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Short communication

NMR studies of *N*-(benzimidazol-2-yl)pyridinium derivatives: QSAR with the anti-leishmanial activity and their carbon-13 NMR chemical shifts

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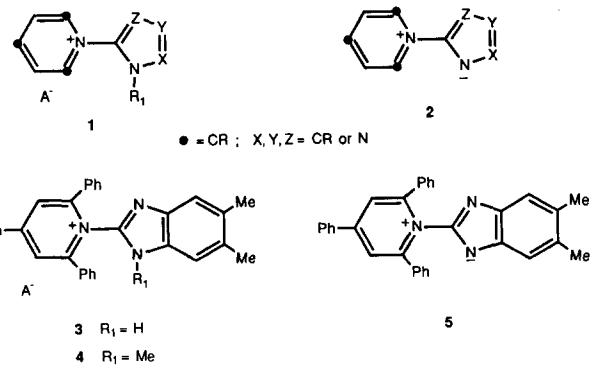
Summary — Quantitative structure–activity - relationships between the *in vitro* anti-leishmanial activity of *N*-benzimidazolyl-2,4,6-triphenylpyridinium salts **6** and pyridinium benzimidazolate betaines **7** and their ^{13}C-NMR chemical shifts have been studied, in order to ascertain the influence of the benzimidazole substituents upon anti-leishmanial activity. The calculated ^{13}C-chemical shifts allow the selection of a representative subset of compounds. Several new *N*-benzimidazolylpyridinium derivatives **6** and **7** have been prepared. Among them, 5-methoxy-1-methylbenzimidazole **21** and 6-methoxy-1-methylbenzimidazole **22** derivatives have high anti-leishmanial activity *in vitro* and compound **22** shows an interesting activity *in vivo* although host toxicity is present.

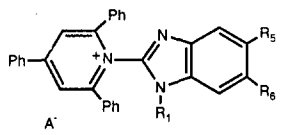
Résumé — Étude RMN de dérivés *N*-(benzimidazol-2-yl)pyridinium: établissement du QSAR entre l'activité anti-leishmania et les déplacements chimiques en ^{13}C RMN. *Une étude de QSAR entre l'activité anti-leishmania* in vitro *des sels de* N-*benzimidazolyl-2,4,6-triphénylpyridinium* **6** *et des bétaïnes de benzimidazolate-pyridinium* **7** *et de leurs déplacements chimiques ^{13}C-RMN a été réalisée afin de voir l'influence du substituant sur l'activité anti-leishmania. Ceci permet la sélection d'un groupe d'analogues des composés* **6** *et* **7** *représentatifs. Quelques nouveaux dérivés de* N-*benzimidazolylpyridinium* **6** *et benzimidazolate pyridinium* **7** *ont été synthétisés. Entre tous, les dérivés 1-méthyl-5-méthoxybenzimidazole* **21** *et 1-méthyl-6-méthoxybenzimidazole* **22**, *ont une grande activité anti-leishmania* in vitro. *Le composé* **22** *montre également une activité intéressante* in vivo, *bien qu'il soit toxique pour l'hôte.*

***N*-(benzimidazol-2-yl)pyridinium salts / anti-leishmanial activity / ^{13}C NMR chemical shifts / QSAR**

In a previous paper [1], we reported the synthesis and antiprotozoal evaluation of *N*-azolylpyridinium salts **1** and mesomeric betaines of pyridinium azolate **2**. Among them, compounds **3**–**5** exhibited interesting activity against *Leishmania donovani*.



Pursuing our study of heterocyclic betaines and their derivatives with potential biological applications, several new *N*-benzimidazolyl-2,4,6-triphenylpyridinium salts **6** and pyridinium benzimidazolate betaines **7** have been prepared in order to ascertain the influence of the benzimidazole moiety upon anti-leishmanial activity. The substituents at 1, 5 or/and 6 positions in the benzimidazole ring have been selected by means of a quantitative - structure–activity -relationships (QSAR) study between the *in vitro* anti-leishmanial activity of these compounds **6** and **7** and their ^{13}C-NMR chemical shifts as the only structure parameters. In this connection, ^{13}C-NMR chemical shifts have infrequently been applied in QSAR and only a few studies have been published [2, 3]. In contrast, the transmission of the substituent effects on the ^{13}C-NMR chemical shifts in several series of benzimidazoles has been investigated and is by now a quite well documentated subject [4–7].



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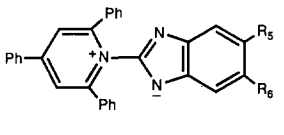

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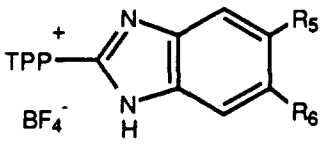
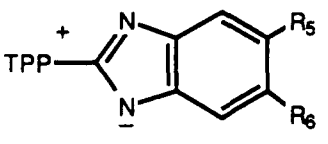
QSAR analysis

We here describe the QSAR with the *in vitro* anti-leishmanial activity of *N*-(1*H*-benzimidazol-2-yl)-2,4,6-triphenylpyridinium tetrafluoroborates **3**, **8–11**, pyridinium benzimidazolate inner salts **5**, **12–15** as well as *N*-(1-methylbenzimidazol-2-yl)-2,4,6-triphenylpyridinium salts **4**, **16–23** and their ^{13}C-NMR chemical shifts of the benzimidazole nucleus.

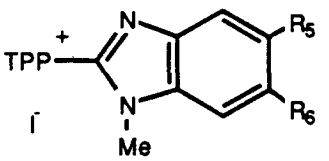
upfield shifts for carbons C-5 and C-6, in comparison to those of the corresponding *N*-benzimidazolylpyridinium salts. On the other hand, the ^{13}C chemical shifts show that C–4 moves to a lower field and C–7 moves to a higher field, while C–5 and C–6 depend on the presence of substituents for moving from unsubstituted benzimidazoles (NH) to *N*-methylbenzimidazoles (NMe). However, the difference values of the ^{13}C chemical shifts for C–5 and C–4, $\Delta\delta$ (5–4), move to a higher field on going from benzimidazoles (NH) to betaines and to *N*-methylbenzimidazoles (NMe). The difference values for C–6 and C–7, $\Delta\delta$ (6–7), also move to a higher field on going from benzimidazoles (NH) to mesomeric betaines, but move to a lower field on going from benzimidazoles (NH) to *N*-methylbenzimidazoles (NMe).



3, 8 - 11

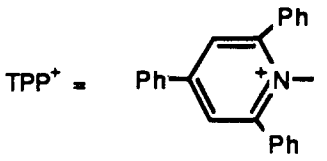


5, 12 - 15



4, 16 - 23

Compounds	R_5	R_6
8, 12, 16	H	H
3, 4, 5	Me	Me
9, 13, 17	PhCO	H
18	H	PhCO
10, 14, 19	NO_2	H
20	H	NO_2
11, 15, 21	MeO	H
22	H	MeO
23	MeO	MeO
24	NO_2	NO_2



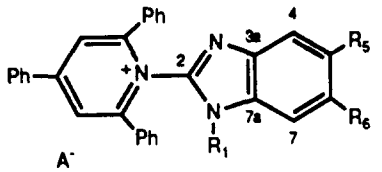
The substituent influence on the chemical shifts of substituted benzimidazoles, their cations and their anions has been extensively investigated [4–7], and for 5(6)-substituted benzimidazoles, excellent correlations of ^{13}C chemical shifts with various Hammett σ parameters were observed. Therefore, in attempting to quantify the structural properties of this series of compounds and with the aim of rationalizing the structural properties, we turned our attention to the results of ^{13}C-NMR.

Since the results reported in table I represent a homogeneous set of NMR data of benzimidazoles (**3** and **8–11**), it is possible to compare the effect produced on the ^{13}C chemical shifts by formation of betaines (**5** and **12–15**) or by *N*-methylation (**4** and **16–22**). From the examination of data in table I it is clear that all of the mesomeric betaines show ^{13}C downfield shifts for carbons C–4 and C–7, and ^{13}C

This study led us to consider the difference values of the ^{13}C chemical shifts as the independent variables in a QSAR analysis using multiple linear regression analysis as the statistical method. It has previously been pointed out that substituted cephalosporins show linear correlations between reactivity and the difference values of the ^{13}C chemical shifts of two adjacent carbon atoms [8], and also that a parabolic relationship exists between the $\Delta\delta$ values and activity.

In undertaking our QSAR study, we considered the *MIC* values (in μg/ml) at 72 h for the set of *N*-methylbenzimidazoles (**4** and **16–20**), and for benzimidazoles, betaines and *N*-methylbenzimidazoles together (**3–5**, **8–10**, **12–14** and **16–20**), both having been previously described [1]. The following equations summarized the results of QSAR analysis for the training sets:

Table I. ^{13}C-NMR chemical shifts (δ, ppm) in DMSO-d_6 of the carbon atoms of the benzimidazole ring and *in vitro* anti-leishmanial activity of *N*-(benzimidazol-2-yl)-2,4,6-triphenylpyridinium salts **3**, **4**, **8–11**, **16–23** and pyridinium benzimidazolate inner salts **5**, **12–15**.



Compd	R-1	R-5	R-6	A⁻	C-2	C-3a	C-4	C-5	C-6	C-7	C-7a	Inh %[a,b]	MIC_{50}[c]
8	H	H	H	BF_4^-	141.3	136.5	116.2	123.8	123.8	116.2	136.5	27	48
3	H	Me	Me	BF_4^-	140.3	136.0	115.5	133.2	133.2	115.5	136.0	67	32
9	H	PhCO	H	BF_4^-	143.6	136.1	119.2	131.4	125.4	116.1	139.1	41	48
10	H	NO_2	H	BF_4^-	146.4	137.0	113.7	143.5	118.8	116.6	140.9	52	37
11	H	MeO	H	BF_4^-	140.3	136.0	97.2	156.8	114.0	118.1	131.7	69	19
12	-	H	H	-	152.3	145.0	116.8	117.9	117.9	116.8	145.0	27	48
5	-	Me	Me	-	151.7	143.8	117.3	125.9	125.9	117.3	143.8	57	43
13	-	PhCO	H	-	155.3	144.2	120.8	127.4	121.1	116.7	149.2	35	52
14	-	NO_2	H	-	150.4	140.0	113.6	143.1	114.4	116.7	143.6	47	8
15	-	MeO	H	-	151.7	145.2	99.4	153.3	108.5	117.1	139.6	61	36
16	Me	H	H	I^-	141.3	138.9	119.9	123.6	124.7	111.2	133.5	49	30
4	Me	Me	Me	I^-	140.4	137.6	119.7	132.5	134.0	110.0	131.5	70	5
17	Me	PhCO	H	I^-	143.2	138.4	122.2	132.9	126.2	111.7	136.3	58	21
18	Me	H	PhCO	I^-	143.8	141.7	119.9	125.3	133.1	113.6	133.2	60	32
19	Me	NO_2	H	I^-	144.6	137.9	116.3	144.2	120.0	112.6	137.4	71	3
20	Me	H	NO_2	I^-	145.5	142.8	120.9	118.9	144.3	108.7	133.2	67[d]	16
21	Me	MeO	H	I^-	141.2	140.2	101.7	156.6	112.0	115.2	128.0	e	-
22	Me	H	MeO	I^-	140.0	133.4	120.7	113.7	157.3	93.9	134.5	e	-
23	Me	MeO	MeO	I^-	139.0	132.6	101.8	147.6	148.6	93.7	127.6	e	-

[a] *In vitro* growth inhibition percentages of *Leishmania donovani* promastigote forms. Average number at concentrations of 100 and 10 μg/ml at 24, 48 and 72 h. [b] For compounds **3–5**, **8–10**, **12–14** and **16–20**, from data previously reported [1]. [c] MIC_{50} values at 72 h in μg/ml were calculated from growth inhibition percentages at the doses of 100, 10 and 1 μg/ml. [d] This value corresponds to a mixture of 19 + 20 (60–40%). [e] See table V.

$$\log 1/MIC = -2.29\ (\pm 0.27) + 0.03\ (\pm 0.009)\ \Delta\delta\ (6\text{–}7) + 0.06\ (\pm 0.009)\ \Delta\delta\ (5\text{–}4) \quad (1)$$
$$n = 6\ r = 0.960\ s = 0.154\ F_{2,3} = 17.8\ (P = 0.02)$$

$$\log 1/MIC = -1.97\ (\pm 0.22) + 0.03\ (\pm 0.009)\ \Delta\delta\ (6\text{–}7) + 0.03\ (\pm 0.009)\ \Delta\delta\ (5\text{–}4) \quad (2)$$
$$n = 14\ r = 0.682\ s = 0.317\ F_{2,11} = 4.8\ (P = 0.03)$$

where n represents the number of compounds considered, r is the correlation coefficient, s is the standard deviation from the regression, F represents the overall statistical significance of the equation, and the values between brakets give the 95% standard error of the parameters.

In order to reduce the experimental error, the analysis was carried out using the average of the inhibition percentages of *Leishmania donovani* at the doses of 100 and 10 μg/ml at 24, 48 and 72 h (Inh %), as the dependent variable. The result of this regression analysis is shown with equation (3) for the *N*-methylbenzimidazole set and with equation (4) for the complete set.

$$\text{Inh \%} = 31.40\ (\pm 2.60) + 1.06\ (\pm 0.09)\ \Delta\delta\ (6\text{–}7) + 1.12\ (\pm 0.09)\ \Delta\delta\ (5\text{–}4) \quad (3)$$
$$n = 6\ r = 0.991\ s = 1.469\ F_{2,3} = 81.3\ (P < 0.01)$$

$$\text{Inh \%} = 22.10\ (\pm 5.13) + 1.45\ (\pm 0.23)\ \Delta\delta\ (6\text{–}7) + 1.06\ (\pm 0.23)\ \Delta\delta\ (5\text{–}4) \quad (4)$$
$$n = 14\ r = 0.891\ s = 7.430\ F_{2,11} = 21.2\ (P < 0.01)$$

which show an improvement of the statistical significance and the values between brackets give the 99% standard error of the parameters.

The equations found allowed us, on a simple basis, to design other monosubstituted *N*-methylbenzimidazoles and predict their biological activity prior to synthesis. In this respect, the ^{13}C-NMR chemical shifts of several *N*-(1-methylbenzimidazol-2-yl)-2,4,6-triphenylpyridinium salts were calculated with data from other benzimidazoles [5] and data of substituent effects on aromatic compounds [8]. Table II reports the calculated and observed chemical shifts of two monosubstituted compounds (**21** and **22**) as well as the predicted inhibitory effect against *Leishmania donovani* using calculated and observed δ through equations (3) and (4), together with the observed activity values for comparison. Compounds **21** and **22** have been synthesized and the highest activity has been observed in this series (see below and table V). By including the observed values (activity and ^{13}C chemical shifts) of **21** and **22** in the regression of the equation (3) and of **11**, **15**, **21** and **22** in the regression of equation (4), we obtained the following equations:

$$\text{Inh \%} = 27.50\ (\pm 2.48) + 1.16\ (\pm 0.07)\ \Delta\delta\ (6\text{–}7) + 1.35\ (\pm 0.07)\ \Delta\delta\ (5\text{–}4) \quad (5)$$
$$n = 8\ r = 0.993\ s = 2.335\ F_{2,5} = 184.3\ (P < 0.01)$$

$$\text{Inh \%} = 24.03\ (\pm 5.20) + 1.27\ (\pm 0.19)\ \Delta\delta\ (6\text{–}7) + 1.05\ (\pm 0.16)\ \Delta\delta\ (5\text{–}4) \quad (6)$$
$$n = 148\ r = 0.885\ s = 9.688\ F_{2,15} = 27.2\ (P < 0.01)$$

Equation (5) shows that the addition of compounds **21** and **22** improves significance. The values between brackets of equations (5) and (6) give the 99.9% standard error of the parameters. On the other hand, the independent variables Δδ (6–7) and Δδ (5–4) are correlated with values lower than 0.5. These correlations are: 0.438 for equations (1) and (3); 0.398 for equations (2) and (4); and only 0.025 for equation (5) and 0.001 for equation (6).

In order to evaluate the role played on activity by a 5,6-disubstituted benzimidazole we calculated ^{13}C chemical shifts of compound **23** (table II), and equations (5) and (6) predicted 1-methyl-5,6-dimethoxybenzimidazole **23** as the most potent analogue of these *N*-benzimidazolylpyridinium derivatives against *Leishmania donovani* (see table II). Compound **23** has been synthesized and tested. Although it shows an interesting activity, its potency is inferior to that of the remaining members of the series.

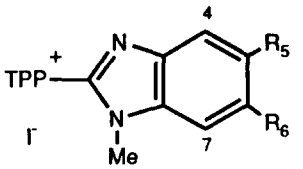
The above QSAR studies nevertheless provide a valuable guide to the synthesis and anti-leishmanial evaluation of the monomethoxy benzimidazoles **21** and **22** as well as the dimethoxy derivative **23** described in the following sections. Furthermore, these studies have allowed obtention of the most active compounds of the series, which present electron-donating substituents in the benzimidazole nucleus (MeO) in contrast with some of the most active compounds previously synthesized, which contained electron-withdrawing groups (*ie* NO_2).

Chemistry

The target *N*-(1-methylbenzimidazol-2-yl)-2,4,6-triphenylpyridinium salts **21** and **22** (5 and 6-methoxy) together with the 5,6-dimethoxybenzimidazole derivative **23**, required for this investigation, were synthesized according to a general procedure previously applied to this class of compounds [1]. As is outlined in schemes 1 and 2, 2-aminobenzimidazoles **27** and **31** served as starting material for the synthesis of the desired compounds **21**, **22** and **23** respectively.

2-Amino-5-methoxybenzimidazole **27** was prepared from 4-methoxy-2-nitroaniline **25**, by reduction with sodium hydrosulphite, followed by cyclization of **26** with cyanogen bromide using a modification of the methods described by Bergeim *et al* [10] and Basaglia *et al* [11] respectively. On the other hand, a search of the literature has revealed four reports of the synthesis of 2-amino-5,6-dimethoxybenzimidazole **31** [12–15], three of them being included in patents. Nevertheless, the information encountered was somewhat confusing. The starting compound **31** was conveniently prepared from 1,2-dimethoxy-4-nitrobenzene **28**

Table II. Calculated and observed ^{13}C chemical shifts (δ, ppm), and inhibitory effect against *Leishmania donovani* of compounds **21–23**.



Compd	R_5	R_6		C-4	C-5	C-6	C-7	Inh %[a] Calc Eq(3)	Calc Eq(4)	Calc Eq(5)	Calc Eq(6)	Obs
21	MeO	H	Calc	105.2	153.8	110.0	112.1	84	71	91	72	100
			Obs	101.7	156.6	112.0	115.2	89	76	98	78	
22	H	MeO	Calc	120.8	108.9	154.9	96.5	80	94	79	86	92
			Obs	120.7	113.7	157.3	93.9	91	107	92	97	
23	MeO	MeO	Calc	102.6	139.1	142.2	100.5	116	121	125	115	82
			Obs	101.8	147.6	148.6	93.7	141	150	153	142	

[a]Average number at concentrations of 100 and 10 μg/ml at 24, 48 and 72 h.

(scheme 2). A mild nitration of **28** afforded 1,2-dimethoxy-4,5-dinitrobenzene **29** (95%), followed by catalytic reduction with H_2/Pd-C, cleanly furnished the unstable 1,2-arylidenediamine **30** which was then rapidly used in the subsequent reaction with cyanogen bromide to form compound **31** (93%).

Reaction of 2-aminobenzimidazole **27** and **30** with 2,4,6-triphenylpyrylium tetrafluoroborate gave the *N*-benzimidazolylpyridinium salts **11** and **32** which were transformed into the corresponding mesomeric betaines of pyridinium benzimidazolate **15** and **33** respectively using an anionic ion-exchange resin (OH^- form) (schemes 1 and 2). Finally, *N*-methylation of the betaines **15** and **33** with methyl iodide/acetone under neutral, mild conditions furnished the two isomeric *N*-methylmonomethoxybenzimidazole derivatives **21** (5-methoxy) and **22** (6-methoxy) as well as the *N*-methyldimethoxybenzimidazole **23**. Synthetic details are given in the experimental section. The structure of all the new compounds were confirmed by spectroscopic methods, and their ^1H-NMR chemical shifts and their physical data are set out in tables III and IV, respectively.

Results and discussion

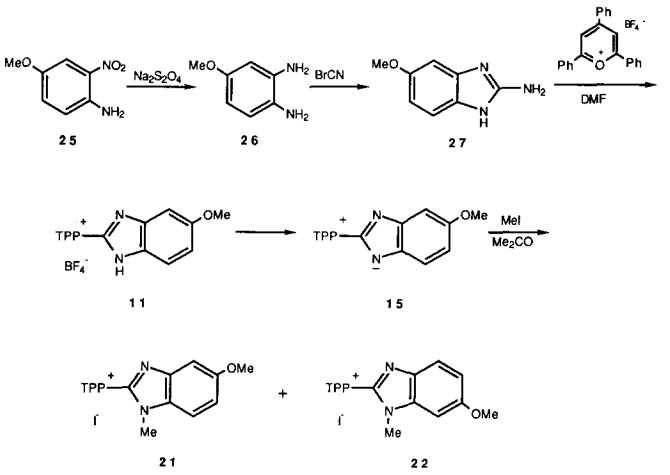
Results in table V show that compounds **21–23** exhibited a high *in vitro* anti-leishmanial activity, which lends credence to the QSAR analysis performed.

Compound **22** has been tested against *Leishmania donovani* in mice and its activity has been confirmed, although host toxicity is present at the dose levels used (table VI and experimental protocols).

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. ^1H-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 TMS as internal standard or the central peak of



Scheme 1.

dimethylsulfoxide-d_6. TLC was performed on SiO_2 (silica gel 60 F_{250}, Merck), in the following solvent systems: A, methanol-diethyl ether (8:2); B, diethyl ether-methanol (9.5:0.5); C, chloroform-methanol (8.5:1.5) 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) [16]. 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.

4-Methoxy-2-nitroaniline **25** and 1,2-dimethoxy-4-nitrobenzene **28** are commercially available. 2,4,6-triphenylpyrylium tetrafluoroborate [17], *N*-benzimidazolyl-2,4,6-triphenylpyridinium salts **3**, **4**, **8–10**, **16–20** and betaines of pyridinium azolate **5**, **12–14** were prepared as previously described [1].

4-Methoxy-1,2-phenylenediamine ***26***

This diamine has previously been described by Bergeim *et al* [10] and was prepared by reduce 4-methoxy-2-nitroaniline **25** with stannous chloride with a 42% yield.

We prepared the base **26** in pure form and with an improved yield (60%) by reducing compound **25** with sodium hydrosulfite.

To a suspension of 4-methoxy-2-nitroaniline **25** (15 g, 0.089 mmol) and sodium sulfate decahydrated (28.75 g, 0.089 mmol) in 715 ml of water and 267 ml of 15 N ammonium hydroxide solution was added 440 ml of methanol to dissolve the solid upon warming. The solution was then cooled in an ice-bath and sodium hydrosulfite (41.43 g, 0.238 mol) was added in four portions at 10 min intervals. After the spontaneous reaction had subsided the solution was boiled gently for 19 h and allowed to stand at room temperature for 36 h.

The solution was concentrated under reduced pressure until the methanol and part of the water has been evaporated to induce precipitation of the starting compound **25**, which was collected.

After removal of the methanol and part of the water under reduced pressure, compound **25** (0.77 g) was removed by filtration. The aqueous filtrate was washed with diethyl ether (5 x 30 ml) and extracted continuously with diethylether for 48 h. The organic layer was dried and the solvent removed under reduced pressure to give the desired compound **26** pure as an oil 7.01 g (60%), which must be stored at low temperature in an atmosphere of nitrogen, and even so compound **26** decomposed with time. TLC solvent system C.

2-Amino-5-methoxy-1H-benzimidazole ***27***

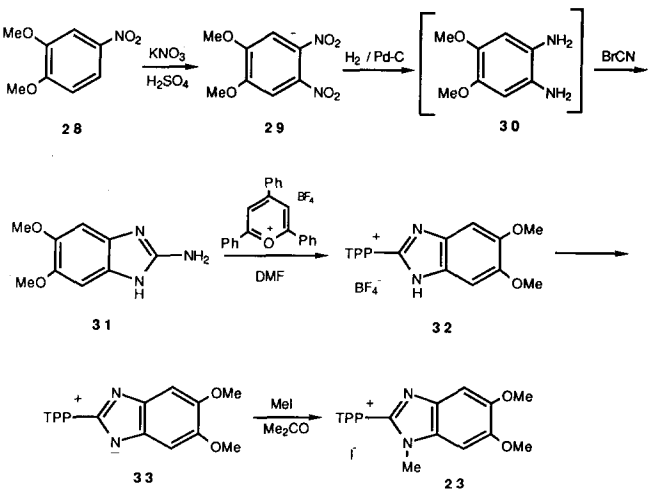
Cyanogen bromide (4.39 g, 4.14 mmol) was added portionwise to a cooled solution of 4-methoxy-1,2-phenylenediamine **26** (3.91 g, 2.76 mmol) in water (60 ml) under stirring and was heated at 50°C for 4 h. The mixture was made alkaline by the addition of 2 N sodium carbonate (250 ml) and a dark solid removed by filtration. The aqueous solution was washed with diethyl ether (2 x 25 ml) and was then extracted continuously with diethyl ether for 48 h. The organic layer was dried and evaporated under pressure to give 2.64 g (59%) of a white crystalline solid **27**: mp = 198–199°C. Lit [11] 198–200°C. TLC solvent system C. ^{1}H-NMR (DMSO-d_6): 6.76 (1H, H-7), 6.5 (1H, H-3), 6.25 (1H, H-6), 5.83 (2H, NH_2), 3.58 (6H, MeO).

1,2-Dimethoxy-4,5-dinitrobenzene ***29***

1,2-Dimethoxy-4-nitrobenzene **28** (10 g, 54.0 mmol) was dissolved in concentrated sulfuric acid (80 ml, d = 1.83), cooled to – 5°C and potassium nitrate (5.52 g, 54.0 mmol) was added portionwise, keeping the temperature between – 5–0°C. After standing for half an hour at 0°C, the solution was poured into ice water (350 ml) and yellow solid precipitated, yield 96%, mp = 114–115°C. Lit [18] 125–132°C, yield 95%. TLC solvent system C. ^{1}H-NMR (DMSO-d_6): 3.8 (6H, MeO), 7.5 (2H, H-3,6).

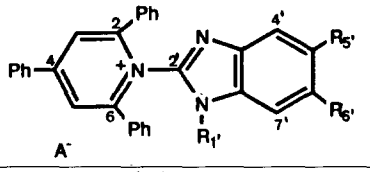
2-Amino-5,6-dimethoxybenzimidazole ***31***

A solution of 1,2-dimethoxy-4,5-dinitrobenzene **29** (4.4 g, 19.2 mmol) in methanol (350 ml), on PdC 10% (1.16 g) was shaken under hydrogen at room temperature and pressure until absorption ceased. The catalyst was filtered off under an atmosphere of nitrogen. The filtrate has shown, by TLC and by ^{1}H-NMR of an aliquot one spot and signals corresponding to 4,5-diamino-1,2-dimethoxybenzene **30**, which immediately decomposed in air.



Scheme 2.

Table III. ^{1}H-NMR chemical shifts (δ, ppm) of compounds **11**, **15**, **21**, **22**, **23**, **32–33**.



Compd	R-1'	R-5'	R-6'	A⁻	Ph-2,6	Ph-4 Ho	Ph-4 Hm,p	H-3,5	R-1'	H-4'	H-7'	R-5'	R-6'
11	H	MeO	H	BF_4^-	7.35-7.73	8.41	7.35-7.73	8.76	a	6.98	7.60	3.72	6.79
15	-	MeO	H		7.19-7.73	8.32	7.19-7.73	8.48		6.71	7.05	3.63	6.38
21	Me	MeO	H	I^-	7.36-7.74	8.46	7.36-7.74	8.87	3.43	7.08	7.36-7.74	3.72	6.92
22	Me	H	MeO	I^-	7.32-7.80	8.45	7.32-7.80	8.86	3.40	7.46	6.95	3.74	6.85
23	Me	MeO	MeO	I^-	7.38-7.75	8.41	7.38-7.75	8.82	3.45	7.10	7.00	3.73*	3.76*
32	H	MeO	MeO	BF_4^-	7.41-7.71	8.32	7.41-7.71	8.74	12.5[b]	7.04	7.04	3.72	3.72
32[c]	H	MeO	MeO	BF_4^-	7.25-7.60	8.22	7.25-7.60	8.60	a	6.93	6.93	3.64	3.64
33	-	MeO	MeO		7.50-7.78	8.25	7.50-7.78	8.43		6.79	6.79	3.66	3.66

*Values can be interchanged. [a]Signal not observed. [b]Broad band. [c]80% DMSO-d_6-20% CF_3COOH.

In view of the relative ease of decomposition of compound **30**, cyanogen bromide (3.07 g, 28.9 mmol) was added to the methanolic solution after removing the catalyst and it was stirred at room temperature for 21 h. The reaction mixture was evaporated to dryness, and the residue made basic with 2 N sodium carbonate. The aqueous solution was washed with chloroform (5 x 25 ml) and then evaporated to dryness. The crude product **31** was treated with absolute ethanol (80 ml) and insoluble material filtered off. The ethanolic solution was evaporated to dryness to give 3.47 g (93%) of compound **31** mp = 173–175°C. TLC solvent system C. ^{1}H-NMR (DMSO-d_6): 3.5 (6H, MeO), 5.6 (2H, NH_2), 6.6 (2H, H-4,7).

Procedures for N-(benzimidazol-2-yl)-2,4,6-triphenylpyridinium salts and pyridinium benzimidazolate inner salts (table V)

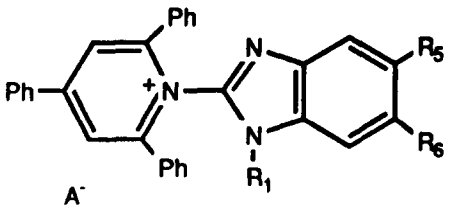
Method A To a solution of 2-aminobenzimidazole **27** or **31** (12 mmol) in 10 ml of anhydrous DMF was added the 2,4,6-triphenylpyrylium tetrafluoroborate (4.0 g, 10 mmol). The reaction mixture was refluxed under stirring for the time specified in table VI and then cooled and washed with ethyl ether (3 x 15 ml) to give an oil that solidified on trituration with water. The precipitate of compound **11** was recrystallized (table V) while compound **32** was dissolved in 70% ethanol (50 ml) and then passed through the hydroxide form of an anion-exchange resin (method B). The orange-red eluate was evaporated to dryness and recrystallized in absolute ethanol with addition of a few drops of tetrafluoroboric acid (54% in diethyl ether) to give yellow crystals of **32** (table IV) TLC solvent system A.

Method B A solution of *N*-benzimidazolylpyridinium salts **11** and **32** in 70% ethanol was passed through a column packed with anion-exchange resin Amberlite IRA-401 (OH^- form). The neutral orange to red eluates were evaporated to dryness and then recrystallized (table IV). This experimental procedure has been previously reported in detail [1, 16].

Method C A solution of methyl iodide (1.04 g, 7.28 mmol) in anhydrous acetone (10 ml) was added dropwise at 0–5°C to a stirred suspension of 2,4,6-triphenylpyridinium benzimidazolate inner salts **15** or **33** (1.82 mmol) in anhydrous acetone (50 ml) under an atmosphere of nitrogen, and then allowed to stand at room temperature for the time specified in table IV.

The resulting solution was evaporated to dryness, and washing the residue with diethyl ether gave a mixture of the *N*-methyl isomers **21** (5-methoxy) and **22** (6-methoxy), the ^{1}H-NMR in DMSO-d_6 of an aliquot of the residue has shown that it was a mixture of the *N*-methyl isomers **21** (45%) and **22** (55%), TLC (THF-ethyl ether 7.5:2.5). In the same way compound **23** (5,6-dimethoxy) was obtained, TLC solvent system B. The mixture of **21** + **22** was recrystallized twice from absolute ethanol to give compound **22** in a pure form. The mother liquor from the crystallization was evaporated to dryness and the residue which is enriched with the isomer **21** was recrystallized successively from carbon tetrachloride to afford 9 mg of compound **21** (table IV). It is noteworthy that compound **21** decomposes under the conditions of recrystallization (compound **21** has not been possible to obtain suitable amount which precludes the *in vivo* anti-leishmanial evaluation).

Table IV. Physical data of *N*-benzimidazolylpyridinium salts and pyridinium benzimidazolate inner salts.



Compd	R_1	R_5	R_6	A^-	m.p.,°C[a]	recrys solvent[b]	yield[c] (%)	Method[d]	reaction time(h)	mol. formula[e]
11	H	MeO	H	BF_4^-	163-4	A	48	A	0.75	$C_{31}H_{24}N_3OBF_4\ H_2O$
15	-	MeO	H	-	237-8	B	96	B	f	$C_{31}H_{23}N_3O\ H_2O$
21+22	Me	{MeO;H}		I^-	--	-	89	C	15	----
21	Me	MeO	H	I^-	--	f	-	-	-	----
22	Me	H	MeO	I^-	232	C	-	-	-	$C_{32}H_{26}N_3OI\ H_2O$
32	H	MeO	MeO	BF_4^-	244-5	B	47	A	0.75	$C_{32}H_{26}N_3O_2BF_4$
33	-	MeO	MeO	-	121-2	C	97	B	f	$C_{32}H_{25}N_3O_2$
23	Me	MeO	MeO	I^-	217-8	C	80	C	17	$C_{33}H_{28}N_3O_2I\ 2H_2O$

[a]Melting points are uncorrected. [b]A = 2-propanol-hexane, B = 70% ethanol, C = absolute ethanol. [c]Yields not optimized. [d]See *Chemistry* section. [e]Elemental analysis for C, H and N were within ± 0.4% of theoretical values. [f]See *Experimental protocols* section.

Biological evaluation

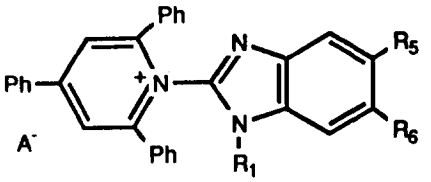
Determination of *in vitro* anti-leishmanial activity was carried out as described previously [1].

Activity *in vivo* of compound **22** was determined with *Leishmania donovani* in Balb/c mice by the method of Hunter and Coombs [19]. Compound **22** proved considerably more toxic than the previous ones in the series reported in the previous paper [1]. Thus, when infected mice were treated intraperitoneally with **22** at 200 mg (kg body weight)$^{-1}$ suspended in gum tragacanth, all six were dead by the next morning. Consequently, a group of five infected mice were treated with the compound at 50 mg (kg body weight)$^{-1}$. All mice showed signs of drug toxicity and therefore drug treatment was stopped. The experiment was allowed to run its course and the parasite load determined in the usual way.

The results are given in table VI. Four of the five treated mice had a lower parasite load than the control mice, although in no case were all parasites eliminated. The results are expressed as the percentage reduction in the number of parasites per 100 host cell nuclei compared with the mean parasite burden of the control group.

Treatment regime: four daily doses of pentostam at 100 mg (kg body weight)$^{-1}$ and one dose of compound **22** at 50 mg (kg body weight)$^{-1}$.

Table V. Growth inhibition percentages of *Leishmania donovani* promastigote forms[a,b].



					24 h			48 h			72 h		
µg/ml					100	10	1	100	10	1	100	10	1
Compound	A^-	R-1	R-5	R-6									
11	BF_4^-	H	MeO	H	84.4	23.5	10.0	83.5	29.6	12.0	89.5	58.0	28.0
15		-	MeO	H	27.5	3.5	0	29.0	6.6	0	38.0	14.0	5.0
21	I^-	Me	MeO	H	100	100	100	-	-	-	-	-	-
22	I^-	Me	H	MeO	100	55.6	44.5	100	96.1	69.2	100	100	100
32	BF_4^-	H	MeO	MeO	77.9	47.0	27.9	94.9	41.6	12.2	91.3	20.7	0
33		-	MeO	MeO	55.9	44.1	32.3	86.3	42.0	37.9	95.0	46.0	41.7
23	I^-	Me	MeO	MeO	85.3	63.2	32.2	100	77.7	39.2	100	67.4	51.4
Glucantime					25.0	7.0	2.0	20.0	13.0	7.0	39.5	20.4	10.5

[a]Average number from 5 different experiments. SME > 10%. [b]See *Experimental protocols* section.

Drug suspended in gum tragacanth and administered by the intraperitoneal route. Parasite load determined five days after termination of pentostam treatment.

Table VI. Anti-leishmanial *in vivo* activity of compound **22**. See *Experimental protocols* section.

Compd	*Group*	*Parasite load parasites (100 host cell nuclei)$^{-1}$ means ± SD*
None	6	106 ± 18
22	5	51 ± 29
Pentostam	6	0

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References

1 Alcalde E, Dinarés I, Elguero J, Frigola J, Osuna A, Castanys S (1990) *Eur J Med Chem* 25, 309–319

2 Hambloch H, Frahm AW, Wiedemann B (1985) *Eur J Med Chem* 20, 71–77

3 Sparatore F, Caliendo G, La Rotonda MI, Novellino E, Silipo C, Vittoria A (1988) *Quant Struc Act Relat* 7, 178–183
4 Mathias LJ, Overberger CG (1978) *J Org Chem* 43, 3526–3530
5 Fruchier A, Pappalardo L, Elguero J (1980) *An Quim* 76, 79–84
6 Lopyrev VA, Larina LI, Vakul'skaya TI, Larin MF, Nefedova OB, Shibanova EF, Voronkov MG (1981) *Org Magn Reson* 15, 219–224
7 Lopyrev VA, Larina LI, Vakul'skaya TI, Larin MF, Shibanova EF, Titova IA, Voronkov MG (1985) *Magn Reson Chem* 23, 301–304
8 Nishikawa J, Tori K (1984) *J Med Chem* 27, 1657–1663
9 Breitmaier E, Voelter W (1987) *In: Carbon-13 NMR Spectroscopy* 3rd ed, Verlag Chemie, Weinheim
10 Bergeim FH, Losee K, Lott WA(1947) *J Am Chem Soc* 69, 583–587
11 Basaglia L, Mariani B (1963) *Ann Chim* 53, 755–763
12 Bellasio E, Campi A, Trani A, Baldoni E, Caravaggi AM, Nathansohn G (1973) *Il Farmaco, Ed Sc* 28, 164–182
13 Joseph J (1963) *J Med Chem* 6, 601
14 Bellasio E, Ger Offen 2,353,163 (1974) (*CA* 81, 77920e)
15 Skaletzky LL US 3,928,596 (1975) (*CA* 84, 74270u)
16 Alcalde E, Dinarés I, Elguero J, Fayet JP, Vertut MC, Miravitlles C, Molins E (1987) *J Org Chem* 52, 5009–5015
17 Dimroth K, Reichardt C, Vogel K (1969) *Org Synth* 49, 121–124
18 Weinberger L, Day AR (1959) *J Org Chem* 24, 1451–1455
19 Hunter CA, Coombs GH (1987) *Med Sci Res* 15, 1233–1234