Amodiaquine analogs. Synthesis and anti-leishmanial activity

Elaine S. Coimbra1, Adilson D. da Silva2, Rafael M. P. Dias2, Roberta C. N. R. Corrales1,2, Marcelle de L. F. Bispo3,4, Carlos R. Kaiser4, Marcus V. N. de Souza3,4*

1Departamento de Parasitologia, Microbiologia e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, Cidade Universitária, 36036-900, Juiz de Fora, MG, Brazil.
2Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Juiz de Fora, Cidade Universitária, 36036-900, Juiz de Fora, MG, Brazil.
3Fundação Oswaldo Cruz, Instituto de Tecnologia em Fármacos-Far Manguinhos, 21041-250, Rio de Janeiro, RJ, Brazil.
4Universidade Federal do Rio de Janeiro, Instituto de Química, Departamento de Química Orgânica, CP 68563, 21945-970, Rio de Janeiro, RJ, Brazil.

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 antimalarial1,2,3, antiviral4, antibacterial5, antifungal6, anti-inflammatory7 and antitumoral8. Recently activity Amodiaquine (AQ) (Figure 1) against different species of Leishmania sp at µM concentration has been reported9,10,11. 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 intercalation12, metal chelation13 and generation of metal ion–induced radical intermediates14-16, which are common intracellular processes that could affect the parasite survival.

*corresponding author: Marcus V. N. de Souza
E-mail: marcos_souza@far.fiocruz.br
http://dx.doi.org/10.13171/mjc.1.3.2011.26.09.22
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\textsuperscript{17}, L. amazonensis has been associated to all clinical forms of leishmaniasis\textsuperscript{17,18}, L. braziliensis usually caused mucocutaneous disease\textsuperscript{17,19} and L. major, an agent causal of cutaneous form in the Old World\textsuperscript{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\textsuperscript{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.

| Compounds | Substituents | Antileishmanial activity | Macrophages |
|-----------|--------------|--------------------------|-------------|
|           | R1          | R2          | R3          | 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 ± 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 ± 0.74 | >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 ± 0.64 | 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           | NO₂         | >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\textsuperscript{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\textsuperscript{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 weight ≤ 500, number of hydrogen bond 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.

Acknowledgments

This work was supported by FAPEMIG, CAPES, CNPq and UFJF. We are grateful to Dr. Maria Aparecida de Souza from Universidade Federal of Uberlândia, Brazil, for the promastigotes of *Leishmania* species.

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 (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-quinolylhydrazones derivatives 7a-h were previously synthesized by our research group (Scheme 1)\textsuperscript{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, 50°C, 5h, 67-93%; (b) N$_2$H$_4$.H$_2$O (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-
quinoxinylhydrazones derivatives 7a-h.

| Entry | Substituents | Yield (%) | mp (°C) |
|-------|--------------|-----------|---------|
| 1     | OH H H       | 91        | 150–151 [19] |
| 2     | COOH H H     | 70        | 304-305 [20] |
| 3     | H H OH COOH  | 67        | 322-323 [22] |
| 4     | 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 OMe   | 85        | 144-145 [18] |
| 7f    | H H NO₂      | 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 BHI²⁵, 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 dehydrogenase²⁴,²⁶. The screening was performed in 96-well microtiter plates maintained at 24 °C. 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: IC₅₀ 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 °C and 5% CO₂ 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.

References

1- B. E. Evans, K. E. Rittle, M. G. Bock, R. M. DiPardo, R. M. Freidinger, W. L. Whitter, G. F. Lundell, D. F. Veber, P. S. Anderson, R. S. L. Chang, V. J. Lotti, D. J. Cerino, T. B. Chen, P. J. Kling, K. A. Kunkel, J. P. Springer, J. Hirshfield, J Med Chem, 1988, 31, 2235-2246.

2- A. A. Patchett, R. P. Nargund, Annu Rep Med Chem, 2000, 35, 289-298.

3- E. M. Scholar The chemotherapy of malaria. In: Scholar EM., Pratt WB, editors. The Antimicrobial Drugs. New York, USA: Oxford University Press, 2000, 375-418.

4- M. Font, A. Monge, I. Ruiz, B. Heras., Drug Design Discov, 1997, 14, 259-272.

5- D. Kaminsky, R. I. Meltzer, J Med Chem, 1968, 11, 160-163.

6- R. Musiol, J. Jampilek, V. Buchta, L. Silva, H. Niedbala, B. Podeszwa, A. Palka, K. Majerz-Maniecka, B. Oleksys, J. Polanski, Bioorg Med Chem, 2006, 14, 3592-3598.

7- A. E. Sloboda, D. Powell, J. F. Poletto, W. C. Pickett, Jr. J. J. Gibbons, D. H. Bell, A. L. Oronsky, S. S. Kerwar., J. Rheumatol.

8- T. Nakamura, M. Oka, K. Aizawa, H. Soda, M. Fukuda, K. Terashi, K. Ikeda, Y. Mizuta, Y. Noguchi, Y. Kimura, T. Tsuruo, S. Kohno, Biochem Bioph Res Co.

9- E. S. Coimbra, R. Carvalhaes, R. M. Grazul, P. A. Machado, M. V. N. De Souza, A. D. Da Silva, Chem. Biol. Drug Des., 2010, 75, 628-631.

10- S. Guglielmo, M. Bertinaria, B. Rolando, M. Crosetti, R. Fruttero. V. Yardley. S. L. Croft, A. Gasco, Eur J Med Chem, 2009, 44, 5071-5079.

11- H. Mello, A. Echevarria, A. M. Bernardino, M. Canto-Cavalheiro, L. L. Leon, J Med Chem, 2004, 47, 5427-5432.

12- T. B. Chaston, R. N. Watts, J. Yuan, D. R. Richardson, Clin Cancer Res, 2004, 10, 7365-7374.

13- K. N. Zelenin, L. A. Khorseeva, V. V. Alekseev, Pharm Chem. J., 1992, 26, 395-405.

14- S. Gemma, L. Savini, M. Altarelli, P. Tripaldi, L. Chiasseneri, S. S. Coccone, V. Kumar, C. Camodeca, G. Campiani, E. Novellino, S. Clarizio, G. Delogu, S. Butini, Bioorg Med Chem, 2009, 17, 6063-6072.

15- D. R. Richardson, P. C. Sharpe, D. B. Lovejoy, D. Senaratne, D. S. Kalinowski, M. Islam, P. V. Bernhardt, J Med Chem, 2006, 49, 6510-6521.

16- D. O. Santos, C. E. Coutinho, M. F. Madeira, C. G. Bottino, R. T. Vieira, S. B. Nascimento, A. Bernardino, S. C. Bourguignon, S. Corte-Real, R. T. Pinho, C. R. Rodrigues, H. C. Castro, Parasitol Res, 2008, 4, 21-32.

17- L. L. Leon, G. M. C. Machado, L. E. Carvalho-Paes, G. J. Grimaldi, Trans R Soc Trop Med Hyg., 1990, 84, 678-680.

18- C. Bern, J. H. Maguire, J. Alvar, PLoS Negl Trop Dis., 2008, 2, e313.

19- P. Escobar, S. Matu, C. Marques, S. L. Croft, Acta Trop., 2002, 81, 151-157.

20- A. G. Tempone, C. Martins de Oliveira, R. G. Berlink, Planta Med., 2011, 77, 572-85.

21- R. M. Lawrence, K. C. Dennis, P. M. O’Neill, D. U. Hahn, M. Roeder, C. Struppe, Org Process Res Dev., 2008, 12, 294-297.
23- A. L. P. Candéa, M. L. Ferreira, K. C. Pais, L. N. F. Cardoso, C. R. Kaiser, M. G. M. O. Henriques, M. C. S. Lourenço, F. A. F. M. Bezerra; M. V. N. de Souza., Bioorg Med Chem Lett 2009, 19, 6272-6274.
24- F. G. Braga, E. S. Coimbra, M. O. Matos, A. M. L. Carmo, M. D. Cancio, A. D. Da Silva, Eur J Med Chem 2007, 42, 530-537.
25- F. H. Rodrigues, S. R. Afonso-Cardoso, M. A. B. Gomes, M. E. Beletti, A. Rocha, A. H. B. Guimarães, I. Candeloro, M. A. Souza, Vet Parasitol 2006, 139, 37-46.