Antimicrobial natural products from *Plectranthus* plants

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The growing crisis of antibiotic-resistant infections urges for new antimicrobial agents with novel modes of action. A renewed interest in natural product scaffolds as a source of antimicrobial drug candidates has grown in the recent years. Medicinal plants have long been recognized as a valuable resource of natural antimicrobial compounds that can lead to new and more effective therapeutic agents. This review summarizes briefly the efforts taken in the screening, isolation, identification and evaluation of antimicrobial activities of the diterpenes isolated from *Plectranthus* plants having the potential to become promising anti-infective agents.

Keywords antimicrobial activity; natural products; *Plectranthus* spp.; diterpenes

1. Introduction

1.1. Drug resistance and the antibiotic crisis

Infection diseases are still among the main causes of mortality in developing countries while healthcare-acquired infections (HAI) with resistant microorganisms are a major cause of death worldwide [1, 2]. The successes of the golden age (1955-1985) of antibiotics, which revolutionized medicine, saving millions of human lives, led to a considerable excitement until antibiotic resistance began to emerge. The use and misuse of the antimicrobial agents over the past decades have further stressed this intrinsic resistance ability [1, 2]. The human pathogen *Staphylococcus aureus* that is a commensal microorganism is commonly cited as being a major hospital-acquired (nosocomial) pathogen [1]. Strains of this species that are resistant to β-lactams, namely the methicillin-resistant *Staphylococcus aureus* (MRSA) strains, have been described from clinical sources for over forty years. As a result, the glycopeptide vancomycin has become the antibiotic of last resort for the treatment of Gram-positive bacterial infections in hospitals. However, the occurrence of a fully vancomycin resistant strain of MRSA, in the US in 2002, indicates that the successful treatment of MRSA strains by the use of this glycopeptide antibiotic is not guaranteed [1]. Actually, the emergence of nosocomial vancomycin-resistant *Staphylococcus aureus* (VRSA), vancomycin-resistant *Enterococcus* (VRE) and multidrug-resistant (MDR) strains of these microorganisms have become a common occurrence [3, 4].

Antimicrobial resistance to conventional antibiotics is attributed to the altered bacterial growth phase, in particular, the decreased susceptibility by halted division, genetic polymorphisms, and over-expression of efflux pumps. At the beginning of the millennium, the World Health Organization (WHO) had identified antimicrobial resistance as a public health threat, a consequence of the rapidly emergence and spread of drug resistant pathogens that cannot be treated with currently available antibiotics [2]. Meanwhile the pipeline for new antimicrobial drugs is almost empty, once the development of antimicrobial drugs gives a low return on investment, contributing to the current crisis in fighting against drug-resistant microorganisms. The design and the discovery of antimicrobial drugs with new or combined mechanisms of action to reduce the likelihood of acquired resistance and with improved efficacy are thus urgent requirements.

1.2. Antimicrobial drug discovery from medicinal plants

Natural products are compounds with a wide structural diversity and remain an important source of new chemical entities, new drug prototypes and new drugs, despite the recent interest in molecular modelling, combinatorial chemistry, and diverse synthetic chemistry technologies by pharmaceutical companies and funding organizations [5-12]. Newman and Cragg [7] that accompanied, for more than 30 years, the evolution of natural products as sources for new drugs believe that, though a multidisciplinary approach to drug development is needed, the exploration of natural products as sources of new bioactive agents is indubitable actual.

Medicinal plants have in the last few decades been the subject of intense pharmacological studies, many concerning to their antimicrobial action [13-22]. About 80% of the population in developing countries rely on traditional medicine for their primary health care and, additionally, a market of minimally processed medicinal plants exists in Europe and America [14]. Diterpenes, a type of natural products widespread in the botanical Lamiaceae family, are either well represented in the *Plectranthus* genus. The diterpenes, owing to a great structural variability and a diverse range of oxidised patterns may be promising sources of antimicrobial prototype compounds [23-30]. The analysis of the

antimicrobial activities of closely related diterpenes may offer insights into the structural requirements for the bioactivity of these compounds and, therefore, contribute to the design of new antimicrobial molecules [16, 21, 31].

1.3. Scope of the review

The pressing need to discover and characterize novel and more effective antibiotics, preferably with new modes of action, to overcome drug resistance is driving exploitation of plant sources as antimicrobials. The rationale is based on the fact that plants produce natural compounds as a chemical defence against microbes of their environment. This review will focus on the antimicrobial activity of natural products – secondary metabolites – obtained from *Plectranthus* species, which belong to the Lamiaceae family also known as the Mint family. Some species are medicinal plants with potential use in primary healthcare, which have aroused considerable interest in the exploration of their potential medicinal and economic uses [14, 20, 25]. The diterpene metabolites isolated from *Plectranthus* plants that displayed antimicrobial properties will be highlighted as promising antimicrobial prototype compounds – lead compounds – to design and synthesize new anti-infective agents.

2. *Plectranthus* as promising medicinal plants

2.1. Distribution

Plectranthus is a genus belonging to the Mint family with about 350 known species which are widely distributed in Africa, Madagascar, India, Australia and a few Pacific islands [25]. Some of the African native *Plectranthus* species were, probably, brought to the New World after the pioneer voyages of the Portuguese discoverers in the 16th century, a few being used, for example, in the traditional medicine of Brazil [25]. The genus *Plectranthus* was first described by the French botanist L´Heritier in 1788 [25] and since then the number of species there included have been increasing [32]. Nowadays, *Plectranthus* plants (Fig. 1) are known all over the world due to their horticultural uses since they are fast-growing, produce lovely flowers and are resistant to most pests and plant diseases.

Fig. 1 Images of (a) *Plectranthus ornatus*; (b) *P. grandidentatus*; (c) *P. hadiensis*.

2.2. Ethnopharmacological uses

A number of *Plectranthus* species are plants of medicinal interest and their ethnobotanical uses have recently been carefully reviewed [25]. Traditional herbal preparations are used against a vast array of complaints including skin, respiratory, digestive and infectious ailments [14, 25]. It may be emphasized the wide use of *P. barbatus* for the treatment of several infections in Africa, Brazil and India [25]. Some plants are said to have antimicrobial (*P. barbatus*, *P. amboinicus*, *P. fruticosus*, *P. ecklonii* and *P. montanus*) and antiviral (*P. barbatus*, *P. amboinicus*, *P. lexiflorus*, *P. glandulosus* and *P. parviflorus*) activities [25]. This medicinal use comprises the treatment of gastrointestinal, genitourinary, eye or ear infections. The targeted infectious pathogens may be bacteria, mycobacteria, fungi, protozoa or Herpes and HIV virus [25, 29].

2.3. Bioactive metabolites

The isolation of the secondary metabolite compounds from the *Plectranthus* spp. is important to validate the popular uses of these plants. The bioactive components, with an emphasis on the antimicrobial ones, reported by the majority of the phytochemical studies on these species, are mostly diterpene compounds. It is of utmost interest to gather scientific information about the contribution of these metabolites for the bioactivity of *Plectranthus* species [25, 27-30, 33].

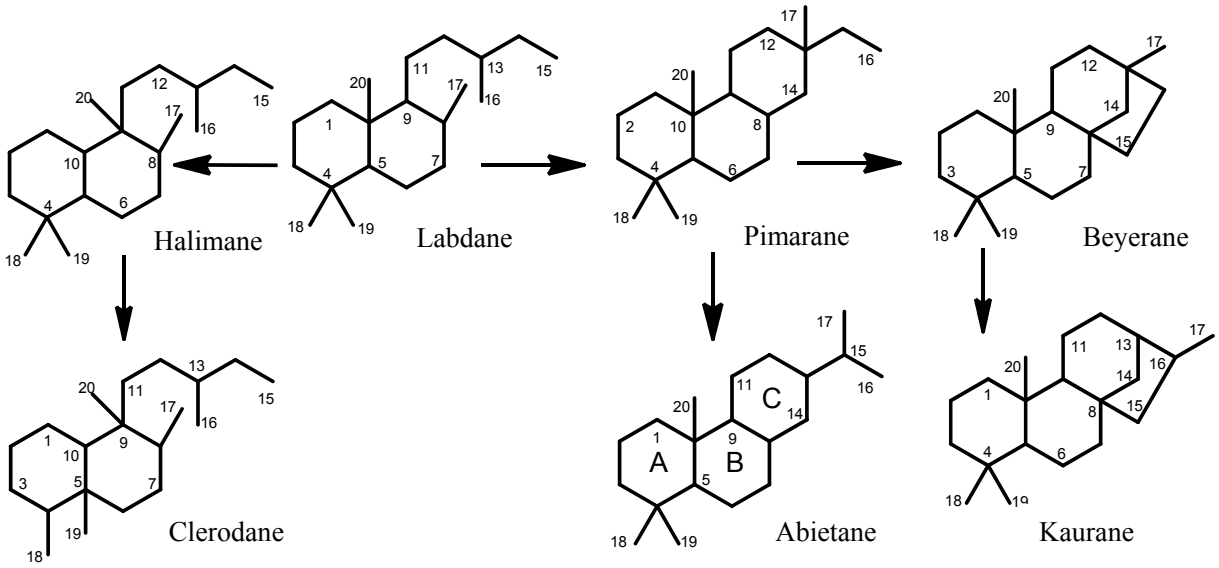
Diterpenes are a major terpene type of plant metabolites with diverse roles in the plant growth and development, and in the resistance to environmental stresses, which are also of chemotaxonomical interest [23, 24, 34]. Some of the most well-known bioactive diterpenes are taxol (anti-cancer properties), phorbol esters (tumour promoters), ginkgolides (platelet-activating factor inhibitors) and forskolin that was isolated from *P. barbatus* (synonym *Coleus forskohlii*), one

of the most studied *Plectranthus*-derived compounds [24, 25]. This diterpene shows a wide range of pharmacological properties, including cardiotonic, bronchodilator, antihypertensive, anti-inflammatory, platelet aggregation inhibitory effects and also stimulation of adenyl cyclase activity, which may explain many of the assorted medicinal uses of *P. barbatus* [24, 25, 35].

3. Antimicrobial diterpenes from *Plectranthus* spp.

3.1. Diterpene chemical structures

Diterpenes are natural compounds with a hydrocarbon skeleton with twenty carbon atoms, corresponding to four isoprene unities – a five carbon chemical structure –, and so belonging to the large terpene metabolite class. They may be acyclic but mostly they are cyclic compounds and, importantly, have high and differently oxygenated chemical features. Diterpenes are the second largest type of terpenes, with over two thousand compounds belonging to more than one hundred distinct skeletal structures [24]. It is the diversity of cyclisation mode (bicyclic, tricyclic, tetracyclic or macrocyclic) of the hydrocarbon skeleton allied to a wide range of oxygen functional groups (e.g. hydroxyls, carbonyls, epoxydes, quinones, acids and acid derivatives) that sets the multiplicity of pharmacological abilities of the diterpenes. In the Lamiaceae family more than ninety different cyclic diterpene skeletons are known [23, 34]. In Scheme 1 it is represented the biosynthetic grid of the diterpenes, classified according to their skeleton, found in the *Plectranthus* species – abietane, beyerane, clerodane, halimane, kaurane, labdane and pimarane.



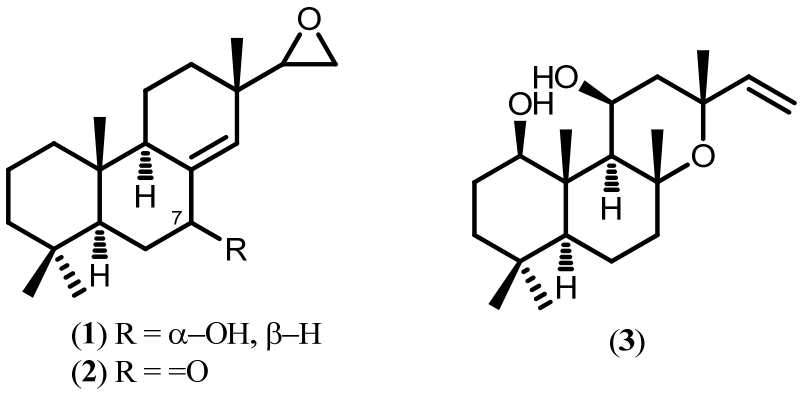
Scheme 1 Metabolic pathway for common diterpene skeletons found in *Plectranthus* spp.

Abietanes are tricyclic diterpenic secondary metabolites with high occurrence and the most widespread in the Lamiaceae family. They have been often isolated from *Plectranthus* spp. [30, 33].

3.2. Diterpenes with antimicrobial activity

3.2.1. Pimarane, labdane, neoclerodane, halimane and abietane diterpenes

The ability of some *Plectranthus* species to produce antimicrobial metabolites led to several phytochemical studies. Gibbons S. *et al.* investigated the chemistry and antibacterial activity of the extracts from *Plectranthus ernstii* [36] and have isolated three antimicrobial diterpenes. Two of them were the pimaranes 15,16-epoxy-7α-hydroxypimar-8,14-ene (**1**) and 15,16-epoxy-7-oxopimar-8,14-ene (**2**), and the other the labdane 1,11-dihydroxy-8,13-epoxylabd-14-ene (**3**) (Fig. 2). Compound (**1**) exhibited a moderate antibacterial activity against a range of MRSA and MDR *S. aureus* strains with a minimum inhibitory concentration (MIC) value of 32 μg/mL. All the three diterpenes exhibited antimycobacterial activity. The compound (**1**) with a hydroxyl group on C-7 is more active against *Staphylococcus* than compound (**2**) that has a carbonyl group on C-7 probably as a result of an increased lipophilicity and a lesser cell uptake [36].

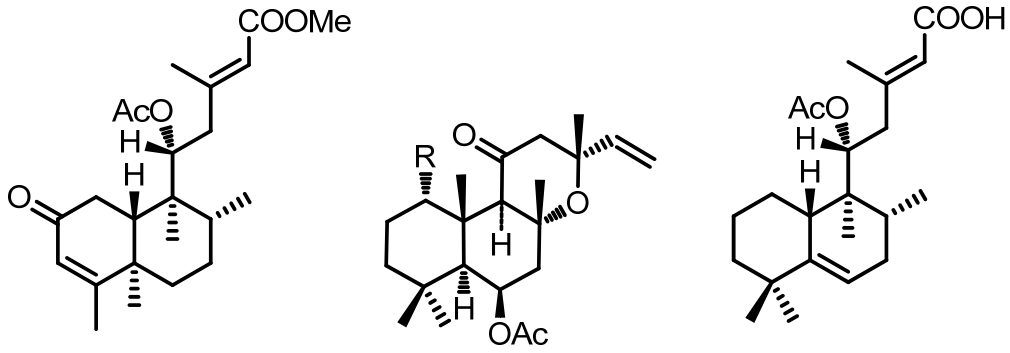


(**1**) R = α–OH, β–H
(**2**) R = =O

(**3**)

Fig. 2 Antibacterial diterpenes isolated from *P. ernstii*.

Several diterpenes were isolated [37-41] from *P. ornatus* Codd. (synonym *P. comosus* Hochst. ex Gürke) namely a neoclerodane derivative, plectrornatin A (**4**) [37, 38] and two labdane derivatives, plectrornatins B (**5**) and C (**6**) [37] (Fig. 3). While plectrornatin B was devoid of antimicrobial activity, the plectrornatins A and C showed low activity against *Candida albicans* [37] (Table 1). The new halimane diterpene, 11-acetoxyhalima-5,13-dien-15-oic acid (**7**) (Fig. 3) was also isolated and exhibited antibacterial activity against several *Staphylococcus* and *Enterococcus* strains (MIC 15.62-62.50 μg/mL) [40, 41] (Table 1). Some derivatives of **7** were prepared but they showed to be less active against the same bacteria, than the prototype halimane **7** [41]. Diterpenes with a halimane skeleton are rarely isolated and their pharmacological properties remain scarcely known. In Brazil, the leaves of *P. ornatus* are used for stomach and liver diseases as substitute of *P. barbatus* (both known as 'boldo'). Both are used for their antibiotic and anti-inflammatory properties [25, 38].



(**4**)

(**5**) R = OH
(**6**) R = OAc

(**7**)

Fig. 3 Diterpenes isolated from *P. ornatus*.

From the ethyl acetate extract of the *P. ecklonii* plants were isolated two known abietanes, parvifloron D (**8**) and parvifloron F (**9**) [42] (Fig. 4). Van Zyl *et al.* [43] also isolated those two abietane diterpenes (**8**, **9**) from a *P. ecklonii* dichloromethane extract. In another study parvifloron D (**8**) was also isolated together with sugiol (**10**) (Fig. 4) from the acetone extract of *P. ecklonii* [44]. Both **8** and **9** compounds were active against *Listeria monocytogenes* [45]. Parvifloron D (**8**) inhibited the growth of *S. aureus*, *Enterococcus faecalis* [44] and *M. tuberculosis* [45] (Table 1). In addition, both compounds were evaluated as antiplasmodial agents and both inhibited β-haematin formation [43]. *P. ecklonii* is used in Zimbabwe to treat skin infections and in South African Republic to treat meningitis [45]. *P. ecklonii* aqueous extracts also showed an inhibitory effect on the biofilm formation of *Strepcococcus sobrinus* and *S. mutans*, which was attributed to the presence of the phenolic rosmarinic acid [46].

Parvifloron D (**8**), after treatment with acid-washed molecular sieves, yielded the rearranged abietane **11** that is the 2β-(4-hydroxy)benzoyloxy derivative of the microstegiol (**12**) (Fig. 4). This derivative **11** showed antibacterial activity against some *Staphylococcus* and *Enterococcus* strains with MIC values in the range 3.91-7.81 μg/mL [44] (Table 1).

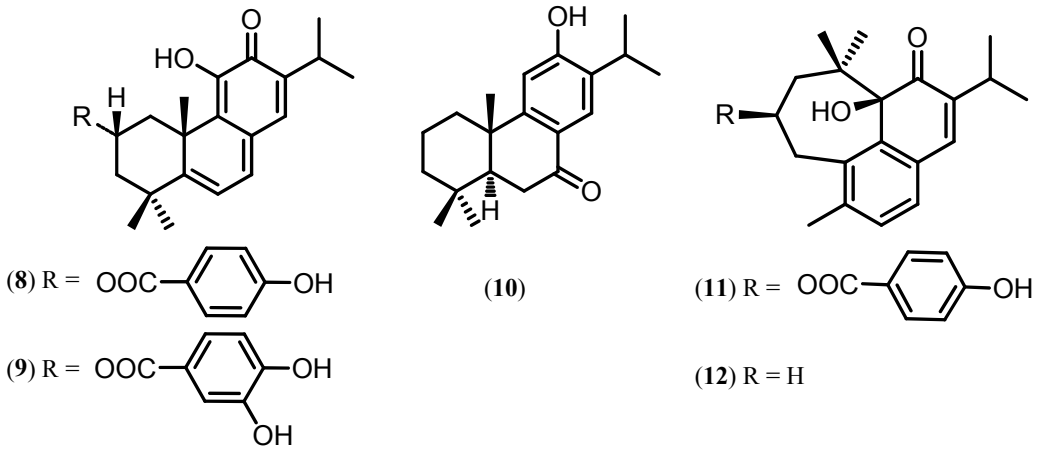


Fig. 4 Antimicrobial diterpenes isolated from *P. ecklonii*.

3.2.2. Royleanone abietanes

Royleanones are abietane-12-hydroxy-11,14-dione diterpenes which have frequently been identified from *Plectranthus* plant extracts. Horminone (**13**) (Fig. 5) is known as an antibacterial abietane and was isolated from *Plectranthus* spp. [47-50]. Its 16-*O*-acetoxy derivative (**14**), isolated from *P. hereroensis*, also shows antibacterial activity [49]. 7α-Acetoxy-6β-hydroxyroyleanone (**15**) (Fig. 5) is a bioactive abietane that was isolated from *P. grandidentatus* and *P. hereroensis* [33, 47, 48, 51-54] and is a common metabolite found in *Salvia* species [22, 26]. Royleanone **15** has been extensively studied showing a variety of biological activities, including antimicrobial activity against *Staphylococcus* and *Enterococcus* [47, 52], and *Mycobacterium* [51] species. Its *in vitro* antiproliferative activity against several human cancer cell lines [54] and T-lymphocyte proliferation inhibition [54] were also studied. Table 1 summarizes the antimicrobial activity of some of the diterpenes discussed until now, against selected human pathogens of healthcare interest.

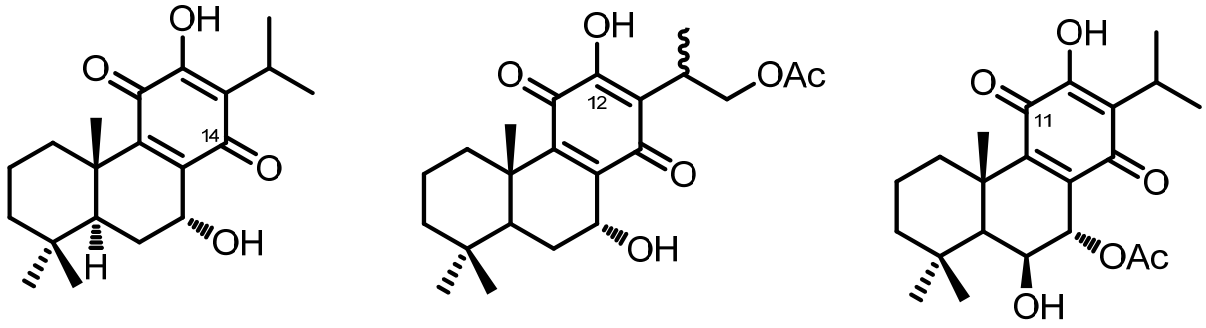


Fig. 5 Antimicrobial abietane royleanones isolated from *P. grandidentatus* and *P. hereroensis*.

Quinone-hydroquinone compounds are sources of free radical reactions that may cause cell damage, they also may act as Michael acceptors that may complex with cellular macromolecular sites, such as proteins and nucleic acids. All bioactive royleanones, being quinone compounds, may act as antiproliferative against prokaryote and eukaryote cells due to these biomechanisms of action. The broad activities shown and the low MIC values (3.12-15.63 μg/mL), revealed in the inhibition of Gram-positive microorganisms, allied to their abundance in plant sources drove to the selection of 7α-acetoxy-6β-hydroxyroyleanone (**15**) as antimicrobial lead compound. It is of utmost interest to know how royleanones cross cell membranes and identify the mechanisms of action by which they interact with the prokaryote and eukaryote cell macromolecules. Further studies and antiproliferative assays on a wide set of new derivatives will answer to these issues and give information about their antimicrobial activity *versus* cell mammalian toxicity.

Table 1 Antimicrobial diterpenes isolated from *Plectranthus* spp.

Active diterpene	Plant	Microorganism	MIC[a] (μg/mL)	Ref.
15,16-Epoxy-7α-hydroxypimar-8,14-ene (**1**)	*P. ernstii*	*S. aureus* ATCC 25923	32	[36]
		S. aureus RN4220	32	[36]
		S. aureus SA-1999B	32	[36]
		S.aureus XU-212	32	[36]
		M. smegmatis	16	[36]
		M. phlei	8	[36]
15,16-Epoxy-7-oxopimar-8,14-ene (**2**)	*P. ernstii*	*M. smegmatis*	128	[36]
		M. phlei	64	[36]
1,11-Dihydroxy-8,13-epoxylabd-14-ene (**3**)	*P. ernstii*	*M. smegmatis*	128	[36]
		M. phlei	128	[36]
Plectrornatin A (**4**)	*P. ornatus*	*C. albicans* CIP 3153A	62.5	[37]
Plectrornatin C (**6**)	*P. ornatus*	*C. albicans* CIP 3153A	62.5	[37]
11-Acetoxyhalima-5,13-dien-15-oic acid (**7**)	*P. ornatus*	*S. aureus* ATCC 25923	31.25	[41]
		S. aureus CIP 106760	15.63	[41]
		E. faecalis ATCC 51299	15.63	[41]
		E. faecalis CIP 104476	62.50	[41]
		E. flavescens ATCC 49996	15.63	[41]
Parvifloron D (**8**)	*P. ecklonii*	*S. aureus* ATCC 43866	15.62	[44]
		S. aureus CIP 106760	15.62	[44]
		E. faecalis ATCC 51299	7.81	[44]
		M. smegmatis	39.06	[45]
		M. tuberculosis	15.6	[45]
		Listeria monocytogenes	190	[45]
Parvifloron F (**9**)	*P. ecklonii*	*M. smegmatis*	39.06	[45]
		M. tuberculosis	31.2	[45]
		Listeria monocytogenes	95	[45]
2β-(4-Hydroxy)benzoyloxy-microstegiol (**11**)	prepared from **8**	*S. aureus* ATCC 43866	3.90	[44]
		S. aureus CIP 106760	7.81	[44]
		S. epidermis ATCC 12228	7.81	[44]
		E. faecalis ATCC 51299	7.81	[44]
		E. hirae ATCC 10541	7.81	[44]
Horminone (**13**)	*Plectranthus* spp.	*S. aureus* ATCC 65388	6.5	[47]
		S. epidermis ATCC12226	1.5	[47]
		Bacillus subtilis ATCC 6633	1.5	[47]
		E. faecalis ATCC 29212	14	[47]
16-Acetoxy-7α-hydroxy royleanone (**14**)	*P. hereroensis*	*S. aureus* ATCC 25923	31.2	[49]
		V. cholerae ATCC 11623	15.6	[49]
7α-Acetoxy-6β-hydroxyroyleanone (**15**)	*P. grandidentatus*, *P. hereroensis*	*S. aureus* ATCC 25923	31.25	[52]
		S. aureus ATCC 43866	15.63	[52]
		S. aureus CIP 106760	7.81	[52]
		E. faecalis ATCC 51299	15.63	[52]
		E. casseliflavus ATCC 49996	7.81	[52]
		M. tuberculosis H_{37}Rv ATCC 27294	25	[51]
		M. tuberculosis MDR	3.12	[51]

[a] MIC value is the minimum inhibitory concentration of the compounds effecting 100% of inhibition.

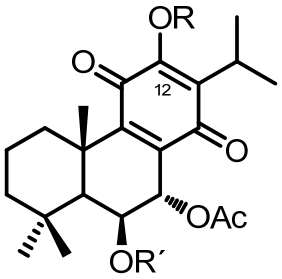
3.3. Structure-activity relationship analysis (SARA)

Abietane diterpenes, and in particular the oxidized abietanes as royleanone diterpenes, have multiple pharmacological activities as previously stated. However, relationships between the chemical features of the royleanones and their bioactivity are rare including the structure-activity relationships (SARs) studies that may influence their antimicrobial activities. In general, all the antimicrobial compounds referred in this text have a lipophilic framework bearing polar oxygenated chemical functions. Comparing this characteristics with former research works that aimed to know the chemical substructures contributing and/or enhancing the antimicrobial activity of other diterpenes [31, 55] it was expectable they have a similar behaviour. This means that the hydrophobic hydrocarbon skeleton, corresponding to the three A, B and C rings (Scheme 1), will be required for the insertion of the royleanones into the prokaryotic cell membrane. Additionally, oxygenated functions such as hydroxyl, carbonyl or esters behave either as hydrogen bond acceptor or hydrogen bond donors and interact with hydrogen bond donors or hydrogen bond acceptor groups, respectively, of the cell membrane [31, 55].

Further SARA of natural antimicrobial royleanones, isolated from *P. grandidentatus* and *P. hereroensis*, led to the deduction that the presences of a 12-hydroxy-*p*-benzoquinone feature in the C ring plus an oxygen atom on the C-6/C-7 positions of the B ring were important for the antibacterial activity [47]. Seeking to modulate the antimicrobial activity of the natural royleanone **15** (7α-acetoxy-6β-hydroxyroyleanone, Fig. 5) and to extend the SARA studies some derivatives were prepared [51, 52]. Benzoyl derivatives were prepared having in mind the findings that the 12-benzoylhorminone was more active than horminone (**13**) against *S. aureus* and *Streptococcus faecalis* [22] and that the addition of a benzyl group to some phenolic abietanes influenced their anti-MRSA activities [56]. Therefore, aromatic mono- and diester derivatives (Fig. 6) of the promising antimicrobial lead **15** were synthesized and their activity evaluated against a panel of *M. tuberculosis* H_{37}Rv and MDR-TB, and MRSA and sensitive *S. aureus* (MSSA), and sensitive *Enterococcus* and VRE strains [51, 52]. However, the chromophoric system of the lead royleanone **15**, i.e. the 6-*O*-hydroxy-7-*O*-acetoxy-12-*O*-hydroxy-11,14-dioxo-quinone motif (Fig. 5 and 6), was kept once it was observed that only natural diterpenes with a quinone framework showed antimicrobial activity against TB, as reported in reference [51]. The modulation of lipophilicity was achieved by esterification at HO-C_6 and/or HO-C_{12} (Fig. 6).

The aromatic monoesters 12-chlorobenzoyl, 12-methoxybenzoyl and 12-nitrobenzoyl esters (**16-18**), along with the 6,12-dibenzoyl ester (**19**), were found to be active (MIC 0.39–3.12 μg/mL) against a MDR TB strain and more active than the lead **15** (Table 2). All were more active than the first-line antituberculostatic isoniazid and rifampicin drugs [51]. Considering the potency and the selectivity observed for royleanone derivative **17**, it was proposed as a new prototype compound for modulation of its antimycobacterial activity [51] (Table 2).

Interestingly, the ester derivatives **16** and **17** were also the most active against the Gram-positive human pathogens assayed in a study carried out by Simões MF group [52] (Table 2). Another derivative of **15**, 7α-acetoxy-6β-propionyloxy-12-*O*-propionylroyleanone (**20**), was equally active against the five MSSA and MRSA, and against the four sensitive and low resistant *Enterococcus* strains assayed (MIC 3.91–7.81 μg/mL) [52]. Considering the potency (median value of MIC 4.8 μg/mL) (Table 2) and the selectivity [51] the 12-*O*-(4-chloro)benzoyl derivative **16** was suggested as a new lead suitable for further modulation of the antibacterial activities against *S. aureus* and *Enterococcus* strains [52].



(**16**) R = 4-Cl-C_6H_4-CO, R´ = H
(**17**) R = 4-MeO-C_6H_4-CO, R´ = H
(**18**) R = R´ = PhCO
(**19**) R = R´ = PhCO
(**20**) R = R´ = CH_3CH_2CO

Fig. 6 Antimicrobial semi-synthetic royleanone derivatives.

The introduction of extra-lipophilic features in the structure of **15** led to more active derivatives, especially in the case of **17** against MDR TB and in the case of **16** against *S. aureus* and *Enterococcus* spp., seems to increase the hydrophobic interactions with the assayed microorganisms targets. Moreover, in the small set of the derivatives studied

it appears that the methoxy group of the 12-methoxybenzoyl ester **17** provides a beneficial extra hydrogen bond acceptor and that the extra chlorine atom of the 12-chlorobenzoyl ester **16** brings a more efficient lipophilic interaction with, respectively the MDR TB and the Gram-positive bacteria assayed.

Table 2 Antimicrobial semi-synthetic diterpenes (**16-20**) prepared from lead royleanone (**15**).

Compound / Microorganisms[a)]	(15)	(16)	(17)	(18)	(19)	(20)	Ref.
	MIC (μg / mL)						
M. tuberculosis $H_{37}Rv$	25	25	3.12	> 25	> 25	25	[51]
MDR TB	3.12	0.78	0.39	3.12	1.56	6.25	[51]
MSSA	15.63-31.25	3.91-15.63	3.91-62.50	7.81-62.50	15.63-62.50	7.81-15.63	[52]
MRSA	7.81	3.91-7.81	3.91	15.63	15.63-125	3.91-7.81	[52]
E. faecalis	15.63	3.91	1.95	1.95	7.81	3.91	[52]
VRE (low)	7.81	1.95	0.98	3.91	7.81	3.91	[52]
E. casseliflavus	7.81	0.98	3.91	3.91	7.81	7.81	[52]
E. faecium	15.63	1.95	0.98	3.91	15.63	3.91	[52]

[a)] *M. tuberculosis* $H_{37}Rv$ ATCC 27294; MDR TB: multidrug-resistant *M. tuberculosis*, a clinical isolate [51]; MSSA: *S. aureus* ATCC 25925, ATCC 43866 and ATCC 700699; *E. faecalis* ATCC 51299; low VRE: *E. faecalis* FFHB 427483; *E. casseliflavus* ATCC 49996; *E. faecium* FFHB 435628; FFHB species are clinical isolates from Hospital do Barreiro and deposited on the Microbiology Laboratory of the Faculty of Pharmacy at the University of Lisbon [52].

4. Mode of action of antimicrobial diterpenes

The mode of action of the diterpenes here reported on prokaryote cells is unknown. Only a few associations with the rare studies describing other antimicrobial diterpenes may be compiled. The general conclusion is that the twenty carbon skeletons arranged in a bicycle or tricycle manner is necessary to guarantee the hydrophobic interactions with the microbial targets that remain unknown. A few examples have enhanced the benefit of an increasing lipophilic property on the rise of the antimicrobial activities. Hydrophilic features brought by the oxygen chemical functions, such as alcoholic, phenolic, ketone, quinone, carboxyl acid and ester groups, may determine the bioactivity. Their ability to establish hydrogen bonds, as donor or acceptor atoms, with the microbial targets and the importance of their position on the hydrocarbon skeleton have been studied and handful conclusions were reported [31, 57-68]. Such are the cases of abietic acid, totarol behaving as efflux pump inhibitors, and horminone (**13**) exhibiting bacteriostatic activity [31, 55, 56, 58-61, 67, 68].

As previous referred, the postulated microbial targets of the studied antimicrobial diterpenes, which show an amphipathic character, might be the cytoplasmic membrane and, if the membrane is penetrated, inside the cell the macromolecules such as the ribosomal RNA, as described for horminone (**13**), inhibiting the protein synthesis [67, 68].

5. Conclusion

The antimicrobial drug resistance is a natural and predictable phenomenon requiring for its management a continuous effort in the antimicrobial drug discovery to reduce the crisis of pathogen resistance. The medicinal plants and the natural plant metabolites, above all, are fundamental sources of new chemical entities that hopefully will be promising drug candidates in key therapeutic areas such as the infectious diseases. In the quest for new antimicrobials, the authors expect that the development of novel antimicrobial agents from *Plectranthus* metabolites will circumvent resistance and thus contribute to the development of novel therapeutic agents with potent activities and appropriate mechanisms of action against a range of key pathogens, allied to an optimum therapeutic index.

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