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Original article

Synthesis, spectroscopic and biological properties of bis(3-arylimidazolidinyl-1)methanes. A novel family of antimicrobial agents

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Abstract

Synthesis, spectroscopic and biological properties of new bis(3-arylimidazolidinyl-1)methanes are described. These compounds were synthesized by condensation reaction between *N*-arylethylenediamines and formaldehyde. Chemical structures were confirmed by means of their ^{1}H- and ^{13}C-NMR and mass spectroscopic data. Investigation of in vitro antimicrobial activity was performed using Gram-negative and Gram-positive bacteria as well as antifungal studies against *Aspergillus niger* and *Candida albicans*. Minimal inhibitory concentrations of active compounds were determined.

Keywords: Imidazolidines; Bis(3-arylimidazolidinyl-1)methanes; Aminals; Antimicrobial activity

1. Introduction

Tetrahydroimidazoles (imidazolidines) result interesting compounds due to their bioactivity, such as strogenic activity [1] and mammary tumor inhibition [2], anti inflammatory and analgesic activity [3]. Fungicide, bactericide and antiviral activities had also been reported [3,4]. On the other hand, they had been employed as carriers of pharmacologically active ethylenediamines [5,6] or carbonyl compounds [7]. Stable 1,3-disubstituted imidazolidines are obtained from *N*,*N*′-disubstituted ethylenediamines and aldehydes, whatever substituents are present in reactants [8]. Instead, in the reaction of *N*-monosubstituted ethylenediamines with two or more carbon aldehydes, the obtained imidazolidine is part of a tautomeric equilibria with the corresponding aminoimine (being one or both species detected by ^{1}H-NMR) [8a,9]. To our knowledge, there are no precedent studies of reaction between *N*-monosubstituted ethylenediamines and formaldehyde.

In this work we present the synthesis of a series of unpublished bis(3-arylimidazolidinyl-1)methanes **1a–j** (Table 1) obtained by reaction of *N*-arylethylenediamines **2** with an excess of formaldehyde, their spectroscopic characterization and microbiological assays which prove their bioactivity. Compounds **1** are representative of a group of aminals characterized by the presence of two saturated 1,3-azole ring connected by a methylene bridge, some of them having antimicrobial activity [10].

2. Chemistry

Bis(3-arylimidazolidinyl-1)methanes **1a–j** were synthesized by condensation of *N*-arylethylenediamines **2** with an excess aqueous formaldehyde (37%) in ethanol under reflux. Precursors *N*-arylethylenediamines **2** were prepared by aminolysis of 2-bromoethylamine (hydrobromide) with the corresponding arylamine (Fig. 1) [11].

Attainment of bis(3-arylimidazolidinyl-1)methanes **1** may be considered as a result of the condensation between

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Table 1
Melting points, yields (%) and molecular formula of bis (3-arylimidazolidinyl-1)methanes **1a–j**

Compound	Ar	M.p. (°C)	Yield (%)	Molecular formula
1a	C_6H_5	141–143	78	$C_{19}H_{24}N_4$
1b	4-$CH_3C_6H_4$	138–140	75	$C_{21}H_{28}N_4$
1c	4-$CH_3OC_6H_4$	144–146	84	$C_{21}H_{28}N_4O_2$
1d	4-ClC_6H_4	190–192	79	$C_{19}H_{22}Cl_2N_4$
1e	3,4-Cl_2 C_6H_3	153–155	81	$C_{19}H_{20}Cl_4N_4$
1f	4-$NO_2C_6H_4$	191–193	85	$C_{19}H_{22}N_6O_4$
1g	4-BrC_6H_4	178–180	79	$C_{19}H_{22}Br_2N_4$
1h	3-ClC_6H_4	131–133	86	$C_{19}H_{22}Cl_2N_4$
1i	3-BrC_6H_4	138–140	89	$C_{19}H_{22}Br_2N_4$
1j	β-$C_{10}H_7$	192–194	87	$C_{27}H_{28}N_4$

N-arylethylenediamines and formaldehyde which leads to aminoimines **3**. When aryl substituents allow subsequent cyclization, NH imidazolidines **3** should be obtained, which by intermolecular condensation with formaldehyde should lead to bisimidazolidines **1** as final products. When at least one of the *ortho* positions of the arylamine is substituted (2-NO_2, 2-Cl, 2-CH_3), cyclization reaction does not occur, and final products are the corresponding aminoimines **3** (Fig. 1).

The basic structures of compounds **1** were confirmed by ^{1}H-NMR and ^{13}C-NMR spectral data (Table 2). Assignment of signals was performed by comparison with spectra of properly 1,3-disubstituted imidazolidines [12–14].

Excepting compounds **1f** and **1i**, mass spectra of all compounds display the molecular ion peak, having generally low intensity. The main fragments as well as their relative intensities are given in Table 3. The probable fragmentation pathways are depicted in Fig. 2.

3. Pharmacology

Screening of in vitro antimicrobial activity of the synthesized bis(3-arylimidazolidinyl-1)methanes **1** was performed using the disk diffusion method employing representative Gram-positive and Gram-negative bacteria, and filamentous, fungi and yeasts. Those compounds which presented any inhibition zone were evaluated by their minimal inhibitory concentration (MIC) using the dilution technique. Results are shown in Table 4.

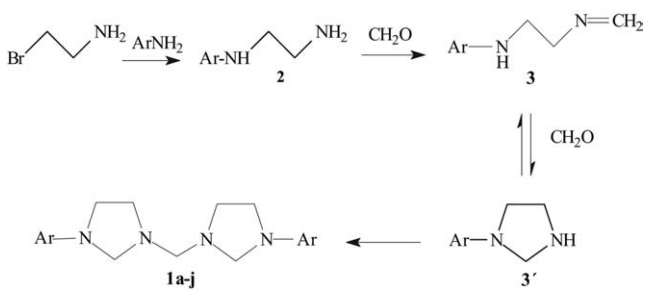
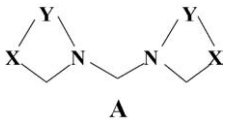


Fig. 1. Synthetic pathways to compound **1**.

4. Results and discussion

Ten compounds (**1**) were screened for their antimicrobial activity. Respect to antifungal activity, results shown that all compounds were inactive frente a *Candida albicans*. However, all derivatives resulted effective against *Aspergillus niger*, being compounds **1a** (MIC 16 ug/ml), **1e** (MIC 4 ug/ml) and **1b** (MIC 1 ug/ml) the most active. A moderate activity (MIC 16–32 ug/ml) was observed for compounds **1c**, **d**, **f–i**.

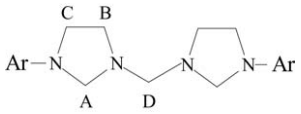
All synthesized compounds showed antibacterial activity against *Escherichia coli*, *Micrococcus luteus*, *Bacillus subtilis*, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Compounds **1e** and **1f** proved to be the most active for *E. coli* and *M. luteus*, respectively. Such activity may be associated to the bactericidal action of related compounds, due to a potential capability to liberate formaldehyde. Compounds of general formula **A** such as methylene-bis heterocycles (**A**, Y = alkylene, arylene, etc.; X = O, S) were described with bactericidal and fungicidal activity [10]. In particular, bis(5-methy1,3-oxazolidin-3-yl)methane (**A**, Y = CH_2–$CH(CH_3)$, X = O) was proposed as fungicide for lubricants and starch pastes [10b].



A

An analogous compound, taurolidine (bis-(1,1-dioxoperhydro-1,2,4-thiazinyl-4)-methane) (**A**, X = NH, Y = $SO_2CH_2CH_2$) showed an important activity against *P. aeruginosa*, *E. coli*, *Proteus vulgaris* and *Salmonella typhimurium* [15]. Such activity is based in the capability of this compound to act as carrier of non-toxic formaldehyde, donating methylol groups to bacterial proteins and endotoxins [16] causing their denaturalization and poly-condensation. Recently, interest for this compound in prevention and treatment of infections related to intravascular catheterization has grown up [17].

Table 2
^{1}H- and ^{13}C-NMR data of compounds **1a–j**



	^{1}H-NMR (δ ppm) (J = Hz)						^{13}C-NMR (δ ppm)					
Compound	A	B	C	D	Aromatics	others	A	B	C	D	Aromatics	others
1a	4.16, s	3.13, t J = 6.31	3.43, t J = 6.31	3.46, s	6.50 (d, 4H) J = 7.68 6.71 (t, 2H) J = 7.12 7.24 (dd, 4H) J_1 = 7.68, J_2 = 7.12		69.0	50.9	45.6	74.4	111.7, 113.8, 129.2, 146.5	
1b	4.14, s	3.12, t J = 6.37	3.39, t J = 6.37	3.46, s	6.47 (d, 4H) J = 8.43 7.04 (d, 4H) J = 8.43	2.25, s CH_3	69.3	50.9	45.9	74.6	111.8, 125.5, 129.7, 144.6	20.2 CH_3
1c	4.12, s	3.12, t J = 6.36	3.37, t J = 6.36	3.47, s	6.50 (dd, 4H) J_1 = 6.80, J_2 = 2.30 6.83 (dd, 4H) J_1 = 6.80, J_2 = 2.30	3.75, s OCH_3	69.7	50.9	46.4	74.7	112.7, 114.9, 141.5, 151.3	55.8 OCH_3
1d	4.11, s	3.12, t J = 6.27	3.37, t J = 6.27	3.44, s	6.43 (d, 4H) J = 8.90 7.17 (d, 4H) J = 8.90		69.0	50.8	45.8	74.1	112.6, 121.2, 129.0, 145.2	
1e	4.10, s	3.12, t J = 6.18	3.36, t J = 6.18	3.43, s	6.33 (dd, 2H) J_1 = 8.72, J_2 = 2.80 6.56 (s, 2H) 7.23 (d, 2H) J = 8.72		68.8	50.7	45.7	73.1	111.2, 112.9, 119.0, 130.5, 132.8, 145.6	
1f	4.25, s	3.19, t J = 6.41	3.52, t J = 6.41	3.52, s	6.44 (d, 4H) J = 9.32 8.13 (d, 4H) J = 9.32		68.5	50.5	45.6	73.3	110.3, 126.3, 137.1, 150.3	
1g	4.10, s	3.11, t J = 6.40	3.36, t J = 6.40	3.45, s	6.49 (d, 4H) J = 8.71 7.26 (d, 4H) J = 8.71		68.9	50.8	45.7	74.1	111.5, 113.2, 131.8, 145.3	
1h	4.13, s	3.12, t J = 6.37	3.39, t J = 6.37	3.44, s	6.36 (dd, 2H) J_1 = 8.30, J_2 = 1.97 6.48 (s, 2H) 6.67 (dd, 2H) J_1 = 7.18, J_2 = 1.97 7.12 (t, 2H) J = 8.10		68.8	50.7	45.6	74.0	109.8, 111.5, 116.2, 130.1, 135.0, 147.3	
1i	4.12, s	3.11, t J = 6.42	3.38, t J = 6.42	3.43, s	6.42 (dd,2H) J_1 = 7.95, J_2 = 2.25 6.64 (t, 2H) J = 2.25 6.81 (dd, 2H) J_1 = 7.69, J_2 = 2.25 7.06 (t, 2H) J = 7.95		68.8	50.7	45.5	74.0	110.2, 114.3, 119.1, 123.3, 130.4, 147.4	
Ij	4.30, s	3.20, t J = 6.45	3.54, t J = 6.45	3.58, s	6.74 (d, 2H) J = 2.20 6.90 (dd, 2H) J_1 = 9.06, J_2 = 2.20 7.20 (t, 2H) J = 6.90 7.37 (td, 2H) J_1 = 6.90, J_2 = 1.30 7.60-7.75 (m, 6H)		69.2	50.9	45.9	74.2	105.5, 115.3, 121.7, 125.2, 125.8, 126.3, 127.6, 129.0, 135.0, 144.3	

Table 3
Select fragments in the EI mass spectra of compounds **1a–j**

Compound	M+•	A	B [a]	C [b]	D	E	F	Others
1a	308 (24.4)	161 (100)	162 (55.5)	148 (34.4)	147 (41.1)	106 (33.3)	56 (12.2)	107 (28.5)
1b	336 (55.5)	175 (100)	176 (22.2)	162 (27.8)	161 (28.6)	120 (13.5)	56 (8.4)	121 (10.6)
1c	368 (7.9)	191 (100)	192 (10.5)	178 (25.5)	177 (27.4)	136 (18.8)	56 (12.1)	137 (21.1)
1d	376 (0.6)	195 (100)	196 (31.4	182 (–)	181 (40.8)	140 (25.1)	56 (11.3)	
	378 (0.4)	197 (32.2)	)198 (9.1)	184 (–)	183 (12.5)	142 (7.8)		
1e	444 (0.02)	229 (58.1)	230 (100)	216(31.3)	215 (17.1)	174 (53.2)	56 (25.8)	43 (19.0)
	446 (0.04)	231 (43.9)	232 (56.4)	218 (19.2)	217 (14.6)	176 (34.0)		
	448 (0.01)							
1f	398 (–)	206 (3.4)	207 (4.9)	193 (24.2)	192 (17.4)	151 (100)	56 (–)	43 (74.7)
								105 (42.7)
								194 (41.6)
1g	464 (0.03)	239 (100)	240 (31.5	226 (12.8	225 (27.2)	184 (37.4)	56 (37.8)	160 (16.5)
	466 (0.13)	241 (94.1)	242 (–) [c]	228 (10.0)	227 (21.1)	186 (37.0)		
	468 (0.02)							
1h	376 (1.7)	195 (64.7)	196 (100)	182 (–)	181 (46.0)	140 (4.4)	56 (7.8)	
	378 (0.7)	197 (29.3)	198 (30.8)	184 (–)	183 (14.2)	142 (1.6)		
1i	464 (–)	239 (100)	240 (–) [d]	226 (25.8)	225 (42.3)	184 (51.2)	56 (11.9)	
	466 (–)	241 (97.2)	242 (12.0)	228 (16.6)	227 (26.4)	186 (53.0)		
	468 (–)							
1j	408 (0,9)	211 (100)	212 (30.2)	198 (36.9)	197 (52.9)	156 (93.3)	56 (11.0)	127 (15.7)

[a] This ion has the same mass that the isotopic peak of A.
[b] This ion has the same mass that the isotopic peak of D.
[c] The absence of *m*/*z* 242 indicates that *m*/*z* peak 240 do not correspond to B ion.
[d] The absence of *m*/*z* 240 indicates that *m*/*z* peak 242 do not correspond to B ion.

5. Experimental protocols

5.1. Chemistry

Melting points were determined with a Büchi capillary apparatus and are uncorrected. NMR spectra were recorded on a Bruker MSL 300 MHz spectrometer using deuteriochloroform as the solvent. Chemical shifts are reported in ppm relative to TMS as an internal standard. Splitting multiplicities are reported as singlet (s), doublet (d), double doublet (dd) and triplet (t). Mass spectra (EI) were recorded with a GC–MS Shimadzu QP-1000 spectrometer operating at 20 eV. Microanalytical data (C, H, N) agreed with the proposed structures within ± 0.4% of the theoretical values. TLC analyses were carried out on aluminum sheets silica gel 60 F_{254}.

*5.1.1. Bis(3-arylimidazolidinyl-1)methanes **1a–j**. General procedure*

Aqueous formaldehyde (3 mmol) was added dropwise to a stirred solution of the appropriate *N*-arylethylenediamine

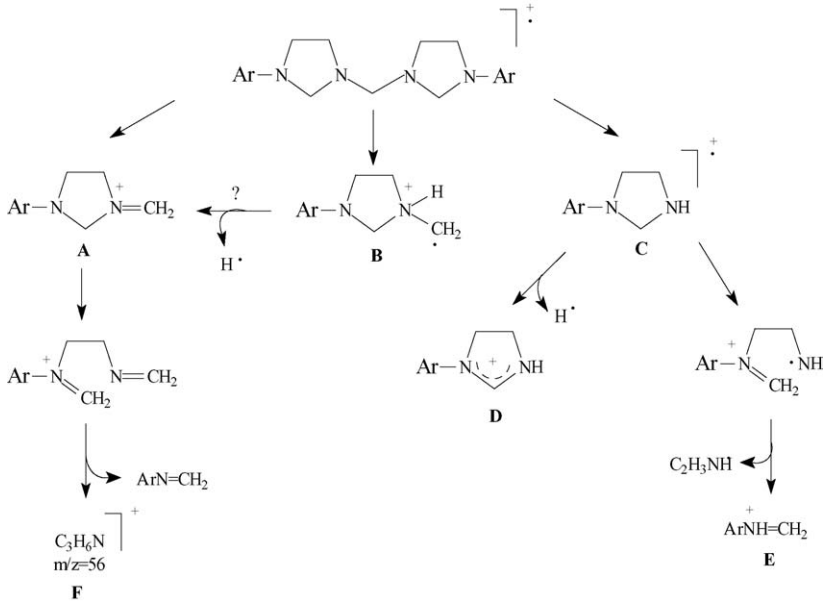


Fig. 2. Probable fragmentation pathways of compound **1**.

Table 4
MIC (ug/ml) values of compounds **1a–j**

Microorganism	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**	**1i**	**1j**
E. coli ATCC 11105 CCM-A-424	32	32	32	32	8	32	128	128	128	>256
M. luteus ATCC 9341 CCM-A-45	16	16	8	8	8	4	32	32	32	>64
B. subtilis ATCC 6633 CCM-A-10	32	32	32	16	16	32	128	128	128	>256
L. monocytogenes ATCC 9027 CCM-A-39	32	32	32	32	16	16	128	128	128	>256
P. aeruginosa ATCC 9027 CCM-A-39	32	32	32	32	32	32	128	128	32	>128
S. aureus ATCC 6538P CCM-A-424	32	32	32	32	16	32	128	128	128	>256
A. niger ATCC 16404	16	1	64	32	4	32	64	64	64	>256

[11] (1 mmol) in ethanol. Reaction was monitored by TLC using benzene/methanol (9:1) as elution solvent until disappearance of the starting diamine (ca. 20 min). Compounds **1** crystallize by cooling the mixture. The solid was filtered and recrystallized from ethanol. Melting points, yields and molecular formula of bis(3-arylimidazolidinyl-1)methanes **1a–j** are given in Table 1.

*5.1.2. 2-Arylamino-1-methyleneaminoethanes (**3**)*

These compounds were obtained from formaldehyde and *N*-(*ortho*-substituted phenyl)ethylenediamines employing the procedure described above.

*5.1.2.1. 2-(2-Nitrophenylamino)-1-methyleneaminoethane (**3**, Ar = 2-nitrophenyl).* M.p. 118–120 °C. ^{1}H-NMR ($CDCl_3$) δ ppm: 2.90 (t, 2H), 3.30 (t, 2H), 3.65 (s, 2H), 6.60 (t, 1H), 6.80 (d, 1H), 7.45 (t, 1H), 8.10 (d, 1H), 8.40 (bs, 1H). MS: *m/z* 193 ($M^+\bullet$).

*5.1.2.2. 2-(2-Chlorophenyl)-1-methyleneaminoethane (**3**, Ar = 2-chlorophenyl).* This compound was isolated as an oil.

^{1}H-NMR ($CDCl_3$) δ ppm: 2.90 (t, 2H), 3.15 (t, 2H), 3.50 (s, 2H), 5.00 (bs, 1H), 6.60 (m, 2H), 7.10 (t, 1H), 7.40 (d, 1H). MS: *m/z* 182 ($M^+\bullet$).

*5.1.2.3. 2-(2-Methylphenyl)-1-methyleneaminoethane (**3**, Ar = 2-methylphenyl).* This compound was isolated as and oil.

^{1}H-NMR ($CDCl_3$) δ ppm: 2.10 (t, 2H), 2.15 (s, 3H), 2.90 (t, 2H), 3.20 (s, 2H), 4.30 (bs, 1H), 6.60 (d, 1H), 6.75 (t, 1H), 7.20-7.35 (m, 2H). MS: *m/z* 162 ($M^+\bullet$).

5.2. Antimicrobial activity

Antimicrobial activity was tested by the disk diffusion method, with Antibiotic Medium number 1 (pH 6.5) and 11 (pH 7.9). The assayed microorganisms were *B. subtilis* ATCC 6633 CCM-A-10, *P. aeruginosa* ATCC 9027 CCM-A-39, *M. luteus* ATCC 9341 CCM-A-45, *S. aureus* ATCC 6538P CCM-A-424, *L. monocytogenes* ATCC 9027 CCM-A-39, *C. albicans* ATCC 10231, *A. niger* ATCC 16404 and *E. coli* ATCC 11105 CCM-A-424. Determination of the MIC was performed in solid media according to the methods proposed by NCCLS [18]. Employed concentration varied between 64 and 0.25 μg/ml and cultures were incubated at 24 h at 37 °C.

Acknowledgements

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