Biometals (2011) 24:595–601
DOI 10.1007/s10534-011-9407-8

Investigation on the pharmacological profile of antimony(III) complexes with hydroxyquinoline derivatives: anti-trypanosomal activity and cytotoxicity against human leukemia cell lines

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Received: 30 September 2010 / Accepted: 2 January 2011 / Published online: 9 January 2011

Abstract Complexes $[Sb(QN)_2Cl]$ (**1**), $[Sb(QC)_2Cl]$ (**2**) and $[Sb(QI)_2Cl]$ (**3**) were obtained with 8-hydroxyquinoline (HQN), 5-chloro-8-hydroxyquinoline (HQC) and 5-chloro-7-iodo-8-hydroxyquinoline (clioquinol, HQI). The quinoline derivatives and their antimony(III) complexes were evaluated for their anti-trypanosomal activity as well as for their cytotoxicity against HL-60 and Jurkat human leukemia cell lines. Upon coordination to antimony(III) the anti-trypanosomal activity of HQC and HQI increases, the highest improvement being observed for complex (**3**), which was the most active among all studied compounds against both epimastigote and trypomastigote forms of *Trypanosoma cruzi.* All quinoline derivatives proved to be cytotoxic against both leukemia cell lineages. Upon coordination to antimony(III) the cytotoxicity of HQN improved against Jurkat leukemia cells. While $SbCl_3$ proved to be cytotoxic against HL-60 cells, it was not active against Jurkat cells. However, its coordination to the quinoline derivatives resulted in complexes with significant cytotoxicity against Jurkat cells.

Keywords 8-Hydroxyquinoline · 5-Chloro-8-hydroxyquinoline · 5-Chloro-7-iodo-8-hydroxyquinoline (clioquinol) · Antimony(III) complexes · Cytotoxicity · Leukemia · Anti-trypanosomal

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Introduction

Human african trypanosomiasis, Chagas disease, and leishmaniasis, collectively a widespread group of parasitic diseases, are a daily threat to more than 550 million people in Mexico, Central America, South America, Sub-Saharan Africa, the Middle East, Indonesia, and India, and cause nearly 150,000 deaths annually. These diseases are caused by unicellular trypanosomatid parasites of the genera *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania* sp. *Trypanosoma cruzi* is the causative agent of Chagas disease, an endemic infection that affects mainly Central and South America, where 15 million people are infected (Tonin et al. 2010).

Unfortunately, drug therapy for these diseases has not changed significantly in the past 50 years. Currently used agents are far less than satisfactory due to extreme toxicity, or the emergence of resistant parasitic strains (Woster 2007). Nifurtimox (nfx) and

benznidazole (bnz), the drugs used in the treatment of Chagas disease, are effective only against the acute infection and require long-term treatment. Moreover, these compounds can cause systemic toxicity and serious side effects (Tonin et al. 2010).

Leishmania is a protozoan parasite responsible for several pathologies collectively known as leishmaniasis, an important public-health problem in Latin America. Leishmaniasis is caused by parasites that are injected into mammals via sandflies and is manifested as cutaneous and visceral lesions (Herwaldt 1999). It is estimated that 2 million of new cases occur each year, with at least 15–20 million infected people. The major clinical use of antimony compounds is as a treatment for leishmaniasis. However the literature reports that antimony(III) complexes with various organic ligands also display in vitro as well as in vivo anticancer activity (Tiekink 2002).

The chemistry of quinoline derivatives has attracted special interest due to their therapeutic properties. It has been shown that 8-hydroxyquinoline (HQN) and some of its derivatives exhibit substantial cytotoxic activity against cancer cells (Nordenberg et al. 1990). 5-Chloro-7-iodo-8-hydroxyquinoline, clioquinol (HQI), has been previously used as an antibiotic in animals and humans and shows anticancer activity both in vitro and in vivo (Yu et al. 2009). It has been reported that when mixed with copper, HQN and clioquinol inhibited proteasomal activity and proliferation in cultured human cancer cells (Zhai et al. 2010). Clioquinol was shown to induce apoptosis, and its actions can be potentiated by both copper and zinc (Ding et al. 2005). In addition clioquinol is being investigated for the treatment of Alzheimer's disease (AD) as a metal chelating agent, to be used in therapies based on modulation of metal metabolism (Filiz et al. 2008).

Trypanosomes and leishmania show several biochemical similarities such as the abundance of stage-regulated cysteine proteinases which play important roles in parasite virulence, in modulation of the host's immune response and in parasite differentiation (Mottram et al. 1998). Taking into consideration that antimony-based drugs are used in the treatment of leishmaniasis, in the present work we investigated the anti-trypanosomal activity of antimony(III) complexes with HQN, HQC (5-chloro-8-hydroxyquinoline) and HQI.

In a previous work we demonstrated that antimony(III) complexes with thiosemicarbazones present significant anti-leukemia activity and were able to induce cell death through apoptosis (Reis et al. 2010). Since quinoline-based compounds show cytotoxic as well as antitumoral properties we also evaluated the effect of the HQN derivatives and their antimony(III) complexes on HL60 and Jurkat leukemia cell lines.

Experimental

Materials and methods

All common chemicals were purchased from Aldrich and used without further purification. Cisplatin, [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT), propidium iodide (PI), sodium citrate, triton X-100, dimethylsulfoxide (DMSO), Roswell Park Memorial Institute (RPMI) 1460 medium and L-glutamine were purchased from Sigma Chemicals. Antibiotic/antimicotic solution and fetal calf serum (FCS) were purchased from Gibco (Grand Island, NY).

Partial elemental analyses were performed on a Perkin Elmer CHN 2400 analyzer. An YSI model 31 conductivity bridge was employed for molar conductivity measurements. Infrared spectra were recorded on a Perkin Elmer FT-IR Spectrum GX spectrometer using KBr plates (400–400 cm^{-1}) and Nujol mulls between CsI plates (400–200 cm^{-1}). NMR spectra were obtained with a Bruker DPX-200 Avance (200 MHz) spectrometer using DMSO-d_6 or DMF-d_7 as the solvent and TMS as internal reference.

Syntheses of the antimony(III) complexes

$SbCl_3$ (1.0 mmol) powder was added to 3.0 mmol of the desired quinoline dissolved in 10.0 ml of ethanol. The mixture was kept at room temperature with stirring for 4 h. The obtained solids were filtered off then washed with diethylether and dried.

*Bis(8-hydroxiquinolinato)(chloro)antimony(III) [$Sb(QN)_2Cl$] (**1**)*

Yellow solid. Anal. Calc. ($C_{18}H_{12}ClN_2O_2Sb$): C, 48.53; H, 2.71; N, 6.29. Found: C, 47.91; H, 2.71; N, 6.06%. FW: 445.51 g mol^{-1}. IR (Kbr, cm^{-1}): ν(C=N) 1552s, ν(CO) 1098m, ρ (py) 626m. IR

(CsI/Nujol, cm^{-1}): ν(M–Npy) 277w, ν(M–Cl) 243w, ν(M–O) 522w. ^{1}H NMR [200 MHz, DMSO-d_6, (ppm), J(Hz)] main signals: 9.09 [s,1H, H(2)], 7.81 [m, 1H, H(3), 4.72], 8.70 [d, 1H, H(4), 8.33], 7.48 [d, 1H, H(5), 7.70], 7.61 [t, 1H, H(6), 7.67], 7.19 [d, 1H, H(7), 6.76]. ^{13}C NMR [200 MHz, DMSO-d_6, δ(ppm)] main signals: 146.71 [C(2)], 122.25 [C(3)], 140.40 [C(4)], 118.85 [C(5)], 129.37 [C(6)], 113.99 [C(7)]. $\Lambda_M = 23.32\ \Omega^{-1}\ cm^2\ mol^{-1}$ in DMF. Melting point: 271.1–275.6°C. Yield 67.6%.

*Bis(5-chloro-8-hydroxyquinolinato)(chloro) antimony(III), [Sb(QC)$_2$Cl]·EtOH (**2**)*

Yellow solid. Anal. Calc. ($C_{20}H_{16}Cl_3N_2O_3Sb$): C 42.86; H, 2.88; N, 5.00. Found: C, 43.01; H, 2.77; N, 5.06%. FW: 560.47 g mol^{-1}. IR (Kbr, cm^{-1}): $\nu(OH_{EtOH})$ 3436s, ν(C=N) 1546m, ν(CO) 1085m, ρ(py) 647m. IR (CsI/Nujol, cm^{-1}): ν(M–Npy) 277w, ν(M–Cl) 236w, ν(M–O) 542w. ^{1}H NMR [200 MHz, DMSO-d_6, δ(ppm), J(Hz)] main signals: 9.08 [s, 1H, H(2)], 7.86 [m, 1H, H(3), 4.01], 8.67 [d, 1H, H(4), 8.36], 7.69 [d, 1H, H(6), 8.33], 7.15 [d, 1H, H(7), 8.37]. ^{13}C NMR [200 MHz, DMSO-d_6, δ(ppm)] main signals: 147.33 [C(2)], 123.43 [C(3)], 135.01 [C(4)], 118.10 [C(5)], 128.49 [C(6)], 113.01 [C(7)], 152.70 [C(8)], 137.26 [C(9)], 126.23 [C(10)]. $\Lambda_M = 30.36\ \Omega^{-1}\ cm^2\ mol^{-1}$ in DMF. Melting point: 298.7–300.2°C. Yield 97%.

*Bis(5-chloro-7-iodo-8-hydroxyquinolinato)(chloro) antimony(III), [Sb(QI)$_2$Cl] (**3**)*

Yellow solid. Anal. Calc. ($C_{18}H_8Cl_3I_2N_2O_2Sb$): C, 28.22; H, 1.05; N, 3.66. Found: C, 28.21; H, 1.08; N, 3.80%. FW: 766.19 g mol^{-1}. IR (Kbr, cm^{-1}): ν(C=N) 1566m, ν(CO) 1097m, ρ(py) 647m. IR (CsI/Nujol, cm^{-1}): ν(M–Npy) 280w, ν(M–Cl) 232w, ν(M–O) 570w. ^{1}H NMR [200 MHz, DMF-d_7, δ(ppm), J(Hz)] main signals: 9.40 [s, 1H, H(2)], 7.96 [s, 1H, H(3)], 8.94 [s, 1H, H(4)], N.O [s, 1H, H(6)], N.O. [s, 1H, H(7)]. ^{13}C NMR [200 MHz, DMF-d_7, δ(ppm)] main signals: 147.80 [C(2)], 124.16 [C(3)], 137.29 [C(4)], 118.39 [C(5)], N.O. [C(6)], 83.13 [C(7)], N.O. [C(8)], N.O. [C(9)], 127.67 [C(10)]. $\Lambda_M = 97.14\ \Omega^{-1}\ cm^2\ mol^{-1}$ in DMF. Melting point: 271.1–273.3°C. Yield 51%. NO, non-observed.

Cytotoxicity in spleen cells and anti-trypanosomal activity

Animals

Male BALB/c mice (6–8 weeks old) were raised at the animal facilities of the Oswaldo Cruz Foundation (Rio de Janeiro, Brazil) and maintained at the animal facilities of the Aggeu Magalhães Research Center of the Oswaldo Cruz Foundation in Recife, Brazil. All mice were sacrificed and treated in accordance with the Oswaldo Cruz Foundation Commission for Experiments with Laboratory Animals (Ministry of Health, Brazil, 0266/05).

Obtaining spleen cells

Spleen cells were obtained according to the literature (Pereira et al. 2004). After euthanizing the animal with CO_2 gas, the spleen of each mouse was removed aseptically and placed in Falcon tubes containing RPMI 1640 with 10% of FCS (complete medium). In a vertical flow, each spleen was transferred to a Petri dish where they were macerated. The obtained cell suspensions were transferred to Falcon tubes containing approximately 10 ml of incomplete medium, centrifuged at 4°C, 200×g for 5 min. After discarding the supernatant, distilled water was added to the sediment to promote lysis of red blood cells. The supernatant, containing no cellular debris was collected and centrifuged at 4°C, 200×g for 5 min. The sediment (containing cells) was re-suspended in complete RPMI 1640. An aliquot of each cell suspension was separated, diluted in trypan blue to be quantified in a Neubauer chamber and the cell viability was determined.

In vitro cytotoxicity assay

The cytotoxicity of the compounds was determined using BALB/c mice splenocytes (6×10^5 cells · $well^{-1}$) cultured in 96-well plates in RPMI 1640 media (Sigma Chemical Co., St. Louis, MO) supplemented with 10% of FCS (Cultilab, Campinas, SP, Brazil) and 50 μg ml^{-1} of gentamycin (Novafarma, Anápolis, GO, Brazil). Each compound was evaluated in six concentrations (1, 5, 10, 25, 50 and 100 μg ml^{-1}), in triplicate on two independent assays. Cultures were incubated in the presence of

^{3}H-thymidine (Amersham Biosciences) (1 μCi well^{-1}) for 24 h at 37°C and 5% CO_2. After this period, the content of the plate was harvested to determine the ^{3}H-thymidine ([^{3}H]TdR) incorporation using a beta-radiation counter (β-matrix 9600, Packard). The cytotoxicity of the compounds was determined by comparing the percentage of ^{3}H-thymidine incorporation (as an indicator of cell viability) of imidazolidines-treated wells in relation to untreated wells. Non-cytotoxic concentrations were defined as those causing a reduction of ^{3}H-thymidine incorporation below 30% in relation to untreated controls.

Inhibition of *T. cruzi* growth

Epimastigotes of *T. cruzi* (Y strain) were cultivated at 26°C in Liver Infusion Tryptose medium (LIT) supplemented with 10% FCS, 1% hemin, 1% R9 medium and 50 μg ml^{-1} gentamycin. Parasites (10^6 ml^{-1}) were cultured in a fresh medium in the absence or in the presence of the compounds being tested (from stock solution in DMSO). Cell growth was determined after 11 days of culture by counting viable forms in a hemacytometer, in triplicate. The compounds used were from a stock solution in DMSO. To determine the IC_{50}, cultures of Y strain epimastigotes in the presence of different concentrations of the compounds were evaluated after 11 days as described above. IC_{50} calculation was carried out using non-linear regression on PRISM 4.0 GRAPHPAD software. Y strain *T. cruzi* trypomastigotes were obtained from culture supernatants of Vero cell line at 37°C and placed in 96-well plates (4×10^5 well^{-1}) in a RPMI 1640 medium supplemented with 10% FCS and 50 μg ml^{-1} gentamycin. Viable parasites were counted in a hemacytometer 24 h after addition of the studied compounds by way of trypan blue exclusion. The percentage of inhibition was calculated in relation to untreated cultures.

Cytotoxic activity against HL60 and Jurkat leukemia cell lines

Jurkat cells (human immortalized line of T lymphocyte) and HL60 cells (human promyelocytic leukemia HL-60 cells) were kindly given by Dr. Gustavo Amarante-Mendes (São Paulo University, Brazil). All lineages were maintained in the logarithmic phase of growth in RPMI 1640 medium supplemented 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin (GIBCO BRL, Grand Island, NY) enriched with 2 mM of L-glutamine and 10% of fetal bovine serum. All cultures were maintained at 37°C in a humidified incubator with 5% CO_2 and 95% air. The media were changed twice weekly and they were regularly examined.

The leukemia cells were inoculated at 50,000 cells of per well. The plates were pre-incubated for 24 h at 37°C to allow adaptation of cells prior to the addition of the test compounds. Freshly prepared solutions of the different substances were tested at 10 μM. Subsequently, the plates were inoculated for 48 h in an atmosphere of 5% CO_2 and 100% relative humidity. Control groups included treatment with 0.1% DMSO (negative control) and 10 μM of cisplatin (positive control). Cell viability was estimated by measuring the rate of mitochondrial reduction of MTT. All substances were dissolved in DMSO, prior to dilution. The compounds that produced a growth inhibition more than 50% in the screen were tested using decreasing concentrations, i.e. six or seven consecutive ten-fold dilutions ranging from 1×10^{-5} to 1×10^{-11} M. The IC_{50} values were determined graphically from concentration-effect curves using Prism 5.0 (GraphPad Software Inc.). For comparison, the cytotoxicity of cisplatin was evaluated under the same experimental conditions both with and without 0.1% of DMSO. All compounds were tested in triplicate, in three independent experiments.

MTT is a standard colorimetric assay, in which mitochondrial activity was measured by splitting tetrazolium salts with mitochondrial dehydrogenases in viable cells only (Mosmann 1983). Briefly, after 4 h of the end of incubation of cells with different compounds, 20 μl of MTT solution (5 mg ml^{-1} in phosphate-buffered saline) were added to each well, the supernatant was removed and 200 μl of 0.04 M HCl in isopropyl alcohol were added to dissolve the formazan crystal. The optical densities (OD) were evaluated in a spectrophotometer at 570 nm. Controls included drug-containing medium (background) and drug-free complete medium. Drug-free complete medium was used as control (blank) and was treated in the same way as the drug-containing media. Results were expressed as percentage of cell proliferation, comparing with 0.1% DMSO control and were calculated as follows: viability (%) = (mean OD treated − mean OD background)/mean OD

untreated cultured, i.e. 0.1% DMSO − mean OD blank wells) × 100. Interactions of compounds and media were estimated on the basis of the variations between drug-containing medium and drug-free medium to escape from false-positive or false-negative (Ulukaya et al. 2004).

All experiments were performed at least three replicates per compound and results shown are the average of three independent experiments. Data are represented as mean ± SEM. Significance was tested by Student's test *t*.

Results and discussion

Microanalyses were compatible with the formation of $[Sb(QN)_2Cl]$ (**1**), $[Sb(QC)_2Cl].EtOH$ (**2**) and $[Sb(QI)_2Cl]$ (**3**). In **1–3** there are two anionic ligands and one chloride ion in the metal coordination sphere. In complex (**2**) one crystallization ethanol molecule is present, as confirmed by its thermogravimetric curve which shows a weight loss of 7.8% (calcd. 8.2%) in the 60–110°C range, corresponding to the loss of one ethanol molecule.

Spectroscopic characterization

The vibrations attributed to ν(C=N) at 1587–1604 cm^{-1} in the infrared spectra of the free bases shift to 1546–1566 cm^{-1} in the spectra of complexes (**1–3**), in agreement with coordination of the nitrogen (Wagner et al. 2007). The in-plane deformation mode of the pyridine ring at 575–634 cm^{-1} in the spectra of the free hydroxyquinolines shifts to 636–647 cm^{-1} in complexes (**1–3**), suggesting coordination of the heteroaromatic nitrogen (Wagner et al. 2007). The ν(C–O) absorption observed at 1059–1084 cm^{-1} in the spectra of the uncomplexed HQN derivatives shifts to 1085–1097 cm^{-1} in the spectra of complexes (**1–3**), indicating coordination of the oxygen (Yurdakul and Arýcý 2004). New absorptions at 277–280 and 522–570 cm^{-1} in the spectra of **1–3** were attributed to the $\nu(Sb–N_{py})$ and ν(Sb–O) modes, respectively, and absorptions at 232–243 cm^{-1} were assigned to the ν(Sb–Cl) vibration (Yin and Zhai 2009; Nakamoto 1970).

The NMR spectra of HQN and of HQC were recorded in DMSO-d_6. The spectrum of HQI was recorded in DMF-d_7 because its antimony(III) complex (**3**) is more soluble in this solvent than in DMSO-d_6. The spectra of complexes (**1**) and (**2**) were recorded in DMSO-d_6. The ^{1}H resonances were assigned on the basis of chemical shifts and multiplicities. The carbon type (C, CH) was determined by using distortionless enhancement by polarization transfer (DEPT 135) experiments. The assignments of the protonated carbons were made by 2D heteronuclear multiple quantum coherence experiments (HMQC).

The ^{1}H NMR spectra of complexes (**1–3**) do not show the signal of C(8)–OH, in accordance with coordination of two anionic ligands. In the ^{13}C NMR spectrum the signals of the pyridine carbons and those of the phenolic carbons undergo significant shifts upon complexation, in accordance with coordination through the pyridine nitrogen and the phenolate oxygen. In the ^{1}H NMR spectrum of **2** the signals attributed to ethanol were observed, in accordance with the proposed formulation.

Anti-trypanosomal activity

The HQN derivatives inhibited the growth of both epimastigote and trypomastigote forms of *T. cruzi* (see Table 1). HQN and HQC showed higher activity against the trypomastigote form than HQI. However, HQI was the most active derivative against the epimastigote form. Upon coordination to antimony(III) the activity of HQC and HQI increases, the highest improvement being observed for complex

Table 1 Anti-trypanosomal activity of 8-hydroxyquinoline derivatives, their antimony(III) complexes, $SbCl_3$ and the reference compounds benznidazole and nifurtimox

Compound	Cytotoxicity (μmol l^{-1})	$IC_{50\ tripo}$ (μmol l^{-1})	$IC_{50\ epi.}$ (μmol l^{-1})
HQN	<6.89	4.47	3.37
HQC	<5.57	3.95	3.28
HQI	<3.27	21.96	2.65
$[Sb(QN)_2Cl]$ (**1**)	<2.24	4.20	1.68
$[Sb(QC)_2Cl]$ (**2**)	1.72	1.22	0.93
$[Sb(QI)_2Cl]$ (**3**)	<1.30	1.28	0.56
$SbCl_3$	<4.38	65.75	6.79
bnz	96.06	6.26	6.65
nfx	3.48	2.75	1.88

bnz benznidazole, *nfx* nifurtimox

(**3**), which was the most active among all studied compounds against both forms of *T. cruzi*. Unfortunately all compounds revealed to be cytotoxic at doses similar to or lower than their IC_{50} values. However, it is worth noticing that the reference drug nfx was less active than complex (**3**), and exhibited a similar cytotoxic effect.

Cytotoxic activity against leukemia cells

All quinoline derivatives showed cytotoxic activity against the two leukemia cell lineages (see Table 2). HQN and HQC presented higher activity against HL-60 cells while HQI was slightly more cytotoxic against Jurkat cells. Upon coordination to antimony(III) the cytotoxicity of HQN improved against Jurkat leukemia cell lines while those of HQC and HQI did not undergo significant modification, considering the presence of two quinoline ligands per metal in the complexes. Interestingly while the starting antimony salt $SbCl_3$ proved to be cytotoxic against HL-60 cells, it was not cytotoxic against Jurkat cells. However, coordination to the quinoline derivatives resulted in complexes with significant cytotoxicity against Jurkat cells.

Although cisplatin proved to be more active against the two leukemia cell lines, the presently studied compounds revealed to be interesting, taking into consideration that a significant proportion of patients chronically treated with antitumoral drugs develop resistance.

Table 2 Cytotoxic activity (IC_{50}) of 8-hydroxyquinoline derivatives and their antimony(III) complexes on HL60 and Jurkat cell lines

Compound	HL60	Jurkat
HQN	8.84 ± 1.73	23.37 ± 1.59
$[Sb(QN)_2Cl]$ (**1**)	4.95 ± 2.55	2.34 ± 0.89
HQC	4.88 ± 0.04	14.88 ± 0.31
$[Sb(QC)_2Cl]$ (**2**)	3.46 ± 1.66	5.85 ± 0.99
HQI	15.80 ± 0.86	10.07 ± 1.13
$[Sb(QI)_2Cl]$ (**3**)	6.37 ± 1.69	6.63 ± 2.29
Cisplatin	0.74 ± 0.16	1.36 ± 0.69
$SbCl_3$	1.84 ± 1.11	>100

HL60 and Jurkat cells were treated with different concentrations of compounds for 48 h and proliferation/cell viability was evaluated by MTT assay. Representative data of three independent experiments performed in triplicate

Acknowledgments This work was supported by CNPq and INCT-INOFAR (Proc. CNPq 573.364/2008-6).

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