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6-Hydroxyquinolinium salts differing in the length of alkyl side-chain: Synthesis and antimicrobial activity

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ABSTRACT

Quaternary ammonium salts substituted with a long alkyl chain exemplify a trustworthy group of medicinal compounds frequently employed as antifungal and antibacterial agents. A great asset of these surfactants underlying their widespread use is low local and system toxicity in humans. In this Letter, a series of novel quaternary 6-hydroxyquinolinium salts with varying length of *N*-alkyl chain (from C_{10} to C_{18}) was synthesized and tested for in vitro activity against pathogenic bacterial and fungal strains. 6-Hydroxyquinolinium salt with C12 alkyl chain seems to be very interesting candidate due to a high antimicrobial efficacy and cytotoxic safety.

Nosocomial infections, known as 'hospital-acquired infections', are predominantly acquired within healthcare settings, constituting *de facto* major cause of death and increased morbidity among hospitalized patients. Undoubtedly, one of the key factors behind these worldwide infections is poor immunity status of the patients (e.g., those undertaking chemotherapy, organ or bone-marrow transplantation, etc.).[1] Those with a compromised immune defence system are highly susceptible to extensive fungal infection, especially by *Candida albicans* and related strains.[2] Unfortunately, the threat of ravaging infection is buttressed by the absence of a strict glove-change regime and the use of improperly or inadequately sterilized medical instruments in some hospital facilities.[3] Amongst the group of ordinary antiseptics and disinfectants that have been used to treat such infections we can also include cationic surfactants, particularly quaternary ammonium salts.[4] Cationic surfactants consist of a hydrophilic part (e.g., a quaternary nitrogen moiety) able to interact with polar chemical milieu, and a hydrophobic part (e.g., a long alkyl chain) which can, on the other hand, penetrate into non-polar molecular agglomerates. Besides being of a high medicinal relevance, these amphiphilic compounds offer a huge number of applications in industry. The most important substances used in industrial fields are derived from *N*-alkylpyridinium and *N*-benzalkonium salts.[5] *N*-Alkylpyridinium quaternary compounds are often used in analytical chemistry as auxiliary solubilizers of water-insoluble substances,[6] or for instance, as antiplaque agents in chewing gums.[7,8] Moreover, these compounds are known for their competitive inhibitory activity on acetylcholinesterase[9] and antagonistic effect on nicotinic receptors.[10] With respect to the capability to form an interface between polar and non-polar phases, the use of *N*-alkylpyridinium salts as carriers to improve absorption of oligonucleotides through the cellular phospholipid membrane has been described.[11,12]

Quaternary ammonium salts containing long alkyl chains exert bactericidal and fungicidal properties. Taking into account their low toxicity in humans, these compounds have been widely applied in the form of sprays, eye drops or solutions such as for rinsing open wounds and as pre-operative disinfectants.[13–16] *N*-Dodecylbenzalkonium (C_{12}) salts have proved to have a potent fungicidal effect, while C_{14} and C_{16} *N*-benzalkonium homologs

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exhibited improved activity against Gram-positive and Gram-negative bacteria, respectively.[17] A potent antimicrobial activity has also been observed for some quinoline derivatives.[18] Even though the mechanism of the surfactant's antimicrobial activity is not known in deep detail, it seems that molecules carrying strong hydrophilic and hydrophobic functionality can change the physicochemical properties of cellular membranes,[19] and as a result of altered membrane permeability eventually cytolysis occurs.

In this Letter, we describe the synthesis, biological activity and LC–MS analysis of a new group of cationic surfactants: 6-hydroxyquinolinium salts with different lengths of alkyl side-chain (C_{10}–C_{18}). Due to a generally accepted consensus of the antimicrobial activity of quaternary ammonium salts, antibacterial and antifungal efficiency has been evaluated only for C_{12}, C_{14} and C_{16} alkylated 6-hydroxyquinolinium, and then compared to the biological activities of the analogous series of C_{12}–C_{16} alkyl homologues of *N*-benzalkonium and *N*-alkylpyridinium compounds. *N*-Benzalkonium analogues are very common components of commercially available disinfectants. The purpose of the comparison of single quaternary ammonium analogues on different microbial strains is to deduce a simple structure–activity relationship which can serve for the preparation of mixtures customized against chosen pathogens.

The preparation of alkyl derivatives of *N*-benzalkonium and *N*-alkylpyridinium used in the present Letter has already been published previously, but without reporting their antibacterial and antifungal activities.[17,20] Since the in vitro biological activity of the quaternary ammonium salts is likely dependent on their lipophilicity, a simple LC–MS analytical method was developed to determine the retention times, which can approximate this important physicochemical property.[21]

The new quaternary surfactants **7**–**11** were prepared by reaction of 6-hydroxyquinoline (**1**) with 1-bromoalkane (**2**–**6**) in acetonitrile (CH_3CN), as shown in Figure 1 (for further details see Supplementary data). The mixture of 6-hydroxyquinoline and 1-bromoalkane was stirred under reflux for 48 h. The prepared salts were obtained as brown crystals by crystallization from acetone. The alkyl chains were selected to have length from C_{10} to C_{18}. The lipophilicity of the prepared compounds was estimated by means of retention times determined by Liquid Chromatography (LC). Yields, melting points, retention times and calculated log*P* (*C*log*P*) of the prepared compounds (by HyperChem (TM) 7.52 Professional, Florida, USA) are shown in Table 1. The products were characterized by ^{1}H NMR, ^{13}C NMR and High-resolution Mass Spectrometry (HRMS) analyses. According to LC with UV detection (λ = 254 nm), the purity of all prepared compounds was ⩾99%.

To determine lipophilicity and HRMS of the studied compounds, an analytical LC–MS method was developed (Supplementary data available).

The in vitro antibacterial activity of compounds **8**–**10** was assayed for the following eight bacterial strains: *Staphylococcus aureus* CCM 4516/08 (SA), Methicillin-resistant *Staphylococcus aureus* H 5996/08 (MRSA), *Staphylococcus epidermidis* H 6966/08 (SE), *Enterococcus* sp. J 14365/08 (ES), *Escherichia coli* CCM4517 (EC), *Klebsiella pneumoniae* D 11750/08 (KP), Extended spectrum beta lactamase-producing *Klebsiella pneumoniae* J 14368/08 (ESBL), and *Pseudomonas aeruginosa*

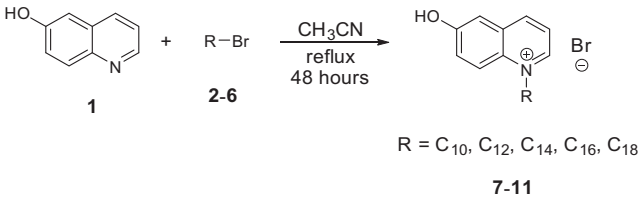

Figure 1. Preparation of 6-hydroxyquinolinium salts (**7**–**11**).

Table 1
Yields, melting points, retention times and *C*log*P* of prepared 6-hydroxyquinolinium salts (**7**–**11**)

Compd	R	Yield (%)	Mp (°C)	HPLC R_t[a] (min)	*C*log*P*[b]
7	C_{10}	69	84.0–86.0	0.65/8.64	5.71
8	C_{12}	51	120.1–122.1	0.95/9.34	6.50
9	C_{14}	41	105.2–107.2	1.65/10.05	7.29
10	C_{16}	45	130.0–132.0	3.30/10.77	8.09
11	C_{18}	32	112.0–114.0	7.06/11.47	8.88

[a] The retention times were determined by isocratic (the first value) and gradient elution (the second value).
[b] *C*log*P* was calculated in HyperChem 7.52 according to the method of Ghose, Pritchett and Crippen.

CCM 1961 (PA). Listed in Table 2, there are obtained minimum inhibitory concentrations (MICs) after 24 h and 48 h of incubation, and the minimum bactericidal concentrations (MBCs) after 48 h of incubation. For the sake of comparison, six similar surfactants containing the same length of carbon chain but different quaternary nitrogen scaffold (*N*-alkylpyridinium (P) and *N*-benzalkonium (B)) were tested for antibacterial activity on the same microbes. The set of additional surfactants involved: *N*-benzyl-*N*,*N*-dimethyl-*N*-dodecylammonium bromide (B_{12}), *N*-benzyl-*N*,*N*-dimethyl-*N*-tetradecylammonium bromide (B_{14}), *N*-benzyl-*N*,*N*-dimethyl-*N*-hexadecylammonium bromide (B_{16}), *N*-dodecylpyridinium bromide (P_{12}), *N*-tetradecylpyridinium (P_{14}) and *N*-hexadecylpyridinium bromide (P_{16}).

It was found that the Gram-positive (G+) bacteria (first four strains) are most sensitive to substances having an alkyl length of C_{12}. 6-Hydroxyquinolinium derivative (**8**) showed the exact same efficacy as its *N*-benzalkonium analogue (B_{12}) against G+ strains, whereas P_{12} exerted substantially lower efficacy except against SA and SE where the efficacy was similar to **8** and B_{12}. As regards the length of alkyl chain, no clear trend has been observed. With the exception of ES, 6-hydroxyquinolinium derivatives showed the lowest potency at C_{14} alkyl chain (**9**), whereas *N*-alkylpyridinium derivatives tended to increase the potency with the length of carbon chain especially in the case of MRSA and SE. In contrast, Gram-negative (G−) bacteria showed a substantial sensitivity decrease with the prolongation of the alkyl side chain. This trend is especially obvious in new 6-hydroxyquinolinium series, where we can assume that the substance **10** (C_{16}) has no or only a little antimicrobial activity against G− strains. In summary, the most potent and most versatile compound seems to be the B_{12} analogue effectively aiming both G+ and G− bacteria and by that confirming its commercial success. Furthermore, this compound could be supplemented or replaced by compound **8** specifically for the prevention of microbial resistance or for targeting G− bacteria, and especially for resistant strains such as MRSA.

A set of eight microorganisms (i.e., *Candida albicans* ATCC 44859 (CA), *Candida tropicalis* 156 (CT), *Candida krusei* E28 (CK), *Candida glabrata* 20/I (CG), *Trichosporon asahii* 1188 (TA), *Aspergillus fumigatus* 231 (AF), *Absidia corymbifera* 272 (AC) and *Trichophyton mentagrophytes* 445 (TM)) was used to evaluate the antifungal activity of the prepared compounds **8**–**10**. A summary of the determined antifungal MICs after 24/48 h of incubation and minimum fungicidal concentrations (MFC) is given in Table 3. To assess the antifungal activities of **8**–**10**, six analogous surfactants containing quaternary nitrogen were tested for biological activity. The result of antifungal activity for two groups—the yeasts (first four strains) and the filamentous fungi are showed in Table 3. As with the antibacterial assessment, no clear correlation between the alkyl chain length and activity against the fungal strains has been observed. However, again the highest activity was determined for the compounds with the C_{12} alkyl chain, whereas C_{14} analogues tended to possess the lowest efficacy. The yeast subgroup showed