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Original article

Looking for new antileishmanial derivatives in 8-nitroquinolin-2(1*H*)-one series

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ABSTRACT

From a recently identified antileishmanial pharmacophore, a structure–activity relationship study was conducted by introducing various aminated, phenoxy or thiophenoxy moieties at position 4 of the 8-nitroquinolin-2(1*H*)-one scaffold, using S_NAr reactions. Thus a series of 47 derivatives was synthesized and evaluated *in vitro* on the promastigote stage of *Leishmania donovani*. In parallel, the cytotoxicity of the active molecules was tested on the human HepG2 cell line. The results we obtained showed that the introduction of a substituent at position 4 of the antileishmanial pharmacophore can either lead to inactive or active derivatives, depending on the nature of the substituent. Aminated moieties appear as very unfavorable toward antileishmanial activity, while phenoxy or thiophenoxy moieties were shown to maintain the *in vitro* antileishmanial profile, especially when the phenyl ring of these moieties was substituted at the *para* or *ortho* position by a halogen atom (except fluorine), a trifluoromethyl group or a methyl group. Most of these derivatives showed a lack of solubility in the culture media which hindered the *in vitro* determination of both their cytotoxicity and activity against the intracellular amastigoste stage of *L. donovani*.

1. Introduction

Leishmaniasis is an infectious disease caused by protozoan parasites belonging to the genus *Leishmania*. Leishmaniasis presents various clinical aspects including cutaneous leishmaniasis (CL), the most common form, muco-cutaneous leishmaniasis (MCL) and visceral leishmaniasis (VL), the most severe form, lethal in untreated patients. This disease is mainly endemic in developing countries of the inter-tropical area but also in southern Europe, especially around the Mediterranean area. According to the WHO, 310 million people from 98 countries are at risk of getting infected and 6 countries are reporting over 90% VL cases (Bangladesh, Brazil, Ethiopia, India, Nepal, South Sudan and Sudan) [1]. The annual incidence of leishmaniasis is 1.3 million people with about 20,000 to 30,000 estimated deaths due to its visceral form. Parasites are transferred from host to host by the bite of an infected female sandfly (*Phlebotominae*), when taking a blood meal. The majority of cases of VL are caused by *Leishmania donovani*, *Leishmania infantum* and *Leishmania chagasi* (in Latin America) [2]. *L. infantum* and *L. chagasi* mainly affect children and immunocompromised individuals and are zoonotic parasites with canines being a major reservoir. *L. donovani*, on the other hand, is an anthroponotic parasite that affects a broad range of ages [3]. *Leishmania* parasites exist in two major morphological stages: extracellular flagellated promastigotes in the digestive tract of their sandfly vector, which is

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the infective stage for mammals, and immotile intracellular amastigotes in the cells of their host' mononuclear phagocytic system.

Waiting for successful vector management and effective vaccines in humans, control of the disease is based on chemotherapy. Unfortunately, there are nowadays very few drugs available for treating leishmaniasis [4]. The oldest historical drugs, such as pentavalent antimonials (sodium stibogluconate and meglumine antimoniate) and pentamidine, present major adverse effects like cardiotoxicity and kidney toxicity [5]. Miltefosine, the only orally active antileishmanial agent, suffers from teratogenicity [6]. Moreover, resistant parasites have emerged in several endemic areas: some publications reported that up to 60% of newly diagnosed VL cases in India did not respond to antimonials [7,8]. Drug-resistance to miltefosine was also reported in the Indian Subcontinent and the overall probability of treatment failure was 21% [9]. In addition, cross-resistance may be emerging among these drugs [10]. Amphotericin B remains, by far, the most effective molecule, but its use was initially limited by a major kidney toxicity. Liposomal amphotericin B presents a more favorable toxicological profile, but the expensive cost of this formulation and its non-oral route of administration are very problematical to treat people in endemic areas [11]. Consequently, new molecules are urgently needed so as to try to overcome this neglected disease. Furthermore, except for some preventive vaccines, there is still no curative treatment for canines, which is both a human health and veterinary concern.

About new molecules with antileishmanial potential, recent reviews show that some small synthetic heterocycles are good early candidates with a view to design new efficient drugs [12]. On the one hand, quinolines are a class of compounds that have shown a good potential as antileishmanials [13]. As an example, sitamaquine, a 4,6,8-trisubstituted quinoline derivative, is in phase IIb/III of development as an oral antileishmanial drug for the treatment of VL [14] (Fig. 1A). Some of recently reported polysubstituted quinolines, like 4-(quinolin-4-yl)morpholine derivatives [15,16] (Fig. 1B.) or 8-hydroxyquinoline derivatives [17] (Fig. 1C.), are also promising candidates. One the other hand, there is renewed interest into nitro-heterocyclic compounds for treating parasitic infectious disease. For instance, fexinidazole will soon enter a phase II clinical study for the treatment of VL in Sudan [18] (Fig. 2A). Recently, diverse nitrated compounds belonging to different chemical series have also shown anti-infectious properties in an early stage development, such as nitroimidazopyridines [19] (Fig. 2B).

Our research teams work on the synthesis, screening improvement [20] and development of new anti-infective agents targeting *Plasmodium falciparum* [21–24], *Rhinovirus* [25], but also *Leishmania donovani* [19,26]. In this context, starting from a previous chemical study focusing on new quinoline derivatives with anti-infective potential [27,28], we previously identified a promising *in vitro* antileishmanial hit-molecule, based on a quinoline ring bearing a nitro group: the 8-nitroquinolin-2(1*H*)-one [29] (Fig. 2C). In order to explore the SARs of this basic molecular scaffold and searching for new antileishmanial hit-compounds, we present herein its pharmacomodulation at position 4 of the quinoline ring by using various S_NAr reactions. A series of 47 molecules was screened *in vitro* on the promastigote stage of *L. donovani*. The resulting hit-compounds were then tested on the human HepG2 cell line, to evaluate their cytotoxicity.

2. Results and discussion

2.1. Synthesis of starting material 3

In a view to explore the pharmacomodulation of the 8-nitroquinolin-2(1*H*)-one on the C-4 position of the quinoline ring, starting material **3** was prepared in two steps (Scheme 1). Nitration of commercial 2,4-dibromoquinoline, at RT, led to the two nitrated position isomers **1** and **2**. Then, 4-bromo-8-nitroquinolin-2(1*H*)-one **3** was prepared from **1** by a S_NAr reaction involving a 65% perchloric acid acetonitrile solution, according to a previously described procedure [30]. Initially, without microwave irradiation, the operating conditions required 72 h at 100 °C for the substrate to be totally consumed. The use of a monomode microwave irradiation heating at 100 °C, in a sealed vial, permitted to reduce the reaction time from 72 h to 30 min.

2.2. Synthesis and screening of new 4-substituted 8-nitroquinolin-2(1H)-one derivatives

2.2.1. Synthesis and evaluation of the aminated derivatives

We started our investigation by introducing various amines (primary, secondary and aromatic) at the C-4 position of the 8-nitroquinolin-2(1*H*)-one scaffold. From our experience in using the S_NAr reaction with amines for anti-infectious pharmacomodulation studies [31], we synthesized 6-aminated derivatives **5**–**10**. Thus, **3** was reacted in a 30% aqueous ammoniac solution under microwave irradiation according to a previously described procedure [32], to obtain 4-amino-8-nitroquinolin-2(1*H*)-one **4** in 30% yields. Compounds **5**–**8** were prepared in medium to very good yields, by S_NAr reactions with primary or secondary amines in *n*-propanol. **9** was obtained from **8** after deprotection of the terminal amine-function in classical acidic conditions. Because of the lower reactivity of aromatic amines, the reaction of **3** with aniline required to use a Buchwald-Hartwig approach to provide the expected product **10** in a low reaction yield (15%), using a previously described procedure [33] (Scheme 2).

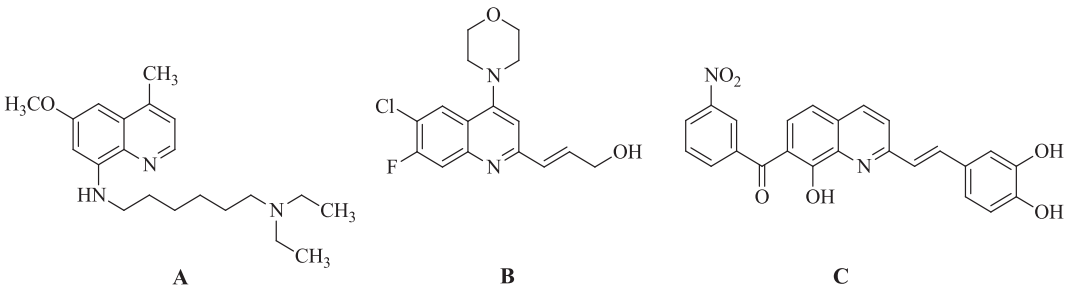


Fig. 1. Structures of some quinoline derivatives displaying antileishmanial properties.

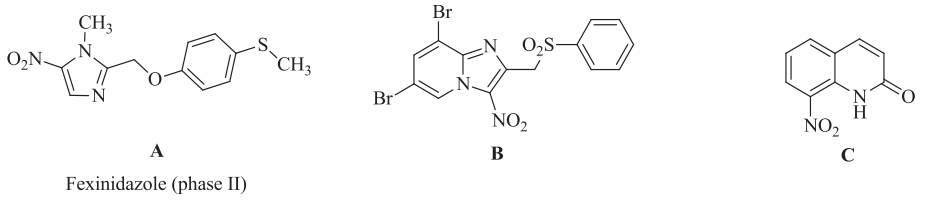
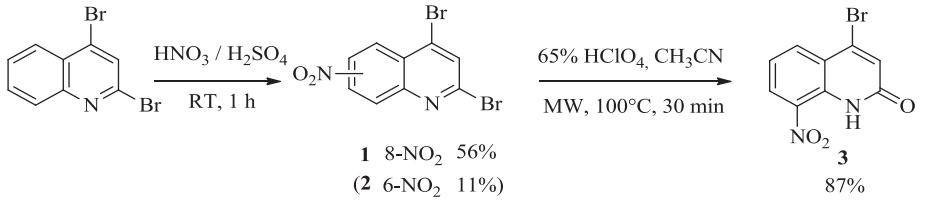
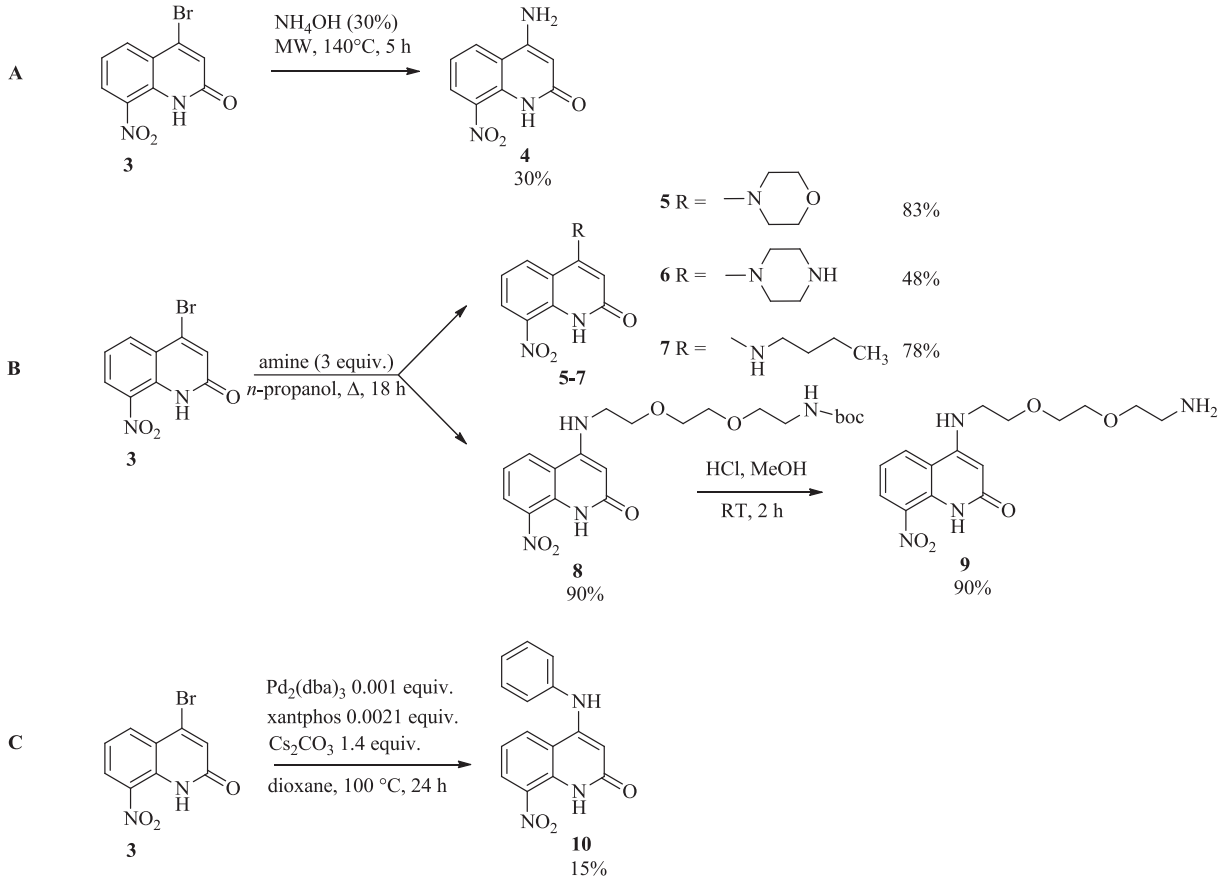


Fig. 2. Examples of some aromatic nitrated heterocycles with antileishmanial activity.



Scheme 1. Two-step synthesis of substrate **3**.



Scheme 2. Synthesis of aminated derivatives **4**–**10**.

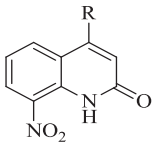
These 7 molecules were then screened *in vitro* on the promastigote stage of *Leishmania donovani* by determining their inhibitory concentrations 50% (IC_{50}) and comparing them to the ones of pentamidine, miltefosine and amphotericin B, chosen as antileishmanial reference drugs. In order to assess their selectivity of action, these compounds were also tested *in vitro* on the human HepG2 cell line, to evaluate their cytotoxicity.

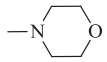
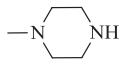
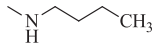
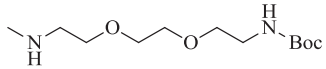
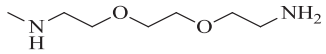
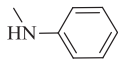
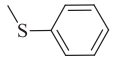
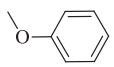
All tested aminated derivatives (**4**–**10**) presented $IC_{50} > 20$ μM, demonstrating a lack of efficacy of these molecules on the promastigote stage of *L. donovani* (Table 1). Moreover, compound **6** and aniline derivative **10** showed significant *in vitro* cytotoxicity (CC_{50} = 1.8–2.4 μM). Consequently, we did not consider appropriate to follow our investigations on this aminated series.

2.2.2. Synthesis and evaluation of thiophenol derivative 11 and phenol derivative 12

Then, we replaced the aniline group by a thiophenol or phenol moiety, using antiparasitic pharmacomodulation approaches that

Table 1
Reaction yields, *in vitro* antileishmanial activity and cytotoxicity of compounds **4**–**12**.



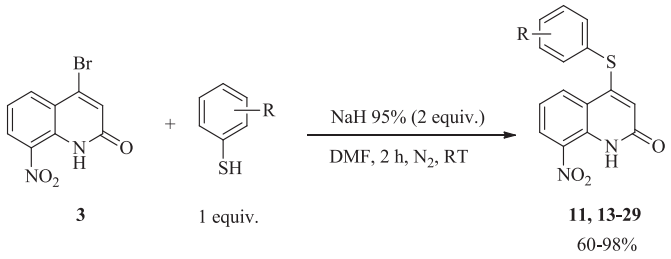
N°	R-	*L. donovani* promastigotes IC_{50} (μM)[a]	HepG2 cytotoxicity CC_{50} (μM)[a]
4	$-NH_2$	>50[c]	>125[c]
5		42.1 (±8.7)	86.2 (±10.2)
6		>50[c]	2.4 (±0.3)
7		21.4 (±3.9)	12.2 (±1.9)
8		>50[c]	34.8 (±1.3)
9		>50[c]	122.9 (±18.5)
10		>50[c]	1.8 (±0.6)
11		**9.3 (± 1.6)**	**>12.5**[d]
12		**14.0 (± 3.4)**	**>12.5**[d]
Pentamidine[b]		6.0 (±0.8)	2.3 (±0.5)
Miltefosine[b]		3.1 (±0.06)	50.3 (±4.1)
Amphotericin B[b]		0.06 (±0.02)	8.8 (±0.6)

In bold: hit-compounds.
[a] The values are means ± SD of three independent experiments.
[b] Pentamidine, Miltefosine and Amphotericin B were used as antileishmanial drug-compounds of reference.
[c] No activity or toxicity noted at the highest tested concentration.
[d] Molecules could not be tested at higher concentrations due to a lack of solubility in the culture medium.

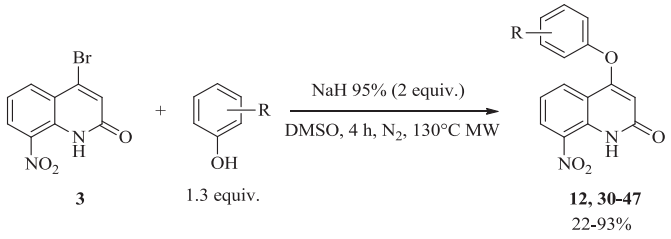
we previously reported [34,35], in order to study the biological properties of the corresponding sulfur or oxygen-containing analogs. Compound **11** was prepared by a S_NAr reaction in 86% yield: the thiophenolate anion, obtained by adding sodium hydride to an anhydrous DMF solution of thiophenol, was reacted with substrate **3** under nitrogen atmosphere at RT (Scheme 3).

We then applied these experimental conditions for synthesizing compound **12**, using phenol instead of thiophenol, but the reaction was unsuccessful. Due to the lower nucleophilicity of the phenol reagent, the reaction did not take place at RT. After a few trials monitored by LC/MS, the operating conditions were defined, requiring 4 h at 130 °C, 1.3 equiv. of phenol, 2 equiv. of NaH and the use of DMSO as an alternative to DMF. Moreover, microwave irradiation was necessary for the substrate to be fully consumed after 4 h (Scheme 4). Compound **12** was finally obtained in 30% yield, because of a difficult purification of the reaction product, requiring an alumina chromatography column, instead of a traditional silica chromatography column.

It is important to note that, contrary to the results obtained with the aniline-substituted derivative **10** which was inactive (IC_{50} > 50 μM) and cytotoxic (CC_{50} = 1.8 μM), thiophenol-



Scheme 3. Preparation of compounds **11** and **13**–**29** by S_NAr reactions involving thiophenols.



Scheme 4. Synthesis of compounds **12** and **30**–**47** by microwave-assisted S_NAr reactions.

substituted derivative **11** and phenol-substituted derivative **12**, showed attractive *in vitro* antileishmanial IC_{50} values activity (9μM < IC_{50} < 14 μM) despite a lack of solubility in the culture medium (Table 1). As a consequence, we decided to focus our attention on the preparation and study of 2 new chemical series: 4-phenylthio-8-nitroquinolin-2(1*H*)-ones and 4-phenoxy-8-nitroquinolin-2(1*H*)-ones. 37 additional molecules were then prepared to explore their antileishmanial potential and structure–activity relationships.

2.3. Synthesis of 4-phenylthio-8-nitroquinolin-2(1H)-one derivatives and 4-phenoxy-8-nitroquinolin-2(1H)-one derivatives

Thus, in order to study the influence of the substitution of the thiophenol moiety, a series of 17 new 4-phenylthio-8-nitroquinolin-2(1*H*)-one derivatives **13**–**29** was synthesized from substrate **3**. The expected products were obtained quickly at RT in good to very good yields (60%–98%), according to Scheme 3 and Table 2.

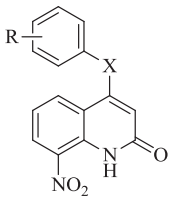
As we proceeded in the thiophenol series, 18 new phenols derivatives **30**–**47** were prepared from **3**, according to Scheme 4. Because phenols are less nucleophilic than thiophenols, the operating conditions required heating at 130 °C, longer reaction times and the assistance of microwave irradiation. Compounds **30**–**47** were obtained 22%–93% yields (Table 2). The low yields obtained for compounds **30**, **33**, **35** and **46** are due to a difficult purification requiring an alumina chromatography column, instead of a traditional silica one.

2.4. In vitro antileishmanial evaluation of 4-phenylthio-8-nitroquinolin-2(1H)-ones and 4-phenoxy-8-nitroquinolin-2(1H)-ones

All synthesized compounds were first tested *in vitro* on the promastigote stage of *Leishmania donovani* (Table 2). Then, only molecules presenting IC_{50} values inferior or equal to 10 μM were evaluated for their cytotoxicity toward the HepG2 cell line.

Among the 18 tested molecules in the thiophenol-containing series, 6 showed an IC_{50} value <10 μM. The IC_{50} values (ranging

Table 2
Reaction yields, *in vitro* antileishmanial activity of the thiophenol and phenol-containing derivatives **11**–**47**.



N°	X	R	*L. donovani* promastigotes IC_{50} (μM)[a]	N°	X	R	*L. donovani* promastigotes IC_{50} (μM)
11	**S**	**H**	**9.3 (±1.6)**	**12**	**O**	**H**	**14.0 (±3.4)**
13	S	2′-OCH_3	>10[c]	**30**	O	2′-OCH_3	>10[c]
14	S	3′-OCH_3	>10[c]	**31**	O	3′-OCH_3	>10[c]
15	S	4′-OCH_3	>10[c]	**32**	O	4′-OCH_3	>10[c]
16	**S**	**2′-CF_3**	**3.5 (± 1.5)**	**33**	**O**	**2′-CF_3**	**10 (± 1.2)**
17	S	3′-CF_3	>10[c]	**34**	**O**	3′-CF_3	>10
18	**S**	**4′-CF_3**	**3.8 (± 1.1)**	**35**	**O**	**4′-CF_3**	**8.4 (± 0.8)**
19	**S**	**2′-Cl**	**3.5 (± 1.7)**	**36**	O	2′-Cl	>10[c]
20	S	3′-Cl	>10[c]	**37**	O	3′-Cl	>10[c]
21	S	4′-Cl	>10[c]	**38**	**O**	**4′-Cl**	**3.7(± 1.4)**
22	S	**2′,4′-diCl**	**3.8 (± 1.7)**	**39**	**O**	**2′-Br**	**8.1(± 1.1)**
23	**S**	**4′-Br**	**4.2 (± 1.7)**	**40**	O	3′-Br	>10[c]
24	S	2′-F	>10[c]	**41**	O	4′-Br	>5[d]
25	S	3′-F	>10[c]	**42**	O	2′-F	>10[c]
26	S	4′-F	>10[c]	**43**	O	3′-F	>10[c]
27	S	2′-CH_3	>10[c]	**44**	O	2′-CH_3	>10[c]
28	S	4′-CH_3	>10[c]	**45**	O	3′-CH_3	>10[c]
29	S	2′-NH_2	>10[c]	**46**	**O**	**4′-CH_3**	**6.0 (± 0.4)**
		Pentamidine[b]	6.0 (±0.8)	**47**			
O	**3′-CH_3-4′-Cl**		**6.0 (± 1.0)**				
		Miltefosine[b]	3.1 (±0.06)				
		Amphotericin B[b]	0.06 (±0.02)				

In bold: most active molecules.
[a] The values are means ± SD of three independent experiments.
[b] Pentamidine, Miltefosine and Amphotericin B were used as antileishmanial drug-compounds of reference.
[c] No activity noted at the highest tested concentration.
[d] Molecule **41** could not be tested at higher concentrations due to a lack of solubility in the culture medium.

from 3.5 μM to 4.2 μM) of the 5 most active compounds (**16**, **18**, **19**, **22**, **23**) were comparable with the ones of pentamidine (6.0 μM) and miltefosine (3.1 μM). Molecules including a methyl, methoxy, fluorine or amine substituent (respectively **27**–**28**, **13**–**15**, **24**–**26** and **29**) did not display significant activity (Table 2), in comparison with compound **11** which presents an unsubstituted thiophenol moiety (IC_{50} = 9.3 μM), whereas bromine, chlorine and trifluoromethyl groups are the substituents which increase antileishmanial activity. Considering that both the Br donating group and the CF_3 attracting group led to more active molecules, it can be hypothesized that the common hydrophobic character of these substituents could participate to improving the antileishmanial activity. It also appeared that the position of the substituents on the thiophenol moiety played an important role toward biological activity: only *ortho* or *para*-substituted molecules displayed activity against the parasite.

Among the 19 compounds belonging to the phenol-containing series, the screening realized on the promastigote stage of the parasite highlighted 8 active molecules (Table 2) presenting $IC_{50} \leq 10$ μM (**12**, **33**–**35**, **38**, **39**, **46**, **47**). As already noted in the thiophenol series, bromine, chlorine and trifluoromethyl substituents on the phenol moiety were favorable for providing antileishmanial activity. Comparing compounds **33**, **34** and **35**, it appeared that the *ortho* or *para* substitution of the phenol moiety was probably the most suitable for preserving antiparasitic activity. Nevertheless, in contrast with the results obtained with thiophenol derivatives, substitution of the phenol ring by a methyl group at the *para* position led to an active derivative, suggesting again that the hydrophobic character of the substituent could be responsible for increasing the antileishmanial activity. The IC_{50} values of the 8 most active derivatives (IC_{50} = 3.7–14 μM) were close to the ones of pentamidine (6.0 μM) and miltefosine (3.1 μM) and were, on average, twice as high as the IC_{50} obtained in the 4-phenylthio-8-nitroquinolin-2(1*H*)-one series.

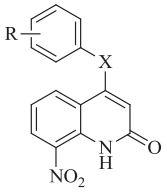
2.5. Cytotoxicity and intracellular anti-amastigote evaluation of the most active compounds

All compounds displaying significant antileishmanial activity were then tested *in vitro* on the human HepG2 cell line in order to explore their cytotoxicity (Table 3). This latter cell line allows a thorough evaluation of the cytotoxic behavior of the tested molecules, in addition to the one of some of their metabolites. The corresponding CC_{50} values were compared with those of pentamidine, miltefosine and amphotericin B chosen as antileishmanial reference drugs. Doxorobucin was chosen as a reference cytotoxic agent. Selectivity indexes (SI) were finally calculated, according to the formula: $SI = CC_{50}/IC_{50}$. All tested derivatives showed a lack of solubility in the HepG2 culture medium which made it difficult to interpret the cytotoxicity results (Table 3). Nevertheless, molecules **18** and **46** displayed respective SI of >3.3 and >4.2, ranging between the ones of the antileishmanial drugs pentamidine (SI = 0.4) and miltefosine (SI = 16.2). Finally, the most selective compounds in each series (**18** and **46**) were evaluated *in vitro* against the intracellular amastigote stage of *Leishmania donovani*. In addition, these 2 derivatives were parallely assessed for their cytotoxicity on the THP-1 macrophage cell line, this last being used in the intracellular infection assay. Unfortunately, because of their lack of solubility in the biological media, no IC_{50} value could be determined for any of these 2 molecules which precipitated above 12.5 μM, enabling the full determination of their antileishmanial potential (Table 3).

3. Conclusion

In a view to investigate their antileishmanial activity, a series of new 8-nitroquinolin-2(1*H*)-one derivatives, bearing an aminated, phenoxy or thiophenoxy group at position 4 of the quinoline ring, was prepared from 4-bromo-8-nitroquinolin-2(1*H*)-one, using SNAr reactions. The *in vitro* evaluation of these molecules toward the promastigote stage of *Leishmania donovani* showed that the introduction of aminated moities led to inactive or cytotoxic derivatives while the substitution of the position 4 of the antileishmanial scaffold by a thiophenol or phenol moiety generated several bioactive molecules, displaying IC_{50} values comparable to the ones of pentamidine and miltefosine. Moreover, SARs revealed that, in both active series, substitution of the phenyl ring by hydrophobic substituents such as Cl, Br, CF_3 at position *ortho* or *para* (or a methyl group at the *para* position in the phenol series) was increasing antileishmanial activity. Finally, the *in vitro* cytotoxicity

Table 3
In vitro antileishmanial activity, cytotoxicity and selectivity index of the most active compounds.



N°	X	R	*L. donovani* promastigotes IC_{50} (μM)[a]	Cytotoxicity CC_{50} (μM)[a] HepG2	Selectivity index ($CC_{50\ HepG2}/IC_{50\ L.\ dono.\ pro.}$)	Intracellular *L. donovani* amastigote IC_{50} (μM)[a]	Cytotoxicity CC_{50} (μM)[a] THP-1
11	S	H	9.3 (±1.6)	>12.5[d]	>1.3	–	–
12	O	H	14.0 (±3.4)	>12.5[d]	>0.9	–	–
16	S	2′-CF_3	3.5 (±1.5)	>7.8[d]	>2.22	–	–
18	**S**	**4′-CF_3**	**3.8 (± 1.1)**	**> 12.5**[d]	**> 3.3**	**> 12.5**[d]	**> 12.5**[d]
19	S	2′-Cl	3.5 (±1.7)	>1.25[d]	>0.4	–	–
22	S	2′,4′-diCl	3.8 (±1.7)	>7.8[d]	>2.1	–	–
23	S	4′-Br	4.2 (±1.7)	>3.9[d]	>0.9	–	–
33	O	2′-CF_3	10 (±0.0)	>15.6[d]	>1.6	–	–
34	O	3′-CF_3	11.4 (±2.5)	>7.8[d]	>0.7	–	–
35	O	4′-CF_3	8.4 (±0.8)	>7.8[d]	>0.9	–	–
38	O	4′-Cl	3.7 (±1.4)	>3.9[d]	>1.1	–	–
39	O	2′-Br	8.1 (±1.1)	>7.8[d]	>0.96	–	–
46	**O**	**4′-CH_3**	**6.0 (± 0.4)**	**> 25.0**[d]	**> 4.2**	**> 12.5**[d]	**> 12.5**[d]
47	O	3′–CH_3 –4′-Cl	6.0 (±1.0)	>15.6[d]	>2.6	–	–
Pentamidine[b]			6.0 (±0.8)	2.3 (±0.5)	0.4	>20	31.2 (±3.2)
Miltefosine[b]			3.1 (±0.06)	50.3 (±1.5)	16.2	6.8 (±0.9)	–
Amphotericin B[b]			0.06 (±0.02)	8.8 (±0.6)	146.7	0.16 (±0.04)	8.4 (±0.8)
Doxorubicin[c]			–	0.2 (±0.05)	–	–	0.008 (±0.001)

In bold: most selective molecules.
[a] The values are means ± SD of three independent experiments.
[b] Pentamidine, Miltefosine and Amphotericin B were used as antileishmanial drug-compounds of reference.
[c] Doxorubicin was used as a cytotoxic drug compound of reference.
[d] Molecules could not be tested at higher concentrations due to lack of solubility in the culture medium.

evaluation of the most active derivatives toward the human HepG2 (+/− THP-1) cell line(s), along with their *in vitro* evaluation against intracellular *L. donovani* amastigotes, revealed that all phenoxy or thiophenoxy-containing molecules showed a lack of solubility in the culture media, hindering the full determination of their biological profile. From the potential of these derivatives, additional chemistry work is under progress so as to synthesize new derivatives with improved aqueous solubility so as to optimize the antileishmanial activity of the 8-nitroquinolin-2(1*H*)-one pharmacophore.

4. Experimental section

4.1. Chemistry

4.1.1. General

Microwave-assisted reactions were performed in 10–20 mL sealed vials using a Biotage Initiator Microwave oven; temperatures were measured with an IR-sensor and reaction times are given as hold times. Commercial reagents were used as received without additional purification. Melting points were determined in open capillary tubes with a Büchi apparatus and are uncorrected. Elemental analysis and HRMS were carried out at the Spectropole, Faculté des Sciences de Saint-Jérôme, Marseille, France. ^1H and ^{13}C NMR spectra were determined on a Bruker Avance 200 or 400 MHz instrument, at the Faculté de Pharmacie de Marseille and Faculté des Sciences de Saint Jérôme. Chemical shifts are given in d values referenced to the solvent. The following adsorbent was used for column chromatography: silica gel 60 (Merck, particle size 0.063–0.200 mm, 70–230 mesh ASTM) or aluminum oxide gel (Sigma–Aldrich, pore size 58 Å, 150 mesh). TLC was performed on 5 cm × 10 cm aluminum plates coated with silica gel 60F-254 (Merck) in an appropriate eluant. Visualization was made with ultraviolet light (234 nm). HRMS spectra were recorded on QStar Elite (Applied Biosystems SCIEX) spectrometer. PEG was the matrix for HRMS. The experimental exact mass was given for the ion which has the maximum isotopic abundance. Purity of synthesized compounds was checked with LC-MS analyses which were realized at the Faculty of Pharmacy of Marseille with a Thermo Scientific Accela High Speed LC System® coupled with a single quadrupole mass spectrometer Thermo MSQ Plus®. The RP-HPLC column used is a Thermo Hypersil Gold® 50 × 2.1 mm (C18 bounded), with particles of 1.9 μm diameter. The volume of sample injected on the column was 1 μL. The chromatographic analysis, total duration of 8 min, is made with the gradient of following solvents: $t = 0$ min, water/methanol 50/50; $0 < t < 4$ min, linear increase in the proportion of water to a ratio water/methanol 95/5; $4 < t < 6$ min, water/methanol 95/5; $6 < t < 7$ min, linear decrease in the proportion of water to return to a ratio 50/50 water/methanol; $6 < t < 7$ min, water/methanol 50/50. The water used was buffered with 5 mM ammonium acetate. The retention times (t_R) of the molecules analyzed are indicated in min. 2,4-dibromoquinoline was purchase from Sigma Aldrich.

4.1.2. Procedure for the preparation of 2,4-dibromo-8-nitroquinoline (1) and 2,4-dibromo-6-nitroquinoline (2)

To a solution of 2,4-dibromoquinoline (10.5 mmol, 3 g) in concentrated sulfuric acid (15 mL), nitric acid 96% (31.4 mmol,

1.3 mL) was added. The reaction mixture was stirred for 1 h at RT. The reaction mixture was then slowly poured into 50 mL of an ice-water mixture and was made alkaline (pH 8) by addition of $NaHCO_3$. The aqueous layer was extracted three times with CH_2Cl_2, and the organic layer was washed twice with water, dried over Na_2SO_4, filtered and evaporated. The crude residue was purified by chromatography on a silica gel (cyclohexane/acetone 9:1) to give compound **1** (2.0 g, 56%) and compound **2** (400 mg, 11%) as white powders.

*4.1.2.1. 2,4-Dibromo-8-nitroquinoline (**1**) mp.* 129 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 7.72–7.76 (m, 1H), 7.98 (s, 1H), 8.07 (dd, J = 1.4 Hz and J = 8.5 Hz, 1H), 8.39 (dd, J = 1.4 Hz and J = 8.5 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 125.5, 127.1, 127.7, 131.0, 131.1, 135.1, 139.8, 143.7, 147.5. LC/MS (ESI$^+$): t_R 3.23 min, m/z [M+H]$^+$ 330.8/332.9/334.8. Anal. calcd for $C_9H_4Br_2N_2O_2$: C 32.56, H 1.21, N 8.44, found: C 32.87, H 1.25, N 8.48.

*4.1.2.2. 2,4-Dibromo-6-nitroquinoline (**2**) mp.* 182 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 8.01 (s, 1H), 8.19 (d, J = 9.2 Hz, 1H), 8.54 (dd, J = 2.4 Hz and J = 9.2 Hz, 1H), 9.11 (d, J = 2.4 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 123.9, 125.0, 126.3, 130.9, 131.2, 136.4, 144.9, 146.5, 150.5. LC/MS (ESI$^+$): t_R 3.93 min. Anal. calcd for $C_9H_4Br_2N_2O_2$: C 32.56, H 1.21, N 8.44, found: C 32.54, H 1.25, N 8.40.

*4.1.3. Preparation of 4-bromo-8-nitroquinolin-2(1H)-one (**3**)*

Based on a previously reported procedure described by Andreev et al. [30], to a sealed flask containing 2,4-dibromo-8-nitroquinoline **1**, (3.01 mmol, 1 g) acetonitrile (10 mL) and perchloric acid 65% were added (18.03 mmol, 1.6 mL). The reaction mixture was heated under microwave irradiation (100 °C) for 30 min. Water was then added and the mixture was extracted twice with CH_2Cl_2 and twice with AcOEt. The organic layer was washed with water, dried over Na_2SO_4 filtered, and evaporated. The crude residue was purified by chromatography on a silica gel (CH_2Cl_2/AcOEt 9:1) to give compound **3** as a yellow powder (705 mg, 87%).

Yellow solid. mp: 240 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 7.21 (d, J = 2.0 Hz, 1H), 7.36–7.44 (m, 1H), 8.36 (d, J = 8.0 Hz, 1H) 8.58 (dd, J = 2.0 Hz and J = 8.0 Hz, 1H), 11.44 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 121.6, 121.7, 127.1, 129.1, 133.2, 136.1, 136.6, 159.6. One non-visible carbon under these experimental conditions. LC/MS (ESI$^+$): t_R 1.06 min m/z [M+H]$^+$ 269.10/271.10. Anal. calcd for $C_9H_5Br_2N_2O_3$: C 40.18, H 1.87, N 10.41, found: C 40.21, H 1.84, N 10.39.

*4.1.4. Preparation of 4-amino-8-nitroquinolin-2(1H)-one (**4**)*

According to a previously described procedure [31], to a sealed flask containing 4-bromo-8-nitroquinolin-2(1*H*)-one **3**, (0.74 mmol, 200 mg), 3 mL of a 30% aqueous ammonia solution were added. The reaction mixture was heated under microwave irradiation (140 °C) for 5 h. The solvent was then evaporated *in vacuo* and the crude residue was purified by chromatography on a silica gel (CH_2Cl_2/MeOH/NH_4OH 90:9:1). Compound **4** was obtained as a yellow powder (46 mg, 30%).

Yellow solid. mp > 250 °C. ^{1}H NMR (200 MHz, DMSO-d_6) = δ (ppm) 5.57 (s, 1H), 6.98 (s, 2H), 7.29–7.37 (m, 1H), 8.34–8.44 (m, 2H), 10.34 (bs, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) = δ (ppm) 92.9, 116.6, 119.8, 127.9, 130.6, 133.5, 134.0, 153.2, 161.6. LC/MS (ESI$^+$): t_R 0.66 min m/z [M+H]$^+$ 206.36. HRMS m/z [M+H]$^+$ calcd for $C_9H_7N_3O_3$: 206.0560, found: 206.0560.

*4.1.5. General procedure for preparation of compounds (**5**) to (**8**)*

To a solution of 4-bromo-8-nitroquinolin-2(1*H*)-one **3** (0.74 mmol, 200 mg) in *n*-propanol (20 mL), 3 equiv. of appropriate amine (2.22 mmol) were added. The mixture was then refluxed for 18 h. Water was then added and the mixture was extracted three times with CH_2Cl_2. The organic layer was washed with water, dried over Na_2SO_4 filtered, and evaporated. The crude residue was purified by chromatography on a silica gel and/or recrystallization to give the expected products **5** to **8**.

*4.1.5.1. 4-Morpholino-8-nitroquinolin-2(1H)-one (**5**).* was obtained after purification by chromatography on a silica gel (eluant: CH_2Cl_2/AcOEt 3:2) and recrystallization from isopropanol, as a yellow solid in 83% yield (169 mg). mp: 238 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 3.11–3.16 (m, 4H), 3.62–3.97 (m, 4H), 6.20 (s, 1H), 7.30–7.34 (m, 1H), 8.11 (d, J = 7.9 Hz, 1H), 8.50 (d, J = 7.9 Hz, 1H), 11.19 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 52.3 (2 CH_2), 66.5 (2 CH_2), 107.8, 119.3, 120.5, 128.1, 132.4, 133.7, 134.6, 159.0, 162.5. LC/MS (ESI$^+$): t_R 0.94 min, m/z [M+H]$^+$ 276.22. Anal. calcd for $C_{13}H_{13}N_3O_3$: C 56.70, H 4.76, N 15.27, found: C 56.79, H 4.72, N 15.13.

*4.1.5.2. 4-(Piperazin-1-yl)-8-nitroquinolin-2(1H)-one (**6**).* was obtained after purification by chromatography on a silica gel (eluant: MeOH) as a yellow solid in 48% yield (98 mg). mp: 196 °C. ^{1}H NMR (200 MHz, DMSO-d_6) = δ (ppm) 2.94–2.97 (m, 8H), 3.33 (bs, 1H), 6.75 (bs, 1H), 6.02 (s, 1H), 7.37 (t, J = 8.1 Hz, 1H), 8.12 (dd, J = 1.1 Hz and J = 8.1 Hz, 1H), 8.40 (dd, J = 1.1 Hz and J = 8.1 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) = δ (ppm) 45.3 (2 CH_2), 52.9 (2 CH_2), 106.0, 118.5, 120.7, 127.6, 132.5, 133.8, 134.4, 159.3, 161.6. LC/MS (ESI$^+$): t_R 1.04 min, m/z [M+H]$^+$ 275.25. HRMS m/z [M+H]$^+$ calcd for $C_{13}H_{14}N_4O_3$: 275.1138, found: 275.1139.

*4.1.5.3. 4-Butylamino-8-nitroquinolin-2(1H)-one (**7**).* was obtained after recrystallization from isopropanol, as a yellow solid in 78% yield (151 mg). mp: 213 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 0.95 (t, J = 8.0 Hz, 3H), 1.66 (sex, J = 8.0 Hz, 2H), 1.75 (qt, J = 8.0 Hz, 2H), 3.30 (t, J = 8.0 Hz, 2H), 5.73 (s, 1H), 6.21 (bs, 1H), 7.32 (t, J = 8.1 Hz, 1H), 8.32 (d, J = 8.0 Hz, 1H), 8.51 (d, J = 8.0 Hz, 1H), 11.09 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 13.8, 20.3, 30.2, 43.3, 90.6, 117.2, 120.8, 128.5, 129.6, 133.7, 133.9, 152.0, 163.0. LC/MS (ESI$^+$): t_R 2.10 min, m/z [M+H]$^+$ 262.23. Anal. calcd for $C_{13}H_{15}N_3O_3$: C 59.76, H 5.79, N 16.08, found: C 59.06, H 5.72, N 15.73.

*4.1.5.4. Tert-butyl-2-{2-[2-(8-nitro-2-oxo-1,2-dihydroquinolin-4-ylamino)ethoxy]-ethoxy}-ethylcarbamate (**8**).* was obtained after purification by chromatography on a silica gel (eluant CH_2Cl_2/MeOH/NH_4OH 98:1.9:0.1) as a yellow solid in 90% yield (291 mg). mp: 120 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 1.41 (s, 9H), 3.31–3.81 (m, 12H), 5.00 (bs, 1H), 5.63 (s, 1H), 5.88 (bs, 1H), 7.21–7.26 (m, 1H), 8.02–8.06 (m, 1H), 8.45 (d, J = 8.3 Hz, 1H), 10.86 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 28.3 (3 CH_2), 40.2, 42.8, 68.0 (2 CH_2), 70.1 (2 CH_2), 79.3, 92.2, 117.2, 120.1, 128.0, 128.9, 133.4, 134.1, 151.1, 156.0, 162.9. LC/MS (ESI$^+$): t_R 2.48 min, m/z [M+H]$^+$ 437.02. Anal. calcd for $C_{20}H_{28}N_4O_7$: C 55.04, H 6.47, N 12.84, found: C 55.10, H 6.66, N 12.44.

*4.1.6. Preparation of 4-{2-[2-(2-aminoethoxy)ethoxy]ethylamino}-8-nitroquinolin-2(1H)-one (**9**)*

In a 50 mL flask, tert-butyl-2-(2-(2-(8-nitro-2oxo-1,2-dihydroquinolin-4-ylamino)ethoxy)ethoxy)-ethylcarbamate **8** (0.46 mmol, 200 mg) was introduced with a HCl/diethylether solution (5 mL) and methanol (5 mL). The reaction mixture was stirred at RT for 2 h. The solvent was then evaporated *in vacuo* and the crude residue was washed five times with diethylether (10 mL). Compound **9** was obtained as a yellow powder (139 mg, 90%).

Yellow solid. mp 173 °C. ^{1}H NMR (200 MHz, DMSO-d_6) = δ (ppm) 2.92 (t, J = 5.4 Hz, 2H), 3.38–3.75 (m, 10H), 5.62 (s, 1H), 6.83 (bs, 2H), 7.34–7.42 (m, 1H), 8.14 (bs, 1H), 8.44 (d, J = 8.2 Hz, 1H), 8.70 (d, J = 7.9 Hz, 1H), 10.69 (bs, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) = δ

(ppm) 38.3, 42.6, 66.6, 67.7, 69.7, 70.5, 89.2, 116.7, 120.5, 128.2, 130.6, 133.2, 134.1, 152.0, 161.7. LC/MS (ESI^+): t_R 0.80 min m/z $[M+H]^+$ 337.22. HRMS *m/z* $[M+H]^+$ calcd for $C_{15}H_{20}N_4O_5$: 337.1506, found: 337.1507.

*4.1.7. Preparation of 4-phenylamino-8-nitroquinolin-2(1H)-one (**10**)*

As previously reported [33], to a sealed flask containing 4-bromo-8-nitroquinolin-2(1*H*)-one **3** (0.63 mmol, 170 mg), $Pd_2(dba)_3$ (0.006 mmol, 5.8 mg), xantphos (0.013 mmol, 7.7 mg), and cesium carbonate (0.88 mmol, 290 mg) were successively added. Then, under N_2 atmosphere, dry dioxane (20 mL) was injected. The solution was stirred at 100 °C for 24 h. Water was then added and the mixture was extracted three times with CH_2Cl_2. The organic layer was washed three times with water, dried over Na_2SO_4, filtered, and evaporated. The crude residue was purified by chromatography (silica gel, AcOEt/CH_2Cl_2 3:7) to give the compound **10** (15%, 27 mg) as a yellow solid.

Yellow solid. mp: 302 °C. ^{1}H NMR (200 MHz, DMSO-d_6) = δ (ppm) 5.72 (s, 1H), 7.24–7.47 (m, 6H), 8.45–8.61 (m, 2H), 8.96 (s, 1H), 10.55 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) = δ (ppm) 93.9, 116.9, 120.3, 124.2 (2 CH), 125.1, 127.9, 129.5 (2 CH), 130.4, 133.9, 139.3, 150.3, 161.5. One non-visible carbon under these experimental conditions. LC/MS (ESI^+): t_R 1.77 min m/z $[M+H]^+$ 282.22. HRMS *m/z* $[M+H]^+$ calcd for $C_{15}H_{11}N_3O_3$: 282.0873, found: 282.0881.

*4.1.8. Preparation of 4-phenylthio-8-nitroquinolin-2(1H)-one derivatives (**11**) and (**13 to 29**)*

4.1.8.1. General procedure. To a sealed flask containing NaH (1.11 mmol, 27 mg), dry DMF (2 mL) and appropriate thiophenol (0.56 mmol) were added under N_2 atmosphere. The reaction mixture was stirred at RT for 30 min. Then a solution of 4-bromo-8-nitroquinolin-2(1*H*)-one **3** (0.56 mmol, 150 mg) in dry DMF (3 mL) was injected. The reaction mixture was stirred at RT for 2 h. The reaction mixture was then poured into a water-ice-mixture. The yellow precipitate was collected by filtration, washed three times with water, dried under reduced pressure and purified by column chromatography or recrystallization, affording compounds **11**, **13**–**29**.

*4.1.8.2. 4-Phenylthio-8-nitroquinolin-2(1H)-one (**11**).* was obtained without purification as a yellow solid in 86% yield (144 mg). mp: 204 °C. ^{1}H NMR (200 MHz, DMSO-d_6) = δ (ppm) 5.74 (s, 1H), 7.46 (t, J = 8.0 Hz, 1H), 7.63–7.70 (m, 5H), 8.32 (d, J = 8.0 Hz, 1H), 8.48 (d, J = 8.0 Hz, 1H), 10.95 (bs, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) = δ (ppm) 116.1, 119.3, 121.6, 126.8, 128.4, 130.6 (2 CH), 130.8, 131.1, 132.3, 134.2, 135.6 (2 CH), 152.1, 158.9. LC/MS (ESI^+): t_R 3.36 min *m/z* $[M+H]^+$ 299.10. Anal. calcd for $C_{15}H_{10}N_2O_3S$: C 60.39, H 3.38, N 9.39, S 10.75, found: C 60.08, H 3.36, N 9.08, S 10.32.

*4.1.8.3. 4-(2-Methoxyphenylthio)-8-nitroquinolin-2(1H)-one (**13**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 98% yield (180 mg). mp: 219 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 3.86 (s, 3H), 5.95–5.96 (m, 1H), 7.04–7.11 (m, 2H), 7.31–7.40 (m, 1H), 7.51–7.58 (m, 2H), 8.37 (d, J = 7.9 Hz, 1H), 8.54 (d, J = 8.4 Hz, 1H), 11.17 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 56.1, 112.1, 113.9, 116.0, 120.8, 120.9, 122.1, 128.2, 132.0, 133.3 (2 CH), 138.0 (2 CH), 151.8, 160.1, 160.2. LC/MS (ESI^+): t_R 3.34 min *m/z* $[M+H]^+$ 329.15. Anal. calcd for $C_{16}H_{12}N_2O_4S$: C 58.53, H 3.68, N 8.53, S 9.77, found: C 57.67, H 3.59, N 8.24, S 9.69.

*4.1.8.4. 4-(3-Methoxyphenylthio)-8-nitroquinolin-2(1H)-one (**14**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 99% yield (184 mg). mp: 221 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 3.84 (s, 3H), 6.06–6.07 (m, 1H), 7.04–7.19 (m, 3H), 7.31–7.39 (m, 1H), 7.40–7.46 (m, 1H), 8.31 (dd, J = 1.3 Hz and J = 8.1 Hz, 1H), 8.55 (dd, J = 1.3 Hz and J = 8.1 Hz, 1H), 11.17 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 55.5, 116.7, 116.9, 120.6, 121.0, 121.1, 127.5, 128.0, 128.4, 131.3, 131.7, 133.2, 133.3, 153.2, 160.1, 160.8. LC/MS (ESI^+): t_R 3.53 min *m/z* $[M+H]^+$ 329.16. Anal. calcd for $C_{16}H_{12}N_2O_4S$: C 58.53, H 3.68, N 8.53, S 9.77, found: C 58.78, H 3.57, N 8.26, S 9.46.

*4.1.8.5. 4-(4-Methoxyphenylthio)-8-nitroquinolin-2(1H)-one (**15**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 75% yield (138 mg). mp: 229 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 3.88 (s, 3H), 5.96–5.97 (m, 1H), 7.01–7.05 (m, 2H), 7.35 (t, J = 8.2 Hz, 1H), 7.47–7.52 (m, 2H), 8.31 (d, J = 8.2 Hz, 1H), 8.54 (dd, J = 0.9 Hz and J = 8.2 Hz, 1H), 11.15 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 55.5, 116.2 (2 CH), 116.4, 116.6, 120.7, 121.0, 128.3, 131.6, 132.5, 133.2, 133.3, 137.8 (2 CH), 154.4, 161.8. LC/MS (ESI^+): t_R 3.55 min *m/z* $[M+H]^+$ 329.10. Anal. calcd for $C_{16}H_{12}N_2O_4S$: C 58.53, H 3.68, N 8.53, S 9.77, found: C 58.21, H 3.65, N 8.41, S 9.69.

*4.1.8.6. 4-(2-Trifluoromethylphenylthio)-8-nitroquinolin-2(1H)-one (**16**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 89% yield (183 mg). mp: 233 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 5.86 (s, 1H), 7.38 (t, J = 8.2 Hz, 1H), 7.66–7.78 (m, 3H), 7.90–7.94 (m, 1H), 8.34 (d, J = 8.2 Hz, 1H), 8.57 (dd, J = 0.9 Hz and J = 8.2 Hz, 1H), 11.22 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 117.9, 120.5, 121.2, 123.0 (q, J = 273 Hz), 125.9, 128.2 (q, J = 15.7 Hz), 128.6, 131.3, 131.8, 133.2, 133.4, 133.5, 134.7 (q, J = 30.0 Hz), 139.6, 152.1, 159.9. LC/MS (ESI^+): t_R 3.68 min *m/z* $[M+H]^+$ 367.06. Anal. calcd for $C_{16}H_9F_3N_2O_3S$: C 52.46, H 2.48, N 7.65, S 8.75, found: C 52.58, H 2.43, N 7.83, S 8.54.

*4.1.8.7. 4-(3-Trifluoromethylphenylthio)-8-nitroquinolin-2(1H)-one (**17**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 92% yield (189 mg). mp: 200 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 6.08 (s, 1H), 7.38 (t, J = 7.9 Hz, 1H), 7.63–7.70 (m, 1H), 7.77–7.83 (m, 2H), 7.88–7.89 (m, 1H), 8.30 (dd, J = 1.0 Hz and J = 7.9 Hz, 1H), 8.57 (dd, J= 1.0 Hz and J = 7.9 Hz, 1H), 11.23 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 117.7, 120.4, 121.2, 123.1 (q, J = 273 Hz), 127.7 (q, J = 3.7 Hz), 128.5, 128.7, 131.0, 131.7, 132.5 (q, J = 3.7 Hz), 132.7 (q, J = 30.0 Hz), 133.3, 133.4, 139.3, 151.9, 159.8. LC/MS (ESI^+): t_R 3.88 min *m/z* $[M+H]^+$ 367.11. Anal. calcd for $C_{16}H_9F_3N_2O_3S$: C 52.46, H 2.48, N 7.65, S 8.75, found: C 52.48, H 2.69, N 7.51, S 8.65.

*4.1.8.8. 4-(4-Trifluoromethylphenylthio)-8-nitroquinolin-2(1H)-one (**18**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 74% yield (152 mg). mp: 197 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 6.08–6.09 (m, 1H), 7.38 (t, J = 7.9 Hz, 1H), 7.69–7.79 (m, 4H), 8.30 (d, J = 7.9 Hz, 1H), 8.57 (d, J = 7.9 Hz, 1H), 11.23 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 118.3, 120.5, 121.2, 123.4 (q, J = 273 Hz), 127.3 (2 CH, q, J = 3.6 Hz), 128.7, 131.8, 132.1, 132.7 (q, J = 30 Hz), 133.3, 133.4, 135.8 (2 CH), 151.4, 159.8. LC/MS (ESI^+): t_R 3.77 min *m/z* $[M+H]^+$ 367.09. Anal. calcd for $C_{16}H_9F_3N_2O_3S$: C 52.46, H 2.48, N 7.65, S 8.75, found: C 52.35, H 2.24, N 7.32, S 8.66.

*4.1.8.9. 4-(2-Chlorophenylthio)-8-nitroquinolin-2(1H)-one (**19**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 90% yield (168 mg). mp: 225 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 5.99 (s, 1H), 7.32–7.51 (m, 5H), 8.29 (d, J = 7.3 Hz, 1H), 8.56 (d, J = 7.3 Hz, 1H), 11.19 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 117.1, 120.5, 121.1, 125.1, 128.6, 130.8 (2 CH), 131.6, 133.2, 133.3, 137.3 (2 CH), 137.6, 152.7, 159,9. LC/MS (ESI^+): t_R

3.59 min *m/z* $[M+H]^+$ 333.12/335.16. Anal. calcd for $C_{15}H_9ClN_2O_3S$: C 54.14, H 2.73, N 8.42, S 9.64, found: C 53.22, H 2.61, N 8.14, S 9.37.

*4.1.8.10. 4-(3-Chlorophenylthio)-8-nitroquinolin-2(1H)-one (**20**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 60% yield (112 mg). mp: 237 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 6.05 (s, 1H), 7.33–7.60 (m, 5H), 8.29 (d, *J* = 8.0 Hz, 1H), 8.56 (dd, *J* = 1.0 Hz and *J* = 8.0 Hz, 1H), 11.21 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 117.5, 120.5, 121.1, 128.6, 128.7, 131.1, 131.5, 131.7, 133.1, 133.3, 134.0, 135.5, 136.1, 152.2, 159.9. LC/MS (ESI^+): t_R 3.77 min *m/z* $[M+H]^+$ 333.06/335.15. Anal. calcd for $C_{15}H_9ClN_2O_3S$: C 54.14, H 2.73, N 8.42, S 9.64, found: C 53.88, H 2.59, N 8.04, S 9.59.

*4.1.8.11. 4-(4-Chlorophenylthio)-8-nitroquinolin-2(1H)-one (**21**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 65% yield (121 mg). mp: 209 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 5.94 (s, 1H), 7.33–7.54 (m, 3H), 7.61–7.71 (m, 2H), 8.34 (d, *J* = 8.0 Hz, 1H), 8.56 (d, *J* = 8.0 Hz, 1H), 11.21 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 117.2, 120.5, 121.1, 126.2, 128.5 (CH and C), 131.3, 131.9, 132.5, 133.4, 138.1, 139.8, 150.5, 159.9, one non-visible carbon under these experimental conditions. LC/MS (ESI^+): t_R 3.73 min *m/z* $[M+H]^+$ 333.11/335.13. HRMS *m/z* $[M+H]^+$ calcd for $C_{15}H_9ClN_3O_3$: 333.0095, found: 333.0095.

*4.1.8.12. 4-(2,4-Dichlorophenylthio)-8-nitroquinolin-2(1H)-one (**22**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 74% yield (152 mg). mp: 199 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 5.93–5.94 (m, 1H), 7.34–7.42 (m, 2H), 7.59–7.65 (m, 2H), 8.30 (d, *J* = 8.4 Hz, 1H), 8.56 (dd, *J* = 1.2 Hz and *J* = 8.4 Hz, 1H), 11,23 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 117.5, 120.4, 121.2, 124.9, 128.6, 128.9, 131.2, 131.8, 133.4, 138.4, 138.6, 140.0, 150.0, 159.8, one non-visible carbon under these experimental conditions. LC/MS (ESI^+): t_R 4.03 min *m/z* $[M+H]^+$ 367.06/369.01/371.01. Anal. calcd for $C_{15}H_8Cl_2N_2O_3S$: C 49.06, H 2.20, N 7.63, S 8.73, found: C 48.57, H 2.20, N 7.66, S 8.38.

*4.1.8.13. 4-(4-Bromophenylthio)-8-nitroquinolin-2(1H)-one (**23**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 78% yield (156 mg). mp: 213 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 6.00 (s, 1H), 7.32–7.48 (m, 3H), 7.64–7.69 (m, 2H), 8.29 (d, *J* = 8.0 Hz, 1H), 8.55 (dd, *J* = 1.0 Hz and *J* = 8.0 Hz, 1H), 11.20 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 117.1, 120.5, 121.1, 125.8, 125.9, 128.6, 131.7, 133.2, 133.3, 133.8 (2 CH), 137.4 (2 CH), 152.5, 159.0. LC/MS (ESI^+): t_R 3.83 min *m/z* $[M+H]^+$ 376.87/378.87. Anal. calcd for $C_{15}H_9BrN_2O_3S$: C 47.76, H 2.40, N 7.43, S 8.50, found: C 47.45, H 2.41, N 7.29, S 8.51.

*4.1.8.14. 4-(2-Fluorophenylthio)-8-nitroquinolin-2(1H)-one (**24**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 68% yield (106 mg). mp: 237 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 6.01 (s, 1H), 7.24–7.42 (m, 3H), 7.53–7.64 (m, 2H), 8.34 (dd, *J* = 1.2 Hz and *J* = 8.1 Hz, 1H), 8.56 (dd, *J* = 1.2 Hz and *J* = 8.1 Hz, 1H), 11.21 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 113.9 (d, *J* = 18.6 Hz), 116.8, 117.3 (d, *J* = 22.4 Hz), 120.5, 121.1, 125.9 (d, *J* = 4.0 Hz), 128.5, 131.8, 133.3, 133.7 (d, *J* = 8.0 Hz), 137.9, 150.9, 159.9, 162.8 (d, *J* = 251.8 Hz) one non-visible carbon under these experimental conditions. LC/MS (ESI^+): t_R 3.22 min *m/z* $[M+H]^+$ 317.10. Anal. calcd for $C_{15}H_9FN_2O_3S$: C 56.96, H 2.87, N 8.86, S 10.14, found: C 56.96, H 2.77, N 8.56, S 10.16.

*4.1.8.15. 4-(3-Fluorophenylthio)-8-nitroquinolin-2(1H)-one (**25**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 86% yield (152 mg). mp: 223 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 6.07 (s, 1H), 7.21–7.56 (m, 5H), 8.29 (dd, *J* = 1.2 Hz and *J* = 8.1 Hz, 1H), 8.57 (dd, *J* = 1.2 Hz and *J* = 8.1 Hz, 1H), 11.21 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 117.6, 118.1 (d, *J* = 20.9 Hz), 120.5, 121.1, 122.7 (d, *J* = 22.3 Hz), 128.6, 128.7, 128.8, 131.7, 131.8 (d, *J* = 11.7 Hz), 133.3 (d, *J* = 4.0 Hz), 152.2, 160.1, 163.2 (d, *J* = 253.2 Hz) one non-visible carbon under these experimental conditions. LC/MS (ESI^+): t_R 3.30 min *m/z* $[M+H]^+$ 317.24. Anal. calcd for $C_{15}H_9FN_2O_3S$: C 56.96, H 2.87, N 8.86, S 10.14, found: C 57.15, H 2.77, N 8.58, S 10.14.

*4.1.8.16. 4-(4-Fluorophenylthio)-8-nitroquinolin-2(1H)-one (**26**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 98% yield (174 mg). mp: 208 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 5.95–5.96 (m, 1H), 7.18–7.23 (m, 2H), 7.36 (t, *J* = 8.1 Hz, 1H), 7.54–7.62 (m, 2H), 8.29 (d, *J* = 8.1 Hz, 1H), 8.55 (dd, *J* = 1.2 Hz and *J* = 8.1 Hz, 1H), 11.18 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 116.6, 117.9 (2 CH, d, *J* = 22.3 Hz), 120.5, 121.1, 121.8 (d, *J* = 3.6 Hz), 128.5, 131.6, 133.2, 133.3, 138.4 (2 CH, d, *J* = 8.8 Hz), 153.2, 159.9, 164.4 (d, *J* = 253.2 Hz). LC/MS (ESI^+): t_R 3.21 min *m/z* $[M+H]^+$ 317.09. HRMS *m/z* $[M+H]^+$ calcd for $C_{15}H_9FN_2O_3S$: 317.0391, found: 317.0388.

*4.1.8.17. 4-(2-Tolylthio)-8-nitroquinolin-2(1H)-one (**27**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 96% yield (168 mg). mp: 236 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 2.42 (s, 3H), 5.85 (s, 1H), 7.33–7.59 (m, 5H), 8.37 (d, *J* = 7.1 Hz, 1H), 8.54–8.58 (dd, *J* = 1.2 Hz and *J* = 7.1 Hz, 1H), 11.17 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 20.5, 115.8, 120.7, 121.0, 125.7, 128.0, 128.4, 131.5, 131.8, 131.9, 133.3, 137.3, 143.4, 152.3, 160.1, one non-visible carbon under these experimental conditions. LC/MS (ESI^+): t_R 3.76 min *m/z* $[M+H]^+$ 313.11. Anal. calcd for $C_{16}H_{12}N_2O_3S$: C 61.53, H 3.87, N 8.97, S 10.27, found: C 61.30, H 3.81, N 8.79, S 10.37.

*4.1.8.18. 4-(4-Tolylthio)-8-nitroquinolin-2(1H)-one (**28**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 90% yield (157 mg). mp: 220 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 2.43 (s, 3H), 5.97–5.98 (m, 1H), 7.30–7.48 (m, 5H), 8.32 (d, *J* = 7.8 Hz, 1H), 8.55 (dd, *J* = 1.2 Hz and *J* = 7.8 Hz, 1H), 11.15 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 21.4, 116.5, 120.7, 121.0, 122.9, 128.4, 131.3 (2 CH), 131.7, 133.2, 136.1 (2 CH), 141.5, 153.9, 160.1, one non-visible carbon under these experimental conditions. LC/MS (ESI^+): t_R 3.87 min *m/z* $[M+H]^+$ 313.07. Anal. calcd for $C_{16}H_{12}N_2O_3S$: C 61.53, H 3.87, N 8.97, S 10.27, found: C 61.24, H 3.83, N 8.87, S 10.28.

*4.1.8.19. 4-(2-Aminophenylthio)-8-nitroquinolin-2(1H)-one (**29**).* was obtained after purification by chromatography (eluant AcOEt/CH_2Cl_2 1:4) as a yellow solid in 70% yield (123 mg). mp: 217 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 4.28 (bs, 2H), 6.01–6.03 (m, 1H), 6.79–6.87 (m, 2H), 7.24–7.42 (m, 3H), 8.33–8.37 (dd, *J* = 1.2 Hz and *J* = 8.1 Hz, 1H), 8.52–8.57 (m, 1H), 11.16 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 107.8, 116.1, 116.2, 119.6, 120.8, 121.0, 128.3, 131.8, 133.1, 133.3, 133.4, 137.8, 149.2, 150.3, 160.0. LC/MS (ESI^+): t_R 2.80 min *m/z* $[M+H]^+$ 314.18. Anal. calcd for $C_{15}H_{11}N_3O_3S$: C 57.50, H 3.54, N 13.41, S 10.23, found: C 57.57, H 3.56, N 12.96, S 10.08.

*4.1.9. Preparation of 4-phenoxy-8-nitroquinolin-2(1H)-one derivatives (**12**) and (**30 to 47**)*

4.1.9.1. General procedure. To a sealed flask containing NaH (1.34 mmol, 33 mg), dry DMSO (5 mL) and appropriate phenol (0.975 mmol) were added under N_2 atmosphere. The reaction mixture was stirred at RT for 30 min. Then a solution of 4-bromo-8-nitroquinolin-2(1*H*)-one **3** (0.75 mmol, 200 mg) in dry DMSO (5 mL) was injected. The reaction mixture was heated at 130 °C under microwave irradiation for 4 h. The reaction mixture was then poured into a water-ice-mixture and for compounds **31**, **32**, **34**, **36**,

38–**40**, **43**–**45** the expected product precipitated and the solid was collected by filtration, washed three times with water dried under reduced pressure and purified by chromatography on a silica or alumina gel and/or recrystallization. For compounds **12**, **30**, **33**, **35**, **37**, **41**, **42**, **46**, **47** the mixture was extracted three times with AcOEt. The organic layer was washed three times with water, dried over Na_2SO_4, filtered, and evaporated. The crude residue was purified by chromatography on a silica or alumina gel and/or recrystallization.

*4.1.9.2. 4-Phenoxy-8-nitroquinolin-2(1H)-one (**12**).* was obtained after purification by chromatography on an alumina gel (eluant CH_2Cl_2) as a yellow solid in 30% yield (64 mg). mp: 170 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 5.76 (s, 1H), 7.15–7.20 (m, 2H), 7.27–7.53 (m, 4H), 8.48–8.60 (m, 2H), 11.07 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 101.1, 118.1, 121.0, 121.3 (2 CH), 126.7, 128.9, 130.5 (2 CH), 131.1, 133.1, 133.8, 152.6, 162.8, 163.3. LC/MS (ESI$^+$): t_R 2.82 min *m/z* [M+H]$^+$ 283.20. Anal. calcd for $C_{15}H_{10}N_2O_4$: C 63.83, H 3.57, N 9.92, found: C 63.86, H 3.65, N 9.59.

*4.1.9.3. 4-(2-Methoxyphenoxy)-8-nitroquinolin-2(1H)-one (**30**).* was obtained after purification by chromatography on an alumina gel (eluant CH_2Cl_2/AcOEt 9:1) as a yellow solid in 25% yield (59 mg). mp: 190 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 3.78 (s, 3H), 5.67–5.68 (m, 1H), 6.99–7.19 (m, 3H), 7.27–7.41 (m, 2H), 8.52–8.60 (m, 2H), 11.08 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 55.7, 100.3, 113.0, 118.0, 121.0, 121.4, 122.7, 127.8, 128.7, 131.3, 133.1, 133.8, 140.8, 151.1, 162.7, 163.2. LC/MS (ESI$^+$): t_R 2.87 min *m/z* [M+H]$^+$ 313.08. Anal. calcd for $C_{16}H_{12}N_2O_5$: C 61.54, H 3.87, N 8.97, found: C 61.22, H 3.73, N 8.75.

*4.1.9.4. 4-(3-Methoxyphenoxy)-8-nitroquinolin-2(1H)-one (**31**).* was obtained after purification by chromatography on a silica gel (eluant CH_2Cl_2/AcOEt 9:1) as a yellow solid in 65% yield (152 mg). mp: 195 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 3.83 (s, 3H), 5.82–5.83 (m, 1H), 6.69–6.77 (m, 2H), 6.85–6.90 (m, 1H), 7.34–7.42 (m, 2H), 8.49 (dd, J = 1.3 Hz and J = 8.1 Hz, 1H), 8.58 (dd, J = 1.3 Hz and J = 8.1 Hz, 1H), 11.08 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 55.6, 101.2, 107.3, 112.3, 113.2, 118.1, 121.1, 128.9, 130.9, 131.1, 133.1, 133.8, 153.6, 161.3, 162.9, 163.2. LC/MS (ESI$^+$): t_R 3.07 min *m/z* [M+H]$^+$ 313.17. Anal. calcd for $C_{16}H_{12}N_2O_5$: C 61.54, H 3.87, N 8.97, found: C 61.52, H 3.75, N 8.77.

*4.1.9.5. 4-(4-Methoxyphenoxy)-8-nitroquinolin-2(1H)-one (**32**).* was obtained after purification by chromatography on a silica gel (eluant CH_2Cl_2/AcOEt 9:1) as a yellow solid in 65% yield (152 mg). mp: 203 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 3.85 (s, 3H), 5.75–5.76 (m, 1H), 6.95–7.11 (m, 4H), 7.37 (t, J = 8.1 Hz, 1H), 8.50 (dd, J = 1.3 Hz and J = 8.1 Hz, 1H), 8.57 (dd, J = 1.3 Hz and J = 8.1 Hz, 1H), 11.08 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 55.7, 100.7, 115.4 (2 CH), 118.1, 121.0, 122.2 (2 CH), 128.9, 131.1, 133.1, 133.8, 145.9, 157.9, 162.9, 163.8. LC/MS (ESI$^+$): t_R 2.91 min *m/z* [M+H]$^+$ 313.12. Anal. calcd for $C_{16}H_{12}N_2O_5$: C 61.54, H 3.87, N 8.97, found: C 61.41, H 3.77, N 8.77.

*4.1.9.6. 4-(2-Trifluoromethylphenoxy)-8-nitroquinolin-2(1H)-one (**33**).* was obtained after purification by chromatography on an alumina gel (eluant CH_2Cl_2/AcOEt 9:1) as a yellow solid in 24% yield (64 mg). mp: 216 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 5.76–5.77 (m, 1H), 7.30–7.52 (m, 3H), 7.67–7.83 (m, 2H), 8.49 (dd, J = 1.3 Hz and J = 8.1 Hz, 1H), 8.60 (dd, J = 1.3 Hz and J = 8.1 Hz, 1H), 11.15 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 101.4, 117.7, 121.4, 122.7 (q, J = 273 Hz), 123.3, 123.9, 126.9, 128.5 (q, J = 5.1 Hz), 128.6 (q, J = 21.6 Hz), 129.1, 131.0, 133.2, 133.8, 133.9, 149.9 (q, J = 1.8 Hz), 162.6. LC/MS (ESI$^+$): t_R 3.34 min *m/z* [M+H]$^+$ 351.06. Anal. calcd for $C_{16}H_9F_3N_2O_4$: C 54.87, H 2.59, N 8.00, found: C 54.40, H 2.79, N 7.44.

*4.1.9.7. 4-(3-Trifluoromethylphenoxy)-8-nitroquinolin-2(1H)-one (**34**).* was obtained after purification by recrystallization from *n*-propanol as a yellow solid in 84% yield (221 mg). mp: 215 °C. ^{1}H NMR (400 MHz, $CDCl_3$) = δ (ppm) 5.74 (s, 1H), 7.38–7.47 (m, 3H), 7.62–7.67 (m, 2H), 8.47 (d, J = 7.8 Hz, 1H), 8.59 (d, J = 8.3 Hz, 1H), 11.11 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) = δ (ppm) 101.7, 117.8, 118.7 (q, J = 3.7 Hz), 121.2, 123.2 (q, J = 273 Hz), 123.6 (q, J = 3.7 Hz), 124.8, 129.1, 130.8, 131.3, 133.3, 133.4 (q, J = 33 Hz), 133.9, 152.9, 162.4, 162.7. LC/MS (ESI$^+$): t_R 3.43 min *m/z* [M+H]$^+$ 351.08. Anal. calcd for $C_{16}H_9F_3N_2O_4$: C 54.87, H 2.59, N 8.00, found: C 54.99, H 2.46, N 7.67.

*4.1.9.8. 4-(4-Trifluoromethylphenoxy)-8-nitroquinolin-2(1H)-one (**35**).* was obtained after purification by chromatography on an alumina gel (eluant CH_2Cl_2/AcOEt 9:1) as a yellow solid in 22% yield (60 mg). mp: 182 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 5.71 (s, 1H), 7.19 (s, 1H), 7.24–7.28 (m, 1H), 7.32 (t, J = 7.9 Hz, 1H), 7.70–7.72 (m, 2H), 8.39 (dd, J = 0.9 Hz and J = 7.9 Hz, 1H), 8.52 (dd, J = 0.9 Hz and J = 7.9 Hz, 1H), 11.03 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 101.8, 117.7, 121.2, 121.8 (2 CH), 123.6 (q, J = 273 Hz), 128.0 (2 CH, q, J = 3.7 Hz), 129.1 (q, J = 33 Hz), 129.2, 130.9, 133.2, 133.9, 155.2, 162.4, 162.6. LC/MS (ESI$^+$): t_R 3.42 min *m/z* [M+H]$^+$ 351.09. HRMS *m/z* [M+H]$^+$ calcd for $C_{16}H_9F_3N_2O_4$: 351.0587, found: 351.0585.

*4.1.9.9. 4-(2-Chlorophenoxy)-8-nitroquinolin-2(1H)-one (**36**).* was obtained after purification by chromatography on an alumina gel (eluant CH_2Cl_2/AcOEt 9:1) as a yellow solid in 51% yield (121 mg). mp: 236 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 5.66–5.67 (m, 1H), 7.24–7.46 (m, 4H), 7.53–7.58 (m, 1H), 8.54 (dd, J = 1.4 Hz and J = 8.1 Hz, 1H), 8.60 (dd, J = 1.4 Hz and J = 8.19 Hz, 1H), 11.14 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) = δ (ppm) 100.1, 117.6, 121.2, 123.4, 127.0, 128.0, 128.7, 129.0, 131.1, 131.4, 133.2, 133.8, 148.3, 162.0, 162.7. LC/MS (ESI$^+$): t_R 3.22 min *m/z* [M+H]$^+$ 317.17/319.10. Anal. calcd for $C_{15}H_9ClN_2O_4$: C 56.89, H 2.86, N 8.85, found: C 56.91, H 2.75, N 8.65.

*4.1.9.10. 4-(3-Chlorophenoxy)-8-nitroquinolin-2(1H)-one (**37**).* was obtained after purification by chromatography on a silica gel (eluant CH_2Cl_2/AcOEt 9:1) and recrystallization from *n*-propanol as a yellow solid in 44% yield (105 mg). mp: 236 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 5.79–5.80 (m, 1H), 7.07–7.12 (m, 1H), 7.20–7.22 (m, 1H), 7.31–7.48 (m, 3H), 8.46 (dd, J = 1.5 Hz and J = 8.3 Hz, 1H), 8.59 (dd, J = 1.5 Hz and J = 8.3 Hz, 1H), 11.11 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) = δ (ppm) 101.5, 117.8, 119.6, 121.2, 122.0, 127.0, 129.1, 130.9, 131.3, 133.1, 133.8, 135.8, 153.1, 162.5, 162.8. LC/MS (ESI$^+$): t_R 3.40 min *m/z* [M+H]$^+$ 317.17/319.10. Anal. calcd for $C_{15}H_9ClN_2O_4$: C 56.89, H 2.86, N 8.85, found: C 56.44, H 2.78, N 8.59.

*4.1.9.11. 4-(4-Chlorophenoxy)-8-nitroquinolin-2(1H)-one (**38**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 65% yield (154 mg). mp: 185 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 5.76 (s, 1H), 7.10–7.16 (m, 2H), 7.38 (t, J = 8.0 Hz, 1H), 7.44–7.49 (m, 2H); 8.46 (d, J = 8.0 Hz, 1H), 8.58 (dd, J = 1.3 Hz and J = 8.0 Hz, 1H), 11.09 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) = δ (ppm) 101.2, 117.8, 121.1, 122.7 (2 CH), 129.1, 130.6 (2 CH), 131.0, 132.3, 133.1, 133.8, 151.0, 162.0, 163.0. LC/MS (ESI$^+$): t_R 3.22 min *m/z* [M+H]$^+$ 317.06/319.10. Anal. calcd for $C_{15}H_9ClN_2O_4$: C 56.89, H 2.86, N 8.85, found: C 56.29, H 2.73, N 8.53.

*4.1.9.12. 4-(2-Bromophenoxy)-8-nitroquinolin-2(1H)-one (**39**).* was obtained after recrystallization from *n*-propanol as a yellow solid in 44% yield (105 mg). mp: 233 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 5.65 (s, 1H), 7.21–7.28 (m, 2H), 7.37–7.49 (m, 2H), 7.69–7.74 (m, 1H), 8.54 (dd, J = 1.2 Hz and J = 8.1 Hz, 1H), 8.60 (dd,

J = 1.2 Hz and J = 8.1 Hz, 1H), 11.12 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) = δ (ppm) 101.1, 116.1, 117.6, 121.2, 123.4, 128.3, 129.0, 129.4, 131.1, 133.2, 133.9, 134.4, 149.6, 161.9, 162.7. LC/MS (ESI$^+$): t_R 3.22 min m/z [M+H]$^+$ 361.03/363.07. Anal. calcd for $C_{15}H_9BrN_2O_4$: C 49.59, H 2.51, N 7.76, found: C 49.97, H 2.49, N 7.89.

4.1.9.13. 4-(3-Bromophenoxy)-8-nitroquinolin-2(1H)-one (***40***). was obtained after purification by chromatography on a silica gel (eluant CH_2Cl_2/AcOEt 7:3) and recrystallization from *n*-propanol as a yellow solid in 44% yield (105 mg). mp: 230 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 5.78–5.79 (m, 1H), 7.11–7.16 (m, 1H), 7.33–7.51 (m, 4H), 8.45 (dd, J = 1.3 Hz and J = 8.0 Hz, 1H), 8.59 (dd, J = 1.3 Hz and J = 8.0 Hz, 1H), 11.10 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) = δ (ppm) 101.5, 117.7, 120.1, 121.2, 123.4, 124.8, 129.1, 130.0, 130.9, 131.6, 133.1, 133.8, 153.1, 162.6, 162.8. LC/MS (ESI$^+$): t_R 3.47 min m/z [M+H]$^+$ 361.08/363.09. Anal. calcd for $C_{15}H_9BrN_2O_4$: C 49.59, H 2.51, N 7.76, found: C 50.07, H 2.48, N 7.83.

4.1.9.14. 4-(4-Bromophenoxy)-8-nitroquinolin-2(1H)-one (***41***). was obtained after purification by chromatography on a silica gel (eluant CH_2Cl_2) and recrystallization from *n*-propanol as a yellow solid in 48% yield (130 mg). mp: 193 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) = 5.76 (s, 1H), 7.05–7.11 (m, 2H), 7.38 (t, J = 8.2 Hz, 1H), 7.58–7.64 (m, 2H), 8.47 (dd, J = 1.3 Hz and J = 8.2 Hz, 1H), 8.58 (dd, J = 1.3 Hz and J = 8.2 Hz, 1H), 11.10 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) = δ (ppm) 101.3, 117.8, 119.9, 121.1, 123.1 (2 CH), 129.1, 131.0, 133.1, 133.6 (2 CH), 133.8, 151.6, 162.6, 162.9. LC/MS (ESI$^+$): t_R 3.31 min m/z [M+H]$^+$ 361.08/363.14. Anal. calcd for $C_{15}H_9BrN_2O_4$: C 49.59, H 2.51, N 7.76, found: C 49.66, H 2.52, N 8.17.

4.1.9.15. 4-(2-Fluorophenoxy)-8-nitroquinolin-2(1H)-one (***42***). was obtained after purification by chromatography on a silica gel (eluant CH_2Cl_2) as an orange solid in 69% yield (150 mg). mp: 201 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 5.76 (s, 1H), 7.26–7.33 (m, 4H), 7.40 (t, J = 8.2 Hz, 1H), 8.51 (d, J = 8.2 Hz, 1H), 8.59 (dd, J = 1.1 Hz and J = 8.2 Hz, 1H), 11.12 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) = δ (ppm) 100.8, 117.6, 117.8 (d, J = 17.5 Hz), 121.2, 123.6, 125.4 (d, J = 4.0 Hz), 128.2 (d, J = 6.9 Hz), 129.0, 131.1, 133.1, 133.8, 139.7 (d, J = 12.4 Hz), 154.0 (d, J = 250.3 Hz), 162.3, 162.7. LC/MS (ESI$^+$): t_R 2.77 min m/z [M+H]$^+$ 301.13. Anal. calcd for $C_{15}H_9FN_2O_4$: C 60.01, H 3.02, N 9.33, found: C 60.29, H 2.94, N 8.84.

4.1.9.16. 4-(3-Fluorophenoxy)-8-nitroquinolin-2(1H)-one (***43***). was obtained after purification by recrystallization from *n*-propanol as a yellow solid in 93% yield (209 mg). mp: 211 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 5.81 (s, 1H), 6.90–7.12 (m, 3H), 7.35–7.53 (m, 2H), 8.46 (dd, J = 1.2 Hz and J = 8.3 Hz, 1H), 8.59 (dd, J = 1.2 Hz and J = 8.3 Hz, 1H), 11.12 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) = δ (ppm) 101.5, 109.4 (d, J = 24.2 Hz), 113.8 (d, J = 20.9 Hz), 117.1 (d, J = 3.3 Hz), 117.8, 121.2, 129.1, 130.9, 131.4 (d, J = 9.5 Hz), 133.1, 133.8, 153.4 (d, J = 10.6 Hz), 162.6, 162.8, 163.5 (d, J = 250.3 Hz). LC/MS (ESI$^+$): t_R 2.81 min m/z [M+H]$^+$ 300.85. Anal. calcd for $C_{15}H_9FN_2O_4$: C 60.01, H 3.02, N 9.33, found: C 60.67, H 3.00, N 9.11.

4.1.9.17. 4-(2-Methylphenoxy)-8-nitroquinolin-2(1H)-one (***44***). was obtained after purification by chromatography on a silica gel (eluant CH_2Cl_2/AcOEt 9:1) as a yellow solid in 47% yield (104 mg). mp: 190 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 2.19 (s, 3H), 5.65–5.66 (m, 1H), 7.07–7.10 (m, 1H), 7.24–7.44 (m, 4H), 8.50 (dd, J = 1.2 Hz and J = 8.0 Hz, 1H), 8.59 (dd, J = 1.2 Hz and J = 8.0 Hz, 1H), 11.10 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 15.7, 100.3, 117.9, 121.1, 121.5, 126.9, 127.9, 128.9, 130.2, 131.0, 132.1, 133.1, 133.9, 150.7, 162.6, 163.0. LC/MS (ESI$^+$): t_R 3.20 min m/z [M+H]$^+$ 297.17. Anal. calcd for $C_{16}H_{12}N_2O_4$: C 64.86, H 4.08, N 9.46, found: C 64.96, H 4.22, N 8.89.

4.1.9.18. 4-(3-Methylphenoxy)-8-nitroquinolin-2(1H)-one (***45***). was obtained after purification by chromatography on a silica gel (eluant CH_2Cl_2/AcOEt 9:1) as a yellow solid in 43% yield (96 mg). mp: 170 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 2.40 (s, 3H), 5.78 (s, 1H), 6.94–6.97 (m, 2H), 7.12–7.16 (m, 1H), 7.31–7.41 (m, 2H), 8.49 (dd, J = 1.3 Hz and J = 8.1 Hz, 1H), 8.57 (dd, J = 1.3 Hz and J = 8.1 Hz, 1H), 11.15 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 21.4, 101.0, 118.2, 121.1, 121.8, 127.5, 128.9, 130.2, 131.1, 133.2, 133.8, 141.0, 152.6, 163.0, 163.5, one non-visible carbon under these experimental conditions. LC/MS (ESI$^+$): t_R 3.42 min m/z [M+H]$^+$ 297.12. Anal. calcd for $C_{16}H_{12}N_2O_4$: C 64.86, H 4.08, N 9.46, found: C 64.23, H 4.12, N 9.06.

4.1.9.19. 4-(4-Methylphenoxy)-8-nitroquinolin-2(1H)-one (***46***). was obtained after purification by chromatography on an alumina gel (eluant CH_2Cl_2/Petroleum ether 2:8) as a yellow solid in 30% yield (66 mg). mp: 197 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 2.40 (s, 3H), 5.77 (s, 1H), 7.02–7.06 (m, 2H), 7.25–7.29 (m, 2H), 7.37 (t, J = 8.2 Hz, 1H), 8.50 (dd, J = 1.2 Hz and J = 8.2 Hz, 1H), 8.57 (dd, J = 1.2 Hz and J = 8.2 Hz, 1H), 11.07 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) δ 20.9, 100.9, 118.2, 121.0 (3 CH), 128.9, 130.9 (2 CH), 131.1, 133.1, 133.8, 136.5, 150.3, 162.9, 163.5. LC/MS (ESI$^+$): t_R 3.40 min m/z [M+H]$^+$ 297.17. Anal. calcd for $C_{16}H_{12}N_2O_4$: C 64.86, H 4.08, N 9.46, found: C 65.01, H 4.43, N 8.73.

4.1.9.20. 4-(4-Chloro-3-methylphenyloxy)-8-nitroquinolin-2(1H)-one (***47***). was obtained after purification by chromatography on a silica gel (eluant CH_2Cl_2/ACOEt 9:1) and recrystallization from *n*-propanol as a yellow solid in 59% yield (146 mg). mp: 198 °C. ^{1}H NMR (200 MHz, $CDCl_3$) = δ (ppm) 2.41 (s, 3H), 5.77 (s, 1H), 6.93–6.98 (m, 1H), 7.05–7.06 (m, 1H), 7.34–7.46 (m, 2H), 8.46 (dd, J = 1.2 Hz and J = 8.0 Hz, 1H), 8.58 (dd, J = 1.2 Hz and J = 8.0 Hz, 1H), 11.10 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) = δ (ppm) 20.3, 101.2, 117.9, 119.9, 121.1, 123.6, 129.0, 130.8, 131.0, 132.3, 133.1, 133.8, 138.8, 150.9, 162.7, 163.1. LC/MS (ESI$^+$): t_R 3.83 min m/z [M+H]$^+$ 331.15/333.15. Anal. calcd for $C_{16}H_{11}ClN_2O_4$: C 58.11, H 3.35, N 8.47, found: C 58.08, H 3.27, N 8.33.

4.2. Biology

4.2.1. Antileishmanial evaluation on L. donovani promastigotes

The *Leishmania* species used in this study was *L. donovani* MHOM/IN/00/DEVI provided by the CNR *Leishmania* (Montpellier, France). The effects of the tested compounds on the growth of *L. donovani* promastigotes were assessed by MTT assay [36]. Briefly, promastigotes in log-phase in Schneider's medium supplemented with 20% fetal 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), in duplicate. Appropriate controls treated by DMSO, pentamidine, miltefosine or amphotericin B (reference 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. Each plate-well was then microscope-examined for detecting possible precipitate formation. 20 μL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (5 mg/mL in PBS) were added to each well followed by incubation for another 4 h. The enzyme reaction was then stopped by addition of 100 μL of 50% isopropanol − 10% sodium dodecyl sulfate [37]. Plates were shaken vigorously (300 rpm) for 10 min The absorbance was finally measured at 570 nm in a BIO-TEK ELx808 Absorbance Microplate

Reader. Inhibitory concentration 50% (IC_{50}) was defined as the concentration of drug required to inhibit by 50% the metabolic activity of *L. donovani* and promastigotes compared to the control. IC_{50} were calculated by nonlinear regression analysis processed on dose–response curves, using TableCurve 2D V5.0 software. IC_{50} values represent the mean value calculated from three independent experiments.

4.2.2. Antileishmanial activity on intracellular amastigotes *L. donovani*

The effects of the tested compounds on the growth of *Leishmania* amastigotes were assessed according to the method of da Luz et al. [38] 500 μL of THP-1 cells were seeded in sterile chamber-slides at an average density of $5 \cdot 10^4$ – cells/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. THP-1 cells were then infected by acidified promastigotes at an average density of 5.10^5 cells/mL (10:1 ratio) and chamber-slides incubated for 24 h at 37 °C. Then, in duplicate, the medium containing various concentrations of tested-compounds was added (final DMSO concentration being inferior to 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} were calculated by non-linear regression analysis processed on dose–response curves, using TableCurve 2D V5.0 software. IC_{50} values represent the mean value calculated from three independent experiments.

4.2.3. Cytotoxicity evaluation on the HepG2 human cell line

The evaluation of the tested molecules cytotoxicity on the HepG2 cell line (hepatocarcinoma cell line purchased from ATCC, ref HB-8065) was performed according to the method of Mosman [36] with slight modifications. Briefly, cells in 100 μL of complete RPMI medium, [RPMI supplemented with 10% FCS, 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. After 24 h incubation, 100 μL of medium with various product concentrations dissolved in DMSO (final concentration less than 0.5% v/v) were added and the plates were incubated for 72 h at 37 °C. Duplicate assays were performed for each sample. Each plate-well was then microscope-examined for detecting possible precipitate formation before the medium was aspirated from the wells. 100 μL of MTT solution (0.5 mg/mL in medium without FCS) were then added to each well. Cells were incubated for 2 h at 37 °C. 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 spectrophotometer using a BIO-TEK ELx808 Absorbance Microplate Reader. 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 by using the TableCurve 2D V5.0 software.

4.2.4. Cytotoxicity evaluation on the THP-1 cell line

The evaluation of the tested molecules cytotoxicity on the THP-1 cell line (acute monocytic leukemia cell line purchased from ATCC, ref TIB-202) was performed according to the method of Mosman [36] with slight modifications. Briefly, cells in 100 μL of complete RPMI medium, were incubated at an average density of 5.10^4 cells/mL in sterile 96-well plates with various concentrations of compounds dissolved in DMSO (final concentration less than 0.5% v/v), in duplicate. The plates were incubated for 72 h at 37 °C. Each plate-well was then microscope-examined for detecting possible precipitate formation before the medium was aspirated from the wells. 100 μL of MTT solution (0.5 mg/mL in medium without FCS) were then added to each well. Cells were incubated for 2 h at 37 °C. 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 10 min. The absorbance was measured at 570 nm with 630 nm as reference wavelength spectrophotometer using a BIO-TEK ELx808 Absorbance Microplate Reader. 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 by using the TableCurve 2D V5.0 software.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.12.056.

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