

Antimicrobial Activities of Novel Bis-Piperidinium Compounds

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Abstract

The antimicrobial activity of two new series of bis-piperidinium compounds, with alkyl chains of different length, against bacterial (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*) and fungal strains (*Aspergillus flavus*, *Aspergillus niger*, *Rhodolorula rubera*, *Lipomyces lofofera* and *Candida albicans*), are described. Antimicrobial activities of the synthesized compounds were compared to that of dodecyltrimethylammonium chloride. Bis-piperidinium salts containing 12–16 carbon side chains showed better antimicrobial properties when compared to the standard dodecyltrimethylammonium chloride.

Key words: bis-piperidinium, quaternary ammonium, piperidinium, antimicrobial activity

The quaternary nitrogen group is an essential component for many biologically active compounds synthesized so far. Quaternary ammonium compounds (QACs) play an important role in living processes such as transmethylation reaction of lipid metabolism (cholin), nerve impulse transmission (acetylcholine) or metabolism of carbohydrates (from vitamins to carboxylases) (Burger and Wolff, 1995). QACs have relatively low toxicity and broad antimicrobial range, therefore are widely used in water sanitization, textile treatment, coating and food industry.

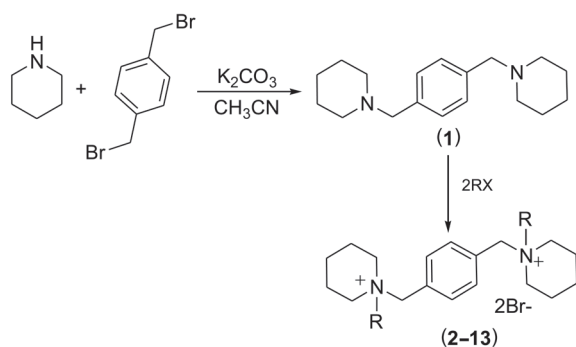
Antibacterial activity of long-chain quaternary ammonium compounds was reported for the first time by Domagk (1935). He pointed out that a large aliphatic chain attached to the quaternary nitrogen improved the antibacterial activity (Domagk, 1935). This work prompted many researchers and in relatively short time, the number of publications and patents on quaternary ammonium rapidly expanded. Bis-quaternary ammonium compounds contain two quaternary ammonium groups linked through a spacer group. Besides their phase-transfer catalytic properties, these compounds are now being studied for their biological applications (Kourai *et al.*, 2006). Some studies on antimicrobial effects of bis-quaternary ammonium compounds have been already published. Mlynarčík *et al.* studied the influence of the length of alkyl chains in the derivatives of 1,3-propanediamine and its 2-hydroxy substituted

analogue used as antibacterial agents on wool fibers (Mlynarčík *et al.*, 1979). The results show clearly the efficacy of bis-quaternary ammonium compounds to protect the wool fabrics against the microorganisms (Infante *et al.*, 1996). Similarly, another study focused on the applications of bis-quaternary ammonium compounds as wood preservatives (Zabielska-Matejuk *et al.*, 2004). Antibacterial properties of bis-quaternary ammonium compound prepared from amines and epichlorohydrin (Chlebicki *et al.*, 2005) hydroxypyridine were also reported (Kourai *et al.*, 2006). Bis-quaternary ammonium compounds have also been used as phase-transfer catalysts in organic synthesis (Saif *et al.*, 2010).

Given this very brief literature review, little data describing the antimicrobial properties of piperidinium compounds is available. To study antibacterial and antifungal properties of bis-piperidinium compounds, we synthesized two new series of compounds (2–13 and 15–26) and investigated their properties.

Twenty four bis-piperidinium compounds (2–13 and 15–26) were synthesized from readily available starting materials and tested for their antimicrobial properties. The synthesis of bis-piperidinium compounds are shown as Figures 1 and 2. 1,4-Bis(piperidin-1-ylmethyl) benzene (1) was prepared as an intermediate product by substituting the bromine atoms at 1,4-bis(bromomethyl)benzene with piperidine in the presence of a base.

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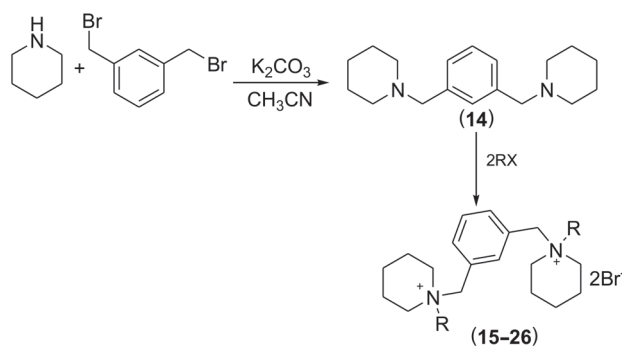
Compound	R	% Yield
2	-CH ₃	81
3	-CH ₂ CH ₃	88
4	-CH ₂ CH=CH ₂	81
5	-(n-CH ₂) ₃ CH ₃	91
6	-(n-CH ₂) ₅ CH ₃	79
7	-(n-CH ₂) ₇ CH ₃	74
8	-(n-CH ₂) ₁₁ CH ₃	78
9	-(n-CH ₂) ₁₅ CH ₃	63
10	-(n-CH ₂) ₁₇ CH ₃	59
11	-benzyl	84
12	4-bromobenzyl	80
13	3-bromobenzyl	81

Fig. 1. Preparation of bis-piperidinium compounds (2-13)

Compounds (2-13) were prepared by quaternization of 1,4-bis(piperidin-1-ylmethyl)benzene (1) by using different alkyl halides. The most common procedure used for the quaternization of tertiary amines involves the addition of alkyl halide to the solution of tertiary amine in an appropriate solvent. 1,1'-(1,4-Phenylenebis(methylene))bis(1-methylpiperidinium)diiiodide (2) was prepared by quaternization of 1,4-bis(piperidin-1-ylmethyl)benzene (1) in tetrahydrofuran (THF) with iodomethane. Rest of the compounds in the series were prepared using similar experimental protocols and suitable alkylating agents. 1,3-Substituted bis-piperidinium compounds were prepared from 1,3-bis(piperidin-1-ylmethyl)benzene (14) according to scheme presented on Fig. 2.

Bis-piperidinium compounds (2-13 and 15-26) prepared during this work were screened for the antimicrobial activity against bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*) and fungal strains (*Aspergillus flavus*, *Aspergillus niger*, *Rhodolorula rubera*, *Lipomyces lofofera* and *Candida albicans*). The results are summarized in Tables I and II.

We focused on bacterial and fungal strains that are either pathogenic or cause opportunistic infections. The microorganisms were exposed to different concentrations of the investigated compounds. The antimicrobial



Compound	R	% Yield
15	-CH ₃	87
16	-CH ₂ CH ₃	78
17	-CH ₂ CH=CH ₂	89
18	-(n-CH ₂) ₃ CH ₃	70
19	-(n-CH ₂) ₅ CH ₃	76
20	-(n-CH ₂) ₇ CH ₃	63
21	-(n-CH ₂) ₁₁ CH ₃	61
22	-(n-CH ₂) ₁₅ CH ₃	54
23	-(n-CH ₂) ₁₇ CH ₃	42
24	-benzyl	83
25	4-bromobenzyl	84
26	3-bromobenzyl	78

Fig. 2. Preparation of bis-piperidinium compounds (15-26)

activity of the tested compound was estimated as the lowest concentration that exhibits an inhibitory effect. Dodecyltrimethylammonium chloride (DTAC) was used as a reference compound. Test microbial strains exhibited the maximum sensitivity to compounds 8 and 21 that contain 12 carbons alkyl chain substitution of quaternary nitrogen. The activities of compounds 7, 9, 10, 20, 22 and 23 with shorter hydrophobic alkyl chain were slightly lower. Compounds 2-6 and 15-19 with short alkyl chain length (< 8 carbons) showed little activity. Compounds 11-13 and 24-26 with an aromatic ring also showed poor antibacterial activity. We can conclude that shortening of the side chain or replacement by aromatic ring resulted in loss of antimicrobial activity.

Previously Ahlstrom also reported that quaternary ammonium compounds with a C₁₆ lipophilic alkyl chain demonstrated higher antibacterial activity when compared to compounds containing shorter alkyl chains (Ahlstrom, 1999). This observation possibly can be attributed to long chain interacting with the fatty acid portion of lipid A and quaternary ammonium compounds are able to cause cell leakage and membrane damage upon their adsorption to the bacterial membrane (Hamilton, 1968). In our work, we observed variable activity of piperidinium compounds against different microorganisms. The differences in cell wall composition of Gram-negative (*P. aeruginosa*) and

Table I
Antibacterial activity of bis-piperidinium compounds
(2–13 and 15–26)

Compound No.	Minimum lethal concentration of the compound [$\mu\text{g/ml}$]			
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. niger</i>	<i>B. Subtilis</i>
2	5000	> 5000	5000	> 5000
3	5000	> 5000	5000	> 5000
4	5000	> 5000	5000	> 5000
5	2500	5000	2500	> 5000
6	1250	5000	5000	2500
7	625	625	1250	1250
8	150	150	300	150
9	150	300	625	300
10	625	625	1250	625
11	5000	5000	5000	5000
12	5000	5000	5000	5000
13	5000	5000	5000	5000
15	5000	> 5000	> 5000	> 5000
16	5000	> 5000	> 5000	> 5000
17	5000	5000	> 5000	> 5000
18	2500	5000	> 5000	5000
19	1250	5000	5000	2500
20	625	625	1250	1250
21	150	150	300	150
22	150	300	625	300
23	625	625	1250	625
24	5000	5000	5000	> 5000
25	5000	5000	5000	5000
26	2500	5000	2500	5000
DTAC*	300	2500	1250	1250

* DTAC = Dodecyltrimethylammonium chloride

Table II
Antifungal activity of bis-piperidinium compounds
(2–13 and 15–26)

Compound No.	Minimum lethal concentration of the compound [$\mu\text{g/ml}$]				
	<i>A. flavu</i>	<i>R. rubera</i>	<i>S. aureus</i>	<i>L. lofofera</i>	<i>C. albicans</i>
2	> 5000	> 5000	5000	> 5000	> 5000
3	> 5000	> 5000	5000	> 5000	> 5000
4	5000	> 5000	5000	> 5000	> 5000
5	> 5000	5000	2500	> 5000	> 5000
6	5000	2500	1250	2500	5000
7	1250	1250	625	1250	1250
8	300	150	150	150	300
9	300	300	150	300	625
10	625	625	625	625	625
11	5000	5000	2500	5000	5000
12	5000	5000	2500	5000	5000
13	5000	5000	2500	5000	5000
15	> 5000	> 5000	5000	> 5000	> 5000
16	> 5000	> 5000	5000	> 5000	> 5000
17	5000	5000	5000	5000	5000
18	5000	5000	2500	5000	5000
19	2500	2500	1250	2500	2500
20	1250	1250	625	1250	1250
21	300	150	150	300	300
22	300	300	150	300	300
23	625	625	300	625	625
24	5000	5000	2500	5000	5000
25	> 5000	5000	5000	5000	> 5000
26	5000	5000	2500	5000	5000
DTAC*	625	300	300	625	625

* DTAC = Dodecyltrimethylammonium chloride

Gram-positive bacteria (*S. aureus*) may be a cause of different resistance levels to the tested compounds. For example, the relatively low permeability of the outer membrane of Gram-negative bacteria may play a key role in the defense of bacteria as the penetration of antibacterial substances is slowed down.

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