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Amodiaquine analogs. Synthesis and anti-leishmanial activity

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Abstract: In this work, we report the synthesis and antileishmanial evaluation of 13 amodiaquine (AQ) derivatives (4-aminoquinoline-aryl and 4-quinolinylhydrazones series). The compounds were tested against four *Leishmania* species and murine macrophages. The appreciable activity of these compounds can be considered an important finding for the rational design of new leads for antileishmania compounds.

Keywords: 4-aminoquinoline, 4-quinolinylhydrazones, antileismanial, amodiaquine.

Introduction

Quinoline nucleus is a significant example of privileged structure with a wide range of biological activities including antimalarial[1,2,3], antiviral[4], antibacterial[5], antifungal[6], anti-inflammatory[7] and antitumoral[8].

Recently activity Amodiaquine (AQ) (Figure **1**) against different species of *Leishmania* sp at µM concentration has been reported[9,10,11]. Therefore, AQ could be considered a good start point to develop new active compounds against leishmaniasis.

In this work, we proposed the synthesis and antileishmanial evaluation of two different series of AQ analogs: 4-aminoquinoline-aryl derivatives (series **a**; **1-5**) and 4-quinolinylhydrazones (series **b**; **7a-h**) (Figure **1**). Both series were designed with the conservation of 7-chloro-quinoline nucleus, however in series **b** the 4-amine-linker, present in AQ and in series **a**, was changed by a 4-hydrazone-linker. This scaffold is widely used in medicinal chemistry due to its ability in interacts with DNA by intercalation[12], metal chelation[13] and generation of metal ion–induced radical intermediates[14-16], which are common intracellular processes that could affect the parasite survival.

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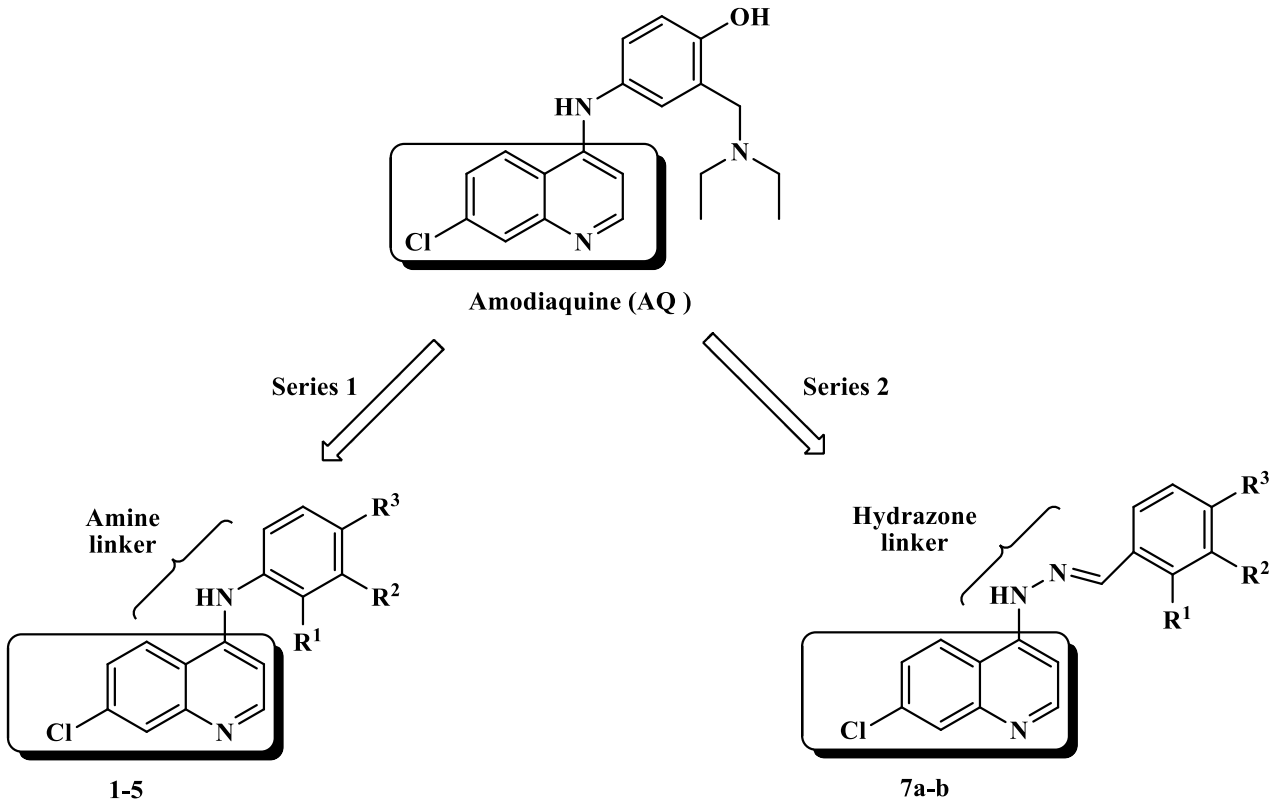

Figure 1. Design of two series of Amodiaquine analogs with potential antileishmanial activities.

Results and Discussion

All synthesized AQ analogs 4-aminoquinoline-aryl (**1-5**) and 4-quinolinylhydrazones (**7a-h**) derivatives were assayed against murine peritoneal macrophages and four *Leishmania* species promastigotes, which are three different species of *Leishmania* from the New World (*L. braziliensis*, *L. chagasi* and *L. amazonensis*) and one species from the Old World (*L. major*) (Table **1**). *L. chagasi*, has been related as the major agent of fatal visceral leishmaniasis in Latin America[17], *L. amazonensis* has been associated to all clinical forms of leishmaniasis[17,18], *L. braziliensis* usually caused mucocutaneous disease[17,19] and *L. major*, an agent causal of cutaneous form in the Old World[19]. The numerous of *Leishmania* species associated to human disease has important implications for clinical treatment and the sensitivity of each species should be considered in both experimental and clinical studies[20]. With this in mind, it is important to point that the compounds with leishmanicidal activity shown be effective against all *Leishmania* species tested.

Table 1: IC_{50} values (µg/mL) of the compounds on promastigotes of *Leishmania* species and murine macrophages.

Compo-unds	Substituents			Antileishmanial activity				Macropha-ges
	R^1	R^2	R^3	*L. amazonensis*	*L. braziliensis*	*L. chagasi*	*L. major*	
1	OH	H	H	20.1 ± 0.98	12.1 ± 1.36	7.9 ± 0.83	25.0 ± .52	>40
2	COOH	H	H	>40	>40	>40	>40	>40
3	H	H	OH	20.3 ± 0.93	12.9 ± 0.32	7.08 ± 1.45	14.6 ± .74	>40
4	H	OH	COOH	>40	>40	>40	>40	28.7 ± 0.28
5	H	H	H	10.1 ± 1.75	4.2 ± 0.65	9.8 ± 0.07	13.1 ± .64	24.7 ± 0.28
7a	H	H	F	>40	>40	>40	>40	>40
7b	H	H	Cl	>40	>40	>40	>40	>40
7c	H	H	Br	>40	>40	>40	>40	>40
7d	H	H	OH	>40	>40	>40	>40	>40
7e	H	H	OMe	>40	>40	>40	>40	>40
7f	H	H	NO_2	>40	>40	>40	>40	36.8 ± 2.51
7g	H	H	CN	>40	>40	>40	>40	>40
7h	H	H	H	2.4 ± 0.49	4.1 ± 1.03	4.03 ± 1.65	19.4 ± 0.26	14.4 ± 7.56
AQ*				14.5 ± 0.74	15.3 ± 0.56	7.5 ± 1.10	23.9 ± 0.04	-
AmB*				0.4 ±0.05	0.3 ± 0.09	1.9 ± 0.25	0.3 ± 0.09	-

*AQ (amodiaquine) and AmB (amphotericin B) were used as reference drugs for antileishmanial tests. IC_{50} values were obtained of at least two independent experiments performed in duplicate.

These results showed that in the 4-aminoquinoline-aryl derivatives (series **a**), the compounds **1** and **3**, containing hydroxyl group in the aromatic ring, displayed a good activity against all promastigotes of *Leishmania* species tested. Another important observation is that the introduction of a carboxyl group in phenyl ring afforded inactive compounds (**2** and **4**). In this series, the compound **5** showed the best leishmanicidal activity (*L. braziliensis* with IC_{50} value of the 4.2 µg/mL). Whereas, in the series **2**, the compound **7h** displayed a significant activity against promastigote forms of *Leishmania* species (IC_{50} values of 2.4 µg/mL, 4.0 µg/mL and 19.4 µg/mL). Both compounds **5** and **7h** were more effective than the AQ and they have no substituents in phenyl ring that could suggest that these compounds are very susceptible to electronic and bulk effects. Furthermore, among the thirteen compounds tested, only four compounds (**4**, **5**, **7f** and **7h**) were cytotoxic against murine macrophages.

Despite the significant leishmanicidal activity, these results should be considered preliminary because the promastigotes are the extracellular form of parasite and live in the gut of the host vector[21]. Amastigote forms are found in mammalian cells and are responsible for all clinical manifestations in humans. So, the use of an intracellular assay will provide more information on the effectiveness of the compounds[21].

In addition, we performed theoretical studies of pharmacokinetic and toxicity properties using Osiris Property Explorer (http://www.organic-chemistry.org/) and Molinspiration program (http://www.molinspiration.com/cgi-bin/properties). Firstly, we calculated some important parameters, such as cLogP, molecular weight (MW), number of hydrogen bond

donors (HBD) and number of hydrogen bond acceptors (HBA), which are related to the oral bioavailability, with the aim to verify if the active compounds fulfilled Lipinski ‘‘Rule of Five’’ (Table **2**).

Table **2**: Lipophilicity (cLog P), molecular weight (MW), number of hydrogen bound donor groups (HBD), number of hydrogen bound acceptor groups (HBA) and solubility (Log S) calculated for active compounds (**1**, **3**, **5** and **7h**) and standard drugs (**AQ** and **AmB**).

Compounds	**Parameters**				
	cLogP	**MW**	**HBD**	**HBA**	**cLogS**
1	4.49	270	2	3	-4.34
3	4.49	270	2	3	-4.34
5	4.78	254	1	2	-4.64
7h	**5.42**	281	1	3	-4.59
AQ	**5.35**	341	2	4	-4.98
AmB	2.38	**924**	**13**	**18**	-5.08

Compounds **1**, **3** and **5** fulfilled Lipinski ‘‘Rule of Five’’ (cLog P ≤5, molecular weigh t ≤500, number of hydrogenbond donors ≤ 5 and number of hydrogen-bond acceptors ≤ 10) [28], which indicates a good theoretical oral bioavailability. Compound **7h** and **AQ** showed one violation; since they have cLogP values ≤5. Whereas the standard drug AmB displayed three violations (MW, HBD and HBA).

After that, we performed a theoretical toxicity risks study (mutagenic, tumorigenic, irritant and teratogenic), which indicated low theoretical toxicity risks for all active compounds, except for the compound **1** that presented a mutagenic profile (Figure **2**). It is important to be mentioned that compounds **3**, **5** and **7h** presented a better profile than our prototype **AQ**, which is also an antimalarial drug currently in the market. Finally, we calculated the drug score values of these substances, which vary from 0 to 1 and indicate the compound's overall potential to qualify for a drug (Figure **2**). All the derivatives presented drug score values higher than the market antimalarial AQ and antiprotozoan AmB (Figure **2**), reinforcing the potential of these new prototypes as lead compounds for continuing the SAR of this class of compounds.

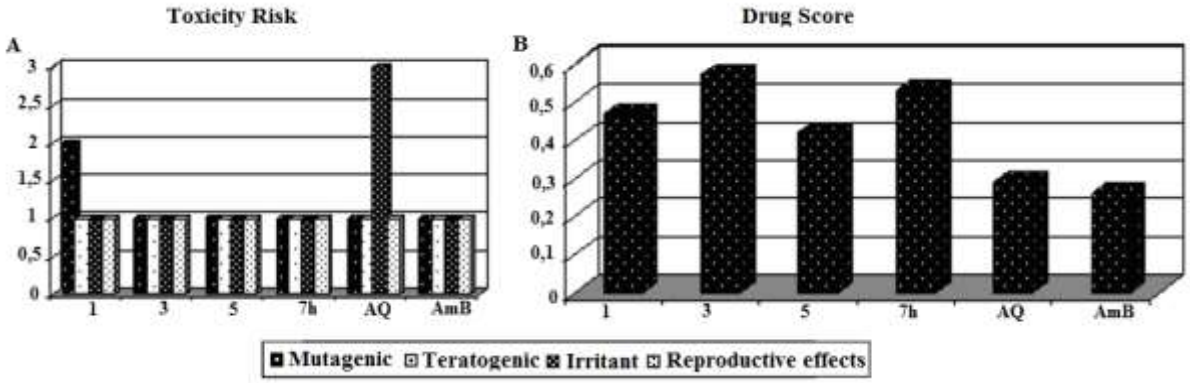

Figure **2**. Toxicity risk and Drug score of active compounds and of the standard drugs AQ and AmB calculated using Osiris Property Explorer program.

Conclusion

In summary, the syntheses of a series of 4-aminoquinoline-aryl and 4-quinolinyl-hydrazones derivatives have been described. Some compounds have exhibited promising antileishmanial activities. Among them, derivatives **5** and **7h** were more effective than the AQ, but they displayed cytotoxicity against macrophages. However, this study is important information about the structure-activity of AQ analogs and could provide a better direction in the management of antileishmanial activity and cytotoxicity in this class of compounds.

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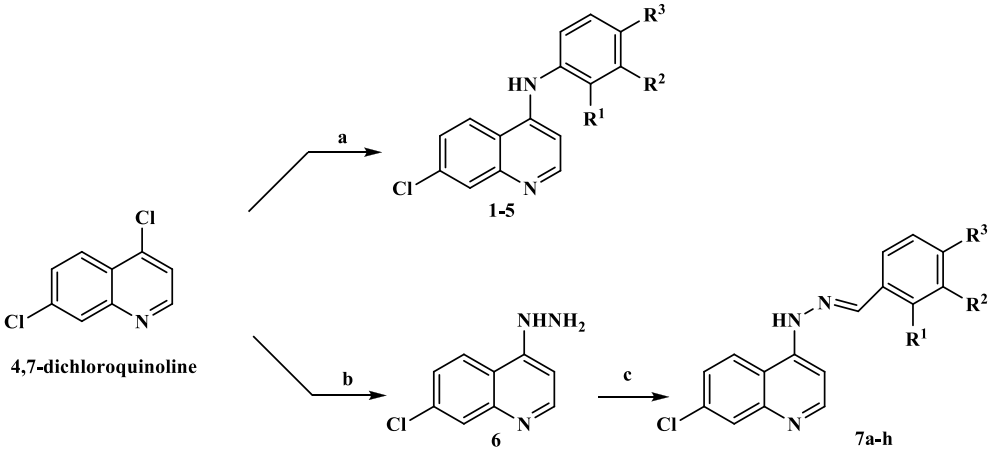
Experimental

Chemistry

The synthesis of all compounds of both series **1** and **2** started from the same precursor 4,7-dichloroquinoline. Hence, different nucleophilic displacements of the halogen present at C(4)-position are performed by several amines (series **1**) or hydrazine hydrate (series **2**). Firstly, the 4-aminoquinoline-aryl derivatives (**1-5**) (Scheme **1**) were prepared in 67-93% yield by treatment of 4,7-dichloroquinoline with appropriated aromatic amines[22] (Table **1**). In general, the ^{1}H NMR spectra showed the characteristic signal for the H-3 proton (shielding effect) at 6.47-6.81 ppm. Furthermore, the IR spectra showed N-H stretching vibrations at 3224-3313 cm^{-1}.

The 7-chloro-4-quinolinylhydrazones derivatives **7a-h** were previously synthesized by our research group (Scheme **1**)[23]. Firstly, 7-Chloro-4-hydrazinoquinoline **6** was prepared from 4,7-dichloroquinoline using hydrazine hydrate (80%) in ethanol under reflux. After that, the compounds **7a-h** were obtained through reaction between the compound **6** and appropriated benzaldehydes (Table **1**). The ^{1}H NMR spectra showed the characteristic signal for the N=CH proton at 8.37-8.81 ppm. Furthermore, the IR spectra showed N-H and N=C stretching vibrations at 3197-3247 and 1570-1585 cm^{-1}, respectively.



Scheme 1. *Reagents and conditions:* (a) corresponding amine, EtOH, 50ºC, 5h, 67-93%; (b) $N_2H_4.H_2O$ (80%), EtOH, 80°C, 2h, 80%; (c) corresponding benzaldehyde, EtOH, r.t., 4-24h, 64-91%.

Table 3 : Yields and melting points of 7-chloro-4-aminoquinoline-aryl (1-5) and 7-chloro-4-quinolinylhydrazones derivatives **7a-h**.

Entry	Substituents			Yield (%)	mp (°C)
	R^1	R^2	R^3		
1	OH	H	H	91	150 –151 [19]
2	COOH	H	H	70	304-305 [20]
3	H	H	OH	93	256-258 [21]
4	H	OH	COOH	67	322-323 [22]
5	H	H	H	85	289-291 [23]
7a	H	H	F	82	225-226 [24]
7b	H	H	Cl	84	198-199 [18]
7c	H	H	Br	74	245-246 [24]
7d	H	H	OH	80	219-220 [18]
7e	H	H	OMe	85	144-145 [18]
7f	H	H	NO_2	70	188-190 [18]
7g	H	H	CN	82	230-231[18]
7h	H	H	H	70	223-225 [25]

Biological Assays

Antileishmanial activity

Four species of *Leishmania* were used: *L. chagasi* (MHOM/Br/74/PP75), *L. braziliensis* (MHOM/Br/75/M2903), *L. major* (MRHO/SU/59/P) and *L. amazonensis* (IFLA/Br/67/PH8). Promastigotes of *L. amazonensis* and *L. braziliensis* were cultured in Warren's medium (brain heart infusion- BHI- plus hemin and folic acid)[24], promastigotes of *L. major* were maintained in Medium BHI[25] , and promastigotes of *L. chagasi* were maintained in Medium 199, both supplemented with 10% fetal bovine serum at 24 °C. Fetal bovine serum was purchased from Cultilab (Campinas, São Paulo, Brazil); brain heart infusion (BHI) from Himédia (Mumbai, Indian), hemin and folic acid were purchased from Sigma Chemical Co (St. Louis, MO, USA).

Antileishmanial activity was determined by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method based on tetrazolium salt reduction by mitochondrial dehydrogenase[24,26]. The screening was performed in 96-well microtiter plates maintained at 24 ºC. Briefly, promastigotes from a logarithmic phase culture were suspended to yield 2 millions of cells/mL (*L. amazonensis*) or 3 millions of cells/mL (*L. chagasi*, *L. braziliensis* and *L. major*) after Neubauer chamber counting. The analysis was made in duplicate. The parasites were exposed to increasing concentration of the compound (at minimum six serial dilutions) for 72h at 24°C. Controls containing 0.5% DMSO and medium alone were also included. The viability of promastigotes was assessed by MTT colorimetric method and the absorbance was measured at 570 nm (Multiskan MS microplate reader, LabSystems Oy, Helsink, Finland). For data analysis: IC_{50} values were obtained of at least two independent experiments performed in duplicate by using GraFit version 5 software (Erithacus Software Ltd., Horley, UK). Amphotericin B (supplied by Cristália, São Paulo, Brazil) and AQ **(**supplied by Ellipse Pharmaceuticals, Pessac, France) were used as the reference drug**.**

Cytotoxicity on macrophages

Mouse peritoneal macrophages in a concentration of 1 x 10^6 cells/mL, were plated in 96-well culture plates and incubated for 72 h at 37 ^oC and 5% CO_2 atmosphere. The culture medium was composed of RPMI-1640 supplemented with 10 % of fetal bovine serum and different concentrations of the tested compounds in 0.5% DMSO. The viability of the macrophages was determined with the MTT assay, as described above, and was confirmed by comparing the morphology with the control group via light microscopy.

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