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Synthesis and leishmanicidal activity of new 1-methyl-2-alkoxymethylimidazoles

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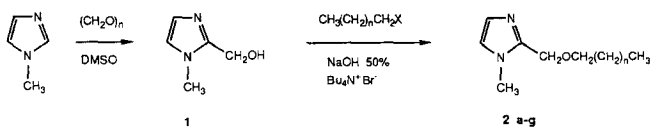
1-methyl-2-alkoxymethylimidazoles / phase-transfer catalysis / leischmanicidal activity

Introduction

The pharmacological importance of the imidazole ring has been established. The 5-nitroimidazoles are extensively used for the chemotherapy of anaerobic bacteria and protozoal diseases and also for the radiosensitization of hypoxic tumors [1]. The 1-substituted imidazoles are effective anti-fungal agents [2]. Recently the good antibacterial activity of a series of 1-alkylimidazoles has been reported [3]. Our interest in imidazole chemistry [4, 5] led us to synthesize new imidazole derivatives characterized by the presence of a long alkoxymethyl chain at the 2 position and explore their activity on 3 *Leishmania* strains.

Chemistry and pharmacology

The new imidazole derivatives **2a–g** are obtained in 2 steps: hydroxymethylation of 1-methylimidazole and alkylation of 1-methyl-2-hydroxymethylimidazole **1** with the appropriate alkyl halides under phase-transfer catalysis.



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The 1-methyl-2-hydroxymethylimidazole **1** was synthesized for the first time by Grindley and Pyman [6] by reacting 1-methylimidazole and 40% aqueous formaldehyde solution at 140°C in a sealed tube with 63% yield. Jocelyn [7] has modified this experimental procedure by bubbling formaldehyde through 1-methylimidazole at 160–170°C, but the yield decreased (36%). We heated at 110°C 1-methylimidazole with an excess of paraformaldehyde in dimethylsulfoxide in a sealed tube for 48 h and obtained 1-methyl-2-hydroxymethylimidazole **1** with 75% yield.

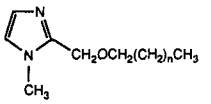
The phase transfer catalysed reaction of alkoxide or phenoxide anions and halides has been widely used to prepare a variety of mixed alkyl or aryl alkyl ethers [8–10]. We have investigated the etherification of 1-methyl-2-hydroxymethylimidazole by phase-transfer catalysis. We used a 25-fold excess of 50% aqueous NaOH to alcohol and tetrabutylammonium bromide (TBAB) as catalyst (0.1 equiv for 1 equiv of alkyl halide). By using a stoichiometric amount of alkyl halide and alcohol, the reaction was completed in 8 h at 80°C, giving in good yields the new 1-methyl-2-alkoxymethylimidazoles **2a–g** (table I).

The new compounds were identified by ^{1}H NMR analysis and their purity established by TLC and microanalysis. These compounds were tested *in vitro* against promastigotes of 3 *Leishmania* strains (table I).

Results and discussion

All the compounds showed good activity against the 3 *Leishmania* strains. The highest activity on *Leish-*

Table I. Properties and leishmanicidal activities against *Leishmania* strains of 1-methyl-2-alkoxymethylimidazoles **2**.



No	*n*	*bp (°C/mmHg) or mp (°C)*	*Yield (%)*	*MIC (mg/l)*		
				Leishmania tropica	*Leishmania infantum* Strain 1	*Leishmania infantum* Strain 2
2a	7	132/0.04	92	10	10	10
2b	8	140/0.03	92	1	1	1
2c	9	138/0.05	93	1	1	1
2d	10	160/0.04	92	1	1	1
2e	11	36	90	0.5	0.5	0.5
2f	12	40	95	1	1	1
2g	13	36	92	5	5	5
2h	14	50	95	10	10	10
Pentamidine				5	5	5

mania was displayed by the compound **2e** (MIC = 0.5 mg/l), 10-fold more active than the reference compound, pentamidine (MIC = 5 mg/l). Derivatives **2b**, **2c**, **2d**, **2f** were also of interest with MIC value 5-fold lower than that of pentamidine. The derivative **2g** was as active and **2a** and **2h** 50% as active as pentamidine.

The results showed the influence of chain length at the 2 position of the imidazole ring. The leishmanicidal activity is greater for the compound **2e** which bears an alkoxymethyl chain with 14 carbon atoms. Activity is reduced by increasing or decreasing the chain length. These structure–activity relationships suggest that the ability of the imidazole ring to bind with the active site and to adopt a conformation able to promote the migration through the membranes of *Leishmania* may play a role in determining the leishmanicidal activity of this series [3].

In conclusion, we have described the preparation of a new series of imidazole derivatives which exhibit good *in vitro* leishmanicidal activity. Further studies on these agents are in progress.

Experimental protocols

Chemistry

Melting points were taken on a Büchi apparatus using glass capillary tubes and are uncorrected.

^{1}H NMR spectra were recorded on a Bruker 200 MHz instrument and chemical shifts are reported in δ units (ppm) relative to internal TMS. Microanalyses for C, H, N were performed by the microanalytical section of the St-Jérôme Faculty and were within ± 0.4% of theoretical values.

1-Methyl-2-hydroxymethylimidazole ***1***

A mixture of 0.366 mol (30 g) of 1-methylimidazole and 0.6 mol (18 g) of paraformaldehyde in 100 ml of dimethylsulfoxide was heated in a sealed tube at 110°C for 48 h. The dimethylsulfoxide was removed completely at reduced pressure, 1-methyl-2-hydroxymethylimidazole crystallized and was recovered by filtration and washed with acetone. Recrystallization from ethanol gave 30.74 g (75%) of product. mp = 114°C (Lit [6] mp = 116°C). ^{1}H NMR ($CDCl_3$) δ 3.59 (s, 3H, NCH_3); 4.42 (s, 2H, CH_2); 5.34 (m, 1H, OH); 6.70 (d, J = 1.1 Hz, H imidazole); 7.00 (d, J = 1.1 Hz, H imidazole).

General procedure for the preparation of 1-methyl-2-alkoxymethylimidazole ***2***

In a 100-ml round-bottom flask were placed NaOH pellets (30 g, 0.75 mol) in water (30 ml).

1-Methyl-2-hydroxymethylimidazole (3.36 g, 0.03 mol) was then added, and maintained for 1 h at 80°C with stirring until the mixture became homogeneous. Tetrabutylammonium bromide (0.96 g, 0.0003 mol) and alkyl halide (0.03 mol) were added and stirring continued for 8 h at 80°C. The organic layer was separated, washed twice with 5-ml portions of water and dried over $MgSO_4$. The filtered solution was concentrated under reduced pressure and the residue was purified by distillation or chromatography on silica gel with ethyl acetate. For example, compound **2b** showed characteristic ^{1}H NMR spectrum: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 6.4 Hz, 3H, CH_2CH_3); 1.25 (m, 14H, CH_2); 1.56 (quint, J = 6.4 Hz, 2H, CH_2OCH_2-CH_2); 3.43 (t, J = 6.4 Hz, 2H, $CH_2OCH_2CH_2$); 3.69 (s, 3H, NCH_3); 4.56 (s, 2H, CH_2O); 6.86 (d, J = 1.1 Hz, H imidazole); 6.93 (d, J = 1.1 Hz, H imidazole).

Biology

Leishmania strains

The *Leishmania* strains were *Leishmania infantum*, strain 1 (MCAN/FR/74 LPMA 57. WHO); strain 2 (MCAN/ FR/73/ LPMA 56. WHO); and one strain of *L tropica* (MHOM/FR/65/ LPMA 59. WHO).

Isolation
Leishmania infantum was originally isolated from ganglia of dogs in Marseille and *Leishmania tropica* from a human case of cutaneous leishmaniasis with numerous typical ulcerations. These isolates containing numerous *Leishmania* amastigotes were cultivated in NNN (Novy, Mac Neal, Nicolle) [11] and Tobie [12] media where they were transformed into promastigote forms.

Experimental techniques
After 5 to 10 subcultures, the parasites were adapted to these media, after which they could be cultivated in sterile liquid nutrient broth medium (nutrient broth (Oxoid) 13 g, sodium chloride 3 g, distilled water 1 l) complemented by fresh rabbit blood [13] for screening the compounds.

Preparation of rabbit blood
This was aseptically collected by intracardiac puncture, quickly defibrinated and, before it was added to the culture medium, streptomycin (50 mg/l) and penicillin G (50 units/ml) were added (these concentrations did not affect *Leishmania* growth). The treated blood (1 ml) was then distributed to each tube containing medium.

Maintenance of strains in nutrient broth medium
After the addition of 1 ml of a solution containing 10^6 *Leishmania* promastigotes, to the medium, the tubes were incubated at 24°C. Subcultures were made once a week, each subculture being 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 antiparasitic tests
The test compounds were first dissolved in dimethylformamide (10 mg/ml) then distributed to the culture tubes to obtain final concentrations of 100; 50; 25; 10; 5; 1; 0.5 mgl/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 7 days in culture by checking microscopically (x 40) for the presence or absence of promastigotes. 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, determined under the same conditions.

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