European Journal of Medicinal Chemistry 54 (2012) 75–86

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Discovery of a new antileishmanial hit in 8-nitroquinoline series

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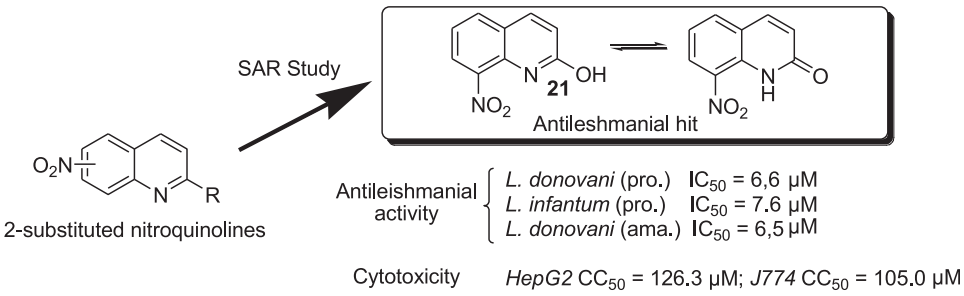
GRAPHICAL ABSTRACT



HIGHLIGHTS

- ► A series of 2-substituted nitroquinolines was prepared and studied concerning their *in vitro* antileishmanial properties.
- ► The 2-hydroxy-8-nitroquinoline (molecule **21**) appeared as a hit molecule on both pro- and amastigotes stages of *L. donovani*.
- ► Hit molecule 21, non-toxic on 2 different cell lines also appeared as a selective anti-infectious agent among protozoa.

ARTICLE INFO

Article history:
Received 28 February 2012
Received in revised form
18 April 2012
Accepted 20 April 2012
Available online 2 May 2012

Keywords:
8-Nitroquinoline
Visceral leishmaniasis
Leishmania donovani
Leishmania infantum

ABSTRACT

A series of nitrated 2-substituted-quinolines was synthesized and evaluated *in vitro* toward *Leishmania donovani* promastigotes. In parallel, the *in vitro* cytotoxicity of these molecules was assessed on the murine J774 and human HepG2 cell lines. Thus, a very promising antileishmanial hit molecule was identified (compound **21**), displaying an IC_{50} value of 6.6 µM and CC_{50} values $\geq$ 100 µM, conferring quite good selectivity index to this molecule, in comparison with 3 drug-compounds of reference (amphotericin B, miltefosine and pentamidine). Compound **21** also appears as an efficient *in vitro* antileishmanial molecule against both *Leishmania infantum* promastigotes and the intracellular *L. donovani* amastigotes (respective IC_{50} = 7.6 and 6.5 µM). Moreover, hit quinoline **21** does not show neither significant antiplasmodial nor antitoxoplasmic *in vitro* activity and though, presents a selective antileishmanial activity. Finally, a structure–activity relationships study enabled to

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0223-5234/$ – see front matter
doi:10.1016/j.ejmech.2012.04.029

Promastigotes
Amastigotes
hepG2 cytotoxicity
Selectivity index

define precisely the antileishmanial pharmacophore based on this nitroquinoline scaffold: 2-hydroxy-8-nitroquinoline.

1. Introduction

Leishmaniasis is one of the most important parasitic diseases worldwide as regards of its critical impact on human health and also because of the rapid emergence and spreading of resistant parasites. It is encountered in 97 countries, mainly in tropical and sub-tropical regions, but also in southern Europe, especially around the Mediterranean area. These parasitic infections are caused by a protozoan of the *Leishmania* genus transmitted to its mammal hosts (humans, dogs, monkeys, rodents…) by the bite of an infected sandfly (*Phlebotominae*). *Leishmania* parasites present two major morphological stages: extracellular flagellated promastigotes in the digestive tract of their sandfly vector and non-motile amastigotes inside the cells of their hosts' mononuclear phagocytic system. Leishmaniasis pathologies depend on the *Leishmania* species and range from restraint cutaneous leishmaniasis to very severe visceral leishmaniasis. There are an estimated 14 million people infected by leishmaniasis worldwide with an annual incidence of 2 million and about 50.000 estimated deaths due to its visceral form caused mainly by *Leishmania donovani*, considering that the number of cases is certainly under-evaluated as leishmaniases are reportable disease in only 40 countries [1].

The current first line drugs against leishmaniases are pentavalent antimonials (sodium stibogluconate and meglumine antimoniate), amphotericin B, pentamidine, paromomycin and miltefosine. Currently, these drugs used in the treatment of leishmaniasis present several problems including non-oral routes of administration (except for miltefosine), expensive cost of liposomal amphotericin B, high toxicity of antimonials, pentamidine, paromomycin and amphotericin B and adverse events of most clinically used molecules, leading to patients withdrawing from treatment and emergence of resistant strains [2,3]. During the last decade, antimonial resistance reached epidemic dimension in Bihar, India, where about 60% of newly diagnosed visceral leishmaniasis do not respond to these molecules nowadays [4]. Evaluation of the *in vitro* susceptibility of Indian *L. donovani* patient isolates to antimonials, amphotericin B and miltefosine indicates that cross-resistance may be emerging among these three drugs. Thus, efficacious and cheap oral antileishmanial agents to use in combination therapy are needed to safeguard against the expanding resistance problem and to overcome miltefosine's shortcomings [5].

During the last decade, only a few clinical studies about new antileishmanial drugs were carried out, even if several novel potential drug targets were identified in biochemical and molecular biology studies. Among these new targets, trypanothione reductase, cysteine peptidases, ornithine decarboxylase and cyclin-depend kinases appeared as particularly relevant [6]. Concerning the new chemical entities with antileishmanial potential, recent reviews showed that small synthetic heterocycles were good early candidates with a view to design new efficient drugs [5,7]. Among these heterocyclic molecules, studies focussing on quinoline derivatives have led to the identification of various interesting molecular scaffolds, bearing a large diversity of substituents at several positions of the quinoline ring. Bis-quinolines [8], some amodiaquine analogues [9], 2-substituted quinolines [10–12], 3-substituted quinolines [13], 4-substituted quinolines [14,15] and 8-substituted quinolines [16,17] are to mention. Sitamaquine, a 4,6,8-trisubstituted quinoline derivative (Fig. 1) targeting the parasitic succinate dehydrogenase even reached phase IIb of clinical trials as an oral antileishmanial drug for the treatment of visceral leishmaniasis [18].

Our research team, has been working on the synthesis of new heterocycles with pharmaceutical potential for years [19–25], and has developed a specific competency in the preparation of original antiprotozoal molecules targeting *Trichomonas* [26], *Plasmodium* [27], or *Leishmania* [28]. In direct continuation of preliminary studies [29–31] which were conducted in our lab on the synthesis and structural analysis of quinoline derivatives bearing antiparasitic potential, we prepared a series of nitrated quinoline derivatives and started to evaluate both their *in vitro* activity toward *L. donovani* promastigotes and their cytotoxicity on two different cell lines: J774 murine macrophage and HepG2 human hepatic cell lines, aiming at identifying original and selective antileishmanial quinolines.

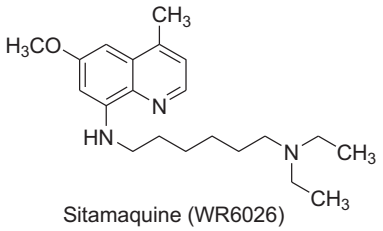
2. Results and discussion

2.1. Organic synthesis

In addition to the bibliographical data which we previously presented about antileishmanial 2-substituted-quinolines, a few nitroaromatic compounds have been reported in the literature as interesting antileishmanial compounds [5]. In the present study, 38 quinolines were evaluated among which 32 were prepared in our lab (6 were purchased). Apart molecules **2**, **35** and **36**, all synthesized molecules were quinolines bearing both a substituent at position 2 and a nitro group, mainly at position 8. Thus, a structural homogeneous quinoline series was obtained, aiming at analysing the antileishmanial structure–activity relationships in optimal conditions.

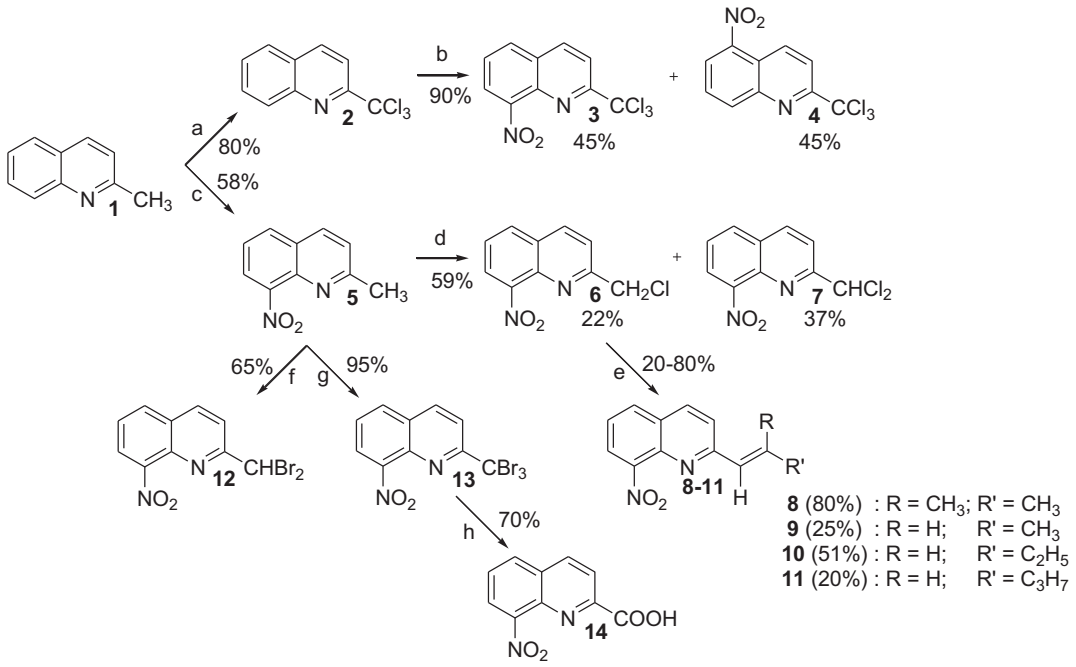
Molecules **2**, **3** and **4** were obtained in good yields, successively by trichlorination [32] and classical nitration reactions. Molecules **5**, **6** and **7** were easily prepared by a nitration reaction followed by a radical chlorination reaction using *N*-chlorosuccinimide (NCS) in carbon tetrachloride, under nitrogen atmosphere and light irradiation. Original vinylic derivatives **8**–**11** were synthesized from **6** by a $S_{RN}1$-E_2 reaction sequence [19,20,33] in variable yields, depending on the nitroalkane used. Compounds **12** and **13** were obtained by a bromination reaction in pure acetic acid, respectively by saturating the reaction medium with non-anhydrous or anhydrous sodium acetate, while molecule **14** was prepared from **13** by an acidic hydrolysis (Scheme 1).

Molecules **15**–**19**, belonging to the 6-nitroquinoline series, were synthesized from commercial quinoline **15** *via* a radical



Sitamaquine (WR6026)

Fig. 1. Sitamaquine, an antileishmanial quinoline undergoing phase IIb clinical trial.



Scheme 1. Synthesis of compounds **1**–**14**. a: PCl_5 (5 equiv), $POCl_3$, reflux for 48 h; b and c: HNO_3 (3 equiv), H_2SO_4, rt for 1 h; d: NCS (3 equiv), AIBN, CCl_4, reflux for 1 h; e: TBAOH 40% in water (3 equiv), CH_2Cl_2, nitroalkane (3 equiv), N_2, hν, rt for 1 h; f: refluxing acetic acid saturated with hydrated sodium acetate, Br_2 (2.5 equiv), reflux for 1 h; g: refluxing acetic acid saturated with anhydrous sodium acetate, Br_2 (5 equiv), reflux for 1 h; h: H_2SO_4 20%, reflux for 48 h.

dichlorination or an ionic trichlorination reaction, followed by $S_{RN}1$-$E_{RC}1$ reactions (Scheme 2) [29].

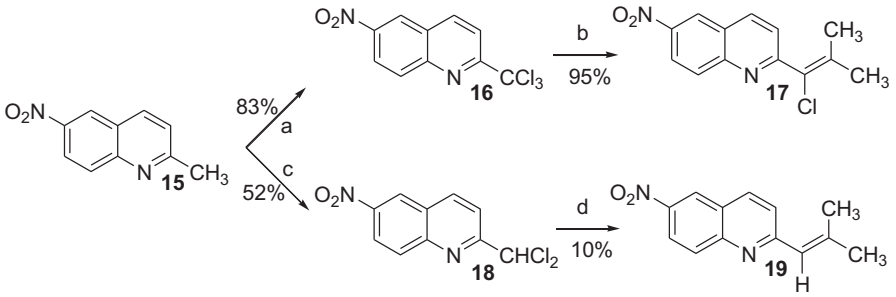
8-Nitro-2-trichloromethylquinoline **3** is an ideal substrate for operating simple pharmacomodulation reactions. Its trichloromethyl group can react with 2-nitropropane through consecutive $S_{RN}1$-$E_{RC}1$ reactions, to afford vinylic chloride **20** [29]. As a leaving group, its reactivity with hydroxide or methoxide anions also led to the substitution products **21** and **22** and, finally, its "activated carboxylic acid-like" reactivity permitted to obtain corresponding amides **23**–**25**. Molecule **21**, hit compound in the series, participates to a lactam–lactim prototropic tautomerism equilibrium (Scheme 3).

Compounds **27**–**38** were synthesized after identifying that molecule **21** was the series' hit, in order to study precisely the role played by the nitro group and the hydroxyl substituent of the quinoline ring, aiming at defining a pharmacophore. Molecules **27**, **28**, **30**, **31**, **35**, **36** and **38** were easily prepared by classical nitration reactions at room temperature. Quinoline derivatives **32** and **33** were obtained by S_NAr reactions from corresponding 2-chlorinated substrates **30** and **31**, using ammonia or acidic aqueous medium (Scheme 4).

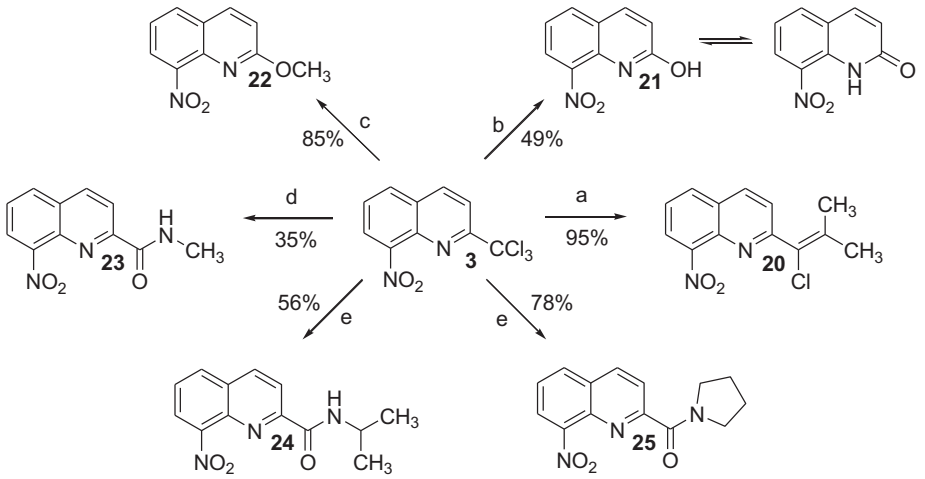
2.2. Biological evaluation

In a first time, molecules **1**–**25** were screened *in vitro* on *L. donovani* promastigotes by determining their inhibitory concentration 50% (IC_{50}) and, in parallel, were evaluated one the J774 and HepG2 cell lines, in order to assess their cytotoxicity *via* the determination of their cytotoxic concentrations 50% (CC_{50}). The J774 mouse macrophage cell line was chosen as a non-adherent cell line affording later cellular support for *L. donovani* amastigotes evaluation. The adherent HepG2 cell line was used because of its typical metabolism enzymes, allowing detection of some possible toxic metabolites. Corresponding selectivity indexes (SI) were then calculated according to the following formula: $SI = CC_{50}/IC_{50}$. Amphotericin B, pentamidine and miltefosine were chosen as antileishmanial reference-drugs and doxorubicin was used as a positive control for cytotoxicity evaluation. Results are presented in Table 1.

10 out of these 25 tested quinolines presented an antileishmanial IC_{50} lower than 15 μM. Among these derivatives, brominated molecules **12**, **13** can only be considered as globally aspecific toxic molecules because of their very low CC_{50} values. Interestingly,



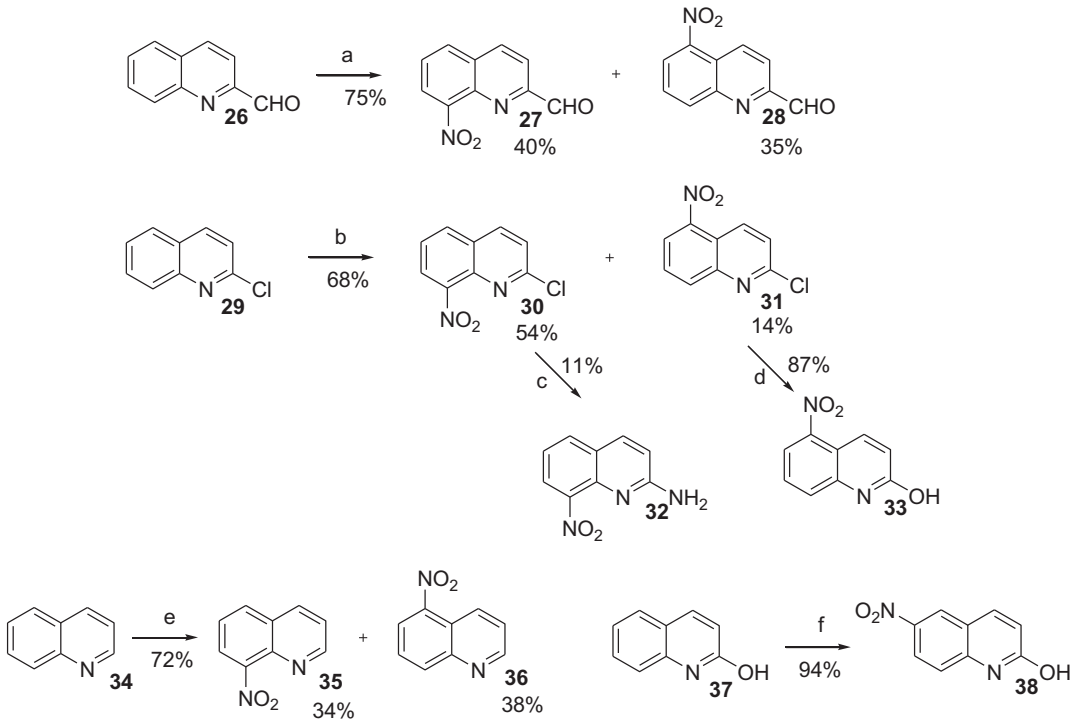
Scheme 2. Synthesis of compounds **15**–**19**. a: PCl_5 (5 equiv), $POCl_3$, 800 W MW for 20 min; b: TBAOH 40% in water (5 equiv), CH_2Cl_2, 2-nitropropane (5 equiv), N_2, hν, rt for 5 h; c: NCS (4 equiv), AIBN, CCl_4, reflux for 72 h; d: 2-nitropropane (3 equiv), N_2, hν, rt for 1 h.



Scheme 3. Synthesis of compounds **20**–**25**. a: TBAOH 40% in water (5 equiv), CH_2Cl_2, 2-nitropropane (5 equiv), N_2, hν, rt for 5 h; b: NaOH (5 equiv), ethanol–water (2:1), reflux for 30 min; c: Sodium methylate (5 equiv), methanol, N_2, reflux for 12 h; d: methylamine 33% in ethanol (15 equiv), sealed reactor, 100 °C for 24 h; e: appropriate amine (15 equiv), dioxane, reflux for 4 h.

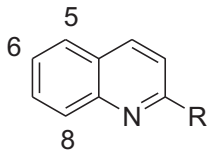
nitrated quinolines **9**–**11**, substituted with various vinylic groups at position 2, exhibited good antileishmanial IC_{50} values despite some significant cytotoxicity. These last molecules share some structural analogy with previously reported antileishmanial quinolines [11,12] bearing a vinylic group at position 2. However, by considering in the same 8-nitro series molecules **8** and **20**, it appears that 2-vinylic-substituted-quinolines whose vinylic double bond is either tri-substituted or including a chlorine atom lose their anti-leishmanial properties. Then, only 2-hydroxylated molecule **21** displays good and selective antileishmanial activity. Its IC_{50} value (6.6 μM) appears as similar to the one of pentamidine (6.0 μM) and twice as much as the one of miltefosine (3.1 μM). However, quinoline **21** is less toxic than all reference-drugs on both tested cell lines and presents good selectivity indexes (**15** and **19**) in comparison with the ones of reference-drugs which range from 0.17 to 88.

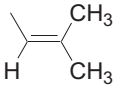
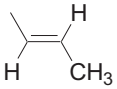
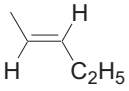
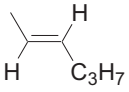
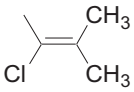
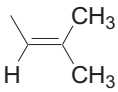
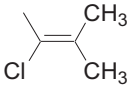
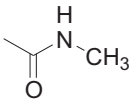
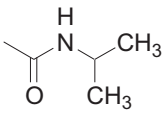
To enrich structure–activity relationships, molecules **26**–**38** were prepared and then tested. The first information emerging from the results obtained is that only the hydroxy group at position 2 confers antileishmanial activity to this quinoline scaffold involved in a prototropic tautomeric equilibrium between quinoline-2-ol and its [1*H*]-quinolin-2-one counterpart. Then, taking into account molecule **37**, it also appears that the nitro group is mandatory for preserving antileishmanial activity. Finally, when comparing molecules **21**, **33** and **38**, it can be concluded that this antileishmanial quinoline pharmacophore is also based on the presence of a nitro group at position 8 of the quinoline ring.



Scheme 4. Synthesis of compounds **26**–**38**. a, b, e, f: HNO_3 (3 equiv), H_2SO_4, rt for 1 h; c: 0.5 M ammonia solution in dioxane, sealed reactor, 110 °C for 48 h; d: HCl 10%, reflux for 1 h.

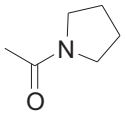
Table 1
In vitro antileishmanial activity and cytotoxicity of quinolines **1**–**38**.



Compound	Nitro group	R-	Antileishmanial activity: *L. donovani* promastigotes IC_{50} (μM)[a]	Cytotoxicity: J774 CC_{50} (μM)[a]	Selectivity index J774 CC_{50}/IC_{50}	Cytotoxicity: HepG2 CC_{50} (μM)[a]	Selectivity index HepG2 CC_{50}/IC_{50}
1	–	$-CH_3$	>50[b]	>100[b]	–	>100[b]	–
2	–	$-CCl_3$	>50[b]	>100[b]	–	>100[b]	–
3	8-Nitro	$-CCl_3$	14	16.3	1.2	25.0	1.8
4	5-Nitro	$-CCl_3$	4.6	4.9	1.1	10.0	2.2
5	8-Nitro	$-CH_3$	>50[b]	>100[b]	–	>100[b]	–
6	8-Nitro	$-CH_2Cl$	7.2	4.3	0.6	18.3	2.5
7	8-Nitro	$-CHCl_2$	>50[b]	4.8	<0.1	21.5	<0.4
8	8-Nitro		>50[b]	44.8	0.9	>100[b]	–
9	8-Nitro		10.2	6.8	0.7	12.0	1.2
10	8-Nitro		6.7	9.9	1.5	10.0	1.5
11	8-Nitro		7.0	11.7	1.7	16.0	2.3
12	8-Nitro	$-CHBr_2$	39	4.9	0.13	1.2	0.03
13	8-Nitro	$-CBr_3$	0.9	2.1	2.3	<0.1	<0.11
14	8-Nitro	$-COOH$	>50[b]	79.9	<1.6	>100[b]	–
15	6-Nitro	$-CH_3$	>50[b]	>100[b]	–	>100[b]	–
16	6-Nitro	$-CCl_3$	1.2	0.6	0.5	10.0	8.3
17	6-Nitro		11.8	16.6	1.4	>25	>2.1
18	6-Nitro	$-CHCl_2$	10.9	2.6	0.24	5.6	0.51
19	6-Nitro		33.7	45.1	1.3	>100[b]	>3.0
20	8-Nitro		28.0	34.9	1.2	>100[b]	>3.6
21	**8-Nitro**	**–OH**	**6.6**	**105.0**	**15.9**	**126.3**	**19.1**
22	8-Nitro	$-OCH_3$	24.2	30.8	1.3	50.0	2.1
23	8-Nitro		40.7	44.7	1.1	>100[b]	>2.5
24	8-Nitro		>50[b]	77.8	<1.6	>100[b]	–

(*continued on next page*)

Table 1 (*continued*)

Compound	Nitro group	R-	Antileishmanial activity: *L. donovani* promastigotes IC_{50} (μM)[a]	Cytotoxicity: J774 CC_{50} (μM)[a]	Selectivity index J774 CC_{50}/IC_{50}	Cytotoxicity: HepG2 CC_{50} (μM)[a]	Selectivity index HepG2 CC_{50}/IC_{50}
25	8-Nitro		>50[b]	43.3	<0.87	>100[b]	–
26	–	–CHO	>50[b]	28.8	<0.58	>100[b]	–
27	8-Nitro	–CHO	>50[b]	7.2	<0.14	40.6	<0.81
28	5-Nitro	–CHO	>50[b]	22.8	<0.46	92	<1.8
29	–	–Cl	>50[b]	>100[b]	–	>100[b]	–
30	8-Nitro	–Cl	>50[b]	>100[b]	–	>100[b]	–
31	5-Nitro	–Cl	>50[b]	>100[b]	–	>100[b]	–
32	8-Nitro	$-NH_2$	47.0	>100[b]	>2.1	47.9	1.0
33	5-Nitro	–OH	>50[b]	>100[b]	–	>100[b]	–
34	–	–H	>50[b]	>100[b]	–	>100[b]	–
35	8-Nitro	–H	>50[b]	>100[b]	–	>100[b]	–
36	5-Nitro	–H	>50[b]	>100[b]	–	>100[b]	–
37	–	–OH	>50[b]	>100[b]	–	>100[b]	–
38	6-Nitro	–OH	>50[b]	83.7	<1.7	>100[b]	–
Ref.	Amphotericin B[c]		0.1	3.1	31	8.8	88
Ref.	Pentamidine[c]		6.0	1.0	0.17	2.3	0.38
Ref.	Miltefosine[c]		3.1	94.6	30.5	50.3	16.2
Ref.	Doxorubicin[d]		–	0.02	–	0.2	–

Bold signifies hit-molecule.
[a] Mean of two independent experiments.
[b] No significant activity or toxicity noted at the highest tested concentration.
[c] Amphotericin B, pentamidine and miltefosine were used as antileishmanial drug-compounds of reference.
[d] Doxorubicin was used as a drug compound of reference for cytotoxicity.

From these initial results, hit compound **21** was then involved in additive studies in order to estimate more precisely its real potential as an efficient and selective antileishmanial molecule. As presented in Table 2, this molecule was tested on *Leishmania infantum* promastigotes and was shown to be as efficacious as pentamidine and miltefosine. More interestingly, **21**, tested on the amastigotes stage of the *L. donovani* parasite, appeared as active as miltefosine and more potent than pentamidine on this intracellular stage of the parasite which target is a key point for identifying relevant agents with real therapeutic potential [4]. Finally, to allow definition of **21** selectivity of action among protozoa, *in vitro* assessments of its antiplasmodial and antitoxoplasmic properties where performed and revealed that it is not active against *Plasmodium falciparum* and *Toxoplasma gondii*, in comparison with the reference-drugs chloroquine and pyrimethamine.

3. Conclusion

A new selective antileishmanial pharmacophore was identified in quinoline series from the *in vitro* evaluation of a series of nitrated quinoline derivatives substituted at position 2 by various groups. Structure–activity relationships showed that, from the quinoline scaffold, such pharmacophore was based on the presence of a nitro group at position 8 and a hydroxy group at position 2. Thus, the hit compound **21** displays quite good activity against both promastigotes (*L. donovani* and *L. infantum*) and amastigotes (*L. donovani*) parasite stages, comparable or better than the ones of miltefosine and pentamidine reference-drugs. Moreover, **21** is not toxic on the two tested cell lines and does not show any antiplasmodial or antitoxoplasmic activity. This set of results make quinoline **21** a very promising selective antileishmanial molecule and a good candidate for further *in vivo* studies.

4. Experimental section

4.1. Chemistry

4.1.1. General

Melting points were determined in open capillary tubes with a Büchi apparatus and are uncorrected. ^1H and ^{13}C NMR spectra

Table 2
In vitro, quinoline **21** is a non-toxic and selective antileishmanial hit molecule acting on both promastigotes and amastigotes stages.

Compound	*L. donovani* promastigotes IC_{50} (μM)[a]	*L. infantum* promastigotes IC_{50} (μM)[a]	*L. donovani* amastigotes IC_{50} (μM)[a]	K1 *P. falciparum* IC_{50} (μM)[a]	*T. gondii* (tachyzoïtes) IC_{50} (μM)[a]	J774 cytotoxicity CC_{50} (μM)[a]	HepG2 cytotoxicity CC_{50} (μM)[a]
21	6.6	7.6	6.5	19.0	56.1	105	126.3
Amphotericin B[c]	0.1	0.05	0.2	–	–	3.1	8.8
Pentamidine[c]	6.0	8.2	>20[b]	–	–	1.0	2.3
Miltefosine[c]	3.1	11.6	6.8	–	–	94.6	50.3
Chloroquine[d]	–	–	–	0.5	–	–	30.0
Pyrimethamine[e]	–	–	–	–	0.8	–	7.1
Doxorubicin[f]	–	–	–	–	–	0.02	0.2

[a] Mean of two independent experiments.
[b] No significant activity or toxicity noted at the highest tested concentration.
[c] Amphotericin B, pentamidine and miltefosine were used as antileishmanial drug-compounds of reference.
[d] Chloroquine was used as antiplasmodial drug-compound of reference.
[e] Pyrimethamine was used as antitoxoplasmic drug-compound of reference.
[f] Doxorubicin was used as a drug compound of reference for cytotoxicity.

were determined on a Bruker Avance 200 MHz instrument, at the Faculté de Pharmacie de Marseille. Chemical shifts are given in δ values referenced to the solvent. Elemental analyses were carried out with a Thermo Finnigan EA 1112 apparatus at the Spectropôle department of the Faculté des Sciences et Techniques de S^t Jérôme. Silica Gel 60 (Merck 70-230) was used for column chromatography. The progress of the reactions was monitored by thin layer chromatography using Kieselgel 60 F254 (Merck) plates.

The 2-methylquinoline **1** was purchased from Acros Organics.

The 2-methyl-6-nitroquinoline **15** was purchased from Maybridge.

Quinoline-2-carbaldehyde **26**, 2-chloroquinoline **29**, quinoline **34**, and 2-hydroxyquinoline **37** were purchased from Alfa Aesar.

4.1.2. Preparation of 2-trichloromethylquinoline **2** [34]

Compound **2** was prepared as described previously [34] and was obtained as a white solid by recrystallization (petroleum ether) in 80% yield; mp 65 °C, Lit: 56 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.56–7.65 (m, 1H), 7.73–7.85 (m, 2H), 8.05 (d, J = 8.7 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 8.26 (d, J = 8.7 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 98.0 (C), 117.4 (CH), 127.3 (CH), 127.8 (C), 128.3 (CH), 130.2 (CH), 130.5 (CH), 138.0 (CH), 145.8 (C), 157.3 (C).

4.1.3. Preparation of 8-nitro-2-trichloromethylquinoline **3** [32]

Compound **3** was prepared as described previously [32] and was obtained as a yellow solid by recrystallization (isopropanol) in 45% yield; mp 127 °C, Lit: 127 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.74 (dd, J = 7.4 and 8.4 Hz, 1H), 8.08–8.20 (m, 3H), 8.43 (d, J = 8.8 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 97.1 (C), 119.4 (CH), 124.8 (CH), 127.2 (CH), 128.4 (C), 131.4 (CH), 136.7 (C), 138.3 (CH), 148.2 (C), 159.0 (C).

4.1.4. Preparation of 5-nitro-2-trichloromethylquinoline **4** [29]

Compound **4** was prepared as described previously [29] and was obtained as a yellow solid by recrystallization (1-propanol) in 45% yield; mp 67 °C, Lit: 67 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.87–7.95 (m, 1H), 8.30 (d, J = 9.3 Hz, 1H), 8.48–8.55 (m, 2H), 9.20 (dd, J = 0.8 and 9.3 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 97.0 (C), 120.5 (CH), 120.9 (C), 126.2 (CH), 128.6 (CH), 134.5 (CH), 137.2 (CH), 145.2 (C), 146.0 (C), 158.6 (C).

4.1.5. Preparation of 2-methyl-8-nitroquinoline **5** [35]

Compound **5** was prepared as described previously [35] and was obtained as a beige solid by recrystallization (1-propanol) in 58% yield; mp 141 °C, lit: 135–137 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.75 (s, 3H), 7.40 (d, J = 8.5 Hz, 1H), 7.47–7.55 (m, 1H), 7.91–7.97 (m, 2H), 8.10 (d, J = 8.5 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 25.7 (CH_3), 123.1 (CH), 123.7 (CH), 124.2 (CH), 127.3 (C), 131.4 (CH), 135.8 (CH), 139.0 (C), 147.9 (C), 162.0 (C).

4.1.6. Preparation of 2-chloromethyl-8-nitroquinoline **6** [33] and 2-dichloromethyl-8-nitroquinoline **7**

To a solution of 1 equiv of **5** in carbon tetrachloride was added 3 equiv of *N*-chlorosuccinimide and a catalytic quantity of azoisobutyronitrile. The mixture was stirred and heated under reflux for 1 h. A residue was eliminated by filtration and the organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*.

Compound **6** was obtained after purification by silica gel column chromatography (eluent: petroleum ether/diethyl ether 1:1) as a pale yellow solid in 22% yield; mp 156 °C, Lit: 156 °C [33]. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 4.84 (s, 2H), 7.58–7.66 (m, 1H), 7.80 (d, J = 8.6 Hz, 1H), 8.00–8.05 (m, 2H), 8.29 (d, J = 8.6 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 46.9 (CH_2), 122.2 (CH), 123.8 (CH), 125.6 (CH), 128.1 (C), 131.5 (CH), 137.2 (CH), 138.5 (C), 159.5 (C). The quaternary carbon atom bearing the nitro group was not observed under these experimental conditions.

Compound **7** was obtained after purification by silica gel column chromatography (eluent: petroleum ether-diethyl ether 1:1) as a pale yellow solid in 37% yield; mp 169 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.85 (s, 1H), 7.64–7.72 (m, 1H), 8.04–8.09 (m, 3H), 8.40 (d, J = 8.7 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 71.5 (CH), 120.2 (CH), 124.3 (CH), 126.6 (CH), 128.7 (C), 131.5 (CH), 137.3 (C), 138.3 (CH), 147.0 (C), 159.8 (C). Anal. Calcd for $C_{10}H_6Cl_2N_2O_2$: C, 46.72; H, 2.35; N, 10.90. Found: C, 46.43; H, 2.32; N, 11.16.

4.1.7. Preparation of 2-(2-methylprop-1-enyl)-8-nitroquinoline **8** [33], (E)-8-nitro-2-(prop-1-enyl)quinoline **9**, (E)-2-(but-1-enyl)-8-nitro-quinoline **10** and (E)-8-nitro-2-(pent-1-enyl)quinoline **11**

To a solution of 3 equiv of corresponding nitroalkane in dichloromethane, 3 equiv of tetrabutylammonium hydroxyde (40% in water) were added under inert atmosphere (N_2) and irradiation with a tungsten lamp (60 W). The mixture was stirred at rt for 30 min and 1 equiv of 2-chloromethyl-8-nitroquinoline **6** was then added. The mixture was stirred for 1 h. The organic layer was washed six times with water, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by chromatography on a silica gel with the appropriate eluent.

Compound **8** was obtained, after purification by column chromatography (eluent: petroleum ether–dichloromethane 1:1) as a white solid in 80% yield; mp 91 °C, Lit: 92 °C [33]. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.01 (d, J = 0.9 Hz, 3H), 2.33 (d, J = 0.8 Hz, 3H), 6.41–6.43 (m, 1H), 7.33 (d, J = 8.6 Hz, 1H), 7.43–7.50 (m, 1H), 7.88–7.97 (m, 2H), 8.08 (d, J = 8.6 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.7 (CH_3), 28.3 (CH_3), 123.4 (CH), 123.8 (CH), 123.9 (CH), 124.4 (CH), 127.0 (C), 131.3 (CH), 135.6 (CH), 139.5 (C), 147.9 (C), 159.5 (C). The quaternary carbon atom bearing the nitro group was not observed under these experimental conditions.

Compound **9** was obtained after purification by column chromatography (eluent: cyclohexane–ethyl acetate 6:4) as a brown oil in 25% yield. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.94 (dd, J = 1.5 and 6.7 Hz, 3H), 6.65 (dd, J = 1.5 and 15.8 Hz, 1H), 6.91–7.05 (m, 1H), 7.59–7.67 (m, 2H), 7.83 (d, J = 8.4 Hz, 1H), 8.15–8.20 (m, 1H), 8.45 (d, J = 8.7 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 18.5 (CH_3), 121.0 (CH), 123.5 (CH), 125.1 (CH), 127.7 (C), 131.5 (CH), 131.8 (CH), 135.7 (CH), 137.0 (CH), 138.4 (C), 147.8 (C), 157.9 (C). HR MS (+ESI): 215.0815 (M + H^+). Calcd for $C_{12}H_{10}N_2O_2$: 214.0742.

Compound **10** was obtained after purification by column chromatography (eluent: dichloromethane–cyclohexane 7:3) as a brown oil in 51% yield. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.07 (t, J = 7.5 Hz, 3H), 2.23–2.35 (m, 2H), 6.61 (d, J = 6.0 Hz, 1H), 6.96–7.11 (m, 1H), 7.59–7.67 (m, 1H), 7.85 (d, J = 8.7 Hz, 1H), 8.17 (d, J = 7.9 Hz, 2H), 8.44 (d, J = 8.7 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 12.9 (CH_3), 25.6 (CH_2), 121.1 (CH), 123.5 (CH), 125.1 (CH), 127.7 (C), 129.2 (CH), 131.7 (CH), 137.0 (CH), 138.4 (C), 142.0 (CH), 147.8 (C), 158.0 (C). HR MS (+ESI): 229.0972 (M + H^+). Calcd for $C_{13}H_{12}N_2O_2$: 228.0899.

Compound **11** was obtained, after purification by column chromatography (eluent: cyclohexane–ethyl acetate 6:4) as a brown oil in 20% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.95–1.02 (m, 3H), 1.52–1.67 (m, 2H), 2.26–2.37 (m, 2H), 6.70 (d, J = 15.9 Hz, 1H), 6.93–7.08 (m, 1H), 7.46–7.60 (m, 2H), 7.92–7.99 (m, 2H), 8.13 (d, J = 8.7 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 13.8 (CH_3), 22.0 (CH_2), 35.1 (CH_2), 120.9 (CH), 123.6 (CH), 124.1 (CH), 128.0 (C), 130.3 (CH), 131.4 (CH), 136.0 (CH), 139.5 (C), 140.6 (CH), 148.1 (C), 158.5 (C). HR MS (+ESI): 243.1128 (M + H^+). Calcd for $C_{14}H_{14}N_2O_2$: 242.1055.

4.1.8. Preparation of 2-dibromomethyl-8-nitroquinoline **12** [35]

A refluxed solution of acetic acid was saturated with hydrated sodium acetate. 1 equiv of **5** was then added followed by 2.5 equiv of bromine and the reaction mixture was stirred for 1 h. Water was then added and the reaction mixture was extracted with

dichloromethane. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*.

Compound **12** was obtained, after purification by column chromatography (eluent: dichloromethane), as a yellow solid in 65% yield; mp 182 °C, Lit: 183 °C [35]. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.76 (s, 1H), 7.62–7.70 (m, 1H), 8.03–8.07 (m, 2H), 8.12–8.16 (m, 1H), 8.35–8.39 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 41.4 (CH), 121.7 (CH), 124.3 (CH), 126.5 (CH), 128.5 (C), 131.4 (CH), 136.8 (C), 138.1 (CH), 148.3 (C), 160.6 (C).

4.1.9. Preparation of 8-nitro-2-tribromomethylquinoline **13** [36]

Compound **13** was prepared as described previously [36] and was obtained after purification by silica gel column chromatography, eluting with dichloromethane/petroleum ether (1:1), as a yellow solid in 95% yield; mp 130 °C, Lit: 130 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.72 (dd, J = 8.2 and 8.3 Hz, 1H), 8.07–8.15 (m, 2H), 8.37 (s, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 88.5 (C), 119.9 (CH), 124.8 (CH), 127.1 (CH), 128.2 (C), 131.2 (CH), 136.3 (C), 137.7 (CH), 148.3 (C), 160.4 (C).

4.1.10. Preparation of 8-nitroquinoline-2-carboxylic acid **14** [36]

Compound **14** was prepared as described previously [36] and was obtained, after purification by column chromatography (eluent: ethyl acetate), as a white solid in 70% yield; mp 181 °C, Lit: 181–182 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.80–7.88 (m, 1H), 8.21–8.25 (m, 1H), 8.30–8.34 (m, 1H), 8.44–8.61 (m, 2H). The proton of the carboxylic acid function was not observed under these experimental conditions. ^{13}C NMR (50 MHz, $CDCl_3$) δ: 121.0 (CH), 126.3 (CH), 128.0 (CH), 130.4 (C), 132.6 (CH), 137.3 (C), 139.4 (CH), 147.9 (C), 163.0 (C). The quaternary carbon atom bearing the nitro group was not observed under these experimental conditions.

4.1.11. Preparation of 6-nitro-2-trichloromethylquinoline **16** [32]

Compound **16** was prepared as described previously [32] and was obtained as a pale yellow solid by recrystallization (isopropanol) in 83% yield; mp 152 °C, Lit: 152 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.24 (d, J = 8.9 Hz, 1H), 8.36 (d, J = 9.3 Hz, 1H), 8.53 (d, J = 8.9 Hz, 1H), 8.58 (dd, J = 2.5 and 9.3 Hz, 1H) 8.85 (d, J = 2.5 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 97.2 (C), 119.5 (CH), 123.8 (CH), 124.0 (CH), 126.8 (C), 132.1 (CH), 139.9 (CH), 146.8 (C), 147.9 (C), 160.5 (C).

4.1.12. Preparation of 2-(1-chloro-2-methylprop-1-enyl)-6-nitroquinoline **17** [29]

Compound **17** was prepared as described previously [29] and was obtained as a white solid by recrystallization (isopropanol) in 95% yield; mp 119 °C, Lit: 119 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.97 (s, 3H), 2.12 (s, 3H), 7.72 (d, J = 8.6 Hz, 1H), 8.19 (d, J = 9.3 Hz, 1H), 8.34 (d, J = 8.6 Hz, 1H), 8.45 (dd, J = 2.5 and 9.3 Hz, 1H), 8.76 (d, J = 2.5 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.8 (CH_3), 22.7 (CH_3), 122.9 (CH), 123.5 (C), 123.7 (CH), 123.9 (CH), 125.5 (C), 131.0 (CH), 136.9 (C), 138.0 (CH), 145.5 (C), 149.0 (C), 160.0 (C).

4.1.13. Preparation of 2-dichloromethyl-6-nitroquinoline **18**

To a solution of 1 equiv of **15** in carbon tetrachloride (20 mL) were added 4 equiv of *N*-chlorosuccinimide and a catalytic amount of azoisobutyronitrile. The mixture was stirred and heated under reflux for 72 h. A residue was eliminated by filtration and the organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (eluent: petroleum ether–dichloromethane 1:1) and product **18** was obtained as a pale yellow solid in 52% yield; mp 128 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.86 (s, 1H), 8.06 (d, J = 8.7 Hz, 1H), 8.21 (d, J = 9.2 Hz, 1H), 8.47–8.55 (m, 2H), 8.81 (d, J = 2.3 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 71.4 (CH), 120.2 (CH), 123.7 (CH), 124.1 (CH), 126.9 (C), 131.5 (CH), 140.0 (CH), 146.3 (C), 148.3 (C), 160.9 (C). Anal. Calcd for $C_{10}H_6Cl_2N_2O_2$: C, 46.72; H, 2.35; N, 10.90. Found: C, 46.60; H, 2.52; N, 10.55.

4.1.14. Preparation of 2-(2-methylprop-1-enyl)-6-nitroquinoline **19** [33]

To a solution of 3 equiv of 2-nitropropane in dichloromethane, 3 equiv of tetrabutylammonium hydroxyde (40% in water) were added. The mixture was stirred at rt for 30 min under inert atmosphere (N_2) and irradiation with a tungsten lamp (60 W) and 1 equiv of **18** was then added. After 1 h of reaction, the organic layer was washed six times with water, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*.

Compound **19** was obtained, after purification by column chromatography (eluent: chloroform–ethyl acetate 9:1) as a yellow solid in 10% yield; mp 108 °C, Lit: 109 °C [33]. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.04 (s, 3H), 2.27 (s, 3H), 6.52 (br s, 1H), 7.43 (d, J = 8.6 Hz, 1H), 8.09–8.23 (m, 2H), 8.42 (dd, J = 2.4 and 9.2 Hz, 1H), 8.71 (d, J = 2.4 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.5 (CH_3), 28.0 (CH_3), 123.0 (CH), 124.2 (CH), 124.5 (CH), 124.9 (CH), 130.6 (CH), 130.9 (C), 137.4 (CH), 144.9 (C), 146.9 (C), 150.0 (C), 160.9 (C).

4.1.15. Preparation of 2-(1-chloro-2-methylprop-1-enyl)-8-nitroquinoline **20** [29]

Compound **20** was prepared as described previously [29] and was obtained as a pale yellow solid by recrystallization (isopropanol) in 95% yield; mp 128 °C, Lit: 128 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.12 (s, 3H), 2.13 (s, 3H), 7.59 (dd, J = 7.5 and 8.3 Hz, 1H), 7.85 (d, J = 8.7 Hz, 1H), 7.97–8.04 (m, 2H), 8.24 (dd, J = 8.8 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 22.4 (CH_3), 23.9 (CH_3), 123.5 (CH), 123.7 (C), 123.8 (CH), 125.3 (CH), 127.5 (C), 131.4 (CH), 136.2 (CH), 138.3 (C), 139.0 (C), 148.1 (C), 158.7 (C).

4.1.16. Preparation of 8-nitroquinolin-2-ol **21** [37]

To a solution of 1 equiv of **3** in an ethanol–water solution (2:1), 5 equiv of NaOH were added. The reaction mixture was refluxed for 30 min. The reaction mixture was then neutralized and extracted twice with dichloromethane. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*.

Compound **21** was obtained, after purification by column chromatography (eluent: ethyl acetate) as an orange solid in 49% yield; mp 163 °C, Lit: 163 °C [37]. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.76 (dd, J = 1.8 and 9.8 Hz, 1H), 7.28–7.36 (m, 1H), 7.80 (d, J = 9.8 Hz, 1H), 7.88 (dd, J = 1.1 and 7.8 Hz, 1H), 8.51 (dd, J = 1.4 and 8.4 Hz, 1H), 11.31 (br s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 121.9 (CH), 122.1 (C), 123.8 (CH), 127.9 (CH), 133.1 (C), 133.9 (C), 135.6 (CH), 139.9 (CH), 161.5 (C).

4.1.17. Preparation of 2-methoxy-8-nitroquinoline **22** [38]

To a solution of 1 equiv of **3** in dry methanol, 5 equiv of sodium methylate were added under nitrogen atmosphere. The reaction mixture was refluxed for 12 h. Water was added and the mixture was extracted twice with dichloromethane. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*.

Compound **22** was obtained as a dark red solid in 85% yield; mp 114 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 4.07 (s, 3H), 7.02 (d, J = 9.0 Hz, 1H), 7.38–7.46 (m, 1H), 7.89–7.99 (m, 2H), 8.05 (d, J = 9.0 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 54.0 (CH_3), 115.0 (CH), 122.5 (CH), 123.9 (CH), 126.1 (C), 131.4 (CH), 138.1 (C), 138.4 (CH), 146.5 (C), 163.7 (C). Anal. Calcd for $C_{10}H_8N_2O_3$: C, 58.82; H, 3.95; N, 13.72. Found: C, 59.37; H, 4.18; N, 13.36.

4.1.18. Preparation of N-methyl-8-nitroquinoline-2-carboxamide **23**

A DMF solution of 8-nitro-2-trichloromethylquinoline **3** (1 equiv) was placed in a sealed reactor. 15 equiv of methylamine (33%

solution in ethanol) were then added. The mixture was stirred and heated under reflux for 24 h. After disappearance of **3** (monitored by TLC), the mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated. The crude residue was purified by chromatography on a silica gel column, eluting with ethyl acetate.

Compound **23** was obtained as a yellow solid in 35% yield; mp 174 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.90 (d, J = 4.2 Hz, 3H), 7.83–7.91 (m, 1H), 8.30 (d, J = 8.6 Hz, 1H), 8.37–8.41 (m, 2H), 8.51 (d, J = 4.2 Hz, 1H), 8.77 (d, J = 8.6 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 26.8 (CH_3), 120.9 (CH), 125.3 (CH), 127.7 (CH), 129.8 (C), 132.8 (CH), 137.4 (C), 139.1 (CH), 148.1 (C), 152.5 (C), 164.2 (C). Anal. Calcd for $C_{11}H_9N_3O_3$: C, 57.14; H, 3.92; N, 18.17. Found: C, 57.32; H, 4.00; N, 18.32.

4.1.19. Preparation of N-isopropyl-8-nitroquinoline-2-carboxamide **24** and [(8-nitroquinolin-2-yl)-pyrrolidin-1-yl]methanone **25**

To a solution of 1 equiv of 8-nitro-2-trichloromethylquinoline **3** in dioxane (30 mL), 15 equiv of appropriate amine were added. The mixture was stirred and heated under reflux for 4 h.

After disappearance of **3** (monitored by TLC), the mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated. The crude residue was purified by chromatography on a silica gel (eluent: ethyl acetate) to give the corresponding amide **24** and **25**.

Compound **24** was obtained as an orange solid in 56% yield; mp 129 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.32 (d, J = 6.6 Hz, 6 H), 4.23–4.40 (m, 1H), 7.71 (dd, J = 1.3 and 8.3 Hz, 1H), 7.96 (br s, 1H), 8.12 (dd, J = 1.3 and 8.3 Hz, 1H), 8.19 (dd, J = 1.3 and 7.4 Hz, 1H), 8.40–8.50 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 22.6 (CH_3*2), 41.7 (CH), 120.6 (CH), 125.0 (CH), 126.5 (CH), 129.8 (C), 132.3 (CH), 137.8 (CH), 147.8 (C), 152.0 (C), 162.3 (C). The quaternary carbon atom bearing the nitro group was not observed under these experimental conditions. Anal. Calcd for $C_{13}H_{13}N_3O_3$: C, 60.22; H, 5.05; N, 16.21. Found: C, 60.07; H, 5.18; N, 15.89.

Compound **25** was obtained as a brown solid in 78% yield; mp 129 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.91–2.03 (m, 4H), 3.73 (t, J = 6.2 Hz, 2H), 4.00 (t, J = 6.7 Hz, 2H), 7.67 (dd, J = 7.9 and 7.9 Hz, 1H), 8.07 (d, J = 7.8 Hz, 2H), 8.24 (d, J = 8.6 Hz, 1H), 8.35 (d, J = 8.6 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 23.8 (CH_2), 26.9 (CH_2), 47.6 (CH_2), 49.5 (CH_2), 123.3 (CH), 123.9 (CH), 126.3 (CH), 128.9 (C), 131.7 (CH), 136.8 (CH), 137.7 (C), 148.4 (C), 155.4 (C), 164.3 (C). Anal. Calcd for $C_{14}H_{13}N_3O_3$: C, 61.99; H, 4.83; N, 15.49. Found: C, 62.10; H, 4.99; N, 15.40.

4.1.20. Preparation of 8-nitroquinoline-2-carbaldehyde **27** [35] and 5-nitro-quinoline-2-carbaldehyde **28** [39]

Pure H_2SO_4 was added onto 1 equiv of **26** at 0 °C. 3 equiv of 65% HNO_3 were then added dropwise and the reaction mixture was stirred at rt for 1 h. After the reaction mixture was poured into water, the solution was neutralized with Na_2CO_3 and extracted twice with dichloromethane. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*.

Compound **27** was obtained, after purification by column chromatography (eluent: dichloromethane–ethyl acetate 8:2), as a beige solid in 40% yield; mp 154 °C, Lit: 152 °C [35]. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.74–7.82 (m, 1H), 8.11–8.18 (m, 3H), 8.43–8.47 (m, 1H), 10.19 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 119.0 (CH), 124.5 (CH), 127.9 (CH), 130.5 (C), 131.8 (CH), 137.7 (CH), 139.0 (C), 146.8 (C), 153.9 (C), 192.9 (CH).

Compound **28** was obtained, after purification by column chromatography (eluent: dichloromethane–ethyl acetate 8:2), as a beige solid in 35% yield; mp 167 °C, Lit: 168 °C [39]. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.89–7.97 (m, 1H), 8.23 (d, J = 9.0 Hz, 1H), 8.50–8.60 (m, 2H), 9.18 (d, J = 9.0 Hz, 1H), 10.23 (d, J = 0.8 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 120.0 (CH), 122.8 (C), 126.8 (CH), 128.5 (CH), 133.7 (CH), 137.3 (CH), 145.6 (C), 147.9 (C), 153.2 (C), 192.7 (C).

4.1.21. Preparation of 2-chloro-8-nitroquinoline **30** [40] and 2-chloro-5-nitroquinoline **31** [41]

Pure H_2SO_4 was added onto 1 equiv of **29** at 0 °C. 3 equiv of 65% HNO_3 were then added dropwise and the reaction mixture was stirred at rt for 1 h. After the reaction mixture was poured into water, the solution was neutralized with Na_2CO_3 and extracted twice with dichloromethane. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*.

Compound **30** was obtained, after purification by column chromatography (eluent: cyclohexane–ethyl acetate 8:2), as a white solid in 54% yield; mp 152 °C, Lit: 149 °C [40]. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.52–7.56 (d, J = 8.6 Hz, 1H), 7.60–7.68 (m, 1H), 8.02–8.11 (m, 2H), 8.20 (d, J = 8.6 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 124.6 (CH), 124.9 (CH), 125.8 (CH), 127.6 (C), 131.8 (CH), 138.6 (CH), 139.0 (C), 147.3 (C), 153.6 (C).

Compound **31** was obtained, after purification by column chromatography (eluent: cyclohexane–ethyl acetate 8:2), as a pale yellow solid in 14% yield; mp 134 °C, Lit: 133–134 °C [41]. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.63 (d, J = 9.2 Hz, 1H), 7.80–7.88 (m, 1H), 8.34 (d, J = 8.5 Hz, 1H), 8.40 (dd, J = 1.1 and 7.7 Hz, 1H), 8.99 (d, J = 9.2 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 119.9 (C), 124.9 (CH), 125.4 (CH), 128.8 (CH), 134.9 (CH), 135.5 (CH), 145.4 (C), 148.0 (C), 152.5 (C).

4.1.22. Preparation of 8-nitroquinolin-2-amine **32** [40]

1 equiv of **30** was put in a sealed reactor. 15 mL of a 0.5 M solution of ammonia in dioxane were injected. The reaction mixture was then stirred at 110 °C for 48 h before the solvent was evaporated.

Compound **32** was obtained, after purification by column chromatography (eluent: dichloromethane–ethyl acetate 1:1), as a crystalline brown solid in 11% yield; mp 160 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.20 (br s, 2H), 6.80 (d, J = 8.9 Hz, 1H), 7.21–7.28 (m, 2H), 7.75–7.80 (m, 1H); 7.86–7.93 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 124.5 (CH), 124.9 (CH), 125.8 (CH), 127.6 (C), 131.8 (CH), 138.7 (CH), 138.9 (C), 147.2 (C), 153.5 (C). MS (+ESI): 190.38 ($M + H^+$). Calcd for $C_9H_7N_3O_2$: 189.05.

4.1.23. Preparation of 5-nitroquinolin-2-ol **33** [42]

Compound **33** was prepared as described previously [42] and was obtained, after purification by column chromatography (eluent: ethyl acetate), as a beige solid in 87% yield; mp 302 °C, Lit: 302 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 6.76 (d, J = 10.1 Hz, 1H), 7.63–7.75 (m, 2H), 7.85–7.90 (m, 1H), 8.25 (d, J = 10.1 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 111.7 (C), 118.7 (CH), 121.2 (CH), 125.5 (CH), 130.3 (CH), 134.4 (CH), 140.4 (C), 146.5 (C), 161.0 (C).

4.1.24. Preparation of the 8-nitroquinoline 35 [43] and 5-nitroquinoline **36** [41]

Pure H_2SO_4 was added onto 1 equiv of **34** at 0 °C. 3 equiv of 65% HNO_3 were then added dropwise and the reaction mixture was stirred at rt for 1 h. The reaction mixture was successively poured into water, neutralized with Na_2CO_3 and extracted twice with dichloromethane. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*.

Compound **35** was obtained as a pale yellow solid in 34% yield; mp 90 °C, Lit: 89–90 °C [43]. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.53–7.66 (m, 2H), 8.04 (d, J = 8.0 Hz, 2H), 8.27 (dd, J = 1.6 and 8.4 Hz, 1H), 9.70 (dd, J = 1.7 and 4.2 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 122.8 (CH), 123.8 (CH), 125.3 (CH), 129.0 (C), 132.0 (CH), 136.1 (CH), 139.5 (C), 148.2 (C), 152.6 (CH).

Compound **36** was obtained by recrystallization (isopropanol) as a pale yellow solid in 38% yield; mp 71 °C, Lit: 71–72 °C [41]. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.61–7.68 (m, 1H), 7.76–7.85 (m, 1H), 8.36–8.45 (m, 2H), 8.98–9.04 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 121.2 (C), 123.9 (CH), 124.6 (CH), 127.5 (CH), 131.9 (CH), 136.6 (CH), 145.5 (C), 148.2 (C), 151.6 (C).

4.1.25. Preparation of 6-nitroquinolin-2-ol ***38*** *[44]*

Pure H_2SO_4 was added onto 1 equiv of **37** at 0 °C. 3 equiv of 65% HNO_3 were then added dropwise and the reaction mixture was stirred at rt for 1 h. After the reaction mixture was poured into water, the solution was neutralized with Na_2CO_3 and extracted twice with dichloromethane. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*.

Compound **38** was obtained, after recrystallization from isopropanol, as a yellow solid in 94% yield; mp 279 °C, Lit: 280 °C [44]. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 6.68 (d, J = 9.6 Hz, 1H), 7.43 (d, J = 9.1 Hz, 1H), 8.12 (d, J = 9.8 Hz, 1H), 8.33 (dd, J = 2.6 and 9.0 Hz, 1H), 8.70 (d, J = 2.6 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 116.3 (CH), 118.7 (C), 124.0 (CH), 124.5 (CH), 125.3 (CH), 140.3 (CH), 141.7 (C), 143.5 (C), 162.1 (C).

4.2. Biology

4.2.1. Antileishmanial evaluation

Leishmania species used in this study were *L. donovani* MHOM/IN/00/DEVI and *L. infantum* MCAN/ES/98/LLM-877, all purchased from CNR *Leishmania* (Montpellier, France).

4.2.1.1. Antileishmanial activity on promastigotes. The effects of the tested compounds on the growth of *L. donovani* promastigotes were assessed by MTT assay [45]. Briefly, promastigotes in late log-phase in Schneider's medium supplemented with 20% foetal calf serum (FCS), 2 mM L-glutamine and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin), were incubated at an average density of 10^6 parasites/mL in sterile 96-well plates with various concentrations of compounds dissolved in DMSO (final concentration less than 0.5% v/v) incorporated in duplicate. Appropriate controls treated by DMSO, pentamidine, miltefosine or amphotericin B (references drugs purchased from Sigma Aldrich) were added to each set of experiments. After a 72 h incubation period at 27 °C, parasite metabolic activity was determined. Plates were centrifuged at 900 g for 10 min and the supernatant removed. After the addition of MTT (0.5 mg/mL in RPMI 1640, 100 μL/well), plates were incubated for 6 h at 27 °C. The plates were subsequently centrifuged at 900 g for 10 min and the supernatant removed. The pellet was dissolved in 100 μl of DMSO and the absorbance measured in a plate reader at 570 nm. Inhibitory concentration 50% (IC_{50} Ld) was defined as the concentration of drug required to inhibit by 50% the metabolic activity of *L. donovani* promastigotes compared to the control. IC_{50} was calculated by non-linear regression analysis processed on dose-response curves, using TableCurve 2D V5 software. IC_{50} values represent the mean value calculated from two independent experiments.

The antileishmanial activity of the hit molecules were subsequently evaluated on promastigotes following the same method as described above.

4.2.1.2. Antileishmanial activity on intracellular amastigotes. The effects of the tested compounds on the growth of *Leishmania* amastigotes were assessed in the following way. Five hundred microlitres of J774A.1 cells were seeded in sterile chamber-slides at an average density of 5×10^4 cell/mL and incubated for 24 h at 37 °C and 6% CO_2. *L. donovani* promastigotes were centrifuged at 900 g for 10 min and the supernatant replaced by the same volume of Schneider 20% FCS pH 5.4 and incubated for 24 h at 27 °C. J774A.1 cells were then infected by acidified promastigotes at an average density of 5×10^5 cell/mL (10:1 ratio) and chamber-slides incubated for 24 h at 37 °C. Afterwards, medium with a range of compound concentrations was added in duplicate (final DMSO concentration less than 0.5% v/v). Appropriate controls treated with or without solvent (DMSO), and various concentrations of pentamidine, miltefosine and amphotericin B were added to each set of experiments. After 120 h incubation at 37 °C and 6% CO_2, well supernatant was removed. Cells were fixed with analytical grade methanol and stained with 10% Giemsa. The percentage of infected macrophages in each assay was determined microscopically by counting at least 200 cells in each sample. IC_{50} was defined as the concentration of drug necessary to produce a 50% decrease of infected macrophages compared to the control. IC_{50} was calculated by non-linear regression analysis processed on dose-response curves, using TableCurve 2D V5 software. IC_{50} values represent the mean value calculated from two independent experiments.

4.2.2. Antiplasmodial evaluation

In this study, a K1 culture-adapted *P. falciparum* strain resistant to chloroquine, pyrimethamine and proguanil was used in an *in vitro* culture. Maintenance in continuous culture was done as described previously by Trager and Jensen [46]. Cultures were maintained in fresh A+ human erythrocytes at 2.5% haematocrit in complete medium (RPMI 1640 with 25 mM HEPES, 25 mM $NaHCO_3$, 10% of A+ human serum) at 37 °C under reduced O_2 atmosphere (gas mixture 5% O_2, 5% CO_2, and 90% N_2). Parasitaemia was maintained daily between 1% and 6%. The *P. falciparum* drug susceptibility test was carried out by comparing quantities of DNA in treated and control cultures of parasite in human erythrocytes according to a SYBR Green I fluorescence-based method [47] using a 96-well fluorescence plate reader. Parasite culture was synchronized at ring stage with 5% sorbitol. Compounds were incubated in a total assay volume of 200 μL (RPMI, 2% haematocrit and 1% parasitaemia) for 72 h in a humidified atmosphere (5% O_2 and 5% CO_2) at 37 °C, in 96-well flat bottom plates. Triplicate assays were performed for each sample. After incubation, 170 μL supernatant was discarded and cells were washed with 150 μL 1X PBS. 15 μL resuspended cells were transferred to 96-well flat bottom non-sterile black plates (Greiner Bio-one) already containing 15 μL of the SYBR Green I lysis buffer (2XSYBR Green, 20 mM Tris base pH 7.5, 20 mM EDTA, 0.008% w/v saponin, 0.08% w/v Triton X-100). Negative control, treated by solvents (DMSO or H_2O) and positive control (chloroquine, purchased from Sigma Aldrich) were added to each set of experiments. Plates were incubated for 15 min at 37 °C and then read on a TECAN Infinite F-200 spectrophotometer with excitation and emission wavelengths at 497 and 520 nm, respectively. The concentrations of compounds required to induce a 50% decrease of parasite growth (IC_{50W2}) were calculated from two independent experiments.

4.2.3. Antitoxoplasmic evaluation

The effects of the tested compounds on the growth of *T. gondii* tachyzoites (PRU-β-Gal strain) [48] were assessed by a colorimetric microtiter assay adapted from McFadden et al. [49]. Briefly, tachyzoites were maintained by serial passage in confluent monolayer of human foreskin fibroblasts HFF (ATCC, Manassas, USA). For assay, 96-well microtiter plates were seeded with 3.10^4 HFF cells per well and allowed to grow to confluence in RPMI 1640 (without phenol red) supplemented with 10% FCS and 1% L-glutamine/penicillin-streptomycin mix at 37 °C with 6% CO_2. Cell monolayers were infected with 1×10^4 parasites per well and incubated for 3 h at 37 °C with 6% CO_2. Then, various concentrations of compounds dissolved in DMSO (final concentration less than 0.5% v/v) were

incorporated in triplicate. Appropriate controls treated by DMSO or the reference drug pyrimethamine (purchased from Sigma Aldrich) were added to each set of experiments. Negative control consisted in cell monolayers incubated without parasite and drug. After a 72 h incubation period at 37 °C with 6% CO_2, cell medium was removed and 100 μL of a 1 mM chlorophenol red-β-D-galactopyranoside (CPRG) solution were added to each well. The plates were incubated at 37 °C with 6% CO_2 for 6 h, at which time β-galactosidase activity was measured by reading plates at 570 and 630 nm on a Biotek microtiter plate reader. Blanking was made on the negative-control wells. The concentration of compounds required to induce a 50% decrease of parasite growth (IC_{50}) was calculated by non-linear regression analysis processed on dose-response curves, using the TableCurve software 2D v.5.0. IC_{50} values represent the mean value calculated from two independent experiments.

4.2.4. Cytotoxicity evaluation

The evaluation of the tested molecules cytotoxicity on the HepG2 (hepatocarcinoma cell line purchased from ATCC, ref HB-8065) J774A.1 (mouse macrophage cell line ECACC, Salisbury UK) and HFF (human foreskin fibroblast) cell lines was done according to the method of Mosmann [45] with slight modifications. Briefly, cells in 100 μL of complete medium, [RPMI supplemented with 10% foetal bovine serum, 1% L-glutamine (200 mM) and penicillin (100 U/mL)/streptomycin (100 μg/mL)] were inoculated into each well of 96-well plates and incubated at 37 °C in a humidified 6% CO_2 with 95% air atmosphere. After a 24 h incubation, 100 μL of medium with various product concentrations was added and the plates were incubated for 72 h. At the end of the treatment and incubation, each plate-well was microscope-examined for detecting possible precipitate formation before the medium was aspirated from the wells. 10 μL of MTT solution (5 mg MTT/mL in PBS) were then added to each well with 100 μL of medium without foetal calf serum. Cells were incubated for 2 h at 37 °C to allow MTT oxidation by mitochondrial dehydrogenase in the viable cells. After this time, the MTT solution was removed and DMSO (100 μL) was added to dissolve the resulting blue formazan crystals. Plates were shaken vigorously (300 rpm) for 5 min. The absorbance was measured at 570 nm with 630 nm as reference wavelength with a microplate spectrophotometer. DMSO was used as blank and doxorubicin (purchased from Sigma Aldrich) as positive control. Cell viability was calculated as percentage of control (cells incubated without compound). The 50% cytotoxic concentration was determined from the dose-response curve, as a mean of two independent experiments.

Acknowledgments

This work was supported by the Centre National de la Recherche Scientifique (CNRS), Aix-Marseille Université and the Assistance Publique-Hôpitaux de Marseille (APHM). L. Paloque is supported by the Region PACA/Yelen BDE PhD fellowship. The authors also thank the French National Leishmaniasis Reference Center of Montpellier (CHU de Montpellier, France) for providing the *Leishmania* strains.

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