Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry
ELSEVIER
Bioorganic & Medicinal Chemistry 12 (2004) 853–857

Synthesis and antimicrobial properties of imidazolium and pyrrolidinonium salts

D. Demberelnyamba,[a] Ki-Sub Kim,[a] Sukjeong Choi,[a] Seung-Yeob Park,[a] Huen Lee,[a,*] Chang-Jin Kim[b] and Ick-Dong Yoo[b]

[a]*Department of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology, 373-1 Guseong-dong, Yuseong-gu, Daejeon 305–701, South Korea*
[b]*Antioxidant Research Lab., Korea Research Institute of Bioscience and Biotechnology, 52 Oeun-dong, Yuseong-gu, Daejeon 305–333, South Korea*

Received 22 October 2003; accepted 6 January 2004

Abstract—For the purpose of developing new disinfectants and antiseptics, we searched for compounds having high bactericidal activity against gram-positive bacteria, gram-negative bacteria, and fungi. Three different series of quaternary imidazolium and pyrrolidinonium salts were synthesized: series A (1-alkyl-3-methylimidazolium chlorides and bromides); series B (1-alkyl-2-methyl-3-hydroxyethylimidazolium chlorides); and series C (*N*-alkyl-*N*-hydroxyethylpyrrolidinonium). Series B and C were newly designed. These three series were tested to evaluate their antibacterial and antifungal properties for the first time. Seven microbial strains were used in the study: *Escherichia coli* KCTC1924, *Salmonella typhimurium* KCTC1926, *Staphylococcus aureus* 209 KCTC1916, *Staphylococcus aureus* R209 KCTC1928, *Bacillus subtilis* KCTC1914, *Candida albicans* KCTC1940, and *Chlorella regularis*. The antimicrobial efficiency was measured by bacterial and fungal growth inhibition expressed as minimal inhibitory concentration (MIC) values. Series A and B imidazolium salts had very good antimicrobial activity against the examined Gram-negative bacteria, Gram-positive bacteria, and fungi. Also the pyrrolidinonium salt was found to have low MIC for some of tested microorganisms. The antibacterial and antifungal active properties of the salts depend upon the structure of functional groups and the alkyl chain length in the imidazolium and pyrrolidinonium ring. Among the synthesized quaternary imidazolium and pyrrolidinonium salts, the imidazolium salts containing a long alkyl chain and the introduction of a hydroxyethyl chain and methyl group into the imidazolium ring structure leads to broad spectrum active antimicrobial agents which not only have bacteriostatic properties but could be powerful bactericides.

1. Introduction

The history of modern antiseptics and disinfectants dates back several decades. The chronological list includes benzalkonium chloride (BAC) and cetylpyridinium chloride (CPC), both of which are quaternary ammonium compounds and are still now broadly used. Quaternary ammonium compounds (QAC) are generally known to be bioactive substances and are used mainly for environmental disinfection, disinfection of medical equipment, and disinfection in hospitals. In 1938, Domagk[1] introduced the first QACs based on the disinfectant known as Zephird. In 1926, Browning et al[2] described the antibacterial and antifungal activity of heterocyclic QACs derivatives. In 1983, Preston[3] detailed the effect of a number of structural features on the efficiency of dialkyl QACs; total carbon atom contents of 22–24 were expected to be the most effective. In 1985, Kourai et al.[4] investigated the heterocyclic QACs and reported that for bacteria there was a quantitative relation between MIC (minimum inhibitory concentration) and water–octanol partition coefficient P, on the basis of *N*-alkylpyridinium iodides. In 1995 and 1996, Pernak[5,6] reported, for quaternary alkylthiomethylimidazolium chlorides, the existence of a quantitative relation between CMC (critical micellar concentration) and MIC against bacteria and the hydrophobicity index (HI).

Keywords: Antimicrobial agents; Antimicrobial activity; Quaternary *N*-alkyl-*N*-methylimidazolium halides; Quaternary *N*-alkyl-*N*-hydroxyethylimidazolium halides; Quaternary *N*,*N*-alkylhydroxyethylpyrrolidinonium halides.
* Corresponding author. Tel.: +82-4286-93917; fax: +82-4286-93910; e-mail: h_lee@kaist.ac.kr

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.01.003

The mode of antimicrobial action of QACs[7–9] consists of their interaction with cytoplasmic membrane of bacteria and the subsequent loss of permeability properties of the membrane. QACs interfere with respiration and ATP synthesis and, in sufficient concentrations, can cause membrane leakage, release of the cellular constituents, and even cell death. Antimicrobial activity of QACs is closely related to surfactant properties.[8] In particular, QACs with alkyl chains below a certain length, and thus with weak surfactant properties, might appear to be ineffective as antimicrobial agents. Moreover, it is noteworthy that the aggregation properties like micellation and other association patterns of amphiphilic QACs may greatly influence the antimicrobial efficiency of these compounds.[9] Even though QACs are widely used because of their relative safety and broad spectrum of biocide activity, their direct applications to some highly demanding fields has been limited by the discovery of microbial resistance phenomena against all types of biocides.[10]

Commercial antiseptics and disinfectants are not sufficiently active at their usual concentrations against causative organisms of nosocomial infections, nor are they active enough against pseudomonas strains that cause opportunistic infections. In order to overcome this increasing resistance phenomenon, it might be urgently needed to develop a significant number of new compounds with original chemical structures.[7] A number of research programs have been aimed at increasing the antimicrobial performances of molecules by introducing new molecular parameters such as heteroatoms,[11] chemical functions,[12,13] and aromatics.[14] The formulation studies of new antibacterial and antifungal agents have been an active research field. In the present research, we propose new and potential agents that have long alkyl chains, methyl, and hydroxyethyl groups in imidazolium and pyrrolidinonium structure which will enhance the impact of microbiological activity. First of all, as initial attempts, three different series of quaternary imidazolium and pyrrolidinonium salts were synthesized: series A (1-alkyl-3-methylimidazolium chlorides and bromides); series B (1-alkyl-2-methyl-3-hydroxyethylimidazolium chlorides); and series C (*N*-alkyl-*N*-hydroxyethylpyrrolidinonium). Their structures are shown in Figures 1–3. They were tested to evaluate their antibacterial and antifungal properties.

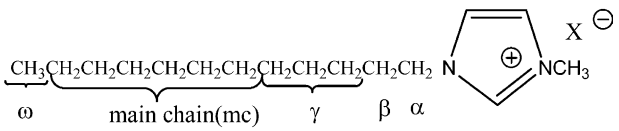


Figure 1. The structure of series A:1-alkyl-3-methylimidazolium halides.

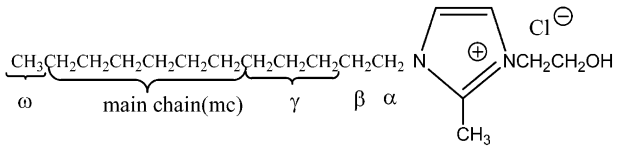


Figure 2. The structure of series B: 1-alkyl-3-hydroxyethyl-2-methylimidazolium chlorides.

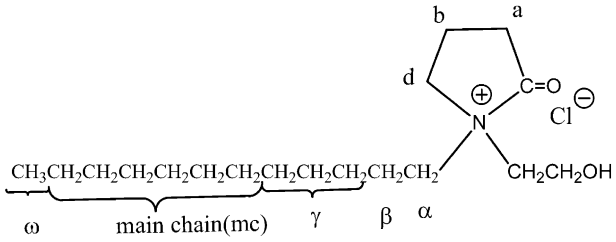


Figure 3. The structure of series C: *N*-dodecyl-*N*-hydroxyethylpyrrolidinonium bromide.

2. Results and discussion

2.1. Synthesis of quaternary imidazolium and pyrrolidinonium salts

We synthesized 9 salts based on various types of cations made by changing the carbon number of alkyl substituents in order to establish the influence of chemical structure on antibacterial and antifungal activity. The 2-methyl substituted imidazolium and *N*-alkyl-*N*-hydroxyethylpyrrolidinonium salts of 1-alkyl-3-methylimidazolium, 1-alkyl-3-hydroxyethyl-2-methylimidazolium halides, and *N*-alkyl-*N*-hydroxyethylpyrrolidinonium chlorides were prepared and examined to test antibacterial and antifungal activity. Alkylimidazole and alkylpyrrolidinone were obtained by deprotonation of imidazole by sodium or sodium ethanoate followed by alkylation in ethanol or acetonitrile.[15] Alkylation of the 1-alkylimidazole and *N*-alkylpyrrolidinone derivatives were conducted by refluxing in methanol or acetonitrile—a solvent chosen for its stability toward strongly alkylating agents, its moderately high boiling point, and the insolubility of the imidazolium and pyrrolidinonium salts in this medium. The longer chain salts are low melting crystalline solids.

The present research results confirm the existence of close interactions among structure, surface active properties, and biological activity against bacteria and fungi for 3 different series (A, B, and C) of quaternary imidazolium compounds: A: 1-alkyl-3-methylimidazolium; B: 1-alkyl-3-hydroxyethyl-2-methylimidazolium chlorides and bromides; and C: *N*-alkyl-*N*-hydroxyethylpyrrolidinonium chlorides. We tested the following Quaternary 1-alkyl-3-methylimidazolium compounds: (A), 1-methyl-3-octylimidazolium bromide (C_8MeImBr, **1a**), 1-methyl-3-decylimidazolium chloride (C_{10}MeImCl, **1b**), 1-methyl-3-dodecylimidazolium bromide (C_{12}MeImBr, **1c**), 1-methyl-3-tetradecylimidazolium chlorides (C_{14}MeImCl, **1d**), 1-methyl-3-tetradecylimidazolium bromide (C_{14}MeImBr, **1e**), 1-methyl-3-hexadecylimidazolium bromide (C_{16}MeImBr, **1f**), and 1-alkyl-2-methyl-3-hydroxyethylimidazolium salts: (B), 1-hydroxyethyl-2-methyl-3-tetradecylimidazolium chloride (C_{14}HEtMeImCl, **2a**), and 1-hexadecyl-3-hydroxyethyl-2-methylimidazolium chloride (C_{16}HEtMeImCl, **2b**); and (C): *N*-dodecyl-*N*-hydroxyethylpyrrolidinonium chloride (C_{12}HEtPyrrBr, **3a**).

All the tested imidazolium chlorides, bromides, and pyrrolidinonium bromides are highly surface active agents

that have two hydrophobic alkyl substituents in the 1 and 3 positions or 1 and 2 positions in the imidazolium ring and one hydrophobic alkyl and one hydrophilic hydroxyethyl substituents in pyrrolidinonium ring.

3. Antimicrobial activity

3.1. MIC results

The efficiencies of these imidazolium and pyrrolidinonium salts series A, B, and C were evaluated against bacterial and fungal strains. The MIC values (μg/mL) of each compound after 1 day of exposure are shown in Table 5. As test strains, *B. subtilis* KCTC1914, *S. aureus* 209 KCTC1916, and a few antibiotic resistant *S. aureus* R209 KCTC1928 were chosen from among gram-positive bacteria. *E. coli* KCTC1924, *S. typhimurium* KCTC1926 was chosen from among gram-negative bacteria. *C. albicans* KCTC1940 was chosen as a representative fungus. *Chllolella regularis* was chosen as a representative algal bacterium.

N-dodecyl-*N*-hydroxyethylpyrrolidinonium chloride (Series C) was found to have low MIC for some of tested bacteria, fungi and algal bacterium.

The 2 series of 1-alkyl-3-methylimidazolium (A) and 1-alkyl-3-hydroxyethyl-2-methylimidazolium (B) quaternary salts were the most efficient salts among these tested. Three series of quaternary salts showed the broadest bactericidial and fungicidal activity. The more important series between the two series was 1-alkyl-3-methylimidazolium salts. Their antibacterial and antifungal activity was greatly affected by their chain length, the type of substituted functional groups, and their position in the imidazolium ring. The variation of these factors can lead to low activity structures (Series C) for some tested strains or high activity compounds (Series A and B). For the tested series A quaternary imidazolium compounds, we found the preferred 1-alkyl group's chain length for the controlling test organisms to be in the C_{12}–C_{16} range. The results show all of the tested imidazolium halides that have two hydrophobic groups in the 1 and 3 positions in the imidazolium ring were the most suitable for antimicrobial activity. Also, the long alkyl chain imidazolium salts with methyl and hydroxyethyl substitution in the 2 and 3 positions of the imidazolium ring have an efficient antimicrobial activity.

In view of the results, it appeared that the quaternary imidazolium salts from series A and B are the most effective products against the tested bacterial and fungal strains. We evaluated the antimicrobial activities of series A and B and compared them with commercially produced and widely used quaternary ammonium substances (benzalkonium chloride and cetylpyridinium chloride) and antibiotics (Gentamycin and Kanamycin).

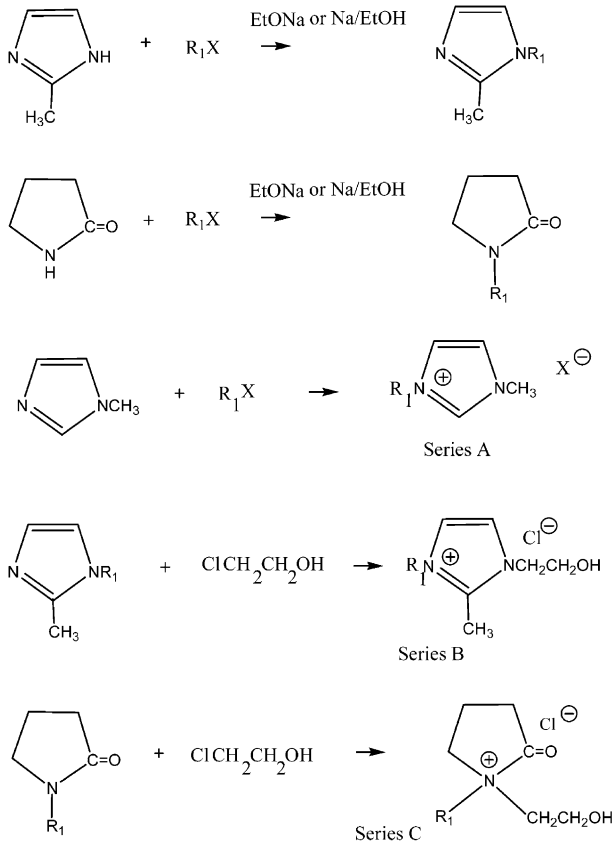
4. Conclusion

The evaluation of antibacterial and antifungal surfactant structures leads us to consider several molecular parameters. The most important parameters were the introduction of a long alkyl chain, and the hydroxyethyl and methyl groups in different positions within the imidazolium and pyrrolidinonium structure. The most efficient structures were 1-alkyl-3-methylimidazolium chlorides and bromides, and 1-alkyl-3-hydroxyethyl-2-methylimidazolium chlorides with a long alkyl chain. Some of these compounds give results globally superior to the commercially available products benzalkonium chloride (BAC) and cetylpyridinium chloride (CPC) and used as references. Using the structure and activity correlation that we have established as a starting point, we are now able to synthesize variations of well defined structure parameters in order to optimize quaternary onium compounds with different heterocyclic ring structures for future development of new antiseptics and disinfectants.

5. Experimental

5.1. MIC measurements

Antibacterial and antifungal activities of imidazolium and pyrrolidinonium salts were evaluated through measurements of minimal inhibitory concentrations (MIC) expressed in μg/mL. Seven microorganisms were used: *E. coli* KCTC1924, *S. typhimurium* KCTC1926, *S. aureus* 209 KCTC1916, *S. aureus* R209 KCTC1928, *B. subtilis* KCTC1914, *C. albicans* KCTC1940, and *C. regularis*.



Scheme 1. Synthesis of quaternary imidazolium and pyrrolidinonium salts.

The MIC determination tests were carried out by the automatic serial dilution method in LB broth (microtitre). Bacterial and fungal inocula were prepared by dilution of an overnight broth culture to give the equivalent of approximately 10^6 cell/mL.[16]

5.2. Synthesis of quaternary imidazolium and pyrrolidinonium salts

The syntheses of the long chain 1-alkyl-3-methylimidazolium, 1-alkyl-2-methyl-3-hydroxyethylimidazolium, and *N*-alkyl-*N*-hydroxyethylpyrrolidinonium salts were carried out more straightforwardly as shown in Scheme 1.

5.3. Preparation of 1-alkyl-2-methylimidazoles and *N*-alkylpyrrolidinones

Sodium (2.3 g/0.1 mol) was dissolved to 200 mL of anhydrous ethanol and mixed with 2-methylimidazole (0.1 mol) or pyrrolidinone (0.1 mol). Then, the appropriate amount of alkyl chloride or alkyl bromide (0.12

Table 1. ^{1}H NMR chemical shift data for long chain quaternary 1-alkyl-3-methyl imidazolium salts

No	Im:H^2	Im:H^5	Im:H^4	N-CH_2	N-CH_3	β	γ	mc	ω
1d	9.06	7.58	7.44	4.29	3.93	1.90	1.35	1.26	0.90
1e		7.55	7.53	4.25	3.93	1.89	1.33	1.26	0.85
1f		7.61	7.60	4.30	3.95	1.91	1.37	1.26	0.85

Table 2. ^{1}H NMR chemical shift data for long chain quaternary 1-alkyl-3-hydroxyethyl-2-methylimidazolium chlorides

No	Im:H^5	Im:H^4	CH_2O	OH	NCH_2	αNCH_2	C^2CH_3	βCH_2	γCH_2	mc	ω
2a	7.42	7.19	4.28	4.16	3.92	3.79	2.78	2.53	1.32	1.26	0.85
2b	7.45	7.21	4.31	4.19	3.93	3.68	2.81	2.56	1.35	1.28	0.87

Table 3. ^{1}H NMR chemical shift data for long chain quaternary *N*-dodecyl-*N*-hydroxyethylpyrrolidinonium bromides

No	CH_2O	NCH_2α	NCH_2	β	d	b	a	γ	mc	ω
3a	4.07–4.05, m	4.07–4.05, m	3.69–3.67, t	3.65–3.6, m	3.61-03.57, t	2.23, m	1.76, t	1.38, m	1.29, br.s	0.89–0.86

Table 4. FAB Mass spectra data

Compd	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**2a**	**2b**	**3a**
m/*z* (cation)	195.0	223.1	251.45	279.1	279.1	307.1	323.2	351.2	300.06

Table 5. The MIC of quaternary imidazolium and pyrrolidinonium salts

Compd no	Substituents			Anion X	MIC (μg/mL)						
	R1	R2	R3		Tested organisms (bacteria and fungi)						
					EC	ST	SA	SAR	BS	CA	CR
1a	C_8H_{17}	H	Me	Br	64	500	64	250	500	250	500
1b	$C_{10}H_{21}$	H	Me	Cl	8	125	16	32	125	250	250
1c	$C_{12}H_{25}$	H	Me	Br	8	32	4	8	8	32	16
1d	$C_{14}H_{29}$	H	Me	Cl	4	8	4	4	4	8	8
1e	$C_{14}H_{29}$	H	Me	Br	4	8	4	4	4	8	16
1f	$C_{16}H_{33}$	H	Me	Br	8	4	8	4	4	8	8
2a	$C_{14}H_{29}$	Me	HEt	Cl	16	32	16	32	16	64	125
2b	$C_{16}H_{33}$	Me	HEt	Cl	16	8	8	8	8	8	32
3a	$C_{12}H_{25}$	HEt		Cl	8	16			4		8
BAC							8	8	8		
CPC							8	8	8		
Genta-mycin					1	0.5	0.25	0.25	1		
Kana-mycin					16	1	2	1	2		

BAC: benzalkonium chloride, CPC:cetylpyridinium chloride. EC: *E. coli* KCTC1924; ST: *S. typhimurium* KCTC1926; SA: *S. aureus* 209 KCTC1916; SAR: *S. aureus* R209 KCTC1928; BS: *B. subtilis* KCTC1914; CA: *C. albicans* KCTC1940 and CR: *C. regularis*.

mol) was added and the mixture was refluxed for 6 h. The precipitated sodium chlorides or bromides were filtered and further ethanol was removed by rotary distillation. The resulting yields were in the range of 70–80%.

5.4. Preparation of quaternary imidazolium salts

Methylimidazole (0.1 mol) or 1-alkyl-2-methylimidazole (0.1 mol) were dissolved in anhydrous acetonitrile and, then, alkyl halide was added (0.12 mol). The reaction was carried out for 6 h under refluxing and the product purified by extraction with hexane and recrystallized from acetonitrile.

5.5. Preparation of quaternary pyrrolidinonium salts

A mixture of freshly synthesized *N*-alkylpyrrolidinones or *N*-hydroxyethylpyrrolidinone (0.1 mol) and 2-chloroethanol or alkyl halide (0.1 mol) in anhydrous acetonitrile was refluxed for 24 h and the mixture was allowed to cool. However, in the case of high homologues of quaternary pyrrolidinonium salts, a white solid was formed. This crude solid was filtered and washed three times by hexane and recrystallized from acetonitrile. In case of low homologues of quaternary pyrrolidinonium salts, the liquid was formed, washed by hexane, and finally dried. The yields were 83–88%. The purities of all synthesized quaternary imidazolium and pyrrolidinonium salts were checked by ^{1}H NMR and High Resolution Mass Spectrometer. The NMR and FAB Mass spectra were recorded on a Bruker AMX FT 500 MHz NMR spectrometer and FAB Mass JMS-HX110A, and their chemical shifts and ion mass (m/z) were summarized in Tables 1–4.

Acknowledgements

This work was performed for the Greenhouse Gas Research Center, one of the Critical Technology-21 Programs, founded by the Ministry of Science and Technology. It also partially supported by both KISTEP and the Brain Korea 21 Project.

References and notes

1. Domagk, G., U.S. Patent, **1938**, *2*, 108 765.
2. Browning, C.H.; Cohen, J.B.; Ellingsworth, S.; Gulbrebsen, R. *Proc. R.. Soc. Med.* **1926**, *100*, 293.
3. Preston, A. F. J. *Am. Oil. Chem. Soc.* **1983**, *60*, 567.
4. Kourai, H. F.; Michikowa, H.; Takeshi, T.; Horie, K.; Takeshi, K.; Shibasaki, J. *J. Antibact. Antifung. Agent* **1985**, *11*, 553.
5. Pernak, J.; Skrzypczak, A. *Eur. J. Med. Chem.* **1996**, *31*, 901.
6. Pernak, J.; Skrzypczak, A.; Bogaski, M. B. *Chem. Pharm. Bull.* **1995**, *43*, 2019.
7. Merianos, J. J. Disinfection, Sterilization, and Preservation; 4th Ed.; Lea & Febiger: Philadelphia, **1991**, p 225
8. Kopecky, F. *Pharmazie* **1996**, *51*, 135.
9. Denyer, S. P.; Stewart, G. S. *Int. Biodet. Biodegrad.* **1998**, *41*, 261.
10. Davies, J. *Nature* **1996**, *383*, 219.
11. Skrzypczak, A.; Brycki, B.; Mirska, I.; Pernak, J. *Eur. J. Med. Chem.* **1997**, *32*, 661.
12. Diz, M.; Manresa, A.; Pinazo, A.; Erra, P.; Infante, M. R. *J. Chem. Soc., Perkin Trans. 2* **1994**, 1871.
13. Pavlikova, M.; Lacko, Y.; Devinsky, F.; Mlynarcik, D. *Coll Czech Chem Comm.* **1995**, *60*, 1213.
14. Pernak, J.; Mirska, I.; Kmiecik, R. *Eur. J. Med. Chem.* **1999**, *43*, 765.
15. Begtrup, M.; Larsen, P. *Acta Chemica Scandinavica* **1990**, *44*, 1050.
16. Lancini, G. C.; Parenti, F.; Gallo, G. G. Antibiotics: A Multidisciplinary Approach; 2nd Ed.; Plenum press, New York and London, **1995**, p 12.