Structure-antimicrobial activity relationships of monoketone curcuminoids

Abstract

Background: Dental caries is a major public health concern worldwide. This pathology is caused by acidicogenic and aciduric bacteria, which produce a structurally and functionally organized biofilm on the tooth surface. Streptococcus mutans is one of the most important colony-forming bacteria present in the buccal microbiota, stands out due its ability of producing substances that favor adhesion and the accumulation of other microorganisms, forming a resistant extracellular matrix that can destroy dental hard tissue. Although chlorhexidine permits unrestricted use, distribution, and build upon your work non-commercially, its use have been limited due to some side effects.

Methods: MKCs (monoketone curcuminoids) are promising compounds with respect to their antimicrobial activity against a panel of cariogenic bacteria, including S. Mutans. We investigated the antimicrobial activity of three synthetic monoketone curcuminoids (MKCs) against a representative panel of cariogenic bacteria and examined some preliminary structure-antimicrobial activity relationships of MKCs.

Results: Compounds 1a and 2 (Scheme 1) were obtained by aldol condensation in acidic conditions. Compounds 1a and 2 were determined by using the broth micro dilution method in 96-well microplates. Chlorhexidine was used as positive control.

Conclusion: Compounds 1 and 2 displays promising antimicrobial activity against some cariogenic bacteria. Our results suggest that these compounds might be promising for the development of new oral care products.

Keywords: antibacterial activity, dental caries, monoketone curcuminoids, oral pathogens, Streptococcus mutans

Introduction

Dental caries constitutes a major public health concern worldwide. This pathology is caused by acidicogenic and aciduric bacteria, which produce a structurally and functionally organized biofilm (dental plaque) on the tooth surface. Streptococcus mutans, one of the most important colony-forming bacteria present in the buccal microbiota, stands out due its ability of producing substances that favor adhesion and the accumulation of other microorganisms, forming a resistant extracellular matrix that can destroy dental hard tissue. Although chlorhexidine, which is currently the most effective anti plaque agent, its use have been recommended by dentists only for short periods due to reversible local side effects.

Recently, monoketone curcuminoids (MKCs), or 1,5-diarylpentadien-3-one derivatives, have been reported as promising compounds with respect to their antimicrobial activity against a panel of cariogenic bacteria, including S. Mutans. Although some preliminary structure-relationships have been previously proposed, the effect of some important structure features on the antimicrobial activity was not evidenced. In this paper, we report on the antimicrobial activity of three MKCs derivatives against cariogenic bacteria aiming to better understand the structure-antimicrobial activity relationships among these compounds.

Materials and methods

Synthesis of monoketone curcuminoids 1-2, 1a-1b

Compounds 1 and 2 (Scheme 1) were obtained by aldol condensation in acidic conditions, as previously reported. Briefly, a mixture of acetone (580mg, 10mmol) and vanillin (20mmol) for compound 1 or 4-hydroxy-benzaldehyde (20mmol) for 2 was slowly added to acetic acid (50mL) saturated with hydrogen chloride at O°C. The reaction mixture was stirred at room temperature for 24h. The crude reaction mixture was poured into ice-cold water (200mL) and then extracted with ethyl acetate (3x30mL). After drying the organic layer over MgSO4, filtration, and solvent removal under reduced pressure, the resulting solid was purified using silica gel flash column chromatography using hexane: ethyl acetate (v/v) (Figure 1). Compounds 1a and 2a were obtained from 1 by catalytic hydrogenation. In this procedure, compound 1 dissolved in HPLC grade ethyl acetate was added to a high-pressure reactor together with Pd/C (10%) and kept at room temperature under stirring, H2 atmosphere, and pressure of 100 psi for 18h.

Chemical structures of compounds 1, 1a, and 2 were confirmed on the basis of NMR, IR, and MS analyses.

(1E,4E)-1,5-bis (4-hydroxy-3-methoxyphenyl)penta-1,4-dien-3-one (1): Yield 21.0%, orange powder. IR (KBr pellet) of 1(C=O): νmax/cm-1: 3411 (νOH), 3005 (νCH), 1714 (νC=O), 1589 (δC=C), 1093 (νC-O). LRESI-MS (m/z, %relative intensity): 327(100) [M+H]+. NMR 1H (400MHz, CDCl3): δ 3.95 (6 H, s, H10=H10'), 6.93 (2 H, d, J1,6=15.8Hz, H2=H2'), 6.94 (2 H, d, J1,6=8.2Hz, H6=H6').

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Abbreviations: MKCs, monoketone curcuminoids
(CH, C5=C5'), 123.5 (CH, C2=C2'), 127.6 (C, C4=C4'), 143.3 (CH, C3=C3'), 146.9 (C, C7=C7'), 189.2 (C=O, C1).

1,5-bis(4-hydroxy-3-methoxyphenyl)pentan-3-one (1a): Yield 37%, white powder. IR (KBr pellet) of 1a (C, H, O), cm⁻¹: 1460 (ν-OH), 2925 (νC-H), 1701 (νC=O), 2999 (νC-H), 1232 (νC−O).

Results and discussion

In a previous work, we reported the antimicrobial activity of twenty-five monoketone curcuminoids, including curcumin A (1), which was demonstrated to be more effective than curcumin against twenty-five monoketone curcuminoids, including curcumin A (1), 1a, 1b, and 2 were dissolved in tryptic soy broth (TSB, Difco) and dimethylsulfoxide (DMSO) (Merck, Darmstadt, Germany) to achieve final concentrations between 0.195 and 400μg/mL. Inoculated microorganisms were cultured in blood agar (Difco Labs, Detroit, MI, USA) at 37°C for 24h. Next, the inoculum quantity was standardized to match 0.5 in the McFarland scale (1.5x10⁸ CFU/mL) using a spectrophotometer (Femto (São Paulo, Brazil)) at 625nm. The microorganism suspensions were diluted to a final concentration of 5x10⁵CFU/mL. Colonies of the selected bacteria were cultured in blood agar (Difco Labs, Detroit, MI, USA) at 37°C for 24h Next, the inoculum quantity was standardized to match 0.5 in the McFarland scale (1.5x10⁸ CFU/mL) using a spectrophotometer (Femto (São Paulo, Brazil)) at 625nm. The microorganism suspensions were diluted to a final concentration of 5x10⁵CFU/mL. Colonies of the selected bacteria were cultured in blood agar (Difco Labs, Detroit, MI, USA) at 37°C for 24h. The in vitro antimicrobial activity of compounds1-2, 1a-1b was evaluated on the basis of their minimum inhibitory concentration (MIC), which was assessed on the basis of the McFarland scale (1.5x10⁸ CFU/mL) using a spectrophotometer (Femto (São Paulo, Brazil)) at 625nm. The microorganism suspensions were diluted to a final concentration of 5x10⁵CFU/mL. Colonies of the selected bacteria were cultured in blood agar (Difco Labs, Detroit, MI, USA) at 37°C for 24h. The in vitro antimicrobial activity of compounds1-2, 1a-1b was evaluated on the basis of their minimum inhibitory concentration (MIC).
S. mutans and S. mitis. However, some important structure-antimicrobial activity of three MKCs against a panel of cariogenic bacterial strains on the basis of their minimum inhibitory concentration (MIC) values.

Classification of the antibacterial activity was based on MIC values, in this way, MIC values lower than 100μg/mL, between 100< MIC<500μg/mL or between 500<MIC<1000μg/mL and higher than 1000μg/mL correspond to promising, moderate, weak and inactive activities, respectively.3,5 In this way, according to these criteria, compounds 1 and 2 displayed the lowest MIC values against S. mutans (MIC=50μg/mL) and S. mitis (MIC=50μg/mL), as well as moderate activity against S. sanguinis (MIC=100μg/mL), S. salivarius (MIC=200μg/mL) and compound 1 was also active against L. casei (MIC=100μg/mL). Compound 2 also had promising activity for L. casei (MIC=25μg/mL) and moderate activity against S. sobrinus (MIC=200μg/mL). Moreover, the compounds 1b also displayed moderate active against S. mutans (MIC=200μg/mL), S. mitis (MIC=100μg/mL), S. sanguinis (MIC=200μg/mL) and L. casei (MIC=200μg/mL). The activity of compound 1 and 2 against S. mutans is a note worthy result because natural compounds capable to inhibit this primary causative agents of dental caries are still scarce.2 None of the tested MKCs was significantly active against Enterococcus faecalis (Table 1).

Table 1 Minimum inhibitory concentration (MIC) values [μg/ml] of the monoketone curcuminoids against selected cariogenic bacteria

| Microorganism                  | 1   | 1a  | 1b  | 2   | Positive control |
|--------------------------------|-----|-----|-----|-----|-----------------|
| Streptococcus mutans (ATCC 25175) | 50  | >400| 200 | 50  | 0.92b           |
| Streptococcus mitis (ATCC 49456)    | 50  | 400 | 100 | 50  | 1.84b           |
| Streptococcus salivarius (ATCC 25975) | 200 | >400| >400| 200 | 1.84b           |
| Streptococcus sanguinis (ATCC 10556) | 100 | 400 | 200 | 100 | 0.92b           |
| Streptococcus sobrinus (ATCC 33478) | 400 | 400 | 400 | 200 | 0.92b           |
| Enterococcus faecalis (ATCC 4082)  | >400| >400| >400| 400 | 7.37b           |
| Lactobacillus casei (ATCC 11578)   | 100 | 400 | 200 | 25  | 0.92b           |

*Chlorhexidinehydrochloride

According to previous reports, MKCs bearing a double bond between C2 and C3, and between C2' and C3' were inactive against the tested microorganisms in the range of concentrations tested, except for compound 1 (curcumin A).3 These results led us to suspect that the higher activity observed for curcumin A could be related to the simultaneous presence of methoxy and hydroxy groups in the aromatic ring, since compounds displaying one, two or three methoxy groups at the aromatic ring were inactive.4 To verify this hypothesis, we synthesized the curcumin A derivatives 1a and 1b, and the hydroxy curcuminoid 2 and evaluated their antimicrobial activity against cariogenic bacteria. Comparison between the MIC values of curcumin A (1) and compounds 1a and 1b revealed that absence of the double bond between reduction of the carbonyl group at C1 and/or the double bond between C2 and C3/C2’-C3’ caused a significative reduction in the antimicrobial activity. In addition, it was observed that compound 2, which does not have a methoxy group in its structure displayed antimicrobial activity very similar to that of compound 1. Compound 2 was more active than curcumin A against S. sobrinus (MIC=200μg/mL) and L. casei (MIC=25 μg/mL). These results revealed that the hydroxy group at the aromatic ring plays a key role for the antimicrobial activity, and its presence is more important for this activity than the methoxyl group.

Data obtained from the present study, in combination with those previously reported, revealed that the carbonyl group at C1 and the double bonds between C2 and C3 are important structure features for the antimicrobial activities of MKCs. However, the nature of the substituent at the aromatic rings plays an important role in this activity. Among the tested compounds, compounds displaying a hydroxyl group (1 and 2) were much more active than those with methoxyl, bromine, chlorine, and methyl groups.3

Conclusion

Compounds 1 and 2 display promising antimicrobial activity against Streptococcus mutans, which is one of the main causative microorganisms of dental caries. The hydroxyl group attached to the aromatic rings, as well as the double bond between C2-C3 and C2C3' and the carbonyl group at C1, are the most important structure features for the antimicrobial activity. Taken together, these results suggest that these compounds might be promising for the development of new oral care products.

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Conflict of interest

The authors declare no conflict of interest.

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