

Short communication

Functional derivatives of 5-benzo[1,3]dioxol-5-yl-1-methyl-1*H*-imidazole-2-carbaldehyde and evaluation of leishmanicidal activity

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Abstract – New imidazole derivatives were obtained after subjecting 5-benzo[1,3]dioxol-5-yl-1-methyl-1*H*-imidazole-2-carbaldehyde to a Knoevenagel reaction. Some of these compounds exhibit significant in vitro leishmanicidal activity. © 2000 Éditions scientifiques et médicales Elsevier SAS

imidazoles / benzodioxole / antiparasitic agents / leishmanicidal activity

1. Introduction

Leishmaniasis is a major parasitic disease: the true incidence and prevalence of leishmaniasis is uncertain because many cases go undiagnosed or unreported in areas where the infection is endemic. Recently, the World Health Organization estimated that 350 million people worldwide were at risk of infection [1]. The causative agents of leishmaniasis are various species of the protozoa *Leishmania*, belonging to the family of Trypanosomatidae, giving rise to skin manifestations and more importantly, systemic involvement of the reticulo-endothelial system [2]. Moreover, visceral leishmaniasis is now a particularly pertinent problem because the leishmaniasis have clearly been shown to be opportunistic manifestations in the acquired immunodeficiency syndrome [3]. If progress has been made in the drug treatment of leishmaniasis, resistance to pentavalent antimonials is increasing and unacceptable toxicity at effective therapeutic doses is described for second-line drugs such as pentamidine and amphotericin B [4, 5]. Also, better drugs are still required.

The pharmacological interest of the imidazole ring has been established, nitroimidazoles being extensively used in therapy against amoebic, trichomonal, giardial and anaerobic infections, or as antabuse agents, or as hypoxic cell radiosensitizers [6]. Metronidazole and *N*-substituted imidazoles (ketoconazole, fluconazole and itraconazole) are well-tolerated drugs that are potentially active against *Leishmania*, but their use in the treatment of cutaneous and visceral leishmaniasis has produced conflicting results [7]. Derivatives of heterocyclic aldehydes have been found to have potent cytotoxic effects against various *Leishmania* strains [8, 9]. Our interest in imidazole chemistry led us to synthesize new functional derivatives of 5-benzo[1,3]dioxol-5-yl-1-methyl-1*H*-imidazole-2-carbaldehyde and evaluate their antiparasitic properties.

2. Chemistry

6-Nitropiperonal was converted into the methylimine **1** by condensation with methylamine in ethanol. The imidazole ring was obtained by reacting equimolar quantities of tosylmethylisocyanide (TosMIC) and aldimine with K₂CO₃ in refluxing methanol [10]. The metalation of heterocyclic ring systems by organolithium reagents is

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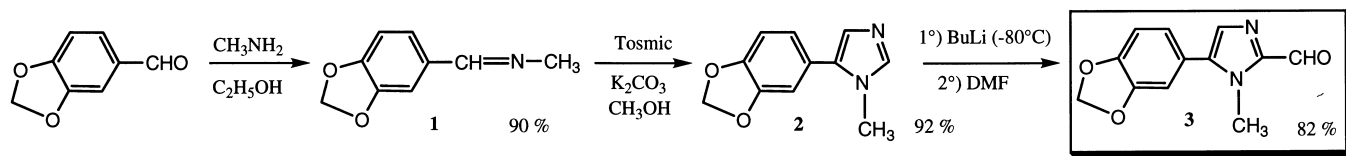


Figure 1. Synthesis of aldehyde **3**.

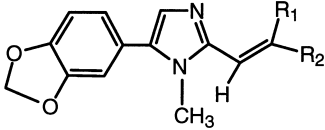
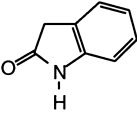
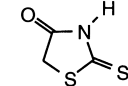
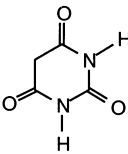
frequently a useful method of placing substituents in positions not available in substitution by most reagents [11]. So, metalation of **2** with *n*-butyllithium followed by addition of dimethylformamide produced the aldehyde **3** (figure 1).

The classical Knoevenagel reaction of **3** with various derivatives containing active methylene groups, in the presence of catalytic amounts of piperidine, and by refluxing in appropriate solvent [12], gave original imidazoles **4a–f** bearing a trisubstituted double bond at the 2 position (table I). The configuration of derivative **4a** has been determined by its ^{13}C sequence with $3J$ coupling: this showed *trans* coupling between the ethylenic proton

and the carbon of the nitrile group (two signals at 116 ppm) and *cis* coupling between the same ethylenic proton and the carbon of the amide group (two signals at 163 ppm). For extension, the *E* configuration has been attributed to other compounds.

For obtaining new, more soluble imidazoles, we have studied the deprotection of some derivatives with boron tribromide at -80°C in dichloromethane [13]. The required diphenols **5a–b** were obtained in good yields and in the case of derivative **5b**, the opening of the dioxole ring was accompanied by hydrolysis of the ester groups (figure 2).

Table I. Experimental conditions for synthesis of derivatives **4**.

					
R ₁	R ₂	Solvent of reaction	Time of reaction (h)	No.	Yield (%)
CN	CONH ₂	ethanol	16	4a	87
COOMe	COOMe	benzene	16	4b	84
CH ₃	NO ₂	ether	48	4c	72
		benzene	0.5	4d	57
					
		benzene	0.5	4e	60
					
		ether	0.5	4f	63
					

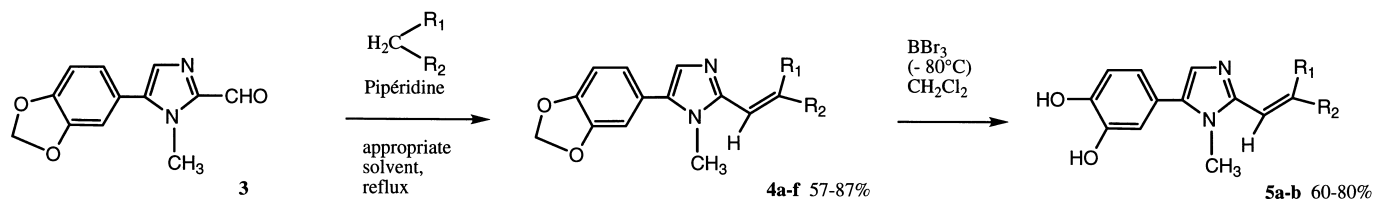


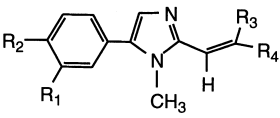
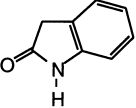
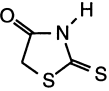
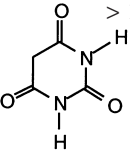
Figure 2. Synthesis of original imidazoles **4a-f** and diphenols **5a-b**.

3. In vitro results and discussion

The new compounds were tested in vitro against promastigotes of two *Leishmania* strains as reported in table II. Derivatives **4b** and **4c** showed considerable leishmanicidal activity: these were, respectively, five and twenty times more active than pentamidine. Derivative **3** was slightly inferior with an MIC five times higher, while other compounds were inactive.

As some derivatives were more efficient against protozoa than the starting aldehyde **3**, this confirms our interest in synthesizing new aldehyde derivatives. The leishmanicidal activity was modulated by the substituent at the 2-position of the imidazole nucleus. Compound **4c** with a nitro group was more efficient than compound **4b** with ester groups. Incorporation of a heterocyclic group decreased the leishmanicidal activity. The presence of an

Table II. Leishmanicidal activity of functional derivatives of 5-benzo[1,3]dioxol-5-yl-1-methyl-1*H*-imidazole-2-carbaldehyde.

						
No.	R ₁	R ₂	R ₃	R ₄	MIC (μg/mL) <i>L. infantum</i> strain 1	MIC (μg/mL) <i>L. infantum</i> strain 2
4a	OCH ₂ O		CN	CONH ₂	> 100	> 100
5a	OH	OH	CN	CONH ₂	> 100	> 100
4b	OCH ₂ O		COOMe	COOMe	1	1
5b	OH	OH	COOH	COOH	> 100	> 100
4c	OCH ₂ O		CH ₃	NO ₂	0.25	0.25
4d	OCH ₂ O				> 100	> 100
4e	OCH ₂ O				> 100	> 100
4f	OCH ₂ O				> 100	> 100
3					25	25
Pentamidine (isethionate)					5	5

amide or nitrile group resulted in similar decreases in the protozoal activity. The importance of a dioxole nucleus was also noted. Deprotection of the dioxole ring or hydrolysis of ester groups were detrimental to leishmanicidal activity, derivative **5b** being inactive.

The high in vitro leishmanicidal activity of **4c** makes this compound a promising lead for the development of an effective therapeutic agent. However, this study will be completed by additional tests in amastigote/macrophage in vitro models or in vivo mouse models. Synthesis of various analogues of **4c** and study of the mechanism of action of these new imidazoles are under active investigation in our laboratories.

4. Experimental protocols

4.1. Chemistry

Melting points were taken on a Büchi capillary melting point apparatus and are uncorrected. Both ^1H - and ^{13}C -NMR spectra were recorded on a Bruker ARX 200 MHz and AMX 400 MHz spectrometer. The ^1H - chemical shifts are reported as parts per million downfield from tetramethylsilane (Me_4Si), and the ^{13}C - chemical shifts were referenced to the solvent peaks: CDCl_3 (77.1 ppm) or $\text{DMSO}-d_6$ (39.6 ppm). Absorptions are reported with the following notations: s, singlet; d, doublet; t, triplet; q, quartet; m, a more complex multiplet or overlapping multiplets. Microanalyses for C, H, N were performed by the Microanalytical Section of St-Jérôme Faculty, Aix-Marseille 3 University and were within $\pm 0.4\%$ of theoretical values.

4.1.1. Benzo[1,3]dioxol-5-ylmethylene-methyl-amine **1**

A solution of benzo[1,3]dioxole-5-carbaldehyde (12 g, 0.08 mol) in ethanol (80 mL) was added dropwise under nitrogen atmosphere to a solution of methylamine (40% solution in water, 10 g, 0.128 mol) in ethanol (160 mL). After 24 h refluxing, the reaction mixture was evaporated. The solid residue was dissolved in water and the aqueous layer was extracted with dichloromethane. The combined organic layers were dried over MgSO_4 and removed under reduced pressure. The crude solid was recrystallized from methanol–water (1:1) to yield 11.78 g (90%) of **1**, beige solid, m.p. 44–46 °C, ^1H -NMR (CDCl_3 , 200 MHz) δ 3.42 (s, 3H, NCH_3); 5.94 (s, 2H, OCH_2O); 6.77 (d, $J = 8.0$ Hz, 1H, arom. H); 7.02 (dd, $J = 8.0$ and 1.6 Hz, 1H, arom. H); 7.28 (d, $J = 1.6$ Hz, 1H, arom. H); 8.09 (s, 1H, $\text{CH}=\text{N}$). Anal. $\text{C}_9\text{H}_9\text{NO}_2$ (C, H, N, O).

4.1.2. 5-Benzo[1,3]dioxol-5-yl-1-methyl-1H-imidazole **2**

Solid K_2CO_3 (3.5 g, 25.3 mmol) was added under nitrogen atmosphere to a solution of **1** (2.08 g, 12.75 mmol) and TosMIC (5 g, 25.6 mmol) in dry methanol (20 mL). After stirring at room temperature for 0.5 h, the reaction mixture was refluxed for 48 h. After cooling, the solvent was removed under reduced pressure and the solid residue was washed with a saturated NaCl solution, followed by dichloromethane. The combined organic layers were dried over MgSO_4 and evaporated. The residue was recrystallized from chloroform to give **2** (2.4 g, 92%), yellow solid, m.p. 154–156 °C, ^1H -NMR (CDCl_3 , 200 MHz) δ 3.61 (s, 3H, NCH_3); 6.00 (s, 2H, OCH_2O); 6.83 (m, 3H, arom. H); 7.00 (s, 1H, H-2 imidazole); 7.46 (s, 1H, H-4 imidazole). Anal. $\text{C}_{11}\text{H}_{10}\text{N}_2\text{O}_2$ (C, H, N, O).

4.1.3. 5-Benzo[1,3]dioxol-5-yl-1-methyl-1H-imidazole-2-carbaldehyde **3**

A solution of *n*-butyllithium 1.6 M in hexane (24 mL) was added dropwise to a cooled (–80 °C) and stirred solution of **2** (5.13 g, 25.37 mmol) in anhydrous tetrahydrofuran (50 mL). After 1.5 h stirring, the solution was quenched with dimethylformamide (2.2 mL, 27.90 mmol). When addition was completed, the reaction was allowed to warm to room temperature and ether (100 mL) was added. The reaction mixture was extracted with 1 N HCl solution. After basification with a sodium hydrogen carbonate solution and extraction with dichloromethane, the solvent was dried over MgSO_4 and evaporated. The crude solid was purified by column chromatography on silica gel with a mixture of chloroform–acetone (7:3) and recrystallized from chloroform to give **3** (4.78 g, 82%) as yellow solid, m.p. 146–148 °C, ^1H -NMR (CDCl_3 , 200 MHz) δ 3.97 (s, 3H, NCH_3); 6.07 (s, 2H, OCH_2O); 6.90 (m, 3H, arom. H); 7.29 (s, 1H, H imidazole); 9.84 (s, 1H, CHO). Anal. $\text{C}_{12}\text{H}_{10}\text{N}_2\text{O}_3$ (C, H, N, O).

4.1.4. General procedure for Knoevenagel reactions

Under nitrogen atmosphere, a mixture of **3** (0.3 g, 1.3 mmol) and the appropriately substituted derivative in ethanol (10 mL), benzene (10 mL), or ether (110 mL), was refluxed in the presence of two drops of piperidine for an appropriate time. Three experimental conditions were used:

– protocol A: after cooling, the reaction mixture was poured into ice and the solid formed was collected and recrystallized from chloroform.

– protocol B: after cooling, the solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel with a mixture of

chloroform–acetone (7:3) and recrystallized from the appropriate solvent.

– protocol C: after cooling, the reaction mixture was poured into water. The organic layer was separated and the aqueous layer was extracted with ether. The combined organic layers were dried over MgSO_4 and removed under reduced pressure. Purification by column chromatography on silica gel with a mixture of chloroform–acetone (7:3) and recrystallization from ethanol– H_2O (1:1) gave the required compound.

4.1.4.1. 3-(5-Benzo[1,3]dioxol-5-yl-1-methyl-1H-imidazol-2-yl)-2-cyano-acrylamide **4a**

Protocol A; yield = 87%; yellow solid, m.p. > 260 °C (CHCl_3); $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, 400 MHz) δ 3.75 (s, 3H, NCH_3); 6.10 (s, 2H, OCH_2O); 7.01 (dd, J = 8.0 and 1.6 Hz, 1H, arom. H); 7.08 (d, J = 8.0 Hz, 1H, arom. H); 7.14 (d, J = 1.6 Hz, 1H, arom. H); 7.43 (s, 1H, H imidazole); 7.70 (broad s, 1H, NH); 7.86 (broad s, 1H, NH); 7.96 (s, 1H, H ethylenic). $^{13}\text{C-NMR}$ ($\text{DMSO-}d_6$, 100 MHz) δ 31.57, 101.61, 104.18, 108.85, 109.10, 116.25, 122.11, 123.06, 131.11, 133.47, 137.49, 141.11, 147.82, 147.89, 163.17. Anal. $\text{C}_{15}\text{H}_{12}\text{N}_4\text{O}_3$ (C, H, N, O).

4.1.4.2. 2-(5-Benzo[1,3]dioxol-5-yl-1-methyl-1H-imidazol-2-ylmethylene)-malonic acid dimethyl ester **4b**

Protocol B; yield = 84%; yellow solid, m.p. = 212–214 °C (*n*-hexane); $^1\text{H-NMR}$ (CDCl_3 , 200 MHz) δ 3.68 (s, 3H, NCH_3); 3.85 (s, 3H, OCH_3); 3.95 (s, 3H, OCH_3); 6.02 (s, 2H, OCH_2O); 6.85 (m, 3H, arom. H); 7.20 (s, 1H, H imidazole); 7.55 (s, 1H, H ethylenic). Anal. $\text{C}_{17}\text{H}_{16}\text{N}_2\text{O}_6$ (C, H, N, O).

4.1.4.3. 3-(5-Benzo[1,3]dioxol-5-yl-1-methyl-1H-imidazol-2-yl)-2-nitro-acrylamide **4c**

Protocol C; yield = 72%; orange–red solid, m.p. = 220–222 °C (ethanol– H_2O , 1:1); $^1\text{H-NMR}$ (CDCl_3 , 200 MHz) δ 2.84 (s, 3H, CH_3); 3.71 (s, 3H, NCH_3); 6.04 (s, 2H, OCH_2O); 6.86 (m, 3H, arom. H); 7.29 (s, 1H, H imidazole); 7.93 (s, 1H, H ethylenic). Anal. $\text{C}_{14}\text{H}_{13}\text{N}_3\text{O}_4$ (C, H, N, O).

4.1.4.4. 3-(5-Benzo[1,3]dioxol-5-yl-1-methyl-1H-imidazol-2-ylmethylene)-1,3-dihydro-indol-2-one **4d**

Protocol B; yield = 57%; yellow solid, m.p. = 252–254 °C (CHCl_3); $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, 200 MHz) δ 3.83 (s, 3H, NCH_3); 6.06 (s, 2H, OCH_2O); 6.93 (m, 3H, H aromatic); 7.35 (m, 2H, H indole); 7.46 (s, 1H, H imidazole); 7.60 (s, 1H, H ethylenic); 8.33 (dd, J = 8.3 and 1.0 Hz, 1H, H indole); 9.62 (dd, J = 8.9 and 1.4 Hz, 1H, H indole). Anal. $\text{C}_{20}\text{H}_{15}\text{N}_3\text{O}_3$ (C, H, N, O).

4.1.4.5. 5-(5-Benzo[1,3]dioxol-5-yl-1-methyl-1H-imidazol-2-ylmethylene)-2-thioxo-thiazolidin-4-one **4e**

Protocol B; yield = 60%; red solid, m.p. > 260 °C (CHCl_3); $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, 200 MHz) δ 3.78 (s, 3H, NCH_3); 6.10 (s, 2H, OCH_2O); 7.07 (m, 3H, H aromatic); 7.44 (s, 1H, H imidazole); 7.51 (s, 1H, H ethylenic); 13.60 (s, 1H, NH). Anal. $\text{C}_{15}\text{H}_{11}\text{N}_3\text{O}_3\text{S}_2$ (C, H, N, O).

4.1.4.6. 5-(5-Benzo[1,3]dioxol-5-yl-1-methyl-1H-imidazol-2-ylmethylene)-pyrimidine-2,4,6-trione **4f**

Protocol B; yield = 63%; red solid, m.p. > 260 °C (CHCl_3); $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, 200 MHz) δ 3.61 (s, 3H, NCH_3); 6.11 (s, 2H, OCH_2O); 7.03 (m, 3H, arom. H); 7.23 (s, 1H, H imidazole); 7.68 (s, 1H, H ethylenic); 9.70 (broad s, 2H, NH). Anal. $\text{C}_{16}\text{H}_{12}\text{N}_4\text{O}_5$ (C, H, N, O).

4.1.5. General procedure for deprotection

A solution of boron tribromide (1 M in dichloromethane, 4 mmol) was added to a cold (–80 °C) and stirred solution of **4a–b** (1 mmol) in dichloromethane (10 mL). The reaction mixture was stirred at –80 °C for 2.5 h and quenched by the addition of methanol (10 mL). After the reaction was allowed to reach room temperature, the solvent was evaporated and the residue was co-evaporated three times with methanol. The final solid was recrystallized from chloroform.

4.1.5.1. 2-Cyano-3-[5-(3,4-dihydroxy-phenyl)-1-methyl-1H-imidazol-2-yl]-acrylamide **5a**

Yield = 60%; orange–red solid, m.p. = 160–162 °C (CHCl_3); $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, 200 MHz) δ 3.77 (s, 3H, NCH_3); 4.77 (broad s, 2H, OH); 6.90 (m, 3H, arom. H); 7.38 (s, 1H, H imidazole); 7.85 (broad s, 1H, NH); 7.90 (broad s, 1H, NH); 8.05 (s, 1H, H ethylenic). Anal. $\text{C}_{14}\text{H}_{12}\text{N}_4\text{O}_3$ (C, H, N, O).

4.1.5.2. 2-[5-(3,4-Dihydroxy-phenyl)-1-methyl-1H-imidazol-2-ylmethylene]-malonic acid **5b**

Yield = 80%; orange solid, m.p. = 230–232 °C (CHCl_3); $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, 200 MHz) δ 3.71 (s, 3H, NCH_3); 6.75 (broad s, 2H, OH); 6.87 (m, 3H, arom. H); 7.08 (s, 2H, COOH); 7.55 (s, 1H, H ethylenic); 7.58 (s, 1H, H imidazole). Anal. $\text{C}_{14}\text{H}_{12}\text{N}_2\text{O}_6$ (C, H, N, O).

4.2. Biology

Leishmanicidal activity was evaluated on *Leishmania infantum* strains (strain 1: MCAN/FR/74 LPMA 57; WHO and strain 2: MCAN/FR/73 LPMA 56; WHO). *Leishmania infantum* was originally isolated from the lymph ganglions of dogs in Marseille. These isolates contained numerous *Leishmania* amastigotes and were cultivated in NNN (Novy, Mac Neal, Nicolle) [14] and

Tobie [15] media where they were transformed into promastigote forms. These strains were maintained in continuous culture in RPMI 1640 (Gibco) containing 10% heat-inactivated foetal calf serum. Streptomycin (50 mg/L) and penicillin G (50 units/mL) were also added (these concentrations did not affect *Leishmania* growth).

Promastigotes (10^6 *Leishmania*/mL) were inoculated into tubes containing 5 mL of the above-described medium and incubated at 24 °C [16]. Subcultures were made once a week and each subculture was checked for abundance and motility of promastigote forms. They were counted with a Malassez cell and the volume of inoculum was adjusted to distribute 10^6 *Leishmania*/mL. The test compounds were first dissolved in dimethylformamide (10 mg/mL), then the appropriate volumes of this solution were added to the culture tubes to obtain final concentrations of 100 (50 µL); 50 (25 µL); 25 (12.5 µL); 10 (5 µL); 5 (2.5 µL); 1 (0.5 µL) and 0.5 (0.25 µL) mg/L. Dimethylformamide was completely inactive on the parasites at these concentrations. Each strain and each concentration was tested in triplicate. The minimal inhibitory concentrations (MIC) of the compounds were determined after the parasites had been in culture for 7 days by checking for the presence or absence of promastigotes microscopically ($\times 400$). The absence of promastigotes in the tubes was confirmed by retroculture. If the parasites did not recover, that concentration of a compound was considered leishmanicidal. The MIC for each compound was then compared with that of pentamidine isethionate (Pentacarinat®) determined under the same conditions.

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