A Novel Ester-bonded Gemini Quaternary Ammonium Salt with Good Antimicrobial Activity and Anti-mold Performance for Wet Blue Leather

by

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Abstract

Herein, we have developed a kind of novel ester-bonded gemini quaternary ammonium salts microbicides named QAS(n+m) for leather application, in which n and m denotes the number of $-CH_2$ between the ester groups on the spacer, and the length of alkyl chain connected to ester groups on the hydrophobic tail chain, respectively. Results indicate that the hydrophobic tail lengths and the spacer lengths in the QAS(n+m) structures affect their antimicrobial activities very differently. QAS(4+10) was used to investigate the antimicrobial mechanism, cytotoxicity, and anti-mold performance for wet blue leather as it exhibits the strongest antimicrobial effect. It shows QAS(4+10) is capable of inactivating microorganisms mainly by disrupting the integrity of their cell membranes. Compared to commercial leather microbicide product 2-thiocyanomethylthiobenzothiazole (TCMTB), QAS(4+10) exhibits comparable mold resistance and lower toxicity. The present work gives positive insight into the development of novel candidate microbicides for the preservation of wet blue leather.

Introduction

Natural leather has been popularly used for fashion, footwear, upholstery and many crafts due to its breathability and durability.[1] Leather is made from hides and skins, which are basically constituted of 60% ~ 70% water, approximately 30% of proteins and a certain amount of fat.[2] These nutrient substances can serve as metabolic substrates for bacteria and fungi. Microorganisms are capable of contaminating hides at any leather processing stage ranging from slaughter to finished leather,[3] resulting in undesirable pigmentation, non-uniformity of dyeing and finishing, fatty acids production, poor physical-mechanical properties of leather.[4] Therefore, the proper use of microbicides, i.e. preservatives and fungicides, is considered as a facile and effective strategy to control microorganisms.[5] Generally, preservatives protect hides and skins against bacterial invasion (such as Halophilic bacteria, *Micrococcus, Bacillus, Pseudomonas, Proteus* and *Escherichia*),[3] while fungicides protect leather from fungal contamination (such as *Aspergillus, Penicillium*, *Paecilomyces, Scopulariopsis, Trichoderma and Rhizopus*).[3] To prevent the growth of these microorganisms, many antimicrobial agents have been used in leather industry, including phenols (e.g. 4-chloro-3-methylphenl, trichlorophenol), esters (e.g. dimethyl fumarate), aldehydes (e.g. glutaraldehyde) heterocyclic compound (e.g. N-octilisotiazolinona and 2-thiocyanomethylthiobenzothiazole), quaternary ammonium salts (QAS) and so on. Among them, the most widely used preservatives pentachlorophenol (PCP) and trichlorophenol (TCP) and fungicides dimethyl fumarate (DMF) have been restricted because of their high toxicity and ecological hazards.[6,7] Nowadays, 2-thiocyanomethylthiobenzothiazole (TCMTB) has become one of the main microbicides used in leather industry due to its excellent antimicrobial property. However, its toxicity and hazard assessments are also attracting great concern. It has been reported that TCMTB has potential sensitization[8] and reproductive toxicity[9] and can cause potential environmental problems.[10,11] More importantly, the TCMTB content in leather products has been limited to below 250.0 mg/kg in the latest testing standards *LEATHER STANDARD by OEKO - TEX®*.[12] Therefore, it is highly desirable to exploit novel nontoxic or low-toxic leather microbicides.

In the past decades, quaternary ammonium salts with single tail and single polar head (QAS) have been intensively investigated owing to their good antimicrobial activity. As developed, gemini QAS which is composed of two conventional QAS connected through the spacer[13] are emerging as the new generation of QAS compounds because they have lower critical micelle concentration (CMC),[14] unusual aggregation behavior,[15] lower cytotoxicity,[16] better water solubility, antimicrobial activity and broad-spectrum compared with the conventional ones.[17,18] Most of studies about gemini QAS focus on synthesis, structures and applications such as in agriculture,[19] textile industry[20] and leather industry.[21] As reported, introducing various functional groups and altering the hydrophobic tail lengths or spacer lengths can magnificently affect the properties of gemini QAS.[22] For instance, the melting points of the gemini QAS will be reduced by introducing hydroxyl groups,[23] and the introduction of heteroatoms into the gemini QAS can decrease its aquatic toxicity.[24,25]

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Currently, ester-bonded gemini QAS has drawn increasing interest owing to their better biodegradability and compatibility with the environment.[26,27] Nevertheless, reports on the studies of gemini QAS containing multiple ester groups are considerably insufficient, the details of relations between the structures especially alkyl chain lengths and their antimicrobial property, and their ability to preserve leather against microbes contamination remain unclear, which is important for their applications in leather industry.

In this study, we synthesized nine novel gemini QAS compounds with four ester groups. In our approach, alcohol compounds were functionalized with acyl group via a nucleophilic substitution reaction, and the ester groups were hereby incorporated into the hydrophobic tail and spacer of gemini QAS. We choose the most common microorganisms (*S. aureus*, *E. coli* and *A. niger)* present in hides or leather as the testing strains to study the antimicrobial effect of gemini QAS. Their antimicrobial mechanism, cytotoxicity and anti-mold performance for wet blue leather were further investigated. Our aim is to provide a novel gemini QAS microbicide with lower toxicity for leather industry.

Materials and Methods

1,2-ethanediol (≥99%), 1,3-propanediol (≥99%), 1,4-butanediol (98%), n-octanol (≥99%), n-decanol (≥99%), n-dodecanol (98%), chloroacetyl chloride (98%), bromoacetyl bromide (97%), diethylamine (≥99%), agar (BR), peptone (BR) as well as beef extract (BR) were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd., China. Dodecyl trimethyl ammonium bromide (DTAB, AR), dodecyl trimethyl ammonium chloride (DTAC, AR), dichloromethane (AR), tetrahydrofuran (AR) and acetone (AR) were provided by Chengdu Kelong Chemical Reagent Co., Ltd., China. Commercial product 2-thiocyanomethylthiobenzothiazole (TCMTB) with an active content of 30 wt% was bought from Fo Shan Lan Feng Auxiliary Limited Co., Ltd. Malt extract broth (MEB, BR) was supplied by Japan Institute of Biological Sciences Co., Ltd. *SYTO 9* (BR) and pyridinium iodide (*PI*, BR) were purchased from Thermo Fisher Biochemical Products Beijing Co., Ltd., China. Dulbecco's modified eagle medium (DMEM, BR) fetal bovine serum (BR) and penicillin/streptomycin antibiotics were obtained from HyClone Co., America.

S. aureus (CICC 21600) and *E. coli* (CICC 10389) were purchased from China Center of Industrial Culture Collection. *A. niger* (ATCC 10864) used in the study was acquired from Biofeng Co., Ltd., China.

Syntheses of gemini quaternary ammonium salts QAS(n+m)

Nine novel gemini QAS compounds with four ester groups were synthesized through a four-step procedure as shown in Figure 1. Firstly, 1,2-ethanediol, 1,3-propanediol and 1,4-butanediol, was reacted with chloracetyl chloride to generate ester-based dichlorosubstitutes ($ClCH_2COO(CH_2)_nOOCCH_2Cl$, n = 2, 3, 4), respectively.[14] Secondly, ester-based dichlorosubstitutes further reacted with diethylamine to obtain intermediate ester-based di-tertiary amine ($((C_2H_5)_2NCH_2COO(CH_2)_nOOCCH_2N(C_2H_5)_2$,

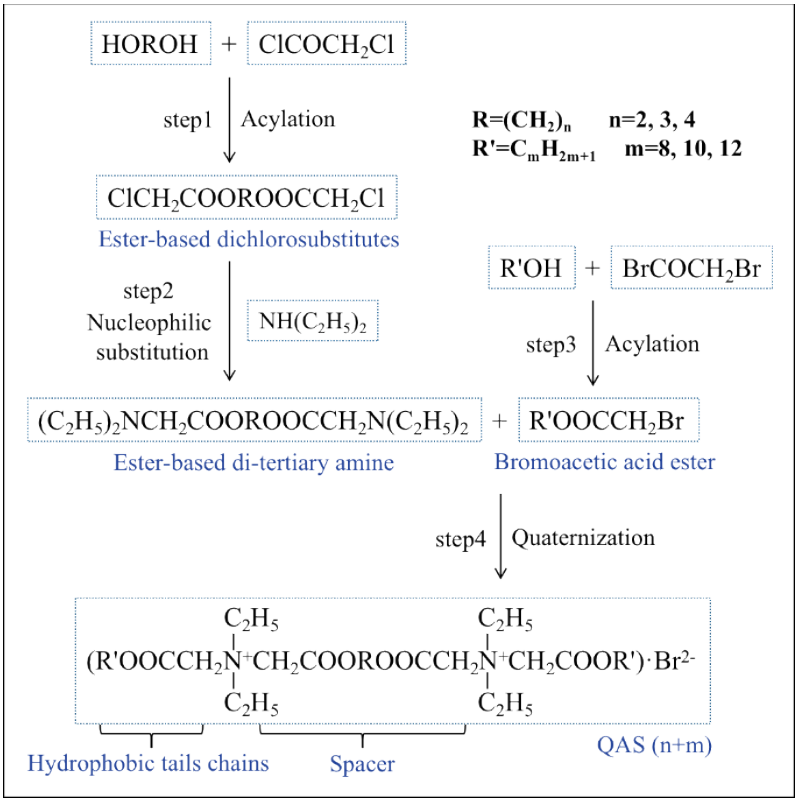

Figure 1. Synthesis process of QAS(n+m)

n = 2, 3, 4). Thirdly, n-octanol, n-decanol and n-dodecanol was esterified with bromoacetyl bromide to form bromoacetic acid ester ($H_{2m+1}C_mOOCCH_2Br$, m =8, 10, 12), respectively.[28] Finally, the gemini QAS(n+m) ($H_{2m+1}C_mOOCCH_2(C_2H_5)_2N^+CH_2COO(CH_2)_nOOCCH_2N^+(C_2H_5)_2CH_2COOC_mH_{2m+1}$)·2Br, n = 2, 3, 4 and m =8, 10, 12) were obtained by quaternization of ester di-tertiary amine with bromoacetic acid ester,[26] where n denotes the number of CH_2 between the ester groups on the spacer, and m denotes the length of alkyl chain connected to ester groups on the hydrophobic tail chains. The resulting QAS(n+m) was purified by recrystallization and drying. The structures of QAS(n+m) have been identified by Fourier transform infrared spectrum (FT-IR), proton nuclear magnetic resonance (^{1}H-NMR) and mass spectrometry (MS).

Antimicrobial activity evaluation

The minimum inhibitory concentration (MIC) of QAS(n+m) determined by the broth dilution method was used to evaluate the antimicrobial activities of QAS(n+m) against Gram-positive bacteria (*S. aureus*), Gram-negative bacteria (*E. col*i, *Escherichia*) and fungi (*A. niger*).[29] The test medium for *S. aureus* and *E. coli* (i.e. beef-peptone liquid medium) was prepared according to the most conventional formula (i.e. 3.0 g beef extract, 10.0 g peptone and 1000 mL water). *S. aureus* and *E. coli* was incubated in beef-peptone liquid medium until the culture reached exponential phase (1×10^{10} CFU/mL), respectively. Then 200 μL bacterial suspension was dispersed in 3.8 mL beef-peptone liquid medium containing different concentrations of QAS(n+m) and incubated for 24 h at 37°C. The final concentrations of QAS(n+8), QAS(n+10) and QAS(n+12) in the experiment was in the range of 0.74~375.00 μM, 0.59~300.00 μM and 0.07~37.50 μM, respectively. *A. niger* was grown on Malt Extract Agar medium (MEA) and incubated for 5 d at 30°C. After the incubation period, the spores of *A. niger* were re-suspended in Malt extract broth (MEB). 200 μL *A. niger* spores suspensions (1×10^6 CFU/mL) was dispersed in 3.8 mL MEB containing different concentrations of QAS(n+m) ($C_{QAS(n+m)}$ = 0.59~300.00 μM) and incubated for 48 h at 30°C. MIC value is defined as the lowest concentration of the QAS(n+m) at which there was no visible growth of microorganisms in the suspension. There was no QAS(n+m) in the negative and positive control and no strains in the negative control. TCMTB was used as test control group for the antifungal activity evaluation.

In addition, we further determined the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of QAS(n+m) by following the procedure previously reported.[30] The test medium for *S. aureus* and *E. coli* (i.e. beef-peptone agar medium) was prepared by following the most conventional formula (i.e. 3.0 g beef extract, 10.0 g peptone, 20.0 g agar and 1000 mL water). After bacterial suspension (1×10^{10} CFU/mL) was treated with different concentrations of QAS(n+m) as described above for 24 h at 37°C, the mixtures were streaked and grown on beef-peptone agar medium for 24 h at 37°C. MBC is defined as the lowest concentration of the QAS(n+m) at which there was no visible growth of bacteria on the agar medium.[30] The MFC values were determined by the same procedure using MEA as the medium and *A. niger* as the testing microorganism.

Antimicrobial mechanism study

Scanning electron microscopy (SEM) observation

To examine the antimicrobial mechanism of gemini QAS, bacteria of exponential phase and A. niger spores suspensions were treated with QAS(4+10), respectively. The bacterial samples were incubated for 4 h at 37°C, and the samples of *A. niger* spores were incubated for 8 h at 30°C. Then the suspensions were centrifuged for 10 min at 8000 rpm to obtain the precipitates and resuspended in 2.5% glutaraldehyde solution for 4 h at 4°C to keep the cell morphology intact. Subsequently, various alcohol concentrations (30%, 60%, 90% and 100%) were used to dehydrate the cells in sequence and each dehydration process was kept for 10 min. Finally, the alcohol solutions containing the cells were dropped on conductive glass for SEM scanning (Inspect F50, FEI Co., Ltd., America). All the samples were sputter-coated with gold before scanning.[31]

Determination of K^+ and soluble proteins content

In addition, to detect the destruction of *A. niger* spores by QAS(4+10), the K^+ content and the soluble proteins content in the supernatant of *A. niger* spores suspensions treated with QAS(4+10) for 8 h were determined by ion chromatography (IC) and Coomassie blue staining method, respectively.

Live/dead bacteria staining assay

200 μL bacterial suspension of exponential phase was mixed with 200 μL QAS(4+10) in 1.5 mL sterile Eppendorf tubes and the final QAS(4+10) concentration of the mixture was kept at MIC values. After culturing at 37°C for 4 h, 5 μL of *SYTO 9* (3.34 μM) and *PI* (20 μM) (v/v = 1:1) were added to the medium and the dying process was kept for 15 min in the dark.[32] Finally, the sample was dropped on the slide grid and observed by fluorescence microscope (CKX53, Olympus Co., Japan).

Cytotoxicity assay (CCK-8 test)

The CCK-8 test was used to evaluate the cytotoxicity of QAS(4+10), antimicrobial agent TCMTB, DTAB and DTAC. L929 cells were stored in the complete medium consisting of 10% fetal bovine serum, 1% penicillin/streptomycin antibiotics and 89% DMEM. 100 μL cell suspensions (1×10^4 cells/well) was put into the 96-well plates and cultured for 24 h at 37°C in CO_2 incubator. Then 100 μL fresh medium with different concentrations of QAS(4+10), TCMTB, DTAB and DTAC was used to replace the original medium, respectively. After incubating for another further 48 h at the same condition, the original medium was removed and replaced with 90 μL fresh complete medium and 10 μL CCK-8 solutions (5 mg/mL). The incubation was further conducted for 1.5 h at the same condition. There was only L929 cells and medium in the positive control and no L929 cells in the negative control.[33] Enzyme-labeled Instrument

(Bajiu Co., Ltd., China) was used to measure the absorbance of each well solution and the wavelength was set at 450 nm. The following equation was used to calculate the relative growth rates (RGRs):

$$\text{Relative growth rates (\%)} = \frac{(OD_t - OD_n)}{(OD_p - OD_n)} \times 100\%$$

Where OD_t, OD_n and OD_p represents the absorbance of the test group, negative control group and positive control group, respectively.

Anti-mold performance of QAS(4+10) for wet blue leather

The anti-mold test of QAS(4+10) for wet blue leather were carried out by following the method described in China light industry standards QB/T 4199-2011.[34] One piece of wet blue leather without any microbicides during the production process was taken as control group. Another two pieces of wet blue leather soaked in 0.5 wt.% TCMTB solution (active content) and 0.5 wt.% QAS(4+10) solution for 24 h respectively, were taken as the test groups. The three leather samples were placed equidistantly on MEA medium, and 30 µL *A. niger* spores suspensions (5×10^5 CFU/mL) was dropped at the center of each piece of leather, respectively. The incubations were conducted at 30°C in a chamber with relative humidity (RH) of 90% for 10 d and the samples were photographed at different incubation days.

Furthermore, the anti-mold performance was also tested by the inhibition zone method. The above wet blue leathers were placed equidistantly on MEA medium which had been completely inoculated with *A. niger* spores suspensions by spread plate method and the plate was incubated at 30°C in a chamber (RH =90%). The samples were observed and photographed accordingly.

Results and Discussion

Antimicrobial activity evaluation

The antimicrobial activity of QAS(n+m) was investigated by MIC, MBC and MFC values as listed in Table I. We chose *S. aureus, E. coli and A. niger* as the test microorganisms because they are common microorganisms on hides or leather. It shows that all the as-prepared gemini QAS exhibit good antimicrobial effect against *S. aureus*, *E. coli* and *A. niger*. The resistance of the three strains to QAS(n+m) varies in the following order: *A. niger* > *E. coli* > *S. aureus,* which is consistent with the previous study.[35] *A. niger* is usually more resistant to chemical disinfectants than bacteria because filamentous Aspergillus have better ability to form biofilm, thus protecting them from damage by biocides.[36,37] Additionally, QAS(n+m) are more active toward *S. aureus* than *E. coli*. It is possibly attributed to the different ultra-structures in their cell walls. Compared with thick but porous cell wall of Gram-positive bacteria (*S. aureus*), the cell wall of Gram-negative bacteria (*E. coli*) composed a thinner peptidoglycan layer and an outer lipopolysaccharide membrane has a stronger tendency to restrict the penetration of small molecules like QAS.[38,39]

From Table I it can be seen that both hydrophobic tail lengths and the spacer lengths affect the antimicrobial activity of QAS(n+m) markedly, and QAS(n+10) demonstrates the stronger antibacterial efficacy. It is possibly because that the hydrophobic tail chain lengths of QAS(n+10) is the most similar to the phospholipid bilayer of bacterial cell membrane, which is favorable for the interactions between QAS(n+10) and bacteria.[40] Likewise, QAS(n+10) also

Table I

The MIC, MBC and MFC values of QAS(n+m) against different microorganisms

QAS(n+m)	*S. aureus*		*E. coli*		*A. niger*	
	MIC	MBC	MIC	MBC	MIC	MFC
QAS(2+8)	46.88	187.50	187.50	>375.00	>300.00	>300.00
QAS(2+10)	18.75	37.50	37.50	75.00	300.00	>300.00
QAS(2+12)	18.75	37.50	>37.50	>37.50	150.00	>300.00
QAS(3+8)	11.72	46.88	46.88	375.00	>300.00	>300.00
QAS(3+10)	2.34	9.38	9.38	9.38	150.00	150.00
QAS(3+12)	18.75	37.50	>37.50	>37.50	150.00	300.00
QAS(4+8)	11.72	23.44	23.44	93.75	300.00	>300.00
QAS(4+10)	2.34	4.69	4.69	9.38	75.00	150.00
QAS(4+12)	9.375	37.5	>37.50	>37.50	75.00	150.00
QAS(5+10)	1.17	2.34	4.69	9.38	37.50	150.00
TCMTB	---	---	---	---	4.69	37.50

Notes: The unit is µM. "---" means no data.

exhibits the stronger antifungal effect, which is closely related to the proper hydrocarbon tail lengths. Either the shorter hydrocarbon tail or the longer tail tend to affect the penetration of QAS molecules into the spores membranes, resulting in lower antimicrobial activity.[35]

As for all the QAS(n+10) (n = 2, 3, 4) compounds, QAS(4+10) exhibits the best antimicrobial activity. On one hand, the electrostatic repulsions between the head groups (N^+) of gemini QAS diminishes when the spacer lengths gets longer, thus contributing to the adsorption of QAS on microbial cell surface.[23] On the other hand, when the spacer-$(CH_2)_n$ group is short (<4), the presence of conjugation effect of two ester groups in the spacers possibly leads to the averaging of electron clouds, thus weakening their induced effect on the head groups (N^+) and unfavorably affecting the adhesion of QAS on the cell surfaces. To verify the above analysis, QAS(5+10) was further synthesized. Its antimicrobial activity against *E. coli* is comparable to that of QAS(4+10) and slightly better than that of QAS(4+10) against *S. aureus* and *A. niger*. It further indicates that the spacer lengths of QAS(n+10) may change the polarity of the head groups of QAS, thus affecting the antimicrobial effect.

Antimicrobial mechanism study

The antimicrobial mechanism of gemini QAS was studied by SEM observation, the live/dead bacteria staining assay and determination of spillage content of *A. niger* spores. As illustrated in Figure 2A, the untreated microbial cells exhibit their own regular and typical morphology, i.e. plump and smooth surface with uniform size and distribution for bacteria,[31,41] and normal shape for *A. niger* spores.[42] In contrast, incomplete or deformed shapes, irregularly wrinkled and coarse outer surfaces are observed for the bacteria strains treated with QAS(4+10) (Figure 2B). The *A. niger* spores treated with QAS(4+10) also shrink (Figure 2B).

Figure 3 shows that fluorescence microscopy observation of the tested bacteria untreated and treated with QAS(4+10). DNA or RNA of microbes can generate red fluorescence when specifically combined with *PI* and green fluorescence when bonded with *SYTO9*.[43] Meanwhile, *PI* staining can identify dead cells as it is impermeant to living cells.[43] As for the *E. coli* and *S. aureus* untreated with QAS(4+10) (Figure 3A), no red fluorescence but much green fluorescence is observed, signifying that almost all the bacteria are alive. As for the bacteria treated with QAS(4+10) at MIC value for 4 h (Figure 3B), the presence of strong red fluorescence indicates the excellent antimicrobial activity of QAS(4+10).

Additionally, after treatment with QAS(4+10), the contents of K^+ and soluble protein in *A. niger* spores suspensions increased up to 0.75 ppm and 0.054 mg/mL, respectively (Table II). It indicates that the membrane structures of *A. niger* spores were damaged by QAS(4+10), leading to the release of the intracellular substances. The above results confirm that as-prepared gemini QAS compounds are membrane active substances and their effective destruction on the integrity of the bacteria cell membranes or plasma membrane of fungi spores cause the death of microbes.

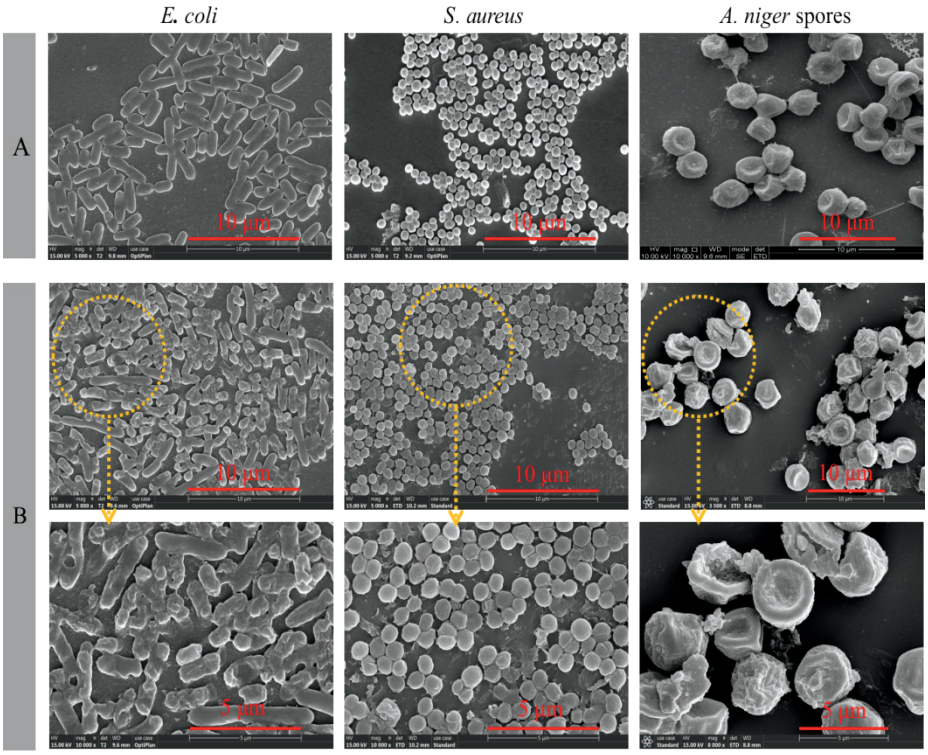

Figure 2. SEM images representing the morphology of the tested microorganisms untreated **(A)** and treated **(B)** with QAS(4+10) at MIC value

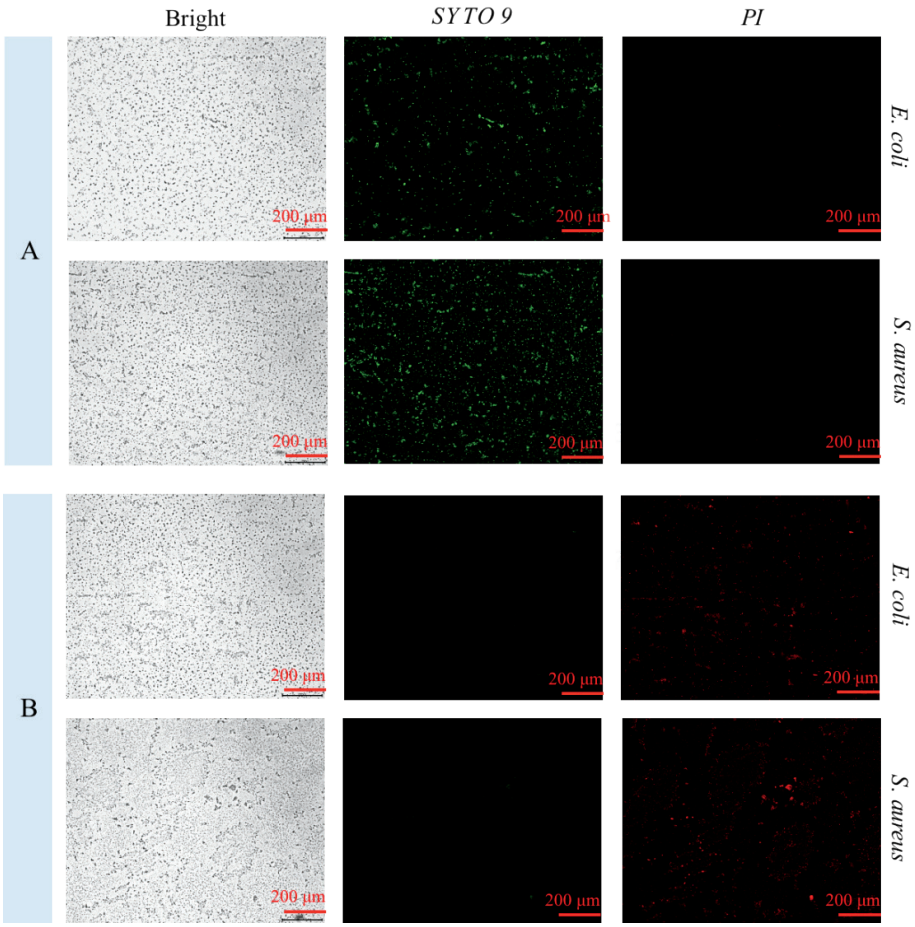

Figure 3. Fluorescence microscopy observation images of the tested bacteria untreated **(A)** and treated **(B)** with QAS(4+10) at MIC value

Cytotoxicity evaluation

Figure 4 and Figure 5 show the cell morphology of L929 and their cell relative growth rates (RGRs) in culture medium containing QAS(4+10), antimicrobial agent TCMTB, traditional quaternary ammonium salts DTAB and DATC, respectively. As for QAS(4+10) treated samples ($C_{QAS(4+10)}$≤75 μM), the cell morphology shows the same typical shuttle-shape as the control group (Figure 4A-D). By contrast, the cell shapes of DTAB, DATC and TCMTB treated samples change obviously, indicating that QAS(4+10) has lower cytotoxicity than these commercial leather microbicides. It can be further quantitatively proven by the results of RGRs.

The colorimetric assay (CCK-8 test) is widely used to examine the cytotoxicity of compounds to the living cells.[33] It is well accepted that the RGRs over 80% indicates non-cytotoxicity of the testing samples.[44] As shown in Figure 5, the cytotoxicity of the microbicides is highly related to their concentrations. The RGRs of L929 cells samples treated with QAS(4+10) ($C_{QAS(4+10)}$≤10 μM) is higher than 80%, revealing that QAS(4+10) at lower concentration exhibits no cytotoxicity. However, DTAB, DTAC (C = 10 μM) and TCMTB (C = 5 μM) have significant cytotoxicity as proved by the lower RGRs. As described in the above Table I, both the MIC and MBC of QAS(4+10) towards *S. aureus* and *E. coli* were lower than 10 μM, meaning that

Table II

The K^+ content and the soluble proteins content in the supernatant of *A. niger* spores with different treatment

The testing components	K^+	Soluble protein
A. niger spores without any treatment	0	0
A. niger spores treated with QAS(4+10)	0.75 ppm	0.054 mg/mL

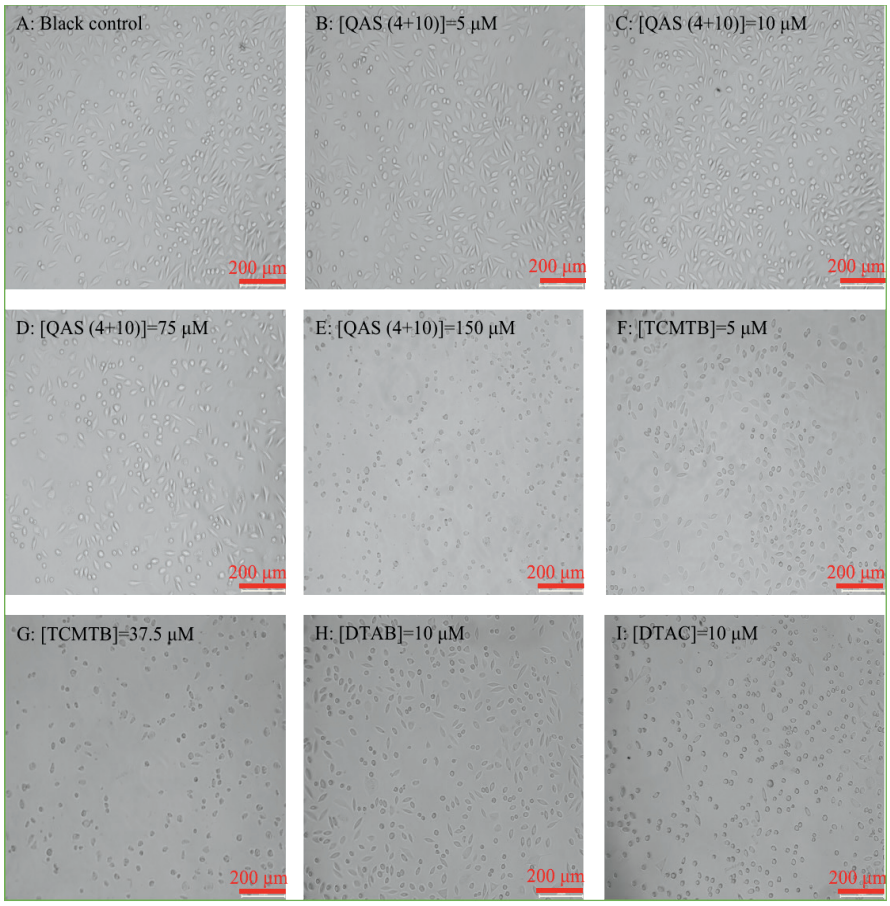

Figure 4. Cell morphology of L929 after 48 h of incubation with the QAS(4+10), TCMTB, DTAB and DATC with different concentrations (**A:** Black control; **B:** [QAS(4+10)]=5 μM; **C:** [QAS(4+10)]=10 μM; **D:** [QAS(4+10)]=75 μM; **E:** [QAS(4+10)]=150 μM; **F:** [TCMTB]=5 μM; **G:** [TCMTB]=37.5 μM; **H:** [DTAB]=10 μM; **I:** [DTAC]=10 μM)

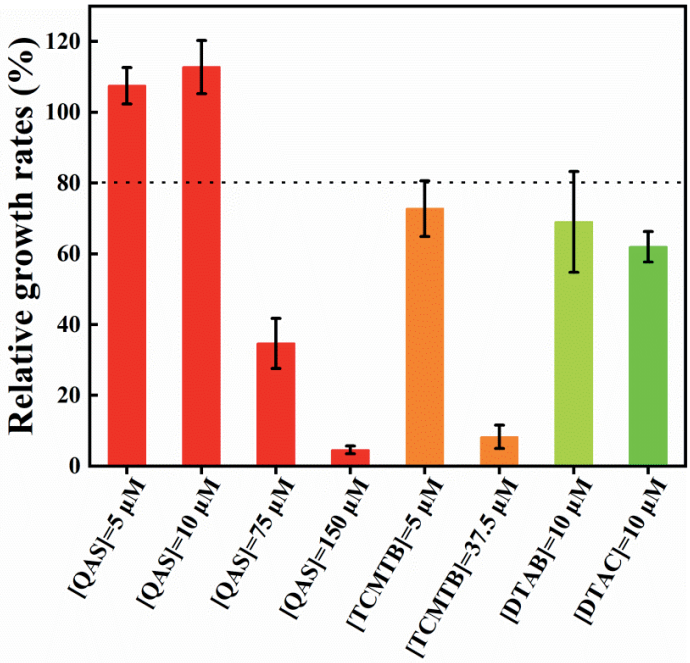

Figure 5. Relative growth rates of L929 after 48 h of incubation with the QAS(4+10), TCMTB, DTAB and DATC with different concentrations

the excellent bactericidal activity and non-toxicity can be achieved by choosing QAS(4+10) as microbicides.

Anti-mold performance of QAS(4+10) for wet blue leather

Figure 6 shows the anti-mold property of QAS(4+10) for wet blue leather within 10 days. *A. niger* are clearly observed on the control group after 5 days incubation, indicating the validity of the anti-mold experiments. In contrast, no *A. niger* appears on the TCMTB or QAS(4+10) treated samples, indicating that the wet blue leather treated with 0.5% microbicides exhibits excellent anti-mold performance. With the time extending (10 d), the control group is totally covered by black colonies of *A. niger*, whereas there is still no mold growth on the microbicides treated samples, indicating that the QAS(4+10) has comparable mold resistance compared with the widely used leather microbicide TCMTB. This result is very encouraging for its potential application in leather industry.

Similar result is also found by the inhibition zone method. As shown in Figure 7, no *A. niger* are observed on the surfaces of both TCMTB and QAS(4+10) treated leather, further affirming the good antifungal activity. Additionally, it is worth noting that a significant inhibitory

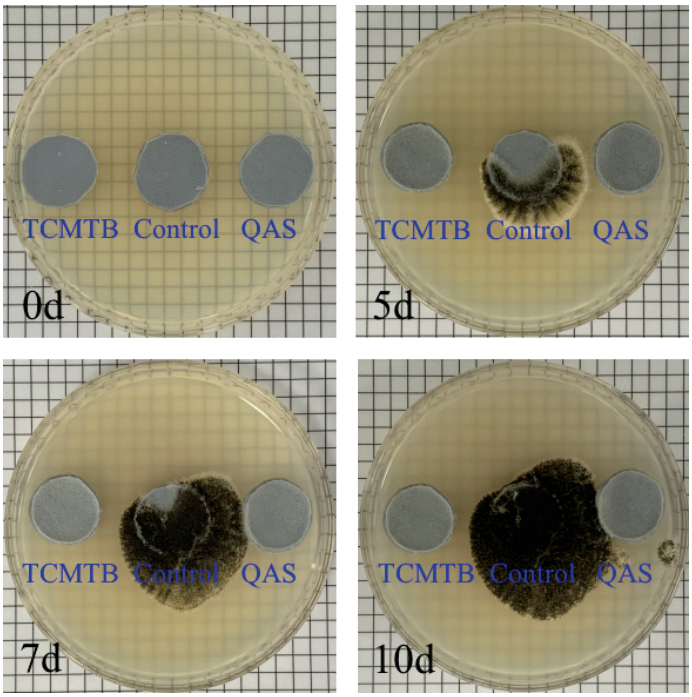

Figure 6. The growth of *A. niger* on the surface of wet blue leather treated with different microbicides: left sample treated with 0.5 wt.% TCMTB, right sample treated with 0.5 wt.% QAS(4+10) and middle sample without any treatment as control group

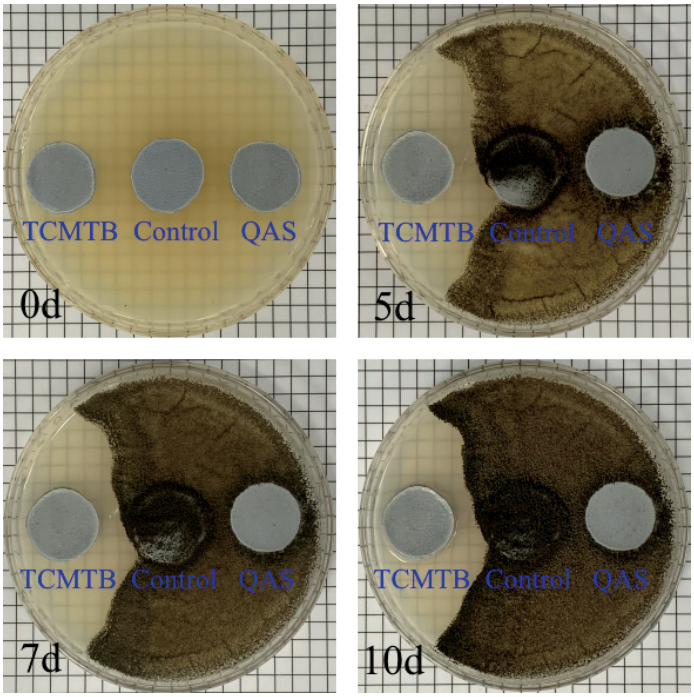

Figure 7. The result of the anti-mold property of QAS(4+10) by inhibition zone method: left sample treated with 0.5 wt.% TCMTB, right sample treated with 0.5 wt.% QAS(4+10) and middle sample without any treatment as control group

circle is observed for TCMTB treated sample, indicating that TCMTB absorbed by wet blue leather is more likely to leach in comparison with QAS(4+10). It is understandable because TCMTB is always used in the form of emulsions due to its poor water solubility, thus demulsificaiton and exudation of TCMTB upon contact with leather is unavoidable. By comparison, hydrophilic QAS(4+10) has stronger binding affinity with leather and is more stable in the practical application, which is favorable to endow leather with long-lasting anti-mold performance.

Conclusion

In conclusion, nine kinds of ester-bonded gemini QAS with different structures were synthesized and their antimicrobial actions, cytotoxicity and anti-mold performance for wet blue leather were studied. Results show that the hydrophobic tail lengths and spacer lengths in the gemini QAS structures can markedly affect their antimicrobial activity. All the as-synthesized QAS exhibit strong antimicrobial effect against *S. aureus*, *E. coli* and *A. niger*, and QAS(4+10) shows the optimal performance. SEM and fluorescence microscopy observation show that the integrity of the bacteria and *A. niger* spores cell membranes are effectively disrupted by QAS(4+10). Compared with the most widely used leather microbicide TCMTB, QAS(4+10) has comparable anti-mold performance for leather and better cyto-compatibility. It is reasonably believed that QAS(4+10) proposed in this study has the potential to be a highly effective and low-toxic leather microbicide.

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