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Antileishmanial activity of a new 8-hydroxyquinoline derivative designed 7-[5′-(3′-phenylisoxazolino)methyl]-8-hydroxyquinoline: preliminary study

Zainaba Dardari [a,*], Meryem Lemrani [b], Abdelmejid Bahloul [c], Abdelfatah Sebban [c], Mohammed Hassar [b], Said Kitane [d], Mohammed Berrada [c], Mohammed Boudouma [a]

[a] *Département de Biologie, Laboratoire de Microbiologie et Biologie Moléculaire, Faculté des Sciences Ben M'sik-Casablanca, Université Hassan II-Mohammedia, Sidi Othmane, Casablanca B.P. 7955, Morocco*
[b] *Laboratoire des leishmanioses, Institut Pasteur du Maroc, Casablanca, Morocco*
[c] *Département de Chimie, Laboratoire de Chimie des Polymères et de Synthèse Organique, Faculté des Sciences Ben M'sik-Casablanca, Université Hassan II-Mohammedia, Casablanca B.P. 7955, Morocco*
[d] *Laboratoire de Chimie Appliquée, Ecole Nationale de L'industrie Minérale, Rabat, Morocco*

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Abstract

7-[5′-(3′-phenylisoxazolino)methyl]-8-hydroxyquinoline a new synthetic 8-hydroxyquinoline derivative, was found, for the first time, to inhibit the multiplication in vitro of *Leishmania tropica, Leishmania major* and *Leishmania infantum* at micromolar concentrations. For each test we calculated a 50% inhibitory concentration (IC_{50}) and the IC_{50} values found after 48 h are: 0.4 μg/ml for *L. tropica*, 0.88 μg/ml for *L. major* and 0.62 μg/ml for *L. infantum*. As positive control, amphotericin B, a standard antileishmanial drug was used.

1. Introduction

Leishmaniasis is a major health problem that affects the population of 88 countries at a rate of 2 million cases per year with a prevalence of 12 million and 350 million at risk around the world. It is a significant cause of morbidity and mortality [1]. A causative agent of this disease are parasites of the genus *Leishmania*, which infect and replicate in macrophage of the vertebrate host. Depending on the parasite species, different forms of leishmaniasis may develop in the mammalian host, ranging from chronic skin ulcers to fatal visceral disease if untreated.

Three different forms of cutaneous leishmaniasis (CL) occur in distinct geographical areas of Morocco. First, zoonotic cutaneous leishmaniasis (ZCL) is epidemic in the South of the country, it is known to be caused by *Leishmania major* (Zymodeme MON25) [2]. Second, a chronic form of CL is encountered in the Center and North of Morocco, it is due to *Leishmania tropica* [3,4]. Third, a sporadic form occurs in the North Morocco, it is caused by *Leishmania infantum*, which is responsible of human and canine visceral leishmaniasis in the same area [5,6].

Chemotherapy is limited to the use of pentavalent antimonials such as sodium stibogluconate (Pentostam), *N*-methylglucamine (Glucantime) or amphotericin B. These drugs are toxic and difficult to administrate because of their long duration of therapy and high cost [7]. Treatment failure, especially in patients with kala-azar, mucosal leishmaniasis, and diffuse CT, is becoming a common problem in many areas of endemicity [8]. Thus, other drugs that are more effective, less toxic, and easier to use are urgently needed.

A large number of 8-hydroxyquinoline derivatives have already been synthesized and shown to be active agents. Indeed, the 8-hydroxyquinoline derivatives have been reported to possess antitumor [9–11], antimicrobial activities [9,12,13]. However, in spite of the existing interest on this class of compounds, studies on other effects of 8-hydroxyquinoline are lacking and virtually nothing in known about its activity on *Leishmania* species.

Thus, in the course of the research completed in our respective laboratories for the development of heterocyclic

* Corresponding author.
E-mail address: dzainaba@yahoo.fr (Z. Dardari).

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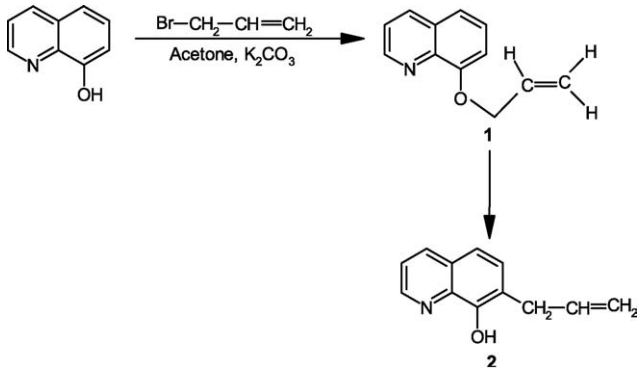


Scheme 1

compounds of both synthetic and biological interest and the increased interest on new antileishmanial agents due to the lack of effective drug, we reported here the synthesis and the antileishmaniasis activity of a new 8-hydroxyquinoline derivative designed 7-[5′-(3′-phenylisoxazolino)methyl]-8-hydroxyquinoline.

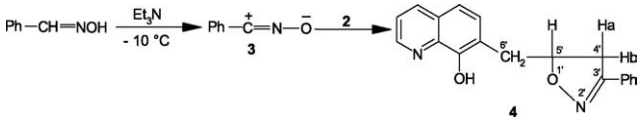
2. Experimental procedures

2.1. Chemistry

The melting points were given with a Buchi 510 apparatus. Spectra NMR were carried out in $CDCl_3$/TMS using Bruker spectroscopin AC200 (200 MHz for ^1H 62 MHz for ^{13}C). The spectra IR were recorded with Perkin Elmer 577, the solid products were pelletized in KBr. The ultimate analysis was carried out by the central service of microanalysis of CNRS Vernaison, France.

2.2. 7-[5′-(3′-phenylisoxazolino)methy1]-8-hydroxyquinoline 4

7-Allyl-8-hydroxyquinoline **2** was obtained by a Williamson synthesis from 8-hydroxyquinoline and allyl bromide via allyloxyquinoline **1** which underwent a Claisen rearrangement to yield **2** [13] (Scheme 1). 7-allyl-8-hydroxyquinoline **2** (13.5 mmol) was dissolved in 50 ml of tetrahydrafurane (THF) and added to an etheric solution enclosing the precursor of benzonitriloxide **3** (Scheme 2) prepared as described [14–17]. The mixture was kept at –10 °C and triethylamine was slowly added. The crude product was then filtered, washed with a mixture of water–chloroform, dried on Na_2SO_4 and recrystallized from ethanol [18].



Scheme 2

Yield of the product was 85%; m.p = 115 °C; ^1H NMR (200 MHz, $CDCl_3$): 3.31(dd, 4′H); 3.19 (dd, 4′H); 5.25 (m, 5′H); 3.38 (d, 6′H); ^{13}C NMR (62 MHz, $CDCl_3$): 39.00 (4′C); 81.00 (5′C); 35.00 (6′C); IR/$\gamma_{C=N}$: 1580 cm^{-1}. Anal. Calcd. for $C_{19}H_{16}N_2O_2$: C, 75.00; H, 5.26. Found: C, 75.24; H, 5.13.

2.3. Microorganisms and growth conditions

The synthetic compound was tested for its antimicrobial activity against the following *Leishmania* species: *L. tropica* (MHOM/SY/86/LIPA154), *L. major* (MHOM/MA/95/9Y) and *L. infantum* (MHOM/FR/78/LEM75) were used in this study. Promastigotes of each species were grown at 25 °C in RPMI-1640 medium (Aqual) supplemented with 15% heat-inactivated fetal calf serum (Aqual) and antibiotics.

2.4. Promastigotes drug susceptibility assay

Promastigotes drug susceptibility determinations were made using a previously described direct counting assay based on growth inhibition [19]. Promastigotes were seeded at an initial concentration equivalent to 1.5×10^6 promastigotes/ml and allowed to multiply for 8 d in medium alone or in the presence of serial dilutions of drug ranging from 1.25 to 10 μg/ml. Amphotericin B was used in the same concentrations as a positive control. The protozoal counts were taken using Thomas haemocytometer.

2.5. Determination of percentage of growth inhibition

Growth rate (GR) is the relation between the number of viable *Leishmania* at 48 h and the number counted at 0 h. the percentage of growth inhibition (%GI) was calculated with respect to the growth control as follows: $\%GI = 1 - (GR_{extract}/GR_{control}) \times 100$.

2.6. Statistical analysis

Statistical analysis was done using Statview software. The log dose–response curves allowed determination of the con-

Table 1
Percentage growth inhibition of *Leishmania* promastigotes by the 7-[5′-(3′-phenylisoxazolino)methyl]-8-hydroxyquinoline

Strains	Time (h)	Compound*				Amphotericin B*			
		1.25	2.50	5	10	1.25	2.50	5	10
L. tropica	24	50	62.5	87	95	76.5	94.5	100	100
	48	80.3	92	97	98	87.2	96	100	100
L. major	24	51	81	94	97	69	92.1	100	100
	48	66	98	97	98.2	80.4	95.3	100	100
L. infantum	24	45	78	94.5	100	73	93	100	100
	48	75.2	93	99.5	100	83.4	94.1	100	100

* Concentration (μg/ml).

Table 2
IC_{50} values after 48 h of promastigotes incubation with 7-[5′-(3′-phenylisoxazolino)methyl]-8-hydroxyquinoline and amphotericin B

Species	IC_{50} (μg/ml)	
	Compound	Amphotericin B
L. tropica	0.4	0.25
L. major	0.88	0.50
L. infantum	0.62	0.34

centration causing a 50% reduction in the promastigotes number (IC_{50}). The standard deviation for the range of IC_{50} values for the compound for assays on promastigotes was determined by least-square regression analysis of the relative growth rate (% control) against the logarithm of the 8-hydroxyquinoline concentration at $P = 0.0281$ for *L. tropica*, $P = 0.2569$ for *L. major* and $P = 0.0780$ for *L. infantum*. The IC_{50} for the amphotericin B were at $P = 0.0211$ for *L. tropica*, $P = 0.0682$ for *L. major* and $P = 0.0322$ for *L. infantum*.

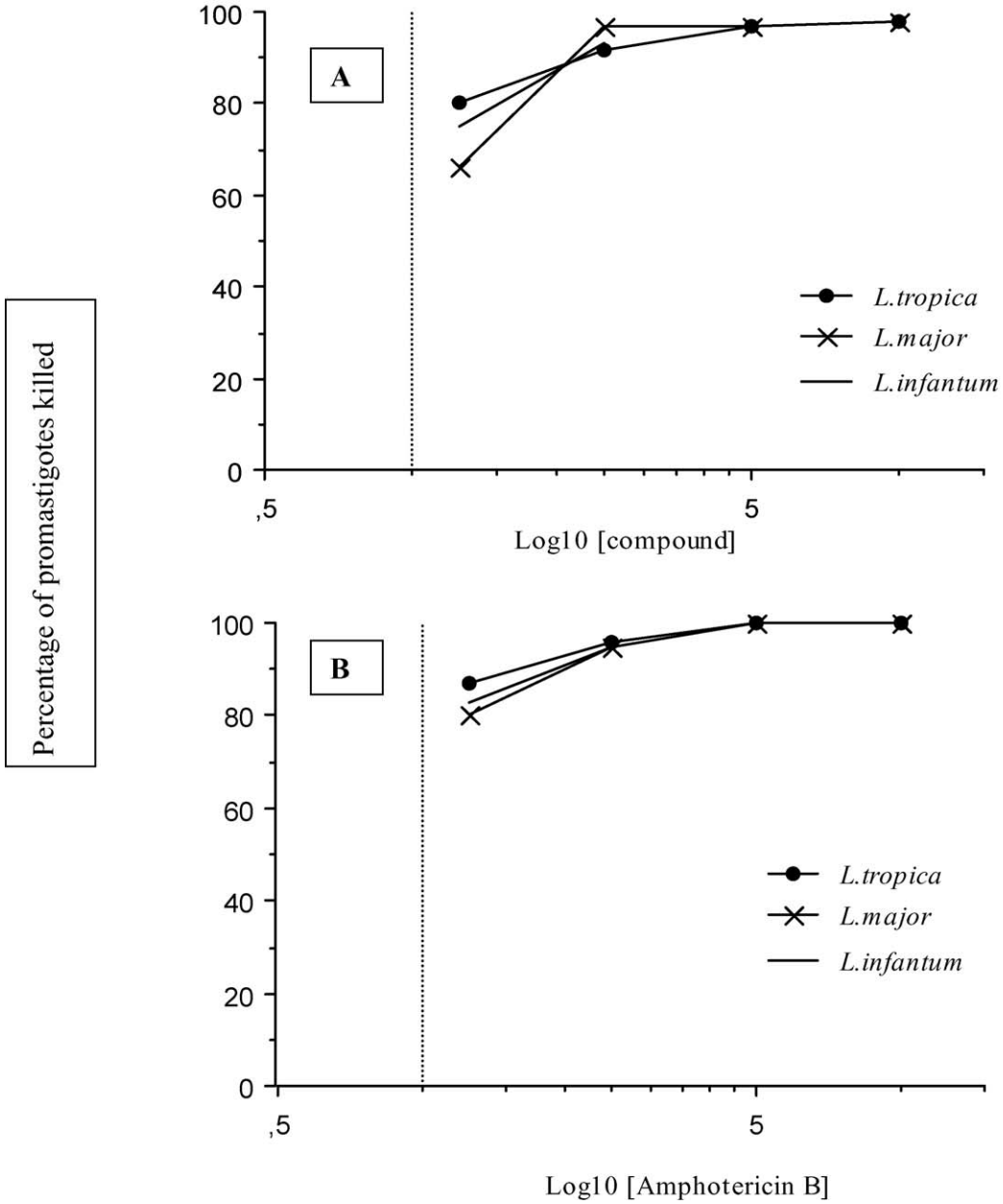


Fig. 1. Effect of different concentrations of the compound and amphotericin B against *L. tropica, L. major* and *L. infantum*. (A): Curve A allowed the determination of IC_{50} of the compound; *L. tropica:* $Y = 75.344 + 51.81X\text{-}29.243X^2$ $R^2 = 0.999$; *L. major:* $Y = 56.514 + 121.867X + 81.936X^2$, $R^2 = 0.934$; *L. infantum:* $Y = 68.197 + 78.352X - 46.9X^2$, $R^2 = 0.994$. (B): Curve B allowed the determination of IC_{50} of the amphotericin B; *L. tropica:* $Y = 83.26 + 41.52X\text{-}24.829X^2$, $R^2 = 1$; *L. major:* $Y = 74.147 + 66.985X - 41.382X^2$, $R^2 = 0.995$; *L. infantum:* $Y = 78.089 + 54.917X - 33.106X^2$, $R^2 = 0.999$.

3. Results

As shown in Table 1, 7-[5′-(3′-phenylisoxazolino)methyl]-8-hydroxyquinoline inhibited leishmanial growth, a concentration of 10 μg/ml exerted a strong inhibitory effect on promastigotes multiplication of the three species tested (i.e. *L. tropica, L. major* and *L. infantum*).

The IC_{50} values of the product and the amphotericin B obtained after 48 h for the promastigotes growth of *L. tropica, L. major* and *L. infantum* were shown in Table 2 and Fig. 1.

4. Discussion

Although members of the class of 8-hydroxyquinoline derivatives have been studied in several areas, they have been reported to have diverse activities. To our knowledge, this is the first report showing an antileishmanial activity of the 8-hydroxyquinoline derivatives. Our results clearly show that 7-[5′-(3′-phenylisoxazolino)methyl]-8-hydroxyquinoline inhibited leishmanial growth at micromolar concentrations. In fact, the assay was based on direct counting of *Leishmania* promastigotes, the result obtained with the compound were homogeneous, and all tested *Leishmania* showed a significant reduction of the promastigotes concentration to 50% after 24 h of incubation in presence of the minimal concentration of the compound (1.25 μg/ml). The percentage of growth inhibition reported in Table 1 showed that a concentration of 10 μg/ml inhibited completely the promastigotes growth of *L. infantum* after 24 h, whereas, only 95% and 97% of promastigotes of *L. tropica* and *L. major* respectively was inhibited. It is of interest to note, that in Mediterranean area, *L. infantum* causes CT [20–24], lethal visceral leishmaniasis in dog and human. Furthermore, this last form is reported more and more frequently in association with the human immunodeficiency virus (HIV) [25].

To further validate the assay, we determined the IC_{50} values for the compound and standard antileishmanial drug (amphotericin B). The IC_{50} values reported in Table 2 indicate that *L. tropica* was the most sensitive species to the compound as well as to the amphotericin B. *L. major* which causes the ZCL was slightly less susceptible.

In the present study, amphotericin B was used as the standard antileishmanial drug and all the *Leishmania* tested were highly susceptible to this compound. The IC_{50} values of amphotericin B are slightly better than those of the 7-[5′-(3′-phenylisoxazolino)methyl]-8-hydroxyquinoline. Amphotericin B is one of the most active antileishmanial agents [26]. In cases of treatment not responding to Pentostam, it was used as a satisfactory substitute [27]. However, drawbacks to amphotericin B include the requirement for infusions, length of therapy, adverse reactions, close laboratory monitoring for potential toxicity and, to some extent cost. In contrast, adequate data on acute toxicity of 8-hydroxyquinoline were rare [28]. Considering the effect of 7-[5′-(3′-phenylisoxazolino)methyl]-8-hydroxyquinoline on *Leishmania* strains which is an interesting finding, we can raise the question of whether this compound may be a substitute to antileishmanial agents used classically. But, to be able to do so more studies are needed.

In conclusion, we have described for the first time the antileishmanial activity of the 7-[5′-(3′-phenylisoxazolino)methyl]-8-hydroxyquinoline against *L. tropica, L. major* and *L. infantum* promastigotes. We also found that the sensitivity of promastigotes to amphotericin B closely parallels the sensitivity to the compound. Moreover, the compound could have more favorable biological properties, requiring further studies. Thus, more investigations should be done, first to test antileishmanial effect using *Leishmania* amastigote (parasite vertebrate stage) and further investigate in vivo activity in experimentally infected animals, second to better define the spectrum of the antimicrobial activity of this compound and to improve the biological activity by a suitable structural modifications.

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