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Novel naphthalene-based bis-pyridinium compounds with pronounced antibacterial activity

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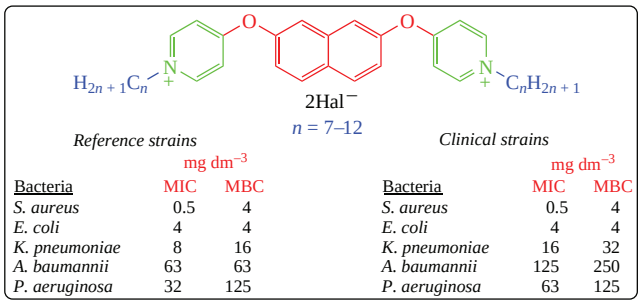
Novel *para*-substituted bis-pyridinium compounds containing 2,7-dioxynaphthalene spacer were synthesized in two simple steps from the corresponding dihydroxynaphthalene. The microbiological study on five reference (*E. coli* ATCC 25922, *K. pneumoniae* ATCC 70060, *S. aureus* ATCC 43300, *P. aeruginosa* ATCC27853, and *A. baumannii* ATCC 15308) and five clinical (*E. coli* B-3421/19, *K. pneumoniae* B-2523/18, *S. aureus* B-8648, *P. aeruginosa* B-2099/18 and *A. baumannii* B-2926/18) bacterial strains showed promising range of antibacterial properties for these biocides, compared to modern sanitizers.



Reference strains

mg dm^{-3}

Bacteria	MIC	MBC
S. aureus	0.5	4
E. coli	4	4
K. pneumoniae	8	16
A. baumannii	63	63
P. aeruginosa	32	125

Clinical strains

mg dm^{-3}

Bacteria	MIC	MBC
S. aureus	0.5	4
E. coli	4	4
K. pneumoniae	16	32
A. baumannii	125	250
P. aeruginosa	63	125

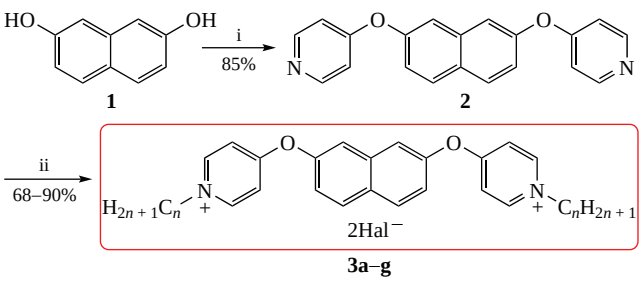
Keywords: quaternary bis-ammonium compounds, pyridinium compounds, alkylation, biological activity, antibacterial agents, biocides.

Although bis-pyridinium compounds (BPCs) as broad-spectrum biocides are known since 1980,[1] interest to this subgroup of quaternary ammonium compounds (QACs) in recent years became actual.[2–6] However, at the moment only one representative of BPCs, namely, octenidine dihydrochloride (OCT), is manufactured in industry.[7] This compound is active against Gram+ and Gram– bacteria, biofilms,[8–10] and mainly applicable as antiseptic. However, over the past ten years, the issue of emerging bacterial resistance to biologically active compounds has become severe, and OCT has not been an exception.[11] Moreover, in light of the still ongoing pandemic, this problem has become more relevant than ever due to the increased demand for antiseptics and disinfectants. For the above reasons, expanding the QAC class as broad-spectrum cationic biocides is an important challenge for modern chemistry. More information about bis-pyridinium compounds and other QACs can be found elsewhere.[12–16]

The presented study is a continuation of our work on the biologically active BPCs.[17–23] Recently, we conducted a microbiological evaluation of new *ortho*-substituted BPCs having 2,7-dioxynaphthalene spacer.[20] These salts possessed good antibacterial properties, however, they were difficult to synthesize due to problems in alkylation process. Herein, we have broadened the range of naphthalene-based BPCs with *para*-substituted salts.

The synthesis route consists of two simple stages. First, 2,7-dihydroxynaphthalene **1** was converted into *para*-bipyridine platform **2** by the Ulman-type condensation (Scheme 1, step i). The reaction was carried out under argon atmosphere at 140 °C for 72 h. This method was used in our previous works and proved to be reliable for different scaffolds. Second, the resulting platform **2** was quaternized with alkyl halides in acetonitrile (step ii). Unlike *ortho*-BPCs, full conversion was observed in 24 h (4 times faster) with slightly better yields. *n*-Alkyl bromides C_7–C_{12} and *n*-alkyl iodide C_8 were used to obtain the desired products **3a**–**g** (for details, see Online Supplementary Materials).

The obtained compounds **3a**–**g** were tested for microbiological activity against ten bacterial strains (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Staphylococcus aureus* ATCC 43300, *Acinobacter Baumannii* ATCC 15308, *Pseudomonas aeruginosa* ATCC 27853, *E. coli* B-3421/19, *K. pneumoniae* B-2523/18, *S. aureus* B-8648, *P. aeruginosa* B-2099/18 and *A. baumannii* B-2926/18). Clinical strains used in the study cause healthcare-associated infections (HAIS). More than 1.4 million people suffer from HAIS all over the world at any given moment, and 5–10% from several infections at the same time.[24] Table 1 shows the values of the minimum



a *n* = 7, Hal = Br, 91%
b *n* = 8, Hal = Br, 77%
c *n* = 8, Hal = I, 74%
d *n* = 9, Hal = Br, 90%
e *n* = 10, Hal = Br, 83%
f *n* = 11, Hal = Br, 85%
g *n* = 12, Hal = Br, 68%

Scheme 1 *Reagents and conditions*: i, 4-chloropyridine hydrochloride (2 equiv.), CuI (1 equiv.), K_3PO_4 (8 equiv.), picolinic acid (0.2 equiv.), DMSO, inert atmosphere, 140 °C, 72 h; ii, alkyl halides (4 equiv.), MeCN, 81 °C, 24 h.

Table 1 MIC/MBC values (mg dm^{-3}) for prepared BPCs.[a]

Compound	MIC/MBC (mg dm^{-3})									
	Reference strains					Clinical strains				
	Sa	*Ec*	*Kp*	*Ab*	*Pa*	*Sa*	*Ec*	*Kp*	*Ab*	*Pa*
3a: *n* = 7, Br	1	8	250	>500	250	2	32	63	250	16
	8	32	500	>500	500	4	63	500	250	16
3b: *n* = 8, Br	0.5	4	8	63	32	0.5	4	16	125	63
	4	4	16	63	125	4	4	32	250	125
3c: *n* = 8, I	0.5	4	16	125	63	0.5	4	16	32	63
	4	4	16	125	250	4	4	32	63	63
3d: *n* = 9, Br	0.5	2	16	32	32	0.5	8	8	32	63
	4	2	32	63	63	4	8	16	63	63
3e: *n* = 10, Br	4	8	32	16	63	4	32	32	32	63
	16	8	63	32	250	16	125	63	32	125
3f: *n* = 11, Br	16	32	63	16	250	16	53	63	32	125
	32	32	250	16	500	63	>500	125	32	250
3g: *n* = 12, Br	63	32	250	16	32	32	125	500	125	>500
	125	63	500	32	500	500	>500	500	>500	>500
BAC	125	4	500	>500	>500	125	500	500	>500	>500
	250	8	>500	>500	>500	500	>500	500	>500	>500
CPC	4	8	63	16	500	2	8	8	32	250
	16	8	250	32	>500	16	63	16	125	500
OCT	0.5	0.5	4	32	8	0.5	2	2	32	32
	2	0.5	8	125	16	2	4	4	125	63

[a] Reference strains: *Escherichia coli* ATCC 25922 (*Ec*), *Klebsiella pneumoniae* ATCC 700603 (*Kp*), *Staphylococcus aureus* ATCC 43300 (*Sa*), *Acinobacter baumannii* ATCC 15308 (*Ab*), *Pseudomonas aeruginosa* ATCC 27853 (*Pa*); Clinical strains – *E. coli* B-3421/19, *K. pneumoniae* B-2523/18, *S. aureus* B-8648, *P. aeruginosa* B-2099/18 and *A. baumannii* B-2926/18. Reference QACs: cetylpyridinium chloride (CPC), benzalkonium chloride (BAC) and octenidine dihydrochloride (OCT).

inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of compounds **3a**–**g** and the references such as cetylpyridinium chloride (CPC), benzalkonium chloride (BAC) and octenidine dihydrochloride (OCT). Detailed biological assay is represented in Online Supplementary Materials. Compound CPC was synthesized by simple alkylation of pyridine with cetyl chloride while BAC was from Acros Organics and OCT was synthesized in four stages by known method.[1]

Based on microbiological study we can highlight a couple of trends and dependencies. Compounds **3a**–**f** possessed activity against both reference and clinical Gram+ *S. aureus*. Activity remains approximately the same in C_7–C_9 range, and then decreases sharply with alkyl lengthening. For Gram– *E. coli* and *K. pneumoniae*, the scope of active compounds was less, namely, for salts **3b**–**e**, with C_8–C_9 alkyl length optimum. Surprisingly uncommon picture was noticed for *A. baumannii* strain. Biocidal properties were better for more long-tailed BPCs **3e**–**g**. Unfortunately, all synthesized compounds showed poor activity against reference *P. aeruginosa*, and failed to compete with OCT. However, compound **3a** had the lowest MIC and MBC on clinical *P. aeruginosa*. Nevertheless, no clear correlation was observed between biological effect on reference and clinical bacterial strains. Overall, compounds **3b**–**d** possessed relatively the same activity, and were better than the others in this series. Concerning reference compounds, synthesized BPCs were more active than mono-QACs BAC and CPC, and comparable to OCT on most strains.

In conclusion, seven new naphthalene-based bis-pyridinium compounds with 2,7-dioxynaphthalene spacer have been synthesized. Their MICs and MBCs were measured on both reference and clinical bacterial strains pathogenic for humans. Hit-compounds **3b**–**d** with C_8-C_9 alkyl chain showed overall best antibacterial activity among all synthesized BPCs and reference mono-QACs (BAC and CPC), and comparable activity to OCT. We will continue further study and development of this class of cationic biocides, adapting the constructed methodology to new scaffolds.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2022.09.012.

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