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Preparation and in vitro antiprotozoan activity of new naphthoimidazolediones

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Summary — The original compound bearing the coplanar quinone and imidazole systems, 2-chloromethyl-4,9-dihydro-1-methyl-1H-naphtho[2,3-*d*]imidazol-4,9-dione reacted with various nitronate anions to afford, in moderate to good yields, new naphthoimidazolediones bearing a trisubstituted ethylenic double bond at the 2-position. These compounds were evaluated as potential antiprotozoan agents. Some derivatives were found to have a significant activity, but the naphthoquinone was found to be a less efficient pharmacophore than the nitro group.

naphthoimidazolediones / antiprotozoan activity / $S_{RN}1$

Introduction

Since the initial proposal by Kornblum [1] and Russell [2] of the radical chain mechanism put forward to explain the C-alkylation of nitronate anions by *p*-nitrobenzyl chloride and its designation as $S_{RN}1$ by Bunnett [3], there has been a marked development of the reaction both from a synthetic and mechanistic point of view. A very attractive feature of the $S_{RN}1$ reactions is that they proceed under mild conditions and produce excellent yields of pure products. Substitution proceeding by an $S_{RN}1$ mechanism at the sp^3 carbons attached to heterocyclic systems has been reported for the synthesis of new pharmacological compounds [4]. In particular, new 5-nitroimidazoles bearing a trisubstituted ethylenic double bond in position 2 have been prepared by reacting 1-methyl-2-chloromethyl-5-nitroimidazole with various nitronate anions by the $S_{RN}1$ mechanism, and these compounds have shown antiparasitic and antianaerobic activity which was clearly greater than that of metronidazole [5–7]. As conflicting toxicological studies have revealed that 5-nitroimidazoles have mutagenic or carcinogenic activities [8, 9], we developed research centered on the discovery of novel lead structures without nitro groups. As we extended the $S_{RN}1$ reactions to anthraquinone [10] and benzoquinone [11] series, we synthesized a new compound which bears the coplanar quinone and imidazole systems: 2-chloromethyl-4,9-dihydro-1-methyl-1H-naphtho[2,3-*d*]-imidazol-4,9-dione **3** and studied its reactivity with various nitronate anions to compare the influence of the quinone group on pharmacological activity. Moreover, synthesis and cytotoxicity of several 1,2-disubstituted naphtho[2,3-*d*]imidazol-4,9-diones have been recently described [12].

Chemistry and pharmacology

The starting material **3** was prepared following established procedures [13, 14] in three steps from the inexpensive and commercially available 2-amino-3-chloro-1,4-naphthoquinone.

This amine reacted with chloroacetylchloride, giving the corresponding amide **1** in 59% yield. Treatment of this amide with methylamine at refluxing ethanol afforded derivative **2**. The cyclization of **2** was obtained with hydrochloric acid generated in situ by acetylchloride and ethanol and yielded the required chloride **3**.

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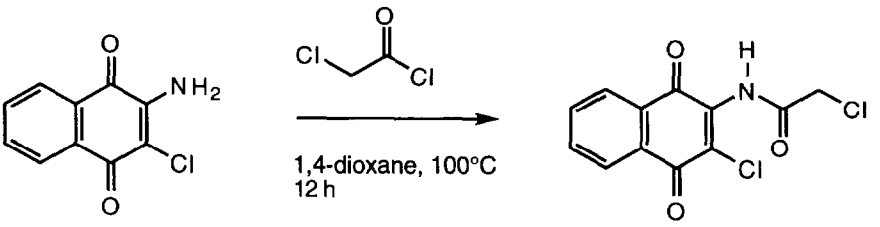
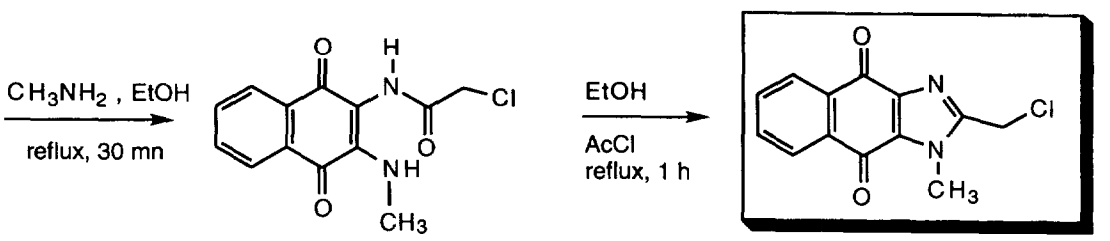


1 59%



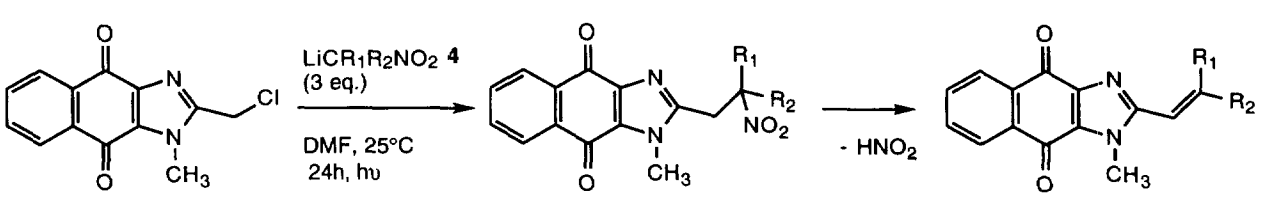
2 54%

3 75%

The nitroalkanes were commercially available (2-nitropropane, nitrocyclopentane, nitrocyclohexane) or prepared by oxidation of the corresponding primary amines with *m*-chloroperbenzoic acid [15] by refluxing in 1,2-dichloroethane for 3 h. The nitroalkane was purified by distillation or recrystallization and converted into lithium salts. Among heterocyclic nitronate anions, the 5-nitro-1,3-dioxane salt **4m** was studied and prepared from 2,2-dimethyl-5-hydroxymethyl-5-nitro-1,3-dioxane by the method [16] described previously from 2-hydroxymethyl-2-nitropropane-1,3-diol, acetone and boron trifluoride diethyl etherate. Treatment with lithium methoxide split off formaldehyde to give the corresponding salt. As 1-methyl-2-(1-methyl-pyrrolidin-2-one-3-ylidenemethyl) 5-nitroimidazole, showed an antiprotozoan and anti-anaerobic activity that was clearly greater than that of metronidazole, we investigated the $S_{RN}1$ reaction of **3** with the corresponding 3-nitrolactam anion. By using lithium diisopropylamide, prepared in situ from diisopropylamine and butyllithium as base, tetrahydrofuran as solvent and *n*-propyl nitrate as the nitrating agent, the lithium salt of 1-methyl-3-nitropyrrolidin-2-one **4n** was obtained from 1-methylpyrrolidin-2-one in 70% yield [17, 18].

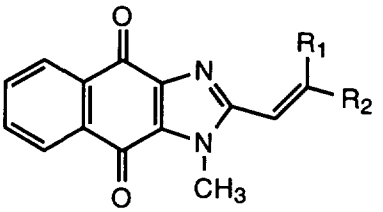
By using **3** as a model compound and an excess of nitronate anions (3 equivalents) at room temperature for 24 h, different experimental conditions conductive to $S_{RN}1$ reactions (inert atmosphere, light catalysis) were used. For aliphatic and cyclic nitronate anions **4a–l**, we utilized the conditions described by Kornblum [1] in dimethylformamide after rigorous degassing known as the freeze-pump-thaw procedure *(Method A)*. With **4m**, the reaction was carried out in methanol *(Method B)* while for **4n**, phase transfer catalysis using tetrabutylammonium bromide, water and toluene *(Method C)* was used.

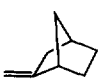
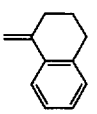
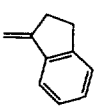
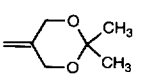
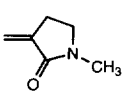
The reaction of **3** with various nitronate anions led in moderate to good yields (32–88%) to the new naphthoimidazolediones **6** bearing a trisubstituted double bond at the 2-position (table I). The weakest yield was obtained with 3-nitrolactam anion **4n** because contrarily to 5-nitroimidazole series [18], the final product **6n** was not partially soluble in the organic solvent and reacted with the nitronate anion, giving resinous products.



3 5 6

Table I. New 2-alkylidenemethyl-4,9-dihydro-1-methyl-1H-naphtho[2,3-*d*]imidazol-4,9-dione.



Compound No	*R_1*	*R_2*	*Isomer*	*Method*	*Yield (%)*	*Mp (°C)*	*Formula*
6a	CH_3	CH_3	–	A	58	210	$C_{16}H_{14}N_2O_2$
6b	$(CH_2)_4$		–	A	68	220	$C_{18}H_{16}N_2O_2$
6c	$(CH_2)_5$		–	A	54	205	$C_{19}H_{18}N_2O_2$
6d	$(CH_2)_6$		–	A	44	212	$C_{20}H_{20}N_2O_2$
6e	$(CH_2)_7$		–	A	56	218	$C_{21}H_{22}N_2O_2$
6f	$(CH_2)_{11}$		–	A	66	210	$C_{25}H_{30}N_2O_2$
6g			–	A	46	168	$C_{20}H_{18}N_2O_2$
6h			E	A	88	234	$C_{23}H_{18}N_2O_2$
6i			E	A	70	281	$C_{22}H_{16}N_2O_2$
6j	CH_3	C_6H_5	E	A	73	186	$C_{21}H_{16}N_2O_2$
6k	CH_3	$(CH_2)_2CH_3$	E	A	65	145	$C_{18}H_{18}N_2O_2$
6l	CH_3	$(CH_2)_3CH(CH_3)_2$	E	A	72	142	$C_{21}H_{24}N_2O_2$
6m			–	B	71	188	$C_{19}H_{18}N_2O_4$
6n			E	C	32	285	$C_{18}H_{15}N_3O_3$

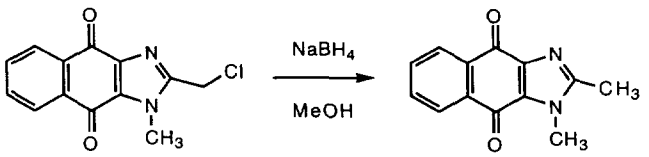
A: Kornblum conditions; B: reaction in methanol; C: phase transfer catalysis.

Under the reaction conditions, the C-alkylation product is not isolated, and the formation of **6** can be rationalized in terms of an initial $S_{RN}1$ reaction to give **5** as a highly reactive product which undergoes nitrous acid elimination, leading to the ethylenic compound **6**. Such behavior has already been observed in analogous 5-nitroimidazole systems [5] where elimination is very easy on position 2.

When the ethylenic derivative was unsymmetrical, only the E isomer was isolated. This selectivity may

be explained by favored conformations: for example in the compound **6j**, the conformation in which the aromatic ring is placed between two hydrogen atoms, is more stable than the conformation in which a steric hindrance is observed between the aromatic ring and quinoneimidazole system.

For comparison to dimetridazole, the chloride **3** was converted into 2-methyl-4,9-dihydro-1-methyl-1H-naphtho[2,3-*d*]imidazol-4,9-dione **3** by $NaBH_4$ in 88% yield.



3 **7** 88%

The new compounds were identified by ^{1}H-NMR analysis and their purity established by determination via TLC and microanalysis. These compounds were tested in vitro against *Trichomonas vaginalis* and *Leishmania infantum*.

Results and discussion

Table II shows the in vitro antiprotozoan activity of 15 target compounds. Compounds **6f**, **6m** and **7** displayed significant activity against *T vaginalis* and *L infantum*. The trichomonacidal activity was two-fold superior to that of reference metronidazole, whereas the leishmanicidal activity was five-fold higher than that of pentamidine. Compound **6n** showed better trichomonacidal activity (MIC = 10 µg/mL), and was inactive against *L infantum*; the contrary was observed with compound **6k**. The other derivatives were inactive.

The new naphthoimidazolediones were less active than the 5-nitroimidazole analogs. For example, when compared with metronidazole (MIC = 5 µg/mL), 1-methyl-2-(1-methyl pyrrolidin-2-one-3-ylidenemethyl)-5-nitroimidazole was 100-fold more effective in vitro against *T vaginalis*, while the derivative analog **6n** was two-fold less potent. Replacement of nitro by the naphthoquinone group produced a substantial decrease in antiprotozoan activity. The functionalization of the methyl group at the 2-position did not modify the antiprotozoan activity in the case of **6f**, **6m**, or resulted in complete loss of activity for other derivatives. The introduction of a heterocyclic moiety at the 2-substitution favored antiprotozoan activity. The size of the substituent was also important for the 2-alkylidenemethyl-4,9-dihydro-1-methyl-1H-naphtho[2,3-*d*]imidazol-4,9-dione because compound **6f** with the dodecyl group was the most active.

Table II. Protozoocidal activity of new naphthoimidazolediones.

Compound No	*MIC (µg/mL) Trichomonas vaginalis*	*MIC (µg/mL) Leishmania infantum*
6a	>100	>100
6b	>100	>100
6c	>100	>100
6d	>100	>100
6e	>100	>100
6f	10	25
6g	>100	>100
6h	>100	>100
6i	>100	>100
6j	>100	>100
6k	>100	25
6l	>100	>100
6m	10	25
6n	10	>100
7	10	25
Metronidazole	5	
Pentamidine		5

MIC: minimal inhibitory concentration.

In conclusion, we have described the preparation of new naphthoimidazolediones bearing a trisubstituted ethylenic double bond at the 2-position. These compounds were evaluated as potential antiprotozoan agents and although some derivatives were found to have a significant in vitro activity, the naphthoquinone was found to be a less efficient pharmacophore than the nitro group.

Experimental protocols

Chemistry

Melting points were recorded 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 (ppm) relative to internal TMS. Microanalyses for C, H, N were performed by the Microanalytical Section of St-Jérôme Faculty, and were within ± 0.4% of theoretical values.

The lithium salts of 2-nitropropane **2a** [19], nitroalkanes **2b–l** [20], 2,2-dimethyl-5-nitro-1,3-dioxane [20] and 1-methyl-3-nitropyrrolidin-2-one [20] were prepared as previously described.

2-Chloroacetylamino-3-chloro-1,4-naphthoquinone 1

Chloroacetylchloride (10 mL, 125 mmol) was added under inert atmosphere to a solution of 2-amino-3-chloro-1,4-naphthoquinone (6.23 g, 30 mmol) in anhydrous 1,4-dioxane (90 mL).

The reaction mixture was stirred at reflux for 16 h and evaporated. The residue was then washed with ethanol and filtered. After addition of activated carbon, the filtrate was heated and filtered. After cooling, the resulting yellow precipitate was filtered and washed with ethanol; the combined solids were recrystallized from ethanol to give 5 g (59%) of 2-chloroacetylamino-3-chloro-1,4-naphthoquinone **1**, mp = 180 °C (lit [21], mp = 182–182.8 °C). ^{1}H-NMR ($CDCl_3$) δ 4.27 (s, 2H); 7.80 (m, 2H); 8.17 (m, 2H); 8.66 (broad s, 1H).

2-Chloroacetylamino-3-methylamino-1,4-naphthoquinone ***2***
To a solution of 2-chloroacetylamino-3-chloro-1,4-naphthoquinone **1** (10 g, 35.2 mmol) in anhydrous ethanol (150 mL) and upon refluxing was added 33% ethanolic methylamine solution (7 mL, 70.4 mmol). After stirring for 30 min, the reaction mixture was cooled to room temperature, whereupon a precipitate was formed. The red solid was filtered, washed with ethanol and recrystallized from ethanol to afford 4.21 g (43%) of 2-chloroacetylamino-3-methylamino-1,4-naphthoquinone **2**, mp = 188 °C. ^{1}H-NMR ($CDCl_3$) δ 3.12 (s, 3H); 4.25 (s, 2H); 6.23 (broad s, 1H); 7.72 (m, 2H); 8.10 (m, 2H); 8.64 (broad s, 1H).

*2-Chloromethyl-4,9-dihydro-1-methyl-1H-naphtho[2,3-*d*]-imidazol-4,9-dione* ***3***
Acetylchloride (20 mL, 281 mmol) was slowly added to a solution of 2-chloroacetylamino-3-methylamino-1,4-naphthoquinone **2** (4.17 g, 15 mmol) in ethanol (100 mL). The mixture was heated to reflux for 1 h and then cooled to room temperature. The resulting precipitate was filtered, washed with ethanol and recrystallized from ethanol to give 2.90 g (74%) of 2-chloromethyl-4,9-dihydro-1-methyl-1H-naphtho[2,3-*d*]-imidazol-4,9-dione **3**, as yellow solid, mp = 210 °C. ^{1}H-NMR ($CDCl_3$) δ 4.19 (s, 3H); 4.81 (s, 2H); 7.76 (m, 2H); 8.18 (m, 1H); 8.26 (m, 1H).

General procedure for $S_{RN}1$ reactions

Method A
To a solution of 2-chloromethyl-4,9-dihydro-1-methyl-1H-naphtho[2,3-*d*]imidazol-4,9-dione **3** (0.52 g, 2 mmol) in dry DMF (30 mL), the lithium salt of nitroalkane (6 mmol) was added under nitrogen and anhydrous conditions. The reaction mixture was then irradiated with two 60 W fluorescent lamps from a distance of 10 cm. After stirring at room temperature for 24 h, the reaction mixture was removed under reduced pressure. The residue was dissolved in dichloromethane (40 mL) and the solvent washed with water (3 x 100 mL), dried over anhydrous $MgSO_4$ and evaporated. Purification by chromatography on a silica gel column eluting with chloroform/ acetone (8:2), and recrystallization from methanol gave the required compound.

*2-Isopropylidenemethyl-4,9-dihydro-1-methyl-1H-naphtho[2,3-*d*]-imidazol-4,9-dione* ***6a***. Yellow solid, 58% yield, mp = 210 °C, ^{1}H-NMR ($CDCl_3$) δ 2.06 (s, 3H); 2.30 (s, 3H); 4.04 (s, 3H); 6.09 (s, 1H); 7.71 (m, 2H); 8.14 (m, 1H); 8.24 (m, 1H).

*2-Cyclopentylidenemethyl-4,9-dihydro-1-methyl-1H-naphtho-[2,3-*d*]imidazol-4,9-dione* ***6b***. Orange solid, 68% yield, mp = 220 °C, ^{1}H-NMR ($CDCl_3$) δ 1.79 (m, 4H); 2.59 (t, *J* = 6.5 Hz, 2H); 3.01 (t, *J* = 7.6 Hz, 2H); 4.06 (s, 3H); 6.29 (s, 1H); 7.71 (m, 2H); 8.12 (m, 1H); 8.22 (m, 1H).

*2-Cyclohexylidenemethyl-4,9-dihydro-1-methyl-1H-naphtho-[2,3-*d*]imidazol-4,9-dione* ***6c***. Orange solid, 54% yield, mp = 205 °C, ^{1}H-NMR ($CDCl_3$) δ 1.59 (m, 6H); 2.34 (t, *J* = 5.6 Hz, 2H); 2.89 (t, *J* = 5.8 Hz, 2H); 4.04 (s, 3H); 6.01 (s, 1H); 7.71 (m, 2H); 8.13 (m, 1H); 8.23 (m, 1H).

*2-Cycloheptylidenemethyl-4,9-dihydro-1-methyl-1H-naphtho-[2,3-*d*]imidazol-4,9-dione* ***6d***. Orange solid, 44% yield, mp = 212 °C, ^{1}H-NMR ($CDCl_3$) δ 1.57 (m, 8H); 2.54 (t, *J* = 5.6 Hz, 2H); 3.03 (t, *J* = 5.8 Hz, 2H); 4.04 (s, 3H); 6.09 (s, 1H); 7.71 (m, 2H); 8.13 (m, 1H); 8.24 (m, 1H).

*2-Cyclooctylidenemethyl-4,9-dihydro-1-methyl-1H-naphtho-[2,3-*d*]imidazol-4,9-dione* ***6e***. Orange solid, 56% yield, mp = 218 °C, ^{1}H-NMR ($CDCl_3$) δ 1.43 (m, 6H); 1.85 (m, 4H); 2.41 (t, *J* = 5.2 Hz, 2H); 3.01 (t, *J* = 5.3 Hz, 2H); 4.24 (s, 3H); 6.07 (s, 1H); 7.72 (m, 2H); 8.14 (m, 1H); 8.26 (m, 1H).

*2-Cyclododecylidenemethyl-4,9-dihydro-1-methyl-1H-naphtho-[2,3-*d*]imidazol-4,9-dione* ***6f***. Orange solid, 66% yield, mp = 210 °C, ^{1}H-NMR ($CDCl_3$) δ 1.24–1.52 (m, 14H); 1.55–1.68 (m, 4H); 2.35 (t, *J* = 6.8 Hz, 2H); 2.82 (t, *J* = 6.8 Hz, 2H); 4.03 (s, 3H); 6.13 (s, 1H); 7.68–7.72 (m, 2H); 8.13 (m, 1H); 8.23 (m, 1H).

*2-(2-Norbornylidenemethyl-4,9-dihydro-1-methyl-1H-naphtho-[2,3-*d*]imidazol-4,9-dione* ***6g***. Orange solid, 46% yield, mp = 168 °C, ^{1}H-NMR ($CDCl_3$) δ 1.34–1.63 (m, 6H); 2.54 (m, 1H); 2.73 (m, 2H); 2.95 (m, 1H); 4.05 (s, 3H); 6.24 (s, 1H); 7.71 (m, 2H); 8.12 (m, 1H); 8.23 (m, 1H).

*2-(1,2,3,4-Tetrahydro-1-naphthalenylidenemethyl-4,9-dihydro-1-methyl-1H-naphtho[2,3-*d*]imidazol-4,9-dione* ***6h***. Red solid, E isomer, 88% yield, mp = 234 °C, ^{1}H-NMR ($CDCl_3$) δ 1.84 (quint, *J* = 6.3 Hz, 2H); 2.78 (t, *J* = 6.2 Hz, 2H); 3.29 (td, *J* = 6.3 Hz and 1.5 Hz, 2H); 4.07 (s, 3H); 7.10–7.23 (m, 5H); 7.65 (m, 2H); 8.09 (m, 1H); 8.19 (m, 1H).

*2-(1-Indylidenemethyl-4,9-dihydro-1-methyl-1H-naphtho[2,3-*d*]-imidazol-4,9-dione* ***6i***. Red solid, E isomer, 70% yield, mp = 281 °C, ^{1}H-NMR ($CDCl_3$) δ 3.13 (t, *J* = 6.2 Hz, 2H); 3.54 (td, *J* = 6.2 Hz and 1.5 Hz, 2H); 4.16 (s, 3H); 6.76 (s, 1H); 7.22–7.39 (m, 4H); 7.68 (m, 2H); 8.12 (m, 1H); 8.23 (m, 1H).

*2-(2-Phenylpropen-1-yl)-4,9-dihydro-1-methyl-1H-naphtho-[2,3-*d*]imidazol-4,9-dione* ***6j***. Orange solid, E isomer, 73% yield, mp = 186 °C, ^{1}H-NMR ($CDCl_3$) δ 2.75 (d, *J* = 1.1 Hz, 3H); 4.12 (s, 3H); 7.35–7.61 (m, 6H); 7.73 (m, 2H); 8.16 (m, 1H); 8.27 (m, 1H).

*2-(2-Methylpenten-1-yl)-4,9-dihydro-1-methyl-1H-naphtho-[2,3-*d*]imidazol-4,9-dione* ***6k***. Orange solid, E isomer, 65% yield, mp = 145 °C, ^{1}H-NMR ($CDCl_3$) δ 0.94 (t, *J* = 7.4 Hz, 3H); 1.52–1.63 (m, 4H); 2.25 (d, *J* = 1.0 Hz, 3H); 4.01 (s, 3H); 7.69 (m, 2H); 8.10 (m, 1H); 8.21 (m, 1H).

*2-(2,5-Dimethylhexen-1-yl)-4,9-dihydro-1-methyl-1H-naphtho-[2,3-*d*]imidazol-4,9-dione* ***6l***. Orange solid, E isomer, 72% yield, mp = 142 °C, ^{1}H-NMR ($CDCl_3$) δ 0.90 (d, *J* = 6.5 Hz, 6H); 1.19–1.27 (m, 3H); 1.51–1.63 (m, 2H); 2.27 (d, *J* = 1.3 Hz, 3H); 4.03 (s, 3H); 6.07 (s, 1H); 7.71 (m, 2H); 8.12 (m, 1H); 8.21 (m, 1H).

Method B
2,2-Dimethyl-5-nitro-1,3-dioxane lithium salt (1 g, 6 mmol) was added to a solution of 2-chloro-methyl-4,9-dihydro-1-methyl-1H-naphtho[2,3-*d*]imidazol-4,9-dione **3** (0.52 g, 2 mmol) in dry methanol (30 mL). The reaction was allowed to proceed

for 24 h at room temperature under nitrogen and in the presence of light (2 x 60 W fluorescent lamps). After 24 h, methanol was distilled off on a rotatory evaporator under reduced pressure and water (50 mL) was added to the residue which was extracted with dichloromethane (3 x 50 mL). The extracts were dried and evaporated. Purification by recrystallization from ethanol gave 0.48 g (71%) of 2-(2,2-dimethyl-1,3-dioxane-5-ylidenemethyl)-4,9-dihydro-1-methyl-1H-naphtho-[2,3-*d*]imidazol-4,9-dione **6m**. Yellow solid, mp = 188 °C, ^{1}H-NMR ($CDCl_3$) δ 1.45 (s, 6H); 4.09 (s, 3H); 4.29 (m, 2H); 4.52 (m, 2H); 6.14 (s, 1H); 7.74 (m, 2H); 8.15 (m, 1H); 8.25 (m, 1H).

Method C

Under a nitrogen atmosphere, the lithium salt of 1-methyl-3-nitropyrrolidin-2-one (3.6 g titrated to 83%, 20 mmol) and tetrabutylammonium bromide (0.32 g, 1 mmol) were dissolved in water (25 mL). After degassing by ultrasounds under an inert atmosphere for 30 min, a solution of 2-chloromethyl-4,9-dihydro-1-methyl-1H-naphtho[2,3-*d*]imidazol-4,9-dione **3** (2.6 g, 10 mmol) in dichloromethane (60 mL) was added, and the mixture stirred for 24 h at room temperature under nitrogen and irradiation with fluorescent lamps. The organic layer was separated and the aqueous layer was extracted with five portions of dichloromethane (50 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and removed under reduced pressure. Purification by chromatography on a silica gel column eluting with dichloromethane/acetone (8:2) and recrystallization from acetone gave 1.02 g (32%) of 2-(1-methyl pyrrolidin-2-one-3-ylidenemethyl)-4,9-dihydro-1-methyl-1H-naphtho[2,3-*d*]-imidazol-4,9-dione **6n**. Yellow solid, E isomer, mp = 285 °C, ^{1}H-NMR ($CDCl_3$) δ 3.10 (s, 3H); 3.55 (m, 2H); 3.60 (t, J = 4.6 Hz, 2H); 4.23 (s, 3H); 7.25 (t, J = 2.4 Hz, 1H); 7.73–7.80 (m, 2H); 8.18–8.31 (m, 2H).

*2-Methyl-4,9-dihydro-1-methyl-1H-naphtho[2,3-*d*]imidazol-4,9-dione 7*. To a solution of 2-chloromethyl-4,9-dihydro-1-methyl-1H-naphtho[2,3-*d*]imidazol-4,9-dione **3** (0.52 g, 2 mmol) in dry methanol (100 mL), sodium borohydride (0.168 g, 6 mmol) was added at 0 °C. After stirring for 1 h, the reaction mixture was poured into water (100 mL) and extracted three times with chloroform (60 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and the solvent evaporated. Recrystallization of the residue obtained from ethanol gave 0.4 g (88%) of 2-methyl-4,9-dihydro-1-methyl-1H-naphtho-[2,3-*d*]imidazol-4,9-dione **7**. Yellow solid, mp = 210 °C, ^{1}H-NMR ($CDCl_3$) δ 2.52 (s, 3H); 3.98 (s, 3H); 7.70 (m, 2H); 8.04 (m, 1H); 8.15 (m, 1H).

Biology

In vitro trichomonacidal activity was performed on a *T vaginalis* wild strain grown in oxoid liquid medium (*Trichomonas* medium code CM 161). The minimal inhibitory concentration (MIC) was determined after 48 h [22] using metronidazole as the reference drug.

Leishmanicidal activity was evaluated on *L infantum* strain (MCAN/FR/74 LPMA 57; WHO). *Leishmania infantum* was originally isolated from the ganglia of dogs in Marseille, France. These isolates contained numerous *Leishmania* amastigotes and were cultivated in NNN (Novy, Mac Neal, Nicolle) [23] and Tobie [24] media, where they were transformed into promastigote forms. This strain was maintained in continuous culture in RPMI 1640 (Gibco) containing 10% heat-inactivated fetal 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 in tubes containing 5 mL of the above-described medium and incubated at 24 °C [25]. Subcultures were made once a week and each subculture was checked for abundance and motility of promastigote forms. They were counted by 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 distributed to the culture tubes to obtain final concentrations of 100; 50; 25; 10; 5; 1 and 0.5 mg/L. Dimethylformamide was completely inactive on the parasites at these concentrations. Each strain and concentration was tested in triplicate. The MIC of the compounds was determined after the parasites had been in culture for 7 days by checking microscopically (x 400) 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 to be leishmanicidal. The MIC for each compound was then compared with that of pentamidine determined under the same conditions.

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