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Design, synthesis and biological evaluation of potential antibacterial butyrolactones

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ABSTRACT

Novel butyrolactone analogues were designed and synthesized based on the known lichen antibacterial compounds, lichesterinic acids (**B-10** and **B-11**), by substituting different functional groups on the butyrolactone ring trying to enhance its activity. All synthesized butyrolactone analogues were evaluated for their in vitro antibacterial activity against *Streptococcus gordonii*. Among the derivatives, **B-12** and **B-13** had the lowest MIC of 9.38 μg/mL where they have shown to be stronger bactericidals, by 2–3 times, than the reference antibiotic, doxycycline. These two compounds were then checked for their cytotoxicity against human gingival epithelial cell lines, Ca9–22, and macrophages, THP-1, by MTT and LDH assays which confirmed their safety against the tested cell lines. A preliminary study of the structure–activity relationships unveiled that the functional groups at the C_4 position had an important influence on the antibacterial activity. An optimum length of the alkyl chain at the C_5 position registered the best antibacterial inhibitory activity however as its length increased the bactericidal effect increased as well. This efficiency was attained by a carboxyl group substitution at the C_4 position indicating the important dual role contributed by these two substituents which might be involved in their mechanism of action.

1. Introduction

The usages of antibiotics on a large scale alongside their misapplication have lead to the emergence of resistant pathogenic bacteria.[1] Both, the infection of these re-emergent strains which has increased the global mortality rate to be a growing concern and the global reduction in antibiotics production open a new era where other potent candidates should be found to fight against bacteria.[2] Indeed, an infinite number of plant species have been tested against a huge number of bacterial strains in vitro. In addition, many phytochemicals found effective against a broad spectrum of microorganisms comprising fungi, yeast and bacteria were uncovered.[3] Throughout the last 2 decades, plants are becoming a famous rich source of antimicrobial substances.[4] Furthermore, many other promising drug sources still need to be explored.[5] Lichens which are symbiotic organisms comprising a fungus and a photosynthetic alga and/or cyanobacterium constitutes a potential source of over 1000 distinct secondary metabolites.[6] They comprise antitumor, antiviral and antimicrobial activities.[6–9] Sensitive as well as several multi-drug resistant bacterial strains were shown to be susceptible to these lichen compounds.[6]

Streptococcus gordonii (*S. gordonii*) is an eminent member of the viridans streptococci large category. Not only was this bacteria described as an agent of septic arthritis but also it can colonise damaged heart valves and represents the primary etiological agent of subacute bacterial endocarditis.[10] In the oral cavity, *S. gordonii* adhere to the salivary pellicle which coats the teeth, proliferate and excrete an extracellular polysaccharide matrix protecting their developing microcolony on which secondary colonizers will adhere.[11] The late colonizing strains such as *Porphyromonas gingivalis* bind the sites provided by *S. gordonii* and form a highly pathogenic complex microbial community.[12,13] *S. gordonii* as a pioneer initial colonizer initiates the formation of dental plaques contributing in turn to the onset of dental caries and periodontal diseases as well as their progression.[14,15] Inhibiting *S. gordonii* might block the successive steps leading to acute oral diseases and this may constitute prevention rather than a risky cure after biofilm formation.

To address this oral issue, we synthesized a natural butyrolactone, L-lichesterinic acid. Cavalito et al. have extracted it from the lichen, *Cetraria islandica*, and shown to have an activity against

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Streptococcus hemolyticus and *Staphylococcus aureus*.[16] Our goal in this study is to evaluate its antibacterial activity against *S. gordonii* in solid and liquid media under anaerobic conditions. Trying to enhance its activity, some derivatives were synthesized and tested (Fig. 1). Finally, the cytotoxic effect of the most active compounds was evaluated on two human cell lines, gingival epithelial cells, Ca9–22, and macrophages, THP-1. To the best of our knowledge, it is the first study to describe some of these synthetic derivatives, their antibacterial activity against *S. gordonii* and their cytotoxic effects.

2. Results and discussion

2.1. Chemistry

Scheme 1 illustrates the synthesis of enantiopure (−)-lichesterinic acid **B-10** and its derivatives. This straightforward asymmetric synthesis has already been described by Braukmüller and Brückner in 2006 for the preparation of paraconic acids.[17] To the best of our knowledge, this strategy had been used only for the synthesis of naturally aliphatic α-methylene butyrolactone (+)-methylenolactocin (R = C_5H_{11}) and (+)-protolichesterinic acid (R = $C_{13}H_{27}$) and their (−) enantiomers (Fig. 2). Based on a six steps method, one additional step is required to obtain a series of lichesterinic acid derivatives by isomerization of the double bond. Moreover, this lactone strategy has been extended to include different alkyl chain lengths R (C_7H_{15}, C_9H_{19}, $C_{15}H_{31}$, and $C_{16}H_{33}$). Briefly, it began with the preparation of hydroxyl lactones **1a**–**e** where the enantiocontrol was imposed by the asymmetric dihydroxylation of *trans*-configured β,γ-unsaturated carboxylic ester with AD mix-α® or AD mix-β®. The resulting lactones were dehydrated giving butenolides **2a**–**e**. For the two next steps we modified the approach according to Perepogu et al.[18] A Gilman addition of a vinyl group was added *trans*-selectively to the C=C bond giving vinyl lactones **3a**–**e,** followed by an oxidation of the double bond allowing access to HO_2C-substituted lactones **4a**–**e**. α-Activation by Stiles' reagent, followed by amino-methylation in situ fragmentation provided the α-methylene butyrolactones **5a**–**e**. Then, the target enantiopure lichesterinic acid derivatives **6a**–**e** were obtained by isomerization of the double bond using NEt_3 in DMF. This synthesis is achieved in seven steps and around 10% overall yield with good enantioselective excess determined by chiral HPLC.

2.2. Biological activity

2.2.1. Antibacterial activity

2.2.1.1. Agar dilution. Seven out of the thirteen butyrolactones screened (Fig. 1) showed an activity with the concentrations tested against *S. gordonii* under anaerobic conditions (Table 1). Compounds **B-2**, **B-4**, **B-5, B-6**, **B-7**, and **B-13** didn't exhibit any activity. The least active compounds were **B-1** and **B-3** showing the highest Minimal Inhibitory Concentration (MIC) of 300 μg/mL. Then, the MIC decreased to be 200 μg/mL for **B-12** and continued decreasing to pass by 150 μg/mL for **B-8** and **B-9** and reaches the lowest value with **B-10** and **B-11** registering 90 μg/mL (Fig. 3A, Table 1). Alongside, doxycycline displayed an MIC of 0.41 μg/mL which was fixed and used always as a positive control (Fig. 3B, Table 1). In addition, the mixture of the solvents (DMSO + methanol) used to dissolute our compounds was found inactive at the highest concentration tested. These results were taken into the liquid medium to confirm and compare.

2.2.1.2. Broth microdilution. Compared to the solid medium, all butyrolactones were found active except **B-7**. At this step, **B-2**, **B-4**, **B-5** and **B-6** joined the antibacterial panel (Table 2).

According to the efficiency of the compounds, they can be distributed into 3 groups. The least efficient were **B-1**, **B-3**, **B-8**, and **B-9**. The most effective were **B-10**, **B-11**, **B-12**, and **B-13**.

The highest inhibitory activity was for **B-10** and **B-11** which registered the same results with MIC = 4.69 μg/mL and Minimal Bactericidal Concentration (MBC) = 18.75 μg/mL. While MIC

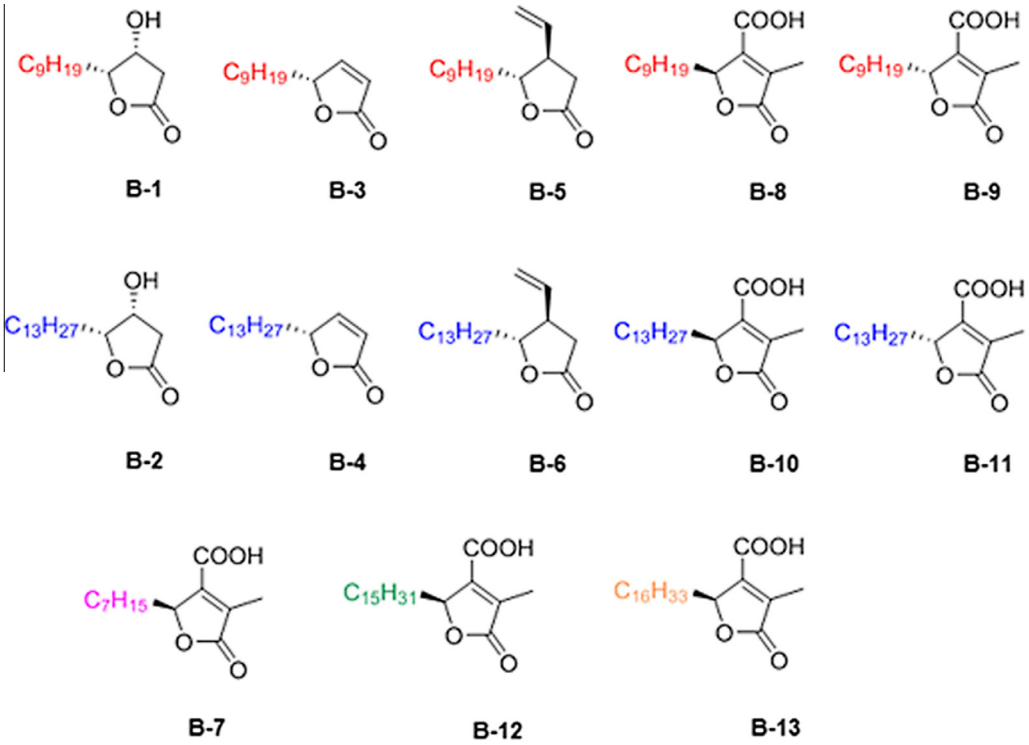

Figure 1. Chemical structures of butyrolactones.