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ScienceDirect
European Journal of Medicinal Chemistry 44 (2009) 717–724
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
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Original article

Preparation and antimicrobial behaviour of quaternary ammonium thiol derivatives able to be grafted on metal surfaces

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Received 6 February 2008; accepted 6 May 2008
Available online 16 May 2008

Abstract

New thiol derivatives containing a quaternary ammonium group bearing variable hydrocarbon chains via an amide connector or not between the sulphur and nitrogen atoms were synthesised with the future aim to be grafted on metal surfaces for obtaining contact-active auto-bactericidal surfaces. Their biostatic and bactericidal activities were evaluated against four microbial strains (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*). The presence of the thiol and amide functions in these surfactants was discussed in relation with the antimicrobial activity along with the influence of the length of alkyl chains in order to determine which molecular parameters are 'critical' for biological activity, and consequently what molecules must be chosen for grafting on metal surface.

Keywords: Preservatives; Quaternary ammonium salts; Surfactants; Thiols; Minimal inhibitory concentration; Minimal lethal concentration

1. Introduction

Because of the ever-growing demand for healthy living, there is a keen interest in materials capable of killing harmful microorganisms [1]. Concerning metallic material, the usual strategy consists in coating the metal surfaces with natural antibacterial polymers or synthetic polymers impregnated with antimicrobial agents or in applicating organic or aqueous dispersion of antimicrobial agents [2–4]. However, these strategies suffer from an important drawback since the potentially active agents are released gradually into the surrounding environment. Consequently, metal surfaces that could kill harmful microorganisms on contact without releasing antimicrobial agents are in a growing field of research from a long time [5,6].

With this aim, the concept of self-assembled monolayers (SAMs), introduced by Nuzzo and Allara [7], of thiol and disulfide molecules covalently bound to metals and bearing potentially biocide moieties could be considered. These metal grafted molecules must bear potentially biocide moieties in their structures. These moieties could be quaternary ammonium salts for which the antimicrobial activity is known for a long time [1,8–12]. So, the final purpose of our research project is to associate this SAMs' concept and the well-known bactericidal properties of quaternary ammonium compounds in order to obtain contact-active antimicrobial metal surfaces. This strategy can be summarized as shown in Scheme 1.

First and foremost it was necessary to demonstrate that molecules bearing a thiol function at one end or a disulfide

Abbreviations: CMCs, Critical micellar concentrations; MIC, Minimal inhibitory concentration; MLC, Minimal lethal concentration; SAMs, Self-assembled monolayers.

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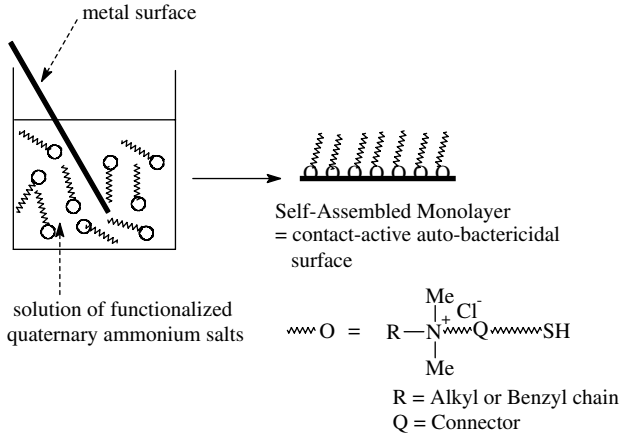
doi:10.1016/j.ejmech.2008.05.007



Scheme 1. Formation of contact-active auto-bactericidal metal surface via SAMs formation with functionalized thiols.

group into their structure and a quaternary ammonium group at the other end are able to be grafted on a metal surface to give an SAM of good quality. In a recent work, we showed that thiols and disulfides bearing internal quaternary ammonium in their structure can absorb on clean gold surface and form organized self-assembled monolayers providing that the hydrocarbon spacer between the charged nitrogen and the grafted sulphur atom is long enough to minimize the repulsive interactions between the charged groups when the structure is grafted [13]. Also, we showed that the presence of the H-donor amido group as connector between the two parts of the structures can also compensate the repulsive interaction of neighbouring charged structures on the surface, thanks to the formation of hydrogen bonding. These results suggested that potential contact-active antimicrobial metal surfaces could be obtained, at least with gold, using appropriate quaternary ammonium thiol or disulfide derivatives [13]. However, one question remains: does the presence of a moderately polar thiol function at one of the ends of these double tailed surfactants that are the dialkyldimethylammonium chlorides schematized in Scheme 1 preserve, avoid or, may enhance the antimicrobial properties of these surfactant homologues to one of the most used commercially available ammonium preservatives, benzylalkonium chloride or BAK®? Indeed, it is well known for a long time that the first effect used to explain the preservative effect of these quaternary ammonium salts is due to their surfactant properties [14–19], and it would be possible that the presence of a thiol function at the end of a tail decreases this surfactant activity.

So, the aim of this intermediate step is to test the bactericidal efficiencies of two series (series **C** and **CQ**) of thiols comparatively to the commercially available preservative BAK50 (50% w/w aqueous solution of BAK) taken as reference in this work, in order, first to check the bactericidal and surfactant properties of these thiols and, secondly, to select the best ones to be grafted on various metal surfaces. The two series synthesised are represented in Scheme 2 along with BAK50.

2. Results and discussion

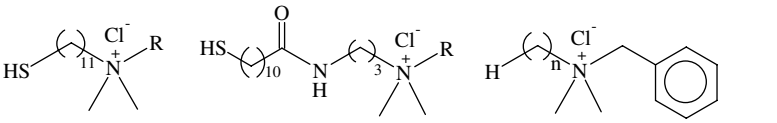
2.1. Chemical synthesis

The synthetic pathway for the preparations of compounds of series **C** is described in Fig. 1.

The starting material for the synthesis of all the compounds of series **C** is 11-bromo-undec-1-ene (Aldrich, 95%, 7766-50-9) on which a simple nucleophilic substitution is performed using trimethylamine ethanolic solution (Fluka, no. 92260) for **C1** and dimethylamine ethanolic solution (Fluka, no. 38950) for the other compounds of series **C**. Excepted for **C1**, the Menschutkin reaction leading to the ethylenic quaternary ammonium bromides is performed at high temperature without solvent using benzyl bromide (Aldrich, 98%, no. B17905), 1-bromodecane (Aldrich, 98%, no. 145785), 1-bromododecane (Aldrich, 97%, no. B65551), and tetradecyl bromide (Aldrich, 97%, no. 195332), respectively for **CBz**, **C10**, **C12** and **C14**. For all compounds of series **C**, the formation of the thiol function and the halogen exchange are achieved from the addition of thioacetic acid (Aldrich, 96%, no. 507095) under light irradiation followed by stirring in a methanolic solution of hydrogen chloride.

The synthetic pathway for the preparation of compounds of series **CQ** is described in Fig. 2.

11-Thioacetylundecanoic acid is obtained from 11-bromoundecanoic acid (Fluka, no. 18628) and potassium thioacetate (Fluka, no. 60595) [20,21]. The amide formation is performed using 3-*N*,*N*-dimethylaminopropylamine (Aldrich, no. D145009), *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide hydrochloride (Aldrich, EDC, no. E7750), and 4-dimethylaminopyridine (Aldrich, DMAP, no. 107700). Generally, the quaternization is achieved using the alkyl bromides previously described, excepted for **CQ1** for which methyl iodide



Series **C**

R = $CH_2C_6H_5$	**CBz**
= CH_3	**C1**
= $C_{10}H_{21}$	**C10**
= $C_{12}H_{25}$	**C12**
= $C_{14}H_{29}$	**C14**

Series **CQ**

R = $CH_2C_6H_5$	**CQBz**
= CH_3	**CQ1**
= $C_{10}H_{21}$	**CQ10**
= $C_{12}H_{25}$	**CQ12**
= $C_{14}H_{29}$	**CQ14**

BAK

mixture with n = 12 (40%)
n = 14 (50%)
n = 16 (10%)

Scheme 2. Quaternized ammonium chlorides used in this work.

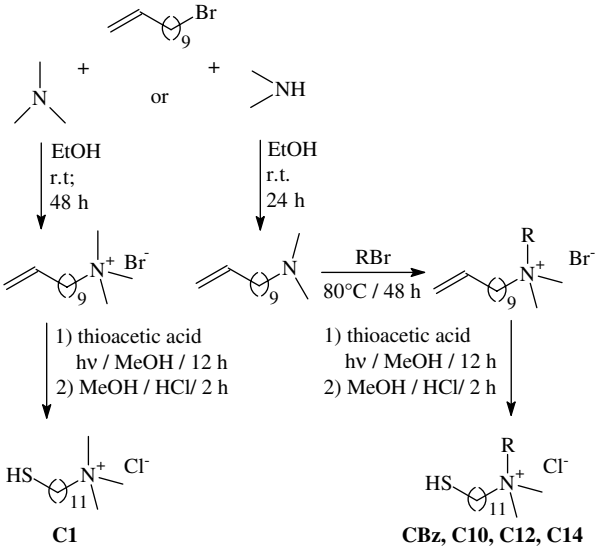


Fig. 1. Synthetic pathways for the series **C**.

(Aldrich, no. I8507) is used. Halogen exchange is performed as previously described for series **C**.

2.2. Antimicrobial activity

Antibacterial and antifungal evaluations of salts of the series **C** and **CQ** were run using MIC (Minimal Inhibitory Concentration) and MLC (Minimal Lethal Concentration) measurements versus four microorganisms: *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Aspergillus niger* (ATCC 6275) and *Candida albicans* (ATCC 10231). Three independent experiments were undertaken for each compound. The strains used are ubiquitous, opportunistic and commonly encountered in biodeterioration material problems or fouling of the industrial processes, in addition these types of strains could be encountered in nosocomial infections.

2.2.1. Surfactant and microbiostatic activities

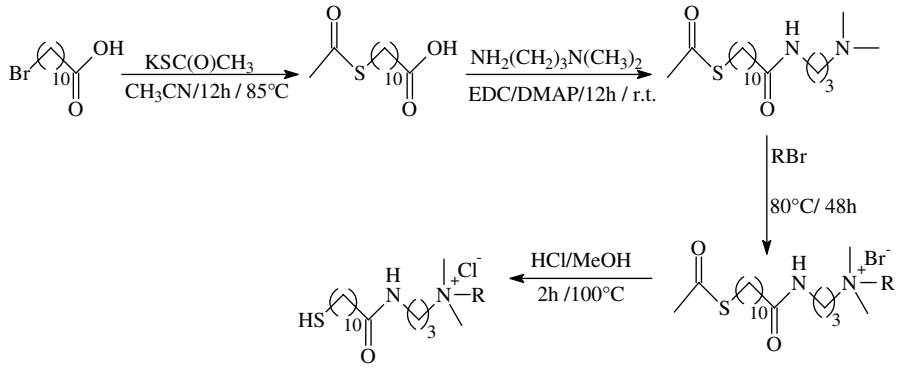
Table 1 summarizes the MIC values for all the molecules tested along with their Critical Micellar Concentrations (CMCs) and their surface tensions (γ_S).

The comparison of the CMC values of compounds **C1** and **CBz** with their homologues bearing a methyl group instead of the thiol function [23] seems to show that, effectively, this later decreases the surfactant efficiency of these molecules; however, compounds **C1** and **CBz** could not be representative of the series **C**. Nevertheless, the comparison of the MIC of **BAK 50** evaluated in the same conditions with those of compounds **C10–C14** confirms a decrease of the microbiostatic activity of these surfactants with the presence of the thiol function.

All the compounds of series **C** are active towards the four microorganisms studied with the exception of **C14** which shows only an acceptable activity towards *A. niger*. Concerning the sub-series formed by **C1** and **C10–C14**, a non-linear dependence of the microbiostatic activity with the length of the variable tail is observed (Fig. 3).

A parabolic dependence of MIC with the length of the tail can be observed with a maximum for **C10**. This type of dependence was previously observed in different studies [24–26]. This phenomenon is called ''cut-off effect''. The origin of this effect is not well explained. Among the various assumptions proposed by Balgavy and Devinsky [25], the concept of free volume could be applied to the quaternary ammonium salts. In solution, the polar ammonium heads interact with those of phospholipids and the hydrocarbon chains are parallel to those of phopholipids of the cell. At this level, the density of the hydrophobic area of the bilayer is necessarily modified and a free volume is formed. When the length of the hydrocarbon chain of ammonium salts is smaller than that of phospholipids, the total free volume created in the bilayer is small. When the length of the surfactant tail becomes comparable to that of phospholipids, the free volume decreases and tends towards zero. Molecules bearing chains between these two extremes lead to the most important free volume inside the bilayer. More the free volume is large; more the decrease of hydrophobicity of the membrane is large. Consequently, a largest destabilization of the membrane is expected and the bactericidal activity increases. Moreover, the smallest CMI value is observed for the most bioactive surfactant **C10**.

As a whole, a large difference is observed between the results obtained with *P. aeruginosa* and those obtained with the three other strains. It is well known that, due to the structure of



CQ1, **CQ Bz**, **CQ10**, **CQ12**, **CQ14**

Fig. 2. Synthetic pathway for the series **CQ**.

Table 1
MIC values of the molecules of series **C** and **CQ** along with their CMC and γ_S

Molecules	MIC (μmol/L)				CMC (mmol/L)	γ_S (mN/m)
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus niger*		
BAK 50	3.2	93	8.36	4.74		
C1	56.6	556.9	1124.8	56.6	40 (11.6)[a]	44.1
CBz	681.6	1055.9	471.6	351.0	6.7 (2.7)[b]	30.3
C10	11.6	308.9	31.6	31.6	1.6	28.3
C12	34.7	326.2	39.5	67.2	0.53	31.0
C14	1306.3	1121.5	1524.6	90.0	0.13	33.2
CQ1	105.4	594.6	>1065.1	105.3	49	45.9
CQBz	592.2	979.8	>1057.3	772.6	5.6	34.1
CQ10	17.5	171.4	>676.8	44.1	1.4	33.7
CQ12	18.7	183.5	>687.6	47.02	0.61	36.1
CQ14	187.7	441.8	>741.6	56.3	0.14	38.4

[a] CMC of the homologue dodecyltrimethylammonium chloride at 25 °C from Ref. [22].
[b] CMC of the homologue benzyldodecyldimethylammonium chloride at 25 °C from Ref. [22].

its outer membrane, this organism possesses the ability to metabolize quaternary ammonium compounds as a source of carbon and nitrogen [27]; therefore, the result observed here is not surprising.

In comparison with compound **CBz** bearing the benzyl substituent largely met in this type of preservatives, compound **C10** demonstrates always a larger microbiostatic activity for all the strains studied.

The results obtained with the series **CQ** confirm those obtained with series **C** with the exception of *C. albicans* for which the MIC values are higher than the detection threshold (Fig. 4).

For this series, the most important feature is the fact that the presence of the amide group as connector between the charged nitrogen and the hydrocarbon chain bearing the thiol function does not largely disturb the microbiostatic activities of these surfactants even if their surface properties (CMC and γ_S) are slightly decreased. So comparatively with the SAMs formed with the compounds of the series **C**, we can expect SAMs of best quality (higher density) with the compounds of series **CQ** due to the presence of hydrogen bonds, and consequently an increase of the microbiostatic activity of the treated metal surface.

2.2.2. Microbicidal activity

Table 2 summarizes the values of MLC evaluated for all the compounds of series **C** and **CQ**. Only three values can be obtained for fungi, and only **C10** shows an MLC for *A. niger* in the range of concentrations used. These MLCs are weak for *S. aureus* and they increase for *P. aeruginosa*. Similar trends are observed for MIC and MLC. In agreement with the literature, the MLC values are generally higher than the MIC values. Indeed, the frontier between microbiostatic activity and microbicidal activity is sometimes a question of concentrations used, and a same product can be microbiostatic at low concentration and microbicidal at higher concentration [14]. For the most active compounds with 10 and 12 carbon atoms, the analogues with the amide connector seem to be slightly active than the compounds of series **C**.

3. Experimental section

3.1. Chemical synthesis

3.1.1. General

^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra were recorded on a Brüker AC 200 spectrometer. Signal attributions were confirmed by HMQC (2D NMR) experiments. The mass spectra were recorded on a Finnigan Mat LCQ Classic Electrospray source API1. Elemental analyses were performed using a Flash EA1112 automatic elemental analyzer from Thermo Scientific equipped with Eager 300 software.

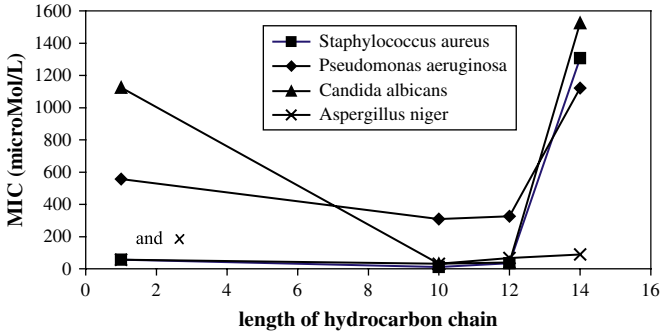


Fig. 3. Variation of the MIC of series **C** as a function of the length of the hydrocarbon chain.

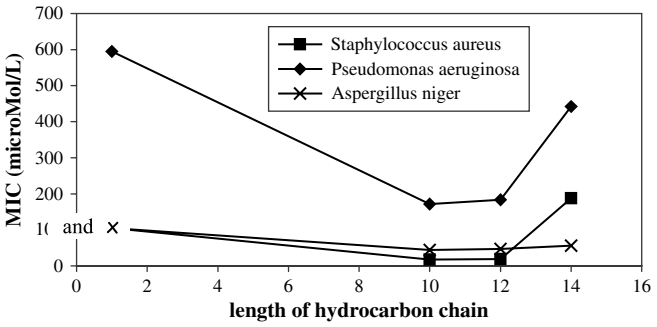


Fig. 4. Variation of the MIC of series **CQ** as a function of the length of the hydrocarbon chain.

Table 2
MLC values of the molecules of series **C** and **CQ**

Molecules	MLC (μmol/L)			
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus niger*
C1	56.6	>1116.88	>1904.81	>131.10
CBz	681.6	>1528.65	>896.2	681.6
C10	47.95	308.9	>61.29	47.95
C12	73.3	326.2	>63.36	>92.22
C14	>1524.6	>1524.6	>1524.6	>123.72
CQ1	>395.2	>864.49	>1065.06	>395.2
CQBz	946.17	>1057.32	>1057.32	>946.2
CQ10	32.50	249.88	>676.80	>60.74
CQ12	38.2	183.5	>687.60	>61.71
CQ14	187.7	598.67	>741.60	>74.16

*3.1.2. General procedures for preparation of thiols without amido connector, **C1**, **CBz**, **C10**, **C12** and **C14***

3.1.2.1. Formation of N,N-dimethylundec-10-enamine. A total of 21 millimoles (mmol) of 11-bromoundec-1-ene is added to 63 mmol of dimethylamine (5.6 M ethanolic solution). The mixture is stirred at room temperature for 24 h, then, a 10% aqueous solution of sodium hydroxide is added and the mixture is extracted three times with diethyl ether. The organic layers were washed, dried and then concentrated. The residue is rectified on Kugelrohr apparatus to afford *N*,*N*-dimethylundec-10-enamine (yield: 93%).

3.1.2.2. Formation of the unsaturated quaternary ammonium bromides. A total of 5 mmol of ethylenic amine previously prepared is added to 6 mmol of the selected 1-bromoalcane, RBr. The mixture is stirred at 80 °C for 48 h. After cooling, 20 mL of diethyl ether are added at room temperature. The precipitate obtained is filtered and washed several times with diethyl ether to give the expected ethylenic quaternary ammonium bromide with yields of 89%, 83%, 83% and 87% for R = $CH_2C_6H_5$, $C_{10}H_{21}$, $C_{12}H_{25}$ and $C_{14}H_{29}$, respectively.

*3.1.2.3. Formation of thiols **CBz**, **C10**, **C12** and **C14***. A total of 10 mmol of the selected unsaturated quaternary ammonium bromide is mixed with 30 mmol of thioacetic acid in a little amount of methanol. The mixture is irradiated for 12 h using a 300-W ultraviolet lamp (high pressure quartz–mercury arc). After solvent and residual thioacetic acid evaporation, the crude product is triturated several times with diethyl ether. Small quantities of methanol and 10% aqueous solution of hydrochloric acid are added. The mixture is stirred at 105 °C for 2 h. Methanol and water are then evaporated under reduced pressure and a suspension of charcoal in methanol is added. The mixture is stirred at room temperature for 4 h. After filtration on celite, the filtrate is concentrated and the residue is lyophilised to give the expected thiol with yields of 81%, 76%, 80% and 76% for **CBz**, **C10**, **C12** and **C14**, respectively.

*3.1.2.4. Formation of thiol **C1***. 11-Bromoundec-1-ene (25 mmol) is added to 82 mmol of trimethylamine (4.2 M ethanolic solution). The mixture is stirred at room temperature for 48 h. After concentration, the residue is dissolved in dichloromethane (20 mL), and then precipitated by addition of hexane (300 mL) to afford, after filtration, *N*,*N*,*N*-trimethylundec-10-enamonnium bromide (yield: 91%). The corresponding thiol **C1** is obtained according to the same pathway as previously described (cf. Section 3.1.2.3) with a yield of 85%.

*3.1.2.4.1. Compound **C1***. Yield: 77%. ^{1}H NMR (200 MHz, MeOD): δ/ppm = 1.32 (m, 14H [−CH_2−]$_7$), 1.59 (m, 2H [SCH_2−CH_2]), 1.81 (m, 2H [N−CH_2−CH_2]), 2.42 (t, 2H [S−CH_2]), 3.19 (s, 9H [N$^+$(CH_3)$_3$]), 3.44 (m, 2H [N−CH_2]). ^{13}C NMR (MeOD): δ/ppm = 24.2 (N−C−*C*), 25.6 (S−*C*), 30.0 (S−C−C−C_7), 35.3 (S−C−*C*), 53.1 (N−*C*H$_3$), 68.6 (N−*C*H$_2$). MS, [M−Cl]$^+$, *m*/*z* (%): 246 (100). Anal. Calcd for $C_{14}H_{32}NSCl$: C 59.64, H 11.44, N 4.97, S 11.37; found: C 59.64, H 11.51, N 4.99, S 11.06.

*3.1.2.4.2. Compound **CBz***. Yield: 67%. ^{1}H NMR (200 MHz, MeOD): δ/ppm = 1.30 (m, 14H [−CH_2−]$_7$), 1.64 (m, 2H [SCH_2−CH_2]), 1.70 (m, 2H [N−CH_2−CH_2]), 2.42 (t, 2H [S−CH_2]), 3.11 (s, 6H [N$^+$(CH_3)$_2$]), 3.40 (m, 4H [CH_2−N−CH_2]), 4.56 (s, 2H, [CH_2−C_6H_5]), 7.60 (m, 5H [C_6H_5]). ^{13}C NMR (MeOD): δ/ppm = 24.0 (N−C−*C*), 25.2 (S−*C*), 30.0 (S−C−C−C_7), 35.5 (S−C−*C*), 51.6 (N−*C*H$_3$), 65.0 (N−*C*H$_2$), 69.8 (N−*C*H$_2$−C_6H_5), 129.2−131.2−132.4−135.4 (*C*$_6$H$_5$). MS, [M−Cl]$^+$, *m*/*z* (%): 322.6 (100). Anal. Calcd for $C_{19}H_{36}NSCl$: C 65.65, H 10.49, N 4.05, S 9.26; found: C 66.09, H 10.82, N 4.07, S 9.41.

*3.1.2.4.3. Compound **C10***. Yield: 59%. ^{1}H NMR (200 MHz, MeOD): δ/ppm = 0.79 (t, 3H [CH_2−CH_3]), 1.30 (m, 28H [−CH_2−]$_7$ and [−CH_2−]$_7$), 1.64 (m, 2H [SCH_2−CH_2]), 1.70 (m, 4H [CH_2−CH_2−N−CH_2−CH_2]), 2.42 (t, 2H [S−CH_2]), 3.11 (s, 6H [N$^+$(CH_3)$_2$]), 3.40 (m, 4H [CH_2−N−CH_2]). ^{13}C NMR (MeOD): δ/ppm = 15.3 (CH$_2$−*C*H$_3$), 24.0 (*C*−C−N−C−C and *C*−CH$_3$), 25.2 (S−*C*), 30.0 (S−C−C−C_7 and N−C−C−C_5), 33.1 (*C*−C−CH$_3$), 35.5 (S−C−*C*), 51.6 (N−*C*H$_3$), 65.0 (N−*C*H$_2$). MS, [M−Cl]$^+$, *m*/*z* (%): 372.4 (100). Anal. Calcd for $C_{23}H_{50}NSCl$: C 67.68, H 12.35, N 3.43, S 7.85; found: C 67.75, H 12.57, N 3.87, S 7.69.

*3.1.2.4.4. Compound **C12***. Yield: 62%. ^{1}H NMR (200 MHz, MeOD): δ/ppm = 0.82 (t, 3H [CH$_2$−CH_3]), 1.30 (m, 32H [−CH_2−]$_7$ and [−CH_2−]$_9$), 1.64 (m, 2H [SCH_2−CH_2]), 1.70 (m, 4H [CH_2−CH$_2$−N−CH$_2$−CH_2]), 2.42 (t, 2H [S−CH_2]), 3.11 (s, 6H [N$^+$(CH_3)$_2$]), 3.40 (m, 4H [CH_2−N−CH_2]). ^{13}C NMR (MeOD): δ/ppm = 15.1 (CH$_2$−*C*H$_3$), 24.0 (*C*−C−N−C−C and *C*−CH$_3$), 25.2 (S−*C*), 30.0 (S−C−C−C_7 and N−C−C−C_7), 33.5 (*C*−C−CH$_3$), 35.5 (S−C−*C*), 51.6 (N−*C*H$_3$), 65.0 (N−*C*H$_2$). MS, [M−Cl]$^+$, *m*/*z* (%): 400.4 (100). Anal. Calcd for $C_{25}H_{54}NSCl$: C 68.83, H 12.48, N 3.21, S 7.35; found: C 68.98, H 12.60, N 4.99, S 7.16.

*3.1.2.4.5. Compound **C14***. Yield: 61%. ^{1}H NMR (200 MHz, MeOD): δ/ppm = 0.78 (t, 3H [CH$_2$−CH_3]), 1.30 (m, 36H [−CH_2−]$_7$ and [−CH_2−]$_{11}$), 1.64 (m, 2H [SCH_2−CH_2]), 1.70 (m, 4H [CH_2−CH$_2$−N−CH$_2$−CH_2]), 2.42 (t, 2H [S−CH_2]), 3.11 (s, 6H [N$^+$(CH_3)$_2$]), 3.40 (m, 4H [CH_2−N−CH_2]). ^{13}C NMR (MeOD): δ/ppm = 15.6 (CH$_2$−*C*H$_3$), 24.0 (*C*−C−N−C−C and *C*−CH$_3$), 25.2 (S−*C*), 30.0 (S−C−C−C_7 and N−C−C−C_9), 33.1 (*C*−C−CH$_3$), 35.5

(S–C–*C*), 51.6 (N–*C*H_3), 65.0 (N–*C*H_2). MS, $[M-Cl]^+$, *m/z* (%): 428.4 (100). Anal. Calcd for $C_{27}H_{58}NSCl$: C 69.85, H 12.59, N 3.02, S 6.90; found: C 69.77, H 12.71, N 3.09, S 6.85.

3.1.3. General procedures for preparation of thiols with amido connector, ***CQ1****,* ***CQBz****,* ***CQ10****,* ***CQ12*** *and* ***CQ14***

3.1.3.1. Thiol protection. 11-Bromoundecanoic acid (5.3 g, 20 mmol) and potassium thioacetate (2.5 g, 22 mmol) are dissolved in acetonitrile (60 mL) under nitrogen and the mixture is heated at 85 °C for 12 h. The volatiles are removed under vacuum and 40 mL of water are added. The solution is extracted three times with diethyl ether. The organic layers are washed with water, dried on Na_2SO_4 and then concentrated under reduced pressure. The residue is purified by column chromatography over silica gel using hexane as the eluant. 11-Thioacetoxyundecanoic acid is obtained as a beige solid (4.3 g, 82%).

3.1.3.2. Amide formation. Previously prepared 15 mmol (3.9 g) of 11-thiacetoxyundecanoic acid, 15 mmol (2.87 g) of *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide hydrochloride and 1.5 mmol (0.18 g) of 4-dimethylaminopyridine are added to 50 mL of dichloromethane. The mixture is stirred for 4 h at room temperature and then 15 mmol (1.53 g) of 3-*N*,*N*-dimethylaminopropylamine are added at 0 °C. After stirring for 24 h at room temperature, the reaction mixture is washed three times with water. The organic layer is separated, dried and then concentrated under reduced pressure. The crude product is obtained as a beige solid (3.6 g, 70%).

3.1.3.3. Quaternization of the protected thiol. Excepted for the trimethyl ammonium derivative, 10 mmol of the resulting tertiary amine are added to 12 mmol of the selected 1-bromoalcane (decyl bromide, dodecyl bromide, tetradecyl bromide or benzyl bromide). The solution is stirred and heated at 80 °C for 2 days. After cooling, 20 mL of diethyl ether and the final product precipitates. The mixture is filtered and the salt obtained is washed several times with diethyl ether. The crude product was obtained as beige solid [4.35 g (77%), 5 mg (84%), 5.15 g (83%) and 313 mg (80%) for $R_H = C_{10}H_{21}$, $C_{12}H_{25}$, $C_{14}H_{29}$ and $CH_2C_6H_5$, respectively].

For the compounds with $R_H = CH_3$, 10 mmol of the precursor are added to a solution of 40 mmol of methyl iodide in 30 mL of anhydrous diethyl ether. The solution was stirred and heated at 40 °C for 2 days. The final product precipitates. The mixture was filtered and the salt obtained was washed several times with diethyl ether. The product **3** was obtained as beige solid (3.93 g, 81%).

3.1.3.4. Thiol deprotection. A total of 7 mmol of the selected protected thiol is dissolved in a minimal volume of methanol and 10% aqueous solution of hydrochloric acid. The solution is stirred at 100 °C for 1 h. After removal of methanol and water under vacuum, a suspension of charcoal in methanol is added to the crude product. The mixture is stirred at room temperature for 4 h. After filtration on celite, the filtrate is concentrated and the residue is lyophilised to give the expected thiol with yields of 94%, 90%, 92%, 91% and 94% for **CQ1**, **CQBz**, **CQ10**, **CQ12** and **CQ14**, respectively.

3.1.3.4.1. Compound ***CQ1****.* Yield: 44%. ^{1}H NMR (200 MHz, MeOD): δ/ppm = 1.3 (m, 12H [S–C–C–(CH_2)$_6$]), 1.62 (m, 4H [S–C–CH_2 and CH_2–C–C(O)]), 1.96 (m, 2H [CH_2–C–N^+]), 2.08 (t, *J* = 7.5 Hz, 2H [CH_2–C(O)]), 2.51 (t, *J* = 7.1 Hz, 2H [S–CH_2]), 3.07 (s, 9H [N(CH_3)$_3$]), 3.35 (m, 2H [NH–CH_2]), 3.43 (m, 2H [CH_2–N^+]). ^{13}C NMR (200 MHz, MeOD): δ/ppm = 24.2 (*C*–C–N^+), 25.1 (S–*C*), 27.2 (*C*–C–CO), 30.0 (S–C–C–C_6), 34.9 (S–C–*C*), 35.3 (*C*–CO), 37.8 (NH–*C*), 53.4 [N(*C*H_3)$_3$], 66.9 (*C*–N^+), 177.4 (*C*O). MS, $[M-Cl]^+$, *m/z* (%): 317.3 (100). Anal. Calcd for $C_{17}H_{35}N_2OSCl$: C 58.17, H 10.05, N 7.98, S 9.13; found: C 58.96, H 9.99, N 7.96, S 9.03.

3.1.3.4.2. Compound ***CQBz****.* Yield: 41%. ^{1}H NMR (200 MHz, MeOD): δ/ppm = 1.3 (m, 12H [S–C–C–(CH_2)$_6$]), 1.60 (m, 4H [S–C–CH_2 and CH_2–C–C(O)]), 1.91 (m, 2H [CH_2–C–N^+]), 2.12 (t, *J* = 7.5 Hz, 2H [CH_2–C(O)]), 2.48 (t, *J* = 7.1 Hz, 2H [S–CH_2]), 3.06 (s, 6H [N(CH_3)$_2$]), 3.30 (m, 4H [NH–CH_2 and CH_2–N^+]), 4.60 (m, 2H [CH_2–C_6H_5]), 7.60 (m, 5H [C_6H_5]). ^{13}C NMR (200 MHz, MeOD): δ/ppm = 24.0 (*C*–C–N^+), 25.2 (S–*C*), 27.2 (*C*–C–CO), 30.0 (S–C–C–C_6), 35.4 (S–C–*C* and *C*–CO), 37.4 (NH–*C*), 51.2 [N(*C*H_3)$_2$], 63.6 (*C*–N^+), 66.9 (*C*–C_6H_5), 129.3–134.8–130.1–132.6 (C_6H_5), 177.8 (*C*O). MS, $[M-Cl]^+$, *m/z* (%): 393.3 (100). Anal. Calcd for $C_{22}H_{39}N_2OSCl$: C 63.66, H 9.47, N 6.75, S 7.56; found: C 63.87, H 9.70, N 6.73, S 7.56.

3.1.3.4.3. Compound ***CQ10****.* Yield: 42%. ^{1}H NMR (200 MHz, MeOD): δ/ppm = 0.79 (t, 3H [C–CH_3]), 1.3 (m, 26H [S–C–C–(CH_2)$_6$ and N^+–C–C–(CH_2)$_7$]), 1.60 (m, 6H [S–C–CH_2, CH_2–C–C(O) and N^+–C–CH_2]), 1.91 (m, 2H [CH_2–C–N^+]), 2.12 (t, *J* = 7.5 Hz, 2H [CH_2–C(O)]), 2.48 (t, *J* = 7.1 Hz, 2H [S–CH_2]), 3.06 (s, 6H [N(CH_3)$_2$]), 3.30 (m, 6H [NH–CH_2 and CH_2–N^+–CH_2]).

^{13}C NMR (200 MHz, MeOD): δ/ppm = 15.4 (C–*C*H_3), 23.1 (N^+–C–*C*), 24.0 (*C*–C–N^+), 24.4 (*C*–CH_3), 25.2 (S–*C*), 27.2 (*C*–C–CO), 30.0 (S–C–C–C_6), 33.2 (*C*–C–CH_3), 35.4 (S–C–*C* and *C*–CO), 37.4 (NH–*C*), 51.2 [N(*C*H_3)$_2$], 63.6 (*C*–N^+), 65.4 (N^+–*C*), 177.8 (*C*O). MS, $[M-Cl]^+$, *m/z* (%): 443.4 (100). Anal. Calcd for $C_{26}H_{53}N_2OSCl$: C 65.44, H 11.19, N 5.87, S 6.72; found: C 65.72, H 11.2770, N 5.64, S 6.59.

3.1.3.4.4. Compound ***CQ12****.* Yield: 42%. ^{1}H NMR (200 MHz, MeOD): δ/ppm = 0.74 (t, 3H [C–CH_3]), 1.3 (m, 30H [S–C–C–(CH_2)$_6$ and N^+–C–C–(CH_2)$_9$]), 1.60 (m, 6H [S–C–CH_2, CH_2–C–C(O) and N^+–C–CH_2]), 1.91 (m, 2H [CH_2–C–N^+]), 2.12 (t, *J* = 7.5 Hz, 2H [CH_2–C(O)]), 2.48 (t, *J* = 7.1 Hz, 2H [S–CH_2]), 3.06 (s, 6H [N(CH_3)$_2$]), 3.30 (m, 6H [NH–CH_2 and CH_2–N^+–CH_2]). ^{13}C NMR (200 MHz, MeOD): δ/ppm = 15.9 (C–*C*H_3), 22.9 (N^+–C–*C*), 24.0 (*C*–C–N^+), 24.2 (*C*–CH_3), 25.2 (S–*C*), 27.2 (*C*–C–CO), 30.0 (S–C–C–C_8), 32.8 (*C*–C–CH_3), 35.4 (S–C–*C* and *C*–CO), 37.4 (NH–*C*), 51.2 [N(*C*H_3)$_2$], 63.6 (*C*–N^+), 65.8 (N^+–*C*), 177.8 (*C*O). MS, $[M-Cl]^+$, *m/z* (%): 471.4

(100). Anal. Calcd for $C_{28}H_{57}N_2OSCl$: C 66.56, H 11.37, N 5.54, S 6.34; found: C 66.70, H 11.49, N 5.30, S 6.27.

3.1.3.4.5. Compound ***CQ14***. Yield: 43%. ^{1}H NMR (200 MHz, MeOD): δ/ppm = 0.82 (t, 3H [C−CH_3]), 1.3 (m, 34H [S−C−C−(CH_2)$_6$) and N^+−C−C−(CH_2)$_{11}$], 1.60 (m, 6H [S−C−CH_2, CH_2−C−C(O) and N^+−C−CH_2]), 1.91 (m, 2H [CH_2−C−N^+]), 2.12 (t, J = 7.5 Hz, 2H [CH_2−C(O)]), 2.48 (t, J = 7.1 Hz, 2H [S−CH_2]), 3.06 (s, 6H [N(CH_3)$_2$]), 3.30 (m, 6H [NH−CH_2 and CH_2−N^+−CH_2]). ^{13}C NMR (200 MHz, MeOD): δ/ppm = 13.1 (C−*C*H$_3$), 22.6 (N^+−C−*C*), 24.0 (*C*−C−N^+), 24.4 (*C*−CH$_3$), 25.2 (S−*C*), 27.2 (*C*−C−CO), 30.0 (S−C−C−*C*$_{10}$), 33.0 (*C*−C−CH$_3$), 35.4 (S−C−*C* and *C*−CO), 37.4 (NH−*C*), 51.2 [N(*C*H$_3$)$_2$], 63.6 (*C*−N^+), 65.7 (N^+−*C*), 177.8 (*C*O). MS, [M−Cl]$^+$, *m/z* (%): 499.4 (100). Anal. Calcd for $C_{30}H_{61}N_2OSCl$: C 67.56, H 11.53, N 5.25, S 6.01; found: C 67.75, H 11.35, N 5.37, S 5.99.

3.2. Surfactant property evaluation

The Critical Micellar Concentrations (CMCs) were obtained by electrical conductivity measurements [28] at 25 °C using a conductimeter apparatus Consort C831. The surface tensions (γ_s) were determined at 25 °C by the Wilhelmy plate method using a tensiometer Krüss K100 [22,29,30]. The substrates being insoluble in pure water, a 10% methanolic aqueous solution was used. It was demonstrated that the CMCs varies only slightly with 10% of methanol in water [31].

3.3. Antimicrobial characteristics

3.3.1. MIC determination

MIC is defined as the lowest concentration of an antimicrobial activity that will inhibit the visible growth of a microorganism after overnight incubation. The MICs were taken as the minimal concentration showing no growth (absence of turbidity) after 24 h of incubation at 30 °C for bacteria and after 72 h of incubation at 25 °C for fungi. MICs were determined using a serial dilution method. The automat Biomek® 1000 (Beckman®) apparatus used for our experiments performed automatically dilutions of the tested antimicrobial agents solutions in 96 well micro-titration plates.

3.3.2. MLC determination

The MLC is defined as the lowest concentration of antimicrobial activity that will prevent the growth of a microorganism after subculture onto antibiotic-free media. The MLC levels were determined by taking (after 5 days incubation for bacteria or 7 days for fungi) from MIC microtiter plates, 100 µl from the three sub-doses wells where no growth was observed, and subculture by streaking onto trypticase soy agar (TSA) or Sabouraud Dextrose Agar (SDA). Plates are incubated as described below and checked after incubation period to determine whether or not viable microorganisms remain.

3.3.3. Cultivation

MIC determinations were run with the third generation of bacteria or fungi. For bacteria, samples were taken during the exponential phase. Bacteria were grown overnight on a TSA slant and inoculated into 20 mL of M9GY at pH 7.0 ± 0.2 culture medium and incubated overnight at 30 °C. The optical density of the suspension was then measured at 660 nm. If necessary, additional M9GY medium was added to the suspension to adjust the optical density at 660 nm to 0.05 corresponding approximately to between 5.10^6 and 5.10^7 cfu/mL.

Fungi were incubated on SDA slants for 5 days at 25 °C. MIC determinations were run with the third generation of fungi; culture samples were taken during the exponential phase of fungal growth. Each fungal strain was cultivated during 5–7 days on SDA at 25 ± 2 °C until sufficient spores were formed. Then fungal spores were harvested by adding 5 mL of M9GY at pH 5.0 ± 0.2 to the SDA slant, which were then gently scraped to suspend the microorganisms. The fungal solution is then filtered with sterile cheesecloth under aseptic conditions to eliminate the residual mycelium. The suspension was adjusted using a counting cell under microscope, to 5.10^6–5.10^7 spores/mL by adding M9GY if necessary.

Initial solution concentration of each antimicrobial agent was prepared in a mixture of methanol/sterile deionised water (ratio 1/1).

4. Conclusion

The two series, **C** and **CQ**, of double tailed cationic surfactants synthesised in this work show antimicrobial activities against the four classical strains studied here. For both series, the best efficiency is observed when the length of the hydrocarbon chain is 10 carbon atoms. It is the ''cut-off effect'' of this type of preservative. So, the future elaboration of contact-active auto-bactericidal metal surfaces via SAMs formation on metal surfaces using these dialkyldimethylammonium chlorides (particularly, **C10**–**C14** and **CQ10**–**CQ14**) bearing a thiol function at the end of one of the alkyl groups can be successfully envisaged. Although the presence of a thiol function at the end of one of the tails slightly decreases the tensioactive properties of these quaternary ammonium salts in comparison with the unsulfurated homologues, their microbiostatic and microbicidal activities against the four strains studied remain. Moreover, the presence of an amido connector between the charged nitrogen and the thiol function (series **CQ** versus series **C**) does not largely affect the antibacterial properties of the most efficient of these preservatives. This is an important point concerning the future formation of SAMs, since one of our previous works showed that the presence of this amido connector, and consequently of possible hydrogen bond formations, seem to be necessary to compensate the repulsive electric force between the charged nitrogen atoms of two neighbouring monomers in the SAMs and consequently to obtain the best SAMs.

The next step of this study is the SAMs formation on various metals (particularly, gold and iron) and the antibacterial evaluation of the treated surfaces obtained.

Acknowledgments

This work was supported by the "Region PACA" Council, the Society ARECO (Grasse), and Rohm and Haas France.

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