Studii şi Cercetări Ştiinţifice 2013, **14** (3), pp. 145 – 153
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER

SYNTHESIS AND ANTIBACTERIAL STUDY OF NOVEL BENZIMIDAZOLIUM DERIVATIVES

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Received: February, 22, 2013
Accepted: June, 21, 2013

Abstract: Condensation of *o*-phenylenediamine with formic acid, acetic acid and glycolic acid gave a series of benzimidazole which on reaction with alkyl halide in phase transfer catalysis afforded the formation of the corresponding 1,3-dialkylbenzimidazolium salts. The structure has been established on the basis of spectroscopic and spectrometric analysis (^{1}H and ^{13}C NMR, IR, MS and single X-ray diffraction). The *in vitro* antibacterial activities of these salts have been investigated.

Keywords: *alkylation, antibacterial activity, benzimidazolium, phase transfer catalysis*

INTRODUCTION

Benzimidazoles are remarkably effective compounds both with respect to their inhibitory activity and their favourable selectivity ratio. Extensive biochemical and pharmacological studies have confirmed that benzimidazole molecules are effective against various strains of microorganisms [1 - 6]. Benzimidazole compounds possess broad spectrum of biological properties and were investigated for their antiviral (anti-HIV) [7], anticancer [8], antibacterial [9], anti-inflammatory [10], antitumor [11], antioxidant [12] and antiemetic activities [13, 14].
It has been shown that some similar quaternary ammonium compounds possess an antibacterial activity [15 - 17] (Figure 1).

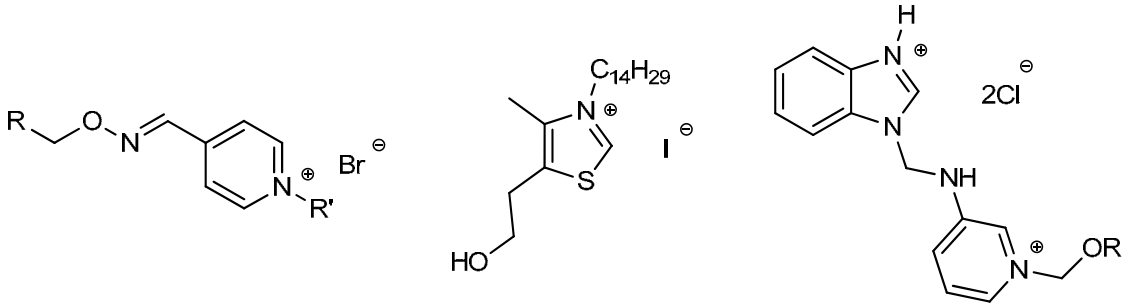


Figure 1. *Structures of antibacterial quaternary ammonium compounds*

Benzimidazole is a constituent of vitamin B_{12}, and is related to the DNA base purine and the stimulant caffeine [18].
In this paper, we report the synthesis and the antibacterial activity of a new series of benzimidazolium salt **4a-e**. The synthesized products have the advantage of being soluble in aqueous and organic media, which gives a good spread in the culture medium.

MATERIALS AND METHODS

Chemistry

Melting points were determined using a capillary melting point apparatus (Electrothermal).
^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution with TMS as an internal reference using an Avance 300 (Bruker) instrument, chemical shifts (δ) are given in ppm downfield from TMS. Multiplicities of ^{13}C NMR resources were assigned by distortion less enhancement by polarization transfer (DEPT) experiments. Mass spectrums were recorded in a SYNAPT G2 HDMS (WATERS) spectrometer in atmospheric pressure ionization (API).

General method for preparation of benzimidazolium salts 4a-e

2-substitued benzimidazole (7.5 mmol), potassium carbonate (1.55 g, 11.25 mmol) and tetra-*n*-butylammonium bromide (0.18 g, 0.75 mmol) were stirred in *N*,*N*-

dimethylformamide (50 mL) for an hour. To this suspension was added alkyl halide (22.5 mmol) and the mixture was stirred for 48 hours at room temperature. The mixture was filtered and the solvent removed under vacuum. The residue was crystallized from ethanol to give 1,3-dialkylbenzimidazolium salts.

1,3-Diethylbenzimidazolium bromide **4a** was obtained as a yellow crystals. Mp = 160°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.70 (t, *J* = 7.2 Hz, 6H, -CH_3), 4.64-4.71 (q, *J* = 7.2 Hz, 4H, -CH_2), 6.1 (m, 2H, =CH), 7.28-7.78 (m, 4H, H_{Ar}), 11.19 (s, 1H, -N_2CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.9 (-CH_3), 42.9 (NCH_2), 113.1-127.2-131.2 (C_{Ar}), 142.0 (N_2C-); MS (API+): m/z = 175.0974.

1,3-Diallylbenzimidazolium bromide **4b** was obtained as a colourless crystals. Mp = 159°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.3-5.51 (m, 8H, =CH_2 and -CH_2), 6.06-6.12 (m, 2H, =CH), 7.58-7.76 (m, 4H, H_{Ar}), 11.23 (s, 1H, -N_2CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 50.2 (NCH_2), 113.8 (=CH), 122.0 (=CH_2); 127.3-129.6-131.4 (C_{Ar}), 142.8 (N_2C-); MS (API+): m/z = 199.0892.

1,3-Diallyl-2-methylbenzimidazolium bromide **4c** was obtained as a brown crystals. Mp = 236°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.19 (s, 3H, -CH_3); 5.17 (d, *J* = 17.1 Hz, 4H, =CH_2), 5.36 (m, 4H, CH_2), (m, 2H, =CH), 7.58-7.76 (m, 4H, H_{Ar}), 11.23 (s, 1H, -N_2CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.6 (CH_3), 48.6 (NCH_2), 112.9 (=CH), 119.7 (=CH_2), 126,9-129.4 (CH_{Ar}), 131.1 (C_q), 151.6 (N_2C-); MS (API+): m/z = 213.1054.

1,3-Dioctyl-2-methylbenzimidazolium bromide **4d** was obtained as a colourless crystals. Mp = 188°C; ^{1}H NMR (300MHz, $CDCl_3$): δ 0.84 (t, *J* = 6.9 Hz, 6H, -CH_3), 1.28-1.35 (m, 20H, -CH_2), 1.78 (m, 4H, CH_2), 4.46 (t, *J* = 7,5Hz, 4H, N-CH_2), 7.61-8.04 (m, 4H, H_{Ar}), 11.23 (s, 1H, -N_2CH); ^{13}C NMR (75MHz, $CDCl_3$): δ 10.9-14.8-40.9 (-CH_3), 22.5, 26.3, 29.0, 29.1, 31.6 (CH_2), 45.5 (NCH_2), 113.4-126,4 (CH_{Ar}), 131.3 (C_q); 151.8 (N_2C-); MS (API+): m/z = 343.2649.

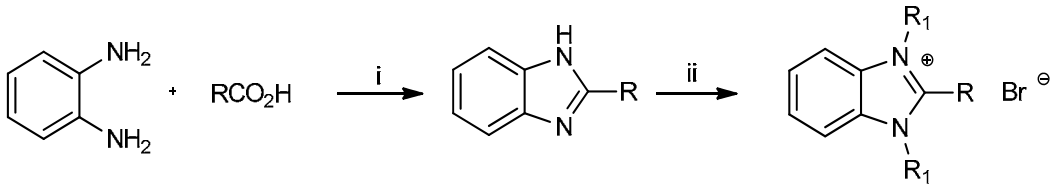
1,3-Diallyl-2-hydroxymethylbenzimidazolium bromide **4e** was obtained as a colourless crystal. Mp = 93°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.32 (d, *J* = 7.6 Hz, 2H, =CH_2), 5.43-5.52 (m, 4H, -CH_2), 6.08-6.17 (m, 2H, =CH), 7.59-7.76 (m, 4H, H_{Ar}), 11.28 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 50.2 (CH_2-OH), 113.8 (=CH), 122.0 (=CH_2), 127.2-129.5 (C_{Ar}); 131.4 (C_q), 142.8 (N_2C-); MS (API+): m/z = 229.1358.

RESULTS AND DISCUSSION

Chemistry

The synthetic pathways for preparation of the targeted compounds listed in Table 1 are shown in Figure 2. The method we have adopted for the synthesis of various benzimidazole derivatives **3a-c** is that described by Phillips [19]. This method involves condensation of *o*-phenylenediamine **1** with carboxylic acids (formic acid **2a**, acetic acid **2b** and glycolic acid **2c**) under reflux of hydrochloric acid (5N) for 5 hours. Products **3a-c** are obtained with good yields. Structure of compounds **3a-c** was confirmed by comparison of its physical and spectral data with the reported ones [20, 21].

This was followed by alkylation for 48 hours at room temperature using different alkyl halides (3 equiv) and tetra-*n*-butylammonium bromide (TBAB) as phase transfer catalyst. The mineral salts were removed by filtration and the solvent was removed under reduced pressure. The residue was recrystallized from ethanol to afford 1,3-dialkylbenzimidazolium salts with a good yield.

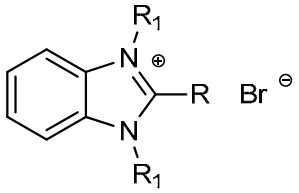


1 **2a**- R = H; **2b**- R = CH_3; **2c**- R = CH_2OH **3a**, **3b**, **3c**

4a- R= H; R_1 = $CH_2CH{=}CH_2$
4b- R= H; R_1 = C_2H_5
4c- R= CH_3; R_1 = $CH_2CH{=}CH_2$
4d- R= CH_3; R_1 = C_8H_{17}
4e- R= CH_2OH; R_1 = $CH_2CH{=}CH_2$

Figure 2*. i) HCl (5N), Δ, 5 hours, ii) K_2CO_3, DMF, TBAB, R_1X, RT, 48 hours*

Table 1. *Structures, melting point and exact mass of compounds* ***4a-e***



Compounds	R	R_1	Mp (°C)	Yield (%)	m/z (API+)
4a	H	C_3H_5	159	51	175.0974
4b	H	C_2H_5	160	40	199.0892
4c	CH_3	C_3H_5	236	54	213.1054
4d	H	C_8H_{17}	100	65	343.2649
4e	CH_2-OH	C_3H_5	160	60	229.1358

These products were characterized using ^{1}H- and ^{13}C NMR, mass spectrometry and X-ray diffraction for compounds **4b** and **4d**.

Compounds **4a-e** were prepared in one step, contrary to literature, where benzimidazolium salts were synthesized in two steps [22 - 24].

The ^{1}HNMR spectra of the compound **4b** showed a triplet signal centred at δ 1.70 ppm which has been assigned to six methyl protons of ethyl group, and a quartet signal ranging from δ 4.64 to 4.71 ppm due to methylene protons. The ^{13}CNMR spectra showed in particular signals due to methyl and methylene groups at δ 14.91 and 42.95 ppm, respectively.

The ORTEP representation of **4b** and **4c** are shown in Figure 3 and 4 and the crystal data and structure refinement for **4b** and **4c** are presented in Table 2.

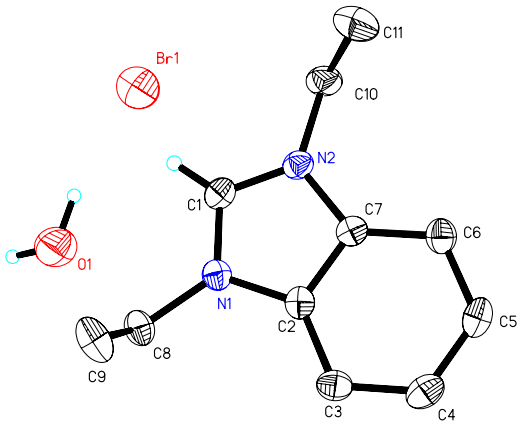


Figure 3. *ORTEP presentation of compound* ***4b****, showing 30% probability displacement ellipsoids*

Table 2. *Crystal data and structure refinement for* ***4b*** *and* ***4c***

Empirical formula	**$C_{11}H_{17}BrN_2O$**	**$C_{23}H_{39}N_2Br$**
Formula weight	273.18	423.46
Crystal system	Monoclinic	Triclinic
Space group	P2(1)/c	P1
Unit cell dimensions	a = 8.7917(2) Å	a = 9.1081(2) α = 76.631(1)
	b = 8.7553(2) Å β= 102.745(2)°	b = 9.1224(2) β = 78.224(1)
	c = 16.2593(4) Å	c = 16.0670(3) γ = 77.786(1)
Volume	1220.71(5) Å3	1252.38(5)
Z	4	2
Density (calculated)	1.486 Mg/m^3	1.123 Mg/m^3
F(000)	560	452
Theta range for data collection	5.14 to 29.57°	2.3 to 27.2°
Index ranges	-12<=h<=11, -12<=k<=11, -22<=l<=21	-11<=h<=11, -11<=k<=11, -20<=l<=20
Reflections collected	12689	35854
Independent reflections	3397 [R(int) = 0.0301]	10854 [R(int) = 0.032
Final R indices [I>2sigma(I)]	R_1 = 0.0311, wR_2 = 0.0737	R_1 = 0.1140, wR_2 = 0.3285
Largest diff. peak and hole	0.468 and -0.362 e.Å^{-3}	5.28 and -0.42 e.Å^{-3}

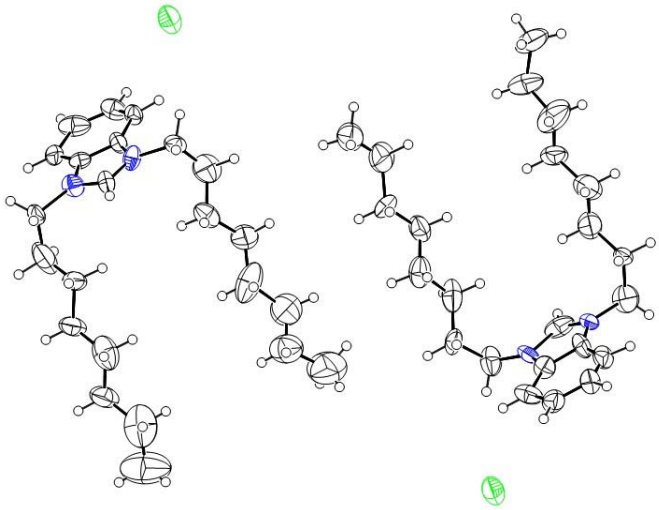


Figure 4. *ORTEP presentation of compound* ***4c****, showing 30% probability displacement ellipsoids*

Antibacterial activity

Each of the benzimidazolium salts derivatives **4a-e** was in vitro tested for antibacterial activity against various strains to evaluate the potential range of prospective medicinal applications. The anti-bacterial activity was carried out using both Gram positive (*Streptococcus sanguis* ATCC 10556; *Streptococcus viridans* ATCC 19950; *Bacillus Substilis* ATCC 6633; *Staphilococcus aures* ATCC 25923; *Listeria Monocitogènes* ATCC 7644) and Gram negative (*Salmonelle enteritidis* ATCC 13076; *Klebsiella oxytoca* ATCC 13182; *Klebsiella pneumoniae* ATCC 13883; *Escherichia Coli* ATCC 25922; *Proteus mirabilis* ATCC 49565) bacteria.

The preliminary antibacterial assay was performed as follows: A Mueller-Hinton Agar medium (MHA, 19 mL) was inoculated with 1mL of strain suspension (previously grown on nutritive solution for 24 hours at 37 °C until 106 CFU/mL). The test product (10 mg) was dissolved in 1 mL of DMSO/water mixture and then diluted to obtain solutions with a range of DMSO concentrations (50 %, 25 %, 10 %). The inoculated MHA was then transferred onto a Petri dish and 50 µL of a diluted solution was introduced in holes (diameter: 6 mm). The antimicrobial activity was observed after 18 hours incubating at 37 °C. As an inhibition of development of the strain within a diameter up than 6 mm around the considered hole area.

The antibacterial screening results (the zone of inhibition), are presented in Table 3.

Biological assay results shown in Table 3, indicate that, with exception of **4a** and **4e**, all newly synthesized compounds have been found active against most bacterial species tested.

Table 3**. Antibacterial activity of 2-substitued benzimidazolium salts **4b-d

Compounds / **Strains**	**4b**				**4c**				**4d**			
	100%	50%	25%	10%	100%	50%	25%	10%	100%	50%	25%	10%
Salmonelle enteritidis	-	-	-	-	-	-	-	-	+ +	+	+	+
Klebsiella oxytoca	+	+	+	-	+	+	+	-	+ + +	+ + +	+ +	+ +
Klebsiella pneumonia	+	+	+	+	+	+	+	+	+ +	+	+	+
Streptococcus sanguis	-	-	-	-	+	-	-	-	+ + + +	+ + +	+ + +	+ +
Streptococcus viridans	-	-	-	-	+ +	+	+	-	+ + +	+ + +	+ +	+ +
Bacillus substilis	+ +	+ +	+ +	+ +	+ +	+	+	+	+ +	+ + + +	+ + + +	+ + +
Escherichia coli	+	+	+	+	+	+	-	+	+ +	+ +	+ +	+
Staphilococcus aures	+	+	-	-	+	+	-	-	+	+	+	+
Listeria monocitogènes	+	-	-	-	+	-	-	-	+ + +	+ +	+ +	+ +
Proteus mirabilis	-	-	-	-	-	+	-	-	+ + +	+ +	+ +	+ +

(-) = 6 mm; (+) = 6-15 mm; (+ +) = 15-25 mm; (+ + +) = 25-35 mm; (+ + + +) = 35-45 mm.

The compound **4d** exhibited more pronounced antibacterial activities than other compounds **4a-c** and **4e**, with better activity against both Gram positive and Gram negative bacteria, until the concentration 10 %. This large difference in activity between compound **4d** and other compounds can be explained by the substitution of allyl group by a long hydrocarbon chain. The compound **4d** is the only one that showed activity against *Salmonella enteritidis*, and it was found more active against Gram positive bacteria in comparison to gram negative.

CONCLUSIONS

Novel benzimidazolium derivatives containing three alkyl groups were synthesized by the treatment of *o*-phenylenediamiane with carboxylic acid followed by an alkylation and quaternization in phase transfer catalysis (PTC) conditions. This method produces the benzimiodazolium derivatives in one step and in good yield. The structures were verified by spectroscopic data. The antibacterial activity, show that the benzimidazolium alkylated at the position 1 and 3 with a long chain alkyl is the most active compound against Gram positive and Gram negative bacteria.

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