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Antibacterial and antifungal activities of 2,3-pyrrolidinedione derivatives against oral pathogens

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ABSTRACT

Among the novel approaches applied to antimicrobial drug development, natural product-inspired synthesis plays a major role, by providing biologically validated starting points.

Tetramic acids, a class of natural products containing a 2,4-pyrrolidinedione ring system, have attracted considerable attention for their antibacterial, antiviral, antifungal and anticancer activities. On the contrary, compounds with a 2,3-pyrrolidinedione skeleton have been considerably less investigated. In this work, we established chemical routes to the substituted 2,3-pyrrolidinedione core, which enabled the introduction of a wide range of diversity. In the perspective of a potential application for oral healthcare, a number of analogues with various substituents on the 2,3-pyrrolidinedione core were investigated for their antimicrobial and antifungal activities. The most promising compound showed a significant antimicrobial activity on *Streptococcus mutans* and *Candida albicans,* comparable to that of chlorhexidine, the gold standard in oral healthcare.

Oral diseases impose a huge economic burden on society[1] and dental caries and oral candidiasis are two of the most common, biofilm-related diseases of the oral cavity worldwide.

Dental caries represents a global health problem still far from being completely eradicated.[2] In this disease, the predominance of *Streptococcus mutans* – a facultative anaerobic, Gram-positive, acidogenic bacterium- interplays with further caries risk factors, such as frequent sugar intake and hyposalivation, leading to demineralization of hard dental tissues.[3] In the case of oral candidiasis, *Candida albicans*, an opportunistic yeast of the oral cavity, proliferates locally producing severe inflammatory lesions of the mucosa, mainly in locally or systemically immunocompromised patients.[4] The yeast presence has also shown to increase *S. mutans* pathogenicity.[5] Oral candidiasis in form of prosthetic stomatitis occurs frequently on the palatal mucosa of elderly patients wearing removable prosthesis, due to a local proliferation of the microorganism. Similarly, pseudo-membranous oral candidiasis is among those oral lesions strongly associated with HIV infection.[2] In the case of dental caries, the gold standard acute therapy consists of carious lesion removal and tooth restoration using dental materials, while for oral candidiasis, appropriate antimycotic therapy is recommended.

A preventive approach via antiseptic agents for the control of dental biofilm formation and composition, has recently been proposed to avert both diseases.[6,7] Chlorhexidine (CHX), a cationic biguanide, is the gold standard for controlling oral biofilm and for oral healthcare, representing the most investigated and prescribed product, commercially available in different forms. However, its use for caries prevention is still largely controversial,[6,8,9] despite its well-reported intense antibacterial action and its ability to firmly adsorb to the tooth structure (pellicle formation on dental hard tissues) and the gingiva (substantivity).[10–12] Indeed, CHX is used in vitro as reference biocidal molecule to assess the efficacy of alternative antiseptic agents, showing excellent antibacterial activity against a plethora of oral pathogens, including *S. mutans*,[13] as well as broad-spectrum antifungal activity against *Candida* spp.[4]

Nonetheless, CHX is not exempt from drawbacks, which include both local side effects (such as dental pigmentation and disgeusia) and potential systemic hypersensitivity reactions.[8] In addition, a reduced level of susceptibility to CHX by pathogens cannot be *a*

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priori excluded, considering also the increasing use of this agent for the oral and hand hygiene.[14] All together, these issues strongly encourage scientific research in finding alternative antiseptic compounds.

The discovery of new antibacterial and antifungal agents with novel mechanisms of action represents an effective strategy to overcome the limitations related to existing drugs. Interestingly, these agents may also be incorporated inside dental materials.[15,16] Among the novel approaches applied to antimicrobial drug development, natural product-inspired synthesis plays a major role, by providing biologically validated starting points. In this perspective, the re-examination of function and availability of natural products allowed the identification of favoured structures, suitable for drug optimization.[17]

A class of natural products that has attracted considerable attention is that of tetramic acids, containing a 2,4-pyrrolidinedione ring system (compound **1**, Fig. 1). A tetramic acid is an attractive skeleton because a combination of substituents at different positions can give a large variety of structurally diverse molecules.[18] Some natural tetramic acid derivatives show remarkable biological activities, ranging from antibacterial and antiviral to antifungal and anticancer ones. Examples include reutericyclin,[19] sintokamide A,[20] lactacystin,[21] streptolydigins,[22] oxazolomycin,[23] janolusimide.[24]

Compared to the numerous studies on bioactivities of tetramic acids, compounds with a 2,3-pyrrolidinedione skeleton (compound **2**, Fig. 1) have been considerably less investigated.

To the best of our knowledge, only one natural product (Leopolic acid A, Fig. 1)[25] and a few synthetic compounds[26–28] have been reported in the literature so far. Therefore, this skeleton can be considered a very attractive target for biological evaluation, offering the possibility of preparing products with several points of diversity, similarly to what occurred for tetramic acid. In this work, we show the results of our preliminary exploration directed towards establishing chemical routes to the substituted 2,3-pyrrolidinedione core, which enabled the introduction of a wide range of diversity. In the perspective of a potential application for oral healthcare, we also assessed the antimicrobial activity of representative compounds on *S. mutans* and *C. albicans*, and preliminary structure-activity relationships (SAR) have emerged.

We envisaged that the most straightforward route to the synthesis of the 2,3-pyrrolidinedione system could be the Michael addition of a suitably protected amine to ethyl acrylate, followed by a Dieckmann cyclization with diethyl oxalate.[29] We opted for the *p*-methoxybenzyl (PMB) protecting group, which could be easily removed by cerium ammonium nitrate (CAN) or 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ).

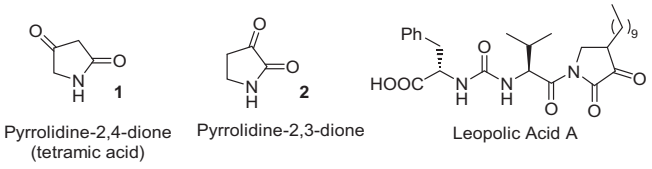


Figure 1. General structure of 2,3-, 2,4-pyrrolidinediones and Leopolic acid A.

Thus, ethyl acrylate **3** was reacted with *p*-methoxybenzylamine to obtain compound **4**, which was then treated with diethyl oxalate to give 2,3-pyrrolidinedione **5** (Scheme 1). NMR observations showed that the compound exists as an enol tautomer (see SI). Indeed, it has been reported[30–32] that apparently all 4-monosubstituted 2,3-dioxopyrrolidines are highly enolized, regardless of the nature of the substituent in position 4.

After obtaining compound **5**, we planned to prepare a series of analogues to obtain a range of diversity around the heterocyclic core. At first, we decided to compare different functionalities at position 4. The enolic OH was first protected with a tertbutylsilyl (TBS) group, by treatment of **5** with TBSCl, to obtain compound **6**.

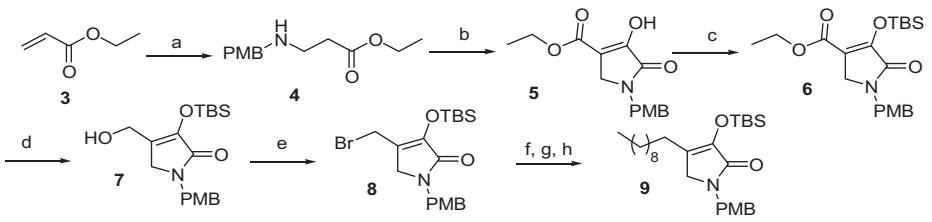
Reduction of **6** with diisobutylaluminium hydride (DIBALH) successfully gave alcohol **7** in 72% yield. Inspired by the 2,3-pyrrolidinedione-containing natural compound Leopolic acid A[25] we planned the synthesis of a 4-alkyl substituted derivative. Thus, alcohol **7** was converted into the corresponding bromide by Appel reaction with PPh_3 and CBr_4. The bromide was reacted with PPh_3 to give a bromonium salt which was then subjected to Wittig reaction with a nine-carbons aldehyde. Reduction of the double bond by catalytic hydrogenation gave compound **9** in 89% yield.

Unfortunately, attempts to remove the PMB group from compounds **6**–**9** (CAN in ACN /water or DDQ, CH_2Cl_2) resulted into unstable compounds which decomposed during purification.

Assuming that this instability could be due to the enol protecting group, we decided to use a benzyl group as an alternative. Thus, compound **5** was treated with benzyl bromide to obtain compound **10** (Scheme 2). To compare the benzyl group-containing compounds to analogues with the TBS group on the enolic OH, we repeated the same sequence reported above (see Scheme 1) using compound **10** instead of compound **6** (Scheme 2). Reduction with DIBALH, followed by Appel bromination and Wittig reaction, allowed the introduction in position 4 of the aliphatic chain (compound **13**).

Deprotection of PMB group from compound **10** was successful and gave **14**. Having the compound with a free NH in hands, we explored the effect of a polar group linked to nitrogen, to be compared with compound **10**, carrying a lipophylic PMB group. Thus, **14** was acylated with activated N-protected valine, to obtain **15**. Removal of the Boc protecting group afforded **16**, containing the free aminoacid residue.

The prepared compounds were investigated for their antimicrobial and antifungal activities. The results are shown in Figure 2 (antibacterial activity against 24 h *S. mutans* biofilm) and Figure 3 (antifungal activity against 24 h *C. albicans* biofilm) and in Table S1 (see SI).



(a) *p*methoxybenzylamine, EtOH, rt, 12h, 98%; (b) diethyloxalate, NaOEt, EtOH, reflux, 3h, 83%; (c) TBSCl, imidazole, DCM, rt, 6h, 86%; (d) DIBAL-H, DCM, -78°C to rt, 1h, 72%; (e) PPh_3, CBr_4, DCM, rt, 12h, 87%; (f) PPh_3, toluene, reflux, 6h, 61%; (g) n-nonanal, LiHMDS, THF, -78°C to rt, 5h, 88%; (h) H_2/Pd/C, EtOAc, rt, 1h, 89%

Scheme 1. Synthesis of compounds **5**–**9**.