Eur. J. Med. Chem. 36 (2001) 313–320

PII: S0223-5234(01)01226-0/FLA

Original article

Synthesis and antimicrobial activities of new pyridinium and benzimidazolium chlorides

Juliusz Pernak[a]*, Jarostaw Rogoża[a], Ilona Mirska[b]

[a]Poznań University of Technology, Skłodowskiej-Curie 2, 60-965 Poznań, Poland
[b]K. Marcinkowski University of Medical Sciences, Sieroca 10, 61-771 Poznań, Poland

Received 3 July 2000; revised 6 February 2001; accepted 8 February 2001

Abstract – A novel class of pyridinium and benzimidazolium chloride has been obtained in high yield. The antimicrobial activities of three homologous series of pyridinium and benzimidazolium chlorides against cocci, rods, fungi and bacillus have been measured. The antimicrobial activities of *N*,*N*′-bis[3-(1-alkoxymethyl)pyridinium chloride]methylenediamines, 1-undecyloxymethyl-3-(1-benzimidazolmethylamino)pyridinium, 1-undecyloxymethyl- and 1-dodecyloxymethyl-3-[1(benzotriazol-1-yl)methylamino]pyridinium chlorides exhibited strong activity and wide antibacterial spectra similar to the activity of benzalkonium chloride. © 2001 Éditions scientifiques et médicales Elsevier SAS

Antimicrobial activities / Benzalkonium chloride / Benzimidazolium chlorides / Pyridinium chlorides / Bispyridinium chlorides

1. Introduction

Pyridinium compounds are an important class of chemicals used widely as biocides, cationic surfactants, drugs, and herbicides. The formal positive charge on the nitrogen atom of these compounds possesses many unique properties, which have been utilized in numerous applications. The first in 1915 Jacobs and Heidelberg [1, 2] published synthesis and antimicrobial activity of the quaternary ammonium compounds (QAC). In 1935, Domagk [3] disclosed the antibacterial activity of the long-chain QAC. Following Domagk's publication, a large number of application areas were developed for QAC as biocides with a wide range of antimicrobial spectra. Disinfectants based on QAC are widely used in the hospital environment and the food industry. Resistance to disinfectants based on QAC is, according to a recent report [4], a potential problem in the food processing industry. QAC are studied all the time for use against various illnesses among other things malaria [5]. Numerous studies of the synthesis and antimicrobial characteristics of different novel QAC have been published recently [6–15].

The present paper reports on the synthesis and antimicrobial activities of new pyridinium, bispyridinium and benzimidazolium chlorides as potential novel biocides.

2. Chemistry

1-Alkoxymethyl-(3-nicotionylaminomethyl)benzimidazolium (**2**), 1-alkoxymethyl-3-(1-benzimidazolmethylamino)pyridinium (**4**), 1-alkoxymethyl-3-[1-(benzotriazol-1-yl)methylamino]pyridinium (**7**) chlorides and *N*,*N*′-bis[3-(1-alkoxymethyl)pyridinium chloride]methylenediamine (**5**) listed in *tables I–III* were synthesized by the method shown in *figure 1*. *N*-Mannich bases (**1**, **3**, **6**) were synthesized in the reaction of azole with 3-substituted pyridine. *N*-(1-*H*-Benzimidazolmethyl)-3-pyridinecarboxamide (**1**) is a new compound, which was obtained in a one-pot condensation reaction of benzimidazole–formaldehyde–nicotinamide. This amide was characterized by ^{1}H- and ^{13}C-NMR. The NH proton resonated at 9.93

* Corresponding author
E-mail address: juliusz.pernak@put.poznan.pl (J. Pernak).

ppm as a triplet and the methine protons appeared at 5.83 ppm as a doublet with a coupling constant in the range of 5.6 and 6.0 Hz, respectively. The chemical shift of the carbonyl carbon appeared at 165.3 ppm. 3-(Benzotriazol-1-ylmethylamino)pyridine (**6**) was prepared earlier by the condensation of 3-aminopyridine with 1-(hydroxymethyl)benzotriazole [16]. We obtained the same compound by direct condensation of benzotriazole–formaldehyde–nicotinamide. For synthesized of 3-(benzotriazol-1-ylmethylamino)-pyridine (**3**) we used the microwave technique with success.

Table I. 1-Alkoxymethyl-3-(nicotionylaminomethyl)benzimidazolium chlorides (**2**) prepared

Chloride	R	Yield (%)	M.p. [a] (°C)	Purity (%)
2a	C_2H_5	85	152–156	
2b	C_3H_7	85	150–154	
2c	C_4H_9	85	133–137	
2d	C_5H_{11}	80	130–134	
2e	C_6H_{13}	80	117–121	95
2f	C_7H_{15}	84	121–125	96
2g	C_8H_{17}	80	118–122	95
2h	C_9H_{19}	75	114–117	96
2i	$C_{10}H_{21}$	75	110–114	95
2j	$C_{11}H_{23}$	75	123–126	97
2k	$C_{12}H_{25}$	70	119–123	94

[a] Solvent for recrystallization $CHCl_3$–$CH_3COOC_2H_5$.

Table II. 1-Alkoxymethyl-3-(1-benzimidazolmethylamino)-pyridinium chlorides (**4**) and *N*,*N*′-bis[3-(1-alkoxymethyl)-pyridinium chloride]methylenediamines (**5**) prepared

Chloride	R	Yield (%)	M.p. [a] (°C)	Purity (%)
4a	C_3H_7	82	143–145	
4b	C_4H_9	75	145–147	
4c	C_5H_{11}	73	144–146	
4d	C_6H_{13}	70	144–146	
4e	C_7H_{15}	67	148–150	
4f	C_8H_{17}	70	141–143	
4g	C_9H_{19}	66	145–147	
4h	$C_{10}H_{21}$	62	146–148	
4i	$C_{11}H_{23}$	65	146–147	
4j	$C_{12}H_{25}$	63	144–147	
5a	C_9H_{19}	85	68–71	198
5b	$C_{10}H_{21}$	83	70–73	198
5c	$C_{11}H_{23}$	84	70–73	198
5d	$C_{12}H_{25}$	87	69–72	198

[a] Solvent for recrystallization MeOH–CH_3COCH_3 (**4a**–**g**), H_2O (**4h**–**j** and **5a**–**d**).

Table III. 1-Alkoxymethyl-3-[1-(benzotriazol-1-yl)methylamino]pyridinium chlorides (**7**) prepared

Chloride	R	Yield (%)	M.p. [a] (°C)	Purity (%)
7a	C_3H_7	80	132–134	
7b	C_7H_9	80	139–140	
7c	C_5H_{11}	82	139–141	
7d	C_6H_{13}	81	146–148	
7e	C_7H_{15}	78	133–135	99
7f	C_8H_{17}	80	132–134	99
7g	C_9H_{19}	80	134–136	99
7h	$C_{10}H_{21}$	78	138–140	99
7i	$C_{11}H_{23}$	77	135–136	99
7j	$C_{12}H_{25}$	78	134–136	99

All chlorides prepared (**2**, **4**, **5** and **7**) were very hygroscopic. The chemical purity was determined by a direct 2-phase titration procedure for chlorides **2**, **5** and **7** with alkyl long chain length (6–12 carbon atoms), presented in *tables I–III*. 1-Alkoxymethyl-3-(1-benzimidazolmethylamino)pyridinium chlorides (**4**) were destroyed in acidic medium which is a two-phase titration method.

3. Antimicrobial activity

All synthesized pyridinium and benzimidazolium chlorides (**2**, **4**, **5** and **7**) were tested for antimicrobial activity against cocci, rods, fungi and bacillus.

4. Results and discussion

The quaternization of the three prepared *N*-Mannich bases (**1**, **3** and **6**) was run with an electrophile like chloromethyl alkyl ether to give good yields of three different final chlorides — benzimidazolium (**2**), bispyridinium (**5**) and pyridinium (**7**). $ROCH_2Cl$ is an excellent reagent but very easily hydrolyzed. In this situation, reaction should be conducted under strictly anhydrous conditions. This quaternization depends on the substituent in the three-position of the pyridine ring. The strong electron-withdrawing group deactivates the nitrogen atom in pyridine and in this situation, the nitrogen atom N-3 in benzimidazole is nucleophilic enough to react with $ROCH_2Cl$. 1-Alkoxymethyl-3-(1-benzimidazolmethylamino)pyri-

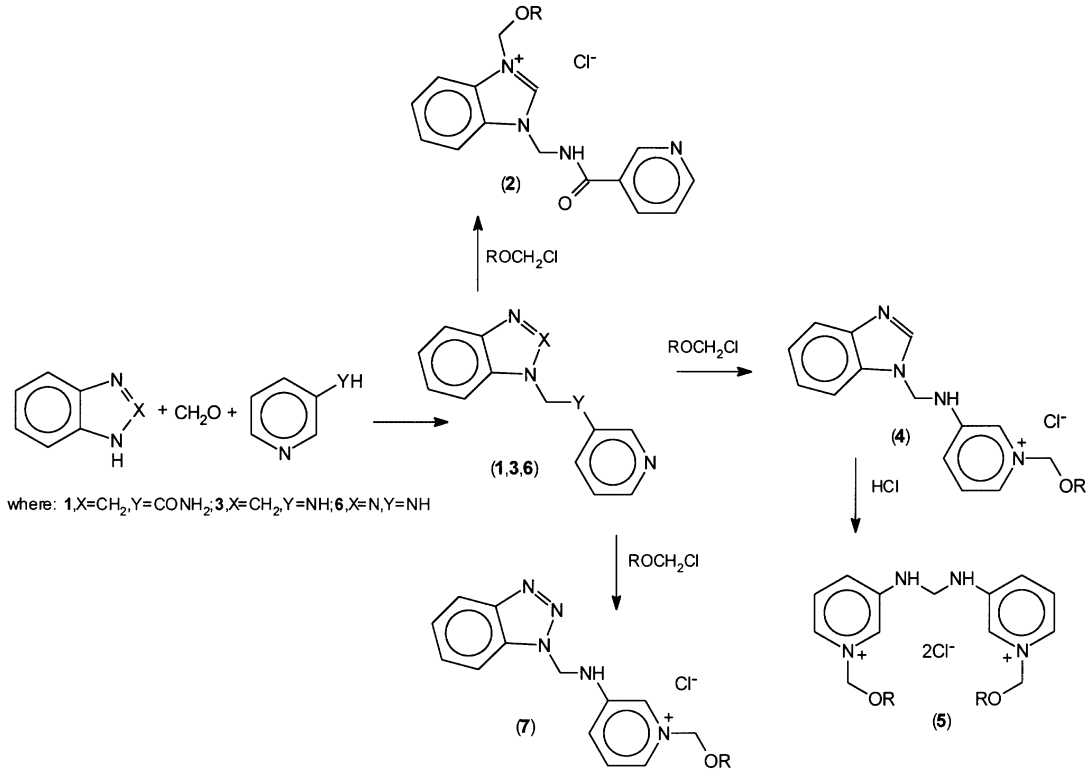

Figure 1. Synthesis of compounds **2**, **4**, **5**, **7**.

dinium chloride (**4**) was converted into *N*,*N*′-bis[3-(1-alkoxymethyl)pyridinium chloride]methylenediamine (**5**). The formation of bispyridinium is probably the result of the attack of HCl on the N-3 of the benzimidazole ring to give an intermediate as shown in *figure 2*, which is unstable and quickly converted to bispyridinium chloride to replace the benzimidazole moiety. In this reaction benzimidazole is a good living group in acidic solution. *N*,*N*′-Bis(3-pyridine)methylnediamine is a well known compound [17], which was prepared by imines as an intermediate. Probably, the iminium salt is the intermediate in the synthesis of bispyridinium chloride [18].

The newly prepared compounds were characterized by their ^{1}H- and ^{13}C-NMR spectra and by elemental analyses. The NH proton resonated at 11.30–11.20 for chlorides **2**, at 9.10–8.95 for chlorides **4**, at 9.55–9.45 for chlorides **5** and at 10.31–9.47 for chlorides **7** as a triplet with a coupling constant in the range 5.7, 6.9, 6.0 and 6.1 Hz, respectively. The methine protons (NCH_2N) appear as a doublet at 6.38–5.90 ($J = 6$ Hz) for chlorides **2**, **4** and **7** and as a triplet at 4.85 ($J = 5.4$ Hz) for chlorides **5**. The chemical shift of the characteristic carbon α between the azole ring and amide or amine fell in the region of 55.10–50.70.

Minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC) values, determined for 11 benzimidazolium chlorides (**2a**–**k**) and for 24 pyridinium chlorides (**4a**–**j**, **5a**–**d** and **7a**–**j**) are given in *tables IV–VI*. The calculated average MIC values for all the strains are presented in *figure 3* as a relationship between the alkyl chain length and antimicrobial activity. As shown by the results, all the chlorides studied are active against

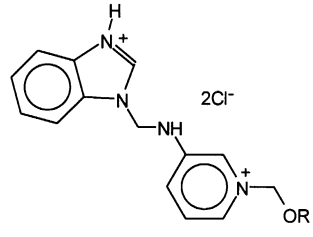

Figure 2. Intermediate backing to bispyridinium chloride.

Table IV. The MIC values [a] of 1-alkoxymethyl-3-(nicotionylaminomethyl)benzimidazolium chlorides (**2**)

Strains		Chlorides										
		2a	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**	**2h**	**2i**	**2j**	**2k**
Cocci												
Micrococcus luteus	MIC	>1443	>1387	1335	1287	311	300	290	141	545	132	257
Staphylococcus aureus	MIC	>1443	>1387	>1335	1287	621	600	581	281	273	132	514
Enterococcus faecalis	MIC	>1443	>1387	>1335	1287	311	300	290	70	34	66	257
Moraxella catarrhalis	MIC	1443	1387	668	322	155	75	72	70	273	265	257
Rods												
Escherichia Coli	MIC	>1443	>1387	>1335	>1287	1242	1200	1161	562	1091	529	1028
Proteus vulgaris	MIC	>1443	>1387	>1335	>1287	1242	1200	1161	562	545	1058	>1028
Klebsiella Pneumoniae	MIC	>1443	>1387	>1335	>1287	>1242	>1200	1161	1125	545	529	1028
Pseudomonas aeruginosa	MIC	>1443	>1387	>1335	>1287	>1242	>1200	>1161	>1125	>1091	>1058	>1028
Fungi												
Candida Albicans	MIC	>1443	>1387	1335	1287	1242	1200	1161	562	1091	265	>1028
Rhodotorula rubra	MIC	>1443	>1387	1335	1287	1242	1200	581	562	1091	265	1028
Bacillus												
Bacillus Subtilis	MIC	>1443	>1387	1335	1287	311	300	145	70	545	33	514

[a] In μM, the number of microorganisms in mL range from 10^4 to 10^5.

cocci, rods, fungi and bacillus. Their activities are greatly affected by an alkyl chain length in the alkoxymethyl substituent and a kind of quaternary ammonium moieties in a molecule. The same correlation is observed for the MIC and the MBC values. The favorite alkyl group is the one which contains 9–12 carbon atoms. In comparison with commercially available benzalkonium chloride (BAC–Aldrich product, in which R represents a mixture of alkyls from C_8H_{17} to $C_{18}H_{37}$) 1-alkoxymethyl-3-nicotionylaminomethyl)benzimidazolium chlorides (**2**) are only slightly effective. The most active chlorides against the studied microorganisms are *N*,*N*′-bis[3-(1-alkoxymethyl)pyridinium chloride]methylenediamines (**5**) and 1-undecyloxymethyl-3-(1-benzimidazolmethylamino)pyridinium (**4i**), 1-undecyloxymethyl- and 1-dodecyloxymethyl-3-[1-(benzotriazol-1-yl)methylamino]pyridinium (**7i** and **7j**) chlorides. The activity of chlorides **5**, **4i**, **7i** and **7j** are similar to the activity of BAC. It is known that compounds that possess two quaternary ammonium moieties in the molecule are strongly active against bacteria and fungi. New observation is that pyridinium salts of formula weight, higher than 445, with four and five nitrogen atoms are potential new biocides.

The most active chlorides **4i**, **5b** and **7i** were tested against *Mycobacterium tuberculosis* strain $H_{37}R_v$. It was found, in the TAACF screening program, that they are active (MIC<6.25 μg/mLand Inh.>92%) and are potential compounds for use in the treatment of mycobacterial infections.

5. Experimental protocols

5.1. Chemistry

Melting points were measured on a Kofter hot stage apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded with a Varian model XL 300 spectrometer with TMS as standard. Elemental analyses CHN were done at the A. Mickiewicz University, Poznań. For all the synthesized compounds, satisfactory microanalyses were obtained C±0.37; H±0.35; and N± 0.31. $ROCH_2Cl$ was prepared via chloromethylation of alcohol. Pyridinium and benzimidazolium chlorides obtained were determined by two-phase back titration of sodium dodecyl sulfate with Hyamine 1622 using diimidium bromide and erioglaucine indicators.

Table V. The MIC and MBC values [a] of 1-alkoxymethyl-3-(1-benzimidazolmethylamino)pyridinium chlorides (**4**) and *N*,*N*′-bis[3-(1-alkoxymethyl)-pyridinium chloride]methylenediamines (**5**)

Strains		Chlorides														
		4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**	**5a**	**5b**	**5c**	**5d**	BAC [b]
Cocci																
M. luteus	MIC	48	46	44	166	10.5	10	38	19	9	35	7	6.5	12	12	7
	MBC	93	46	172	334	21	20	74	37	18	68	7	13	12	24	16
S. aureus	MIC	752	722	1386	1335	322	77	148	72	18	68	14	13	12	24	5
	MBC	1504	1443	1386	1335	322	311	148	144	32	273	53	26	25	46	5
S. epidermidis	MIC	752	722	1386	668	80	40	19	37	18	8.5	7	13	12	12	3
	MBC	1504	1443	1386	1335	160	154	74	144	18	8.5	7	26	25	46	3
Rods																
E. Coli	MIC	376	361	693	1335	644	311	300	290	70	545	27	26	25	46	7
	MBC	752	722	2774	1335	644	311	600	290	70	545	27	52	195	46	10
S. marcescens	MIC	1504	1443	2774	1335	644	621	600	1161	281	545	214	101	195	187	54
	MBC	1504	1443	2774	1335	644	621	1200	1161	281	1090	214	204	195	1495	205
P. vulgaris	MIC	752	722	693	668	322	154	149	72	70	135	53	13	48	93	16
	MBC	1504	1443	1386	668	644	154	300	144	140	1090	53	51	97	187	16
K. Pneumoniae	MIC	1504	722	1386	334	322	154	149	37	140	135	27	26	97	46	11
	MBC	3008	2886	5547	668	644	311	300	144	140	545	27	26	97	46	11
P. aeruginosa	MIC	752	722	1386	1335	322	311	300	290	140	1090	27	51	97	93	54
	MBC	1504	1443	1386	1335	322	311	300	290	140	1090	27	101	97	93	205
Fungi																
C. albicans	MIC	>12030	>11544	>11096	5340	2574	1242	1200	290	70	17.5	13.5	13	195	93	7
	MBC	>12030	>11544	>11096	10681	2574	1242	1200	290	70	17.5	27	13	195	187	16
R. rubra	MIC	>12030	>11544	>11096	2670	1287	621	300	72	32	17.5	7	13	6	6	11
	MBC	>12030	>11544	>11096	2670	1287	621	300	72	32	17.5	27	13	12	6	11
Bacillus																
B. subtilis	MIC	1504	1443	2774	1335	644	154	149	72	32	135	14	13	25	46	11
	MBC															

[a] In μM, the number of microorganisms in mL range from 10^4 to 10^5.
[b] Benzalkonium chloride.

Table VI. The MIC and MBC values [a] of 1-alkoxymethyl-3-[1-(benzotriazol-1-yl)methylamino]pyridinium chlorides (**7**)

Strains		Chlorides										
		7a	**7b**	**7c**	**7d**	**7e**	**7f**	**7g**	**7h**	**7i**	**7j**	BAC [b]
Cocci												
M. luteus	MIC	1499	1439	1383	333	321	154	76.6	74.2	35.9	17.4	7
	MBC	>1499	>1439	1383	666	321	154	76.6	74.2	35.9	69.6	16
S. aureus	MIC	1499	>1439	>1383	666	321	40.0	38.3	37.1	35.9	34.8	5
	MBC	>1499	>1439	>1383	1332	642	79.3	38.3	74.2	71.8	34.8	5
S. epidermidis	MIC	374	173	88.5	21.3	20.5	19.8	<19.2	18.5	18.0	17.4	3
	MBC	750	719	88.5	42.6	82.2	79.3	76.6	144	35.9	34.8	3
Rods												
E. coli	MIC	>1499	>1439	>1383	1332	642	79.3	76.6	74.2	71.8	135	7
	MBC	>1499	>1439	>1383	1332	642	79.3	76.6	74.2	71.8	135	10
S. marcescens	MIC	>1499	>1439	>1383	>1332	>1284	310	299	144	281	272	54
	MBC	>1499	>1439	>1383	>1332	>1284	620	599	579	281	272	205
P. vulgaris	MIC	96.0	92.0	346	165	642	310	149	144	71.8	69.6	16
	MBC	375	360	692	1332	1284	620	599	290	281	272	16
K. pneumoniae	MIC	>1499	>1439	>1383	>1332	1284	620	299	144	139	135	11
	MBC	>1499	>1439	>1383	>1332	>1284	620	599	144	281	272	11
P. aeruginosa	MIC	>1499	>1439	1383	1332	642	79.3	76.6	74.2	139	135	54
	MBC	>1499	>1439	>1383	>1332	1284	154	599	144	139	135	205
Grzyby												
C. albicans	MIC	>1499	>1439	>1383	>1332	>1284	620	299	144	139	69.6	7
	MBC	>1499	>1439	>1383	>1332	>1284	>1239	599	290	561	272	16
R. rubra	MIC	>1499	>1439	>1383	1332	642	310	149	74.2	35.9	17.4	11
	MBC	>1499	>1439	>1383	>1332	642	310	153	74.2	281	17.4	11
Laseczki												
B. subtilis	MIC	1499	719	1383	165	642	154	76.6	74.2	35.9	17.4	11
	MBC	>1499	1439	1383	165	642	154	76.6	74.2	71.8	34.8	11

[a] In μM, the number of microorganisms in mL range from 10^4 to 10^5.
[b] Benzalkonium chloride.

*5.1.1. 1-Alkoxymethyl-3-(nicotionylaminomethyl)-benzimidazolium chlorides (**2**)*

Nicotinamide (30.5 g, 0.25 mol), paraformaldehyde (7.5 g, 0.25 mol), benzimidazole (29.5 g, 0.25 mol) and a few drops of conc. sulfuric acid were refluxed in toluene (500 mL) for 48 h with water removed azeotropically by a Dean–Stark apparatus. Then the toluene was evaporated under reduced pressure (60°C/30 Torr) and the residue was recrystallized from MeOH. *N*-(1 *H*-benzimidazolmethyl)-3-pyridinecarboxamide was obtained in 90% yield (m.p. = 218–220°C). The obtained compound (0.01 mol) was dissolved in anhydr. DMF and the corresponding $ROCH_2Cl$ (0.01 mol) was added. The mixture was stirred at r.t. for 1 h. DMF was removed under reduced pressure, the product was washed with warm hexane and recrystallized.

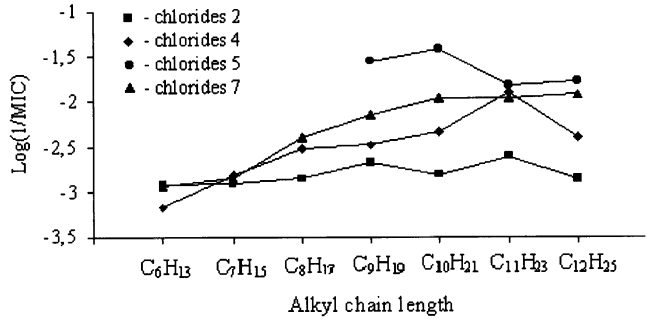

Figure 3. Relation between alkyl chain length and antimicrobial activity.

*5.1.2. Chloride (**2k**)*

^{1}H-NMR (DMSO-d_6) δ ppm = 11.25 (t, J = 6 Hz, NH), 10.38 (s, 1H), 9.22 (s, 1H), 8.80 (d, J = 4.2 Hz, 1H), 8.58 (d, J = 5.8 Hz, 1H), 8.52 (d, J = 6.7 Hz, 1H), 8.08 (d, J = 5.8 Hz, 1H), 7.74 (m, 2H), 7.60 (t, J = 7.7 Hz, 1H), 6.08 (d, J = 7.4 Hz, NCH_2N), 6.06 (s, NCH_2O), 3.57 (t, J = 6.3 Hz, 2H), 1.45 (m, 2H), 1.13 (m, 18H), 0.85 (t, J = 6.6, 3H). ^{13}C-NMR δ ppm = 165.8 (CO), 152.2, 148.3, 143.9, 136.2, 130.6, 130.5, 128.2, 127.0, 123.9, 114.6, 114.2, 76.7 (NCH_2O), 69.0, 51.8 (NCH_2N), 31.3, 29.0, 28.98, 28.92, 28.89, 28.7, 28.0, 28.5, 25.2, 22.1, 14.0.

*5.1.3. 1-Alkoxymethyl-3-(1-benzimidazolmethylamino)-pyridinium chlorides (**4**)*

A mixture of equimolar amounts (0.1 mol) of 3-aminopyridine, paraformaldehyde and benzimidazole was heated between 110–90°C in a microwave reactor (Plazmatronika S.A., Wrocław, Poland). The reaction was carried out under atmospheric pressure with power 850 W and for 10 min. After being cooled to r.t., the crude product was recrystallized from EtOH–H_2O to afford the desired 3-(benzimidazolmethylamino)-pyridine: yield 80%; m.p. 160–162°C, lit. m.p. 163–164°C [19]. The synthesized 3-substituted pyridine (0.01 mol) was dissolved in anhydrous acetone adding an equimolar amount of the appropriate $ROCH_2Cl$. The mixture was stirred for 10–15 min at r.t. The precipitate solid was removed and recrystallized.

*5.1.4. Chloride (**4f**)*

^{1}H-NMR (DMSO-d_6) δ ppm = 8.98 (t, J = 6.9 Hz, NH), 8.75 (s, 1H), 8.69 (s, 1H), 8.36 (d, J = 5.8 Hz, 1H), 8.08 (d, J = 8.7 Hz, 1H), 7.87 (t, J = 8.5 Hz, 1H), 7.64 (d, J = 7.7, 1H), 7.25 (m, 2H), 5.91 (d, J = 6.9 Hz, NCH_2N), 5.52 (s, NCH_2O), 3.45 (t, J = 6.4 Hz, 2H), 1.45 (m, 2H), 1.22 (m, 10 H), 0.84 (t, J = 6.8 Hz, 3H). ^{13}C-NMR δ ppm = 146.0, 144.0, 143.5, 133.0, 131.8, 128.5, 128.0, 126.6, 122.6, 122.1, 119.4, 111.4, 88.6 (NCH_2O), 70.1, 51.9 (NCH_2N), 31.2, 28.6, 25.2, 22.1, 14.0.

*5.1.5. N,N′-Bis[3-(1-alkoxymethyl)pyridinium chloride]methylenediamine (**5**)*

1-Alkoxymethyl-3-(1-benzimidazolmethylamino)pyridinium chloride (**4**) (0.01 mol) after standing in HCl solution (40 mL of H_2O and 10 mL of concentrated HCl) at r.t. slowly changes into *N*,*N*′-bis[3-(1-alkoxymethyl)pyridinium chloride]methylenediamine. After 5 h the precipitate was filtered and recrystallized.

*5.1.6. Chloride (**5c**)*

^{1}H-NMR ($CDCl_3$) δ ppm = 9.51 (t, J = 6.0 Hz, 2NH), 9.38 (s, 2H), 8.16 (d, J = 8.5 Hz, 2H), 7.97 (d, J = 6.0 Hz, 2H), 7.65 (t, J = 8.5 Hz, 2H), 6.07 (s, 4H), 4.87 (t, J = 5.5, 2H), 3.68 (t, J = 6.4 Hz, 4H), 1.61 (m, 4H), 1.32 (m, 32H), 0.88 (t, J = 6.6 H_2, 6H). ^{13}C-NMR δ ppm = 146.7, 130.7, 127.5, 127.0, 125.8, 88.83 (NCH_2O), 71.7, 50.0 (NCH_2N), 31.7, 29.4, 29.3, 29.1,28.95, 25.6, 22.5, 13.9.

*5.1.7. 1-Alkoxymethyl-3-[1-(benzotriazol-1-yl)-methylamino]pyridinium chloride (**7**)*

3-Aminopyridine (9.4 g, 0.1 mol) formaldehyde (powder, 3 g, 0.1 mol) and benzotriazole (11.9 g, 0.1 mol) was refluxed in EtOH (100 mL). After cooling to r.t., H_2O (50 mL) was added and the product was collected by filtration and recrystallized from EtOH–H_2O. The product was 3-(benzotriazol-1-ylmethylamino)pyridine (**6**, m.p. 153–154°C). The obtained compounds (0.01 mol) were dissolved in anhydr. acetone and the corresponding $ROCH_2Cl$ was added. The mixture was stirred at r.t. for 10 min. The solid product removed and recrystallized from H_2O.

*5.1.8. Chloride (**7b**)*

^{1}H-NMR (DMSO-d_6) δ ppm = 9.55 (t, J = 6.1 Hz, NH), 8.70 (s, 1H), 8.44 (d, J = 5.8 Hz, 1H), 8.27 (d, J = 8.2 Hz, 1H), 8.17 (d, J = 8.7 Hz, 1H), 8.04 (d, J = 8.5 Hz, 1H), 7.92 (t, J = 8.5 Hz, 1H), 7.58 (t, J = 7.7 Hz, 1H), 7.4 (t, J = 7.6 Hz, 1H), 6.38 (d, J = 6.0 Hz, NCH_2N), 5.84 (s, NCH_2O), 3.47 (t, J = 6.5 Hz, 2H), 1.45 (m. 2H), 1.20 (m, 2H), 0.75 (t, J = 7.3 Hz, 3H). ^{13}C-NMR δ ppm = 146.2, 145.5, 132.3, 132.1, 128.6, 128.0, 127.7, 126.7, 124.5, 119.3, 111.5, 88.6 (NCH_2O), 69.9, 54.8 (NCH_2N), 30.6, 18.3, 13.4.

5.2. Antimicrobial characteristic

The following microorganisms were used: *Micrococcus luteus* ATCC 9341, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442, *Proteus vulgaris* NCTC 4635, *Klebsiella pneumoniae* ATCC 4352, *Escherichia coli* NCTC 8196, *Serratia marcescens* ATCC 8100, *Candida albicans* ATCC 10231, *Rhodotorula rubra* PhB and *Bacillus subtilis* ATCC 6633. The *R. rubra* was taken from the Department of Pharmaceutical Bacteriology, K. Marcinkowski University of Medical Sciences, Poznań. Antimicrobial activity was determined by the

tube dilution method. Twofold dilutions of the chlorides were prepared in the Mueller–Hinton broth medium (bacteria) or in the Sabouraud broth medium (fungi). A suspension of the standard microorganisms, prepared from 24 h cultures of bacteria in the Mueller–Hinton broth medium and from 48 h cultures in the Sabouraud agar medium for fungi at a concentration of 10^6 cfu/mL, were added to each dilution in a 1:1 ratio. Growth (or its lack) of the microorganisms was determined visually after incubation for 24 h at 37°C (bacteria) or 48 h at 28–30°C (fungi). The lowest concentration at which there was no visible growth (turbidity) was taken as the MIC. Then from each tube, one loopful was cultured on an agar medium with inactivates (0.3% lecithin, 3% polysorbate 80 and 0.1% cysteine L) and incubated for 48 h at 37°C (bacteria) or for 5 days at 28–30°C (fungi). The lowest concentration of the chloride supporting no colony formation was defined as the MBC.

Acknowledgements

This investigation received financial support from the Polish Committee of Scientific Research Grant KBN No 3 T09B 010 15. Antimycobacterial data were provided by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) through a research and development contract with the US National Institute of Allergy and Infections Diseases.

References

[1] (a) Jacobs W.A., Heidelberger M., J. Biol. Chem. 20 (1915) 659–683. (b) Jacobs, W.A., Heidelberger, M., J. Biol. Chem. 20 (1915) 685–694. (c) Jacobs, W.A., Heidelberger, M., J. Biol. Chem. 21 (1915) 103–143.

[2] Jacobs W.A., J. Exp. Med. 23 (1916) 563–568.

[3] Domagk G., Dtsch. Med. Wochenschr. 61 (1935) 829–832.

[4] Heir E., Sundhelm G., Holck A.L., J. Appl. Bacteriol. 79 (1995) 149–156.

[5] Calas M., Cordina G., Bompart J., Bari M.B., Taib Jei, Ancelin M.L., Vial H., J. Med. Chem. 40 (1997) 3557–3566.

[6] Maeda T., Manabe Y., Yamamoto M., Yoshida M., Okazaki K., Nagamune H., Kourai H., Chem. Phrm. Bull. 47 (1999) 1020–1023.

[7] Pernak J., Mirska I., Kmiecik R., Eur. J. Med. Chem. 34 (1999) 765–771.

[8] Khan K.M., Saify Z.S., Khan A., Zeeshan A.M., Saeed M., Abdel-Jalil R.J., Grubler G., Voelter W.Z., Naturforsch. B: Chem. Sci. 54 (1999) 1210–1218.

[9] R. Pi Subirana, G.N. Bonastre, J. Bigorra, Ger. Offen. DE 19,829,743; CA 132, 2000, p. 32166.

[10] Pernak J., Arndt A., Brycki B., Arch. Pharm. Pharm. Med. Chem. 330 (1997) 253–258.

[11] Skrzypczak A., Brycki B., Mirska I., Pernak J., Eur. J. Med. Chem. 32 (1997) 661–668.

[12] Brycki B., Skrzypczak A., Mirska I., Pernak J., Arch. Pharm. Pharm. Med. Chem. 329 (1996) 279–282.

[13] Kourai H., Manabe Y., Matsutani E., Hasegawa Y., Nakagawa K., J. Antibact. Antifung. Agents 23 (1995) 271–280.

[14] Kourai H., Hasegawa Y., Wada K., J. Antibact. Antifung. Agents 22 (1994) 653–661.

[15] Siatra-Papastaikoudi T., Papadaki-Valiraki A., Tsantili-Kakoulidou A., Tzouvelekis L., Mentis A., Chem. Pharm. Bull. 42 (1994) 392–394.

[16] Katritzky A.R., Xiangfu L., Yang J.Z., Denisko O.U., Chem. Rev. 98 (1998) 409–548.

[17] A. Sauleau, Bull. Soc. Chim. Fr. (1973) 2823–2835.

[18] Pernak J., Węglewski J., Szymańska D., Polish J. Chem. 70 (1996) 1135–1142.

[19] Milata V., Kada R., Collect. Czech. Chem. Commun. 59 (1994) 725–730.