In Vitro Antimalarial Activity of a Series of Cationic 2,2′-Bipyridyl- and 1,10-Phenanthrolineplatinum(II) Benzoylthiourea Complexes

Timothy J. Egan,*,† Klaus R. Koch,*,§ Paul L. Swan,† Cailean Clarkson,# Donelly A. Van Schalkwyk,# and Peter J. Smith#

Department of Chemistry, Division of Pharmacology, Department of Medicine, University of Cape Town, Private Bag, Rondebosch 7701, South Africa, and Department of Chemistry, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa

Received December 8, 2003

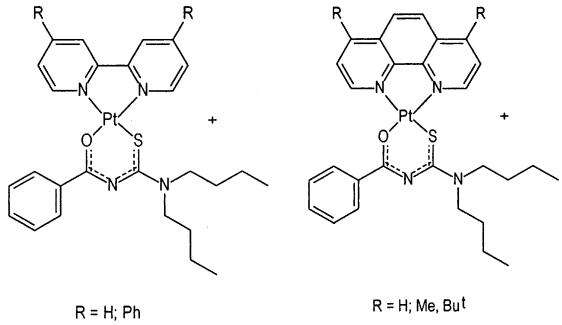
We have synthesized a series of novel 2,2′-bipyridyl and 1,10-phenanthroline benzoylthiourea complexes of platinum(II) with various substituents on the bipyridyl and phenanthroline ligands. All of these square-planar mixed-ligand cationic complexes were found to form moderately strong complexes with ferriprotoporphyrin IX in 40% aqueous DMSO (log *K* ranging from 4.81 to 6.24). The complexes also all inhibit *β*-hematin (synthetic hemozoin or malaria pigment) formation in acetate solution. Four of the compounds were found to exhibit in vitro antimalarial activity, with (*N*-benzoyl-*N*′,*N*′-di(2-hydroxyethyl)thioureato)(4,4′-di-*tert*-butyl-2,2′-bipyridyl)platinum(II) chloride being particularly active. These active complexes exhibited equally strong activity against both the D10 chloroquine sensitive and K1 chloroquine resistant strains of malaria parasite. Cytotoxicity testing of the four most active compounds shows that they exhibit selective activity against malaria parasites with selectivity indices greater than 85. These compounds represent a new family of potential antimalarials.

Introduction

There is now extensive evidence that quinoline antimalarials such as chloroquine form complexes with ferriprotoporphyrin IX (Fe(III)PPIX) in solution[1−4] and that they are capable of specifically inhibiting the incorporation of Fe(III)PPIX into *β*-hematin (synthetic hemozoin or malaria pigment).[4−7] In the malaria parasite, hemozoin formation is a biomineralization-like process[8,9] by which almost all of the heme released in the parasite food vacuole as a result of enzymatic degradation of hemoglobin is detoxified.[10]

A number of recent studies have provided evidence for a quantitative relationship between the strength of inhibition of *β*-hematin formation and antiparasitic activity against the malarial parasite *Plasmodium falciparum* in culture.[4,11−14] In addition, a number of studies have shown that accumulation of antimalarials in the parasite is of direct importance for their biological activity[15−19] and that interaction with Fe(III)PPIX may play a central role in this process.[20−22] We have previously reported the results of two detailed studies on structure−activity relationships in aminoquinoline antiplasmodials.[13,14] On the basis of these studies, we have hypothesized the following requirements for this type of activity against the malaria parasite: (i) accumulation in the food vacuole of the parasite through pH trapping, (ii) association of the quinoline with Fe(III)-PPIX via coplanar stacking of the quinoline and porphyrin rings, (iii) inhibition of hemozoin formation as a result of this interaction, and (iv) resulting death of the parasite as a result of the build-up of toxic hematin (H_2O−Fe(III)PPIX).[14] This hypothesis suggests a strategy for the discovery of novel antimalarials, namely, searching for suitable compounds that form complexes with Fe(III)PPIX and that block *β*-hematin formation. Such compounds can then be investigated for any inherent antiplasmodial activity and subsequently chemically modified to enhance accumulation in the food vacuole through the introduction of basic amine groups to improve their activity. This approach may lead to new non-quinoline antimalarials that avoid cross-resistance with chloroquine.

Chart 1. Two Classes of Previously Reported[23] Mixed-Ligand Cationic *N*-Benzoyl-*N*′,*N*′-di(*n*-butylthioureato-*S*,*O*)platinum(II) Complexes with 2,2′-Bipyridyl and 1,10-Phenanthroline, Which Show Pronounced Self-Association Properties in Acetonitrile Solution at Room Temperature

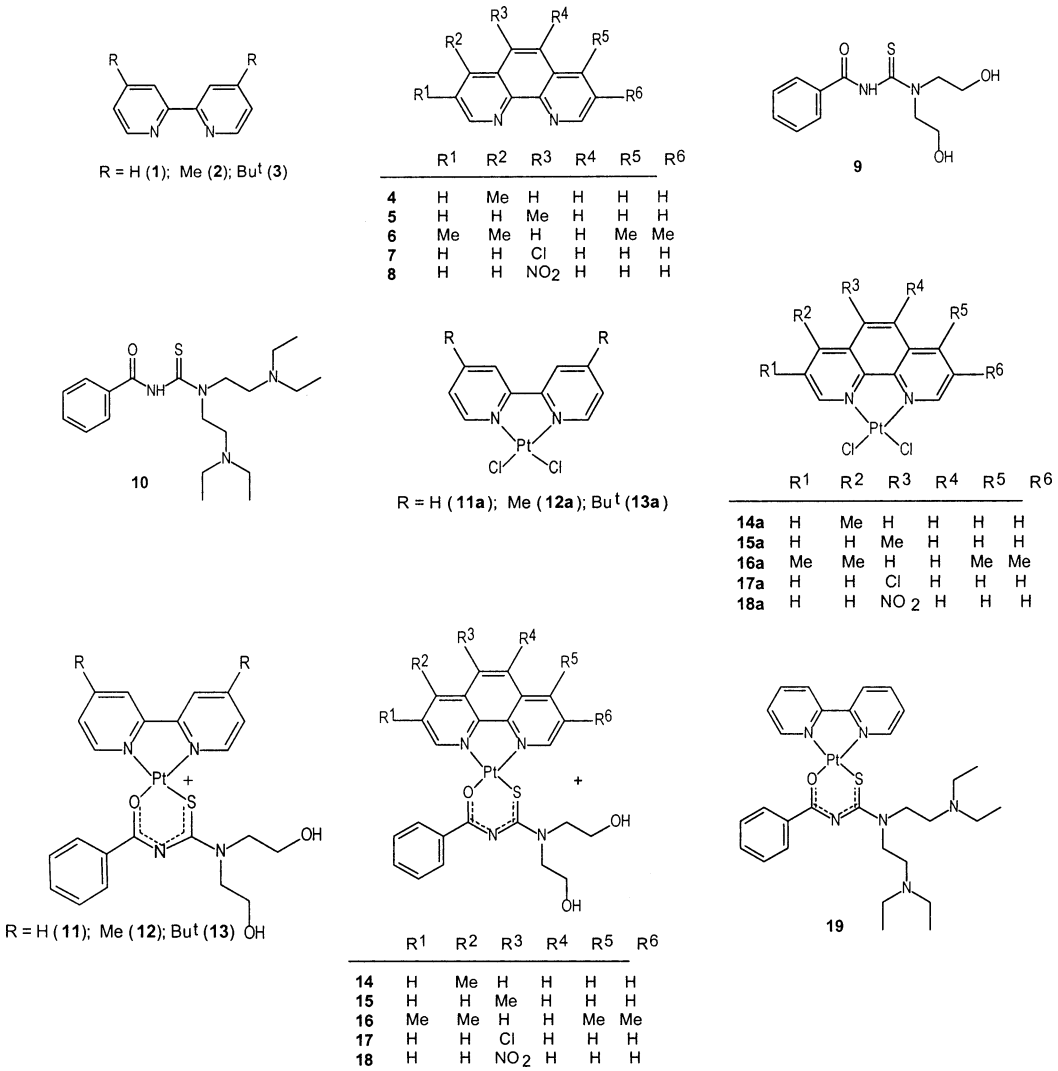


A new class of mixed-ligand cationic *N*-acyl-*N*′,*N*′-dialkylthioureato)diimineplatinum(II) complexes (Chart 1), which show a pronounced tendency to undergo marked, concentration-dependent self-association or aggregation presumably through *π*-cation type interactions in acetonitrile solution, has recently been described

* To whom correspondence should be addressed. For T.J.E.: phone, +27-21-650-2528; fax, +27-21-689-7499; e-mail, tegan@science.uct.ac.za. For K.R.K.: phone, +27-21-808-3020; fax, +27-21-808-3849; e-mail, krk@sun.ac.za.
† Department of Chemistry, University of Cape Town.
§ Department of Chemistry, University of Stellenbosch.
# Department of Medicine, University of Cape Town.

10.1021/jm031132g CCC: $27.50
Published on Web 04/23/2004

Chart 2. Ligands and Pt(II) Complexes Prepared and Investigated in This Study



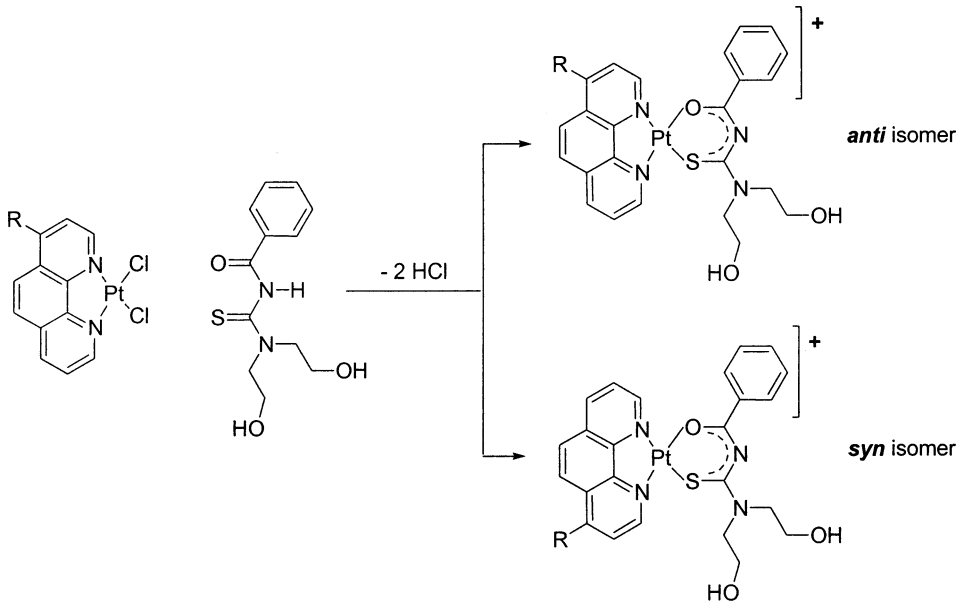
by Koch et al.[23] The self-stacking tendency of these complexes is strikingly similar to that exhibited by porphyrins and suggests that these mixed-ligand Pt(II) complexes may associate with Fe(III)PPIX. Accordingly, we have synthesized a series of similar, but more water-soluble complexes of this general motif (**11**−**19**, Chart 2) and studied their interaction with hematin. We report here the relatively strong in vitro interactions of these Pt(II) complexes with hematin, their ability to inhibit β-hematin formation, and some significant biological activity against *P. falciparum* maintained in culture.

Chemistry

A series of 2,2′-bipyridyl and 1,10-phenanthroline ligands **1**−**8** were reacted with K_2PtCl_4 to result in a series of 2,2′-bipyridyl- and 1,10-phenathrolinedichloroplatinum(II) complexes **11a**−**18a** (Chart 2) according to published methods.[24] All of the bipyridyl and 1,10-phenanthroline ligands except **3** are commercially available. Compound **3** was synthesized from 4-*tert*-butylpyridine using a literature procedure.[25] The benzoylthioureas **9** and **10** were synthesized according to the method of Douglass and Dains.[26] Synthesis of **9** has been reported previously,[27] but **10** is a new compound. All of the synthesized ligands were fully characterized by means of elemental analysis, ^{1}H and ^{13}C as well as 2-D COSY and HSQC NMR spectroscopy, and electrospray mass spectrometry. The sharp melting or decomposition points of these substances further confirmed their purity.

The target molecules **11**−**18** (Chart 2) were prepared as chloride salts by reaction of the complexes **11a**−**18a** with **9** followed by addition of a weak base, triethylamine, to abstract the proton from the thiourea NH group. Substance **19** (Chart 2) was prepared by an analogous reaction of **11a** with **10**. The synthesis and characterization of these complexes closely paralleled that of the analogous *N*-benzoyl-*N*′,*N*′-dialkylthioureato-(diimine)platinum(II) PF_6 complexes previously reported.[23] The target complexes were fully characterized by means of elemental analysis, ^{1}H and ^{13}C as well as 2-D COSY and HSQC NMR spectroscopy, and electrospray mass spectrometry, in concordance with assignments for related substances done previously.[23] None of the mixed-ligand complexes melted, but all exhibited sharp decomposition points. Moreover, the deliberately designed inclusion of either hydroxyl or amino functional groups in, respectively, *N*-benzoyl-*N*′,*N*′-di(2-hydroxyethyl)thiourea or *N*-benzoyl-*N*′,*N*′-bis-diethylaminoethylthiourea to achieve a greater water solubility, as well as the saltlike nature of the resulting mixed-

Scheme 1. Formation of the syn and anti Isomers for Complexes **14**, **15**, **17**, and **18**



ligand platinum(II) complexes, rendered these substances all relatively hygroscopic. Consequently most of the complexes, particularly those derived from 1,10-phenanthroline, absorbed moisture from the atmosphere or from solvents and were generally isolated as hydrated salts. Compound **19**, which contains two basic amino groups, was isolated as a dihydrochloride salt.

As may be seen from Scheme 1, compounds **14**, **15**, **17**, and **18** are expected to form as a pair of syn and anti isomers, which could not be separated chromatographically in our hands. This isomerism arises from the nonsymmetric substitution pattern on ligands **4**, **5**, **7**, and **8**, which is confirmed by the ^{1}H NMR spectra of these compounds in D_2O/CD_3CN solution. Since the ^{1}H NMR spectra of these complexes are both concentration and solvent mixture dependent, the unambiguous assignment of the syn and anti isomers for complexes **14**, **15**, **17**, and **18** is not trivial. These detailed assignments will be published elsewhere.

Determination of Association Constants with Fe(III)PPIX. Shelnutt has previously shown[28−31] that 1,10-phenanthrolines themselves form noncoordinating complexes with metallouroporphyrins. For this reason, we investigated the interactions of all of the bipyridyl and phenanthroline ligands **1**−**8** as well as the target complexes **11**−**19** with Fe(III)PPIX. The interactions were investigated in 40% (v/v) aqueous DMSO, in which Fe(III)PPIX is monomeric. This nonphysiological solvent system is suitable for spectrophotometric titrations and has been used extensively in our laboratory for investigating the interactions of antimalarials and related compounds with Fe(III)PPIX.[3,13,14,32] We have previously pointed out[33] that association constants obtained in this way follow essentially the same trends and are similar in magnitude to those reported in more physiologically relevant acidic aqueous solution.[4] The latter conditions require a titration calorimeter.

The intensity of the Fe(III)PPIX Soret band at 402 nm was determined as a function of concentration of the various compounds **1**−**8** and **11**−**19**. The data were found to fit closely to a 1:1 association model in all of those cases where a change is observed. The association constants obtained from nonlinear least-squares fitting to this model are reported in Table 1. It is immediately apparent from Table 1 that none of the bipyridyl ligands (**1**−**3**) form appreciable complexes with Fe(III)PPIX, while all of the phenanthroline ligands form weak complexes (**4**−**8**) with log K values ranging from 2.88 to 3.32. The acylthiourea ligands either form no complex with Fe(III)PPIX (**9**) or form a very weak complex (**10**, log K = 1.93). By contrast, all of the bipyridyl and phenanthrolineplatinum(II) acylthiourea complexes form strong complexes with Fe(III)PPIX, with log K values ranging between 4.81 (**13**) and 6.24 (**18**). In the cases of **16**−**18**, these complexes are appreciably stronger than those of chloroquine (shown in Table 1 for comparison).

Inhibition of β-Hematin Formation. All of the ligands (**1**−**10**) and the target platinum complexes (**11**−**19**) were assayed for their ability to inhibit the formation of β-hematin. The assay used is essentially qualitative in nature and relies on differences in the infrared spectrum between hematin and β-hematin. We have reported full details of the assay elsewhere,[5] and we and others have used it extensively in other studies.[13,14,32,34] These earlier studies have demonstrated that the assay gives results that are qualitatively in agreement with other assays.[4,35] Nonetheless, direct comparison with β-haematin inhibition IC_{50} values obtained from other assays is difficult, in part owing to the high concentration of Fe(III)PPIX used (4.6 mM). In general a positive result with 4 equiv of a compound relative to Fe(III)-PPIX correlates with strong to very strong inhibition in other assays, while a negative result correlates with very weak to no inhibition (direct comparison of this method with a published quantitative assay is given elsewhere[14]). Inhibition in the presence of more than 4 equiv of a compound in this assay corresponds to very weak inhibition. Results of the assay are reported in Table 1.

As can be seen in Table 1, none of the ligands (**1**−**10**) but all of the platinum target complexes (**11**−**19**) inhibit β-hematin formation. Three of the platinum complexes (**12**, **13**, and **19**) inhibit the process more weakly than the other platinum complexes. Although the assay is

Table 1. Association Constants with Fe(III)PPIX, β-Hematin Inhibitory Activities, and in Vitro Antimalarial Activities against the Chloroquine-Sensitive D10 and Chloroquine-Resistant K1 Strains of *P. falciparum* of the Compounds Studied in This Work

compd[a]	log K [b] ± SEM ($n = 3$)[c]	β-hematin inhibition	D10 IC_{50} (nM) ± SD ($n = 8$)[d]	K1 IC_{50} (nM) ± SD ($n = 8$)[d]
1	NA[f]	−	NT[i]	NT[i]
2	NA[f]	−	NT[i]	NT[i]
3	NA[f]	−	NT[i]	NT[i]
4	3.22 ± 0.03	−	NT[i]	NT[i]
5	2.93 ± 0.03	−	NT[i]	NT[i]
6	3.32 ± 0.01	−	NT[i]	NT[i]
7	3.01 ± 0.03	−	NT[i]	NT[i]
8	2.88 ± 0.02	−	NT[i]	NT[i]
9	NA[f]	−	NT[i]	NT[i]
10	1.93 ± 0.02	−	NT[i]	NT[i]
11	5.05 ± 0.02	+	336 ± 76	295 ± 56
12	5.13 ± 0.01	+/−[h]	295 ± 42	824 ± 102
13	4.81 ± 0.01	+/−[h]	141 ± 29	119 ± 33
14	5.75 ± 0.01	+	282 ± 45	488 ± 130
15	5.80 ± 0.02	+	308 ± 64	557 ± 89
16	5.96 ± 0.02	+	602 ± 44	706 ± 95
17	6.03 ± 0.01	+	594 ± 93	666 ± 79
18	6.24 ± 0.02	+	2927 ± 736	1925 ± 309
19	5.82 ± 0.01	+/−[h]	1958 ± 85	2378 ± 513
CQ[e]	5.52 ± 0.03[g]	+	35 ± 7	156 ± 34

[a] Refer to Chart 2 for structures. [b] In 40% aqueous DMSO (v/v), pH 7.4, 25 °C, 0.020 M HEPES buffer. [c] Standard error of the mean, three determinations. [d] Standard deviation, four independent determinations in duplicate. [e] Chloroquine for comparison. [f] No association. [g] From ref 3. [h] Weaker inhibition. [i] Not tested.

essentially qualitative, it was evident from the infrared spectra that although β-hematin was formed in the presence of 3 equiv of compounds **12**, **13**, and **19** relative to Fe(III)PPIX, the amount is small. In the case of **12**, addition of 8 equiv was found to completely inhibit β-hematin formation, confirming the interpretation that these compounds are weaker inhibitors.

Biological Testing

Because the platinum target complexes (**11**−**19**) all associate strongly with Fe(III)PPIX and inhibit β-hematin formation, these were tested for in vitro antimalarial activity against both a chloroquine-sensitive (D10) and a chloroquine-resistant (K1) strain of *P. falciparum*. Unlike chloroquine, these platinum complexes bear a permanent positive charge. With the exception of **19**, they also lack protonatable groups. For this reason, we expected that they would show at best weak activity. This is because they are likely to diffuse across biological membranes poorly and would thus not access the drug target in the parasite well via passive diffusion and they are unable to accumulate in the food vacuole through pH trapping. Results of antiplasmodial testing are shown in Table 1.

Contrary to expectation, some of these compounds exhibited strong antiplasmodial activity. Compounds **12**−**14** showed particularly promising activity, with compound **13** showing the strongest activity. Furthermore, all of the compounds tested except **12** and **15** showed no significant difference in activity between the chloroquine-sensitive and chloroquine-resistant strains.

Because platinum complexes, including such compounds as cisplatin and carboplatin, are important anticancer drugs,[36] it was considered possible that the complexes under investigation may be simply exhibiting a general cytotoxic effect. For this reason their cytotoxicity against mammalian cells (Chinese hamster ovarian cells) was investigated for the four most active compounds. The results are shown in Table 2. As can be seen, all four compounds exhibit selective activity against malaria parasites, with selectivity indices in excess of 85.

Table 2. In Vitro Cytotoxicity against Chinese Hamster Ovarian Cells of Four of the Most Active Compounds against *P. falciparum*

compd[a]	CHO IC_{50} (μM) ± SD ($n = 6$)[b]	CHO/D10 S_I[c]	CHO/K1 S_I[c]
11	>162	>482	>549
12	78 ± 13	264	95
13	12 ± 3	85	101
14	>140	>496	>287
emetine[d]	0.139 ± 0.007	NT[e]	NT[e]

[a] Refer to Chart 2 for structures. [b] Standard deviation, two independent determinations in triplicate. [c] Selectivity index = cytotoxicity IC_{50}/antiplasmodial IC_{50}. [d] Emetine dihydrochloride control. [e] Not tested.

Discussion

Association with Fe(III)PPIX and Inhibition of β-Hematin Formation. As expected on the basis of previous work by Shelnutt on metal uroporphyrins,[30,31] all of the phenanthroline ligands (**4**−**8**) were found to form complexes with Fe(III)PPIX. The association constants obtained with these compounds are weak and fall in a narrow range. The interaction strength of copper uroporphyrin was reported to increase as electron-withdrawing groups are attached to the phenanthroline nucleus,[30] but a similar correlation is not observed in the current system. For example, 4-methyl-1,10-phenanthroline (**4**) forms a stronger complex with Fe(III)PPIX than does 5-chloro-1,10-phenanthroline (**7**), while the converse was reported with copper uroporphyrin.

By contrast to the phenanthrolines, the bipyridyl ligands (**1**−**3**) do not appreciably interact with Fe(III)-PPIX. We attribute this to the nonplanar nature of these compounds, which are likely to be twisted around the bond linking the two pyridyl rings. The very weak interaction of the benzoylthiourea ligands (**9** and **10**) with Fe(III)PPIX can probably be ascribed to the lack of an extended aromatic system in these ligands.

As initially predicted, all of the cationic mixed-ligand platinum benzoylthiourea complexes interacted strongly with Fe(III)PPIX. The strong association of these complexes with Fe(III)PPIX can probably be ascribed to several factors: (i) the enforced planar conformation of the bipyridyl ligands in the complexes, which now permits coplanar interaction[37] with the porphyrin; (ii) the presence of a greatly extended planar system with strongly delocalized electrons in all of these complexes; (iii) the cationic character of the platinum complexes, possibly leading to cation$-\pi$ interactions[38] with the porphyrin. The stronger interactions of the phenanthroline complexes with Fe(III)PPIX compared to the bipyridyl complexes may be attributed to the larger aromatic planar surface area in the former. More detailed rationalization of the strengths of the various interactions with Fe(III)PPIX is difficult, given the relatively small number of derivatives.

In view of the weak or nonexistent interaction of the various ligands (**1**−**10**) with Fe(III)PPIX, it is unsurprising that none of them inhibit β-hematin formation. Given that strong association with Fe(III)PPIX does not necessarily lead to inhibition of β-hematin formation in the quinolines,[12−14] it is probably more surprising that all of the target platinum complexes (**11**−**19**) exhibit such activity. The somewhat weakened β-hematin inhibitory activity of the substituted bipyridyl complexes (**12** and **13**) can possibly be ascribed to steric effects that may influence the conformation of their complexes with Fe(III)PPIX in solution or may weaken their interaction with surface sites on the nascent β-hematin crystal. The weakening of the inhibitory activity by the presence of amino groups in the benzoylthiourea ligand in **19** is more surprising and merits further investigation.

Biological Activity. In view of the observation that the target platinum complexes (**11**−**19**) associate with Fe(III)PPIX with comparable or greater strength than chloroquine and that they inhibit β-hematin formation, we expected that they might show some antiplasmodial activity. However, we expected such activity to be weak for two reasons. First, these complexes are cationic and so would not be expected to efficiently cross biological membranes without some specific uptake mechanism. Second, with the exception of **19**, they lack basic groups that are needed for pH trapping in the food vacuole. Our earlier studies on aminoquinolines indicated that a basic amino group (together with the basic quinoline N atom) is essential for strong activity against the parasite,[13,14] suggesting that the aminoquinolines cross the food vacuole membrane by passive diffusion and accumulate at the site of action though pH trapping. We were therefore pleasantly surprised to find that some of these cationic platinum compounds exhibit strong in vitro antimalarial activity.

As with the aminoquinolines, there is no obvious correlation between the antiplasmodial activity of these compounds and their association constants with Fe(III)-PPIX. However, unlike the aminoquinolines, their activity does not appear to correlate with their ability to inhibit β-hematin formation. Two of the four most active compounds do not inhibit this process as strongly as the other compounds. It thus seems possible that these compounds act against some other target in the parasite. Given that well-known platinum antitumor drugs act via interaction with DNA,[36] this may be a possible target. Existing antitumor Pt(II)-containing drug−DNA interactions are thought to predominantly occur through direct Pt(II) N-coordination to nucleic acids or by intercalation between nucleic acid bases in the DNA.[36] In addition, some of these compounds as well as other platinum complexes have been shown to exhibit antimalarial activity.[39,40] In the case of the compounds reported in this work however, we reasonably expect the chelated Pt(II) complexes to remain coordinatively intact under biological conditions. Since both ligands are bound in a bidentate mode to the Pt(II) ion, it is unlikely that they would undergo substitution reactions resulting in coordination to nucleic acids. If DNA interaction takes place at all, we believe it is more likely that it occurs via intercalation or major/minor groove binding. Whatever the mode of action is, there must be some mechanism by which these cationic platinum complexes are selectively taken up by the malaria parasite because most are more than 100 times less active against mammalian cells. Clearly, identification of the target of these compounds warrants further investigation.

Interestingly, the one compound (**19**) that incorporates basic groups shows the second weakest antiplasmodial activity. The fully protonated form of this compound should bear a +3 charge, so its weak activity may be a reflection of the fact that its membrane-crossing ability is worse than that of the singly charged species. Alternatively, if these cations gain access to the parasite via ion pumps, **19** may simply be too bulky or bear an inappropriate charge to effectively enter the parasite.

Conclusion

This study has identified a novel family of water-soluble compounds with significant activity against malaria parasites. These compounds are as active against the chloroquine-resistant K1 strain of parasite as against the chloroquine-sensitive D10 strain. One compound is highly active, with an IC_{50} almost in the range of chloroquine. Upon reflection, the relatively high price of platinum as well as the potential toxicity of this heavy metal would seem to rule out such compounds as potential new antimalarial drugs. However, if these compounds prove to be sufficiently active in vivo, the quantity of platinum per dose could conceivably be so low as to contribute very little cost to a single dose. (For example, 0.1 g of compound **13** would cost about $0.60 at current prices.) Given that no attempt has yet been made to optimize the biological activities of this class of compound, it may well be that analogues that are significantly more active may be found, and work in this regard is underway. Furthermore, this class of compound exhibits acceptable selectivity against malaria parasites using the cytotoxicity assay available in our laboratory.

In light of the promising properties of this class of compound, a number of recommendations can be made. First, given the potential toxicity of platinum complexes, it will be necessary to screen them for cytotoxicity in human cell lines before further development can be contemplated. If these compounds are metabolically stable and do not undergo ligand exchange in vivo, they may turn out not to be excessively toxic. Clearly, in vivo

testing will be required to establish this. Second, the presence of isomers is undesirable because these would need to be separated and individually tested. The easiest solution to this is to confine future studies to symmetrically substituted ligands. Third, an additional concern regarding the potential use of such compounds is their tendency to self-aggregate. This could make formulation difficult because their physicochemical and pharmacokinetic properties can be expected to depend on the degree of aggregation. This will require further study.

Finally, given that these compounds inhibit β-hematin formation strongly, it may be possible to design neutral analogues with basic side chains that exhibit activity similar to chloroquine. If it turns out that the cationic platinum complexes examined in this study act against a different target, then such neutral complexes could constitute a second family of platinum antiplasmodials.

Experimental Section

Antiplasmodial Testing. Antiplasmodial testing was performed on both the chloroquine-sensitive *P. falciparum* clone D10 and on the chloroquine-resistant clone K1. Parasites were maintained in continuous culture using the method of Trager and Jensen[41] with minor modifications. Cultures were maintained at a 3−5% hematocrit with a 2−8% parasitemia. Parasitized human O+ red blood cells were suspended in culture flasks containing RPMI medium supplemented with 0.05% Albumax. The flasks were gassed with 3% O_2, 4% CO_2, and 93% N_2.

All of the compounds tested for antiplasmodial activity were dissolved in water. Antiplasmodial activity was determined by assaying the activity of the parasite lactate dehydrogenase enzyme by a slightly modified version of the assay of Makler et al.[42] Antiplasmodial determinations were all performed at 1% hematocrit and 2% parasitemia. The compounds were added at the trophozoite stage, and a period of 48 h was allowed to elapse before the LDH assay was performed.

The data were fitted to dose−response curves by nonlinear least-squares fitting using GraphPad Prism software.[43] The 50% inhibitory concentrations (IC_{50}) were obtained from these fits. The reported IC_{50} values are the result of four independent experiments carried out in duplicate.

In Vitro Cytotoxicity. Cytotoxicity assays were performed on Chinese hamster ovarian (CHO) cells using the MTT colorimetric assay[44] as previously described.[45] Emetine dihydrochloride (Sigma) was used as a positive control. An initial 2 mg/mL stock solution of emetine and the test compounds was prepared in Millipore water and diluted in medium to give six serial 10-fold dilutions with a final concentration range of 100 μg/mL to 1 ng/mL. The 50% inhibitory concentrations (IC_{50}) were determined from the dose−response curves, using nonlinear dose−response curve-fitting analyses with GraphPad Prism software,[43] and are given as the mean $IC_{50} \pm$ standard deviation of two independent experiments each performed in triplicate.

Chemistry. Hemin, potassium thiocyanate, benzoyl chloride, diethanolamine, *N*,*N*,*N*′,*N*′-tetraethyldiethylenetriamine, 4-*tert*-butylpyridine, 2,2′-bipyridyl (**1**), 4,4′-dimethyl-2,2′-bipyridyl (**2**), 4-methyl-1,10-phenanthroline (**4**), 5-methyl-1,10-phenanthroline (**5**), 3,4,7,8-tetramethyl-1,10-phenanthroline (**6**), 5-chloro-1,10-phenanthroline (**7**), and 5-nitro-1,10-phenanthroline (**8**) were all obtained from Sigma-Aldrich, South Africa. Potassium tetrachloroplatinate was obtained on loan from Anglo Platinum Ltd. All reagents were used as supplied without further purification, except for acetone that was distilled over 4 Å molecular sieves and potassium carbonate.

Infrared (IR) spectroscopy was performed on a Perkin-Elmer 983 infrared spectrometer as KBr disks. Proton and carbon NMR spectra were recorded on a Varian Unity 600 MHz, Varian Unity 400 MHz, or a Varian Mercury 300 MHz NMR spectrometer. Solvents are as indicated. Mass spectra were obtained on a VG Quattro Fisons electrospray mass spectrometer at the University of Stellenbosch; ESMS spectra are reported for the most abundant peak for M^+ found only, since in the spectrum for each complex one obtains typically six peaks corresponding to the isotopic distribution of platinum, which occurs naturally as six stable isotopes ranging from ^{190}Pt to ^{198}Pt, the most abundant isotope being ^{195}Pt at 33.7%. Melting points were measured on a Reichert-Jung Thermovar hot-stage microscope attached to a DP-4 digital thermometer and are uncorrected. Microanalyses were performed by the University of Cape Town microanalysis service using a Fisons EA 1108 elemental analyzer.

4,4′-Di-*tert*-butyl-2,2′-bipyridyl (**3**) and *N*-benzoyl-*N*′,*N*′-di-2-hydroxyethylthiourea (**9**) were synthesized according to published methods.[25,27] Both were characterized by melting point determination, elemental analysis, and ^{1}H and ^{13}C NMR and were found to be of acceptable purity for further study. Melting points agreed with literature values within a margin of 3 °C. That for **3** was 159 °C (lit.[25] 156 °C), while that for **9** was 120−121 °C (lit.[27] 120−122 °C). The full synthetic details and characterization for all novel compounds are supplied below.

***N*-Benzoyl-*N*′,*N*′-bisdiethylaminoethylthiourea (10).** Dry potassium thiocyanate (0.367 g, 3.78 mmol) was dissolved in dry distilled acetone by heating under reflux and N_2. To this, benzoyl chloride (0.531 g, 3.78 mmol) was added dropwise with constant stirring leading to the immediate precipitation of KCl. After the mixture was heated under reflux for 40 min, *N*,*N*,*N*′,*N*′-tetraethyldiethylenetriamine (0.814 g, 3.78 mmol) in dry acetone was added dropwise and the solution was heated under reflux for a further 3 h. KCl was removed by filtration and acetone was removed under vacuum to yield a crude oil. After purification by column chromatography on 40 g of silica gel (60 mesh) using 2% ammonia in methanol as eluent and recrystallization from diethyl ether, **10** (0.79 g, 55%) was afforded as a crystalline product: mp 98−99 °C; ^{1}H NMR (acetone-d_6, 300 MHz) δ 7.76 (2H, d, J = 7.6 Hz, Ar−H), 7.54 (1H, t, J = 7.2 Hz, Ar−H), 7.46 (2H, t, J = 7.5 Hz, Ar−H), 3.92 (2H, t, J = 6.8 Hz, $-CH_2-$), 3.87 (2H, m, $-CH_2-$), 2.82−2.87 (4H, m, $-CH_2-$), 2.60 (4H, q, J = 7.2 Hz, $-CH_2-$), 2.57 (4H, q, J = 7.2 Hz, $-CH_2-$), 1.04 (6H, t, J = 7.2 Hz, $-CH_3$), 0.99 (6H, t, J = 7.0 Hz, $-CH_3$); ^{13}C NMR (acetone-d_6, 75.462 MHz) δ 184.02, 169.48, 137.47, 133.13, 129.80, 129.78, 54.40, 53.73, 52.55, 51.54, 50.38, 48.97, 13.51, 12.36; MS *m*/*z* 378.6 (M^+, calcd 378.54). Anal. ($C_{20}H_{34}N_4OS$) C, H, N.

Pt(diimine)Cl_2 Complexes (11a−18a). Potassium tetrachloroplatinate (0.208 g, 0.500 mmol) was dissolved in 15 mL of water. To this was added 0.500 mmol of the appropriate diimine (**1**−**8**), and the mixture was acidified with 1.0 mL of 4 M HCl and heated under reflux for 2 h. The yellow precipitates were filtered off, washed extensively with water followed by small volumes of methanol and diethyl ether, and dried for 24 h at room temperature under vacuum. Products were characterized by elemental analysis. Yields were as given below.

2,2′-Bipyridyldichloroplatinum(II) (11a): yield 0.203 g (96%). Anal. ($C_{10}H_8Cl_2N_2Pt$) C, H, N.

4,4′-Dimethyl-2,2′-bipyridyldichloroplatinum(II) (12a): yield 0.216 g (96%). Anal. ($C_{12}H_{12}Cl_2N_2Pt$) C, H, N.

4,4′-Di-*tert*-butyl-2,2′-bipyridyldichloroplatinum(II) (13a): yield 0.243 g (91%). Anal. ($C_{18}H_{24}Cl_2N_2Pt$) C, H, N.

4-Methyl-1,10-phenanthrolinedichloroplatinum(II) (14a): yield 0.214 g (93%). Anal. ($C_{13}H_{10}Cl_2N_2Pt$) C, H, N.

5-Methyl-1,10-phenanthrolinedichloroplatinum(II) (15a): yield 0.186 g (81%). Anal. ($C_{13}H_{10}Cl_2N_2Pt$) C, H, N.

3,4,7,8-Tetramethyl-1,10-phenanthrolinedichloroplatinum(II) (16a): yield 0.236 g (94%); Anal. ($C_{16}H_{16}Cl_2N_2Pt$) C, H, N.

5-Chloro-1,10-phenanthrolinedichloroplatinum(II) (17a): yield 0.195 g (81%). Anal. ($C_{12}H_7Cl_3N_2Pt$) C, H, N.

5-Nitro-1,10-phenanthrolinedichloroplatinum(II) (18a): yield 0.211 g (86%). Anal. ($C_{12}H_7Cl_2N_3Pt$) H, N. C: calcd 29.35; found 29.82.

(*N*-Benzoyl-*N′*,*N′*-di(2-hydroxyethylthioureato)-*S*,*O*)-(2,2′-bipyridyl)platinum(II) Chloride (11). Reaction of **11a** (0.100 g, 0.237 mmol) dissolved in 14 mL of warm DMF with a 4 mL solution of **9** (0.070 g, 0.261 mmol) and triethylamine (0.036 mL, 0.26 mmol) added dropwise afforded the bright-yellow product after stirring at 80 °C for 4 h. The product was precipitated by addition of 50 mL of acetonitrile, and the filtered product was washed with DMF and diethyl ether and then dried under vacuum at room temperature (0.132 g, 85%): mp 206−207 °C (dec); ^{1}H NMR (CD_3CN/D_2O 1:1, 400 MHz, 0.02 M sample concn) δ 8.33 (1H, d, J = 5.5 Hz, Ar−H), 7.96 (1H, t, J = 8.1 Hz, Ar−H), 7.95 (1H, d, J = 8.4 Hz, Ar−H), 7.92 (1H, t, J = 8.1 Hz, Ar−H), 7.78 (1H, d, J = 7.7 Hz, Ar−H), 7.71 (1H, d, J = 7.7 Hz, Ar−H), 7.56 (1H, t, J = 7.4 Hz, Ar−H), 7.54 (2H, d, J = 7.0 Hz, Ar−H), 7.51 (1H, t, J = 6.0 Hz, Ar−H), 7.30 (2H, t, J = 7.7 Hz, Ar−H), 7.23 (1H, t, J = 6.6 Hz, Ar−H), 3.83 (2H, t, J = 6.3 Hz, $-CH_2-$), 3.71 (2H, t, J = 5.9 Hz, $-CH_2-$), 3.67 (4H, m, $-CH_2-$); MS (ESMS) *m*/*z* 618.1 (M^+, calcd 618.5). Anal. ($C_{22}H_{23}ClN_4O_3PtS$) C, H. N: calcd 8.57; found 8.15.

(*N*-Benzoyl-*N′*,*N′*-di(2-hydroxyethylthioureato)-*S*,*O*)-(4,4′-dimethyl-2,2′-bipyridyl)platinum(II) Chloride (12). Reaction of **12a** (0.080 g, 0.178 mmol) dissolved in 10 mL of warm DMF with a 3 mL solution of **9** (0.053 g, 0.196 mmol) and triethylamine (0.028 mL, 0.26 mmol) added dropwise afforded the yellow product after stirring at 80 °C for 4 h. Addition of 40 mL of acetonitrile to the cooled solution followed by further cooling to −10 °C caused precipitation of the product, which was then washed with DMF and diethyl ether and dried under vacuum at room temperature (0.104 g, 86%): mp 223−225 °C (dec); ^{1}H NMR (CD_3CN/D_2O 1:1, 400 MHz, 0.02 M sample concn) δ 7.91 (1H, d, J = 5.5 Hz, Ar−H), 7.74 (1H, d, J = 5.9 Hz, Ar−H), 7.62 (1H, t, J = 7.3 Hz, Ar−H), 7.48 (1H, s, Ar−H), 7.47 (2H, d, J = 8.1 Hz, Ar−H), 7.37 (1H, s, Ar−H), 7.36 (2H, t, J = 8.1 Hz, Ar−H), 7.19 (1H, d, J = 5.5 Hz, Ar−H), 6.99 (1H, d, J = 5.9 Hz, Ar−H), 3.83 (2H, t, J = 6.3 Hz, $-CH_2-$), 3.69 (2H, t, J = 5.9 Hz, $-CH_2-$), 3.69 (4H, t, J = 5.1 Hz, $-CH_2-$), 2.19 (3H, s, $-CH_3$), 2.11 (3H, s, $-CH_3$); MS (ESMS) *m*/*z* 646.0 (M^+, calcd 646.6). Anal. ($C_{24}H_{27}ClN_4O_3PtS$) H, N. C: calcd 42.27; found 41.77.

(*N*-Benzoyl-*N′*,*N′*-di(2-hydroxyethylthioureato)-*S*,*O*)-(4,4′-di-*tert*-butyl-2,2′-bipyridyl)platinum(II) Chloride (13). Reaction of **13a** (0.200 g, 0.374 mmol) dissolved in 60 mL of warm dry acetone with a 15 mL solution of **9** (0.111 g, 0.411 mmol) and triethylamine (0.044 mL, 0.41 mmol) in acetone added dropwise afforded the bright-yellow product after refluxing for 4 h. Cooling to −10 °C caused precipitation of the product, which was then washed with methanol and diethyl ether and dried under vacuum at room temperature (0.185 g, 65%): mp 206−208 °C (dec); ^{1}H NMR (CD_3CN/D_2O 1:1, 400 MHz, 0.02 M sample concn) δ 8.46 (1H, d, J = 6.2 Hz, Ar−H), 8.26 (1H, t, J = 6.2 Hz, Ar−H), 8.09 (1H, d, J = 2.2 Hz, Ar−H), 7.93 (1H, d, J = 2.2 Hz, Ar−H), 7.70 (2H, d, J = 7.0 Hz, Ar−H), 7.68 (1H, d, J = 5.9 Hz, Ar−H), 7.58 (1H, t, J = 7.3 Hz, Ar−H), 7.48 (1H, dd, J = 6.2, 2.2 Hz, Ar−H), 7.32 (2H, t, J = 7.9 Hz, Ar−H), 4.02 (4H, m, $-CH_2-$), 3.84 (2H, t, J = 5.7 Hz, $-CH_2-$), 3.76 (2H, t, J = 6.3 Hz, $-CH_2-$), 1.47 (9H, s, $-CH_3$), 1.35 (9H, s, $-CH_3$); MS (ESMS) *m*/*z* 730.1 (M^+, calcd 730.7). Anal. ($C_{30}H_{39}ClN_4O_3PtS$) C, H, N.

(*N*-Benzoyl-*N′*,*N′*-di(2-hydroxyethylthioureato)-*S*,*O*)(4-methyl-1,10-phenanthroline)platinum(II) Chloride (14). Reaction of **14a** (0.184 g, 0.400 mmol) suspended in 220 mL of acetonitrile under reflux with a 20 mL solution of **9** (0.119 g, 0.44 mmol) in acetonitrile added dropwise and heated under reflux for 4 h followed by triethylamine (0.047 mL, 0.44 mmol) and heated under reflux for a further 1 h afforded the crude yellow product upon cooling overnight. The product, which consisted of a mixture of inseparable syn and anti isomers (1:9), was recrystallized from a mixture of methanol and acetonitrile. It was washed with acetonitrile and diethyl ether and dried under vacuum at room temperature (0.170 g, 60%): mp 196−197 °C (dec); ^{1}H NMR (CD_3CN/D_2O 1:1, 400 MHz, 0.02 M sample concn) anti isomer δ 8.29 (1H, d, J = 8.4 Hz, Ar−H), 7.66 (1H, m, Ar−H), 7.53 (1H, m, Ar−H), 7.45 (1H, t, J = 7.0 Hz, Ar−H), 7.38 (1H, d, J = 8.8 Hz, Ar−H), 7.18−7.11 (3H, m, Ar−H), 7.11 (1H, d, J = 7.3 Hz, Ar−H), 6.95 (3H, m, Ar−H), 3.76 (2H, t, J = 5.5 Hz, $-CH_2-$), 3.58 (2H, t, J = 5.5 Hz, $-CH_2-$), 3.56 (2H, t, J = 5.5 Hz, $-CH_2-$), 3.43 (2H, t, J = 5.9 Hz, $-CH_2-$), 2.03 (3H, s, $-CH_3$); MS (ESMS) *m*/*z* 656.2 (M^+, calcd 656.6). Anal. ($C_{25}H_{25}ClN_4O_3PtS\cdot H_2O$) C, H, N.

(*N*-Benzoyl-*N′*,*N′*-di(2-hydroxyethylthioureato)-*S*,*O*)(5-methyl-1,10-phenanthroline)platinum(II) Chloride (15). Reaction of **15a** (0.184 g, 0.400 mmol) suspended in 220 mL of acetonitrile under reflux with a 20 mL solution of **9** (0.119 g, 0.44 mmol) in acetonitrile added dropwise and heated under reflux for 4 h followed by triethylamine (0.047 mL, 0.44 mmol) and heated under reflux for a further 1 h afforded the crude yellow product upon cooling overnight. The product, which consisted of a mixture of inseparable syn and anti isomers (1:1), was recrystallized from a mixture of methanol and acetonitrile. It was washed with acetonitrile and diethyl ether and dried under vacuum at room temperature (0.113 g, 40%): mp 194−196 °C (dec); ^{1}H NMR (CD_3CN/D_2O 1:1, 400 MHz, 0.02 M sample concn) anti isomer δ 8.18 (1H, d, J = 7.7 Hz, Ar−H), 7.93 (1H, d, J = 8.1 Hz, Ar−H), 7.77 (1H, d, J = 4.8 Hz, Ar−H), 7.53−7.43 (2H, m, Ar−H), 7.16−7.09 (4H, m, Ar−H), 6.96−6.87 (3H, m, Ar−H), 3.76 (2H, m, $-CH_2-$), 3.56 (4H, m, $-CH_2-$), 3.46 (2H, m, $-CH_2-$), 1.93 (3H, s, $-CH_3$), syn isomer δ 8.09 (1H, d, J = 7.7 Hz, Ar−H), 8.03 (1H, d, J = 8.1 Hz, Ar−H), 7.87 (1H, d, J = 4.8 Hz, Ar−H), 7.53−7.43 (2H, m, Ar−H), 7.26−7.19 (2H, m, Ar−H), 7.16−7.09 (2H, m, Ar−H), 6.96−6.87 (3H, m, Ar−H), 3.76 (2H, m, $-CH_2-$), 3.56 (4H, m, $-CH_2-$), 3.46 (2H, m, $-CH_2-$), 1.99 (3H, s, $-CH_3$); MS (ESMS) *m*/*z* 656.2 (M^+, calcd 656.6). Anal. ($C_{25}H_{25}ClN_4O_3PtS\cdot H_2O$) C, H, N.

(*N*-Benzoyl-*N′*,*N′*-di(2-hydroxyethylthioureato)-*S*,*O*)-(3,4,7,8-tetramethyl-1,10-phenanthroline)platinum(II) Chloride (16). Reaction of **16a** (0.201 g, 0.400 mmol) suspended in 220 mL of acetonitrile under reflux with a 20 mL solution of **9** (0.119 g, 0.44 mmol) in acetonitrile added dropwise and heated under reflux for 4 h followed by triethylamine (0.047 mL, 0.44 mmol) and heated under reflux for a further 1 h afforded the crude yellow product upon cooling overnight. The product was recrystallized from a mixture of methanol and acetonitrile. It was washed with acetonitrile and diethyl ether and dried under vacuum at room temperature (0.15 g, 50%): mp 203−205 °C (dec); ^{1}H NMR (CD_3CN/D_2O 1:1, 400 MHz, 0.02 M sample concn) δ 7.59 (1H, s, Ar−H), 7.57 (1H, s, Ar−H), 7.55 (1H, d, J = 9.0 Hz, Ar−H), 7.50 (1H, d, J = 9.3 Hz, Ar−H), 7.42 (1H, m, Ar−H), 7.29 (2H, d, J = 7.5 Hz, Ar−H), 7.28 (2H, m, Ar−H), 3.84 (2H, t, J = 5.9 Hz, $-CH_2-$), 3.77 (2H, t, J = 5.7 Hz, $-CH_2-$), 3.69 (2H, t, J = 5.7 Hz, $-CH_2-$), 3.64 (2H, t, J = 5.9 Hz, $-CH_2-$), 2.13 (6H, s, $-CH_3$), 2.04 (3H, s, $-CH_3$), 1.99 (3H, s, $-CH_3$); MS (ESMS) *m*/*z* 698.4 (M^+, calcd 698.6). Anal. ($C_{28}H_{31}ClN_4O_3PtS\cdot H_2O$) C, H, N.

(*N*-Benzoyl-*N′*,*N′*-di(2-hydroxyethylthioureato)-*S*,*O*)(5-chloro-1,10-phenanthroline)platinum(II) Chloride (17). Reaction of **17a** (0.192 g, 0.400 mmol) suspended in 220 mL of acetonitrile under reflux with a 20 mL solution of **9** (0.119 g, 0.44 mmol) in acetonitrile added dropwise and heated under reflux for 4 h followed by triethylamine (0.047 mL, 0.44 mmol) and heated under reflux for a further 1 h afforded the crude yellow product upon cooling overnight. The product, which consisted of a mixture of inseparable syn and anti isomers (1.5:8.5), was recrystallized from a mixture of methanol and acetonitrile. It was washed with acetonitrile and diethyl ether and dried under vacuum at room temperature (0.199 g, 68%): mp 192−194 °C (dec); ^{1}H NMR (CD_3CN/D_2O 1:1, 400 MHz, 0.02 M sample concn) anti isomer δ 8.31 (1H, d, J = 7.2 Hz, Ar−H), 8.25 (1H, d, J = 7.8 Hz, Ar−H), 8.11 (2H, m, Ar−H), 7.46−7.41 (3H, m, Ar−H), 7.39 (1H, s, Ar−H), 7.08 (2H, t, J = 7.3 Hz, Ar−H), 6.87 (2H, d, J = 7.7 Hz, Ar−H), 3.83 (2H, t, J = 5.9 Hz, $-CH_2-$), 3.66 (2H, t, J = 5.1 Hz, $-CH_2-$), 3.60 (2H, t, J = 5.1 Hz, $-CH_2-$), 3.53 (2H, t, J = 4.8 Hz, $-CH_2-$); MS (ESMS) *m*/*z* 677.3 (M^+, calcd 677.0). Anal. ($C_{24}H_{22}Cl_2N_4O_3PtS\cdot H_2O$) C, H, N.

(*N*-Benzoyl-*N′*,*N′*-di(2-hydroxyethylthioureato)-*S*,*O*)(5-nitro-1,10-phenanthroline)platinum(II) Chloride (18). Reaction of **18a** (0.100 g, 0.204 mmol) dissolved in 20 mL of warm DMF with a 5 mL solution of **9** (0.061 g, 0.224 mmol) and triethylamine (0.025 mL, 0.23 mmol) in DMF added dropwise and stirred at 60 °C for 8 h afforded the crude orange product upon cooling. Addition of diethyl ether aided precipitation, and after evaporation of ether from the filtrate the product was washed with DMF and diethyl ether. Further addition of diethyl ether to the filtrate yielded a second crop of product, which was filtered and washed. The two batches of product, which consisted of a mixture of inseparable syn and anti isomers (8:2), were recrystallized from a mixture of methanol and acetonitrile. They were washed with acetonitrile and diethyl ether and dried under vacuum at room temperature (0.070 g, 46%): mp 182−183 °C (dec); ^{1}H NMR (CD_3CN/D_2O 1:1, 400 MHz, 0.02 M sample concn) syn isomer δ 8.88 (1H, d, J = 8.4 Hz, Ar−H), 8.76 (1H, d, J = 7.7 Hz, Ar−H), 8.54 (1H, d, J = 4.8 Hz, Ar−H), 8.48 (1H, s, Ar−H), 8.11 (1H, m, Ar−H), 8.01 (1H, t, J = 6.2 Hz, Ar−H), 7.70 (1H, dd, J = 8.7, 3.3 Hz, Ar−H), 7.36 (1H, t, J = 7.0 Hz, Ar−H), 7.00 (2H, t, J = 7.3 Hz, Ar−H), 6.88 (2H, d, J = 7.3 Hz, Ar−H), 3.94 (2H, t, J = 5.5 Hz, $-CH_2-$), 3.83 (2H, t, J = 5.1 Hz, $-CH_2-$), 3.63 (4H, m, $-CH_2-$); MS (ESMS) *m*/*z* 687.3 (M^+, calcd 687.5). Anal. ($C_{24}H_{22}ClN_5O_5PtS\cdot1^1/_2H_2O$) C, N. H: calcd 3.36; found 2.94.

(*N*-Benzoyl-*N′*,*N′*-bisdiethylaminoethylthioureato-*S*,*O*)-(2,2′-bipyridyl)platinum(II) Chloride (19). Reaction of **11a** (0.100 g, 0.237 mmol) partially dissolved in hot acetonitrile with a solution of **10** (0.099 g, 0.261 mmol) in acetonitrile added dropwise afforded the yellow-orange product after stirring at 70 °C for 2 h. The crude product was precipitated by reduction of solvent volume under vacuum. The final hydrochloride product was recrystallized from methanol, extensively washed with acetonitrile, and then dried under vacuum at room temperature (0.122 g, 58%): mp 169−171 °C (dec); ^{1}H NMR (CD_3CN/D_2O 1:1, 400 MHz, 0.02 M sample concn) δ 8.99 (1H, d, J = 5.5 Hz, Ar−H), 8.62 (1H, d, J = 5.9 Hz, Ar−H), 8.39 (2H, m, Ar−H), 8.34 (2H, d, J = 4.0 Hz, Ar−H), 8.14 (2H, d, J = 9.2 Hz, Ar−H), 7.98 (1H, m, Ar−H), 7.76 (1H, t, J = 7.7 Hz, Ar−H), 7.68 (1H, dd, J = 9.9, 5.0 Hz, Ar−H), 7.60 (2H, t, J = 8.1 Hz, Ar−H), 5.52 (2H, t, J = 8.0 Hz, $-CH_2-$), 4.23−4.16 (4H, m, $-CH_2-$), 3.35 (6H, m, $-CH_2-$), 3.21 (4H, q, J = 7.3 Hz, $-CH_2-$), 1.40 (6H, t, J = 7.3 Hz, $-CH_3$), 1.18 (6H, t, J = 7.3 Hz, $-CH_3$); MS (ESMS) *m*/*z* 728.7 (M^+, calcd 728.8). Anal. ($C_{30}H_{41}ClN_6OPtS\cdot2^1/_2H_2O\cdot2HCl$) C, H, N.

Methods used to determine association constants with hematin and to ascertain whether the compounds inhibit β-hematin formation have been described in detail elsewhere.[3,5,32]

Acknowledgment. This work was supported by the National Research Foundation (Grant No. 2061833, T.J.E.), Anglo Platinum (K.R.K.), THRIP (Project 1562, K.R.K.), and University of Cape Town University Research Committee (T.J.E.). Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Research Foundation. We thank Dr. T van der Merwe from the University of Stellenbosch Biochemistry Department for the ESMS spectra.

Supporting Information Available: Elemental analysis data of **11**−**19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM031132G