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Short communication

Highly potent anti-proliferative effects of a gallium(III) complex with 7-chloroquinoline thiosemicarbazone as a ligand: Synthesis, cytotoxic and antimalarial evaluation

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ABSTRACT

A gallium(III) complex with 7-chloroquinoline thiosemicarbazone was synthesized and characterized. The complex proved to be thirty-one times more potent on colon cancer cell line, HCT-116, with considerably less cytotoxicity on non-cancerous colon fibroblast, CCD-18Co, when compared to etoposide. Its anti-malarial potential on 3D7 isolate of *Plasmodium falciparum* was better than lumefantrine.

1. Introduction

Over the past few years, bioorganometallic chemistry has emerged as a rapidly growing area which associates classical organometallic chemistry to biology, medicine, and molecular biotechnology [1]. Among the incredible number and variety of roles that metals play in contemporary medicine; cancer [2] and malaria [3] treatments are appreciated as arguably the most prominent application of metal-based drugs. After the success of ferroquine and cisplatin, the potential of ruthenium, rhodium and gallium has been assessed in an enormous number of studies [4]. Gallium(III)-containing complexes have shown to have particular efficacy against bladder, urothelial carcinomas, malignant glioblastoma and some lymphomas [5]. Gallium(III) nitrate has been approved for the treatment of hypercalcemia of malignancy, but its unfavorable pharmacokinetics has been a major obstacle in its use in systemic chemotherapy [6]. The preparation of gallium(III) complexes with organic ligands thus has become an important strategy for creating tumor-inhibiting therapeutic agents with potential advantages over gallium salts regarding oral bio-availability, hydrolytic stability and membrane penetration ability [7–12].

Thiosemicarbazones (TSCs), especially α-*N*-Heterocyclic thiosemicarbazones are versatile ligands exhibiting various binding modes with transition and some main group metals and possess a wide range of pharmaceutical properties [13]. Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone, 3-AP) is the most prominent representative among these compounds as it has been tested in a variety of tumor cell lines in the preclinical setting

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and is currently undergoing different phase I and II clinical trials [14].

Over the last decade, there has been an increasing interest in metal-containing anti-malarials. Given the importance of iron metabolites in malarial physiology and toxicity, various chelators such as deferoxamine [15] and reversed siderophores [16] have been explored as potential anti-malarials. However, these chelators may bind other essential metals such as zinc, calcium, and magnesium, in intracellular compartments, thereby resulting in deprivation of these vital nutrients that are required for the mammalian host as well. Therefore, metallodrugs that possess an optimal balance of hydrophobicity/hydrophilicity, or relative lipophilicity, for permeation across membrane bilayers, and remain non-metabolized in the intracellular target sites, may offer alternative remedies. Anti-malarial potential of transition metal complexes of chloroquine especially with ruthenium and rhodium complexes have been explored and have shown general improvement on the efficacy of chloroquine with essentially no sign of acute toxicity in experimental animals [17].

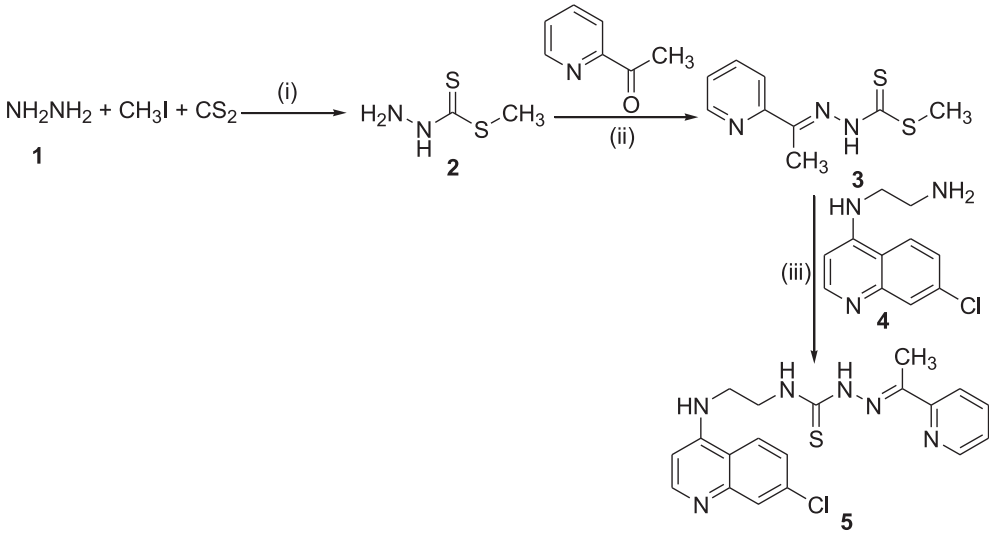
2. Synthetic chemistry and pharmacology

2.1. Synthetic chemistry

In continuation of our interest in the synthesis of scaffolds with medicinal and biological potential [18], the present manuscript explicates the synthesis of 7-chloroquinoline-based thiosemicarbazone **5**, its metal complexation with a gallium(III) metal center **6**, followed by evaluation of its cytotoxic and anti-plasmodial profiles. The preparation of the N^1-(7-Chloro-quinolin-4-yl)-ethylamino-2-acetylpyridine thiosemicarbazone **5** is based on the combination of literature method [19] as depicted in Scheme 1 involving the addition of the hydrazine hydrate to a solution of KOH in water: 2-propanol (1:1) mixture followed by the dropwise addition of carbon disulfide. The reaction mixture was stirred below 10 °C for 2 h with subsequent addition of iodomethane to yield Methyl hydrazinecarbodithioate **2**. The condensation reaction of **2** with 2-acetylpyridine in 2-propanol resulted in the isolation of intermediate **3** which upon refluxing with N^1-(7-Chloro-quinolin-4-yl)-ethane-1,2-diamine **4** in ethanol for 24 h yielded the desired N^1-(7-Chloro-quinolin-4-yl)-ethylamino-2-acetylpyridine-thiosemicarbazone **5**.

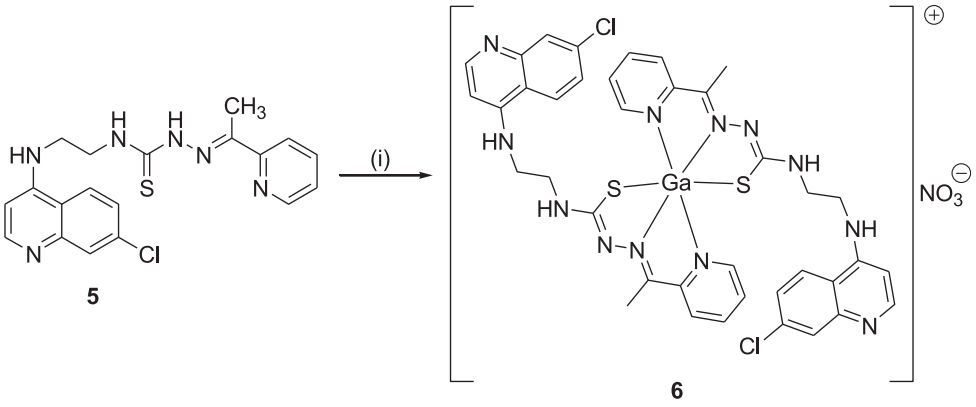
For the synthesis of desired 7-chloroquinoline based gallium(III) complex **6**, small portions of $Ga(NO_3)_3 \cdot xH_2O$ was added to a solution of thiosemicarbazone **5** in ethanol. The resulting yellow solution was refluxed with stirring for 2 h, cooled to room temperature until crystalline yellow solid separates out. The solution was then filtered and washed with cold ethanol twice under reduced pressure to obtain the complex **6** as shown in Scheme 2.

The structure to the ligand and the complex was assigned by using spectral data and analytical evidences. Infrared spectra were acquired for ligand **5** and complex **6** (see Table 1, Supporting Information). Thiosemicarbazones exhibit characteristic bands corresponding to various functional groups in different energy regions. The most significant infrared bands in the region 4000–500 cm^{-1} are found between 3100 and 3500 (assigned to NH stretching vibrations); 1580–1630 (assigned to C=N + NH); and 1100–1300 and 820–900 (assigned to the C=S moiety). Thiosemicarbazones can coordinate as either a neutral (thione) or as a mono-anionic (thiolate) ligand [20]. Ligand **5** in our case appears to be in the thione form as a solid [21,22]. This can be inferred primarily from the absence of a ν(S–H) absorption in the region 2600–2500 cm^{-1}. There are two bands in the ν(N–H) region and the presence of the peak due to the hydrazinic hydrogen confirms the thione formulation. The thiolate form was evident in complex **6** as determined from HRMS. Ligand **5** shows a medium intensity band in the 1620–1605 cm^{-1} region, and this is assigned as the C=N (imine) linkage. On coordination with $Ga(NO_3)_3 \cdot xH_2O$, this band shifts significantly from 1609 to 1614 cm^{-1}. The shift in this band is indicative of the iminic nitrogen being involved in the ligation [23,24]. The ^{1}H NMR spectrum of the ligand exhibited the presence of a singlet at δ 2.41 corresponding to methyl group, a pair of double doublets at δ 3.57 and 3.97 corresponding to two methylenes along with the characteristic quinoline ring protons. Its ^{13}C NMR spectrum exhibited characteristic absorptions at δ 12.3, 154.6 and 178.4 ppm corresponding to the methyl, iminic and thiocarbonyl



Reagents and conditions: (i) equimolar quantites of KOH, 1:1 water:2-propanol, -10 °C, 100 min. (ii) room temp., 2 h (iii) ethanol, reflux, 20 h

Scheme 1. Synthesis of 2-acetylpyridine-7-chloroquinoline based thiosemicarbazone.



Scheme 2. Synthesis of gallium(III) based complex **6**.

groups along with the presence of requisite number of carbons corroborating the assigned structure. The ^{1}H NMR spectrum of the complex **6** exhibited the presence of two singlets at δ 2.43 and 2.66 corresponding to two methyl groups, the presence of two singlets at δ 3.09 and 3.65 corresponding to four methylenes along with the characteristic quinoline ring protons. The appearance of characteristic absorptions at δ 12.3 and 13.3 corresponding to methyl substituents in ^{13}C NMR spectrum of the Ga(III) complex along with the required number of carbons further established the assigned structure. The appearance of molecular ion peak at 399.1112 for ligand **5** and 865.1246 $[M-NO_3]^+$ for complex **6** in High Resolution Mass Spectrum (HRMS) unambiguously confirmed the assigned structure.

2.2. Anticancer evaluation

Complex **6** and etoposide were evaluated for their cytotoxicity against HCT-116, Caco-2, and HT-29 by a colorimetric assay (MTS), which measures mitochondrial dehydrogenase activity as an indication of cell viability. The growth inhibition effects of the complex were also investigated in noncancerous colonic myofibroblast CCD-18Co cells and compared to the colon cancer cell lines. Table 1 lists the antiproliferative effect of complex **6** and etoposide on different cell lines after 24, 48, and 72 h treatment, while the same has been graphically represented in Fig. 1. The results demonstrated a great difference in the inhibition of cell proliferation among cancer lines and the noncancerous CCD-18Co cells. The highest antiproliferative effects on three cancer colon cell lines were observed in case of complex **6** with IC_{50} values ranging from 0.55 ± 0.39 to 7.25 ± 1.27 μM after 72 h on all cancer cells. The complex **6** proved to be thirty-one times more potent on HCT-116, four times more potent on Caco-2 and two and a half times more potent on HT-29 compared to etoposide after 72 h. Complex **6** did not prove to be cytotoxic when compared to noncancerous colon fibroblasts (CCD-18Co) cell line, exhibiting an IC_{50} of 11.81 ± 1.42 μM at 72 h. Further complex **6** proved to be more selective (twenty-one times compared to normal cell line CCD-18Co and eight time compared to etoposide) against HCT-116 cell line as evidenced by calculated selectivity index (SI) shown in Table 1.

2.3. Antimalarial evaluation

Further, the anti-malarial profile of complex **6** was evaluated against 3D7 isolate of *Plasmodium falciparum* and compared with standard drugs *viz*. quinine, artemisinin and lumefantrine using the SYBR Green assay [25]. The graphical representation of the drug concentration and the percentage growth inhibition has been depicted in Fig. 2. As evident, from Fig. 2, the complex **6** showed 100% inhibition at lower concentration in comparison to lumefantrine while other standard drugs have shown better inhibition against *P. falciparum*. The antimalarial profile of Ligand **5** was also evaluated in order to validate the significance of Ga complexation in the present case. Ligand **5** and complex **6** exhibited an average IC_{50} value of 250 and 173 nM against *P. falciparum* suggestive of the enhancement in antimalarial efficacy *via* gallium co-ordination.

3. Conclusion

In conclusion, we describe herein the synthesis of a highly potent anti-proliferative gallium(III) complex with 7-chloroquinoline-thiosemicarbazone as a ligand with anti-malarial efficacy. The synthesized complex showed much better anti-proliferative profile on different colon cancer cell lines compared with the standard drug etoposide while a better anti-malarial profile than 7-Chloroquinoline based thiosemicarbazone **5** and lumefantrine has been observed on 3D7 isolate of *P. falciparum*.

4. Experimental section

Melting points were determined by open capillary using Veego Precision Digital Melting Point apparatus (MP-D) and are uncorrected. IR spectra were acquired on a Shimadzu D-8001 spectrophotometer. ^{1}H NMR spectra were acquired on a Jeol 300 and 400 MHz spectrometer in DMSO-d_6, using TMS as internal standard. Chemical shift values are expressed as parts per million downfield from TMS, and *J* values are in units of Hz. Splitting patterns are indicated as s: singlet, d: doublet, t: triplet, m: multiplet, dd: double doublet, ddd: doublet of a doublet of a doublet, and br: broad peak. ^{13}C NMR spectra were recorded on a Jeol 300 and 400 (75 and 100 MHz) spectrometer in DMSO-d_6 using TMS as internal standard. HRMS were acquired on BRUCKER high resolution mass spectrometer (micrOTOF-QII) and Waters Q-tof Ultima mass spectrometer. Cone voltage was 25 V. The samples were dissolved in the methanol at 1 mg/ml; then the resulting solution was diluted with methanol to 5 ng/μL. The mobile phase was methanol with a flow rate of 50 μL/min. MTS salt [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphenyl)-2*H*tetrazolium salt] and etoposide standard were obtained from Sigma–Aldrich. Analytical or reagent grade chemicals were used throughout this study. All chemicals including solvents were obtained from Sigma–Aldrich

Table 1
Anti-proliferative effects of complex **6** and etoposide on different cell lines after 24, 48, and 72 h treatment. Selectivity index (SI) was calculated after 72 h of treatment. Data are expressed as IC_{50} (μM). IC_{50} is defined as the concentration required to achieve 50% inhibition over control cells (0.5% DMSO); IC_{50} values are shown as mean standard error values taken from three independent experiments.

Cell line	Compound	Time treatment			Selectivity index (SI)
		24 h	48 h	72 h	
HCT-116	Complex **6**	14.26 ± 1.37	2.96 ± 0.74	0.55 ± 0.39	21.47
	Etoposide	38.10 ± 1.20	26.70 ± 1.60	17.10 ± 1.60	2.53
Caco-2	Complex **6**	19.56 ± 1.57	11.96 ± 1.03	4.02 ± 0.78	2.94
	Etoposide	32.90 ± 1.90	19.00 ± 1.80	16.50 ± 1.30	2.62
HT-29	Complex **6**	19.66 ± 1.37	13.63 ± 1.35	7.25 ± 1.27	1.63
	Etoposide	35.10 ± 1.70	21.90 ± 1.60	19.30 ± 1.80	2.24
CCD-18Co	Complex **6**	28.24 ± 1.72	21.13 ± 1.53	11.81 ± 1.42	
	Etoposide	58.90 ± 2.10	49.30 ± 1.60	43.20 ± 1.80	

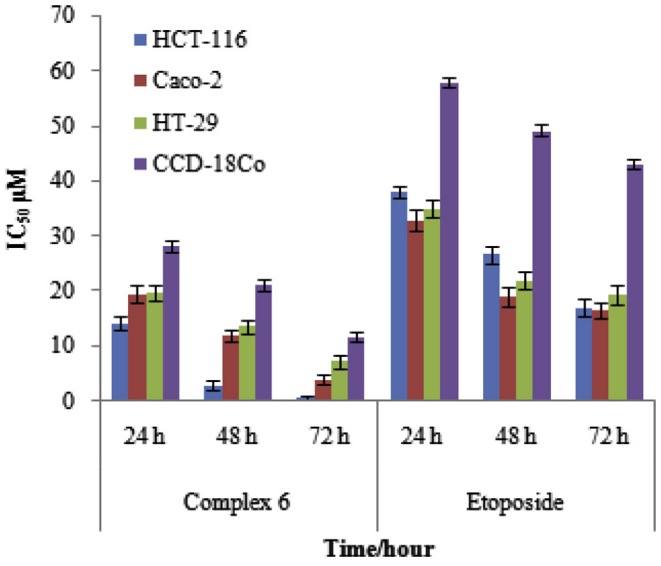


Fig. 1. Effect of complex **6** and etoposide on various cancer cell lines after 24, 48, and 72 h treatment.

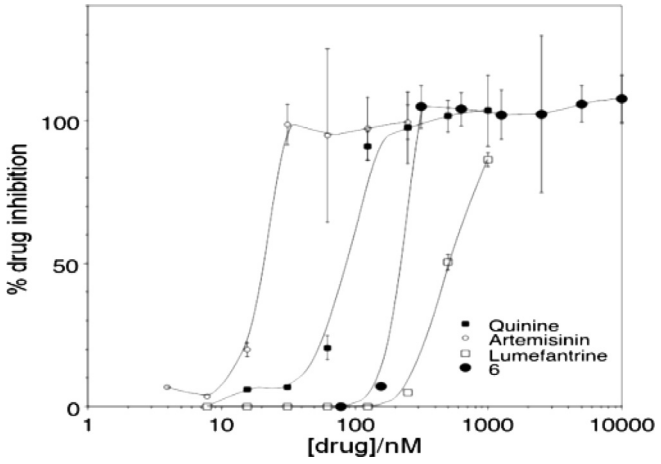


Fig. 2. Dose–response curve of the complex **6** against 3D7 isolate of *P. falciparum*, asexual blood stage.

(St. Louis, MO, USA) or other commercial vendors and used as received. Microanalyses (C, H, N) were performed by CHN analysis by the Microanalysis Laboratory at the University of Illinois Urbana/Champaign, U.S.A. FT IR spectra were acquired in the range 4000–400 cm^{-1} using the ATR accessory (with a diamond crystal) on a Nicolet 6700 FT IR spectrophotometer.

*4.1. Procedure for the synthesis and characterization of N^1-(7-chloro-quinolin-4-yl)-ethylamino-2-acetylpyridine-thiosemicarbazone (**5**)*

To 4.8 g (0.02 mol) of **3** dissolved in 100 ml of EtOH was added 0.02 mol of N^1-(7-Chloro-quinolin-4-yl)-ethane-1,2-diamine **4**. The resulting solution was heated under reflux until the evolution of methyl mercaptan almost completely ceased as detected by the yellow color it imparts to moistened $Pb(OAc)_2$ paper placed at the mouth of the reflux condenser. Reaction time was 20 h and the resulting thiosemicarbazone got crystallized from hot solution during the progress of reaction. The crystals were collected, washed with cold diethyl ether and dried under reduced pressure.

*4.1.1. N^1-(7-chloro-quinolin-4-yl)-ethylamino-2-acetylpyridine-thiosemicarbazone (**5**)*

Yield 82%; Off White Solid; m.p. 203–204 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.41 (s, 3H, $-CH_3$); 3.57 (dd, J = 2.1, 5.7 Hz, 2H, $-CH_2-$); 3.97 (dd, J = 2.1, 5.7 Hz, 2H, $-CH_2-$); 6.77 (d, J = 5.5 Hz, 1H, H^b); 7.41 (m, 1H, PyridylH); 7.47 (dd, J = 2.2, 9.0 Hz, 1H, H^d); 7.52 (t, J = 5.1 Hz, –NH–, exchangeable with D_2O); 7.82 (m, 2H, H^e + PyridylH); 8.29 (d, J = 9.0 Hz, 1H, H^c); 8.32 (d, J = 8.0 Hz, 1H, PyridylH); 8.43 (d, J = 5.4 Hz, 1H, H^a); 8.60 (d, J = 4.13, 1H, PyridylH); 8.87 (t, J = 6.1 Hz, –NH–, exchangeable with D_2O); 10.50 (s, –NH–, exchangeable with D_2O); ^{13}C NMR(DMSO-d_6, 100 MHz): 12.3, 41.9, 98.9, 117.3, 120.7, 123.9, 124.0, 124.1, 127.4, 133.4, 136.3, 148.5, 148.7, 148.8, 150.2, 151.7, 154.6, 178.4; HRMS: Calcd for $C_{19}H_{19}ClN_6S$ $[M+H]^+$ 399.1153, found 399.1112; Anal. Calcd (%) for: C, 57.21; H, 4.80; N, 21.07, found: C, 57.19; H, 4.77; N, 21.10. FT IR (ν/cm^{-1}): 1265(C=S), 1609(C=N).

*4.2. Procedure for the synthesis and characterization of complex (**6**)*

To the stirred solution of ligand **5** (615 mg, 0.771 mmol) in ethanol (60 ml) was added $Ga(NO_3)_3 \cdot xH_2O$ (184 mg, 0.355 mmol) in small portions. The resulting yellow solution was refluxed with stirring for 2 h; cooled to room temperature until crystalline yellow solid separates out. The solution was then filtered and washed with cold ethanol twice under reduced pressure to obtain the complex **6** (609 mg).

*4.2.1. Complex (**6**)*

Yield 45.5%; Yellow solid; m.p. >250 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.43 (s, 3H, $-CH_3$); 2.66 (s, 3H, $-CH_3$); 3.09 (s, 2H, $-NCH_2$); 3.65 (s, 6H, 3xNCH_2); 6.85 (d, J = 6.0 Hz, 1H, CQ-H); 6.98 (d, J = 6.0 Hz, 1H, CQ-H); 7.51 (s, 1H, –NH–); 7.67–7.85 (m, 7H, –ArH); 8.10–8.16 (m, 2H, –ArH); 8.35–8.41 (m, 7H, –ArH); 8.85 (s, 1H, –NH–); 9.38 (s, 1H, –NH–); 10.42 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6, 100 MHz): 12.3, 13.3, 41.7, 42.3, 43.0, 45.0, 98.8, 98.9, 115.0, 115.5, 115.6, 119.0, 119.2, 120.8, 121.7, 124.1, 125.6, 126.9, 127.4, 136.9, 138.1, 138.3, 138.4, 142.8, 143.2, 148.4, 148.6, 149.0, 152.5, 154.1, 156.0, 156.6, 160.8, 162.1, 166.2, 173.8; m/z (HRMS, positive mode): 865.1246 (100%, $[M-NO_3]^+$); CHN: Calcd for $C_{78}H_{178}Cl_2GaN_{13}O_{34}S_2$, C, 45.76; H, 8.76; N, 8.89. Found: C, 45.62; H, 8.71; N, 8.96; FT IR (ν/cm^{-1}): 1614 (C=N), 2929 (NH), 3273 (OH).

4.3. Cell lines and culture conditions

Human colon cancer cell lines Caco-2 (adenocarcinoma), HT-29 (adenocarcinoma) and HCT-116 (carcinoma) and the normal colon cells CCD-18Co were obtained from American Type Culture Collection (Rockville, USA). Caco-2 cells were grown in EMEM

medium supplemented with 10% v/v fetal bovine serum, 1% v/v nonessential amino acids, 1% v/v L-glutamine and 1% v/v antibiotic solution (Sigma). HT-29 and HCT-116 cells were grown in McCoy's 5a medium supplemented with 10% v/v fetal bovine serum, 1% v/v nonessential amino acids, 2% v/v HEPES and 1% v/v antibiotic solution. CCD-18Co cells were grown in EMEM medium supplemented with 10% v/v fetal bovine serum, 1% v/v nonessential amino acids, 1% v/v L-glutamine and 1% v/v antibiotic solution and were used from PDL = 26 to PDL = 35 for all experiments. Cells were maintained at 37 °C in an incubator under a 5% CO_2/95% air atmosphere at constant humidity. The pH of the culture medium was determined using pH indicator paper (pHydrion™ Brilliant, pH 5.5–9.0, Micro Essential Laboratory, NY, USA) inside the incubator. Cells were counted using a hemacytometer and were plated at 5000–3000 cells per well, in a 96-well format for 24 or 48 h prior to crude extract addition depending on the cell line. All of the test samples were solubilized in DMSO (<0.5% in the culture medium) by sonication and were filter sterilized (0.2 μm) prior to addition to the culture media. Control cells were also run in parallel and subjected to the same changes in medium with a 0.5% DMSO. In addition, cells were treated as indicated above for 24, 48, or 72 h.

4.4. Cell proliferation and viability tests (Trypan blue exclusion and MTS assays)

At the end of each treatment, trypsinized cells (2.5 g/L trypsin, 0.2 g/L EDTA) were suspended in culture medium, counted using a Neubauer haemocytometer (Bad Mergentheim, Germany) and viability measured using Trypan blue dye exclusion. Experiments were performed in duplicate. The MTS assay was carried out as described previously [26]. At the end of each day of treatment with serially diluted test samples (ranging from 1 to 200 μg/mL concentrations), 20 μL of the MTS reagent, in combination with the electron coupling agent, phenazine methosulfate, was added to the wells and cells were incubated at 37 °C in a humidified incubator for 3 h. Absorbance at 490 nm (OD_{490}) was monitored with a spectrophotometer (SpectraMax M2, Molecular Devices Corp., operated by SoftmaxPro v.4.6 software, Sunnyvale, CA, USA), to obtain the number of cells relative to control populations. 20 μL of etoposide 4 mg/mL (Sigma) was assayed as a negative control of proliferation. The results are expressed as the concentration that inhibit growth of cell by 50% vs. control cells (control medium used as negative control), IC_{50} Data are presented as the mean ± S.D. of three separated experiments on each cell line (n = 2 plates per experiment; 2 wells per treatment per time point). Etoposide provided consistent IC_{50} values of 15–25 μM for HCT-116 cells, 30–40 μM for Caco-2 and HT-29 cells, and 40–45 μM for the CCD-18Co cells.

4.5. Anti-plasmodial evaluation

The synthesized Ga(III) complex of 7-chloroquinoline-thiosemicarbazone was evaluated for its anti-plasmodial activity. Direct effect of compounds on *P. falciparum* was determined by SYBR green I (Invitrogen-Molecular Probes, Eugene OR) based assay as previously described [25]. Briefly, the inhibition assay was initiated by adding serially diluted compound in 96 well microplates followed by sorbitol synchronized 1% ring stage parasites at 1% hematocrit in RPMI 1640 media with glutamine and hepes supplemented with 10% human serum and 50 mg/mL hypoxanthine. Culture plates were then transferred to 37 °C incubator and maintained in the environment of 5% O_2, 5% CO_2 and 90% N_2 for 72 h. 2x SYBR Green I solution in lysis buffer was added after one cycle of freeze and thaw. Plates were then placed at room temperature in dark for 1–2 h and fluorescence values were quantified on a plate reader at emission and excitation wavelengths of 535 and 485, respectively.

Acknowledgments

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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.2014.08.054.

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