistant and quinolone-resistant *S. aureus*, with an MIC of 3–10 μg mL^{-1}.[17] Piperazines and substituted



piperazines are important pharmacophores that can be found in many marketed drugs. Piperazinyl-linked ciprofloxacin dimers have been reported as potent antibacterial agents against resistant strains.[18] Structurally, **10**, **11**, and **13** incorporate a disulfide bridge in their dioxopiperazine ring, **12** contains a dioxopiperazine ring with two methylsulfanyl groups, while **14** contains disulfide bonding. The difference in activity level and structural features suggest that disulfide bonds are important for the antibacterial activity of these compounds.[19–22] Further, Richard *et al.* reported the mode of action of such kind of compounds with disulfide bridges, and stated that this may be due to expression of certain RNA polymerase subunits and membrane permeabilization.[23]



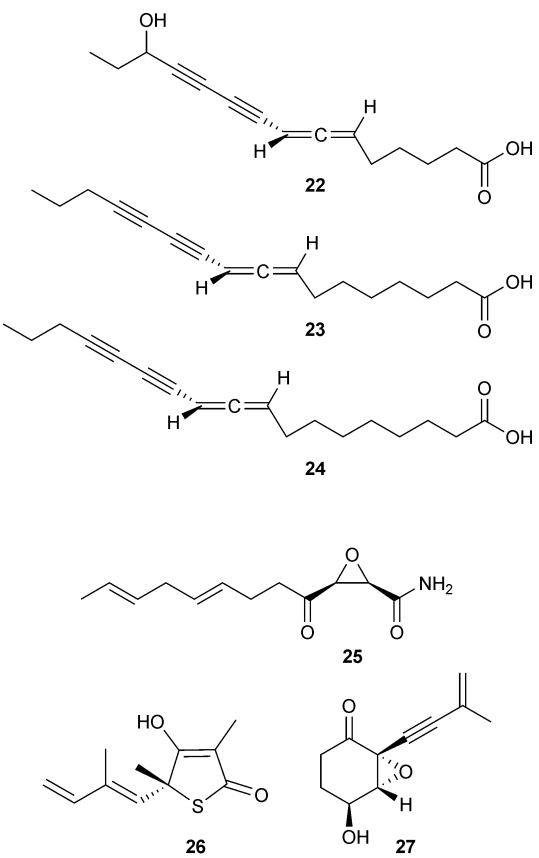
3 Acetylenes

The antifungal and antimicrobial properties of fatty acids have been known for centuries. Compared to saturated fatty acids, unsaturated fatty acids with double and/or triple bonds are, in general, more potent against fungal pathogens.[24] Indeed, the

archetypal unsaturated fatty acid with a single double bond at C-10, undecylenic acid (UDA), is still on the market as a cost-effective antifungal agent, and is the active ingredient of many topical over-the-counter antifungal preparations.[25]

Five 6-acetylenic acids (**15–19**) purified by reverse-phase HPLC were identified as potential antimicrobial chemicals, their tructures being established by LC–MS, NMR and HPLC–ESI–MS studies. Compounds **15–18** showed MICs of 1.108–7.812, 1.170–7.793, 0.588–1.568 and 0.205–0.972 μg mL^{-1}, respectively, while compound **19** was not active. Reference compounds **20** and **21** also failed to show significant activity. Compound **18** was the most active, in particular against the dermatophytes *Trichophyton mentagrophytes* and *T. rubrum* and the opportunistic pathogens *Candida albicans* and *Aspergillus fumigatus*, with MICs comparable to several control drugs.[26] *In vitro* toxicity testing against mammalian cell lines indicated that none of the isolates was toxic at concentrations up to 32 μM. Taking into account the low *in vitro* and *in vivo* toxicities and significant antifungal potencies, these 6-acetylenic acids (**15–21**) may be excellent leads for further preclinical studies.[26]

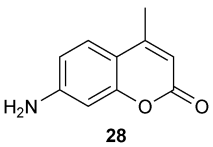
Three other acetylenic acids, phomallenic acids A–C (**22–24**), isolated from a leaf-litter fungus of the *Phoma* genus, exhibited MICs in the range 3.9–7.8 μg mL^{-1} against wild-type *S. aureus*. Phomallenic acid C (**24**), the analogue with the longest chain, exhibited the best overall activity, and was superior to cerulenin (**25**) and thiolactomycin (**26**), the two most studied and commonly used FabF inhibitors.[27] *In vitro* antifungal testing further demonstrated that the antifungal activities of the acetylenic acids were associated with their chain lengths and position of the triple bonds.[26] Another acetylenic compound (**27**) was obtained from a sterile dark ectotrophic fungus isolated from roots of an Australian native grass, *Neurachne alopecuroidea*. The structure of **27** was determined by spectroscopic and X-ray diffraction studies, and it was found to completely inhibit the growth of *Phytophthora cinnamomi* and *Gaeumannomyces graminis* var. *tritici* (Ggt) at 0.98 μg mL^{-1}, *Rhizoctonia solani* at 7.81 μg mL^{-1}, and *Pythium irregulare* and *Alternaria alternata* at 15.63 μg mL^{-1}.[28]



Although the mode of actions of these compounds has not been studied yet, Kenny *et al.* have discussed the mode of action for long-chain unsaturated fatty acids on *S. aureus*, stating that "they involve disruption of the cell membrane, leading to interference with energy production within the bacterial cell".[29] It is important to note, however, that these studies were performed on olefinic fatty acids, not acetylenic fatty acids.

4 Coumarins

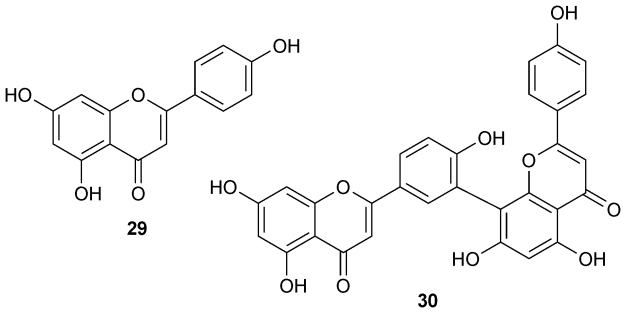
Coumarins are mostly plant metabolites and are widely distributed in members of the Rutaceae family. Very few of the small number from microbial sources have any biological properties. However, an amino-coumarin, 7-amino-4-methylcoumarin (**28**), was found to be a constituent of the culture extracts of an endophytic *Xylaria* fungal species isolated from *Ginkgo biloba*. Compound **28** afforded broad-spectrum antibacterial and antifungal activities *in vitro* against *Staphylococcus aureus*, *Escherichia coli* (MICs both 10 μg mL^{-1}), *Salmonella typhimurium* (MIC 15 μg mL^{-1}), *Salmonella enteritidis* (MIC 8.5 μg mL^{-1}), *Aeromonas hydrophila* (MIC 4 μg mL^{-1}), *Yersinia* sp. (MIC 12.5 μg mL^{-1}), *Shigella* sp. (MIC 6.3 μg mL^{-1}), *Vibrio parahaemolyticus* (MIC 12.5 μg mL^{-1}) and *Candida albicans* (MIC 15 μg mL^{-1}). These results provide promising baseline information for the potential use of this unusual endophytic fungus and its components in the control of food spoilage and food-borne diseases.[30]

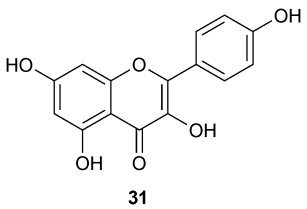


5 Flavonoids and isoflavonoids

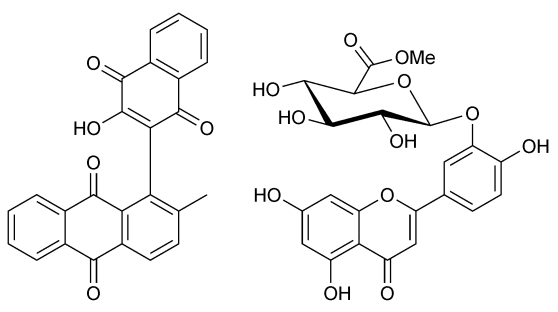
Flavonoids are one of the biggest classes of secondary metabolites, and are distributed in various plant species. They protect the plants from UV radiation and other environmental stresses, and significant antioxidant properties are associated with them. They have also been identified as potent antimicrobial agents. Apigenin (**29**), a common phytochemical, has been identified from *Scutellaria barbata* (Lamiaceae), and exhibited a potent activity (MIC 3.9–15.6 μg ml^{-1}) against 20 strains of MRSA.[31] A dimeric variant of **29**, amentoflavone (**30**) from *Selaginella tamariscina*, exhibited good inhibitory activity (MIC 5 μg mL^{-1}) against the fungal pathogens *C. albicans*, *S. cerevisiae* and *T. beigelii*, indicating its broad-spectrum antibiotic potential.[32] Another common phytochemical, kaempferol (**31**), isolated from the methanolic extract of *Vismia laurentii*, inhibited two Gram-negative and four Gram-positive pathogens, all with an MIC of

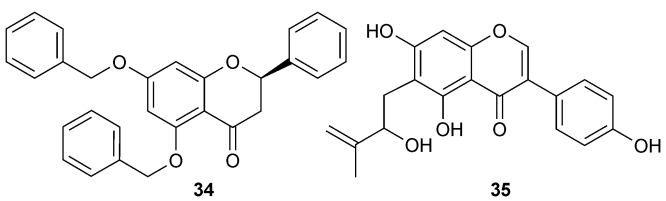
2.4 μg mL^{-1}, and showed activity against *Candida glabrata* (MIC 4.8–9.7 μg mL^{-1}).[33]





The methanolic extract from the root bark of *Newbouldia laevis* produced chrysoeriol (**32**), which has broad-spectrum *in vitro* activity against four Gram-positive and ten Gram-negative bacterial species, as well as three *Candida* species (MIC 1.2–9.76 μg mL^{-1}).[34] Flavone glycoside **33**, from the leaves of *Vitex negundo*, is also known as an antimicrobial agent. Compound **33** showed promising activity against *Trichophyton mentagrophytes* and *C. neoformans* (MICs both 6.25 μg mL^{-1}) when compared to the standard antifungal drug fluconazole (MIC 2.0 μg mL^{-1}).[35] A dibenzyloxyflavone **34** from *Helichrysum gymnocomum* was found to have high activity against *Cryptococcus neoformans* ATCC 90112, with an MIC of 7.8 μg mL^{-1}.[36] Laburnetin (**35**), isolated from the methanolic extract of *Ficus chlamydocarpa*, exhibited potent inhibitory activity against *Mycobacterium smegmatis* and *M. tuberculosis*, with MIC values of 0.61 and 4.88 μg mL^{-1} respectively.[37]

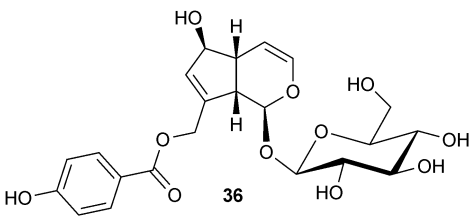


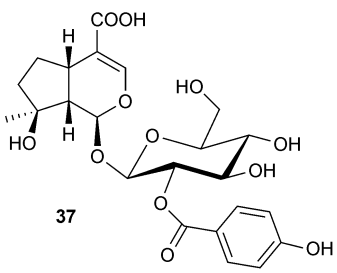


The efficacy of flavonoids against such a variety of pathogens can be attributed to cell-wall permeability and the porins in the outer membrane present in microorganisms – it seems likely that the compounds may block the charges of the amino acids in porins.[38] A comparison of the above-discussed properties reveals that the flavonoids having free hydroxyl groups at C-5 and C-7 in ring A are more active, a conclusion supported by studies of their synthetic analogues.[39] The activity of flavonoids may also be due to their ability to complex with extracellular and soluble proteins and then with bacterial cell walls. The same authors also suggest that the more lipophilic flavonoids may also disrupt microbial membranes.[40]

6 Iridoids

Iridoids are widely distributed in dicotyledonous plant families such as the Apocynaceae, Scrophulariaceae, Diervillaceae, Lamiaceae, Loganiaceae and Rubiaceae. They are known for their biological activities, and recent developments have revealed that they also possess antimicrobial properties.[41] The iridoid glycosides **36** and **37** were purified from an alcoholic extract of leaves of *Vitex negundo*; **37** showed promising activity against *Trichophyton mentagrophytes* and *Cryptococcus neoformans* (MIC for both 6.25 μg mL^{-1}) while **36** inhibited the growth of *C. neoformans* and *T. mentagrophytes* (MIC for both 12.5 μg mL^{-1}).[35]

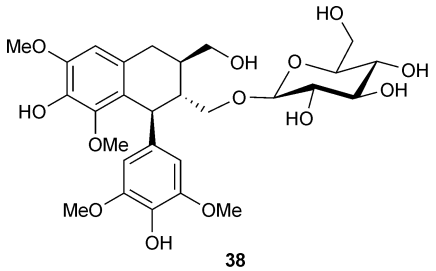




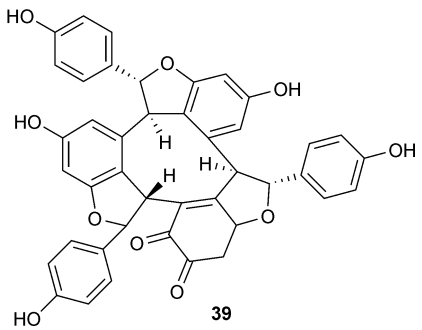
7 Lignans

A lignan, (+)-lyoniresinol-3α-*O*-β-D-glucopyranoside (**38**), isolated from the root bark of *Lycium chinense*, exhibited potent antimicrobial activity against *Staphylococcus aureus* (MIC 2.5–5.0 μg mL^{-1}), and three human-pathogenic fungi, *Candida albicans*, *Saccharomyces cerevisiae* and *Trichosporon beigelii* (MICs 5.0, 5.0 and 10.0 μg mL^{-1}, respectively), without having any hemolytic effect on human erythrocytes. It induced the accumulation of intracellular trehalose on *C. albicans* as stress response to the drug, and disrupted the dimorphic transition that forms pseudo-hyphae caused by the pathogenesis, indicating **38** to be a potential lead compound for the development of antibiotic agents.[42] Another lignan, a resveratrol trimer with an *ortho*-quinone nucleus, hopeanolin (**39**), was isolated and

characterized from the stem bark of *Hopea exalata*. Hopeanolin (**39**) demonstrated high antifungal activity, with MIC values of 1.56 μg mL^{-1} (*Alternaria solani*), 10.6 μg mL^{-1} (*Colletotrichum lagenarium*), 6.22 μg mL^{-1} (*Fusarium oxysporum*), 0.10 μg mL^{-1} (*Pyricularia oryzae*) and 1.55 μg mL^{-1} (*Valsa mali*).[43]

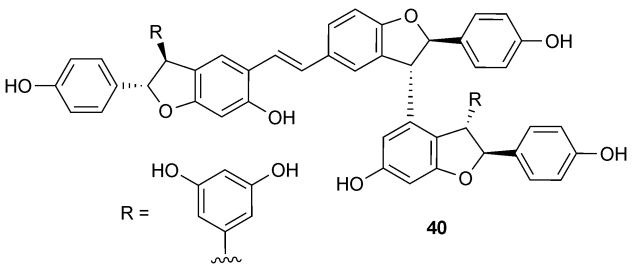


38



39

Heyneanol A (**40**), from the root extract of *Vitis* sp. (grape vine), was identified as antibacterial agent against Gram-positive pathogens. Using the disc diffusion method, it exhibited an MIC of 2.0 μg mL^{-1} towards MRSA and 2.0–4.0 μg mL^{-1} towards *Enterococcus faecium*, *S. aureus*, *Streptococcus agalactiae* and *Streptococcus pyogenes*.[44]

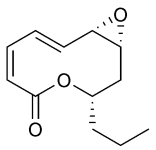


40

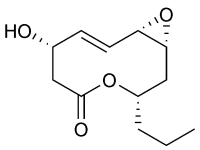
Phenolics usually inactivate microbial adhesins, enzymes, cell envelope transport proteins, *etc.* through non-specific forces like hydrogen bonding, covalent bonding and hydrophobic effects.[45,46] Therefore, antimicrobial lignans may also possess same mode of action.

8 Macrolides

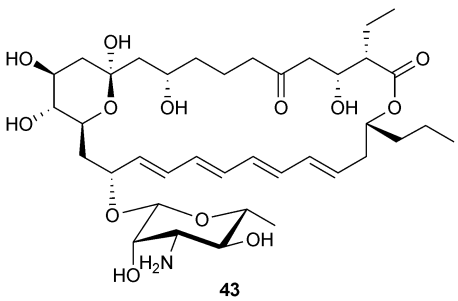
Macrolides are known to be bacterial constituents with significant antimicrobial activities. A number of macrolides have been identified and evaluated for their activities,[47] and in recent years work has been in progress to isolate such potential antimicrobials, mostly from microbial sources. Two 10-membered macrolides, phomolides A (**41**) and B (**42**), were purified from the culture of *Phomopsis* sp. hzla01-1. Both compounds showed significant antimicrobial activities against *Escherichia coli* CMCC44103, *Candida albicans* AS2.538 and *Saccharomyces cerevisiae* ATCC9763, with MICs in the range of 5–10 μg mL^{-1}. Furthermore, these compounds showed significant cytotoxicity against the HeLa cell line at 10 μg mL^{-1}.[48] The cytotoxic tetraene macrolide CE-108 (**43**), a secondary metabolite of *Streptomyces diastaticus* 108, also exhibited potent antimicrobial properties against *Aspergillus cadidus*, *Fusarium oxysporum*, *Aspergillus niger*, *Microscporum gypseum* and *Trichophyton mentagrophytes* (MICs 8.0 μg mL^{-1}), and against *Cryptococcus neoformans*, *Microsporum canis*, *Trichophyton rubrum* and *Trichophyton tonsurans* (MICs 16 μg mL^{-1}). This compound should therefore be a good candidate to develop a broad-spectrum antifungal drug.[49]



41



42

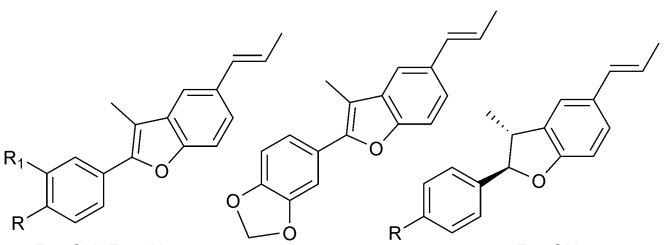


43

9 Phenolics (other than flavonoids and lignans)

The antimicrobial activity of plant phenolics has been intensively studied, and, in addition to controlling invasion and growth of plant pathogens, their activity against human pathogens has been investigated to characterize and develop new healthy food ingredients, medical compounds, and pharmaceuticals.[50–52]

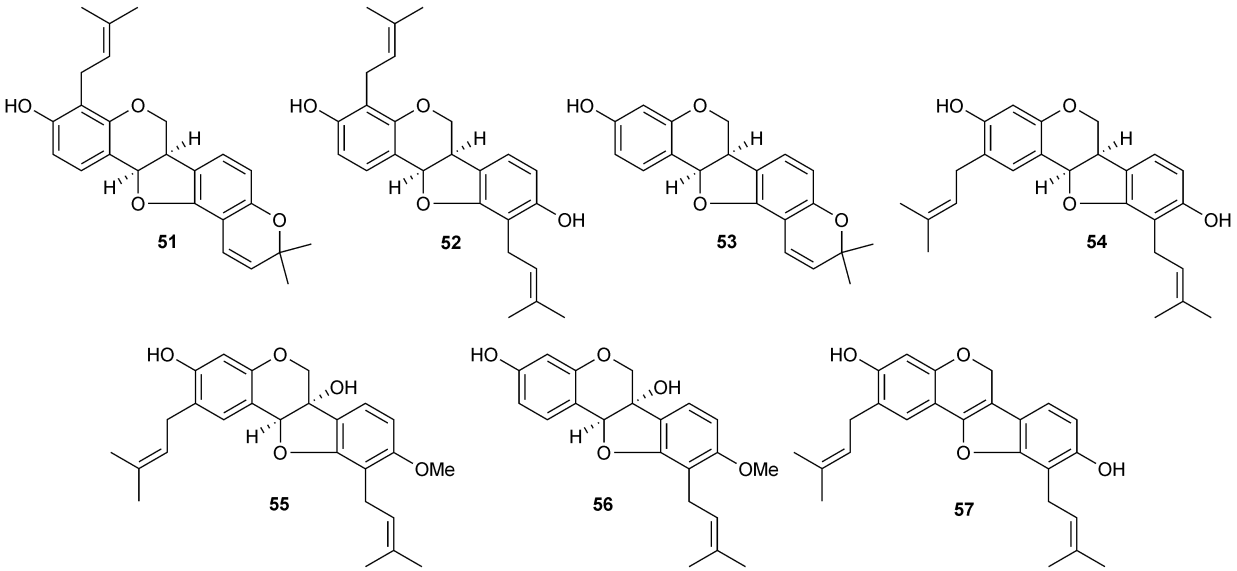
The leaves of *Piper regnellii* produced four phenolics, namely eupomatenoid-6 (**44**), eupomatenoid-5 (**45**), eupomatenoid-3 (**46**), and conocarpan (**47**). Compounds **44** and **45** showed good activity against *Staphylococcus aureus*, with an MIC of 1.56 μg mL^{-1} and 3.12 μg mL^{-1}, respectively. Both compounds had an MIC of 3.12 μg mL^{-1} against *Bacillus subtilis*, while **47** was quite active against *Staphylococcus aureus* and *B. subtilis*, with an MIC of 6.25 μg mL^{-1}.[53] Eupomatenoid-5 (**45**) showed strong activity towards *Trichophyton rubrum*, with an MIC of 6.2 μg mL^{-1}. These results suggest that the plant should be further examined for possible antifungal agents, and provides preliminary scientific



44 R = OH, R_1 = H
45 R = OH, R_1 = OMe
48 R = OMe, R_1 = H
49 R = OMe, R_1 = OMe

46

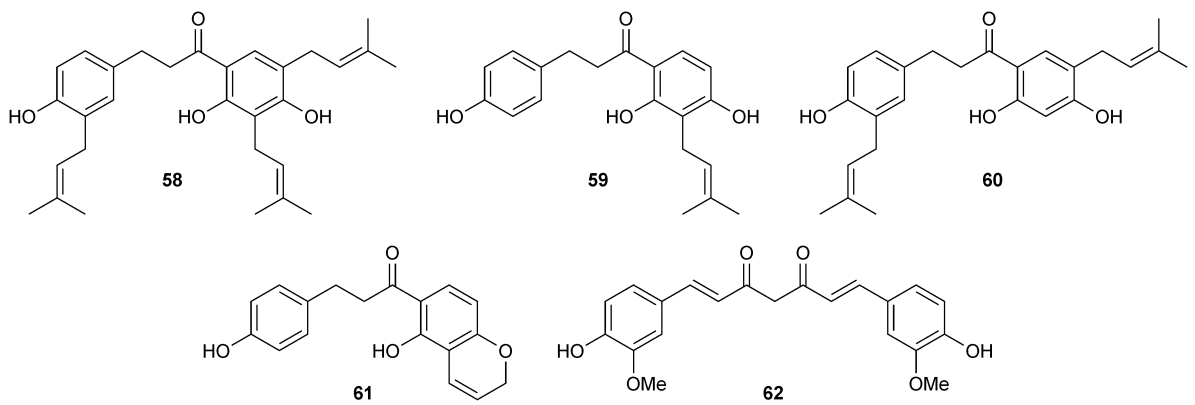
47 R = OH
50 R = OMe

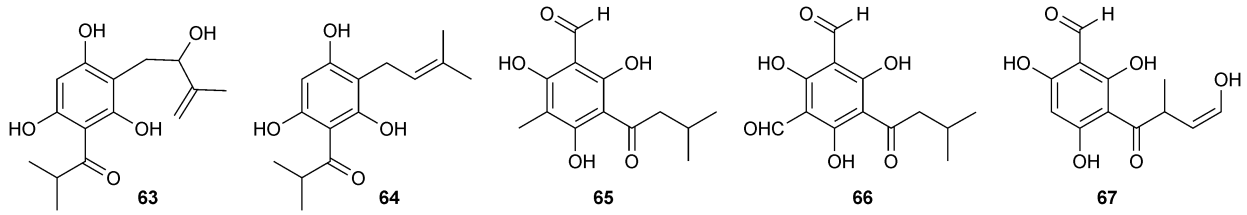


validation for the traditional medicinal use of this plant.[54] The low (or lack of) activity of **48–50** and structural comparison of **44–50** and their activity levels indicates that phenolic functions are important for the compound to be antimicrobial, as was observed for flavonoids.

Other antimicrobial phenolics include pterocarpans, erybraedin B (**51**), erybraedin A (**52**), phaseollin (**53**), erythrabyssin II (**54**), erystagallin A (**55**), erythrabissin-1 (**56**) and erycristagallin (**57**) isolated from the stems of *Erythrina subumbrans* (Leguminosae). Compounds **52** and **54** exhibited the highest degree of activity against *Streptococcus* strains, with an MIC range of 0.78–1.56 μg mL^{-1}, whereas **57** exhibited the highest degree of activity against *Staphylococcus* strains, including drug-resistant strains (MRSA and VRSA), with an MIC range of 0.39–1.56 μg mL^{-1}. Compounds **52**, **54**, **55** and **57** were also reported to be more active against several strains of *Streptococcus* and *Staphylococcus* than the standard antibiotics vancomycin and oxacillin.[55] Compound **57** showed the highest level of activity against *S. aureus* strains resistant to vancomycin and oxacillin, with an MIC range of 0.39–1.56 μg mL^{-1}. These compounds may prove to be potent phytochemical agents for antibacterial activity, especially against MRSA and VRSA.[55] The variation in activity and structural differences in these kinds of compounds shows that the dimethylallyl and phenolic units may play important role – for example, **51** and **53** have a pyran ring and are inactive.

Kanzonols are well-known phyto-phenolics possessing antimicrobial activities,[56] and kanzonol C (**58**) has significant biological activities. Kanzonol C **58** and its analogues, isobavachalcone (**59**), stipulin (**60**) and 4-hydroxylonchocarpin (**61**), were isolated from the extract of the twigs of *Dorstenia barteri*, and showed broad-spectrum inhibitory activities against both Gram-positive and Gram-negative bacteria and fungi, MIC values being determined using the micro-well dilution method. Compound **59** was highly active against *Enterobacter cloacae*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Bacillus stearothermophilus*, *Candida albicans* and *C. glabrata* (MICs 0.3 μg mL^{-1}), *Enterobacter aerogens*, *Morganella morganii*, *Shigella flexneri*, *Bacillus cereus*, *B. megaterium* and *B. subtilis* (MICs 0.6 μg mL^{-1}), *Proteus mirabilis*, *Proteus vulgaris*, *Microsporum audorium* and *Trichophyton rubrum* (MICs 1.2 μg mL^{-1}). Compound **61** inhibited growth of *E. cloacae*, *M. morganii*, *B. megaterium* and *B. stearothermophilus* (MICs 1.2 μg mL^{-1}), *E. aerogens*, *S. flexneri*, *S. faecalis*, *S. aureus*, *B. cereus*, *B. subtilis*, *C. albicans*, *C. glabrata* and *T. rubrum* (MICs 4.9 μg mL^{-1}) and *M. audorium* (MIC 9.8 μg mL^{-1}). Compound **58** showed



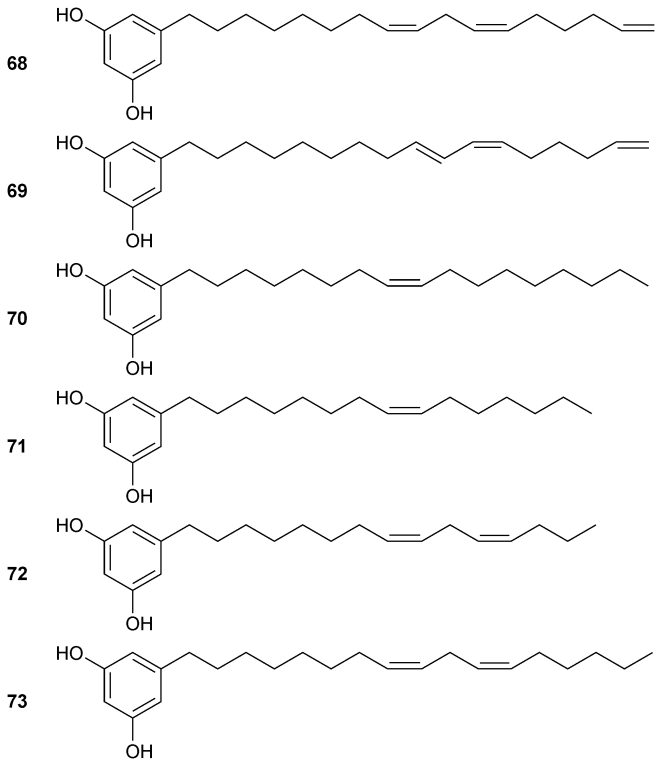


moderate activity against *E. aerogens*, *E. cloacae*, *M. morganii*, *S. flexneri*, *S. faecalis*, *B. megaterium*, *B. stearothermophilus*, *C. albicans* and *C. glabrata* (MICs 4.9 μg mL^{-1}), *P. mirabilis*, *P. vulgaris*, *B. cereus*, *B. subtilis* and *M. audorium* (MIC, 9.8 μg mL^{-1}). The structure–activity relationship was not discussed, but compound **60** (from the same source), which differs from **58** and **59** in the position of isoprene unit, was found to be inactive.[56] Curcumin (**62**), a similar compound, has been reported to induce filamentation in *B. subtilis* 168, suggesting that it inhibits bacterial cytokinesis. Further, **62** strongly inhibited the formation of the cytokinetic *Z*-ring in the protein structure of *B. subtilis*.[57] On that basis, the site of action of the kanzonols can be predicted, but further studies of these antimicrobials are needed.

The structural similarities and antimicrobial activities of two compounds from *Helichrysum gymnocomum*, acylphloroglucinol (**63**) and its analogue **64**, have been examined. Compound **63** afforded high activity against *Cryptococcus neoformans* ATCC 90112 (MIC 7.8 μg mL^{-1}), while **64** was active against *S. aureus* ATCC 12600 (MIC 6.8 μg mL^{-1}), *Enterococcus faecalis* ATCC 29212, methicillin- and gentamycin-resistant *S. aureus* ATCC 33592, *B. cereus* ATCC 11778 (MICs 7.8 μg mL^{-1}), and *S. epidermidis* ATCC 2223 (MIC 9.8 μg mL^{-1}).[36] The natural compounds grandinol (**65**), jensenone (**66**) were synthesized and their synthetic analogue (**67**) also showed high antimicrobial activities. The comparative data revealed that phloroglucinols are known for their various bioactivities and their acyl derivatives possess antimicrobial activities, therefore, they can be good candidates for further clinical studies to develop new antimicrobial dugs.[58]

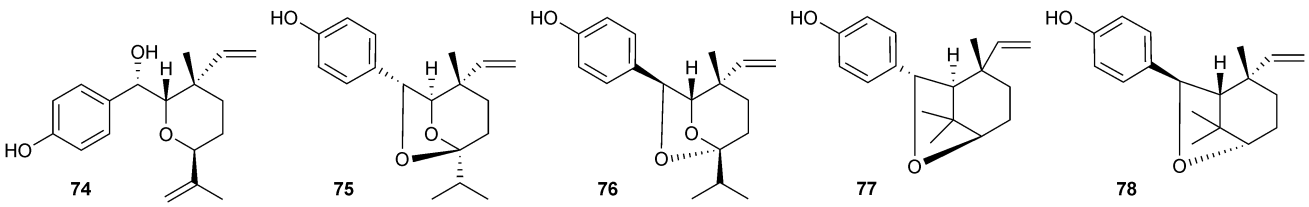
Six isolates of mushroom *Merulius incarnatus*, 5-alkylresorcinols (**68–73**), were characterized by sophisticated NMR techniques. Compound **68** is the first 5-alkylresorcinol derivative that contains a conjugated *trans–cis* double-bond system. Compounds **68–73** were found to inhibit MRSA, with IC_{50} values of 2.5–15.0 μg mL^{-1}.[59]

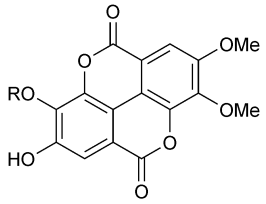
Psoracorylifols A–E (**74–78**) are phenolics isolated from a well-known traditional Chinese medicine, the seeds of *Psoralea corylifolia*. The structures of **74–78**, including their absolute configurations, were established on the basis of spectral methods and biosynthetic reasoning, with that for **74** being confirmed by single-crystal X-ray diffraction. Psoracorylifols D (**77**) and E (**78**) have an unprecedented carbon skeleton. All five compounds



showed significant inhibitory activity against two strains of *Helicobacter pylori* (SS1 and ATCC 43504), with MICs of 12.5–25 μg mL^{-1}. It is remarkable that psoracorylifols A–E (**74–78**) are 5–10 times stronger than the standard drug metronidazole, which is a critical ingredient for combination therapies of *H. pylori* and *Clostridium difficile* infections. Therefore, compounds of this kind could be substitutes for current *H. pylori* and *C. difficile* anti-infective drugs,[60] although their mode of action remains unstudied.

The stem bark of *Irvingia gabonensis* produced 3,3′,4′-tri-*O*-methylellagic acid (**79**) and 3,4-di-*O*-methylellagic acid (**80**). The lowest MIC values for **79** and **80** (both 9.76 μg mL^{-1}) were observed against *E. coli*, *Proteus vulgaris* and *B. subtilis*, while **80** exhibited high activity against *P. aeruginosa* (MIC 4.8 μg mL^{-1}).[61] Again, compounds with more free hydroxyl groups (like **80**) are more active.





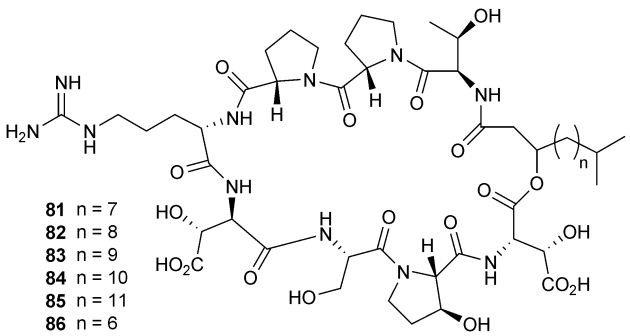
79 R = CH_3

80 R = H

10 Polypeptides

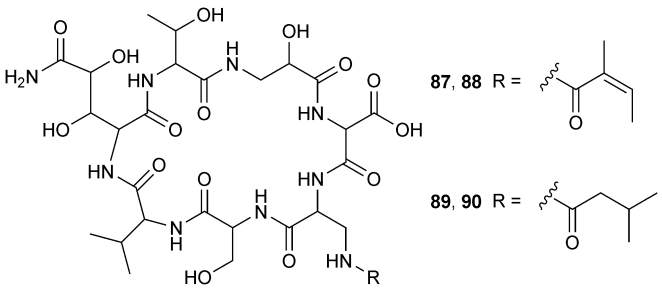
Polypeptides are well-known for their pharmaceutical uses, and have earned recognition as antimicrobial agents, for example in pharmaceutical applications, as well as for preservation, cleaning and disinfection. Polypeptides have in particular been used to treat textiles or laundry (*e.g.*, in detergents), and for odour reduction by reducing microbial growth.[62] Most of these polypeptides are of microbial origin, and in recent years many polypeptides have been discovered to be antimicrobial agents.

Tripropeptins (TPPs) A–E and Z (**81–86**) have been isolated from a culture of *Lysobacter* sp., and all were found to be potent against a wide range of pathogens (MIC 0.39–12.5 μg mL^{-1}). In particular, tripropeptin C (**83**) and tripropeptin D (**84**) showed excellent activities against Gram-positive bacteria, including both MRSA and vancomycin-resistant *Enterococcus* (VRE).[63] The antimicrobial activities of these tripropeptins with longer side-chains were higher, indicating that the side-chain plays an important role in the antimicrobial activities not only of TPPs (**81–86**) but also of other lipopeptides, such as the echinocandinmicafungin group,[64] the daptomycin group (LY146032 and A21978Cs),[65] the polymyxins and the octapeptins.[66] A comparison in the literature of the antimicrobial activities of TPPs (**81–86**) and tripropeptin-like lipopeptides suggests that the C-13 chain seems to be the best for antimicrobial activity.[67]



81 n = 7
82 n = 8
83 n = 9
84 n = 10
85 n = 11
86 n = 6

GE 23077 factors A1 (**87**), A2 (**88**), B1 (**89**) and B2 (**90**) are antibiotics isolated from fermentation broths of an *Actinomadura* species. As a mixture, they inhibited *Escherichia coli* and *Bacillus subtilis* RNA polymerase at a concentration of 0.02 μg mL^{-1}. The RNA polymerase from a rifampicin-resistant strain of *E. coli* was also inhibited at a similar concentration (IC_{50} 0.04 μg mL^{-1}). Individually, **87–90** inhibited *E. coli* RNA polymerase with IC_{50} values of 0.15, 0.035, 0.1 and 0.02 μg mL^{-1}, respectively.[68]

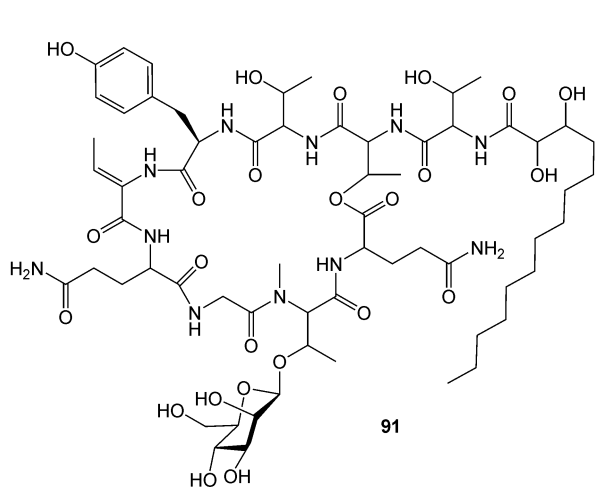


87, **88** R =

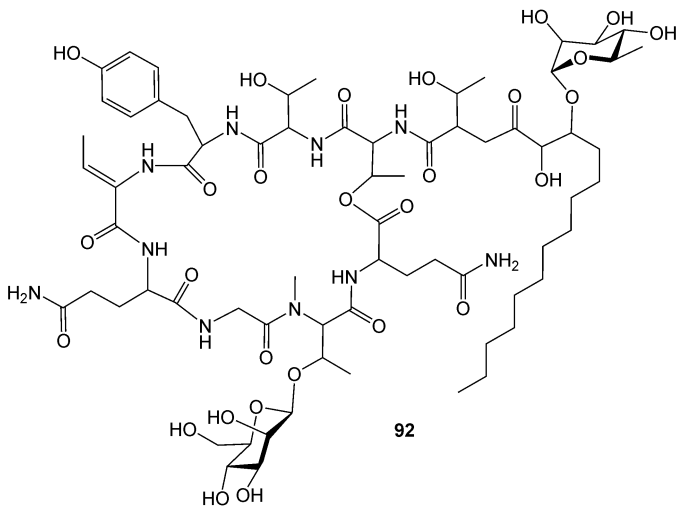
89, **90** R =

Hassallidins A (**91**)[69] and B (**92**),[70] isolated from a cyanobacterium of the genus *Hassallia*, are members of a class of compounds with broad-spectrum antifungal activity. The MICs of the two compounds against 10 species of *Candida* were similar (8.0–16.0 μg mL^{-1}), and the literature suggests that the additional carbohydrate moiety of **92** does not play a decisive role in the antifungal mode of action. However, due to the additional hydrophilic unit, **92** has higher water-solubility, a feature that could be important for bioavailability, making it a promising lead for the development of new antifungal drugs.

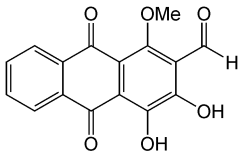
Recently, a review has been published on mechanism of action of various drugs including polypeptides, but different polypeptide drugs display different mechanisms.[71]



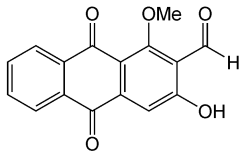
91



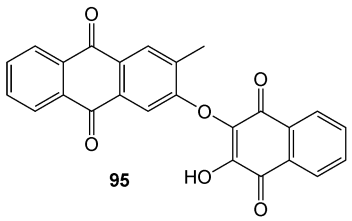
92



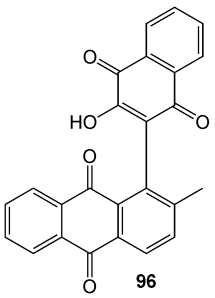
93



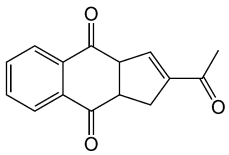
94



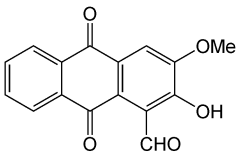
95



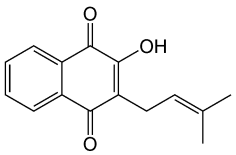
96



97



98



99

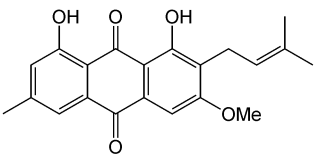
11 Quinones

Quinones from natural sources have a variety of biological activities, and are particularly well-known for their bactericidal properties.[72,73]

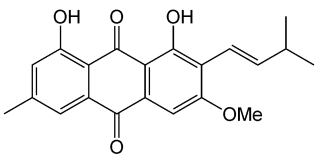
3,4-Dihydroxy-1-methoxyanthraquinone-2-carboxaldehyde (**93**) and damnacanthal (**94**), were identified from the aerial part of *Saprosma fragrans* as antifungal compounds with MIC values as follows: **93**: 12.5 μg mL^{-1} (*Trichophyton mentagrophytes*); **94**: 12.5, 6.25 and 1.56 μg mL^{-1} (*Cryptococcus neoformans*, *Sporothrix schenckii* and *T. mentagrophytes*, respectively).[74] Another anthraquinone containing a naphthaquinone moiety, named newbouldiaquinone A (**95**), is a constituent of the roots of *Newbouldia laevis* (Bignoniaceae). It possesses broad-spectrum antimicrobial activity, and is reported to be 13- and 24-fold more active against *Candida glabrata* and *Enterobacter aerogens*, respectively, than the standard antibiotics nystatin and gentamycin.[75] Against Gram-negative bacteria it showed high potential, with MICs in the range 0.31–9.76 μg mL^{-1}.[75]

The root bark of *Newbouldia laevis* contains newbouldiaquinone (**96**), 2-acetylfuro-1,4-naphthoquinone (**97**), 2-hydroxy-3-methoxy-9,10-dioxo-9,10-dihydroanthracene-1-carbaldehyde (**98**) and lapachol (**99**). Compounds **96–99** have broad-spectrum *in vitro* antimicrobial activity against six Gram-positive and twelve Gram-negative bacterial species, as well as *Candida* strains, the MIC values being in the range 0.076–9.76 μg mL^{-1}.[35]

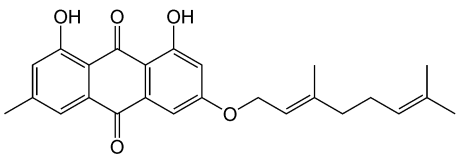
Kuete *et al.* identified four antimicrobial quinones (**100–104**) from the extract of *Vismia laurentii*, these being active against Gram-negative bacteria and two *Candida* species. Compound **100** inhibits the growth of *Streptococcus faecalis* and *Morganella morganii* (both MICs 4.8 μg mL^{-1}) and *Pseudomonas aeruginosa* and *Shigella flexneri* (both MICs 2.4 μg mL^{-1}). Compound **101** had MICs of 4.8 μg mL^{-1} against *M. morganii* and *S. faecalis*, and 2.4 μg mL^{-1} against *P. aeruginosa*, *S. flexneri*, *B. subtilis*, *C. albicans* and *C. glabrata*. Compound **102** was only active against Gram-positive and fungal pathogens (*B. megaterium*, *B. subtilis*, *C. albicans* and *C. glabrata*) with MICs of 2.4 μg mL^{-1}, while compound **103** had MICs of 4.8 μg mL^{-1} against *P. aeruginosa*, *S. flexneri* and *B. subtilis*, and 9.7 μg mL^{-1} against *M. morganii*. Compound **104** was highly active against *B. subtilis* (MIC 1.2 μg mL^{-1}), and also showed significant activity against *S. dysenteriae*, *S. faecalis* and *B. stearothermophilus* (MICs 4.8 μg mL^{-1}), and *B. cereus* (2.4 μg mL^{-1}). The latter also had moderate activity (MIC 19.5 μg mL^{-1}) against *M. morganii*, *P. vulgaris* and *S. aureus*.[33]



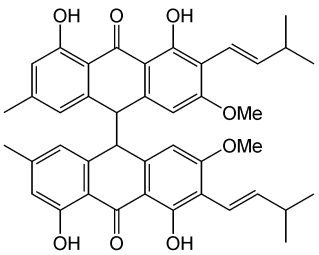
100



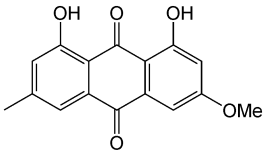
101



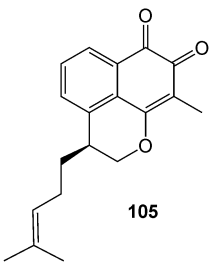
102



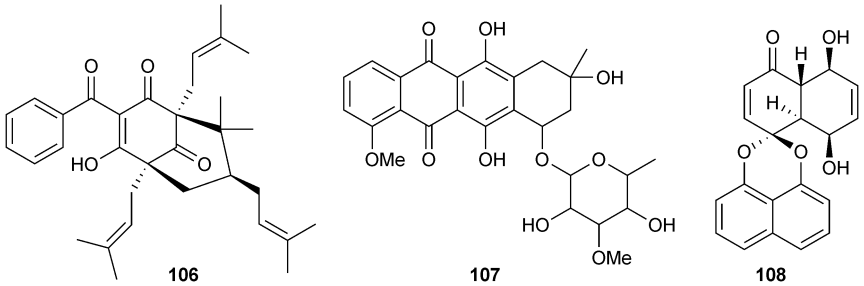
103



104



105



Omicron-naphthoquinone, 9-methyl-3-(4-methyl-3-pentenyl)-2,3-dihydronaphtho[1,8-*bc*]pyran-7,8-dione (**105**), is a constituent of the Australian plant *Eremophila serrulata*, and exhibits antimicrobial activity against *Staphylococcus aureus* ATCC 25923, *Streptococcus pneumoniae* ATCC 49619 and *Streptococcus pyogenes* ATCC 10389, with MICs of 7.8 μg mL^{-1}.[76]

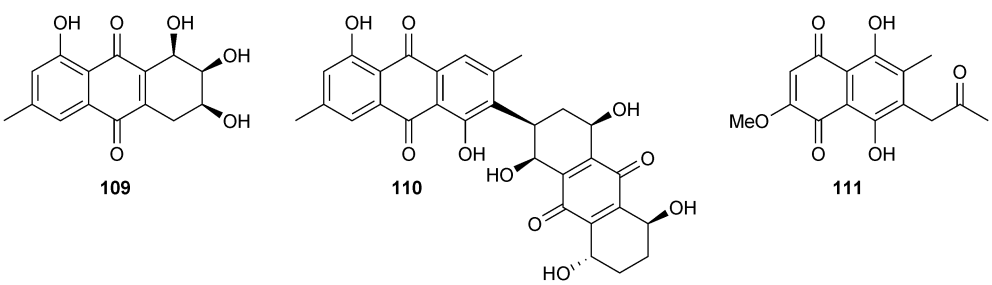
7-*epi*-Clusianone (**106**), identified from the antimicrobial extract of the fruit of *Rheedia brasiliensis*, showed high antibacterial activity at low concentrations (MIC 1.25–2.5 μg mL^{-1}) against *Streptococcus mutans*. This may lead to the use of **106** as a new agent to control *S. mutans* biofilms; however, more studies are needed to further elucidate the mechanism of action.[77] Anthracycline antibiotic mutactimycin C (**107**) was purified by reverse-phase HPLC from a soil isolate (*Saccharothrix* sp.) that inhibited two Gram-positive bacteria (*Micrococcus leteus* and *Klebsiella pneumoniae*) *in vitro* with MICs of 5.0 μg mL^{-1}.[78] Decaspirone A (**108**), related to the palmarumycin class of compounds, was isolated from cultures of the freshwater aquatic fungal species *Decaisnella thyridioides*, and its structure was established by X-ray crystallography. It displayed MIC values of approximately 10 and 5 μg mL^{-1} against *Aspergillus flavus* and *Fusarium verticillioides*, respectively.[79]

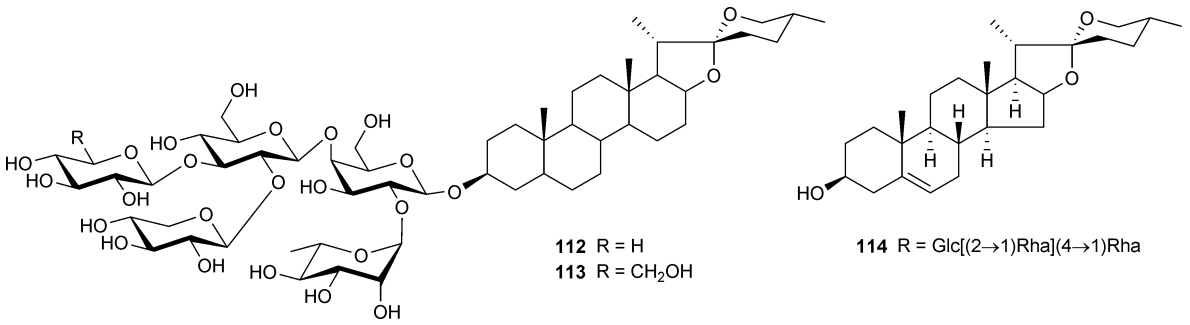
An unidentified endophytic fungus of the order Pleosporales was isolated from *Anthyllis vulneraria* (Fabaceae), and produced pleosporone (**109**) and phaeosphenone (**110**), following isolation by silica gel and Sephadex LH-20 chromatography followed by reverse-phase HPLC. In a two-plate whole-cell differential sensitivity screening assay using an antisense-sensitized *S. aureus* strain,[80,81] **109** exhibited significant activity towards *Streptococcus pneumoniae* and *Haemophilus influenzae* (MICs of 4.0 and 1.0 μg mL^{-1}, respectively) and *Bacillus subtilis* and *E. coli* (MICs of 8.0 and 16 μg mL^{-1}, respectively).[82] Compound **110** showed broad-spectrum antibacterial activity against Gram-positive bacteria, with MIC values ranging from 8 to 64 μg mL^{-1}. It showed the highest activity for *S. pneumoniae* and *C. albicans* (MICs of 8.0 μg mL^{-1}), and modest selectivity for the inhibition of RNA synthesis over DNA and protein synthesis in *S. aureus*.[83] Ribosomal protein S4 (RPSD), a part of the ribosomal small subunit, is one of the proteins that is a part of the ribosomal machinery and is a potential new target for the discovery of antibacterial agents. Therefore, **109** and **110** could be good leads for antibacterial drug development. Another endophytic fungus, *Chloridium* sp., produces javanicin (**111**) under both liquid and solid media culture conditions. This highly functionalized naphthaquinone (**111**), the structure of which was established by X-ray crystallography, was found to have strong antibacterial activity against *Pseudomonas fluorescens* and *P. aeruginosa* (MICs 2.0 μg mL^{-1}), *Rhizoctonia solani* and *Verticillium dahlae* (MICs 10 μg mL^{-1}), and antifungal activity against *Cercospora arachidicola* (MIC 5.0 μg mL^{-1}).[84]

Protein synthesis is one of the best antibacterial targets, and studies in this area have led to the development of a number of highly successful clinical drugs. In addition to providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in proteins,[85] often leading to inactivation of the protein and loss of function. For that reason, the potential range of quinone antimicrobial effects is great. Probable targets in the microbial cell are surface-exposed adhesins, cell-wall polypeptides, and membrane-bound enzymes. Quinones may also render substrates unavailable to the microorganism. The above-discussed results reveal that quinones exhibit a range of antimicrobial efficacies, and the reasons for this may lie in the substituent present on the benzene ring and perhaps in solubility differences.

12 Steroidal saponins

Many plant extracts containing steroidal saponins have been reported to exhibit antimicrobial activities,[86] and this also applies to the purified steroidal saponins.[87] Tigogenin 3-*O*-β-D-xylopyranosyl-(1→2)-[β-D-xylopyranosyl (1→3)]-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-galactopyranoside (TTS-12, **112**) and tigogenin 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (TTS-15,





113) were obtained from the extract of *Tribulus terrestris*, and their *in vivo* activity in a *Candida albicans* vaginal infection model studied. Both **112** and **113** were very active against *C. albicans* (MIC_{80} 10 and 2.3 μg mL^{-1}) and *Cryptococcus neoformans* (MIC_{80} 1.7 and 6.7 μg mL^{-1}). Phase-contrast microscopy and transmission electron microscopy showed that **112** has significant *in vitro* and *in vivo* antifungal activity, weakening the virulence of *C. albicans* and killing fungi through destroying the cell membrane.[88] Another cytotoxic steroidal saponin, dioscin (**114**), isolated from the rhizomes of *Smilacina atropurpurea*, was found to be active against *C. albicans* and *C. glabrata* (minimum fungicidal concentration (MFC) ≤ 5.0 μg mL^{-1}).[89]

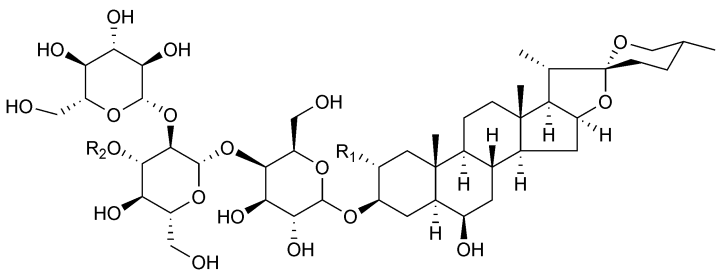
Aginoside (**115**) and (25*R*)-5α-spirostan-3β,6β-diol-3-*O*-{β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} (**116**) were found in the flower of *Allium leucanthum*. In an *in vitro* experiment, **115** and **116** showed antifungal activity against seven *Candida* strains with an MFC ranging from 6.25 to 12.5 μg mL^{-1}.[90]

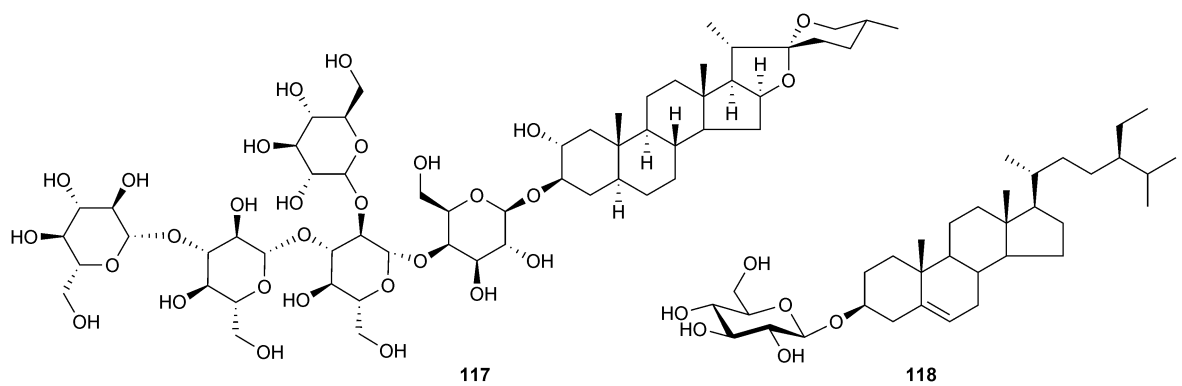
CAY-1 (**117**), a saponin from *Capsicum frutescens*, showed antifungal activity and is reported to be active against 16 different fungal strains, including *Candida* spp. and *Aspergillus fumigatus*, with MICs ranging from 4.0 to 16 μg mL^{-1}. It was especially active against *Cryptococcus neoformans*, exhibiting 90% inhibition at 1.0 μg mL^{-1}. However, it had no significant cytotoxicity against 55 mammalian cell lines at concentrations up to 100 μg mL^{-1}. Importantly, CAY-1 (**117**) appears to act by disrupting the membrane integrity of fungal cells.[91] β-Sitosterol-3-*O*-β-D-glucopyranoside (**118**), a very common phytochemical, was obtained from the root bark of *Newbouldia laevis*, and displayed broad-spectrum *in vitro* antimicrobial activity against three Gram-positive and nine Gram-negative bacterial species, as well as three *Candida* species (MICs 0.61–9.76 μg mL^{-1}).[34]

In general, the mechanism of action of steroids is not yet clear, but their antimicrobial properties might be due to their ability to disrupt membrane integrity.

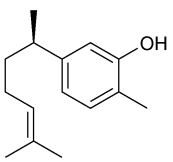
13 Terpenoids

Terpenoids are a class of secondary metabolites made of isoprene units. The important phytochemicals in essential oils are mixtures of mono- and sesquiterpenoids, and are known for their antimicrobial properties – 60% of essential oil derivatives examined to date are inhibitory to fungi, while 30% inhibit bacteria.[92] Many higher terpenoids are also reported to have

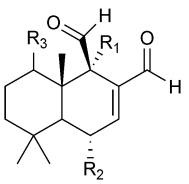




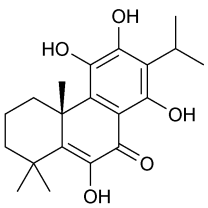
antimicrobial properties. These facts indicate the antimicrobial potential of this important class of compounds. The sesquiterpene xanthorrhizol (**119**), isolated from the ethanol extract of *Curcuma xanthorrhiza*, has MICs against *Bacillus cereus*, *Clostridium perfringens*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Vibrio parahaemolyticus* in the range 8.0–16.0 μg mL^{-1}. In addition, it maintained its antibacterial activity after thermal treatment (121 °C, 15 min) under a wide range of pH conditions (pH 3.0, 7.0 and 11.0), strongly suggesting that it could be effective as a natural preservative to prevent the growth of foodborne pathogens.[93] Cinnamodial (**120**), a diterpenoid isolated from the leaves and bark of *Pleodendron costaricense*, exhibited high activity against *Alternaria alternata* (MIC 3.9 μg mL^{-1}), *Candida albicans* D10 (an azole-resistant strain), and *Wangiella dermatitidis* (MICs 15.6 μg mL^{-1}).[94] Wellsow *et al.* investigated *Plectranthus saccatus* for its antimicrobial secondary metabolites, and isolated coleon U (**121**) and coleon U quinone (**122**). Compound **121** showed high activity against *B. subtilis* and *Pseudomonas syringae*, with an MIC of 3.13 and 6.25 μg mL^{-1}, respectively, and **122** also had high activity against *P. syringae*, with an MIC of 3.13 μg mL^{-1}.[95]



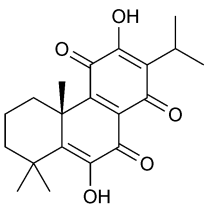
119



120 R_1 = OH, R_2 = OAc, R_3 = H

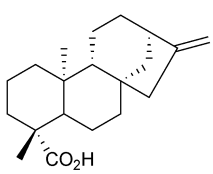


121

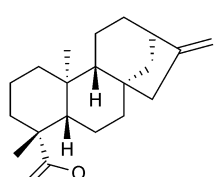


122

A kaurane diterpene, *ent*-kaur-16(17)-en-19-oic acid (**123**) isolated from *Aspilia foliacea*, showed potent activity against oral pathogens, with MIC values of 10.0 μg mL^{-1} against *Streptococcus sobrinus*, *S. mutans*, *S. mitis*, *S. sanguinis* and *Lactobacillus casei*.[96] This is an interesting observation, because very few natural products are known to inhibit the growth of oral pathogens, some of which (including *Streptococcus* spp.) are responsible for dental plaque. Kaurane diterpenes have been reported to exhibit antimicrobial properties,[97,98] and thus **123** could be a lead compound to develop anti-plaque drugs. A glycoside of **123** isolated from *Sagittaria pygmaea*, 18-β-3′,5′-diacetoxyarabinofuranosyl-*ent*-kaur-16-ene (**124**), was characterized by X-ray crystallography, and exhibited significant antibacterial activity against two oral pathogens, *Streptococcus mutans* ATCC 25 175 and *Actinomyces viscosus* ATCC 27 044, with MIC values against both pathogens of 15.6 μg mL^{-1}.[99]



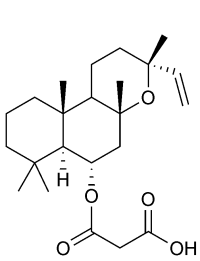
123



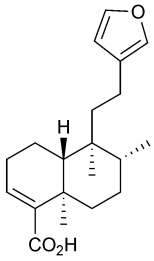
124

R = 3′,5′-diacetoxyarabinofuranoyl

A labdane diterpenoid, 6α-malonyloxymanoyl oxide (**125**) was isolated from the aerial parts of *Stemodia foliosa*, and found to inhibit the growth of *Staphylococcus aureus*, *Bacillus cereus*, *B. subtilis*, *Mycobacterium smegmatis*, and *M. phlei* with MICs of 7.0–15.0 μg mL^{-1}.[100] The diterpenoid hardwickiic acid (**126**), obtained from the stem bark of *Irvingia gabonensis*, exhibited potent activity against five Gram-negative and four Gram-positive bacteria, with MIC values of 1.22–4.8 μg mL^{-1} (the lowest being against *Neisseria gonorrhoeae*).[61]

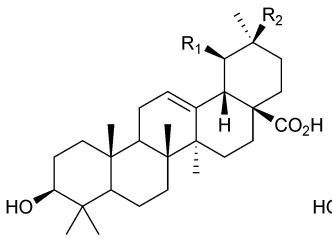


125



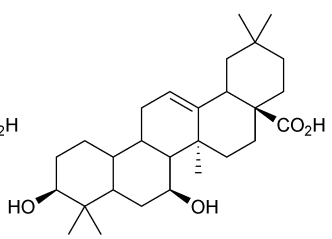
126

Pentacyclic triterpenes also have antimicrobial activities, many of them being found in various plant families. Oleanolic acid (**127**) and ursolic acid (**128**) are well-known phytochemicals, isolated by Horiuchi *et al.* from the extract of the leaves of *Salvia officinalis*. Both **126** and **127** exhibited potent activity against vancomycin-resistant *Enterococcus* (VRE) pathogens, with MICs of 8.0 and 4.0 μg mL^{-1}, respectively. They also showed activity against *Streptococcus pneumoniae* and MRSA, with MICs of 16.0 and 8.0 μg mL^{-1}, respectively.[101] Previously, Woldemichael *et al.* and Kowalewski *et al.* had described the antimicrobial properties of **127** and **128**,[102,103] with quite different results. It seems that such differences are due to differences in assay conditions and in the strains used. Both **127** and **128** have very low toxicity – **127** has already been successfully used as an orally administered drug to treat human liver diseases in China,[104] and so they might be used for the treatment of VRE infections . Compound **127**, together with another pentacyclic triterpenoid, canthic acid (**129**), was isolated from the root bark of *Newbouldia laevis*. Both compounds showed broad-spectrum *in vitro* antimicrobial activity against six Gram-positive and twelve Gram-negative bacterial species, as well as *Candida* strains (MICs 0.038–9.76 μg mL^{-1}); notably, **129** showed activity of 0.038 μg mL^{-1} against *Bacillus subtilis* and *B. cerus*, and 0.076 μg mL^{-1} against *B. megaterium*.[34]



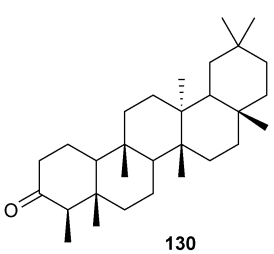
127 R_1 = H, R_2 = CH_3

128 R_1 = CH_3, R_2 = H

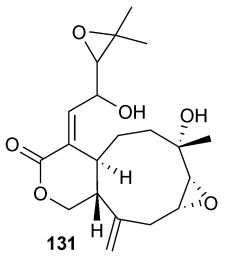


129

Fridelin (**130**), a constituent of *Vismia laurentii*, exhibited broad-spectrum antimicrobial character against six Gram-negative, four Gram-positive and two fungal strains, with MICs of 2.4–9.7 μg mL^{-1}.[33] A diterpene, xeniolide I (**131**), has been isolated from the Kenyan soft coral *Xenia novaebrittanniae*, and possesses antibacterial activity at a concentration of 1.25 μg mL^{-1} against *Escherichia coli* and *Bacillus subtilis*.[105]



130



131

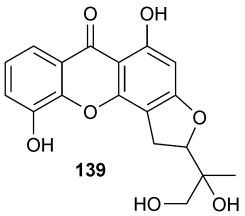
The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by these lipophilic compounds. Mendoza *et al.* found that increasing the hydrophilicity of kaurene diterpenoids by introduction of a 3β-OH group drastically reduced their antimicrobial activity.[106]

14 Xanthones

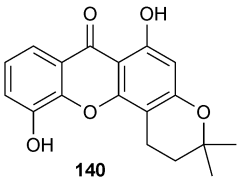
Xanthones related to phytoalexins are thought to be produced as a result of microbial infection in higher plants as a consequence of a passive defense system. Biological evaluation revealed that these compounds have fewer side-effects and a low tendency to result in pathogen resistance compared with conventional antibiotics and synthetic antibacterial agents; natural xanthones are known for their potent MRSA-inhibitory properties.[107]

Two tetraoxygenated xanthones, mangostanin (**132**) and α-mangostin (**133**), were isolated from the crude hexane extract of the fruits of *Garcinia cowa*. Compound **132** is a strong inhibitory agent against the growth of *Staphylococcus aureus* (penicillin-sensitive strain ATCC 25923 and methicillin-resistant strain MRSA SK1), with MIC values of 4.0 μg mL^{-1}, whereas compound **133** inhibited both strains of *S. aureus* with MIC values of 8.0 μg mL^{-1}.[108] Boonsri *et al.* isolated formoxanthone A (**134**), formoxanthone C (**135**), macluraxanthone (**136**), xanthone V1 (**137**) and gerontoxanthone I (**138**) as potential antibiotics from the roots of *Cratoxylum formosum*. Compounds **135** and **136** were quite active against *B. subtilis* (both MICs 4.6 μg mL^{-1}), *S. aureus* (MIC 2.3 and 4.6 μg mL^{-1}, respectively), *S. faecalis* (MIC 18.7 and 2.3 μg mL^{-1}, respectively) and *S. typhi* (MIC 4.6 and 9.3 μg mL^{-1}, respectively). Compound **137** was particularly active, with an MIC for all the tested pathogens of 1.1 μg mL^{-1} (except *P. aeruginosa*, MIC 9.3 μg mL^{-1}), while **138** inhibited the pathogens with MIC values of 1.1–4.6 μg mL^{-1}. All these compounds were also reported to have cytotoxic properties.[109]

Xanthones **139** and **140** are the active constituents of *Vismia laurentii*. Compound **139** inhibited the growth of *Bacillus subtilis* and *Candida glabrata* with an MIC value of 1.2 μg mL^{-1}, whereas **140** showed the same degree of activity against *Bacillus stearothermophilus*, and significant activity against *B. subtilis* (MIC 4.8 μg mL^{-1}).[33]



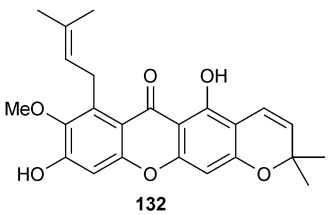
139



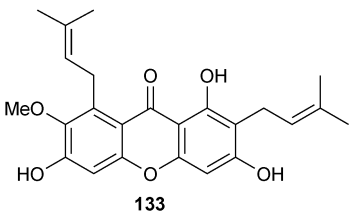
140

15 Miscellaneous compounds

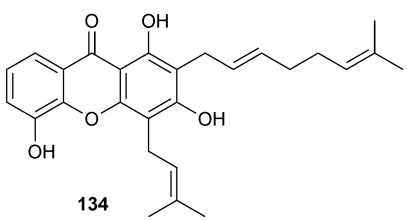
The cytotoxic compound aculeatin D (**141**) was isolated as minor constituent from the rhizomes of *Amomum aculeatum*, and showed very potent activity against two *Plasmodium falciparum* strains (MIC 0.42 μg mL^{-1}), as well as against *Trypanosoma brucei rhodesiense* (MIC 0.20 μg mL^{-1}) and *Trypanosoma cruzi* (MIC 0.49 μg mL^{-1}). The same compound was less potent as an antibacterial, with moderate activity against *Bacillus cereus* (MIC 16 μg mL^{-1}), *Escherichia coli* (MIC 16 μg mL^{-1}) and *Staphylococcus epidermidis* (MIC 8 μg mL^{-1}) .[110] Platensimycin (**142**) is a novel broad-spectrum Gram-positive antibiotic produced by *Streptomyces platensis*, and was discovered by



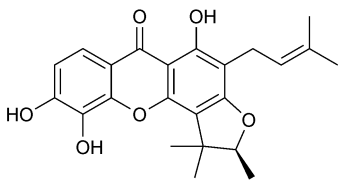
132



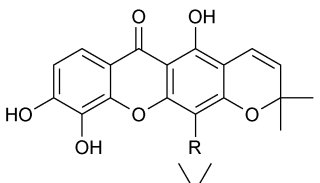
133



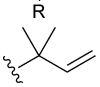
134



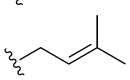
135

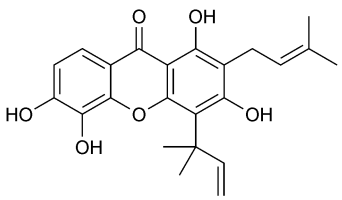


136 R =



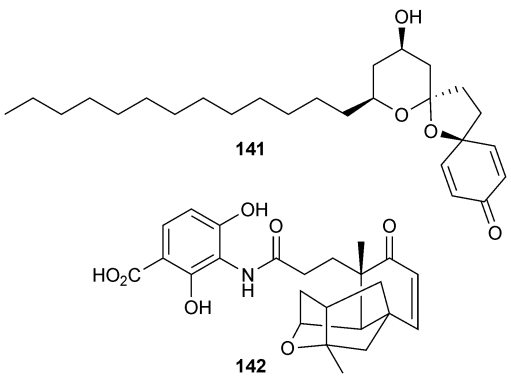
137 R =





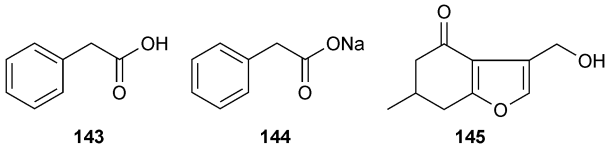
138

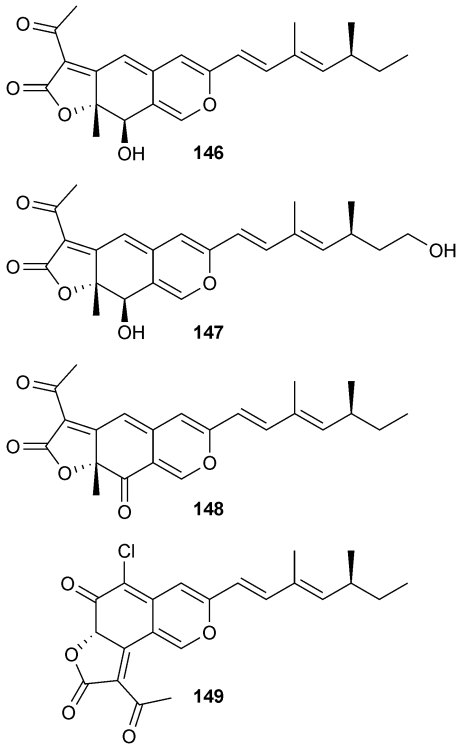
a target-based whole-cell screening strategy using an antisense differential sensitivity assay. It was isolated originally by a three-step isolation method using Amberchrome, Sephadex LH-20, and reverse-phase HPLC chromatographies. The structure was elucidated by 2D NMR methods and confirmed by X-ray crystallographic analysis of a bromo derivative. It inhibits bacterial growth, with IC_{50} values of 48 and 160 nM for *S. aureus* and *E. coli*, respectively. This compound also inhibited phospholipid synthesis (IC_{50} 0.1 μg mL^{-1}), and exhibited MIC values of 0.1–1.0 μg mL^{-1} against MRSA.[111] Mechanistically, it exerts its activity by a novel mode of action that involves specific binding with the acyl enzyme intermediate of the key condensing enzyme FabF of the fatty acid synthesis pathway. Fatty acids are essential for survival of bacteria and are synthesized by a series of enzymes including the elongation enzymes, β-ketoacyl acyl carrier protein synthase I/II (FabF/B). Inhibition of fatty acid synthesis is therefore a target for the discovery and development of antibacterial agents, and **142** could be a candidate for antibacterial drug development.[111]



Phenylacetic acid (**143**) and sodium phenylacetate (**144**) were isolated from *Streptomyces humidus* strain S5-55, both being identified by NMR, EI-MS and ICP-MS. Compounds **143** and **144** completely inhibited the growth of *Pythium ultimum*, *Phytophthora capsici*, *Rhizoctonia solani*, *Saccharomyces cerevisiae*, and *Pseudomonas syringae* pv. *syringae* at concentrations from 10 to 50 μg mL^{-1}. They were also as effective as the commercial fungicide metalaxyl in inhibiting spore germination and hyphal growth of *P. capsici*.[112] A derivative of benzofuran (**145**) was purified from a culture of *Phomopsis* sp. hzla01-1, and showed significant antimicrobial activity against *Escherichia coli* CMCC44103, *Candida albicans* AS2.538 and *Saccharomyces cerevisiae* ATCC9763, with MICs of 5–10 μg mL^{-1}, and strongest activity against *Bacillus subtilis* CMCC63501, with an MIC of 1.25 μg mL^{-1}. None of the three compounds showed significant cytotoxicity against the HeLa cell line at 10 μg mL^{-1}.[48]

Four compounds, rotiorinols A (**146**), C (**147**), (−)-rotiorin (**148**) and rubrorotiorin (**149**), were isolated from the fungus *Chaetomium cupreum* CC3003. The absolute configuration of **146** was determined by the modified Mosher method, along with X-ray analysis of its acetate derivative, as well as by chemical transformation. Compounds **146–149** exhibited antifungal activity against *Candida albicans* with IC_{50} values of 0.6–24.3 μg mL^{-1}, the halogenated compound **149** being the most active.[113]





16 Conclusions and future prospects

In the literature spanning 2000 to 2008, more than 300 secondary metabolites were reported to possess weak to very strong antimicrobial activities, but less than half of this number have significant antimicrobial potential. A year-by-year review of the literature revealed that little work was done on this aspect of antibiotic development in 2000–2004, and although the number of studies in this area continues to increase, it remains insufficient due to the rapid appearance of antibiotic-resistant strains. In the past, most antimicrobials have been derived from microorganisms, and it is expected that microorganisms, together with plants, will continue to be a good source of antimicrobials.[114]

Numerical analysis of the compounds discussed in this review shows that phenolic compounds form the largest group (47, including 7 flavonoids and 3 lignans), the next being quinones (19) and alkaloids (14).

Phenolic compounds are widely distributed in plants, where they protect the plants from microbial infections, UV-radiation and chemical stressors, and plants containing phenolics are receiving great interest at this time. Due to the potential antioxidative properties of phenolics, plants rich in these metabolites are becoming an important part of diets, and current research into these compounds has led to the conclusion that they not only act against oxidative stress, but are also potent anti-infectives. In spite of these facts, however, the absorption and bioavailability of phenolics in humans remain controversial – data on these

aspects of phenolics are scarce, and highlight the need for extensive investigations of the action of the gastrointestinal tract upon phenolics, and their subsequent absorption and metabolism.

Quinones are the other significant group of secondary metabolites with potentially useful activities. The reason for the wide range of antimicrobial effects may lie in the fact that besides providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in proteins, resulting in loss of protein function.[115] On the other hand, the possible toxic effects of quinones obtained from natural sources needs to be thoroughly examined before they can be assessed as potential drugs. In the literature reviewed, 14 alkaloids were shown to possess antimicrobial properties, but no toxicity studies were reported.

Some diverse and unusual structures isolated from natural sources may also be potential drug candidates. For example, platensimycin (**142**), identified from *Streptomyces platensis* as a novel broad-spectrum Gram-positive antibiotic, and rubrorotiorin (**149**), isolated from the fungus *Chaetomium cupreum* CC3003, both show strong antifungal properties.

In conclusion, the results summarised in this review emphasise the potential of antimicrobial natural products, and highlight the need to continue to explore natural sources, using the most sophisticated techniques for metabolite purification, characterization and assessment that are at our disposal.

17 References

1 C. F. Amabile-Cuevas, *Am. Sci.*, 2003, **91**, 138–149.
2 V. Behal, *Fol. Microbiol.*, 2001, **46**, 363–370.
3 E. Omulokoli, B. Khan and S. C. Chhabra, *J. Ethnopharmacol.*, 1997, **56**, 133–137.
4 T. Céline, G. Jean-Charles, D. Philippe, F. Christophe, H. Reynald, F. Maria-Elena, R. A. Antonieta and F. Alain, *Phytother. Res.*, 2003, **17**, 678–680.
5 G. O'Donnell and S. Gibbons, *Phytother. Res.*, 2007, **21**, 653–7.
6 D. Lagoutte, V. Nicolas, E. Poupon, A. Fournet, R. Hocquemiller, D. Libong, P. Chaminade and P. M. Loiseau, *Biomed. Pharmacother.*, 2008, **62**, 99–103.
7 M. Shibazaki, M. Taniguchi, T. Yokoi, K. Nagai, M. Watanabe, K. Suzuki and T. Yamamoto, *J. Antibiot.*, 2004, **57**, 379–382.
8 B. Kunze, R. Jansen, G. Hofle and H. A. Reichenbach, *J. Antibiot.*, 2004, **57**, 151–155.
9 W. Huang, J. Pei, B. Chen, W. Pei and X. Ye, *Tetrahedron*, 1996, **52**, 10131–10136.
10 A. Sato, S. Takahashi, T. Ogita, M. Sugano and K. Kodama, *Annu. Rep. Sankyo Res. Lab.*, 1995, **47**, 1–58.
11 J. M. Turner and A. J. Messenger, *Adv Microb Physiol.*, 1986, **27**, 211–75.
12 J. R. Kerr, *Infect. Dis. Rev.*, 2000, **2**, 184–94.
13 C. Maul and et al, *J. Antibiot.*, 1999, **52**, 1124–1134; K. Yagishita, *J. Antibiot.*, 1960, **13**, 83.
14 S. R. Giddens, Y. Feng and H. K. Mahanty, *Mol. Microbiol.*, 2002, **45**, 769–83.
15 S. R. Giddens and D. C. Bean, *Int. J. Antimicrob. Agents*, 2007, **29**, 93–97.
16 C. Zheng, C.-J. Kim, K. S. Bae, Y.-H. Kim and W.-G. Kim, *J. Nat. Prod.*, 2006, **69**, 1816–1819.
17 C. J. Zheng, S. H. Park, H. Koshino, Y. H. Kim and W. G. Kim, *J. Antibiot.*, 2007, **60**, 61–64.
18 P. Chaudhary, R. Kumar, A. K. Verma, D. Singh, V. Yadav, A. K. Chhillar, G. L. Sharma and R. Chandra, *Bioorg. Med. Chem.*, 2006, **14**, 1819–1826.
19 C. Takahashi, A. Numata, Y. Ito, E. Matsumura, H. Araki, H. Iwaki and K. Kushida, *J. Chem. Soc., Perkin Trans. 1*, 1994, 1859–1864.
20 J.-Y. Dong, H.-P. He, Y.-M. Shen and K.-O. Zhang, *J. Nat. Prod.*, 2005, **68**, 1510–1513.
21 B. K. Joshi, J. B. Gloer and D. T. Wicklow, *J. Nat. Prod.*, 1999, **62**, 730–733.
22 T. Yamada, C. Iwamoto, N. Yamagaki, T. Yamanouchi, K. Minoura, T. Yamori, Y. Uehara, T. Andoh, K. Umemura and A. Numata, *Tetrahedron*, 2002, **58**, 479–487.
23 C. Richard, R. Canon, K. Naghmouchi, D. Bertrand, H. Prévost and D. Drider, *Food Microbiology*, 2006, **23**, 175–183.
24 H. Gershon and L. Shanks, *Can. J. Microbiol.*, 1978, **24**, 593–597.
25 S. J. Jurenka, Undecylenic acid – a monograph, *Altern. Med. Rev.*, 2002, http://www.highbeam.com/Alternative_Medicine_Review/publications.aspx?date_200202.
26 X. C. Li, M. R. Jacobs, S. I. Khan, M. K. Ashfaq, K. S. Babu, A. K. Agarwal, H. N. Elsohly, S. P. Manly and A. M. Clark, *Antimicrob. Agents Chemother.*, 2008, **52**, 2442–2448.
27 J. G. Ondeyka, D. L. Zink, K. Young, R. Painter, S. Kodali, A. Galgoci, J. Collado, J. R. Tormo, A. Basilio, F. Vicente, J. Wang and S. B. Singh, *J. Nat. Prod.*, 2006, **69**, 377–380.
28 H.-J. Kim, F. Vinale, E. L. Ghisalberti, C. M. Worth, K. Sivasithamparam, B. W. Skelton and A. H. White, *Phytochemistry*, 2006, **67**, 2277–2280.
29 J. G. Kenny, D. Ward, E. Josefsson, I.-M. Jonsson, J. Hinds, H. H. Rees, J. A. Lindsay, A. Tarkowski and M. J. Horsburgh, *PLoS One*, 2009, **4**, 1–29.
30 X. Liu, M. Dong, X. Chen, M. Jian, X. Lv and J. Zhou, *Appl. Microbiol. Biotechnol.*, 2008, **78**, 241–247.
31 Y. Sato, S. Suzaki, T. Nishikawa, M. Kihara, H. Shibata and T. Higuti, *J. Ethnopharmacology*, 2000, **72**, 483–488.
32 H. J. Jung, W. S. Sung, S.-H. Yeo, H. S. Kim, I.-S. Lee, E.-R. Woo and D. G. Lee, *Arch. Pharmacal. Res.*, 2006, **29**, 746–751.
33 V. Kuete, J. R. Nguemeving, V. P. Beng, A. G. Azebaze, F. X. Etoa, M. Meyer, B. Bodo and A. E. Nkengfack, *J. Ethnopharmacol.*, 2007, **109**, 372–379.
34 V. Kuete, K. O. Eyong, G. N. Folefoc, V. P. Beng, H. Hussain, K. Krohn and A. E. Nkengfack, *Pharmazie*, 2007, **62**, 552–556.
35 B. Sathiamoorthy, P. Gupta, M. Kumar, A. K. Chaturvedi, P. K. Shukla and R. Maurya, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 239–242.
36 S. E. Drewes and S. F. van Vuuren, *Phytochemistry*, 2008, **69**, 1745–1749.
37 V. Kuete, B. Ngameni, C. C. Simo, P. K. Tankeu, B. T. Ngadjui, J. I. Meyer, N. Lall and J. R. Kuiate, *J. Ethnopharmacol.*, 2008, **120**, 17–24.
38 M. A. Alvarez, N. B. Debattista and N. B. Pappano, *Fol. Microbiol.*, 2008, **53**, 23–28.
39 B. Sherif, G. Abdel, W. Louise, H. Z. Zidan, M. A. Hussein, C. W. Keevil and C. D. B. Richard, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 518–522.
40 H. Tsuchiya, M. Sato, T. Miyazaki, S. Fujiwara, S. Tanigaki, M. Ohyama, T. Tanaka and M. Iinuma, *J. Ethnopharmacol.*, 1996, **50**, 27–34.
41 R. Tundis, M. R. Loizzo, F. Menichini, G. A. Statti and F. Menichini, *Recent Developments. Mini Reviews in Medicinal Chemistry*, 2008, **8**, 399–420.
42 D. G. Lee, H. J. Jung and E.-R. Woo, *Arch. Pharmacal. Res.*, 2005, **28**, 1031–1036.
43 H. M. Ge, B. Huang, S. H. Tan, D. H. Shi, Y. C. Song and R. X. Tan, *J. Nat. Prod.*, 2006, **69**, 1800–1802.
44 S. C. Peng, C. Y. Cheng, F. Sheu and C. H. Su, *J. Appl. Microbiol.*, 2008, **105**, 485–491.
45 E. Haslam, *J. Nat. Prod.*, 1996, **59**, 205–215.
46 J. L. Stern, A. E. Hagerman, P. D. Steinberg and P. K. Mason, *J. Chem. Ecol.*, 1996, **22**, 1887–1899.
47 C. Jaruchoktaweechai, *J. Nat. Prod.*, 2000, **63**, 984–986; T. Nagao, *J. Antibiot.*, 2001, **54**, 333–339.
48 X. Du, C. Lu, Y. Li, Z. Zheng, W. Su and Y. Shen, *J. Antibiot.*, 2008, **61**, 250–253.
49 F. J. Perez-zuniga, E. M. Seco, T. Cuesta, F. Degenhardt, J. Rohr, C. Vallin, Y. Iznaga, E. M. Perez, L. Gonzalez and F. Malpartida, *J. Antibiot.*, 2004, **57**, 197–204.
50 R. Puupponen-Pimiä, L. Nohynek, H. L. Alakomi and K. M. Oksman-Caldentey, *Appl. Microbiol. Biotechnol.*, 2005, **67**, 8–18.
51 H. M. A. Cavanagh, M. Hipwell and J. M. Wilkinson, *J. Med. Food*, 2003, **6**, 57–61.

52 K. M. Chung, T. Y. Wong, C. I. Wei, Y. W. Huang and Y. Lin, *Crit. Rev. Food Sci. Nutr.*, 1998, **38**, 421–464.
53 G. L. Pessini, B. P. Dias Filho, C. V. Nakamura and D. A. G. Cortez, *Mem. Inst. Oswaldo Cruz, Rio de Janeiro*, 2003, **98**, 1115–1120.
54 A. M. Koroishi, S. R. Foss, D. A. Cortez, T. Ueda-Nakamura, C. V. Nakamura and B. P. Dias Filho, *J. Ethnopharmacol.*, 2008, **117**, 270–277.
55 T. Rukachaisirikul, P. Innok, N. Aroonrerk, W. Boonamnuaylap, S. Limrangsun, C. Boonyon, U. Woonjina and A. Suksamrarn, *J. Ethnopharmacol.*, 2007, **110**, 171–175.
56 A. T. Mbaveng, B. Ngameni, V. Kuete, I. K. Simo, P. Ambassa, R. Roy, M. Bezabih, F. X. Etoa, B. T. Ngadjui, B. M. Abegaz, J. I. Meyer, N. Lall and V. P. Beng, *J. Ethnopharmacol.*, 2008, **116**, 483–489.
57 D. Rai, J. K. Singh, N. Roy and D. Panda, *Biochem. J.*, 2008, **410**, 147–155.
58 S. B. Bharate, S. I. Khan, N. A. Yunus, S. K. Chauthe, M. R. Jacob, B. L. Tekwani, I. A. Khan and I. P. Singh, *Bioorg. Med. Chem.*, 2007, **15**, 87–96.
59 W. Jin and J. K. Zjawiony, *J. Nat. Prod.*, 2006, **69**, 704–706.
60 S. Yin, C.-Q. Fan, L. Dong and J.-M. Yue, *Tetrahedron*, 2006, **62**, 2569–2575.
61 V. Kuete, G. F. Wabo, B. Ngameni, A. T. Mbaveng, R. Metuno, F. X. Etoa, B. T. Ngadjui, V. P. Beng, J. I. Meyer and N. Lall, *J. Ethnopharmacol.*, 2007, **114**, 54–60.
62 Y. Debbie and G. Erik, 'Use of polypeptides having antimicriobial activity', *US Pat.*, IPC8 class: AA61K3816FI; USPC class: 514 12.
63 H. Hashizume, M. Igarashi, S. Hattori, M. Hori, M. Hamada and T. Takeuchi, *J. Antibiot.*, 2001, **54**, 1054–1059.
64 M. Hino, A. Fujie, T. Iwamoto, Y. Hori, M. Hashimoto, Y. Tsurumi, K. Sakamoto, S. Takase and S. Hashimoto, *J. Ind. Microbiol. Biotechnol.*, 2001, **27**, 157–162.
65 H. L. Jeremy and E. J. A. Lea, *Biochim. Biophys. Acta*, 1986, **859**, 219–226.
66 R. S. Daniel, K. S. Rosenthal and P. E. Swanson, *Ann. Rev. Biochem.*, 1977, **46**, 723–763.
67 H. Hashizume, S. Hattori, M. Igarashi and Y. Akamatsu, *J. Antibiot.*, 2004, **57**, 394–399.
68 I. Ciciliato, E. Corti, E. Sarubbi, S. Stefanelli, L. Gastaldo, N. Montanini, M. Kurz, D. Losi, F. Marinelli and E. Selva, *J. Antibiot.*, 2004, **57**, 210–217.
69 T. Neuhof, P. Schmieder, K. Preussel, R. Dieckmann, H. Pham, F. Bartl and H. von Döhren, *J. Nat. Prod.*, 2005, **68**, 695–700.
70 T. Neuhof, P. Schmieder, M. Seibold, K. Preussel and H. von Döhren, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 4220–4222.
71 A. M. S. Mayer, A. D. Rodríguez, R. G. S. Berlinck and M. T. Hamann, *Biochim. Biophys. Acta, Gen. Subj.*, 2009, **1790**, 283–308.
72 N. D. Malkina and et al, *J. Antibiot.*, 1994, **47**, 342–348.
73 S. Omura, Y. Iwai, K. Hinotozawa, H. Tanaka, Y. Takahashi and A. Nakagawa, *J. Antibiot.*, 1982, **35**, 1425.
74 D. N. Singh, N. Verma, S. Raghuwanshi, P. K. Shukla and D. K. Kulshreshtha, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 4512–4514.
75 K. O. Eyong, G. N. Folefoc, V. Kuete, V. P. Beng, K. Krohn, H. Hussain, A. E. Nkengfack, M. Saeftel, S. R. Sarite and A. Hoerauf, *Phytochemistry*, 2006, **67**, 605–609.
76 C. P. Ndi, S. J. Semple, H. J. Griesser, S. M. Pyke and M. D. Barton, *Phytochemistry*, 2007, **68**, 2684–2690.
77 L. S. Almeida, R. M. Murata, R. Yatsuda, M. H. Dos Santos, T. J. Nagem, S. M. Alencar, H. Koo and P. L. Rosalen, *Phytomedicine*, 2008, **15**, 886–891.
78 A. Zitouni, H. Boudjella, F. Mathieu, N. Sabaou and A. Lebrihi, *J. Antibiot.*, 2004, **57**, 367–372.
79 P. Jiao, D. C. Swenson, J. B. Gloer, J. Campbell and C. A. Shearer, *J. Nat. Prod.*, 2006, **69**, 1667–1671.
80 K. Young, H. Jayasuriya, J. G. Ondeyka, K. Herath, C. Zhang, S. Kodali, A. Galgoci, R. Painter, V. Brown-Driver, R. Yamamoto, L. L. Silver, Y. Zheng, J. I. Ventura, J. Sigmund, S. Ha, A. Basilio, F. Vicente, J. R. Tormo, F. Pelaez, P. Youngman, D. Cully, J. F. Barrett, D. Schmatz, S. B. Singh and J. Wang, *Antimicrob. Agents Chemother.*, 2006, **50**, 519–526.
81 S. B. Singh, J. W. Phillips and J. Wang, *Curr. Opin. Drug Discovery Dev.*, 2007, **10**, 160–166.
82 C. Zhang, J. G. Ondeyka, D. L. Zink, A. Basilio, F. Vicente, J. Collado, G. Platas, J. Huber, K. Dorso, M. Motyl, K. Byrne and S. B. Singh, *Bioorg. Med. Chem.*, 2009, **17**, 2162–2166.
83 C. Zhang, J. G. Ondeyka, D. L. Zink, A. Basilio, F. Vicente, J. Collado, G. Platas, J. Huber, K. Dorso, M. Motyl, K. Byrne and S. B. Singh, *J. Nat. Prod.*, 2008, **71**, 1304–1317.
84 R. N. Kharwar, V. C. Verma, A. Kumar, S. K. Gond, J. K. Harper, W. M. Hess, E. Lobkovosky, C. Ma, Y. Ren and G. A. Strobel, *Curr. Microbiol.*, 2009, **58**, 233–238.
85 J. L. Stern, A. E. Hagerman, P. D. Steinberg and P. K. Mason, *J. Chem. Ecol.*, 1996, **22**, 1887–1899.
86 U. Abbasołu and S. Türköz, *Pharm. Biol.*, 1995, **33**, 293–296.
87 K. Krishnan, R. T. Ramalingam and K. G. Venkatesan, *J. Appl. Biol. Sci.*, 2008, **2**, 109–112.
88 J.-D. Zhang, Z. Xu, Y.-B. Cao, H.-S. Chen, L. Yan, M.-M. An, P.-H. Gao, Y. Wang, X.-M. Jia and Y.-Y. Jiang, *J. Ethnopharmacol.*, 2006, **103**, 76–84.
89 Y. Zhang, H.-Z. Li, Y.-J. Zhan, M. R. Jacob, S. I. Khan, X.-C. Li and C.-R. Yang, *Steroids*, 2006, **71**, 712–719.
90 L. Mskhiladze, J. Kutchukhidze, D. Chincharadze, F. Delmas, R. Elias and A. Favel, *Georgian Med. News*, 2008, **154**, 39–43.
91 S. Renault, A. J. De Lucca, S. Boue, J. M. Bland, C. B. Vigo and C. P. Selitrennikoff, *Med. Mycol.*, 2003, **41**, 75–81.
92 S. C. Chaurasia and K. K. Vyas, *J. Res. Indian Med. Yoga Homeopath.*, 1977, 24–26.
93 L. Y. Lee, J. S. Shim, Y. Rukayadi and J. K. Hwang, *J. Food Prot.*, 2008, **71**, 1926–1930.
94 V. T. Amiguet, P. Petit, C. A. Ta, R. Nuñez, P. Sánchez-Vindas, L. P. Alvarez, M. L. Smith, J. T. Arnason and T. Durst, *J. Nat. Prod.*, 2006, **69**, 1005–1009.
95 J. Wellsow, R. J. Grayer, N. C. Veitch, T. Kokubun, R. Lelli, G. C. Kite and M. S. J. Simmonds, *Phytochemistry*, 2006, **67**, 1818–1825.
96 S. R. Ambrosio, N. A. Furtado, D. C. de Oliveira, F. B. da Costa, C. H. Martins, T. C. de Carvalho, T. S. Porto and R. C. Veneziani, *Z. Naturforsch. C*, 2008, **63**, 326–330.
97 E. L. Ghisalberti, *Fitoterapia*, 1997, **68**, 303–325.
98 R. Slimestad, A. Marston, S. Mavi and K. Hostettmann, *Planta Med.*, 1995, **61**, 562–563.
99 Y. Shi, B. Yu, I. D. Williams, H. H. Sung, Q. Zhang, J. Y. Liang, N. Y. Ip and Z. D. Min, *Planta Med.*, 2007, **73**, 84–90.
100 L. L. da Silva, M. S. Nascimento, A. J. Cavalheiro, D. H. Silva, I. Castro-Gamboa, M. Furlan and V. S. Bolzani, *J. Nat. Prod.*, 2008, **71**, 1291–1293.
101 K. Horiuchi, S. Shiota, T. Hatano, T. Yoshida, T. Kuroda and T. Tsuchiya, *Biol. Pharm. Bull.*, 2007, **30**, 1147–1149.
102 G. M. Woldemichael, M. P. Singh, W. M. Maiese and B. N. Timmermann, *Z. Naturforsch. C*, 2003, **58**, 70–75.
103 Z. Kowalewski, M. Kortus, W. Kedzia and H. Koniar, *Arch. Immunol. Ther. Exp.*, 1976, **24**, 115–119.
104 J. Liu, *J. Ethnopharmacol.*, 1995, **49**, 57–68.
105 A. Bishara, A. Rudi, I. Goldberg, Y. Benayahu and Y. Kashman, *Tetrahedron*, 2006, **62**, 12092–12097.
106 L. Mendoza, M. Wilkens and A. Urzua, *J. Ethnopharmacol.*, 1997, **58**, 85–88.
107 D. E. Townsend, N. Ashdown, S. Bolton, J. Bradley, G. Duckworth, E. C. Moorhouse and W. B. Grubb, *J. Hosp. Infect.*, 1987, **9**, 60–71.
108 K. Panthong, W. Pongcharoen, S. Phongpaichit and C. T. Walter, *Phytochemistry*, 2006, **67**, 999–1004.
109 S. Boonsri, C. Karalai, C. Ponglimanont, A. Kanjana-opas and K. Chantrapromma, *Phytochemistry*, 2006, **67**, 723–727.
110 J. Heilmann, R. Brun, S. Mayr, T. Rali and O. Sticher, *Phytochemistry*, 2001, **57**, 1281–1285.
111 S. B. Singh, H. Jayasuriya, J. G. Ondeyka, K. B. Herath, C. Zhang, D. L. Zink, N. N. Tsou, R. G. Ball, A. Basilio, O. Genilloud, M. T. Diez, F. Vicente, F. Pelaez, K. Young and J. Wang, *J. Am. Chem. Soc.*, 2006, **128**, 11916–11920.
112 B. K. Hwang, S. W. Lim, B. S. Kim, J. Y. Lee and S. S. Moon, *Appl. Environ. Microbiol.*, 2001, **67**, 3739–3745.
113 S. Kanokmedhakul, K. Kanokmedhakul, P. Nasomjai, S. Louangsysouphanh, K. Soytong, M. Isobe, P. Kongsaeree, S. Prabpai and A. Suksamrarnr, *J. Nat. Prod.*, 2006, **69**, 891–895.
114 G. M. Cragg, D. J. Newman and K. M. Snader, *Nat. Prod. Drug Discovery Dev.*, 1997, **60**, 52–60.
115 J. L. Stern, A. E. Hagerman, P. D. Steinberg and P. K. Mason, *J. Chem. Ecol.*, 1996, **22**, 1887–1899.

