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Original article

Synthesis, characterization, antiparasitic and cytotoxic evaluation of thioureas conjugated to polyamine scaffolds

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ABSTRACT

A series of mono- and multimeric 4-amino-7-chloroquinoline and ferrocenyl thioureas have been prepared by the reaction of a 7-chloroquinoline methyl ester and a ferrocenylimine methyl ester with various amines. These compounds were characterized using standard spectroscopic and analytical techniques. The compounds were evaluated against the NF54 (CQ-sensitive) and Dd2 (CQ-resistant) strains of *Plasmodium falciparum*. The quinoline compounds show enhanced activity compared to the ferrocene compounds against this parasite. Compound **5** displays the most promising activity against the NF54 strain. Compounds **5** and **6** are effective at inhibiting β-hematin formation perhaps due to an increased number of quinoline moieties. The trimeric (**12**) and tetrameric (**13**) ferrocenyl compounds also inhibit β-hematin formation, albeit to a lesser degree compared to the quinoline thioureas. The compounds were also screened against the G3 strain of *Trichomonas vaginalis* and here the ferrocene-containing compounds show a slightly higher parasite growth inhibition compared to the quinoline thioureas. The quinoline compounds were also found to be more cytotoxic compared to the ferrocenyl compounds. Compound **6** displays good cytotoxicity against WHCO1 oesophageal cancer cells.

1. Introduction

Polyamines are compounds that play a vital role in all living systems. These compounds are naturally occurring agents that are found in large amounts in mammalian cells and are mainly responsible for maintaining cell viability [1]. Previous studies reveal that these systems are able to transport cytotoxic drugs into tumor cells [2]. The tumor cells import polyamines by means of a polyamine transporter (PAT) in order to sustain their growth. The transporter is able to tolerate modified polyamines, therefore drug incorporated polyamines are able to penetrate the tumor cells [3]. Although polyamine research has been aimed mainly towards the discovery of selective drugs targeting human tumor cells, these compounds have also been found to exhibit selective uptake by *Plasmodium* infected erythrocytes [4].

Malaria is an infectious disease that affects humans and is considered to be the most common parasitic disease in the world [5]. This disease is caused by protozoans of the genus *Plasmodium*. The most deadly species of the parasite is *Plasmodium falciparum* [6]. According to the World Health Organization (WHO), in 2010, 216 million cases of malaria were reported worldwide. In 2010, 655,000 cases of malaria-related deaths were documented [7]. Many treatments are available to date to combat this disease, historically the most widely used being chloroquine (CQ), (Fig. 1). This drug has been a successful treatment against this disease for many years. The onset of resistance against CQ has led to increased research towards the attainment of more potent compounds that are able to overcome drug resistance. Artemisinin-based combination therapies (ACTs) are the current treatment for this disease and involve treatment using an artemisinin derivative in conjunction with a 4-aminoquinoline or an amino alcohol, in order to delay the onset of resistance [8]. Resistance in many strains of *P. falciparum* is mainly due to mutations in the *P. falciparum*

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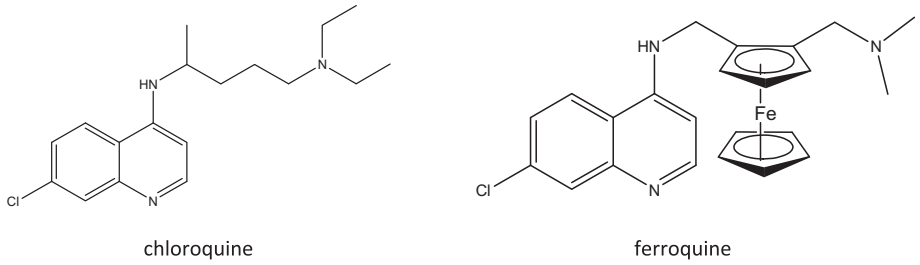


Fig. 1. CQ and FQ, potent 7-chloroquinoline compounds.

chloroquine resistant transporter (PfCRT), a protein involved in drug efflux and proton equilibrium across the membrane of the digestive vacuole. This mutation prevents accumulation of lethal concentrations of chloroquine [9,10]. It is widely accepted that many quinoline-based antimalarials disturb the detoxification of free heme, which is generated upon hemoglobin (Hb) degradation. The oxidized by-product of Hb degradation is ferriprotoporphyrin-IX (hematin), which is toxic towards the parasite. The parasite converts this compound into a less toxic form known as hemozoin (malarial pigment). CQ is believed to form a complex with heme and in turn inhibits hemozoin formation, causing a build up of toxic hematin (Fe(III)PPIX) [11–14]. Hematin is therefore believed to be the target of many quinoline antimalarials, but a study conducted by Buller et al. suggests that hemozoin may be the target. In this study they found that the fastest growing crystal face of β-hematin (synthetic hemozoin) is the binding site for quinoline-based drugs. This in turn leads to inhibition of crystal growth [15].

Thioureas are attractive for the design of antiplasmodial agents. Thiosemicarbazones are a class of thioureas that possess a wide range of biological properties including antimalarial activity [16–20]. Many thiosemicarbazones display activity against protozoans by inhibition of various cysteine proteases as well as other targets [20]. Ferrocenyl compounds have also been shown to possess activity including anticancer, antimalarial and antifungal [21]. In addition to this, thiosemicarbazones (TSCs) containing ferrocenyl moieties have been found to be active against protozoa as well as certain tumors [22,23]. More recently ferrocenyl thiosemicarbazones conjugated to a poly(propyleneimine) dendrimer have been evaluated *in vitro* and display moderate antiplasmodial activity [24]. Incorporation of the ferrocenyl moiety may alter the lipophilic nature of molecules and therefore may enhance the activity of certain compounds. Ferroquine (Fig. 1) is an excellent example of a ferrocenyl-containing molecule that exhibits potent antiplasmodial activity against CQ resistant strains of *P. falciparum* [25]. This study investigates the synthesis and characterization of polyamine–thiourea conjugates. The aims were to (i) investigate the effect of increasing the number of active pharmacophores (and molecule size) on biological activity and (ii) to evaluate whether conjugation to the various polyamines enhances the biological activity of these compounds. A preliminary evaluation of the *in vitro* antiparasitic and antitumor activity is described. In addition to this, the inhibition of β-hematin formation by these compounds is also studied.

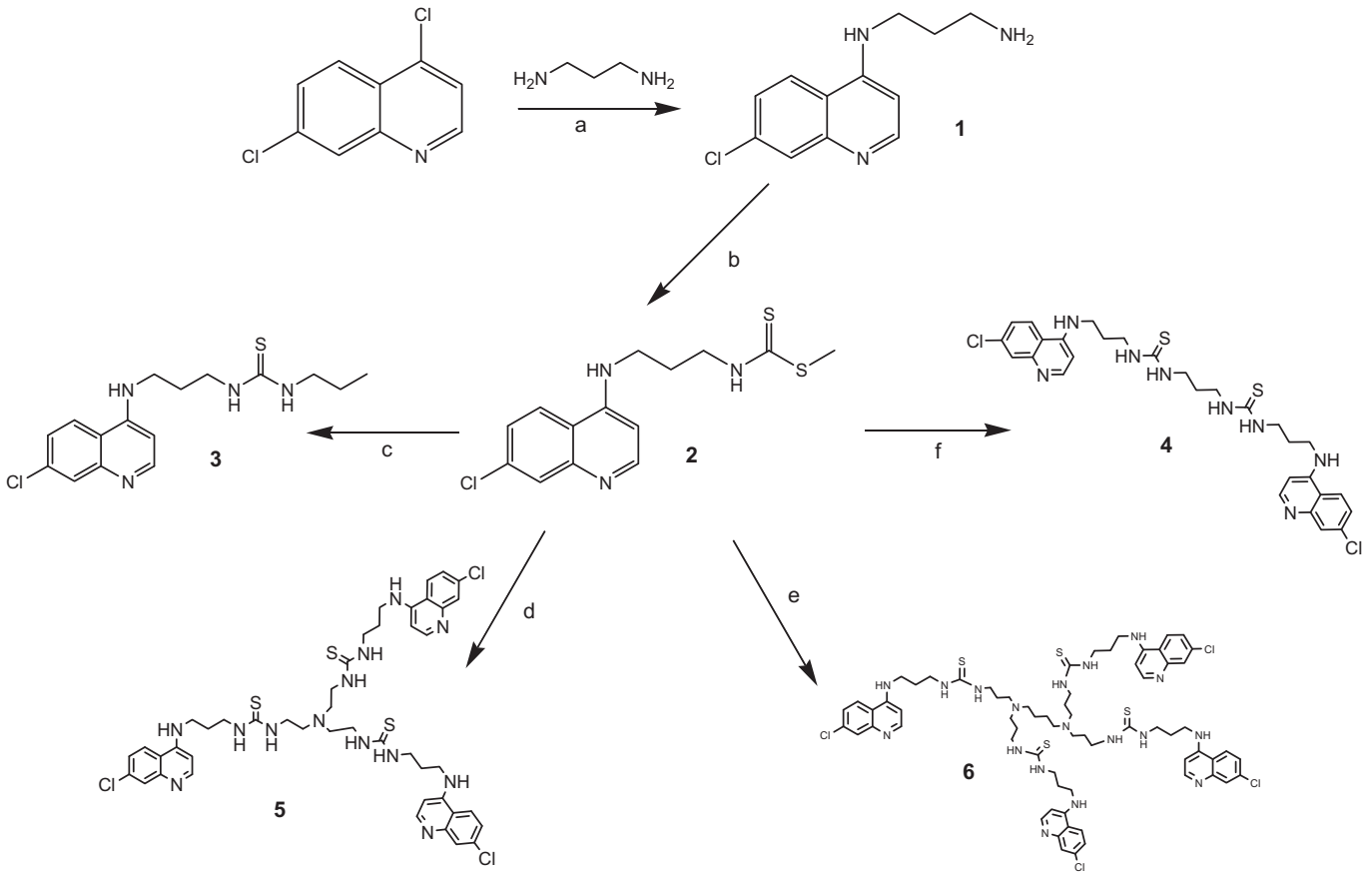
2. Results and discussion

2.1. Chemistry

Four 4-amino-7-chloroquinolines were synthesized and characterized using template reactions. These compounds were prepared as outlined in Scheme 1. *N′*-(7-Chloroquinolin-4-yl)-propane-1,3-diamine (**1**) and 3-(7-chloro-quinolin-4-ylamino)-propyl-dithiocarbamic acid methyl ester (**2**) were prepared according to literature procedures [26,27]. The first step involves reacting 4,7-dichloroquinoline and 1,3-diaminopropane to yield compound **1**. Subsequent reaction of compound **1** with carbon disulfide in the presence of KOH, followed by addition of methyl iodide drop-wise at 0 °C gives rise to compound **2**. Compound **2** was refluxed with *n*-propylamine, 1,3-diaminopropane, tris(2-amino)ethyl amine and *N*,*N*,*N′*,*N′*-tetrakis(3-aminopropyl)-1,4-butanediamine (DAB-Am-4) in MeOH to afford compounds **3**–**6**, respectively, as cream powders. Three ferrocenylimine thioureas were prepared by the procedures outlined in Scheme 2. Methyl hydrazinecarbodithioate (**7**) was prepared using the method described by Klayman et al. [16]. Subsequent reaction of compound **7** with ferrocenecarboxaldehyde in *i*-PrOH gives rise to thioester **9** [28]. Compounds **10** and **12** were prepared by refluxing compound **9** with *n*-propylamine and tris(2-amino)ethyl amine in EtOH, respectively. Compound **11** was prepared by modification of the method outlined by D.M. Wiles et al. [29]. Compound **11** was prepared by reacting ethane-1,2-dithiosemicarbazide (**8**) [30] and ferrocenecarboxaldehyde in water. The ferrocenyl compounds were isolated as orange–red powders.

2.2. Characterization

The structures of all the compounds were confirmed by ^{1}H NMR, ^{13}C{^{1}H} NMR and FTIR spectroscopy, ESI-mass spectrometry and elemental analysis. The lack of a singlet at 2.46 ppm in the ^{1}H NMR spectra of compounds **3**–**6** confirms successful displacement of the $-SCH_3$ group of compound **2** in each case. For compounds **3**–**6**, signals for the newly formed N*H* group occur at approximately 7.19 ppm. Signals for the imine protons of compounds **10**–**12** are observed as sharp singlets at 7.89, 7.90 and 7.90 ppm for **10**–**12** respectively. These shifts are upfield in comparison to the same signal observed for compound **9**. Signals for the hydrazinic protons are observed between 11.11 and 11.29 ppm. Singlets for the newly formed amino group are observed between 8.12 and 8.36 ppm. Signals for the unsubstituted Cp ring of the ferrocenyl moieties are observed as singlets at approximately 4.2 ppm for these compounds. The ^{13}C NMR spectral data displays signals for the thiocarbonyl of compounds **3**–**6** between 181.68 and 182.23 ppm, while these signals appear at 176.73–177.23 ppm for compounds **10**–**12**. The imine carbon signals appear at 143.45–143.96 ppm for the ferrocenyl derivatives. The IR spectra of compounds **3**–**6** display absorption bands for the quinoline C=N between 1611 and 1613 cm^{-1}. Absorption bands attributed to the C=N are observed between 1604 and 1611 cm^{-1} for compounds **10**–**12**. Further confirmation of the attainment of these compounds can be observed by the data obtained from ESI mass spectrometry. The mass spectra of the compounds gave peaks corresponding to $[M + H]^{+}$. Peaks corresponding to $[M + 2H]^{2+}$ and $[M + 2Na]^{2+}$ were also observed for compounds **4** and **5**, respectively.

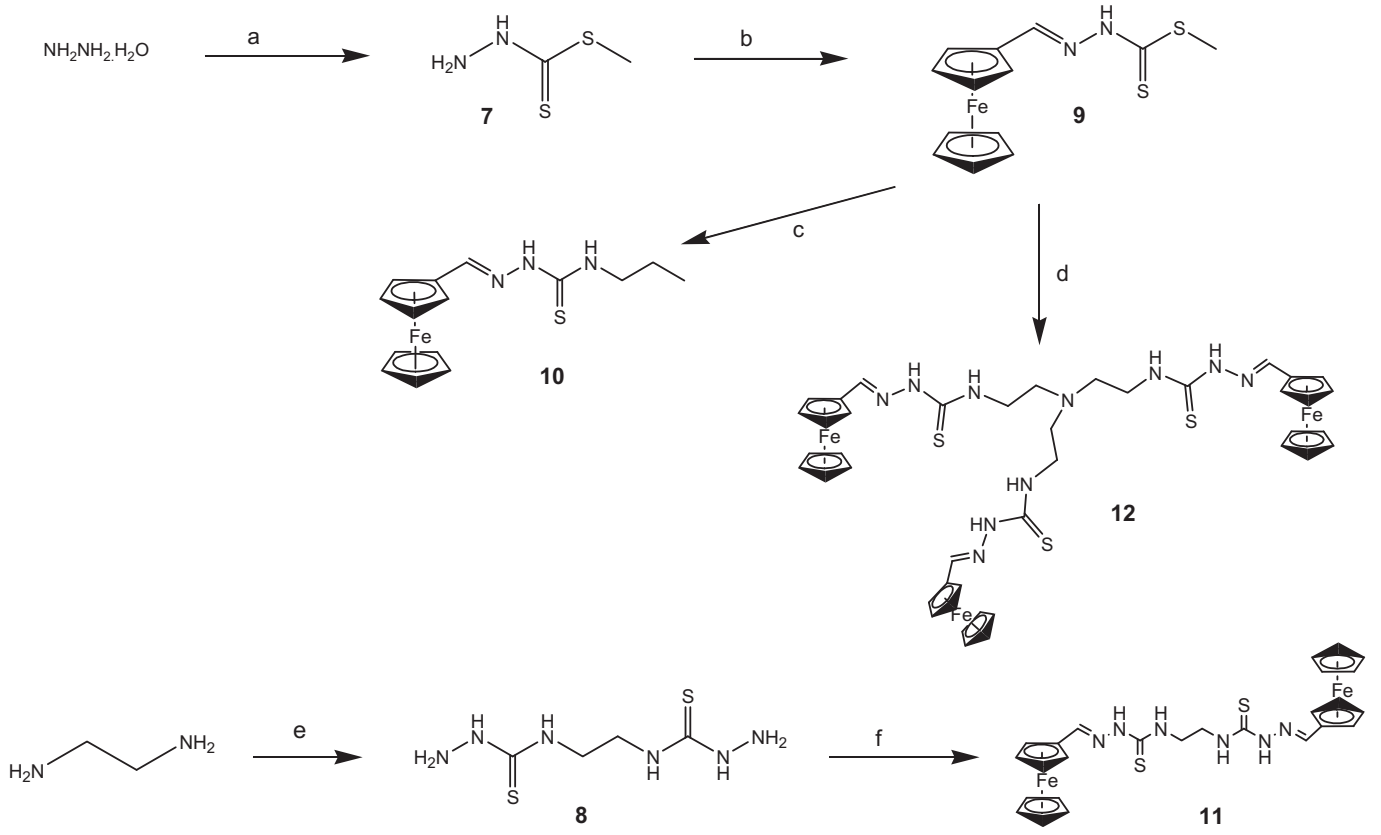


Scheme 1. Synthesis of 4-amino-7-chloroquinoline thioureas. Reagents and conditions: (a) neat; (b) KOH (1 eq.), CS_2 (1 eq.), CH_3I (1 eq.) 0–5 °C; (c) *n*-propylamine, (d) tris(2-amino)ethyl amine; (e) DAB-Am-4; (f) 1,3-diaminopropane MeOH, reflux.

2.3. *In vitro* antiplasmodial activity

Thiourea moieties are active pharmacophores that form part of many biologically active systems [31,32]. More recently, researchers have introduced thiourea moieties as part of the side chain of some 4-aminoquinoline systems. These compounds have been shown to exhibit antiplasmodial activity [26,33]. In addition to this, polyamine conjugates have attracted attention due to their pharmaceutical application [34]. These polyamines may alter the lipophilic nature of compounds which may aid in the transport of drugs across membranes, which is a specifically useful trait for antimalarial agents. In addition to this, incorporation of pharmacophores onto polyamines may result in the attainment of multifunctional systems which may show enhanced activity in comparison to monofunctional derivatives. This has been observed with ruthenium–arene complexes and ferrocenyl thiosemicarbazones conjugated to poly(propyleneimine) dendrimers [24,35,36]. Compounds **3**–**6** and **10**–**12** were evaluated for their *in vitro* antiplasmodial activity against the NF54 CQ sensitive and Dd2 CQ resistant strains of *P. falciparum*. A dose–response experiment was performed on the compounds to determine the IC_{50} or the concentration inhibiting 50% of parasite growth. The results are shown in Table 1.

Compounds **3**–**6** (quinoline derivatives) display better activity against both strains of *P. falciparum* compared to compounds **10**–**12**. This result is comparable to other compounds containing the 4-amino-7-chloroquinoline motif that have been used successfully to combat malaria. The trimeric quinoline, **5**, shows the best activity in the CQ sensitive strain, while the trimeric ferrocenyl compound (**12**), shows the lowest activity. Comparing the activity of compounds **3**, **4** and **5**, the enhanced activity of the latter may be due to incorporation of a basic tertiary amino group (not found in **3** and **4**) that may aid in accumulation of the compound in the digestive vacuole of the parasite. In the case of **6**, conjugation to the 1st generation DAB dendrimer leads to a loss of activity compared to **3** and **5**, however this particular compound is more active than **4**. The slightly lower activity of compound **6**, which also contain tertiary amines, may be attributed to its lipophilicity. Predicted log *P* and log *D* values are shown in Table 1. In the sensitive strain, **5** shows the best activity, suggesting sufficient vacuolar accumulation of this compound which may be a consequence of the log *D* at pH 7.4. Compounds **3** and **6** exhibit similar activity in the sensitive strain but are less active than **5**. This suggests less accumulation which may be a consequence of the lower log $D_{7.4}$ values compared to the rest of the compounds. Compound **4** has a higher log $D_{7.4}$ value than **3** and **6** but appears to accumulate less based on the larger IC_{50} value. The estimated log *P* value of compound **6** indicates it is more lipophilic than the other members of this series (when uncharged) and this factor may hinder its transport as it may be trapped in the lipid membrane and may not accumulate sufficiently inside of the digestive vacuole [38] which may be the reason for the low activity in the resistant strain. The mono- and trimeric ferrocenyl compounds (**10** and **12**) exhibited the lowest activity at the tested concentration, while the dimeric ferrocenyl thiourea (**11**) displayed moderate activity in the CQ sensitive strain. This particular compound displays a log *P* value comparable to chloroquine. The low activity of **12** in the sensitive strain may be a combination of its lipophilic character and the lack of the active quinoline



Scheme 2. Synthesis of ferrocenyl thioureas. Reagents and conditions: (a) KOH (1 eq.), CS_2 (1 eq.), CH_3I (1 eq.) 0–5 °C; (b) ferrocenecarboxaldehyde *i*-PrOH, reflux; (c) *n*-propylamine EtOH reflux; (d) tris(2-amino)ethyl amine EtOH reflux; (e) NaOH, CS_2, $NH_2NH_2{\cdot}H_2O$; (f) ferrocenecarboxaldehyde H_2O r.t.

moiety. The ferrocenyl precursor (**9**), was also tested, but was found to be inactive (not shown). This suggests functionalization with the various amines leads to enhanced activity.

Lipophilicity appears to have little effect on the activity of these compounds in the resistant strain. Compounds **3** and **4** show comparable activity, while compounds **5** and **6** exhibit similar activity in the Dd2 strain. Compounds **3**, **4** and **10** show similar activity in both the sensitive and resistant strains, while compounds **5** and **6** show slightly lower activity in the resistant strain compared to NF54. The activity of compound **11** decreased significantly against Dd2 compared to NF54 which is confirmed by the large Resistance Index (RI) of 27.7. The RI is valuable for the analysis of the potential of compounds as antimalarial drugs. RI values are defined as the quotient of the IC_{50} value obtained in the resistant strain and the IC_{50} value obtained in the sensitive strain. Smaller RI values (<1) indicate that the compound is more active in the resistant strain than in the sensitive strain, while larger values (>1) indicate the opposite. Ideally, the compound should exhibit similar activity in both strains. The activity of compound **12** increased significantly; this particular compound gave an RI of 0.1. In most cases, with the exception of compound **11**, the compounds show a lower RI compared to CQ.

Table 1
In vitro antiplasmodial activity for thioureas **3**–**6** and **10**–**12** against NF54 and Dd2 strains of *P. falciparum*. Selected log *P* and log *D* values are also given.

Compound	IC_{50} (μM) ± SE[a] (NF54)[b]	IC_{50} (μM) ± SE (Dd2)[c]	RI[d]	Log *P*[e]	Log $D_{7.4}$	Log $D_{4.8}$
3	0.16 ± 0.03	0.26 ± 0.01	1.6	2.95	2.71	1.63
4	0.34 ± 0.03 (n = 6)[f]	0.23 ± 0.01	0.7	4.03	3.54	1.40
5	0.09 ± 0.01	0.52 ± 0.06	5.8	5.88	5.02	−0.22
6	0.20 ± 0.01	0.51 ± 0.03	2.5	8.62	2.78	−3.62
10	15.79 ± 1.28	16.22 ± 1.59	1.0	2.99[g]	–	–
11	1.15 ± 0.07	31.88 ± 5.89	27.7	4.78	–	–
12	71.71 ± 9.93	5.63 ± 0.19	0.1	7.66	–	–
CQ	0.02 ± 0.01	0.14 ± 0.01	7	4.63[h]	–	–

[a] IC_{50} represents the micromolar equivalents of test compounds required to inhibit parasite growth by 50%.
[b] NF54 chloroquine-sensitive strain of *P. falciparum*.
[c] Dd2 chloroquine-resistant strain of *P. falciparum*.
[d] Resistance index (RI) = IC_{50}Dd2/IC_{50}NF54.
[e] Log *P* and log *D* values calculated using MarvinSketch V5.9.4.
[f] n = number of data sets averaged, n = 3 (for NF54) and n = 2 (for Dd2), exceptions are specified.
[g] Log *P* of ferrocenyl compounds estimated using methods described in the Supporting information.
[h] Literature log *P* value [37].

2.4. β-Hematin inhibition study

In order to design new antimalarials, the ability of the compounds to inhibit heme aggregation should also be considered (Table 2). The ability of these compounds to inhibit β-hematin (synthetic hemozoin) formation was investigated using an NP-40 detergent mediated assay [39]. In addition to the above-mentioned compounds, a tetrameric ferrocenyl derivative (**13**) shown in Fig. 2, was also evaluated. Compound **13** showed activity against the W2 CQ-resistant strain of *P. falciparum* in a previous study [24]. Compounds **12** and **13** appear to inhibit β-hematin formation giving IC_{50} values of 30.70 and 15.20 μM, respectively. The quinoline compounds inhibit β-hematin formation better than the ferrocenyl thioureas, specifically compound **6** (IC_{50} < 4.5 μM). This may be attributed to an increased number of planar aromatic

Table 2
IC_{50} (μM)[a] values obtained for compounds **3**–**6** and **10**–**13** using an NP-40 detergent mediated β-hematin inhibition assay.[b]

Compound	IC_{50} (μM)[a] ± SE
3	58.17 ± 1.01
4	6.52 ± 1.06
5	4.48 ± 1.04
6	<4.5
10	Inactive
11	Inactive
12	30.70 ± 1.06
13	15.20 ± 1.03
CQ	73.76 ± 1.02

n = number of data sets averaged, $n = 4$.
[a] IC_{50} represents the micromolar equivalents of test compounds required to inhibit β-hematin formation by 50%.
[b] Sigmoidal dose–response curves (variable slope) were plotted using GraphPad Prism V. 5.

Table 3
Percentage parasite growth inhibition (%) obtained for **3**–**6** and **9**–**13** against *T. vaginalis*.

Compound	% Inhibition ± SE
3	34.9 ± 11
4	55.9 ± 9
5	51.4 ± 11
6	58.7 ± 9
9	43.1 ± 9
10	46.2 ± 10
11	59.6 ± 11
12	53.2 ± 10
13	61.5 ± 11
Metronidazole	100

moieties that are able to interact with heme and thus preventing biocrystallization into β-hematin [13]. There appears to be a relationship between the size of these molecules and their ability to inhibit heme aggregation. The larger, tetrameric molecules are able to inhibit β-hematin formation to a greater extent than the monomeric derivatives. No clear correlation is observed between β-hematin inhibition and the *in vitro* antiplasmodial activity. This is probably due to differences in accumulation of these compounds inside the parasite.

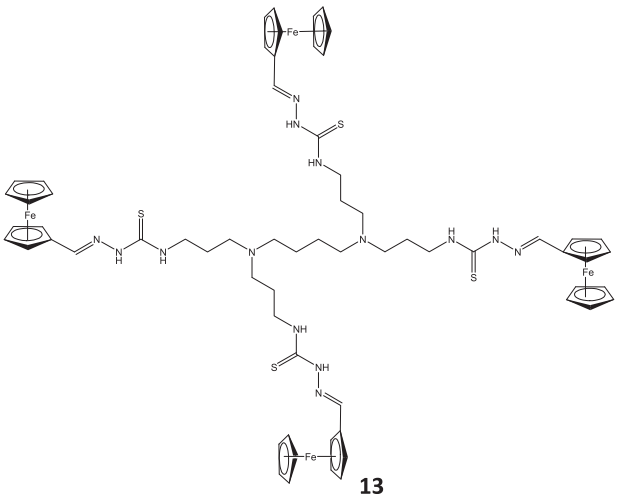
2.5. *T. vaginalis* growth inhibition

Trichomonas vaginalis (*T. vaginalis*) is a protozoan responsible for the sexually transmitted disease trichomoniasis. The current FDA-approved drug, metronidazole (Fig. 2), currently experiences resistance in some patients [40,41]. There has recently been an interest in obtaining a suitable alternative treatment for this infection [41–44]. We have conducted a preliminary study to evaluate the activity of our synthesized compounds against this particular parasite. The compounds were screened against the G3 strain of *T. vaginalis* at a drug concentration of 10 μM. Table 3 shows the data obtained from this screen.

In the case of the quinoline compounds, the monomeric derivative **3** (functionalised with *n*-propylamine) showed the lowest percentage growth inhibition. The tetrameric compound **6**, which was functionalised with DAB-Am-4, showed the best inhibition of the series, followed by compound **4** (functionalized with 1,3-diaminopropane) and then compound **5** (functionalized with tris(2-aminoethyl)amine). A similar trend is observed for the ferrocenyl compounds. The tetrameric compound (**13**) shows the highest percentage inhibition, followed by the dimeric and then the trimeric derivative. The monomeric compounds (**9** and **10**) display the lowest inhibition of the series. Overall, the largest compounds show the highest inhibition. On average the ferrocenyl compounds inhibit parasite growth slightly better than the quinoline compounds. The lipophilic nature of the ferrocene moiety may be a factor contributing to the slightly enhanced activity of the organometallic derivatives. Although many of these compounds are able to inhibit more than 50% of parasite growth, the activity is not comparable to metronidazole.

2.6. *In vitro* cytotoxicity

The *in vitro* antitumor activity of the compounds was also evaluated against WHCO1 oesophageal cancer. This was to elucidate information regarding the selectivity of these compounds. A promising antimalarial candidate should ideally exhibit good antiplasmodial activity, but low cytotoxicity. It has been found that both quinoline and ferrocene containing compounds have been studied as anticancer agents [45,46]. Some ferrocene compounds have been found to be effective against certain cancers [46]. The compounds were evaluated for their antitumor activity using a (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay. With the exception of compounds **11** and **12**, the rest



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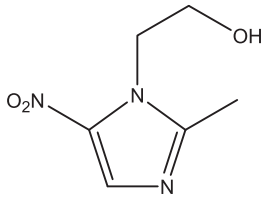

metronidazole

Fig. 2. Tetrameric ferrocenyl thiourea (**13**) and metronidazole, the current treatment for *T. vaginalis*.

Table 4
IC_{50} values obtained for **3**–**6** and **10**–**12** against WHCO1 oesophageal cancer cells.[a]

Compound	IC_{50} (μM) ± SE
3	43.75 ± 1.09
4	38.35 ± 1.12
5	6.21 ± 1.09
6	0.99 ± 1.05
10	55.73 ± 1.28
11	Inactive
12	Inactive
Cisplatin	13.00[b]

n = number of data sets averaged, $n = 3$.
[a] Values were determined from a dose response curve (assayed with MTT), IC_{50} represents the micromolar equivalents of test compounds required to inhibit 50% cell viability.
[b] IC_{50} value obtained for cisplatin against WHCO1 cancer cells [47].

of the compounds appeared to cause decreased cell viability at the tested concentration. Nevertheless, some trends with regards to the antitumor activity are observed and the IC_{50} values are shown in Table 4.

The quinoline compounds show enhanced activity compared to the ferrocenyl thioureas. Compound **6** exhibits promising activity compared to the rest of the series. There appears to be an increase in activity when going from the monomeric quinoline derivative to the tetrameric compound. This phenomenon is also observed for certain dendritic compounds whereby cytotoxicity is often generation-dependant, with higher generation dendrimers showing the best activity [35,36,48]. Studies carried out on naturally occurring polyamines show that these systems are able to transport cytotoxic drugs into tumor cells [2]. In our case, for the synthetic polyamines are under investigation, it has been shown that the polyamine transporter is able to tolerate modified polyamines therefore this compound may be taken up by the cell [3]. This factor in combination with the ability of these quinolines to interact with DNA, possibly by intercalation, may be a reason for the enhanced activity [49]. The activity of the compounds was also compared to cisplatin, a potent metal-containing anticancer agent, compounds **5** and **6** exhibit better activity than this drug. Compound **10** exhibits low cytotoxicity in this cell-line, while compounds **11** and **12** were not active at the concentration tested.

3. Conclusions

A series of 4-amino-7-chloroquinoline (**3**–**6**) and ferrocenyl (**10**–**12**) thioureas have been prepared. These compounds were characterized using standard spectroscopic and spectrometric techniques, confirming the integrity of these molecules. The compounds were evaluated for their *in vitro* antiplasmodial activity against the NF54 CQ sensitive and Dd2 CQ resistant strain of *P. falciparum*, *in vitro* antitumor activity against WHCO1 oesophageal cancer cells and *in vitro* inhibition of *T. vaginalis* growth. The β-hematin inhibition activity of these compounds was also evaluated. The quinoline compounds **3**–**6** display moderate to good activity against both strains of *P. falciparum*, although not as good as chloroquine. There does not appear to be a clear correlation between increasing the number of active moieties and antiplasmodial activity. Various other factors including vacuolar accumulation appear to be a contributing factor. In the case of the ferrocenyl thioureas, functionalization with the various amines does lead to enhanced activity compared to the methyl ester precursor. With regard to cytotoxicity, the monomeric thiourea shows the lowest activity while compound **6**, the tetrameric compound shows promising cytotoxic behavior, better than cisplatin against this particular cell-line. There appears to be a relationship between the size of the molecule and cytotoxicity in the case of the quinoline derivatives. The ferrocenyl thioureas show lower antitumor activity compared to the quinolines. In the case of *T. vaginalis* growth, it appears that the ferrocenyl compounds show slightly enhanced activity compared to the quinolines although not comparable to metronidazole. All of the quinoline compounds were able to inhibit β-hematin formation, but the tetrameric derivative (**6**) displays the best activity which is of interest for further investigation. A size dependant-activity was observed for all compounds for this assay. No clear correlation between the β-hematin inhibition activity and the antiplasmodial activity was observed. However, the data does suggest that 4-amino-7-chloroquinolines remain a potent and important class of compounds towards malaria.

4. Experimental

4.1. General

All reagents and solvents were obtained from commercial sources (Sigma–Aldrich, Merck, and Kimix) and, unless otherwise stated, were used as received. *N′*-(7-Chloroquinolin-4-yl)-propane-1,3-diamine (**1**) [24] and 3-(7-chloro-quinolin-4-ylamino)-propyl-dithiocarbamic acid methyl ester (**2**) [26], ethane-1,2-dithiosemicarbazide (**8**) [27], ferrocenylthioester (**9**) [28] and the tetrameric thiourea (**13**) [30] were prepared by modification of literature procedures. Nuclear magnetic resonance (NMR) spectra were recorded using a Varian Mercury 300 spectrometer (^{1}H at 300.077 MHz, ^{13}C at 75.454 MHz), a Varian Unity 400 spectrometer (^{1}H at 399.953 MHz, ^{13}C at 100.577 MHz) or a Bruker 400 FT spectrometer (^{1}H at 400.200 MHz, ^{13}C at 100.600 MHz). Coupling constants are reported in Hz. Infrared (IR) spectra were determined using a Perkin Elmer Spectrum 100 FT-IR spectrometer and was carried out in the solid state using KBr pellets. Electrospray Ionization (ESI) mass spectrometry was used to further characterize compounds and determinations were carried out using a Waters API Quattro instrument in the positive mode. Elemental analyses of these compounds were performed using a Thermo Flash 1112 Series CHNS-O Analyser.

4.2. Synthetic methods and characterization

4.2.1. Compound *3*

Compound **2** (0.354 g, 1.09 mmol) was dissolved in MeOH (20 ml), to this *n*-propylamine (0.108 g, 1.82 mmol) was added drop-wise with stirring. The solution was refluxed for 24 h and cooled to room temperature. The solvent was removed *in vacuo* and diethyl ether added to the residue. The mixture was washed with diethyl ether and the supernatant decanted. This was repeated until the supernatant remained clear. The oily residue was dried under vacuum giving rise to a cream solid. Yield: 0.220 g, 60%; IR (cm^{-1}): 3435 (N–H); 1613 (C=N); 1136 (C=S); ^{1}H NMR (DMSO-d_6): δ ppm 0.86 (3H, t, $^3J_{H-H} = 7.4$, CH_3); 1.49 (2H, m, CH_2); 1.90 (2H, m, CH_2); 3.30 (4H, m, CH_2); 3.52 (2H, m, CH_2); 6.47 (1H, d, $^3J_{H-H} = 5.4$, Ar–H); 7.19 (1H, br s, NH); 7.35 (2H, m, NH); 7.42 (1H, dd, $^3J_{H-H} = 8.9$, $^4J_{H-H} = 2.2$, Ar–H); 7.80 (1H, d, $^4J_{H-H} = 2.2$, Ar–H); 8.24 (1H, d, $^3J_{H-H} = 9.0$, Ar–H); 8.40 (1H, d, $^3J_{H-H} = 5.4$, Ar–H); ^{13}C NMR (DMSO-d_6): δ ppm 11.1; 21.8; 27.6; 40.3; 41.1; 45.1; 98.5; 117.4; 123.8 (2C); 127.4; 133.2; 148.9; 149.9; 151.7; 181.99. ESI MS: m/z 337.1248 (100%, $[M + H]^+$). $C_{16}H_{21}N_4SCl \cdot 1.5H_2O$ calculated: C 52.81, H 6.65, N 15.40%; found: C 53.06; H 6.96; N 16.21%.

By following the same synthetic procedure as that for **3**, the following compounds were synthesized, with the exception that these compounds were washed repeatedly with acetone (until supernatant remained clear) followed by diethyl ether.

4.2.2. Compound **4**

Compound **4** was prepared from **2** (1.49 g, 4.56 mmol) and 1,3-propanediamine (0.117 g, 1.56 mmol). Yield: 0.818 g, 85%; IR (cm^{-1}): 3258 (N–H); 1613 (C=N); 1138 (C=S); ^{1}H NMR (DMSO-d_6): δ ppm 1.73 (2H, m, CH_2); 1.90 (4H, m, CH_2); 3.30 (4H, m, CH_2); 3.40 (4H, m, CH_2); 3.51 (4H, m, CH_2); 6.46 (2H, d, $^3J_{H-H}$ = 5.4, Ar–H); 7.19 (2H, m, NH); 7.37–7.47 (6H, m, NH, Ar–H); 7.77 (2H, d, $^4J_{H-H}$ = 2.1, Ar–H); 8.24 (2H, d, $^3J_{H-H}$ = 9.0, Ar–H); 8.40 (2H, d, $^3J_{H-H}$ = 5.4, Ar–H); ^{13}C NMR (DMSO-d_6): δ ppm 27.4; 28.5; 40.9–40.9 (6C); 98.4; 117.2; 123.7; 123.9; 127.0; 133.3; 148.7; 149.9; 151.6; 181.7; ESI MS: *m/z* 629.1812 (20%, $[M + H]^+$); 315.0951 (100%, $[M + 2H]^{2+}$). $C_{29}H_{34}N_8S_2Cl_2 \cdot 2H_2O$ calculated: C 52.32, H 5.75, N 16.83%; found: C 52.51; H 5.95; N 15.13%.

4.2.3. Compound **5**

Compound **5** was prepared from Compound **2** (0.415 g, 1.27 mmol) and tris(2-aminoethyl)amine (0.0586 g, 0.400 mmol). Yield: 0.242 g, 58%. IR (cm^{-1}): 3280 (N–H); 1611 (C=N); 1138 (C=S). ^{1}H NMR (CD_3OD-d_4): δ ppm 1.96 (6H, m, CH_2); 2.66 (12H, m, CH_2); 3.44–3.74 (12H, m, CH_2); 6.44 (3H, m, Ar–H); 7.31 (3H, m, Ar–H); 7.71 (3H, m, Ar–H); 7.99 (3H, m, Ar–H); 8.28 (3H, m, Ar–H). ^{13}C NMR (DMSO-d_6): δ ppm 27.5; 41.1; 41.6; 52.9; 54.4; 98.5; 117.3; 123.8 (6C); 127.3; 133.1; 148.9; 149.8; 151.6; 182.2. ESI-MS: *m/z* 980 (5%, $[M + H]^+$); 513 (60%, $[M + 2Na]^{2+}$). $C_{45}H_{54}N_{13}S_3Cl_3 \cdot 3H_2O$ calculated: C 52.29, H 5.85, N 17.62%; found: C 52.00; H 6.38; N 16.60%.

4.2.4. Compound **6**

Compound **6** was prepared from compound **2** (0.888 g, 2.725 mmol) and G1 DAB dendrimer (0.164 g, 0.518 mmol). Yield: 0.092 g, 12%; IR (cm^{-1}): 3272 (N–H); 1611 (C=N); 1138 (C=S). ^{1}H NMR (DMSO-d_6): δ ppm 1.32 (4H, m, CH_2); 1.59 (8H, m, CH_2); 1.89 (8H, m, CH_2); 2.35 (12H, m, CH_2); 3.21–3.45 (16H, m, CH_2); 3.50 (8H, m, CH_2); 6.44 (4H, br d, $^3J_{H-H}$ = 5.4, Ar–H); 7.19 (4H, br s, NH); 7.41 (12H, m, NH, Ar–H); 7.77 (4H, d, $^4J_{H-H}$ = 2.2, Ar–H); 8.25 (4H, m, Ar–H); 8.38 (4H, d, $^3J_{H-H}$ = 5.3, Ar–H); ^{13}C NMR (DMSO-d_6): δ ppm 24.7; 26.8; 28.1; 41.4–42.9 (12C); 51.6; 53.8; 99.1; 117.9; 124.5 (8C); 127.9; 133.8; 149.5; 150.4; 152.3; 182.3; ESI MS: *m/z* 1429 (70%, $[M + H]^+$); $C_{68}H_{88}N_{18}S_4Cl_4 \cdot 5H_2O$ calculated: C 53.81, H 6.51, N 16.61%; found: C 53.90; H 6.42; N 15.02%.

4.2.5. Compound **10**

Compound **9** (0.197 g, 0.618 mmol) was suspended in EtOH (20 ml). *n*-Propylamine (0.0430 g, 0.730 mmol) was added to the suspension and the resulting solution was refluxed under Argon for 12 h. The solvent was removed *in vacuo* and the residue dissolved in DCM (15 ml). The organic layer was washed with water until the water layer remained clear. The organic extracts were dried over $NaSO_4$. The solvent was removed *in vacuo*, to the residue diethyl ether and petroleum ether was added and the product was allowed to precipitate. Yield: 0.062 g, 30%. IR (cm^{-1}): 3371 (N–H); 3129 (N–H); 1604 (C=N); 816 (C=S); 1090 (ferrocene). ^{1}H NMR (DMSO-d_6): δ ppm 0.88 (3H, t, $^3J_{H-H}$ = 7.5, CH_3); 1.59 (2H, m, CH_2); 3.49 (2H, m, CH_2); 4.21 (5H, s, Cp); 4.42 (2H, m Cp); 4.72 (2H, m, Cp); 7.89 (1H, s, HC=N); 8.12 (1H, br s, NH); 11.11 (1H, s, NH); ^{13}C NMR (DMSO-d_6): δ ppm 11.7; 22.7; 45.5; 68.0; 69.4; 70.4; 79.6; 143.4; 176.8. ESI-MS: *m/z* 330.0724 (100%, $[M + H]^+$). $C_{15}H_{19}N_3FeS$ calculated: C 54.72, H 5.82, N 12.76%; found: C 53.99; H 5.73; N 10.84%.

By following the same synthetic procedure as that for **10**, compound **12** was synthesized.

4.2.6. Compound **11**

Compound **11** was prepared from ethane-1,2-dithiosemicarbazide (0.160 g, 0.767 mmol) which was suspended in H_2O (25 ml). To this, a few drops of 10.1 M HCl was added. Ferrocenecarboxaldehyde (0.331 g, 1.55 mmol) was added to the suspension and the reaction mixture stirred for 24 h at room temperature. The precipitate was filtered, washed with water and dried *in vacuo*. Yield: 0.453 g, 98%. IR (cm^{-1}): 3437 (N–H); 3267 (N–H); 1611 (C=N); 816 (C=S). ^{1}H NMR (DMSO-d_6): δ ppm 3.78 (4H, s, CH_2); 4.17 (10H, s, Cp); 4.39 (4H, t, $^3J_{H-H}$ = 1.8, Cp); 4.74 (4H, t, $^3J_{H-H}$ = 1.8, Cp); 7.90 (2H, s, HC=N); 8.36 (2H, br s, NH); 11.29 (2H, s, NH) ppm. ^{13}C NMR (DMSO-d_6): δ ppm 44.0; 68.2; 69.4; 70.5; 79.4; 143.9; 177.2. ESI MS: *m/z* 601.0582 (100%, $[M + H]^+$). $C_{26}H_{28}N_6Fe_2S_2 \cdot H_2O$ calculated: C 50.50, H 4.89, N 13.59%; found: C 50.67, H 4.71, N 13.85%.

4.2.7. Compound **12**

Compound **12** was prepared from compound **9** (0.506 g, 1.59 mmol) and tris(2-aminoethyl)amine (0.0780 g, 0.534 mmol). Yield: 0.226 g, 46%. IR (cm^{-1}): 3431 (N–H); 3366 (N–H); 1606 (C=N); 818 (C=S); 1103 (ferrocene). ^{1}H NMR (DMSO-d_6): δ ppm 2.83 (6H, m, CH_2); 3.71 (6H, m, CH_2); 4.18 (15H, s, Cp); 4.41 (6H, m, Cp); 4.69 (6H, m, Cp); 7.90 (3H, s, HC=N); 8.15 (3H, br s, NH); 11.22 (3H, s, NH) ppm. ^{13}C NMR (DMSO-d_6): δ ppm 43.4; 53.3; 68.0; 69.4; 70.5; 79.4; 143.7; 176.7. ESI-MS: *m/z* 957.1362 (70%, $[M + H]^+$). $C_{42}H_{48}N_{10}Fe_3S_3$ calculated: C 52.73, H 5.06, N 14.64%; found: C 52.63, H 5.54, N 13.98%.

4.3. Biological and biophysical assays

4.3.1. In vitro antiplasmodial assay

The test samples were tested in triplicate on one occasion against chloroquine sensitive (CQS) NF54 strain and chloroquine-resistant (CQR) Dd2 strain of *P. falciparum*. Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen [50]. Quantitative assessment of antiplasmodial activity *in vitro* was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler et al. [51]. The test samples were prepared as a 20 mg/ml stock solution in 100% DMSO and sonicated to enhance solubility. Samples were tested as a suspension if not completely dissolved. Stock solutions were stored at −20 °C. Further dilutions were prepared on the day of the experiment. Chloroquine (CQ) was used as the reference drug in all experiments. A full dose–response measurement was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC_{50} value). Test samples were tested at a starting concentration of 1000 ng/ml, which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 2 ng/ml. The same dilution technique was used for all samples. The highest concentration of solvent to which the parasites were exposed to had no measurable effect on the parasite viability. The IC_{50} values were obtained using a non-linear dose–response curve fitting analysis via GraphPad Prism v.4.0 software. Evaluation is based on means from three independent experiments.

4.3.2. β-Hematin inhibition assay

The β-hematin formation assay was adapted from the method described by Wright and co-workers [39]. Test compounds were prepared as a 10 mM stock solution in 100% DMSO. Test samples were tested at a starting concentration of 500 μM and the lowest drug concentration being 5 μM. The stock solution was serially diluted to give 12 concentrations in a 96 well flat-bottom assay plate. NP-40 detergent was then added to mediate the formation of β-hematin (30.55 μM, final concentration). A 25 mM stock solution of hematin was prepared by dissolving hemin (16.3 mg) in dimethyl sulfoxide (DMSO) (1 ml). A 177.76 μl aliquot of hematin stock was suspended in 20 ml of a 2 M acetate buffer, pH 4.7. The hematin

suspension was then added to the plate to give a final hematin concentration of 100 μM. The plate was then incubated overnight at 37 °C. The assay was analyzed using the pyridine-ferrochrome method developed by Ncokazi and Egan [52]. 32 μl of a solution of 50% pyridine, 20% acetone, 20% water, and 10% 2 M HEPES buffer (pH 7.4) was added to each well. To this, 60 μl acetone was then added to each well and mixed. The absorbance of the resulting complex was measured at 405 nm on a SpectraMax 340PC plate reader. The IC_{50} values were obtained using a non-linear dose–response curve fitting analysis via GraphPad Prism v.5.0 software.

4.3.3. In vitro T. vaginalis assay

Cultures of *T. vaginalis* G3 strain were grown in 5 ml complete TYM Diamond's media in a 37 °C incubator for 24 h. 10 mM stocks of the compounds were made by dissolving in DMSO and were screened against G3 stain of *T. vaginalis*. Cells untreated and inoculated with 5 μL DMSO are used as controls. 5 μl of 10 mM stocks of compound library were inoculated for a final concentration of 10 μM. Results were calculated based on counts utilizing a hemocytometer after 24 h.

4.3.4. In vitro antitumor (MTT) assay

The oesophageal cancer cell line WHCO1, derived from a primary oesophageal squamous cell carcinoma, was provided by Professor Rob Veale (University of the Witwatersrand, Johannesburg, South Africa). IC_{50} determinations were carried out using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. 3000 cells were seeded per well in 96-well plates. Cells were incubated at 37 °C under 5% CO_2 (24 h), after which aqueous DMSO solutions of each compound (10 μl, with a constant final concentration of DMSO of 0.2%) were plated at various concentrations. After 48 h incubation, observations were made, and MTT (10 μl) solution added to each well. After 4 h of incubation, solubilization solution (100 μl) was added to each well, and incubated overnight. Plates were read at 595 nm on a BioTek microplate reader, and IC_{50} values calculated using GraphPad Prism 4.00 Package of GraphPad Software, San Diego, USA.

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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.2013.08.004.

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