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Synthesis and biological evaluation of novel bispyridinium salts containing naphthalene-2,7-diylbis(oxy) spacer

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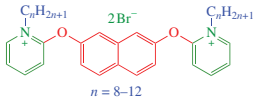
Novel five bispyridinium salts based on a naphthalene-2,7-diylbis(oxy) spacer were synthesized. The obtained compounds showed stronger biological activity against five strains of bacteria and two fungi as compared to modern biocides used in the pharmaceutical market.



Bacteria	MIC/μg ml^{-1}	Fungi	MIC/μg ml^{-1}
MRSA	⩽0.25	*C. albicans*	⩽0.25
E. coli	⩽0.25	*C. neoformans*	⩽0.25
K. pneumoniae	⩽0.25		
A. baumannii	8		
P. aeruginosa	⩽0.25		

n = 10

Keywords: bis-quaternary ammonium compounds, pyridinium salts, biological activity, antibacterial agents, antifungal activity.

From time immemorial bacteria have been one of the main threats to humanity. Due to the lack of measures taken and the thoughtless use of almost the only 'weapon' capable of withstanding this adversity, biocides, by the masses of people the problem has now taken on critical proportions. According to a 2016 report by British scientists, 700 000 people worldwide die from infections caused by resistant bacteria every year, and by 2050 this number can reach 10 million. In other words, thirty years from now, if we do not act, one person will die every three seconds.[1]

Recent studies have shown that some bacterial strains have developed resistance to octenidine and other quaternary ammonium compounds (QACs).[2,3] Due to the abovementioned reasons, the development of new compounds to overcome resistance is one of the main problems in this area. The study of structure–activity relationship is one of the modern interdisciplinary approaches of organic chemistry.[4,5]

The aim of this work was the synthesis and biological study of novel bispyridinium salts (BPSs – subgroup of QACs), containing naphthalene-2,7-diylbis(oxy) spacer. It is known that the spacer nature in BPSs has a significant impact on bis-QACs' manifestation of sertain effects including biocidal effects.[6] The first and main representative of this group of biocides is octenidine dihydrochloride.[7] Over the past decades plenty of spacer variations in bispyridinium salts have been designed, and these derivatives showed antibacterial, antifungal and antimalarial activity.[8–10] Amongst others, BPSs containing benzene ring as a spacer were synthesized (Figure 1).[11–15] The studies on structure/antibacterial activity relationship showed that BPSs containing an aromatic moiety in the linker had high antibacterial and antifungal activity. These compounds have the same activity as octenidine (MIC and MBC), but are better in terms of cytotoxicity (normal human epidermal keratinocytes).[16] In this work, we have synthesized new BPSs containing rigid aromatic naphthalene-2,7-diylbis(oxy) linker.

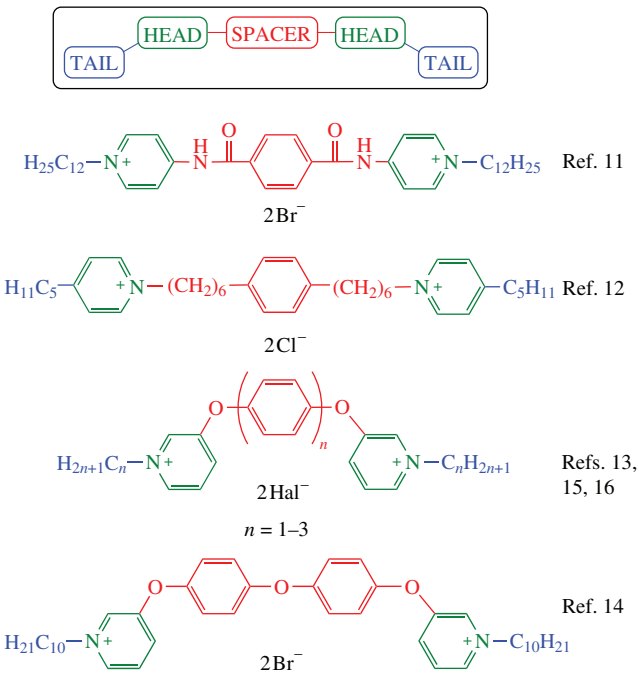
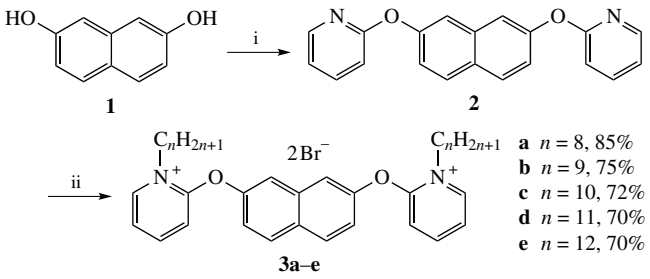


Figure 1 Structure of cationic bispyridinium salts with benzene containing spacer.

The synthesis started with the Ullmann type reaction of available 2,7-dihydroxynaphthalene **1** with 2-bromopyridine in the presence of CuI and K_3PO_4 in DMSO at 90 °C (Scheme 1). This technique allows the synthesis to be carried out at lower temperatures than under standard for reactions of this type conditions.[17] The second stage was the quaternization of the obtained bipyridine 'platform' **2** with linear alkyl bromides in the range from 8 to 12 carbon atoms. This interval was selected in accordance with the results of previous studies.[13–15] The impact of the counterion on the antibacterial and



Scheme 1 *Reagents and conditions*: i, 2-bromopyridine, K_3PO_4, CuI, picolinic acid, DMSO, argon, 90 °C, 24 h; ii, alkyl bromide (4 equiv.), MeCN, 81 °C, 4 days.

antifungal properties of the compounds is negligible, therefore, convenient alkyl bromides were chosen for the synthesis.† All details of the synthesis, isolation and purification of substances, as well as ^1H, ^{13}C NMR spectra and HPLC of the target compounds are provided in Online Supplementary Materials.

All the salts obtained were tested for microbiological activity against a panel of five patogenic bacteria, including Methicillin-resistant *Staphylococcus aureus* (MRSA) and two fungi, cytotoxicity on human kidney embryonic cells, and hemotoxicity on human red blood cells (Table 1). Widely known commercially available antiseptics (benzalkonium chloride (BAC), cetylpyridinium chloride (CPC), chlorhexidine gluconate (CHG), octenidine dihydrochloride (OCT), as well as hit compounds **4**[13] and **5**[14] from our recent studies were selected as reference samples (Figure 2). Microbiological assays were performed by CO-ADD (The Community for Antimicrobial Drug Discovery),[18,19] with full experimental details described in the Online Supplementary Materials.

According to the analysis of microbiological data, most of the compounds obtained exhibit relatively strong antibacterial and antifungal properties. The new salt **3c** is the hit-compound in this series, showing results comparable to octenidine and superior to all other samples. Moreover, **3c** exhibits less cyto- and hematoxicity than octenidine. All salts, except for **3a** showing no

Table 1 MIC and cytotoxicity values (μg ml^{-1}) for prepared BPSs.

Compound	MIC[a]							Cytotoxicity[b]	
	Bacteria					Fungi		HEK-293 (CC_{50})	RBC (HC_{50})
	MRSA	*Ec*	*Kp*	*Ab*	*Pa*	*Ca*	*Cn*		
3a (C_8)	>32	>32	>32	>32	>32	>32	>32	>32	>32
3b (C_9)	⩽0.25	0.5	8	8	4	⩽0.25	⩽0.25	2.25	1.12
3c (C_{10})	⩽0.25	⩽0.25	⩽0.25	8	⩽0.25	⩽0.25	⩽0.25	4.28	0.64
3d (C_{11})	⩽0.25	16	>32	>32	2	⩽0.25	⩽0.25	1.08	1.34
3e (C_{12})	⩽0.25	32	>32	>32	32	⩽0.25	⩽0.25	0.90	1.82
BAC	0.5	16	>32	32	>32	0.5	1	2.8	3.4
CPC	⩽0.25	16	>32	>32	32	⩽0.25	⩽0.25	1.15	1.11
CHG	⩽0.25	1	32	8	8	32	>32	>32	>32
OCT	⩽0.25	⩽0.25	⩽0.25	⩽0.25	⩽0.25	⩽0.25	⩽0.25	1.12	⩽0.25
4	⩽0.25	1	8	2	4	⩽0.25	⩽0.25	3.1	16.9
5	⩽0.25	1	4	2	4	⩽0.25	⩽0.25	3.2	4.3

[a] MRSA, Methicillin-resistant *Staphylococcus aureus* (ATCC 43300); *Ec*, *Escherichia coli* (ATCC 25922); *Kp*, *Klebsiella pneumonia* (ATCC 700603); *Ab*, *Acinetobacter baumannii* (ATCC 19606); *Pa*, *Pseudomonas aeruginosa* (ATCC 27853), *Ca, Candida albicans* (ATCC 90028); *Cn*, *Cryptococcus neoformans var. grubii* (ATCC 208821). [b] HEK-293, Human embryonic kidney cells (ATCC CRL-1573, CC_{50}); RBC, Human red blood cells (HC_{50}).

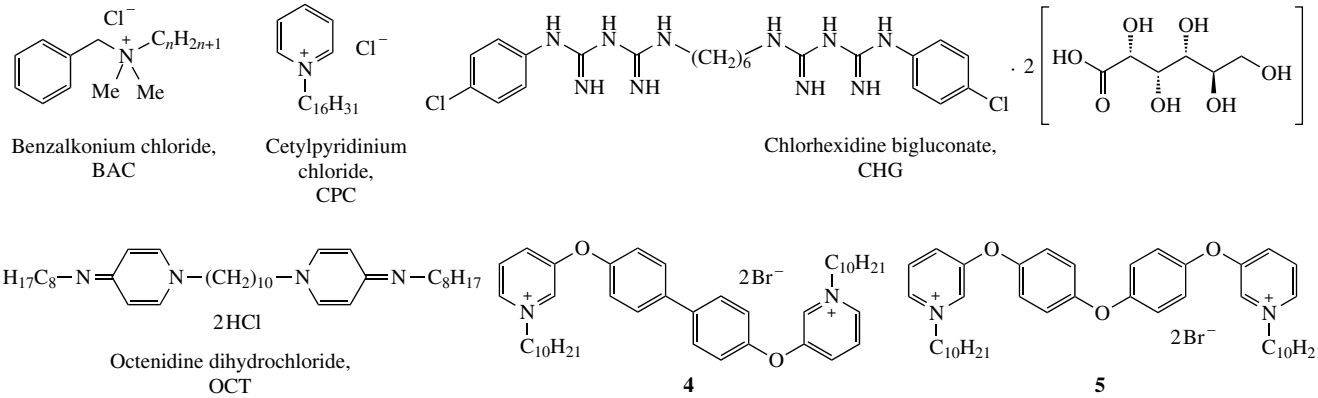


Figure 2 Reference antiseptics.

† *Synthesis of 2,2′-[naphthalene-2,7-diylbis(oxy)]dipyridine* **2**. A mixture of 2,7-dihydroxynaphthalene (1.60 g, 10 mmol), 2-bromopyridine (3.16 g, 20 mmol), potassium phosphate (8.48 g, 40 mmol), copper(I) iodide (1.90 g, 10 mmol) and picolinic acid (0.25 g, 2 mmol) in dry DMSO (50 ml) was heated to 90 °C for 24 h in argon atmosphere. The solvent was removed under reduced pressure, ethyl acetate (50 ml) was added to the crude residue, and this was refluxed for 1 h. The solid was filtered off and the filter cake was washed with hot ethyl acetate (20 ml). The organic filtrate was concentrated under reduced pressure and the residue was purified by recrystallization from heptane to provide a white solid product (2.26 g, 7.2 mmol, 72% yield).

Synthesis of 2,2′-[naphthalene-2,7-diylbis(oxy)]dipyridinium dibromides **3**. To a solution of compound **2** (0.31 g, 1 mmol) in acetonitrile (3 ml), alkyl bromide (4 mmol) was added. The mixture was refluxed for 7 days, cooled and filtered. The filtered solid was washed with cold acetone (10 ml) and dried to afford a white solid product **3**. The yields of **3a–e** were 70–85% depending on the alkyl bromide.

For characteristics of products **3a–e**, see Online Supplementary Materials.

activity, have MIC values of ⩽0.25 on all strains of fungi and MRSA, as well as the hit-compounds from our previous work.[13–15] With raising the length of the alkyl tail from C_8 to C_{10}, the antibacterial activity increases, and from C_{10} to C_{12} it decreases.

In summary, five new bispyridinium salts containing naphthalene-2,7-diylbis(oxy) spacer were synthesized, their antibacterial and antifungal activity was evaluated on five strains of pathogenic bacteria and two fungi, respectively, and cytotoxicity and hemotoxicity were measured. The dependence of biological activity on the length of the alkyl tail was analyzed. Hit-compound with $C_{10}H_{21}$ aliphatic tails displayed better antibacterial properties than benzalkonium chloride, chlorhexidine, cetylpyridinium chloride and comparable with octenidine.

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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.2020.11.004.

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