Heterocyclic Betaines. XXII. Azinium(Azolium) 4-Nitrobenzimidazolate Inner Salts and Their Derivatives with Several Interannular Spacers. Synthesis, Characterization and Antitrichomonal Activity

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The synthesis of an ensemble of pyridinium(imidazolium) 4-nitrobenzimidazolate betaines and their derivatives with several interannular linkages has been explored. Their antiprotozoal activity has also been examined.

Key words azinium benzimidazolate betaine; azolium benzimidazolate betaine; 4-nitrobenzimidazole derivative; antitrichomonal activity

One of the aspects we have been interested in developing in the field of heterocyclic betaines[1] is the potential biological applications[2] of these appealing molecules. We have already reported[3] the synthesis and antiprotozoal evaluation against *Trichomonas vaginalis in vivo* and *in vitro* of a variety of *N*-ylides such as triphenylpyridinium benzimidazolate **1** (Fig. 1), pyridinium benzimidazolate **3**, and imidazolium benzimidazolate **5** and their derivatives **2**, **4**, and **6**, respectively, all of them incorporating a nitro group at position 4 or 5 of the benzimidazole ring. As an extension to our previous work, we studied the effect of the introduction of different linkages between their dipolar charged heterocyclic components on the biological activity of compounds of this kind.

As regards C–C′ bond type, the two 1-*n*-butyl-4-(1*H*-benzimidazol-2-yl)pyridinium salts **12** and **13** were chosen, together with their corresponding 1-*n*-butyl-4-(benzimidazol-2-ylidene)-1,4-dihydropyridines **14** and **15** bearing a nitro group at position 4 or 5 of the benzimidazole ring (Chart 1). They were synthesized by a three-step procedure. The requisite 2-(4-pyridyl)-1*H*-benzimidazoles **9** (4-nitro) and **11** (5-nitro) were prepared by two alternative methods as shown in Chart 1. Thus, compound **9** was obtained by condensation of arylenediamine **8** and isonicotinic acid **7** using polyphosphoric acid as the cyclodehydrating agent,[4a,b] whereas to obtain compound **11**, 2-(4-pyridyl)-1*H*-benzimidazole **10** was nitrated using a similar protocol to that described by Ichikawa and Hisano for similar systems.[4c]

Reaction of compounds **9** and **11** with *n*-butyl iodide or bromide under neutral conditions gave the 1-*n*-butyl-4-benzimidazolylpyridinium salts **12** and **13**, which were then deprotonated with potassium carbonate (to pH *ca.* 8) to provide two new aza-analogues of sesquifulvalene with a betaine character (*vide infra*) **14**A and **15**A.

To complete the study of the *N*-ylides (direct linkage of C–N′ bond type *i.e.* **1**—**6**),[3] several attempts were made to obtain the corresponding thiazolium salt **17** (Chart 2 and Experimental), but we were unable to obtain this compound, probably owing to its intrinsic instability.

The (4-nitrobenzimidazol-2-yl)methylpyridinium **21** and (4-nitrobenzimidazol-2-yl)methylimidazolium salt **22** (Chart 3) were prepared by displacement of the chlorine atom of the 2-chloromethyl-4-nitrobenzimidazole **20**, previously obtained by chlorination of the corresponding 2-hydroxymethyl-4-nitrobenzimidazole **19**, by pyridine or *N*-butylimidazole. The short times required for these reactions indicate the highly electrophilic character of the chloromethyl benzimidazole derivatives, which exhibit similar behavior to benzyl chlorides.[1b,5] The betaines of pyridiniomethyl benzimidazolate **23** and imidazoliomethyl benzimidazolate **24** (Chart 3) were obtained by deprotonation of the quaternary salts using an anion exchange Amberlite IRA-410 resin (OH^- form)[5]; this was found to be the method of choice for this transformation owing to the similar solubility of compounds **21** and **22** and their inner salts **23** and **24** in water and organic solvents.[6]

The higher homologues with an ethylene spacer were then examined, and the 4-nitrobenzimidazolylpyridinium-

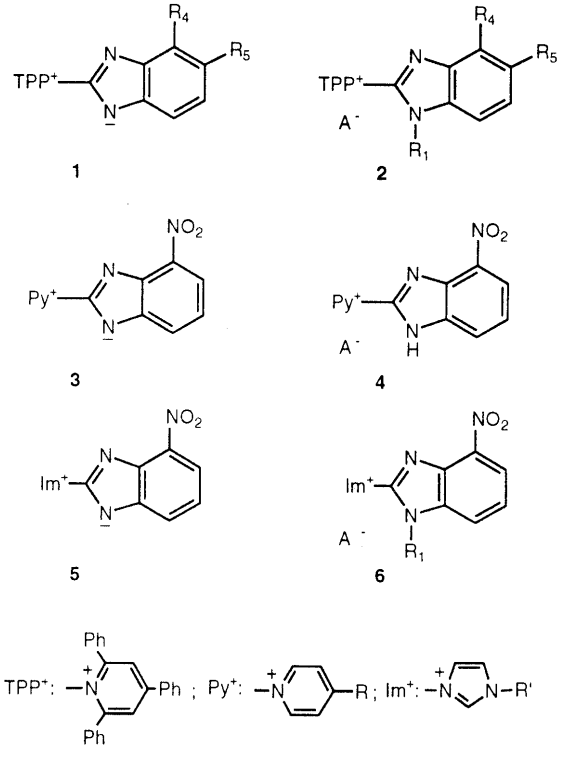

Fig. 1

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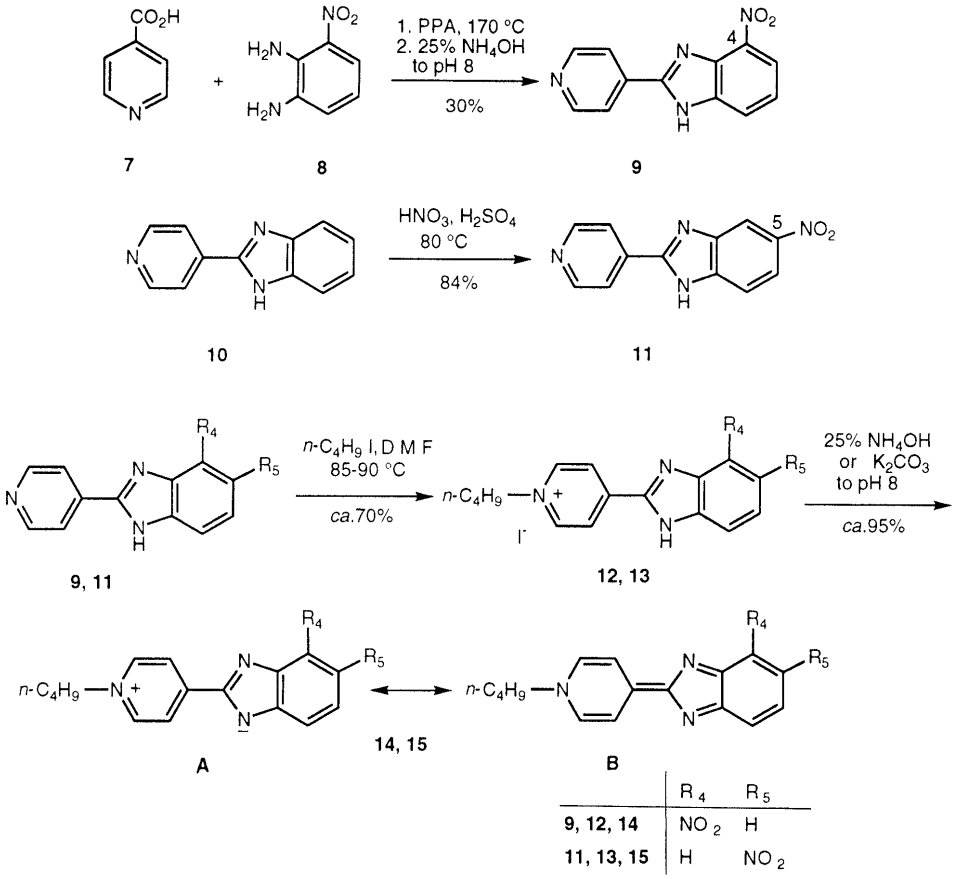


Chart 1

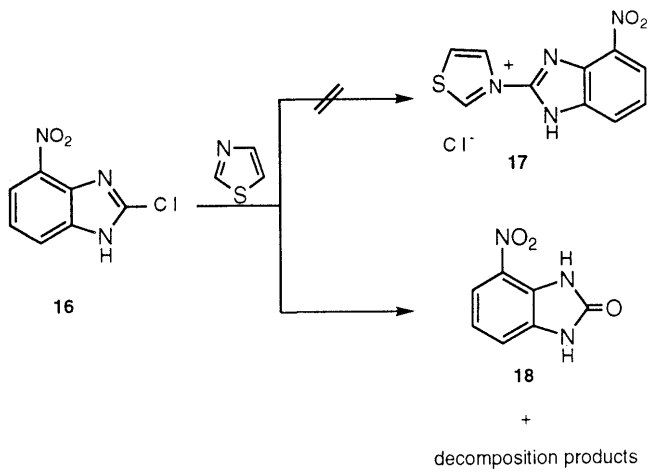


Chart 2

(azolium) salt **25**—**28** (Fig. 2) were chosen as models to study the biological behavior. The preparations of the (benzimidazolylethyl)pyridinium salts **25**[1b,7a] and **26**,[1b,7a] the (benzimidazolylethyl)imidazolium salt **27**[1b,7b] and its ethyleneimidazolium benzimidazolate inner salt **28**[1b,7b] have been reported.

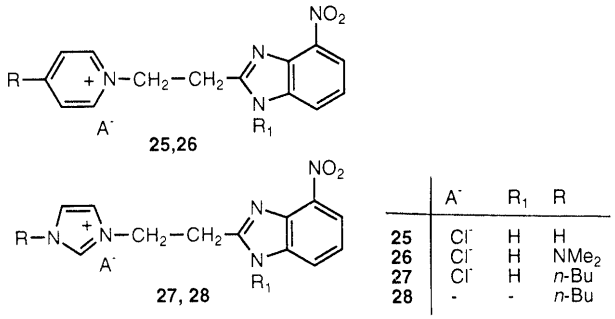
The 4-nitrobenzimidazol-2-ylpyridinium salt **32** containing a thioethylene spacer was then examined (Chart 4). The key intermediate **30** was obtained, along with the 8-nitro-2,3-dihydrothiazolo[3,2-*a*]benzimidazole **31**, only when sodium naphthalenide[8] was used as the base. The use of other bases in the alkylation step only afforded the product of *N*-alkylation **33** in all cases, and the inverse regioselectivity was observed when methyl chloroformate or phenacyl bromide was used as the protecting group, resulting in the formation of compounds **34** and **35** (Chart 4). The treatment of 2-chloroethylthiobenzimidazole **30** with pyridine or *n*-butylimidazole induced cyclization to give **31**, and although we were unable to obtain the salt **32**, the antiprotozoal activity of the intermediates **33**, **34** and **35** was tested, and this route was abandoned.[9a,d]

The physical data of the new compounds described here are listed in Table I and details of their preparation are given in Experimental. The structures of the new products have been unambiguously characterized on the basis of their spectroscopic data, and all of them gave satisfactory elemental analysis.

^{1}H-NMR and ^{13}C-NMR chemical shifts were assigned by comparison with the available data from related structures.[3,5,7,10] Selected ^{1}H-NMR and ^{13}C-NMR chemical shifts are set out in Tables II and III, respectively. Moreover, for compounds **14** and **15**, their physicochemical properties favor the betainic resonance forms **14A** and **15A**, in agreement with data reported for simple structures.[10]

The efficacy of the described compounds **12**—**15**, **21**, **22**, **25**—**28**, **33**—**35** as antitrichomonal agents was tested *in vitro* against *Trichomonas vaginalis*. The activities of the products are presented as the minimum lethal concentration used that killed all the parasites by 24 h (MLC, $\mu g\,ml^{-1}$), and the LD_{50} ($\mu g\,ml^{-1}$), the minimum concentration used that reduced the number of parasites by

at least 50%. Amongst the compounds tested, the LD_{50} of the 1-(4-nitro-2-benzimidazolyl)ethylpyridinium salt **25** showed a weak (MLC, 50; LD_{50}, 50) inhibitory activity as compared with the standard antitrichomonal drug metronidazole (MLC, 1; LD_{50}, 1), as did the intermediates **33**—**35** (MLC, 50; LD_{50}, 10—50). None of the other compounds manifested activity deserving of further study, and for this reason no evaluation of activity *in vivo* was carried out.



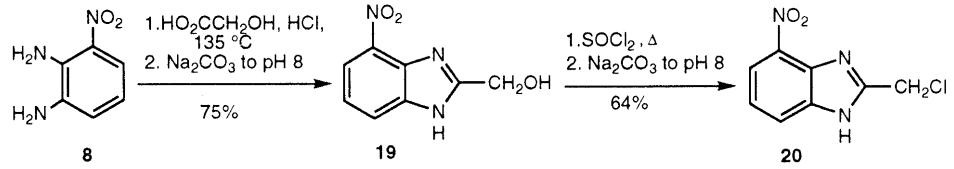
	A⁻	R_1	R
25	Cl^-	H	H
26	Cl^-	H	NMe_2
27	Cl^-	H	*n*-Bu
28	-	-	*n*-Bu

Fig. 2

Experimental

General Methods Melting point (uncorrected): CTP-MP 300 hot-plate apparatus (data in Table I). IR (KBr discs): Perkin-Elmer 1430 spectrophotometer. Absorption maxima are given in cm^{-1}; only significant peaks are quoted. ^{1}H-NMR: Bruker AM-100 or Perkin-Elmer R-24B spectrometer (100 and 60 MHz, respectively. ^{13}C-NMR: Bruker AM-100 Fourier-transform spectrometer (25.1 MHz). NMR spectra were determined in dimethylsulfoxide-d_6 (DMSO-d_6), and chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane (TMS) as an internal standard or to the central peak of dimethylsulfoxide-d_6. TLC: Merck silica gel 60 F_{254} plates; detection under UV light. Ion-exchange chromatography: Amberlite IRA-401 (OH^- form).[1,5] If necessary, the compounds were dried by incubation overnight at 25°C in a vacuum oven. Microanalyses were performed on a Carlo Erba model 1106 element analyzer by the Instituto de Química Bio-orgánica, Barcelona.

Materials 1-Bromo-2-chloroethene, *N*-butylimidazole, *n*-butyl iodide or bromide, hydroxyacetic acid, methyl chloroformate, phenacyl bromide, pyridine, thiazole and isonicotinic acid (**7**) are commercially available. 1,2-Diamino-3-nitrobenzene (**8**),[11] 2-(4-pyridyl)-1*H*-benzimidazole (**10**),[10] 2-chloro-4-nitrobenzimidazole (**16**),[12] 1-[2-(4-nitro-1*H*-benzimidazol-2-yl)ethyl]pyridinium chlorohydrate (**25**),[1b,7a] 4-(*N*,*N*-dimethylamino)-1-[2-(4-nitro-1*H*-benzimidazol-2-yl)ethyl]pyridinium



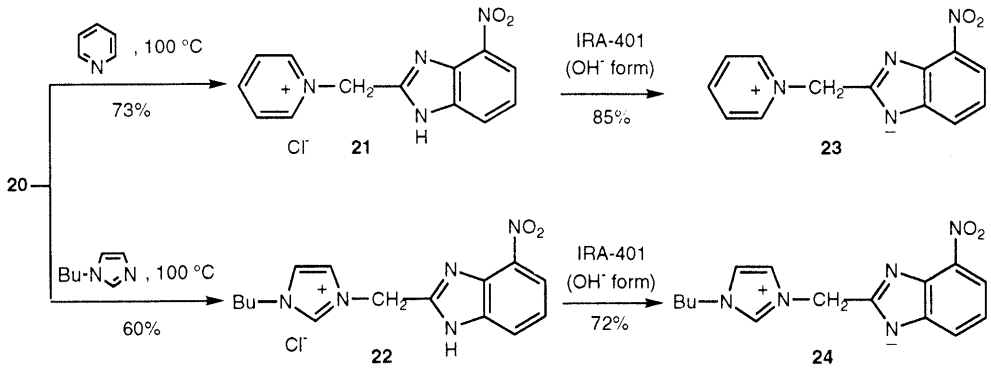


Chart 3

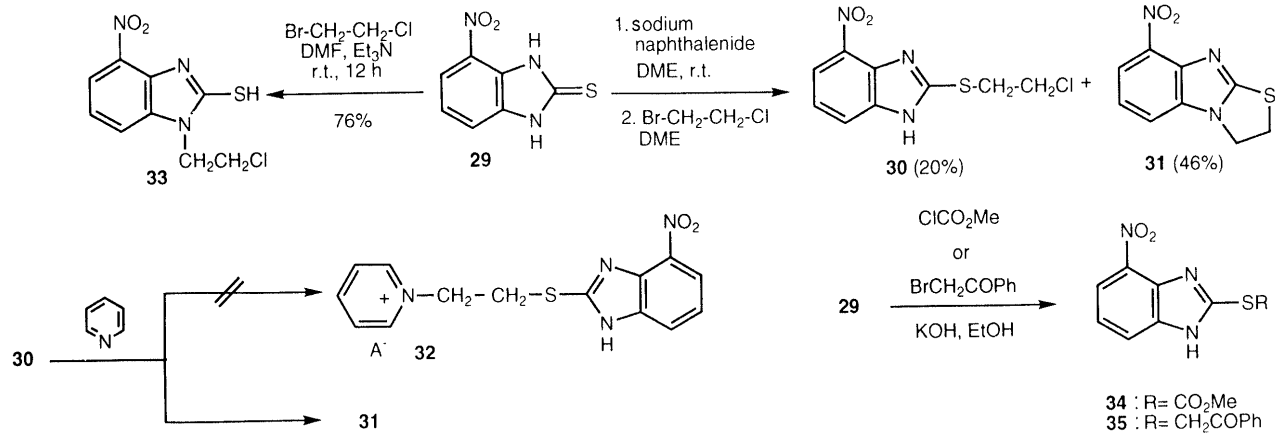
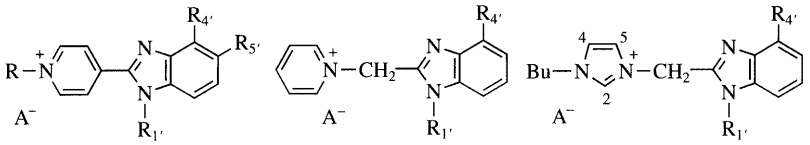


Chart 4

TABLE I. Preparation and Physical Data of 4-Benzimidazolylpyridinium Salts **12** and **13**, *N*-Benzimidazolylmethylpyridinium Salt **21**, *N*-Benzimidazolylmethylimidazolium **22**, and the Corresponding Betaines **14**, **15**, **23** and **24**



A **B** **C**

Compd. No.	Structure	R	$R_{1'}$	$R_{4'}$	$R_{5'}$	A^-	Method	Yield[a] (%)	Time (h)	mp (°C)[b] (Recryst. solv.)[c]
12	**A**	*n*-Bu	H	NO_2	H	I^-	A	67	3	207—208 (iso-PrOH)
14	**A**	*n*-Bu	—	NO_2	H	—	B	90	—	200—202[d]
13	**A**	*n*-Bu	H	H	NO_2	Br^-	A	72	8	218—219 (iso-PrOH)
15	**A**	*n*-Bu	—	H	NO_2	—	B	98	—	221[d]
21	**B**	—	H	NO_2	H	Cl^-	C	73	1	248 (MeCN/MeOH (9 : 1))
23	**B**	—	—	NO_2	H	—	D	85	—	264[d]
22	**C**	*n*-Bu	H	NO_2	H	Cl^-	C	60	1	174—175 (MeCN)
24	**C**	*n*-Bu	—	NO_2	H	—	D	72	—	182[d]

a) Yields were not optimized. *b*) Uncorrected, measured with a CTP-MP hot-plate melting point apparatus. *c*) Crystalline forms were **12**, orange needles; **13**, colorless prisms; **21**, brown needles; **22**, brown prisms. *d*) Recrystallization was not necessary. **14**, yellow needles; **15**, bright yellow prisms; **23**, yellow powder; **24**, orange powder.

TABLE II. ^{1}H-NMR Data[a,b] for 4-Benzimidazolylpyridinium Salts **12** and **13**, *N*-Benzimidazolylmethylpyridinium Salt **21**, *N*-Benzimidazolylmethylimidazolium **22**, and the Corresponding Betaines **14**, **15**, **23** and **24**

Compd. No.	H-2,6	H-4	H-3,5	CH_2	$R_{1'}$	H-4′	H-5′	H-6′	H-7′	R[c]
12	9.28	—	8.97	—	14.00[d]	—	8.29	8.56	8.29	4.65
14	8.90	—	8.70	—	—	—	7.85—7.96	7.07	7.85—7.96	4.49
$\Delta\delta$[e]	−0.38	—	−0.27	—	—	—	−0.38[f]	−1.49	−0.38[f]	−0.16
13	9.30	—	8.80	—	[g]	8.67	—	8.25	7.96	4.66
15	8.92	—	8.69	—	—	8.43	—	7.82	7.59	4.51
$\Delta\delta$[e]	−0.38	—	−0.11	—	—	−0.24	—	−0.43	−0.37	−0.15
21[h]	9.17	8.84	8.33	6.31	[g]	—	7.88	7.26	7.88	—
23[h]	9.15	8.77	8.23	5.93	—	—	7.47	6.74	7.49	—
$\Delta\delta$[e]	−0.02	−0.07	−0.10	−0.38	—	—	−0.41	−0.52	−0.39	—
	H-2	H-4	H-5							
22	9.54	7.93	7.93	5.94	13.74	—	8.14	7.44	8.14	4.27
24	9.38	7.77	7.85	5.57	—	—	7.71	6.89	7.71	4.18
$\Delta\delta$[e]	−0.16	−0.16	−0.08	−0.37	—	—	−0.43	−0.55	−0.43	−0.09

a) In DMSO-d_6. *b*) Recorded on a Bruker AM-100 spectrometer (100 MHz). ^{1}H-NMR chemical shifts are reported in ppm (δ) downfield from TMS in DMSO-d_6. *c*) Only the chemical shifts corresponding to $-CH_2-N^+-$ are quoted. *d*) Broad signal. *e*) $\Delta\delta$: Observed chemical shift difference between betaines **14**, **15**, **23**, **24** and their precursors **12**, **13**, **21** and **22**, respectively. *f*) With respect to the average signal of the betaine structure. *g*) Signal not observed. *h*) In D_2O.

TABLE III. ^{13}C-NMR Data[a,b] for 4-Benzimidazolylpyridinium Salts **12** and **13**, *N*-Benzimidazolylmethylpyridinium Salt **21**, *N*-Benzimidazolylmethylimidazolium **22**, and the Corresponding Betaines **14**, **15**, **23**[c] and **24**

Compd. No.	C-2,6	C-4	C-3,5	CH_2	C-2′	C-3a′	C-4′	C-5′	C-6′	C-7′	C-7a′	R[d]
12	145.2	148.5	124.8	—	144.1	130.5	142.8	120.9	123.1	126.1	134.5	60.3
14	143.8	152.1	123.4	—	158.1	137.6	141.5	117.4	125.3	118.1	150.8	59.4
$\Delta\delta$[e]	−1.4	+3.6	−1.4	—	+14.0	+7.1	−1.3	−3.5	+2.2	−8.0	+16.3	−0.9
13	145.6	149.9	124.4	—	143.9	142.0	113.8	143.0	119.5	115.8[f]	143.2[f]	60.4
15	144.0	151.2	124.2	—	156.3	142.1	115.2	141.5	122.1	118.4	137.6	59.2
$\Delta\delta$[e]	−1.6	+1.3	−0.2	—	+12.4	+0.1	+1.4	−1.5	+2.6	+2.6	−5.6	−1.2
21	148.2	150.2	135.1	59.9	152.2	130.4	146.1	122.7	125.0	128.9	135.1	—
	C-2	C-4[g]	C-5[g]									
22	137.4	123.6	122.5	46.2	151.9	129.1	144.7	119.2	121.7	126.3	133.6	48.9
24	137.0	123.9	123.5	50.4	162.2	136.3	142.0	115.4	116.0	122.4	152.0	48.8
$\Delta\delta$[e]	−0.4	+0.3	+1.0	+4.2	+10.3	+7.2	−2.7	−3.7	−5.7	−3.9	+18.4	−0.1

a) In DMSO-d_6. *b*) Recorded on a Bruker AM-100 spectrometer (25.1 MHz). ^{13}C-NMR chemical shifts are reported in ppm (*d*) downfield from the central peak of DMSO-d_6. *c*) The insolubility of compound **23** in DMSO-d_6 prevented recording of its ^{13}C-NMR. *d*) Only the chemical shifts corresponding to $-CH_2-N^+-$ are quoted. *e*) $\Delta\delta$: Observed chemical shift difference between betaines **14**, **15**, **23**, **24** and their precursors **12**, **13**, **21** and **22**, respectively. *f*) Broad signal due to annular prototopic tautomerism. *g*) Unambiguous assignment for the signals of C-4 and C-5 in the imidazolium ring.[2b]

chlorohydrate (**26**),[1b,7a] 3-butyl-1-[2-(4-nitro-1*H*-benzimidazol-2-yl)-ethyl]imidazolium chlorohydrate (**27**),[1b,7b] 2-[2-(3-butyl-1-imidazolio)-ethyl]4-nitrobenzimidazolate (**28**),[1b,7b] and 2-mercapto-4-nitrobenzimidazole (**29**)[13] were prepared as described in the literature.

4-Nitro-2-(4-pyridyl)-1*H*-benzimidazole (9) A suspension of isonicotinic acid (**7**, 3.8 g, 30.8 mmol) and 3-nitro-*o*-phenylenediamine (**8**, 5.0 g, 32.7 mmol) in polyphosphoric acid (24 g) was stirred and maintained at 170°C (bath temperature) for 4 h. The mixture was then cooled and poured into water, and the resulting suspension was adjusted to pH 8 with a solution of concentrated NH_4OH. The precipitated product was collected by filtration and recrystallized from EtOH to provide **9** (2.2 g, 30% yield), mp 270—272°C (yellow needles). *Anal.* Calcd for $C_{12}H_8N_4O_2$: C, 60.0; H, 3.35; N, 23.3. Found: C, 60.0; H, 3.2; N, 23.2. ^{1}H-NMR (DMSO-d_6) δ: 7.41 (1H, t, H-6), 8.12 (2H, d, H-5 and H-7), 8.24 (2H, AA'BB' system, H-3', 5'), 8.75 (2H, AA'BB' system, H-2', 6'). ^{13}C-NMR (DMSO-d_6) δ: 120.5 (C-5), 121.9 (C-7 and C-3', 6'), 122.6 (C-6), 127.5 (C-7a), 134.1 (C-4), 136.4 (C-4'), 146.2 (C-3a), 152.9 (C-2). IR (KBr): 3280—3060, 1565, 1470, 1310 cm^{-1}.

5-Nitro-2-(4-pyridyl)-1*H*-benzimidazole (11) Concentrated HNO_3 (1.0 ml, d=1.37) was added dropwise to a stirred solution of 2-(4-pyridyl)benzimidazole **10** (2.5 g, 13.0 mmol) in concentrated H_2SO_4 (6.1 ml, d=1.84) at 0°C. The reaction mixture was heated by slowly raising the temperature of the thermostat to 80°C over approximately 1 h, and then allowed to stand at room temperature for another 4 h. The mixture was poured onto ice (100 ml), and the precipitate was collected and recrystallized from EtOH to give **11** (2.6 g, 84% yield), mp 271—272°C (yellow powder). *Anal.* Calcd for $C_{12}H_8N_4O_2$: C, 60.0; H, 3.35; N, 23.3. Found: C, 60.2; H, 3.4; N, 23.5. ^{1}H-NMR (DMSO-d_6) δ: 7.56 (1H, d, H-7), 7.96 (1H, d, H-6), 8.30 (1H, s, H-4), 8.51 (2H, AA'BB' system, H-3', 5'), 8.90 (2H, AA'BB' system, H-2', 6'). ^{13}C-NMR (DMSO-d_6) δ: 112.7 (C-4), 115.1 (C-7), 118.3 (C-6), 120.6 (C-3', 5'), 136.1 (C-4'), 143.5 (C-5), 150.5 (C-2', 6'), 153.3 (C-2). IR (KBr): 3600, 1620, 1520, 1350 cm^{-1}.

Preparation of 1-Butyl-4-(nitro-1*H*-benzimidazol-2-yl)pyridinium Salts (12 and 13) (Table I) Method A: *n*-Butyl iodide or bromide (41.0 mmol) was added dropwise to a stirred solution of nitro-2-(4-pyridyl)benzimidazole **9** or **11** (8.2 mmol) in dry *N*,*N*-dimethylformamide (DMF) (30 and 12 ml respectively) under an atmosphere of nitrogen, and the solution was then maintained in a bath at 85—95°C for the time specified in Table I. Et_2O (70 ml) was added to the cooled mixture, and the orange precipitate was collected by filtration, washed in Et_2O and then recrystallized (Table I). Compound **12**: *Anal.* Calcd for $C_{16}H_{17}IN_4O_2$: C, 45.3; H, 4.0; N, 13.2. Found: C, 45.4; H, 4.3; N, 13.1. IR (KBr) 3300—3100, 1640, 1470, 1340 cm^{-1}. Compound **13**: *Anal.* Calcd for $C_{16}H_{17}BrN_4O_2$: C, 50.9; H, 4.5; N, 14.85. Found: C, 51.1; H, 4.75; N, 14.9. IR (KBr): 3500—3300, 1640, 1520, 1340 cm^{-1}.

Preparation of 1-Butyl-4-(nitrobenzimidazolyl-2-ylidene)-1,4-dihydropyridines (14 and 15) (Table I) Method B: A solution of 1-butyl-4-(nitro-1*H*-benzimidazol-2-yl)pyridinium salts **12** or **13** in H_2O or EtOH–H_2O was adjusted to pH 8 with concentrated NH_4OH or K_2CO_3, and the bright yellow solid was collected, and washed with H_2O (Table I). Compound **14**: *Anal.* Calcd for $C_{16}H_{16}N_4O_2 \cdot H_2O$: C, 61.1; H, 5.8; N, 17.8. Found: C, 61.1; H, 5.8; N, 17.8. IR (KBr): 1620, 1450, 1330 cm^{-1}. Compound **15**: *Anal.* Calcd for $C_{16}H_{16}N_4O_2 \cdot H_2O$: C, 61.1; H, 5.8; N, 17.8. Found: C, 61.3; H, 5.6; N, 17.95. IR (KBr) 1600, 1470, 1300 cm^{-1}.

Attempted Preparation of Hydrochloride 17 Various attempts were made to study the course of the reaction between 4-nitro-2-chlorobenzimidazole **16** and thiazole.

Three equivalents of thiazole were heated in DMSO (90°C, 4 h), dioxane (reflux, 30 h), *n*-butanol (reflux, 2 h), anisole (reflux, 24 h), or in a sealed tube (100°C, 1 h; 135°C, 1 h). The course of the reaction was followed by TLC and by ^{1}H-NMR (DMSO-d_6) of aliquots.

In some cases, the only product isolated was 4-nitro-2-benzimidazolone **18**. When the reaction was carried out in a sealed tube (both at 100 and 135°C), decomposition products were formed.

2-Hydroxymethyl-4-nitro-1*H*-benzimidazole (19) A suspension of 3-nitro-*o*-phenylendiamine **8** (5.0 g, 32.7 mmol), hydroxyacetic acid (2.5 g, 32.7 mmol) and 5 N HCl (13 ml, 65.4 mmol) was stirred and maintained at 135°C for 72 h. The cooled mixture was filtered, and the solid obtained was dissolved in H_2O and adjusted to pH 8 with Na_2CO_3. The precipitated product was collected by filtration, washed with H_2O and recrystallized from iso-PrOH to afford **19** (4.7 g, 75% yield), mp 208°C (brown solid). *Anal.* Calcd for $C_8H_7N_3O_3$: C, 49.7; H, 3.6; N, 21.8. Found: C, 49.4; H, 3.8; N, 21.6. ^{1}H-NMR (DMSO-d_6) δ: 4.40 (s, 2H, CH_2), 7.00 (1H, t, H-6), 7.70 (2H, d, H-5 and H-7). IR (KBr): 3260—3100, 1500, 1340 cm^{-1}.

2-Chloromethyl-4-nitro-1*H*-benzimidazole (20) Thionyl chloride (7.1 ml, 98.0 mmol) was added dropwise to 2-hydroxymethyl-4-nitrobenzimidazole **19** (1.0 g, 5.2 mmol) at 0°C, and the suspension was then heated at reflux for 2 h. The excess of thionyl chloride was removed *in vacuo*, and the residue was poured onto ice and adjusted to pH 8 with Na_2CO_3. The precipitate was filtered off, washed with H_2O and dried to give **20** (0.7 g, 64% yield), mp 164°C (brown solid, lit.[14] 163°C). ^{1}H-NMR (DMSO-d_6) δ: 4.77 (2H, s, CH_2), 7.07 (1H, t, H-6), 7.81 (2H, d, H-5 and H-7). IR (KBr): 3380, 1500, 1340 cm^{-1}.

Preparation of 1-(4-Nitro-1*H*-benzimidazol-2-yl)methylpyridinium and 3-Butyl-1-(4-nitro-1*H*-benzimidazol-2-yl)methylimidazolium Chlorohydrates (21 and 22) (Table I) Method C: A stirred solution of 2-chloro-4-nitromethylbenzimidazole **20** (1.5 g, 7.1 mmol) in 10 eq (71.0 mmol) of pyridine or *N*-butylimidazole was heated to 100°C and maintained at 100°C under an atmosphere of nitrogen for the time specified in Table I. The reaction mixture was cooled to room temperature and treated in Me_2CO (15 ml) to give a white precipitate. The crude product **21** or **22** was collected by filtration, washed with Me_2CO, and recrystallized (Table I). Compound **21**: *Anal.* Calcd for $C_{13}H_{11}ClN_4O_2$: C, 53.7; H, 3.8; N, 19.3. Found: C, 53.7; H, 3.75; N, 19.1. IR (KBr): 3000, 2940, 1630, 1500, 1340, 1270 cm^{-1}. Compound **22**: *Anal.* Calcd for $C_{15}H_{18}ClN_5O_2$: C, 53.65; H, 5.4; N, 20.9. Found: C, 53.7; H, 5.2; N, 20.6. IR (KBr): 3500—3300, 1500, 1330 cm^{-1}.

Preparation of 4-Nitro-2-(1-pyridiniomethyl)benzimidazolate (23) and 2-(3-Butyl-1-imidazoliomethyl)-4-nitrobenzimidazolate (24) (Table I) Method D: A column packed with anion-exchange Amberlite resin IRA-401 was used and the chloride form was converted to the hydroxy form.[5] A solution of 4-nitrobenzimidazolylmethylpyridinium and imidazolium salt **21** or **22** (0.05 mmol) in 85% EtOH (30 ml) was passed through the column. The neutral eluates were concentrated on a rotary evaporator at 30°C to give the corresponding inner salt **23** or **24** (Table I). Compound **23**: *Anal.* Calcd for $C_{13}H_{10}N_4O_2 \cdot H_2O$: 57.3; H, 4.4; N, 20.6. Found: C, 57.0; H, 4.2; N, 20.5. IR (KBr): 1550, 1360 cm^{-1}. Compound **24**: *Anal.* Calcd for $C_{15}H_{17}N_5O_2$: C, 60.2; H, 5.7; N, 23.4. Found: C, 60.3; H, 5.8; N, 23.1. IR (KBr): 1500, 1350 cm^{-1}.

2-(2-Chloroethylthio)-4-nitro-1*H*-benzimidazole (30) Metallic sodium (1.0 g, 43.5 mmol) was added in small portions under an atmosphere of nitrogen to a solution of naphthalene (2.0 g, 15.6 mmol) in dry dimethoxyethane (60 ml). After the suspension became green, stirring was maintained at room temperature for 3 h. The resulting solution was added dropwise under an atmosphere of nitrogen to a solution of **29** (0.6 g, 3.1 mmol) in dry dimethoxyethane until the mixture remained green for a few seconds. Then, 1-bromo-2-chloroethane (2.8 ml, 3.5 mmol) was added and stirring was continued at room temperature for 5 d. H_2O (25 ml) was added, and the solvent was removed *in vacuo*. Purification by column chromatography (SiO_2, $CHCl_3$) afforded **30** (0.16 g, 20%), mp 139°C (yellow powder). *Anal.* Calcd for $C_9H_8ClN_3O_2S$: C, 41.95; H, 3.1; N, 16.3. Found: C, 42.0; H, 3.3; N, 15.9. ^{1}H-NMR (DMSO-d_6) δ: 3.78 (2H, t, CH_2S), 4.01 (2H, t, CH_2Cl), 7.43 (1H, dd, H-6), 8.00 (1H, d, H-7), 8.15 (1H, d, H-5). ^{13}C-NMR (DMSO-d_6) δ: 33.2 (CH_2S), 43.2 (CH_2Cl), 117.6 (C-5), 121.0 (C-7), 124.2 (C-6), 130.3 (C-7a), 132.4 (C-4), 146.0 (C-3a), 154.6 (C-2). IR (KBr): 3200, 1570, 1380 cm^{-1}.

Elution with $CHCl_3$/MeOH (9:1) gave **31** (0.3 g, 46%), mp 122°C (yellow powder, lit.[13] 120°C). ^{1}H-NMR (DMSO-d_6) δ: 4.27 (2H, t, CH_2S), 4.66 (2H, t, CH_2N), 7.50 (1H, t, H-6), 7.89 (1H, d, H-7), 8.19 (1H, d, H-5). ^{13}C-NMR (DMSO-d_6) δ: 34.0 (CH_2S), 42.1 (CH_2N), 114.3 (C-7), 116.1 (C-5), 118.2 (C-6), 132.6 (C-4 and C-7a), 138.2 (C-3a), 160.1 (C-2). IR (KBr); 1570, 1380 cm^{-1}.

Attempted Quaternization of Compound 30 Method E: 2-(2-Chloroethylthio)-4-nitrobenzimidazole **30** (0.5 g, 1.95 mmol) was dissolved in dry pyridine (2.4 ml, 29.3 mmol) and the solution was maintained in a sealed tube at 135°C for 7 d. Analysis of an aliquot of the reaction mixture indicated that the cyclic compound **31** was formed as the major product, along with decomposition products.

Method F: 2-(2-Chloroethylthio)-4-nitrobenzimidazole **30** (0.78 g, 3.0 mmol) was dissolved in *n*-butylimidazole (1.2 ml, 9.0 mmol) and potassium iodide (0.75 g, 4.5 mmol) was added. The mixture was maintained at 60—70°C under an atmosphere of nitrogen for 12 h. The only product detected in an aliquot of the reaction mixture was the cyclic compound **31**.

1-(2-Chloroethyl)-2-mercapto-4-nitro-1*H*-benzimidazole (33) Method

G: 1-Bromo-2-chloroethane (14 ml, 102 mmol) and benzyltriethylammonium bromide (35 mg, 0.15 mmol) were added to a suspension of **29** (3.0 g, 15.4 mmol) in 40% NaOH (10 ml, 100 mmol), and the mixture was heated at reflux for 8 h. After the mixture had cooled down to room temperature, the organic phase was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 ml). The organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo*. The solid residue was subjected to column chromatography [(SiO_2, $CHCl_3$/MeOH (99 : 1)] to afford an orange solid. Recrystallization from $CHCl_3/Et_2O$ (3 : 1) gave **33** (0.9 g, 23%), mp 165 °C (orange prisms). *Anal.* Calcd for $C_9H_8ClN_3O_2S$: C, 41.95; H, 3.1; N, 16.3. Found: C, 42.0; H, 3.3; N, 16.0. ^{1}H-NMR (DMSO-d_6) δ: 3.86 (2H, t, CH_2Cl), 4.60 (2H, t, CH_2N), 7.10 (1H, t, H-6), 7.53 (1H, d, H-7), 7.72 (1H, d, H-5). ^{13}C-NMR (DMSO-d_6) δ: 33.9 (CH_2Cl), 48.7 (CH_2N), 118.6 (C-5), 121.0 (C-7), 125.1 (C-6), 127.6 (C-3a), 133.5 (C-4), 152.6 (C-7a), 162.3 (C-2). IR (KBr): 1510, 1300 cm^{-1}.

Method H: 1-Bromo-2-chloroethane (1.4 ml, 10.2 mmol) and Et_3N (0.85 ml, 10.2 mmol) were added to a solution of **29** (2.0 g, 10.2 mmol) in dry DMF (40 ml). The reaction solution was stirred at room temperature under an atmosphere of nitrogen for 12 h. The reaction mixture was then poured onto ice (100 ml), and the precipitate obtained was collected by filtration, washed with H_2O (2 × 10 ml) and dried to give **33** (2.0 g, 76%).

2-(Methoxycarbonylthio)-4-nitro-1*H*-benzimidazole (34) KOH (0.3 g, 5.1 mmol) was added to a suspension of **29** (1.0 g, 5.1 mmol) in EtOH (60 ml). Methyl chloroformate (0.4 ml, 5.1 mmol) was then added, and the reaction mixture was stirred at room temperature under an atmosphere of nitrogen for 3 h. Addition of H_2O (40 ml) to the reaction mixture afforded a precipitate, which was filtered, dried and characterized as the thiocarbonate **34** (1.0 g, 77%), mp 164 °C (orange solid). *Anal.* Calcd for $C_9H_7N_3O_4S$: C, 42.7; H, 2.8; N, 16.6. Found: C, 42.5; H, 2.75; N, 16.4. ^{1}H-NMR (DMSO-d_6) δ: 3.43 (3H, s, MeO_2C), 7.34 (1H, t, H-6), 7.53 (1H, d, H-7), 8.00 (1H, d, H-5). ^{13}C-NMR (DMSO-d_6) δ: 35.4 (CH_3), 114.2 (C-7), 115.2 (C-5), 119.4 (C-6), 132.1 (C-4), 152.2 (C-2), 182.3 (C=O). IR (KBr): 3400, 1710, 1450, 1330, 1160 cm^{-1}.

2-(Benzoylmethylthio)-4-nitro-1*H*-benzimidazole (35) A solution of KOH (0.6 g, 10.2 mmol) in dry EtOH (20 ml) was added to a suspension of **29** (2.0 g, 10.2 mmol) in dry EtOH (20 ml), and then phenacyl bromide (2.0 g, 10.2 mmol) was added to the solution. The reaction mixture was refluxed under an atmosphere of nitrogen for 6 h, and then cooled down to room temperature; the suspension was filtered, and the solid was washed with EtOH (2 × 5 ml) before being purified by column chromatography [SiO_2, $CHCl_3$/MeOH (95 : 5)] to afford **35** (1.0 g, 31%), mp 177 °C. *Anal.* Calcd for $C_{15}H_{11}N_3O_3S$: C, 57.5; H, 3.5; N, 13.4. Found: C, 57.3; H, 3.65; N, 13.4. ^{1}H-NMR (DMSO-d_6) δ: 5.02 (2H, s, CH_2), 7.10 (1H, t, H-6), 7.50—7.80 (5H, m, Ph), 8.02 (1H, d, H-7), 8.15 (1H, d, H-5). ^{13}C-NMR (DMSO-d_6) δ: 39.1 (CH_2), 115.2 (C-7), 117.3 (C-5), 120.7 (C-6), 133.2—135.5 (Ph), 134.1 (C-4), 154.5 (C-2), 193.2 (C=O). IR (KBr): 3360, 1690, 1450, 1330, 1260 cm^{-1}.

Biological Method The efficacy of the compounds as antitrichomonal agents was tested *in vitro* according to the reported method.[3)]

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References and Notes

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