Eur J Med Chem (1992) 27, 171–176

Synthesis and antitrichomonal activity of azinium (azolium) 4-nitrobenzimidazolate betaines and their derivatives

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(Received 2 July 1990; accepted 5 September 1991)

azinium and azolium benzimidazolate betaines / *N*-4-nitro-benzimidazolylpyridinium and azolium salts / 4-nitrobenzimidazoles / antitrichomonal activity

Introduction

Among the many attractive types of heterocyclic betaines, the inner salts of azinium azolate **1** and azolium azolate **2** are few and scattered. Only a few examples have hitherto been reported, most of them in connection with our research work on the chemistry of heterocyclic betaines [1–3].

This novel ensemble of heterocyclic compounds **1** and **2** as well as their derivatives **3**, **4** is of interest from a theoretical point of view [1, 2] as well as for their potential biological applications [3].

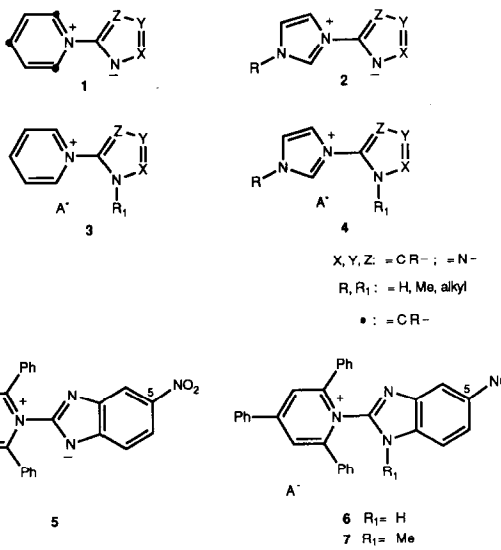
In a previous paper [3] we reported the synthesis and antiprotozoal evaluation of a variety of mesomeric betaines of pyridinium azolate **1** and their *N*-azolylpyridinium salts **3**, and the *N*-5-nitrobenzimidazol-2-ylpyridinium derivatives **5–7** exhibited *in vitro* activity against *Trichomonas vaginalis*. On the other hand, there have been relatively few reports, however, concerning the chemical and biological aspects of 4-nitrobenzimidazole derivatives, although some have been shown to possess antifungal [4–6], antibacterial and anthelminthic activities [7–9].

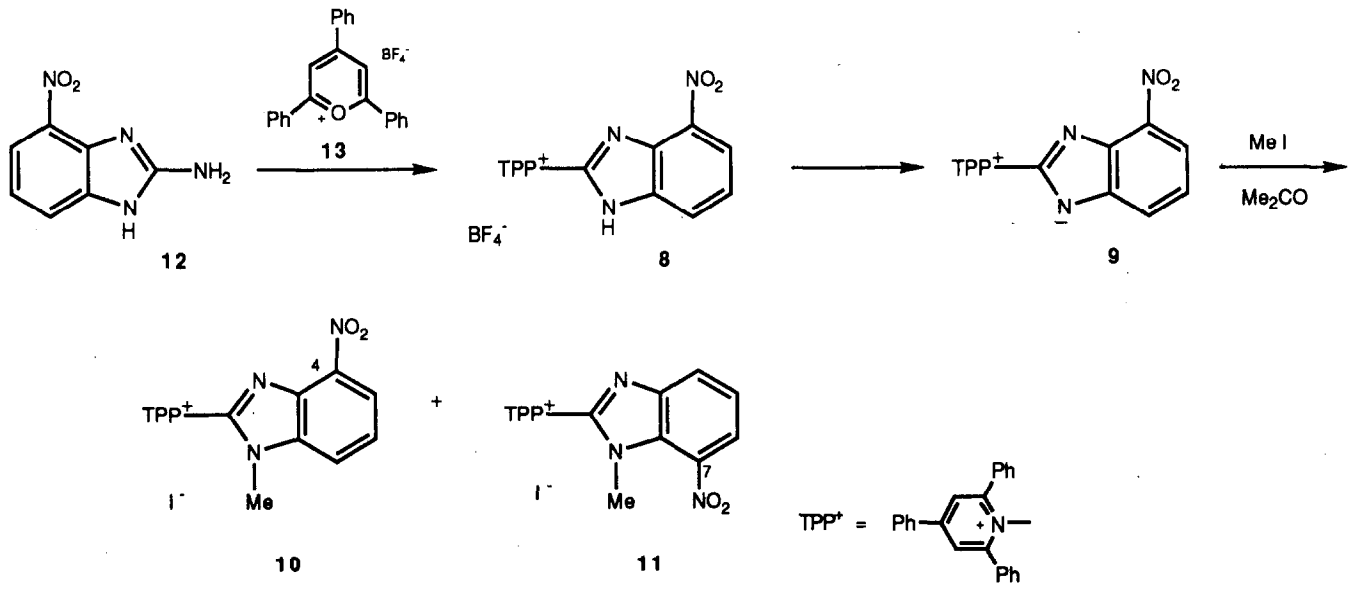
The aim of the present study was to prepare analogues of **5–7** with antitrichomonal activity, and we report in this paper on the synthesis of a series of 4-nitrobenzimidazole derivatives and their activity against *T vaginalis in vitro* and *in vivo*.

Chemistry

The *N*-4-nitrobenzimidazol-2-yl-2,4,6-triphenylpyridinium salts **8**, **10** (4-nitro), **11** (7-nitro) and the betaine **9** were synthesized according to the method reported previously [3] (scheme 1); the 2-amino-4-nitrobenzimidazole **12** reacted with the pyrylium salt **13** to give the desired compound **8** which was transformed into the mesomeric betaine **9** using an anion-exchange resin (OH^- form). *N*-Methylation of the inner salt **9** with methyl iodide/acetone under neutral and mild conditions afforded the 2 isomeric *N*-methylnitrobenzimidazole compounds **10** and **11**, isomeric ratio **10/11** = 45/55.

Alternatively, the 4-nitrobenzimidazol-2-ylpyridinium salts **14**, **15** and betaines **16**, **17** (scheme 2) were prepared from the 2-chloro-4-nitrobenzimid-





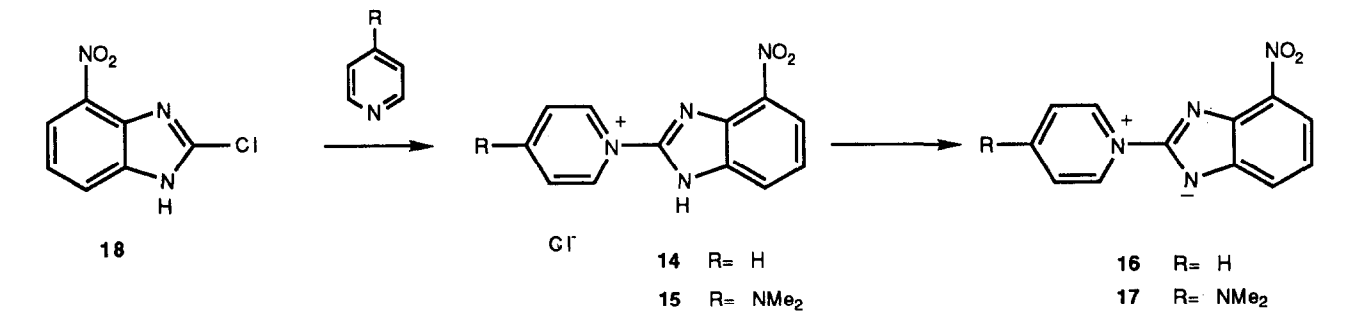
Scheme 1.

azole **18** and displacement of the chlorine atom by pyridines. *N*-4-nitrobenzimidazol-2-ylimidazolium salts **19**, **20** and betaines **21**, **22** were prepared in a similar manner to that of compounds **14**–**17** by reaction of 2-chloro-4-nitrobenzimidazole **18** with alkylimidazoles to give the above-mentioned salts **19** and **20** (scheme 3).

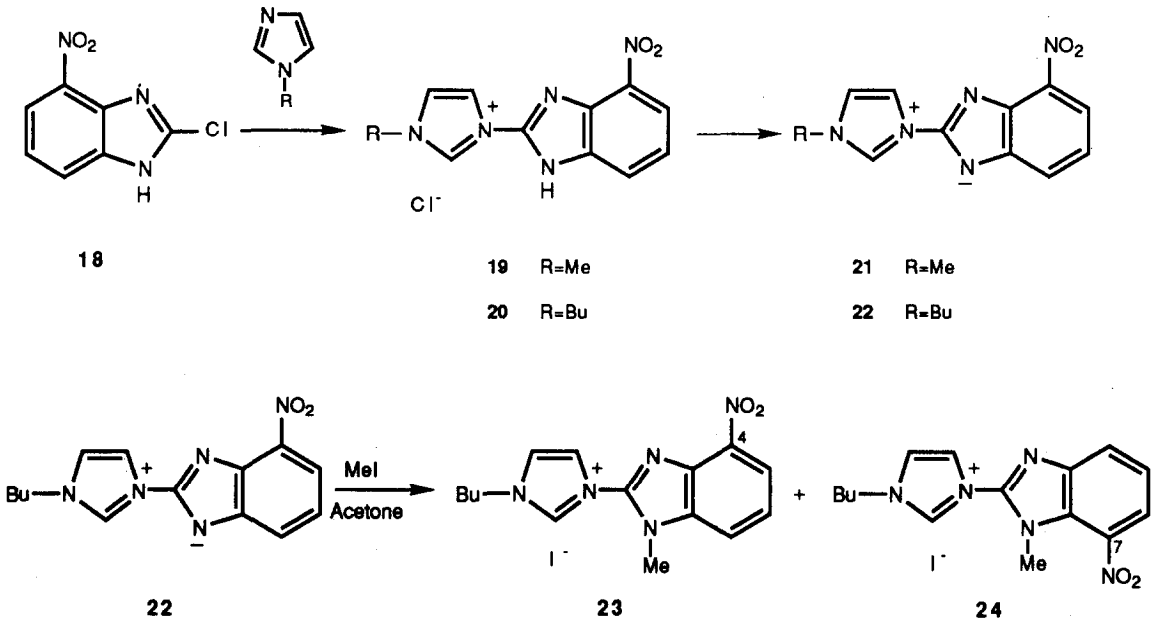
The mesomeric betaines of pyridinium benzimidazolate **16**, **17** (scheme 2) and imidazolium benzimidazolate **21**, **22** (scheme 3) were performed by deprotonation of the quaternary salts using potassium carbonate for neutralization to pH ≈ 8. It is noteworthy that transformation of **20** into the corresponding mesomeric betaine **22** using a strong base anion-exchange (OH^- form) resin proceeded with a low yield (31%). *N*-methylbenzimidazole isomers **23** (4-NO_2) and **24** (7-NO_2) have been obtained in a similar manner as for compounds **10** and **11**, as shown in scheme 1.

The physical data of the new compounds described in this work are listed in table I and details of their preparation are given in the *Experimental protocols*. Their structures have been unambiguously characterized on the basis of their spectroscopic data, and all of them gave satisfactory elemental analysis.

^1H NMR chemical shifts have been assigned by comparison with the available data from structures **1**–**4** [1–3] and if necessary an ^{13}C NMR spectrum was recorded. Selected ^1H NMR chemical shifts are given in table II.



Scheme 2.



Scheme 3.

Table I. Physical data of *N*-benzimidazolylpyridinium salts **8**, **10**, **11**, **14**, **15** imidazolium salts **19**, **20**, **23**, **24** and their corresponding betaines **9**, **16**, **17**, **21** and **22**.

Compd	*Structure*	*R, R'*	*R-1'*	*R-4'*	*R-5'*	*R-7'*	*A⁻*	*mp, °C*[a]	*Recrys solvent*[b]	*Yield*[c] *(%)*	*mol. formula*[d]
8	A	Ph	H	NO_2	–	–	BF_4^-	241–242	A	86	$C_{30}H_{21}N_4O_2BF_4$
9	A	Ph	–	NO_2	–	–	–	306–307	B	90	$C_{30}H_{20}N_4O_2\ H_2O$
10	A	Ph	Me	NO_2	–	–	I^-	311–312	C		$C_{33}H_{23}N_4O_2I\ H_2O$
11	A	Ph	Me	–	–	NO_2	I^-	295–297	[f]		$C_{31}H_{23}N_4O_2I\ H_2O$
14	A	H	H	NO_2	–	–	Cl^-	204	–	78	$C_{12}H_9N_4O_2Cl\ 3/2H_2O$
15	A	R = H R' = NMe_2	H	NO_2	–	–	Cl^-	270	D	95	$C_{14}H_{14}N_5O_2Cl\ 3/4H_2O$
16	A	H	–	NO_2	–	–	–	270	–	91	$C_{12}H_8N_4O_2$
17	A	R = H R' = NMe_2	–	NO_2	–	–	–	300	–	97	$C_{14}H_{13}N_5O_2\ 5/2H_2O$
19	B	Me	H	NO_2	–	–	Cl^-	240–242	E	71	$C_{13}H_{10}N_5O_2Cl\ 1/4H_2O$
20	B	nBut	H	NO_2	–	–	Cl^-	238–240	E	86	$C_{14}H_{16}N_5O_2$
21	B	Me	–	NO_2	–	–	–	268–269	E	86	$C_{11}H_9N_5O_2$
22	B	nBut	–	NO_2	–	–	–	105–106	F	80	$C_{14}H_{15}N_5O_2\ 1/3H_2O$
23	B	nBut	Me	NO_2	–	–	I^-	205–206	B	52	$C_{13}H_{18}N_5O_2I$
23 + 24	B	nBut	Me	NO_2, H	–	H, NO_2	I^-	195–198	–	87	–

[a]Melting points are uncorrected. [b]A = isopropanol-tetrafluoroboric acid, B = 60% ethanol, C = chloroform, D = isopropanol, E = acetonitrile, F = chloroform-diethyl ether (2:1). [c]Yields not optimized. [d]Elemental analysis for C, H and N were within ± 0.4% of theoretical values.

Table II. Selected ^1H NMR chemical shifts (δ, ppm) in DMSO–d_6 of *N*-benzimidazolylpyridinium salts **8**, **10**, **11**, **14**, **15**, imidazolium salts **19**, **20**, **23** and their corresponding betaines **9**, **16**, **17**, **21**, **22**.

Compd	*R-2,6*	*R-4*	*H-3,5*	*H-5'*	*H-6'*	*H-7'*
8	7.35–7.77	8.45 7.35–7.77	8.84	8.14	7.42	8.14
9	7.12–7.75	8.25 7.12–7.75	8.55	7.12–7.75	6.92	7.12–7.75
10	7.32–7.70	8.46 7.32–7.70	8.94	8.25	7.32–7.70	8.05
11	7.41–7.72	8.45 7.41–7.42	8.90	8.05	7.41–7.42	8.05
14	9.87	8.93	8.46	8.31	7.59	8.31
15	9.04	–	7.27	8.10	7.42	8.10
16	10.01	8.70	8.22	7.93	7.17	7.93
17	9.09	–	7.22	7.95	7.24	7.95
	H-2	*H-4*	*H-5*			
19	10.30	8.06	8.69	8.24	7.55	8.17
20	10.38	8.16	8.71	8.25–8.15	7.54	8.25–8.15
21	9.94	7.84	8.33	7.83	7.04	7.79
22	10.33	7.95	8.37	7.84–7.78	7.04	7.84–7.78
23	10.27	8.21	8.43	8.32	7.68	8.25

Results and discussion

It was found that some of the 4-nitrobenzimidazole derivatives had activity against *T vaginalis in vitro* (table III). Notably, the *N*-4-nitrobenzimidazol-2-ylpyridinium derivatives **8**, **9**, **14–17** showed inhibitory activity in relation with the LD_{50} to the standard antitrichomonal drug metronidazole [10]. The lack of activity of *N*-1*H*-benzimidazol-2-ylpyridinium chlorohydrate **25** and *N*-5,6-dimethyl-1*H*-benzimidazol-2-yl-pyridinium chlorohydrate **26** previously described [11] confirmed that the nitro group is necessary for antitrichomonal activity.

A logical extension of the study was to consider molecular modifications in the quaternary heteroaromatic moiety in order to establish a possible structure–activity relationship. Thus, the *N*-2,4,6-triphenylpyridinium group has been replaced by *N*-pyridinium, *N*-4-dimethylaminopyridinium (scheme 2) and *N*-alkylimidazolium (scheme 3). In all cases, they were 4-nitrobenzimidazoles. *N*-4-nitrobenzimidazol-2-ylimidazolium derivatives **19–22** showed moderate antichromonal activity, although the N-methyl derivative **23** was inactive.

The antitrichomonal activity of compounds **9**, **15–17**, **20** and **22** were studied further using an *in vivo* assay and the results are given in table IV. 4-Nitro-2-

Table III. Activity against *Trichomonas vaginalis* growing axenically *in vitro*.

Compound	*MLC (μg ml^{-1})*	*LD_{50} (μg ml^{-1})*
8	> 100	10
9	> 5[a]	2
14	> 100	1
15	25	10
16	> 100	1
17	25	10
19	> 100	25
20	100	5
21	> 100	50
22	50	5
23	> 100	> 100
25	> 100	> 100
26	> 100	> 100
Metronidazole	1	1

[a]Greater concentrations could not be used because of solubility problems. > 100 signifies that even at 100 μg ml^{-1} the compound did not kill all the parasites/reduce the number of parasites by 50%. The figures given are in μg ml^{-1}.

Table IV. Activity against *Trichomonas vaginalis* growing subcutaneously in mice. The figures are the number of mice with lesions of the size indicated. The benzimidazoles were given on 3 occasions at 300 mg (kg body weight)$^{-1}$. Metronidazole was administered at 15 mg (kg body weight)$^{-1}$.

Compound	*Size of lesions after 120 h*			
	Large	*Medium*	*Small*	*Undetectable*
None	5	1	–	–
11	5	1	–	–
18	–	3	3	–
19	–	1	3	2
20	1	4	1	–
25	–	–	2	4
27	–	2	2	2
Metronidazole	–	–	1	5

(1-pyridinio)benzimidazolate **16** and 1-(1*H*-benzimidazol-2-yl)-3-butylimidazolium salt **20** have shown antitrichomonal activity at 300 mg (kg body weight)$^{-1}$, and metronidazole being the reference drug. The results at other time points showed a similar trend. Compounds **16** and **20** were also tested at lower concentrations, at which they were less active (data not shown).

Experimental protocols

Chemistry

Melting points were determined on a CTP-MP 300 hot-plate apparatus and are uncorrected. IR spectra were recorded as KBr disks on a Perkin–Elmer 1430 spectrophotometer. ^{1}H NMR spectra were obtained either with a Bruker AM-100 or Perkin–Elmer R-24B spectrometer operating at 100 and 60 MHz respectively. ^{13}C NMR spectra were run on a Bruker AM-100 Fourier transform spectrometer operating at 25.1 MHz. NMR spectra were determined in dimethylsulfoxide–d_6, and chemical shifts are expressed in parts per million (δ) relative to the central peak of dimethylsulfoxide–d_6. TLC was performed on SiO_2 (silica gel 60 F_{250}, Merck), in the following solvent systems: A, methanol–diethyl ether (1.5:8.5); B, tetrahydrofuran–diethyl ether (7:3); C, chloroform–methanol (9:1) as developing solvent, and the spots were located with UV light. Ion-exchange chromatography was carried out on an anionic (OH^- form) ion-exchange resin (Amberlite IRA-401) [11]. If necessary, the compounds were dried by heating overnight at 110°C in a vacuum oven. Where microanalyses are indicated by symbols of the elements, the analytical results were within ± 0.4% of the theoretical values; they were performed on a Carlo Erba 1106 analyzer by the Instituto de Química Bio-orgánica, Barcelona.

Pyridine, *N*-dimethylamino-4-pyridine, *N*-methyl and *N*-butylimidazole are commercially available. 2-Amino-4-nitrobenzimidazole **12** [12], 2,4,6-triphenylpyrylium tetrafluoroborate **13** [13], 4-nitro-2-chlorobenzimidazole **18** [14], *N*-1*H*-benzimidazol-2-ylpyridinium chlorohydrate **25** [11] and *N*-5,6-dimethyl-1*H*-benzimidazol-2-yl-pyridinium chlorohydrate **26** [11] were prepared as in the literature.

1-(4-Nitro-1H-benzimidazol-2-yl)-2,4,6-triphenylpyridinium tetrafluoroborate 8

To a solution of 2-amino-4-nitrobenzimidazole **12** (2.04 g, 11.46 mmol) in 7 ml of anhydrous dimethylformamide was added 2,4,6-triphenylpyrylium tetrafluoroborate **13** (3.80 g, 9.57 mmol), and the reaction mixture refluxed under stirring for 1 h. After cooling, diethyl ether was added (25 ml) to give a yellow solid **8**, 4.26 g (86% yield); mp 235–236°C. Recrystallization from 2-propanol and few drops of tetrafluoroboric acid afforded an analytical sample of **8**; mp 241–242°C. TLC solvent system A (R_F 0.15).

4-Nitro-2-(2,4,6-triphenyl-1-pyridinio)benzimidazolate 9

A column packed with anion-exchange Amberlite resin IRA-401 was used and the chloride form was converted to the hydroxide form [11]. A solution of *N*-benzimidazolylpyridinium salt **8** (4.40 g, 7.89 mmol) in 70% ethanol (300 ml) was passed through the column. The neutral eluates were concentrated on rotary evaporator at 45°C to give a bright red solid, which was recristallized from 60% ethanol to afford 3.33 g (90% yield) of the betaine **9**: mp 306–307°C.

N-Methylation of the mesomeric betaine 9

A solution of methyl iodide (2.14 g, 15.02 mmol) in dry acetone (10 ml) was added dropwise at 0–5°C to a stirred solution of betaine **9** (2.00 g, 4.27 mmol) in 190 ml of dry acetone under an atmosphere of nitrogen, and then allowed to stand at room temperature for 15 h. The progress of the reaction was monitored by TLC (solvent B) and by ^{1}H NMR of aliquots. The resulting solution was evaporated to dryness, and washing the residue with diethylether afforded a mixture of the *N*-methyl isomers **10** (4-NO_2) and **11** (7-NO_2) as an orange solid (2.26 g, 87% yield). Isomeric ratio **12/13** = 45/55 (^{1}H NMR).

The mixture **10** + **11** was recrystallized from chloroform to give compound **10** in a pure form; mp 311–312°C (46% yield). The mother liquor from recrystallization was evaporated to dryness and the residue, was triturated with diethylether to afford compound **11**, mp 295–297°C (23% yield). TLC solvent system B, **10** (R_F 0.3) and **11** (R_F 0.45). Compound **10**: ^{13}C NMR (DMSO–d_6) 159.1 (C-4), 156.4 (C-2,6), 144.3 (C-2'), 138.4 (C-4'), 135.7 (C-7'a), 131.9 (C-3'a), 125.8 (C-3,5), 124.5 (C-7'), 120.6 (C-6'), 118.7 (C-5'), 31.3 (N-Me). Compound **11**: ^{13}C NMR (DMSO–d_6) 159.1 (C-4), 156.4 (C-2,6) 144.4 (C-2'), 141.7 (C-3'a), 136.2 (C-7'), 127.0 (C-4'), 126.0 (C-3,5), 125.4 (C-7'a), 123.3 (C-5'), 122.5 (C-6'), 34.5 (N-Me). δC of the phenyl rings carbons in 2,4,6-triphenylpyridinium were found in the range of 128.6 to 133.4 ppm.

Procedures for N-benzimidazolylpyridinium and imidazolium salts and their corresponding mesomeric betaines (table I)

A stirred solution of 4-nitro-2-chlorobenzimidazole **18** (3.0 g, 15.2 mmol) in 3 equivalents (45.6 mmol) of the corresponding anhydrous pyridines or *N*-alkylimidazoles was heated at 130°C under an atmosphere of nitrogen for a time among 1 min to 0.75 h. The reaction mixture was cooled and treated with diethylether (50–75 ml) to give yellow to orange precipitates. The crude products **14**, **15**, **19**, and **20** were filtered, washed with diethylether, and recrystallized.

A stirred solution of 4-nitro-2-chlorobenzimidazole **18** (2.0 g, 10.0 mmol) in 8 ml of pyridine was heated at 130°C under an atmosphere of nitrogen for 45 min. The mixture was cooled and treated with chloroform (15 ml) to give golden brown crystals, which were filtered and washed with 2 x 10 ml portions of ethanol to provide 2.2 g of 4-nitro-2-(1-pyridinio)benzimidazolate **16**. A solution of the mesomeric betaine **16** (1.0 g, 4.2 mmol) was dissolved in ethanol (400 ml) and then aqueous 10 % hydrochloric acid (20 ml) added. The solution was concentrated to give 0.9 g of 1-(4-nitro-1*H*-benzimidazol-2-yl)pyridinium hydrochloride **14** (table I).

Preparation of azinium and azolium benzimidazolate betaines 17, 21 and 22

A solution of *N*-benzimidazolylpyridinium hydrochloride **15** or *N*-benzimidazolylimidazolium hydrochlorides **19**, **20** in water or ethanol–water was neutralized with concentrated NH_4OH or K_2CO_3 to pH 8, and the bright yellow solids were collected, washed with water, and recrystallized (table I).

To a stirred solution of the mesomeric betaine **22** (1.0 g, 3.5 mmol) in dry acetone (40 ml) was added dropwise under an atmosphere of nitrogen at 0–5°C a solution of methyl iodide (0.76 ml, 12.2 mmol). When the addition was complete, the mixture was heated under reflux for 20 h and allowed to cool to room temperature. The progress of the reaction was monitored by TLC system C by ^{1}H NMR of aliquots.

The solvent was removed in the rotary evaporator to give a brown solid that was a mixture of *N*-methyl derivatives **23** and **24**, ratio 77/23 (^{1}H NMR in DMSO-d_6). The mixture was recrystallized twice from ethanol giving 0.78 g of pure **23** (4-nitro) (table I). TLC system C, **23** (R_F 0.40) and **24** (R_F 0.45). Compound **23**: ^{13}C NMR (DMSO-d_6) 143.5 (C-2'), 138.4 (C-7'a), 138.1 (C-2), 137.7 (C-4'), 132.6 (C-3'a), 123.5 (C-6), 123.4 (C-5), 122.8 (C-4), 119.9 (C-7), 118.6 (C-5), 32.2 (N-Me), 49.6, 30.9, 18.7, 13.2 (*n*-But).

Biological evaluation

Determining the efficacy of the compounds as antitrichomonal agents

Trichomonas vaginalis (clone G3) was maintained axenically at 37°C in modified Diamond's medium as described previously [15]. The activity of the benzimidazoles towards the parasite *in vitro* was determined in duplicate using doubling dilutions in the range 100–101 μg ml^{-1} with incubation at 37°C under anaerobic conditions in microtitre plates for 24 h as described previously [18]. Solutions of the test compounds were made in DMSO; the concentration of DMSO (0.25%) included in the experiments was shown not to affect growth of the parasite. The activities of the compounds are presented as the MLC, the minimum concentration used that killed all the parasites by 24 h, and the LD_{50}, the minimum concentration used that reduced the number of parasites by at least 50%.

The activity of the compounds against *T vaginalis in vivo* was determining using the subcutaneous assay as described previously [16]. The mice were used in groups of 6. The experimental compounds were administered orally as suspensions in gum tragacanth at 2, 18 and 24 h post-infection. Metronidazole was given as an aqueous solution. The size of the subcutaneous lesions resulting from inoculation of the parasites were monitored daily.

Acknowledgments

This work was supported by Laboratorios Dr Esteve, Barcelona (Project No 302, Fundació Bosch i Gimpera-Universitat de Barcelona). We thank Laboratorios Dr Esteve for a studentship to LP. The present study is part of a research work awarded by the Sociedad Española de Química Terapéutica (Premio Rhône Poulenc, 1991).

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