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Synthesis and biological evaluation of novel bis-quaternary ammonium compounds with *p*-terphenyl spacer

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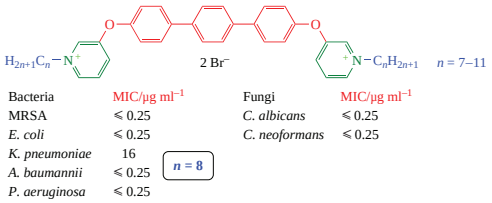
Novel bis-quaternary ammonium compounds with a *p*-terphenyl spacer between two pyridinium heads were synthesized. Hit compound with C_8H_{17} aliphatic substituent is superior to known benzalkonium chloride, miramistin and chlorhexidine bisgluconate in MIC values against five pathogenic bacteria.



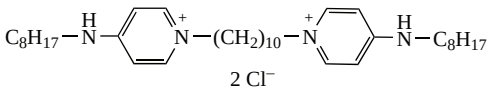
Keywords: bis-quaternary ammonium compounds, pyridinium salts, terphenyls, alkylation, antibacterial agents, fungicides, antifungal activity, methicillin resistance, *Staphylococcus aureus*.

Quaternary ammonium compounds (QACs) possessing positively charged nitrogen atom with four substituents or with three substituents and one double bond showed themselves as potential antibacterial agents in 1916 in the study of hexamethylenetetramine derivatives[1] and secured this title with the discovery of benzalkonium chloride (BAC) in 1935,[2] which is widely used up to now. Further, the study of this class led to the discovery of many useful properties of QACs which found application as surfactants, personal care products, cosmetics, softeners, dyes, biological stains, and, of course, broad-spectrum antiseptics and disinfectants.[3–5] An important subgroup of QACs is bispyridinium salts (BPSs) exhibiting antibacterial activity even at low concentrations and having antifungal and antiviral effects. An example of such a dimeric pyridinium salt is one of the most effective anticeptics, namely, octenidine dihydrochloride.[6]

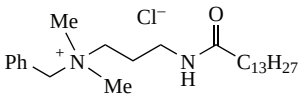
Recent studies have shown that some bacterial strains possess developed resistance to octenidine and other QACs.[7,8] Such resistance is possible due to the production of efflux pumps in bacterial cells. Furthermore, genes encoding the production of efflux pumps can be transmitted between bacteria through plasmids, or generated by mutations.[9] Hence, the development of new structures to overcome resistance is one of the main problems in this area.

The study of structure–activity relationship is among the modern interdisciplinary approaches of organic chemistry.[10,11] The spacer nature in BPSs has a significant impact on bis-QACs' manifestation of certain effects including biocidal effects.[12] Amongst others, bis-QACs containing a benzene ring in the spacer were obtained, for instance, salt **1**.[13] Compounds of this type are not inferior to octenidine in effectiveness (MIC and MBC) but exceed it in cytotoxicity against normal human epidermal keratinocytes.[14] Recently we have synthesized structural analogues of salt **1**, *viz.* compounds **2**,**3** with other types of spacers. Minimal inhibitory concentration (MIC) values of **2** and **3** were higher than that of **1** and well-known antibacterial agents benzalkonium chloride (BAC) and chlorhexidine digluconate (CHG).

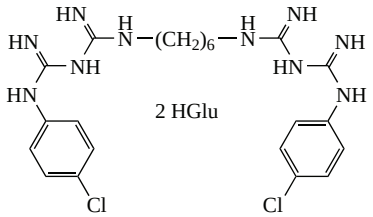
The aim of this work was to synthesize novel BPSs containing *p*-terphenyl in spacer, study their antibacterial and antifungal properties, toxicity, identify the hit compound and compare the data obtained with known standards (BAC, miramistin and CHG). The synthesis started with the bromination of *p*-terphenyl **4** under



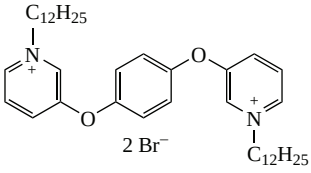
Octenidine



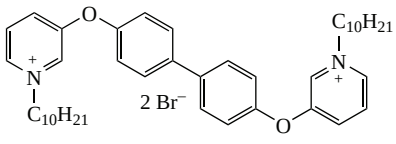
Miramistin



Chlorhexidine bisgluconate



1



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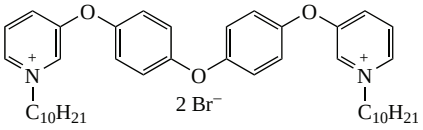

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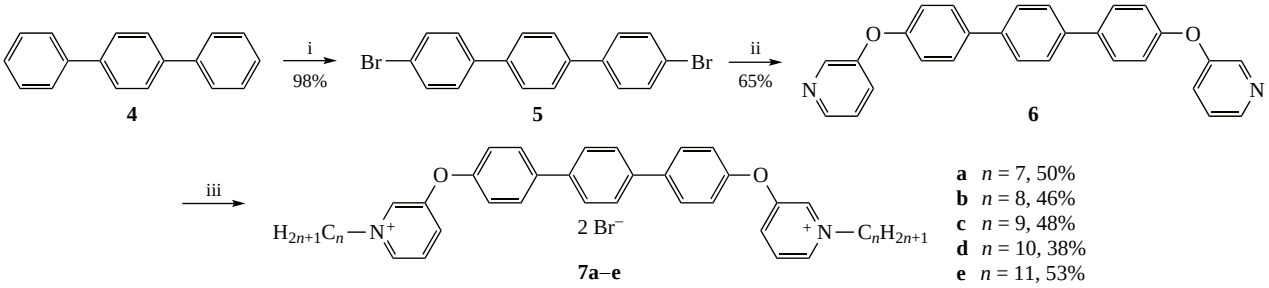

Scheme 1 *Reagents and conditions*: i, Br_2 (2.5 equiv.), H_2O_2 (25%), H_2O, CH_2Cl_2, reflux, 4 h; ii, 3-hydroxypyridine, Cu (powder), K_2CO_3, DMA, argon, reflux, 3 days; iii, alkyl bromide (2.4 equiv.), DMF, 120 °C, 4 days.

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

Compound (alkyl)	MIC[a]							Cytotoxicity[b]	
	Bacteria					Fungi		HEK-293 (CC_{50})	RBC (HC_{50})
	MRSA	*E. coli*	*K. pneumoniae*	*A. baumannii*	*P. aeruginosa*	*C. albicans*	*C. neoformans*		
7a (C_7)	0.25	4	> 32	16	32	⩽ 0.25	⩽ 0.25	0.5	0.6
7b (C_8)	⩽ 0.25	⩽ 0.25	16	⩽ 0.25	⩽ 0.25	⩽ 0.25	⩽ 0.25	⩽ 0.25	⩽ 0.25
7c (C_9)	⩽ 0.25	4	> 32	2	8	⩽ 0.25	⩽ 0.25	1.0	0.3
7d (C_{10})	⩽ 0.25	4	> 32	16	16	⩽ 0.25	⩽ 0.25	0.8	1.6
7e (C_{11})	⩽ 0.25	> 32	> 32	> 32	> 32	⩽ 0.25	⩽ 0.25	11.7	0.7
BAC	0.5	16	> 32	32	> 32	0.5	1	2.8	3.4
Miramistin	8	32	32	32	32	32	16	24.3	11.9
CHG	⩽ 0.25	1	32	8	8	32	> 32	> 32	> 32
1	⩽ 0.25	4	16	4	8	⩽ 0.25	⩽ 0.25	3.4	4.1
2	⩽ 0.25	1	8	2	4	⩽ 0.25	⩽ 0.25	3.1	16.9

[a] MRSA, Methicillin-resistant *Staphylococcus aureus* (ATCC 43300); *E. coli*, *Escherichia coli* (ATCC 25922); *K. pneumoniae*, *Klebsiella pneumoniae* (ATCC 700603); *A. baumannii*, *Acinetobacter baumannii* (ATCC 19606); *P. aeruginosa*, *Pseudomonas aeruginosa* (ATCC 27853); *C. albicans*, *Candida albicans* (ATCC 90028); *C. neoformans*, *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}).

standard conditions (Scheme 1). Next, the Ulmann-type reaction of compound **5** with 3-hydroxypyridine gave 3,3′-[*p*-terphenyl-4,4′′-diylbis(oxy)]dipyridine **6**. This procedure was optimized compared with our previous work.[15] Dipyridine **6** is insoluble in methyl isobutyl ketone (solvent for quaternization of close analogues[15]); therefore, its quaternization with alkyl bromides was carried out in DMF. However, complete conversion of compound **6** was achieved only in 4 days. The target compounds **7** were isolated by simple filtration.[†]

Compounds **7a**–**e** were tested for biological activity against five pathogenic bacteria, including Gram-positive [methicillin-resistant S*taphylococcus aureus* (MRSA) strain ATCC 43300] and Gram-negative (*Escherichia coli*, ATCC 25922; *Klebsiella pneumoniae*, ATCC 700603; *Acinetobacter baumannii*, ATCC 19606; P*seudomonas aeruginosa*, ATCC 27853) strains. Two fungi (*Candida albicans*, ATCC 90028; *Cryptococcus neoformans var. grubii*, ATCC 208821) were also tested. The commercially available BAC, CHG as well as hit compounds ⩽ **1** and **2** from our recent studies[16] were selected as reference samples. Microbiological assays were performed by CO-ADD (The Community for Antimicrobial Drug Discovery),[15,16] with full experimental details described in Online Supplementary Materials.

[†] *Synthesis of 3,3′-[*p*-terphenyl-4,4′′-diylbis(oxy)]dipyridine* **6**. A mixture of 4,4′′-dibromo-*p*-terphenyl **5** (3.88 g, 10 mmol), 3-hydroxypyridine (2.09 g, 22 mmol), potassium carbonate (6.07 g, 44 mmol), and copper powder (2.81 g, 44 mmol) in dry DMA (100 ml) was refluxed for 3 days under argon atmosphere. The solvent was removed under reduced pressure, ethyl acetate (200 ml) was added to the crude residue, and the mixture was refluxed for 1 h. Then the mixture was filtered and washed with hot ethyl acetate (50 ml). The organic filtrate was concentrated under reduced pressure, and the residue was purified by recrystallization from ethyl acetate to provide a white solid product **6** (2.7 g, 6.5 mmol, 65% yield).

The data summarized in Table 1 demonstrate some clear trends in MIC on bacteria and fungi. The optimum bioactivity of BPSs **7a**–**e** was observed with octyl chains at pyridinium nitrogens. Thus, compound **7b** with C_8H_{17} substituent showed the lowest value of the MIC among the tested samples against all bacterial strains, except *K. pneumonia*. At the same time, this compound is the most toxic to HEK and RBC. Amphiphiles with shorter and longer alkyl chains displayed less activity. All new salts were effective against fungi and MRSA (MIC ⩽ 0.25 ug ml^{-1}), as well as the previously obtained BPSs **1** and **2** with one and two benzene rings as spacer, respectively. Compounds **7c** and **7d** showed better MIC values compared to BAC and miramistin, and compound **7c** exhibited activity comparable to CHG (except for fungi against which CHG is not active). When switching from C_{10} to C_{11} or from C_8 to C_7 tails, a decrease in activity against strains of *E. coli*, *A. baumannii* and *P. aeruginosa* was observed, but the rates on fungi (*C. albicans*, *C. neoformans*) and MRSA remain the same.

In general, with an increase in the number of benzene rings in the aromatic spacer, the following trends were noticed. (1) MIC values for hit compounds in each series are decreased, but toxicity is increased. (2) The length of the alkyl chains of the hit compounds becomes shorter by two carbon atoms with each additional ring: for phenyl it is C_{12}, for biphenyl – C_{10}, for terphenyl – C_8. (3) Activity on fungi and MRSA remains unchanged.

In summary, five novel BPSs were synthesized, and their antibacterial, antifungal, hemolytic and cytotoxic activities were assessed. The series of compounds showed potent MIC values against five bacterial strains and two fungi. A clear relationship between antimicrobial activity and the tail length was noted. Hit compound **7b** with C_8H_{17} aliphatic tails displayed better antibacterial properties than benzalkonium chloride, miramistin, chlorhexidine and the closest structural analogues **1** and **2**. The

dependence of antibacterial activity on the number of benzene rings in the aromatic spacer was determined.

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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.2020.07.006.

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