Ruthenium metallotherapeutics: novel approaches to combatting parasitic infections.

Nicole S. Britten[1] and Jonathan A. Butler[1*]

[1]Faculty of Science and Engineering, Manchester Metropolitan University, Manchester, United Kingdom

*Corresponding author

E-mail: jonathan.butler@mmu.ac.uk (JB)

Abstract

Human parasitic infections cause a combined global mortality rate of over onemillion people per annum and represent some of the most challenging diseases for medi-cal intervention. Current chemotherapeutic strategies often require prolonged treatment,coupled with subsequent drug-induced cytotoxic morbidity to the host, while resistancegeneration is also a major concern. Metals have been used extensively throughout the his-tory of medicine, with more recent applications as anticancer and antimicrobial agents.Ruthenium metallotherapeutic antiparasitic agents are highly effective at targeting arange of key parasites, including the causative agents of malaria, trypanosomiasis, leish-maniasis, amoebiasis, toxoplasmosis and other orphan diseases, while demonstrating low-er cytotoxicity profiles than current treatment strategies. Generally, such compounds alsodemonstrate activity against multiple cellular target sites within parasites, including inhi-bition of enzyme function, cell membrane perturbation, and alterations to metabolic path-ways, therefore reducing the opportunity for resistance generation. This review providesa comprehensive and subjective analysis of the rapidly developing area of ruthenium met-al-based antiparasitic chemotherapeutics, in the context of rational drug design and poten-tial clinical approaches to combatting human parasitic infections.

Running title: Ruthenium-based compounds as antiparasitic agents.

Key words: Antiparasitic, Ruthenium, Malaria, Trypanosomiasis, Leishmaniasis, Amoebiasis.

1. Introduction

Ruthenium (Ru) is a transition metal found in Group 8 of the periodic table, with an atomic mass of101.07. Specifically, Ru is among the platinum group of transition metals, also known as precious metals, due to their rarity. Ru is currently used in chemistry as a catalyst for various reactions [1] as well as in histology as a polycationic stain called Ruthenium Red. It is typically used to stain mucopolysaccharide structures but may also be used to trace cellular mechanisms for their study [2].

An emerging research area is the use of Ru-based compounds within the anticancer chemotherapy field, which display activity in a similar manner to the first transition metal to be used in modern medicine - platinum at the core of the cisplatin anti-tumour compound (Fig. 1a). However, known issues with platinum drugs are the cytotoxic effects on the host, which often result in a wide variety of clinical side effects. The redox potential of Ru permits the metallotherapeutic to be delivered in the most inert form, which would be less widely toxic to the host [3]. Once at the target site, the intra-cellular environment of diseased tissue, for example, reduced oxygen and low pH, permits the reduction of Ru into more biologically active ionic derivatives [4]. Furthermore, Ru and Iron (Fe) have similar redox potential where Ru can interact with essential metalloproteins in preference to Fe. One such example is the transferrin Fe-binding protein, which is often sequestered by diseased/infected cells due to the increased need for Fe. This process further enables preferential targeting and accumulation of Ru in key cell types, which enhances potential anticancer and antimicrobial activity within the host [4].

In the 1960s, the antimicrobial activity of Ru (II) mononuclear complexes was first studied against Gram-positive, Gram-negative, and acid-fast bacteria [5]. The initial compound, $[Ru(phen)_3]^{2+}$, was found to have little to no activity against all bacteria. However, the addition of methyl groups to the bidentate phenanthroline ligands, creating $[Ru(Me_4Phen)_3]^{2+}$, caused a significant increase in antibacterial activity, most notably against Gram-positive bacteria. It was also demonstrated that resistance generation was less likely when compared to traditional antibiotics [5]. Subsequent studies discovered that the antibacterial effects were due to the compound binding to the major groove of DNA [6].More recently, dinuclear poly pyridyl ruthenium(II) compounds have been investigated, mainly due to their higher affinity for DNA, with subsequent increased DNA binding ability. These compounds were found to be highly active against a number of bacteria [7]. A di-verse range of other antibacterial Ru-based compounds have now been synthesised, all featuring a common Ru-based elemental core [8].

More recently, the anti-parasitic activity of Ru-based compounds has been explored using either single compounds or those coordinated with established antiparasitic agents, such as the use of the anti-malarial drug

chloroquine (Fig. 1b) in the form of $[RuCl_2(chloroquine)]_2$. [9] This demonstrated significantly increased activity when compared to chloroquine alone [9]. Ru-based compounds are considered a potential treatment option due to lower host cytotoxicity, effective biodistribution, and different mechanisms of antiparasitic action compared to current chemotherapeutics [10].

Current antiparasitic treatment options often cause significant side effects [11], and require prolonged treatment regimens [12]. One study showed 89.8% of 176 patients treated with benznidazole (Fig. 1c) and nifurtimox (Fig. 1d) for trypanosomiasis experienced ad-verse effects, with mucocutaneous and digestive symptoms being recorded, respectively [12]. Metronidazole (Fig. 1e) which is used to treat amoebiasis is tolerated well in the host, however minor side effects such as nausea, diarrhoea, vomiting, and mouth dryness are common and potentially result in premature termination of treatment [13]. Indeed, longer-term use of metronidazole is linked to more serious side effects involving neurological disorders such as peripheral neuropathy. Melarsoprol (Fig. 1f) treatment for African trypanosomiasis has a 50% fatality rate due to complications such as posttreatment reactive encephalopathy (P-TRE) that can occur 1-10 days after starting treatment in 5-10% of patients caused by the rapid destruction of the parasite within the central nervous system [14].

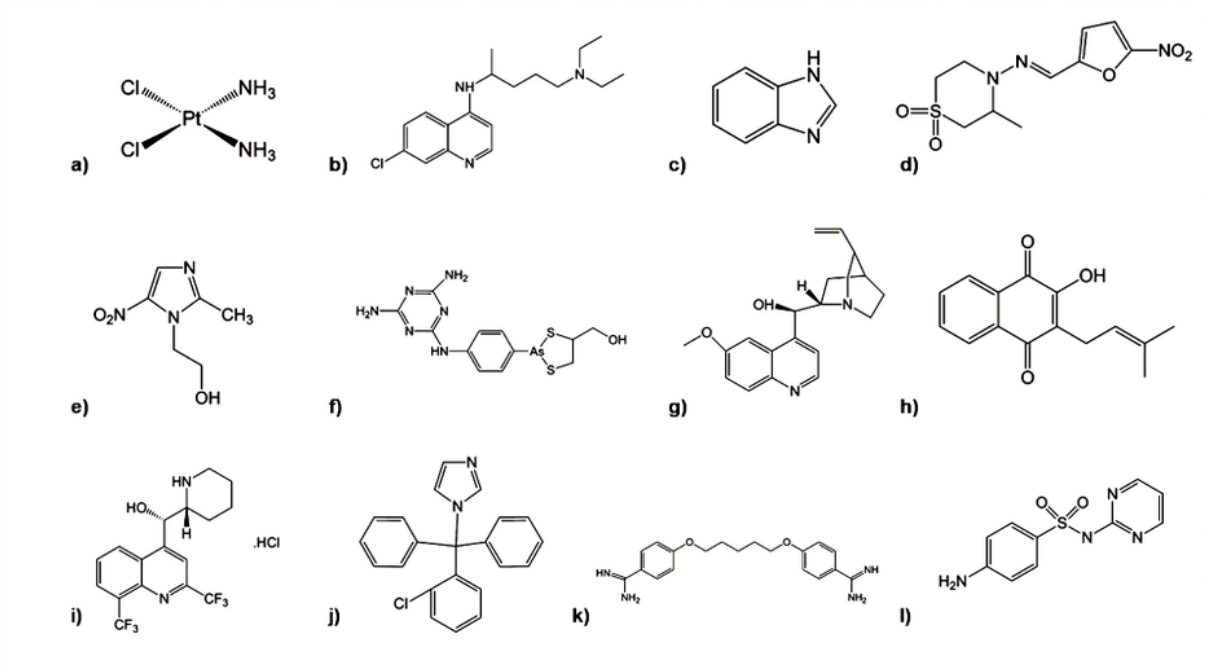

Fig. (1). Current antiparasitic treatments (a) Cisplatin, (b) Chloroquine, (c) Benznidazole, (d) Nifurtimox, (e) Metronidazole,(f) Melarsoprol, (g) Quinine, (h) Lapachol, (i) Mefloquine, (j) Clotrimazole, (k) Pentamidine, (l) Sulfadiazine.

Resistance generation to parasitic treatments is also a serious concern. Efflux systems are present within the membrane of some parasites, which contribute to antimicrobial resistance, such as the multidrug resistance protein A (MRPA) that can confer melarsoprol resistance in *Trypanosoma brucei* parasites [14].

This review focuses on recent developments in the use of novel Ru-based antiparasitic chemotherapy deployed against key neglected parasitic infections, including trypanosomiasis, leishmaniasis, amoebiasis and toxoplasmosis. Clinical applications and host cytotoxicity are explored alongside future perspectives in this emerging antiparasitic research field.

2. Methods

The search for suitable literature was conducted using PubMed (National Center for Biotechnology Information, National Library of Medicine) and Google Scholar by applying individual search terms for the specific tropical disease (‘malaria’ ‘trypanosomiasis’,‘leishmaniasis’, ‘amoebiasis’, ‘toxoplasmosis’, ‘lymphatic filariasis’, ‘schistosomiasis’, ‘strongyloidiasis’,‘trichuriasis’) with the Boolean operator AND ‘ruthenium’. Individual parasitic genera were also included in secondary search criteria, including ‘Plasmodium’ ‘Trypanosoma’, ‘Leishmania’, ‘Entamoeba’, ‘Toxoplasma’, ‘Setaria’, ‘Schistosoma’, ‘Strongyloides’,‘Trichuris’ AND ‘ruthenium’.

Studies were considered eligible if there was evidence of antiparasitic activity through the inclusion of half-maximal inhibitory concentration (IC_{50}) or half--maximal effective concentration (EC_{50}) values against the respective parasites. Secondary parameters included synergy with current treatment options, the potential for disease progression, and the mechanism of anti-parasitic activity. Inclusion of publications was limited to those written in English and authored as full manuscripts with no restriction on publication year.

3. Malaria

Plasmodium falciparum is one of the main species in the Plasmodium genus that causes malaria [15], affecting 228 million people worldwide and causing 405,000 deaths in 2018 [16, 17]. As one of the most prevalent diseases in Africa, there are five human infective *Plasmodium* species, including *P. falciparum, Plasmodium knowlesi, Plasmodium malariae, Plasmodium ovale* and *Plasmodium vivax*. Although parasitic treatments and prophylactic therapy are available, the overall global infective trend is increasing, from 214 million to 219 million cases in 2015 and 2017, respectively [15]. Malaria typically is asymptomatic, with few experiencing symptoms such as head and muscle aches, fever, fatigue, and chills allowing for undetected progression to fatal

multi organ failure [17]. Current treatments involve quinine (Fig. 1g) derived therapeutics as well as doxycycline, which target parasitic DNA replication, protein synthesis, and cell membranes [15]. Additionally, the parasite biocrystallizes haematin (a component of haemoglobin) to haemozoin which is less toxic. Current drug treatments are also de-signed to inhibit this process, thus resulting in an in-crease in the concentration of haematin and subsequent death of the parasitic cell via oxidative stress [18, 19]. However, resistance is now emerging to chloroquine, a first-line treatment, which is conferred through a chloroquine resistance transporter responsible for causing an efflux of the active compound from the parasitic digestive vacuole [20].

Increased biological activity has been observed when organic compounds are conjugated with a metal pharmacophore [21]. For example, cyclometallated complexes composed of Ru with benzimidazole have a strong M-Cσ bond within its chelating ring, which may prevent reduction and ligand exchange reactions from occurring [22]. These complexes demonstrate effective anti-plasmodial activity, which is significantly enhanced when conjugated with Ru, potentially proving to be more efficient than current treatments. This is thought to occur due to higher numbers of transmembrane or direct interactions between the metal complex and a plasmodium target. Ru-based compounds are al-so thought to mimic iron and interact with serum albumin and transferrin [23]. N-propyl Ru cyclometalated compounds act by stimulating the generation of reactive oxygen species (ROS) in P. falciparum, inhibiting kinase and thioredoxin reductase enzyme function impairing DNA and protein function within the parasite through intercalation and methylation disruption [24]. Consequently, these compounds demonstrate differential toxicity against the parasite compared to host mammalian cells [21].

Chloroquine is a common treatment option for malarial diseases. Ru-based compounds coordinated with chloroquine analogues and a, N,O-chelating salicyladiminate ligand demonstrate the highest anti-plasmodial activity to date [25]. One such compound, [Ru(II)-chloroquine]$_2$, demonstrated a 4.5-fold increase in in vitro activity compared to chloroquine diphosphate [26]. Likewise, a trinuclear complex comprised of [Ru(p-cymene)Cl_2]$_2$ containing polypyridyl ester ligands (monodentate donors) (Fig. 2a) and benzene-1,3,5-tricarboxylic acid tripyridin-4-ylmethyl ester also proved to be effective anti-plasmodial compounds with low cytotoxicity against HEK cell lines, proving to have a 4.5 fold increased effect on the parasites than the currently used drug chloroquine [27] (Table 1).

Ru compounds containing chloride ligands in the aromatic ring have proved to be effective against malarial parasites. The addition of silicon to available anti-malarial drugs increased the lipophilicity and pharmacological activity of the overall compound with de-creased cytotoxicity, making it more desirable for use [28, 29]. Compounds such as organosilane thiosemicarbazones and their metal complexes demonstrated a vari-able range

of antiparasitic activity, where one example,η^6-iPrC$_6$H$_4$Me)Ru(μ-Cl)Cl]$_2$, where R_1 = Ferrocene, R_2= CH_3, X = Si (Fig. 2b), demonstrated half-maximal inhibitory concentration (IC_{50}) values of 7.81 ± 0.56 μM against P. falciparum strain NF54 (Table 1). However, organosilane thiosemicarbazone Ru complexes have been shown to be more selective against the parasite than non infected cell lines and in this example, the addition of silicon improved differential toxicity and potency [19].

RAPTA complexes contain a monodentate 1,3,5-triaza-7-phospaadamantane (PTA) and η^6 arene ligand coupled to a Ru core to form [Ru(η^6-p-arene)Cl2(P-TA)]. These compounds can protonate in low pH environments, such as the conditions seen within the digestive vacuole of the parasite, thus making these ideal antiparasitic agents. A series of RAPTA 7-chloroquino-line derivatives were synthesised with all demonstrating IC_{50} values of <0.40 μM and between 1.5 and 4.5μM against the chloroquine (Fig. 1b) sensitive *P. falciparum* NF54 and resistant P. falciparum K1 strains respectively [18]. The lowest IC_{50} values were observed following exposure to (η^6-p-cymene)(N-(2-((5-fluoro-2-hydroxyphenyl)methylimino)propyl)-7-chloroquinolin-4-amine)PTA ruthenium(II) hexafluorophosphate (Fig. 2c), where values of 0.10 μM ± 0.069 (against strain NF54) and 3.8 μM ± 0.68 (against strain K1) were observed, compared to chloroquine alone (0.031 μM ± 0.004 and 0.36 μM ± 0.07 respectively). Observed cytotoxicity against a CHO cell line model with this derivative was >100 μM [18]. The protonation of complexes at low pH represents a promising drug delivery system for novel metal-chloroquine metallotherapuetic agents.

In a further study, $RuCl_2$(Lap)(dppb) complexes were found to be more potent and have more selective antiparasitic efficacy compared to lapachol (Fig. 1h) alone, which failed to inhibit *P. falciparum*. Compounds such as [Ru(Lap)(PPh_3)$_2$(phen)]PF_6 (Fig. 2d) and [$RuCl_2$(Lap)(dppb)] were found to be 50 times more potent than lone lapachol and only 5 times less potent than the currently used drug mefloquine (Fig.1i) making it ideal for use [11].

Ru-containing compounds with an antifungal clotrimazole (Fig. 1j) component are 50-fold more potent as anti-plasmodial agents compared to unmodified compounds [30]. The presence of a dimethylaminopropoxyside-chain further increased the effectiveness of the drug, with the compounds showing an IC_{50} value of 0.7and 2.2 μM. However, the presence of hydroxyl moieties in a para position or hydrolysable ester group resulted in increased compound cytotoxicity, which further demonstrates the importance of rational drug design [24, 31].

Overall, the presence of Ru compounds coordinated with traditional treatment options improves the effectiveness of anti-plasmodial compounds. The addition of ligands such as chloride or η6 arene considerably improves drug specificity to the Plasmodium para-site with minimal cytotoxic effects on the host, which demonstrates the

potential to reduce side effects within patients. Furthermore, as these compounds have multiple mechanisms of antiparasitic activity, resistance generation is less likely to occur, and novel metal com-pounds could provide alternatives to developing entirely new classes of drugs to combat malarial infections.

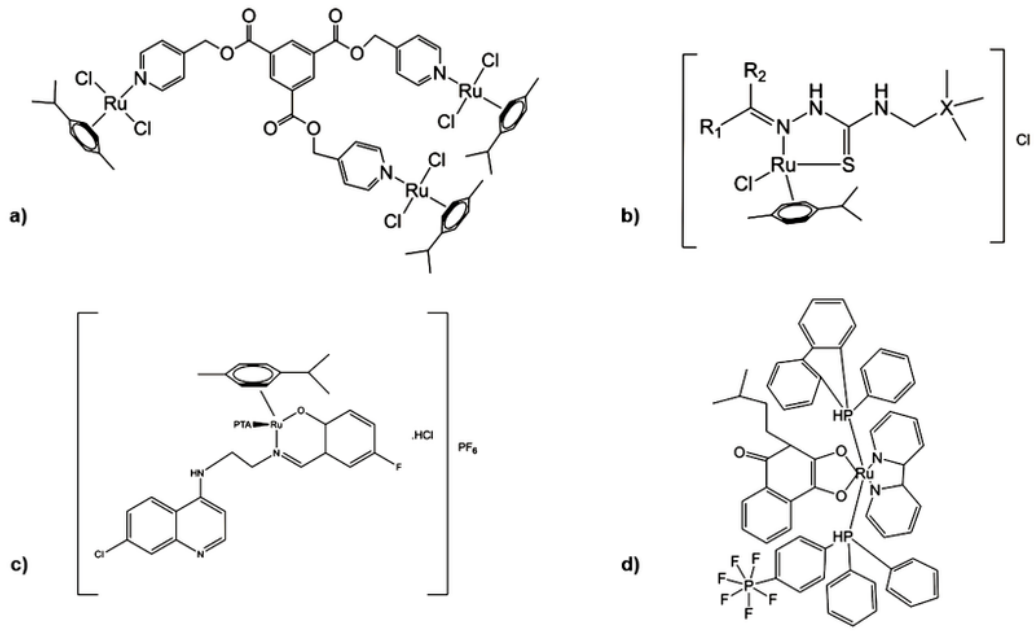

Fig. (2). Ruthenium-based compounds which demonstrate antiparasitic activity against *Plasmodium* species. (a) $[Ru(p\text{-}cymene)Cl_2]_2$ with benzene-1,3,5-tricarboxylic acid, tripyridin-4-ylmethyl ester, [27] (b) η^6-$iPrC_6H_4Me)Ru(\mu\text{-}Cl)Cl]_2$, where R_1 =Ferrocene, R_2 = CH_3, X = Si [19] (c) (η^6-p-cymene)(N-(2-((5-fluoro-2-hydroxyphenyl)methylimino)propyl)-7-chloroquino-lin-4-amine)PTA ruthenium(II) hexafluorophosphate [18] (d) $[Ru(Lap)(PPh_3)_2(phen)]PF_6$. [34]

4. Trypanosomiasis

4.1 *American trypanosomiasis*

Chagas disease, additionally known as American trypanosomiasis, affects up to 7 million people world-wide, predominantly in Latin America [32, 33]. *Trypanosoma cruzi* protozoan infection may be asymptomatic in the acute phase, which affects 10 million people worldwide, however, progression can result in cardiac and gastric diseases such as megacolon, megaesophagus, heart insufficiency and arrhythmias [34].Chagas disease is a major neglected tropical disease [35] and is transmitted by triatomine insects that penetrate the mucous membranes, eyes, and broken skin of the host [36]. In addition to vector-borne infections, transmission is associated with the exchange of contaminated blood or organs and through perinatal transmission [36, 37].

Currently, only two medications are routinely used in clinical practice, nifurtimox and benznidazole, but these are only effective in the acute dis-ease phase and are ineffective during the chronic stage. There are significant side effects associated with these treatment regimes due to the duration of exposure required to have any clinical impact [34].

Current treatments for infection with *T. cruzi* generally target enzymes present within the parasite such as trypanothione reductase, superoxide dismutase, cysteine protease [39, 40] DNA topoisomerase [35], and kinase protease [40]. These enzymes are vital to the metabolic processes of the parasite and therefore disrupting these represents a key target for treatment [35].Specific drug target sites include lanosterol 14-demethylase and transsialidase enzymes [41]. A cysteine protease termed cruzain also represents a potential unique drug target. This enzyme is responsible for the proteolytic activity, survival, and growth of the parasite. Nitric Oxide (NO) inactivates cruzain by S-nitrosylation of the binding site, therefore, it has been proposed that Ru-nitrosyl-containing compounds could demonstrate selective and pleiotropic activity by inhibiting the action of glyceraldehyde 3-phosphate dehydrogenase [42]. Furthermore, compounds with a general formula $[RuCl_2(ATZ)(COD)]$ (COD = 1,5-cyclo-octadi-ene) have high activity against the trypomastigote form of *T. cruzi* with an IC_{50} range between 3.3 and 27.2 μM after 24-hour exposure, whereas benznidazole is effective at 5.0 μM. One of the more active compounds is dichloro[2-(para-bromophenylthio-(Z)-ethylidene)hydrazone-1,3-thiazol-4(5H)-one] cyclooctadiene ruthenium(II) (Fig. 3a) which had an IC_{50} value against trypomastigotes of 5.5 μM could prove more effective than current treatment options [43].

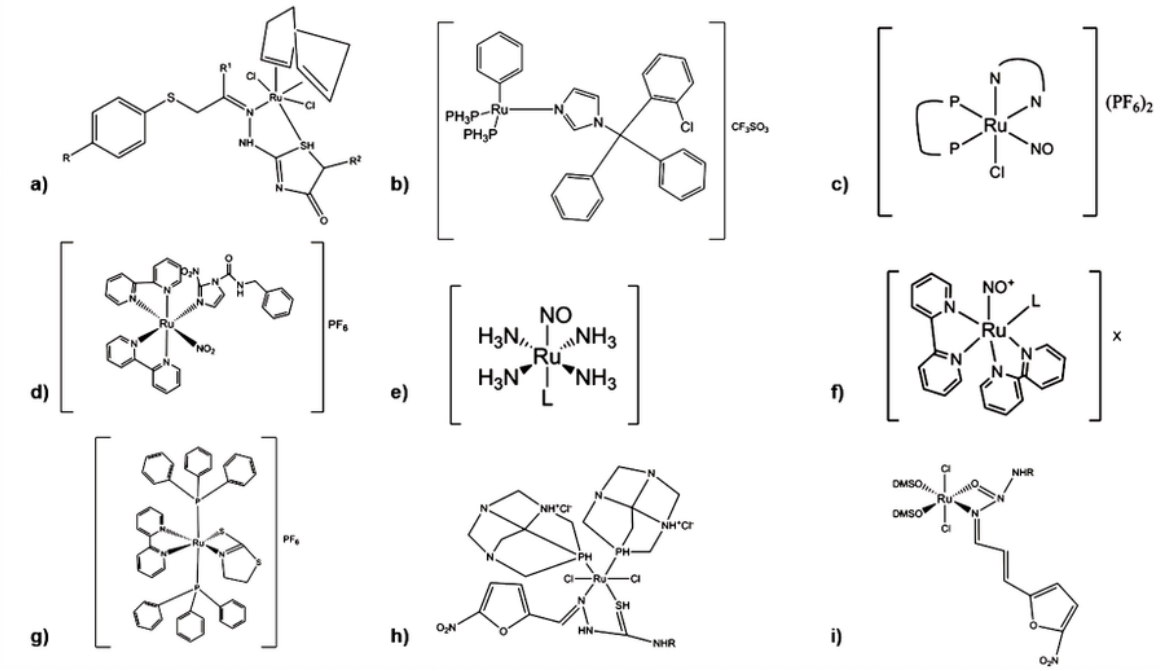

Fig. (3). Ruthenium-based compounds which demonstrated antiparasitic activity against *Trypanosoma cruzi*. (a)[$RuCl_2$(ATZ)(COD)], where R = Br, R_1 = H, R_2 = H [43], (b) [RuCp(PPh_3)$_2$(CTZ)](CF_3SO_3)] [46], (c) ct-[RuCl(NO)(dpp-b)(5,5=-mebpy)](PF_6)$_2$ [42], (d) cis-[Ru(NO_2)(bpy)$_2$(Bz)](PF_6) [34], (e) trans-[RuII(NO)(NH_3)$_4$(L)]X_3, where L = imidazole co-ordinated through nitrogen (imN) or imidazole coordinated through carbon (imC), pyridine (py), L-histidine (L-hist), sulphite (SO_3^{2-}), pyrazine (pz), nicotinamide (nic), 4-picoline (4-pic), triethyl-phosphite ([P(OEt)$_3$]), isonicotinamide (isn), isonicotinicacid (ina), X = BF_4^-, Cl^- or PF_6^- [47], (f) cis-[RuII(NO)(bpy)$_2$(L)]X_3, where L = imidazole (imN), 1-methylimidazole (1-miN),or sulfite ion (SO_3^{2-}) and X = BF_4^-, Cl− or PF_6^- [50], (g) trans-[Ru(tzdt)(PPh_3)$_2$(bpy)]PF_6 [10], (h) [$RuCl_2$(HL)(HPTA)$_2$]Cl_2 [37], (i) [RuIICl_2(dmso)$_2$L [56].

Table **1**: Comparison of IC_{50} values of Ru metallotherapeutics against respective parasite and mammalian cell lines (with cell type in brackets), coupled with the proposed mechanisms of antiparasitic activity for each compound.

Compound	IC_{50} of parasite (μM)	IC_{50} Cell cytotoxicity (μM)	Activity	Reference
Malaria				
[Ru(*p*-cymene)Cl_2]$_2$ with benzene-1,3,5-tricarboxylic acid tripyridin-4-ylmethyl ester	5.87 ± 0.58 (NF54)	98.1 ± 2.0 (HEK)	Inhibit haemozoin	[27]
η^6-^{i}PrC_6H_4Me)Ru(μ-Cl)Cl]$_2$, where R_1 = Ferrocene, R_2 = CH_3, X = Si	7.81 ± 0.56 (NF54)	ND	Increased lipophilicity.	[19]
(η^6-*p*-cymene)(*N*-(2-((5-fluoro-2-hydroxyphenyl)methylimino)propyl)-7-chloroquinolin-4-amine)PTA ruthenium(II) hexafluorophosphate	0.10 ± 0.069 (NF54) 3.8 ± 0.68 (K1)	>100 (CHO)	Protonate in low pH environments.	[18]
[Ru(Lap)(PPh_3)$_2$(phen)]PF_6	43.5 ± 0.71	0.33 ± 0.08 (J774 macrophages)	Inhibit parasitic proliferation.	[11]
American trypanosomiasis				
mer-[$RuCl_3$(dmso)(H_2O)(tmtp)]·2H_2O	43.2 ± 3.5	2150 ± 172.0 (J774) 808.2 ± 64.7 (Vero)	Inhibit proliferation and Fe-SOD.	[35]
trans-[Ru(tzdt)(PPh_3)$_2$(bipy)]PF_6	0.010 ± 0.001	0.9 ± 0.9	Interacts with cDNA.	[10]

	(trypomastigotes)	(DU-145 cells) 3.3 ± 1.3 (MCF-7 cells)		
$[RuCl_2(HL_4)(HPTA)_2]Cl_2$	84.2 ± 1.3 (epimastigotes) 85.2 ± 1.9 (trypomastigotes)	>200 (murine macrophage RAW 264.7)	Induce oxidative stress, interacts with DNA.	[37]
African trypanosomiasis				
$[RuCl(\eta^6\text{-}p\text{-cym})(1,10\text{-phenanthroline-5,6-dione})][PF_6]$	0.19 ± 0.5	1.26 ± 0.78 (HL60)	Coil and kink plasmid	[58]
$[RuCl_2(\eta^6\text{-}p\text{-cym})(phenanthridine)]$	165.0 ± 45.5	>100 (HL60)	Affects DNA replication	
$[RuCl(\eta^6\text{-}p\text{-cym})(5\text{-amine-1,10-phenanthroline})][PF_6]$	2.7 ± 0.3	44.63 ± 7.35 (HL60)	Knots and kinks plasmids at sharp angles.	
$[Ru_2(p\text{-cymene})_2(L1)_2]Cl_2$	2.9	>100 (J774)	Inhibit α-14 C demethylase	[60]
$[Ru_2(p\text{-cymene})_2(L4)_2]Cl_2$	0.5	26 (J774)		
Leishmaniasis				
cis-$[RuII(\eta^2\text{-}O_2CR)(dppm)_2]PF_6$, where R = 4-bu-tylbenzoate (bbato)	7.52 (*L. amazonesisis*) 9.09 (*L. braziliensis*)	8.73 (RAW 264.7 macrophages)	Interact with DNA covalently	[61]
cis,*fac*-$[RuCl_2(dmso)_3(tmtp)]$	9.2 ± 0.7	330.8 ± 26.5 (J774) 335.7 ± 26.9 (Vero)	High selectivity for Fe-SOD	[35]
$[Ru(\eta^6\text{-}p\text{-cymene})Cl_2(CTZ)]$	LD_{50} 0.015 ± 0.004 (*L. major*)	>7.5 (Human osteoblast)		[30]
$[Ru(Lap)(PPh_3)_2(Me\text{-bipy})]PF_6.CH_3OH$	0.18 ± 0.04	LC_{50} 1.0 ± 0.46 (J774)	Inhibit promastigote proliferation	[11]
$[RuCl_2(Lap)(dppb)]$	0.14 ± 0.04	LC_{50} >10 (J774)		
cis-$[Ru(bpy)_2SO_3(NO)]PF_6$	30 - 60 (*L. amazonesisis*)	Not toxic at 10-60 (BALB)	NO donor	[64]
$[RuCl(CTZ)(\eta_6\text{-}p\text{-cymene})(PPh_3)]PF_6$	0.24 ± 1.65 (*L. amazonesisis*)	Cytotoxic at 1. (murine macrophages)	Decrease flagella length, mitochondria swelling and leakage, decreased cell size	[68]
$[RuCl(KTZ)(\eta^6\text{-}p\text{-cymene})(PPh_3)]PF_6$	0.08 ± 2.62 (*L. amazonesisis*)			
Amoebiasis				

[Ru(metronidazole)$_2$(Cl)$_2$(H$_2$O)$_2$]	0.51 ± 0.06	ND	Produce nitro radicals, bind to DNA and enzymes.	[71]
[Ru(acac)(pdto)]Cl	0.06 ± 0.005	>100 (Human peripheral blood lymphocytes)	Interact with DNA and bidentate ligands.	[69]
[Ru(pdto)(acetylacetonate)]Cl	0.06 μMol/L	ND	Increase ROS	[70]
Toxoplasmosis				
[Ru(η^6-*p*-cymene)(tBu$_2$acac)(P(OiPr)$_3$)][BF$_4$]	18.7	3 (Human foreskin fibroblasts)	Create lipid inclusions, distort nuclear membrane.	[81]
[Ru(η^6-*p*-cymene)(tBu$_2$acac)(P(OEt)$_3$)][BF$_4$]	41.1	10 (HFF)		
[(η^6-*p*-Me-C$_6$H$_4$Pr^i)Ru$_2$(μ-Cl)Cl$_2$]SR (where R is 4-C$_6$H$_4$CH$_3$)	34 ± 4	800 (HFF)	Distort mitochondria and overall parasite morphology	[82]
[(η^6-*p*-Me-C$_6$H$_4$Pr^i)Ru$_2$(μ-Cl)Cl$_2$]SR (where R is 4-C$_6$H$_4$Bu^t)	62 ± 10	>1000 (HFF)		
[(η^6-*p*-Me-C$_6$H$_4$Pr^i)$_2$Ru$_2$(μ$_2$-SCH$_2$-C$_6$H$_4$-R)$_2$Cl$_2$]$_2$ (where R is 4-C$_6$H$_4$CH$_3$)	1.2 ± 0.5	5129 (HFF)	Interferes with adhesion, invasion, proliferation and intracellular establishment and interact with ribosomal proteins.	

The sterol biosynthesis pathway is another potential drug target site eliciting differential toxicity as it is unique to the *T. cruzi* parasite. Compounds containing azole functional groups have demonstrated inhibitory activity against cytochrome P450 14DM (CYP450),which is responsible for the enzymatic reaction of lanosterol 14α-demethylation through binding to the N3 site of the imidazole group within the enzyme [36].To address this potential target, Sanchez-Delgado *et al*. [44] combined the sterol biosynthesis inhibiting proper-ties of the antifungal agent clotrimazole (CTZ) with Ru as the central metal ion to produce [RuCl$_2$(CTZ)$_2$].This exhibited 90% growth inhibition of *T. cruzi* at 10-5 M with no cytotoxicity observed in mammalian vero cell lines. Another study found [RuCl$_2$(CTZ)2] to be 10 fold more active against T. cruzi than CTZ alone. However, this compound was found to have low solubility, therefore bipy (bpy = 2,2′-bipyridine) ligands were used instead of chloride, but this resulted in reduced anti-protozoal activity [44]. The original compound is therefore thought to hydrolyse inside the parasite releasing the CTZ ligand to inhibit the activity of CYP450, leaving the remaining

compound [$RuCl_2$]to interact with the DNA. Subsequent studies found that a Ru(II) compound containing p-cymene and CTZ ligands had an LD_{50} of 0.1 μM, which is 58-fold higher activity than free CTZ and 6 fold higher than the Ru compound with just the CTZ ligand [30]. In comparison, the addition of a ketoconazole ligand to form Ru-KTZ (where KTZ = ketoconazole) exhibited higher solubility and lower cytotoxicity to host cells, but had reduced antiparasitic activity than Ru-p-cymene-CTZ [36, 45]. A further compound with a CTZ ligand, [$RuCp(PPh_3)_2(CTZ)](CF_3SO_3)$] (Fig. 3b), was additionally found to exhibit a high IC_{50} value of 0.25 μM against epimastigotes, which is 30-fold higher than the commonly used drug nifurtimox. [$RuCp(PPh_3)_2(CTZ)](CF_3SO_3)$] also demonstrated molecular inhibition towards the biosynthetic pathway where squalene is converted to squalene oxide [46].

The antiparasitic activity of compounds containing both NO and phosphine ligands coordinated to Ru has also been explored. One example, *ct*-[RuCl(NO)(dpp-b)(5,5=-mebipy)]$(PF_6)_2$ (Fig. 3c), was the most effective in a series and resulted in a significant increase inNO release with subsequent induced intracellular vacuole formation. Using murine infection modelling, *ct*-[RuCl(NO)(dppb)(5,5=-mebipy)]$(PF_6)_2$ proved to be a more efficient treatment than the standard benznidazole with an EC_{50} of 2.1 ± 0.6 μM (Table 1). The compound was found to cause parasitic shrinking, cell membrane fragmentation and discontinuity in 76% of the parasitic cells, mitochondria swelling, and nuclear membrane loss leading to necrosis of the parasitic cell. Multiple mechanisms of antiparasitic activity on differing areas of the cell could prove beneficial in reducing resistance generation. When *ct*-[RuCl(NO)(dpp-b)(5,5=-mebipy)]$(PF_6)_2$ was used synergistically with benznidazole at 75 μmol/Kg and 38 μmol/Kg, 100% survival rate and lower parasitaemia was observed than with the individual treatments [42].

The compound *trans*-[$Ru(Bz)(NH_3)_4$-$SO_2](CF_3SO_3)_2$ has been shown to exhibit antiparasitic effects at low concentrations. It is also capable of catalysing nitrite to nitrosyl conversion at a low concentration of 0.4 μmol/kg and decreasing the number of parasites in the heart. *Cis*-[$Ru(NO_2)(bpy)_2(Bz)](PF_6)$ (Fig. 3d) also demonstrated high anti-trypanocidal activity and low cytotoxicity in mouse cells. Following mouse infection studies, less damage to the heart, less inflammation, and fewer parasites residing in the myocardium was observed following treatment, which was more effective than benznidazole [34]. Compounds reported by Toledo *et al*., 2005 [47] with a general formula *trans*-[RuII(NO)$(NH_3)_4$(L)]X_3 (where L = imidazole coordinated through nitrogen (imN) or imidazole coordinated through carbon(imC)), pyridine (py),L-histidine(L-hist), sulphite (SO_3^{2-}), pyrazine (pz), nicotinamide(nic), 4-picoline (4-pic), triethylphosphite ([$P(OEt)_3$]), isonicotinamide (isn), isonicotinic acid (i-na), X = BF_4^-, Cl^- or PF_6^-) (Fig. 3e) donate NO, have high solubility and are resistance to oxidation reactions[36, 47]. Antiparasitic assays with *trans*-[RuI-I(NO)$(NH_3)_4$(L)]X_3 compounds exhibited an IC_{50} value

of 244 μM (Table 1) [48], however the compounds were highly toxic to vero cell lines and less effective than current treatment options. Many other trans compounds with the same formula were found to be as effective as sodium nitroprussid (SNP) (NO donor reference), with 60% of those with the compound [Ru(NO)isn] (where isn = isonicotinamide) surviving for more than 120 days in murine modelling. The glycolysis pathway for ATP production is also vital in *T. cruzi* [49] and a study conducted by Silva *et al*., 2010 [50] confirmed that Ru compound *cis*-[RuII(NO)$(bpy)_2$(L)]X_3, where L = imidazole (imN), 1-methylimi-dazole (1-miN), or sulfite ion (SO_3^{2-}) and X = BF_4^-, Cl^-or PF_6^- (Fig. 3f) [50], affected the glyceraldehy-de-3-phosphatedehydrogenase enzyme which plays avital part in this pathway [49]. Compounds such as *cis*-[Ru$(NO_2)(bpy)_2(Bz)](PF_6)$ exhibited high efficacy at low concentrations, which was characterised by the ability to release NO into the intracellular compartments of the parasite, with no demonstrable cytotoxicity against host cells. Such compounds with high efficacy should be considered in further drug studies [34].

The compound, *trans*-[Ru$(Bz)(NH_3)_4SO_2](CF_3SO_3)_2$, has also been found to mimic iron [35], binding to transferrin and albumin and has shown high hydrosolubility against *T. cruzi* than benznidazole alone [51]. Compound *mer*-[$RuCl_3$(dmso)(H_2O)(tmt-p)]·$2H_2O$ exhibited a 21-fold higher activity than benznidazole, proving to be highly selective against T.cruzi and displayed the ability to inhibit proliferation. The compound is lipophilic as hydrophilic Tmtp can cross the membrane (log Po/w=−1.65) and active up-take facilitates transport through the cell membrane. The antioxidant enzyme Fe-SOD was significantly inhibited at 50% following the addition of *mer*-[$RuCl_3$(dmso)(H_2O) (tmtp)]·$2H_2O$, while decreased inhibition of human CuZn-SOD was observed, showing that these compounds exhibited target specificity with-out affecting comparable human enzymes [35].

Other Ru-based compounds were evaluated for their toxicity using cisplatin as a reference, where the results showed toxicity against cancer cells and it was hypothesized that these compounds interact with ctDNA by forming ternary complexes [52]. A Ru complex *trans*-[Ru$(tzdt)(PPh_3)_2$(bipy)]PF_6 (Fig. 3g) displayed the highest antiparasitic activity against T. cruzi in a concentration-dependent manner compared to the other agents examined in this study and had a high selectivity index. Additionally, the compound displayed a high ctDNA binding constant of 4.9 $x10^3$ M^{-1}. It was further suggested that the presence of bipy ligands and a net molecular positive charge contributed to the overall antiparasitic efficacy. At 0.1 μM, this compound had similar activity to benznidazole and reduced the number of parasites infecting macrophages. Synergistic studies with benznidazole showed a further reduction in macrophage infection rate [10].

316 The compound $[RuCl_2(HL_4)(HPTA)_2]Cl_2$, where HL = bioactive 5-nitrofuryl containing thiosemicarbazones and
317 PTA=1,3,5-triaza-7-phosphaadamantan (Fig. 3h), demonstrated high selectivity and inhibitory activity against T.
318 cruzi with a half-maximal inhibitory concentration of 84.2 ±1.3 μM and 85.2 ±1.9 μM for *T. cruzi* epimastigotes
319 and trypomastigotes respectively [37, 53]. This compound was presumed to induce oxidative stress within the
320 parasite and interact with the parasitic DNA [54]. Synergy between the metal complexes and commonly used
321 medication could pro-vide useful combination therapy, thus preventing the possibility of resistance evolution and
322 proving a more effective treatment against one of the most deadly tropical diseases known to WHO [55]. The
323 addition of thiosemicarbazone is thought to allow intracellular reduction of a nitro moiety and production of
324 ROS that can damage the parasitic cells and improve the com-pound's overall effect. The lipophilicity of these
325 com-pounds increases as the N-substituent changes from hydrogen to phenyl. $[RuCl_2(HL_4)(HPTA)_2]Cl_2$ proved
326 to be the most effective compound out of those examined, with 30% parasitic inhibition, due to the production of
327 free radicals and oxidative stress. In turn, the parasitic cell experienced shrinking within the cytoplasm and
328 reduced overall cell size. DNA damage was observed in in vitro assays as the Ru complexes were effective at
329 binding to calf thymus DNA where intercalation occurs, lengthening DNA helix and allowing for covalent
330 bonding to bend the helix reducing its viscosity [37]. 5-Nitrofuran derivatives also demonstrated activity by
331 reducing the nitro group and releasing ROS resulting in subsequent oxidative stress within the parasite [36].
332 $[RuIICl_2(dmso)2L$ (Fig. 3i) caused DNA binding and free radical production in vivo with *T. cruzi* with high
333 hydrophilicity and capacity to bind to proteins [56].
334 In summary, Ru compounds act upon *T. cruzi* in many ways, including NO release and donation, mimicking iron
335 to enter the cell through specific channels, and the presence of a high positive charge, which potentially
336 facilitates DNA intercalation. Targeting multiple sites within the parasite leads to improved efficacy, which
337 would potentially reduce the concentration and duration of treatment.
338
339
340 **4.2** ***African trypanosomiasis***
341 African trypanosomiasis is a sleeping sickness caused by *T. brucei gambiense* and *T. brucei*
342 *rhodesiense* parasites affecting 60 million people and a third of livestock [57]. The infection is vector-borne and
343 mediated by the tsetse fly, and following exposure, para-sites can cross the blood-brain barrier causing
344 significant morbidity and mortality. Melarsoprol B and Eflornithine are some of the drugs commonly used for

treatment [37]. Another current treatment is aminophenylarsenic acid but it must be administered in high doses for a prolonged duration. However, a significant side effect is a blindness due to atrophy of the optic nerve [58].

A series of Ru compounds were synthesised to target DNA replication within *T. brucei* by intercalating polycyclic aromatic ligands between β-form helix base pairs, allowing subsequent interaction with the parasitic DNA [59]. [RuCl(η^6-p-cym)(1,10-phenanthro-line-5,6-dione)][PF_6] (Fig. 4a) caused coiling and kinking of DNA, [$RuCl_2$(η^6-p-cym)(phenanthridine)] (Fig.4b) caused knots and kinks with sharper angles; [Ru-Cl(η^6-p-cym)(5-amine-1,10-phenanthroline)][PF_6](Fig. 4c) knotted and created breaking points thus affecting DNA replication, preventing essential proteins being made leading to parasite death. [RuCl(η^6-p-cym)(1,10-phenanthroline-5,6-dione)][PF_6] was the most potent of the compounds examined, with an IC_{50} of 190 nM (Table 1) and had similar mammalian cytotoxicity profiles to the well-characterized anti-cancer platinum-based metallotherapeutic cisplatin, rendering it viable for use in humans [57].

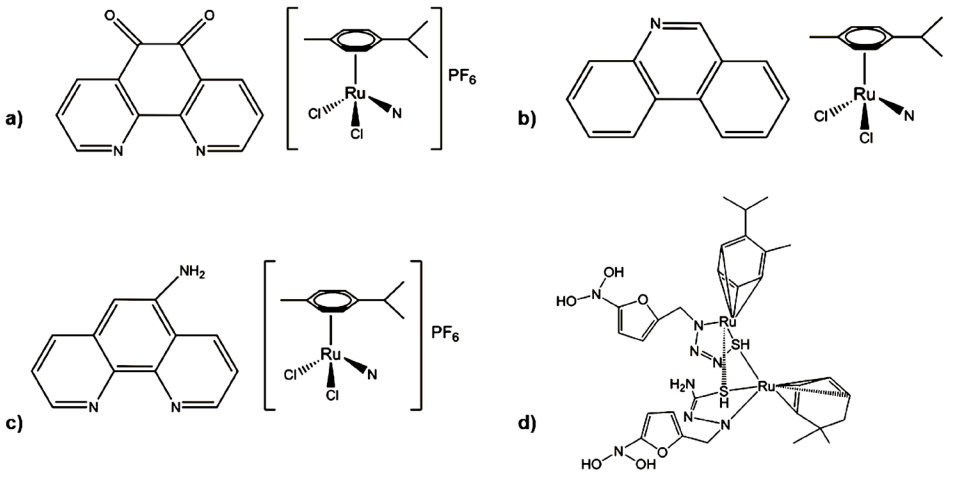

Fig. (4). Ruthenium metallotherapeutics which demonstrated antiparasitic activity against *Trypanosoma brucei*, (a) [RuCl(η^6-p-cym)(1,10-phenanthroline-5,6-dione)][PF_6], (b) [$RuCl_2$(η^6-p-cym)(phenanthridine)], (c) [RuCl(η^6-p-cym)(5-amine-1,10-phenanthroline)][PF_6] [57], (d) [Ru_2(*p*-cymene)$_2$(L)$_2$]X_2. [60]

T. brucei uses a receptor-mediated endocytic mechanism to incorporate cholesterol into its cytoplasm [59]. Due to the increased concentrations of intracellular cholesterol, the Ru compound RuCpCTZ is 40-fold higher against the infective form of T. brucei with an IC_{50} of 0.6 μM. This suggests that within the CTZ complex the RuCP moiety is the determining factor for the overall observed antiparasitic activity. Additionally, RuCpCTZ

demonstrated a lower IC_{50} than previously documented $[Ru_2(p\text{-cymene})_2(L)_2]X_2$ complexes, where L = 5-nitrofuryl containing thiosemicarbazones and X = Cl^- or PF_6^- (Fig. 4d) against T. brucei brucei strain number 427 [60]. The mechanism of antiparasitic action of the CTZ complex involves the inhibition of α-14 C demethylase (an essential enzyme in the sterol membrane biosynthesis pathway), causing the conversion of lanosterol to ergosterol to cease and lanosterol to accumulate, altering the permeability of the cell wall.

5. Leishmaniasis

Leishmaniases are a group of diseases caused by the *Leishmania* protozoan parasites, which cause vector-borne infection through the bite of an infected female plebotomine sandfly [37, 61]. There are twenty *Leishmania* species and it is estimated that infection results in over 70,000 deaths annually [11]. There are three forms, cutaneous where persistent ulcers or nodules form on the body, mucocutaneous lesions destroy mucous membranes such as the nose, mouth, and genital areas [62] and visceral, which exhibits symptoms such as weight loss, fever, anaemia, hepatosplenomegaly resulting in subsequent mortality [63]. Coinfection with HIV and drug abuse increases the risk of contracting the disease in visceral and cutaneous forms [63]. Current treatments are complex and have a high propensity for resistance generation, combined with un-wanted side effects, high toxicity, and financial burden [63]. Treatments include antimonials, amphotericin B, pentamidine (Fig. 1k), and meglumine antimoniate, which have been used for over 40 years with little further progress in treatment options [35]. *Leismania* species have effective mechanisms to evade the host immune response, including the suppression of inducible NO synthase in macrophages leading to a decrease in NO production, thus allowing parasitic replication [64].

The use of Ru-based treatment therapy in Leishmania infections has been explored due to its low toxicity and high efficacy. They also have the ability to exchange ligands in vivo, which changes the activity of the compounds [65]. The octahedral geometry of Ru permits coordination to molecular targets [56], variable redox potentials, and the ability to bind biomolecules such as serum proteins and DNA [66].

Compounds with a general formula *cis*-$[RuII(\eta^2\text{-}O_2CR)(dppm)_2]PF_6$, where dppm = *bis*(diphenylphos-phino)methane and R = 4-butylbenzoate (bbato) (Fig.5a), have demonstrated high levels of activity towards different *Leishmania* species except *L. braziliensis* and were also found to have low cytotoxicity (10%) against host macrophages [61]. Exchanging chloride groups for chelating ligands increased the biological activity of the compound by increasing the overall molecular positive charge, therefore permitting covalent interactions with DNA more readily.

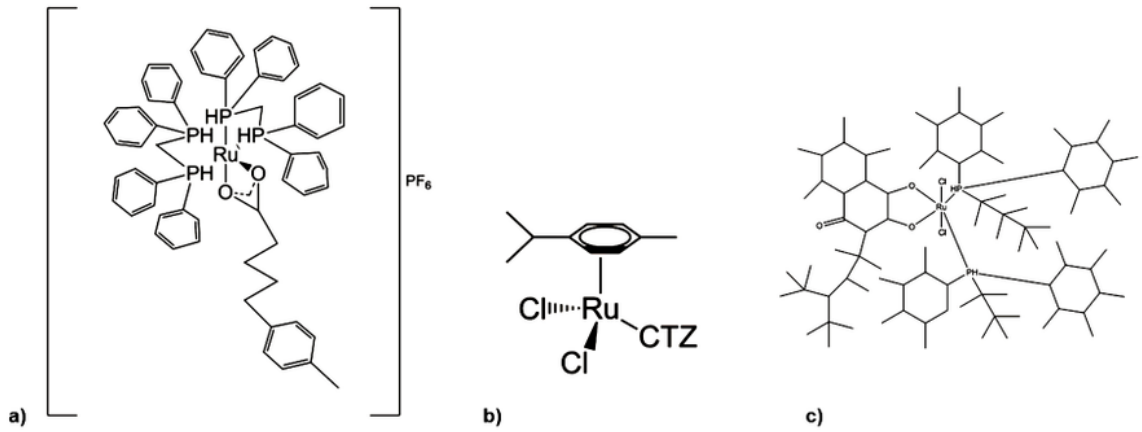

Fig. (5). Ruthenium metallotherapeutics which demonstrated antiparasitic activity against *Leishmania* species, (a) cis-[RuII(η^2-O_2CR)(dppm)$_2$]PF_6 [61], (b) [Ru(η^6-p-cymene)Cl_2(CTZ)] [30], (c) [$RuCl_2$(Lap)(dppb)] [11].

Superoxide dismutase (SOD) antioxidant enzymes are found within mitochondria and are differentiated by metal cofactors. SOD with iron cofactors has also been the target for the development of novel anti-leishmania treatments [67]. The compound *cis,fac*-[$RuCl_2$(dmso)$_3$(tmtp)] demonstrated a 3-fold increase in anti-leishmania activity compared to meglumine antimoniate against *L. brasiliensis* and proved to be less toxic to macrophages and more lipophilic. This com-pound was also found to display high selectivity to Fe--SOD and caused 70% of SOD activity [35]. A further compound, [Ru(η^6-p-cymene)Cl_2(CTZ)] (Fig. 5b), demonstrated high efficacy against *L. major* compared to treatment with CTZ alone and was also less toxic to the human host proving to be a promising compound for progression to further studies [30]. Lapachol-Ru complexes have also been explored and were biologically more active than the free ligand as they have the ability to inhibit *L. amazonensis* promastigote proliferation [11].

Two compounds [Ru(Lap)(PPh_3)$_2$(Me-bipy)]PF_6.CH_3OH and [$RuCl_2$(Lap)(dppb)] (Fig. 5c) had similar antiparasitic activity to amphotericin B but were shown to be non-cytotoxic and exhibit higher selectivity indexes [11].As previously discussed, some Ru compounds act as NO donor/releasing compounds, such as *cis*-[Ru(bpy)$_2$$SO_3$(NO)]$PF_6$, which also has high water solubility to enable entry into parasitic cells, while

maintaining low cytotoxicity to host cells thus making them ideal candidates against *Leishmania* species [64]. The increase in intracellular NO levels caused by NO-re-leasing compounds has been shown to result in a decrease in promastigote levels to the point of eliminating the infection, while maintaining host macrophage activity during the treatment course [64]. Further studies using electron microscopy showed that Ru-based compounds such as [RuCl(CTZ)(η^6-p-cymene)(PPh_3)]PF_6 and [RuCl(KTZ)(η^6-p-cymene)(PPh_3)]PF_6 have physical effects on *Leishmania* species. These observations included reduced flagella length, swelling of the mitochondria with subsequent leakage, kinetoplast disorganisation, abnormal condensation of chromatin, the appearance of vacuoles containing cellular debris and an overall reduction in cell size. Additionally, membrane protrusions were visible, but further work is required to demonstrate the in vivo effects of these compounds [68].

6. Amoebiasis

Amoebiasis is caused by the protozoan *Entamoeba histolytica* and is an amoebic dysentery disease that affects 50% of the population in developing countries and can lead to liver abscess formation and results in over 100,000 deaths annually, being the 4th leading cause of parasitic death [69]. Additionally, it is the second leading cause of death by a protozoan parasite after malaria. Infection occurs through the consumption of contaminated food sources [70]. Current first-line drug treatments such as metronidazole and nitroimidazole generate nitroso free radicals to combat infection, however these cause unwanted side effects and an increase in resistance [69]. Metronidazole is commonly used in high dosages for prolonged periods causing host peripheral neuropathy with sensory disturbances and resistance is now emerging due to treatment stipulations [71]. Likewise, treatment with nitroimidazoles results in significant host morbidities such as irritation of the gastric mucus lining, headache, vomiting, diarrhoea, haematuria and occasional toxicity within the central nervous system [70].

Ru (III) ionic derivatives of compounds become active in a hypoxic environment, such as the colon, due to the subsequent reduction and conversion into the more biologically active Ru (II) state. Given *E. histolytica* colonises the colon, Ru (III)-based compounds have been explored as treatment options for amoebiasis infections [72].

Under aerobic conditions, a reduction reaction with metronidazole treatment generates nitro radicals which bind to DNA and enzymes within the parasite. Metals are thought to reduce the likelihood of enzymatic degradation of metronidazole, therefore combined with the Ru metallotherapeutic, [Ru(metronidazole)$_2$(Cl)$_2$(H_2O)$_2$] (Fig. 6a), this exhibited an IC_{50} value of 0.51μM against *E. histolytica* which was lower than metronidazole alone

(IC_{50} = 1.81 μM) showing that combining Ru compounds with metronidazole produced greater effects against parasites [71].

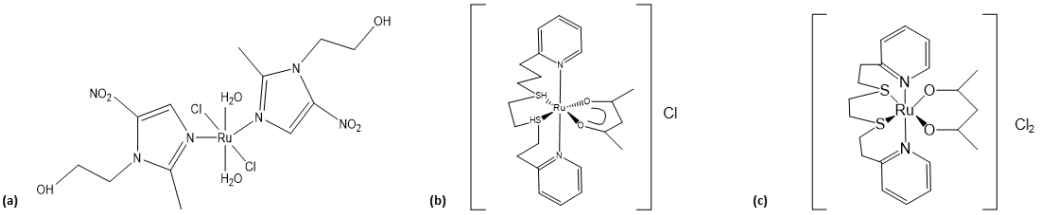

Fig. (6). Ruthenium metallotherapeutics which demonstrated antiparasitic activity against *Entamoeba histolytica*, (a) [Ru(metronidazole)$_2$(Cl)$_2$(H_2O)$_2$] [71], (b) [Ru(acac)(pdto)]Cl [69], (c) [Ru(pdto)(acetylacetonate)]Cl [70].

As previously discussed, Ru-based compounds are known to interact with DNA and bidentate ligands. In a study by Toledano-Magaña *et al*., 2017 [69], trophozoites of *E. histolytica* were targeted with [Ru(a-cac)(pdto)]Cl, where pdto =2,2′-[1,2-ethanediylbis-(sulfanediyl-2,1-ethanediyl)]dipyridine and acac = acetylacetonate (Fig. 6b), and a 50-100-fold increase in efficacy was observed when compared to traditional treatments. This compound caused rounding of the parasite and nuclear membrane damage, thus inducing apoptosis in 90% of amoeba cells. In murine models, 100% of parasites were eliminated after 24 hours with a dose of 5 mg/kg administered every 12 hours. Crucially, there was no evidence of parasitic infection after 5 days post treatment and there was a reduction in observed inflammation within the liver [69].

In another study [70], twenty Ru compounds were tested with a general formula of [Ru(pdto)(E-E)]Clx(E-E bidentate, either neutral or negatively charged ligands). [Ru(pdto)(acetylacetonate)]Cl (Fig. 6c) was the most effective of the treatments studied with an IC_{50} of 0.06 μmol/L due to the metal having high water solubility. Low mammalian cytotoxicity was observed, despite an increase in the production of parasitic intra-cellular ROS. Ru compounds with an O-O and N-O donor were more readily oxidised and exhibited better antiparasitic activity than N-N ligands [70].

7. Toxoplasmosis

Toxoplasmosis is caused by the parasite *Toxoplasma gondii*, which when latent will reside within the host central nervous system. Transmission can be conveyed through food, water, and the placenta where it can replicate within the mammalian gut [73]. In the acute form, tachyzoites, fast replication allows the parasite to invade and target host cells and additionally secrete proteins to allow survival and immune evasion. Colonisation of monocytes results in hypermigration, permitting systemic dissemination through the host [74, 75]. This includes invading epithelial cells within the blood-brain barrier where parasitic replication causes cells to lyse enabling entry to the brain parenchyma [76]. Conditions caused by *T. gondii* include myocarditis, encephalitis, and blindness, and treatment is normally through the administration of sulfadiazine (Fig.1l) and pyrimetham, with leucovori supplementation which inhibits dihydrofolate reductase [77, 78]. Treatment for toxoplasmosis is complex as tachyzoites can establish drug-resistant bradyzoite cysts leading to persistent infection [79]. As 80% of primary infections are asymptomatic, there is significant potential for disease progression [80].

Metallotherapeutic Ru compounds could provide an alternative treatment option, in addition to combatting potentially drug-resistant forms of the disease. Two hydrolytically stable Ru phosphite compounds with additional hydrocarbon exteriors [Ru(η^6-p-cymene)(tBu2acac)(P(OiPr)$_3$)][BF_4] (Fig. 7a) and [Ru(η^6-p-cymene)(tBu2acac)(P(OEt)$_3$)][BF_4] (Fig. 7b) were synthesised for use against *T. gondii* and demonstrated IC_{50} values of 18.7 and 41.1 nM respectively. However, [Ru(η^6-p-cymene)(tBu2acac)(P(OiPr)$_3$)][BF_4] required a prolonged treatment course of between 22 and 27 days of tachyzoites in a human foreskin fibroblast model. These compounds functioned at multiple sites within the parasite and impaired the metabolic activity of the tachyzoites by creating lipid-containing or empty inclusions, in addition to causing a distorted nuclear membrane. While highly effective, the observed duration of treatment in vitro may be prohibitively long for in vivo applications [81].

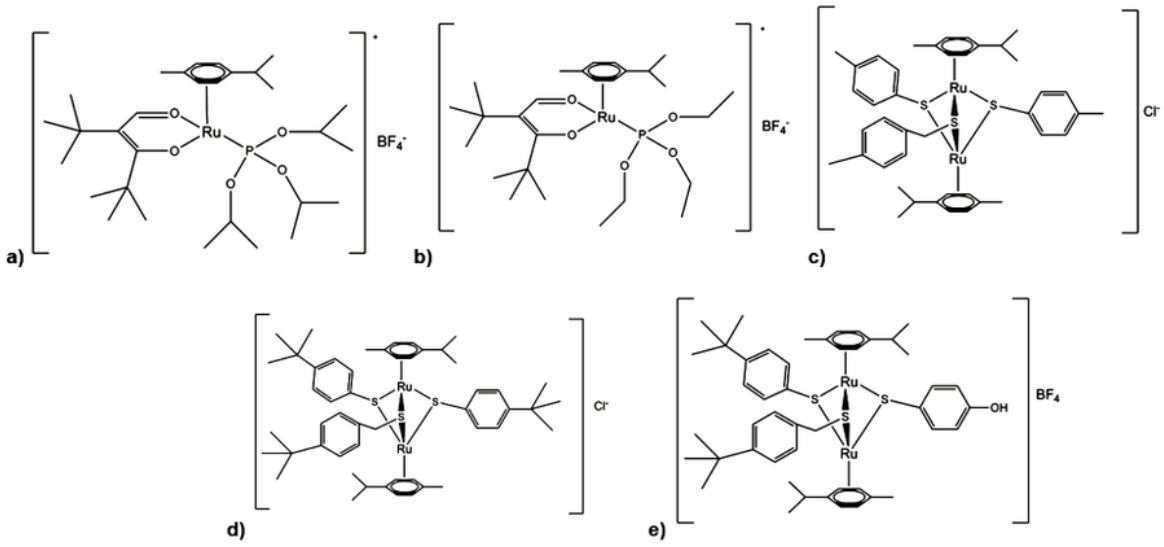

Fig. (7). Ruthenium metallotherapeutics which demonstrated antiparasitic activity against *Toxoplasma gondii*. (a) [Ru(η^6-p-cymene)(tBu_2acac)($P(OiPr)_3$)][BF_4], (b) [Ru(η^6-p-cymene)(tBu_2acac){$P(OEt)_3$}][BF_4] [82], (c) [(η^6-*p*-Me-$C_6H_4Pr^i$)Ru_2(μ-Cl)Cl_2]S4-$C_6H_4CH_3$. (d) [(η^6-*p*-Me-$C_6H_4Pr^i$)Ru_2(μ-Cl)Cl_2]S4-$C_6H_4Bu^t$, (e) [(η^6-*p*-Me-$C_6H_4Pr^i$)$_2Ru_2$(μ_2-SCH_2-C_6H_4-R)$_2Cl_2$]$_2$ Where SCH_2R is 4-$C_6H_4CH_3$. [82]

Compounds [(η^6-p-Me-C_6H_4Pri)Ru_2(μ-Cl)Cl_2]SR(where R is 4-$C_6H_4CH_3$) (Fig. 7c), [(η6-p-Me-C_6H_4Pri)Ru_2(μ-Cl)Cl_2]SR (where R is 4-C_6H_4But) (Fig.7d), and [(η6-p-Me-C_6H_4Pri)$_2Ru_2$(μ2-SCH_2-C_6H_4-R)$_2Cl_2$]$_2$ (where SCH2R is 4-$C_6H_4CH_3$) (Fig. 7e) were the most effective compounds in a study by Basto *et al*. (2017) [82], where IC_{50} values were observed of 34nM, 62 nM, and 1.2 nM respectively. However, [(η6-p-Me-C_6H_4Pri)Ru_2(μ-Cl)Cl_2]S4-$C_6H_4CH_3$ was cytotoxic to human foreskin fibroblasts (HFF) with an IC50 against the parasite of 800 nM whereas [(η6-p-Me-C_6H_4Pri)Ru_2(μ-Cl)Cl_2]S4-C_6H_4But had an IC_{50} of >1mM. Interestingly, compound [(η6-p-Me-C_6H_4Pri)$_2Ru_2$(μ2-SCH_2-C_6H_4-R)$_2Cl_2$]$_2$ demonstrated the lowest IC_{50} against the parasite of 1.2 nM but was the least cytotoxic to cell lines with an observed IC_{50} against HFF of >5 mM indicating that this has the potential for future therapeutic applications. Furthermore, [(η6-p-Me-C_6H_4Pri)$_2Ru_2$(μ2-SCH_2-C_6H_4-R)$_2Cl_2$]$_2$ interacted with TgTEF1α, which is specific to the importation of mitochondrial tRNA [83, 84], and was able to inhibit the invasion of host cells by the parasite [85]. Although biologically relevant IC_{50} values were observed, it was further shown that the three compounds in this series acted in a parasitostatic manner, which might limit the

application as an independent treatment option. However, further studies regarding the potential synergistic activity between these novel metallotherapeutics combined with traditional treatments could be explored with a view to reducing disease progression [82].

Ru metallotherapeutics as anti-toxoplasmosis agents act through the targeting of parasite-specific mitochondria, distorting membranes and inhibiting invasion of host cells. However, more research is needed to reduce treatment duration and identify ligands with improved bioactivity to elicit improved treatment responses.

8. Orphan Diseases

8.1 *Lymphatic filariasis*

Setaria cervi is the causative agent of filariasis in the bovine host where it resembles the antigenic pattern and nocturnal periodicity as the human parasite *Wuchereria bancrofti* [86]. Some Ru-based com-pounds have been found to inhibit topoisomerase II in Setaria cervi with varying efficacy depending on the number of uncoordinated and coordinated nitrogen atoms and mononuclear or binuclear configuration. These compounds include the carbon monoxide-releasing molecules $[RuH(CO)(PPh_3)_2(paa)]BF_4$, $[RuH(CO)\ (PPh_3)_2(pbp)]PF_6$, $[RuH(CO)(PPh_3)_2(\text{-}\mu\text{-bbp})RuH(CO)\text{-}(PPh_3)_2](BF_4)_2$, $[RuH(CO)(PPh_3)_2(\eta^2\text{-tptz})]BF_4$, $[RuH(CO)(PPh_3)_2(\eta 2\text{-bppz})]BF_4$ and $[RuH(CO)(PPh_3)_2(\eta^2\text{-bp-pz})]PF_6$, which have all been found to exhibit inhibitory activity against topoisomerase II [87].

8.2 *Schistosomiasis*

Trans-$[Ru(bpy)_2(NO)SO_3](PF_6)\text{-}PF_6$ has been investigated due to the NO-releasing potential of the molecule with a view to treating infections caused by the parasitic flatworm *Schistosoma mansoni* [88]. Both eggs and worms were eliminated upon treatment and infection-associated liver inflammation was also reduced. The NO released by the donor stimulated the production of ROS and caused nitration and nitrosilation to render the parasite nonviable [89]. The action of *trans*-$[Ru(bpy)_2(NO)SO_3](PF_6)\text{-}PF_6$ increased mitochondrial NADH oxidation and subsequently caused the opening of permeability transition pores, releasing cytochrome c, causing cell death [89]. Another two compounds, Rubb12-tri and Rubb7-tnl, both demonstrated high adult parasite mortality (EC_{50} values 3.489 ±0.532 μM and 6.829 ± 0.625 μM, respectively) and reduced egg hatching upon incubation at 50 μM due to an increase in the inhibition of acetylcholinesterase [90].

8.3 *Strongyloidiasis*

NO-releasing molecules can also be used against strongylodiasis, caused by the parasite *Strongyloides* species. In a study conducted by Ruano *et al*. (2015) [91], treatment with NO donors reduced infection caused by

Strongyloides venezuelensis in murine models and prevented hyperinfection caused by traditional treatment with dexamethasone.

8.4 *Trichuriasis*

In over 100 countries, 4.5 billion people are at risk from a range of parasitic infections causing chronic and insidious effects to the host rather than death [92]. Acetylcholinesterases are the main virulence factor of the parasitic *Trichuris sp*. nematodes, which cause trichuriasis and target human nerve cells. Compounds based on Ru polypyridal complexes such as$[Ru(phen)_2(bxbg)]^{2+}$ (where phen = 1,10 phenanthroline, bxbg = bis(o-xylene)bipyridine glycoluril) have been found to inhibit the action of these enzymes, proving to be a promising therapy [93]. These compounds exhibit their activity by electrostatically and hydrophobically interacting with the peripheral anionic site on the enzyme and not interacting directly with the active site, thus proving less toxic to mammalian cells. Compounds such as Rubb7-tl and Rubb12-tl demonstrated a range of activity against nematodes of 18-76%, with the greatest inhibitory action against both worms and faecal eggs of *T. muris* as shown by murine modelling [94]. As *T. muris* studies are used as a model for *T. trichuris* infection in humans [95], it is possible that Rubb type compounds could be effective against human disease.

9. Conclusion

The development of effective antiparasitic chemotherapy remains a challenge, with very few novel therapeutics being developed. Indeed, many current treatment options were identified and/or developed over forty years ago. Identifying novel drug targets within parasites further remains a challenge due to the complexity of the eukaryotic cell and requirements for differential toxicity within the host. Due to the length of treatment required to resolve parasitic infections, coupled with reduced differential toxicity leads to significant drug-induced morbidity. Many Ru metallotherapeutics described in this review exhibit low IC_{50} values and demonstrate high selectivity indices. Due to the propensity for Ru coordination chemistry, it is possible to use intelligent drug design to modify lead drug candidates to improve efficacy and minimize off-target effects. Although some Ru-based compounds are cytotoxic to mammalian cells and have not yet been tested on murine models, the initial results (Table 1) are promising with most compounds exhibiting low cytotoxicity against cell lines with low concentrations of the com-pound required to produce significant antiparasitic effects. The most promising current lead Ru therapeutic candidates include RAPTA 7-chloroquinoline derivatives and [Ru(Lap)(PPh3)2(phen)]PF6 for the treatment of malaria, *trans*-$[Ru(tzdt)(PPh_3)_2(bipy)]PF_6$ for treatment of American trypanosomiasis, $[RuCl(\eta^6\text{-p--cym})(5\text{-amine-1,10-phenanthroline})][PF_6]$ for African trypanosomiasis,

567 *cis-fac*-[$RuCl_2(dmso)_3(tmtp)$] for leishmaniasis and [Ru(acac)(pdto)]Cl for amoebiasis. The synergy between
568 novel Ru-based compounds and conventional antiparasitic agents may provide a solution to the issue of drug-
569 induced host cytotoxicity by reducing the required treatment concentration and/or therapeutic exposure period.
570 Coordinating current treatments such as CZT to Ru scaffold structures also represents a promising avenue for
571 further research to develop the next generation of novel metallotherapeutics treatments to help combat neglected
572 parasitic infections in the future.

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