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Original article

Synthesis and antikinetoplastid activities of 3-substituted quinolinones derivatives

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ABSTRACT

A new family of quinolinone derivatives has been synthesized and evaluated for their antikinetoplastid activities against *Leishmania donovani* and *Trypanosoma brucei brucei*. Results from these structure–activity relationship studies enabled identification of compounds **3a** and **4g** as the most active compounds against *L. donovani* promastigotes and amastigotes parasites (IC_{50} values in a range of 2–11 μM). Additionally, compound **3b** has emerged from this study as the most active compound in the series against *T. b. brucei* with a MEC value of 12 μM. These three compounds are worth of further *in vivo* evaluation.

1. Introduction

Leishmaniases constitute one of the six priority diseases of the "Tropical Diseases Research" program of the World Health Organisation. The leishmaniases are a complex of diseases caused by at least 17 species of the protozoan parasite *Leishmania* that affect 12 million people and that threaten 350 million people worldwide [1,2]. Eighty-nine countries are affected by leishmaniasis all over the five continents, with 2 million new cases each year. Leishmaniasis encompasses three forms: cutaneous, mucosal and visceral, the last being the second largest parasitic killer in the world which is usually fatal if patients are untreated. Visceral leishmaniasis is caused by three related species or subspecies *Leishmania donovani*, *Leishmania donovani infantum*, *Leishmania donovani chagasi*, which make up the *L. donovani* complex. These protozoan parasites produce a systemic and life-threatening infection by infecting reticuloendothelial cells and macrophages in all organs [3]. More than 90% of visceral leishmaniasis cases occur in Bangladesh, Brazil, India and Sudan. It is a zoonotic disease using different reservoirs and sand fly vectors in different parts of the world. In addition, according to WHO, co-infection *Leishmania*/HIV is increasing and underscoring the seriousness and urgency of treatment [4,5]. Even with proper treatment, patients with both infections suffer from relapses. They are especially more severe forms of cutaneous and mucosal leishmaniasis and the outcome are often fatal. So, AIDS and visceral leishmaniasis (VL) are mutually reinforcing. So, leishmaniasis represents a real scourge in less economically developed countries, where deadly epidemics of visceral leishmaniasis periodically flare up.

Five different drugs are mainly used in the treatment of leishmaniasis including pentavalent antimony compounds (sodium stibogluconate and meglumine antimoniate), Amphotericin B (AmB) and its liposomal form (AmBisome®), Paromomycin and Miltefosine. In the past 10 years, lipid formulations of AmB, Miltefosine and Paromomycin have been accepted in first-line medicines for the treatment of visceral leishmaniasis. The toxicity of these molecules is an established fact [6]. Antimonial resistance is well described whereas resistance to Miltefosine is beginning to emerge and the AmB resistance is at risk, and only Miltefosine can be administered orally [4–7]. These facts highlight the urgent need to develop new and more effective drugs to combat visceral leishmaniasis. Despite the necessity of treating leishmaniasis, and notably visceral leishmaniasis, no really satisfying treatment exists, mainly due to drug expensiveness, drug toxicity and drug-resistances appearance. Thus, the development of new drug by the discovery of new therapeutic targets seems hopeful. One of these targets could be the 90-kDa heat shock proteins (HSP90). HSP90 is a molecular chaperone

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whose functions include assisting in protein folding, in both stress responses and regular cell signalling. Despite being up regulated under stress conditions, HSP90 accounts for at 1–2% of the total cell proteins under normal conditions. HSP90 is an emerging target of therapeutic interest for the treatment of cancer and others diseases [8]. In the case of *Leishmania* parasites, their genomes contain multicopy gene clusters of HSP90 and it is the most abundant heat shock protein, ~2.5% of its entire proteome. A fundamental function associated with HSP90 in *L. donovani* is sensing the thermal shift during the transition from the sand fly vector to the mammalian host, triggering the morphological differentiation between the promastigote to the amastigote forms. The inhibition of parasite HSP90 leads to growth arrest and induces the parasite apoptosis [9]. Moreover, treatment of promastigote parasites with HSP90 inhibitors induces the synthesis of amastigote-specific marker proteins and a morphological alteration similar to axenic amastigote differentiation [10]. Other HSP90 studies in other parasites, showed the importance of this protein and its species specificity. Additionally, HSP90 inhibitors displays a greater affinity towards HSP90 of *Plasmodium falciparum* over than that of human HSP90 [11].

The starting point for this exploration (Scheme 1 and Table 1) of the antileishmanial activity of 3-substituted quinolinones may be considered as these analogues are potent HSP90 inhibitors as we previously reported [12]. In our continuing efforts to discover novel HSP90 inhibitors [13], and in combination with our previous structure–activity relationships (SAR) studies, we have examined the ability of these new quinolinone derivatives to inhibit *L. donovani* and *T. b. brucei* growth in culture. The present paper describes the syntheses, *in vitro* antileishmanial and trypanocidal activities and the structure–activity relationship (SAR) studies for a designed series of 3, 4-substituted quinolin-2-ones.

2. Chemistry

The target 4-substituted amidoquinolinones **4** were prepared from 3-nitroquinolinones **2** by a one-pot hydrogenation/peptidic coupling sequence according to our previously reported conditions [12]. In a typical experiment, we achieved this transformation in a sequential way by mixing, in a first step, **2a**,**b** with Pd/C in THF, under hydrogen atmosphere. After completion, PyBop, DIEA, and aromatic or aliphatic carboxylic acids were introduced in a second step at room temperature for 12 h. Thus, under this protocol, the desired 3-amidoquinolinones **4a**–**i** were isolated in good to excellent overall yields (20–94%). To complete our small library of quinolinone derivatives, **4a**–**i** were subjected to tosylation reaction by using tosyl chloride and triethylamine in dichloromethane as the solvent. The resulting compounds **5a**–**j** were obtained in excellent 70–98% yields (Scheme 1).

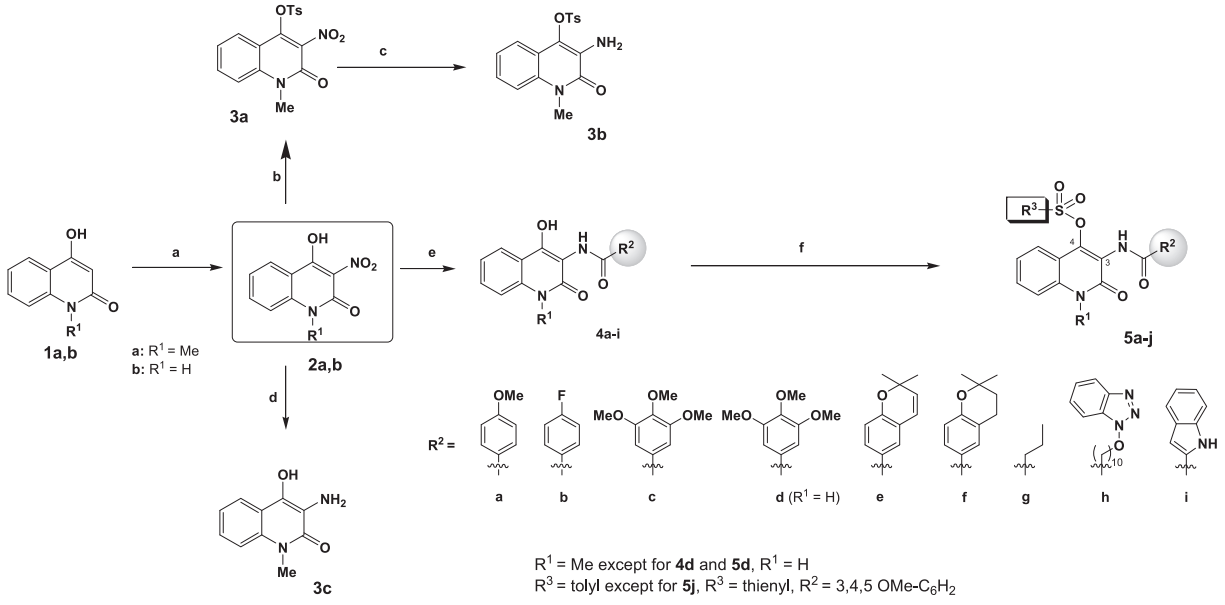
Simplified 3-(*N*-substituted)aminoquinolinone **7a**–**d** having no substituent at the C4 position of the heterocycle nucleus were synthesized by the palladium-catalyzed C–N coupling reaction of nitrogen nucleophiles with 3-bromoquinolin-2-(1*H*)-ones **6a**–**b** according to our previously reported conditions (Scheme 2) [14]. The reactions take place rapidly in 1,4-dioxane and proceeded in good to excellent yields using palladium acetate as the catalyst, Xantphos as the ligand and Cs_2CO_3 as the base.

Among a variety of bioisosteres of the amide moiety, 1,2,3-triazoles have gained increasing attention in drug discovery [15]. Triazoles can mimic the topological and electronic features of an amide bond, and they can actively participate in hydrogen bonding as well as dipole–dipole interactions. Based on these considerations, we decided to replace the amide bond of quinolinones **7** by the 1,2,3-triazole functionality as a non-hydrolyzable bioisostere. Thus, the requisite azidoquinolinone **6d** for the synthesis of triazoles **8**, was prepared from 3-bromoquinolinone **6a** according to our published procedure [16]. Subsequent Huisgen's 1,3-dipolar cycloaddition of **6d** with various terminal alkynes in the presence of copper(I) thiophene-2-carboxylate (CuTC) [17] and eco-friendly ethanolic conditions provided the desired 1,3-triazolyl-2(1*H*)-quinolinones **8a**–**d** in excellent yields (60–91%). All the triazole products were rapidly isolated by simple filtration from the reaction mixture with no further purification.

3. Biolology

3.1. Materials and methods

The compound susceptibility was determined by measuring their IC_{50} against *L. donovani* promastigote and axenic amastigote



Scheme 1. Synthetic strategy to target quinolinone **4** and **5**. *Reagents and conditions*: (a) AcOH, HNO_3, 88–94%; (b) TsCl, Et_3N, CH_2Cl_2 95%; (c) Fe, AcOH, EtOH/H_2O (5:2), 67%; (d) H_2, Pd/C, MeOH; (e) One-pot: (*i*) H_2, Pd/C, cat. HCl, THF; (*ii*) R^2CO_2H, PyBOP, DIPEA, 20–94% (overall yield two steps); (f) R^3SO_2Cl, Et_3N, CH_2Cl_2, 70–98%.

Table 1
Antiproliferative effect of compounds **1**–**8** against *L. donovani* and *T. b. brucei* and their antiproliferative effect against macrophages or MCF7 cancer cell line.

Cpds	*L. donovani* promastigotes IC_{50} [μM]	*T. b. brucei* MEC [μM]	Viability [%][a]		Cpds	*L. donovani* promastigotes IC_{50} [μM]	*T. b. brucei* MEC [μM]	Viability [%][a]	
			MCF7	Raw264.7				MCF7	Raw264.7
1a	>100	>200		70[b]	**5d**	18	>200	55[c]	
2a	>100	>200		80[b]	**5e**	30	>200	37[c]	
2b	>100	>200		90[b]	**5f**	30	>200	100[c]	
3a	3	>200		100[b]	**5g**	50	>200	55[c]	
3b	30	12.5	24[c]		**5h**	37	>200	69[c]	
4a	11	>200	81[c]		**5i**	42	>200	50[c]	
4b	>100	>200	74[c]		**5j**	14	>200	77[c]	
4c	3.5	>200	100		**7a**	>100	>200	89[c]	
4d	>100	>200	76[c]		**7b**	>100	>200	20[c]	
4e	53	>200	100[c]		**7c**	>100	>200	22[c]	
4f	56	>200	98[c]		**7d**	>100	>200	10[c]	
4g	11	>200	97[c]		**8a**	nd[d]	–	–	
4h	34	200	72[c]		**8b**	nd[d]	–	–	
4i	>100	>200	107[c]		**8c**	nd[d]	–	–	
5a	25	>200	82[c]		**8d**	nd[d]	–	–	
5b	49	>200	57[c]		Miltefosine	3.7	–		85[b]
5c	41	>200	84[c]		Pentamidine	2.84	5		75[b]

[a] Value of the antiproliferative effect (% of viable cells compared to untreated cells 100%) of analogues **1**–**8** at a concentration of 100 μM.
[b] Antiproliferative effect against macrophages.
[c] Antiproliferative effect against MCF7 cancer cell line determined in a previous study [12].
[d] Nd: no determined: compound precipitate at a concentration of 100 μM.

forms from wild-type *L. donovani* (MHOM/ET/67/HU3) line named LV9 WT strain and against *T. brucei brucei* CMP (Châtenay–Malabry Parasitologie) strain by determining the MEC (Minimum Effective Concentration) values.

3.1.1. Parasite lines and culture

Promastigote LV9 WT forms were grown in M-199 medium supplemented with 40 mM HEPES, 100 μM adenosine, 0.5 mg/L haemin, 10% heat-inactivated foetal bovine serum (FBS) and 50 μg/mL gentamycin at 26 °C in a dark environment under an atmosphere of 5% CO_2. All the experiments were performed with parasites in their logarithmic phase of growth.

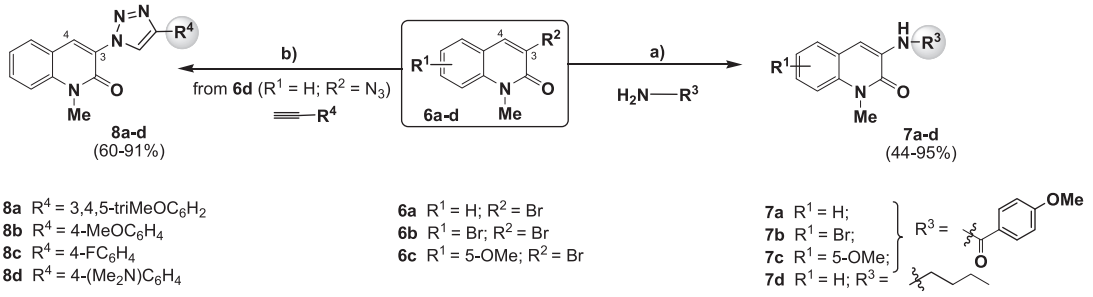
Differentiation of promastigotes into axenic amastigotes was achieved by dilution of 1×10^6 promastigotes in 5 mL of axenic amastigote media (15 mM KCl; 8 mM glucose; 5 mM glutamine, 1 × M-199, 2.5% BBL™ trypticase™ peptone, 4 mM haemin, and 20% FBS). The pH was adjusted to pH 5.5. Axenic amastigotes were grown at 37 °C in 5% CO_2.

3.1.2. In vitro antileishmanial activity on promastigote forms

L. donovani Promastigote forms from logarithmic phase culture were suspended to yield 10^6 cells/mL. Miltefosine was used as antileishmanial reference compound. Compounds to be evaluated and Miltefosine were distributed in the plates by making a serial dilution. The final concentrations used were between 100 μM and 50 nM. Triplicates were used for each concentration. After a 3-day incubation period at 27 °C in the dark and under a 5% CO_2 atmosphere, the viability of the promastigotes was assessed using the tetrazolium-dye (MTT) colourimetric method, which measures the reduction of a tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells. After incubation of the cells with the MTT reagent, a detergent solution (Triton X100, HCl) was added to lyse the cells and dissolve the coloured crystals. The absorbance at 570 nm, directly proportional to the number of viable cells, was measured using an ELISA plate reader. The results are expressed as the concentrations inhibiting parasite growth by 50% (IC_{50}) ± SD after a 3-day incubation period.

3.1.3. In vitro antileishmanial activity on axenic amastigote forms

L. donovani Amastigote forms were suspended to yield 10^7 cells/mL. The final compound concentrations used were 100 μM, 10 μM, 1 μM, 500 nM and 50 nM. Duplicates were used for each concentration. Cultures were incubated at 37 °C for 72 h in the dark and under a 5% CO_2 atmosphere, then the viability of the amastigotes was assessed using the SYBR® green I (Invitrogen, France) incorporation method. Parasite growth is determined by using SYBR® Green I, a dye with marked fluorescence enhancement upon contact with parasite DNA. Parasites were lysed following Direct PCR–Cell Genotyping without DNA isolation protocol (Euromedex, France). 10 μl of SYBR Green I was added to each well, and the contents were mixed. Fluorescence was measured with Mastercycler® ep realplex (Eppendorf, France). Fluorescence obtained was compared to those from the range obtained with different parasite densities.



Scheme 2. Synthetic strategy to target quinolinones **7** and **8**. *Reagents and conditions*: (a) $Pd(OAc)_2$ (5 mol%), Xantphos (5 mol%), Cs_2CO_3 (2 equiv), dioxane, 100 °C, 44–95%; b) CuTC (10 mol%), EtOH, 40 °C, 60–91%.

3.1.4. In vitro trypanocidal activity evaluation

The drug incubation infectivity test (DIIT) was used for compound evaluation following the Loiseau et al. protocol [18].

3.1.5. Cytotoxicity

The mouse monocyte/macrophage cell line RAW 264.7 was maintained in DMEM supplemented with 10% heat-inactivated foetal bovine serum. RAW 264.7 cells were seeded into a 24-well microtiter plate at a density of 5×10^4 cells/well in 750 μL of DMEM. After incubation in a 5% CO_2 incubator at 37 °C for 24 h, the culture medium was replaced with 750 μL of fresh DMEM containing various concentrations of compounds (10 μM, 1 μM and 100 nM). The number of alive RAW 264.7 macrophages, after treatment, was determined by the trypan blue dye exclusion assay. After an additional 24 h incubation, the cells were released with a cell scraper, incubated with trypan blue, and counted using a hemocytometer.

The cytotoxicity against MCF7 was determined in a previous study [12].

4. Results and discussion

4.1. Antileishmanial activity

The *in vitro* antileishmanial activity of newly synthesized quinolinones was first evaluated against promastigote forms of *L. donovani* and against *T. b. brucei*. The IC_{50} values corresponding to the concentration of studied compounds leading to 50% growth inhibition of *L. donovani* and *T. b. brucei cultures* are presented in Table 1. In addition, the *in vitro* toxicity of these quinolinones was evaluated by their growth-inhibitory potency in MCF7 cell line or Raw 264.7 macrophages at concentration of 100 μM. The quantification of cell survival was established by using MTT assays after 72 h exposure. Results summarized in Table 1 showed that the pharmacological properties of the compounds greatly depended on the number and the chemical nature of the substituents. Compound **3a** bearing a nitro group at the C-3 position of the quinolinone scaffold and a tosyl group at the C4 position, exerted a strong antiproliferative activity on *L. donovani* promastigote (IC_{50} = 3 μM), whereas it was deprived of cytotoxicity towards macrophages (100% survival) and *T. b. brucei* (MEC > 200 μM). Its amino derivative 3b showed high antiproliferative activity against human MCF7 cancer cells (24% survival) and displayed 10-fold lower activity against *L. donovani* promastigotes (IC_{50} = 30 μM). In addition, among all the compounds synthesized, **3b** exhibited the best activity against *T. b. brucei* (MEC = 12.5 μM). Compounds **4a** and **4g**, bearing a 4-methoxybenzamide group and a butyramide group at the C3 position of the quinolinone nucleus, respectively, demonstrated a strong activity on *L. donovani* promastigotes with an IC_{50} of 11 μM, while it exhibited low antiproliferative activity against *T. b. brucei* (MEC >200 μM) and human cancer cell line MCF7. However, substitution of the methoxy group on the benzamide moiety of **4a** by a fluorine atom (**4b**) considerably reduced the activity against *L. donovani* promastigotes (IC_{50} > 100 μM). Compound **4c** with a 3,4,5-trimethoxybenzamide group at the C3 position, exerted a strong activity against *L. donovani* parasite (IC_{50} = 3.5 μM), but was far less active on *T. b. brucei* (MEC >200 μM). This result indicates that antileishmanial properties of these compounds greatly depended on the substituents on the C-3 amide group. One can note that **4d**, in which the nitrogen atom is non-substituted (compare to **4c**), lost any activity against both *L. donovani* (IC_{50} > 100 μM), suggesting that the presence of the methyl substituent at the N-1 position of the quinolinone moiety is important for antileishmanial activity. In contrast to analogues **4**, compounds **5a**–**j** with a tosyl group at the C4 position of the quinolinone nucleus exhibited only slight effects against both *L. donovani* and *T. b. brucei*, and only slightly affected the growth of MCF7 cells.

Despite an important structural similarity between compounds **3**–**5** and **7**–**8**, the difference of activity is quite noteworthy. The replacement of the hydroxyl or tosyl group at the C4 position of compounds **3**–**5** with a hydrogen atom (Compounds 7**a**–**d**) resulted in decreased activities. This finding indicates that the substituent at the C4 position plays a critical role in antileishmanial properties. For example, **7d** (also named 6BrCaQ) [12], the most active compound against MCF7 cell line (10% survival at a concentration of 100 μM IC_{50} = 7 μM), recently identified in our laboratory as an inhibitor of the HSP90 protein folding machinery, showed low inhibition towards both *Leishmania* parasites (IC_{50} > 100 μM for *L. donovani*, and IC_{50} > 200 μM against *T. b. brucei*).

Further screening revealed that triazoles **8a**–**d** were inactive against *Leishmania* parasites probably due to their poor solubility in aqueous solutions. The overall activity profile of the tested compounds demonstrated that the biological activity was highly influenced by the substituent at the C-3 and/or C4 positions of the quinolinone moiety.

Next, compounds **3a**, **3b**, **4a**, **4c** and **4g** which have shown strong inhibition against promastigotes (IC_{50} around 10 μM), were further screened against amastigote model and their IC_{50} were given in Table 2. Among them, compounds **3a** and **4g** were found to be the most active compounds against *L. donovani* amastigote forms with an IC_{50} of 2–3 μM (Table 2). Compound **4a** and **4c** having an aromatic amide chain at the C3 position of the quinolinone moiety, which displayed IC_{50} values of 11 and 3.5 μM against promastigote form, respectively (Table 1), exhibited decreased activity against *L. donovani* amastigote forms (IC_{50} > 100 μM). On the basis of above discussion it is evident that the antiamastigote activity was noticeably influenced by the type of substituent attached at the C3 position of the quinolinone nucleus. From an attempt of structure-analysis, no clear-cut correlation significantly emerged. Anyway, the level of *in vitro* activity of compounds **3a** and **4g** on the *L. donovani* axenic amastigote assay justifies further *in vivo* evaluation of these compounds on the *L. donovani*/Balb/c mice model.

On the basis of above discussion it is evident that the antiamastigote activity was noticeably influenced by the type of substituent attached at the C3 position of the quinolinone nucleus. From an attempt of structure-analysis, no clear-cut correlation significantly emerged. Anyway, the level of *in vitro* activity of compounds **3a** and **4g** on the *L. donovani* axenic amastigote assay justifies further *in vivo* evaluation of these compounds on the *L. donovani*/Balb/c mice model.

4.2. Physico–chemical properties calculations

Analysis of the Lipinski descriptors for bioavailability estimation using SciFinder® program "Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994–2011 ACD/Labs)" (https://

Table 2
Antileishmanial effect of **3a**–**b**, **4a**, **4c** and **4g** derivatives against *L. donovani*.

Cpds	Promastigotes *L. donovani* IC_{50} [μM]	Amastigotes *L. donovani* IC_{50} [μM]	Viability [%][a]	
			MCF_7	Raw264.7.
3a	3	2		100[b]
4a	11	>100	81[c]	
4c	3.5	>100	100[c]	
4g	11	3	97[c]	
Miltefosine	3.7	5		85[b]
Pentamidine	2.84	6.6		75[b]

[a] Value of the antiproliferative effect (% of viable cells compared to untreated cells 100%) of analogues **3a**–**b**, **4a**, **4c** and **4g** at a concentration of 10^{-4} M.
[b] Antiproliferative effect against macrophages.
[c] Antiproliferative effect against MCF7 cancer cell line determined in a previous study [12].

Table 3
Physico–chemical and absorption properties for the most active compounds.

Compound	LogP[a]	MW[b]	*n*OHNH donors[c]	*n*ON acceptors[d]	Lipinski's violations
3a	2.208	374	0	8	0
3b	1.923	344	2	6	0
4a	1.512	324	2	6	0
4c	0.886	384	2	8	0
4g	0.848	260	2	5	0
Miltefosine	5.67	509	0	7	2

[a] LogP: logarithm of compound partition coefficient between *n*-octanol and water.
[b] MW: molecular weight.
[c] *n*-OHNH: number of hydrogen bond donors.
[d] *n*-ON: number of hydrogen bond acceptors.

scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf) was also carried out (Table 3). These parameters describe molecular properties important for drug pharmacokinetics in the human body. According to "Lipinski rules", an orally active drug must not violate more than one of the following criteria: ≤5 hydrogen donors (nOHNH), ≤10 hydrogen acceptors (nON), MW ≤ 500 and clogP ≤ 5.

Compounds **3a** (clogP = 2.20), **3b** (clogP = 1.923), **4a** (clogP = 1.51), **4c** (clogP = 0.88) and **4g** (clogP = 0.84) meet these claims. However, one of the reference drugs for pharmacological testing, Miltefosine (clogP = 5.67, MW = 509, nOHNH = 0, nON = 7), which was also subjected to Explorer Properties does not fulfil two out of four Lipinski descriptors.

5. Conclusion

In summary, a series of 3-substituted quinolinone derivatives was synthesized and evaluated for their *in vitro* antikinetoplastid activity against *L. donovani* and *T. b. brucei* parasites. The anti-leishmanial effect was dramatically dependent on the nature of the substituents attached on the C3 position of the quinolinone nucleus. Among these analogues, compounds **3a**, **3b** and **4g** were identified to be the most potent representatives of this newly simplified quinolinones. Our results demonstrate that **3a**, **3b** and **4g** which exerted similar biological profile as the reference compounds Miltefosine and Pentamidine may be considered as promising candidates for the development of more potent analogues. Further studies and optimization leading to novel analogues with superior biological properties are currently underway in our laboratories.

6. Experimental

6.1. General considerations

Tetrahydrofuran (THF) and diethylether were distilled from sodium–benzophenone ketyl. Piperidine, and triethylamine were distilled from potassium hydroxide under argon prior to use. The compounds were all identified by usual physical methods, i.e. ^{1}H NMR, ^{13}C NMR, IR, MS and elemental analysis. ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ with a Bruker Avance 300. ^{1}H chemical shifts are reported in ppm from an internal standard TMS or of residual chloroform (7.27 ppm). The following abreviation are used: m (multiplet), s (singlet), bs (broad singlet), d (doublet). ^{13}C chemical shifts are reported in ppm from the central peak of deuteriochloroform (77.14). IR spectra were measured on a Bruker Vector 22 spectrophotometer (neat, cm^{-1}). Elemental analyses were performed with a Perkin–Elmer 240 analyser. Mass spectra were obtained with a LCT Micromass spectrometer. Analytical TLC was performed on Merck precoated silica gel 60F plates. Merck silica gel 60 (230–400 mesh) was used for column chromatography.

Materials: DMF distilled from BaO, CH_2Cl_2 distilled from CaH_2, and usual solvents were purchased from SDS (Paris, France). Liquid chromatography was performed on Merck silica gel 60 (70/30 mesh), and TLC was performed on silica gel, 60F-254 (0.26 mm thickness) plates. Visualisation was achieved with UV light and phosphomolybdic acid reagent unless otherwise stated. All other reagents were of high grade and were used without further purification. Analogues 1, 3, 4 6, and 7 were prepared according to our previously reported procedure [12,14,19].

6.2. General protocol for one-pot amide formation

A round bottom flask, light protected by an aluminium sheet, was charged with 110 mg of Pd/C 10%, the 4-hydroxy-3-nitroquinolinone **2a**–**b** (1 mmol), HCl conc. (12 N, few drops) and freshly distilled dry THF (7 mL). The flask was sealed and purged with three cycles vacuum/nitrogen. The vacuum outlet was replaced with two balloons of hydrogen. The reaction was stirred at room temperature for 8 h, until complete as judged by TLC.

Afterwards, DIPEA (10 equiv) was added dropwise, under nitrogen atmosphere. The coupling agent PyBOP (1.2 mmol) and the acid (1.2 mmol) were added portion wise. This operation was done under light protection. After the mixture was stirred 12 h at room temperature, ethyl acetate was added. The combined organics were filtered through a plug of Celite by flushing with EtOAc. After concentration under vacuum, the organic layer was washed with aqueous HCl (1 M) and twice with brine, dried over Na_2SO_4, and concentrated under vacuum. The crude was purified by column chromatography on silica gel to yield the corresponding amide **4** as a yellow solid.

6.2.1. 11-(1H-Benzo[d][1,2,3]triazol-1-yloxy)-N-(4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)undecanamide (**4h**)

Yellow solid (20%); m.p.: 88–90 °C; TLC: R_f: 0.82 (CH_2Cl_2/EtOAc: 8/2); IR (neat, cm^{-1}): 3317, 2920, 1640, 1612, 1587, 1527, 1467, 1414, 1357, 1312, 1241, 1094, 957, 750, 763, 719, 677, 650, 614, 584, 568, 552; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 12.8 (s, 1H), 8.67 (s, 1H), 8.06 (s, 1H, ϑ= 8,0 H), 7.94 (dd, 1H, J_1 = 8.4 Hz, J_2 = 0.7 Hz), 7.54–7.39 (m, 3H), 7.30 (t, 1H, J_1 = 7.4 Hz, J_2 = 0.8 Hz), 7.26–7.22 (m, 2H), 4.47 (t, 2H, J = 6.6 Hz), 3.66 (s, 3H), 2.45 (t, 2H, J = 7.6 Hz), 1.82–1.64 (m, 4H), 1.51–1.42 (m, 2H), 1.39–1.21 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 173.8, 159.5, 148.8, 143.4, 136.6, 130.3, 127.8, 127.4, 124.6, 124.5, 122.5, 120.2, 117.2, 113.7, 109.2, 108.6, 81.0, 36.9, 29.9, 29.3, 29.2, 29.1 (2C), 29.0, 28.0, 25.7, 25.5. MS (APCI$^+$) m/z: 492.1 $[M + H]^+$.

6.2.2. 3-(11-(1H-Benzo[d][1,2,3]triazol-1-yloxy)undecanamido)-1-methyl-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzenesulfonate (**5h**)

To an ice-cooled solution of 4-hydroxyquinolinone **4h** (1 mmol) in 20 mL of dry dichloromethane, Et_3N (2 mmol) was added dropwise, under argon atmosphere. After the mixture was stirred 10 min, *p*-toluensulfonic chloride (1.5 mmol) was added portion wise under argon. After the mixture was stirred 4–12 h at room temperature, ethyl acetate was added, the organic layer washed with aqueous HCl (1 M), dried over Na_2SO_4, and concentrated under vacuum. The crude was purified by column chromatography on silica gel to yield 4-tosylquinolinones **5h** in 85% yield as a yellow solid.

6.2.3. 3-(11-(1H-Benzo[d][1,2,3]triazol-1-yloxy)undecanamido)-1-methyl-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzenesulfonate (**5h**)

Yellow solid (85%); m.p.: 61–63 °C; TLC: R_f: 0.47 (CH_2Cl_2/AcOEt : 8/2); IR (neat, cm^{-1}): 3297, 2929, 2853, 1677, 1650, 1637, 1602, 1517, 1497, 1466, 1371, 1313, 1261, 1193, 1179, 1071, 862, 806, 769, 731, 699,

654, 615, 583, 559. Anal. Calcd for $C_{34}H_{39}N_5O_6S$ (645.77): C 63.24, H 6.09, N 10.84, found: C 62.91, H 6.27, N 10.44; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.94 (d, 1H, $J = 8.3$ Hz), 7.77 (d, 2H, $J = 8.3$ Hz), 7.62 (dd, 1H, $J_1 = 8.1$ Hz, $J_2 = 1.2$ Hz), 7.52–7.40 (m, 3H), 7,32–7,26 (m, 5H); 7.13 (t, 1H, $J = 8.0$ Hz), 4.46 (t, 2H, $J = 6.6$ Hz), 3.67 (s, 3H), 2.39 (s, 3H), 2.09 (t, 2H, $J = 7.9$ Hz), 1.78 (q, 2H, $J = 8.0$ Hz), 1,54–1,41 (m, 4H), 1,36–1,18 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 170.7, 160.2, 145.8 (2C), 144.8, 136.9, 133.9, 130.8, 129.9 (2C), 128.2 (2C), 127.9, 127.5, 124.6, 124.3, 123.0, 120.2, 119.7, 117.5, 114.0, 108.7, 81.0, 36.4, 30.4, 29.4, 29.3 (2C), 29.2 (2C), 28.0, 25.6, 25.0, 21.8; MS (APCI$^+$) m/z: 646.2 $[M + H]^+$.

6.3. General procedure for the preparation of 3-(N-substituted) aminoquinolinones **7**

A flame-dried resealable Schlenk tube was charged with $Pd(OAc)_2$ (7.5 mg, 0.05 mmol, 5 mol%), Xantphos (29 mg, 0.05 mmol, 5 mol%), the solid reactant(s) (1.0 mmol of the bromoquinolin-2(1*H*)-one **6**, 1.5 mmol of appropriate amine or amide) and Cs_2CO_3 (651 mg, 2 mmol). The Schlenk tube was capped with a rubber septum, evacuated and backfilled with argon; this evacuation/backfill sequence was repeated one additional time. The liquid reactant(s) and 1,4-dioxane (2 mL) were added through the septum. The septum was replaced with a teflon screwcap. The Schlenk tube was sealed, and the mixture was stirred at 100 °C for 10 h. The resulting suspension was cooled to room temperature and filtred through celite eluting with ethyl acetate, and the inorganic salts were removed. The filtrate was concentrated and purification of the residue through a silica gel column chromatography gave the expected product **7**.

6.3.1. 4-Methoxy-N-(6-methoxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)benzamide (**7c**)

Brown solid (77%); m.p.: 181–183 °C; TLC: R_f: 0.69 (CH_2Cl_2); IR (neat, cm^{-1}): 1690, 1597, 1529, 1496, 1367, 1317, 1236, 1174, 1030, 899, 843, 755, 622; ^{1}H NMR (300 MHz, $CDCl_3$): 9.33 (s, 1H), 8.78 (s, 1H), 7.91 (d, 2H, $J = 8.7$ Hz), 7.26–7.24 (m, 1H), 7.08–7.04 (m, 2H), 6.97 (d, 2H, $J = 8.7$ Hz), 3.86 (s, 3H), 3.84 (s, 3H), 3.78 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 165.2, 162.7, 157.5, 155.3, 129.9, 129.1 (2C), 128.3, 126.4, 121.9, 119.5, 117.1, 115.1, 113.9 (2C), 110.1, 55.5, 55.4, 30.3; MS (APCI$^+$) m/z: 339.0 $[M + H]^+$.

6.4. General procedure for the preparation of triazoles **8**

A flame-dried resealable 2–5 mL Pyrex reaction vessel was charged with the solid reactant(s): CuTc (10 mol%), 3-azidoquinolin-2(1*H*)-one **6d** (1 mmol), alkyne (2 mmol) in EtOH (6 mL). The reaction vessel was capped with a Teflon screwcap. The reaction vessel was sealed, and then heated at 40 °C (time: see experimental section). The resulting suspension was cooled to room temperature, filtered and washed with H_2O and *c*-hexane to afford the expected triazoles **8**.

6.4.1. 1-Methyl-3-(4-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-1-yl)quinolin-2(1H)-one (**8a**)

Brown solid (64%); m.p. 191–193 °C. $R_f = 0.38$ (CH_2Cl_2/EtOAc 9:1). 1H NMR ($CDCl_3$, 300 MHz, δ, ppm): $\delta = 9.01$ (s, 1H), 8.57 (s, 1H), 7.70 (d, 1H, $J = 8.0$ Hz), 7.59 (m, 1H), 7.40 (d, 1H, $J = 8.5$ Hz), 7.31 (t, 1H, $J = 7.5$ Hz), 7.10 (s, 1H), 6.67 (d, 1H, $J = 10.0$ Hz), 3.88 (s, 6H), 3.83 (s, 3H), 3.82 (s, 3H) ppm. 13C NMR ($CDCl_3$, 75 MHz, δ, ppm): δ = 156.5, 153.6 (2C), 153.1, 147.4, 138.7, 131.7, 129.9, 126.5, 126.0, 123.5, 120.9, 119.2, 114.4, 109.7, 102.9 (2C), 61.0, 56.2, 56.1, 30.3 ppm. MS (APCI+): $m/z = 393.0$ $[M + H]^+$. IR: $\nu = 1651, 1593, 1471, 1235, 1126, 1002$ cm^{-1}. Anal. Calcd for $C_{21}H_{20}N_4O_4$ (392.14): C 64.28, H 5.14, N 14.28; found: C 64.97, H 5.69, N 14.77.

6.4.2. 3-(4-(4-Methoxyphenyl)-1H-1,2,3-triazol-1-yl)-1-methylquinolin-2(1H)-one (**8b**)

Brown solid (91%); m.p. 200–202 °C. $R_f = 0.29$ (CH_2Cl_2). 1H NMR ($CDCl_3$, 300 MHz, δ, ppm): $\delta = 8.93$ (s, 1H), 8.54 (s, 1H), 7.78 (d, 2H, $J = 8.8$ Hz), 7.68 (dd, 1H, $J_1 = 8.0$, $J_2 = 1.0$ Hz), 7,60 (m, 1H), 7.38 (d, 1H, $J = 8.5$ Hz), 7.29 (m, 1H), 6.91 (d, 2H, $J = 8.8$ Hz), 3.80 (s, 3H), 3.78 (s, 3H) ppm. 13C NMR ($CDCl_3$, 75 MHz, δ, ppm): δ = 159.6, 156.5, 147.4, 138.7, 131.6, 129.8, 127.2 (2C), 126.7, 123.4, 123.1, 120.3, 119.2, 114.3, 114.2 (3C), 55.3, 30.3 ppm. MS (APCI+): $m/z = 333.0$ $[M + H]^+$. IR: $\tilde{\nu} = 1650, 1597, 1470, 1245, 1021$ cm^{-1}. Anal. Calcd for $C_{19}H_{16}N_4O_2$ (332): C 68.66, H 4.85; N 16.86; found: C 68.97, H 4.99; N 17.13.

6.4.3. 3-(4-(4-Fluorophenyl)-1H-1,2,3-triazol-1-yl)-1-methylquinolin-2(1H)-one (**8c**)

Yellow solid (60%); m.p. 228–230 °C. $R_f = 0.24$ (CH_2Cl_2). 1H NMR ($CDCl_3$, 300 MHz, δ, ppm): $\delta = 9.00$ (s, 1H), 8.57 (s, 1H), 7.84 (m, 1H), 7.70 (d, 1H, $J = 7.8$ Hz), 7.62 (t, 1H, $J = 7.2$ Hz), 7.60 (s, 1H), 7.41 (d, 1H, $J = 8.4$ Hz), 7.31 (t, 1H, $J = 7.1$ Hz), 7.08 (t, 2H, $J = 8.8$ Hz), 3.82 (s, 3H) ppm. 13C NMR ($CDCl_3$, 75 MHz, δ, ppm): δ = 163.5, 160.2, 155.7, 145.8, 137.9, 130.8, 129.1, 129.1, 126.8, 126.6, 125.8, 122.6, 120.0, 118.4, 115.1, 114.8, 113.5, 29.5 ppm. MS (APCI+): m/z = 321.0 $[M + H]^+$. IR: ν = 1650, 1597, 1495, 1648, 1402, 1216, 1044, 809, 755 cm^{-1} Anal. Calcd for $C_{18}H_{13}FN_4O$ (320.10): C 67.49 H 4.09 N 17.49; found: 67.98 H 4.61 N 17.91.

6.4.4. 3-(4-(4-(Dimethylamino)phenyl)-1H-1,2,3-triazol-1-yl)-1-methylquinolin-2(1H)-one (**8d**)

Yellow solid (75%); m.p. 210–212 °C. $R_f = 0.84$ (CH_2Cl_2). 1H NMR ($CDCl_3$, 300 MHz, δ, ppm): $\delta = 8.95$ (d, 1H, $J = 1.9$ Hz), 8.62 (s, 1H), 7.84–7.75 (m, 3H), 7.67 (t, 1H, $J = 7.9$ Hz), 7.46 (d, 1H, $J = 8.4$ Hz), 7.36; (t, 1H, $J = 7.5$ Hz), 6.81 (d, 2H, $J = 8.5$ Hz), 3.88 (s, 3H), 3.00 (s, 6H) ppm. 13C NMR ($CDCl_3$, 75 MHz, δ, ppm): $\delta = 156.6, 150.4, 148.0, 138.7, 131.4, 129.8, 129.6, 126.8$ (2C), 123.3, 119.5, 119.3, 118.6, 114.3, 112.5 (2C), 40.5 (2C), 30.3 ppm (one carbon is missed). (APCI+): $m/z = 346.0$ $[M + H]^+$. IR: $\nu = 1656, 1597, 1224, 1022, 806, 764$ cm^{-1}. Anal. Calcd for $C_{20}H_{1ç}NO$ (345.0): C 69.55, H 5.54, N 20.28; found: C 69.98, H 5.89, N 20.97.

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