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Studies on quinones. Part 39: Synthesis and leishmanicidal activity of acylchloroquinones and hydroquinones☆

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Abstract—Acylhydroquinone-based compounds are attractive targets for the design of new leishmanicidal drugs. We have previously described sesquiterpene quinones and hydroquinones series, which exhibit different degree of potency against *Leishmania amazonensis*. The present study details the preparation of acylchloroquinones and hydroquinones possessing lipophilic substituents and examines their in vitro activity against intracellular *L. amazonensis* amastigotes. The quinone or hydroquinone nucleus is essential for the activity of the members of the series. The lipophilicity of the cycloaliphatic systems in these members seems to attenuate the cytotoxical effect and increases the selectivity of those compounds containing the norbornene system.

1. Introduction

Leishmaniasis is a widespread parasitic disease caused by protozoan parasites of the genus *Leishmania*. The disease is endemic in some tropical areas of the world and in underdeveloped countries, directly affecting about 2 million people annually, with approximately 350 million people who live at risk of contracting the infection worldwide.[2,3] Leishmaniasis is an opportunistic infection that affects HIV infected individuals. This co-infection involves the visceral form of leishmaniasis, which is one of its most severe form.[4] The drugs, which have been used frequently in the treatment of the leishmaniases are the pentavalent antimonials Pentostam and Glucantime.[5,6] AmBisome, a liposomal formulation of amphotericin B,[7] which overcomes the toxic limitations of the parent drug, was developed for the treatment of visceral leishmaniasis. However, because of its cost, it is not suitable for use in underdeveloped countries. More recently the drug Miltefosine[8,9] has been introduced in chemotherapy of visceral leishmaniasis with promising results. Due to the toxic side-effects and variable efficacy of the drugs used in antileishmanial chemotherapy, efforts are being made to find new and inexpensive compounds as new leads.

Natural and synthetic naphthoquinones have been described for many years for their antiprotozoal activity.[10] In addition, quinones fused to a variety of heterocyclic rings (e.g., furan, thiophene, pyridine, imidazole, and pyran) were also described as antiprotozoal agents.[11] Biological activities of quinones emerge due to their ability to act as potent inhibitors of electron transport, as uncouplers of oxidative phosphorylation, as DNA intercalating agents, as bioreductive alkylating agents and as producers of reactive oxygen radicals, by redox cycling, under aerobic conditions. In all these cases, the mechanisms require bioreduction of the quinone nucleus as the first activating step.[12–14]

As part of our current research program toward the synthesis of potential bioactive quinones,[1,11,15–19] we have reported the synthesis and in vitro leishmanicidal activity of sesquiterpene quinones and hydroquinones.[1,15] Among the members of this family, compounds **1**–**3**

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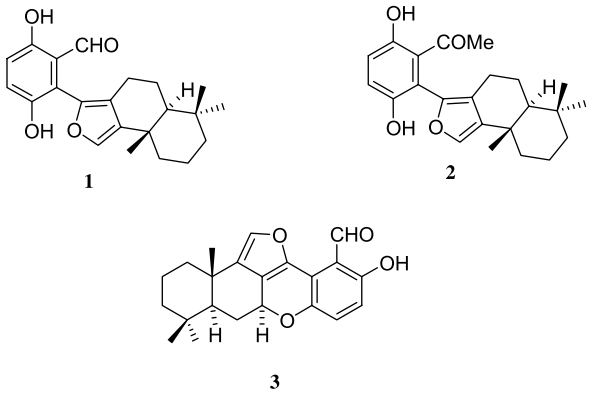


Figure 1.

(Fig. 1) showed promising activities against macrophages. These studies also revealed that the activity depends on the acylquinone or acylhydroquinone core and that the sesquiterpene fragment is not an essential requirement but, in some cases, appears to cause a slight increase of selectivity toward macrophages.

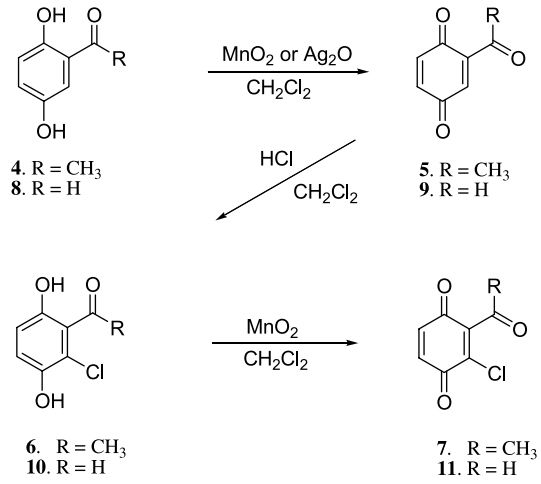
These observations prompted us to investigate the synthesis of new compounds containing the biologically active acylhydroquinone or acylquinone framework fused to lipophilic cycloaliphatic fragments. Here we wish to report the synthesis and leishmanicidal activity of acylchlorobenzoquinones, acylchlorohydroquinones and cycloaliphatic fused derivatives. The in vitro biological studies have been performed using intracellular *Leishmania amazonensis* amastigotes stage in mouse peritoneal macrophages and Miltefosine as the reference drug.

2. Results and discussion

2.1. Chemistry

On the basis of the results of Kayser et al.[20] on the increase of the leishmanicidal activity of naphthoquinones by introducing an alicyclic ring at the quinone nucleus, we designed 6-acyl-7-chloro-1,4-alkanonaphthalenes **13**, **14**, **16**, and **17** for this study. For the synthesis of the target compounds we took advantage of a previous work on the Diels–Alder reaction of 2-acyl-3-chloro-1,4-benzoquinones with 1,3-butadienes.[21] The requisite precursors **6**, **7**, **10**, and **11** were prepared by the route described in Scheme 1. Acetylbenzoquinone **5** was obtained from **4** and manganese dioxide and then reacted with hydrogen chloride in dichloromethane to give addition compound **6** in 82% yield. Further oxidation of **6** with manganese dioxide in dichloromethane afforded 2-acetyl-3-chloro-1,4-benzoquinone **7** in 70% yield.

Formylbenzoquinone **9** prepared from **8** and silver(II) oxide was reacted with hydrogen chloride in dichloromethane to give addition product **10** in 69% yield. Preparation of 2-formyl-3-chloro-1,4-benzoquinone **11** by oxidation of **10** with silver(II) oxide was attempted;



Scheme 1. Synthesis of 2-acyl-3-chloro-1,4-benzoquinones **7** and **11**.

however, isolation of **11** was unsuccessful due to its unstability even in dichloromethane solution.

Cycloaddition reaction of quinone **7** with cyclopentadiene gave Diels–Alder adduct **12**, which after column chromatography on silica gel, was isolated as enolization product **13**. The synthesis of compound **13** was achieved by cycloaddition of quinone **7** with cyclopentadiene followed by treatment of the crude with silica gel in dichloromethane at room temperature. Under these conditions, compound **13** was isolated in 80% total yield.

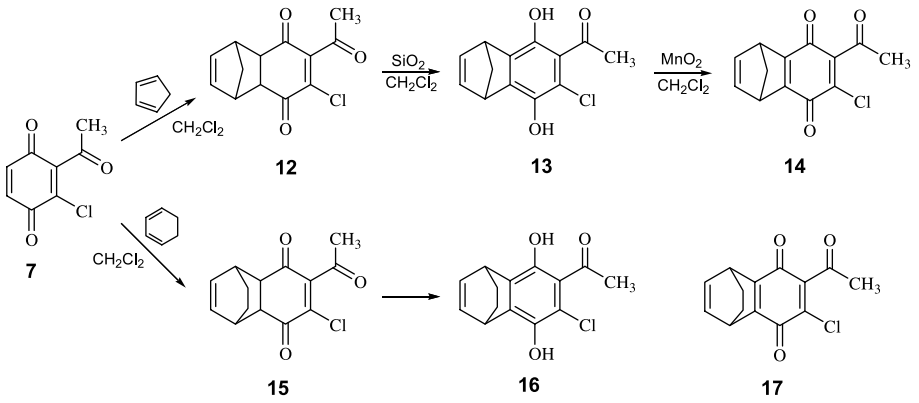
The synthesis of compound **16** was performed by following the procedure described for the preparation of compound **13** (Scheme 2). Cycloaddition of **7** with cyclohexa-1,3-diene followed by enolization of Diels–Alder adduct **15**, afforded compound **16** in 78% total yield.

The 1,4-dihydro-1,4-alkanonaphthalene-5,8-diones **14** and **17** were obtained in 97% and 89% yields, respectively, by oxidation of compounds **13** and **16** with manganese dioxide in dichloromethane.

2.2. Biology

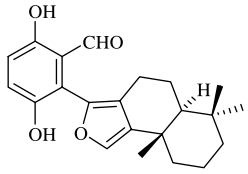
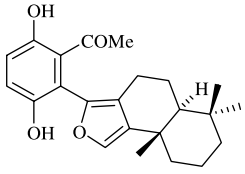
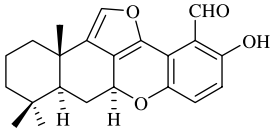
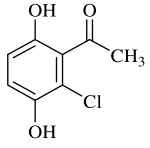
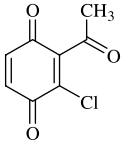
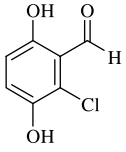
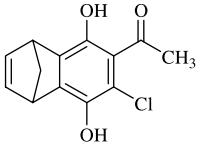
The acylchlorobenzoquinones and hydroquinones were tested in vitro against intracellular *L. amazonensis* amastigotes stage in mouse peritoneal macrophages. The results of the biological evaluation are indicated in Table 1. The IC_{50} and TC_{50} of compounds **1**–**3**, previously reported,[15] and that of 1,4-naphthoquinone also are included in Table 1 in order to compare their antileishmanicidal activity and cytotoxic effects with those of the new compounds.

Although almost all of the tested compounds showed leishmanicidal activity, none was as effective as miltefosine used here as the reference drug against *L. amazonensis* intracellular amastigotes. All compounds showed a weak selectivity (i.e., the ratio of between cytotoxic and leishmanicidal effect).



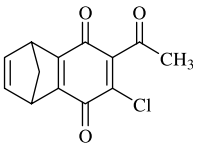
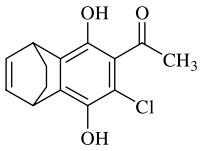
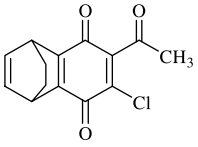
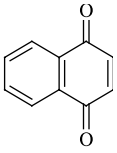
Scheme 2. Synthesis of 6-acetyl-7-chloro-1,4-alkanonaphthalenes **13**, **14**, **16**, and **17**.

Table 1. Inhibitory concentrations IC_{50} and cytotoxicity TC_{50} of compounds **6**, **7**, **10**, **11**, **13**, **14**, **16**, and **17** against *Leishmania amazonensis* macrophages

Entry	Compound	Structure	IC_{50} (μM)	TC_{50} (μM)	Log P[a]
1	**1**		9	25	6.76
2	**2**		16	8	7.76
3	**3**		18	13	7.68
4	**6**		50	6.3	0.96
5	**7**		51	6.4	3.08
6	**10**		55	27.2	1.40
7	**13**		18.7	9.4	4.55

(*continued on next page*)

Table 1 (*continued*)

Entry	Compound	Structure	IC_{50} (μM)	TC_{50} (μM)	Log P[a]
8	**14**		18.7	9.4	5.07
9	**16**		35.5	8.9	4.73
10	**17**		>100	8.9	5.54
11	—		5.0	2.5	1.75
12	—	Miltefosine	3.12	12.5	—

[a] The values of lipophilicity (log pK_a) were calculated using the Dixon method in the Spartan package.[22]

Comparison of quinones **7**, **14**, and **17** (entries 5, 8, and 10) indicates that cytotoxicity decreases with lipophilicity and a similar trend is observed for hydroquinones **6**, **13**, and **16** (entries 4, 7, and 9). Furthermore, comparison of compounds **1**, **6**, and **10** (entries 1, 4, and 6) shows that selectivity increases with the lipophilicity.

It is noteworthy that cycloaliphatic substituted quinones **14** and **17** are less cytotoxic than 1,4-naphthoquinone (entries 11 and 12) probably due to the high lipophilicity of the former two. It seems possible that substituents on **14** and **17** play two roles in the parasitic activity: (i) by lowering the oxidation potential of the quinone nucleus with respect to 1,4-naphthoquinone and (ii) preventing bioreductive alkylation reactions.

Among compounds **7**, **14**, **17**, and **6**, **13**, **16** those possessing the lipophilic norbornene system (**13** and **14**) are the most active. It is noteworthy that compounds **13** and **14** show the highest leishmanicidal activity of the series, which are similar to those exhibited by compounds **2** and **3** (entries 2 and 3).

These observations could be interpreted by considering that the in vitro leishmanicidal activity of the compounds depends on the redox capability of the quinone and hydroquinone nucleus and on the presence of the lipophilic cycloaliphatic rings, which seemed to increase the leishmanicidal effect and attenuate the cytotoxical effect. These results are encouraging and further in vitro experiments with acylquinones and hydroquinones must be performed to understand the mechanism of drug toxicity in *L. amazonensis*.

3. Conclusion

In conclusion, the high yield synthesis of 6-acetyl-7-chloro-5,8-dihydroxy-1,4-alkanonaphthalenes **13** and **17**, and its corresponding quinones **14** and **16**, were performed from 2,5-dihydroxycetophenone **4** through the sequence: (a) oxidation of **4** followed by conjugate addition of hydrogen chloride, (b) oxidation of the addition product **6**, (c) Diels–Alder reaction of **7** with cyclopentadiene and cyclohexa-1,3-diene, (d) enolization of the Diels–Alder adducts **12** and **15**, and (e) oxidation. The compounds reported here exhibit weak to moderate leishmanicidal activity and high toxicity compared with Miltefosine. Regarding molecular structure, these in vitro studies show that the antileishmanial activity is determined by the presence of quinone or hydroquinone groups and lipophilic fragments. The lipophilicity of the cycloaliphatic fragments in these members seems to attenuate the cytotoxical effects and, in the case of the norbornene derivatives (**13** and **14**), increases the leishmanicidal activity compared with their monocyclic precursors **6** and **7**.

4. Experimental

4.1. Chemical synthesis

All reagents were of commercial quality, reagent grade and were used without further purification. Melting points (mp) were determined on a Köfler hot-stage apparatus and are uncorrected. The IR spectra were recorded on a FT Bruker vector22-FT spectrophotometer using KBr discs and the wave numbers are given in

cm^{-1}. ^1H NMR spectra were measured on Bruker AM-200 and AM-400 in $CDCl_3$. Chemical shifts are expressed in ppm downfield relative to TMS (δ scale) and the coupling constants (J) are reported in hertz. ^{13}C NMR spectra were acquired in deuteriochloroform at 50 and 100 MHz on Bruker AM-200 and AM-400 spectrometers. The elemental analyses were performed in a Fison SA, model EA-1108 apparatus. Silica gel Merck 60 (70–230 mesh), and TLC aluminum foil 60 F_{254} were used for preparative column and analytical TLC, respectively.

4.1.1. 2,5-Dihydroxy-6-chloroacetophenone (6). A suspension of 2,5-dihydroxyacetophenone (**4**) (500 mg, 3.29 mmol), manganese dioxide (3.0 g, 34.5 mmol), magnesium sulfate (500 mg), and dichloromethane (50 mL) was vigorously stirred for 30 min at room temperature. The mixture was filtered through Celite and the solids were washed with dichloromethane (15 mL). Dry hydrogen chloride was bubbled through the filtrate for 5 min and the resulting solution was left overnight at room temperature. Evaporation of the solvent left a solid, which was purified by column chromatography on silica gel (dichloromethane) to give pure **6** (497 mg, 82%); mp 96–98 °C (lit.[23] mp 94–96 °C); IR (KBr): ν_{max} 3418 (OH), 1635 (C=O); ^1H NMR (200 MHz, $CDCl_3$): δ 11.91 (s, 1H, C_2-OH), 7.19 (d, 1H, $J = 9$ Hz, 3-H or 4-H), 6.92 (d, 1H, $J = 9$ Hz, 4-H or 3-H), 5.49 (s, 1H, C_5-OH), 2.83 (s, 3H, $COCH_3$); ^{13}C NMR (50 MHz, $CDCl_3$): δ 204.0, 157.3, 144.9, 123.4, 118.9, 118.8, 117.9, 33.4.

4.1.2. 2,5-Dihydroxy-6-chlorobenzaldehyde (10). A suspension of 2,5-dihydroxybenzaldehyde (**8**) (170 mg, 1.23 mmol), silver(II) oxide (510 mg, 2.2 mmol), magnesium sulfate (500 mg), and dichloromethane (35 mL) was vigorously stirred for 30 min for 2 h. The mixture was filtered and the solids were washed with dichloromethane (15 mL). Dry hydrogen chloride was bubbled through the filtrate for 5 min and the resulting solution was left overnight at room temperature. The solvent was removed and the residue was chromatographed over silica gel (dichloromethane) to give pure **10** as a gold yellow solid (146 mg, 69%); mp 155–157 °C; IR(KBr): ν_{max} 3271 (OH), 2773 and 2719 (C=O); ^1H NMR (200 MHz, $CDCl_3$): δ 11.50 (s, 1H, C_2-OH), 10.35 (s, 1H, CHO), 7.26 (d, 1H, $J = 9$ Hz, 3-H or 4-H), 6.88 (d, 1H, $J = 9$ Hz, 4-H or 3-H), 5.41 (s, 1H, C_5-OH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 194.7, 157.9, 144.5, 125.9, 120.6, 117.8, 115.8. Anal. Calcd for $C_7H_5O_3Cl$: C, 48.72; H, 2.92. Found: C, 48.87; H, 2.65.

4.1.3. 2-Acetyl-3-chloro-1,4-benzoquinone (7). A suspension of **6** (150 mg, 0.8 mmol), manganese dioxide (730 mg, 8.4 mmol), magnesium sulfate (500 mg) and dichloromethane (15 mL) was vigorously stirred for 30 min at room temperature. After the usual work-up, and column chromatography over silica gel (dichloromethane), compound **7** was obtained as an orange solid (104 mg, 70%); mp 110–114 °C (lit.[23] mp 110–112 °C); IR (KBr): ν_{max} 1717 (COMe), 1679, 1655 (C=O quinone); ^1H NMR (200 MHz, $CDCl_3$): δ 6.98 (d, 1H, $J = 10$ Hz, 5-H or 6-H), 6.84 (d, 1H, $J = 10$ Hz, 6-H or 5-H), 2.48 (s, 3H, $COCH_3$); ^{13}C NMR (50 MHz, $CDCl_3$): δ 207.0, 196.0, 183.0, 178.7, 143.1, 137.8, 136.3, 136.1.

4.1.4. 6-Acetyl-7-chloro-5,8-dihydroxy-1,4-dihydro-1,4-methanonaphthalene (13). A solution of quinone **7** (197 mg, 1.07 mmol), cyclopentadiene (100 mg, 1.5 mmol), and dichloromethane (10 mL) was left for 24 h at room temperature. Silica gel (3 g) was added to the mixture and the suspension was stirred for 24 h at room temperature. The suspension was filtered and the solid was washed with dichloromethane. The filtrate was evaporated to afford compound **13** (216 mg, 80%). Column chromatography of the crude, eluting with dichloromethane gave pure **13** (145 mg, 54%) as a pale yellow solid, mp 156–158 °C; IR (KBr): ν_{max} 3390 (OH), 1605 (COMe); ^1H NMR (400 MHz, $CDCl_3$): δ 11.73 (s, 1H, C_1-OH), 6.89 (dd, 1H, $J = 5.2$; 3.1 Hz, 6-H or 7-H), 6.80 (dd, 1H, $J = 5.2$; 3.1 Hz, 7-H or 6-H), 5.44 (s, 1H, C_4-OH), 4.27 (m, 1H, 5-H or 8-H), 4.23 (m, 1H, 8-H or 5-H), 2.77 (s, 3H, COMe), 2.29 (dt, 1H, $J = 7.3$; 1.5 Hz, 9-H or 9-H′), 2.23 (dt, 1H, $J = 7.3$; 1.5 Hz, 9-H′ or 9-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 204.5, 151.2, 147.8, 144.0, 142.0, 140.6, 140.5, 117.8, 117.0, 70.6, 48.7, 47.3, 33.8. Anal. Calcd for $C_{13}H_{11}O_3Cl$: C, 62.29; H, 4.42. Found: C, 62.25; H, 4.40.

4.1.5. 6-Acetyl-7-chloro-5,8-dihydroxy-1,4-dihydro-1,4-ethanonaphthalene (16). A solution of quinone **7** (197 mg, 1.07 mmol), cyclohexa-1,3-diene (100 mg, 1.25 mmol), and dichloromethane (10 mL) was left for 24 h at room temperature. Silica gel (3 g) was added to the mixture and the suspension was stirred for 24 h at room temperature. The suspension was filtered and the solid was washed with dichloromethane. Evaporation of the solvent afforded crude compound **16** (221 mg, 78%); further column chromatography (dichloromethane) gave pure **16** (207 mg, 73%) as yellow crystals, mp 150–152 °C; IR (KBr): ν_{max} 3404 (OH), 1624 (COMe); ^1H NMR (400 MHz, $CDCl_3$): δ 12.25 (s, 1H, C_1-OH); 6.54 (m, 1H, 6-H or 7-H), 6.46 (m, 1H, 7-H or 6-H), 5.47 (s, 1H, C_4-H), 4.53 (m, 1H, 5-H or 8-H), 4.48 (m, 1H, 8-H or 5-H), 2.79 (s, 3H, COMe), 1.54 (m, 2H, 9-H and 10-H), 1.46 (m, 2H, 9-H′ and 10-H′); ^{13}C NMR (50 MHz, $CDCl_3$): δ 204.2, 151.4, 140.3, 139.6, 135.6, 133.8, 133.3, 116.0, 115.0, 34.6, 33.4, 32.9, 24.7, 24.6. Anal. Calcd for $C_{14}H_{13}O_3Cl$: C, 63.52; H, 4.95. Found: C, 63.68; H, 4.85.

4.1.6. 6-Acetyl-7-chloro-1,4-dihydro-1,4-methanonaphthalene-5,8-dione (14). A suspension of **13** (100 mg, 0.4 mmol), manganese dioxide (367 mg, 4.2 mmol), magnesium sulfate (0.5 g), and dichloromethane (10 mL) was stirred for 30 min at room temperature. The suspension was filtered, the solids were washed with dichloromethane, and the solvent was removed under vacuum to give crude quinone **14**. Column chromatography of the crude afforded pure **14** (96 mg, 97%) as an orange solid, mp 145–147 °C. IR (KBr): ν_{max} 1722 (COMe), 1672, 1648 (C=O quinone); ^1H NMR (400 MHz, $CDCl_3$): δ 6.89 (m, 2H, 6-H and 7-H), 4.18 (m, 1H, 5-H or 8-H), 4.12 (m, 1H, 8-H or 5-H), 2.46

(s, 3H, $COCH_3$), 2.39 (dt, 1H, $J = 7.3$; 5.0 Hz, H-9 or H′-9), 2.32 (dt, 1H, $J = 7.3$; 1.5 Hz, 9-H′ or 9-H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 197.0, 179.7, 175.4, 161.3, 160.7, 142.4, 141.9, 137.0, 74.1, 49.2, 48.7, 31.0, 29.7. Anal. Calcd for $C_{14}H_{13}O_3Cl$: C, 62.79; H, 3.65. Found: C, 62.41; H, 3.62.

4.1.7. 6-Acetyl-7-chloro-1,4-dihydro-1,4-ethanonaphthalene-5,8-dione (17). A suspension of **16** (150 mg, 0.57 mmol), manganese dioxide (520 mg, 6.0 mmol), magnesium sulfate (500 mg), and dichloromethane (15 mL) was stirred for 30 min at room temperature. Work up of the mixture followed by column chromatography afforded pure quinone **17** (134 mg, 89%) as yellow crystals, mp 144–146 °C; IR (KBr): ν_{max} 1724 (COMe), 1673, 1646 (C═O quinone); ^1H NMR (400 MHz, $CDCl_3$): δ 6.41 (m, 2H, 6-H and 7-H), 4.43 (m, 1H, 5-H or 8-H), 4.34 (m, 1H, 8-H or 5-H), 2.46 (s, 3H, $COCH_3$), 1.53 (m, 2H, 9-H and 10-H), 1.38 (m, 2H, 9-H′ and 10-H′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 197.2, 179.9, 175.8, 150.0, 148.9, 142.5, 137.2, 134.0, 133.9, 35.0, 34.4, 31.3, 25.0, 24.9. Anal. Calcd for: $C_{14}H_{13}O_3Cl$: C, 64.01; H, 4.22. Found: C, 63.94; H, 4.08.

4.1.8. In vitro activity against intracellular *L. amazonensis* amastigotes. Female BALB/c mice aged 2–4 months were obtained from the breeding center of the Pasteur Institute. *L. amazonensis* strain LV79 (MPRO/BR/1972/M1841) was propagated in BALB/c mice. *L. amazonensis* amastigotes were isolated from lesions and purified as described earlier.[24] Bone marrow plugs from tibias and femurs of BALB/c mice were suspended in RPMI 1640 medium (Seromed) supplemented with 10% heat-inactivated fetal calf serum (FCS, Dutscher, Brumath, France), 50 mg/mL of streptomycin, 50 IU/mL of penicillin (culture medium) and with 15% L-929 fibroblast-conditioned medium. Cells were then distributed in bacteriologic Petri dishes (Greiner, Germany) and were incubated at 37 °C in a 5% CO_2 atmosphere. Five days later, adherent macrophages were washed with Dulbecco's phosphate buffered solution (PBS) and taken off by treatment for 20 min at 37 °C with Ca^{2+} and Mg^{2+}-free Dulbecco's PBS containing 2 mg/mL of glucose. Recovered macrophages were suspended in culture medium and they were then deposited in flat-bottom 96-well plates (Tanner, Switzerland) at a density of 4×10^4 cells/well. Twenty-four hours after replating, macrophages were infected at a multiplicity of five amastigotes per host cell and were incubated at 34 °C, which is the permissive temperature for the survival and multiplication of LV79 strain amastigotes. In most instances, more than 95% of the macrophages were found to be infected.

For all drugs, stock solutions were prepared in DMSO at a concentration of 500 μg/mL. Twofold serial dilutions were made from 250 μg/mL in culture medium supplemented with 0.5% DMSO final. Twenty-four hours after infection, freshly prepared drugs were added to the infected cultures in triplicate. The first final drug concentration was 25 μg/mL and the final DMSO concentration 0.1%. This DMSO concentration was proven to have no effect on control cultures.

Thirty-hours after drug addition, infected cultures were examined using an inverted phase contrast Zeiss microscope (magnification of 400). Toxic effects in the macrophages were evidenced by the change in morphological features, that is, loss of refringency, vacuolation of cytoplasm or loss of cytoplasmic material.

Leishmanicidal effects of drugs are easily detectable looking at the regression of parasitophorous vacuoles and the overall decrease in parasite number. For some drugs, a complete clearance of amastigotes was achieved.

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