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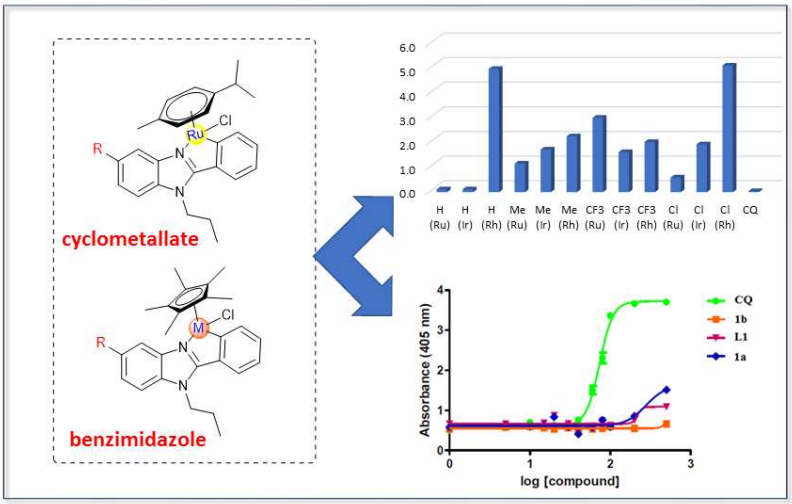


Structure-activity relationship studies of antiplasmodial cyclometallated ruthenium(II), rhodium(III) and iridium(III) complexes of 2-phenylbenzimidazoles

Laa-iqa Rylands[a], Athi Welsh[a], Keletso Maepa[b], Tameryn Stringer[a], Dale Taylor[b], Kelly Chibale[a,c], Gregory S. Smith[a,*]

[a]*Department of Chemistry, University of Cape Town, Rondebosch, Cape Town, 7701, South Africa*
[b]*Department of Medicine, Division of Clinical Pharmacology, University of Cape Town, Observatory, Cape Town, 7925, South Africa*
[c]*South African Medical Research Council Drug Discovery and Development Research Unit, Department of Chemistry and Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Rondebosch, Cape Town, 7701, South Africa*

Abstract

Benzimidazoles, such as albendazole, thiabendazole and omeprazole have antiplasmodial activity against *Plasmodium falciparum* and are widely used as scaffolds for metal-based drug research. Incorporating substituents with various lipophilic and electronic properties can influence trans-membrane interactions and concomitantly improve the biological activity. To study structure-activity relationships, a series of 2-phenylbenzimidazole and their corresponding Ru(II), Ir(III) and Rh(III) cyclometallated complexes were synthesized and evaluated for antiplasmodial activity against the chloroquine-sensitive (NF54) strain of the human malaria parasite *Plasmodium falciparum*. Selected metal complexes were further screened against the multidrug-resistant (K1) strain. In general, the 2-phenylbenzimidazole ligands showed weak antiplasmodial activities (IC_{50} ~ 17.66 – 22.32 µM) while an enhancement of antiplasmodial activity was observed upon coordination of the ligands with either ruthenium, iridium or rhodium. The new cyclometallated complexes were found to be active against both parasite strains, with IC_{50} values in the low to submicromolar range (0.12 - 5.17 µM). In addition, the metal complexes have relatively low cytotoxicity against mammalian Chinese Hamster Ovarian (CHO) cells.

Keywords: Bioorganometallic chemistry, Benzimidazoles, Half-sandwich complexes, antimalarials

1. Introduction

Malaria is one of the most widespread parasitic diseases, largely affecting developing countries of sub-Saharan Africa. According to the World Health Organisation, over 216 million cases and a resultant 445 000 deaths were reported in 2016 [1]. The current recommended treatment for malaria is the artemisinin-based combination therapy (ACT) regimen, which is based on combining a short duration acting artemisinin derivative with along acting partner drug [2]. However, reports of decreased efficacy, reduced parasite clearance time in case of the ACT regimen, and widespread resistance by Plasmodium parasites to previously effective drugs, necessitates the exploration of new chemotypes which are chemically and structurally diverse. In this regard, the benzimidazole chemotype has been identified as a promising drug development scaffold. Compounds containing the benzimidazole moiety have previously displayed interesting biological activities as antitumor agents, antiretrovirals and antimalarials [3-6]. Several recent publications reported various benzimidazole derivatives with different substitution

*Corresponding author.
Email address: gregory.smith@uct.ac.za

patterns, possessing potent antiplasmodial activities [4, 7, 8]. The mechanism of action of these benzimidazoles is still unclear, with no conclusive mode of action reported. However, there are reports of benzimidazoles and related compounds targeting the haemoglobin degradation pathway, [9] dihydroorotate dehydrogenase (DHODH) [10] and plasmepsins [11]. In addition to promising biological activities, benzimidazoles also provide the prospect for structural diversification through metal complexation, by virtue of the presence of several donor atoms.

Over the past decade, the field of bioorganometallic chemistry has provided promising alternatives to traditional organic drugs. The success of cisplatin and related platinum-based drugs has demonstrated that the use of metals in medicine may be a useful strategy in drug design and development [12]. Consequently, this sparked research efforts into the synthesis of new transition metal complexes with interesting and occasionally, multifunctional biological properties [13-16]. It has been well-documented that the biological activity of an organic compound may be enhanced upon conjugation of a metal with a particular pharmacophore [17, 18]. With regard to antimalarial drugs, several research articles have reported the synthesis and promising antimalarial activity of various metal complexes of chloroquine [19, 20]. This is exemplified by ferroquine, an organometallic ferrocenyl analogue of chloroquine that is currently one of the pipeline candidates in the Medicines for Malaria Venture (MMV) portfolio and is in phase IIb combination clinical trials with artefenomel, a trioxalane [21]. There is clear evidence that the introduction of a metal can be advantageous, through which various oxidation states, geometries and reactivities can be accessed [19]. Indeed, exploring the effect of metal complexation remains one of the major driving forces for bioorganometallic studies [21].

In recent years, the synthesis and biological evaluation of cyclometalated complexes have been extensively investigated [22-24]. The strong M-C σ bond contained within the chelating ring of a cyclometalated complex may help prevent biological reduction and ligand exchange reactions [25]. Moreover, structural and electronic properties can be fine-tuned by modifying the bidentate (*C,N*-) ligand and/ or other ancillary ligands [25], which may result in the enhancement of the overall activity of the compound. Thus, cyclometalated complexes may aid in stabilising the metal complex under biological conditions while also introducing chemical diversity.

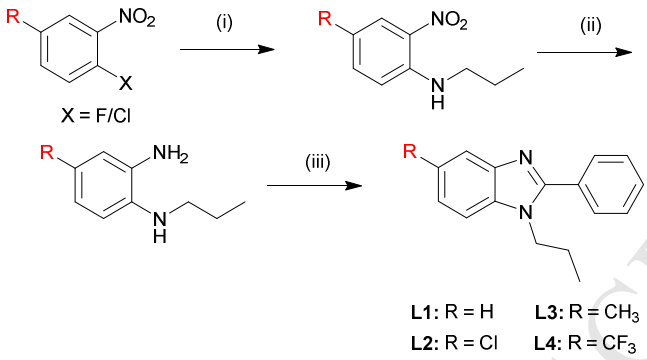
Herein we report on the structure-activity relationship studies of 2-phenylbenzimidazole ligands and their cyclometalated complexes, varying the electron-withdrawing/ donating/hydrophobicity properties at the 5-position of the benzimidazole ring. 2-Phenylbenzimidazoles were chosen specifically because of their known ability to readily form stable metal complexes through cyclometallation, which further enabled an investigation into the effects of metal complexation on bioactivity.

2. Results and discussion

2.1 Synthesis and characterisation

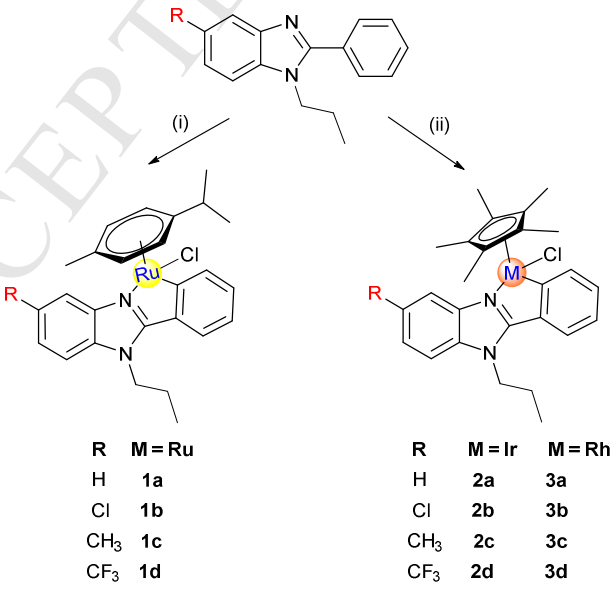
Earlier work performed in our labs and those of others [4,7,8] have revealed benzimidazoles as promising scaffolds to target *Plasmodium falciparum*. In this study, we focused on halogen, methyl trifluoromethyl and cyano-substituted 2-phenylbenzimidazoles. These were chosen from the Craig Plot which is often used in rational drug design, and which allows for probing variation in terms of hydrophobicity and electron-withdrawing/donating properties. The general synthetic strategy towards the phenylbenzimidazole ligands involved the condensation and cyclisation of substituted 1,2-

diaminobenzenes with various aromatic benzaldehydes [26], to produce the required 2-phenylbenzimidazoles, as outlined in Scheme 1. The first step towards the synthesis of the 2-phenylbenzimidazole ligands involved the formation of an appropriate nitroaniline derivative, that was reduced using a zinc in the presence of ammonium chloride to afford the required substituted 1,2-diaminobenzenes. The diamines were reacted with benzaldehyde, in a condensation/cyclisation reaction, to afford the substituted 2-phenylbenzimidazole ligands (**L1-L4**) in moderate yields (37 – 62%).



Scheme 1 General synthetic approach to 2-phenylbenzimidazole ligands. Reagents and conditions: (i) *n*-$PrNH_2$/ DMF/ rt; (ii) Zn/ NH_4Cl/ MeOH/ rt; (iii) Benzaldehyde/ TFA/ $MgSO_4$/ EtOH/ rt.

The 2-phenylbenzimidazole ligands (**L1** – **L4**) were cyclometallated via sodium acetate-mediated C-H activation reaction to produce the corresponding cyclometalated complexes (**1** – **3**, Scheme 2). To achieve this transformation, the 2-phenylbenzimidazole ligands were dissolved in dichloromethane and reacted with 1.2 equivalents of NaOAc and 0.5 equivalents of either the dichloro(*p*-cymene)ruthenium(II) dimer or the pentamethylcyclopentadienyliridium(III) /rhodium(III) dimer. The ruthenium, iridium and rhodium complexes (**1-3**) were isolated as either green, yellow or red solids in good to excellent yields (40 – 90%) and were characterized using standard spectroscopic and analytical techniques.



Scheme 2 Transformation of 2-phenylbezimidazoles to Ru, Ir and Rh cyclometallated complexes. Reagents and conditions: (i) $[RuCl(p\text{-cymene})]_2$/ NaOAc/ DCM/ rt; (ii) $[RhCl(\eta^5\text{-}C_5H_5)]_2$ or $[IrCl(\eta^5\text{-}C_5H_5)]_2$ / NaOAc/ DCM/ rt.

Evidence of metal coordination is provided in the aromatic region of the ^{1}H NMR spectra, integrating for a total of seven protons confirming that cyclometallation had indeed taken place. From the ^{1}H NMR spectra of the ruthenium complexes, four separate doublets between 6.02 – 5.29 ppm, a multiplet at 2.15 ppm, a singlet at 1.96 ppm and two doublets between 0.82 and 0.72 ppm were observed. These resonances correspond to the p-cymene ancillary ligand and confirmed the formation of the ruthenium complex. Interestingly, as a result of the p-cymene ligand having less freedom of rotation in the metal-benzimidazole complex, the aromatic protons of the p-cymene moiety appeared as four separate doublets (6.02, 5.79, 5.59, 5.29 ppm) as opposed to two doublets observed in the precursor metal dimer. Similarly, the methyl protons of the isopropyl group appear as two distinct doublets (0.82 and 0.72 ppm), due to the chirality induced by the metal centre. In the ^{1}H NMR spectrum of the Ru and Ir complexes, two distinct peaks were also observed at 4.45 and 4.31 ppm and were subsequently assigned to the methylene protons adjacent to the tertiary amine. Heteronuclear single quantum coherence spectroscopy (HSQC) confirmed that the two proton signals are assigned to one carbon peak. The change from an apparent triplet before complexation to two distinct signals is likely due to the methylene protons being diastereotopic, because of the stereogenic centre induced at the metal after complexation.

Similarly, in the ^{1}H NMR spectrum of the Ir/Rh Cp* cyclometallated benzimidazole complexes, the absence of one aromatic proton, relative to the benzimidazole starting material, supported metal complexation. Additionally, the appearance of a singlet at 1.76 ppm, which integrates for fifteen protons was due to the Cp* ligand and further confirmed that the metal was coordinated and that the Ir/Rh complexes were successfully synthesised. In the ^{13}C{^{1}H} NMR spectra of the Ir(III) complexes, a distinct peak assigned to the methyl substituents on the Cp* moiety was observed at approximately 9.00 ppm. IR spectroscopic data was obtained for the metal complexes, which revealed a weak absorption band around 1586 – 1577 cm^{-1}, corresponding to the C=N stretching frequencies. Normally, the C=N absorption band of the ligand is observed at ~1627 cm^{-1}, after complexation, however, a shift to ~1586 cm^{-1} is observed. A frequency shift of the C=N absorption band observed for the corresponding benzimidazoles to lower frequencies was a strong indicator of successful coordination of the nitrogen to the metal centre, a phenomenon explained by the synergistic effect.

2.2 Crystallography

The molecular structures of the Ir(III) complexes (**2c**, **2d**) and Rh(III) complexes (**3a**, **3c**) were elucidated by single crystal X-ray diffraction. Single crystals of each complex were obtained by the slow diffusion of a concentrated chloroform/ethyl acetate mixture (Figures 1-4). The crystallographic data and refinement parameters for the four complexes are presented in Table 1 and selected bond distances and angles are listed in Table 2. The iridium complexes, **2c** and **2d** crystallised as red blocks in the monoclinic space group $P2_{1/c}$ and as orange blocks in the triclinic space group $P\bar{1}$, respectively. The rhodium complexes **3a** and **3c** crystallised as red blocks in the monoclinic space group C2/c and $P2_{1/c}$, respectively. Figures 2-5 show the molecular structures of the complexes (**2c**, **2d**, **3a** and **3c**). The Ir(III) and Rh(III) complexes adopt a “three-legged piano-stool” structure, which is commonly observed in many previously reported literature articles involving rhodium and iridium half-sandwich complexes [22, 27]. In this case, the pentamethylcyclopentadienyl (Cp*) ligand forms the seat of the piano-stool and the three legs of the stool are formed from the chloride and chelating (*C,N*-) benzimidazole ligand.

The crystallographic data shows that the Rh atom (**3a**) is disordered over two positions and the Cp* ligand disordered over two places (Figure 3). The crystallographic data further suggests that the geometry around the metal centre is pseudo-octahedral, which is typically observed in piano-stool structures. The bond distances obtained for complexes **2c**, **2d**, **3a** and **3c** are comparable. The distance between the metal centre and the carbon atoms of the Cp* ligand ranges between 2.143 and 2.265 Å. The bond distance between the metal centre and the chloride ligand is approximately 2.4 Å, whereas the distance between the metal centre and the two chelating atoms ranges between 2.050 and 2.079 Å.

Table 1
Crystallographic data and refinement parameters for complexes **2c**, **2d**, **3a** and **3c**.

	Complex			
	2c	**2d**	**3a**	**3c**
Chemical formula	$C_{27}H_{32}ClIrN_2$	$C_{27}H_{29}ClF_3IrN_2$	$C_{26}H_{30}ClN_2Rh$	$C_{27}H_{32}ClN_2Rh$
Formula weight	612.22	666.19	508.88	522.90
Crystal system	monoclinic	triclinic	monoclinic	monoclinic
Space group	$P2_1/c$ (No. 14)	$P\bar{1}$ (No. 2)	C2/c (No. 15)	$P2_1/c$ (No. 14)
a, b, c (Å)	10.9895(8), 12.3159(9), 18.2121(13)	8.9926(7), 11.817(1), 12.0582(10)	14.6558(10), 17.8972(13), 17.6608(11)	10.9353(6), 12.3347(7), 18.2520(11)
α, β, γ (˚)	90, 99.465(2), 90	82.592(1), 77.641(1), 84.652(1)	90, 96.436(1), 90	90, 99.3740(10), 90
V ($Å^3$)	2431.4(3)	1238.38(18	4603.2(5)	2429.0(2)
Z	4	2	8	4
D ($g.cm^{-3}$)	1.673	1.787	1.469	1.430
μ (mm^{-1})	5.618	5.540	0.873	0.830
F (000)	1208	652	2096	1080
Crystal size (mm)	0.21 x 0.26 x 0.32	0.21 x 0.26 x 0.31	0.12 x 0.14 x 0.18	0.03 x 0.07 x 0.18
T (K)	173	173	173	173
Scan range/ ˚	1.9 < Θ < 28.4	1.7 < Θ < 28.3	2.1 < Θ < 28.3	1.9 < Θ < 28.2
Unique reflections	6077	5949	5742	6004
R_{int}	0.073	0.039	0.079	0.063
Reflections used [I > 2σ(I)]	5003	5248	4122	4757
R indices (all data)	R 0.0253, wR2 0.0532, S 1.04	R 0.0275, wR2 0.0517, S 1.02	R 0.0600, wR2 0.1541, S 1.04	R 0.0328, wR2 0.0855, S 1.04
Goodness-of-fit	1.040	1.021	1.042	1.043
Max, Min Δρ (e $Å^{-3}$)	-0.93, 1.62	-1.30, 1.00	-1.47, 0.89	-0.55, 1.05

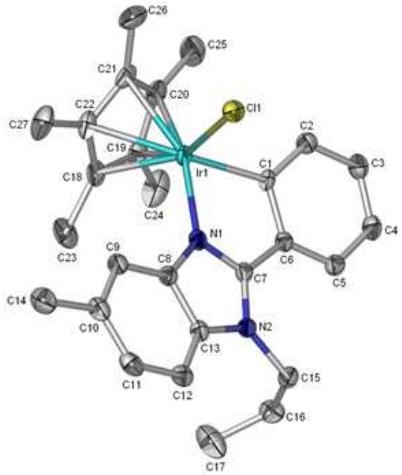


Fig. 1 Molecular structure complex **2c**, where thermal ellipsoids are drawn at 50% probability level.

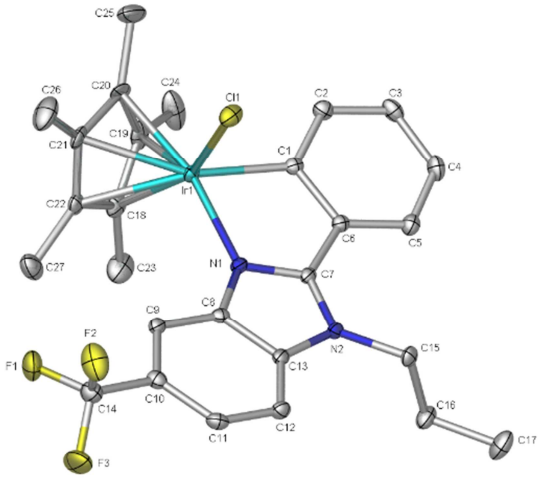


Fig. 2 Molecular structure of complex **2d**, where thermal ellipsoids are drawn at 50% probability level.

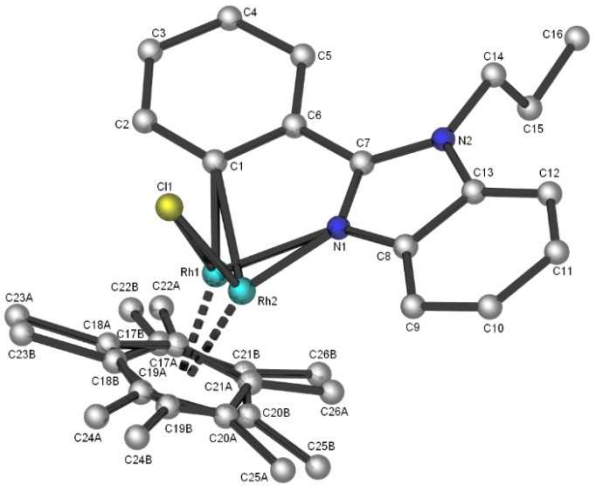


Fig. 3 Molecular structure of complex **3a**, where thermal ellipsoids are drawn at 50% probability level.

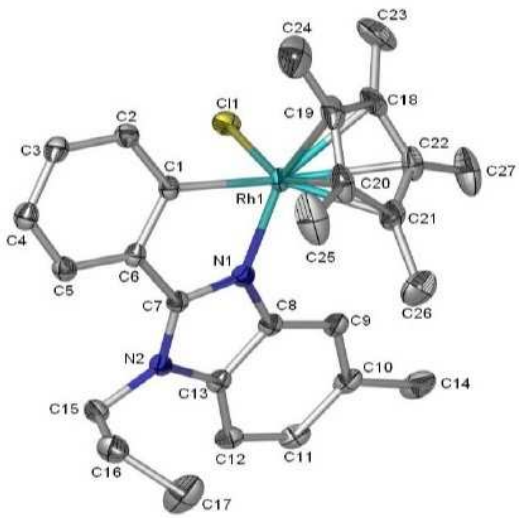


Fig. 4 Molecular structure of complex **3c**, where thermal ellipsoids are drawn at 50% probability level.

Table 2

Selected bond distances and angles.

	Complex			
	2c (Ir)	**2d** (Ir)	**3a** (Rh)	**3c** (Rh)
Selected bond distances (Å)				
M(1) – Cl(1)	2.4114(9)	2.4054(10)	2.4000(19)	2.4089(8)
M(1) – N(1)	2.072(2)	2.079(2)	2.082(5)	2.070(2)
M(1) – C(1)	2.062(3)	2.050(4)	2.029(6)	2.043(2)
N(1) – C(7)	1.345(4)	1.337(4)	1.327(6)	1.345(3)
M(1) – C(18)	2.173(3)	2.157(4)	_	2.207(4)
M(1) – C(19)	2.143(3)	2.159(4)	_	2.183(4)
M(1) – C(20)	2.177(4)	2.144(4)	_	2.140(3)
M(1) – C(21)	2.205(4)	2.247(4)	_	2.182(2)
M(1) – C(22)	2.257(3)	2.265(4)	_	2.260(3)
Selected bond angles (˚)				
Cl(1) – M(1)– C(1)	89.04(9)	87.40(10)	92.56(16)	90.72(7)
N(1) – M(1) – C(1)	77.09(12)	77.05(12)	77.6(2)	77.78(9)
Cl(1) – M(1) – N(1)	88.86(7)	86.40(7)	90.01(12)	91.85(6)

2.3 In vitro antiplasmodial activity and cytotoxicity evaluation

The synthesised C5-substituted 2-phenylbenzimidazole ligands were initially screened *in vitro* against the chloroquine-sensitive (NF54) strain of *Plasmodium falciparum* (Table 3), using the pLDH (Plasmodium lactate dehydrogenase) assay. Based on the antiplasmodial activity observed for the 2-phenylbenzimidazole ligands, the corresponding metal complexes (Ru, Ir and Rh) were also evaluated against the chloroquine-sensitive (NF54) strain and selected complexes were then further screened against the chloroquine-resistant (K1) strain (Table 4).

Table 3

In vitro antiplasmodial evaluation of 2-phenylbenzimidazole ligands (**L1**-**L4**) against the chloroquine-sensitive (NF54) strain of *P. falciparum*.

Compound	R	IC_{50} (µM) ± SD
CQ	-	0.044
L1	H	22.32 ± 0.16
L2	Cl	20.64 ± 0.81
L3	CH_3	17.66 ± 1.30
L4	CF_3	20.08 ± 1.59

NA = Not active at the tested concentration

The 2-phenylbenzimidazole ligands (**L1 – L4**) generally show weak to moderate antiplasmodial activity against the chloroquine-sensitive (NF54) strain of P. falciparum with IC50 values in the same range. Although variation of the substituents in the 5-position did not have a significant effect on the antiplasmodial activity of the ligands, it is worth noting that the electron-donating methyl substituent does slightly improve the antiplasmodial activity, as **L3** shows slightly enhanced antiplasmodial activity across the studied ligand series.

Table 4

In vitro antiplasmodial data for the ruthenium (**1**), iridium (**2**) and rhodium (**3**) cyclometallated benzimidazole complexes against the chloroquine-sensitive (NF54) and chloroquine-resistant (K1) strains of *P. falciparum* as well as cytotoxicity against CHO cells.

Compound	R	M/ L_n	IC_{50} (μM) ± SD			RI[a]	SI[b]
			NF54	K1	**CHO**		
CQ	_	_	0.044	0.29 ± 0.04		6.59	_
1a	H	Ru/ *p*-cymene	0.12 ± 0.01	2.68 ± 0.15	50.92 ± 0.81	22.33	424
1b	Cl		0.59 ± 0.04	1.85 ± 0.27	_	3.14	_
1c	CH_3		1.16 ± 0.02	0.97 ± 0.12	_	0.84	_
1d	CF_3		3.02 ± 0.15	1.04 ± 0.14	_	0.34	_
2a	H	Ir/ Cp*	0.19 ± 0.01	4.31 ± 0.27	20.71 ± 0.23	22.68	109
2b	Cl		1.94 ± 0.04	4.20 ± 0.18	_	2.16	_
2c	CH_3		1.73 ± 0.13	2.25 ± 0.15	_	1.30	_
2d	CF_3		1.62 ± 0.04	1.20 ± 0.23	_	0.74	_
3a	H	Rh/ Cp*	5.00 ± 1.53	_	_	_	_
3b	Cl		5.17 ± 0.58	_	_	_	_
3c	CH_3		2.26 ± 0.20	_	_	_	_
3d	CF_3		2.04 ± 0.03	_	_	_	_
Emetine	_	_	_	_	0.05 ± 0.03	_	_

[a] (IC_{50} K1/ IC_{50} NF54). [b] (IC_{50} CHO/ IC_{50} NF54)

The biological data obtained for the cyclometallated complexes reveal good antiplasmodial activity in the low micromolar range and there are a few discernible trends. Firstly, the antiplasmodial activity was significantly enhanced upon complexation of the either the ruthenium, iridium or rhodium metal centres with the 2-phenylbenzimidazole ligands, which suggests that the metal entity plays a vital role in improving the antiplasmodial activity. The enhanced activity may be attributed to increased trans-membrane interactions brought about by the presence of the biofriendly metal entities, or possibly through the interaction of the metal complex with the one or more targets via yet unknown mechanisms of action. Secondly, the Ru(II) cyclometallated complexes are more active in comparison with the Ir(III) and Rh(III) complexes. This is consistent acroos all three series of cyclometallated complexes. For example, in the chloroquine-sensitive strain (NF54), the IC_{50} value for the chloro-substituted ruthenium complex (**1b**) was found to be 0.59 μM, whereas that for the corresponding iridium (**2b**) and rhodium complex (**3b**) was found to be 1.94 and 5.17 μM, respectively. Ruthenium-based drugs are thought to mimic iron and interact with proteins such as serum albumin and transferrin [28,29]. However, the nature of the substituents does not seem to greatly influence the antiplasmodial activity. In general, the rhodium complexes are the least active compared to both the ruthenium and iridium cyclometallated complexes.

Consequently, only the Ru(II) and Ir(III) metal complexes were evaluated further against the chloroquine-resistant (K1) strain, a multi-drug resistant strain. The antiplasmodial data obtained for the K1 strain emphasizes the superiority of the Ru(II) complexes over the analogous Ir(III) complexes, where the antiplasmodial activity of the Ru(II) complexes was approximately 2-fold greater than the corresponding Ir(III) complexes. The low activity observed in the K1 strain compared to the NF54 strain for **1a**, **1b**, **2a** and **2b** suggests that the unsubstituted Ru/Ir

complexes (**1a** and **2a**) may be significantly less effective at targeting resistant strains of *P. falciparum* when compared with compounds **1b** and **2b**, which lose activity slightly. The relatively low resistance indices of the trifluoromethyl-substituted complexes **1d** (RI = 0.34) and **2d** (RI = 0.74) implies that these compounds maintain their antiplasmodial activity in the resistant strain and show no cross resistance. They are more active in the chloroquine-resistant strain relative to the chloroquine-sensitive strain. Both RI values are significantly lower than that of the chloroquine standard (6.59). On the contrary, the unsubstituted complexes **1a** and **2a** have relatively large RI values (22.33 and 22.68, respectively), which infers that the introduction of an appropriate substituent to the phenylbenzimidazole scaffold may be important to target resistant strains of the malaria parasite.

Cytotoxicity studies were performed using complexes **1a** and **2a** to determine whether the synthesised metal complexes are toxic to mammalian cells and to gain insight into the selectivity of the compounds towards the malaria parasites. The compounds were tested against the mammalian Chinese Hamster Ovarian (CHO) cell-line and the results are also shown in Table 4, where emetine was used as the standard control. The results indicate that the tested compounds are relatively non-cytotoxic compared to the emetine standard. The Ru(II) complex (**1a**) displayed the highest selectivity, as evidenced by its relatively high IC_{50} value (50.92 µM), and the compound's SI value (424). This highlights the selectivity of the Ru(II) complexes towards the malarial parasites over non-parasitic mammalian cells.

2.4 β-Haematin inhibition studies

The mechanism of action for quinoline-based antimalarial drugs, such as chloroquine, is thought to target the erythrocytic stage of the malaria parasites life cycle in which these quinoline-based drugs target and prevent the detoxification of the host's hematin (a toxic product of the hemoglobin degradation process) to hemozoin in the parasite's food vacuole. Planar compounds, such as chloroquine, bond to hematin via π-stacking interactions [28]. Preventing further conversion of hematin to hemozoin results in the death of the parasite due to the toxic build-up of free hematin. The inhibition of the formation of hemozoin or its synthetic counterpart, β-hematin, is therefore regarded as an attractive approach to preliminarily elucidate a possible antiplasmodial mechanism of action of metal-based compounds. The NP40 detergent-mediated assay, developed by Sandlin *et al.*, was used in this study to determine β-haematin inhibition activity [29]. This method involves the use of NP40, a neutral detergent, which mimics lipids and mediates the formation of β-haematin in this assay.

Since the benzimidazole-based compounds have moderate antiplasmodial activity against both strains of *P. falciparum* and the mechanism of action of benzimidazoles remains unknown, we thought it important perform β-haematin inhibition studies using selected compounds (**L1**, **1a** and **1b**) to shed light on a potential contributing mode of action of the synthesised 2-phenylbenzimidazole-based compounds. Figure 5 shows the respective log-based dose-response curves of the tested compounds and chloroquine (CQ), which were screened to a concentration of 500 µM.

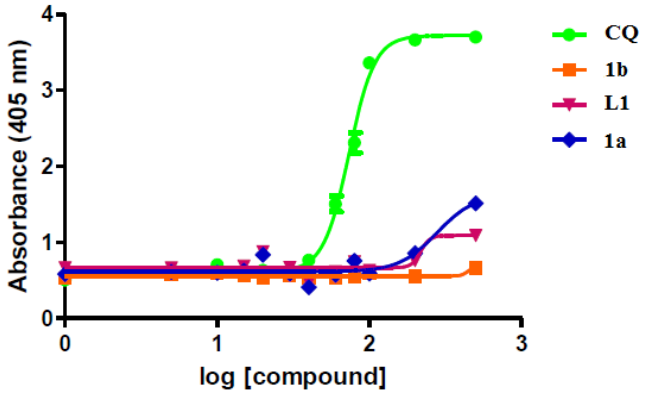


Fig. 5 β-Haematin inhibition studies of selected compounds (**L1**, **1a**, **1b** and **CQ**).

The results reveal that the compounds (**L1**, **1a**, **1b**) do not show any inhibition b-hematin at the tested concentrations. Since the absorbance is directly proportional to the concentration of free haem, CQ, as anticipated, was effective at inhibiting β-haematin formation over the tested range. However, in the case of the benzimidazole compounds, the presence of β-haematin was still observed over the tested concentration range (low absorbance, hence little to no free haem present). These results suggest that the compounds are not β-haematin inhibitors using this cell-free assay and may therefore exert their antiplasmodial effect via a different mechanism of action in the parasite, which may include, amongst others, the disruption of the Reactive Oxygen Species (ROS) balance in the parasite and/or parasite Dihydroorotate dehydrogenase (DHODH) inhibition.

3. Conclusions

A series of C5 substituted 2-phenylbenzimidazole ligands (**L1** – **L4**) and corresponding Ru(II), Ir(III) and Rh(III) cyclometallated complexes were successfully synthesised and fully characterised using spectroscopic and analytical techniques. The substituted 2-phenylbenzimidazole ligands displayed very weak activity against the chloroquine-sensitive (NF54) strain of *Plasmodium falciparum*. On the other hand, the antiplasmodial activity of 2-phenylbenzimidazoles were significantly enhanced upon cyclometallation with ruthenium, iridium and rhodium. The Ru(II) complexes generally displayed better antiplasmodial activity compared to both the Ir(III) and Rh(III) complexes. Since the rhodium complexes generally displayed weaker activity compared to both the ruthenium and iridium complexes, rhodium complexes were excluded from further screening against the chloroquine-resistant (K1) strain. A similar trend was observed in the CQR K1 strain, where the Ru(II) complexes were found to display better antiplasmodial activity than the Ir(III) complexes. Interestingly, the complexes also displayed lower resistance index values relative to CQ which, by extension, suggests that the metal complexes do not show cross-resistance with CQ. In light of their promising activity, select compounds were screened against Chinese Hamster Ovarian (CHO) cells to determine selectivity. The complexes displayed low cytotoxicity and generally high selectivity indices, supporting that the compounds are selective towards the malaria parasites.

In terms of a potential mechanism of action, the benzimidazole compounds did not show β-haematin inhibition activity in the cell-free assay at the tested concentration of 500 μM, suggesting that the compounds are unlikely candidates for hemozoin inhibition in the parasite and may therefore exert their pharmacological effect by a different mechanism. In conclusion, the compounds presented here, particularly the metal complexes, have been found to display promising antiplasmodial activity over the metal-free ligands, however, the nature of the substituent does not seem to influence the antiplasmodial effect. The striking activity maintained in the resistant strain for the ruthenium complexes warrant further investigations as a potential new class of antimalarials whose unique structure may aid in overcoming resistance mechanisms.

4. Experimental

4.1. Materials and methods

All reagents and solvents were purchased from commercial sources (Sigma Aldrich, Combi blocks, Kimix and Merck) and were used without further purification. Reactions were carried out under an inert nitrogen or argon atmosphere, unless otherwise stated. The [RuCl(p-cymene)]$_2$, [IrCl(η^5-C_5H_5)]$_2$ and [RhCl(η^5-C_5H_5)]$_2$ dimeric precursors were prepared following literature methods [30, 31]. The ligand precursors, 2-Nitro-*N*-propylaniline [32], 4-Chloro-2-nitro-*N*-propylaniline [33], 4-Methyl-2-nitro-*N*-propylaniline [34], 3-Nitro-4(propylamino)benzonitrile [35], 2-Nitro-*N*-propyl-4-(trifluoromethyl)aniline [36], N^1 Propylbenzene-1,2-diamine [32], 4-Methyl-N^1-propylbenzene-1,2-diamine [35], 3-Amino-4 (propylamino)benzonitrile [35], N^1-Propyl-4-(trifluoromethyl)benzene-1,2-diamine [37], **L1** [38] and **L3** [39] were synthesised following methods adapted from the literature.

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker XR600 MHz spectrometer (^{1}H at 599.95 MHz and ^{13}C{^{1}H} at 151.0 MHz), a Bruker XR400 MHz spectrometer (^{1}H at 399.95 MHz and ^{13}C{^{1}H} at 100.6 MHz) or a Bruker XR300 MHz (^{1}H at 299.95 MHz) using tetramethylsilane (TMS) as the internal standard. Infrared (IR) spectroscopy was performed on a Perkin-Elmer Spectrum 100 FT-IR spectrometer using Attenuated Total Reflectance (ATR) with vibrations measured in units of cm^{-1}. Mass Spectrometry (MS) determinations were carried out using Electron Impact (EI) on a JEOL GCmateII instrument or Electrospray Ionisation (ESI) on a Waters API Quattro Micro triple quadrupole mass spectrometer with data recorded using the positive mode. Elemental analysis (C, H, N) were performed using a Fissions EA 110 CHNS apparatus or an Elementar Vario EL Cube Analyser. Purity was also determined using an analytical Agilent HPLC 1260 equipped with an Agilent infinity diode array detector (DAD) 1260 UV-Vis detector, with an absorption wavelength range of 210 – 640 nm. Melting points were obtained using a Büchi Melting Point Apparatus B-540 and are uncorrected.

4.2. General synthetic procedure for the synthesis of 1,2-diamine derivatives

Nitroaniline compounds (1.eq.) were dissolved in anhydrous MeOH, under an argon atmosphere at room temperature. Ammonium chloride (10 eq.) was then added to the solution and left to stir for 5 min. Thereafter, Zinc dust (20 eq.) was added to the reaction flask. The

reaction was allowed to stir vigorously for 30 – 45 min. After the reaction was complete, the reaction mixture was filtered through Celite, and washed with copious amounts of MeOH. Excess solvent was concentrated *in vacuo* and saturated $NaHCO_3$ (aq.) was added to the resultant residue and extracted with EtOAc (2 x 30 mL). The combined organic layers were collected, dried over anhydrous Na_2SO_4 and the solvent removed to afford the product or purified further via column chromatography using EtOAc:Petroleum ether as an eluent to afford the desired products.[40]

4.2.1. 4-Chloro-N^1-propylbenzene-1,2-diamine

4-Chloro-2-nitro-*N*-propylaniline (0.739 g, 3.44 mmol) was dissolved in MeOH (100 mL). NH_4Cl (1.84 g, 34.4 mmol) and Zinc (4.52 g, 69.1 mmol) was then added to the solution. Product: Brown oil (0.214 g, 34%); R_f (EtOAc: Pet. Ether, 2:8): 0.39. Elemental Analysis for $C_9H_{13}ClN_2$ (184.67 g.mol^{-1}): Found (%) C, 57.86; H, 6.78; N, 14.02; Calcd. (%) C, 58.54; H, 7.10; N, 15.17. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.76 (dd, $^3J_{HH}$ = 8.4 and $^4J_{HH}$ = 2.4 Hz, 1H, ArH), 6.69 (d, $^4J_{HH}$ = 2.4 Hz, 1H, ArH), 6.54 (d, $^3J_{HH}$ = 8.4 Hz, 1H, ArH), 3.37 (br s, 3H, N<u>H</u>, N<u>H$_2$</u>), 3.04 (t, $^3J_{HH}$ = 6.9 Hz, 2H C<u>H$_2$</u>), 1.02 (t, $^3J_{HH}$ = 7.5 Hz, 3H, C<u>H$_3$</u>), 1.79 – 1.58 (m, 2H, C<u>H$_2$</u>). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ (ppm): 136.5, 135.5, 123.1, 120.0, 116.1, 112.6, 46.2, 22.8, 11.7
. MS (EI, *m/z*): 184.0871 (80%, $[M]^+$).

*4.3. General synthetic procedure for the synthesis of 2-phenylbenzimidazole ligands (**L1** – **L4**)*

The synthesised 1,2-diamines (1 eq.) were dissolved in EtOH. Benzaldehyde (1.2 eq.), TFA (0.1 eq.) and $MgSO_4$ (6 eq.) was added to the reaction flask, under an argon atmosphere at 25 °C. The reaction was then left to stir, open to air, for 24 h. The reaction mixture was filtered and the solvent reduced *in vacuo*. The resultant residue was dissolved in DCM (30 mL) and washed with H_2O (2 x 20 mL) and sat. NaCl (20 mL). The organic layers were collected, dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was then purified via column chromatography using EtOAc:Petroleum ether as the eluent to afford the desired compounds.[23]

*4.3.1. 5-Chloro-2-phenyl-1-propyl-1H-benzo[d]imidazole (**L2**)*

4-Chloro-N^1-propylbenzene-1,2-diamine (0.190 g, 1.03 mmol) was dissolved in EtOH (10 mL). Benzaldehyde (0.13 mL, 1.23 mmol), TFA (11.7 mg, 0.103 mmol) and $MgSO_4$ (0.743 g, 6.17 mmol) was then added to the reaction flask. Product: Brown solid (0.174 g, 62%); R_f (EtOAc:Pet. Ether, 5:5): 0.63. Mp: 84.8 – 88.6 °C. FT-IR (ATR) *v* (cm^{-1}) = 1595 (imine, C=N). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.79 (d, $^4J_{HH}$ = 1.8 Hz, 1H, ArH), 7.72 – 7.65 (m, 2H, ArH), 7.56 – 7.48 (m, 3H, ArH), 7.32 (d, $^3J_{HH}$ = 8.6 Hz, 1H, ArH), 7.26 (dd, $^3J_{HH}$ = 8.6 and $^4J_{HH}$ = 1.8 Hz, 1H, ArH), 4.26 – 4.08 (m, 2H, C<u>H$_2$</u>), 1.93 – 1.71 (m, 2H, C<u>H$_2$</u>), 0.85 (t, $^3J_{HH}$ = 8.6 Hz, 3H, C<u>H$_3$</u>).^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ (ppm): 155.1, 144.1, 134.4, 130.4, 130.1, 129.4, 128.9, 128.1, 123.2, 119.8, 111.0, 46.6, 23.3, 11.3. Purity 100% by LC (t_R 3.50 min); MS (EI, *m/z*): 270.01 (100%, $[M]^+$).

*4.3.2. 2-Phenyl-1-propyl-5-(trifluoromethyl)-1H-benzo[d]imidazole (**L4**)*

N^1-propyl-4-(trifluoromethyl)benzene-1,2-diamine (1.26 g, 5.79 mmol) was dissolved in EtOH (10 mL). Benzaldehyde (0.71 mL, 6.95 mmol), TFA (65.9 mg, 0.579 mmol) and $MgSO_4$ (4.15 g, 34.7 mmol) was then added to the reaction flask. Product: Off-white solid (0.814 g, 46%); R_f

(EtOAc:Pet. Ether, 2:8): 0.33. Mp: 64.7 – 66.0 °C. FT-IR (ATR) ν(cm^{-1}) = 1627 (imine, C=N), 1323 (Ar amine, C-N). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.12 – 8.08 (m, 1H, ArH), 7.75 – 7.66 (m, 2H, ArH), 7.60 – 7.52 (m, 4H, ArH), 7.49 (d, $^{3}J_{HH}$ = 8.4 Hz, 1H, ArH), 4.27 – 4.19 (t, $^{3}J_{HH}$ = 7.8 Hz, 2H, C$\underline{H}_2$), 1.94 – 1.76 (m, 2H, C$\underline{H}_2$), 0.87 (t, $^{3}J_{HH}$ = 7.2 Hz, 3H, C$\underline{H}_3$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ (ppm): 155.9, 142.8, 136.8, 130.3, 129.5, 129.0, 126.4 – 123.7 (d, $^{1}J_{CF}$ = 270.1 Hz, Ar-$\underline{C}F_3$), 125.2 – 124.9 (d, $^{2}J_{CF}$ = 32.1 Hz), 119.7, 117.9, 110.6, 46.7, 23.3, 11.3. ^{19}F NMR (377 MHz, $CDCl_3$) δ (ppm): -60.67. Purity 100% by LC (t_R 3.56 min); MS (*m/z*): 305.1 (100%, $[M+H]^+$).

4.4. General synthetic procedure for synthesis of Ru, Ir and Rh cyclometalated complexes

The selected 2-phenylbenzimidazole (1 eq.) was dissolved in dry DCM (6 mL) under argon. Sodium acetate (1.2 eq.) was then added, and the reaction mixture left to stir at rt for 10 min. Thereafter, the appropriate metal dimer (2 eq.) was added, in one portion. The reaction mixture was allowed to stir at rt, under argon, for 24 h, after which the reaction mixture was filtered through Celite and washed with DCM. The solvent was concentrated and diethyl ether (10 mL) was added to the residue. The mixture was then cooled to 0 °C and left to stir for 10 min. The precipitate that formed was filtered by suction and dried under vacuum.[23]

*4.4.1. N-Propyl Ru cyclometalated complex (**1a**)*

2-Phenyl-1-propyl-*1H*-benzo[*d*]imidazole **L1** (77.2 mg, 0.327 mmol) was dissolved in DCM (6 mL). Sodium acetate (32.1 mg, 0.392 mmol) and dichloro(*p*-cymene)ruthenium(II) dimer (100 mg, 0.0.163 mmol) was then added to the solution. Product: Green solid (77.0 mg, 46%). Mp: 239.3 – 247.7 °C. Elemental Analysis for $C_{26}H_{29}ClN_2Ru$ (506.054 g.mol^{-1}): Found (%) C, 60.73; H, 5.46; N, 5.78; Calcd. (%) C, 61.70; H, 5.20; N, 5.50. FT-IR (ATR) ν (cm^{-1}) = 1577 (imine, C=N). ^{1}H NMR (300 MHz,$CDCl_3$) δ (ppm): 8.34 (d, $^{3}J_{HH}$ = 7.5 Hz, 1H, ArH), 7.93 (d, $^{3}J_{HH}$ = 7.8 Hz, 1H, ArH), 7.63 (d, $^{3}J_{HH}$ = 7.8 Hz, 1H, ArH), 7.45 – 7.29 (m, 3H, ArH), 7.20 (td, $^{3}J_{HH}$ = 7.2, 1.2 Hz, 1H, ArH), 7.12 – 7.03 (td, $^{3}J_{HH}$ = 7.8, 1.2 Hz, 1H, ArH), 5.86 (d, $^{3}J_{HH}$ = 5.7 Hz, 1H, $Ar_{p\text{-}cy}$), 5.67 (d, $^{3}J_{HH}$ = 5.4 Hz, 1H, $Ar_{p\text{-}cy}$), 5.37 (d, $^{3}J_{HH}$ = 6.0 Hz, 1H, $Ar_{p\text{-}cy}$), 5.14 (d, $^{3}J_{HH}$ = 5.7 Hz, 1H, $Ar_{p\text{-}cy}$), 4.49 (m, 1H), 4.36 (m, 1H), 2.33 – 2.21 (m, 1H), 2.10 (s, 3H), 1.98 (m, 2H, C$\underline{H}_2$), 1.03 (t, $^{3}J_{HH}$ = 7.5 Hz, 3H, C$\underline{H}_3$), 0.90 (d, $^{3}J_{HH}$ = 6.9 Hz, 3H), 0.76 (d, $^{3}J_{HH}$ = 6.9 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ (ppm): 183.8, 157.8, 141.5, 140.7, 136.1, 133.9, 128.8, 124.0, 123.2, 123.0, 122.5, 117.2, 110.0, 101.5, 98.5, 89.3, 89.1, 82.0, 81.3, 46.2, 31.2, 23.1, 22.6, 21.7, 18.9, 11.2. MS (HR-ESI, *m/z*): 471.1377 (100%, $[M\text{-}Cl]^+$), requires 471.1374.

*4.4.2. N-Propyl (chloro-) Ru cyclometalated benzimidazole complex (**1b**)*

5-Chloro-2-phenyl-1-propyl-*1H*-benzo[*d*]imidazole **L2** (93.5 mg, 0.345 mmol) was dissolved in DCM (6 mL). Sodium acetate (33.9 mg, 0.414 mmol) and dichloro(*p*-cymene)ruthenium(II) dimer (106 mg, 0.173 mmol) was then added to the solution. Product: Orange solid (77.5 mg, 42%). Mp: Decomp. 197.7 °C. Elemental Analysis for $C_{26}H_{28}Cl_2N_2Ru$ (540.49 g.mol^{-1}): Found (%) C, 57.18; H, 5.32; N, 5.12; Calcd. (%) C, 57.78; H, 5.22; N, 5.18. FT-IR (ATR) ν (cm^{-1}) = 1578 (imine, C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.32 (d, $^{3}J_{HH}$ = 7.2 Hz, 1H, ArH), 7.88 (d, $^{4}J_{HH}$ = 1.8 Hz, 1H, ArH), 7.83 (d, $^{3}J_{HH}$ = 8.7 Hz, 1H, ArH), 7.79 (d, $^{3}J_{HH}$ = 8.7 Hz, 1H, ArH), 7.42 (d, $^{3}J_{HH}$ = 8.7, 1.8 Hz, 1H, ArH), 7.11 (m, 2H, ArH), 6.04 (d, $^{3}J_{HH}$ = 6.0 Hz, 1H, $Ar_{p\text{-}cy}$), 5.76 (d, $^{3}J_{HH}$ = 6.0 Hz, 1H, $Ar_{p\text{-}cy}$), 5.63 (d, $^{3}J_{HH}$ = 6.0 Hz, 1H, $Ar_{p\text{-}cy}$), 5.30 (d, $^{3}J_{HH}$ = 6.0 Hz, 1H, $Ar_{p\text{-}cy}$), 4.62 (t, $^{3}J_{HH}$ = 6.9 Hz 2H), 2.19 – 2.04 (m, 1H), 1.97 (s, 3H), 1.80 (m, 2H, C$\underline{H}_2$), 0.87 (t, $^{3}J_{HH}$ = 7.2 Hz, 3H, C$\underline{H}_3$), 0.78 (d, $^{3}J_{HH}$ = 6.9

Hz, 3H), 0.69 (t, $^{3}J_{HH}$ = 6.9 Hz, 3H). $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$) δ (ppm): 184.2, 159.1, 142.3, 140.9, 134.9, 133.6, 129.2, 129.1, 124.3, 123.6, 122.8, 117.0, 111.1, 102.0, 98.5, 89.7, 89.1, 82.3, 81.3, 46.5, 31.1, 23.1, 22.7, 21.9, 19.1, 11.3. MS (HR-ESI, *m/z*): 541.0596 (50%, $[M+H]^+$), requires 541.0751.

*4.4.3. N-Propyl (methyl-) Ru cyclometalated benzimidazole complex (**1c**)*

5-Methyl-2-phenyl-1-propyl-*1H*-benzo[*d*]imidazole **L3** (102 mg, 0.406 mmol) was dissolved in DCM (6 mL). Sodium acetate (39.0 mg, 0.487 mmol) and dichloro(*p*-cymene)ruthenium(II) dimer (124 mg, 0.202 mmol) was then added to the solution. Product: Green solid (0.101 g, 48%). Mp: Decomp. 212.0 – 214.9 °C. Elemental Analysis for $C_{27}H_{31}ClN_2Ru$ (520.08 g.mol^{-1}): Found (%) C, 62.01; H, 6.04; N, 5.37; Calcd. (%) C, 62.36; H, 6.01; N, 5.39. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.33 (d, $^{3}J_{HH}$ = 7.6 Hz, 1H, ArH), 7.70 (s, 1H, ArH), 7.62 (d,^{3} J_{HH} = 8.0 Hz, 1H, ArH), 7.24 (d, ^{3}J = 8.4 Hz, 1H, ArH), 7.22 – 7.13 (m, 2H, ArH), 7.06 (t, $^{3}J_{HH}$ = 7.6 Hz 1H, ArH), 5.86 (d, $^{3}J_{HH}$ = 5.6 Hz, 1H, $Ar_{p\text{-}cy}$), 5.66 (d, $^{3}J_{HH}$ = 6.0 Hz, 1H, $Ar_{p\text{-}cy}$), 5.39 (d, $^{3}J_{HH}$ = 6.0 Hz, 1H, $Ar_{p\text{-}cy}$), 5.13 (d, $^{3}J_{HH}$ = 5.6 Hz, 1H, $Ar_{p\text{-}cy}$), 4.47 (m, 1H), 4.32 (m, 1H), 2.59 (s, 3H, Ar-C$\underline{H}_3$), 2.26 (m, 1H), 2.11 (s, 3H), 1.96 (m, 2H, C$\underline{H}_2$), 1.02 (t, $^{3}J_{HH}$ = 7.6 Hz, 3H, C$\underline{H}_3$), 0.89 (d, $^{3}J_{HH}$ = 7.2 Hz, 3H), 0.76 (d, $^{3}J_{HH}$ = 6.8 Hz, 3H). $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$) δ (ppm): 183.5, 157.6, 141.7, 140.6, 134.2, 134.0, 133.0, 128.6, 124.5, 123.8, 122.5, 117.0, 109.6, 101.7, 98.1, 89.5, 88.9, 82.2, 81.1, 46.2, 30.9, 29.7, 22.6, 21.9 (C$\underline{H}_3$), 21.8, 19.0, 11.3. MS (HR-ESI, *m/z*): 485.1545 (70%, $[M\text{-}Cl]^+$), requires 485.1530.

*4.4.4. N-Propyl (trifluoromethane-) Ru cyclometalated benzimidazole (**1d**)*

2-Phenyl-1-propyl-5-(trifluoromethyl)-*1H*-benzo[*d*]imidazole **L4** (95.0 mg, 0.312 mmol) was dissolved in DCM (6 mL). Sodium acetate (38.1 mg, 0.464 mmol) and dichloro(*p*-cymene)ruthenium(II) dimer (95.6 mg, 0.156 mmol) was then added to the solution. Product: Green solid (71.2 mg, 40%). Mp: Decomp. 214.4 – 218.7 °C. Elemental Analysis for $C_{27}H_{28}ClF_3N_2Ru$ (574.05 g.mol^{-1}): Found (%) C, 56.32; H, 5.15; N, 4.94; Calcd. (%) C, 56.49; H, 4.92; N, 4.88. FT-IR (ATR) ν (cm^{-1}) = 1580 (imine, C=N). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.36 (m, 1H, ArH), 8.19 (s, 1H, ArH), 7.63 (m, 1H, ArH), 7.55 (dd, $^{3}J_{HH}$ = 8.8, 1.2 Hz, 1H, ArH), 7.44 (d, $^{3}J_{HH}$ = 8.4 Hz, 1H, ArH), 7.23 (td, $^{3}J_{HH}$ = 7.6, 1.2 Hz 1H, ArH), 7.09 (td, $^{3}J_{HH}$ = 7.2, 1.2 Hz, 1H, ArH), 5.86 (dd, $^{3}J_{HH}$ = 6.0, 0.8 Hz, 1H, $Ar_{p\text{-}cy}$), 5.73 (dd, $^{3}J_{HH}$ = 6.0, 1.2 Hz, 1H, $Ar_{p\text{-}cy}$), 5.33 (dd, $^{3}J_{HH}$ = 6.0, 1.2 Hz, 1H, $Ar_{p\text{-}cy}$), 5.12 (dd, $^{3}J_{HH}$ = 6.0, 1.2 Hz, 1H, $Ar_{p\text{-}cy}$), 4.50 – 4.26 (m, 2H), 2.31 (m, 1H), 2.10 (s, 3H), 1.96 (m, 2H, C$\underline{H}_2$), 1.01 (t, $^{3}J_{HH}$ = 7.6 Hz, 3H, C$\underline{H}_3$), 0.95 (d, $^{3}J_{HH}$ = 7.2 Hz, 3H), 0.79 (d, $^{3}J_{HH}$ = 7.2 Hz, 3H). $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$) δ (ppm): 184.8, 160.0, 141.1, 141.0, 138.3, 133.4, 129.5, 126.2, 126.0, 125.6, 124.6, 122.9, 120.1, 114.6, 110.8, 101.4, 100.1, 89.7, 89.4, 82.0, 81.4, 46.7, 31.1, 23.1, 22.6, 21.9, 19.1, 11.3. MS (HR-ESI, *m/z*): 539.1236 (100%, $[M\text{-}Cl]^+$), requires 539.1248.

*4.4.5. N-Propyl Ir cyclometalated benzimidazole complex (**2a**)*

2-Phenyl-1-propyl-*1H*-benzo[*d*]imidazole **L1** (59.3 mg, 0.251 mmol) was dissolved in DCM (6 mL). Sodium acetate (24.7 mg, 0.301 mmol) and pentamethylcyclopentadienyliridium(III) chloride dimer (100 mg, 0.126 mmol) was then added to the solution. Product: Yellow solid (76.0 mg, 51%). Mp: 312.2 – 315.9 °C. Elemental Analysis for $C_{26}H_{30}ClIrN_2$ (598.212 g.mol^{-1}): Found (%) C, 51.73; H, 4.51; N, 4.21; Calcd. (%) C, 52.20; H, 5.05; N, 4.73. FT-IR (ATR) ν (cm^{-1}) = 1578 (imine, C=N). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.00 (dd, $^{3}J_{HH}$ = 7.5, 0.9 Hz, 1H, ArH), 7.71 – 7.62 (m, 2H, ArH), 7.40 – 7.27 (m, 3H, ArH), 7.19 (td, $^{3}J_{HH}$ = 7.5, 1.2

Hz, 1H, ArH), 7.06 (td, $^{3}J_{HH}$ = 7.5, 1.2 Hz, 1H, ArH), 4.60 – 4.35 (m, 2H, C$\underline{H}_2$), 2.14 – 1.95 (m, 2H, C$\underline{H}_2$), 1.76 (s, 15H, Cp*C$\underline{H}_3$), 1.09 (t, $^{3}J_{HH}$ = 7.5 Hz, 3H, C$\underline{H}_3$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ (ppm): 165.8, 161.9, 139.8, 137.4, 136.3, 134.3, 130.6, 123.8, 123.2, 123.0, 122.0, 116.9, 110.2, 46.4, 23.4, 11.5, 9.8. MS (HR-ESI, *m/z*): 563.2047 (100%, [M-Cl]$^{+}$), requires 563.2038.

*4.4.6. N-Propyl (chloro-) Ir cyclometalated benzimidazole complex (**2b**)*

5-Chloro-2-phenyl-1-propyl-*1H*-benzo[*d*]imidazole **L2** (93.7 mg, 0.346 mmol) was dissolved in DCM (6 mL). Sodium acetate (36.5 mg, 0.445 mmol) and pentamethylcyclopentadienyliridium(III) chloride dimer (138 mg, 0.173 mmol) was then added to the solution. Product: Yellow solid (0.198 g, 90%). Mp: 304.2 – 305.3 °C. Elemental Analysis for $C_{27}H_{32}Cl_2IrN_2 \cdot H_2O$: Found (%) C, 48.21; H, 4.58; N, 4.41; Calcd. (%) C, 48.71; H, 5.15; N, 4.21. FT-IR (ATR) *v* (cm^{-1}) = 1579 (imine, C=N). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.00 (dd, $^{3}J_{HH}$ = 7.8, 1.2 Hz, 1H, ArH), 7.67 – 7.65 (m, 1H, ArH), 7.63 (dd, $^{3}J_{HH}$ = 7.8, 0.6 Hz, 1H, ArH), 7.28 – 7.25 (m, 1H, ArH), 7.24 (m, 1H, ArH), 7.20 (td, $^{3}J_{HH}$ = 7.8, 1.2 Hz, 1H, ArH), 7.10 – 7.03 (td, $^{3}J_{HH}$ = 7.8, 1.2 Hz, 1H, ArH), 4.48 – 4.40 (m, 1H), 4.36 (m, 1H), 2.06 – 1.97 (m, 2H, C$\underline{H}_2$), 1.76 (s, 15H, Cp*C$\underline{H}_3$), 1.06 (t, $^{3}J_{HH}$ = 7.8 Hz, 3H, C$\underline{H}_3$). ^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$) δ (ppm): 166.3, 163.1, 140.5, 137.4, 134.9, 133.7, 131.0, 129.1, 124.0, 123.4, 122.2, 116.7, 111.2, 88.3, 46.5, 23.3, 11.4, 9.8. MS (HR-ESI, *m/z*): 670.1050 (10%, [M-Na]$^{+}$), requires 670.1470.

*4.4.7. N-Propyl (methyl-) Ir cyclometalated complex (**2c**)*

5-Methyl-2-phenyl-1-propyl-*1H*-benzo[*d*]imidazole **L3** (62.8 mg, 0.251 mmol) was dissolved in DCM (6 mL). Sodium acetate (26.8 mg, 0.327 mmol) and pentamethylcyclopentadienyliridium(III) chloride dimer (100 mg, 0.126 mmol) was then added to the solution. Product: Yellow solid (0.126 g, 80%). Mp: 280.1 – 281.5 °C. Elemental Analysis for $C_{28}H_{35}ClIrN_2$ (627.27 $g.mol^{-1}$): Found (%) C, 53.29; H, 5.31; N, 4.68; Calcd. (%) C, 53.61; H, 5.62; N, 4.47. FT-IR (ATR) *v* (cm^{-1}) = 1580 (imine, C=N). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.99 (dd, $^{3}J_{HH}$ = 7.8, 1.2 Hz, 1H, ArH), 7.64 (d, $^{3}J_{HH}$ = 7.8 Hz, 1H, ArH), 7.48 (s, 1H, ArH), 7.24 (d, $^{3}J_{HH}$ = 8.4 Hz, 1H, ArH), 7.17 (td, $^{3}J_{HH}$ = 7.2, 0.6 Hz, 1H, ArH), 7.14 – 7.10 (dd, , $^{3}J_{HH}$ = 7.8, 1.2 Hz, 1H, ArH), 7.08 – 7.01 (td, $^{3}J_{HH}$ = 7.8, 1.2 Hz, 1H, ArH), 4.50 (m, 1H), 4.40 (m, 1H), 2.53 (s, 3H, Ar-C$\underline{H}_3$), 2.04 (m, 2H, C$\underline{H}_2$), 1.76 (s, 15H, Cp*C$\underline{H}_3$), 1.07 (t, $^{3}J_{HH}$ = 7.8 Hz, 3H, C$\underline{H}_3$). ^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$) δ (ppm): 165.5, 161.6, 140.0, 137.3, 134.4, 133.0, 130.5, 124.4, 123.6, 122.0, 116.8, 109.8, 88.1, 46.4, 23.4, 21.8 (Ar-$\underline{C}H_3$), 11.5, 9.9. MS (HR-ESI, *m/z*): 577.2216 (100%, [M-Cl-CH_3]$^{+}$), requires 577.2195.

*4.4.8. N-Propyl (trifluoromethyl-) Ir cyclometalated benzimidazole complex (**2d**)*

2-Phenyl-1-propyl-5-(trifluoromethyl)-*1H*-benzo[*d*]imidazole **L4** (76.5 mg, 0.251 mmol) was dissolved in DCM (6 mL). Sodium acetate (25.3 mg, 0.308 mmol) and pentamethylcyclopentadienyliridium(III) chloride dimer (100 mg, 0.126 mmol) was then added to the solution. Product: Yellow solid (0.112 g, 67%). Mp: 309.4 – 310.2 °C. Elemental Analysis for $C_{28}H_{32}Cl_2F_3IrN_2$ (681.24 $g.mol^{-1}$): Found (%) C, 49.05; H, 4.48; N, 4.26; Calcd. (%) C, 49.37; H, 4.73; N, 4.11. FT-IR (ATR) *v* (cm^{-1}) = 1586 (imine, C=N). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.03 (dd, $^{3}J_{HH}$ = 7.8, 0.9 Hz, 1H, ArH), 7.99 (s, 1H, ArH), 7.65 (dd, $^{3}J_{HH}$ = 8.1, 1.2 Hz, 1H, ArH), 7.50 (dd, $^{3}J_{HH}$ = 8.7, 1.2 Hz, 1H, ArH), 7.43 (d, $^{3}J_{HH}$ = 8.7 Hz,

1H, ArH), 7.23 (td, $^{3}J_{HH}$ = 7.5, 1.2 Hz, 1H, ArH), 7.13 – 7.05 (td, $^{3}J_{HH}$ = 7.8, 1.2 Hz, 1H, ArH), 4.40 (m, 2H, C$\underline{H}_2$), 2.14 – 1.91 (m, 2H, C$\underline{H}_2$), 1.77 (s, 15H, Cp*C$\underline{H}_3$), 1.06 (t, $^{3}J_{HH}$ = 7.5 Hz, 3H, CH_3). $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$) δ (ppm): 166.6, 164.1, 139.4, 138.2, 137.4, 133.5, 131.3, 126.1, 125.8, 125.5, 124.3, 122.3, 120.0, 114.4, 110.9, 88.4, 46.6, 23.3, 11.4, 9.8. MS (HR-ESI, *m/z*): 707.2049 (45%, $[M\text{-}Cl+IsoProp+H]^{+}$), requires 707.2800.

*4.4.9 N-Propyl Rh cyclometalated benzimidazole complex (**3a**)*

2-Phenyl-1-propyl-*1H*-benzo[*d*]imidazole **L1** (77.0 mg, 0.320 mmol) was dissolved in DCM (8 mL). Sodium acetate (32.0 mg, 0.390 mmol) and pentamethylcyclopentadienylrhodium(III) chloride dimer (100 mg, 0.160 mmol) was then added to the solution. Product: Orange solid (0.070 g, 42%). Mp: 255.4 – 260.0 °C. Elemental Analysis for $C_{26}H_{30}ClN_2Rh$ (508.895 g.mol^{-1}): Found (%) C, 57.87; H, 5.69; N, 4.19; Calcd. (%) C, 61.40; H, 5.90; N, 5.50. FT-IR (ATR) v (cm^{-1}) = 1574 (imine, C=N). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.98 (dd, *J* = 7.6, 1.2 Hz, 1H, ArH), 7.76 (dd, *J* = 7.2, 1.2 Hz, 1H, ArH), 7.62 – 7.58 (dd, *J* = 7.6, 0.8 Hz, 1H, ArH), 7.40 – 7.23 (m, 4H, ArH), 7.12 – 7.06 (t, *J* = 7.6 Hz, 1H, ArH), 4.49 – 4.34 (m, 2H), 2.03 (m, 2H), 1.67 (s, 15H), 1.07 (t, *J* = 7.2 Hz, 3H). $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$) δ (ppm): 180.8, 157.7, 140.2, 138.1, 136.5, 134.7, 129.8, 123.6, 123.0, 123.0, 122.8, 117.4, 110.2, 46.5, 23.3, 11.4, 9.9. MS (HR-ESI, *m/z*): 473.1463 (100 %, $[M\text{-}Cl]^{+}$), requires 473.1464.

*4.4.10. N-Propyl (chloro-) Rh cyclometalated benzimidazole complex (**3b**)*

5-Chloro-2-phenyl-1-propyl-*1H*-benzo[*d*]imidazole **L2** (57.0 mg, 0.187 mmol) was dissolved in DCM (6 mL). Sodium acetate (15.7 mg, 0.191 mmol) and pentamethylcyclopentadienylrhodium(III) chloride dimer (56.0 mg, 0.0906 mmol) was then added to the solution. Product: Orange solid (39 mg, 88%), Mp: 257.3 °C (decomp.). Elemental Analysis for $C_{26}H_{29}Cl_2N_2Rh$ (542.08 g.mol^{-1}): Found (%) C, 54.80; H, 6.03; N, 3.37; Calcd. (%) C, 57.48; H, 5.38; N, 5.16. FT-IR (ATR) *v* (cm^{-1}): 1605 (imine, C=N). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.97 (d, *J* = 6.8 Hz,1H, ArH), 7.74 (s, 1H, ArH), 7.58 (d, *J* = 6.8 Hz,1H, ArH), 7.30 – 7.23 (m, 3H, ArH), 7.10 (t, *J* = 7.2 Hz, 1H, ArH), 4.44 – 4.37 (m, 2H), 2.05 – 1.97 (m, 2H), 1.68 – 1.62 (s + s, 15H), 1.07 (t, *J* = 7.4 Hz, 3H). $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$) δ (ppm): 181.1 (d, $J_{C\text{-}Rh}$ = 31.4 Hz), 158.9, 140.9, 138.0, 134.8, 130.0, 128.8, 123.6, 123.3, 122.8, 117.0, 111.0, 95.8, 94.2, 46.5, 23.3, 11.0, 9.7. MS (HR-ESI, *m/z*): 507.1074 (100 %, $[M\text{-}Cl]^{+}$), requires 507.1074.

*4.4.11. N-Propyl (methyl-) Rh cyclometalated benzimidazole complex (**3c**)*

5-Methyl-2-phenyl-1-propyl-1H-benzo[d]imidazole L3 (39.0 mg, 0.156 mmol) was dissolved in DCM (4 mL). Sodium acetate (40.0 mg, 0.488 mmol) and pentamethylcyclopentadienylrhodium(III) chloride dimer (39 mg, 0.0631 mmol) was then added to the solution. Product: Orange solid (26 mg, 71%). Mp: 235.1 °C (decomp.). Elemental Analysis for $C_{27}H_{32}ClN_2Rh$ (522.13 g.mol^{-1}): Found (%) C, 59.60; H, 7.90; N, 3.97; Calcd. (%) C, 62.00; H, 6.17; N, 5.36. FT-IR (ATR) v (cm^{-1}): 1575 (imine, C=N). ^{1}H NMR (300 MHz, CDCl3) δ (ppm): 7.96 (d, J = 6.7 Hz, 1H, ArH), 7.59 – 7.55 (m, 2H, ArH), 7.23 (m, 2H, ArH), 7.12 – 7.05 (m, 2H, ArH), 4.44 – 4.37 (m, 2H), 2.52 (s, 3H), 2.05 – 1.98 (m, H). 1.67 (s, 15H, Cp*), 1.06 (t, J = 7.3 Hz, 3H). $^{13}C\{^{1}H\}$ NMR (101 MHz, CDCl3) δ (ppm): 180.77 (d, J C-Rh = 31.3 Hz), 137.91, 134.80, 132.64, 129.48, 124.33, 123.25, 122.61, 117.24, 109.62, 95.63, 46.36, 23.14, 21.60, 11.40, 9.74. MS (HR-ESI, *m/z*): 487.1623 (100 %, $[M\text{-}Cl]^{+}$), requires 487.1621.

*4.4.12. N-Propyl (trifluoromethyl-) Rh cyclometalated benzimidazole complex (**3d**)*

2-Phenyl-1-propyl-5-(trifluoromethyl)-*1H*-benzo[*d*]imidazole **L4** (0.208 g, 0.682 mmol) was dissolved in DCM (10 mL). Sodium acetate (73.0 mg, 0.890 mmol) and pentamethylcyclopentadienylrhodium(III) chloride dimer (0.181 g, 0.293 mmol) was then added to the solution. Product: Orange solid (23.6 mg, 50%). Elemental Analysis for $C_{27}H_{29}ClF_3N_2Rh$ (576.10 g.mol^{-1}): Found (%) C, 58.20; H, 7.94; N, 3.40; Calcd. (%) C, 56.21; H, 5.07; N, 4.86. FT-IR (ATR) ν (cm^{-1}): 1546 (imine, C=N). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.06 (s, 1H, ArH), 8.00 (d, J = 7.7 Hz, 1H, ArH), 7.62 (d, J = 7.8 Hz, 1H, ArH), 7.47 – 7.39 (m, 2H, ArH), 7.30 (t, J = 7.8 Hz, 1H, ArH), 7.13 (t, J = 7.8 Hz, 1H,ArH), 4.48 – 4.43 (m, 2H), 2.08 – 2.01 (m, 2H), 1.69 – 1.62 (s + s, 15H), 1.11 (t, J = 7.5 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ (ppm): 181.7 (d, $J_{C\text{-}Rh}$ = 33 Hz), 139.7, 138.3, 138.0, 133.9, 130.3, 123.9, 122.9, 119.8, 114.8, 110.6, 95.9, 46.7, 23.1, 11.1, 9.2.

4.5. Single-crystal X-ray structure analysis

Suitable single crystals of complexes **2c, 2d, 3a** and **3c** were grown from a mixture of chloroform and EtOAc which were allowed to evaporate over 3 – 5 days. Single-crystal X-ray data were collected on a Bruker KAPPA APEX II DUO diffractometer using graphite-monochromated Mo-Kα radiation (χ = 0.71073 Å). Data collection was carried out at 173(2) K. Temperature was controlled by an Oxford Cryostream cooling system (Oxford Cryostat). Cell refinement and data reduction were performed using the program SAINT. The data were scaled and absorption correction performed using SADABS.[41] The structure was solved by direct methods using SHELXS-97 and refined by full-matrix least-squares methods based on F using SHELXL-2014 and using the graphics interface program X-Seed.[41, 42] The programs X-Seed and POV-Ray were both used to prepare molecular graphic images. CCDC 1850798 (**2c**), 1850799 (**3a**), 1850800 (**2d**) and 1850801 (**3c**).

4.6. In vitro antiplasmodial assay

The test samples were tested in triplicate on one or two separate occasions against chloroquine-sensitive (NF54) and chloroquine-resistant (K1) 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.[43] Quantitative assessment of antiplasmodial activity *in vitro* was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler.[44] The test samples were prepared to a 20 mg/mL stock solution in 100% DMSO. 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 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 100 μg/mL, which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 0.2 μg/mL. The same dilution technique was used for all samples. The IC_{50} values were obtained using a non-linear dose response curve fitting analysis via Graph Pad Prism v.4.0 software.

4.7 In vitro cytotoxicity

Compounds were tested for *in vitro* cytotoxicity against the mammalian cell-line, Chinese Hamster Ovarian (CHO), using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay.[45, 46] The MTT assay is used as a colorimetric assay for cellular growth and survival, and compares well with other available assays. The test samples were tested in triplicate on one occasion. The test samples were prepared to a 2 mg/mL stock solution in 10% MeOH or 10% DMSO and were tested as a suspension if not completely dissolved. Test compounds were stored at -20°C until use. Emetine was used as the reference drug in all experiments. The initial concentration of emetine was 100 μg/mL, which was serially diluted in complete medium with 10-fold dilutions to give 6 concentrations, the lowest being 0.001 μg/mL. The same dilution technique was applied to all the test samples. The highest concentration of solvent to which the cells were exposed had no measurable effect on cell viability. IC_{50} values were obtained from full dose-response curves using a non-linear dose-response curve fitting analysis via GraphPad Prism V.4 software.

4.8 β-Hematin inhibition assay

The β-haematin inhibition assay was modified from the method described by Sandlin *et al*.[29] 10mM Stock solutions of the compounds were in DMSO. The compounds were delivered to a 96-well plate in triplicate and were tested at a starting concentration of 500 μM, where the lowest drug concentration was 5 μM. The stock solution was serially diluted to give 12 concentrations in the 96-well flat-bottom assay plate. NP-40 detergent was then added to mediate the formation of β-haematin (30.55 μM, final concentration). A 25mM stock solution of haematin was prepared by dissolving hemin (16.3 mg) in dimethyl sulfoxide (1 mL). A 177.76 μL aliquot of haematin stock was suspended in 20 mL of a 2 M acetate buffer, pH 4.7. The haematin suspension was then added to the plate to give a final haematin concentration of 100 μM. The plate was then incubated for 16 h at 37 °C. The compounds were analysed using the pyridineferrochrome method developed by Ncokazi and Egan.[47] 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 UV-Vis 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* Graph Pad Prism v.5.0 software.[48]

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Abbreviations used

CQ, chloroquine; CHO, Chinese hamster ovarian; NMR, nuclear magnetic resonance; TLC,

thin layer chromatography; RI, resistance index; SI, selectivity index

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Highlights

- 2-Phenylbenzimidazole ligands and their cyclometallated Ru(II), Rh(III) and Ir(III) complexes have been prepared.
- The compounds were characterized using several spectroscopic and analytical techniques.
- The compounds were evaluated as antiplasmodial agents against *Plasmodium falciparum*.
- The metal complexes were more active than the ligands and are selective to malaria parasites over mammalian cells.