Antibacterial Activity of Radial Compounds with Peripheral Quaternary Ammonium Units

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Quaternary ammonium functionalized radial compounds **n-C₁₀I** (*n* = 3, 4, 8, 16), **n-C₁₀Br** (*n* = 3, 4) and their reference non-radial compounds **1-C₁₀X** (*X* = Br, I) were synthesized and their antibacterial properties for gram-positive bacteria (*Methicillin-resistant Staphylococcus aureus*; *MRSA*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) were investigated. The antibacterial activity for tested bacteria depended on the number of the terminal quaternary ammonium unit, and the influence of the counter anion for antibacterial activity was not observed. The antibacterial activity of **3-C₁₀X** (*X* = I, Br) for *MRSA* was the highest in tested bacteria.

Key words: Antibacterial activity, Quaternary ammonium salts, *MRSA*

1. INTRODUCTION

The radial compounds, such as dendrimers [1], provide a very high number of functional groups in a compact space. It is reasonable to expect that these compounds will play a major role as antimicrobial agents because their performance depends on high local concentration [2, 3] as shown in Fig. 1.

Figure 1. A non-radial antimicrobial agent and a radial antimicrobial agent.

However, there are few studies about the relation between the number of the terminal unit and the antibacterial activity in the radial compounds [2, 3]. Therefore, quaternary ammonium functionalized radial compounds **n-C₁₀I** (*n* = 3, 4, 8, 16), **n-C₁₀Br** (*n* = 3, 4) and their reference compounds **1-C₁₀X** (*X* = Br, I) were synthesized and their antibacterial properties for gram-positive bacteria (*MRSA*) and gram-negative bacteria (*E. coli* and *P. aeruginosa*) were investigated. Quaternary ammonium compounds have been widely used as disinfectants. They are effective biocidal agents. The structural formulas of **n-C₁₀I** (*n* = 1, 3, 4, 8, 16) and **n-C₁₀Br** (*n* = 1, 3, 4) are shown in Fig. 2.

Figure 2. Structural formulas of **n-C₁₀I** (*n* = 1, 3, 4, 8, 16) and **n-C₁₀Br** (*n* = 1, 3, 4)

2. EXPERIMENT

2.1 Synthesis

n-C₁₀I (*n* = 1, 3, 4, 8, 16) and **n-C₁₀Br** (*n* = 1, 3, 4) were obtained by the reaction of 1-harodecane and the corresponding dimethylaminopropyl ester derivatives (Scheme 1).

Scheme 1

The preparation of **3-C₁₀I** was typical for all **n-C₁₀X** and involved the corresponding dimethylaminopropyl ester derivatives and 1-iododecane. 0.19 g (0.39 mmol) of tris(3-dimethylaminopropyl)-3,3',3''-nitritotripropanoate and 0.41 g (1.9 mmol) of 1-iododecane were added to a stirred solution of 20-ml chloroform. The stirring was maintained for 2 days under nitrogen atmosphere at 65°C. After this, the solution was concentrated. The concentrated solution was precipitated in hexane. The mixture was filtered and dried in vacuo, and the product was obtained as a yellow solid material (yield 72%). The ¹H and ¹³C NMR spectra of **n-C₁₀I** (*n* = 1, 3, 4, 8, 16) and **n-C₁₀Br** (*n* = 1, 3, 4) were appropriate. The ¹H NMR spectra of **n-C₁₀I** (*n* = 3, 4, 8, 16) in CDCl₃ are shown in Fig. 3 as typical examples. The ¹H NMR spectra of **n-C₁₀Br** (*n* = 1, 3, 4) were the same as those of **n-C₁₀I** (*n* = 1, 3, 4). The electron-spray ionization mass spectra (ESI-MS) of **1-C₁₀X** (*X* = Br, I) showed corresponding molecular ion peaks (*m/z* 228.3 ([*M* - *X* (*X* = Br, I)]⁺).

Figure 3. ¹H NMR spectra of **n-C₁₀I** (*n* = 3, 4, 8, 16) in CDCl₃

2.2 Antibacterial activity test [4, 5]

Antibacterial activity was investigated in vitro on gram-positive bacteria (*MRSA*) and gram-negative bacteria (*E. coli* and *P. aeruginosa*). Overnight bacterial culture was diluted with Mueller-Hinton broth to the density of 1.0×10^6 CFU (colony forming unit)/ml. Tested compounds (**n-C₁₀I**; *n* = 1, 3, 4, 8, 16; *X* = I or Br) were dissolved in dimethyl sulfoxide and diluted to the concentration 5-1000 µg/ml with Mueller-Hinton broth. Then, each petri dish was inoculated with the bacterial suspension and incubated at 37°C for 24 h. The lowest concentration at which there was no visible growth was taken as the minimal inhibitory concentration (MIC).

3. RESULTS AND DISCUSSION

The antibacterial activity of **n-C₁₀X** (*n* = 1, 3, 4, 8, 16; *X* = I or Br) was investigated on three different bacteria in culture. Minimal inhibitory concentration (MIC) of **n-C₁₀X** (*n* = 1, 3, 4, 8, 16; *X* = I or Br) against *MRSA*, *E. coli*, and *P. aeruginosa* were shown in Table 1.

Table 1. Antibacterial activity (MIC) of **n-C₁₀I** (*n* = 1, 3, 4, 8, 16) and **n-C₁₀Br** (*n* = 1, 3, 4) against *MRSA*, *E. coli*, and *P. aeruginosa*

	<i>MRSA</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
1-C₁₀I	60	80	a)
1-C₁₀Br	60	80	a)
3-C₁₀I	4	20	500
3-C₁₀Br	4	20	500
4-C₁₀I	30	70	a)
4-C₁₀Br	30	70	a)
8-C₁₀I	>80	>80	a)
16-C₁₀I	>80	>80	a)

MIC = Minimal inhibitory concentration in µg/ml.

a) inactive

The influence of the counter anion for antibacterial activity was not observed. The most sensitive and least sensitive of the **n-C₁₀X** (*n* = 1, 3, 4, 8, 16; *X* = I or Br) were the *MRSA* and *P. aeruginosa*, respectively. In fact, in *P. aeruginosa*, all the tested compounds except **3-C₁₀X** (*X* = I, Br) were inactive. The reason for more potent against gram-positive bacteria (*MRSA*) and less active against gram-negative bacteria (*E. coli* and *P. aeruginosa*) was structural difference between gram-positive bacteria and gram-negative bacteria. Gram-positive bacteria tend to have a loose cell wall, while gram-negative bacteria have an outer membrane structure in the cell wall forming an additional barrier for foreign molecules (Fig. 4). It is known that quaternary ammonium compounds are typically more potent against gram-positive bacteria and less active against gram-negative bacteria [3]. In comparison of between radial compounds (**n-C₁₀X**; *n* = 3, 4, 8, 16; *X*

Figure 4. Structural difference between gram-positive bacteria and gram-negative bacteria

= I or Br) and non-radial compounds (**1-C₁₀X**; X = Br, I) for antibacterial activity, the order of the MIC was **3-C₁₀X** << **4-C₁₀X** < **1-C₁₀X** < **8-C₁₀I** and **16 C₁₀I** for all tested bacteria. Antibacterial activity of the radial compounds **n-C₁₀X** (n = 3, 4; X = I, Br) was higher than that of the non-radial compound **1-C₁₀X** (X = I, Br), but antibacterial activity decreased with increasing of the terminal quaternary ammonium unit number in the radial compounds. The reason for this decreasing antibacterial activity would be cause of steric hindrance. The antibacterial activity of **3-C₁₀X** (X = I, Br) was the highest in all tested bacteria. In particular, the antibacterial activity for *MRSA* was excellent. The MIC of **3-C₁₀X** (X = I, Br) was 4 µg/ml.

4. CONCLUSIONS

Quaternary ammonium functionalized radial compounds **n-C₁₀I** (n = 3, 4, 8, 16), **n-C₁₀Br** (n = 3, 4) and their reference non-radial compounds **1-C₁₀X** (X =

Br, I) were synthesized and their antibacterial properties for gram-positive bacteria (*MRSA*) and gram-negative bacteria (*E. coli* and *P. aeruginosa*) were investigated. The influence of the counter anion for antibacterial activity was not observed. Antibacterial activity of the radial compounds **n-C₁₀X** (n = 3, 4; X = I, Br) was higher than that of the non-radial compound **1-C₁₀X** (X = I, Br), but antibacterial activity decreased with increasing of the terminal quaternary ammonium unit number in the radial compounds. The antibacterial activity of **3-C₁₀X** (X = I, Br) for *MRSA* was excellent. These compound will applied as antibacterial agent or antibacterial materials.

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