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Macrolides from rare actinomycetes: Structures and bioactivities

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Abstract

Rare actinomycetes are the sources of numerous biologically active secondary metabolites with diverse structures. Among them are macrolides, which have been shown to display several antibiotic activities. In this review, twenty-six groups of macrolides from rare actinomycetes are presented, with their bioactivities and structures of representatives from each group. It has been divided according to the classes of macrolides. The most interesting groups with a wide range of biological activities are ammocidins, bafilomycins, neomaclafungins, rosaramicins, spinosyns, and tiacumicins. Most macrolides are obtained from genus, *Micromonospora*, with smaller contributions from genera such as *Saccharothrix*, *Amycolatopsis*, *Nocardiopsis* and *Catenulispora*. These macrolides display unique cytotoxic, antibacterial, antifungal, antimicrobial, insecticidal, anti-trypanosomal, antimalarial, antiprotozoal, antimycobacterial and anti-herpetic activity. Based on their noticeable bioactivities and diverse structures, macrolides from rare actinomycetes deserve to be investigated further for future applications in medicine. This work highlights the bioactivities and structures of important classes of macrolides from rare actinomycetes, which could be used in medicine in the future or which are already in the market.

Keywords: Rare actinomycetes; Macrolide; Cytotoxic; Antibacterial, Antifungal, Antimicrobial

1. Introduction

Actinomycetes are well known as sources of novel bioactive compounds with significant therapeutic applications [1]. They are common micro-organisms existing

in terrestrial and most aquatic ecosystems with extreme diversity and more than 350 genera are known to date. They are generally unicellular, aerobic and Gram-positive bacteria, with a high content of guanine–cytosine (more than 60%) in their DNA. Communities of actinomycetes are one of the dominant bacterial phyla in soil, with about one millions cells per gram of soil. Species from the genus *Streptomyces* account for >95% of all actinomycete strains isolated from soil. Many of the antimicrobial compounds used today were originally identified from actinomycetes, especially from the genus *Streptomyces* [2, 3]. Recently, it has become more difficult for researchers to obtain novel compounds with potential antibacterial activity from *Streptomyces*, because of increasing resistance to these compounds. On the other hand, many successful antibacterial compounds, including macrolides, have been produced using rare actinomycetes [4].

The strains of actinomycetes, whose isolation frequencies are much lower than strains within *Streptomyces* isolated by traditional methods, are normally named as rare actinomycetes or the non-*streptomycete* actinomycetes [5]. Rare actinomycetes are widely distributed in both terrestrial and marine ecosystems. The environmental conditions are extremely different between both ecosystems. It is accepted that marine rare actinomycetes represent significant sources of novel secondary metabolites because of the extreme and vast diversity of environmental conditions. To accelerate the identification and isolation of rare actinomycetes, our understanding the distribution of such untapped groups of micro-organisms must be expanded [5]. The highest numbers of the bioactive compounds isolated from rare actinomycetes were obtained from the genera *Micromonospora*, *Salinispora* and *Nocardiopsis*, with smaller numbers from other genera such as *Amycolatopsis* and *Saccharopolyspora*.

The richest source of distinctive natural products has been derived from the different members of the genus *Micromonospora* [6, 7].

A number of bioactive compounds have been isolated and identified from this group of organisms, including classes such as aminoglycosides, oligosaccharides, macrolides, peptides, anthracyclines, lactones, amino-acids, nucleosides, ansamycins, polycyclic ethers, polyenes and terpenoids. Herein, we focused on macrolides as a promising group of bioactive compounds.

Macrolides get their name from their macrocyclic lactone ring, which may be attached to one or more sugar moieties *via* glycosidic bonds. The ring usually contains between twelve and sixteen atoms, but it can be much larger and is substituted by hydroxy and / or alkyl groups. Macrolides can be classified based on the number of atoms in the macrocyclic ring lactone. The most important macrolides, in terms of bioactivity, are 14-, 15- and 16-membered-ring compounds [8, 9]. Macrolides from rare actinomycetes have been partly reviewed, with more focus on *Micromonospora* spp. as this genus has proven to be a specific source for these compounds. It is strongly believed that outstanding macrolides from rare actinomycetes deserve to be investigated further for future applications in medicine [8, 10]. Most of the isolated macrolides possess a range of biological activities, including cytotoxic, anti-trypanosomal, antimycobacterial, antibacterial, antifungal, antimalarial, antimicrobial ect. However, few of them are used in medicine field [10]. This review focuses on bioactive macrolides obtained from rare actinomycetes, classifies them in terms of their chemical structures, and covers the literature published until mid-2021. A total of 193 macrolides, in 26 groups, derived from fifteen known and two unidentified rare

actinomycete are presented. Chemical structures of representatives from each group; the sources and biological activities of the bioactive macrolides are also presented.

2. Chemical and biological diversity of macrolides from rare actinomycetes

2.1. Isolation and cultivation of rare actinomycetes

Rare actinomycetes inhabit different ecological niches; although they have been mostly, isolated from soil they can also be found in sediments, stones, plants, water and different animals [11]. They are problematic to isolate from the environment and difficult to cultivate and maintain under traditional conditions, in contrast to the common *Streptomyces* species which can be isolated and cultivated very easily [5-6, 11]. They require different pre-treatment methods for isolation, selective fermentation media for the production of metabolites and use different strategies for selection methods [7]. For selective isolation of rare actinomycetes from soil samples, various physical and chemical pre-treatment techniques have been developed, in combination with different media. These treatments eliminate fast-growing bacteria, fungi, and common *Streptomyces.* Pre-treatment methods generally include dilution with deionised/distilled water, natural/artificial seawater, wet-dry heating, shaking with glass beads, freezing, exposure to ultrasonic waves and centrifugation before starting the inoculation. [11-12]. Treatment with chemicals, such as 1.5% phenol, in the presence of some selective antimicrobial agents like novobiocin or gentamicin reduces the number of unwanted bacteria and increases the number of rare actinomycete-like colonies. Other compounds which have been used effectively in chemical pre-treatment are benzethonium chloride, chlorhexidine gluconate and sodium dodecyl sulfate. Significant increases in the populations of rare actinomycetes colonies have occurred when sediment samples were treated with calcium carbonate,

as the powder alters the pH in favour of the growth of the culture. For selective isolation of marine rare actinomycetes, media low in nutrients have proven to be more efficient than nutrient-rich media. Different carbon and combined carbon-nitrogen sources, such as starch, chitin, oatmeal, casein, hair hydrolysate, *etc*., have been successfully supplemented to the isolation media for selective isolation of marine rare actinomycete taxa [12-15]. Several reviews have discussed these techniques in detail and limitations of space preclude further discussion here.

2.2. 9- and 10-Membered macrolides

2.2.1. Branimycins

Nargenicins and branimycins are a group of 9- or 10-membered antimicrobial macrolides which have been isolated from different bacterial species. Branimycin was initially isolated by the Laatsch research group from the streptomyces strain GW 60/1571. The structure of the only 9-membered macrolide in the nargenicin family was characterised by a *cis*-fused dehydrodecalin system with a unique oxygen-bridge [16, 17]. Two nargenicin macrolides, branimycins B (**1,** Fig. 1a) and C were identified from a culture of the rare actinomycete *Pseudonocardia carboxydivorans* M-227. Both compounds showed antibacterial activity against the Gram-positive bacteria *Corynebacterium urealyticum*, *Clostridium perfringens* and *Micrococcus luteus* with MIC values ranging from 1 to 16 µg mL^{-1} and lower activity against Gram-negative bacteria *Neisseria meningitides* with MIC values of 32 and 64 µg mL^{-1}, respectively. Branimycin B was additionally active against other Gram-negative bacteria, *Bacteroides fragilis*, *Haemophilus influenzae* and *Escherichia coli*, with MIC values ranging from 32 to 64 µg mL^{-1}. Moreover, branimycin C exhibited antimicrobial

activity against *Enterococcus faecalis* 10544 and *Staphylococcus aureus* ATCC 25923 with MIC values of 64 and 32 µg mL^{-1}, respectively [18, 19].

2.2.2. Saccharothriolides

Investigation on secondary metabolites from the rare actinomycete *Saccharothrix* sp. strain A1506 led to the isolation of the phenyl-substituted 10-membered macrolides saccharothriolides A–F. Only saccharothriolide B (**2**, Fig. 1a) showed weak antibacterial activity against *S. aureus* and moderate cytotoxic inhibitory effects towards human tumour cell lines, including HeLa and HT1080 with IC_{50} values of 17.9 and 13.9 µg mL^{-1}, respectively [20]. The phenolic hydroxy group and the stereochemical configuration at the carbon next to the lactone had a great impact on the cytotoxicity of the compound, as revealed by a structure-activity relationship study [21]. Saccharothriolide C2, along with the previously reported saccharothriolides, was produced by combined-culture of *Saccharothrix* sp. strain A1506 and a mycolic acid-containing bacterium, *Tsukamurella pulmonis* TP-B0596. The cytotoxic activity of saccharothriolide C2 was evaluated against human fibrosarcoma HT1080 cells but no activity was observed, even at 100 µM [22].

2.3. 12-Membered macrolides

2.3.1. Actinoallolides

Four 12-membered macrolides actinoallolides A-D, along with the 14-membered macrolide actinoallolide E, were isolated from the rare actinomycete *Actinoallomurus fulvus* strain MK10-036 extracted from the roots of the tabasco pepper, *Capsicum frutescens* [23]. The compounds had no antibacterial activity but showed moderate anti-trypanosomal activity against *Trypansoma brucei brucei* strain (IC_{50} 0.11−1.01 µg mL^{-1}). Actinoallolide A (3, Fig. 1b) displayed the most potent and selective anti-

trypanosomal activity *in vitro* towards *T. b. brucei* strain, with IC_{50} 4.9 ng mL^{-1}, without cytotoxicity against MRC-5 cells (IC_{50} > 100 µg mL^{-1}). Moreover, actinoallolidea A displayed anti-trypanosomal activity *in vitro* towards *Trypansoma b. rhodensiense* STIB900 and *T. cruzi* Tulahuen C4C8 strain, similar to the commonly used therapeutic drug, benznidazole, with IC_{50} values of 0.086 and 0.226 µg mL^{-1}, respectively. The IC_{50} values and the selectivity of actinoallolide A indicated that the ketone at C-21 and the five-membered hemiacetal may improve the anti-trypanosomal activity of these compounds [23,24].

2.3.2. Spinosyns

The insecticidal spinosyns are a group of natural products produced by *Saccharopolyspora spinosa*. They are 12-membered macrolides that contain tetracyclic lactones attached by glycosidic bonds to two deoxysugars, tri-O-methyl rhamnose and the amino-sugar forosamine [25]. Twenty-five spinosyns A-Y were isolated and reported from *Saccharopolyspora spinosa* strains, with spinosyn A (**4**, Fig. 1b) and spinosyn D as the major components [25-27]. Both compounds showed a high level of insecticidal activity and selectivity towards several crop pests such as southern armyworm (*Spodoptera eridania*) and tobacco budworm (*Heliothis virescens*). Additionally, spinosyns A and D appeared to cause little or no harm to a broad range of non-target insects and mammals [28]. A total of thirty-one structurally related insecticidal butenyl-spinosyns were discovered from cultures of the rare actinomycete *Saccharopolyspora pogona*. Most of these substances have a but-1-enyl group at C-21 of the macrolide ring, in place of the ethyl group of normal spinosyns. The insecticidal activities of butenyl-spinosyns are similar to that of the structurally related spinosyn A. This observation suggested that the side-chain at C-21, along with the forosamine and methyl rhamnose moieties, were critical for spinosyns to display

insecticidal activity, even though that activity can vary from one insect species to another [29, 30].

2.4. 14-Membered macrolides

2.4.1. Megalomicins

The rare actinomycete *Micromonospora megalomicea* produced the first 14-membered antibiotic macrolide from the genus *Micromonospora*. The structure of megalomicins consists of 14-membered lactone ring attached to two amino-sugars, rhodosamine, desosamine, and one dideoxysugar, mycarose. These compounds are structurally related to the antibiotic macrolide erythromycins produced by *Streptomyces erythreus* but differ at C-6 sugar moiety. The structure of the compounds was revised later and the amino-sugar at C-6 was renamed into L-megosamine based on detailed ^{13}C NMR and X-ray crystallographic analysis [31, 32]. Megalomicin A, B, C1 and C2 showed antibacterial, antiparasitic and antiviral activity. The antibacterial activities of these substances were evident against *S. aureus*, *Streptococcus faecalis* and *Streptococcus pyogenes*, with MICs ranging from 0.01 to 0.75 µg mL^{-1} [33]. Moreover, megalomicin A (**5**, Fig. 2a) showed strong-to-moderate antiparasitic activities against *T. cruzi*, *Plasmodium falciparum*, *T. brucei*, *Leishmania donovani* and *Leishmania major* with IC_{50} values of 0.2, 1, 2, 3, and 8 µg mL^{-1}, respectively. It also showed antiparasitic activity *in vivo* against *T. brucei* by slowing the replication of the parasitaemia which resulted from reducing its viability [34]. Furthermore, megalomicin C1 was found to have a potent antiviral activity against swine fever virus and herpes simplex virus type 1 (HSV-1) at 50 µM concentration [35]. The antiparasitic activity may be due to the amino-sugar moiety located at C-6, as erythromycin does not have this group in its structure and does not show any parasitic activity [36].

2.4.2. Rustmicins

Rustmicin (**6**, Fig. 2a) was originally obtained from *Micromonospora narashinoensis* strain 980-MC1. Shortly thereafter, an identical compound named galbonolide A was reported from the fermentation broth of *Streptomyces galbus*. NMR analysis showed that rustmicin lacks any sugar substituent. No antibacterial activity was reported for the 14-membered macrolide, but it showed potent fungicidal activity against the wheat stem rust fungus *Cryptococcus neoformans* at concentrations < 1 ng mL^{-1} [37-39]. It also displayed potent to moderate antifungal activity against *Candida tropicalis* MY1012 (MIC 0.03 µg mL^{-1}) and *C. albicans* MY1055 (MIC 6.25 µg mL^{-1}) but was inactive against *Aspergillus fumigatus* [40]. Two years later, it was found that rustamicin showed fungicidal activity by inhibiting fungal biosynthesis of inositol phosphoceramide and that resulted in diminution of synthesis of sphingolipids [41]. Strains 1302-AV2 and 1304-AV3 of the rare actinomycete *Micromonospora chalcea* yielded four rustmicin analogues, named neorustmicins A-D (neorustmycin A **7**, Fig. 2a) [42]. All compounds exhibited significant activity towards wheat stem rust fungus (*Puccinia grannnis f. sp. tritici*) at 0.2, 1, 4 and 5 µg mL^{-1}, respectively. Neorustmicin A showed antimicrobial activity against several plant pathogens, including *Diaporthe citri*, *Cochliobolus miyabeanus* and *Colletotrichum lagenarium* at 100 µg mL^{-1} [43].

2.5. 16-Membered macrolides

2.5.1. Bafilomycins

These groups of 16-membered macrolides are known to show a variety of biological activities, including antitumour and antifungal activity. The structure of the bafilomycins consists of a 16- membered lactone ring, containing a tetraene, attached

240 to a tetrahydropyran by a three-carbon spacer. The carbonyl group of the
241 macrolactone ring is linked to the hydroxy group at C-17 of the sidecha in by a unique
242 hydrogen-bond [44, 45]. Bafilomycin C1-amide (**8**, Fig. 2b), along with four known
243 bafilomycin-1, -3, -4 and -5 derivatives, was isolated from the phenotypically closely
244 related *Stereptomyces* strains *Kitasatospora cheerisanensis*. All compounds exhibited
245 significant cytotoxic activity towards non-small cell lung cancer (A549), melanoma
246 (SK-Mel-2), ovarian cancer (SKOV-3), colon cancer (HCT-15) and central nervous
247 system cancer cells (XF-495), with MIC values ranging from 0.008 to 1.93 ng mL^{-1}.
248 Bafilomycin C1-amide had the stronger antifungal activity against the plant pathogens
249 *Botrytis cinerea*, *Colletotrichum lagenarium*, *Rhizoctonia solani* and *Sclerotinia*
250 *sclerotiorum* in comparison to the other compounds [46]. A bafilolide named
251 R176502 was isolated from a novel *Micromonospora* sp., along with three known
252 bafilomycins A1, B1 and B2. Compound R176502 showed potent antiproliferative
253 activity against a variety of human tumour cell lines, including human breast
254 carcinoma MCF-7, colon carcinoma HT-29, leukaemia cell K562/C1,000 and
255 melanoma Malme-3M, with IC_{50} values ranging between 0.57 nM and 18 nM [47]. A
256 bafilomycin-concanamycin named formamicin was isolated from *Saccharothrix* sp.
257 strain MK27-91F2. Formamicin was reported to have strong antifungal activity
258 against *Pyricularia oryzae*, *Rhizoctonia solani*, *Penicillium digitatum* and *Diaporthe*
259 *citri*, with MIC values ranging from 0.39 to 1.56 µg mL^{-1} [48]. Moreover, it displayed
260 moderate antibacterial activity against *S. aureus* FDA 209P, *B. cereus* ATCC10702
261 and *Micrococcus luteus* IFO3333, with MIC values ranging from 6.25 to 12.5 µg mL^{-1}.
262 It also exhibited strong cytotoxicity towards various leukaemia cell lines (P388,
263 L1210, EL4) *in vitro* with IC_{50} values of 0.15, 0.13 and 0.13 ng mL^{-1}, respectively
264 [48, 49]. Another three bafilomycin-type macrolide micromonospolides A–C were

isolated from an unidentified actinomycete, *Micromonospora* strain. All compounds exhibited inhibitory activity against gastrulation in star-fish embryos with MIC values 0.01, 0.011 and 1.6 µg mL^{-1},respectively [50]. Setamycin, another bafilomycin-type macrolide, was isolated from the rare actinomycete *Kitasatosporia setae* strain KM-6054. This macrolide exhibited weak antimicrobial activity against *S. aureus* FDA 209P and *B. subtilis* PCI 219. Additionally, it showed antiprotozoal activity against the pine wood nematode *Bursaphelenchus ligniclus* with IC_{50} value of 10 µg mL^{-1} [51].

2.5.2. Catenulisporidins

Catenulisporidins A (**9**, Fig. 2b) and B are hygrolidin macrolides isolated from the rare actinobacterium strain *Catenulispora* sp. KCB13F192, along with known hygrolidins. Catenulisporidin A has a tetrahydrofuran ring between C-7 and C-10, while catenulisporidin B has a 1,2-diol at C-10 and C-11. These natural modifications (tetrahydrofuran ring and diol) in the macrolide ring in both compounds made them the first reported example of natural hygrolidin derivatives featuring those modifications. Inhibition of proliferation of cervical carcinoma HeLa cells was evaluated for the three compounds. Hygrolidin displayed moderate antiproliferative activities, with an IC_{50} value of 11.8 µM, while catenulisporidin A and B showed lesser cytotoxic activities (IC_{50} value > 30 µM. This clearly indicated the importance of the rigid macrocyclic system on the antiproliferative activity [52].

2.5.3. Mycinamicins

The rare actinomycete *Micrononospora griseorubida* is a source of several types of 16-membered macrolide mycinamicins. These compounds possess an aglycone and two sugar moieties, desosamine and mycinose, in their structures. Kinoshita *et al*.

289 reported the isolation of eighteen mycinamicins I-XVIII (X: **10**, Fig. 2b) from *M.*
290 *griseorubida* [53, 54]. Among these metabolites, mycinamicin X displayed potent
291 antibacterial activity against *S. aureus* ATCC, *S. aureus* MS353, *S. epidermidis* sp-al-
292 1, *Streptococcus pyogenes* N.Y.5, *Micrococcus luteus* ATCC9341, *M. luteus* ATCC
293 10240 and *B. subtilis* ATCC6633 with MICs ranging from 0.05 to 0.4 μg mL^{-1}. Most
294 of these mycinamicins I- XVIII showed lesser but significant antibacterial activity
295 against various pathogenic bacteria: *S. aureus* FDA 209P JC-1 (MICs 0.1–3.12 μg
296 mL^{-1}), *S. pyogenes* N.Y. 5 (MICs 0.2–3.13 μg mL^{-1}), *M. luteus* ATCC9341 (MICs
297 0.1–1.56 μg mL^{-1}) and *B. subtilis* ATCC 6633 (MICs 0.39–25 μg mL^{-1}). Neither of
298 these compounds were active against *E. coli* NIHJ JC-2 or *P. aeruginosa* IAM 1095
299 [54, 55]. Mycinamicin X contains an N-acetyl-L-cysteine moiety at C-11 and that
300 may play an important role for showing potent antibacterial activity.

301 *2.5.4. Rosaramicins*

302 Rosaramicin (formerly named rosamicin), juvenimicins and izenamicins are all
303 structurally closely related 16-membered macrolides. The structure of these
304 compounds is characterised by 16-membered macrolactone rings attached to a
305 deoxyhexose sugar, desosamine, at C-5. Rosamicin (**11**, Fig. 2b) was isolated from
306 *Micromonospora rosaria* and it was found that it had antibacterial activity against *S.*
307 *aureus*, *S. epidermidis*, *Enterococci* and *Viridans* streptococci, with MIC ranging
308 from 0.02 to 4.0 μg mL^{-1} [56, 57]. It also exhibited an activity equal to the antibiotic
309 erythromycin against *Neisseria meningitidis* (MIC 0.25 μg mL^{-1}), *H. influenzae* (MIC
310 0.5 μg mL^{-1}) and *N. gonorrhoeae* (MIC 0.03 μg mL^{-1}) [58]. A series of 16-membered
311 macrolides named juvenimicins A1-A4 and B1-B4 were isolated from
312 *Micromonospora chalcea* var. *izumensis*. These compounds showed antibacterial
313 activity against *E. coli*, *Proteus vulgaris*, *Xanthomonas oryzae*, *Haemophilus*

gallinarum and *Mycoplasma gallisepticum*, with juvenimicin A3 as the most potent (MIC 0.1-0.3 μg mL^{-1}) [59, 60]. Juvenimicin C was later obtained from the culture of the rare actinomycete *Micromonospora* sp. It was found that juvenimicin C had cancer chemopreventive activity, as it induced the activity of the enzyme quinone reductase 1 (QR1) and glutathione levels by two-fold with doubling concentrations (CD values) of 10.1 and 27.7 μM, respectively [61]. Seven rosaramicin analogues, named izenamicin A1-A3 and B1-B4 were isolated from *Micromonospora* YS-02930K strain. Izenamicin A3 and B3 showed potent antimicrobial activity against *S. aureus* ATCC 6538P (MIC 1.56, 0.78 μg mL^{-1}), *S. epidermidis* IDD 866 (MIC 0.78, 0.39 μg mL^{-1}), *B. subtilis* ATCC 6633 (MICs 1.56–3.13 μg mL^{-1}) and *Streptococcus pyogenes* Cook (MIC 0.39, 0.39 μg mL^{-1}) [62].

2.5.5. Tianchimycins

Tianchimycins A and B (B: **12**, Fig. 2b) were isolated from the strain of *Saccharothrix xinjiangensis* NRRL B-24321, along with the known compound swalpamycin. The structure of these compounds consists of a 16-membered macrolide aglycone that featured a conjugated diene. Tianchimycin B possesses an additional D-aldgaroside sugar unit attached to C-5. Strong and selective antibacterial activity was observed for swalpamycin against *Enterococcus faecalis* ATCC 29212 with MIC 6.25 μg mL^{-1}. Both tianchimycins showed moderate antibacterial activity towards *E. faecalis* ATCC 29212 (MIC 50 μg mL^{-1}) but weak activity against other bacteria [63]. This observation suggested that the presence of the sugar moiety mycinose at C-14 was critical for swalpamycin to display better antibacterial activity.

2.6. 18-Membered macrolides

2.6.1. Borrelidins

The 18-membered macrolides borrelidins C–E, along with the known borrelidin (**13**, Fig. 3a), were isolated from the rare halophilic actinomycete *Nocardiopsis* sp. strain HYJ128. They comprise a nitrile-carrying macrocyclic lactone attached to a cyclopentane ring at C17. Borrelidin displayed activity against *Enterococcus faecium* ATCC 19434, *E. faecalis* ATCC 19433, *Proteus hauseri* NRBC 3851, *Klebsiella pneumoniae* ATCC 10031 and *Salmonella enterica* ATCC 14028 with MIC values ranging from 0.51-65 µM. Borrelidins C and D showed moderate antibacterial activity against *S. enterica* ATCC 14028 with MIC values of 16-63 µM, respectively. Borrelidin E did not exhibit antibacterial activity against the bacteria tested. The authors also reported that borrelidins C and D displayed cytotoxic activity against stomach cancer (SNU638) and leukaemia (K562) cell lines, with IC_{50} values of 5.5-5.7 µM and 8.7-6.7 µM, respectively, while borrelidin E did not show any cytotoxic activity [64]. In a recent investigation by Shin in 2021 [65], borrelidins showed inhibitory activity against the aggregation of amyloid-β (Aβ) and tau (τ) fibrils, which play critical roles in development of Alzheimer's disease (AD). Among all compounds, borrelidin displayed the highest ability to dissociate aggregations of both Aβ and τ fibrils. It was revealed that hydroxylation at C-7 or C-20 and on the cyclopentane ring are all required for the varied activities of the borrelidins [64, 65].

2.6.2. Tiacumicins

The antibiotic tiacumicins represent a class of complex macrolides. Most of the isolated compounds are characterised by an 18-membered macrolactone attached to two sugars, noviose and rhamnose. Both sugar moieties are esterified by a different substitution pattern such as 3-methylpropanoate, propanoate or acetate esters or homodichloroorsellinic acid at different positions [66]. Six compounds, named tiacumicins A-F, were isolated from the broth of *Dactylosporangium aurantiacum*

363 subsp. *hamdenensis* subsp. *nov*. Tiacumcin B (**14**, Fig. 3a) was the major component
364 [67]. Structural comparison studies revealed that tiacumicin B has identical structure
365 to clostomicin B1 and lipiarmycin A3 which were isolated from *Micromonospora*
366 *echinospora* subsp. *armenica* subsp. *nov*. and *Actinoplanes deccanensis* A/10655,
367 respectively [68]. Tiacumicins B and F displayed strong antimicrobial activity
368 towards *Micrococcus luteus* 4678 (MIC 0.78, 0.05 µg mL^{-1}). Additionally, these two
369 compounds displayed moderate activity against S. aureus ATCC 6538P (MIC 6.2,
370 12.5 µg mL^{-1}), *Streptococcus pyogenes* EES 61 (MIC 12.5, 6.2 µg mL^{-1}) and *E.*
371 *faecium* (MIC 6.5, 6.5 µ g mL^{-1}). Other compounds in this group had lower activity
372 with MIC values ranging from 25 to 100 µg mL^{-1} [67]. Recently tiacumicin B was
373 approved by the US FDA as a medicinal drug for the treatment of the otherwise fatal
374 intestinal infections associated with *Clostridium difficile*, under the generic name
375 fidaxomicin. This is the subject of many other detailed comprehensive reviews [68-
376 70].

377 *2.7. 20-Membered macrolides*

378 *2.7.1. Ammocidins*

379 The glycosylated polyketide ammocidins, amycolatopsins and apoptolidins are all
380 structurally closely related macrolides. The structure of the ammocidins consists of a
381 20-membered aglycone that features two trienes, a six-membered cyclic hemiacetal
382 side chain, deoxyglucose and a D-olivomycose-β-D-digitoxose disaccharide. The
383 culture broth of the rare actinomycete *Saccharothrix* sp. AJ9571 produced the 20-
384 membered macrolides ammocidins A-D. Ammocidins A (**15**, Fig. 3b) and B both
385 showed potent cytotoxic activities against human lung carcinoma cells A549, human
386 breast carcinoma cells MCF-7 and human colon carcinoma cells HCT116 with IC_{50}
387 values (0.058, 0.14, 0.11 µM) and (0.073, 0.10, 0.38 µM), respectively, while

388 ammocidins C and D displayed lower activity with IC_{50} values between 1.8 and 23
389 μM. Moreover, ammocidin A enhanced apoptotic cell death in Ras-dependent Ba/F3-
390 V12 cells with an IC_{50} value of 66 ng mL^{-1} [71-73]. Amycolatopsins A–C, three
391 structurally related 20-membered macrolides, were obtained from a strain of the rare
392 actinomycete *Amycolatopsis* sp. MST-108494. Amycolatopsins A and C showed
393 antimycobacterial activity against *Mycobacterium bovis* (BCG) and *M. tuberculosis*
394 (H37Rv) with IC_{50} values of (0.4, 0.27 μM) and (4.4, 5.7 μM), respectively.
395 Amycolatopsins A and B displayed significant cytotoxicity against human colon
396 carcinoma (SW620) and lung carcinoma (NCIH-460) cell lines with IC_{50} values (0.08
397 and 0.14 μM) and (1.2 and 0.28 μM), respectively, while amycolatopsin C was less
398 cytotoxic. This observation suggested that the presence of the disaccharide moiety
399 was critical for better cytotoxicity. These macrolides were not active against bacteria
400 such as *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa* or the fungus *C. albicans* [74].
401 *Nocardiopsis* sp. and *Amycolatopsis* sp., are both sources of 20-membered apoptolidin
402 macrolides. Wender *et al*. demonstrated that apoptolidins A-F isolated from
403 *Nocardiopsis* sp., exhibited significant and selective anticancer and antitumor
404 activities [75]. Apoptolidin B displayed no cytotoxic activity against normal cells
405 even at high concentrations (100 μM) but it selectively inhibited the growth of H292
406 human lung carcinoma cells with a GI_{50} value of 11 nM. Apoptolidins A, C and D
407 were less active with GI_{50} values of 35, 29 and 110 nM, respectively [76, 77]. As
408 another source of macrolides, *Amycolatopsis* sp. ICBB 8242 produced two new
409 apoptolidins, named 2′-O-succinyl-apoptolidin A and 3′-O-succinyl-apoptolidin,
410 along with the previously known apoptolidins A–D. Both succinyl compounds
411 exhibited inhibitory activity against the viability of human lung and cervical cancer
412 cells (NCI-H292, HeLa) with IC_{50} values of (0.09, 0.24 μM) and (0.08, 0.26 μM),

413 respectively [78]. It was seen that the sugar moieties and the hydroxy group at C-16
414 may play an important role for the cytotoxicity of the 20-membered macrolides.

415 *2.7.2. Levantilides*

416 The levantilides are 20-membered macrocyclic lactones structurally related to the
417 cytotoxic macrolide amphidinolide A, which lacks any attached sugar and has a
418 hexyl-side chain at C-19. Levantilides A (**16**, Fig. 3b) and B were isolated from the
419 rare deep-sea actinomycete *Micromonospora* strain M71-A77. Levantilide C was
420 obtained from another collection of the actinomycete *Micromonospora* strain FIM07-
421 0019 found in shallow coastal waters. Levantilide A displayed moderate
422 antiproliferative activity towards gastric tumour GXF 251L, lung tumour LXFL 529L,
423 mammary tumour MAXF 401NL, melanoma MEXF 462NL, pancreatic tumour
424 PAXF 1657L, renal tumour RXF 486L, human colonic cancer SW620 and human
425 hepatoma SMMC7721 cell lines, with IC_{50} values ranging between 20.7 and 52.4 µM.
426 Levantilide C had moderate activities against human leukaemia HL-60, human
427 colonic cancer SW620, human mammary tumour MDA-MB-231 and human
428 hepatoma SMMC7721 cell lines (IC_{50} 16.4 to 39.9 µM). Antimicrobial and antifungal
429 activities were not observed for levantilides A and B [79, 80].

430 *2.8. 22- and 24-Membered macrolides*

431 *2.8.1. Hamuramicins*

432 According to Suga *et al*., the 22-membered macrolide hamuramicins A (**17**, Fig. 4a)
433 and B were isolated from the culture broth of the rare endophytic actinomycete
434 *Allostreptomyces* sp. K12-0794 [81]. The structure of the hamuramicins consists of a
435 22-membered lactone ring that features a triene, a trienone and an alkyl side chain.
436 Both substances showed antimicrobial activity against *Kocuria rhizophia* ATCC9341

with an MIC of 4.0 µg mL^{-1}, and *Xanthomonas oryzae* pv. *Oryzae* KB88 (MICs 4.0, 2.0 µg mL^{-1}). Neither of the hamuramicins were active against *B. subtilis* ATCC6633 and *S. aureus* ATCC6538P. Additionally, isolated hamuramicins A and B displayed cytotoxicity towards human cervical cancer HeLa S3, human colorectal adenocarcinoma HT29, human adenocarcinoma, derived from lung cancer A549, human non-small-cell lung carcinoma H1299 and human cells derived from pancreatic cancer PANC-1 cell lines, with IC_{50} values ranging between 1.1 and 17.8 µM [81].

2.8.2. Maduralide

The 24-membered macrolide named maduralide (**18**, Fig. 4a) was obtained from the culture broth of an unclassified marine bacterium of the order actinomycetales. Maduralide possesses an aglycone attached to a rare deoxytalose sugar at C-13. This compound displayed weak antimicrobial activity against *B. subtilis* ATCC 6633 [82].

2.9. 26-Membered macrolides

2.9.1. Arenicolides

The polyene macrolide arenicolides are 26-membered macrocyclic lactones containing three conjugated dienes and a side chain at C-25. Three 26-membered macrolide arenicolides A-C were isolated from the large-scale fermentation of the rare actinomycete *Salinispora arenicola* strain CNR-005. Arenicolide A (**19**, Fig. 4b) showed moderate cytotoxicity against human colon adenocarcinoma cell line HCT-116 with an IC_{50} value 30 µg mL^{-1}; however, it did not exhibit any antimicrobial activity against *S. aureus* and *E. faecium* [83]. The stereochemical configurations at the oxirane and 12-C were later resolved by unequivocal synthesis [84, 85].

2.9.2. Catenulisporolides

The catenulisporolides are a group of macrolides containing a 26-membered triene aglycone, with an isovaleric acid starter unit which has been encountered very rarely among structures of macrolides. Catenulisporolides A-D (A: **20**, Fig. 4b) were produced by the actinomycete *Catenulispora* sp. KCB13F217. All compounds showed antimalarial activity against *Plasmodium falciparum* strains, including 3D7 and two chloroquine resistant strains Dd2 and K1, with the most potent being catenulisporolide D (IC_{50} values of 2.9, 2.8, 2.1 µM, respectively, against the three strains). None of these compounds displayed antimicrobial or antifungal activity or cytotoxicity towards mammalian cells [86].

2.9.3. Neomaclafungins

This group of macrolides belongs to the antibiotic oligomycin family, which is characterised by a 26-membered lactone ring with a spiroketal. Nine compounds were isolated from the broth of *Actinoalloteichus* sp. NPS702 and were named neomaclafungins A–I (A: **21**, Fig. 4b). These compounds displayed significant antifungal activity in vitro against Trichophyton mentagrophytes ATCC 9533 with MIC concentrations ranging between 1 and 3 µg mL^{-1}. The study suggested that the substituent at C-24 and the absence of ketones at the lactone ring were critical for better antifungal activity [87]. Another spiroketal macrolide related to the oligomycin family, named IB-96212, was obtained from the broth of *Micromonospora* sp. strain L-25-ES25-008. This 26-membered macrolide possesses antimicrobial activity towards *Micrococcus luteus* (MIC of 0.4 µg mL^{-1}). It also showed significant cytotoxic effects against lymphoma cell P388 (IC_{50} value of 0.1 ng mL^{-1}) but lower

484 activity against human colon cancer HT-29, lung adenocarcinoma A-549, and skin
485 cancer SK-MEL-28 cell lines [88].

486 *2.9. 28-Membered macrolides*

487 *2.10.1. Neaumycin B*

488 To the class of 28-membered macrolides belongs the highly cytotoxic
489 spiroketal-containing neaumycin B (**22**, Fig. 5a), which was produced in low yield by
490 the *Micromonospora* sp. strain CNY-010. Neaumycin B is related to the cytovaricin-
491 ossamycin-oligomycin class macrolides. In preliminary testing, neaumycin B
492 displayed potent activity against blood-borne cancers, with moderate selectivity
493 toward the myeloma cell line RPMI-8226. Unfortunately, the compound was
494 unstable, which hampered further testing *in vivo*. It was found later that neaumycin B
495 displayed significant potency and selectivity towards human glioblastoma (U87) cell
496 lines *in vitro* with an LD_{50} value of 6.4 pM [89]. Takeshita *et al.* have recently
497 developed a stereo-controlled synthesis of the C3−C17 fragment of neaumycin B
498 [90].

499 *2.11. 30-Membered macrolides*

500 *2.11.1. Epemicins*

501 This group of compounds is structurally related to the antibiotic aculeximycin,
502 which is characterised by a 30-membered lactone ring attached to a tri-saccharide
503 aculexitriose, vancosamine and mannose. Epemicins A (**23**, Fig. 5a), and B were
504 produced by the rare actinomycete *Kutzneria* sp. CA-103260. The antibiotic activity
505 was evaluated for both compounds against *S. aureus* (MRSA) MB5393. Both
506 epemicins A and B displayed significant antimicrobial potency, with MICs of 2-4 µg
507 mL^{-1} and 1-2 µg mL^{-1}, respectively [91].

508 *2.12. 34-Membered macrolides*

509 *2.12.1. Marinisporolides*

510 Marinisporolides are 34-membered macrolides with an internal spiroketal or
511 hemiketal in their lactone ring. Marinisporolides A (**24**, Fig. 5a) and B were obtained
512 from the marine actinomycete *Marinispora*, strain CNQ-140. Marinisporolide A
513 exhibited weak activity against C. albicans with an MIC of 22 µM. Neither of these
514 compounds displayed any activity against *S. aureus* or the human colon carcinoma
515 cell line HCT-116 [92]. The stereochemical configurations of this oxopolyene
516 macrolide were later resolved by total synthesis of its derivative marinisporolide C
517 [93].

518 2.13. 36-Membered macrolide

519 *2.13.1. Primycin*

520 The actinomycete *Micromonospora galeriensis* produced the natural antibiotic
521 primycin (**25**, Fig. 5b), which is a 36-membered marginolactone macrolide with
522 guanidine and arabinose moieties in the sidechain [94]. Primycin displayed strong
523 activity against *S. aureus*, *S. epidermidis* and *Streptococcus faecalis* (MICs ranging
524 from 0.12 to 0.5 µg mL^{-1}) and one strain of *Listeria monocytogenes* (MIC 0.25 µg
525 mL^{-1}) [95]. Many other studies indicated that primycin acts against multidrug-
526 resistant Gram-positive bacteria, *Staphylococcus* sp., *Bacillus* sp., *Streptococcus* sp.,
527 *Mycobacterium* sp., *Listeria* sp., *Sarcina* sp., *Sporosarcina* sp., and
528 *Propionibacterium* sp. (MIC ranging from 0.02 to 0.1 µg mL^{-1}). It also showed weak
529 activity towards Gram-negative bacteria: *Neisseria* sp., *Enterococcus* sp., *Shigella* sp.,
530 *Vibrio* sp., *Serratia* sp., and *Pasteurella* sp. Moreover, it displayed antifungal
531 activities *in vitro* against *C. albicans*, with an MIC of 0.25 µg mL^{-1} [96-98]. Primycin

has been used for many years in the Hungarian market as a topical antimicrobial agent in dermatology under the trade name Ebrimycin® gel.

2.14. 40-Membered macrolides

2.14.1. Mathemycins

Mathemycins A (**26**, Fig. 5b) and B were obtained from the broth of an *Actinomycete* sp. culture Y-8620959. The stereochemical configurations of mathemycins A have only been determined for the alkenes and the sugars [99, 100]. The 40-membered macrocyclic lactone, mathemycin A exhibited moderate activity against *Fusarium culmorum* 100, *Alternaria mali* P37, *Botrytis cinerea* AO6, *Botrytis cinerea* DO1, *Pellicularia sasakii* JO3, *Leptosphaeria nodorum* JO2, *Pyricularia oryzae* KO2, *Pseudocercosporella herpotrichoides* 008 and *Phytophthora infestans* JO8 with minimum active concentrations ranging from 62.5 to 250 µg mL^{-1}. Mathemycin B showed lower inhibitory activity [101].

2.15. 60-Membered macrolides

2.15.1. Quinolidomicins

The 60-membered benzoquinone-containing macrolides, the quinolidomicins, are considered as the largest macrocyclic lactones to be isolated from terrestrial sources to date. Quinolidomicin A1 (**27**, Fig. 6), A2 and B1 were obtained from the actinomycete *Micromonospora* sp. strain JY-16. Both quinolidomicins A1 and B1 displayed significant cytotoxic activity against various tumour cells lines, including murine leukaemia P388, human colorectal adenocarcinoma HT-29 and human gastric adenocarcinoma MKN28, with IC_{50} values ranging between 25 and 327 nM. Quinolidomicin A2 was inactive against these cell lines and that clearly indicated the

555 importance of the benzoquinone group for the cytotoxic activity of these macrolides
556 [102].

557 In conclusion, 193 macrolides derived from fifteen known and two unidentified
558 rare actinomycetes have been summarised in this review. Rare actinomycetes
559 produced twenty-six groups of macrolides, with cytotoxic, antibacterial, antifungal
560 and insecticidal bioactivities. Among the sets of macrolides summarised in Table 1,
561 *Micromonospora* are the dominant producing genera, yielding twenty of these fifty
562 compounds. The other thirty were produced by other genera such as *Saccharothrix*,
563 *Amycolatopsis*, *Nocardiopsis* and *Catenulispora*. Fifteen of these macrolides show
564 cytotoxic activity, twelve are antibacterial, seven have antimicrobial activity, seven
565 are antifungal, two are insecticidal, two have anti-trypanosomal activity, two act
566 against malaria parasites and others showed antiprotozoal, antimycobacterial and anti-
567 herpetic bioactivities. Cytotoxicity is the most significant activity of these compounds
568 which clearly indicates that macrolides from rare actinomycetes could provide an
569 excellent resource for the discovery of new anticancer agents. Most significant
570 anticancer and antitumour activities have been reported for apoptolidins, bafilomycins
571 and IB-96212. As potent antibacterial agents, mycinamicin X, rosaramicin and
572 primycin have shown comparable activity to the famous antibiotic erythromycin
573 against many Gram-positive and negative bacteria. As for potent antimicrobial agents,
574 tiacumicins B and F displayed potent activities. More importantly, tiacumicins B have
575 been approved by the US FDA for the treatment of diarrhea caused by *Clostridium*
576 *difficile* under the generic name fidaxomicin. Primycin is another excellent macrolide
577 which has been used for many years in the Hungarian market as a topical
578 antimicrobial agent. Some of these macrolides, including rustmicin, formamicin and
579 neomaclafungin, displayed surprisingly strong antifungal activity. In terms of

macrolide class, both 16-membered and 14-membered compounds made up the largest number of the bioactive compounds which indicates the importance of these classes as a potential source of bioactive compounds. This review and other research show that macrolides from rare actinomycetes display a wide range of bioactivities which make this class of compounds of great interest for future development of treatments for cancer, bacterial, fungal and many other infections [103]. The large number of cytotoxic and antibacterial macrolides isolated from rare actinomycetes clearly indicates the importance of these classes as a new source of anticancer and antibiotic agents.

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