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Synthesis and SAR Studies of Acyl-Thiourea Platinum(II) Complexes Yield Analogs with Dual-Stage Antiplasmodium Activity

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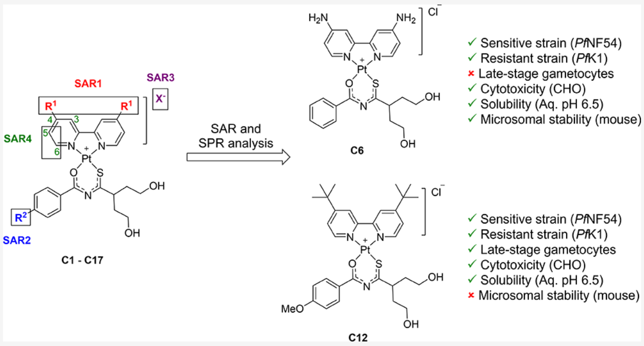

ABSTRACT: Mixed-ligand platinum(II) complexes incorporating bipyridine and acyl-thiourea ligands were synthesized and evaluated for their *in vitro* growth inhibitory activity against the human malaria parasite *Plasmodium falciparum* (*Pf*). The substituents at four distinct sites were varied to identify structure−activity relationships for this series. Most complexes displayed potent *Pf*NF54 activity with IC_{50} values in the nanomolar range and favorable cytotoxicity profiles. Five complexes (**C1**, **C11**, **C12**, **C15**, and **C17**) exhibited activity against both the asexual blood and sexual (gametocyte) stage parasites, with another complex (**C8**) exhibiting activity against late-stage gametocytes only. In addition, the complexes showed comparable ABS potency against the *Pf*K1 multidrug-resistant strain. The pharmacokinetic parameters of one analog (**C6**), which displayed good solubility and mouse microsomal metabolic stability, were measured. This work demonstrates the potential of acyl-thiourea platinum(II) complexes as selective, multistage-active antiplasmodium compounds as part of the search for new antimalarial agents.

KEYWORDS: *Platinum(II) complexes, antiplasmodium, malaria, gametocyte, acyl-thiourea, bipyridine*

The continuous emergence of drug-resistant strains of the most virulent human malaria parasite, *Plasmodium falciparum* (*Pf*), calls for the urgent development of new antimalarial chemotherapeutics.[1,2] Within this context, it is vital to develop drugs that are active against resistant strains and across multiple stages of the parasite life cycle, particularly the asexual blood stage (ABS, symptomatic stage) and gametocyte (transmission stage) parasites.[3] This is especially important since patients treated with drugs that target parasites in the ABS, or even those who are asymptomatic carriers, contain sufficient gametocytes to transmit the parasite from its human host to the mosquito vector, contributing to the spread of the disease.[4] To accelerate drug discovery efforts, chemotypes successfully used in other disease areas can be exploited and optimized as novel compounds against malaria. In this regard, thioureas have gained recognition for their potential medicinal application. In the context of malaria, Verlinden et al.[5] reported a series of alkylated (bis)urea and (bis)thiourea polyamine analogs exhibiting nanomolar *in vitro* antiplasmodium activity against both chloroquine-resistant (CQR) strains (*Pf*W2 and *Pf*HB3) and a chloroquine-sensitive (CQS) strain (*Pf*3D7). Additionally, Pingaew et al.[6] reported a (bis)thiourea compound that exhibited antiplasmodium activity against the CQR *Pf*K1 strain with an IC_{50} value of 1.92 μM.

A less-explored approach in malaria drug discovery is the use of metal-containing drugs. Metallodrugs can be used to detect and treat several diseases and have important biological applications in cellular systems.[7] Metal centers may also be added to organic drugs to improve their efficacy,[8] as is seen with ferroquine (FQ), a ferrocenyl derivative of the 4-aminoquinoline drug chloroquine (CQ). In a study conducted by Dive and Biot, FQ showed a 1.7-fold increase in activity compared to CQ against the *Pf*3D7 strain and a 25-fold increase in activity against *Pf*K1.[9] FQ acts via the same mechanism of action as CQ (inhibition of hemozoin formation) and causes oxidative stress due to the ferrocenyl moiety.[10] FQ is currently in phase IIb clinical trials in combination with artefenomel.[11] Additionally, FQ is also in phase II clinical trials in combination with ZY-19489, a

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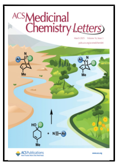


triaminopyrimidine identified from a high-throughput screen against ABS *Pf* parasites.[12]

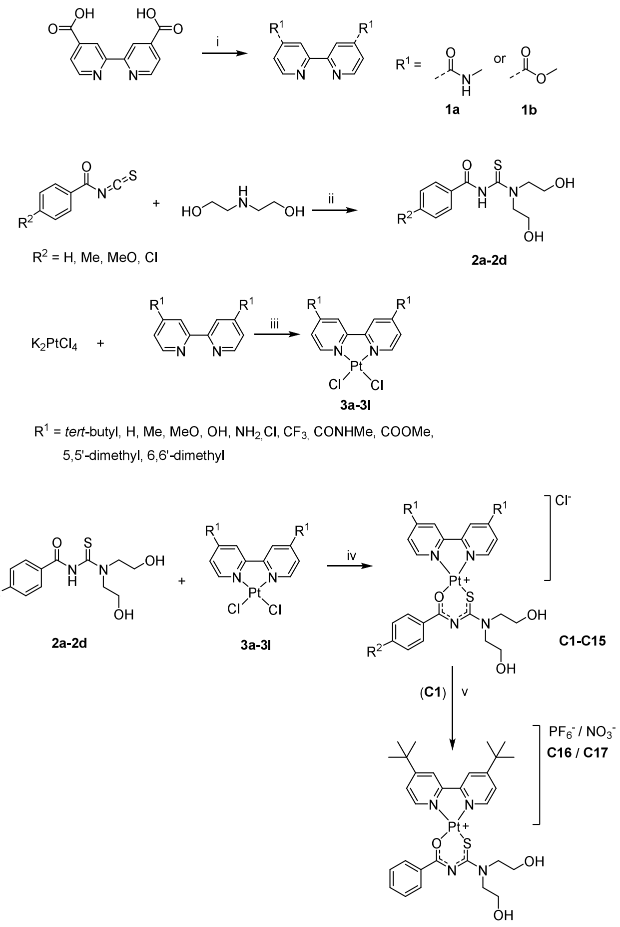
The success of FQ highlights the potential of using transition metals in the search for antimalarials with novel mechanisms of action. Metal complexes that have been studied for their antiplasmodium activities are mainly based on the 4-aminoquinoline scaffold of CQ. Sanchez-Delgado et al. were one of the first groups to synthesize transition metal (Rh and Ru) complexes with a CQ moiety that exhibited comparable antiplasmodium activity to CQ.[13] Although CQ derivatization has dominated the metals-in-malaria field, one of the advantages of complexation chemistry is the relative ease of varying the ligand. Because of their stability toward air and moisture, 2,2′-bipyridine (bpy) ligands and derivatives thereof have been among the most frequently used ligands in complexation chemistry.[14] The bpy ligands have also been used in several applications ranging from supramolecular and macromolecular chemistry to electrochemistry and photochemistry.[15] Transition metal complexes using bpy as a ligand have been reported to show anticancer activity,[16] antimicrobial activity,[17] and antiplasmodium activity.[18]

Previous studies from our group showed that mixed-ligand platinum(II) complexes exhibited appreciable antiplasmodium activity.[19] The current study expands on the previously used bpy and acyl-thiourea scaffolds and further explores the varying substituents on these moieties. A series of platinum(II) complexes were synthesized for evaluation against the CQS *Pf*NF54 and CQR *Pf*K1 strains. In this study, we analyze structure−activity relationships (SARs) for this series and, for the first time, evaluate the activity of the platinum(II) complexes against both ABS and sexual stages of *Pf* parasites as well as their cytotoxicity and the pharmacokinetic properties of a front-runner.

The synthetic procedures for complexes **C1−C17** and their precursors follow modifications of methods reported in the literature (Scheme 1).[19,20] Compounds **1a** and **1b** were synthesized via the condensation of [2,2′-bipyridine]-4,4′-dicarboxylic acid in dimethylformamide (DMF) or methanol (MeOH) to form the methyl amide and ester, respectively. For the acyl-thiourea ligands **2a−2d**, the respective benzoyl isothiocyanates were reacted with diethanolamine under argon.

The [$PtCl_2$(diimine)] precursor complexes were synthesized according to the method described by Morgan and Burstall,[21] wherein the relevant bpy was added to a solution of potassium tetrachloroplatinate in either acidified or neutral water at 95 °C. The bpy−acyl-thiourea ([Pt(diimine)(L-O,S)]$^+$) complexes were synthesized via the dropwise addition of the relevant acyl-thiourea and triethylamine (Et_3N) in DMF, acetone, or acetonitrile (MeCN) to a solution of the respective [$PtCl_2$(diimine)] complex in either DMF, acetone, or MeCN. To determine the influence of the Cl^- counterion, salt metathesis was used for the counterion exchanges between the Cl^--containing *tert*-butyl analog (**C1**) and NH_4PF_6 or $AgNO_3$ (Scheme 1). The complexes were characterized via ^1H and ^{13}C NMR spectroscopy (Figures S1−S37). The *in vitro* antiplasmodium activities of the synthesized compounds against the ABS parasites and early- and late-stage gametocytes were evaluated using previously reported methods[22−24] (Table 1). The antimalarial compounds CQ, methylene blue (MB), and MMV390048 (a clinically validated *Plasmodium* phosphatidylinositol 4-kinase inhibitor[25]) served as positive controls for these assays. For the combined metal complexes, four sites for SAR analysis (SAR1−SAR4) were explored (Figure 1).

Scheme 1. Synthesis of Mixed-Ligand Platinum(II) Complexes and Their Precursors[a]

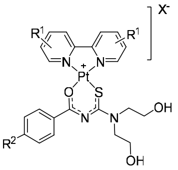


[a]Reagents and conditions: (i) Et_3N, DMF, HATU, 2 h, 27 °C/Et_3N, MeOH, HATU, 24 h, 50 °C; (ii) anhydrous DCM, 0 °C, argon, 4−20 h; (iii) H_2O, 4 M HCl/H_2O, 95 °C, 2−24 h; (iv) Et_3N, acetone, DMF or MeCN, 60 °C/80 °C, 4−24 h; (v) NH_4PF_6, anhydrous DCM/EtOH (1:1), argon, 1 h/$AgNO_3$, anhydrous MeOH, argon, 3 h.

In SAR1 (complexes **C1−C10**), the substituent at the 4,4′-positions of the bpy was varied while the substituent on the acyl-thiourea moiety remained fixed as phenyl (R^2 = H). Complexes **C1−C4** and **C6** displayed good to moderate antiplasmodium activity against the *Pf*NF54 and *Pf*K1 strains, with **C6** being the most potent against *Pf*NF54 (IC_{50} = 36.3 nM). These findings suggest that potency against *Pf* is positively influenced by the electron-donating ability of the substituent in the 4,4-positions (**C1−C4** and **C6**) of the bpy ligand, with *tert*-butyl (**C1**), hydrogen (**C3**), and the primary amine (**C6**) substituents resulting in the highest potencies. On the other hand, complexes containing an electron-withdrawing substituent, such as chloro (**C7**), trifluoromethyl (**C8**), methyl amide (**C9**), and methyl ester (**C10**) on the bpy did not show appreciable ABS whole-cell potency (IC_{50} > 5 μM).

For SAR2 (**C11−C13**), the substituent on the bpy was kept constant (*tert*-butyl), and the *para* position of the phenyl ring of the acyl-thiourea ligand was substituted with a methyl, methoxy, or chloro group. The addition of a methyl (**C11**) or methoxy (**C12**) functionality at the R^2 position resulted in complexes that displayed equipotent activity against *Pf*NF54 compared to the parent complex (**C1**). However, with a chloro

Table 1. Biological and Pharmacological Data including IC_{50} Values for Whole-Cell Potency against *Pf*NF54 and *Pf*K1 ABS Parasites,[a] Early (II/III) and Late (IV/V) Gametocyte Stage Parasites,[b] and Cytotoxicity against the CHO Cell Line, as well as an Evaluation of Solubility and Microsomal Metabolic Stability



General structure of **C1-C17**

Compound	R^1	X^-	R^2	*Pf*NF54 IC_{50} (nM)	*Pf*K1 IC_{50} (nM)	RI	EG/LG IC_{50} (nM)	CHO IC_{50} (μM)	Aqueous solubility (μM) pH 6.5	Microsomal metabolic stability (% remaining after 30 min)	
										Mouse	Human
2*				>5000	ND	NA	ND	>50	ND	ND	ND
K_2PtCl_4*				>5000	ND	NA	ND	>50	ND	ND	ND
3a*				>5000	ND	NA	ND	>50	ND	ND	ND
C1	*tert*-butyl	Cl	H	151 ± 17	433 ± 37	2.9	6362 / 3539 ± 1639	17	95	37	24
C2	Me	Cl	H	1415 ± 276	541 ± 44	0.4	>20 000/ >20 000	>50	170	72	70
C3	H	Cl	H	142 ± 12	184 ± 22	1.3	>20 000/ >20 000	>50	140	75	72
C4	MeO	Cl	H	266 ± 29	164 ± 7	0.6	>20 000/ >20 000	>50	195	88	63
C5	OH	Cl	H	>5000	ND	NA	>20 000/ >20 000	>50	ND	ND	ND
C6	NH_2	Cl	H	36.3 ± 0.6	102 ± 13	2.8	ND/>20 000	>50	100	94	43
C7	Cl	Cl	H	>5000	ND	NA	>20 000/ >20 000	>50	ND	ND	ND
C8	CF_3	Cl	H	>5000	ND	NA	>20 000/380 ± 198	>50	ND	46	47
C9	CONHMe	Cl	H	>5000	ND	NA	>20 000/ >20 000	>50	ND	ND	ND
C10	COOMe	Cl	H	>5000	ND	NA	>20 000/ >20 000	>50	<5	ND	ND
C11	*tert*-butyl	Cl	Me	114 ± 8	29 ± 10	0.3	3030/216 ± 56	30	30	37	41
C12	*tert*-butyl	Cl	OMe	224 ± 13	13 ± 10	0.1	4610/405 ± 75	25	25	45	51
C13	*tert*-butyl	Cl	Cl	52 ± 4	252 ± 83	4.9	>20 000/ >20 000	23	23	47	33
C14	5,5-dimethyl	Cl	H	241 ± 40	886 ± 59	3.7	ND	>50	>50	ND	ND
C15	6,6-dimethyl	Cl	H	2908	2764	0.9	9509/1413 ± 35	5	5	ND	ND
C16	*tert*-butyl	PF_6	H	124 ± 37	252 ± 94	2.0	>20 000/8922	11	11	ND	ND
C17	*tert*-butyl	NO_3	H	14 ± 8	135 ± 41	9.6	3184/3647	16	16	ND′	ND
CQ				11 ± 2	143 ± 3	12.7	ND	ND	ND	ND	ND
MB				NA	NA	NA	190/900	ND	ND	ND	ND
MMV390048				NA	NA	NA	215/134	ND	<5	>95	>95

[a] SYBR Green I-based proliferative assay over 96 h. Precursor compounds with an asterisk were tested by using a 72 h pLDH assay. [b] Luciferase reporter assay over 48 h. Shown are mean IC_{50} ± S.E. values from three independent biological repeats ($N = 3$), each performed in technical triplicates ($n = 3$). Absence of S.E. indicates $N = 1$. RI is the resistance index (RI = *Pf*K1 IC_{50}/*Pf*NF54 IC_{50}). Cytotoxicity IC_{50} was determined using the activity of the mixed-ligand platinum(II) against the mammalian CHO cell line ($N, n = 1, 3$). The solubility and microsomal metabolic stability of selected compounds against humans and mice are also reported. Abbreviations: CQ, chloroquine; MB, methylene blue; *Pf*, *Plasmodium falciparum*; EG, early-stage gametocytes; LG, late-stage gametocytes; ND, not determined; NA, not applicable.

substituent in this position (**C13**), the whole-cell potency improved 3-fold against *Pf*NF54 compared to **C1**. This contrasts with what was observed for SAR1, in which analogs with an electron-withdrawing substituent were less potent against the ABS. Furthermore, complexes **C11** and **C12** were better tolerated in *Pf*K1 with resistance indices (RIs) of <1, demonstrating that they are not cross-resistant with CQ.

For SAR3, salt metathesis was performed to exchange the Cl^- counterion with a hexafluorophosphate (PF_6^-) or nitrate (NO_3^-) ion. While the PF_6^- analog (**C16**) showed comparable activity to the Cl^- derivative (**C1**), interestingly the NO_3^- analog (**C17**) showed an 11-fold increase in activity, which could potentially arise from increased nitric oxide (NO) produced from NO_3^-. NO_3^- is an oxidant of NO[26] and can be produced via the nitrate−nitrite−NO synthase pathway in

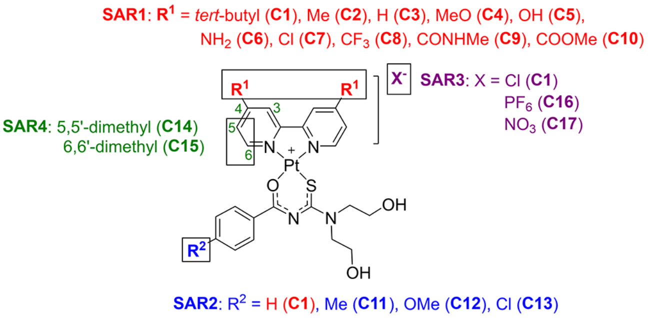


Figure 1. Mixed-ligand platinum(II) complexes showing the four SAR sites and their respective substituents.

mammalian cells.[27] NO protects against severe malaria, highlighting its therapeutic potential.[28] However, to the best of our knowledge, there are no reports on the effects of counterion exchanges from Cl^- to NO_3^- in compounds tested *in vitro* for antiplasmodium activity.

Lastly, the pharmacological effect of substitution with a methyl group at different positions on the bpy ring was determined (SAR4). Changing from the 4,4′-positions (**C2**) to the 5,5′-positions (**C14**) improved the *Pf*NF54 activity by 5-fold, whereas changing to the 6,6′-positions (**C15**) decreased the activity by 2-fold. It is noteworthy that the metal salt (K_2Cl_4Pt) and precursor compounds **2a** and **3a** did not inhibit *Pf* proliferation at a maximum concentration of 5 μM against *Pf*NF54, highlighting the structural importance of combining the two moieties to observe appreciable antiplasmodium activity.

All the complexes were tested for their early- and late-stage gametocyte activity in a dual-point assay (1 μM and 5 μM). Complexes with inhibition of viability of >50% at 1 μM and >70% at 5 μM were further tested in full dose−response assays to determine their IC_{50} values (Table S2). Most of the compounds containing the bulky hydrophobic *tert*-butyl (**C1**, **C11**, **C12**, and **C17**) or trifluoromethyl (**C8**) substituent on the bpy rings exhibited gametocidal activity (IC_{50} < 5 μM). Additionally, complex **C15** with a 6,6-dimethyl substituent on the bpy was active against both early-stage gametocytes (EGs) and late-stage gametocytes (LGs), with a moderate potency of 1413 ± 35 nM against the LGs. Complexes **C11** (R^2 = Me) and **C12** (R^2 = MeO) showed a ≥1.4-fold improvement in activity against EGs compared to **C1**. Additionally, complexes **C11** and **C12** had a >10-fold increase in LG activity compared to EG activity, with their LG IC_{50} values below 1 μM. Complex **C8** did not exhibit ABS activity but showed potent activity against LGs with an IC_{50} of 380 ± 198 nM, an uncommon phenotype for compounds active against gametocytes. Therefore, within this series of compounds, three have potent ABS activity with moderate gametocidal activity (**C1**, **C16**, and **C17**), two have potent dual-stage activity (**C11** and **C12**), and one is selective toward late-stage gametocytes (**C8**). A difference in the potencies of the complexes against the ABS parasites compared to gametocytes is often observed for antimalarials and can arise due to several factors.[29,30] These factors include differences in the stage-specific expression of the target of the compound and its capacity to diffuse across the cell membrane of the parasite in a particular life cycle stage.[31] These results are promising for the potential future development of new dual-stage-acting antimalarials capable of disease treatment and blocking the transmission of mature gametocytes from the human host to the mosquito vector.

Additionally, the cytotoxicity of the metal complexes against the Chinese hamster ovary (CHO) cell line was determined (Table 1). These data indicate that all the complexes with submicromolar ABS activity are selective for *Pf* (selectivity indices > 100) and have a negligible effect on the mammalian CHO cells tested.

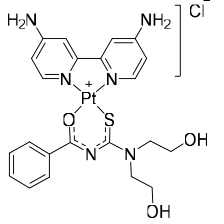
An HPLC-based method was used to determine the kinetic solubility of the selected synthesized metal complexes. All the compounds tested from SAR1 exhibited good solubility with values ranging between 95 and 195 μM (Table 1). The complexes belonging to SAR2 were poorly soluble, except for **C13**, which recorded a solubility of 150 μM. In a series of organic compounds, a decrease in lipophilicity generally increases solubility. However, in the case of these metal complexes, the solubility of the compound increased with higher lipophilicity of the bpy substituent (based on the Craig plot). This deviation from the trend seen in organic compounds could potentially be linked to the dissociation capacity of the anion (Cl^-) and the cationic species, which likely varies between the different analogs. For this series, compounds containing the bulkier *tert*-butyl substituent were less soluble than those with the smaller substituents (Me, H, OH, and NH_2), but no trend was seen within these smaller moieties. Complex **C4**, containing the water-solubilizing MeO substituent at the 4,4′-positions on the bpy, was the most soluble.

A subset of complexes with an aqueous solubility above 10 μM were profiled for microsomal metabolic stability. A range of metabolic stabilities were observed for the tested complexes, with <50% being considered poor and >85% being considered good (Table 1). The microsomal stability of the most active complex against *Pf*NF54, **C6**, was determined to be 94% in the presence of mouse liver microsomes (MLM) but only 43% in the presence of human liver microsomes (HLM), highlighting the species differences in the metabolism of this complex. Unsurprisingly, the analogs containing the *tert*-butyl substituent were moderately to poorly stable in the presence of both MLM and HLM, presumably via oxidative metabolism, as previously reported.[32]

Given the suitable microsomal metabolic stability profile of **C6** (MLM, 94%), good aqueous solubility (100 μM), and potent whole-cell activity (36.3 nM), an *in vivo* pharmacokinetic (PK) experiment was conducted with this analog. In this regard, three replicates of female Balb/C mice were dosed at either 3 mg/kg (intravenously, i.v.) or 10 mg/kg (orally, p.o.). Blood was sampled at specific time intervals over 48 h. The corresponding PK parameters were calculated using noncompartmental analysis (Table 2). PK analysis showed that **C6** exhibited a high steady-state volume of distribution (V_{ssu} = 4560 mL/kg (i.v.)) and high clearance (CL_u > 1000 mL min^{-1} kg^{-1} (p.o.)) of the unbound fraction, contributing to the low bioavailability (3.8% (p.o.)) which results in a lower amount of the test compound in systemic circulation.

It is known that Fe^{2+}/Fe^{3+} redox chemistry has an important role in the bioactivity of ferrocene derivatives.[33] The production of reactive oxygen species (ROS) is one of the modes of action of ferroquine and other ferrocene derivatives[34] and is linked to the correlation between their bioactivity and electrochemical behavior. We determined whether a similar correlation could be made for the mixed-ligand platinum(II) complexes reported herein given that metal centers have the potential to be oxidized and reduced. The electrochemistry of the complexes was studied using cyclic voltammetry (CV),

Table 2. Pharmacokinetic, Permeability, and Protein Plasma Binding Parameters of Complex **C6**[a]



V_{ss} (L/kg) i.v.	45.6
V_{ssu} (L/kg) i.v.	4560
CL_s (mL/min/kg) i.v.	46.4
CL_u (mL/min/kg) i.v.	>1000
$T_{1/2}$ (h) p.o/i.v.	12.0/10.8
Bioavailability (%)	3.8
Permeability ($LogP_{app}$)	-4.68
PPB (% bound)	99%
$\mathbf{F_u}$	0.01

[a] Abbreviations: V_{ss}, steady-state volume of distribution; V_{ssu}, steady-state volume of distribution of unbound fraction ($V_{ssu} = V_{ss}/F_u$); CL_s, clearance; CL_u, clearance of unbound fraction ($Cl_u = Cl_s/F_u$); $T_{1/2}$, half-life; P_{app}, apparent permeability; PPB, plasma protein bound; F_u: fraction unbound ($F_u = (100 - PPB)/100$).

which uses a triangular potential waveform. The CV experiments were performed in either dry MeOH or DMF containing tetrabutylammonium chloride (TBACl) as the supporting electrolyte. The working electrode was glassy carbon with a platinum wire as the counter electrode and Ag/AgCl as the reference. The anodic (E_{pa}) and cathodic (E_{pc}) peak potentials at 0.1 V/s and half-wave potentials ($E_{1/2}$) were measured (Table 3). All of the tested complexes undergo reversible one-electron $Pt^{2+/}Pt^{+}$ reductions (Figures S38−S46). Complexes **C2**, **C4**, and **C8** undergo a second nonreversible reduction (E_{pc2}), which is most likely ligand-based and linked to reductions of the substituent on the bpy. Looking at SAR2, the more electron-withdrawing the substituent, the lower the $E_{1/2}$ (V vs Fe/Fe^{+}) value was. This trend was expected, as a more electron-withdrawing substituent reduces the electron density around the metal center, making it easier for the metal to be reduced from Pt^{2+} to Pt^{+}. The same trend is seen for most of the complexes in SAR1, as the $E_{1/2}$ increases in going from the more electron-withdrawing substituent to the less electron-withdrawing substituent (**C8** < **C3** < **C2** < **C1**). However, unlike ferrocenyl compounds, these complexes do not undergo oxidation, suggesting that the production of ROS is unlikely to be one of the mechanisms of action of these complexes. This is also the case for other bpy-containing platinum(II) complexes that only undergo reductions.[35,36]

Furthermore, no correlation between the half-wave potentials and whole-cell potency of the complexes was observed, and their electrochemical properties are unlikely to contribute to the differences in their pharmacological response.

Although the data suggest that these complexes may not generate ROS via their own redox chemistry, they could potentially disrupt the redox equilibrium within the cell via an alternative mechanism. Platinum complexes are well-known for their capacity to bind to sulfur-containing molecules and proteins, including glutathione (GSH), although this often leads to reduced drug potency.[37] Conversely, GSH can also act as an activator to reduce the oxidation state of metal complexes, subsequently forming a more active species.[38] This is likely to occur via oxidation of GSH to glutathione disulfide (GSSG), which can simultaneously also produce ROS.[38] In the ABS of *P. falciparum*, GSH is an important antioxidant that removes toxic free radicals, often produced during the mitochondrial electron transport chain or heme detoxification processes.[39] Thus, GSH contributes to maintaining the redox equilibrium within the parasite. We therefore evaluated the capacity of selected complexes (**C1** and **C6**) to bind to GSH using a UV−vis absorption titration protocol as previously described.[40] Despite an increasing GSH concentration, the UV−vis spectra show a minimal decrease in absorbance (Figures S47 and S48), indicative of weak binding. Thus, these GSH binding studies show that the tested platinum analogs only bind weakly to GSH with log *K* values of 2.45 ± 0.05 and 2.58 ± 0.19 for **C1** and **C6**, respectively (Table S1). Consequently, it is unlikely that these platinum complexes will elicit their biological efficacy via disruption of the GSH-mediated equilibrium of ROS within the mitochondrion.

Additionally, platinum complexes such as oxaliplatin and cisplatin can cause hemolysis and in turn hemolytic anemia.[41,42] Therefore, to evaluate the *in vitro* hemolytic activity of a subset of the platinum(II) complexes reported herein (**C1**, **C6**, and **C17**), a hemolysis assay was conducted in a 96-well plate with a starting drug concentration of 10 μM. Triton X-100, a known hemolytic agent, and CQ, which does

Table 3. Electrochemical Data for Mixed-Ligand Platinum(II) Complexes[a]

Compound	E_{pa1} (V)	E_{pc1} (V)	E_{pc2} (V vs Ag/Ag^{+})	$E_{1/2}$ (V vs Ag/Ag^{+})	$E_{1/2}$ (V vs Fe/Fe^{+})
C1	−1.233	−1.127		−1.252	−1.670
C2	−1.074	−1.016	−1.084	−1.045	−1.585
C3	−1.210	−1.014		−1.131	−1.530
C4	−1.136	−1.064	−1.515	−1.100	−1.640
C6	−1.138	−1.068		−1.103	−1.643
C8	−0.710	−0.607	−1.272	−1.183	−1.076
C11	−1.239	−1.128		−1.190	−1.601
C12	−1.247	−1.133		−1.190	−1.608
C13	−1.233	−1.119		−1.176	−1.594

[a] $E_{1/2}$ was corrected to ferrocene for comparison. Abbreviations: E_{pc}, cathodic peak potential; E_{pa}, anodic peak potential; $E_{1/2}$, half-wave potential.

not induce hemolysis *in vitro*, were used as the controls. Triton X-100 causes a dose-dependent increase in the percentage of lysed red blood cells (RBCs) over a 72 h period (Figure S49). Contrastingly, complexes **C1**, **C6**, and **C17** do not induce hemolysis when tested up to 10 μM (Figure S49). Furthermore, the hemolytic activity of these compounds remained relatively constant when RBCs were incubated for both 1 and 72 h. Therefore, these complexes are unlikely to elicit their biological activity by causing morphological damage to RBCs.

In summary, a series of mixed-ligand platinum(II) complexes, most of which are novel compounds (**C4**−**C17**), were synthesized. Four SAR sites were explored, and it was found that, for SAR1, ABS activity was highly dependent on the presence of an electron-donating group at the 4,4′-positions of the bpy ligand. For SAR2, substituting hydrogen with chloride improved the potency 3-fold compared to the parent compound **C1**. Generally, the *Pf*K1 strain was more sensitive to these SAR2 analogs than the *Pf*NF54 strain. For SAR3, the counterion exchange of Cl$^-$ with NO$_3^-$ improved the activity 11-fold, highlighting the importance of exploring this SAR in metal complexes. Changing the methyl substituent from the 4,4′-positions (**C2**) to the 5,5′-positions (**C14**) on the bpy improved the *Pf*NF54 activity by 6-fold, whereas changing it to the 6,6′-positions (**C15**) decreased the activity by 2-fold. This suggests that having a substituent at the 6,6′-positions on the bpy may result in steric hindrance. Furthermore, six analogs showed inhibition of late-stage gametocyte viability at <5 μM. Unlike FQ, these complexes do not undergo oxidation and are therefore unlikely to act via oxidative stress. Additionally, no correlation was observed between the half-wave potentials of the tested complexes and their whole-cell potencies, suggesting that the activity is not significantly affected by the redox properties of the different analogs. This was further supported by GSH UV−vis titrations, which showed negligible binding of the tested complexes to GSH. Furthermore, these complexes do not induce *in vitro* hemolysis over a 72 h incubation period. The aqueous solubility of all tested complexes was good (≥ 95 μM), and the microsomal metabolic stability ranged from 24−94% remaining after 30 min of incubation in the presence of both mouse and human liver microsomes. Most of these reported compounds exhibited no CQ cross-resistance, with five complexes showing greater activity in the *Pf*K1 strain, underscoring the importance of considering metal complexes in the fight to overcome antimalarial drug resistance. The ABS growth inhibition data along with the gametocyte activity suggest that the SAR2 complexes in particular can be further optimized as potent dual-stage antiplasmodium agents.

Safety. No unexpected safety hazards were encountered during this work. For the synthesis of the platinum complexes, the standard precautions were taken, and protective clothing was worn when working with toxic and corrosive chemicals such as triethylamine and hydrochloric acid. The solvents used in this study, dimethylformamide, methanol, ethanol, dichloromethane, acetone, and acetonitrile, are flammable and hazardous. They should be kept away from heat, sparks, open flames, and other sources of ignition. All biological studies were performed under approved biosafety protocols at the BSL-2 level.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.4c00545.

Full experimental details, compound characterization data, cyclic voltammograms, glutathione binding and hemolysis studies (PDF)

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Author Contributions

F.-Z.I. synthesized the complexes, analyzed SARs, performed CV, binding and hemolysis experiments, and wrote the manuscript. D.C., S.T., M.L., S.d.R., and L.-M.B. performed all ABS and gametocyte assays. M.N. and L.G. performed and analyzed the pharmacokinetic and physicochemical studies. J.G.W. and K.J.W. edited the manuscript with input from all authors. J.G.W., the late T.J.E., K.J.W., and K.C. conceived or supervised the work.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

^{1}H and ^{13}C NMR, proton and carbon nuclear magnetic resonance; ABS, asexual blood stage; bpy, 2,2′-bipyridine; CHO, Chinese hamster ovary; Cl_u, clearance; CQ, chloroquine; CQR, chloroquine-resistant; CV, cyclic voltammetry; DMF, dimethylformamide; Et_3N, triethylamine; FQ, ferroquine; F_u, fraction unbound; GSH, glutathione; GSSG, glutathione disulfide; HLM, human liver microsomes; i.v., intravenously; MB, methylene blue; MeCN, acetonitrile; MeOH, methanol; MLM, mouse liver microsomes; p.o., orally; P_{app}, apparent permeability; *Pf*, *Plasmodium falciparum*; PK, pharmacokinetic; pLDH, plasmodium lactate dehydrogenase; PPB, plasma protein bound; RI, resistance index; ROS, reactive oxygen species; SAR, structure−activity relationship; $T_{1/2}$, half-life; V_{ss}, steady-state volume of distribution

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